Some Studies on Structure, Synthesis and Biosynthesis of Natural Products

A THESIS SUBMITTED TO THE
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DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

BY

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Certified that all the corrections have been incorporated in the thesis.

External examiner

JUNE 2001

Research Guide

Date: 21.05.2002

DEDICATED

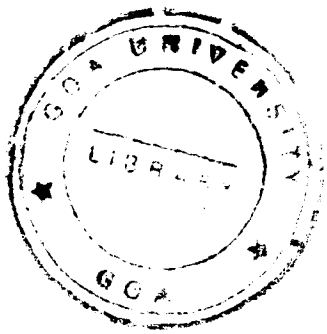
TO

MY PARENTS

T-214

Statement required to be submitted Under the Ordinance OB - 9.9 of Goa University

The Thesis entitled "Some Studies on the Structure, Synthesis and Biosynthesis of Natural products" submitted by the candidate, Shri. Ratnakar Nakul Asolkar for the degree of Doctor of Philosophy in Chemistry is a record of the research work carried out independently by him during the period of study under my guidance. I further state that the research work presented in the thesis has not previously formed the basis for the award of any degree, diploma or other similar titles to the candidate.



Ratnakar Nakul Asolkar
Candidate

Dr. V.P. Kamat
Research Guide

Date: 30.06.2001

Place: Taleigao Plateau, Goa.

CERTIFICATE

This is to certify that the thesis entitled "Some Studies on Structure, Synthesis and Biosynthesis of Natural Products" submitted by Mr. Ratnakar Nakul Asolkar for the award of the degree of Doctor of Philosophy in Chemistry is based on the literature survey/laboratory experiments carried out by him under my supervision. The experimental work presented in this thesis has been carried out independently by him and due acknowledgment has been made whenever outside facilities have been availed of.

Date : 30.06.2001

Dr. V. P. Kamat
(Research Guide)

CONTENTS

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Ratnakar N. Asolkar

GENERAL REMARKS

1. All chart, table, scheme, structure, figures and references in the chapter refer to that particular chapter 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 ^{0}- 80 ^{0}C.
4. Ether refers to diethyl ether.
5. Silica gelused for normal chromatography was of 60-120 mesh size while for the flash column chromatography was 70 - 270 mesh (Mecherey-Nagel & Co).
6. Thin layer chromatography was done on glass plates coated with TLC grade silica gel with 13 % $CaSO_4$ as binder. Also, precoated plastic sheets Polygram SIL G $_{UV254}$ (Mecherey-Nagel & Co., Duren, Germany) were also used. Visualisation of the spots were done by developing the plates in I_2 chamber, and also by using various spraying reagents.
7. Spectral data on the compounds were obtained through the courtesy of various institutions. No details of individual instruments are therefore given. These have been suitably acknowledged.
7. The chemical shift parameters in the ^{1}H NMR and ^{13}C NMR spectra are expressed in δ ppm, with TMS as the internal standard. IR absorption bands are expressed in cm^{-1}. UV absorption signals are expressed in nm.
9. The ^{1}H NMR spectra presented are obtained in normal form and the *J* values reported are of the resolved form.
10. All known compounds were identified by direct comparison of spectral data and the physical constant reported in literature. Molecular formulae of the compound were assigned on the basis of the molecular weight as obtained by mass spectrometry or elemental analysis.
11. **High performance liquid chromatography (HPLC): Instrument I:** Knauer HPLC – equipment containing: monitor, spectral–digital–photometer A0293, 2 pumps type 64 A0307, HPLC – Software V 2.12, mixing chamber A0285, injection valve 6/1 A0263 (type Rheodyne). Preparative: Knauer preparative flow

through cuvette (∅ = 2 mm), sample loop 500 µl; Eurochrom Eurospher RP C18, 100 Å, 5µm, 16 × 250 mm with 16 × 30 mm precolumn.

Instrument II: Jasco HPLC – equipment containing diode array–multiwavelength detector Jasco MD – 910 with time acc. 800 ms, wave acc. 4 nm, start-wavelength 195 nm, end-wavelength 650 nm, 2 pumps type Jasco PU – 987. Analytic: sample loop 20µl, ODS Hypersil 60 Å, 5 µm, 4.6 × 125 mm. Preparative: sample loop 500 µl , Eurochrom Eurospher RP C18, 100 Å, 5µm, 16 X 250 mm with 16 × 30 mm precolumn. HPLC-solvent: acetonitril / water – azeotrop (83.7% MeCN / 16.3% H_2O, bp.: 78.5 °C). The azeotrop was redistilled, filtered through a membrane filter pore Ø: 0.45µm, regenerated cellulose, Sartorius, Göttingen) and then degassed by ultrasonic irradiation.

12. **Spray reagents**

Anisaldheyde / sulphuric acid: 1 ml anisaldheyde was added to a 100 ml solution containing 85 ml methanol, 14 ml acetic acid and 1 ml sulphuric acid.

NaOH or KOH solution: 2 N NaOH or KOH solution was used.

CHAPTER 1

BIOACTIVE SECONDARY METABOLITES FROM MICROORGANISMS

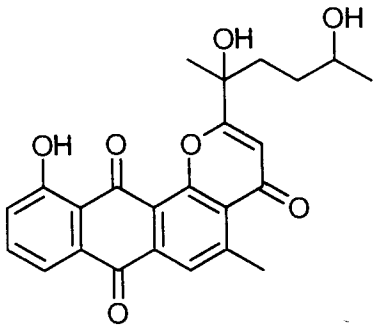
INTRODUCTION

The microorganisms have played a profound role in medicinal science ever since the discovery that, they are not only the cause of infection, but also produce organic substances which can cure the infection. The discovery of penicillin in 1929 heralded the era of antibiotics which ultimately paved the way for our realization that the microorganisms are a rich source of clinically useful natural products[1]. Since then, around 50,000 natural products have been discovered from the microorganisms, and of these substances, more than 10,000 compounds are biologically active. Among the active ones, more than 8000 compounds are antibiotics[1,2]. This tremendous rate of discovery is a testament to the inherent ability of microorganisms to produce bioactive metabolites and the heavy investment made by the pharmaceutical industry in tapping the microbial resources. Due to this investment, over 100 microbial products are in use today as antibiotics, antitumor agents and agrochemicals. Despite many investigations, most antibiotics of microbial origin came from terrestrial bacteria belonging to one taxonomic group-the order *Actinomycetales*. Although the said bacterial group continued to be studied extensively, it is evident that the rate of discovery pertaining to new and novel metabolites from terrestrial *Actinomycetales* is on the decline and hence new sources for bioactive natural products must be explored.

Marine microorganisms are one of such resources, now being explored for their potential as producers of biomedically relevant metabolites. It is now known firmly that marine microorganisms are capable of producing unusual natural products not usually found in terrestrial sources. Marine bacteria, fungi, cyanobacteria and symbiotic microorganisms have been particularly the productive sources of bioactive natural products. Many of these compounds have antibiotic and other useful activities and it is clear that a greater investment in the development of marine microbiology as a renewable source for biomedically relevant substances is required in the present time.

Various studies have shown that the metabolites obtained from the microorganisms are structurally diverse, exhibiting interesting bioactivity[3]. To cite few examples, a marine *Streptomyces sp.* (strain B 8300) from a lagoon in the Gulf of Mexico contained a new pluramycin class antibiotic, δ-indomycinone[5] (**1**), together with the other known me-

tabolites[4]. A new isocoumarin antitumour agent (**2**) known as PM - 94128, was obtained from the culture broth of *Bacillus sp.* PLM-PHD-090 that had been isolated from the marine sediments[6]. Four additional minor antimicrobial agents, thiomarinols D-G, (**3** - **6**) have been obtained from *Alteromonas rava sp.* nov. SANK 73390[7]. The marine bacterium *Micrococcus luteus*, isolated from a new Caledonian sponge of the genus *Xestospongia*[8], produced the glycolipid luttoside (**7**) .



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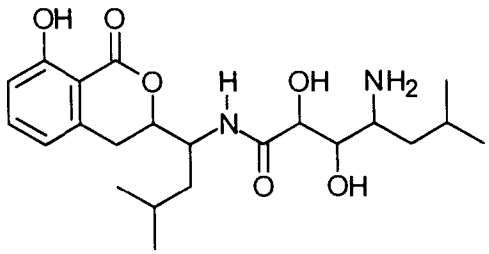

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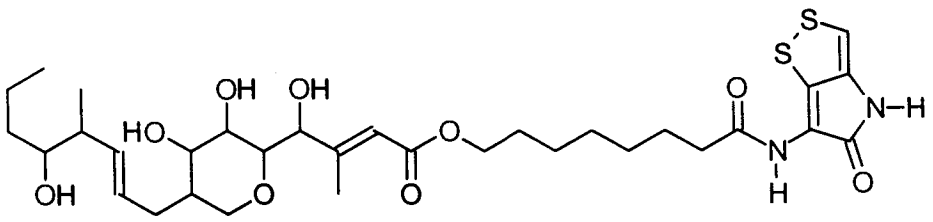

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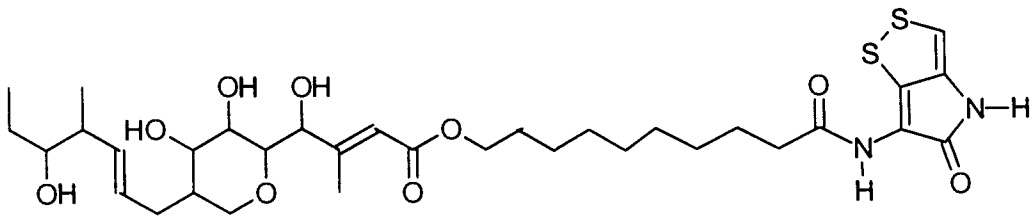

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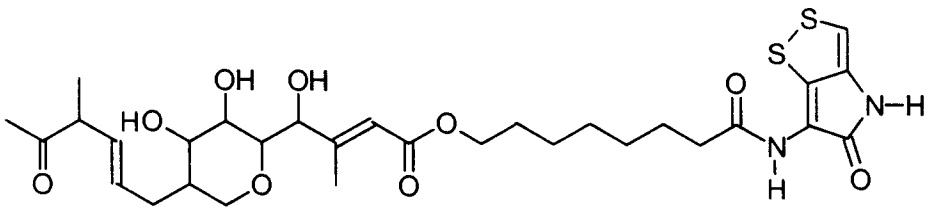

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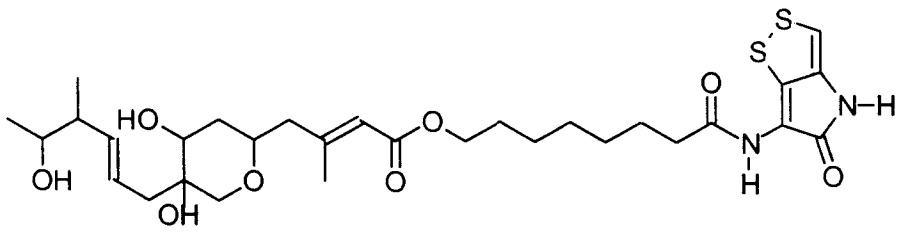

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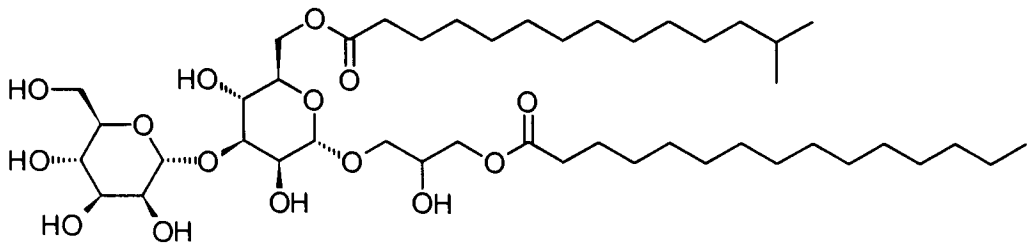

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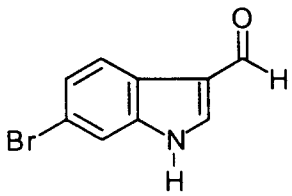
Both the ascidian *Stomozoa murrayi* and an associated bacterium *Actinetobacter sp.* contained 6-bromoindole-3-carbaldehyde (**8**), which had previously been isolated from a marine *Pseudomonas* sp[9]. The natural product **8**) inhibited *in-vitro* settlement of barnacle larvae and also showed moderate antibacterial properties[10]. Three new anticancer antibiotics of the phenazine class, namely pelagiomicins A-C (**9** - **11**) together with the known metabolite griseoluteic acid[11], were obtained from the Gram-negative bacterium *Pelagiobacter variabilis* that was isolated from a Palauan macro alga *Pocockiella variegata*[12]. Pelagiomicin A (**9**) and griseoluteic acid (**12**) were also obtained from a new halophilic marine bacterium (LL-141352) that was isolated from an unidentified orange tunicate from Fiji. Both the phenazines **9** and **12** exhibited activity in a BIA assay to detect DNA damaging agents[13].



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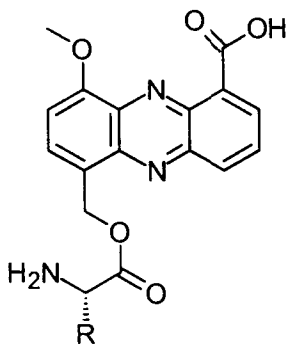
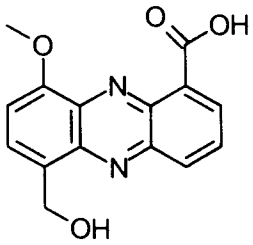


9 **R = CMe_2OH**

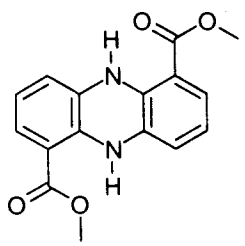
10 **R = $CH Me_2$**

11 **R = H**



12

A marine *Streptomyces* sp. (strain B 8251) isolated from the marine sediment sample in the Gulf of Mexico contained the antimicrobial alkaloid dihydrophencomycin methyl ester (**13**), together with several known metabolites[14]. Actinoflavoside (**14**) is a flavanoid-like glycoside from a *Streptomyces* sp. found in an estuary sediment in New Zealand[15]. Hydroxyakalom (**15**) is a new xanthene oxidase inhibitor obtained from the fermentation broth of the marine bacterium *Agrobacterium aurantiacum* N- 81106[16]. Four middlely cytotoxic acyldepsipeptides, Kailuins A-D (**16 - 19**), were isolated from the liquid cultures of a Gram-negative bacterium (BH- 107) obtained from driftwood collected at Kailua beach, Oaher[17].



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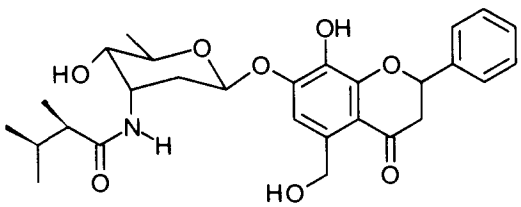

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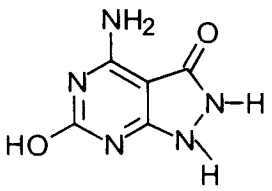

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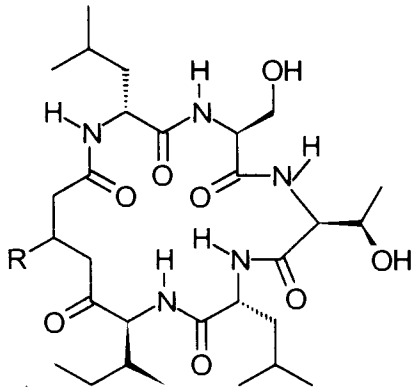


16 R = C_7H_{15}, **17** R = C_9H_{19}
18 R = $(CH_2)_6CH(CH_3)_2$
19 R = $(CH_2)_3CH{=}CH\text{-}C_6H_{13}$

The above mentioned selective reports pertaining to the investigations on few bacterial strains are cited only to exemplify the vast potential associated with the said group of microorganisms for future exploitation.

From the foregoing literature precedents, it is amply clear that this microbial diversity of the marine biosphere offers enormous scope for the discovery of novel natural products. During the course of a research programme carried out at the Department of Organic Chemistry, University of Goettingen in Germany, there was an opportunity to work on two bacterial strains, a fresh water strain designated under the code GBF 90a and a marine *Streptomycete* strain designated under the code B 7064. The crude extracts derived from these strains were subjected to a detailed examination in an effort to isolate and characterise the metabolites produced by the organisms. The full details of this work are presented in both the sections of this chapter.

SECTION I

BIOACTIVE SECONDARY METABOLITES FROM THE LIMNIC STRAIN GBF 90a (*NOCARDIOPSIS*)

The strain GBF 90a originates from a collection of fresh water bacteria and was procured from Dr. I. Wagner-Döbler, Gesellschaft für Biotechnologische Forschung, Brunswick, Germany. The said limnic strain, identified as *Nocardiopsis*, was subcultured on Luria-Bertani agar plates and grown in shaken cultures in the same medium. In the preliminary screening using the agar diffusion method, the crude extract of the said strain showed activities against a host of test pathogens, namely, *Escherichia coli* (21 mm), *Bacillus subtilis* (23 mm), *Staphylococcus aureus* (21 mm), *Mucor miehei* (20 mm), *Candida albicans* (15 mm), *Streptomyces viridochromogenes,* Tü 57 (22 mm), *Chlorella vulgaris* (42 mm), *Chlorella sorokiniana* (22 mm) and *Scenedesmus subspicatus* (18 mm). This interesting bio-activity of the crude culture extract shown during the course of the biological screening encouraged us to undertake the work described in this section which was essentially planned with a hope of isolating the active constituents. The details regarding the same are presented in the following pages of this chapter.

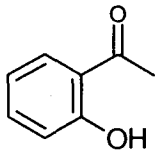
The limnic strain GBF 90a (*Nocardiopsis*) was cultivated on agar (Luria-Bertani-medium) in petri-dishes for 3 days at 29 ^{0}C. Then it was inoculated into Erlenmeyer flasks on the same medium and incubated at 29 ^{0}C for 3 days at 95 rpm. The fermentation on a 20 litre scale was carried out under similar conditions. The ethyl acetate extract, obtained after worked-up of the culture, was defatted with cyclohexane to give 3.3 g of crude residue.

The crude residue obtained after defatting was chromatographed on a flash silica gel column and eluted with a MeOH : $CHCl_3$ gradient system to give a total of ten fractions. The collected fractions were processed on individual basis to provide the following compounds.

The fractions 1 and 2 so collected were found to contain some fatty material as indicated by ^{1}H-NMR and hence were not purified further.

The fraction 3 upon concentration and purification with Sephadex LH 20 column chromatography ($CHCl_3$: CH_3OH, 6 :4) provided a colourless oil (16.8 mg). On the basis of a detailed spectral analysis, it was concluded that the oily compound is *o*-

hydroxyacetophenone (**20**). This was further confirmed by comparison with an authentic sample.



20

Identification of a substituted Azine dimer (Limnazine) (21)

The residue obtained from the fourth fraction was further purified from other impurities/fatty acids by using Sephadex LH 20 to afford a light yellow coloured solid **2**, 8 mg, m.p. 204 ^{0}C, which is designated limnazine . The IR spectrum (fig. 1.01) showed characteristic bands at 1610, 1480 cm^{-1}, in addition to other absorption bands to indicate the aromatic nature of the molecule. The molecular formula of this compound was derived to be $C_{24}H_{28}O_2N_2$ on the basis of HR-EIMS (molecular ion at *m/z* = 376.3) (fig.1.02). In confirmation, the CI mass spectrum (fig. 1.03) showed peaks at *m/z* = 377.3 $(M+1)^+$ and *m/z* = 753.6 $(2M+1)^+$. The molecular formula so emerged indicated the probability that the said crystalline compound may contain 12 double bond equivalents. The ^{1}H-NMR spectrum (fig. 1.04) of this compound showed singlet at δ 1.4 (6 H) corresponding to a *gem*-dimethyl group and a singlet at δ 2.35 (3 H), corresponding to an aromatic methyl group. The presence of an isolated methylene group was also indicated by a singlet at δ 2.96. An ABX-type of signals for three aromatic protons appeared at δ 6.78 (d, J = 8 Hz, 1 H), 7.14 (dd, J = 8 Hz, 2 Hz, 1 H) and 7.95 (d, J = 2 Hz, 1 H) indicating the presence of 1,2,4- (or 1,3,4-) trisubstituted benzene ring in the molecule. The ^{13}C-NMR spectrum (fig. 1.05) exhibited a total of 11 signals, comprising of seven sp^2 and four sp^3 carbon resonances. Among the aromatic ring carbons, the signal which appeared at δ 154.1 was assigned to an oxygen bearing carbon. Thus, all the signals observed in the ^{13}C-NMR spectrum could be accounted for except the signal appearing at δ 155.4. Usually, the signals due to -COOH or -COOR are observed above δ 165, thereby indicating that the signal at δ 155.4 could neither be due to an acid carbonyl nor due to an ester group.

With the help of heteronuclear shift correlation spectrum (fig. 1.06), in the ^{1}H-^{13}C long-range shift correlation experiment (fig. 1.07), the aromatic methyl signal at δ 2.35

was correlated through three-bond coupling with the carbon signals at δ 124.8 and δ 133.1, (hence could be assigned to C-5 and C-7), and the same methyl signal also correlated through two-bond coupling with the carbon signal at δ 119.3, (and hence could be attributed to C-6). Similarly, the aromatic proton at δ 7.95 was correlated through three-bond coupling with the carbon signals at δ 20.1, 133.1, 154.1, 155.4. All the above information could be put together to arrive at the part-structure as depicted in fig. 1a.

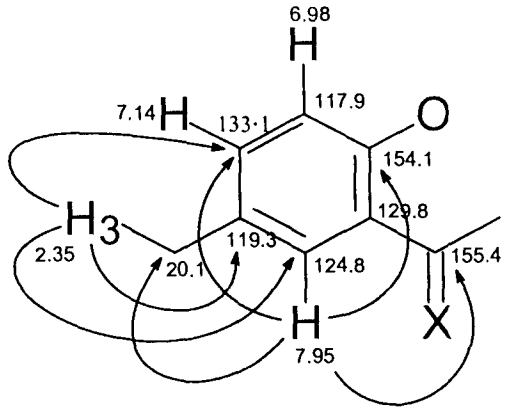


Fig. 1a

Likewise, the proton at δ 7.14 was also correlated through three-bond coupling with the carbon signals at δ 154.1, 119.3, 124.8, 20.1, while the proton at δ 6.78 correlated with the carbon signals at δ 133.1, 154.1, 129.8, thereby confirming the substitution pattern in the benzene moiety (Fig. 1b).

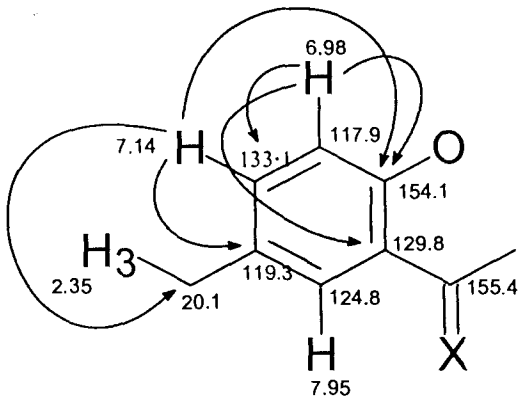


Fig. 1b

Similarly, the methylene signal at δ 2.96 showed correlations through three-bond coupling with carbon signals at δ 129.8, 26.8 and through two-bond coupling with carbon signals at δ 155.4 and 75.9. The above observation permitted to elaborate the said part structure as shown in fig. 1c.

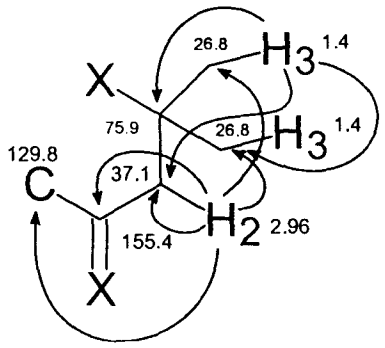
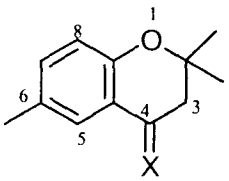


Fig. 1c

Obviously, the above part structures could be elaborated further either by connecting the carbon atom which was showing the signal at δ 155.4 with the carbon atom appearing at δ 37.1, or by connecting the former carbon atom with the carbon showing a signal at δ 75.9 to provide the two possible arrangements as shown in **21a** and **21b**.



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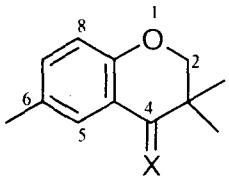
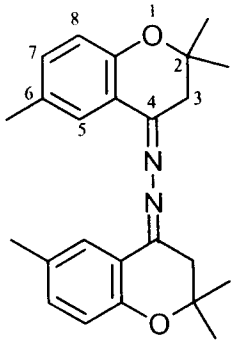


21b

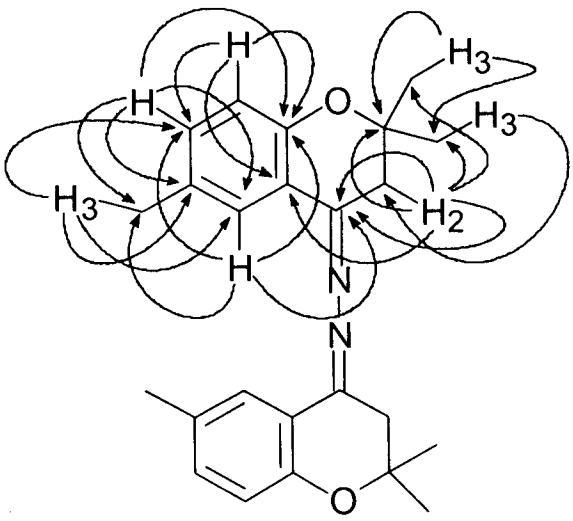
From the ^{1}H-^{13}C long range shift correlation experiment, it was observed that there exists a two-bond coupling between the methylene carbon at δ 37.1 and the carbon signals at δ 155.4 as well as a three-bond coupling with carbon signal at δ 129.8 and a three-bond coupling between aromatic proton at δ 7.95 and the carbon signal at δ 155.4. Of the two possible structures **21a** and **21b**, the latter could be excluded on the basis of above said three and two-bond coupling data.

The molecular weight of the compound was found to be 376.3 daltons. As the molecular weight was even, compound **21** may contain an even number of nitrogen atoms or no nitrogen at all. The molecular weight of the partial structure **21a** was calculated to be 174. Hence, it was assumed that two such units might be existing and linked to each

other through two nitrogen atoms. Indeed, this assumption turned into reality when it was realised that the calculated molecular weight by having an dimeric structure **21** matched with the observed molecular weight. Further support came from HRMS study which provided a molecular weight of 376.2156, which was in agreement with the molecular weight calculated for the molecular formula $C_{24}H_{28}O_2N_2$. Thus a dimeric structure **21** could be assigned to the yellow coloured crystalline compound. The said azine structure accounts for all the spectral characteristics described above.



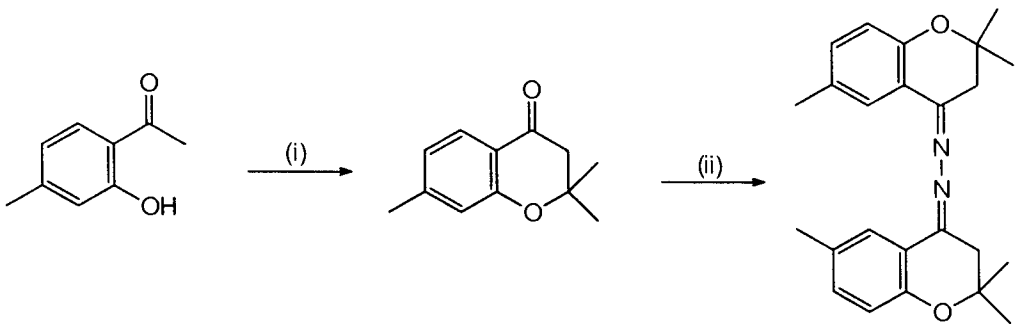
21



It may be noted that, so far, only 1 hydrazine derivative have been reported from the natural sources[18,19]. Although the chromanone based derivatives have already been reported as fungal metabolites[20,21,22], the present report pertaining to the isolation of the hydrazine derivative **21** constitutes the first example involving a dimeric chroman structure.

Finally, in order to confirm the assigned structure, the synthesis of **21** was carried out by an unambiguous route as illustrated in the scheme- I.

Scheme - I



22 **23** **21**

Key : (i) acetone, piperidine, pyridine, reflux 72 h. (ii) Hydrazine hydrate (99 %), refluxed for 2 h

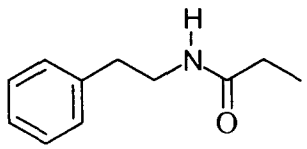
The chromanone **23** was required in connection with the synthesis of a natural product, described separately in the later part of this thesis. The said chromanone **23** on refluxing with excess of hydrazine hydrate gave the corresponding azine derivative **21** as a crystalline compound. The m.p. and the spectral data (IR, ^{1}H NMR & MS) of the synthetic sample **21** were in good agreement with the data reported on the natural product limnazine (**21**) thereby confirming the assigned structure.

Identification of N-(2′-phenylethyl)-propanamide (24)

The residue obtained from fraction 5 showed a single spot on tlc and gave no colouration when sprayed with anisaldehyde/H_2SO_4 reagent. Since the ^{1}H-NMR spectrum indicated that the said residue was a mixture of several aromatic compounds, it was further purified by HPLC to provide three pure compounds designated as 5A, 5B and 5C.

Compound 5A was obtained as a low melting solid (19 mg), m.p. 45 - 46 ^{0}C. The IR spectrum showed bands at 3275 and 1648, in addition to the other absorption bands, indicating the presence a amide group in the molecule. The CIMS (fig. 1.08) showed peaks at m/z = 178.1 $(M+1)^+$ and m/z = 195.1 $(M + 18)^+$ in addition to other fragment ions. The ^{1}H-NMR spectrum (fig. 1.09) showed a multiplet with a relative intensity of 5 protons in

the aromatic region, thereby indicating that the compound contains a monosubstituted aromatic ring. Further, a triplet at δ 1.1 (J = 8 Hz) with the relative intensity of 3 protons and a multiplet at δ 2.18 (J = 8 Hz) with a relative intensity of two protons was attributed to the presence of an ethyl group in the molecule. In addition to this, there existed a triplet at δ 2.82 (J = 8 Hz) and a multiplet at δ 3.52 (J = 8 Hz) both having a relative intensity of two protons each. The multiplet at δ 3.52 (J = 8 Hz) after D_2O exchange appeared as a triplet thereby suggesting the presence of an adjacent NH or OH group. The broad signal at δ 5.5 (1 H) was found to be D_2O exchangeable and was assigned for a NH group, which was further confirmed by the sharp band at 3275 cm^{-1} in the IR spectrum. Besides other signals, the ^{13}C-NMR spectrum showed an amide carbonyl signal at δ 174. The spectrum also showed 4 signals for the six aromatic carbons and 4 in the aliphatic region. The above spectral analysis led to the conclusion that the isolated compound is N-(2′-phenylethyl)-propanamide (**24**). The structure was further confirmed by comparing it with an authentic sample of **24** prepared[20] by the condensation of β-phenylethyl amine and propanoyl chloride.

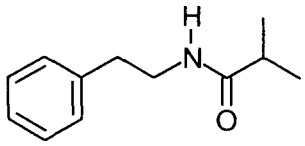


24

Identification of N-(2′-phenylethyl)-2-methylpropanamide (25)

The compound 5B (25 mg) was obtained as a colourless solid with m.p. 82 ^{0}C. The IR spectrum of this colourless crystalline compound showed absorption bands at 3299 and 1640 cm^{-1}, in addition to the other absorption bands, suggesting the presence of a amide group in the molecule. The CI-mass spectrum showed peaks at m/z = 192.1 $(M + 1)^+$ and m/z = 209.1 $(M + NH_4)^+$. The ^{1}H-NMR spectrum (fig. 1.10) of this compound showed almost the same pattern as in the case of compound **24** except for the signals at δ 1.15 (J = 8 Hz) which appeared as a doublet with relative intensity of 6 protons and a signal at δ 2.3 (J = 8 Hz) appearing as a multiplet with relative intensity of 1 proton. On the basis of this information, it is concluded that the isolated compound is N-(2′-phenylethyl)-2-

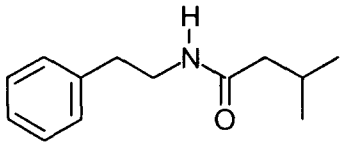
methylpropanamide (**25**). The identity is further confirmed by comparing it with an authentic[20] sample of **25**.



25

Identification of N-(2′-phenylethyl)-3-methylbutanamide (26)

On purification by HPLC, compound 5C was obtained as a colourless solid (24 mg) with m.p. 51 ^{0}C. The IR spectrum showed strong and sharp absorption bands at 3302 and 1639 cm^{-1} to suggest the presence of a amide group in the molecule. The CI- mass spectrum showed peaks at *m/z* = 206.1 $(M + H)^+$, 223.1 $(M + 18)^+$, 240.1 $(M + 18 + 17)^+$, 411.1 $(2M + H)^+$, 428.1 $(2M + 18)^+$. The ^{1}H-NMR spectrum (fig. 1.12) of this compound was also similar to that of compounds **24** and **25**, except for two signals, *viz* a doublet at δ 0.92 (J = 8 Hz) with relative intensity of 6 protons and a one proton multiplet at δ 2.06 (J = 8 Hz). Comparison of the said spectral characteristics with the data of earlier reported compounds, *viz*, **24** and **25**, established its identity as N-(2′-phenylethyl)-3-methylbutanamide (**26**). Further support in favour of structure **26** came from the comparison study with an authentic sample prepared by the condensation of β-phenylethyl amine with 3-methylbutanoyl chloride.



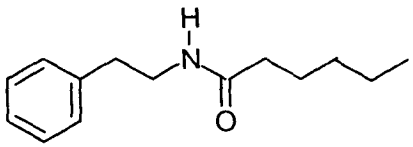
26

Identification of N-(2′-phenylethyl)-hexanamide (27)

The fraction 7 gave two compounds 7A (15 mg) and 7B (45 mg). The compound 7A was found to be identical with N-(2′-phenylethyl)-propanamide (**24**) which was de-

scribed earlier.

The compound 7B was obtained as a colourless solid, m.p. 77-78 °C. The IR spectrum of this compound showed absorption bands at 3303 and 1640 cm^{-1}, indicating the presence of a amide group. The CI-mass spectrum gave a molecular ion peak at *m/z* = 220 $(M + H)^+$. In addition to the said peak, there were other peaks at *m/z* = 237 $(M + 18)^+$, 439 $(2M +1)^+$ and 456 $(2M + 18)^+$. The ^{1}H-NMR spectrum (fig. 1.14) of this compound showed a pattern which was comparable to that of other amides described above. The difference was obvious only in the aliphatic region of the spectrum in the said eompound. A quartet at δ 3.5 (*J* = 8 Hz, 2 H) which on D_2O exchange, appeared as a triplet, and a triplet at δ 2.8 (*J* = 8 Hz, 2 H) were clearly seen thereby indicating the presence of a $PhCH_2CH_2NH$- moiety in the molecule. Similarly, other signals at δ 2.1 (t, *J* = 8 Hz, 2 H), 1.6 (q, *J* = 8 Hz, 2 H), 1.28 (m, 4 H) and 0.85 (t, *J* = 8 Hz, 3 H) in the spectrum suggested the presence of $-CH_2CH_2CH_2CH_3$ side chain in the molecule. With the help of the above spectral information, it was concluded that the said crystalline compound is also an amide having structure **27**, further confirmed by comparison with an authentic sample.



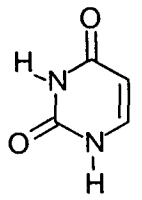
27

It is worthwhile to mention here that the isolation of the amides (**24 - 27**) during the present work represents the first report of their natural occurrence in a fresh water bacterium though the said compounds are known in the literature.

Identification of Uracil (28)

The 8th fraction on purification over Sephadex LH 20 gave 8 mg of a solid, m.p. 334 °C. The EIMS showed a molecular ion peak at *m/z* = 112, in addition to the other fragments. The ^{1}H-NMR spectrum showed two broad singlets at δ 10.95 and δ 10.74 with a relative intensity of one proton each which were exchangeable with D_2O and hence were assigned to two -NH groups. Further, the spectrum showed two doublets at δ 7.36 (*J* =

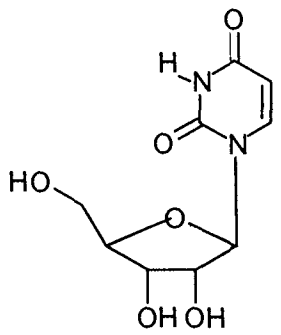
7.5 Hz) and δ 5.43 (J = 7.5 Hz) corresponding to one proton each. These spectral characteristics lead to the conclusion that the crystalline compound **28** is uracil. Further confirmation was done by comparison with the spectral data reported in the literature and also with an authentic sample.



28

Identification of Uridine (29)

The fraction 9 was purified using PTLC (10 % methanol / chloroform) and then through Sephadex LH 20 column to give 20 mg of a white solid, m.p. 165 °C. The EIMS showed a molecular ion at m/z = 244.1. Further, it also showed fragments at m/z (%) = 226.1 (20), 171 (20), 141 (24), 133 (64) and 113 (100). The ^{1}H-NMR spectrum showed a sharp singlet at δ 11.24 (1 H) which was exchangeable with D_2O. Further, a pair of signals at δ 7.84 (d, J = 8.5 Hz, 1 H) and δ 5.64 (dd, J = 8.5 Hz, 3 Hz, 1 H) were seen, thereby indicating the presence of a uracil nucleus in the molecule in analogy with the earlier observation. In addition, the signals at δ 5.3 (d, 1 H) and δ 5.02 (m, 2 H) provided evidence in favour of hydroxyl groups. Another signal at δ 5.78 which appeared as a doublet (1 H), was attributed to an α-anomeric proton. This was further confirmed by looking at the ^{13}C-NMR spectrum, which showed a signal at δ 87.7 as the α-anomeric protons are usually accounted below δ 100. In addition, other signals at δ 4.02 (t, J = 7 Hz, 1 H), 3.98 (t, J = 7 Hz, 1 H), 3.84 (m, 1 H) were also observed. The coupling pattern in the NMR spectrum indicated the presence of a -CH-CH-CH- moiety. The signal at δ 3.62 (ddd, 2 H) on D_2O exchange, collapsed into a dd, implying thereby the presence of a CH_2 group adjacent to a -OH group and a chiral carbon. All the above spectral characteristics could be accounted for only if the crystalline compound is uridine (**29**). Further confirmation of the assigned structure was done by comparison with an authentic sample (commercially available) of uridine (TLC and ^{1}H NMR).



29

Though the initial fractions obtained from the main separation were showing bio-activities against the various test pathogens, the pure compounds, obtained as described above, when tested, did not show any activity against the above test pathogens.

Thus, the limnic bacterial strain derived from *Norcardiopsis* provided eight different metabolites as the result of detailed chemical investigation. The structure elucidation of these metabolites lead to identify them as the known *o*-hydroxyacetophenone and the amides, namely, N-(2′-phenylethyl)-propanamide, N-(2′-phenylethyl)-2-methyl-propanamide, N-(2′-phenylethyl)-3-methylbutanamide and N-(2′-phenylethyl)-hexanamide, besides uracil and uridine. In addition, a novel hydrazine dimer, designated as limnazine, was also isolated during the present study. The structure assigned to limnazine has also confirmed by an unambiguous synthesis.

SECTION II

BIOACTIVE SECONDARY METABOLITES FROM THE MARINE *STREPTOMYCETES* SP. B 7064

As stated in the beginning of this chapter, the marine bacteria represents a rich and diverse source of secondary metabolites. Specially, the *Actinomycetes* and the *Streptomycetes* are the two important groups of organisms from which many useful compounds have been isolated[25]. The marine *Streptomycetes* strain has attracted immense attention owing to their secondary metabolites which exhibited characteristic bio-activities across a broad spectrum of disease producing pathogens[26]. It is an established fact that the discovery of the 16-membered macrolide antibiotic substances from various *Streptomycetes* has provided the necessary impetus towards the search for identifying a number of related substances in addition to the commercially available drugs like Carbomycin[27], Leucomycin[28], Tylosin[29] & Spiramycin[30-32].

As states earlier, we had an opportunity to work on a unidentified marine *Streptomycetes* strain designated under the code no. B 7064 which originated from the collection of marine bacteria of the Alfred-Wegner-Institute für Polar and Meeresforschung in Bremerhaven, Germany. The said strain was subcultured in M_2^+ + 50 % synthetic sea water* on agar plates and grown in shaken cultures using the same medium. In the course of preliminary screening using the agar diffusion method, the crude extract of the B 7064 culture showed promising activities against various bacteria, fungi and algae, namely *Escherichia coli* (20 mm), *Bacillus subtilis* (36 mm), *Staphylococcus aureus* (13 mm), *Mucor miehei* (22 mm), *Candida albicans* (12 mm), *Streptomyces viridochromogenes* Tü 57 (14 mm), *Chlorella vulgaris* (20 mm), *Chlorella sorokiniana* (20 mm) and *Scenedesmus subspicatus* (20 mm).

The said promising bioactivity exhibited by the crude culture extract against a variety of test pathogens called for a detailed chemical investigation and accordingly, the present work was initiated on the said marine *Streptomycetes* strain. The results obtained in the course of the present work are presented in the following part.

The marine *Streptomycetes* strain B 7064 was inoculated into Erlenmeyer flasks on malt extract / yeast extract / glucose in 50 % synthetic sea water* and incubated for 3 days at 28 ^{0}C. Fermentation was carried out in a 20 liters jar fermenter under similar

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* Natural sea water was not easily available (at the Dept. of Organic Chemistry, University of Göttingen, Germany), hence synthetic sea water was used. The detail regarding its preparation is given in the experimental part.

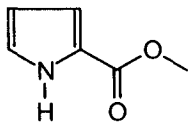
conditions. The ethyl acetate extract residue of the entire culture was dissolved in methanol and defatted with cyclohexane to give 1.95 g of crude residue.

The crude residue obtained from the methanolic extract was chromatographed on a flash silica gel column and eluted with a MeOH : $CHCl_3$ gradient system to give a total of five fractions which on removal of solvent fumished the individual compounds. The separated compounds on further purification provided the pure metabolites which were characterised as per the following.

The fractions no. 1 and 2 so collected from the silica gel column did not show any activity and were found to contain some fatty material as indicated by the ^{1}H-NMR and hence same were not purified/examined further.

Identification of pyrrole-2-carboxylic acid ester (30)

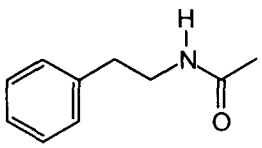
Fraction no. 3 after concentration and then purification by preparative thin layer chromatography [PTLC] ($CHCl_3/CH_3OH$), gave a colourless solid **30** (12 mg), with m.p. 68 ^{0}C. The IR spectrum showed the sharp bands at 3280 and 1679 cm^{-1}, (in addition to other absorption bands) demonstrating the presence of a NH and a carbonyl group in the molecule. The molecular formula of the compound was determined to be $C_6H_7NO_2$ by HR-MS (molecular ion peak at *m/z* = 125). The EI-MS gave a molecular ion at *m/z* = 125 (M^+, 88) and other fragment ions at *m/z* = 94 (M^+ - OCH_3, 100), 93 (40) and 66 (30). The ^{1}H NMR spectrum of this compound disclosed a signal at δ 3.81 (s, 3H), which was assigned to a methoxy group. Further, the spectrum showed three characteristic proton signals at δ 6.24 (dd, 1 H, *J* = 6.0 Hz, 1.5 Hz), 6.5 (d, 1 H, *J* = 6.0 Hz) and 6.8 (d, 1 H, *J* = 6.0 Hz), attributed for the presence of a pyrrole nucleus. In addition to the above, there existed a signal at δ 9.16 (br s, 1H), exchangeable with D_2O, which could be assigned to the NH group. On the basis of the above spectral data, it could be concluded that the isolated compound was pyrrole-2-carboxylic acid methyl ester (**30**). Further, the assigned structure was confirmed by comparing with an authentic sample.



30

Identification of N-(2′-phenylethyl)-ethanamide (31)

The fraction no. 4 on concentration followed by purification through Sephadex LH 20 gave a colourless solid (25.9 mg), m.p. 85 ^{0}C. The IR spectrum showed absorption bands at 3275 and 1650 cm^{-1} (in addition to the other absorption bands) indicating the presence of a amide group in the molecule. The CIMS showed peaks at *m/z* = 164 (M +1)$^+$, 327 (2M +1)$^+$, 181 (M +18)$^+$, 198 (M +18 +17)$^+$, 327 (2M + 1)$^+$ and 344 (2M +18)$^+$. The EIMS showed a molecular ion at *m/z* = 163.1 and additional peaks at *m/z* = 148.1 (M^+ - CH_3), 104.1 (M^+ - CH_3CONH_2), 91 ($CH_2NHCOCH_3$). The ^{1}H-NMR spectrum showed a multiplet at δ 7.26 with a relative intensity of 5 proton in the aromatic region, to indicate that the compound isolated contained a monosubsitituted aromatic ring. Further, a triplet at δ 2.82 and a multiplet at δ 3.56, both having a relative intensity of two protons each were also observed. The multiplet at δ 3.56 after D_2O exchange appeared as a triplet thereby suggesting the presence of a adjacent -NH group. In conformity, the broad signal at δ 5.6 (1 H, -NH) was found to be D_2O exchangeable. In addition, another singlet at δ 1.96 with a relative intensity of 3 protons, indicated the presence of a methyl group in the molecule. From the above spectral data, it could be inferred that the said crystalline compound is N-(2′-phenylethyl)-ethanamide (**31**). Further, the assigned structure was confirmed by comparing it with an authentic sample of **31**.



31

Identification of Chalcomycin and Chalcomycin B

The fifth fraction collected showed a very high level of antibacterial activity, specially against *Escherichia coli* (30 mm), *Bacillus subtilis* (36 mm) and *Candida albicans* (33 mm). The tlc of this crude fraction showed two interesting UV-visible spots [R_f = 0.65 & 0.55 ($CHCl_3/CH_3OH$, 95 : 5)] which turned brown after staining with anisaldeyde/H_2SO_4. This fraction was therefore purified using PTLC ($CHCl_3/CH_3OH$, 95 : 5) to give two subfractions. Both of these subfractions were further purified by processing separately through two Sephadex LH 20 columns ($CHCl_3/CH_3OH$, 60 : 40) to afford two compounds designated as 5A (8.2 mg) and 5B (7.8 mg).

Identification of Chalcomycin (32)

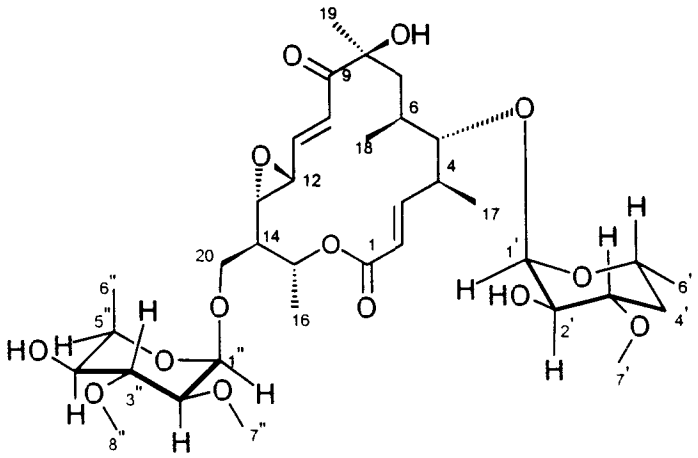
The compound 5A was obtained as a colourless solid, with melting point 122 ^{0}C. The IR spectrum showed absorption bands at 3484, 1718, 1630 and 1084 cm^{-1}, in addition to other bands. The bands at 3484 and 1084 cm^{-1} suggested the presence of a glycoside ring while the bands at 1718 and 1630 cm^{-1} indicated the presence of unsaturated ester and unsaturated ketone carbonyl groups in the molecules. The molecular formula of this compound was calculated as $C_{35}H_{56}O_{14}$ by looking at the HR-MS which showed a molecular ion at m/z = 700. The ESI mass spectrum showed peaks at m/z = 723.4 [M + Na]$^+$ and 1422.7 [2M + Na]$^+$. Thus, on the basis of the molecular formula this compound needs to contain 8 double bonds equivalence (DBE). The ^{1}H NMR (fig. 1.16) and ^{13}C NMR spectrum (fig. 1.17) as well as the APT experiment on compound 5A indicated the presence of 35 carbon atoms and 53 nonexchangeable protons bonded directly to these carbons. Further, from the molecular formula, the number of exchangeable protons were found to be three. The ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY data (Table 1) disclosed the presence of one α,β-unsaturated ketone, one α,β-unsaturated ester carbonyl and 20 methines, one quaternary carbon, three methylenes, three methoxy and six methyl groups. Since, four out of the anticipated eight sites of unsaturations were accounted for, the compound 5A was inferred to contain four rings.

The search in the Antibase[18] with the above described spectral information concluded that the compound 5A is the known macrolide Chalcomycin (**32**) previously isolated from *Streptomyces bikiniensis*[33]. The identity was further confirmed by a direct

comparison with an authentic sample of **32**. The spectral data (^{1}H NMR, ^{13}C NMR, ^{1}H-^{1}H-COSY) obtained on 5A agreed well with those reported in the literature[33] for Chalcomycin (**32**).

Table 1 : ^{1}H NMR, ^{13}C NMR and ^{1}H-^{1}H COSY data of Chalcomycin (32) in $CDCl_3$.

Position (C)	δ_C	δ_H	^{1}H-^{1}H COSY
1	165.3	----	----
2	120.7	5.78	3
3	151.6	6.64	4,2
4	41.7	2.7	5,3,18
5	87.8	3.18	6,4,7
6	34.0	1.23	5,7
7	36.7	2.02, 1.24	5,6
8	78.4	----	----
9	200.2	----	----
10	124.8	6.60	11
11	146.5	6.58	10,12
12	58.7	3.29	11
13	58.9	3.12	14
14	49.5	1.37	13,16,15
15	68.7	5.3	14,17
16	18.3	1.3	14,16
17	18.6	1.17	4
18	19.2	0.98	6
19	27.8	1.35	----
20	66.95	4.16, 3.62	14,17
1′	103.2	4.18	2′
2′	75.0	3.3	1′, 3′
3′	80.4	3.18	2′, 4′
4′	36.9	1.9	3′, 5′
5′	67.8	3.42	4′, 6′
6′	20.9	1.19	5′
7′	56.7	3.38	----
1″	100.9	4.57	2″
2″	81.9	3.04	1″, 3″
3″	79.6	3.75	2″, 4″
4″	72.7	3.16	3″, 5″, 6″
5″	70.7	3.5	4″, 6″
6″	17.8	1.22	3″, 5″
7″	59.7	3.52	----
8″	61.8	3.59	----



32

It may be worthwhile to mention here that, the compound Chalcomycin (**32**) is a 16-membered ring macrolide antibiotic showing activity against the gram-positive organisms. Woo P. W. K. *et al*[33,34]., were the first to come out with the structure of Chalcomycin followed by the details regarding its stereochemistry through X-ray crystallographic analysis[35]. As stated earlier, the macrolide antibiotics have played an important role in medicinal chemistry over the past five decades[36-39]. As we know, the macrolide antibiotics are a group of complex natural products broadly classified into two general classes namely, the basic and the neutral macrolides. Although the basic macrolides consist mostly of the antibacterials which share immense commercial interest in the human health area, the neutral members of this family are of considerable interest as lead structures in semisynthetic programs. Macrolides have stimulated the development of new synthetic methodologies & consequently have led to the total syntheses of several novel compounds possessing multiple chiral centres[39]. They have also prompted the chemists & the technologists to undertake the biosynthetic & molecular genetics studies to provide extensive insight into the fundamental mechanism, as well as the promise for effecting selective genetic combinations in an effort to create useful compounds[40].

Structure elucidation of Chalcomycin B (33)

The compound 5B was obtained as a crystalline solid, (7.8 mg), with melting point 98-100 ^{0}C. The IR spectrum (fig. 1.18) showed absorption bands at 3446, 1740, 1635

and 1080 cm^{-1} in addition to other bands. The bands at 1740, 1730, 1640 and 1635 cm^{-1} suggested the presence of four carbonyl groups, while the bands at 3446 and 1080 cm^{-1} indicated the presence of glycoside residue in the molecule. The molecular formula of this compound was calculated to be $C_{41}H_{64}O_{16}$ by HR-EIMS (fig. 1.19) on the basis of an molecular ion at *m/z* = 812. The ESI mass spectrum (fig. 1.20) exhibited peaks at *m/z* = 835.5 $[M + Na]^+$ & 1647 $[2M + Na]^+$. The molecular formula thus emerged indicated that the compound 5B inherits 10 DBE. The ^1H (fig. 1.21) and ^{13}C NMR spectrum (fig. 1.32) indicated the presence of 41 carbons along with 63 nonexchangeable protons bonded directly to the carbon atoms. Thus, the number of exchangeable protons in the molecule was found to be one. The ^1H and ^{13}C NMR data (Table 2) disclosed the presence of a ketone group, three ester carbonyls, 20 methine carbons of which 12 were oxygen bearing, one quaternary carbon, 5 methylene, three methoxy and eight methyl groups.

Table 2: ^1H NMR, ^{13}C NMR, ^1H-^1H COSY & HMBC data of Chalcomycin B (33) in $CDCl_3$.

Position (C)	δ_C	δ_H	^1H-^1H COSY	HMBC
1	165.3	----	----	----
2	120.8	5.716	3	1,4
3	151.3	6.568	2,4	1,2,4,5
4	41.1	2.51	3,5,6,17	2,3,5,17
5	88.5	3.045	5	1′
6	34.0	1.13	4,7,18	3,4,17
7	37.7	1.8, 1.9	6,18	6,8,9,18,19
8	78.4	----	----	----
9	200.4	----	----	----
10	125.4	6.503	12	9,11,12
11	146.5	6.492	---	9,10,12,13
12	58.7	3.24	10	10,11,13
13	59.0	3.085	14	11,14
14	49.5	1.34	13,15,20	12,13,15
15	68.8	5.272	14,16	1,12,13,14
16	18.3	1.27	14,15	14,15
17	18.2	1.02	4	3,4,5
18	19.4	0.94	6,7	5,6,7
19	27.8	1.31	----	7,8,9
20	67.1	4.12, 3.61	14	13,14,15,4‴
1′	101.6	4.22	2′	3′,5,5′
2′	74.2	4.78	1′	1′,1″,3′

3′	78.8	3.265	4′	2′,7′
4′	37.1	2.04,1.35	3′, 6′,7′	2′, 3′,6′
5′	67.7	3.42	4′, 6′	1′, 3′
6′	20.8	1.19	5′	5′,4′
7′	56.7	3.25	4′	3′
1″	173.2	----	----	----
2″	27.7	2.32	3″	1″, 3″
3″	9.2	1.10	2″	1″
1‴	100.9	4.57	2″	3‴, 20
2‴	80.6	3.025	1″, 3″	1‴,7‴,3‴
3‴	77.6	3.85	2‴, 4‴,8‴	1‴,4‴,5‴,8‴
4‴	74.6	4.40	3‴, 5‴	1⁗,1‴,3‴,5‴
5‴	67.4	3.88	4‴, 6‴	3‴
6‴	17.4	1.12	5‴	5‴
7‴	59.4	3.48	----	2‴
8‴	61.6	3.46	3‴	3‴
1⁗	173.6	----	-----	-----
2⁗	27.7	2.32	3⁗	1⁗,3⁗
3⁗	9.18	1.08	2⁗	2⁗

A detail study/comparison of the ^{1}H and ^{13}C NMR spectrum of Chalcomycin (**32**) and the crystalline compound 5B under investigation, indicated that the later possess the same 16-membered macrolide unit present in the former. But the only difference observed was in the chemical shifts of the signals due to two sugar moieties. When the ^{13}C NMR spectrum of compound 5B was compared with Chalcomycin, six additional carbon signals were observed in the spectrum of compound 5B. These additional signals appeared as two sets of closely placed carbon resonances at δ 173.2, 173.6; and δ 9.18, 9.2 and another lone resonance at δ 27.7. The molecular weight of Chalcomycin is 700 while, that of compound 5B was found to be 812, the difference in the molecular weight between these two being 112 units. Further, the compound 5B contained only one hydroxyl group, while Chalcomycin had three hydroxyl groups in the molecule. To account for all the above described information/observation, it was envisaged that one hydroxyl group in each of the two sugar residue present in chalcomycin (**32**) is involved in the formation of a propionate derivative to provide the corresponding chalcomycin dipropionate ester. In conformity, it was soon realised that the calculated molecular weight of the correspond-

ing propionate derivative matched well with the observed molecular weight in case of compound 5B. Additional support in favour of the dipropionate derivative came from the high resolution mass spectrometry. The electron impact mass spectrum showed two characteristics mass fragments with mass units 247.1586 and 217.146 corresponding to the molecular formula $C_{11}H_{19}O_5$ and $C_{10}H_{17}O_4$ respectively. Further, the assigned structure was confirmed by 2 D NMR study.

In the ^1H - ^{13}C long-range shift correlation, the anomeric proton appearing at δ 4.22 was correlated through a three-bond coupling with the carbon signals at δ 78.8, 67.7 and also with δ 88.5, consequently, the said chemical shifts were ascribed to 3′, 5′ and 5 carbon atoms respectively with the help of heteronuclear shift correlation spectrum. Similarly, the proton at δ 4.78, correlated through a three-bond coupling with the carbon signal at δ 173.2 was assigned to the ester carbonyl in the propionate moiety (C-1″), while it is also coupled through a two-bond coupling with carbon signals at δ 101.6 and δ 78.8. Like wise, the proton at δ 1.10 also showed a three-bond coupling with the carbon signal at C-1″. The other coupling pattern/values observed are shown in the fig. **I**.

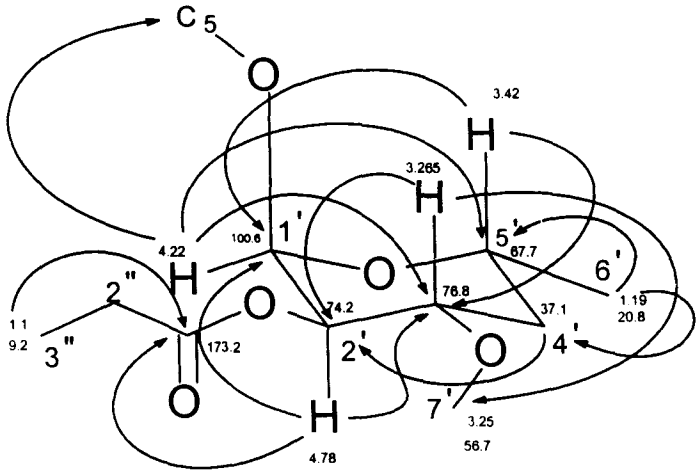


fig. I

Additional support in favour of the above part structure came from the ^1H-^1H COSY experiment. In the ^1H-^1H COSY spectrum, the proton signal at δ 4.22 showed COSY cross-peaks at H-2′ (δ 4.78). Despite the overlapping signals, the ^1H-^1H COSY spectrum showed cross-peaks between H_2-4′ (δ 2.04, 1.35), H-3′ (δ 3.265), H-5′ (δ 3.42) and H-6′ (δ 1.19). In addition, the proton at δ 1.19 showed a three-bond COSY cross-peak to H-5′

(δ 3.42), while the H_2-2″ (δ 2.32) showed COSY cross-peak to H_3-3″ (δ 1.10).

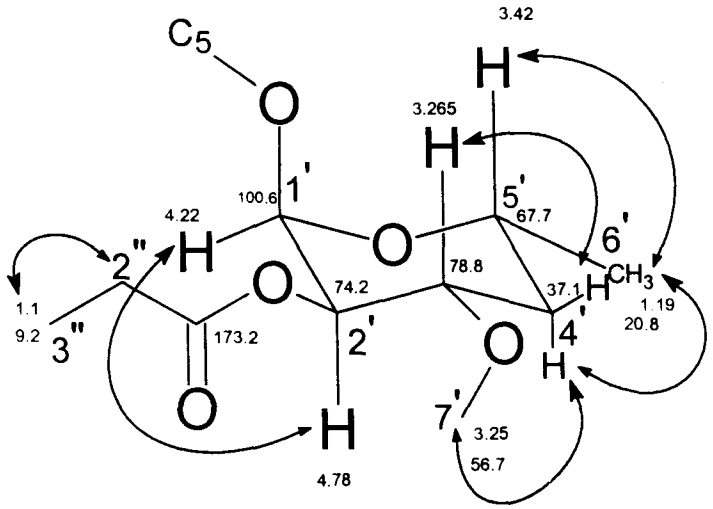


fig. II

Similarly, in the ^{1}H - ^{13}C long-range shift correlation, the anomeric proton at δ 4.57, showed a three-bond coupling with the carbon signals at δ 77.6 and 67.1, and hence the said chemical shifts were assigned to C-3‴ and C-20 respectively. Like wise, the proton at δ 4.40, correlated through a three-bond coupling with the carbon signals at δ 173.6 was assigned to an ester carbonyl of the propionate (C-1⁗) while it also coupled through a two-bond coupling with the carbon signals at δ 77.6 and 67.4 and through four-bond coupling with the anomeric carbon at δ 100.9. The proton at δ 1.08 showed a three-bond coupling with the carbon signal at C-1⁗. The other couplings that were evident in the ^{1}H - ^{13}C long-range spectrum are shown in the fig. III.

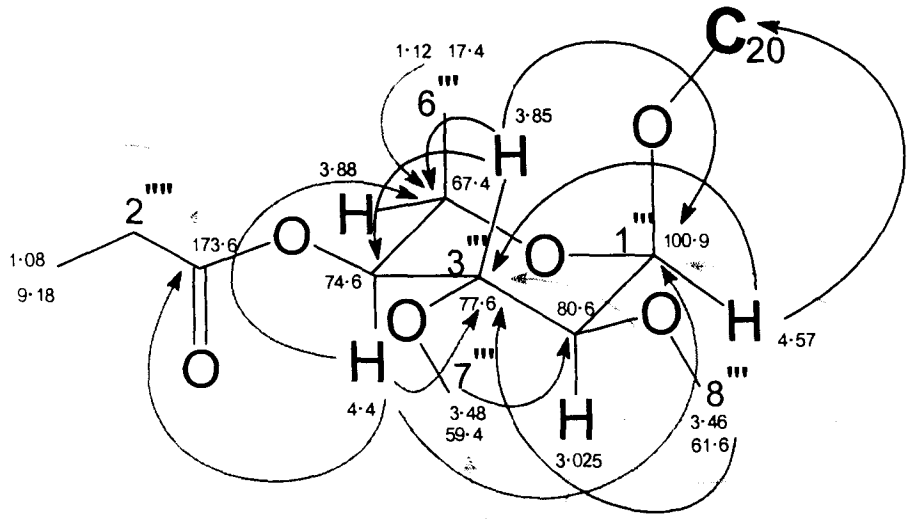


fig. III

Similarly, in the ^1H-^1H COSY experiment, the proton signal at δ 3.025 showed COSY cross-peak to H-2''' (δ 4.57) and H- 3''' (δ 3.85). In addition, the proton signal at δ 4.4 showed COSY cross-peak at H-3''' (δ 3.85) and H-5''' (δ 3.88) whereas the proton signal at δ 3.88 showed correlation with H-4''' (δ 4.4) and H-6''' (δ 1.12). The H_2-2'''' showed COSY correlation with H_3-3'''' (δ 1.08) as indicated in fig. IV.

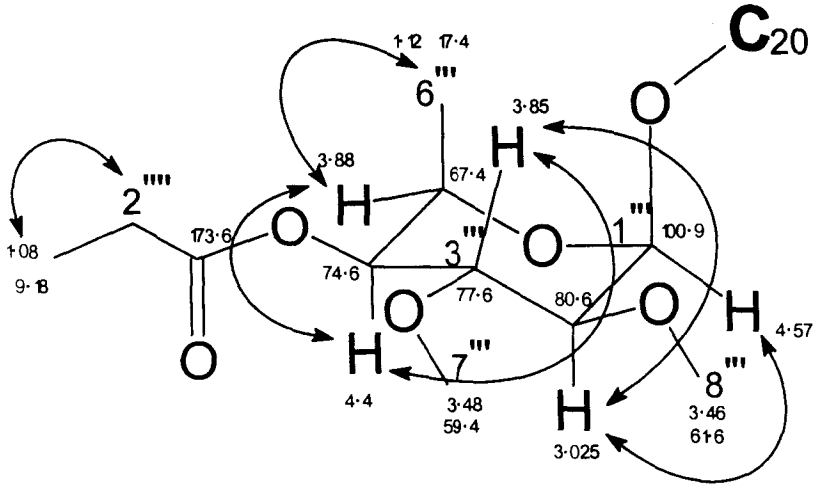
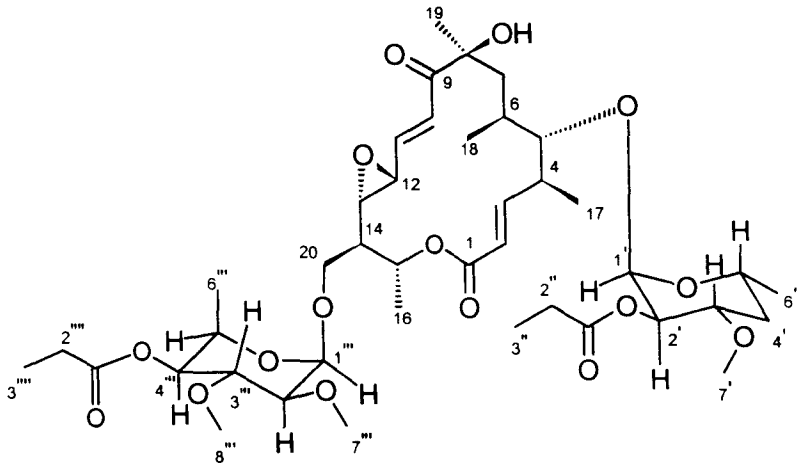


fig. IV

Thus, the long range coupling and the ^1H-^1H COSY experiments provided sufficient evidences to conclude that the crystalline compound 5B isolated during the present work

is the Chalcomycin dipropionate ester, which was designated as Chalcomycin B (**33**). Structure **33** accounts for all the spectral characteristics described in the forgoing pages.



33

The present work represents the first report of the natural occurrence of such a dipropionate ester though the parent macrolide Chalcomycin has been reported earlier[33]. Unfortunately the Chalcomycin B (**33**) did not show any bio-activity though the crude extract was exhibiting the activity against several test pathogens. Similar was the situation with other pure metabolites isolated during the present study.

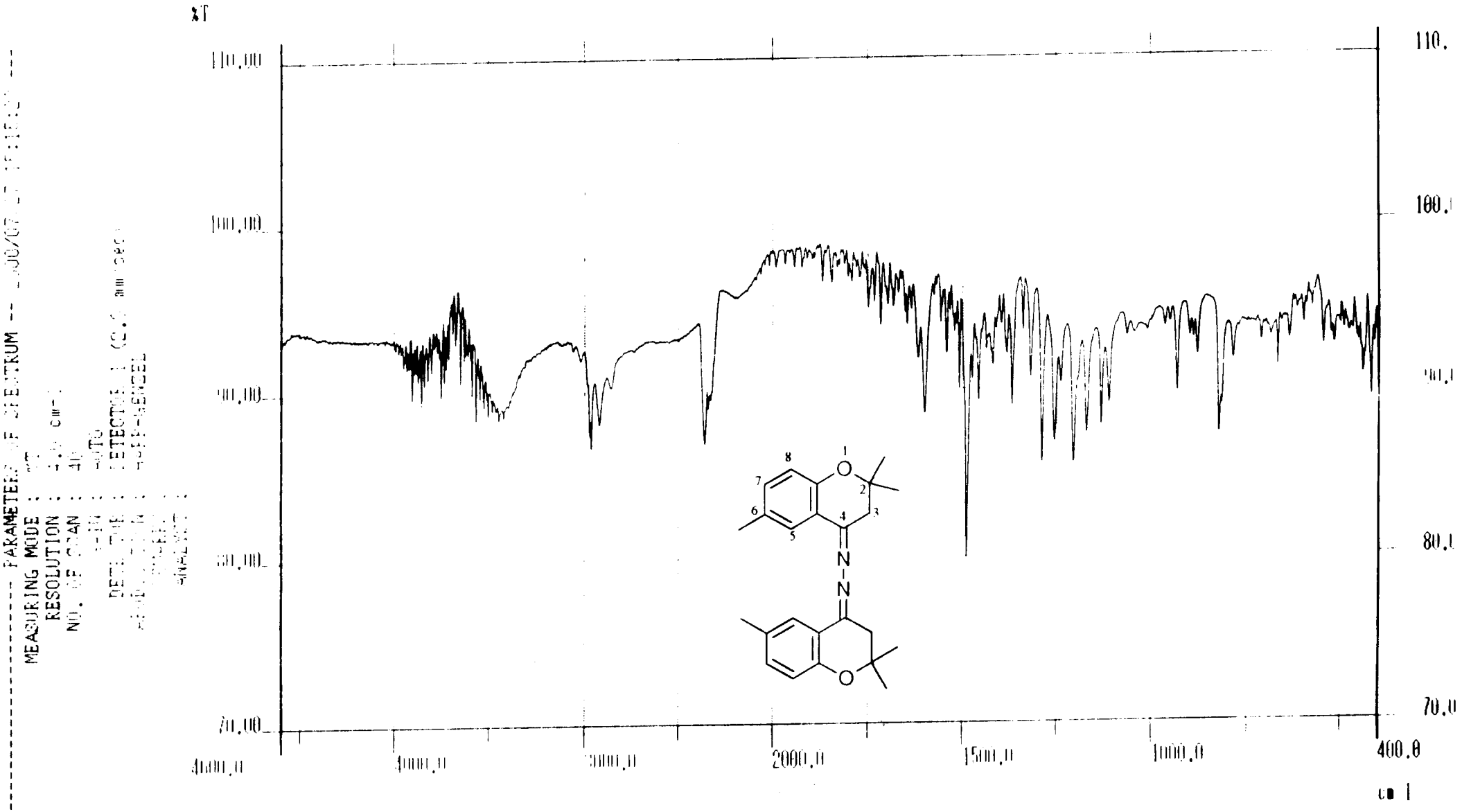


Fig. 1.01 : IR spectrum of limnazine (21)

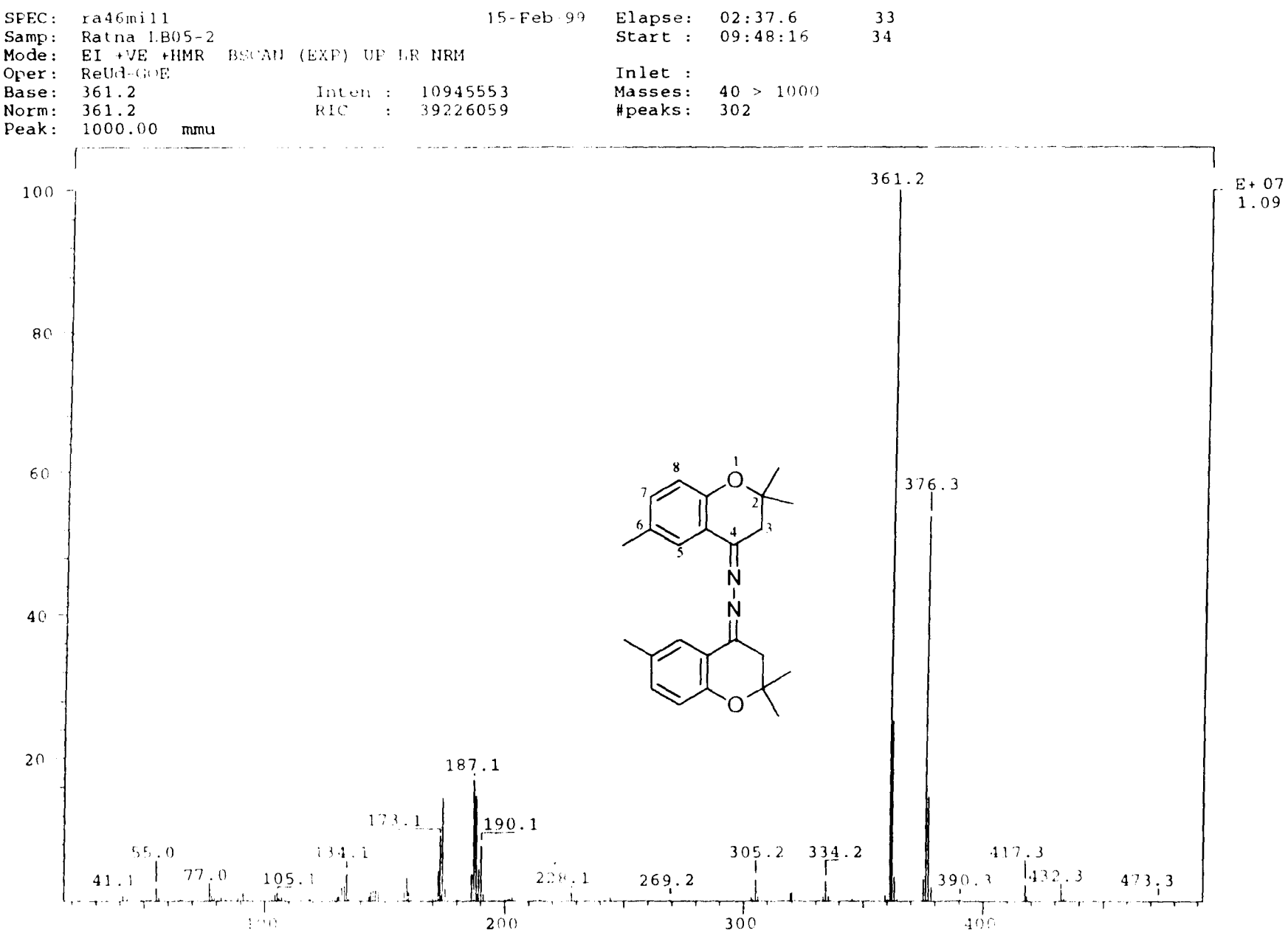


Fig. 1.02 : EI Mass spectrum of limnazine (21)

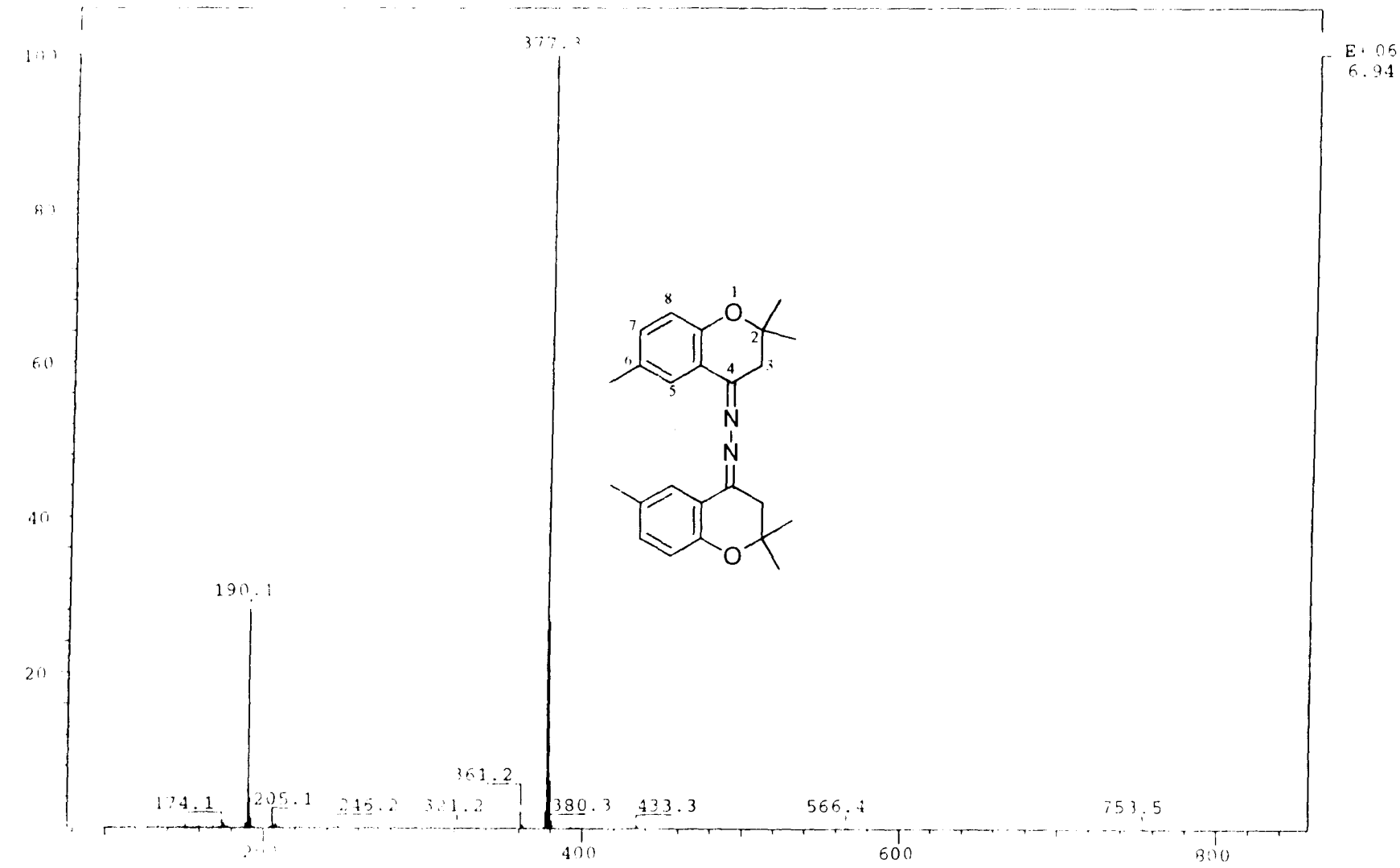


Fig. 1.03 : CI Mass spectrum of limnazine (21)

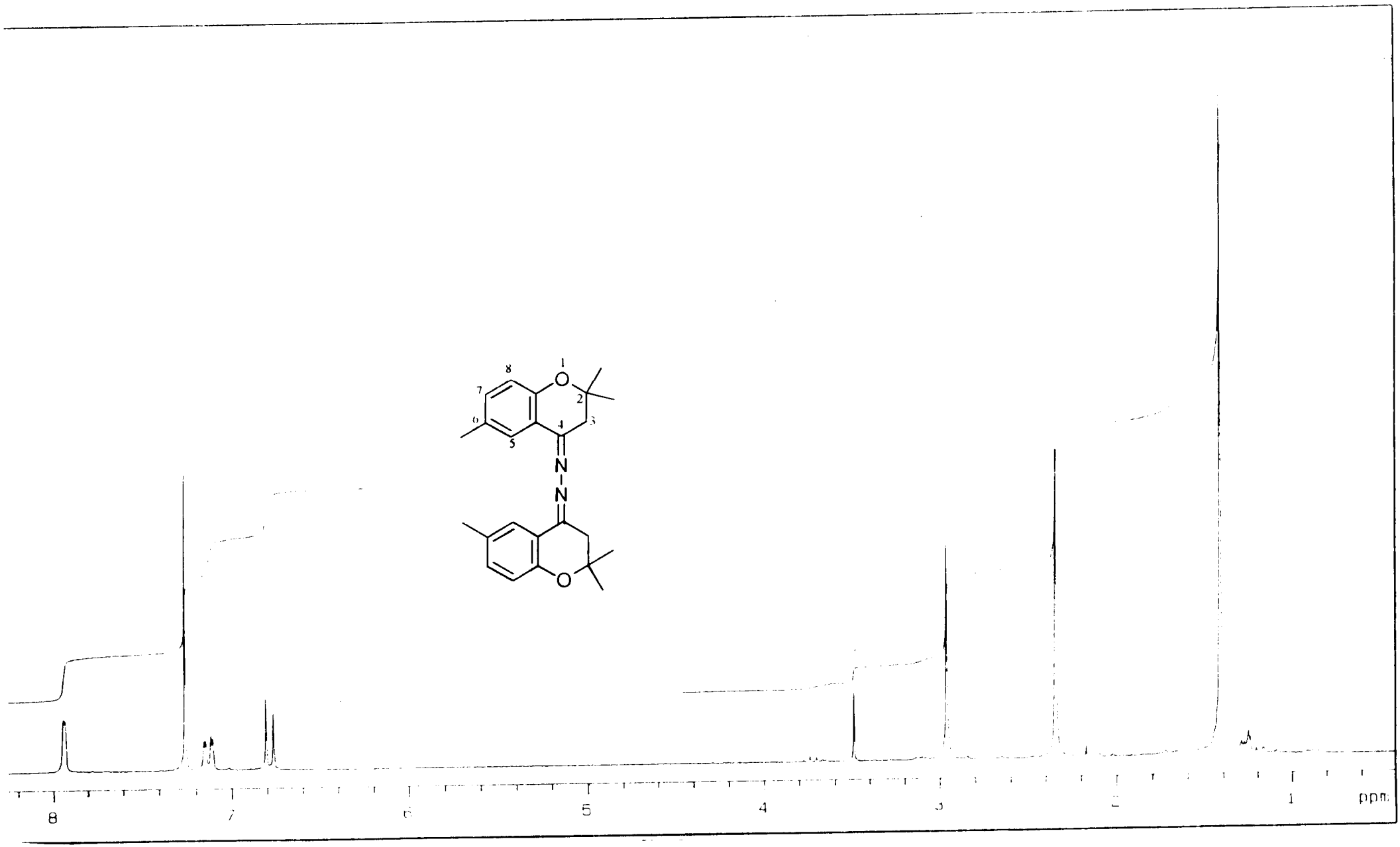


Fig. 1.04 : ^{1}H NMR spectrum of limnazine (21) in $CDCl_3$

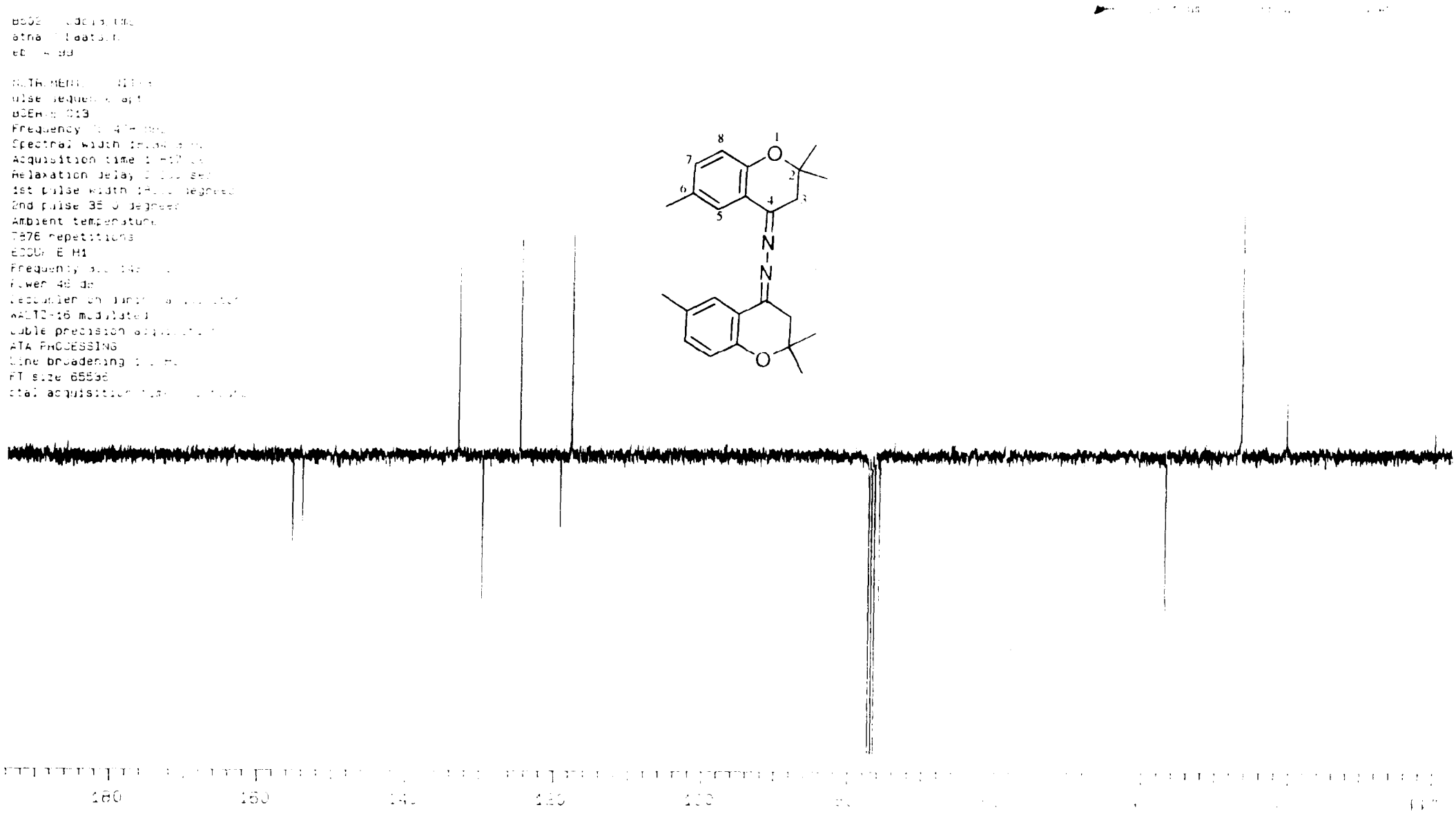
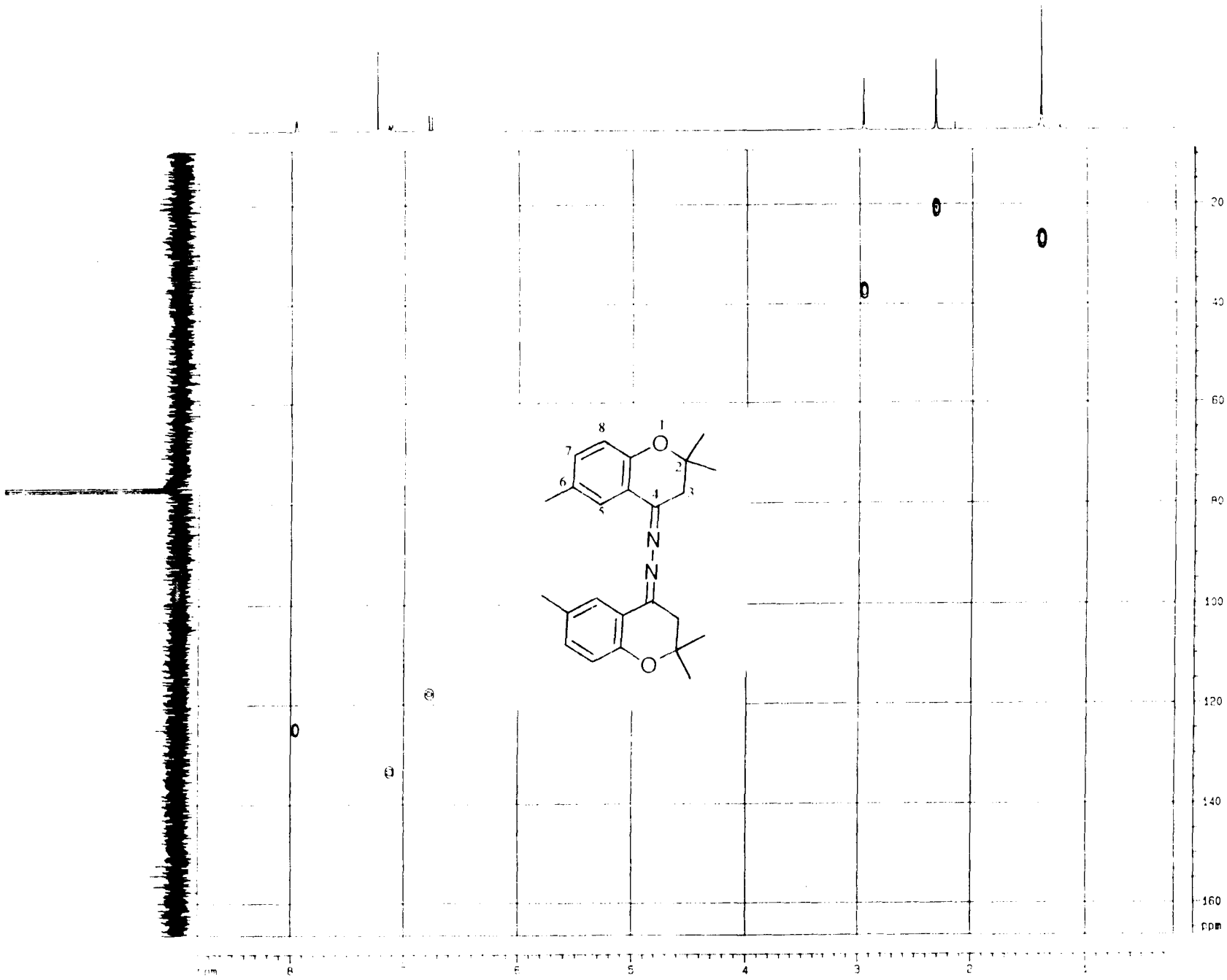


Fig. 1.05 : ^{13}C NMR spectrum of limnazine (21) in $CDCl_3$



Current Data Parameters

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PROCNO	1

Acquisition Parameters

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FIDRES	5.033827 Hz
DW	194.0 usec
RG	4096
NUC1	1H
PL1	3 dB
D1	1.5000000 sec
P1	8.7 usec
D2	0.0034483 sec
PC	17.4 usec
P4	22.4 usec
SFO1	75.4752127 MHz
D7	0.4840000 sec
P3	11.2 usec
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D13	0.0000030 sec
DL0	15.00 dB
DE	277.1 usec
SFO1	300.1347257 MHz
SWH	2577.32 Hz
TD	512
P31	60.0 usec
NS	8
DS	4
IN0	0.0000210 sec

F1 - Acquisition parameters

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TD	165
SFO1	75.47521 MHz
FIDRES	72.150192 Hz
SW	157.731 ppm

F2 - Processing parameters

SI	512
SF	300.1333733 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0
PC	1.40

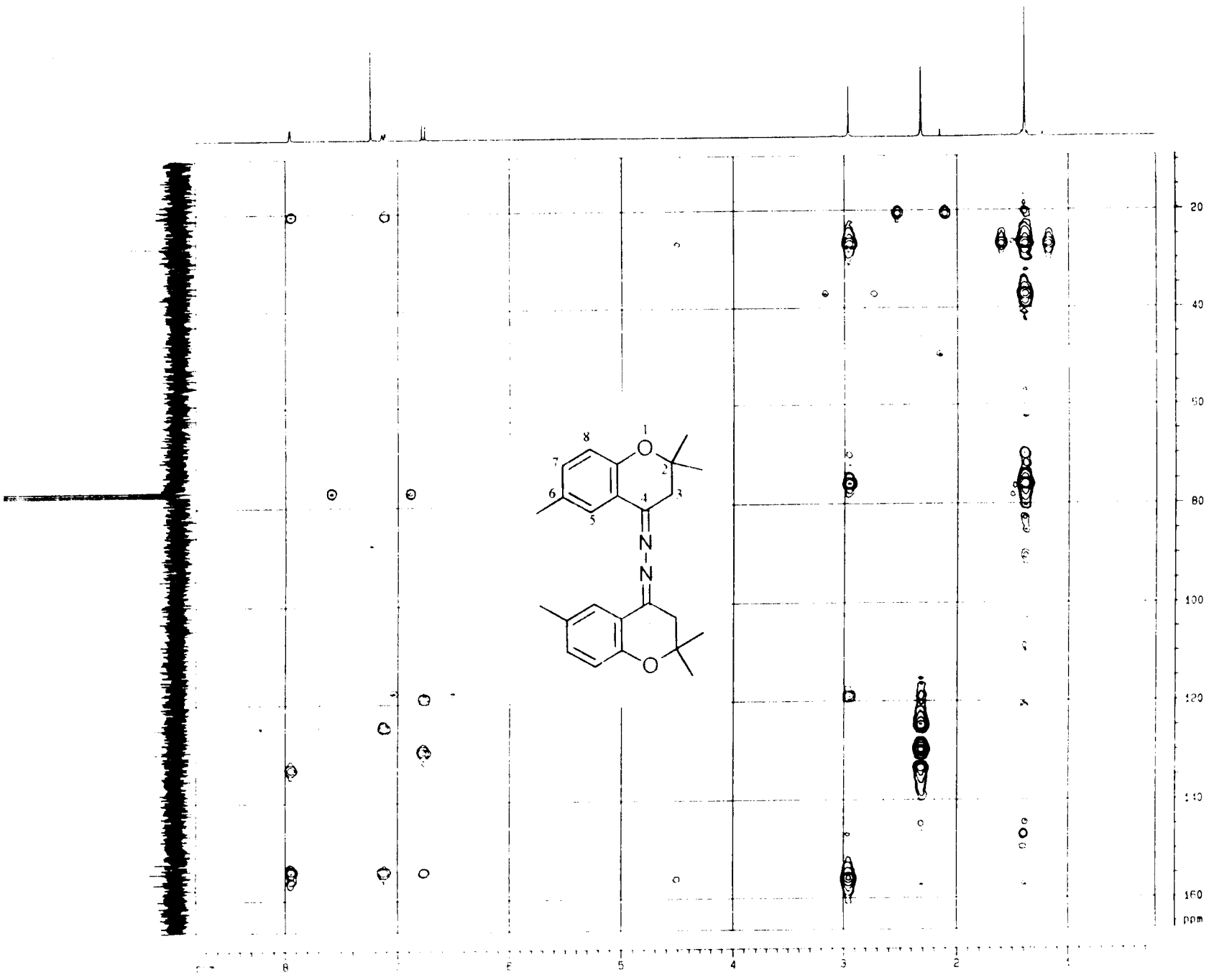
F1 - Processing parameters

SI	256
MC2	TPPI
SF	75.4685952 MHz
WDW	QSINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PPL0	8.800 ppm
F2L0	2641.04 Hz
F2PPHI	8.212 ppm
F2HI	83.72 Hz
F1PPL0	166.650 ppm
F1L0	12569.00 Hz
F1PPHI	0.813 ppm
F1HI	885.00 Hz
F2PPMCM	0.34340 ppm/cm
F2HZCM	103.00278 Hz/cm
F1PPMCM	7.00724 ppm/cm
F1HZCM	595.23914 Hz/cm

Fig. 1.06 : HMQC spectrum of limnazine (21) in $CDCl_3$



SAMPLE
LB502 cdcl3
Rathod Laatsch

Current Data Parameters

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EXPNO	6
PROCNO	1

F2 - Acquisition Parameters

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FIDRES	5.033827 Hz
DW	194.0 usec
RG	2048
NUC EMS	1H
HL1	3 dB
D1	1.5000000 sec
P1	8.7 usec
D2	0.0034483 sec
P3	11.2 usec
SFO2	75.4752127 MHz
DF	0.0600000 sec
D0	0.0000030 sec
P2	17.4 usec
DE	277.1 usec
SFO1	300.1347257 MHz
SW	2577.32 Hz
TD	512
NS	128
DS	4
IN0	0.0000420 dec

F1 - Acquisition parameters

ND0	2
TD	165
SFO1	75.47521 MHz
FIDRES	72.149132 Hz
SW	157.729 ppm

F2 - Processing parameters

SI	512
SF	300.1333733 MHz
WDW	QSINE
SSB	0
LB	0.00 Hz
GB	0
PC	1.40

F1 - Processing parameters

SI	256
MC2	QF
SF	75.4685852 MHz
WDW	SINE
SSB	2
LB	0.00 Hz
GB	0

2D NMR plot parameters

CX2	25.00 cm
CX1	20.00 cm
F2PL0	8.000 ppm
F2L0	2641.04 Hz
F2PH1	0.212 ppm
F2H1	63.72 Hz
F1PL0	166.557 ppm
F1L0	12569.79 Hz
F1PH1	8.814 ppm
F1H1	665.18 Hz
F2PPMCM	0.34349 ppm/cm
F2HZCM	103.08278 Hz/cm
F1PPMCM	7.88713 ppm/cm
F1HZCM	595.23041 Hz/cm

