Chemical Examination of *Uvaria narum* and Transformation Reactions of Carbocyclic Compounds

A THESIS SUBMITTED TO THE

GOA UNIVERSITY

FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

CHEMISTRY

BY

Kamalesh Pundalik Pai Fondekar M.Sc

Department of Chemistry,

Goa University,

Goa

MAY 1997

To

My Mother

STATEMENT REQUIRED TO BE SUBMITTED UNDER ORDINANCE 19.8

OF THE GOA UNIVERSITY

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

(S. K. Paknikar)
Research Guide

(Kamalesh P. Pai Fondekar)
Candidate

CERTIFICATE

This is to certify that the thesis entitled " Chemical Examination of *U.narum* and transformation reactions of carbocyclic compounds" submitted by Mr. Kamalesh P. Pai Fondekar for the award of degree of Doctor of Philosophy in Chemistry is based on literature survey/laboratory experiments carried out by him under my supervision. The thesis or any part thereof has not previously been submitted for any other degree or diploma.

Date : 15th May 1997

Dr. S. K. Paknikar,

(Research Guide)

Acknowledgments

I am proud for having the privilege to work with Dr. S.K.Paknikar, former Professor and Head, Department of Chemistry and Dean, Faculty of Natural Sciences, Goa University as my research guide and mentor during the course of my research work. I express my deep sense of gratitude to him for his continuous help, valuable and inspiring guidance. This work would not have been completed without his resourcefulness and imaginative approach.

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I owe a deep sense of gratitude to Harikaka, Shamalkaki, Yatin and my family members for their constant encouragement and to Rajesh and Mr. R. M. Shevade for their help during the final stages of my work.

Kamalesh P. Pai Fondekar

CONTENTS

Chapter one

Chapter two

Chapter three

Synthetic and structural studies of some natural products

-

Chapter four

Transformations of some carbocyclic compounds

GENERAL REMARKS

1. All chart, scheme, table, structure, figures and reference numbers in the chapter refer to that particular chapter only.
2. Organic extracts were dried over anhydrous $MgSO_4$ unless otherwise stated.
3. All melting and boiling points were recorded in degree Celsius and are uncorrected.
4. Petroleum ether refers to the fraction boiling between the range 60°-80°C.
5. Ether refers to diethyl ether.
6. Silica gel used for column chromatography was of 60-120 mesh size.
7. Thin layer chromatography was done on glass plates coated with TLC grade silica gel with 13% $CaSO_4$ as binder. Visualisation of the plates were done by developing the plates in I_2 chamber, unless otherwise stated.
8. 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.
9. The chemical shift parameters in the ^{1}HNMR and ^{13}CNMR 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.
10. The ^{1}HNMR spectra presented are obtained in normal form and the J values reported are of the resolved form.
11. All known compounds were identified by direct comparison of spectral data and 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.

1.1 Chemistry of the Genus *Uvaria* : A overview

Annonaceae is a large family of aromatic trees, shrubs, climbers comprising of about 120 genera and more than 2000 species. The plants belonging to this family have been commercially known for their edible fruits, edible oils, alcohol production, perfumery preparations and medicinal properties. Inspite of its large size and commercial applications, annonaceous plants did not receive noticeable attention till late sixties.[1] Because of the reported medicinal properties, the plant species belonging to the genus *Uvaria* have been appearance of the first authenticated report in 1968. During the last thirty years around 100 papers appeared in various reputed journals.[2]

In connection with our chemical investigation of the Indian plant *Uvaria narum* (annonaceae), we have carried out a survey of literature till December 1996 on the chemical constituents of the genus *Uvaria*. It is felt that a overview of its present status would serve as prelude to presentation of our contribution to the chemistry of the genus *Uvaria*.

The plants belonging to the genus *Uvaria* are known for their medicinal properties and collective outcome of the studies carried out by various independent groups shows that these are rich sources of bioactive molecules having novel carbon frameworks. The genus *Uvaria* contains about 150 species and their habitats are restricted to certain geographical regions such as Asia, Australasia, Africa, Madagascar and USA.

The chemical constituents isolated from various *Uvaria* species are grouped according to their classification and presented in the form of charts (chart-1 to chart-14). Below each chemical structure, the species from which it is isolated is given with the literature reference. Readers interested in knowing further details are requested to consult the original paper/s.*

This review is divided in to two parts. In the first part, the chemical constituents isolated and characterized from *uvaria* species other than *U.narum* are presented. The second part deals with the chemical constituents of *U.narum* known prior to and/or independently isolated and characterized by other investigators while our study was in progress. For the sake of brevity the medicinal properties of ten representative species of *uvaria* are presented in Table-1.

Table-1 : Medicinal properties of some specific representatives of genus *Uvaria*

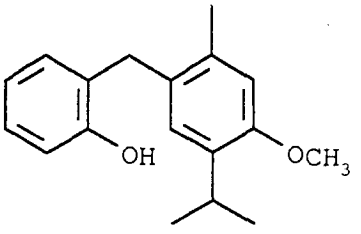
	Species	Part of the plant	Activity
1	*U. tonkinesis*	root bark	cytotoxic to human tumour cells
2	*U. chamae*	stem bark	cytotoxic
3	*U. afzelii*	root bark	antimicrobial
4	*U. elliotiana*	stem bark	antibiotic activity against some fungi
5	*U. angolensis*	root bark	antimicrobial and cytotoxic
6	*U. lucida*	stem and root bark	antimalerial
7	*U. pande*	stem and root bark	antimalerial
8	*U. scheffleri*	root bark	antimalerial
9	*U. accuminata Oliv*	root bark	activity against P-388 lymphocytic leukaemia
10	*U. laptocladon*	root bark	activity against cerebral malaria

* We have practically all papers with us and would be glad to supply the details if required.

Part A :

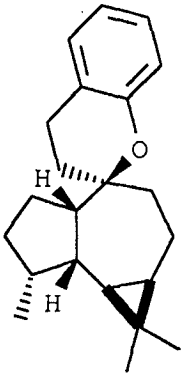
1. Terpenoids :

Several novel terpenoids have been isolated and fully characterized. These include meroterpenoids having a mixed biosynthetic origin. The striking feature of the compounds listed in chart-1, is C-orthohydroxy benzylation of the terpenoid part (e.g. chamanene **1**) and those having benzopyranyl part structure (tanzanene **2**, lucidene **3**, uvarisesquiterpenes A **4**, B **5** and C **6** respectively). It is proposed that benzopyranyl terpenoids are biogenetically derived by a 4+2 cycloaddition of a o-benzoquinone methide **7**, to the olefinic linkages of the corresponding terpenoid.



1

U. chamae[3]



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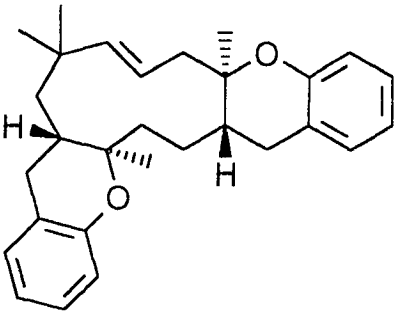
U. tanzania[4]



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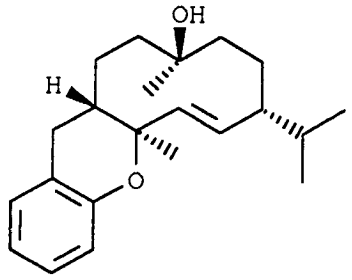
U. lucida[5]



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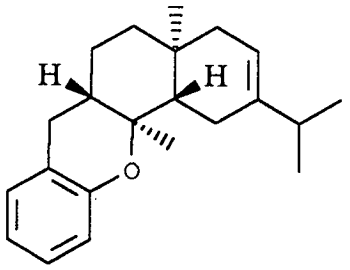
U. angolensis[6]



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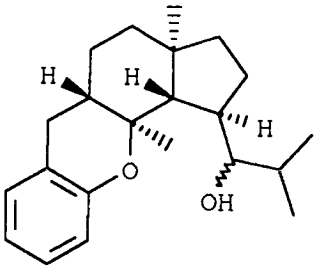
U. angolensis[6]



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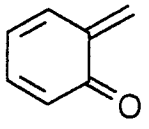
U. angolensis[6]

Chart-1



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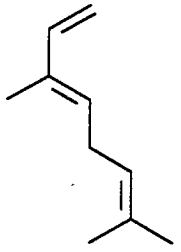

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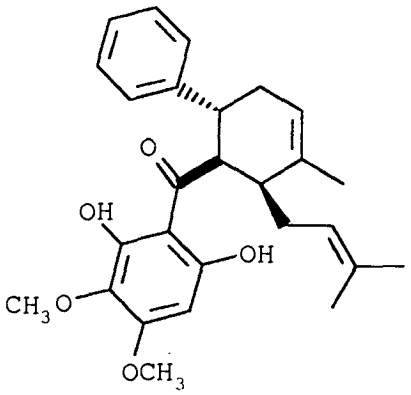
The other meroterpenoids include schefflerin **8** and isoschefflerin **9** (chart-2) which are derived by a Diels-Alder addition of ocimene **10** to the conjugated double bond of highly oxygenated chalcones.



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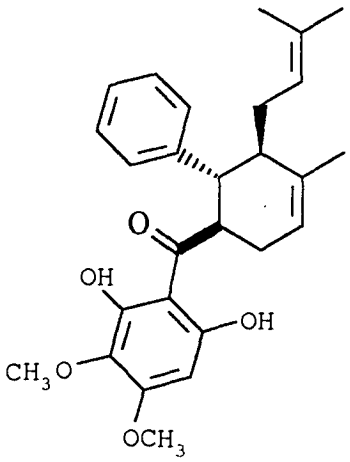
U. scheffleri[7]

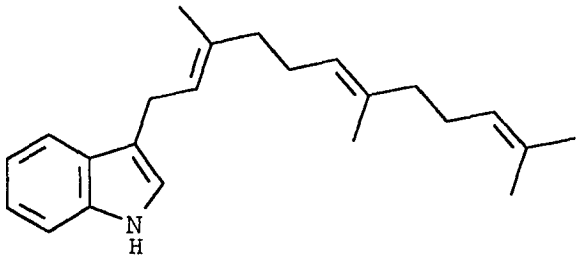


9

U. scheffleri[7]

Chart-2

3-Farnesyl indole **11** and its oxygenated derivatives **12** and **13** have been characterized. Similarly attachment of two C_5 units at C-3 and C-6 of indole nucleus as shown in **14** has been observed (chart-3).



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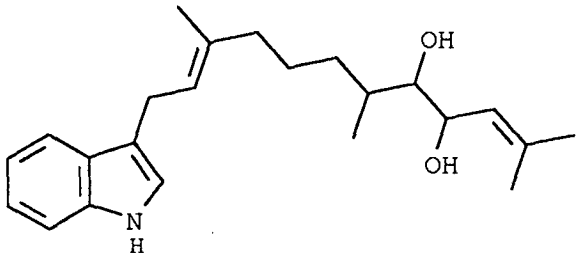

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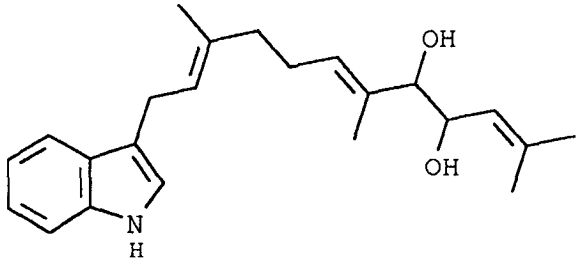

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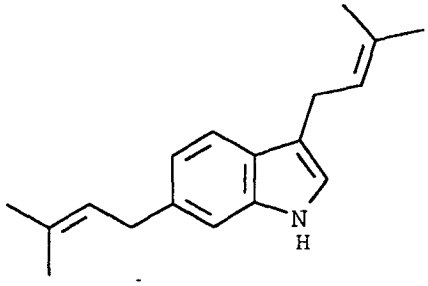

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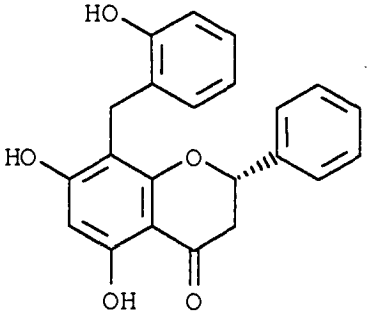
U.elliotianna[8]

* *U.pandensis*[9,10] + *U.scheffeleri*[7]

Chart-3

2. Flavanoids and Chalcones :

Chamanetin **15**, chamanetin-5-methyl ether **16**, chamanetin-5,7-dimethyl ether **17**, isochamanetin**18**, dichamanetin **19**, dichamanetin-5-methyl ether **20**, uvarinol **21**, (+) 6,8-dimethyl pinocembrin-5-methyl ether **22**, demethoxy matteucinol **23**, 2`-hydroxy demethoxy matteucinol **24** have been isolated from various *Uvaria* species. Pinocembrin **25** and pinostrombin **26**, the parent flavanoids are also found to occur naturally in *U.chamae* (chart-4) .C-orthohydroxy benzylation at the activated site of the parent flavanoid pinocembrin and/or its 5-methyl ether derivative appears to be the characteristic features of *Uvaria* flavanoids and perhaps responsible for the observed cytotoxic activity of these molecules.



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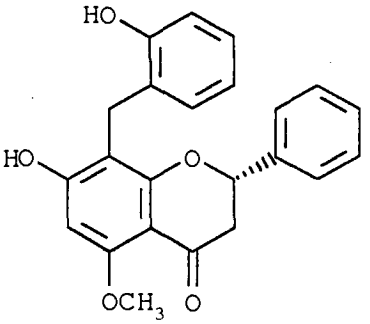

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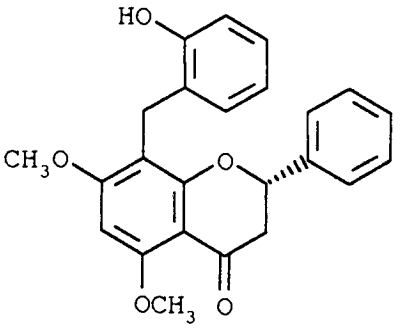

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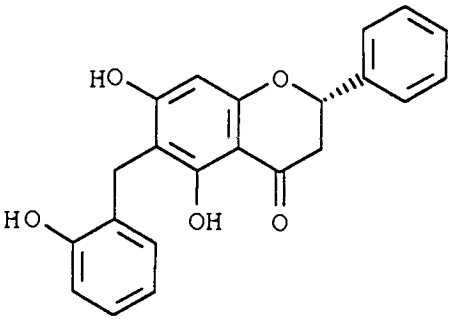

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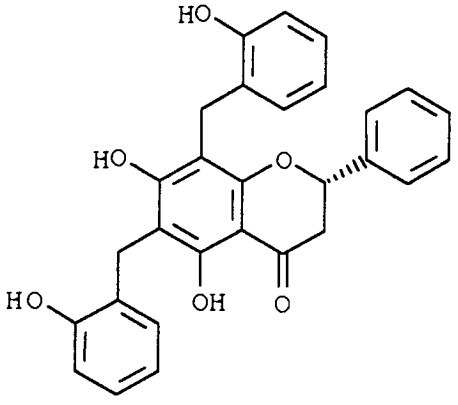

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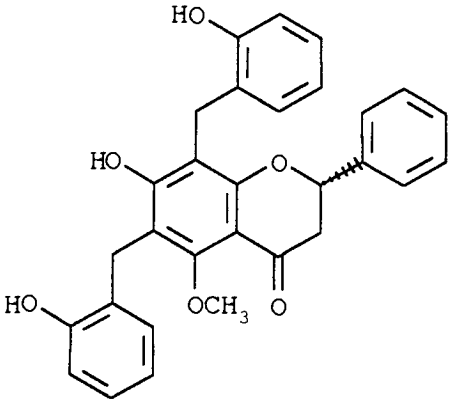

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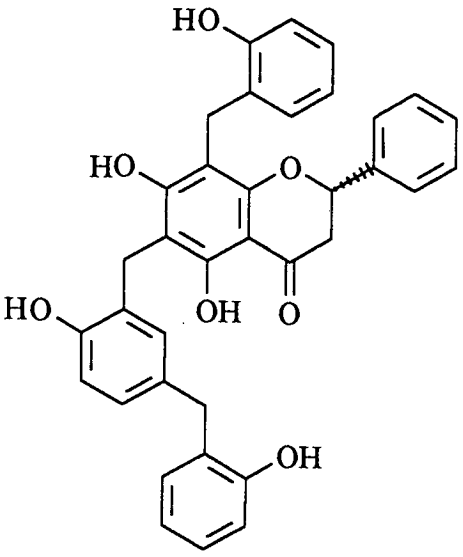

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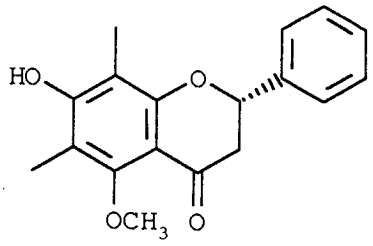

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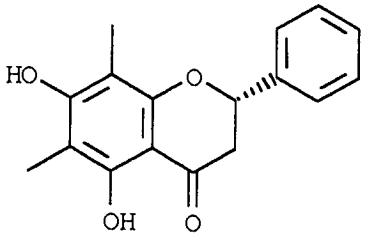

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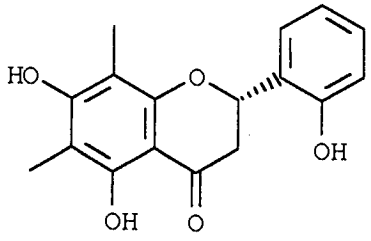

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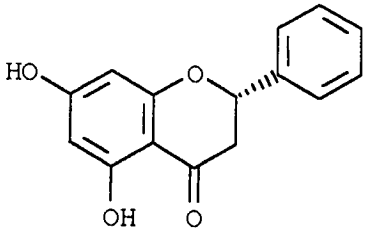

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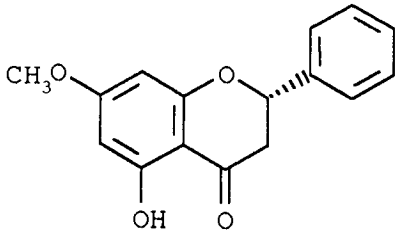

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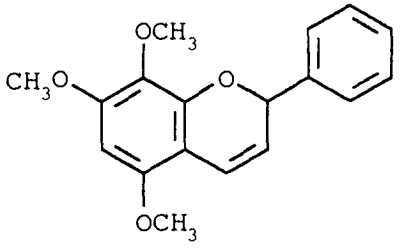
*

*U.chamae [11,12,13] # U.ferruginea [14] $ U.angolensis[15] @U.afzelii[16]

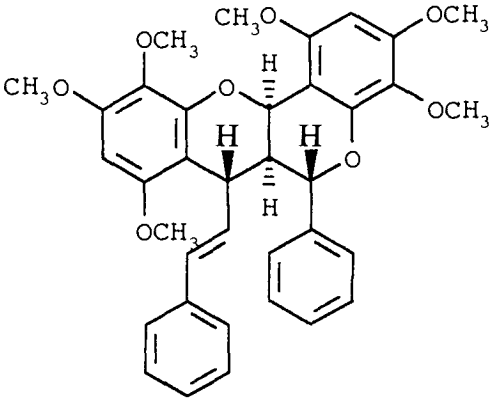
Chart-4

The isolation of 5,7,8-trimethoxy flav-3-ene **27** and the dimeric benzopyran dependesin **28** (chart-5) from *U.dependens* is of special interest. The flavene derivative appears to be derived by enzymatic reduction of C-4 carbonyl of the flavanone precursor followed by dehydration. Acid catalysed dimerisation of **27** produces dependesin **28**.



27

U.dependens[17]

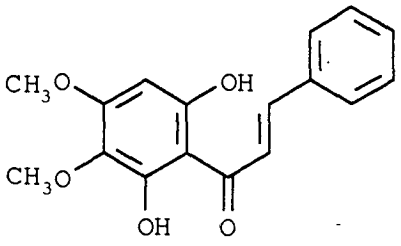


28

U.dependens[17]

Chart-5

It is well-known that chalcones are immediate precursors of flavanoids. Isolation of chalcones **29** and **30** (chart-6) from various *Uvaria* species is therefore understandable.



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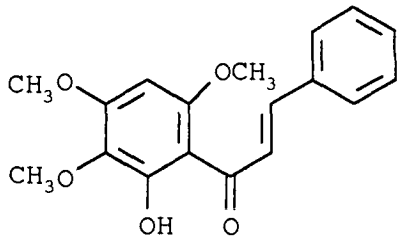

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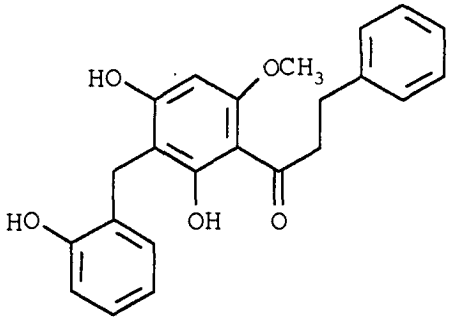
* #

*

* *U.scheffleri*[9] #*U.dependens*[17]

Chart-6

The natural occurrence of dihydrochalcones is quite rare and restricted to certain plant families. It has been shown that uvaretin **31** a dihydrochalcone is an antimalerial compound and also active against P-388 lymphocytic leukemia. Similarly diuvaretin **33** has been shown to posses cytotoxic and antimalerial activities. The dihydrochalcones isolated from various *Uvaria* species (isouvaretin **32**, anguvetin **34**, angoluvarin **35**, chamuvaritin **36**, uvangoletin **37**, angoletin **38**, flavakawin B **39**, triuvaretin **40**, isotriuvaretin **41**) are presented in chart-7.



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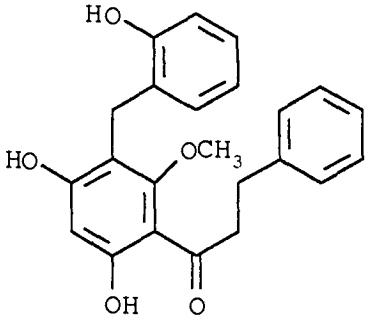

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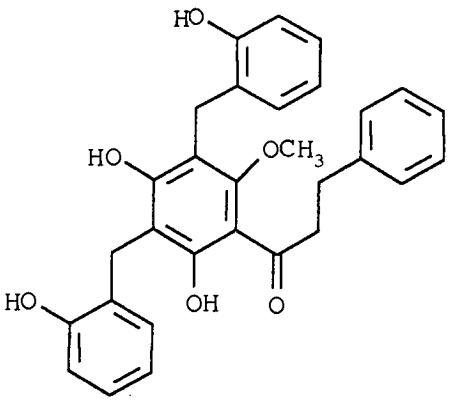

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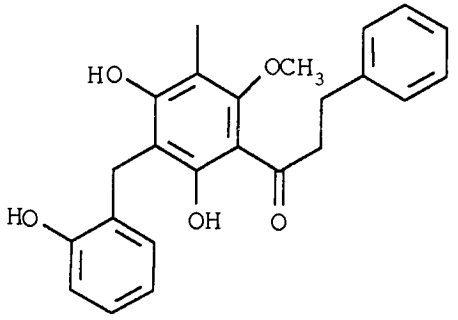

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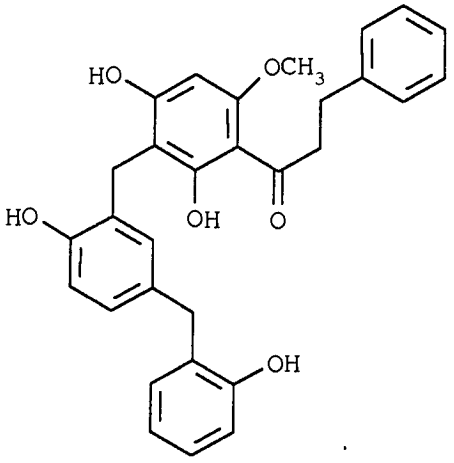
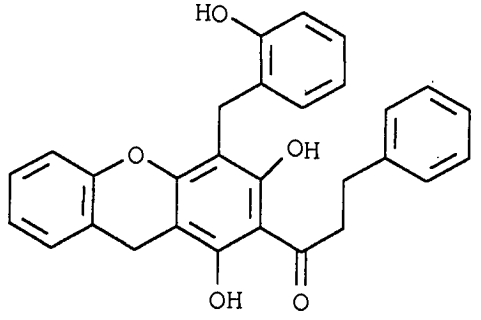


35

** +



36

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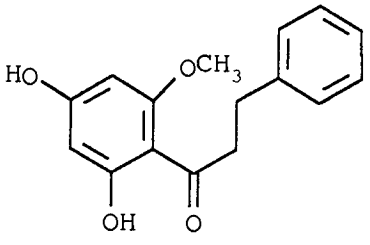

37

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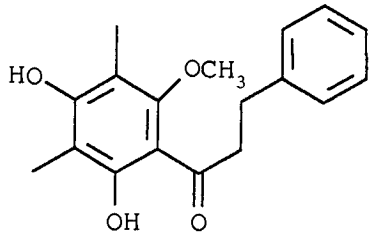

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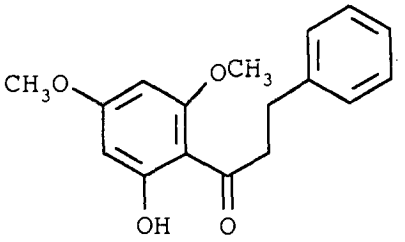

39

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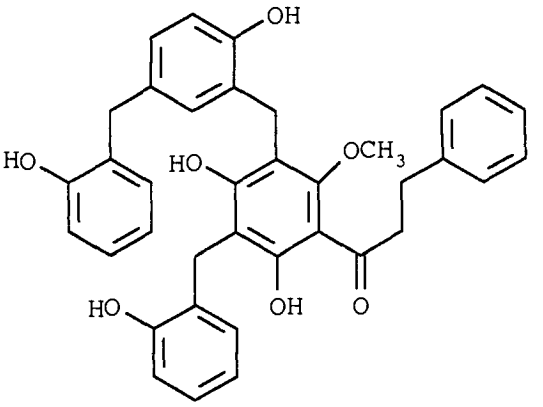

40

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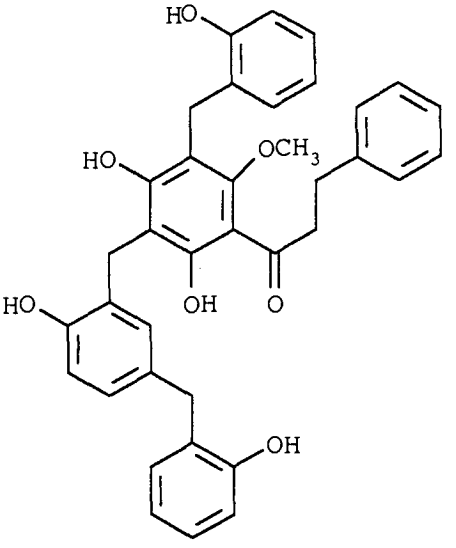


41

+

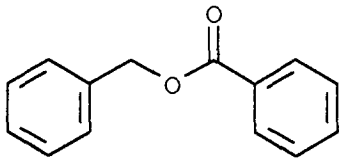
* *U.accuminata*[18] + *U.laptocladon*[19] # *U.chamae*[12] @ *U.lucida*[5]

$ *U.tanzania*[4] ** *U.angolensis*[20,21,22] ++ *U.kirki*[23]

Chart-7

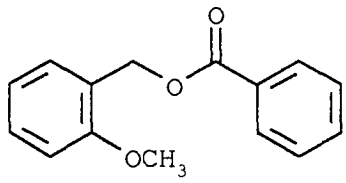
3. Benzoate esters and highly oxygenated cyclohexane derivatives :

These compounds are grouped together because of their structural relationship. Benzyl benzoate **42** and its several oxygenated derivatives **43** to **51** have been shown to be the constituents of the genus *Uvaria* (chart-8).



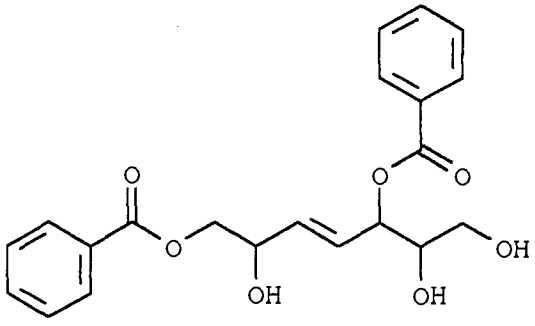
42

$ * @ +



43

$ * @ #



44

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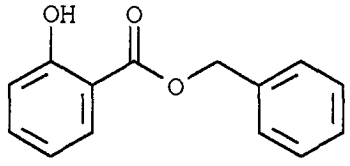

45

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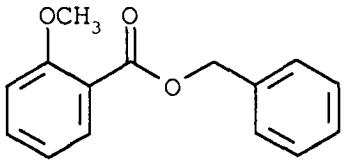

46

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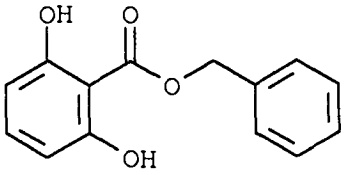

47

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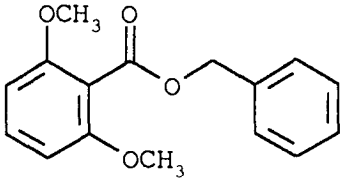

48

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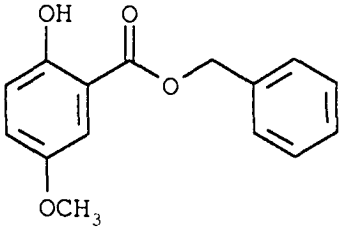

49

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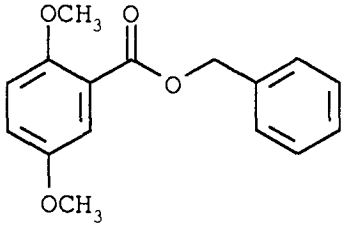

50

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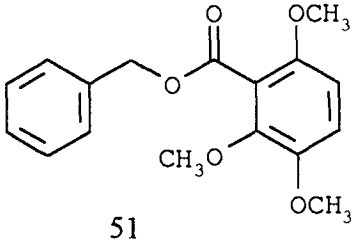


51

**

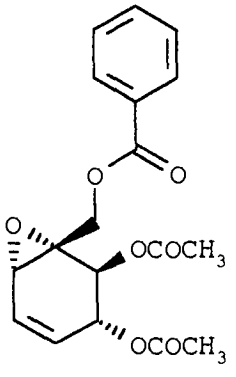
u.chamae[3] $ *u.kirki*[23] * *u.ferruginea*[13] @ *u.purpurea*[24]

\+ *u.scheffleri*[7] ** *u.ovata*[25] ++ *u.rufas blume*[26]

Chart-8

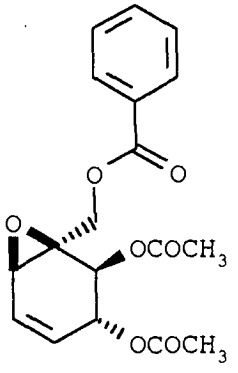
The seven carbon atoms of the straight chain of dibenzoate **44** belongs to the benzyl group of the benzyl benzoate precursor.

A very large number of polyoxygenated cyclohexane derivatives have been isolated and characterized which include α-senepoxide **52**, β-senepoxide **53**, seneol **54**, (+)-pipoxide **55**, (-)-pipoxide **56**, (+)-pandoxide **57**, tingtanoxide **58**, zeylenol **59**, 1-epizeylenol **60**, zeylena **61**,(-) 1,6-desoxy pipoxide **62**, 1,6-desoxy senpoxide **63**, 1,6-desoxy tingtanoxide **64** uvarigranols A, B, C, D, E, F **65**, **66**, **67**, **68**, **69**, and **70** respectively, These are presented in chart-9.



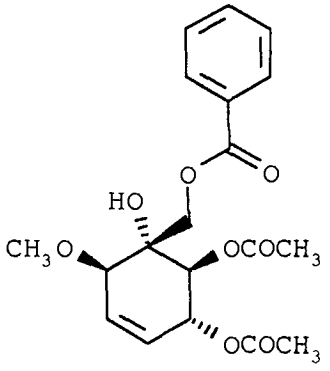
52

*



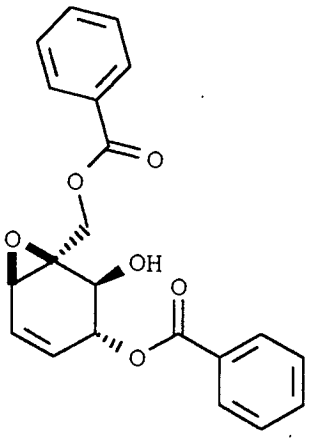
53

@



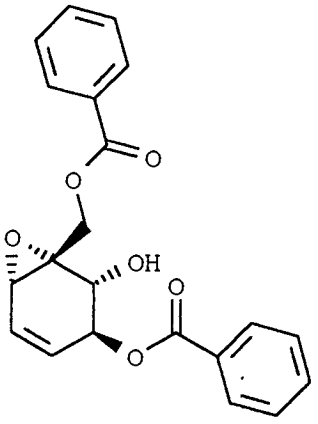
54

*



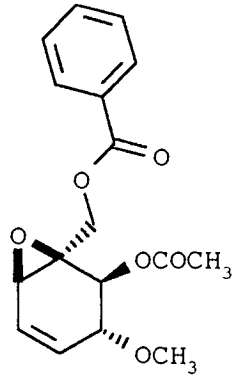
55

$



56

@



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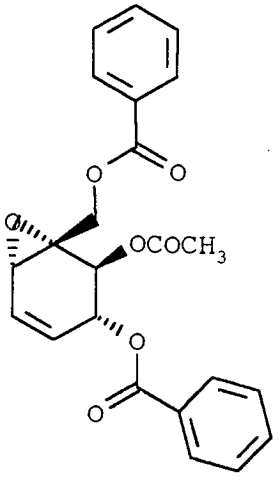
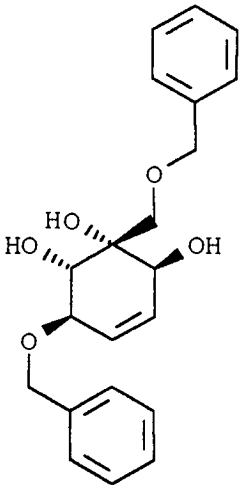


58

+



59

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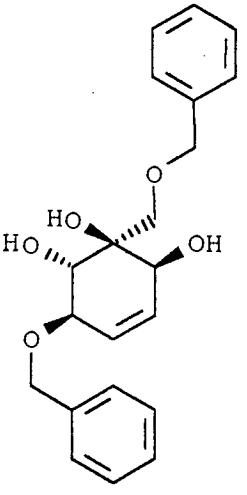

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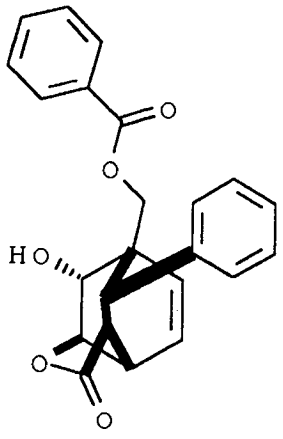

61

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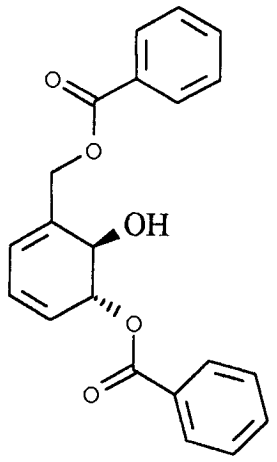
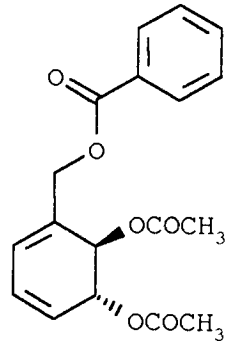


62

$



63

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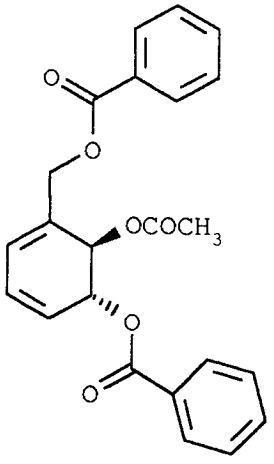
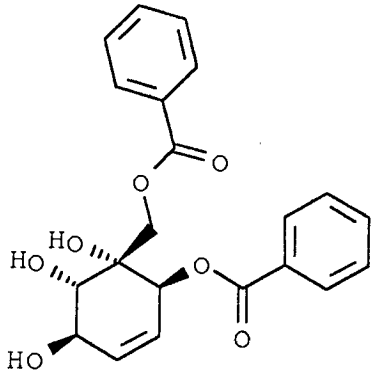


64

+



65

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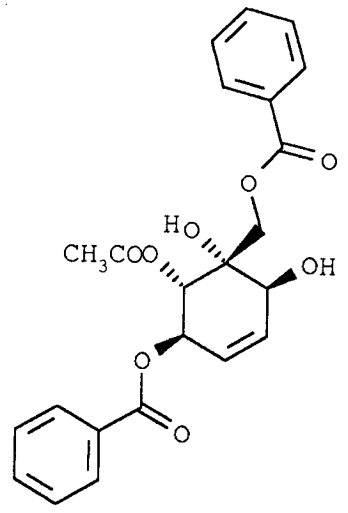

66

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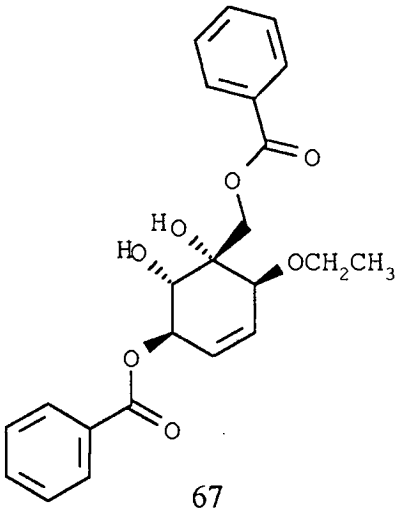

67

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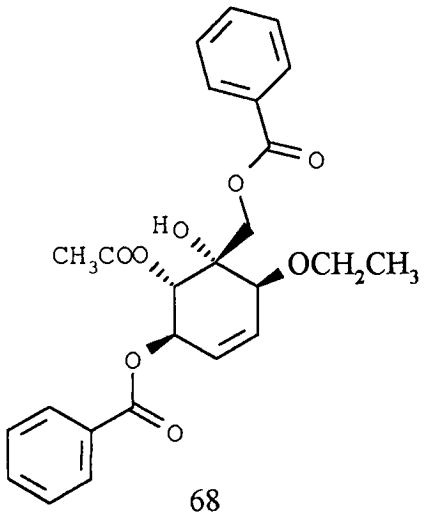

68

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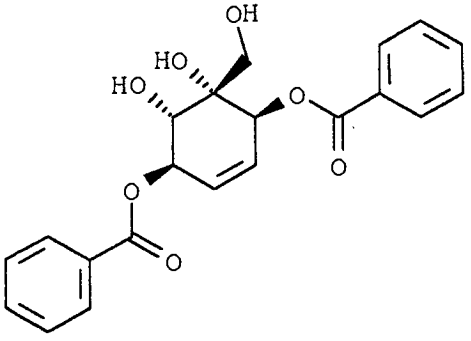

69

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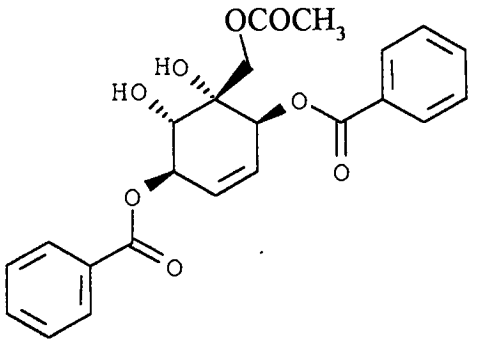


70

**

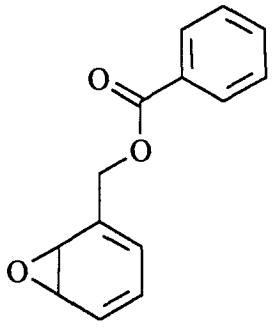
*U.catocarpa[27] + U.ferruginea[13] # U.zeylanca[28,33]

$ U.purpurea[29] @ U.pandensis[30] **

U.grandiflora[31,32]

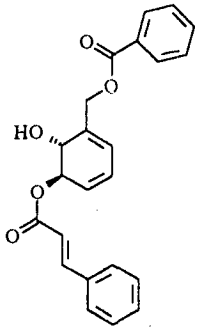
Chart-9

An attractive proposal regarding their biogenesis has been made by Bates and co-workers.[33] It is suggested that they are biosynthesised via arene oxide intermediate **71**. Moreover it appears that the C_7 unit responsible for benzylation of terpenes, chalcones, dihydrochalcones, indole etc. is also derived from **71**.



71

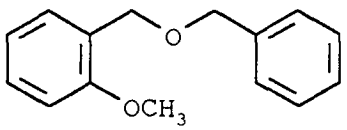
The isolation of (+)- and (-)-pipoxide from *U.purpurea* and *U.pandensis* respectively is of special interest because they are formed via arene oxide intermediates. Another optically active and novel compound, zeylena **61** has been considered to be biosynthesised via an intramolecular cycloaddition of a hypothetic diene intermediate **72**. This suggestion finds an experimental support in the form of a biomimetic synthesis of **61**.[34]



72

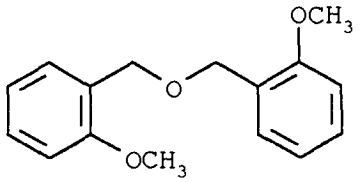
4. Benzyl ethers :

o-Methoxy benzyl ether **73** and also di-o-methoxy benzyl ether **74** have been shown to occur in *U.chamae*(chart-10). There is no report of these compounds from any other *Uvaria* species.



73

U.chamae[3]



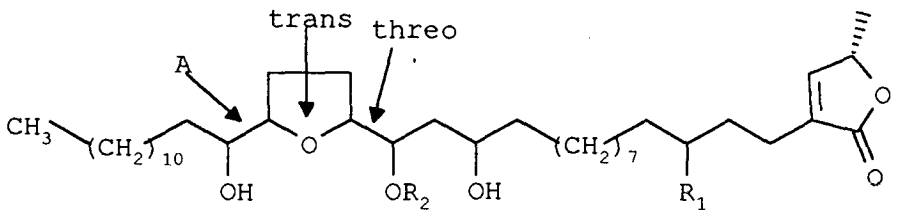
74

U.chamae[3]

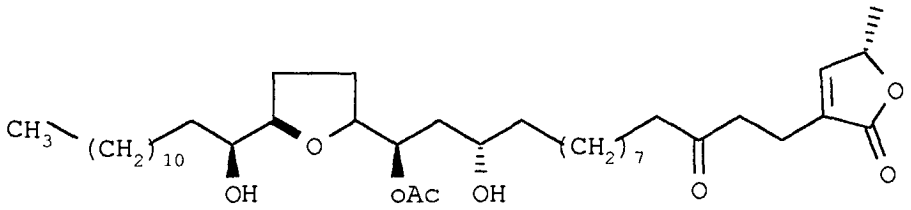
Chart-10

5. Acetogenins :

Seven acetogenins, tonkinins A, B and C (**75**, **76** and **77**) and tonkenensin A, B and C (**78**, **79** and **80**) along with tonkinecin **81** have been isolated from *U.tonkinesis*(chart-11). The bioactivity of these molecules is due to presence of α,β-unsaturated- γ-lactone and the tetrahydrofuran ring. Uvaricin **82** and deaetyluvaricin **83**(chart-11) are structurally similar to the acetogenins of *U.tonkinesis* but contain an additional tetrahydrofuran ring.

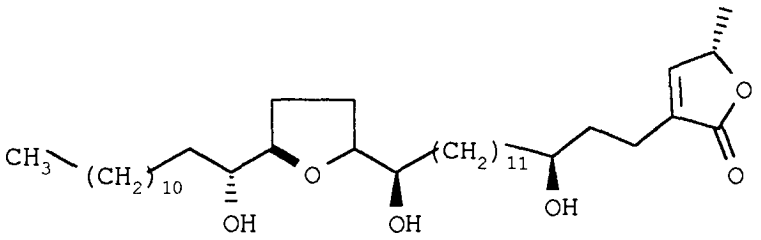


	R_1	R_2	A
75 ----------	=O	—H	erythro
76 --------	=O	—H	threo
78 -------	—OH	—H	erythro
79 --------	—OH	—H	threo
80 ---------	—OH	—Ac	erythro



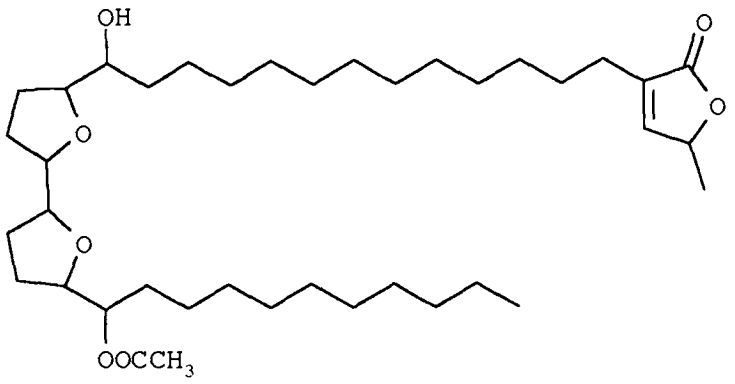
77

U.tonkinesis[35]



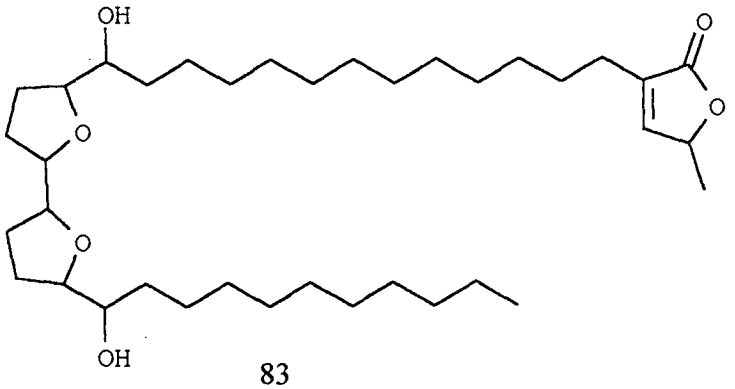
81

U.tonkinesis[36]



82

U.accuminata[37]



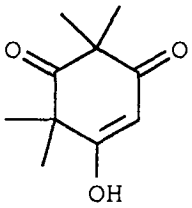
83

U.accuminata[38]

Chart-11

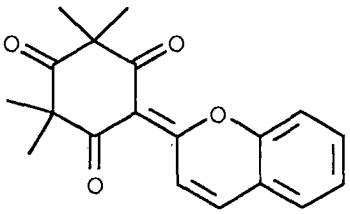
6. Methylated phloroglucinol based natural products :

Syncarpic acid **84**, emorydone **85**, uvafzelic acid **86**, vafzelin **87**, uvafzelin **88** and 2-hydroxy-7,8-dehydro grandiflorone **89** have been isolated from *U.afzelii*. It is proposed that vafzelin **87** can be derived by C-acylation of **84** by o-hydroxyl cinnamoyl moiety, followed by conjugate addition. In fact the origin of all compounds listed in chart-12 can be explained on similar lines.



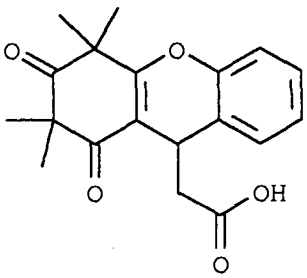
84

U.afzelii[39]



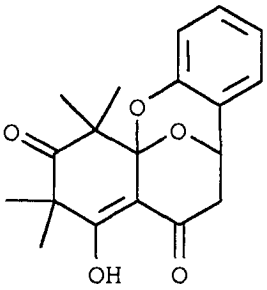
85

U.afzelii[39]



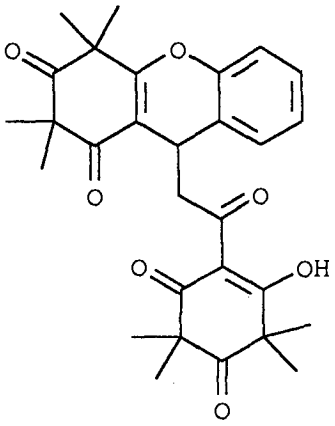
86

U.afzelii[39]



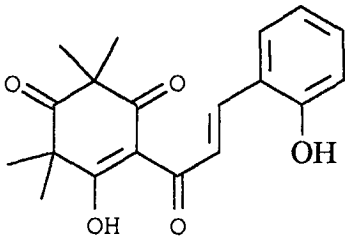
87

U.afzelii[40]



88

U.afzelii[40]



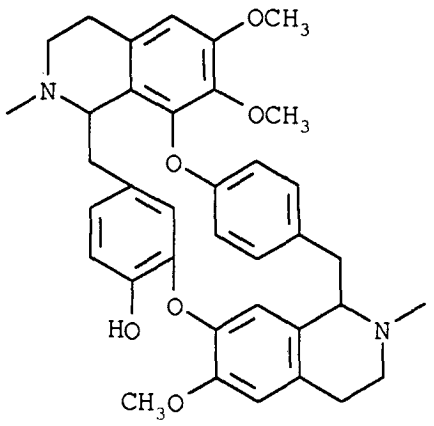
89

U.afzelii[39,40]

Chart-12

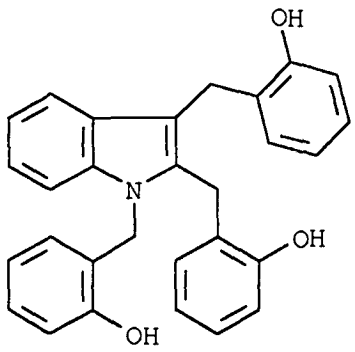
7. Alkaloids :

Chondrofoline **90** was the first alkaloid isolated from *Uvaria* species (*U.ovata*)[41] and belongs to the bis benzyl isoquinolene group.



90

Reference is already made to the isolation of [illegible] terpene units attached to C-3 and C-3, C-6 positions of indole. These are regarded as meroterpenoids and hence listed separately (chart-3). Several indole based alkaloids such as uvarindole A,B, C, D and E (**91**, **92**, **93**, **94** and **95** respectively) have been isolated and characterized from *U.angolensis*. The characteristic feature is C-orthohydroxy benzylation at various reactive sites of indole nucleus. These are depicted in chart-13.



