Some Studies in the Synthesis of Natural Products & their Analogues

A THESIS SUBMITTED TO THE

GOA UNIVERSITY

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

BY

Sulaksha J. Parab, M.Sc.

Department of Chemistry

Goa University

Goa

January 2009

Dedicated
To
My Grandparents

STATEMENT REQUIRED TO BE SUBMITTED UNDER ORDINANCE 19.8 OF THE GOA UNIVERSITY

No part of this thesis has been submitted for a degree or diploma or other academic award. The literature concerning the problems investigated has been surveyed and all the necessary references are incorporated in this thesis. The experimental work has been carried out independently and due acknowledgment has been made wherever outside facilities have been availed of.

(Prof. S. P. Kamat)
Research Guide

(Sulaksha J. Parab)
Candidate

CERTIFICATE

This is to certify that the thesis entitled "Some Studies in the Synthesis of Natural Products and their Analogues" submitted by Ms. Sulaksha J. Parab for the award of degree of Doctor of Philosophy in Chemistry is based on literature survey/laboratory experiments carried out by her under my supervision. The thesis or any part thereof has not previously been submitted for any other degree or diploma.

Date: 20.01.2009

Prof. S. P. Kamat
(Research Guide)

Acknowledgements

At the onset I wish to express my sincere thanks and gratitude to my research guide Dr. S. P. Kamat, Professor of Organic Chemistry, Department of Chemistry, Goa University for his constant support, encouragement, dedication and above all his understanding nature. I will cherish the memories of my research work under his guidance throughout my life.

I wish to thank Prof. S. K. Paknikar, for his valuable guidance and constant encouragement. His new ideas and discussions contributed substantially to the completion of this thesis.

I'm thankful to Prof. K. S. Rane, Prof. J. B. Fernandes and Prof. S. P. Kamat former Heads, Department of Chemistry, Goa University for extending necessary facilities.

I owe special thanks to Dr. Andre Chaperon, CIBA Specialty chemicals, Switzerland, for his guidance, encouragement and constant help throughout my research work.

I thank Prof. George Varvounis, University of Ioannina, Greece, Dr. Prakash P. Wadgaonkar & Mr. I. Shivakumar, NCL, Pune, Prof. M. Mar Herrador del Pino, Universidad De Granada, Spain, Prof. J. A. S. Cavaleiro, University of Aveiro, Portugal and Dr. Kamalesh P. P. Fondekar, CIBA, Goregaon, Mumbai, for their valuable help in spectral measurements and for elemental analysis.

A word of appreciation to Dr. C. G. Naik, Dr. Lizette D'Souza, Dr. S. Parameswaram and Dr. Solimabi Wahidullah, Bio-organic Division, COD, NIO, Goa, for their interest, encouragement and help in spectral measurements.

I thank Dr. Ramachandra Kandre, ETH Zurich, Switzerland, Dr. Asha D'Souza, CSIRO, Australia, Dr. Supriya Tilvi, IISc, Bangalore and Dr. Vaishali

Vohra, Rohm and Haas Company, Marlborough, MA, USA, for their help in Chemical Abstract searches and references.

I thank Dr. J. K. Kirtany, Dr. V. S. Nadkarni, Dr. S. G. Tilve and other faculty members of the Chemistry Department, Goa University, for all the help during the course of this work.

I gratefully acknowledge the assistance and co-operation extended to me by the Goa University Library and the Office staff and also by the Laboratory staff of the Chemistry Department.

A big thank you to my friends Jose and Savia for all their help, constant support, encouragement and cheerful company throughout the course of this work.

I am thankful to Rajesh, Divya (Bio-organic Division, COD, NIO), Mahesh, Vinod, all my research colleagues from the Department of Chemistry and my friends for all their help, support and cheerful company.

Finally, I do not have adequate words to express what I owe to my family members for innumerable acts of advice, moral support, understanding and untiring help.

Sulaksha J. Parab

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Chapter three

GENERAL REMARKS

1. All schemes, tables, structures, figures and reference numbers in the chapter/section, refer to that particular chapter/section only.
2. All melting and boiling points were recorded in degree Celsius and are uncorrected.
3. Petroleum ether refers to the fraction boiling between the range 60-80°C unless otherwise stated.
4. Ether refers to diethyl ether.
5. Silica gel used for column chromatography was of 60-120 mesh size, unless otherwise stated.
6. Thin layer chromatography was done on glass plates coated with TLC grade silica gel with 13% $CaSO_4$ as binder. Visualization of the plates were done by developing the plates in I_2 chamber, unless otherwise stated.
7. IR spectra were recorded on FTIR-8101A Shimadzu spectrophotometer.
8. Spectral data on the compounds were obtained through the courtesy of various institutions. No details of individuals are therefore given. These have been suitably acknowledged.
9. IR absorption bands are expressed in cm^{-1}. UV absorption signals are expressed in nm.
10. The chemical shift parameters in the ^{1}H and ^{13}C NMR spectra are expressed in δ ppm, with TMS as the internal standard.

    DEPT - Distortionless Enhancement by Polarization Technique.

    COSY – ^{1}H-^{1}H Correlation Spectroscopy.

    HMQC – ^{1}H-^{13}C Heteronuclear Multiple Quantum Coherance.

    HMBC – ^{1}H-^{13}C Heteronuclear Multiple Bond Correlation.

    NOE – Nuclear Overhauser Effect

11. Complete spectroscopic data of only new compounds is included in the thesis. Molecular formulae of the compound were assigned on the basis of the molecular weight as obtained by elemental analysis and mass spectrometry.
12. All known compounds were identified by direct comparison of spectral data and physical constants reported in literature.

Chapter One

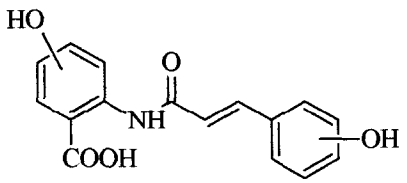
Synthesis of Avenanthramides Constituents of Oats (Avena sativa L)

Introduction

What are Avenanthramides?

Oats have been considered to be a good source of antioxidants for a long time[1] and several compounds in oats are known to have this antioxidant activity. These compounds are flavonoids, Vitamin E (tocopherols and tocotrienols), phenolic acids in free form (derivatives of benzoic and cinnamic acids) and esterified forms[2].

Recently[3] a group of amides trivially named avenanthramides has been isolated from oats. Interestingly, it has been shown that this antioxidant activity and the fresh taste of oat products is mainly due to the presence of these avenanthramides[2]. In fact, avenanthramides are the major phenolic constituents[4] occurring in relatively high concentrations, about 0.2-0.8 mg/g, in the outer regions of the oat kernel e.g. bran and sub-aleurone layers[5].



avenathramide

They are low molecular weight soluble phenolic compounds, a group of nitrogen-containing constituents, collectively named as avenanthramides which are not present in any other cereal grains[3].

Chemically avenanthramides are substituted *N*-cinnamoylanthranilate derivatives[6] i.e. they are substituted hydroxycinnamic acid amides with simple anthranilic acid or its 5- or 4-hydroxy derivatives.

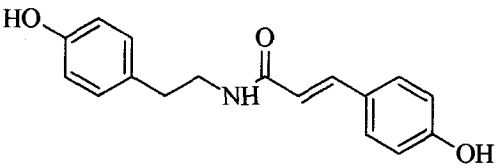
These avenanthramides are phytoalexins produced when oat leaves are infected or inoculated with an incompatible race of crown rust fungus (*Puccinia coronate* f. sp. *Avenae*)[7,8] or by the treatment of oat leaves with various elicitors

including chitin fragments[9], a host specific toxin[10] victorin C, heavy metal ions[11] and Ca ionophore[12]. Although avenanthramides have also been isolated from oat groats and hulls[3], none appear to be present in healthy leaves prior to inoculation with pathogens[6].

What are Phytoalexins?

Plants express a variety of resistance responses to parasites, including fungal infections through various defence reactions. One of these reactions is the production of inducible secondary metabolites, antifungal substances, such as phytoalexins[13]. These phytoalexins are shown to be important in preventing the growth of micro organisms at the site of the infection or rejecting the pathogens on the basis of their toxicity.

Avenanthramides belong to a sub-class of hydroxycinnamic acid amides[6]. These amides are suggested to play important role in reinforcement of cell walls. For instance hydroxycinnamic acid amide of tyramine found in solanaceous plants have been indicated to be a component of suberin that accumulates in mechanically damaged tissues[14].



N-p-coumaroyltyramine

Avenanthramides may have been recruited as phytoalexins from those compounds that function to reinforce cell walls because of their toxicity to pathogens and may still have dual role to play that is as phytoalexins in chemical defence and as substrates for the reinforcement of cell walls in physical defence[15]. The degree of contribution to these roles is dependent on the species of avenanthramides. However, it may be noted that phytoalexins are not necessarily the end products of plant metabolism.

Source of Avenathramides

Avenanthramides are unique to oats and are not present in any other cereal grains[5]. Oat phytochemicals can be roughly divided[4] into low molecular weight, readily soluble "free phenols" and high molecular weight covalently linked to complex, insoluble cell components "bound phenols".

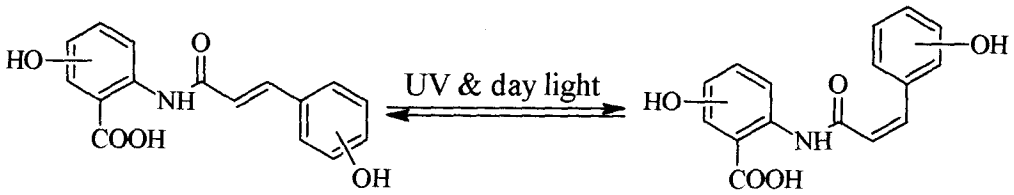
In addition to avenanthramides, the low molecular weight soluble oat phenolics include[4] tocopherols, tocotrienols, flavonoids, hydroxycinnamates, ferulic acid, caffeic acid, vanillic acid, *p*-hydroxyphenylacetic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, sinapic acid, etc.

The "free phenols" appear to represent readily absorbed sources of antioxidants in the human diet, while the insoluble "bound phenols" such as lignin, cell wall polysaccharides, structural and/or storage proteins etc. present different challenges in the attempt to evaluate their long term efficacy since they require further metabolism before absorption from the gastrointestinal tract[4].

Characteristic Physicochemical Properties of Avenanthramides

The natural avenanthramides are pale yellow to yellow-green crystalline substances having comparatively high melting points. They are soluble in organic solvents like ethylacetate, diethyl ether and aqueous mixture with acetone or the lower alcohols but are relatively insoluble in chloroform, benzene or water[3]. They are resistant to acid hydrolysis but are slowly hydrolysed with some decomposition in alkali to the corresponding substituted cinnamic and anthranilic acids[3]. Avenanthramides have an intermediate lipophilicity and seem to be heat stable[5].

In daylight and UV light, they may easily undergo *Z-E* rearrangement but without prior exposure to UV light, both *E* and *Z* isomers exist in solution as a photomediated interconvertable mixture[3].



E-isomer *Z*-isomer

To find out whether the *E* isomer only or both isomers are naturally occurring in oats would require that extraction, purification and estimation be carried out in the absence of UV and daylight[3].

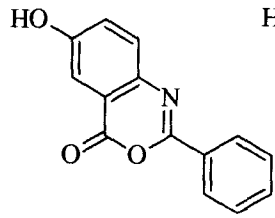
Detection, Identification and Isolation of Naturally Occurring Avenanthramides

The silica gel two dimensional TLC analysis of the extracts of oat groats and hulls based on chromatographic diagnostic colour responses and preliminary MS data has revealed that there are at least 40 chromatographically distinct avenanthramides in addition to other related phenolics[3]. This diversity of closely related large number of phenolics coupled with their individual occurrence in very small quantities has made detection, isolation and identification of avenanthramides difficult[3]. Therefore very few avenanthramides have been individually 'isolated' and characterized.

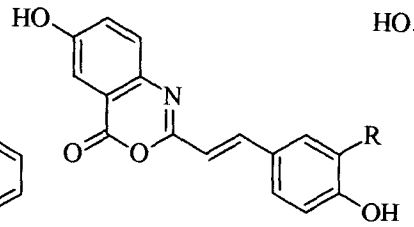
To our knowledge, till date, only 17 different avenanthramides isolated from oats are known in the literature with their structures and spectroscopic data. In fact, it is not proper to say that they have been isolated because except one i. e. avenanthramide A which was isolated by Miyagawa et al[12], as yellow amorphous powder (15.7 mg), all other remaining 16 avenanthramides have been detected in oat extracts and identified[4] by comparison of the retention times and UV and or MS data of their individual peaks in the HPLC chromatograms with those of synthetic standards.

Avenanthramides were formerly called avenalumins (benzoxazin-4-one derivatives) by Mayama et al who first reported[7] in 1981 the occurrence of nitrogen containing phytoalexins in oat leaves. They were the first to isolate 100 mg (from 10 kg of infected fresh oat leaves) of a major oat phytoalexin and

named it as avenalumin I followed by related minor components[7] avenalumin II and III.

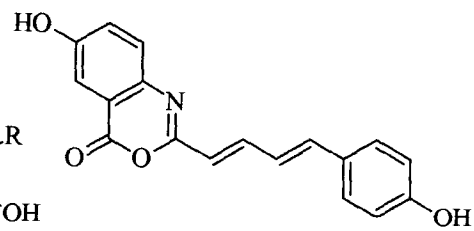


4*H*-3,1-benzoxazin-4-one



avenalumin I R = H

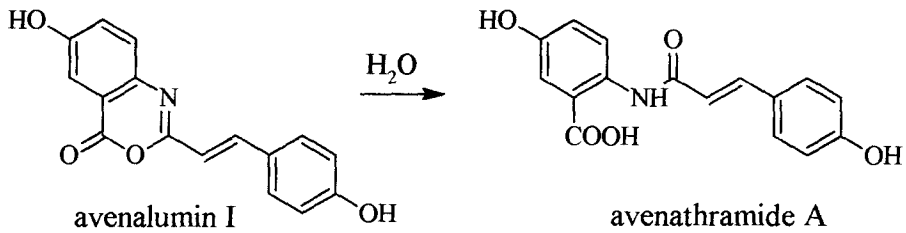
avenalumin II R= OCH_3



avenalumin III

Later on in 1989, Collins[3] carried out fractionation of the methanolic extracts of oat groats and hulls using ion exchange column chromatography followed by analytical and preparative TLC and showed for the first time the presence of a group of closely related oat phytoalexins as open amides and trivially named them avenanthramides.

He also observed[3] that 2-aryl-1,3-benzoxazin-4*H*-ones readily undergo hydrolysis in aqueous media to give the corresponding (*N*-aroylamido)benzoic acids and anticipated that the major biologically active phytoalexin may be the open amide (avenanthramide) rather than the benzoxazinone derivative (avenalumin) as reported[7] by Mayama et al.



avenalumin I

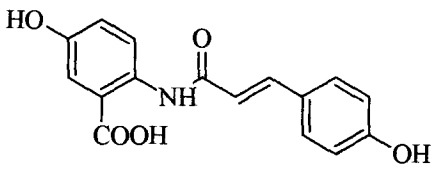
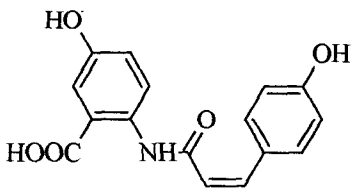
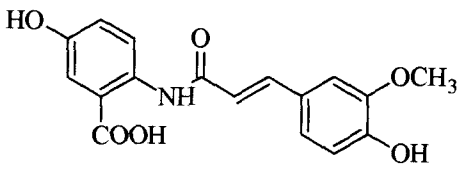
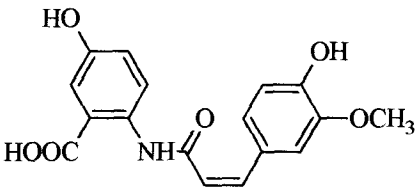
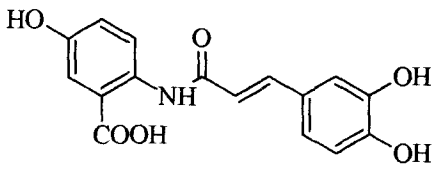
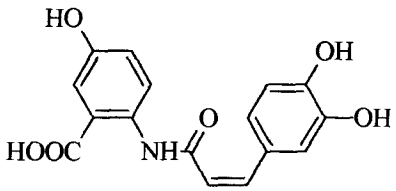
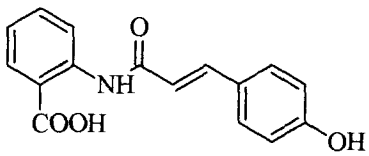
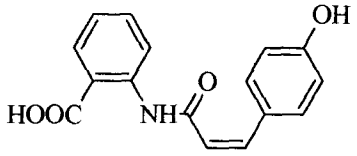
avenathramide A

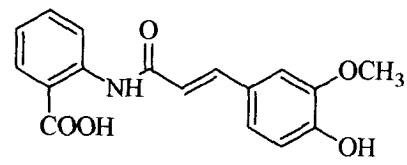
It may be noted that avenalumins were isolated[7] from oat leaves and not from oat grain. Experiments using non-aqueous extraction solvents and/or rapid identification procedures may be necessary to establish the presence of avenalumins in the oat grain.

However, in 1990 Crombie and Mistry[8], recorded spectroscopic data on avenalumins prepared by them and comparison of this data with that reported by Mayama et al[7] clearly indicated that the natural phytoalexin from oat leaves isolated[7] is in fact the avenanthramide A and not avenalumin I.

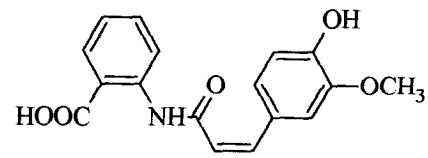
Further, Collins[3] succeeded in separation, purification and identification of ten avenanthramides using ^{1}H & ^{13}C NMR, MS & UV and this constituted the first report on the isolation of avenanthramides from the oat extracts (Table 1).

Table 1: First report[3] on isolation of avenanthramides from oats

 avenanthramide A	 avenanthramide A-1
 avenanthramide B	 avenanthramide B-1
 avenanthramide C	 avenanthramide C-1
 avenanthramide D	 avenanthramide D-1

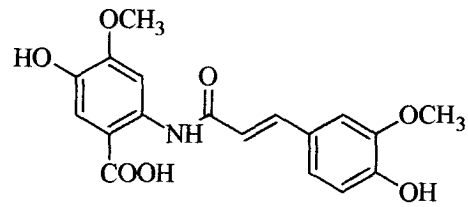


avenanthramide E



avenanthramide E-1

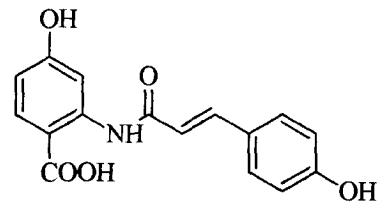
The only avenanthramide having -OCH_3 and -OH groups at C_4 and C_5 positions of the anthranilic acid moiety named avenanthramide A-2 was detected and identified along with avenanthramide B from the extracts of oat bran and was shown to have the structure shown below[5]. Although the concentration of A-2 was too low to be estimated, it was found to have significantly higher antioxidant activity than that of caffeic acid, ferulic acid and vanillin.



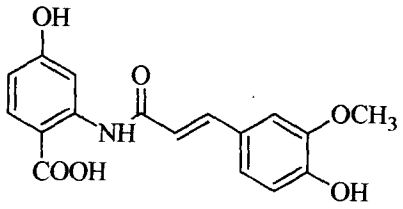
avenanthramide A-2

While studying the action of avenanthramides in the modulation of the inflammatory process associated with the development of atherosclerocis, the following four avenanthramides G, H, K and P were detected[4] by using analytical HPLC. Individual peaks were identified by comparison of their retention times and UV spectra with authentic standards.

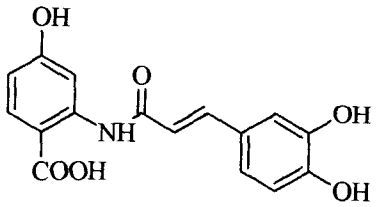
Formation of avenanthramides G and L was observed[6] during the study of the biosynthesis of avenanthramides by administering labelled putative precursors to oat leaves.



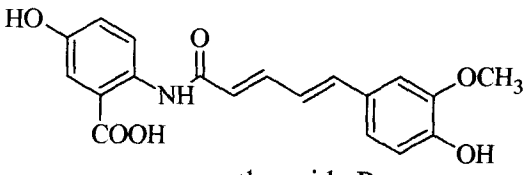
avenanthramide G



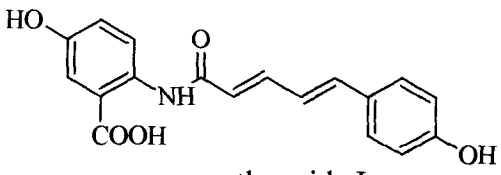
avenanthramide H



avenanthramide K

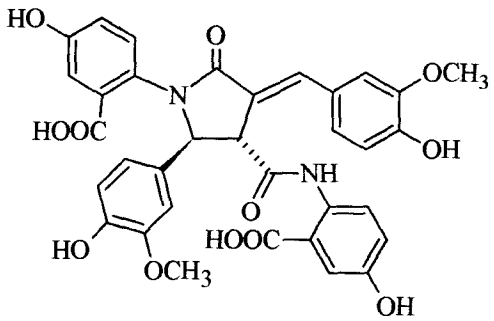


avenanthramide P



avenanthramide L

Recently a new dimerized avenanthramide named bisavenanthramide B was found to accumulate when oat leaves were treated with elicitors due to a reaction of avenanthramide B (an oat phytoalexin) with peroxidase in the presence of hydrogen peroxide[16].



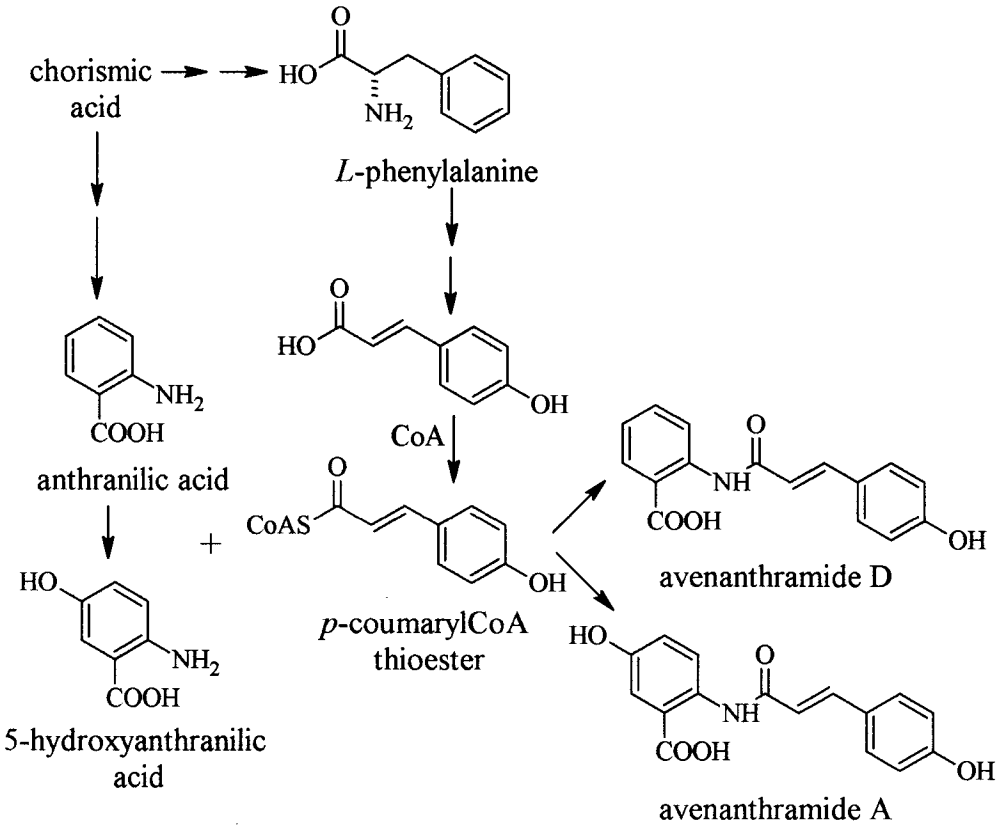
bisavenanthramide B

Biosynthesis of Avenanthramides

The chemical structure of avenanthramides suggests that these compounds are biosynthesised by the condensation of cinnamic acid derivatives with anthranilates or hydroxyanthranilates[6]. It has been demonstrated by administering labelled putative precursors to oat leaf segments that

avenanthramides are *de novo* synthesised from primary metabolites and phenylpropanoid metabolism is involved in their biosynthesis[6].

The biosynthesis of avenanthramides has been investigated with feeding experiments and measurement of enzyme activities. Feeding of elicited oat leaves with labelled *L*-phenylalanine and anthranilic acid revealed that avenanthramides are produced from these precursors[6]. Anthranilic acid and *L*-phenylalanine are derived from chorismate biosynthesised via the shikimate pathway as shown below in a proposed biosynthetic pathway to avenanthramides.



Scheme 1: Proposed[6] biosynthetic pathway to avenanthramides

Anthranilate synthase and chorismate mutase, which catalyse the first reactions leading to anthranilate and phenylalanine, respectively, have been shown to be regulated by elicitor treatment[6].

Moreover, hydroxycinnamoyl-CoA:hydroxyanthranilate *N*-hydroxy-cinnamoyl transferase (HHT), which catalyses the final condensation reaction,

was identified[6] in oat leaves treated with oligo-*N*-acetylchitooligosaccharides and victorin C.

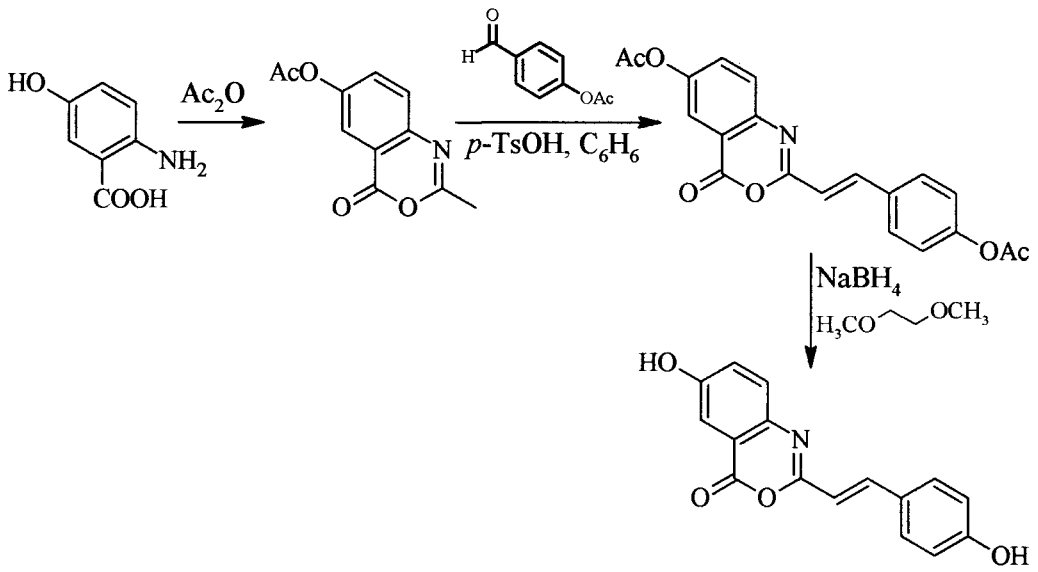
All the putative precursors of a series of avenanthramide found in the elicited leaves, acted to a greater or lesser extent, as substrates, suggesting that each of the avenanthramide was individually synthesized by the condensation of the corresponding substituted anthranilic acid and the substituted cinnamoyl-CoA thioesters[17].

Reported Methods of Synthesis of Avenanthramides

Avenanthramides have been prepared in milligram quantities mainly for the purpose of detection and identification in oat extracts[2,3], structure-antioxidant activity studies[2] and other bioactivity studies[4,18]. They are also prepared for providing support to the structure assigned[3].

First Synthesis of Avenalumin

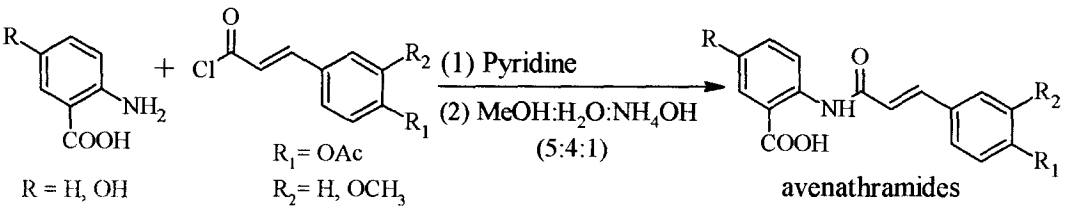
Mayama et al[7] were the first to isolate in 1981 the major phytoalexin, avenalumin I from oat leaves and confirm its structure (2-styryl-1,3-benzoxazin-4*H*-one) by its synthesis from 5-hydroxyanthranilic acid in 45% yield.



However, when Crombie and Mistry[8] repeated this synthetic scheme of avenalumin I reported by Mayama et al[7], they obtained the open amide avenanthramide A instead of the reported avenalumin I. Of course they did succeed in preparing the cyclized products (avenalumins) from the corresponding avenanthramides which were prepared by using Collin's method[3] described below. Spectroscopic data especially IR and ^{13}C NMR recorded on both avenalumins and avenanthramides indicated that the natural phytoalexin from oat leaves isolated and then synthesized by Mayama et al[7] is in fact the avenanthramide A and not avenalumin I.

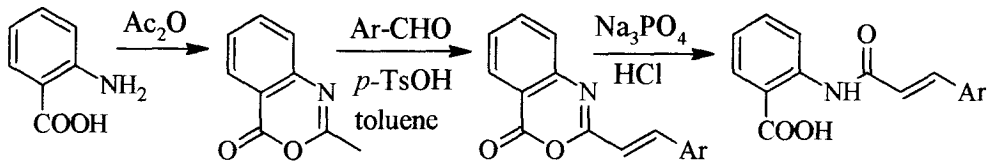
First Synthesis of Avenathramides

Collins[3] who first isolated the oat phytoalexins as open amide and named them 'avenanthramides', confirmed their structures by synthesis using a modification of the Bain and Smalley's procedure[19] in which suitably protected substituted cinnamoyl chlorides are condensed with anthranilic acid in presence of pyridine followed by treatment with mild alkali to remove the protecting group. However, these methods[3,8] involve additional protection and deprotection steps and the yields varied from 55-83%.



The structure of avenanthramide G identified[12] as a stress compound in oats, induced by victorin, a host specific toxin from *Helminthosporium victoriae* was confirmed by its synthesis using above mentioned Collin's procedure[3]. The required 4-hydroxyanthranilic acid was prepared from 2,4-dinitrobenzoic acid and condensed with 4-acetylcinnamoyl chloride prepared from 4-hydroxycinnamic acid.

Bratt et al[2] used a modified version of the method described by Mayama et al[7] and synthesized nine avenanthramides in comparatively low (≈ 40%) yields to identify them in oat extracts and to study the structure-activity relationship.



avenanthramides

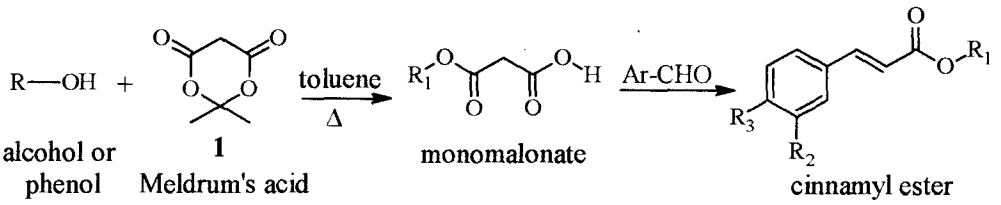
Thus there are only two methods reported in the literature for the synthesis of avenanthramides one of Collins[3] modification of Bain and Smalley[19] and the other of Bratt et al[2] modification of Mayama et al[7]. Others have used either of these two methods for the synthesis of avenanthramides.

Synthesis of Avenanthramides Using a Two-Step General Procedure

Although avenanthramides have been synthesized[2,3] in 40-80% yields, the only two methods reported utilize acid chlorides and also additional protection-deprotection steps are involved which make the synthetic process non-economical. Therefore, a need of a general and efficient method was felt to prepare these naturally occurring oat phytoalexins, avenanthramides, in good yields so that they can be easily detected in oat extracts and further to have them in sufficient quantities to study their biological activity.

In our laboratory a simple two-step general procedure[20] was developed for the synthesis of natural cinnamyl esters and their analogues in good yields by condensation of monomalonates with substituted benzaldehyde derivatives.

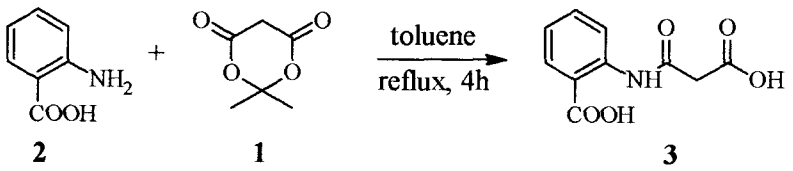
These monomalonates were obtained in high yields by simply heating Meldrum's acid **1** with alcohols or phenols[21] in benzene[20] or toluene[22].



The salient advantage of this method is that it does not involve any type of chromatography for separation and purification. The intermediate monomalonates can be chemically separated and purified. As such, this method can be conveniently scaled up.

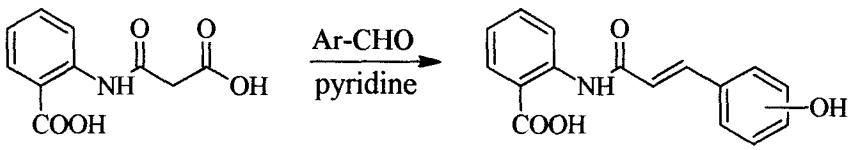
Therefore, we decided to extend this two-step method previously used for the preparation of cinnamyl esters, to prepare avenanthramides by replacing alcohols and phenols with amines as shown below.

First step



2 **1** **3**

Second step

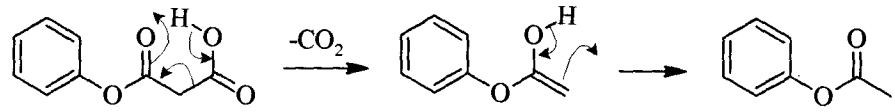


3 avenathramides

Taking note of the possible nucleophilic attack by the $-NH_2$ group of the amine at the carbonyl carbon in Meldrum's acid **1**, we envisaged the formation of the half amide of malonic acid from **1**. The reaction of anthranilic acid **2** with Meldrum's acid **1** was of particular interest as it would give the monomalonamic acid **3**, a key-starting material for the synthesis of naturally occurring avenanthramides and their analogues. Indeed, the reaction did proceed as expected to give **3** in 90% yield and the details are discussed below. To our knowledge compound **3** and its preparation is being reported for the first time.

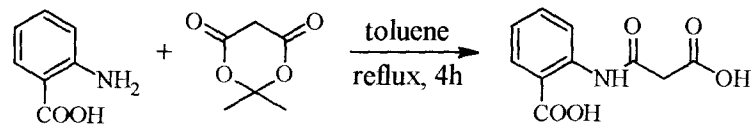
Meldrum's acid **1** was prepared from malonic acid and acetone using the literature procedure[23].

To begin with we decided to use benzene as the solvent and maintain the temperature of the reaction around 65°C because it was observed[20] during synthesis of monomalonates that higher temperatures leads to the formation of acetates by decarboxylation of the monomalonate being a β-keto acid.



Therefore, an equimolar mixture of Meldrum's acid **1** and anthranilic acid **2** in dry benzene was heated while maintaining the temperature between 60 to 70°C. The progress of the reaction was periodically monitored by TLC which indicated the presence of unreacted starting compounds even after heating for a period of 48 hrs. Hence the reaction temperature was raised to 80°C but we could get only 38% yield of a brown solid (m.p. 174°C).

However, when an equimolar mixture of Meldrum's acid **1** and anthranilic acid **2** in dry toluene was refluxed (110°C) and monitored by TLC complete conversion took place in 4 hrs. On cooling to room temperature, a white solid separated out which was first purified chemically (by dissolving in $NaHCO_3$ solution and reprecipitating it by 1:1 HCl) followed by recrystallization from hot water to give white solid in 90% yield, m.p. 174°C.



2 **1** **3**

When acetonitrile was used as the solvent and heated under reflux (80°C), we obtained 61% yield of the white solid (m.p. 174°C) and it took 6 hrs to complete the reaction.

The IR spectrum of the white solid showed bands at 3118 (NH), 1720 (-CH_2COOH), 1685 (NHCO) and 1643 (Ar-COOH) cm^{-1} as expected for the monomalonamic acid **3**.

The molecular formula of the monomalonamic acid **3** was determined to be $C_{10}H_9NO_5.1/4H_2O$ on the basis of its elemental analysis.

In the ^1H NMR spectrum, the characteristic singlet at δ 3.44 integrated for 2 protons indicated the presence of the methylene group flanked by two carbonyls, one of the carboxyl and the other of the amide group supporting the formation of the expected 2-[(carboxyacetyl)amino]benzoic acid **3**. The remaining four aromatic protons were observed as doublet of doublets between δ 7.08 to 8.56.

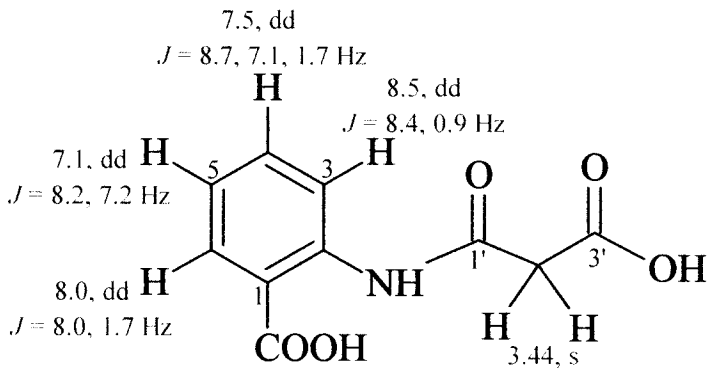


Figure I: ^1H NMR assignments for the various protons of **3**

The ^{13}C NMR spectrum of **3** showed 9 signals for the 10 carbons present. The signal due to the methylene carbon was unexpectedly missing although the two methylene protons are vividly observed as a downfield singlet at δ 3.44 in the ^1H NMR spectrum of **3** (see Figure I). The three carbonyl carbons, two of the carboxyl groups at δ 164.9 & 169.0 and one of the amide group at δ 169.6 were observed in the ^{13}C NMR spectrum of **3**.

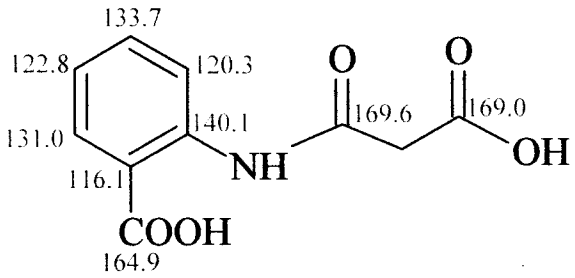
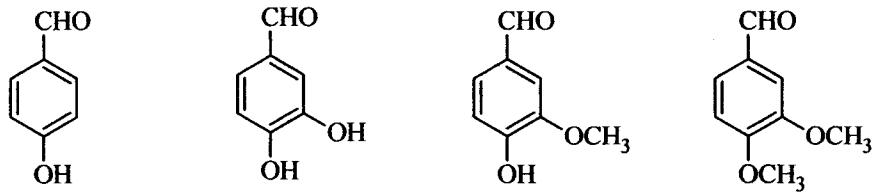


Figure II: ^{13}C NMR assignments for the various carbons in **3**

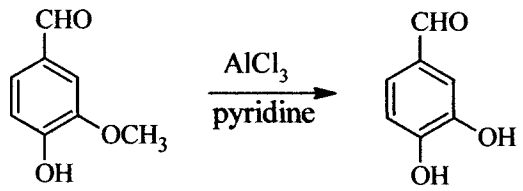
The next step involved condensation of monomalonamic acid **3** with various aromatic substituted aldehydes using modified Knoevenagel condensation[24] to give the different avenanthramides in a single step.

The selection of the avenanthramides to be synthesised was mainly based on the availability of the required aldehydes in the store. Of course we could prepare two of them using reported procedures. Our priority was to prepare some of the natural avenanthramides which can be obtained by condensation of monomalonamic acid **3** with *p*-hydroxybenzaldehyde **4**, 3,4-dihydroxy-benzaldehyde (protocatechualdehyde) **5**, 4-hydroxy-3-methoxybenzaldehyde (vanillin) **6** and 3,4-dimethoxybenzaldehyde (veratraldehyde) **7**.



4 **5** **6** **7**

3,4-Dihydroxybenzaldehyde **5** was prepared[25] by Lewis acid ($AlCl_3$) catalyzed demethylation of 4-hydroxy-3-methoxybenzaldehyde (vanillin) **6**.

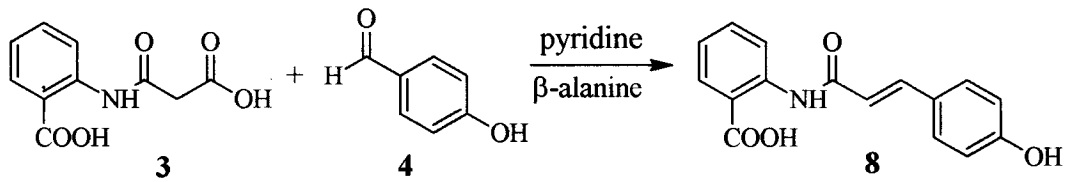


6 **5**

Synthesis of Natural Avenanthramides

To begin with we decided to carry out this condensation reaction using one single aldehyde (*p*-hydroxybenzaldehyde) **4** and optimize the reaction conditions so as to obtain maximum yield of the products (avenanthramides).

Thus condensation of monomalonamic acid **3** with purified *p*-hydroxy-benzaldehyde **4** (equimolar amount) in the presence of dry pyridine and β-alanine as a cocatalyst using Verley-Doebner modification of Knoevenagel condensation[24] followed by acidification with conc. HCl gave yellow solid in 85% yield. Recrystallization from hot water and acetone mixture gave pale yellow crystals having melting point 220°C. The reported[3] melting point for the expected avenanthramide D **8** is 219°C, thus indicating its identity with the previously synthesized[3] (83%) and the natural avenanthramide D which was further confirmed by comparison of its spectroscopic data (IR, UV and ^{1}H NMR) with that reported for *N*-[4′-hydroxy-(*E*)-cinnamoyl]anthranilic acid **8**.

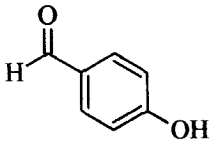
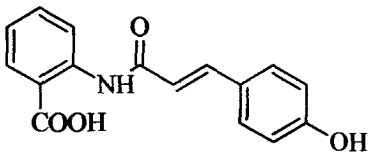
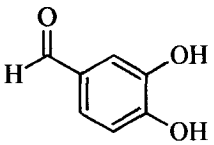
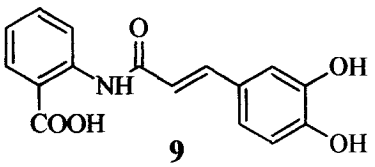
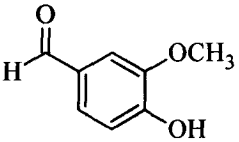
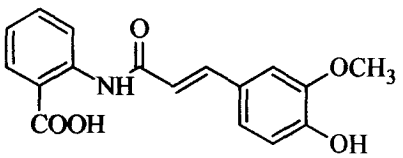
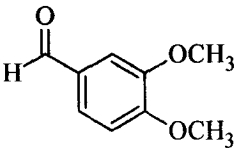
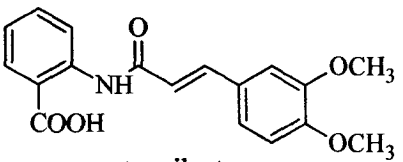


In its IR spectrum the presence of diagnostic bands at 3120 cm^{-1} (-NH-group) and 1665 cm^{-1} (conjugated amide carbonyl) supported the formation of **8**.

The ^{1}H NMR spectrum of **8** showed the presence of two downfield 1H doublets at δ 6.61 and 7.51 (J = 15.6 Hz) characteristic of α,β-unsaturated olefinic protons of the *trans*-cinnamyl double bond. The two doublets integrated for 2H each at δ 6.81 and 7.54 (J = 8.4 Hz) confirmed the presence of *p*-substituted benzene ring of the cinnamyl moiety. The remaining four aromatic protons of the anthranilic acid moiety showed three doublets and a triplet integrating for one proton each at δ 7.61 (d, J = 8.1 Hz, C_4-H), 7.98 (d, J = 7.8 Hz, C_6-H), 8.55 (d, J = 8.1 Hz, C_3-H) and 7.16 (t, J = 7.5 Hz, C_5-H) respectively.

Similarly condensation of monomalonamic acid **3** with 3,4-dihydroxy-benzaldehyde **5**, 4-hydroxy-3-methoxybenzaldehyde (vanillin) **6**, and 3,4-dimethoxybenzaldehyde (veratraldehyde) **7** in the presence of dry pyridine and β-alanine using Verley-Doebner modification of Knoevenagel condensation[24] gave avenanthramides **9** to **11** in good yields ranging from 74-85%.

Table 2: Natural avenanthramides synthesized using our method

Benzaldehyde derivatives	Natural avenanthramides	Yield %	Nature & m.p.
 **4**	 avenanthramide D **8**	85	Pale yellow crystals m.p. 220°C (Lit.[3] 219°C)
 **5**	 **9**	75	Yellow crystals m.p. 230-234°C (Lit.[2] 221-230°C)
 **6**	 avenanthramide E **10**	85	Pale yellow crystals m.p. 212°C (Lit.[3] 235°C)
 **7**	 tranilast **11**	74	Yellow crystals m.p. 184°C

In the UV spectra of natural avenanthramides **8** to **10**, the λ_{max} was observed in a narrow range of 336-338 nm except the avenanthramide **11** showed λ_{max} at 208 nm.

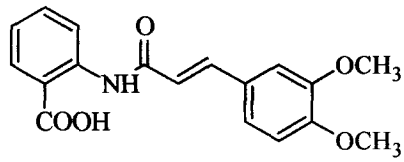
In the IR spectra of all, the amide carbonyl frequency was invariably observed within a range of 1660 to 1687 cm^{-1}.

Similarly in the ^1H NMR spectra of all the four avenanthramides **8** to **11,** the characteristic α,β-unsaturated olefinic cinnamyl protons appeared as two doublets between δ 6.52 and 7.55 with J = 15.3 to 15.9 Hz.

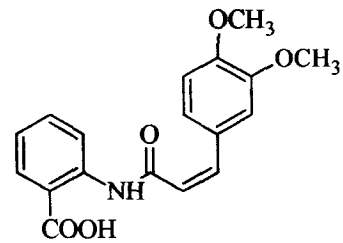
The spectral data (UV, IR & ^1H NMR) recorded on our synthetic avenanthramides **8** to **10** was found to be identical in all respects with that reported in literature[2,3] on the respective natural avenanthramides. The m.p. (212°C) of the avenanthramide E **10** recorded by us did not match with that reported[3] (235°C).

The avenanthramide *N*-[3′,4′-dimethoxy-(*E*)-cinnamoyl]anthranilic acid **11** is a natural anthranilic acid amide occurring in Chinese medicinal plant *Nandina domestica*[26] and is the only natural avenanthramide which has not been detected in oat extracts so far.

It is marketed under the trade names of Tranilast and Rizaben and is used in case of allergic reactions, such as bronchial asthma, allergic rhinitis, allergic conjunctivitis, food allergies, urticaria or atopic dermatitis[3].



E-isomer



Z-isomer

Interestingly, the *Z*-isomer of **11** has been shown to possess over 10 times the antiallergic activity of the *E*-isomer[27].

Recently **11** has been shown[4] to prevent restenosis after percutaneous transluminal coronary angioplasty. Tranilast **11** when taken orally is rapidly absorbed, transported to the liver and demethylated at the 4′-position producing the active metabolite *N*-(4′-hydroxy-3′-methoxycinnamoyl)anthranilic acid **10** (avenanthramide E).

The avenanthramide **11** was described[27] in pharmaceutical patents much before this class of compounds were detected, isolated, synthesized and called avenanthramides by Collins in 1989. However, neither melting point nor spectroscopic data was available in the patented[26] and subsequent literature[3,4] on **11**. However, we could find its m.p. (211-213°C) from internet. Therefore, we have recorded its UV, IR and ^{1}H NMR data and is included in the experimental section so as to have complete spectral data on these compounds at one place.

The assignments of signals for the various protons in the ^{1}H NMR spectrum of Tranilast **11** are shown below (see Figure-III).

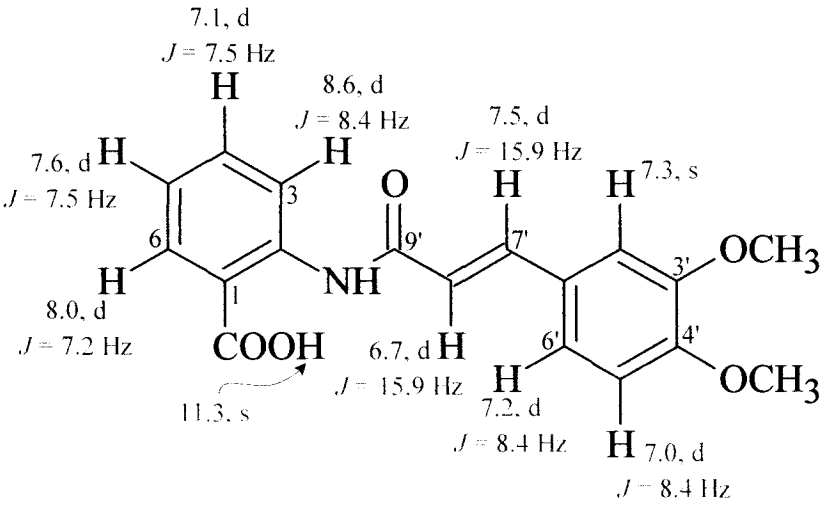


Figure III: ^{1}H NMR assignments for the various protons of **11**

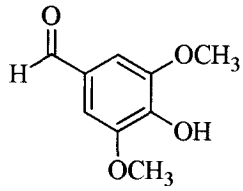
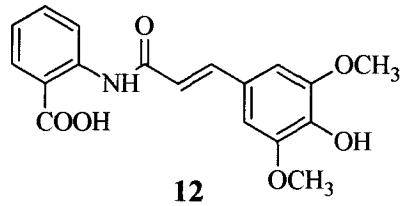
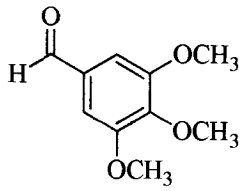
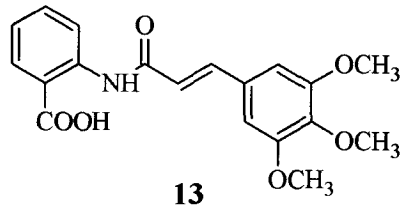
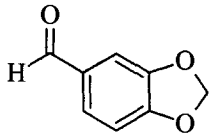
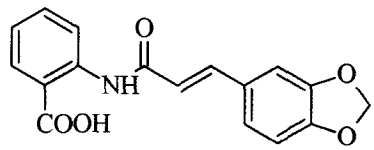
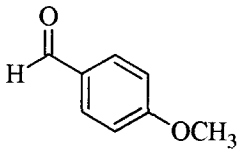
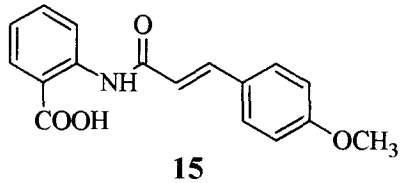
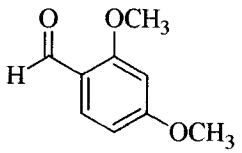
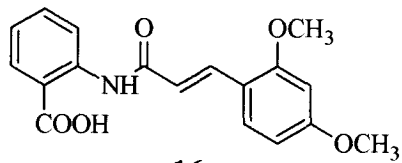
Application of the two-step General Procedure for the Synthesis of many more Reported and New Avenanthramides

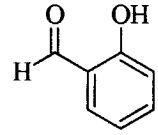
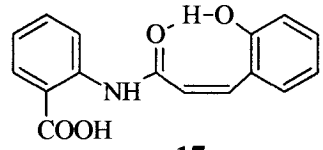
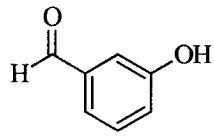
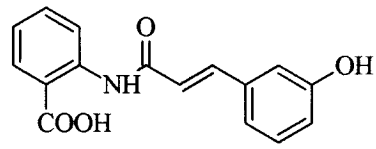
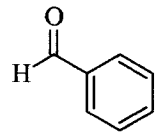
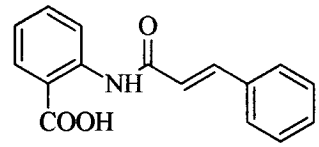
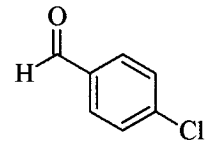
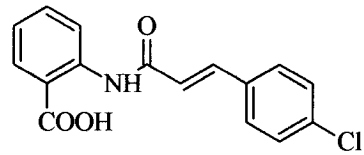
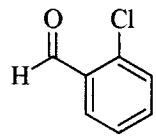
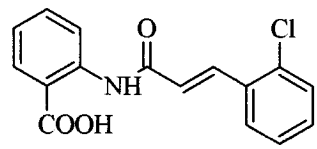
It may be noted that several phenolics such as *p*-hydroxybenzoic, protocatechuic, vanillic, syringic, *p*-coumaric, caffeic, ferulic, sinapic, etc. are also isolated as free acids or their esters from the bran layer of oat grains[28]. Moreover, the silica gel two dimensional TLC analysis of the mixture of avenanthramides isolated from oat groat extracts is found to contain at least 40 chromatographically distinct avenanthramides[3] of which very few have been individually isolated and characterized as they are present in very small quantities. Our method can be efficiently used to prepare these remaining (not isolated but detected) avenanthramides in sufficient quantities and subsequently used to detect their presence in oat extracts and also study their bioactivity.

The generality of our method has been exemplified by preparing several more reported and new avenanthramides **12** to **21**.

Thus condensation of monomalonamic acid **3** with 4-hydroxy-3,5-dimethoxy-benzaldehyde (syringaldehyde) **22**, 3,4,5-trimethoxybenzaldehyde **23**, 3,4-methylenedioxybenzaldehyde (piperonal) **24**, 4-methoxybenzaldehyde (anisaldehyde) **25**, 2,4-dimethoxybenzaldehyde **26**, 2-hydroxybenzaldehyde (salicylaldehyde) **27**, 3-hydroxybenzaldehyde **28**, benzaldehyde **29**, 4-chlorobenzaldehyde **30** and 2-chlorobenzaldehyde **31** in the presence of dry pyridine and β-alanine using Verley-Doebner modification of Knoevenagel condensation[24] gave avenanthramides **12** to **21** (Table 3) in good to excellent yields.

Table 3: Avenanthramides synthesized using our method

Benzaldehyde derivatives	Natural avenanthramides	Yield %	Nature & m.p.
 **22**	 **12**	65	Bright yellow crystals m.p. 214°C (Lit.[2] 199-200°C)
 **23**	 **13**	65	Yellow crystals m.p. 168°C
 **24**	 **14**	71	Pale yellow crystals m.p. 202°C (dec)
 **25**	 **15**	70	Pale yellow crystals m.p. 190°C
 **26**	 **16**	86	Orange crystals m.p. 194-98°C (dec)

 **27**	 **17**	73	White cottony threads m.p. 290°C (dec)
 **28**	 **18**	95	White shiny crystals m.p. 242°C (dec)
 **29**	 **19**	66	Light geen flakes m.p. 188°C
 **30**	 **20**	65	White crystals m.p. 200°C (dec)
 **31**	 **21**	74	Pale yellow crystals m.p. 220°C (dec)

The above ten avenanthramides **12** to **21** were obtained as crystalline solids having a wide range of colours ranging from white to pale yellow to bright yellow to orange to light green and in yields ranging from 65-95%.

In the UV spectra of avenanthramides **14**, **16**, **17**, **18**, **19**, **20**, **21** & **23**, the λ_{max} was observed in a narrow range of 207-214 nm except the avenanthramides **12**, **13** & **15** showed λ_{max} in range of 326-339 nm.

In the IR spectra of all, the amide carbonyl frequency was invariably observed within a range of 1660 to 1697 cm^{-1}.

In the ^1H NMR spectra of all the ten avenanthramides **12** to **21** the characteristic α,β-unsaturated olefinic cinnamyl protons appeared as two doublets between δ 6.52 and 7.93 with J = 15.5 to 16.0 Hz except the avenanthramide **17** showed the α,β-unsaturated olefinic cinnamyl protons at δ 7.53 and 8.68 with J = 8.2 Hz indicating it to be a *Z*-isomer.

Similarly in the ^{13}C NMR spectra of all the ten avenanthramides **12** to **21** the characteristic signals due to the carboxyl carbonyl carbon (Ar-C̲OOH) and the amide carbonyl carbon (Ar-NH-C̲O-) appeared between δ 162.9 and 171.9 except the avenanthramide **17** showed the signals due to the carboxyl carbonyl carbon (Ar-C̲OOH) at δ 159.7 and the amide carbonyl carbon (Ar-NH-C̲O-) at δ 167.6.

These avenanthramides **12** to **21** have not been detected in oat extracts nor have they been isolated from other natural sources so far. However, in view of the presence of several hydroxy and methoxy derivatives of cinnamic acids in the phenolic portion of oat extracts, the occurrence of at least some of them like **12** to **15** cannot be ruled out at this stage. For the same reason (to detect its presence in oat extracts) the avenanthramide **12** was previously prepared by Bratt et al[2] using modified version of the method described by Mayama et al[7].

The avenanthramide **12** on recrystallization from water and acetone mixture gave bright yellow crystals having m.p. 214°C (Lit.[2] 199-200°C). Although the m.p. did not match its spectroscopic data (UV, IR and ^1H NMR) matched well with that reported[2] for *N*-[4′-hydroxy-3′,5′-dimethoxy-(*E*)-cinnamoyl]anthranilic acid **12**.

Its methyl ether, *N*-[3′,4′,5′-trimethoxy-(*E*)-cinnamoyl]anthranilic acid **13**, prepared by condensation of monomalonamic acid **3** with 3,4,5-trimethoxy-benzaldehyde **23** displayed ^1H NMR spectrum having pattern similar to that of avenanthramide **12** but was having an additional 3H singlet at δ 3.89 due to the methoxy group at $C_{4'}$-position which was further supported by a signal at δ 60.1 ($C_{4'}$-O$\underline{C}H_3$) in the ^{13}C NMR spectrum of **13**.

Finally **13** being a new compound its structure was confirmed by its HRFABMS data which showed a peak at *m/z* 380.1113 $[M + Na]^+$ as expected and its molecular formula was determined to be $C_{19}H_{19}NO_6$.

The structure of each new avenanthramide was confirmed by ^1H, ^{13}C NMR spectroscopy and mass spectrometry.

The avenathramide **14** obtained by condensation of monomalonamic acid **3** with piperonal **24** is a new compound. Therefore, we recorded its complete spectroscopic (UV, IR, ^1H & ^{13}C NMR and MS) data which is in perfect agreement with the assigned structure **14**.

The molecular formula of **14** was determined to be $C_{17}H_{13}NO_5$ on the basis of its LCMS data which showed a peak at *m/z* 334.2902 for $[M + Na]^+$ as expected.

The ^1H NMR assignments for the various protons of **14** are shown below in figure IV.

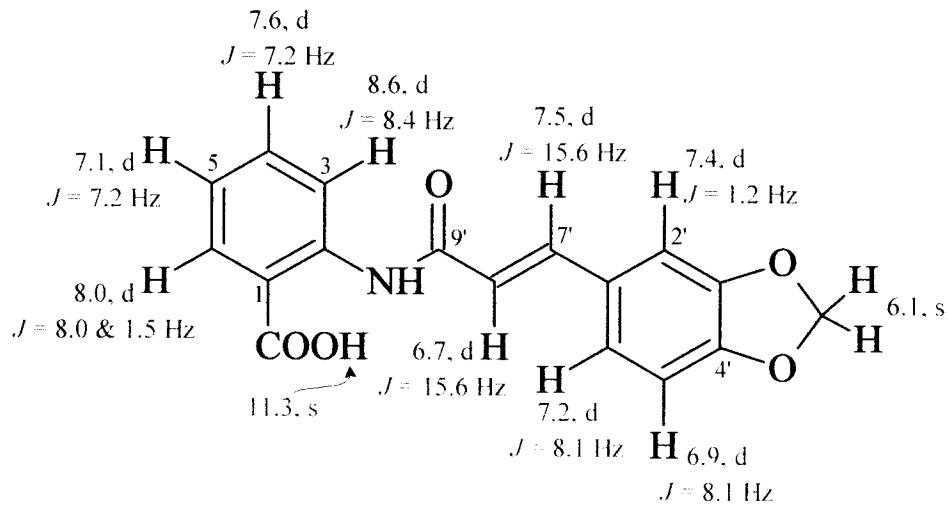


Figure IV: ^1H NMR assignments for the various protons of **14**

The assignments of the ^{13}C NMR signals for the various carbons of **14** are shown below in figure V

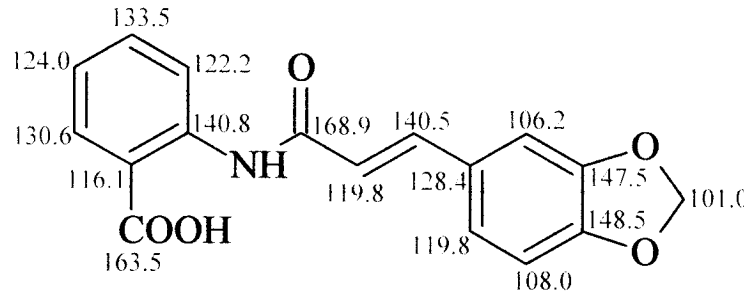
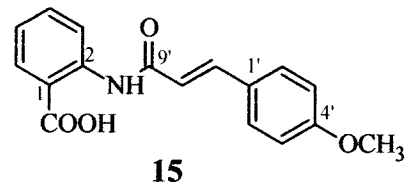


Figure V: ^{13}C NMR assignments for the various carbons of **14**

The avenanthramide **15** is the methyl ether of the naturally occurring avenanthramide D **8** and was prepared by condensation of monomalonamic acid **3** with *p*-methoxybenzaldehyde (anisaldehyde) **25** in 70% yield. Recrystallization from acetone and water mixture gave pale yellow needles having m.p. 190°C.



15

Its structure was established to be *N*-(4′-methoxy-(*E*)-cinnamoyl)anthranilic acid **15** by comparison of its ^{1}H and ^{13}C NMR data with that of the avenanthramide D **8**. In the ^{1}H NMR spectrum of **15** the 3H singlet due to the methoxy group was not observed as it got buried under the DMSO signal however, the ^{13}C NMR spectrum of **15** showed the presence of methoxy carbon signal at δ 56.2 (-O<u>C</u>H$_3$).

Condensation of monomalonamic acid **3** with 2,4-dimethoxy-benzaldehyde **26** gave the avenanthramide **16** as orange coloured solid in 86% yield. Recrystallization from water-methanol mixture gave orange crystals having m.p. 194-98°C (dec). The required 2,4-dimethoxybenzaldehyde **26** was

prepared[†] in our lab by formylation of 1,3-dimethoxybenzene using *N*,*N*-dimethylformamide and $POCl_3$.

Avenanthramide **16** is also a new compound and its structure was shown to be *N*-[2′,4′-dimethoxy-(*E*)-cinnamoyl]anthranilic acid **16** mainly on the basis of its ^1H NMR, ^{13}C NMR and MS data.

The ^1H NMR spectrum of **16** showed two 3H singlets at δ 3.89 and 3.82 indicating the presence of two methoxy groups. The detail assignments of the chemical shifts with *J* values for the various protons of **16** are presented below in figure VI.

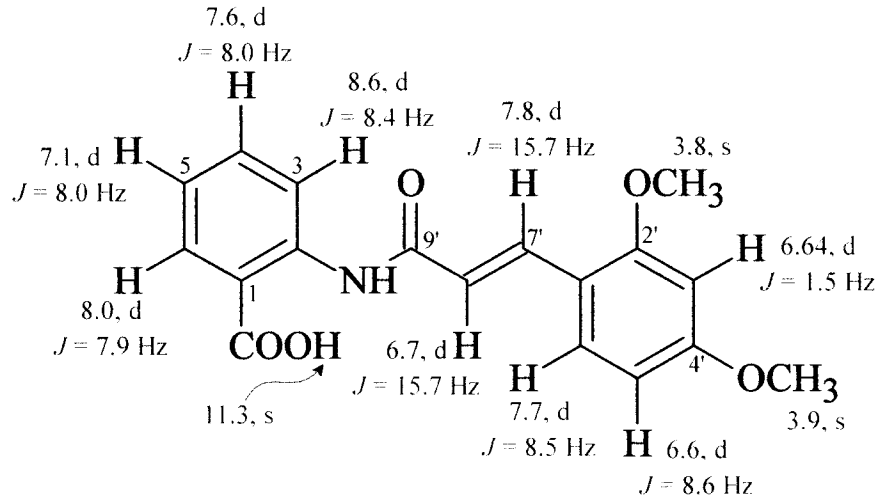


Figure VI: ^1H NMR assignments for the various protons of **16**

The ^{13}C NMR spectrum showed in all 18 distinct signals as expected and their assignments for all the 18 carbons of **16** are shown below in figure VII.

[†] We are thankful to Dr. Asha D'Souza for a sample of 2,4-dimethxybenzaldehyde

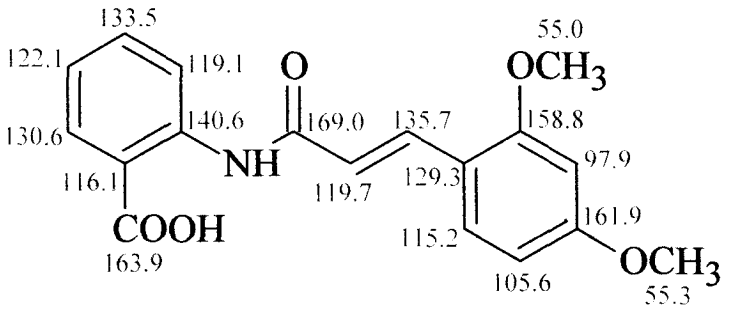


Figure VII: ^{13}C NMR assignments for the various carbons of **16**

The structure **16** was confirmed by recording its LCMS data which showed a peak at *m/z* 350.6357 for $[M + Na]^+$ indicating its molecular formula to be $C_{18}H_{17}NO_5$ as expected.

Condensation of monomalonamic acid **3** with 2-hydroxybenzaldehyde (salicylaldehyde) **27** and then with 3-hydroxybenzaldehyde‡ **28** gave the two avenanthramides **17** and **18** as white solids in 73 and 95% yields respectively.

These two avenanthramides being new compounds, their structures were established mainly on the basis of their ^1H NMR, ^{13}C NMR and MS data.

In the ^1H NMR spectrum of **17** the α,β-unsaturated olefinic protons were observed at δ 7.53 and 8.68 with J = 8.2 Hz indicating *Z* configuration for the cinnamyl double bond. Probably the phenolic $C_{2'}$-OH is getting chelated with the amide carbonyl through hydrogen bonding forcing the cinnamyl double bond to remain in the *Z* configuration.

The ^1H NMR assignments for the various protons of **17** are shown below in figure VIII.

‡ We are thankful to Dr. S. G. Tilve, Goa University, for providing a sample of 3-hydroxybenzaldehyde.

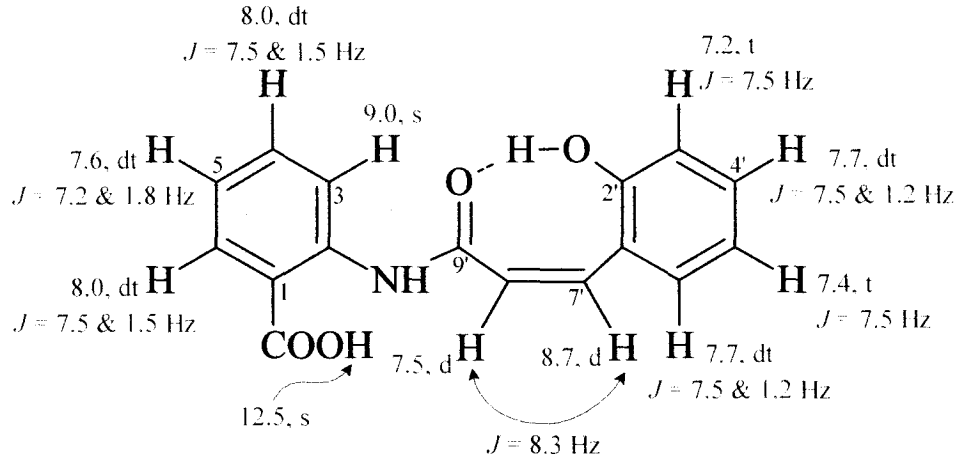


Figure VIII: ^{1}H NMR assignments for the various protons of **17**

The ^{13}C NMR spectrum of the avenanthramide **17** showed in all 16 signals as expected and their assignments are shown below in figure IX which are consistent with the structure *N*-[2′-hydroxy-(*Z*)-cinnamoyl]anthranilic acid **17**.

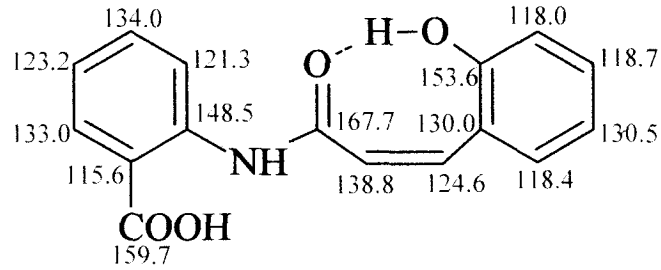
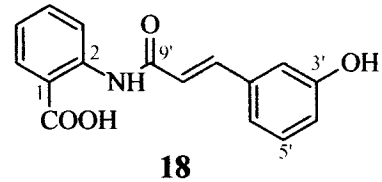


Figure IX: ^{13}C NMR assignments for the various carbons of **17**

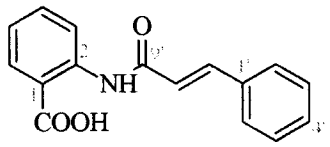
The avenanthramide **18** was obtained as white shiny crystals (m.p. 242°C with decomp). Its molecular formula was determined to be $C_{16}H_{13}NO_4$ on the basis of its LCMS data which showed a peak at *m/z* 306.0243 for $[M + Na]^+$ as expected.



18

Its ^{1}H and ^{13}C NMR data is fully consistent with the assigned structure *N*-[3'-hydroxy-(*E*)-cinnamoyl]anthranilic acid **18** and is given in the experimental section.

Condensation of monomalonamic acid **3** with simple benzaldehyde **29** gave the avenanthramide **19** as pale green solid in 66% yield. Recrystallization from hot water and acetone mixture gave light green flakes having m.p. 188°C.



19

Its molecular formula was determined to be $C_{16}H_{13}NO_3$ on the basis of its HRFABMS data which showed a peak at *m/z* 290.0791 for $[M + Na]^+$ as expected. Its structure was established to be *N*-(*E*)-cinnamoylanthranilic acid **19** on the basis of its ^{1}H and ^{13}C NMR data and is given in the experimental section.

The ^{13}C NMR spectrum of **19** showed in all 14 distinct signals for 16 carbons as expected.

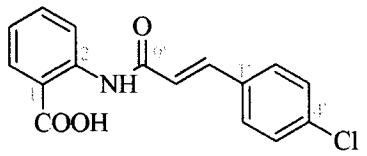
Although preparation of avenanthramides **10**, **15** and **19** has been reported[29] by Ashok Kumar and coworkers, their melting points and the ^{1}H NMR data reported[29] only on **10** clearly shows its non-identity with **10** prepared in the present study as well as with that reported in the literature[3]. Moreover, our attempts to prepare **10** using the procedure of Ashok Kumar and coworkers[29] did not work and instead gave the starting *N*-acetylanthranilic acid back with no trace of **10**. No NMR data was reported[29] on **15** and **19**.

Condensation of the monomalonamic acid **3** with 4-chlorobenzaldehyde **30** and 2-chlorobenzaldehyde[§] **31** gave avenanthramides **20** as pale yellow crystals m.p. 220°C (dec) and **21** as white crystals m.p. 200°C (dec) in 74 and 65% yields respectively.

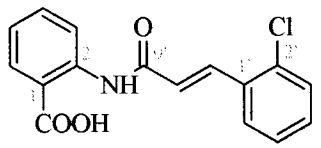
[§] We are thankful to Dr. S. G. Tilve, Goa University, for a sample of 2-chlorobenzaldehyde.

The study of their ^{1}H NMR data clearly indicated the 1,4- and 1,2-substitution pattern in the cinnamyl moiety of **20** and **21** respectively.

The ^{13}C NMR spectrum of **20** showed 14 distinct signals for 16 carbons while that of **21** showed 16 signals for 16 carbons as expected.



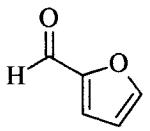
20



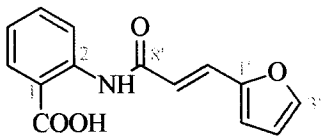
21

The molecular formula of both **20** and **21** was determined to be $C_{16}H_{12}NO_3Cl$ on the basis of their LCMS data which gave a peak at *m/z* 324.0379 $[M + Na]^+$ for **20** and at *m/z* 324.0572 $[M + Na]^+$ for **21**. Thus the structures of these two new chloroavenanthramides were confirmed to be *N*-[4′-chloro-(*E*)-cinnamoyl]anthranilic acid **20** and *N*-[2′-chloro-(*E*)-cinnamoyl]anthranilic acid **21** respectively.

Finally to establish the generality of this prcedure we caried out one reaction of monomalonamic acid **3** with 2-furaldehyde** **32** and obtained a new compound **33** as greyish white flakes m.p. 184°C (dec) in 74% yield.



32



33

The structure of this compound was established on the basis of its spectroscopic (IR, ^{1}H & ^{13}C NMR and MS) data.

The assignments of the ^{1}H NMR signals for the various protons of **33** are shown below in figure X.

** We are thankful to Dr. S. G. Tilve, Goa University, for a sample of 2-furaldehyde

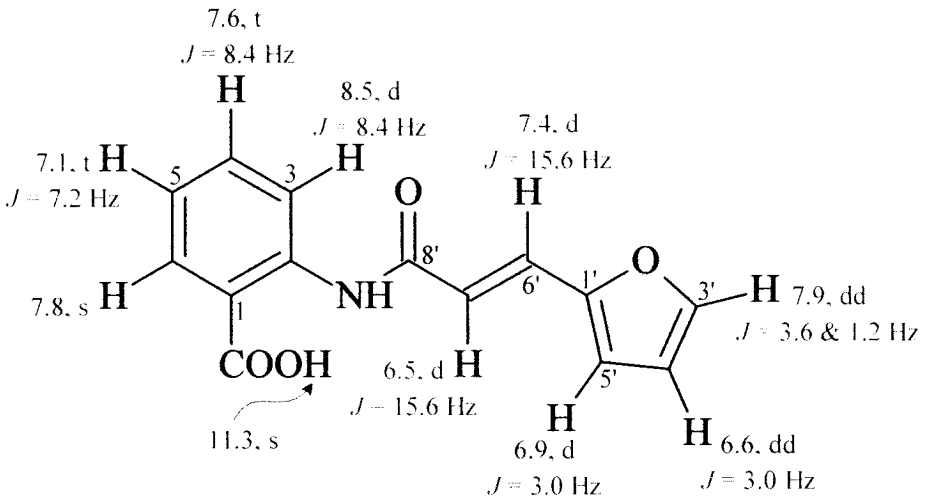


Figure X: ^{1}H NMR assignments for the various protons of **33**

The ^{13}C NMR spectrum of **33** showed 14 distinct signals for 14 carbons and the assignments of the signals to various carbons of **33** are shown below in figure XI.

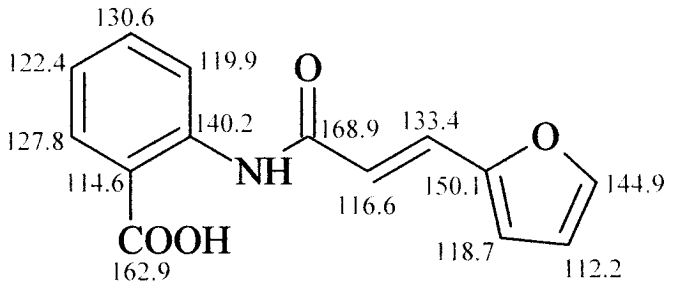


Figure XI: ^{13}C NMR assignments for the various carbons of **33**

Its molecular formula was determined to be $C_{14}H_{11}NO_4$ on the basis of its LCMS data which showed a peak at *m/z* 280.0580 $[M + Na]^+$ as expected.

Salient Advantages of our Method

It's a two-step general method and does not involve protection-deprotection steps as in reported methods.

Products are obtained in good to excellent yields (65 to 95%)

The major advantage of our method is that the intermediate monomalonamic acid **3** can be chemically separated and purified.

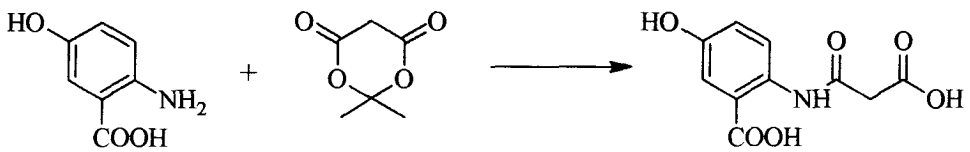
Secondly, this method does not involve any type of time and chemicals consuming chromatography for separation and purification.

We have carried out all these reactions on microscale using 40 to 100 mg quantities of the starting materials and the yields reported are the actual isolated yields for these microscale quantities.

However, the reactions can be conveniently scaled up to gram quantities without affecting either the yields or the quality of the products.

Drawbacks of this method

The reaction of 5-hydroxyanthranilic acid **34** with Meldrum's acid **1** to give the corresponding monomalonamic acid did not work as we failed to isolate the expected product **35**.



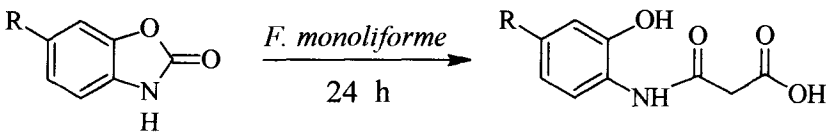
34 **1** **35**

Synthesis of monomalonamic acids

Introduction

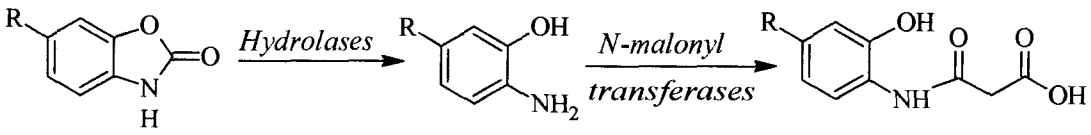
Literature survey indicated that the β-dicarbonyl derivatives belonging to the monomalonamic acid family are very important compounds having interesting pharmacological properties, including antihypertensive[30], sedative, anticonvulsant[31], anti-inflammatory[32], analgesic[33,34] and central nervous system stimulating[35] activities.

N-arylmalonamic acids are formed from benzoxazolinone derivatives by fungal transformation[36] and these acids have been shown to possess both plant growth regulatory and fungicidal activity[37].



R = H, OCH_3 R = H, OCH_3

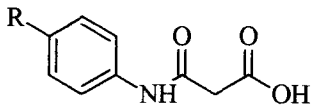
The metabolism of 2-benzoxazolinone involves the cleavage of the amide linkage leading to the formation of *o*-aminophenol intermediate. The aminophenol is then converted into the corresponding malonamic acid by the enzyme *N*-malonyl transferase[38].



R = H, OCH_3 R = H, OCH_3 R = H, OCH_3

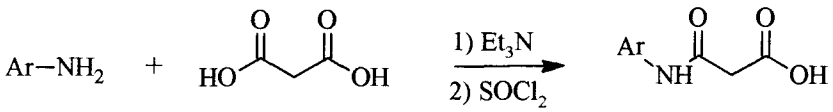
Reported methods for the preparation of *N*-arylmalonamic acids

N-arylmalonamic acids have been synthesized from malonic acid and its derivatives with or without using a solvent. Khetan et al[39] synthesized malonamic acids from appropriate aniline and malonic acid derivative using either pyridine or acetic acid as solvents at 100°C.



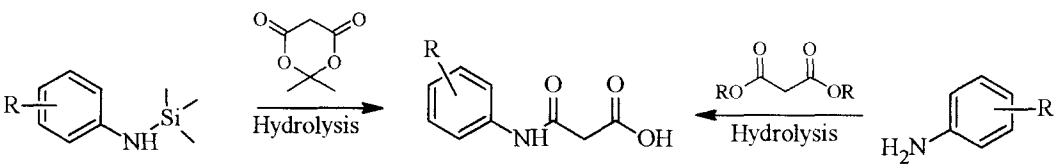
R = Br, I, OCH_3, OEt

A convenient one-pot synthesis of *N*-arylmalonamic acid has been demonstrated based on the *in-situ* generation of malonyl monoacyl chloride, followed by reaction with aniline[40].



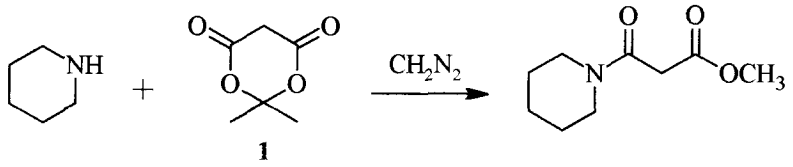
N-arylmalonamic acids have also been prepared by refluxing the appropriate aniline with malonic acid or with diethyl malonate[41] or with ethyl malonyl chloride[42] followed by alkaline hydrolysis of the ester group.

Alternatively the silylated aniline[43] is treated with Meldrum's acid **1** followed by hydrolysis of the malonic silyl ester to give *N*-arylmalonamic acid.



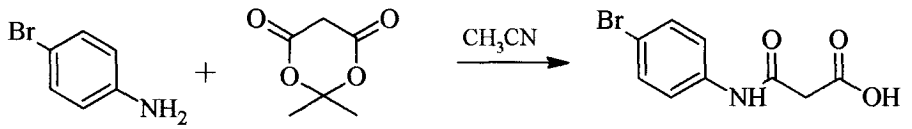
Matoba et al[44] demonstrated that when diazomethane (CH_2N_2) is added to a methanolic solution of Meldrum's acid **1**, violent evolution of N_2 gas occurs

with the formation of dimethyl malonate in quantitative yield. But when CH_2N_2 was added to a solution of **1** in piperidine, *N*-(methoxycarbonylacetyl) piperidine was obtained in 59% yield.



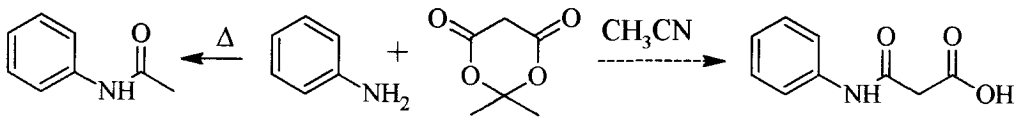
1

Hurd et al[45], during investigation studies on Meldrum's acid **1**, prepared *N*-*p*-bromophenylmalonamic acid from *p*-bromoaniline and Meldrum's acid **1** by heating them under reflux in acetonitrile solvent (yield was not reported).



1

Hurd et al[45] also made a statement in his article that "if Meldrum had performed his experiment with aniline using CH_3CN as the solvent, he would undoubtedly have obtained the *N*-phenylmalonamic acid **36** instead of acetanilide".



1 **36**

Interestingly, this reaction of aniline with Meldrum's acid **1** was neither carried out by Meldrum[46] nor by Hurd et al[45]. Hence we decided to investigate this reaction using our procedure and the results obtained are discussed below.

Present work

Our successful attempt to prepare[20] half esters of malonic acid by the reaction of Meldrum's acid **1** with all types of alcohols and phenols led us to prepare successfully monomalonamic acid **3** by the reaction of Meldrum's acid **1** with anthranilic acid **2**. We also found that compound **3** and its preparation is not reported previously.

Although the reaction of 5-hydroxyanthranilic acid **34** with Meldrum's acid **1** to give the corresponding monomalonamic acid **35** did not work as we failed to isolate the expected product, the results obtained by Hurd et al[45] especially with *p*-bromoaniline and **1** tempted us to investigate this reaction of **1** with several amines (including aniline) using our procedure involving benzene[20] or toluene[22] as the solvents.

Preparation of *N*-phenylmalonamic acid 36

To begin with we carried out the reaction of aniline with Meldrum's acid **1** in anhydrous CH_3CN using reaction conditions reported[45] for *p*-bromoaniline and obtained the expected product **36** (m.p. 130°C, Lit[47] 132°C) in 40% yield.

When benzene was used as the solvent, the optimum yield of **36** was 60% if the temperature was maintained between 60-70°C. Refluxing temperature (80°C) of benzene gave < 60% yield due to the formation of acetanilide by decarboxylation.

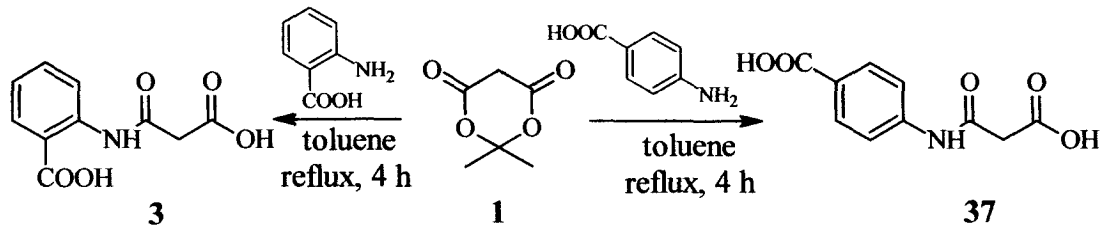
However, when dry toluene was used as the solvent at its reflux temperature, acetanilide (m.p. 110°C, Lit[48a] 113-115°C) was obtained exclusively with no trace of the expected *N*-phenylmalonamic acid **36**.

Preparation of 4-[(carboxyacetyl)amino]benzoic acid 37

Since the reaction of anthranilic acid **2** with Meldrum's acid **1** in refluxing toluene gave us 90% yield of the required monomalonamic acid **3**, we decided to use *p*-aminobenzoic acid (PABA) which is used in pharmaceutical preparations,

forms a part of the structure of folic acid (vitamin B_9) and a precursor in the biosynthesis of folic acid.

An equimolar mixture of *p*-aminobenzoic acid and **1** when heated under reflux in dry toluene gave the expected 4-[(carboxyacetyl)amino]benzoic acid **37** as a white solid in 91% yield. Recrystallization from ethanol afforded white shiny crystals m.p. 260°C (with decomposition).



In the IR spectrum of **37** bands at 3273 (NH), 1720 (CH_2COOH), 1678 (NHCO), 1664 (Ar-COOH) cm^{-1} indicated the functional groups present.

The ^1H NMR spectrum of **37** showed only three signals, two *o*-coupled 2H doublets centred at δ 7.68 and 7.9 indicating the presence of *p*-anthranilic acid moiety and a 2H singlet at δ 3.4 due to the methylene group flanked by two carbonyls confirming the formation of **37**.

This was further supported by the presence of 8 distinct signals for the 10 carbons in the ^{13}C NMR spectrum of **37**.

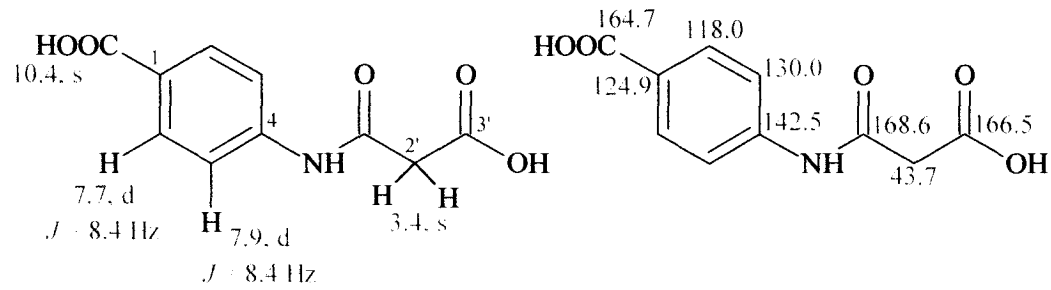
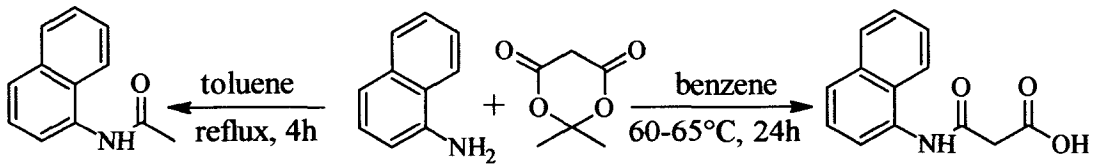


Figure XII: Assignments of ^1H & ^{13}C NMR signals for the Hs & Cs of **37**

Preparation of *N*-(1-naphthyl)malonamic acid 38

Reaction of 1-naphthylamine with Meldrum's acid **1** using toluene as the solvent gave the unwanted *N*-naphthylacetamide (m.p. 152°C, Lit[48b] 160°C) in 77% yield.



N-naphthylacetamide 1-naphthylamine **1** **38**

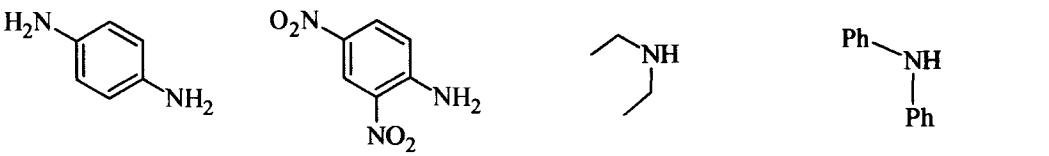
However, when benzene was used as the solvent and the temperature maintained between 60-65°C, the expected *N*-(1-naphthyl)malonamic acid **38** was obtained in 69% yield. Recrystallization from ethanol gave buff coloured needles having m.p. 144°C.

The IR spectrum of **38** showed bands at 3260 (NH), 1732 (CH_2COOH) and 1710 (NHCO) cm^{-1} indicating the presence of the functional groups present.

The ^1H NMR spectrum of **38** displayed the characteristic 2H singlet at δ 3.55 for the two methylene protons flanked by carbonyl groups.

Its ^{13}C NMR spectrum showed 13 signals (including two carbonyls) as expected.

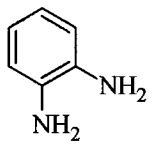
In the reaction of the following four amines[††] with Meldrum's acid **1** either with toluene or benzene we failed to isolate the expected product but instead obtained their acetamide derivatives.



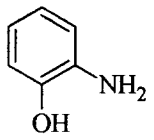
p-diaminobenzene 2,4-dinitroaniline *N*,*N*-diethylamine *N*,*N*-diphenylamine

[††] We are thankful to Dr. B. R. Srinivasan, Goa University, for providing samples of 1,4-diaminobenzene and 1,2-diaminobenzene.

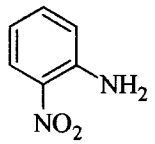
Surprisingly, the following four aromatic amines neither gave the expected product nor the acetate and the starting material was recovered back.



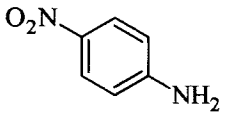
o-diaminobenzene



o-aminophenol

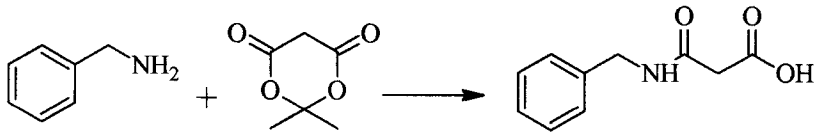


o-nitroaniline



p-nitroaniline

However, when benzylamine was condensed with **1**, *N*-benzylmalonamic acid **39** was obtained as colourless solid in 13-40% yield depending upon the solvent used (details in the experimental section). The maximum yield of 40% of **39** was obtained when the reaction was carried out in pyridine and diethyl ether. Recrystallization from benzene gave colourless crystals (m.p. 86-88°C).



1 **39**

The *N*-benzylmalonamic acid **39** was characterized by the study of its IR, ^{1}H NMR and ^{13}C NMR data.

Its IR spectrum showed bands at 3287 (NH) and 1745 cm^{-1}.

In the ^{1}H NMR spectrum of **39** three signals were observed, a 2H singlet at δ 3.32, a 2H doublet at δ 4.46 (J = 5.4 Hz) and a 5H multiplet between δ 7.25 to 7.37. Their assignments are shown below in figure XIII.

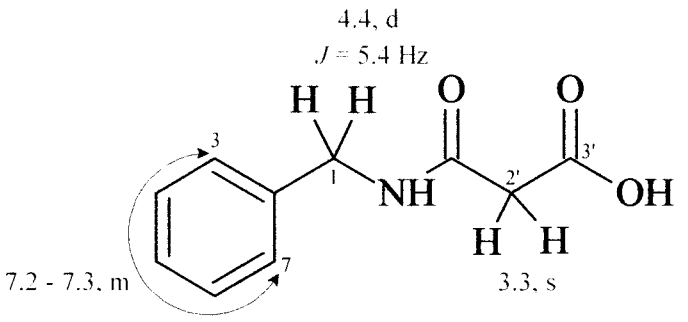


Figure XIII: ^{1}H NMR assignments for the various protons of **39**

In the ^{13}C NMR spectrum of **39** only 7 signals were observed for 10 carbons. Three sp^2 carbons of the benzene ring are overlapping at δ 127.8 and their assignments are shown below in figure XIV.

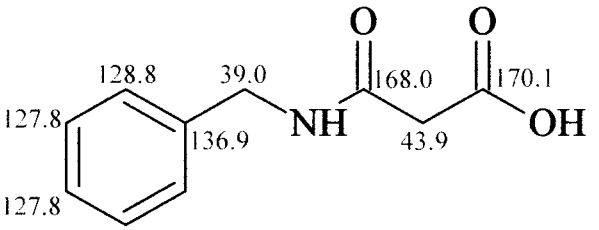


Figure XIV: ^{13}C NMR assignments for the various carbons of **39**

To our knowledge the compounds **37**, **38** and **39** as well as their preparations are being reported for the first time.