Fig. 1.07 ; HMBC spectrum of limnazine (21) in $CDCl_3$

Fig. 1.08 : CI mass spectrum of 24

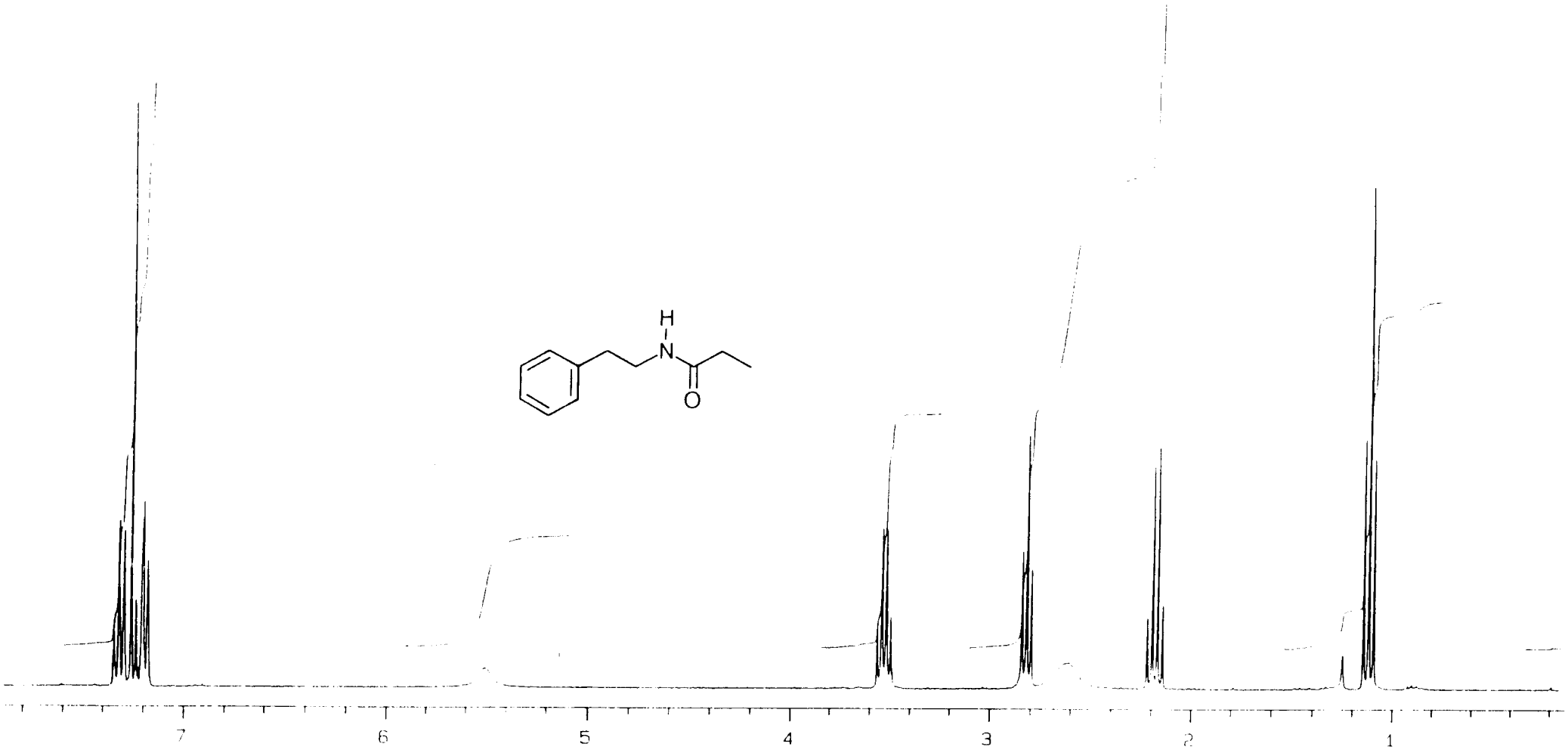

Fig. 1.09 : ^{1}H NMR spectrum of 24 in $CDCl_3$

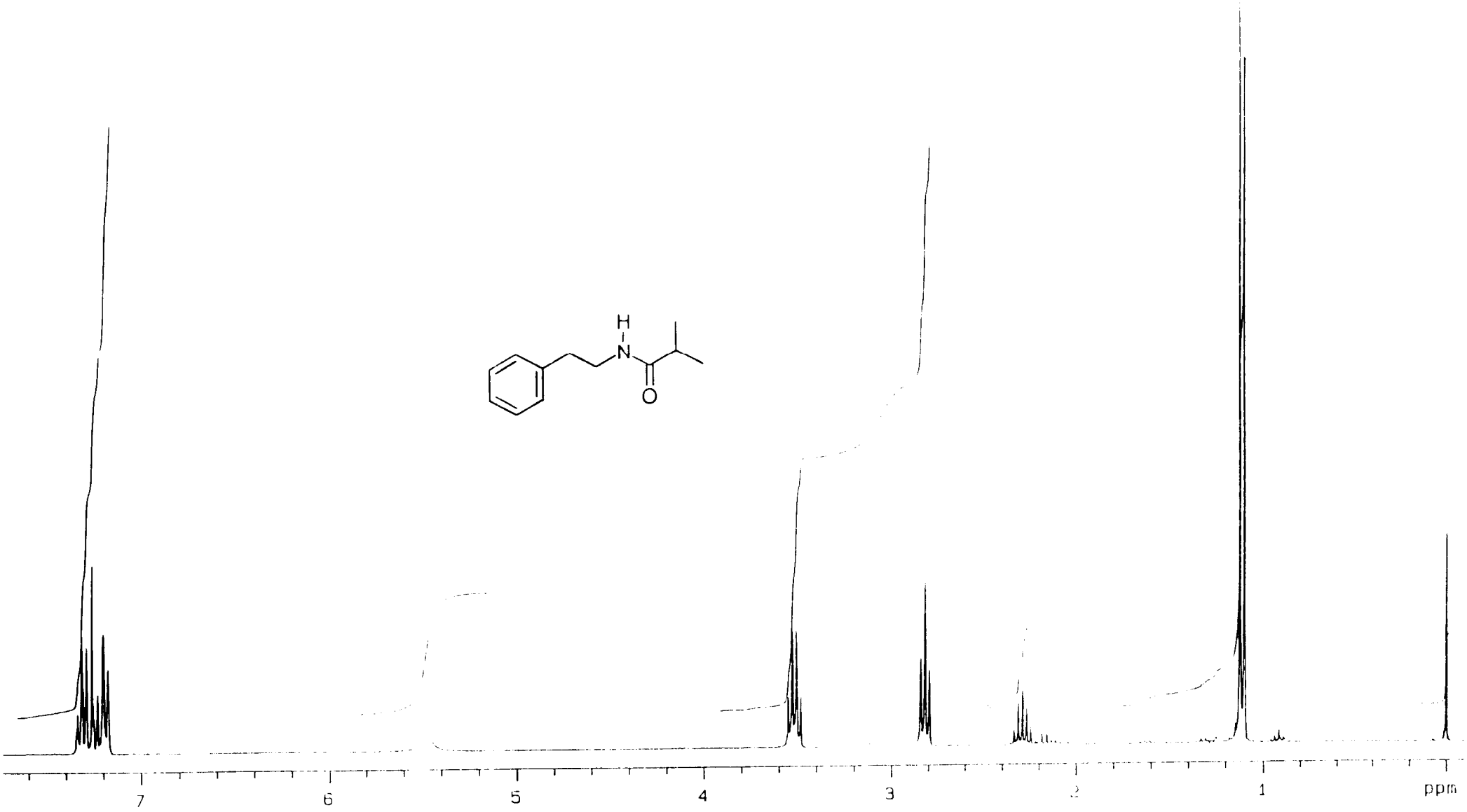

Fig. 1.10 : ^{1}H NMR spectrum of 25 in $CDCl_3$

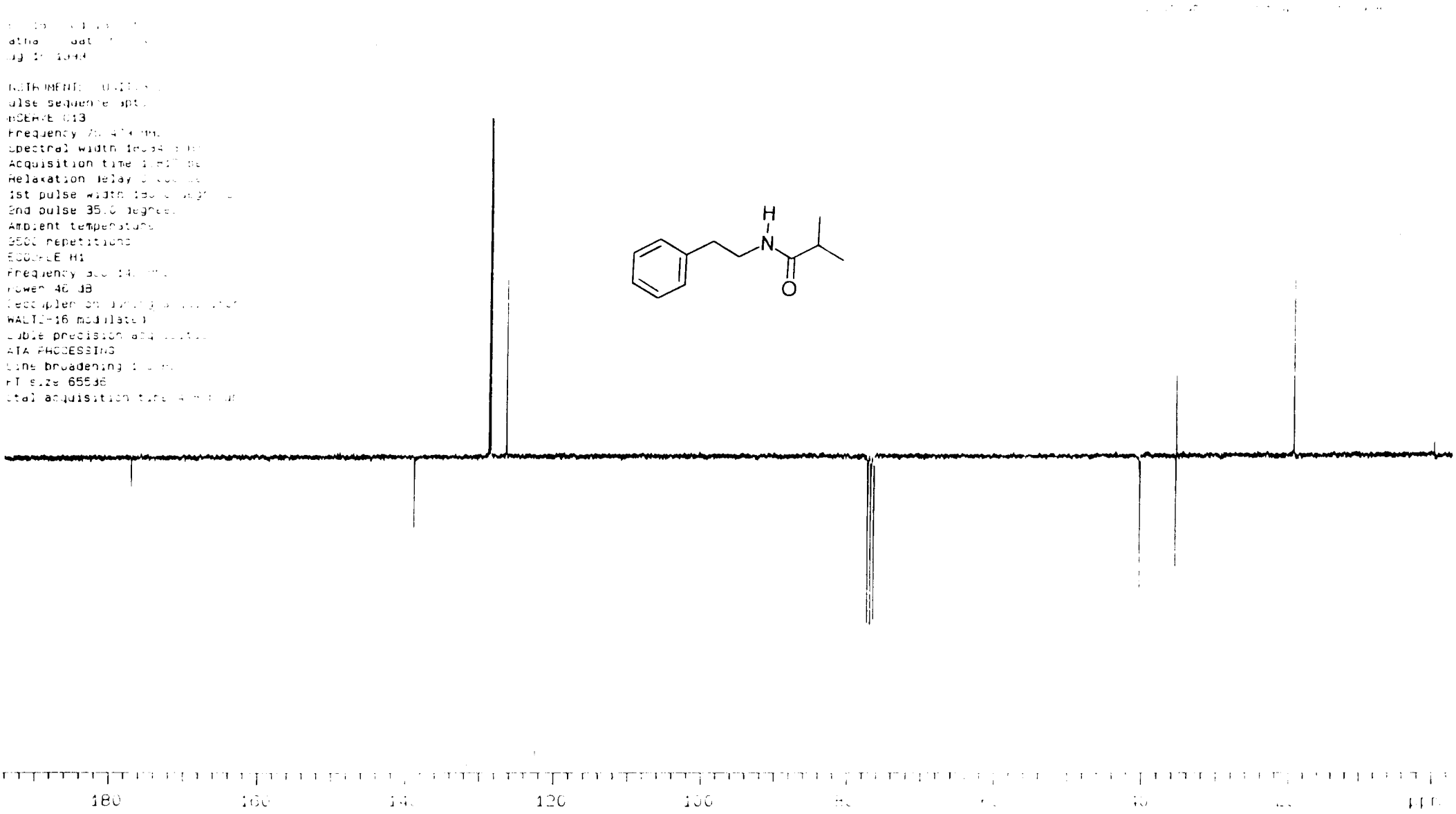

Fig. 1.11 : ^{13}C NMR spectrum of 25 in $CDCl_3$

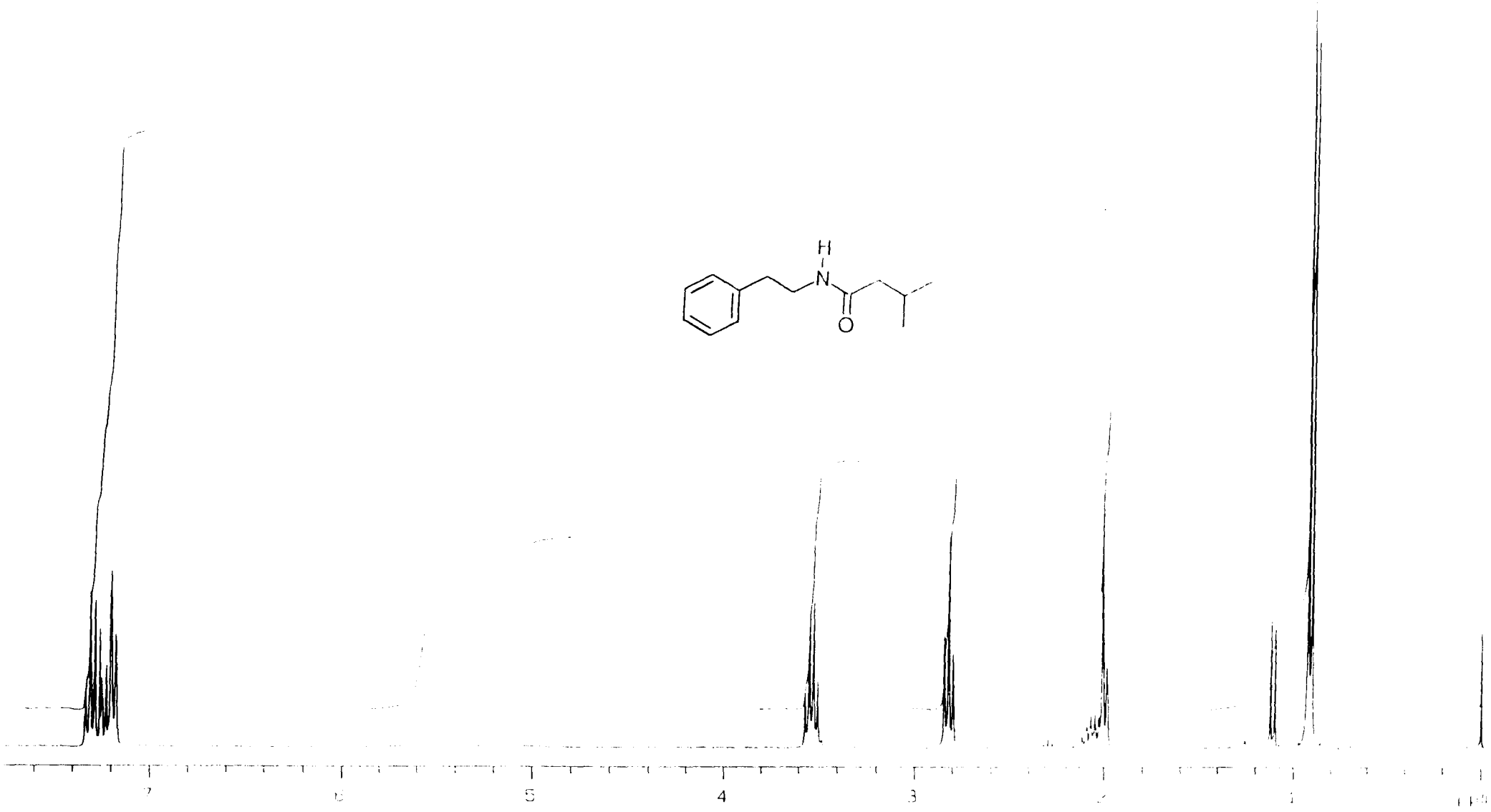

Fig. 1.12 : ^{1}H NMR spectrum of 26 in $CDCl_3$

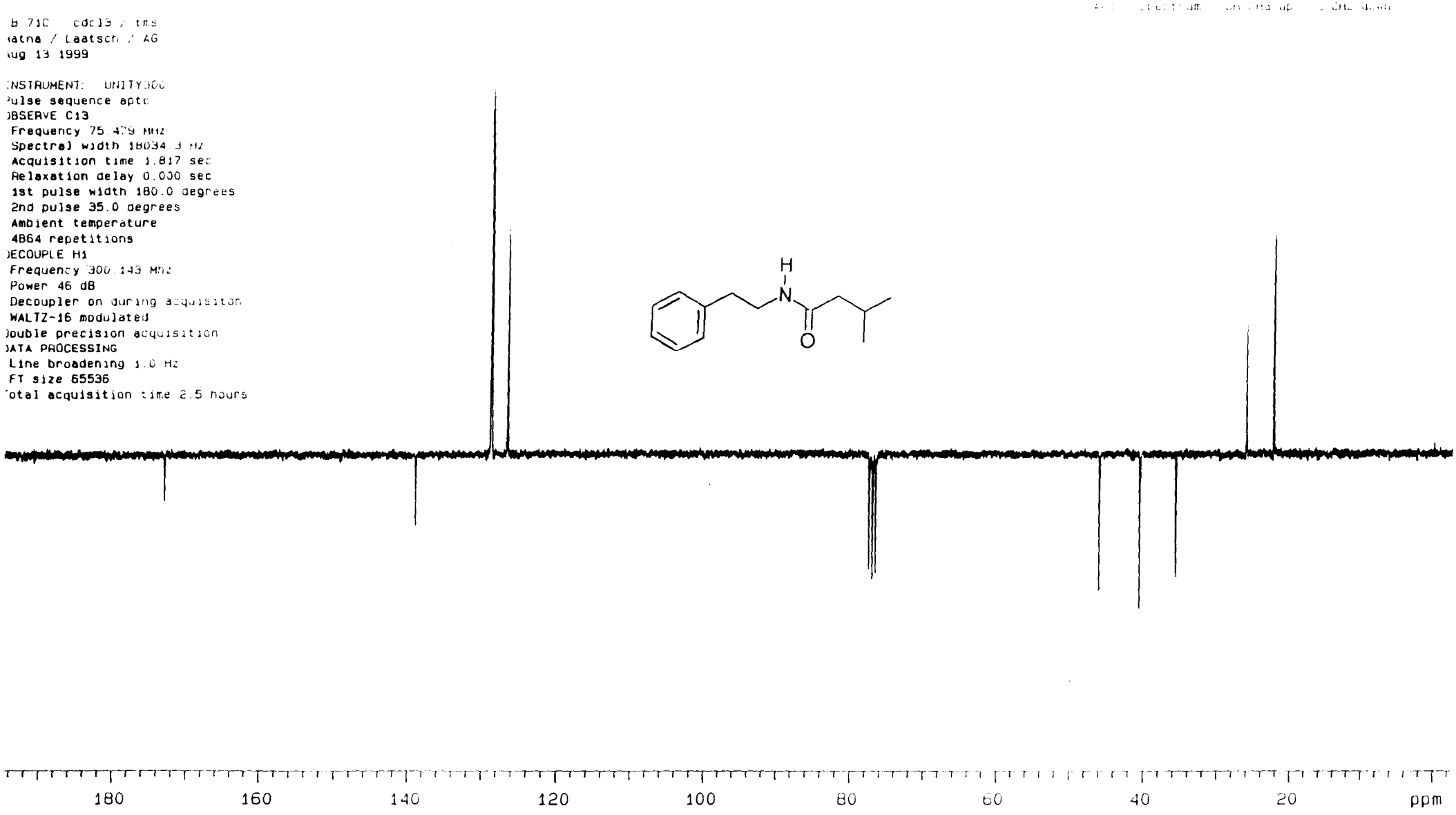

Fig. 1.13 : ^{13}C NMR spectrum of 26 in $CDCl_3$

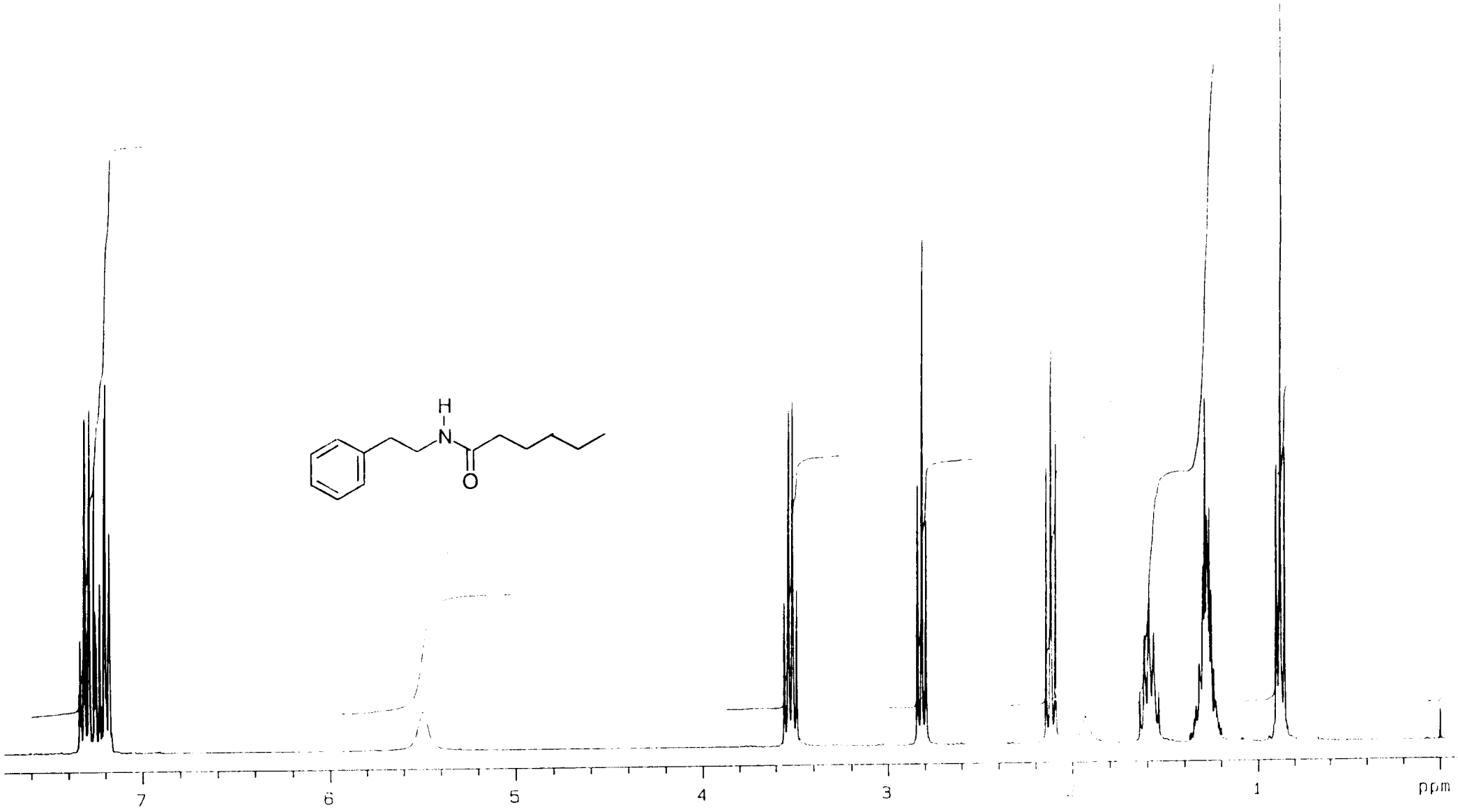

Fig. 1.14 : ^{1}H NMR spectrum of 27 in $CDCl_3$

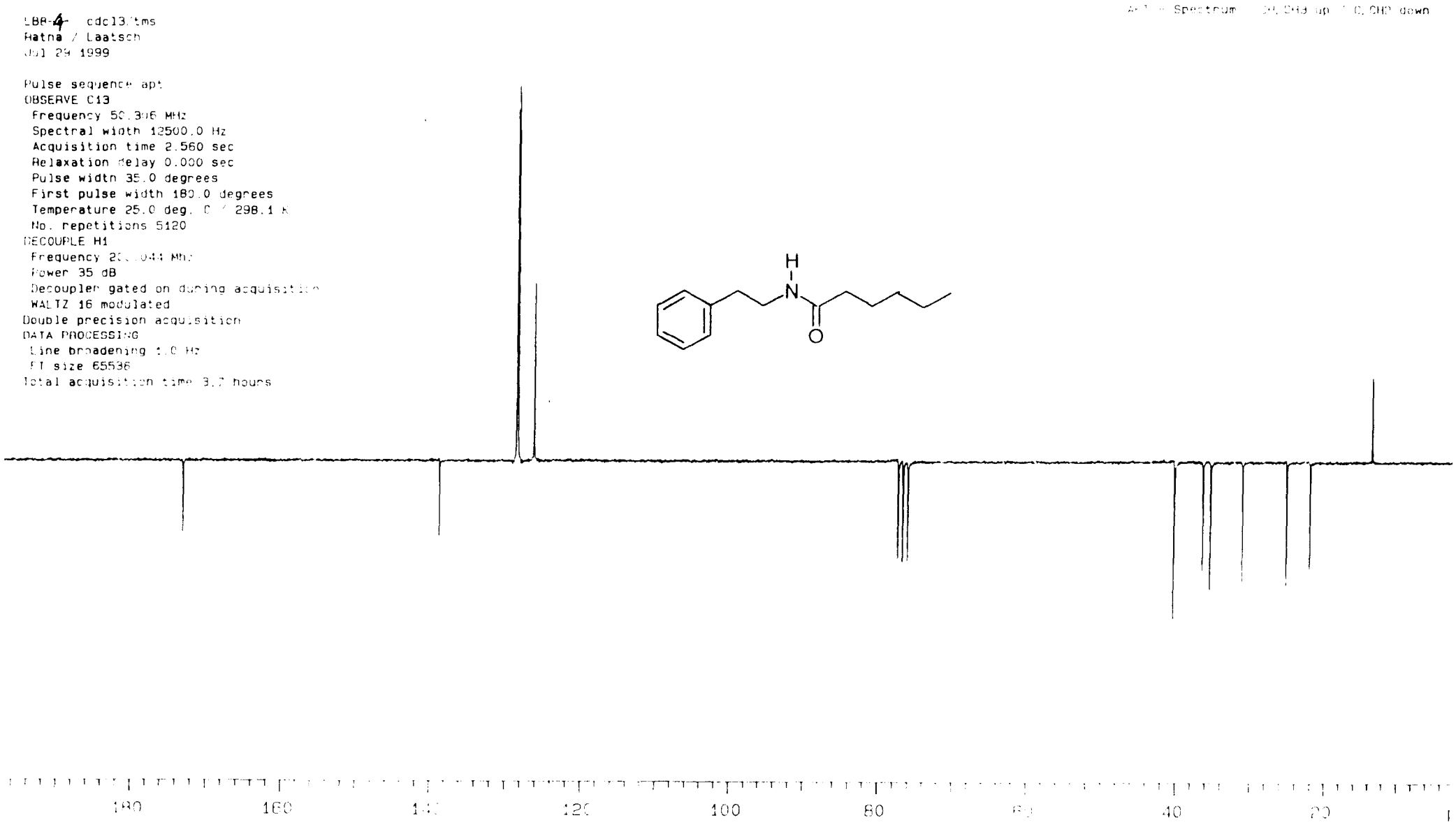

Fig. 1.15 : ^{13}C NMR spectrum of 27 in $CDCl_3$

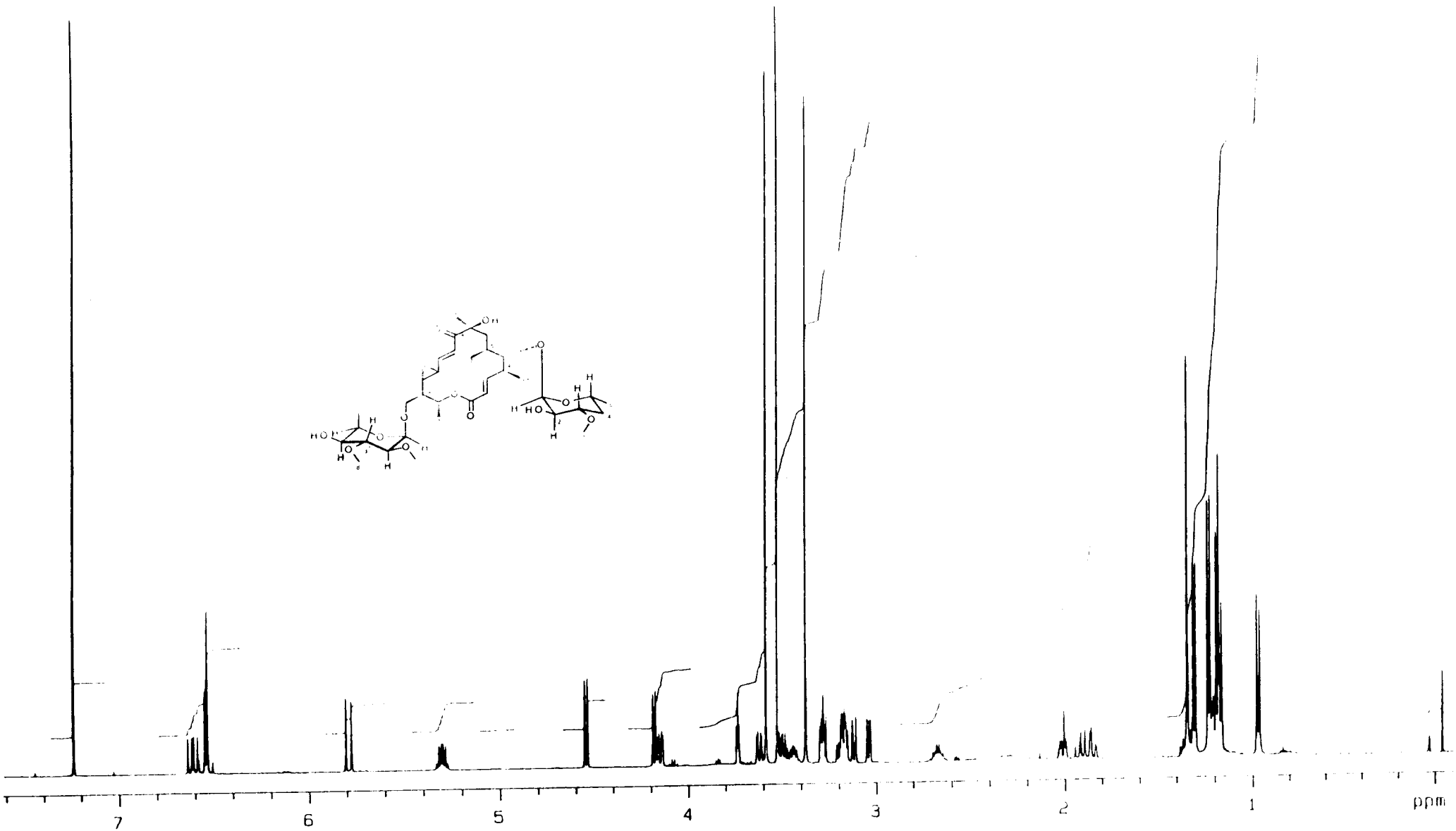

Fig. 1.16 : ^{1}H NMR spectrum of Chalcomycin (32) in $CDCl_3$

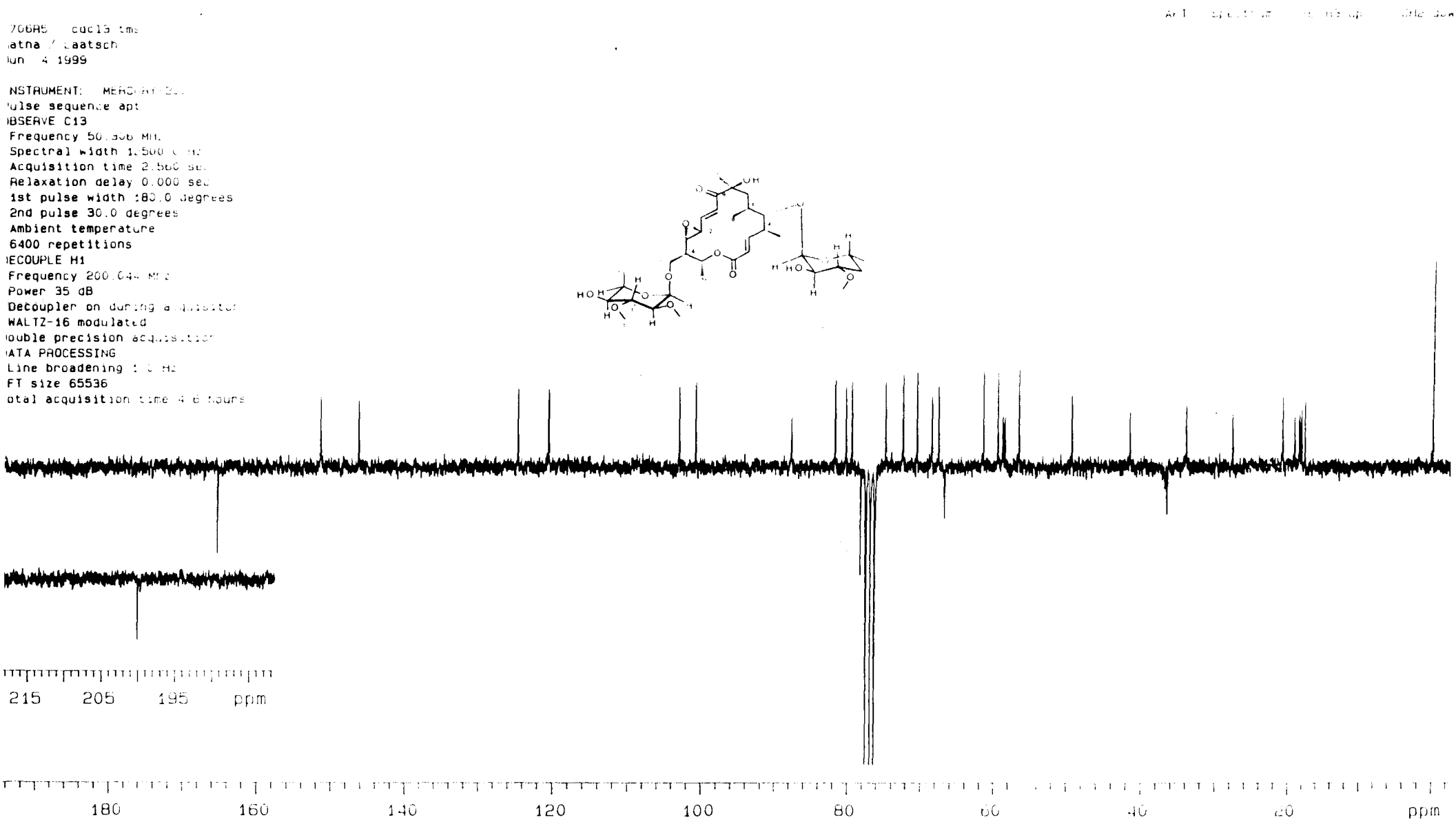

Fig. 1.17 : ^{13}C NMR spectrum of Chalcomycin (32) in $CDCl_3$

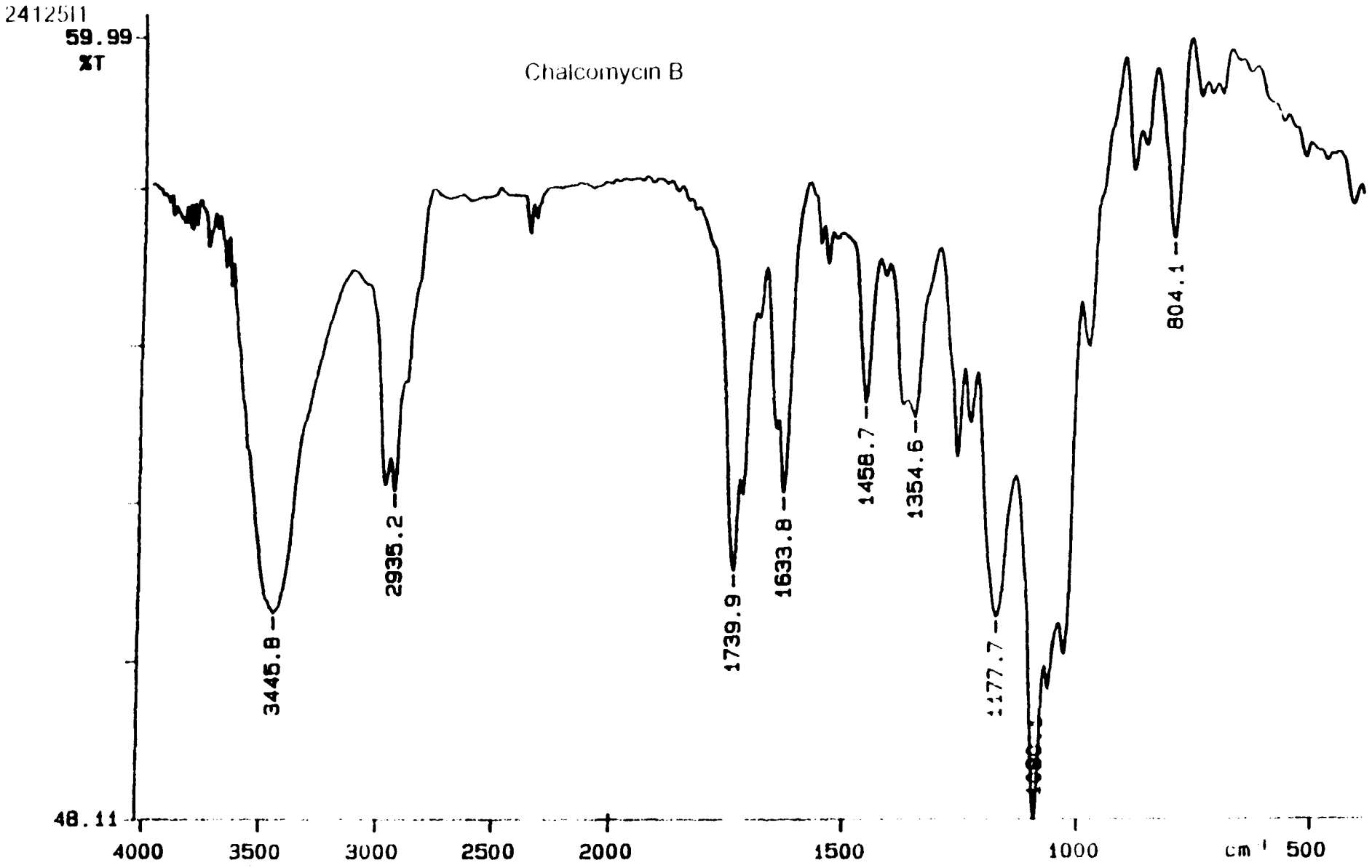

Fig. 1.18 : IR spectrum of Chalcomycin B (33)

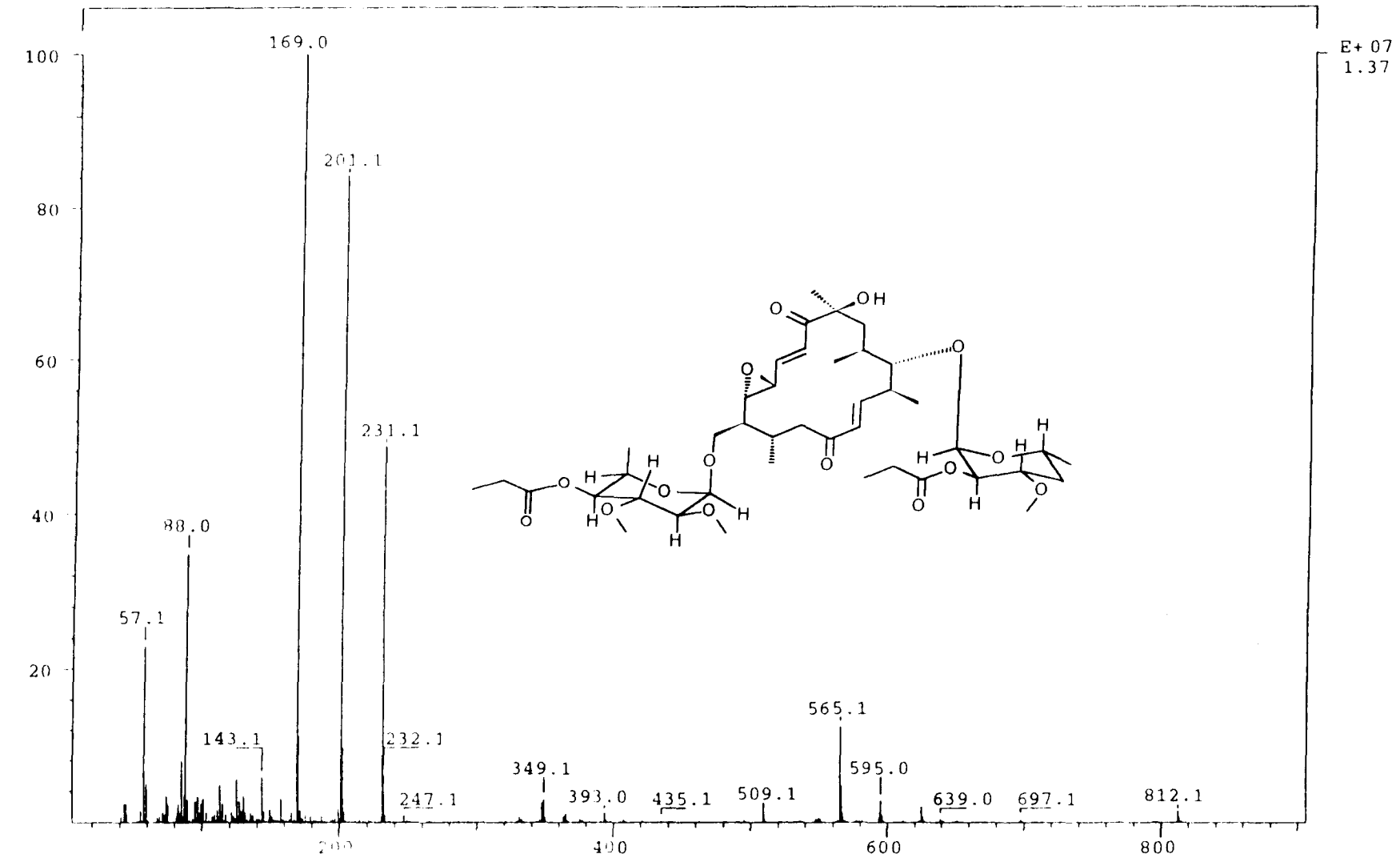

Fig. 1.19 : EI mass spectrum of Chalcomycin B (33)

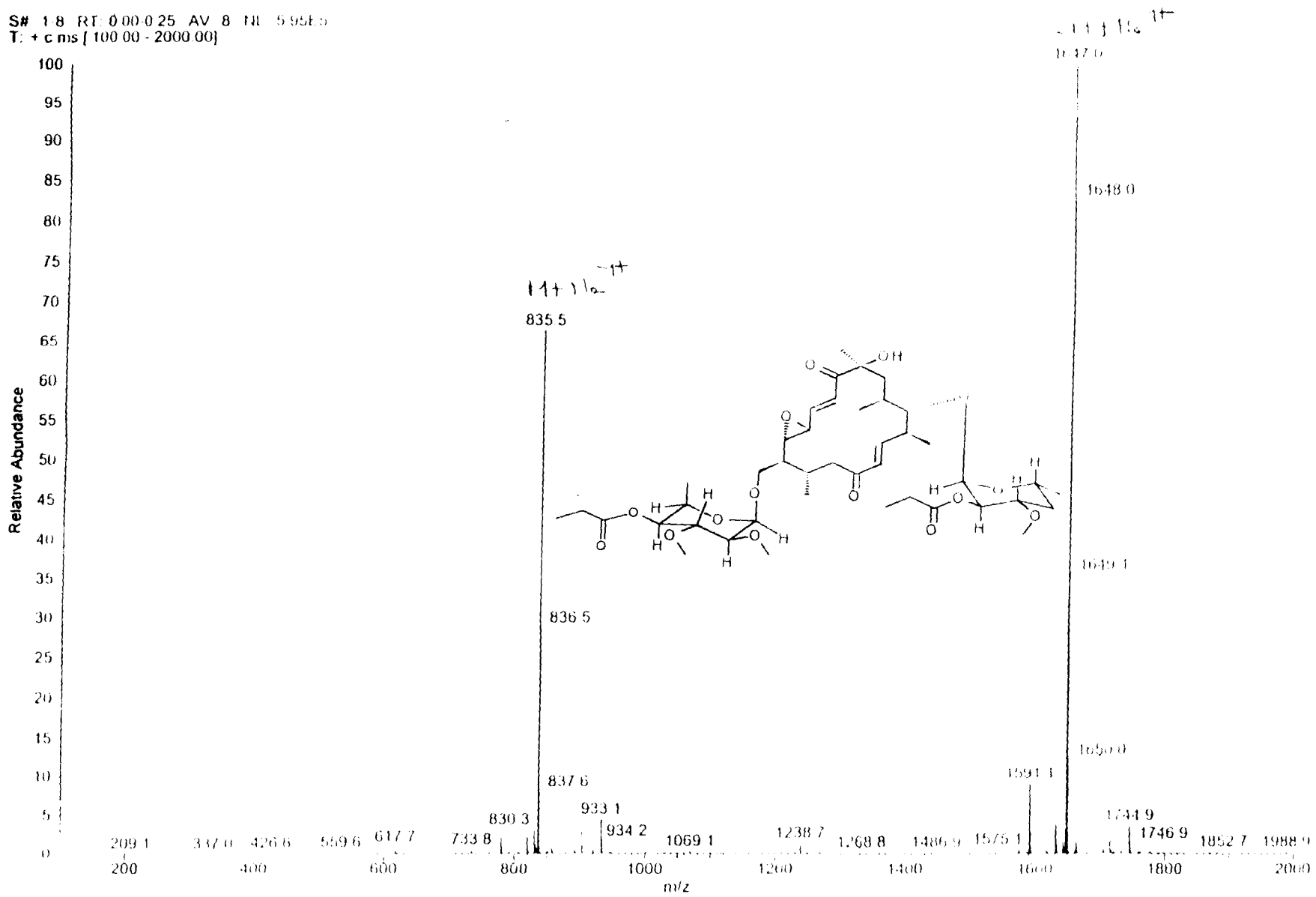

Fig. 1.20 : ESI mass spectrum of Chalcomycin B (33)

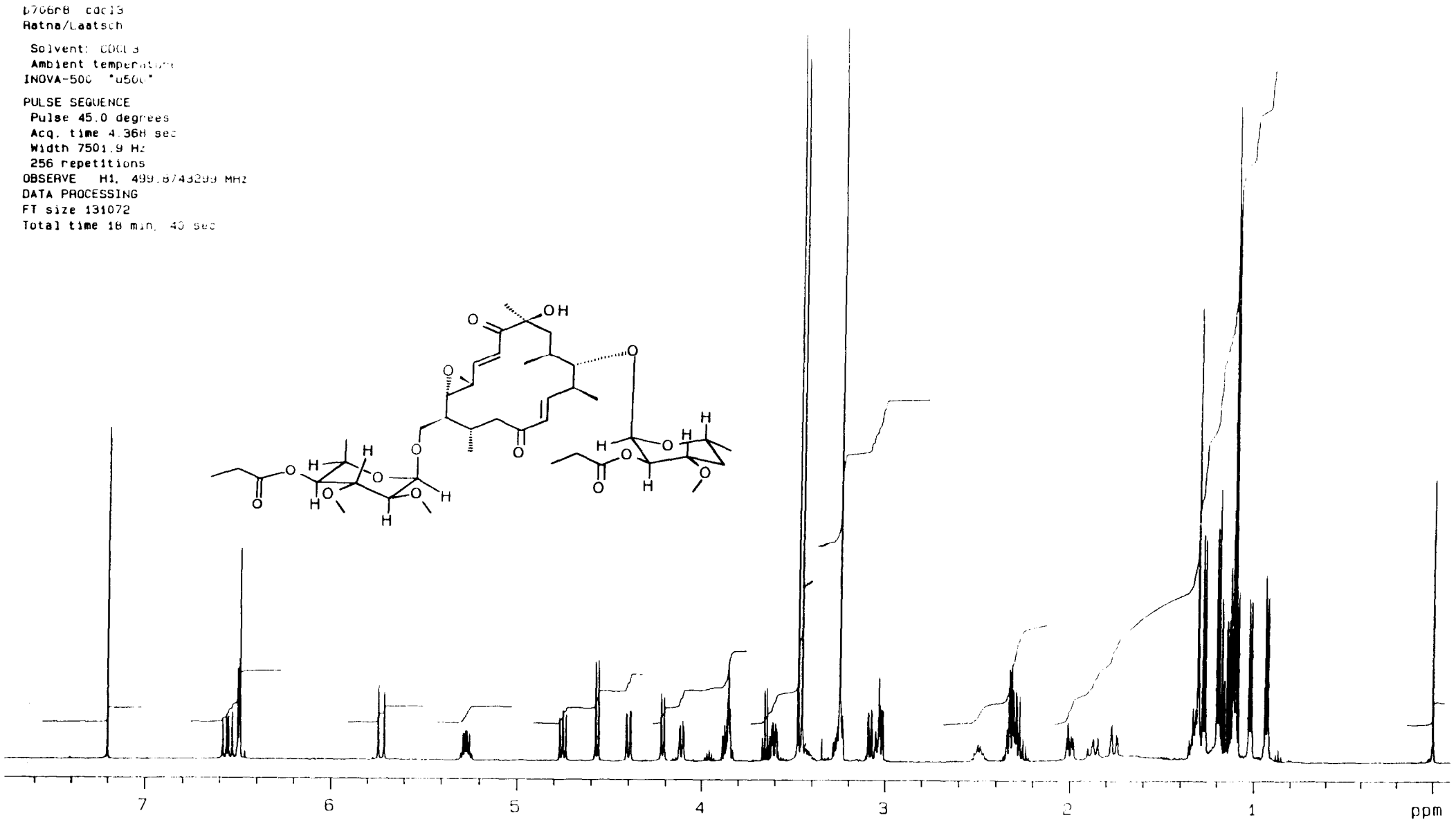

Fig. 1.21 : ^{1}H NMR spectrum of Chalcomycin B (33) in $CDCl_3$

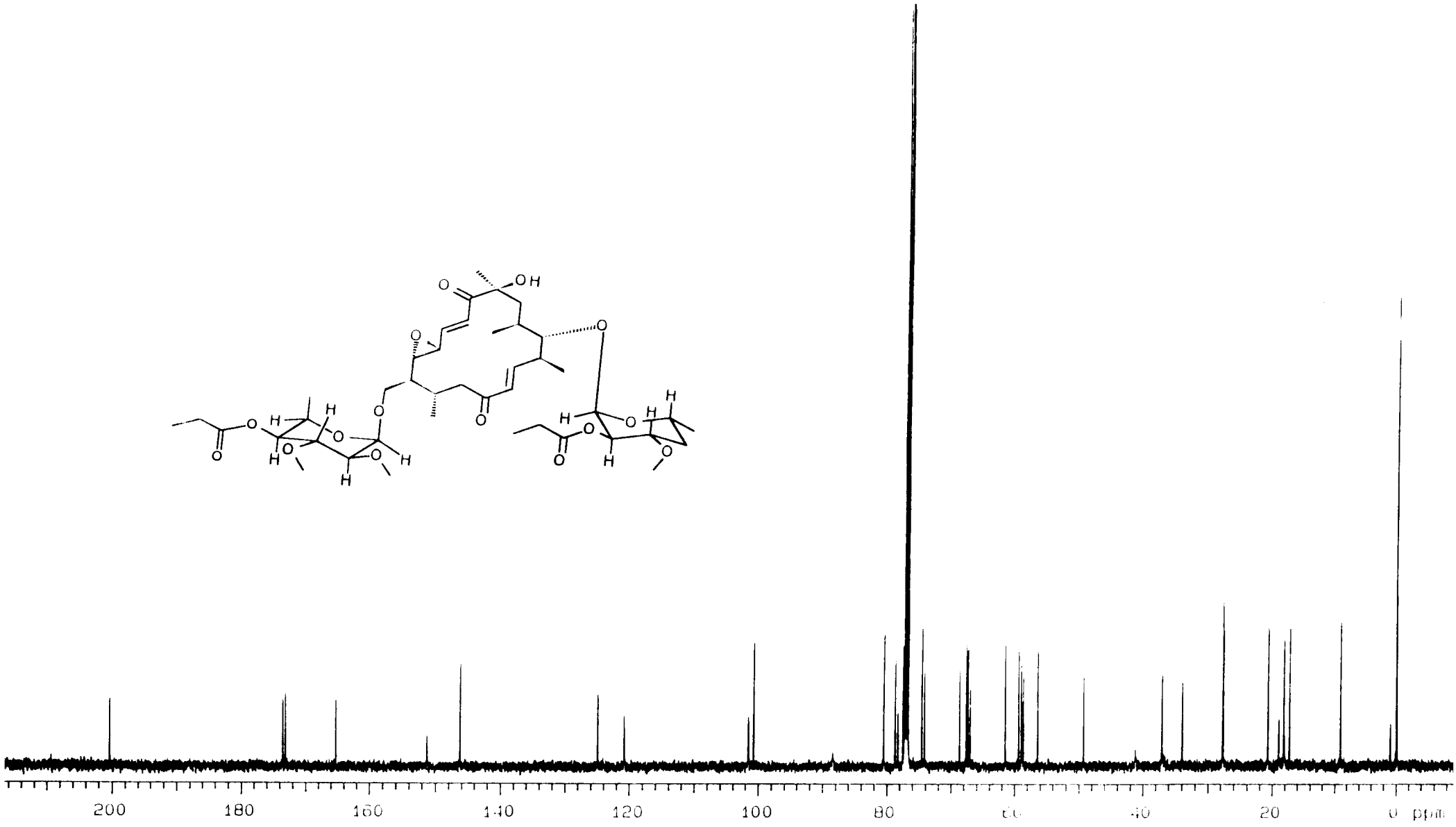

Fig. 1.22 : ^{13}C NMR spectrum of Chalcomycin B (33) in $CDCl_3$

b706r8 cdcl3
Ratna/Laatsch
Jul 21 1999

INSTRUMENT: INOVA 500
Pulse sequence relayh
OBSERVE H1
Frequency 499.876 MHz
Spectral width 3750.9 Hz
2D Spectral width 3750.9 Hz
Acquisition time 0.136 sec
Relaxation delay 1.500 sec
COSY 90-45
Temperature 27.0 deg C
4 repetitions
256 increments
Double precision acquisition
DATA PROCESSING
Sine bell 0.068 sec
F1 DATA PROCESSING
Sine bell 0.034 sec
FT size 1024 x 1024
Total acquisition time 28 minutes

VS= 400
TH= 2

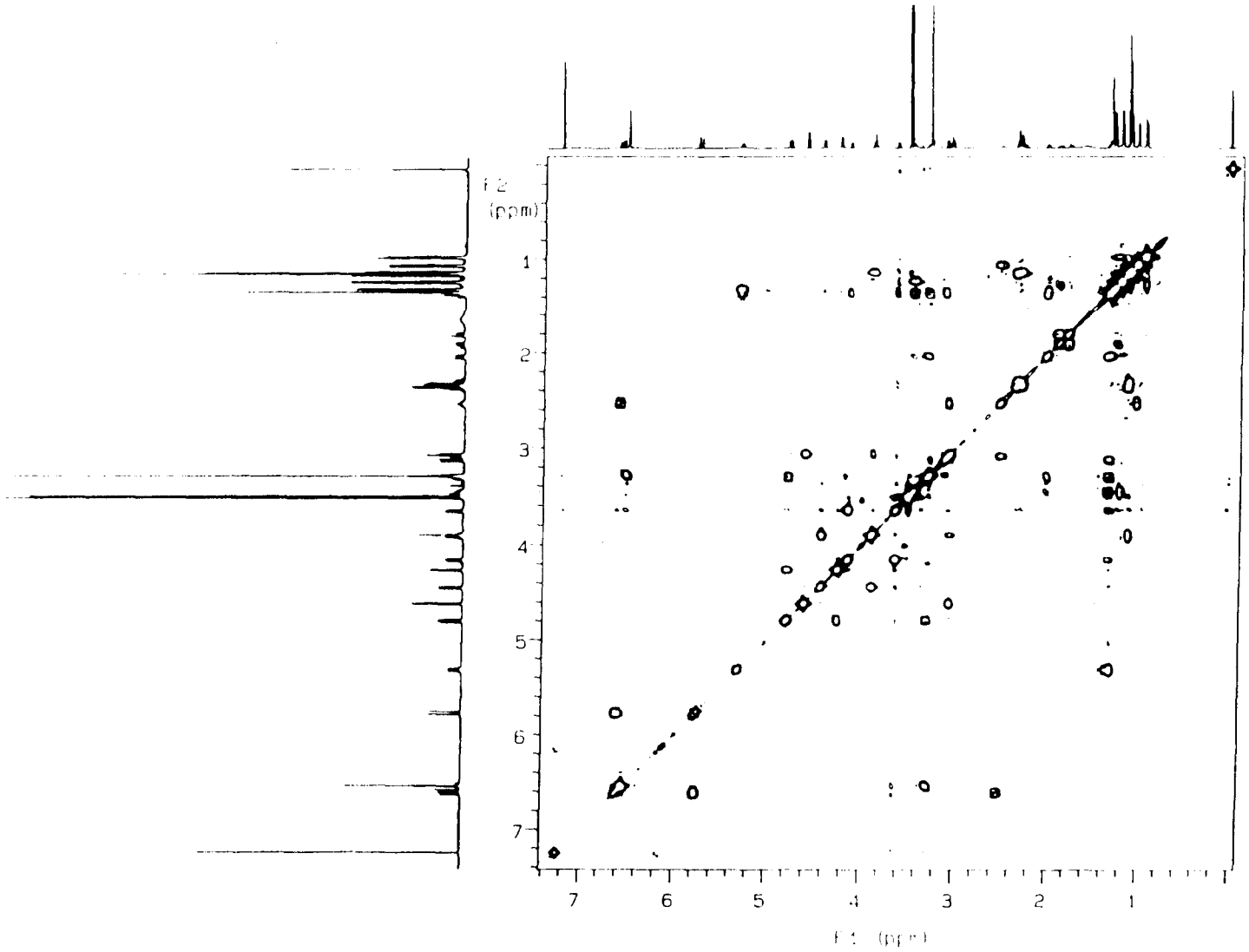

Fig. 1. 23 : ^{1}H - ^{1}H COSY spectrum of Chalcomycin B (33)

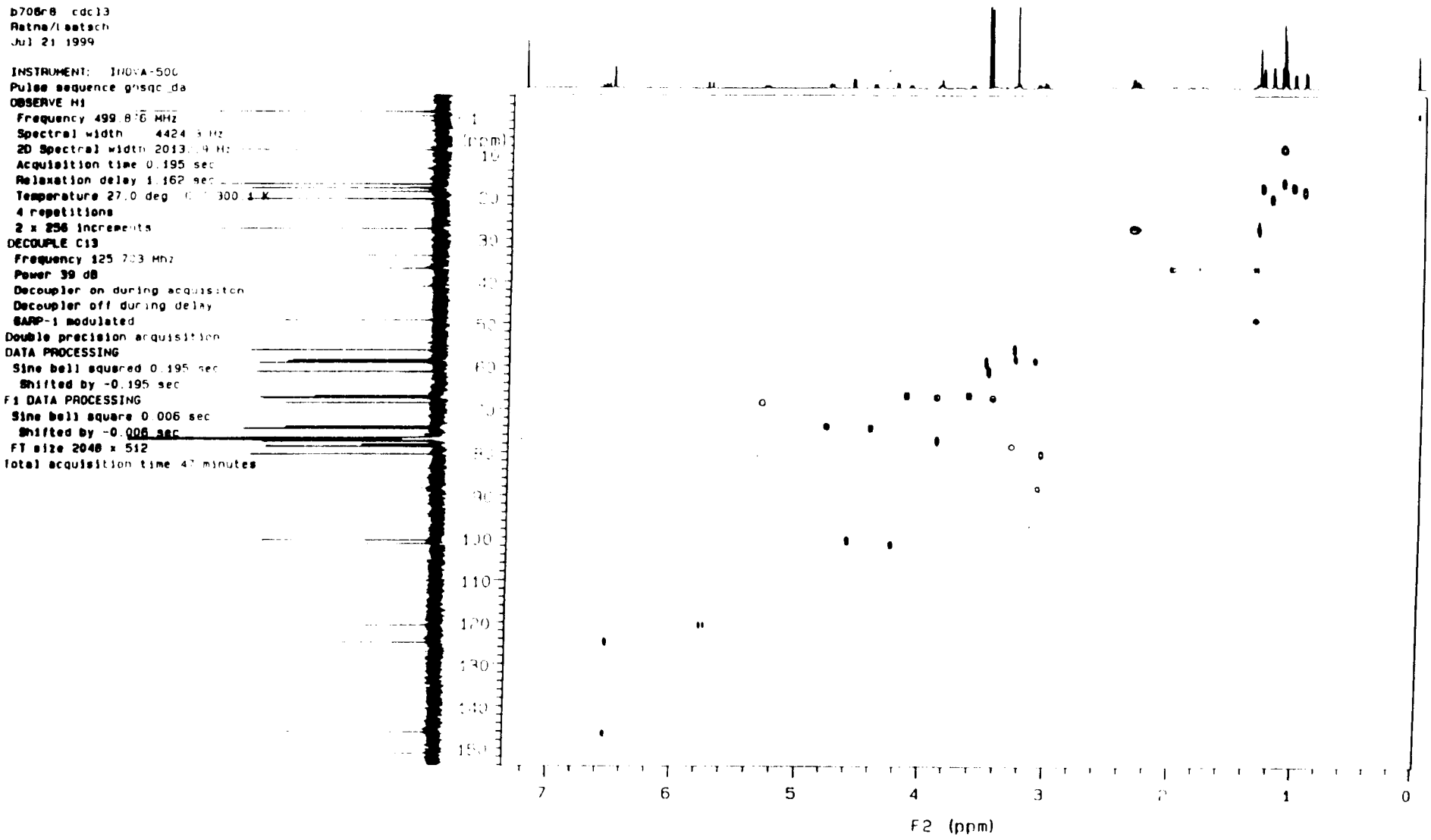

Fig. 1.24 ; HMQC spectrum of Chalcomycin B (33) in $CDCl_3$

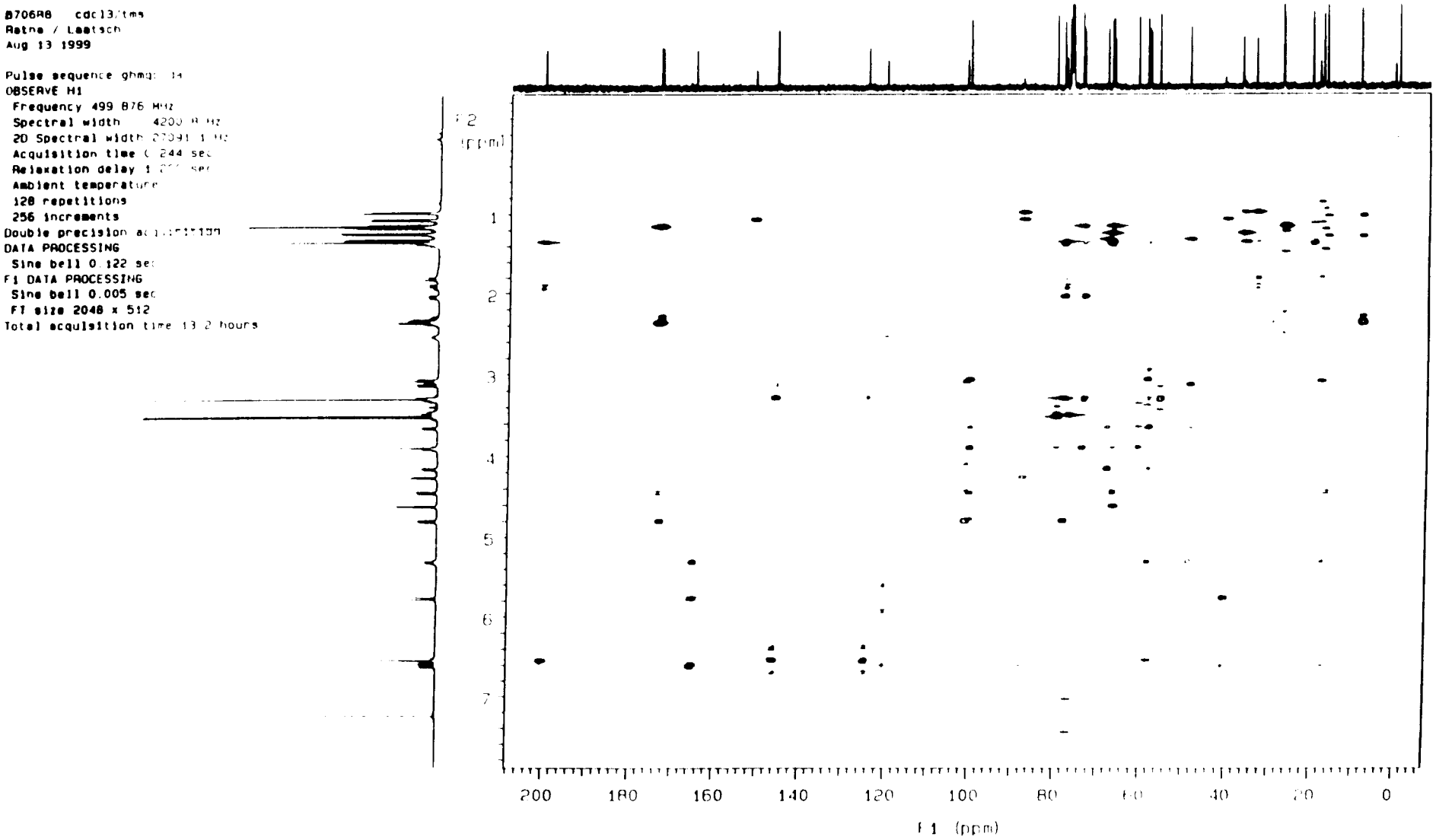

Fig. 1.25 : HMBC spectrum of Chacomycin B (33) in $CDCl_3$

EXPERIMENTAL PART

Artificial Seawater:

Fe-citrate	2.0	g
NaCl	389.0	g
$MgCl_2.6H_2O$	176.0	g
Na_2SO_4	64.8	g
$CaCl_2$	36.0	g
Na_2HPO_4	0.16	g
SiO_2	0.3	g
Trace element solution	20	ml
Salt solution	200	ml

The above ingredients are dissolved in tap water and made to 20 litres.

Trace Element Solution:

H_3BO_3	0.611	g
$MnCl_2$	0.389	g
$CuSO_4$	0.056	g
$ZnSO_4.7H_2O$	0.056	g
$Al_2 (SO_4)_3.18H_2O$	0.056	g
$NiSO_4.6H_2O$	0.056	g
$Co(NO_3).6H_2O$	0.056	g
TiO_2	0.056	g
$(NH_4)_6Mo_7O_{24}.H_2O$	0.056	g
LiCl	0.028	g
$SnCl_2$	0.028	g
KI	0.028	g

The above ingredients are dissolved in tap water to make 1 l of solution.

Salt Solution:

KCl	110	g
$NaHCO_3$	32	g
KBr	16	g
$SrCl_2.6H_2O$	6.8	g
H_3BO_3	4.4	g
NaF	0.48	g
NH_4NO_3	0.32	g

The above ingredients are dissolved in tap water to make 2 l of solution.

Preparation of Luria-Bertani-Medium

Tryptone (E. Merck)	10 g
Yeast extract (E. Merck)	5 g
NaCl	10 g

The above components were dissolved in 1 litre of tap water and the pH of the medium was adjusted to 7.2 with the addition of 2N NaOH and sterilised for 33 min at 121 ^{0}C in autoclave.

Preparation of Luria-Bertani Agar Plates

Tryptone (E. Merck)	10 g
Yeast extract (E. Merck)	5 g
NaCl	10 g
Agar-Agar (E. Merck)	18 g

The above components were dissolved in one litre of tap water and the mixture was stirred for 30 min at room temp. and then heated at 50-60 °C in a micro-wave oven for 30 min. Prior to autoclaving, the pH of the solution was adjusted to 7.2 by addition of 2N NaOH. After autoclaving in sterile condition, it was transferred to the petri-dishes by keeping the thickness of the agar (in the petri-dishes) to around 1 cm and after cooling to room temperature, the agar plates were kept in cold room maintained at 4 °C.

Preparation of M_2^+ 50 % S. W. medium

Malt extract (E. Merck)	10 g
Yeast extract (E. Merck)	4 g
Glucose (E. Merck)	4 g
Synthetic Sea water	500 ml
Tap water	500 ml

The pH of the resulting solution was adjusted to 8 with the addition of 2N NaOH and sterilised for 33 min at 121 ^{0}C in autoclave.

Preparation of M_2^+ 50 % S. W. Agar plates

Malt extract	10 g
Yeast extract	4 g
Glucose	4 g
Agar	18 g
Synthetic Sea water	500 ml
Tap Water	500 ml

The resulting solution was stirred for 30 min at room temp. and then heated at 50-60 °C in a microwave oven for 30 min. Prior to autoclaving, the pH of the solution was set to 8 by addition of 2N NaOH. After autoclaving, in sterile condition, it was transferred to the petri-dishes by keeping the thickness of the agar (in the petri-dishes) to around 1 cm and after cooling to room temperature, the agar plates were kept in cold room at 4 °C.

Limnic strain GBF 90a

Culture, Fermentation and Work-up

The limnic strain GBF 90a, which was identified as *Nocardiopsis* was cultivated on agar (Luria-Bertani-medium) in petri-dishes for 3 days at 29 ^{0}C. Three litres of Luria-Bertani broth were equally distributed in 15 1-Litre-Erlenmeyer flasks, then sterilised and inoculated with pieces of a well grown agar-culture. After retaining for a period of 3 days at 29 ^{0}C and 95 rpm, all flasks with optimum culture growth were used to inoculate a 20 litres fermenter with Luria-Bertani-medium, which was kept for 72 h at 29 ^{0}C. The resulting culture broth was filtered through celite. The aqueous phase as well as the mycelium and the celite used for filtration were extracted three times with ethyl acetate. The combined organic phase was collected and evaporated *in vacuo* at 30 ^{0}C to get 4.12 g of the crude extract. The resulting crude extract was dissolved in methanol and defatted with cyclohexane and the said methanol extract was concentrated under vacuum to give 3.3 g of crude residue.

Isolation

The methanolic extract after concentration was subjected to flash silica gel column chromatography (65 × 2.0 cm) utilising the solvent system $CHCl_3$ / CH_3OH in the proportion (0 :100, 1L ; 1 : 99, 0.5 L ; 2 : 98, 0.5 L; 3 : 97, 0.6 L; 4 : 96, 0.6 L; 5 : 95, 0.8 L; 6 : 94, 0.8 L; 8 : 92, 0.8 L; 10 : 90, 1L; 13 : 87, 0.7L; 20 : 80, 1.5L). Various fractions (10 ml each) were collected and the separation was monitored through tlc, the visualisation of the spot was carried out either by UV light or with spraying reagent. - Identical individual fractions were combined and concentrated to give ten main fractions.

***o*-Hydroxyacetophenone (20)**

The residue obtained after concentration of fraction 3 was chromatographed on a Sephadex LH 20 column (50 × 1 cm, chloroform / methanol, 6 : 4), to furnish **20**, 18 mg as a oily liquid.

EIMS (70 eV): ***m/z*** **(%)** = 136 (M^+, 50), 121 (M^+ - CH_3, 100), 93 (12), 65 (20) and 43 (14).

^{1}HNMR ($CDCl_3$, 200 MHz): δ = 12.25 (s, exchangeable with D_2O, 1 H, -OH), 7.75 (d, *J* = 8 Hz, 1 H, 6-H), 7.55 (t, *J* = 8 Hz, 1 H, 5-H), 6.95 (d, *J* = 8 Hz, 1 H, 4-H), 6.82 (t, *J* = 8 Hz, 1 H, 3-H) and 2.65 (s, 3 H, 8-H_3).

^{13}C/APTNMR ($CDCl_3$, 50.3 MHz): δ = 204.5 (1C, $C_{quat.}$), 162.3 (1C, $C_{quat.}$), 136.4 (1C, CH), 130.7 (1C, CH), 119.9 (1C, $C_{quat.}$), 118.4 (1C, CH) and 26.7 (1C, CH_3).

Limnazine (21)

The residue derived from fraction 4 was purified using a Sephadex LH 20 column (50 × 1 cm, chloroform / methanol 6 :4) to give **21** (8 mg) of a light yellow coloured solid, m.p. 204 °C.

IR (KBr): ν **(fig. 1.01)** = 2995, 2920, 1610, 1540, 1490, 1450, 1325, 1290, 1260, 1210, 1160, 1120, 920, 820 and 615 cm^{-1}.

EIMS (70 eV): ***m/z*** **(%) (fig. 1.02)** = 376.3 (M^+, 56), 361.2 (M^+ - CH3, 100), 190.1 (10), 187.1 (20) and 173.1 (10).

DCIMS (NH_3): ***m/z*** **(%) (fig. 1.03)** = 377.3 ([M + H^+], 100) and 753.5 ([2M + H^+], 10).

^{1}H NMR ($CDCl_3$, 200 MHz): δ **(fig. 1.04)** = 7.95 (d, *J* = 2 Hz, 2 H, 5,5′-H), 7.14 (dd, *J* = 8 Hz, *J* = 2 Hz, 2 H, 7,7′-H), 6.78 (d, *J* = 8 Hz, 2 H, 8,8′-H), 2.96 (s, 4 H, 3,3′-CH_2), 2.35 (s, 6 H, 11,11′-CH_3) and 1.4 (s, 12 H, 2,2′ -$(CH_3)_2$).

^{1}H NMR ([D_6]acetone, 200 MHz): δ = 7.92 (d, *J* = 2 Hz, 2 H, 5,5′-H), 7.15 (dd, *J* = 8 Hz, *J* = 2 Hz, 2 H, 7,7′-H), 6.77 (d, *J* = 8 Hz, 2 H, 8,8′-H), 3.0 (s, 4 H, 3,3′-CH_2), 2.3 (s, 6 H, 11,11′-CH_3) and 1.37 (s, 12 H, 2,2′-$(CH_3)_2$).

^{13}C/APT NMR ($CDCl_3$, 75.5 MHz): δ **(fig. 1.05)** = 155.4 (2C, $C_{quat.}$), 154.1 (2C, $C_{quat.}$), 133.1 (2C, CH), 129.8 (2C, $C_{quat.}$), 124.8 (2C, CH), 119.3 (2C, $C_{quat.}$), 117.9 (2C, CH), 75.9 (2C, $C_{quat.}$), 37.1 (2C, 2 x CH_2), 26.8 (4C, 2 x $(CH_3)_2$) and 20.1 (2C, 2 x CH_3).

HMQC (inverse CH-COSY, $CDCl_3$, INVBTP, F 1 75.5 MHz, F 2 300.1 MHz) (H →C) (fig. 1.06) : 5-H → C-5; 7-H →C-7; 8-H →C-8; 3-H →C-3; 9-H →C-9; 10-H →C-10 and 11-H →C-11.

HMBC (inverses COLOC, $CDCl_3$, INV4LPLRND, F 1 75.5 MHz, F 2 300.1 MHz) (H→C)(fig. 1.07) : 5 H ^3J →C-11; 5 H ^3J →C-7; 5 H ^3J →C-8a; 5 H ^3J →C-4; 7 H ^3J →C8a; 7 H ^2J →C-6; 7 H ^3J →C-5; 7 H ^3J →C-11; 8 H ^2J →C-7; 8 H ^2J →C8a; 8 H ^3J →C-4a; 11 H ^2J →C-6; 11 H ^3J →C-5; 11 H ^3J →C-7; 3 H ^2J →C-4; 3 H ^3J →C-4a; 3 H ^3J →C-9, C-10; 3 H ^2J →C-2; 9 H ^2J →C-2; 9 H ^3J →C-10; 9 H ^3J →C-3; 10 H ^2J →C-2; 10 H ^3J →C-11 and 10 H ^3J →C-3.

Preparation of Limnazine (21) from Chromanone (23)

A mixture of chromanone (**23**), (0.1 g, mmol) and hydrazine hydrate[23] (10 ml) was heated under reflux for 2 h. The excess hydrazine hydrate remaining in the reaction mixture was distilled off and the residue after cooling was poured on crushed ice, extracted with diethyl ether (3 x 25 ml). The combined ether extract was washed with water, saturated aqueous sodium chloride and dried (Na_2SO_4). The solvent was removed and the crude product was purified by a silica gel column chromatography using petroleum ether : ether (1 : 19) as the eluent to give a light yellow coloured crystalline solid **21** (0.065 gm, 58 %), with m.p.204 ^{0}C. The m.p and the spectral data recorded on the synthetic sample were in perfect agreement with those recorded for the natural product.

Fraction 5 on concentration followed by purification by HPLC provided three differ-

ent crystalline compounds namely **24**, (19 mg) m.p. 45 - 46 ^{0}C, **25**, (25 mg) m.p. 82 ^{0}C and **26**, (24 mg) m.p. 52 ^{0}C .

N-(2′-phenylethyl)-propanamide (24)

IR (KBr): ν = 3375, 3081, 2974, 1648, 1557, 1455, 1428, 1375, 1247, 1198, 1048, 748, 700, 497 and 465 cm^{-1}.

CIMS (NH_3): *m/z* (%) (fig. 1.08) = 178.1 ([M + H]$^+$, 38), 195.1([M + NH_4]$^+$, 100) and 355.1 ([2M + H]$^+$, 25).

^{1}HNMR ($CDCl_3$, 300 MHz): δ (fig. 1.09) = 7.28 (m, 5 H, Ar-H), 5.5 (br.s, exchangeable with D_2O; 1 H, N-H), 3.52 (q, J = 8 Hz, 2 H, 1′-H_2), 2.82 (t, J = 8 Hz, 2 H, 2′-H_2), 2.18 (q, J = 8 Hz, 2 H, 2-H_2) and 1.1 (t, J = 8Hz, 3 H, 3-H_3).

^{13}C/APT-NMR ([D_6]acetone, 75.5 MHz): δ = 174 (1C, $C_{quat.}$), 140.5 (1C, $C_{quat.}$), 129.5 (2C, CH), 129.1 (2C, CH), 126.9 (1C,CH), 41.1 (1C, CH_2), 41.3 (1C, CH_2), 36.5 (1C, CH_2) and 10.2 (1C, CH_3).

N-(2′-Phenylethyl)-2-methylpropanamide (25)

IR (KBr): ν = 3299, 2967, 2870, 1640, 1548, 1458, 1363, 1242, 1195, 1100, 748, 698 and 486 cm^{-1}.

CIMS (NH_3): *m/z* (%) = 192.1 ([M + H]$^+$, 30), 209.1 ([M + NH_4]$^+$, 100) and 383.2([2M + H]$^+$, 10). **^{1}H NMR ($CDCl_3$, 300 MHz):** δ **(fig. 1.10)** = 7.25 (m, 5 H, Ar-H), 5.5 (br.s, exchangeable with D_2O; 1 H, N-H), 3.5 (q, J = 8 Hz, 2 H, 1′-H_2), 2.82 (t, J = 8 Hz, 2 H, 2′-H_2), 2.3 (h, J = 8 Hz, 1 H, 2-H) and 1.15 (d, J = 8Hz, 6 H, 3-H_3,4-H_3).

^{13}C/APT NMR ($CDCl_3$, 75.5 MHz): δ **(fig. 1.11)** = 174 (1C, $C_{quat.}$), 140.5 (1C, $C_{quat.}$), 128.8 (2C, CH), 128.6 (2C, CH), 126.5 (1C, CH), 40.5 (1C, CH_2), 35.7 (1C, CH_2), 35.6 (1C, CH) and 19.6 (1C, CH_3).

N-(2′-phenylethyl)-3-methyl-butanamide (26)

IR (KBr): ν = 3302, 2959, 2868, 1639, 1545, 1456, 1368, 1308, 1198, 1031, 748, 699, 605 and 497 cm^{-1}.

CIMS (NH_3): *m/z* (%) = 206.1([M + H]$^+$, 28), 223.1([M + NH_4]$^+$, 100), 240,1([2M + NH_4 + NH_3]$^+$, 15), 411.1 ([2M + H]$^+$, 28) and 428.1 ([2M + NH_4 + NH_3]$^+$, 42).

^{1}H NMR ($CDCl_3$, 300 MHz): δ (fig. 1.12) = 7.28 (m, 5 H, Ar-H), 5.56 (br.s, exchangeable with D_2O; 1 H, N-H), 3.56 (q, *J* = 8 Hz, 2 H, 1′-H_2), 2.82 (t, *J* = 8 Hz, 2 H, 2′-H_2), 2.06 (sx, *J* = 8 Hz, 1 H, 3-H), 1.98 (d, *J* = 8 Hz, 2 H, 2-H_2) and 0.92 (d, *J* = 8 Hz, 6 H, 4-H_3, 5-H_3).

^{13}C/APT NMR ($CDCl_3$, 75.5 MHz): δ (fig. 1.13) = 172.9 (1C, $C_{quat.}$), 138.9 (1C, $C_{quat.}$), 128.7 (2C, CH), 128.6 (2C, CH), 126.5 (1C,CH), 46.1 (1C, CH_2), 40.6 (1C, CH_2), 35.7 (1C, CH_2), 26.2 (1C, CH) and 22.4 (2C, CH_3).

N-(2′-phenylethyl)-hexanamide (27)

Fraction 7 on evaporation of the solvent followed by purification furnished compound **24** (15 mg) and another crystalline compound **27** (35 mg), m.p. 77-78 °C.

IR (KBr): ν = 3303, 2929, 2864, 1640, 1548, 1455, 747, 699 and 497 cm^{-1}.

CIMS (NH_3): *m/z* (%) = 220 ($[M + H]^+$, 32), 237 ($[M + NH_4]^+$, 100), 439 ($[2M + H]^+$, 20) and 456 ($[2M + NH_4]^+$, 18).

^{1}H NMR ($CDCl_3$, 300 MHz): δ (fig. 1.14) = 7.24 (m, 5 H, Ar-H), 5.48 (br.s, exchangeable with D_2O, 1 H, N-H), 3.5 (q, J = 8 Hz, 2 H, 1′-H_2), 2.8 (t, *J* = 8 Hz, 2 H, 2′-H_2), 2.1 (t, *J* = 8 Hz, 2 H, 2-H_2), 1.6 (q, *J* = 8 Hz, 2 H, 3-H_2), 1.28 (m, 4 H, 4-H_2, 5-H_2) and 0.85 (t, *J* = 8 Hz, 3 H, 6-H_3).

^{13}C/APT NMR ($CDCl_3$, 75.5 MHz): δ (fig. 1.15) = 173.3 (1C, $C_{quat.}$), 138.5 (1C, $C_{quat.}$), 128.7 (2C, CH), 128.6 (2C, CH), 126.5 (1C,CH), 40.6 (1C, CH_2), 36.7 (1C, CH_2), 35.7 (1C, CH_2), 31.4 (1C, CH_2), 26.5 (1C, CH_2), 22.4 (1C, CH_2) and 13.9 (1C, CH_3).

Uracil (28)

Fraction 8 was concentrated and the residue obtained was purified using Sephadex LH 20 to afford the colourless compound **28** (8 mg), m.p.334 °C (lit.[24] m.p. 335 °C).

EIMS (70 eV): *m/z* (%) = 112 (M^+, 84) and 69(43).

^{1}H NMR ($[D_6]$DMSO, 300 MHz): δ = 10.95 (br. s, exchangeable with D_2O, 1 H, N-H), 10.74 (s br, exchangeable with D_2O, 1 H, NH), 7.36 (d, *J* = 7.5 Hz; 1 H, 6-H) and 5.43 (d, *J* = 7.5 Hz; 1 H, 5-H).

Uridine (29)

Fraction 9 upon evaporation followed by purification provided a crystalline compound **29** (20 mg), m.p. 165 ^{0}C (lit.[24] m.p. 165-166 ^{0}C).

EIMS (70 eV): *m/z* (%) = 244.1 (M^+, 10), 226.1 (M^+ - H_2O, 20), 171 (20), 141 (24), 133 (64), 113 (100), 73 (74) and 57 (48).

^{1}HNMR ([D_6]DMSO, 300 MHz): δ = 11.24 (s, exchangeable with D_2O, 1 H, N-H), 7.84 (d, J = 8.5 Hz; 1 H, 6-H), 5.78(d, J = 7 Hz; 1 H, 1'-H), 5.64 (dd, J = 8.5 Hz, J = 3 Hz; 1 H, 5-H), 5.3 (d, exchangeable with D_2O; 1 H, OH), 5.02 (m, exchangeable with D_2O; 2 H, 2 x OH), 4.02 (t, J = 7 Hz; 1 H, 4'-H), 3.98 (t, J = 7 Hz; 1 H, 3'-H), 3.84 (m, 1 H, 2'-H) and 3.62 (m, 2 H, 5'-H_2).

^{13}C/APT-NMR ([D_6]DMSO, 75.5 MHz): δ = 163.1 (1C, $C_{quat.}$), 150.8 (1C, $C_{quat.}$), 140.7 (1C, CH), 101.8 (1C, CH), 87.7 (1C, CH), 84.8 (1C, CH), 73.6 (1C, CH), 69.9 (1C, CH) and 60.9 (1C, CH_2).

General procedure for the synthesis of N-(2'-phenylethyl)-amides [20]:

A mixture of β-phenylethylamine (25 mmol) in dichloromethane (25 ml) and 10 % aqueous sodium hydroxide (25 ml) was stirred vigorously and treated dropwise with the appropriate acid chloride (30 mmol) over a period of 25 min. After the addition, the mixture was stirred for an additional 15 min and then poured into water (40 ml). The aqueous layer was separated and the organic layer was washed with water, 5 % aqueous HCl, water and dried ($MgSO_4$). The solid residue obtained after evaporation of the solvent was recrystallised from suitable solvents to give the respective amides in pure form.

Marine *Streptomycetes* strain B 7064

Culture, Fermentation and Work-up

The marine *Streptomycetes* strain B 7064 was cultivated on agar (M_2^+ 50 % S. W. medium) in petri dishes for 3 days at 28 ^{0}C. Three liters of these broth were equally distributed in 15 1-L-Erlenmeyer flasks, then sterilised and inoculated with pieces of a well grown agar-culture. After retaining for a period of 3 days at 28 ^{0}C and 95 rpm, ten flasks with optimum culture growth were used to inoculate a 20 liter fermenter with (M_2^+ 50 %

S. W. medium), which was kept for 72 h at 28 ^{0}C. The resulting culture broth was filtered through celite. The aqueous phase as well as the mycelium and the celite used for filtration were extracted three times with ethyl acetate. The combined organic phase was collected and evaporated *in vacuo* at 30 ^{0}C to get 2.6 g of the crude extract. The resulting crude extract was dissolved in methanol and defatted with cyclohexane followed by concentration *in vacuo* to give 1.95 g of crude residue.

Isolation

The methanolic residue obtained as above was chromatographed on a flash silica gel column (65 X 2.0 cm) and eluted using a gradient solvent system consisting of $CHCl_3$: CH_3OH in the proportions (100 : 0, 1L; 99 : 1, 0.7L; 97 : 3, 0.6L; 95 :5, 0.5L; 92 :8, 0.6L; 90 :10, 1L; 88 :12, 0.8 L; 85 :15, 0.7 L; 80 :20, 0.5 L, 75 : 25, 1.5 L). Various fractions (10 ml each) were collected and the extent of separation was monitored through silica gel tlc, the visualisation of the spots was carried out either in UV light or by spraying with anisaldehyde / sulphuric acid. Identical fractions were combined to give five major fractions, which were concentrated in *vacuo* and the residue obtained from each fraction was further purified either by using PTLC or Sephadex LH 20 column to provide the following compounds.

Pyrrole-2-carboxylic acid methyl ester (30)

Fraction no. 3 after concentration and then purification by PTLC furnished **30** (12 mg), m.p 68. ^{0}C (lit.[41] m.p.72 ^{0}C).

IR (KBr): ν = 3280, 2952, 1679, 1557, 1448, 1405, 1323, 1200, 1170, 1120, 1088, 1029, 979, 748 and 606 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 125 (M^+ 88), 94 (M^+ - $COCH_3$, 100), 93 (M^+ - CH_3OH, 40) and 66 (M^+ - $COOCH_3$, 30).

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 9.16 (br s, H/D exchangeable, 1 H, -NH), 6.8 (d, J = 6 Hz, 1 H, 3-H), 6.5 (d, J = 6 Hz, 1 H, 4-H), 6.24 (dd, J = 6 Hz, 1.5 Hz, 1 H, 5-H) and 3.81 (s, 3 H, -OCH_3).

N-(2′-phenylethyl)-ethanamide (31)

The residue obtained after concentration of fraction 4 was chromatographed on a Sephadex LH 20 column to give **31** (25.9 mg) as colourless solid, m.p. 85 ^{0}C.

IR (KBr). ν = 3275, 2970, 1650, 1558, 1496, 1455, 1430, 1370, 1240, 1195, 1045, 745, 700 and 500 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 163.1 (M^+, 68), 148.1 (M^+ - CH_3, 5), 120.1 (M^+ - CH_3CO, 10), 105 (M^+ - CH_3CONH, 100) and 91 (M^+ - $CH_3CONHCH_2$, 30).

CIMS (NH_3): *m/z* (%) = 164 ([M + H^+], 28), 181 ([M + NH_4]$^+$, 100) and 198 ([M + NH_4 + NH_3], 8).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.26 (m, 5 H, Ar-H), 5.6 (br.s, H/D-exchangeable; 1 H, N-H), 3.56 (q, *J* = 8 Hz, 1 H, H-1′), 2.82 (t, *J* = 8 Hz, 2 H, H_2-2′) and 1.96 (s, 3 H, H_3-2).

^{13}C/APT NMR ([D_6]acetone, 50.5 MHz): δ = 174 (1C, $C_{quat.}$), 140.8 (1C, $C_{quat.}$), 129.5 (2C, CH), 129.1 (2C, CH), 126.9 (1C, CH), 41.3 (1C, CH_2), 36.4 (1C, CH_2) and 22.8 (1C, CH_3).

Chalcomycin (32)

Fraction no. 5 on evaporation of solvent followed by purification of the residue by PTLC and then finally purified by Sephadex LH 20 column to give Chalcomycin (**32**) (7.8 mg), m.p. 122 ^{0}C (lit.[33] m.p. 121-123 ^{0}C).

IR (KBr): ν = 3490, 2930, 1718, 1630, 1458, 1350, 1236, 1170, 1083, 982, 890 and 726 cm^{-1}.

ESIMS: *m/z* (%) = 723 ([M + Na]$^+$, 100) and 1422.7 ([2M + Na]$^+$.

^{1}H NMR ($CDCl_3$, 500 MHz): δ **(fig. 1.16)** = 6.62 (dd, *J* = 10.6 Hz, 5.3 Hz, 1 H, H-3), 6.58 (d, *J* = 3.8 Hz, 1 H, H-10), 6.52 (s, 1 H, H-11), 5.78 (d, *J* = 14.8 Hz, 1 H, H-2), 5.3 (m, 1 H, H-15), 4.57 (d, *J* = 8.2 Hz, 1 H, H-1″), 4.18 (d, *J* = 8.2 Hz, 1 H, H-1′), 4.16 (dd, *J* = 10.7 Hz, 3.3 Hz, 1 H, H-20), 3.75 (t, *J* = 3.3 Hz, 1 H, H-3″), 3.62 (dd, *J* = 10.7 Hz, 3.3 Hz, 1 H, H-20), 3.59 (s, 3 H, H_3-8″), 3.52 (s, 3 H, H_3-7″), 3.5 (m, 1 H, H-5″), 3.42 (m, 1 H, H-5′), 3.38 (s, 3 H, H_3-7′), 3.3 (m, 1 H, H-2′), 3.29 (m, 1 H, H-12), 3.18 (m, 1 H, H-5), 3.18 (m, 1 H, H-3′), 3.16 (m, 1 H, H-4″), 3.12 (dd, *J* = 8.2 Hz, 3.3 Hz, 1 H, H-13), 3.04 (dd, *J* = 8.2 Hz, 3.3 Hz, 1 H, H-2″), 2.07 (m, 1 H, H-4), 2.02 (m, 1 H, H-7), 1.9 (m, 2 H, H_2-4′), 1.37 (m, 1 H, H-14), 1.35 (s, 3 H, H_3-19), 1.3 (d, *J* = 8.2 Hz, 3 H, H_3-16),

1.22 (d, J = 8.2 Hz, 3 H, H_3-6″), 1.21 (m, 1 H, H-7), 1.2 (m, 1 H, H-6), 1.19 (d, J = 8.2 Hz, 3 H, H_3-5′), 1.17 (d, J = 8.2 Hz, 3 H, H_3-17) and 0.98 (d, J = 8.2 Hz, 3 H, H_3-18).

^{13}C/APT-NMR ($CDCl_3$, 50.3 MHz): δ (fig. 1.17) = 200.2 (C-9, $C_{quat.}$), 165.3 (C-1, $C_{quat.}$), 151.6 (C-3, CH), 146.5 (C-11, CH), 124.8 (C-10, CH), 120.7 (C-2, CH), 103.2 (C-1′, CH), 100.9 (C-1″, CH), 87.8 (C-5, CH), 81.9 (C-2 ″, CH), 80.4 (C-3′, CH), 79.6 (C-3″, CH), 78.4 (C-8, $C_{quat.}$), 75 (C-2′, CH), 72.7 (C-4″, CH), 70.7 (C-5″, CH), 68.7 (C-15, CH), 67.8 (C-5′, CH), 66.9 (C-20, CH_2), 61.8 (C-8″, OCH_3), 59.7 (C-7″, OCH_3), 58.9 (C-13, OCH), 58.7 (C-12, OCH), 56.7 (C-7′, OCH_3), 49.5 (C-14, CH), 41.7 (C-4, CH), 36.9 (C-4′, CH_2), 36.7 (C-7, CH_2), 34 (C-6, CH), 27.8 (C-19, CH_3), 20.9 (C-6′, CH_3), 19.2 (C-18, CH_3), 18.6 (C-17, CH_3), 18.3 (C-16, CH_3) and 17.8 (C-6″, CH_3).

^{1}H,^{1}H-COSY ($CDCl_3$, 300.1 MHz) (H↔H): 2-H↔3-H, 3-H↔4-H, 4-H↔5-H, 4H↔18-H, 5-H↔6-H, 5-H↔7-H, 6-H↔7-H, 10-H↔12-H, 13-H↔14-H, 14-H↔15-H, 14-H↔16-H, 15-H↔17-H, 16-H↔15-H, 17-H↔4-H, 18-H↔7-H, 18-H↔6-H, 20-H↔14-H, 1′-H↔2′-H, 2′-H↔3′-H, 3′-H↔4′-H, 3′-H↔2′-H, 4′-H↔5′-H, 5′-H↔6′-H, 1″-H↔2′′-H, 2″-H↔3″-H, 3″-H↔4″-H, 3″-H↔2″-H, 4″-H↔5″-H, 4″-H↔6″-H, 5″-H↔6″-H, and 6″-H↔3″-H.

HMQC (inverse CH-COSY, $CDCl_3$, INVBTP, F1 75.5 MHz, F2 300.1 MHz) (H →C): 2-H → C-2; 3-H →C-3; 4-H →C-4; 5-H →C-5; 6-H →C-6; 7-H →C-7; 10-H → C-10; 11-H →C-11; 12-H →C-12; 13-H →C-13; 14-H →C-14; 15-H →C-15; 16-H →C-16; 17-H → C-17; 18-H →C-18; 19-H →C-19; 20-H →C-20; 1′-H →C-1′; 2′-H → C-2′; 3′-H →C-3′; 4′-H →C-4′; 5′-H →C-5′; 6′-H →C-6′; 7′-H →C-7′; 1″-H → C-1″; 2″-H → C-2″; 3″-H →C-3″; 4″-H →C-4″; 5″-H →C-5″; 6″-H →C-6″; 7″-H →C-7″ and 8″-H →C-8″.

Chalcomycin B (33)

Fraction no. 5 after concentration and purification by PTLC and then by Sephadex LH 20 gave Chalcomycin B (**33**) (7.8 mg) as crystalline solid, m.p. 98 - 100 °C.

IR (KBr): ν (fig. 1.18) = 3445, 2935, 1740, 1730, 1640, 1634, 1459, 1355, 1178, 1080, 1040, 1010, 980, 850 and 804 cm^{-1}.

$[\alpha]^{20}_{D}$ = - 0.1° (c 0.1095, MeOH)

EIMS (70 eV): *m/z* (%) (fig. 1.19) = 812.1 (M^+, 5), 595 (M^+ 8), 565.1 (12), 231.1 (48), 201.1 (86) and 169 (100).

(+)-ESIMS: *m/z* (%) (fig. 1.20) = 1647 ([2M + Na]$^+$, 100) and 835.5 ([M + Na]$^+$, 67); – (+)-ESI-Daughter-ion-MS (from 836): *m/z* (%) = 836.4 ([M + H]$^+$, 65), 761.4 (80) and 605.4 (100); – (+)-ESI-Daughter-ion-MS (from 605.4): *m/z* (%) = 605.4 (M^+, 20), 587.3 (100), 575.3 (45); – (+)-ESI-Daughter-ion-MS (from 761.4): *m/z* (%) = 761.4 (M^+, 10), 729.3 (50), 687.4 (80), 617.3 (100) and 531.3 (90).

^{1}H NMR ($CDCl_3$, 500 MHz): δ (fig. 1.21) = 6.568 (dd, *J* = 10.6 Hz, 5.3 Hz, 1 H, H-3), 6.503 (d, *J* = 3.8 Hz, 1 H, H-10), 6.492 (s, 1 H, H-11), 5.716 (d, *J* = 14.8 Hz, 1 H, H-2), 5.272 (m, 1 H, H-15), 4.78 (t, *J* = 8.2 Hz, 1 H, H-2′), 4.57 (d, *J* = 8.2 Hz, 1 H, H-1‴), 4.4 (dd, *J* = 9.8 Hz, 2.5 Hz, 1 H, H-4‴), 4.22 (d, *J* = 8.2 Hz, 1 H, H-1′), 4.12 (dd, *J* = 11.5 Hz, 3.3 Hz, 1 H, H-20), 3.88 (m, 1 H, H-5‴), 3.85 (m, 1 H, H-3‴), 3.61 (dd, *J* = 11.5 Hz, 3.3 Hz, 1 H, H-20), 3.48 (s, 3 H, H_3-7‴), 3.46 (s, 3 H, H_3-8‴), 3.42 (m, 1 H, H-5′), 3.265 (m, 1 H, H-3′), 3.25 (s, 3 H, H_3-7′), 3.24 (dd, *J* = 5.9 Hz, 1.65 Hz, 1 H, H-12), 3.085 (dd, *J* = 9.2 Hz, 2 Hz, 1 H, H-13), 3.045 (d, *J* = 9.9 Hz, 1 H, H-5), 3.025 (dd, *J* = 7.9 Hz, 3 Hz, 1 H, H-2‴), 2.51 (m, 1 H, H-4), 2.32 (m, 4 H, H_2-3″, H_2-3‴), 2.04 (m, 1 H, H-4′), 1.8, 1.9 (dd, *J* = 14.9 Hz, 3.3 Hz, 2 H_2, H-7), 1.34 (m, 1 H, H-14), 1.31 (s, 3 H, H_3-19), 1.27 (d, *J* = 8.2 Hz, 3 H, H_3-16), 1.19 (d, *J* = 8.2 Hz, 3 H, H_3-5′),1.13 (m, 1 H, H-6), 1.12 (d, *J* = 8.2 Hz, 3 H, H_3-6‴), 1.1 (d, *J* = 8.2 Hz, 3 H, H_3-3″), 1.08 (d, *J* = 8.2 Hz, 3 H, H_3-3‴), 1.02 (d, *J* = 8.2 Hz, 3 H, H_3- 17) and 0.94 (d, *J* = 8.2 Hz, 3 H, H_3-18).

^{13}C/APT NMR ($CDCl_3$, 125.5 MHz); δ (fig. 1.22) = 200.4 (C-9, $C_{quat.}$), 173.6 (C-1‴″, $C_{quat.}$), 173.2 (C-1″, $C_{quat.}$), 165.3 (C-1, $C_{quat.}$), 151.3 (C-3, CH), 146.3 (C-11, CH), 125 (C-10, CH), 120.9 (C-2, CH), 101.6 (C-1′, CH), 100.8 (C-1‴, CH), 88.5 (C-5, CH), 80.6 (C-2‴, CH), 78.8 (C-3′, CH), 78.4 (C-8, $C_{quat.}$), 77.6 (C-3‴, CH), 74.6 (C-4‴, CH), 74.2 (C-2′, CH), 68.8 (C-15, CH), 67.7 (C-5′, CH), 67.4 (C-5‴, CH), 67.1 (C-20, CH_2), 61.6 (C-8‴, OCH_3), 59.4 (C-7‴, OCH_3), 59 (C-13, OCH), 58.7 (C-12, OCH), 56.5 (C-7′, OCH_3), 49.4 (C-14, CH), 41.2 (C-4, CH), 37.1 (C-7, C-4′, CH_2), 34 (C-6, CH), 27.7 (C-2″, C-2‴″, CH_2), 20.8 (C-6′, CH_3), 19.1 (C-18, CH_3), 18.3 (C-16, CH_3), 18.2 (C-17, CH_3), 17.4 (C-6‴″, CH_3), 9.2 (C-3″, CH_3) and 9.1 (C-3‴″, CH_3).

^{3}J →C-1‴; 4‴-H ^{2}J →C-3‴; 4‴-H ^{2}J →C-5‴; 4‴-H ^{3}J →C-1⁗; 5‴-H ^{3}J →C-3‴; 6‴ -H ^{2}J →C-5‴; 7‴-H ^{3}J →C-2‴; 8‴-H ^{2}J →C-3‴; 2⁗-H ^{2}J →C-1⁗; 2⁗-H ^{2}J →C-3⁗ and 3⁗-H ^{2}J →C-2⁗.

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CHAPTER 2

SECONDARY METABOLITES FROM MARINE ORGANISMS

INTRODUCTION

A number of medicinal and bio-chemical agents in use today are either the natural products themselves or their active derivatives and the modified templates. Over the years, the natural product chemistry has experienced a dramatic development due to the high degree of technological advancement. The advent of sophisticated chromatographic (HPLC, gel filtation etc) and spectroscopic methods, like high field PMR, CMR and mass spectrometeric techniques coupled with molecular force field calculations and X-ray crystallography etc, have markedly enhanced our ability in the isolation and structure elucidation of complex and diverse natural products. The robotic advances that have taken place in the bioassay guided separation and the rapid & precise evaluation of the biological activity of crude mixture as well as the purified compounds from natural sources have helped to gather an increased number of new medicinal compounds, insecticides, herbicides and other biological agents[1]

The marine environment is the abode for about 5,00,000 species of living organisms belonging to approximately 30 phyla[2]. Among the marine flora, the microorganisms (bacteria, fungi), phytoplanktons, algae (blue-green, brown, red and green), sponges, coelenterates (soft corals), gorgonians, sea anemones, tunicates and echinoderms (sea stars and sea cucumbers) have served as the attractive targets for detailed chemical examination[3]. The marine organisms are considered as a renewable source for novel organic compounds exhibiting a variety of biological activities.

Early investigation on natural product chemistry was largely restricted to the terrestrial plants. However, gradually over the years, the focus has been shifted towards the studies of natural products- the secondary, non-primary metabolites produced by orgainisms that live in sea, specially for the treatment of human diseases. Thus, the explorations in the area of marine natural products have likewise undergone an evolution over the past three and half decades; begining with the early investigations on toxins, followed by studies in cytotoxicity, anti-tumor, etc, to the present day anti-AIDS drugs wherein a myriad of activities based on whole animal model and receptor binding assay are being pursued[4]. Besides, several compounds with useful pharmacological properties have been isolated from various marine plants and animals during the past three and half decades, and quite a few among them have been exploited as useful

drugs[5]. This includes compounds such as tetrodotoxin, tedanolide, saxitoxin, halitoxin, kainic acid, cephalosporin C, Didemin B, Bryostastin etc. The list is thus exhaustive and is ever growing as vouched by the plethora of publications appearing on this topic lately[6].

A look at the literature revealed that sponges, algae and coelenterates are the most widely studied animal species of marine origin. A steady number of marine natural products are being targeted for synthesis as well as for in-depth investigations of their biological properties, both from the ecological (biofouling) and biomedicinal viewpoints[7]. Thus, the marine natural product chemistry continues to be rigorously pursued with futher room to expand its influence. Keeping in view the relative importance of marine organisms[8], the principal focus of the present study was laid at the isolation and structure elucidation of novel bioactive molecules from marine organisms found in Indian seas.

Studies on organisms of Indian seas

The Indian region (Arabian sea, Gulf of Mannar, Lakshadweep islands, Andaman and Nicobar islands and the Bay of Bengal) accounts for a wide spread biodiversity and is rich in marine flora and fauna. Since marine organisms are known for exhibiting extensive variations in their chemical compositions depending on the region of their occurrence, the present study was undertaken as a part of the research programme on the isolation of bioactive natural products from the marine organisms pertaining to the Indian ocean region at the Chemical Oceanography Division, National Institute of Oceanography, Dona Paula Goa. The first section of this chapter describes the isolation and structure elucidation of various secondary metabolites obtained from the soft coral *Sinularia capillosa*. Similarly, the second section deals with the secondary metabolites isolated from the green seaweed *Cladophora prolifera* collected from Anjuna beach, Goa, India.

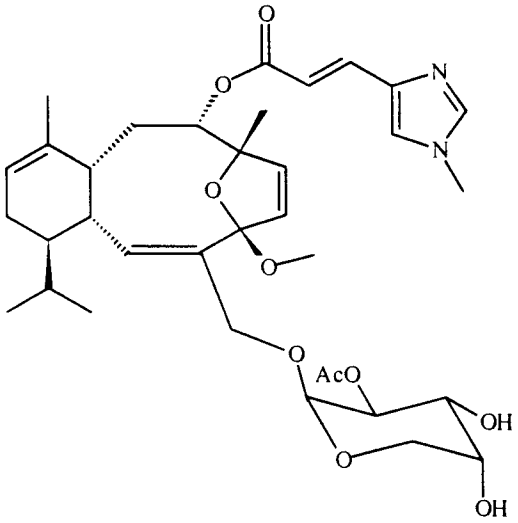
SECTION I

SECONDARY METABOILTES FROM THE SOFT CORAL *SINULARIA CAPILLOSA*

SOFT CORALS

The phylum Cnidaria consists of a very large group of aquatic animals, most of them being marine in habitat. They are acoelomate metazoans, diploblastic and often with radical symmetry. Cnidaria have no anus, their internal cavity opens into a mouth often surrounded by tentacles bearing specialized stinging organs. An amazing variety of fixed, free swimming, solitary or colonial forms, ranging from very large animals to microscopic orgainsms are found in the phylum Cnidaria.

The order Alcyonacea has been the subject of extensive chemical studies during the past three and half decades[9]. The results of these studies have shown that a wide array of structurally distinct cembranoids are found within the order Alcyonacea[10]. One of the important discoveries reported in the recent years is that of eleutherobin (**1**), a cytotoxin, which stabilizes the microtubules like the way Taxol[11,12] does it. Eleutherobin (**1**) was isolated from a western Australian *Eleutherobia*[13] sp. The synthesis of eleutherobin (**1**) and the related compound sarcodictyin A (**2**), which is a cytotoxin from the Mediterranean stoloniferan coral *Sarcophyton roseum*[14] sp. were reported soon after their isolation in view of their immense potential as pharmaceuticals[15,16]. Similarly, the *Eleutherobia aurea* sp. form South Africa contained two additional xenicane diterpences[17] namely, 7,8-epoxyzahavin A (**3**), which inhibited superoxide production in rabbit-cell neutrophils, and xeniolide C (**4**). Likewise, the Indonesian soft coral *Nephthea chabrolii* was reported to provide hydroxycolorenone (**5**) and methoxycolorenone (**6**), which are insecticidal towards the larvae of the pest *Spodoptera littoralis*[18].



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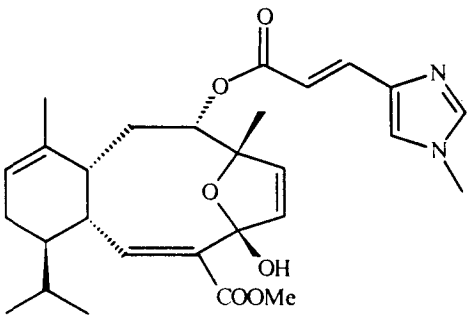

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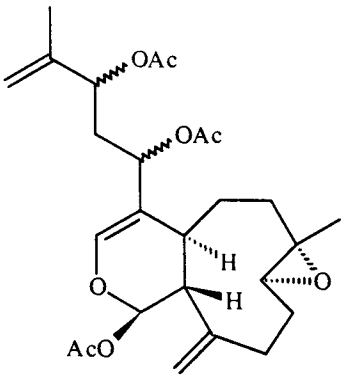

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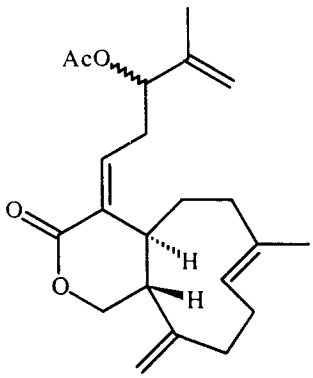

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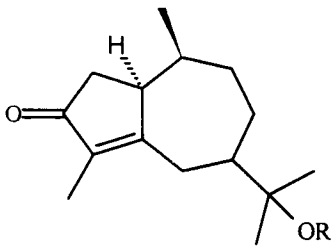
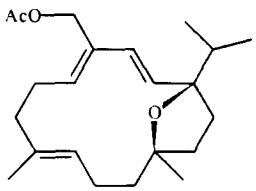


5: R = H,

6: R = Me

Likewise the Lobophynins A-C (**7-9**), of which only lobophynin C (**9**) causes ichthyotoxicity and brine shrimp lethality, are the three new cembranoids isolated from the Japanese soft coral *Lobophytum schoedei*[19]. Similarly, six new polyhydroxylated steroids 10-15 have been isolated from the soft coral *Pieterfaurea unilobata*[20]. The structures of preclavulones 1 (**16**) and 2 (**17**), which are the new prostanoids isolated from the soft coral *Clavularia viridis* from Okinawa, were confirmed by their total syntheses from (S)-malic acid[19]. The first total syntheses of 1,2-dehydronephthenol (**18**), which is a simple cembranoid alcohol from *Lobophytum pauciflorum*[22], and (-)-

trans-cembranolide (**19**), which is a metabolite of *Sinularia mayi*[23], have also been reported[24,25].



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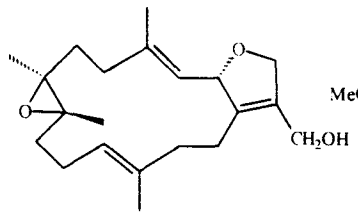

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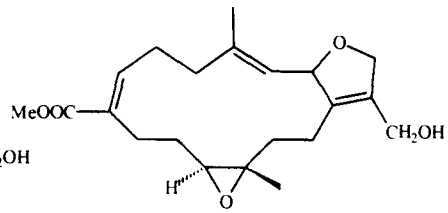

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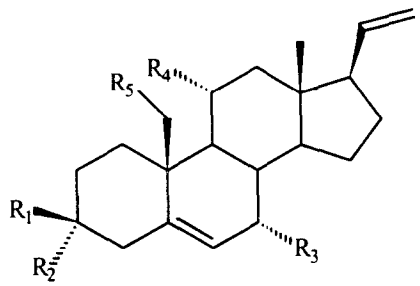


10: $R_1 = R_4 = R_5$ =H, R_2 = OAc, R_3 = OH

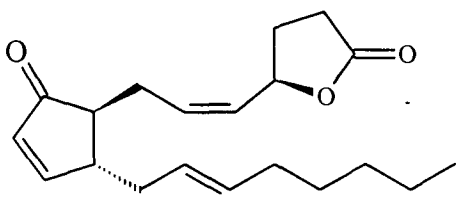
11: R_1 = OH, $R_2 = R_4 = R_5$ = H, R_3 = OAc

12: $R_1 = R_5$ = H, R_2 = OAc, $R_3 = R_4$ = OH

13: $R_1 = R_5$ = H, $R_2 = R_3$ = OAc, R_4 = OH

14: $R_1 = R_4$ =H, $R_2 = R_5$ = OAc, R_3 = OH

15: R_1 = H, $R_2 = R_3 = R_5$ = OAc, R_4 = OH



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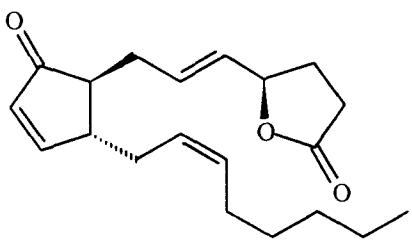

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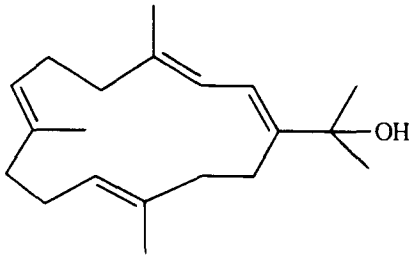

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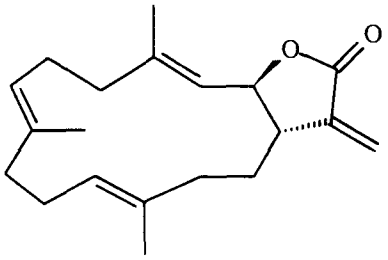
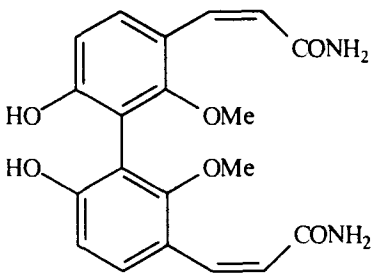


19

***Sinularia* species**

The taxonomic identification of the genus *Sinularia* is relatively straightforward. In this genus, there are almost 90 species on which a very good taxonomic literature is available[26] thereby permitting their easy identification. Till today, 33 species of this genus have been chemically examined[27]. The chemistry of this genus is quite rich and varied. Many of the *Sinularia* species examined contain bioactive and toxic molecules. For example, an unusual cinnamide dimer (**20**) was isolated as a minor metabolite of the Indian Ocean soft coral *Sinularia flexibilis*[28]. Similarly, the same sinularia species has been reported to contain sandensolide monoacetate (**21**) and flexibolide (**22**) together with other known metabolites[29]. In addition, four rare aromadendrane diterpenoids (**23 - 25**) were also isolated from an Indian Ocean soft coral of the genus *Sinularia*[30].