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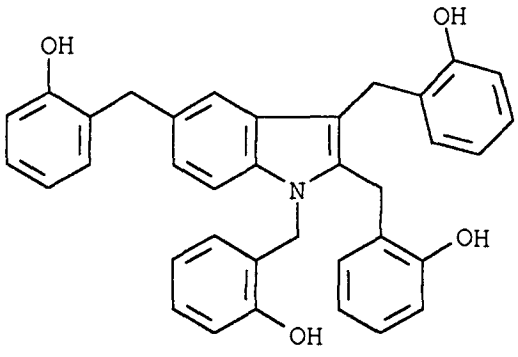

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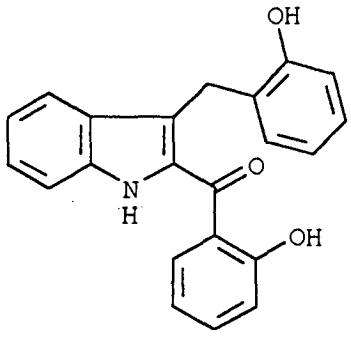

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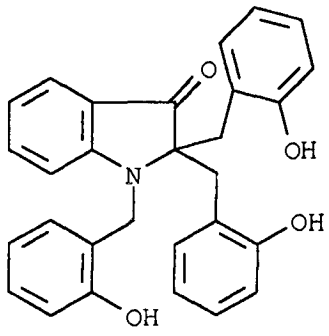

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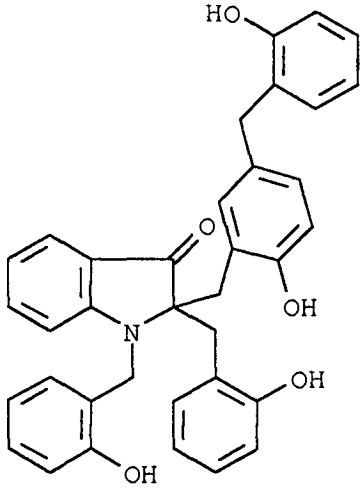
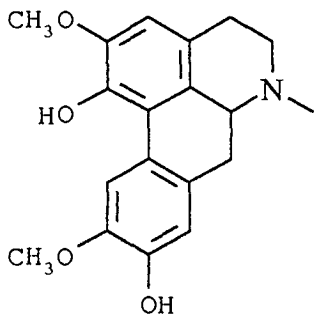


95

U.angolensis[42,43,44]

Chart-13

The more familiar type of alkaloids of genus *Uvaria* (*U.chamae*) are aporphines and proaporphines which includes isoboldine **96**, asimilobine **97**, glaziovine **98**, pronuciferene **99**, thaliporphine **100** and glaucine **101**. The structures of these alkaloids are shown in chart-14.



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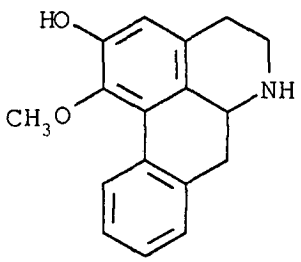

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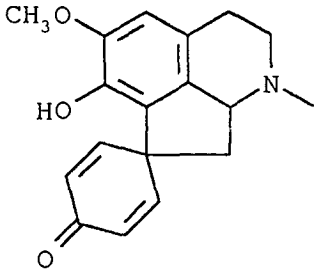

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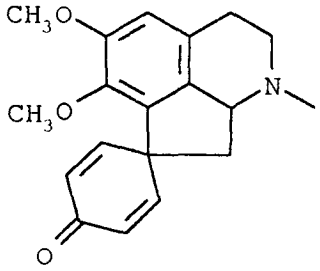

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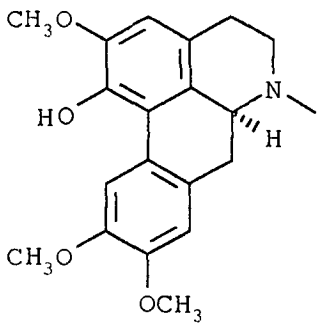

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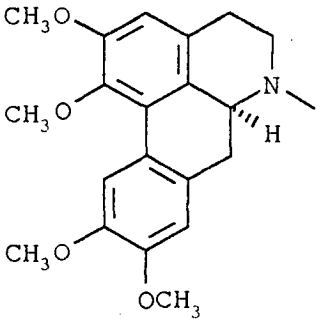
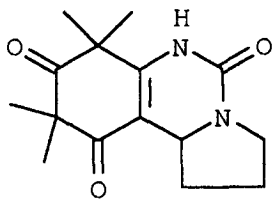


101

U.chamae[45]

Chart-14

The alkaloid syncarpurea **102** has been isolated from *U. afzelii*[46] along with syncarpic acid (chart-12). It is apparently derived by C-alkylation of syncarpic acid **84** by an intermediate **103** followed by cyclisation. Interestingly alkaloids having structures **104** and **105** have been isolated and characterized from *Hexbolus crispiflorus*.[47]



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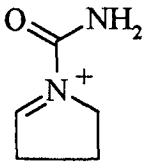

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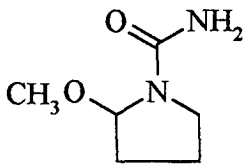

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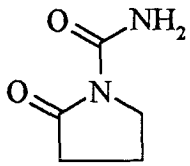
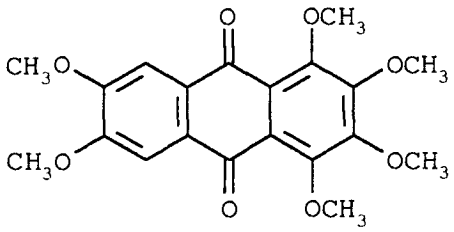


105

8. Miscellaneous compounds :

1,2,3,4,6,7-Hexamethoxy xanthone **106** and 7-methyl julgone **107** have been isolated from *U.kirki*.[48]



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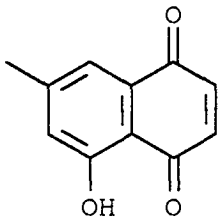

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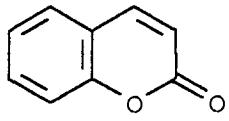
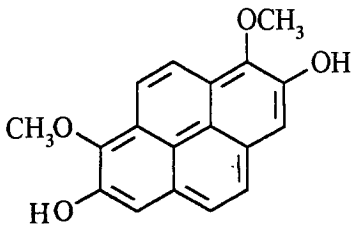


108

Coumarin **108** without any substitution in the aromatic ring has been shown to be a constituent of *U.afzelii*[39].

Very recently Achenbach and co-workers have reported the isolation of two new oxygenated pyrenes **109** and **110** from *U.lucida* ssp. lucida[49]. Another interesting compound and perhaps the biogenetic precursor of pyrenes **111** has also been isolated from same source and fully characterized.



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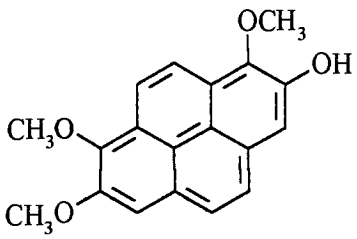

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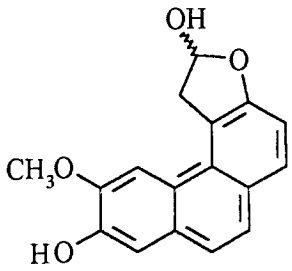


111

Part B

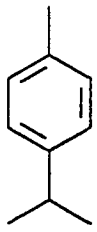
In part A of this review, the present status of the chemistry of various *Uvaria* species other than *U.narum* and *U.hookeri* is presented. The reason for not including these species is two fold, i) *U.narum* and *U.hookeri* are found to occur in India and have been subjected to chemical examination before and ii)the author of this thesis has done further work on *U.narum* and observed characteristic differences in the nature of chemical constituents of these *Uvaria* species and those described in part A.

About 18 species are reported to grow in India but *U.narum* and *U.hokeri* are widely known for their medicinal properties to the herbalists. A decoction of the root bark of *U.narum* is used to control fits in pregnant women at the time of delivery. It is also used foe treating rheumatic pains, bowel disorders and skin inflammations.

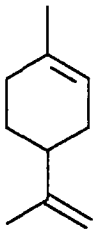
Iyengar and co-workers carried out the bioactivity studies on the essential oil obtained from the root bark of *U.narum* and identified some of the constituents by GCMS analysis[50]. This study was followed by further investigations by Hisham and collaborators.[52] The only other species which received attention is *U.hookeri*. There is some doubt, however, whether *U.narum* and *U.hookeri* are really distinctly different species. Some morphological features which can distinguish u.narum and *U.hookeri*

have been found by Manilal[51]. It is out of place to go in to further details. From the chemotaxonomic point of view, these two species appear to be identical.

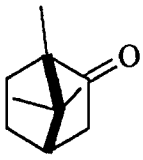
The essential oil obtained from the root bark of *U.narum* is mainly composed of mono and sesquiterpenes[52] (chart-15).



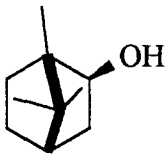
p-cymene



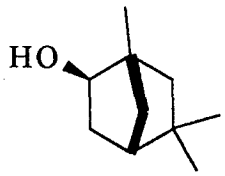
limonene



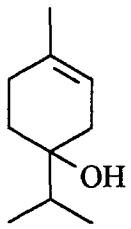
camphor



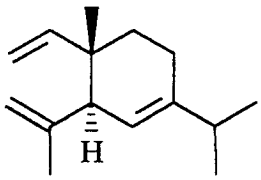
borneol



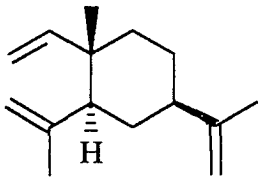
α-fenchyl alcohol



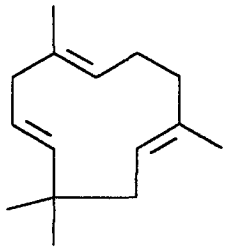
4–terpineol



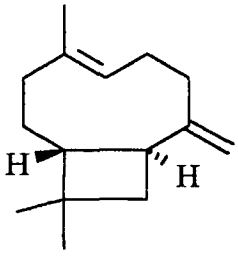
δ-elemene



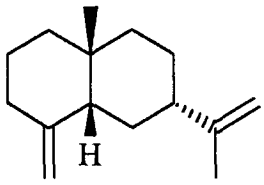
β-elemene



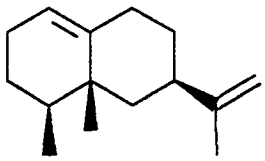
humulene



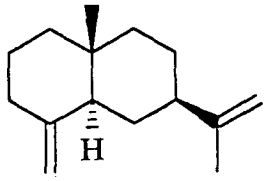
caryophyllene



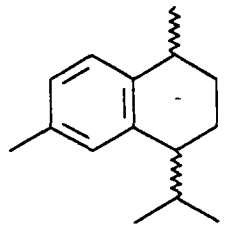
7-epi-β-selinene



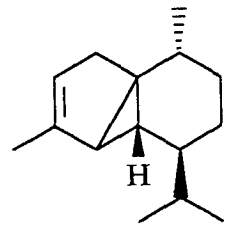
eremophilene



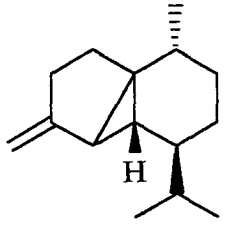
β- selinene



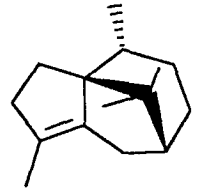
calamenene



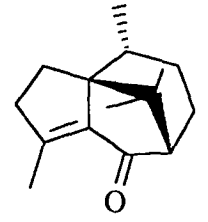
α-cubenene



β-cubenene



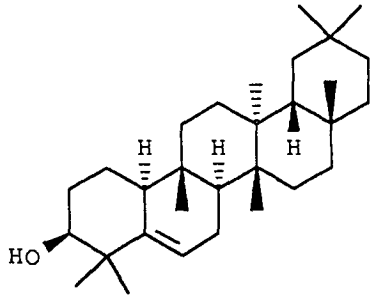
cyperene



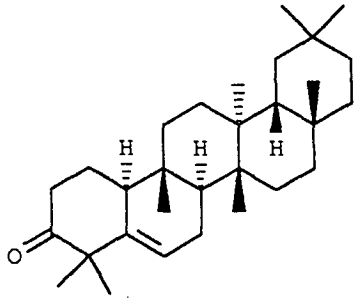
patchoulenone

Chart - 15

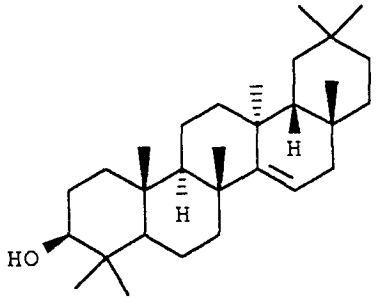
Petroleum ether extract of the air dried leaves has been shown to contain the triterpene glutinol **112**, and C_{55} terpenoid betulapren-11-ol **113**[53] while glutinone **114** and teraxerol **115** are reported from the root bark along with glutinol **112**[54]. The compounds are listed in chart-16.



112



114

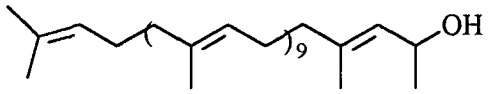


115

U.scheffleri[7] *U.narum*[54]

U.scheffleri,U.dependens.[7,17] *U.narum*[54]

U.scheffleri, U.dependens.[7,17] *U.narum*[54]

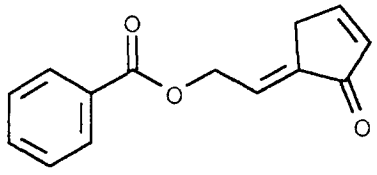


113

U.narum[53]

Chart - 16

Benzyl benzoates and the compounds derived by the oxidative modifications of the aromatic ring are not so abundant in *U.narum* or *U.hookeri** . In fact, in contrast to the other uvaria species, the presence of benzyl benzoate could be detected only by GCMS. Though present in trace quantities, unambiguous characterization of new benzoic acid ester **116** from *U.narum* has been reported by Parmar et.al.[53] There is no doubt that **116** is biogenetically derived by oxidative modification of aromatic ring of the benzyl group.



116

U.narum[53]

Bates and co-workers have proposed that the precursor for the C-benzylated products of *Uvaria* species is probably derived from benzyl benzoates. Since benzyl benzoates are present in trace quantities, absence of benzopyranyl terpenoids in *U.narum* is understandable.

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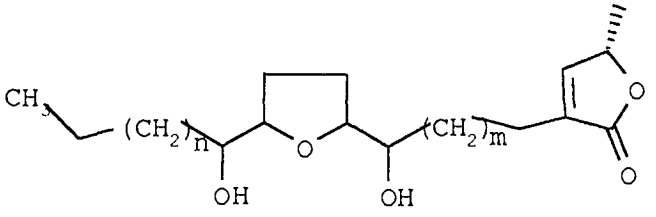
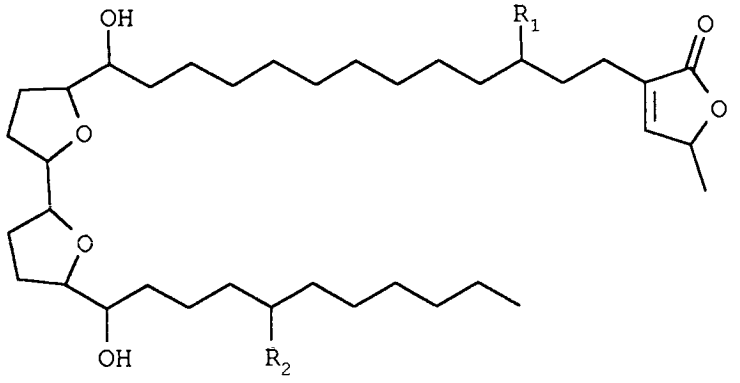
* There is only one report on chemical investigation of *U.hookeri*.[55]

From the published literature as well as our own work on *U.narum* another interesting fact is the absence of chalcones, dihydrochalcones, flavanoids and nitrogen bases. The reported medicinal properties (CNS depressent, antibacterial, antifungal of *U.narum* and *U.hookeri*) must be due to the uvariamicins and other reported acetogenins. Acetogenins isolated from *U.narum* includes uvariamicins I, II, III (**117**, **118** and **119** respectively), isodesacetyluvaricin **120**, squamocin-28-one **121**, narumicins I and II (**122** and **123** resp.), squamocin **124** and panalicin **125** which are reported in chart-17.[54,56,57] The individual screening of the acetogenins exhibited antimicrobial and anthelmintic activities.



117 --- n=12, m=11, threo-trans-threo (relative stereochemistry of THF core)
118 --- n=10, m=13, threo-trans-threo
119 --- n=8, m=15, threo-trans-threo



		R_1	R_2	
120	---	—H	—H	--- threo-trans-threo-trans-threo
121	---	—H	=O	--- threo-trans-threo-trans-erytho
122	---	—OH	—H	--- threo-trans-threo-trans-threo
123	---	—OH	—H	--- threo-trans-threo-trans-erytho
124	---	—H	—OH	--- threo-trans-threo-trans-erytho
125	---	—OH	—OH	--- threo-trans-threo-trans-erytho

U.narum[55,56,57]

Chart - 17

1.2 Chemical examination of methanolic leaves extract of *Uvaria narum*

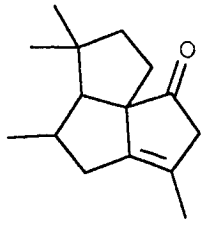
Present status of the chemistry of genus *Uvaria* is presented briefly in the first section of this chapter. No attempt is made to present a comprehensive survey but the leading references given at the end of this chapter would prove useful for anyone who is interested in getting further details about a particular species.

After the initial whirling movement, the work on the isolation of natural products and their structure determination suffered a temporary setback. The chances of isolating a new bioactive natural product were becoming bleak and perhaps was the main reason for organic chemists to look for their availability through synthesis. Naturally more emphasis was on synthetic aspects. Several bioactive and/or structurally complex natural products were synthesised. Newer organic reagents and synthetic methodologies were developed. However, from the view point of economy, availability, toxicity, tolerance and environmental problems, the plant drugs once again looked much superior to their synthetic counterparts.

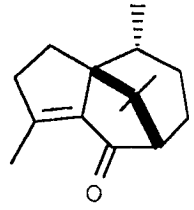
The unbelievable developments which were taking place in analytical chemistry, particularly in separation techniques and instrumental analysis made it possible for organic chemists to look back again to the plant kingdom for bioactive molecules which can serve as natural drugs or lead molecules.

During the search for new antitumor agents the C-benzylated dihydrochalcones uvaretin **31** and chamuvaretin **36** were found to be active against P-388 lymphocytic leukemia and were isolated from *U.accuminata*[18] and *U.chamae*[58] (annonaceae) respectively. This resulted in a hectic activity in investigating various species of the genus *Uvaria* for their chemical constituents. The collective outcome of these studies is presented earlier (section 1.1).

For us, the southern parts of Goa (Canacona and nearby areas) and northern parts of Karnataka provided one of the well characterized Indian species, *Uvaria narum* and hence we selected it for our studies.* When our studies were undertaken, we came across a research paper which described the isolation and characterisation of a sesquiterpene ketone, narcinone **126** having a new carbon framework.[59] The assigned structure was based on a detailed spectral analysis and posed an intriguing problem from biogenetic point of view. Since narcinone **126** was stated to be one of the main constituent of *U.narum*, other structurally related sesquiterpenes were expected to be present in this source. This expectation was short lived as Hisham and co-workers established the identity of narcinone **126** with patchoulenone **127**,[60] a well characterized constituent of *Cyperus rotundus*[61] and other *Cyperus* species.



126



127

* We thank Goa state council for science and technology for financial support.

The results obtained during our chemical examination of the methanol extract of the leaves, stem and roots are presented.** The leaves and stem of the plant material was collected near udupi (Karnataka state) about 100 kms. South of Goa while roots of the plant was collected from calicut (Kerala state). Previous studies by Hisham et.al.[52, 54-57,60] and Parmar et.al.[53] were restricted to the leaves and root bark of *U.narum*. The examination of the stem bark has been carried out for the first time.

Chemical examination of the methanol extract of the leaves of *Uvaria narum*

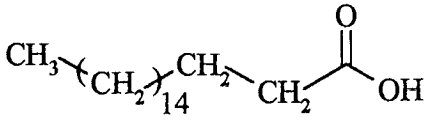
The methanol extract of the leaves was chromatographed on silica gel and individual components were isolated by elution with petroleum ether and increasing the polarity by addition of diethyl ether and finally elution with diethyl ether alone.

From the least polar fractions, a waxy solid designated as compound **A**, m.p. 63-65°C was obtained. Its IR spectrum (KBr) showed it to be a saturated hydrocarbon. Its ^{1}HNMR spectrum (fig-1.01) further confirmed the conclusions. The m.p. differed from tritricontane (m.p.73°C) previously isolated from *u.narum*[53] but matched well with that reported for tricontane. Mixture melting points with authentic triacontane also showed no depression. GCMS analysis, however showed it to be a mixture of several straight chain alkanes ($C_{22}H_{46}$ to $C_{33}H_{68}$). Untriacontane, $C_{31}H_{64}$ was found to be the major component (62.9%) accompanied by $C_{29}H_{60}$ (14.81%) and tritriacontane $C_{33}H_{68}$ (19%). Compound **A** was therefore found to be mixture of

** Characterisation of all the constituents is almost entirely based on ^{1}HNMR data. We thank Professor R. B. Bates for the spectral data and valuable suggestions and help in identification of the individual components. No instrumental facilities were available and the investigation was carried out in collaboration with Professor Bates.

straight chain alkanes. It is to be noted that mixture melting point determination cannot be used as a criteria for establishing the identity in case of such compounds.

Compound **B** which is eluted from the column with petroleum ether-ether (90:10) was obtained as a solid, m.p. 78°C (single spot, TLC). Further purification was done by repeated column chromatography before measuring its spectral data. Surprisingly, ^{1}HNMR spectrum (fig-1.02) showed it to be a 1:1 mixture of two compounds having R-CH_2-COOH and R`-CH_2-CH_2-OH. The methylene next to carboxyl group appeared as a triplet at δ 2.36, while the methylene of the primary alcohol showed a downfield triplet (δ 3.65). The triplet due to terminal methyl (δ 0.88) and a broad singlet (δ 1.25) confirmed the presence of CH_3-$(CH_2)_n$- and the integration count clearly showed it to be a 1:1 mixture of stearic acid **128** and stearyl alcohol **129**. We believe that the hydrolysis of steryl sterate **130** took place while purification.*



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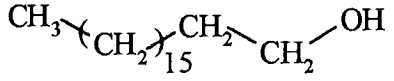

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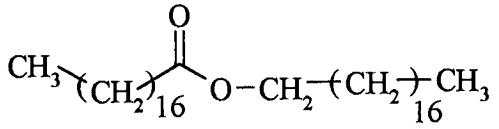


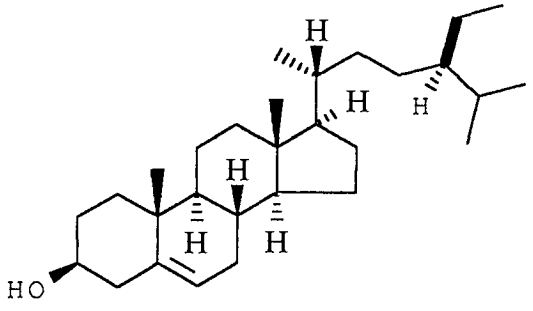
130

* A mixture of stearic acid and stearyl alcohol is expected to show two spots on TLC.

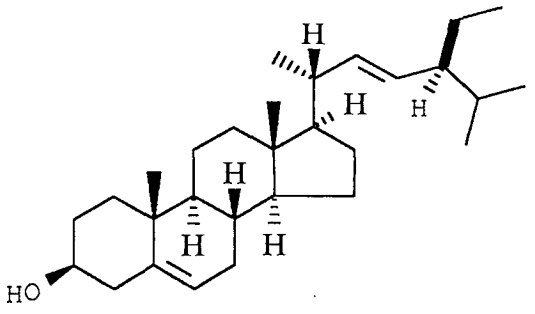
Compound **C** was isolated as a crystalline solid, m.p.83°C by elution of the column with petroleum ether-ether (1:1). Spectral data on this compound could not be obtained so far and still remains uncharacterised.

Further fractions deposited a solid, m.p.122°C which turned out to be identical with benzoic acid (IR, ^{1}HNMR, mix m.p.). Since benzyl benzoates are known to be the constituents of genus *Uvaria*, isolation of benzoic acid is understandable.

When column is eluted with ether (100%), a solid m.p. 165°C (compound **D**) was obtained. TLC showed it to be composed of two closely spaced spots but further separation could not be effected. Its ^{1}HNMR spectrum (fig-1.03) showed it to be a mixture of phytosterols and comparison of the chemical shifts with those reported for β-sitosterol **131** and stigmasterol **132** matched very well. Moreover, integration values of C_{22} and C_{23} vinyl protons (δ 5.0 and δ 5.14 respectively) showed that compound **D** is a 60:40 mixture of β-sitosterol **131** and stigmasterol **132**.



131



132

1.3 Chemical examination of methanolic root bark extract of *Uvaria narum*

In the preceding section, the isolation and characterisation of the chemical constituents of the methanol extract of the leaves of *U.narum* is reported. This section describes the identification of some of the individual constituents of the methanol extract of the roots, mainly through ^{1}HNMR spectral analysis. Wherever possible, the identification is supported by other data. At the end of this section the information regarding the constituents of the stem bark and GCMS data on the total essential oil of *U.narum* is presented.

The total methanol extract was fractionated using silica gel column chromatography and the individual fractions so obtained were further subjected to repeated column chromatography. The isolation and purification was monitored by TLC. Inspite of these efforts, only few constituents could be obtained in pure state. Some fractions which appeared pure (TLC) turned out to be still to be a mixtures of two closely related compounds from ^{1}HNMR or GCMS analysis. Nevertheless, since ^{1}HNMR spectra data for the previously characterised *Uvaria* constituents was readily available for comparison, it was possible to establish identity whenever we had inseparable mixtures.* Our studies confirmed the previous findings and added further information to the existing knowledge of *U.narum*.

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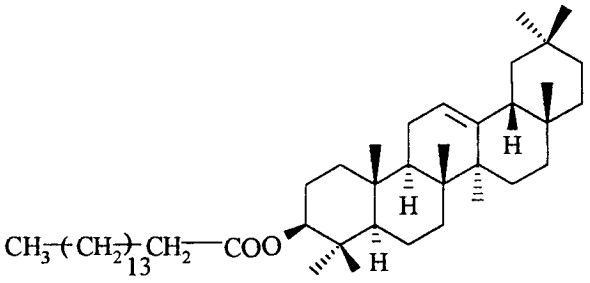
* We had no access to the modern preparative separation techniques.

The least polar fraction was found to be a colourless oil and composed of hydrocarbon mixture. The mono and sesquiterpene hydrocarbons characterised previously from *U.narum* are shown in chart-15. We did not attempt to isolate individual constituents as GCMS analysis of the of u.narum was made available to us.**

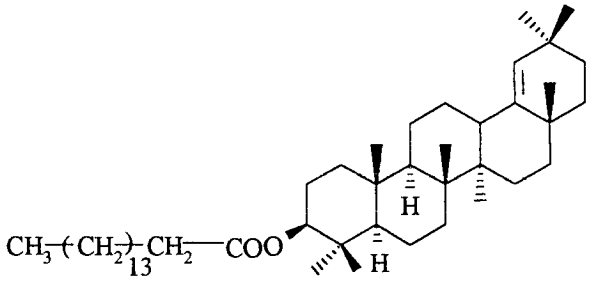
Fractions eluted with petroleum ether-benzene (90:10) on further purification yielded a low melting solid (m.p. 45-50°C). Its IR spectrum showed a band at 1730 cm^{-1} due to an ester carbonyl group. ^{1}HNMR spectrum (fig. 1.04) showed it to be a fatty acid ester of a triterpene having 8 tertiary methyl groups. The olefinic region showed two equal intensity signals (δ 4.8, s ; δ 5.18, t) together integrating for one proton. The one proton multiplet at δ 4.5 is clearly due to an axial C-3 methine. The ^{1}HNMR also showed all the typical signals of a fatty acid ester. The number of methylenes in the ester part showed it to be a palmitate ester. Thus we are handling a mixture of two pentacyclic triterpenes whose 3-β-hydroxyl group is esterified with palmatic acid.

The chemical shift and the splitting pattern showed that we are handling a 1:1 mixture of β-amyrin palmitate **133** and germancyl palmitate **134**. The sample was too small to carry out the hydrolysis experiments. It may be noted that a mixture of α-amyrin and β-amyrin palmitate has been shown to exhibit a strong antihapatotoxic activity. The natural occurrence of β-amyrin palmitate and germancyl palmitate is therefore of significance considering the reported medicinal properties of *U.narum*.

** We thank Dr. C. S. Narayanan for transferring the information to us on knowing our interest in *U.narum*.

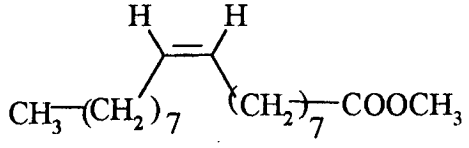


133



134

Fractions eluted with petroleum ether-benzene (80:20) were pooled and subjected to repeated column chromatography yielded a yellowish oil. Its IR spectrum showed a presence of a strong band at 1743 cm^{-1}. Typical of an ester carbonyl. The ^{1}HNMR spectrum (fig. 1.05) revealed it to be an unsaturated methyl ester (δ 3.67, 3H and δ 5.43, m). The triplet due to -$C\underline{H}_2$-$COOCH_3$ appeared at δ 2.30 (J=7.6 Hz). The olefinic pattern was exactly identical with the one observed for a -CH_2-CH=CH-CH_2- having a **Z** configuration. The ratio of the integration of the olefinic protons and -OCH_3 group clearly showed that the unsaturated ester is 60% and remaining component (40%) is saturated ester. On the basis of chemical shifts and integration values, we conclude that this fraction is composed of a mixture of methyl oleate **135** (60%) and methyl sterate **136** (400%). It may be noted that steryl sterate **130** has been shown to be a constituent of the leaves of *U.narum*.



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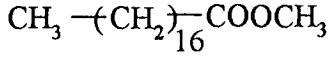
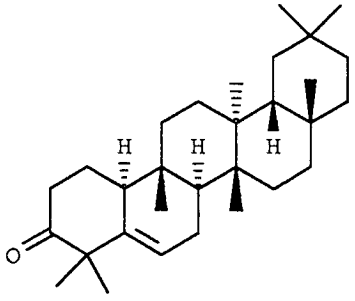


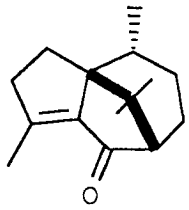
136

Fractions eluted with petroleum ether-benzene (1:1) afforded a high melting crystalline solid m.p. 234°C. Its ^{1}HNMR spectrum (fig.1.06) showed the presence of 8 tertiary methyl groups (8 x 3H, singlets) in the region (δ 0.8-1.25), a methylene next to a carbonyl group (δ 2.44, 2H, m) and a single olefinic proton (δ 5.68). all the chemical shifts (see experimental) showed identical values with those reported for glutinone **114**.[54] It may be noted that glutinone **114** has been shown to be a constituent of *U.narum* and other *Uvaria* species.



114

The next compound which followed glutinone **114** was obtained as a low melting solid, m.p. 45°C and identified as patchoulenone **127** ^{1}HNMR spectrum (fig.1.07) clearly showed two tertiary methyl singlets (δ 0.89, 1.02), a secondary methyl, a vinyl β with respect to carbonyl function (δ 2.06) and no signals above δ 2.9 .Comparison of the chemical shifts with those reported for patchoulenone **127**[60] unambiguously established their identity.



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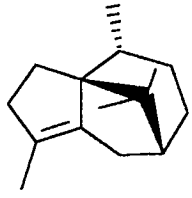
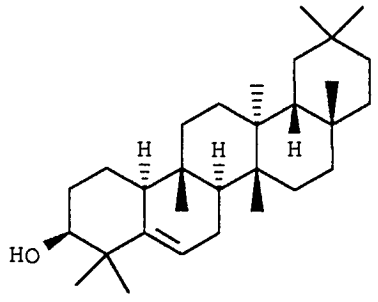


137

Patchoulenone **127**, was first isolated by Sorm and co-workers from *Cyperus rotundus*[61] which contains cyprene **137** as the major sesquiterpene hydrocarbon. Incidentally cyprene **137** has also been shown to be a constituent of the essential oil obtained from the root bark of *U.narum*. Co-occurrence of patchoulenone **127** and cyprene **137** is therefore understandable.

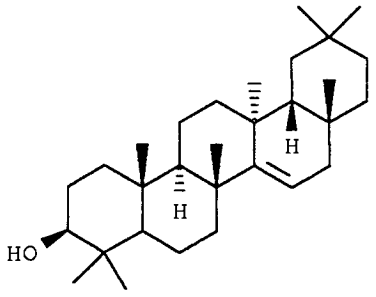
The chromatographic fractions which immediately followed patchoulenone **127** did not show any carbonyl absorption in the IR spectra but showed the presence of hydroxyl functionality. A crystalline solid was obtained from these fractions and was further purified by recrystallisation from $CHCl_3$-MeOH, m.p. 207°C in pure state. This compound was identified as glutin-5-ene-3-β-ol (glutinol) **112**. Its ^{1}HNMR spectrum is presented in fig.1.08 and the chemical shift values which matched with those reported for glutinol **112**[54] are given in experimental section.



112

Repeated chromatography of the benzene-ether (90:10) eluted fractions afforded another crystalline compound, m.p. 270°C in pure state. The ^{1}HNMR spectrum (fig.1.09) showed that this also belongs to the pentacyclic triterpene group having 8 tertiary methyl groups, a C-3 methine proton (δ 3.19, m) and a olefinic

proton (δ 5.53, m).The melting point and ^{1}HNMR chemical shifts were fully identical with those reported for taraxerol **115**.[54] This identification also confirmed the previous report on the natural occurrence of **115** in the root bark of *U.narum*.



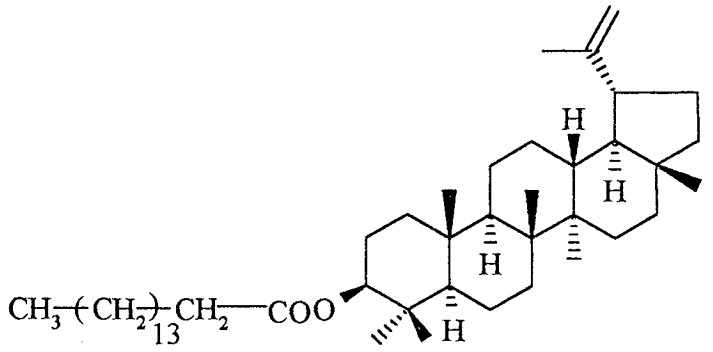
115

Besides characterisation of the compounds presented above, we have also found that the methanol extract of the root bark contains benzoic acid and a 60:40 mixture of β-sitosterol **131** and Stigmasterol **132**. These compounds were found to be the constituents of the leaves of *U.narum*.

From the tail fractions, we have isolated two more compounds one of which appears to a fatty acid and the other, a high melting solid, m.p.267°C, practically insoluble in all organic solvents but slightly soluble in pyridine. Its ^{1}HNMR measured in pyridine showed signals of the solvent. The signals coming from the protons of the compound are too weak to draw any conclusions regarding the structural features. Perhaps derivatisation may help in improving its solubility.

During the present study, we had extracted the powdered stem bark of *U.narum* with methanol. By initial column chromatography and subsequent rechromatography of individual fractions, three triterpenes were isolated. These have

been identified as lupeol palmitate **138**, lupeol **139** and lupenone **140**.* There is no previous report on the isolation of lupane based triterpenes from any species of the genus uvaria. Lupeol palmitate 138 has been isolated before from *Sambucus formosana* Nakai (caprifoliaceae) and shown to posses antihepatotoxic activity.



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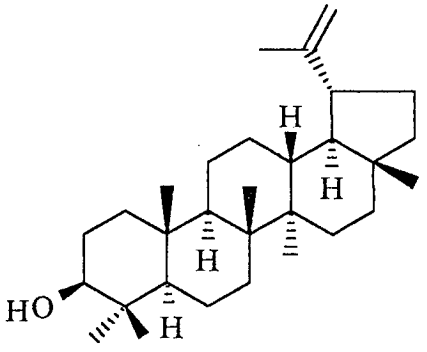

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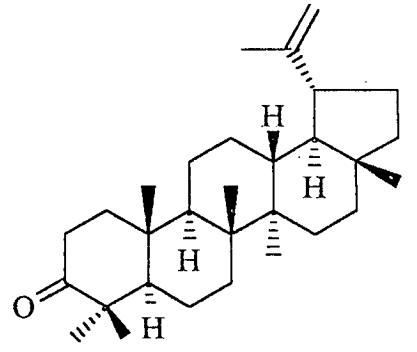
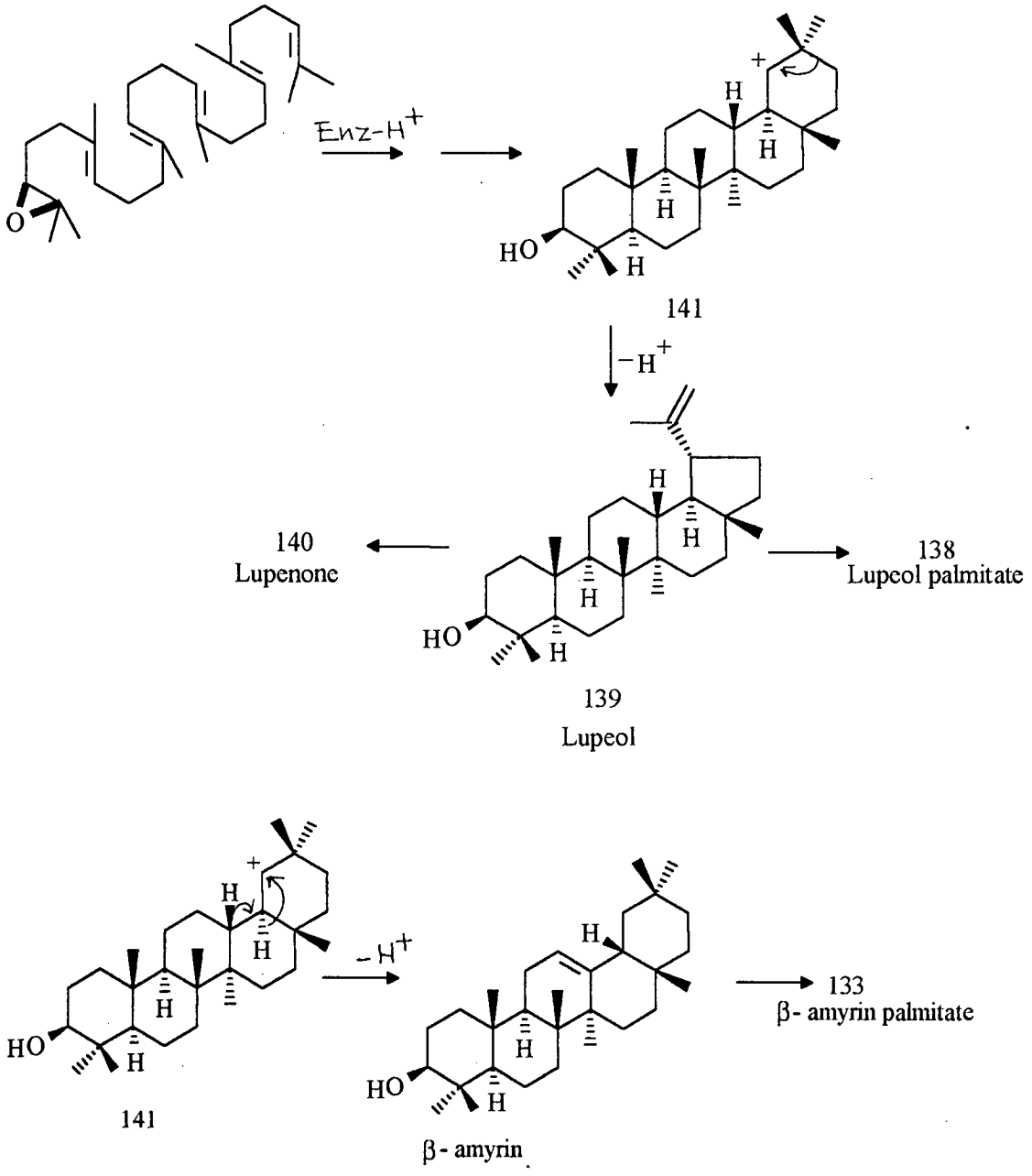
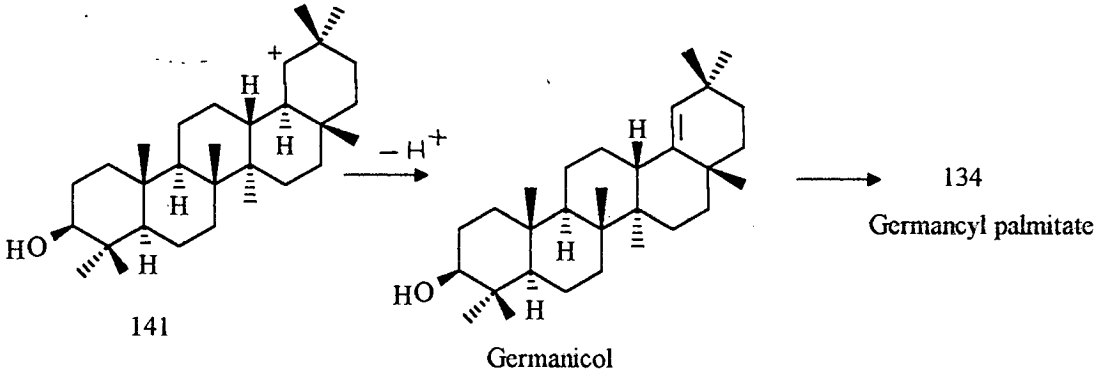


140

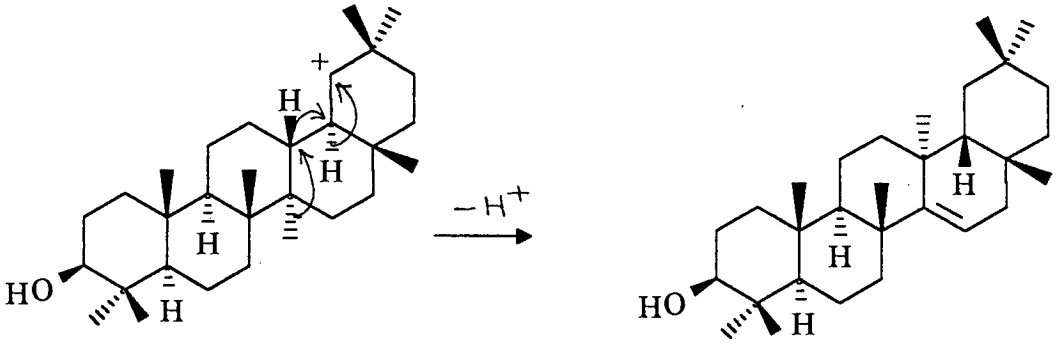
* The chromatographic separation and identification was done by Ms Usha Kelkar as a part of M.Sc. project (Goa University, 1994) and hence spectral details are not included here.

The pentacyclic triterpenes isolated from *Uvaria narum* (leaves / root bark / stem bark) are interesting from biogenetic viewpoint. All of them can be derived from the intermediate carbocation **141** which in turn is known to be derived from squalene epoxide. For the sake of clarity the intermediate **141** is drawn separately and formation of the end product is shown with the help of curved arrows.

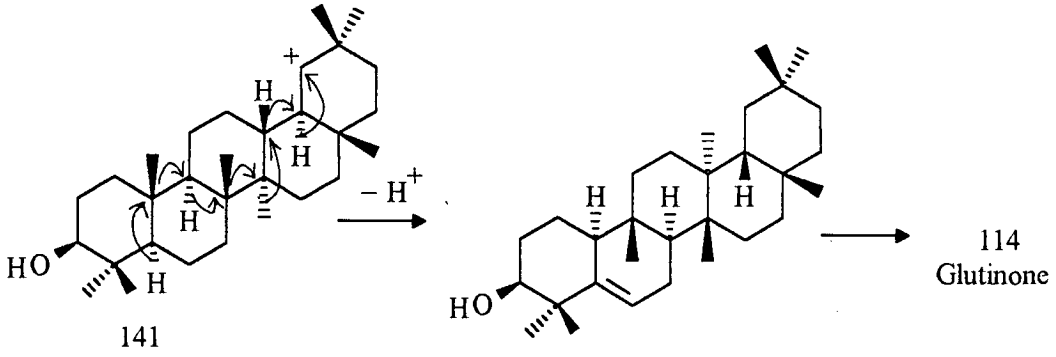




141 Germanicol 134 Germancyl palmitate



141 115 Taraxerol



141 112 Glutinol 114 Glutinone

Based on the biogenetic considerations, it would be possible to predict the natural occurrence of other pentacyclic triterpenes in *U.narum* which can be derived from the carbocation intermediate.

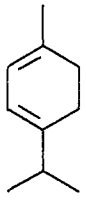
In view of the antihapatotoxic activity of lupeol palmitate **138**, it would be worthwhile to prepare palmitate esters of other easily available triterpene alcohols for testing their bioactivity.

GCMS analysis of the root bark essential oil of *Uvaria narum*

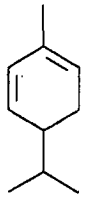
Preliminary work on the GCMS analysis of the essential oil of the root bark was reported by Nanda et.al. Caryophyllene 142 was unambiguously identified while tentative identification of 4 other compounds was also reported. In a more detailed study, Hisham et.al. detected at least 52 components and identified 22 of them (chart-15). Bornyl acetate and patcholenone **127** were found to be the major components.

The purpose of our investigation was to scrutinize the data more carefully and gain further insight in to the nature of other constituents. We have now tentatively identified 12 more terpenoids by matching the mass spectral data with the reference spectra on known compounds. The newly identified compounds are listed in chart-18.