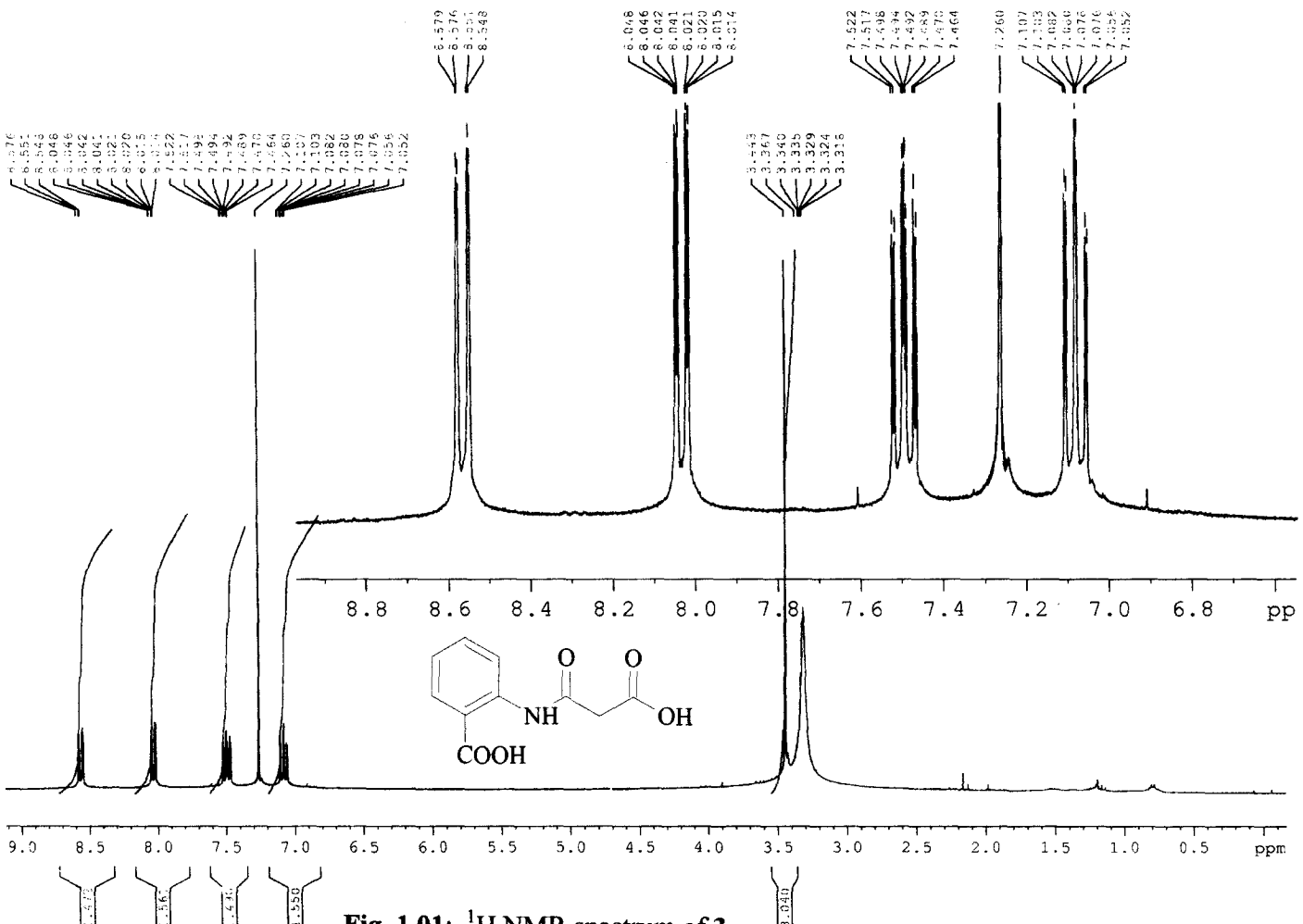


Fig. 1.01: ^{1}H NMR spectrum of **3**

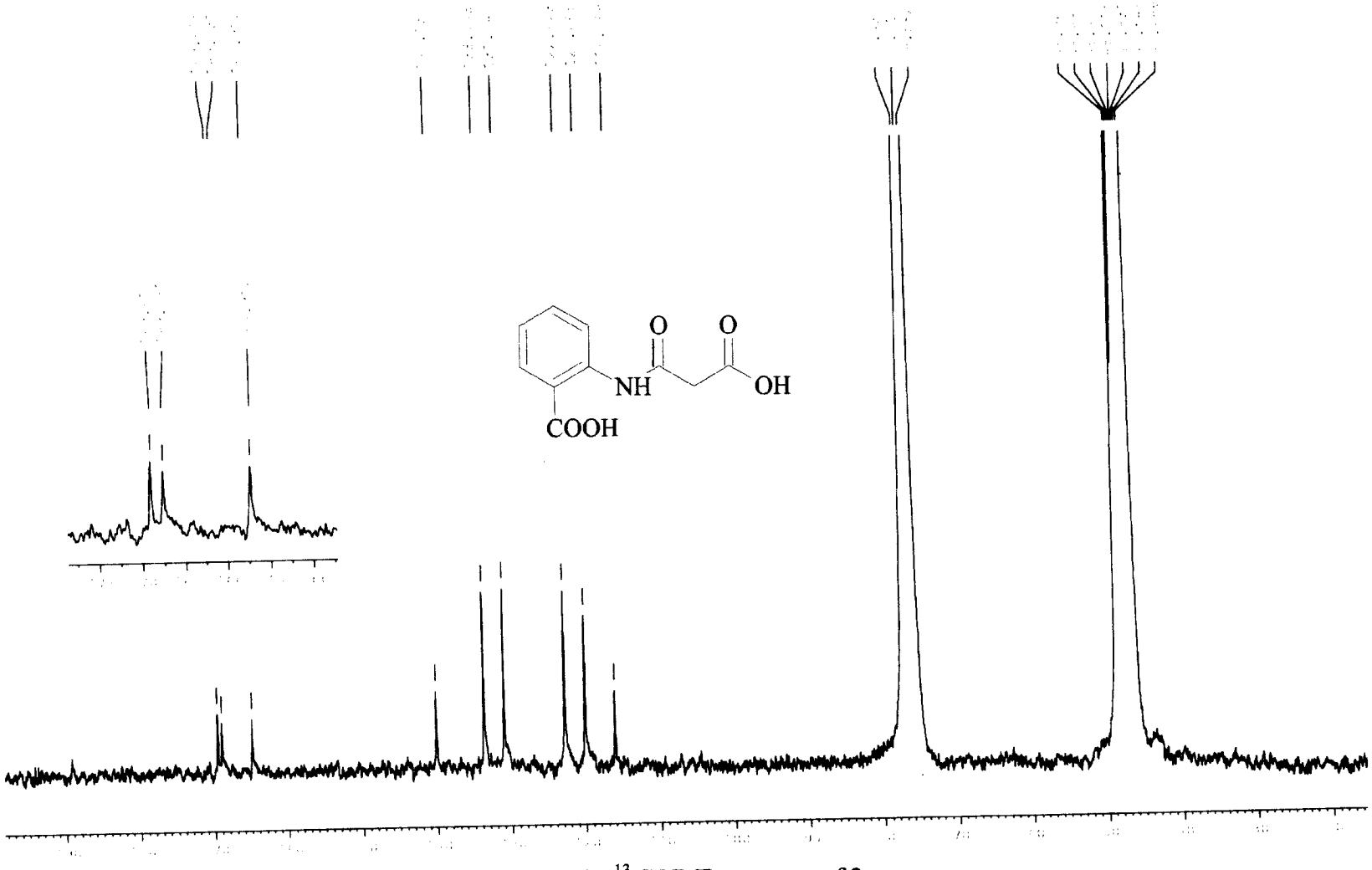


Fig. 1.02: ^{13}C NMR spectrum of **3**

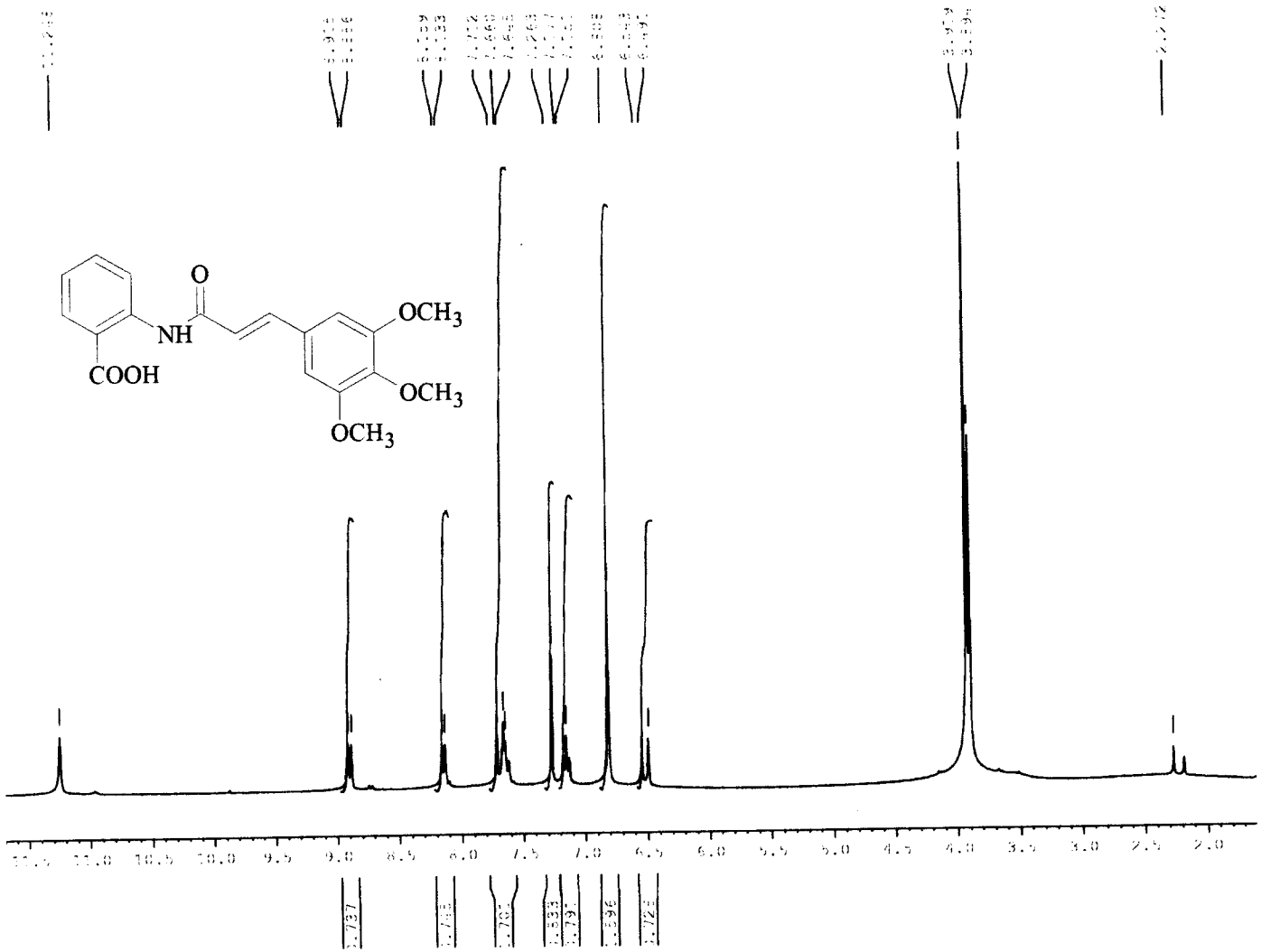


Fig. 1.03: ^{1}H NMR spectrum of **13**

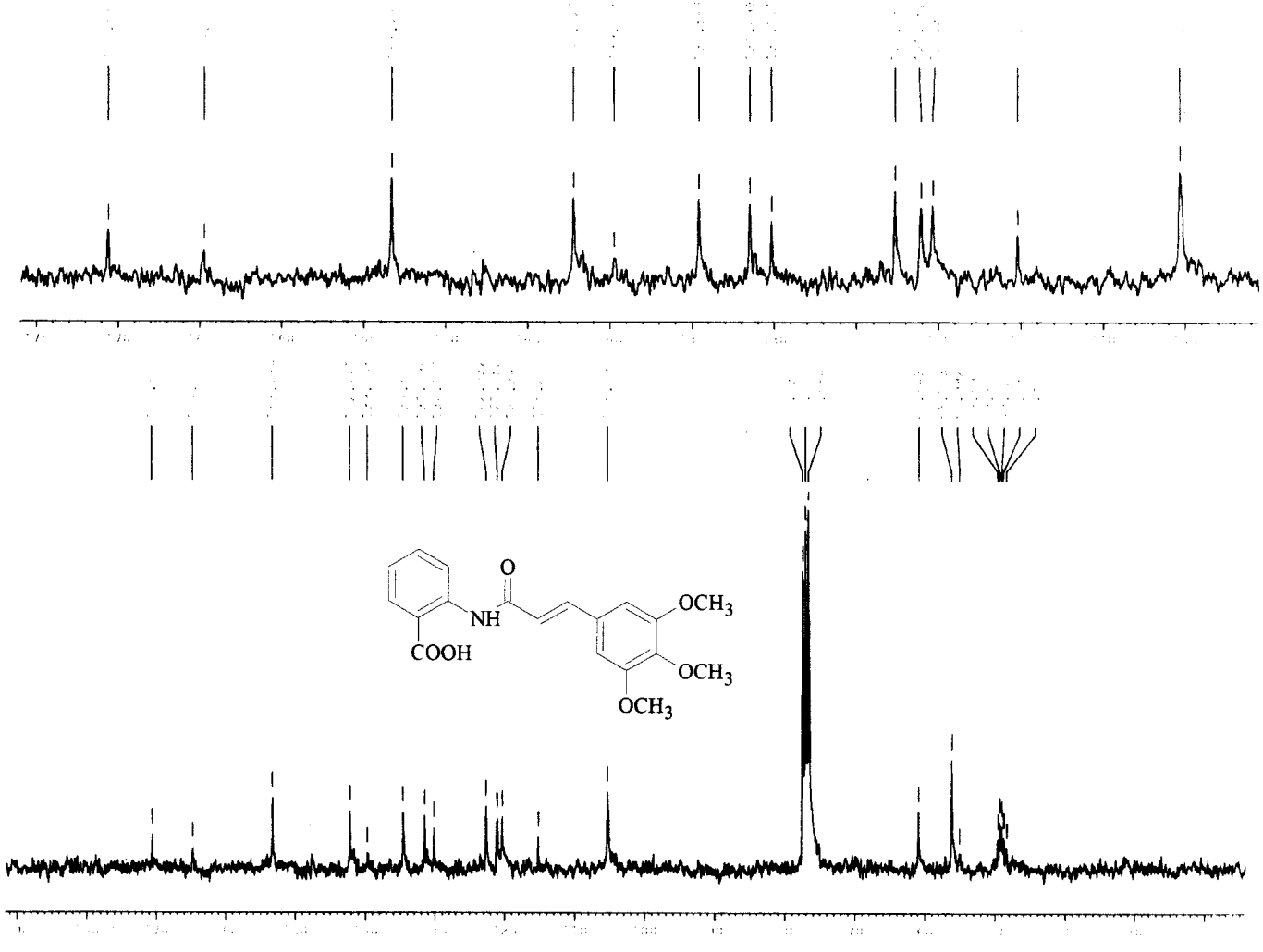


Fig. 1.04: ^{13}C NMR spectrum of **13**

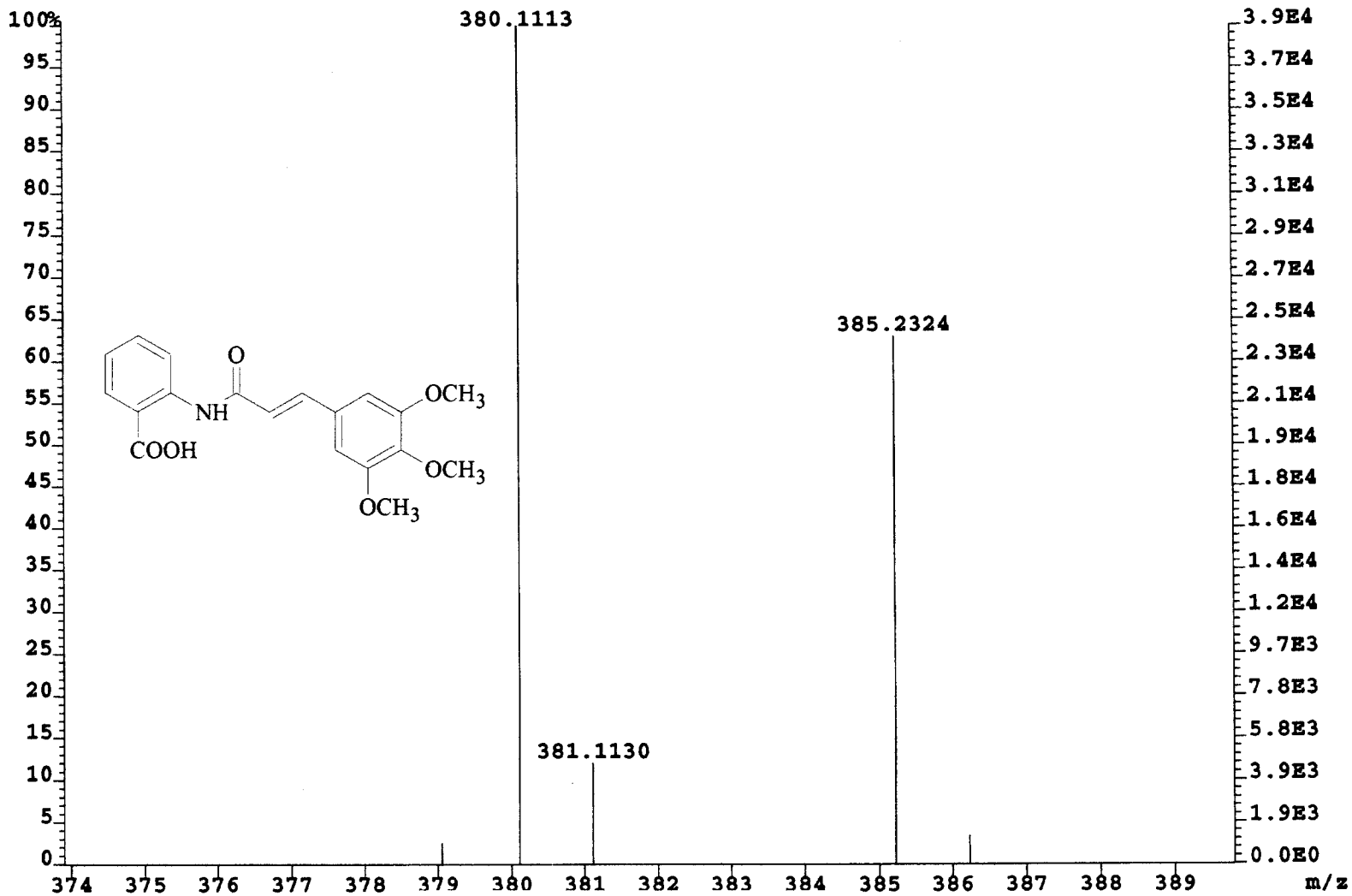


Fig. 1.05: HRFABMS spectrum of **13**

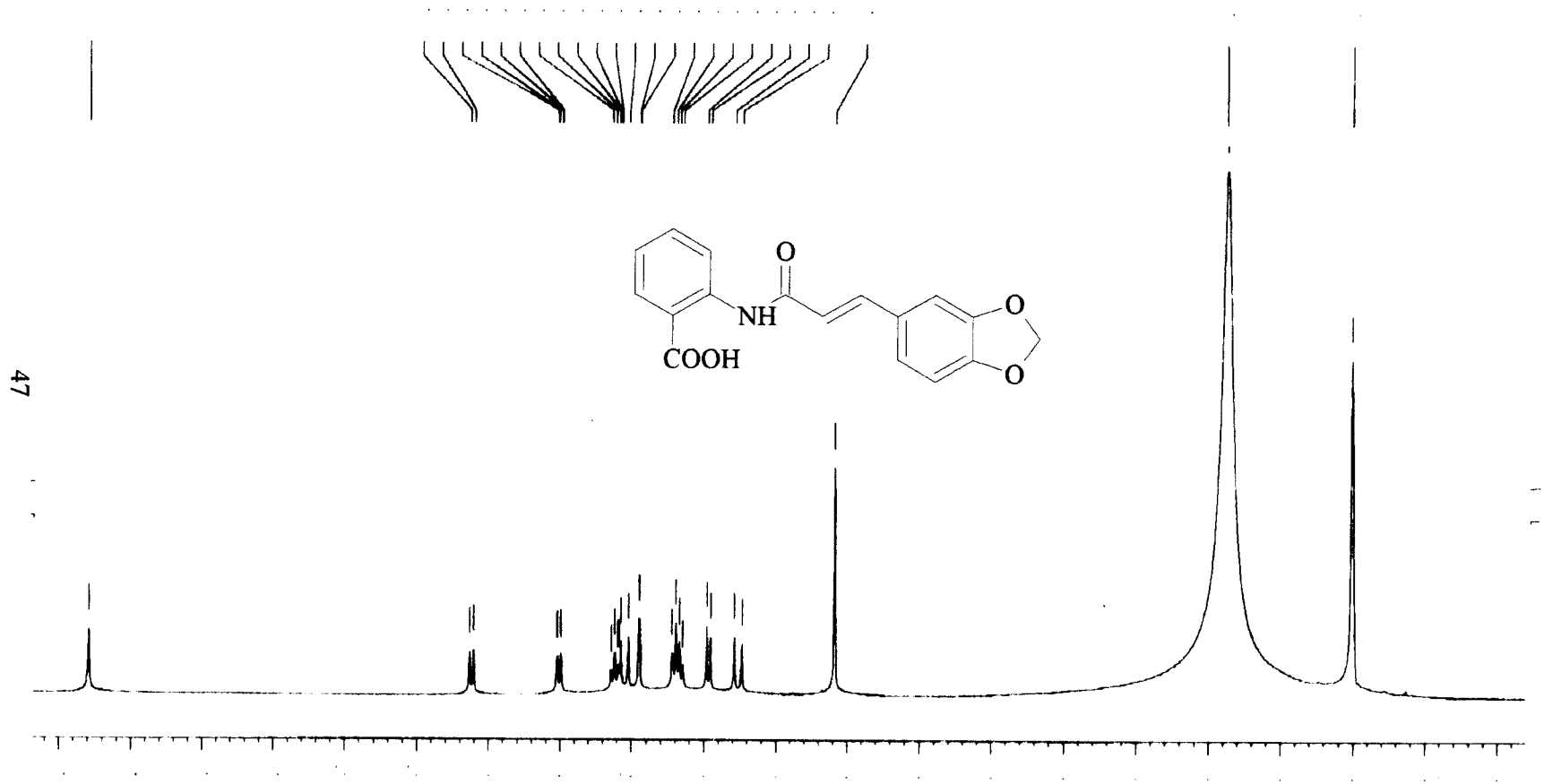


Fig. 1.06: ^{1}H NMR spectrum of **14**

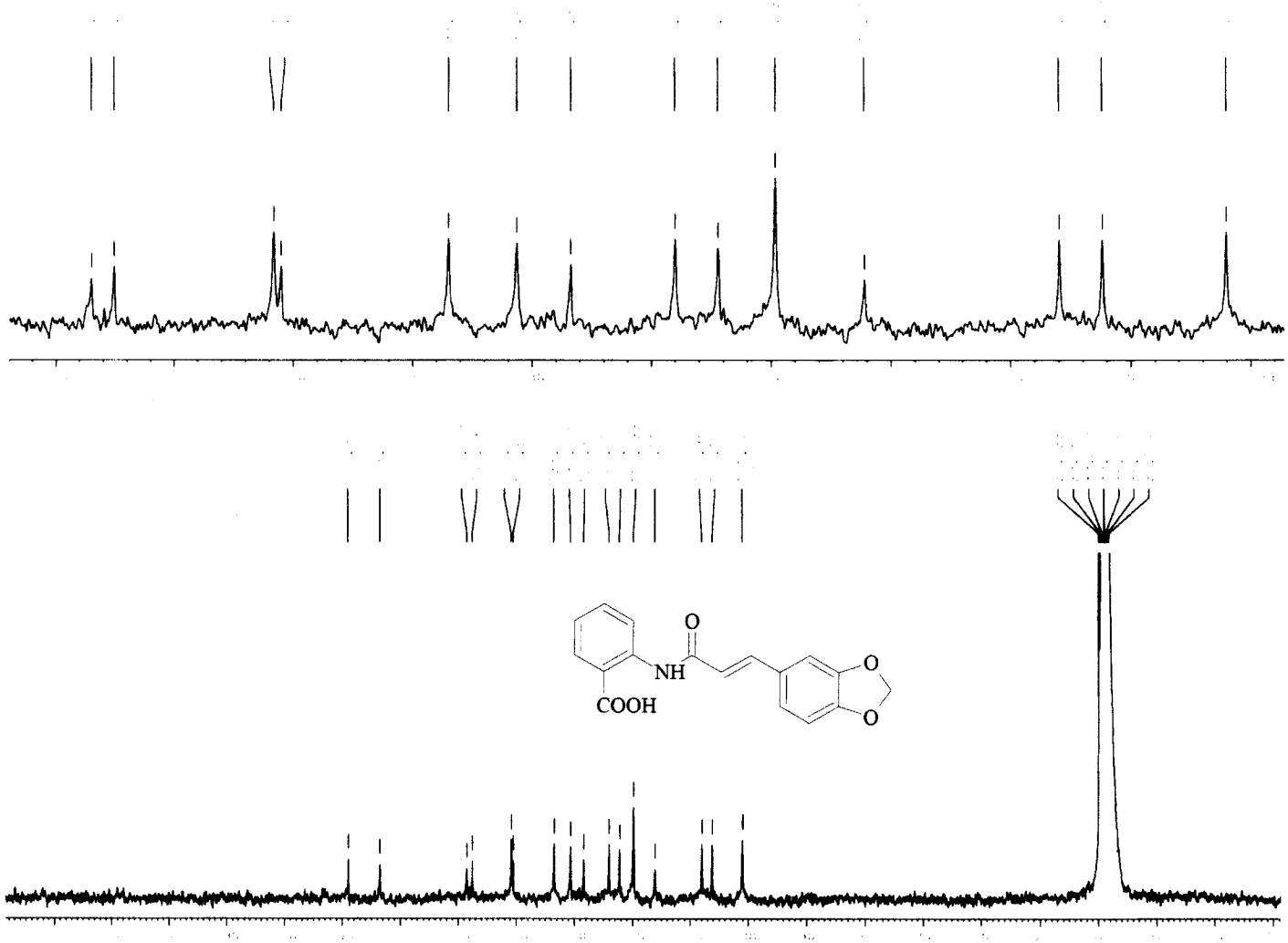


Fig. 1.07: ^{13}C NMR spectrum of **14**

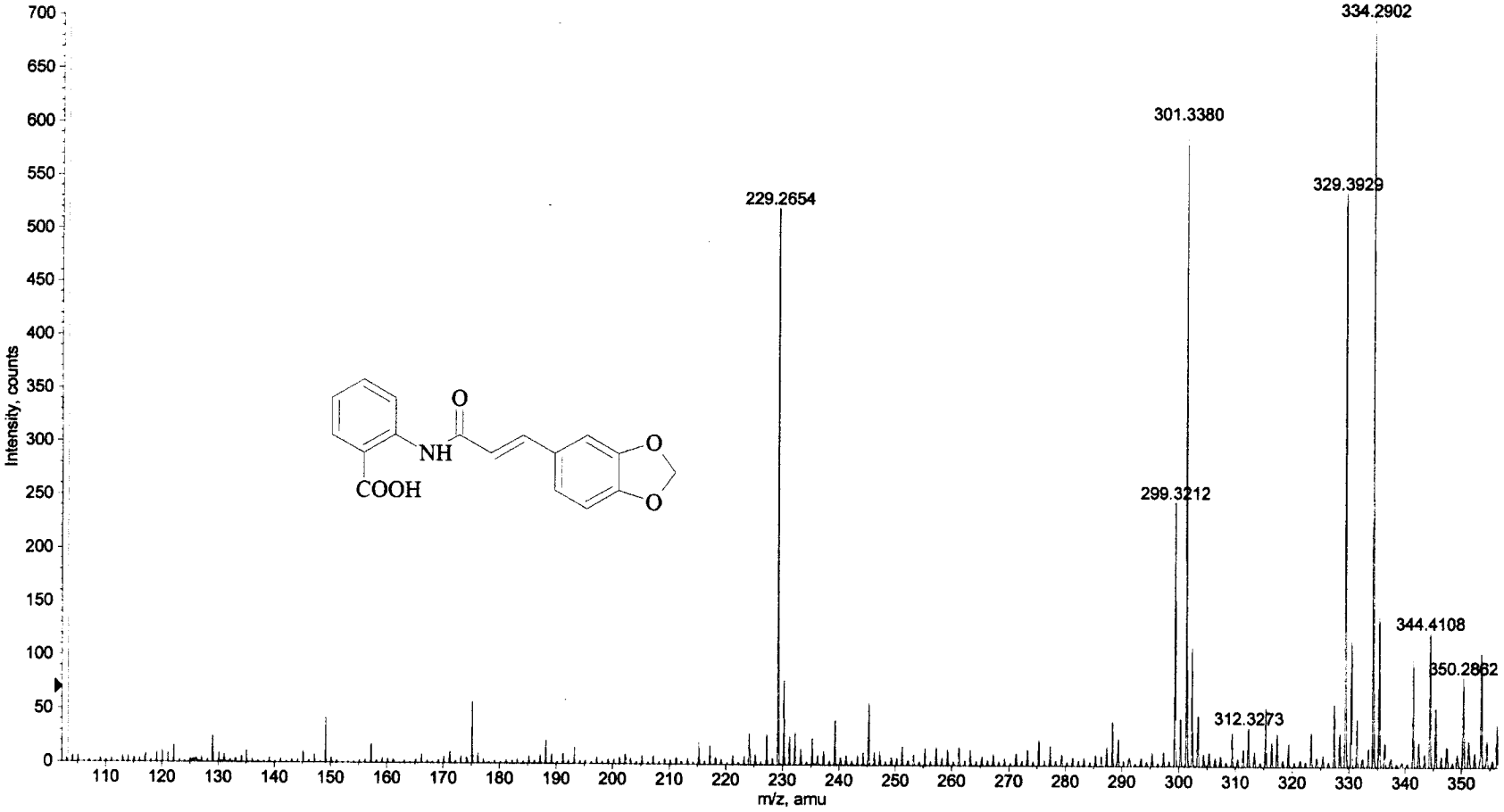


Fig. 1.08: LCMS spectrum of **14**

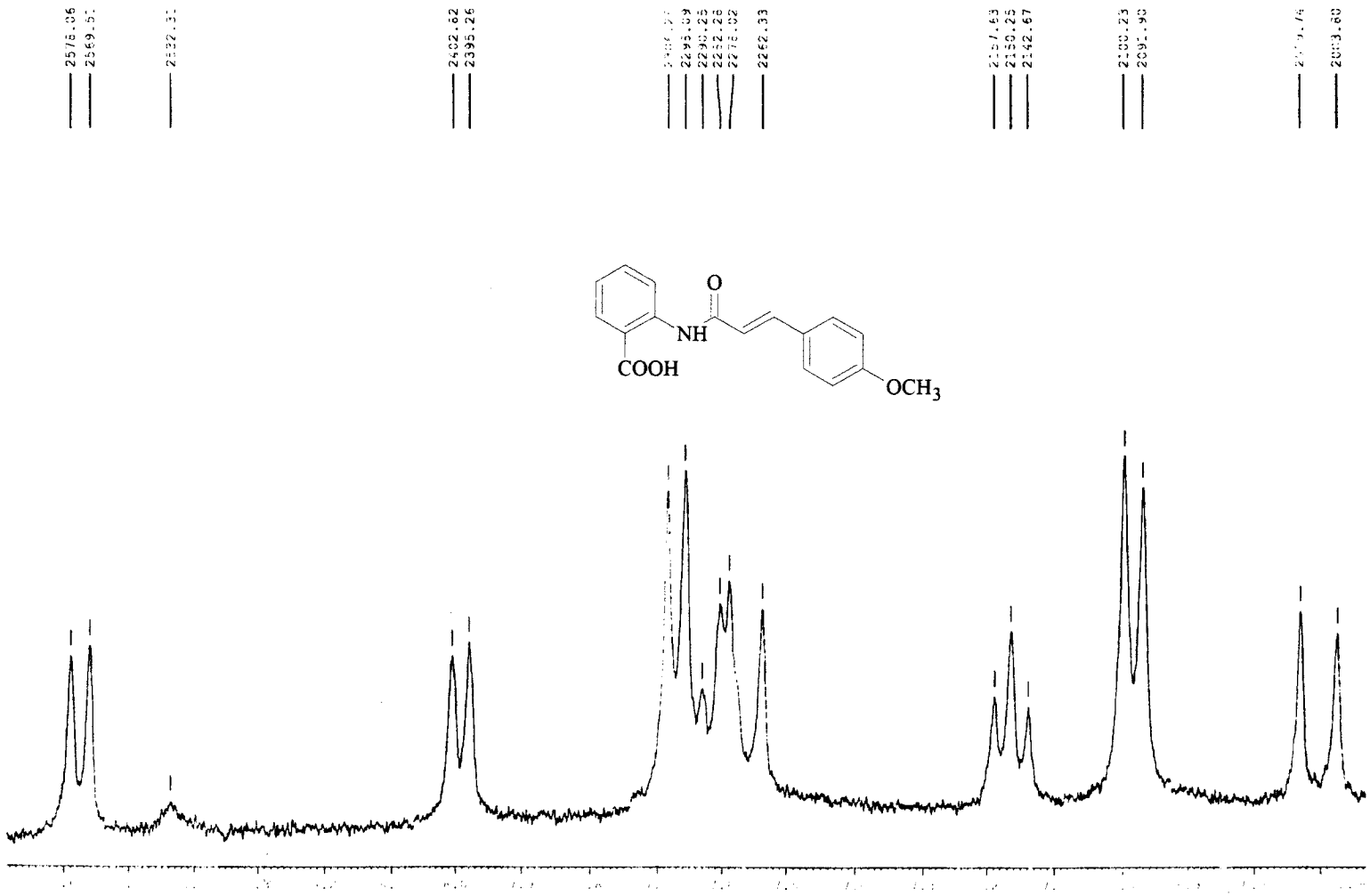


Fig. 1.09: ^{1}H NMR spectrum of **15**

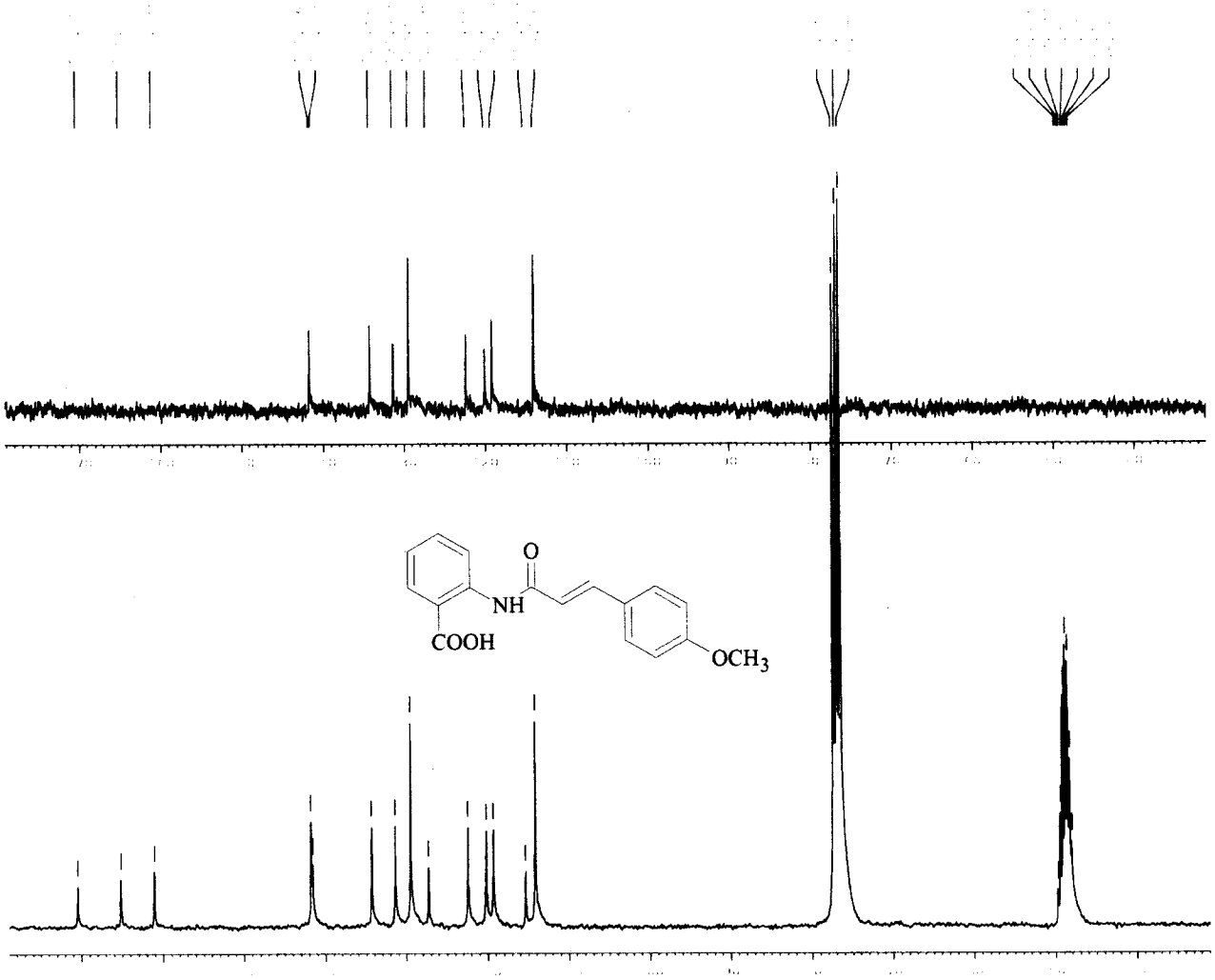


Fig. 1.10: ^{13}C NMR spectrum of **15**

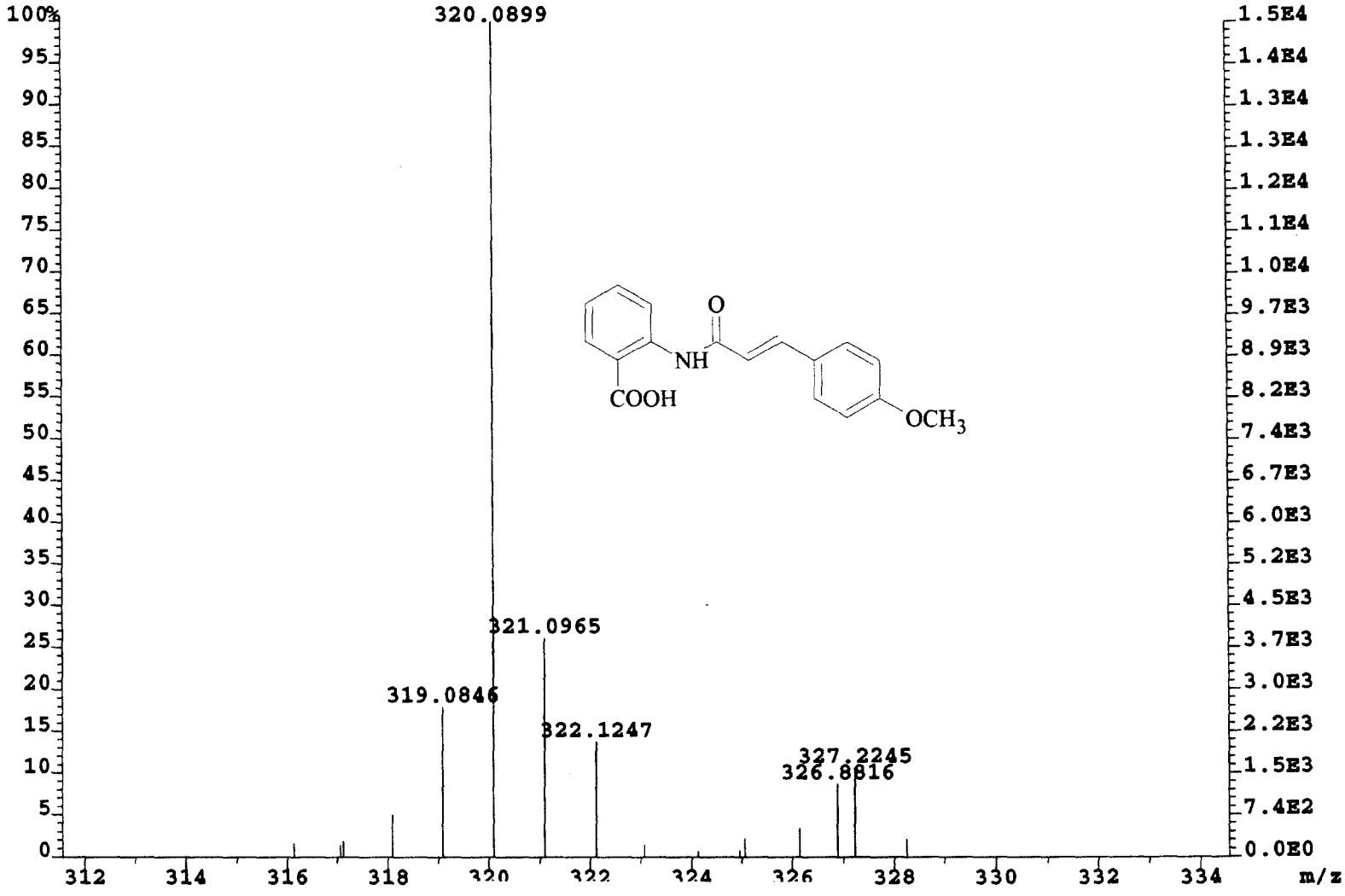


Fig. 1.11: HRFABMS spectrum of **15**

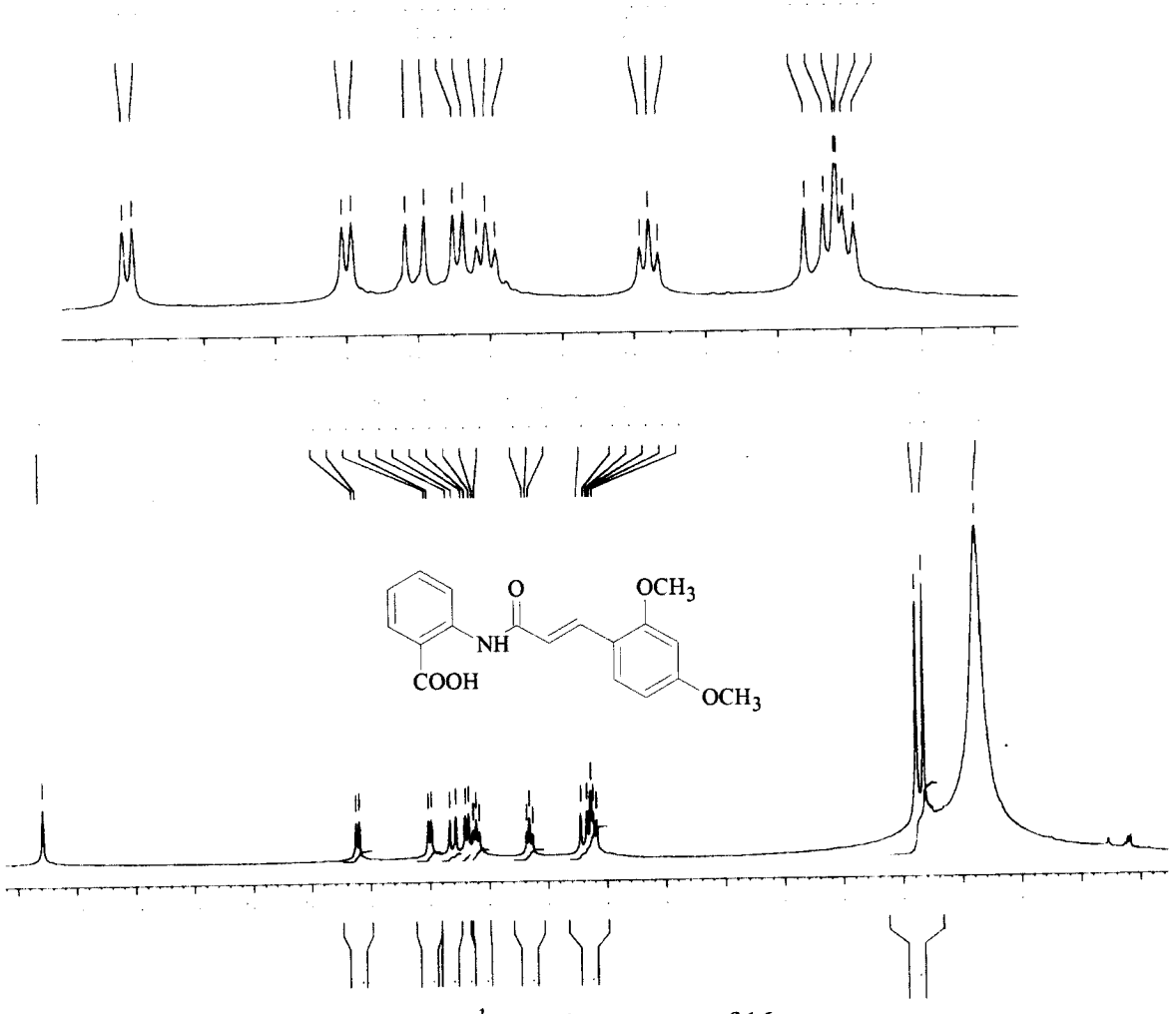


Fig. 1.12: ^{1}H NMR spectrum of **16**

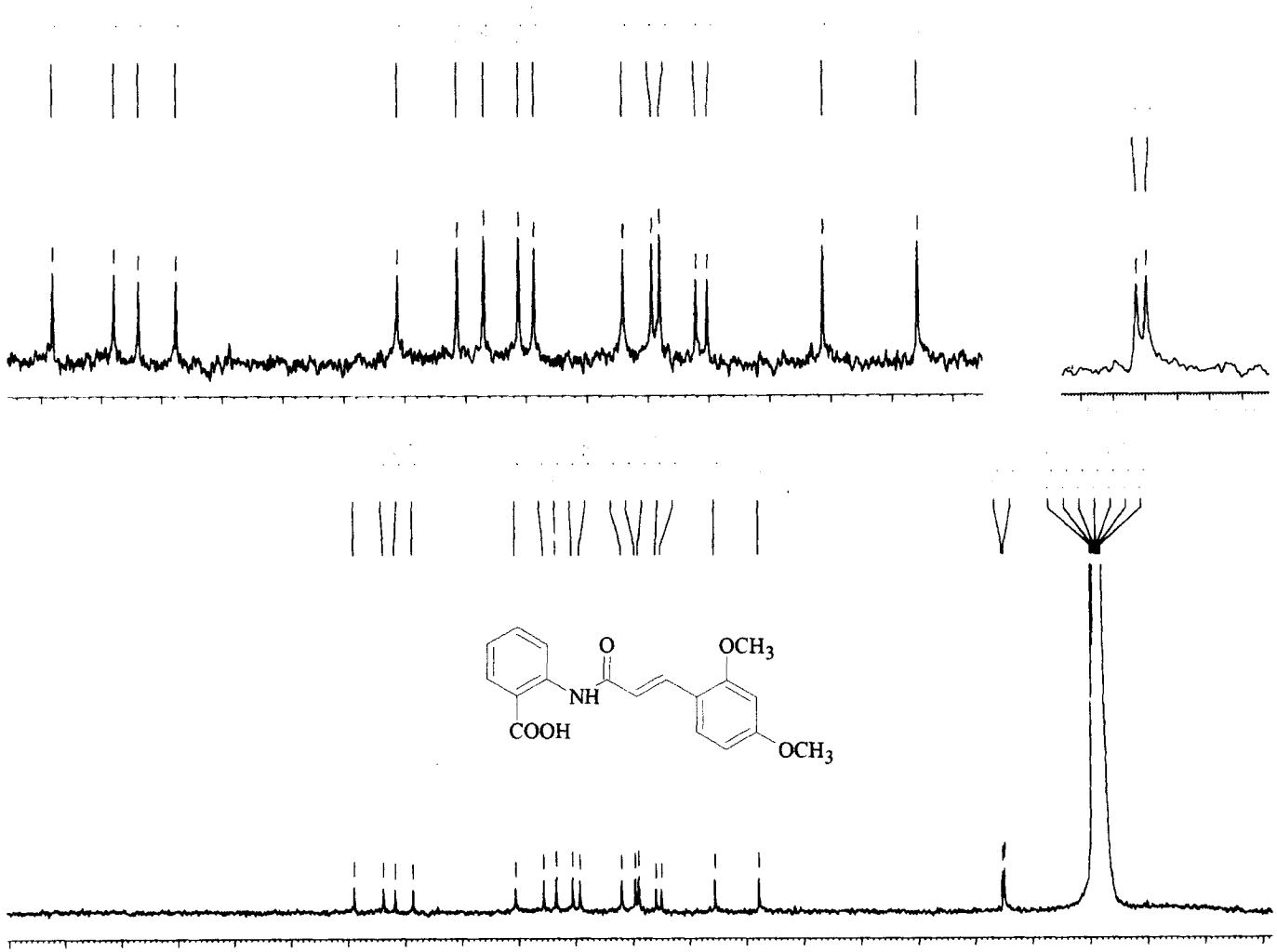


Fig. 1.13: ^{13}C NMR spectrum of **16**

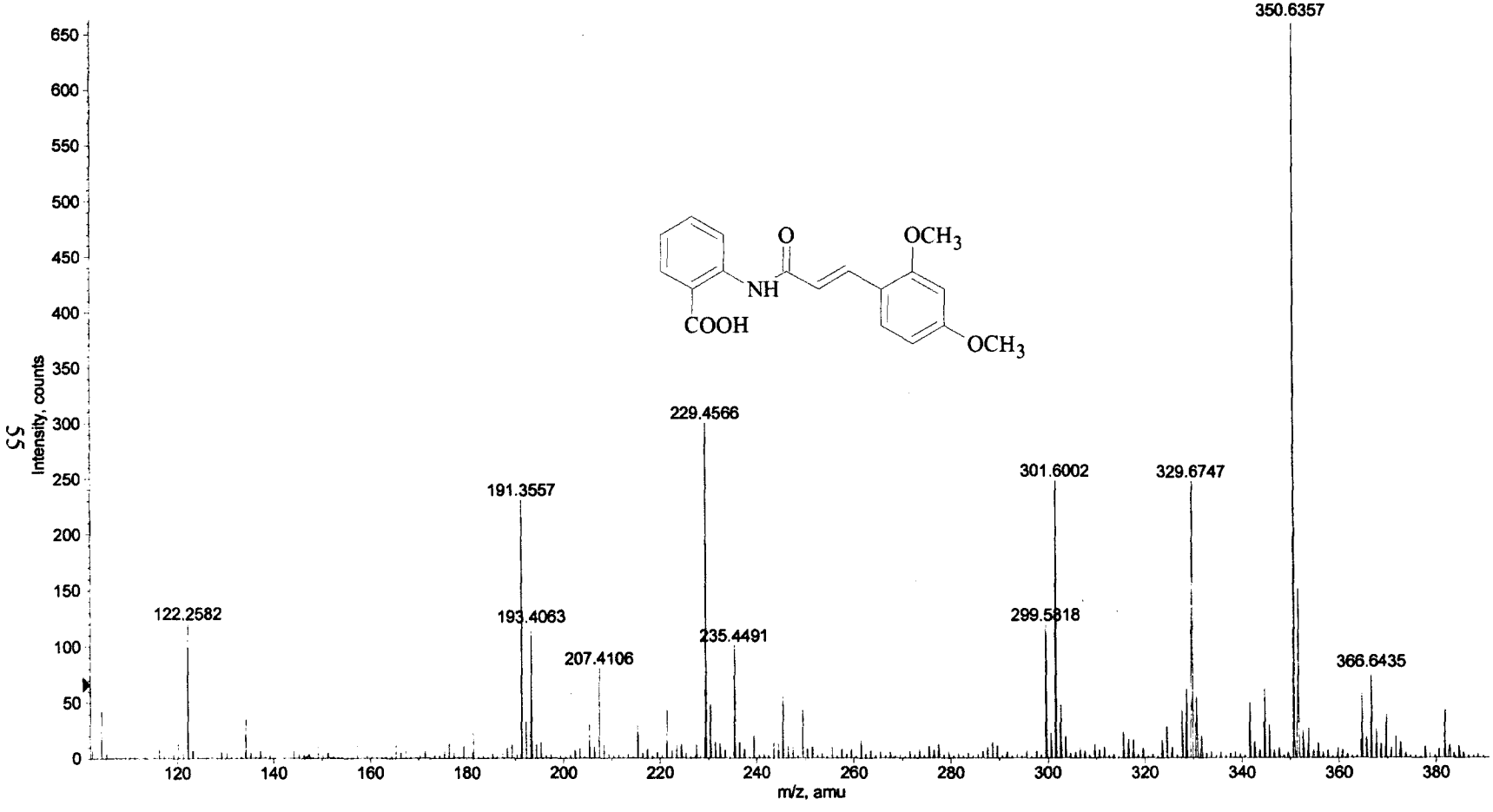


Fig. 1.14: LCMS spectrum of **16**

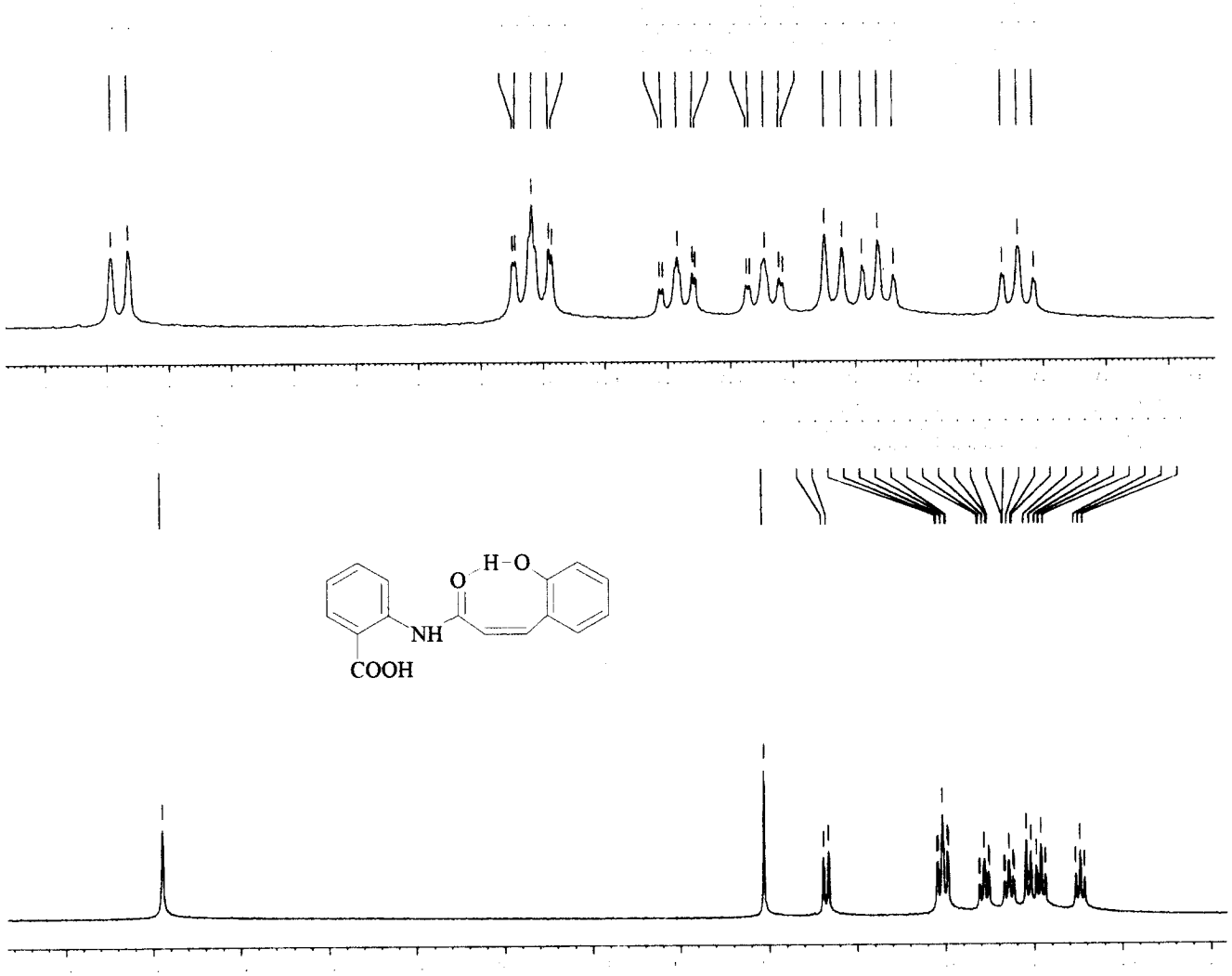


Fig. 1.15: ^{1}H NMR spectrum of **17**

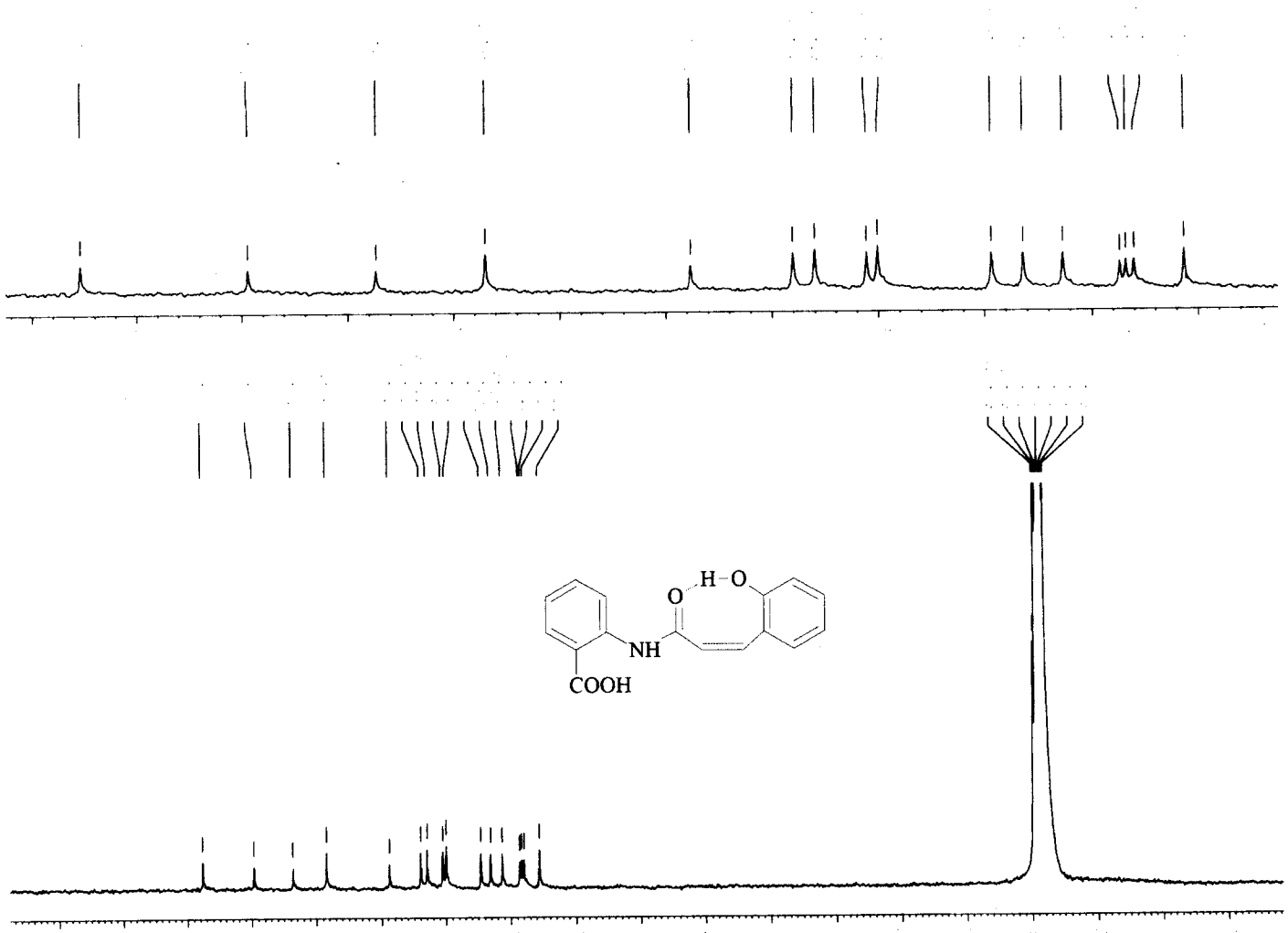


Fig. 1.16: ^{13}C NMR spectrum of **17**

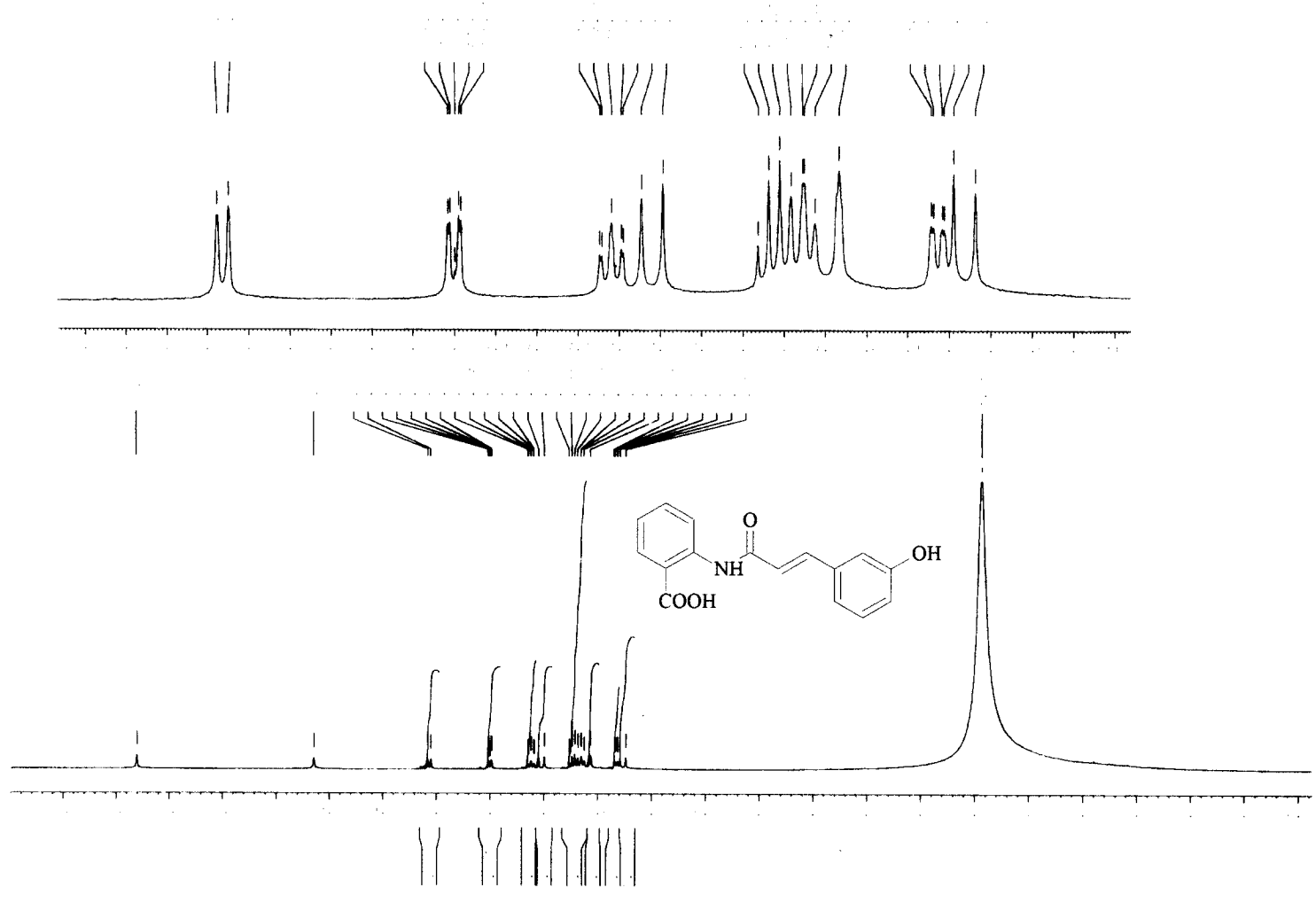


Fig. 1.17: ^{1}H NMR spectrum of **18**

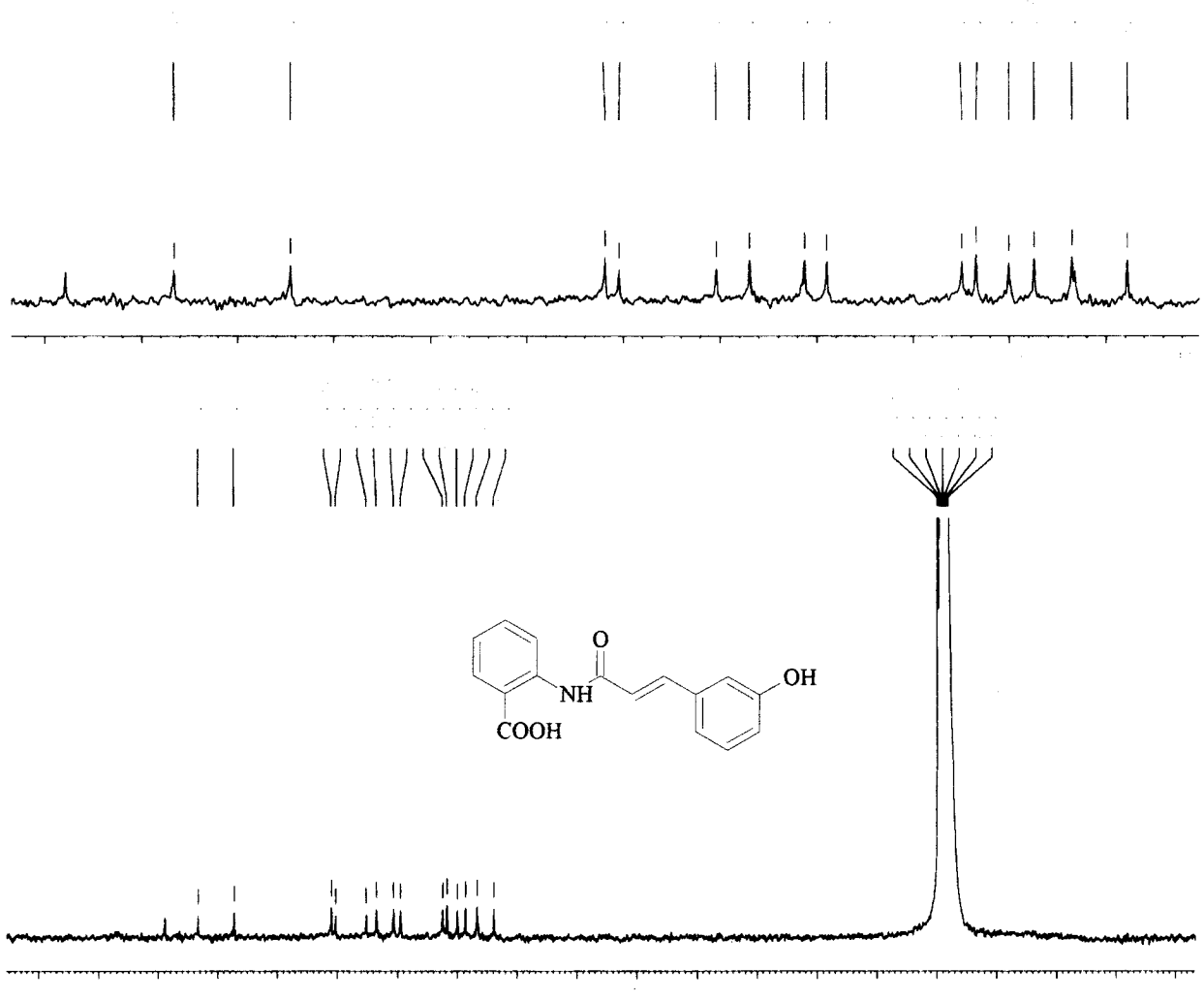


Fig. 1.18: ^{13}C NMR spectrum of **18**

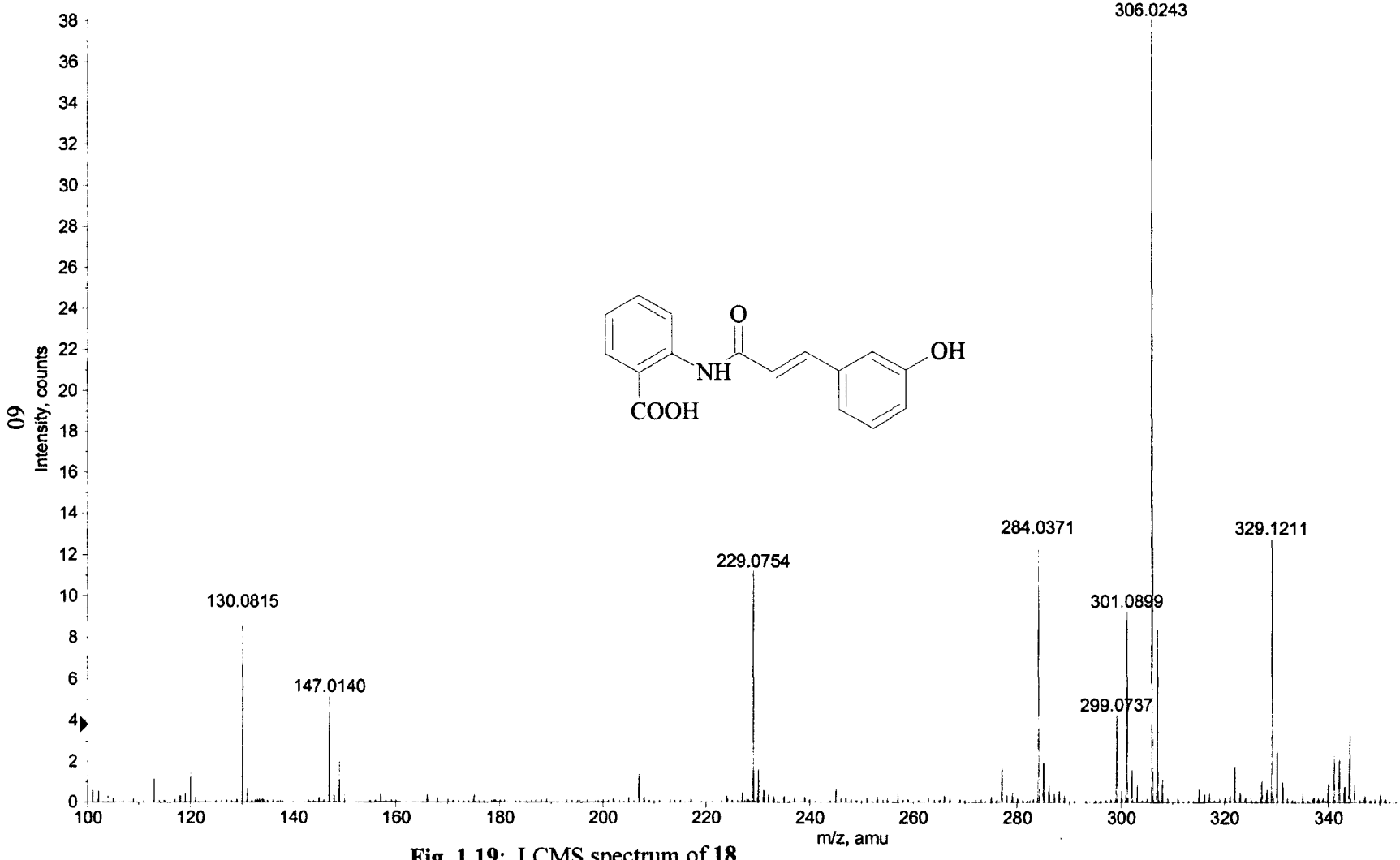


Fig. 1.19: LCMS spectrum of **18**

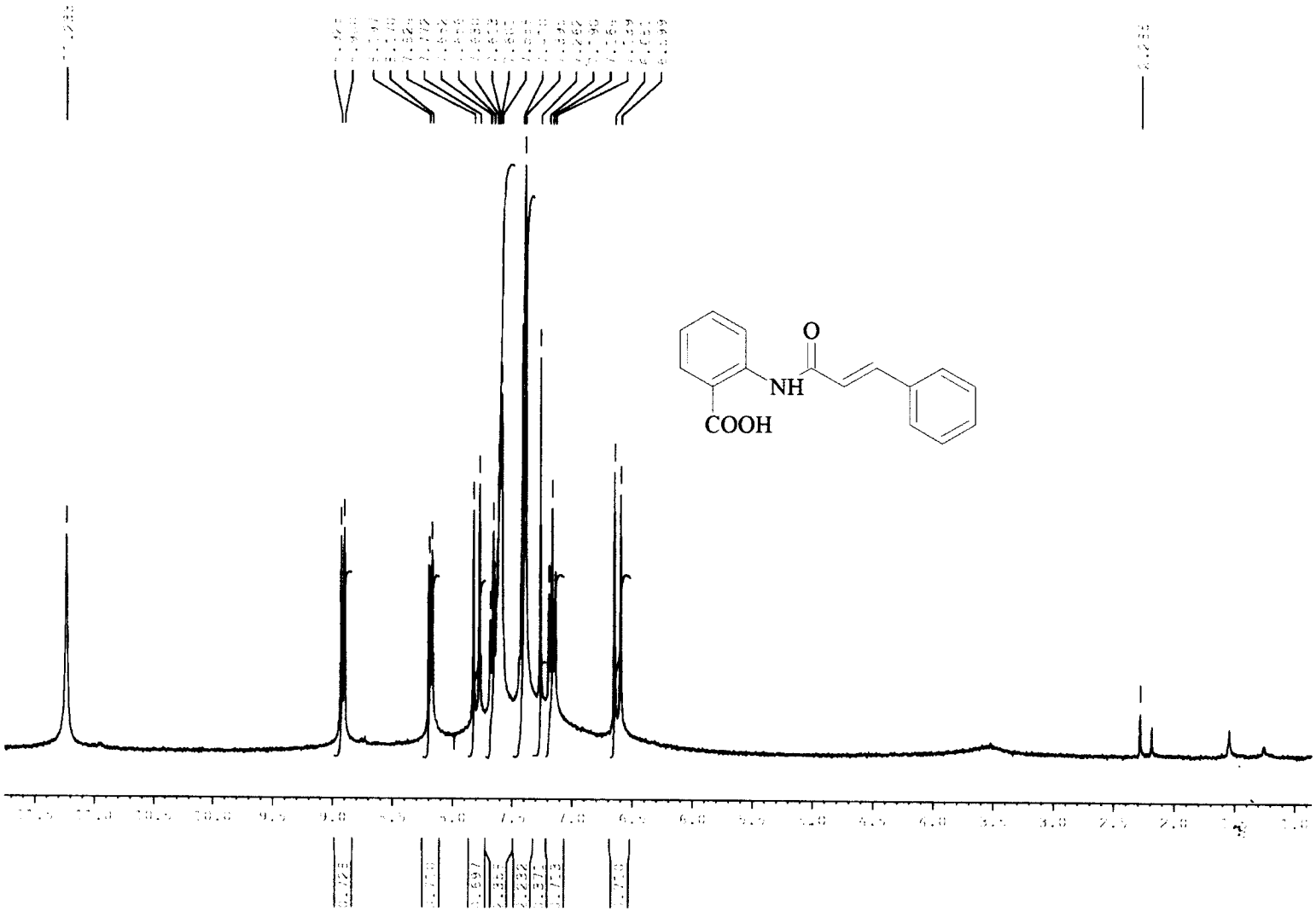


Fig. 1.20: ^{1}H NMR spectrum of **19**

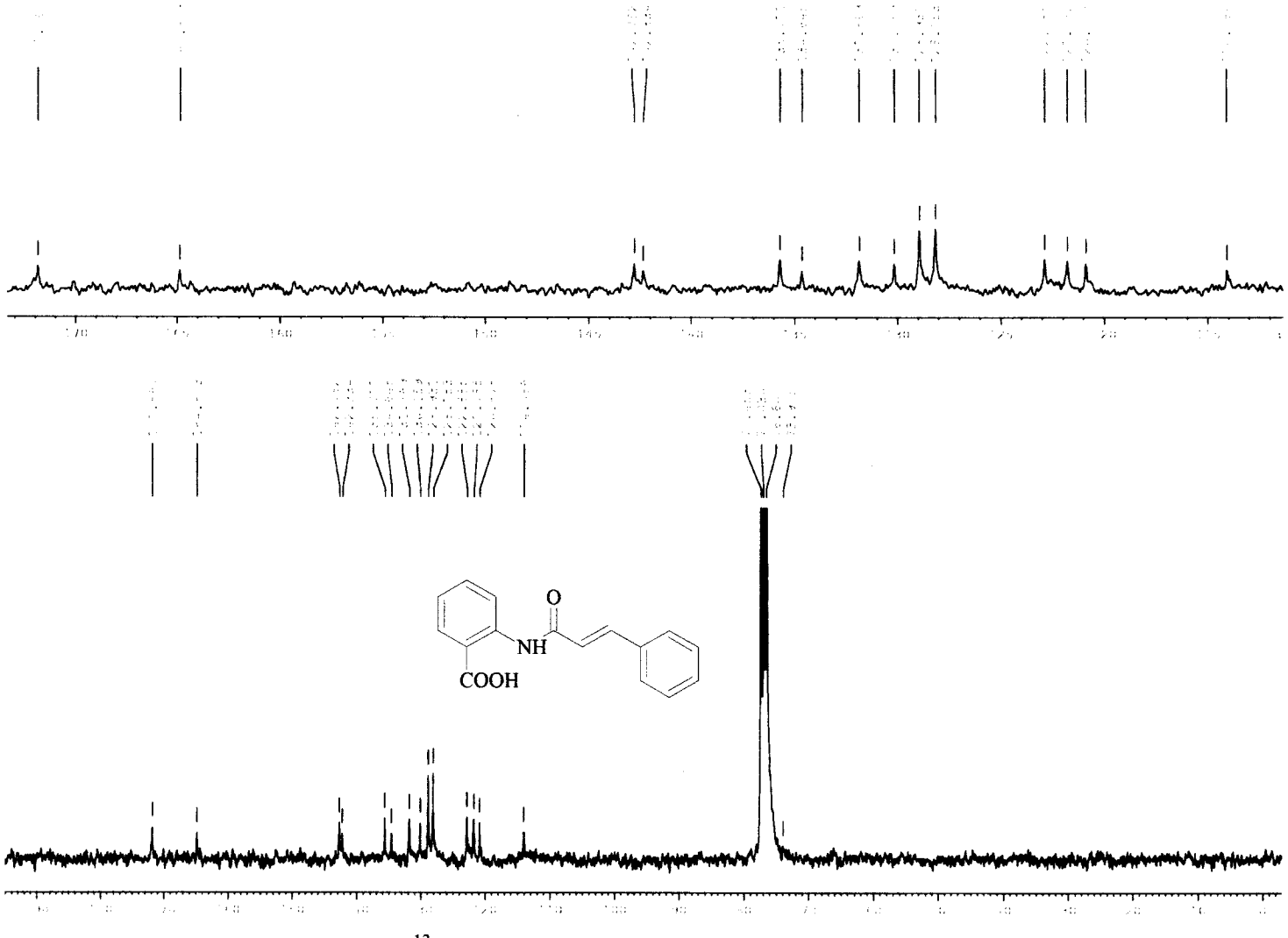


Fig. 1.21: ^{13}C NMR spectrum of **19**

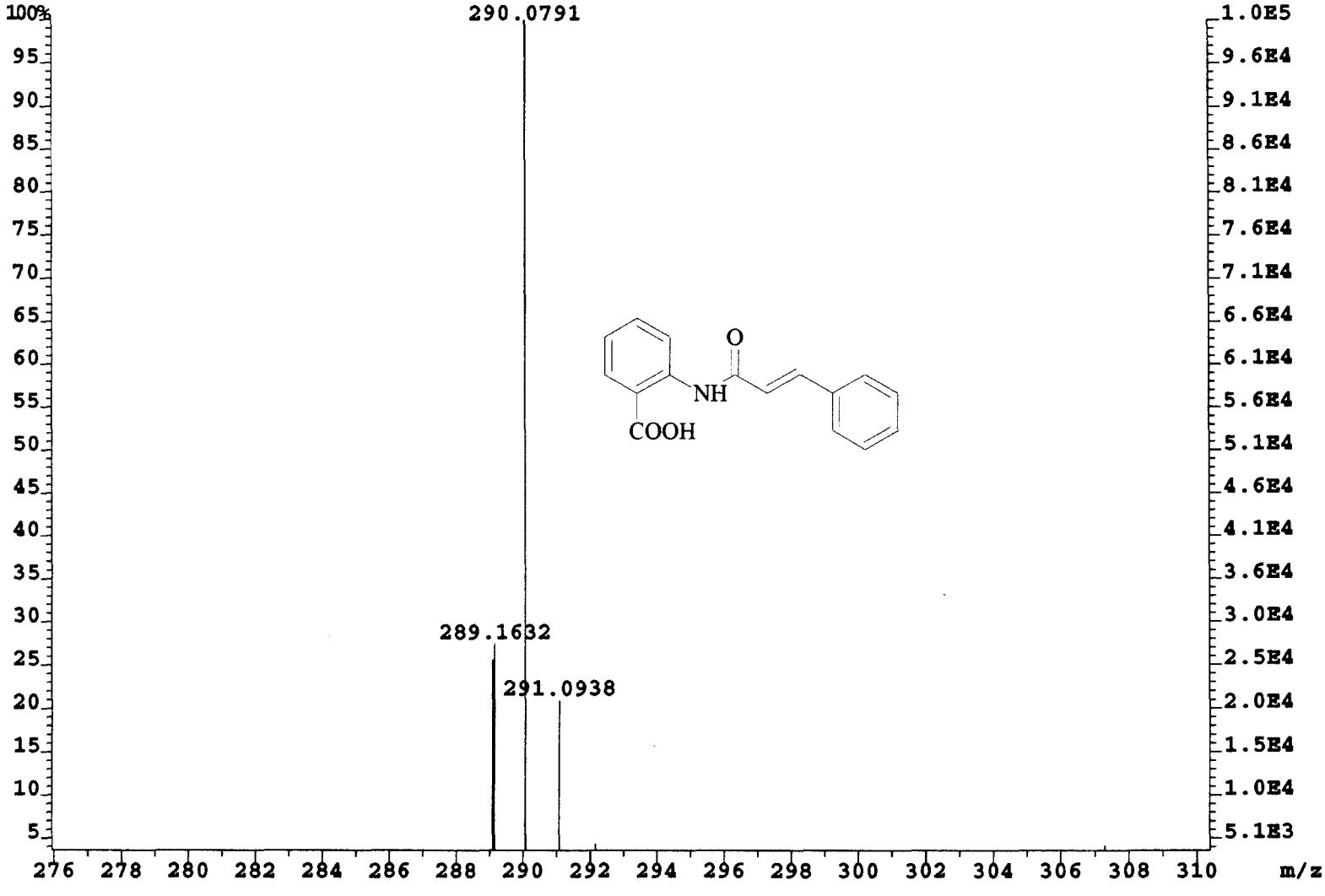


Fig. 1.22: HRFABMS spectrum of **19**

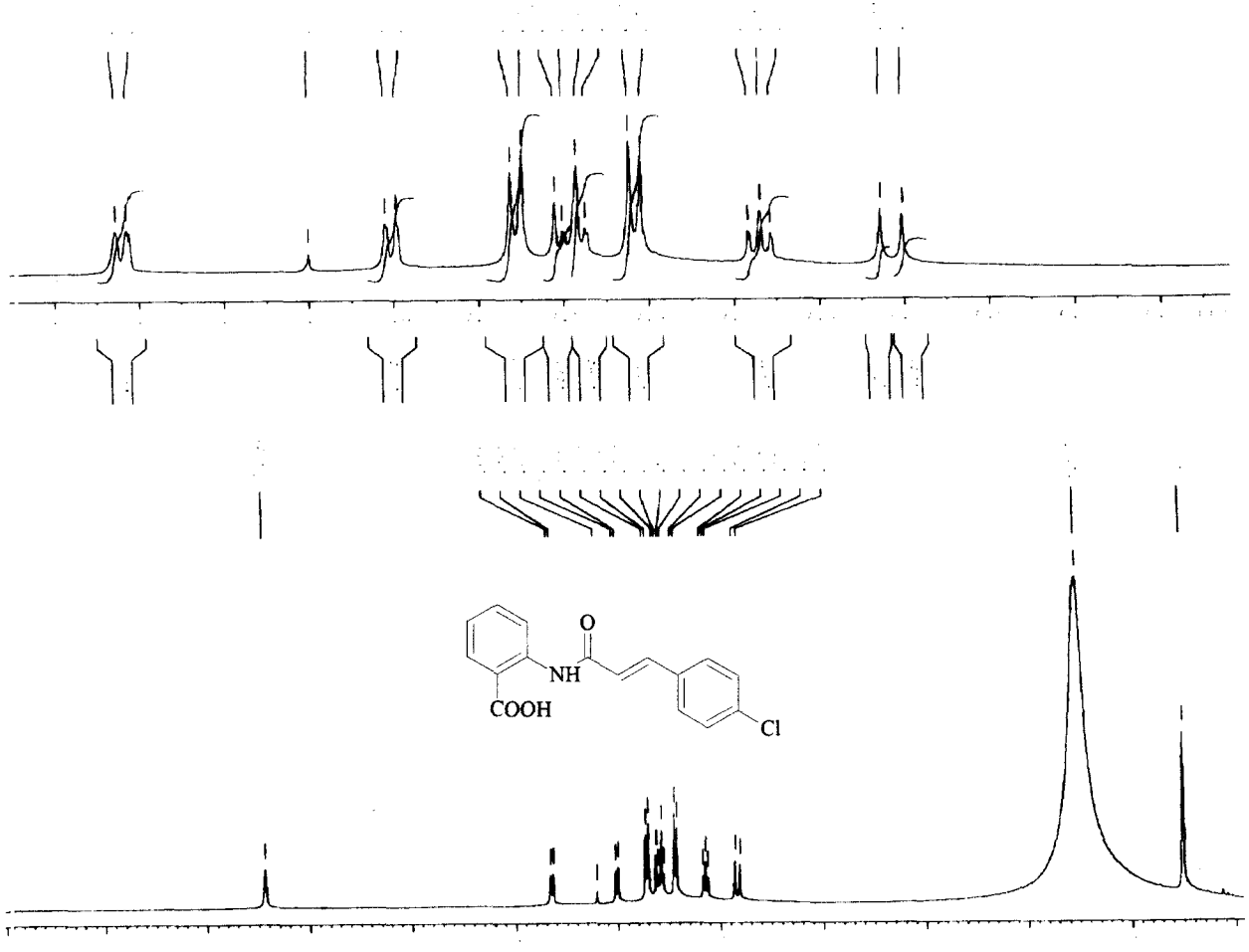


Fig. 1.23: ^{1}H NMR spectrum of **20**

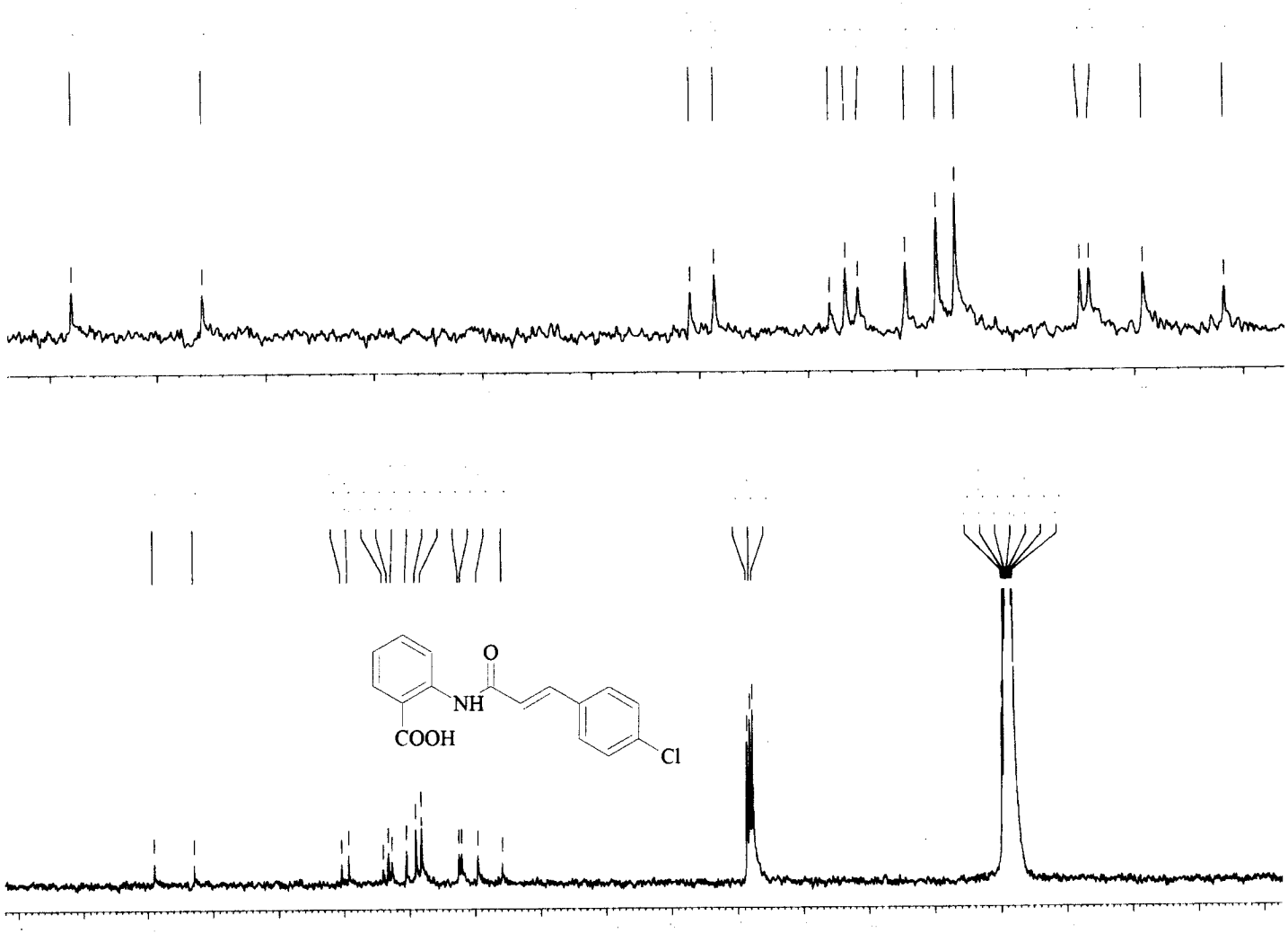


Fig. 1.24: ^{13}C NMR spectrum of **20**

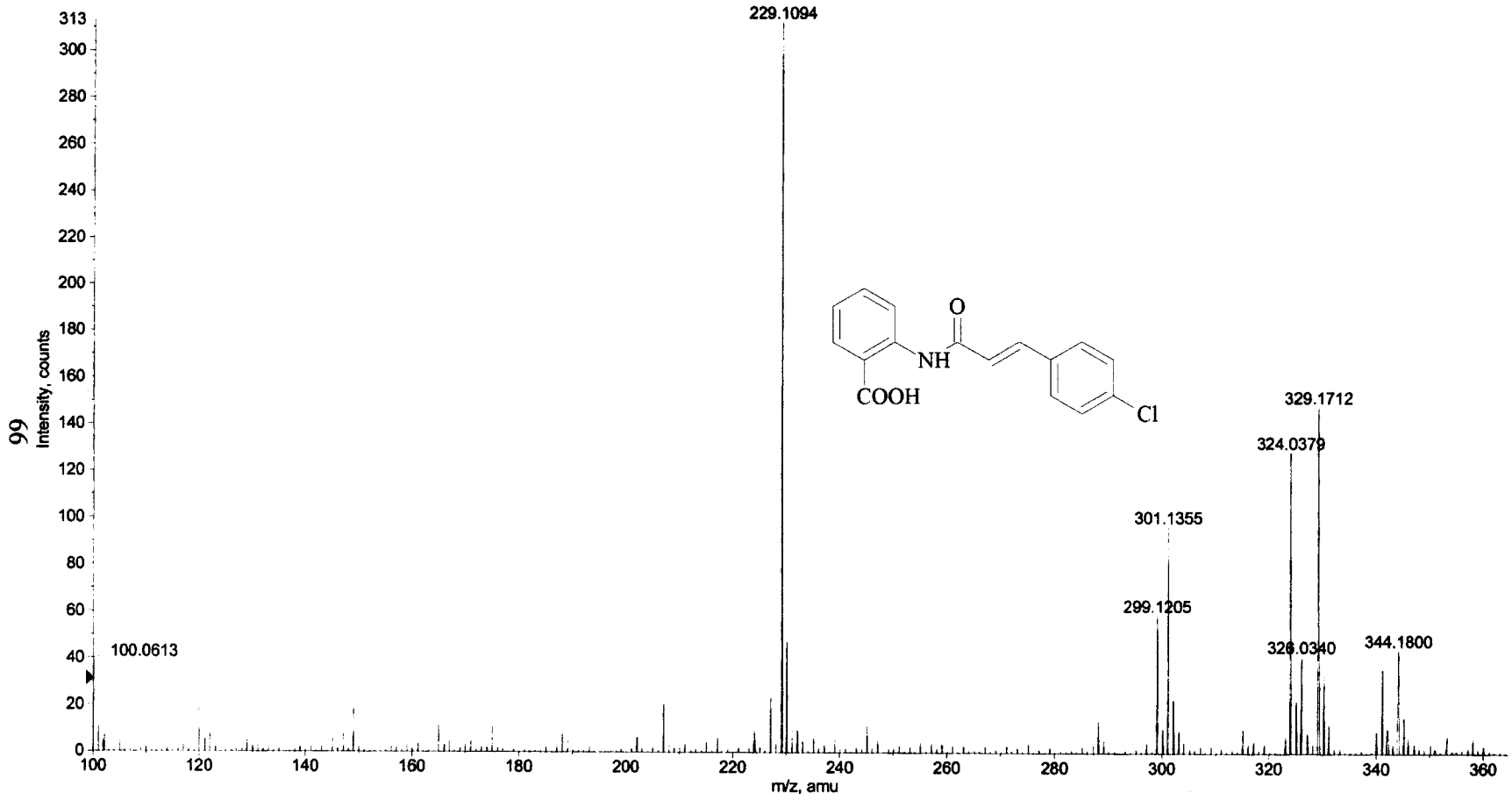


Fig. 1.25: LCMS spectrum of **20**

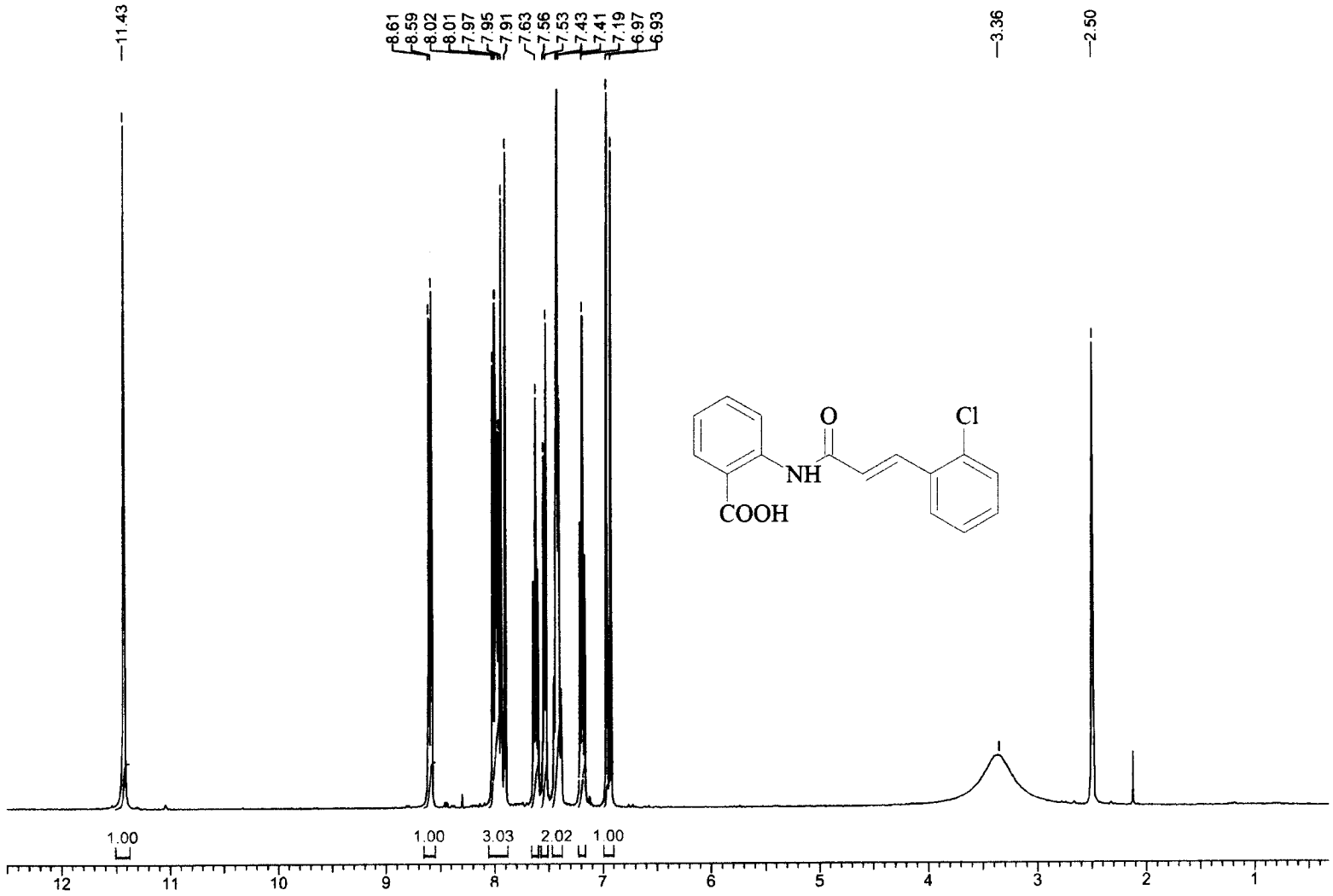


Fig. 1.26: ^{1}H NMR spectrum of **21**

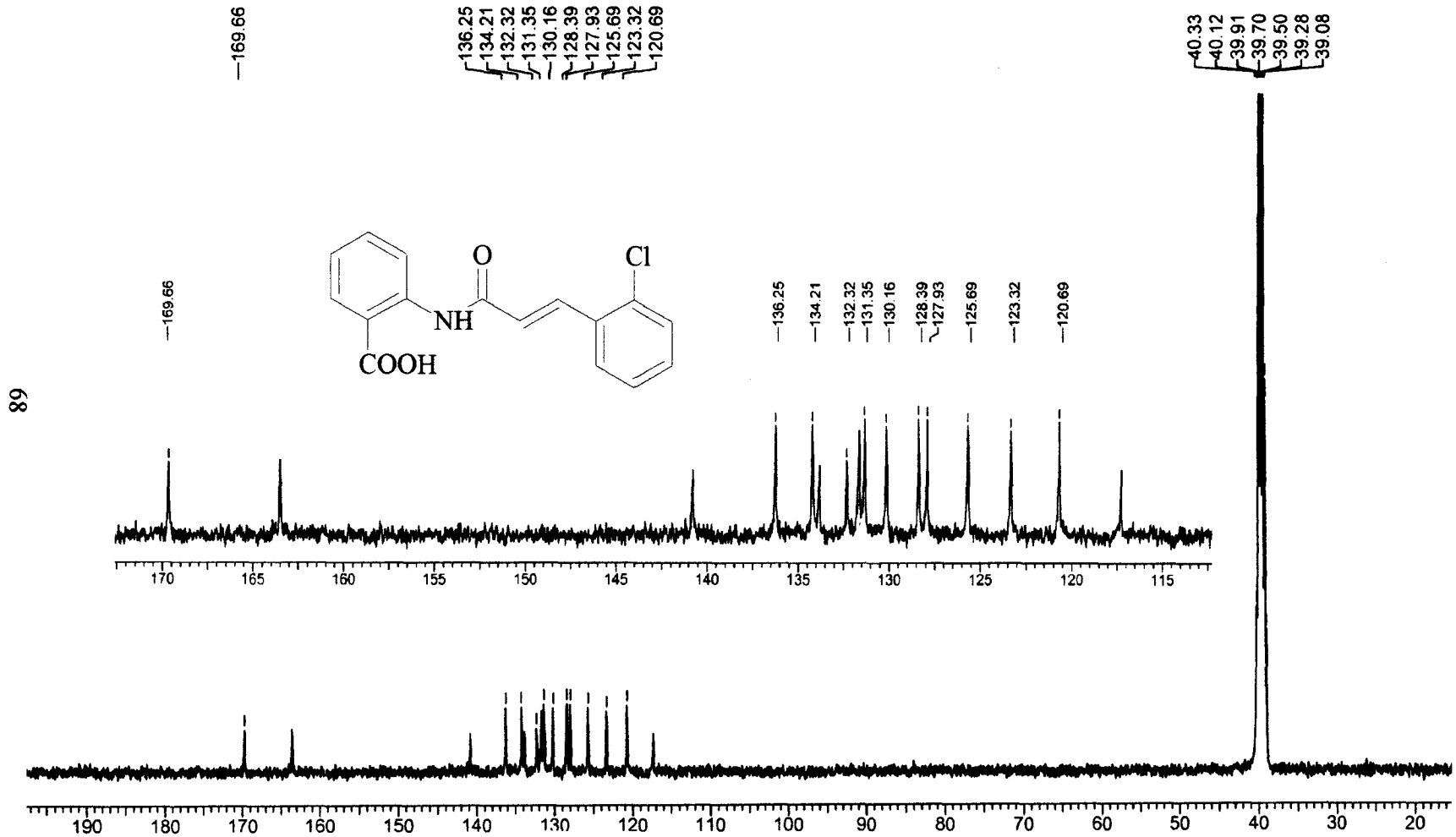


Fig. 1.27: ^{13}C NMR spectrum of **21**

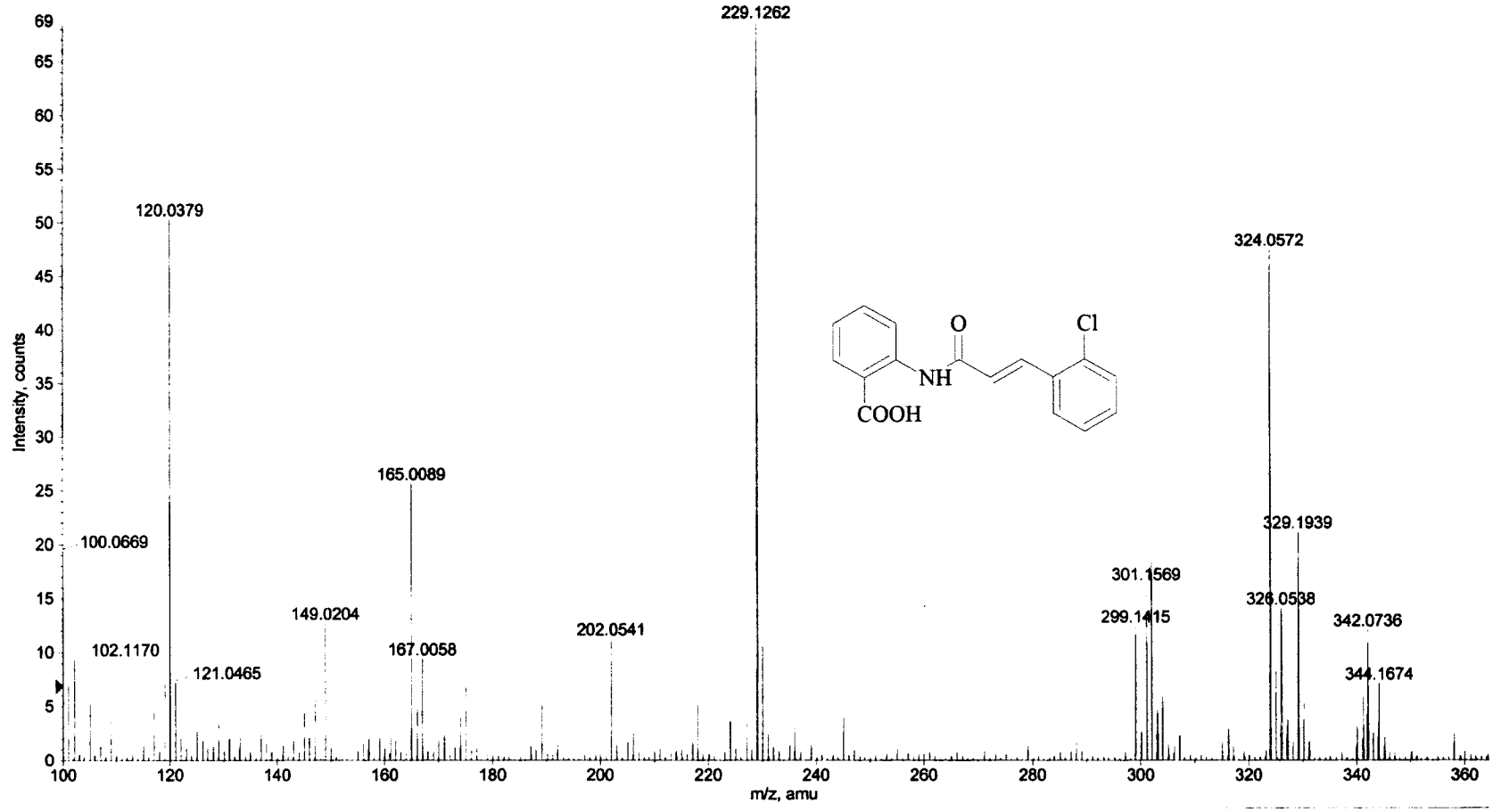


Fig. 1.28: LCMS spectrum of **21**

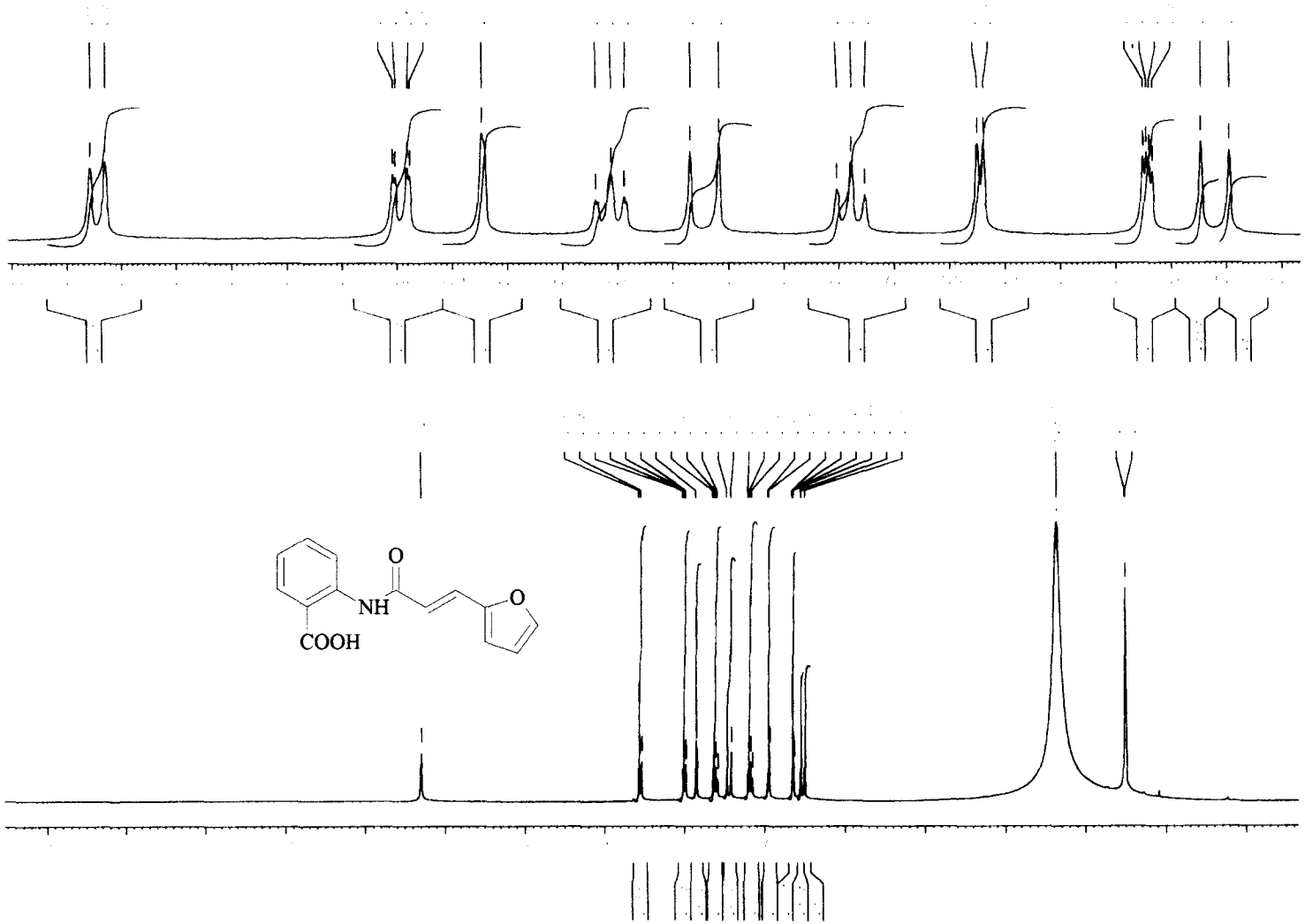


Fig. 1.29: ^{1}H NMR spectrum of **33**

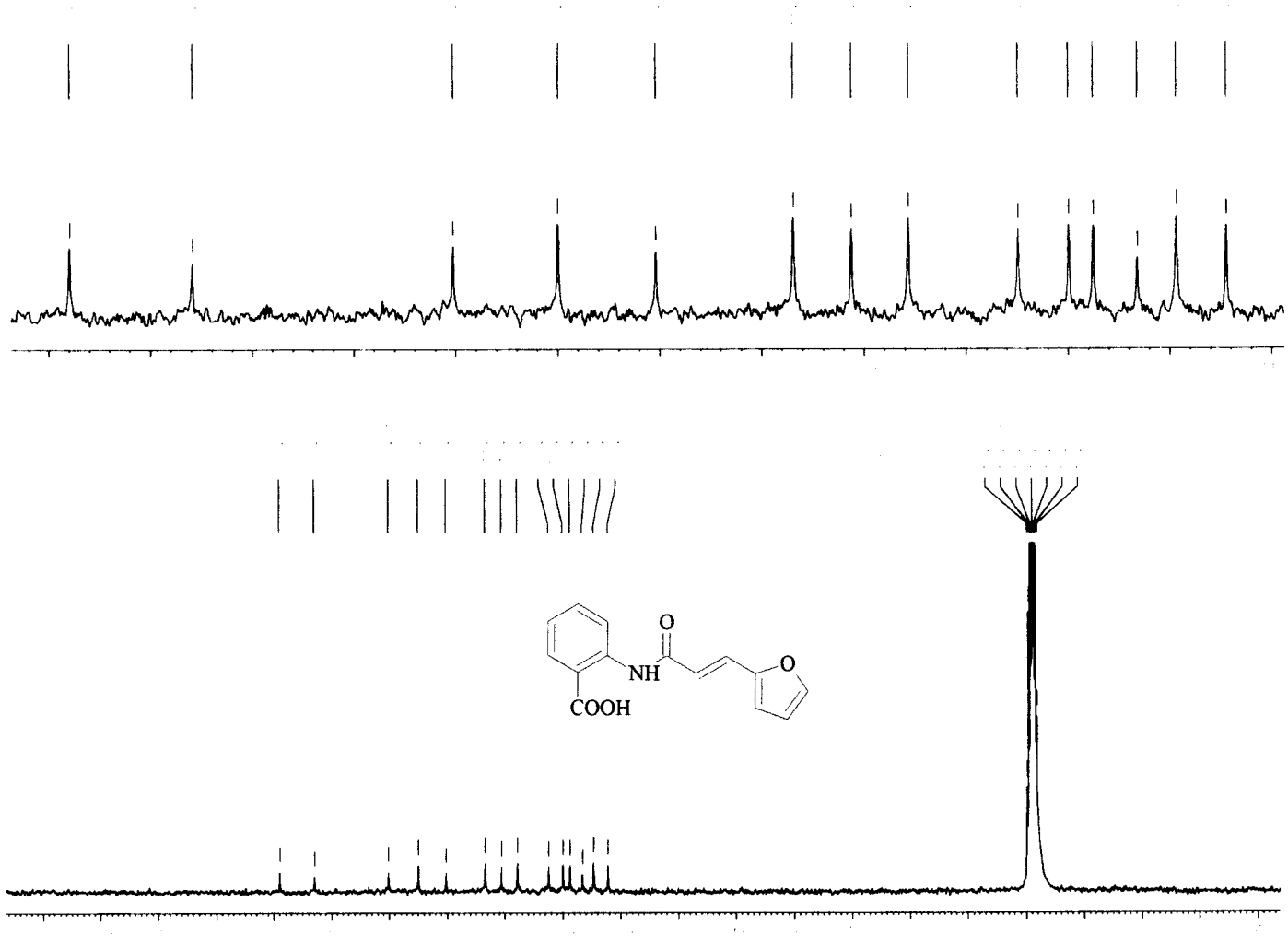


Fig. 1.30: ^{13}C NMR spectrum of **33**

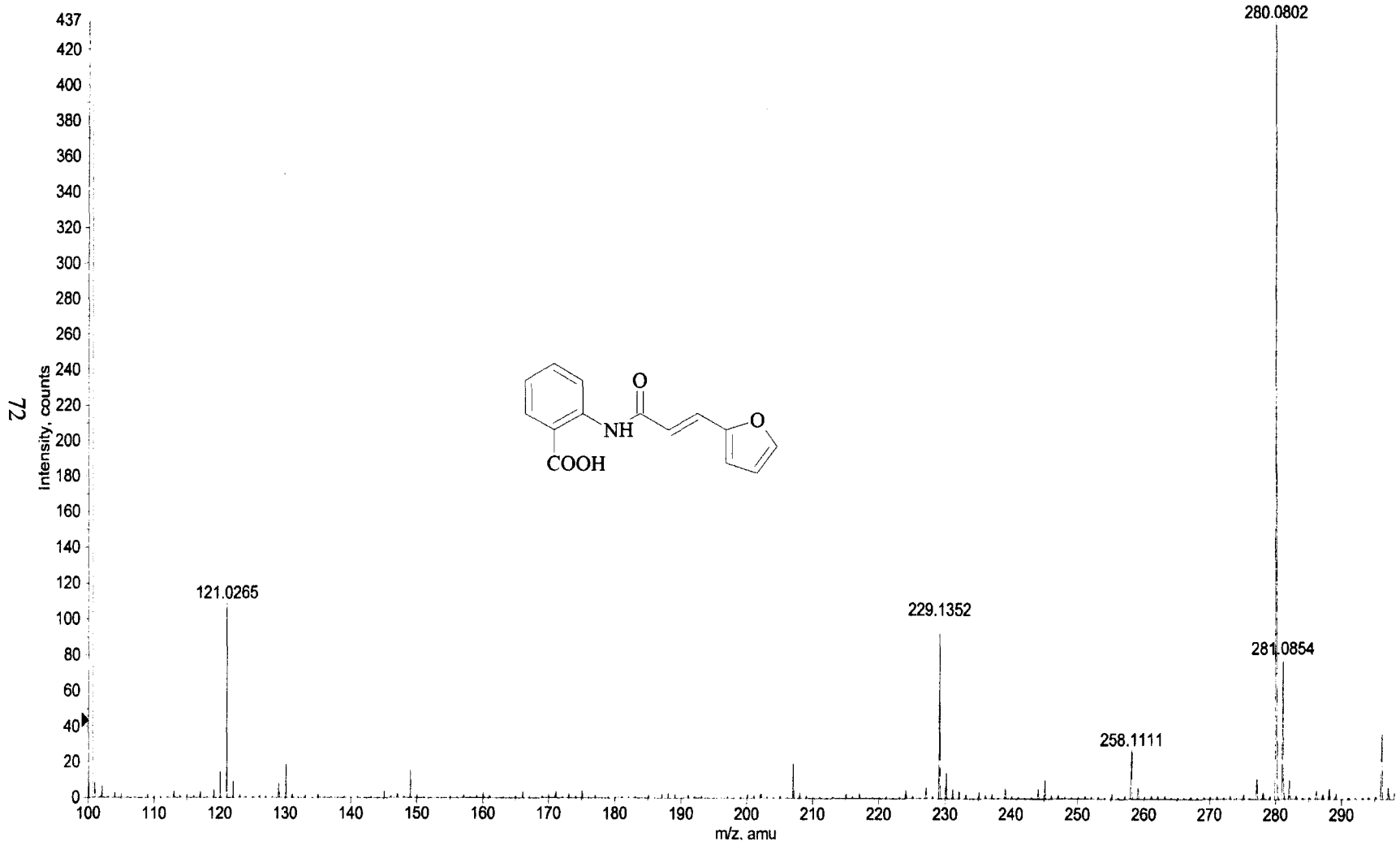


Fig. 1.31: LCMS spectrum of **33**

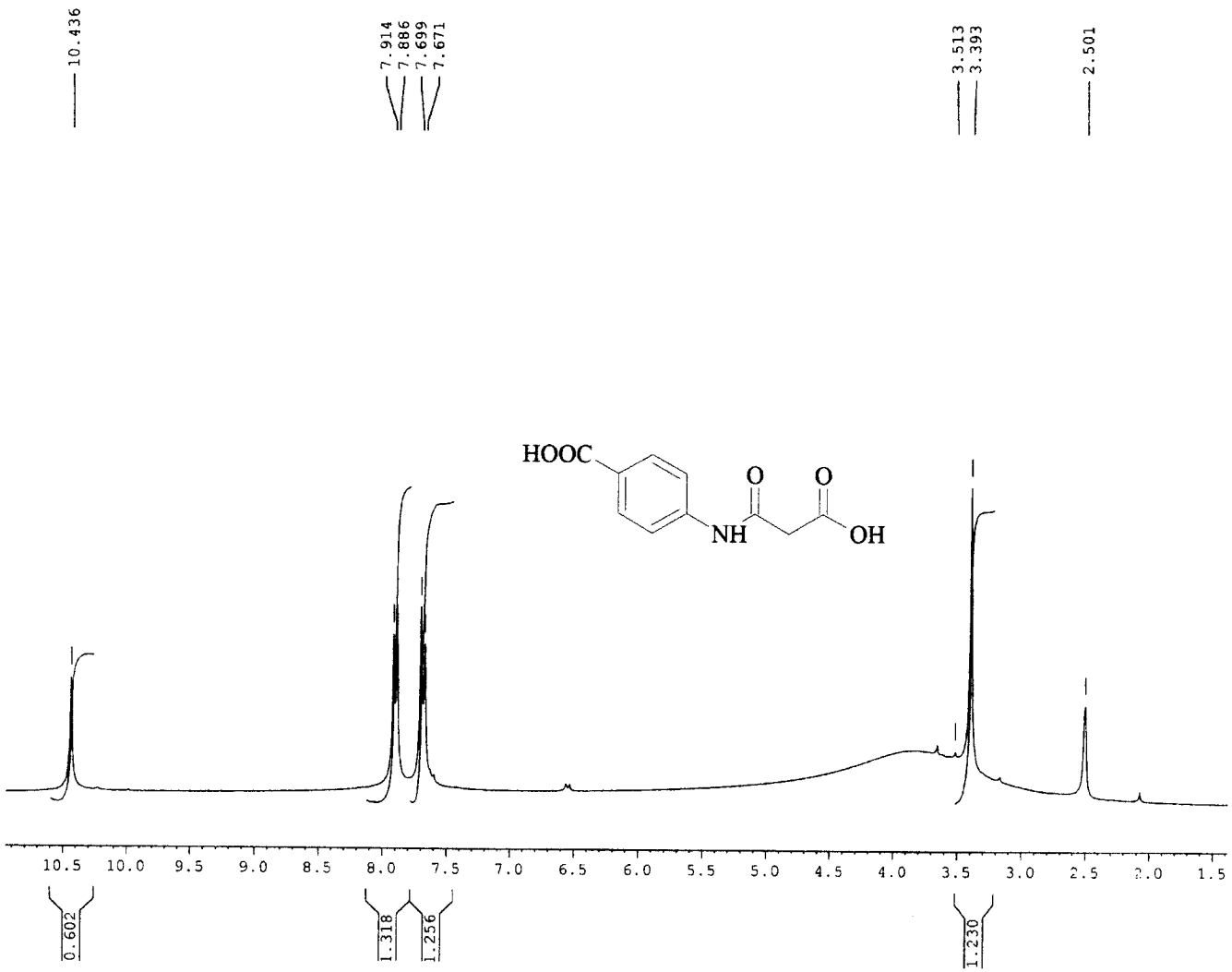


Fig. 1.32: ^{1}H NMR spectrum of **37**

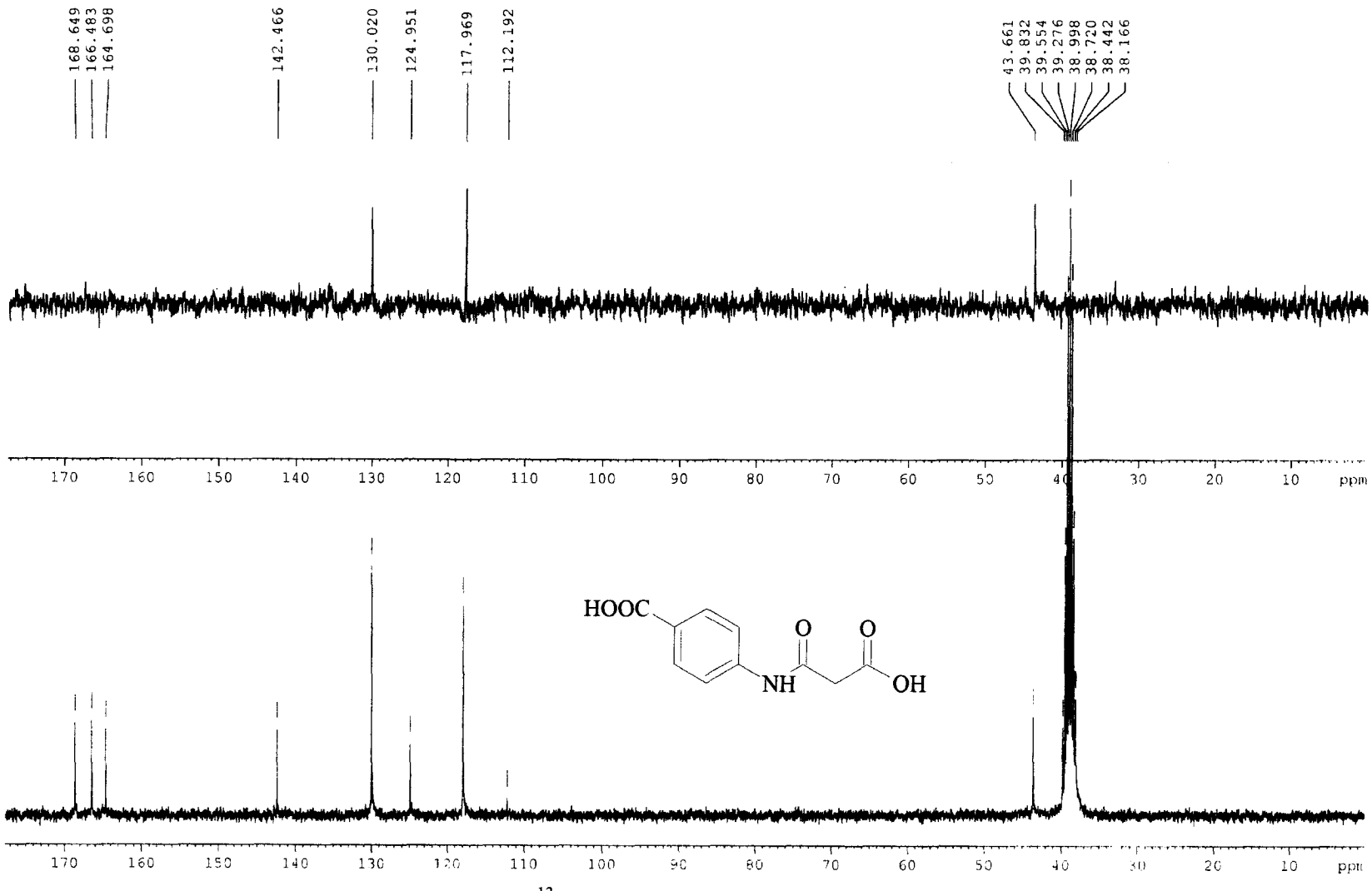


Fig. 1.33: ^{13}C NMR spectrum of **37**

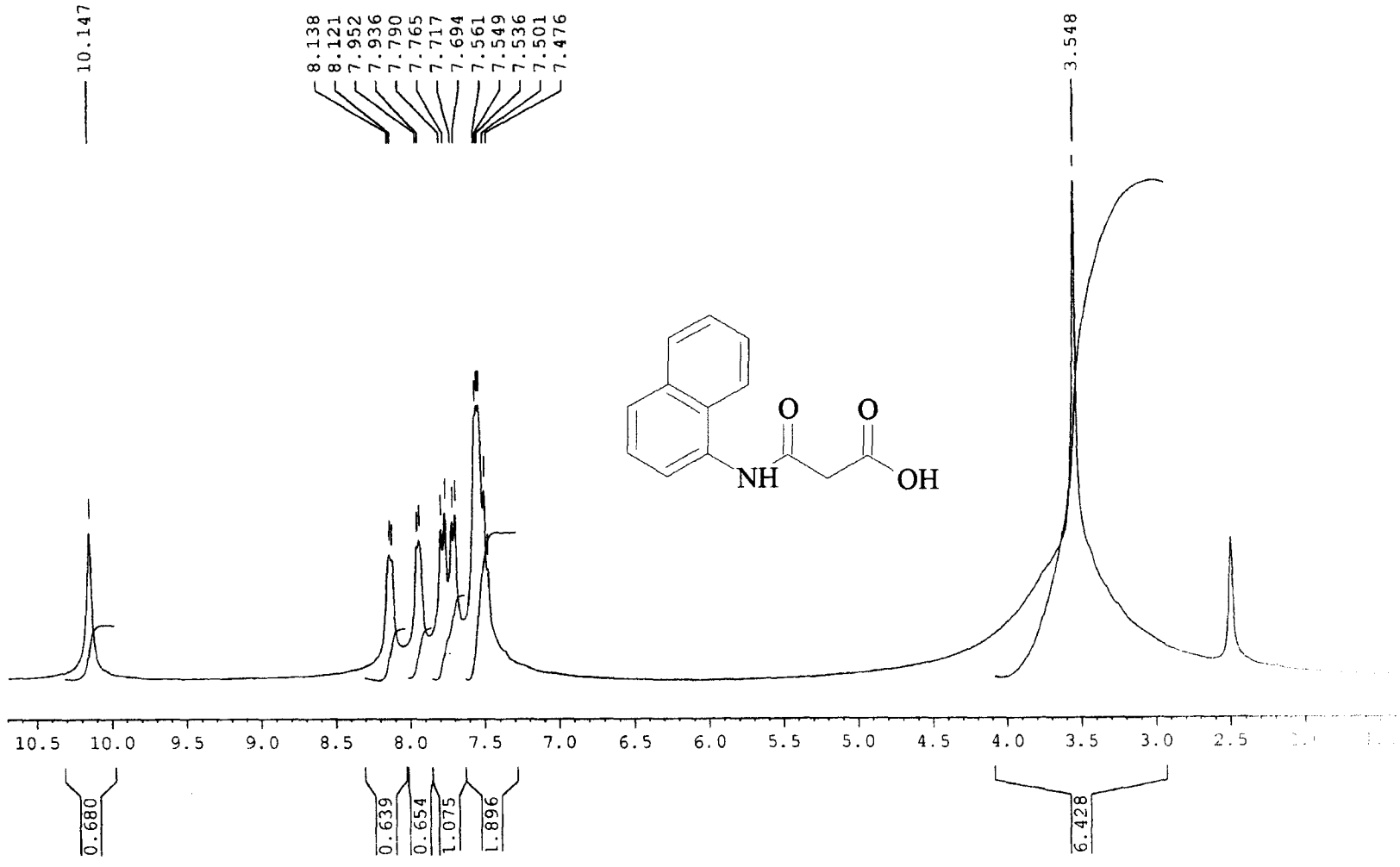


Fig. 1.34: ^{1}H NMR spectrum of **38**

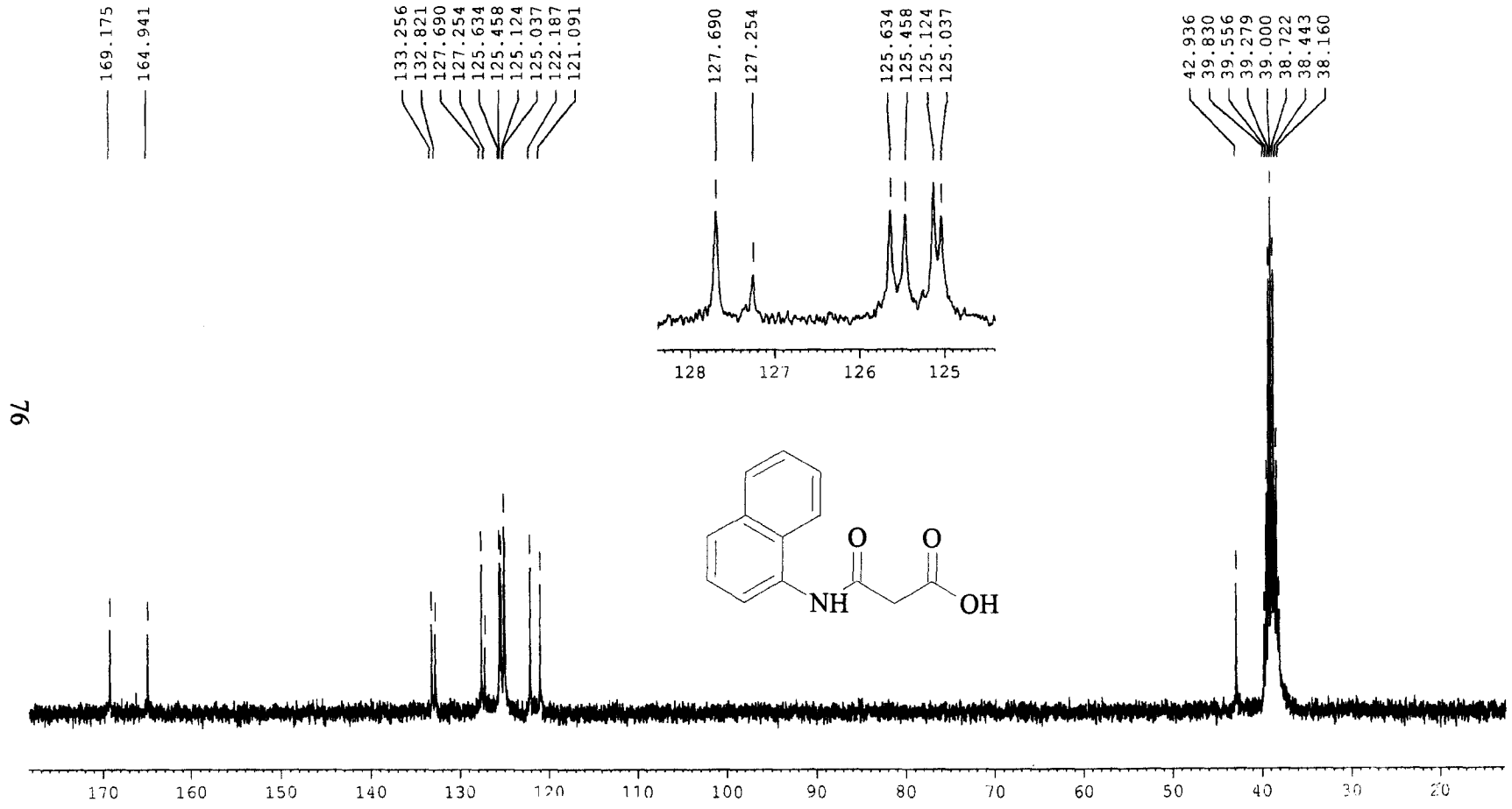


Fig. 1.35: ^{13}C NMR spectrum of **38**

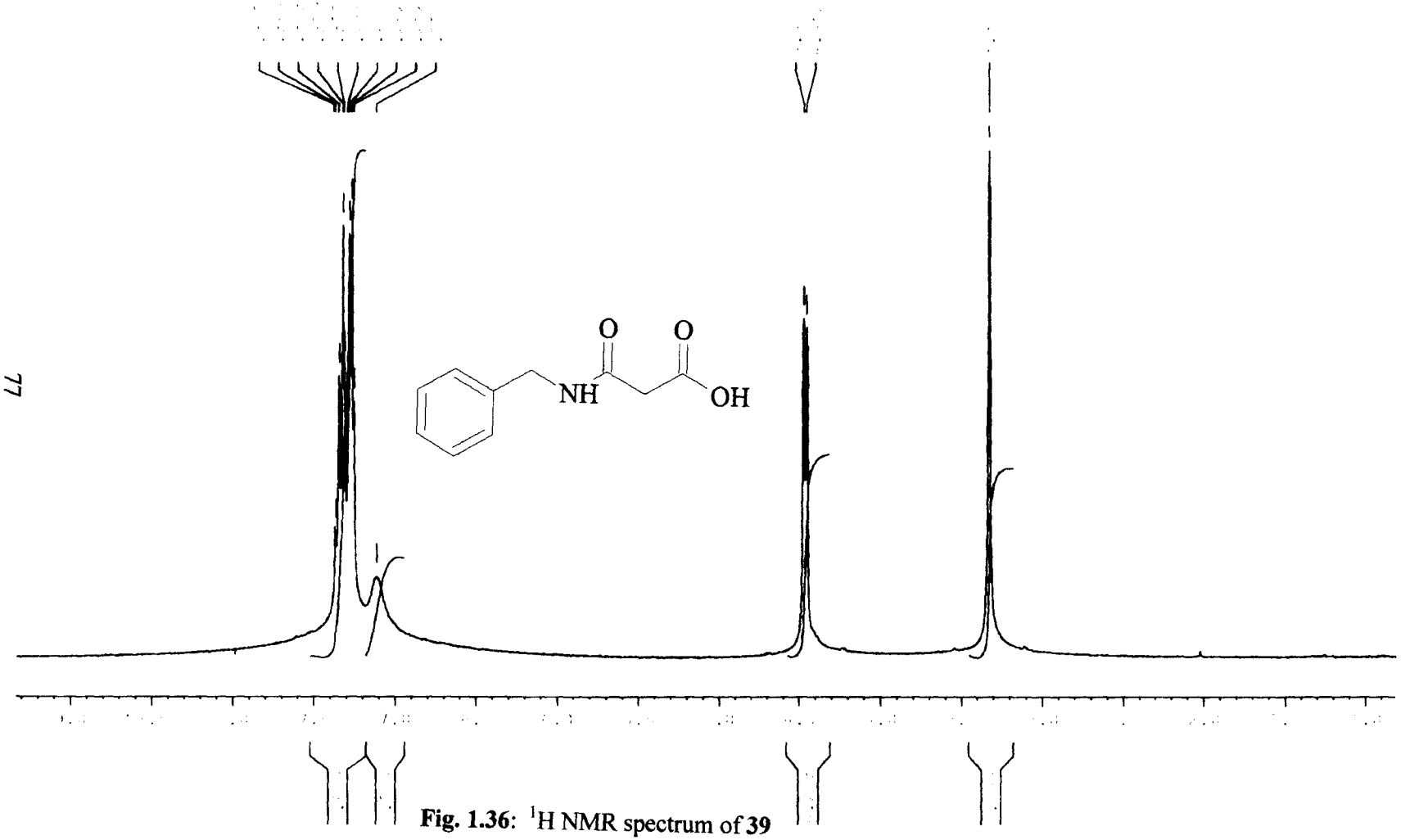


Fig. 1.36: ^{1}H NMR spectrum of **39**

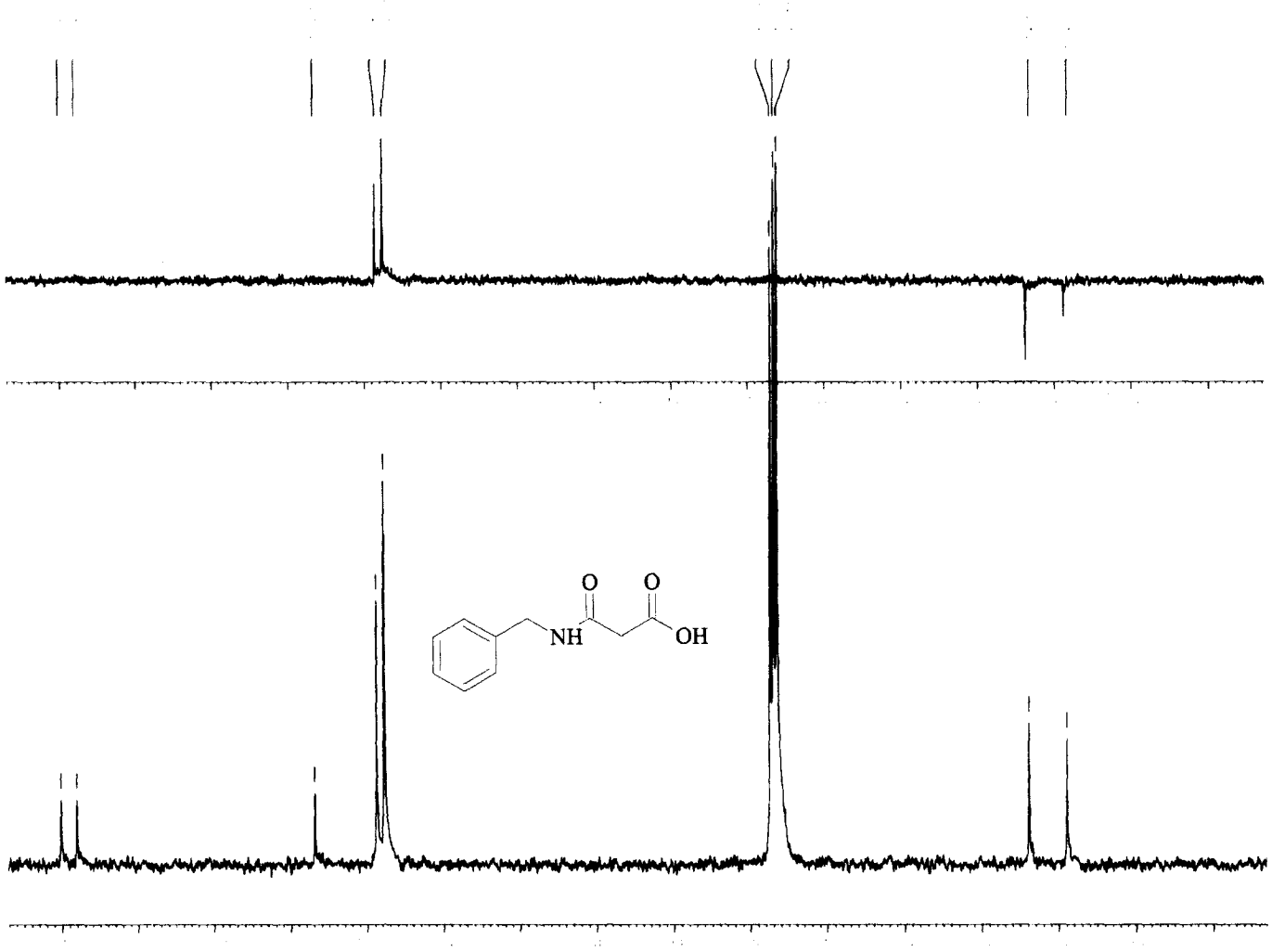
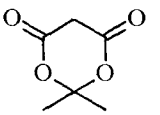


Fig. 1.37: ^{13}C NMR spectrum of **39**

Experimental

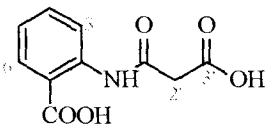
Preparation of Meldrum's acid **1**



1

Prepared according to the reported[23] literature procedure.

Preparation of 2-[(carboxyacetyl)amino]benzoic acid **3**



3

Using benzene as the solvent

An equimolar mixture of Meldrum's acid **1** (1.44 g, 10 mmole) and anthranilic acid **2** (1.37 g, 10 mmole) in dry benzene (10 mL) was heated under reflux while maintaining the temperature between 60 to 70°C. The progress of the reaction was periodically monitored by TLC which indicated the presence of unreacted starting compounds even after 48 hrs. Hence the temperature was raised to 80°C. The reaction mixture was cooled to room temperature, extracted with saturated $NaHCO_3$ solution, regenerated using 1:1 HCl. Brown solid separated was filtered under suction, washed with water and dried at 100°C to give **3** (0.848 g, 38%), m.p. 174°C.

Using toluene as the solvent

An equimolar mixture of Meldrum's acid **1** (1.44 g, 10 mmole) and anthranilic acid **2** (1.37 g, 10 mmole) in dry toluene (10 mL) was refluxed for 4 hrs. On cooling to room temperature, the malonamic acid **3** separated out as white solid which was filtered and washed with water. It was then chemically purified by

dissolving in saturated $NaHCO_3$ solution, regenerated using 1:1 HCl, filtered under suction, washed with water and dried at 100°C to give **3** (2.01 g, 90%). Recrystallization from hot water afforded white solid, m.p. 174°C.

IR ν_{max} (KBr): 3118 (NH), 1720 (CH_2COOH), 1685 (NHCO), 1643 (Ar-COOH), 1608, 1591, 1296 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.01): For assignments refer **Figure I**, pg 15.

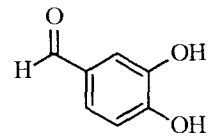
^{13}C NMR (75 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.02): For assignments refer **Figure II**, pg 16.

Elemental analysis: Calcd. for $C_{10}H_9NO_5.1/4H_2O$: C, 52.96; H, 4.22; N, 6.27. Found: C, 52.74; H, 4.17; N, 6.15.

Using acetonitrile as the solvent

An equimolar mixture of Meldrum's acid **1** (1.44 g, 10 mmole) and anthranilic acid **2** (1.37 g, 10 mmole) in dry CH_3CN (10 mL) was refluxed for 6 hrs. The reaction mixture was cooled to room temperature, extracted with saturated $NaHCO_3$ solution, regenerated using 1:1 HCl. White solid separated was filtered under suction, washed with water and dried at 100°C to give **3** (1.362 g, 61%) having m.p. 174°C.

3,4-Dihydroxybenzaldehyde (*protocatechualdehyde*) **5**



5

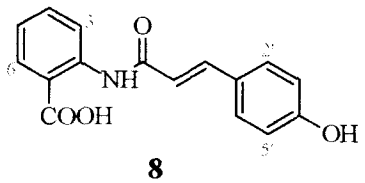
Prepared according to the reported[25] literature procedure.

General procedure for the preparation of avenanthramides

A mixture of 2-[(carboxyacetyl)amino]benzoic acid **3** (0.45 mmole), the corresponding benzaldehyde derivatives (0.45 mmole) and catalytic amount of β-alanine (10 mg) was refluxed in pyridine (0.5 mL) for 110 min. The reaction mass was cooled in ice and acidified with conc HCl (1.0 mL). The solid product that separated out was filtered, washed with water and recrystallized using hot water-acetone mixture to give the respective avenanthramide.

For the hydroxybenzaldehydes, the reaction mixtures were just kept in dark at room temperature in loosely stoppered Erlenmeyer flasks for a period of two weeks and then worked up as given above.

Avenanthramide D **8** *N-[4'-Hydroxy-(E)-cinnamoyl]anthranilic acid*



8

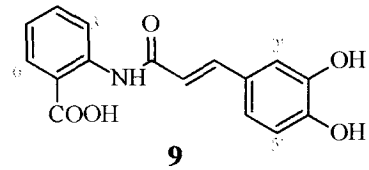
Reaction of monomalonamic acid **3** (0.10 g, 0.45 mmole) and 4-hydroxy-benzaldehyde **4** (0.055 g, 0.45 mmole) gave avenanthramide D **8** as yellow solid (0.110 g, 85%). Recrystallization from water-acetone mixture gave pale yellow crystals, m.p. 220°C, Lit.[3] 219°C.

UV λ_{max} (MeOH): 328, 211 nm.

IR ν_{max} (KBr): 3120, 1665 (NHCO), 1610 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.61 (d, J = 15.6 Hz, 1H, $C_{8'}$-H), 6.81 (d, J = 8.4 Hz, 2H, $C_{3',5'}$-H), 7.16 (t, J = 7.5 Hz, 1H, C_5-H), 7.51 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 7.54 (d, J = 8.4 Hz, 2H, $C_{2',6'}$-H), 7.61 (d, J = 8.1 Hz, 1H, C_4-H), 7.98 (d, J = 7.8 Hz, 1H, C_6-H), 8.55 (d, J = 8.1 Hz, 1H, C_3-H), 10.15 (s, 1H, OH), 11.26 (s, 1H, COOH).

Avenanthramide **9** *N-[3',4'-dihydroxy-(E)-cinnamoyl]anthranilic acid*



9

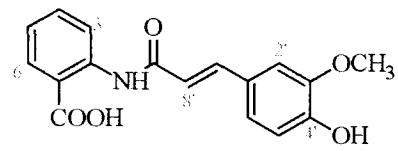
Reaction of **3** (0.10 g, 0.45 mmole) and 3,4-dihydroxybenzaldehyde **5** (0.062 g, 0.45 mmole) gave avenanthramide **9** as yellow solid (0.10 g, 75%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 230-234°C, Lit.[2] 221-230°C.

UV λ_{max} (MeOH): 338, 211 nm.

IR ν_{max} (KBr): 3260, 1665 (NHCO), 1600, 1590, 1270 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.52 (d, J = 15.3 Hz, 1H, $C_{8'}$-H), 6.79 (d, J = 8.1 Hz, 1H, $C_{5'}$-H), 7.00 (d, J = 8.1 Hz, 1H, $C_{6'}$-H), 7.10 (s, 1H, $C_{2'}$-H), 7.18 (t, J = 7.8 Hz, 1H, C_5-H), 7.44 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 7.61 (t, J = 7.8 Hz, 1H, C_4-H), 8.00 (d, J = 8.1 Hz, 1H, C_6-H), 8.55 (d, J = 8.1 Hz, 1H, C_3-H), 9.34 (s, 1H, OH), 9.68 (s, 1H, OH), 11.29 (s, 1H, COOH).

Avenanthramide **10** *N-[4'-hydroxy-3'-methoxy-(E)-cinnamoyl]anthranilic acid*



10

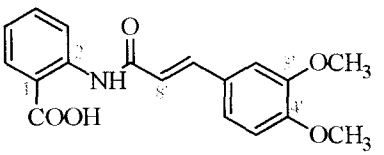
Reaction of **3** (0.10 g, 0.45 mmole) and 4-hydroxy-3-methoxybenzaldehyde (vanillin) **6** (0.069 g, 0.45 mmole) gave avenanthramide **10** as yellow solid (0.12 g, 85%). Recrystallization from water-acetone mixture gave pale yellow crystals, m.p. 212°C, Lit.[3] 235°C.

UV λ_{max} (MeOH): 336, 211 nm.

IR ν_{max} (KBr): 3515, 1660 (NHCO), 1600, 1520, 1270 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.68 (d, J = 15.6 Hz, 1H, $C_{8'}$-H), 6.81 (d, J = 8.1 Hz, 1H, $C_{5'}$-H), 7.12 (d, J = 8.4 Hz, 1H, $C_{6'}$-H), 7.17 (d, J = 7.5 Hz, 1H, C_5-H), 7.30 (s, 1H, $C_{2'}$-H), 7.51 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 7.60 (t, J = 7.8 Hz, 1H, C_4-H), 8.00 (d, J = 7.8 Hz, 1H, C_6-H), 8.56 (d, J = 8.4 Hz, 1H, C_3-H), 9.71 (s, 1H, OH), 11.25 (s, 1H, COOH).

Avenanthramide **11** *(Tranilast) N-[3',4'-dimethoxy-(E)-cinnamoyl]anthranilic acid*



11

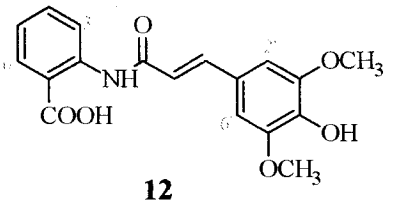
Reaction of **3** (0.10 g, 0.45 mmole) and 3,4-dimethoxybenzaldehyde **7** (0.074 g, 0.45 mmole) gave avenanthramide **11** as yellow solid (0.109 g, 74%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 184°C.

UV λ_{max} (MeOH): 336, 208 nm.

IR ν_{max} (KBr): 3200, 1687 (NHCO), 1597, 1260, 1192 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.77 (d, J = 15.9 Hz, 1H, $C_{8'}$-H), 7.00 (d, J = 8.4Hz, 1H, $C_{5'}$-H), 7.17 (d, J = 7.5 Hz, 1H, C_4-H), 7.23 (d, J = 7.5 Hz, 1H, $C_{6'}$-H), 7.34 (s, 1H, $C_{2'}$-H), 7.55 (d, J = 15.9 Hz, 1H, $C_{7'}$-H), 7.63 (d, J = 8.4 Hz, 1H, C_5-H), 8.00 (d, J = 7.2 Hz, 1H, C_6-H), 8.58 (d, J = 8.4 Hz, 1H, C_3-H), 11.28 (s, 1H, COOH).

Avenanthramide **12** *N-[4'-hydroxy-3',5'-dimethoxy-(E)-cinnamoyl]anthranilic acid*



12

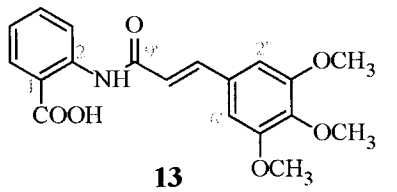
Reaction of **3** (0.10 g, 0.45 mmole) and 4-hydroxy-3,5-dimethoxybenzaldehyde **22** (0.082 g, 0.45 mmole) gave avenanthramide **12** as yellow solid (0.10 g, 65%). Recrystallization from water-acetone mixture gave bright yellow crystals, m.p. 214°C, Lit.[2] 199-200°C.

UV λ_{max} (MeOH): 339, 211 nm.

IR ν_{max} (KBr): 3503, 1665 (NHCO), 1609, 1270 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6): δ 6.71 (d, J = 15.5 Hz, 1H, $C_{8'}$-H), 6.98 (s, 2H, $C_{2',6'}$-H), 7.14 (t, J = 7.5 Hz, 1H, C_5-H), 7.50 (d, J = 15.5 Hz, 1H, $C_{7'}$-H), 7.58 (t, J = 7.85 Hz, 1H, C_4-H), 7.97 (d, J = 7.0 Hz, 1H, C_6-H), 8.55 (d, J = 8.4 Hz, 1H, C_3-H), 8.90 (s, 1H, O<u>H</u>), 11.23 (s, 1H, COO<u>H</u>).

Avenanthramide **13** *N-[3',4',5'-trimethoxy-(E)-cinnamoyl]anthranilic acid*



13

Reaction of **3** (0.10 g, 0.45 mmole) and 3,4,5-trimethoxybenzaldehyde **23** (0.088 g, 0.45 mmole) gave avenanthramide **13** as yellow solid (0.104 g, 65%). Recrystallization from water-acetone mixture gave yellow crystals, m.p. 168°C.

UV λ_{max} (MeOH): 339, 211 nm.

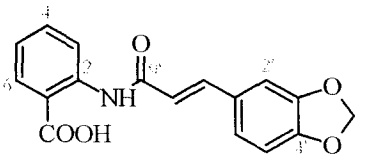
IR ν_{max} (KBr): 3580, 1660 (NHCO), 1600, 1583, 1267 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, **Fig**. 1.03): δ 3.89 (s, 3H, $C_{4'}$-OCH_3,), 3.92 (s, 6H, $C_{3',5'}$-OCH_3), 6.52 (d, J = 15.6 Hz, 1H, $C_{8'}$-H), 6.81 (s, 2H, $C_{2',6'}$-H), 7.15 (t, J = 7.8 Hz, 1H, C_5-H), 7.65 (t, J = 4.5 Hz, 1H, C_4-H), 7.69 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 8.15 (d, J = 7.8 Hz, 1H, C_6-H), 8.90 (d, J = 8.7 Hz, 1H, C_3-H), 11.25 (s, 1H, COOH).

^{13}C NMR (75 MHz, $CDCl_3$, **Fig**. 1.04): δ 56.1 (3',5'-OCH_3), 60.1 (4'-OCH_3), 105.3 (C-2',6'), 115.3 (C-1), 120.3 (C-8'), 121.0 (C-3), 122.6 (C-5), 130.2 (C-1'), 131.5 (C-6), 134.6 (C-4), 139.8 (C-2), 141.8 (C-3',5'), 142.2 (C-7'), 153.3 (C-4'), 164.7 (C-7), 171.0 (C-9').

HRFABMS (**Fig**. 1.05): *m/z* $[M + Na]^+$ calcd for $C_{19}H_{19}NO_6Na$ 380.1110, Found 380.1113.

Avenanthramide **14** *N-[3',4'-methylenedioxy-(E)-cinnamoyl]anthranilic acid*



14

Reaction of **3** (0.10 g, 0.45 mmole) and 3,4-methylenedioxybenzaldehyde (piperonal) **24** (0.067 g, 0.45 mmole) gave avenanthramide **14** as yellow solid (0.22 g, 71%). Recrystallization from water-methanol mixture gave pale yellow crystals, m.p. 202°C (dec).

UV λ_{max} (MeOH): 337, 207 nm.

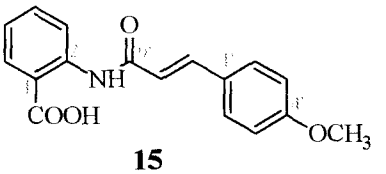
IR ν_{max} (KBr): 1691 (NHCO), 1600, 1450, 1211 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.06): For assignments refer **Figure IV**, pg 25.

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.07): For assignments refer **Figure V**, pg 26.

LCMS (Fig. 1.08): *m/z* $[M + Na]^+$ calcd for $C_{17}H_{113}NO_5Na$ 334.2832, Found 334.2902.

Avenanthramide **15** *N-[4'-methoxy-(E)-cinnamoyl]anthranilic acid*



15

Reaction of **3** (0.10 g, 0.45 mmole) and 4-methoxybenzaldehyde (anisaldehyde) **25** (0.061 g, 0.45 mmole) gave avenanthramide **15** as yellow solid (0.093 g, 70%). Recrystallization from water-acetone gave pale yellow crystals, m.p. 190°C.

UV λ_{max} (MeOH): 326, 211 nm.

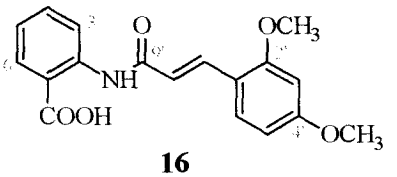
IR ν_{max} (KBr): 3310, 1670 (NHCO), 1600, 1255cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.09): δ 6.70 (d, J = 16 Hz, 1H, $C_{8'}$-H), 6.98 (d, J = 8.4 Hz, 2H, $C_{3',5'}$-H), 7.16 (t, J = 7.5 Hz, 1H, C_5-H), 7.56 (d, J = 16 Hz, 1H, $C_{7'}$-H), 7.62 (d, J = 8.1 Hz, 2H, $C_{2',6'}$-H), 7.67 (d, J = 8.1 Hz, 1H, C_4-H), 8.00 (d, J = 7.5 Hz, 1H, C_6-H), 8.58 (d, J = 8.7 Hz, 1H, C_3-H), 11.28 (s, 1H, COOH).

^{13}C NMR (75 MHz, $CDCl_3$, **Fig**. 1.10): δ 56.2 (4'-OCH_3), 114.2 (C-3',5'), 115.4 (C-1), 119.3 (C-8'), 120.2 (C-3), 122.4 (C-5), 127.3 (C-1'), 129.5 (C-2',6'), 131.4 (C-6), 134.3 (C-4), 141.6 (C-2), 141.8 (C-7'), 161.1 (C-4'), 165.2 (C-7), 170.4 (C-9').

HRFABMS (Fig. 1.11): *m/z* $[M + Na]^+$ calcd for $C_{17}H_{15}NO_4Na$ 320.0899, Found 320.0898.

Avenanthramide **16** *N-[2',4'-dimethoxy-(E)-cinnamoyl]anthranilic acid*



16

Reaction of **3** (0.10 g, 0.45 mmole) and 2,4-dimethoxybenzaldehyde **26** (0.075 g, 0.45 mmole) gave avenanthramide **16** as orange solid (0.281 g, 86%). Recrystallization from water-methanol mixture gave orange crystals, m.p. 194-98°C (dec).

UV λ_{max} (MeOH): 339, 208 nm.

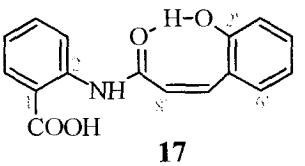
IR ν_{max} (KBr): 1693 (NHCO), 1660, 1598, 1288 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.12): For assignments refer **Figure VI**, pg 27.

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.13): For assignments refer **Figure VII**, pg 28.

LCMS (Fig. 1.14): *m/z* $[M + Na]^+$ calcd for $C_{18}H_{17}NO_5Na$ 350.3258, Found 350.6357.

Avenanthramide **17** *N-[2'-hydroxy-(Z)-cinnamoyl]anthranilic acid*



17

Reaction of **3** (0.10 g, 0.45 mmole) and 2-hydroxybenzaldehyde **27** (0.055 g, 0.45 mmole) gave avenanthramide **17** as a white solid (0.206 g, 73%). Recrystallization from water-acetone mixture gave white cotton like threads, m.p. 290°C (dec).

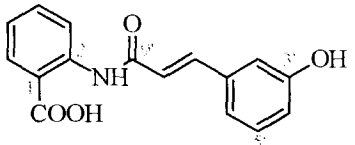
UV λ_{max} (MeOH): 207 nm.

IR ν_{max} (KBr): 3074 (br), 1726, 1693 (NHCO), 1674, 1608, 1531, 1261 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.15): For assignments refer **Figure VIII**, pg 29.

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.16): For assignments refer **Figure IX**, pg 29.

Avenanthramide **18** *N-[3'-hydroxy-(E)-cinnamoyl]anthranilic acid*



18

Reaction of **3** (0.10 g, 0.45 mmole) and 3-hydroxybenzaldehyde **28** (0.055 g, 0.45 mmole) gave avenathramide **18** as white solid (0.269 g, 95%). Recrystallization from water-acetone mixture gave white shiny crystals, m.p. 242°C (dec).

UV λ_{max} (MeOH): 322, 207 nm.

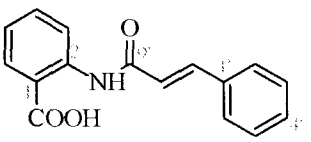
IR ν_{max} (KBr): 3169, 1693 (NHCO), 1612, 1514, 1288 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.17): δ 6.76 (d, J = 15.7 Hz, 1H, $C_{8'}$-H), 6.83 (dd, J = 7.9 & 1.6 Hz, 1H, C_5-H), 7.06 (s, 1H, $C_{2'}$-H), 7.12-7.26 (m,, 3H, $C_{4',5',6'}$-H), 7.52 (d, J = 15.7 Hz, 1H, $C_{7'}$-H), 7.62 (dt, J = 7.9 & 1.5 Hz, 1H, C_4-H), 8.00 (dd, J = 7.9 & 1.6 Hz, 1H, C_6-H), 8.56 (d, J = 8.4 Hz, 1H, C_3-H), 11.30 (s, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.18): δ 113.9 (C-2'), 116.6 (C-1), 116.8 (C-4'), 118.7 (C-5'), 120.0 (C-8'), 121.7 (C-3), 122.5 (C-5), 129.5 (C-6'), 130.6 (C-1'), 133.5 (C-6), 135.2 (C-4), 140.2 (C-2), 141.0 (C-7'), 157.2 (C-3'), 163.3 (C-7), 168.9 (C-9').

LCMS (Fig. 1.19): *m/z* $[M + Na]^+$ calcd for $C_{16}H_{13}NO_4Na$ 306.2728, Found 306.0243.

Avenanthramide **19** *N-(E)-cinnamoylanthranilic acid*



19

Reaction of **3** (0.10 g, 0.45 mmole) and benzaldehyde **29** (0.048 g, 0.45 mmole) gave avenanthramide **19** as light green solid (0.080 g, 66%). Recrystallizaion from water-acetone gave light green flakes m.p. 188°C.

UV λ_{max} (MeOH): 311, 211 nm.

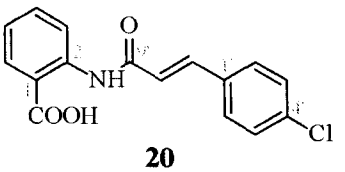
IR ν_{max} (KBr): 3141, 1668 (NHCO), 1611, 1548, 1223 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig.** 1.20): δ 6.63 (d, J = 15.6 Hz, 1H, $C_{8'}$-H), 7.16 (t, J = 7.5 Hz, 1H, C_5-H), 7.40-7.64 (m, 5H, Ar-H), 7.66 (t, J = 7.8 Hz, 1H, C_4-H), 7.80 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 8.12 (d, J = 8.1 Hz, 1H, C_6-H), 8.91 (d, J = 8.4 Hz, 1H, C_3-H), 11.23 (s, 1H, COOH).

^{13}C NMR (75 MHz, $CDCl_3$, **Fig.** 1.21): δ 114.1 (C-1), 120.9 (C-8′), 121.8 (C-3), 122.9 (C-5), 128.2 (C-2′,6′), 128.9 (C-3′,5′), 130.2 (C-4′), 131.9 (C-1′), 134.7 (C-6), 135.7 (C-4), 142.3 (C-2), 142.8 (C-7′), 164.9 (C-7), 171.9 (C-9′).

HRFABMS (Fig. 1.22): *m/z* $[M + Na]^+$ calcd for $C_{16}H_{13}NO_3Na$ 290.0793, Found 290.0791.

N-[4'-chloro-(E)-cinnamoyl]anthranilic acid **20**



20

Reaction of **3** (0.10 g, 0.45 mmole) and 4-chlorobenzaldehyde **30** (0.063 g, 0.45 mmole) gave chloroavenanthramide **20** as pale yellow solid (0.223 g, 74%). Recrystallization from water-methanol mixture gave pale yellow crystals, m.p. 220°C (dec).

UV λ_{max} (MeOH): 317, 208 nm.

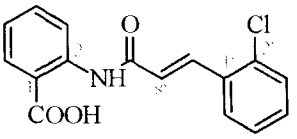
IR ν_{max} (KBr): 3329, 1672 (NHCO), 1608, 1531, 1259 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.23): δ 6.83 (d, J = 15.6 Hz, 1H, $C_{8'}$-H), 7.14 (t, J = 8.4 Hz, 1H, C_5-H), 7.43 (d, J = 8.4 Hz, 2H, $C_{3',5'}$-H), 7.57 (t, J = 8.0 Hz, 1H, C_4-H), 7.60 (d, J = 15.6 Hz, 1H, $C_{7'}$-H), 7.71 (d, J = 8.4 Hz, 2H, $C_{2',6'}$-H), 8.00 (d, J = 7.8 Hz, 1H, C_6-H), 8.61 (d, J = 8.4 Hz, 1H, C_3-H), 11.44 (s, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.24): δ 115.9 (C-1), 119.6 (C-8'), 122.1 (C-3), 122.5 (C-5), 128.3 (C-2',6'), 129.1 (C-3',5'), 130.5 (C-6), 132.7 (C-1'), 133.3 (C-4'), 134.0 (C-4), 139.3 (C-7'), 140.4 (C-2), 162.9 .(C-7), 169.0 (C-9').

LCMS (**Fig**. 1.25): *m/z* $[M + Na]^+$ calcd for $C_{16}H_{12}NO_3ClNa$ 324.7185, Found 324.0379.

Avenanthramide **21** *N-[2'-chloro-(E)-cinnamoyl]anthranilic acid*



21

Reaction of **3** (0.10 g, 0.45 mmole) and 2-chlorobenzaldehyde **31** (0.063 g, 0.45 mmole) gave chloroavenanthramide **21** as white solid (0.196 g, 65%). Recrystallization from water-methanol mixture gave white crystals, m.p. 200°C (dec).

UV λ_{max} (MeOH): 318, 214 nm.

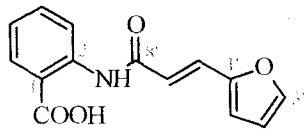
IR ν_{max} (KBr): 3064, 1678 (NHCO), 1608, 1531, 1228 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.26): δ 6.95 (d, J = 16.0 Hz, 1H, $C_{8'}$-H), 7.21 (t, J = 8.0 Hz, 1H, C_5-H), 7.43 (t, J = 8.0 Hz, 2H, $C_{4',5'}$-H), 7.54 (d, J = 8.0 Hz, 1H, $C_{6'}$-H), 7.64 (t, J = 8.0 Hz, 1H, $C_{3'}$-H), 7.93 (d, J = 16.0 Hz, 1H, $C_{7'}$-H), 8.00 (d, J = 8.0 Hz, 1H, C_4-H), 8.01 (d, J = 8.0 Hz, 1H, C_6-H), 8.60 (d, J = 8.0 Hz, 1H, C_3-H), 11.43 (s, 1H, COOH).

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.27): δ 117.3 (C-1), 120.7 (C-8'), 123.3 (C-3), 125.7 (C-5), 127.9 (C-5'), 128.4 (C-6'), 130.2 (C-6), 131.4 (C-4'), 131.7 (C-3'), 132.3 (C-1'), 133.9 (C-2'), 134.2 (C-4), 136.3 (C-7'), 140.8 (C-2), 163.5 (C-7), 169.7 (C-9').

LCMS (**Fig**. 1.28): *m/z* $[M + Na]^+$ calcd for $C_{16}H_{12}NO_3ClNa$ 324.7185, Found 324.0572.

N-(E)-furanoylanthranilic acid **33**



33

Reaction of **3** (0.10 g, 0.45 mmole) and 2-furaldehyde **32** (0.043 g, 0.45 mmole) gave **33** as greyish white solid (0.0885 g, 74%). Recrystallization from water-acetone mixture gave grey flakes, m.p. 184°C (dec).

UV λ_{max} (MeOH): 325, 208 nm.

IR ν_{max} (KBr): 1697 (NHCO), 1658, 1604, 1531, 1217 cm^{-1}.

^{1}H NMR (300 MHz, DMSO-d_6, **Fig**. 1.29): For assignments refer **Figure X**, pg 32.

^{13}C NMR (75 MHz, DMSO-d_6, **Fig**. 1.30): For assignments refer **Figure XI**, pg 32.

LCMS (**Fig**. 1.31): *m/z* $[M + H]^+$ calcd for $C_{14}H_{12}NO_4$ 258.0760, Found 258.1111; $[M + Na]^+$ calcd for $C_{14}H_{11}NO_4Na$ 280.0580, Found 280.0802.

Preparation of Avenanthramide **10** *N-[4'-hydroxy-3'-methoxy-(E)-cinnamoyl]anthranilic acid using procedure reported*[29] *by Ashok Kumar et al*

N-Acetyl anthranilic acid

Prepared according to the reported literature procedure[49].

Reaction of N-acetyl anthranilic acid with vanillin **6**

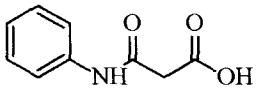
To a solution of *N*-acetyl anthranilic acid (0.25 g, 1.4 mmole) in methanol (8 mL) was added vanillin **6** (0.175 g, 1.4 mmole) in aqueous NaOH (2%, 5 mL) and the reaction mixture was stirred at room temperature for 10 hrs and then heated under reflux for 6 hrs. Methanol was distilled off and ice cold water was added to the

residue. Acidification of the aqueous layer gave *N*-acetyl anthranilic acid (identified by comparison of m.p. & IR) as yellow solid which was filtered, washed with water and dried.

Conclusion

Under the reaction conditions reported[29] by Ashok Kumar et al for the preparation of **10**, the staring material *N*-acetyl anthranilic acid was recovered unchanged.

N-Phenylmalonamic acid **36**



36

Using acetonitrile as the solvent

An equimolar mixture of Meldrum's acid **1** (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry acetonitrile (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a $CaCl_2$ guard tube. The reaction mixture was heated under reflux and the progress of the reaction was monitored by TLC. After 6 hrs, when no more change in TLC was observed, the reaction mixture was cooled to room temperature, extracted with saturated $NaHCO_3$ solution and regenerated using 1:1 HCl. The solid separated was filtered, washed with water and dried at 100°C to give *N*-phenylmalonamic acid **36** (0.075 g, 40%). Recrystallization from ethanol afforded white shiny crystals, m.p. 130°C, Lit.[47] 132°C.

Using benzene as the solvent

An equimolar mixture of Meldrum's acid **1** (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry benzene (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a $CaCl_2$ guard tube. The reaction

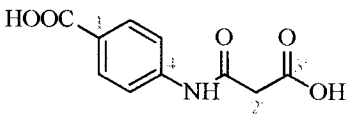
mixture was heated to 60-65°C and the progress of the reaction was monitored by TLC. After 24 hrs, when no more change in TLC was observed, the reaction mixture was cooled to room temperature, extracted with saturated $NaHCO_3$ solution and regenerated using 1:1 HCl. The solid separated was filtered, washed with water and dried at 100°C to give *N*-phenylmalonamic acid **36** (0.113 g, 60%). Recrystallization from ethanol afforded white shiny crystals, m.p. 130°C, Lit.[47] 132°C.

IR ν_{max} (KBr): 3118 (NH), 1720 ($CH_2\underline{C}OOH$), 1685 (NH$\underline{C}O$), 1643 (Ar-$\underline{C}OOH$), 1608, 1591, 1296 cm^{-1}.

Using toluene as the solvent

An equimolar mixture of Meldrum's acid **1** (0.155 g, 1.076 mmole) and aniline (0.098 g, 1.076 mmole) in dry toluene (3 mL) was taken in a round bottomed flask fitted with a Leibig condenser and a $CaCl_2$ guard tube. The reaction mixture was heated under reflux. TLC of the reaction mixture indicated formation of acetanilide. The reaction mixture was worked up as before to afford acetanilide as white solid (0.0924 g, 65%), m.p. 110°C, Lit[47a] 113-115°C.

4-[(Carboxyacetyl)amino]benzoic acid **37**



37

An equimolar mixture of Meldrum's acid **1** (0.288 g, 2 mmole) and *p*-amino-benzoic acid (0.274 g, 2 mmole) in dry toluene (3 mL) was heated under reflux and the progress of the reaction monitored by TLC. After 4 hrs on cooling the reaction mixture to room temperature a white solid separated out, this was filtered and washed with water. It was then chemically purified by dissolving in saturated $NaHCO_3$ solution, regenerated using 1:1 HCl, filtered under suction,

washed with water and dried at 100°C to give a white solid (0.407 g, 91.25%). Recrystallization from ethanol afforded white shiny crystals of 4-[(carboxyacetyl)amino]benzoic acid **37**, m.p. 260°C (decomp).

IR ν_{max} (KBr): 3273 (NH), 1720 (CH_2COOH), 1678 (NHCO), 1664 (Ar-COOH), 1537, 1176, 933 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.32): For assignments refer **Figure XII**, pg 38.

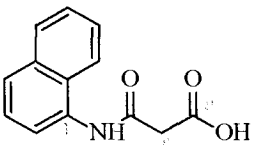
^{13}C NMR (75 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.33): For assignments refer **Figure XII**, pg 38.

*Reaction of Meldrum's acid **1** with 1-naphthylamine*

Formation of N-naphthylacetamide

An equimolar mixture of Meldrum's acid **1** (0.216 g, 1.5 mmole) and 1-naphthyl amine (0.2 g, 1.4 mmole) was heated to reflux in dry toluene (5 mL) for 4 hrs. On cooling to room temperature, white solid separated out which was filtered, washed with water and dried at 100°C to give *N*-(1-naphthyl)acetamide as white solid (0.2 g, 77.29%). Recrystallization from $CHCl_3$-MeOH afforded colourless crystals m.p. 152°C, Lit.[48b] 160°C.

N-(1-Naphthyl)malonamic acid **38**



38

An equimolar mixture of Meldrum's acid **1** (0.216 g, 1.5 mmole) and 1-naphthyl amine (0.2 g, 1.4 mmole) in dry benzene (5 mL) was heated to 60-65°C for 24 hrs. On cooling to room temperature, the half amide separated out as white solid which was filtered and washed with water. It was then chemically purified by

dissolving in saturated $NaHCO_3$ solution, regenerated using 1:1 HCl, filtered under suction, washed with water and dried at 100°C to give white solid (0.22 g, 68.75%). Recrystallization from benzene afforded colourless crystals m.p. 144°C (decomp) of *N*-(1-naphthyl)malonamic acid **38**.

IR ν_{max} (KBr): 3260 (NH), 1732 (CH_2COOH), 1710 (NHCO), 1550, 792 cm^{-1}.

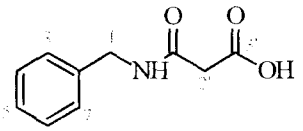
^{1}H NMR (300 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.34): δ 3.55 (s, 2H, $C_{2'}$-H), 7.47-7.56 (m, 3H), 7.68 (d, J = 6.9 Hz, 1H), 7.77 (d, J = 7.5 Hz, 1H), 7.94 (d, J = 4.8 Hz, 1H), 8.13 (d, J = 5.1 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.35): δ 42.93 (C-2′), 121.09, 122.19, 125.03, 125.12, 125.46, 125.63, 127.25, 127.69, 132.82, 133.25, 164.94 (C-1′), 169.17 (C-3′).

*Reaction of Meldrum's acid **1** with a) p-phenylenediamine, b) 2,4-dinitroaniline, c) N,N-diethylamine and d) N,N-diphenylamine.*
Formation of respective N-acetamide derivatives.

An equimolar mixture of Meldrum's acid **1** (1 mmole) and the respective amine (1 mmole) in dry toluene (3 mL) was refluxed for 4 hrs. On cooling to room temperature the reaction product was worked up as before to give the respective acetamide derivatives: (a) *N,N′*-benzene-1,4-diyldiacetamide as pale brown solid m.p. 302°C, Lit.[47c] 301-303°C; (b) *N*-(2,4-dinitrophenyl)acetamide as yellow crystals, m.p. 116°C, Lit.[47d] 121°C; (c) *N,N*-diethylacetamide as yellow oil; (d) *N,N*-diphenylacetamide as colourless plates m.p. 100°C, Lit.[47e] 100°C.

N-Benzylmalonamic acid **39**



39

An equimolar mixture of Meldrum's acid **1** (0.36 g, 2.5 mmole) and benzylamine (0.265 g, 2.5 mmole) in appropriate solvent (5 mL) was refluxed for 24 hrs as indicated in the table.

Solvent	Temperature °C	Yield %
Toluene	Reflux	Nil
C_6H_6	R.T.	13
C_6H_6	40-50	16
C_6H_6	60-70	20
CH_3CN	80	23
Ether	Reflux	20
Pyridine/ether	Reflux	40

On cooling to room temperature, the organic layer was extracted with saturated $NaHCO_3$ (3 × 3 mL). The $NaHCO_3$ extract was acidified with conc. HCl and extracted with ether (3 × 3 mL). The combined organic extracts were washed with H_2O (2 × 3 mL), dried over Na_2SO_4 and evaporated to give white solid (0.188 g, 53%). Recrystallization from benzene gave colourless crystals (m.p. 86-88°C) of *N*-benzylmalonamic acid **39**.