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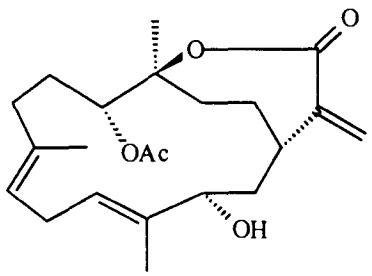

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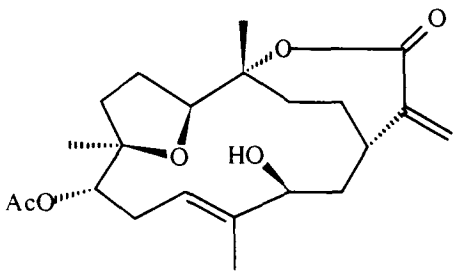

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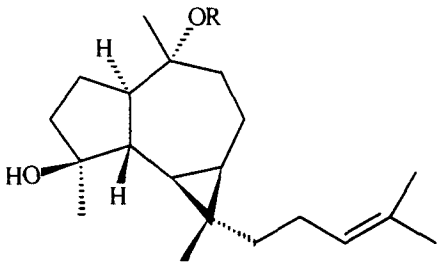
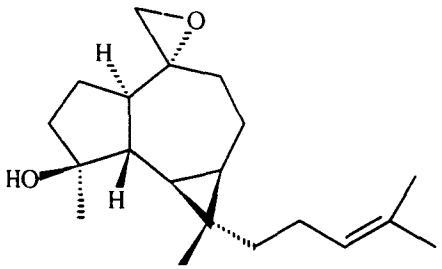


23: R = H ; 24: R = Me



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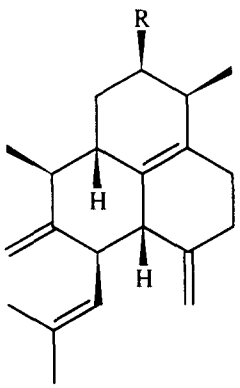
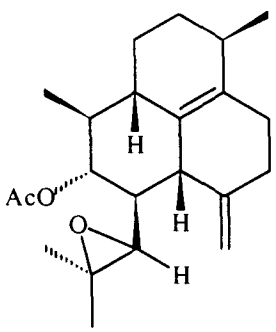
Another variety *viz. Sinularia nanolabata* from Japan was found to contain the cytotoxic amphilectane-type diterpenes namely sinulobatins[31] A-D (**26 - 29**). Similarly, the acylated spermidine derivative (**30**) and other related compounds were obtained from the cytotoxic constituents of a soft coral, *Sinularia compacya* collected from Nauru[32], and also the new furanosesquiterpene (**31**) along with twelve related derivatives have been reported from the Australian soft coral *Sinularia capillosa*[33]. The literature pertaining to the chemical examination of *Sinularia* species has been excellently reviewed[34].



26: R = OAc , 27: R = H



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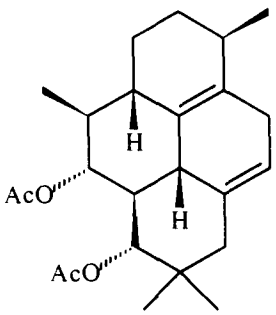

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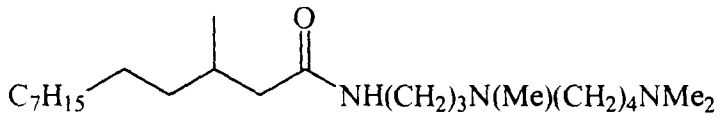

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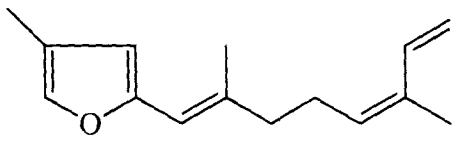


31

PRESENT STUDY

CHEMICAL EXAMINATION OF THE SOFT CORAL *SINULARIA CAPILLOSA*

The above cited literature reports pertaining to the soft coral *Sinularia* species represents only a sample survey relating to the isolation of bio-active metabolites. By taking into account, the rich structural diversity and the interesting pharmacological properties exhibited by the metabolites, it was our interest to subject the soft coral *Sinularia capillosa* collected from Okha coast, Gujarat for a detail chemical examination during the present study. The results obtained during this exercise are described in this section.

Systematic description of the soft coral:

Phylum : Cnidaria(Coelenterate)

Class : Anthozoa

Order : Alcyonacea

Family : Alcyoniidae

Genus : *Sinularia*

Species : *capillosa*

The soft coral *Sinularia capillosa*, grows to a reasonable biomass on rocky substratum with mat like configuration.

Occurrence : *Sinularia* sp. is prominent in Indo-Pacific region and occurs quite widely in Indian waters. In the present study collection were made from the Okha coast (Gujarat state) during the month of October 1993.

Extraction :

The organisms after collection were immediately washed with fresh water, made into thin slices and preserved in excess of methanol until workup. Just before work-up, the aqueous methanol was decanted and the sliced coral material was reextracted with fresh methanol.The concentrated methanol extract was partitioned with ethyl acetate. The residue obtained from the ethyl acetate extract was subjected to silica gel column chromatography and eluted with various proportions of petrolum ether-ethyl acetate mixture to afford different fractions.

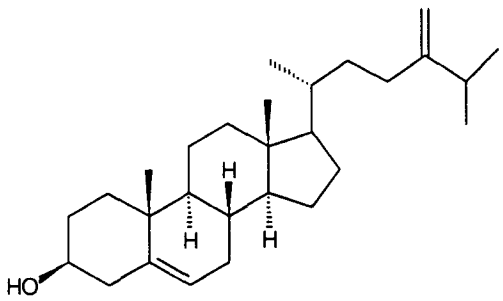
Separation & identification of the metabolites:

The initial two fractions on concentration provided a semi-solid and an oily compound which were further purified and identified as hexadecanoic acid (**32**) and octadecanoic acid (**33**) respectively.

$CH_3(CH_2)_{14}COOH$ $CH_3(CH_2)_{16}COOH$

32 **33**

Similarly, the subsequent two fractions on concentration followed by purification provided two known steroids namely, 24-methylene cholesterol[35] (**34**) & cholesterol (**35**).



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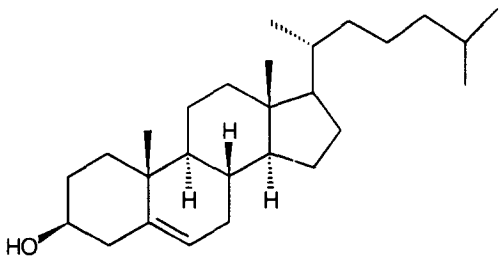
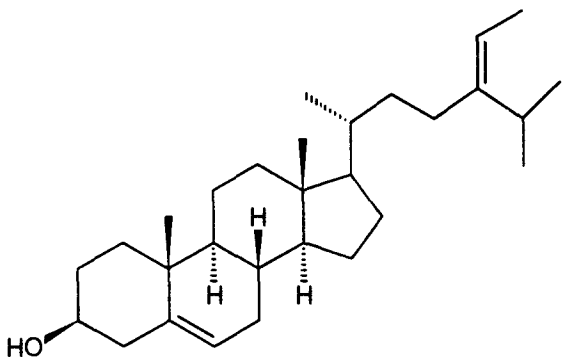


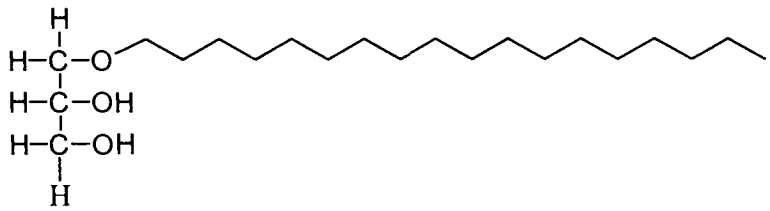
35

The next fraction collected on evaporation of the solvent furnished a semi-solid residue which on purification by HPLC gave a colourless solid (25 mg), m.p.201 ^{0}C. The IR spectrum of this solid showed strong absorption band at 3400 indicating the presence of hydroxyl group in the molecule. The molecular weight of the compound was found to be 414 by EIMS, which corresponds to a molecular formula $C_{29}H_{48}O$. The steroidal nature of the isolated compound was demonstrated by its typical mass spectral fragmentation pattern which showed prominent peaks at m/z 397 (M^{+} - 15), 379 (M - 33)$^{+}$, 314 (M - C_7H_{14})$^{+}$, 273 (M - Side chain)$^{+}$ and 255 (273 - 18). In conformity with above, the ^{1}H NMR spectrum also revealed the presence of 3-hydroxy-5-ene system in addition to the usual angular methyl group signals at δ 0.69 and 1.01 (s, 3 H, each) which could be assigned to C-18 and C-19 methyls respectively. Further, the presence of the ethylidene group in the side chain at C-24 was indicated by the signals at δ 5.18 (q, 1 H, J = 6.6 Hz) and 1.57 (d, 3 H, J = 6.6 Hz) which was further supported by the chemical shift values of C-26 proton and C-27 proton as well as C-21 proton, observed at δ 0.975 (6 H, d, J = 6.6 Hz) and 0.985 (3 H, d, J = 5.4 Hz) respectively. On the basis of above described spectral data, it was concluded that the crystalline compound which was isolated with m.p.201^{0}C is fucosterol (**36**), which has also been previously reported from a soft coral *Sinularia sipalosa*[36].



36

The subsequent fraction which was collected as described earlier, on concentration of the solvent gave a light yellow coloured residue, which was further purified to give 69 mg of a colourless solid. On the basis of spectral analysis, it was identifed as the octadecyl glycerol (batyl alcohol)(**37**), which has also been reported from the soft coral *Sinularia conferta*[37].



37

The last fraction which was collected with 100 % ethyl acetate as the eluent on evaporation of the solvent gave a solid residue, which was purified further by silica gel column chromatography using petroleum ether : ethyl acetate (1 : 3) as the solvent system to give a colourless solid, crystallised from chloroform-methanol as colourless flakes (34 mg), with melting point 88 ^{0}C. The IR spectrum of this solid compound showed strong absorption bands at 3360 and 1065 cm^{-1}, to indicate the presence of a glycosidic moiety[38] in the molecule. The ESI mass spectrum showed a peak at m/z 947 (2M + Na). The ^{1}H NMR spectrum (fig. 2.01) of the said solid compound gave evidences in favour of a sugar derivative with the presence of a anomeric proton signal appearing at δ 5.4, and other three oxymethine protons at δ 4.6, 4.5 and 4.34. In addition, the signal for two oxymethylene protons at δ 4.03 & 4.24 and 4.2 & 3.95 were also observed. Further, the signals at δ 0.86 and 1.28 indicated the presence of a straight aliphatic side chain. The ^{13}C NMR spectrum (fig. 2.02) showed nine oxygenated carbon resonances, in the form of five doublets at δ 101.4, 70.9, 70.7, 70.2 and 69.9 and four triplets at δ 64.3, 71.1, 71.8 and 73.4. The presence of an arabinose moiety was confirmed by the signals at δ 69.9, 70.2, 70.7, 70.9 and 101.4. Thus, the observed aliphatic methylene signals and the other four oxygen bearing carbon signals indicated that the said crystalline compound could be a disubstituted glycerol derivative. To derive further information, the said compound was subjected to acetylation reaction.

On acetylation, it formed a tetra-acetate, the ESI spectrum (fig. 2.04) of which showed a peak at m/z 1283 (2M + Na) corresponding to the molecular formula $C_{33}H_{58}O_{11}$. The formation of tetra-acetyl derivative indicated that the parent molecule has four hydroxyl groups. The IR spectrum (fig. 2.03) showed bands at 3350, 1750 and 1650 cm-1, in addition to the other bands. The acetyl derivative so formed was easily soluble in chloroform compared to the parent compound. The ^{1}H NMR spectrum (fig.

2.05) of the acetate displayed a doublet at δ 5.3 (1 H, J = 3.0 Hz) for an anomeric proton. The coupling constant of (3.0 Hz) observed in case of the anomeric proton confirmed that the adjacent proton on the carbon has axial orientation embodying a cyclic system with restricted rotation. In the case of ^{13}C NMR spectrum (fig. 2.06), the signal due to anomeric carbon after acetylation was shifted upfield at δ 96.7. The EIMS of the acetyl derivative did not register the molecular ion but the fragment ion at m/z 371 (M^+ - $C_{11}H_{15}O_7$) as the base peak, confirmed the presence of a triacetyl arabinose moiety in the molecule.

In the CH-COSY spectra (fig. 2.08) of the acetyl compound, it was observed that the three signals which appeared at δ 3.51, 5.08 and 3.57/3.65 were directly connected to the oxygen atom. The signals at δ 3.57/3.65 and 3.51 showed a germinal coupling (J = 11 Hz) which could be due to the restricted molecular rotation as anticipated earlier. In the ^1H-^1H COSY spectrum (fig. 2.07), the signal at δ 5.08 (for CH-OAc) coupled with the signal at δ 3.57/3.65 and 3.51. Similarly, the signal at δ 3.38 (due to CH_2O) coupled with the aliphatic chain part (δ 3.38 (m)→ 1.34(m) → 1.22(m) → 0.87 (t). Thus, with the help of the said information, the presence of -O-CH_2-(CHAc)-CH_2-O- fragment in the tetra acetyl derivative could be confirmed (as shown in the fig. 1a).

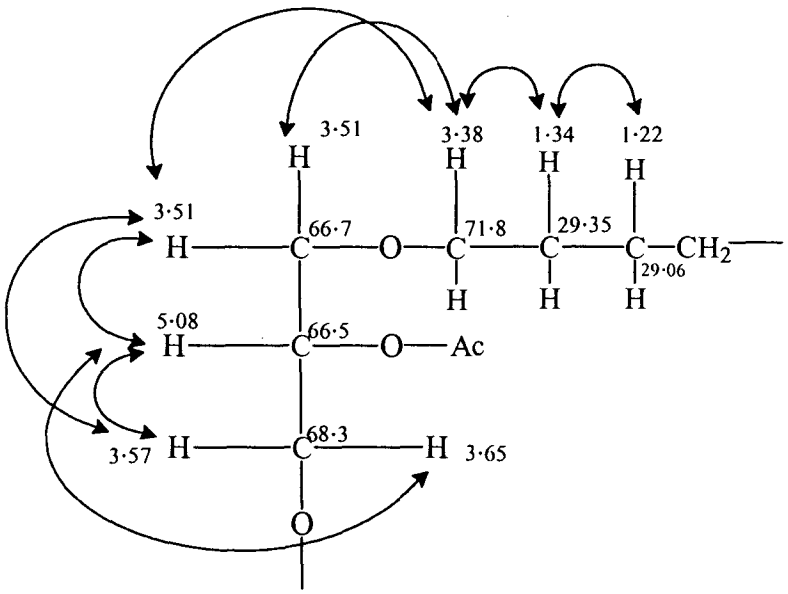


fig 1a

Similarly, it was also observed that the signal at δ 5.05 coupled with the signals at δ 5.12, 5.27 and 5.3, while the signal at δ 5.12 coupled with the signals at δ 5.27 and 5.3. Similarly, the signal at δ 5.27 coupled with the signals at δ 3.88, 5.05 and 5.12 as shown in the fig 1b.

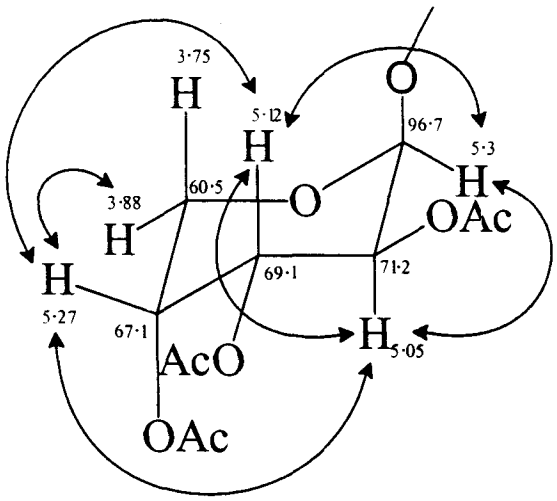
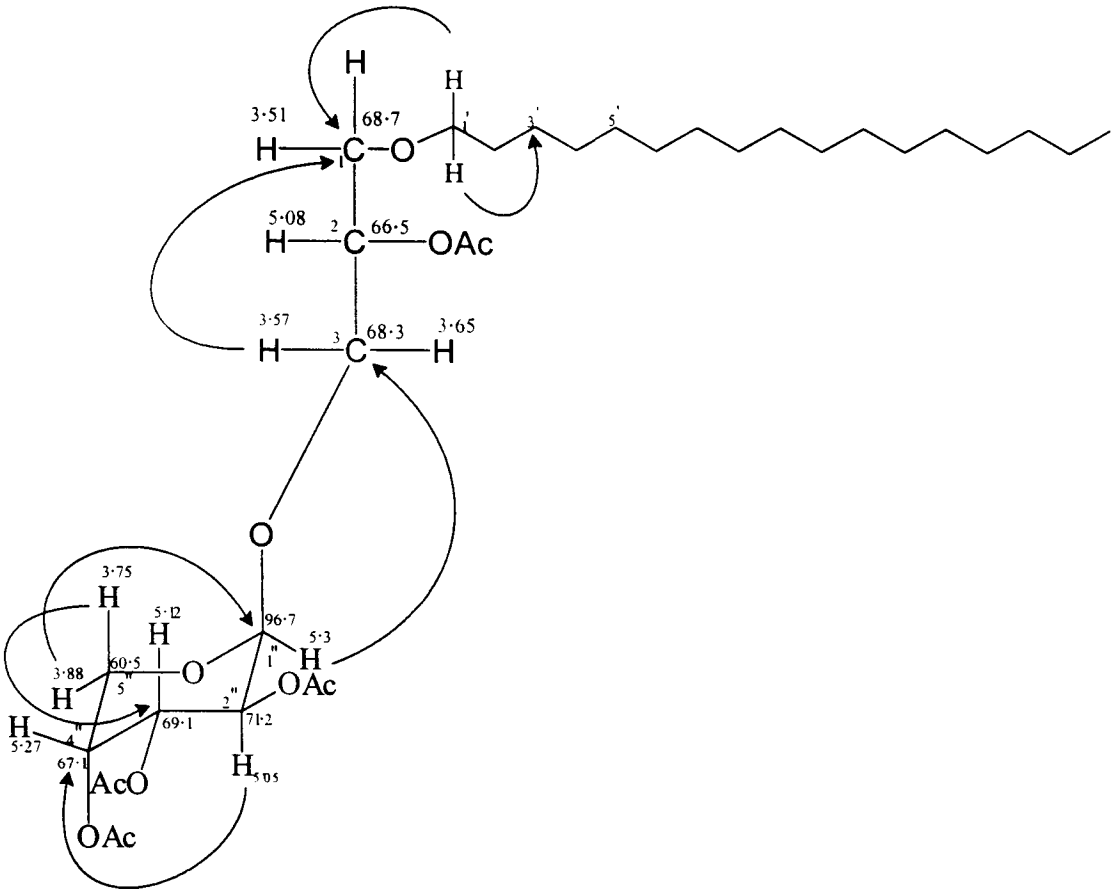


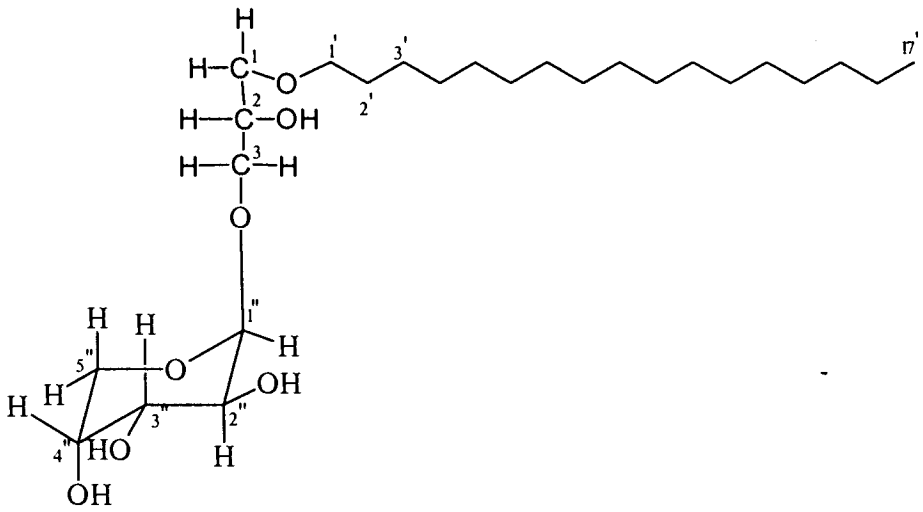
Fig 1b

In the long range coupling (fig. 3.11), the signal at δ 3.38 correlates through three bond coupling with the glycerol carbon at δ 68.7 and also with carbon at δ 25.06. Similarly, the signal at δ 5.3 correlated through three bond coupling with carbon signal at δ 68.3 while the signal at δ 3.57/3.65 correlates through three bond coupling with the carbon at δ 68.7. The other long range couplings observed for the sugar moiety are shown in **39** below.



39

The configuration at the anomeric carbon in the parent compound (**38**) was considered as β, since β anomeric carbon[39] usually show a carbon resonance signal above δ 100 as the observed value was in the present case 101.4. On the basis of above, it is concluded that the tetraacetyl derivative which is formed by the acetylation of the parent arabinoside could be assigned the structure (**39**). Consequently, the parent glycoside is represented as 2-hydroxy-3-(heptadecyloxy)-propyl-β-D-arabinopyranoside (**38**). It may be noted that (**38**) is a new glycoside reported for the first time as a metabolite from the *Sinularia* species. Unfortunately, the compound (**38**) did not exhibit any bioactivity.



38

Thus, the chemical examination of the soft coral *Sinularia capillosa* collected from the Okha coast, in Gujarat state provided two known acids, namely the hexadecanoic acid and octadecanoic acid together with three sterols namely fucosterol, 24-methylene cholesterol and cholesterol besides the known octadecyl glycerol. In addition, the crystalline compound obtained in the ethyl acetate extract of the organism was identified as a new glycoside, namely, 2-hydroxy-3-(heptadecyloxy)-propyl-β-D-arabinopyranoside (**38**) on the basis of a detailed spectral analysis and further confirmed by the preparation of the corresponding tetra acetyl derivative.

SECTION II

SECONDARY METABOLITES FROM THE MARINE GREEN ALGA *CLADOPHORA PROLIFIERA*

ALGAE

Systematic description of algae

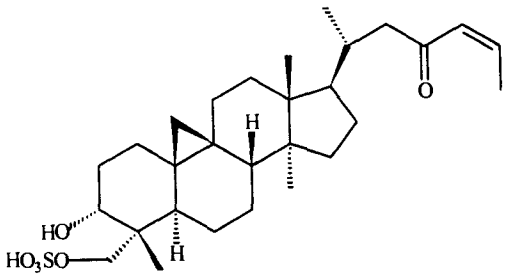
The Algae includes all chlorophyll bearing and oxygen evolving photosynthetic thalloid organisms having no true roots, stems and leaves or leaf-like organisms except bryophytes and vascular plants. Many colourless organisms are also referable to algae on the basis of their similarity to photosynthetic forms with respect to structure, life history and the nature of the cell wall and storage products[40]. In size and complexity, the algae range from single cells having 0.5 microns in diameter and to multicellular structure of ten meters in length. Algae requires an aquatic environment and they can inhabit practically all habitable environments from desert to polar snows. Algae make up for most of the world biomass and therefore are truly global in nature and importance. There are roughly about 30,000 known algae species, but the actual number of existing species[41] may probably exceed this number. The alga are classified into following seven groups[42].

Classification of Algae

Group	*Class*
1) Chlorophyta	Chlorophyceae
2) Euglenophyta	Euglenophyceae
3) Chrysophyta	Xanthophyceae
	Chrysophyceae
	Bacillariophyceae
4) Pyrrophyta	Desmophyceae
	Dinophyceae
	Cryptophycrae
5) Phaeophyta	Phaeophyceae
6) Cyanophyta	Cyanophyceae
7) Rhodophyta	Rhodophyceae

The natural substances from marine sources and especially from the seaweeds have been used in folk medicine since ancient times. Over the times, the development of modern chemical, pharmacological and engineering technology has facilitated the

investigation and exploitation of hitherto untapped drug resources from the oceans[43]. During the last four decades, several bioactive compounds have been isolated from marine sources including the sea weeds; thus accounting for a wide spectrum of metabolites, *viz* amino acids, lipids, organic acids, carbohydrates, proteins, peptides, steroids etc. To, cite few examples, specially concerned with sea weeds, a new cycloartenol sulfate (**40**), which is an inhibitor of VZV and CMV proteases was isolated from a green algae *Tuemoya* sp[44]. Similarly, the Sulfonoquinovosyl dipalmitoyl glyceride (**41**), which is a P-selectrin receptor inhibitor was isolated from the green algae *Dictyochloris* fragrans[45]. Likewise, a new ceramide sertularamide (**42**) has been reported from *Caulerpa sertularioides*[46], while α-1-glyceryl-D-mannoside-4-ammonium salt (**43**) was isolated from the green algae *Cologlossa lepieurii*[47], which is used in traditional Chinese medicine as an anthelmintic. Similarly, a new bisindole alkaloid, caulersin (**44**) has been isolated from the *Caulerpa serrulata*[48].



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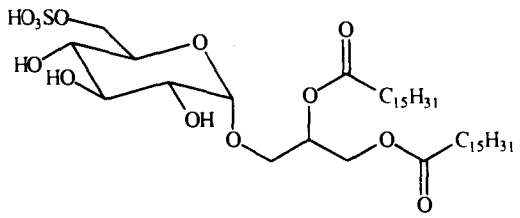

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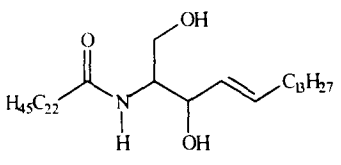

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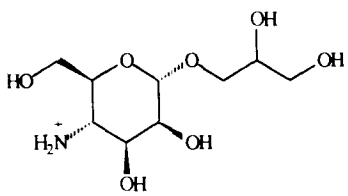

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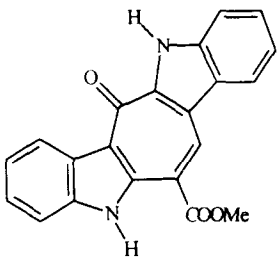
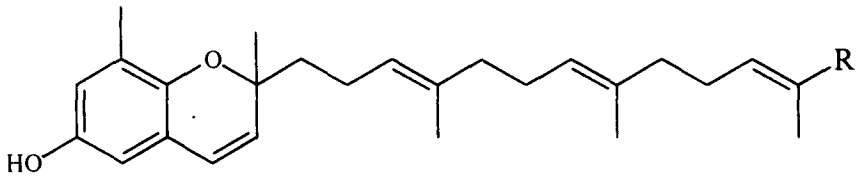


44

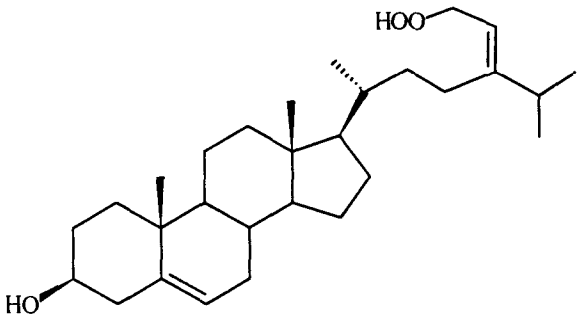
Further, the chromenols (**45**) and (**46**) have been isolated along with the known meroditerpenoids from *Desmarestia menziesii*[49] collected from King George Island in

the Antarctic. Similarly, the brown algae *Turbinaria ornata*[50] was reported to contain the new sterol hydroperoxide, 29-hydroperoxystigmasta-5,24(28)-dien-3β-ol (**47**). The major arsenic compound in the edible red alga *Chondria crassicaulis* (yuna) was found to be 1-deoxy-1-dimethylarsinoylribitol-5-sulfate (**48**), which has been synthesized from ribitol[51]. The cytotoxic sterols **49 - 52** have also been reported from another variety of red alga *Galaxaura marginata*[52]. Thus, the various marine algal species act as a renewable source of many interesting and useful organic metabolites.



45 R = -CH_2OH

46 R = -COOH



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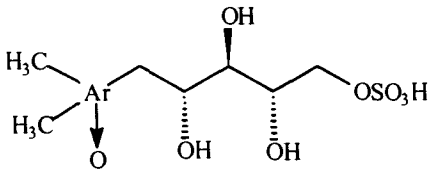

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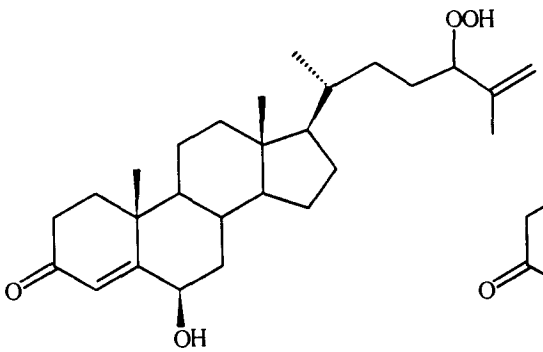

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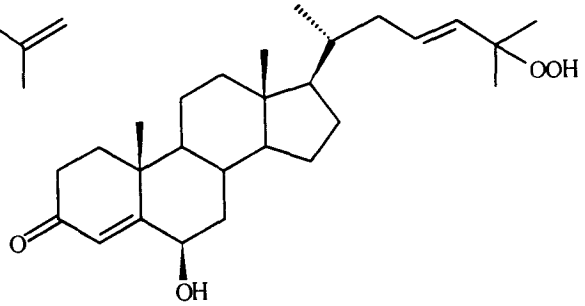

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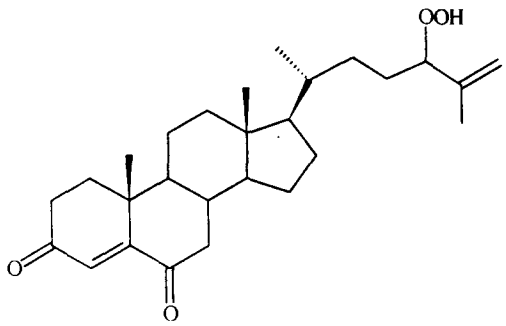

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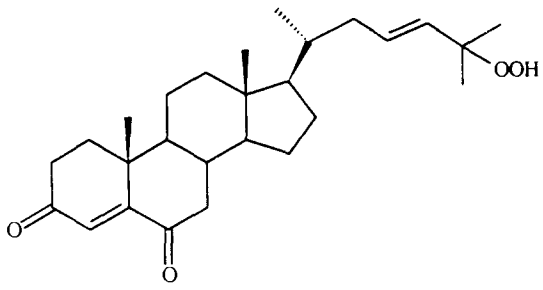


52

PRESENT STUDY

CHEMICAL EXAMINATION OF A GREEN SEAWEED *CLADOPHORA PROLIFIERA*

Systematic description:

Phylum :Euglenophyta

Class :Euglenophyceae

Family :Cladophoraceae

Genus :*Cladophora*

Species :*Prolifiera*

During the last thirty five years, a great deal of chemical work has been carried out on Indian seaweeds[53]. However, this work was confined mostly to the mineral constituents, carbohydrates with special reference to alginates, carrageinin, agar and other organic chemicals[54]. As stated earlier, in connection with a research project aimed at the isolation and structure elucidation of bioactive compounds from marine sources, we had an opportunity to under take a detail chemical investigation of the green alga *Cladophora prolifiera* belonging to the family *Cladophoraceae*. Though, there have been reports[55 - 58] pertaining to the isolation of several steroidal derivatives from few marine algal species, the *Cladophora prolifiera*, belonging to the family Cladophoraceae, is hitherto remained mostly unexamined for its chemical constituents and hence the present work was initiated. The results obtained during the present study is described in this section.

Occurrence : *Cladophora* sp is generally found in tropical and subtropical oceans. It was collected from the intertidal zone of Anjuna beach, (Goa, west coast of India) during the pre-monsoon period in 1997.

Extraction :The algae after collection was washed with fresh water, air dried and powdered. About 10 kg of this powder was extracted thrice with methanol. The combined methanol extract was concentrated under reduced pressure to give the crude methanolic extract residue. The screening results indicated that the crude extract shows promising antibacterial activity. Further extraction of the residue with various other organic solvents and follow-up study resulted in the location of the activity in the petroleum ether and chloroform soluble fractions.

Petroleum ether soluble fraction

The residue obtained from the petroleum ether extract was subjected to silica gel chromatography and eluted with a gradient solvent system consisting of petroleum ether in ethyl acetate to afford 6 different fractions.

The initial fraction so collected on concentration gave a semi-solid residue which on further purification gave a colourless solid of which the physical and spectroscopic properties were identical with those reported for quadradecanoic acid (**53**)[59].

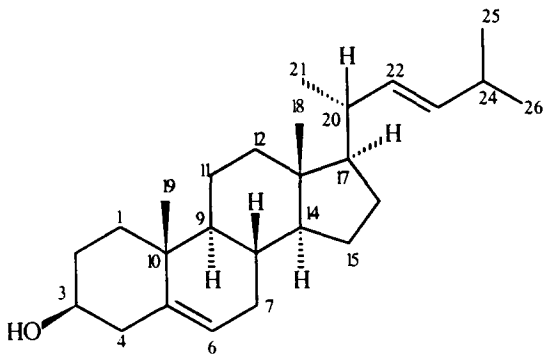
$$CH_3(CH_2)_{14}COOH$$

53

The subsequent fraction after evaporation of the solvent give a semi-solid residue, which was purified further to give a colourless solid melting point 138 ^{0}C. The IR spectrum of the solid compound exhibited absorption bands at 3440 and 1049 cm^{-1}, indicating the presence of a hydroxyl group. The EIMS showed molecular ion peak at m/z 370 corresponding to the molecular formula $C_{26}H_{42}O$. The said molecular formula, indicated the presence of six double bond equivalents, four of which could be assigned to the four rings of the steroidal carbocyclic nucleus and the remaining two could be ascribed to two double bonds, since the carbonyl group was absent. In addition to the molecular ion peak in the mass spectrum, the diagnostically important peaks were observed at m/z 273 (M^+ - SC), 352 (M^+ - H_2O), 255 (M^+ - [SC + H_2O]) and 213 (255 -

ring D) which were characteristic of a steroidal skeleton. Further, the ion fragments at m/z 255, 69, 83 and 112 indicated that hydroxyl group was located in ring A at C_3 in analogy with other steroids.

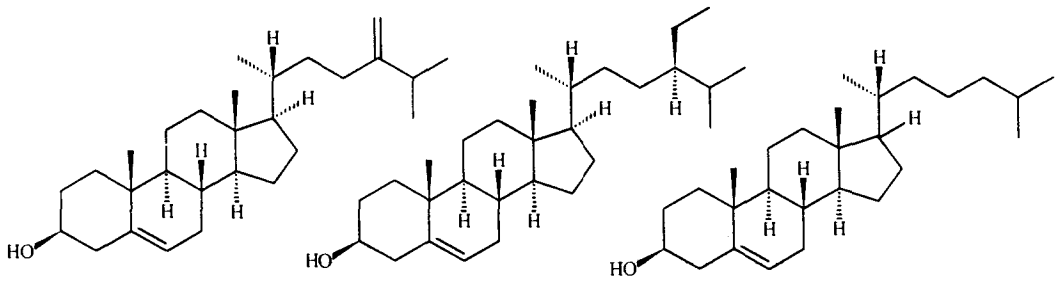
The ^{1}H NMR spectrum displayed signals at δ 0.7 and 1.01 (each 3 H) which were assigned to C_{18} and C_{19} methyl groups respectively. Three doublets at δ 0.82, 0.88 and 0.92 (each 3 H) were assigned to C_{25}, C_{26} and C_{21} methyls, a multiplet centered at δ 3.52 was attributed to C_3 α-methine proton and the signal at δ 5.18 was assigned to $C_{22/23}$ olefinic protons. The broad singlet at δ 5.35 was accounted for the C_6 olefinic proton. Thus, the ^{1}H NMR spectral data and the mass fragmentation pattern of the crystalline compound agreed well with those reported for 24-nor-cholesta-5,22-diene-3β-ol (**54**) in the literature[60] there by suggesting their identity. It may be noted that the C_{26} sterols are widely spread in marine invertebrates and have been reported in five marine phyla namely, the Coelenarates, Echinoderms, Molluscs, Tunicates and Porifera[61]. Though known to be a constituent of marine invertebrates, this is the first report of the occurrence of 24-nor-cholesta-5,22-diene-3β-ol (**54**) in Cladophoraceae family. This sterol has also been reported from the red alga, *Rhodymenia palmata*[62].



54

The next fraction after concentration give an oily residue, which was purified further to give a colourless crystalline solid with m.p.125 ^{0}C. The spectral data and physical constant of this compound agreed well with those reported for 24-methylene cholesterol (**55**)[63 - 65] suggesting their identity.

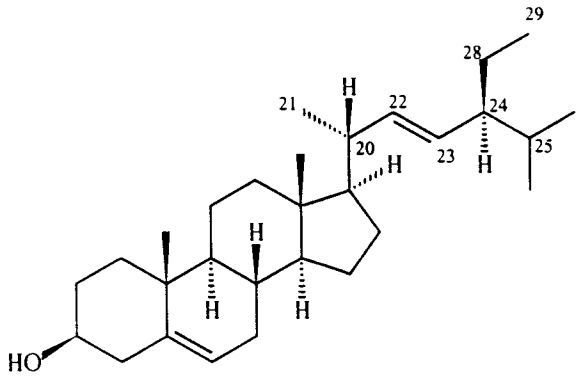
Similarly, the subsequent two fractions upon concentration and purification provided two crystalline compounds which were identified as β-sitosterol[66] (**56**) and cholesterol[67] (**57**) respectively.



55 **56** **57**

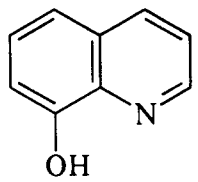
The main chloroform fraction collected earlier by the extraction of the crude residue was chromatographed on the silica gel column and eluted with a gradient solvent system consisting of chloroform in ethyl acetate to give various fractions.

One of the middle fraction after evaporation of the solvent followed by purification of the residue obtained by silica gel chromatography gave a colourless crystalline solid with melting point 169 ^{0}C. The IR spectrum showed absorption bands at 3490, 1465, 1045 and 960 cm^{-1}. Its EIMS showed molecular ion peak at m/z 412 and other diagnostic fragments at m/z 394, 383, 369, 271, 255, 240 and 231 there by indicating that the compound under investigation is a steroid. Further, in addition to other characteristic signals, the NMR spectrum showed a multiplet centered at δ 5.18 (2 H) with J = 7.5 Hz (coupling between 22-20 H and 23-24 H) and J = 15 Hz (coupling between 22-23 H, trans) there by indicating a $\Delta^{22(23)}$ double bond in the steroidal side chain. The configuration at C-24 could be deduced as 'R' on the basis of a triplet at δ 0.857 and in analogy with the literature report[68]. The above described spectral data suggested that the crystalline compound under examination is stigmasterol (**58**) and further confirmed by comparison with the data reported in the literature[68].



58

The latter fractions on concentration provided a brown coloured residue, which was purified further by silica gel column chromatography to give a crystalline solid with melting point 73 ^{0}C. The m.p and the spectral data recorded on this compound established its identity with 8-hydroxyquinoline. Though this quinoline derivative is a known compound, its natural occurrence in *Cladophora* speices is not reported so far. However, the related derivative, 7-Hydroxyquinoline (**59**) has been reported from the soft coral *Sinularia microclavata*[69].



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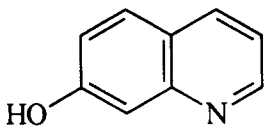
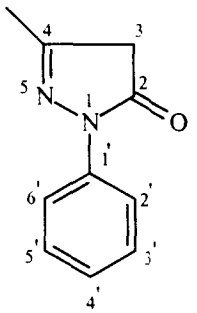


59

The last fraction after evaporation of the solvent followed by purification gave a crystalline solid with melting point 127 ^{0}C. The IR spectrum showed a absorption band at 3420 cm^{-1}, accounting for a CO-N grouping while the bands at 1670 and 755 cm^{-1} suggested the presence of a carbonyl group and the monosubstituted aromatic ring. The EIMS showed molecular ion peak at m/z 174 corresponding to the molecular formula $C_{10}H_{10}ON_2$. The ^{1}H NMR spectrum of the crystalline solid exhibited two singlets at δ 2.21 (3 H) and 3.42 (2 H) corresponding to a methyl and a methylene group respectively. Further, five aromatic proton signals at δ 7.86 (dd, J = 8.5 Hz, 1.5 Hz, 2 H), 7.38 (t, J = 8.5 Hz, 2 H), 7.18 (dd, J = 8.5 Hz, 1.5 Hz, 1H) were observed to indicate the presence of a monosubstituted benzene ring in the compound. The ^{13}C NMR

spectrum displayed signals at δ 43.1 and 16.9 which were accounted for the methylene and the methyl group respectively. Further, the signals at δ 170.5 and 156.1 were indicative of a carbonyl carbon at (C-2) and the quaternary carbon to which the methyl group is attached. In addition, the signals at δ 138.7, 128.7, 124.9 and 18.9 represented the benzeniod carbons. From the above spectral information, the said crystalline compound was identified as 4-methyl-1-phenyl-pyrazole-2-one (**60**) and further confirmed by comparison with an authentic sample[70]. Though **60** is a known compound, it has been isolated for the first time from the *Cladophora* species.



60

As stated earlier, none of the isolated compounds in the present study exhibited any specific activity, though the crude extract obtained in the beginning was showing promising antibacterial activity prior to the separation of individual metabolites.

Thus, in conclusion, a detailed chemical examination of the sea weed *Cladophora prolifiera* from Goa, the west coast of India provided all together eight different known metabolites. Out of these, the steroid derivative 24-Nor-cholesta-5,22-diene-3β-ol (**54**) has been reported for the first time in Cladophoraceae family. Similarly, two heterocyclics namely 8-hydroxyquinoline (**58**) and 4-methyl-1-phenyl-pyrazole-2-one (**60**) have been found and this is the first report of their natural occurrence in this species.

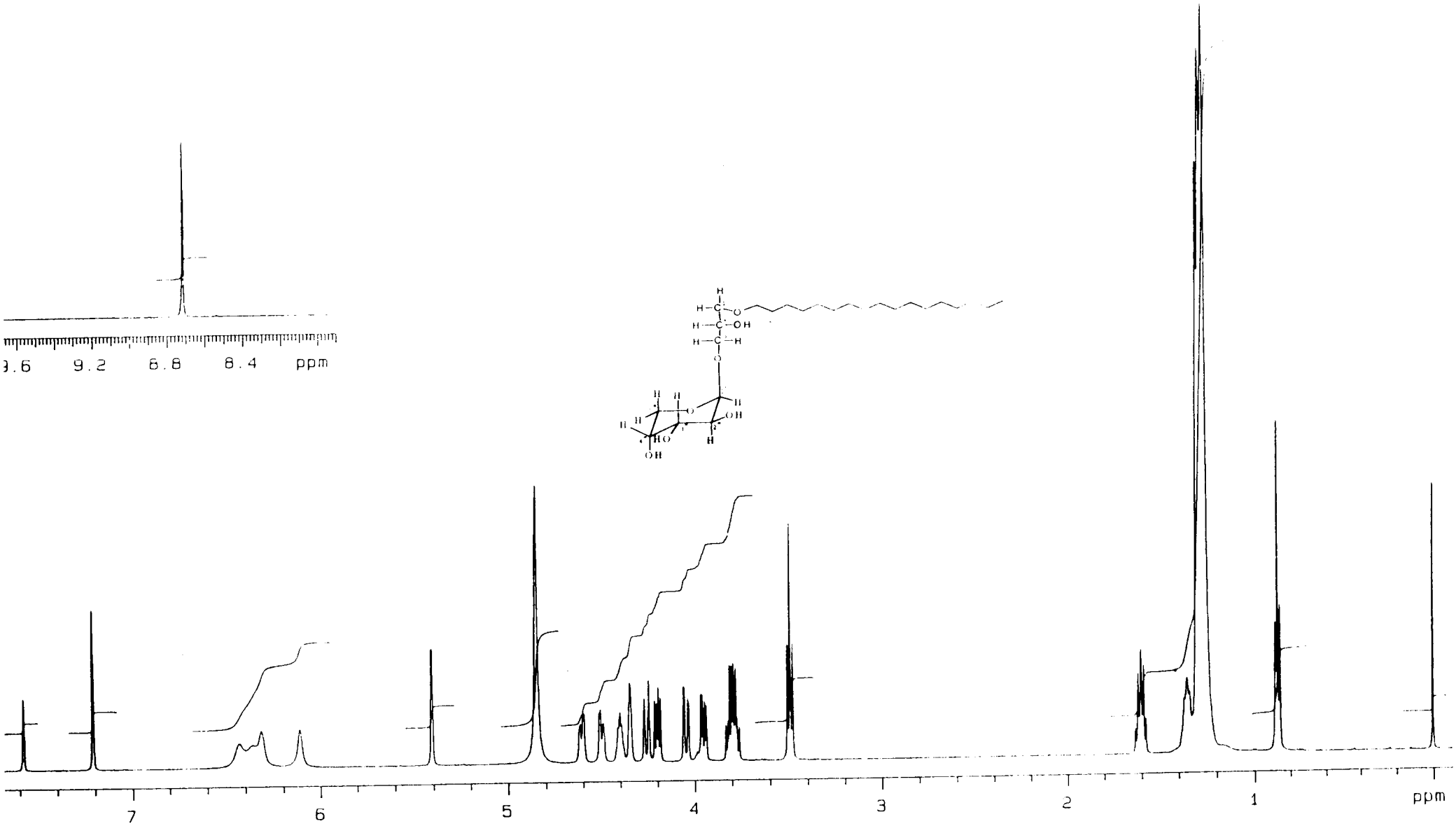


Fig. 2.01 : ^{1}H NMR spectrum of compound 38 in DMSO-d_6

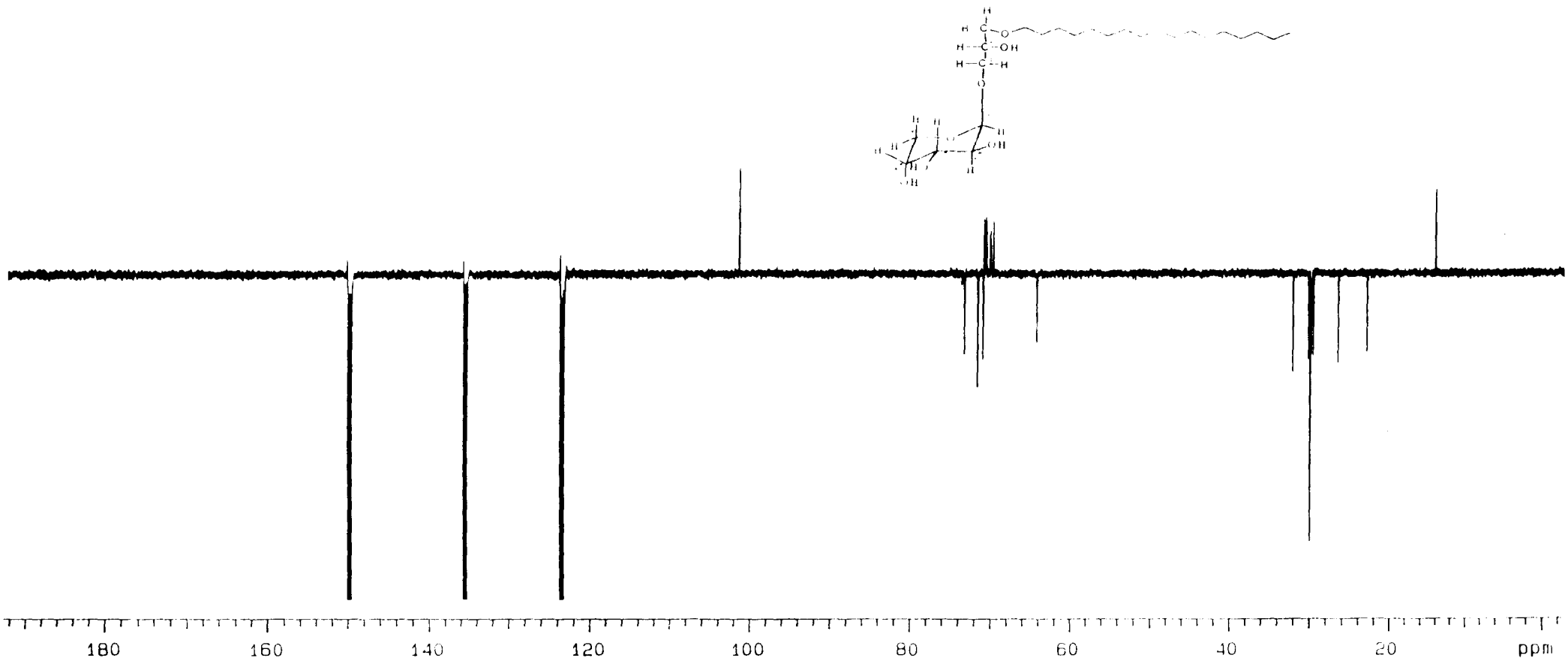


Fig. 2.02 : ^{13}C NMR spectrum of compound 38 in DMSO-d_6

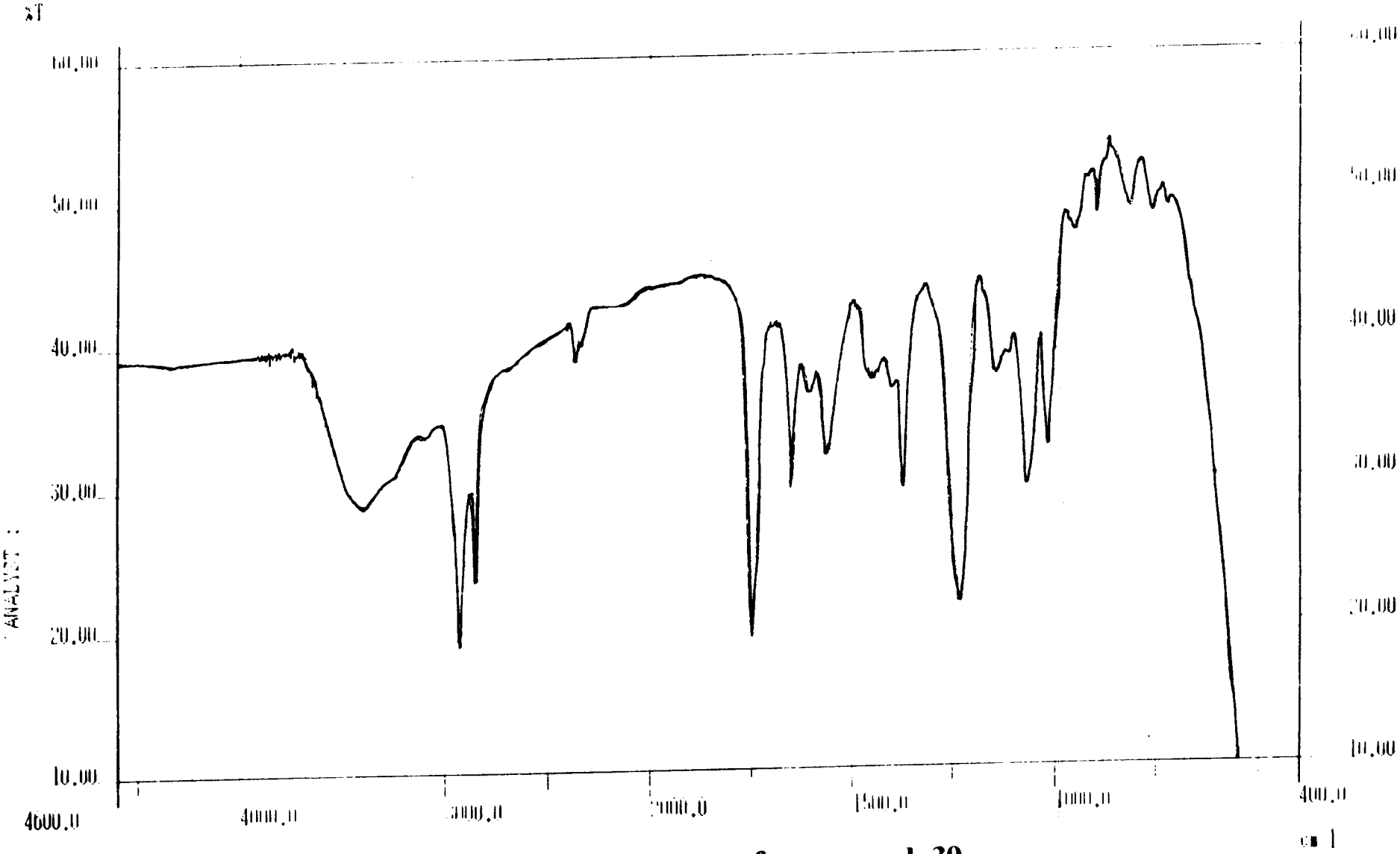


Fig. 2.03 : IR spectrum of compound 39

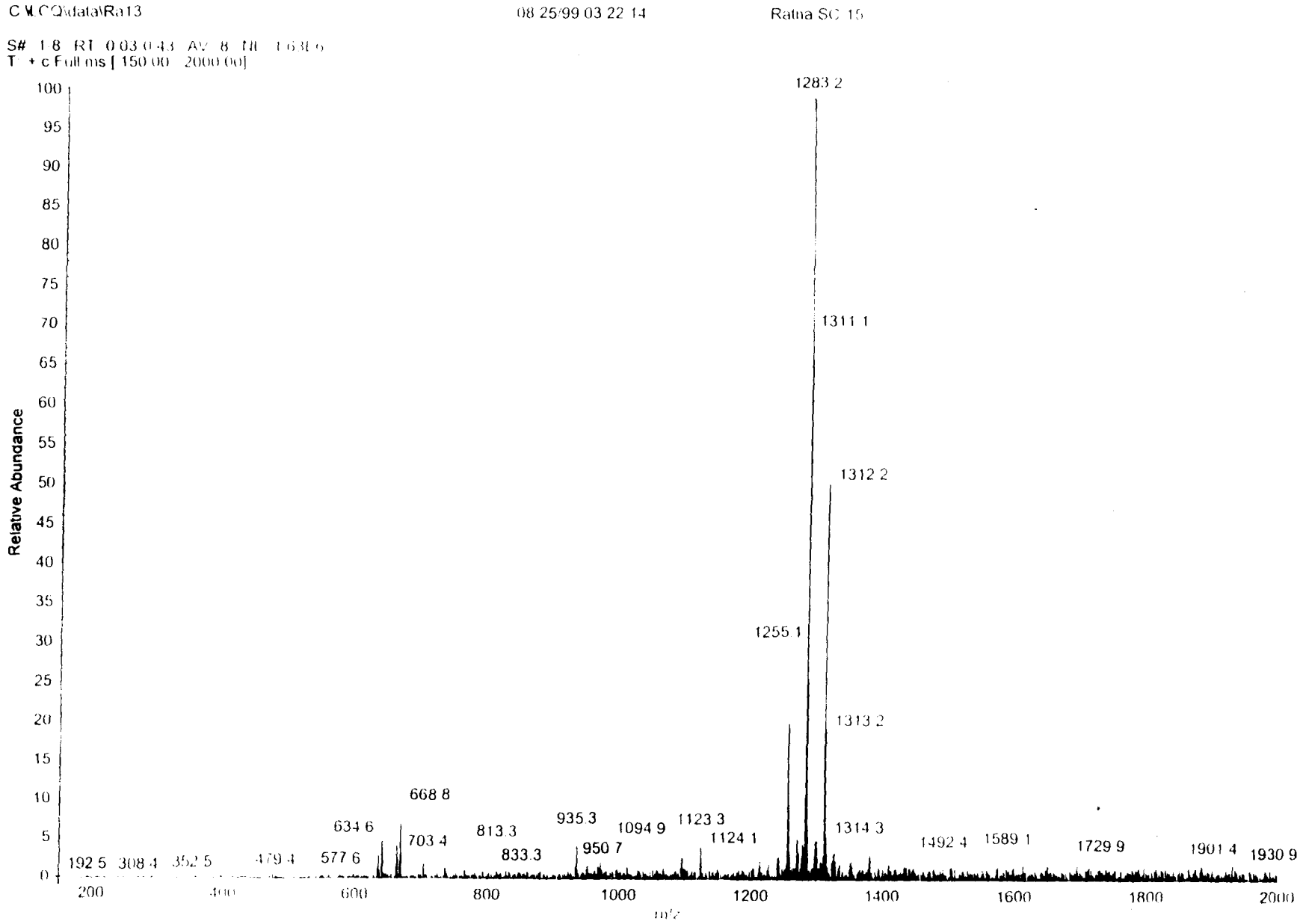


Fig. 2.04 : ESI mass spectrum of compound 39

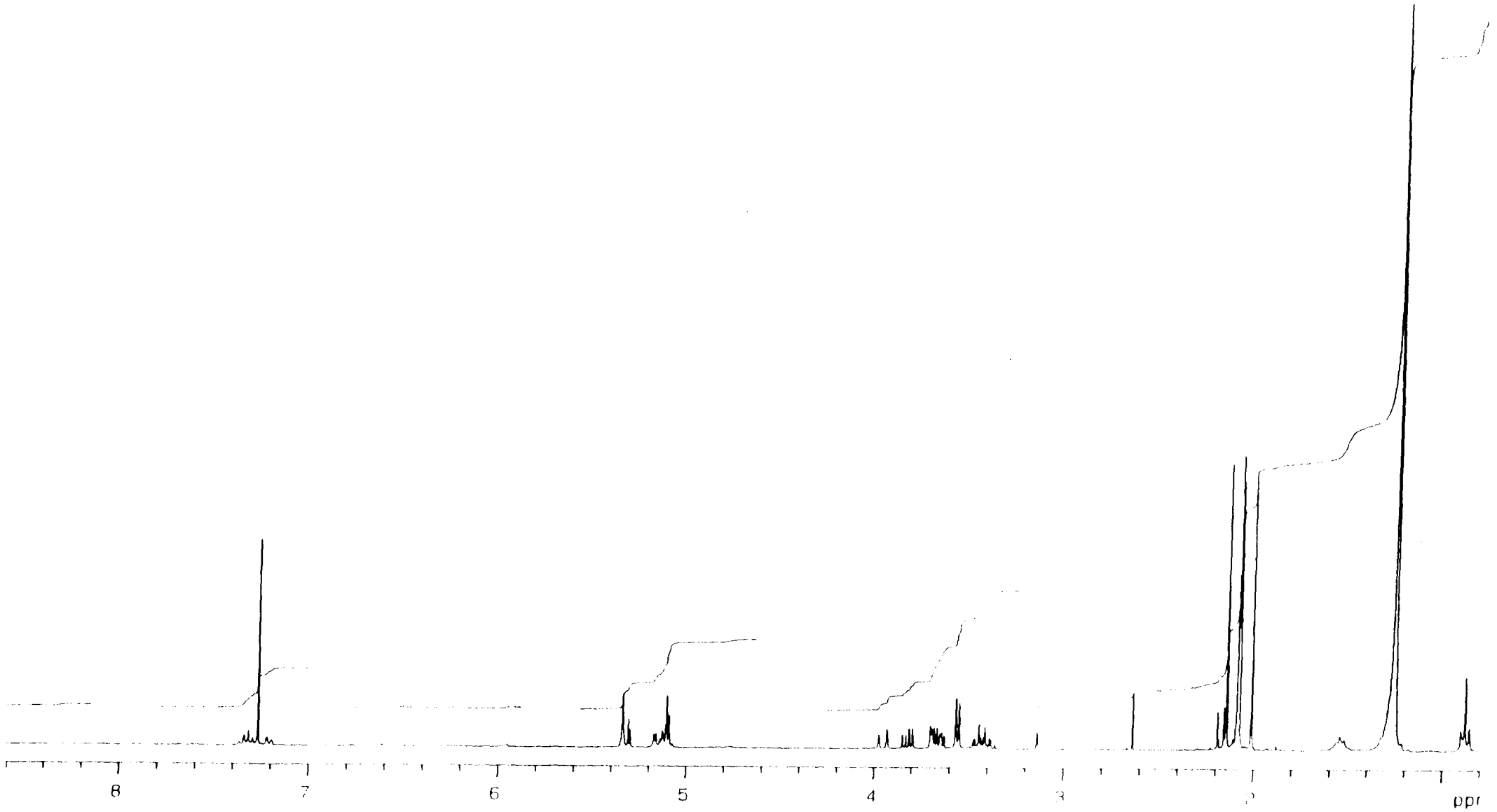


Fig. 2.05 : ^{1}H NMR spectrum of compound 39 in $CDCl_3$

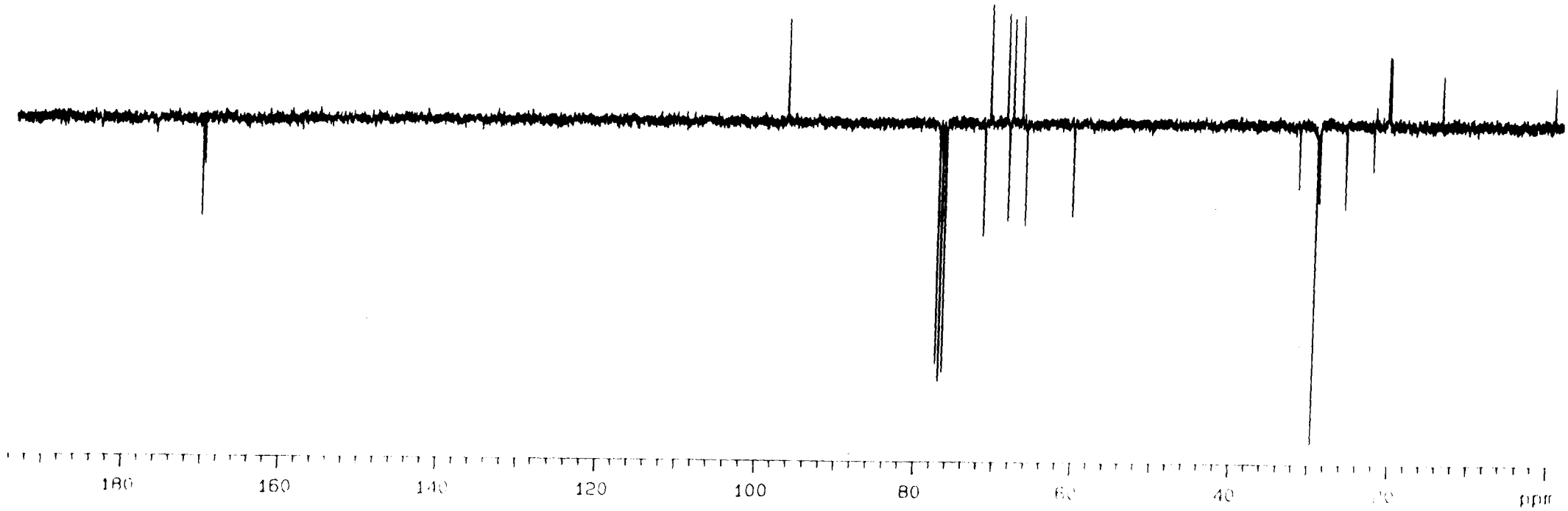


Fig. 2.06 : ^{13}C NMR spectrum of compound 39 in $CDCl_3$

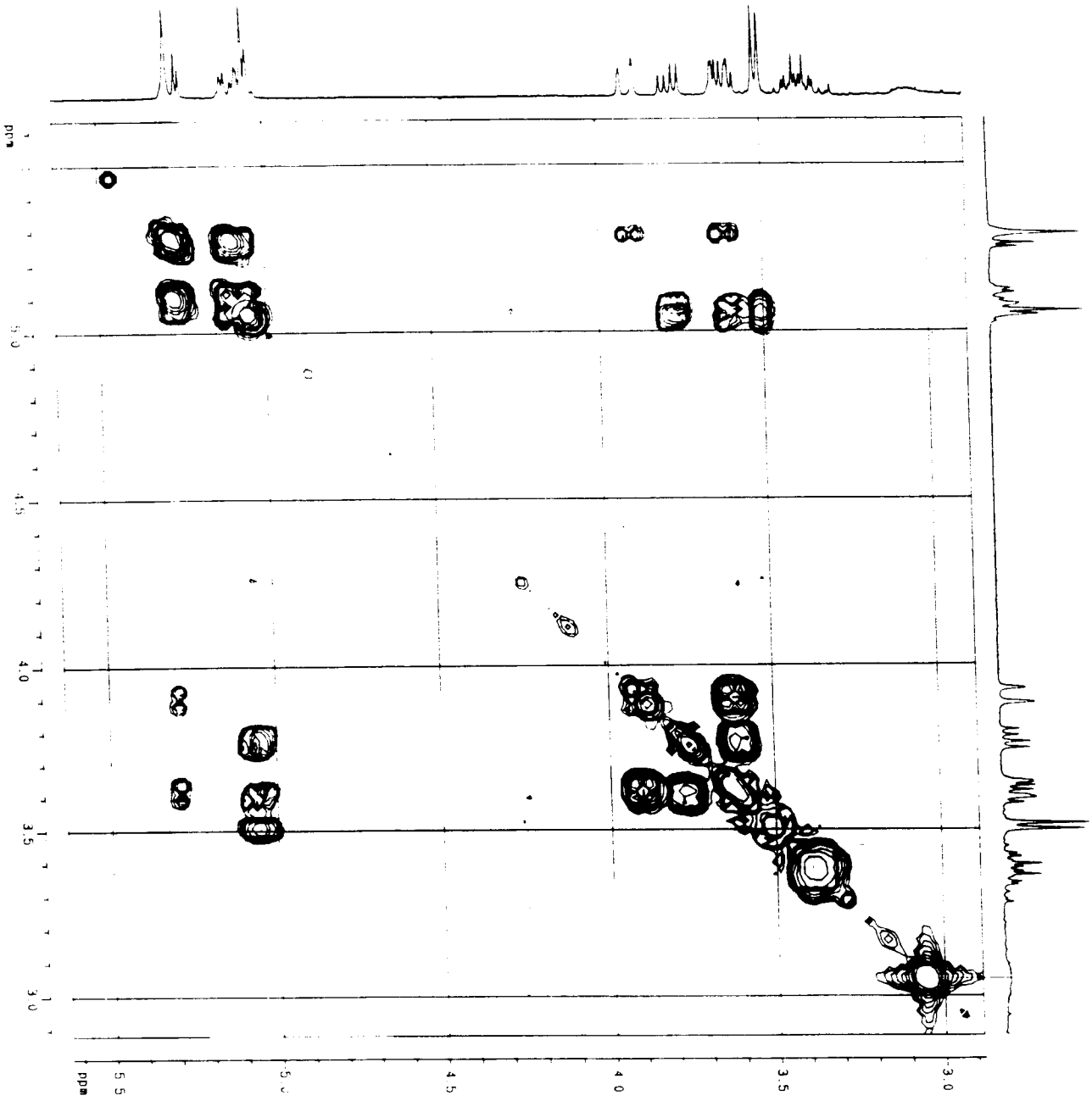


Fig. 2.07 : ^{1}H-^{1}H COSY spectrum of compound 39 $CDCl_3$

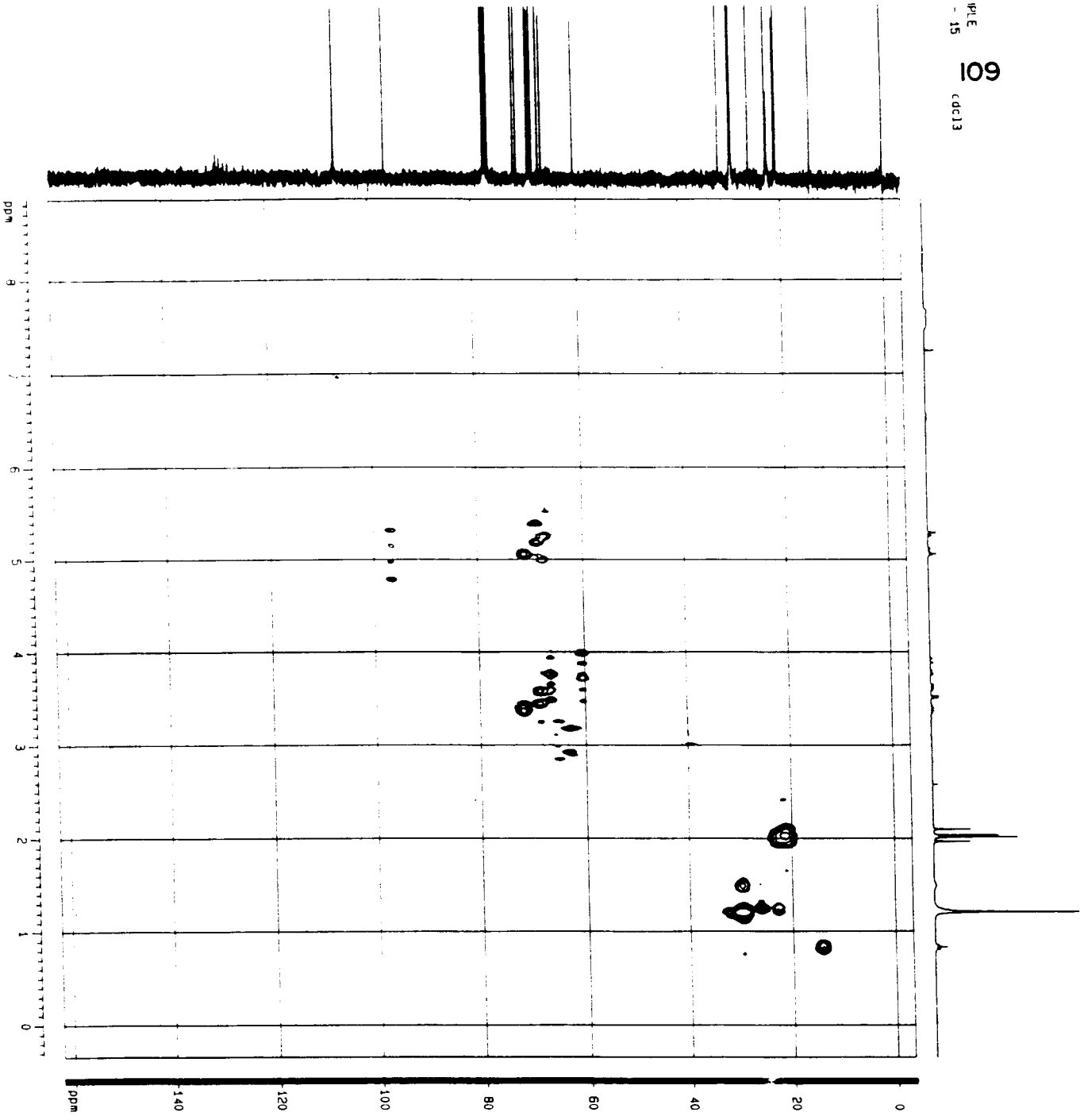


Fig. 2.08 : HMQC spectrum of compound 39 in $CDCl_3$

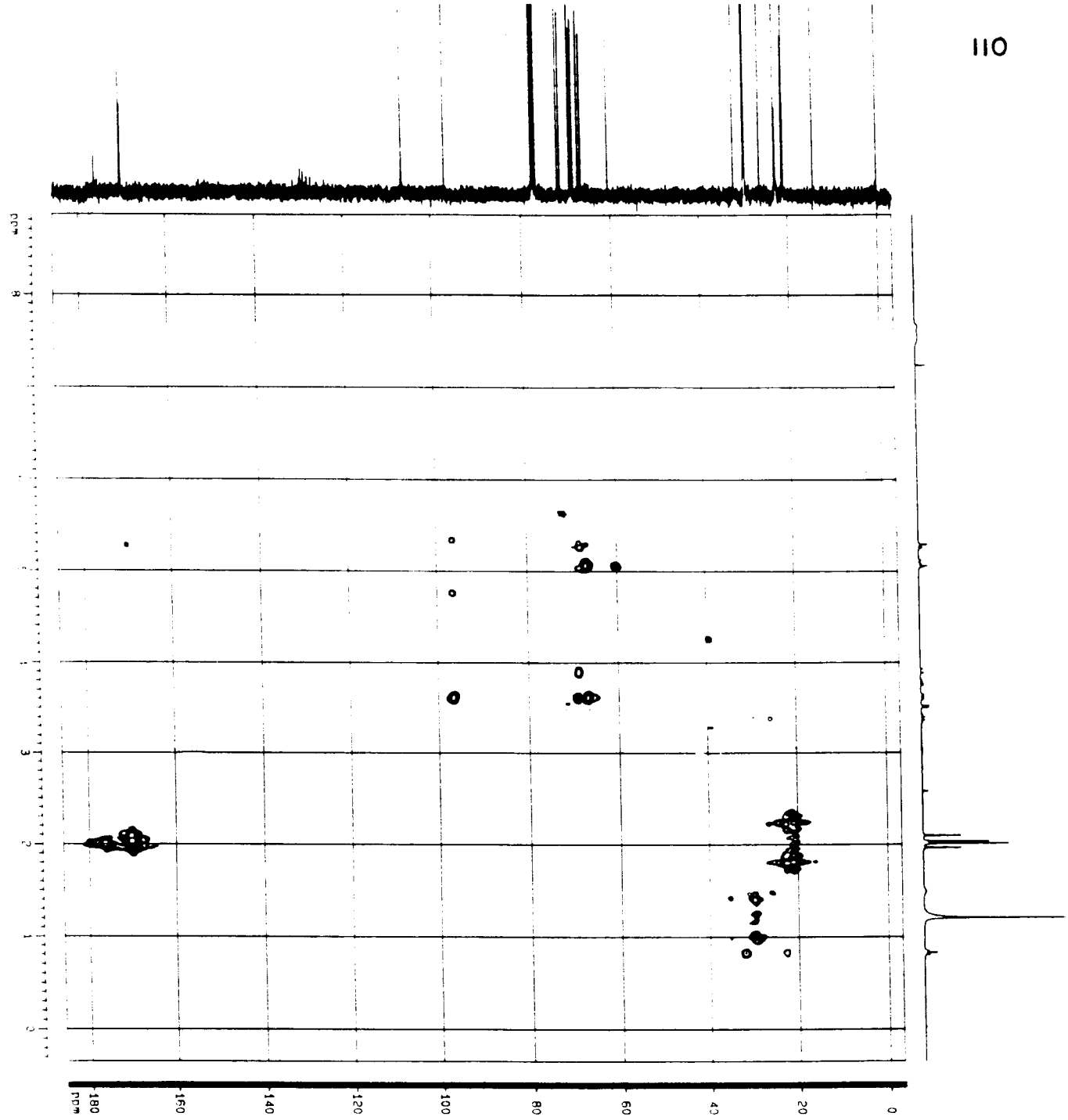
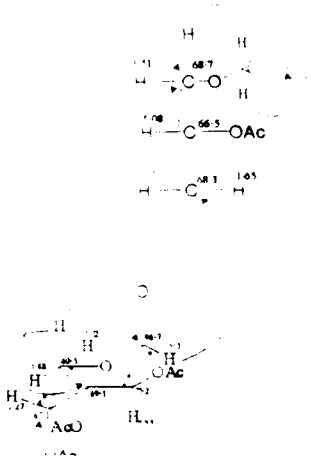


Fig. 2.09 : HMBC spectrum of compound 39 in $CDCl_3$



EXPERIMENTAL

Soft coral *Sinularia capillosa*

The soft coral *Sinularia capillosa* (2.5 kg, dry wt) was collected from the intertidal rocky region at the Okha coast, Gujarat, India during October 1993 using SCUBA diving. The collected material was washed with fresh water, made into thin slices and preserved in methanol at room temperature until workup.

Identification :

The collected organism was identified as *Sinularia capillosa* by Dr. (Mrs) V. Jayasree, NIO, Goa, India. A Voucher specimen of the same was deposited at the National Museum, NIO, Goa.

Extraction and Identification :

The sliced coral material was extracted with methanol (3 x 5 L) at room tempertaure. The combined extract was filtered and the solvent was removed under reduced pressure to give a dark brown gummy substance. The concentrated residue was re-dissolved in aq. methanol and extracted with ethyl acetate, the ethyl acetate layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated to give 8.2 gms of crude residue.

The ethy acetate extract residue was chromatographed over silica gel and eluted using petroleum ether, followed by increasing amount of ethyl acetate/petroleum ether mixture and finally with 100 % ethyl acetate. Various fractions (20 ml each) were collected and the separation was monitored through silica gel tlc. The different fractions on concentration followed by further purification furnished the following compounds.

Hexadecanoic acid (32)

The evaporation of the solvent from the relevant fraction followed by purification of the semi-solid gave a colourless crystalline solid, 30 mg, m.p.94 ^{0}C.

IR (KBr) ν = 3420, 2950, 1700, 1465, 1465, 1430, 1300, 940, 730, 690 and 550 cm^{-1}.

EIMS (70 ev) (m/z) = 256 (M^+, 10), 171 (10), 129 (20), 91(15), 69 (45), 55 (75) and 44 (100).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 8.4 (br s, exchangeable with D_2O, 1 H, -OH), 2.35 (t, 2H), 1.3 (br s, aliphatic CH_2) and 0.9 (t, 3 H).

^{13}C NMR ($CDCl_3$, 100Hz) δ = 179.7 (1C, $C_{quat.}$), 34.04 (1C, CH_2), 31.9 (1C, CH_2), 29.6 to 29.1 (10 C, CH_2), 24.7 (1C, CH_2), 22.6 (1C, CH_2) and 14.05 (1C, CH_3).

Octadecanoic acid (33)

The crude residue obtained after concentration of the solvent was purified further to give a colourless crystalline solid, 22 mg, m.p.68 ^{0}C.

IR (KBr) ν = 3420, 2950, 1700, 1465, 1465, 1430, 1300, 940, 730, 690 and 550 cm^{-1}.

EIMS (70 ev) (m/z) = 284 (M^+, 19), 256 (M^+, - CO, 30), 213 (11), 129 (24), 73 (70), 55 (80) and 42 (100).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 2.35 (t, 2 H), 1.65 (t, 2 H), 1.3 (br s, 28 H) and 0.88 (t, 3 H)

Fucosterol (36)

The semi-solid residue obtained after evaporation of the solvent was further purified by HPLC to give a colourless crystalline solid, 25 mg, m.p.201 ^{0}C

IR (KBr) ν = 3400, 3050, 1464, 1380, 1220, 780 and 650 cm^{-1}.

EIMS (70 ev) (m/z) = 412 (M^+, 10), 397 (M^+ - CH_3, 10), 379 (M^+ - [H_2O + CH_3], 10), 315 (15), 314 (M^+ - C_7H_{14}, 30), 313 (70), 299 (25), 273 (M^+ - SC, 20), 255 (40), 231 (60) and 213 (10).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.35 (br d, 1 H, H-6), 5.18 (q, J = 6.5 Hz, 1 H, H-28), 5.52 (m, 1 H, H-3α), 1.57 (d, J = 6.8 Hz, 3 H, H_3-29), 1.01 (s, 3 H, H_3-19), 0.985 (d, J = 5.5 Hz, 3 H, H_3-21), 0.975 (d, J = 6.0 Hz, 6 H, H_3-26 & H_3-27) and 0.69 (s, 3 H, H_3-18))

Octadecyl glycerol (batyl alcohol)(37)

The light yellow coloured residue obtained after evaporation of the solvent was further purified to give 69 mg of colourless solid, m.p. 70 ^{0}C (lit.[39] m.p. 70 ^{0}C).

IR (KBr) ν = 3400, 2820, 1560, 1445, 1350, 1240, 1135, 700 and 620 cm^{-1}.

EIMS (70 ev) (m/z) = 286 (M^+, 10), 258 (M^+ - CO, 10), 183 (25), 172 (100), 154(10) and 89 (15).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 3.86 (q, 1H, H-2), 3.72 (q, 2 H, H_2-3), 3.65 (m, 2 H, H_2-1), 3.51 (t, 2 H, H_2-1′), 1.55 (t, *J* = 5 Hz, 2 H, H_2-2′), 1.25 (br s, 30 H, H_2-3′ to H_2-17′), 0.85 (t, *J* = 7 Hz, 3 H, H_3-18′)

^{13}C NMR ($CDCl_3$, 100Hz) δ = 72.5 (1C, CH_2), 71.9(1C, CH), 70.5 (1C, CH_2), 64.2 (1C, CH_2), 31.9 (1C, CH_2), 29.6- 29.3 (aliphatic CH_2), 26.1(1C, CH_2), 22.6 (1C, CH_2), 18.4 (1C, CH_2) and 14 (1C, CH_3).

2-hydroxy-3-(heptadecyloxy)-propyl-β-D-arabinopyranoside (38).

The fraction obtained by eluting the column with 100 % ethyl acetate, on evaporation of the solvent *in vacuo* followed by further purification by silica gel column chromatography gave a colourless solid (34 mg), m.p 88 °C.

IR (KBr) ν = 3340, 2930, 1215, 1180, 1160, 850, 800 and 640 cm^{-1}.

ESI(70 ev) (m/z) = 947 (2M + Na, 100)

^{1}H NMR (Pyridine-d_6, 400 MHz) δ **(fig. 2.01)** = 5.4 (d, *J* = 3 Hz, 1 H, H-1″), 4.6 (dd, *J* = 10 Hz, 3 Hz, 1 H, H-2″), 4.5 (dd, *J* = 10 Hz, 3 Hz, 1 H, H-3″), 4.34 (t, *J* = 4.5 Hz, 1 H, H-2), 4.34 (s, 1 H, H-4″), 4.24 & 4.03 (dd, *J* = 10 Hz, 3 Hz, 2 H, H_2-5″), 4.2 & 3.95 (dd, *J* = 10 Hz, 5 Hz, 2 H, H_2-3), 3.8 (m, 2 H, H_2-1), 3.48 (t, *J* = 10 Hz, 4 Hz, H_2-1′), 1.6 (pent, *J* = 7 Hz, 2H, H_2-2′), 1.35 (t, *J* = 4.5 Hz, 2 H, H_2-3′), 1.28 (br s, 28 H, H_2-4′ to H_2-16′) and 0.86 (t, *J* = 7 Hz, 3 H, H_3-17′).

^{13}C NMR (Pyridine-d_6, 100Hz) δ **(fig. 2.02)** = 101.4 (1C, CH), 73.4 (1C, CH_2), 71.8 (1C, CH_2), 71.1 (1C, CH_2), 70.9 (1C, CH), 70.7 (1C, CH), 70.2 (1C, CH), 69.9 (1C, CH), 64.3 (1C, CH_2),32.1 (1C, CH_2), 30.2 to 26.54 (aliphatic 13 CH_2), 22.9 (1C, CH_2) and 20.3 (1C, CH_3).

2-Acetyloxy-3-(heptadecyloxy)-propyl-β-D-arabinotriacetyloxypyranoside (39).

The compound **38** (15 mg) was dissolve in pyridine (5 ml) and then acetic anhydride (5 ml) was added. The mixture was warmed slightly on a water bath for 10

min and then allowed to stand at room temperature over night. The reaction mixture was worked-up as usual and, the product obtained was purified by crystallization from a mixture of chloroform and methanol to give the tetra acetate (**39**).

IR (KBr) ν (fig. 2.03) = 3360, 2910, 2820, 1750, 1650, 1610, 1565, 1450, 1370, 1225, 1135, 1065 and 1015 cm^{-1}.

(+)ESI MS m/z (%) (fig. 2.04) = 1283.2 [$(2M + Na)^+$, 100].

^{1}H NMR ($CDCl_3$, 300 MHz) δ (fig. 2.05) = 5.3 (d, J = 3 Hz, 1 H, H-1″), 5.27 (d, J = 8.5 Hz, 1 H, H-4″), 5.12 (d, J = 8.5 Hz, 3 Hz, 1 H, H-3″), 5.08 (m, 1 H, H-2), 5.05 (dd, J = 8.5Hz, 3 Hz, 1 H, H-2″), 3.88/3.75 (m, H_2-5″) 3.65/3.57 (dd, J = 11 Hz, 2 H, H_2-3), 3.51 (d, J = 11 Hz, 2 H , H_2-1), 3.38 (t, J = 4.8 Hz, 2 H, H_2-1′), 1.97, 1.99, 2.02 & 2.08 (s, each 3 H, 4 x -O-CO-CH_3), 1.45 (m, 2 H, H_2-2′), 1.28 (br s, 28 H, H_2-3′ to H_2-16′) and 0.82 (t, J = 7 Hz, 3 H, H_3-17′).

^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (fig. 2.06) = 176.2 (1C, $C_{quat.}$), 170.4 (1C, $C_{quat.}$), 170.3 (1C, $C_{quat.}$), 170.1 (1C, $C_{quat.}$), 96.7 (1C, CH), 71.8 (1C, CH_2), 71.2 (1C, CH), 69.1 (1C, CH), 68.7 (1C, CH_2), 68.3 (1C, CH), 67.1 (1C, CH), 66.5 (1C, CH_2), 60.5 (1C, CH_2), 31.9 (1C, CH_2), 29.7 to 22.69 (15C, 15 x CH_2),21 (1C, $OCOCH_3$), 20.9 (1C, $OCOCH_3$), 20.8 (1C, $OCOCH_3$), 20.7 (1C, $OCOCH_3$) and 14.1 (1C, CH_3).

^{1}H,^{1}H-COSY ($CDCl_3$, 499.9 MHz) (H↔H) (fig. 2.07) : 1-H↔2-H, 1-H↔3-H, 1-H↔1′-H, 2-H↔3-H, 1′-H↔2′-H, 2′-H↔3′-H, 1″-H↔2″-H, 1″-H↔3″-H, 2″-H↔3″-H, 2″-H↔4″-H and 3″-H↔4″-H.

HMQC (inverse CH-COSY, $CDCl_3$, INVBTP, F1 125.5 MHz, F2 499.9 MHz) (H →C) (fig. 2.08) : 1-H → C-1; 2-H →C-2; 3-H →C-3; 1′-H →C-1′; 2′-H →C-2′; 3′-H →C-3′; 4′-H to 16′-H→C-4′ to C-16′, 17′-H → C-17′; 1″-H →C-1″; 2″-H →C-2″; 3″-H →C-3″; 4″-H →C-4″; 5″-H →C-5″; 7″-H →C-7″;9″-H →C-9″;11″-H → C-11″; 13″-H →C-13″ and 2-H‴ →C-2‴.

HMBC (inverses COLOC, $CDCl_3$, INV4LPLRND, F1 125.5 MHz, F2 499.9 MHz) (H→C) (fig. 2.09) : 3-H ^3J →C-1; 1′-H ^3J →C-1; 1′-H ^3J →C-3′; 16′-H ^2J →C-17′; 2‴-H ^3J →C-1‴; 1″-H ^3J →C-3; 2″-H ^2J →C-3″; 2″-H ^3J →C-4″; 5-H″ ^3J →C-3″; 5″-H ^3J →C-1″; 7″-H ^2J →C-6″; 9″ H ^2J →C-8″ and 10″-H ^2J →C-9″.

Cladophora prolifera

Collection of the sea weed:

The *Cladophora prolifiera* was collected from the intertidal zone of Anjuna beach, (Goa, west coast of India) during premonsoon period in 1997. After collection, the sea weeds were washed with fresh water, dried and powdered.

Extraction and Identification :

About 10 kg of dried and powdered sea weeds were extracted thrice with methanol. The combined methanol extract was concentrated under reduced pressure to give crude methanolic extract residue. The screening results at this stage indicated that the crude extract has promising antibacterial activity. The crude residue was therefore re-dissolved in aq. Methanol and extracted first with petroleum ether followed by chloroform, the organic layer in each case were washed with water, dried over anhydrous Na_2SO_4 and concentrated to give 12 gms and 7 gms of crude residue respectively. The antibacterial activity was traced in both part of the crude residues.

The crude residue obtained from the petroleum ether extract was chromatographed over silica gel and eluted by using increasing proportion of ethyl acetate in petroleum-ether /ethyl acetate solvent system. Various fractions (20 ml each) were collected and the separation was monitored through silica gel tlc. The different fraction upon removal of solvent furnished the respective compounds which were further purified through silica gel column chromatography to provide the following pure compounds.

Quadradecanoic acid (14)

The appropriate fraction after evaporation of the solvent followed by purification gave a colourless solid **14** 23 mg.

IR (KBr) ν = 3400, 2920, 1700, 1460, 1465, 1430, 1300, 935, 730, 690 and 540 cm^{-1}.

EIMS (70 eV) m/z = 228.2 (M^+, 50), 213.2 (M^+ - CH_3, 24), 200.2 (18), 185.2 (24), 171.1 (18), 129.1 (56) and 97.1 (20).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 8.5 (br s, exchangeable with D_2O, 1 H, -OH), 2.35 (t, J = 8 Hz, 2 H, CH_2), 1.62 (m, 2 H, CH_2), 1.24 (br s, 20 H, 10 x CH_2) and 0.88 (t, J = 6 Hz, 3 H, CH_3).

^{13}C NMR ($CDCl_3$, 300 MHz) δ = 180 (1C, $C_{quat.}$), 34.1 (1C, CH_2), 31.9 (1C, CH_2), 30 (1C, CH_2), 29.9 to 29.08 (aliphatic CH_2), 24.7 (1C, CH_2), 22.7 (1C, CH_2) and 14.1 (1C, CH_3).

24-Nor-cholesta-5,22-diene-3β-ol (15)

The subsequent fraction after concentration of the solvent gave a residue which was further purified by silica gel chromatography to give **15** as colourless solid 20 mg, with m.p. 138 ^{0}C.

IR (KBr) ν = 3440, 2940, 2850, 1462, 1376, 1050 and 750 cm^{-1}.

EIMS (70 eV) m/z = 370 (M^+, 15), 355 (M^+ - H_2O, 30), 273 (M^+ - SC, 18), 255 (M^+ - [SC + H_2O], 35), 213 (255 - ring D, 40), 112 (35), 83 (68), 69 (50) and 55 (80).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 5.35 (br s, 1 H, H-6), 5.18 (m, 2 H, H-22/23), 3.52 (m, 1 H, H-3α), 1.01 (s, 3 H, H_3-19), 0.92 (d, 3 H, H_3-21), 0.88 (d, *J* = 6. 8 Hz, 3 H, H_3-25/26), 0.82 (d, *J* = 6.8 Hz, 3 H, H_3-25/26) and 0.7 (s, 3 H, H_3-19).

24-Methylene cholesterol (16)

This was obtained as a colourless crystalline solid 25 mg, m.p.125 ^{0}C.

IR (KBr) ν = 3500, 2940, 2850, 1450, 1376,1099, 1050, 980and 750 cm^{-1}.

EIMS (70 eV) m/z = 398 (M^+, 10), 383 (M^+ - CH_3, 12), 380 (M^+ - H_2O, 6), 365 (M^+ - [H_2O + CH_3], 8), 314 (M^+ - C_6H_{12},), 273 (M^+ - SC, 10), 271 (M^+ - [SC + 2H], 42) and 255 (M^+ - [SC + H_2O],15).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.35 (br s, 1 H, H-6), 6.64, 4.75 (br s, 2 H, H-28), 3.55 (m, 1 H, H-3α), 1.08 (s, 3 H, H_3-19), 1.0 (d, J = 6.4 Hz, 3 H, H_3-21), 0.98 (d, *J* = 6. 8 Hz, 3 H, H_3-26/27), 0.96 (d, *J* = 6.8 Hz, 3 H, H_3-26/27) and 0.68 (s, 3 H, H_3-18).

β-Sitosterol (17)

This was obtained as a crystalline solid 1.25 g, m.p. 135 ^{0}C.

IR (KBr) ν = 3410, 2940, 1435, 1450,1250, 1040, 970 and 750 cm^{-1}.

EIMS (70 eV) m/z = 414 (M^+ , 64), 399 (M^+ - CH_3, 30), 396 (M^+ - H_2O, 40), 381 (M^+ - [H_2O + CH_3], 30), 329 (M^+ - C_6H_{13}, 50), 308 (M^+ - $C_7H_{11}O$, 40), 273 (M^+ - SC, 30), 255 (M^+ - [SC + H_2O],40) and 213 ([255 - ring D],50).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.36 (br s, 1 H, H-6), 3.55 (m, 1 H, H-3α), 1.01 (s, 3 H, H_3-19), 0.95 (d, J = 6.4 Hz, 3 H, H_3-21), 0.85 (t, *J* = 7.2 Hz, 3 H, H_3-29), 0.83 (d, *J* = 6.8 Hz, 3 H, H_3-26/27), 0.81 (d, J = 6.8 Hz, 3 H, H_3-27/26), and 0.68 (s, 3 H, H_3-18).

Cholesterol (18)

Obtained as a colourless crystalline compound 28 mg.

IR (KBr) ν = 3460, 2940, 2850, 1455, 1380,1080, 1040,780 and 750 cm^{-1}.

EIMS (70 eV) m/z = 386 (M^+ ,17), 371 (M^+ - CH_3, 10), 368 (M^+ - H_2O, 12), 353 (M^+ - [H_2O + CH_3], 10), 275 (M^+ - $C_7H_{11}O$, 25), 273 (10), 231 (18)and 213 (10).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 5.35 (br s, 1 H, H-6), 3.55 (m, 1 H, H-3α), 1.01 (s, 3 H, H_3-19), 0.95 (d, *J* = 6.4 Hz, 3 H, H_3-21), 0.83 (d, *J* = 6.8 Hz, 3 H, H_3-26/27), 0.81 (d, J = 6.8 Hz, 3 H, H_3-27/26), and 0.68 (s, 3 H, H_3-18).

Stigmasterol (19).

The evaporation of the concerned fraction followed by purification gave a colourless crystalline solid, 35 mg, m.p. 169 °C.

IR (KBr) ν = 3480, 2940, 1470, 1375, 1045, 960, 730 and 600 cm^{-1}.

EIMS (70 eV) m/z = 412 (M^+, 10), 397 (M^+,- CH_3, 18), 394 (M^+,- H_2O, 15), 383 (M^+,- C_2H_5,20), 369 (M^+,- C_3H_7,12), 271 (25), 255 (M^+, - [H_2O + SC], 20), 240 (M^+ - [SC + H_2O + CH_3],18), 231 (15), 229 (26), 213 (40), 159 (30) and 121 (35).

^{1}H NMR ($CDCl_3$, 300 MHz) δ = 5.36 (br s, 1 H, H-6), 5.18 (m, *J* = 15 Hz , 7.5 Hz, 2 H, H-22, H-23), 3.55 (m, 1 H, H-3α), 1.08 (s, 3 H, H_3-19), 1.0 (d, *J* = 6 Hz, H_3-21), 0.95 (d, *J* = 6.8 Hz, 2 H, H_3-26/27), 0.857 (t, *J* = 7 Hz, 3 H, H_3-29) and 0.7 (s, 3 H, H_3-18)

8-Hydroxyquinoline (20)

The residue obtained after concentration of the relevant fraction was purified further to give colourless crystalline solid, m.p. 73 °C (lit.[70] m.p. 70 °C).

IR (KBr) ν = 3450, 2980, 1580, 1510, 1470, 1410, 1370, 1270, 1265, 1220, 1210, 810, 760 and 740 cm^{-1}.

EIMS (70 eV) m/z = 145.1 (M^+, 100), 117.1 (40), 116 (10), 89 (16) and 63 (15).

^{1}H NMR ($CDCl_3$, 200 MHz) δ = 8.7 (dd, *J* = 4.5 Hz, 1.5 Hz, 1 H, H-7), 8.15 (dd, *J* = 8.2 Hz, 1.5 Hz,1 H, H-2), 7.48 (dd, *J* = 7.2 Hz, 2.5 Hz, 1 H, H-3), 7.43 (dd, *J* = 4.2 Hz, 1.5 Hz, 1 H, H-6), 7.32 (dd, *J* = 4.5 Hz, 1.5 Hz, 1 H, H-5) and 7.2 (dd, *J* = Hz, 1.5 Hz, 1 H, H-4).

^{13}C NMR ($CDCl_3$, 75 MHz) δ = 152.4 (1C, $C_{quat.}$), 147.8 (1C, CH), 138.4 (1C, $C_{quat.}$), 128.6 (1C, CH), 127.7 (1C, CH) , 121.6 (1C, CH), 117.8 (1C, CH) and 110.1 (1C, CH).

4-Methyl-1-phenyl-pyrazole-2-one (22)

This was obtained after concentation of the concerned fraction followed by purification of the crude residue to give 15 mg of a solid, m.p. 127 ^{0}C (lit. [70] m.p. 127 ^{0}C).

IR (KBr) ν = 3420, 2910, 1670, 1610, 1525, 1510, 1460, 1430, 1370,1000,755 and 700 cm^{-1}.

EIMS (70 eV) m/z = 174 (M^+, 100), 132 (M^+ - $COCH_2$, 10), 105 (M^+ - $COCH_2CCH_3$, 25), 91 (M^+ - [105 + N], 40) and 77 (40).

^{1}H NMR ($CDCl_3$, 400 MHz) δ = 7.86 (dd, *J* = 8.5 Hz, 1.5 Hz, 2 H, H-2′, H-6′), 7.38 (t, *J* = 8.5 Hz, 2 H, H-3′, H-5′), 7.18 (dd, *J* = 8.5 Hz, 1.5 Hz, 1 H, H-4′), 3.42 (s, 3 H, H_3-7) and 2.17 (s, 2 H, H_2-3).

^{13}C NMR ($CDCl_3$, 75 MHz) δ = 170.5 (1C, $C_{quat.}$), 156.1 (1C, $C_{quat.}$), 138.7 (1C, $C_{quat.}$), 128.7 (2C, 2 x CH), 124.9 (1C, CH), 118.9 (2C, 2 x CH), 43.1 (1C, CH_2) and 16.9 (1C, CH_3).

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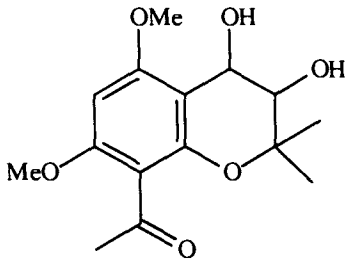
CHAPTER 3

SYNTHETIC STUDIES ON CHROMANONES AND CHROMONES

INTRODUCTION

The benzopyranone (chromanone) analogues have widespread occurrence and are of considerable significance due to their potential as important pharmaceuticals[1]. In nature, the benzopyranones are frequently encountered in different sources exhibiting diverse bio-activity. For example, the aerial parts of *Euodia lunu-ankenda* Merr. (an Indian plant) yielded the diol[2] (**1**), which is reported to possess antibacterial activity. Further, an antibiotic substance LL-D253α, possesing the structure **2** has been isolated from the mycotoxin-producing strains of fungi belonging to the genus *Phoma*[3,4,5]. Similarly, the novel 2-substituted chromanones (**3**) & (**4**) have also been isolated from the brown alga *Zonaria tournefortii*[6].



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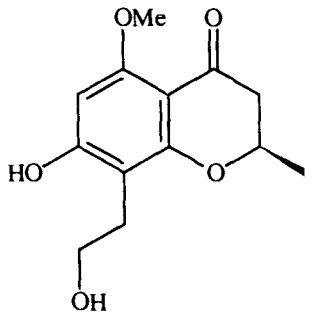

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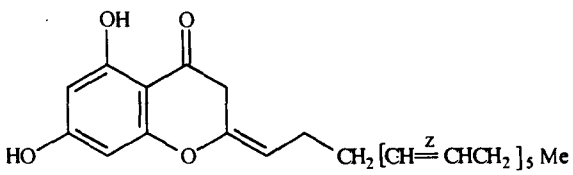

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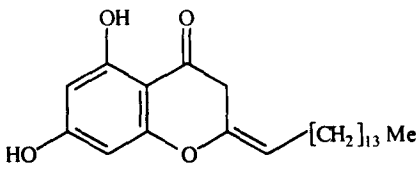

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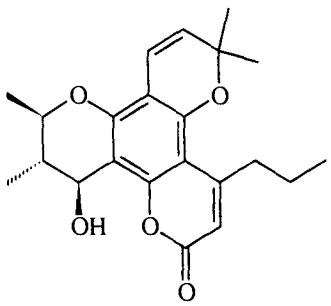
In addition, the chromanones also form the part-structure of several biologically active natural products[7] besides serving as the synthetic intermediates for chromans, chromenes, chromanols etc. which exhibited diverse pharmacological activities[8]. With the isolation of the anti-HIV agent calanolide A[9] (**5**), which bears a chiral chromanol moiety, there was a renewed interest in the chemistry of chromanones. Similarly, the natural product khellin[10] (**6**) provided lead to the discovery of a totally synthetic drug namely disodium cromoglycate[11,12,13] and further stimulated interest in the synthesis of novel heterocyclic linked chromones as the possible antiallergy and antiviral drugs[14,15].

The pharmacologically important derivatives such as the K^+ channel opener cromakalim[16] (**7**), the LTB_4 antagonist R_0 25-3562[17] (**8**), the class III antiarrhythmic RP-58866[18] (**9**), and the aldose reductase inhibitor sorbinil[19] (**10**), all exemplify the importance of possessing a benzopyran structural unit in such clinically useful molecules.



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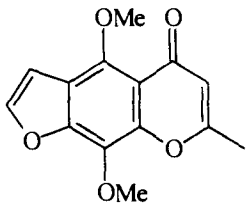

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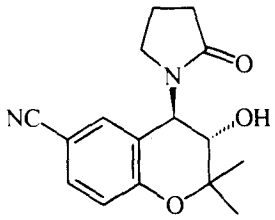

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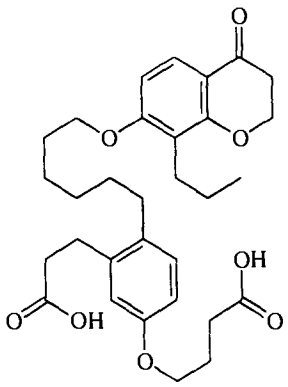

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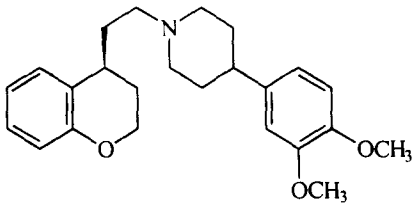

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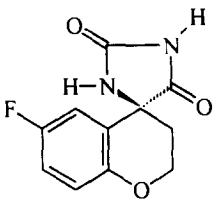

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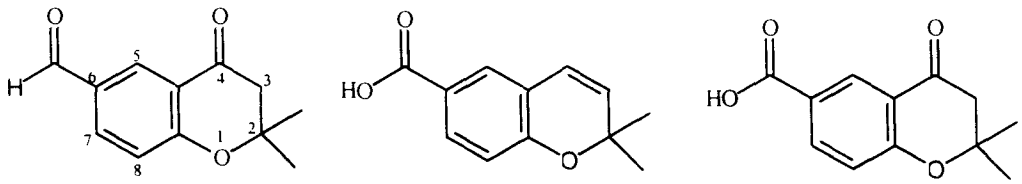
The above were the few discrete examples cited and it may be stated that the literature available in this connection is replete with full information regarding the natural occurrence, pharmacological significance and synthetic studies pertaining to a variety of substituted chroman-4-one analogues[1].

The ongoing section of this chapter describes the details of the synthesis pertaining to lactarochromal, a new chromanone isolated as fungal metabolite.

SECTION I

A SHORT AND FACILE SYNTHESIS OF LACTAROCHROMAL, THE FUNGAL METABOLITE

Recently, Ayer & Trifonov[20] examined the metabolites produced by the mycorhizal fungus *Lactarius deliciosus* when it was grown in liquid culture medium. During the said investigation, they reported the isolation of a new chroman-4-one derivative and designated it as lactarochromal and assigned the structure **11** on the basis of spectroscopic analysis. In addition, it was also reported[20] that the said fungus produced Anofinic acid (**12**)[21] along with 3-hydroxy acetyl indole and other known compounds.