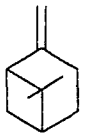
Monoterpenes



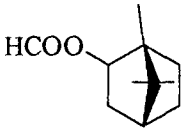
α-terpinene



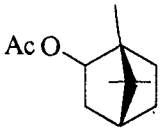
α-phellandrene



β-pinene



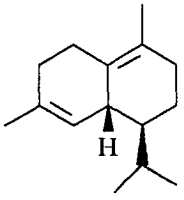
bornyl formate



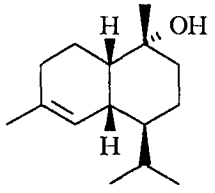
isobornyl acetate

1,8-cineole

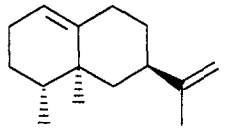
Sesquiterpenoids



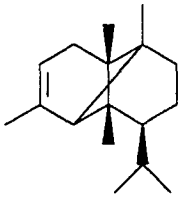
δ-cadinene



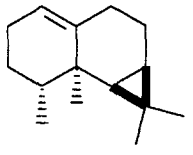
T-cadinol



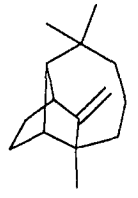
valancene



copaene



β-gurjunene



longifolene

Table-18

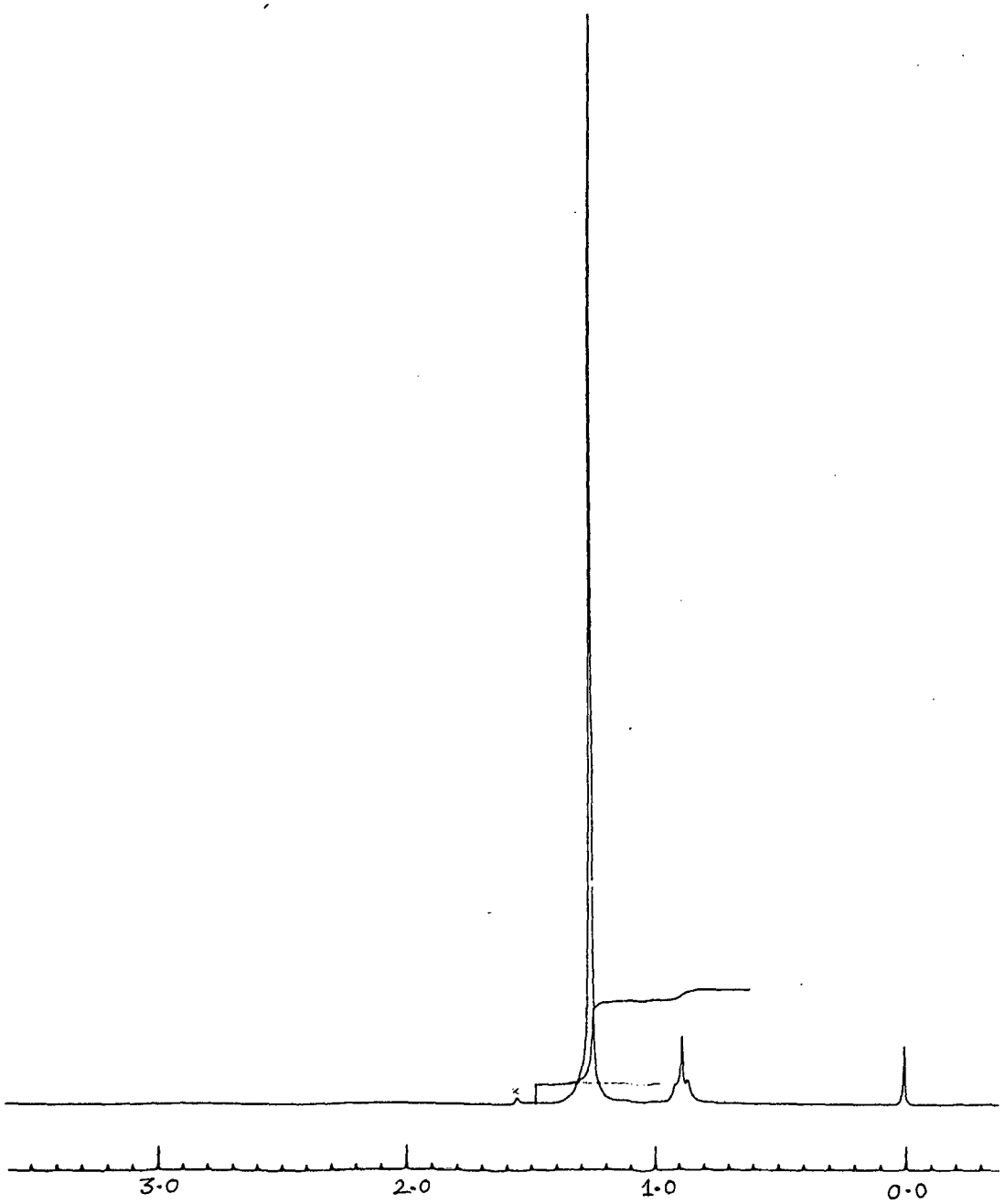


Fig. 1.01 : ^{1}H NMR spectrum of Compound A

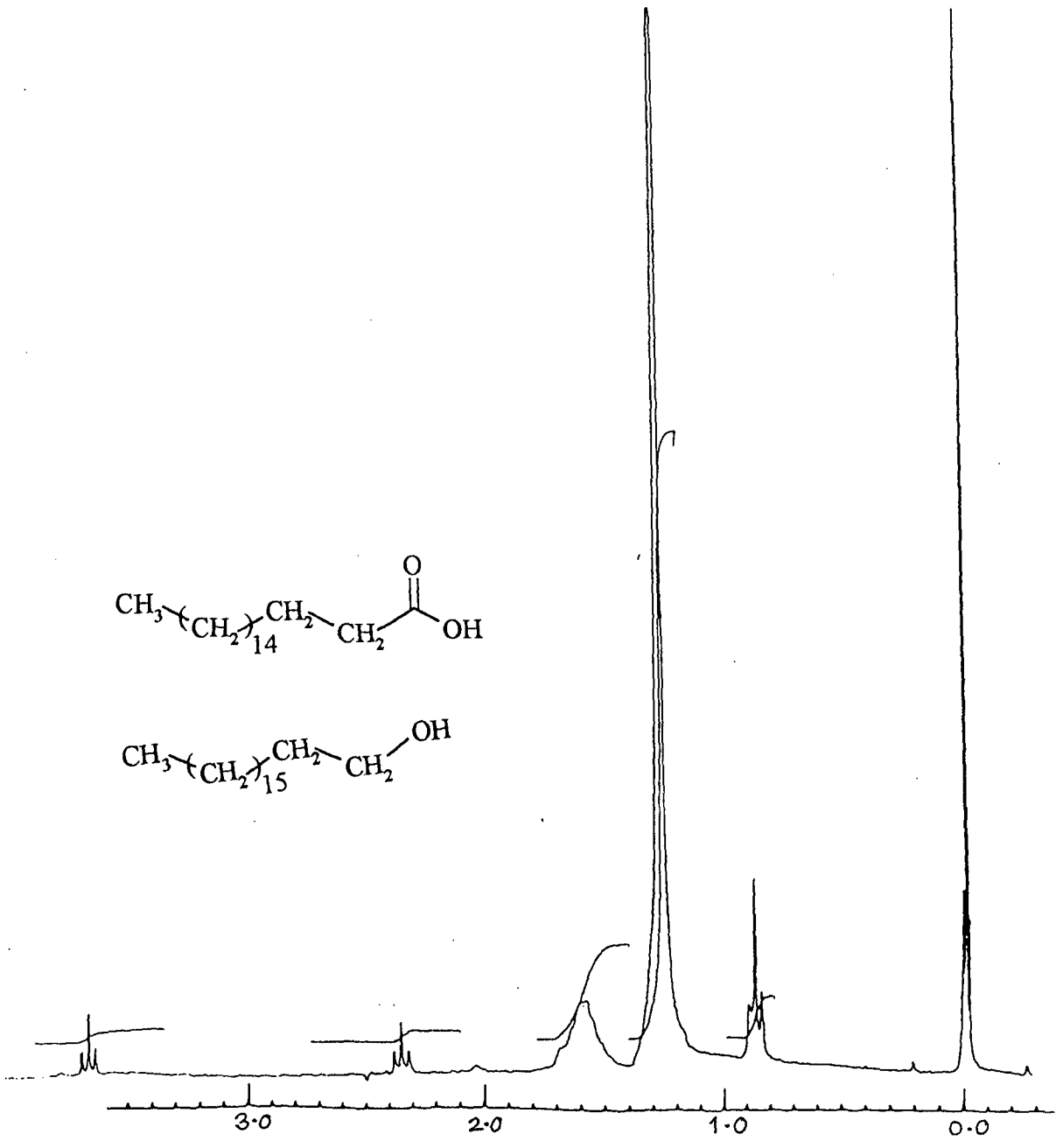


Fig. 1.02 : ^{1}H NMR spectrum of 128 + 129

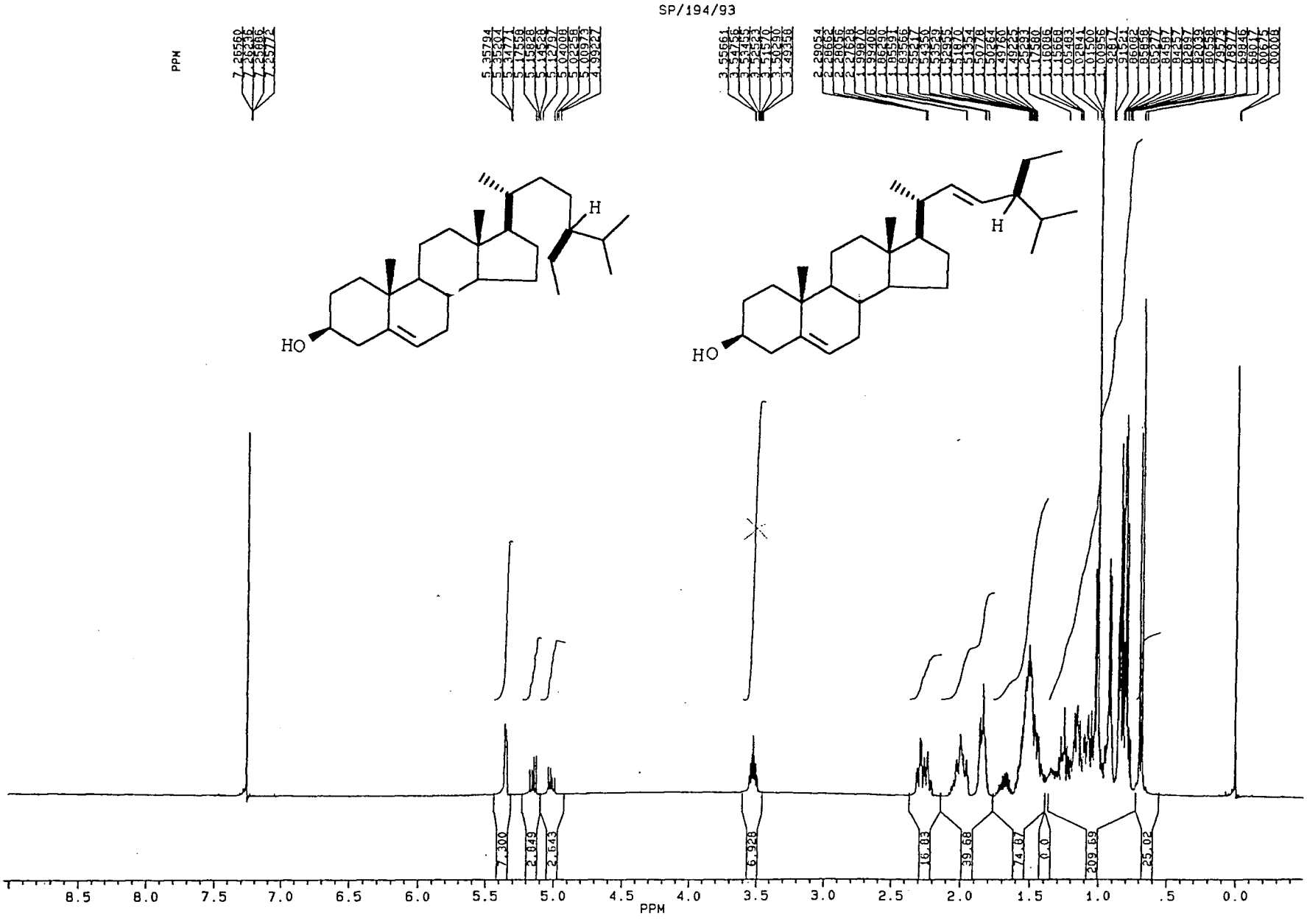


Fig. 1.03 : ^{1}H NMR spectrum of 131 + 132

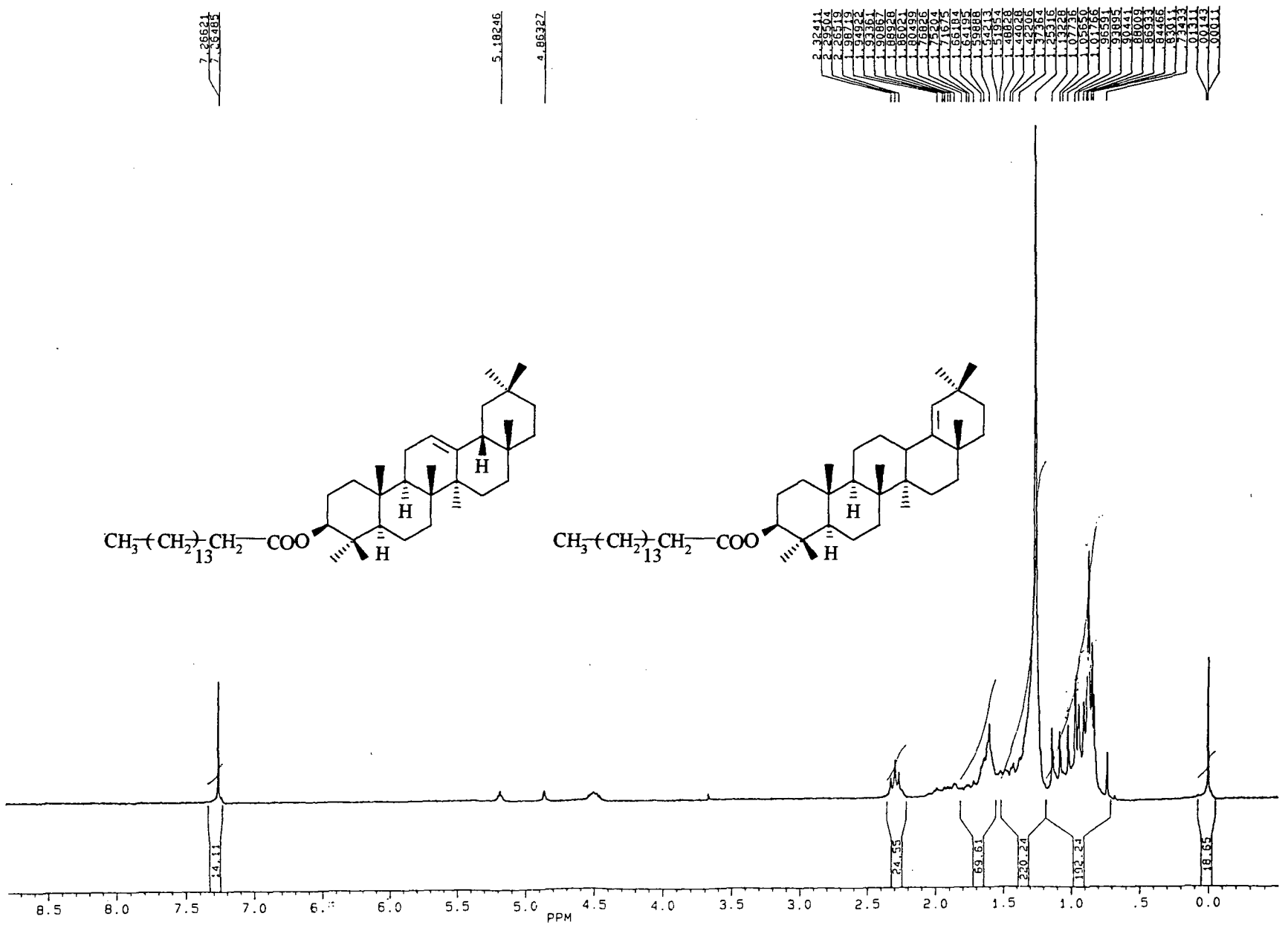


Fig. 1.04 : ^{1}H NMR spectrum of 133 + 134

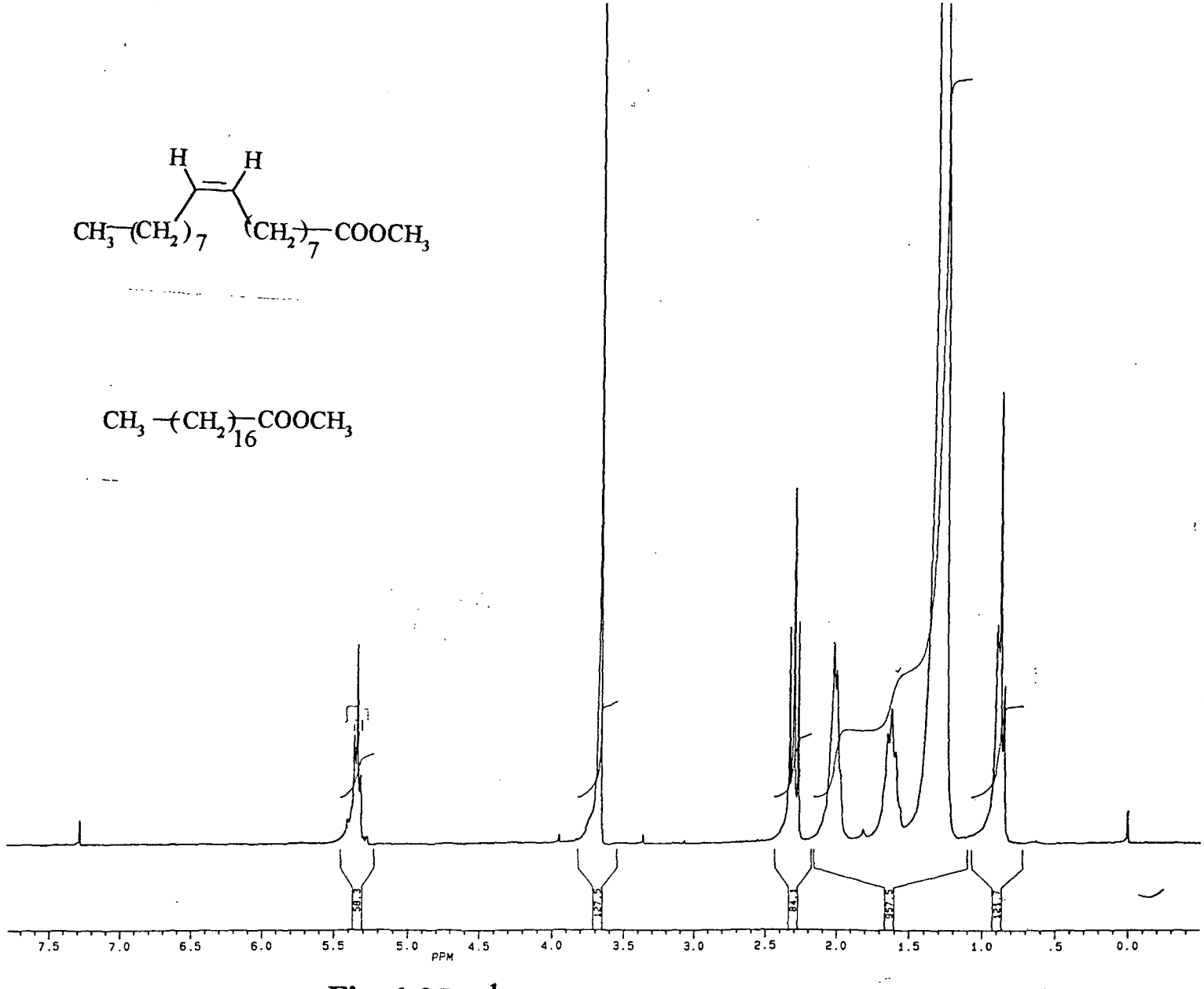


Fig. 1.05 : ^{1}H NMR spectrum of 135 + 136

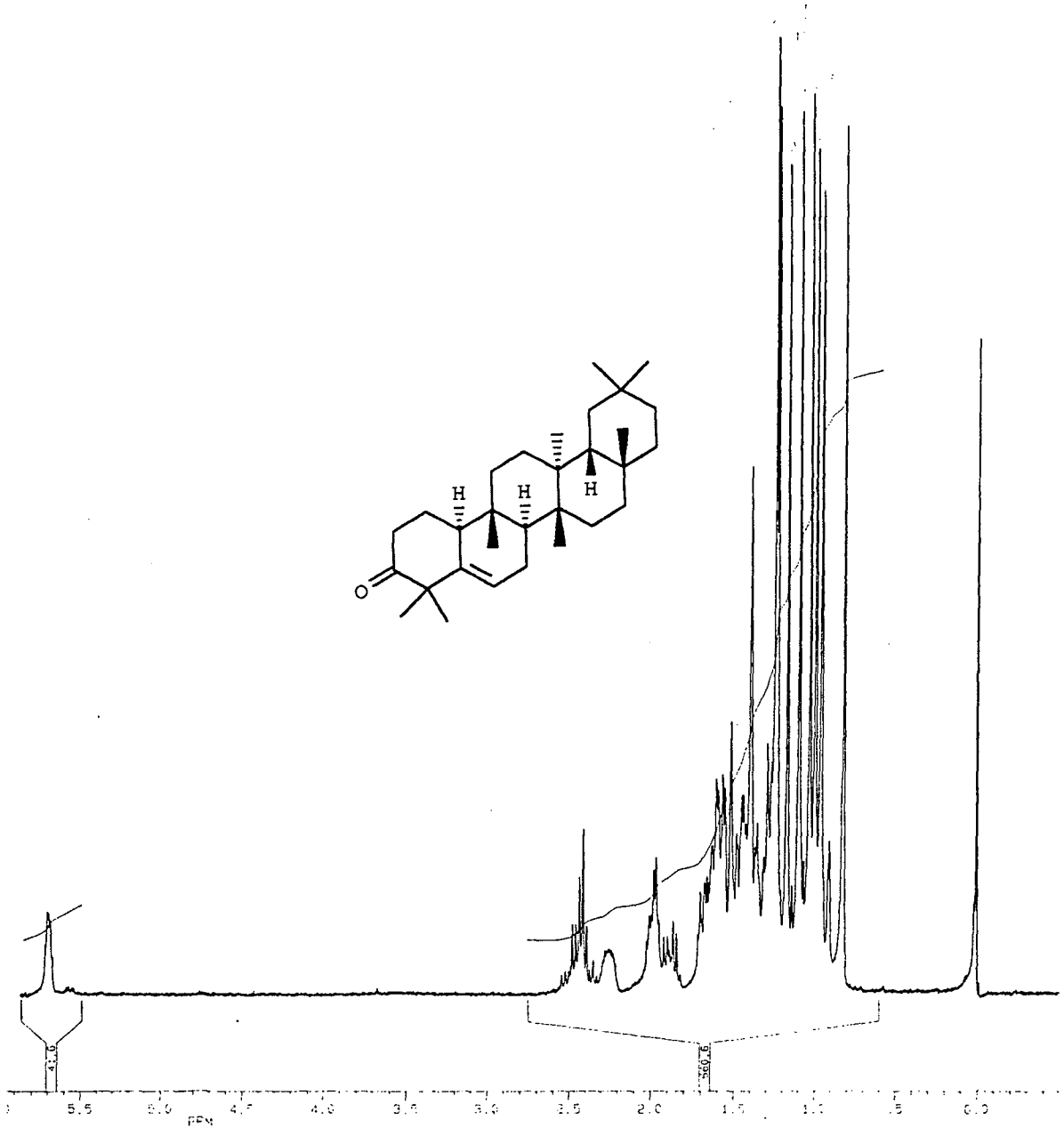


Fig. 1.06 : ^{1}H NMR spectrum of 114

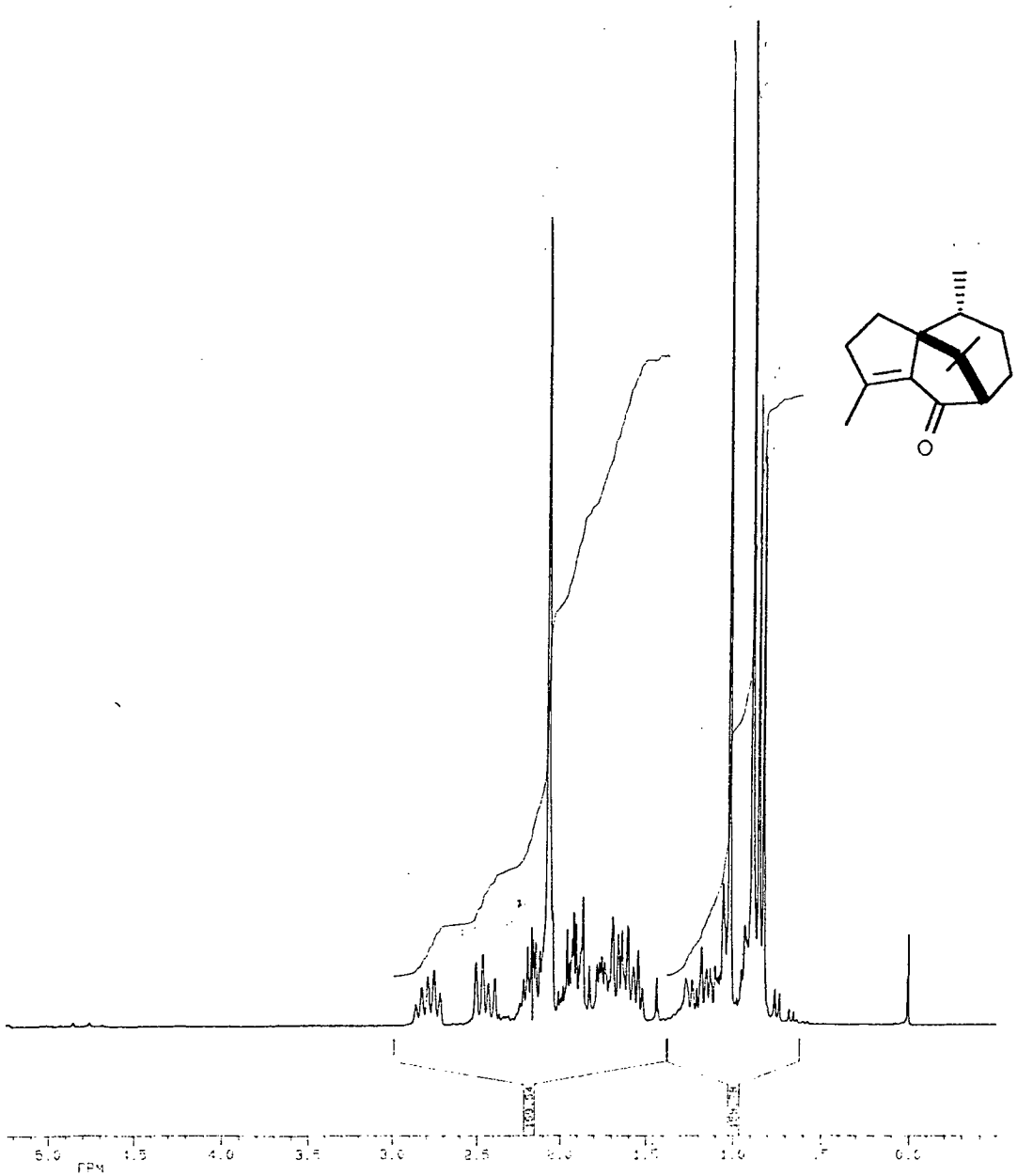


Fig. 1.07 : ^{1}H NMR spectrum of 127

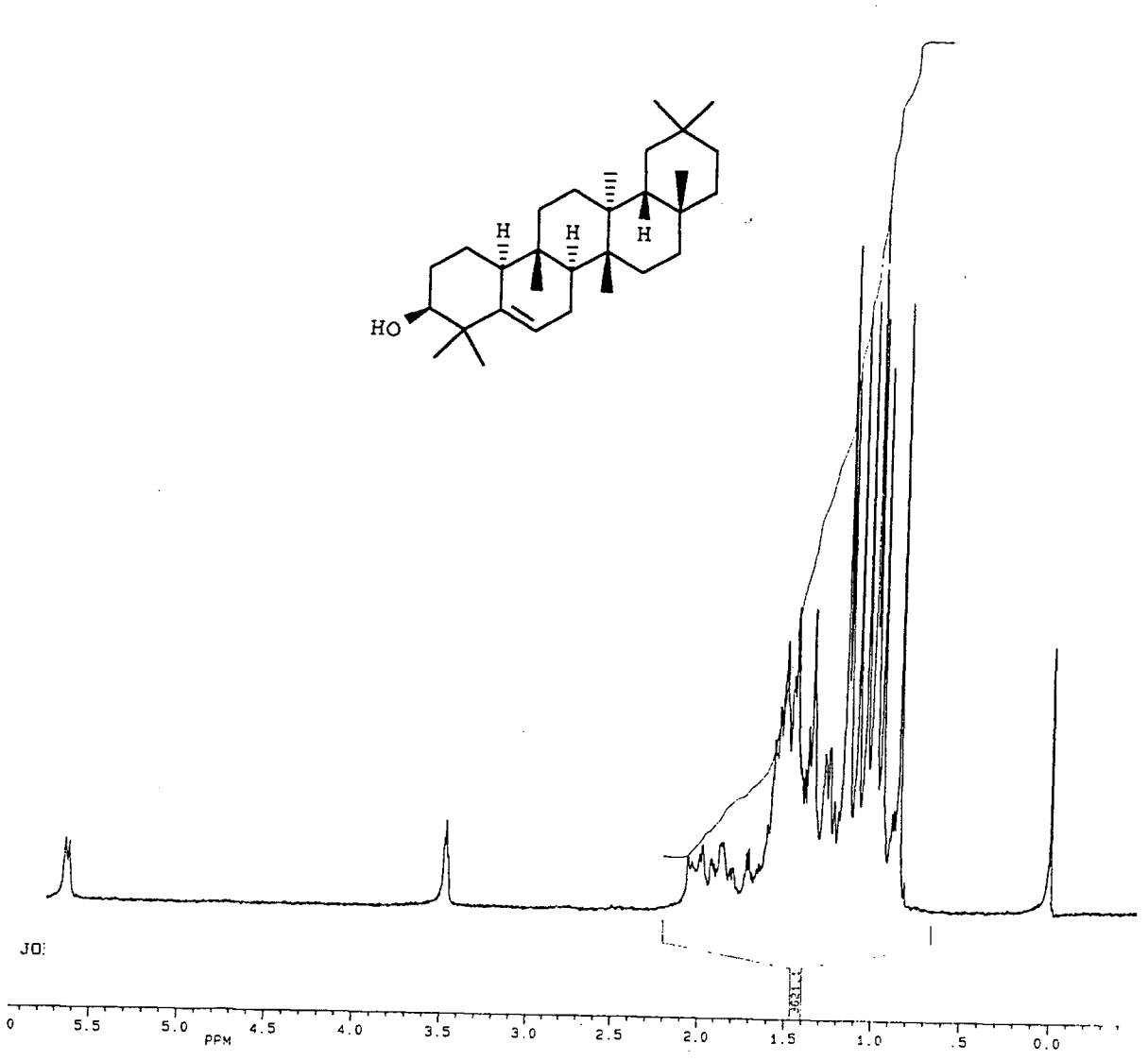


Fig. 1.08 : ^{1}H NMR spectrum of 112

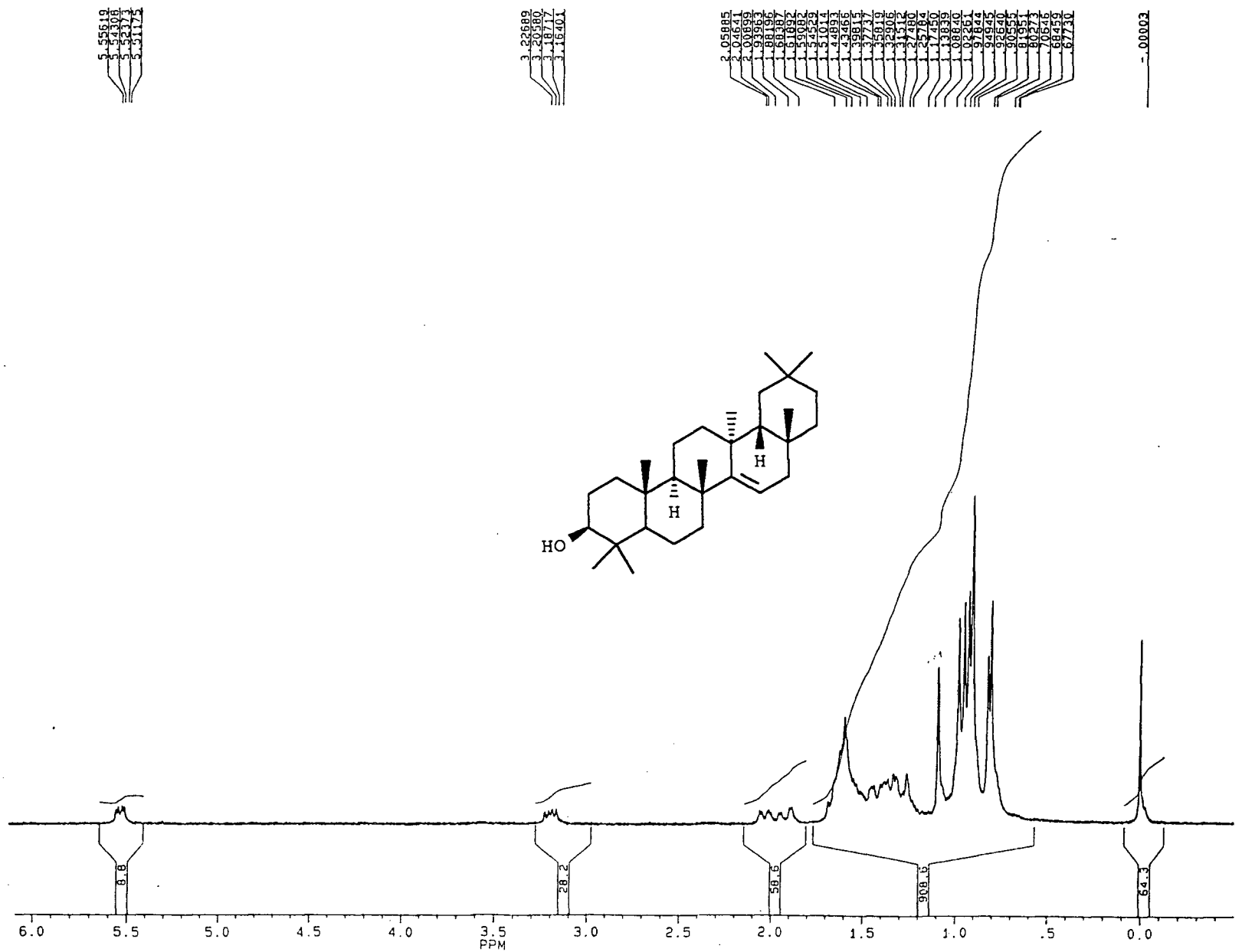
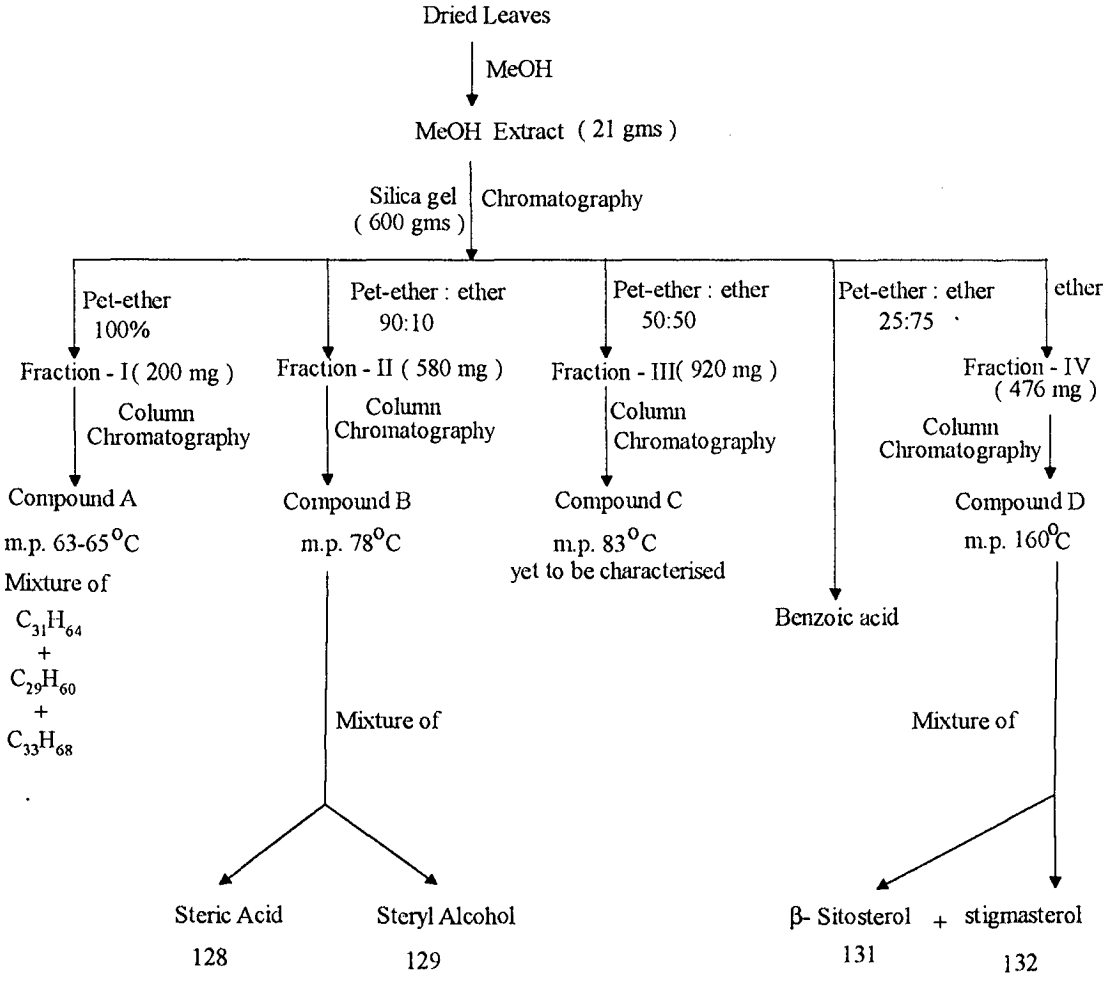


Fig. 1.09 : ^{1}H NMR spectrum of 115

Experimental :

Chemical examination of *Uvaria narum* wall (Annonaceae). :

The leaves of *Uvaria narum* were collected from Udupi (Karnataka state) and identified by Dr. M. Janarthanan of Botany department, Goa University where voucher specimen is deposited. The leaves (1.3 kg) were shade dried, grinded and extracted with methanol. The extract was concentrated under reduced pressure to a greenish viscous mass. The crude methanolic extract (21 gm) was fractionated over silica gel (600 gm) using successively pet.ether and ether in the increasing order of polarity.



Compound A : m.p.63-65 °C

IR : ν_{max} (KBr) : 2850, 1465 cm^{-1}

GCMS analysis :

R.T.	%	M.F.
13.56	14%	n- $C_{29}H_{60}$
14.49	3%	n- $C_{30}H_{62}$
15.89	60%	n- $C_{31}H_{64}$
18.96	18%	n- $C_{33}H_{68}$

Steric acid **128** + Steryl alcohol **129** (1:1 mix) : m.p. 78 °C

Steric acid **128** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.88 (3H, t, J = 6.5 Hz, $-CH_3$), 1.51-1.64 (2H, br, β-CH_2), 2.36 (2H, t, J = 7.4 Hz, α-CH_2), 1.25 (14 x 2H, all other protons).

Steryl alcohol **129** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.88 (3H, t, J = 6.5 Hz, $-CH_3$), 1.51-1.64 (2H, br, β-CH_2 and -OH), 3.65 (2H, t, J =6.5 Hz, α-CH_2), 1.25 (15 x 2H, all other protons).

Compound C : m.p. 83 °C

Yet to be characterised

β-Sitosterol **131** + Stigmasterol **132** (40:60 mixture) : m.p. 160°C

β-Sitosterol **131** :

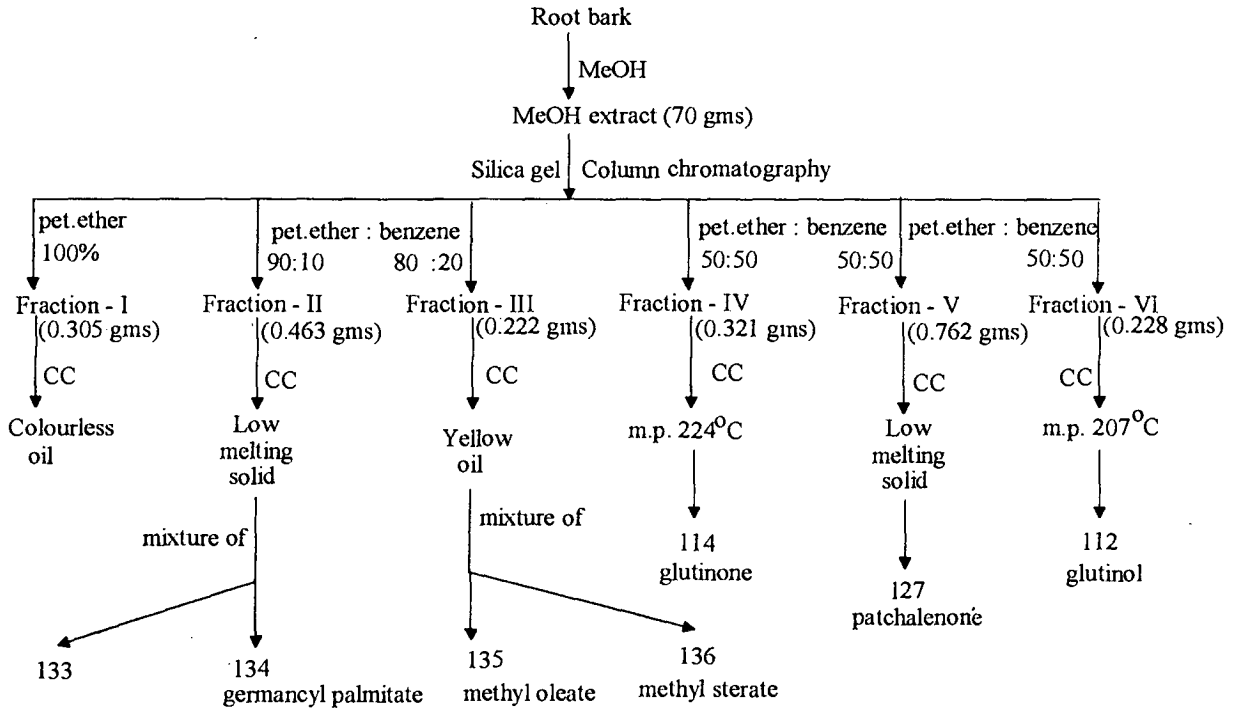
^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.68, 0.81, 0.83, 0.84, 0.92, 1.01 (3H each, 2 x s, 3 x d, 1 x t, 6 x CH_3), 3.51 (1H, m, C_3-H), 5.35 (1H, t, C_5-H), 0.8-2.3 (m, all other protons).

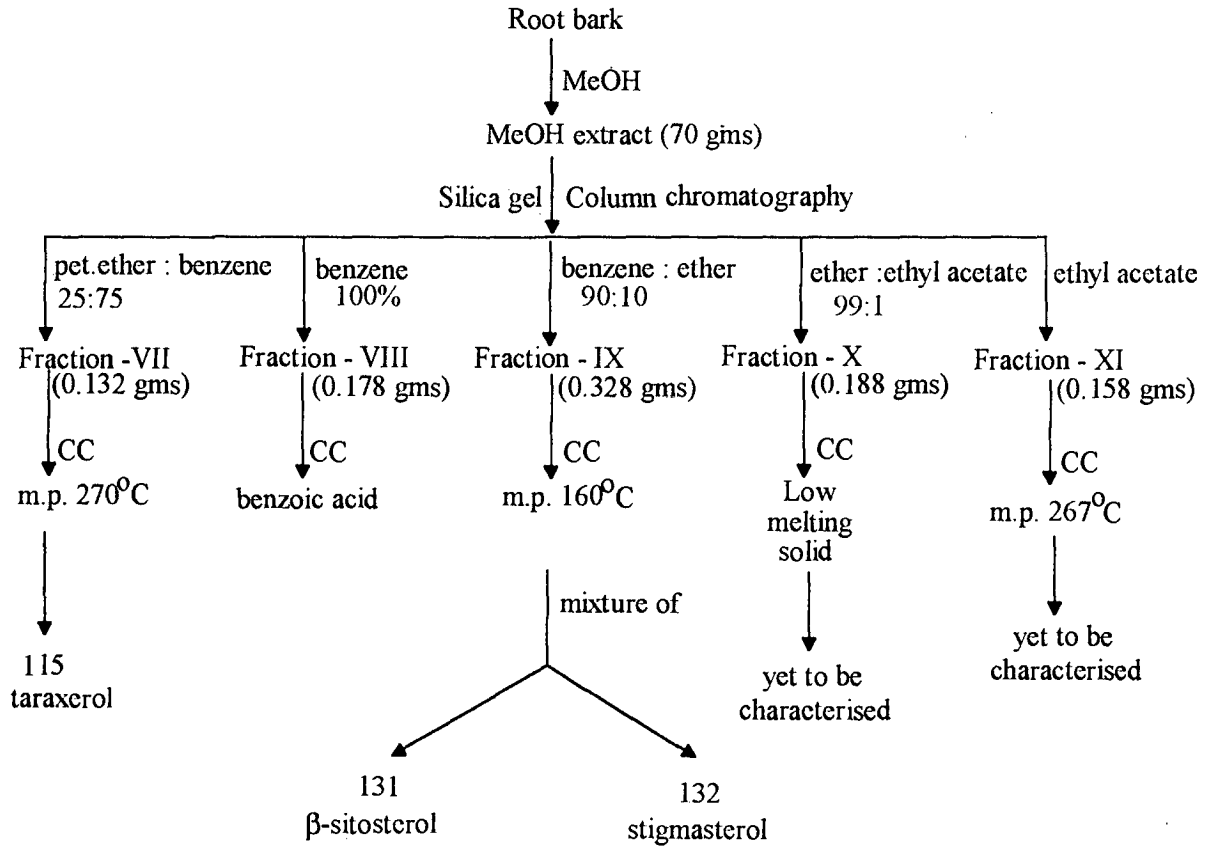
Stigmasterol **132** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.70, 0.79, 0.80, 0.84, 0.92, 1.01 (3H each, 2 x s, 3 x d, 1 x t, 6 x CH_3), 3.52 (1H, m, C_3-H), 5.00 (1H, m, C_{23}-H), 5.14 (1H, m, C_{22}-H), 5.35 (1H, t, C_5-H), 0.8-2.3 (m, all other protons).

Chemical examination of root bark of *Uvaria narum* (Annonaceae) :

The roots of Uvaria narum was collected from the campus of Calicut University (Kerala state). The plant was identified by Dr. M. Janarthanan and vaucher specimen is deposited in the Botany department of Goa University. The shade dried root bark (2 kg.) was powdered and soaked in methanol. The methanolic extract was concentrated under reduced pressure to a brownish viscous mass. The crude methanolic extract (70 gms) was fractionated over silica gel (800 gms) successively with petroleum ether, benzene, ether and ethyl acetate.


Root bark
MeOH
MeOH extract (70 gms)
Silica gel
Column chromatography
pet.ether 100%
pet.ether : benzene 90:10
80 :20
pet.ether : benzene 50:50
50:50
pet.ether : benzene 50:50
Fraction - I
(0.305 gms)
Fraction - II
(0.463 gms)
Fraction - III
(0.222 gms)
Fraction - IV
(0.321 gms)
Fraction - V
(0.762 gms)
Fraction - VI
(0.228 gms)
CC
Colourless oil
Low melting solid
Yellow oil
m.p. 224°C
Low melting solid
m.p. 207°C
mixture of
114 glutinone
127 patchalenone
112 glutinol
133
134 germancyl palmitate
135 methyl oleate
136 methyl sterate


Root bark
MeOH
MeOH extract (70 gms)
Silica gel
Column chromatography
pet.ether : benzene 25:75
benzene 100%
benzene : ether 90:10
ether :ethyl acetate 99:1
ethyl acetate
Fraction -VII
(0.132 gms)
Fraction - VIII
(0.178 gms)
Fraction - IX
(0.328 gms)
Fraction - X
(0.188 gms)
Fraction - XI
(0.158 gms)
CC
m.p. 270°C
benzoic acid
m.p. 160°C
Low melting solid
m.p. 267°C
mixture of
115 taraxerol
yet to be characterised
yet to be characterised
131 β-sitosterol
132 stigmasterol

β-Amyrin palmitate **133** + Germancyl palmitate **134** (1:1 mixture) : Low melting solid.

IR : ν_{max} (KBr): 2921, 1743, 1463, 1377 and 1172 cm^{-1}

β-Amyrin palmitate **133** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.83, 0.87, 0.90, 0.94, 0.97, 1.02, 1.05, 1.13 (3H each, 8 x s, 8 x CH_3), 0.88 (3H, t, $\underline{CH_3}$-$(CH_2)_n$-COOR), 1.25 (13 x 2H, s, CH_3-$(\underline{CH_2})_{13}$-CH_2-COOR), 2.30 (2H, t, α-CH_2), 4.5 (1H, m, C_3-H), 5.18 (1H, m, olefinic proton), 0.8-2.0 (m, all other protons).

Germancyl palmitate **134** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.73, 0.84, 0.87, 0.90, 0.94, 0.97, 1.01, 1.07 (3H each, 8 x s, 8 x CH_3), 0.88 (3H, t, $\underline{CH_3}$-$(CH_2)_n$-COOR), 1.25 (13 x 2H, s, CH_3-$(\underline{CH_2})_{13}$-CH_2-COOR), 2.30 (2H, t, α-CH_2), 4.5 (1H, m, C_3-H), 4.86 (1H, m, olefinic proton), 0.8-2.0 (m, all other protons).

Methyl oleate **135** + Methyl sterate **136** (60 : 40 mixture) : Yellow oil

Methyl oleate **135** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.88 (3H, t, J = 6.8 Hz, $\underline{CH_3}$-$(CH_2)_n$-COOR), 1.61 (2H, t, J = 7 Hz, β-CH_2), 2.00 (2H, d, J = 5.5 Hz, α-CH_2 to olefinic carbon), 2.30 (2H, t, J = 7.6 Hz, α-CH_2), 3.67 (3H, s, -OCH_3), 5.34 (2H, m, olefinic protons), 1.26 (s, all other protons).

Methyl sterate **136** :

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.88 (3H, t, J = 6.8 Hz, $\underline{CH_3}$-$(CH_2)_n$-COOR), 2.30 (2H, t, J = 7.6 Hz, α-CH_2), 3.67 (3H, s, -OCH_3), 1.26 (s, all other protons).

Glutinone **114** : m.p. 224°C ($CHCl_3$ - MeOH)

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.82, 0.96, 0.99, 1.03, 1.09, 1.16, 1.22, 1.24 (3H each, 8 x s, 8 x CH_3), 2.43 (2H, m, C_2-H), 5.69 (1H, br, C_6-H), 0.9-2.00 (m, all other protons).

Patcholenone **127** : Low melting solid

0.89, 1.02 (3H each, 2 x s, C_7-CH_3), 0.84 (3H, d, C_9-CH_3), 2.06 (3H, s, C_3-CH_3), 0.8-2.9 (10 H, m, all other protons).

Glutinol **112** : m.p. 207°C

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.85, 0.95, 0.99, 1.00, 1.04, 1.09, 1.14, 1.16 (3H each, 8 x s, 8 x CH_3), 3.47 (1H, br, C_3-H), 5.63 (1H, d, J = 5.7 Hz, C_5-H), 0.82-2.07 (m, all other protons).

Taraxerol **115** : m.p. 277°C (CHCl3-MeOH)

IR : ν_{max} (film) : 3300, 2998, 2361, 2342, 1473, 1038, 816 cm^{-1}

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) : 0.80, 0.82, 0.90, 0.92, 0.95, 0.98, 1.03, 1.07 (3H each, 8 x s, 8 x CH_3), 3.20 (1H, m, C_3-H), 5.53 (1H, d, J = 5.7 Hz, C_{14}-H), 0.70-2.06 (m, all other protons).

References :

1. Laboeuf M, Cave A, Bhaumik P K, Mukherjee B, Mukherjee R, Phytochemistry, **21**, 2783 (1982).
2. Parmar V S, Tyagi O D, Malhotra A, Singh S K, Bisht K S, Jain R, Nat. Prod. Rep. 219 (1994).
3. Lasswell W L, Hufford C D, Phytochemistry, **16**, 1439 (1977).
4. Weenen H, Nkunya M H H, Mgani Q A, Posthumus M A, Waibel R, Achenbach H, J. Org. Chem., **56**, 5865 (1991).
5. Weenen H, Nkunya M H H, El-Fadl A A, Harkema S, Zwanenburg B, J. Org. Chem., **55**, 5107 (1990).
6. Waterman P G, Mohammad I, J. Nat. Prod., **51**, 719 (1988).
7. Nkunya M H H, Achenbach H, Renner C, Waibel R, Weenen H, Phytochemistry, **29**, 1261 (1990).
8. Achenbach H, Raffelsberger B, Tet. Lett., **28**, 2571 (1979).
9. Nkunya M H H, Weenen H, Koyi N J, Phytochemistry, **26**, 2402 (1987).
10. Nkunya M H H, Weenen H, Phytochemistry, **28**, 2217 (1989).
11. Hufford C D, Lasswell W L, Lloydia, **41**, 156 (1978).
12. Lasswell W L, Hufford C D, J. Org. Chem., **42**, 1295 (1977).
13. Hufford C D, Lasswell W L, Hirotsu K, Clardy J, J. Org. Chem., **44**, 4709 (1979).
14. Kodpind M, Thebtaranonth C, Thebtaranonth Y, Phytochemistry, **24**, 3071 (1985).
15. Hufford C D, Oguntimein B O, Phytochemistry, **19**, 2036 (1980).

16. Hufford C D, Oguntimein B O, Baker J K, J. Org. Chem., **46**, 3073 (1981).

17. Nkunya M H H, , Waibel R, Achenbach H, Phytochemistry, **34**, 853 (1993).

18. Cole J R, Torrence S J, Wiedhopf R M, Arora S K, Bates R B, J. Org. Chem., **41**, 1852 (1976).

19. Nkunya M H H, Weenen H, Renner C, Waibel R, Achenbach H, Phytochemistry, **32**, 1297 (1993).

20. Okorie D A, Phytochemistry, **16**, 1591 (1977).

21. Muhammad I, Waterman P G, J. Nat. Prod., **48**, 571 (1985).

22. Hufford C D, Oguntimein B O, Shoolery J N, J. Org. Chem., **52**, 5286 (1987).

23. Nkunya M H H, J. Nat. Prod., **48**, 999 (1985).

24. Kodpnd M, Sadavongvivad C, Thebtaranonth C, Thebtaranonth Y, Phytochemistry, **23**, 199 (1984).

25. Panichpol K, Waigh R D, Waterman P G, J. Pharm. Pharmacol., **28**, Suppl, 71P (1971).

26. Chayunkiat A, Wiriyachitra P, Taylor W, J. Sci. Soc. Thai., **10**, 239 (1984).

27. Hollands R, Becher D, Geudemer A, Polosky J, Ricroch N, Tetrahedron, **24**, 1633 (1968).

28. Jolad S D, Hoffmann J J, Cole J R, Tempesta M S, Bates R B, Phytochemistry, **23**, 935 (1984).

29. Holbert G W, Ganem B, Engen D V, Clardy J, Borsub L, Chantrapromma K, Sadavongvivad C, Thebtaranonth Y, Tet. Lett.,**8**, 715 (1979).

30. Nkunya M H H, Weenen H, Koyi N J, Thijs L, Zwanenburg B, Phytochemistry, **26**, 2563 (1987).

31. Pan X P, Yu D Q, Phytochemistry, **40**, 1709 (1995).

32. Pan X P, Yu D Q, Lee K H, Chin. Chem. Lett., **7**, 241 (1996).

33. Jolad S D, Hoffmann J J, Schram K H, Cole J R, Tempesta M S, Bates R B, J. Org. Chem., **46**, 4267 (1981).

34. Ogawa S, Takagaki T, J. Org. Chem. **50**, 5075 (1985).

35. Chen Y, Chen R Y, Yu D Q, Phytochemistry, **43**, 793 (1996).

36. Chen Y, Yu D Q, J. Nat. Prod., **59**, 507 (1996).

37. Tempesta M S, George R, Bates R B, J. Org. Chem., **47**, 3151 (1982).

38. Jolad S D, Hoffmann J J, Cole J R, Barry C E III, Bates R B, Linz G S, Konig W A, J. Nat. Prod., **48**, 644 (1985).

39. Hufford C D, Oguntimein B O, Baker J K, J. Org. Chem., **46**, 3073 (1981).

40. Hufford C D, Oguntimein B O, Engen D V, Muthard D, Clardy J, J. Am. Chem. Soc., **102**, 7365 (1980).

41. Panichpol K, Waigh R D, Waterman P G, Phytochemistry, **16**, 621 (1977).

42. Waterman P G, Mohammad I, J. Chem. Soc. Chem. Commun., 1280 (1984).

43. Mohammad I, Waterman P G, J. Nat. Prod., **48**, 571 (1985).

44. Muhammad I, Hasan C M, Anisuzzuman A M T, J. Bangladesh Acad. Sci., **11**, 195 (1987).

45. Labceuf M, Cave A, Plant Med. Phytother., **14**, 143 (1980).

46. Hufford C D, Oguntimein B O, Martin M, Tet. Lett., **25**, 371 (1984).

47. Achenbach H, Pure and App. Chem., **58**, 653 (1986).

48. Tammami B, Torrence S J, Fabela F V, Wiedhopf R M, Cole J R, Phytochemistry, **16**, 2040 (1977).

49. Achenbach H, Hohn M, Waibel R, Nkunya M H H, Jonker S A, Muhie S, Phytochemistry, **44**, 459 (1997).

50. Nanda A, Narayana C S, Kulkarni D R, Iyengar M A, Ind. Drugs, **23**, 647 (1986).

51. Manilal K S, Flora of Silent Valley, Pg. 3 (1988), Mathrubhumi Press.

52. Hisham A, Pieters L, Schepens P, Vlietinck A, J. Essent. Oil Res. **4**, 475 (1992).

53. Parmar V S, Bisht K S, Malhotra A, Jha A, Errington W, Howarth O W, Tyagi O D, Stein P C, Jensen S, Boll P M, Olsen C E, Phytochemistry, **38**, 951 (1995).