IR ν_{max} (KBr): 3287 (NH), 1745 (CH_2COOH), 1632 (NHCO), 1604, 1566, 1193 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.36): For assignments refer **Figure XIII**, pg 41.

^{13}C NMR (75 MHz, $CDCl_3$, MeOH in traces, **Fig**. 1.37): For assignments refer **Figure XIV**, pg 41.

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Chapter Two

Section 2.1

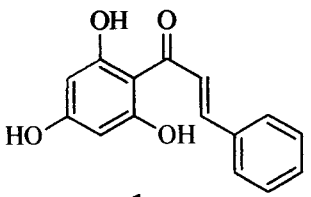
The Interesting Chemistry Encountered During the Synthesis of a Novel 5′-bromo-2′-hydroxy-4,4′,6′-trimethoxychalcone Constituent of *Garcinia nervosa*

Introduction

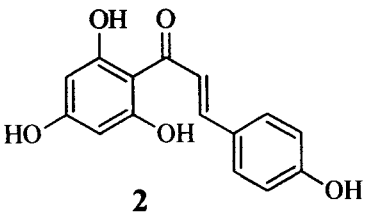
Chalcones form a major class of natural products with widespread distribution throughout the plant kingdom[1]. Natural chalcones occur mainly as petal pigments and have also been found in heartwood, bark, leaves, fruits and roots of many trees, plants and vegetables[2]. They are also responsible for the yellow colour of many plant organs[3].

They are structurally and biogenetically related to phenyl propenoids[1] which are precursors in flavanoid biosynthesis. The enzymatic cyclisation of 6′-hydroxychalcones leads to the formation of flavanones and subsequently to a large number of flavanoids, including flavones, flavanols, dihydroflavanols, aurones and isoflavones[3,4]. All the natural biflavonoids[5] are produced in plants by the oxidative coupling of chalcones[6].

Naturally occurring and synthetic chalcones show interesting biological activities such as anticancer or anti-infective[2], chemopreventive, antiproliferative, antimicrobial[3], antioxidant[7], anti-inflammatory, anti-platelet, anti-tumor, anti-mutagenic[8], antifungal, insect anti-feedant and prostaglandin binding[9].



1

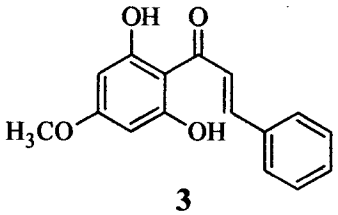


2

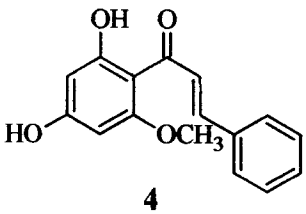
Although the most simple unsubstituted chalcone has not been isolated as a natural product, numerous hydroxy and methoxy derivatives are known. 2′,4′,6′-Trihydroxychalcone **1** (commonly called pinocembrin chalcone) a major

component of *Populus* bud exudates[10], also isolated from *Boesnbergia pandurata* Schult[11] and *Helichrysum trilincatum* DC, acts as antibacterial[12], hypochloretic, hypotensive and anticoagulating agent[13].

2′,4′,6′,4-Tetrahydroxychalcone **2** (isosaliprupol or chalcononaringenin) is the most active 2,2-diphenyl-1-pyrril hydrazil (DPPH) radical scavenger[3] and its methyl ether derivatives are widely distributed in plants[10]. For example, 2′,6′-Dihydroxy-4′-methoxychalcone **3**, isolated from *Bosenbergia pandurata* Schultz[11], is selectively toxic to parasites (*Leishmania amazonesis*)[1]. Cardamonin **4**, isolated from *Bosenbergia pandurata* Schultz[11], *Aplinia speciosa*[14] and *Piper species*[15], exhibit an appreciable anti-HIV-1 PR[1] and invitro antitumor promoting activity[16].

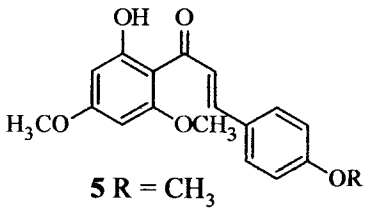


3

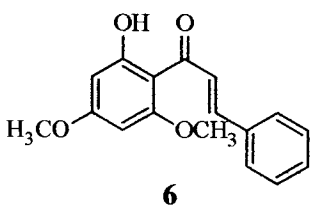


4

Flavokawain A **5**, flavokawain B **6** and flavokawain C **7**, constituents of *Piper species*[17-20], are psychoactive compounds and are among the key active constituents of the ceremonial beverage called Kava. The herb Kava-Kava is used as a traditional medicine for treating gonorrhoea, menstrual pain, tuberculosis, respiratory tract infection, chronic inflammation of urinary tract and chronic pain related to gout and arthritis[21]. Flavokawain B **6** is the most active cyclooxygenase (COX-I) inhibitor[21] while flavokawain C **7** acts as DPPH radical scavenger[22].



5 R = CH_3
7 R = H

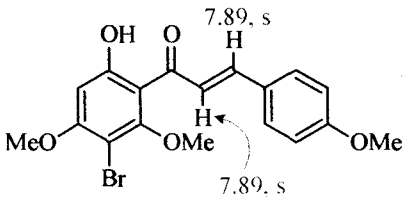


6

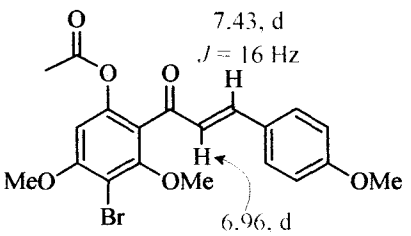
Chalcones, similar to other flavonoids, can also mimic estrogens and andrenal androgens, consequently they demonstrate abilities to bind to the estrogen receptor or to inhibit aromatase (i.e. estrogen synthase), thus decreasing estrogen production and subsequently inhibit proliferation of the hormone dependent breast cancer cells[3]. The anti-estrogenic activity of chalcones has also been correlated to their redox potential, measured by the capacity to inhibit the DPPH radical[3]. Structure-activity studies have revealed the absolute requirement of an olefinic function and also the position & number of hydroxyl groups for their biological activity[8].

Present Study

Sometime back we came across a report[23] of a novel 5′-bromo-2′-hydroxy-4,4′,6′-trimethoxychalcone **8** being isolated from the leaves of *Garcinia nervosa*. Although brominated natural compounds are reported from marine sources, this constituted the first report of the occurrence of a brominated natural product in non-marine plants. Secondly, this appears to be the first report of the isolation of a chalcone from *Garcinia species* which are known as a source of xanthones, benzophenones and biflavanoids. *Garcinia species* are well known for their medicinal properties such as anti-inflammatory, anti-immunosuppressive, antimicrobial activities and for their use in healing skin infections and wounds.



8

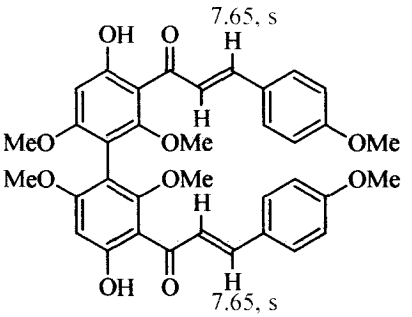


9

The structure **8** was assigned on the basis of its detailed spectral analysis (IR, ^{1}H NMR, ^{13}C NMR, HMQC, NOE and MS).

The ^{1}H NMR spectrum of **8** showed a singlet for the *trans*- α, β olefinic protons at δ 7.89. However its acetate **9** showed two independent doublets of one proton each at δ 6.96 for H-α and δ 7.43 for H-β with J = 16 Hz.

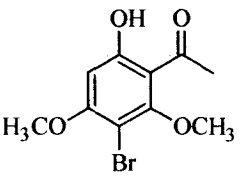
Chalcones having substitution pattern similar to that of **8** usually show two doublets with AB pattern each for H-α and H-β with $J \approx 16$ Hz[24]. We have not come across any other report on chalcones showing a singlet for the *trans*-α, β olefinic protons except the bichalcone **10**[6] which is reported to show a singlet at δ 7.65 for the four (H-α, α′, β, β′) protons.



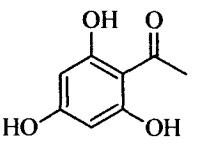
10

The simple structure of the bromochalcone **8** and the unusual observation in its ^{1}H NMR spectrum attracted our attention to synthesize **8** and verify its ^{1}H NMR data with that reported for the natural **8**.

The substitution pattern of **8** suggested that it can be easily obtained by the aldol condensation of 3-bromo-2,4-dimethoxy-6-hydroxyacetophenone **11** and *p*-methoxybenzaldehyde. The bromo compound **11** in turn can be prepared from phloroacetophenone **12**.

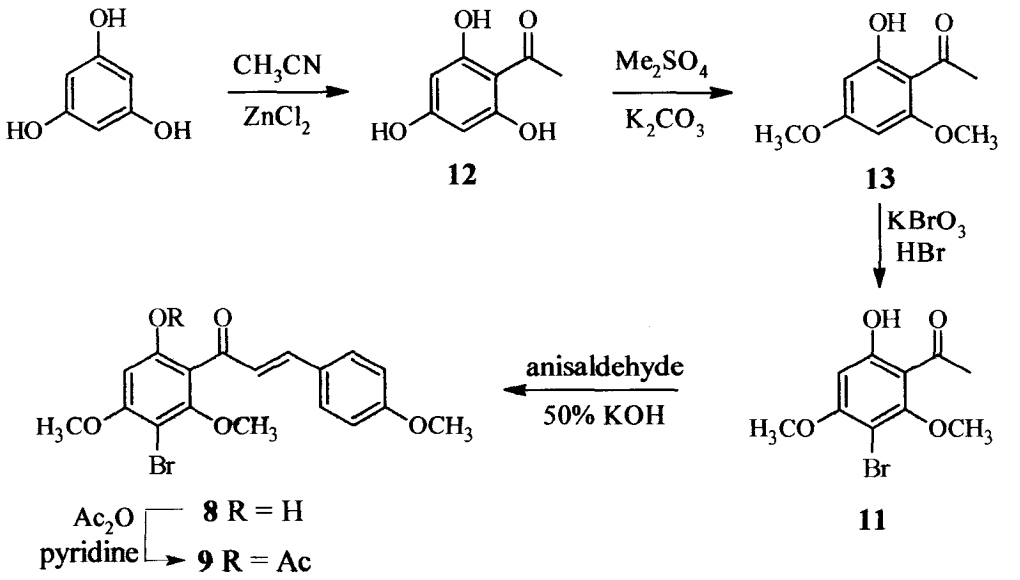


11



12

The synthetic scheme towards **8** was planned from **12** which was prepared from phloroglucinol[25] as shown in scheme 1.

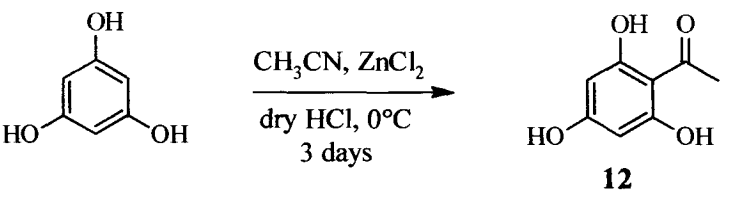


Scheme 1

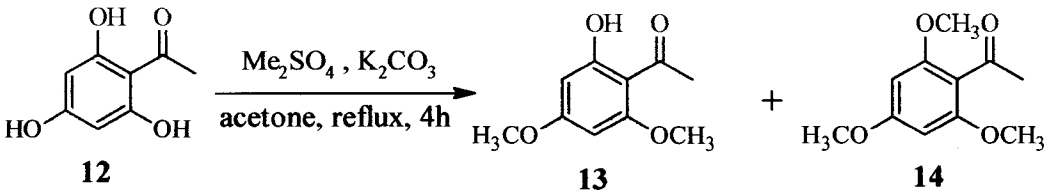
Although the synthetic route towards **8** appeared to be very simple and straight forward, we had tough time in completing this scheme especially in putting the Br atom at the required position and also ascertaining its position. Different strategies used and the interesting results obtained to accomplish the goal of synthesizing the chalcone **8** are discussed in the following part of this section.

Results and Discussion

Acylation of phloroglucinol with CH_3CN and $ZnCl_2$ using the reported procedure[25] gave phloroacetophenone **12** as pale yellow needles, m.p 220°C (Lit.[25] 219°C).



Methylation[9] of **12** with Me_2SO_4 in the presence of anhydrous K_2CO_3 in dry acetone gave a pale yellow solid which on purification by silica gel column chromatography using petroleum ether as eluent followed by recrystallization from the same solvent gave cream coloured needles of 2,4-dimethoxy-6-hydroxyacetophenone **13** (m.p. 78°C, Lit.[26] 82°C) in 80% yield. Further elution with petroleum ether-diethyl ether (8:2) gave colourless plates of 2,4,6-trimethoxyacetophenone **14** (m.p. 103°C, Lit.[26] 103°C, 9%).



12 **13** **14**

Bromination[27] of **13** using $KBrO_3$ and 48% HBr in glacial AcOH gave yellow solid which was recrystallized from benzene to give pale brown needles m.p. 186-188°C. Recrystallization from $CHCl_3$-petroleum ether gave bright yellow needles, m.p. 200°C.

The IR spectrum of the yellow solid showed bands at 1632 (chelated C=O), 1580 (Ar-C=C), 1284, 1136 (C-O-C) cm^{-1}.

Its ^1H NMR spectrum showed 5 signals, all of them being singlets: three of 3H each and two of 1H each accounting for all the 11 protons as expected. Moreover the presence of a 3H singlet at δ 2.63 (-CO-C<u>H</u>$_3$) and a 1H singlet at δ 6.0 (Ar-<u>H</u>) revealed nuclear bromination of **13**.

Condensation of the bromo derivative of **13** with *p*-methoxybenzaldehyde in the presence of 50% ethanolic KOH at room temperature for 72 hrs[9] gave the chalcone as bright yellow solid in quantitative yield. Recrystallization using $CHCl_3$-MeOH gave yellow flakes, m.p. 180°C (Lit.[23] m.p. of **8** is also 180°C).

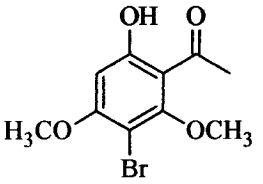
Since the m.p. matched well with that reported for the natural **8**, the chalcone obtained was acetylated[23] using acetic anhydride and pyridine. Usual

workup furnished a greenish yellow solid which on recrystallization gave greenish flakes, m.p. 178-180°C (Lit.[23] 120-122°C).

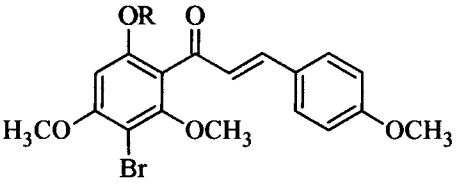
Although the m.p. of the solid obtained after acetylation was almost the same as that of the chalcone before acetylation, the IR and the ^{1}H NMR spectra clearly indicated the presence of the acetate group as follows.

Bands at 1770 cm^{-1} (-O-CO-CH$_3$) and 1660 cm^{-1} (Ar-CO-CH$_3$) in its IR spectrum and the absence of a singlet at δ 14.09 (-OH) and appearance of a 3H singlet at δ 2.16 (-O-CO-CH$_3$) in its ^{1}H NMR spectrum supported the presence of the acetate group.

Although the melting points of the chalcones (synthetic and natural) were identical (180°C) the melting points of their respective acetates differed by 60 degrees. Therefore it became necessary to characterize the chalcone and/or the intermediate bromo derivative of **13** (we prepared) and confirm the position of the Br atom in the aromatic nucleus because depending upon the position (C_3 or C_5) the bromo derivative of **13** can have structures **11** or **15** and hence chalcones can have the structures **8** or **16**.

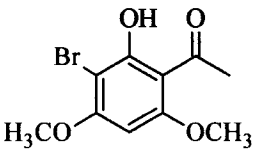


11



8 R = H, m.p. 180°C

9 R = Ac, m.p. 120-122°C



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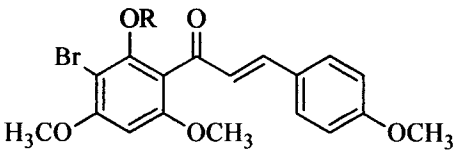
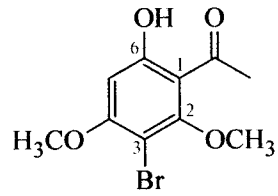


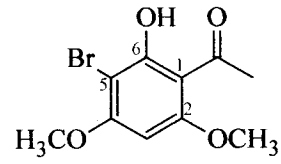
16 R = H, m.p. 180°C

17 R = Ac, m.p. 180°C

We decided to analyse the bromo derivative of **13** rather than the chalcone as it contained less number of atoms.



11



15

Simple ^{1}H and ^{13}C NMR data was not enough to say conclusively that the bromo derivative of **13** is having structure **11** or **15**. Hence the position of the Br atom was determined and confirmed from HMBC and NOE experiments* as follows:

In the HMBC data, the hydroxyl proton on C_6 correlated to C_1, C_5 and C_6 while the proton on C_3 correlated to C_1, C_2, C_3, C_4 and C_5. The fact that the OH proton did not correlate to the carbon which is directly attached to the lone aromatic proton at C_3 and the C_3-H also did not show correlation to C_6 indicated that the aromatic proton is in *p*-position with respect to the OH group. Thus the bromo derivative was assigned the structure 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15**.

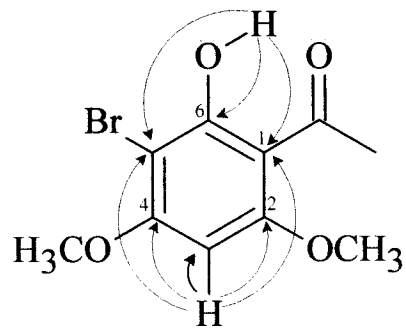


Figure I: HMBC of **15**

* We are thankful to Dr. O'Connell, University of North Carolina, NC, USA for recording HMBC and NOE data on **15**.

Further, the ^{13}C NMR assignments and the confirmation of the structure was made by the study of its NOE data. Inversion of acetyl methyl group C_8-H gave a strong and weak NOE to methoxy protons C_9-H and C_{10}-H. The strong interaction with the more upfield of the two methoxy peaks leads to the assignment of that peak to C_9-H. Hence C_2-OCH_3 was assigned the peak at δ 55.72 and C_4-OCH_3 at δ 56.27. The equivalence of the NOE from C_3-H to both the methoxy groups (at C_2 and C_4) confirmed that the lone aromatic proton is at C_3-position and hence the structure of the bromo derivative was confirmed to be 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15**.

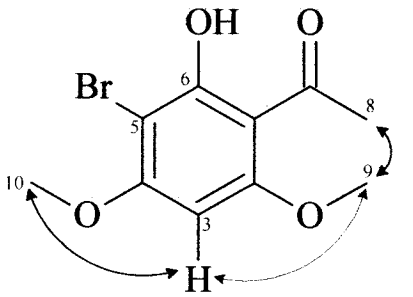
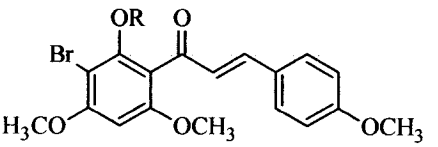


Figure II: NOE of **15**

Consequently the chalcone prepared from the bromo derivative **15** was assigned the structures **16** and hence its acetate the structure **17**.



16 R = H
17 R = Ac

Chalcone **16** was fully characterised on the basis of its ^1H NMR, ^{13}C NMR, COSY, HMQC and HMBC data. In the ^1H NMR spectrum of **16** the two characteristic 1H each doublets having AB pattern at δ 7.76 and 7.84 with J = 15.4 Hz were due to the *trans*-α, β olefinic protons respectively. Moreover, the

lone aromatic proton showed a singlet at δ 6.07. Whereas in the case of the natural chalcone[23] a singlet was observed for the *trans*-α, β olefinic protons and the singlet due to the lone aromatic proton appeared at δ 6.36.

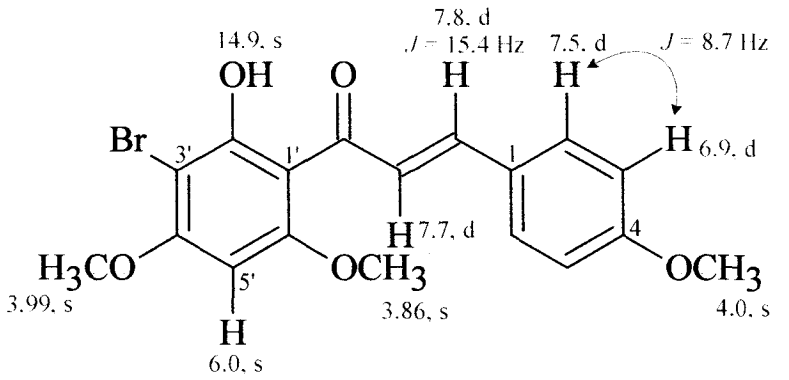


Figure III: Assignments of ^{1}H NMR signals for the characteristic protons of **16**

Striking differences were observed in the ^{13}C NMR spectrum which showed downfield signal at δ 163.2 for C-2′ and three upfield signals for C-1′, C-3′ and C-5′ at δ 106.93, δ 91.98 and δ 87.16 respectively as compared to those of the natural chalcone[23].

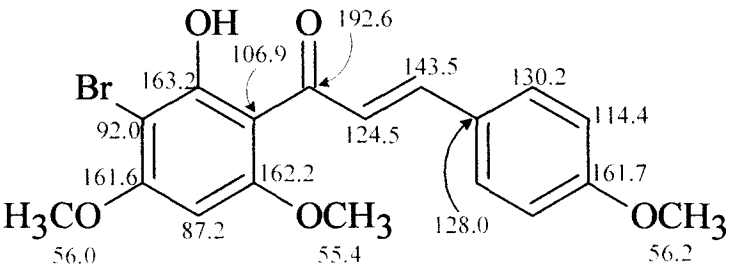


Figure IV: Assignments of ^{13}C NMR signals to the various carbons of **16**

In the HMBC the OH proton at C-2′ showed correlation to C-1′, C-2′ & C-3′ while the proton at C-5′ correlated to C-1′, C-3′, C-4′, C-5′ & C-6′. This supported the previous discussion and the structure **16** for our synthetic chalcone.

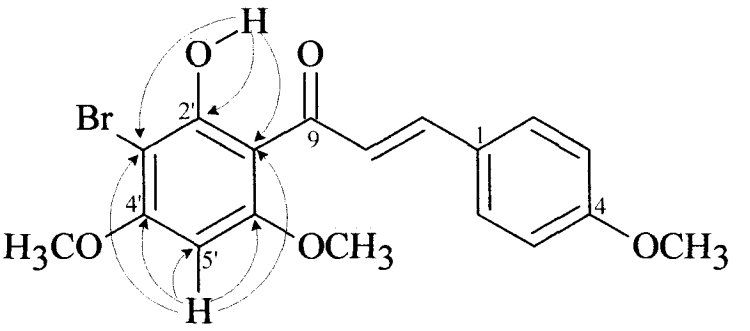


Figure V: Selected HMBC of **16**

Since bromination of **13** using $KBrO_3$ and HBr[27] did not give the required bromo derivative **11** it was decided to alter the brominating reagent and/or the reaction conditions.

b) Using Bromine in acetic acid[28] and Bromine in water[29]

Various reagents are reported in the literature for bromination of organic substrates under acidic, alkaline and neutral conditions. Br_2 in acetic acid[28] is the most widely used reagent which gives mono-, di- and polybrominated products depending on the reaction conditions and the molar concentration of Br_2 used. Stirring an equimolar mixture of Br_2 and **13** at room temperature for 30 mins gave a yellow solid which was characterized as **15** by comparison of its m.p. and IR with that obtained earlier. Similar results were obtained when AcOH was replaced by H_2O as solvent for bromination[29].

c) Using Oxone in combination with KBr[30]

Meanwhile we came across a report[30] in which a triple salt ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$), commercially called 'Oxone'[†] was used in combination with aqueous halides like NaBr for regioselective bromination of moderately activated aromatic compounds.

Slow addition of Oxone to an equimolar mixture of **13** and KBr gave after usual workup, a yellow solid which was identified as 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** by comparison of its m.p. and IR data with that obtained earlier.

d) Using KBr, H_2O_2 and ammonium molybdate[31]

Bromination of activated aromatic compounds in the presence of ammonium molybdate as catalyst, H_2O_2 as reoxidant and KBr as bromine source has been reported to proceed rapidly to afford high yields of the corresponding monobromo derivatives[31].

Slow addition of H_2O_2 to an equimolar mixture of **13** and KBr in the presence of ammonium molybdate afforded a solid which was identified as **15** (m.p. and IR).

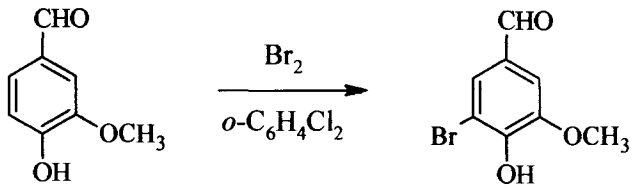
e) Using KBr and H_2O_2[32]

Selective nuclear monobromination is also reported in the absence of a catalyst by using NH_4Br as a bromine source and H_2O_2 as the oxidant[32]. Stirring an equimolar mixture of **13** and KBr (in place of NH_4Br) in the presence of H_2O_2 gave a solid which was again identified as **15** by comparison of its m.p. and IR.

[†] We are thankful to Dr. C. G. Naik, Bio-Organic Division, NIO, for providing a sample of Oxone.

f) Using Br_2 and *o*-dichlorobenzene[33]

Bromine in *o*-dichlorobenzene[33] has been used for the following conversion of vanillin to 5-bromovanillin.



Vanillin 5-Bromovanillin

However, reaction **13** with Br_2 in *o*-dichlorobenzene did not prove fruitful as the product obtained was identified as **15** (m.p. and IR).

g) Using *N*-bromosuccinimide (NBS) in *N,N*-dimethylformamide (DMF)[34]

N-bromosuccinimide (NBS) in *N,N*-dimethylformamide (DMF) is a mild, selective nuclear brominating reagent for reactive aromatic compounds[34]. Stirring an equimolar mixture of **13** and NBS in dry DMF gave a solid which was identified as **15** by comparison of its m.p. and IR.

h) Using NBS in the presence of H_2SO_4[35]

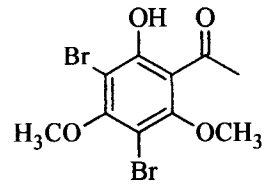
Regioselective bromination of substituted acetophenones[35] using NBS in the presence of catalytic amount of H_2SO_4 in aqueous medium has been reported to give good yield of bromo compounds. Using this reagent we did get 99% yield but of the same not required bromo derivative **15**.

i) Using NBS in the presence of diisopropylamine[36]

Regioselective *ortho*-bromination of **13** using NBS in the presence of a secondary amine (diisopropylamine)[36] gave crude solid, a mixture of two compounds as indicated by TLC. Purification by column chromatography

followed by recrystallization from the same solvent gave pista green flakes m.p. 102°C which showed a band at 1620 (C=O) cm^{-1} in its IR spectrum.

Absence of peaks in the aromatic region in its ^1H NMR spectrum indicated it to be a fully substituted 3,5-dibromo-2,4-dimethoxy-6-hydroxyacetophenone **18**.



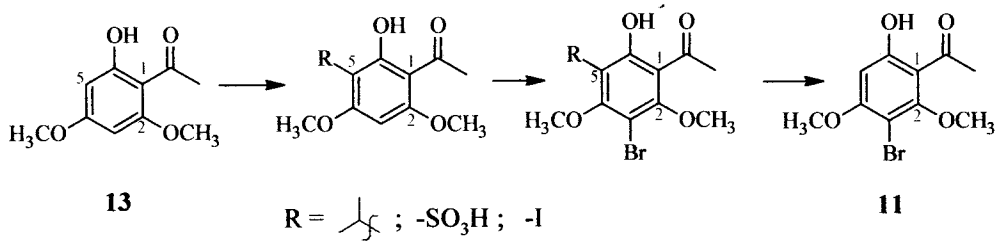
18

Further elution with petroleum ether-diethyl ether (8:2) gave **15** as a pale yellow solid. Formation of **18** in this case indicated that the bromination must have occurred first at the C_5-position followed by that at the C_3-position in the presence of excess brominating reagent.

Second strategy was to block the C_5-position and introduce Br at the desired C_3-position.

Hence it was decided to block the C_5-position by a suitable group which can be easily removed after introducing Br at the desired C_3-position. Several procedures for different types of groups have been reported in the literature to protect and deprotect specific positions on the aromatic nucleus depending upon the substituents present.

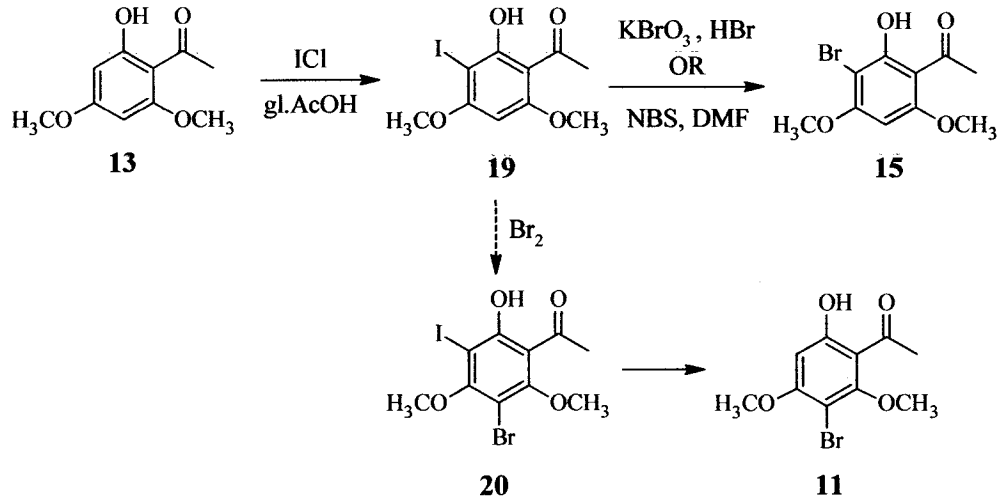
To begin with we decided to protect the more reactive C_5-position either by an isopropyl[37] or a sulfonic acid[38] ($-SO_3H$) or an iodo[39] group whichever was giving satisfactory results followed by bromination at the C_3-position and deprotection of the blocking group to give the required bromo derivative **11** as depicted in scheme 2.



Scheme 2

Introduction of isopropyl group as well as $-SO_3H$ group at the more reactive C_5 position using literature reported[37,38] procedures did not give the expected products.

So the next alternative was to use iodine as the blocking group.



Scheme 4

Reaction of **13** with ICl in glacial acetic acid[39] gave canary yellow solid melting at 216°C. In its ^1H NMR spectrum, the presence of a 3H singlet at δ 2.64 (-CO-C<u>H</u>$_3$) and a 1H singlet at δ 6.01 (Ar-<u>H</u>) indicated that the iodine atom must have occupied one of the two vacant positions. Based on our previous experience with bromination of **13** to give only **15**, the iodo compound was presumed to be

5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19**. Moreover this was supported by the chemical shift (δ 6.01) of the C_3-H.

Bromination[28] of **19** using Br_2 in glacial acetic acid gave a yellow solid, m.p. 202°C, identical (IR and ^{1}H NMR) with **15**. No trace of the expected 3-bromo-2,4-dimethoxy-5-iodo-6-hydroxyacetophenone **20** was obtained. It appears that under the conditions used for bromination (HBr in AcOH[40]) iodine gets knocked off and is liberated as I_2 giving way for Br to occupy C_5-position and thus form **15** instead of the expected **20**. Indeed during workup of the reaction mixture crystals of I_2 were seen deposited in the beaker containing the aqueous medium.

Changing the brominating reagent to NBS in DMF[34] did not prove fruitful as it also gave the unwanted **15** as the sole product.

Since iodine was getting knocked off under acidic conditions, it was decided to carry out bromination of the iodo compound **19** under non acidic or neutral conditions. Using KBr with Oxone[30] in acetonitrile-water mixture as well as KBr with ammonium molybate[31] did not affect any change and the starting material **19** was recovered back.

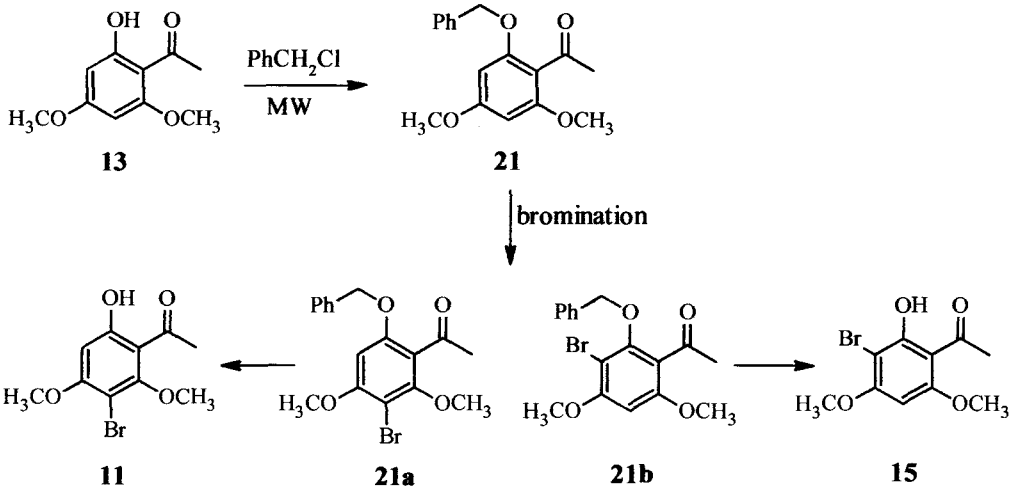
Thus blocking the more reactive C_5-position by isopropyl, (-SO_3H) or iodine did not prove fruitful to give **11**. Therefore, it was decided to protect the OH group in **13** by a bulky group so as to make the C_5-position sterically hindered.

Third strategy was to protect the OH group in 13 by a bulky group so as to make the more reactive C_5-position sterically hindered and hence less reactive.

The following protecting groups such as benzyl, acetyl and *p*-toluene-sulfonyl were tried and the results obtained are discussed below.

1) Benzyl as the protecting group

To begin with we decided to protect the chelated OH group of **13** as its benzyl ether which are comparatively easy to prepare and also to cleave.



Scheme 5

Heating to reflux an equimolar mixture of **13**, benzyl chloride and anhydrous K_2CO_3 in dry acetone for 4 hrs followed by usual workup ended in total recovery of the starting material (TLC).

However, heating an equimolar mixture of **13** and benzyl chloride in excess anhydrous K_2CO_3 and TBAB using Microwave irradiation for 3 min after workup gave dark yellow oil. Purification by silica gel column chromatography and elution with petroleum ether-diethyl ether (6:4) gave yellow oil.

A strong peak at 1693 cm^{-1} (-CO-CH_3) in its IR spectrum was indicative of loss of chelation. Further in its ^1H NMR spectrum absence of a signal due to the OH group, appearance of a downfield 2H singlet at δ 4.94 and a 5H singlet at δ 7.2 confirmed the formation of the benzyl ether.

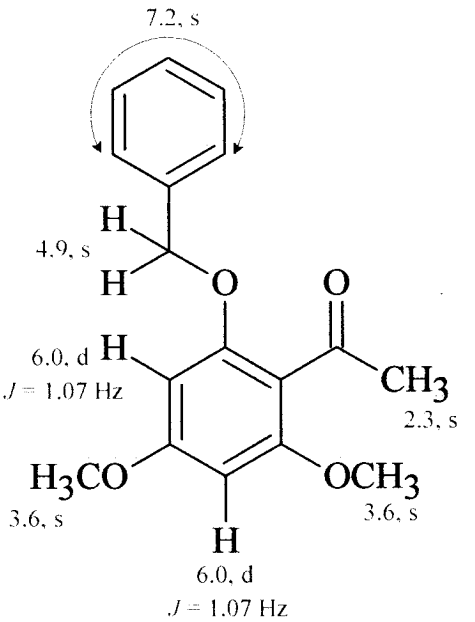


Figure VI: Assignments of the ^1H NMR signals for various protons of **21**

The ^1H NMR data clearly suggested it to be 2-benzyloxy-4,6-dimethoxyacetophenone **21** and was further supported by its ^{13}C NMR spectrum which showed in all 15 distinct signals for the 17 carbons present in **21**.

In its ^{13}C NMR DEPT spectrum, the characteristic signal at δ 70.52 (Ph-CH_2-O-Ar) confirmed the formation of **21** and the assignments for the various carbons of **21** are shown below in figure VII.

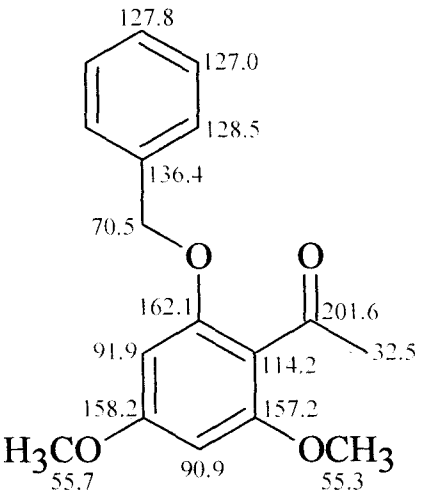
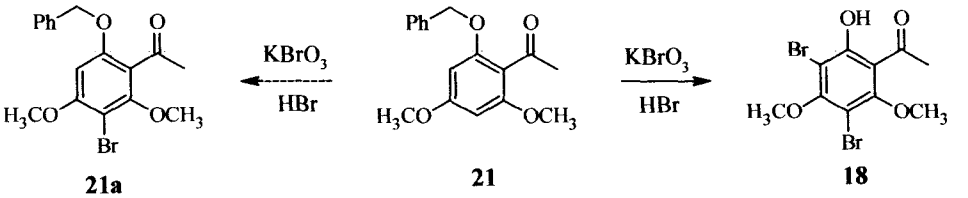


Figure VII: Assignments of ^{13}C NMR signals to various carbons of **21**

Bromination of **21** was expected to give 5-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21a**. However, bromination of **21** using $KBrO_3$ and HBr[27] gave after usual workup a viscous oil which on purification by silica gel column chromatography gave greenish yellow solid having m.p. 102°C and was identified as 3,5-dibromo-2,4-dimethoxy-6-hydroxyacetophenone **18** by comparison of its IR with that obtained earlier.

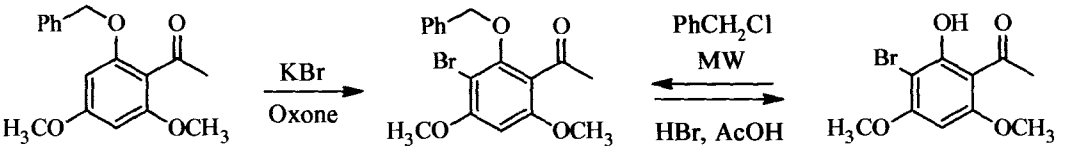


Further elution with petroleum ether-diethyl ether (6:4) gave a pale yellow solid identified as **15** on the basis of its TLC, m.p. and IR.

Formation of **15** and **18** clearly indicated that under the acidic conditions of bromination (HBr, AcOH) the benzyl ether **21** must have cleaved to regenerate the phenol **13** which then underwent bromination to give a mixture of **15** and **18**.

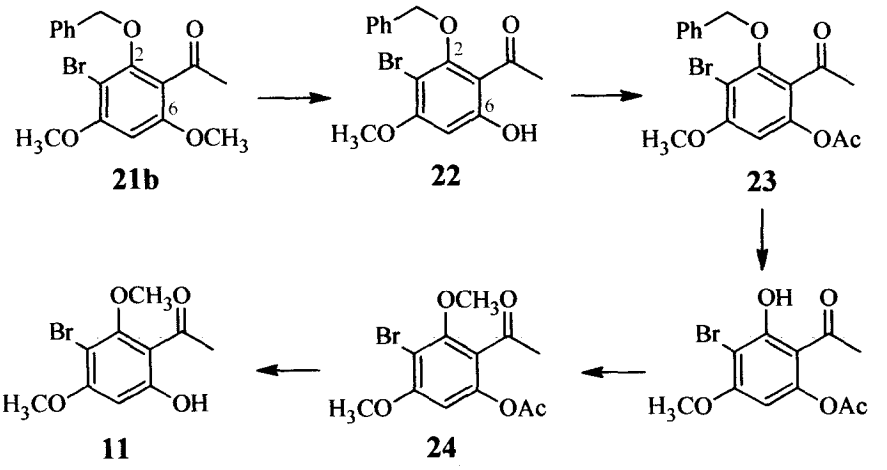
Therefore it was decided to carry out bromination under neutral conditions. Thus bromination of **21** using KBr and Oxone[30] in acetonitrile and water after usual workup gave colourless oil. Trituration with petroleum ether gave colourless plates, m.p. 102°C.

In its ^{1}H NMR spectrum a 2H singlet at δ 4.98 (Ph-C<u>H</u>$_2$-O-), a 2H doublet at δ 7.50 (J = 7.2 Hz, Ar-<u>H</u>) and a 3H multiplet at δ 7.33-7.40 (Ar-<u>H</u>) indicated that the benzyloxy group was intact. Moreover, a 1H singlet for the lone aromatic proton at δ 6.34 (C_3-H or C_5-H), indicated monobromination in the more activated benzene ring which was further supported by ^{13}C NMR and DEPT experiments. The position of Br was determined by the cleavage of the labile benzyl group using HBr in glacial AcOH as well as conversion of **15** to its benzyl ether which indicated that bromination occurred at the same unwanted α to the benzyloxy group resulting in the formation of 2-benzyloxy-3-bromo-4,6-dimethoxyacetophenone **21b**. The required **21a** was not formed.



21 **21b** **15**

Since direct bromination of **21** did not give the desired product it was decided (scheme 6) to selectively demethylate the C_6-OMe group of **21b** to give **22** and protect the OH group so generated as its acetate **23** followed by the cleavage of the benzyl ether and methylation of the OH to give the desired 2-acetoxy-5-bromo-4,6-dimethoxyacetophenone **24** which can be hydrolysed to **11**.

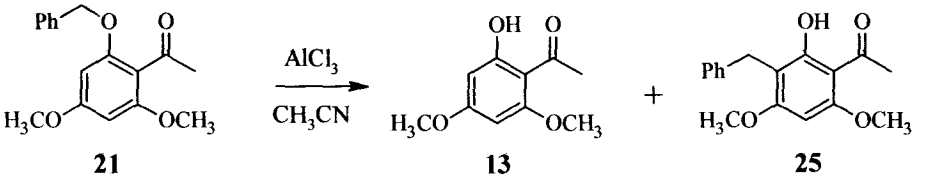


Scheme 6

Demethylation of the methoxy group ortho to an aldehydic or a ketonic group is reported using $AlCl_3$ in dichloromethane[41]. Thus stirring **21b** with $AlCl_3$ in dry CH_2Cl_2 at room temperature gave the debenzylated product **15** instead of the expected phenol **22.**

Selective demethylation of methyl ethers in the presence of benzyl ethers using $AlCl_3$ in refluxing acetonitrile is reported[42]. However, **21b** under these reaction conditions also gave **15** instead of the expected phenol **22.**

Alternatively when **21** was subjected to the above reaction conditions ($AlCl_3$ in refluxing CH_3CN)[42] it gave a viscous residue after usual workup, TLC of which showed it to be a mixture of two compounds. Purification by column chromatography using petroleum ether as eluent gave a cream coloured solid (m.p. 78°C) identified as **13** by comparison of its m.p. and IR.



Further elution with petroleum ether-diethyl ether (95:5) gave a white crystalline solid, m.p.114-116°C.

GCMS analysis showed a peak at *m/z* 286 indicating the molecular formula of the compound to be $C_{17}H_{18}O_4$ which is same as that of the starting **21** but its IR spectrum showed a strong band at 1620 cm^{-1} indicating the presence of a chelated Ar-CO-CH_3. Therefore we recorded its NMR data.

In its ^1H NMR spectrum a 5H multiplet at δ 7.11-7.29 and a 2H singlet at δ 3.92 (Ph-CH_2-Ar) indicated that the benzyl group is present but probably attached to a carbon and not to oxygen [observed δ 4.98 for (Ph-CH_2-O-)]. Secondly, the benzyl group must have migrated (from oxygen to carbon) either to *ortho*- or *para*-position with respect to the OH group. This assumption was further supported by the presence of a downfield 1H singlet at δ 14.01 (chelated OH) and a comparatively upfield 1H singlet at δ 5.96 (only Ar-H between two Ar-OCH_3 groups). Hence the structure 5-benzyl-2,4-dimethoxy-6-hydroxyaceto-phenone **25** was assigned to this white crystalline solid having m.p.114-116°C. Cresyl benzyl ethers in the presence of $AlCl_3$ are known to undergo rearrangement wherein the benzyl group migrates to the vacant *o*- or *p*-position[43].

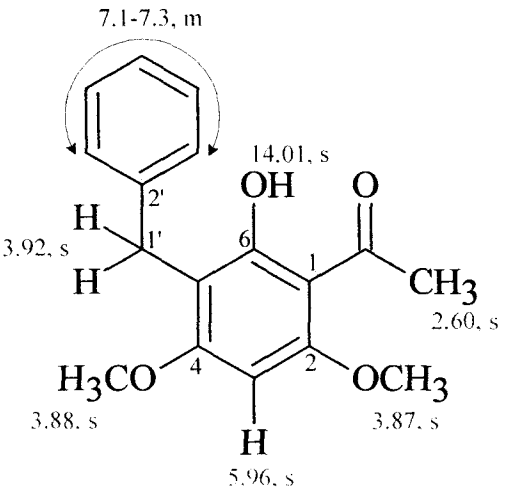


Figure VIII: Assignments of ^1H NMR signals to various protons of **25**

The structure **25** was further supported by ^{13}C NMR and DEPT experiments.