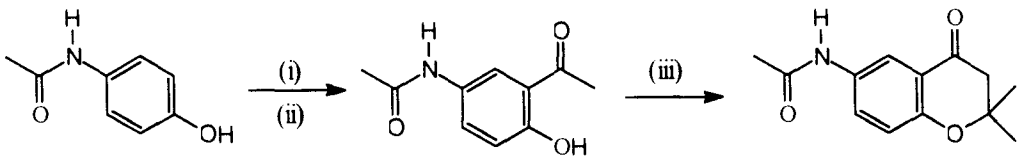


11 **12** **13**

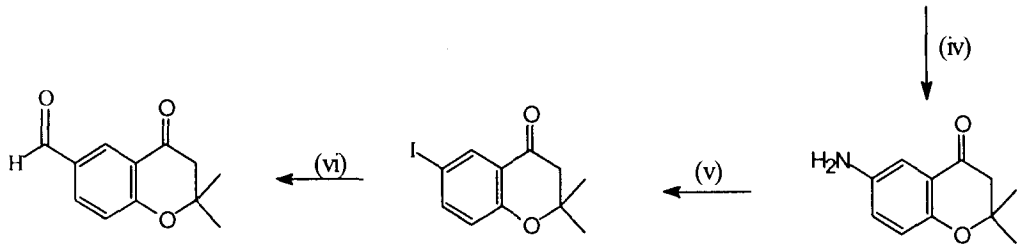
Since, the structure **11** was based only on spectral data, an unambiguous synthetic support to confirm the assigned structure seemed desirable. Further, in the absence of any information provided by the above workers, we were also interested in examining whether the natural product **11** possesses any biological activity since the chromanones are known to exhibit diverse activities as stated earlier. With these objectives, we undertook the present work and the details along with the preparation of the corresponding acid **13**, which is also a natural product[22] are described in this section.

To start with, since lactarochromal (**11**) is an carboxaldehyde of the corresponding benzopyran-4-one, we sought to prepare the required iodo chromanone (**18**) as depicted in scheme **1**.

Scheme 1



14 **15** **16**



11 **18** **17**

Reagents and conditions : (i) CH_3COCl, warming on oil bath (ii) $AlCl_3$, Nitrobenzene, heat (iii) Acetone, piperidine, pyridine, reflux 72h (iv) 5N HCl, ethanol, reflux (v) H_2SO_4, $NaNO_2$, water, KI (vi) n-butyllithium, N-formylpiperidine, N_2 atmosphere, HCl.

Accordingly, the *p*-acetoxy acetanilide prepared from *p*-acetaminophenol[23] when subjected to Fries rearrangement according to the literature procedure[24] gave 2-acetyl-4-acetamidophenol (**15**). The latter was condensed with excess of acetone in presence of piperidine and pyridine to give 6-acetylamino-2,2-dimethylchroman-4-one (**16**). The melting point and the spectral data recorded on **16** agreed well with those reported in the literature[25]. Deprotection of the amino group in **16** with 5 N HCl gave the 6-amino-2,2-dimethylchroman-4-one (**17**). The IR spectrum (fig. 3.01) of this amino compound showed absorption bands at 3300, 1690 cm^{-1} to indicate the presence of the amino and carbonyl groups respectively. The EIMS (fig. 3.02) showed molecular ion peak at m/z 191 which corresponds to the molecular formula $C_{11}H_{13}O_2N$. The ^1H NMR spectrum (fig. 3.03) showed a broad singlet at δ 4.18 (exchangeable with D_2O) which was assigned to the amino group in the molecule. The two singlets at δ 2.64 (2 H) and 1.2 (6 H) were assigned to the isolated methylene and two methyl groups respectively. Further, a pair of ABX-type signals at δ 7.18 (d, J = 2.5 Hz, 1 H), 6.9 (dd, J = 8.5 Hz, 2.5 Hz 1 H) and 6.76 (d, J = 8.5 Hz, 1 H) were observed for the aromatic protons, indicating the presence of 1,3,4 (or 1,2,4) -trisubstituted benzene nucleus in the molecule. The ^{13}C NMR spectrum (fig. 3.04) exhibited 11 carbon signals which comprised of five sp^3 and six sp^2 carbon resonances including those derived from one methylene and two methyl groups. All the above described spectral characteristics are in conformity with the structure **17**, for the said amino compound.

The next step was to convert the compound **17** into the required 2,2-dimethyl-6-iodo-chroman-4-one (**18**) by the reaction of the diazonium salt derived from **17** with KI. When the said reaction was carried out, the desired compound **18** was obtained in 56 % yield as a light yellow coloured oil along with two other crystalline compounds obtained as minor products of the reaction. The IR (fig. 3.05) spectrum of compound **18** exhibited band at 1690 cm^{-1} along with other absorption bands, thereby demonstrating the presence of a carbonyl group. The EIMS (fig. 3.06) showed molecular ion peak at m/z 302, corresponding to the molecular formula $C_{11}H_{11}O_2I$. The ^1H NMR spectrum (fig. 3.07) showed singlets at δ 2.72 (2 H) and 1.45 (6 H) for a isolated methylene and two methyl groups respectively. In addition, the observed signals at δ 8.15 (d, J = 2.5 Hz, 1 H), 7.7 (dd, J = 8.5 Hz, 2.5 Hz 1 H) and 6.72 (d, J = 8.5 Hz, 1 H) indicated the presence three aromatic protons in the molecule. The ^{13}C NMR spectrum (fig. 3.08) accounted for all eleven carbon atoms in the form of five sp^3 and six sp^2 carbon resonances. The above described spectral analysis were in conformity with the structure **18** for the said yellow coloured oil.

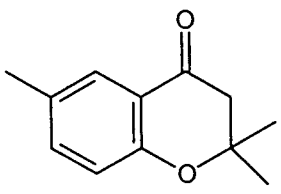
Similarily, the other two crystalline compounds obtained as the minor products during the preparation of **18** were identified (see experimental part) as 2,2-dimethyl-chroman-4-one (**19**) and 2,2-dimethyl-3,6-di-iodo-chroman-4-one (**20**) on the basis of spectral analysis.

Finally, to complete the synthetic sequence, the formyl group was introduced on the chromanone moiety by treating the lithium salt of **18** with N-formylpiperidine. After the usual work up, the residue obtained was purified to give a crystalline solid with melting point 91 ^0C. The IR spectrum of this compound (fig. 3.09) showed bands at 1700 and 1687 cm^{-1} to indicate the presence of two carbonyl groups. The molecular formula of this compound was determined to be $C_{11}H_{12}O_3$ by HR-EIMS (molecular ion at *m/z* = 204.0788) (fig. 3.10). The ^1H NMR spectrum (fig. 3.11) showed two singlets at δ 1.48 (6 H) and 2.76 (2 H) to indicate the presence of two methyl and one isolated methylene groups respectively in the molecule. Another singlet at δ 9.89 (1 H) was accounted for the presence of an aldehyde group. In addition, the aromatic proton signals observed at δ 7.03 (d, J = 8.8 Hz, 1 H), 8.0 (dd, J = 8.8 Hz, 2.0 Hz, 1 H) and 8.32 (d, J = 2.0 Hz, 1 H) confirmed the presence of trisubstituted benzene nucleus in the molecule. The ^{13}C NMR

spectrum (fig. 3.12) showed signals at δ 26.5 and 48.4 which were assigned to the two methyl and one methylene groups respectively in the molecule. The signals due to the aldehyde and the ketonic groups were observed at δ 191.1 and 190.1 respectively. The presence of three benzenoid carbon as singlets at δ 164.3, 129.8 and 119.8 and another three as doublets at δ 134.8, 131.1 and 119.6 were all in conformity with the structure **11** for the said crystalline compound. The melting point and the spectral data (IR, ^{1}H NMR, ^{13}C NMR & MS) of the synthetic **11** were in good agreement with those reported for the natural lactarochromal[20], which thus confirmed the structure assigned to the natural product by the earlier workers.

After having realised the synthesis of the natural product **11** invariably by following a relatively longer route starting from *p*-acetaminophenol (**14**) as described above, we were tempted to conceive an alternate route to effect a short and facile synthesis of lactarochromal. For this purpose, we envisaged 2,2,6-trimethylchroman-4-one (**21**) as the intermediate compound which could be then oxidised at the aromatic methyl group to afford the desired natural product **11** in just one step. Accordingly, we directed our efforts for the preparation of **21** as per the following procedures.

In an attempt to prepare the required chromanone (**21**) in a single step, the *p*-cresol (**22**) was condensed with β,β-dimethylacrylic acid in presence of PPA[26]. The reaction product after work-up was found to be a inseparable (2:1) mixture consisting of the desired chromanone (**21**) and another compound, presumably the coumarin derivative (**23**). Attempted separation/purification of this mixture through routine laboratory methods as well as *via* the semicarbazone derivative did not yield any satisfactory results.



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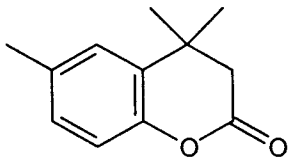
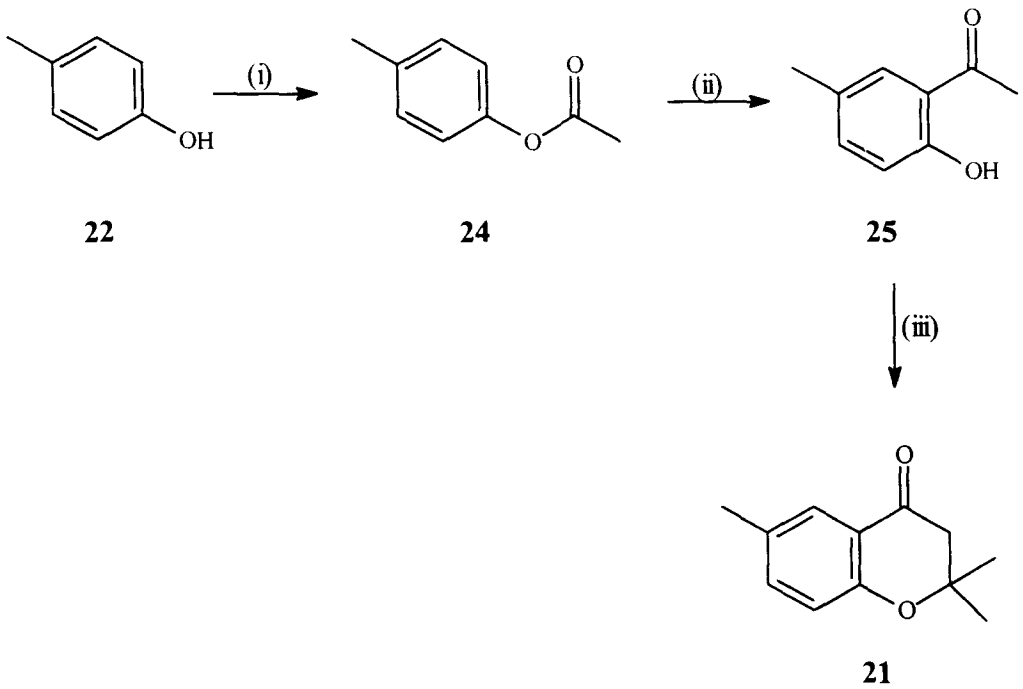


23

Repeating the said condensation reaction by resorting to $ZnCl_2$ & $POCl_3$ instead of PPA as reported by Patel[27] did not provide any solution in respect of obtaining **21** in pure form.

Thus, having failed to obtain the required chromanone (**21**) in pure form by employing the above procedure, we decided to proceed *via* the simple phenolic ester, *p*-cresyl acetate, as shown below:

Scheme 2



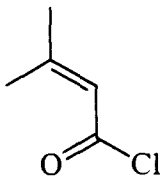
22 **24** **25** **21**

Reagents and conditions: (i) $(CH_3CO)_2O$, pyridine (ii) $AlCl_3$, Heat, 120 ^0C (iii) acetone, piperidine, pyridine, reflux, 72 h.

Accordingly, *p*-cresyl acetate (**24**), prepared by acetylation of *p*-cresol (**22**) with acetic anhydride in presence of pyridine, was subjected to Fries reaction as described in the literature[24] to afford 2'-hydroxy-5'-methylacetophenone (**25**). The latter was refluxed with excess of acetone in presence of piperidine and pyridine for 72 h. The reaction mixture after usual work-up and purification, provided the desired chromanone **21** in 77 % yield. The IR spectrum (fig. 3.13) of compound **21** exhibited band at 1687 cm^{-1}, in addition to the other absorption bands, demonstrating the presence of a carbonyl group.

The EIMS (fig. 3.14) showed the molecular ion peak at m/z 190 corresponding to the molecular formula $C_{12}H_{14}O_2$. The ^1H NMR spectrum (fig. 3.15) showed the presence of three tertiary methyl groups at δ 1.44 (s, 6 H) and 2.3 (s, 3 H). The signal at δ 2.72 was assigned to a isolated methylene group. Further, three aromatic protons were seen at δ 6.82 (d, J = 8.5 Hz, 1 H), 7.28 (dd, J = 8.5 Hz, 2.0 Hz, 1 H) and 7.65 (d, J = 2.0 Hz, 1 H) as expected. The above described spectral characteristics were in conformity with the assigned structure **21** for the said compound.

Alternatively, **21** may also be prepared by another procedure starting from 4-methyl-phenyl-β,β-dimethylacrylate (**27**) which was obtained by the reaction of *p*-cresol and β,β-dimethylacryloyl chloride (**26**). The resulting phenolic ester (**27**) was subjected to Fries reaction to afford the intermediate 1-(2'-hydroxy-5'-methylphenyl)-3-methyl-2-buten-1-one (**28**) which when stirred with NaOH at 50 °C temperature for 5 h underwent cyclization to provide the desired chromanone (**21**).



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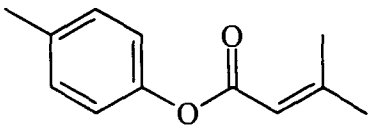

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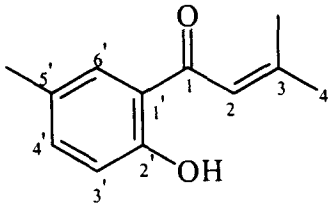

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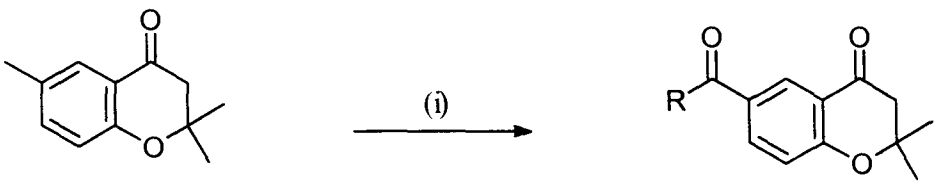
The oxidation of the aromatic methyl group in **21** was accomplished by potassium persulfate and Cu(II) ions in aqueous acetonitrile[28] to furnish lactarochromal (**11**) which was identical in all respects with the synthetic sample obtained earlier.

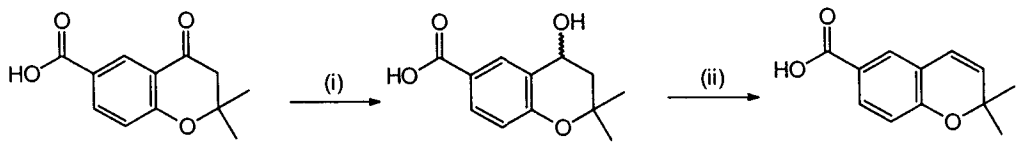


21 **11: R = -H**

13: R = -OH

Reagents and conditions : (i) $K_2S_2O_8$-Cu(II), CH_3CN, H_2O, 75-80 °C, 1 h.

In addition, the above reaction also provided a crystalline compound which was identified as an acid based on its solubility in aq. $NaHCO_3$ solution. The IR spectrum (fig. 3.16) of this acid exhibited characteristic absorption bands at 3450 and 1710 cm^{-1}. The HR-EIMS (fig. 3.17) showed a molecular ion peak at m/z 220.0735 which was consistent with the molecular formula $C_{12}H_{12}O_4$. The ^1H NMR spectrum (fig. 3.18) showed almost similar pattern to that of lactarochromal (**11**), except for the aldehyde proton signal. The ^{13}C NMR spectrum (fig. 3.19) was consistent with the proposed structure **13**. The search of literature with this information revealed that compound **13** has already been reported as a natural product from *Chrysothamnus viscidiflorus*[29]. The spectral data recorded on the synthetic sample **13** agreed well with those reported on the natural product[29], thus constituting a formal synthesis of the acid **13**. It is noteworthy that the synthetic acid **13** was obtained as a crystalline solid in contrast to the naturally occurring material which has been reported as an oil. The acid (**13**) was also converted into Anofinic acid (**12**)[21] *via* the corresponding alcohol (**29**) obtained by $NaBH_4$ reduction followed by dehydration.



13 **29** **12**

Reagents and Conditions: (I) $NaBH_4$, methanol, 0 ^0C , stirred for 40 min (ii) *p*-TsOH, N_2 atmosphere, reflux for 20 h.

The lactarochromal and the corresponding acid were tested against various test pathogens but both were found to be inactive.

Thus, in conclusion, the fungal metabolite lactarochromal (**11**) has been synthesized by two independent routes starting from *p*-acetaminophenol and 2,2,6-trimethylchroman-4-one respectively. The latter route constituted a short and facile synthesis of the natural product. The present work has also resulted in the formal synthesis of the acid **13**, a natural product reported earlier from *Chrysothamnus viscidiflorus*[22]. In addition, the

compound **13** has also been transformed into Anofinic acid (**12**), another natural product from *Anodendron affine*[21] and also a fungal metabolite from *Lactarius deliciosus*.

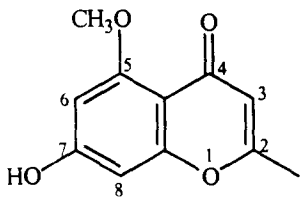
The above described work has already been published[29].

SECTION II

SYNTHESIS OF MARITIMIN, A CHROMONE FROM *PANCRATIUM MARITIMUM*

Oxygenated 2-methylchromones are widespread in Nature and found to possess a wide range of Pharmacological activities[30]. The crude extracts of the plant-material containing these constituents are used in folk medicine for a variety of ailments[31]. For example, the ethanolic extract obtained from the leaves of *Cassia multijuga*, possessing antibacterial activity has been used in African folk medicine[32]. Similarly, Tsui and Brown[33] reported the isolation of three new 2-substituted chromones from the aerial parts of *Baeckea frutescens* L., which is an aromatic low-growing shrub widespread in the grasslands of Hong Kong and is used in traditional Chinese medicine for the treatment of rheumatism and snake-bites[34]. The literature covering the natural occurrence of chromone and its derivatives together with the pharmacological activities exhibited etc. have appeared from time to time in reviews[30,35] and publications[36].

In a recent communication, Youssef, *et al*[37]., reported the isolation and structure elucidation of a new chromone derivative designated as maritimin along with two other new polyoxygenated acetophenones (**31**) & (**32**) together with few known flavanoids from the ethanolic extract of the fresh flowering bulbs of *Pancratium maritimum* L (family Amaryllidaceae, subfamily Amaryllidoidae). The newly reported chromone, maritimin was shown by spectral analysis to be 7-hydroxy-5-methoxy-2-methylchromone (**30**).



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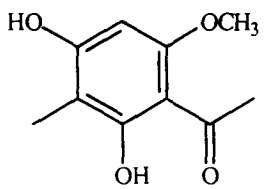

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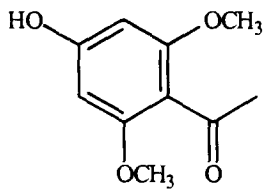

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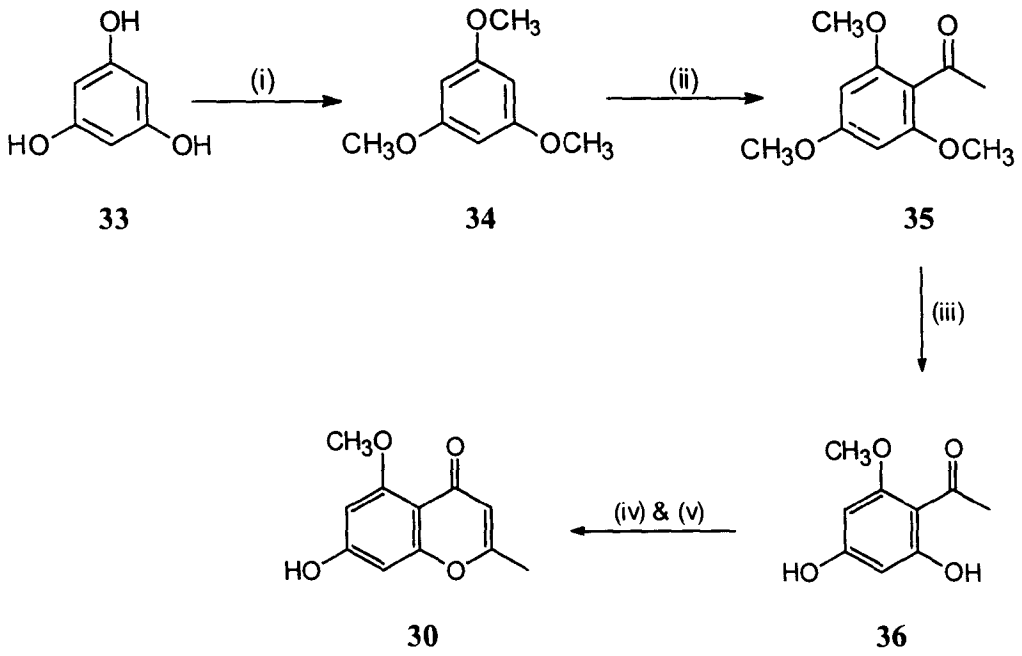
Since the synthesis of maritimin has not been reported and the structure **30** was based only on spectral data, a synthetic support to confirm the assigned structure seemed desirable. With the said aim in mind, we undertook the synthesis of this simple natural product maritimin (**30**) as per the scheme 1.

Scheme 1



Reagents & conditions: (i) DMS, acetone, K_2CO_3, reflux (ii) CH_3COCl, anhydrous $AlCl_3$, -5 ^{0}C to -10 ^{0}C (iii) anhydrous $AlCl_3$, chlorobenzene, reflux 1 h (iv) Na, EtOAc, N_2 atmosphere (v) MeOH, H_2SO_4, reflux 30 min.

In general, the 2-methyl chromones are prepared *via* the corresponding *o*-substituted acetophenones as the versatile intermediates[38,39,40]. Hence, in the present case, the intermediate *o*-hydroxyacetophenone derivative (**36**) could lead maritimin (**30**). Accordingly, to prepare the said intermediate, the easily available starting material namely phloroglucinol (**33**) was converted into its trimethyl ether (**34**), which on acetylation[41] using AcCl/$AlCl_3$ furnished the crystalline acyl trimethyl ether (**36**) with melting point 110 ^{0}C (lit.[42] m.p. 110 ^{0}C). The infrared spectrum of this compound exhibited the carbonyl absorption in the region at 1700 cm^{-1} in addition to other absorption bands. Its ^{1}H NMR spectrum showed signals at δ 3.8 (s, 3H), 3.56 (s, 6 H) and δ 2.45 (s, 3 H), to demonstrate the presence of three methoxy and one methyl groups in the molecule. In the aromatic region, there was a singlet at δ 6.103 with the intensity

of two protons, to indicate the presence of two aromatic protons in the molecule. The EIMS showed the molecular ion peak at 210 which is consistent with the molecular formula $C_{11}H_{14}O_4$. Thus, the above described data were in conformity with the assigned structure **35** for the said crystalline compound.

The compound **35** on partial demethylation[42] afforded 2′,4′-dihydroxy-6′-methoxyacetophenone (**36**) with melting point 205 °C (lit.[42] 205-207 °C). The IR spectrum of this compound showed the bands at 3450 and 1645 cm^{-1} in addition to other absorption bands, thereby indicating the presence of a phenolic hydroxyl and an aryl carbonyl group respectively. The ^1H NMR spectrum showed the presence of a methoxy group at δ 3.82 (s, 3 H) and further a singlet at δ 2.52 (3 H) was attributed for a methyl group. The signal at δ 13.78 (1 H) was found to be exchangeable with D_2O, there by confirming the presence of hydroxyl group in the molecule. Further, two doublets which appeared at δ 5.96 (J = 2.1 Hz, 1 H) and δ 5.86 (J = 2.1 Hz, 1 H) were assigned to the two aromatic protons (H-5, & H-3). The ^{13}C NMR spectrum confirmed the presence of a methoxy group at δ 55.7 and a methyl group at δ 32.4 respectively. The presence of five benzenoid carbon singlets at δ 202.1, 166.1, 165.6, 163.2 & 104 in addition to another pair of the same as doublets at δ 95.5 & 91.2 were all in conformity with the structure **36**. Additional proof for the structure **36** came from the EIMS, which showed the molecular ion peak at m/z 182.1 which was consistent with the molecular formula $C_9H_{10}O_4$.

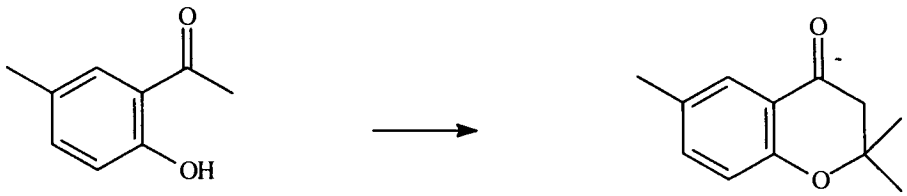
Finally, **36** on reaction with Na & EtOAc followed by acid-catalyzed cyclization of the resulting β-diketone[43] gave a crystalline compound **30**, with melting point 114 °C (lit.[37] m.p. 112-113 °C). The IR spectrum (fig. 3.20) of this compound showed characteristic bands at 3450 and 1665 cm^{-1} for the hydroxyl and benzpyrone moiety, respectively. The HRMS (fig. 3.21) showed a molecular ion peak at m/z 206.0556 consistent with the molecular formula $C_{11}H_{10}O_4$. The ^1H NMR spectrum (fig. 3.22) showed signals due to two aromatic (meta split) proton doublets at δ 6.35 and 6.33 with J = 2.4 Hz, ascribed to C-8 and C-6 protons respectively and two three-proton singlets which appeared at δ 3.84 and 2.37 accounting for an aromatic methoxy group and methyl

group respectively. Saturation of the signal pertaining to the C-2 methyl caused collapse of the signal at δ 6.02 of the H-3 resonance into a sharp singlet. The diagnostic signal for the presence of a chelated hydroxyl group was not observed, thereby indicating that the methoxy group was present at C-5 and the hydroxyl group was placed at C-7. The ^{13}C NMR spectrum (fig. 3.23) confirmed the presence of a methyl and a methoxy group at δ 20.8 and 56.9 respectively. The presence of six benzenoid carbons as singlets at δ 180.5, 167, 163.3, 160.8, 158 and 104.3 and another three carbons as doublets at δ 108.7, 97.1 and 94.9 proved beyond doubt the structure **30** for the said crystalline compound. Thus, the spectral data (IR, ^{1}H NMR & MS) and the m.p. reported on the synthetic sample were in good agreement with the reported data on maritimin[8]. The ^{13}C NMR spectrum, not measured earlier on natural maritimin, has now been recorded on our synthetic sample and is fully consistent with the structure **30**. Incidentally, this is the first report of the synthesis of maritimin.

SECTION III

ON THE STRUCTURE AND SYNTHESIS OF CYCLOHEXENONE DERIVATIVES

In connection with the synthesis of a fungal metabolite-lactarochromal, (the details of which has been described in the first section of this chapter), the compound 2,2,6-trimethylchroman-4-one (**21**) was required as the key intermediate. Accordingly, by following a literature report[45], an attempt was made to obtain the compound **21** by refluxing 2′-hydroxy-5′-methylacetophenone (**25**) with pyrrolidine in excess of acetone for 5 h.

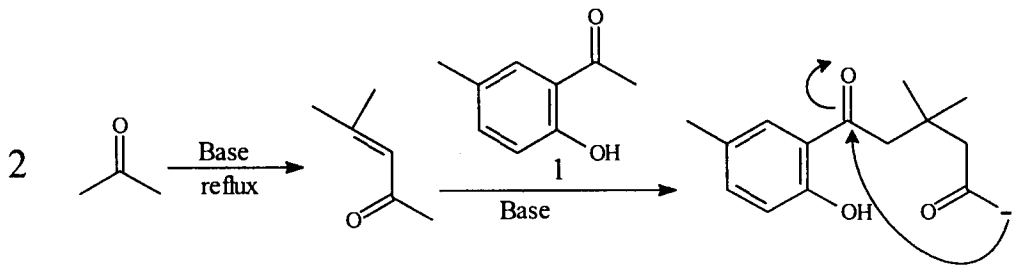


25 **21**

However, surprisingly, the reaction mixture after work-up provided a crystalline compound with melting point 145 ^{0}C. The IR spectrum (fig. 3.24) of this crystalline compound showed bands at 3450 and 1635 cm^{-1} (in addition to the other absorption bands) accounting for the presence of a hydroxyl group and the α,β-unsaturated carbonyl group respectively in the molecule. The EIMS (fig. 3.25) showed the molecular ion peak at m/z 230, corresponding to the molecular formula $C_{15}H_{18}O_2$. The chemical ionization mass spectrum showed peaks at m/z 231.1 ($M^+ + 1$), 248.1 ($M^+ + NH_4$), 462.3 ($2M^+ + 1$), 478.4 ($2M^+ + NH_4$) and 708.5 ($3M^+ + NH_4$), which further confirmed the above said molecular formula for the crystalline compound. The ^{1}H NMR spectrum (fig. 3.26) showed an aromatic methyl group appearing at δ 2.28 (3 H) and a gemdimethyl group at δ 1.13 (6 H). The signal at δ 7.78 (1 H) was found to be exchangeable with D_2O, and hence was assigned to the phenolic hydroxyl group present in the molecule. The presence of two isolated methylene groups were indicated by the signals at δ 2.67 (s, 2H) and 2.37 (s, 2 H). Further, the ABX-type signals for three aromatic protons at δ 7.05 (d, J = 2.0 Hz, 1 H), 7.01 (dd, J = 8.3 Hz, 2.0 Hz, 1 H) and 6.88 (d, J = 8.3 Hz, 1 H) indicated the presence of an 1,3,4 (or 1,2,4)-trisubstituted aromatic ring in the molecule. Further, a singlet at δ 6.78 (1 H) was assigned to a olefinic proton. The ^{13}C NMR spectrum (fig. 3.27) confirmed the presence of two methylene groups appearing at δ 50.7 and 43.8. It

also confirmed the presence of two methyl groups appearing at δ 28.3 attached to a quaternary carbon, besides the signal at δ 20.5 was assigned to the methyl on the aromatic ring while the signal at δ 34.01 was accounted for the quarternary carbon on which the gemdimethyl group is placed. Six benzenoid carbons, three of them appearing as doublets at δ 131.2, 128.7 and 125.5 while other three appearing as singlets at δ 158.7, 129.2 and 125.5 were also observed. The signals at δ 202.4, 152.2 and 116.5 were assigned to an α,β-unsaturated carbonyl carbon, a C-3 tertiary carbon and the C-2 olefinic carbon respectively. On the basis of above described spectral data, it can be concluded that the crystalline compound obtained during the reaction of **25** with excess of acetone in presence of pyrrolidine at reflux temperature is 3-(2′-hydroxy-5′-methylphenyl)-5,5-dimethyl-cyclohex-2-en-1-one (**37a**) and not the expected chromanone derivative (**21**). However, it may be noted that **21** was obtained when pyrrolidine was replaced with a mixture of pyridine and piperidine as the base in the reaction.

The formation of **37a** as the product of the reaction in the present case could be easily rationalized. Generation of mesityl oxide from acetone and then, condensation with **25** to provide the diketo intermediate **38** (as shown in the chart-1) which will further undergo intramolecular aldol cyclization followed by dehydration to provide the cyclohexenone derivative (**37a**) in a one-pot operation.



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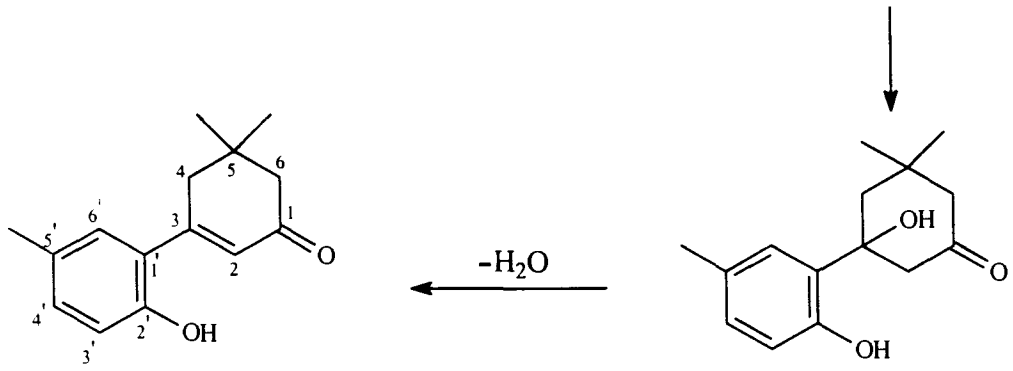
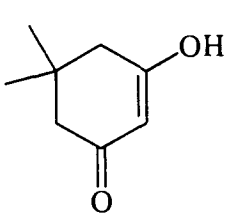


37a

Chart-I

It is well known that, the α- and β- substituted cyclohexenone derivatives serve as valuable intermediates in organic synthesis[46]. These intermediates, whenever needed are generally prepared in multiple steps depending upon the synthetic plan envisaged for their onward use. The formation of the cyclohexenone derivative (**37**) in one step from the corresponding acetophenone and acetone during the present study prompted us to investigate the said reaction further.

A look at the literature revealed the presence of few isolated reports[47-49] pertaining to the preparation of 3-phenyl substituted cyclohexenones. In one such report, Woods[47,48] had examined the reaction of dimedone (**39**) with phenyl magnesium bromide and reported the formation of 5,5-dimethyl-3-phenyl-Δ^2-cyclohexenone (**40**) as one of the product in 31 % yield. Similarly, Broquet[49] investigated the reaction between the Wittig reagent (**41**) and the aliphatic α, β- unsaturated ketones and reported the formation of substituted cyclohexenones (**42**).



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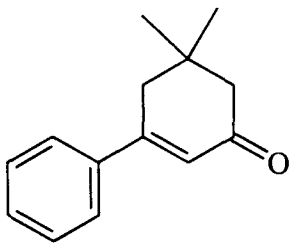

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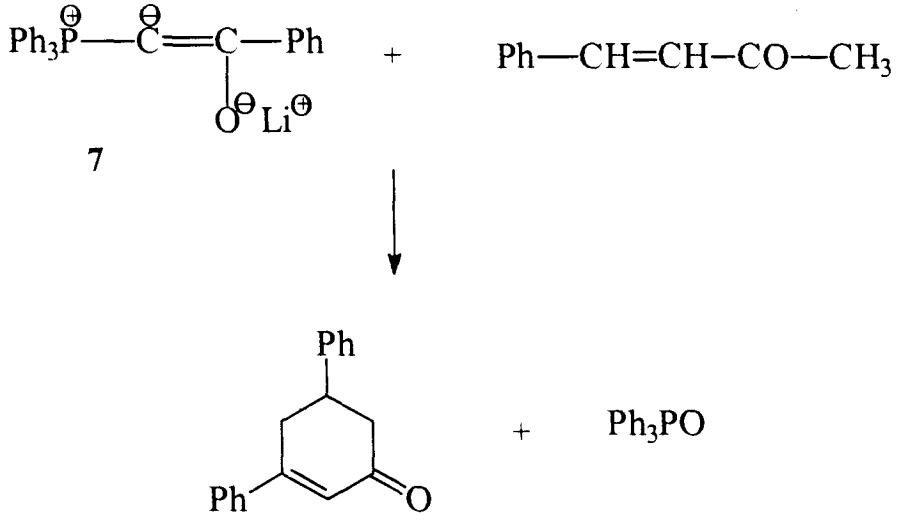

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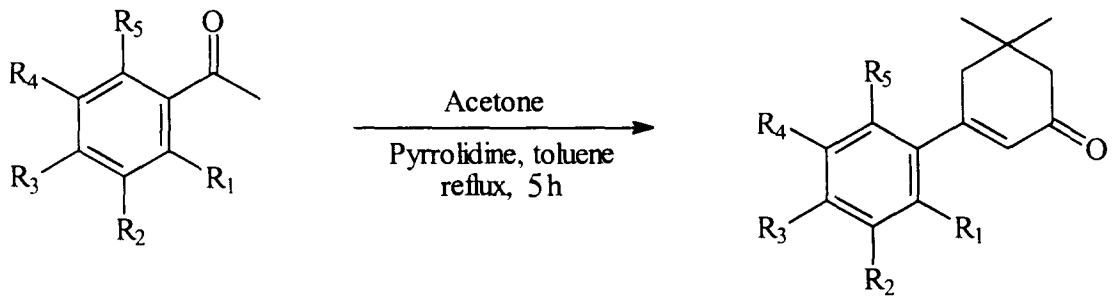
42

Thus, by taking into consideration, the simplicity of the procedure by which **37** was produced in one-pot compared to the above cited literature methods, we were curious to find out wether the same could be exploited further towards the preparation of such 3-aryl substituted cyclohexenone analogues in general. Encouraged by the one-pot synthesis of 3-(2′-hydroxy-5′-methylphenyl)-5,5-dimethyl-cyclohex-2-en-1-one (**37a**) as described above, we desired to test the above procedure on other substituted acetophenone derivatives and thus extended the said methodology to the preparation of two more cyclohexenone derivatives as shown in the Scheme 1 & table 1.

Scheme 1



25 **37**

Table 1

Compound	R_1	R_2	R_3	R_4	R_5	Yield (%)
37a	OH	H	H	CH_3	H	43
37b	OH	H	H	H	H	58
37c	OH	H	H	$NHCOCH_3$	H	37
37d	CH_3	H	CH_3	H	CH_3	---
37e	H	Cl	Cl	H	H	---

Accordingly, the other acetophenone derivatives employed in the present study were 2-hydroxyacetophenone (**25b**) and 2-hydroxy-5-acetaminoacetophenone (**25c**). The acetophenone (**25b**), when subjected to the said conditions, provided 3-(2′-hydroxyphenyl)-5,5-dimethyl-cyclohex-2-en-1-one (**37b**) as a light yellow coloured crystalline solid, melting point 121 ^{0}C in 58 % yield. The IR spectrum (fig. 3.28) of this compound showed bands at 3420 and 1630 cm^{-1}, (in addition to other absorption bands) thereby indicating the presence of a phenolic hydroxyl and the α,β-unsaturated carbonyl group respectively. The molecular weight of the compound was found to be 216 by EIMS (fig. 3.29), corresponding to the molecular formula $C_{14}H_{16}O_2$. The ^{1}H NMR spectrum (fig. 3.30) showed signals at δ 8.12 (br s, 1 H) which was found to be exchangeable with D_2O, conforming the presence of a hydroxyl group. It further disclosed two tertiary methyl signals at δ 1.15 (6 H) which was assigned to the gemdimethyl group. The presence of two isolated methylene groups and the olefinic proton were indicated by the signals at δ 2.68 (s, 2 H), 2.38 (s, 2 H) and 6.81 (s, 1 H) respectively. Further, the signals at δ 7.26 (d, J = 8 Hz, 1 H), 7.22 (dd, J = 8 Hz, 2.0 Hz, 1 H), 6.99 (t, J = 8 Hz, 1 H) and 6.9 (d, J = 8 Hz, 1 H) which were observed in the aromatic region, indicated the presence of four aromatic protons and consequently a 1,2-disubstituted aromatic ring in the molecule. The ^{13}C NMR spectrum (fig. 3.31) confirmed the presence of two methylene groups which appeared at δ 50.7 and 43.6. It also confirmed the presence of two methyl groups appearing at δ 28.3 attached to a quaternary carbon, while the signal at δ 34.01 accounted for the quaternary carbon to which

gemdimethyl group is attached. Further, the signals at δ 202.6, 154.5 and 116.9 were assigned to an α,β-unsaturated carbonyl carbon, the C-3 carbon to which the aryl substitutent is attached and the C-2 olefinic carbon respectively. The presence of two benzanoid carbons, as singlets at δ 158.6 and 126.1 and another set of four as doublets at δ 13.7, 128.5, 126.6 and 120.1 were all in conformity with the structure **37b** for the said crystalline compound.

Similarly, the acetophenone (**25c**) furnished the 3-(2′-hydroxy-5′-acetamino-phenyl)-5,5-dimethyl-cyclohex -2-en-1-one (**37c**) as a light yellow crystalline solid, melting point 229 ^{0}C, in 37 % yield. The IR spectrum (fig. 3.32) exhibited bands at 3290, 3190 and 1645 cm^{-1}, (in addition to other absorption bands) indicating the presence of hydroxyl, imino and the α,β-unsaturated carbonyl group respectively. The EIMS (fig. 3.33) showed the molecular ion peak at m/z 273.1 corresponding to the molecular formula $C_{16}H_{19}O_3N$. The ^{1}H NMR spectrum (fig. 3.34) disclosed the singlets at δ 1.99 (3 H) & 2.2 (6 H) which could be conveniently assigned to an -NHCO**CH$_3$** group and to the gemdimethyl group respectively. The presence of an imino and a hydroxyl groups were confirmed by the presence of signals at δ 9.69 (s, 1 H, disappeared on addition of D_2O) and 9.58 (br s, 1 H, disappeared on addition of D_2O) respectively. The signals at δ 6.15 (s, 1 H), 2.62 (s, 2 H) and 2.25 (s, 2 H) were assigned for the olefinic proton and the two isolated methylene groups respectively. In addition, the signals at δ 7.45 (d, J = 2 Hz, 1 H), 7.4 (dd, J = 8 Hz, 2.0 Hz, 1 H) and 6.82 (d, J = 8 Hz, 1 H) appearing in the aromatic region, confirmed the presence of three aromatic protons in the molecule. The ^{13}C NMR spectrum (fig. 3.35) confirmed the presence of two methylene groups which appeared at δ 50.5 and 43 and two methyl groups appearing at δ 27.7 attached to a quaternary carbon. The signal at δ 23.6 was assigned to the methyl of the amido group on the aromatic ring while the signal at δ 34.01 was accounted for the quaternary carbon to which the gemdimethyl group is attached. Similarly, the other signals appearing at δ 198.8, 167.6, 150.6 and 116.1 were assigned to the α,β-unsaturated carbonyl carbon, amide carbonyl carbon, a tertiary carbon (at C-3) and the olefinic carbon (C-2) respectively. Further, the presence of three benzenoid carbon which appeared as singlets at δ 156.1, 131.2 and 126.4 and another three carbons as doublets at δ 125.9, 12.5 and 119.5 were also

observed. All the above described spectral data were in perfect agreement with the structure **37c** for the said crystalline compound.

In case of 2,4,6-trimethylacetophenone (**25d**), however, it was observed that the reaction did not proceed further and the same resulted in the recovery of starting material. Similar was the situation with respect to 3,4-dichloroacetophenone (**25e**). These results indicated that the acetophenone substrates employed must be fairly reactive/activated for the reaction to take place under the experimental conditions employed. Nevertheless, the present reported method has the necessary potential to emerge as a convenient method for the simple and facile preparation of 3-aryl substituted-cyclohex-2-en-1-one starting from the appropriately substituted acetophenone derivatives, and thus, it may be concluded that during the present study, the said method has been successfully used for the one-pot preparation of three new cyclohexenone derivatives in fair yields.

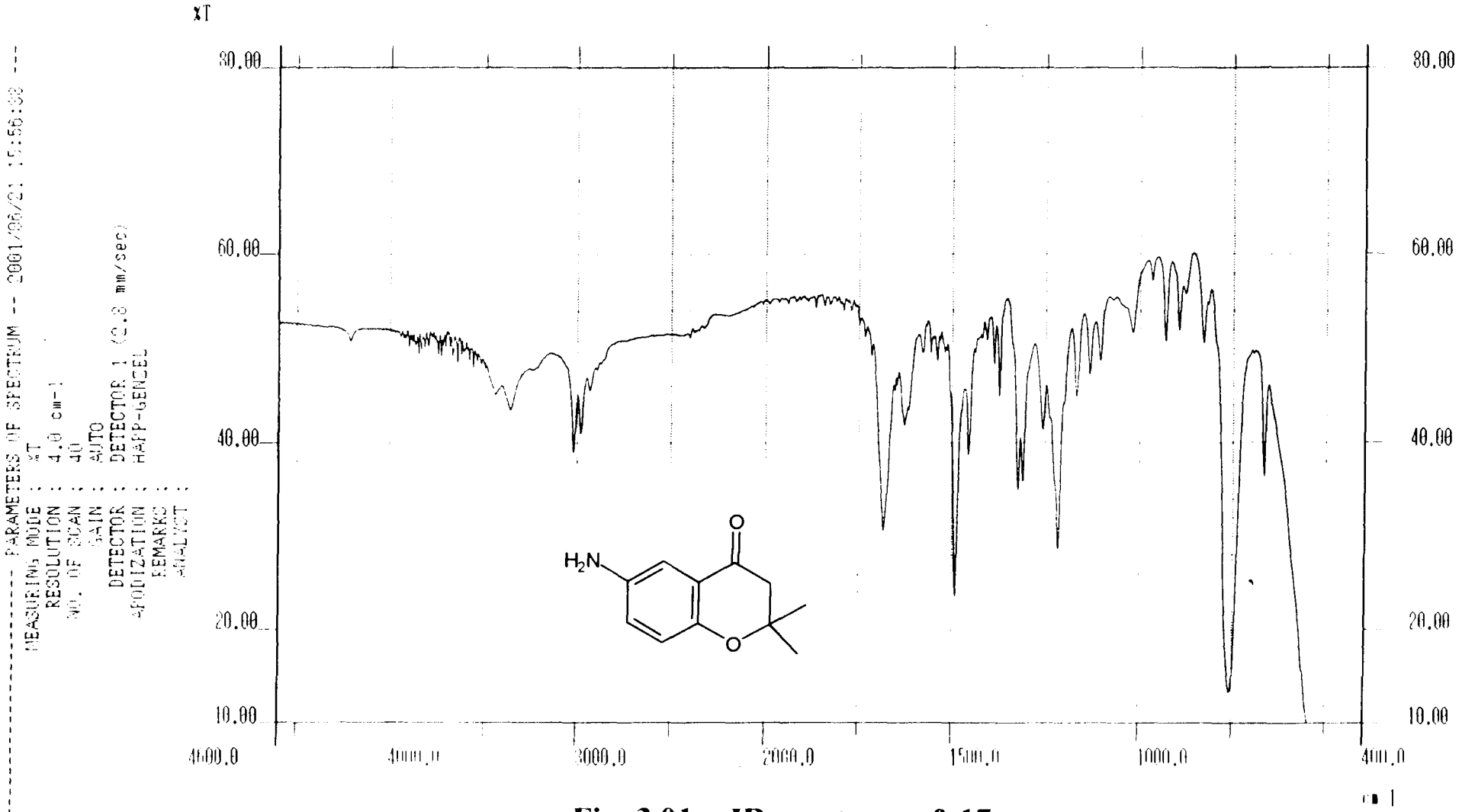


Fig. 3.01 : IR spectrum of 17

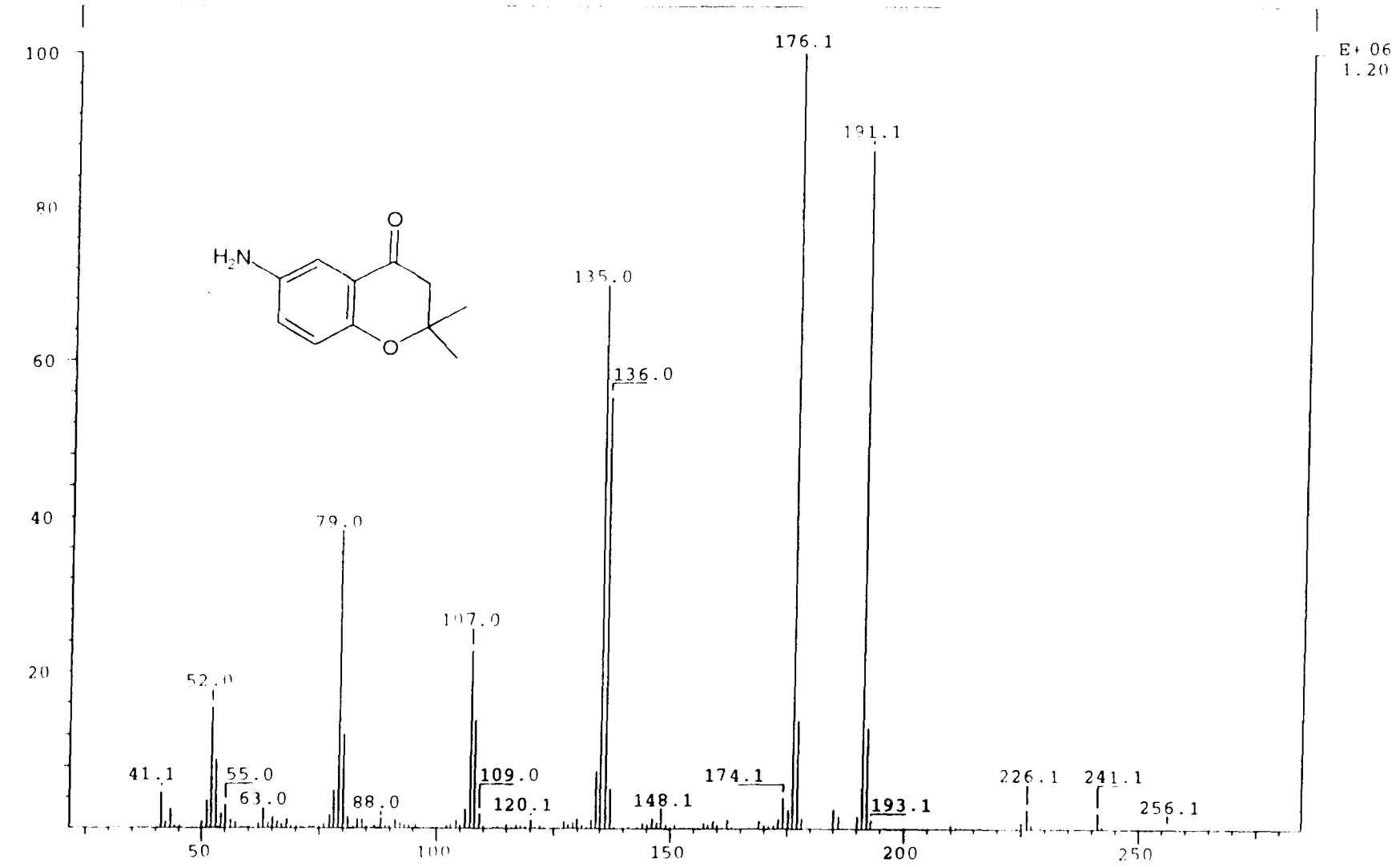


Fig. 3.02 : EI mass spectrum of 17

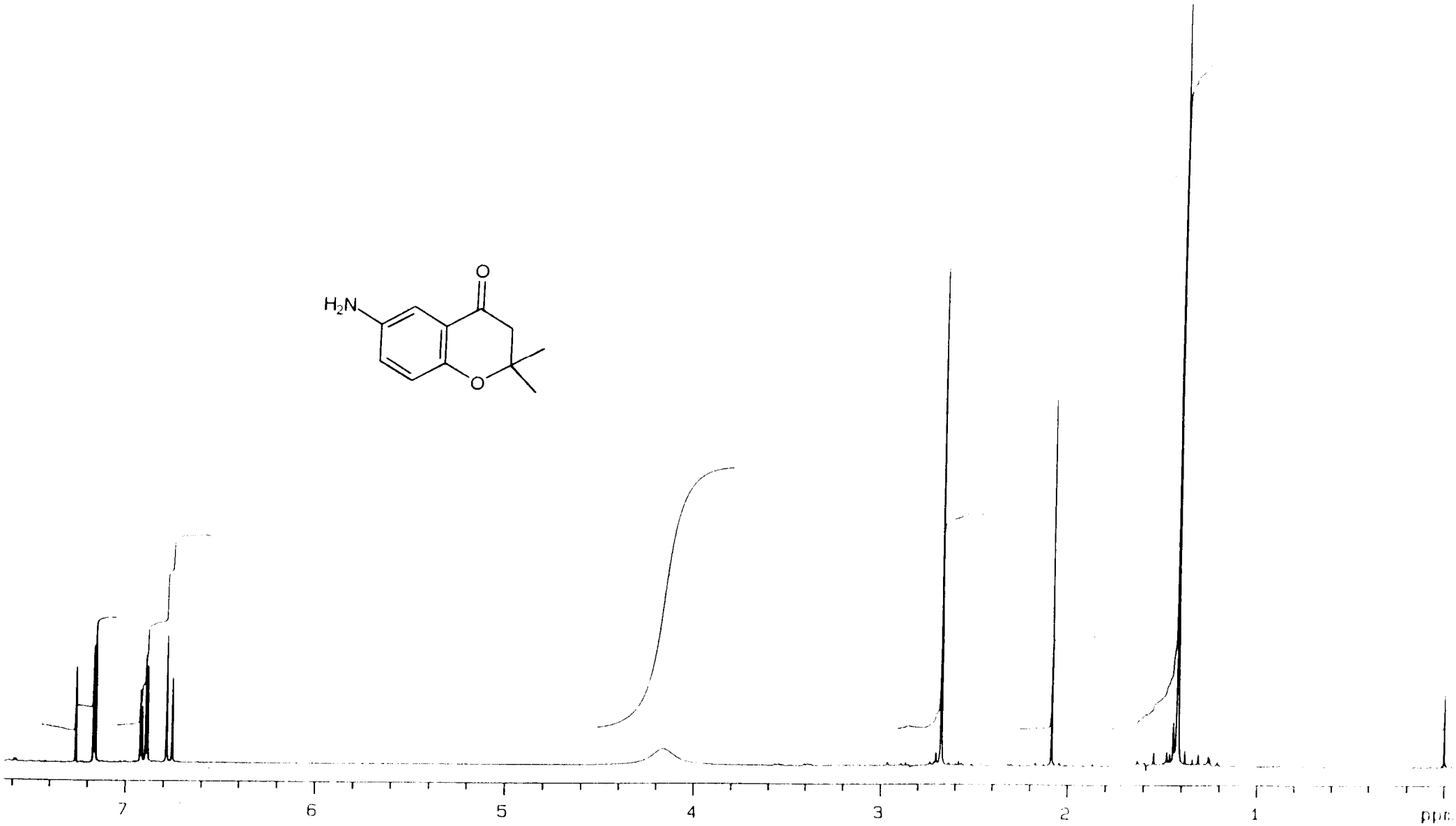


Fig. 3.03 : ^{1}H NMR spectrum of 17 in $CDCl_3$

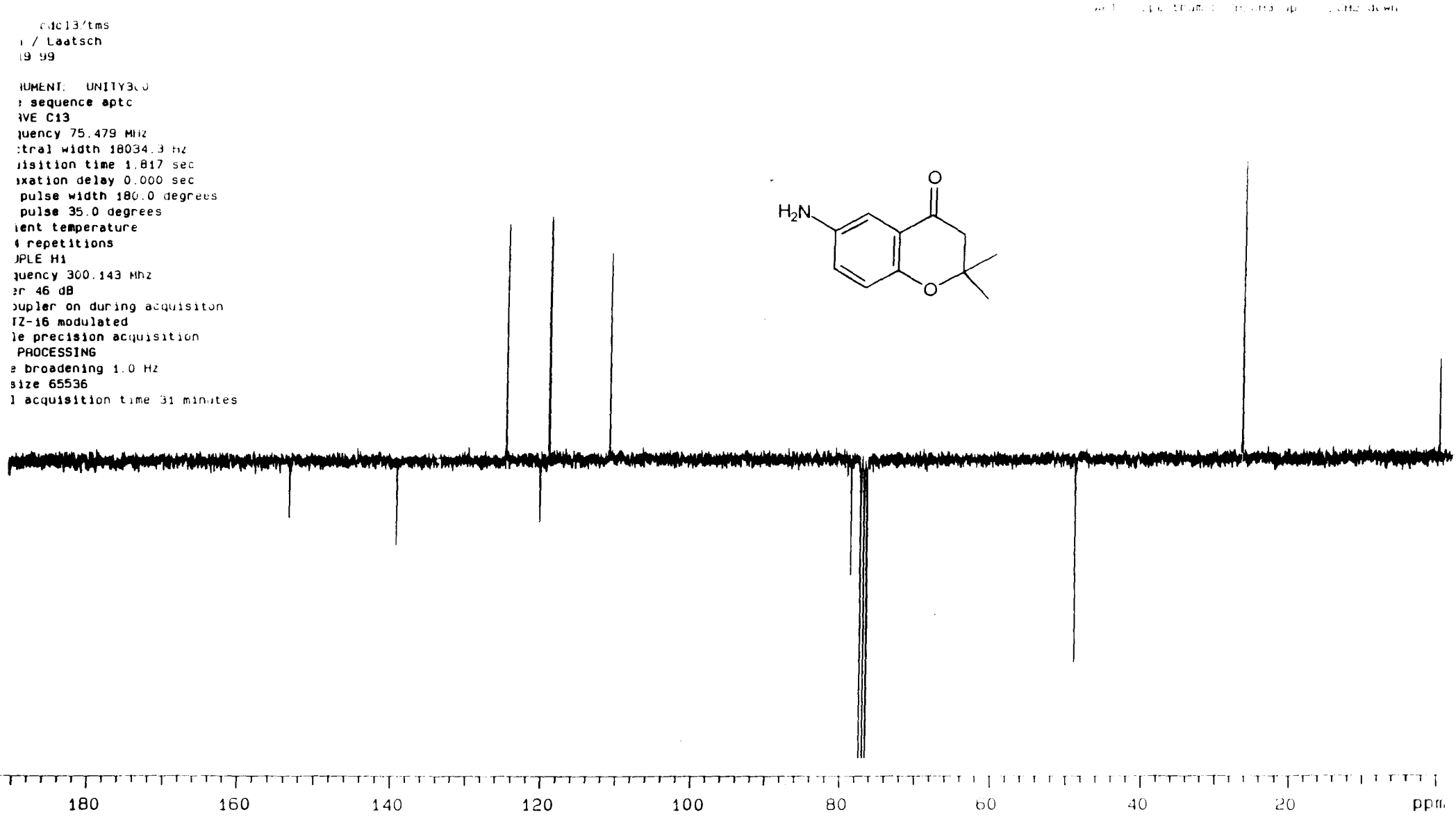


Fig. 3.04 : ^{13}C NMR spectrum of 17 in $CDCl_3$

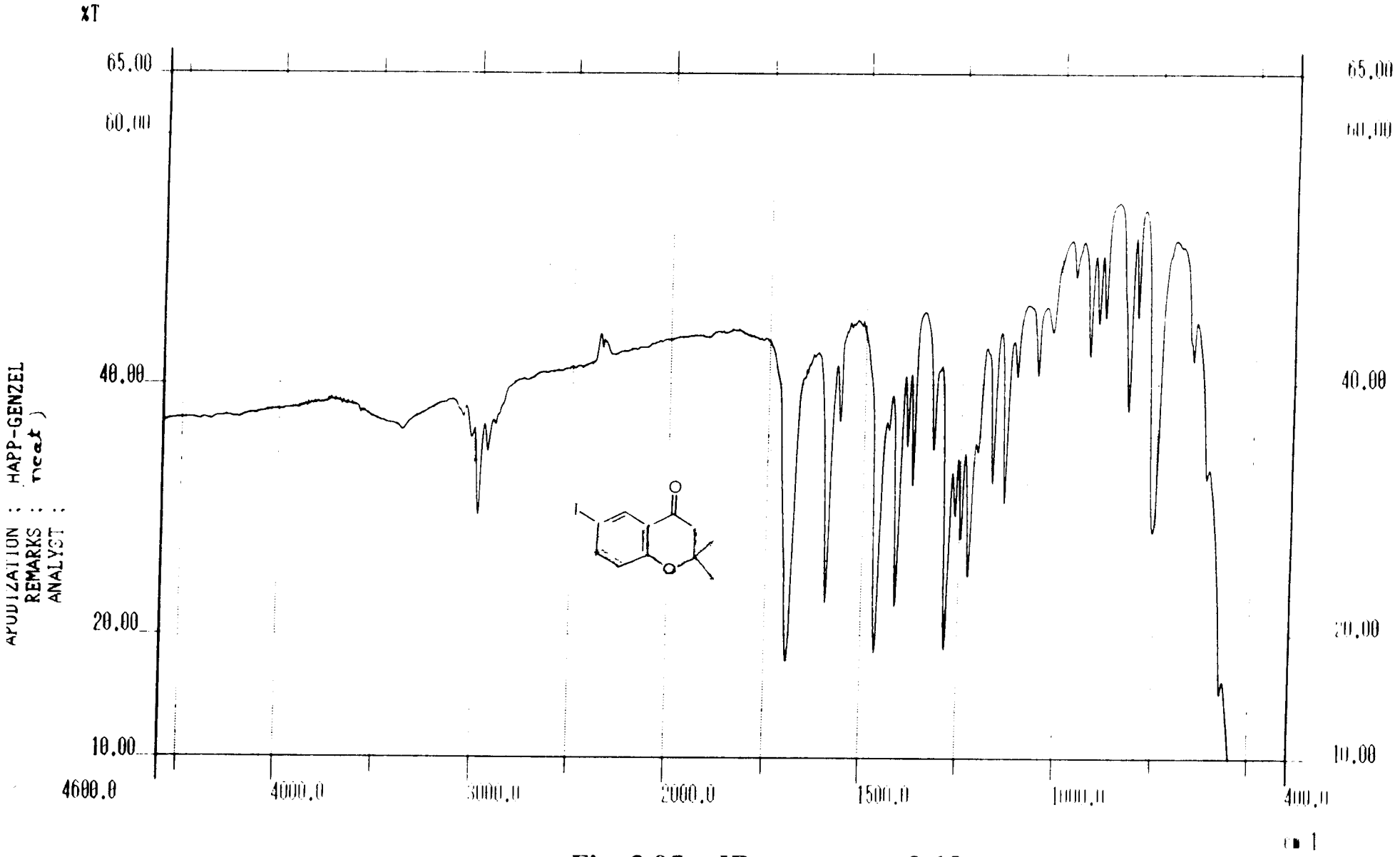


Fig. 3.05 : IR spectrum of 18

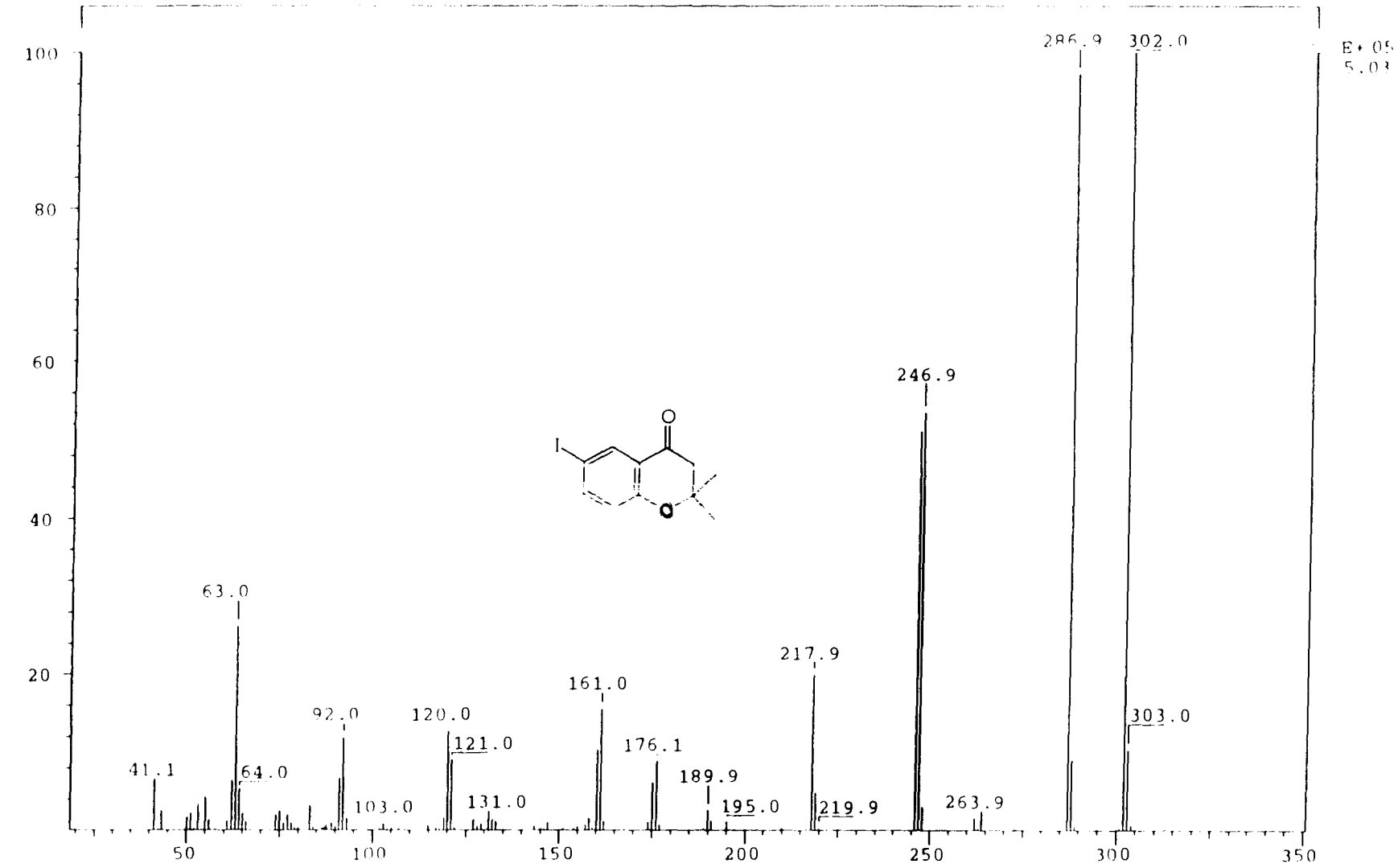


Fig. 3.06 : EI mass spectrum of 18

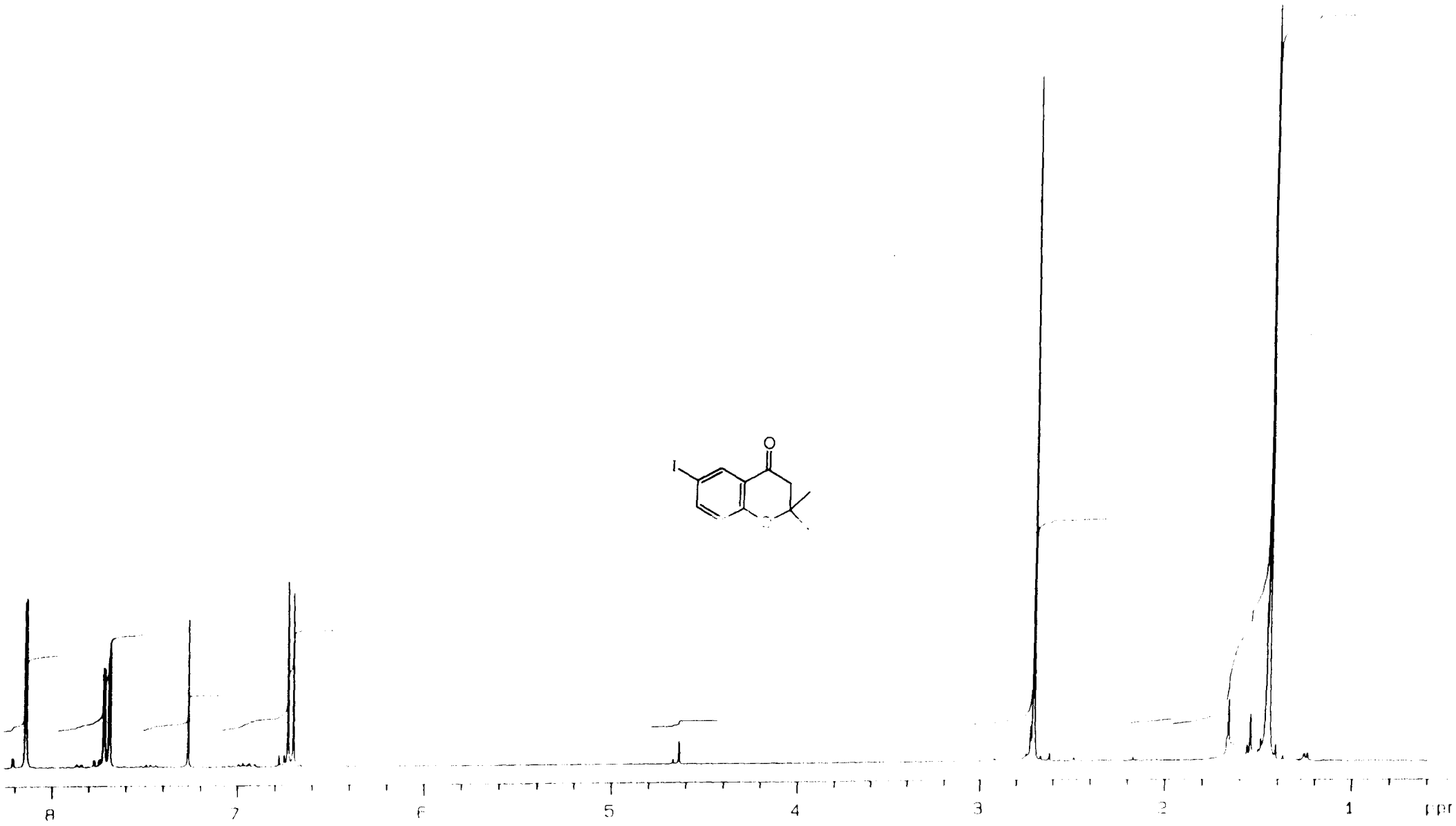


Fig. 3.07 : ^{1}H NMR spectrum of 18 in $CDCl_3$

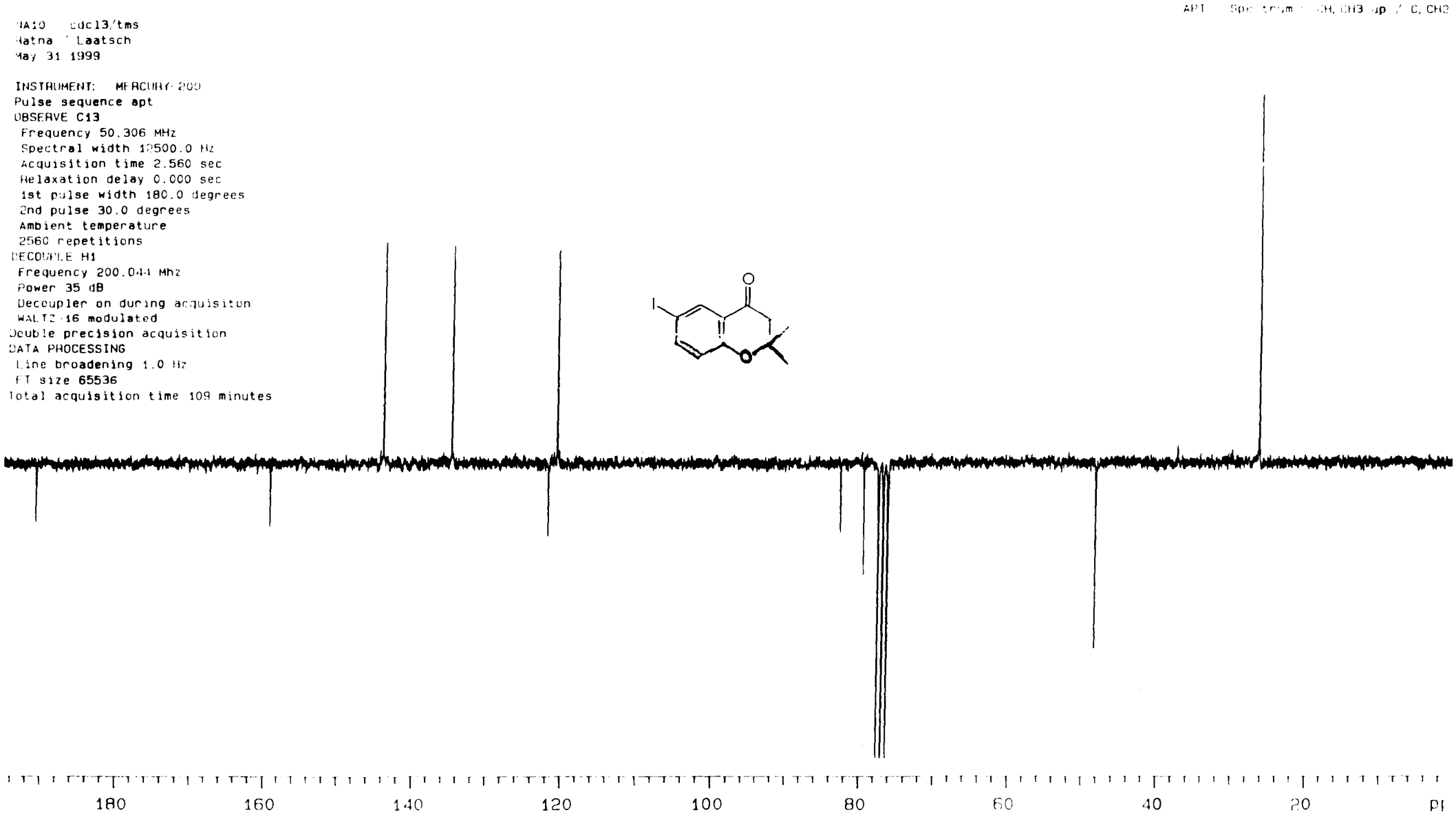


Fig. 3.08 : ^{13}C NMR spectrum of 18 in $CDCl_3$

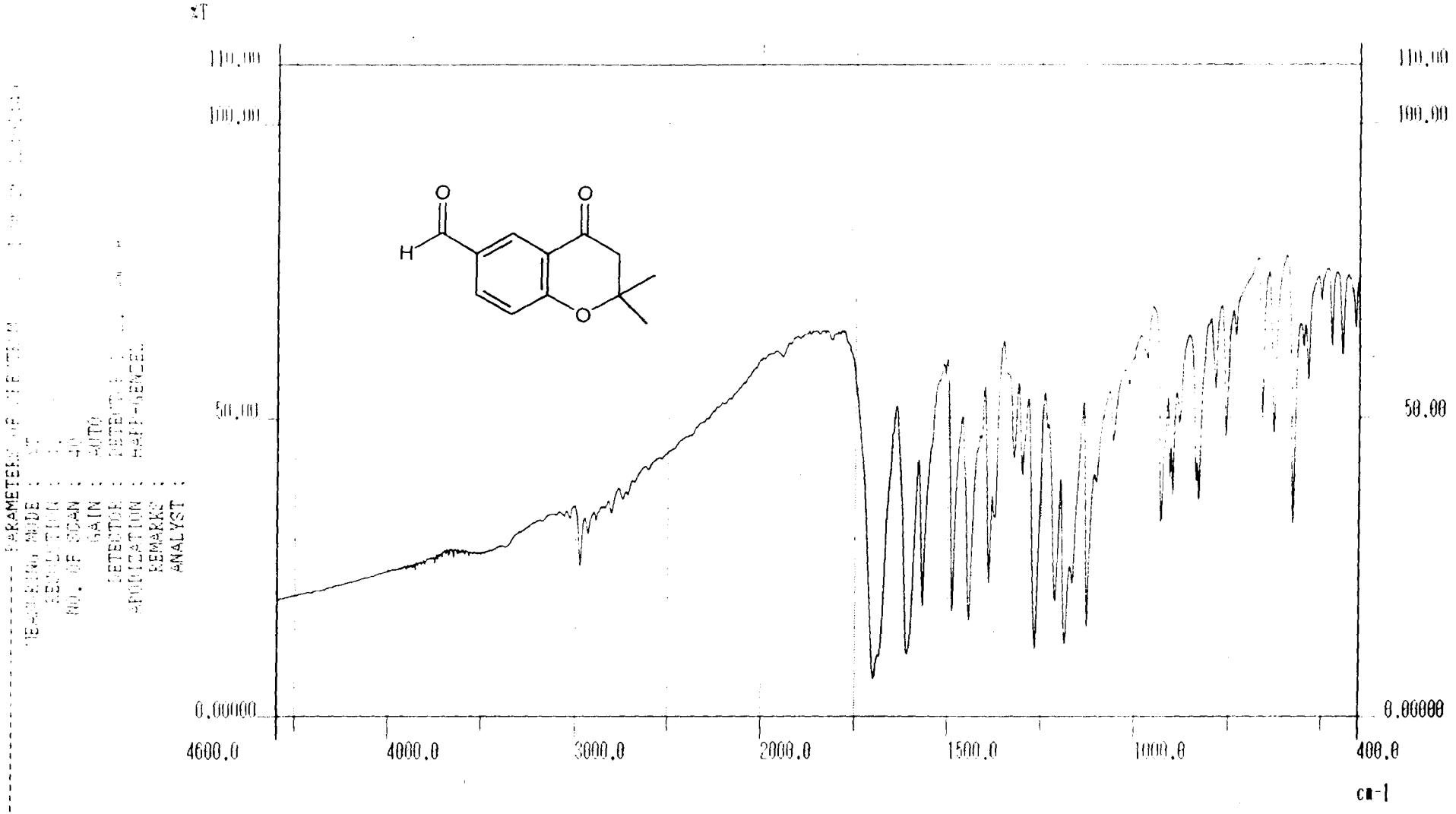


Fig. 3.09 : IR spectrum of lactarochromal (11)

Fig. 3.10 : EI mass spectrum of lactarochromal (11)