54. Hisham A, Pieters L A C, Claeys M, Vanden Heuvel H, Esmans E, Dommisse R, Vlientinck A J, Phytochemistry, **30**, 2373 (1991).

55. Padmaja V, Thankamany V, Hisham A, J. Ethnopharmacol., **40**, 181 (1993).

56. Hisham A, Pieters L A C, Claeys M, Esmans E, Dommisse R, Vlientinck A J, Tet.Lett.,31, 4649 (1990).

57. Hisham A, Pieters L A C, Claeys M, Esmans E, Dommisse R, Vlientinck A J, Phytochemistry, **30**, 545 (1991).

58. Owaifo A O, Okorie D A, Bababunmi E A, Cancer Lett., **8**, 87 (1979).

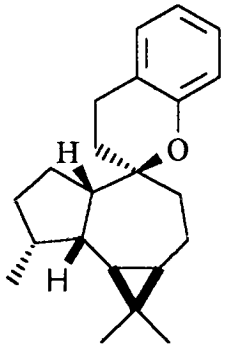
59. Hisham A, Pieters L A C, Dommisse R, Esmans E, Vlientinck A J, Planta Med., **56**, 542 (1990).

60. Hisham A, Wray V, Pieters L, Claeys M, Dommisse R, Vlientinck A J, Mag. Res. Chem., **30**, 295 (1992).

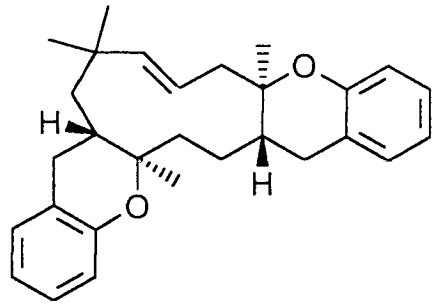
61. Trivedi B, Motl O, Herout V, Sorm F, Collect. Czech. Chem. Commun. **29**, 1675 (1964).

2.1 Thermal reactions of saligenin with olefins : A simple approach to benzopyranyl terpenoids

The plants belonging to the genus *Uvaria* (Fam : Annonaceae) have been subjected to extensive chemical studies because of their reported medicinal properties. We have reported striking features of genus *uvaria* along with the chemical examination of *Uvaria narum* in chapter-1. The novel benzopyranylsesquiterpenoids such as tanzanene **1**[1],lucidene **2**[2], uvarisesquiterpenes A, B and C (**3**, **4** and **5** respectively)[3] have attracted our attention because of their novel structural features and their proposed biogenetic origin by a non-enzymatic 4+2 cycloaddition of o-benzoquinone methide **6** to the olefinic linkages of previously well characterized sesquiterpenes. Though this biogenetic proposal looks highly attractive as the unambiguously established stereochemical features of **1** and **2** fit well with the proposed 4+2 cycloadditon, the stereochemistry established for uvarisesquiterpenes B and C (**4** and **5**) indicates that addition of the C_7 fragment need not be via 4+2 cycloaddition and ionic intermediates may well be involved.



1



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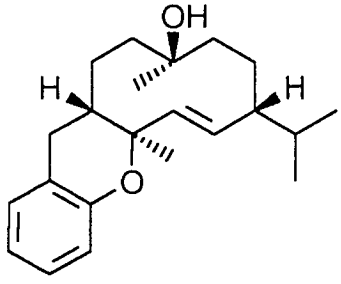

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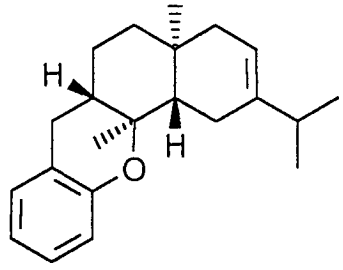

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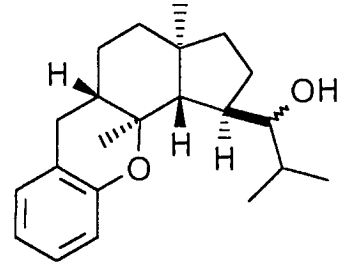

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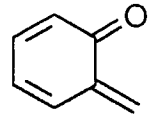
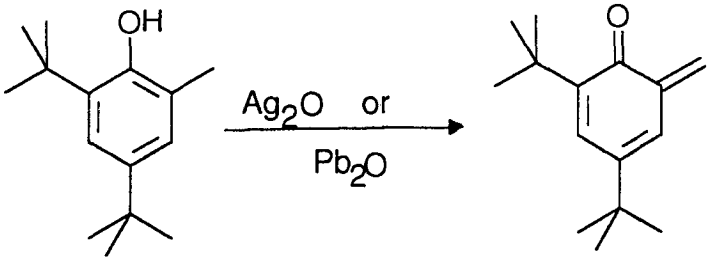


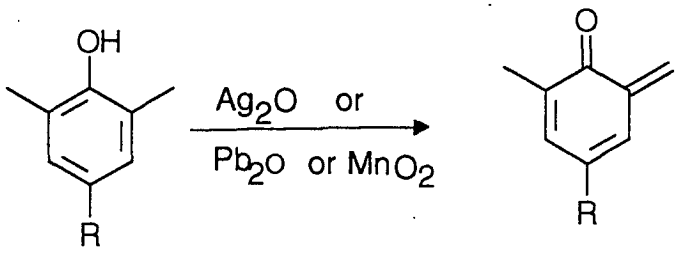
6

The simplest way to synthesize these compounds is the reaction of the corresponding sesquiterpene with *insitu* generated o-benzoquinone methide **6**. There are previous reports of generation of **6**(o-methylene-2,4-cyclohexadiene-1-one) which are presented in chart-1.

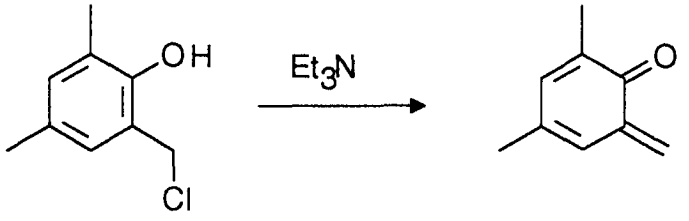
Moore and Waters[4]



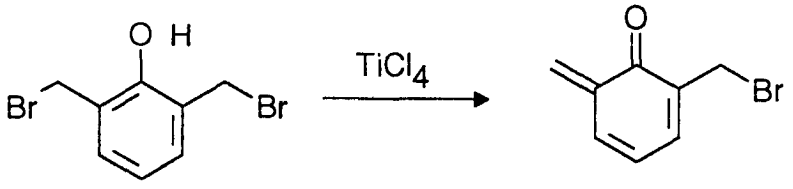
Bolon D A[5]



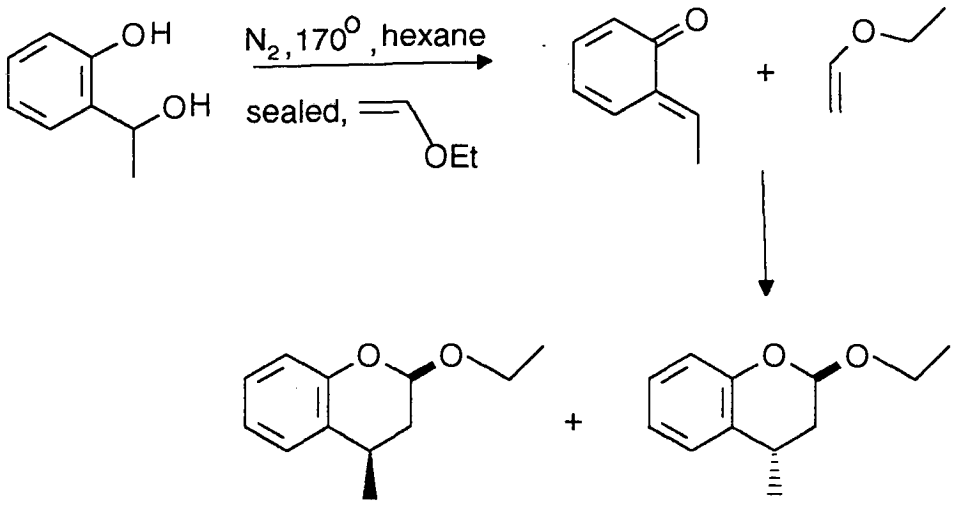
Dean et.al.[6]



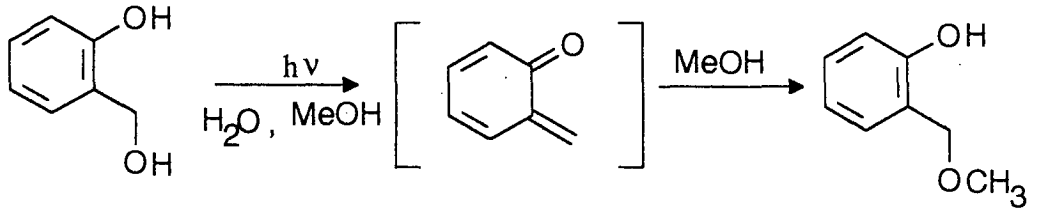
Bavoux et.al[7]



Arduni et.al.[8]



Wan et.al.[9]



Hultzsch K[10]

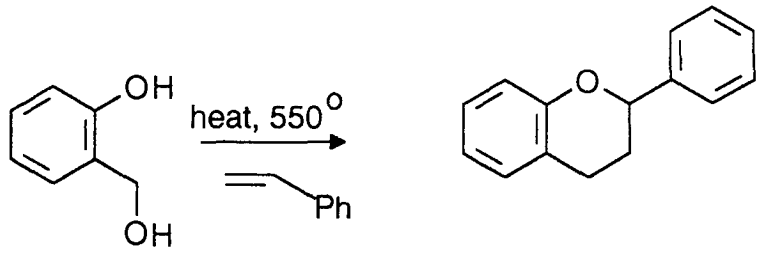
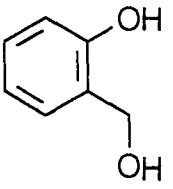


Chart -1

The most convenient starting material for the generation of **6** is the commercially available saligenin **7** (o-hydroxy benzyl alcohol) and the reported thermal reaction of **7** and styrene to produce 2-phenyl chroman **8**[10] further encouraged us to

use it as a precursor for **6**. However, the high temperature used for this reaction were of limited value especially where the starting terpene substrate is thermally sensitive.

Secondly, under high temperatures retro-Diels-Alder reaction was also considered likely thus affecting the overall yields of the adducts. Generation of **6** under mild conditions seemed desirable if the reaction is to be of general application.



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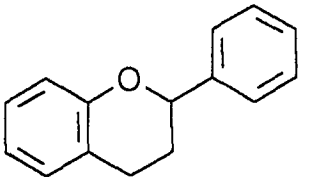
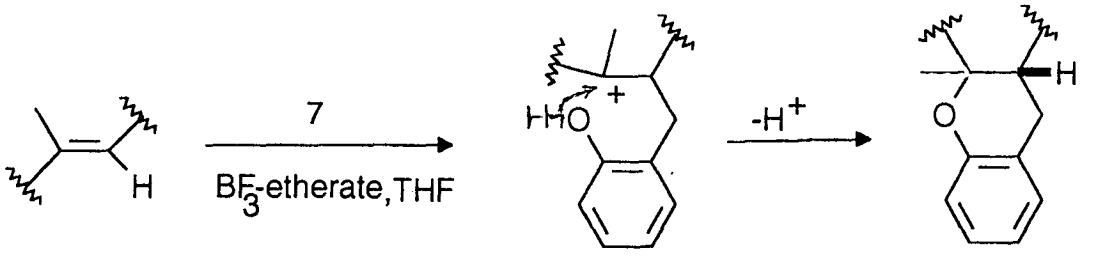


8

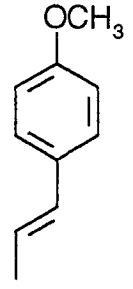
Reaction of phenols with saligenin **7** in the presence of BF_3 -etherate in the THF is known to produce ortho-benzylated products[11] and can be considered as an alternative procedure for the laboratory preparation of benzopyranyl terpenes as the intermediate **9** can produce the desired product by simple elimination of a proton as shown in Scheme-1



9

Scheme - 1

Incidentally, though the previous proposal of 4+2 cycloaddition for the biogenesis of the benzopyranyl terpenoids is quite logical and attractive, it is equally likely that these novel meroterpenoids might involve the ionic intermediate **9**. Initially, we chose to study the thermal reaction of saligenin **7** and the unsaturated substrates at reflux temperatures using different anhydrous solvents. While benzene and toluene did not prove useful, refluxing an equimolar mixture of **7** and the unsaturated substrate in dry xylene produced the desired compounds in fair to good yields. In this section we describe the characterization of the products derived by heating an equimolar mixture of **7** and the unsaturated substrates viz. anethole **10**, (+)-limonene **11**, camphene **12**, (-)-α- phellandrene **13**, (-)-α- zingiberene **14** and (-)- caryophylene **15** at the reflux temperature of xylene.



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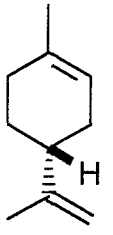

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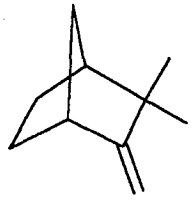

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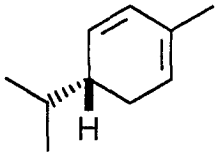

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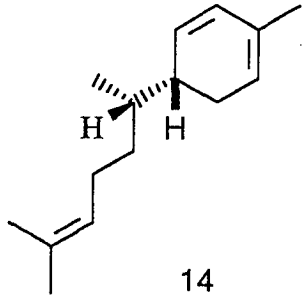

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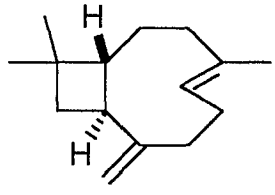
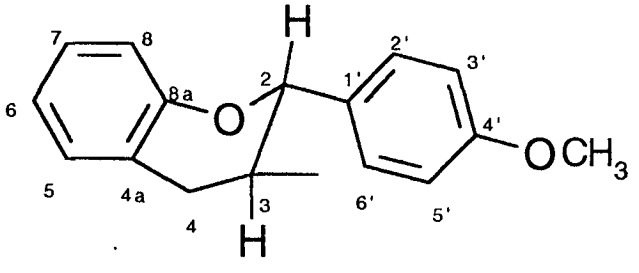


15

The reaction of anethole **10** was of particular interest as we anticipated the formation of adduct **16** on mechanistic considerations. An equimolar mixture of anethole **10** and saligenin **7** in dry xylene was refluxed for 12 hr. while monitoring the reaction by TLC. Most of the solvent was removed under reduced pressure and the residue was subjected to chromatography over silica gel. After recovery of the starting material, a crystalline compound $C_{17}H_{22}O$, m.p 110 ^{0} was obtained and was shown by spectral analysis to posses structure **16** as anticipated. Its IR spectrum did not show any bands in the hydroxyl region while the most characteristic feature in the ^{1}HNMR was the doublet integrating for one proton at δ 4.63 (J=9 Hz). This is obviously due to the C_2 -H (see numbering on structure **16**) and the coupling constant showed that C_2-H and C_3-H have trans relationship. All other chemical shifts of the ^{1}HNMR spectrum could be assigned and similarly ^{13}CNMR spectral data was fully consistent with structure **16**. The assignments of the various protons are given in the experimental section while ^{13}CNMR data is presented in fig-1.



16

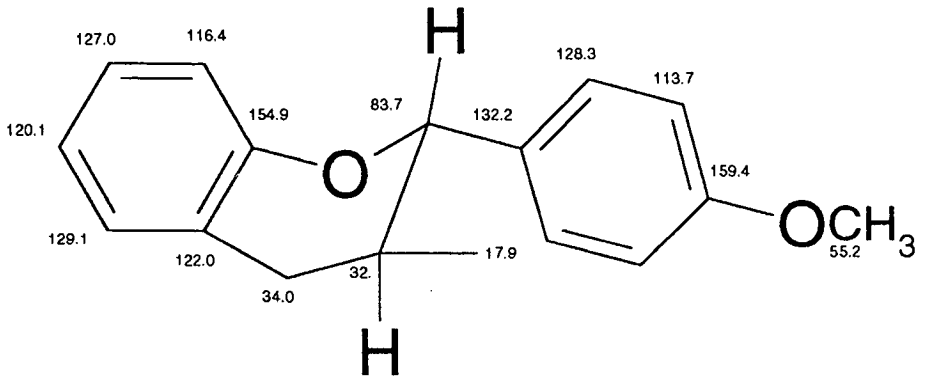
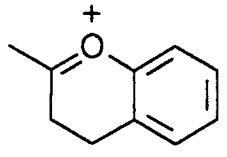
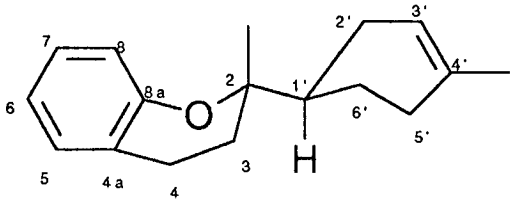


Fig -1

In the case of (+)-limonene **11** , the adduct was obtained as colourless liquid and though appeared homogeneous was shown to be a mixture of four compounds (2 major + 2 minor) by GCMS and corresponded to the molecular formula $C_{17}H_{22}O$. The mass spectra of the two major compounds were identical except for the differences in heights of some peaks and besides molecular ion at m/z 242 showed the base peak at m/z 147 due to fragment ion **17** and in turn suggested that the major adducts can be represented by structures **18** and **19** and epimeric at C-2.



17



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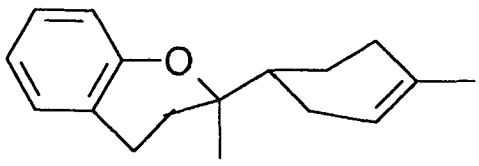

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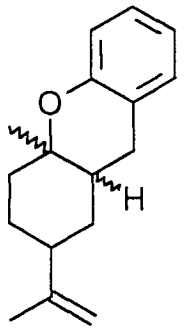
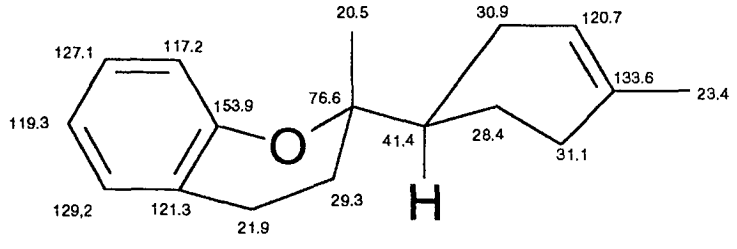


20

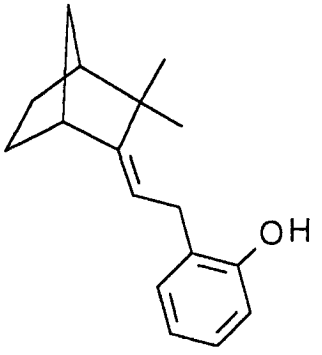
Similarly mass spectra of the two minor components were identical but distinctly different from those recorded on major components (**18** & **19**), the characteristic difference being the base peak at m/z 107 due to $(HO\text{-}Ph\text{-}CH_2)^+$. Obviously the minor compounds are diasteriomers represented by the gross structure **20**. Repeated and careful preparative TLC afforded one of the major compounds but still not in analytically pure state as could be seen from its ^{1}HNMR spectrum. Structure **18** is assigned to this major compound on the basis of ^{1}H,^{13}C NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR spectra. The assignments for the various protons are given in the experimental section while ^{13}CNMR assignments are presented in fig-2.



18

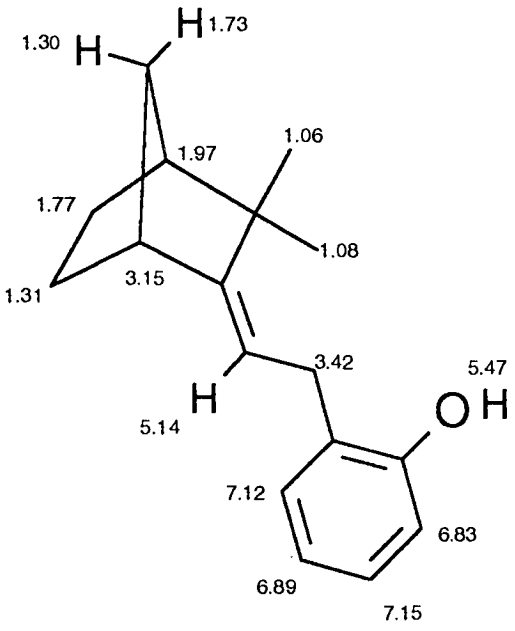
Fig - 2

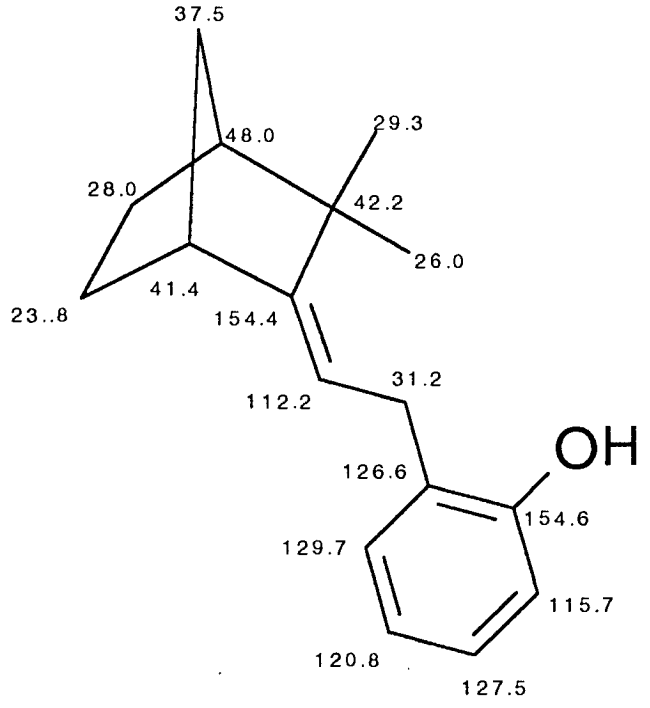
Thermal reaction of camphene **12** with saligenin **7** yielded after chromatographic purification a colourless oil, $C_{17}H_{22}O$, deduced from ^{13}CNMR data combined with GCMS which showed it to be a single compound. It exhibited in its IR spectrum a diagnostic band at 3450 cm^{-1} due to a hydroxyl group. Its ^{1}HNMR spectrum showed two singlets, three protons each at δ 1.06 and δ 1.08 for the gem dimethyl group. Benzylic protons were observed much downfield (δ 3.42) compared to the normal benzylic protons (δ 2.4-2.9) of benzopyranyl system. A single olefinic proton was observed at δ 5.14 in place of two in the starting compound **12**. A broad peak at δ 5.47 further supported the presence of hydroxyl group. The above observations were confirmed by the ^{13}CNMR spectrum which revealed the presence of two quartets (δ 26.0 and 29.3), four triplets (δ 23.8, 28.0, 31.2 and 37.5), seven doublets (δ 41.4, 48.0, 112.2, 115.7, 120.8, 127.5 and 129.7) and four singlets (δ112.2, 126.6, 154.4 and 154.6). Complete analysis of the data with the help of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR suggested structure **21** for the adduct.



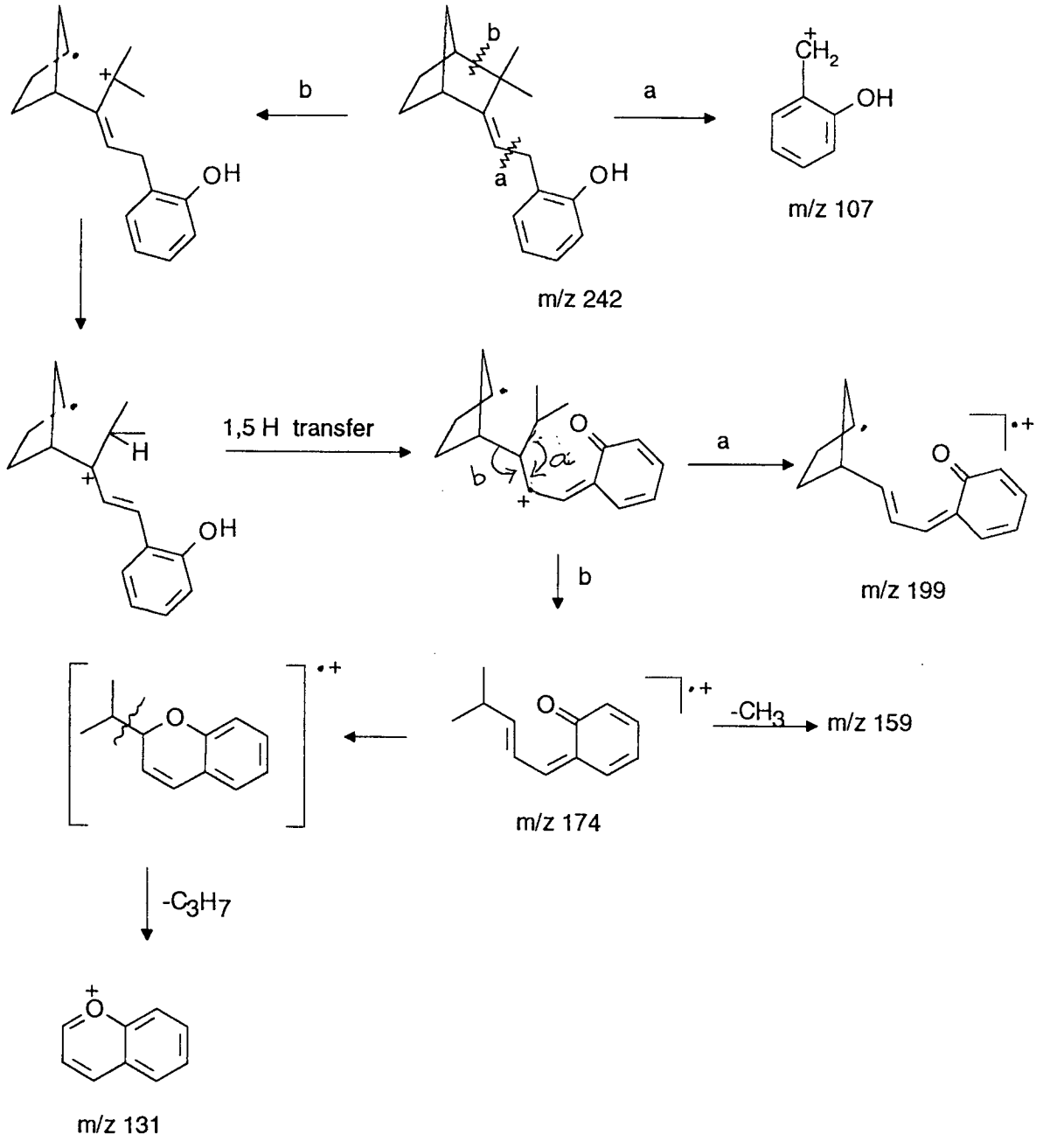
21

The assignments of the ^{1}HNMR and ^{13}CNMR are shown below.





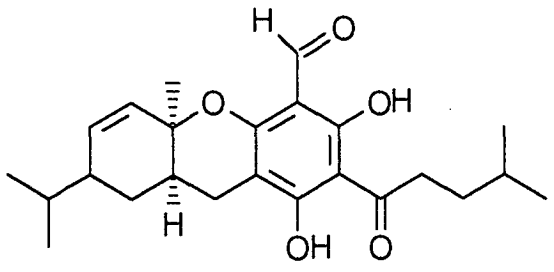
The mass spectrum of the adduct **21** showed a base peak at m/z 131 and other significant peaks at m/z 199,174, 159 and 107. The probable genesis of these fragment ions is shown in scheme-2.



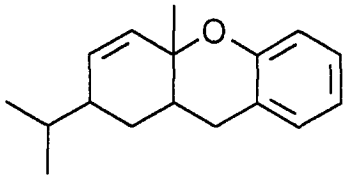
Scheme - 2 : Mass spectral fragmentation of **21**

The formation of o-hydroxy benzylated derivative **21** is understandable due to the hindered nature of the olefinic linkage of camphene. Moreover, it also suggested that the under the experimental conditions used C_7 unit is most probably not added in the 4+2 cycloaddition pathway considered earlier.

The isolation of Euglobal II C **22** from *Eucalyptus globulas* and *E. goroticormis*[12] suggested that α-phellandrene would serve as another model compound during the present study. The thermal reaction of saligenin **7** and phellandrene **13** is expected to produce the adduct having the gross structure **23**. The stereochemical assignment would then clarify the structure of the reactive intermediate generated thermally (xylene, reflux temp.) from saligenin.



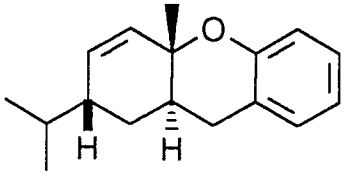
22



23

Refluxing a mixture of **7** and **13** for 12 hr. in dry xylene followed by usual workup and chromatography afforded a colourless oil, having the gross structure **23**. The IR spectrum did not show any bands in hydroxyl region and suggested the formation of the expected benzopyranyl system. The ^{1}HNMR spectrum showed the characteristic two olefinic proton signals at δ 5.75, a singlet methyl signal at δ 1.45,

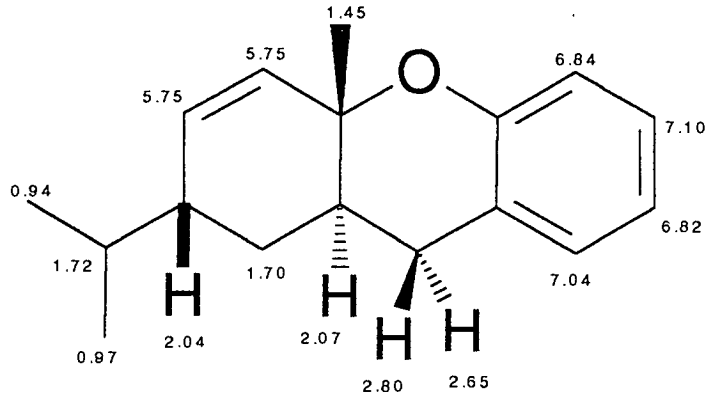
isopropyl methyl doublets (δ 0.94 and 0.96). It also displayed the four aromatic protons (δ 6.82, 6.84, 7.04 and 7.10), two benzylic protons (δ 2.65 and 2.80) and two methine signals (δ 2.04 and 2.07).The assignments for individual signals could be made without any problem. The ^{13}CNMR spectrum showed 17 signals, 3 quartets (δ 20.0, 20.2 and 27.9), 2 triplets (δ 27.0 and 27.9), 9 doublets (δ 31.1, 34.6, 38.9, 116.6, 119.6, 126.9, 129.0, 131.7 and 132.3) and 3 singlets (δ 75.0, 121.5 and 153.1). The stereochemistry of **23** as shown in **23a** as derived by NOE experiments as presented below.

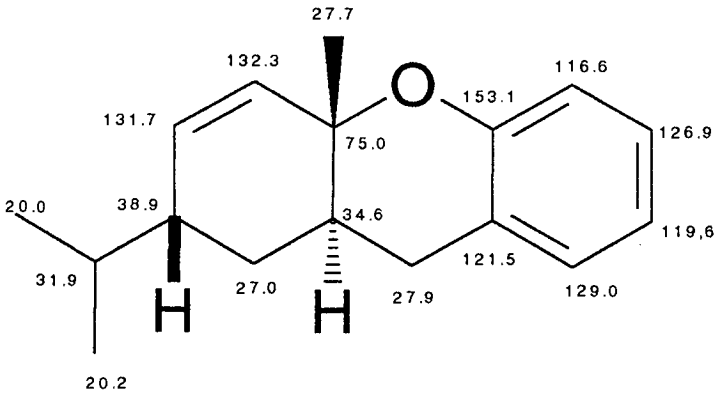


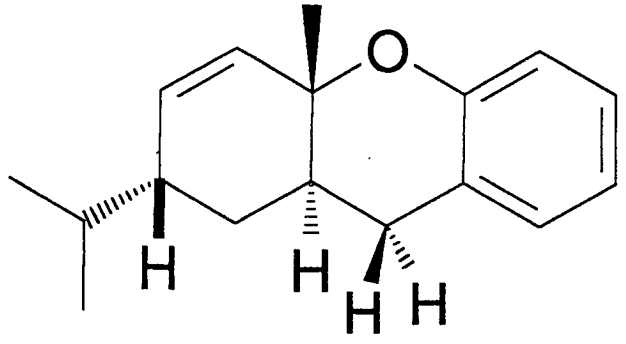
23a

Irradiation of the signal of angular methyl at δ 1.45 enhanced the signal intensity of benzylic proton Ha at δ 2.80 but the signal intensity of C_2 methine proton at the ring junction at δ 2.07 remained unaffected. Irradiation of the signal of C_2 methine proton at δ 2.07 enhanced only the signal intensity of benzylic proton Hb at δ 2.65. Irradiation of the signals of benzylic protons Ha and Hb further supported the above observation. Irradiation of the signal of the C_2 methine proton at δ 2.07 did not affect C_4 methine proton signal at δ 2.04. From these NOE experiments it was concluded that the angular methyl is *cis* to benzylic proton Ha and *trans* to C_2 methine proton. Further, C_2 methine proton is *trans* to both benzylic proton Ha and C_4 methine proton. In (-)-α- phellandrene **13**, C_4-H is known to have *S*-configuration and remains

unchanged in the reaction. We can conclude therefore that the structure **23a** of the α-phellandrene adduct represent the absolute stereochemistry.

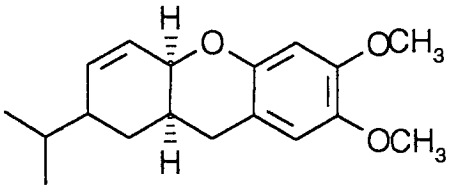






23a

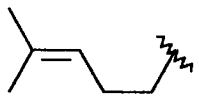
The *trans* stereochemistry of C_1 methyl and C_2-H in **23a** clearly shows that it is not formed by a 4+2 of quinone methide **6** to the trisubstituted olefinic linkage of **13** since in a similar reaction the substituted quinone methide generated by an unambiguous method produces the *cis* adduct **24** when reacted with α-phellandrene **13**.



24

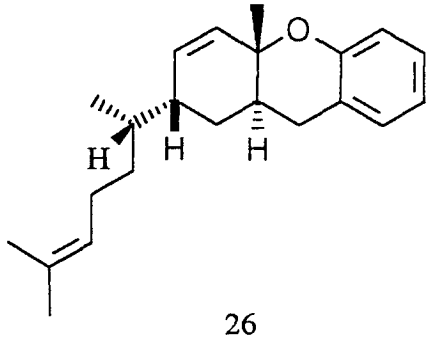
(-)-α-Zingiberene **14**, the isoprenelogue of α-phellandrene was then selected as another model compound to check the outcome of the thermal reaction.

(-)-α-Zingiberene **14** a was refluxed with saligenin **7** for 12 hr. in dry xylene followed by usual workup and chromatographic separation afforded a colourless oil, $[\alpha]_D^{25} = -66.5^0$. GCMS showed it to be essentially single component (90%) with four other minor components (10%) having molecular formulas $C_{22}H_{30}O$. The ^{1}HNMR spectrum had all the structural features of α -phellandrene **23a**, and as expected only one secondary methyl (δ 0.88) and part structure **25** in place of other secondary methyl.



25

^{13}CNMR (APT) spectrum of the adduct exhibited four quartets (δ16.2, 17.8, 25.8, 27.9), four triplets (δ 26.0, 26.6, 27.8, 34.4), ten doublets (δ 35.0, 36.3, 36.9, 116.6, 119.6, 124.5, 126.9, 128.9, 132.3 and 132.3) four singlets (δ 75.3, 121.8, 131.3 and 153.2). The assignments of the signals in the ^{1}H and ^{13}C NMR data have been made with the help of ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR established structure **26** is therefore assigned for the adduct obtained from (-)-zingiberene.



26

The stereostructure of the adduct **26** is based on the comparison of the ^{1}H and ^{13}C NMR chemical shifts with that of **23a**. The ^{1}HNMR values are given in experimental section while ^{13}CNMR values are presented fig-3.

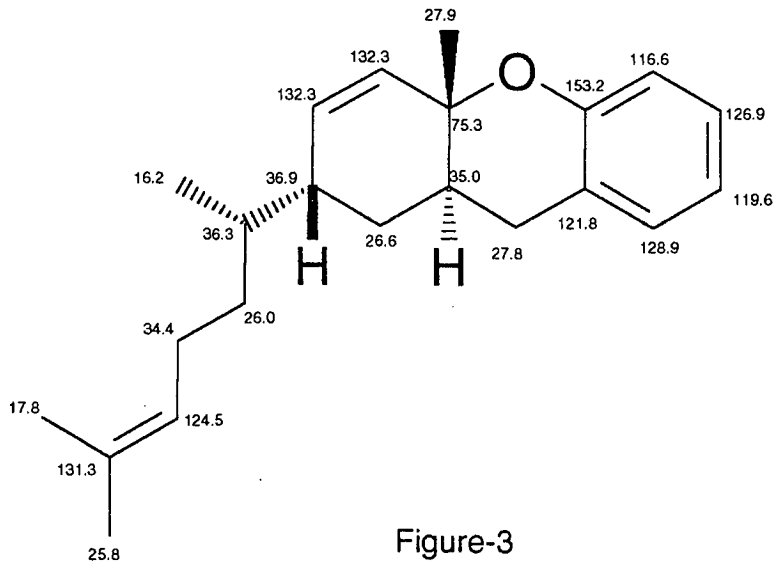
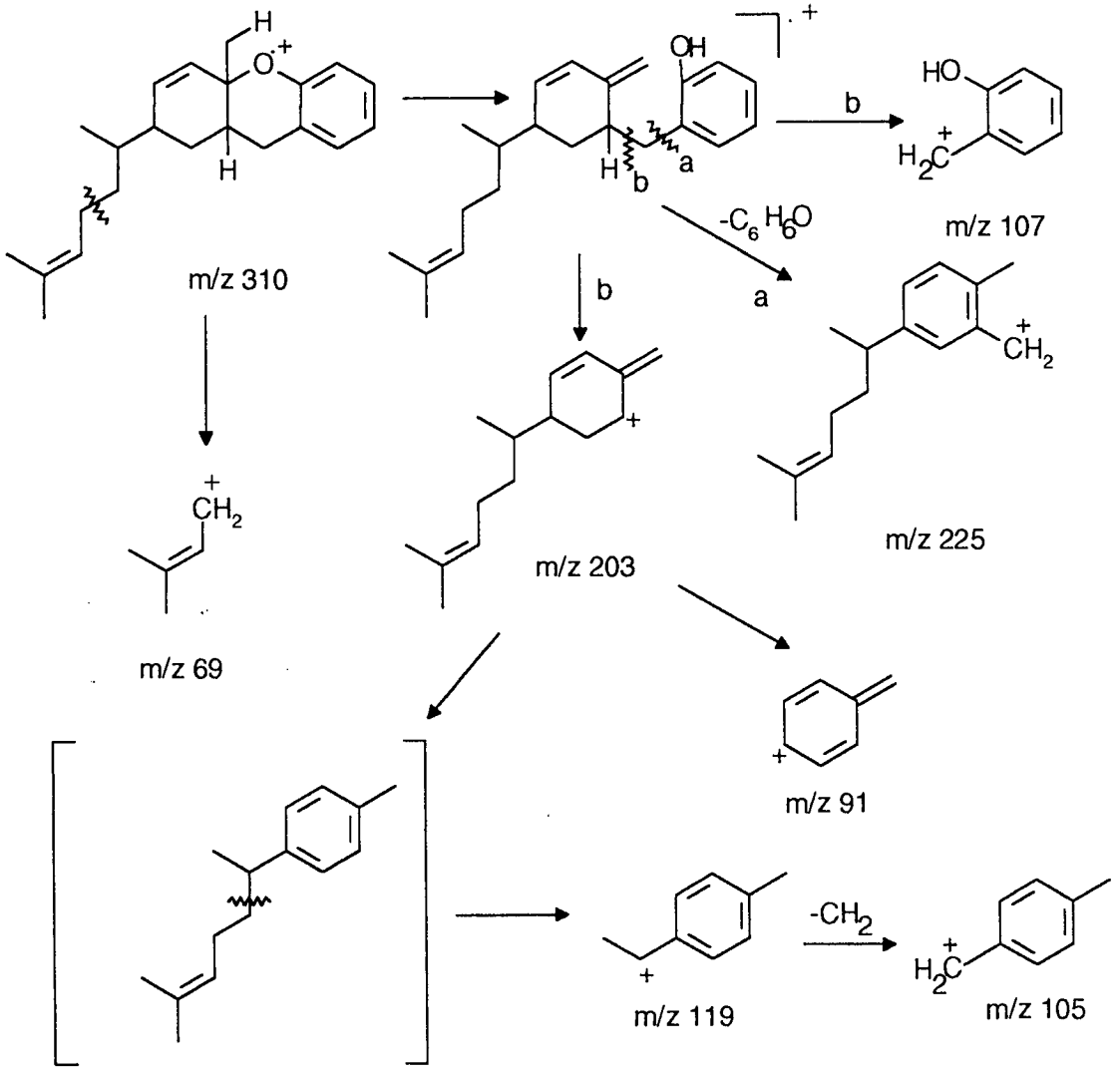


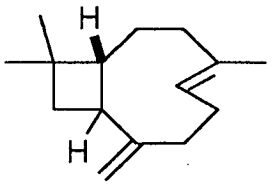
Figure-3

The mass spectral fragmentation is consistent with the proposed structure **26** and the genesis of the major fragment ions is presented in scheme-3.

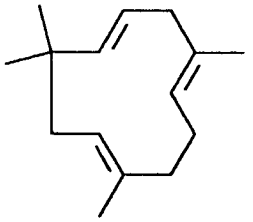


Scheme - 3 : Mass spectral fragmentation of **26**

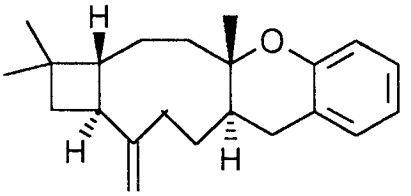
Reaction of (-) caryophyllene **15** with saligenin **7** was of particular interest considering its structural relationship with humulene **27**, the sesquiterpene part of lucidene **2**. Under identical experimental conditions we anticipated the formation of adduct **28** based on the reported reactivities of the double bonds of the caryophyllene.



15



27



28

The adduct obtained from (-)-caryophyllene **15** and saligenin **7** after purification showed it to be a single compound by GCMS having molecular formula $C_{22}H_{30}O$. The IR spectrum showed characteristic bands at 890 and 1780 cm^{-1} due to the exocyclic olefinic linkage and 750, 1585 and 1610 cm^{-1}due to aromatic ring. The ^{1}HNMR spectrum was consistent with structure **28** which showed the presence of three tertiary methyls (δ 0.99,1.02 and 1.18), two benzylic hydrogens (δ 2.66 and 2.86), two olefinic protons of the exocyclic methylene (δ 4.86 and 4.90) and four aromatic protons of the 1,2-disubstituted benzene grouping (δ 6.22 to 7.28). The D_2O exchanged ^{1}HNMR further confirmed the absence of hydroxyl group. The ^{13}CNMR data supported the structure **28** assigned for the adduct which is presented in figure-4.

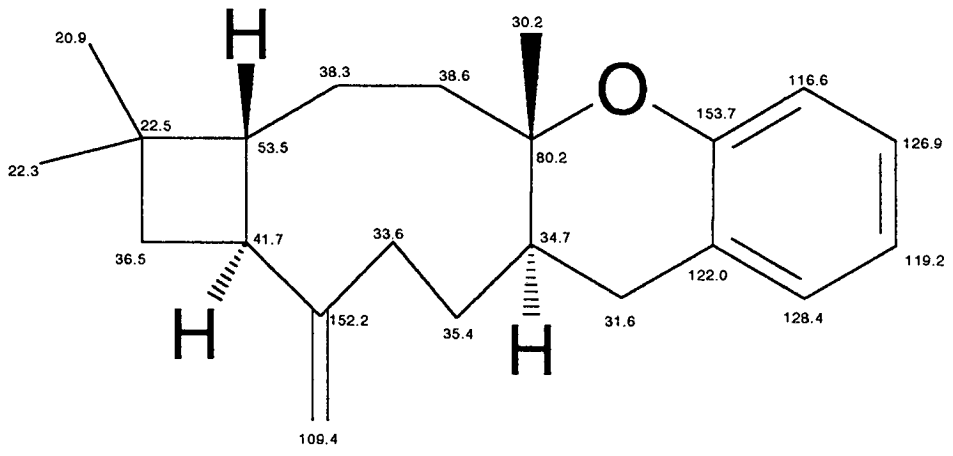
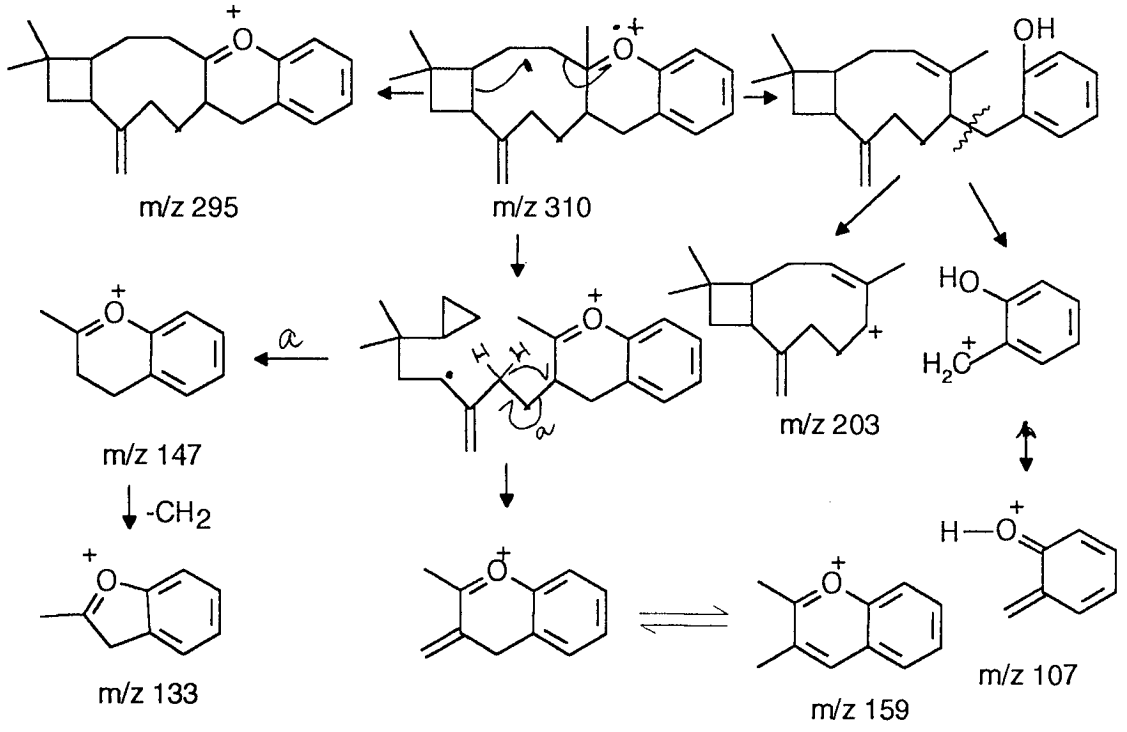


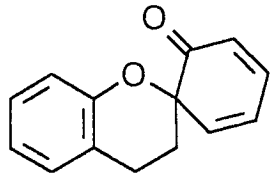
Figure-4

The mass spectral fragmentation is consistent with the proposed structure **28** and the genesis of the major fragment ions is presented in scheme-4.

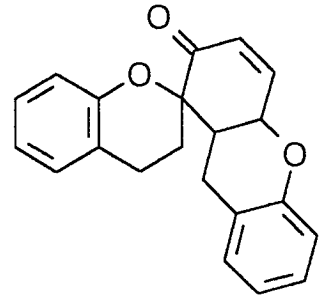


Scheme - 4 : Mass spectral fragmentation of **28**

The results presented above, particularly those obtained in the thermal reaction of saligenin with camphene **12**, (-)-α–phellandrene **13** and (-)-zingiberene **14** made us to firmly believe that the C_7 unit is added to the olefinic linkage via ionic intermediates and not by concerted 4+2 cycloaddition process as envisaged in the early stages. In order to probe in to the nature of reactive species generated by reaction of saligenin at reflux temperature using xylene (138°C) we examined the more polar fraction of each reaction in an effort to isolate and characterize the other compounds formed. It is known that o-quinone methide when generated alone undergoes self condensation via a hetero Diels-Alder reaction. Dimer **29** and trimer **30** have been fully characterized[13].

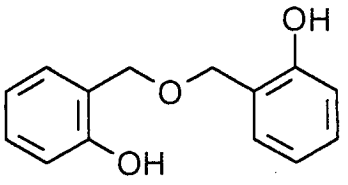


29

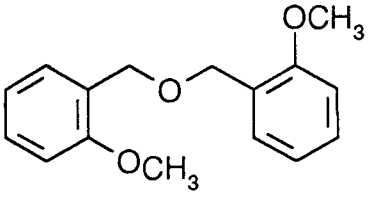


30

From the polar fraction of each reaction, a crystalline compound, m.p.119°C, $C_{14}H_{14}O_3$ [M^+ 258] was isolated. The molecular formula spectral analysis clearly showed that this compound has structure **31** and is derived from two molecules of saligenin but not identical with the dimer **29**. Characterization of this compound gave a clue to the most likely structure of the reactive intermediate. We have utilized this compound for the synthesis of natural product **32** previously isolated from *Uvaria chamae*[11] and the results are presented in chapter 3 (section-1) of this thesis.



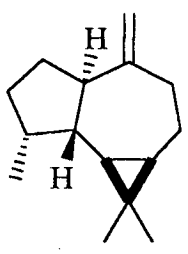
31



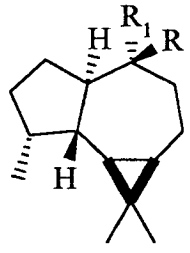
32

2.2 Biomimetic synthesis and absolute configuration of (-) - tanzanene

(-) - Alloaromadendrene, a naturally occurring tricyclic sesquiterpene has been shown to be a constituent of *Eucalyptus globulus*[14], *Pervoskia srophullariaefolia*[14] and *Metrosiderous scandeus*[15]. This hydrocarbon belongs to a small group of tricyclic sesquiterpenes (**33** to **38**) which contain a cyclopropane ring fused to a hydroazulene skeleton.