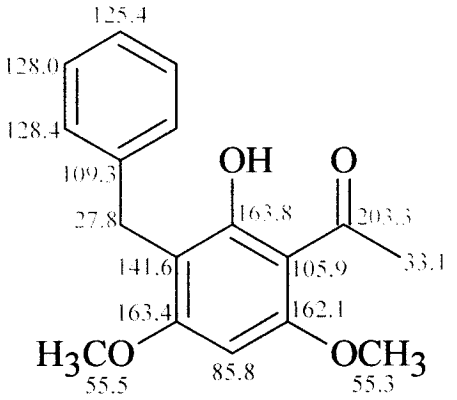
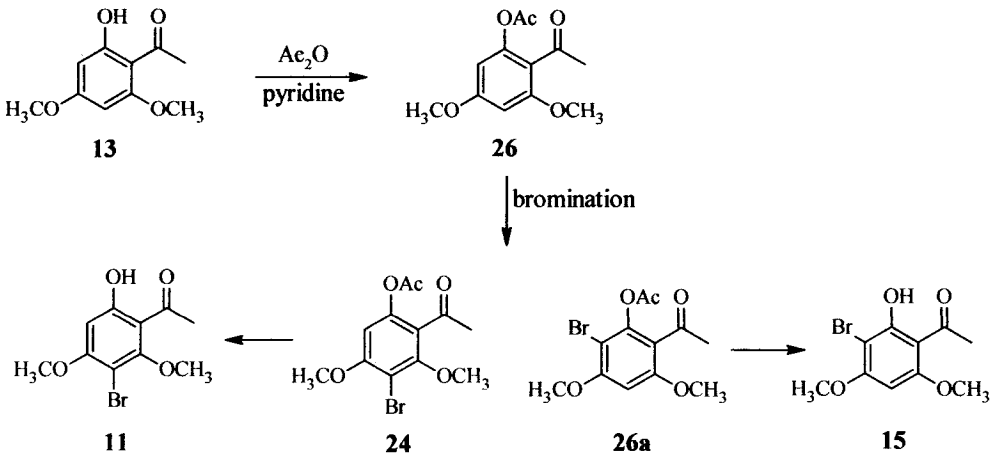


Figure IX: Assignments of ^{13}C NMR signals to various carbons of **25**

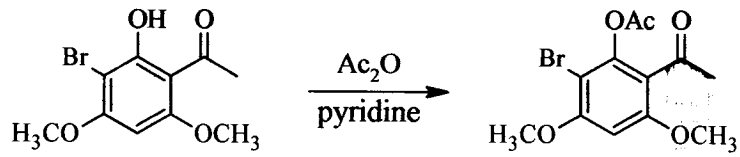
2) Acetate as the protecting group

In the second approach the chelated OH group of **13** was protected as its acetate followed by bromination.



Reaction of **13** with acetic anhydride in pyridine[23] gave 2-acetoxy-4,6-dimethoxyacetophenone **26** m.p. 106-108°C (Lit.[26] 107°C). Bromination[27,29] of **26** was carried out first with $KBrO_3$ in HBr and glacial acetic acid and then with Br_2 in water. In both reactions after usual workup, gave the bromo derivative as white solid in 97% yield. Recrystallization from $CHCl_3$-petroleum ether gave colourless cubes having m.p. 150°C.

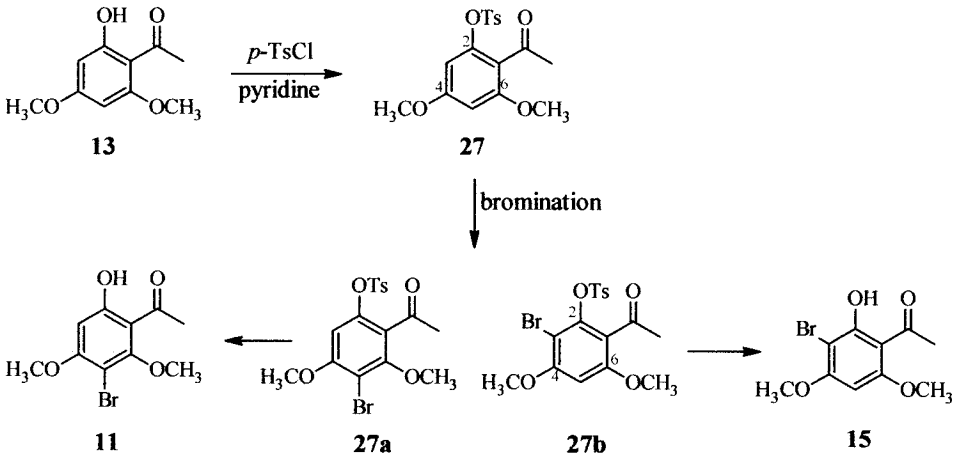
In its ^1H NMR spectrum a 3H singlet at δ 2.30 (-OCO-C<u>H</u>$_3$), a 3H singlet at δ 2.47 (-CO-C<u>H</u>$_3$) and a 1H singlet at δ 6.40 (the only Ar-H) supported nuclear bromination. The position of Br was determined by comparison (m.p & IR) of the acetate prepared by acetylation of **15** which indicated that the bromo derivative obtained is the unwanted 2-acetoxy-3-bromo-4,6-dimethoxyaceto-phenone **26a** and the required **24** was not formed.



15 **26a**

3) *p*-Toluenesulfonate as the protecting group

In the third approach the chelated OH group of **13** was protected as its *p*-toluenesulfonyl ester followed by bromination.



Heating under reflux an equimolar mixture of **13**, *p*-TsCl and anhydrous K_2CO_3 in dry acetone[44] for 4 hrs followed by usual workup gave a crude solid which on purification by column chromatography gave a white solid. Recrystallization from $CHCl_3$-petroleum ether gave colourless needles having m.p. 150-152°C.

IR spectrum showed bands at 1687 cm^{-1} (not chelated -CO-CH_3), 1367 cm^{-1} (S=O). In its ^1H NMR spectrum absence of a downfield singlet at δ 14.0 and appearance of a 3H singlet at δ 2.35 due to 4′-CH_3, two 2H doublets at δ 7.33 & 7.76 with J = 8.1 Hz and a (2H) singlet at δ 6.36 for C_3-H & C_5-H supported the formation 2-*p*-toluenesulfonyloxy-4,6-dimethoxyacetophenone **27**.

Bromination[27] of **27** using $KBrO_3$ and HBr in glacial AcOH gave the bromo derivative as colourless solid in 98% yield. Recrystallization from $CHCl_3$-petroleum ether gave colourless needles m.p. 176-178°C. A 1H singlet at δ 6.43 in its ^1H NMR spectrum confirmed nuclear bromination. The position of Br was determined by alkaline hydrolysis[44] of the bromo derivative which gave **15**

indicating it to be again the unwanted 3-bromo-4,6-dimethoxy-2-*p*-toluenesulfonyloxy-acetophenone **27b** and the required **27a** was not formed.

Attempted selective demethylation[41,42] of the C_6-OMe group in **27b** using $AlCl_3$ in dry CH_2Cl_2 at room temperature for 2 hrs or in CH_3CN resulted in the ester hydrolysis product to give **15**.

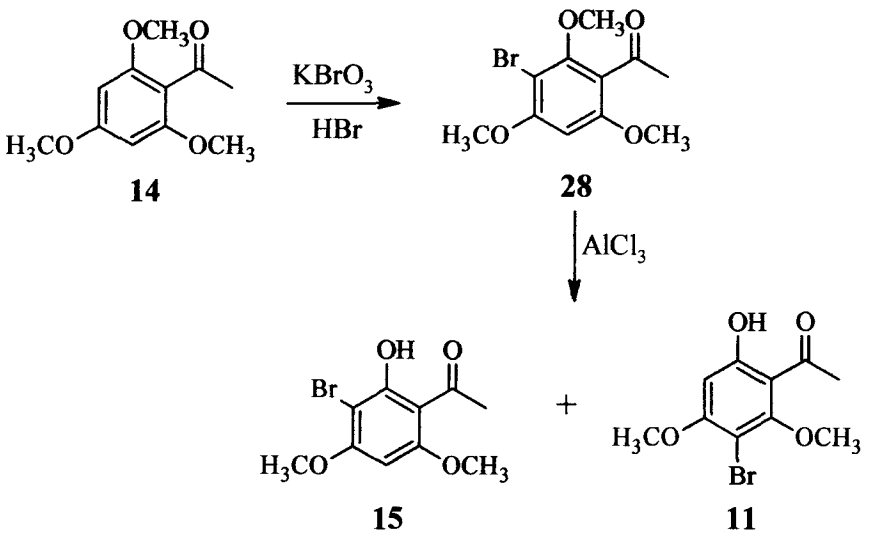
Thus our attempts towards the synthesis of **11** by using the following three strategies did not give the expected results.

1) Bromination of 2,4-dimethoxy-6-hydroxyacetophenone **13** by various brominating reagents gave only the unwanted 5-bromo-2,4-dimethoxyacetophenone **15**.
2) Blocking the more reactive C_5-position by isopropyl, ($-SO_3H$) or iodine followed by bromination also did not prove fruitful to give **11**.
3) Protection of the OH group in **13** by a bulky group so as to make the C_5-position sterically hindered also did not give expected results.

Therefore, a fourth strategy was planned as follows:

Fourth strategy involved monobromination of symmetrical 2,4,6-trimethoxy-acetophenone 14 followed by monodemethylation.

Monobromination of symmetrical 2,4,6-trimethoxyacetophenone **14** can give only **28** followed by its monodemethylation was expected to give the required bromo derivative **11** or the unwanted **15** or both as shown below.



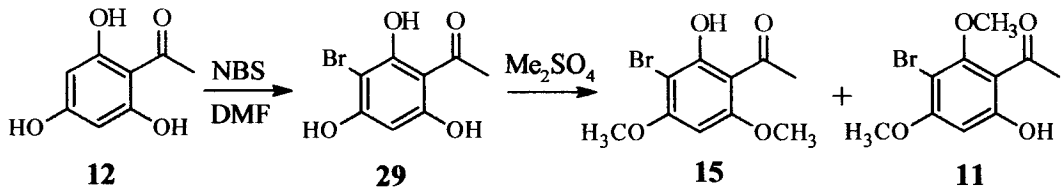
Scheme 7

Bromination[27] of 2,4,6-trimethoxyacetophenone **14** with $KBrO_3$ and HBr in glacial AcOH gave a colourless solid. Recrystallization from petroleum ether gave colourless cubes m.p. 78°C. A singlet for the lone aromatic proton at δ 6.3 (C_5-H) in its ^1H NMR spectrum supported the formation of 3-bromo-2,4,6-trimethoxyacetophenone **28**.

Demethylation of **28** was carried out first by using reported procedure[45] wherein $AlCl_3$ in CH_2Cl_2 in the presence of pyridine was heated to reflux at 45°C for 24 hrs. Second time the reaction mixture was just stirred at room temperature for 2 hrs. In both cases only the unwanted bromo derivative **15** was obtained.

Fifth strategy involved bromination of phloroacetophenone 12 followed by methylation.

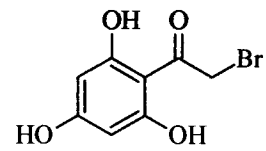
Since all the above attempts to synthesize **11** failed by the bromination of the dimethyl ether **13** or trimethyl ether **14** it was decided to carry out nuclear bromination of **12** followed by methylation as in scheme 8.



12 **29** **15** **11**

Scheme 8

Bromination[27] of phloroacetophenone **12** using $KBrO_3$, HBr and AcOH gave the starting material unchanged. But bromination[34] of **12** using NBS in dry DMF gave a brown viscous residue which on standing gave a brick red solid, a mixture of two compounds (TLC). Purification by silica gel column chromatography and elution with $CHCl_3$-petroleum ether (1:1) gave a yellow solid (TLC single spot) but the ^1H NMR spectrum revealed it to be a mixture of two compounds, the required 3-bromo-2,4,6-trihydroxyacetophenone **29** along with α-bromo-2′,4′,6′-trihydroxyacetophenone **30**.



30

Purification of the yellow solid (mixture of **29** & **30**) by flash column chromatography[‡] using $CHCl_3$ as eluent gave **29** as greenish yellow crystals, m.p. 191°C.

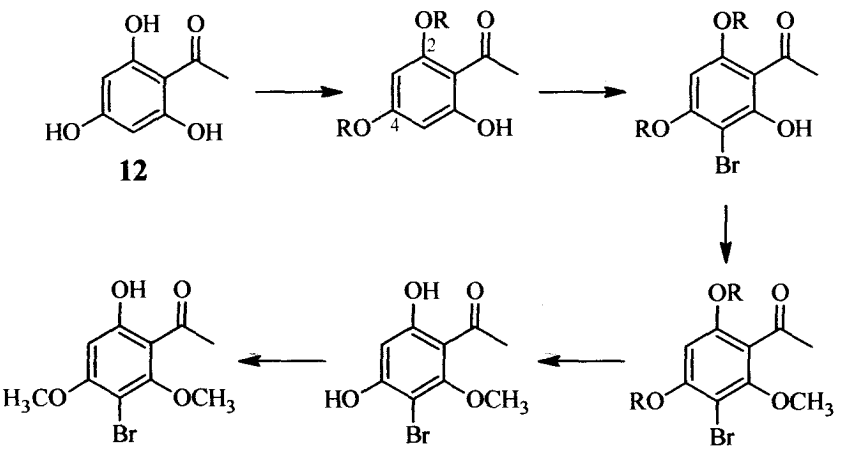
[‡]We are thankful to Dr. C. G. Naik, Bio-Organic Division, NIO, Goa for his help.

In its ^{1}H NMR spectrum a 3H singlet at δ 2.68 (-CO-C<u>H</u>$_3$) and a 1H singlet at δ 6.02 supported the formation of **29**.

Methylation[9,46] of **29** with Me_2SO_4 using either K_2CO_3 in dry acetone or with Claisen's alkali gave a yellow solid which was identified as the unwanted bromo derivative **15**.

Sixth strategy involved protection of the two OH groups of phloroacetophenone 12

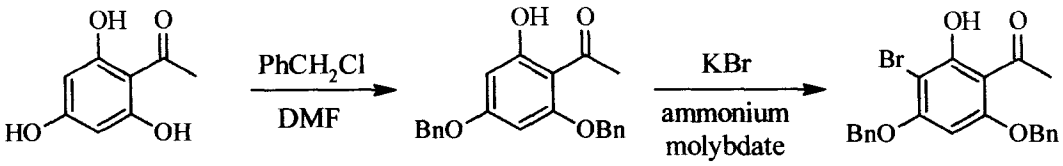
Since all the previous attempts to synthesise **11** failed an altogether different strategy was planned wherein the two OH groups in **12** were first protected either as benzyl ether or as *p*-tosyl ester followed by bromination, methylation of the chelated OH, deprotection of the protecting groups at C_2 & C_4 and finally selective monomethylation of C_4-H to yield **11**.



Scheme 9

1) Protection by benzyl group as benzyl ether

The protecting group used was benzyl group as the benzyl ethers can be easily cleaved[47] by hydrogenolysis over Pd-C.



12 **31** **32**

Scheme 10

Reaction of **12** with 2 equivalents of benzyl chloride, anhydrous K_2CO_3 in dry DMF gave 2,4-dibenzyloxy-6-hydroxyacetophenone **31** as white solid in 45% yield. Recrystallization from petroleum ether gave white crystals m.p.96°C (Lit.[48] 96-98°C).

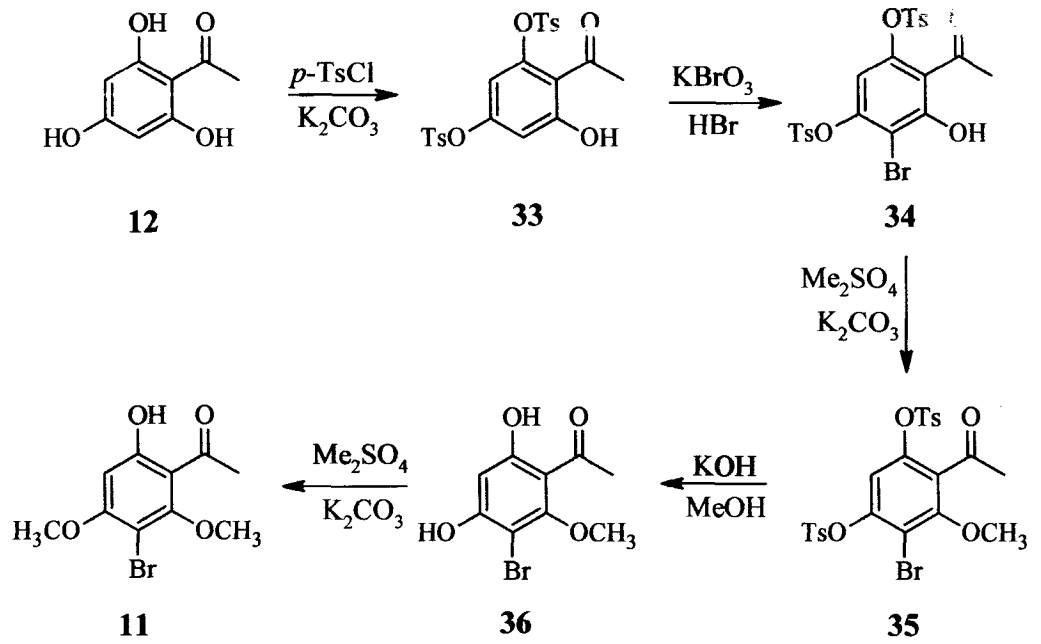
Since benzyloxy group is susceptible to cleavage under acidic conditions of HBr, the bromination was carried out using KBr and ammonium molybdate in glacial AcOH[31]. Usual workup gave a solid which on recrystallization from $CHCl_3$-petroleum ether gave yellow needles, m.p. 118°C.

In its ^{1}H NMR spectrum a broad 10H singlet at δ 7.39, two 2H singlets at δ 5.06 & 5.17 indicated that the benzyl groups are intact. Further a singlet for the lone aromatic proton, C_3-H, at δ 6.11 supported nuclear bromination. It was characterised as 5-bromo-2,4-dibenzyloxy-6-hydroxyacetophenone **32** on the basis of the chemical shift of C_3-H.

Low yields of the starting **31** and inconsistent results of the bromination reaction made us to opt for the *p*-tosyloxy group as the protecting group.

2) Protection by *p*-tosyloxy group as *p*-tosyl ester

This route involved protection of the two free OH groups of **12** as *p*-tosyl ester which can be easily hydrolysed under alkaline conditions to regenerate the phenol. The detail scheme to prepare **11** from **12** in five steps is illustrated below.



Scheme 11

Refluxing a mixture of **12**, two equivalents of *p*-TsCl and anhydrous K_2CO_3 in dry acetone[44] for 4 hrs after usual workup gave a pale brown semi-solid. Purification by column chromatography using petroleum ether-ethyl acetate (8:2) as eluent gave white solid. Recrystallization from petroleum ether gave colourless plates having m.p. 78°C.

IR showed bands at 1634 (C=O), 1595 (ArC=C) and 1377 (S=O). In its ^{1}H NMR spectrum it showed two ortho coupled doublets for 4H each at δ 7.32 & 7.72 and two (3H) singlets at δ 2.46 & 2.49 for the methyls of the *p*-tosyl group indicating the presence of the di-*p*-tosyl grouping. Further a downfield 1H

singlet at δ 12.74 (chelated OH) and two *meta*-coupled doublets of 1H each at δ 6.35 & 6.44 for H-5 & H-3 respectively confirmed the formation of 2,4-di-*p*-tosyloxy-6-hydroxyacetophenone **33**.

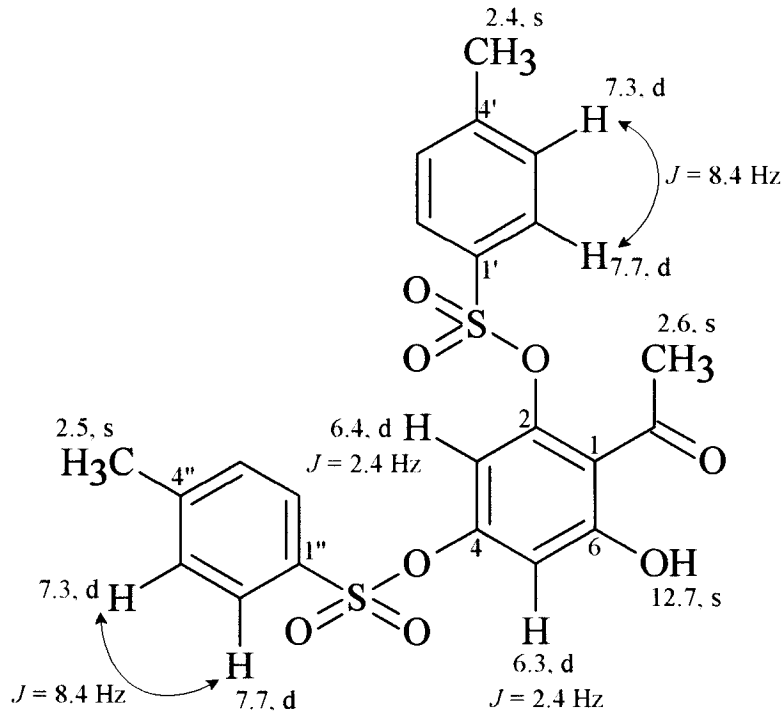


Figure X: Assignments of ^{1}H NMR signals for various protons of **33**

The structure **33** was further supported by ^{13}C NMR spectrum which showed 18 signals (nine quarternary carbons including a carbonyl at δ 203.2, six methines and three methyl carbons) as expected.

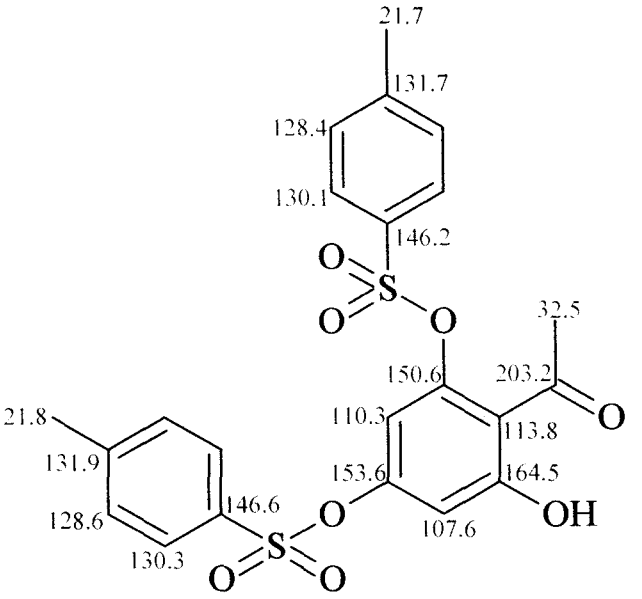


Figure XI: Assignments of ^{13}C NMR signals for various carbons of **33**

ESIMS of **33** showed a molecular ion peak at *m/z* 477.07362 $[M + H]^+$ as expected.

Bromination of **33** using $KBrO_3$ and HBr in glacial AcOH[27] for 30 mins at room temperature gave a yellow solid. Recrystallization from petroleum ether-$CHCl_3$ mixture gave lemon yellow plates, m.p. 114°C.

Appearance of a singlet at δ 6.63 integrating for 1H due to H-3 in its ^1H NMR spectrum confirmed nuclear monobromination.

ESIMS showed a molecular ion peak at *m/z* 554.9823 $[M + H]^+$ and a peak at *m/z* 556.9765 $[M + 2 + H]^+$ almost equal in intensity to the molecular ion peak. The compound was characterised as 5-bromo-2,4-di-*p*-tosyloxy-6-hydroxyacetophenone **34**. The position of bromine was considered on the basis of the results of bromination of **13**.

Refluxing **34** with 1 equivalent of Me_2SO_4, anhydrous K_2CO_3 in dry acetone[9] for 4 hrs gave after usual workup a white solid which on recrystallization from petroleum ether-benzene mixture gave white shiny cubes having melting point 128°C. Disappearance of a signal due to chelated OH and

appearance of a 3H singlet at δ 3.72 due to methoxy group in its ^{1}H NMR spectrum supported its formation. It was characterised as 5-bromo-2,4-di-*p*-tosyloxy-6-methoxyacetophenone **35**. The position of bromine was confirmed by HMBC.

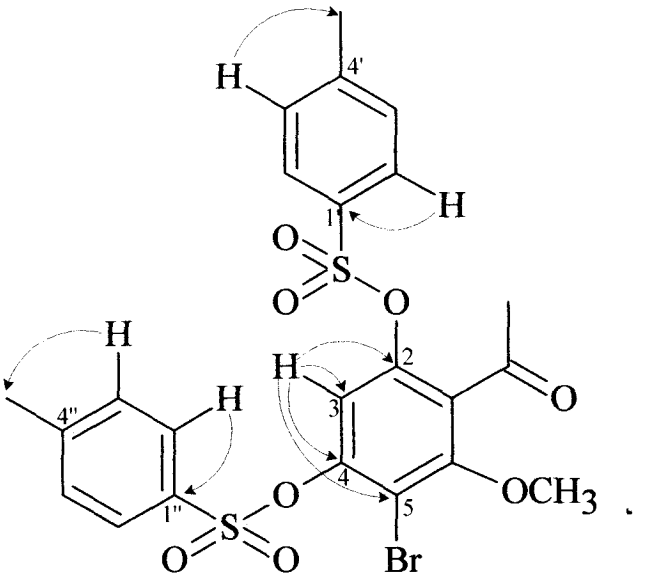


Figure XII: HMBC of **35**

In the HMBC the lone aromatic proton at C-3 correlated to C-2, C-3, C-4 and C-5. The fact that the proton at C-3 does not show correlation to the carbon carrying the methoxy group i.e.C-6 confirmed that the lone aromatic proton is para and the bromine is ortho with respect to the methoxy group.

Alkaline hydrolysis[44] of **35** using KOH and aqueous MeOH gave a brown viscous product which on purification by column chromatography and elution with petroleum ether-ethyl acetate (9.8:0.2) gave colourless plates, m.p. 116°C. A 3H singlet at δ 3.89 of methoxy group, a singlet for the lone aromatic proton, H-5, at δ 6.45, a 1H singlet at δ 6.25 due to C_4-OH and a upfield singlet for C_6-OH at δ 13.22 confirmed the hydrolysis of the di-*p*-tosyl groups. The compound was characterised as 3-bromo-4,6-dihydroxy-2-methoxyacetophenone **36**.

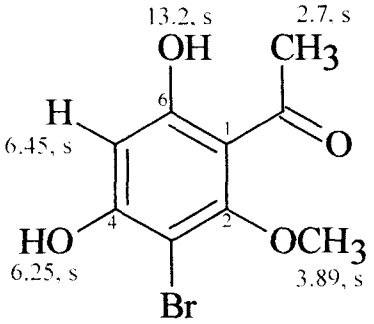


Figure XIII: Assignments of the ^{1}H NMR signals for the various protons of **36**

The structure of **36** was further supported by its ^{13}C NMR spectrum which showed in all nine signals (six quarternary, one methine and two methyls) as expected and their assignments are shown below.

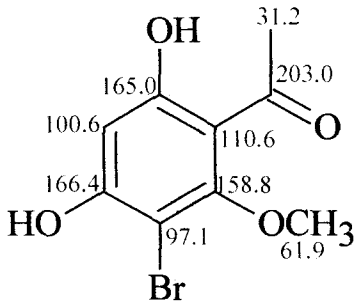


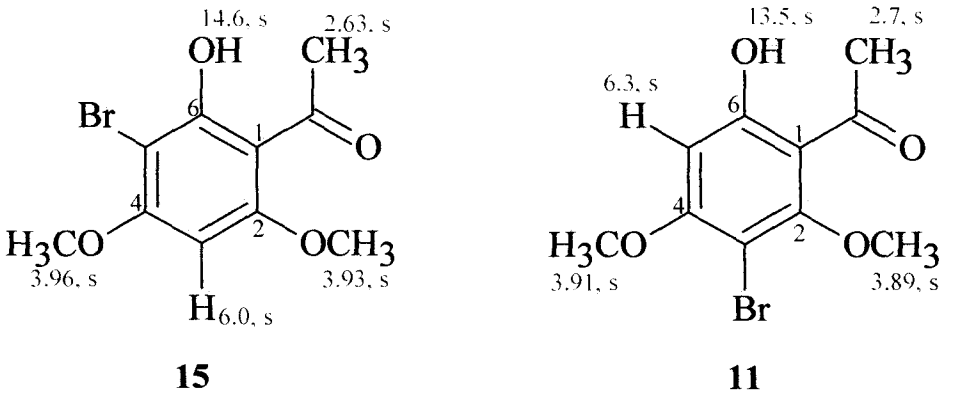
Figure XIV: Assignments of the ^{13}C NMR signals for all the 9 carbons of **36**

ESIMS data showed a molecular ion peak at *m/z* 260.9802 $[M + H]^+$ and a peak at *m/z* 262.9788 $[M + 2 + H]^+$ almost equal in intensity to the molecular ion peak.

Selective monomethylation of **36** using Me_2SO_4 and anhydrous K_2CO_3 by refluxing an equimolar mixture in dry acetone[9] for 3 hrs gave after usual workup a brown residue which was purified by column chromatography. Elution with petroleum ether-diethyl ether (9:1) gave colourless solid. Recrystallization from petroleum ether gave colourless plates, m.p. 102°C.

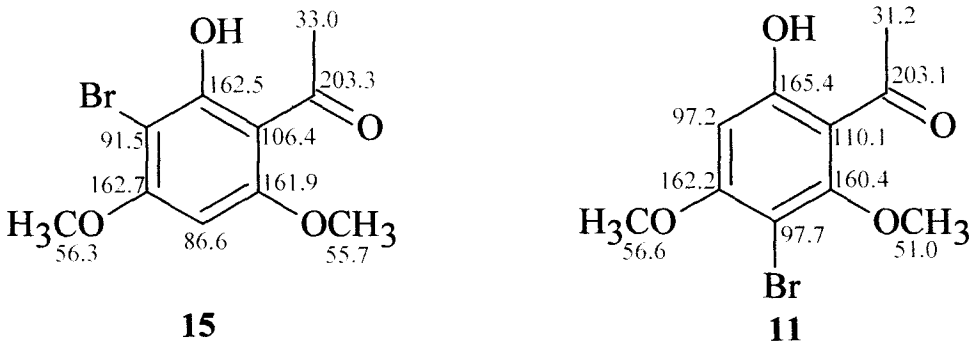
Its IR spectrum was different from that of the earlier bromo compound **15**. Moreover in its ^{1}H NMR spectrum the singlet due to the lone aromatic proton was observed at δ 6.32 which is comparatively downfield to that observed for the bromo compound **15** (at δ 6.0). It was characterised as 3-bromo-2,4-dimethoxy-6-hydroxyacetophenone **11**.

A comparative ^{1}H & ^{13}C NMR data of **15** & **11** is shown below.



15 **11**

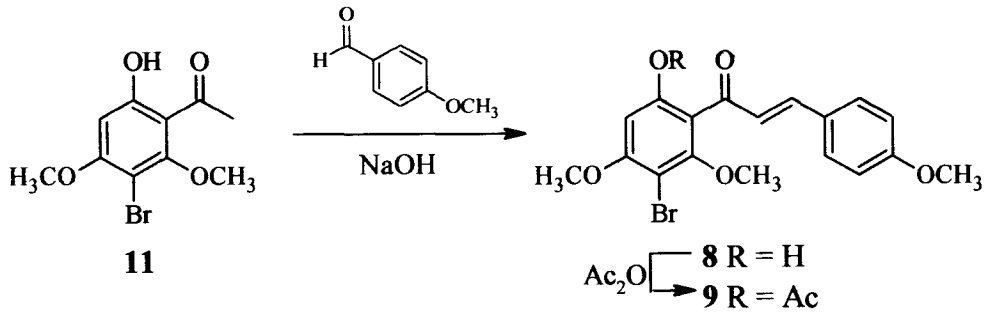
Figure XV: Comparison of the ^{1}H NMR data of **15** and **11**



15 **11**

Figure XVI: Comparison of the ^{13}C NMR data of **15** and **11**

The difference in the m.p., IR and ^{1}H and ^{13}C NMR values confirmed the non-identity of **11** with **15**.



Scheme 12

Condensation[49] of 3-bromo-2,4-dimethoxy-6-hydroxyacetophenone **11** with *p*-methoxybenzaldehyde in 50% aqueous KOH at 45°C for 1 hr gave 5′-bromo-4,4′,6′-trimethoxy-2′-hydroxychalcone **8** as yellow solid, m.p. 180°C (Lit.[23] 180°C) in 70% yield.

Interestingly, our synthetic chalcone **8** also showed a singlet at δ 7.90 for the *trans* olefinic (H-α and H-β) protons in its ^{1}H NMR spectrum which was in agreement with that observed at δ 7.87 for the natural chalcone.

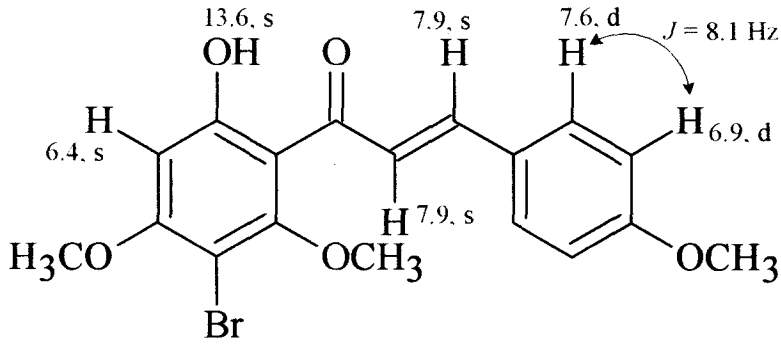


Figure XVII: Assignments of ^{1}H NMR signals to some characteristic protons of **8**

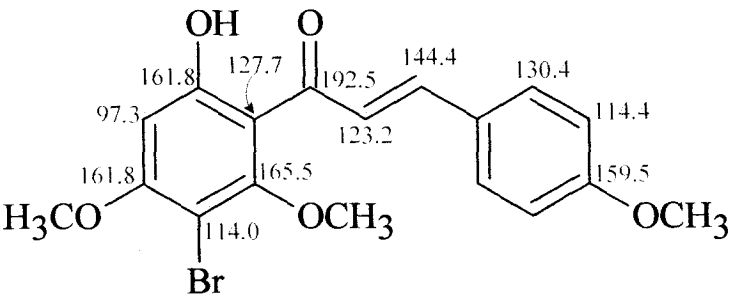


Figure XVIII: ^{13}C NMR assignments for the various carbons of **8**

Further, acetylation of **8** with Ac_2O in pyridine[23] gave **9** as pale yellow solid (m.p. 126-128°C, Lit.[23] 120-122°C) in 72% yield.

In its ^1H NMR spectrum the 2H singlet for the trans olefinic protons observed in **8** at δ 7.90 was resolved into two individual AB doublets each integrating for 1H each at δ 7.40 & 6.92 for H-β and H-α respectively with $J = 16$ Hz as reported[23].

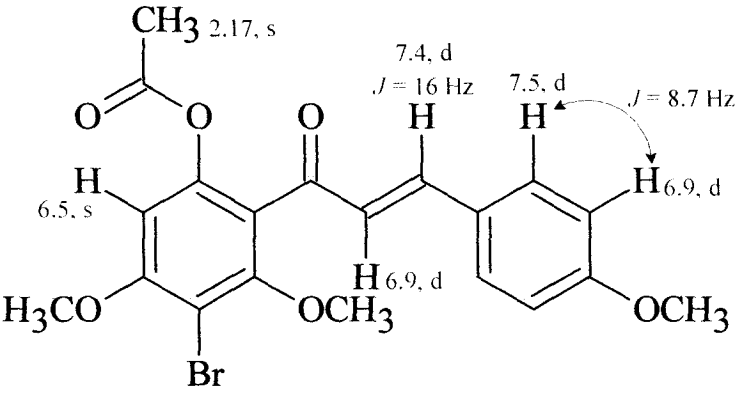


Figure XIX: Assignments of ^1H NMR signals to some characteristic protons of **9**

In conclusion we have successfully synthesized the natural chalcone **8** and its acetate **9** identical in all respects including the chemical shifts observed for the *trans*-α, β-olefinic protons with that isolated from *Garcinia nervosa.*