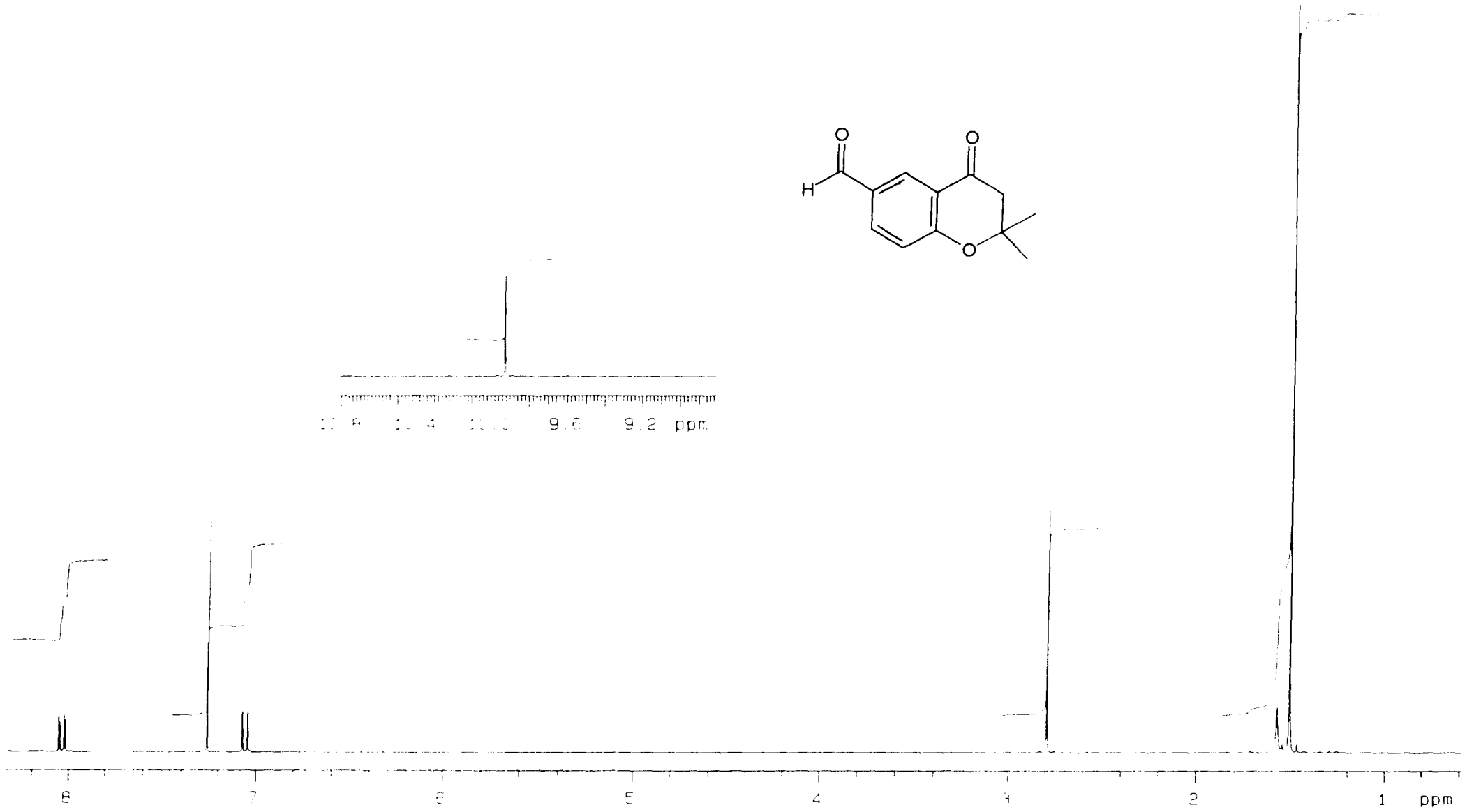


Fig. 3.11 : ^{1}H NMR spectrum of lactarochromal (11) in $CDCl_3$

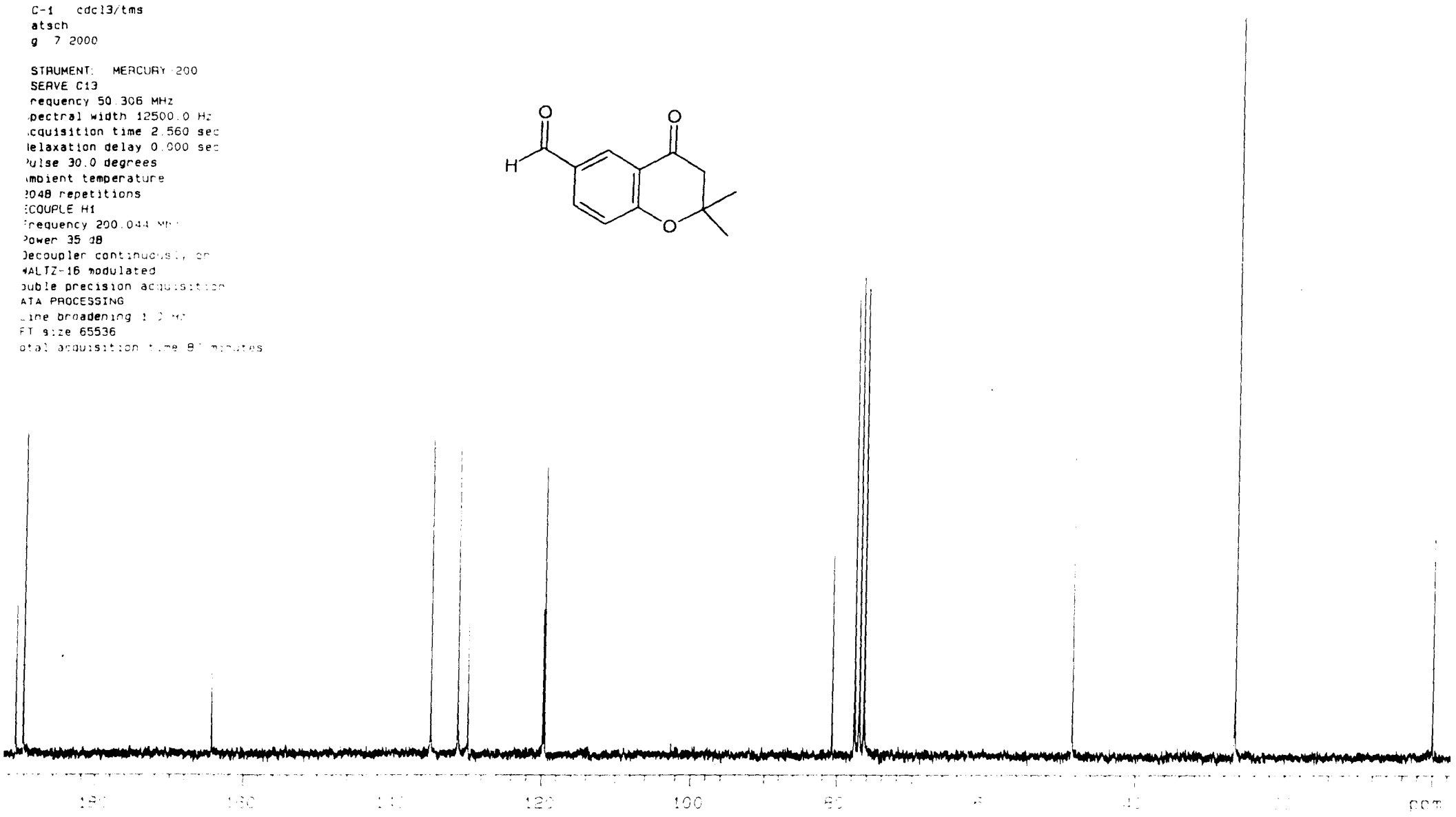


Fig. 3.12 : ^{13}C NMR spectrum of lactarochromal (11) in $CDCl_3$

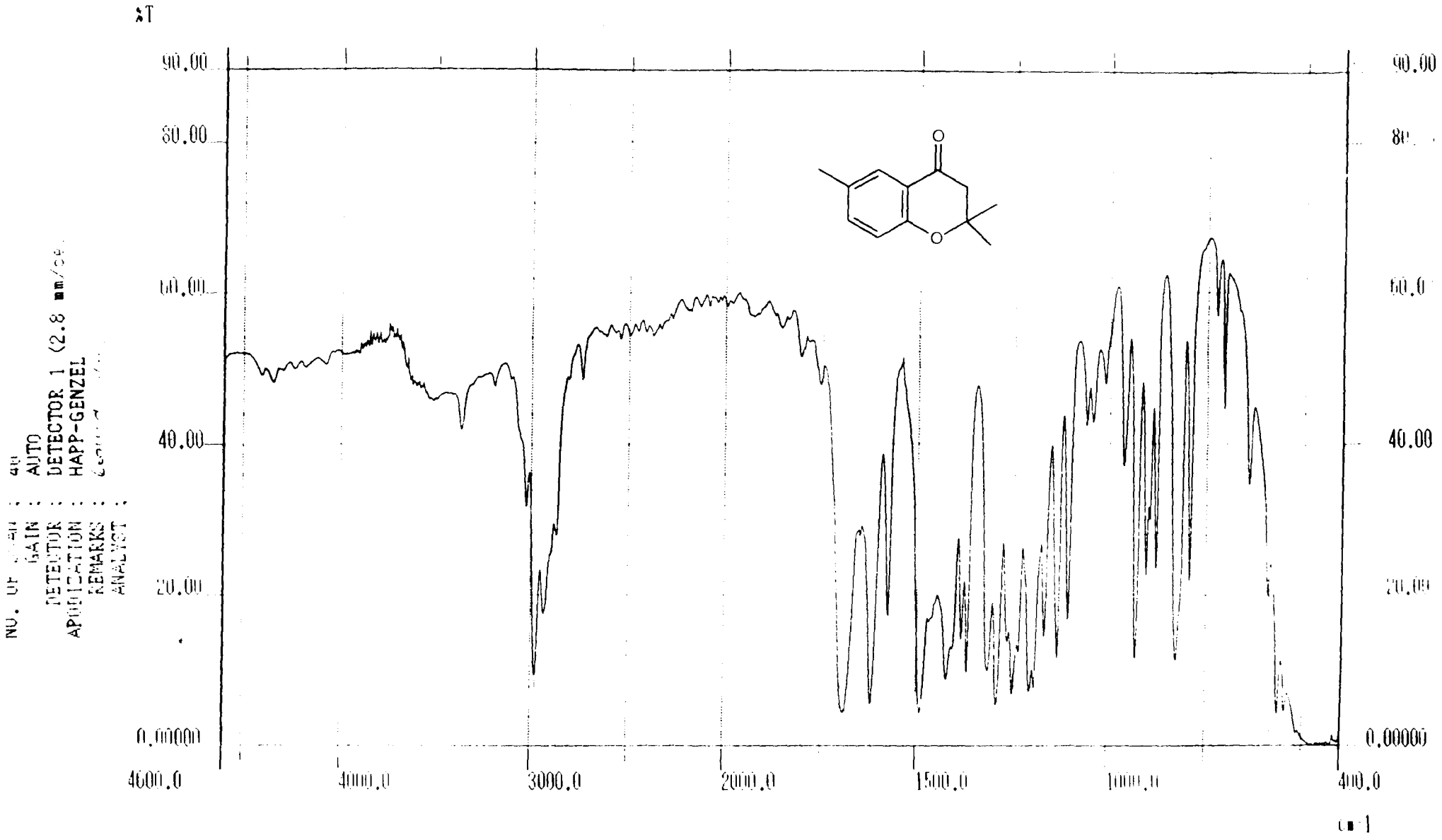


Fig. 3.13 : IR spectrum of 21

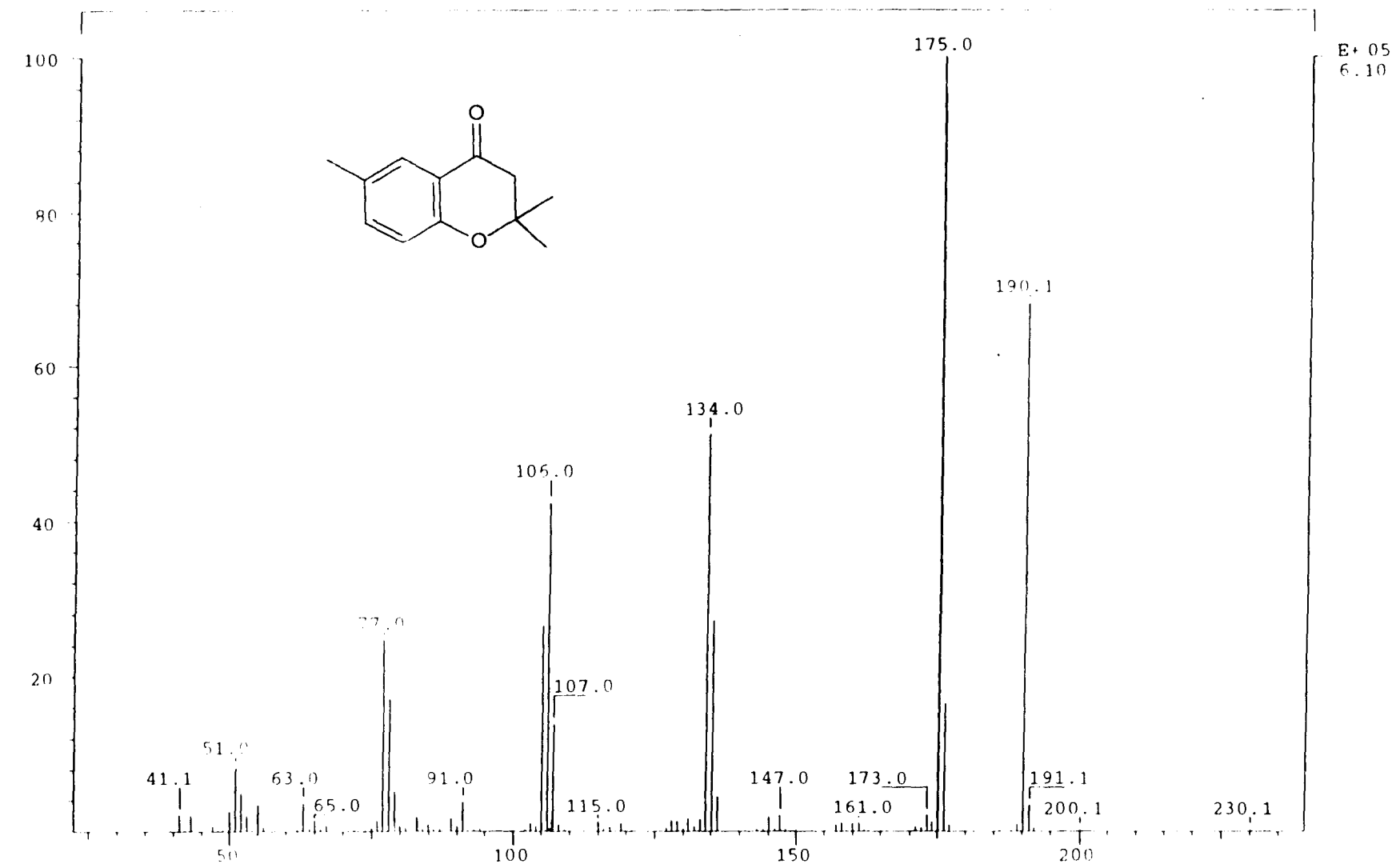


Fig. 3.14 : EI mass spectrum of 21

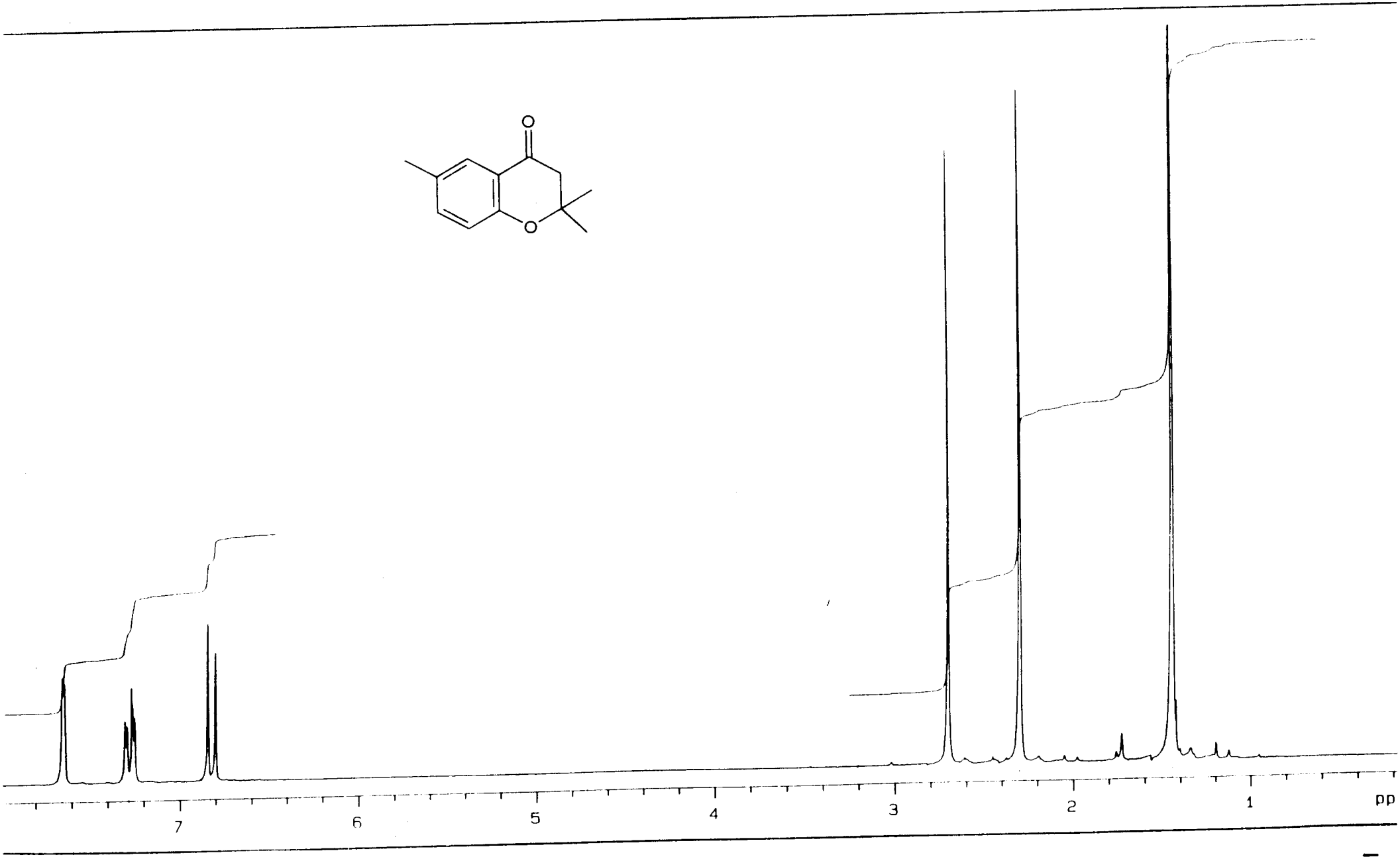


Fig. 3.15 : ^{1}H NMR spectrum of 21 in $CDCl_3$

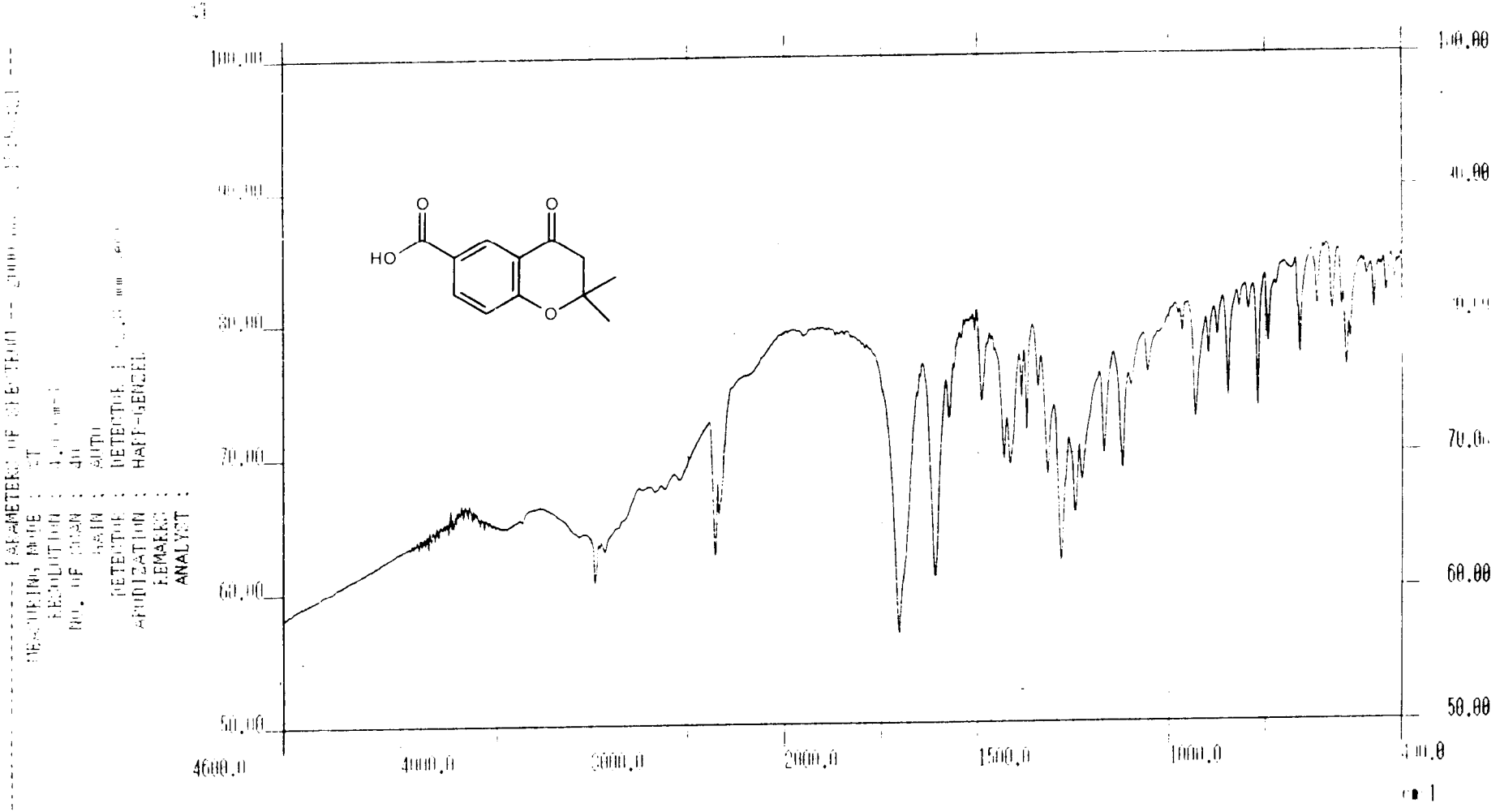


Fig. 3.16 : IR spectrum of 13

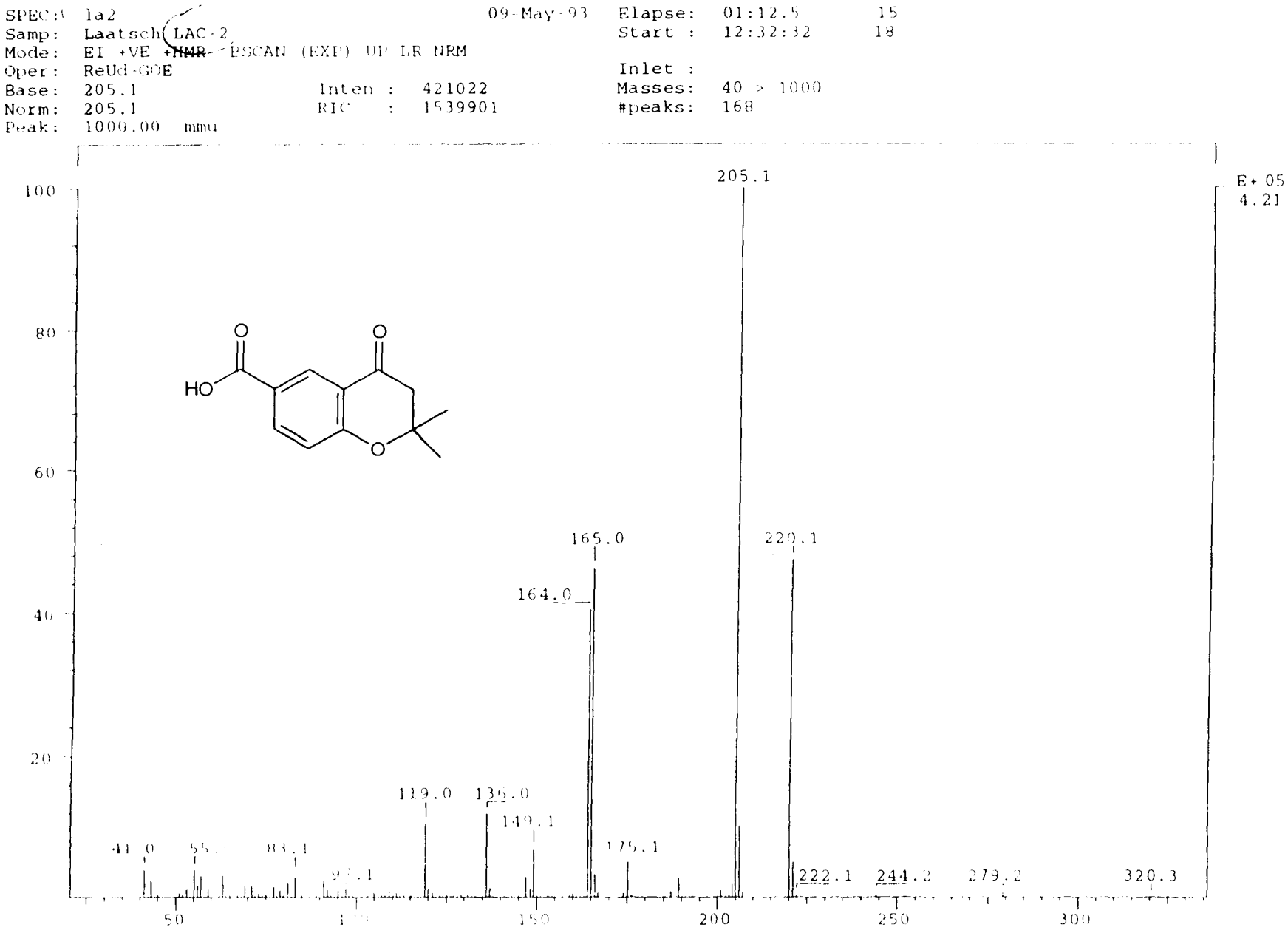


Fig. 3.17 : EI mass spectrum of 13

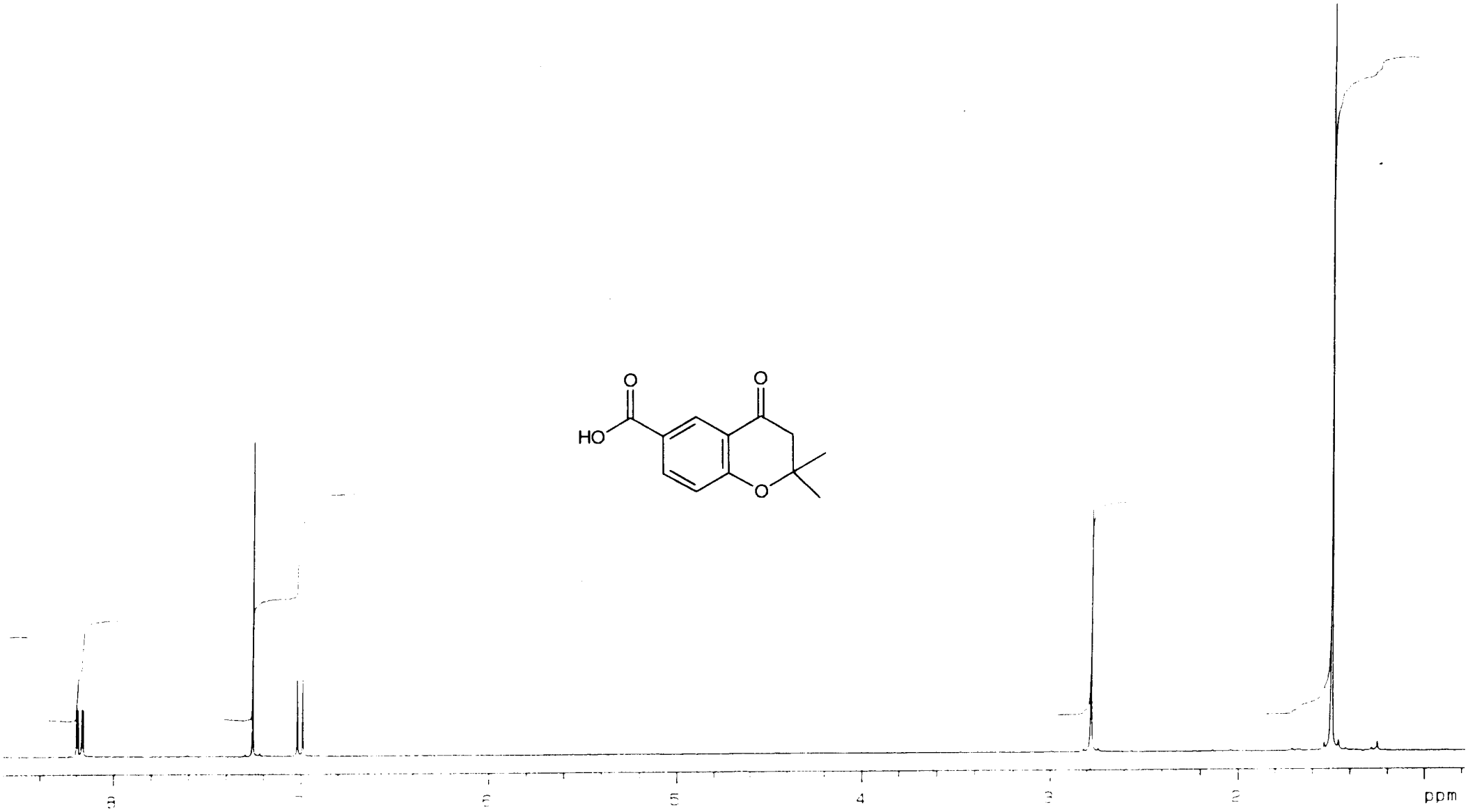


Fig. 3.18 : ^{1}H NMR spectrum of 13 in $CDCl_3$

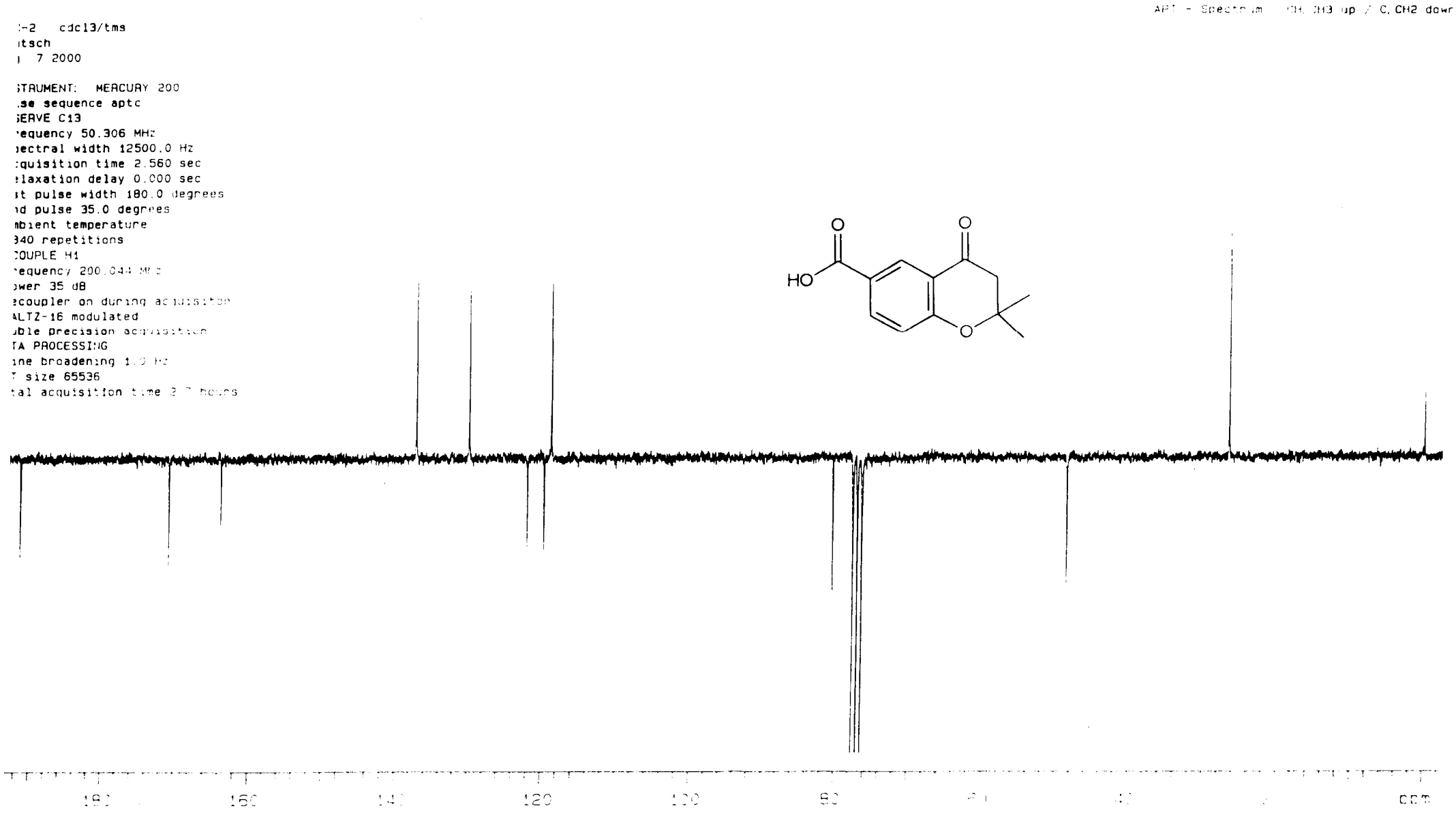


Fig. 3.19 : ^{13}C NMR spectrum of 13 in $CDCl_3$

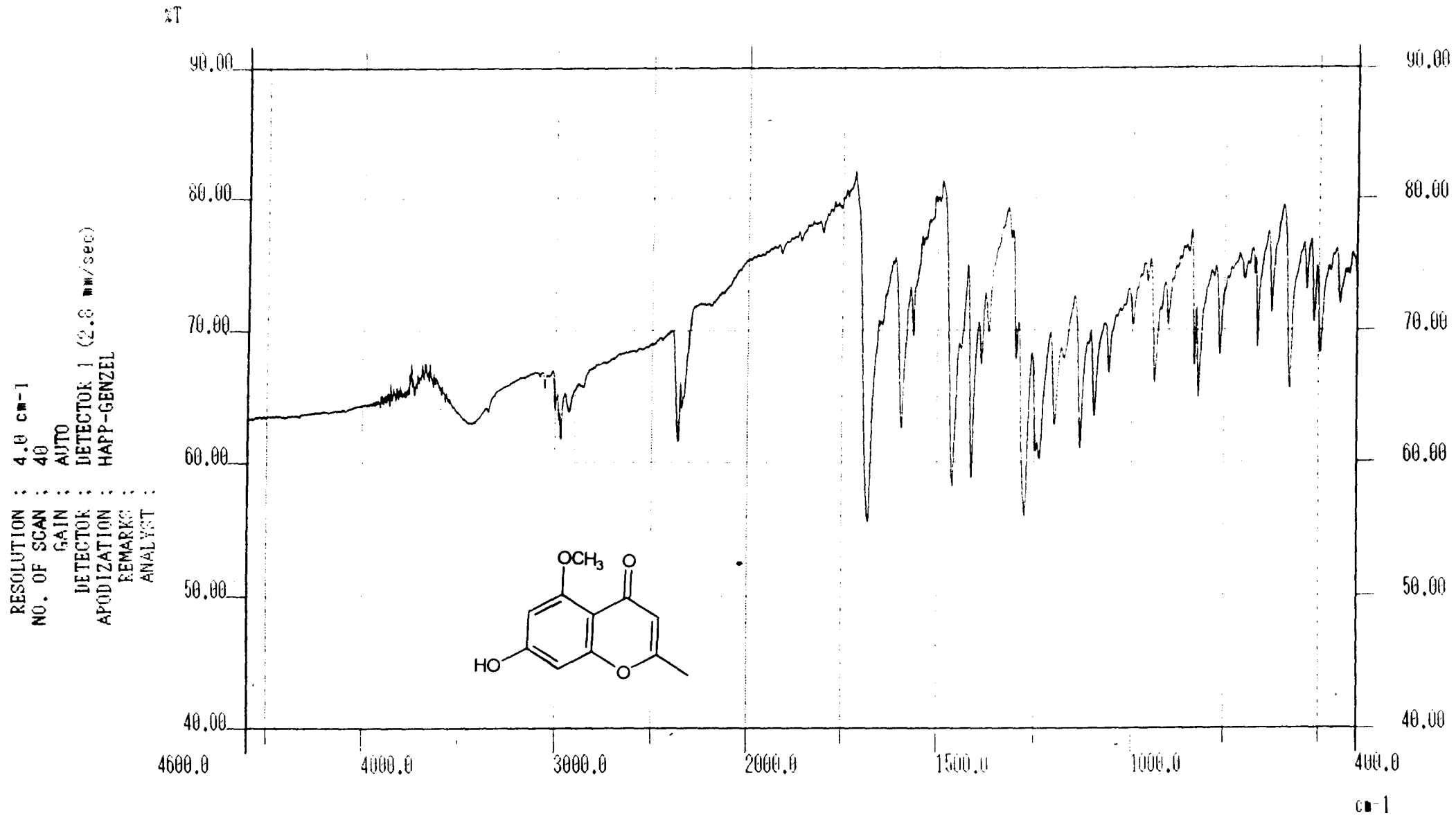


Fig. 3.20 : IR spectrum of 30

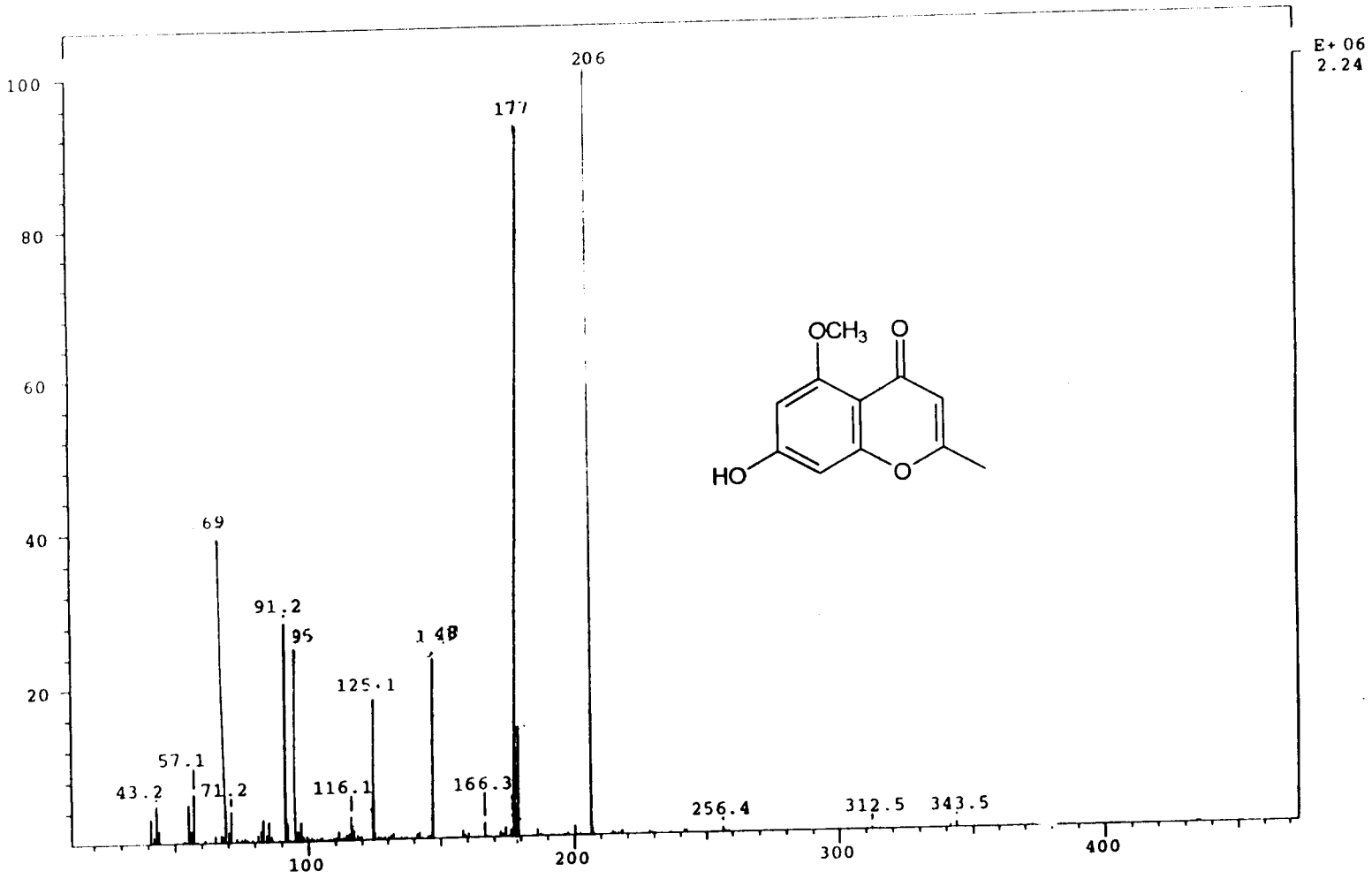


Fig. 3.21 : EI mass spectrum of 30

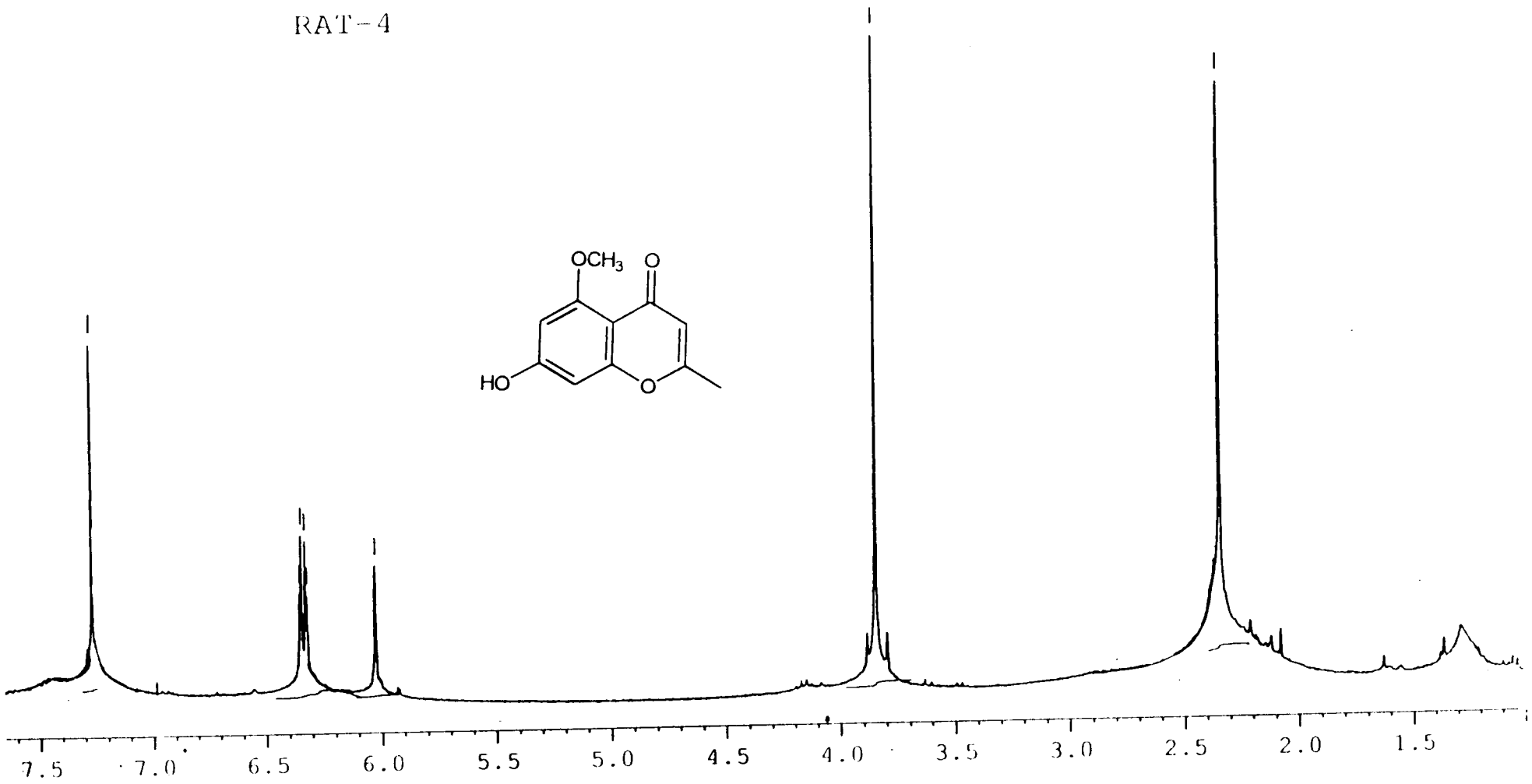


Fig. 3.22 : ^{1}H NMR spectrum of 30 in $CDCl_3$

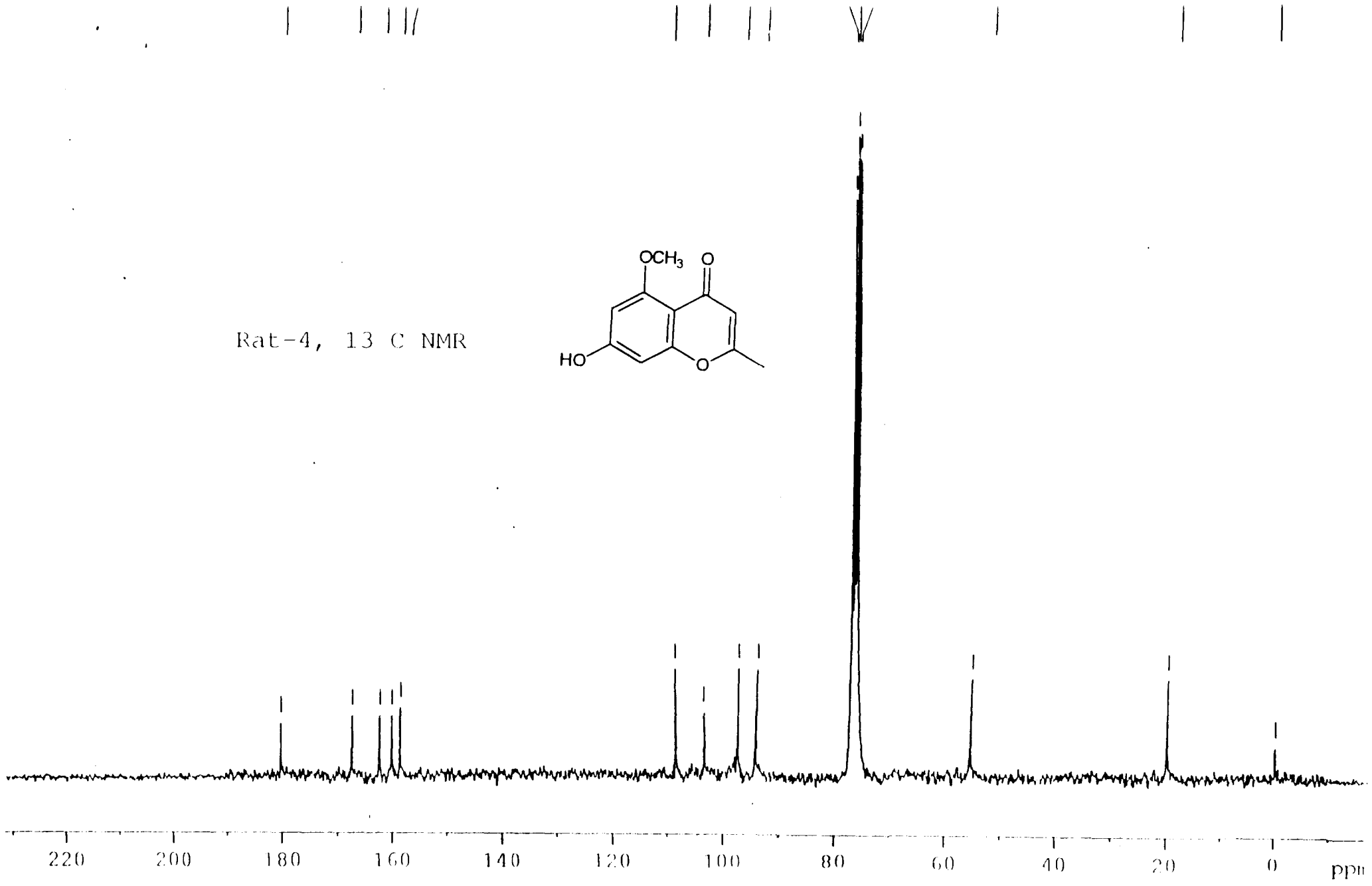


Fig. 3.23 : ^{13}C NMR spectrum of 30 in $CDCl_3$

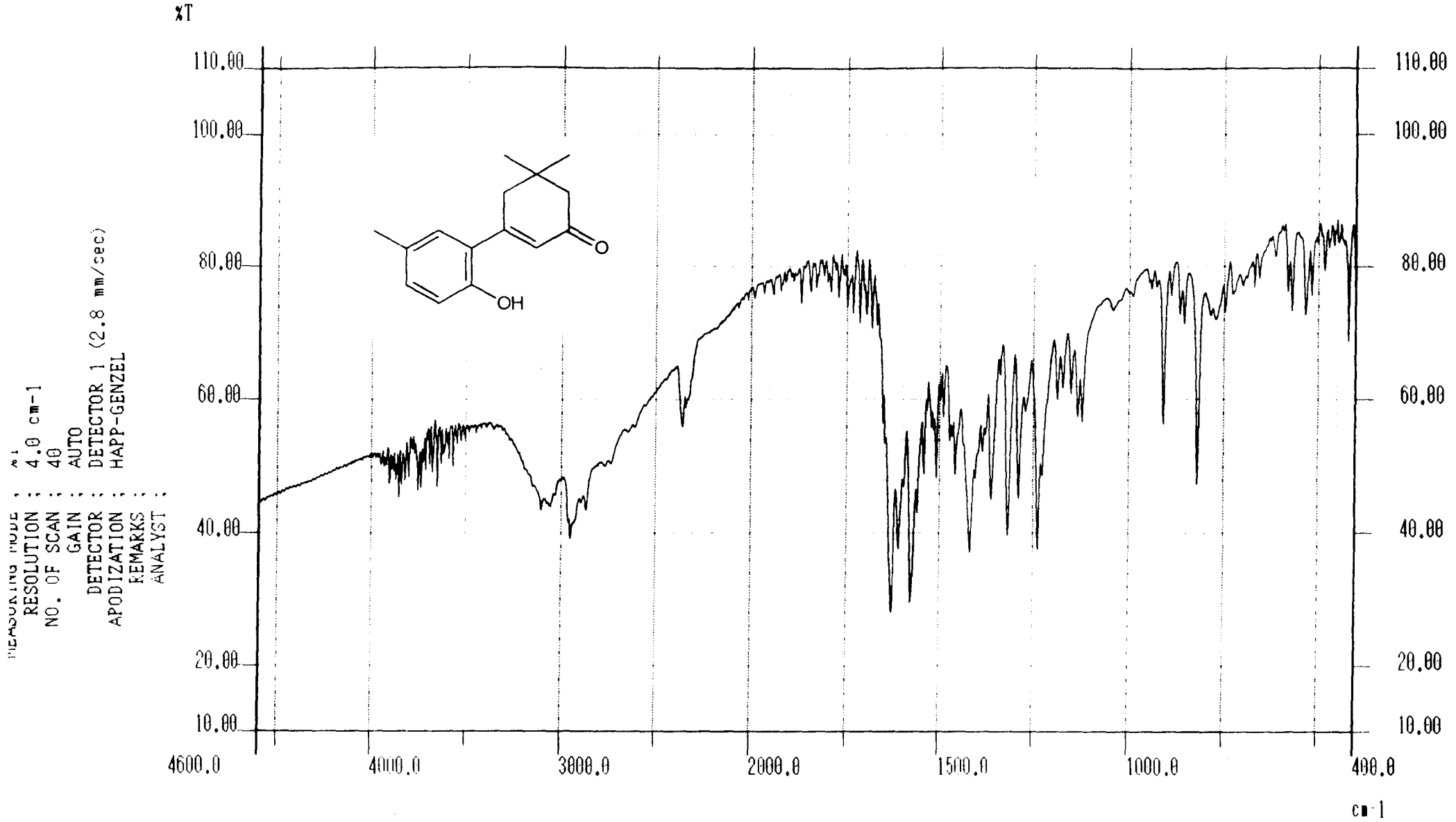


Fig. 3.24 : IR spectrum of 37a

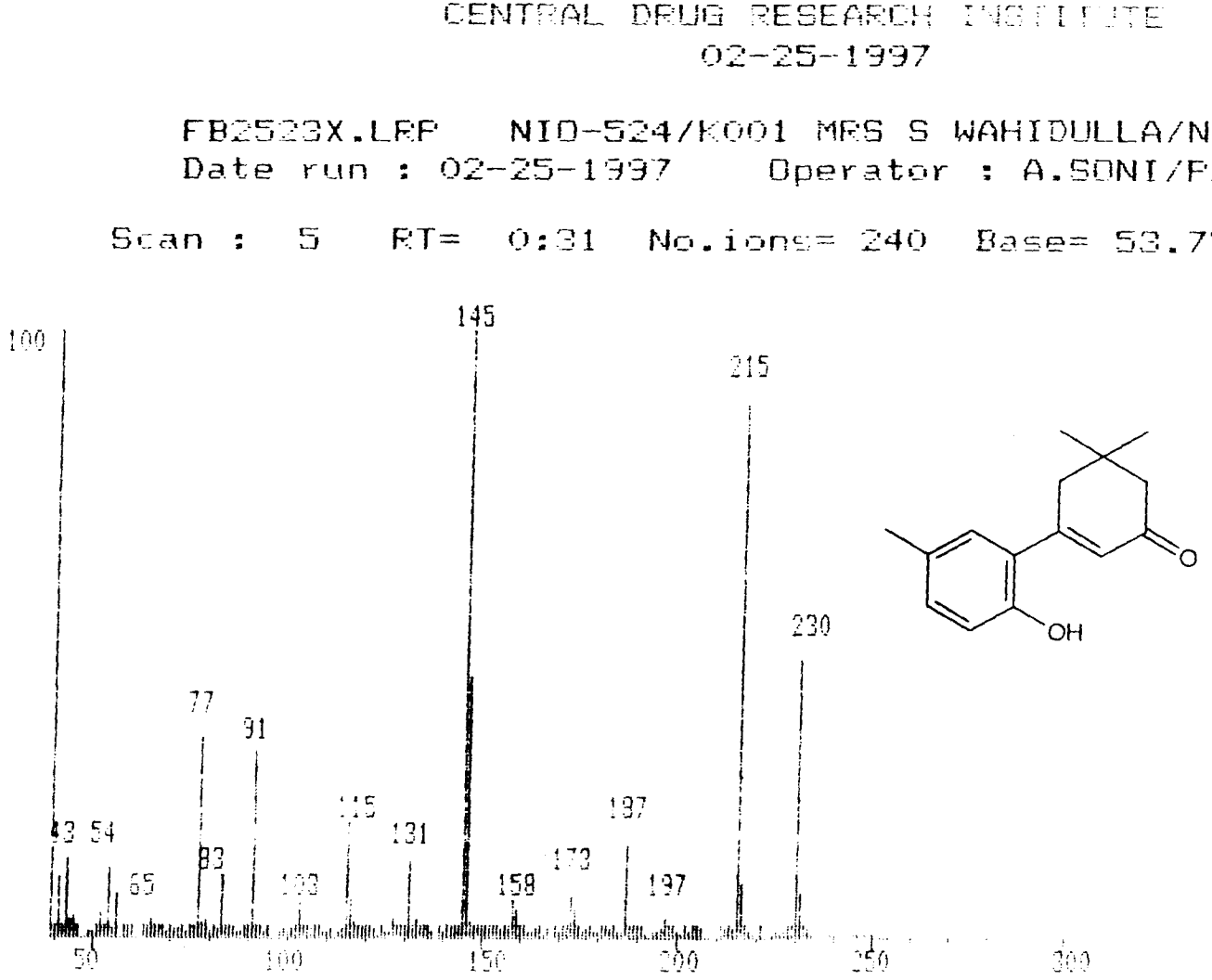


Fig. 3.25 : EI mass spectrum of 37a

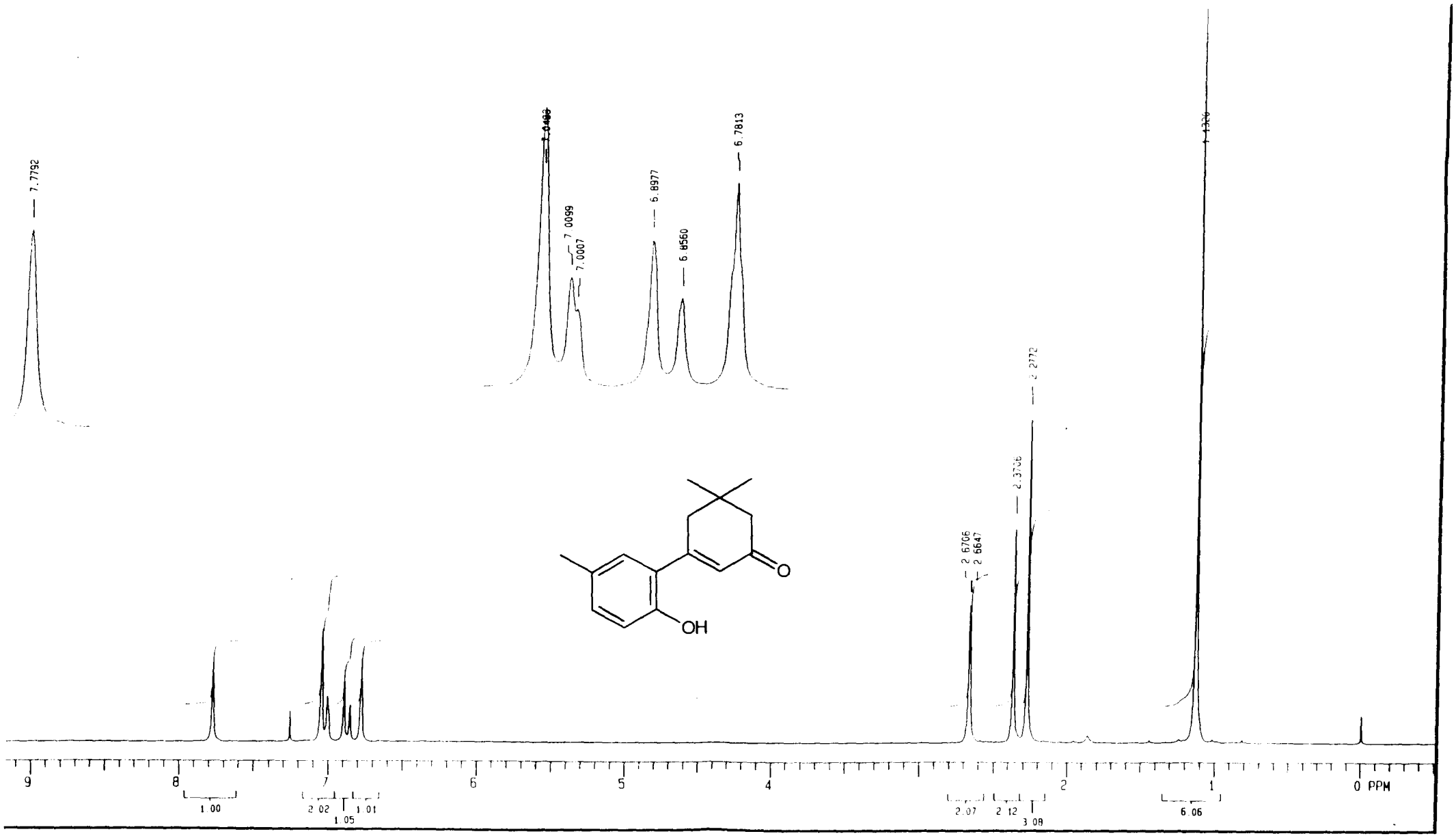


Fig. 3.26 : ^{1}H NMR spectrum of 37a in $CDCl_3$

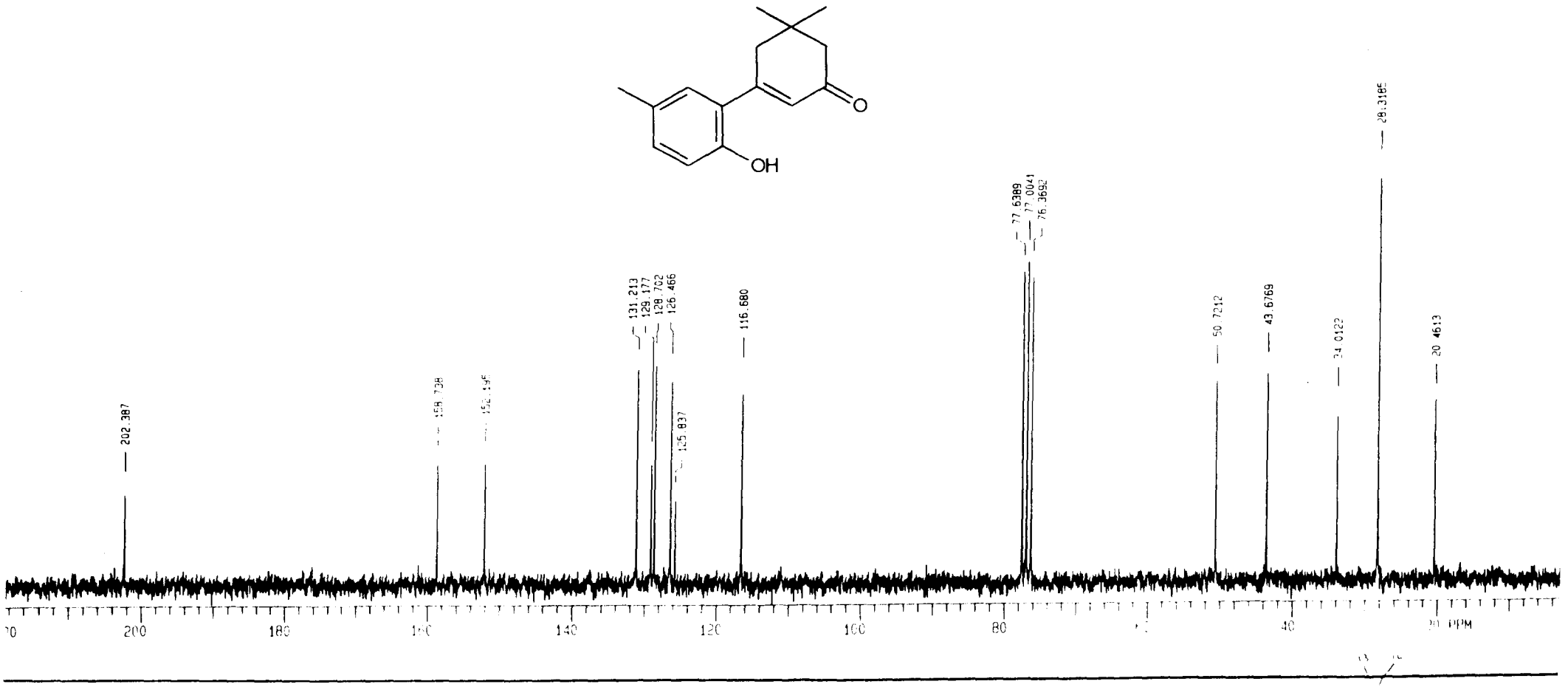


Fig. 3.27 : ^{13}C NMR spectrum of 37a in $CDCl_3$

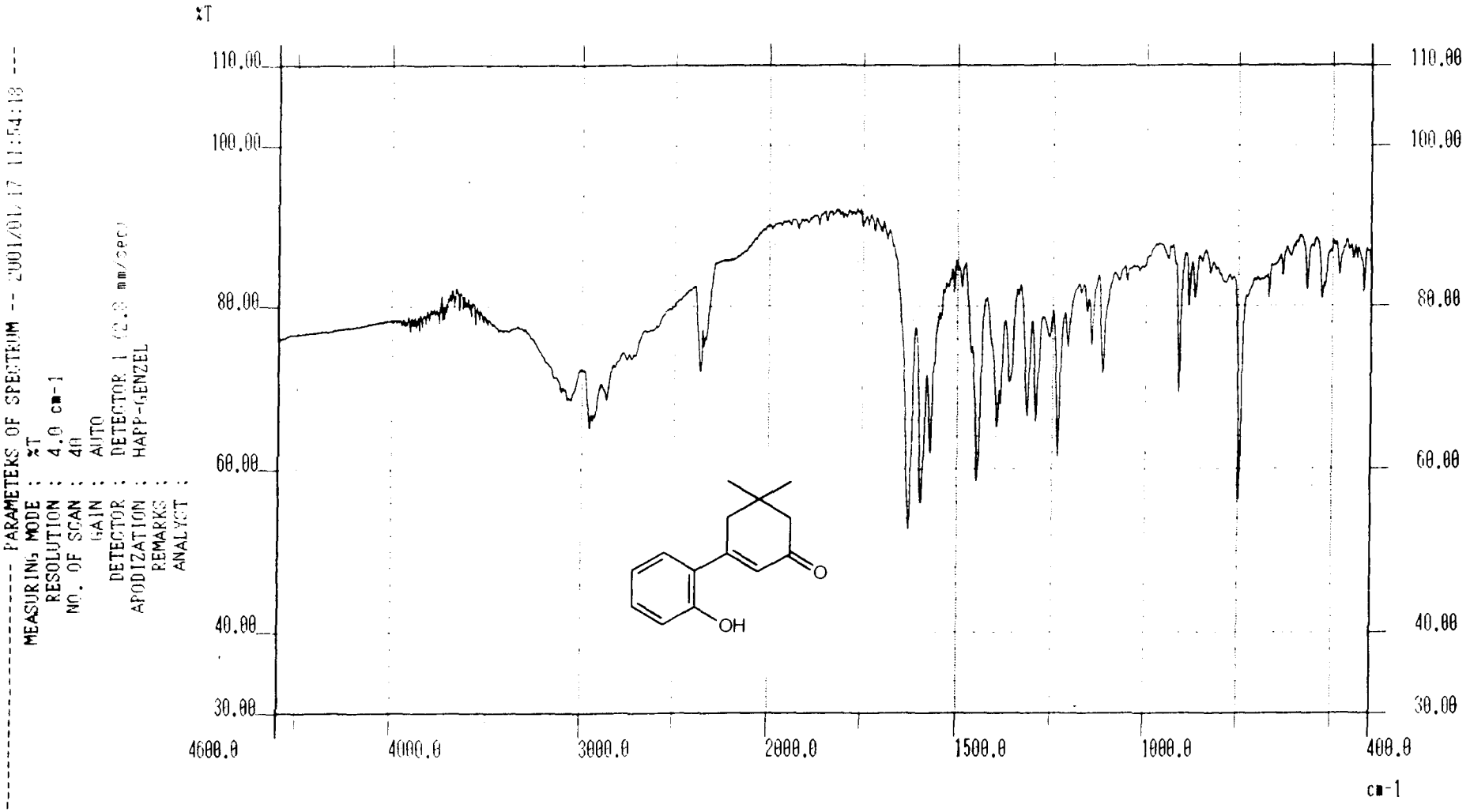


Fig. 3.28 : IR spectrum of 37b

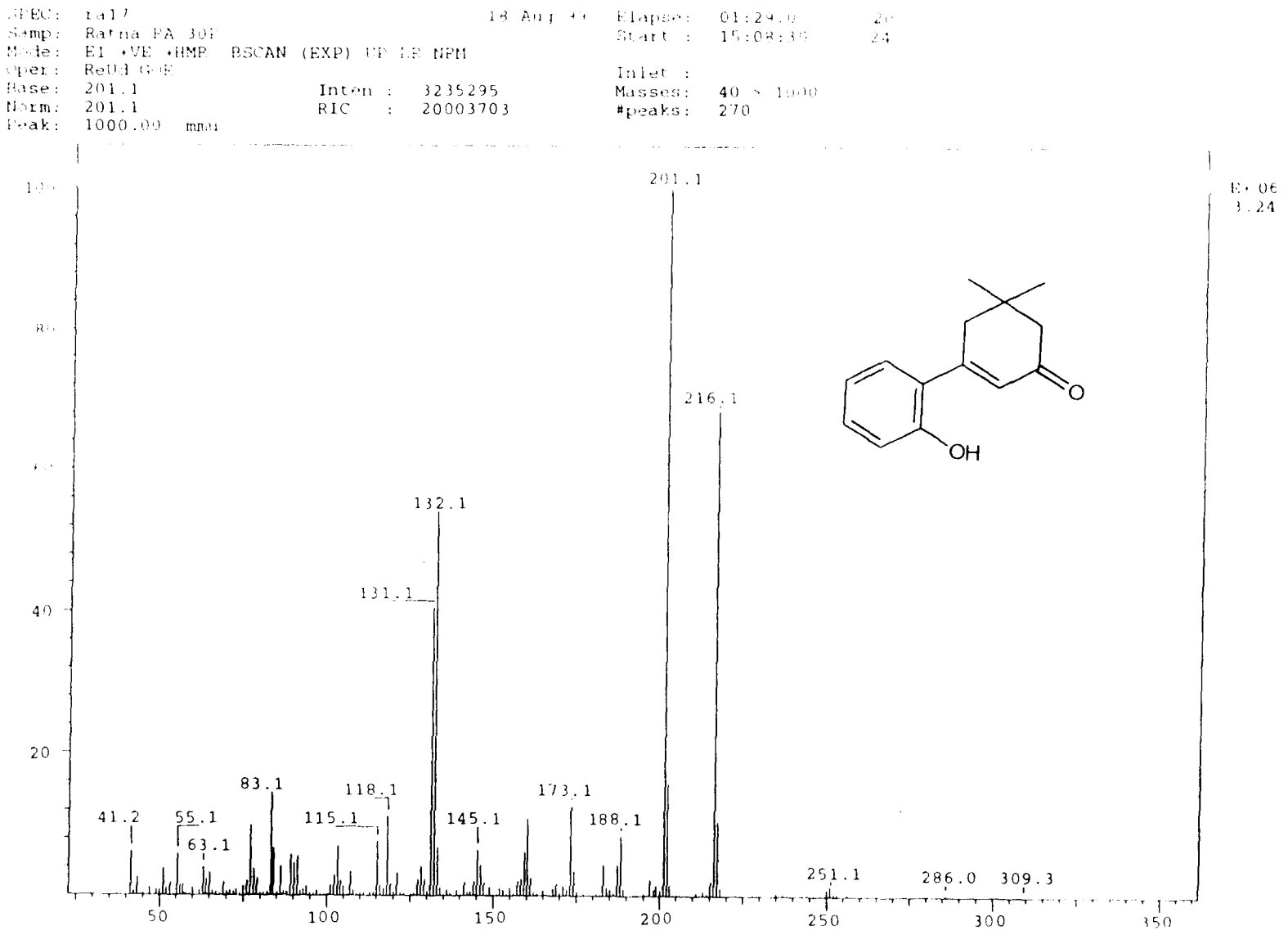


Fig. 3.29 : EI mass spectrum of 37b

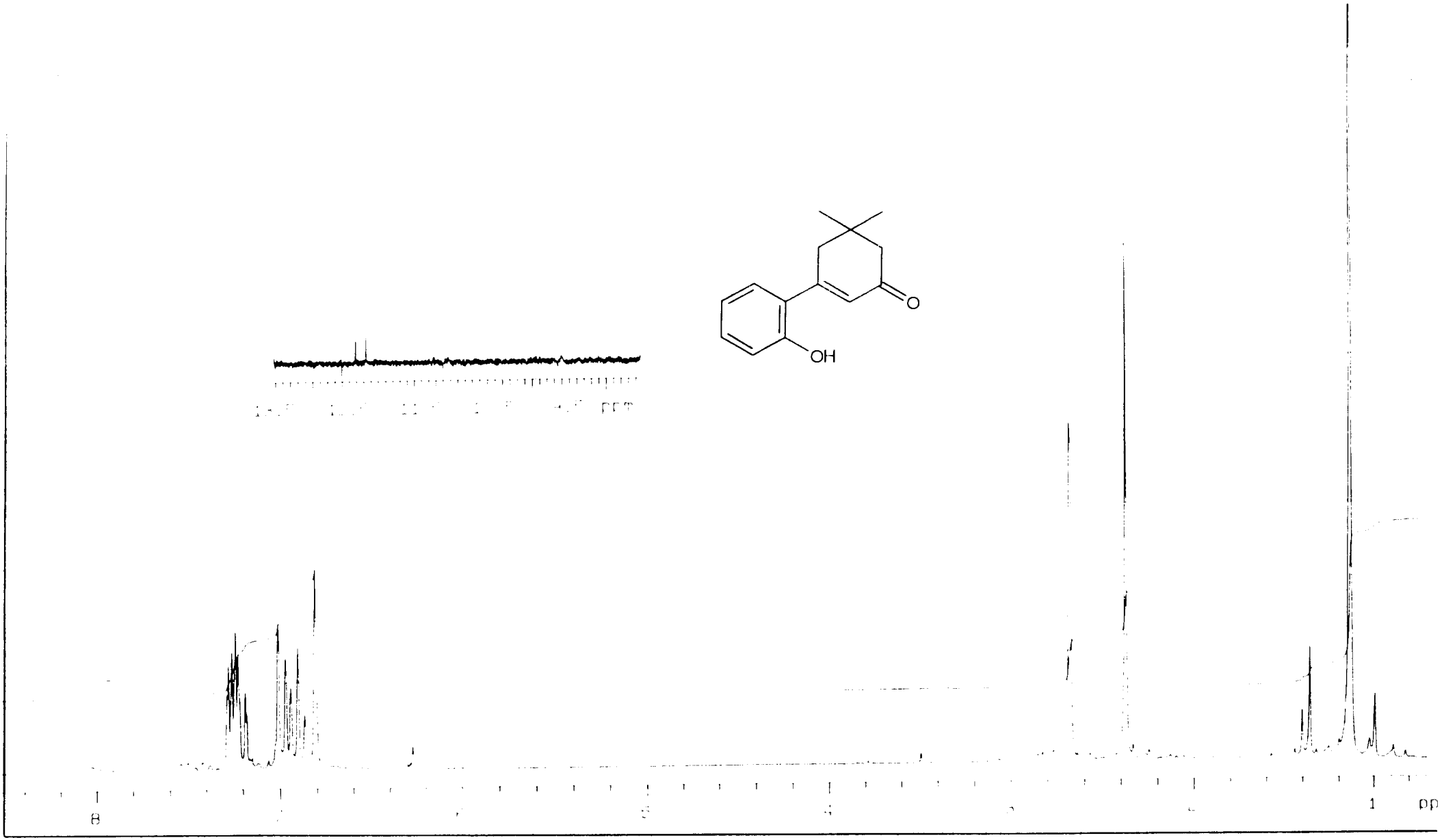


Fig. 3.30 : ^{1}H NMR spectrum of 37b in $CDCl_3$

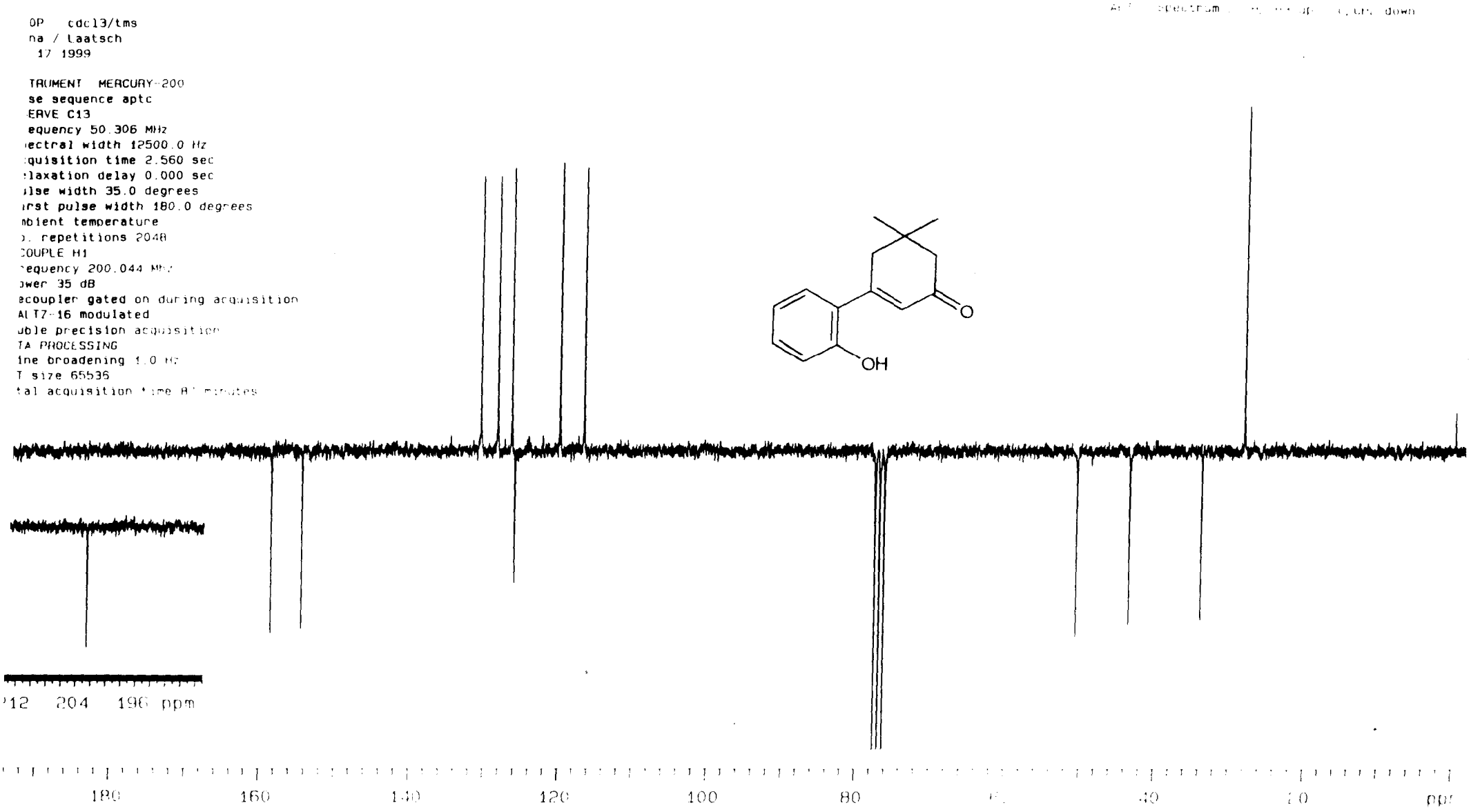


Fig. 3.31 : ^{13}C NMR spectrum of 37b in $CDCl_3$

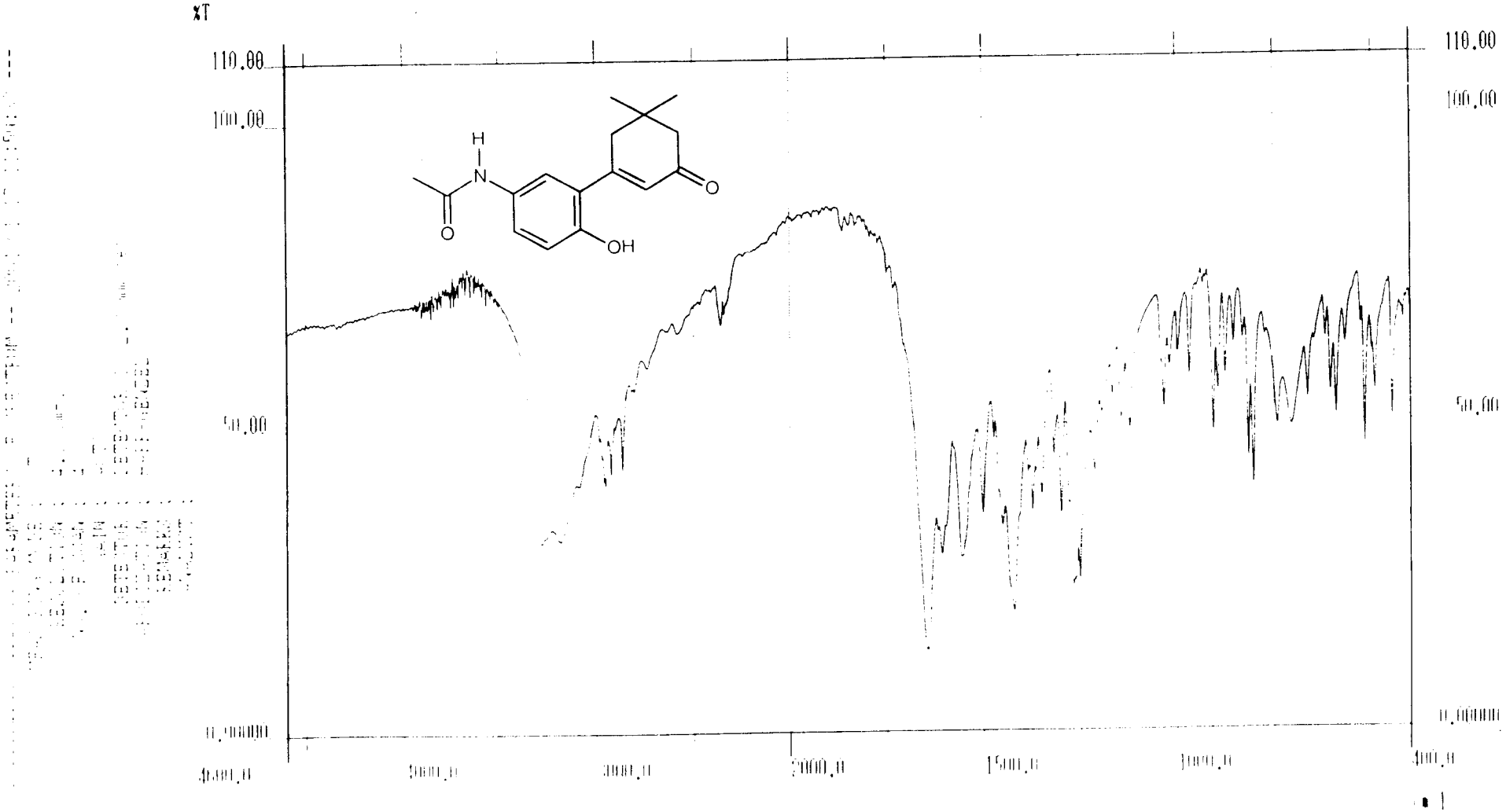


Fig. 3.32 : IR spectrum of 37c

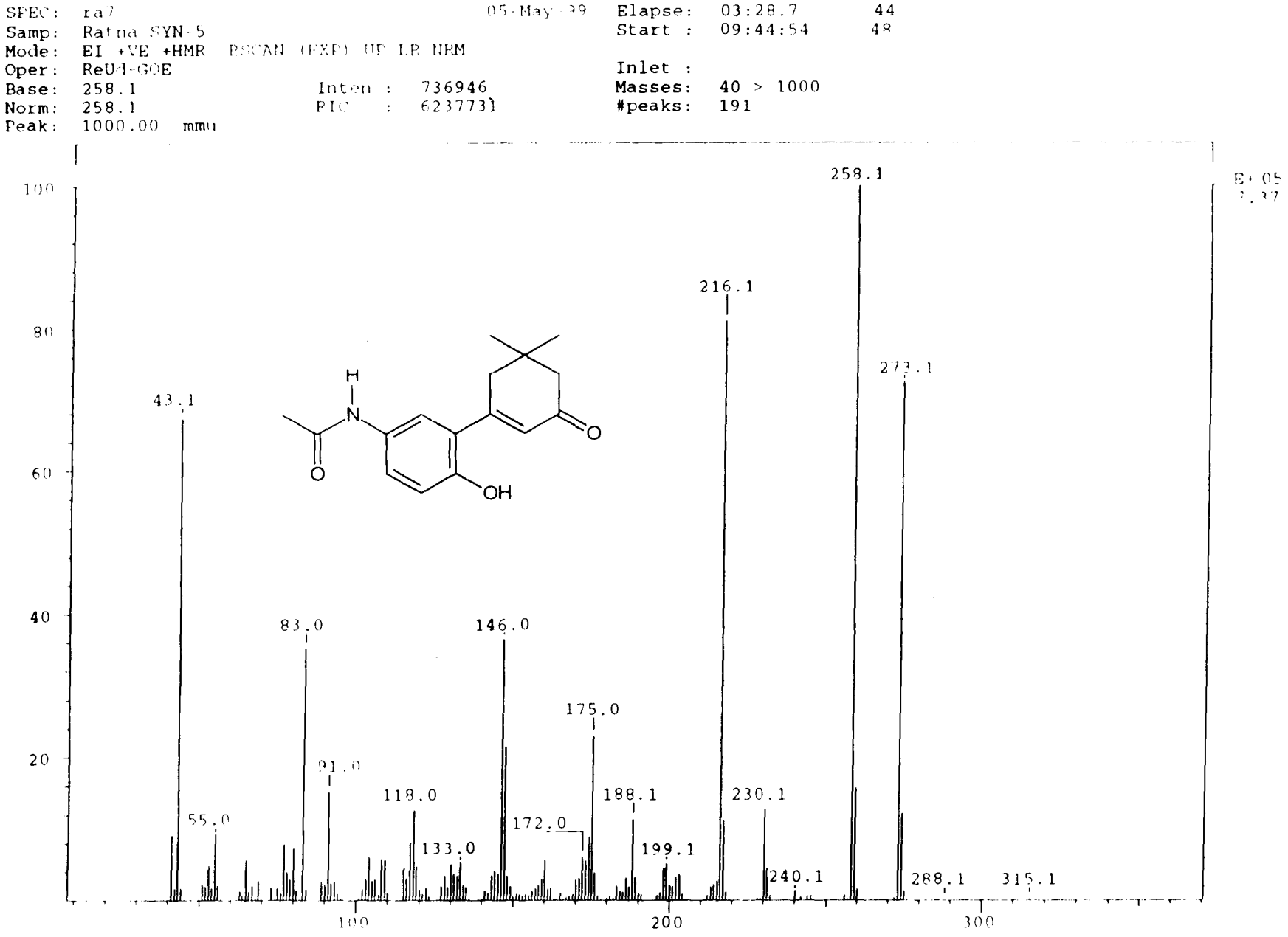


Fig. 3.33 : EI mass spectrum of 37c

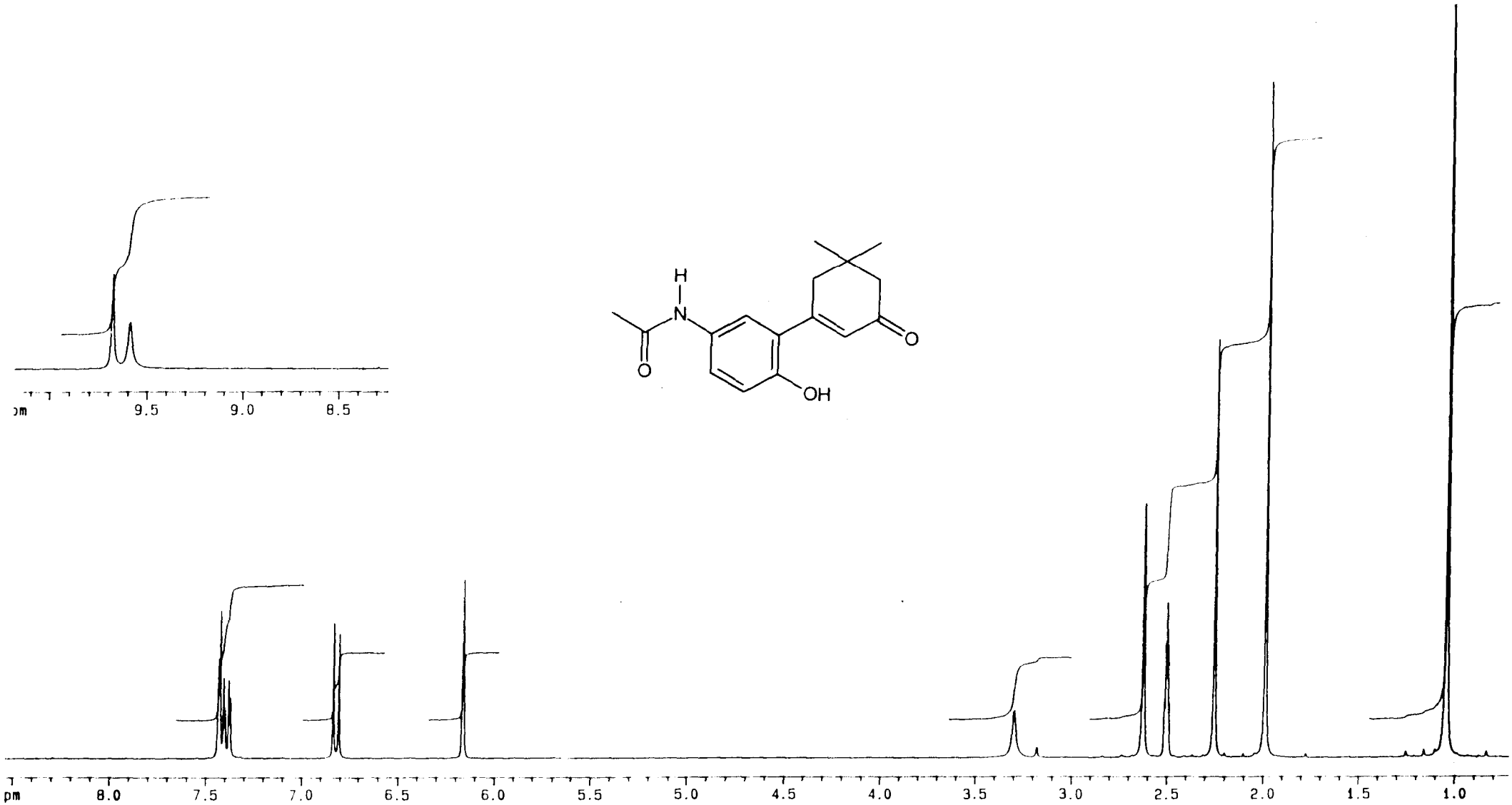


Fig. 3.34 : ^{1}H NMR spectrum of 37c in DMSO-d_6

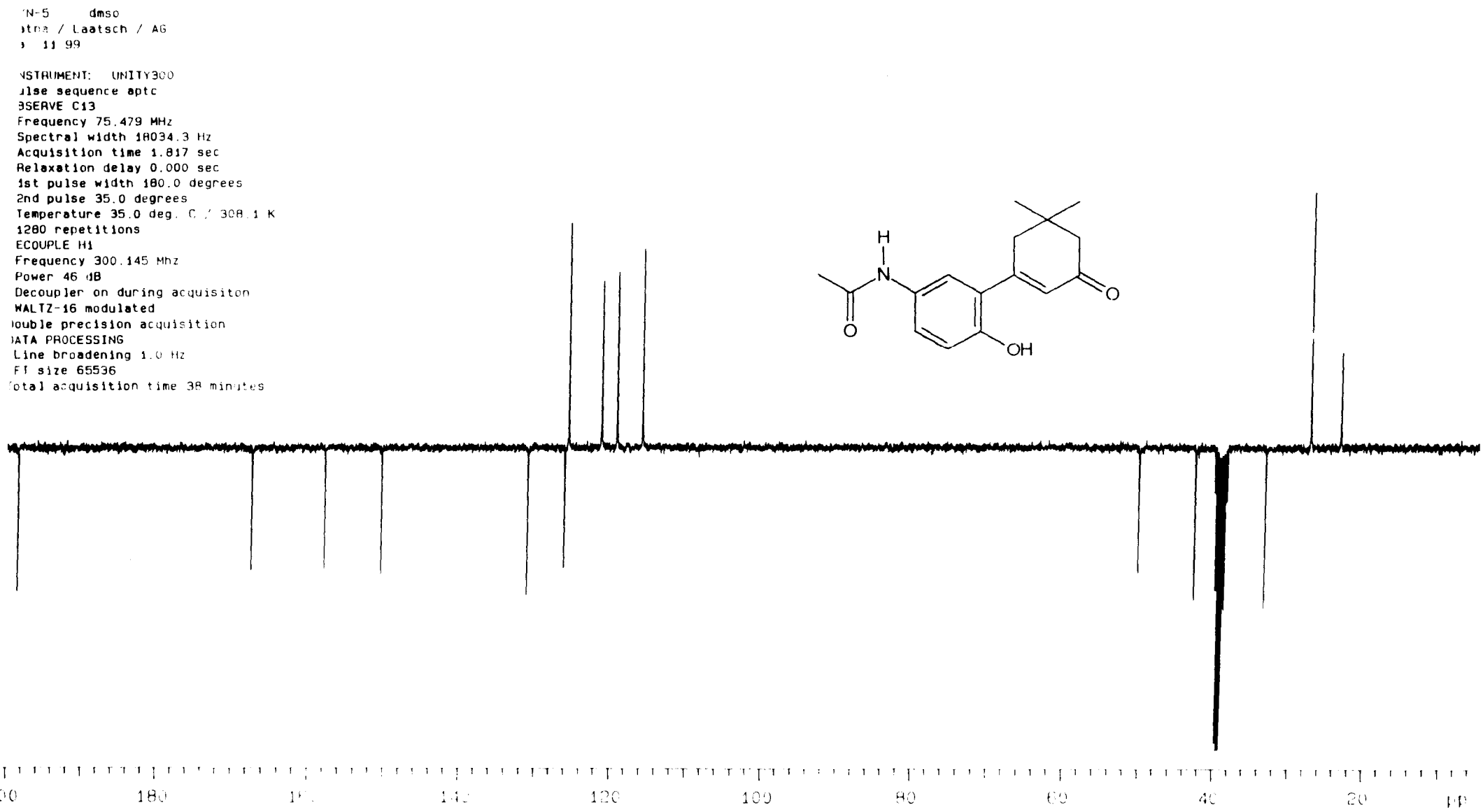


Fig. 3.35 : ^{13}C NMR spectrum of 37c in DMSO-d_6

EXPERIMENTAL

Preparation of *p*-acetoxy acetanilide

p-Acetaminophenol (**14**) (3.02 g, 20 mmol) and acetylchloride (1.5 g, 30 mmol) were mixed together and gently heated for 30 min on a oil bath with constant shaking. The product obtained was crystallized from water to give *p*-acetoxy acetanilide (3.85 g, 87 %) as colourless crystalline solid with m.p. 150 ^{0}C.

IR (KBr) ν = 3380, 2940, 1750, 1690, 1610, 1540, 1510, 1410, 1360, 1315, 1240, 1190, 1015, 920 and 700 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 193.1 (M^+ 16), 151 (M^+, 60) and 109 (M^+, 100).

^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.72 (br s, 1 H, exchangeable with D_2O, N-H), 7.48 (d, 2 H, *J* = 8.5 Hz, H-3, H-5), 7.0 (d, 2 H, *J* = 8.5 Hz, H-2, H-6), 2.3 (s, 3 H, CH_3) and 2.12 (s, 3 H, CH_3).

Preparation of 2-acetyl-4-acetaminophenol (15)

A mixture of *p*-acetoxy acetanilide (2 g, 10.3 mmol) and $AlCl_3$ (2.8 g, 20.91 mmol) in dry nitrobenzene (10 ml) was heated at 100 ^{0}C for 16 h. The mixture was cooled, poured into a solution of 2N HCl and ice and stirred for 1 h. The product, solidified on standing, was filtered, washed with water and dried. The organic layer was extracted with 5 % NaOH (100 ml x 2) and the resulting basic solution was acidified by conc. HCl, the precipitated solid was filtered and dried. The crude product was further purified by flash silica gel chromatography using $CHCl_3$: MeOH (95 : 5) as the eluent, to yield a colourless crystalline solid **15** (1.7 g, 85 %), m.p. 168-169 ^{0}C.

IR (KBr) ν = 3250, 3050, 2990, 1660, 1640, 1620, 1570, 1490, 1370, 1310, 1290, 1250, 1160, 1020, 900, 860, 800 and 620 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 193.1 (M^+, 100), 178 (M^+ - CH_3, 10), 151.1 (M^+, 88), 136 (M^+, 60), 133 (28), 108 (20) and 43.1 (15).

^{1}H NMR (200 MHz, $CDCl_3$): δ = 12.1(s, exchangeable with D_2O, 1 H, -OH), 8.18 (d, *J* = 2.5 Hz, 1 H, H-3), 7.42 (br s, exchangeable with D_2O, 1 H, N-H), 7.32 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-5), 6.9 (d, *J* = 8.5 Hz, 1 H, H-6), 2.62 (s, 3 H, CH_3) and 2.18 (s, 3 H, CH_3).

Cyclization of (15) to 6-acetylamino-2,2-dimethylchroman-4-one (16)

A mixture of 2-acetyl-4-acetaminophenol (**15**) (1 g, 5.18 mmol), acetone (2 ml), piperidine (0.5 ml, 5.18 mmol) and pyridine (1.16 ml) was heated at reflux for 72 h. The reaction mixture was concentrated under reduced pressure and poured into a mixture of 2N NaOH and ice. The reaction product was extracted with ethyl acetate (3 x 40 ml), the combined extracts were washed with water, dried and concentrated. The resulting light coloured yellow crude product was then chromatographed on a flash silica gel column and eluted with $CHCl_3$: MeOH (97 : 3) to give **16** (1.05 g, 88 %) as light yellow crystals with m.p. 164-165 ^{0}C (lit.[27] 162-164 ^{0}C).

IR (KBr)ν = 3300, 2980, 2920, 1690, 1670, 1590, 1480, 1370, 1310, 1250, 1220, 830 and 720 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 233 (M^+, 18) and 135 (100).

CIMS (NH_3): *m/z* (%) = 484.2 ($[2M + NH_4]^+$, 50), 251.1($[M + NH_4]^+$, 100) and 234 ($[M + H]^+$, 10).

^{1}H NMR (200 MHz, $CDCl_3$) : δ = 7.68 (dd, *J* = 9.0 Hz, 2.5 Hz, 1 H, H-7), 7.68 (d, *J* = 2.5 Hz, 1 H, H-5), 7.56 (br s, 1 H, N-H), 6.90 (d, *J* = 9.0 Hz, 1 H, H-8), 2.74 (s, 2 H, H_2-3), 2.2 (s, 3 H, CH_3) and 1.42 (s, 6 H, 2 x CH_3).

Hydrolysis of (16) to 6-amino-2,2-dimethylchroman-4-one (17)

A solution of 6-acetylamino-2,2-dimethylchroman-4-one (**16**) (0.6 g, 2.575 mmol) in ethanol (10 ml) was refluxed with 5 N HCl (3 ml) for 6 h. The resultant red coloured reaction mixture was cooled and poured into water and extracted with ethyl acetate and dried over Na_2SO_4. The crude red coloured residue obtained after concentration was purified on a flash silica gel column and eluated with $CHCl_3$: MeOH (95 : 5) to give light orange coloured solid **17** (0.439 g, 89 %), m.p. 143-144 ^{0}C.

IR (KBr)ν **(fig. 3.01)** = 3300, 2960, 1690, 1660, 1600, 1490 and 720 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.02)= 191.1 (M^+, 88), 176.1 (M^+ - CH_3, 100), 136 (56), 135 (72), 107 (24) and 79 (36).

^{1}H NMR (200 MHz, $CDCl_3$): δ **(fig. 3.03)** = 7.18 (d, *J* = 2.5 Hz, 1 H, H-5), 6.9 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-7), 6.76 (d, *J* = 8.5 Hz, 1 H, H-8), 4.18 (br s, exchangeable with D_2O, 2 H, NH_2), 2.64 (s, 2 H, H_2-3) and 1.2 (s, 6 H, 2 x CH_3).

^{13}C/APT-NMR ($CDCl_3$, 75.5 MHz): δ (fig. 3.04) = 192.9 (1C, $C_{quat.}$), 155.5 (1C, $C_{quat.}$), 139.4 (1C, $C_{quat.}$), 124.9 (1C, CH), 120.3 (1C, $C_{quat.}$), 119.1 (1C, CH), 111 (1C, CH), 78.7 (1C, $C_{quat.}$), 49.1 (1C, CH_2) and 26.6 (2C, 2 x CH_3).

Preparation of 6-iodo-2,2-dimethylchroman-4-one (18)

The amine (**17**) (0.4 g, 2.094 mmol) was dissolved in a mixture of H_2SO_4 (5 ml) and water (12 ml) with warming. The resultant clear solution was cooled to 0 °C and treated with a solution of $NaNO_2$ (0.148 g, 2.144 mmol) in water (2.5 ml) dropwise with stirring. The mixture was stirred for an additional 1 h at 0 °C, and then a solution of KI (0.5 g, 3.017 mmol) in water (3 ml) was added dropwise. The reaction mixture was stirred overnight at room temperature. Extraction of the organic part with diethyl ether followed by evaporation of the solvent gave a red gum, which was chromatographed on silica gel and eluted with a gradient solvent system consisting of chloroform and methanol to give **19** (0.115 g, 31 %) m.p. 70 °C, **18** (0.35 g, 56 %) as yellow oil and **20** (0.125 g, 14 %) m.p. 98 °C.

2,2-dimethylchroman-4-one (19)

IR (KBr)ν = 2995, 1690, 1660, 1610, 1575, 1460, 1375, 1330, 1310, 1225, 1225, 1200, 1570, 1150, 1075, 1020, 920 and 760 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 176.1 (M^+, 48), 161.1 (M^+ - CH_3, 100), 121.1 (62), 120.1 (64), 93.1 (16), 92.1 (64), 65 (24) and 64 (22).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.83 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-5), 7.48 (m, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-6), 6.96 (t, *J* = 8.5 Hz, 1 H, H-7), 6.92 (d, *J* = 8.5 Hz, 1 H, H-8), 2.72 (s, 2 H, H_2-3) and 1.42 (s, 6 H, 2 x CH_3).

^{13}C/APT-NMR ($CDCl_3$, 75.5 MHz): δ = 192.6 (1C, $C_{quat.}$), 159.9 (1C, $C_{quat.}$), 136.1 (1C, CH), 126.5 (1C, CH), 120.6 (1C, CH), 120.2 (1C, $C_{quat.}$), 118.3 (1C, CH), 79.1 (1C, $C_{quat.}$), 48.9 (1C, CH_2) and 26.6 (2C, 2 x CH_3).

2,2-dimethyl-6-iodo-chroman-4-one (18)

IR (neat)ν **(fig. 3.05)** = 2990, 1690, 1590, 1465, 1410, 1375, 1280, 1220, 1140, 825 and 755 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.06) = 302 (M^+, 100), 286.9 (M^+ - CH_3, 96), 246.9 (56), 245.9 (54), 217.9 (20), 176.1 (16) and 161 (18).

^{1}H NMR (300 MHz, $CDCl_3$): δ (fig. 3.07) = 8.15 (d, *J* = 2.5 Hz, 1 H, H-5), 7.7 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-7), 6.72 (d, *J* = 8.5 Hz, 1 H, H-8), 2.72 (s, 2 H, H_2-3) and 1.45 (s, 6 H, 2 x CH_3).

^{13}C/APT-NMR ($CDCl_3$, 50.4 MHz): δ (fig. 3.08) = 191.1 (1C, $C_{quat.}$), 159.5 (1C, $C_{quat.}$), 144.5 (1C, CH), 135.1 (1C, CH), 121.9 (1C, $C_{quat.}$), 120.8 (1C, CH), 82.8 (1C, $C_{quat.}$), 79.6 (1C, $C_{quat.}$), 48.4 (1C, CH_2) and 26.5 (2C, 2 x CH_3).

2,2-dimethyl-3,6-di-iodo- chroman-4-one (20)

IR (KBr)ν = 3300, 2950, 1675, 1660, 1590, 1460, 1410, 1270, 1240, 1200, 1135, 1100, 940, 820, 810 and 760 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 427.9 (M^+, 36), 301 (M^+ - I, 100), 246.9 (64), 217.9 (12) and 174.1 (M^+ - 2I, 24).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.2 (d, *J* = 2.5 Hz, 1 H, H-5), 7.75 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-7), 6.75 (d, *J* = 8.5 Hz, 1 H, H-8), 4.64 (s, 1 H, H-3), 1.65 (s, 3 H, CH_3) and 1.58 (s, 3 H, CH_3).

^{13}C/APT-NMR ($CDCl_3$, 75.5 MHz): δ = 186.5 (1C, $C_{quat.}$), 158 (1C, $C_{quat.}$), 144.8 (1C, CH), 135.3 (1C, CH), 120.5 (1C, CH), 119.2 (1C, $C_{quat.}$), 83.7 (1C, $C_{quat.}$), 79.8 (1C, $C_{quat.}$), 37.4 (1C, CH), 30.1 (1C, CH_3) and 20.4 (1C, CH_3).

Preparation of lactarochromal (11)

To a stirred and cooled (0 °C) solution of **18** (0.2 g, 0.663 mmol) in dry ether (25 ml), n-butyllithium (0.7 ml, 1.13 mmol, 1.6 M in hexane) was added dropwise, under nitrogen atmosphere. After 1 h of stirring, N-formyl-piperidine (100 mg) was added. The reaction mixture was stirred for additional 2 h followed by acidification with an ice cooled solution of HCl, and extracted with ether (3 x 50 ml). The combined ether layer was washed with a solution of 10 % $NaHCO_3$, dried (Na_2SO_4) and evaporated. The crude residue was purified further by column chromatography on silica gel, using pet ether :

ether (9 : 1) to give **11** (0.08 g, 68 %) as crystalline solid with m.p. 91 ^{0}C (lit.[20] m.p. 89-91 ^{0}C).

IR (KBr) ν (fig. 3.09) = 2990, 1700, 1687, 1610, 1565, 1475, 1440, 1390, 1270,1220, 1185, 1170, 1125 and 830 cm^{-1}.

EIMS (70 eV) m/z (fig. 3.10) = 204.1 (M^+, 66), 189 (M^+ - CH_3, 100), 149. (50), 148 (48), 147 (14) and 119 (16).

^{1}H NMR (500 MHz, $CDCl_3$) δ (fig. 3.11) = 9.89 (s, 1 H, CHO), 8.32 (d, *J* = 2.0 Hz, 1 H, H-5), 8.00 (dd, *J* = 8.8, 2.0 Hz, 1 H, H-7), 7.03 (d, *J* = 8.8 Hz, 1 H, H-8), 2.76 (s, 2 H, CH_2) and 1.48 (s, 6 H, 2 x CH_3).

^{13}C NMR (125.5 MHz, $CDCl_3$) δ (fig. 3.12) = 191.1 (1C, $C_{quat.}$), 190.1 (1C, CH), 164.3 (1C, $C_{quat.}$), 134.8 (1C, CH), 131.1 (1C, CH), 129.8 (1C, $C_{quat.}$), 119.8 (1C, $C_{quat.}$), 119.6 (1C, CH), 80.6 (1C, $C_{quat.}$), 48.4 (1C, CH_2) and 26.5 (2C, 2 x CH_3).

Attempted preparation of 2,2,6-trimethyl-2H-1-benzopyran-4-one (21) using polyphosphoric acid

To *o*-phosphoric acid (18.8 ml), P_2O_5 (30 g) was added and the mixture was stirred well on a stirrer for 30 min below 85 ^{0}C. Then, a mixture of *p*-cresol (5.4 g, 50 mmol) and β,β-dimethylacrylic acid (5 g, 50 mmol) was added and the resulting thick mass was heated on a water bath for 1 h. The resultant product was cooled and extracted with ether. The ether extract was washed with dilute HCl (1 : 1), water, cold 5 % aqueous NaOH and finally with water. The ether extract was dried over Na_2SO_4 and evaporated in vaccum. The reddish brown oily residue obtained was purified on a silica gel column by using ether : pet. ether (1 : 19) to give a light yellow oil (8.2 g, 85 %).

IR (neat) ν = 3010, 2990, 1770, 1687, 1610, 1590, 1520, 1495, 1410, 1365, 1260, 1020 and 915 cm^{-1}.

The IR spectra of this oily product indicated the presence of desired compound **21** along with another by-product which was formed during the reaction. In order to separate **21** from the unwanted by-product, the whole mixture was subjected to semicarbazide hydrochloride to get a solid semicarbazone derivative, m.p.182 ^{0}C. Regeneration of the

ketone from semicarbazone followed by the running of the IR spectra indicated that the oily product is still impure in nature.

Attempted preparation of of 2,2,6-trimethylchroman-4-one (21) using $ZnCl_2$ & $POCl_3$

A mixture of *p*-cresol (2.5 g, 23.15 mmol), β,β-dimethylacrylic acid (2 g, 20 mmol), freshly fused and finely powdered zinc chloride (12 g) and phosphorous oxychloride (18 ml) was kept at room temperature for 24 h, with occasional stirring. The reaction mixture was poured over crushed ice. The reaction product was extracted with ethyl acetate, washed with water, and stirred in presence of aq. NaOH (10 %, 45 ml) for 6 h at 50 ^{0}C. The product was extracted with ethyl acetate, washed with water and dried. The crude residue obtained was purified on a silica gel column by using ether : pet. ether (1 : 19) to give a light yellow oil (3.2 g, 71 %). The IR spectra of this oily product indicated the presence of same type of impurity as observed in case of PPA reaction.

IR (neat) ν = 3010, 2990, 1770, 1687, 1610, 1590, 1520, 1495, 1410, 1365, 1260, 1020 and 915 cm^{-1}.

Preparation of *p*-cresyl acetate (24)

p-Cresol (20 g, 0.189 mol) was dissolved in pyridine (15 ml) and to this mixture, excess of freshly distilled acetic anhydride (60 ml) was added. The resulting mixture was refluxed for 15 h, and then subjected to distillation, the fraction distilling below 160 ^{0}C was rejected. The higher boiling fraction was taken in ether and washed succesively with dil. HCl (2N), water and 2N NaOH. The ether layer after drying over anhydrous $MgSO_4$ was evaporated to provide *p*-cresyl acetate (**24**) (25 g, 88 %), b.p. 212 ^{0}C (lit[24]. 212-213 ^{0}C).

Fries rearrangement of *p*-cresyl acetate (24) to 2′-hydroxy-5′-methylacetophenone (25)

A mixture of *p*-cresyl acetate (10 g, 66.66 mmol) and anhydrous $AlCl_3$ (11 g) was heated for 30 min at 120 ^{0}C. After cooling, the semi solid mass was poured in crushed ice

containing con. HCl (20 ml). The yellowish brown liquid separated out immediately and got solidified on cooling to 10 ^{0}C. Repeated crystallization from petroleum ether gave 2′-hydroxy-5′-methylacetophenone (**25**) (8 g, 80 %) m.p. 60 ^{0}C (lit[24]. m.p. 60 ^{0}C).

IR (KBr) ν = 3450, 2950, 1760, 1640, 1620, 1490, 1370, 1320, 1298, 1220, 1200, 920 and 700 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 150 (M^+, 40), 136 (24), 135 (M^+ - CH_3, 100), 107 (18) and 77 (22).

^{1}H NMR (300 MHz, $CDCl_3$) δ = 12.1 (s, exchangeable with D_2O, 1 H, -OH), 7.51 (d, *J* = 2.1Hz, 2 H, H-6′), 7.29 (dd, *J* = 8.4 Hz, 2.1 Hz, 1 H, H-4′), 6.89 (d, *J* = 8.4 Hz, 1 H, H-3′), 2.62 (s, 3 H, CH_3) and 2.32 (s, 3 H, CH_3).

Preparation of 2,2,6-Trimethylchroman-4-one (21)

A mixture of 2′-hydroxy-5′-methylacetophenone (**25**) (1 g, 6.66 mmol), acetone (2.5 ml), piperidine (0.56 ml, 6.66 mmol) and pyridine (2.5 ml) was heated at reflux for 72 h. The mixture was then cooled, concentrated under reduced pressure and poured into a mixture of 2N NaOH (25 ml) and ice. The resultant solution was extracted with ethyl acetate, the organic layer was washed with water, dried over anhydrous $MgSO_4$ & concentrated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel (petroleum ether-diethyl ether, 19:1) to afford **21** (0.98 g, 77 %) as a light yellow oil.

IR (neat) ν (fig. 3.13) = 2990, 1687, 1610, 1565, 1475, 1440, 1375, 1320, 1270, 1250, 1210, 1190, 1170, 1130, 1110, 930, 900 and 840 cm^{-1}.

EIMS(70 eV) m/z (fig. 3.14) = 190 (M^+, 70), 175 (M^+ - CH_3, 100), 134 (50), 133 (28), 107 (16), 106 (44) and 105 (28).

^{1}H NMR:(200 MHz, $CDCl_3$) δ (fig. 3.15) = 7.65 (d, *J* = 2.0 Hz, 1 H, H-5), 7.28 (dd, *J* = 8.5, 2.0 Hz, 1 H, H-7), 6.82 (d, *J* = 8.5 Hz, 1 H, H-8), 2.72 (s, 2 H, CH_2), 2.3 (s, 3 H, CH_3) and 1.44 (s, 6 H, 2 x CH_3).

Preparation of 4-methyl-phenyl-β,β-dimethylacrylate (27)

β,β-dimethylacryloyl chloride (**26**) was prepared by treating β,β-dimethylacrylic acid with excess of thionyl chloride. The said acryloyl chloride (**26**) (5 g, 42.19 mmol) was added dropwise to *p*-cresol (4 g, 37 mmol), over a period of one h. The reaction mixture was heated on a steam-bath for 4 h and then cooled, water (50 ml) was added, and the resultant reaction product was extracted with diethyl ether. The reddish brown oily product obtained after evaporation of the solvent was purified by a silica gel column chromatography to give 4-methyl-phenyl-β,β-dimethylacrylate as a light yellow oil (**27**) (5.2 g, 74 %).

IR (neat) ν = 2995, 1735, 1650, 1610, 1600, 1510, 1440, 1350, 1380, 1200, 1160, 1120, 1060 and 960 cm^{-1}.

Fries rearrangement of 4-methyl-phenyl-β,β-dimethylacrylate (27) to 1-(2′-hydroxy-5′-methylphenyl)-3-methyl-2-buten-1-one (28)

4-methyl-phenyl-β,β-dimethylacrylate (**27**) (4.2 g, 30 mmol) was added slowly to powdered anhydrous aluminium trichloride (4.14 g, 27.79 mmol), and the mixture kept at 90 ^{0}C for 2 h. After cooling, 2 N HCl (45 ml) was added, and the yellow coloured reaction product was extracted with diethyl ether (3 x 30 ml), washed with water, dried over $MgSO_4$ and concentrated. The dark yellow coloured solid obtained was purified by silica gel column chromatography to give 1-(2′-hydroxy-5′-methylphenyl)-3-methyl-2-buten-1-one (**28**) (2.89 g, 68 %) as yellow coloured solid, m.p. 49 ^{0}C.

IR (KBr) ν = 3450, 2990, 1660, 1615, 1575, 1450, 1380, 1350, 1295, 1225, 1210, 1080, 1000 and 795 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 190 (M^+, 5), 175 (M^+ - CH_3, 100) and 135 (M^+- C_4H_7, 12).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 12.63 (br s, exchangeable with D_2O, 1 H, -OH), 7.55 (d, *J* = 1.5 Hz, 1 H, H-6′), 7.22 (dd, *J* = 9.0 Hz, 1.5 Hz, 1 H, H-4′), 6.88 (d, *J* = 9.0 Hz, 1 H, H-3′), 6.77 (s, 1 H, H-2), 2.34 (s, 3 H, H-7′), 2.2 (s, 3 H, H_3-5) and 2.05 (s, 3 H, H_3-4).

^{13}C NMR ($CDCl_3$, 100 MHz): δ = 196.3 (1C, $C_{quat.}$), 161.2 (1C, $C_{quat.}$), 157.1 (1C, $C_{quat.}$), 136.8 (1C, CH), 129.6 (1C, CH), 127.6 (1C, $C_{quat.}$), 120.4 (1C, $C_{quat.}$), 120.2 (1C, CH), 110.2 (1C, $C_{quat.}$), 28.0 (1 C, CH_3), 21.3 (1C, CH_3) and 20.5 (1C, CH_3).

Cyclization of 1-(2'-hydroxy-5'-methylphenyl)-3-methyl-2-buten-1-one (28) to Chromanone (21)

A solution of **28** in aqueous base was prepared by dissolving (**28**, 0.150 g, 0.78 mmol) in 10 ml of 5 % aqueous sodium hydroxide. The resulting bright yellow solution was stirred at 50 ^{0}C for 5 h. After cooling to room temperature the solution was diluted with water and extracted with diethyl ether (3 x 25 ml), washed with brine and dried ($MgSO_4$). Evaporation of the ether under reduced pressure provided viscous oil which was subjected to purification by silica gel chromatography to give chromanone (**21**) (0.1 g, 65 %) as a light yellow oil having identical spectral properties as described earlier.

Lactarochromal (11) and the corresponding acid (13)

Chromanone (**23**) (0.4 g, 2.106 mmol) in acetonitrile (15 ml) was added to a solution of potassium persulfate (1.25 g, 4.2 mmol) in water (15 ml) & copper sulfate (0.127 g, 5 mmol). The resulting solution was stirred at 75-80 ^{0}C for 1 h and then cooled. The cold mixture was extracted with diethyl ether (3 x 50 ml) and the combined ethereal layer was washed with 60 % sodium bicarbonate solution (3 x 30 ml). Acidification of the bicarbonate washings with dil HCl gave a colourless crystalline solid **13** (0.09 g, 19 %), m.p. 182 ^{0}C.

IR (KBr) ν (fig. 3.16) = 3450, 3000, 1710, 1615, 1565, 1475,1440, 1415, 1360, 1325, 1280, 1245, 1225, 1120, 930, 840 and 770 cm^{-1}.

EIMS (70 eV) m/z (fig. 3.17) = 220.1 (M^+, 48), 205.1 (M^+ - CH_3, 100), 175.1 (10), 165 (44), 164 (40), 149.1 (12), 136 (15) and 119 (14).

^{1}H NMR (300 MHz, $CDCl_3$) δ (fig. 3.18) = 8.63 (d, *J* = 2.0 Hz, 1 H, H-5), 8.19 (dd, *J* = 8.5, 2.0 Hz, 1 H, H-7), 7.0 (d, *J* = 8.5 Hz, 1 H, H-8), 2.85 (s, 2 H, CH_2) and 1.49 (s, 6 H, 2 x CH_3).

^{13}C NMR (50 MHz, $CDCl_3$) δ (fig. 3.19) = 191.2 (1C, $C_{quat.}$), 191.2 (1C, $C_{quat.}$), 170.8 (1C, $C_{quat.}$),163.2 (1C, $C_{quat.}$), 137.1 (1C, CH), 129.8 (1C, CH), 121.5 (1C, $C_{quat.}$), 119.8 (1C, $C_{quat.}$), 119.2 (1C, CH), 48.2 (1C, CH_2) and 26.2 (2C, 2 x CH_3).

The remaining ether extract was washed with water, dried over anhydrous Na_2SO_4 & evaporated under reduced pressure. The crude solid product obtained was further purified using a silica gel column (petroleum ether-diethyl ether, 9:1) to give a colourless crystalline solid **11** (0.26 g, 61 %), m.p. 91°C (lit.[20] m.p. 89-91°C). The m.p. and the spectal data (IR, ^{1}H NMR, ^{13}C NMR and MS) recorded on this synthetic sample were in perfect agreement with those reported for lactarochromal[20].

Transformation of (13) to 2,2-dimethyl-6-carboxylchroman-4-one (29)

$NaBH_4$ (0.016 g, 0.42 mmol) was added to a cold stirred suspension of **13** (0.08 mg, 0.364 mmol) in methanol (5 ml) at 0 °C and the resultant reaction mixture was stirred at this temperature for 40 min. After stirring for additional 24 h at room temperature, 2M HCl was added and the product was extracted with ethyl acetate (3 x 25 ml). The ethyl aceatate extract was washed with water, dried over ($MgSO_4$) and concentrated. The resulting semi-solid residue was then chromatographed on a silica gel column and eluted with $CHCl_3$: MeOH (2 : 3) to give **29** (0.06 g, 76.8 %) as a colourless crystalline solid with m.p.159-160 °C.

IR (KBr) ν/max = 3400, 2980, 1690, 1610, 1575, 1490,1450, 1415, 1370, 1310, 1290, 1265, 1195, 1115, 1070, 920, 905 and 770 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ = 8.12 (d, *J* = 2.1 Hz, 1 H, H-5), 7.71 (dd, *J* = 8.6, 2.1 Hz, 1 H, H-7), 6.73 (d, *J* = 8.6 Hz, 1 H, H-8), 4.72 (m, 1 H, H-4), 2.1/1.75 (dd, J = 9.7 Hz, 5.9 Hz, 2 H, H_2-3), 1.38 (s, 3 H, CH_3) and 1.25 (s, 3 H, CH_3).

^{13}C NMR (75.5 MHz, $CDCl_3$ + DMSO-d_6) δ = 165.6 (1C, $C_{quat.}$), 155.1 (1C, $C_{quat.}$), 128.7 (1C, CH),128.2 (1C, CH), 124.1 (1C, $C_{quat.}$), 120.6 (1C, $C_{quat.}$), 114.7 (1C, CH), 74.7 (1C, $C_{quat.}$), 40.1 (1C, CH_2), 27.4 (1C, CH_3) and 24 (1C, CH_3).

Preparation of Anofinic acid (12)

A mixture of acid **29** (0.05 g, 0.225 mmol) and TsOH (0.01 g, 0.55 mmol) in toluene (10 ml) was refluxed under nitrogen atmosphere for 20 h. The reaction mixture was cooled and concentrated under reduced pressure. The residue obtained was chromatographed on silica gel and eluted with petroleum ether : ether (1 : 4) to give **12** (0.03 g, 66.7 %), m.p. 154-155 °C (lit.[20] m.p. 150-156 °C).

IR (KBr):ν = 3450, 2990, 1675, 1610, 1600, 1560, 1490, 1430, 1370, 1260, 1210, 1160, 960, 820 and 710 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ =8.12 (dd, *J* = 8.5 Hz, 2.5 Hz, 1 H, H-7), 7.93 (d, *J* = 2.5 Hz, 1 H, H-5), 7.02 (d, *J* = 8.5 Hz, 1 H, H-8), 6.5 (d, *J* = 11 Hz, 1 H, H-4), 5.78 (d, *J* = 11 Hz, 1 H, H-3) and 1.5 (s, 6 H, 2 x CH_3).

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ = 171.9 (1C, $C_{quat.}$), 158.7 (1C, $C_{quat.}$), 131.9 (1C, CH), 131.2 (1C, CH), 128.8 (1C, CH), 121.8(1C, CH), 120.9 (1C, $C_{quat.}$), 116.6 (1C, CH), 76.8 (1C, $C_{quat.}$) and 28.8 (2C, 2 x CH_3).

Preparation of 1,3,5-trimethoxybenzene (34)

Anhydrous K_2CO_3 (11 g, 80 mmol) was added to a solution of 1,3,5-trihydroxybenzene (**33**) (3 g, 24 mmol) in dry acetone (22.5 ml). To this slurry was added dimethyl sulfate (7.8 mL, 80 mmol) while stirring with an overhead stirrer over a period of 1 h. The reaction mixture was then refluxed for 24 h, with simultaneous stirring. This was then cooled and filtered. The residual K_2CO_3 was washed twice with acetone and filtered. Evaporation of the solvent from the combined filtrate gave a brown solid which on chromatography over silica gel using petroleum ether : diethyl ether (9 : 1) furnished a white solid. Recrystallisation from petroleum ether gave white needles of **34** (3.2 g, 80 %) with m.p. 54 °C (lit.[44] m.p. 54-5 °C).

IR (KBr) ν = 2900, 1630, 1615, 1475, 1450, 1210, 1200, 1160, 1075 and 950 cm^{-1}.

Acylation of (34) to 2′,4′,6′-trimethoxyacetophenone (35)

Anhydrous $AlCl_3$ (3.5 g) was added portionwise at -10 °C to a solution of 1,3,5-trimethoxybenzene (**34**) (2.02 g, 12 mmol) in dichloromethane (150 ml). Then, acetyl chloride (2 ml) was added dropwise by maintaining the reaction temperature to below -5 °C. After stirring overnight, the mixture was poured into ice-water, and extracted with dichloromethane (3 x 40 ml). The combined organic layer was successively washed with saturated $NaHCO_3$ and brine, then dried over Na_2SO_4. Evaporation of the solvent gave a brown coloured residue which was further purified by silica gel column chromatography to give 2′,4′,6′-trimethoxyacetophenone (**35**) (1.76 g, 70 %) as a light orange coloured solid with m.p. 110 °C (lit.[42] m.p. 110 °C).

IR (KBr) ν = 2900, 1700, 1620, 1600, 1475, 1450, 1415, 1275, 1210, 1175, 1125 and 900 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 210.2 (M^+, 28), 195.1 ($M^+ - CH_3$, 100), 180.1 (8) and 137 (8).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.103 (s, 2 H, H-3, H-5), 3.80 (s, 3 H, OCH_3), 3.56 (s, 6 H, 2 x OCH_3) and 2.45 (s, 3 H, CH_3).

Partial demethylation of (35) to 2′,4′-dihydroxy-6′-methoxyacetophenone (36)

A mixture of 2′,4′,6′-trimethoxy-acetophenone (**35**) (1.5 g, 7.14 mmol), anhydrous $AlCl_3$ (3.5 g) and chlorobenzene (15 ml) were refluxed for 1 h. Chlorobenzene was then removed by steam distillation, and the residual solution was cooled to give the cream-coloured needles. Recrystallisation from ethanol gave **36** (0.5 g, 38.5 %), m.p. 205 °C (lit[42]. m.p. 205-207 °C).

IR (KBr) ν = 3450, 2900, 1645, 1620, 1610, 1475, 1450, 1280, 1210, 1160 and 1110, cm^{-1}.

EIMS (70 eV): *m/z* (%) = 182.1 (M^+, 44), 167.1 ($M^+ - CH_3$, 100), 152.1 (10) and 124.1 (8).

^{1}H NMR (300 MHz, DMSO-d_6): δ = 13.78 (br s, exchangeable in D_2O, 1 H, -OH), 5.96 (d, J = 2.1 Hz, 1 H, H-5), 5.86 (d, J = 2.1 Hz, 1 H, H-3), 3.82 (s, 3 H, OCH_3) and 2.52 (s, 3 H, CH_3).

^{13}C NMR (75.5 MHz, DMSO-d_6): δ = 202.1 (1C, $C_{quat.}$), 166.1 (1C, $C_{quat.}$), 165.6 (1C, $C_{quat.}$), 163.2 (1C, $C_{quat.}$), 104 (1C, $C_{quat.}$), 95.5 (1C, CH), 91.2 (1C, CH), 55.7 (1C, OCH_3) and 32.4 (1C, CH_3).

Preparation of 7-hydroxy-5-methoxy-2-methylchromone (maritimin 30)

A solution of 2′,4′-dihydroxy-6′-methoxyacetophenone (**36**) (0.1 g, 0.549 mmol) in dry ethyl acetate (1.2 ml) under N_2 atmosphere was added to pulverized sodium (0.12 g) with cooling. After 15 min, the reaction mixture was maintained at 0 °C for 48 h. The excess ethyl acetate was then removed under reduced pressure and the residue was treated with a mixture of crushed ice and HCl. The precipitated solid (β-diketone) was filtered, and dissolved in methanol containing a trace of sulfuric acid and heated under reflux for

30 min. The reaction mixture after cooling was diluted with water to give the brown coloured solid, which was further purified by silica gel column chromatography, eluent-petroleum ether : diethyl ether (4 : 1) to give a colourless crystalline solid **30** (0.018 g, 32 %) with m.p. 114 ^{0}C (lit[37]. m.p. 112 -113 ^{0}C).

IR (KBr) ν (fig. 3.20) = 3450, 1665, 1610, 1565, 1200 and 900 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.21) = 206 (M^+, 100), 178 (M^+ - CO, 16), 177(M^+ - [CO + H], 90), 148 (30), 123 (20), 95 (30) and 69 (40).

HRMS : found 206.0556, calculated for $C_{11}H_{10}O_4$: 206.0579.

^{1}H NMR (300 MHz, $CDCl_3$): δ (fig. 3.22) = 6.35 (d, J = 2.4 Hz, 1 H, H-8), 6.33 (d, J = 2.4 Hz, 1 H, H-6), 6.02 (s, 1 H, H-3), 3.84 (s, 3 H, OCH_3) and 2.37 (s, 3 H, CH_3).

^{13}C NMR (75.5 MHz, $CDCl_3$): δ (fig. 3.23) = 180.5 (1C, $C_{quat.}$), 167 (1C, $C_{quat.}$), 163.3 (1C, $C_{quat.}$), 160.8 (1C, $C_{quat.}$), 158 (1C, $C_{quat.}$), 108.7 (1C, CH), 104.3 (1C, $C_{quat.}$), 97.1 (1C, CH), 94.9 (1C, CH), 56.9 (1C, OCH_3) and 20.8 (1C, CH_3).

Further elution of the silica gel column with petroleum ether : diethyl ether (3 : 2) gave cream-coloured needles of recovered (**36**) (0.050 g).

General procedure for the preparation of cyclohexenone derivatives

Preparation of 3-(2′-hydroxy-5′-methylphenyl)-5,5-dimethyl-cyclohex-2-en-1-one (37a)

A mixture of 2′-hydroxy-5′-methylacetophenone (**25a**) (1.428 g, 9.52 mmol), acetone (5.6 ml) and pyrrolidine (3 ml) in 30 ml of toluene was refluxed by using Dean Stark Apparatus for 5 h. After cooling, dil HCl (15 ml) was added and the resultant mixture was stirred for 30 min and extracted with diethyl ether (3 x 30 ml). The combined ether layer was washed with water and dried over $MgSO_4$. The concentration of the organic part gave brown coloured residue which was purified using a silica gel column to give 3-(2′-hydroxy-5′-methylphenyl)-5,5-dimethyl-cyclohex-2-en-1-one (**37a**) (0.92 g, 43 %) as a colourless crystalline solid, m.p. 145 ^{0}C.

IR (KBr) ν (fig. 3.24) = 3450, 2980, 1635, 1600, 1570, 1420, 1355, 1315, 1235, 915 and 825 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.25) = 230 (M^+, 48), 215 (M^+, - CH_3, 88), 187 (19), 145 (100).

CIMS (NH_3): *m/z* (%) (fig. 3.26) = 231.1 ([M + 1]$^+$, 18), 248.1 ([M + 18]$^+$, 38), 461.3 ([2M + 1]$^+$, 100), 478.4 ([2M + 18]$^+$, 28) and 708.5 ([3M + 18]$^+$, 10).

^{1}H NMR (200 MHz, $CDCl_3$) δ (fig. 3.27) = 7.78 (s, exchangeable with D_2O, 1 H, -OH), 7.05 (d, *J* = 2.0 Hz, 1 H, H-6′), 7.01 (dd, *J* = 8.3 Hz, 2.0 Hz, 1 H, H-4′), 6.88 (d, *J* = 8.3 Hz, 1 H, H-3′), 6.78 (s, 1 H, H-2), 2.67 (s, 2 H, H_2-6), 2.37 (s, 2 H, H_2-4), 2.28 (s, 3 H, H_3-7′), 1.13 (s, 6 H, 2 x CH_3).