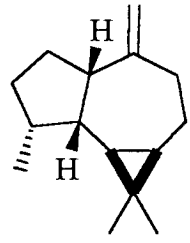


33

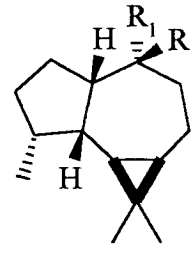


34 R = CH_3, R_1 = OH

35 R = OH, R_1 = CH_3



36

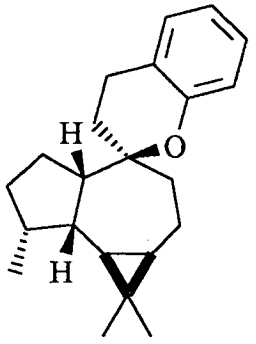


37 R = CH_3, R_1 = OH

38 R = OH, R_1 = CH_3

The degradative work carried out by various groups finally culminated in the correct structure assignment for aromadendrene and alloaromadendrene as shown in **33** and **36** respectively. There was some confusion in the literature regarding the stereochemical relationship of aromadendrene and alloaromadendrene but the elegant degradative and correlative work of Sorm and coworkers clearly established that they differ in the stereochemistry at C-1[14]. Subsequent synthetic work of Buchi's group led

to the unambiguous assignment of stereostructures (including absolute stereochem.) to **36, 37** and **38** to (-) - alloaromadendrene, (-) - ledol and (-) - viridiflorol respectively[16].



1

Very recently, Weenen et al. reported the isolation of yet another sesquiterpene, (-) - tanzanene **1** structurally and stereochemically related to (-) - alloaromadendrene from the root bark of *Uvaria tanzaniae* Vedc[1]. The assigned structure including the relative stereochemistry was established by a detailed spectral analysis (IR, UV, MS, ^{1}H & ^{13}C NMR). ^{1}HNMR decoupling experiments gave evidence that o-hydroxy benzyl group is linked to the seven membered ring in a spiro fashion. This was further supported by long range ^{13}C-^{1}H correlation experiments. Decouplig experiments conclusively supported an alloaromadendrene **34** skeleton having a quaternary C-10 and no exocyclic olefinic linkage. The C-H correlation spectra and NOE experiments established the relative stereochemistry at C-4, C-5, C-6 and C-7. Based on the small coupling constant (J=3.5 Hz) and an identical value for the coupling between the same protons (H-1 and H-5) of virdiflorol **38** fixed the *cis* fusion of the rings. Further calculated values for alkylation of CH_3-15 and phenyl ether formation at -OH group were found in excellent agreement with the β-configuration for C-10 -O- linkage as in virdiflorol **38**. This established the relative stereochemistry

at all chiral centers of (-) - tanzanene **1**. The absolute stereochemistry, however was not established. When this work was undertaken, no synthesis was also reported.

Having developed a general and simple method for the preparation of benzopyranyl compounds from anethole, limonene etc. (section 2.1, this thesis), we envisaged a one-pot biomimetic synthesis of optically active tanzanene starting from (-) - alloaromadendrene **36** which is commercially available* . If the reaction proceeds in the desired manner we anticipated that the synthetic tanzanene would be either (-) - tanzanene or its enantiomer. This would enable to establish the absolute configuration of the natural product.

Refluxing equimolar mixture of saligenin **7**, (-) - alloaromadendrene **36** in dry xylene for 12 hr followed by chromatography over silica gel resulted in the recovery of unchanged **36** and isolation of a highly crystalline compound (yield 45% based on recovered **36**), m.p. 84°C (lit 84-85°C), $[\alpha]_D^{25}$ = -7.1° (c=0.182, $CHCl_3$). A direct comparison of the IR, UV, ^1H, $^{13}CNMR$ spectra of the synthetic compound and those reported on natural tanzanene **1**, co-TLC and mixture m.p. determination unambiguously established their identity. In addition, the sign and magnitude of specific rotation of the synthetic tanzanene established the absolute configuration of natural (-) - tanzanene as shown in **1**. It is of interest to note that we failed to detect formation of isomeric product **39** in this reaction.

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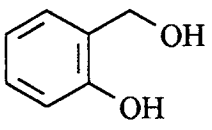
* We thank M/s Fluka AG for a precious gift of (-) - alloaromadendrene.



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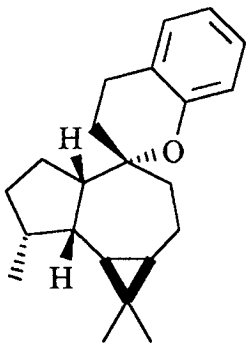
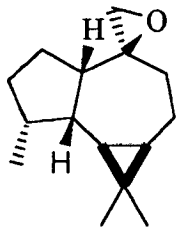


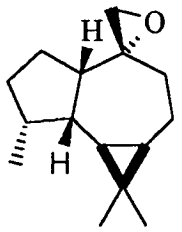
39

(-) - Alloaromadendrene **36** is known to produce a mixture of epoxide **40** and **41**[14*] . This would suggest that approach of peracid from both faces seems possible. In view of this observation, regioselective formation of a single isomer is rather surprising. Though we had proposed earlier that in the present synthesis (-) - tanzanene is formed via 4+2 cycloaddition , results obtained in case of (-) - α - phellandrene and camphene and formation of 2,2'-dihydroxy bibenzyl ether clearly showed that our earlier conclusion is not correct and the benzopyranyl system is introduced via ionic intermediates. Our efforts to detect the formation of compounds like **42** and **43** also failed. At present we have no explanation to offer for these observations.

* Ratio of these isomeric epoxides was not reported.



40



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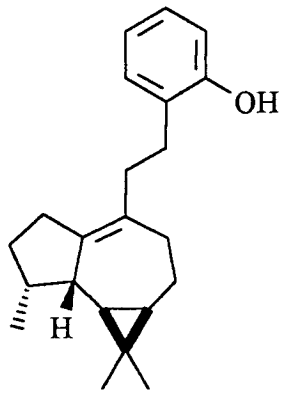

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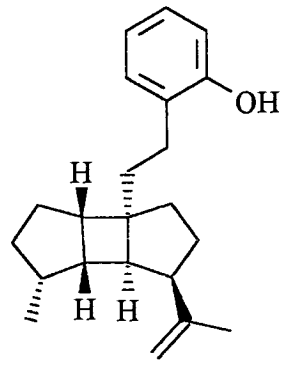
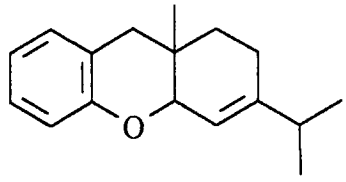


43

Natural occurrence of **44**[17,18] and o-hydroxy benzylation at the activated sites of the aromatic rings would indirectly suggests that the incorporation of o-hydroxy benzyl units and the formation of benzopyranyl terpenoids takes place in the nature via ionic intermediates. In all probability the benzopyranyl sesquiterpenes (-) - tanzanene **1** and lucidene **2** are not derived by non-enzymatic 4+2 cycloaddition process as proposed earlier. As an yet another incidence of serendipity, our synthesis can still be considered as biomimetic synthesis as claimed earlier.

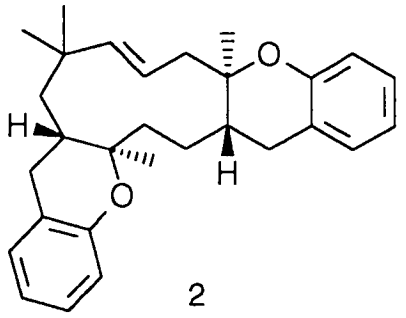


44

2.3 Synthesis of Lucidene, a Bis(benzopyranyl) sesquiterpene

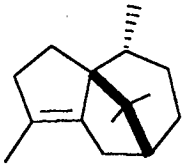
The genus *Uvaria* of the family annonaceae has been known as a rich source of a several bioactive compounds. The most attractive feature being the attachment of a o-hydroxy benzyl moiety to flavanoids, chalcones, dihydrochalcones etc. The elegant studies of several groups have resulted in the isolation of novel terpenoids having a benzopyranyl part structure.

Nkunya and co-workers carried out a systematic chemical investigation of the petroleum ether extract of *Uvaria lucida*[2] and besides the previously known uvaretin, diuvaretin, chemuvaretin, isolated a crystalline sesquiterpene lucidene, m.p. 208-10°C and assigned structure **2** to it. The structure assignment was based on a detailed spectral analysis (^{1}H, $^{13}CNMR$ and MS) and single crystal X-ray crystallography. Naturally occurring lucidene is optically inactive and crystallises in enantiomeric pairs in the space groups P1. X-ray analysis showed that the unit cell contains two independent molecules with approximately the same conformations, one of which is shown in **2a**.



2

Nkunya and coworkers further found by GC-MS analysis that the hydrocarbon fraction contains cyperene **45** (39.3%), β-selinene **46** (17.9%), α-selinene **47** (5.5%) humulene **27**(<1%) and several unidentified compounds* .



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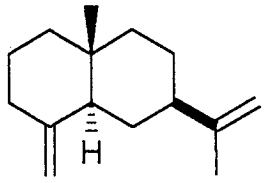
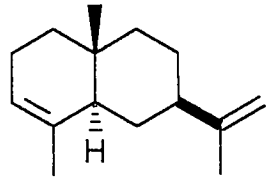


46



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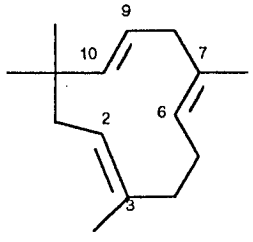
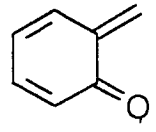


27



6

Since natural lucidene is optically inactive, Nkunya and co-workers considered that its biosynthesis may involve a non-enzymatic double Diels-Alder reaction of **27** with two molecules of o-benzoquinone methide **6**. In order to explain the natural

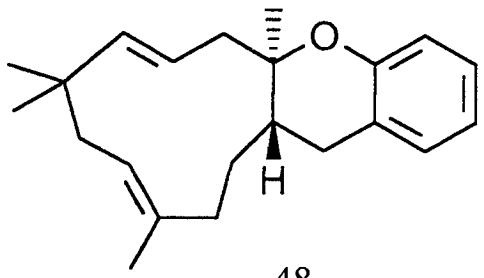
* of the combined non polar fractions.

occurrence of compounds containing o-hydroxybenzyl groups, it is considered that o-benzoquinone methide **6** can also react as a Michael acceptor.

The general method developed during the present study for addition of the C_7 unit to the olefinic linkages of terpene hydrocarbons led us to synthesise (-)- tanzanene and established its absolute configuration (section 2.2, this thesis).

The observation that the hydrocarbon fraction contains less than 1% of humulene **27** suggested its high reactivity with **27** to produce lucidene **2**. Commercial availability of humulene **27** made us to check the generality of the method developed by us for the practical synthesis of benzopyanyl terpenoids. We have achieved the synthesis lucidene **2** and found that it is possible to isolate the intermediate where only one C_7 unit is added to C_6-C_7 double bond of humulene. The details of this study are presented in this section.

Refluxing a mixture of humulene **27** and saligenin **7** in 1:1 molar ratio in dry xylene for 12 hr, we obtained a highly crystalline compound, m.p.113°C in 75 % yield. Its mass spectrum exhibited the molecular ion peak at m\z 310 corresponding to molecular formula $C_{22}H_{30}O$. ^{1}HNMR spectrum displayed three singlets (3H each) at δ 1.03, 1.07 and 1.11, a vinylic methyl group (δ 1.64), a benzylic methylene (δ 2.46 and 2.93), three vinyl hydrogens (δ 5.03, 5.13 and 5.20) and four aromatic hydrogens (δ 6.81-7.15). This data clearly showed that only one C_7 unit is added to the trisubstituted double bond of humulene and considering the preferential reactivity of the C_6-C_7 double bond of the humulene, structure **48** is assigned to it. Since only one benzopyranyl group is present in place of two in lucidene we have named this intermediate as semilucidene.



48

The UV (λ_{max}, MeOH) absorptions at 284, 277, 202 nm and IR spectra (bands at 1610, 1590, 1250 and 760 cm^{-1}) established the presence of benzopyran moiety. The twin IR bands at 1380 and 1360 are due to the gem dimethyl group and a significant absorption band at 965 cm^{-1} is the characteristic of the *trans* disubstituted double bond. Its ^{13}C NMR is fully consistent with the proposed structure and showed the presence of four quartets (δ 17.3, 20.2, 24.4 and 30.4), five triplets (δ 29.6, 37.8, 38.3, 41.5 and 43.2), eight doublets (δ 35.7, 116.9, 119.2, 120.9, 123.0, 127.1, 128.6 and 141.9) and five singlets (δ 30.5, 80.2, 121.7, 136.4 and 153.9). The ^{13}C NMR chemical shifts have been fully assigned and shown in fig-5.

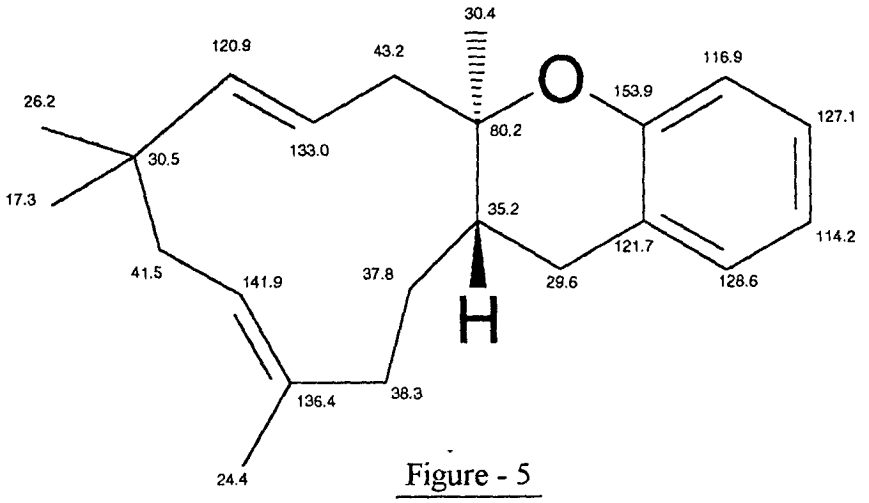
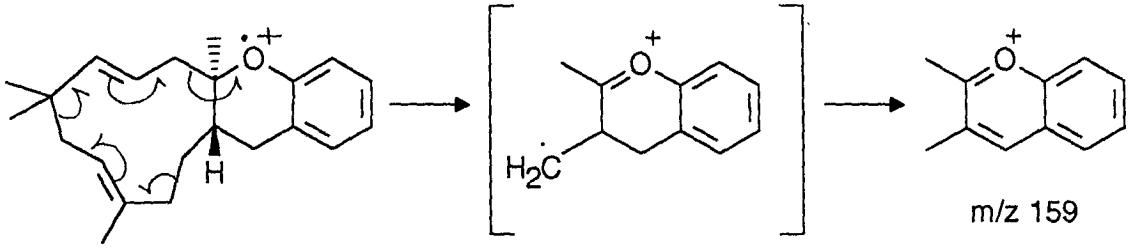


Figure - 5

The EIMS spectrum of semilucidene **46** showed a base peak at m/z 159 besides the molecular ion peak at m/z 310. The genesis of the base peak is presented below (scheme-5).



Scheme - 5

Though there is no report on the natural occurrence of semilucidene in *Uvaria lucida* or any other species of the genus uvaria, we anticipate its natural occurrence particularly from the genus *uvaria* and or *Xylopia*[*] .

[*] The genus *Xylopia* is known to contain o-hydroxy benzylated compounds which are common to the genus *uvaria*.

We anticipated that refluxing semilucidene **48** with saligenin **7** in dry xylene would produce lucidene **2**. Indeed, the reaction did proceed in the desired manner and afforded a crystalline solid on filtration through a short column of silica gel and elution with petroleum ether. Further purification by repeated crystallisation from petroleum ether gave a pure substance, m.p. 213°C. Refluxing humulene **27** with four fold quantity of saligenin **7** in dry xylene for 36 hr. afforded the same compound, m.p. 213°C. Comparison of the UV, IR and Mass spectra for the synthetic compound with that of natural lucidene **2**[**] showed complete identity. However, comparison of the ^{1}H and ^{13}C NMR though showed almost identical chemical shift values, we observed minor differences and hence

our synthetic sample was sent to Professor Nkunya for a direct comparison. Unfortunately we did not receive any communication informing the outcome of the direct comparison of the synthetic and natural lucidene. At this stage, we felt that the synthetic compound may be a stereoisomer of lucidene and a further proof is desirable for a decisive stereochemical assignment.

Some intriguing observations were made regarding the optical activity of our synthetic compound. Humulene **27**, the starting material used in the present synthesis is known to be optically inactive as it contains no chiral centre. Refluxing it with saligenin in dry xylene is therefore expected to produce lucidene **2** or a stereoisomer in optically inactive form. When the sample was sent for ^{1}H and ^{13}C NMR analysis,[*] as a

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[**] We thank Prof. Nkunya for the copies of the spectra on natural lucidene.

[*] Instrumental facilities are not available in our laboratories and the sample are send to other laboratories for spectral analysis.

routine practice the specific rotation of the synthetic compound was measured. To our surprise it showed specific rotation ($[\alpha]_D^{25}$ +13.2°, c, 0.75, $CHCl_3$). Rechecking this unusual observation confirmed the unusual findings. In order to confirm this, another sample which was obtained from the mother liquor was sent to Professor Sharpless for the measurement of specific rotation. Though the magnitude of specific rotation was somewhat lower ($[\alpha]_D^{20}$ +3.42°, c, 0.38, $CHCl_3$, average of ten measurements). It did suggest that the chirality has been introduced. These observations are most intriguing especially because we did not use any chiral catalyst or a chiral column in our synthesis or at the time of separation and purification respectively. Moreover the intermediate product, semilucidene **48** showed no rotation as expected. It is therefore safe to assume that the chirality is introduced after the formation of semilucidene.

In order to establish the structure (including the stereochemistry) unambiguously, we carried out a single crystal X-ray analysis.* Our sample crystallised (from petroleum ether) in a different space group (orthorhombic, $P2_12_12_1$). The unit cell contains only one molecule as against two observed in case of natural lucidene. The X-ray analysis unambiguously showed that we are handling a single enantiomer and not a racemic mixture. Moreover the stereochemistry is exactly the same as the assigned to natural lucidene. The X-ray crystal structure (fig 6) also showed that the synthetic sample used for the structure determination is a single enantiomer. The confirmations of the racemic (natural lucidene fig 2a) and the single enantiomorph (synthetic lucidene, fig 6) are nearly identical. However the absolute stereochemistry of the synthetic (+)- isomer remains to be established.

* In collaboration with Dr. Niger of University of Bonn. We thank him for the X-ray analysis.

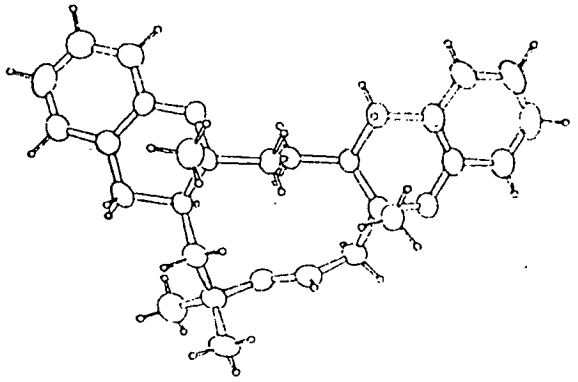


Fig. 2a : X-ray structure of natural Lucidene

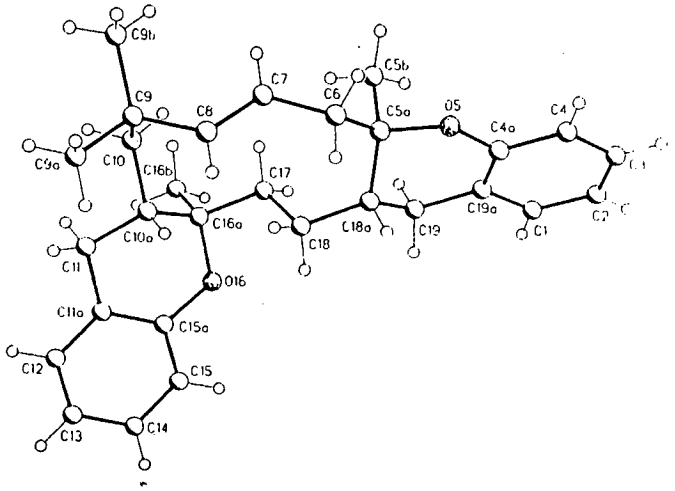
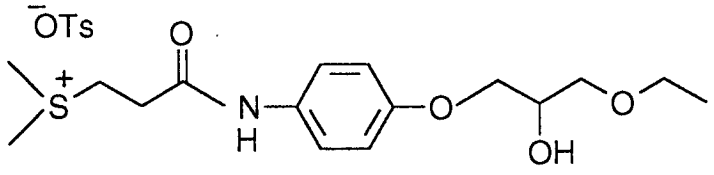


Fig. 6 : X-ray Structure of synthetic Lucidene

Any explanation to the isolation of optically active lucidene would be incomplete and unacceptable to most of the chemists at this stage. However, in all probability, the resolution is taking place due to spontaneous crystallisation of one enantiomer. This explanation would find some support from the recent findings of Tamura and coworkers[19++] . who have demonstrated unambiguously the unusual enantiomeric resolution by recrystallisation of a racemic compound **49**.



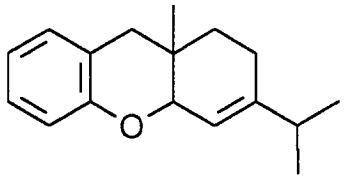
49

Efforts are being made to prepare lucidene in substantial quantities and subject it to the process of recrystallisation and measuring rotation after each step and check the enantiomeric enrichment. We hope to offer a definite answer to this problem in the near future.

In conclusion, we have achieved a simple synthesis of lucidene **2** confirming the generality of the method developed by us.

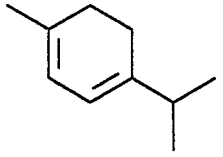
Recently, another benzopyranyl monoterpene, 3-isopropyl-9a-methyl-1,2,4a,9a-tetrahydroxanthene **44** has been isolated from xylopia africana by Ekpa and co-workers[17,18].

++Enantiomeric resolution by recrystallisation of racemic compounds has been believed to be totally infeasible.[20]



44

The assigned structure is based on spectral analysis (MS, ^{1}H & ^{13}C NMR and DEPT), and the X-ray analysis of the dibromo derivative. It appears to be derived by addition of C_7 fragment (most likely $HO\text{-}C_6H_4\text{-}CH2^{+}$) to α-terpenene **50**. We believe that a simple synthetic confirmation to the assigned structure would be possible by using the general method developed by us. We have initiated the work in this direction.



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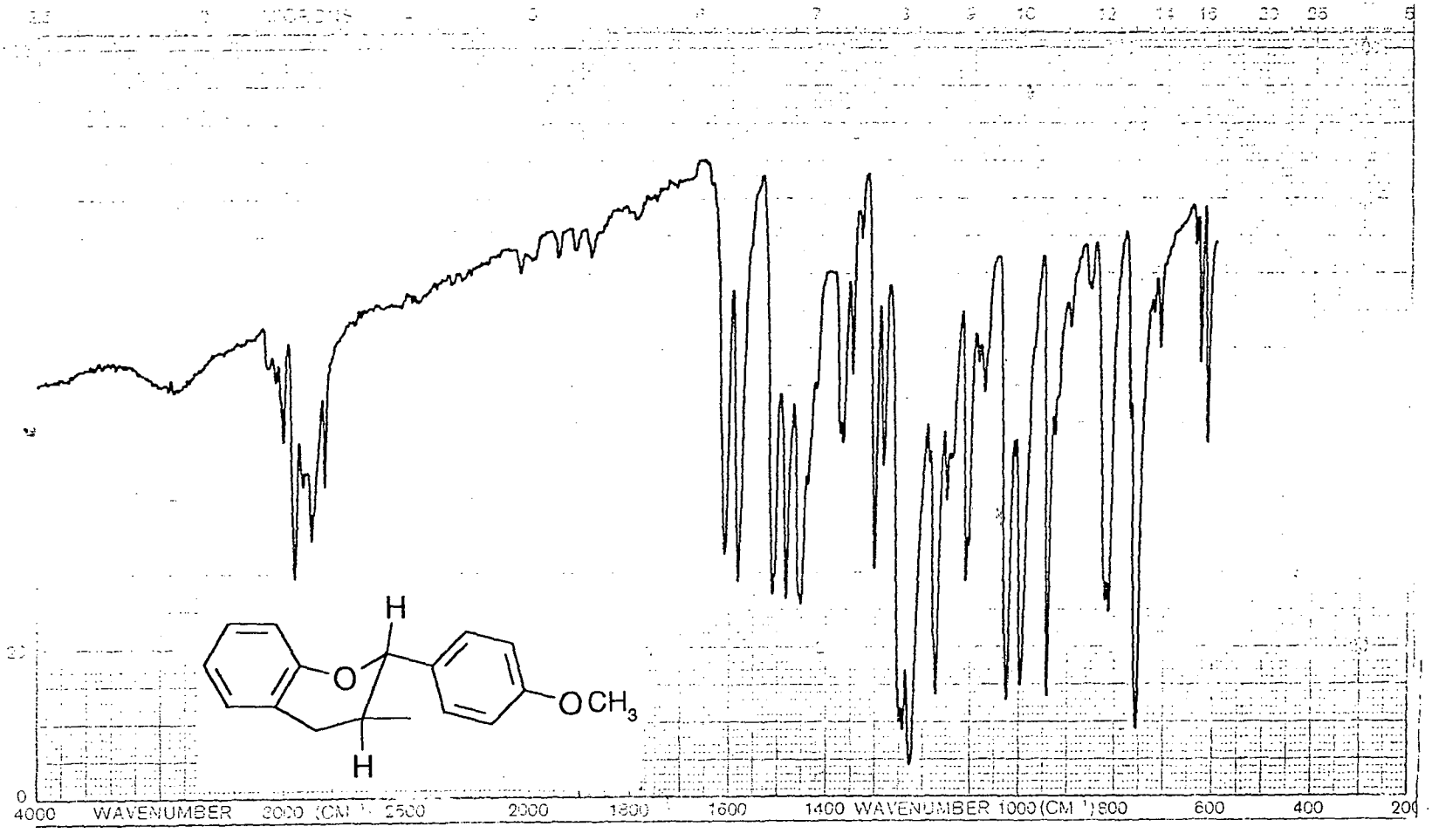