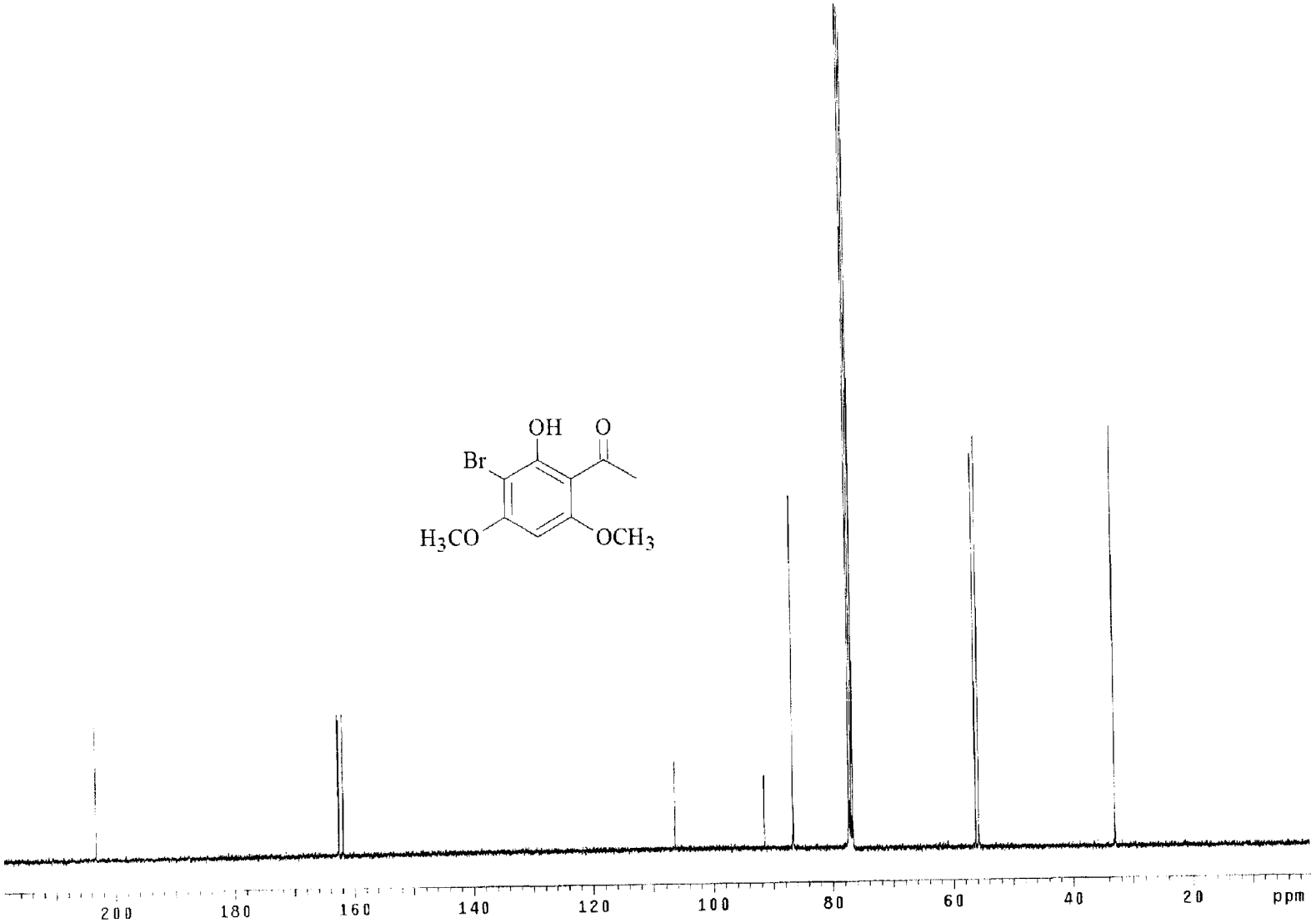


Fig. 2.01: ^{1}H NMR spectrum of **15**

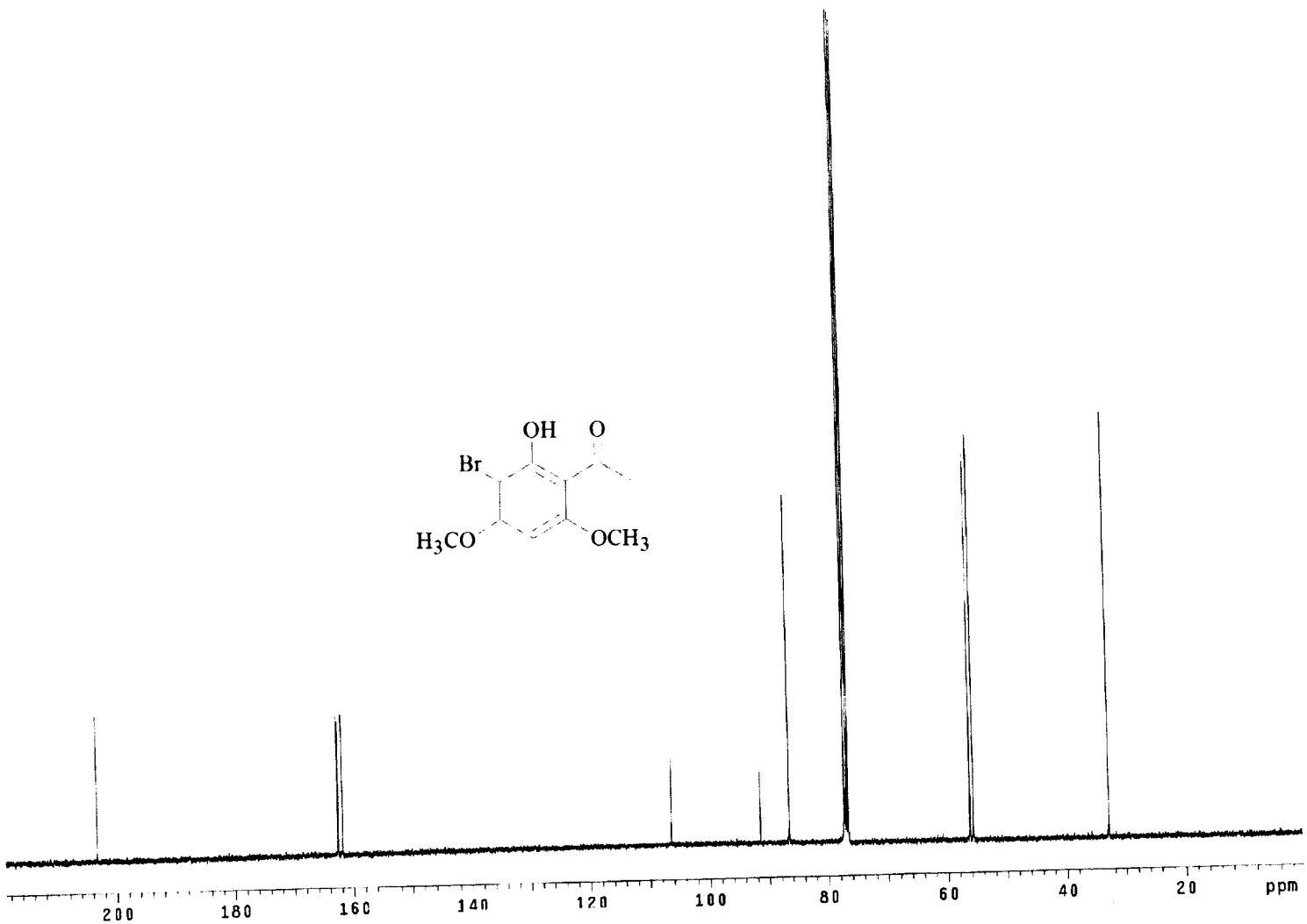


Fig. 2.02: ^{13}C NMR spectrum of **15**

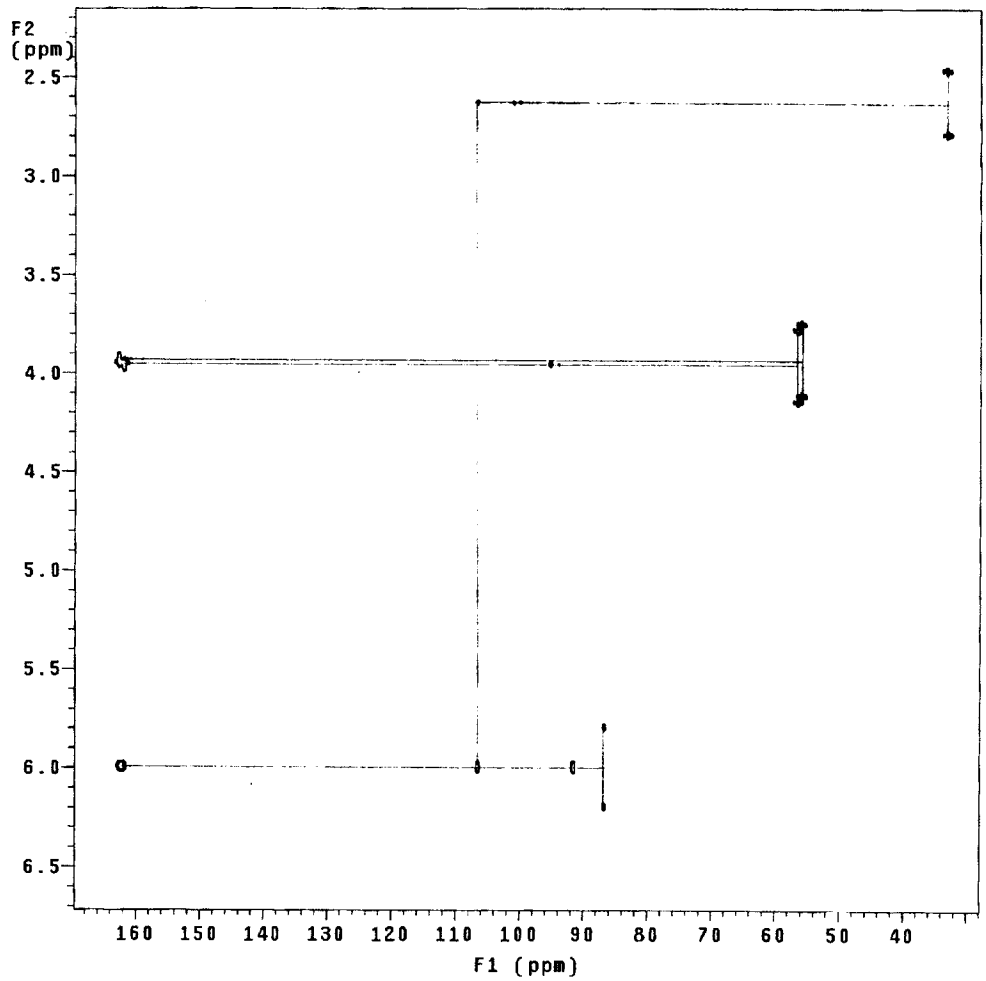


Fig. 2.03: ^{1}H-^{13}C HMBC spectrum of **15**

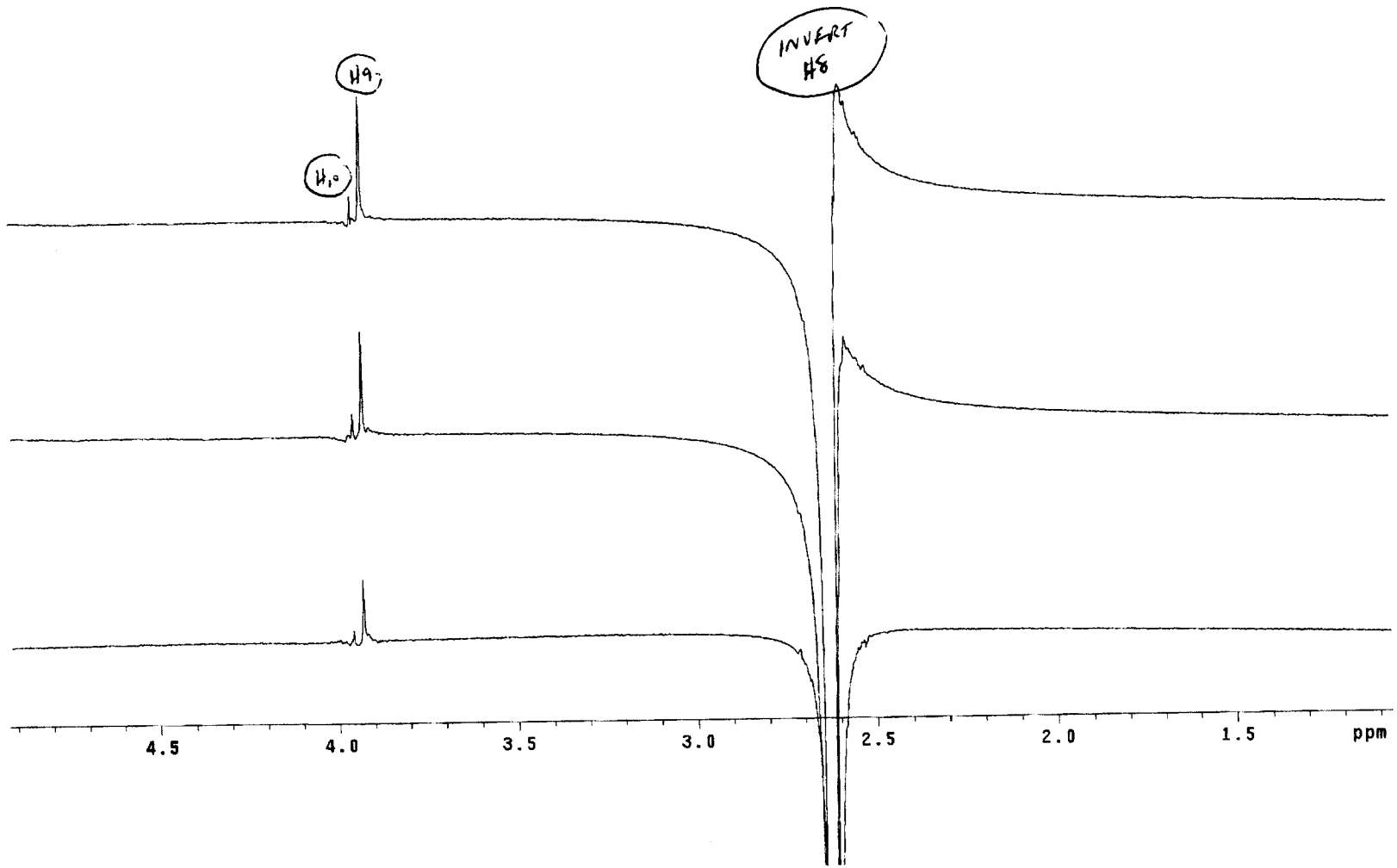


Fig. 2.04: NOE spectrum of **15**, inversion of H-8

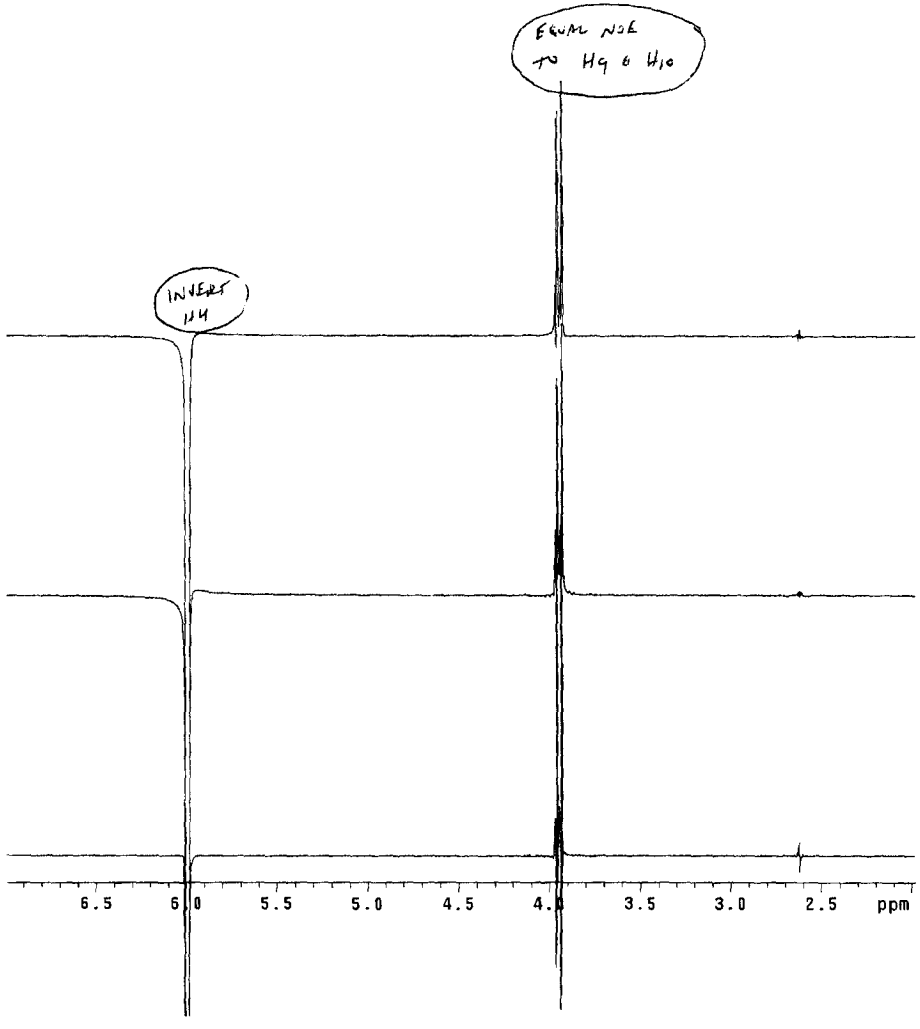


Fig. 2.05: NOE spectrum of **15**, inversion of H-3

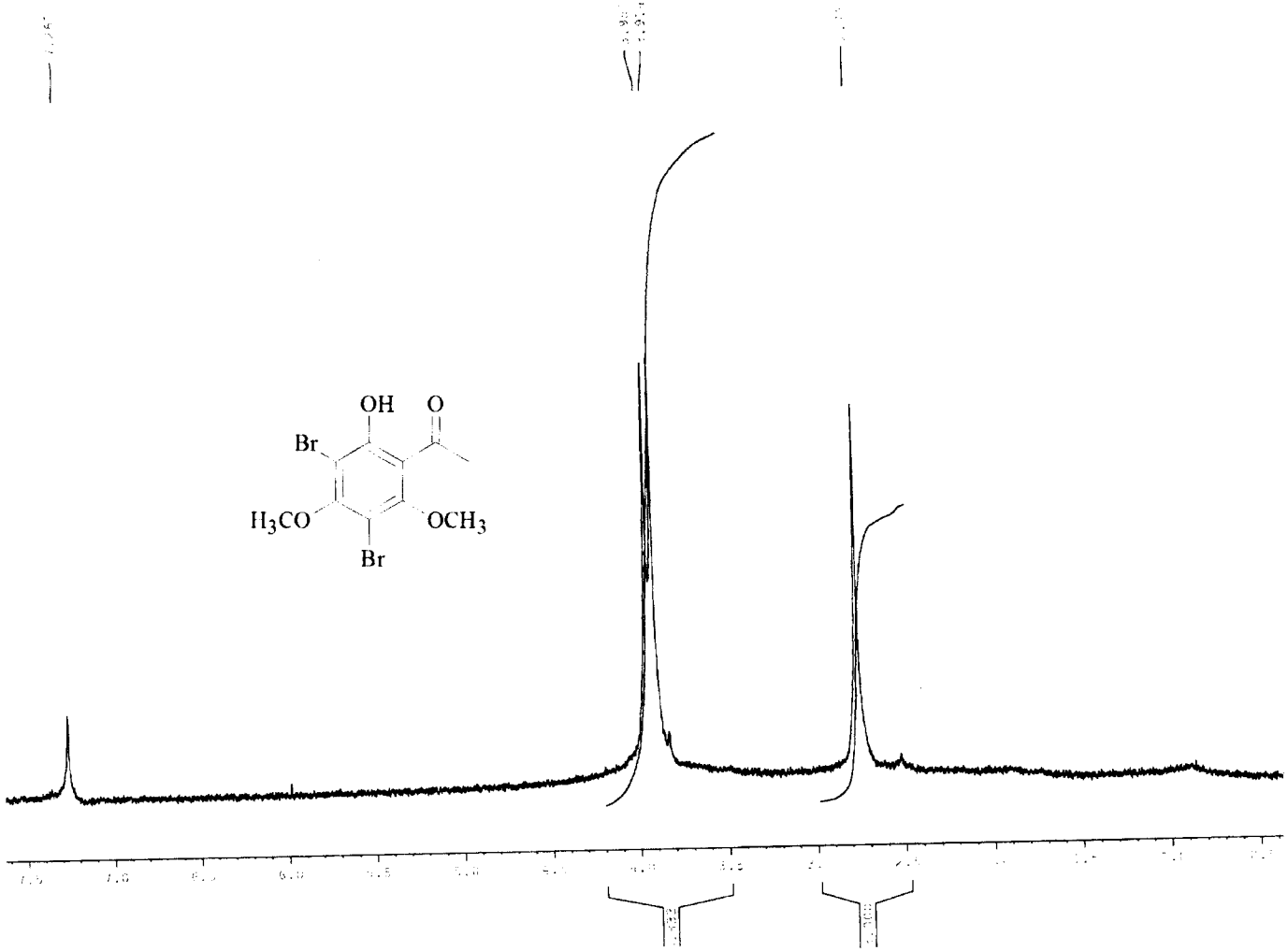


Fig. 2.06: ^{1}H NMR spectrum of **18**

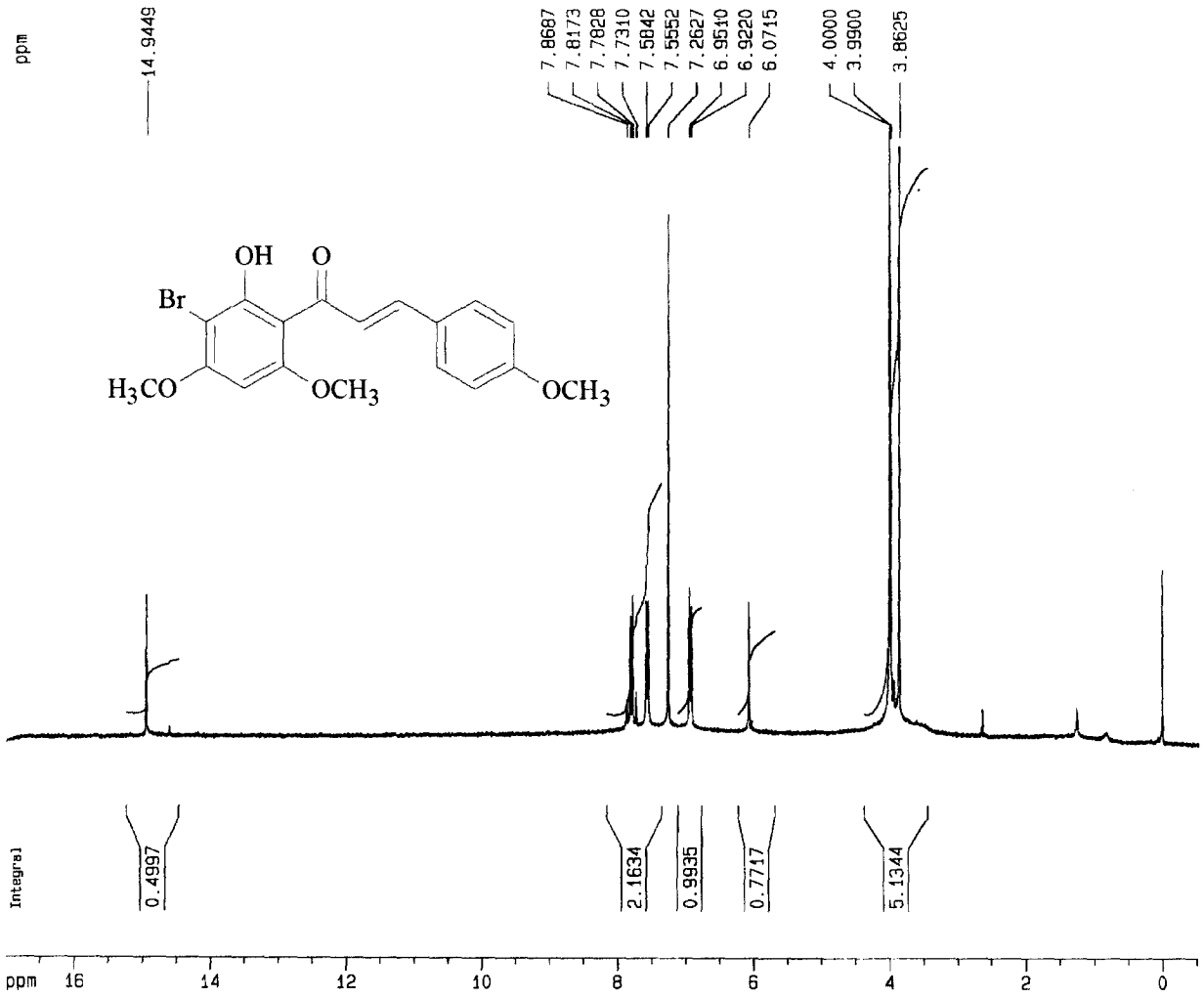


Fig. 2.07: ^{1}H NMR spectrum of **16**

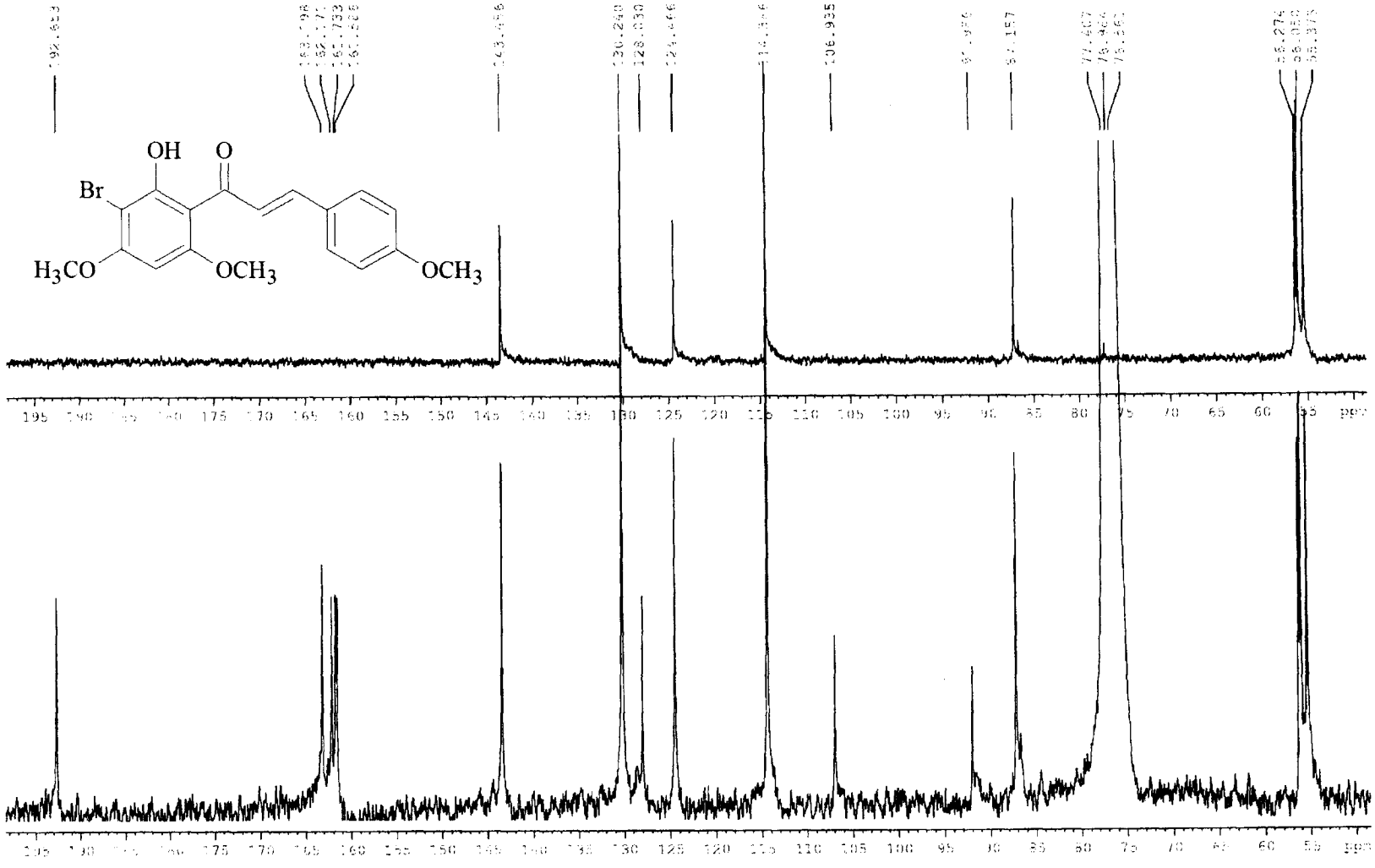


Fig. 2.08: ^{13}C NMR spectrum of **16**

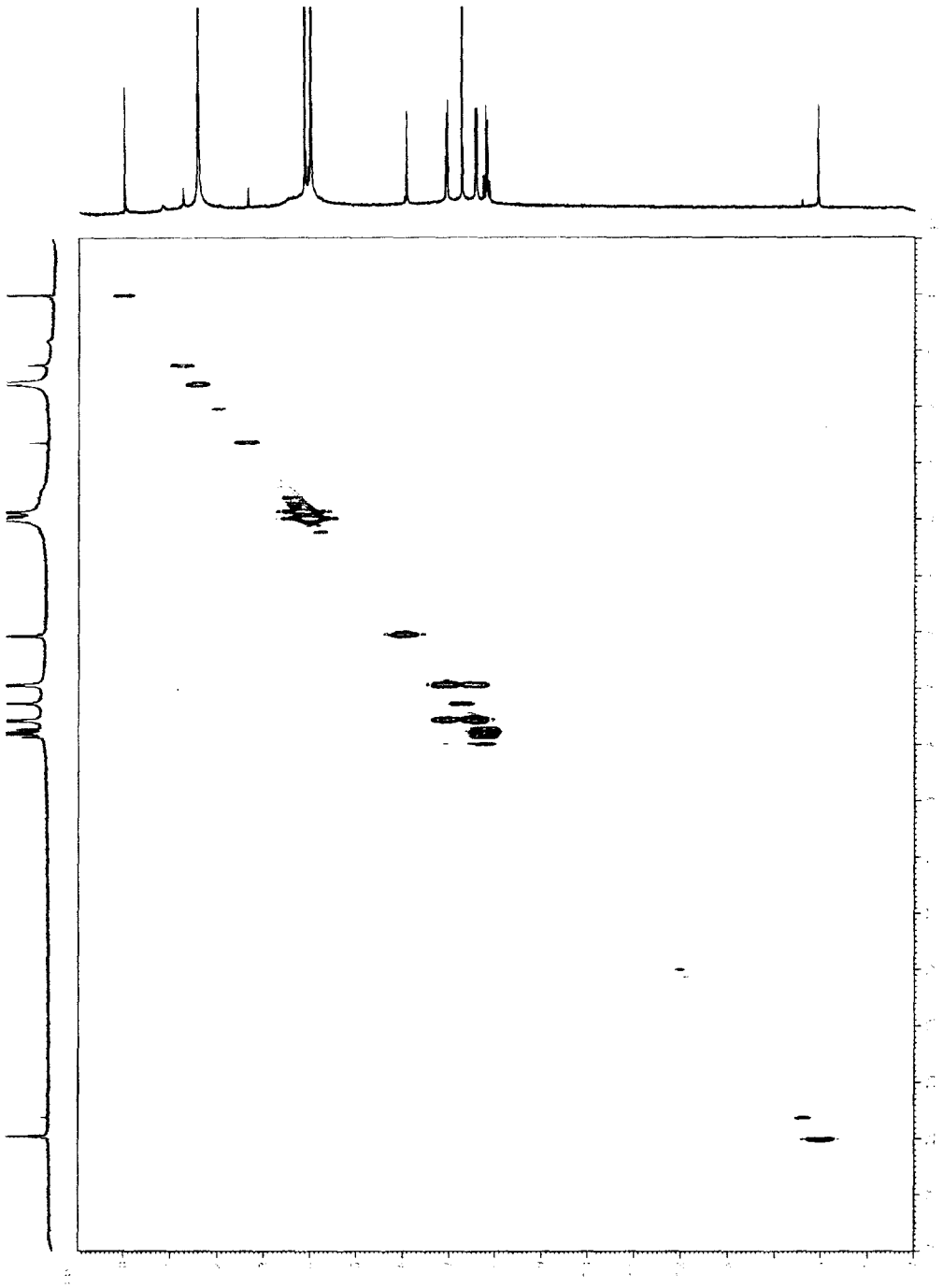


Fig. 2.09: COSY spectrum of **16**

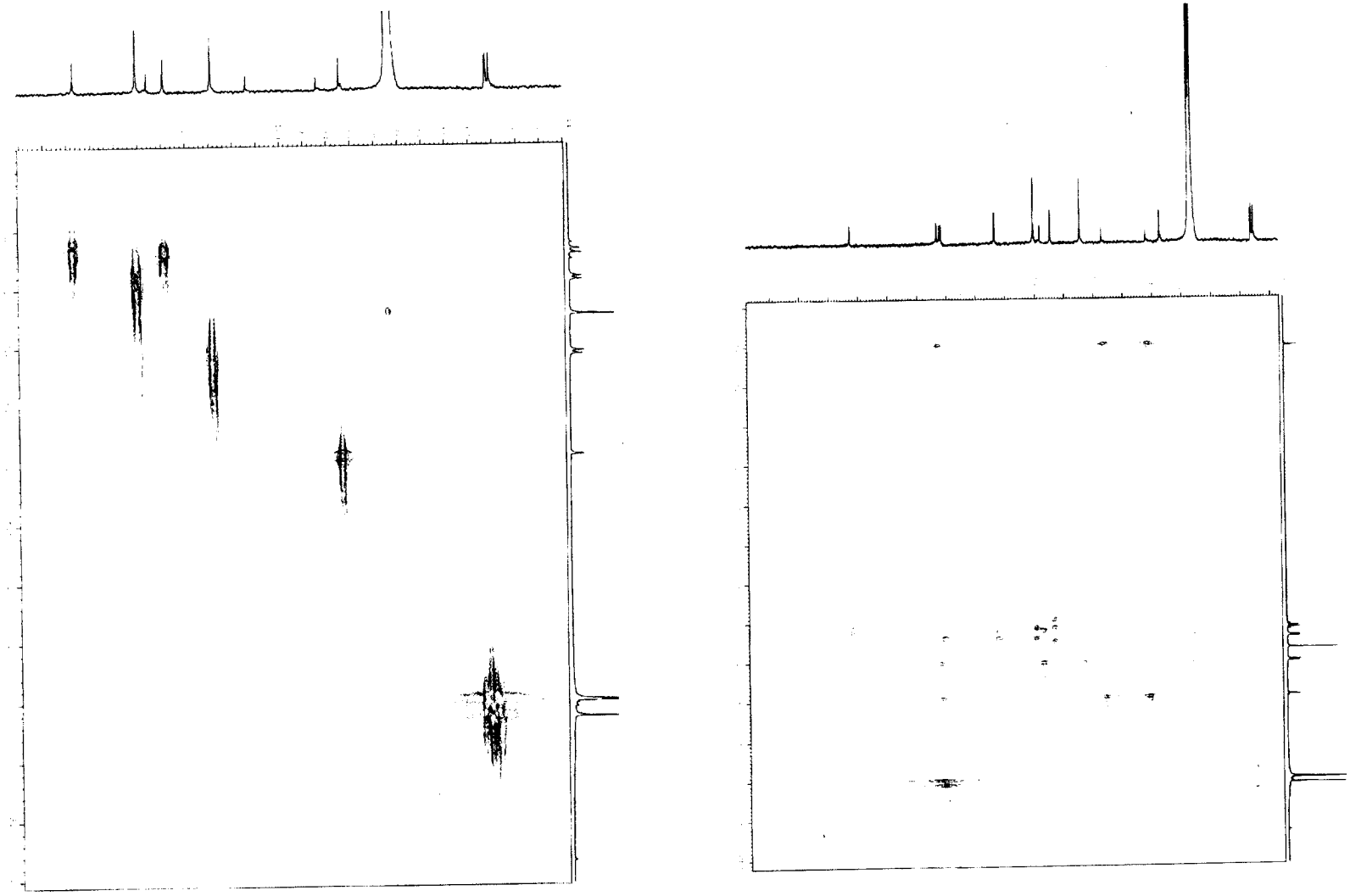


Fig. 2.10: ^{1}H-^{13}C HMBC spectrum of **16**

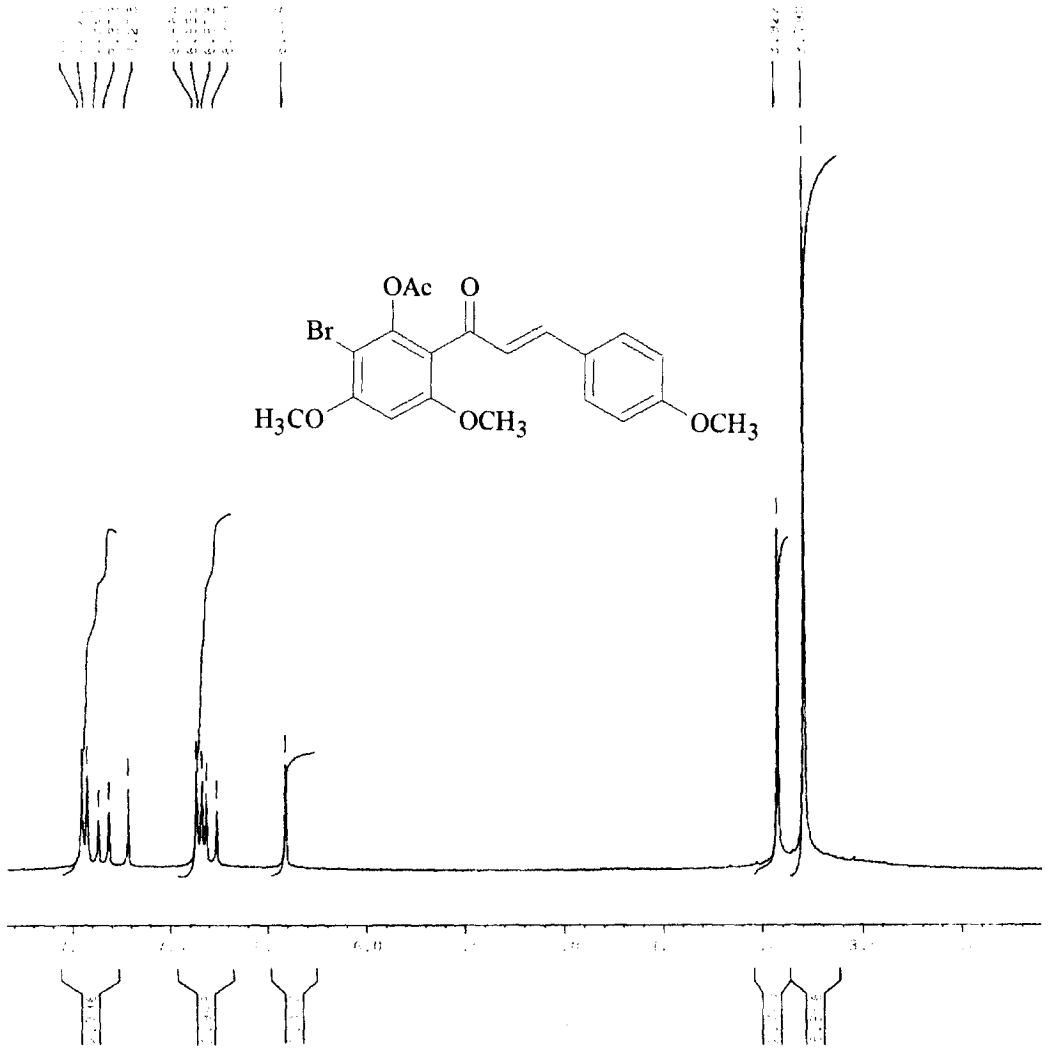


Fig. 2.11: ^{1}H NMR spectrum of **17**

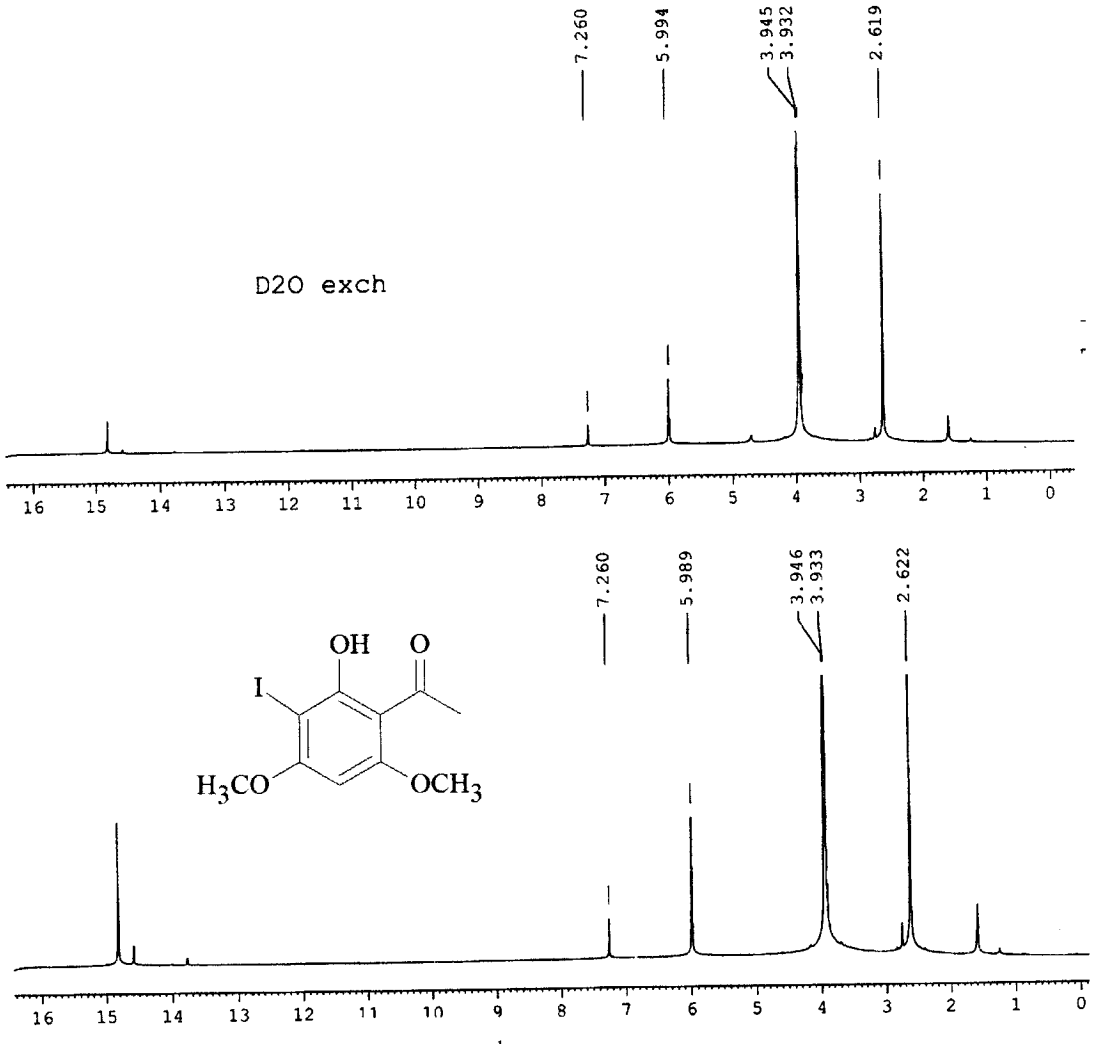


Fig. 2.12: ^{1}H NMR spectrum of **19**

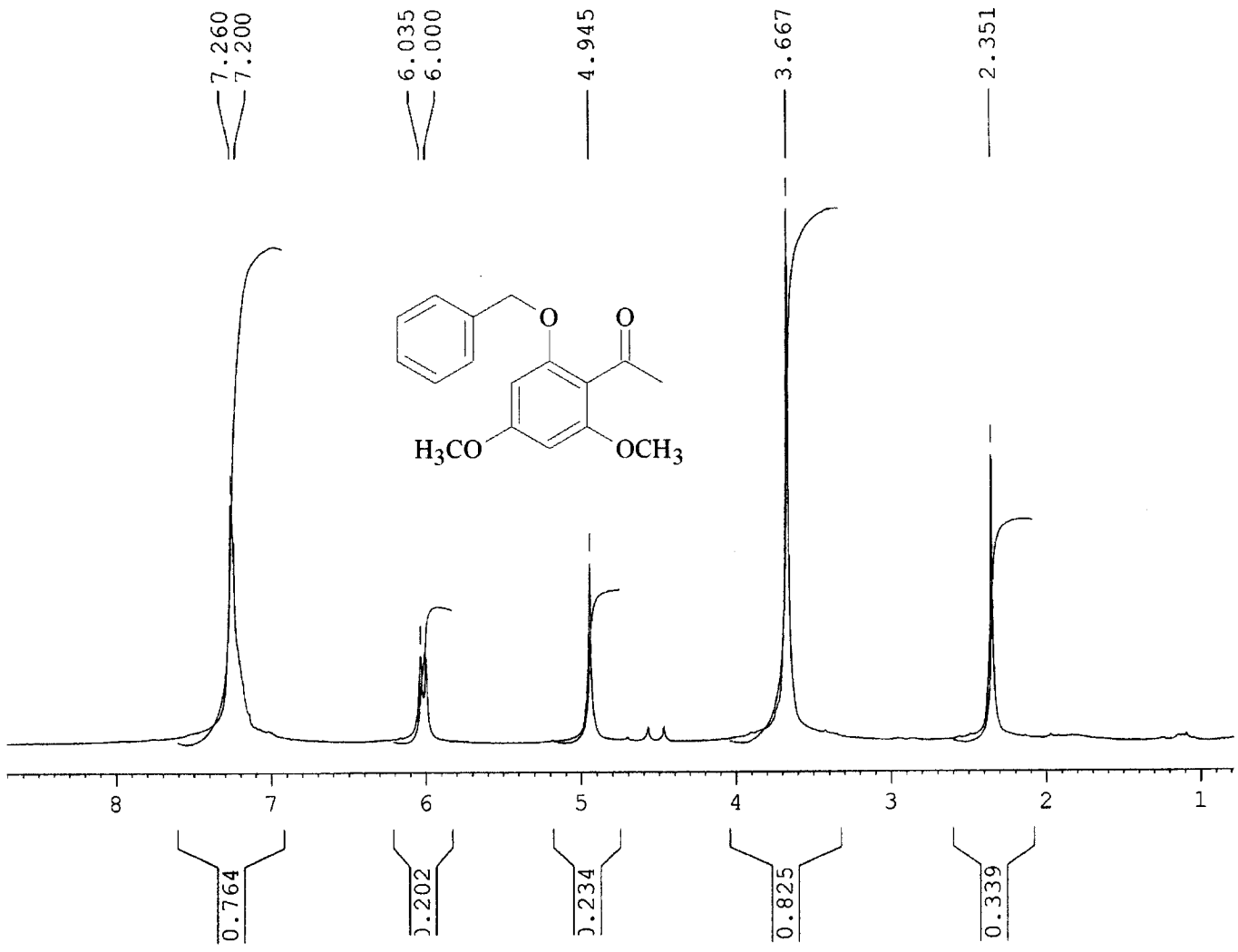


Fig. 2.13: ^{1}H NMR spectrum of **21**

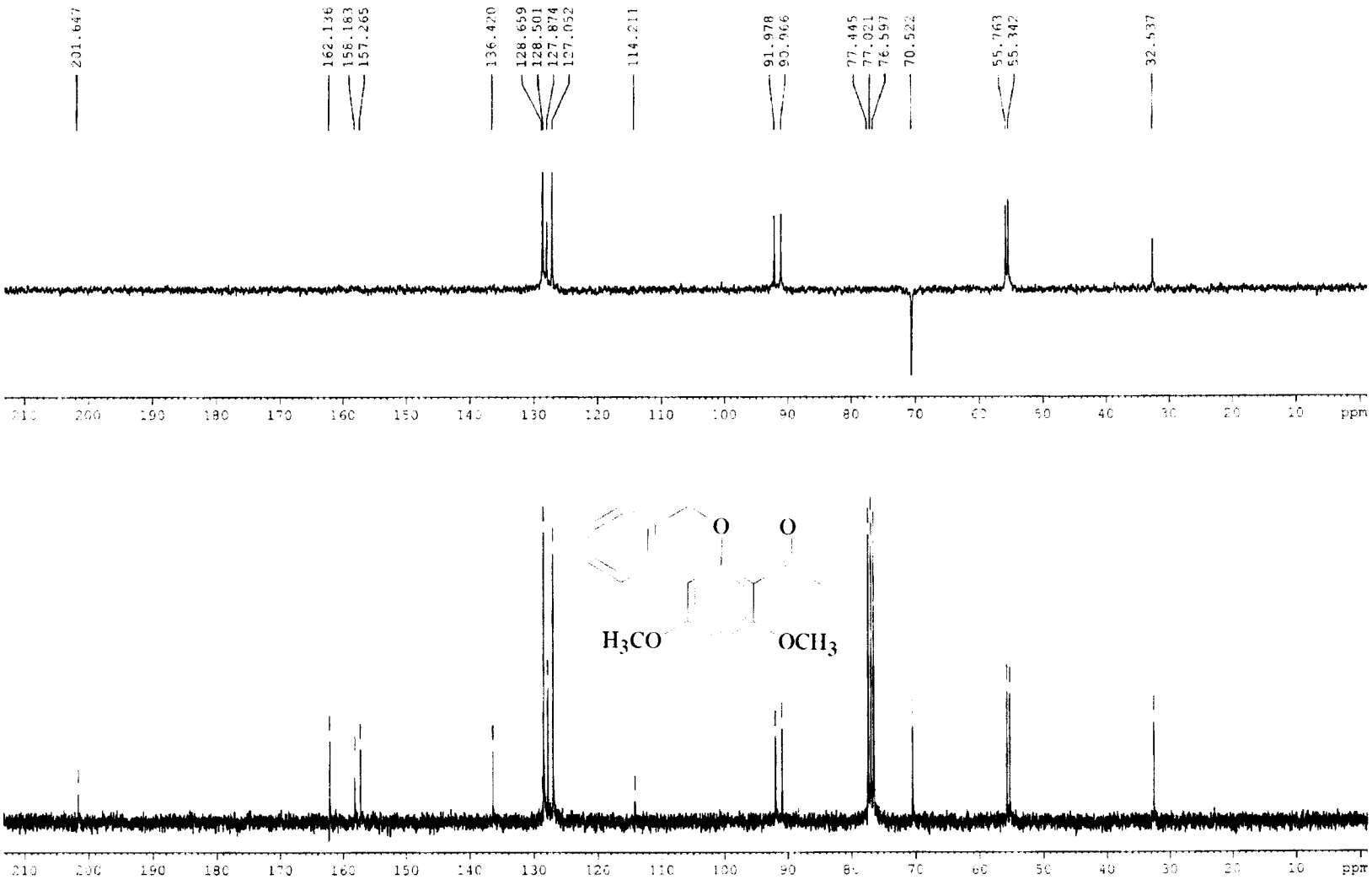


Fig. 2.14: ^{13}C NMR spectrum of **21**

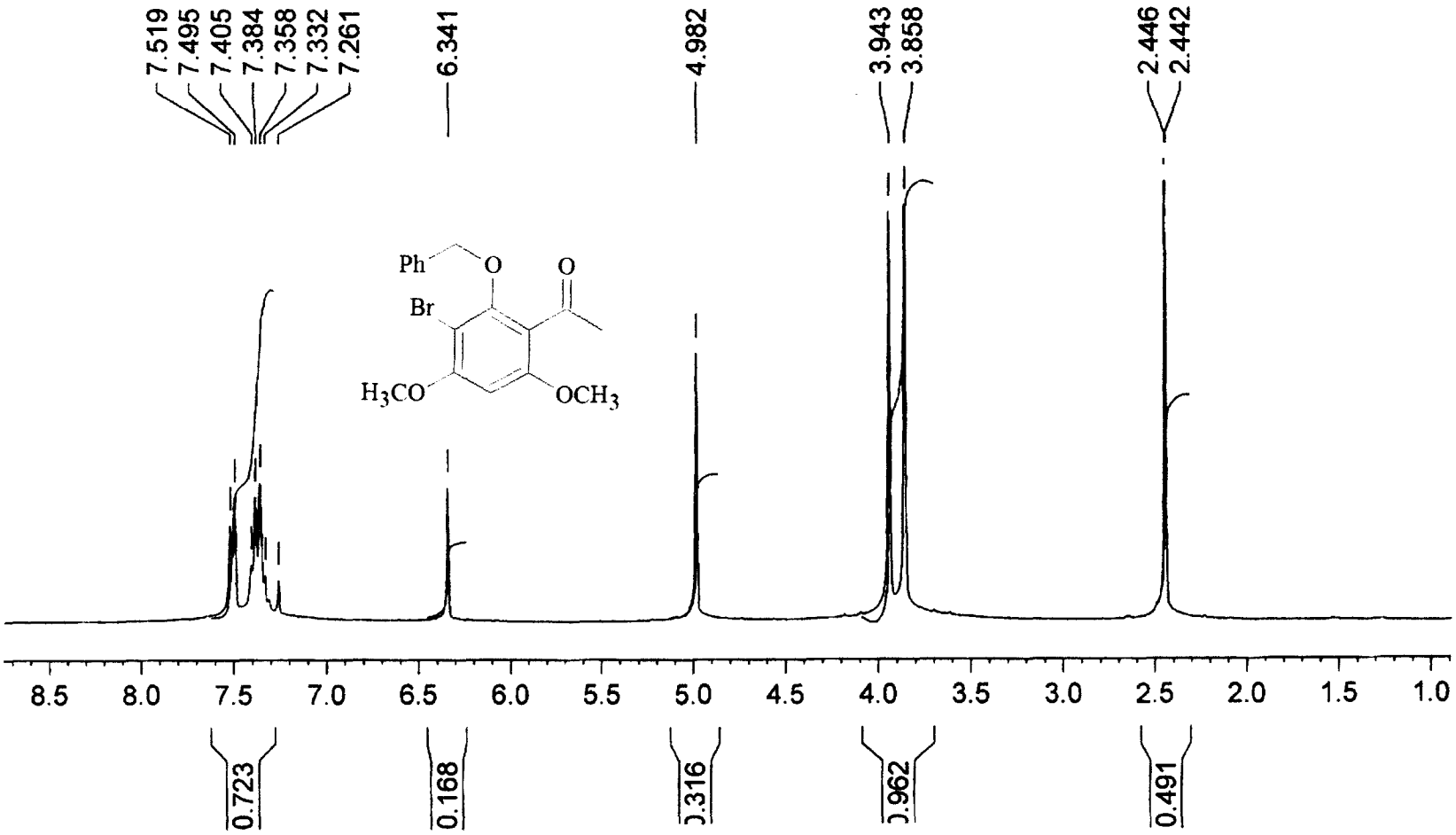


Fig. 2.15: ^{1}H NMR spectrum of **21b**

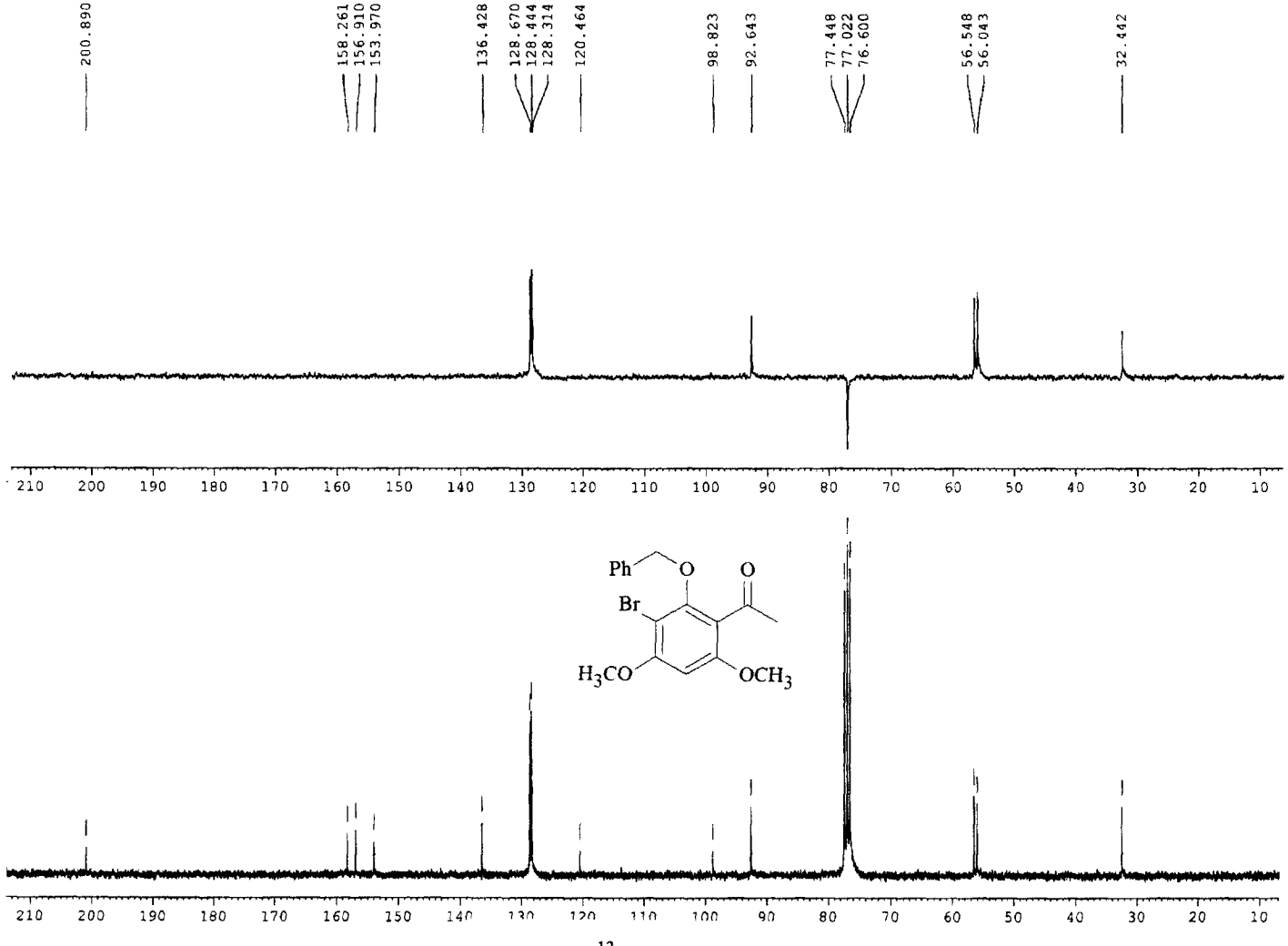


Fig. 2.16: ^{13}C NMR spectrum of **21b**

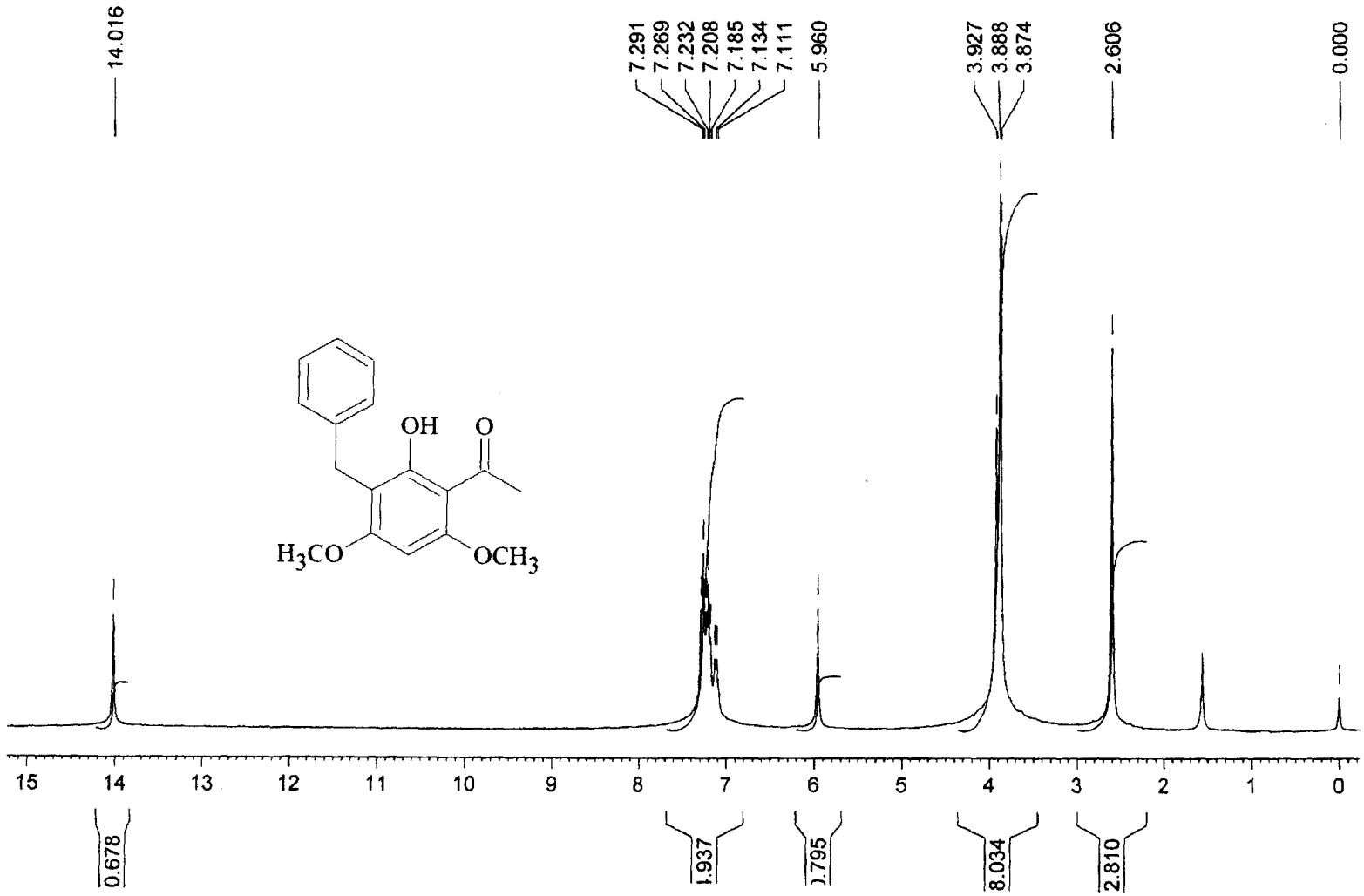


Fig. 2.17: ^{1}H NMR spectrum of **25**

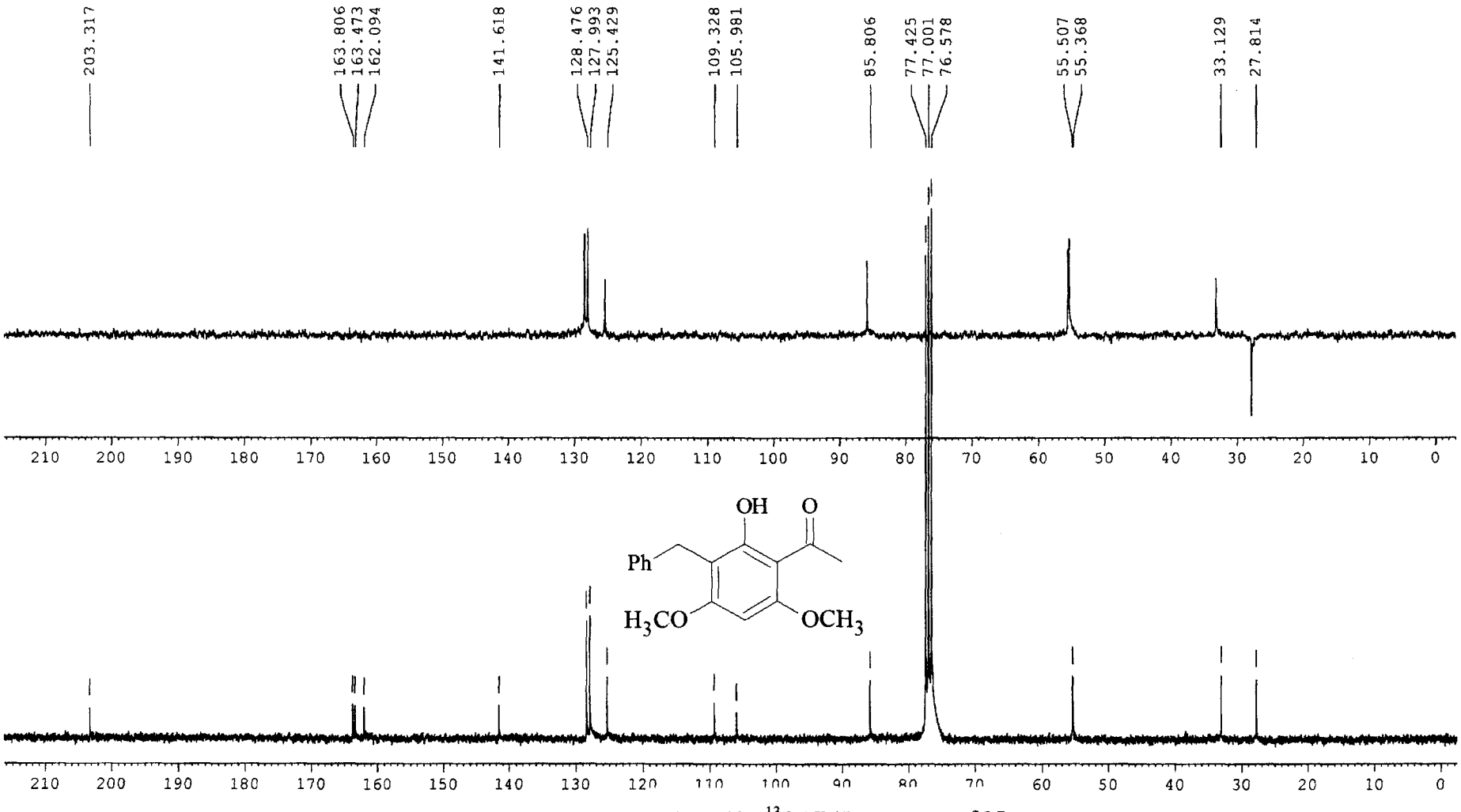


Fig. 2.18: ^{13}C NMR spectrum of **25**

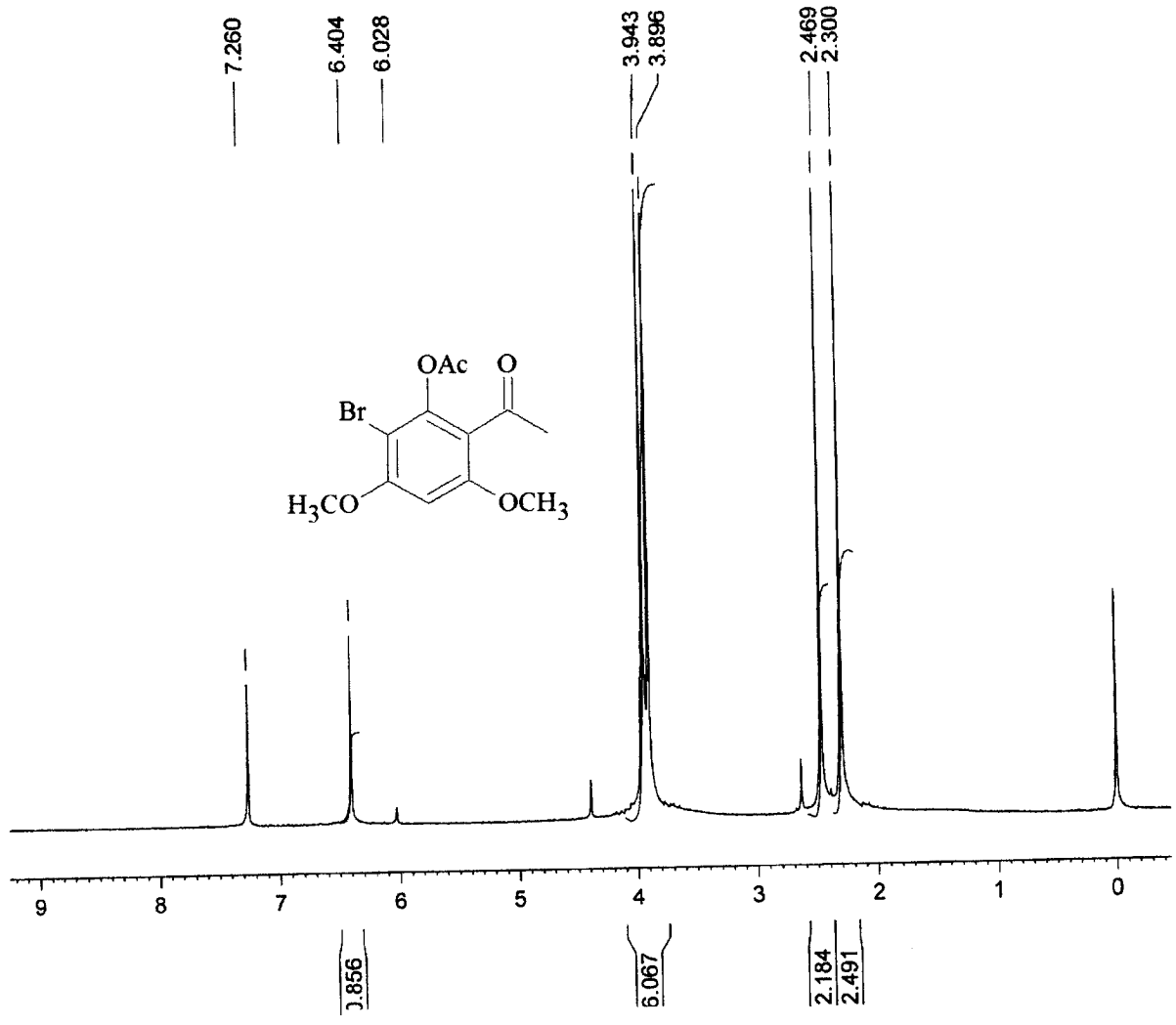


Fig. 2.19: ^{1}H NMR spectrum of **26a**

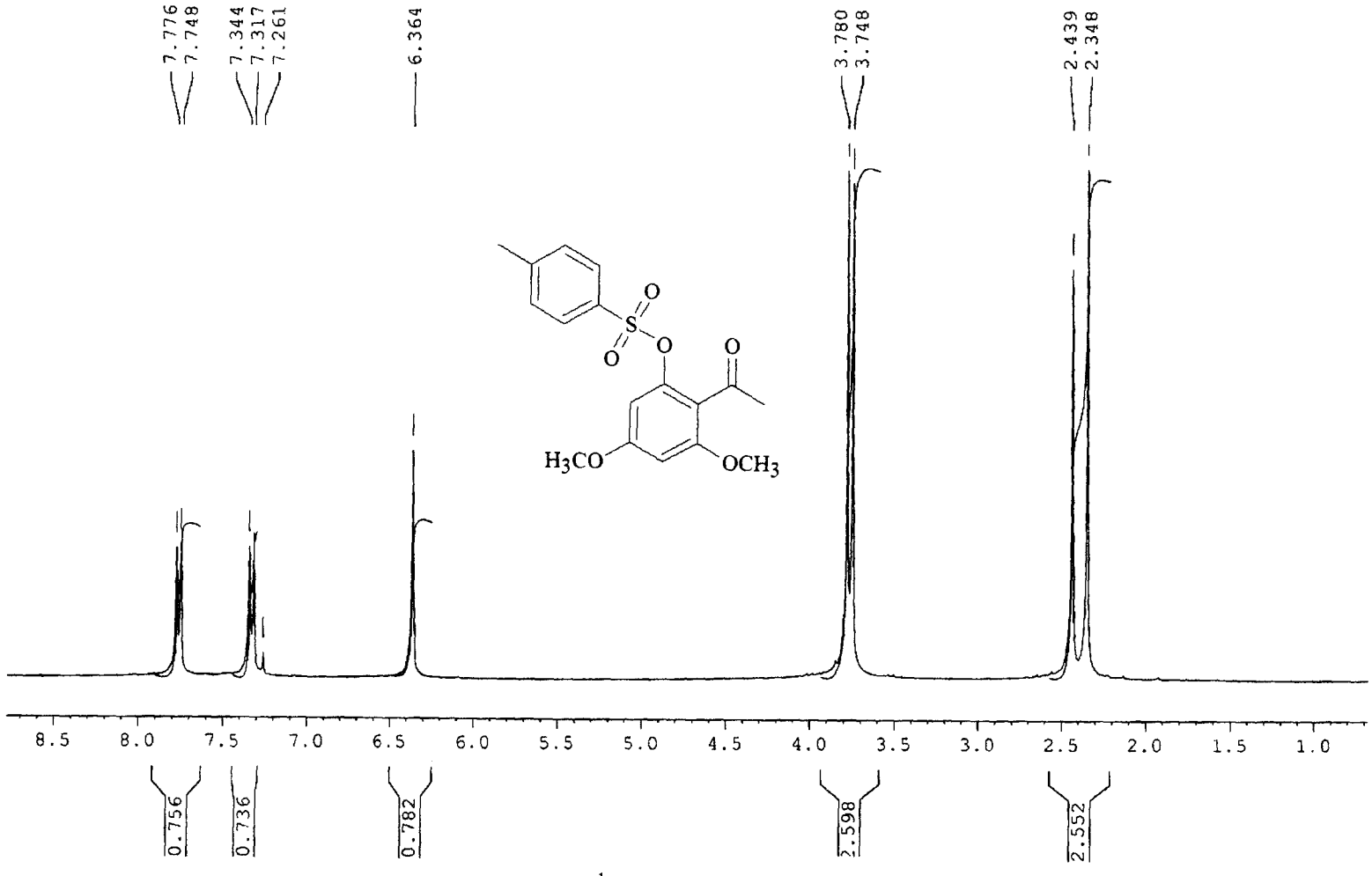


Fig. 2.20: ^{1}H NMR spectrum of **27**

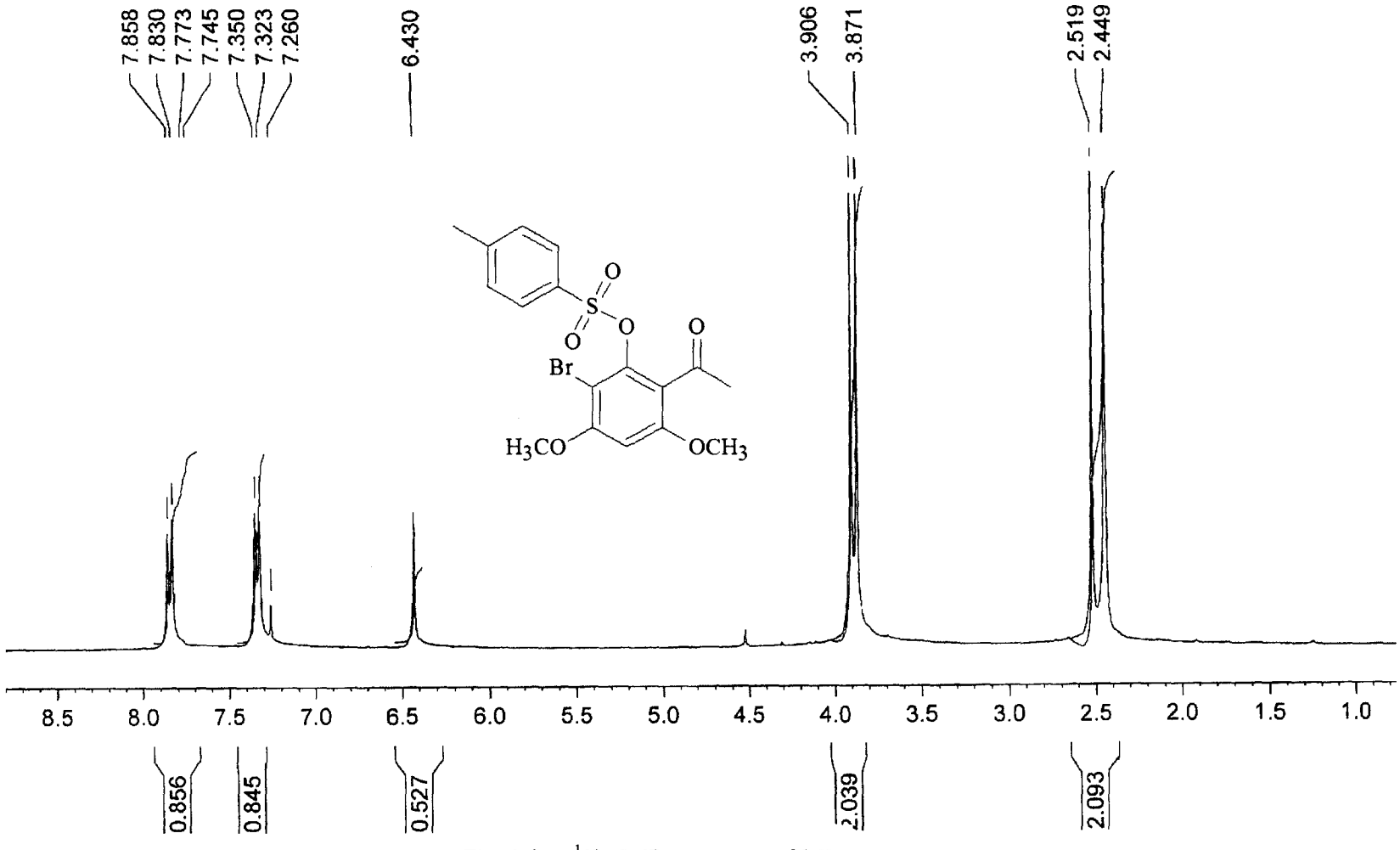


Fig. 2.21: ^{1}H NMR spectrum of **27b**

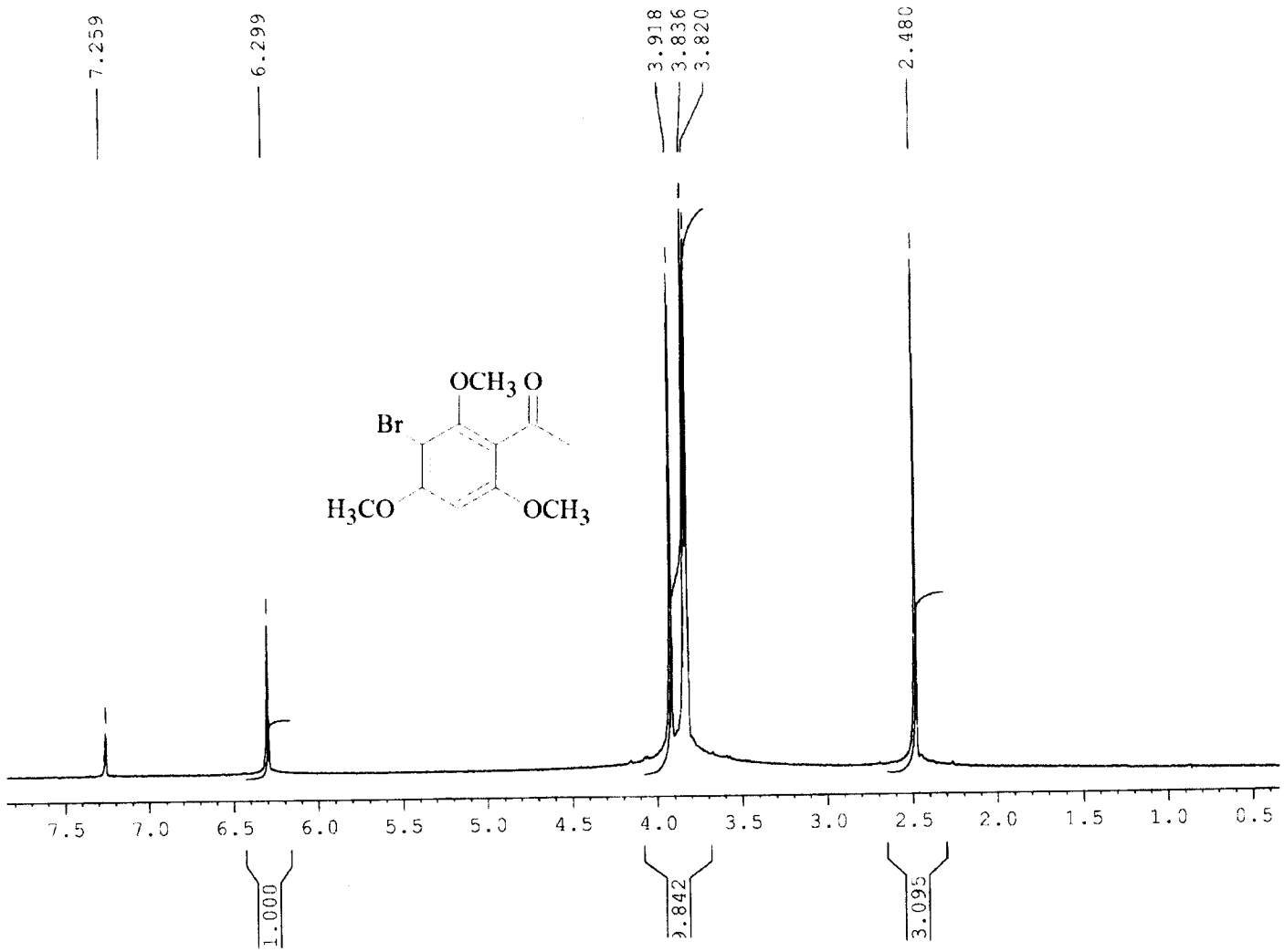


Fig. 2.22: ^{1}H NMR spectrum of **28**

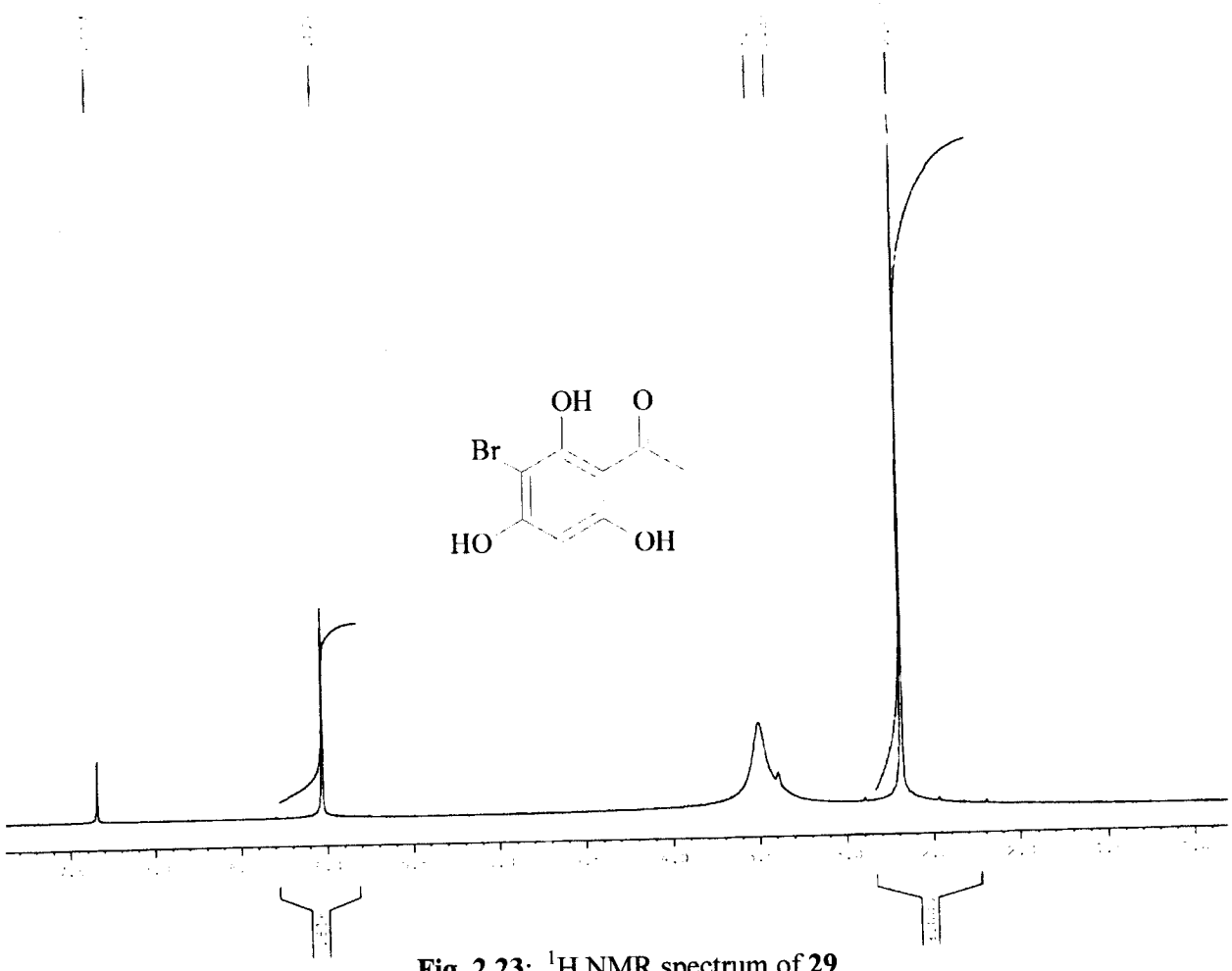


Fig. 2.23: ^{1}H NMR spectrum of **29**

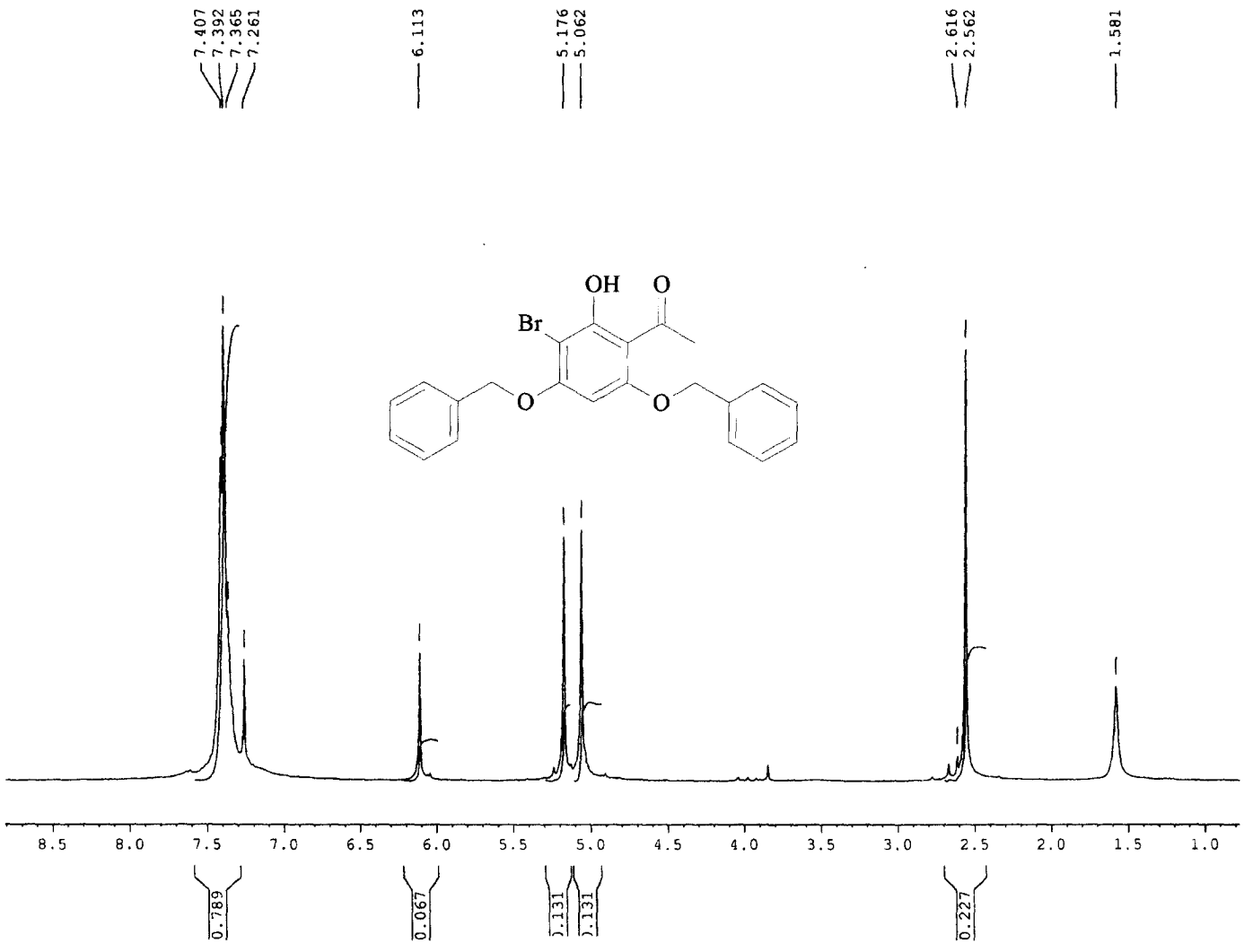


Fig. 2.24: ^{1}H NMR spectrum of **32**

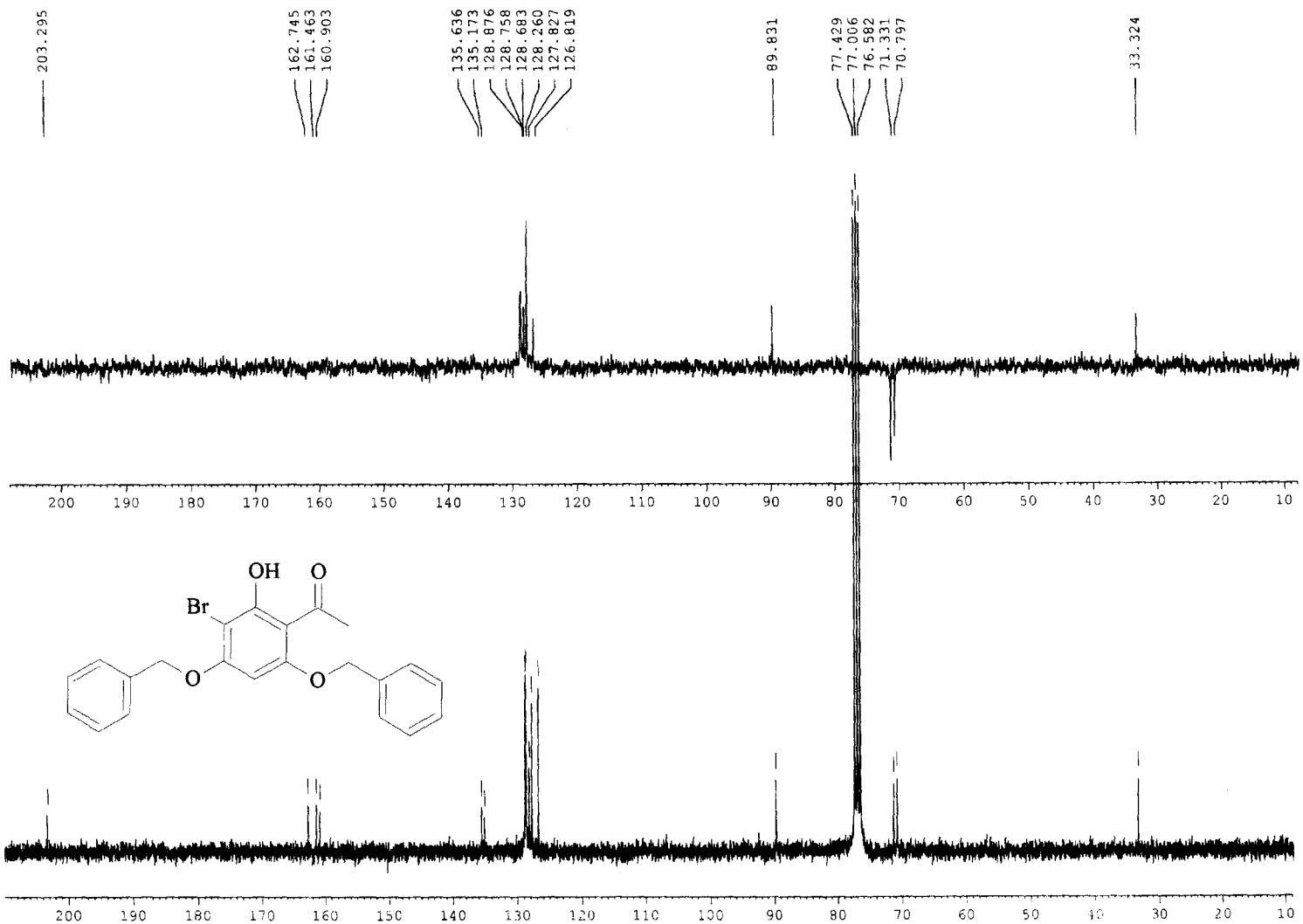


Fig. 2.25: ^{13}C NMR spectrum of **32**

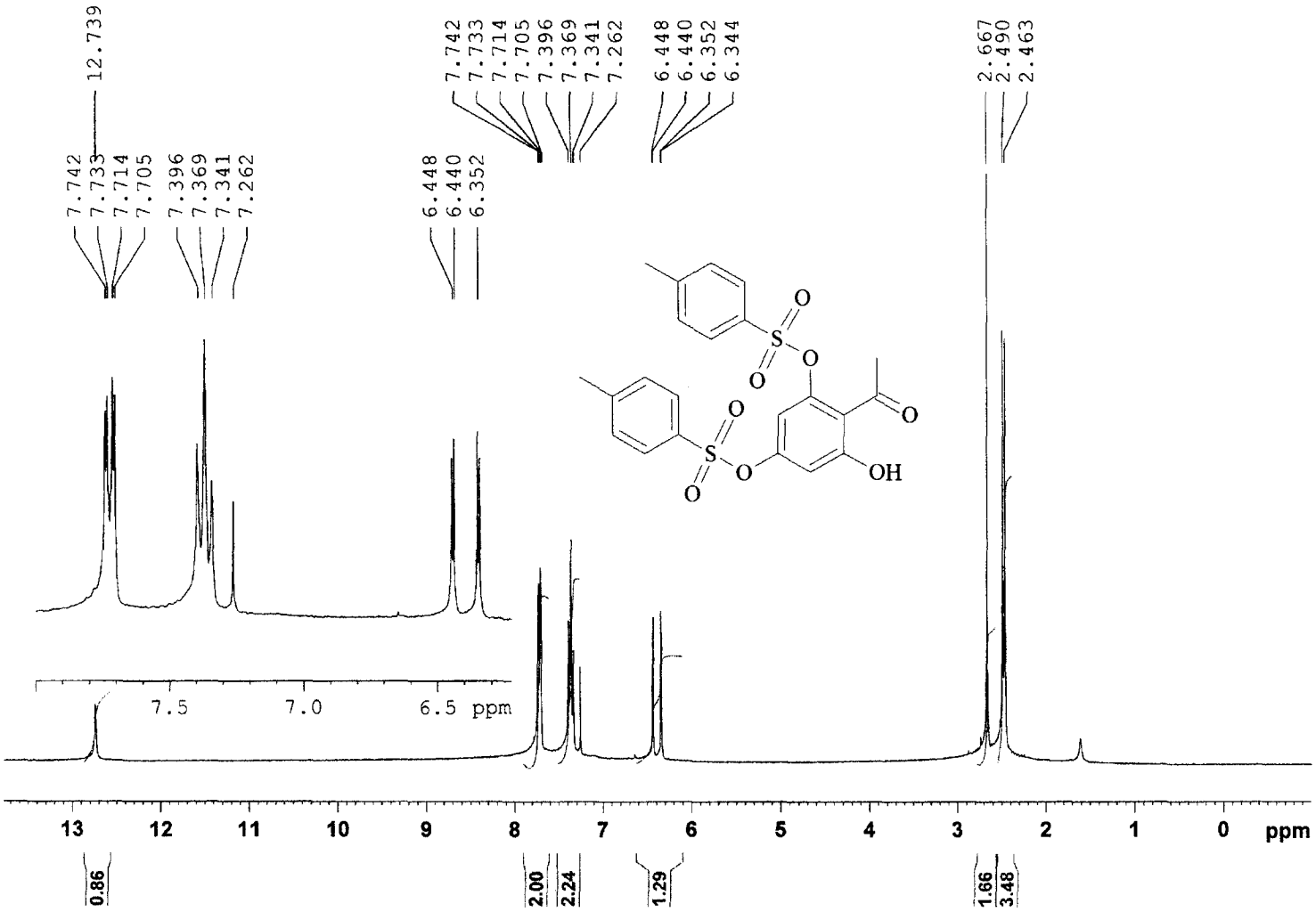


Fig. 2.26: ^{1}H NMR spectrum of **33**

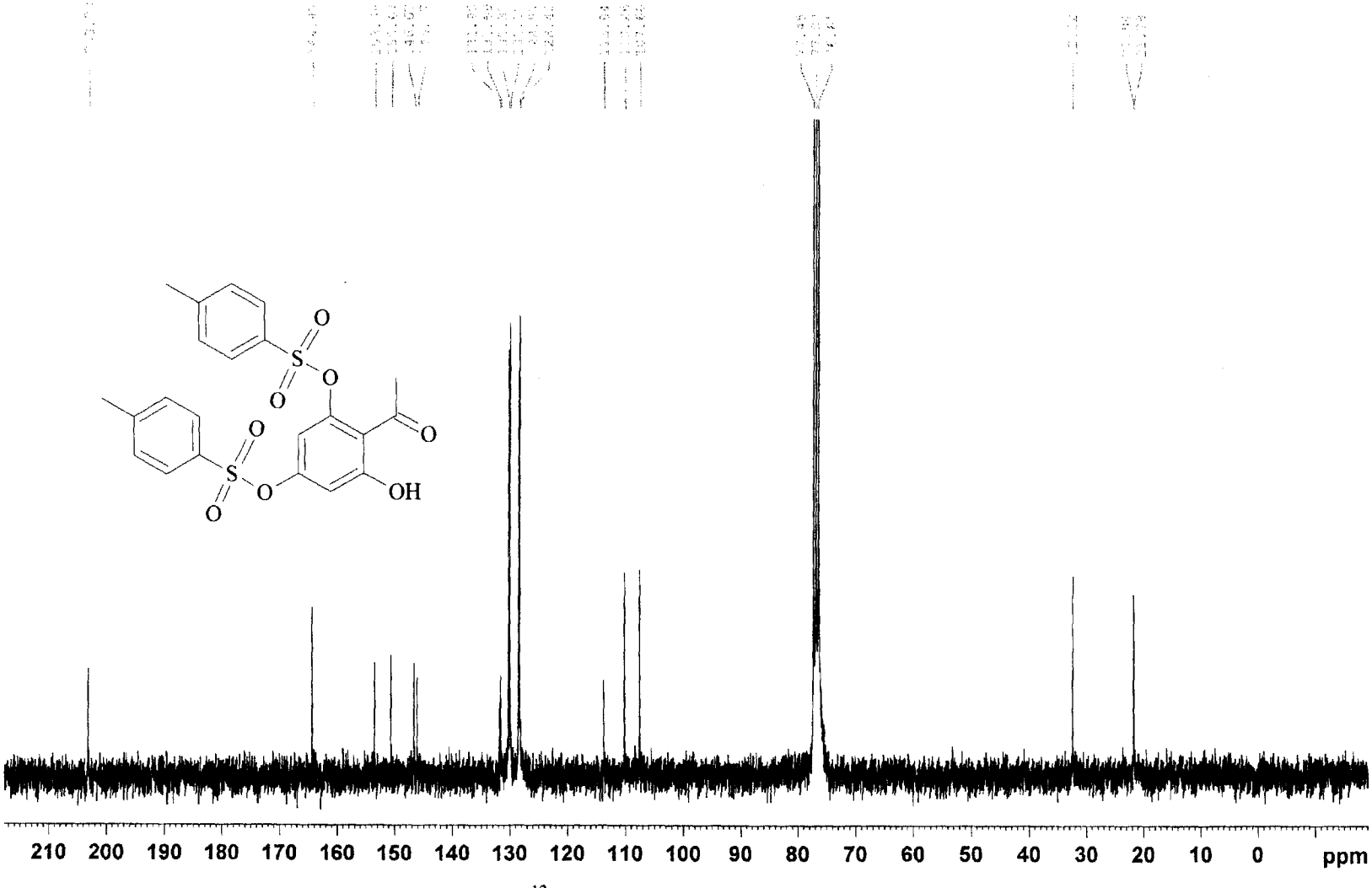


Fig. 2.27: ^{13}C NMR spectrum of **33**

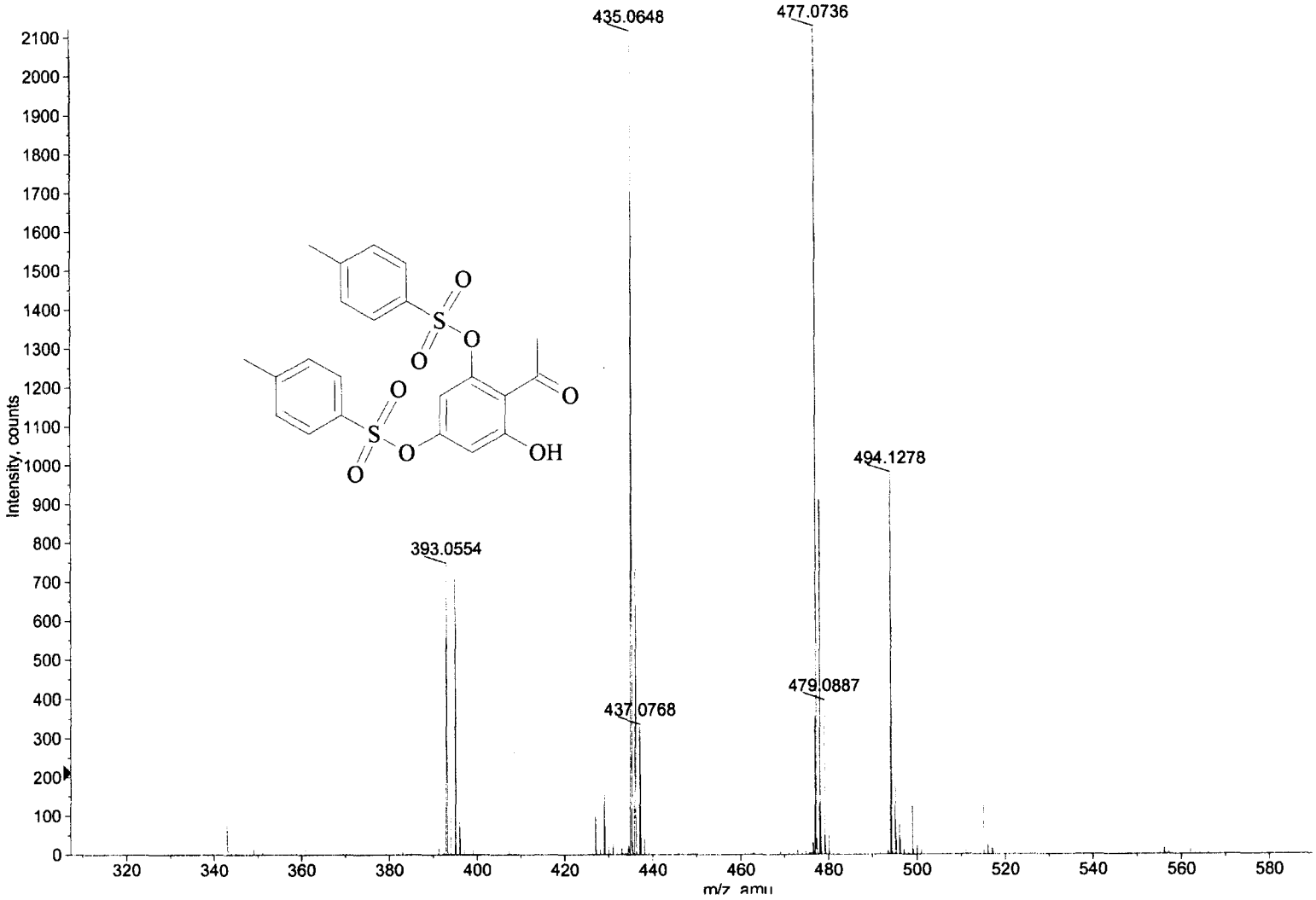


Fig. 2.28: ESIMS spectrum of **33**

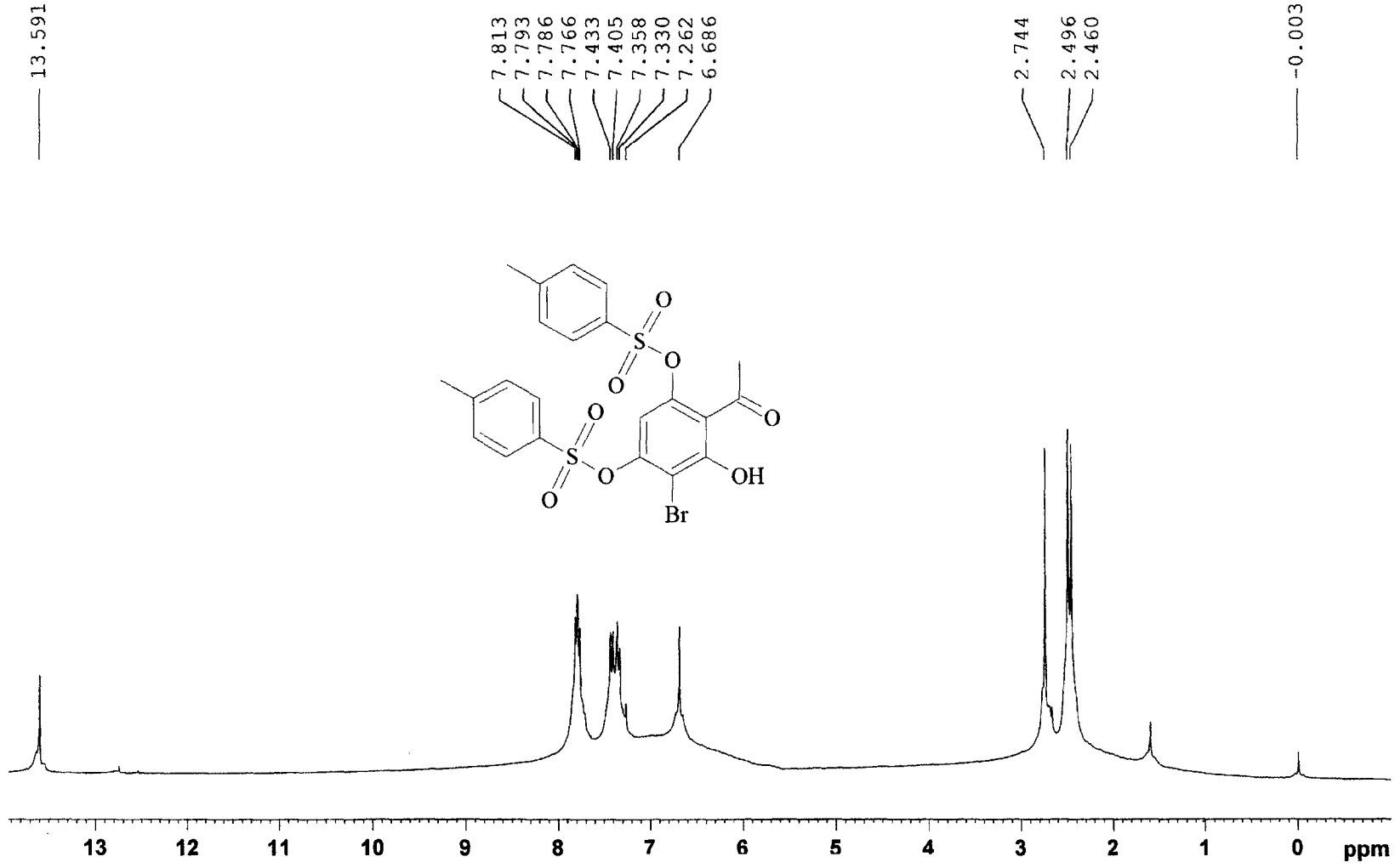


Fig. 2.29: ^{1}H NMR spectrum of **34**

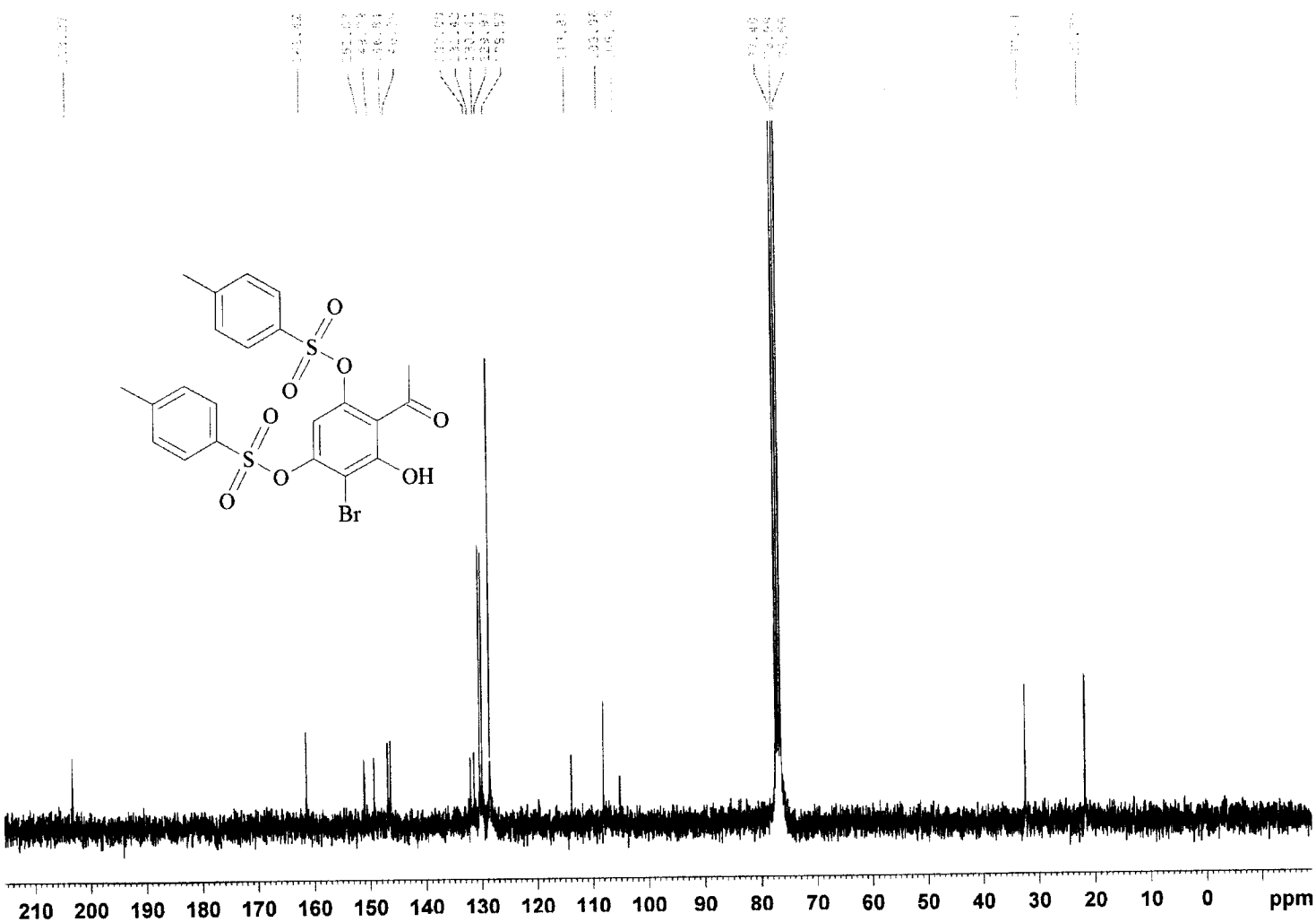


Fig. 2.30: ^{13}C NMR spectrum of **34**

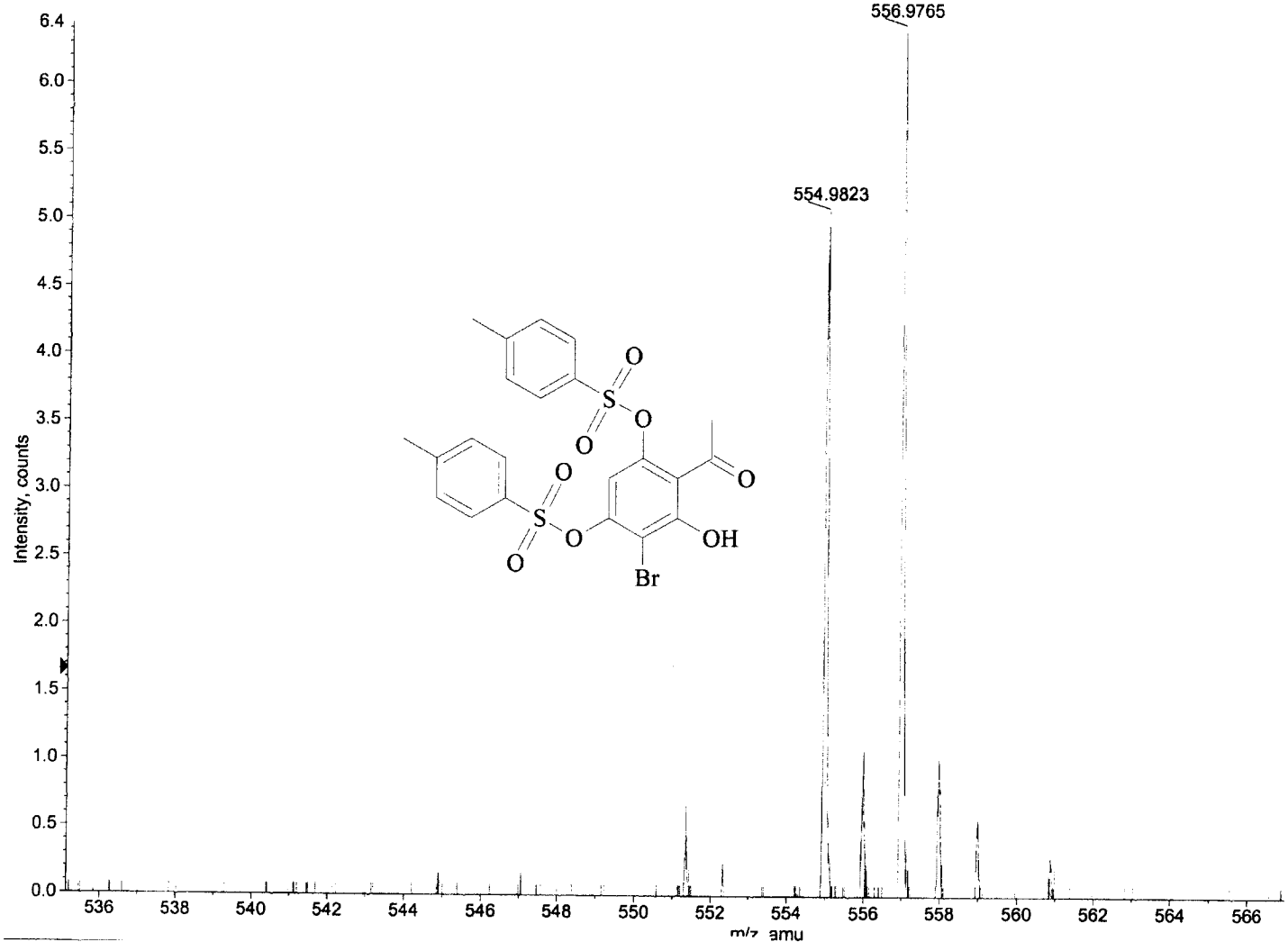


Fig. 2.31: ESIMS spectrum of **34**

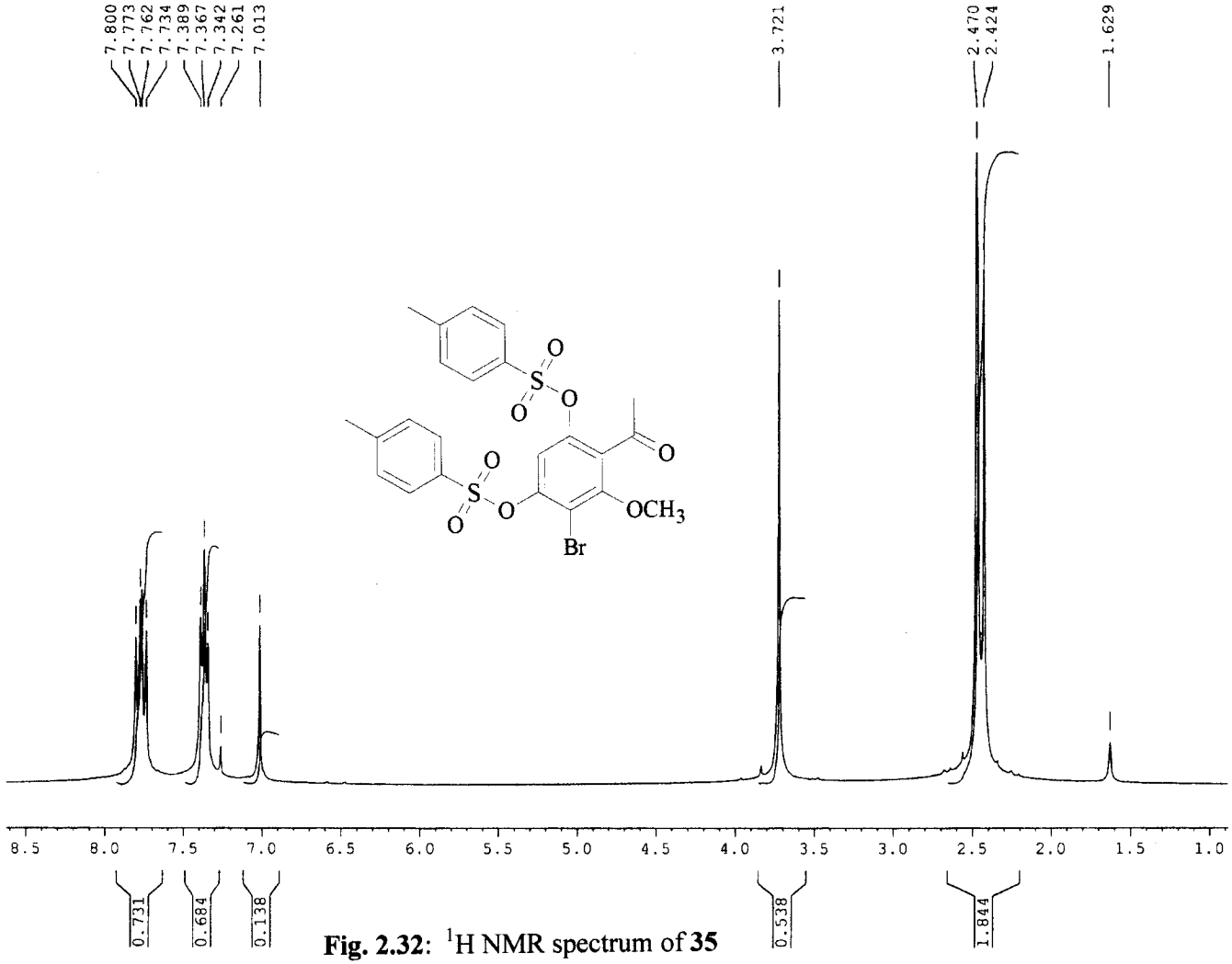


Fig. 2.32: ^{1}H NMR spectrum of **35**

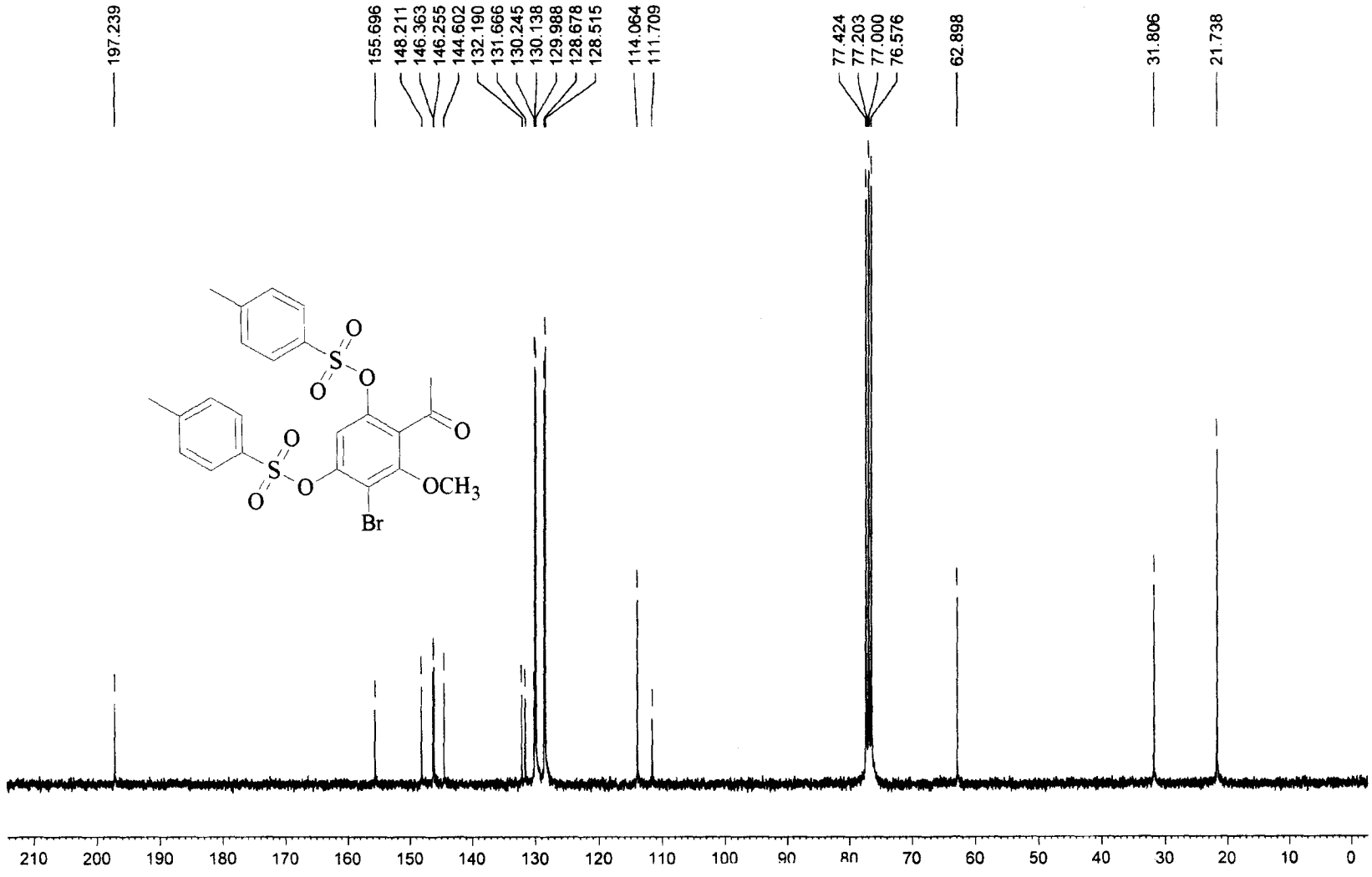


Fig. 2.33: ^{13}C NMR spectrum of **35**

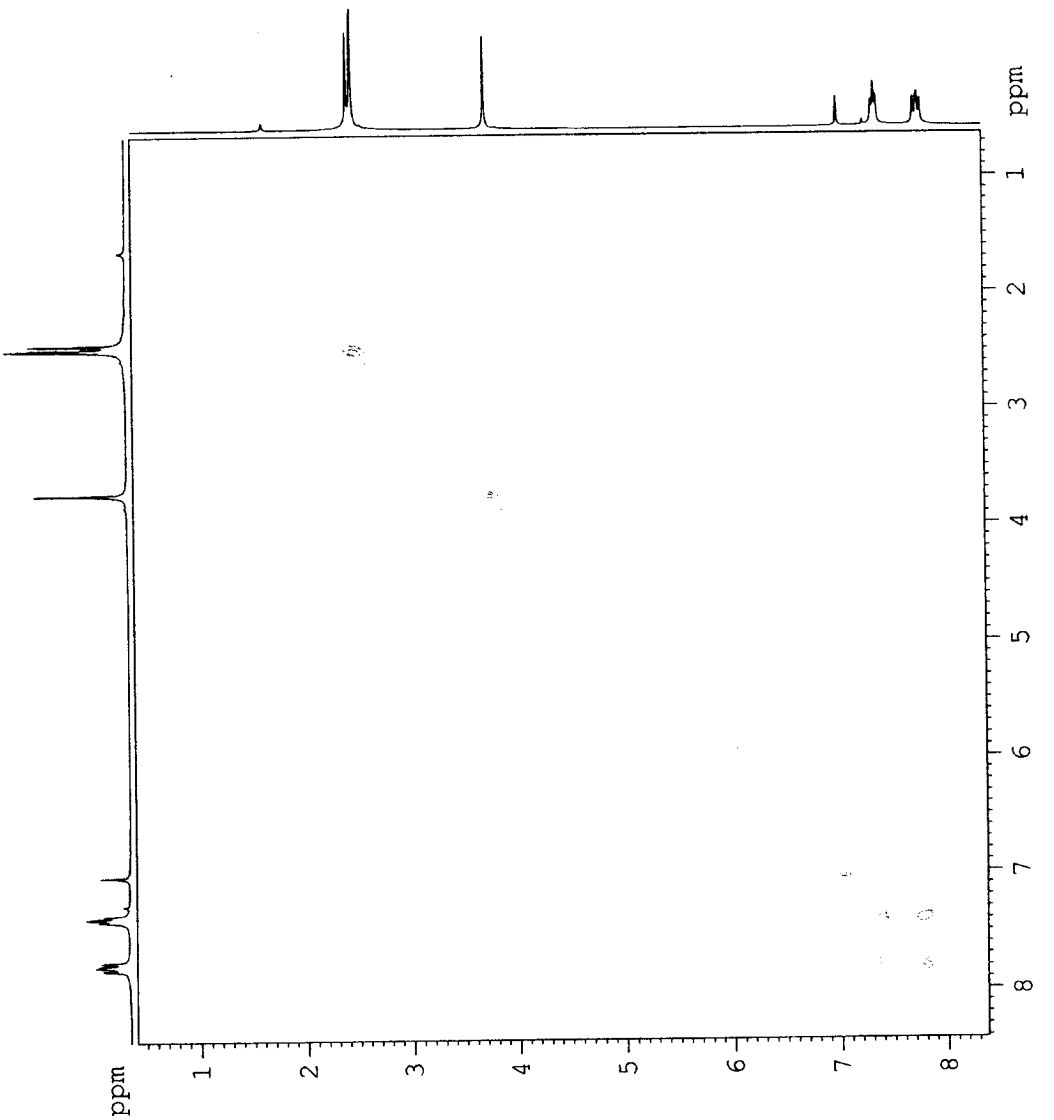


Fig. 2.34: COSY spectrum of **35**

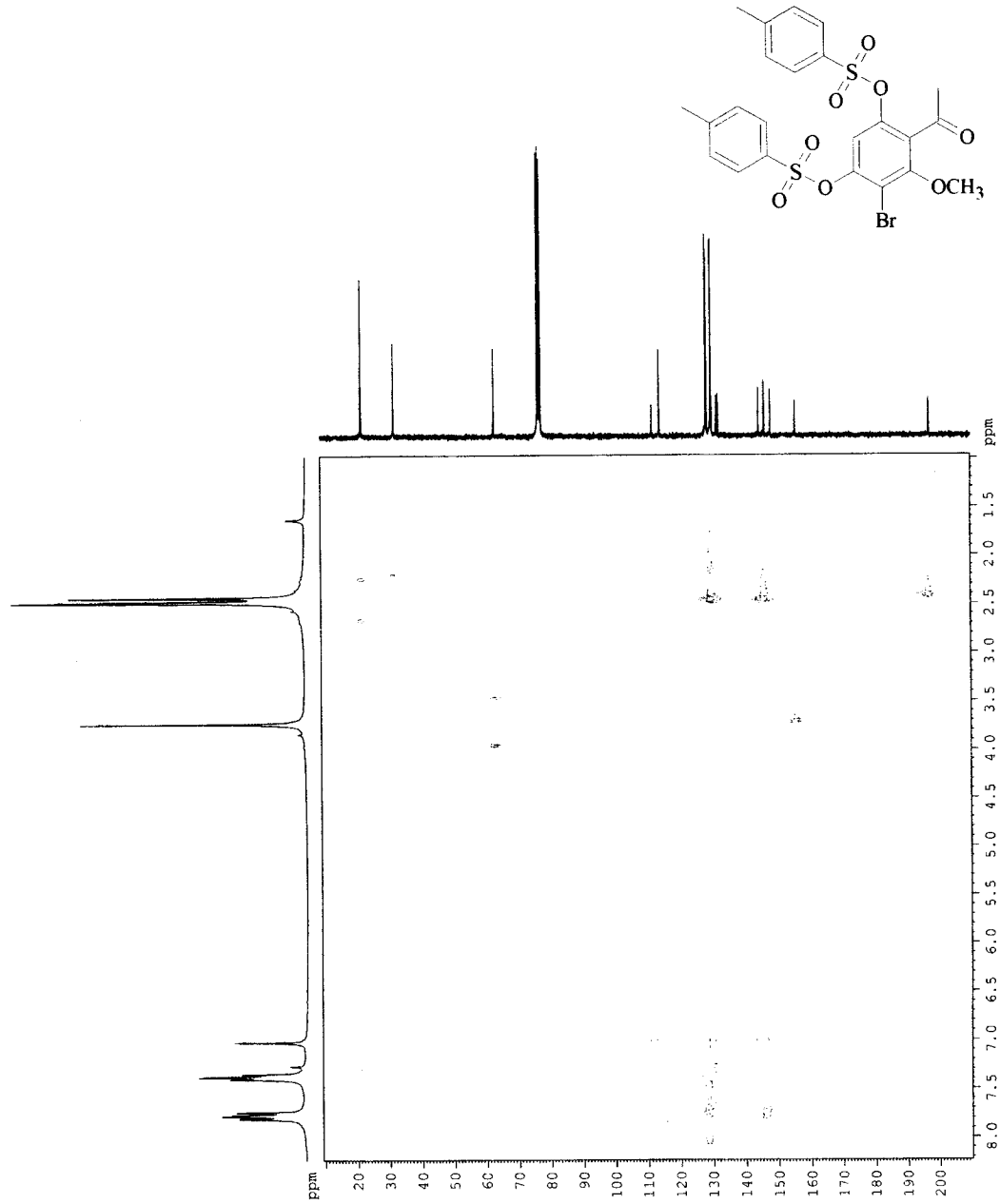


Fig. 2.35: ^{1}H-^{13}C HMBC spectrum of **35**

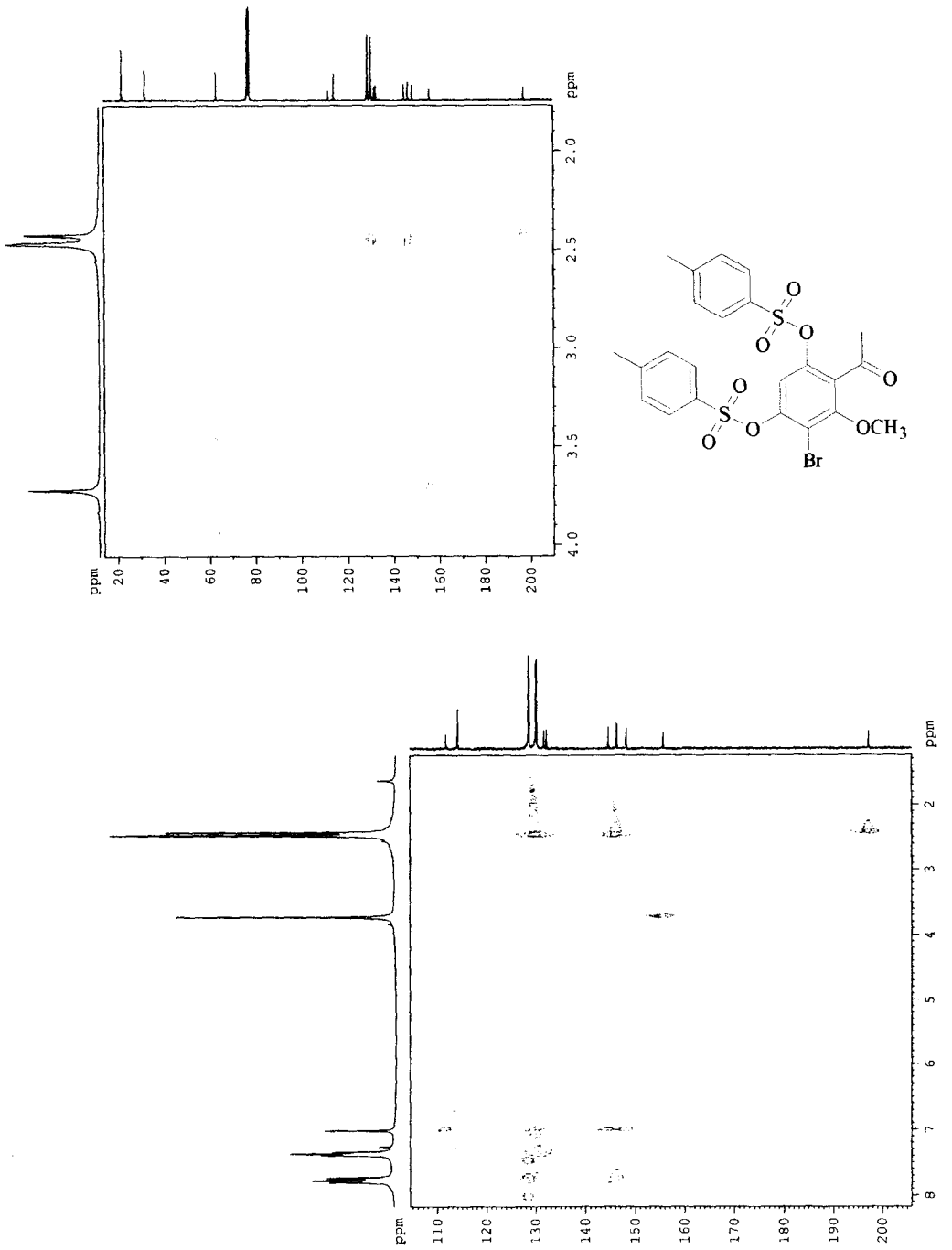


Fig. 2.36: ^{1}H-^{13}C HMBC spectrum (expansion) of **35**

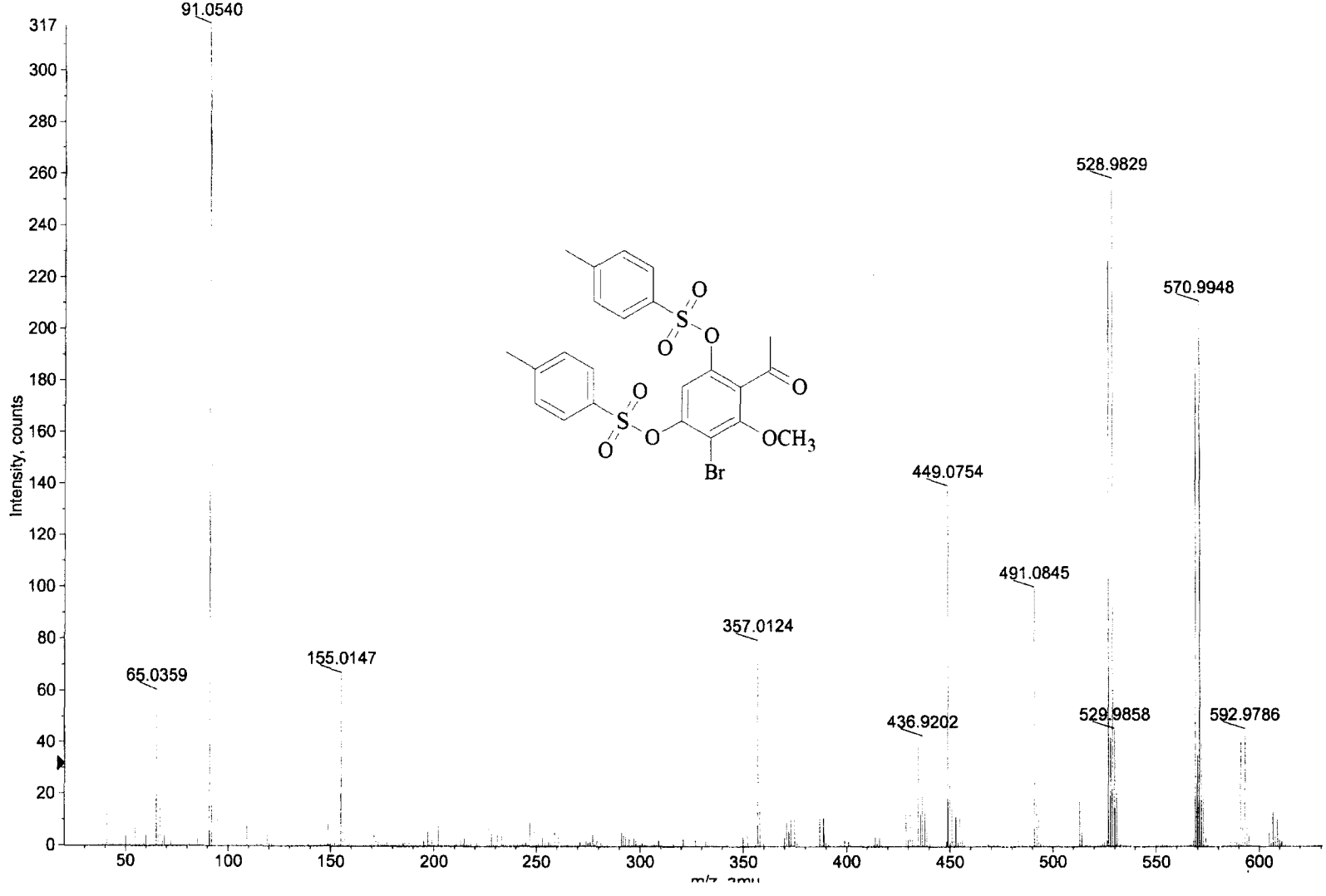


Fig. 2.37: ESIMS spectrum of **35**

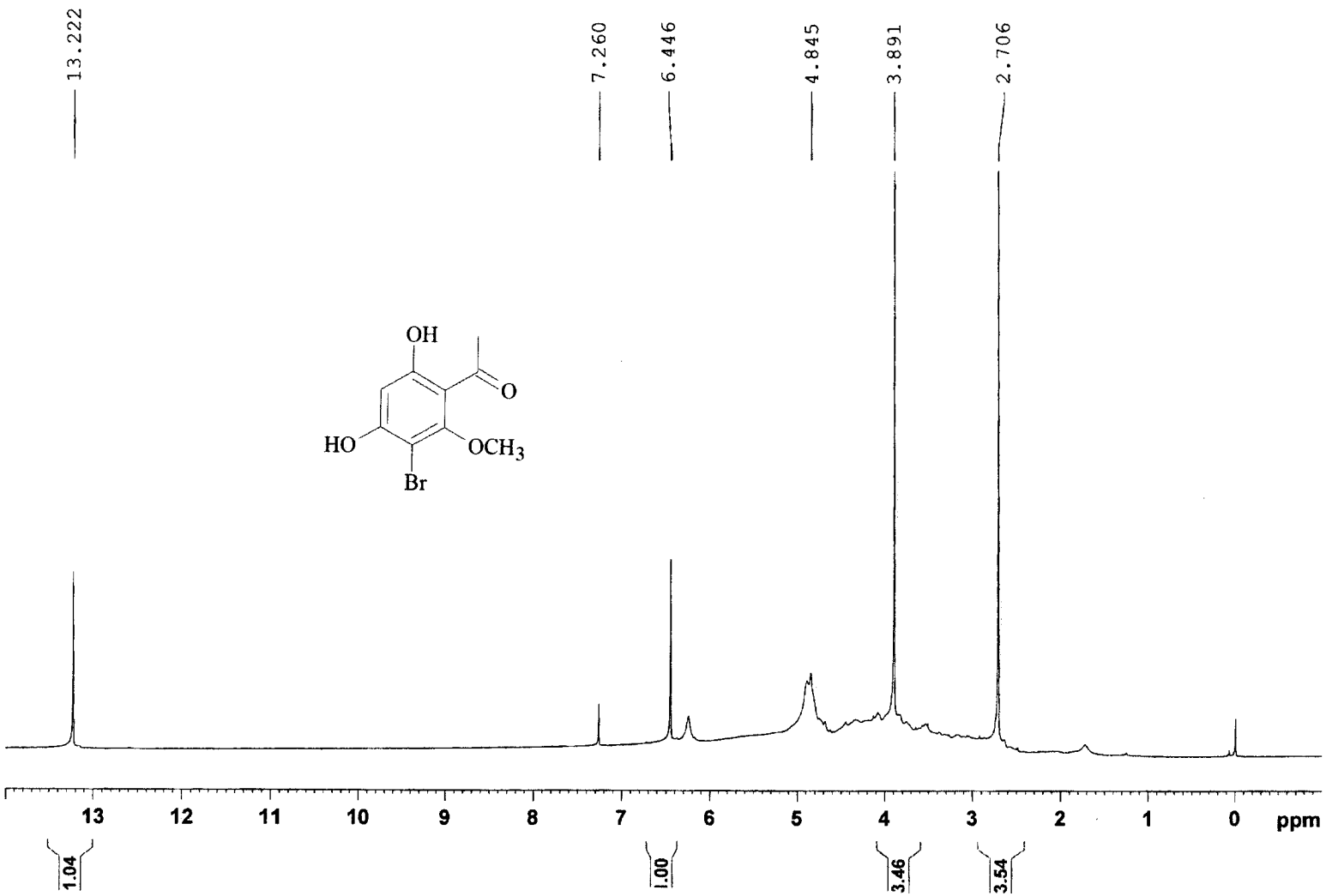


Fig. 2.38: ^{1}H NMR spectrum of **36**

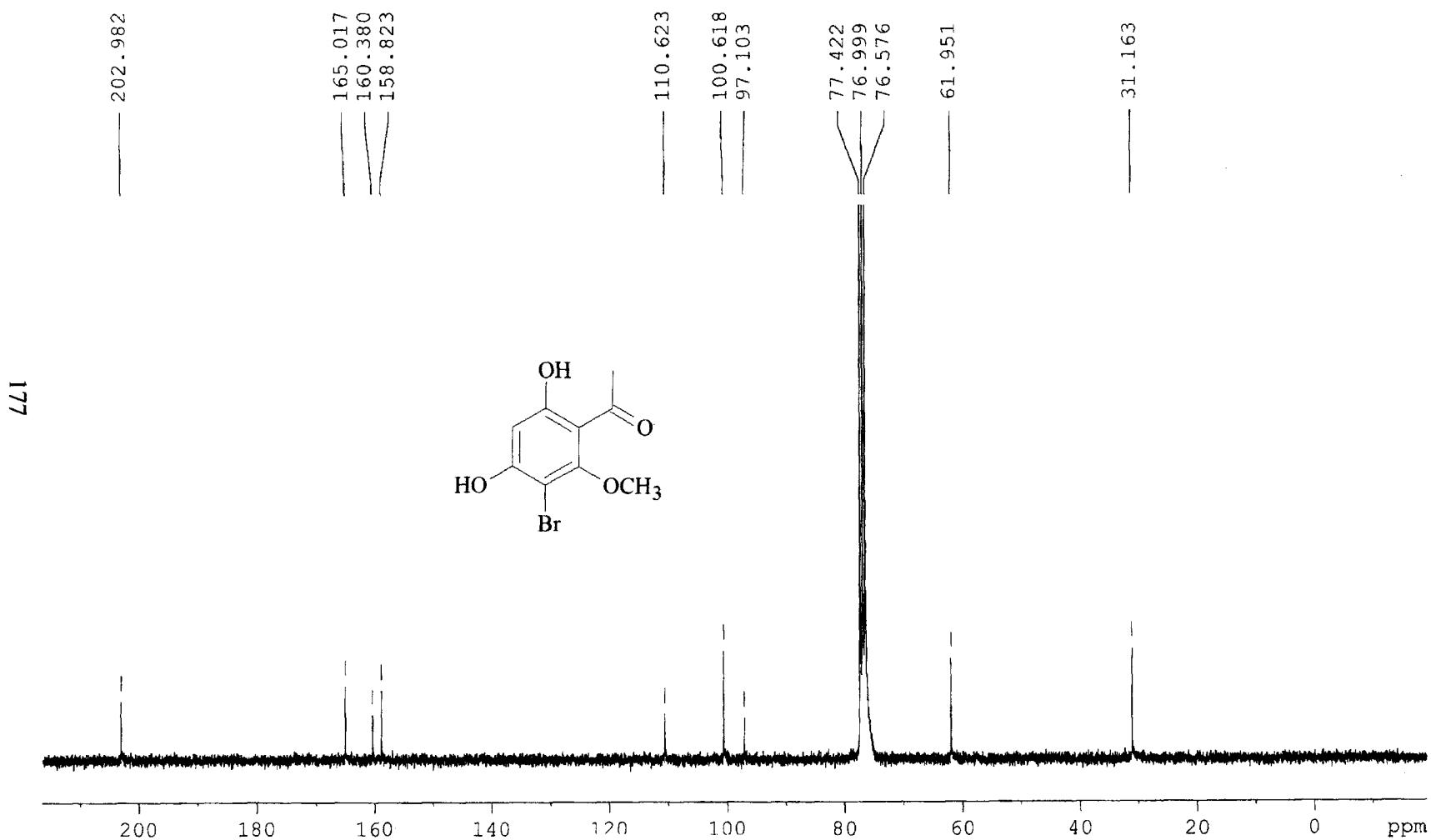


Fig. 2.39: ^{13}C NMR spectrum of **36**

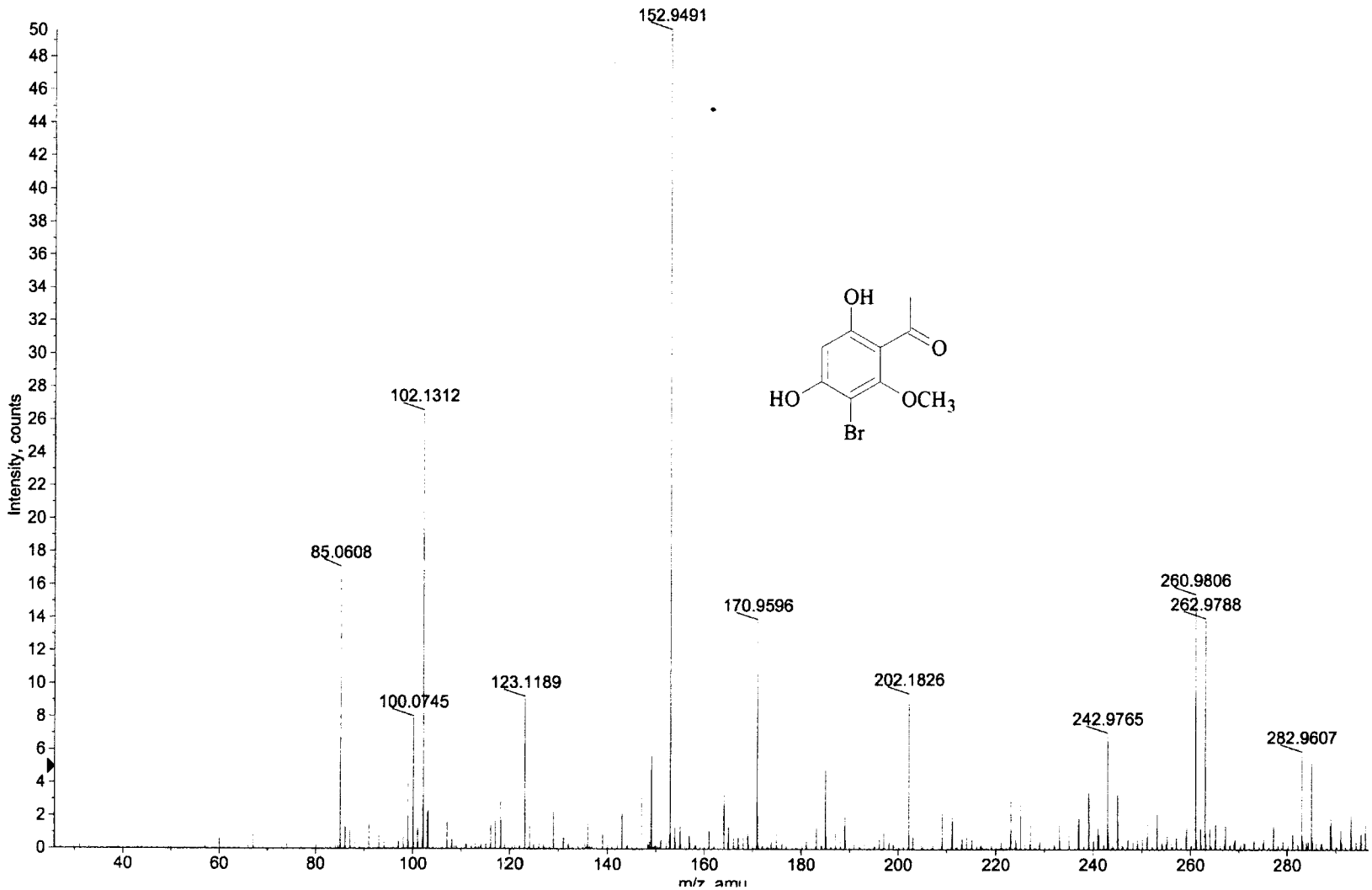


Fig. 2.40: ESIMS spectrum of **36**

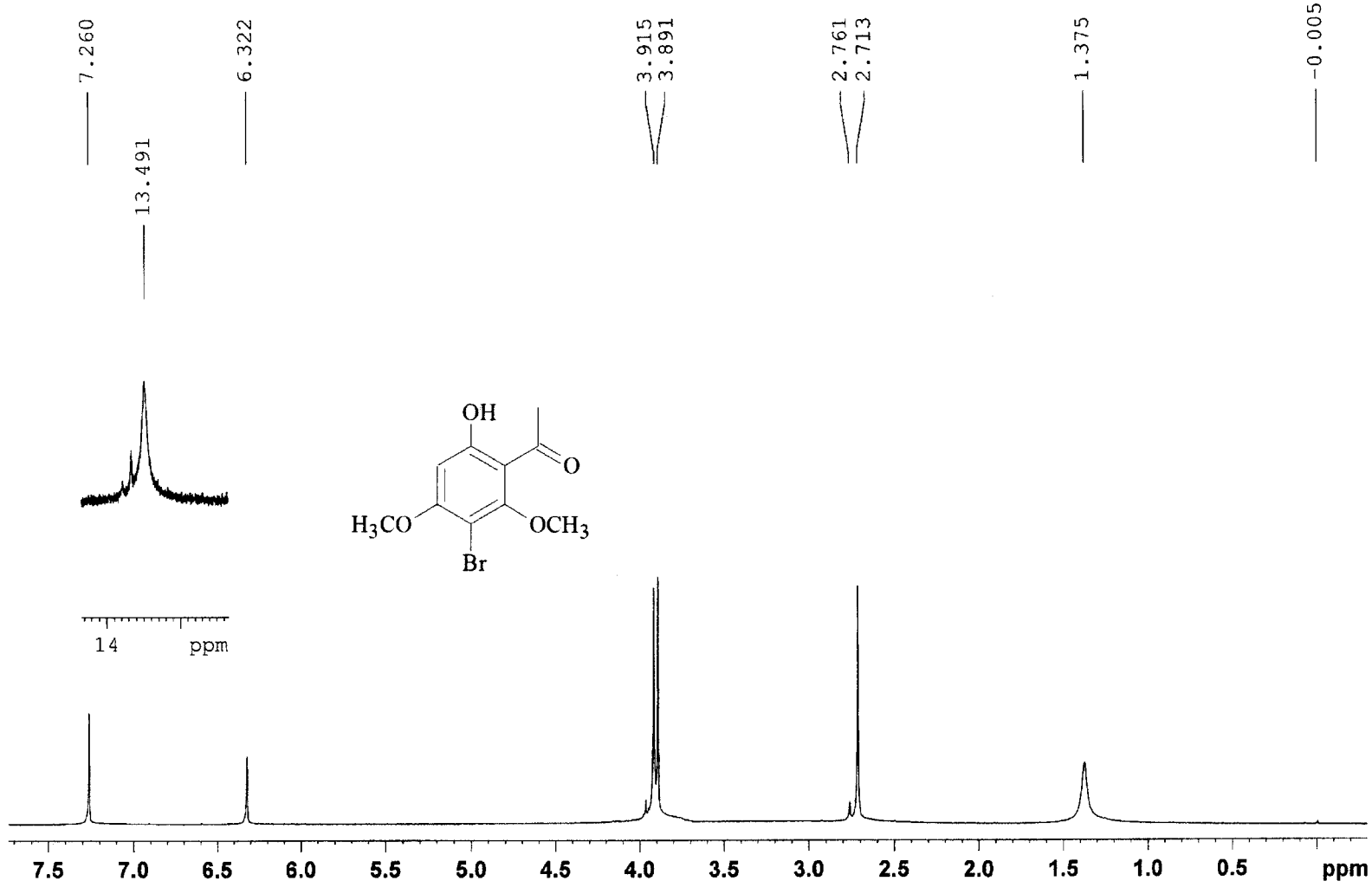


Fig. 2.41: ^{1}H NMR spectrum of **11**

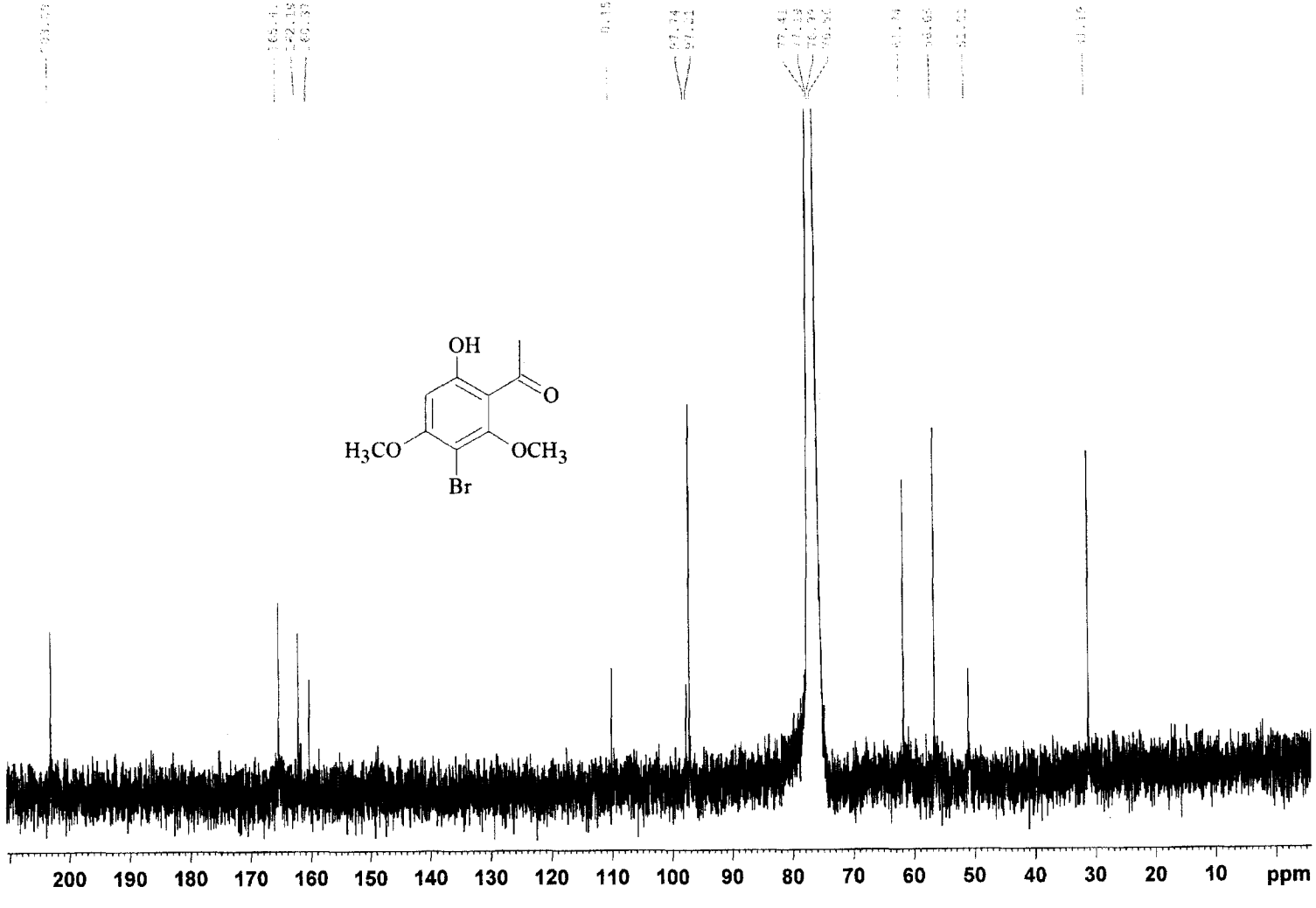


Fig. 2.42: ^{13}C NMR spectrum of **11**

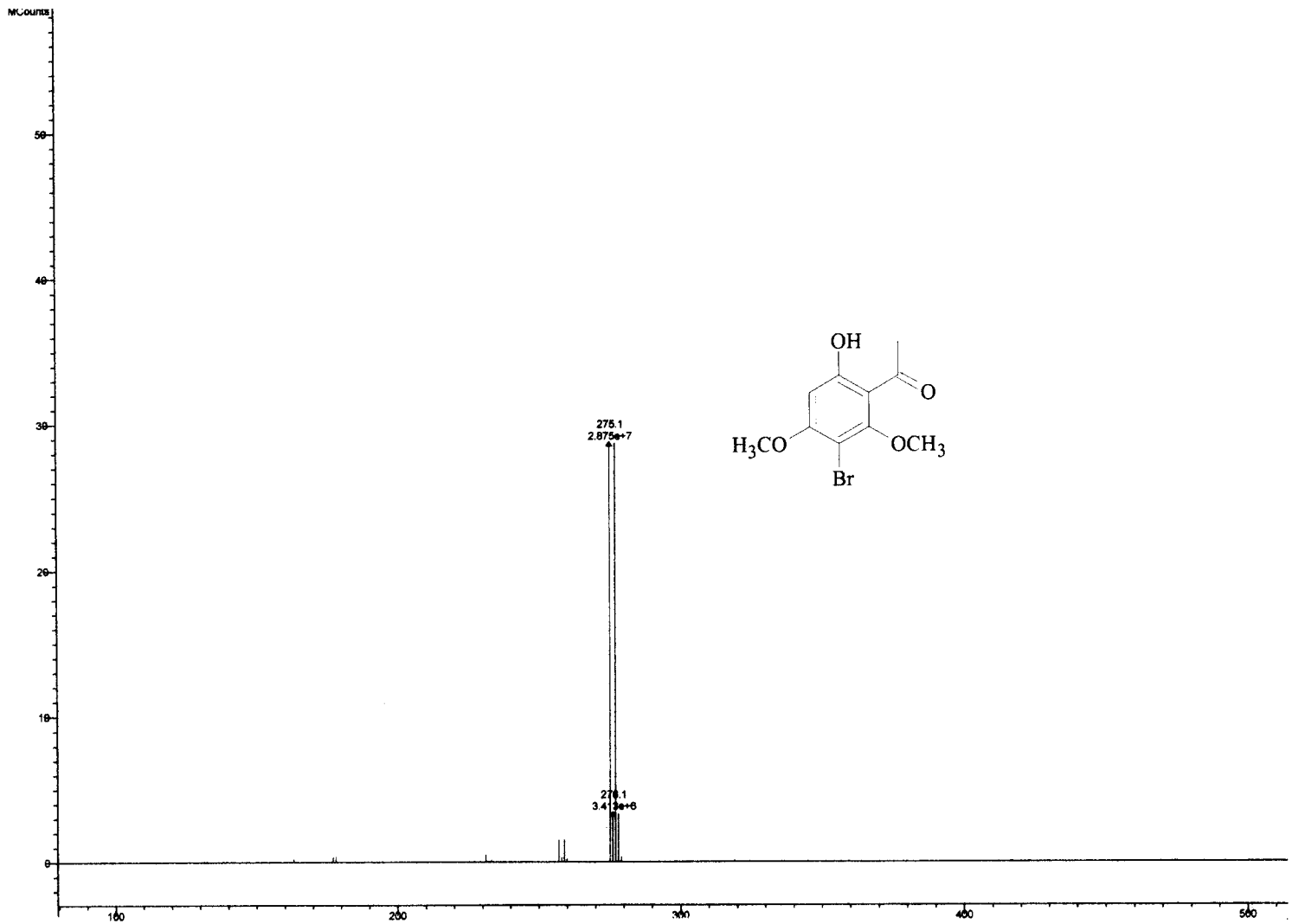


Fig. 2.43: ESIMS spectrum of **11**

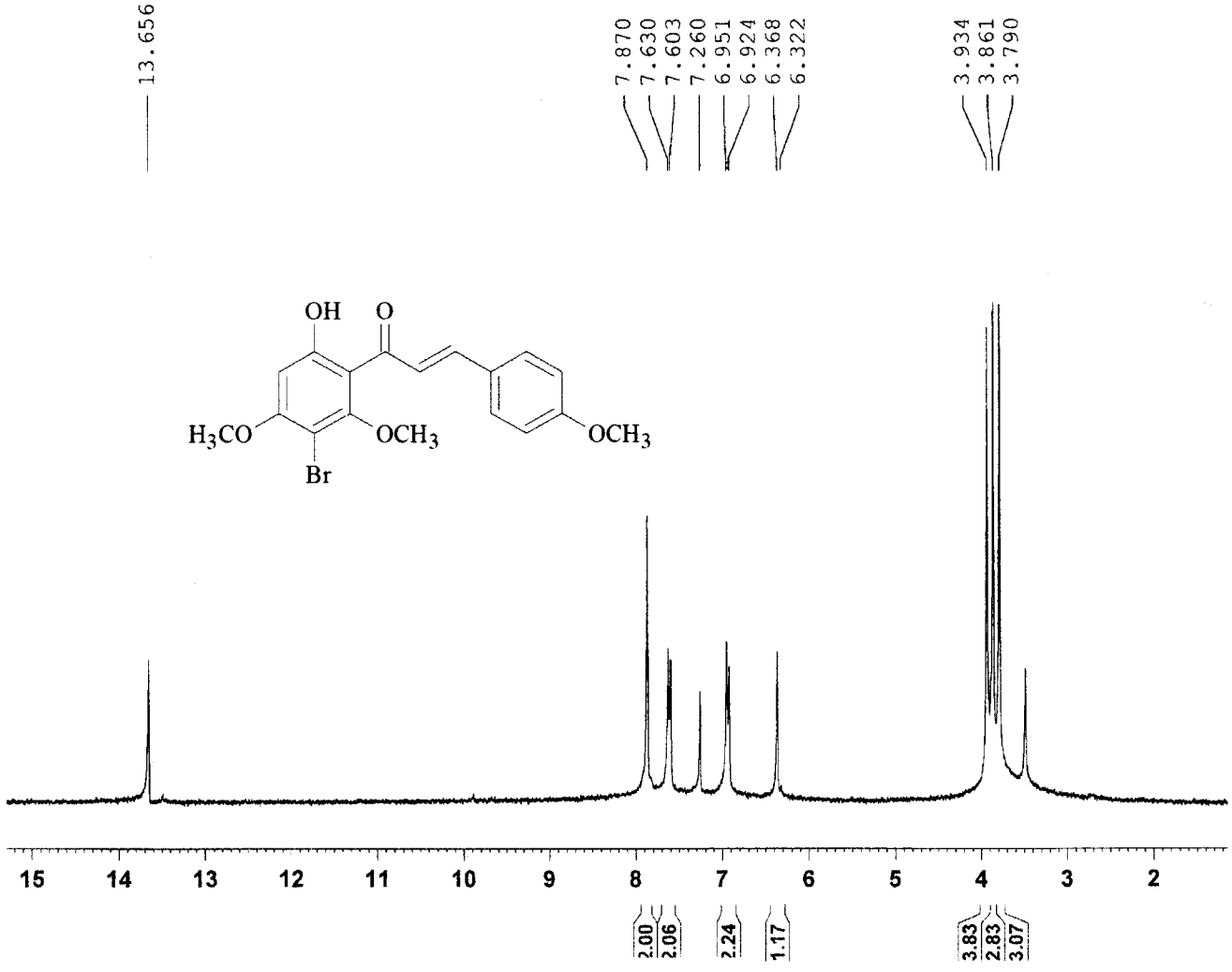


Fig. 2.44: ^{1}H NMR spectrum of **8**

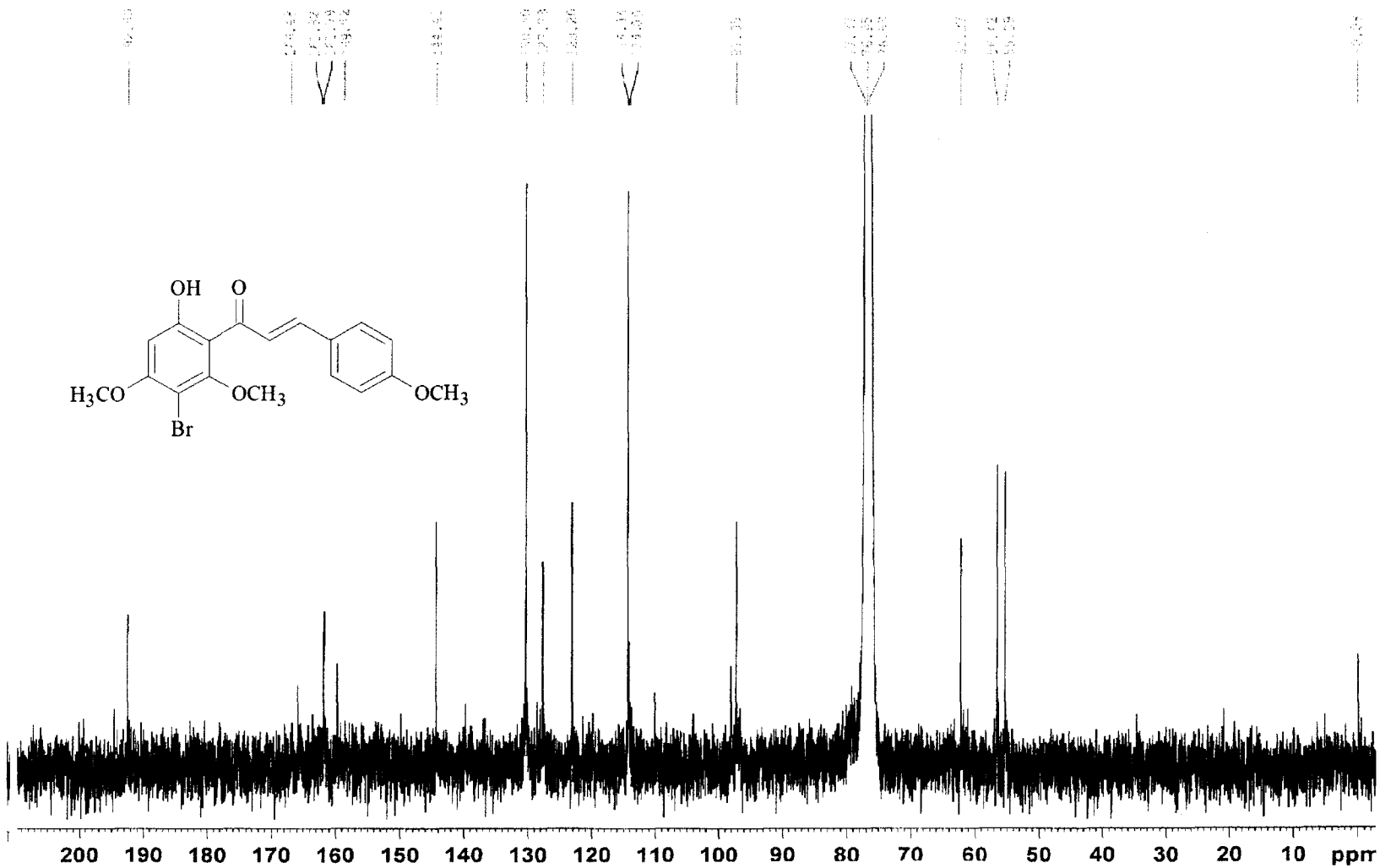


Fig. 2.45: ^{13}C NMR spectrum of **8**

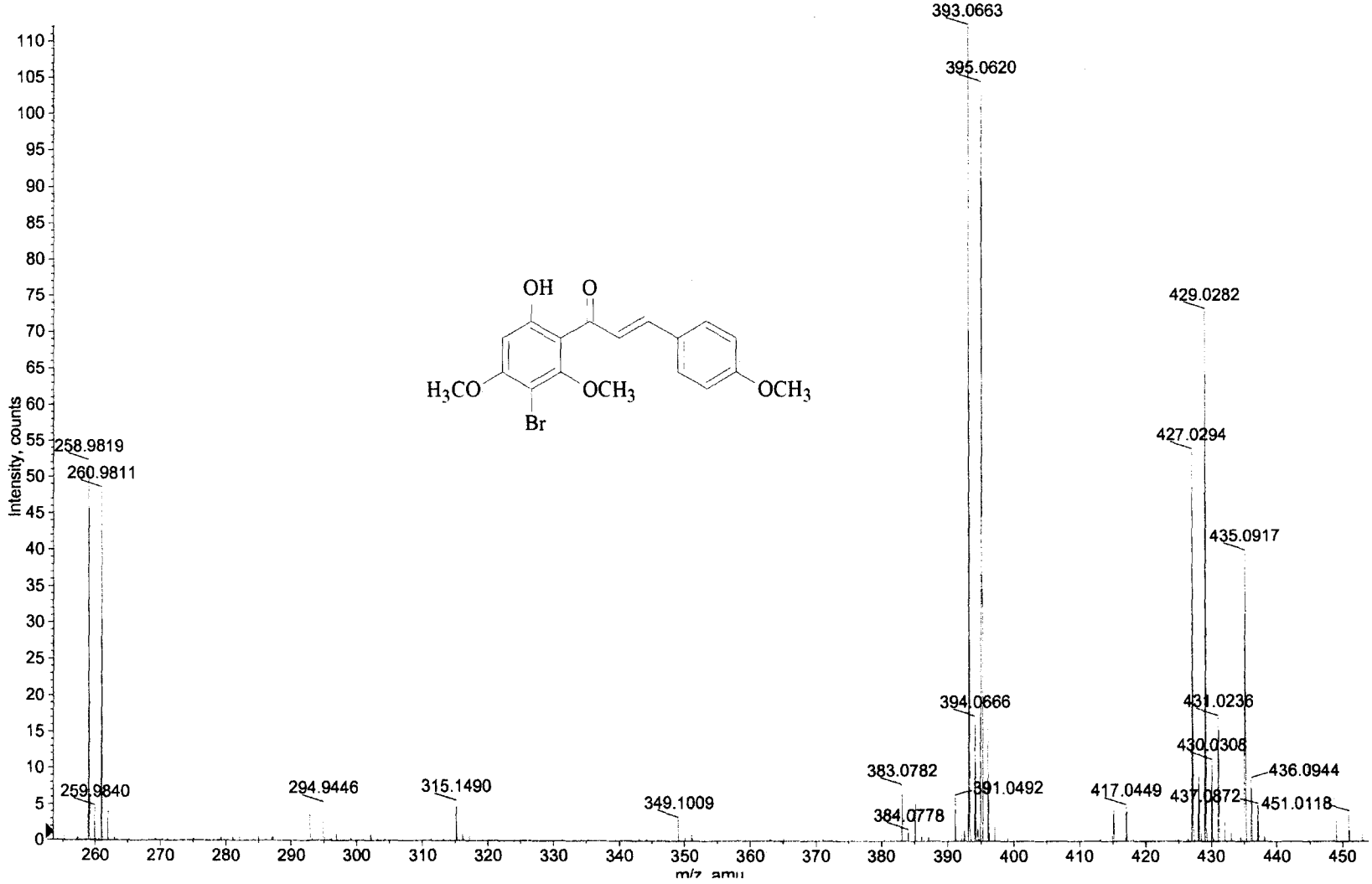


Fig. 2.46: ESIMS spectrum of **8**

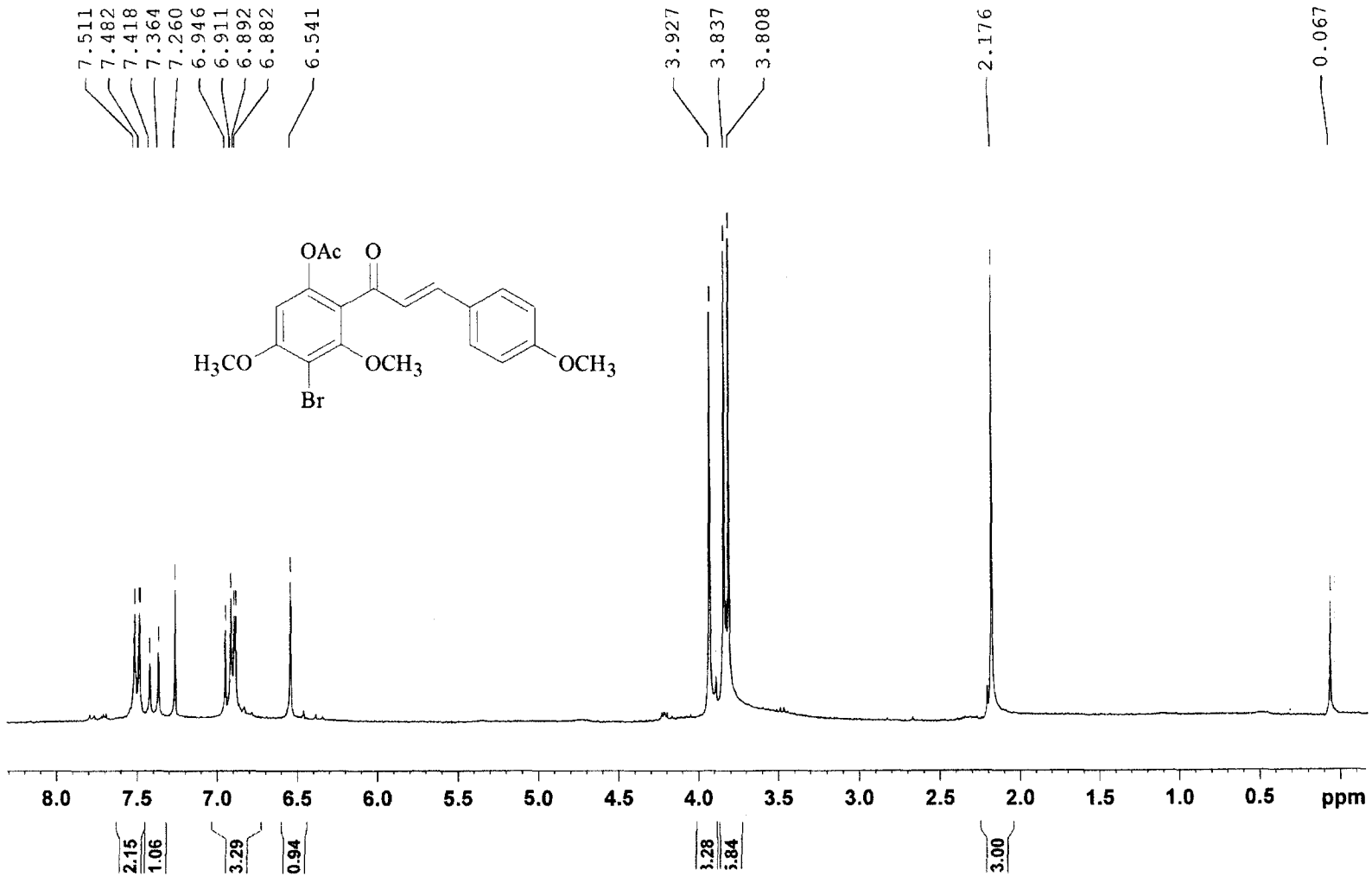
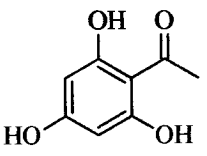


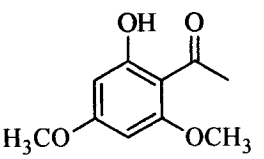
Fig. 2.47: ^{1}H NMR spectrum of **9**

Experimental

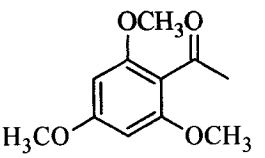
Phloroacetophenone **12**, *2,4-Dimethoxy-6-hydroxyacetophenone* **13**[9] *and 2,4,6-trimethoxyacetophenone* **14**



12



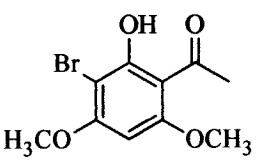
13



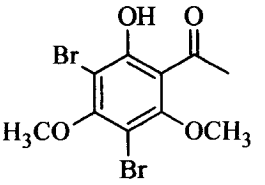
14

Prepared according to the reported literature procedure[25,26].