^{13}C NMR ($CDCl_3$, 75.5 MHz): δ (fig. 3.28) = 202.4 (1C, $C_{quat.}$), 158.7 (1C, $C_{quat.}$), 152.2 (1C, $C_{quat.}$), 131.2 (1C, CH), 129.2 (1C, $C_{quat.}$), 128.7 (1C, CH),126.7 (1C, CH), 125.5 (1C, $C_{quat.}$), 116.5 (1C, CH), 50.7 (1C, CH_2), 43.8 (1C, CH_2), 34.01 (1C, $C_{quat.}$), 28.3 (2C, CH_3) and 20.5 (1C, CH_3).

3-(2′-hydroxyphenyl)-5,5-dimethyl-cyclohex -2-en-1-one (37b)

The compound (**37b**) was prepared from 2-hydroxyacetophenone (**25b**) (1.3 g, 9.558 mmol) acetone (5.4 ml), pyrrolidine (2.5 ml) and toluene (30 ml) as per the general procedure described above to provide 3-(2′-hydroxyphenyl)-5,5-dimethyl-cyclohex -2-en-1-one (**37b**) (1.2 g, 58 %) as a light yellow crystalline solid, m.p. 121 ^{0}C.

IR (KBr) ν (fig 3.29) = 3420, 2950, 1630, 1590, 1570, 1450, 1395, 1320, 1290, 1235, 1210, 910 and 745 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.30) = 216 (M$^+$, 78), 201 (M$^+$, - CH_3, 100), 188 (M$^+$, - CO, 8), 173(M$^+$, - [$COCH_3$], 12), 132 (56) and 133 (40).

^{1}H NMR (200 MHz, $CDCl_3$) δ (fig. 3.31) = 8.12 (br s, exchangeable with D_2O, 1 H, -OH), 7.26 (d, *J* = 8.0 Hz, 1 H, H-6′), 7.22 (dd, *J* = 8.0 Hz, 2.0 Hz, 1 H, H-5′), 6.99 (d, *J* = 8.0 Hz, 1 H, H-4′), 6.9 (d, J = 8 Hz, 1 H, H-3′), 6.81 (s, 1 H, H-2), 2.67 (s, 2 H, H_2-6), 2.37 (s, 2 H, H_2-4), 2.28 (s, 3 H, H_3-7′) and 1.13 (s, 6 H, 2 x CH_3).

^{13}C/APT NMR ($CDCl_3$, 50 MHz): δ (fig. 3.32) = 202.6 (1C, $C_{quat.}$), 158.6 (1C, $C_{quat.}$), 154.5 (1C, $C_{quat.}$), 130.7 (1C, CH), 128.5 (1C, CH), 126.6 (1C, CH),126.1 (1C, $C_{quat.}$), 120.1 (1C, CH), 116.9 (1C, CH), 50.7 (1C, CH_2), 43.6 (1C, CH_2), 34.01 (1C, $C_{quat.}$) and 28.3 (2C, CH_3).

3-(2′-hydroxy-5′-acetamino-phenyl)-5,5-dimethyl-2-cyclohexen-1-one (37c)

The compound (**37c**) was prepared from 2-hydroxyacetophenone (**25c**) (1.1 g, 5.699 mmol) acetone (2.7 ml) pyrrolidine (1.15 ml) and toluene (30 ml) as per the general procedure described above to provide 3-(2′-hydroxy-5′-acetamino-phenyl)-5,5-dimethyl-cyclohex -2-en-1-one (**37c**) (0.57 g, 37 %) as a light yellow crystalline solid, m.p. 229 ^{0}C.

IR (KBr) ν (fig. 3.33) = 3290, 3190, 2950, 1645, 1610, 1555, 1500, 1450, 1425, 1375, 1350, 1320, 1300, 1275, 1250, 1210, 1125,1035, 910 and 805 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 3.34) = 273.1 (M^+, 76), 258.1 (M^+, - CH_3, 100), 230.1 (M^+, - CO, 8), 216.1 (82), 188.1 (12), 175 (20), 146 (38), 118 (16), 83 (36) and 41.1 (66).

^{1}H NMR (200 MHz, DSMO-d_6): δ (fig. 3.35) = 9.69 (s, exchangeable with D_2O, 1 H, -NH), 9.58 (br s, exchangeable with D_2O, 1 H, -OH), 7.45 (d, J = 2.0 Hz, 1 H, H-6′), 7.4 (dd, J = 8.0 Hz, 2.0 Hz, 1 H, H-4′), 6.82 (d, J = 8.0 Hz, 1 H, H-3′), 6.15 (s, 1 H, H-2), 2.62 (s, 2 H, H_2-6), 2.25 (s, 2 H, H_2-4), 1.99 (s, 3 H, CH_3NH) and 1.05 (s, 6 H, 2 x CH_3).

^{13}C/APT NMR (50 MHz, DMSO-d_6): δ (fig. 3.36) = 198.8 (1C, $C_{quat.}$), 167.6 (1C, $C_{quat.}$), 158.1 (1C, $C_{quat.}$), 150.6 (1C, $C_{quat.}$), 131.2 (1C, $C_{quat.}$), 126.4 (1C, $C_{quat.}$), 125.9 (1C, CH),121.5 (1C, CH), 119.5 (1C, CH), 116.1 (1C, CH), 50.5 (1C, CH_2), 43.0 (1C, CH_2), 33.7 (1C, $C_{quat.}$), 28.3 (2C, CH_3) and 23.6 (1C, CH_3).

Attempted preparation of the 3-(2′,4′,6′-trimethyl-phenyl)-5,5-dimethyl-cyclohex-2-en-1-one (37d)

A mixture of 2,4,6-trimethylacetophenone (**25d**) (1.88 g, 10 mmol), acetone (4.5 ml), pyrrolidine (0.9 ml) and toluene (60 ml) were refluxed as described in the general procedure. However, the reaction after usual work-up resulted in the recovery of 2,4,6-trimethylacetophenone (**25d**).

Attempted preparation of the 3-(3′,4′-dichloro-phenyl)-5,5-dimethyl-cyclohex-2-en-1-one (37e)

A mixture of 3,4-dichloroacetophenone (**25e**) (1.5 g, 7.94 mmol), acetone (3.8 ml), pyrrolidine (0.8 ml) and toluene (60 ml) were refluxed as described in the general

procedure and work-up as usual to provide back the starting material- 3,4-dichloroacetophenone (**25e**).

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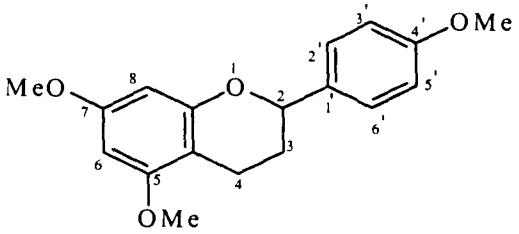
CHAPTER 4

MISCELLANEOUS SYNTHETIC STUDIES

SECTION I

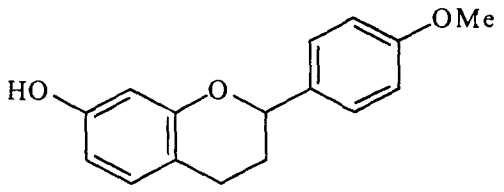
SYNTHESIS OF FLAVANS: ONE-POT SYNTHESIS OF FLAVANS FROM 4-METHYLCOUMARINS

The flavans constitute an important group having widespread natural occurrence[1-4]. The term flavan is applied, collectively, to a large group of compounds possessing a 3,4-dihydro-2-phenyl-2H-1-benzopyran (2-phenylchroman) nucleus. It has been observed that the flavans substituted in the heterocyclic ring are frequently encountered in nature, but the unsubstituted ones have rarely been found presumably due to their instability in solutions leading to polymeric products. Many of the naturally occurring flavans are reported to be lipid-soluble and appear to be the leaf-surface constituents[5]. The first naturally occurring flavan isolated was the 5,7,4′-trimethoxyflavan (**1**) from the methylated resin of the Australian 'blackboy' grass (*Xanthorrhea preissii*) by Birch *et al*[6]., in 1964. Subsequently, other red resins from *Dracaena draco*[7] & *Daemonorops draco*[8] were also investigated to yield a variety of mono- & dimeric flavans. Since then, a number of naturally occurring flavans have been reported in the literature[9,10].



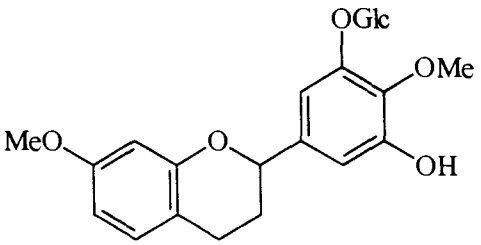
1

Naturally occurring flavans exhibit a number of important biological activities like coronary vasodilatary[11], fiscidal[12], anti-tumor[13] and anti-feedant activity[14]. The antibacterial action of flavans and the defensive role played by these compounds against microorganisms have been well studied[15]. For example, the flavan Broussin (**2**) isolated from the wounded xylum tissue of paper mulberry (*Broussonetia papyrifera* Vent) shoots has been ascribed as a phytoalexin[15].



2

Auriculoside (**3**) was the first flavan glycoside to be investigated pharmacologically followed by diffutin (**4**) and both of these compounds were found to be associated with adaptogenic activities[16,17]. Similarly, two new methylated flavan-3-ol glucosides (**5**) and (**6**), together with other known compounds were isolated from the methanol extract of the stem-bark of *Maytenus senegalensis*[18]. The aqueous extract of this plant is used as an important herbal remedy in folk medicine. Thus, the naturally occurring flavans continued to attract the attention of chemists in view of their interesting pharmacological properties.



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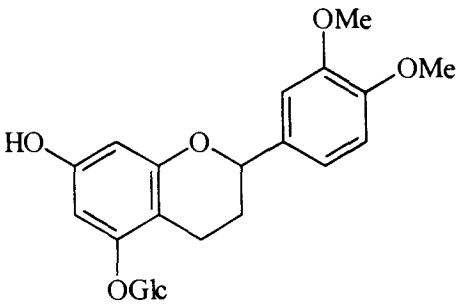

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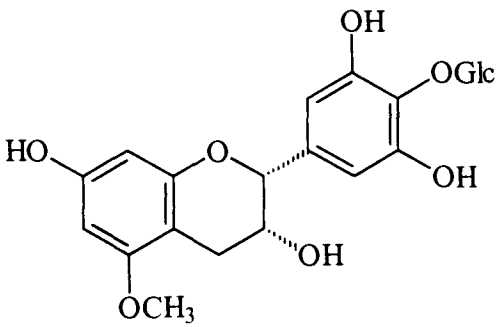

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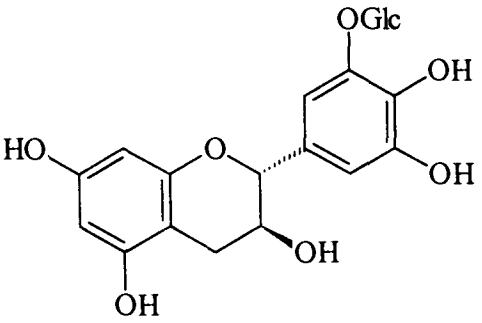
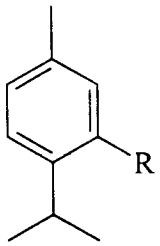


6

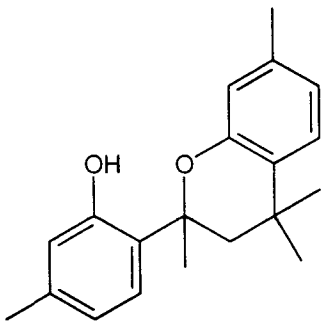
Recently, Yoshida, *et al*[19]., isolated from the roots of *Inula nervosa* (compositae), a thymol dimer named Inulavosin possessing a heterocyclic skeleton along with other two known compounds namely, thymol (**7**) and its isobutylate (**8**). Based on the spectral analysis, Inulavosin was assigned the structure **9a** by the said workers. Since the structure **9a**, assigned to the natural product was based only on spectral data, a synthetic support to prove unambiguously the said assignment

seemed desirable. In addition, **9a** was shown to be a piscicide, and since piscicides often exhibit other biological activities like insecticidal[20], anti-tumor promotion[21], aphrodisiac[22], tumor promotion[23] and antifungal properities[24,25], we were attracted towards the said newly reported natural product to effect its convenient synthesis.



7 R = OH

8 R = $OCOCH(CH_3)_2$

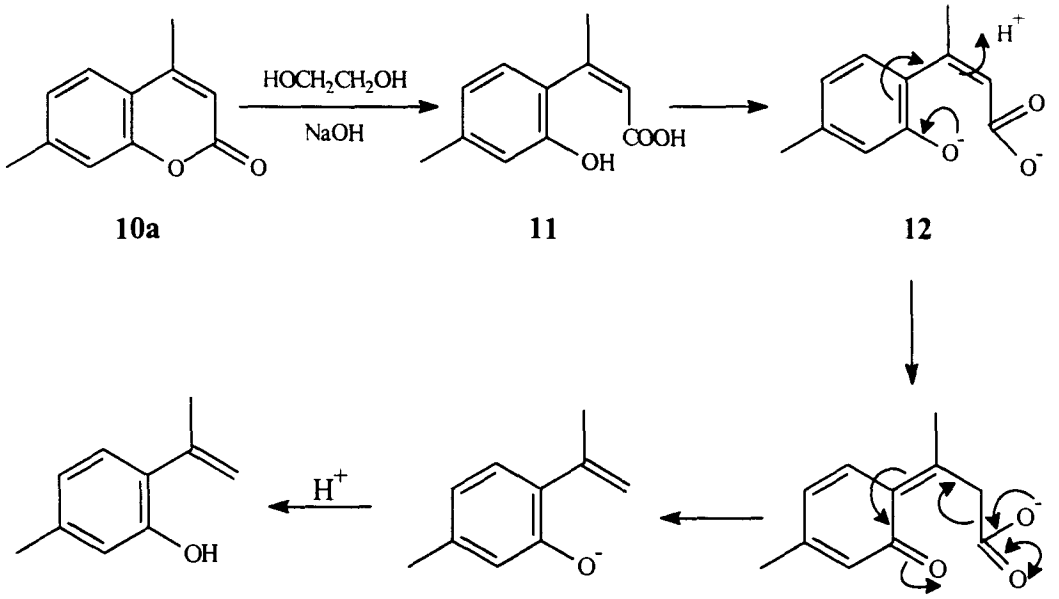


9a

A random survey of literature revealed that few such structurally related compounds have been prepared earlier by the dimerization of the corresponding *o*-isopropenylphenols. The *o*-isopropenylphenols required for the said purpose were generally prepared *via* the dehydration of the corresponding tertiary alcohols as reported by Baker *et al*[26]., and Still and Snodin[27] or by heating 2-hydroxy 4: β-dimethylcinnamic acid as demonstrated by Fries and Finkworth[28]. Similarly, Baker *et al*[29]., had also reported the preparation of such compounds from phenol and acetone in presence of dry HCl gas. More interestingly, Divakar and Rao[30] had observed that refluxing a mixture of 4-methylcoumarin, ethanediol and NaOH furnished the isopropenylphenol which they required in connection with the thermal rearrangement of some oxiranes. In all the above cases, the intermediate *o*-isopropenylphenol so formed was dimerized either by subjecting it to pyrolysis or by employing acidic reaction conditions to provide the corresponding flavan derivatives in low yields.

Accordingly, by considering the flavan based carbocyclic frame work present in the natural product, we anticipated that inulavosin (**9a**) could be synthesized by the dimerization of the appropriate *o*-isopropenylphenol. Thus, by taking a hint

from the work reported by Rao *et al*[30]., we envisaged that inulavosin (**9a**) could be prepared in a single step if optimum conditions are developed to effect *in situ* dimerization of the intermediate *o*-isopropenylphenol formed from the appropriate 4-methylcoumarins. Mechanistically, it was considered that[31] 4,7-dimethylcoumarin (**10a**) when refluxed with NaOH and ethanediol would initially furnish the hydroxy acid (**11**) by opening of the lactone ring followed by protonation of the dianion (**12**) to provide the quinone methide (**13**), which was then expected to decarboxylate readily to give the phenolate anion (**14**) which on acidification would provide the corresponding 5-methyl-2-isopropenylphenol (**15**) (Scheme- 1).



10a **11** **12**

15 **14** **13**

Scheme - 1

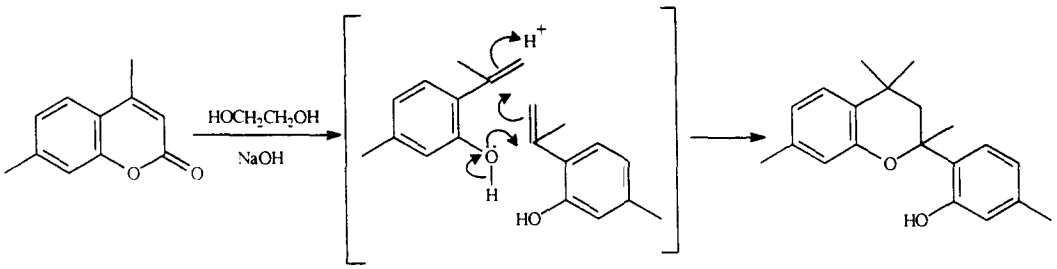
Thus, in agreement with above, when a mixture of 4,7-dimethylcoumarin (**10a**), ethanediol and sodium hydroxide was refluxed at 210 ^{0}C for two and half hour under nitrogen atmosphere, followed by acidification of the reaction mixture with dilute hydrochloric acid, it furnished a crystalline solid. The IR spectrum (fig. 4.01) of this solid compound showed a strong absorption band at 3410 cm^{-1} to demonstrate the presence of a phenolic hydroxyl group. The EIMS (fig. 4.02) showed a molecular ion peak at m/z 296, corresponding to the molecular formula $C_{20}H_{24}O_2$. The ^{1}H

NMR spectrum (fig. 4.03) showed the presence of five tertiary methyl groups at δ 1.18, 1.43, 1.69, 2.27 and 2.3 , the latter two being the aromatic methyl groups. The signal at δ 8.18 (1 H) was found to be exchangeable with D_2O, and hence was assigned to the phenolic hydroxyl group present in the molecule. The presence of an isolated methylene group was indicated by the signal at δ 2.54 and 2.06 (each 1 H, d, J = 15 Hz). Further, two pairs of ABX-type signals at δ 7.18 , 7.04 (d, J = 8 Hz, 1 H each), 6.82, 6.67 (dd, J = 8 Hz, 2.5 Hz 1 H each) and 6.75, 6.65 (d, J = 1.5 Hz, 1 H each) were observed in the aromatic region, indicating the presence of 1,3,4 (or 1,2,4) -trisubstituted benzene nucleus in the molecule. The ^{13}C NMR spectrum (fig. 4.4) exhibited 20 carbon signals comprised of eight sp^3 and twelve sp^2 carbon resonances. All the above described spectral characteristics were in accordance with the structure **9a** thereby confirming unambiguously the structure assigned to Inulavosin by the earlier workers[19]. It may be pertinent to note that Baker *et al*[26] in 1952, while investigating the condensation product formed by the reaction of *m*-cresol with acetone had reported the formation of a dimeric compound and identified the same as 2'-hydroxy-2,4,4,7,4'-pentamethylflavan based on its chemical degradation studies. However, it is surprising to note that Yoshida *et al*[19]., did not make any reference to the said work of Baker *et al*[26]., while reporting their work on the structure elucidation of inulavosin.

The spectral data (^1H, ^{13}C NMR, MS) recorded on the said crystalline solid agreed well with those reported on the natural inulavosin (**9a**). A point of interest to note here was that our synthetic sample was a crystalline solid having m.p. 76 ^0C in contrast to the natural product which was reported to be an oil.

It is interesting to note that the required natural product **9a** was obtained directly from **10a** in a single step rather than proceeding *via* two steps i.e. isolating the intermediate **15** followed by subsequent dimerization. This aspect has also avoided the difficulty in handling of the relatively unstable **15**, which has the tendency to undergo polymerization under the acidic condition. Thus, the 5-methyl-2-isopropenylphenol (**15**) generated *in situ* gets dimerized to the corresponding flavan **9a** during the course of the reaction as shown in the Scheme - 2. In other

words, this reaction leads directly to a one-pot synthesis of Inulavosin (**9a**) from the corresponding 4-methylcoumarin (**10a**).



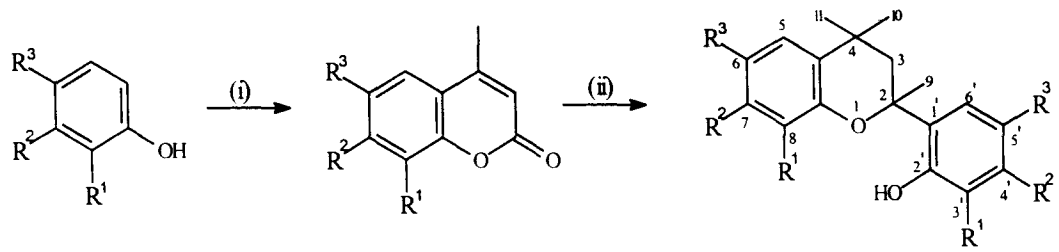
10a **9a**

Scheme - 2

It may be noted that the information pertaining to the preparation of these structurally related flavan derivatives have appeared more often in the patent literature than in the scholarly journals. By taking into account the importance attributed to these compounds, it was worthwhile to develop a convenient procedure for the preparation of such flavan derivatives in general starting from the appropriate phenols *via* the corresponding 4-methylcoumarins.

Encouraged by the one-pot synthesis of the natural flavan derivative Inulavosin (**9a**) as described above, we extended the said methodology to the preparation of four more flavans **9b** - **9f** as shown in the Scheme 3 & table 1.

Scheme - 3



16 **10** **9**

Reagents and conditions : (i) EAA, H^+ (ii) NaOH, $HOCH_2CH_2OH$, Reflux at 210 ^0C, N_2 atm., then H^+.

Table 1

Compound	R^1	R^2	R^3	Yield (%)
9a	H	CH_3	H	68
9b	H	OH	H	64
9c	H	H	CH_3	80
9e	CH_3	CH_3	H	61
9f	H	CH_3	CH_3	78

The required 4-methylcoumarin derivatives (**10**) were prepared from the respective phenols and ethylacetoacetate by using the standard reaction conditions[32]. Thus, 7-hydroxy-4-methylcoumarin (**10b**) provided the corresponding flavan (**9b**) in 64 % yield. The physical constant and the spectral data recorded on **9b** were in perfect agreement with the data reported on the compound which was formed by the acid catalyzed reaction of acetone with excess of resorcinol[33], the structure of which was revised subsequently by Livant and Xu[34]. Similarly, 4,6-dimethylcoumarin (**10c**) furnished 2′-hydroxy-2,4,4,6,5′-pentamethylflavan (**9c**) in 80 % yield. The acetate **9d** of this flavan was found to be identical in all respects with a compound isolated previously by Dinge *et al*[35]., who reported its formation as a minor product while attempting the synthesis of isothymol, a naturally occurring phenol isolated from *Nemolaene oaxacere*[36]. The other coumarins employed in the present study were 4,7,8-trimethylcoumarin (**10e**) and 4,6,7-trimethylcoumarin (**10f**) which were prepared from 2,3-dimethylphenol (**16e**) and 3,4-dimethylphenol (**16f**) respectively. The coumarin (**10e**), when subjected to the said condition, provided 2′-hydroxy-2,4,4,7,8,3′,4′-heptamethylflavan (**9e**) as a oily liquid in 60 % yield. The IR spectrum (fig. 4.12) showed bands at 3400 cm^{-1}, in addition to other absorption bands thereby indicating the presence of a phenolic hydroxyl group. The ^1H NMR spectrum (fig. 4.13) showed signals at δ 8.73 (1 H) which was found to be exchangeable with D_2O, confirming the presence of a hydroxyl group. It further disclosed seven tertiary methyl signals at δ 1.23, 1.46, 2.18, 2.19, 2.26 & 2.27, the

latter four were the aromatic methyl groups. The presence of an isolated methylene group was indicated by the signals at δ 2.5 & 2.05 (each 1 H, d, J = 15 Hz). In addition, it also showed aromatic proton signals at δ 7.03, 6.93 (each 1 H, d, J = 8 Hz) and 6.84, 6.69 (each 1 H, d, J = 8 Hz) indicating the presence of 1,2,3,4 (or 1,4,5,6)-tetrasubstituted benzene ring in the molecule. Thus, the above described spectral data were in conformity with the assigned structure **9e** for the said oily compound.

Similarly, the coumarin (**10f**) furnished the corresponding 2'-hydroxy-2,4,4,6,7,4',5'-heptamethylflavan (**9f**) as a crystalline solid, m.p. 157 ^{0}C, in 78 % yield. The IR spectrum (fig. 4.14) exhibited band at 3410 cm^{-1}, in addition to other absorption bands thereby indicating the presence of a hydroxyl group. The ^{1}H NMR spectrum (fig. 4.15) disclosed the methyl singlets at δ 1.21 (3 H), 1.43 (3 H), 1.67 (3 H), 2.18 (6 H) & 2.2 (6 H), indicating the presence of a gem-dimethyl and a tertiary methyl together with four aromatic methyl groups. The presence of a hydroxyl group and an isolated methylene groups were indicated by the signals at δ 8.18 (s, 1 H, disappearing on addition of D_2O) and 2.52, 2.05 (each 1 H, d, J = 15 Hz) respectively. In addition, it also showed two pairs of signals at δ 7.02, 6.86 (each 1 H, s) and 6.72, 6.65 (each 1 H, s) suggesting the presence of four aromatic protons in the molecule. The ^{13}C NMR spectrum (fig. 4.16) confirmed the presence of a methylene group at δ 48.05 and seven methyl groups at δ 18.97, 19.07, 19.26, 29.35, 30.76, 32.2 and 33.17. The presence of eight benzenoid carbons appearing as singlets at δ 152.38, 148.15, 137.1, 135.76, 130.07, 124.04, 118.9 & 118.62 and another set of four as doublets at δ 127.59, 127.47, 127.16 & 127.08 were all in agreement with the structure **9f** for the said crystalline compound.

It may be noted that the compounds **9e** and **9f** obtained during the present study represent two new additions to the said group of flavan derivatives.

In summary, it may be concluded that the present work has led to a one-pot synthesis of Inulavosin (**9a**) isolated from the roots of *Inula nervosa* and

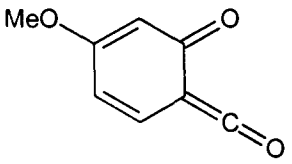
consequently has confirmed the structure that was assigned to the said natural product by the earlier workers. In addition, it has also provided a simple procedure which is easy to perform in a one-pot operation and will be generally applicable to the synthesis of a large number of flavan derivatives having the structure **9**.

The present work has already been published[37].

SECTION II

SYNTHESIS OF THE UNUSUAL PRODUCT OF THE PHOTOLYSIS OF 6-METHOXYBENZOFURAN-2,3-DIONE AND STYRENE

In an effort to develop a new general route to benzopyrone derivatives, Gray *et al*[38]., examined the possibility of generating the keto-ketene intermediate **17** by irradiation of 6-methoxybenzofuran-2,3-dione (**18**) and then trapping it by cyclo-addition to various alkenes and alkynes. Accordingly, they investigated the photolysis of 6-methoxybenzofuran-2,3-dione (**18**) and styrene (**19**) and obtained three products, out of which two were characterized as 6-methoxyisoaurone (**20**) and 2-hydroxy-4-methoxybenzoic acid (**21**). The third product, a crystalline solid, left unidentified by these authors, was assigned structure **22** by Paknikar & Kirtany[39] based on published spectral data and mechanistic considerations.



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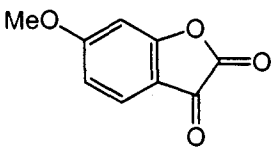

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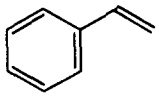

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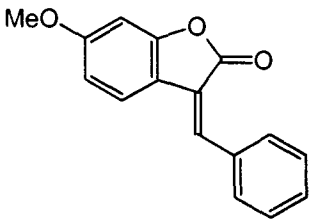

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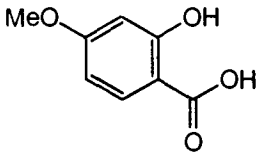
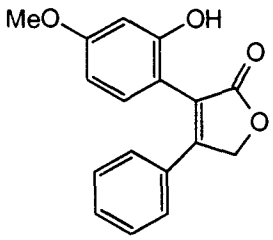


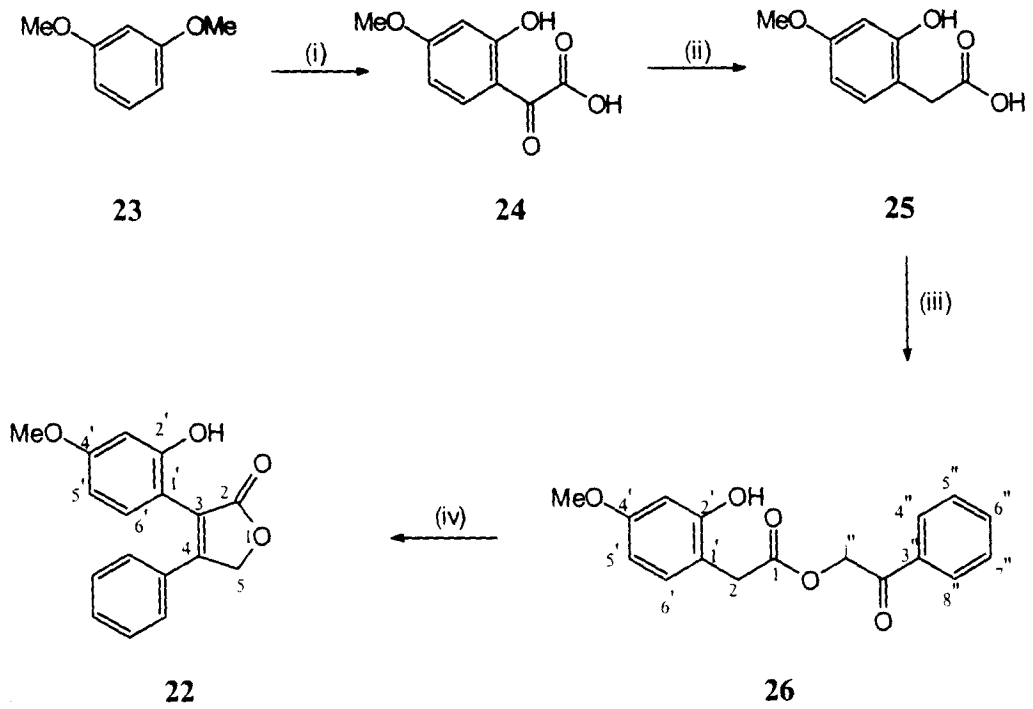
21



22

It was our interest to provide a synthetic proof to confirm the assigned structure **22** to the said product and also to prove that the reported lower value 1723 cm^{-1} of butenolide carbonyl absorption in the IR spectrum of **22** as compared to the normal value (1750 cm^{-1}) for such type of compounds is due to the influence of intramolecular hydrogen bonding involving the nearby hydroxyl function. With the said aim in mind, we undertook the synthesis of **22** as per the scheme 1.

Scheme 1



Key: (i) a) $C_2H_5OCOCOCl$, $AlCl_3$ b) aq. NaOH (ii) $NH_2NH_2.H_2O$ (99 %), NaOEt, N_2 atmp, Δ 200 ^{0}C, 15 min. (iii) $PhCOCH_2Br$, $(C_2H_5)_3N$, CH_3CN (iv) NaH, THF

The required 2-hydroxy-4-methoxyphenylacetic acid (**25**) was prepared *via* the corresponding glyoxylic acid (**24**) by following the literature procedure[38]. Accordingly, the readily available 1,3-dimethoxybenzene (**23**) was acylated in presence of anhydrous $AlCl_3$ catalyst utilizing ethyl oxalylchloride followed by hydrolysis of the ester thus formed to provide 2-hydroxy-4-methoxyphenylglyoxylic acid (**24**). The Wolff-Kishner reduction of the acid **24** gave the corresponding acid (**25**) with m.p.131 ^{0}C (lit[38] m.p. 131-3 ^{0}C). The infrared spectra of this acid exhibited carbonyl absorption in the region at 1699 cm^{-1} in addition to other absorption bands. The acid **25** was then condensed with phenacyl bromide in the presence of triethyl amine in acetonitrile[40] to provide the ketoester (**26**), m.p. 150 ^{0}C. The IR spectrum of **22** (fig. 4.17) showed a strong absorption

band at 3330 cm^{-1} to indicate the presence of a phenolic hydroxyl group while the other two bands at 1743 cm^{-1} and 1690 cm^{-1} demonstrated the presence of ester carbonyl and aryl carbonyl groups respectively. The ^1H NMR spectrum (fig. 4.18) of this compound showed the presence of a methoxy group at δ 3.78 (s, 3 H), and two singlets at δ 5.4 (2 H) and δ 3.81 (2 H) which were assigned to two methylene groups. Further, the signals which appeared at δ 7.9 (dd, J = 7.5 Hz, 1.5 Hz, 2 H), 7.52 (d, J = 7.5 Hz, 1 H) and 7.5 (dd, J = 7.5 Hz, 1.5 Hz, 2 H) were assigned to 4″, 8″, 6″, 5″ and 7″ aromatic hydrogens respectively. The signal at δ 7.05 (1 H) was found to be exchangeable with D_2O, and hence was assigned to the phenolic hydroxyl group present in the molecule. An ABX-type of signal pattern appeared at δ 7.06 (d, J = 7.5 Hz, 1 H), 6.55 (d, J = 2.5 Hz, 1H) and 6.49 (dd, J = 7.5 Hz, 2.5 Hz, 1 H), indicated the presence of 1,2,4-(or 1,3,4-) trisubstituted benzene ring in the molecule. The ^{13}C NMR spectrum (fig. 4.19) confirmed the presence of a methoxy group at δ 55.32 and two methylene groups at δ 66.66 and δ 55.32 respectively. The presence of eight benzenoid carbons (doublets at δ 134.2, 131.5, 128.9 (2 C), 127.8 (2 C), 107 and 103.7) and another set of six benzenoid carbons (singlets at δ 191.86, 172.75, 160.54, 155.91, 133.78 and 113.03) were all in conformity with the structure **26** for the said crystalline compound.

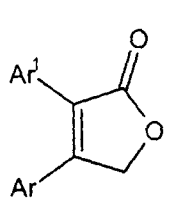
Having the required ketoester (**26**) in hand, we then turned our attention to effect the intramolecular cyclization in an effort to get the cyclized product **22**. Attempted cyclization reaction with Et_3N/CH_3CN according to the reported procedure[40] resulted in the recovery of the starting material. The desired cyclization was achieved through NaH as the base. Thus, the intramolecular cyclization of the keto ester (**26**) with sodium hydride in THF[41] gave the desired furanone (**22**) with m.p. 179 °C (lit.[38] 182-184 °C). The IR spectrum (fig. 4.20) of this compound showed bands at 3280 and 1723 cm^{-1} in addition to other absorption bands indicating the presence of a phenolic hydroxyl and a ester carbonyl group respectively.

The ^{1}H NMR spectrum (fig. 4.21) showed a signal at δ 7.68 (1 H) which was found to be exchangeable with D_2O, and hence corresponds to the hydroxyl group. A singlet at δ 7.36 (5 H) was assigned to a phenyl group, while the signal at δ 6.96 (d, J = 8.6 Hz, 1 H), 6.58 (d, J = 2.5 Hz, 1 H) and 6.38 (dd, J = 8.6 Hz, 2.5 Hz, 1 H) suggested the presence of 1,2,4-trisubstituted benzene ring. The singlets at δ 5.3 (2 H) and 3.80 (3 H) were assigned for the methylene and a methoxy group respectively. All the said spectral data were in complete agreement with the suggested structure **22** for the cyclized compound.

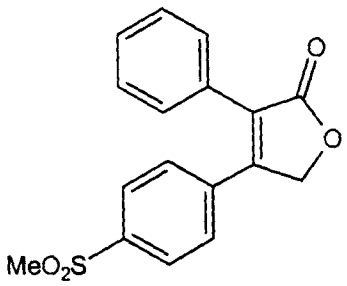
Especially noteworthy was the carbonyl absorption at 1723 cm^{-1} which was in perfect agreement with the reported value for the photoproduct by Gray *et al*[38]. Incidentally, this observation confirmed that the lower value of carbonyl absorption obtained in the present case is due to the hydrogen bonding involving the hydroxyl group situated on the aromatic ring. Thus, the m.p. & spectral data (IR & PMR) of the synthetic sample agreed well with the corresponding data reported for the photoproduct by Gray *et al*[1]., there by confirming unambiguously, the earlier proposed[39] structure **22** for the same.

The work described in this section has already been published[44].

It may be appropriate to mention here that though, the unsymmetrical 3,4-disubstituted 2(5H)-furanones occur rarely in nature[45], they are covered quite extensively as drugs and biocides in patent literature[46-48]. In particular, the unsymmetrical 3,4-diaryl disubstituted 2(5H)-furanones of the type **27** have been reported to be useful in the treatment of inflammation and other cyclooxygenase-2 mediated diseases[46,47].



27



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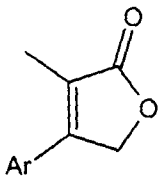

29

One such compound is Rofecoxib (Vioxx) (**28**), an anti-inflammatory drug launched by the Merck company recently[46]. On the other hand, several simple 4-aryl-3-methyl-2(5H)-furanones (**29**) have been reported to be highly effective as antifungal agents[48] for plants having agronomic importance and as a result, a number of methods have been developed for the synthesis of such compounds[49]. Against this background, the addition of a unsymmetrically disubsituted furanone derivative (**22**) to the existing list of such useful compounds (as a result of the present synthesis) may prove significant.

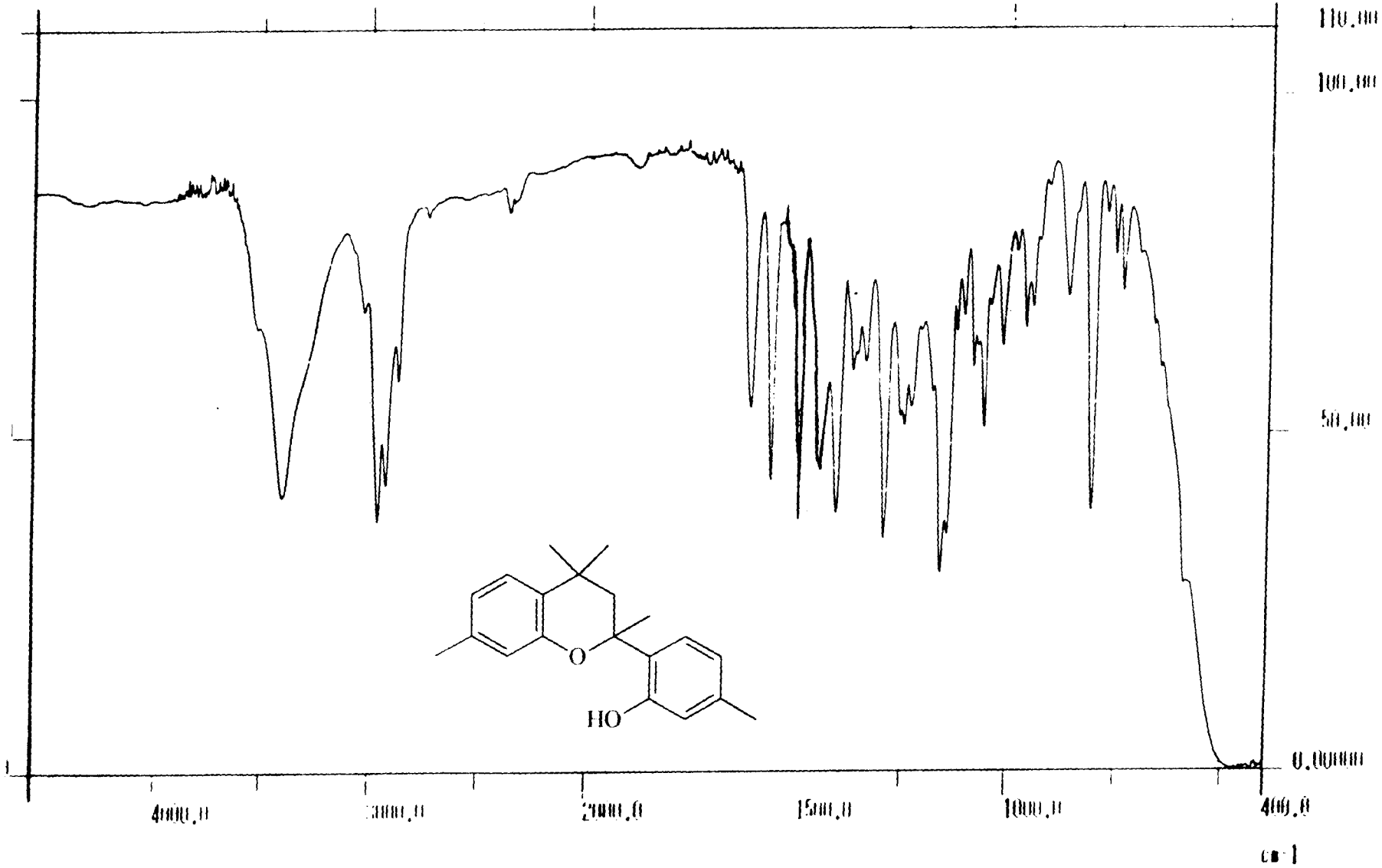

Fig. 4.01 : IR spectrum of inulavosin (9a)

CENTRAL DRUG RESEARCH INSTITUTE
02-25-1997

FB2525X.LRP NIO-524/K003 MRS S WAHIDULLA/NIO #7974
Date run : 02-25-1997 Operator : A.SONI/PRAKASH/SUNIL

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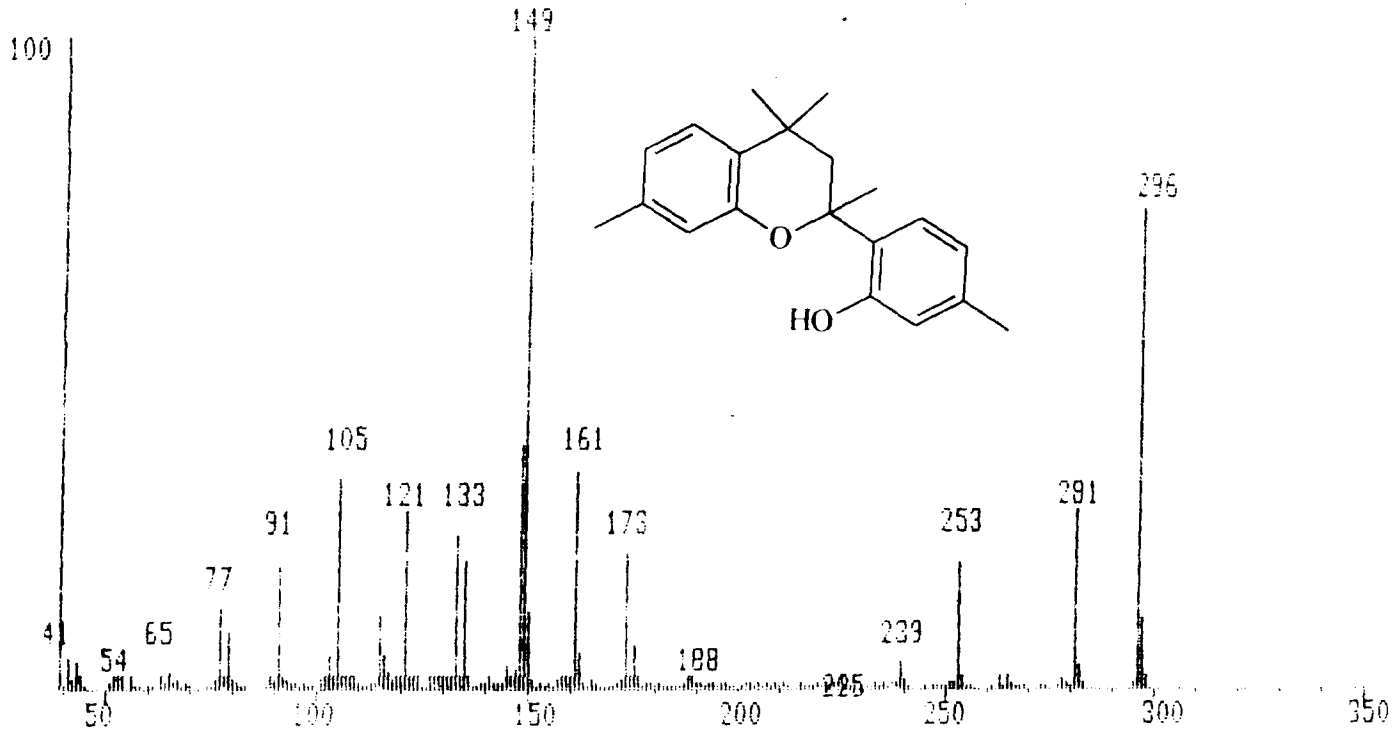

Fig. 4.02 : EI mass spectrum of inulavosin (9a)

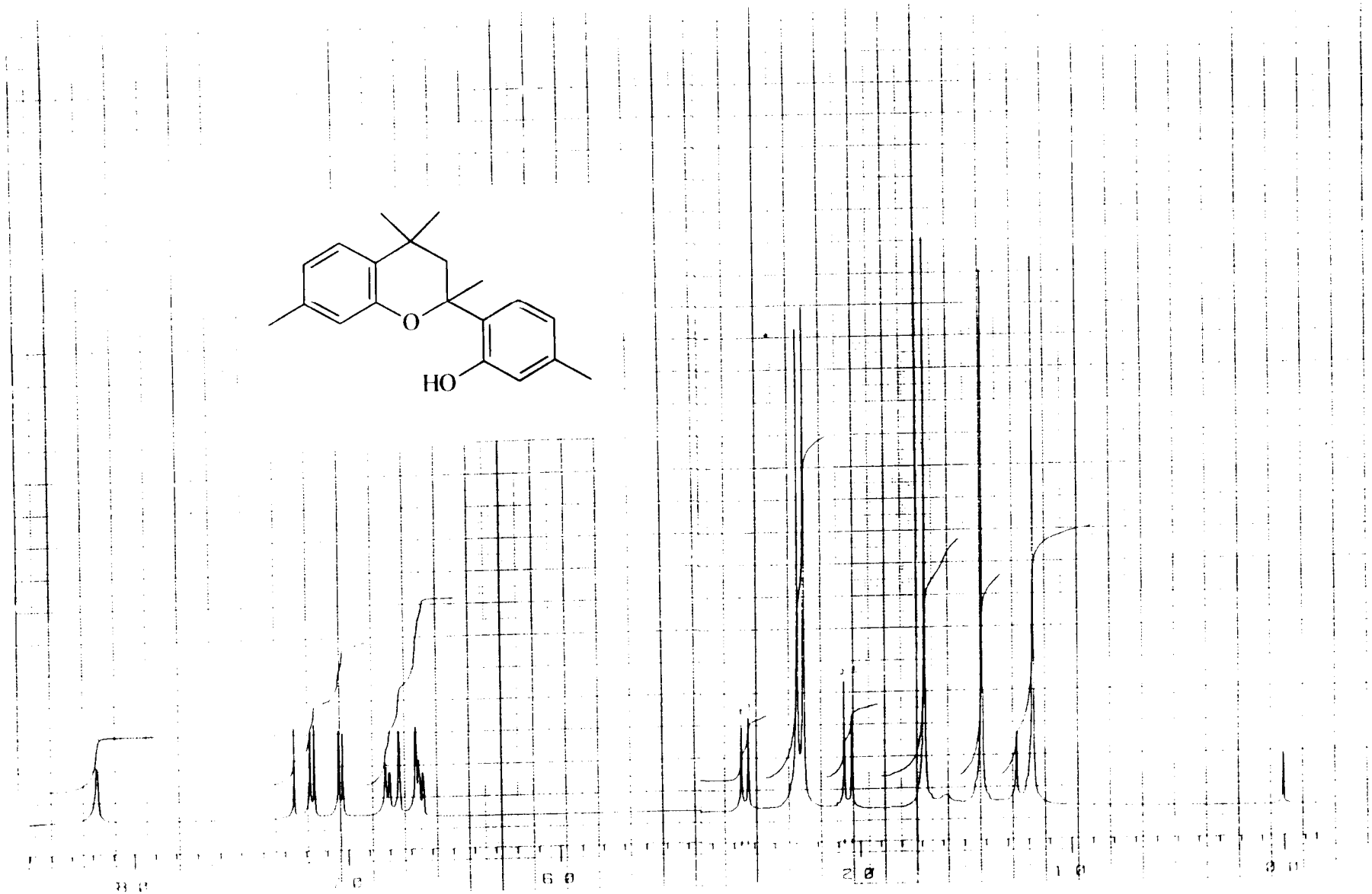

Fig. 4.03 : ^{1}H NMR spectrum of inulavosin (9a) in $CDCl_3$

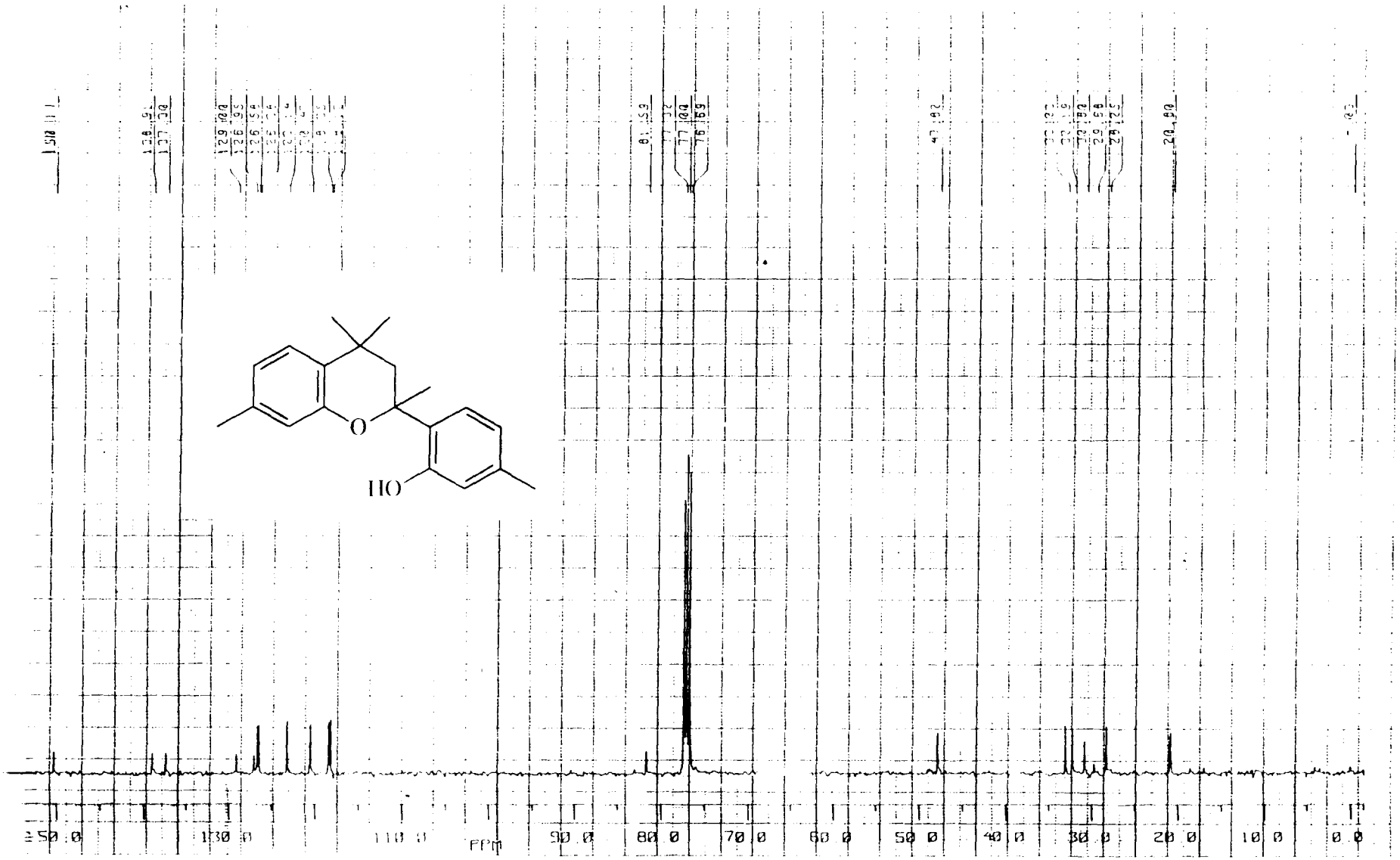

Fig. 4.04 : ^{13}C NMR spectrum of inulavosin (9a) in $CDCl_3$

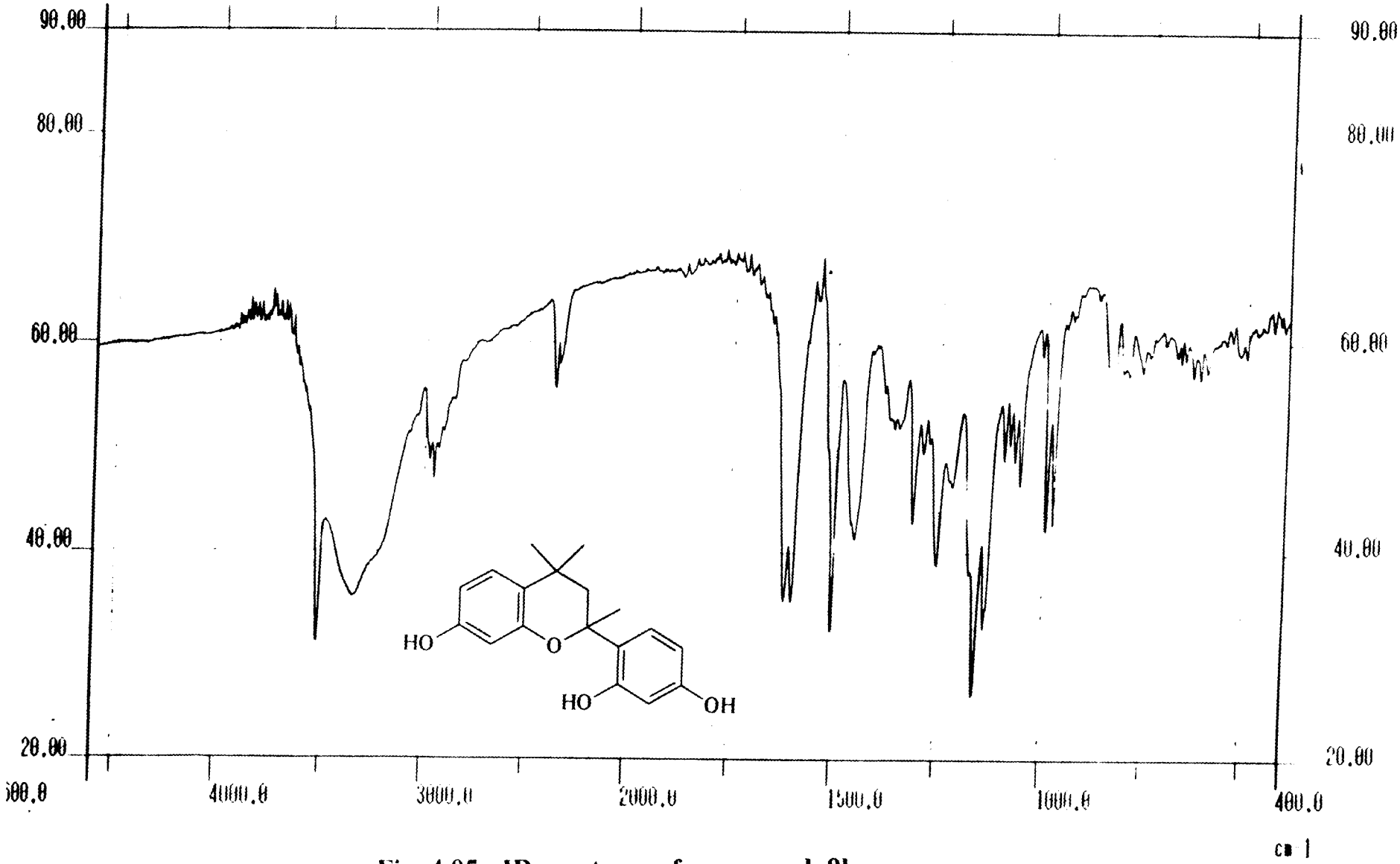

Fig. 4.05 : IR spectrum of compound 9b

CENTRAL DRUG RESEARCH INSTITUTE
04-30-1997

AP3009X.LRP KO022/DR S Y KAMAT/N10 #9132
Date run : 04-30-1997 Operator : A SONI/PRAKASH/SUNIL

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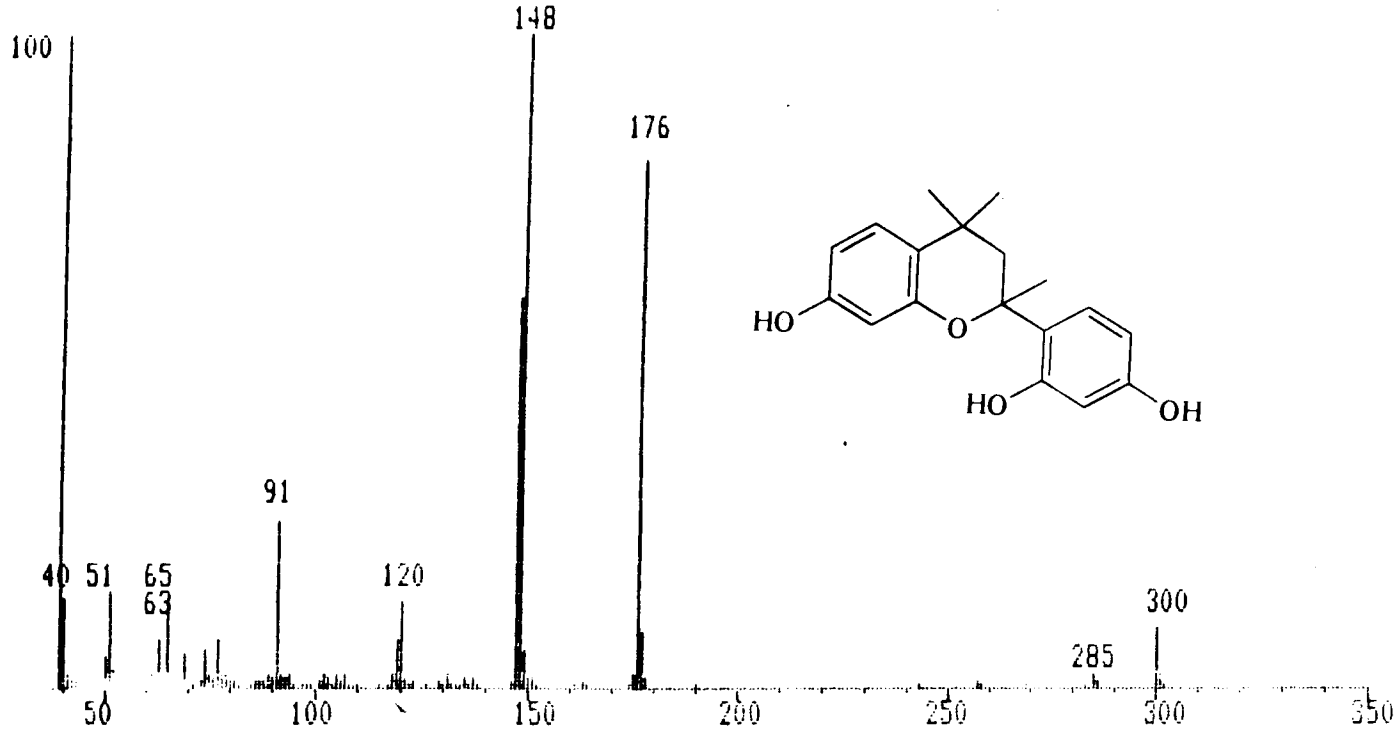

Fig. 4.06 : EI mass spectrum of compound 9b

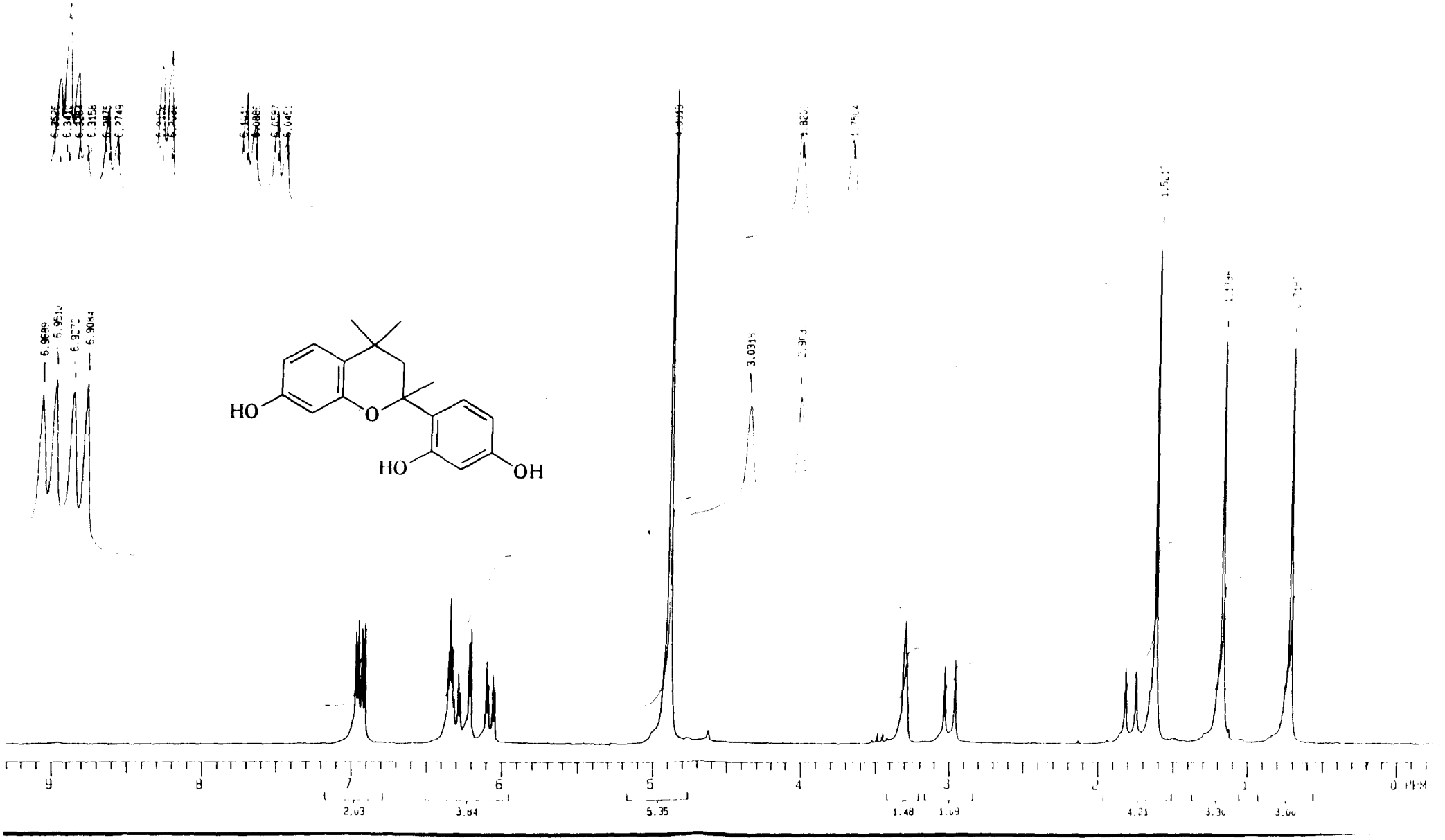

Fig. 4.07 : ^{1}H NMR spectrum of compound 9b in CD_3OD_3

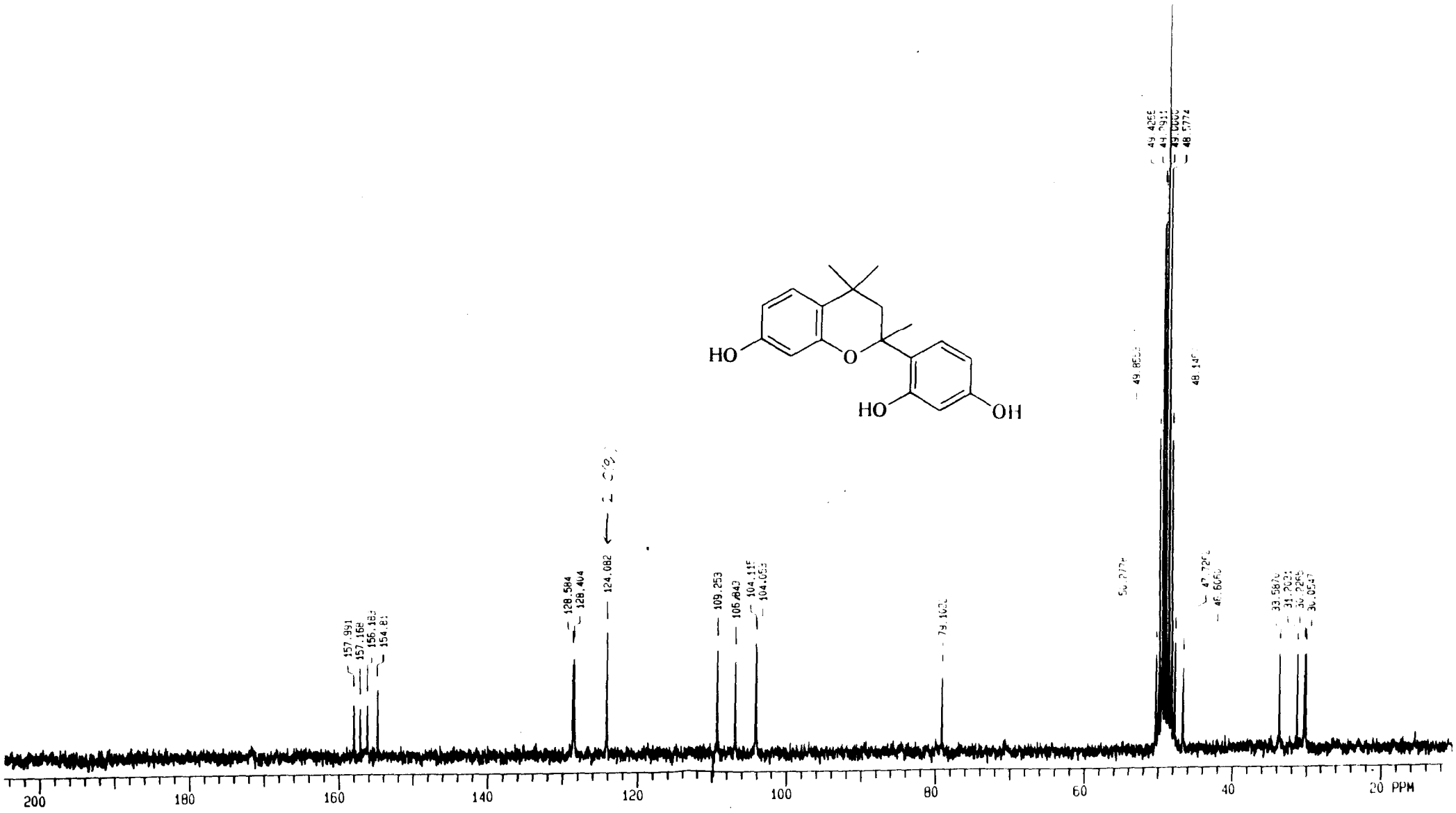

Fig. 4.08 : ^{13}C NMR spectrum of compound 9b in CD_3OD_3

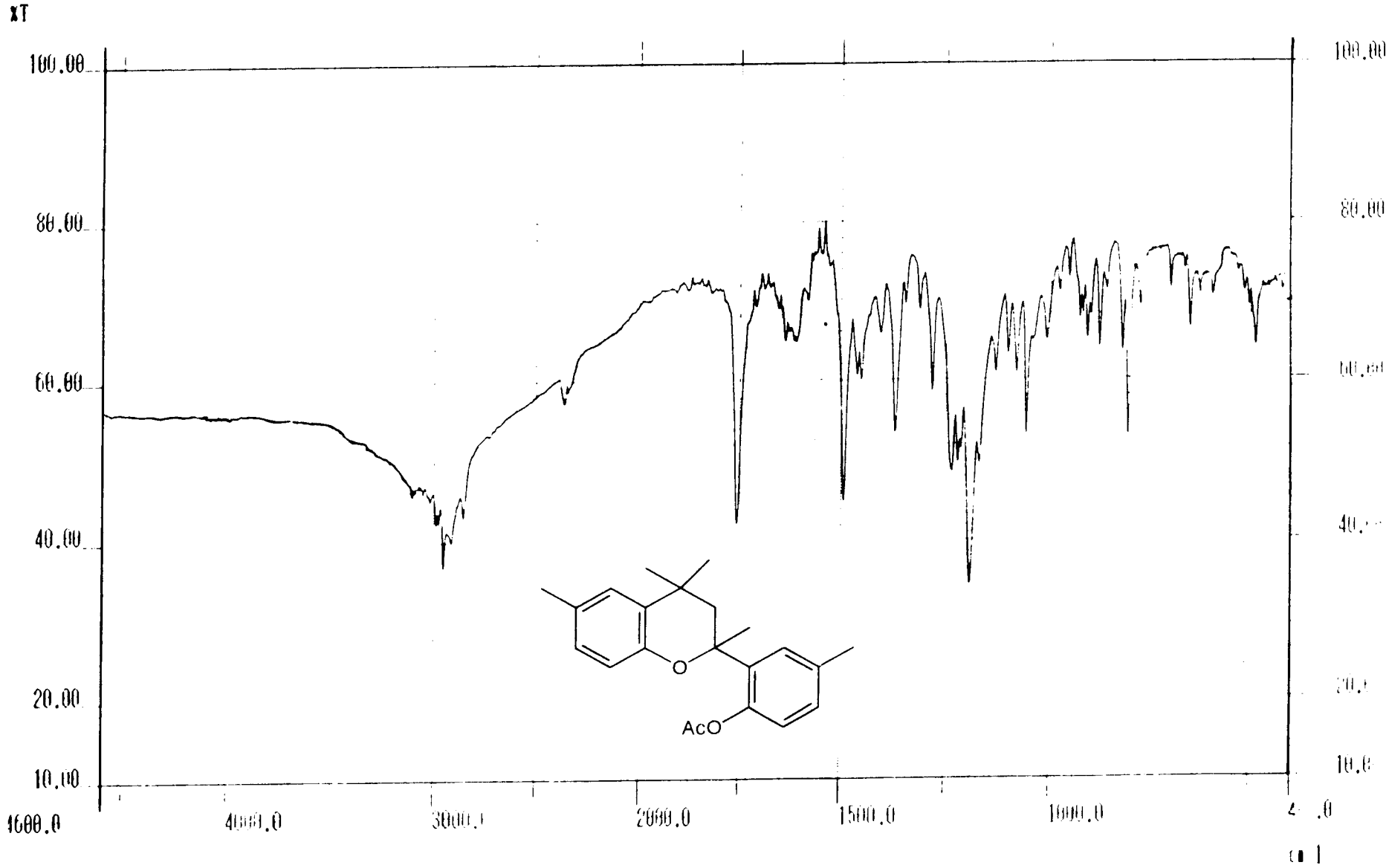

Fig. 4.09 : IR spectrum of compound 9d

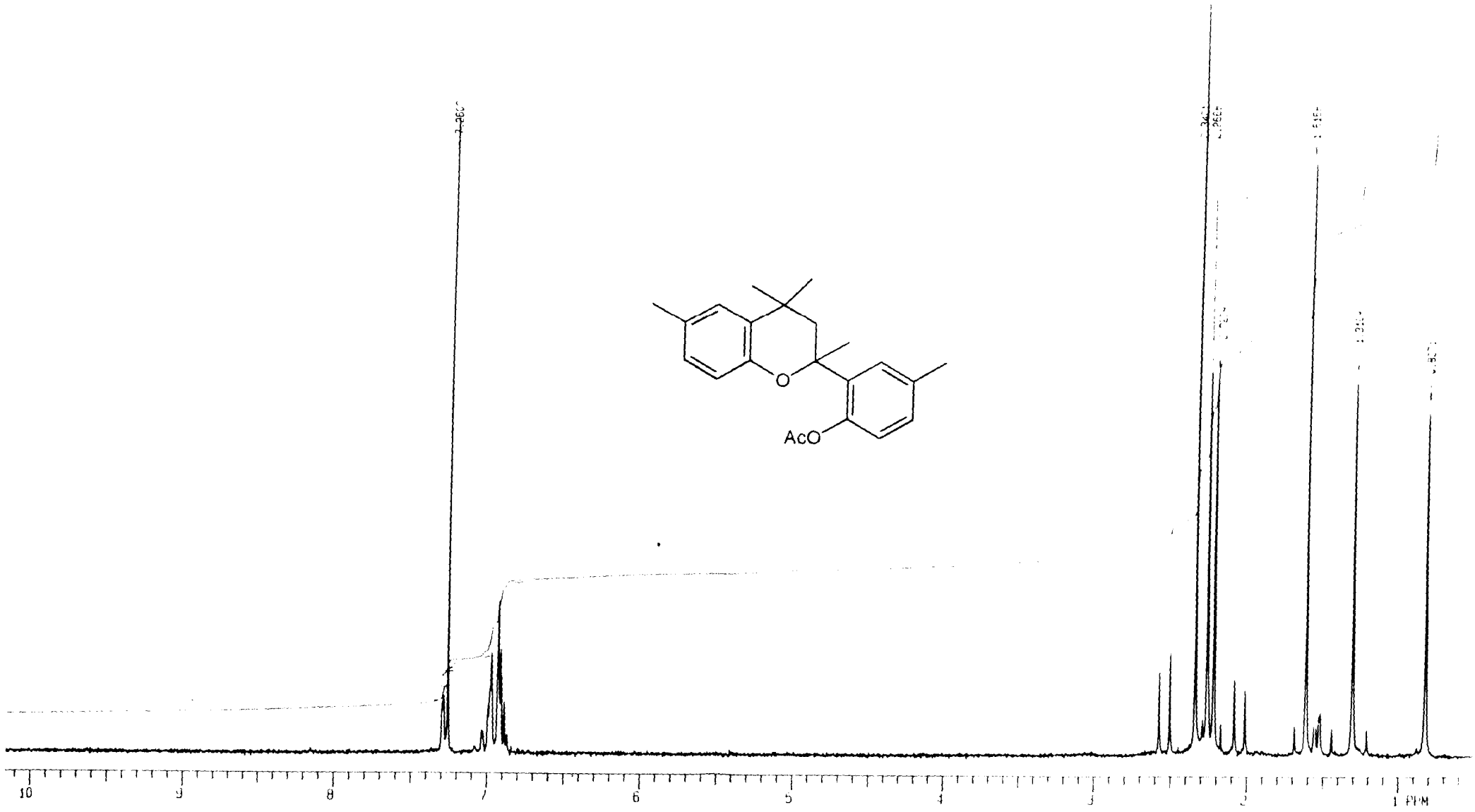

Fig. 4.10 : ^{1}H NMR spectrum of compound 9d in $CDCl_3$

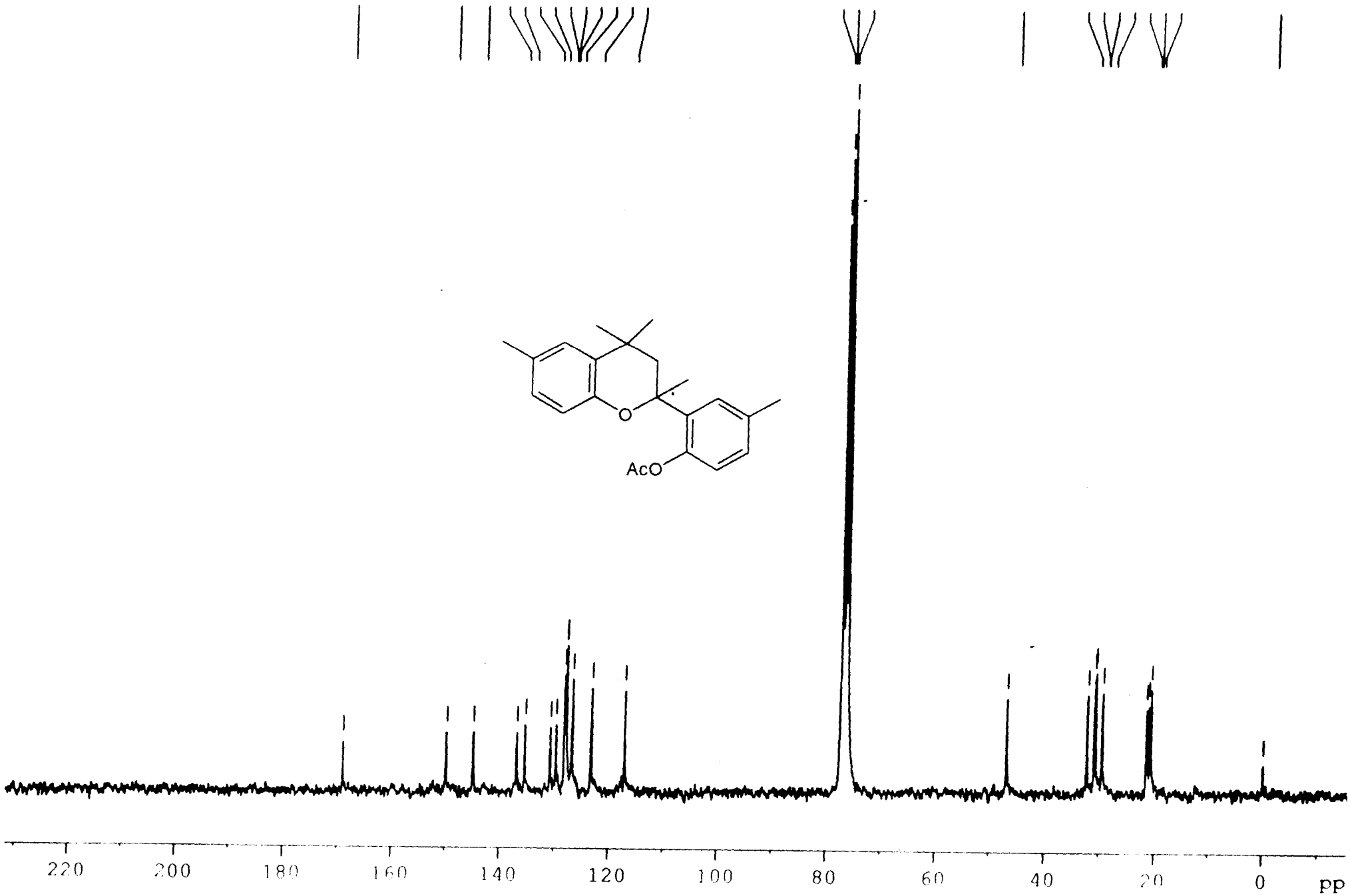

Fig. 4.11 : ^{13}C NMR spectrum of compound 9d in $CDCl_3$

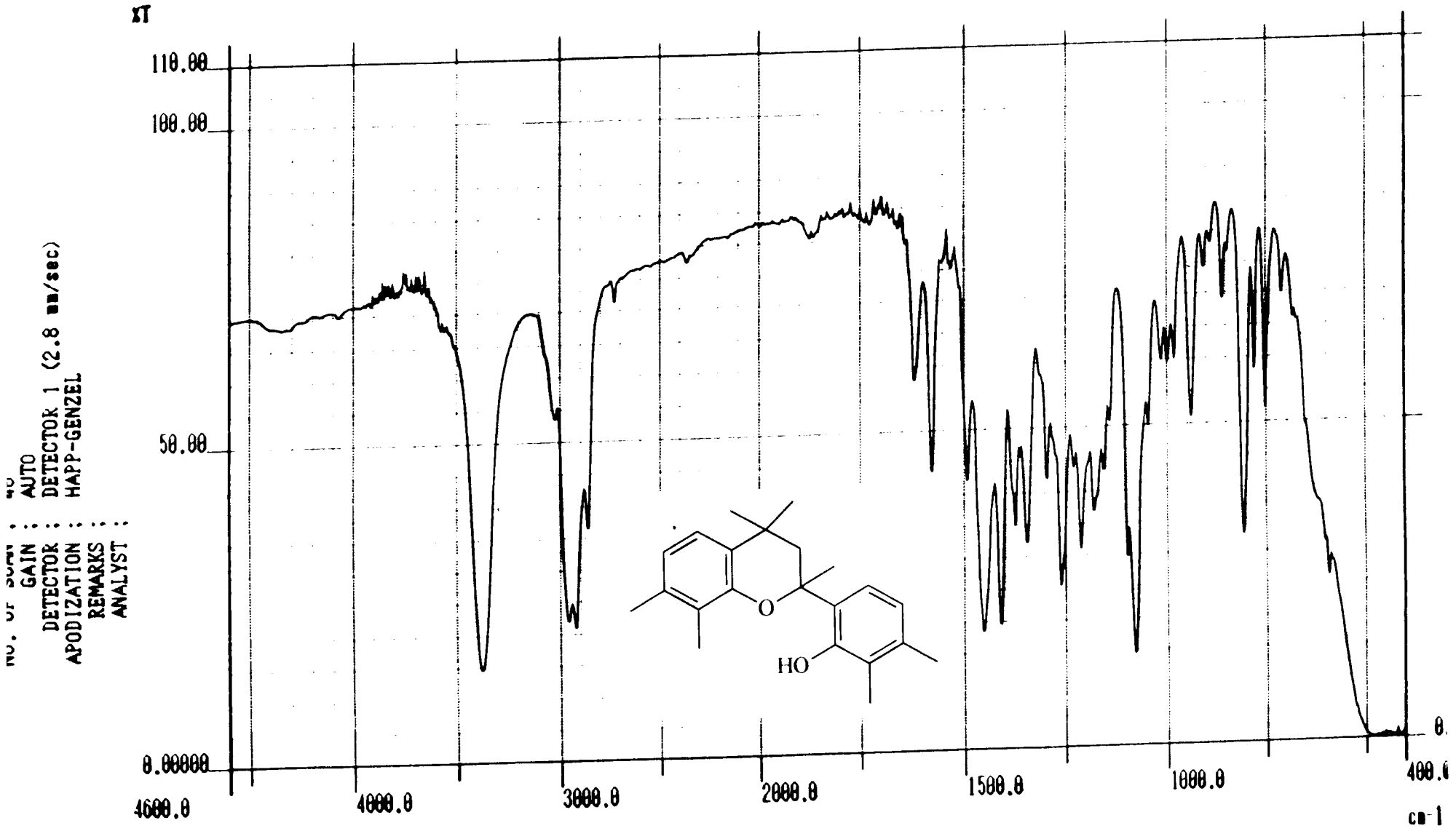

Fig. 4.12 : IR spectrum of compound 9e

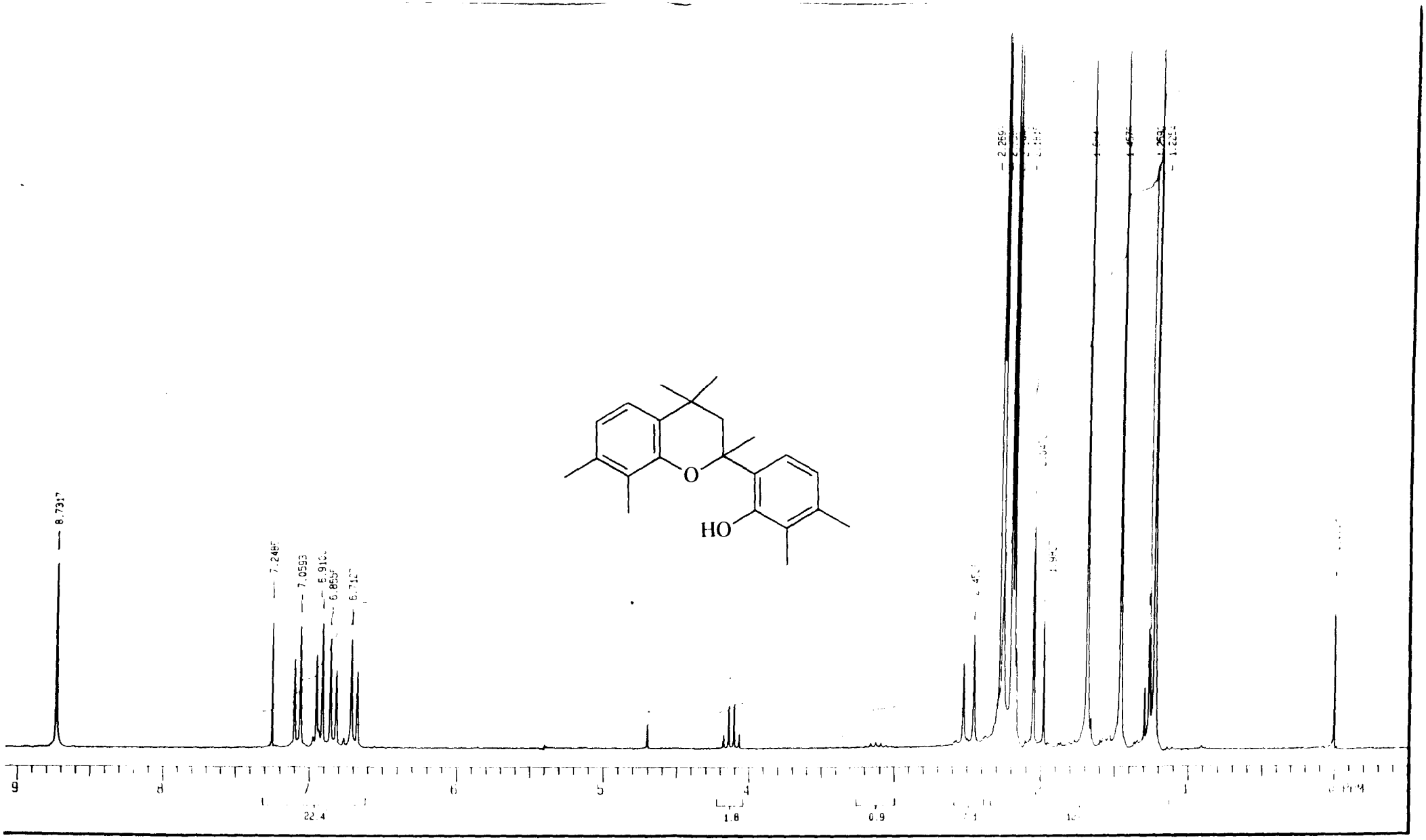

Fig. 4.13 : ^{1}H NMR spectrum of compound 9e in $CDCl_3$

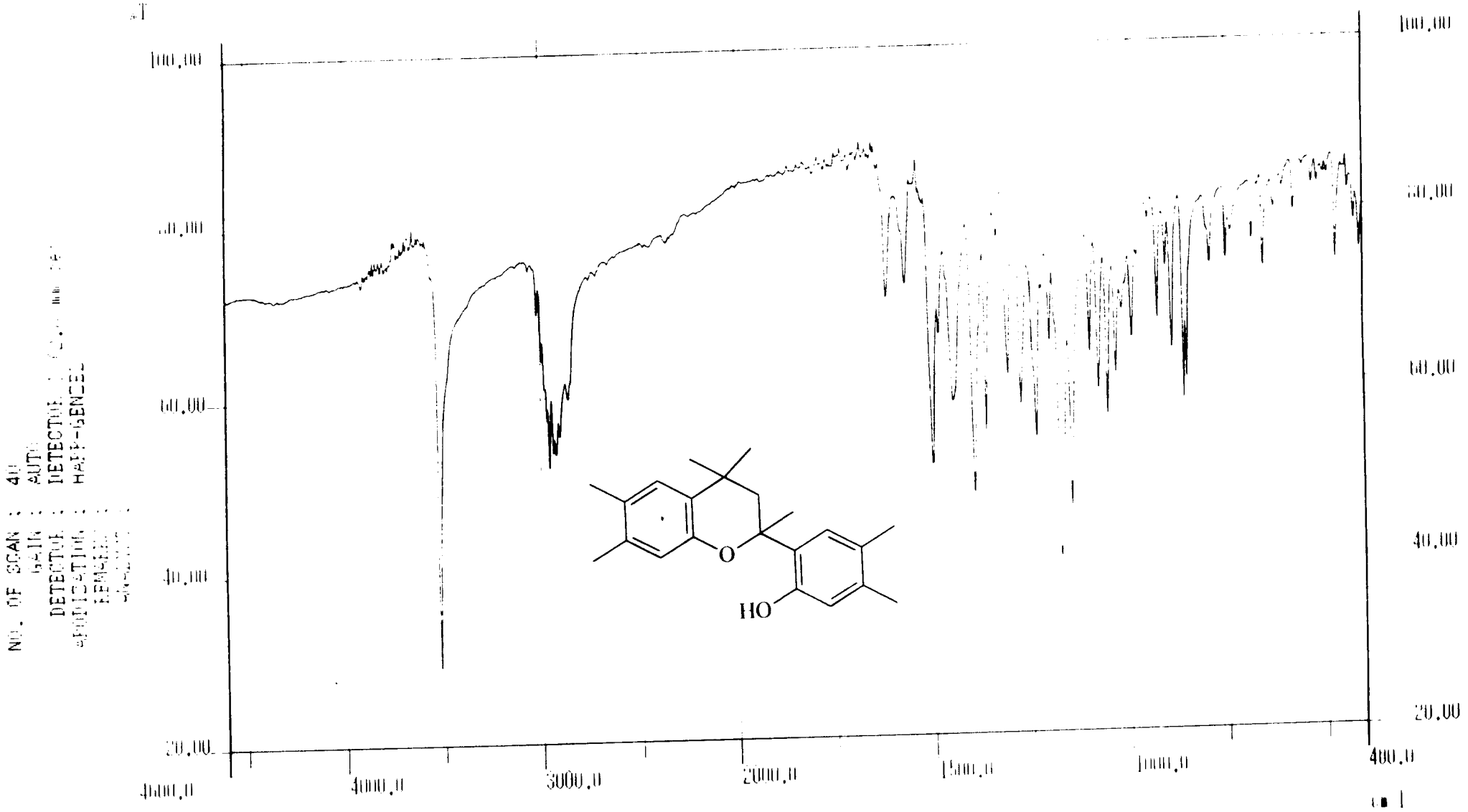

Fig. 4.14 : IR spectrum of compound 9f

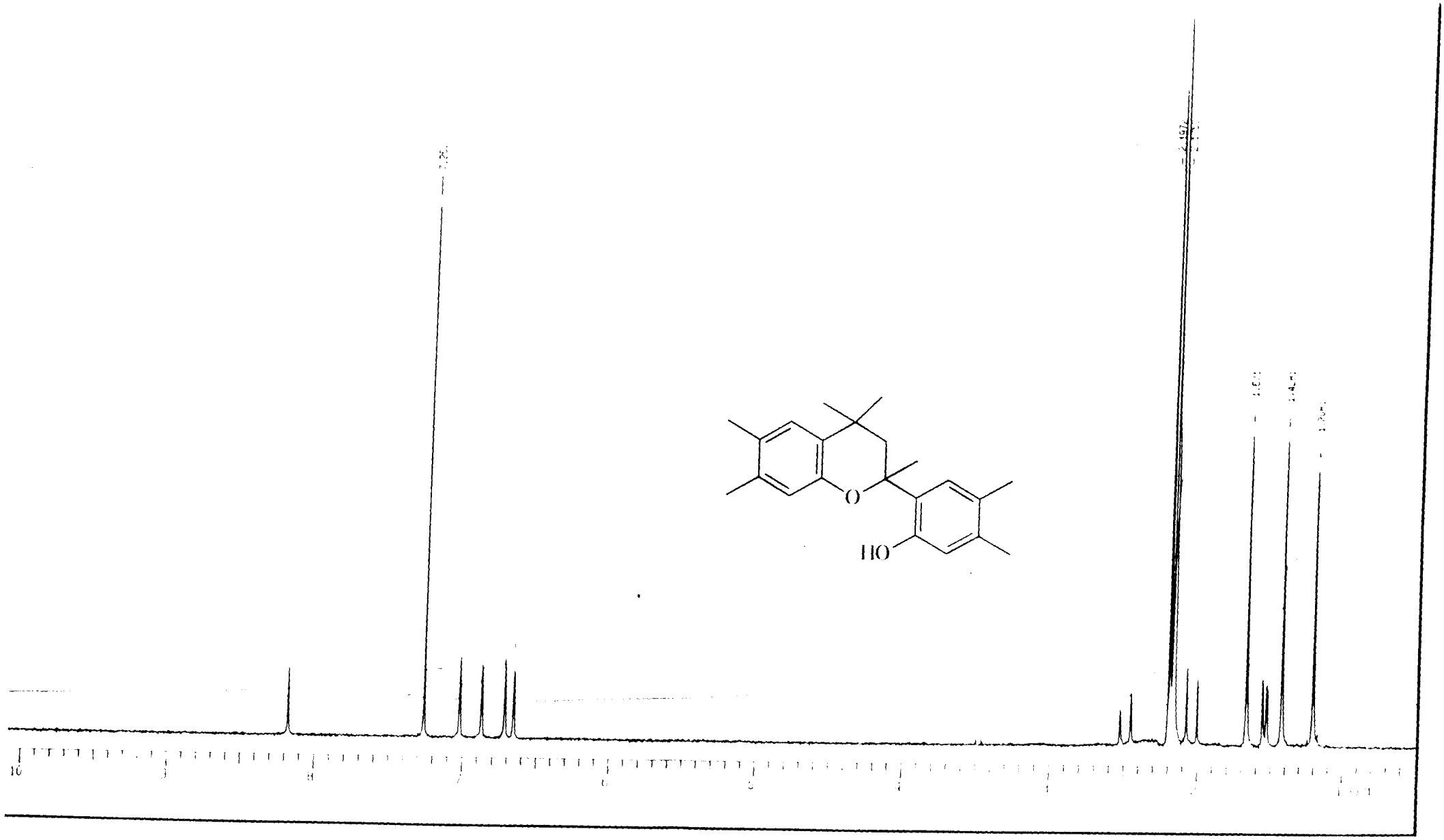

Fig. 4.15 : ^{1}H NMR spectrum of compound 9f in $CDCl_3$

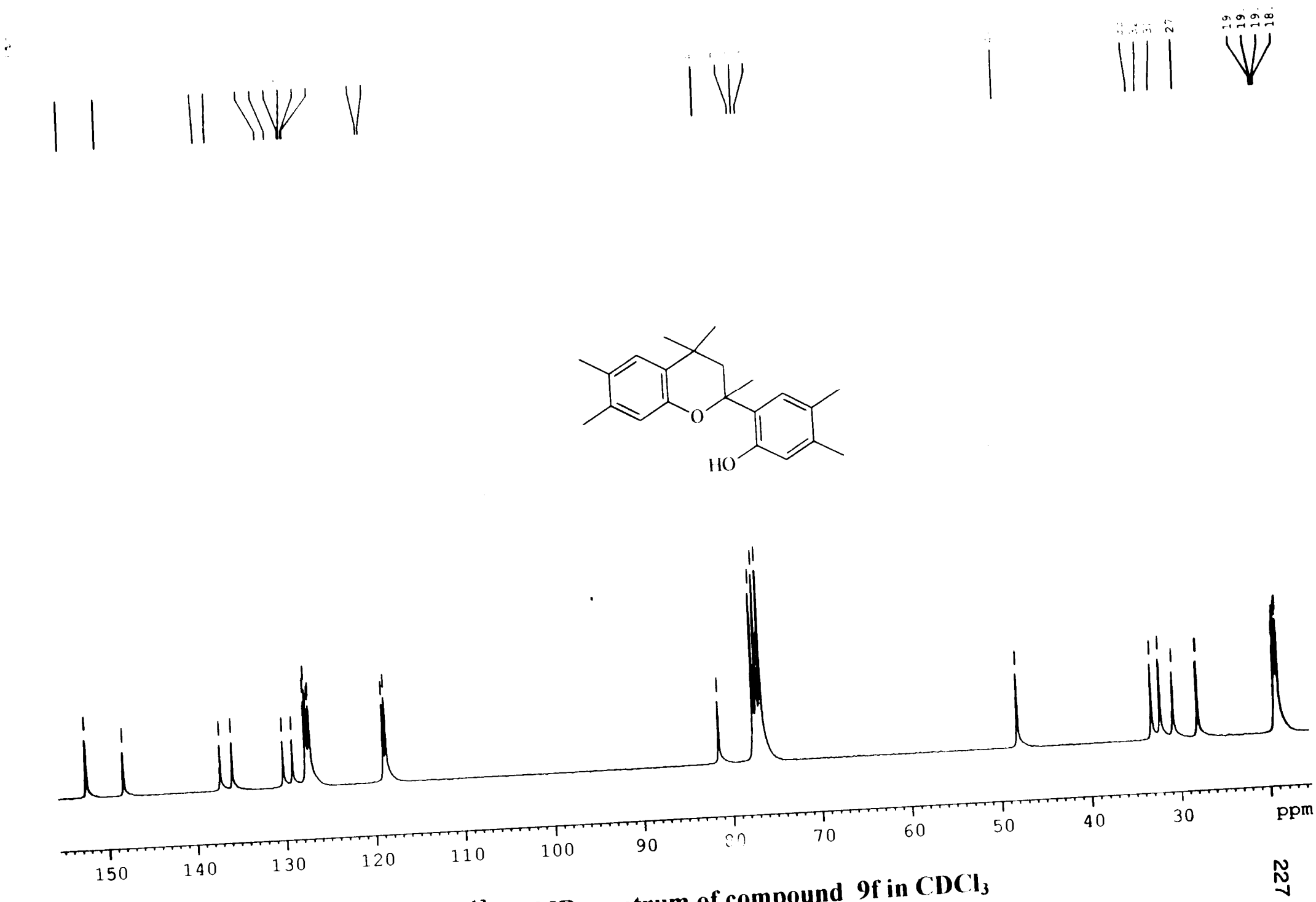

Fig. 4.16 : ^{13}C NMR spectrum of compound 9f in $CDCl_3$

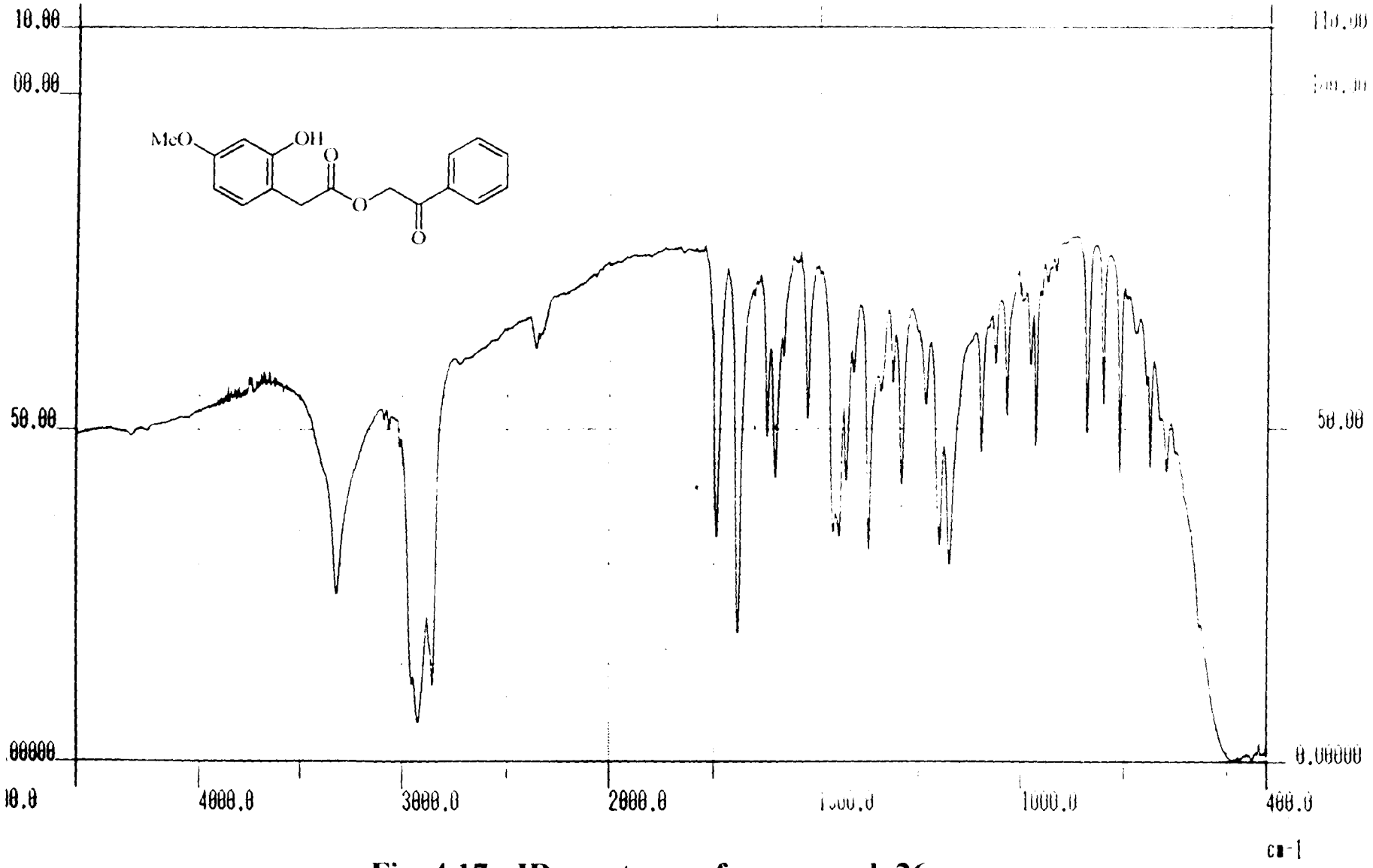

Fig. 4.17 : IR spectrum of compound 26

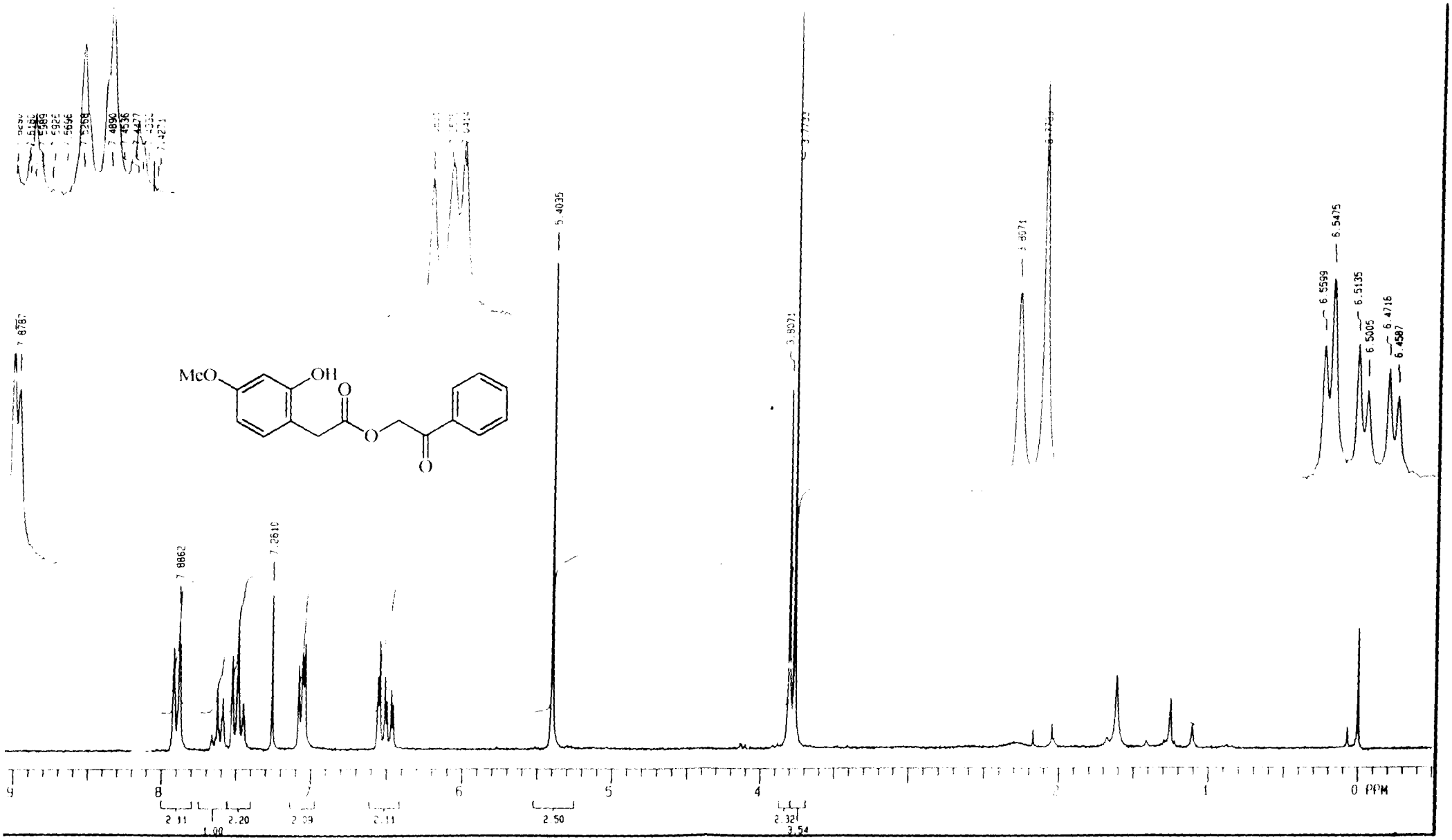

Fig. 4.18 : ^{1}H NMR spectrum of compound 26 in $CDCl_3$

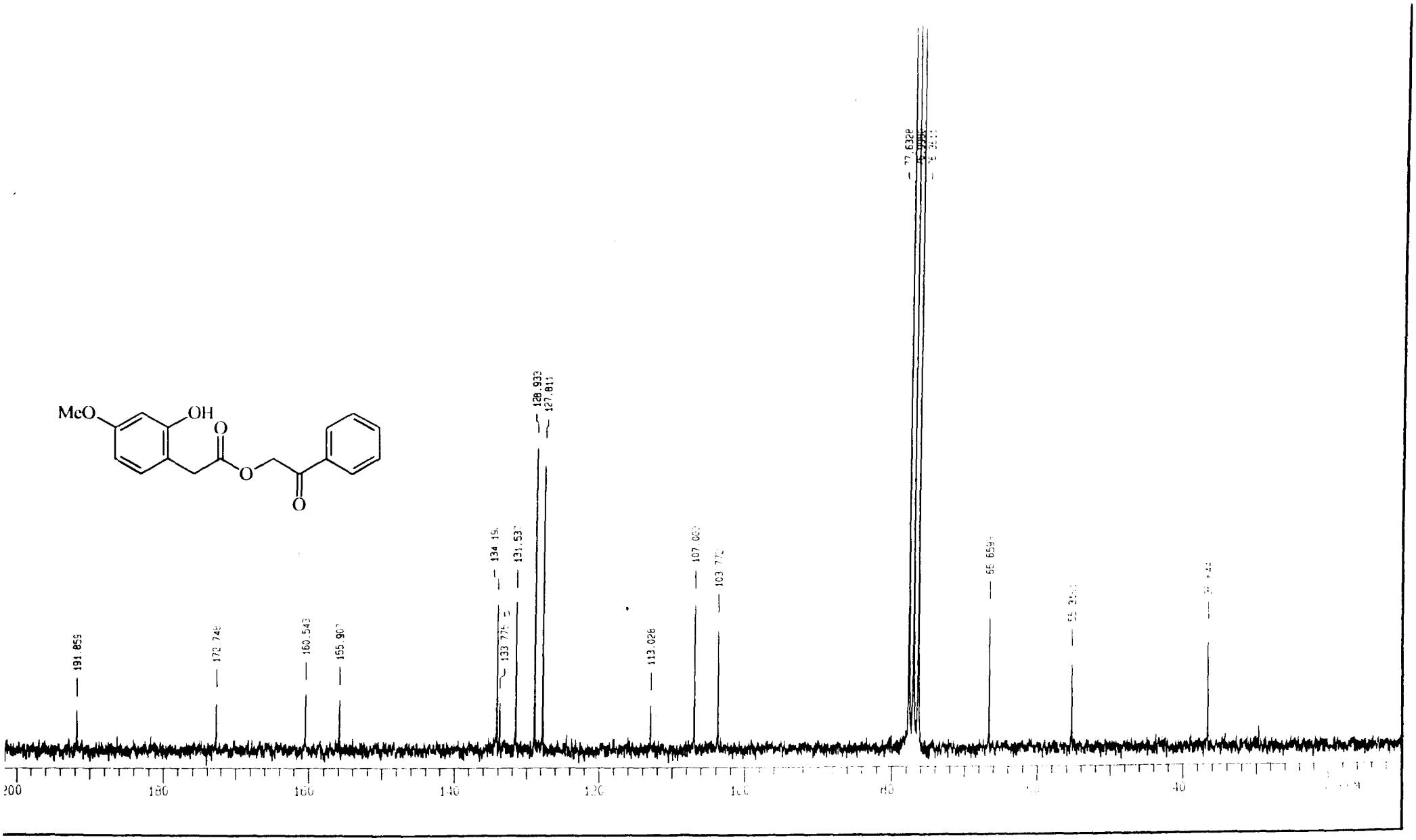

Fig. 4.19 : ^{13}C NMR spectrum of compound 26 in $CDCl_3$

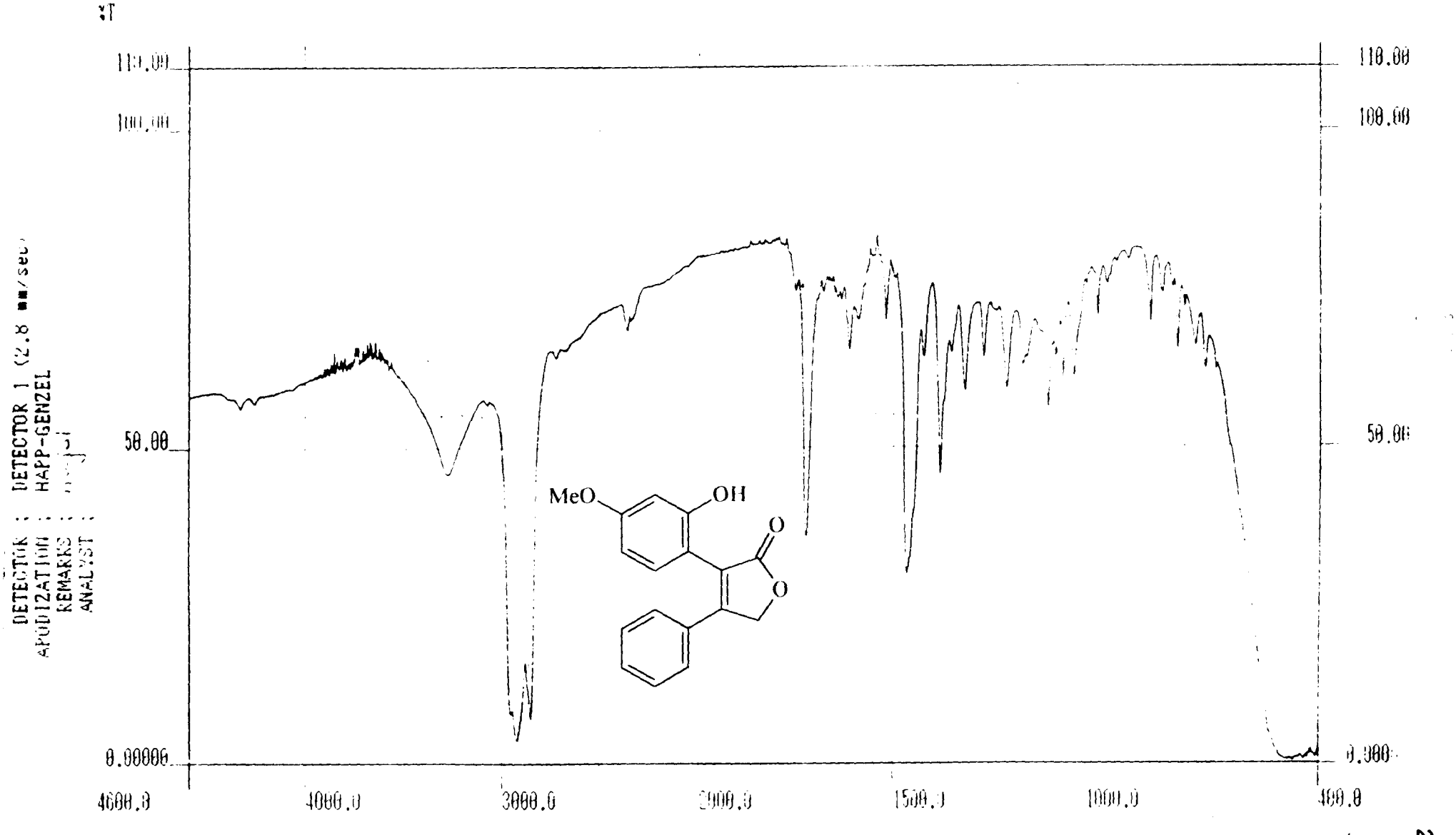

Fig. 4.20 : IR spectrum of compound 27

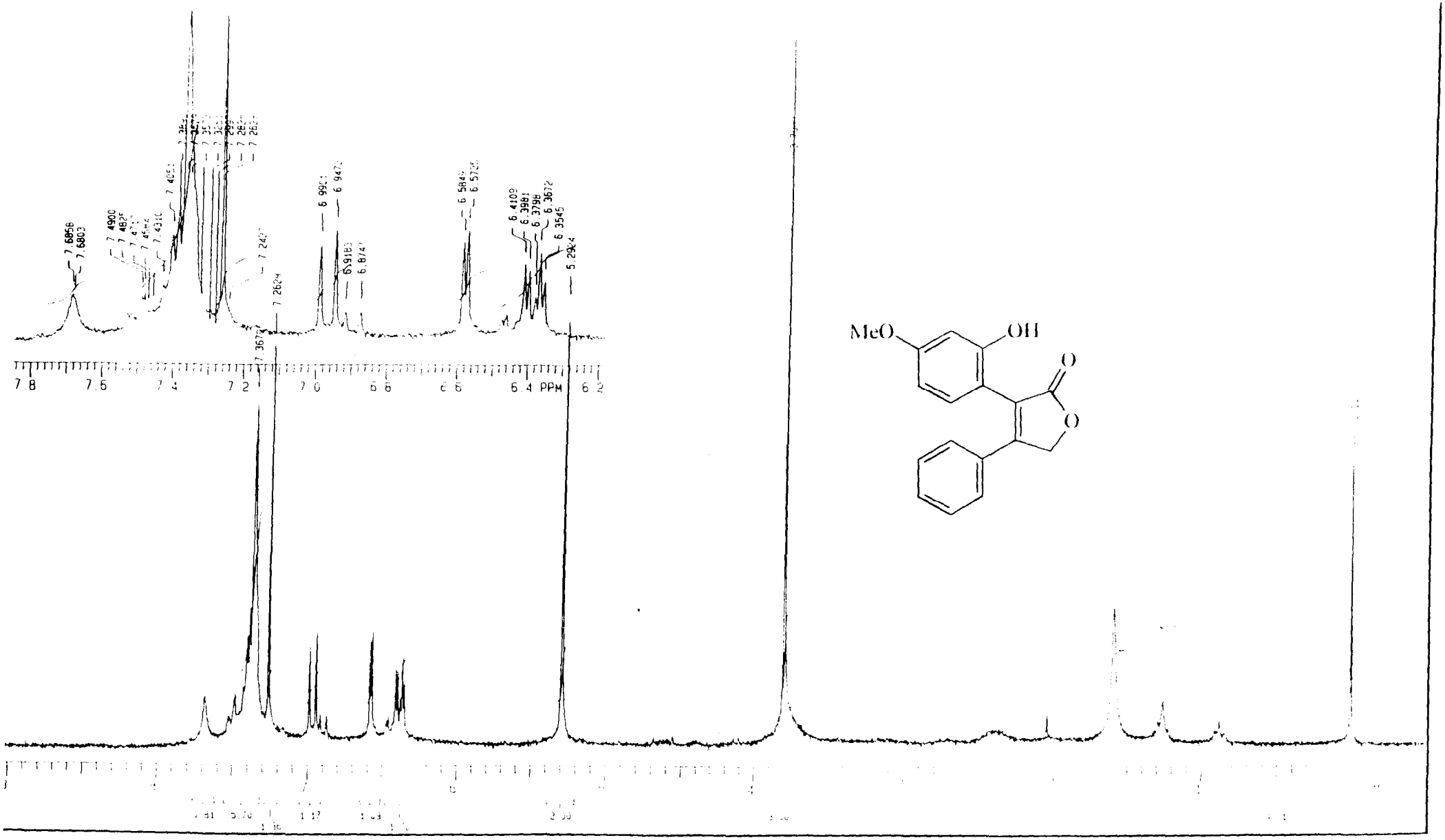

Fig. 4.21 : ^{1}H NMR spectrum of compound 27 in $CDCl_3$

EXPERIMENTAL:

General procedure for the preparation of flavans (9)

Preparation of 2'-hydroxy-2,4,4,7,4'-pentamethylflavan (9a)

A mixture of 4,7-dimethylcoumarin (**10a**) (3.0 g, 17.24 mmol), sodium hydroxide (3.5 g, 86.2 mmol) and ethanediol (20 ml) was refluxed at 210 ^{0}C under nitrogen atmosphere for two and half hours, then cooled, diluted with water followed by addition of diethyl ether (50 ml). Dilute HCl (aq) was then added with stirring till pH of the reaction mixture was adjusted to 2. The organic layer was separated and aqueous layer was extracted with diethyl ether (25 ml x 3). The combined diethyl ether extracts were washed with water and dried ($MgSO_4$). After removal of solvent, the residue was chromatographed on a silica gel column and eluted using pet. ether : diethyl ether (97 :3) as eludent, to furnish 2'-hydroxy-2,4,4,7,4'-pentamethylflavan (**9a**) (1.728 g, 68 %) as a crystalline solid m.p. 76 ^{0}C, (lit.[19] colourless oil).