Fig. 2.01 : IR spectrum of 16

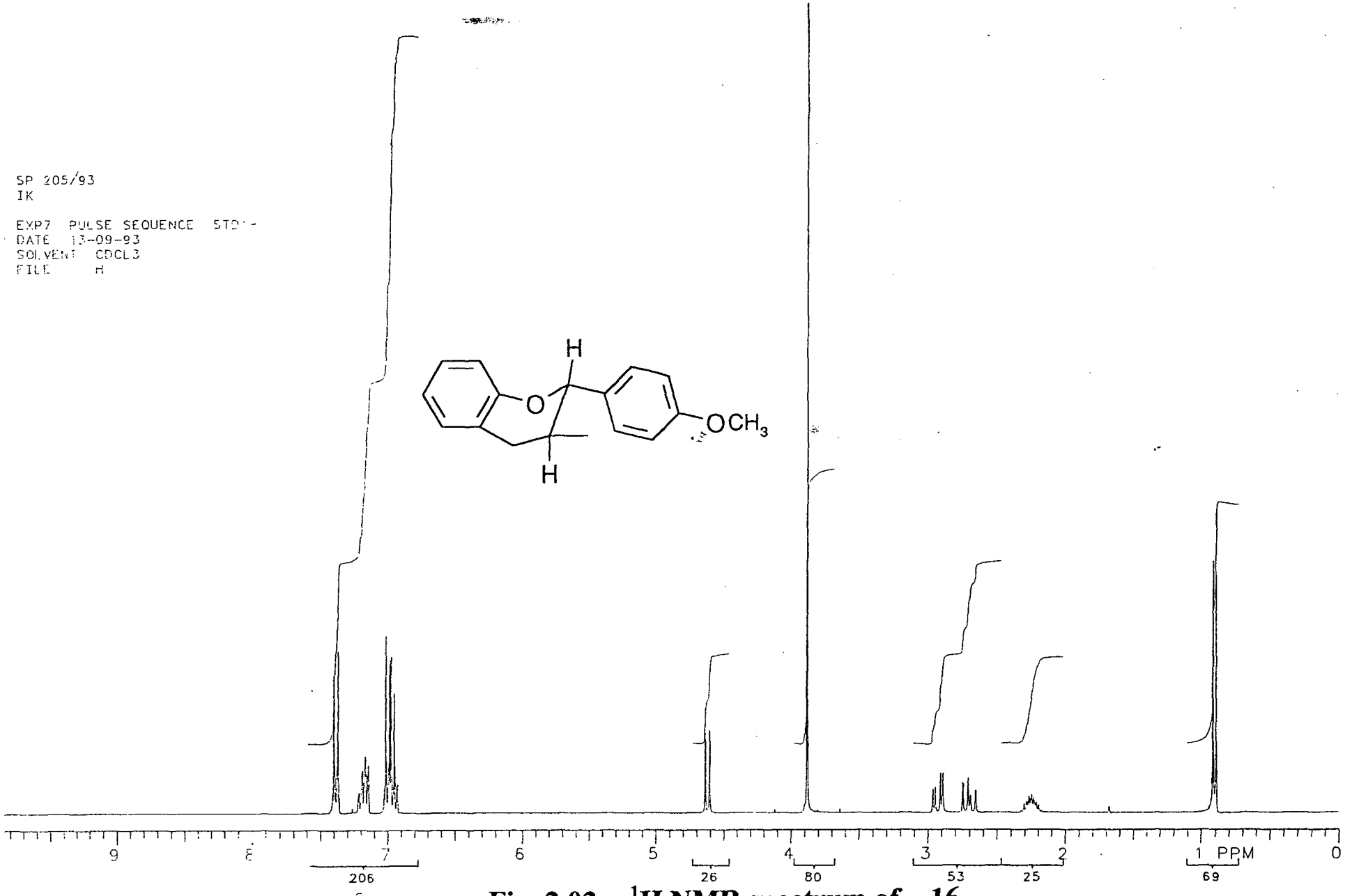


Fig. 2.02 : ^{1}H NMR spectrum of 16

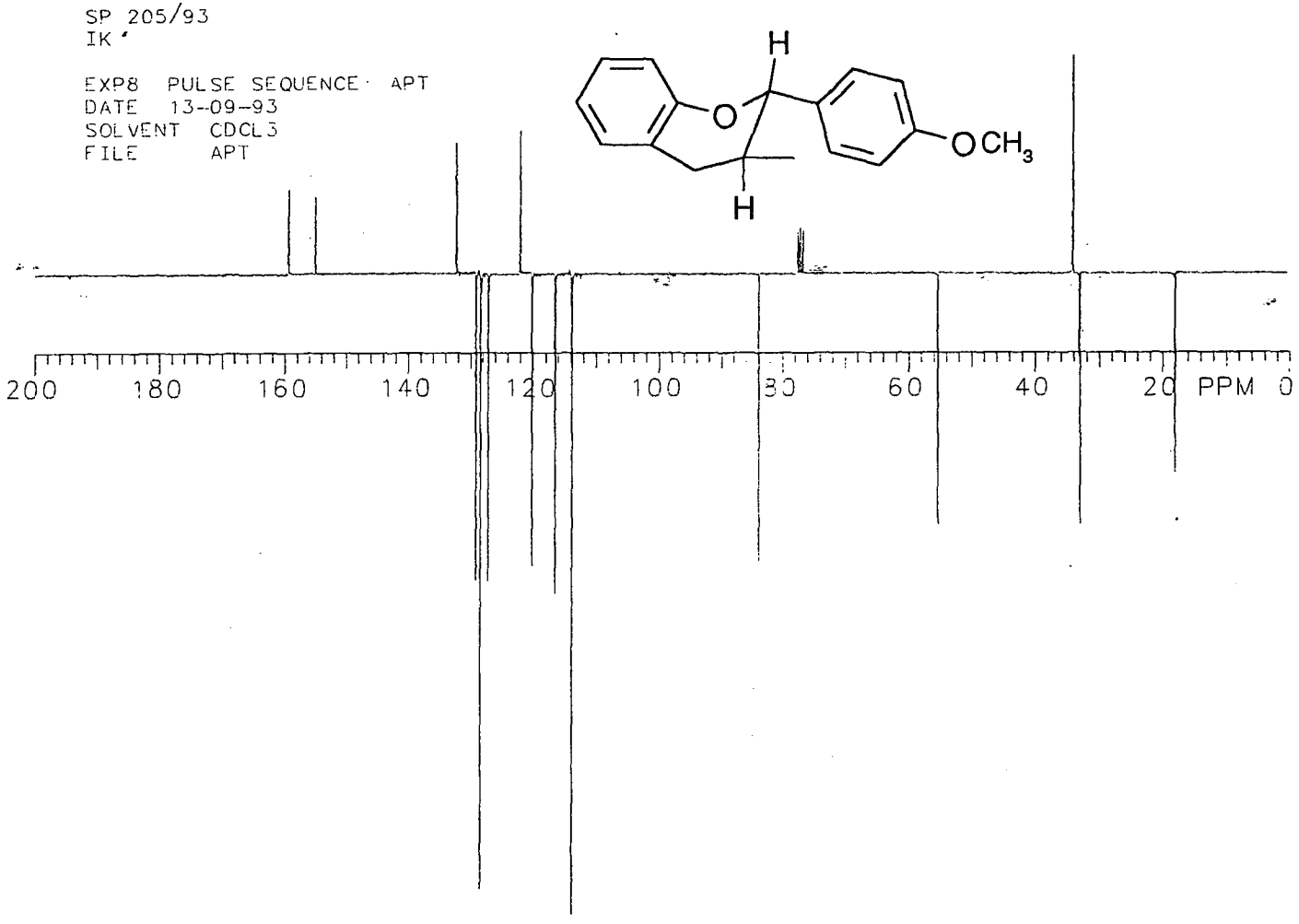


Fig. 2.03 : ^{13}C NMR spectrum of 16

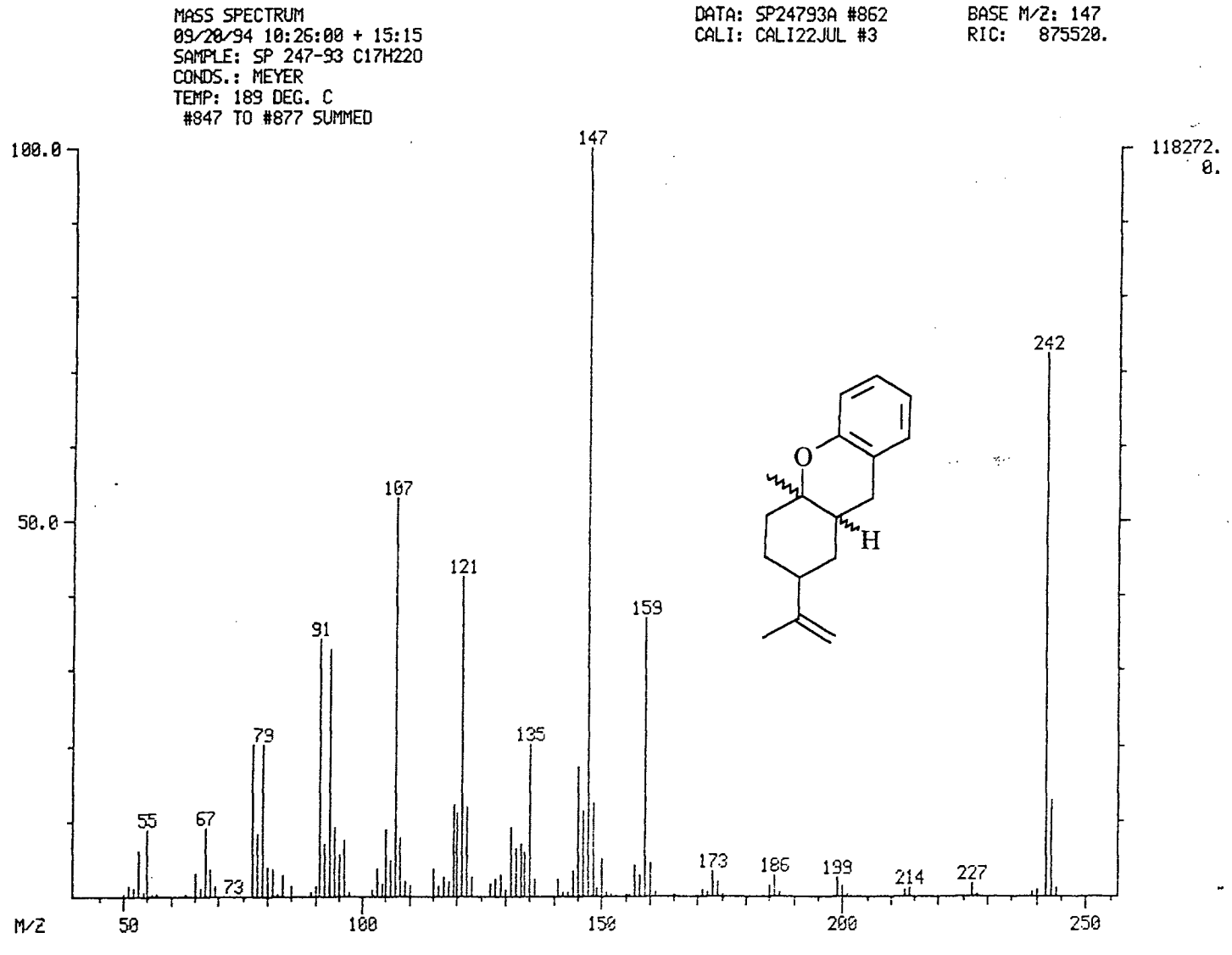


Fig. 2.04 : Mass spectrum of 20

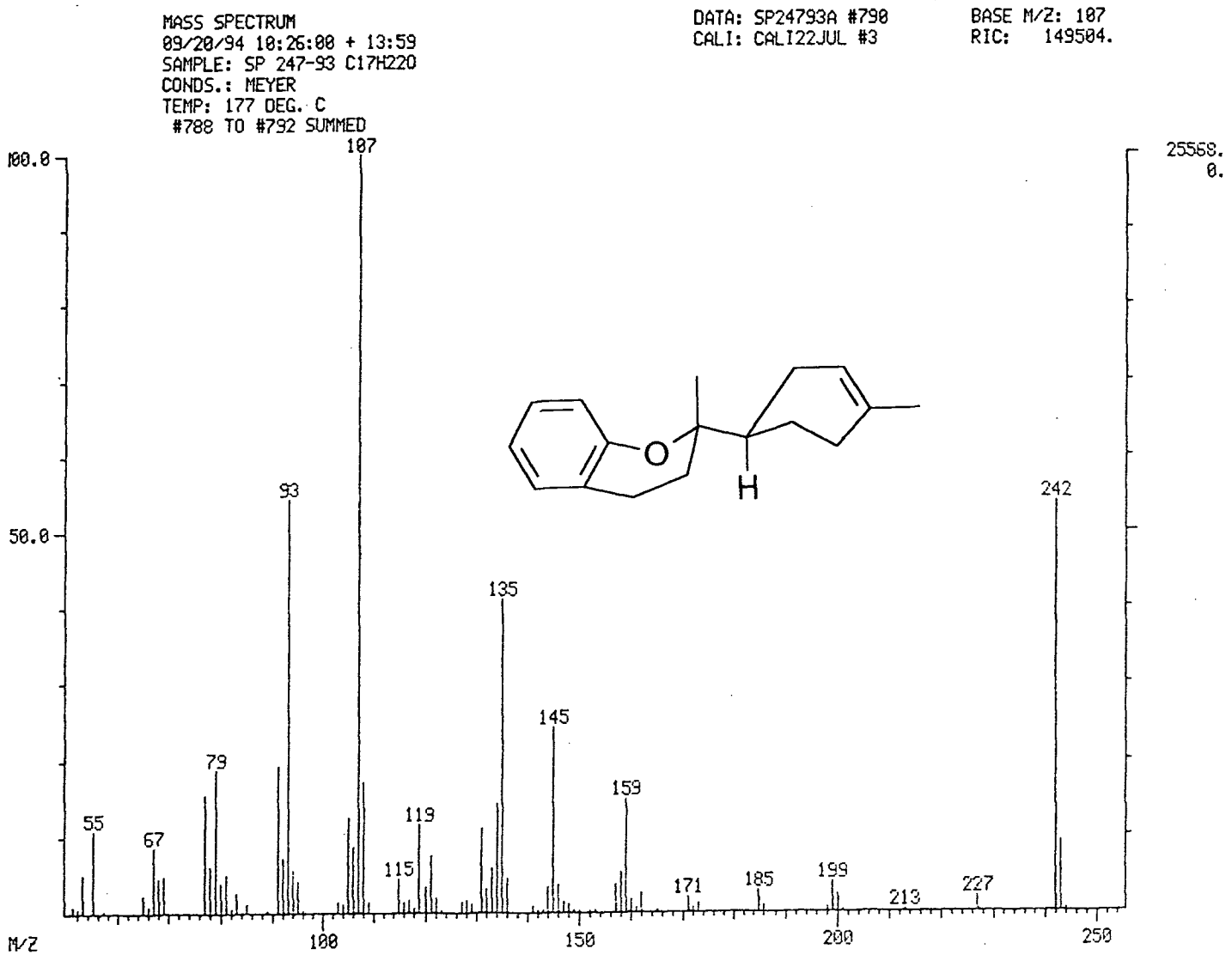


Fig. 2.05 : Mass spectrum of 18

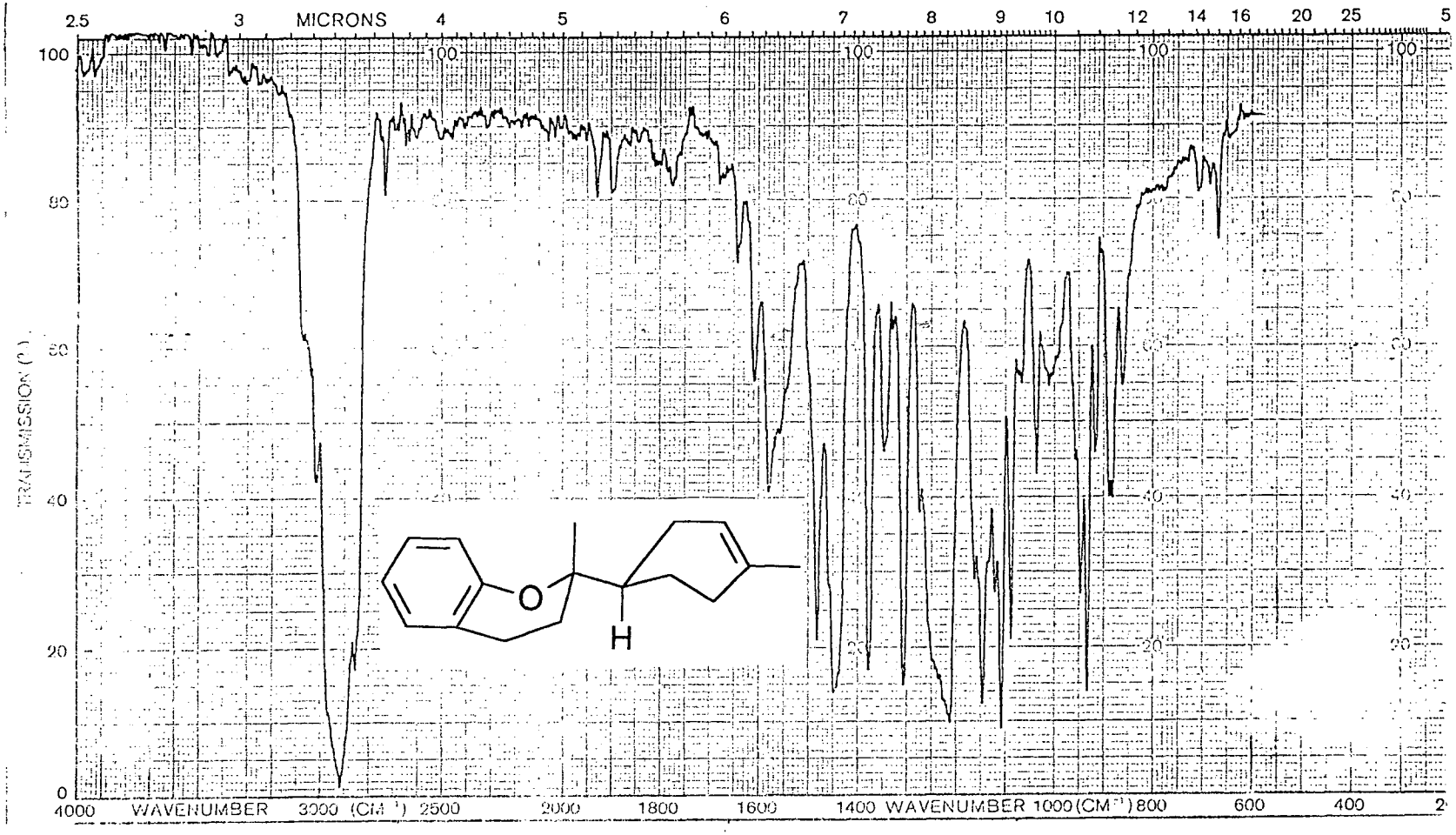


Fig. 2.06 : IR spectrum of 18

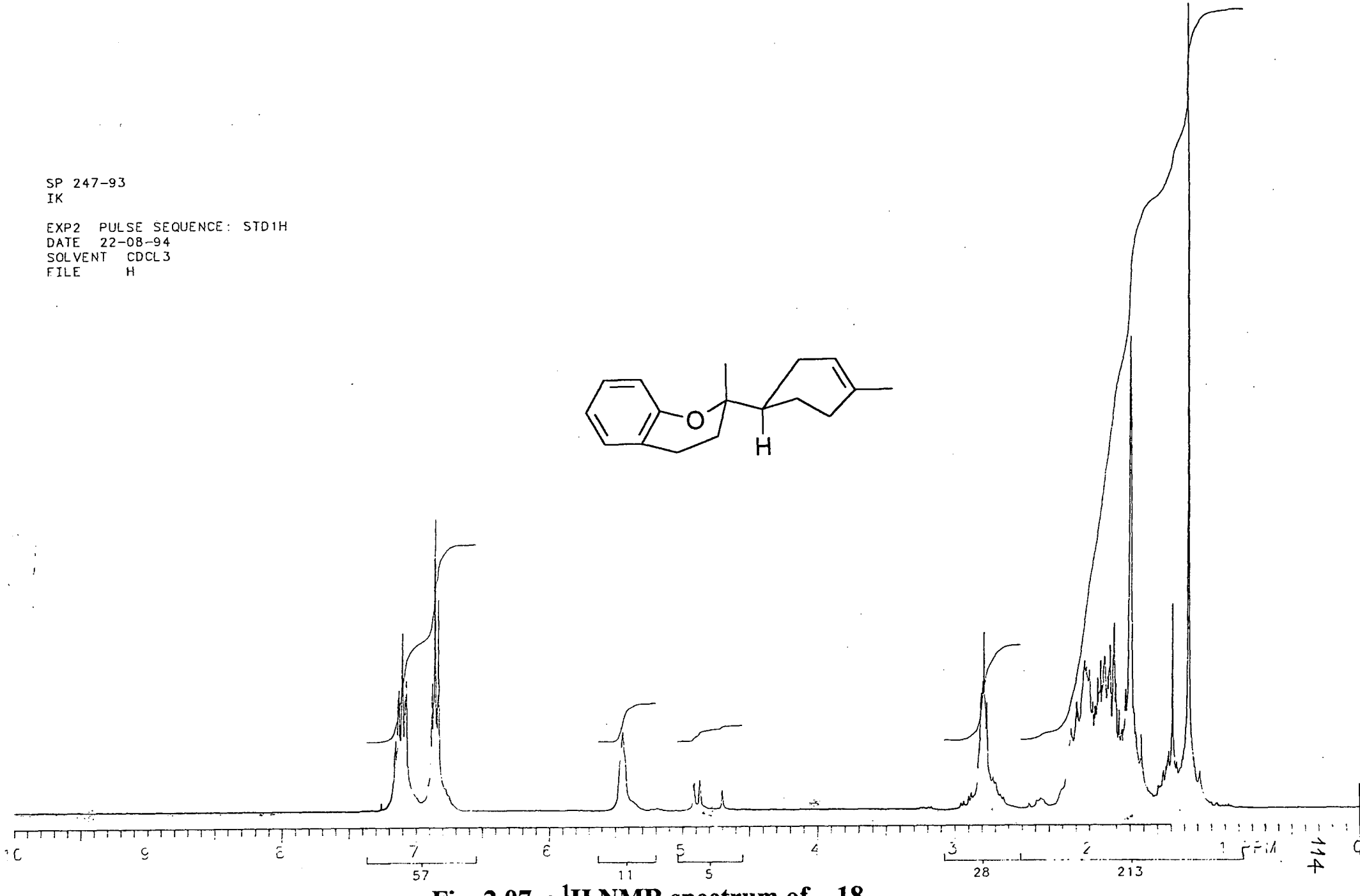


Fig. 2.07 : ^{1}H NMR spectrum of 18

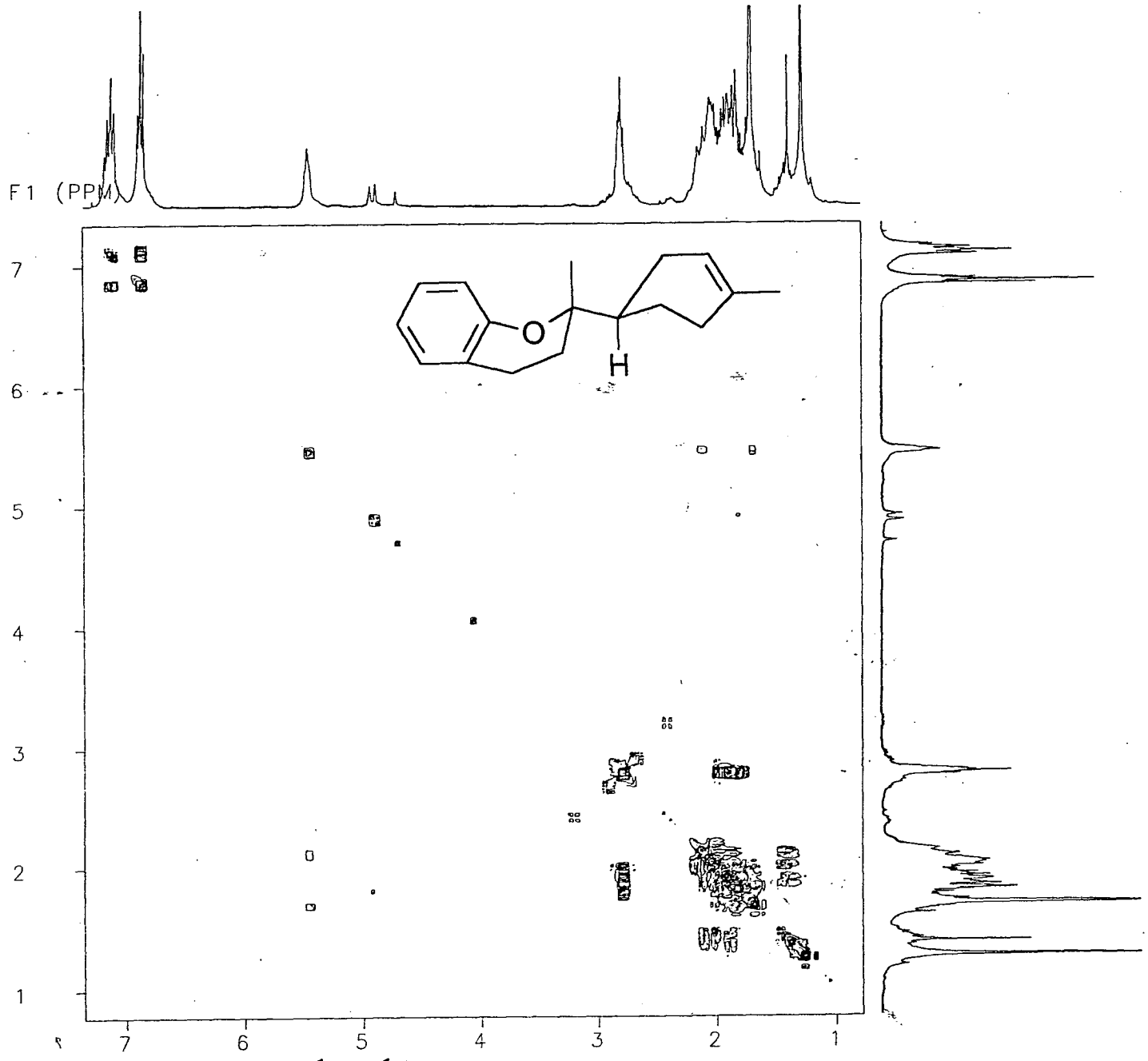


Fig. 2.08 : ^{1}H - ^{1}H COSY spectrum of 18

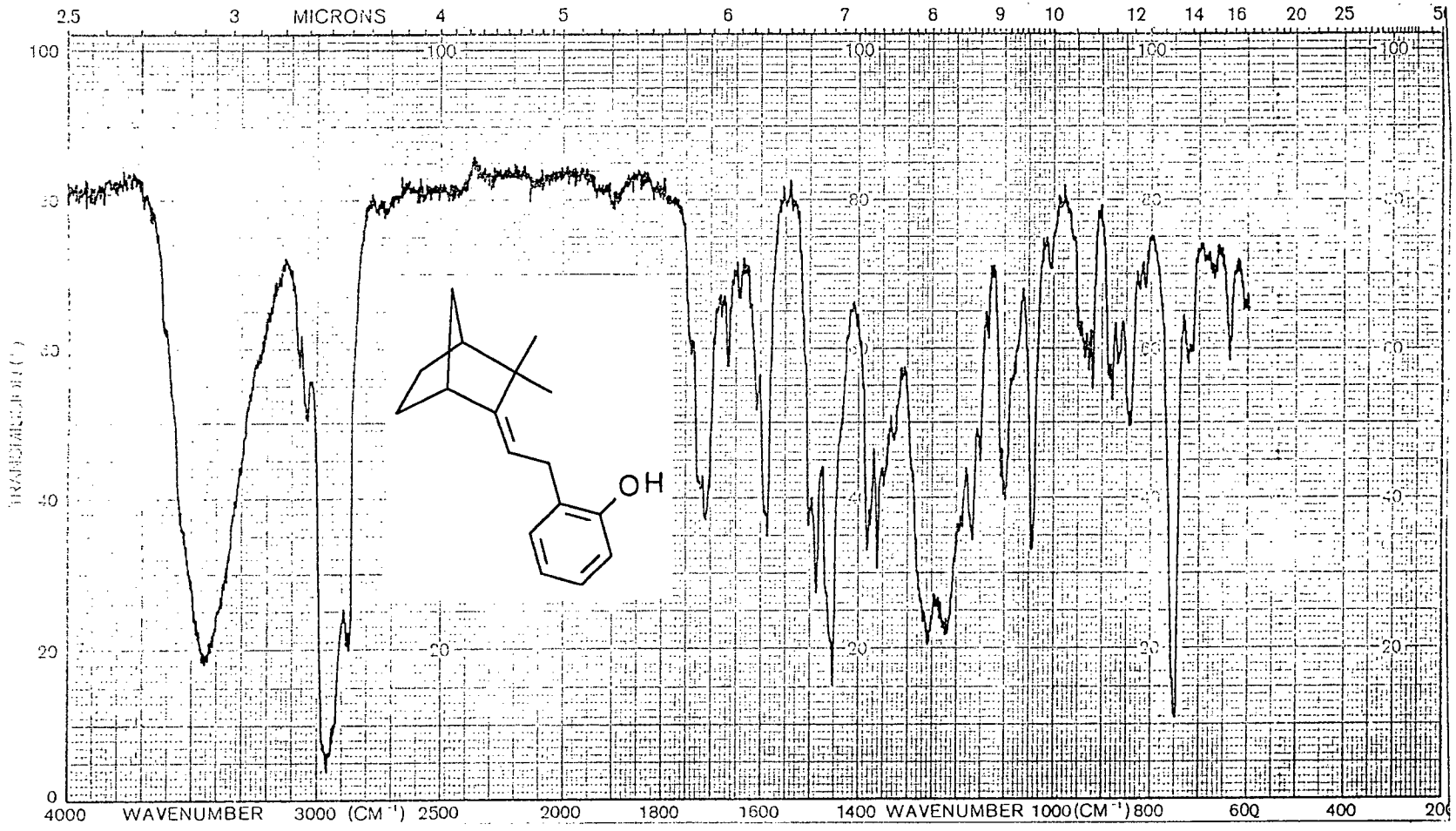


Fig. 2.09 : IR spectrum of 21

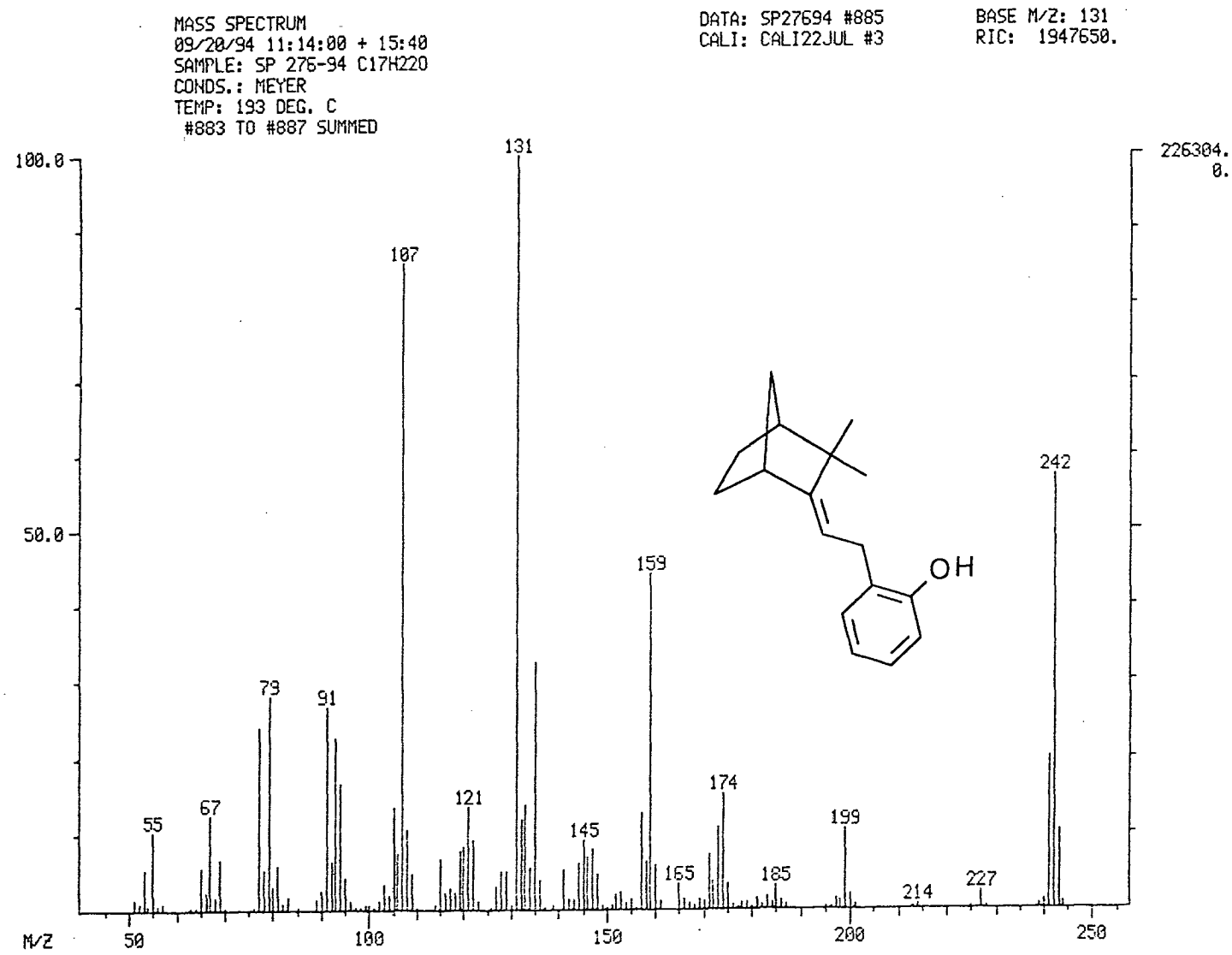


Fig. 2.10 : Mass spectrum of 21

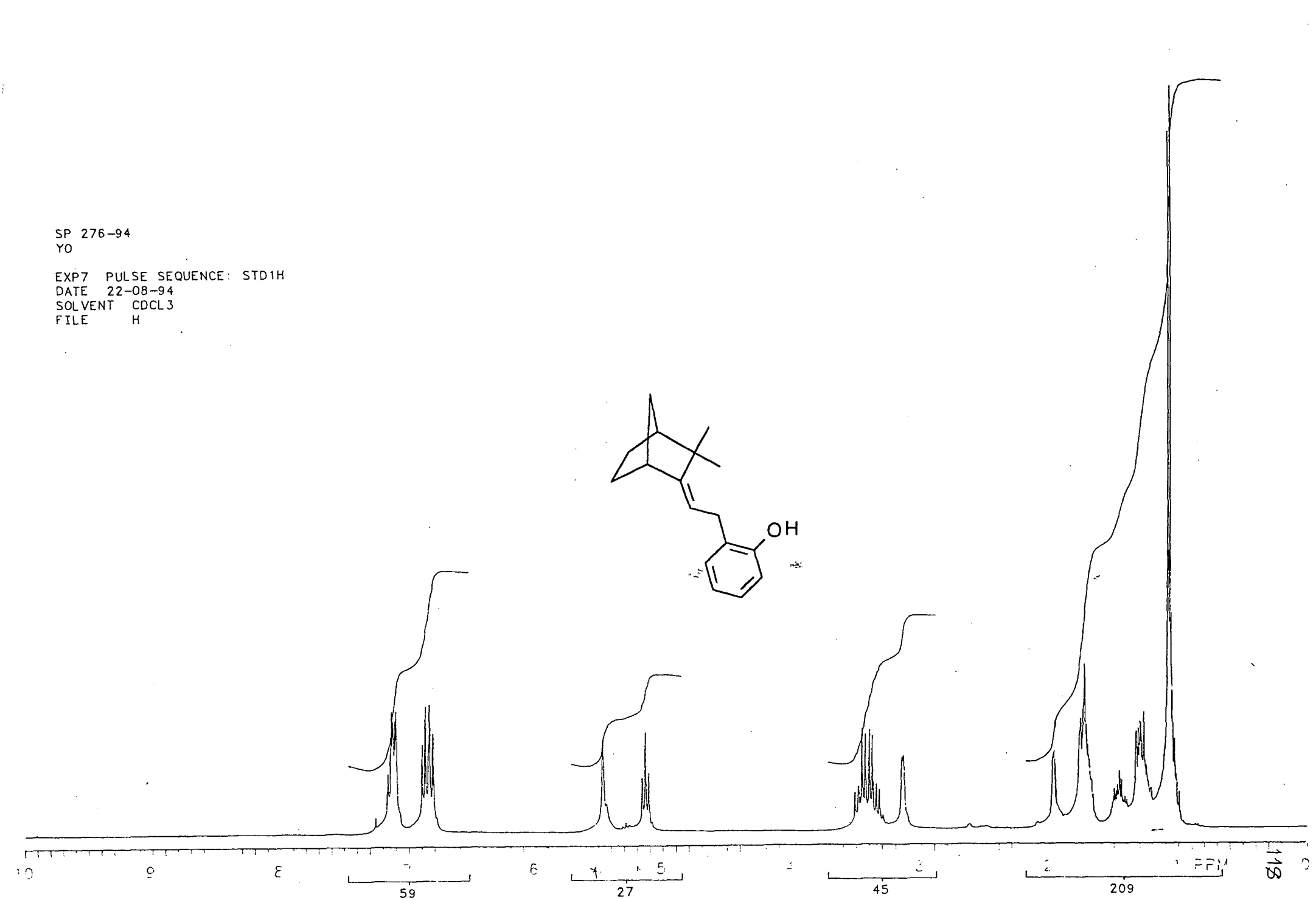


Fig. 2.11 : ^{1}H NMR spectrum of 21

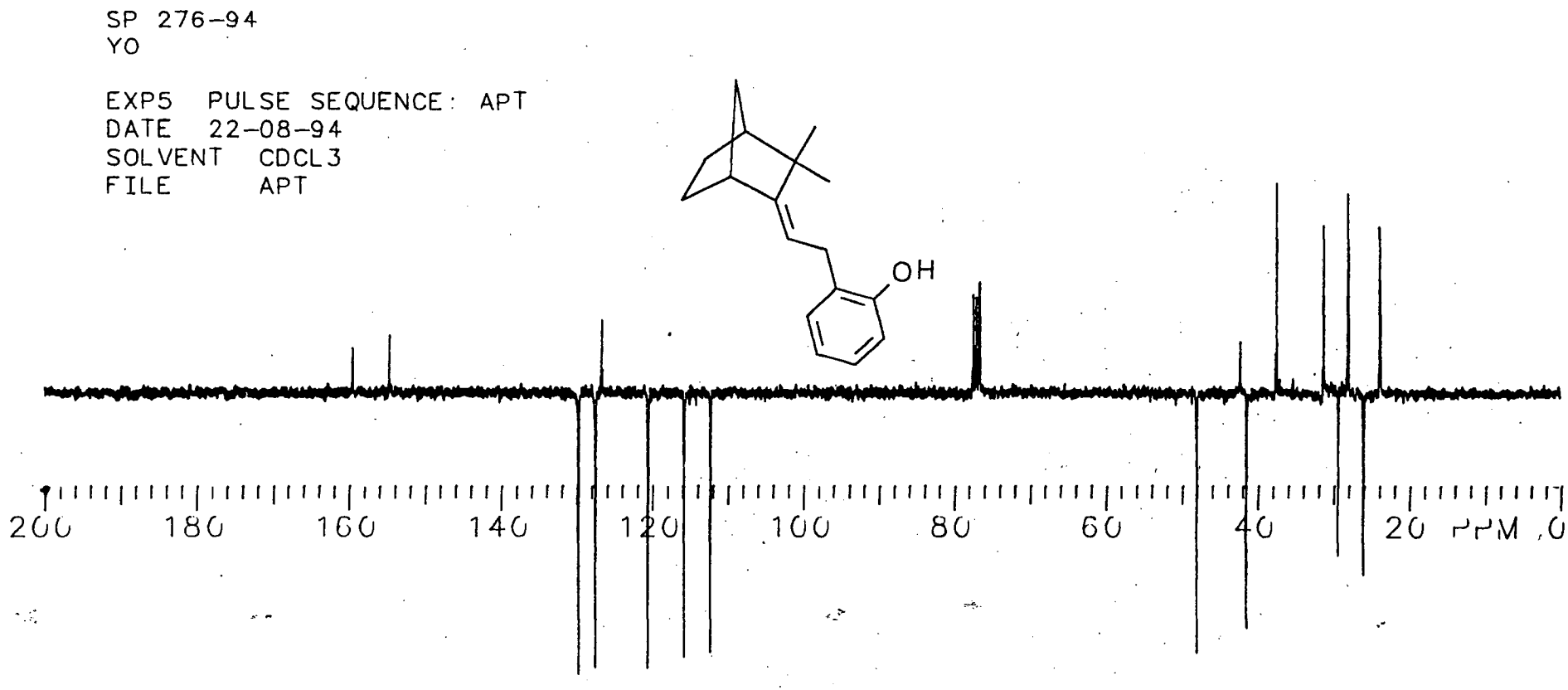


Fig. 2.12 : ^{13}C NMR spectrum of 21

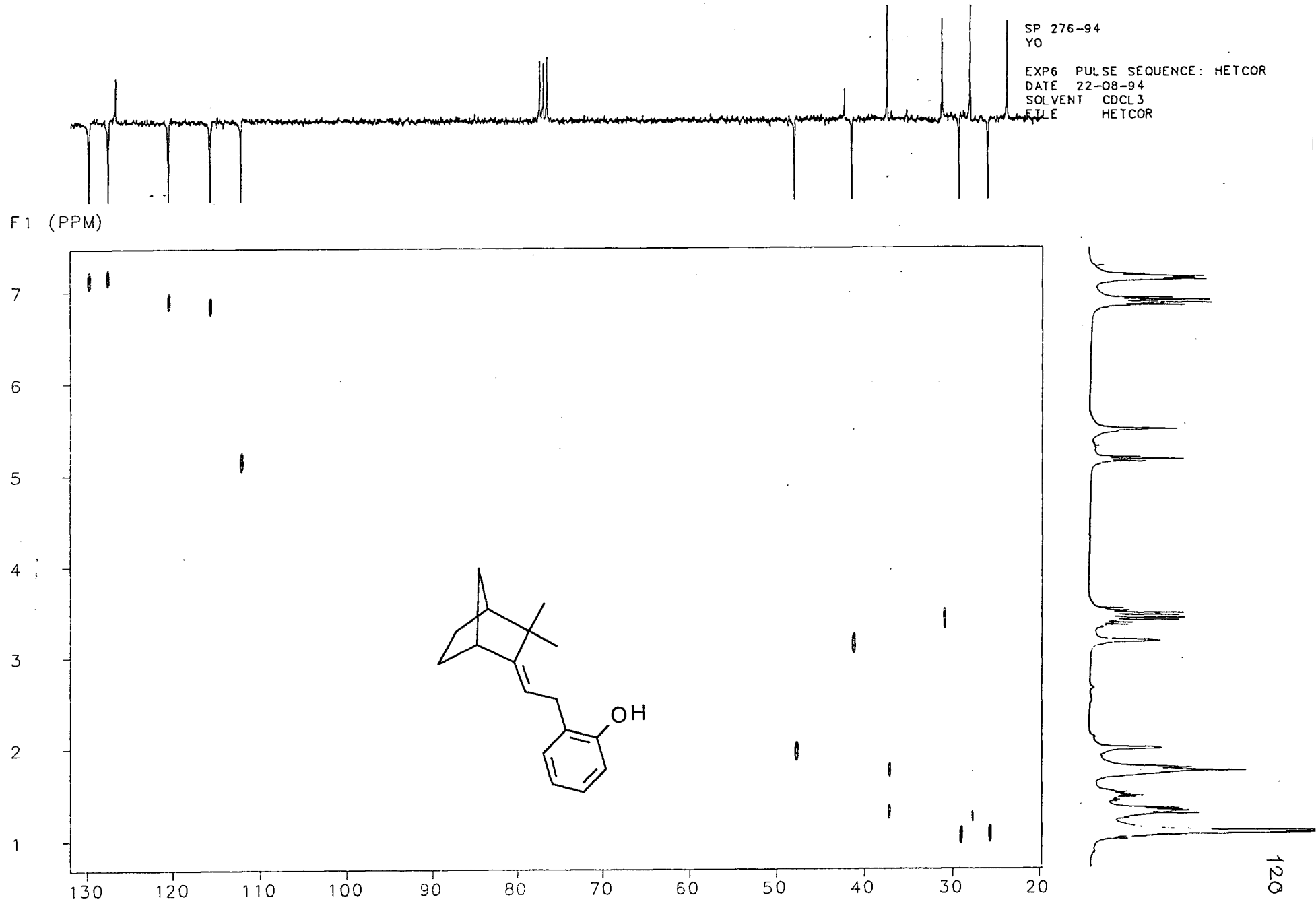


Fig. 2.13 : ^{1}H - ^{13}C HETCOR spectrum of 21

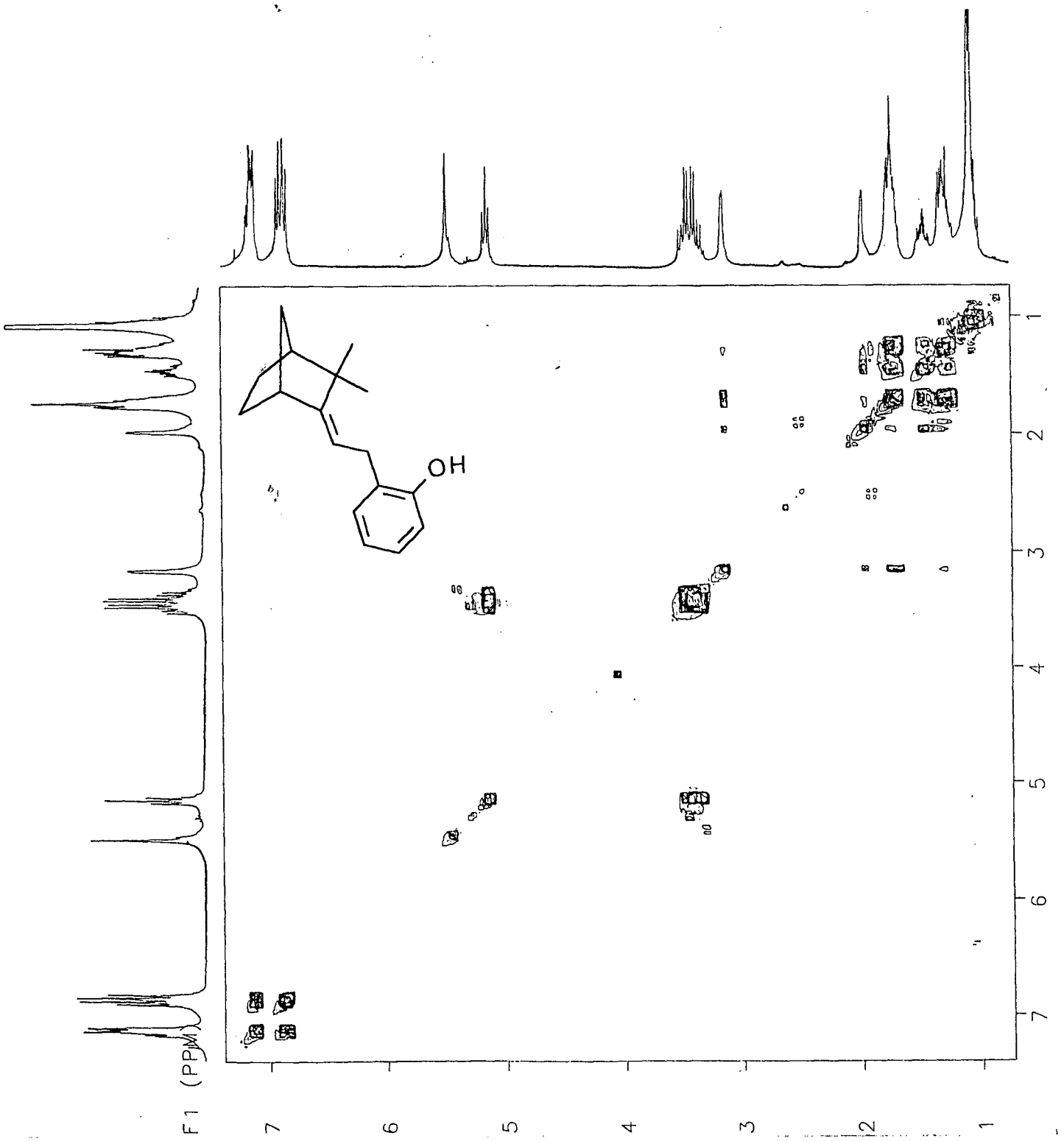


Fig. 2.14 : ^{1}H - ^{1}H COSY spectrum of 21

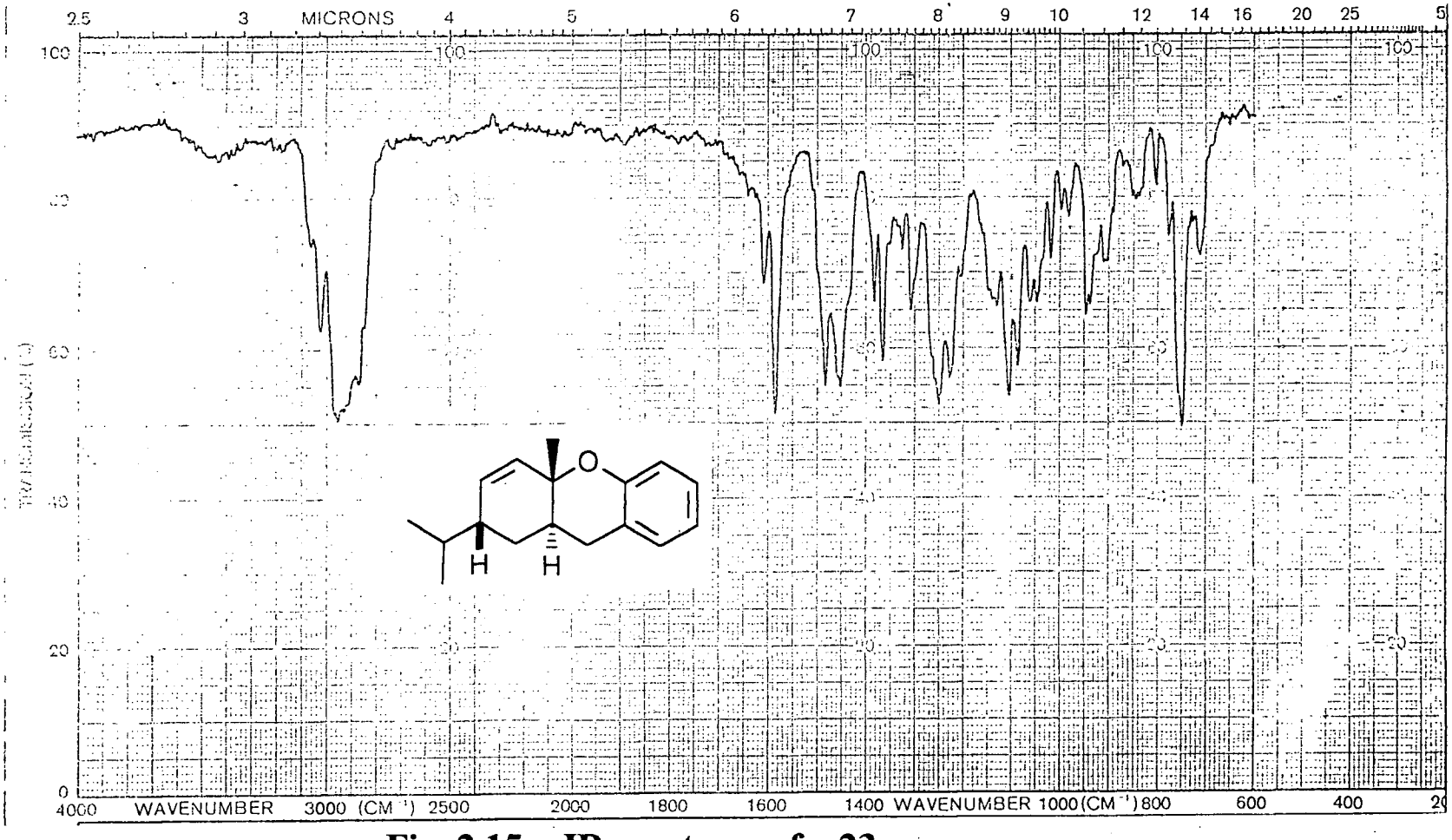


Fig. 2.15 : IR spectrum of 23

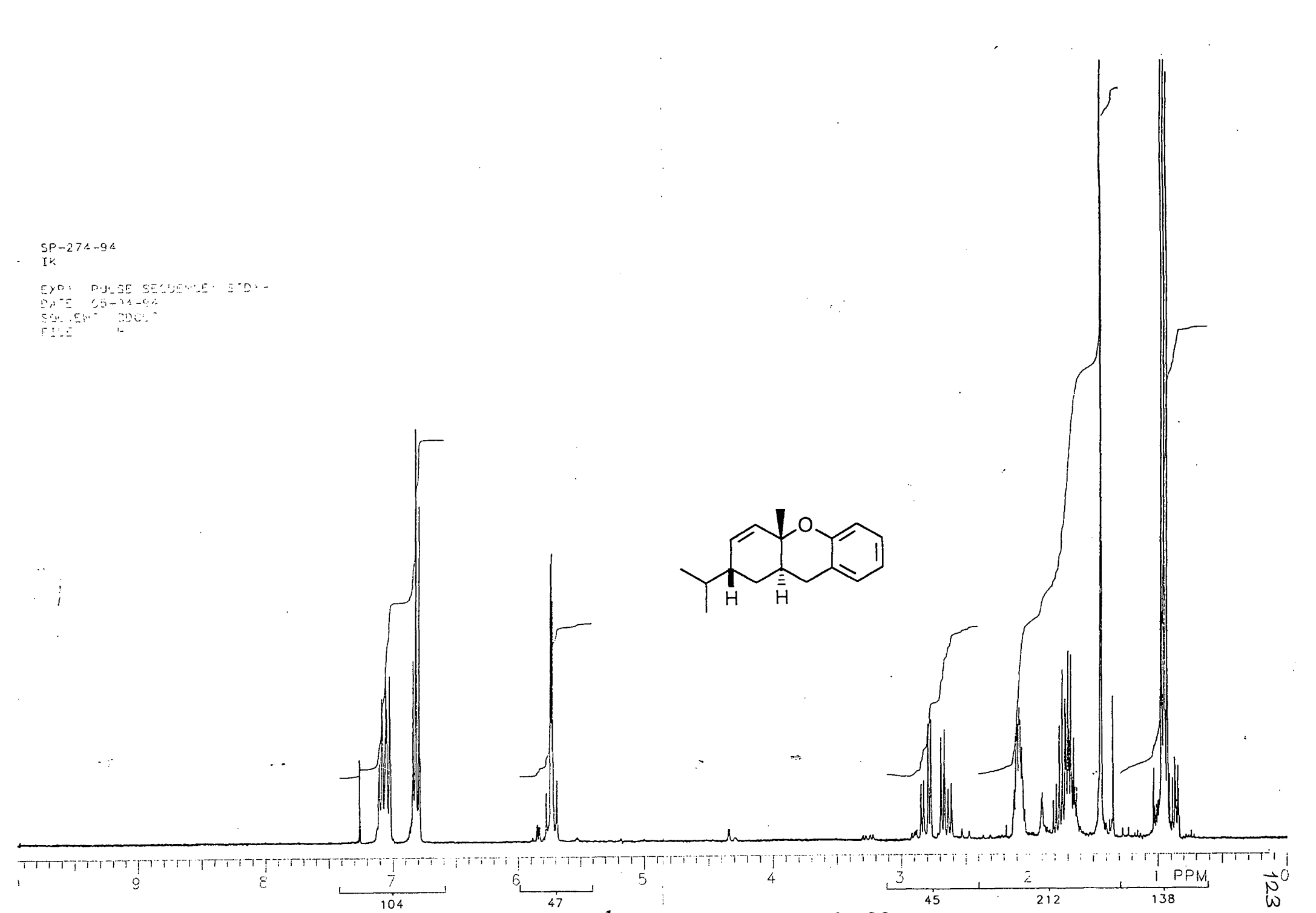


Fig. 2.16 : ^{1}H NMR spectrum of 23

Fig. 2.17 : ^{13}C NMR spectrum of 23

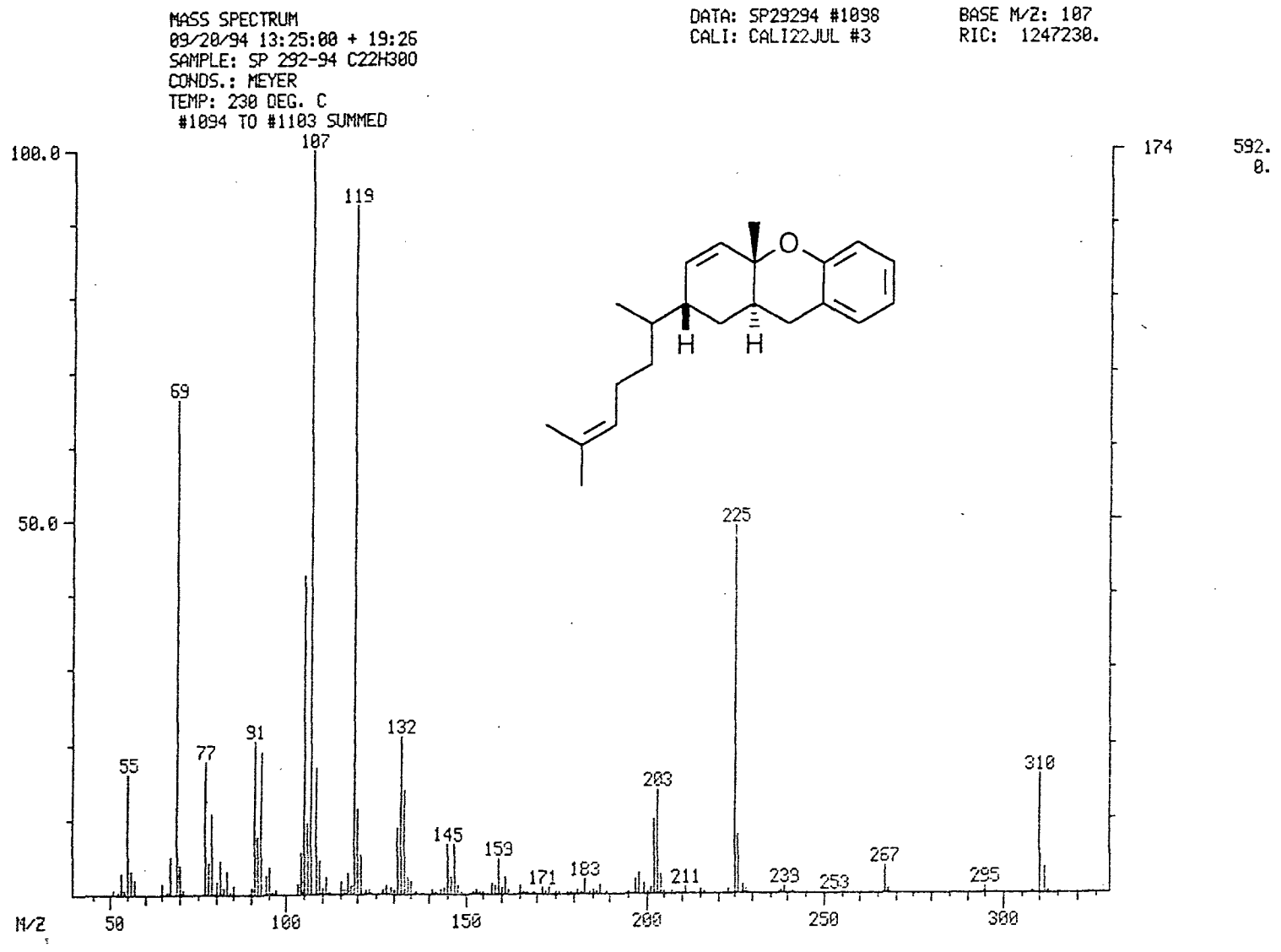


Fig. 2.18 : Mass spectrum of 26

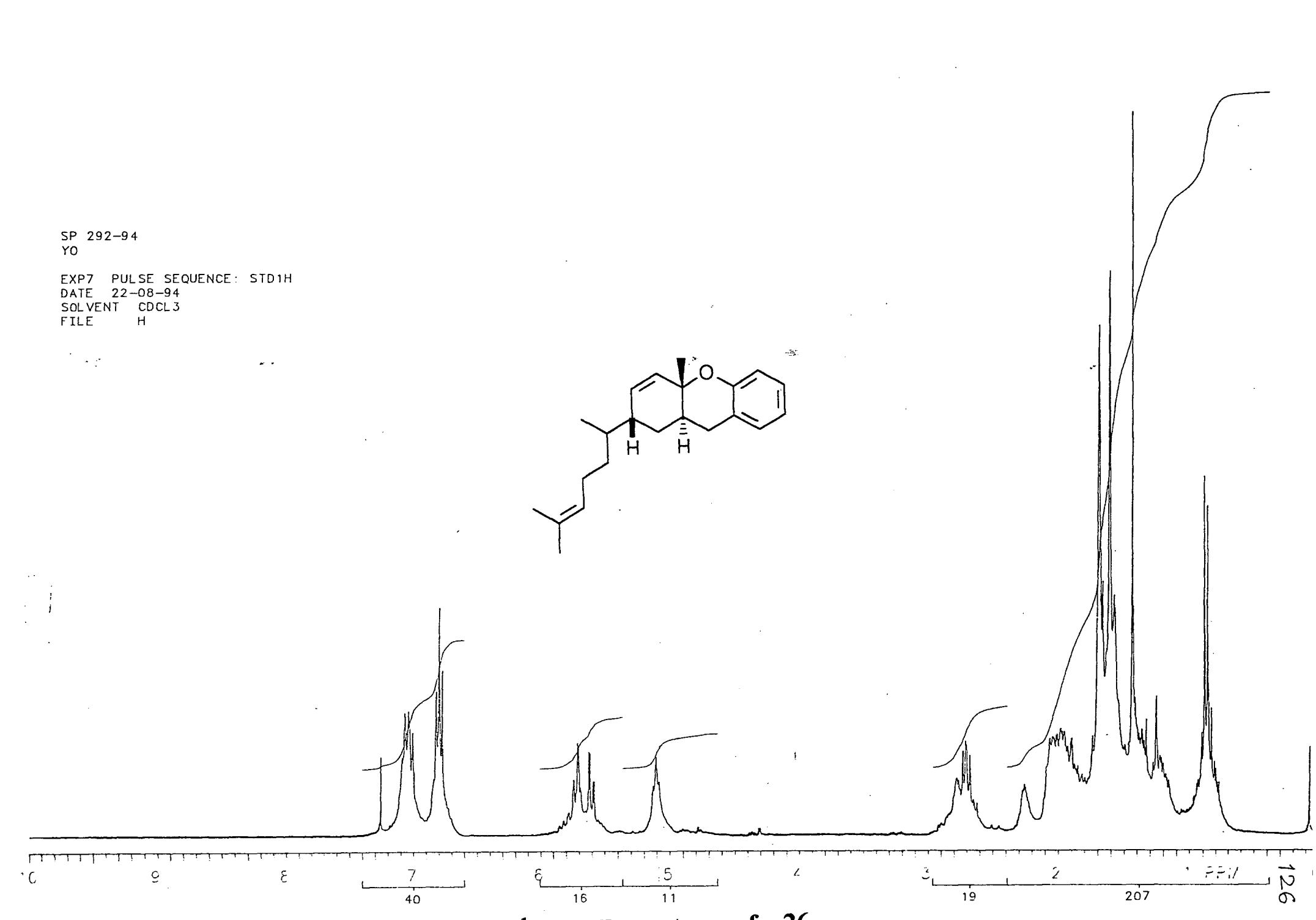


Fig. 2.19 : ^{1}H NMR spectrum of 26

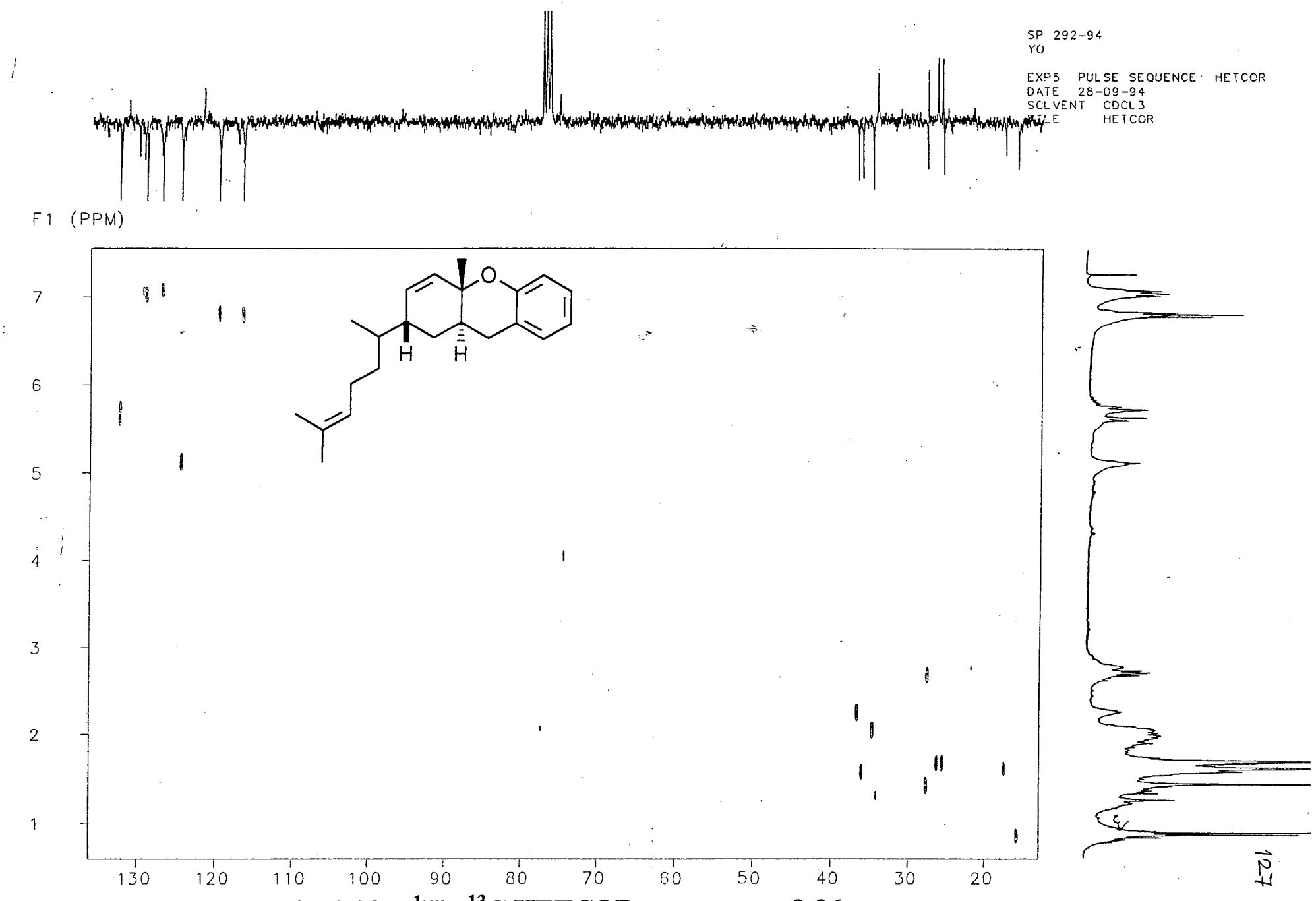


Fig. 2.20 : ^{1}H - ^{13}C HETCOR spectrum of 26

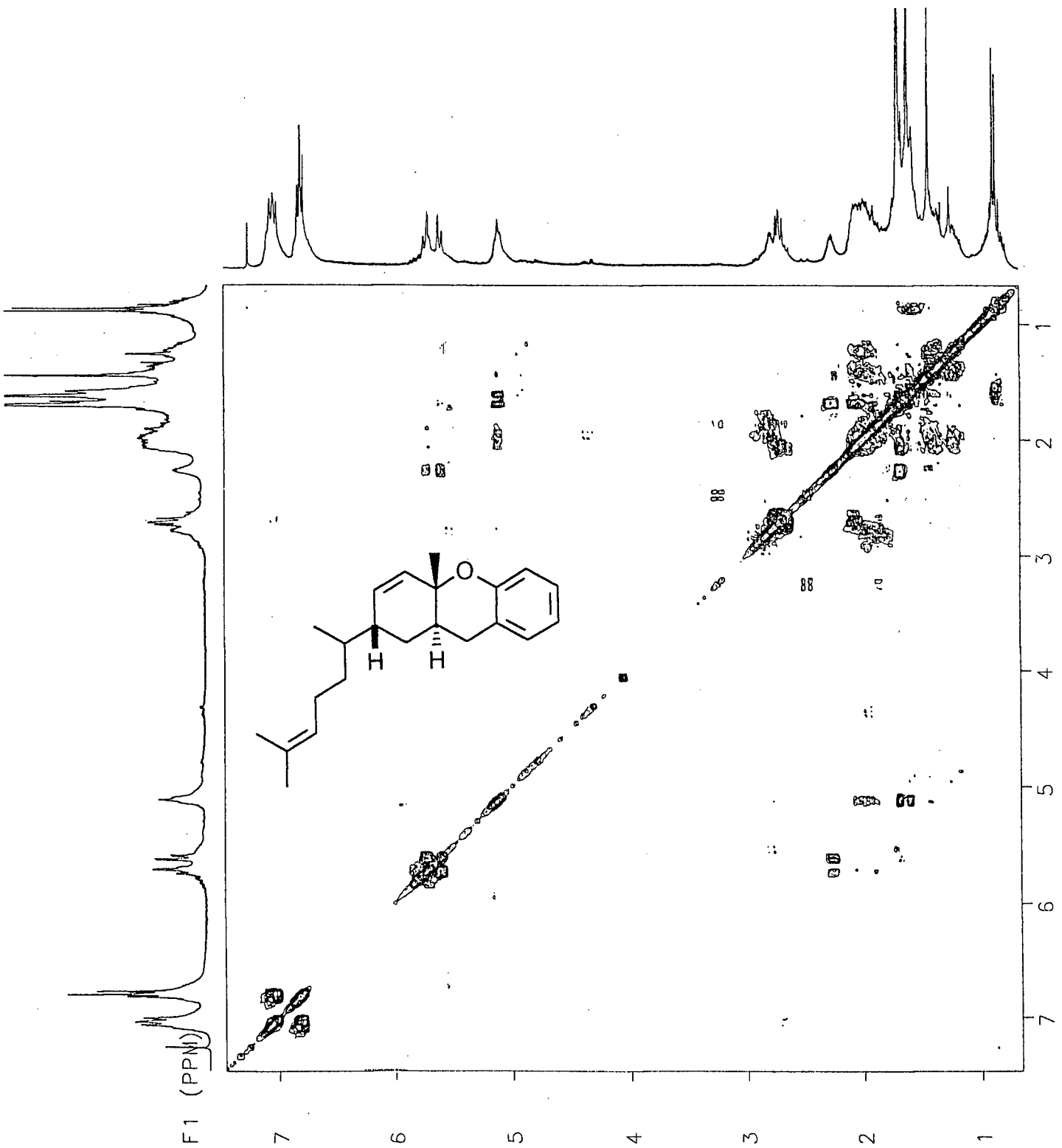


Fig. 2.21 : ^{1}H - ^{1}H COSY spectrum of 26

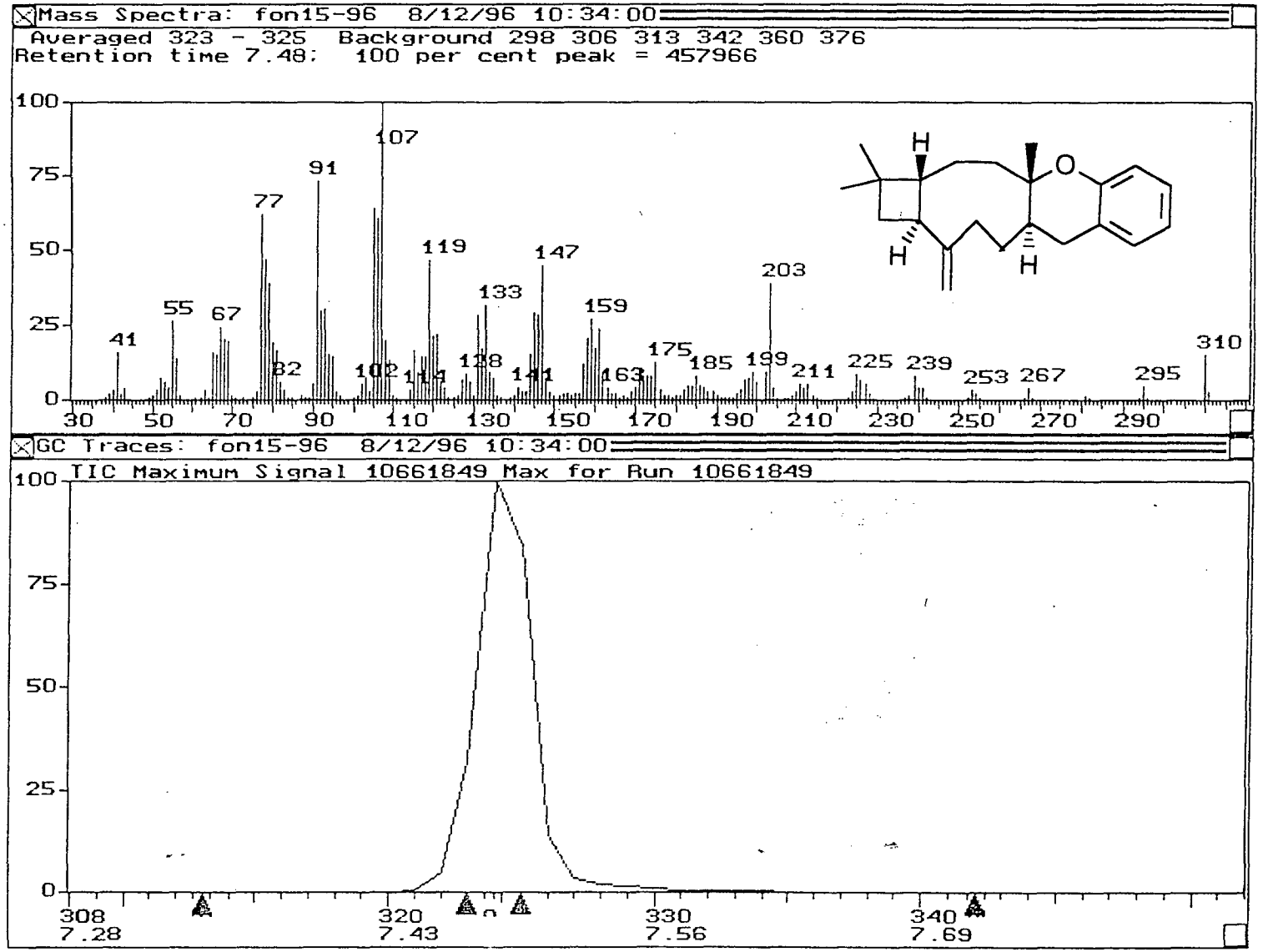


Fig. 2.22 : Mass spectrum of 28

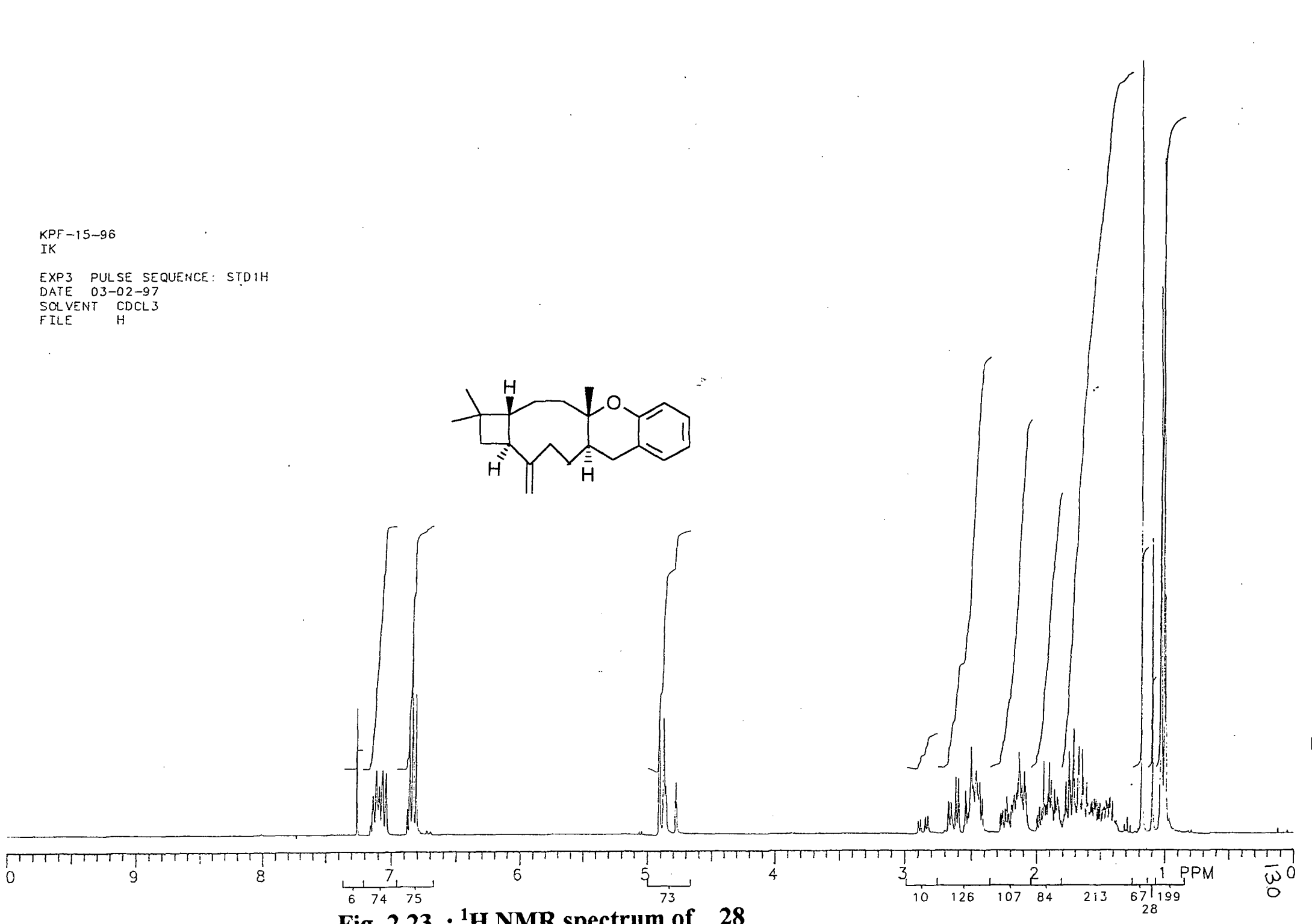


Fig. 2.23 : ^{1}H NMR spectrum of 28

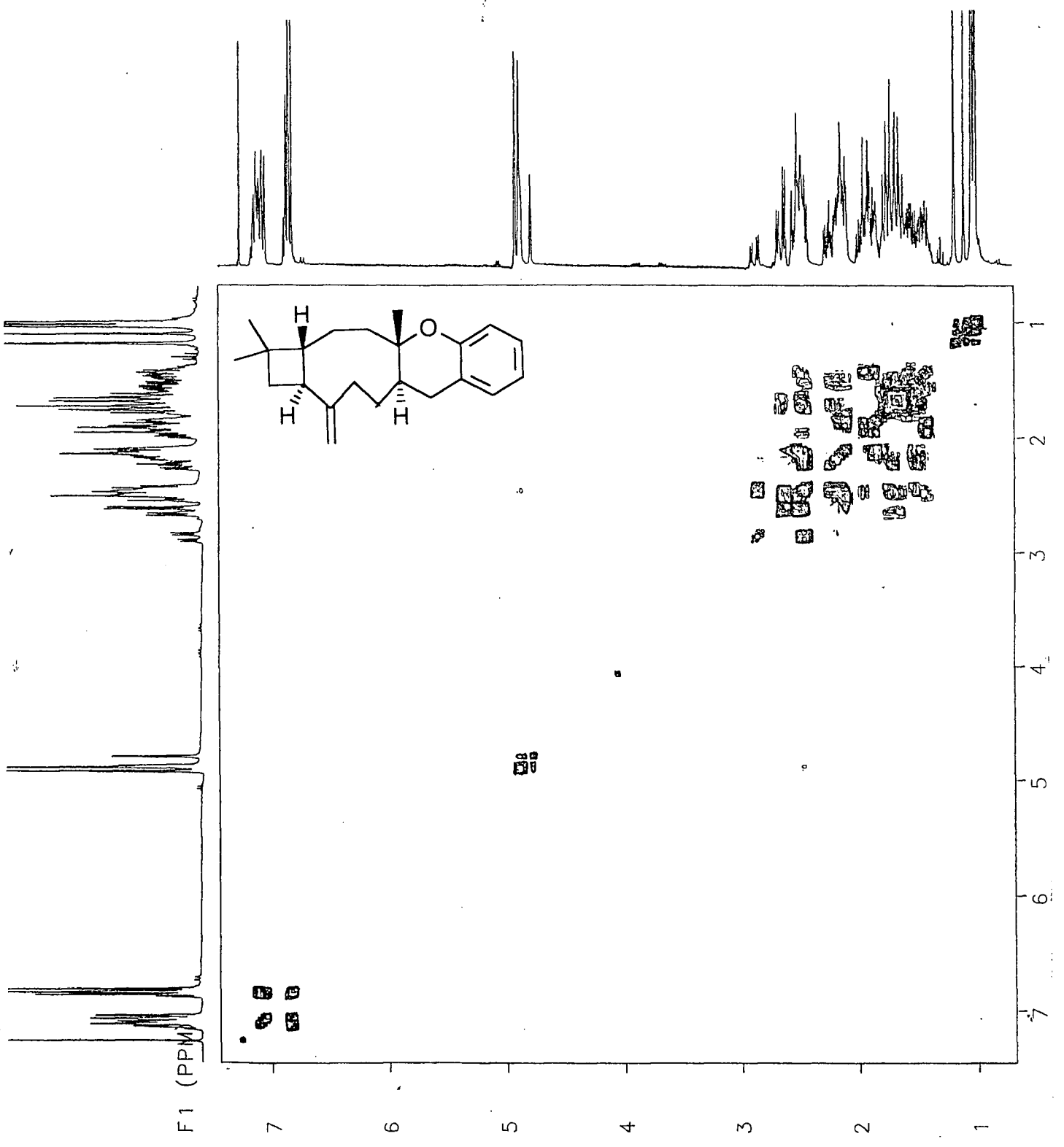


Fig. 2.24 : ^{1}H - ^{1}H COSY spectrum of 28

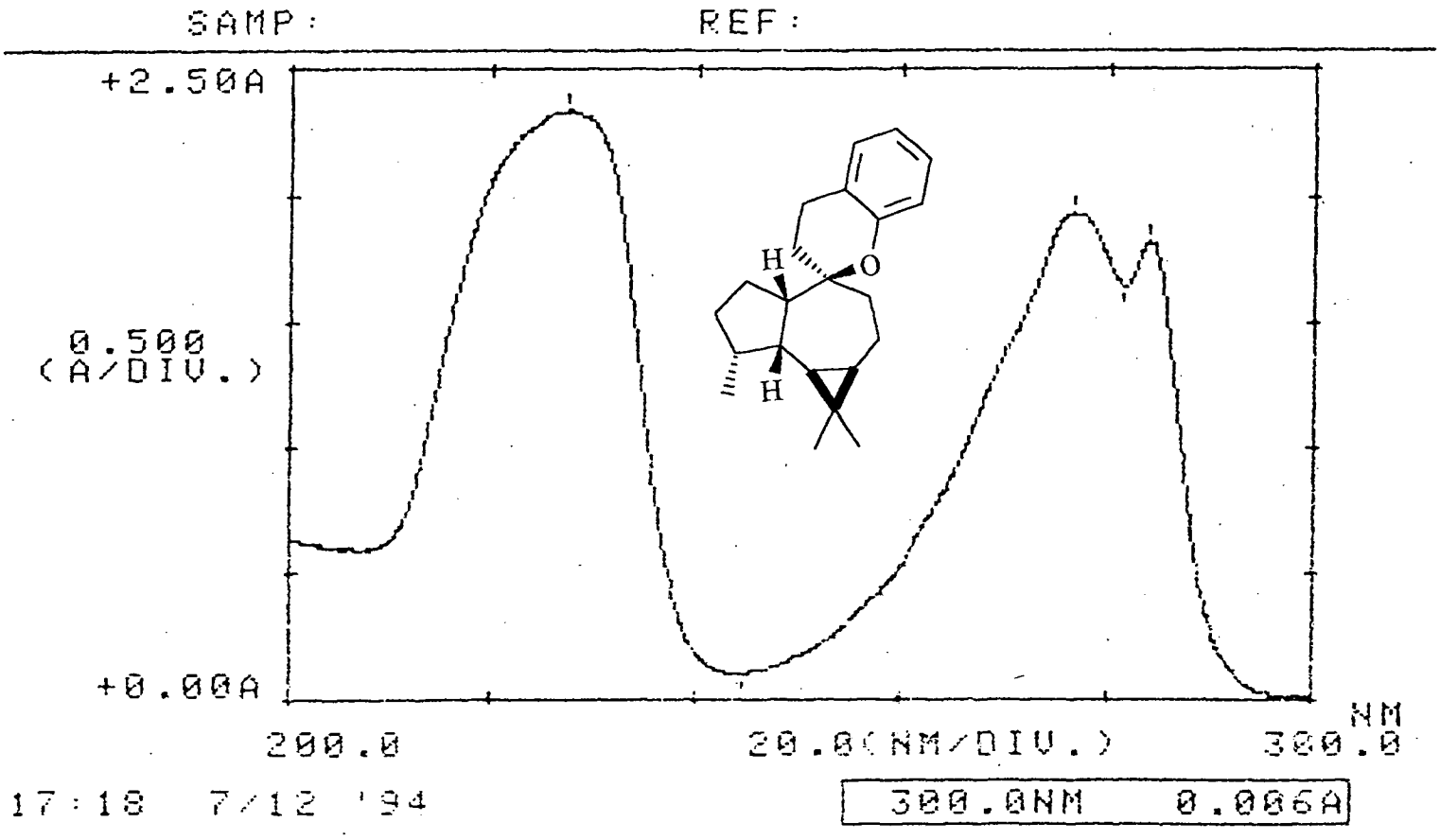


Fig. 2.25 : UV spectrum of 1

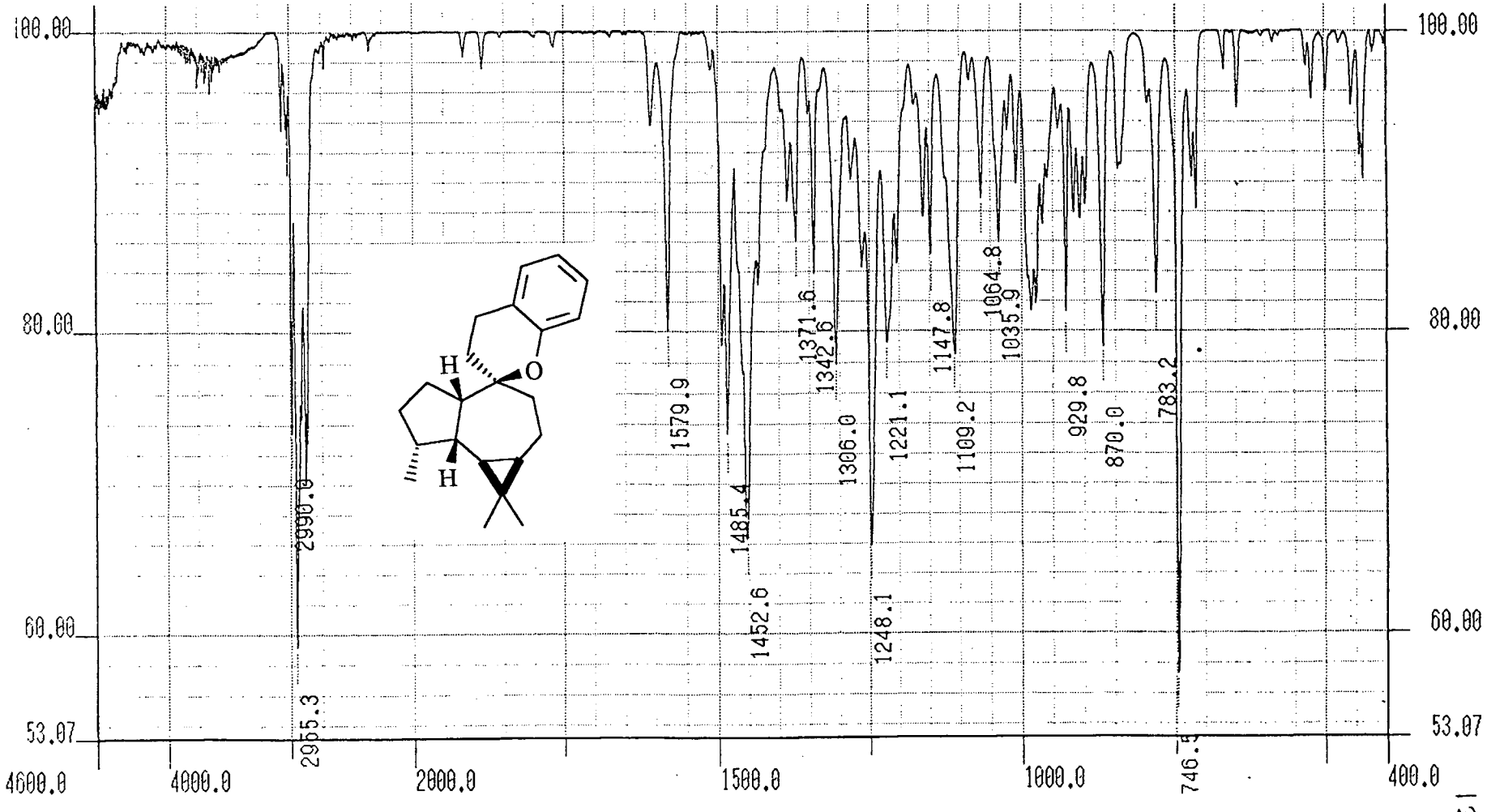


Fig. 2.26 : IR spectrum of 1

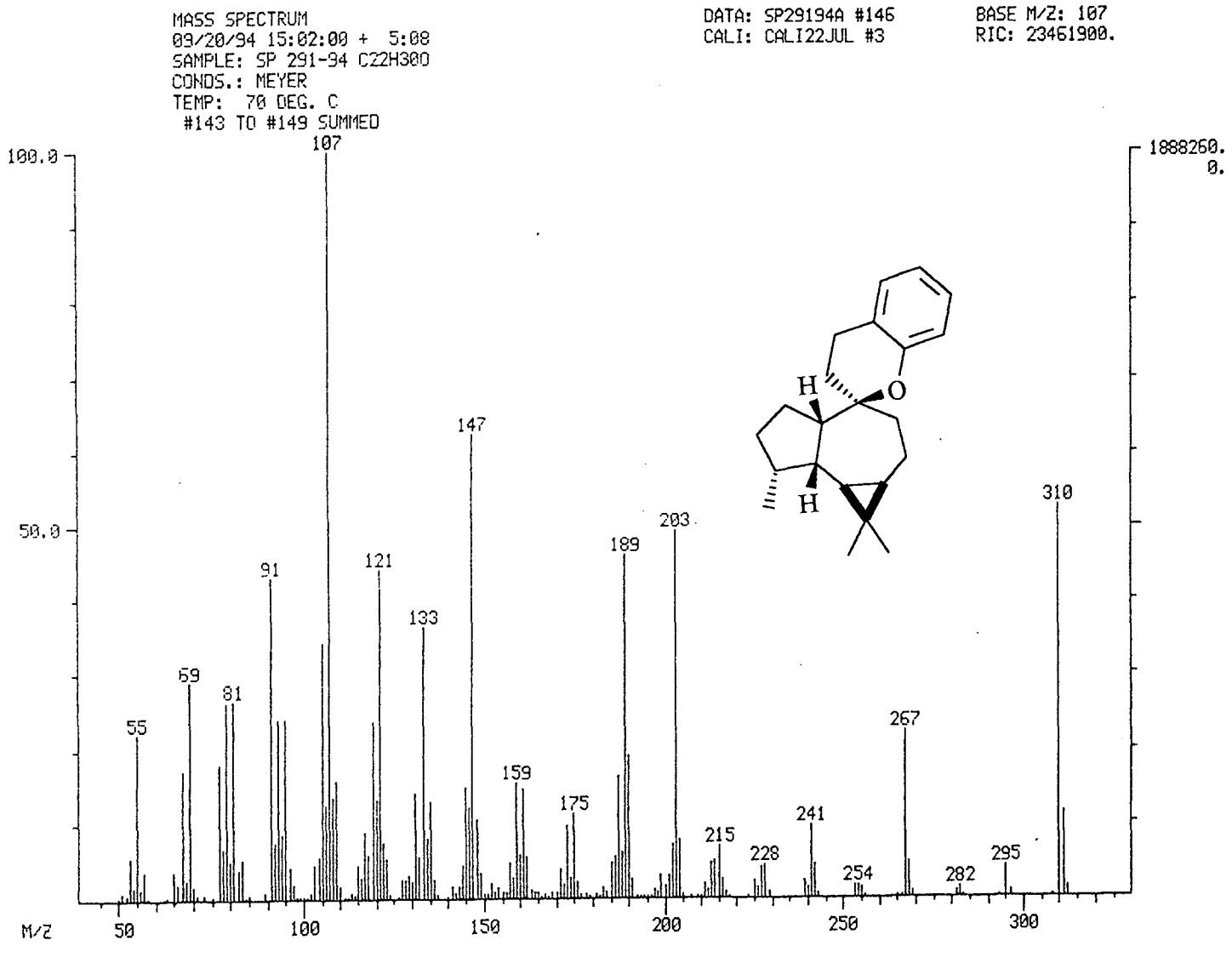


Fig. 2.27 : Mass spectrum of 1

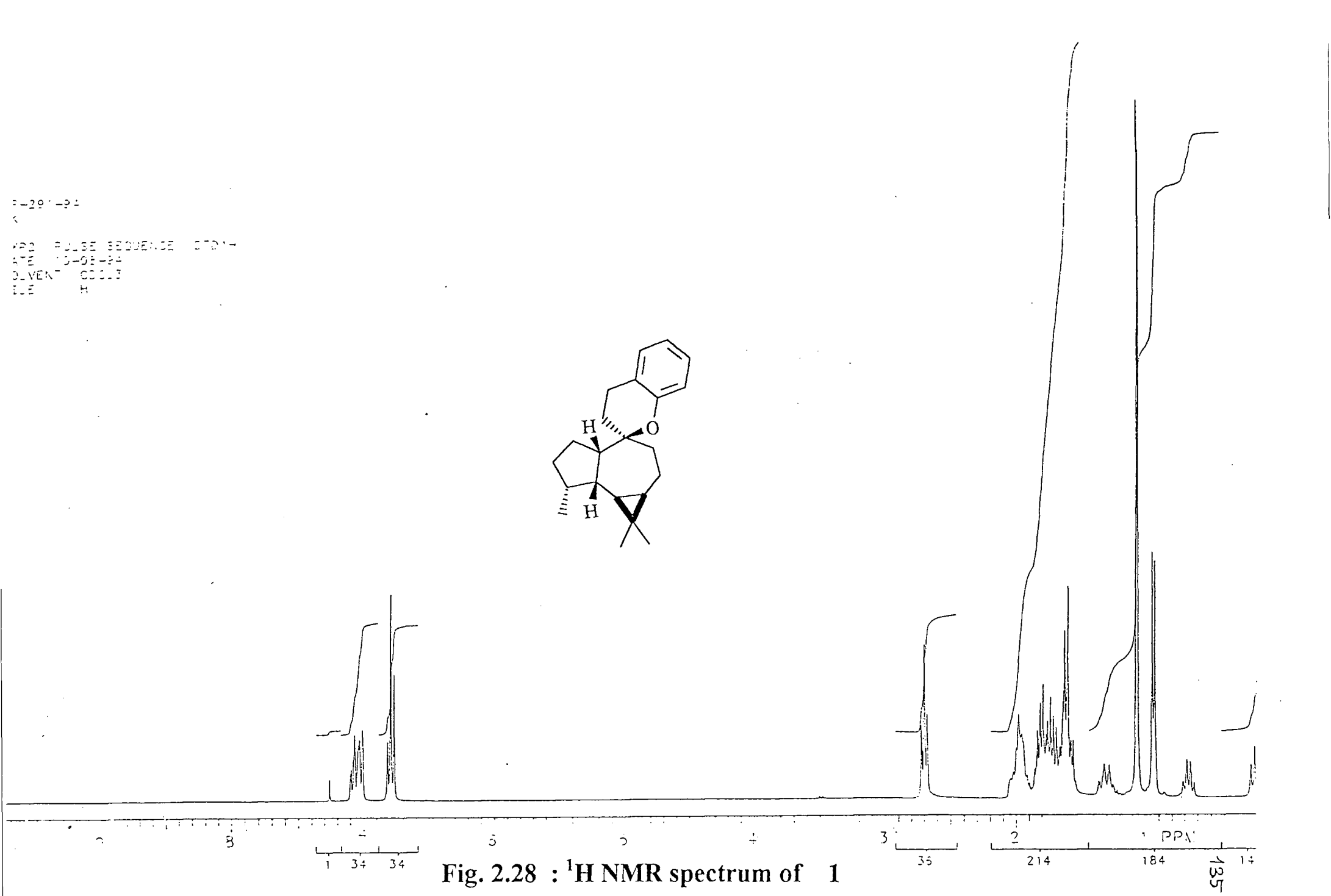


Fig. 2.28 : ^{1}H NMR spectrum of 1

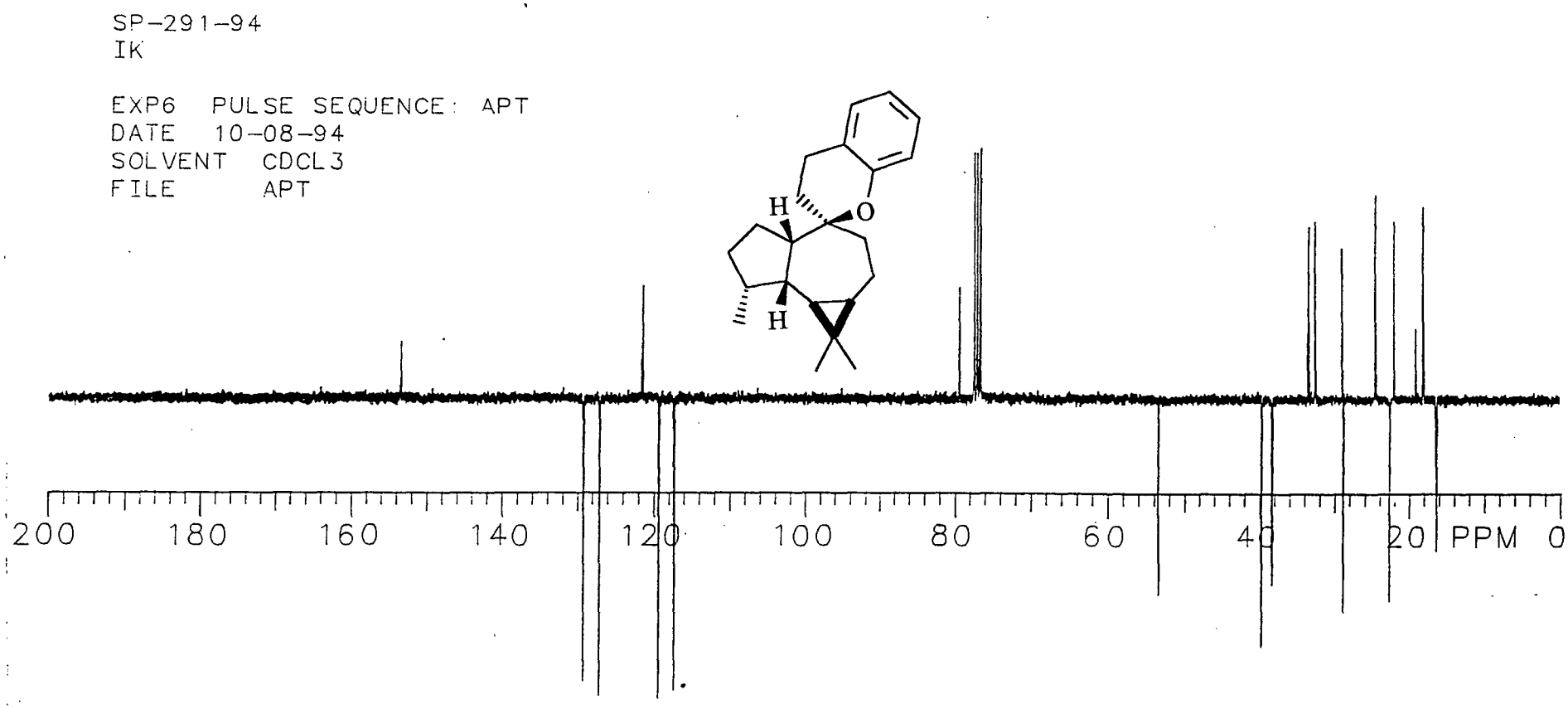


Fig. 2.29 : ^{13}C NMR spectrum of 1

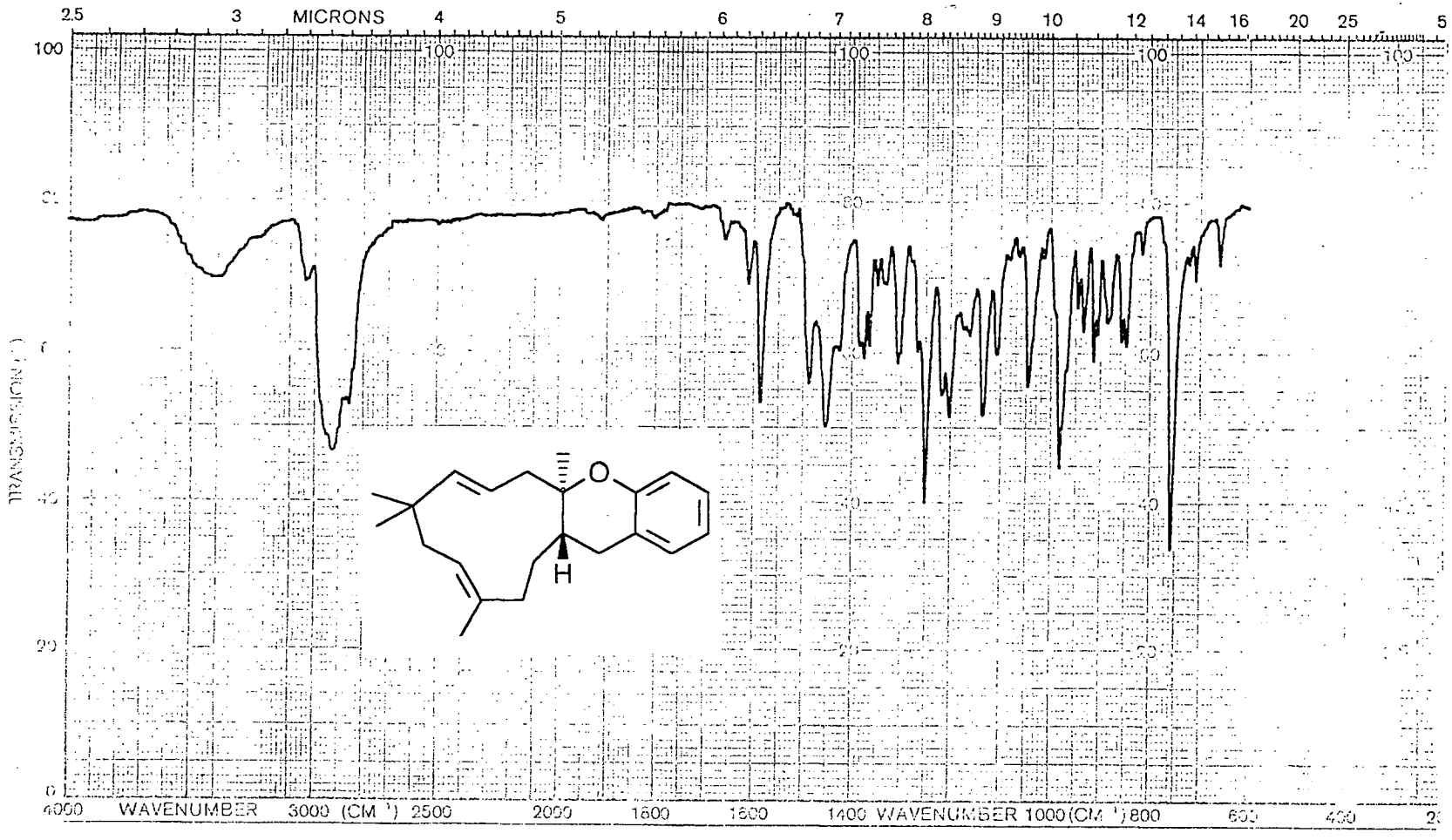


Fig. 2.30 : IR spectrum of 48

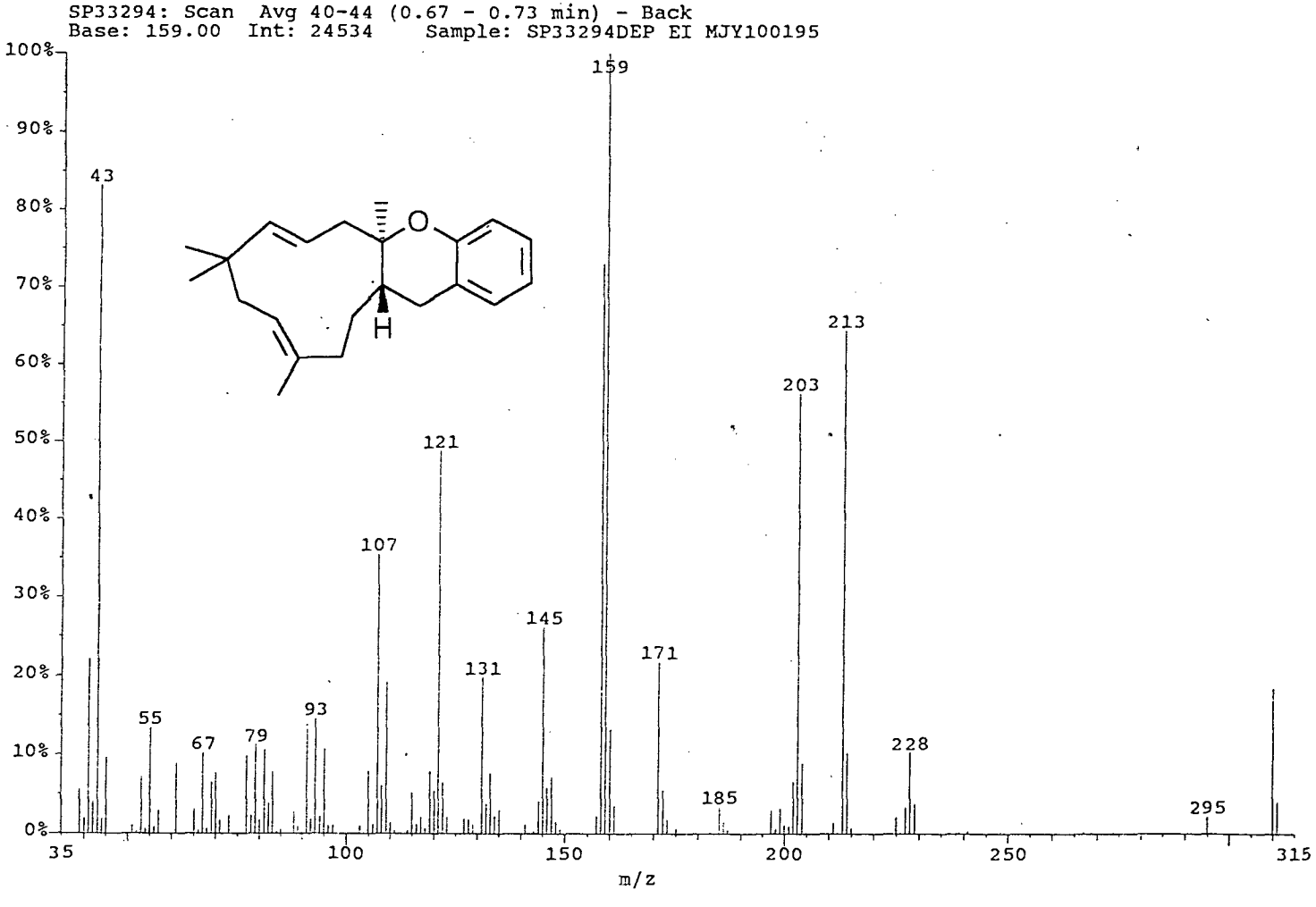


Fig. 2.31 : Mass spectrum of 48

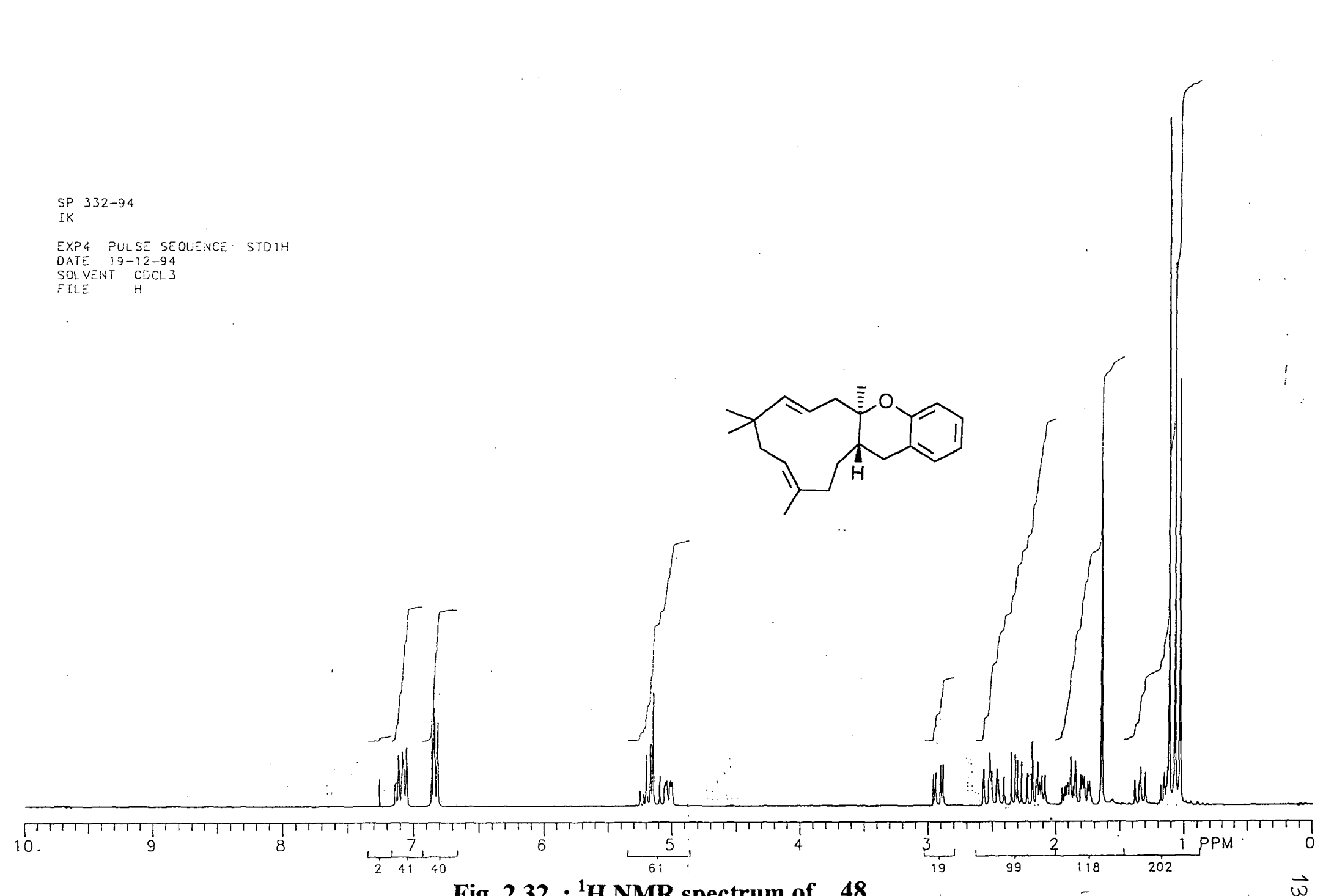


Fig. 2.32 : ^{1}H NMR spectrum of 48

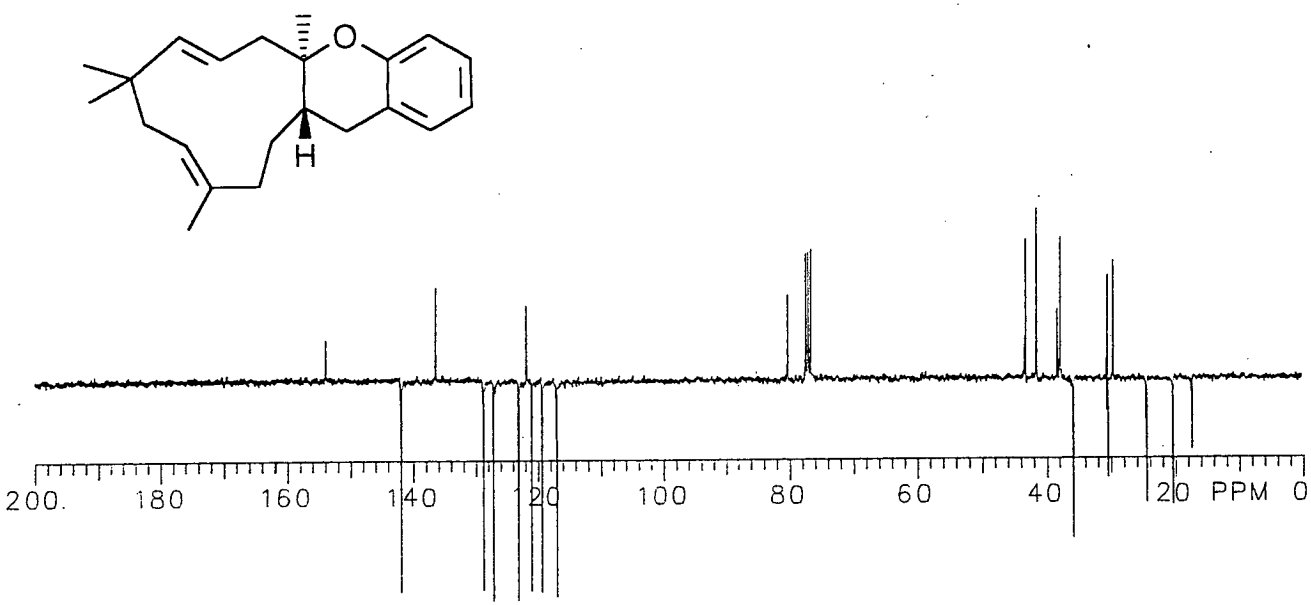


Fig. 2.33 : ^{13}C NMR spectrum of 48

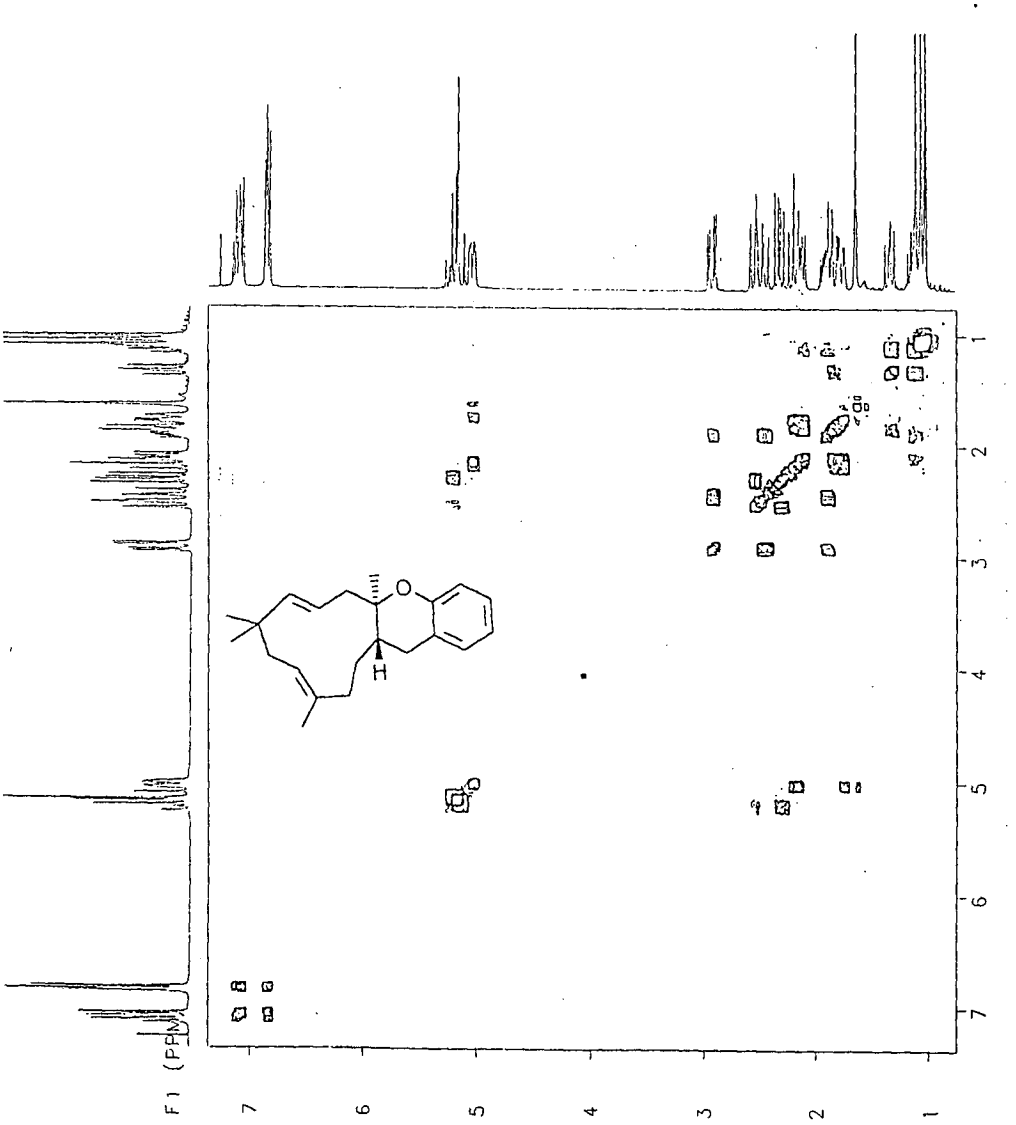


Fig. 2.34 : ^1H - ^1H COSY spectrum of 48

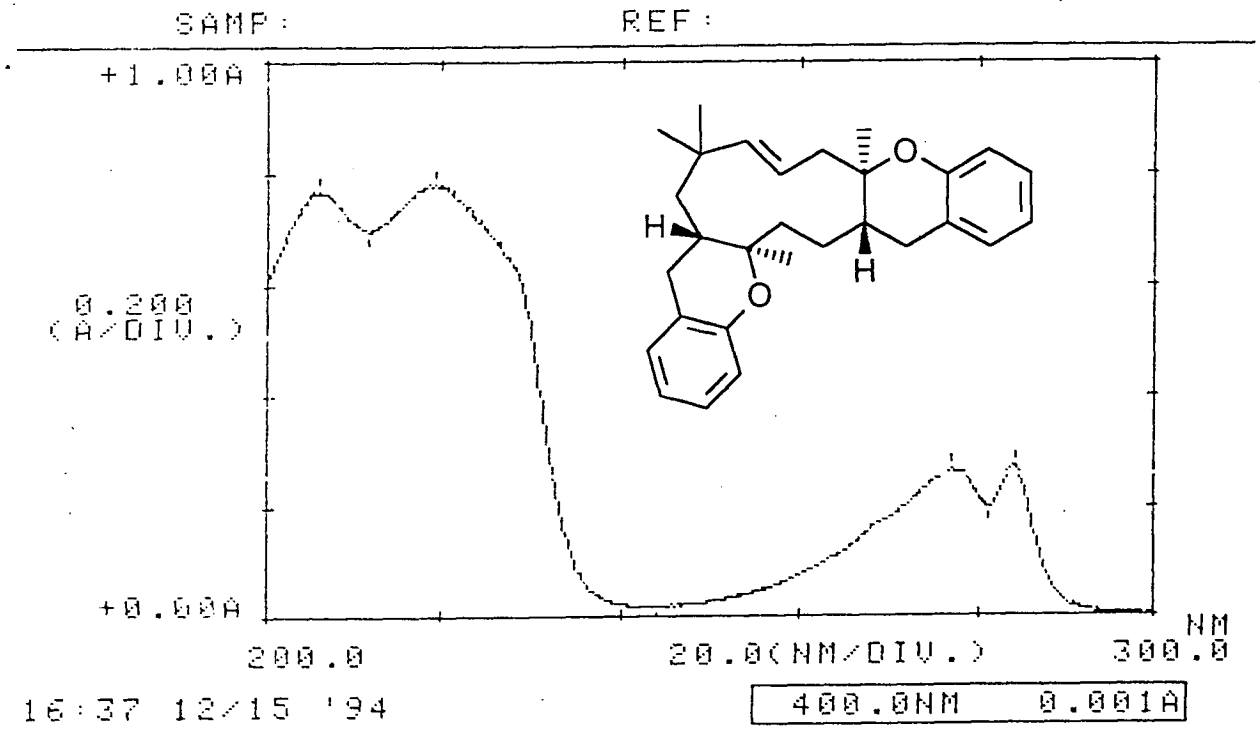


Fig. 2.35 : UV spectrum of 2

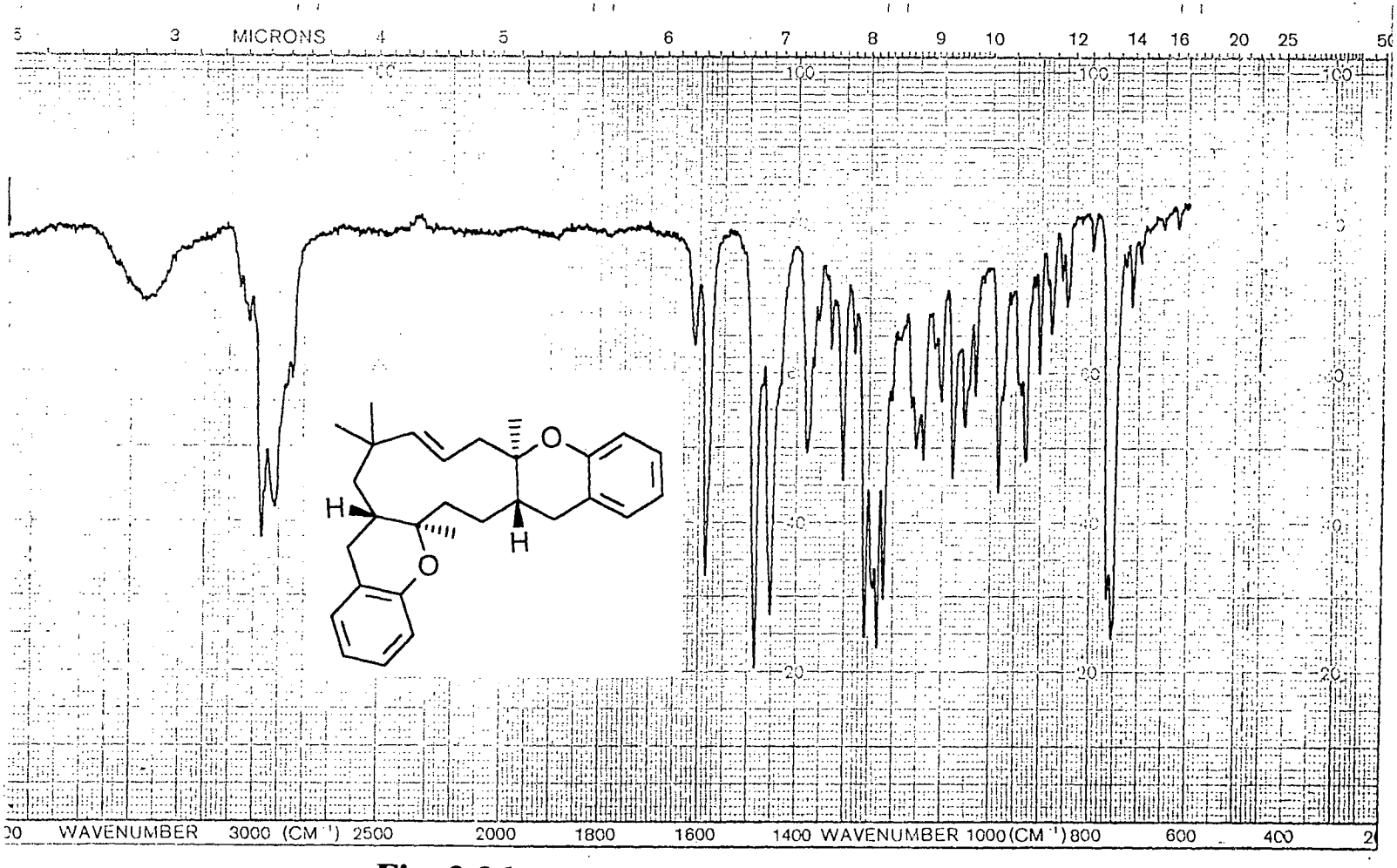


Fig. 2.36 : IR spectrum of 2

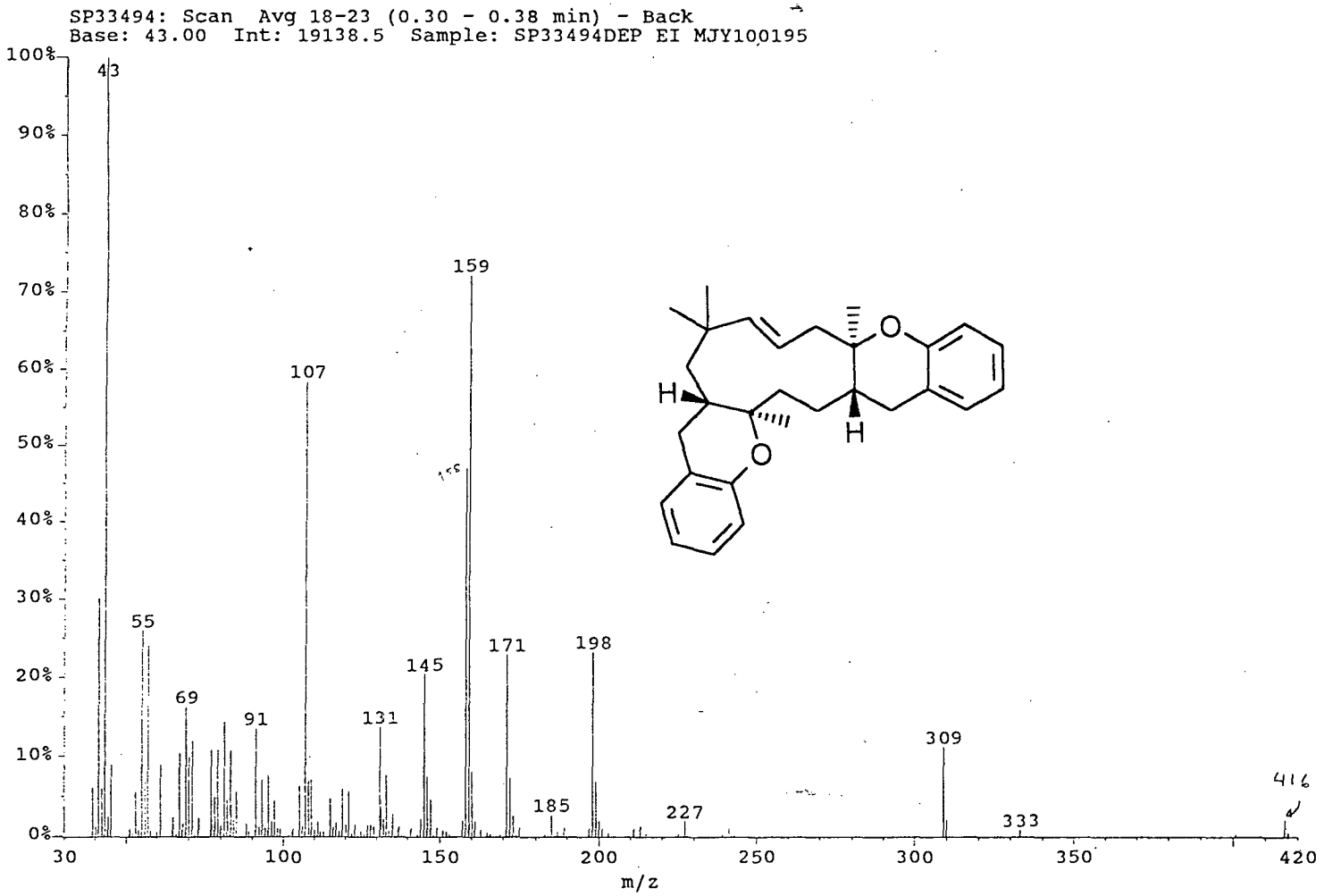


Fig. 2.37 : Mass spectrum of 2

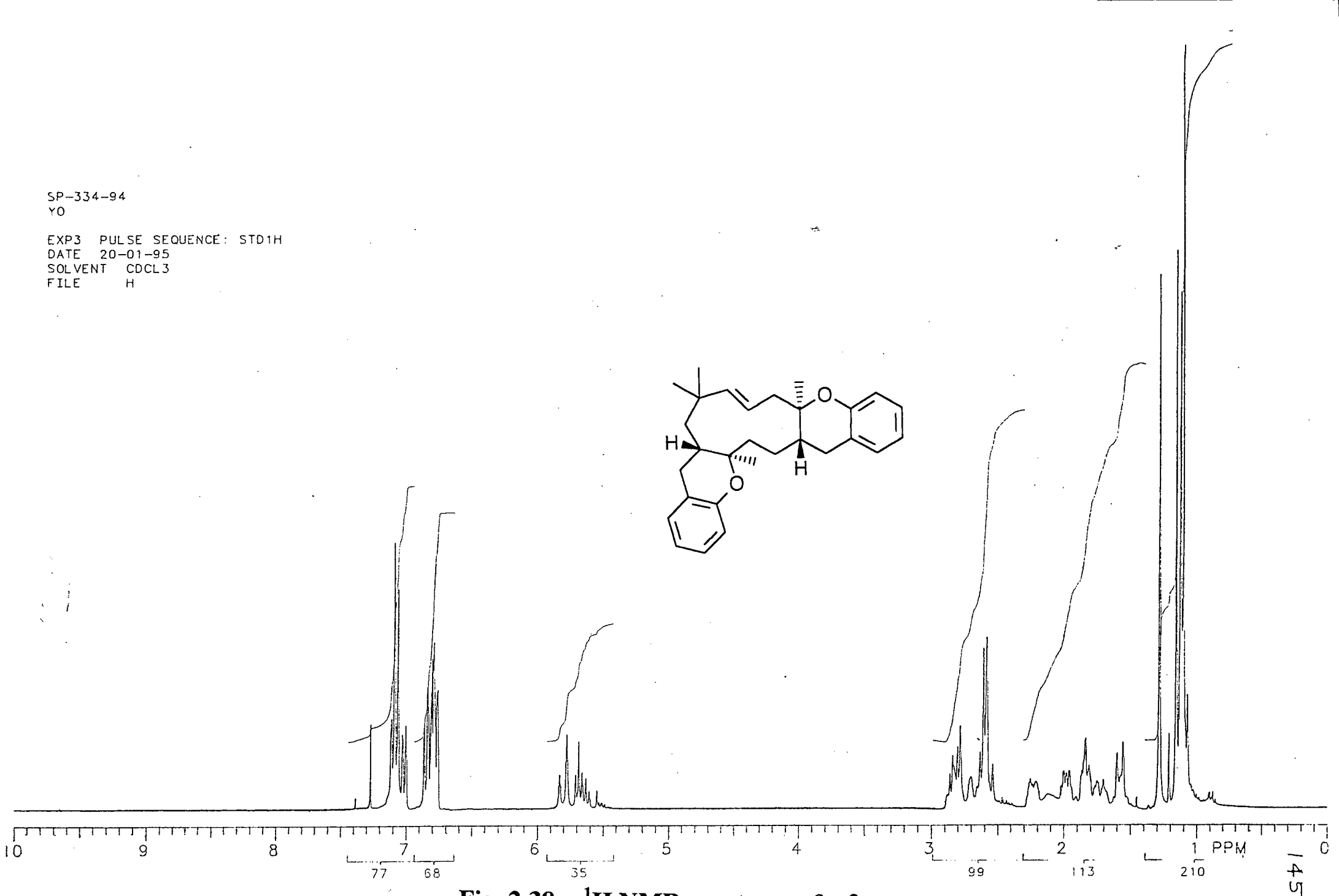


Fig. 2.38 : ^{1}H NMR spectrum of 2

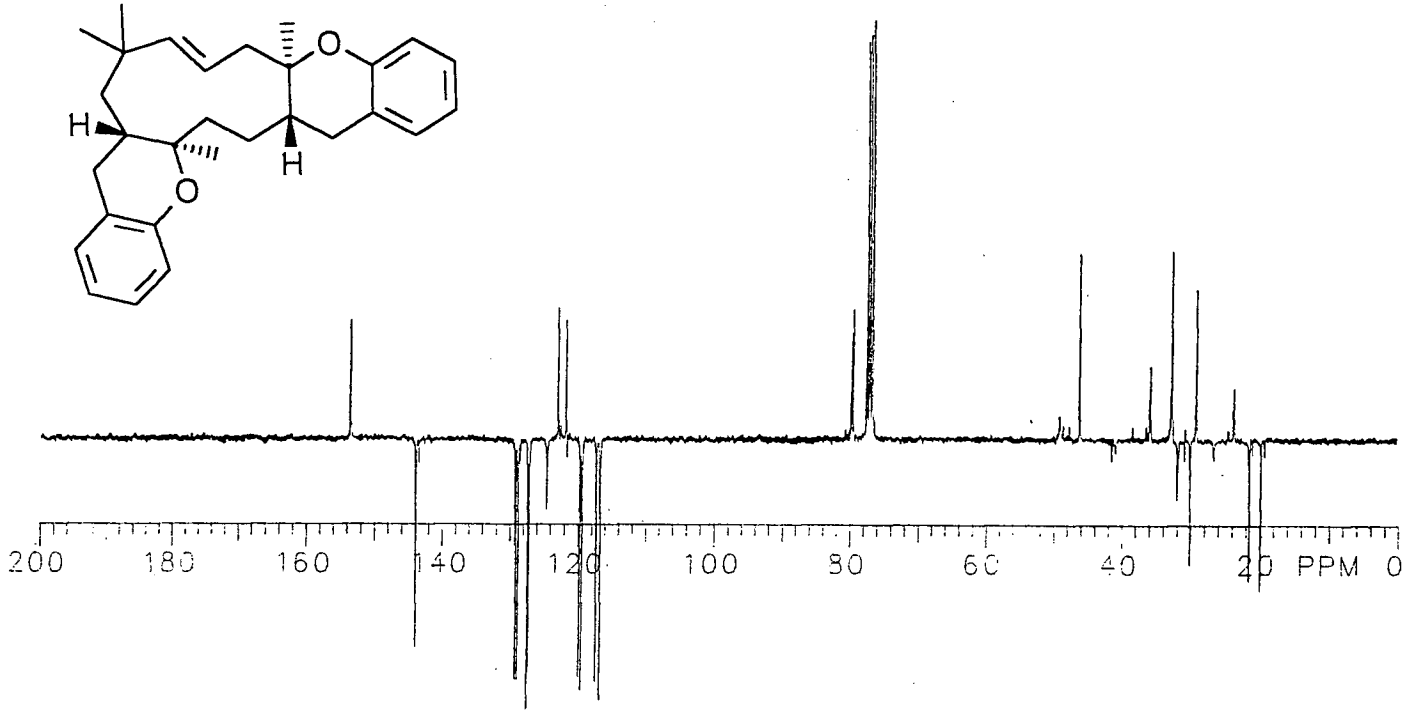


Fig. 2.39 : ^{13}C NMR spectrum of 2

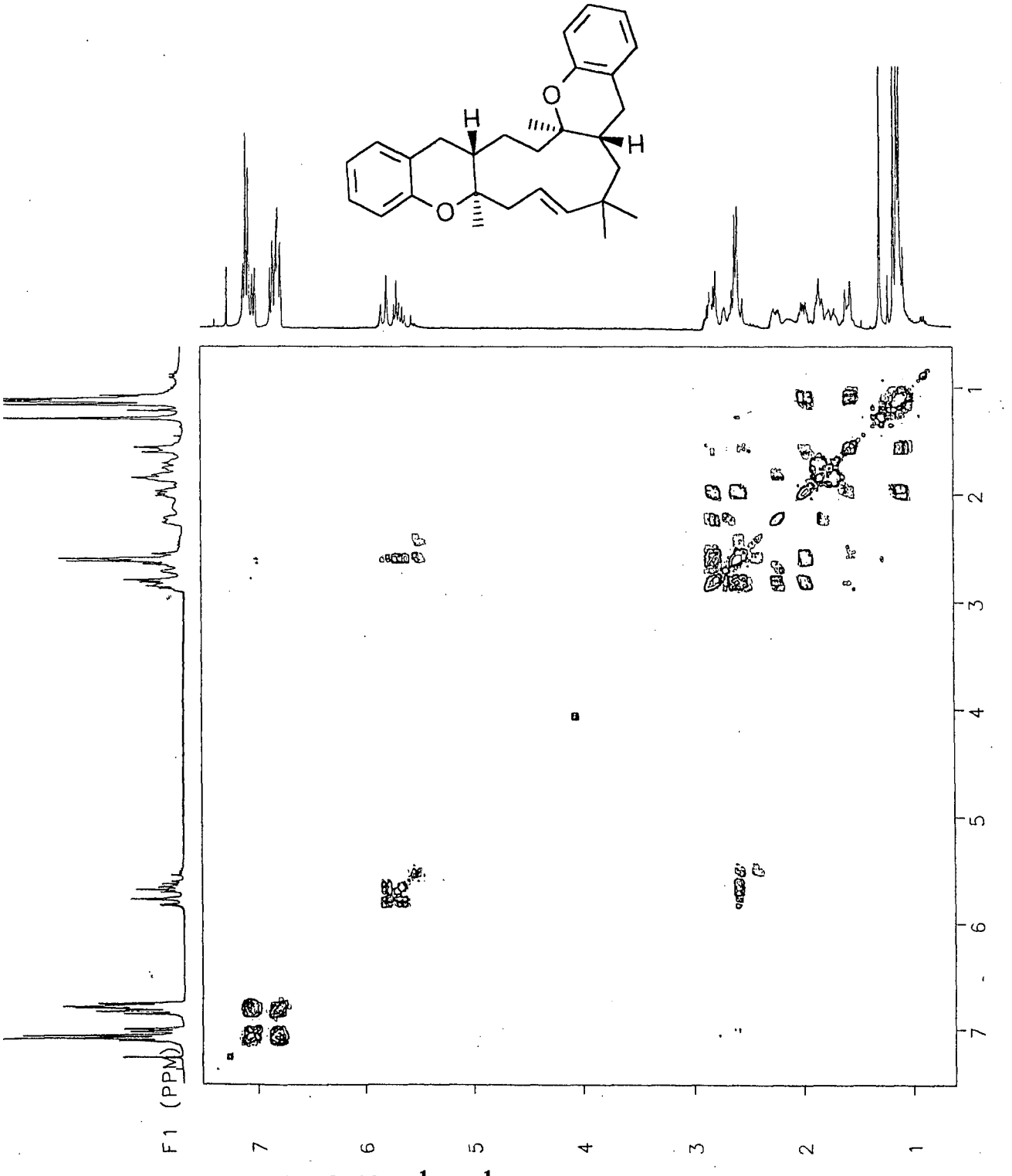


Fig. 2.40 : ^{1}H - ^{1}H COSY spectrum of 2

Experimental :

General procedure for the thermal reaction of saligenin 7 with olefins :

An equimolar mixture (0.015 mole) of saligenin 7 and unsaturated substrates in dry xylene (5 mL) was refluxed for 12 hr. At the end of this period the TLC pattern remained unchanged. Solvent was removed under reduced pressure, residue chromatographed over silica gel and eluted with pet.ether. The initial fractions contained unreacted olefins. Further elution with the same solvent or increasing polarity with benzene afforded desired adducts. Further elution with benzene gave 2,2'-dihydroxy dibenzyl ether **29** as colourless needles, m.p. 119°C in all cases. All the Yields reported in this chapter are calculated on the basis of recovered unreacted olefin.

Thermal reaction of anethole **10** with saligenin 7

compound **16**, m.p. 110°C , yield : 32%.

IR : ν_{max} (KBr) (fig. 2.01): 2980, 1610, 1580, 1510, 1485, 1450, 1370, 1300, 1250, 1230, 1170, 1110, 1000,940, 820, 760, 610 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) (fig. 2.02) : 0.90(3H, d, J=7 Hz, C_3 - CH_3), 2.25(1H, m, C_3 - H), 2.70 (1H,dd, J=16.0,11.0 Hz, C_4 - Ha), 2.93(1H, dd, J=16.0, 5.0 Hz, C_4 - He), 3.87(3 H , s, $C_{4'}$ - OCH_3), 4.63(1H, d, J=9.0 Hz, C_2 - H), 6.97 (1H, d, J=8 Hz , C_8 - H), 7.0(3H, d, J=8.5 Hz, C_6 - H, $C_{3'}$ - H,C5' - H), 7.15(1H, dd, J=8.0, 2.5, 0.5 Hz, C_5 - H), 7.20(1H, dd, J=8.0, 2.5 Hz, C_7 - H), 7.40(2H, d, J=8.5 Hz, $C_{2'}$ - H, $C_{6'}$ - H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 2.03) : For assignments see page 77

Thermal reaction of (+) - limonene **11** with saligenin **7**

A diastereomeric mixture of adducts **18** and **20** was obtained by following the general procedure.

GCMS (HR) of **20**, (fig. 2.04) m/z :242 (M^+), 159, 147 (100%), 135, 121, 107, 91, 77, 67, 55.

GCMS (HR) of **18**, (fig. 2.05) m/z :242 (M^+), 159, 145, 135, 119, 107 (100%), 93, 79, 67, 55.

Spectral data on the major compound **18** obtained by further purification by preparative TLC as colourless oil, yield : 28%, $[\alpha]_D^{25}$+37.8° (c, 4.68, $CHCl_3$)

IR : ν_{max} (film) (fig. 2.06) : 2920, 1605, 1580, 1450, 1375, 1345, 1305, 1210, 1145, 1105, 1090,945 and 935cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz,^{1}H - ^{1}H COSY,^{1}H - ^{13}C HETCOR) (fig. 2.07): 1.28(3H, bs, C_2 - CH_3), 1.70(3H, bs, C_4 - CH_3), 1.72 - 2.18(9H, complex pattern, C_3 - Ha, He, C_1 - H,C_2 - Ha, He, C_5 - Ha, He and C_6 -Ha, He), 2.80(2H, m, C_4 - Ha, He), 5.45 (1H, m, C_3 - H), 6.82 - 6.88 (2H, m, C_6 - H and C_8- H), 7.06 -7.17(2H, m, C_5- H and C_7 - H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT,^{1}H - ^{13}C HETCOR) : For assignments see page 78

Thermal reaction of camphene **12** with saligenin **7**

The compound **21**,after purification was obtained as a colourless oil (53%)

IR : ν_{max} (film) (fig. 2.09) :3450, 2980, 1710, 1585, 1490, 1455, 1260, 1222, 1045, 750 cm^{-1}.

GCMS (HR) (fig. 2.10) : m/z 242 (M^+), 199, 174, 159, 131(100%), 107, 91, 79.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz,^{1}H - ^{1}H COSY,^{1}H - ^{13}C HETCOR) (fig. 2.11) : 1.06 (3H, s, C_{17}-CH_3), 1.08 (3H, s, C_{16}-CH_3), 1.30 (1H, d, J=14 Hz, C_7-H_a), 1.31 (2H, td J=9.0, 1.5 Hz, C_4-H), 1.74 (1H, d, J=14 Hz, C_7-H_b), 1.75 (2H, m, C_5-H), 1.97 (1H, dd, J=4.0, 1.5 Hz, C_3-H), 3.15 (1H, dd, J=4.5, 1.5 Hz, C_6-H), 3.42 (2H, m, C_9-H), 5.14 (1H, t, J=7.0 Hz, C_8-H), 5.47 (1H, br s, -OH), 6.83 (1H, dd, J=8.0, 1.5 Hz, C_{12}-H), 6.88 (1H, ddd, J= 8.0, 7.5, 1.5 Hz, C_{14}-H), 7.11 (1H, ddd, J= 7.5, 1.5, 0.5 Hz, C_{15}-H), 7.15 (1H, dd, J=7.5, 1.5 Hz, C_{13}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT,^{1}H - ^{13}C HETCOR) (fig. 2.12): For assignments see page 80

Thermal reaction of (-)-α-phellandrene **13** with saligenin **7**

Compound **23** was obtained as a colourless oil (29%)

IR : ν_{max} (film) (fig. 2.15) : 2960, 1610, 1585, 1485, 1455, 1385, 1367, 1250, 1230, 940, 750 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) (fig. 2.16) : 0.94 (3H, d, J=6.5 Hz, C_7-CH_3), 0.96 (3H, d, J=6.5 Hz, C_7-CH_3), 1.45 (3H, s, C_1-CH_3), 1.70 (2H, m, C_3-H), 1.72 (1H, m, C_7-H), 2.04 (1H, m, C_4-H), 2.07 (1H, m, C_2-H), 2.65 (1H, dd, J=16.0, 6.5 Hz, C_{11}-H_α)2.80 (1H, dd, J=16.0, 6.0 Hz, C_{11}-H_β), 5.75 (2H, m, C_5-H, C_6-H), 6.82 (1H, dd, J=8.0, 7.5 Hz, C_{13}-H), 6.84(1H, dd, J=6.5, 1.5 Hz, C_{15}-H), 7.04 (1H, m, C_{12}-H), 7.10 (1H, m, C_{14}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz) (fig. 2.17): For assignments see page 84

Thermal reaction of (-)-zingiberene **14** with saligenin **7**

Compound **26** was obtained as a colourless oil (44%), $[\alpha]_D^{25} = -66.5°$ (c,3.02.$CHCl_3$).

IR : ν_{max} (film) : 2960, 1585, 1485, 1450, 1380, 1250, 1100, 750 cm^{-1}.

GCMS (HR) (fig. 2.18) : m/z 310 (M^+), 225, 203, 119, 105, 107(100%), 91, 69.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz,^{1}H - ^{1}H COSY,^{1}H - ^{13}C HETCOR) (fig. 2.19): 0.88 (3H, d, J=7.0 Hz, C_7-CH_3), 1.44 (3H, s, C_1-CH_3), 1.62 (3H, s, C_{12}-CH_3), 1.69 (2H, m, C_3-H), 1.70 (3H, s, C_{12}-CH_3), 1.71 (2H, m, C_9-H), 2.05 (1H, m, C_2-H), 2.25 (2H, m, C_{10}-H), 2.69 (1H, d, J=11 Hz, C_{16}-Ha), 2.72 (1H, d, J=11 Hz, C_{16}-Hb), 5.11 (1H, m, C_{11}-H), 5.61 (1H, dd, J=10.0, 3.0 Hz, C_5-H), 5.72 (1H, m, C_6-H), 6.79 (1H, dd, J=8.0, 1.5 Hz, C_{18}-H), 6.82 (1H, dd, J=7.0, 1.5 Hz, C_{20}-H), 7.02 (1H, dd, J=8.0, 2.0 Hz, C_{16}-H), 7.06 (1H, m, J=8.0, 7.5 Hz, C_{19}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT,^{1}H - ^{13}C HETCOR) : For assignments see page 87

Thermal reaction of (-)caryophyllene **15** with saligenin **7**