5-Bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** *and 3,5-Dibromo-2,4-dimethoxy-6-hydroxyacetophenone* **18**



15



18

a) *Using $KBrO_3$ and HBr*[27]

To a stirred solution of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.290 g, 1.48 mmole) and $KBrO_3$ (0.085 g, 0.50 mmole) in glacial acetic acid (2 mL) was added HBr (48%, 0.3 mL, 2.6 mmole) drop by drop and the mixture was stirred at room temperature for 30 min, diluted with cold water (5 mL) and stirred for further 15 min. Dull yellow solid was collected by filtration, washed with dilute sodium bisulfite, water and dried at 100°C to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.313 g, 83%). Recrystallization from benzene gave pale brown needles, m.p. 186-188°C while recrystallization from $CHCl_3$-petroleum ether mixture afforded pale yellow needles, m.p. 200°C.

IR ν_{max} (KBr): 1632, 1581,1419,1284,1136,786 cm^{-1}.

^{1}H NMR ($CDCl_3$, 400 MHz, **Fig.** 2.01): For assignments refer **Figure XV**, pg 136.

^{13}C NMR ($CDCl_3$, 100 MHz, **Fig.** 2.02): For assignments refer **Figure XVI**, pg 136.

b) *Using Br_2 in acetic acid*[28] and *Br_2 in water*[29]

Br_2 (0.1632 g, 0.06 mL, 1.02 mmole) in glacial acetic acid (0.5 mL) was added drop by drop to a stirred suspension of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.2 g, 1.02 mmole) in glacial acetic acid (5.5 mL). The reaction mixture was stirred for 30 min at room temperature, diluted with water (5 mL) & stirred for a further 15 min. The precipitated solid was filtered, washed with dilute sodium bisulfite, water and dried at 100°C to give 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15** (0.2281 g, 60.48%). Recrystallization from $CHCl_3$-petroleum ether mixture afforded pale yellow needles, m.p. 200°C.

To a stirred solution of KBr (0.397 g, 3.31 mmole) and Br_2 (0.171 g, 0.055 mL, 1.07 mmole) in water (4.0 mL), 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.2 g, 1.02 mmole) was added and the reaction mixture was stirred at room temperature for 1 hr. The pale yellow solid was filtered, washed with dilute sodium bisulfite, water and dried to give 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15** (0.226 g, 60%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 200°C.

c) *Using KBr and Oxone*[30]

To a stirred solution of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.07 g, 0.4 mmole) in acetonitrile:water (2:1, v/v, 1.5 mL), KBr (0.05 g, 0.42 mmole) was added followed by dropwise addition of Oxone (0.26 g, 0.4 mmole) in H_2O (1 mL) and the reaction mixture was stirred for 30 min at room temperature. Solid precipitated was collected by filtration, washed with water and dried to give

5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.263 g, 95.63%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 198°C.

d) *Using KBr, ammonium molybdate and H_2O_2*[31]

To a stirred suspension of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.196 g, 1 mmole), KBr (0.131 g, 1.1 mmole), ammonium molybdate (0.0155 g, 0.0125 mmole) in glacial AcOH (3 mL), H_2O_2 (30%, 0.2 mL) was added and the reaction mixture was stirred for 30 min at room temperature. The yellow coloured reaction mixture was neutralized with saturated $NaHCO_3$ and the precipitated solid was filtered, washed with water and dried to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.21 g, 76%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow crystals, m.p. 198-200°C.

e) *Using KBr and H_2O_2*[32]

To a stirred solution of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.196 g, 1 mmole) and KBr (0.131 g, 1.1 mmole) in glacial AcOH (2 mL) was added 30% H_2O_2 (0.2 mL) and the reaction mixture stirred at room temperature for 2 hrs, neutralised with saturated $NaHCO_3$ and extracted with ether. The organic extracts were dried over Na_2SO_4 and evaporated to leave 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15** as yellow solid (0.2 g, 75%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow crystals, m.p. 198-200°C.

f) *Using Br_2 in o-dichlorobenzene*[33]

To a stirred suspension of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.49 g, 2.5 mmole) in *o*-dichlorobenzene (2 mL), Br_2 (0.41 g, 0.13 mL, 2.6 mmole) was added at such a rate that the temperature of the reaction mixture was kept below 40°C. The reaction mixture was stirred for 30 min, diluted with water (5 mL).

The aqueous layer decanted and to the emulsion ether (10 mL) was added, the precipitated solid was filtered, washed with dilute sodium bisulfite, water, ether and dried to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.582 g, 84.65%). Recrystallization from $CHCl_3$-petroleum ether mixture afforded yellow needles, m.p. 200°C.

g) *Using N-bromosuccinamide (NBS) in N,N-dimethylformamide (DMF)*[34]

Equimolar mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.49 g, 2.5 mmole) and NBS (0.455 g, 2.5 mmole) in dry DMF (5 mL) was stirred at room temperature for 24 hrs, diluted with distilled water (10 mL). The contents were cooled and stirred for further 15 min. The solid separated was collected by filtration, washed with cold water and dried at 100°C to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.662 g, 96.29%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 200°C.

h) *Using N-bromosuccinamide (NBS) and sulphuric acid*[35]

Equimolar mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.05 g, 0.25 mmole) and NBS (0.0418 g, 0.25 mmole) in water (2.5 mL) was heated to 60°C. Aqueous solution of H_2SO_4 (40%, 0.03 mL, 0.5 mmole) was then added and the reaction mixture was maintained at 60°C for additional 2 hrs, cooled to room temperature, extracted with $CHCl_3$ (2 × 3 mL), organic extracts were washed with water (2 × 2 mL), dried over Na_2SO_4 and evaporated to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as yellow solid (0.068 g, 99%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 200°C.

i) *Using N-bromosuccinamide (NBS) and diisopropylamine*[36]

To a solution of NBS (0.267 g, 1.5 mmole) and 2,4-dimethoxy-6-hydroxy-acetophenone **13** (0.196 g, 1 mmole) in CH_2Cl_2 (5 mL) was added a solution of

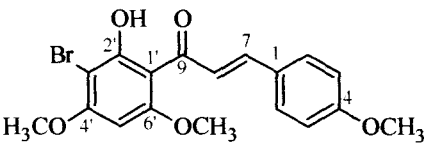
diisopropylamine (2 drops) in CH_2Cl_2 (5 mL) at 20°C and stirred at this temperature for 7 hrs. The reaction mixture was diluted with CH_2Cl_2 (5 mL), organic layer washed with HCl (0.1N, 2 × 10 mL), water (2 × 10 mL), dried over Na_2SO_4 and evaporated to leave crude solid (0.3122 g). TLC indicated it to be a mixture (2 spots), purification by column chromatography using petroleum ether as eluent gave 3,5-dibromo-2,4-dimethoxy-6-hydroxy-acetophenone **18** as pista green solid (0.279 g, 78.84%). Recrystallization from petroleum ether gave pista green flakes, m.p. 102°C.

IR ν_{max} (KBr): 1612, 1572, 1537, 1388, 1286, 1093, 960, 702 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.06): δ 2.76 (s, 3H, -COC<u>H</u>$_3$), 3.91 (s, 3H, C_2-OC<u>H</u>$_3$), 3.95 (s, 3H, C_4-OC<u>H</u>$_3$), 13.78 (s, 1H, -O<u>H</u>).

Further elution with petroleum ether-diethyl ether (8:2) gave 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as yellow solid (0.2212 g, 80.4%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 198°C.

3′-Bromo-2′-hydroxy-4,4′,6′-trimethoxychalcone **16**



16

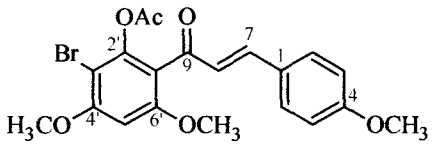
A mixture of 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.115 g, 0.41 mmole) and anisaldehyde (0.056 g, 0.050 mL, 0.4 mmole) in alcoholic KOH (50%, 2.5 mL) was kept at room temperature for 72 hrs. The dark yellow mass was diluted with water (3 mL) and acidified with conc. HCl. Yellow solid separated was filtered, washed with water and dried to give 3′-bromo-2′-hydroxy-4,4′,6′-trimethoxychalcone **16** (0.142 g, quantitative). Recrystallization from $CHCl_3$-MeOH mixture gave yellow flakes, m.p. 180°C.

IR ν_{max} (KBr): 3420, 2920, 2340, 1625, 1415, 1210, 1120, 560 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.07): For assignments refer **Figure III**, pg 110.

^{13}CNMR ($CDCl_3$, 75 MHz, **Fig.** 2.08): For assignments refer **Figure IV**, pg 110.

2'-Acetoxy-3'-bromo-4,4',6'-trimethoxychalcone **17**



17

3'-Bromo-2'-hydroxy-4,4',6'-trimethoxychalcone **16** (25 mg, 0.0636 mmole) in acetic anhydride (2 mL) and dry pyridine (1 mL) was heated on a boiling water bath for 2 hrs. The reaction mixture was cooled, poured over crushed ice containing conc. HCl (few drops) and the solid separated out was collected by filtration, washed with water and dried in oven at 100°C to give 2'-acetoxy-3'-bromo-4,4',6'-trimethoxychalcone **17** (0.0162 g, 73.17%). Recrystallization from $CHCl_3$-MeOH mixture gave greenish shiny flakes, m.p. 178-180°C.

IR ν_{max} (KBr): 1770, 1660, 1597, 1184, 1095, 827 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.11): δ 2.16 (s, 3H, -OCOC$\underline{H}_3$), 3.79 (s, 6H, 2 × -OC$\underline{H}_3$), 3.92 (s, 3H, -OC$\underline{H}_3$), 6.40 (s, 1H, $C_{5'}$-H), 6.78 (d, J = 16 Hz, 1H, C_α-H), 6.85 (d, J = 9 Hz, 2H, $C_{3,5}$-H), 7.43 (d, J = 9 Hz, 2H, $C_{2,6}$-H), 7.37 (d, J = 16 Hz, 1H, C_β-H).

Introduction of isopropyl group[37]

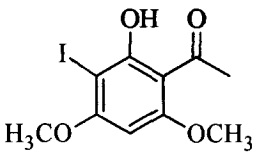
To a suspension of anhydrous $AlCl_3$ (0.25 g, 1.87 mmole) in dry CH_2Cl_2 (5 mL) was added with stirring 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.25 g, 1.2 mmole) at 0°C. The suspension was then cooled to -10°C and isopropyl

bromide (0.159 g, 0.121 mL, 1.3 mmole) was added. The reaction mixture was then warmed to room temperature and stirred at this temperature for 5 hrs, quenched with dilute HCl and extracted with ether (3 × 5 mL). The combined organic extracts were washed with water (2 × 3 mL), dried over Na_2SO_4 and evaporated to give 0.3038 g of crude residue identified as the starting material **13**.

Introduction of sulfonic acid group followed by bromination and hydrolysis[38]

A mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.392 g, 2 mmole) and conc. H_2SO_4 (0.2 mL) was heated on a boiling water bath with continuous stirring for 3 hrs. The reaction mixture was then placed in an ice bath and NaOH (0.9 g, 22.5 mmole) in water (2 mL) was added. Reaction mixture was allowed to attain room temperature, Br_2 (0.1 mL, 0.31 g, 1.9 mmole) was added & the temperature was allowed to increase to 40-50°C and stirred at this temperature for 1 hr. The flask was then kept in an oil bath at 140-150°C to evaporate the solution. A thick greyish black mass was obtained. It was cooled, conc. H_2SO_4 (1 mL) added and the resulting reaction mixture was heated in an oil bath at 190-210°C and distilled with steam. The steam distillate and the residue left over were separately extracted with ether. The residual extractions showed a complex TLC pattern.

5-Iodo-2,4-dimethoxy-6-hydroxyacetophenone **19**



19

To a stirred suspension of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.5 g, 2.55 mmole) in glacial acetic acid (8 mL) and water (2 mL) was added ICl (0.5181 g, 0.16 mL, 3.188 mmole) in glacial acetic acid (2 mL) drop by drop over a period of 10 min. The reaction mixture was stirred for 15 min at room temperature and then heated over steam bath for further 15 min. Conc. aqueous

sodium thiosulfate (2 mL), water (1 mL) was added and the reaction mixture was allowed to attain room temperature slowly, cooled in an ice bath, the canary yellow precipitate was filtered, washed with dilute acetic acid, water and dried to give 5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19** (0.786 g, 93.5%), m.p. 216°C.

IR ν_{max} (KBr): 1626, 1586, 1413, 1284, 1134, 787 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.12): δ 2.64 (s, 3H, -$COCH_3$), 3.95 (s, 3H, C_2-OCH_3), 3.97 (s, 3H, C_4-OCH_3), 6.01 (s, 1H, C_3-H), 14.9 (s, 1H, -OH).

Bromination on 5-Iodo-2,4-dimethoxy-6-hydroxyacetophenone **19**

a) *Using Br_2 in acetic acid*[28]

To a stirred suspension of 5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19** (0.125 g, 0.388 mmole) in glacial acetic acid (2.5 mL) was added Br_2 (0.062 g, 0.02 mL, 0.388 mmole) in glacial acetic acid (0.5 mL). The reaction mixture was stirred at room temperature for 30 min, diluted with water (2 mL) and stirred for further 10 min. The precipitated solid was filtered, washed with dilute sodium bisulphite, water and dried to give 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15** (0.101 g, 64.94%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 202°C.

b) *Using N-bromosuccinamide (NBS) in N,N-dimethylformamide (DMF)*[34]

Equimolar mixture of 5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19** (0.125 g, 0.388 mmole) and NBS (0.07 g, 0.388 mmole) in dry DMF (5 mL) was stirred at room temperature for 24 hrs, diluted with distilled water (10 mL). The contents were cooled while stirring for 15 min, the solid separated was collected by filtration, washed with cold water and dried at 100°C to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as a yellow solid (0.11 g, 71%), recrystallization from $CHCl_3$-petroleum ether mixture gave yellow needles, m.p. 202°C.

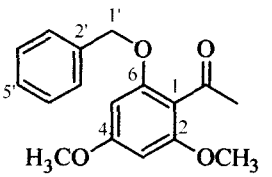
c) *Using KBr and Oxone*[30]

To a stirred solution of 5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19** (0.064 g, 0.2 mmole) in acetonitrile:water (2:1, v/v, 3.0 mL) mixture was added KBr (0.024 g, 0.21 mmole) followed by drop by drop addition of Oxone (0.0123 g, 0.2 mmole) in H_2O (2 mL) and the reaction mixture was stirred for 2 hrs. Solid precipitated was collected by filtration, washed with water and dried to give 0.063 g identified as the starting material **19**.

d) *Using KBr, ammonium molybdate and* H_2O_2[31]

To a stirred suspension of 5-iodo-2,4-dimethoxy-6-hydroxyacetophenone **19** (0.0196 g, 0.06 mmole), KBr (0.0086 g, 0.075 mmole), ammonium molybdate (0.003 g, 0.0025 mmole) in glacial acetic acid (1 mL) was added H_2O_2 (30%, 0.2 mL) drop by drop and the reaction mixture stirred for 30 min at room temperature. The yellow coloured reaction mixture was neutralized with saturated $NaHCO_3$. The precipitated solid was filtered, washed with water and dried to give 0.015 g identified as the starting material **19**.

2-Benzyloxy-4, 6-dimethoxyacetophenone **21**



21

a) *Conventional method*

A mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.294 g, 1.5 mmole), benzyl chloride (0.3036 g, 2.4 mmole) and anhydrous K_2CO_3 (0.957 g, 6.9 mmole) in dry acetone (15 mL) was refluxed for 4 hrs. Acetone was filtered and evaporated to give yellow oil. TLC indicated it to be a mixture of **13** and benzyl chloride.

b) *Using Microwave*[N]

A mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.294 g, 1.5 mmole), benzyl chloride (0.3036 g, 2.4 mmole), anhydrous K_2CO_3 (0.957 g, 6.9 mmole) and TBAB (catalytic amount) was subjected to microwave at power level 2 for 3 min. The contents were cooled to room temperature, added H_2O (10 mL) and extracted with ether (3 × 4 mL). The organic extracts were washed with water (3 × 4 mL), dried over Na_2SO_4 and evaporated to leave a dark yellow oil which on purification by column chromatography using petroleum ether-ethyl acetate (6:4) gave 2-benzyloxy-4,6-dimethoxyacetophenone **21** (0.639 g, 89%) as yellow oil.

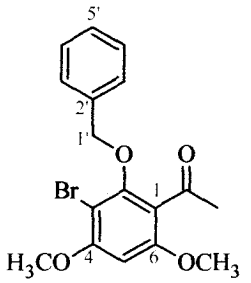
IR ν_{max} (KBr): 1693, 1604, 1587, 1417, 1247, 1226, 1157, 738, 698 cm^{-1}.

^{1}**H NMR** ($CDCl_3$, 300 MHz, **Fig**. 2.13): For assignments refer **Figure VI**, pg 118.

^{13}**C NMR** ($CDCl_3$, 75 MHz, **Fig**. 2.14): For assignments refer **Figure VII**, pg 119.

[N] IFB industries Ltd, Model: Electron, Power max. 600 W, Microwave frequency 2450 MHz.

2-Benzyloxy-3-bromo-4, 6-dimethoxyacetophenone **21b**



21b

a) *Using $KBrO_3$ and HBr*[27]

To a mixture of 2-benzyloxy-4, 6-dimethoxyacetophenone **21** (0.5 g, 1.74 mmole) & $KBrO_3$ (0.0973 g, 0.583 mmole) in glacial acetic acid (4 mL) was added HBr (48%, 0.517 g, 6.4 mmole) and the reaction mixture was stirred at room temperature for 30 min, diluted with water (5 mL) and the viscous mass separated was extracted with $CHCl_3$ (3 × 5 mL). The organic extracts were washed with dilute sodium bisulphite (2 × 3 mL), water (2 × 3 mL), dried over Na_2SO_4 and evaporated to give a viscous oil (0.73 g). Purification by silica gel column chromatography using petroleum ether as eluent gave 3,5-dibromo-2,4-dimethoxy-6-hydroxyacetophenone **18** as pista green solid (0.15 g, 31%). Recrystallization from petroleum ether gave pista green flakes, m.p. 102°C.

Further elution with petroleum ether-diethyl ether (6:4) gave 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as pale yellow solid (0.12 g, 32%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 198°C.

b) *Using KBr and Oxone*[30]

To a stirred solution of 2-benzyloxy-4,6-dimethoxyacetophenone **21** (0.095 g, 0.33 mmole) in acetonitrile:water (2:1, v/v, 3.0 mL) mixture was added KBr (0.04 g, 0.4 mmole) followed by drop by drop addition of Oxone (0.216 g, 0.4 mmole) in water (2 mL) and the reaction mixture was stirred for 30 min.

White sticky mass which separated out was extracted with ether (3 × 3 mL). The organic extracts were washed with H_2O (2 × 2 mL), dried over Na_2SO_4 and evaporated to give a white sticky mass (0.1 g, 83%) which on trituration with petroleum ether gave 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** as white plates, m.p. 102°C.

IR ν_{max} (KBr): 1697, 1593, 1566, 1406, 1367, 1244, 1217, 1101, 910 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.15): δ 2.44 (s, 3H, -COC$\underline{H}_3$), 3.86 (s, 3H, C_2-OC$\underline{H}_3$), 3.94 (s, 3H, C_4-OC$\underline{H}_3$), 4.98 (s, 2H, $C_{1'}$-H), 6.34 (s, C_5-H), 7.33-7.40 (m, 3H, $C_{4',5',6'}$-H), 7.50 (d, J = 7.2 Hz, 2H, $C_{3',7'}$-H).

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.16): δ 32.44 (-CO$\underline{C}H_3$), 56.04 (6-O$\underline{C}H_3$), 56.55 (4-O$\underline{C}H_3$), 77.02 (C-1′), 92.64 (C-5), 98.82 (C-3), 120.46 (C-1), 128.31 (C-5′), 128.44 (C-4′,6′), 128.67 (C-3′,7′), 136.42 (C-2′), 153.97 (C-6), 156.91 (C-4), 158.26 (C-2), 200.89 ($\underline{C}O$).

3-Bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** *from* **15**

A mixture of 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.533 g, 1.93 mmole), benzyl chloride (0.245 g, 1.93 mmole) and anhydrous K_2CO_3 (3.0 g, 21.7 mmole) in catalytic TBAB was subjected to microwave irradiation at power level 2 for 3 min. The contents were cooled to room temperature, added water (10 mL) and extracted with ether (3 × 4 mL). The organic extracts were washed with water (3 × 4 mL), dried over Na_2SO_4 and evaporated to leave 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** (0.532 g, 75%) as colourless oil which on trituration with petroleum ether gave white plates, m.p. 102°C.

Deprotection of 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** *using HBr in glacial acetic acid*

To a solution of 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** (0.03 g, 0.082 mmole) in glacial acetic acid (2 mL) was added HBr (48%, 1 mL) and the

mixture stirred for 15 min at room temperature, diluted with water (5 mL), the precipitated solid was filtered, washed with water and dried to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.018 g, 81.81%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles m.p. 198°C.

Demethylation of 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b**
Formation of 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15**

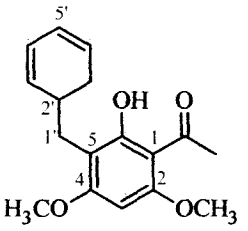
a) *Using anhydrous $AlCl_3$ in dichloromethane*[41]

To a solution of anhydrous $AlCl_3$ (0.074 g, 0.555 mmole) in dry CH_2Cl_2 (2 mL) was added 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** (0.135 g, 0.37 mmole) in dry CH_2Cl_2 (2 mL), the reaction mixture stirred at room temperature for 2 hrs, acidified with dilute HCl, extracted with CH_2Cl_2 (2 × 3 mL). The combined organic extracts washed with water (2 × 3 mL), dried over Na_2SO_4 and evaporated to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.40 g, 72.7%) as yellow solid. Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles m.p. 196°C.

b) *Using anhydrous $AlCl_3$ in acetonitrile*[42]

To a solution of anhydrous $AlCl_3$ (0.138 g, 1.03 mmole) in dry CH_3CN (2 mL) was added 3-bromo-2-benzyloxy-4,6-dimethoxyacetophenone **21b** (0.3757 g, 1.03 mmole) in dry CH_3CN (8 mL) & the contents were refluxed for 8 hrs, acidified with dilute HCl, extracted with CH_2Cl_2 (2 × 3 mL). The combined organic extracts were washed with water (2 × 3 mL), dried over Na_2SO_4 and evaporated to leave 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.2 g, 71%) as yellow solid. Recrystallization from petroleum ether-$CHCl_3$ mixture gave pale yellow needles, m.p. 196°C.

Demethylation on 2-benzyloxy-4, 6-dimethoxyacetophenone **21** *using anhydrous* $AlCl_3$ *in acetonitrile*[42]*, Formation of* **13** *and 5-benzyl-2,4-dimethoxy-6-hydroxyacetophenone* **25**



25

To a solution of anhydrous $AlCl_3$ (0.11 g, 0.82 mmole) in dry CH_3CN (3 mL) was added 2-benzyloxy-4, 6-dimethoxyacetophenone **21** (0.21 g, 0.73 mmole) in dry CH_3CN (2 mL) and the contents were refluxed for 8 hrs, acidified with dilute HCl, extracted with CH_2Cl_2 (2 × 3 mL). The combined organic extracts were washed with H_2O (2 × 3 mL), dried over Na_2SO_4 and evaporated to give (0.18 g) crude solid. TLC indicated it to be a mixture of 2 spots. Purification by column chromatography using petroleum ether as eluent gave 2,4-dimethoxy-6-hydroxy-acetophenone **13** (0.082 g, 57%). Further elution with petroleum ether-diethyl ether (95:5) gave 5-benzyl-2,4-dimethoxy-6-hydroxyacetophenone **25** (0.67 g, 32%). Recrystallization from petroleum ether-diethyl ether gave colourless crystals, m.p. 114-116°C.

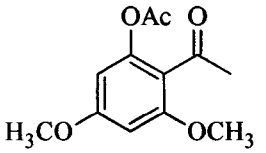
IR ν_{max} (KBr): 1620, 1593, 1462, 1275, 1116, 788, 879, 702 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.17): For assignments refer **Figure VIII**, pg 122.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.18): For assignments refer **Figure IX**, pg 123.

GCMS: *m/z* 286 $[M]^+$.

2-Acetoxy-4,6-dimethoxyacetophenone **26**

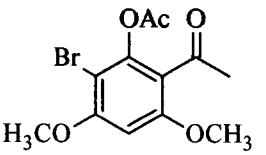


26

A solution of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.196 g, 1 mmole) in acetic anhydride (0.2 mL) and dry pyridine (0.4 mL) was heated on a boiling water bath for 2 hrs. The reaction mixture was cooled to room temperature and poured over crushed ice containing HCl (6N, 2 mL). White solid separated was collected by filtration, washed with water and dried to give 2-acetoxy-4,6-dimethoxyacetophenone **26** (0.085 g, 70%) m.p. 106-108°C, Lit.[26] 107°C.

IR ν_{max} (KBr): 1758, 1670, 1608, 1253, 1152, 825 cm^{-1}.

2-Acetoxy-3-bromo-4,6-dimethoxyacetophenone **26a**



26a

a) *Using Br_2 in water*[29]

To stirred solution of KBr (0.139 g, 1.16 mmole) in water (1.5 mL) and Br_2 (0.062 g, 0.02 mL, 0.39 mmole) was added 2-acetoxy-4,6-dimethoxy-acetophenone **26** (0.085 g, 0.357 mmole) and the reaction mixture stirred at room temperature for 1 hr. The solid was collected by filtration, washed with water and dried to give crude solid (0.10 g). Purification by column chromatography and elution with petroleum ether-$CHCl_3$ (8:2) gave 2-acetoxy-3-bromo-4,6-dimethoxyacetophenone **26a** as white solid (0.1 g, 88.5%). Recrystallization from $CHCl_3$-petroleum ether mixture gave white shiny cubes, m.p. 150°C.

IR ν_{max} (KBr): 1766, 1682, 1599, 1219, 1105, 879, 605 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig**. 2.19): δ 2.30 (s, 3H, -OCOC$\underline{H}_3$), 2.47 (s, 3H, -COC$\underline{H}_3$), 3.89 (s, 3H, C_6-OC$\underline{H}_3$), 3.94 (s, 3H, C_4-OC$\underline{H}_3$), 6.40 (s, 1H, C_5-H).

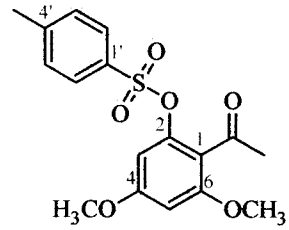
b) *Using $KBrO_3$ and HBr*[27]

To a stirred mixture of 2-acetoxy-4,6-dimethoxyacetophenone **26** (0.2085 g, 0.876 mmole), $KBrO_3$ (0.0487 g, 0.292 mmole) in glacial acetic acid (5 mL) was added HBr (48%, 0.253 g, 3.21 mmole) and the reaction mixture stirred for 30 min, diluted with water (10 mL) and stirred for a further 15 min. The bromo derivative 2-acetoxy-3-bromo-4,6-dimethoxyacetophenone **26a** separated as white solid was collected by filtration, washed with dilute sodium bisulfite, water and dried to give 0.268 g, 96.61%. Recrystallization from $CHCl_3$-petroleum ether mixture gave white cubes, m.p.148°C.

c) *Acetylation*[23] *of 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone* **15**

A solution of 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.1 g, 0.363 mmole), Ac_2O (2 mL) and dry pyridine (1 mL) was heated on a boiling water bath for 2 hrs, cooled to room temperature and poured over crushed ice containing conc. HCl. The acetyl derivative 2-acetoxy-3-bromo-4,6-dimethoxy-acetophenone **26a** separated out as white solid (0.11 g, 95%). Recrystallization from $CHCl_3$-petroleum ether mixture gave white cubes, m.p. 150°C.

2-p-Toluenesulfonyloxy-4,6-dimethoxyacetophenone **27**



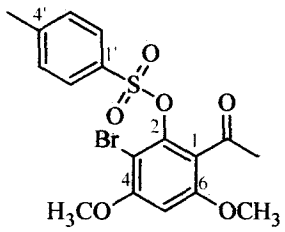
27

A mixture of 2,4-dimethoxy-6-hydroxyacetophenone **13** (0.392 g, 2 mmole), *p*-toluenesulfonyl chloride (0.6 g, 3.15 mmole), anhydrous K_2CO_3 (3.0 g, 21.73 mmole) in dry acetone (10 mL) was refluxed for 8 hrs. The reaction mixture was cooled to room temperature, filtered and the solvent evaporated to give a pale grey solid. TLC indicated it to be a mixture which on purification by column chromatography using petroleum ether-diethyl ether (1:1) as eluent gave 2-*p*-toluene-sulfonyloxy-4,6-dimethoxyacetophenone **27** (0.5 g, 72%) as white solid. Recrystallization from $CHCl_3$-petroleum ether mixture gave colourless needles, m.p. 150-152°C.

IR ν_{max} (KBr): 1687, 1612, 1572, 1367, 1251, 1151, 1087, 945, 777, 671 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.20): δ 2.35 (s, 3H, $C_{4'}$-C<u>H</u>$_3$), 2.44 (s, 3H, -COC<u>H</u>$_3$), 3.75 (s, 3H, C_2-OC<u>H</u>$_3$), 3.78 (s, 3H, C_4-OC<u>H</u>$_3$), 6.36 (s, 2H, $C_{3,5}$-H), 7.33 (d, J = 8.1 Hz, 2H, $C_{3',5'}$-H), 7.76 (d, J = 9 Hz, 2H, $C_{2',6'}$-H).

3-Bromo-4,6-dimethoxy-2-p-toluenesulfonyloxyacetophenone **27b**



27b

To a mixture of 2-*p*-toluenesulfonyloxy-4,6-dimethoxyacetophenone **27** (0.4 g, 1.14 mmole), $KBrO_3$ (0.08 g, 0.048 mmole) was added HBr (48%, 0.53 mL, 4.6 mmole) and the contents were stirred for 1 hr. Usual workup gave 3-bromo-4,6-dimethoxy-2-*p*-toluenesulfonyloxyacetophenone **27b** (0.48 g, 98%) as white solid. Recrystallization from $CHCl_3$-petroleum ether mixture gave tiny white crystals, m.p. 176-178°C.

IR ν_{max} (KBr): 1687, 1595, 1456, 1379, 1217, 1176, 813, 765, 740, 549 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.21): δ 2.45 (s, 3H, $C_{4'}$-C<u>H</u>$_3$), 2.52 (s, 3H, -COC<u>H</u>$_3$), 3.87 (s, 3H, C_2-OC<u>H</u>$_3$), 3.90 (s, 3H, C_4-OC<u>H</u>$_3$), 6.43 (s, 2H, C_5-H), 7.34 (d, J = 8.1 Hz, 2H, $C_{3',5'}$-H), 7.84 (d, J = 9 Hz, 2H, $C_{2',6'}$-H).

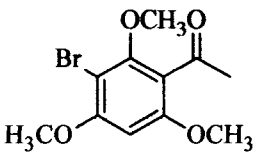
Alkaline hydrolysis[44] *of 3-bromo-4,6-dimethoxy-2-p-toluenesulfonyloxy acetophenone* **27b**

A mixture of 3-bromo-4,6-dimethoxy-2-*p*-toluenesulfonyloxyacetophenone **27b** (0.342 g, 0.79 mmole) and KOH (0.45 g, 8.0 mmole) in methanol (7.5 mL) & water (7.5 mL) was heated to reflux for 1 hr, cooled and neutralised with acetic acid, solid separated was filtered, washed with water, dried to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.178 g, 81.25%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 198°C.

Demethylation of 3-bromo-4,6-dimethoxy-2-p-toluenesulfonyloxy acetophenone **27b** *using anhydrous* $AlCl_3$

To a solution of anhydrous $AlCl_3$ (0.05 g, 0.36 mmole) in dry CH_2Cl_2 (5 mL) was added 3-bromo-4,6-dimethoxy-2-toluenesulfonyloxyacetophenone **27b** (0.103 g, 0.24 mmole) in dry CH_2Cl_2 (5 mL) & the reaction mixture was stirred at room temperature for 2 hrs. Usual workup gave 5-bromo-2,4-dimethoxy-6-hydroxy-acetophenone **15**, m.p. 198°C.

3-Bromo-2,4,6-trimethoxyacetophenone **28**



28

Equimolar mixture of 2,4,6-trimethoxyacetophenone **14** (0.30 g, 1.42 mmole) & NBS (0.255 g, 1.42 mole) in dry DMF (4 mL) was stirred at room temperature for 24 hrs, diluted with distilled water (6 mL). The contents were cooled with stirring for 15 min. The solid separated was filtered, washed with cold water & dried to give 3-bromo-2,4,6-trimethoxyacetophenone **28** (0.349 g, 84.5%) as white solid. Recrystallization from petroleum ether gave white plates, m.p. 78°C.

IR ν_{max} (KBr): 1685, 1587, 1386, 1242, 1111, 823, 609 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.22): δ 2.48 (s, 3H, -COC<u>H</u>$_3$), 3.82 (s, 3H, -OC<u>H</u>$_3$), 3.83 (s, 3H, -OC<u>H</u>$_3$), 3.91 (s, 3H, -OC<u>H</u>$_3$), 6.3 (s, 1H, C_5-H).

Demethylation of 3-bromo-2,4,6-trimethoxyacetophenone **28**

a) *Using anhydrous $AlCl_3$ and pyridine*[45]

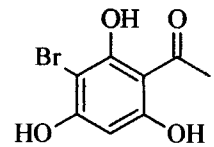
To a suspension of 3-bromo-2,4,6-trimethoxyacetophenone **28** (0.1 g, 0.346 mmole) in dry CH_2Cl_2 (5 mL) was added dry pyridine (0.12 g, 0.13 mL, 1.52 mmole) and heated to reflux for 24 hrs at 45°C. The reaction mixture was

cooled to room temperature and acidified with 20% HCl till acidic to Congo red. The organic layer was separated, washed with water (2 × 2 mL), dried over Na_2SO_4 and the solvent evaporated to give white solid (0.0768 g). Recrystallization from petroleum ether gave **28** as white plates, m.p.78°C. The aqueous portion was extracted with ether (3 × 3 mL), washed with water (2 × 3 mL), dried over Na_2SO_4 and evaporated to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.015 g, 14%). Recrystallization from $CHCl_3$-petroleum ether mixture gave pale yellow needles, m.p. 198°C.

b) *Using anhydrous $AlCl_3$ in dichloromethane*[41]

To a stirred solution of $AlCl_3$ (0.075 g, 0.2595 mmole) in dry CH_2Cl_2 (2 mL) was added 3-bromo-2,4,6-trimethoxyacetophenone **28** (0.066 g, 0.22 mmole). The reaction mixture was stirred at room temperature for 2 hrs, acidified with 20% HCl till acidic to Congo red. The organic layer was separated out and the aqueous layer was extracted with ether (3 × 3 mL). The combined organic portion was washed with water (2 × 3 mL), dried over Na_2SO_4, solvent evaporated to give 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** (0.059 g, 94%). Recrystallization from $CHCl_3$-petroleum ether mixture gave yellow needles, m.p. 196°C.

3-Bromophloroacetophenone **29**



29

a) *Using $KBrO_3$ and HBr*[27]

To a stirred solution of phloroacetophenone **12** (0.063 g, 0.375 mmole) and $KBrO_3$ (0.022 g, 0.13 mmole) in glacial acetic acid (1 mL) was added HBr (48%, 0.08 mL, 0.7 mmole) and the reaction mixture was stirred for 30 min at room

temperature, diluted with cold water (5 mL). Solid separated was filtered, washed with dilute sodium bisulfite, water and dried at 100°C to give the starting material **12**, m.p. 219°C, Lit.[26] 218-219°C.

b) *Using N-bromosuccinamide (NBS) in N,N-dimethylformamide(DMF)*[34]

An equimolar mixture of phloroacetophenone **12** (3.36 g, 0.02 mmole) & NBS (3.56 g, 0.018 mmole) in dry DMF (15 mL) was stirred at room temperature for 24 hrs. The wine coloured reaction mixture was diluted with water (50 mL) & extracted with CH_2Cl_2 (5 × 10 mL). The organic extracts were washed with water (3 × 10 mL), dried over Na_2SO_4 and evaporated to leave a brick red solid. Purification by flash chromatography using $CHCl_3$ as eluent gave pale green crystals of 3-bromophloroacetophenone **29** in 50% yield, m.p. 191°C (decomp).

IR ν_{max} (KBr): 1610, 1580, 1215, 1155, 829, 754 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig**. 2.23): δ 2.68 (s, 3H, -COC<u>H</u>$_3$), 6.02 (s, 1H, C_5-H).

Methylation of 3-bromophloroacetophenone **29**

a) *Using dimethyl sulphate and Claisen's alkali*[46]

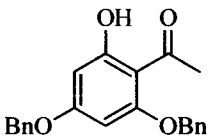
To a well stirred and cooled solution of 3-bromophloroacetophenone **29** (0.080 g, 0.32 mmole) in Claisen's alkali (1 mL, 0.35 g KOH dissolved in 0.25 mL H_2O & 0.75 mL MeOH), was added Me_2SO_4 (0.088 g, 0.06 mL, 0.69 mole) maintaining the temperature at 10°C. After completion of addition the reaction mixture was refluxed for 2 hrs, cooled to room temperature, diluted with water (2 mL) & extracted with ether (3 × 3 mL), the organic extracts were washed with water (2 × 3 mL), dried over Na_2SO_4, solvent evaporated to give a brown residue which was purified by column chromatography. Elution with $CHCl_3$ gave 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as pale brown solid. Recrystallization

from benzene gave pale brown needles, m.p. 186°C. Recrystallization from $CHCl_3$-petroleum ether gave pale yellow needles, m.p. 200°C.

b) *Using dimethyl sulphate and K_2CO_3*[9]

A mixture of 3-bromophloroacetophenone **29** (0.1 g, 0.4 mmole), anhydrous Me_2SO_4 (0.106 g, 0.08 mL, 0.84 mmole) & anhydrous K_2CO_3 (0.4 g, 2.9 mmole) was refluxed in dry acetone (2 mL) for 3 hrs. The reaction mixture was cooled, diluted with distilled water (5 mL), extracted with ether (3 × 3 mL), the organic layer washed with water (2 × 3 mL), dried over Na_2SO_4 & evaporated to give a solid residue. Purification by column chromatography using petroleum ether-C_6H_6 (6:4) as eluent gave 5-bromo-2,4-dimethoxy-6-hydroxyacetophenone **15** as pale yellow solid, m.p. 186°C.

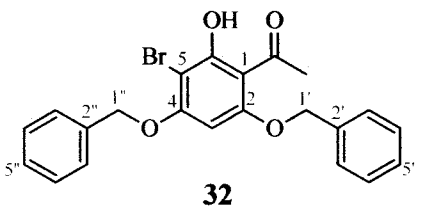
2,4-Dibenzyloxy-6-hydroxyacetophenone **31**



31

Prepared according to the reported[48] literature procedure.

5-Bromo-2,4-dibenzyloxy-6-hydroxyacetophenone **32**



32

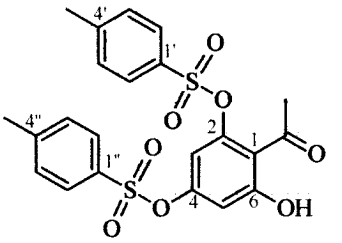
To a stirred suspension of 2,4-dibenzyloxy-6-hydroxyacetophenone **31** (0.115 g, 0.368 mmole), KBr (0.048 g, 0.405 mmole), ammonium molybdate (0.0155 g, 0.0125 mmole) in glacial acetic acid (3 mL) was added H_2O_2 (30%, 0.2 mL) and

the reaction mixture was stirred for 30 min at room temperature. The yellow coloured reaction mixture was neutralized with saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic extracts were washed with water (2 × 3 mL), brine (2 × 3 mL), dried over Na_2SO_4, solvent evaporated to give 5-bromo-2,4-dibenzyloxy-6-hydroxyacetophenone **32** as pale yellow solid (0.1 g, 70%). Recrystallization from $CHCl_3$-petroleum ether gave colourless needles, m.p. 118°C.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.24): δ 2.56 (s, 3H,-$COC\underline{H}_3$), 5.06 (s, 2H, $C_{1'}$-H), 5.17 (s, 2H, $C_{1''}$-H), 6.11 (s, 1H, C_3-H), 7.39 (brs, 10H, Ar-H).

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.25): δ 33.32 (-$CO\underline{C}H_3$), 70.8 (C-1′), 71.33 (C-1″), 89.83 (C-3), 126.82 (C-5′), 127.83 (C-5″), 128.6 (C-4′,6′), 128.68 (C-4″,6″), 128.76 (C-3′,7′), 128.87 (C-3″,7″), 135.17 (C-2′), 135.64 (C-2″), 160.9 (C-2), 161.46 (C-4), 162.75 (C-6), 203.3 (<u>C</u>O).

2,4-Di-p-toluenesulfonyloxy-6-hydroxyacetophenone **33**



33

A mixture of phloroacetophenone **12** (1.008 g, 6 mmole), *p*-toluenesulfonyl chloride (2.286 g, 12 mmole) and anhydrous K_2CO_3 (2.484 g, 18 mmole) in dry acetone (25 mL) was heated under reflux for 5 hrs. Usual workup gave crude solid (3.374 g). TLC indicated it to be a mixture (2 spots). Purification by column chromatography using petroleum ether-ethyl acetate (8:2) gave 2,4-di-*p*-toluene-sulfonyloxy-6-hydroxyacetophenone **33** (2.016 g, 71%) as white solid. Recrystallization from petroleum ether gave white crystals, m.p. 78°C.

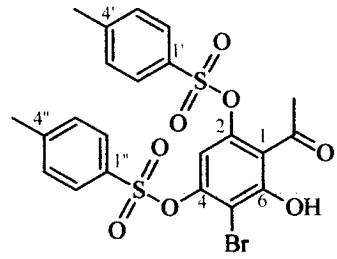
IR ν_{max} (KBr): 1634, 1595, 1377, 1246, 1174, 1045, 995 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.26): For assignments refer **Figure X**, pg 132.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.27): For assignments refer **Figure XI**, pg 133.

ESIMS (Fig. 2.28): *m/z* $[M + H]^+$ calcd. for $C_{22}H_{21}O_8S_2$ 477.5268, Found 477.0736.

5-Bromo-2,4-di-p-toluenesulfonyloxy-6-hydroxyacetophenone **34**



34

To a mixture of 2,4-di-*p*-toluenesulfonyloxy-6-hydroxyacetophenone **33** (0.5044 g, 1.05 mmole) and $KBrO_3$ (0.06 g, 0.35 mmole) in glacial acetic acid (2 mL) was added HBr (48%, 0.2 mL, 1.84 mmole) and the reaction mixture was stirred for 30 min at room temperature. Usual workup gave 5-bromo-2,4-di-*p*-toluene-sulfonyloxy-6-hydroxyacetophenone **34** (0.57 g, 97%) as yellow solid which on recrystallization from $CHCl_3$-petroleum ether mixture gave lemon yellow plates m.p. 114°C.

IR ν_{max} (KBr): 1627, 1595, 1387, 1192, 1064, 861, 781 cm^{-1}.

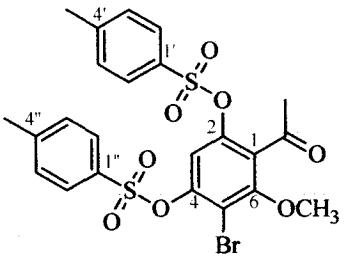
^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.29): δ 2.46 (s, 3H, $C_{4'}$-C<u>H</u>$_3$), 2.50 (s, 3H, $C_{4''}$-C<u>H</u>$_3$), 2.74 (s, 3H, -COC<u>H</u>$_3$), 6.68 (s, 1H, C_3-H), 7.34 (d, J = 8.4 Hz, 2H, $C_{3',5'}$-H), 7.42 (d, J = 8.4 Hz, 2H, $C_{2',6'}$-H), 7.79 (dt, J = 7.1 & 2.1 Hz, 4H, $C_{2'',3'',5'',6''}$-H), 13.6 (s, 1H, -O<u>H</u>).

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.30): δ 21.79 (4',4''-<u>C</u>H$_3$), 32.44 (-CO<u>C</u>H$_3$), 105.19 (C-3), 108.06 (C-5), 113.81 (C-1), 128.57 (C-2',3',5',6'), 129.97

(C-3″,5″), 130.41 (C-2″,6″), 131.42 (C-4′), 132.08 (C-4″), 146.35 (C-1′), 146.84 (C-1″), 149.29 (C-2), 151.07 (C-4), 161.42 (C-6), 203.27 (CO).

ESIMS (Fig. 2.31): *m/z* $[M + H]^+$ calcd. for $C_{22}H_{20}Br^{79}O_8S_2$ 554.9777, Found 554.9823; $[M + 2 + H]^+$ calcd. for $C_{22}H_{20}Br^{81}O_8S_2$ 556.4229, Found 556.9765.

5-Bromo-2,4-di-p-toluenesulfonyloxy-6-methoxyacetophenone **35**



35

A mixture of 5-bromo-2,4-di-*p*-toluenesulfonyloxy-6-hydroxyacetophenone **34** (1.32 g, 2.378 mmole), anhydrous Me_2SO_4 (0.2996 g, 2.378 mmole) & anhydrous K_2CO_3 (0.984 g, 7.134 mmole) in dry acetone (20 mL) was heated to reflux for 4 hrs. Usual workup gave 5-bromo-2,4-di-*p*-toluenesulfonyloxy-6-methoxyacetophenone **35** (1.586 g, 84.6%) as white solid. Recrystallization from benzene-petroleum ether mixture gave shining white cubes, m.p. 128°C.

IR ν_{max} (KBr): 1710, 1585, 1377, 1355, 1193, 1053, 933, 871 cm^{-1}.

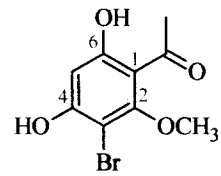
^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.32): δ 2.42 (s, 3H, $C_{4'}$-C<u>H</u>$_3$), 2.47 (s, 3H, $C_{4''}$-C<u>H</u>$_3$), 2.56 (s, 3H, -COC<u>H</u>$_3$), 3.72 (s, 3H, -OC<u>H</u>$_3$), 7.00 (s, 1H, C_3-H), 7.36 (d, J = 6.6 Hz, 4H, $C_{2',3',5',6'}$-H), 7.74 (d, J = 8.1 Hz, 2H, $C_{3'',6''}$-H), 7.78 (d, J = 8.7 Hz, 2H, $C_{2'',6''}$-H).

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.33): δ 21.73 (4′,4″-<u>C</u>H$_3$), 31.80 (-CO<u>C</u>H$_3$), 62.89 (-O<u>C</u>H$_3$), 111.73 (C-5), 114.08 (C-3), 128.48 (C-3′,5′), 128.65 (C-2′,6′), 129.97 (C-3″,5″), 130.12 (C-2″,6″), 130.22 (C-1), 131.55 (C-4′), 132.07 (C-4″),

144.54 (C-1′), 146.26 (C-1″), 146.37 (C-2), 148.16 (C-4), 155.65 (C-6), 197.28 (CO).

ESIMS (Fig. 2.37): *m/z* $[M + 2 + H]^+$ calcd. for $C_{23}H_{22}Br^{81}O_8S_2$ 570.4494, Found 570.9948.

3-Bromo-4,6-dihydroxy-2-methoxyacetophenone **36**



36

A mixture of 5-bromo-2,4-di-*p*-toluenesulfonyloxy-6-methoxyacetophenone **35** (0.958 g, 1.683 mmole) and KOH (3.76 g, 0.0673 mmole) in a mixture of ethanol (30 mL) & water (30 mL) was heated to reflux for 4 hrs, cooled, neutralised with glacial acetic acid and extracted with ether (4 × 5 mL). The organic extracts were washed with saturated $NaHCO_3$ (2 × 5 mL), water (2 × 5 mL), dried over Na_2SO_4 and evaporated to give (0.3875 g, 88%) oily residue. Purification by column chromatography using petroleum ether-ethyl acetate (98:2) gave 3-bromo-4,6-dihydroxy-2-methoxyacetophenone **36** (0.3483 g, 79%) as white solid. Recrystallization from petroleum ether gave white flakes m.p. 116°C.

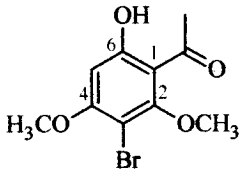
IR ν_{max} (KBr): 3302, 1581, 1469, 1413, 1261, 1157, 970, 825, 759 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.38): For assignments refer **Figure XIII**, pg 135.

^{13}CNMR ($CDCl_3$, 75 MHz, **Fig.** 2.39): For assignments refer **Figure XIV**, pg 135.

ESIMS (Fig. 2.40): *m/z* $[M + H]^+$ calcd. for $C_9H_{10}Br^{79}O_4$ 260.9757, Found 260.9802; $[M + 2 + H]^+$ calcd. for $C_9H_{10}Br^{81}O_4$ 262.0767, Found 262.9788.

3-Bromo-2,4-dimethoxy-6-hydroxyacetophenone **11**



11

A mixture of 3-bromo-4,6-dihydroxy-2-methoxyacetophenone **36** (0.095 g, 0.362 mmole), anhydrous Me_2SO_4 (0.0456 g, 0.362 mmole) & anhydrous K_2CO_3 (0.15 g, 1.086 mmole) in dry acetone (10 mL) was heated to reflux for 3 hrs. Usual workup gave (0.1068 g) as crude residue. Purification by column chromatography using petroleum ether-diethyl ether (9:1) gave 3-bromo-2,4-dimethoxy-6-hydroxyacetophenone **11** (0.0662 g, 67%) as white solid. Recrystallization from petroleum ether gave white needles, m.p. 102°C.

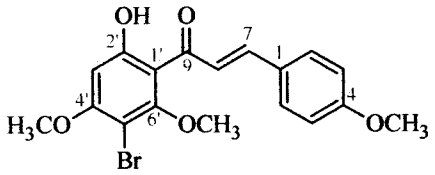
IR ν_{max} (KBr): 1627, 1585, 1436, 1361, 1253, 1105, 819, 748 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.41): For assignments refer **Figure XV**, pg 136.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.42): For assignments refer **Figure XVI**, pg 136.

ESIMS (Fig. 2.43): *m/z* $[M + 2]^+$ calcd. for $C_{10}H_{11}Br^{81}O_4$ 275.0959, Found 275.1.

5'-Bromo-2'-hydroxy-4,4',6'-trimethoxychalcone **8**



8

To a mixture of 3-bromo-2,4-dimethoxy-6-hydroxyacetophenone **11** (0.02 g, 0.0727 mmole) and anisaldehyde (0.01 g, 0.073 mmole) in ethanol (1 mL) was added dropwise KOH (0.1 g) in water (1 mL) and the reaction mixture was stirred for 4 hrs at 45°C. The yellow orange mixture was diluted with water (3 mL) & acidified with conc. HCl. Yellow solid separated was filtered, washed with water and dried to give 5'-bromo-2'-hydroxy-4,4',6'-trimethoxychalcone **8** (0.02 g, 70%). Recrystallization from $CHCl_3$-MeOH mixture gave yellow flakes, m.p. 178°C, Lit.[23] 180°C.

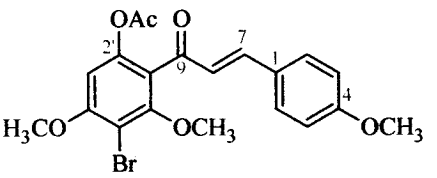
IR ν_{max} (KBr): 2963, 1626, 1605, 1261, 1102, 801, 657 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.44): δ 3.79 (s, 3H, -OC<u>H</u>$_3$), 3.86 (s, 3H, -OC<u>H</u>$_3$), 3.93 (s, 3H, -OC<u>H</u>$_3$), 6.36 (s, 1H, $C_{3'}$-H), 6.93 (d, J = 8.1 Hz, 2H, $C_{3,5}$-H), 7.62 (d, J = 8.1 Hz, 2H, $C_{2,6}$-H), 7.87 (s, 2H, $C_{\alpha,\beta}$-H), 13.65 (s, 1H, -O<u>H</u>).

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 2.45): δ 55.39 (6'-O<u>C</u>H$_3$), 56.62(4'-O<u>C</u>H$_3$), 62.27 (4-O<u>C</u>H$_3$), 97.35 (C-3'), 114.0 (C-5'), 114.44 (C-3,5), 123.20 (C-8), 127.73 (C-1'), 130.4 (C-2,6), 144.41 (C-7), 159.52 (C-4), 161.79 (C-2'), 161.82 (C-4'), 165.48 (C-6'), 192.5 (C-9).

ESIMS (Fig. 2.46): *m/z* $[M + H]^+$ calcd. for $C_{18}H_{18}Br^{79}O_5$ 393.0332, Found 393.0663; $[M + 2 + H]^+$ calcd. for $C_{18}H_{18}Br^{81}O_5$ 394.2359, Found 395.0620.

5'-Bromo-2'-acetoxy-4,4',6'-trimethoxychalcone **9**



9

A mixture of 5'-bromo-2'-hydroxy-4,4',6'-trimethoxychalcone **8** (7.5 mg, 0.019 mmole), acetic anhydride (1 mL) and dry pyridine (0.5 mL) was heated on a boiling water bath for 2 hrs. The reaction mixture was cooled, poured over crushed ice containing conc. HCl (few drops) and the solid separated out was collected by filtration, washed with water and dried to give 5'-bromo-2'-acetoxy-4,4',6'-trimethoxychalcone **9** (0.006 g, 72%) as yellow solid m.p. 126-28°C, Lit.[23] 122-26°C.

IR ν_{max} (KBr): 2931, 1766, 1638, 1599, 1251, 1177, 837, 552 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 2.47): δ 2.17 (s, 3H, -OCOC<u>H</u>$_3$), 3.80 (s, 3H, -OC<u>H</u>$_3$), 3.83 (s, 3H, -OC<u>H</u>$_3$), 3.93 (s, 3H, -OC<u>H</u>$_3$), 6.54 (s, 1H, $C_{3'}$-H), 6.90 (d, J = 8.7 Hz, 2H, $C_{3,5}$-H), 6.92 (d, J = 16.0 Hz, 1H, C_α-H), 7.39 (d, J = 16.0 Hz, 1H, C_β-H), 7.49 (d, J = 8.7 Hz, 2H, $C_{2,6}$-H).

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Section 2.2

Synthesis of 6-Cinnamylchrysin a Constituent of *Chinese Propolis*

Introduction

Propolis, referred to as 'bee glue', is a sticky material that honeybees collect from buds and exudates of plants used in the construction and adaptation of bee hives[1].

There is a long history of the use of propolis for various purposes dating back to at least 300 BC[1] and even now in the 21st century, it is used in home remedies and personal products. Propolis has also gained wide popularity as a health food in various parts of world including United Sates, European Union, and Japan where it is claimed to improve human health and prevent diseases such as inflammation, heart disease, diabetes and even cancer[2].

Several biological activities[3] such as anticancer, antioxidant, antiinflammatory, antiseptic, antimycotic, bacteriostatic, astringent, choleric, spasmolytic and anaesthetic properties have been reported for propolis and its constituents.

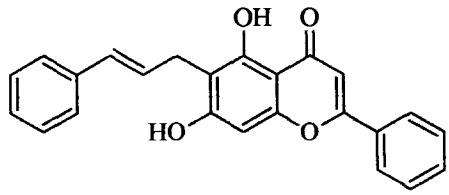
More than 300 constituents have been identified so far from propolis, among which phenolic compounds such as flavonoids and cinnamic acid derivatives have been reported as major constituents of propolis[4].

Due to these purported beneficial effects, there is a renewal of interest not only in the composition and biological activities of the constituents of propolis but also in the synthesis of these active constituents so that they can be easily detected in propolis extracts and further to have them in sufficient quantities to study their biological activities and also use them in useful pharmaceutical preparations.

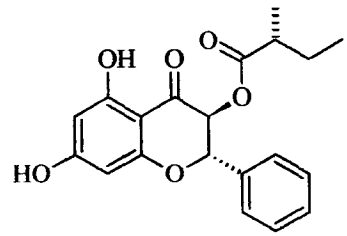
In our laboratory we have developed a general two-step procedure for the synthesis of natural cinnamyl esters and their analogues using Meldrum's acid. Several natural cinnamyl esters isolated from propolis[5] and natural

avenanthramides isolated from oats† have been synthesized using this two-step procedure in high yields ranging from 64-96%.

That is how we got interested in propolis and while investigating the constituents of propolis especially with respect to new natural cinnamyl esters, we came across an article reporting isolation of two new flavonoids, 6-cinnamyl-chrysin **1** and 3-*O*-[(*S*)-2-methylbutyroyl] pinobanksin **2** from the methanolic extract of *Chinese propolis* along with known flavanoids and cinnamyl esters[2].



1



2

Cinnamic acid and it derivatives are widely distributed in the plant kingdom and there are several reports of the attachment of the cinnamyl group with other classes of compounds[4,6,7]. For example several cinnamylphenols, which form a comparatively small group of flavonoid-related phenolics i.e. having structural units (C6 + C3 + C6) biogenetically analogous to flavonoids, have been isolated. They have been shown to possess a range of biological activities and are valuable as potential cancer chemopreventive agents (anti-tumor promoters)[8].

The parent chrysin moiety **3** present in 6-cinnamylchrysin **1** is a widely distributed natural flavonoid[9], having many different biological activities such as anti-oxidant, anti-viral, anti-diabetogenic, anti-cancer activity and anti-anxiolytic effect.

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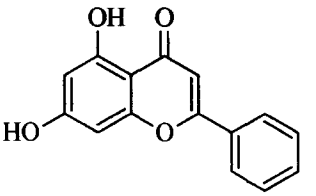
† Synthesis of avenanthramides, constituents of oats, have been described in chapter 1 of this thesis.



3

Although there are several reports on the isolation of cinnamylphenols[4,6,7], isolation of 6-cinnamylchrysin **1** constituted the first report of the occurrence of a flavone with a cinnamyl moiety from propolis[2].

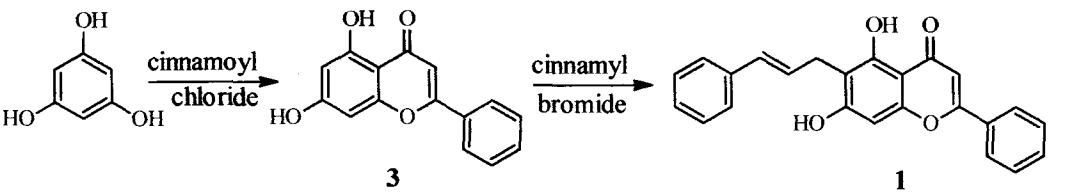
It may be noted that only 30.5 mg of **1** were isolated[2] as yellow amorphous powder (m.p. not reported) after repeated column chromatography followed by preparative TLC.

Although **1** was shown[2] to possess weak antiproliferative activity, we feel that this unique compound, a combination of cinnamyl phenol and a flavonoid, both having a wide range of biological activities may possess some interesting synergetic bioactivity and needs to be investigated.

Moreover, there has been no report on the synthesis of **1** since its isolation (2002). Therefore, we decided to synthesize **1** starting with phloroglucinol and our synthetic strategy is discussed below.

The phloroglucinol motif is ubiquitous in all natural flavonoid structures. As such, it constitutes the ultimate starting material for the synthesis of several natural polyphenols and their analogues[10].

Our original plan was to prepare 6-cinnamylchrysin **1** in just two key-steps by *C*-acylation of the phloroglucinol[11] with cinnamoyl chloride followed by *C*-alkylation of the chrysin **3** formed with cinnamyl bromide as shown below.



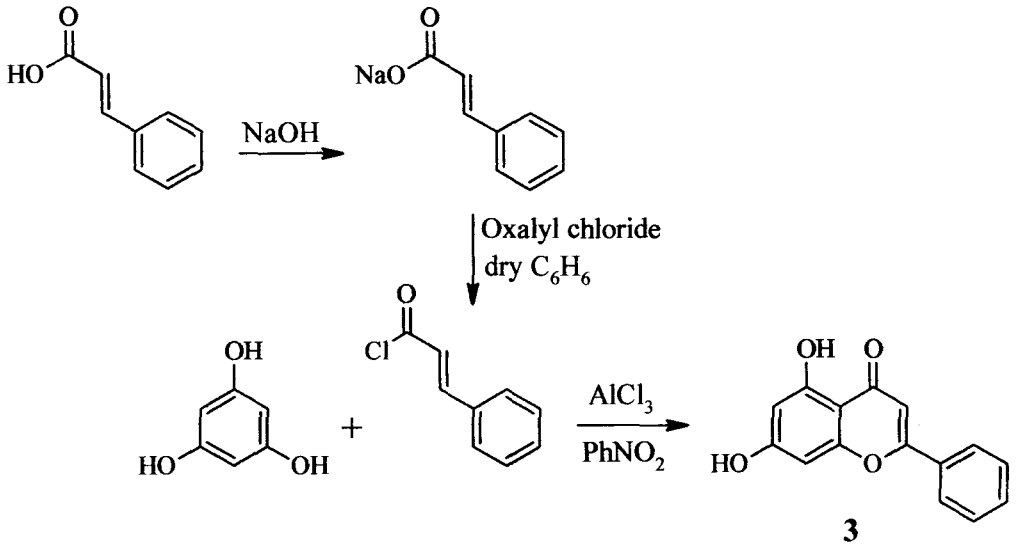
3 **1**

Scheme 1

Cinnamoyl chloride[11] was prepared by refluxing a mixture of sodium cinnamate (prepared from cinnamic acid and NaOH) and oxalyl chloride* in dry benzene. Distillation of the residue under reduced pressure gave cinnamoyl chloride as yellow liquid.

Similarly cinnamyl bromide[12] was prepared from cinnamyl alcohol as follows. Commercially available cinnamyl alcohol was treated with Br_2 and PPh_3* in anhydrous acetonitrile. Distillation under reduced pressure gave cinnamyl bromide as pale yellow liquid.

The first step in the synthesis of **1** involed the preparation of chrysin **3** from phloroglucinol and cinnamoyl chloride as shown below.



3

The second step involved the *C*-alkylation of chrysin **3** using cinnamyl bromide in NaOH.

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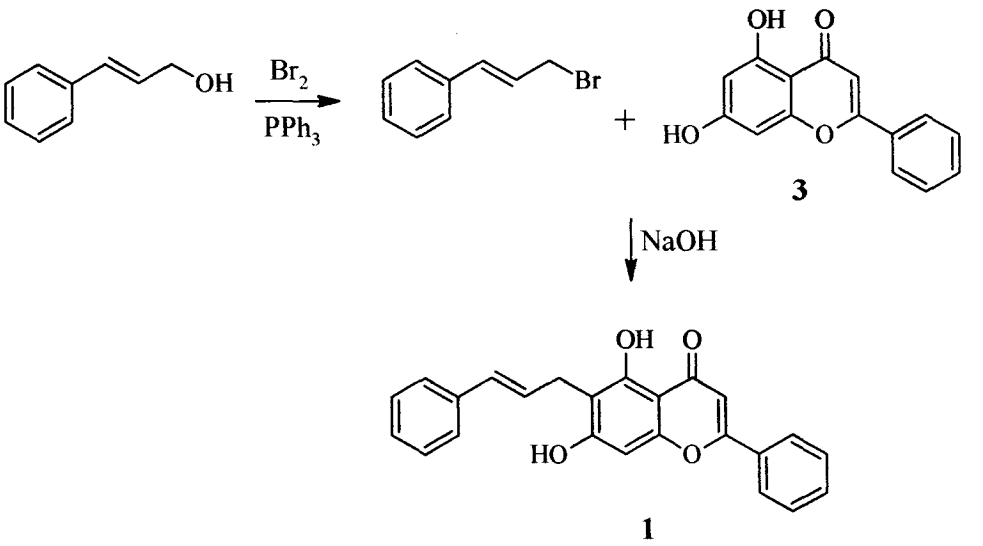
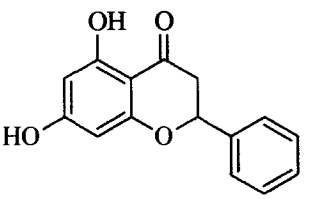
* We are thankful to Dr. S. G. Tilve for providing oxalyl chloride and PPh_3

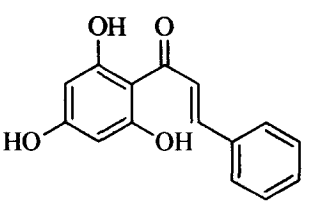


Friedel Craft's acylation of phloroglucinol with cinnamoyl chloride[11] is known to give the flavone, chrysin **3**. Stirring an equimolar mixture of phloroglucinol and cinnamoyl chloride with excess of anhydrous $AlCl_3$ in nitrobenzene (purified by steam distillation) at room temperature overnight gave an oily residue which on purification by silica gel column chromatography gave 5,7-dihydroxy flavanone (pinocembrin) **4** in only 8% yield, m.p. 210°C (Lit.[13] 203-204°C).

However slight modification[14] of the reaction conditions, i.e. by heating the reaction mixture to 50 to 60°C for 150 hrs, gave 2′,4′,6′-trihydroxychalcone (pinocembrin chalcone) **5** as pale yellow needles, m.p. 192°C (Lit.[13] 189-190°C) in 35% yield.



4

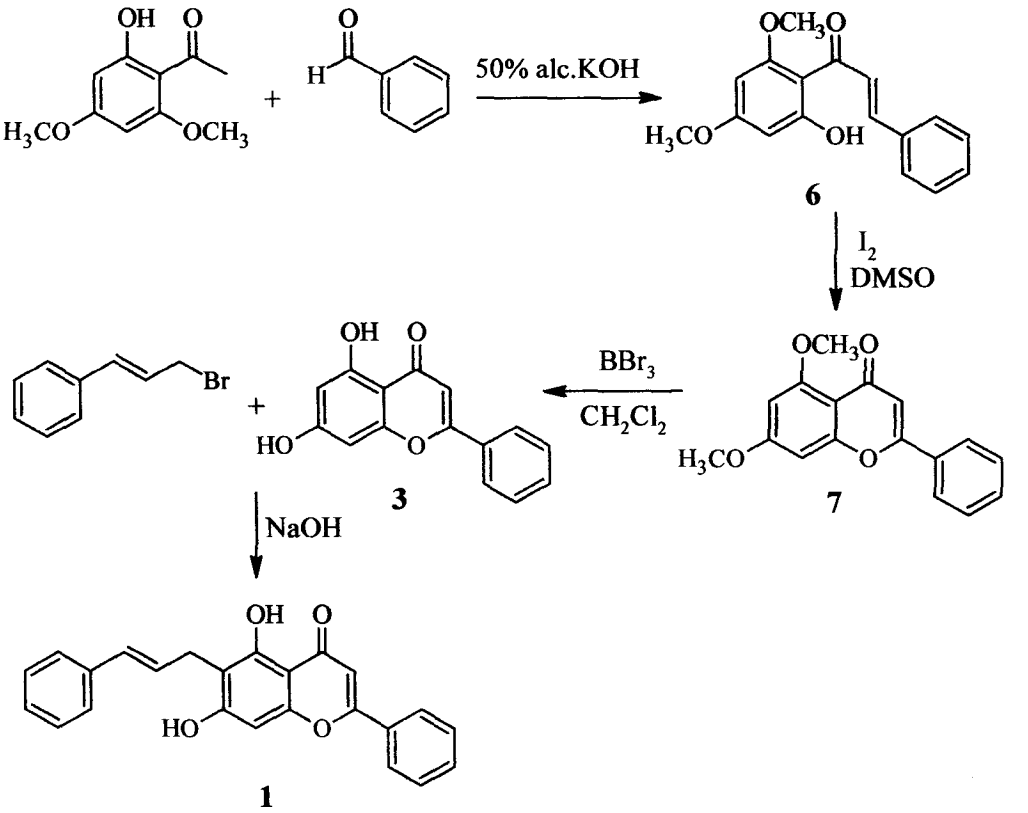


5

Thus we failed to get the required chrysin **3** directly in a single step (scheme 1) and instead obtained the flavanone **4** and the chalcone **5** that too in low yields.

Therefore, a slightly longer route was thought of, which in fact is the usual method employed to prepare **3** (scheme 2).

As phloroacetophenone cannot be converted[15] directly into chalcone **5**, its dimethyl ether[‡] (prepared by methylation of phloroacetophenone using dimethyl sulphate) was used and subsequently converted into chalcone **6**.



Scheme 2

[‡] Dimethyl ether of phloroacetophenone used was prepared for the work included in section 2.1

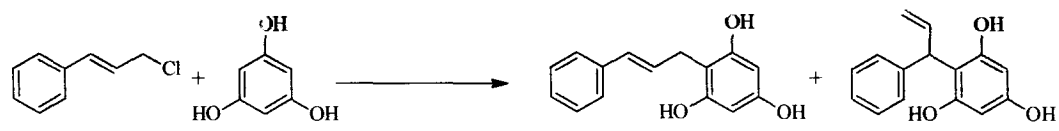
Condensation of 2,4-dimethoxy-6-hydroxyacetophenone with benzaldehyde gave 4′,6′-dimethoxy-2′-hydroxychalcone **6** as yellow solid. Recrystallization from petroleum ether gave bright yellow plates of **6** having m.p. 92°C (Lit.[16] 91.5-92°C) in 78% yield.

Cyclization[17] to the corresponding flavone was carried out by refluxing chalcone **6** in DMSO in presence of catalytic amount of I_2. Usual workup gave 5,7-dimethoxyflavone **7** as buff coloured solid. Recrystallization from petroleum ether:ethyl acetate mixture gave pale yellow needles (0.445 g, 91.3%) of **7** having m.p. 136-138°C (Lit.[18] 142°C).

Demethylation[19] of **7** using BBr_3 in anhydrous CH_2Cl_2 gave crude viscous residue which was indicated to be a mixture of two compounds by TLC. Purification by silica gel column chromatography using petroleum ether:ethyl acetate (8:2) as eluent gave unreacted flavone **7** (co-TLC, m.p. & IR). Further elution with petroleum ether:ethyl acetate (6:4) gave chrysin **3** as yellow amorphous powder, m.p. 275°C (Lit.[20] 278°C) in 51% yield.

The last step in the synthesis of 6-cinnamylchrysin **1** involved introduction of the cinnamyl group at the C_6 position of chrysin **3**.

Interestingly, the selective *C*-alkylation of phloroglucinol has never been reported, the only examples found in the literature were concerning reactions with protected versions of phloroglucinol[21]. However, we came across a comparatively recent (2004) article in which mono-selective and *C*-specific alkylation of phloroglucinol has been achieved[10] with various activated alkyl halides in buffered aqueous solutions which were shown to be crucial to control the regio-selectivity of the reaction.



2 equiv	phosphate buffer 0.2 M, pH 7.8	35%	35%
1.25 equiv	NaOH (1.25 equiv), H_2O/EtOH (15/85, v/v)	69%	traces

We used this procedure for *C*-alkylation of chrysin **3** which was dissolved in ethanolic solution of aqueous NaOH and stirred with excess of cinnamyl bromide[12] at room temperature for 3 hrs as reported[10]. However, TLC of the reaction mixture indicated that the reaction was incomplete. Stirring was continued at room temperature for another 3 hrs. Workup of the reaction mixture left a sticky solid which was washed successively with hexane and finally with ethyl acetate to give yellow amorphous solid having m.p. 268°C (dec) in 69% yield. Melting point of **1** was not reported[2]. The yellow solid obtained was fully characterised by recording its IR, ^{1}H NMR, ^{13}C NMR and ESIMS data and was found to be identical in all respects except some discrepancies observed in the ^{1}H NMR spectrum of natural and our synthetic **1** which are discussed below.

Its IR spectrum showed the presence of hydroxyl (3196 cm^{-1}) and carbonyl (1649 cm^{-1}) groups as expected.

Besides the signals for the ten aromatic protons of the two phenyl groups, the ^{1}H NMR spectrum of our synthetic **1** showed three 1H each singlets and two 2H each singlets.

The three 1H singlets were as expected and reported at δ 6.36 (C_8-H, Ar-H), 6.96 (C_3-H, -CO-C<u>H</u>=C) and 13.15 (C_5-O<u>H</u>, chelated to the CO).

But the two singlets, each for 2H were not as expected and reported. Surprisingly, no coupling was observed between the two olefinic cinnamyl protons as both of them appeared as a 2H singlet at δ 6.36. However, the ^{13}C NMR spectrum did show two signals at δ 127.1 and 129.0 as reported for the natural **1**. Similarly, the two methylene protons at C_1'' neither showed geminal nor vincinal coupling and appeared as a 2H singlet at δ 3.44.

The assignments for the various protons of **1** are shown below in figure I.

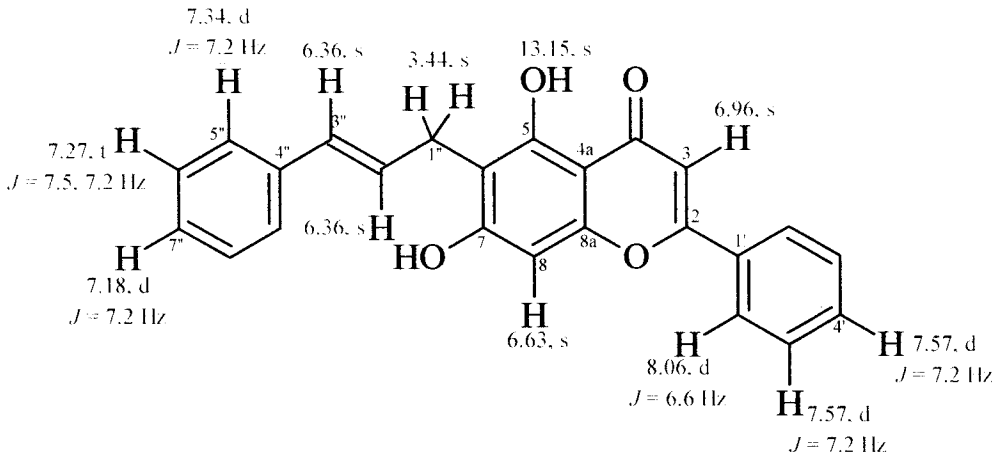


Figure I: ^{1}H NMR assignments for the various protons of synthetic **1**

However, the ^{13}C NMR spectrum of our synthetic **1** showed 20 signals for all the 24 carbons present in the molecule as expected and all the ^{13}C values for the respective carbons matched well with that reported[6] without having any discrepancy.

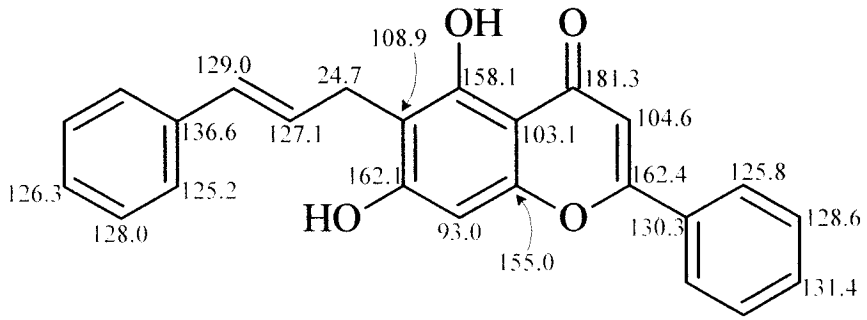


Figure II: ^{13}C NMR spectral assignments of **1**

Table 1: Comparison of ^{1}H and ^{13}C NMR data for synthetic and natural **1**

Position	^{1}H NMR of **1** (δ_H)		^{13}C NMR of **1** (δ_C)	
	synthetic	natural	synthetic	natural
2	—	—	162.4	162.7
3	6.96, s	6.90, s	104.6	105.4
4	—	—	181.3	181.7
4a	—	—	103.1	103.7
5	—	—	158.1	158.6
6	—	—	108.9	109.3
7	—	—	162.1	162.1
8	6.63, s	6.57, s	93.0	93.2
8a	—	—	155.0	155.4
5-OH	13.15, s	13.09, s	—	—
7-OH	—	10.86, s	—	—
1′	—	—	130.3	130.7
2′,6′	8.06, d J = 6.6 Hz	8.03, d J = 6.4 Hz	125.8	126.1
3′,5′	7.57, d J = 7.2 Hz	7.56, m	128.6	128.8
4′	7.57, d J = 7.2 Hz	7.56, m	131.4	131.5
1″	3.44, s	3.45, d J = 5.1 Hz	24.7	25.1
2″	6.36, s	6.31, dt J = 16.1, 5.1 Hz	127.1	127.5
3″	6.36, s	6.37, d J = 16.1 Hz	129.0	129.4
4″	—	—	136.6	137.1
5″,9″	7.34, d J = 7.2 Hz	7.31, d J = 7.3 Hz	125.2	125.5
6″,8″	7.27, t J = 7.5, 7.2 Hz	7.25, dd J = 7.6, 7.3 Hz	128.0	128.2
7″	7.18, d J = 7.2 Hz	7.15, br t J = 7.6 Hz	126.3	126.5

In the ESIMS spectrum of **1** a peak at *m/z* 371.2079 $[M + H]^+$ and 393.1883 $[M + Na]^+$ indicated its molecular formula to be $C_{24}H_{18}O_4$, further confirming the structure of **1**.

In conclusion we have succeeded in synthesizing the novel natural compound 6-cinnamylchrysin **1** in good yield. To our knowledge this appears to be the first and the only report of its synthesis.

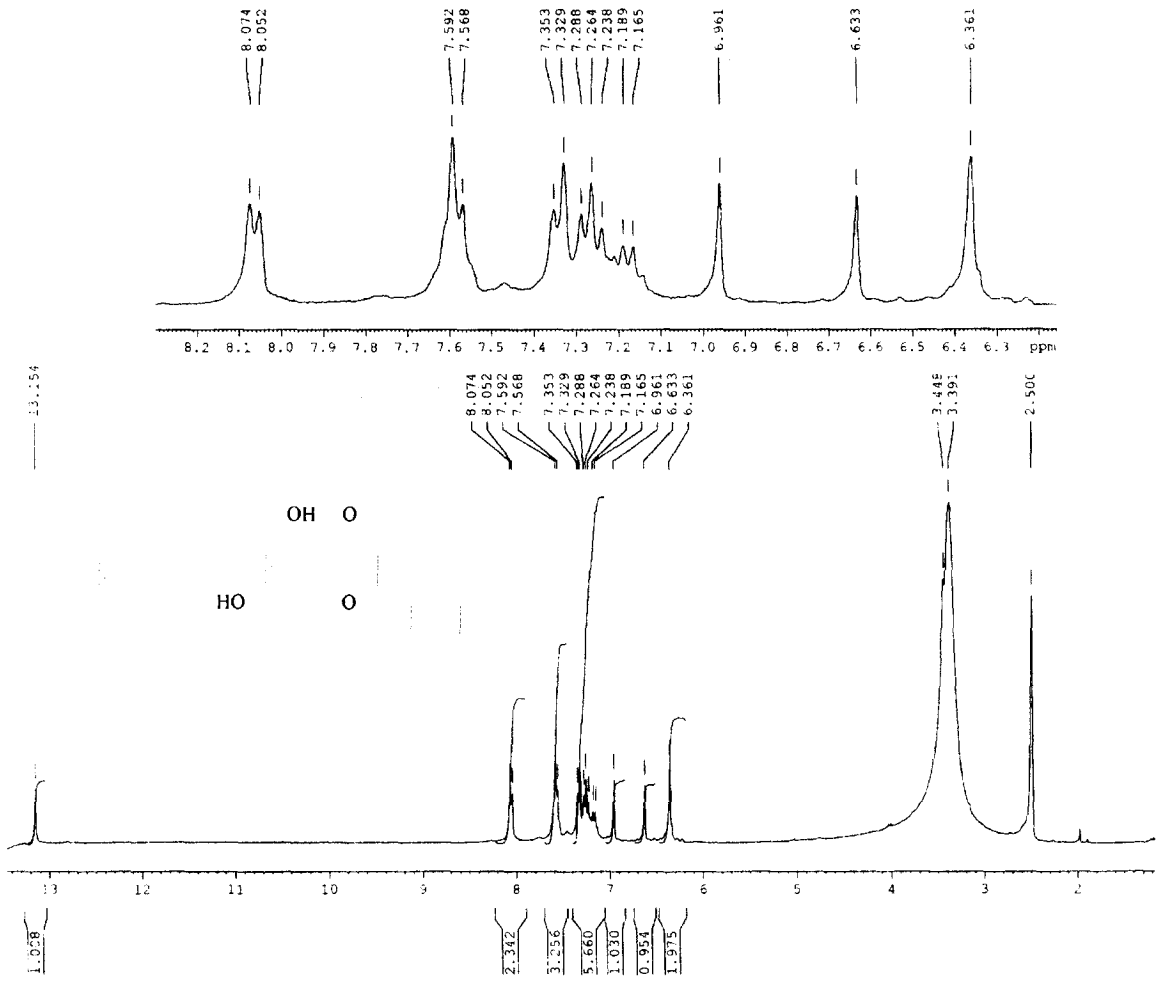


Fig. 2.48: ^{1}H NMR spectrum of **1**

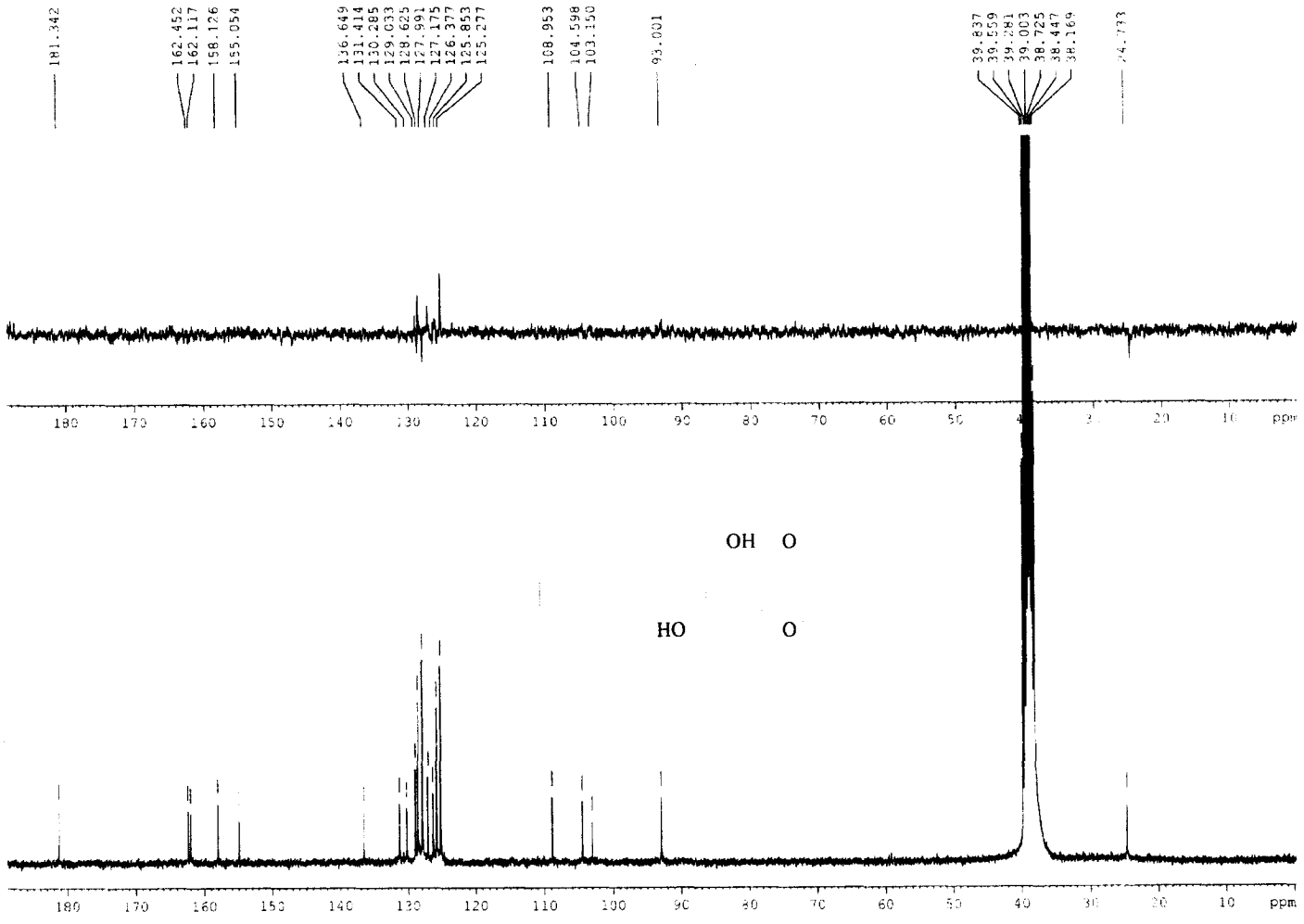


Fig. 2.49: ^{13}C NMR spectrum of **1**

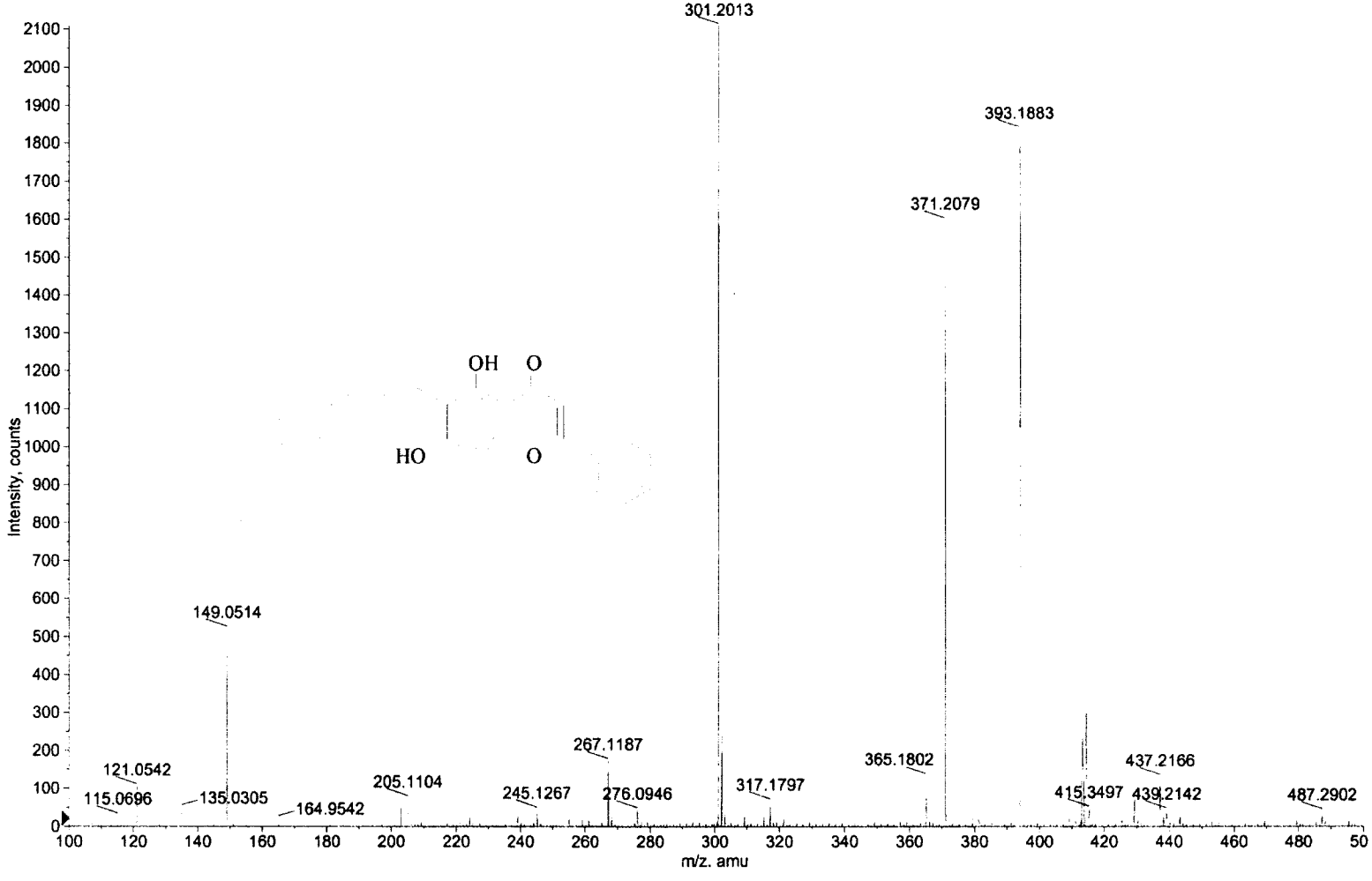
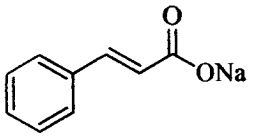
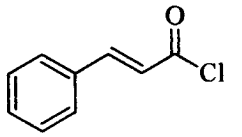


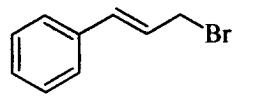
Fig. 2.50: ESIMS spectrum of **1**

Experimental

Sodium cinnamate, Cinnamoyl chloride[11] and *Cinnamyl bromide*[12]

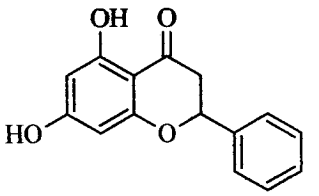






Prepared according to the reported literature procedures[11,12].

5,7-Dihydroxyflavanone (*Pinocembrin*) **4**

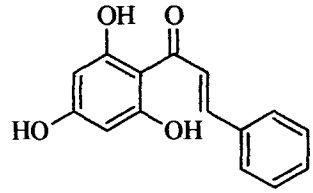


4

Nitrobenzene was purified by steam distillation followed by extraction in diethyl ether solvent and drying.

Phloroglucinol (0.504 g, 4 mmole), cinnamoyl chloride (0.666 g, 4 mmole) and anhydrous $AlCl_3$ (1.335 g, 10 mmole) in nitrobenzene (7 mL) was stirred at room temperature overnight. The reddish syrupy liquid obtained was poured over crushed ice containing conc. HCl (2 mL). Bright yellow solid that separated out turned into a sticky mass on standing. It was subjected to steam distillation and the residue left over was cooled and extracted with CH_2Cl_2 (3 × 5 mL). The combined organic extracts were washed with water (2 × 5 mL), dried over Na_2SO_4 and evaporated to leave oily residue (0.9906 g). Purification by silica gel column chromatography using petroleum ether:$CHCl_3$ as eluent gave the pinocembrine **4** as yellow solid (0.079 g, 8%) having m.p. 210°C, Lit.[13] 203-204°C.

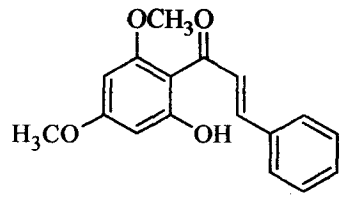
2',4',6'-Trihydroxy chalcone (*Pinocembrin chalcone*) **5**



5

To a solution of phloroglucinol (1.0 g, 7.93 mmole) and cinnamoyl chloride (1.3 g, 7.8 mmole) in nitrobenzene (20 mL) was added drop by drop a solution of anhydrous $AlCl_3$ (1.49 g, 0.01 mole) in nitrobenzene (10-15 mL). After completion of addition, the reaction mixture was stirred at 50-60°C for 150 hrs. The dark coloured reaction mixture was poured onto crushed ice containing conc. HCl. The mixture was then subjected to steam distillation to remove nitrobenzene. On cooling tiny yellow crystals were seen deposited in the residual mass. It was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with water (2 × 10 mL), dried over Na_2SO_4 and evaporated to leave crude residue (1.958 g). Purification by silica gel column chromatography using petroleum ether:$CHCl_3$ as eluent gave pinocembrin chalcone **5** as pale yellow solid (0.719 g, 36%). Recrystallization from $CHCl_3$ gave pale yellow needles m.p. 192°C, Lit.[13] 189-190°C.

2'-Hydroxy-4',6'-dimethoxychalcone **6**



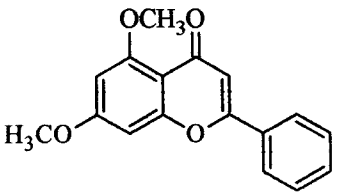
6

A solution of 2-hydroxy-4,6-dimethoxyacetophenone (1.009 g, 5.14 mmole) and benzaldehyde (0.6 g, 5.64 mmole) in ethanol (20 mL) and aqueous NaOH (50%, 10 mL) was stirred at 45°C for 1 hr and then at room temperature overnight. The

yellow coloured reaction mixture was diluted with water and acidified with conc. HCl with cooling. Yellow solid that separated out was filtered, washed with water and dried to give 2′-hydroxy-4′,6′-dimethoxychalcone **6** (1.1421 g, 78.11%). Recrystallization from petroleum ether gave yellow plates having m.p. 92°C, Lit.[16] 91.5-92°C.

IR ν_{max} (KBr): 1620, 1585, 1344, 1219, 1112, 974, 817 cm^{-1}.

5,7-Dimethoxyflavone **7**

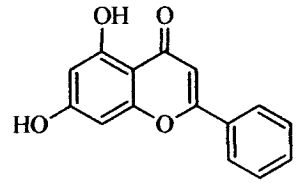


7

A solution of 2′-hydroxy-4′,6′-dimethoxychalcone **6** (0.491 g, 1.7 mmole) in dry DMSO (6 mL) was heated to reflux in presence of catalytic amount of I_2 for 30 mins. The reaction mixture was cooled to room temperature and diluted with water (10 mL). The aqueous portion was extracted with $CHCl_3$ (3 × 10 mL). The combined organic extracts were washed with dilute $Na_2S_2O_3$ (2 × 5 mL) to remove excess of I_2, water (2 × 5 mL), dried over Na_2SO_4 and evaporated to leave 5,7-dimethoxyflavone **7** (0.4446 g, 91.3%) as buff coloured solid. Recrystallization from petroleum ether-ethyl acetate mixture gave pale yellow needles having m.p. 136-138°C, Lit.[18] 142°C.

IR ν_{max} (KBr): 2980, 1643, 1606, 1348, 1217, 1161, 744 cm^{-1}.

5-7-Dihydroxyflavone (Chrysin) **3**

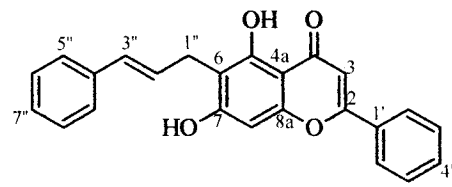


3

To a well stirred and cooled solution of 5,7-dimethoxyflavone **7** (0.6 g, 2.12 mmole) in dry CH_2Cl_2 (25 mL) was added BBr_3 (20 mL, 1M solution in hexane) drop by drop. The reaction mixture was stirred for 2 hrs at 10°C followed by stirring at room temperature for 48 hrs. The orange coloured reaction mixture was poured onto crushed ice and the solid that separated out was filtered, washed with water and dried over Na_2SO_4 to give crude product (0.458 g). TLC of the crude product indicated it to be a mixture of two compounds. Purification by silica gel column chromatography using petroleum ether:ethyl acetate (9:1) as eluent gave 5,7-dimethoxyflavone **7** (0.08 g). Further elution with petroleum ether:ethyl acetate (7:3) gave chrysin **3** (0.276 g, 51%) as yellow powder m.p. 275°C, Lit.[20] 278°C.

IR ν_{max} (KBr): 1651, 1612, 1581, 1489, 1354, 1174, 1095, 802 cm^{-1}.

6-Cinnamylchrysin **1**



1

To a stirred solution of chrysin **3** (0.1 g, 0.4 mmole) in ethanol (2 mL) was added NaOH (0.0196 g, 0.5 mmole) in H_2O (0.2 mL). Cinnamyl bromide (0.097 g, 0.488 mmole) in ethanol (2 mL) was then added drop by drop and stirring was continued for further 6 hrs at room temperature, diluted with water (5 mL) and

extracted with CH_2Cl_2 (2 × 5 mL). The combined organic extracts were washed with brine (2 × 5 mL), dried over Na_2SO_4 and evaporated to leave a dark yellow sticky solid. The sticky solid was washed successively with hexane and ethyl acetate to give 6-cinnamylchrysin **1** as yellow amorphous solid (0.1 g, 69%), m.p. 268°C (decomp).

IR ν_{max} (KBr): 3196, 1649, 1614, 1581, 1490, 1356, 1305, 1195, 1095 cm^{-1}.

^{1}H NMR (DMSO-d_6, 300 MHz, **Fig.** 2.48): For assignments refer **Figure I**, pg 226.

^{13}C NMR (DMSO-d_6, 75 MHz, **Fig.** 2.49): For assignments refer **Figure II**, pg 226.

ESIMS (Fig. 2.50): *m/z* $[M + H]^+$ calcd for $C_{24}H_{19}O_4$ 371.1277, Found 371.2079 $[M + Na]^+$ calcd for $C_{24}H_{18}NaO_4$ 393.1097, Found 393.1883.

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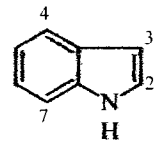
Chapter Three

Section 3.1

Green Synthesis of 2,3-Disubstituted Indoles and 1,2,3,4-Tetrahydrocarbazoles

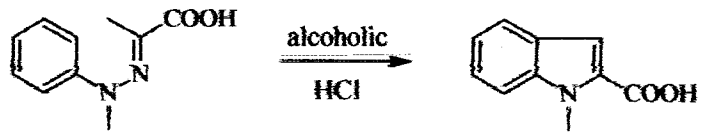
Introduction

Since the first synthesis of indole by Bayer in 1866, more synthetic routes to indoles have probably been published compared to any other heterocyclic or carbocyclic ring system and amongst these routes, the classical Fischer method has been the mainstay of chemists involved in the synthesis of indoles and their derivatives.



Indole

The first report on indolization of an arylhydrazone was published by Fischer and Jourdan in 1883 and was achieved by the reaction of pyruvic acid 1-methylphenylhydrazone with alcoholic HCl. However, it took almost a year for Fischer and Hess to identify the product of this reaction as 1-methylindole-2-carboxylic acid[1].

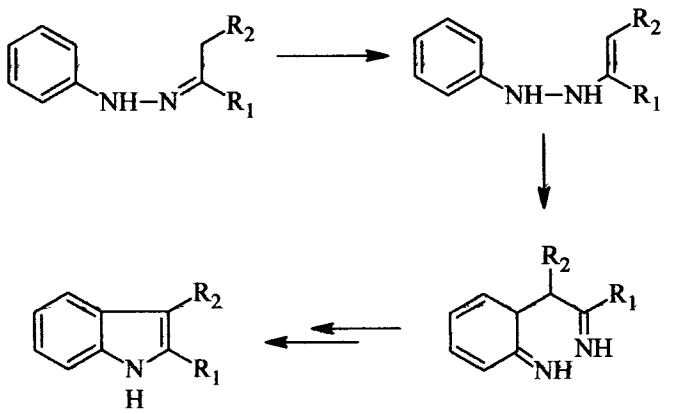


Since this discovery, the reaction has been extensively used for the last more than 120 years and is the most versatile method for the preparation of indoles even today.

There are elaborate review articles reported[1] in the literature on Fischer indole synthesis, its mechanism and applications. Secondly, lots of variations have been reported in the original Fisher indole synthesis.

In its simplest form, the Fischer indole synthesis involves the rearrangement of hydrazones, prepared from arylhydrazines and enolizable ketones, upon heating in acid with loss of ammonia to afford indoles. The use of acid catalyst could be avoided if sufficiently high temperatures are used[1].

The process involves initial tautomerization to an ene-hydrazine that undergoes a [3,3]-sigmatropic rearrangement followed by ring closure and aromatization[2].

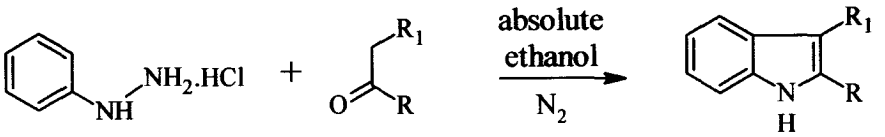


Normally Fischer indole synthesis involves acids as catalysts and acidic workup. Of course, use of acid catalyst is apparently not essential if sufficiently high temperatures are used[1]. Several phenylhydrazones were successfully indolized by non-catalytic thermal reaction. Phenylhydrazones have also been thermally rearranged to the corresponding indoles in the presence of NaOH with or without solvent[1].

Our present work is in fact a minor modification of the reported[3] Fischer-indole synthesis so as to make the process green. This method avoids the use of customary higher temperatures and corrosive mineral and Lewis acids as catalysts or during workup. Instead we have used ethanol as the solvent and acetic acid for acidification of the reaction mixture before workup. The

temperatures involved are just the refluxing temperature of ethanol which is 77°C. Therefore we would like to consider this method to be a green process which neither makes use of high temperatures nor any toxic or corrosive chemicals.

It involved heating to reflux a mixture phenylhydrazine hydrochloride **1** (3.45 mmole), the appropriate ketone (3.28 mmole) in absolute ethanol (25 mL) under N_2 atmosphere for 6 hrs. The *in-situ* generated acid catalyzes the formation of indole nucleus. The workup of the reaction mixture was carried out using acetic acid instead of HCl[3] affording the indole derivatives in pure form which were further recrystallised using appropriate solvent system.



1 **2** to **5** **6** to **9**

Synthesis of 2,3-disubstituted indoles

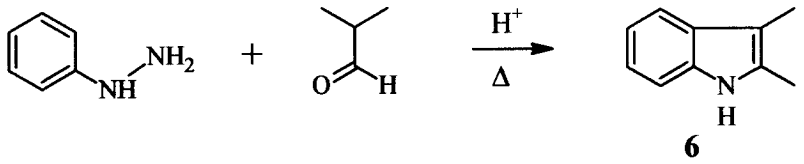
To begin with we used this method to prepare successfully four 2,3-disubstituted indole derivatives (**6** to **9**) in yields ranging from 70% to quantitative.

1) 2,3-Dimethylindole 6

Heating to reflux a mixture of phenylhydrazine hydrochloride **1** and butanone **2** in absolute ethanol under N_2 atmosphere for 6 hrs followed by neutralisation of the cooled reaction mixture with glacial acetic acid gave 2,3-dimethylindole **6** as pale pink solid in **85% yield**. Recrystallization from petroleum ether afforded pale pink shiny flakes, m.p. 102°C (Lit.[4] 104-106°C).

2 6

2,3-Dimethylindole **6** has been synthesized[5] from phenylhydrazine and 2-methylpropanaldehyde in **50% yield**.



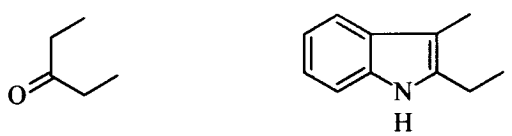
6

It has also been prepared[1] by *p*-toluenesulfonic acid catalyzed indolization of butanone phenylhydrazone in the presence of acetic anhydride followed by treatment with acid or distillation from Zn dust.

Indolization of phenylhydrazones of methyl ketones are known to give exclusively the corresponding 3-substituted-2-methyl indoles[1]. However, indolization of butanone phenylhydrazone has been reported[6] to give not only 2,3-dimethylindole **6** but also small amounts of 2-ethylindole.

2) 2-Ethyl-3-methylindole 7

Similarly reaction of phenylhydrazine hydrochloride **1** with 3-pentanone **3** gave, after workup with glacial acetic acid, 2-ethyl-3-methylindole **7** as a viscous oil which solidified on standing in **quantitative yield**.

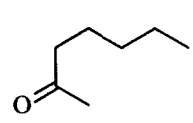


3 7

Recrystallization from hexane gave **7** as cream coloured flakes having m.p. 66°C (Lit.[4] 64-66°C).

3) 3-Butyl-2-methylindole 8

Reaction of 2-heptanone **4** and phenylhydrazine hydrochloride **1** gave dark yellow oil[8] of 3-butyl-2-methylindole **8** in **95% yield**.



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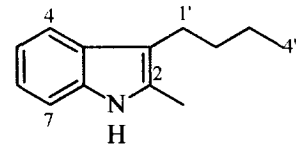


8

The indole **8** being a liquid its structure was determined by the analysis of its ^{1}H NMR data which agreed well with that reported[2].