IR (KBr) : ν (fig. 4.01) = 3410, 2990, 2930, 1620, 1570, 1510, 1450, 1420, 1300, 1250, 1160, 1145, 1075, 1020 and 910 cm^{-1}.

EIMS (70 eV): m/z (%) (fig. 4.02) = 296 (M^+, 75), 281 (M^+ - CH_3, 31), 253 (23), 175 (10), 173 (24), 161 (36), 149 (100), 148 (40), 135 (23), 133 (27) and 121 (30).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (fig. 4.03) = 8.18 (s, exchangeable with D_2O, 1 H, -OH), 7.18 (d, J = 8 Hz, 1 H, H-5), 7.04(d, J = 8 Hz, 1 H, H-6'), 6.81 (dd, J = 8 Hz, 2.5 Hz, 1 H, H-6), 6.75 (d, J = 2.5 Hz, 1 H, H-5'), 6.67 (d, J = 2.5 Hz, 1 H, H-8), 6.65 (dd, J = 8 Hz, 2.5 Hz, 1 H, H-3'), 2.54 & 2.06 (d, J = 15 Hz, 2 H, H_2-3), 2.3 (s, 3 H, H_3-7), 2.27 (s, 3 H, H_3-4'), 1.69 (s, 3 H, H_3-10), 1.43 (s, 3 H, H_3-11) and 1.18 (s, 3 H, H_3-9).

^{13}C NMR ($CDCL_3$, 100 MHz): δ (fig. 4.04)= 154.44 (1C, $C_{quat.}$), 150.17 (1C, $C_{quat.}$), 138.91 (1C, $C_{quat.}$), 137 (1C, $C_{quat.}$), 129 (1C, $C_{quat.}$), 126.95 (1C, $C_{quat.}$), 126.58 (1C, CH), 126.38 (1C, CH), 123.14 (1C, CH), 120.47 (1C, CH), 118.35 (1C, CH), 118.12 (1C, CH), 81.59 (1C, $C_{quat.}$), 47.82 (1C, CH_2), 33.03 (1C, CH_3), 32 (1C, CH_3), 30.8 (1C, $C_{quat.}$), 29.68 (1C, CH_3) and 20.8 (2C, CH_3).

7,2',4'-trihydroxy-2,4,4-trimethylflavan (9b)

The compound **9b** was prepared from 7-hydroxy-4-methylcoumarin (**10b**) (2.6 g, 15.85 mmol), sodium hydroxide (3.22 g, 79.27 mmol) and ethanediol (25 ml) as per the general procedure described above to provide 7,2',4'-trihydroxy-2,4,4-trimethylflavan (**9b**) (1.5 g, 64 %) as a colourless crystalline solid, m.p. 234 ^{0}C (lit.[34] m.p. 231 - 232 ^{0}C).

IR (KBr) : ν (fig. 4.05) = 3510, 3320, 2950, 1620, 1600, 1505, 1450, 1310, 1250, 1210, 1185, 995 and 840 cm^{-1}.

EIMS (70 eV): m/z (%) (fig. 4.06) = 300 (M^+, 12), 285 (M^+ - CH_3, 8), 177 (12), 176 (M^+ - $C_9H_{10}O_2$, 81), 149 (10), 148 (M^+ - $C_8H_8O_3$, 100), 147 (61) and 120 (17).

^{1}H NMR (CD_3OD, 400 MHz): δ (fig. 4.07) = 6.96 (d, J = 8.5 Hz, 1 H, H-5), 6.92 (d, J = 8.5 Hz, 1 H, H-6'), 6.35 (d, J = 2.5 Hz, 1 H, H-8), 6.30 (dd, J = 8.5 Hz, 2.5 Hz, 1 H, H-6), 6.21 (d, J = 2.5 Hz, 1 H, H-3'), 6.07 (dd, J = 8.5 Hz, 2.5 Hz, 1 H, H-5'), 3.0, 1.79 (d, J = 13.8 Hz, 2 H, H_2-3), 1.6 (s, 3 H, H_3-10), 1.17 (s, 3 H, H_3-11) and 0.72 (s, 3 H, H_3-9).

^{13}C NMR (CD_3OD, 50 MHz): δ (fig. 4.08) = 157.99 (1C, $C_{quat.}$), 157.17 (1C, $C_{quat.}$), 156.18 (1C, $C_{quat.}$), 154.82 (1C, $C_{quat.}$), 128.58 (1C, CH), 128.4 (1C, CH), 124.08 (2C, $C_{quat.}$), 109.25 (1C, CH), 106.84 (1C, CH), 104.12 (1C, CH), 104.05 (1C, CH), 79.1 (1C, $C_{quat.}$), 46.61 (1C, CH_2), 33.59 (1C, CH_3), 31.2 (1C, $C_{quat.}$), 30.23 (1C, CH_3) and 30.05 (1C, CH_3).

2'-hydroxy-2,4,4,6,5'-pentamethylflavan (9c)

The compound **9c** was prepared from 4,6-dimethylcoumarin (**10c**) (1.5 g, 8.62 mmol), sodium hydroxide (1.75 g, 43.2 mmol) and ethanediol (20 ml) as per the general procedure described above to give 2'-hydroxy-2,4,4,6,5'-pentamethylflavan (**9c**) (1.02 g, 80 %) as an oil.

IR (neat) : ν = 3410, 2980, 1610, 1510, 1460, 1420, 1380, 1340, 1260, 1190, 1160,1075, 810 and 770 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 8.15 (s, exchangeable with D_2O, 1 H, -OH), 7.08 (s, 1 H), 6.85 (m, 5 H, Ar-H), 2.55 & 2.08 (d, J = 15 Hz, 2 H, H_2-3), 2.29 (s, 3 H,

H_3-6), 2.26 (s, 3 H, H_3-5′), 1.69 (s, 3 H, H_3-10), 1.44 (s, 3 H, H_3-11) and 1.22 (s, 3 H, H_3-9).

2′-acetyl-2,4,4,6,5′-pentamethylflavan (9d)

The compound **9c** (0.5 g) was dissolve in pyridine (10 ml) and then acetic anhydride (10 ml) was added. The reaction mixture was warmed slightly on a water bath for 15 min and then allowed to stand at room temperature over night. The reaction mixture was worked-up as usual and the product obtained was purified by crystallization from a mixture of petroleum ether diethyl ether to give 2′-acetyl-2,4,4,6,5′-pentamethylflavan (**9d**) as crystalline solid with m.p. 148 ^{0}C (lit.[35] m.p. 147-148 ^{0}C).

IR (KBr) : ν (fig. 4.09) = 2950, 1760, 1610, 1490, 1370, 1280, 1240, 1190, 1065 and 810 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz): δ (fig. 4.10) = 7.31 (slightly bs, 1 H), 6.95 (m, 5 H, Ar-H), 2.34 (s, 3 H, H_3-6), 2.55 & 2.07 (d, J = 15 Hz, 2 H, H_2-3), 2.27 (s, 3 H, H_3-5′), 2.22 (s, 3 H, H_3-10), 1.62 (s, 3 H, H_3-11), 1.31 (s, 3 H, H_3-9) and 0.83 (s, 3 H, OCOC**H_3**).

^{13}C NMR ($CDCl_3$, 75 MHz): δ (fig. 4.11) = 169.01 (1C, $C_{quat.}$), 149.95 (1C, $C_{quat.}$), 144.94 (1C, $C_{quat.}$), 136.94 (1C, $C_{quat.}$), 135.45 (1C, $C_{quat.}$), 130.82 (1C, $C_{quat.}$), 129.73 (1C, $C_{quat.}$),128.24 (1C, CH), 128.03 (1C, CH), 127.91 (1C, CH), 126.82 (1C, CH), 123.31 (1C, CH), 117.15 (1C, CH), 76.6 (1C, $C_{quat.}$), 46.97 (1C, CH_2), 32.28 (1C, CH_3), 30.94 (1C, $C_{quat.}$), 30.82 (1C, CH_3), 29.55 (1C, CH_3), 21.53 (1C, CH_3), 21.19 (1C, CH_3) and 20.76 (1C, CH_3).

2′-hydroxy-2,4,4,7,8,3′,4′-heptamethylflavan (9e)

The compound **9e** was prepared from 4,7,8-trimethylcoumarin (**10e**) (3.133 g, 16.66 mmol), sodium hydroxide (4.05 g, 99.98 mmol) and ethanediol (40 ml) as per the general procedure described above to provide 2′-hydroxy-2,4,4,7,8,3′,4′-heptamethylflavan (**9e**) (1.639 g, 61 %) as a viscous oil after purification through silica gel column.

IR (neat) : ν (fig. 4.12) = 3400, 2950, 2920, 1625, 1575, 1490, 1460, 1410, 1340, 1260, 1210, 1130, 940 and 800 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz): δ (fig. 4.13) = 8.73 (s, exchangeable with D_2O, 1 H, -OH), 7.03 (d, *J* = 8 Hz, 1 H, H-5), 6.93 (d, *J* = 8 Hz, 1 H, H-6′), 6.84 (d, *J* = 8 Hz, 1 H, H-6), 6.69 (d, *J* = 8 Hz, 1 H, H-5′), 2.5 & 2.05 (d, *J* = 15 Hz, 2 H, H_2-3), 2.27 (s, 3 H, H_3-7), 2.26 (s, 3 H, H_3-4′), 2.19 (s, 3 H, H_3-8), 2.18 (s, 3 H, H_3-3′), 1.69 (s, 3 H, H_3-10), 1.46 (s, 3 H, H_3-11) and 1.23 (s, 3 H, H_3-9).

Anal. Calculated for $C_{22}H_{28}O_2$: C, 81.48; H, 8.64. Found : C, 81.54; H, 8.47.

2′-hydroxy-2,4,4,6,7,4′,5′-heptamethylflavan (9f)

The compound **9f** was prepared from 4,6,7-trimethylcoumarin (**10f**) (3 g, 15.95 mmol), sodium hydroxide (3.9g, 96.98 mmol) and ethanediol (40 ml) as per the general procedure described above to give 2′-hydroxy-2,4,4,6,7,4′,5′-heptamethylflavan (**9f**) (2.02 g, 78 %) as a crystalline solid from silica gel column chromatography, m.p. 157 °C.

IR (KBr) : ν (fig. 4.14) = 3410, 2980, 1610, 1510, 1460, 1420, 1380, 1340, 1260, 1190, 1160,1075, 810 and 770 cm^{-1}.

^{1}H NMR ($CDCl_3$, 200 MHz): δ (fig. 4.15) = 8.18 (s, exchangeable with D_2O, 1 H, -OH), 7.02 (s, 1 H, H-5), 6.86 (s, 1 H, H-6′), 6.72 (s, 1 H, H-8), 6.65 (s, 1 H, H-3′), 2.52 & 2.05 (d, *J* = 15 Hz, 2 H, H_2-3), 2.2 (s, 6 H, H_3-6, H_3-5′), 2.18 (s, 6 H, H_3-7, H_3-4′), 1.67 (s, 3 H, H_3-10), 1.43 (s, 3 H, H_3-11) and 1.21 (s, 3 H, H_3-9).

^{13}C NMR ($CDCl_3$, 75 MHz): δ (fig. 4.16) = 152.38 (1C, $C_{quat.}$), 148.15 (1C, $C_{quat.}$), 137.1 (1C, $C_{quat.}$), 135.76 (1C, $C_{quat.}$), 130.07 (1C, $C_{quat.}$), 129.04 (1C, $C_{quat.}$), 127.59 (1C, CH), 127.47 (1C, CH), 127.16 (1C, CH), 127.08 (1C, CH), 118.90 (1C, $C_{quat.}$), 118.62 (1C, $C_{quat.}$), 81.38 (1C, $C_{quat.}$), 48.05 (1C, CH_2), 33.17 (1C, CH_3), 32.2 (1C, CH_3), 30.76 (1C, CH_3), 27.97 (1C, $C_{quat.}$), 19.35 (1C, CH_3), 19.26 (1C, CH_3), 19.07 (1C, CH_3) and 18.97 (1C, CH_3).

Anal. Calculated for $C_{22}H_{28}O_2$: C, 81.48; H, 8.64. Found : C, 81.03; H, 8.74.

Preparation of 2-hydroxy-4-methoxyphenylglyoxylic acid (24):

Powdered anhydrous $AlCl_3$ (27 g, 0.2 mol) was added at 0 ^{0}C to a mixture of 1,3-dimethoxybenzene (**23**) (13.8 g, 0.1 mol) and ethyloxalyl chloride (13.6 g, 0.1 mol) in 1,2-dichloroethane (150 ml). The mixture was refluxed for 1 h, cooled, and poured into acidified ice water. The organic layer was separated, dried, and evaporated under reduced pressure. The residue was warmed with 8 % NaOH (50 ml) for 30 min, cooled, acidified and extracted with dichloromethane. The organic extract was dried, evaporated under reduced pressure, and the residue recrystallized from water to give the glyoxylic acid (**24**) (8.5 g, 44 %), m.p. 121 ^{0}C (lit.[42] 120-122 ^{0}C).

IR (KBr): ν = 3117, 2500, 1742, 1621, 1567, 1508 and 1463 cm^{-1}.

^{1}H-NMR (300 MHz, $CDCl_3$): δ = 11.77 (br s, exchangeable with D_2O, 1 H, -OH), 8.4 (d, J = 9.0 Hz, 1 H, H-3), 8.1(br s, exchangeable with D_2O, -OH), 6.53 (dd, J = 7.0 Hz, 2.4 Hz, 1 H, H-5), 6.47 (d, J = 2.4 Hz, 1 H, H-6) and 3.9 (s, 3 H, OCH_3).

^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 184.3 (1C, $C_{quat.}$), 168.8 (1C, $C_{quat.}$), 168.3 (1C, $C_{quat.}$), 162 (1C, $C_{quat.}$), 135.1 (1C, CH), 110.4 (1C, $C_{quat.}$), 109.8 (1C, CH), 100.8 (1C, CH) and 50.9 (1C, OCH_3).

Reduction of (24) to 2-hydroxy-4-methoxyphenylacetic acid (25):

2-Hydroxy-4-methoxyphenylglyoxylic acid (**24**) (2 g, 10 mmol) was dissolved in dry ethanol (20 ml) and hydrazine hydrate (3 g, 99 %, 60 mmol) was added. The resultant solution was then added to a solution of sodium (3 g) in dry ethanol (30 ml) and the mixture was heated slowly under N_2 on an oil-bath until the bath temperature reached 200 ^{0}C at which it was maintained for 15 min. The mixture was then cooled, dissolved in 50 % HCl (60 ml), and extracted with diethyl ether. The ether layer was dried and evaporated under reduced pressure to yield a reddish brown semi-solid (1 g). The purification of this semi-solid residue was carried out by using a silica gel column chromatography with gradient solvent system consisting of petroleum ether : ether. The fraction eluted with ether : petroleum ether (1 : 1) gave 2-hydroxy-4-methoxyphenylacetic acid (**25**), 300 mg (23 %) as a colourless crystalline solid with m. p. 131 ^{0}C (lit.[43] 131-313 ^{0}C).

IR (KBr) :ν = 3359, 3150, 1699, 1666, 1621, 1525, 1508 and 1445 cm^{-1}.

^{1}H-NMR (200 MHz, $CDCl_3$): δ = 7.02 (d, *J* = 7.95 Hz, 1 H, H-6), 6.49 (dd, *J* = 7.95 Hz, 2.56 Hz, 1 H, H-5), 6.45 (d, *J* = 2.56 Hz, 1 H, H-3), 4.65 (br s, exchangeable with D_2O, 1 H, OH), 3.77 (s, 3 H,) and 3.65 (s, 2 H).

^{13}C-NMR (75.5 MHz, $CDCl_3$): δ = 178.2 (1C, $C_{quat.}$), 160.6 (1C, $C_{quat.}$), 155.6 (1C, $C_{quat.}$), 131.7 (1C, CH), 112.3 (1C, $C_{quat.}$), 106.8 (1C, CH), 103.1 (1C, CH), 55.4 (1C, CH_2) and 36.3 (1C, OCH_3).

The further elution of the column with ether : pet. ether (3 :2) gave 500 mg 2-hydroxy-4-methoxyphenylglyoxylic acid (**24**).

Preparation of keto ester (26):

2-Hydroxy-4-methoxyphenylacetic acid (**25**) (40 mg, 0.22 mmol) was dissolved in acetonitrile (2 ml) containing triethylamine (0.2 ml) and this brown coloured solution was added to phenacyl bromide (Aldrich, 98 %) (45 mg, 0.226 mmol) with stirring at room temperature. The reaction mixture was stirred for 90 min at room temperature and then the acetonitrile was removed under reduced pressure. The dark brown oily coloured residue obtained was dissolved in dichloromethane (10 ml) and washed successively with 2N HCl, 5 % aq. sodium bicarbonate, brine and dried (anhydrous Na_2SO_4). The solvent was removed under reduced pressure and the residue was filtered through a pad of silica gel followed by recrystallization of the solid mass obtained using ethyl acetate/hexane (1 : 3) to afford keto ester (**26**) (45 mg, 68 %) as a white crystalline solid, m.p. 150 °C.

IR (Nujol) : ν (fig. 4.17) = 3333, 1743, 1690, 1621, 1525, 1508 and 1445 cm^{-1}.

^{1}H-NMR (200 MHz, $CDCl_3$): δ **(fig. 4.18)** = 7.9 (dd, *J* = 7.5 Hz, 1.5 Hz, 2 H, H-4″, H-8″), 7.52 (d, *J* = 7.5 Hz, 1 H, H-6″), 7.50 (dd, *J* = 7.5 Hz, 1.5 Hz, 2 H, H-5″, H-7″), 7.06 (d, *J* = 7.5 Hz, 1 H, H-6′), 7.05 (s, exchangeable with D_2O, 1 H, -OH), 6.55 (d, *J* = 2.5 Hz, 1 H, H-3′), 6.49 (dd, *J* = 7.5 Hz, 2.5 Hz, 1 H, H-5′), 5.4 (s, 2 H, H_2-1″), 3.81 (s, 2 H, H_2-2), and 3.78 (s, 3 H, H_3-4′).

^{13}C-NMR (50.5 MHz, $CDCl_3$): δ **(fig. 4.19)** = 191.86 (1 C, $C_{quat.}$), 172.75 (1C, $C_{quat.}$), 160.54 (1C, $C_{quat.}$), 155.91 (1C, $C_{quat.}$), 134.2 (1C, CH), 133.78 (1C, $C_{quat.}$),

131.54 (1C, CH), 128.9 (2C, CH, CH), 127.81 (2 C, CH, CH), 113.03 (1C, $C_{quat.}$), 107 (1C, CH), 103.77 (1C, CH), 66.66 (1C, CH_2), 55.32 (1C, OCH_3) and 36.65 (1C, CH_2).

Cyclization of keto ester (26) to 3-(2'-hydroxy-4'-methoxyphenyl)-4-phenyl-2-(5H)-furanone (22):

Keto ester (**26**) (25 mg, 0.083 mmol) in dry THF (1 ml) was added under N_2 atmosphere to a suspension of degreased NaH (60 %) (8 mg) in dry THF (2 ml). The resulting dark red coloured solution was stirred at room temperature for 1 h and diluted with 1N HCl till *p*H of the reaction mixture was adjusted to 1. The organic part was extracted with ethyl acetate (3 x 25 ml), washed with brine and dried with Na_2SO_4. The residue obtained after evaporation of the solvent was filtered through a pad of silica gel and subjected to purification using HPLC (pre-packed column, 4 cm x 5 cm, 50 % EtOAc / Hexane) to afford **22** (10 mg, 42 %), as a crystalline compound m.p. 179 °C (lit.[1] 182 - 184 °C).

IR (Nujol) : ν (fig. 4.20) = 3280, 1723, 1621, 1525, 1508 and 1445 cm^{-1}.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (fig. 4.21) = 7.68 (br s, exchangeable with D_2O, 1 H, -OH), 7.36 (s, 5 H), 6.96 (d, *J* = 8.6 Hz, 1 H, H-6'), 6.58 (d, *J* = 2.5 Hz, 1 H, H-3'), 6.38 (dd, *J* = 8.6 Hz, 2.5 Hz, 1 H, H-5'), 5.3 (s, 2 H, H_2-5) and 3.80 (s, 3 H, H_3-4').

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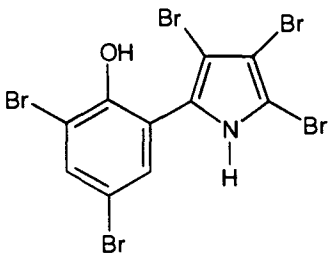
CHAPTER 5

SOME STUDIES ON THE BIOSYNTHESIS OF THE MARINE ANTIBIOTIC PENTABROMOPSEUDILIN

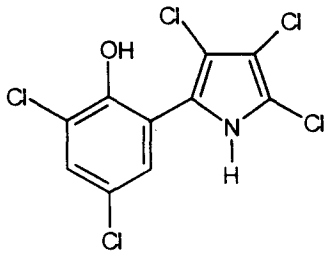
INTRODUCTION

The marine environment covers more than 70 % of the earth's surface and includes a vast array of diverse habitats ranging from tropical, shallow water coral reefs to sub-zero, deep-ocean trenches. Within these habitats reside diverse plant species and invertebrate phyla, the majority of which are uniquely marine. We are aware that the basis for all live processes to sustain is the metabolism, which proceeds through a network of complex bio-chemical changes. As we know, the primary metabolism in plants (photosynthesis) starts with the consumption of carbon dioxide and ends with the generation of a variety of sugars, amino acids and peptides[1,2]. The primary metabolism is of utmost significance because it is responsible for the life to sustain on this earth. Similarly, the secondary metabolism terminates with the production of organic compounds having diverse structural features. Till today, about 1,30,000 different secondary metabolites are known from all kinds of organisms[3]. In most cases, a direct benefit to the producer is not known. However, it is most likely that these compounds are produced by the organisms for their survival or for communicating purpose among them.

An interesting example of a parallel development of very similar target molecule is the formation of Pentabromopseudilin[4] (**1**) and Pentachloropseudilin[5] (**2**). Though, both of these phenylpyrroles are structurally very similar, their producers belong to two different genera. The compound **1** is produced by a marine *Alteromonas* sp. (Chromobacteria), while **2** by a terrestrial *Actinoplanes*- strain. Besides their structural relationship, both compounds exhibit strong antibiotic action. The structure of **1** was determined by X-ray crystallography[6] and further confirmed by three independent syntheses[7,8,9]. Compound **1** is a stronger antibiotic than penicillin, besides showing antitumor activity and is also highly phytotoxic. Its mechanism of action has been elucidated by structure-activity studies[10]. The interesting biological properties and the high bromine content (over 70 %) combined with a straightforward carbon skeleton for which no obvious precursor could be foreseen has made **1** a challenging target for biosynthetic investigations.



1



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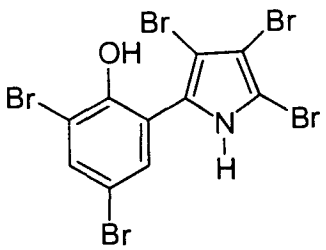
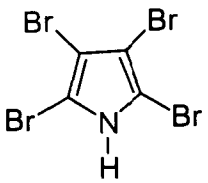
CONSTITUENTS FROM *ALTEROMONAS LUTEOVIOLACEUS*

As stated earlier, Andersen[4] isolated Pentabromopseudilin (**1**) together with two other highly brominated heterocycles from *Chromobacterium violacem*, a marine bacterium that originated in the north Atlantic sea water sample. This bacterium was subsquently re-assigned as *Alteromonas luteoviolaceus*. Infact Burkholder[12] on the other hand had isolated **1** as the first natural compound from the marine micro-organism *Pseudomonas bromoutilis* eight years prior to Andersen's report. Besides exhibiting a potent antibiotic activity against gram-postive and gram-negative bacteria, **1** also inhibited different enzyme systems and showed strong *in vitro* activity against leukaemia and melanoma cell lines. The other compounds isolated from the same source were tetrabromopyrrole (**3**) and hexabromobipyrrole (**4**) with estimated 79 % bromine content each. Whereas compound **3** exhibited an even broader activity than **1** the bipyrrole (**4**) showed activity only against a restricted band of marine test strains. In addition, the other non-halogenated compounds, namely, *p*-hydroxybenzaldehyde (**5**), *p*-hydroxy benzoic acid-*n*-propylester (**6**) and indole-3-carbaldehyde (**7**) have also been isolated from the same source.



1



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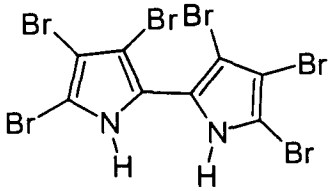

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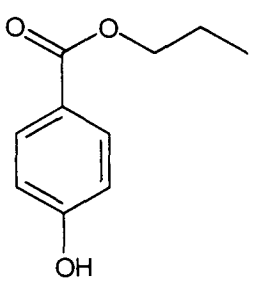

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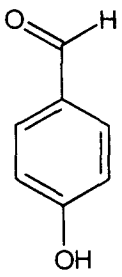

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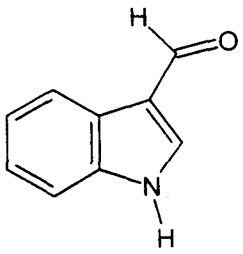
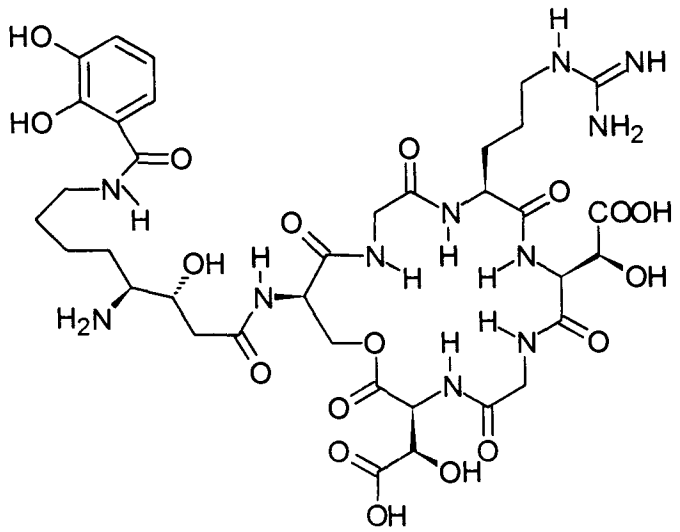


7

It may also be interesting to note that the strongest known siderophore[14], namely, Alterobactin A[15] (**8**) with a building constant of 10^{49} to 10^{51} has been characterised from *Alteromonas luteoviolaceus*. In addition, the open chain analogue, *viz.*, Alterobactin B (**9**) which co-ordinates iron only weakly has also been isolated from the same source.



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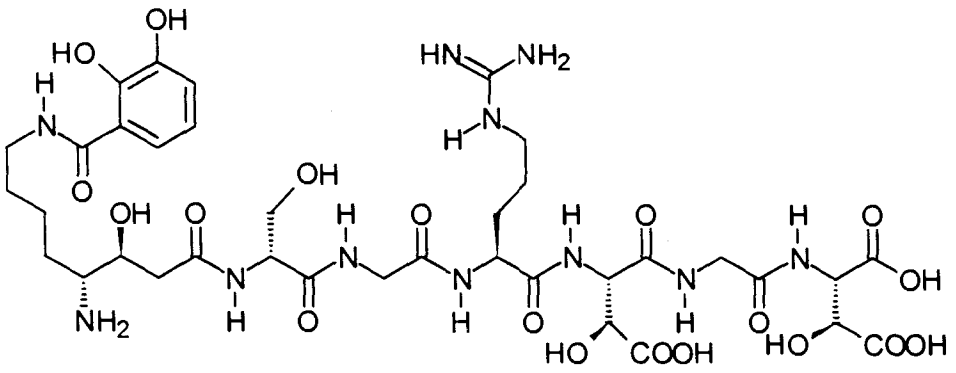

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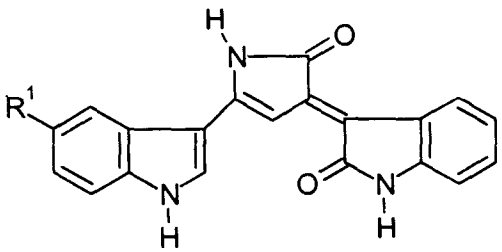
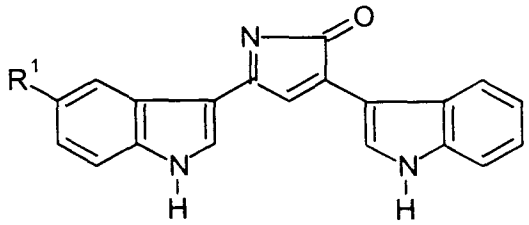
Quite astonishing observation was the dark violet colour exhibited by *Alteromonas luteoviolaceus* which was subsequently attributed to violacein (**10a**) and deoxyviolacein (**10b**),[12,13] produced during the cultivation stage.



10 a : $R^1 = OH$

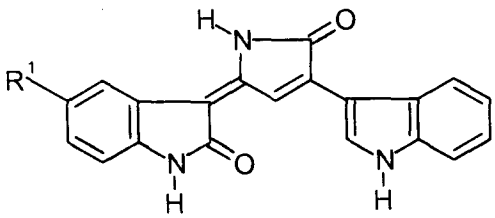
b : $R^1 = H$

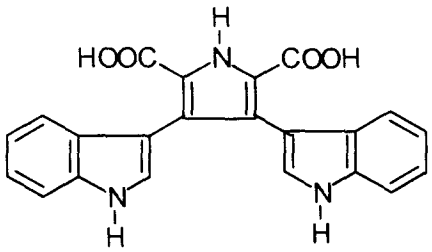
Likewise, a number of compounds have been isolated from *Chromobacterium violacem* involving the bacterial strains ATCC 53434 and 12472. The intensive investigation carried out in connection with the biosynthesis of violacein (**10a**) using one of above said strains resulted in the characterization of several intermediate products[16]. The cultivation of *Chromobacterium violacem* in the presence of sodium-N,N-diethyldithiocarbamate, produced four structurally related indoles derivatives namely prodeoxyviolacein (**11a**), proviolacein (**11b**), pseudodeoxyviolacein (**12a**) and pseudoviolacein (**12b**)[16].



11 **a** : $R^1 = H$

b : $R^1 = OH$





12 **a** : $R^1 = H$; **b**: $R^1 = OH$ 13

In addition, a structurally related compound, the chromopyrrole acid (**13**) has also been isolated, though the same has no connection with the biosynthesis of **10a**, as has been proved.

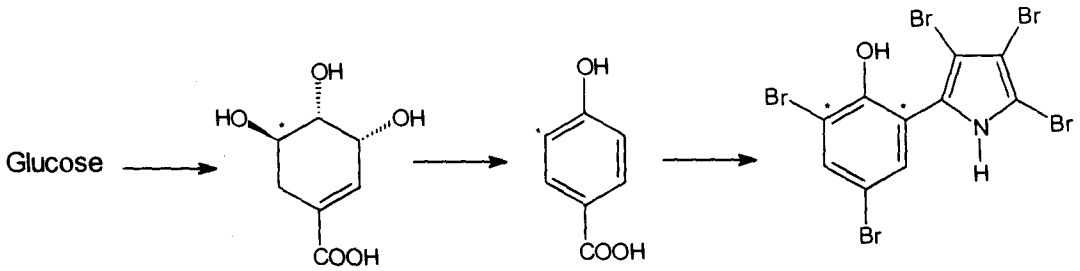
PREVIOUS INVESTIGATIONS ON THE BIOSYNTHESIS OF PENTABROMOPSEUDILIN (1)

As we know, various methods are available in the literature[11] to aid the understanding of the biosynthetic process. One of the common method used / practised in most cases is the identification of the appropriate building blocks/units by employing suitable precursors in the feeding experiments. For this purpose, the acetate, amino acids and/or sugars are labelled with either radioactive (^{14}C, ^{34}P, ^{3}H) or stable (^{13}C, ^{15}N, ^{2}H, ^{18}O) isotopes and fed. Then, by means of suitable procedures, the labelled atoms/sites in the target molecule are located. Once the basic units are identified in this way, further incorporating experiments with components which are nearer in the biosynthetic pathway to the target molecules are carried out.

Alternately, the examination of the participating enzyme systems associated with the biosynthetic sequences are also exploited to provide valuable information. The

blocking of single reaction step, for example, by mutation, can give important information and in such cases, the intermediates, which are no more transformed, can be enriched and finally identified.

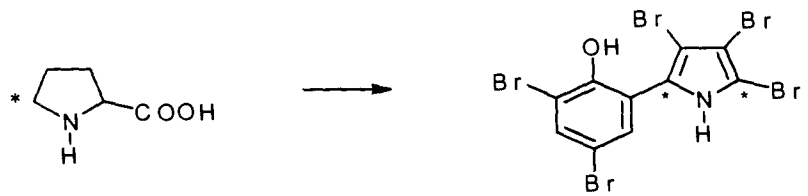
The maiden investigation on the biosynthesis of pentabromopseudilin (**1**) was carried out by Hanefeld[17] by employing feeding experiments in a complex medium utilising ^{13}C-acetate, various amino acids and differently labelled glucose molecules. On the basis of the said investigation, they established that the benzene ring in **1** originated from shikimate pathway *via p*-hydroxybenzoic acid as a direct precursor.



14 **15** **1**

Fig. 1: Biosynthesis of the Benzene ring in Pentabromopseudilin (**1**)

Similarly, by another feeding experiment employing D,L-(5-^{13}C)Proline (**16**), Peschke[18] observed that after a good growth of the strain in amino acid medium, 16 mg/l of **1** was isolated, with enrichment of labeled material. In this way, proline (**16**) was identified as the biosynthetic precursor for the pyrrole unit in **1**. In addition it was also felt that there must be the involvement of a symmetrical intermediate in the biosynthetic pathway, because the C-2 as well as the C-5 in the pyrrole unit were equally enriched with labelled material.



16 **1**

Fig. 2: Biosynthesis of the Pyrrole ring in Pentabromopseudilin (**1**)

Peschke's[18] first attempt to rationalize the involvement of the symmetrical intermediate as visualized above was through the compound, tetrabromopyrrole (**3**). Accordingly, when the feeding experiments were carried out by the addition of **3** to the culture medium, the compound **1** was obtained in better yield compared to the experiments employing only the amino acids. Though this was a hint for tetrabromopyrrole serving as a possible symmetrical intermediate, unfortunately, feeding with ^{15}N-pyrrole did not gave any satisfying results, i.e., no incorporation of the labelled material was observed in **1**. Thus, the said feeding experiment did not prove conclusively whether the fed **3** was transported into the cell by the bacteria or not. On the other hand, **3** being a secondary metabolite reported from the same bacteria, could be co-related with the biosynthesis of **1** obviously without being considered as a direct pre-step.

PRESENT STUDY

In continuation of the work carried out by Hanefeld and Peschke, we undertook the present investigation on the biosynthesis of pentabromopsudilin (**1**) during the stay at the Department of Organic Chemistry, University of Goettingen, Germany,. Accordingly, the culture of the marine bacteria *Alteromonas luteoviolaceus* strain 1893 was fed with tetrachloropyrrole (**17**) in anticipation of deriving indirect evidences in favour of tetrabromopyrrole (**3**) serving as the symmetrical precursor in the biosynthesis of **1**. Further, the cultures of the same bacteria were fed with 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) and 3-chloro-4-hydroxybenzoic acid (**20**) to probe whether the bromination at ortho position with respect to the hydroxy function in *p*-hydroxybenzoic acid (**15**) precedes the formation of the biaryl structure. Further, to lend additional support to the results reported by Hanefeld[19], feeding experiments with 2-chlorophenol (**22**) and 4,6-dibromo-2-chlorophenol (**23**) were also carried out in different media and at different conditions.

The ongoing paragraphs of this chapter describe the methodology used for the above mentioned feeding experiments, the details regarding the isolation and structure elucidation of the newly formed metabolites together with the discussion and conclusions derived as a result of present study.

RESULTS AND DISCUSSION

Structure elucidation of various metabolites isolated in the feeding experiments

The feeding experiments in the present study were carried out by two different methods and in three different media. The details regarding the same have been described fully in the experimental section of this chapter. For the sake of clarity and ease of comparison, the results obtained from various feeding experiments have been presented in the tabular form below (Table 1).

Table: 1 Results of the Feeding Experiments with various substrates.

Expt No	Precursor fed	Constituents in aq. medium 2L	Compounds isolated
1.	Tetrachloropyrrole 85 mg	Glucose-Glycine[a]	2,4-dibromophenol (**25**), 2,4,6-tribromophenol (**26**), pentabromopseudilin (**1**), *p*-hydroxybenzaldehyde (**6**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**).
2.	Tetrachloropyrrole 90 mg	Amino acid[b]	2,4-dibromophenol (**25**), tetrachloropyrrole (**17**), pentabromopseudilin (**1**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**).
3.	Tetrachloropyrrole 100 mg	Glucose-Glycine + *p*-hydroxybenzoic acid (100 mg)	pentabromopseudilin (**1**), tetrachloropyrrole (**17**), *p*-hydroxybenzaldehyde (**6**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**), *p*-hydroxybenzylalcohol (**37**).

4.	3-Chloro-4-hydroxybenzoic acid. 200 mg	M_1^{+c}	3′-chlorotetrabromopseudilin (**27**), 5,5′-dibromo-3,3′-dichloro-2,2′-dihydroxybiphenyl (**28**), Indole-3-carbaldehyde (**7**).
5.	2-Chloro-phenol 105 mg	M_1^+	1,8-dihydroxyanthraquinone (**29**), 4-bromo-2-chlorophenol (**30**), 4,6-dibromo-2-chlorophenol (**23**), pentabromopseudilin (**1**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**).
6.	2-Chloro-phenol 115 mg	Amino acid with excess of proline (200mg)	1,8-dihydroxyanthraquinone (**29**), 4-bromo-2-chlorophenol (**30**), pentabromopseudilin (**1**), *p*-hydroxybenzylalcohol (**41**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**).
7.	4,6-Dibromo-2-chlorophenol 90 mg	M_1^+	4,6-dibromo-2-chlorophenol (**23**),1,8-dihydroxyanthraquinone (**29**),*p*-hydroxybenzaldehyde (**6**), 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**).
8.	4,6-Dibromo-2-chlorophenol 100 mg	Amino acid	1,8-dihydroxyanthraquinone (**29**), 4,6-dibromo-2-chlorophenol (**23**), *p*-hydroxybenzaldehyde (**6**).

9.	3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid. 125 mg	M_1^-	14-methylpentadecanoic acid (**31**), 2,4,6-tribromo-2,5-dideuterophenol (**32**), pentabromopseudilin (**1**), 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**), Indole-3-carbaldehyde (**7**), 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**).
10.	3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid. 135 mg	Amino acid	2,4,6-tribromo-2,5-dideuterophenol (**32**), pentabromopseudilin (**1**), 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**), deoxyviolacein (**10b**), violacein (**10a**).

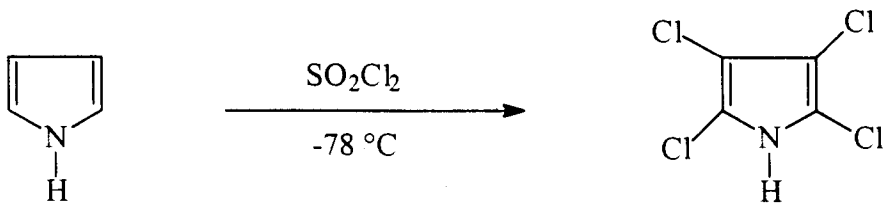
[a] medium consisting of Glucose, Glycine, L-Tyrosine & KBr dissolved in a mixture of synthetic sea water* and tap water.

[b] medium consisting of L-Tyrosine, Histidine hydrochloride, L-Ornithine hydrochloride, Glycin, L-Proline & KBr dissolved in a mixture of synthetic sea water and tap water.

[c] medium consisting of Peptone, Yeast extract & KBr dissolved in a mixture of synthetic sea water and tap water.

* Procedure for the preparation of Synthetic sea water is described in the experimental part pertaining to chapter 1 of this thesis.

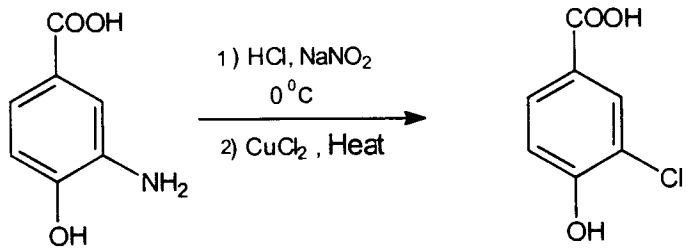
The 2,3,4,5-tetrachloropyrrole (**17**) which was required for the feeding experiment was prepared by the addition of an ethereal solution of pyrrole to sulfuryl chloride in ether at - 78 ^{0}C and then allowing it to warm to room temperature followed by stirring for 3 days.



18 **17**

In an effort to find out whether 2,3,4,5-tetrachloropyrrole (**17**) serves as a symmetrical precursor for the formation of pyrrole ring during the biosynthesis of **1**, the feeding expt. No 1, 2 and 3 were carried out (table 1). If **17** is involved as a symmetrical precursor, then formation of 3′,5′-dibromo-2,3,4-trichloro-2′-hydroxypseudilin could be expected as a metabolite. However, no incorporation/utilization of tetrachloropyrrole (**17**) was observed in any of the said experiments since 3′,5′-dibromo-2,3,4-trichloro-2′-hydroxypseudilin was not isolated/formed. On the other hand, compounds such as 2,4-dibromophenol (**25**), 2,4,6-tribromphenol (**26**) and *p*-hydroxybenzyl alcohol (**6**) were isolated for the first time in such feeding experiments, though these compounds have already been reported as the secondary metabolites from *Alteromonas luteoviolaceus*. In addition, pentabromopseudilin (**1**) and 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) were also isolated from all the three feeding experiments. Thus, no positive evidence came in favour of 2,3,4,5-tetrachloropyrrole seving as a symmetrical precursor for the biosynthesis of pyrrole ring in **1**.

The next series of experiments were carried out by utilising 3-chloro-4-hydroxybenzoic acid (**20**) as the feed. The acid (**20**) required for the said experiment was prepared[25] from 3-amino-4-hydroxybenzoic acid (**33**).

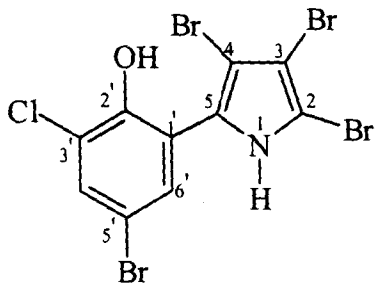


33 **20**

In this feeding experiment, three pure compounds other than the pentabromopseudilin (**1**) were isolated and subjected to structure elucidation as described below.

Identification of 3′-Chlorotetrabromopseudilin (27)

The residue obtained in the second fraction after the usual work-up procedure was purified using a Sephadex LH 20 to provide a crystalline solid (6.5 mg). The said compound showed similar R_f = 0.75 (chloroform/methanol; 98 : 2) as pentabromopseudilin (**1**). The EI-MS (fig. 5.09) showed *m/z* = 504.7 and the isotope splitting pattern indicated that it had 4 bromine and 1 chlorine atoms. This was confirmed by the high resolution mass spectrum, which indicated the molecular formula as $C_{10}H_4Br_4ClON$. In addition, other fragment ions were also seen, which resulted from the loss of one bromine atom (*m/z* = 425.8), two bromine atoms (*m/z* = 346.9) and the loss of bromine and HCN (*m/z* = 398.8). The ^{1}H-NMR spectrum (fig. 5.10) measured in [D_6]DMSO showed two broad singlets at δ 7.68 and δ 7.36 and other two D_2O exchangeable signals at δ 12.56 and δ 9.86. All the four signals had a relative intensity of 1 proton each. The compound showed a comparable spectrum with that of pentabromopseudilin (**1**) except for a slight difference in the proton chemical shift values.



27

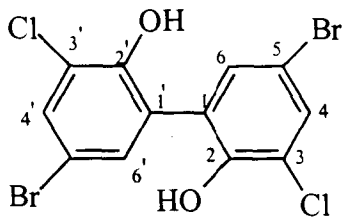
The ^{13}C-NMR spectrum (fig. 5.11) of the said compound looked very similar to that of **1**. Except for one carbon atom, the chemical shift values of all other signals agreed well with those reported for **1**. This deviation with respect to one resonance signal could be attributed to the influence exerted by the halogen atom bonded directly to the carbon atoms. Consequently, only one carbon atom in the molecule under investigation had to have a significantly different δ value, which is possible

only when one bromine atom in **1** is substituted by a chlorine atom. As expected, one singlet at δ 112, corresponding to the normal position where C-3′ and C-5′ of pentabromopseudilin (**1**) would have its ^{13}C signals, was missing in the ^{13}C-NMR spectrum. However, the crystalline compound isolated showed a singlet at δ 122 in its ^{13}C-NMR spectrum. After comparing the same with the spectrum of pentabromopseudilin, it was concluded that this was the expected position of the signal for a chlorinated C-atom on a phenol ring. The δ value of the carbon atom no 5′ in the phenyl ring indicated that it is bonded to a bromine atom as in the case of **1**. However, compared to **1**, the deviation in the chemical shift value of C-5′ in the present case was only 0.65 ppm. Based on above, it could be concluded that the crystalline compound isolated during the present feeding experiment was 3′-chlorotetrabromopseudilin (**27**), a new pseudilin derivative not reported so far.

Identification of 5,5′-Dibromo-3,3′-dichloro-2,2′-dihydroxybiphenyl (28)

The residue obtained from the third fraction was purified by using Sephadex LH 20 to give 2.5 mg of a white solid. The EIMS (fig. 5.12) of this compound showed *m/z* at 409.9. The fragmentation pattern in the mass spectrum indicated that the compound under investigation had two bromine and two chlorine atoms. Moreover, a fragment ion was observed at *m/z* = 252, which indicated the loss of two bromine atoms during its formation. The high resolution mass spectrum gave the molecular formula as $C_{12}H_6Br_2Cl_2O_2$.

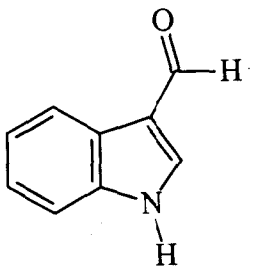
In the ^{1}H-NMR spectrum (fig. 5.13), only two doublets were seen in the aromatic region, with characteristic *meta* coupling pattern. The ^{13}C-NMR spectrum of this compound showed almost the same pattern as that of 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) which has reported earlier as a metabolite from *Alteromonas luteoviolaceus*. Only one carbon atom in the spectrum, however, had a different chemical shift, ie, one of the signals at δ 122 was shifted to the deep down field area. The reason that could be attributed to this shift was the replacement of one of the bromine atom in **24** by a chlorine atom, and consequently, the compound isolated during the present study from the feeding experiment could be formulated as 5,5′-dibromo-3,3′-dichloro-2,2′-dihydroxybiphenyl (**28**).



28

Identification of Indole-3-carbaldehyde (7)

The third compound which was obtained as a light brown coloured solid, m.p. 197 ^{0}C from the feeding experiment was identified as indole-3-carbaldehyde (**7**) on the basis of spectral analysis (described in the experimental part) and further confirmed by comparision with an authentic sample. It may be noted that **7** co-occurs with tetrabromobiphenol in *Alteromonas luteoviolaceus* as stated earlier.



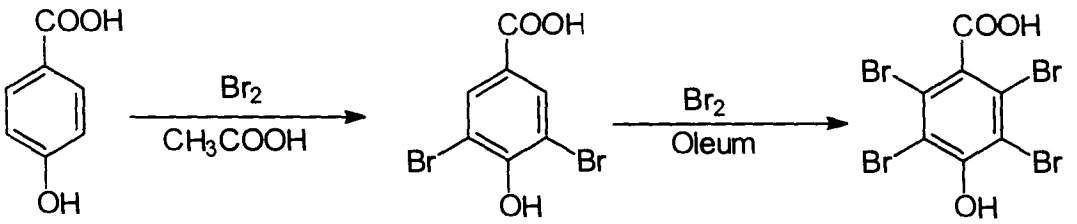
7

Next, the feeding experiments were carried out by utilising the 2-chlorophenol with the aim of examining whether the same would be incorporated to provide the corresponding 3′-chlorotetrabromopseudilin (**27**) and the corresponding biphenol (**28**). However, neither **27** nor **28** was isolated in this feeding experiment. On the contrary, other compounds such as 1,8-dihydroxyanthraquinone (**29**), 4-bromo-2-chlorophenol (**30**) and 4,6-dibromo-2-chlorophenol (**23**) were isolated for the first time in these feeding experiments. In addition, pentabromopseudilin (**1**) and 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) were also isolated from all these feeding experiments.

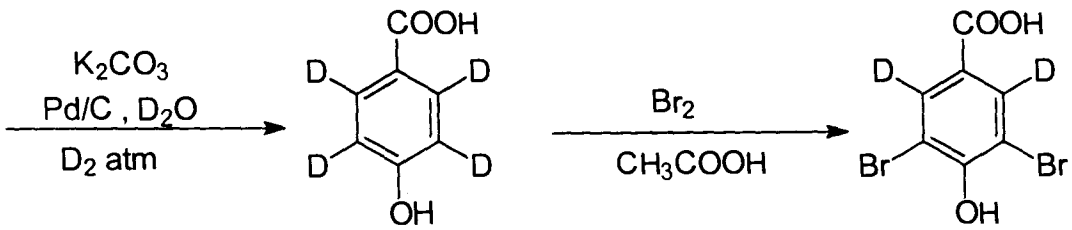
Since the feeding experiment by employing *p*-chlorophenol did not provide the desired results with incorporation of chlorine atom in the pseudilin skeleton, the

experiments no 7 and 8 were carried out by utilising 2-chloro-4,6-dibromophenol. Contrary to our expectation, neither 3'-chlorotetrabromopseudilin (**27**) nor the corresponding biphenol (**28**) was isolated. However, as observed in the earlier experiment, the compounds, *viz.*, 1,8-dihydroxyanthraquinone (**29**), 4-bromo-2-chlorophenol (**30**) and 4,6-dibromo-2-chlorophenol (**23**) were isolated along with 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**) as the product of these feeding experiments.

Next, we tried the feeding experiment by employing 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) as the substrate. The deuteriated acid (**19**) required for the said feeding experiment was prepared from 4-hydroxybenzoic acid (**15**) as per the following scheme.



15 **34** **35**



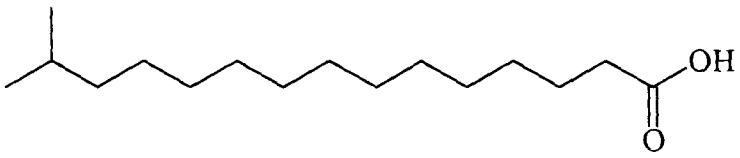
36 **19**

In this case, the feeding experiment was carried out in two media (M_1 medium and amino acid medium) and the cultures were harvested after 72 h by extraction with ethyl acetate. The feeding experiment in M_1^+ medium gave (258 mg) of crude extract

which on further purification on silica gel, PTLC and Sephadex gave six compounds, which were isolated and characterised as below.

Identification of 14-Methylpentadecanoic acid (31)

The first fraction was found to contain a fatty acid (42 mg) . The EI-MS gave a molecular ion at *m/z* = 252 (M^+ 100%) and other fragmentation ions at 213 (M^+ - C_3H_7). The ^{1}H-NMR spectrum showed a doublet at δ 0.92 (6 H, 2-CH_3), a multiplet at δ 1.08 (2 H, CH_2), a broad singlet at δ 1.3 (18 H, 9 CH_2), multiplet at δ 1.5 (1 H, CH), triplet at δ 1.6 (2 H, CH_2) and a triplet at δ 2.53 (2 H, CH_2). From this information, it was concluded that the isolated compound was 14-methylpentadecanoic acid (**31**).

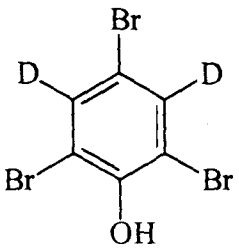


31

Isolation of 2,4,6-Tribromo-3,5-dideutero-phenol (32)

The second fraction was purified on Sephadex to give 30 mg of a white solid. The EI-MS (fig. 5.18) spectrum showed molecular ions at *m/z* = 330, 332, 334 and 336. The relative intensity of these peaks were in the ratio of 1 : 3 : 3 : 1, which indicated that the compound contained 3 bromine atoms. Further, it also showed fragment ions at *m/z* = 250, 252, 254 (M^+- HBr). The ^{1}H-NMR spectrum (fig. 5.19) showed a sharp singlet at δ 5.9 (1 H, exchangeable with D_2O). There were no other signals in the proton NMR spectrum. These spectroscopic data indicate that the compound isolated might be tribromophenol, in which the remaining two hydrogens are substituted by deuterium atoms. The ^{13}C-NMR spectrum (fig. 5.20) showed only three signals, among which the signal at δ 148.86 was assigned for the phenolic carbon. The signal at δ 112.42 had a relative intensity of two carbons and hence was assigned to the carbons bearing the deuterium atoms, while the signal at δ 110.20 with a relative intensity of three was assigned to the carbons bearing the three bromine atoms. Thus,

from the above spectroscopic data, it could be concluded that the compound isolated in the feeding experiment was 2,4,6-tribromo-3,5-dideutero-phenol (**32**).



32

The third fraction on purification gave 8 mg of pentabromopseudilin (**1**) whose identity was confirmed by co-chromatography (TLC), ^{1}H-NMR and also by EI-MS.

The fourth fraction was purified by Sephadex column to give 24.5 mg of 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**), which was identified on the basis of (TLC), ^{1}H-NMR and EI-MS.

The fifth fraction was separated from other impurities by PTLC using 12% CH_3OH : $CHCl_3$ to give 3.3 mg of indole-3-carbaldehyde (**7**).

The last fraction was purified by using HPLC to give 5.2 mg of 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) and 7.3 mg of 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**).

As in the above case, the feeding experiment in amino acid medium, provided 125 mg of the crude extract which was chromatographed on a silica gel column and eluted using a gradient solvent system consisting of cyclohexane : ethyl acetate to give a total of five fractions.

The first fraction on purification gave 20 mg of solid, which was identified as 2,4,6-tribromo-3,5-dideutero-phenol (**32**).

The second and third fractions on purification using Sephadex column gave 4.5 mg of pentabromopseudilin (**1**) and 15 mg of 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**).

The fourth fraction was found to contain a mixture of two blue-violet coloured compounds, which were separated by PTLC using 10 % CH_3OH : $CHCl_3$, to give two individual pure compounds designated as 4A and 4B. The final purification was done through Sephadex column.

The compound 4A (1.2 mg) was characterised as deoxyviolacein (**10b**) by comparison of the 1H-NMR spectrum with that reported in the literature[13,23]. In a similar way, the compound 4B (6 mg) was identified as violacein (**10a**) by comparing the IR, UV and ^{1}H-NMR data with those reported in the literature[13,23]. It may be noted that both the above compounds **10a** and **10b** have been isolated previously from *Alteromonas luteoviolaceus*.

DISCUSSION

As state earlier, Hanefeld[19] by his feeding experiments with differently labeled glucose sample showed that the benzene ring of **1** is derived from carbohydrate metabolism. Furthermore, it was concluded that the shikimic acid is converted *via* a symmetrical intermediate into the benzene ring of **1**. The feeding experiment with *p*-hydroxy-(2,3,5,6-D_4)-benzoic acid confirmed that it is the direct precursor for this moiety. Peschke[18] observed in his feeding experiment by employing D,L-(5 ^{13}C) proline that **1** was enriched with labelled material. In this way, proline was identified as the starting precursor for the pyrrole unit biosynthesis in **1**. In addition, it was also inferred that there must be a symmetrical intermediate involved in the biosynthetic pathway because C-2 as well as C-5 positions in the pyrrole unit were enriched in **1**.

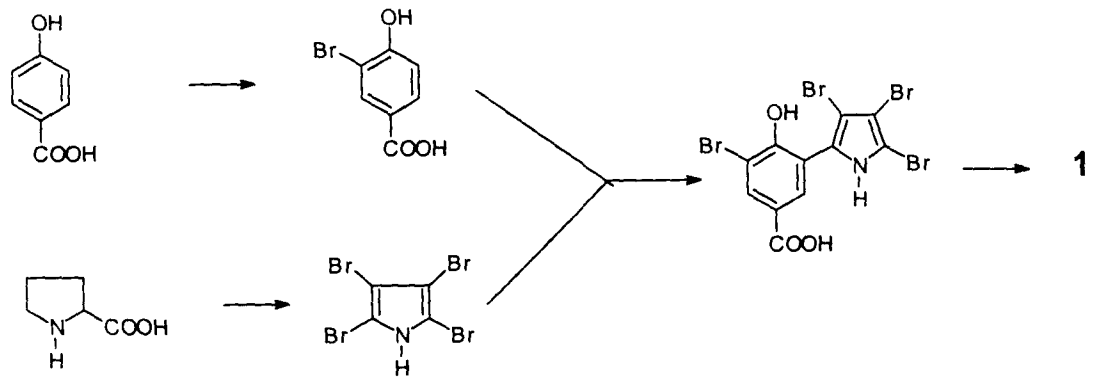
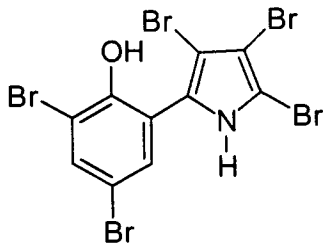


Fig. 3 : Postulated biosynthesis of Pentabromopseudilin (**1**) by the earlier workers.

The results obtained during the present feeding experiment with 3-chloro-4-hydroxy benzoic acid re-confirmed the earlier observation that *p*-hydroxy benzoic acid is the basic precursor for the benzene fragment in **1**. In addition, a new pseudilin derivative, *viz.*, 3'-chloro-tetrabromopseudilin (**27**) was also isolated along with the corresponding biphenyl (**28**)during this study. It is seen that pentabromopseudilin (**1**) is a mixed biaryl formed by the combination of a phenol and a pyrrole unit. Depending on the growth conditions, the *Alteromonas luteoviolaceus* strain also produces two additional biaryls resulting from the coupling of two identical units to provide the products **4** and **24** respectively, besides the corresponding precursor units *viz* **3** and **25**. This could lead to the assumption that the individual monomer units are formed first which will then couple/cross couple together in a separate step to provide the corresponding biaryls.



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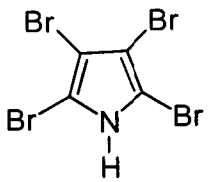

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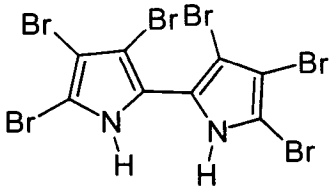

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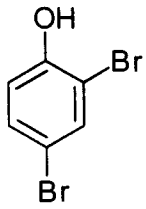

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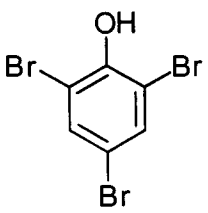

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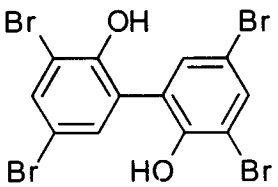
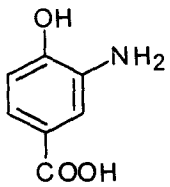
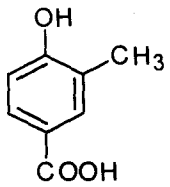


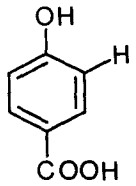
24

Similarly, in analogy with the oxidative coupling reactions[20,21,23], it may be appropriate to believe that the pentabromopesudilin (**1**) is formed *via* a biaryl coupling of the respective monomeric fragments. Probably, it would be possible that a phenol oxidase oxidizes the phenol moiety as well as the pyrrole fragment to generate the respective radicals which will further react to form a biaryl.

However, one important aspect to be noted is that the oxidation potentials of phenol (or hydroxy benzoic acid) and pyrrole are so different that never a cross coupling product could be expected. That means the nature has to bring the oxidation potentials of both fragments closer to an optimum level so that the desired coupling reaction could take place. One of the ways to achieve this is *via* the bromination of pyrrole. It is known that in case of two substances having different oxidation potentials, it is always the one which has the higher potential oxidises first and will react further independently. Consequently, to obtain the pseudilin based skeleton through coupling of the corresponding radicals, both the coupling precursors units must have similar oxidation potentials. The AM1 calcualtion[24] for the HOMO-energies of substituted *p*-hydroxy benzoic acids as well as the pyrrole derivatives showed that tetrabromopyrrole has a near similar oxidation potential and therefore could be the coupling partner for such a phenol / acid to give the corresponding biaryls.

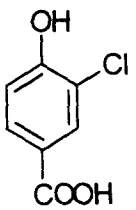


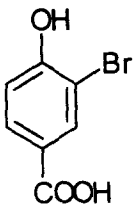


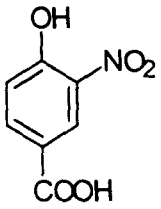


HOMO-Energies -9.081 -9.592 -9.608

in eV

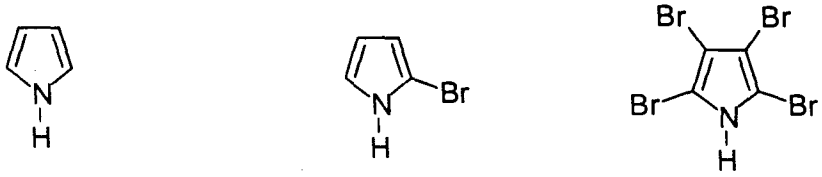






HOMO-Energies -9.701 -9.899 -10.327

in eV

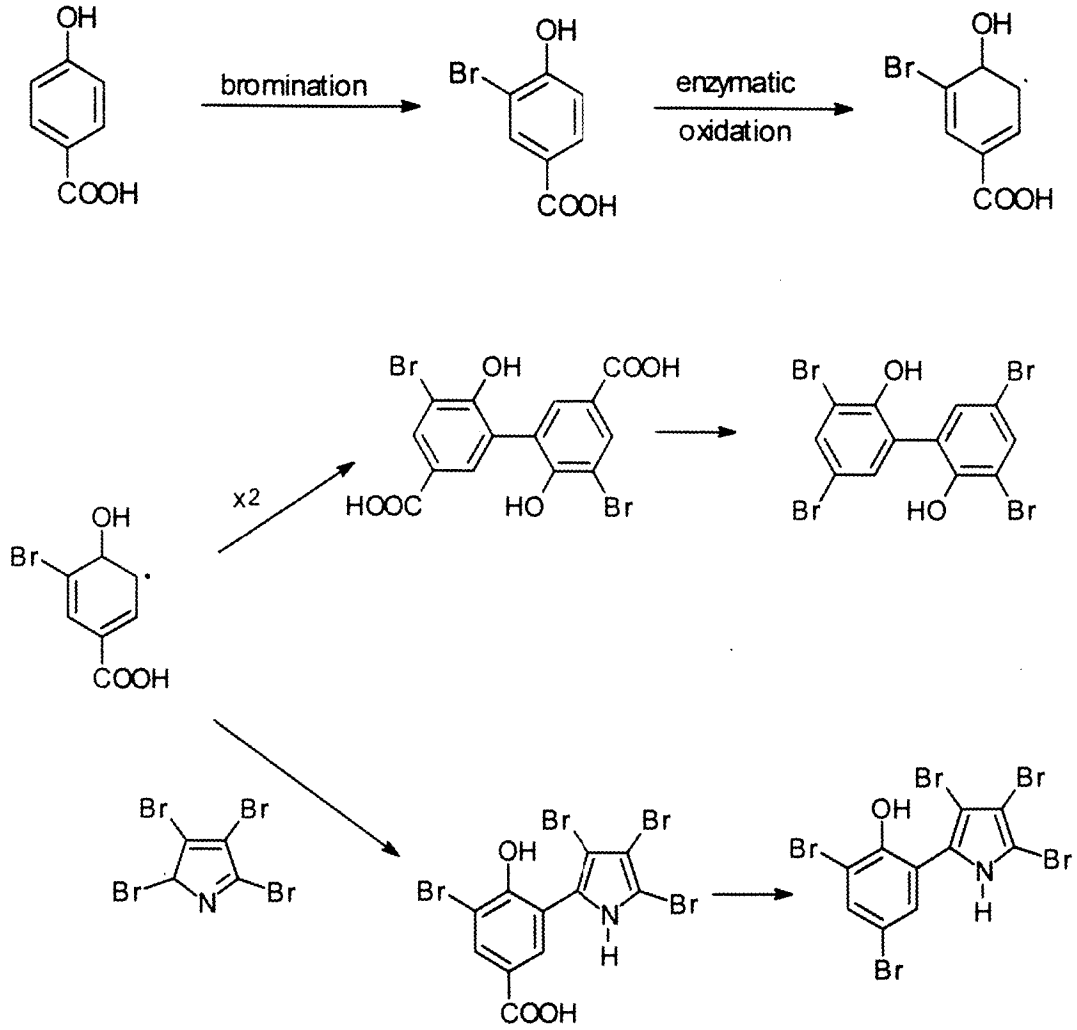


HOMO-Energies	-8.657	-8.800	-9.228
in eV			

Fig. 4: HOMO - energies of pyrroles and substituted phenols

Thus, it could be visualised that the cross coupling of such a brominated pyrrole with hydroxybenzoic acid may first yield a carboxylated pseudilin which subsequently should lose carbon dioxide. This reaction as well may be facilitated by electron withdrawing substituents, however, it is difficult to predict at this stage, the exact decarboxylation sequence, ie whether it is at the stage of the uncoupled hydroxybenzoic acid or later after the stage of pseudilin formation. Consequently, two sequences may operate *viz*: a) the hydroxybenzoic acid is brominated first followed by decarboxylation and then the coupling reaction takes place or b) the hydroxybenzoic acid is brominated first and then coupling takes place followed by decarboxylation and eventually again brominated to provide the corresponding biaryls.

If decarboxylation of the bromo acid takes place prior to the coupling reaction, then bromination of the resulting monobromophenol should follow immediately to give the dibromophenol in order to enhance its oxidation potential for further transformation as explained earlier. Therefore, it implies that the cell should be capable of taking up the mono, di, or tribromophenols for the onward biosynthetic process and this can be checked/examined by employing labelled bromophenols in the feeding experiments. However, it is known that such bromophenols are not easy to prepare and hence the said exercise could be not be undertaken.



Scheme- I

To find out which of the above mentioned decarboxylation reaction sequence operates during the biosynthesis of **1**, some feeding experiments were carried out. Accordingly, with an aim to insert one chlorine atom *via* the feed precursor and then look for a monochloro-tetrabromopseudilin molecule, we carried out the following feeding experiments. If the coupling occurs with a brominated hydroxybenzoic acid during the biosynthesis process, then feeding with chloro-hydroxybenzoic acid may also lead to the same result. In confirmation, the feeding experiment with 3-chloro-4-hydroxybenzoic acid provided 6.5 mg of 3'-chloro-tetrabromopseudilin (**27**) and 2.5 mg of dichloro-dibromobiphenol (**28**). These results provided evidences in favour c the sequence (b) operating in preference to (a), ie, the hydroxybenzoic acid is fi brominated, followed by enzymatic oxidation and then coupling reaction and fina

decarboxylation and eventually again bromination to provide the corresponding biaryls in the course of biosynthesis as shown in the scheme- I.

The formation of 3'-chloro-tetrabromopseudilin (**27**) and dichloro-dibromobiphenol (**28**) can be conveniently explained on the basis of above pathway/sequence of the reactions, in which the phenol radical plays a key role. The formation of these two products depend on the coupling of two chlorophenol radicals to produce the biphenol (**28**) or instead with a pyrrole radical to give 3'-chlorotetrabromopseudilin (**27**). The formation of **27** and **28** and not pentabromopseudilin (**1**) and tetrabromobiphenol (**24**) indicated that 3-chloro-4-hydroxybenzoic acid is incorporated into the benzene ring of **1**, which further confirmed that *p*-hydroxybenzoic acid is the direct precursor of **1** as reported earlier.

In other feeding experiment with *o*-chlorophenol and 4,6-dibromo-2-chlorophenol, it may be seen that 3'-chlorotetrabromopseudilin (**27**) and dichloro-dibromobiphenol (**28**) were not formed, but pentabromopseudilin (**1**) and tetrabromobiphenol (**24**) were isolated in addition to 1,10-dihydroxyanthraquinone (**29**), 4-bromo-2-chlorophenol (**30**) and 4,6-dibromo-2-chlorophenol (**23**). The formation of pentabromopseudilin (**1**) and tetrabromobiphenol (**24**) indicated that *o*-chlorophenol and 4,6-dibromo-2-chlorophenol are not incorporated into the benzene ring of **1**, which further suggested that the carboxylic group plays a major role in the biosynthesis. Similarly, in the case of feeding experiment with 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**), no pentabromopsudilin enriched with deuterium was formed, but a high yield of pentabromopseudilin (**1**) and tetrabromobiphenol (**24**) was observed. Hence, this feeding experiment could not prove whether the fed **19** was transported into the cell since no labelling could be detected in the products isolated. Consequently, it is thought that the substrate **19** was unable to penetrate the cell membrane of *Alteromonas luteoviolaceus*. This problem could possibly be overcome by employing mutation techniques.

In summary, the present investigation involving the feeding experiments has enabled us to draw certain positive conclusions with respect to the biosynthesis of the marine antibiotic pentabromopseudilin as described above. However, further studies are necessary to supplement the above described results. Specially, the presumed

involvement of the five membered symmetrical intermediate during the biosynthesis of pyrrole ring and the observed enhancement in the yields of pentabromopseudilin on feeding the culture with tetrabromopyrrole in absence of direct evidence to its incorporation needs to be probed further. Hence the need of additional feeding experiments by utilising intermediate compounds like pyrrolidine, pyrrole-2-carboxylic acid, 3,4,5-tribromopyrrole-2-carboxylic acid etc have been felt to draw definite conclusions in this regard. Paucity of the time did not permit us to undertake the said work at this stage. However, the same will be taken up for investigation in near future.

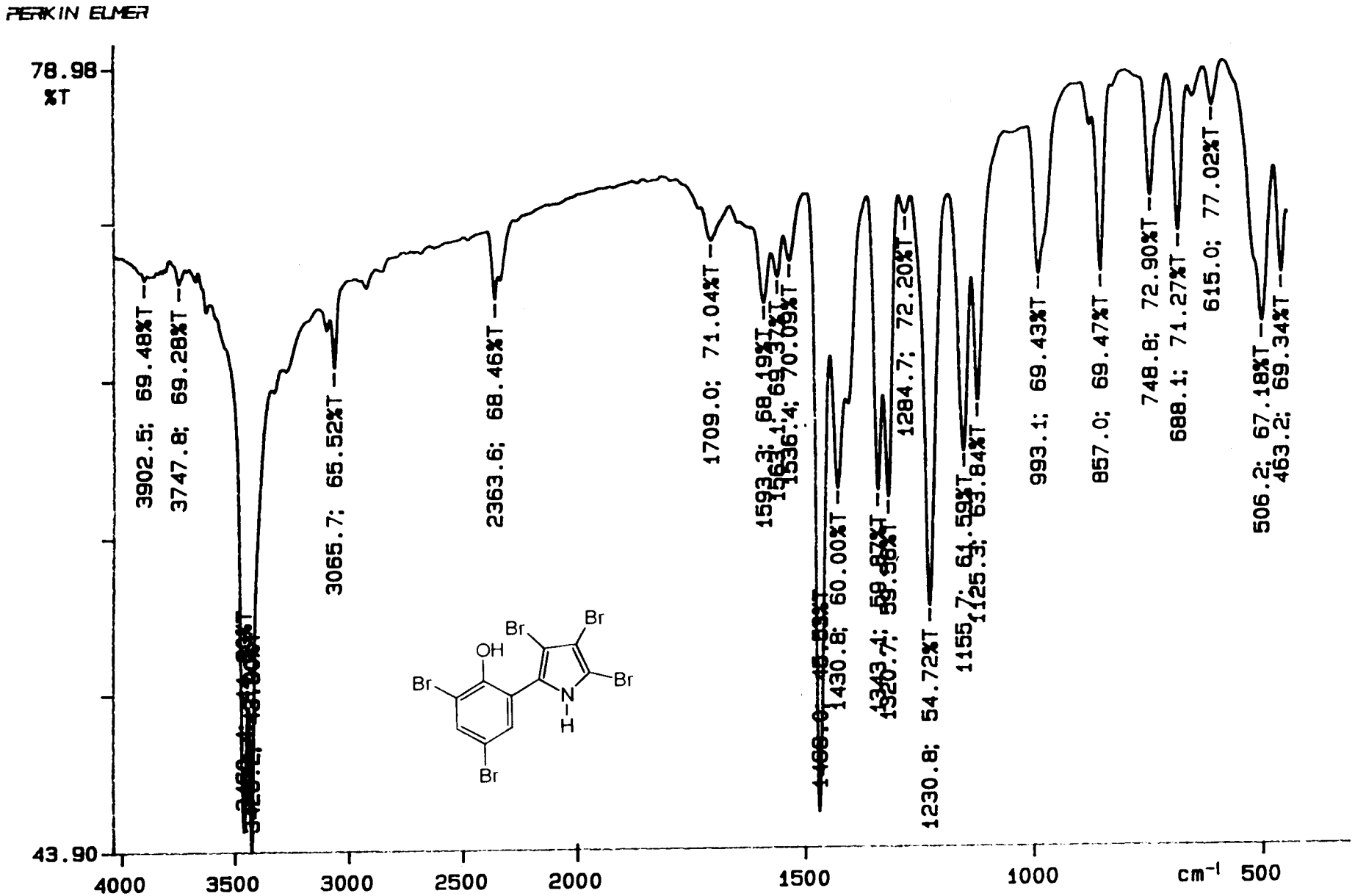


Fig. 5.01 : IR spectrum of Pentabromopseudilin (1)

SPEC: ra9 20-Jul-98 Elapse: 01:38.6 22
Samp: Ratna RA-8 Start : 15:05:58 24
Mode: EI +VE +HMR BSCAN (EXP) UP LR NRM
Oper: ReUd-Goe Inlet :
Base: 473.7 Inten : 55481 Masses: 40 > 1000
Norm: 473.7 RIC : 694829 #peaks: 218
Peak: 1000.00 mmu

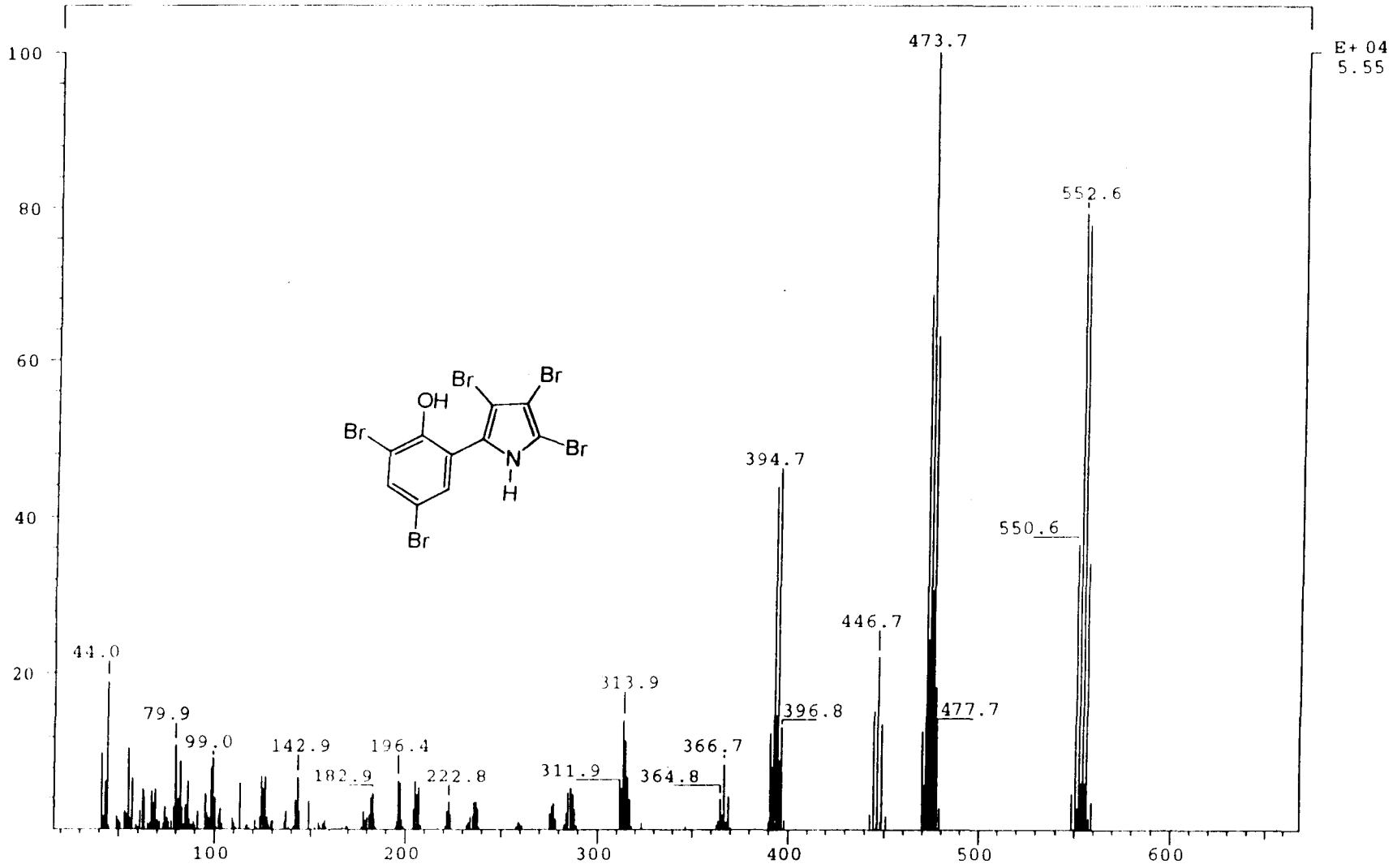


Fig. 5.02 : EI mass spectrum of Pentabromopseudilin (1)

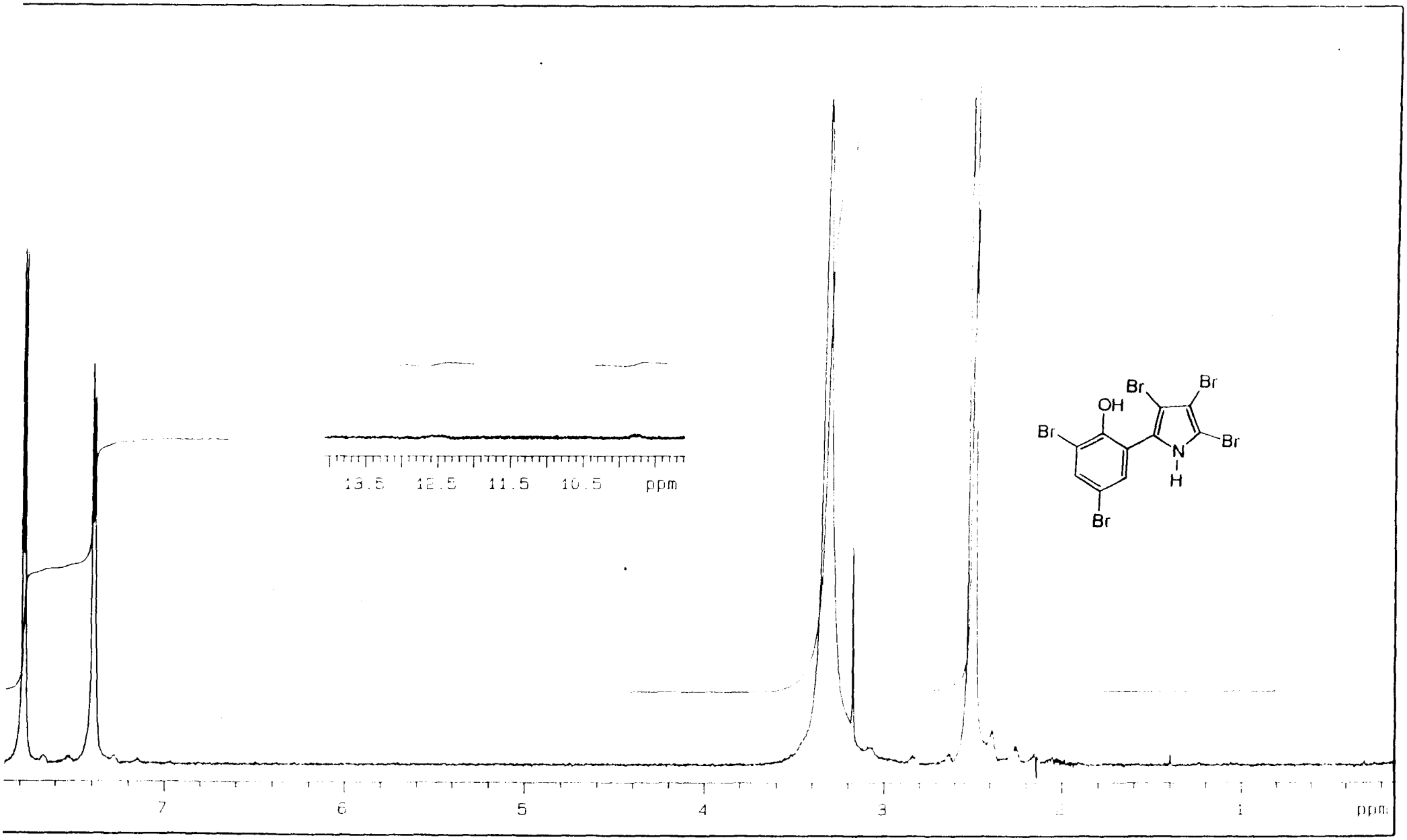


Fig. 5.03 : ^{1}H NMR spectrum of Pentabromopseudilin (1) in DMSO-d_6

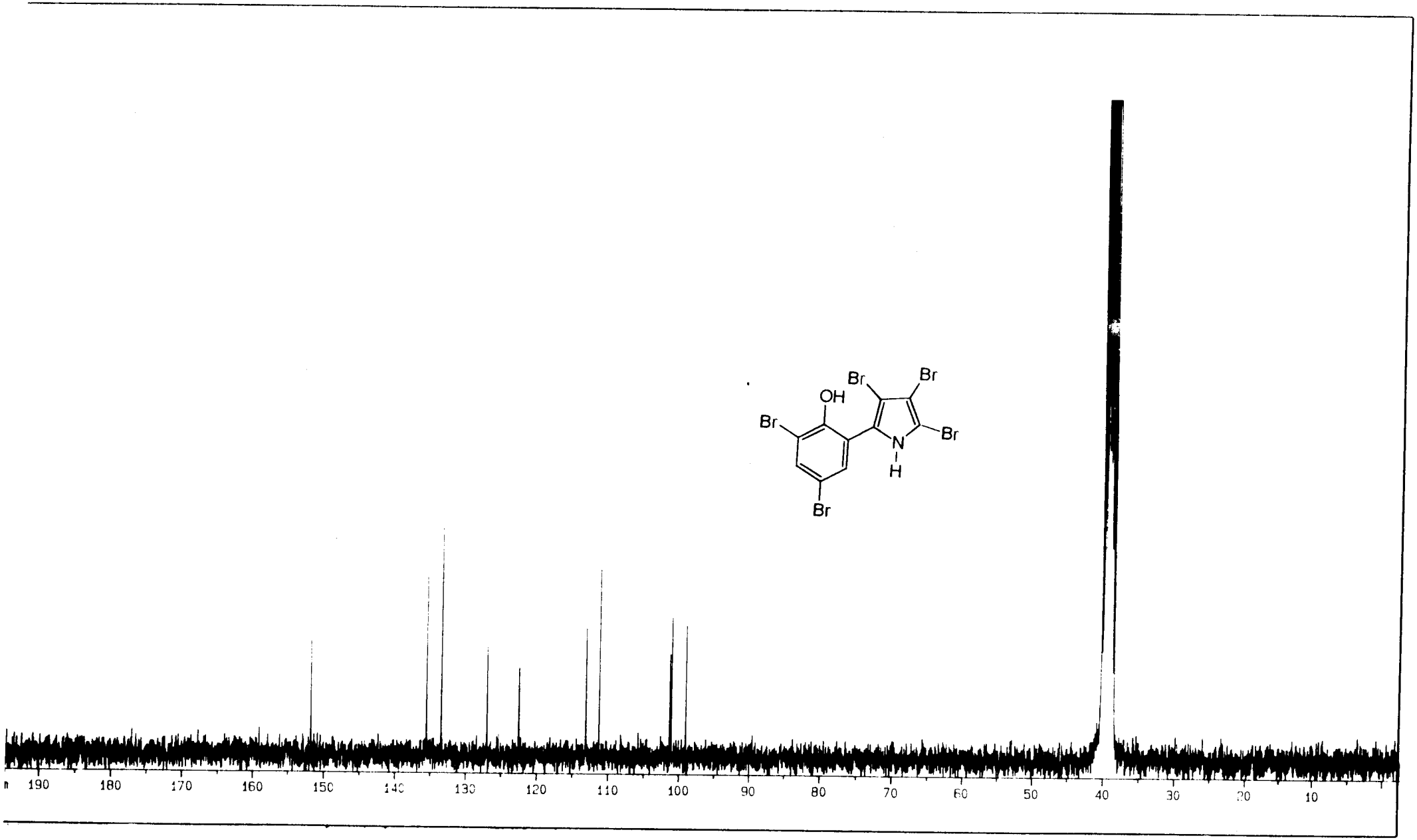


Fig. 5.04 : ^{13}C NMR spectrum of Pentabromopseudilin (1) in DMSO-d_6

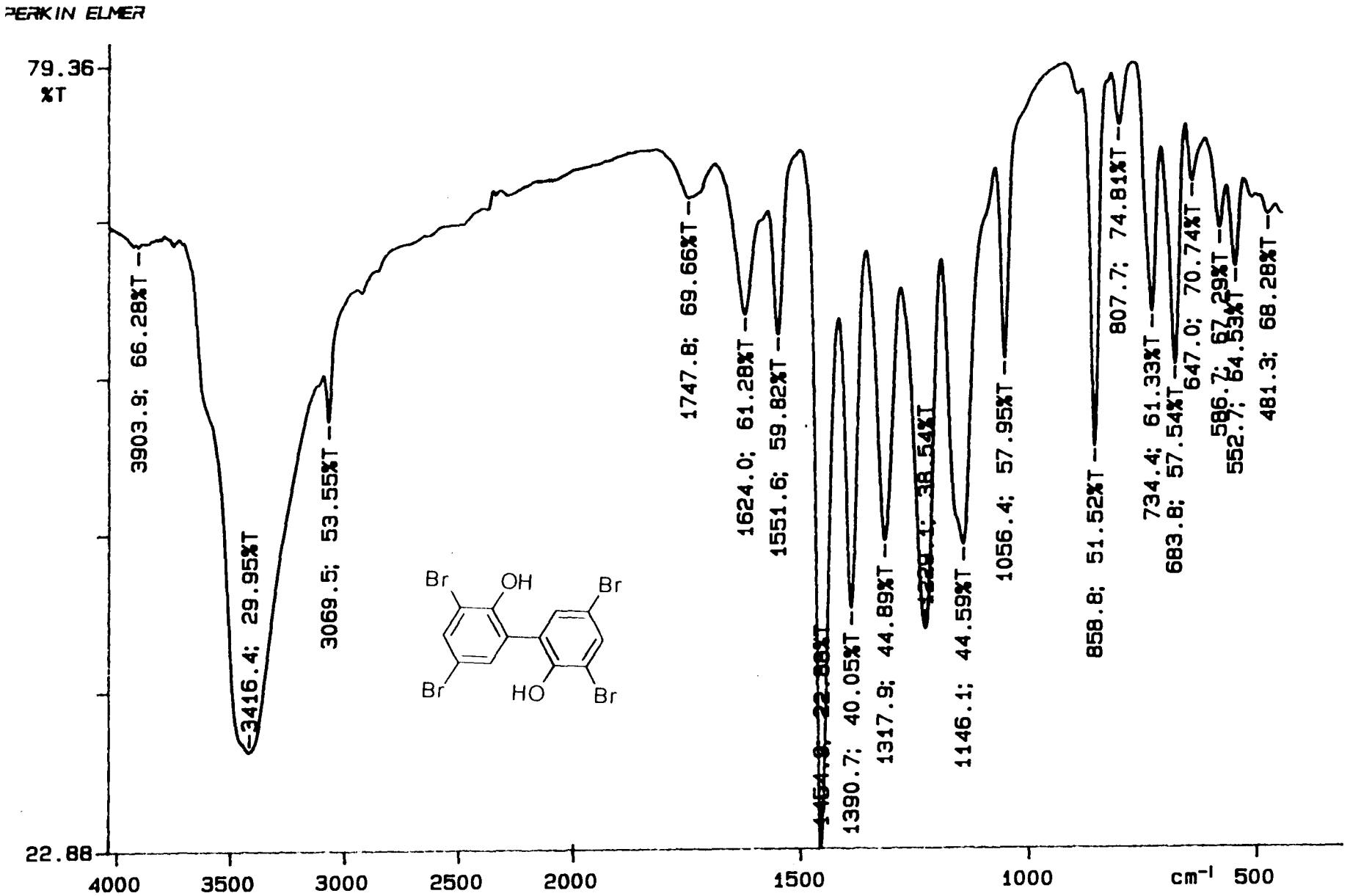


Fig. 5.05 : IR spectrum of compound 24

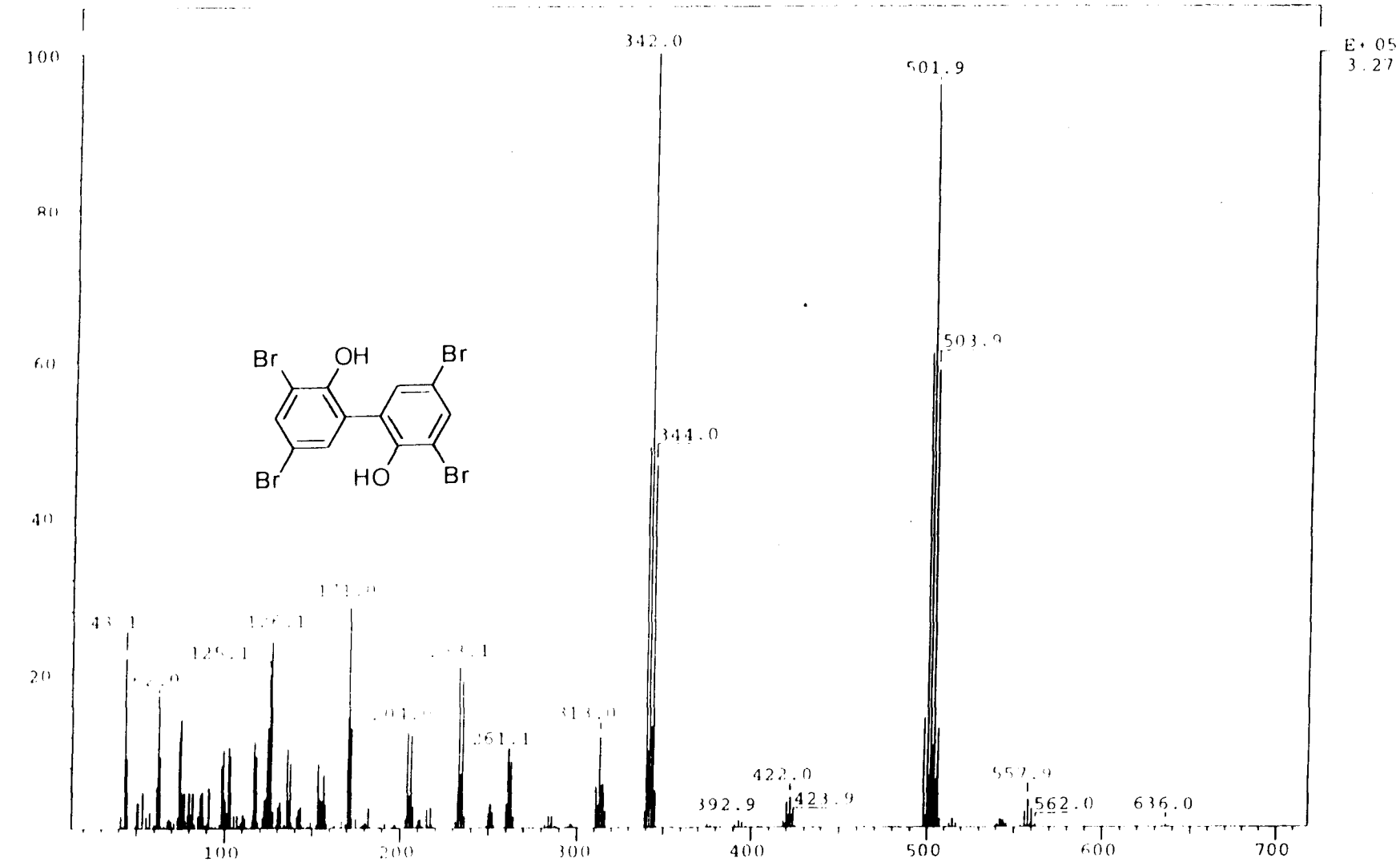


Fig. 5.06 : EI mass spectrum of compound 24

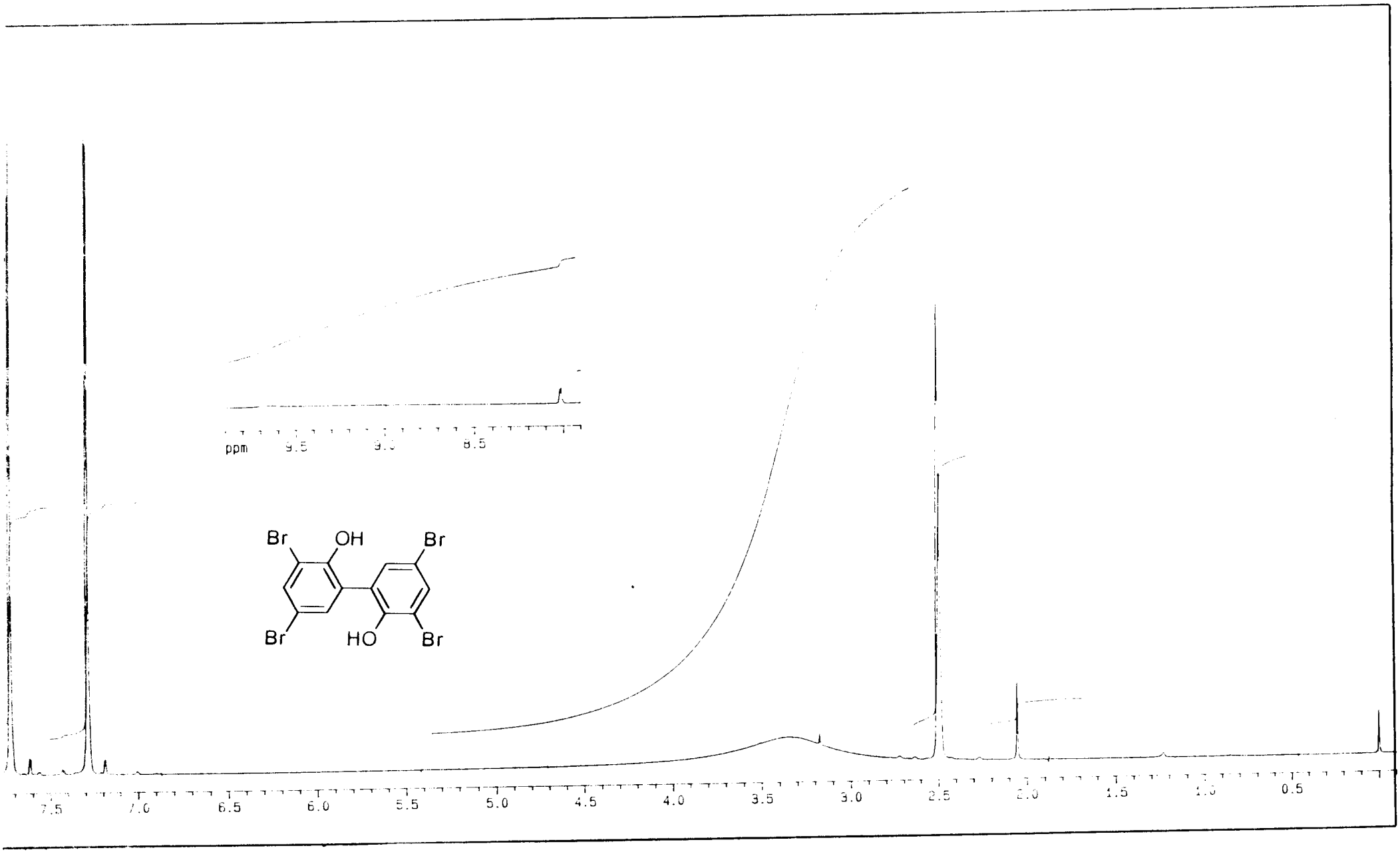


Fig. 5.07 : ^{1}H NMR spectrum of compound 24 in DMSO-d_6

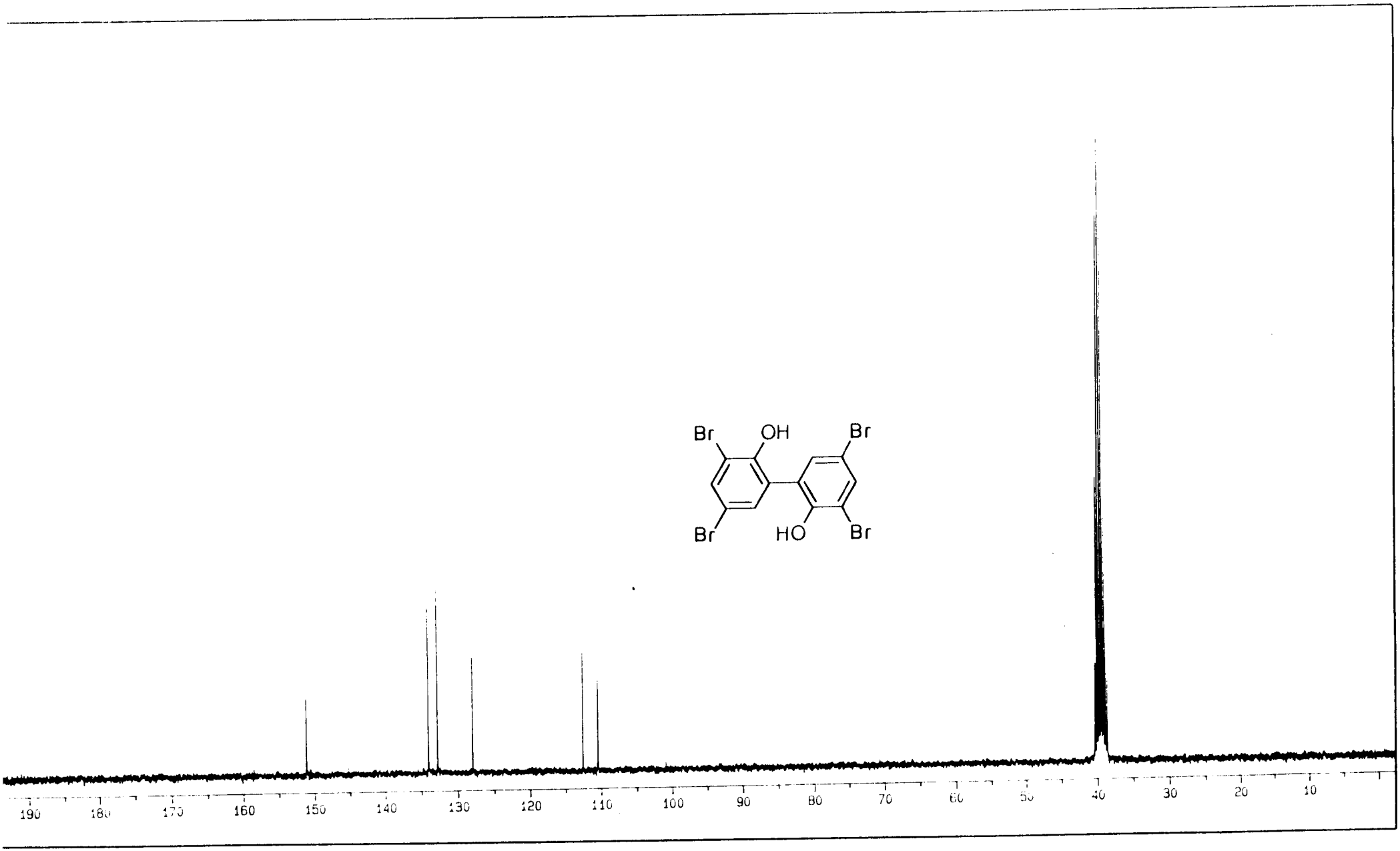


Fig. 5.08 : ^{13}C NMR spectrum of compound 24 in DMSO-d_6

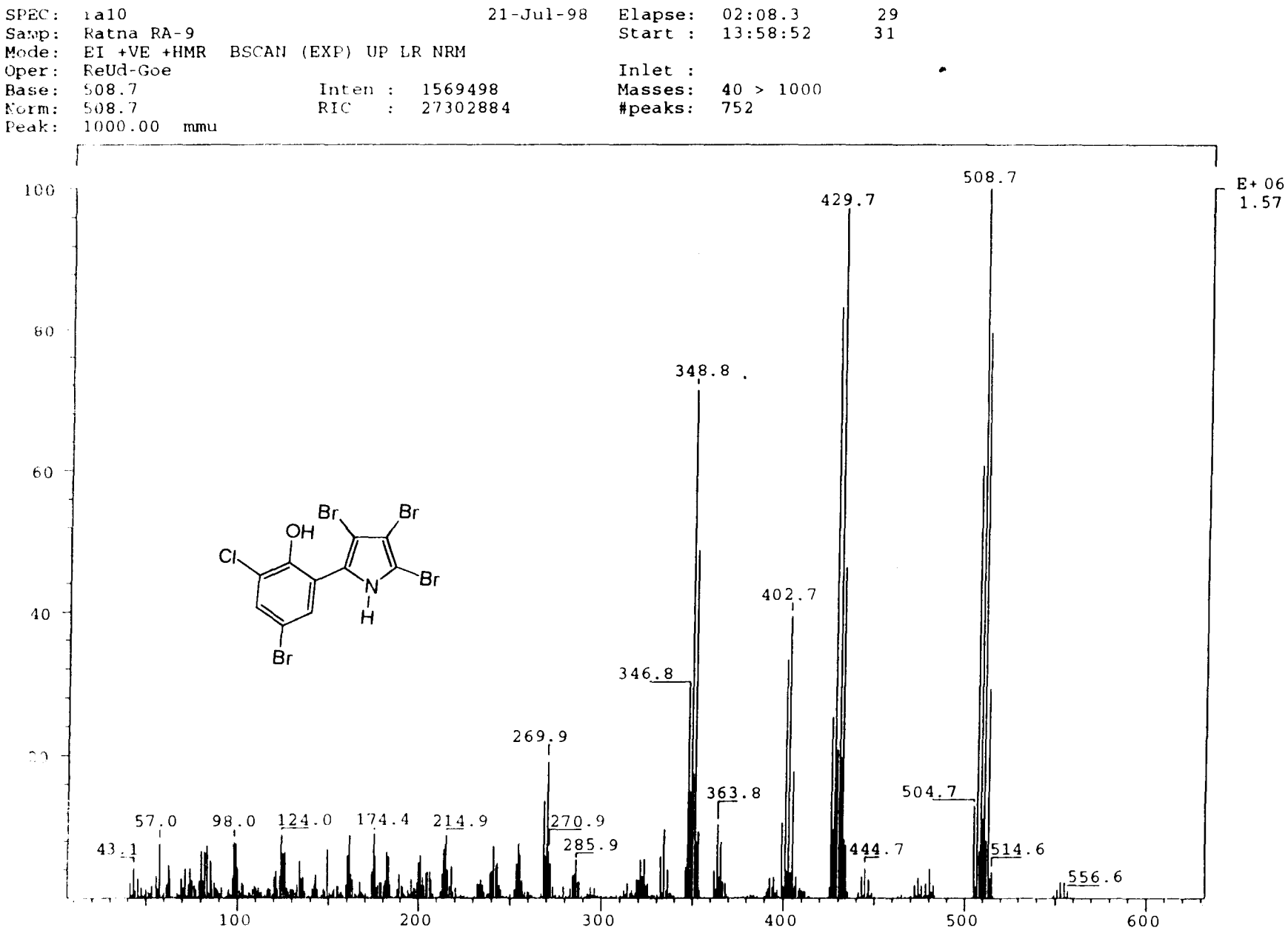


Fig. 5.09 : IR spectrum of 3′-chlorotetrabromopseudilin (27)