The reaction was carried out for 26 hr gave compound **28**. The purified reaction product was obtained as a colourless oil (61%), $[\alpha]_D^{25} = -39.8°$ (c,1.040,$CHCl_3$).

IR : ν_{max} (film) : 2920, 1630, 1585, 1485, 1430, 1370, 1250, 1205, 1055, 885, 750 cm^{-1}.

GCMS (HR) (fig. 2.22): m/z 310 (M^+), 295, 203, 159, 147, 107(100%).

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz ^{1}H - ^{1}H COSY) (fig. 2.23): 0.99 (3H, d, J=2.5 H, C_8-CH_3), 1.02 (3H, d, J=2.5 H, C_8-CH_3), 1.18 (3H, s, C_1-CH_3), 1.41-2.55 (13 H, complex pattern, C_2-H, C_3-H, C_4-H, C_6-H, C_7-H, C_9-H, C_{10}-H, C_{11}-H), 2.66 (1H, dd, J=16.5, 5.5 Hz, C_{16}-Ha), 2.86 (1H, dd, J=16.5, 5.5 Hz, C_{16}-Hb), 4.86 (1H, d, J=1.5 Hz, C_{11}-Ha), 4.90 (1H, d, J=1.5 Hz, C_{11}-Hb), 6.83 (1H, ddd, J=7.5, 7.5, 1.5 Hz, C_{18}-H), 6.87 (1H, dd, 7.0, 1.5 Hz, C_{20}-H), 7.05 (1H ddd, J=7.5, 7.5, 0.5 Hz, C_{17}-H), 7.11 (1H ddd, J=7.5, 7.5, 0.5 Hz, C_{19}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz) : For assignments see page 90

Synthesis of (-)-tanzanene **1** :

Thermal reaction of (-) alloaromadendrene **36** with saligenin **7** for 12 hr gave (-) tanzanene **1**, m.p. 84°C (CH_3OH) (lit[1] 84-85°C) , yield : 45%, $[\alpha]_D^{25} = -7.1°$ (c,0.182,$CHCl_3$) (lit.[1] $[\alpha]_D^{25} = -4.5°$ (c,0.182,$CHCl_3$).

UV (fig. 2.25): λ_{max} (EtOH) : 284, 276, 227 nm.

IR : ν_{max} (KBr) (fig. 2.26) : 2990, 2965, 1610, 1580,1485, 1452, 1248, 1109 and 796 cm^{-1}.

GCMS (HR) (fig. 2.27) : m/z 310 (M+), 267, 241, 215, 203, 189, 147, 133, 121, 107 (100%), 91, 81 and 69.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) (fig. 2.28) : 0.16 (1H, dd, J= 10.0, 9.0 Hz, C_6-H), 0.67 (1H, ddd, J= 10.0, 9.5, 8.0 Hz, C_7-H), 0.92 (3H, d, J= 6.5 Hz, C_4-CH_3), 1.06 (3H, s, C_{11}-CH_3), 1.07 (3H, s, C_{11}-CH_3), 1.30 (1H, m, C_3-Ha), 1.53 - 2.06 (12H, complex pattern, C_1-H, C_2-H, C_3-H, C_4-H, C_5-H, C_8-H, C_9-H, C_{15}-H), 2.70 (2H, t, J=6.5 Hz, C_{16}-H), 6.78 (1H, d, J=7.5 Hz, C_{17}-H), 6.81 (1H, dd, J=7.5, 1.5 Hz, C_{18}-H), 7.02 (1H, dd, J=7.5, 1.5 Hz, C_{20}-H), 7.07 (1H, ddd, J=7.5, 7.5, 1.5 Hz, C_{19}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 2.29): 16.2 (q, C_{12}), 16.3 (q, C_{14}), 18.1 (t, C_8), 19.1 (d, C_{11}), 20.0 (t, C_{16}), 22.6 (d, C_6), 24.5 (t, C_2), 28.8 (d, C_{13}), 28.8 (d, C_7), 28.9 (t, C_3), 32.5 (t, C_{15}), 33.4 (t, C_9), 38.3 (d, C_4), 39.7 (d, C_5), 53.4 (d, C_4), 79.4 (s, C_{10}), 117.3 (d, C_{20}), 119.3 (d, C_{18}), 121.5 (s, C_{16a}), 127.0 (d, C_{19}), 129.2 (d, C_{17}), 153.4 (s, C_{20a}).

Preparation of semilucidene **48** :

Thermal reaction of humulene **27** with saligenin **7** for 12 hr. gave semilucidene **46**, white crystalline solid,(75%), m.p. 113°C, $[\alpha]_D^{25} = 0°$.

UV : λ_{max} (MeOH) : 284, 277, 202 nm.

IR : ν_{max} (KBr) (fig. 2.30) :2960, 1590, 1490, 1455, 1380, 1300, 1250, 1200, 1135, 1045, 985, 760 cm^{-1}.

HRMS (fig. 2.31): m/z 310 (M^+), 295, 228, 213, 203, 171, 159 (100%), 158, 145, 131, 121, 107, 93, 79, 67 and 55.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz,^{1}H - ^{1}H COSY) (fig. 2.32) : 1.03 and 1.07 (3H each ,s,C_{11} - CH_3), 1.11(3H, s,C_7 - CH_3) , 1.15-1.40 (2H, m, C_5 - CH_2), 1.64 (3H, t, J = 1.0 Hz, C_3 - CH_3), 1.72-2.58 (7H, m, allylic hydrogens and C_6 - H), 2.46 (1H, dd,

J=16.0,11.0 Hz, C_{16} - Ha), 2.93 (1H, dd, J=16.0, 5.0 Hz ,C_{16} - He), 5.03 (1H, m, C_2 - H), 5.13 (1H, dd, J=16.0,1.0 Hz, C_{10} - H), 5.20 (1H, ddd, J=16.0,9.0,2.0 Hz, C_9 - H), 6.81- 6.87 (2H, m,C_{18} - H, C_{20}- H), 7.04 -7.15 (2H, m, C_{17} - H and C_{19} - H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 2.33) : For assignments see page 102

Synthesis of lucidene **2** :

Thermal reaction of semilucidene **48** with saligenin **7** for 15 hr. gave lucidene **2**, white crystalline solid (petroleum ether) (29%), m.p. 213°C (lit.[2] m.p.210°C), $[\alpha]_D^{25}$ +13.2°(c, 0.75, $CHCl_3$) {lit.[2] $[\alpha]_D^{25}$ 0°}, numbering is same as reported for natural lucidene[2].

Lucidene **2** could be obtained directly from humulene **27** without isolation of semilucidene **48** following the procedure presented below.

A solution of humulene **27** (0.106 gm., 0.5 mmole), saligenin **7**(0.248 gm., 2.0 mmole) in dry xylene (5 mL) was refluxed for 35 hr. Solvent was removed under reduced pressure, concentrated and chromatographed on silica gel. Elution with pet.ether-benzene (9:1) yielded semilucidene **48** (10 mg), identified by CO-TLC and mix. m.p. Further elution with pet.ether-benzene (8:2) yielded lucidene 0.45 gm.(21%) as a crystalline solid which was further purified by recrystallisation from petroleum ether, m.p. 213°C.

UV : λ_{max} (hexane) (fig. 2.35): 284, 277, 219 and 206 nm.

IR : ν_{max} (KBr) (fig. 2.36):1610, 1585, 1485, 1450, 1380, 1305,1255, 1220, 1080, 985, 925, 750 cm^{-1}.

HRMS (fig. 2.37): 310 [M+],198, 171, 159, 158, 145, 131,107, 91, 69, 55, 43 (100%).

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz,^{1}H - ^{1}H COSY) (fig. 2.38) : 1.10 (3H, s, C_9 -CH_3), 1.11 (1H, dd, J =16.0, 6.0 Hz, C_{10} - Ha), 1.13 (3H, s, C_9 - CH_3), 1.16 (3H, s, C_{16a} - CH_3), 1.29 (3H, s, C_{5a} - CH_3), 1.57 (1H, dd, J=16.0, 1.5 Hz, C_{10} - Hb), 1.72 (1H, dd, J=13.0, 4.0 Hz, C_{17} - Ha), 1.79 (1H, dd, J=16.0, 3.0 Hz, C_{17} - Hb), 1.85 (1H, br m, C_{18} - Ha), 1.98 (1H, dddd, J=12.0, 6.0, 5.5, 1.5 Hz , C_{10a} - H), 2.10 (1H, V br s, C_{18} - Hb), 2.22 (1H, m, C_{18a} - H), 2.56 (2H, d, J=7.0Hz, C_6 - H), 2.61 (1H, dd, J=16.0, 7.5 Hz, C_{11}- Hα), 2.71 (1H, br d, C_{19} - Hα), 2.81 (1H, br d, C_{19} - Hβ), 2.82 (1H, dd, J=16.0, 6.0 Hz, C_{11} - Hβ), 5.80 (1H, d, J=16.0 Hz, C_8 - H), 5.65 (1H, ddd, J=16.0, 7.5, 7.5 Hz, C_7 - H), 6.75 - 7.12 (8H, complex pattern, Ar - H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 2.39) : 19.7 (q, C_9 - CH_3), 21.45 (q, C_9 - CH_3), 23.77 (s, C_9), 29.20 (t, C_{19}), 29.20 (t, C_{11}), 30.10 (q, C_{16a} - CH_3), 30.10 (q, C_{17a} - CH_3), 31.90 (d, C_{10a}), 31.90 (d, C_{18a}), 32.74 (t, C_{18}), 35.83 (t, C_{17}), 46.14 (t, C_6), 46.14 (t, C_{10}), 79.46 (s, C_{6a}), 79.72 (s, C_{17a}), 116.62 (d, C_{15}), 117.16 (d, C_4), 119.25 (d, C_{13}), 119.61 (d, C_2), 121.63 (s, C_{11a}), 122.80 (s, C_{19a}), 124.43 (d, C_7), 127.01 (d, C_{14}), 127.25 (d, C_3), 128.65 (d, C_{12}), 129.07 (d, C_1), 143.86 (d, C_8), 153.4 (s, C_{4a}), 153.4 (s, C_{15a}).

Single crystal X-ray data :

Empirical formula	$C_{29}H_{36}O_2$
F. W.	416.58
Habit	plates
Colour	colourless
Crystal size	0.23 x 0.23 x 0.13 mm
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Unit cell dimentions	a = 9.012 (2) A° α=90° b = 14.933 (4) A° β=90° c=17.800 (6) A° γ=90°
Volume	2395.5 (12) A^{o3}
Z	4
Density (calculated)	1.155 mg/m^3
Absorption coefficient	0.541 mm^{-1}
F (000)	904
Wave length	1.54178 Ao (CuKα)
Temperature	293 K
Monochromator	graphite
range for data collection	3.86 to 59.99°
Scan-type	two-theta/omega-scans
No. of standards	2 (orientation), 3 (intensity)
Interval of standards	120 min (intensity)
Decay of standards	none
Reflection collected	4151
Independent reflections	3515 (R_{int} = 0.0307)
Absorption correction	semi-emperical from s-scans
Max & min transmissions	0.919 and 0.797
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3515 / 0 / 280
Goodness of fit on F^2	1.031
Final R indices	R_1 = 0.597, wR_2 = 0.1170
R indices (all data)	R_1 = 0.1894, wR_2 = 0.1589
Absolute structure parameters	0.43 (71)
Largest diff. peak and hole	0.165 and -0.235 eA^{o-3}

References :

1. Weenen H, Nkunya M H H, Mgani Q A, Posthumus M A, Waibel R, Achenbach H, J. Org. Chem. **56**, 5865, (1991).
2. Weenen H, Nkunya M H H, El Fadl A A, Harkema S, Zwanenburg B, J. Org. Chem. **55**, 5107, (1990).
3. Muhammad I, Waterman P G, J. Nat. Prod. **51**, 719, (1988).
4. Moore R F, Waters W A, J. Chem Soc. 243, (1953).
5. Bolon D A, J. Org Chem. **35**, 3666, (1970).
6. Chauhan M S, Dean F M, McDonald S, Robinson M S, J Chem. Soc. Per.trans.1, 359, (1973).
7. Bavoux C, J Chem. Soc. Per.trans.1, **12**, 2059, (1989).
8. Arduini A, Bosi A, Pochini A, Ungaro R, Tetrahedron, **41**, 3095, (1985).
9. Wan P, Baker B, Diao L, Fischer M, Shi Y, Yang C, Can. J. Chem. **74**, 465, (1996).
10. Hultzsch K, J. Prakt. Chem. **158**, 275, (1941).
11. Lasswell W L, Hufford C D, Phytochemistry, **16**, 1439, (1977).
12. Kuzuka M, Sawada T, Kasahara F, Mizuta E, Amano T, Komiya T, Goto M, Chem. Pharm. Bull. **30**, 1952, (1982).
13. Turner A B, Quart. Rev. **28**, 347, (1964).
14. Dolejs L, Motl O, Soucek M, Herout V, Sorm F, Coll. Czech. Chem. Commun., **25**, 1483 (1960).

15. Birch A J, Grimshaw J, Speake R N, Gascoigne R M, Hellyer R O, Tetrahedron, **3**, 15 (1959).