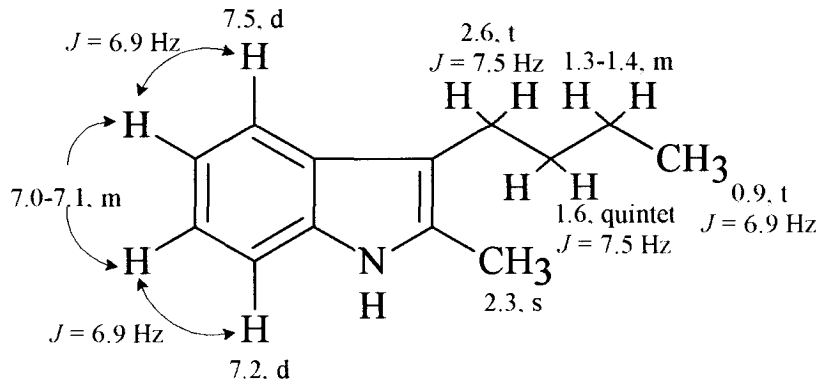
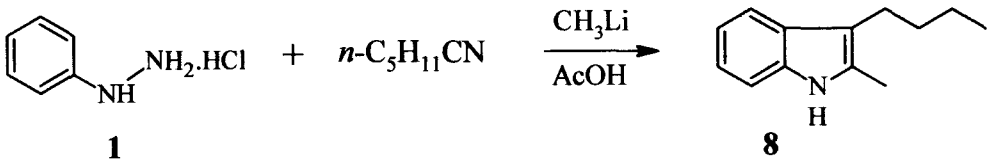


Figure I: Assignments of ^{1}H NMR signals for the various protons of **8**

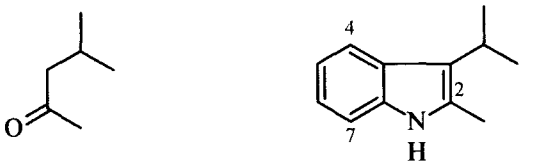
3-Butyl-2-methylindole **8** has been synthesized[2] recently (2005) but in only **54% yield** from hexanenitrile, methyl lithium and **1**. Moreover, this method requires expensive commercially available organolithium reagents or they are to be freshly prepared.



1 **8**

4) 3-Isopropyl-2-methylindole 9

Reaction of 4-methyl-2-pentanone **5** with phenylhydrazine hydrochloride **1** gave 3-isopropyl-2-methylindole **9** as dark yellow oil in only 29% yield. However, we could increase the yield up to **70%** using excess of **1** and increasing the reaction time to 24 hrs.



5 **9**

The indole **9** being a liquid its structure was determined by the analysis of its ^{1}H NMR data which agreed well with that reported[7].

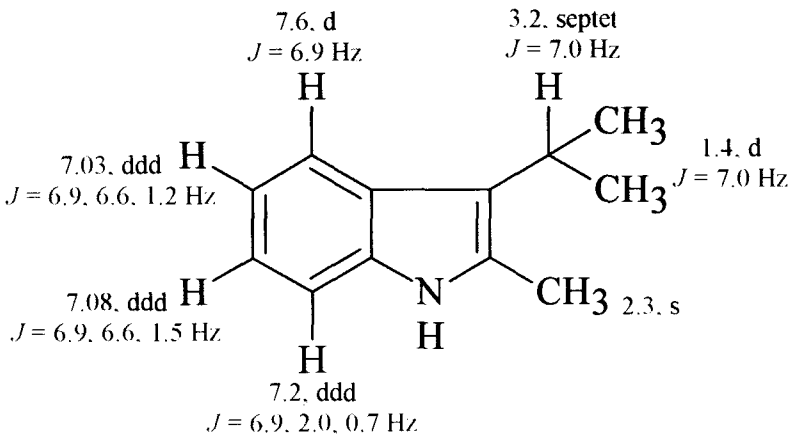
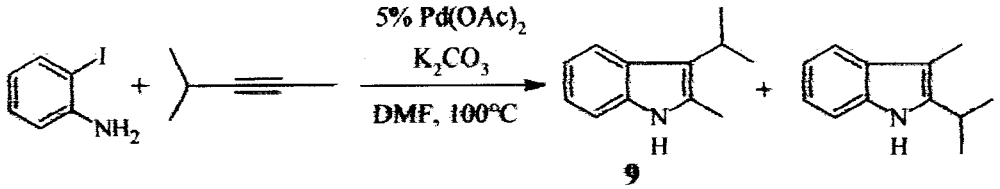


Figure II: Assignments of ^{1}H NMR signals for the various protons of **9**

3-Isopropyl-2-methylindole **9** has been prepared[7] (1998) along with 2-isopropyl-3-methylindole by the Pd catalyzed reaction of 2-iodoaniline and 4-methylpent-2-yne in DMF at 100°C in only **25% yield.**



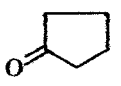
9

We have successfully extended this method for the syntheses of five already reported tetrahydrocarbazole derivatives (**10** to **14**) and three new (**15** to **17**) tetrahydrocarbazole derivatives in yields ranging from 67% to quantitative.

Synthesis of tetrahydrocarbazole derivatives (10 to 14)

5) 1,2,3,4-Tetrahydrocyclopenta[*b*]indole 10

Reaction of cyclopentanone **18** with phenylhydrazine hydrochloride **1** gave after workup 1,2,3,4-tetrahydrocyclopenta[*b*]indole **10** as violet solid in **quantitative yield.** Recrystallization from petroleum ether gave violet crystals having m.p. 94°C.



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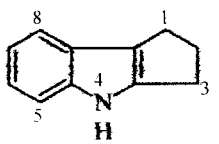


10

Indole **10** has been synthesized[8] (1999) in **75% yield** using phenylhydrazine and cyclopentanone using zeolite as catalyst. We could not find

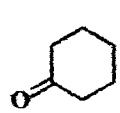
the m.p. of the indole **10** reported in the literature hence it was characterised by IR and ^{1}H NMR spectral data.

The ^{1}H NMR spectrum of **10** showed three 2H singlets at δ 3.93 (C_3-H), 3.86 (C_1-H) and 3.79 (C_2-H). Further a 1H singlet at δ 6.37 (C_7-H), two 1H doublets (J = 8.7 Hz) at δ 6.94 (C_6-H) & 7.62 (C_5-H) and a 1H singlet at δ 7.87 (C_8-H) supported the formation of **10**.

6) 2,3,4,9-Tetrahydro-1*H*-carbazole 11 and

7) 5,6,7,8,9,10-Hexahydrocyclohepta[*b*]indole 12

Similarly the reaction of cyclohexanone **19** and cycloheptanone **20** with phenylhydrazine hydrochloride **1** gave 2,3,4,9-tetrahydro-1*H*-carbazole **11** and 5,6,7,8,9,10-hexahydrocyclohepta[*b*]indole **12** as solids in quantitative and 72% yield respectively.



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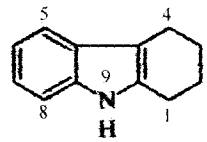
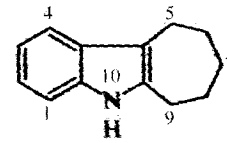


11



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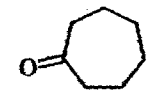
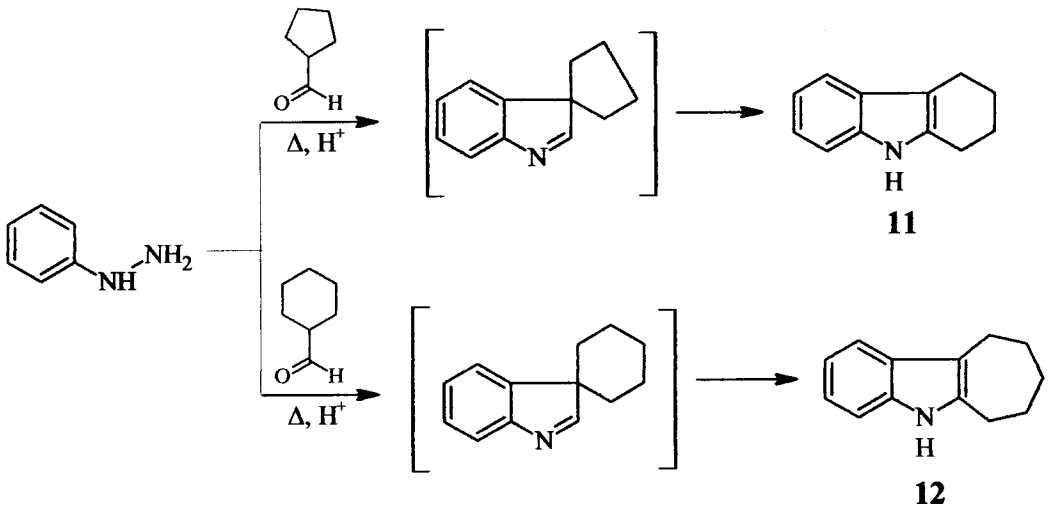


20

Recrystallization from hexane gave colourless plates of **11** having m.p. 110°C (Lit.[4] 110-114°C).

Hexahydrocarbazole **12** was obtained as pale yellow plates from ethanol having m.p. 134°C.

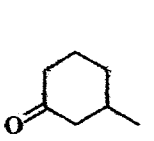
Compounds **11** and **12** have been synthesized[5] (2006) in 54% and 55% yields by the reaction of phenylhydrazine with cyclopentanaldehyde and cyclohexanaldehyde respectively as shown below.



The m.p. of **12** is not reported[5] and hence it was characterised by comparison[5] of its NMR data which showed a 4H triplet (J = 2.7 Hz) at δ 1.86 ($C_{6,7}$-H), a 2H doublet (J = 5.1 Hz) at δ 1.97 (C_8-H), two 2H triplets (J = 6.0 Hz) at δ 2.83 (C_5-H) & 2.9 (C_9-H), a multiplet between 7.18-7.22 ($C_{2,3}$-H), a 1H doublet (J = 7.2 Hz) at δ 7.28 (C_1-H) and a 1H doublet (J = 8.4 Hz) at δ 7.57 (C_4-H) supporting the formation of **12**. The data recorded was in accordance with that reported on **12** in the literature[5].

8) 2-Methyl-2,3,4,9-tetrahydro-1*H*-carbazole 13

Some conflicting results are reported[9] for the indolization of 3-methylcyclohexanone phenylhydrazone claiming that both the possible isomers 2-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **13** and 4-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **13a** were formed. According to Grammaticakis[10] the indolization of 3-methylcyclohexanone phenylhydrazone affords only **13**, but in two other reports, the 2-methyl isomer **13** was isolated in low[11] and unspecified yield[12] suggesting that the 4-methyl isomer **13a** might have been produced but was not isolated.



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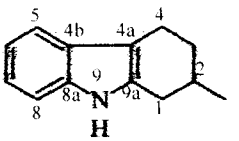
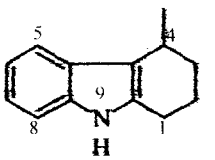


13



13a

Interestingly when we carried out this reaction of 3-methylcyclohexanone **21** with phenylhydrazine hydrochloride **1** using our method we got 2-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **13** as wine red solid **in quantitative yield.** Recrystallization from petroleum ether afforded **13** as wine red cubes having m.p. 90°C. Neither m.p. nor the spectral data was available on **13** in the literature[11]. Therefore, we characterized it fully by recording its spectral data.

The ^{1}H and ^{13}C NMR assignments for the various protons and carbons of **13** are shown below in figures III & IV.

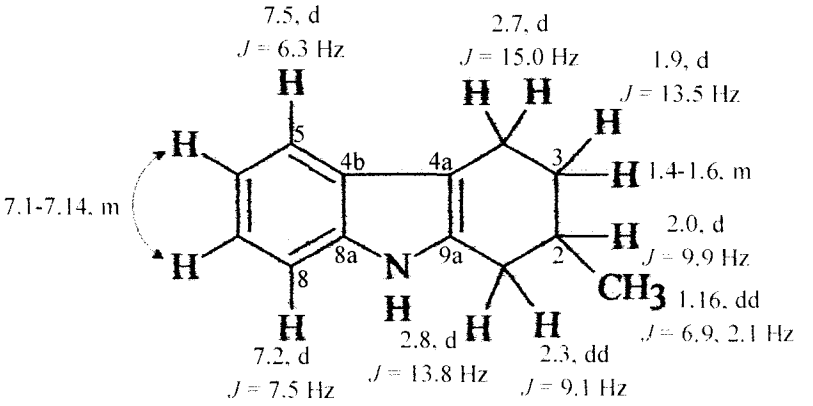


Figure III: Assignments of ^{1}H NMR signals for the various protons of 13

In the ^{13}C NMR spectrum of **13**, thirteen distinct signals (four quarternary, five methines, three methylenes and a methyl) were observed for all the 13 carbons of **13** as expected.

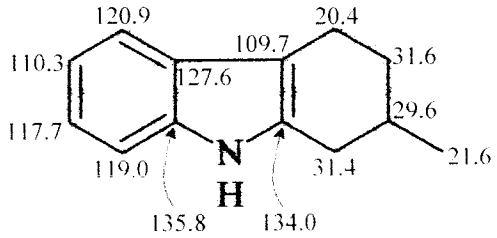


Figure IV: Assignments of ^{13}C NMR signals for the various carbons of **13**

To confirm the position of the methyl group HMBC and HMQC correlation studies were carried out which fully supported the structure **13**.

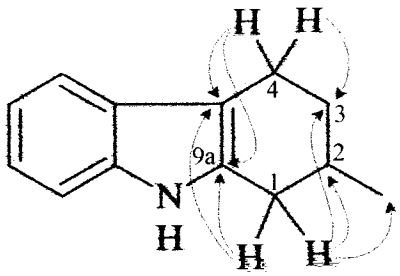


Figure V: Selected HMBC of **13**

The ^{1}H and ^{13}C NMR assignments were made on the basis of HMBC correlations. The possibility of formation of **13a** was ruled out since in the HMBC correlation spectrum, two methylene groups (C_1 & C_4) showed correlations to C-4a & C-9a which is possible only if the methyl group is at C-2. This was further supported by the correlation of C_1-H to C-2 and C_2-CH_3. Thus confirming the formation of **13**.

9) 5,11-Dihydro-6*H*-benzo[*a*]carbazole 14

Reactions of 1-tetralone* **22** with phenylhydrazine hydrochloride **1** gave after workup 5,11-dihydro-6*H*-benzo[*a*]carbazole **14** as colourless solid in **76% yield**.

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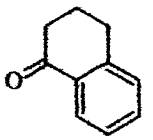
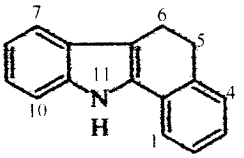
*1-Tetralone **22** was prepared by Jones oxidation of tetralin. We are thankful to Savia P. Torres for a sample of **22**.



22



14

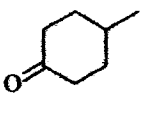
Recrystallization from petroleum ether-$CHCl_3$ gave colourless cubes having m.p. 158°C [Lit.[13] (1930) m.p. 160-161°C].

Its ESIMS data showed a peak at *m/z* 242.1 $[M + Na]^+$ indicating its molecular formula to be $C_{16}H_{13}N$ as expected.

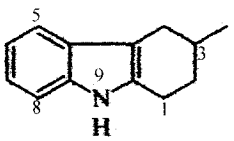
Synthesis of new tetrahydrocarbazole derivatives (15 to 17)

10) 3-Methyl-2,3,4,9-tetrahydro-1*H*-carbazole 15

Reaction of 4-methylcyclohexanone **23** with phenylhydrazine hydrochloride **1** gave 3-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **15** as colourless solid in **93% yield**. Recrystallization from hexane gave colourless plates having m.p. 118°C.



23



15

The pattern of signals observed in the ^1H NMR spectrum of **15** was similar to that obtained for 2-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **13** and the assignments of various protons in **15** are shown in figure VI.

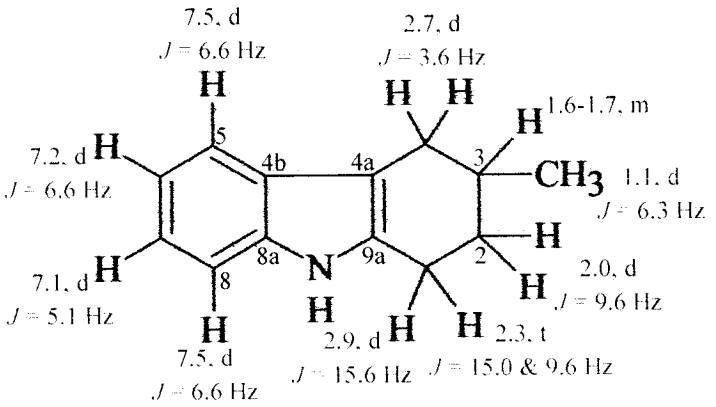


Figure VI: Assignments of ^{1}H NMR signals for the various protons of 15

The ^{13}C NMR spectrum of **15** was also similar that of **13** and showed thirteen distinct signals for all the 13 carbons and their assignments are shown below in figure VII.

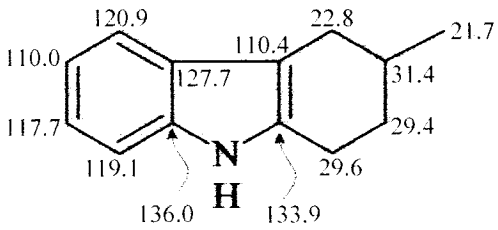


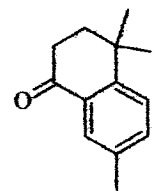
Figure VII: Assignments of ^{13}C NMR signals for the various carbons of **15**

The ESIMS data on **15** showed a peak at *m/z* 185.5 [M^{+}] indicating its molecular formula to be $C_{13}H_{15}N$ as expected.

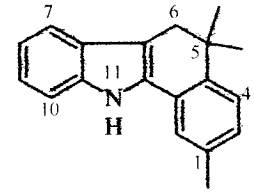
11) 2,5,5-Trimethyl-2,3,4,5,6,11-hexahydro-1*H*-benzo[*a*]carbazole 16.

Reaction of 4,4,7-trimethyl-1-tetralone[†] **24** with phenylhydrazine hydrochloride **1** gave after workup 2,5,5-trimethyl-2,3,4,5,6,11-hexahydro-1*H*-benzo[*a*]carbazole **16** as a brown solid in **67% yield**.

[†] 4,4,7-Trimethyl-1-tetralone **24** was prepared by Jones oxidation of ionene which in turn was prepared from α- & β-ionones. We are thankful to Savia P. Torres for a sample of **24**.



24



16

Recrystallization from petroleum ether-$CHCl_3$ gave pale brown flakes, **m.p. 190°C. It is a new indole derivative and hence was fully characterized by** recording its MS, ^1H, and ^{13}C NMR data.

The ESIMS data of 16 showed a peak at *m/z* 261.5 [M^+] indicating its molecular formula to be $C_{19}H_{19}N$ as expected.

The ^1H NMR spectrum of 16 showed a 6H singlet at δ 1.4 (*gem*-dimethyl group at C_5), a 3H singlet at δ 2.5 (Ar-C_2-$\underline{CH_3}$), and a 2H singlet at δ 2.9 (-$\underline{CH_2}$- at C_6). **The ^{13}C NMR spectrum of 16 showed 18 signals as expected. The assignments of the ^1H & ^{13}C NMR signals are shown below in figures VIII & IX.**

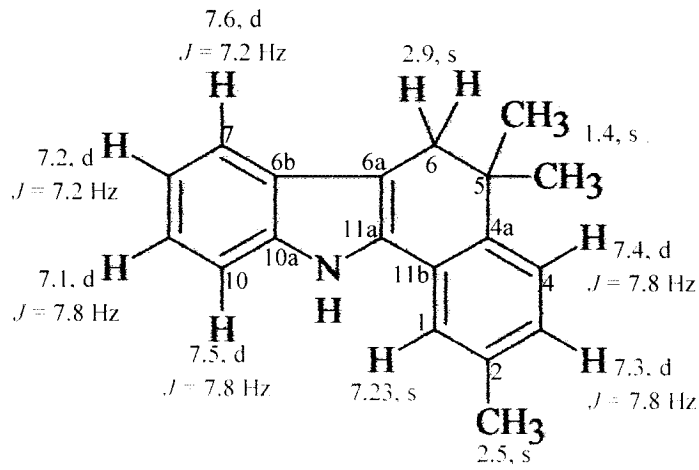


Figure VIII: Assignments of ^1H NMR signals for the various protons of **16**

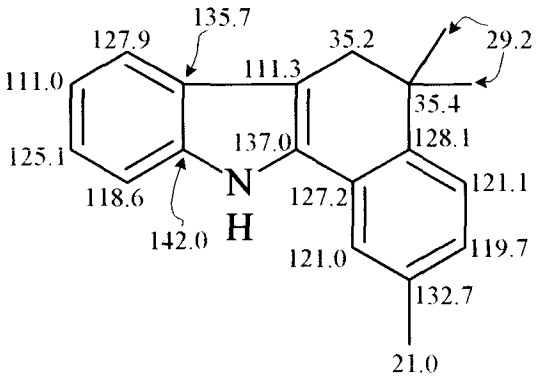
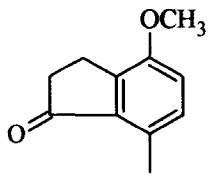


Figure IX: Assignments of ^{13}C NMR signals for the various carbons of **16**

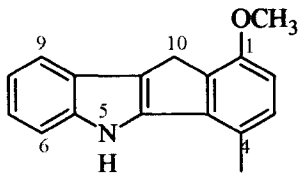
12) 1-Methoxy-4-methyl-5,10-dihydro-indeno[1,2-*b*]indole 17

Reaction of 4-methoxy-7-methyl-1-indanone‡ **25** with phenylhydrazine hydrochloride **1** gave 1-methoxy-4-methyl-5,10-dihydro-indeno[1,2-*b*]indole **17** as pale yellow solid in **quantitative yield**.

Recrystallization from petroleum ether gave pale yellow cotton like threads, m.p. 128-130°C (decomp).



25



17

Compound **17** is also new and was fully characterized by the study of its spectral data. Its ^1H NMR spectrum showed 3 characteristic singlets at δ 2.63 (3H, Ar-C<u>H</u>$_3$), δ 3.54 (2H, -C$_{10}$<u>H</u>$_2$-) and δ 3.96 (3H, Ar-OC<u>H</u>$_3$). The assignments for the various protons in **17** are shown below in figure X.

‡ 4-Methoxy-7-methyl-1-indanone **25** was prepared from 6-methylcoumarin in 3 steps. We are thankful to Jose C Menezes for a sample of **25**.

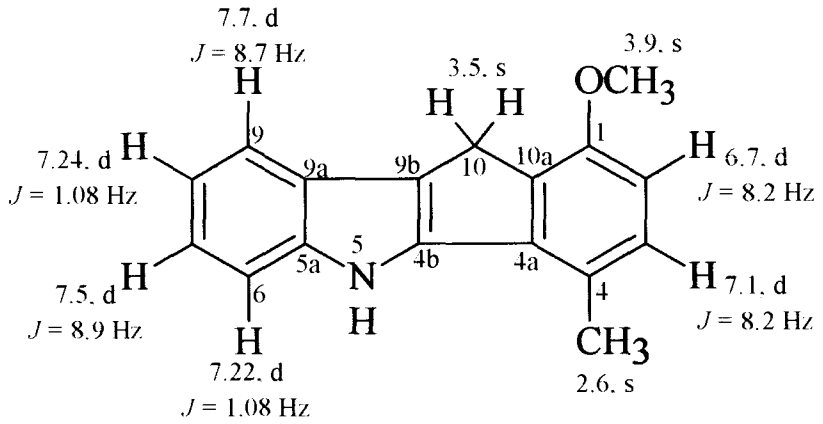


Figure X: Assignments of ^{1}H NMR signals for the various protons of **17**

Its ^{13}C NMR spectrum showed seventeen signals for the seventeen carbons and the assignments for the various carbons are shown below in figure XI.

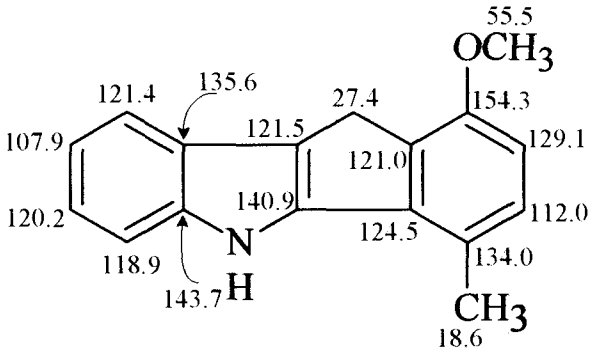
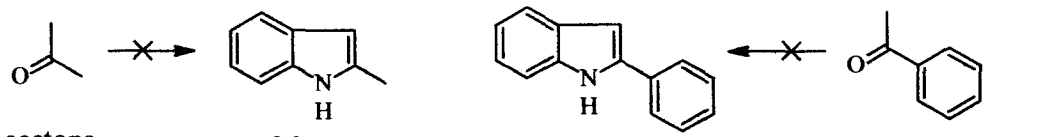


Figure XI: Assignments of ^{13}C NMR signals for the various carbons of **17**

The ESIMS data of **17** showed a peak at *m/z* 249.5 [M^{+}] indicating it to have the molecular formula $C_{17}H_{15}NO$.

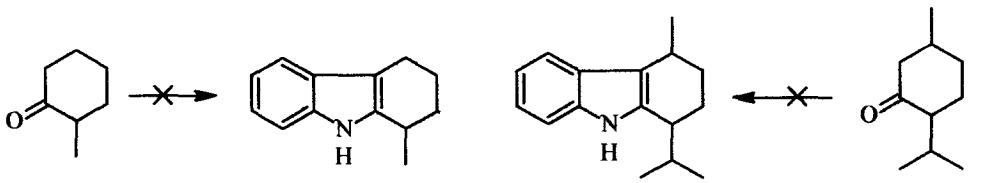
2-Methylindole **26** and 2-phenylindole **27** have been prepared[4] by desulfurization of 3-thioalkoxyindoles in 79 and 74% yield respectively.

Attempts to prepare **26** from acetone and **27** from acetophenone using our above discussed method did not work and instead gave tarry residue after workup.



acetone **26** **27** acetophenone

Similar results were obtained with 2-methylcyclohexanone and menthone.



2-methylcyclohexanone menthone

The formation of dark tarry by-products in some cases during Fischer indolization have been reported[1]. Camphor when subjected to similar reaction conditions was recovered unchanged.

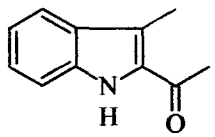
Synthesis of Salvadoricine, constituent of *Salvadora persica*

Introduction

A large number of indole alkaloids have been isolated from a variety of plants and among them 2-acylindole alkaloids form one of the representatives[14]. Moreover, substituted 2-acylindoles also form a class of pharmacologically active compounds.

The plant *Salvadora Persica* (Salvadoraceae) is known in Pakistan as "Peelu" and is used in folk medicine for the treatment of human ailments. The leaves of this plant are used as a reputed diuretic and also as an odontological remedy (dentifrice to detoxify and strengthen the weakened gums)[15].

From the ethanolic extracts of the fresh (not dried) leaves (10 kg) of *Salvadora persica* only 12 mg of the alkaloid salvadoricine (2-acetyl-3-methylindole) **28**, was isolated[15] as a white crystalline solid, m.p. 143-144°C. Its structure was elucidated by spectral analysis and confirmed by synthesis using previously reported method[16] and obtained only 32% yield.

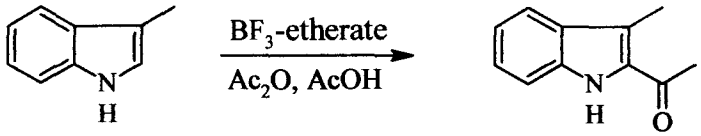


28
Salvadoricine

This is the first and the only report on the isolation of **28** as a natural product, although the compound 2-acetyl-3-methylindole **28** was prepared before[14]. We have presented below all the available syntheses of 2-acetyl-3-methylindole **28** reported before and after it was called salvadoricine so as to make a comparison with our method and also to have them at one place for ready reference.

Reported syntheses of 2-acetyl-3-methylindole 28

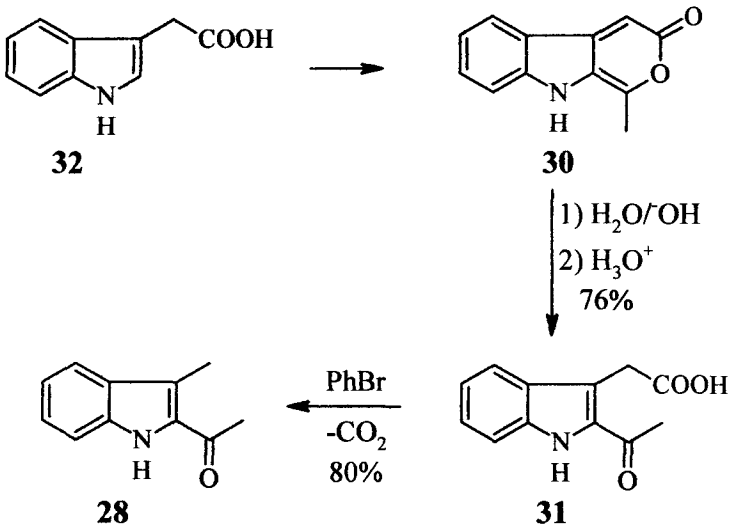
Prior to isolation of salvadoricine alkaloid, Jackson et al[16], during their studies on the acetylation of 3-methyl indole **29** using BF_3-etherate obtained 2-acetyl-3-methylindole **28** in quantitative yield. However, the required starting 3-methylindole **29** had to be prepared or purchased.



29 **28**

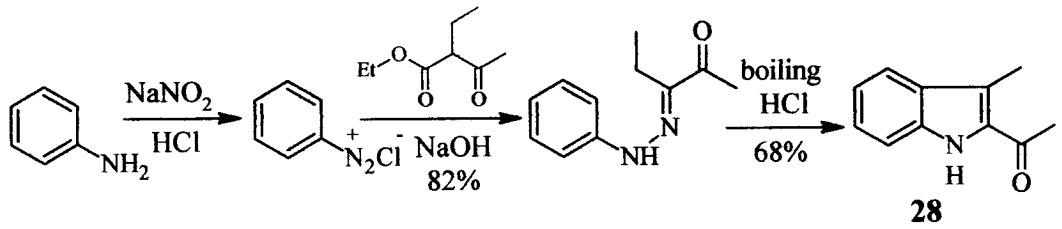
Reported syntheses of salvadoricine 28

1) Salvadoricine was synthesized by Pindur and Abdoust[17] in three steps as shown below. Hydrolysis of methylated pyranoindolone **30** gave 2-acetylindole-3-acetic acid **31** in 76% yield. Decarboxylation of **31** using bromobenzene gave salvadoricine **28** in 80% yield. However, the required starting pyranoindolone **30** had to be prepared from indole-3-acetic acid **32** using reported method[18].



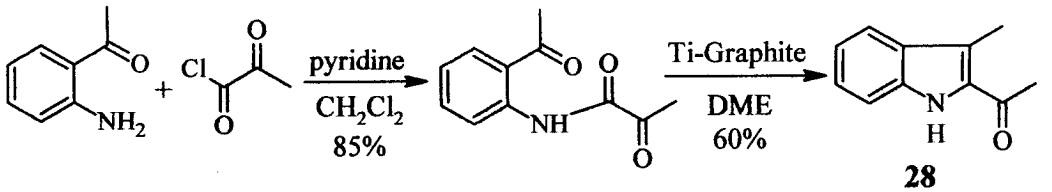
32 **30** **28** **31**

2) During the synthesis of 3,5-disubstituted-2-acetylindoles, Rajur and coworkers[19] prepared 2-acetyl-3-methylindole **28** without knowing that it is salvadoricine in three steps as shown below.



28

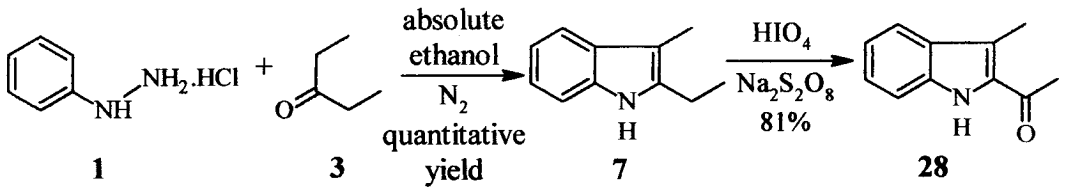
3) Furstner and Jumbam[20] have used the titanium on graphite induced reductive coupling of carbonyl compounds (McMurry reaction) to prepare **28** in two steps as shown below.



28

Our Synthesis of 2-acetyl-3-methylindole (salvadoricine) 28

The method developed for the synthesis of various 2,3-disubstituted indoles in the preceding part was successfully extended for the synthesis of salvadoricine in two steps as shown below.

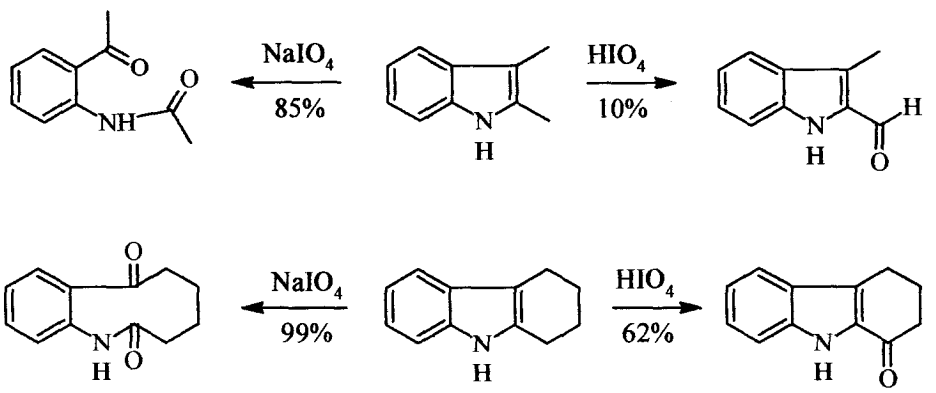


1 **3** **7** **28**

The first step involving the reaction of phenylhydrazine hydrochloride **1** with 3-pentanone **3** was carried out in the preceding part and we obtained 2-ethyl-3-methylindole **7** in quantitative yield.

The second step involved conversion of 2-ethyl-3-methylindole 7 into 2-acetyl-3-methylindole **28.**

Literature[21] survey indicated that periodic acid has been used to selectively oxidize 2,3-disubtituted indoles and also tetrahydrocarbazoles at position 1 to give 2-acylindoles in good yields. Surprisingly oxidation with sodium periodate cleaves the indole double bond to give the corresponding ketoamides as shown below.



Therefore we oxidized **7** with periodic acid and obtained the required 2-acetyl-3-methylindole **26** in 81% yield.

A solution of 2-ethyl-3-methyl indole **7** in methanol was added to excess HIO_4 in methanol-water mixture. Usual workup left a brown residue which was purified by column chromatography. Elution with $CHCl_3$ gave fine pale yellow needles having m.p. 196-198°C.

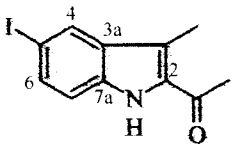
In its IR spectrum bands at 3315 (NH) and 1627 (CO) cm^{-1} were observed.

Its ^1H NMR spectrum showed only five distinct signals instead of the expected six signals. Two 3H singlets at δ 2.50 (C_3-$\underline{CH_3}$) and 2.54 (-$COC\underline{H_3}$)

was indicative of 2-acetyl-3-methyl grouping. Further a 1H doublet at δ 7.24 (J = 8.7 Hz, Ar-H), a 1H doublet of doublet at δ 7.49 (J = 8.7, 1.5 Hz, Ar-H) and a 1H singlet at δ 8.05 indicated it to be a mono-substituted (on the benzene moiety) 2-acetyl-3-methyl indole.

The ^{13}C NMR spectrum showed 11 signals as expected for the 11 carbons present but one of the sp^2 carbon obviously of the benzene moiety is attached to an electronegative atom probably the I atom.

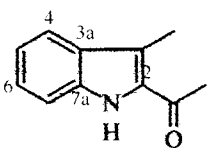
Its ESIMS data showed a peak at m/z 299.9983 $[M + H]^+$ which suggested the molecular formula of this compound to be $C_{11}H_{10}INO$ indicating that iodine is incorporated most probably at the C_5-position giving 2-acetyl-5-iodo-3-methyl-indole **33** which could account for the observed NMR data.



33

The formation of the iodo derivative **33** of the required indole **28** was due to excess of HIO_4 liberating molecular I_2. Hence it was necessary to trap the liberated I_2 and this was achieved by the addition of sodium thiosulfate to the reaction mixture.

A solution of 2-ethyl-3-methyl indole **7** in methanol was added to a solution of sodium thiosulfate and excess of HIO_4 in methanol-water mixture. Usual workup gave a white solid. Recrystallization from petroleum ether gave white cottony threads having m.p. 142°C (Lit.[15] 143-144°C).



28

Its IR spectrum showed bands at 3325(NH) and 1632(CO) cm^{-1}.

In its ^1H NMR spectrum two 3H singlets at δ 2.64 (C_3-C<u>H</u>$_3$) & 2.64(C_2-COC<u>H</u>$_3$) and four signals due to 4 aromatic protons, at δ 7.14 (dd, J = 6.9, 1.8 Hz, C_5-H), 7.37 (d, J = 6.6 Hz, 2H $C_{6,7}$-H) and 7.69 (d, J = 8.4 Hz, C_4-H) supported the formation of salvadoricine **28** and the data was in accordance with that reported[15] for the natural product.

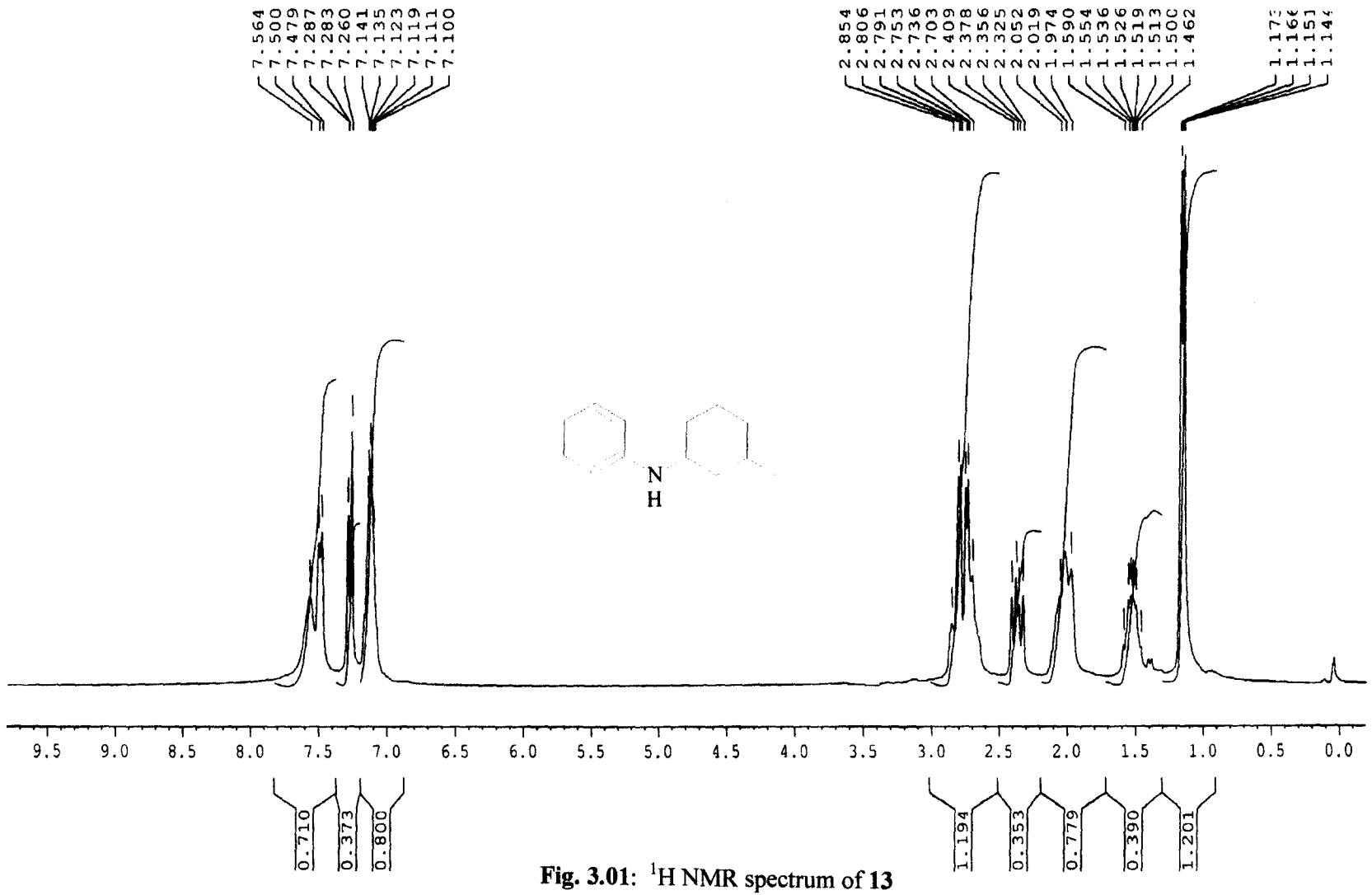


Fig. 3.01: ^{1}H NMR spectrum of **13**

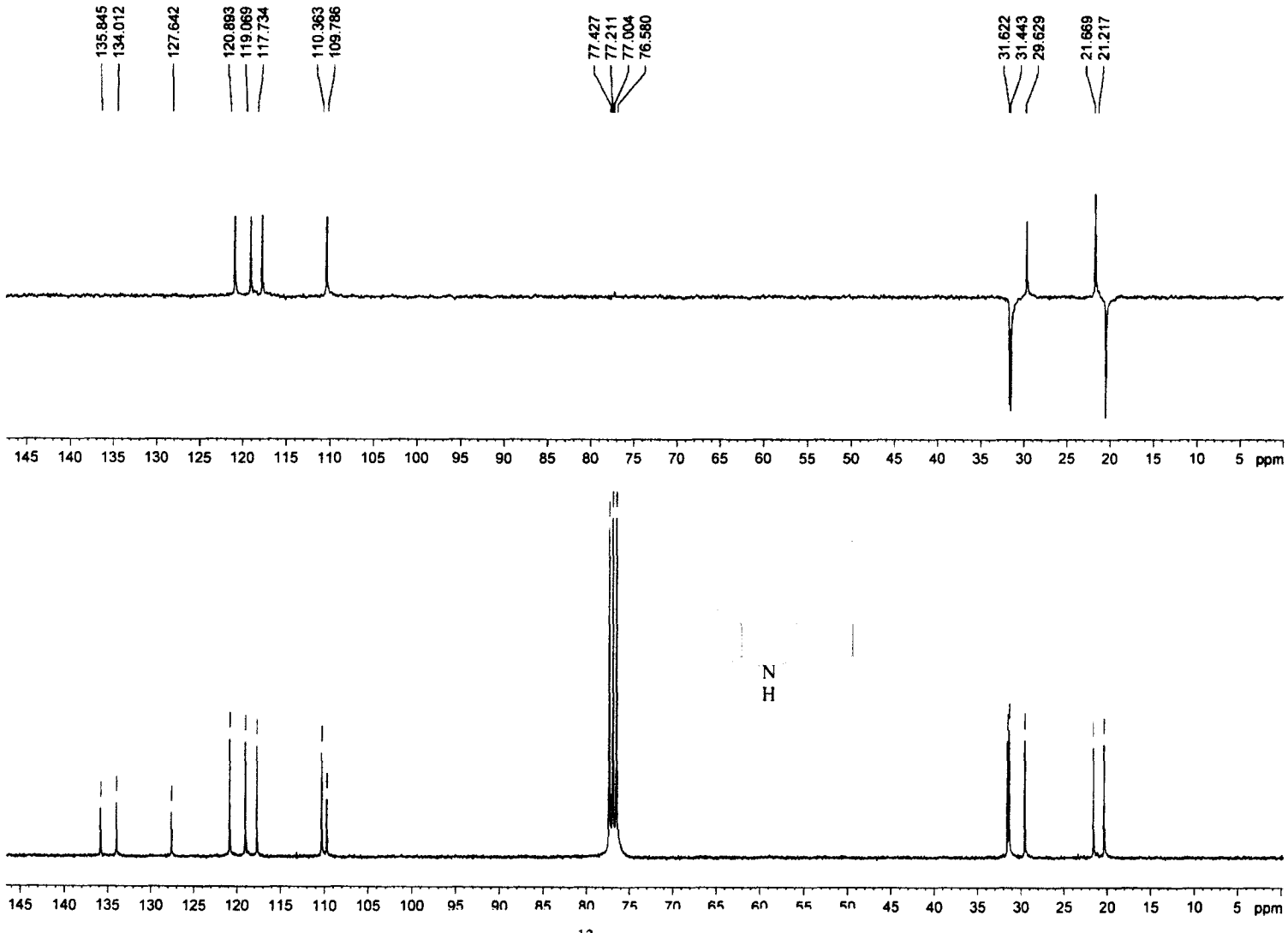


Fig. 3.02: ^{13}C NMR spectrum of **13**

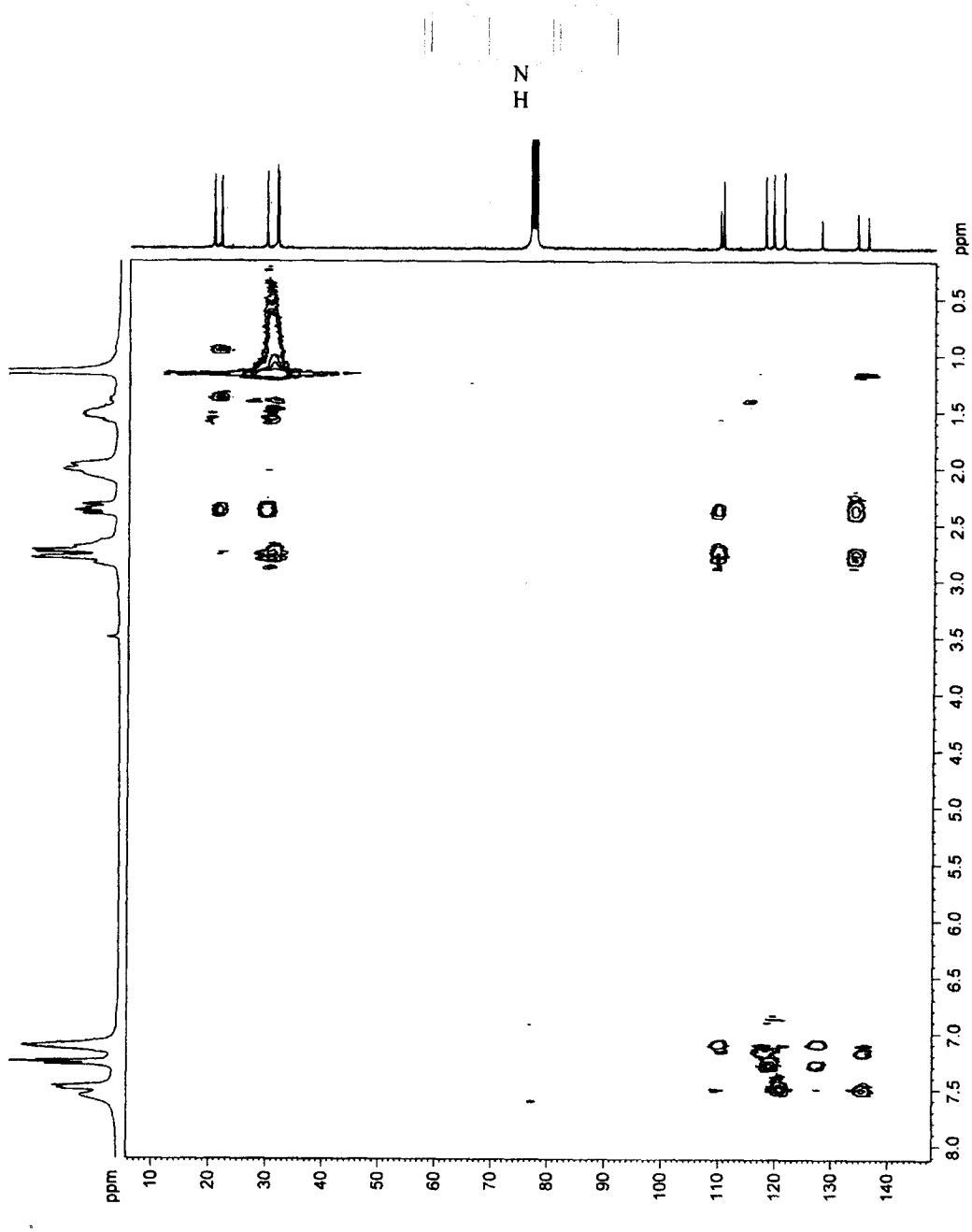


Fig. 3.03: ^{1}H-^{13}C HMBC spectrum of **13**

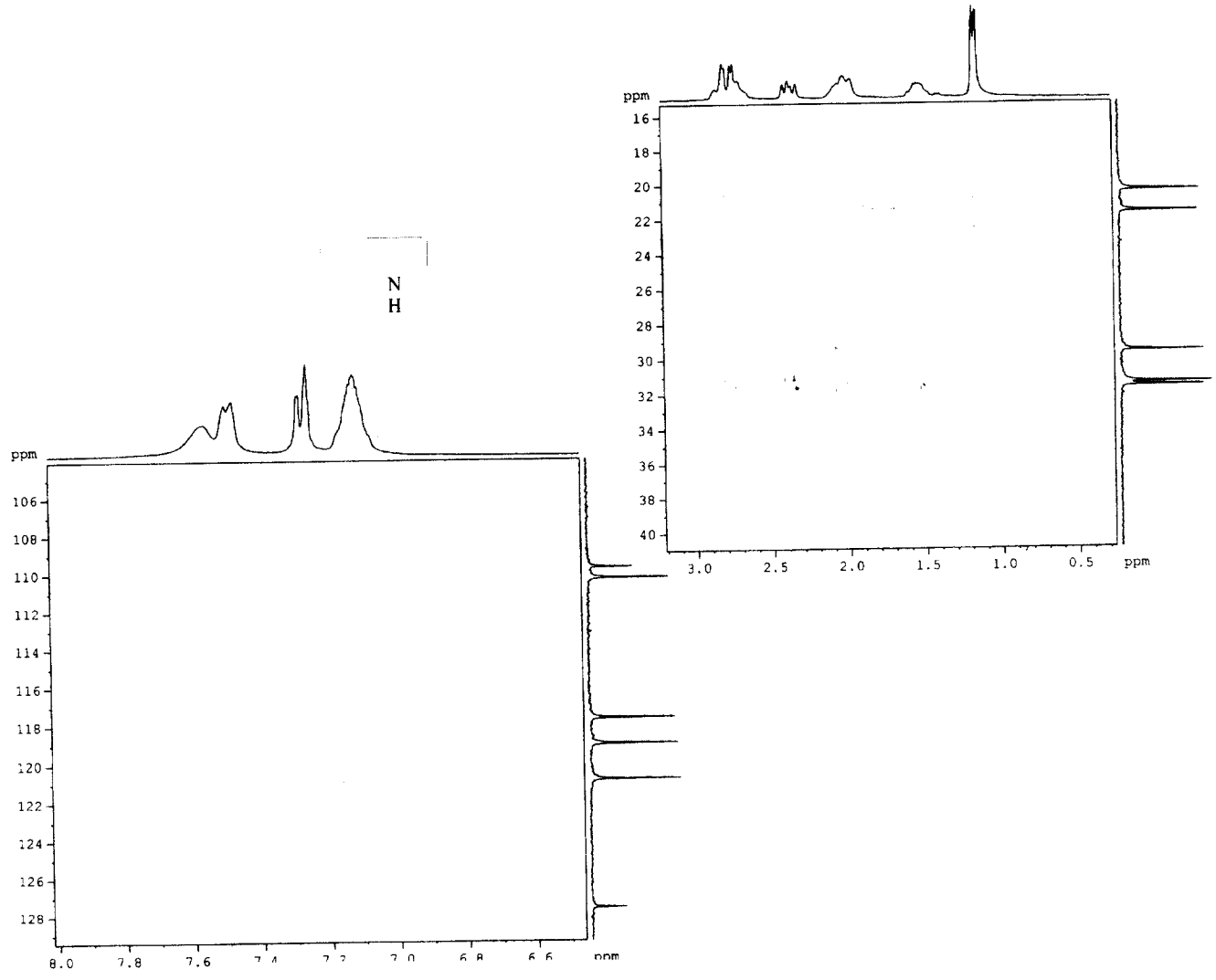


Fig. 3.04: ^{1}H-^{13}C HMQC spectrum of **13**

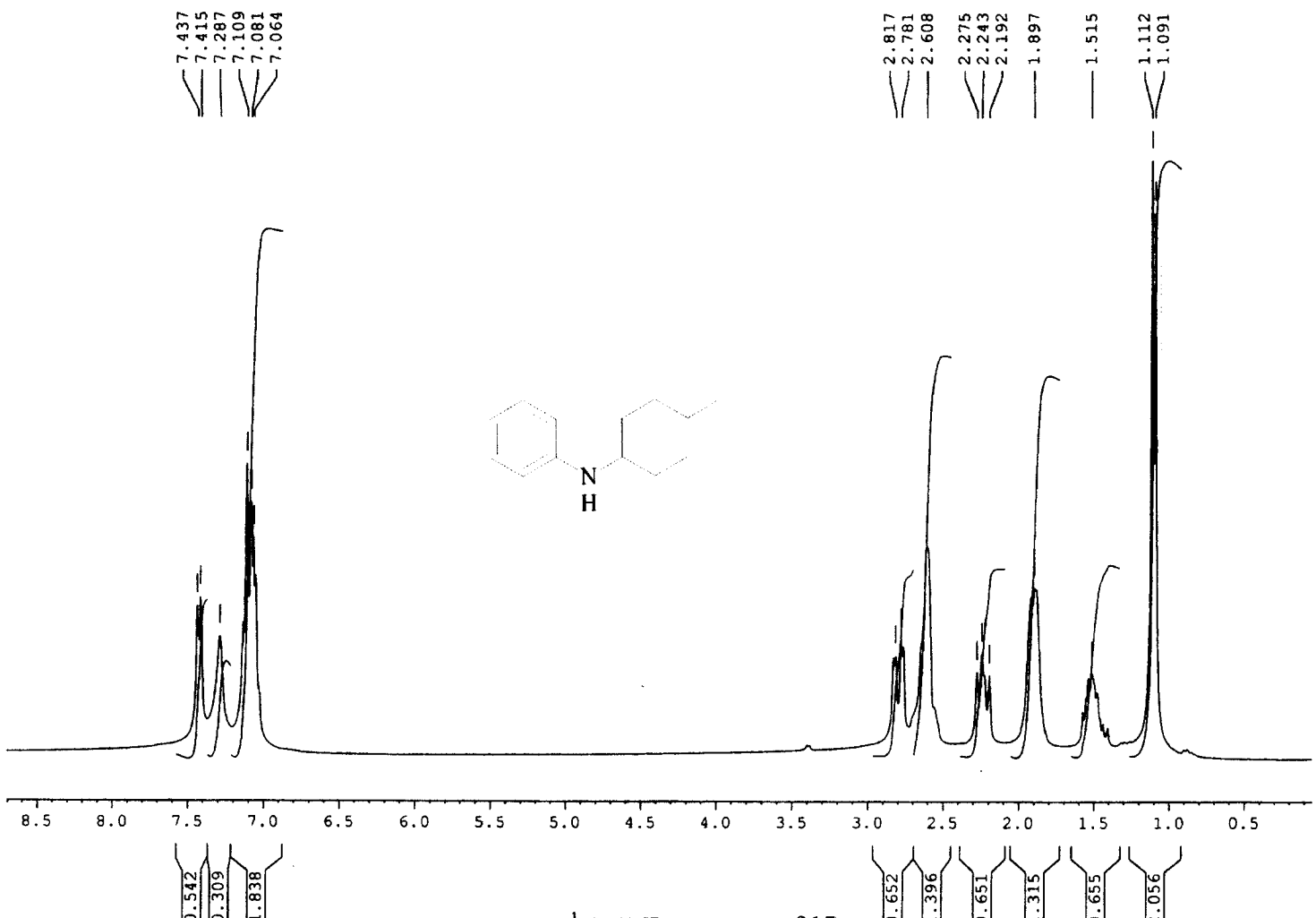


Fig. 3.05: ^{1}H NMR spectrum of **15**

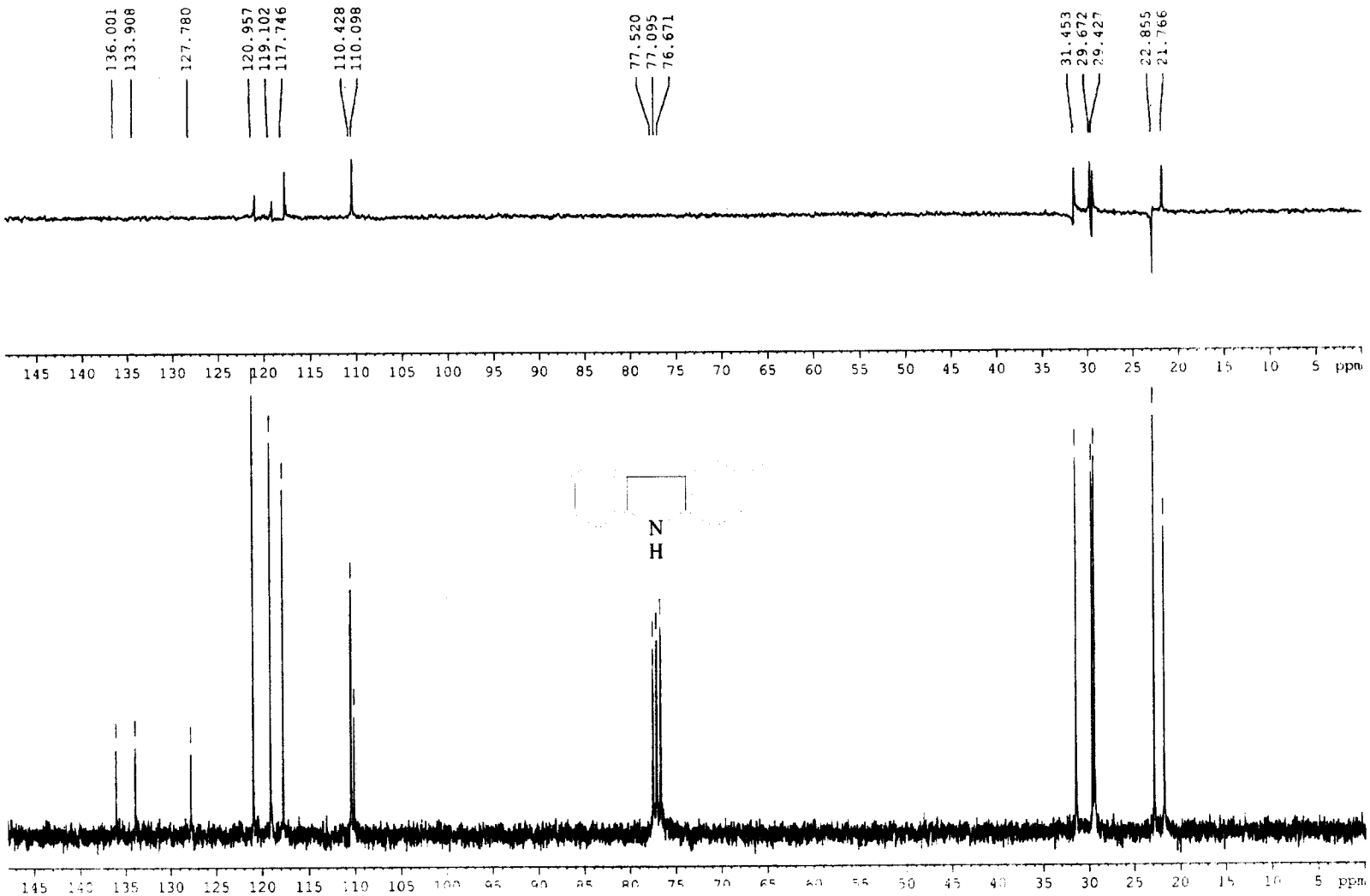


Fig. 3.06: ^{13}C NMR spectrum of **15**

Fig. 3.07: ^{1}H NMR spectrum of **16**

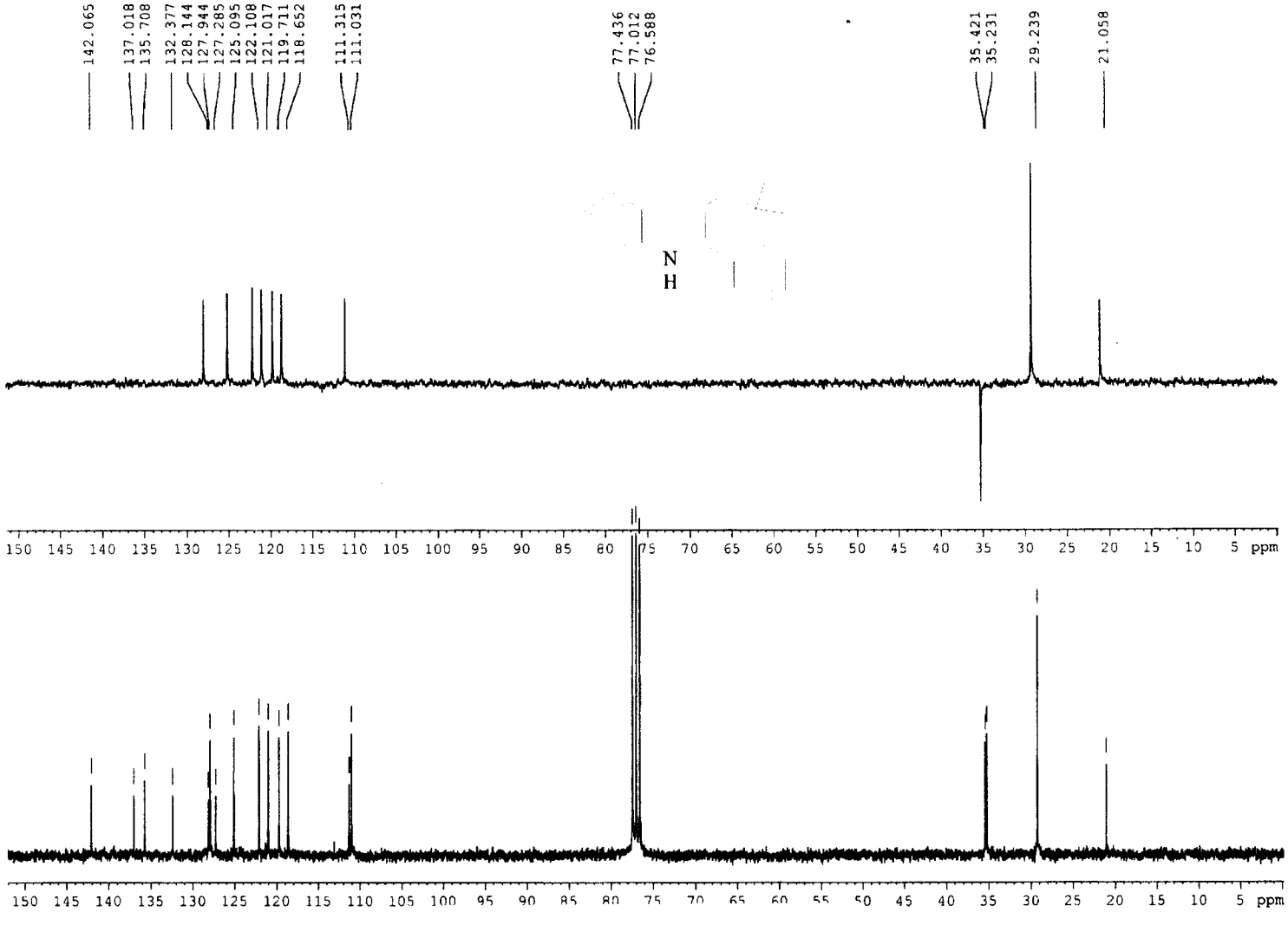


Fig. 3.08: ^{13}C NMR spectrum of **16**

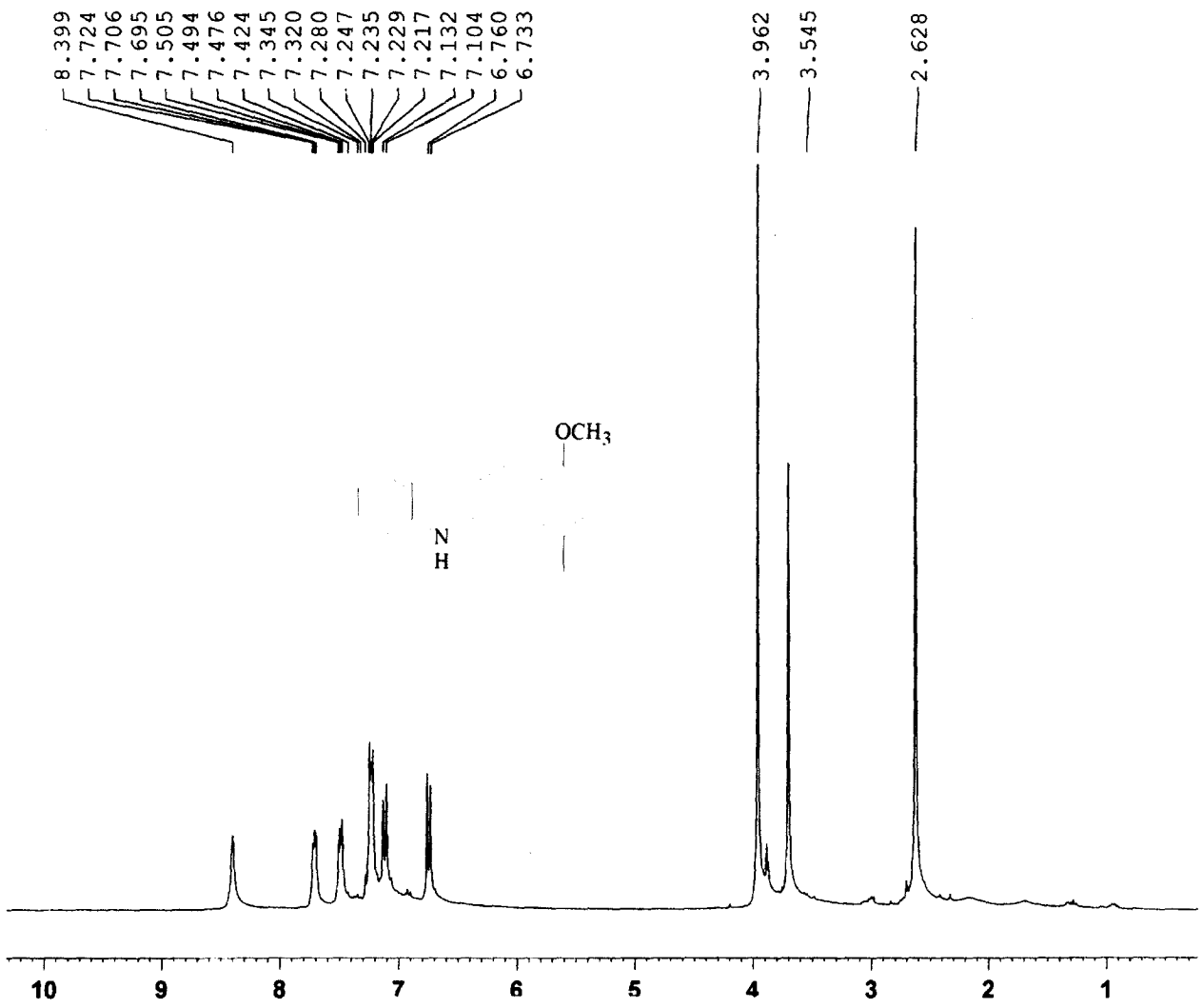


Fig. 3.09: ^{1}H NMR spectrum of **17**

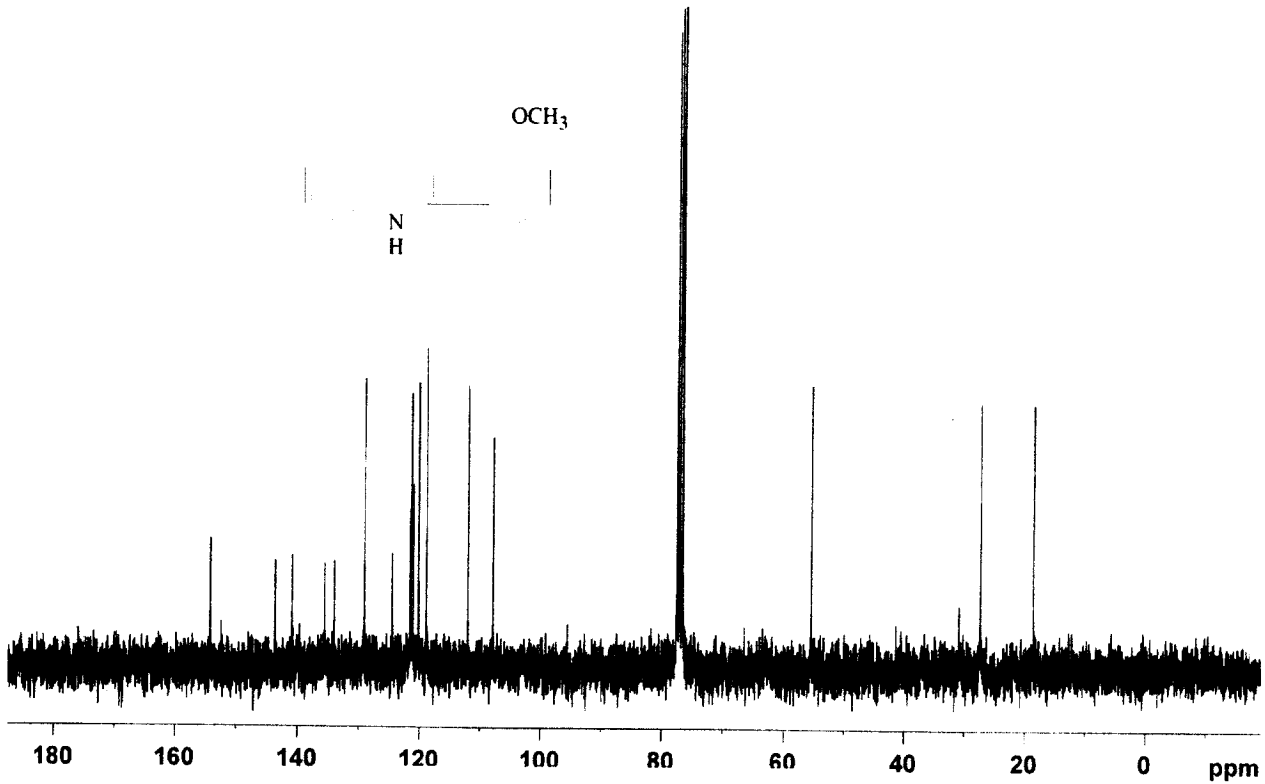


Fig. 3.10: ^{13}C NMR spectrum of **17**

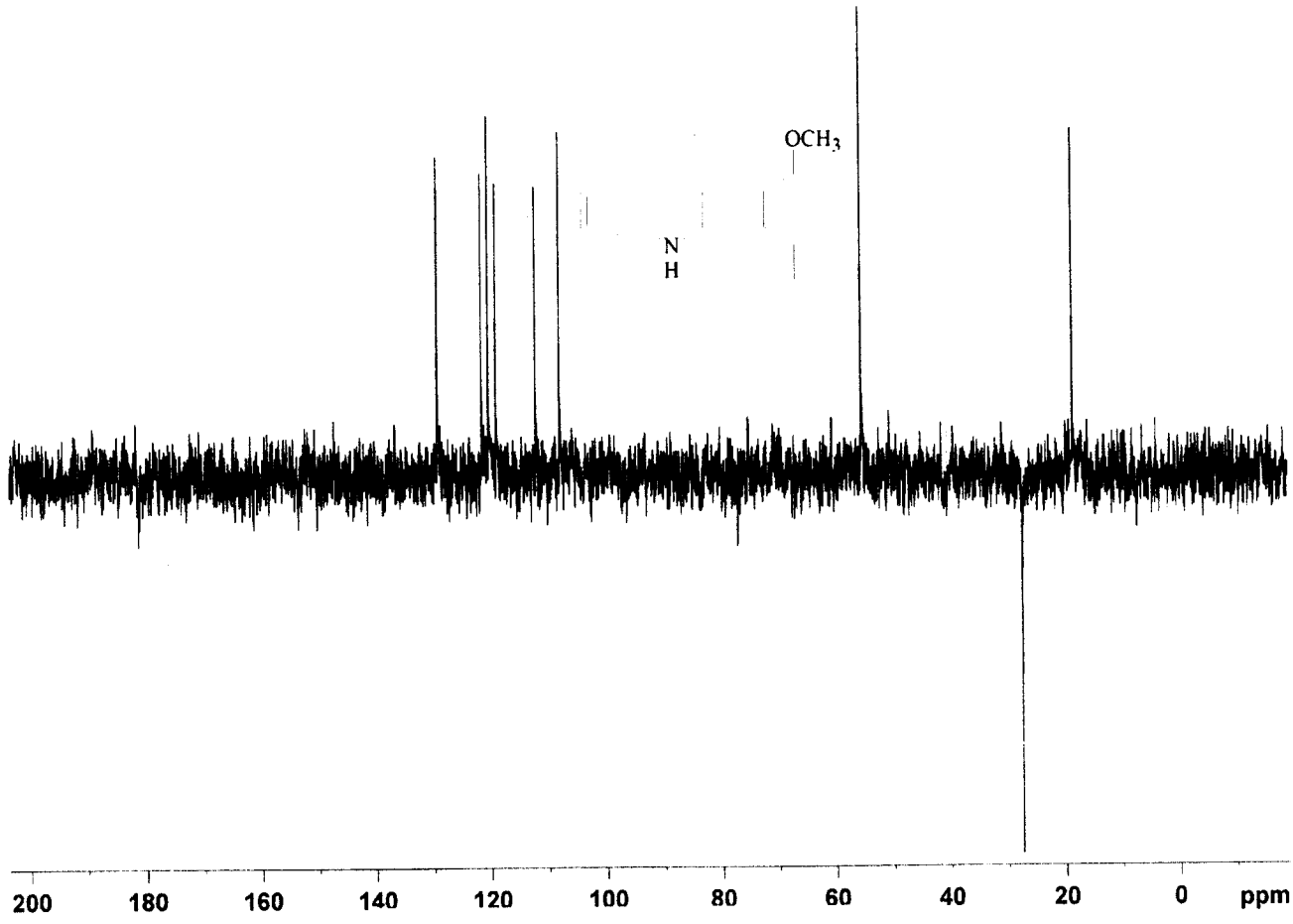


Fig. 3.11: DEPT spectrum of **17**

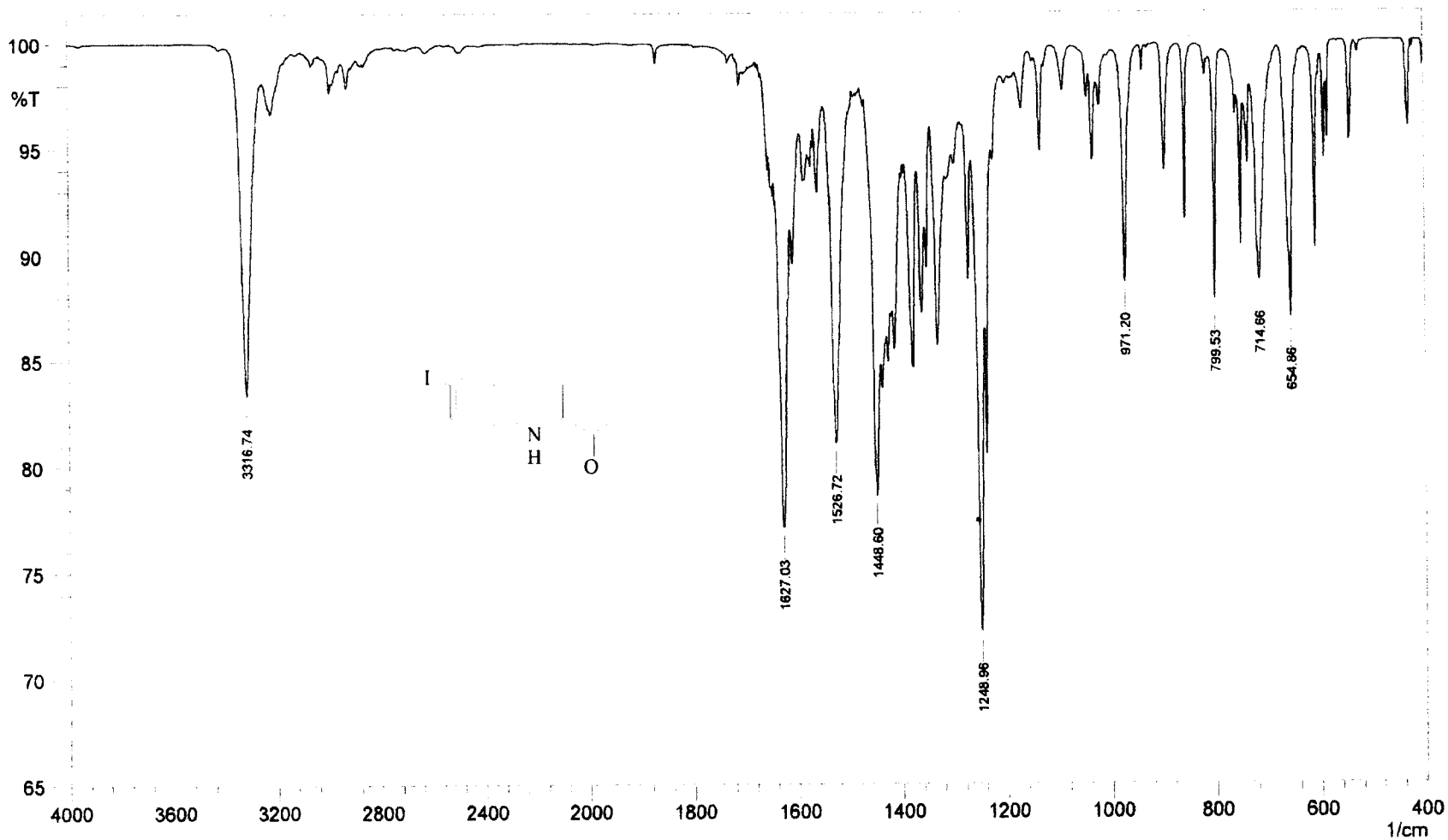


Fig. 3.12: IR spectrum of **33**

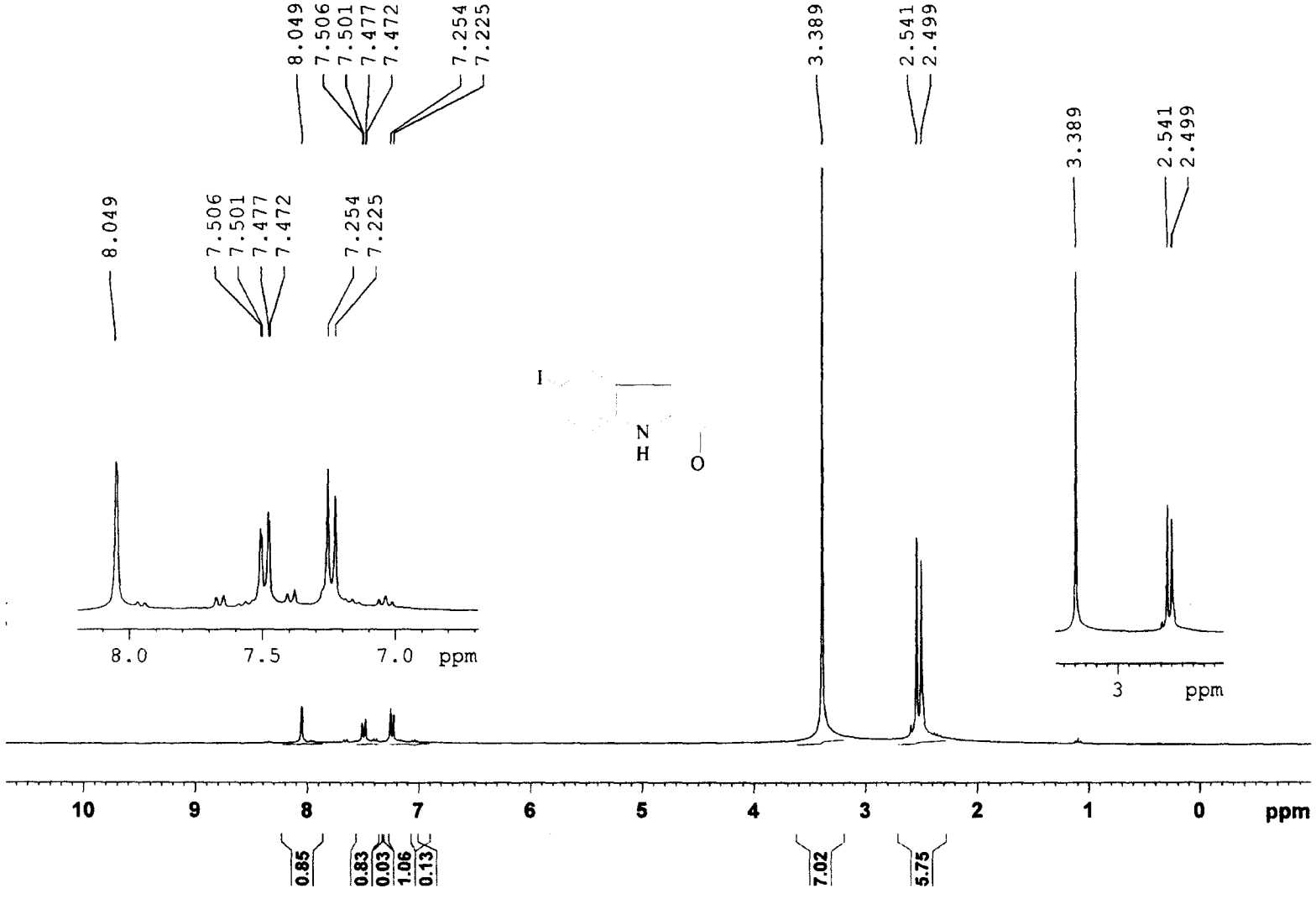


Fig. 3.13: ^{1}H NMR spectrum of **33**

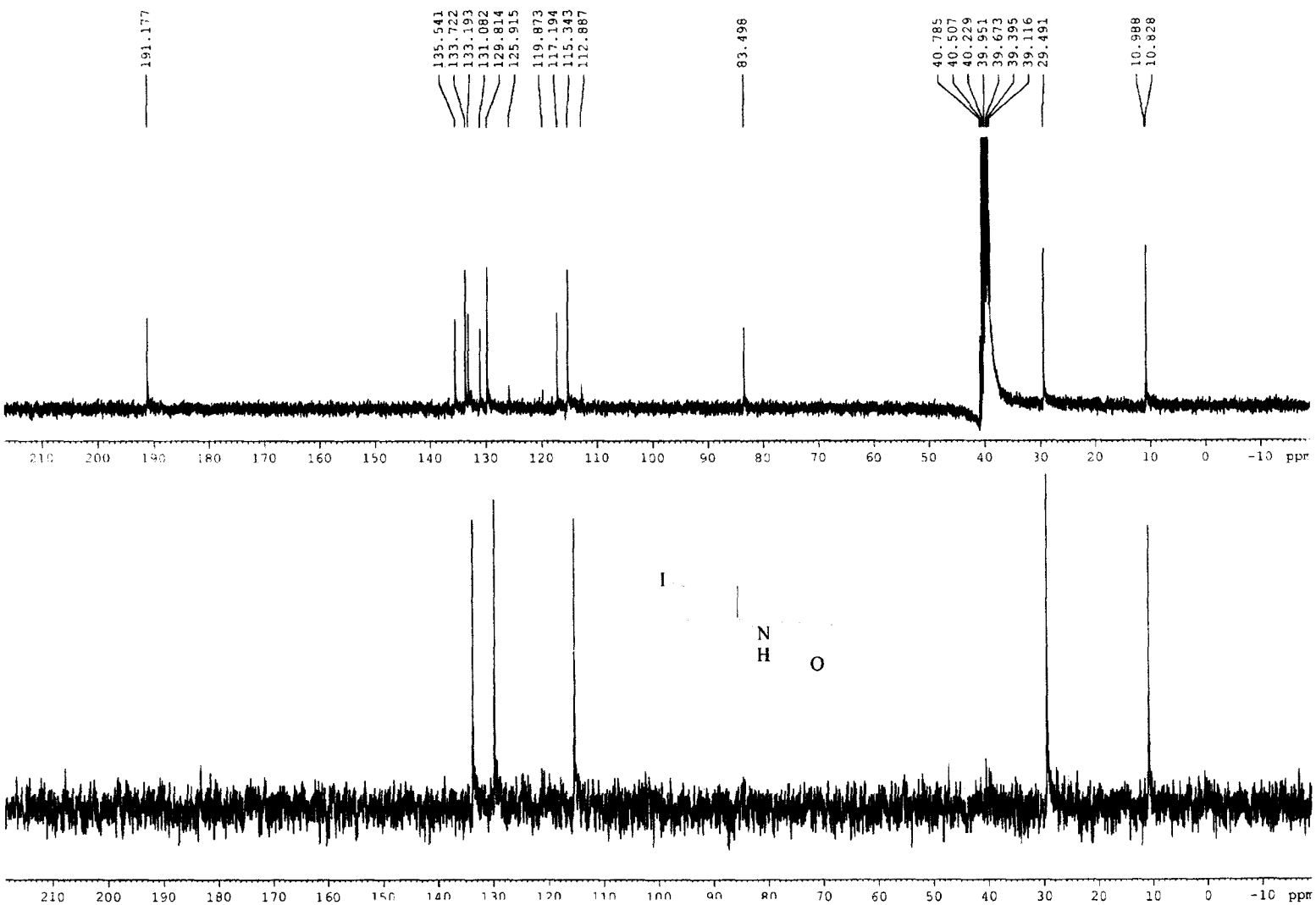


Fig. 3.14: ^{13}C NMR spectrum of **33**

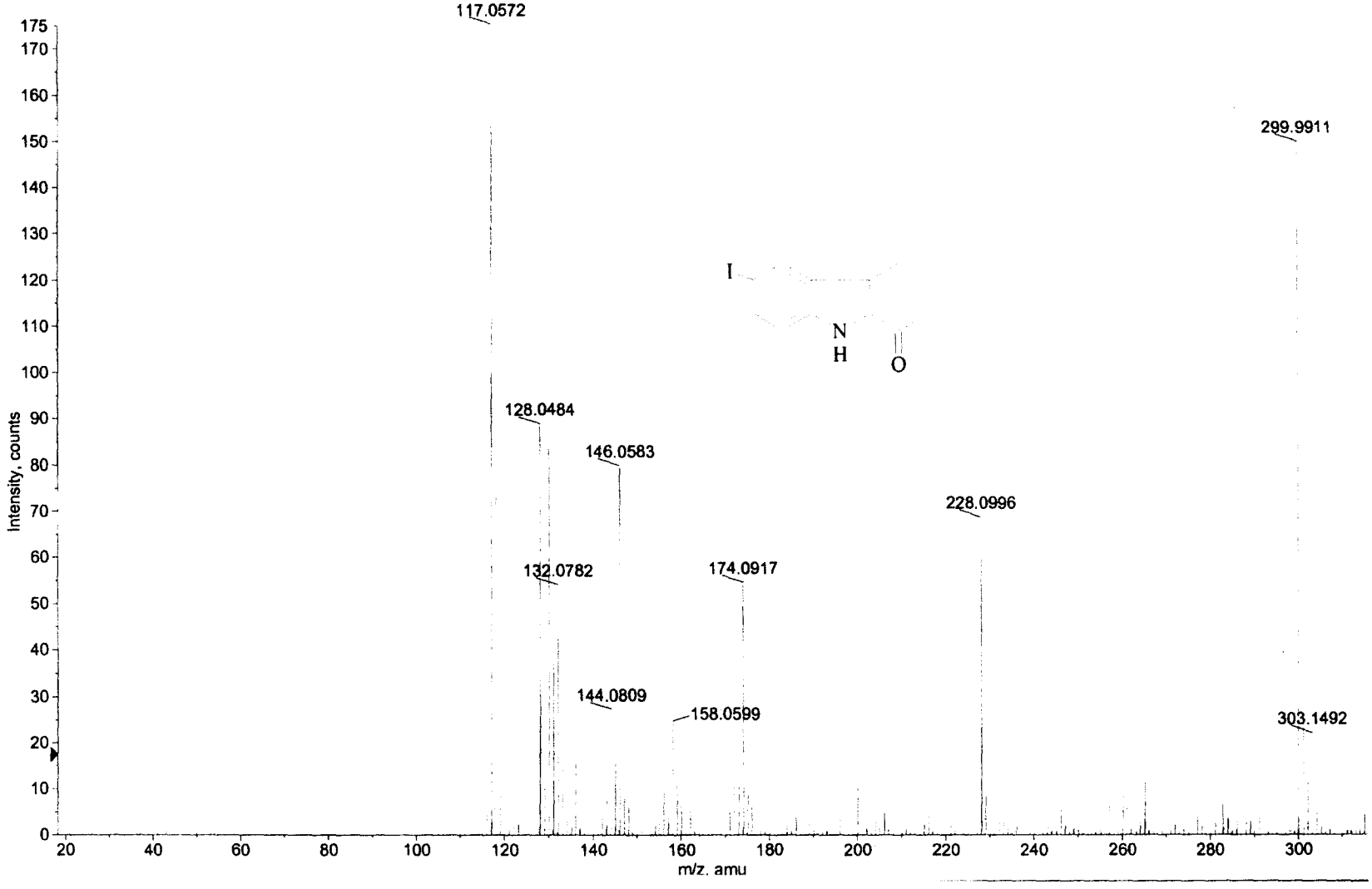


Fig. 3.15: ESIMS spectrum of **33**

Experimental

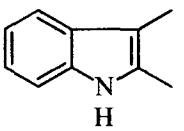
General procedure for the preparation of indoles

A mixture of phenylhydrazine hydrochloride **1** (3.45 mmole) and the appropriate ketone (3.28 mmole) in absolute ethanol (25 mL) was heated to reflux under N_2 atmosphere for 6 hrs. The reaction mixture was cooled to room temperature and poured in glacial acetic acid.

In those cases where the indole derivatives separated out as solids were filtered, washed with 5% Na_2CO_3, water and dried. Pure crystalline indole derivatives were obtained by recrystallization of the solids using appropriate solvents mentioned under respective indole derivative.

In cases where they separated out as oil, the reaction product was extracted with $CHCl_3$, washed with 5% Na_2CO_3, water, dried over Na_2SO_4 and the solvent evaporated to give the corresponding indole derivatives.

2,3-Dimethylindole **6**

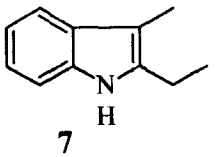


6

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with butanone **2** (0.235 g, 3.28 mmole) gave 2,3-dimethylindole **6** as a pink solid (0.402 g, 85% yield). Recrystallization from petroleum ether gave pink coloured shiny flakes, m.p. 102°C, Lit.[4] 104-106°C.

IR ν_{max} (KBr): 3390 (NH), 1617, 1465, 740 cm^{-1}.

2-Ethyl-3-methylindole **7**

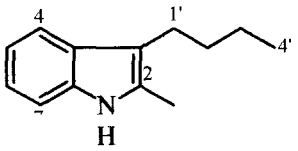


7

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with 3-pentanone **3** (0.281 g, 3.28 mmole) gave 2-ethyl-3-methylindole **7** as off-white solid (0.519 g) in quantitative yield. Recrystallization from hexane gave cream coloured flakes, m.p. 66°C, Lit.[4] 64-66°C.

IR ν_{max} (KBr): 3398 (NH), 2964, 1618, 1462, 744 cm^{-1}.

3-Butyl-2-methylindole **8**



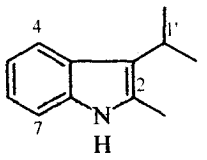
8

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) and 2-heptanone **4** (0.374 g, 3.28 mmole) gave 3-butyl-2-methylindole **8** in the form of dark yellow oil as reported[2] (0.595 g, 95% yield).

IR ν_{max} (KBr): 3400 (NH), 1617, 1454, 738 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): For assignments refer **Figure I**, pg 243.

3-Isopropyl-2-methylindole **9**



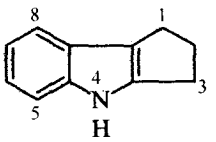
9

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with 4-methyl-2-pentanone **5** (0.328 g, 3.28 mmole) for 24 hrs gave 3-isopropyl-2-methylindole **9** in the form of dark yellow oil as reported[4] (0.397 g, 70% yield).

IR ν_{max} (KBr): 3404 (NH), 2951, 1469, 734 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): For assignments refer **Figure II**, pg 244.

1,2,3,4-Tetrahydrocyclopenta[b]indole **10**



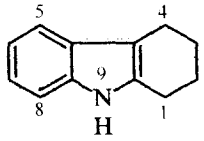
10

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with cyclopentanone **18** (0.374 g, 3.28 mmole) gave 1,2,3,4-tetrahydrocyclopenta[*b*]-indole **10** as a violet coloured solid (0.515 g) in quantitative yield. Recrystallization from petroleum ether gave violet coloured crystals m.p. 94°C.

IR ν_{max} (KBr): 3400 (NH), 1617, 1454, 738 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.79 (s, 2H, C_2-H), 3.86 (s, 2H, C_1-H), 3.93 (s, 2H, C_3-H), 6.37 (s, 1H, C_7-H), 6.94 (d, J = 8.7 Hz, 1H, C_6-H), 7.62 (d, J = 8.7 Hz, 1H, C_5-H), 7.87 (s, 1H, C_8-H).

2,3,4,9-Tetrahydro-1H-carbazole **11**



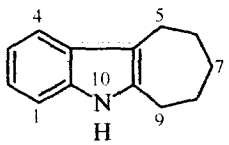
11

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with cyclohexanone **19** (0.321 g, 3.28 mmole) gave 2,3,4,9-tetrahydro-1*H*-carbazole **11** as white solid (0.56 g) in quantitative yield. Recrystallization from hexane gave colourless plates m.p. 110°C, Lit.[4] 110-114°C.

IR ν_{max} (KBr): 3390 (NH), 1617, 1468, 742 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.91 (d, J = 4.5 Hz, 4H, $C_{2,3}$-H), 2.74 (t, J = 6.0 Hz, 4H, $C_{1,4}$-H), 7.1 (d, J = 6.5 Hz, 1H, C_6-H), 7.15 (d, J = 7.2 Hz, 1H, C_7-H), 7.28 (d, J = 7.2 Hz, 1H, C_8-H), 7.5 (dd, J = 6.3, 1.5 Hz, 1H, C_5-H).

5,6,7,8,9,10-Hexahydrocyclohepta[b]indole **12**



12

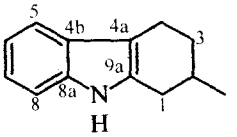
Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with cycloheptanone **20** (0.368 g, 3.28 mmole) gave 5,6,7,8,9,10-hexahydrocyclohepta-[*b*]indole **12** as light yellow solid (0.597 g, 72% yield). Recrystallization from ethanol gave pale yellow plates of **12**, m.p. 134°C.

IR ν_{max} (KBr): 3394 (NH), 2912, 1617, 1466, 740 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.86 (t, J = 2.7 Hz, 4H, $C_{6,7}$-H), 1.97 (d, J = 5.1 Hz, 2H, C_8-H), 2.83 (t, J = 6.0 Hz, 2H, C_5-H), 2.9 (t, J = 5.7 Hz, 2H, C_9-H), 7.18-

7.22 (m, 2H, $C_{2,3}$-H), 7.28 (d, J = 7.2 Hz, 1H, C_1-H), 7.57 (d, J = 8.4 Hz, 1H, C_4-H).

2-Methyl-2,3,4,9-tetrahydro-1H-carbazole **13**



13

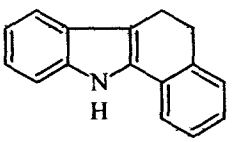
Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with 3-methylcyclohexanone **21** (0.367 g, 3.28 mmole) gave 2-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **13** as wine red coloured solid (0.606 g) in quantitative yield. Recrystallization from petroleum ether gave wine red coloured cubes m.p. 90°C.

IR ν_{max} (KBr): 3404 (NH), 2949, 1487, 1300, 740 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.01): For assignments refer **Figure III**, pg 248.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 3.02): For assignments refer **Figure IV**, pg 249.

5,11-Dihydro-6H-benzo[a]carbazole **14**



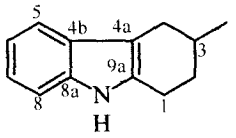
14

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) and 1-tetralone **22** (0.475 g, 3.28 mmole) gave 5,11-dihydro-6*H*-benzo[*a*]carbazole **14** as white solid (0.507 g, 76% yield). Recrystallization from petroleum ether and $CHCl_3$ mixture gave colourless cubes m.p. 158°C, Lit.[13] 160-161°C.

IR ν_{max} (KBr): 3427 (NH), 1462, 1303, 741 cm^{-1}.

ESIMS: *m/z* $[M + Na]^+$ calcd for $C_{16}H_{13}NNa$ 242.0940, Found 242.1.

3-Methyl-2,3,4,9-tetrahydro-1H-carbazole **15**



15

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with 4-methylcyclohexanone **23** (0.367 g, 3.28 mmole) gave 3-methyl-2,3,4,9-tetrahydro-1*H*-carbazole **15** as white solid (0.564 g, 93% yield). Recrystallization from hexane gave colourless plates m.p. 118°C.

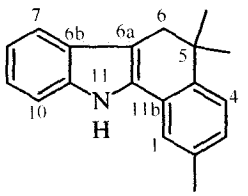
IR ν_{max} (KBr): 3400 (NH), 2920, 1480, 740 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.05): For assignments refer **Figure VI**, pg 251.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 3.06): For assignments refer **Figure VII**, pg 251.

ESIMS: *m/z* $[M]^+$ calcd for $C_{13}H_{15}N$ 185.1205, Found 185.5.

2,5,5-Trimethyl-5,11-Dihydro-6H-benzo[a]carbazole **16**



16

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) with 4,4,7-trimethyl-1-tetralone **24** (0.544 g, 3.28 mmole) gave **16** as brown solid (0.502 g,

66.5% yield). Recrystallization from petroleum ether and $CHCl_3$ mixture gave pale brown flakes m.p. 190°C.

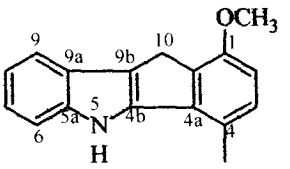
IR ν_{max} (KBr):3402 (NH), 1515, 1466, 746 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.07): For assignments refer **Figure VIII**, pg 252.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 3.08): For assignments refer **Figure IX**, pg 253.

ESIMS: *m/z* $[M]^+$ calculated for $C_{19}H_{19}N$ 261.1517, Found 261.5.

1-Methoxy-4-methyl-5,10-dihydro-indeno[1,2-b]indole **17**



17

Reaction of phenylhydrazine hydrochloride **1** (0.5 g, 3.45 mmole) and 4-methoxy-7-methyl-1-indanone **25** (0.577 g, 3.28 mmole) gave **17** as yellow solid (0.816 g) in quantitative yield. Recrystallization from petroleum ether gave pale yellow crystals like cotton threads, m.p. 128-130°C (decomp.).

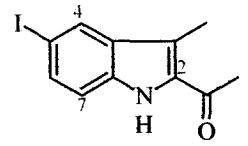
IR ν_{max} (KBr): 3400 (NH), 1590, 1458, 737 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.09): For assignments refer **Figure X**, pg 254.

^{13}C NMR-DEPT ($CDCl_3$, 75 MHz, **Fig.** 3.10 & **Fig.** 3.11): For assignments refer **Figure XI**, pg 254.

ESIMS: *m/z* $[M]^+$ calcd for $C_{17}H_{15}NO$ 249.1153, Found 249.5.

2-Acetyl-5-iodo-3-methylindole **33**



33

To a solution of HIO_4 (1.434 g, 6.58 mmol) in 1:1 methanol:water mixture (15 mL) was added drop by drop 2-ethyl-3-methylindole **7** (0.5 g, 3.14 mmol) in methanol (4 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 hr and then at room temperature for 1 hr. The dark coloured reaction mixture was decanted and the aqueous portion was extracted with $CHCl_3$ (3 × 10 mL). The organic extracts were then washed with saturated Na_2CO_3 (3 × 10 mL), dilute sodium bisulfite solution (3 × 10 mL), water (3 × 10 mL), dried over Na_2SO_4 and evaporated to leave a crude brown residue (0.7 g). The residue was purified by column chromatography using silica gel (200-400 mesh) and elution with $CHCl_3$ gave 2-acetyl-5-iodo-3-methylindole **33** (0.327 g, 60%) as fine pale yellow needles m.p. 196-198°C.

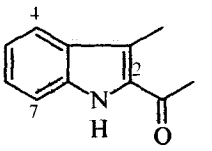
IR ν_{max} (KBr, **Fig.** 3.12): 3315(NH), 1627(CO) cm^{-1}.

^{1}H NMR (DMSO-d_6, 300 MHz, **Fig.** 3.13): δ 2.50 (s, 3H, C_3-C<u>H</u>$_3$), 2.54 (s, 3H, C_2-COC<u>H</u>$_3$), 7.24 (d, J = 8.7 Hz, 1H, C_6-H), 7.49 (dd, J = 8.7, 1.5 Hz, 1H, C_7-H), 8.05 (s, 1H, C_4-H).

^{13}C NMR (DMSO-d_6, 75 MHz, **Fig.** 3.14): δ 10.82 (C_3-<u>C</u>H$_3$), 29.49 (C_2-CO<u>C</u>H$_3$), 83.5 (C-5), 115.34 (C-3), 117.19 (C-7), 129.81 (C-6), 131.08 (C-4), 133.19 (C-3a), 133.72 (C-2), 135.54 (C-7a), 191.18 (<u>C</u>O).

ESIMS (Fig. 3.15): *m/z* $[M + H]^+$ calcd for $C_{11}H_{11}INO$ 299.987975, Found 299.9983.

2-Acetyl-3-methylindole **28** *(salvadoricine)*



28

To a solution of HIO_4 (1.434 g, 6.58 mmol) & sodium thiosulfate (50 mg) in methanol:water (1:1, 15 mL) was added drop by drop 2-ethyl-3-methylindole **7** (0.5 g, 3.14 mmol) in methanol (4 mL) at 0°C. The reaction mixture was stirred at 0°C for 1 hr and then at room temperature for 1 hr. The dark coloured reaction mixture was decanted and the aqueous portion was extracted with diethyl ether (3 × 10 mL). The organic extracts were then successively washed with saturated Na_2CO_3 (3 × 10 mL), dilute sodium bisulfite (3 × 10 mL), water (3 × 10 mL), dried over Na_2SO_4 and evaporated to leave 2-acetyl-3-methyl-indole **28** as white solid (0.442 g, 81% yield). Recrystallization from petroleum ether gave white crystals like cotton threads, m.p. 142°C (Lit.[15] 143-144°C).

IR ν_{max} (KBr): 3325 (NH), 1632 (CO) cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.64 (s, 3H, C_3-C$\underline{H}_3$), 2.64 (s, 3H, -COC$\underline{H}_3$), 7.14 (dt, J = 6.9, 1.8 Hz, 1H, C_5-H), 7.37 (d, J = 6.6 Hz, 2H, $C_{6,7}$-H), 7.69 (d, J = 8.4 Hz, 1H, C_4-H).

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Section 3.2

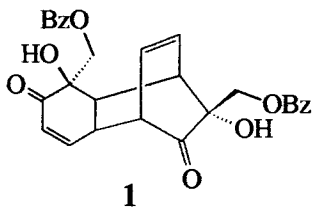
Studies towards the Synthesis of Grandifloracin, a Constituent of *Uvaria grandiflora*

Characterization of Two New Compounds Similar to Grandifloracin

Introduction

The genus Uvaria of the family *Annonaceae* has been known to be a rich source of bioactive compounds. Several compounds with novel carbon framework have been isolated by the elegant studies of various groups.

Yong-Hong and coworkers[1] carried out a systematic chemical investigation of the CH_2Cl_2 extract of *Uvaria grandiflora* and isolated a crystalline biscyclohexeneoxide grandifloracin **1** (m.p. 161-163°C) in addition to two cyclohexeneoxides zeylenone and grandiflorone. The structure **1** including the relative stereochemistry assigned to it was based on the detailed spectral analysis (UV, IR, ^1H, ^{13}C NMR, COSY, HMBC, MS).



1

Results and discussion

The carbon framework of **1** attracted our attention and it was proposed* that **1** is derived from two molecules of **2** by Diels-Alder self-dimerization. Due to our continued interest in the synthesis of naturally occurring compounds derived from Diels-Alder self-dimerization it was suggested[2] that the ideal route

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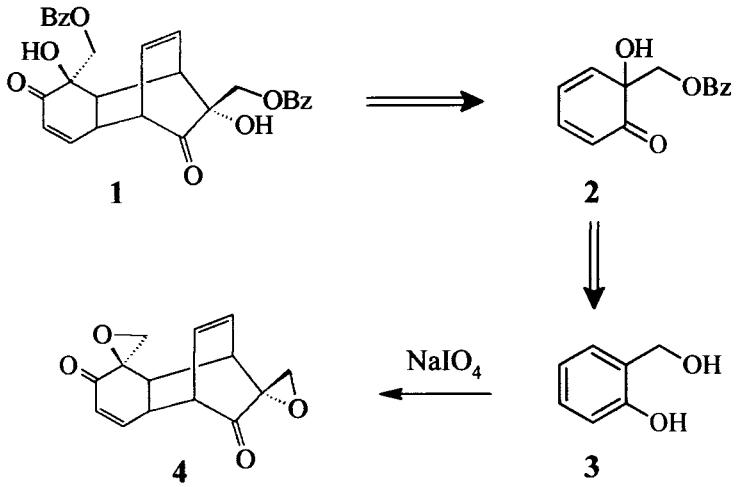
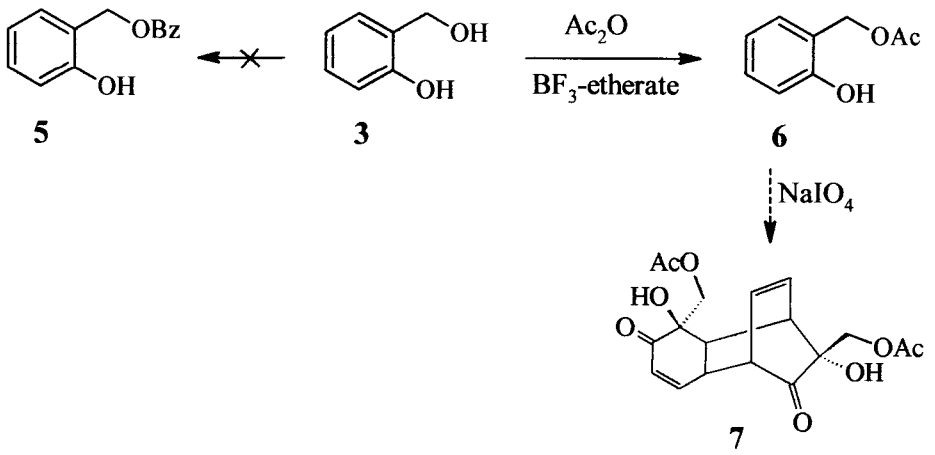
* We are grateful to Prof. S. K. Paknikar for his interest & contribution in proposing biogenesis and biogenetic type synthesis of natural products and making useful suggestions during this work.

towards the synthesis of **1** could be saligenin **3** which is known[3] to dimerize upon oxidation with sodium metaperiodate ($NaIO_4$) to the corresponding dimer **4**.