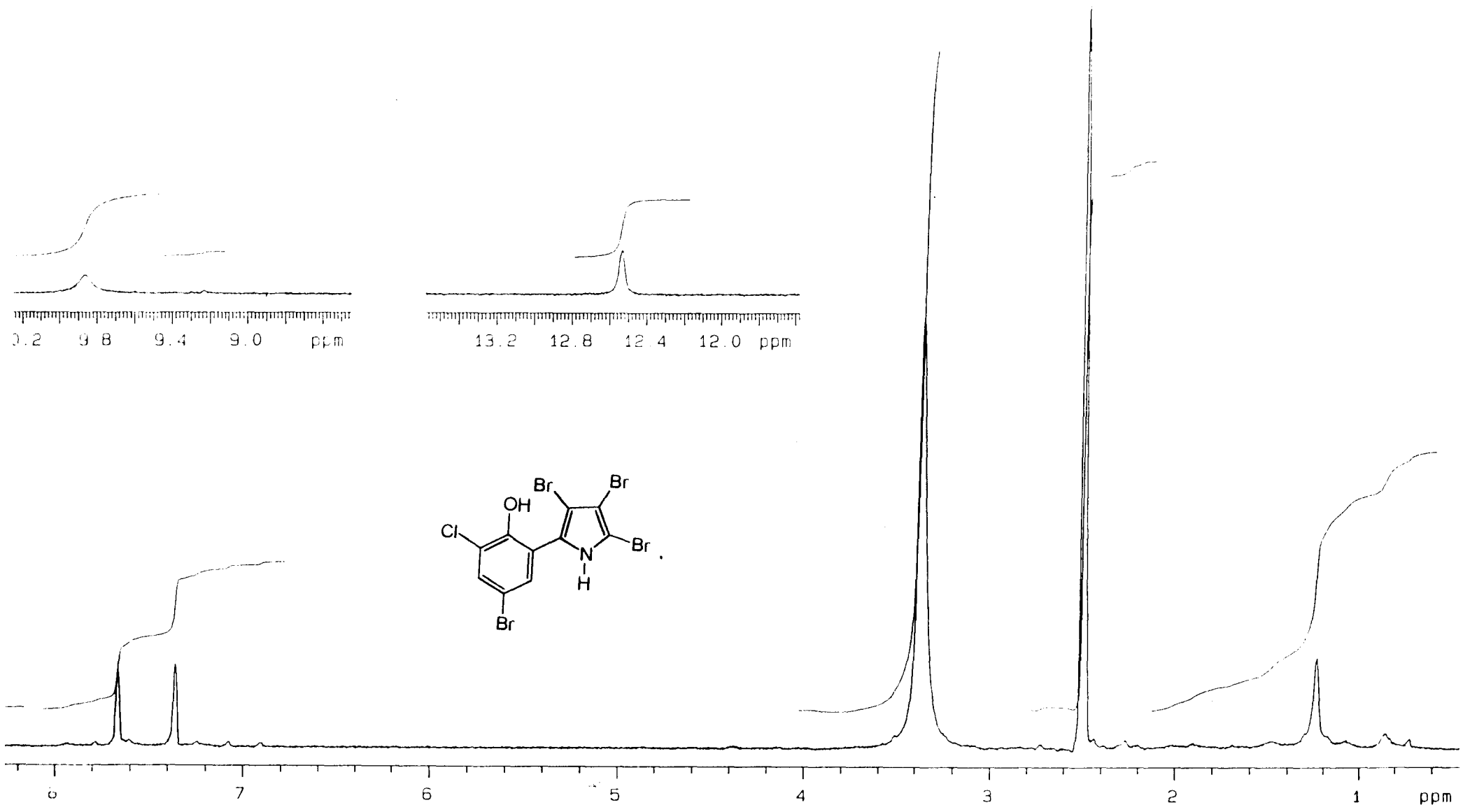


Fig. 5.10 : ^{1}H NMR spectrum of 3′-chlorotetrabromopseudilin (27) in DMSO-d_6

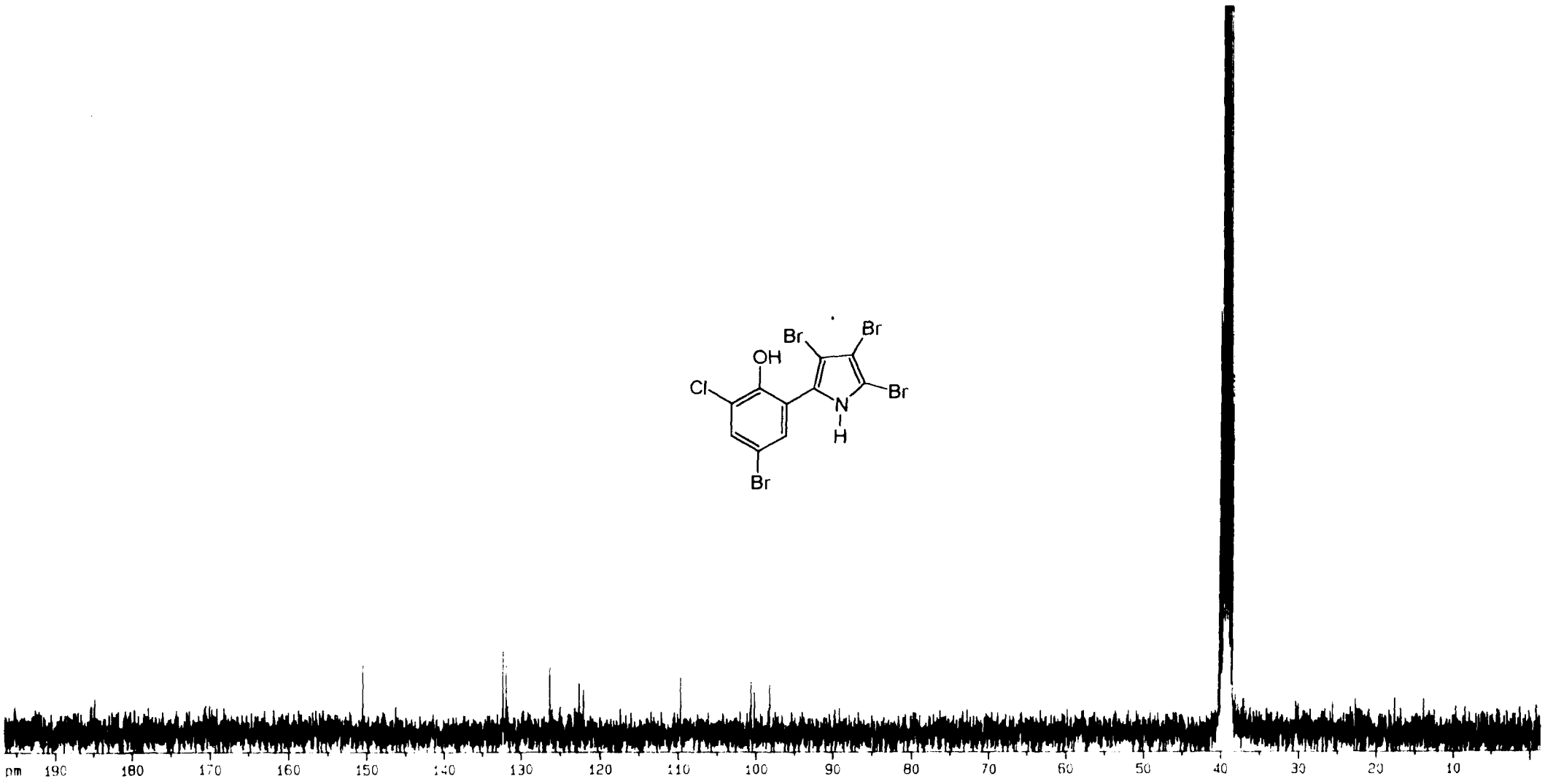


Fig. 5.11 : ^{13}C NMR spectrum of 3′-chlorotetrabromopseudilin(27) in DMSO-d_6

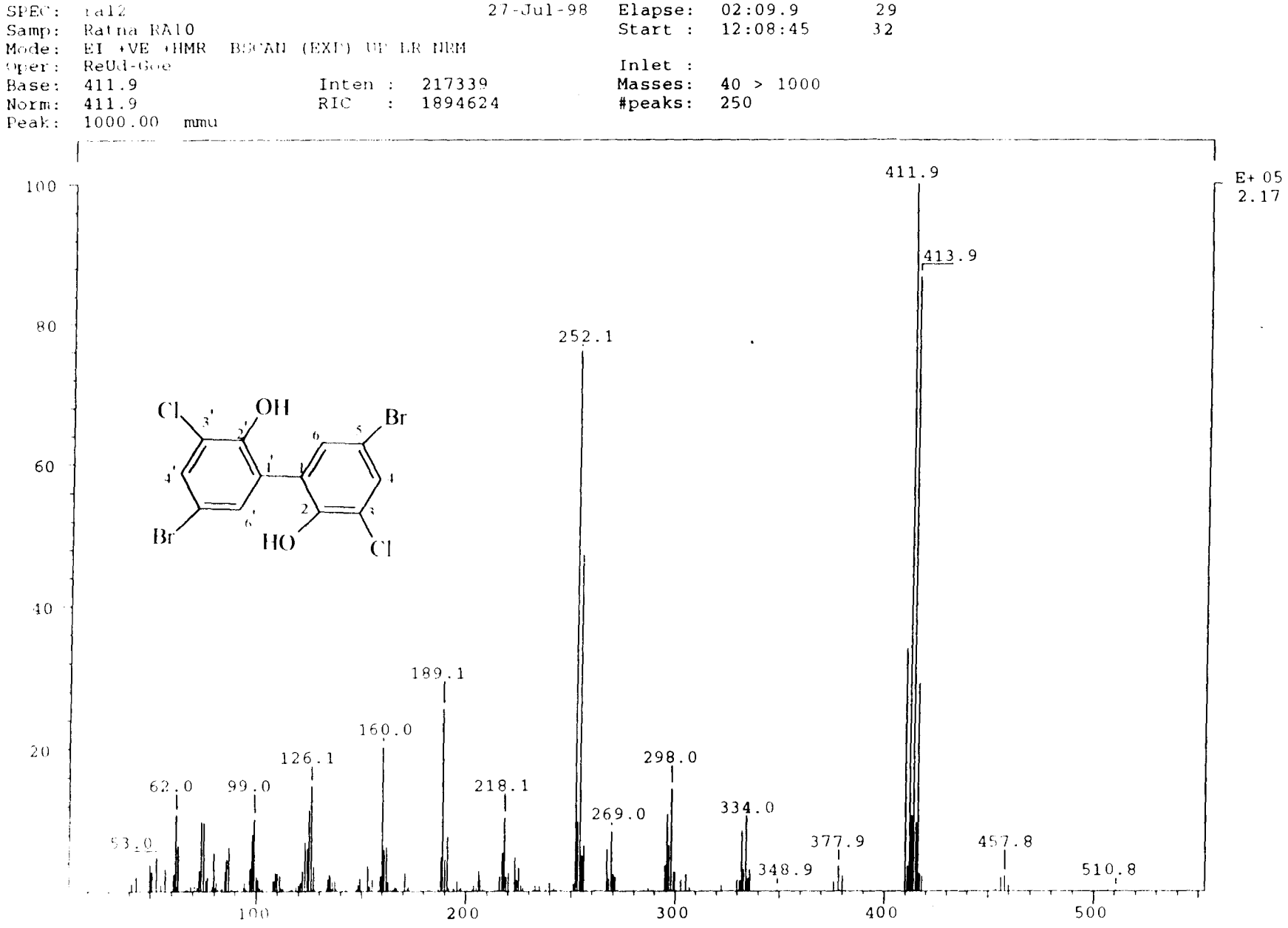


Fig. 5.12 : EI mass spectrum of compound 28

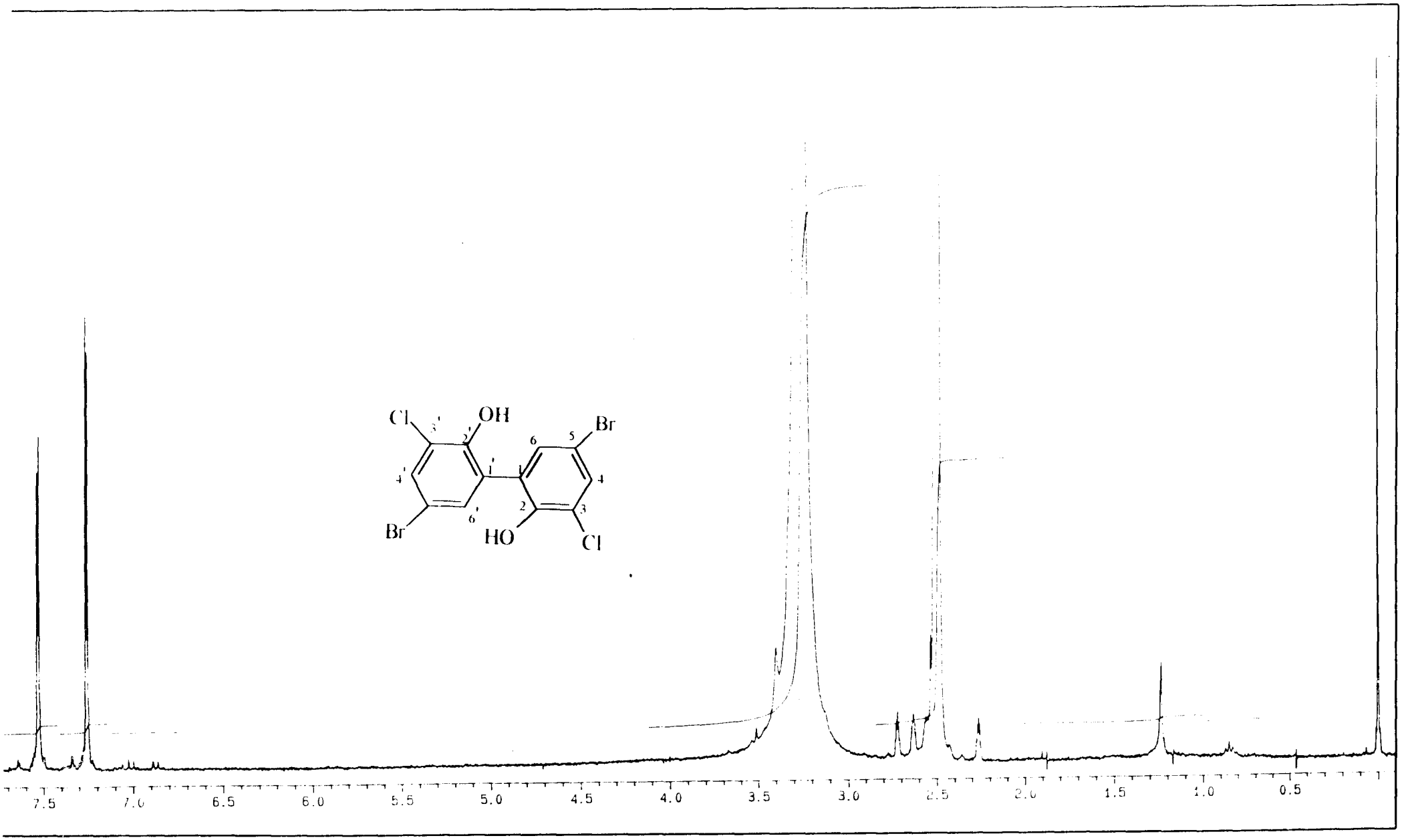


Fig. 5.13 : ^{1}H NMR spectrum of compound 28 in DMSO-d_6

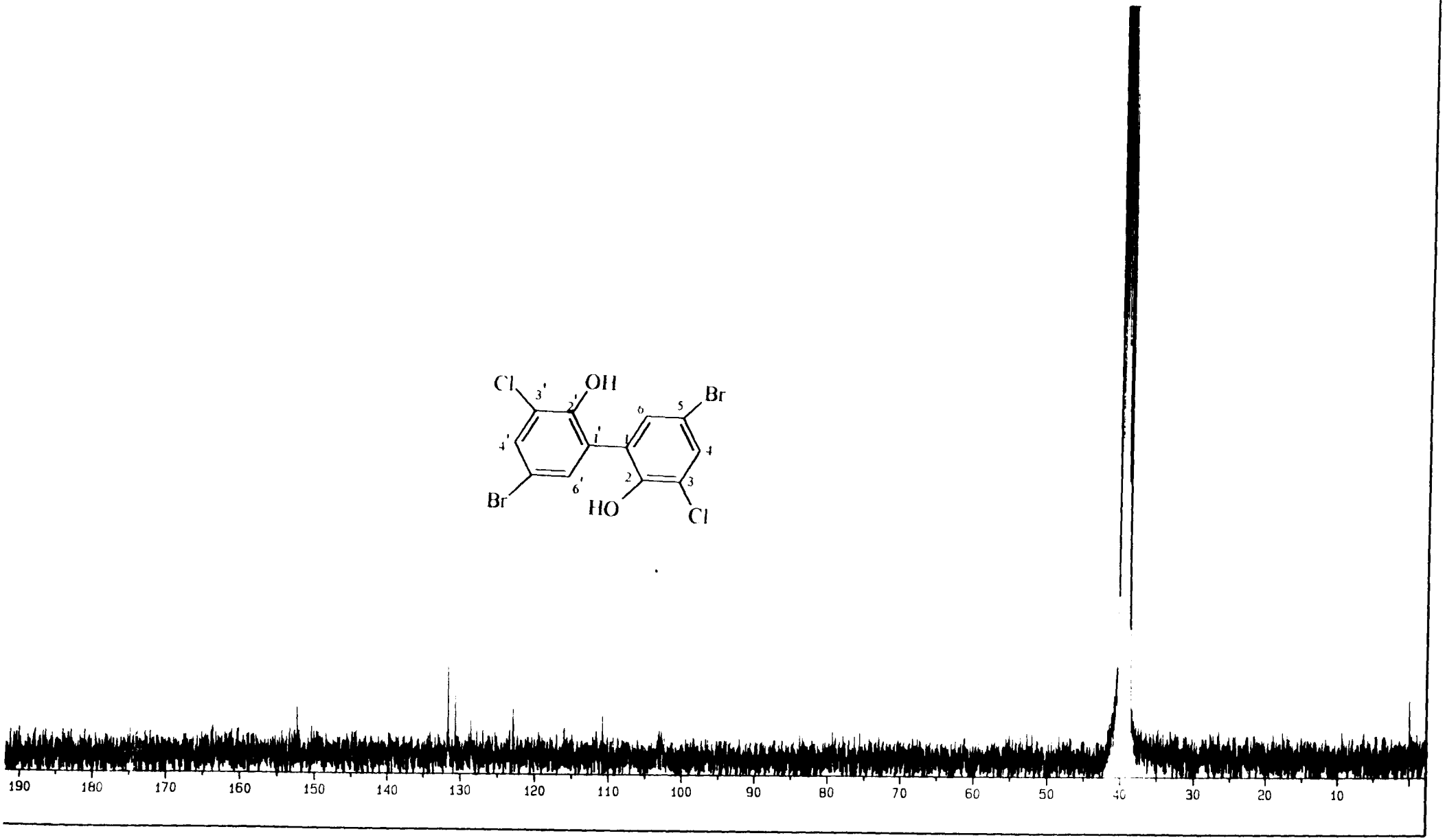


Fig. 5.14 : ^{13}C NMR spectrum of compound 28 in DMSO-d_6

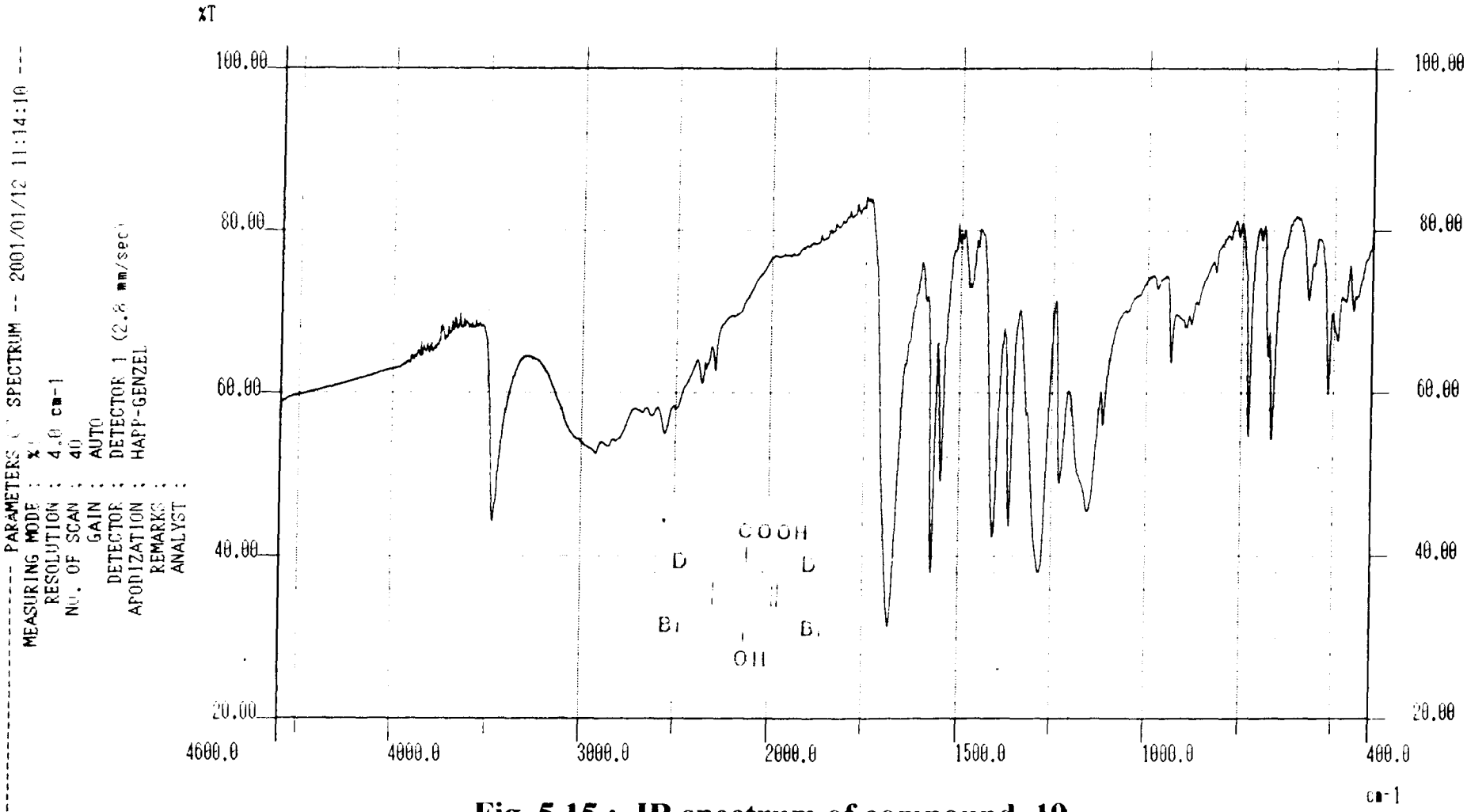


Fig. 5.15 : IR spectrum of compound 19

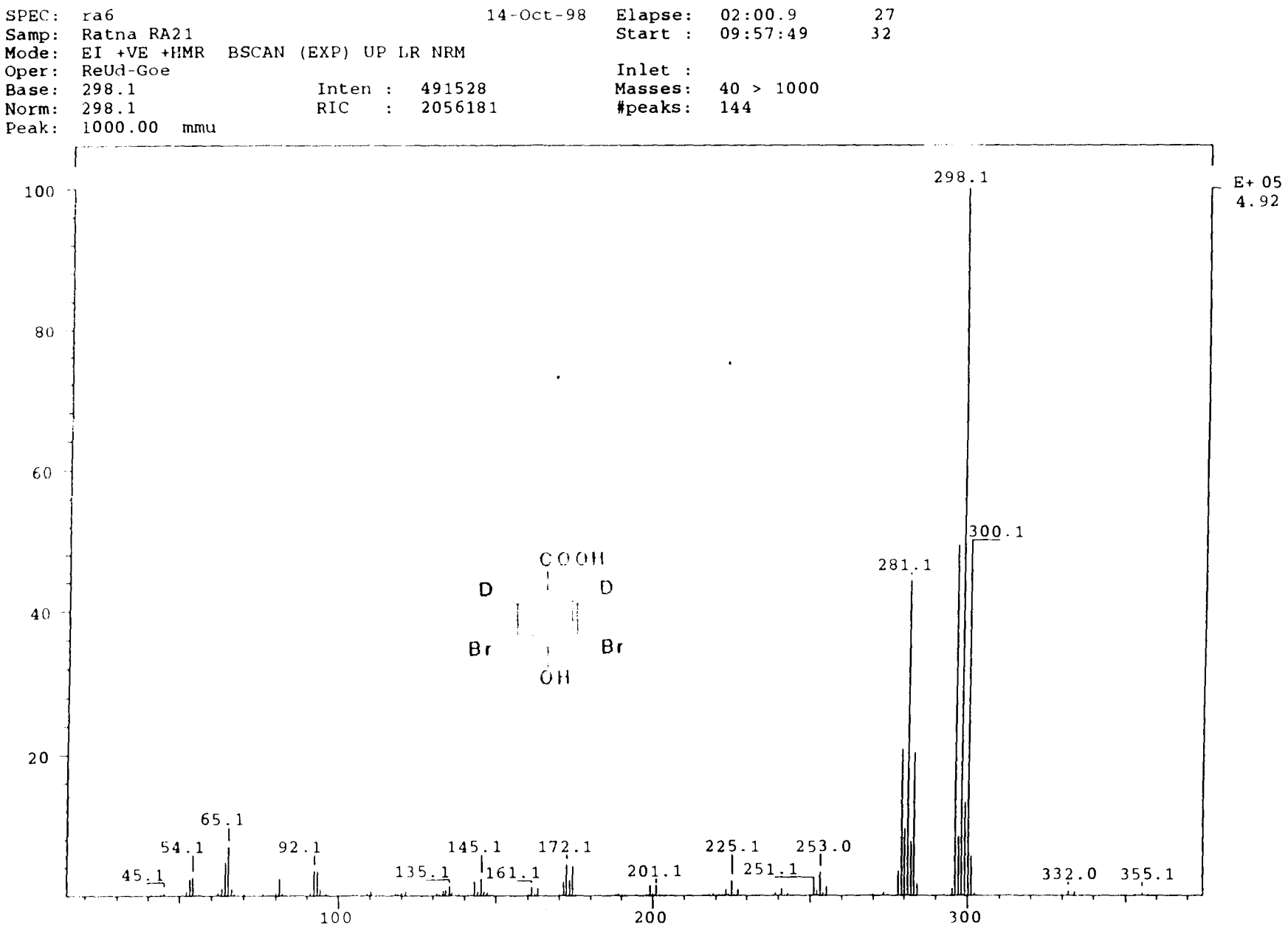


Fig. 5.16 : EI mass spectrum of compound 19

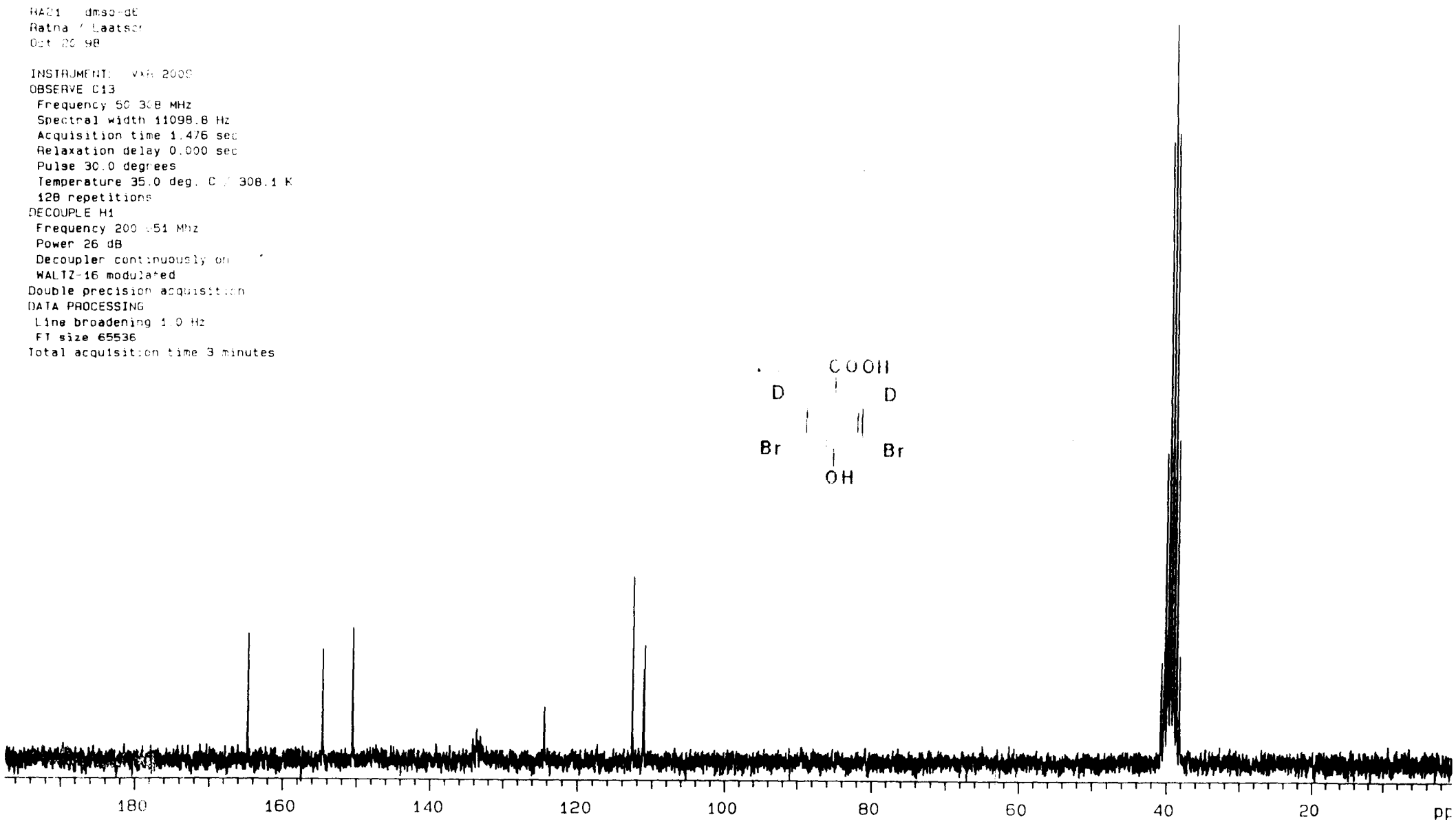


Fig. 5.17 : ^{13}C NMR spectrum of compound 19 in DMSO-d_6

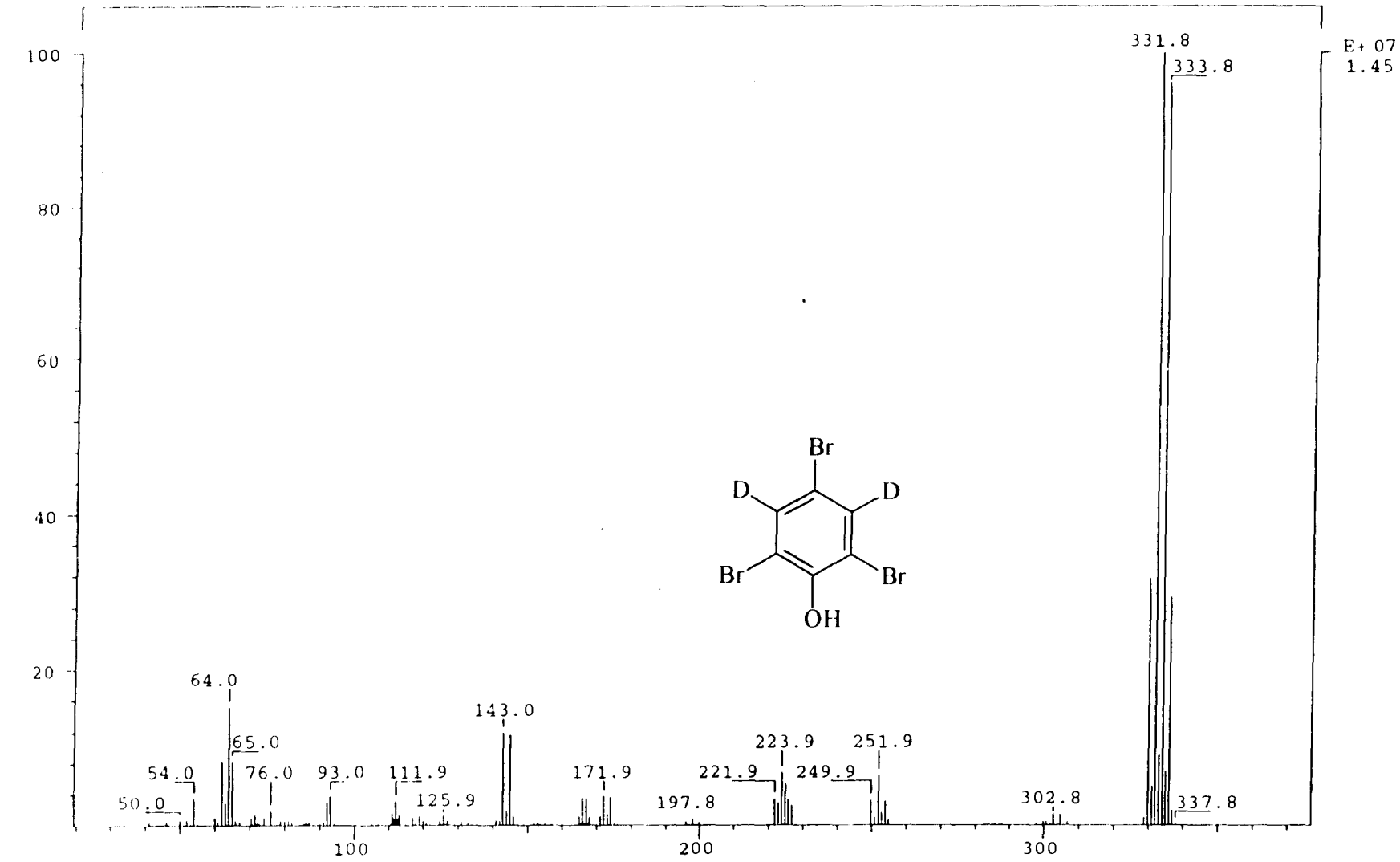


Fig. 5.18 : EI mass spectrum of compound 32

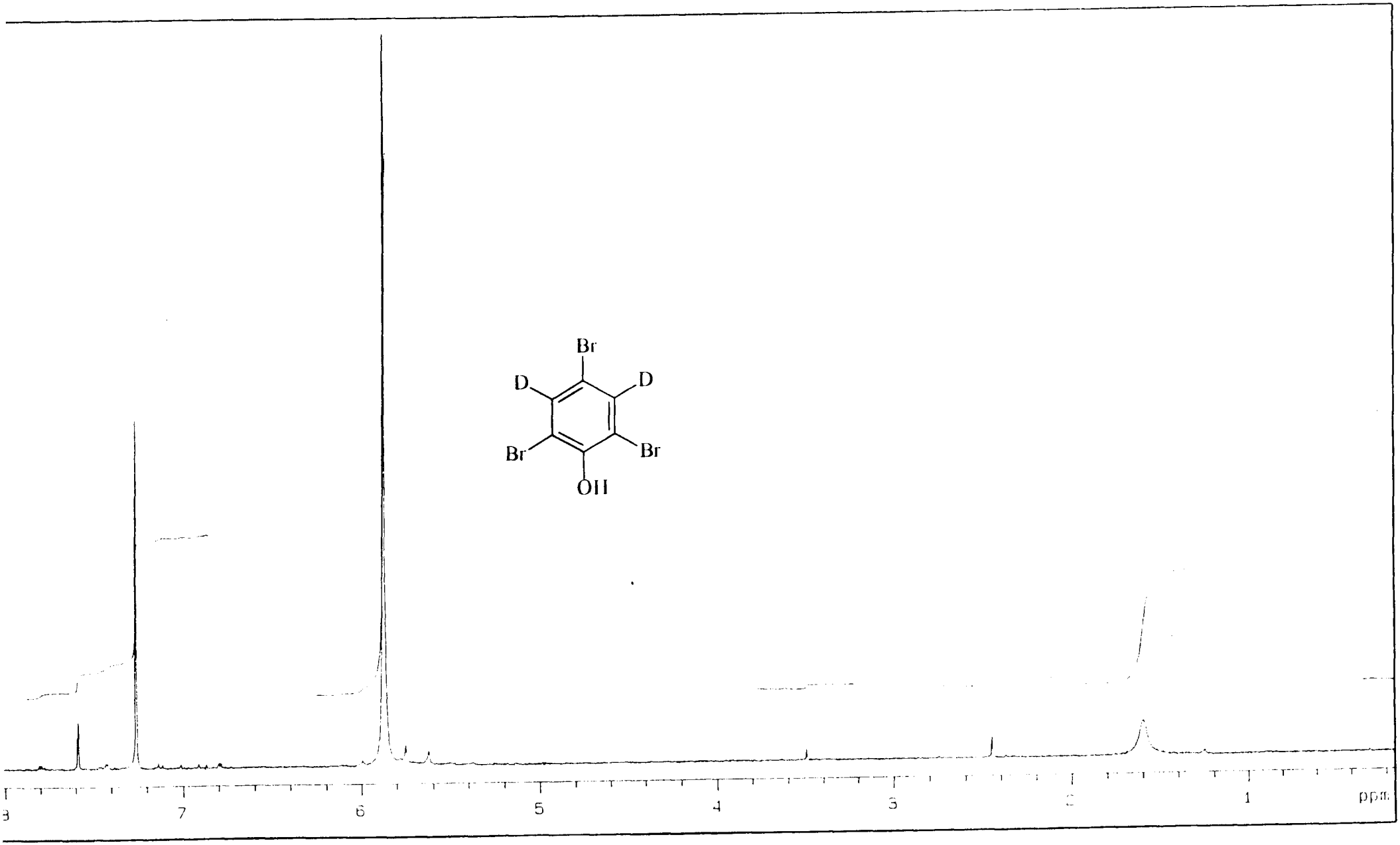


Fig. 5.19 : ^{1}H NMR spectrum of compound 32 in $CDCl_3$

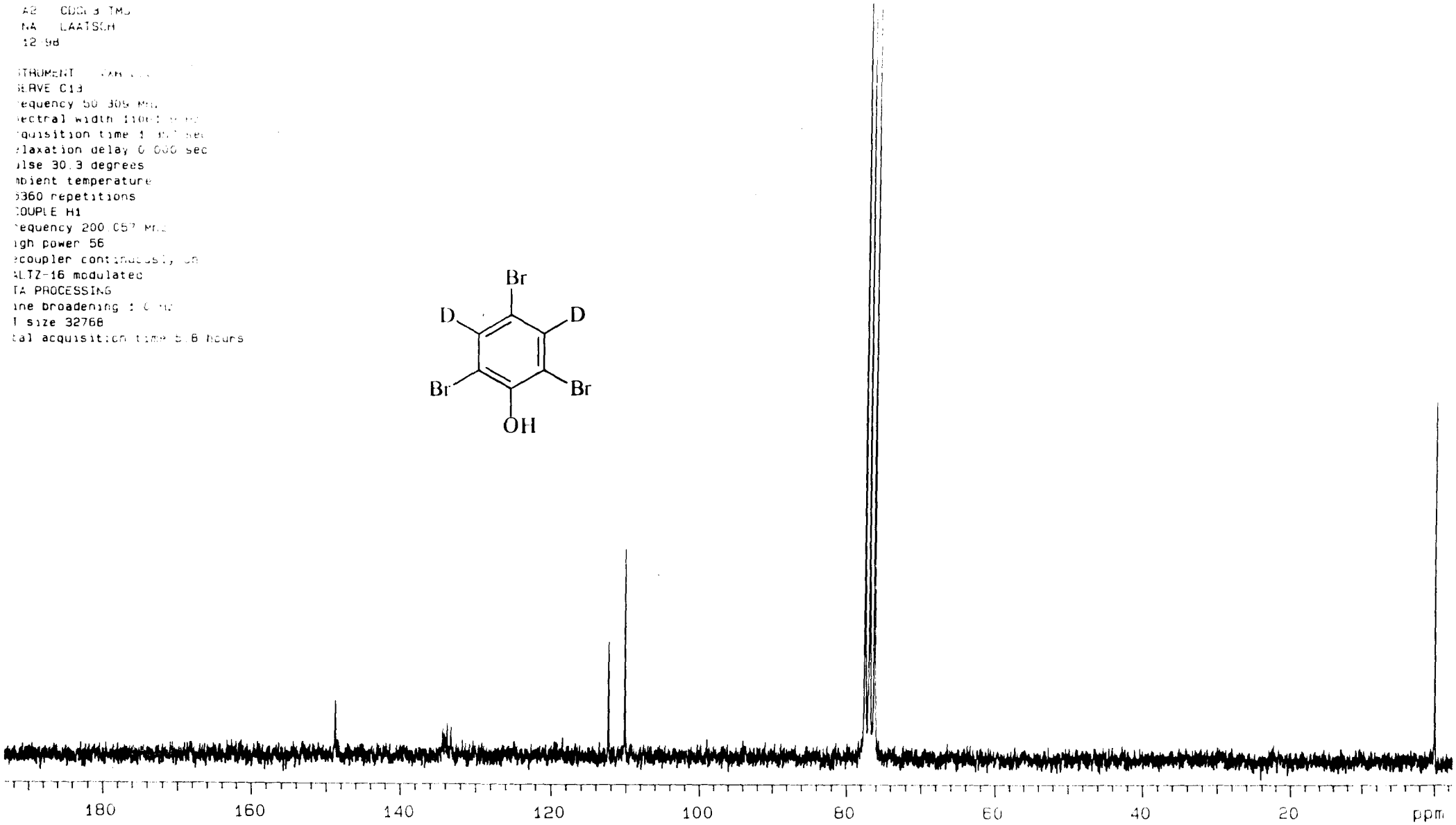


Fig. 5.20 : ^{13}C NMR spectrum of compound 32 in $CDCl_3$

EXPERIMENTAL PART

Microbiologic Materials/ Equipments:

Fermenter: 20 l fermenter by Meredos GmbH (Göttingen, Germany) containing: 20 l culture container with magnetic propeller stirrer and cooling loop (with thermostat); control desk with pH–controller, antifoam controller.

Shaker: Infors AG (CH 4103 Einsbach) type ITE.

Autoclave: Fa. Küster GmbH, Stuttgart. Broth was autoclaved for 33 min at 125 °C. For 20 l fermenter: VST 40 / 60, Fa. Zirbus, Osterode. Broth was autoclaved for 82 min at 125 °C.

Antibiotica–Assay–Disc: ∅ = 9 mm, Schleicher & Schüll n° 321, 261.

Culture Medium: Glucose (E. Merck, Darmstadt), yeast extract and malt extract from Difco, (Henselwerk GmbH, Magstadt; RdH GmbH & Co., Seelze).

Antifoam Solution: Niax PPG 2025; Union Carbide Belgium N. V. (Zwijndrecht).

Celite: Celite france S. A., Rueil-Malmaison Cedex.

Formulations:

Glucose-Glycin Medium for Strain 1893

D-Glucose	5.0	g
Glycin	1.5	g
L-Tyrosine	0.5	g
KBr	1.0	g

The above components were dissolved in 750 ml of synthetic sea water and 250 ml of tap water and the pH of the medium was set to 8 by the addition of 2N NaOH before autoclaving.

Amino Acid Medium for Strain 1893

L- Tyrosine	0.37	g
L-Histidine-Hydrochloride	0.37	g
L-Ornithine-Hydrochloride	0.12	g
Glycin	0.41	g
L- Prolin	0.05	g

KBr	1.00	g

The above components were dissolved in 750 ml of synthetic sea water and 250 ml of tap water and the pH of the medium was set to 8 by the addition of 2N NaOH before autoclaving.

M_1^+ Medium for Shaker-Culture

Peptone (E. Merck)	5.0	g
Hefeextract (E. Merck)	1.0	g
KBr	1.0	g

The above components were dissolved in 750 ml of synthetic sea water and 250 ml of tap water and the pH of the medium was set to 8 with the addition of 2N NaOH before autoclaving.

M_1^+–Agar

Peptone (E. Merck)	5.0	g
Hefeextract (E. Merck)	1.0	g
Agar (E. Merck)	18.0	g

The above components were dissolved in 750 ml of synthetic sea water and 250 ml of tap water and the mixture was stirred for 30 min at room temp. and then heated at 50-60 °C in a micro-wave oven for 30 min. Prior to autoclaving, the pH of the solution was set to 8 by the addition of 2N NaOH. After autoclaving, in sterile condition, it was transferred to the petri-dishes by keeping the thickness of the agar (in the petri-dishes) to around 1 cm and after cooling to room temperature, the agar plates were kept in cold room which was maintained at 4 °C.

M_2^+–Agar

Malz-extract (E. Merck)	10.0	g
D-Glucose (E. Merck)	4.0	g
Hefeextract (E. Merck)	4.0	g
Agar (E. Merck)	18.0	g

The above components were dissolved in 500 ml each of synthetic sea water and tap water and the mixture was stirred for 30 min at room temp. and then heated at 50-60 °C in a micro-wave oven for 30 min. Prior to autoclaving, the pH of the solution

was set to 8 by addition of 2N NaOH. After autoclaving, in sterile condition, it was transferred to the petri-dishes by keeping the thickness of the agar (in the petri-dishes) to around 1 cm and after cooling to room temperature, the agar plates are kept in cold room which was maintained at 4 °C.

General Procedure for the feeding experiment:

Method A:

2 litres of fermentation medium distributed in ten 1000 ml flasks (200 ml in each flask) were autoclaved for 33 min at 121 °C. The flasks were inoculated under sterile conditions with five agar petri-dishes (M_1^+-Agar) of one day old pre-cultures of *Alteromonas luteoviolaceus* (transfered by washing every petri-dish with 5-7 ml of sterile solution of 60-80 % synthetic sea water). The cultures were shaken at 95 rpm and 24 °C on shaker and after 24, 36 and 48 h, the appropriate substances were fed to the cultures under sterile conditions.

The cultures were harvested after 72 h by extraction with ethyl acetate, until the ethyl acetate phase remained colourless. The organic phases were combined and evaporated *in vacuo* at room temperature. The crude extract thus obtained was further purified by using chromatography on silica gel, Sephadex LH 20, PTLC or HPLC.

Method B:

2 litres of fermentation medium distributed in ten 1000 ml flasks (200 ml in each flask) were autoclaved for 33 min at 121 °C. The flasks were inoculated under sterile conditions with five agar petri-dishes (M_1^+-Agar) of one day old pre-cultures of *Alteromonas luteoviolaceus* (by transfering 2-4 cm^2 of agar from Petri-dish). The cultures were shaken at 95 rpm and 24 °C on shaker, after 24, 36 and 48 h, the appropriate substances were fed to the cultures under sterile condition. After 72 h, these cultures were harvested as described above, under Method A.

All the fractions so collected were concentrated and the residue obtained upon purification by chromatography on a 20 g Sephadex LH 20 (column : 50 x 1 cm, chloroform / methanol 6 : 4), or PTLC / HPLC to furnished the following compounds.

Feeding Experiment with Tetrachloropyrrole in Glucose-Glycin Medium:

To a well grown 2 liter culture in glucose-glycin medium, 90 mg of tetrachloropyrrole (**17**) was fed as per the method A. The pH of the harvested broth was found to be 8.5. The silica gel column chromatography of the crude extract (250 mg) gave 5 fractions which were found to contain the following compounds.

2,6-dibromophenol (43)

The fraction 1 upon purification provided 2,6-dibromophenol (**43**), 4 mg, m.p 56 ^{0}C (lit.[26] m.p. 56-57 ^{0}C).

EIMS (70 eV): *m/z* (%) = 250, 252, 254 (M^+ 42, 100, 40), 109, 111 (16, 14), 95, 97 (M^+- Br, -OH, 22, 30), 91 (M^+ - 2Br, 38), 55, 57 (62, 66) and 41, 43 (50, 58).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.45 (d, J = 8.2 Hz, 2 H, 3-H, 5-H), 6.7 (t, J = 8.2 Hz, 1 H, 4-H) and 5.85 (s, exchangeable with D_2O, 1 H, OH).

Identification of 2,4,6-tribromophenol (26) and pentabrompseudilin (1)

Fraction 2 upon purification gave two compounds *viz* (**26**) (4.5 mg) and (**1**) (35 mg).

2,4,6-tribromophenol (26)

Obtained as colourless solid, m.p. 103 ^{0}C.

EIMS (70 eV): *m/z* (%) = 328, 330, 332, 334 (M^+ 34, 100, 98, 30), 248, 250, 252 (M^+- HBr, 2, 6, 4, 2) and 170,172 (M^+-2 Br, 4, 4).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.59 (s, 2 H, 3-H, 5-H) and 5.5 (s br, exchangeable with D_2O, 1 H, OH).

Pentabrompseudilin (1)

Obtained as crystalline solid, 2.6 mg, m.p 178 ^{0}C (lit.[4,5] m.p.178-179 ^{0}C).

IR (KBr): ν (fig. 5.01) = 3460, 3420, 3065, 1593, 1537, 1460, 1430, 1343, 1320, 1230, 1155, 1125, 993, 857, 748, 688 and 506 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 5.02) = 550.6, 552.6, 554.6, 558.6 (M^+ 40, 82, 80, 38), 471.7, 473.7, 475.7, 477.7 (M^+ 64, 100, 60, 18), 390.7, 392.7, 394.7, 396.7 (M^+ 18, 40, 44, 20).

^{1}H NMR ($CDCl_3$, 300 MHz): δ **(fig. 5.03)** = 9.5 (s br, exchangeable with D_2O, 1 H, OH), 8.12 (d, J = 2.4 Hz, 1 H, 4'-H), 7.58 (d, J = 2.4 Hz, 1 H, 6'-H) and 6.09 (s br, exchangeable with D_2O, 1 H, NH).

^{1}H NMR (DMSO-d_6, 200 MHz): δ = 12.51 (s, exchangeable with D_2O, 1 H, OH), 9.74 (s, exchangeable with D_2O, 1 H, NH), 7.78 (d, J = 2.2 Hz, 1 H, 4′-H) and 7.38 (d, J = 2.2 Hz, 1 H, 6′-H).

^{13}C NMR (DMSO-d_6, 75.5 MHz): δ (fig. 5.04) = 151.9 (1C, $C_{quat.}$), 135.6 (1C, CH), 133.5 (1C, CH), 127 (1C, $C_{quat.}$), 122.6 (1C, $C_{quat.}$), 113.2 (1C, $C_{quat.}$), 111.3 (1C, $C_{quat.}$), 101.3 (1C, $C_{quat.}$), 101.1 (1C, $C_{quat.}$) and 99.1 (1C, $C_{quat.}$).

Similarly, fraction 3 also on purification provided the pentabromopseudilin (**1**), 35 mg.

p-Hydroxybenzaldehyde (6)

The fraction 4 provided *p*-hydroxybenzaldehyde (**6**), 27 mg, m.p. 115 ^{0}C (lit.[27] m.p. 115 - 116 ^{0}C).

IR (KBr): ν = 3210, 1670, 1600, 1515, 1455, 1395, 1315, 1290, 1240, 1215, 1160, 1110, 835, 770, 710 and 600 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 122 (M^+, 88), 121 (M^+- H, 100), 94 (M^+- CO, 4), 93 (M^+- CHO, 36) and 65 (24).

^{1}H-NMR (acetone-d_6, 300 MHz): δ = 9.88 (s, 1 H, CHO), 9.45 (s br, exchangeable with D_2O, 1 H, OH), 7.8 (d, J = 8.2 Hz, 2 H, 2-H, 6-H) and 7.0 (d, J = 8.2 Hz, 2 H, 3-H, 5-H).

3,3′,5,5′-Tetrabromo-2,2′-dihydroxybiphenyl (24)

Fraction 5 furnished 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**), 4.3 mg as a crystalline solid, m.p.205 ^{0}C (lit.[28] m.p. 205-207 ^{0}C).

IR (KBr): ν (fig. 5.05) = 3410, 3067 1623, 1551, 1454, 1390, 1316, 1227, 1144, 1055, 857, 734, 683 and 554 cm^{-1}.

EIMS (70 eV): *m/z* (%) (fig. 5.06) = 497.9, 499.9, 501.9, 503.9, 505.9 (M^+, 18, 60, 96, 58, 16), 418, 420, 422, 424 (M^+- HBr, 2, 6, 8, 5) and 340, 342, 344 (M^+- 2Br, 50, 100, 48).

^{1}H NMR (DMSO-d_6, 300 MHz): δ (fig. 5.07) = 7.75 (d, J = 2.4 Hz, 2 H, 4-H, 4-H′) and 7.3 (d, J = 2.4 Hz, 2 H, 6-H, 6-H′).

^{13}C NMR (DMSO-d_6, 75.5 MHz): δ (fig. 5.08) = 151.1 (2C, $C_{quat.}$), 134.1 (2C, CH), 132.8 (2C, CH), 128.0 (2C, $C_{quat.}$), 112.6 (2C, $C_{quat.}$) and 110.4 (2C, $C_{quat.}$).

Feeding Experiment with Tetrachloropyrrole in Amino Acid Medium

To a 2 liter culture in amino acid medium, 85 mg of tetrachloropyrrole (**17**) was fed by using the method B and the well grown cultures were harvested after 72 h. After the usual work-up, followed by chromatographic purification lead to the isolation of four pure compounds namely, 2,4-dibromophenol (**25**), recovered tetrachloropyrrole (**17**) (20 mg), pentabrompseudilin (**1**) (15 mg) and 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**) (13 mg).

2,4-dibromophenol (25)

Obtained as a crystalline solid, 5.2 mg, m.p.39 ^{0}C (lit.[26] m.p.40 ^{0}C).

EIMS (70 eV): *m/z* (%) = 250, 252, 254 (M^+ 42, 100, 40), 170,172 (M^+- HBr, 10, 12) and 92 (M^+- 2Br, 42).

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.60 (d, J = 2.5 Hz, 1 H, 3-H), 7.34 (dd, J = 7.5 Hz, J = 2.5 Hz, 1 H, 5-H), 6.9 (d, J = 7.5 Hz, 1 H, 6-H) and 5.5 (s, exchangeable with D_2O, 1 H, OH).

Feeding Experiment with Tetrachloropyrrole in Glucose-Glycin medium with excess of *p*-hydroxybenzoic acid

To a well grown 2 liter culture in glucose-glycin medium containing 150 mg of *p*-hydroxybenzoic acid (**15**) 100 mg of tetrachloropyrrole (**17**) was fed as per the procedure described under method A. The pH of the broth was found to be 9.0 when harvested. The silica gel column chromatography of the crude extract (220 mg) gave 5 fractions. Fraction 1 to 4 on purification gave pentabrompseudilin (**1**) (4.3 mg), recovered tetrachloropyrrole (**17**) (13.5 mg), *p*-hydroxy-benzaldehyde (**6**) (15 mg) and 3,3'5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**) (2.5 mg), while fraction 5 on purification gave 4-hydroxybenzyl alcohol (**41**) (7.0 mg).

4-hydroxybenzyl alcohol (41)

Obtained as a crystalline solid, m.p.125 ^{0}C, (lit.[26] m.p. 124.5-125.5 ^{0}C).

EIMS (70 eV): *m/z* (%) = 124 (M^+ 100), 123 (M^+- H, 52), 107 (M^+- H, H_2O, 36), 106 (M^+- H_2O, 20), 95 (68), 77 (58) and 67, 65 (M^+ - OH, - CH_2OH, 10, 12).

^{1}H-NMR (acetone-d_6, 300 MHz): δ = 8.25 (s, exchangeable with D_2O, 1 H, OH), 7.18 (d, J = 8.4 Hz, 2 H, H-2, H-6), 6.8 (d, J = 8.4 Hz, 2 H, H-3, H-5), 4.5 (d, J = 3 Hz, 2 H, 7-H_2) and 3.98 (t, exchangeable with D_2O, J = 3 Hz, 1 H, OH).

^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 9.14 (s, exchangeable with D_2O, 1 H, OH), 7.12 (d, J = 8.4 Hz, 2 H, H-2, H-6), 6.68 (d, J = 8.4 Hz, 2 H, H-3, H-5), 4.34 (d, J = 3 Hz, 2 H, 7-H_2) and 4.84 (t, exchangeable with D_2O, J = 3 Hz, 1 H, OH).

Feeding experiment with 3-Chloro-4-hydroxybenzoic acid

To a 1.6 litre culture in M_1^+-medium, 200 mg of 3-chloro-4-hydroxybenzoic acid (**20**) was added as per the procedure described under method A. The well grown cultures with pH = 9.0 were worked-up and chromatographed on a silica gel column. The whole elutant was collected into 3 fractions, to afford the following compounds.

3'-chlorotetrabrompseudilin (27)

The first fraction provided 3'-chlorotetrabrompseudilin (**27**), as a crystalline soild, 6.5 mg.

EIMS (70 eV): *m/z* (%) (fig. 5.09) = 504.7, 506.7, 508.7, 510.7, 512.7, 514.7 (M^+12, 60, 100, 82, 30, 6), 425.7, 427.7, 429.7, 431.7, 433.7 (M^+- Br, 20, 80, 96, 46, 10), 398.7, 400.7, 402.7, 404.7, 406.7 (M^+- Br- HCN, 12, 38, 42, 20, 4), 346.8, 348.8, 350.8, 352.8 (M^+- 2 Br, 30, 74, 50, 10), 267.9, 269.9, 271.9 (M^+- 3 Br, 12, 20, 8), 214.9 (10), 174.4 (10), 124.9 (10) and 98 (10).

^{1}HNMR (DMSO-d_6, 300 MHz): δ **(fig. 5.10)** = 12.54 (s br, exchangeable with D_2O, 1 H, OH), 9.86 (s br, exchangeable with D_2O, 1 H, NH), 7.68 (s br, 1 H, 4'-H) and 7.36 (s br, 1 H, 6'-H).

^{13}CNMR (DMSO-d_6, 75.5 MHz): δ **(fig. 5.11)** = 150.6 (1C, $C_{quat.}$), 132.4 (1C, CH), 131.9 (1C, CH), 126.4 (1C, $C_{quat.}$), 122.7 (1C, $C_{quat.}$), 122.2 (1C, $C_{quat.}$), 109.7 (1C, $C_{quat.}$), 100.8 (1C, $C_{quat.}$), 100.3 (1C, $C_{quat.}$) and 98.2 (1C, $C_{quat.}$).

5,5'-dibromo-3,3'-dichloro-2,2'-dihydroxybiphenyl (28)

Fraction 2 upon purification gave 5,5'-dibromo-3,3'-dichloro-2,2'-dihydroxybiphenyl (**28**), 2.5 mg.

EIMS (70 eV): *m/z* (%)(fig. 5.12) = 409.9, 411.9, 413.9, 415.9, 417.9 (M^+ 36, 100, 88, 30, 4), 298 (16), 254, 256, 258 (M^+- 2 Br, 78, 48, 8), 189 (26), 160 (22), 126 (16) and 99 (11).

^{1}HNMR (DMSO-d_6, 300 MHz): δ **(fig. 5.13)** = 7.52 (d, J = 2.4 Hz, 1 H, 4-H) and 7.25 (d, J = 2.4 Hz, 1 H, 6-H).

^{13}CNMR (DMSO-d_6, 75.5 MHz): δ **(fig. 5.14)** = 152.2 (2C, $C_{quat.}$), 131.7 (2C, CH), 130.7 (2C, $C_{quat.}$), 128.6 (2C, $C_{quat.}$), 123.7 (2C, $C_{quat.}$) and 110.7 (2C, $C_{quat.}$).

Indole-3-carbaldehyde (7)

Fraction 3 on purification using PTLC (chloroform / methanol 22 : 3) gave indole-3-carbaldehyde (**7**), 12 mg as crystalline light orange coloured solid, m.p.197 °C (lit.[29] m.p.197-198 °C).

IR (KBr): ν = 3180, 2920, 1635, 1620, 1575, 1520, 1500, 1445, 1395, 1335, 1290, 1245, 1120, 1080, 1040, 790, 760 and 640 cm^{-1}.

EIMS (70 eV): ***m/z*** **(%)** = 145.1 (M^+, 85), 144.1 (M^+- H, 100), 116.1 (M^+- CHO, 36), 89.1 (M^+-CHO, HCN, 50) and 63 (26).

^{1}H NMR ([D_6]DMSO, 300 MHz): δ = 12.09 (s br, exchangeable with D_2O, 1 H, NH), 9.95 (s, 1 H, CHO), 8.26 (s, 1 H, 2-H), 8.13 (dd., *J* = 6.8 Hz, *J* = 2.4 Hz, 1 H 7-H), 7.52 (dd., *J* = 6.8 Hz, *J* = 2.4 Hz, 1 H 4-H) and 7.25 (m, 2 H, 5-H, 6-H).

^{13}C NMR ([D_6]DMSO, 75.5 MHz): δ = 184.8 (1C, CH), 138.2 (1C, CH), 136.9 (1C, $C_{quat.}$), 124.1 (1C, $C_{quat.}$), 123.3 (1C, CH), 121.9 (1C, CH), 120.7 (1C, CH), 118.1 (1C, $C_{quat.}$) and 112.3 (1C, CH).

Feeding experiment with 2-Chlorophenol in M_1^+-Medium

To a 2 litre culture in M_1^+-medium, 120 mg of 2-chlorophenol (**22**) was fed by using the method A and the well grown cultures with pH = 8.5 were worked-up and chromatographed on a silica gel column. The whole elutant was collected into 4 fractions and the same provided the following compounds.

1,8-dihydroxyanthraquinone (29)

Fraction 1 on purification furnished 1,8-dihydroxyanthraquinone (**29**), 30 mg as a dark yellow coloured solid, m.p.192.5 °C (lit.[26] m.p. 192 °C).

EIMS (70 eV): ***m/z*** **(%)** = 240.1 (M^+ 100), 223.1 (4), 212.1 (8), 184.1 (8), 138.1 (8) and 92.1 (4).

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 12.1 (s br, exchangeable with D_2O, 2 H, OH), 7.86 (dd, *J* = 8.4 Hz, *J* = 2.4 Hz, 2 H, 4-H, 5-H), 7.72 (t *J* = 8.4 Hz, 2-H, 3-H, 6-H) and 7.3 (dd., *J* = 8.4 Hz, *J* = 2.4 Hz, 2 H, 2-H, 7-H).

^{13}C NMR ($CDCl_3$, 50.309 MHz): δ = 193 (1C, $C_{quat.}$), 181.7 (1C, $C_{quat.}$), 162.5 (2C, $C_{quat.}$)137.2 (2C, CH), 133.6 (2C, $C_{quat.}$), 124.6 (2C, CH), 120 (2C, CH) and 115.8 (2C, $C_{quat.}$).

Fraction 2 was separated into two fraction A and B using PTLC and then the two subfraction were purified separately using Sephadex LH 20 column to give 4-bromo-2-chlorophenol (**30**) and 4,6-dibromo-2-chlorophenol (**23**).

4-bromo-2-chiorophenol (30)

Obtained as a colourless solid, 2.0 mg.

EIMS (70 eV): ***m/z*** **(%)** = 206, 208, 210 (M^+ 78, 100, 22), 170 (M^+- Cl, 4), 142, 144 (4, 4), 127.1 (M^+- Br, 4) and 99 (6).

^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.44 (d, J = 2.2 Hz, 1 H, 3-H), 7.28 (dd., J = 7.5 Hz, J = 2.2 Hz, 1 H, 5-H), 6.9 (d, J = 7.5 Hz, 1 H, 6-H) and 5.5 (s, exchangeable with D_2O, 1 H, OH).

4,6-dibromo-2-chlorophenol (23)

Obtained as a crystalline solid, 4.0 mg , m.p.105 °C.

IR (KBr): ν = 3500, 3000, 1560, 1465, 1385, 1315, 1265, 1235, 1150, 850 and 765 cm^{-1}.

EIMS (70 eV): ***m/z*** **(%)** = 284, 286, 288, 290 (M^+ 44, 100, 68, 16).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 7.55 (d, J = 2.5 Hz, 1 H, 3-H), 7.45 (d, J = 2.5 Hz, 1 H, 5-H) and 5.89 (s, exchangeable with D_2O, 1 H, OH).

Fraction 3 on purification provided pentabrompseudilin (**1**), 4.0 mg.

Fraction 4 on purification gave 3,3′5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**), 2.8 mg.

Feeding experiment with 2-Chlorophenol in excess of prolin in amino acid medium

This feeding experiment was carried out in amino acid medium with excess of proline (300 mg). After usual worked up, followed by silica gel chromatography, 5 fractions were obtained, which on further purification by using Sephadex LH 20 or PTLC give 1,8-dihydroxyanthraquinone (**29**) (10 mg), 4-bromo-2-chlorophenol (**30**) (1.8 mg), pentabromopseudilin (**1**) (3.2 mg) and 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (2.0 mg).

Feeding experiment with 4,6-Dibromo-2-chlorophenol (23) in M_1^+ medium

To a 2 litre culture in M_1^+-medium, 120 mg of 4.6-dibromo-2-chlorophenol (**23**) was fed as per the procedure described under method A. The well grown cultures with pH = 9.0 were worked-up and chromatographed on a silica gel column. The whole elutant was collected into 4 fractions. The purification of these fractions yielded, 4,6-dibromo-2-chlorophenol (**23**) (30 mg), 1,8-dihydroxyanthraquinone (**29**) (5.2 mg), *p*-hydroxybenzaldehyde (**6**) (2.5 mg), 3,3',5,5'-tetrabromo-2,2'-dihydroxybiphenyl (**24**) (1.3 mg).

Feeding experiment with 4,6-dibromo-2-chlorophenol (49) in amino acid medium

In this feeding experiment, after usual worked up and purification of the fractions thus obtained gave 1,8-dihydroxyanthraquinone (**29**) (10 mg), 4,6-dibromo-2-chlorophenol (**23**) (25 mg) and *p*-hydroxybenzaldehyde (**6**) (1.8 mg).

Feeding experiment with 3,5-Dibromo-2,6-dideutero-4-hydroxybenzoic acid

a) In M_1^+ medium

To a well grown 2 litres culture of *A. luteo-violaceus* in M_1^+ medium, 120 mg of 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) was fed as per the procedure described under method B. The pH of the broth was found to be 9.0. The silica gel column chromatography of the crude extract (258 mg) gave 6 fractions containing the following compounds.

14-methylpentadecanoic acid (31)

Fraction 1 on purification gave 14-methylpentadecanoic acid (**31**) as a crystalline solid, 42 mg.

EIMS (70 eV): *m/z* (%) = 256 (M^+100), 213 (M^+- C_3H_7, 15), 185 (10), 129 (18) and 73 (25).

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 2.52 (t, 2 H, 2-H_2), 1.6 (t, 2 H, 12-H_2), 1.5 (m, 1 H, 14-H), 1.3 (s br, 18 H, 9 x-CH_2), 1.08 (t, 2 H, 11-H) and 0.92 (d, 6 H, 13-CH_3, 14-CH_3).

2,4,6-tribromo-3,5-dideutero-phenol (32)

Fraction 2 on purification 2,4,6-tribromo-3,5-dideutero-phenol (**32**), 30 mg as crystalline solid.

EIMS (70 eV): *m/z* (%) (fig. 5.18) = 329.9, 331.9, 333.9, 335.9 (M^+ 30, 100, 94, 24), 249.9, 251.9, 253.9 (M^+- HBr, 4,10, 6), 220.9, 222.9, 224.9, 226.9 (4, 6, 10, 6, 4), 143, 145, 147 (40, 38, 6), 62, 64, 66 (46, 76, 38).

^{1}H NMR ($CDCl_3$, 200 MHz): δ (fig. 5.19) = 5.9 (s, exchangeable with D_2O, 1 H, OH).

^{13}C NMR ($CDCl_3$, 50.309): δ (fig. 5.20) = 148.9 (1C, $C_{quat.}$), 112.4 (2C, $C_{quat.}$), 110.2 (3C, $C_{quat.}$).

Fraction 3 on purification gave pentabrompseudilin (**1**) (7.2 mg).

Fraction 4 was further purified over Sephadex LH 20 (column : 50 × 1 cm, chloroform/ methanol 6 : 4). To provides pentabrompseudilin (**1**) (2.0 mg) and 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (24.2 mg).

Fraction 5 was purified using PTLC (chloroform/ methanol 88 : 12), to give two sub fractions A and B. Fraction 5A yielded 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (3.2 mg). Fraction 5B gave indol-3-carboxaldehyde (**7**) (3.3 mg).

Fraction 6 was separated by HPLC to afford two compounds A and B. Compound 6A was identified as 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) (5.0 mg) and the compound 6B turned out as 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (5.2 mg) based on the spectral data as described earlier.

b) Amino acid medium

To a well grown 2 litre culture of *A. luteo-violaceus* in amino acid medium 120 mg of 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid (**19**) was fed using the method B. The pH of the broth was found to be 9.0 when harvested. Silica gel column chromatography of the crude extract (125 mg) gave 4 fractions containing the following compounds.

Fraction 1 gave 2,4,6-tribromo-3,5-dideutero-phenol (**32**) (20 mg).

Fraction 2 gave 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (2 mg) and pentabrompseudilin (**1**) (2 mg).

Fraction 3 gave 3,3′,5,5′-tetrabromo-2,2′-dihydroxybiphenyl (**24**) (15 mg).

Fraction 5 gave deoxyviolacein (**10b**) (1.2 mg) and violacein (**10a**) (2.5 mg), which were identified based on the following spectral data.

Deoxyviolacein (10b)

UV (CH3OH) : 208, 365 and 570.

IR (KBr): ν = 3424, 2910, 1693, 1612, 1545, 1442, 1360, 1269, 1226, 1133, 1022, 953, 869, 802, 764, 729 and 592 cm^{-1}.

^{1}HNMR (DMSO-d_6, 300 MHz): δ = 11.8 (s br, exchangeable with D_2O, 1 H, N-H), 10.68 (s br, exchangeable with D_2O, 1 H, N-H), 10.55 (s, exchangeable with D_2O, 1 H, N-H), 8.94 (d, *J* = 6.5 Hz, 1 H, 19-H), 8.16 (d, *J* = 2.5 Hz, 1 H, 2-H), 7.85 (dd., *J* = 6.0 Hz, *J* = 2.5 HZ, 1 H, 5-H), 7.64 (d, *J* = 2.5 Hz, 1 H, 13-H), 7.58 (d, *J* = 2.5 Hz, 1 H, 8-H), 7.32 (d, *J* = 2.5 Hz, 2 H, 6-H, 7-H), 7.21 (m, 1 H, 21-H), 6.95 (t, *J* = 6.4 Hz, 1 H, 20-H) and 7.82 (d, *J* = 6 HZ, 1 H, 22-H).

Violacein (10a).

^{1}H-NMR (DMSO-d_6, 300 MHz): δ = 10.66 (s br, exchangeable with D_2O, 1 H, N-H), 10.55 (s br, exchangeable with D_2O, 1 H, N-H), 9.28 (s br, exchangeable with D_2O, 1 H, N-H), 8.92 (d, *J* = 6 Hz, 1 H, 19-H), 8.06 (s, 1 H, 2-H), 7.58 (s 1 H, 13-H), 7.36 (d, *J* = 6 Hz, 1 H, 5-H), 7.25 (d, *J* = 1.5 Hz, 1 H, 8-H), 7.0 (t, *J* = 6 Hz, 1 H, 21-H), 6.96 (t, *J* = 6 Hz, 1 H, 20-H), 6.8 (t, *J* = 6 Hz, 1 H, 7-H) and 6.78 (d, 1 H, 22-H)

Synthesis of 3-Chloro-4-hydroxybenzoic acid (20)

Following the literature procedure, 3-chloro-4-hydroxybenzoic acid (**20**) was prepared from 3-Amino-4-hydroxybenzoic acid (**33**) to afford acid **20**, 0.7 g (57 %), m.p. 169-170 ^{0}C (Lit.m.p. 169-171 ^{0}C).

IR (KBr): ν = 3277, 3078, 2941, 1689, 1593, 1513, 1441, 1374, 1288, 1248, 1207, 1152, 1058, 898, 838, 771, 695 and 617 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 172, 174 (M^+, 90, 27), 155, 157 (M^+ - OH, 100, 31), 127, 129 (M^+- COOH, 11, 4), 99, 101 (M^+ - COOH – CO, 12, 4), 63 (17), 61 (25) and 53 (20).

^{1}H NMR (DMSO-d_6, 200 MHz): δ = 12 (s br, exchangeable with D_2O, 2 H, COOH, OH), 7.81 (d, *J* = 2.4 Hz, 1 H, 2-H), 7.72 (dd., *J* = 8.8 Hz, 2.4 Hz, 1 H, 6-H) and 7.02 (d, *J* = 8.8 Hz, 1 H, 5-H).

^{13}C-NMR (DMSO-d_6, 50.3 MHz): δ = 166 (1C, $C_{quat.}$), 157.1 (1C, $C_{quat.}$), 131.1 (1C, CH), 129.7 (1C, CH), 122.6 (1C, $C_{quat.}$)119.6 (1C, $C_{quat.}$) and 116.3 (1C, CH).

Synthesis of 2,3,4,5-tetrachloropyrrole (17)

A solution of pyrrole (1.94 g, 2 ml, 28.9 mmol) in dry diethyl-ether (10 ml) was added dropwise over a period of 1 h to a solution of sulfurylchloride (15.6 g, 4.34 ml, 115 mmol, 4 equivalents) in dry diethyl ether at – 78 °C. The resultant solution was then stirred at – 78 °C for 3 days. Then the reaction mixture was warmed to room temperature and stirred for 5 h followed by the addition of sodium bicarbonate solution. The reaction product was extracted with diethyl ether (3 × 75 ml) and dried over $MgSO_4$. Evaporation of the solvent and then chromatography (silica gel, $CHCl_3$) yielded **17** as a white crystalline solid (5.15 g, 92.3 %), m. p. 108-109 °C.

IR (KBr): ν = 3533, 3396, 2958, 1547, 1481, 1410, 1356, 1269, 1176, 1063, 1002, 938, 694, 610 and 572 cm^{-1}.

EIMS (90 eV): *m/z* (%) = 203, 205 (M^+ 80,100), 168, 170 (M^+- Cl, 52, 50), 141, 143 (M^+ -NHCCl, 52, 50), 132, 134 (M^+-2Cl, 22, 16), 106, 108 (46, 30), 100, 102 (24, 18), 71, 73 (46, 18), 62, 64 (32, 10) and 47 (22).

^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.2 (s br, exchangeable with D_2O, 1 H, NH).

^{13}C NMR ($CDCl_3$, 50.3 MHz): δ = 110.3 (2C, $C_{quat.}$) and 109.5 (2C, $C_{quat.}$).

Synthesis of 3,5-Dibromo-4-hydroxybenzoic acid (34)

To a solution of 4-hydroxybenzoic acid (**15**) (6.9 g, 50 mmol) in glacial acetic acid (50 ml), bromine (5.1 ml, 100 mmol) in glacial acetic acid (8 ml) was added in six portion, with stirring. The solution was then heated within 90 min to 80 °C. After cooling, another 0.5 ml of bromine (10 mmol) in glacial acetic acid (4 ml) was added and the resultant solution was heated to 90 °C for 90 min. After cooling, it was poured ointo 200 ml of ice water. The precipitate formed was filtered off and dried. The resultant compound was recrystallised from water to give colourless needles of **34**, 13.05 g (88.8 %) with m. p. 267 °C (Lit. m.p.268 °C).

EIMS (70 eV): *m/z* (%) = 294, 296, 298 (M^+ 52, 100, 50), 277, 279, 281 (M^+- OH, 32, 60,30), 249, 251, 253 (M^+- COOH, 8, 10, 8), 170, 172 (M^+- [Br + COOH] 8, 10), 143, 145 (10, 12), 90, 92 (10, 12) and 61, 62, 63 (16, 24, 20).

^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8 (s, 2 H, 2-H, 6-H).

Synthesis of 2,3,5,6-Tetrabromo-4-hydroxybenzoic acid (35)

To a solution of 3,5-dibromo-4-hydroxybenzoic acid (**34**) (7 g, 23.7 mmol) in oleum (70 % SO_3, 40 ml), bromine (2.6 ml, 50 mmol) was added in 6 portions. It was stirred for 24 h at room temperature and was poured into 2000 ml of ice water. The precipitate formed was filtered off, dried and was purified on a silica gel column. The first eluted fraction from the column upon concentration gave the pentabromophenol, 0.5 g (4.8 %), m.p.230 °C (lit m.p. 229.5 °C).

Pentabromophenol

EIMS (70 eV): *m/z* (%) = 492, 490, 488, 486, 484 (M^+ 8, 52, 100, 93, 47, 10), 414, 412, 410, 408, 406, 404 (M^+ - Br, 4, 11, 13, 7, 2), 303, 301, 299, 297 (M+ - 2Br, - OH 4, 13, 11, 4) and 141, 139 (13, 13).

^{1}H NMR (DMSO-d_6, 200 MHz): δ = 10.8 (s br, exchangeable with D_2O, 1 H, OH).

^{13}C-NMR (DMSO-d_6, 50.309 MHz): δ = 152.4 (1C, $C_{quat.}$), 127.3 (2C, $C_{quat.}$), 118.5 (1C, $C_{quat.}$) and 115.5 (2C, $C_{quat.}$).

2,3,5,6-Tetrabromo-4-hydroxybenzoic acid (35)

The compound eluted in the second fraction gave (**35**) 7.2 g (67.2 %) as a crystalline solid with m.p. 226-227 °C (Lit. 228 °C)

IR (KBr): ν = 3280, 3120, 2990, 1735, 1690, 1545, 1520, 1375, 1355, 1325, 1265, 1215, 1190, 1150, 1105, 960 and 640 cm^{-1}.

EIMS (70 eV): *m/z* (%) = 450, 452, 454, 456, 458 (M^+ 17, 68, 100, 65, 16), 433, 435, 437, 439, 441, (M^+ - OH 6, 30, 50, 28, 12) and 406, 408, 410, 412, 414 (M^+ - COOH).

^{13}C NMR (DMSO-d_6, 50.308 MHz): δ = 166.2 (1C, $C_{quat.}$), 153.3 (1C, $C_{quat.}$), 133.4 (1C, $C_{quat.}$), 120.5 (2C, $C_{quat.}$) and 115.4 (2C, $C_{quat.}$).

Synthesis of 2,3,5,6-tetradeuterio-4-hydroxybenzoic acid (36)

A solution of 2,3,5,6-tetrabromo-4-hydroxybenzoic acid (**35**) (2.64 g, 5.82 mmol) and K_2CO_3 (5.64 g, 40.8 mmol) in D_2O (50 ml) was shaken with 200 mg Pd/C (10 %Pd) for 10 h under D_2–atmosphere. The catalyst was filtered off. To the filtrate, 20 ml conc. HCl was added and extracted with diethyl ether (4 × 40 ml). The combined extracts were dried over $MgSO_4$, the solvent was distilled off and the residue was recrystallised from water, to give **36**, 775 mg (94.5 %) as colourless crystals with m.p. 218 °C.

IR (KBr): ν = 3390, 2920, 2880, 1675, 1570, 1460, 1390, 1280, 1190, 1080, 940, 810, 700, 600 and 540 cm^{-1}.

EIMS (70 eV): ***m/z*** **(%)** = 142 (M^+ 92), 125 (M^+-OH, 100), 97 (M^+ -COOH, 20), 69 (15), and 41 (8).

^{13}C NMR (DMSO-d_6, 50.309 MHz): δ = 167.1 (1C, $C_{quat.}$), 161.5 (1C, $C_{quat.}$), 131.2 (1C, $C_{quat.}$), 130.8 (1C, $C_{quat.}$), 121.2 (1C, $C_{quat.}$), 115.2 (1C, $C_{quat.}$) and 114.7 (1C, $C_{quat.}$).

Synthesis of 3,5-Dibromo-2,6-dideuterio-4-hydroxybenzoic acid (19)

To a solution of **36** (800 mg, 6 mmol) in glacial acetic acid (6 ml) with stirring, bromine (0.65 ml, 12 mmol) in 1 ml of glacial acetic acid, was added in 6 portions. The solution was heated to 80 °C within 90 min. After cooling, another 0.07 ml (1.2 mmol) of bromine in (0.5 ml) of glacial acetic acid was added, the solution was heated to 90 °C for 1.5 h and was poured after cooling into 150 ml ice water. The precipitated substance was filtered off and recrystallised from water to give **19**, 1.2 g (71 %) with m.p. 243-245 °C.

IR (KBr): ν **(fig. 5.15)** = 3460, 2920, 2880, 1680, 1570, 1545, 1410, 1365, 1280, 1230, 1150, 940, 730 and 670 cm^{-1}.

EIMS (70 eV): ***m/z*** **(%)(fig. 5.16)** = 296, 298, 300 (M^+ 48, 100, 48), 279, 281, 283 (M^+ - OH 20, 44, 20), 251, 253 (-COOH) and 65 (10).

^{13}C-NMR (DMSO-d_6, 50.308 MHz): δ **(fig. 5.17)** = 164.8 (1C, $C_{quat.}$), 154.7 (1C, $C_{quat.}$), 124.4 (1C, $C_{quat.}$), 115.6 (1C, $C_{quat.}$), 112.7 (1C, $C_{quat.}$), 111.2 (1C, $C_{quat.}$) and 111.1 (1C, $C_{quat.}$).

Synthesis of 4,6-Dibromo-2-chlorophenol (23)

To a solution of 2-chlorophenol (**22**) (6.4 g, 50 mmol) in glacial acetic acid (50 ml) was added bromine (5.1 ml, 100 mmol) in 8 ml of glacial acetic acid with stirring. Then, the reaction mixture was heated to 80 °C for 90 min. After cooling, another 0.5 ml (10 mmol) of bromine in 4 ml of glacial acetic acid was added and the resulting solution was heated to 90 °C for 90 min. After cooling, it was poured into ice (300 ml), the precipitate was filtered off and washed with water several times and dried to give **23** as a crystalline solid (8.55 g, 60.2 %) with m. p. 105 °C.

IR (KBr): ν = 3500, 3000, 1560, 1465, 1385, 1315, 1265, 1235, 1150, 850 and 765 cm^{-1}.

EIMS (90 eV): ***m/z*** **(%)** = 284, 286, 288, 290 (M^+ 40, 100, 62, 16), 206, 208 (8, 8), 141, 143, 144 (8, 10, 8) and 126 (M^+ - (OH, + Cl, + Br), 4).

^{1}H NMR ($CDCl_3$, 200 MHz): δ = 7.75 (d, J = 2.4 Hz, 1H, 5-H) and 7.65 (d, J = 2.4 Hz, 1H, 3-H).

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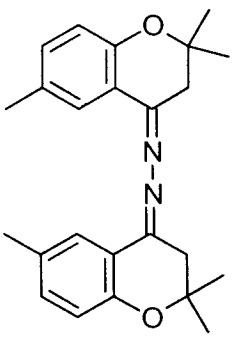
Summary

The Ph. D. thesis entitled "Some Studies on Structure, Synthesis and Biosynthesis of Natural Products" is divided into five chapters.

CHAPTER 1: Bioactive secondary metabolites from the Microorganisms

This chapter is divided into two sections. The first section describes in detail the isolation and structure elucidation of eight secondary metabolites obtained from the limnic bacterial strain *Nocardiopsis* (GBF 90a). The structure elucidation of these metabolites lead to identify them as the known o-hydroxyacetophenone and the amides namely, N-(2′-phenylethyl)-propanamide, N-(2′-phenylethyl)-2-methyl-propanamide, N-(2′-phenylethyl)-3-methyl-butanamide and N-(2′-phenylethyl)-hexanamide, besides uracil and uridine. In addition, a novel hydrazine dimer, designated as limnazine, was also isolated during the present study and the structure **1** assigned to it was confirmed by an unambiguous synthesis. It may be pertinent to state that the natural occurrence of such hydrazine derivatives is rare and till date only three such compounds have been reported[1].



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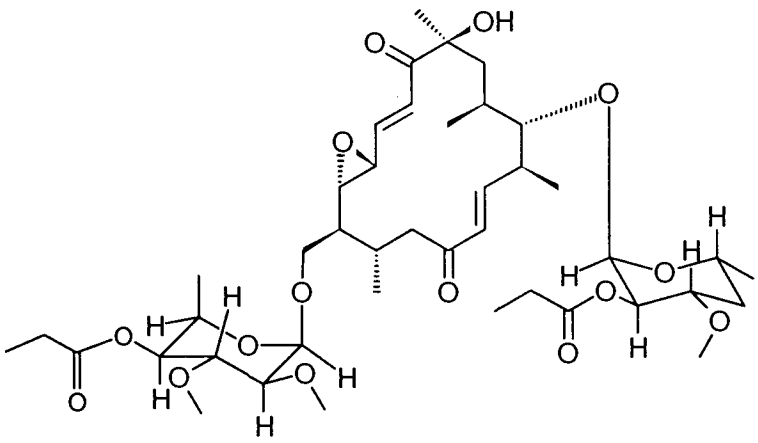

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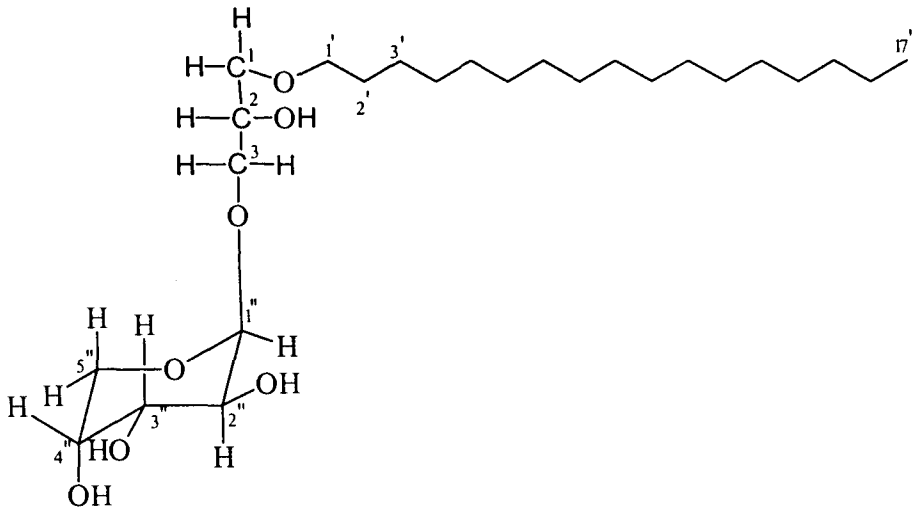
In the second section, the isolation and structure elucidation of the secondary metabolites obtained from the culture of the marine *Streptomycetes* sp. B 7064 strain has been described. Out of the four compounds isolated, two were the known amide and ester and the third one was found to be the macrolytic antibiotic chalcomycin, reported earlier from *Streptomyces bikiniensis*[2,3,4]. The fourth crystalline compound isolated during the present study, is a chalcomycin B (**2**) derived from the parent chalcomycin and the same has been reported for the first time.

CHAPTER 2 : Secondary Metabolites from the Marine Organisms

This chapter is divided into two sections. The first section describes in detail the isolation and structure determination of the various secondary metabolites obtained from the soft coral *Sinularia capillosa*. The chemical examination of this soft coral collected from the Gujarat State provided two known acids namely the hexadecanoic acid and octadecanoic acid together with three sterols namely fucosterol, 24-methylene cholesterol and cholesterol, besides octadecyl glycerol. In addition, the crystalline compound obtained from the ethyl acetate extract of the organism was identified as 2-hydroxy-3-(heptadecyloxy)-propyl-β-D-arabinopyranoside (**3**), a new glycoside. The latter has been reported for the first time from this source.

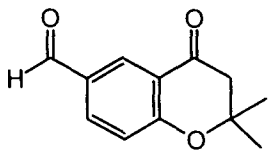


The second section deals with the secondary metabolites isolated from the green seaweed *Cladophora prolifera* that provided eight different known compounds. Out of these compounds, the sterol derivative, namely, 24-nor-cholesta-5,22-diene-3β-ol has been reported for the first time from Cladophoraceae family. Similarly, two heterocyclics, *viz.*, 8-hydroxyquinoline and 4-methyl-1-phenyl-pyrazole-2-one have been found and this is the first report of their natural occurrence in this species.

CHAPTER 3: Synthetic studies on Chromanones and Chromones.

This chapter is divided into three sections.

In the first section, the details of the synthesis pertaining to lactarochromal (**4**), a metabolite isolated from the phytopathogenic fungus *Lactarious deliciosus*[5] has been described. The said natural product has been synthesized by two independent routes starting from p-acetaminophenol and 2,2,6-trimethylchroman-4-one respectively. The latter route constituted a short and facile synthesis of the natural product. This work has also lead to the formal synthesis of the corresponding acid (**5**), which is a natural product reported earlier from *Chrysothamnus viscidiflorus*[6].



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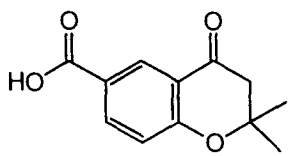

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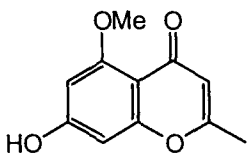
The second section describes the first synthesis of maritimin (**6**), a chromone derivative isolated from the bulbs of *Pancratium maritimum* L[7] (family Amaryllidaceae) starting from phloroglucinol.

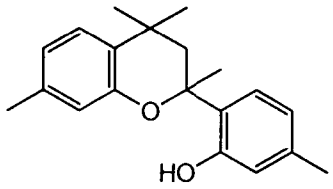


6

The third section describes the simple and facile synthesis of 3-aryl substituted cyclohex-2-en-1-one derivatives starting from the appropriately substituted acetophenones in one-pot operation.

CHAPTER 4 : Miscellaneous synthetic studies

This chapter is divided into two sections. The first section describes one-pot preparation of flavans starting from 4-methylcoumarins. This work has also lead to the synthesis of a thymol dimer, inulavosin **(7)** isolated from the roots of *Inula nervosa*[8].



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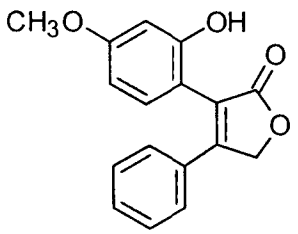
In the second section the synthesis of 3-(2′-hydroxy-4′-methoxyphenyl)-4-phenyl-2-(5H)-furanone **(8)**, one of the products obtained earlier during the photolysis of 6-methoxybenzofuran-2,3-dione and styrene[9] has been described. The present work confirmed the structure **8** that has been proposed[10] on the basis of the earlier published spectral data.



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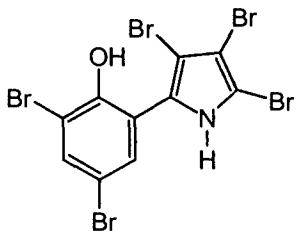
CHAPTER 5 : Some studies on the biosynthesis of the marine antibiotic Pentabromopseudilin.

The work carried out in connection with the biosynthesis of pentabromopseudilin (**9**)[11,12,13], a marine antibiotic has been described in this chapter. The biosynthetic studies were investigated in *Altermonas luteoviolaceus* grown in amino acid and M_1 media by employing feeding experiments with tetrachloropyrrole, 3,5-dibromo-2,6-dideutero-4-hydroxybenzoic acid, 3-chloro-4-hydroxybenzoic acid, 2-chlorophenol and 2-chloro-3,5-dibromophenol. In conformity with the earlier observation[14], the results obtained from these feeding experiments provided additional support to the conclusion that the benzene ring in **9** originated from the shikimate pathway *via* p-hydroxybenzoic acid functioning as a direct precursor. No incorporation of the feeded precursor was observed in the pyrrole ring of **9** during thses experiments. The isolation of a new pseudilin derivative namely 3′-chloro-tetrabromopseudilin (**10**) from the feeding experiment during the present study clarified the most likely decarboxylation sequence while utilizing the *p*-hydroxybenzoic acid precursor for the biosynthesis of pentabromopseudilin molecule.



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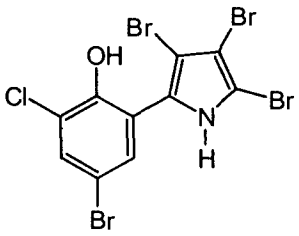

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