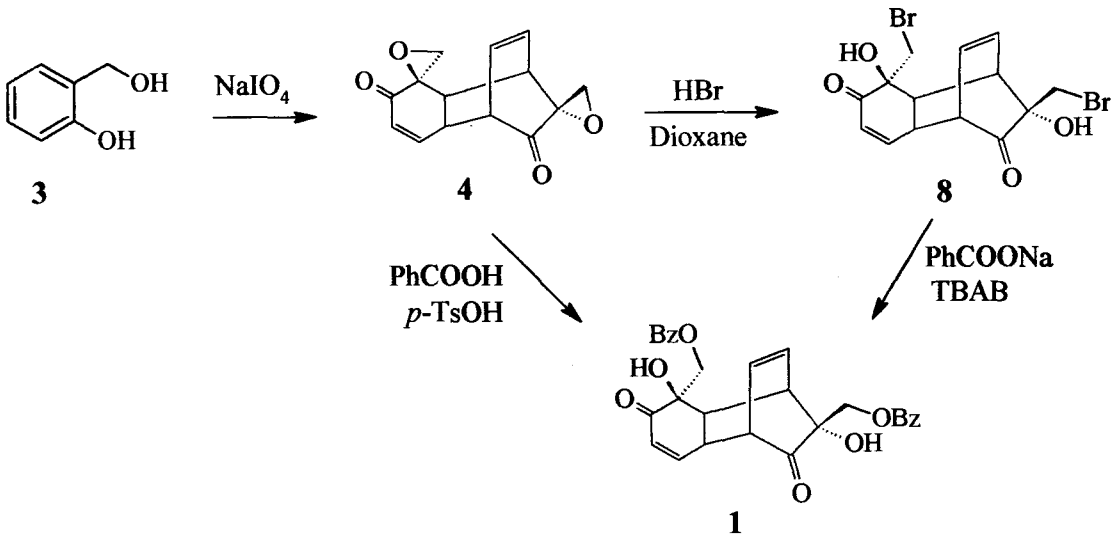


This work involving biogenetic type synthesis of **1**, starting with saligenin **3** was initiated in our Laboratory by Dr. Asha D'Souza in 1999. However, all her attempts to prepare **5**, the monobenzoate of **3** did not yield to. Of course, she did succeed in preparing **6**, the monoacetate of **3** but unfortunately, $NaIO_4$ as well as HIO_4 oxidation of **6** did not yield the expected dimer **7** but resulted in the total recovery of the starting material. For details please refer to her thesis[2].



The dimer **4** is also reported[3] to give a crystalline compound **8**, when treated with HBr, by the nucleophilic oxirane ring opening. The structural features of **4** and **8** are ideally suitable for their conversion into **1** either by opening of the oxirane rings or by displacement of the bromide by the benzoate nucleophile.

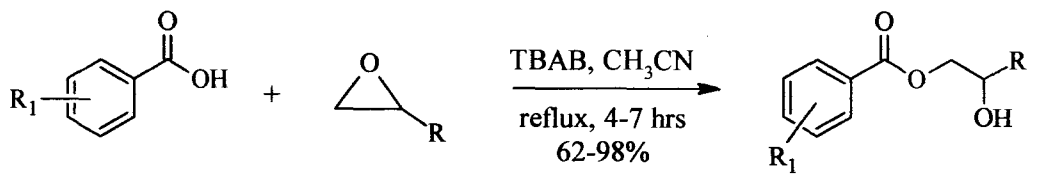
These reactions were also carried out but with partial success[2] i.e the reaction of **8** with sodium benzoate in the presence of TBAB did give an impure product (mixture of two compounds) having NMR data close to that of **1**.



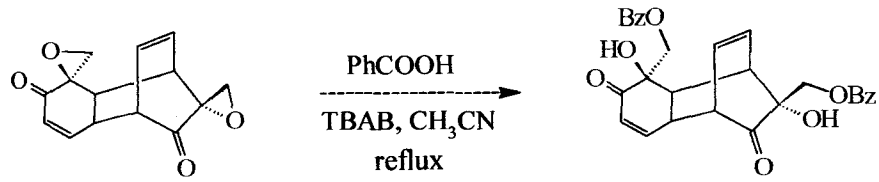
3 **4** **8** **1**

Therefore we decided to retry the synthesis of grandifloracin **1** by making necessary modifications in the previously used reaction conditions, reagents etc. mainly because until then (2004) the synthesis of **1** was not reported.

Moreover, we came across a regioselective ring opening reaction of epoxides with benzoic acid and its derivatives[4] in the presence of catalytic amount of tetrabutylammonium bromide (TBAB) in anhydrous acetonitrile to give benzoylated 1,2-diols from terminal epoxides.



Simple reaction conditions and good yields encouraged us to try the above reaction on the saligenin dimer **4** which also being a substituted terminal diepoxide should give the required grandifloracin **1** in single step.



4 **1**

Thus a solution of **4** and excess of benzoic acid in anhydrous CH_3CN was refluxed for 8 hrs. Usual workup of the reaction mixture gave a brown viscous residue. Purification by column chromatography and elution with $CHCl_3$:MeOH (9.8:0.2) gave a white solid having m.p. 164-66°C. Although the m.p. of the white solid obtained was close to that of natural grandifloracin **1** (161-163°C), it got raised to 182°C on recrystallization from acetonitrile.

Although its IR spectrum showed the presence of two OH groups as expected for **1**, their frequencies at 3570 & 3400 cm^{-1} did not match with those reported for **1**. Moreover, only two carbonyl bands at 1726 cm^{-1} (ester CO) and at 1687 cm^{-1} (probably the conjugated CO) were observed. The third carbonyl band seen at around 1700 cm^{-1} in the IR of **1** was missing.

Therefore, we determined its molecular formula to be $C_{21}H_{18}O_6$ on the basis of it's ESIMS data which showed a peak at *m/z* 367.1499 $[M + H]^+$. This clearly indicated that one out of the two expected benzoate groups ($C_7H_5O_2$) present in **1** was missing.

The ^1H NMR spectrum of the white solid having m.p. 182°C showed the presence of three OH groups, one monosubstituted benzene ring, two additional aromatic protons, only two olefinic protons and two methylene groups attached to oxygen atom.

Its ^{13}C NMR spectrum showed 19 signals, out of which eight were quaternary (including two of carbonyl carbons at δ 201.05 & 165.03), two

methylenes (δ 67.94 & 53.65) and nine methines as indicated by its ^{13}C NMR DEPT spectrum.

On the basis of the above spectroscopic information and also by comparison of the IR, ^{1}H and ^{13}C NMR data reported for **1**, and that of the white solid, we could arrive at the following structure **9** which can account for all the signals observed in the NMR data and their assignments are shown below in figures I and II.

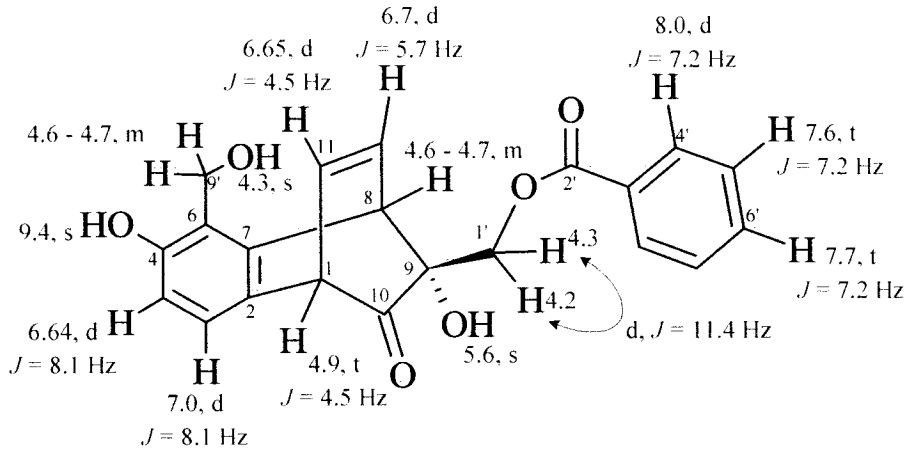


Figure I: Assignments of ^{1}H NMR signals for the various protons of **9**

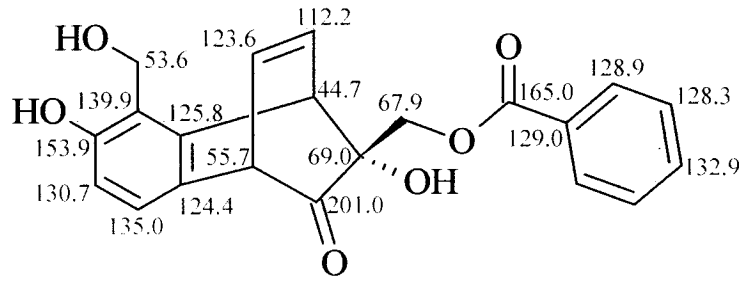


Figure II: Assignments of ^{13}C NMR signals for the various carbons of **9**

The structure **9** was further supported by HMBC experiments. In the HMBC the tertiary hydroxyl proton correlated to C-1, C-10 & C-1′ indicating that

the tertiary hydroxyl is at C-9; correlation of $C_{1'}$-H to C-9, C-10 & C-2′ indicated that the -CH_2-COOPh is attached to C-9. Further correlation of C_4-H and C_3-H to C-5 established the position of the phenolic OH at C-5.

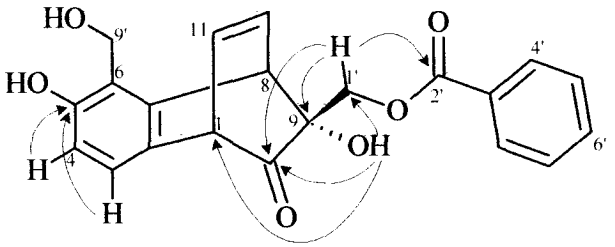


Figure III: Selected HMBC of **9**

The EIMS fragmentation pattern also supports structure **9**. In addition to the peak at *m/z* 367 $[M + H]^+$ its mass spectrum showed significant peaks at *m/z* 349 (100%), 321 and 199. The possible mode of fragmentation and the likely structures for the major fragment ions are shown in chart-1.

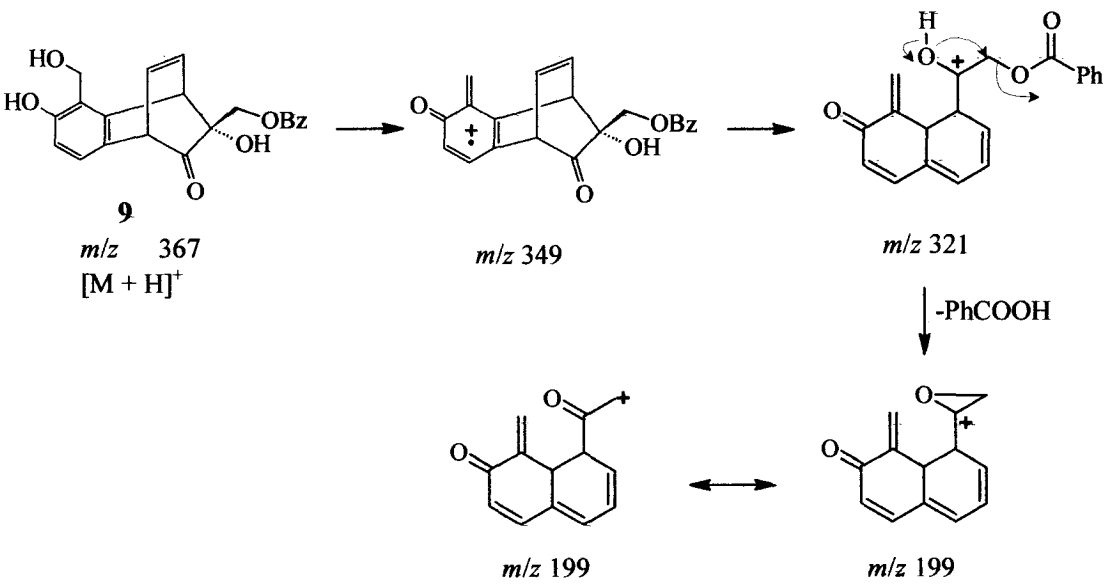
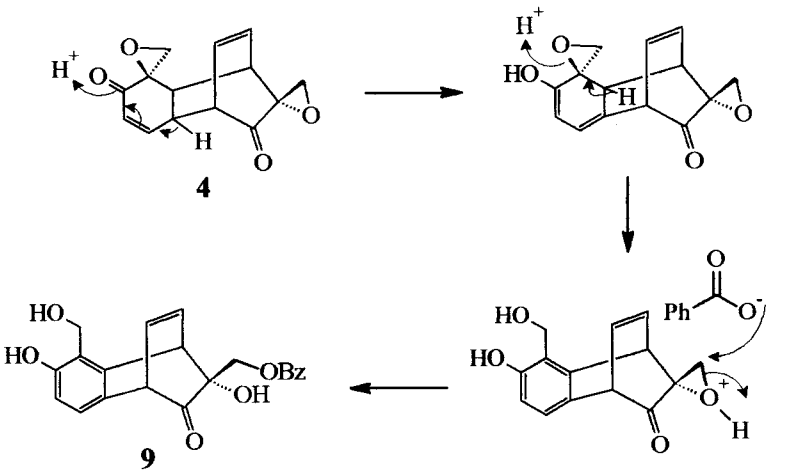


Chart-1: Mass spectral fragmentation pattern of **9**

The probable mechanism for the formation of **9** from the dimer **4** is rationalised below in scheme 1:

It appears that the reaction is initiated by protonation of the conjugated carbonyl with H^+ in the presence of benzoic acid leading to dienol epoxide which immediately undergoes aromatization as shown below in scheme 1. The other epoxide is opened up by the nucleophilic attack of the benzoate anion.



Scheme 1: Probable mechanism for the formation of **9** from **4**

Meanwhile we came across another literature report[5] for the synthesis of esters of benzoic acid wherein bromide is displaced by the benzoate anion. The method involved heating a mixture of potassium salt of benzoic acid with appropriate bromo-compound in either DMF or DMSO as solvents.

Thus a mixture of dibromide **8** and potassium benzoate in dry DMF was heated to 85°C for 2 hrs. Workup of the reaction mixture gave dark yellow viscous oil. Purification by silica gel column chromatography using $CHCl_3$:MeOH (9.8:0.2) as eluent afforded white solid having m.p. 164-66°C. Recrystallization from CH_3CN gave white crystals (m.p. 182°C) identical with **9** (m.p., co-TLC and IR).

Similar results were obtained when the reaction was carried out using anhydrous DMSO as solvent at 100-105°C.

Having failed to obtain the required compound **1** we thought of repeating the reaction of the dibromide **8** and sodium benzoate in the presence of TBAB as catalyst. As mentioned before, this reaction was carried out previously in our laboratory[2] and the product obtained had striking similarities in its NMR data with that of natural grandifloracin **1**.

Reaction of the dibromide **8** with potassium benzoate in refluxing benzene and water mixture in the presence of TBAB after usual workup gave a pale brown residue. TLC (C_6H_6:Et_2O; 95:5) indicated it to be a mixture of two compounds, dimer **4** and a more polar component. Purification by column chromatography using C_6H_6 as eluent gave dimer **4** (co-TLC, m.p. and IR).

Further elution with C_6H_6:Et_2O (8:2) gave a white solid. Careful observation of TLC (C_6H_6:Et_2O; 6:4) indicated it to be a mixture of two closely spaced spots which could be resolved using $CHCl_3$:EtOAc (9:1) as solvent system. The white solid was reloaded on silica gel column and purified by using $CHCl_3$:EtOAc (98:2) as eluent to give a white solid. Recrystallization from the same solvent gave white shiny flakes having m.p. 180°C. Although the m.p. of the solid obtained was very close to that of **9** (m.p. 182°C), its TLC and IR clearly indicated its non-identity with **9** and it was given number **10**.

IR spectrum of **10** showed the presence of three carbonyls (1736, 1708 & 1691 cm^{-1}) as reported[1] for grandifloracin **1** but showed a band at 3500 cm^{-1} indicating the presence of only one OH group in **10**.

The ESIMS spectrum of **10** showed two molecular ion peaks at *m/z* 389.1416 $[M + Na]^+$ and 367.3094 $[M + H]^+$. Thus its molecular formula was determined to be $C_{21}H_{18}O_6$ same as that of the compound **9**.

The ^1H NMR spectrum of **10** showed striking similarities to **1**, however the aromatic region showed signals accounting for only nine protons (five of the benzene ring and four olefinic protons) indicating the presence of only one mono-substituted benzene ring. Further two doublets at δ 2.94 and 3.1 (J = 6 Hz) integrated for 1H each indicated the presence of an oxirane ring.

The ^{13}C NMR and DEPT spectra of **10** showed in all 19 signals of six quaternary carbons (including three of carbonyl carbons), two methylenes and eleven methines for 21 carbons present in **10**.

Thus on the basis of the above IR, NMR & MS data, the compound **10** was assigned the following structure which can account for all the signals observed in the ^{1}H and ^{13}C NMR spectra of **10** and their assignments for the various protons and carbons of **10** are shown below in figures IV and V.

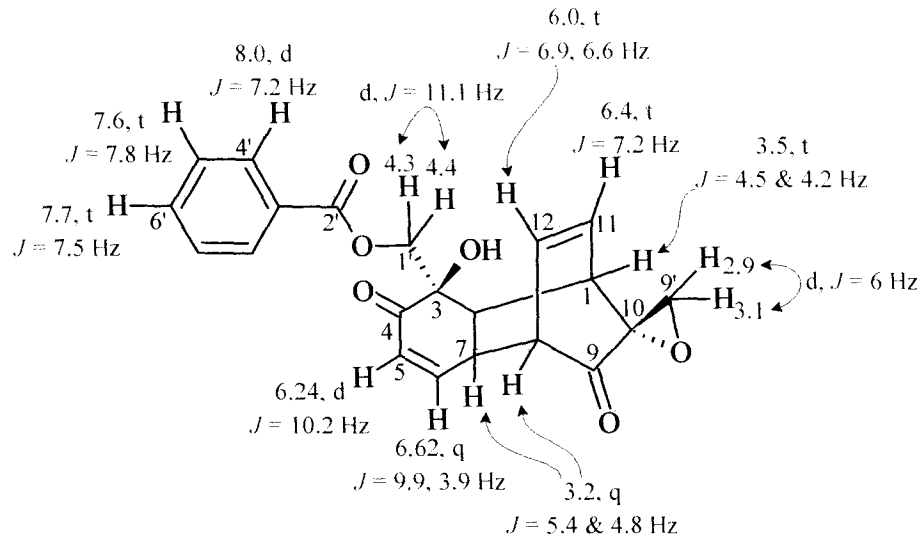


Figure IV: Assignments of the ^{1}H NMR signals for the various protons of **10**

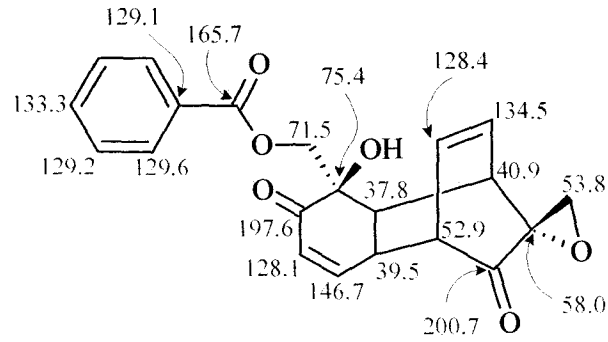


Figure V: Assignments of the ^{13}C NMR signals for the various carbons of **10**

The structure was further supported by HMBC experiments (figure VI). In the HMBC spectrum, the $C_{1'}$-H correlated to C-2′ and C-4 indicating that the -CH_2-COOPh is attached to C-3. The C_5-H correlated to C-7 & C-3 and the C_6-H correlated to C-4, C-2 & C-8. Further correlation of $C_{1'}$-H to C-9 & C-9′ indicated oxirane ring at C-10.

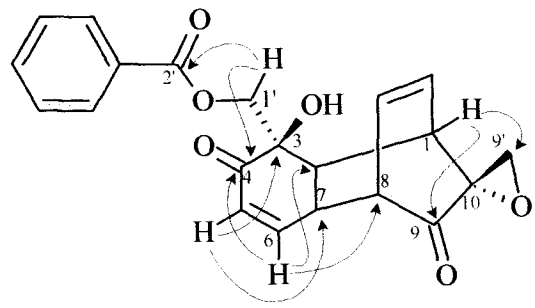
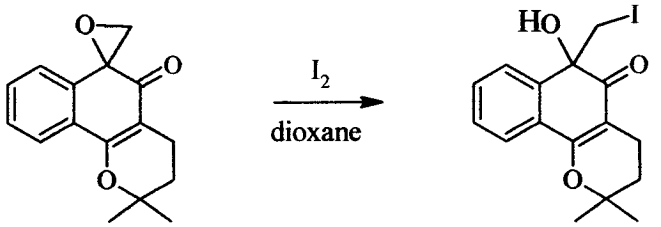


Figure VI: HMBC of **10**

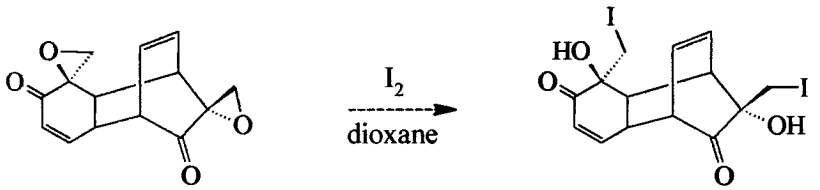
Thus the compound **10** of which NMR data appeared[2] very close to that of **1** is in fact having all the structural features of **1**, except one benzoate unit and a tertiary OH group. Instead there is an oxirane ring. Although **10** is formed from dibromide **8** and not from the dimer **4**, it appears as if the structure **10** is derived by opening of one of the two oxirane rings of the dimer **4** and not both the rings to give **1**. Therefore we may name it as semi-grandifloracin.

During its formation from dibromide **8** only one bromine got displaced by the external benzoate nucleophile, while the other bromine was displaced by the internal tertiary OH nucleophile. And this was always observed whenever the dibromide **8** was used; we obtained invariably some amount of the dimer **4** as the side product by intramolecular nucleophilic displacement of the bromide.

Iodine being a better leaving group than bromine we thought of preparing the di-iodo compound **11** so that the nucleophilic displacement of iodine by benzoate may facilitate the formation of **1**. Moreover, we came across a report[6] wherein oxirane ring was opened up using I_2 in dioxane.

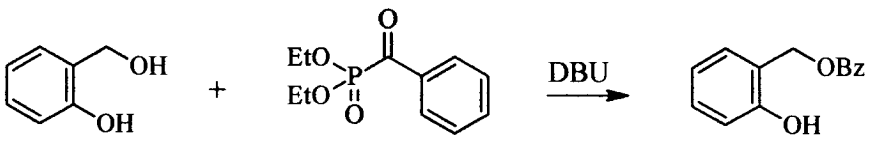


Thus the reaction of the dimer **4** with two equivalents of I_2 in dioxane using reported[6] conditions should give the di-iodo compound **11**. However, when this reaction was carried out we could neither isolate the product nor recover the starting dimer **4**.



4 **11**

Literature survey revealed that there are only two reports for the preparation of 2-hydroxybenzyl benzoate **5**. The first report[7] was on its preparation from the azide of saligenin **3** and the second[8] by selective monobenzoylation of **3** using diethylbenzoyl phosphonate **12**.



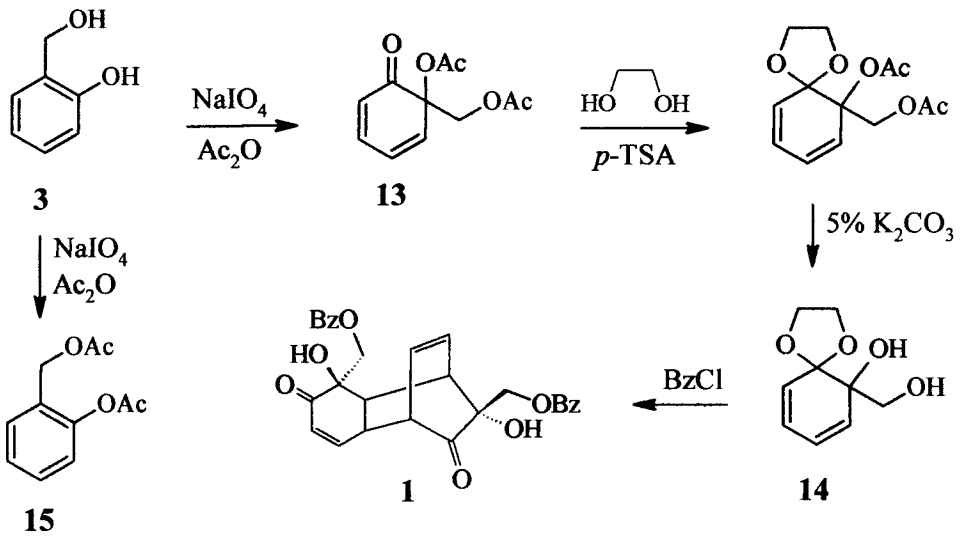
3 **12** **5**

We thought of preparing **5** as per the second report using diethybenzoyl phosphonate **12**.

The required diethylbenzoyl phosphonate **12** was prepared by the reaction of benzoyl chloride with triethyl phosphite* at 0°C followed by reduced pressure distillation to give yellow oil (b.p. 140-142°C at 3 mm) as reported[9].

The reaction of saligenin **3** with **12** in the presence of 1,5-diazabicyclo[5,4,0]undec-5-ene (DBU)* after usual workup gave sticky residue insoluble in common organic solvents and was not investigated.

Meanwhile we came across a report[10] wherein saligenin **3** was oxidised to diacetoxycyclohexadienone **13** using $NaIO_4$ in Ac_2O. Therefore, we decided to prepare grandifloracin **1** as per the scheme 2 in which the acetylative oxidation of **3** would give the dieneone diacetate **13**. Protection of the carbonyl followed by base catalyzed hydrolysis would give the protected diene diol **14**. Selective benzoylation of the primary alcohol with simultaneous deprotection of the carbonyl would give *in-situ* the required *o*-quinol **2** that would immediately dimerize to **1**.



Scheme 2

* We are thankful to Dr. S. G. Tilve, Goa University, for providing triethyl phosphite and DBU

However, after several attempts even with different hands, oxidation of **3** gave the diacetate **15** as yellow oil but we never got even a trace of the reported product **13** indicating that the reported reaction[10] was not reproducible.

Thus in conclusion, although we did not succeed in synthesizing the targeted molecule grandifloracin **1**, we could obtain and fully characterize two compounds **9** & **10**, both having the same molecular formula $C_{21}H_{18}O_6$ and especially the compound **10** is in fact semi-grandifloracin.

It may be noted that the work towards the synthesis of grandiflarcin **1** was initiated in our laboratory in 1999 soon after its isolation report in 1997 in the right direction i.e. by self Diels-Alder dimerization of *o*-quinol **2**. However, we did not succeed mainly because we could not prepare 2-hydroxybenzyl benzoate **5** in our laboratory. The synthesis of grandiflarcin **1** has been recently (2007) reported[11] that too by self Diels-Alder dimerization of 2-hydroxybenzyl benzoate **5** via SIBX-mediated hydroxylative phenol dearomatization in 30% yield.

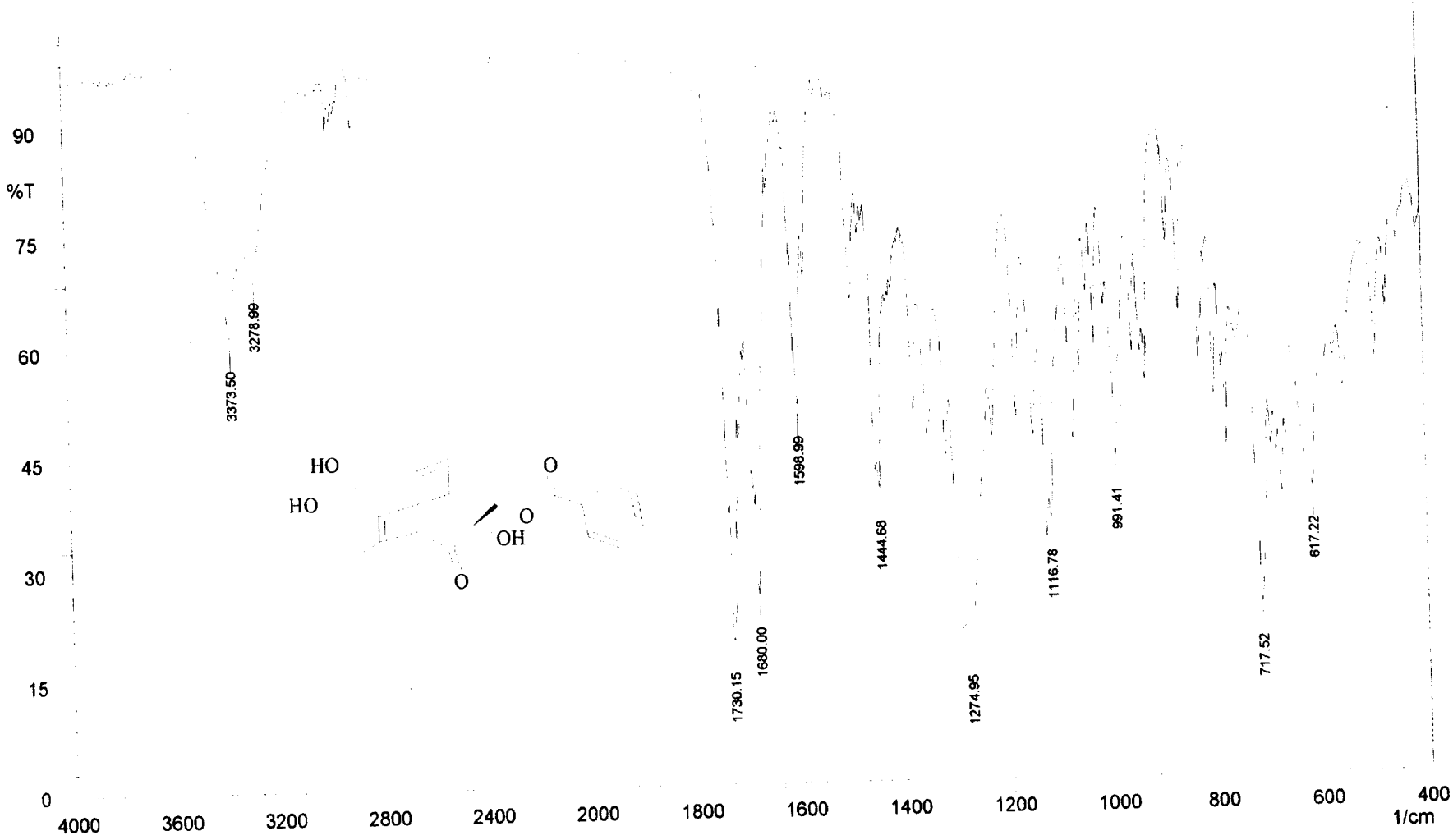


Fig . 3.16: IR spectrum of **9**

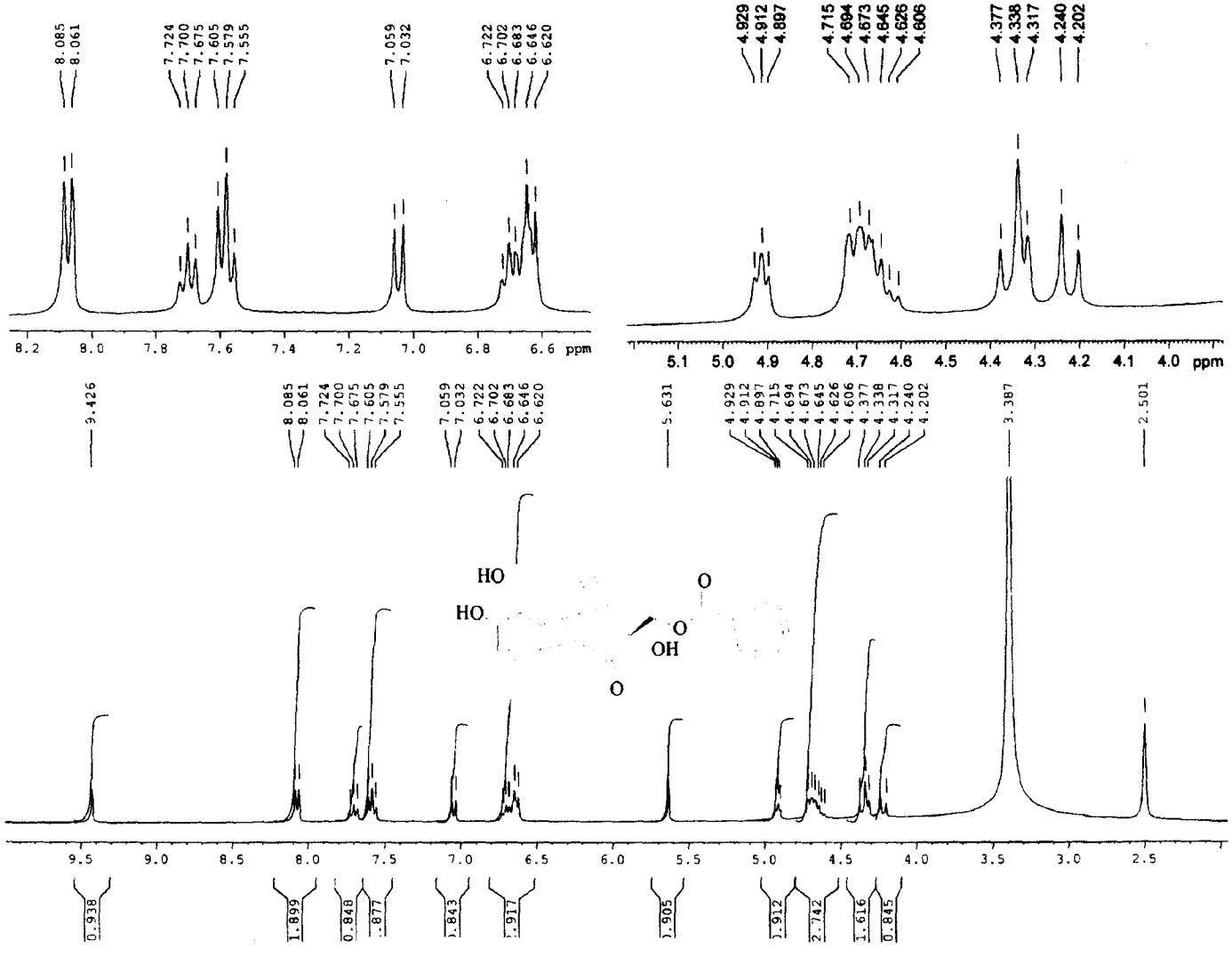


Fig 3.17: ^{1}H NMR spectrum of **9**

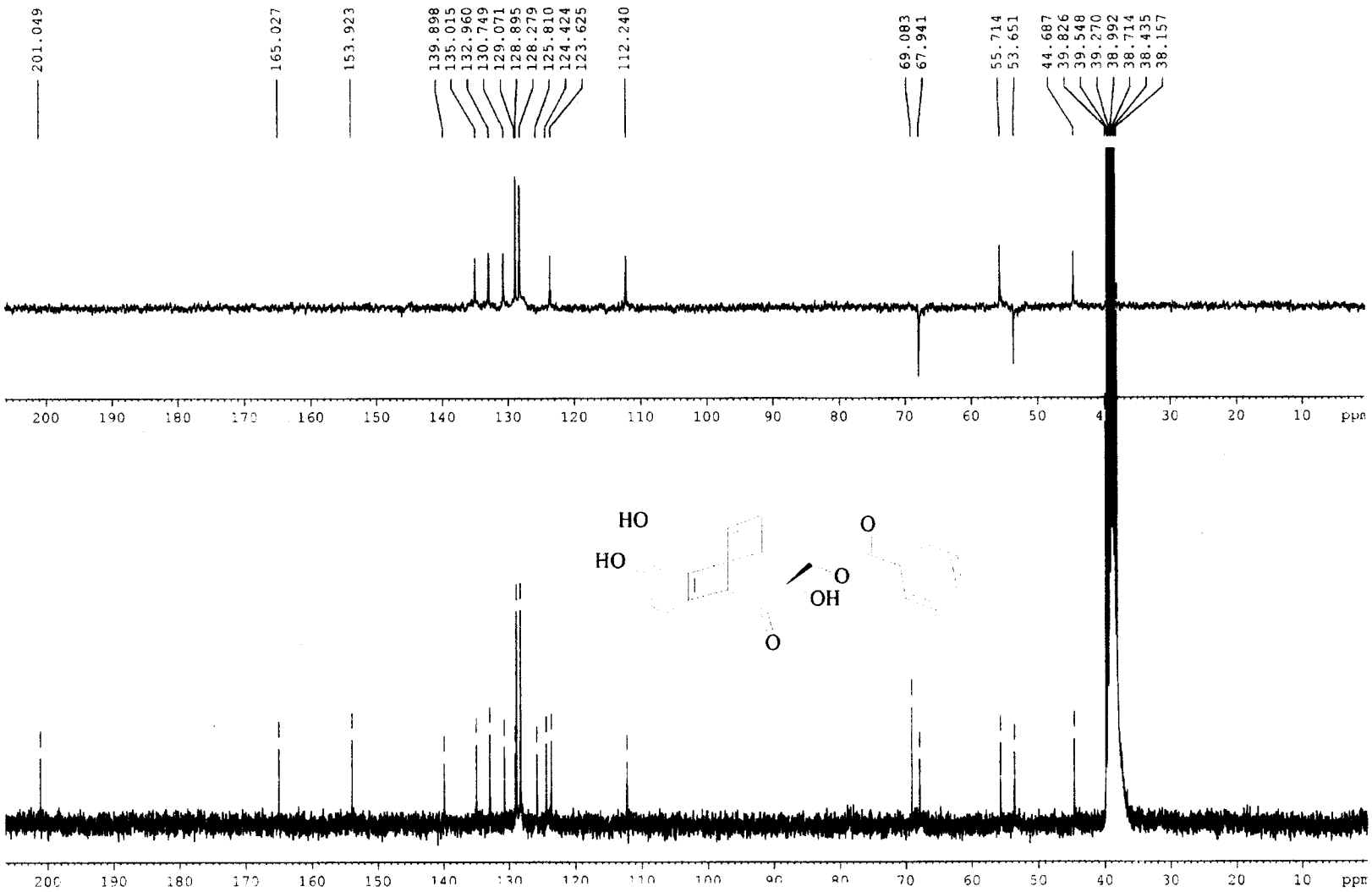


Fig 3.18: ^{13}C NMR spectrum of **9**

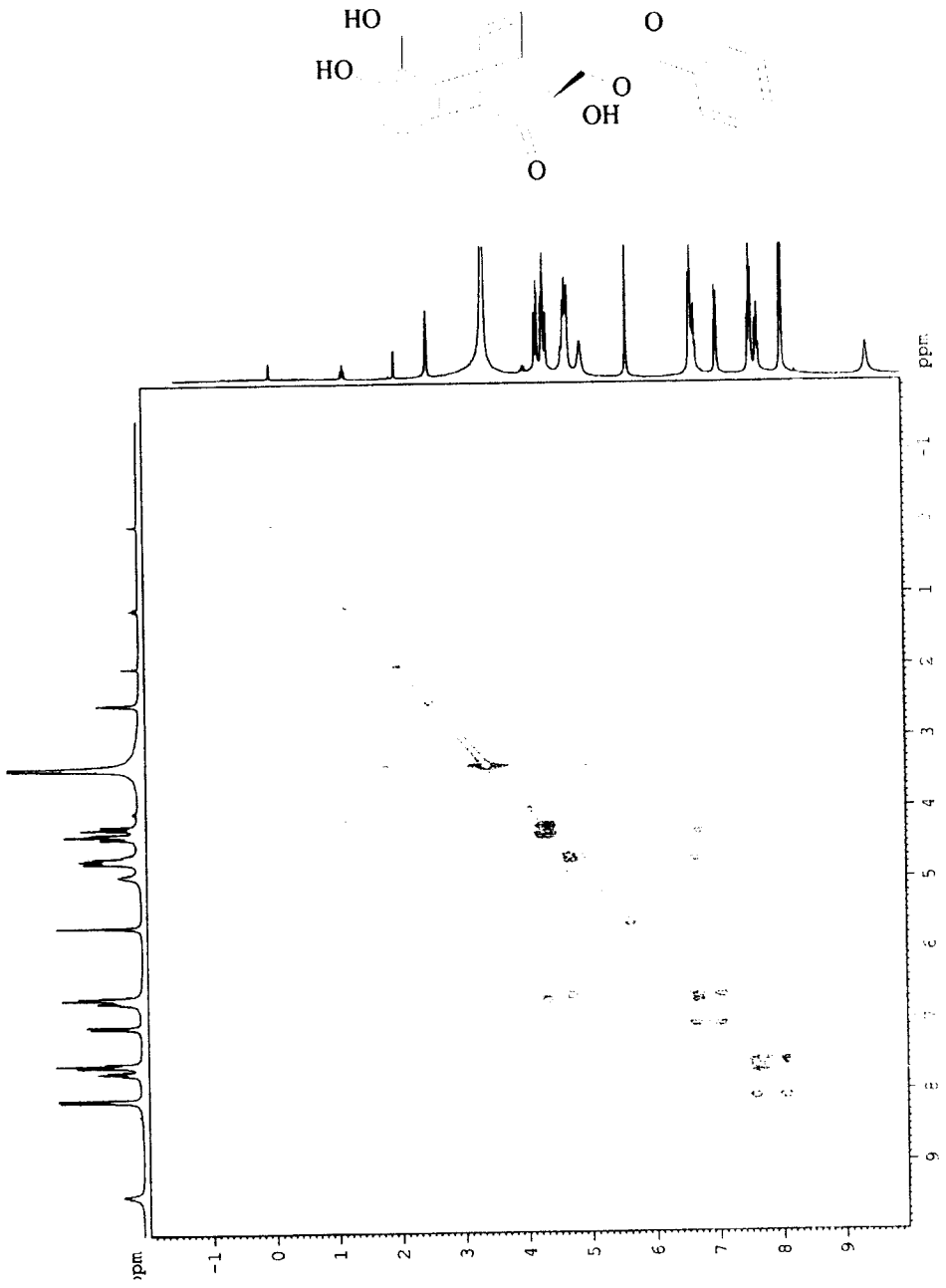


Fig. 3.19: ^{1}H-^{1}H COSY spectrum of **9**

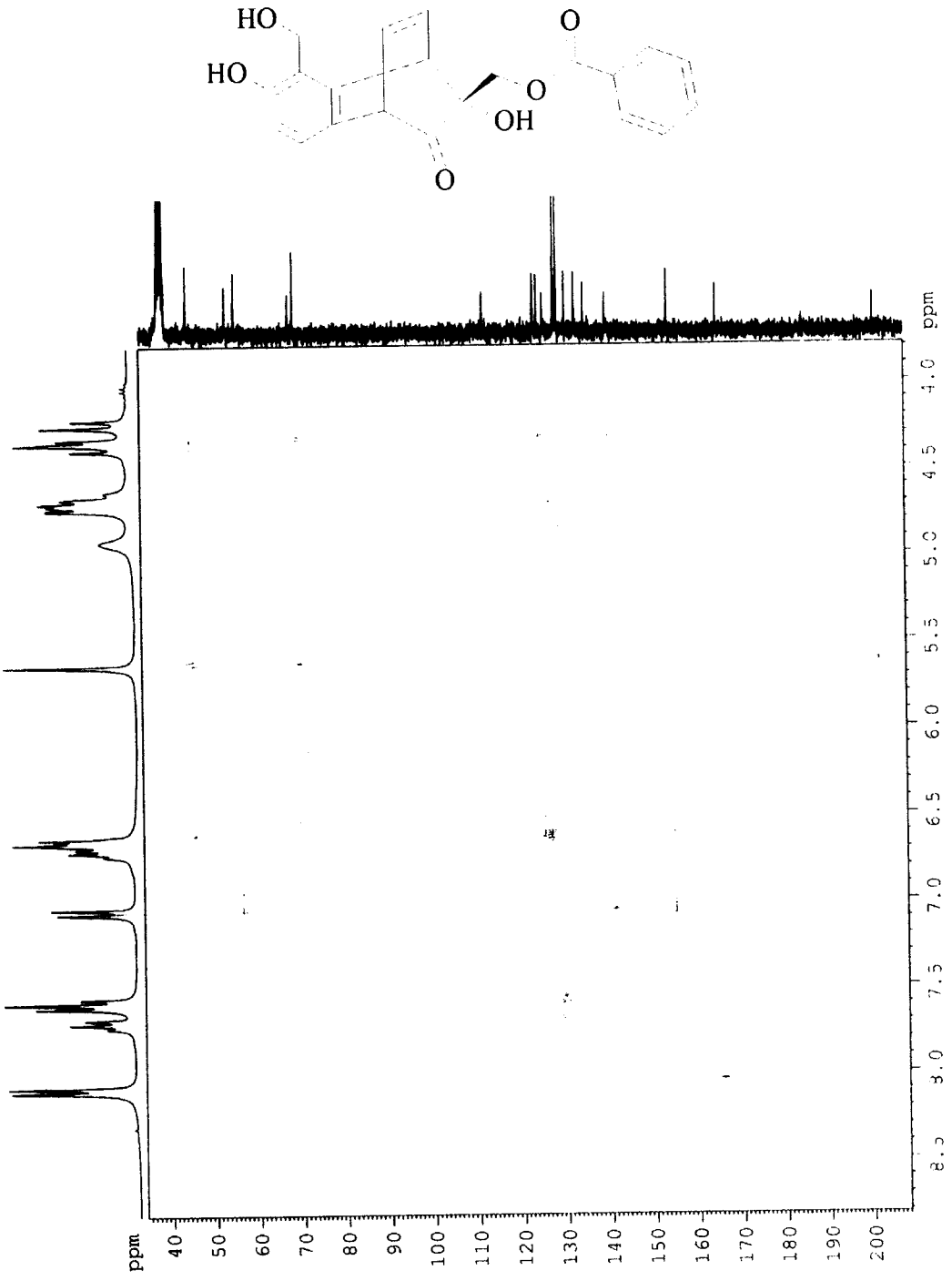


Fig. 3.20: ^{1}H-^{13}C HMBC spectrum of **9**

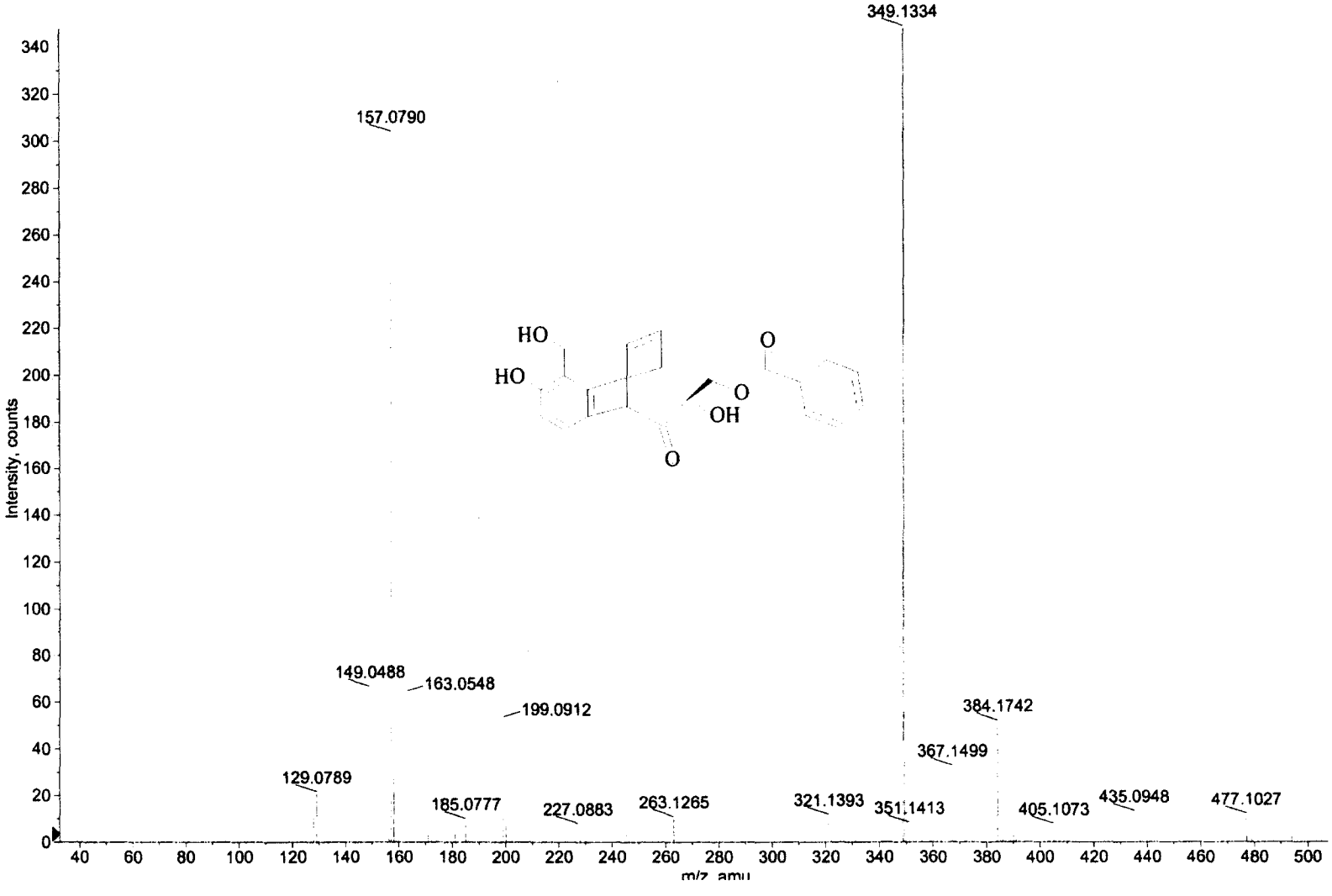


Fig 3.21: ESIMS spectrum of **9**

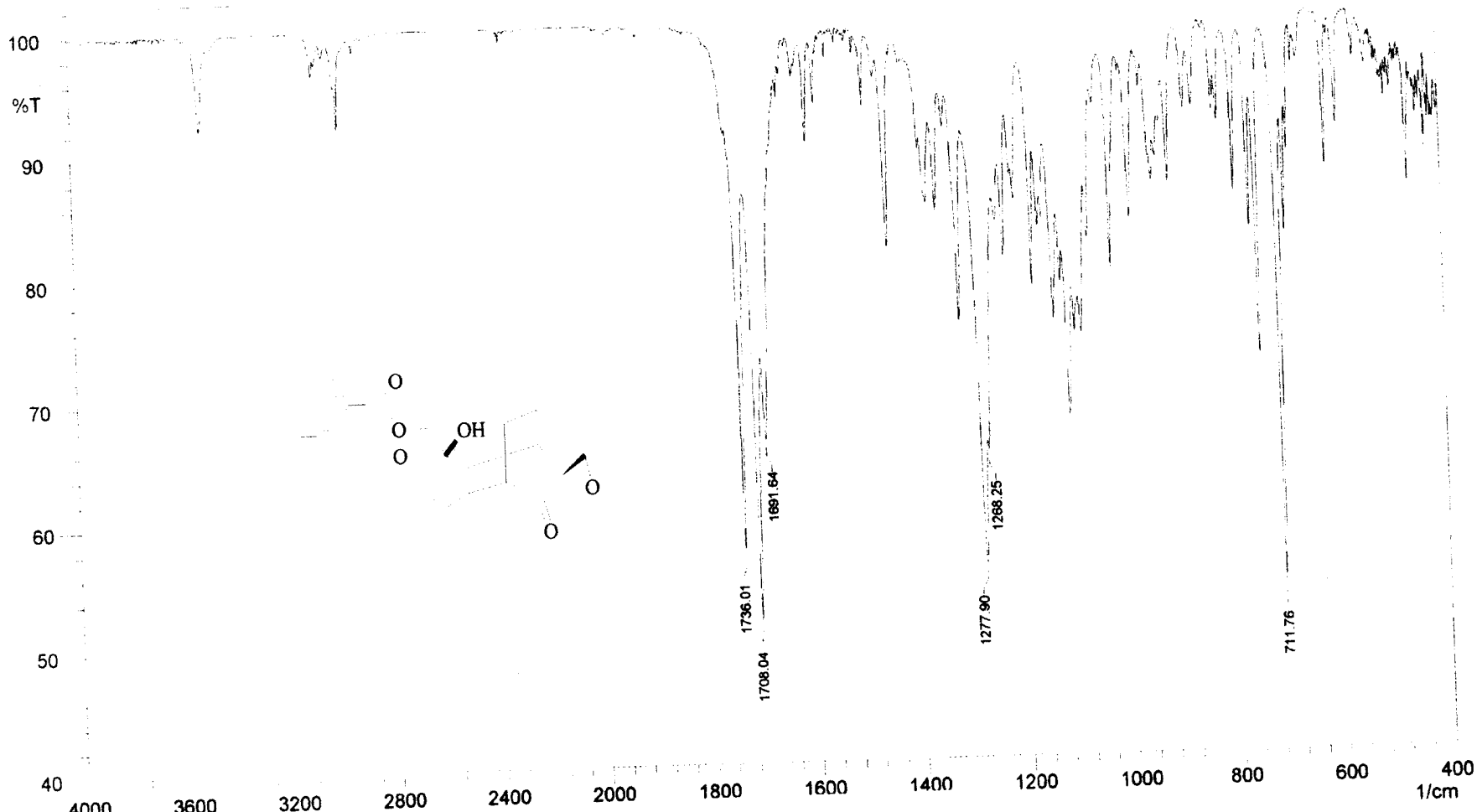


Fig 3.22: IR spectrum of **10**

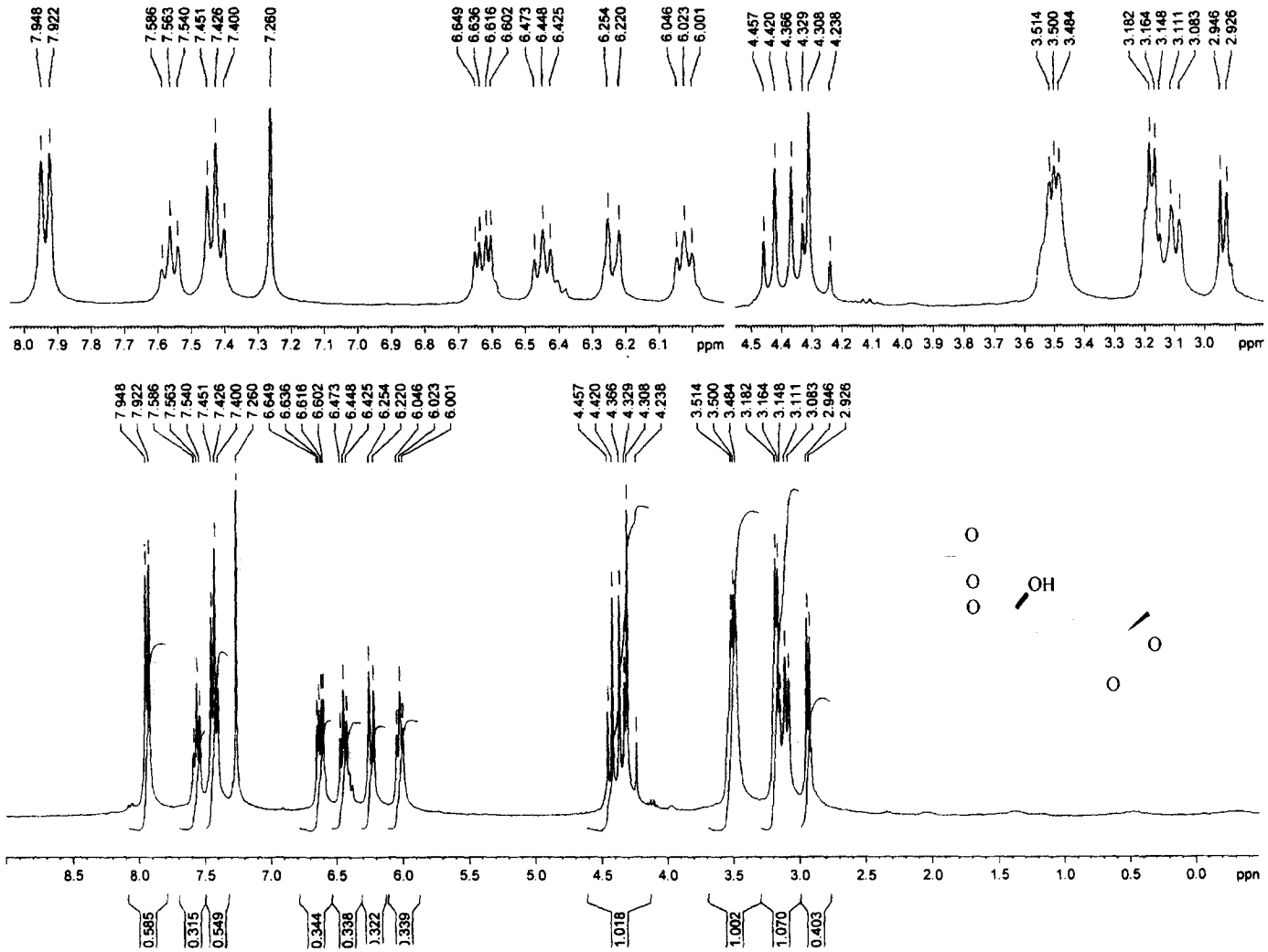


Fig 3.23: ^{1}H NMR spectrum of **10**

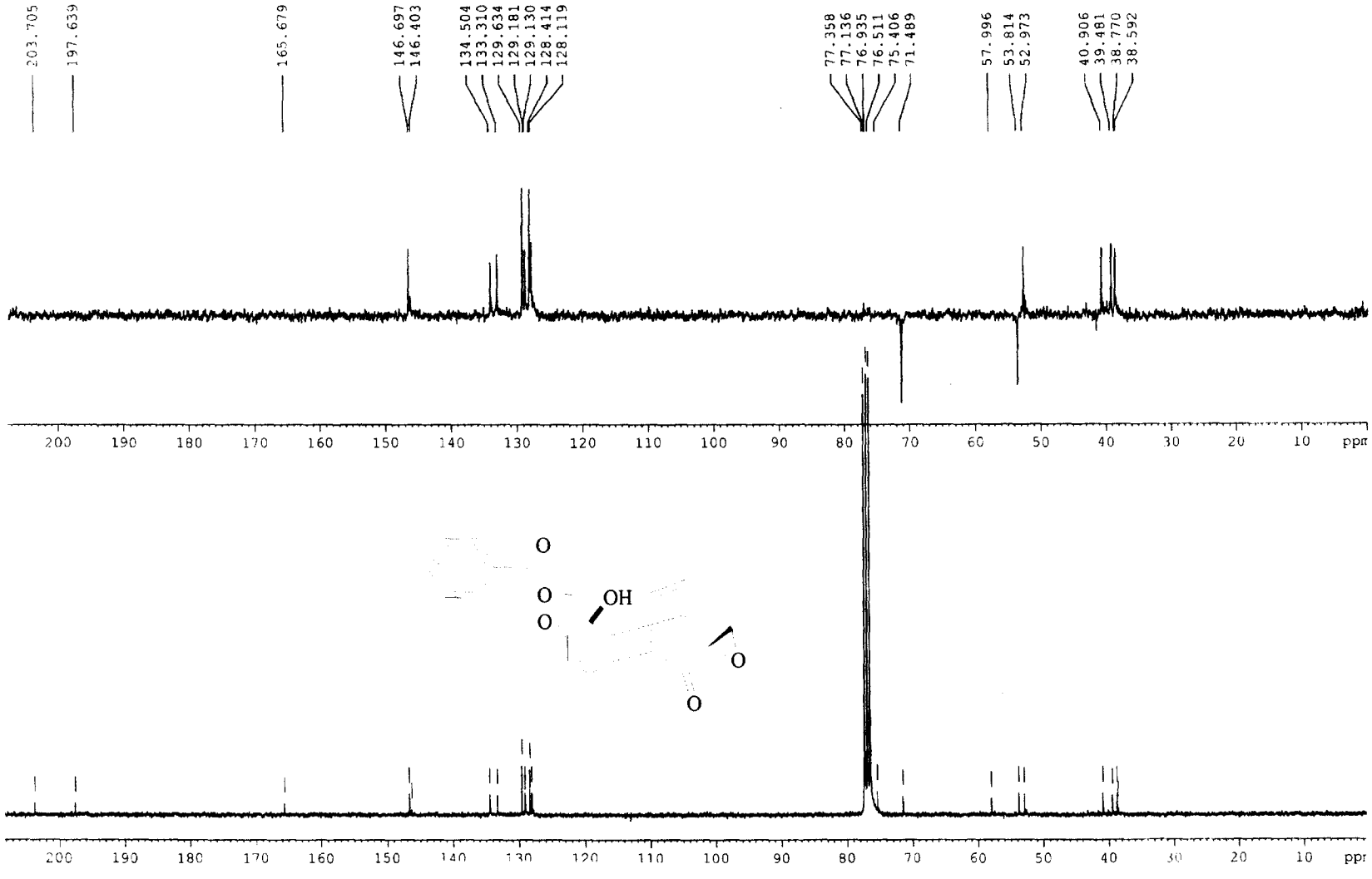


Fig 3.24: ^{13}C NMR spectrum of **10**

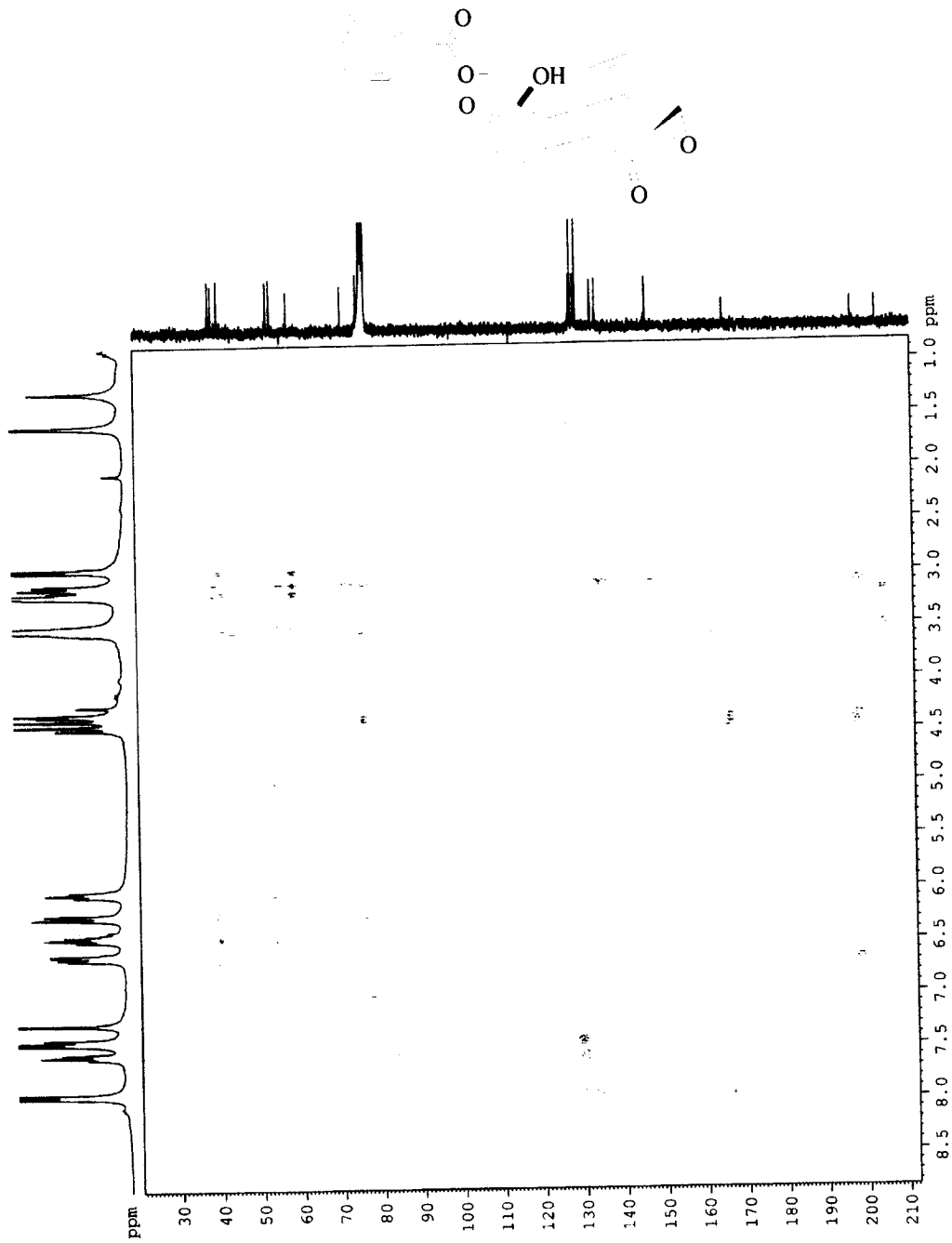


Fig. 3.25: ^{1}H-^{13}C HMBC spectrum of **10**

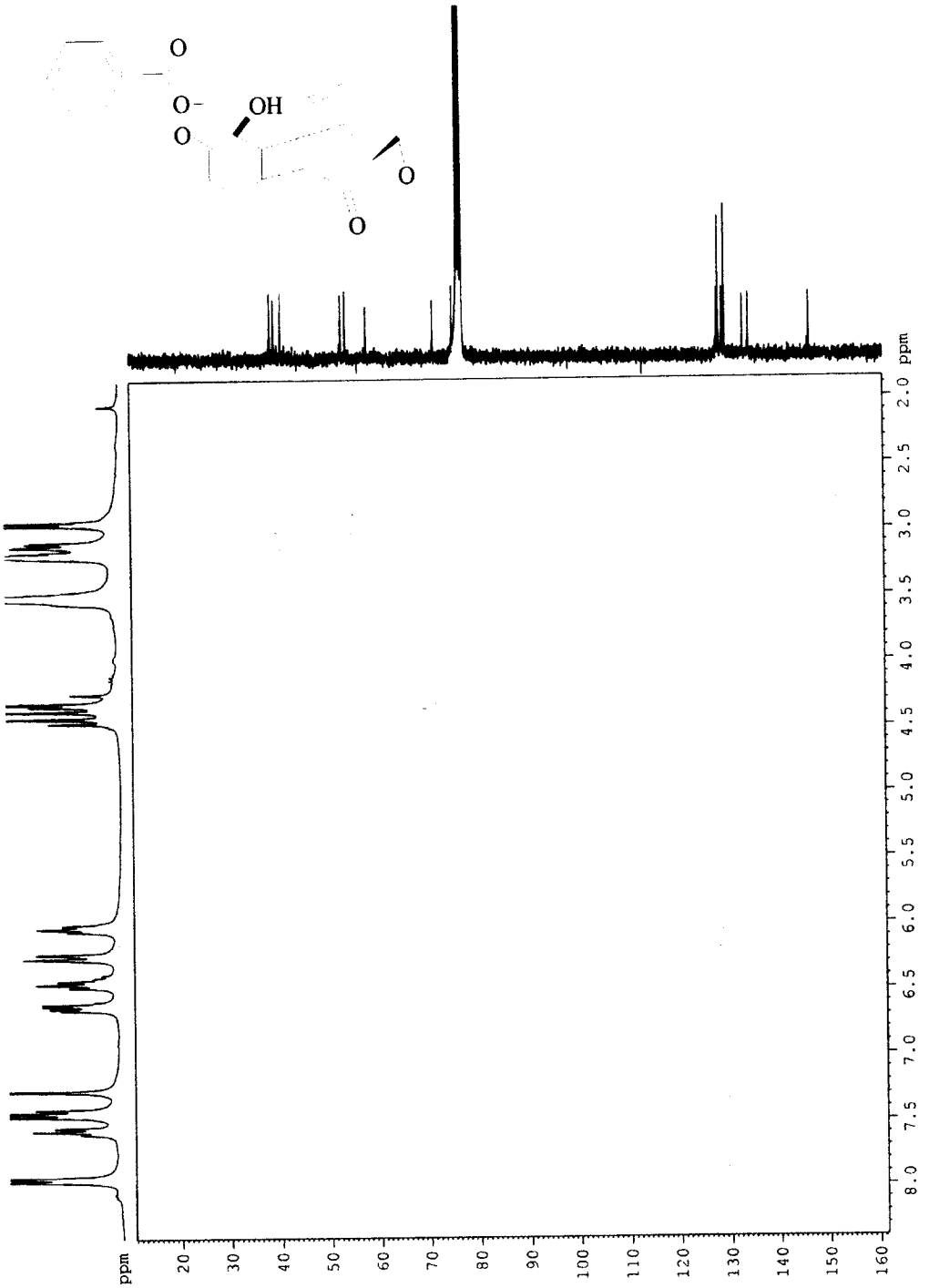


Fig. 3.26: ^{1}H-^{13}C HMBC spectrum (expansion) of **10**

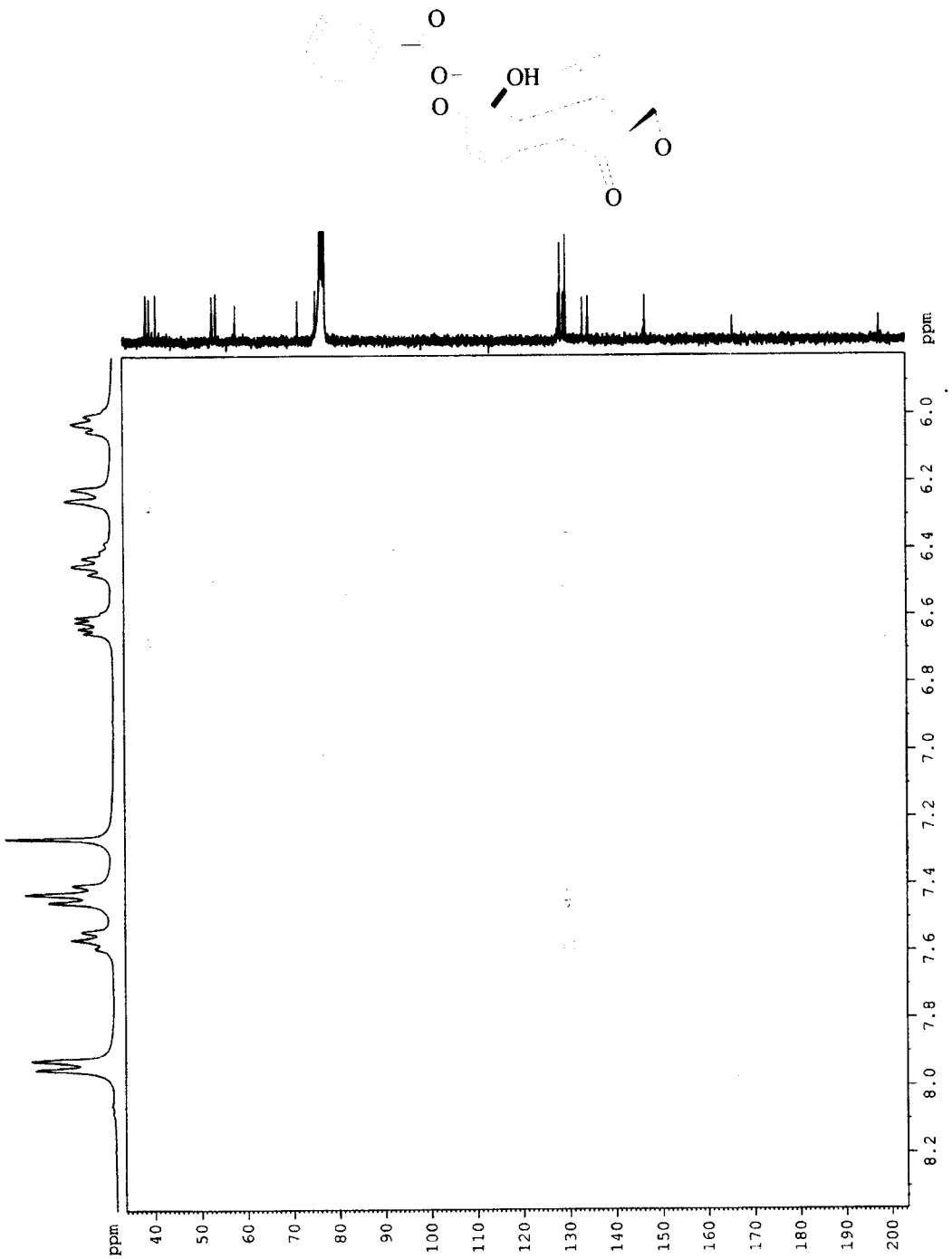


Fig. 3.27: ^{1}H-^{13}C HMBC spectrum (expansion) of **10**

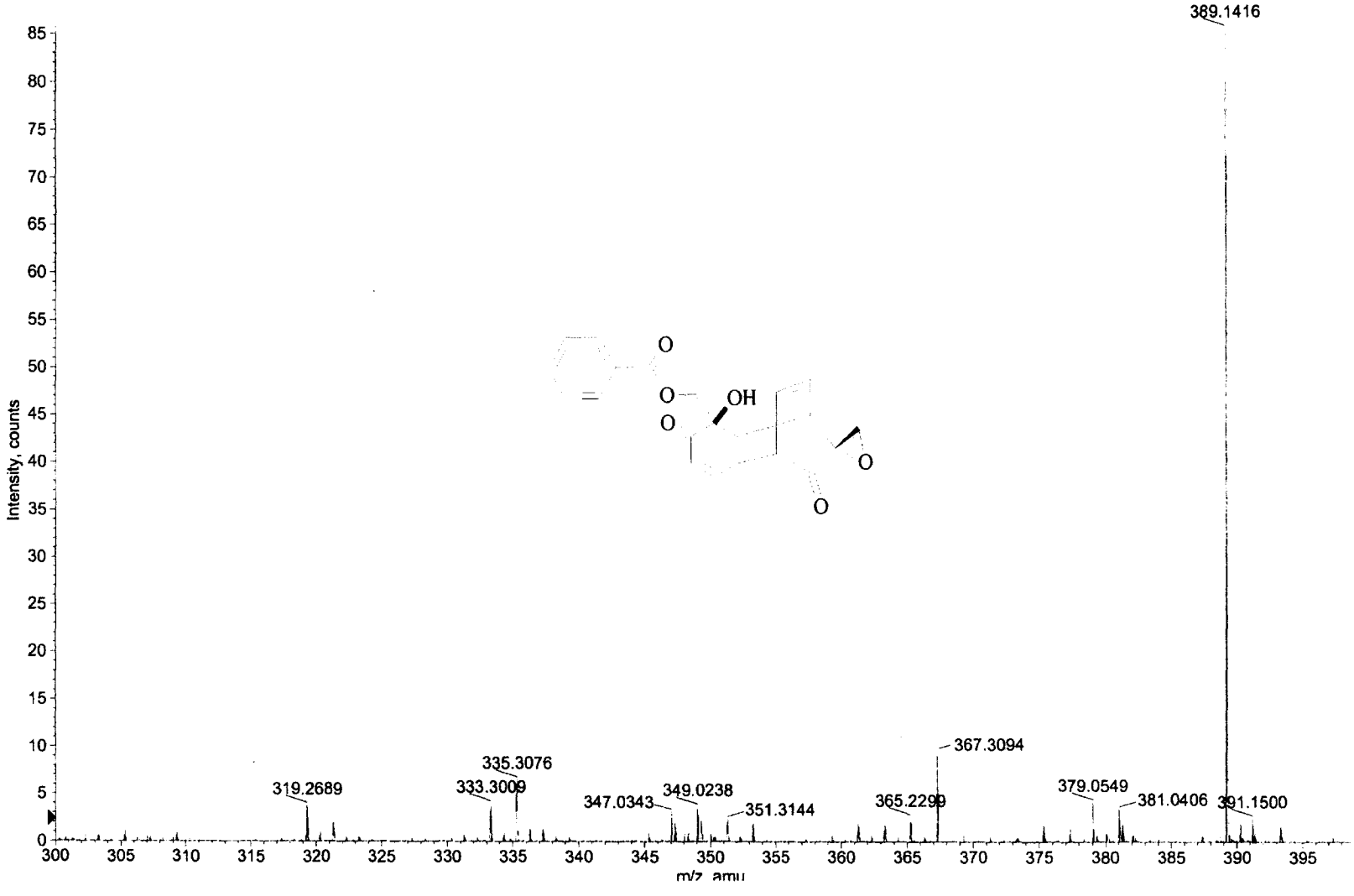
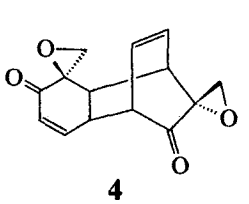


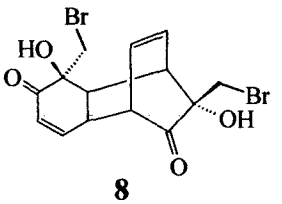
Fig 3.28: ESIMS spectrum of **10**

Experimental

1,3,4,4a,5,8a-Hexahydro-1,4-ethenonaphthalene-3,5-bisspirooxirane-2,6-dione **4** and *1,3,4,4a,5,8a-Hexahydro-3,5-bis(bromomethyl)-3,5-dihydroxy-1,4-etheno-naphthalene-2,6-dione* **8**



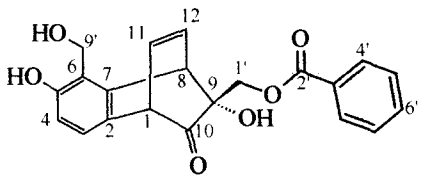
4



8

Prepared according to the reported[2] literature procedure.

Reaction of dimer **4** *with benzoic acid in acetonitrile; formation of [(9R)-5,9-dihydroxy-6-(hydroxymethyl)-10-oxotricyclo[6.2.2.0^{2,7}]dodeca-2,4,6,11-tetraen-9-yl]methylbenzoate* **9**



9

A mixture of dimer **4** (0.244 g, 1 mmole), benzoic acid (0.366 g, 3 mmole) and TBAB (10 mg) was heated to reflux in dry acetonitrile (10 mL) for 8 hrs. The reaction mixture was cooled to room temperature and extracted with $CHCl_3$. The organic extracts were washed with saturated $NaHCO_3$, water, dried over Na_2SO_4 and the solvent evaporated to leave a brown viscous residue (0.402 g). Purification using column chromatography by eluting with $CHCl_3$:MeOH (9.8:0.2) gave **9** as white solid, m.p. 164-66°C. Recrystallization from CH_3CN gave colourless crystals, m.p. 182°C.

IR ν_{max} (KBr, **Fig.** 3.16): 3570 (OH), 3400 (OH), 1726 (ester C=O), 1687 (C=O), 1597, 1292, 983 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.17): For assignments refer **Figure I**, pg 291.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 3.18): For assignments refer **Figure II**, pg 291.

ESIMS (Fig. 3.21): *m/z* $[M + H]^+$ calcd for $C_{21}H_{19}O_6$ 367.1176, Found 367.1499.

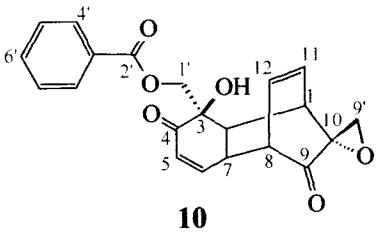
Reaction of dibromide **8** *with potassium benzoate in DMF; formation of* **9**

A mixture of the dibromide **8** (0.244 g, 1 mmole) and potassium benzoate (0.48 g, 3 mmole) was heated to 85°C in dry DMF (10 mL) for 2 hrs. The reaction mixture was cooled to room temperature and extracted with $CHCl_3$. The organic extracts were washed with saturated $NaHCO_3$, water, dried over Na_2SO_4 and the solvent evaporated to leave dark yellow viscous oil (0.1288 g). Purification by silica gel column chromatography and elution with $CHCl_3$:MeOH (9.8:0.2) gave **9** as white solid having m.p. 164-66°C. Recrystallization from CH_3CN gave colourless crystals having m.p. 182°C.

Reaction of dibromide **8** *with potassium benzoate in DMSO; formation of* **9**

A mixture of dibromide **8** (0.244 g, 1 mmole) and potassium benzoate (0.48 g, 3 mmole) was heated to 100-105°C in dry DMSO (10 mL) for 2 hrs. The reaction mixture was cooled to room temperature and extracted with $CHCl_3$. The organic extracts were washed with saturated $NaHCO_3$, water, dried over Na_2SO_4 and the solvent evaporated to leave dark yellow viscous oil (0.158 g). Purification by silica gel column chromatography and elution with $CHCl_3$:MeOH (9.8:0.2) gave **9** as white solid having m.p. 164-66°C. Recrystallization from CH_3CN gave colourless crystals having m.p. 182°C.

Reaction of dibromide **8** *with potassium benzoate in benzene-water mixture using TBAB as catalyst; formation of (3R)-Hydroxy-4,9-dioxo-10,10-epoxymethyltricyclo[6.2.2.0^{2,7}]dodeca-5,11-dien-3-ylmethylbenzoate* **10**



10

A mixture of dibromide **8** (0.244 g, 1 mmole), potassium benzoate (0.48 g, 3 mmole) and TBAB (10 mg) was refluxed in water (0.5 mL) and benzene (15 mL) mixture for 15 hrs. The reaction mixture was cooled to room temperature, the organic layer was separated and washed with saturated $NaHCO_3$, water and dried over Na_2SO_4. Evaporation of the solvent afforded a pale brown residue. Purification by silica gel column chromatography using C_6H_6 as eluent gave dimer **4**. Further elution with C_6H_6:Et_2O (8:2) gave white solid. Careful TLC observation of the white solid (C_6H_6:Et_2O; 6:4) indicated it to be a mixture of two closely spaced spots which could be resolved (TLC) using $CHCl_3$:EtOAc (9:1) as solvent system. Further purification of the white solid by silica gel column chromatography using $CHCl_3$:EtOAc (98:2) as eluent gave **10** as white solid. Recrystallization from the same solvent gave white shiny flakes having m.p. 180°C.

IR ν_{max} (KBr, **Fig.** 3.22): 3500 (OH), 1736 (ester C=O), 1708 (C=O), 1691 (-HC=CH-CO-), 1600, 1480, 1277, 1100, 760, 711 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz, **Fig.** 3.23): For assignments refer **Figure IV**, pg 295.

^{13}C NMR ($CDCl_3$, 75 MHz, **Fig.** 3.24): For assignments refer **Figure V**, pg 295.

ESIMS (Fig. 3.28): *m/z* $[M + H]^+$ calcd for $C_{21}H_{19}O_6$ 367.1176, Found 367.3094; $[M + Na]^+$ cald for $C_{21}H_{18}O_6Na$ 389.09956, Found 389.1416

Reaction of saligenin dimer **4** *with* I_2 *in dioxane*

To a solution of the saligenin dimer **4** (0.5 g, 2.05 mmole) in dioxane (60 mL) was added iodine (1.07 g, 4.1 mmole). The reaction mixture was stirred for 24 hrs at room temperature followed by addition of saturated solution of sodium thiosulfate (50 mL). The mixture was stirred vigorously for 1 hr. The organic phase was separated, washed with water (2 × 15 mL), dried over Na_2SO_4 and concentrated under reduced pressure. No appreciable amount of residue was left in the flask after total removal of the solvent.

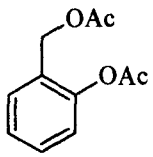
Diethylbenzoyl phosphonate **12**

Triethyl phosphite (9.1 g, 0.05 mole) was added drop by drop over a period of 30 mins to benzoyl chloride (7.02 g, 0.05 mole) at 0°C. The mixture was stirred at room temperature for 30 mins and then distilled under reduced pressure to give pure diethylbenzoyl phosphonate **12** (8.54 g, 54.5%) as yellow oil[12], b.p. 140-42°C at 3 mm.

Reaction of saligenin **3** *with diethylbenzoyl phosphonate* **12**

To a solution of saligenin **3** (0.198 g, 1.6 mmole) in dry CH_2Cl_2 (10 mL) was added diethylbenzoyl phosphonate **12** (0.4065 g, 1.68 mmole) and DBU (1 equiv) and the mixture was refluxed for 5 hrs. The organic portion was separated and concentrated under reduced pressure to give pale brown sticky residue insoluble in common organic solvents.

Acetylative oxidation of saligenin **3**, *formation of 2-(acetyloxy)benzyl acetate* **15**



15

To a stirred solution of **3** (0.661 g, 5.3 mmole) in Ac_2O (3 mL) was added $NaIO_4$ (1.395 g, 6.4 mmole) in portions over a period of 1 hr. Stirring was further continued for 5 hrs at room temperature. The reaction mixture was then poured into a saturated solution of $NaHCO_3$ and stirred vigorously to neutralise excess acetic acid. The aqueous layer was extracted with ethyl acetate (3 × 5 mL) and combined organic extracts were washed successively with saturated $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL), followed by drying over Na_2SO_4. Removal of the solvent furnished a residue. Purification by silica gel column chromatography using petroleum ether:ethyl acetate (8:2) as eluent gave 2-(acetyloxy)benzyl acetate **15** (0.753 g, 73%) as yellow oil.

IR ν_{max} (KBr): 1766, 1737, 1228, 1176, 754 cm^{-1}.

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Summary

The Thesis is divided into three chapters which are further subdivided into sections.

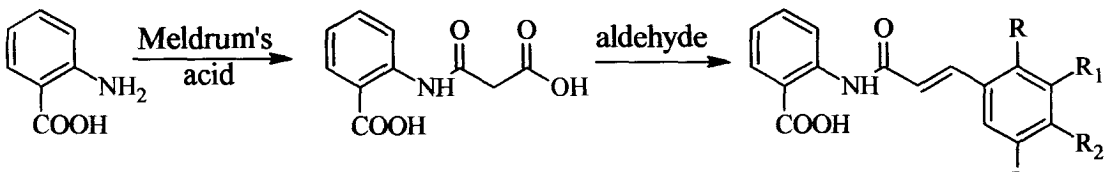
Chapter 1

Deals with the synthesis of avenanthramides, constituent of oats, using a two-step general procedure.

Oats have been considered to be a good source of antioxidants for a long time[1] and recently it has been shown that, this antioxidant activity and the fresh taste of oat products is mainly due to the presence of a group of amides called avenanthramides[2]. Chemically avenanthramides are substituted *N*-cinnamoylanthranilate derivatives. They are phytoalexins and are produced when oat leaves are infected or inoculated with an incompatible race of crown rust fungus[3,4].

Although 40 different natural avenanthramides have been detected (by GCMS analysis and 2D chromatography) in oat extracts[2], very few have been isolated that too in mg quantities. Some of them have been synthesized by using conventional methods for the purpose of identification in oat extracts, structure-antioxidant activity studies[3] and for providing support to the structure assigned but the yields are not satisfactory.

We have developed a general method to synthesize malonamic acids from amines which are further converted into avenanthramides by the modified Knoevenagel reaction of malonamic acid with benzaldehyde derivatives.



1 **2** avenanthramides **3-15**

All the natural avenanthramides (**3-6**) prepared were characterized by comparison of their m.p. and spectral data (IR & ^{1}H NMR) with that reported in the literature and the synthetic analogues of natural avenanthramides (**7-16**) prepared were fully characterized by the study of their IR, ^{1}H NMR, ^{13}C NMR & MS or elemental analysis.

Table 1

Avenanthramides	R	R_1	R_2	R_3	% yield
3	H	H	OH	H	85
4	H	OH	OH	H	75
5	H	OCH_3	OH	H	85
6	H	OCH_3	OCH_3	H	74
7	H	OCH_3	OH	OCH_3	65
8	H	OCH_3	OCH_3	OCH_3	65
9	H	O-CH_2-O		H	71
10	H	H	OCH_3	H	70
11	OCH_3	H	OCH_3	H	86
12	OH	H	H	H	73
13	H	OH	H	H	95
14	H	H	H	H	66
15	H	H	Cl	H	74
16	Cl	H	H	H	65

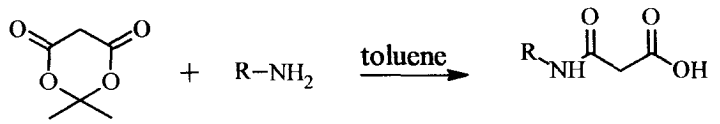
In conclusion the simple two-step method developed has two distinct advantages.

1) Intermediate malonamic acid can be chemically separated and purified.
2) Does not involve any type of chromatography for separation and purification. As such, this method can be conveniently scaled up.

The next part deals with the synthesis of malonamic acids.

β-Dicarbonyl derivatives belonging to the malonamic acid family are very important compounds having interesting pharmacological properties, including

antihypertensive, sedative, anticonvulsant, anti-inflammatory, analgesic and CNS stimulating activities[5].

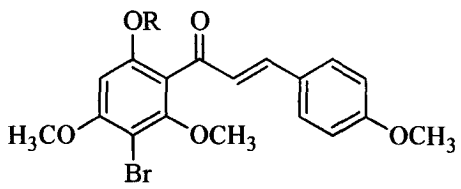


Meldrum's acid Amine Malonamic acid

Condensation of aniline, anthranilic acid, PABA, 1-naphthylamine, benzylamine, etc. gave the corresponding malonamic acid derivatives. All the malonamic acids prepared in the present study were characterised by IR, ^{1}H NMR and ^{13}C NMR.

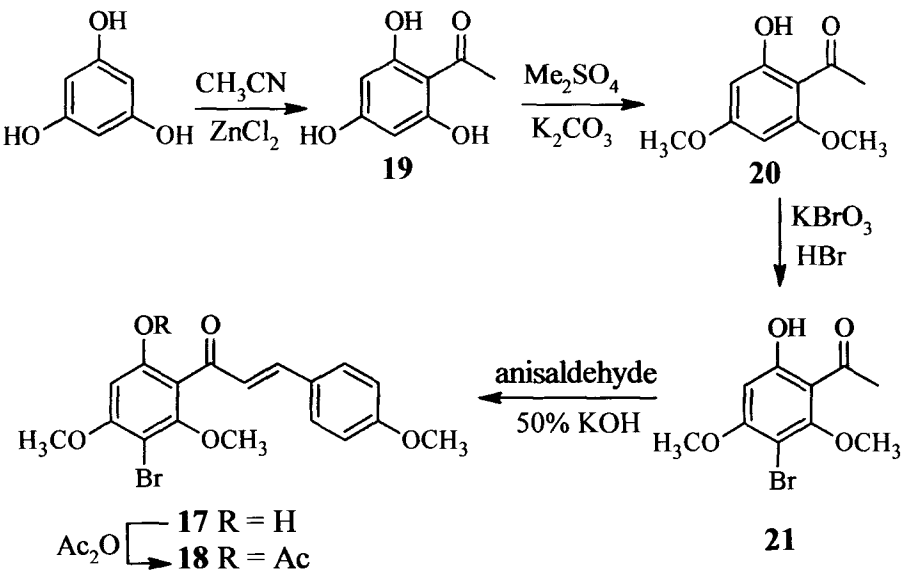
Chapter 2 is divided into two sections.

2.1 Deals with the synthesis of a novel 5′-bromo-2′-hydroxy-4,4′,6′-trimethoxychalcone **17** isolated[6] from the leaves of *Garcinia nervosa*. Although brominated natural compounds are reported from marine sources, this constituted the first report of the occurrence of a brominated natural product in non-marine plants. The simple structure of **17** and the unusual observation in its ^{1}H NMR spectrum attracted our attention. It showed a singlet for the *trans* α, β-olefinic protons which was resolved into two individual doublets (J = 16 Hz) in its acetate **18**.

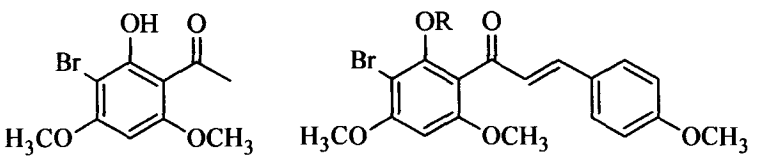


17 R= H
18 R= Ac

Based on the retrosynthetic analysis the following scheme was planned.



Although the melting point of the synthetic and the natural chalcone were identical (180°C), the ^{1}H and ^{13}C NMR spectra were found to be different in a way that the chalcone prepared by us showed two doublets (J = 15 Hz) for the *trans* α,β-olefinic protons instead of the reported[6] singlet. Moreover the observed m.p. 180°C of the acetate of the chalcone prepared was not matching with the m.p. of the acetate of the natural product (m.p. 122-124°C). Due to this discrepancy, the intermediate bromo derivative and the chalcone were fully characterised by NOE, HMBC and HMQC experiments leading to the revision of their structures as **22** and **23** respectively. Consequently the structure of the acetate became **24**.

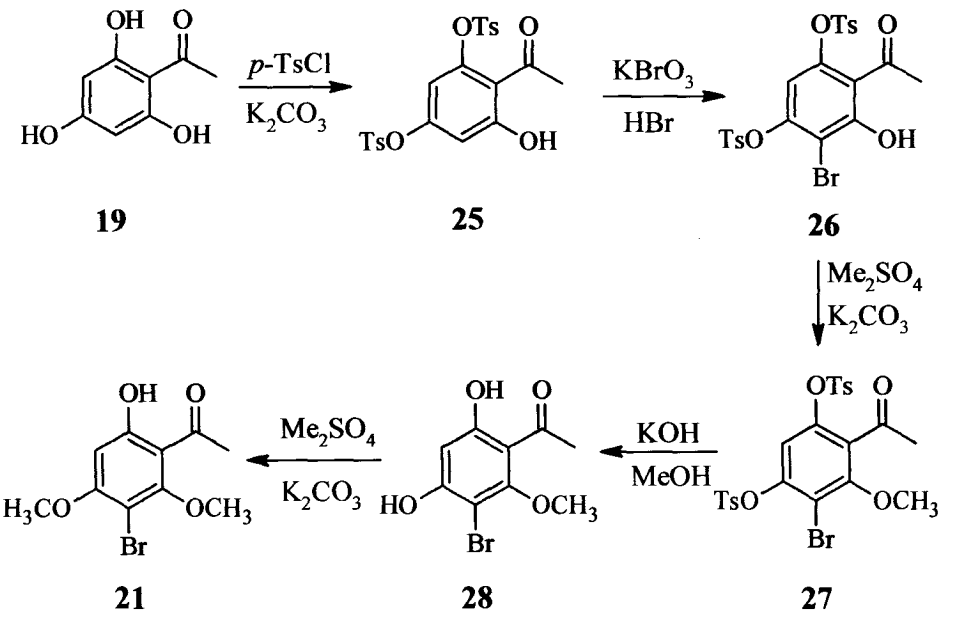


22

23 R=H
24 R=Ac

Various brominating reagents such as Br_2, AcOH; Br_2, H_2O; Br_2, *o*-$Cl_2C_6H_4$; KBr, Oxone; KBr, H_2O_2; KBr, ammonium molybdate, NBS, DMF; NBS, H_2SO_4 and NBS, diisopropylamine, different blocking groups like isopropyl bromide, $AlCl_3$; H_2SO_4; ICl, etc. and some protecting groups such as benzyl, acetoxy, *p*-tosyloxy, etc. were tried on **20** but all gave the bromo derivative **22** instead of the required **21**.

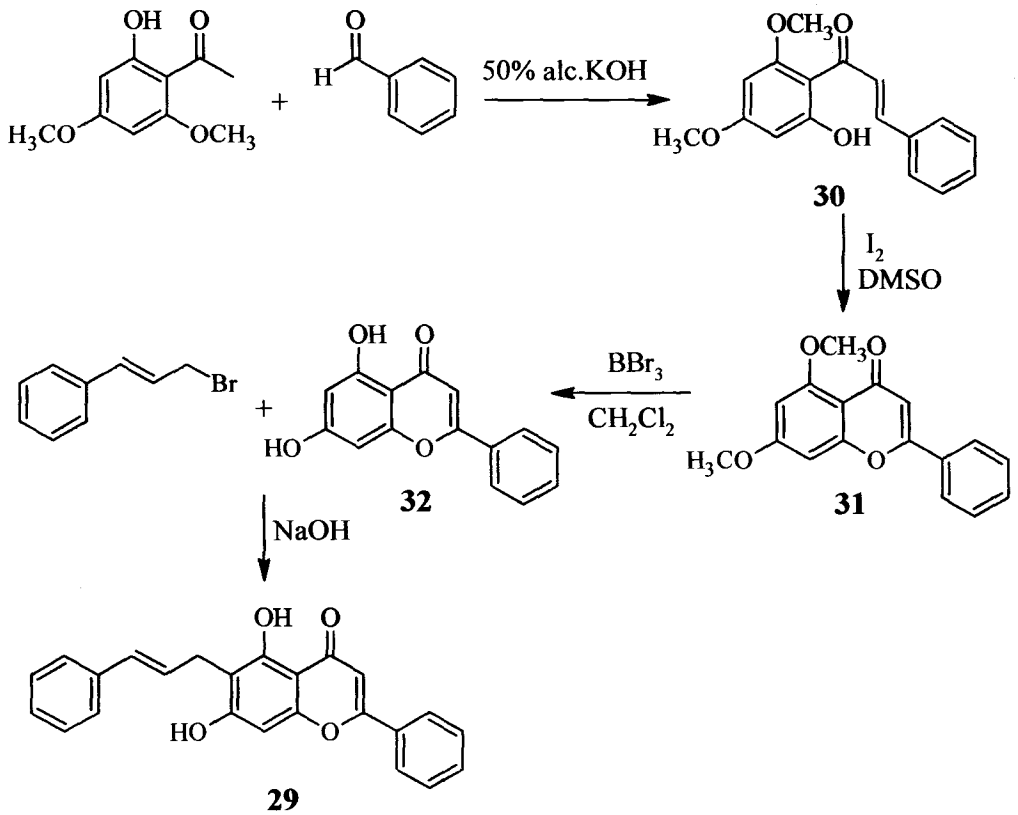
Alternative routes to **21** involved protection of two OH groups of **19**. Protection as dibenzyl ethers of **19** did not yield the expected **21**. But protection of OH groups as di-*p*-tosyl esters did work to give the required **21** as shown below.



19 **25** **26** **27** **28** **21**

All the compounds prepared in this scheme are new and not reported in literature. Hence they were fully characterised on the basis of their NMR and MS data. Condensation of **21** with *p*-methoxy benzaldehyde gave the chalcone **17** whose ^1H NMR showed a singlet for the *trans* olefinic α, β-protons as reported for the natural chalcone and its acetate data (m.p. & NMR) also matched well.

2.2 Deals with the synthesis of 6-cinnmaylchrysin isolated[7] from *Chinese propolis*. Propolis commonly called 'bee glue' a sticky material collected by honeybees from buds and exudates of plants is used in the construction and adaptation of bee hives[8]. Propolis is extensively used particularly in herbal cosmetics and in medicine due to its versatile biological activities[9]. The isolation of 6-cinnamylchrysin **29** constitued the first report of a flavanoid having a cinnamyl group. To our knowledge this appears to be the first and the only report of its synthesis.



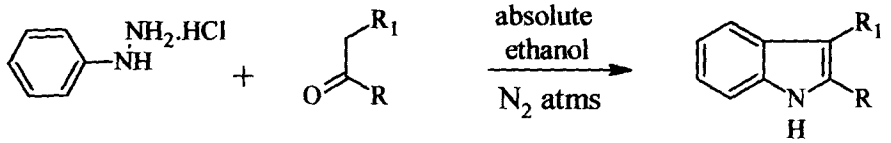
All the intermediates (**30**, **31** & **32**) prepared in the above scheme are known in literature and were characterized by comparison of their m.p. with that reported in literature. The IR, ^{1}H NMR, ^{13}C NMR and MS data of **29** are in agreement with that reported for the natural **29**.

Chapter 3 is divided into two sections.

3.1 The first part deals with a green approach for the synthesis of 2,3-disubstituted indoles and 1,2,3,4-tetrahydrocarbazoles.

Indole derivatives occur widely in nature and have unique biological activities. Many synthetic methods for construction of the indole ring have been reported[10] but the classical Fischer method has been the mainstay of chemists involved in the synthesis of indoles and their derivatives[11].

In the present study the Fischer method for the synthesis of indoles and their derivatives has been modified and involves refluxing phenyl hydrazine hydrochloride **33** and ketone **34-45** in ethanol under N_2 atmosphere followed by pouring the reaction mixture in glacial acetic acid to give twelve indole derivatives in yields ranging from 66.5% to quantitative.

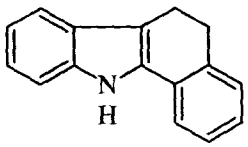


33 **34-45** **46-57**

Table 2

Indoles and 1,2,3,4-tetrahydrocarbazoles	R	R_1	% yield
46	CH_3	CH_3	85
47	CH_3CH_2	CH_3	quantitative
48	CH_3	$CH(CH_3)_2$	70
49	CH_3	$CH_2(CH_2)_2CH_3$	97
50	$CH_2CH_2CH_2$		quantitative
51	$CH_2(CH_2)_2CH_2$		quantitative
52	$CH_2(CH_2)_3CH_2$		93.42
53	$CH_2CH(CH_3)CH_2CH_2$		quantitative
54	$CH_2CH_2CH(CH_3)CH_2CH_2$		93

These indole derivatives (**46-53 & 55**) were characterized by comparison of m.p, IR & ^{1}H NMR data with that reported in literature. Similarly indole derivatives **55, 56** and **57** were prepared, by the reaction of **33** with the respective ketones i.e. tetralone, 4,4,7-trimethyl-1-tetralone and 4-methoxy-7-methyl-1-indanone respectively, in good yields. These indole derivatives **54, 56 & 57** are new compounds and characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR & MS) data.



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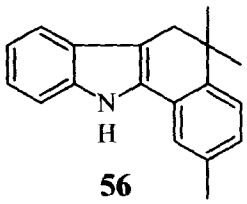

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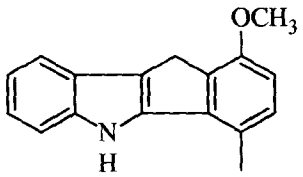
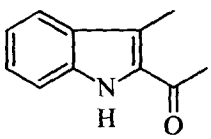
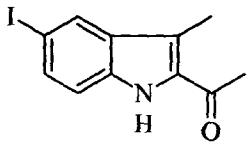


57

The second part deals with the synthesis of Salvadoricine, a natural alkaloid, constituent of *Salvadora persica*[12]. 2-Ethyl-3-methyl indole **47** prepared in the previous section was efficiently converted into Salvadoricine **58** in 81% yield and characterized by comparison of m.p., IR and ^{1}H NMR with that of the natural[12].



58

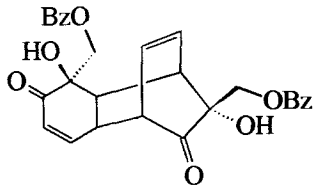


59

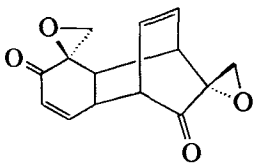
2-Acetyl-5-iodo-3-methyl indole **59** is a new compound and characterized by spectral (IR, ^{1}H NMR, ^{13}C NMR & MS) data.

3.2 Deals with the studies towards the synthesis of grandifloracin **60** isolated[13] from the stem and leaves of *Uvaria grandiflora* and characterization of two new compounds similar to **60**.

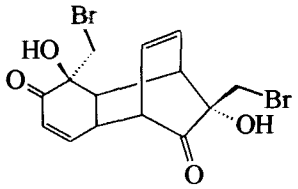
In our lab a simple two step biogenetic type synthesis of **60** from saligenin dimer **61** or the dibromide **62** was previously attempted with partial success[14].



60



61

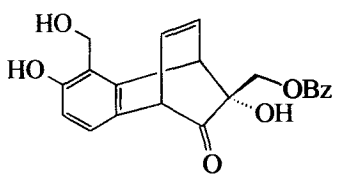


62

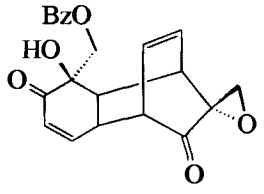
The details of the experimental work carried out and the results obtained by modification of the previously used reaction conditions, reagents, etc are discussed in this section.

Reaction of dimer **61** & benzoic acid or dibromide **62** & potassium benzoate in either CH_3CN[15] or DMF[16] as solvents gave a new product **63** similar to **60** but having one ring aromatic was obtained. The structure **63** was fully supported by its detail spectral (IR, ^1H, ^{13}C NMR, HMQC & ESIMS) data.

While reaction of bisbromohydrin **62** with potassium benzoate in refluxing benzene and water mixture in the presence of TBAB gave monobenzoate **64** having m.p. and NMR data very close to that of grandifloracin **60**. The structure **64** was fully supported by its detail spectral (IR, ^1H, ^{13}C NMR & ESIMS) data.



63



64

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