16. Buchi G, Hofheinz W, Paukstelis J, J. Am. Chem. Soc., **91**, 6473 (1969).

17. Anam E M, Ekpa O D, Gariboldi P V, J. Ind. Chem. Soc. **71**, 179 (1994).

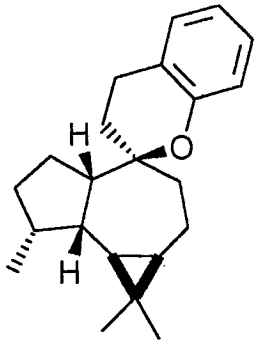
18. Anam E M, Ind. J. Chem. **36B**, 118 (1997).

19. Ushio T, Tamura R, Takahashi H, Azuma N, Yamamoto K, Angew. Chem. Int. Ed. Engl., **35**, 2372 (1996).

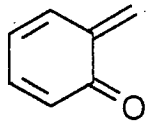
20. . Ushio T, Tamura R, Takahashi H, Azuma N, Yamamoto K, Angew. Chem. Int. Ed. Engl., **35**, 2372 (1996) and references cited therein.

3.1 Synthesis of di-o-methoxy benzyl ether, a constituent of *Uvaria chamae* and comment on the reactive species formed by heating saligenin in xylene at reflux temperature

Following the attractive suggestion that benzopyranyl sesquiterpenoid tanzanene **1** is formed by a non-enzymatic hetero Diels-Alder reaction of o-benzoquinone methide **2** to the exocyclic double bond of alloaromadendrene **3**[1], we reported a simple biomimetic synthesis of (-) tanzanene by refluxing in xylene, a mixture of (-) alloaromadendrene **3**, a sesquiterpene with established absolute configuration and saligenin **4**[2]. This synthesis also defined the absolute configuration of (-) tanzanene as shown.



1



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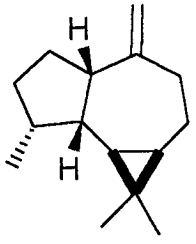
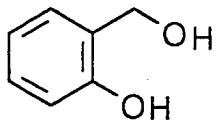


3

It was assumed that the o-quinone methide **2** generated *insitu* from saligenin **4** by loss of water reacts with the exocyclic double bond of (-) alloaromadendrene **3** in

a 4+2 cycloaddition process as suggested. Further extension of this work indicated the generality of the reaction and led to the biomimetic synthesis of lucidene 6* starting from humulene **5**.



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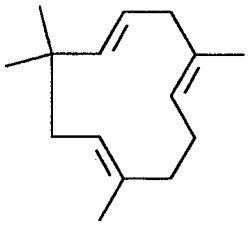

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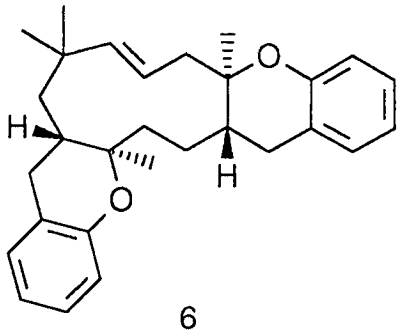

6

The results obtained with other olefins such as (-)-α-phellandrene **7** and camphene **8** raised doubts about the involvement of **2** as reactive intermediate in the above thermal reactions. The products **9** and **10** formed from (-)-α-phellandrene **7** and camphene **8** respectively further suggested that the addition of the C_7 unit of **4** takes place via ionic intermediates. A close look at the methods for the thermal generation **2** or its derivative **11** shows that much higher temperatures are required. Further, o-benzoquinone methide **2** is known to be highly reactive and at temperatures above 20°C undergoes self Diels-Alder condensation to produce mainly the trimer **12**[3]. Formation and characterisation of dimer **13** is also reported[4].

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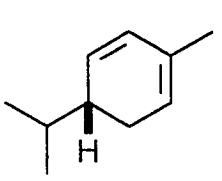
* Lucidene obtained was found to be optically active as against the racemic nature of the natural product. Details of this work are presented in chapter-2 (section-1, this thesis).



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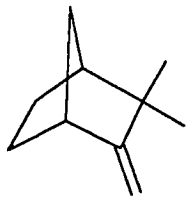

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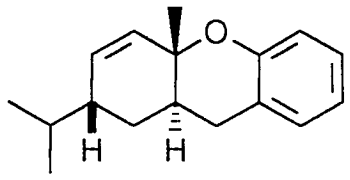

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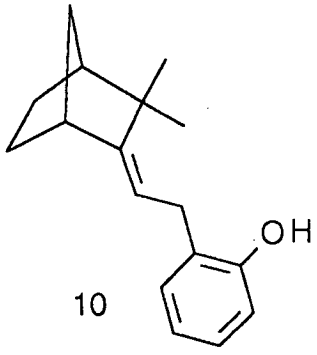

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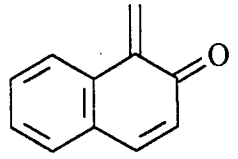

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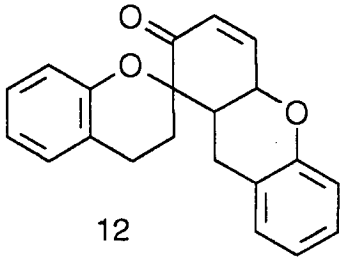

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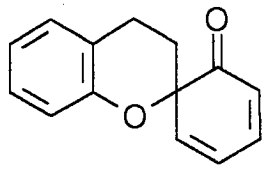


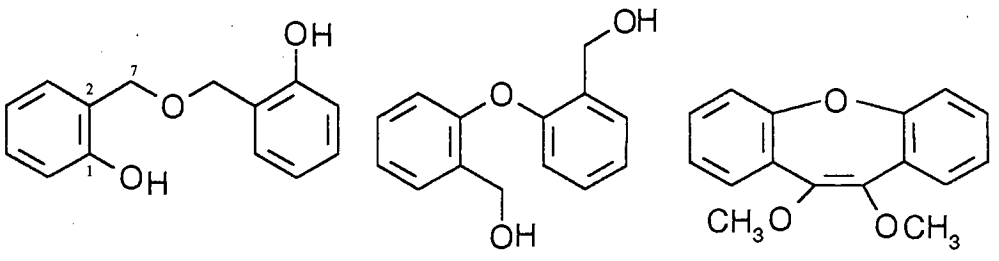
13

In order to probe into the nature of the reactive species generated from saligenin **4** in refluxing xylene it became necessary to look for other products of each reaction, particularly those derived by self condensation of the reactive intermediate obtained from saligenin **4**.

It was found that in each reaction, irrespective of the nature of the unsaturated compound used, a crystalline compound, $C_{14}H_{14}O_3$ (M^+ 230.0941), m.p.119°C was formed. When saligenin **4** was refluxed alone with xylene, the same product could be isolated. This clearly shows that it is derived from two molecules of **4**. The IR

spectrum of compound, m.p.119°C showed bands due to hydroxyl (3300cm^{-1}) and aromatic rings (1497 and 1600cm^{-1}). Absence of carbonyl absorption clearly showed that it is not dimer **13**, though the molecular formula is as expected for **13**. In fact we did not get any fraction having carbonyl absorption in its IR spectrum. This observations clearly established that o-benzoquinone methide **2** is not the reactive intermediate.

Based on the molecular formula, and spectral data (IR, ^{1}HNMR) two alternative structures **14** and **15** were considered.



14 15 16

Compound, m.p. 119°C did not undergo methylation when treated with excess diazomethane at room temperature and led us initially to believe the absence of phenolic hydroxyl groups and structure **15** was therefore preferred over **14**. A literature search indicated that bis(2-hydroxy methyl phenyl)ether **15** is known compound and was used recently by Wong and co-workers[5] for the synthesis of dibenzoxepin derivative **16**. An authentic sample of **15** was made available for a direct

comparison* . Since ^{13}CNMR and IR spectra of **15** were not recorded by Wong and co-workers** ,we therefore measured the IR and ^{13}CNMR on the authentic sample made available to us. A direct spectral comparison (IR,^{1}H and ^{13}C NMR) and co-TLC conclusively established their non identity. Moreover the melting points are also different (lit[6] m.p.98-99°C).

Having ruled out the possibility of structure **15** for the thermal self condensation product derived from saligenin, we then considered structure **14** for it. Interestingly Ziegler and Lercher[7] had reported its preparation by heating saligenin in a sealed tube at 120°C. In 1962 Yeddanapalli and Francis[8] confirmed the findings of Ziegler and Lercher but did not record the spectral data. The IR and ^{1}HNMR are consistent with the assigned structure **14** and are reproduced in figures 3.01 and 3.02 respectively. The ^{13}CNMR showed seven signals and indicated its symmetrical nature. The assignments are given in table-1.

Table-1

Carbon	Chemical shift (δ ppm)	Multiplicity
C1 & C1'	155.5	s
C2 & C2'	122.1	s
C3 &C3'	129.3	d
C4 & C4'	120.3	d
C5 & C5'	129.9	d
C6 & C6'	116.2	d
C7 & C7'	70.6	t

* We thank Dr. H. N. C. Wong for the sample of diol **15** and a copy of it ^{1}HNMR spectrum.

** Personal communication to Prof. S. K. Paknikar.

Further support for structure **14** was found in mass spectral data. The characteristic feature of EIMS is the fragment ion m/z 212 (M^+-18), obviously due to loss of water. The genesis of this and other significant peaks is shown in chart-1.

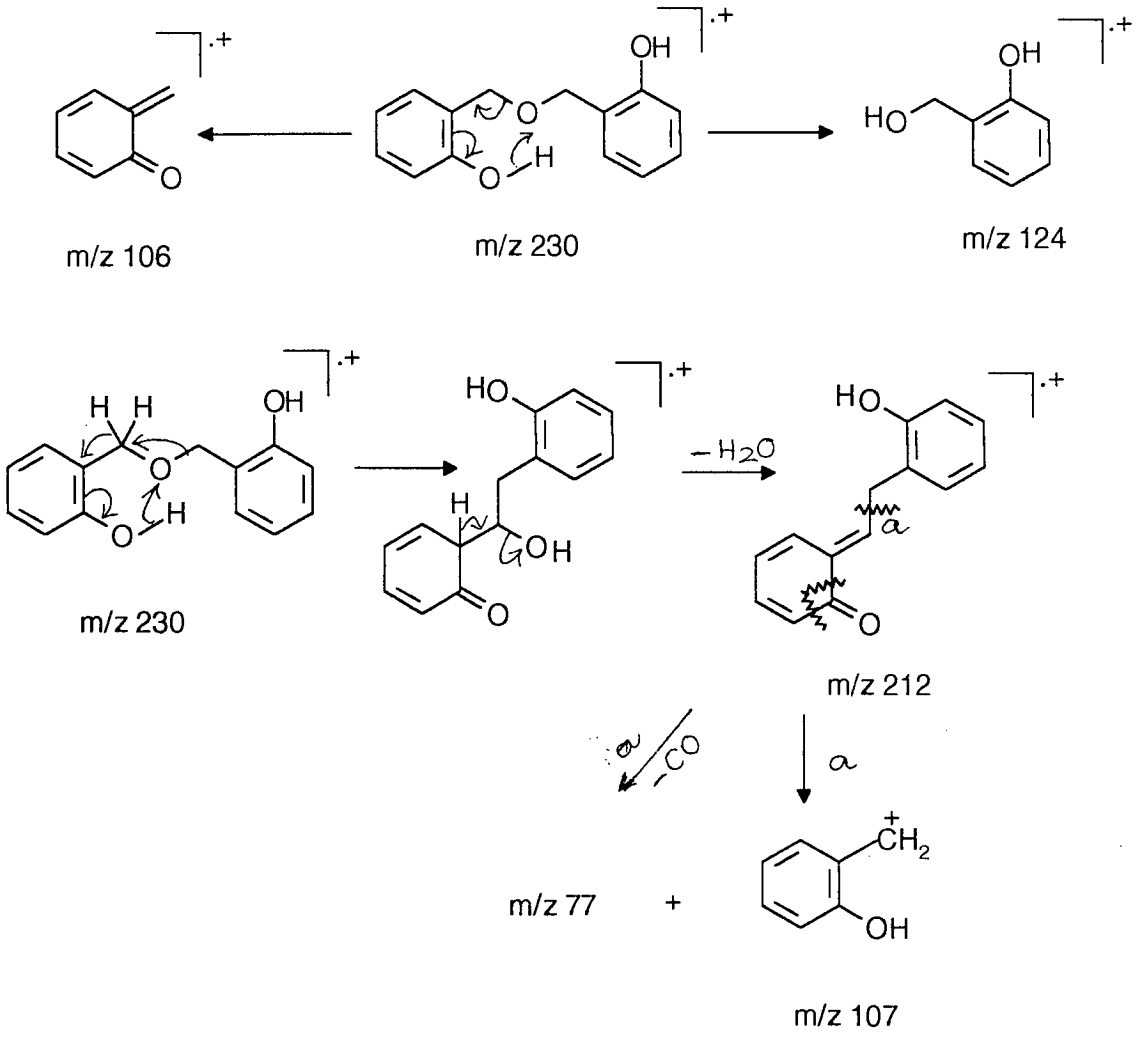
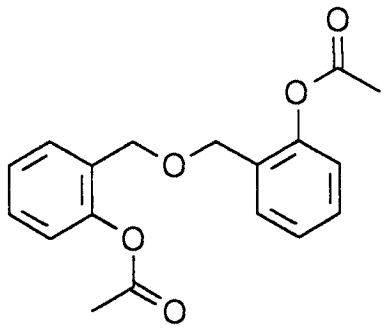


Chart - 1 : Mass spectral fragmentation of 2,2'-dihydroxy bibenzyl ether **14**

On treatment with AC_2O-pyr, **14** gave a liquid diacetate **17**, $C_{18}H_{18}O_5$. The IR spectrum showed bands at 1756 and 1206 cm^{-1} due to phenolic acetate group. The

^{1}HNMR showed a singlet at δ 2.19(6H, acetoxy methyls),another singlet at δ 4.44(4H, Ar-CH_2-O-) and a multiplet centered at δ 7.3 (8H, Ar-H).



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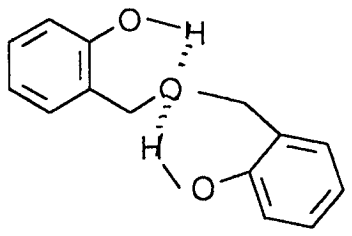
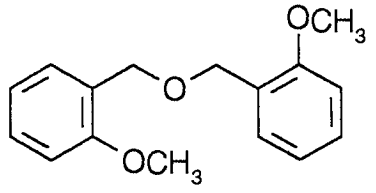


14a

Failure of **14** to undergo methylation with diazomethane is most likely due to the hydrogen bonding as shown in **14a**. Further 2,2'-Dihydroxy bibenzyl ether slowly dissolved in NaOH as expected.



18

Interestingly Lasswell and Hufford had reported the isolation of di-o-methoxy bibenzyl ether **18** from *Uvaria chamae*[9]. Methylation of **14** would then constitute a simple synthesis of this natural product. Refluxing **14** with dimethyl sulphate and aq. NaOH for 2 hr. and usual workup afforded a mixture (mainly mono and dimethyl ethers) from which only the major compound could be isolated in pure form. In our

hands the major product was obtained as a solid,[+] $C_{16}H_{18}O_3$ (M^+ 258.1255) confirming it to be the dimethyl ether derivative **18** of **14**. The spectral data was found to be completely identical with that reported on the natural product. The ^{13}CNMR spectrum (not reported earlier) was fully consistent with the structure. The assignments are shown in table-2.

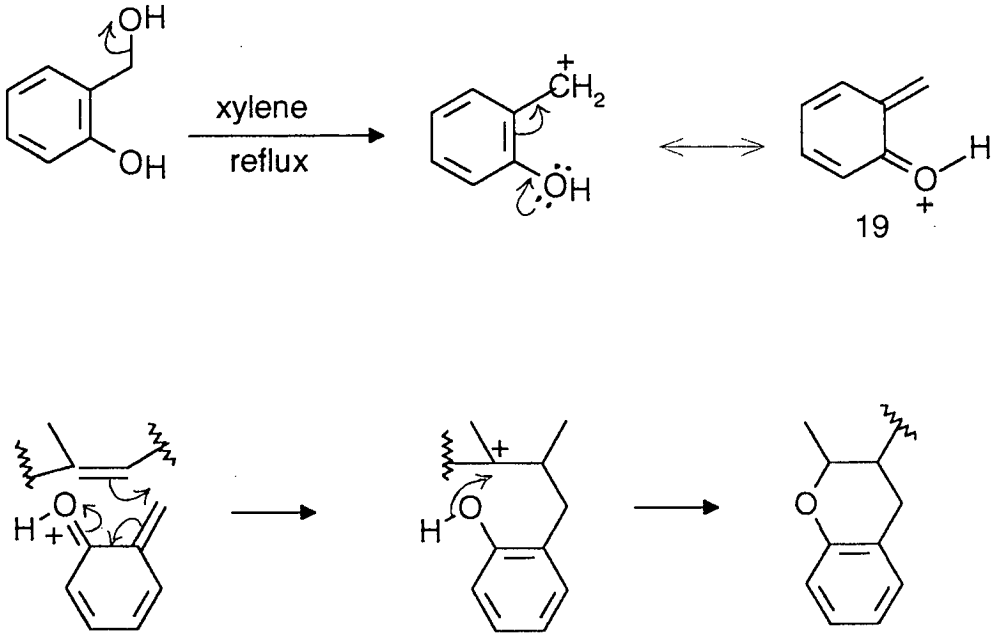
Table-2

Carbons	Chemical shifts δ (ppm)	Multiplicity
C_1 & $C_{1'}$	156.9	s
C_2 & $C_{2'}$	127	s
C_3 & $C_{3'}$	128.3	d
C_4&$C_{4'}$	120.3	d
C_5 & $C_{5'}$	128.7	d
C_6 & $C_{6'}$	110	d
C_7 & $C_{7'}$	67.3	t

Absence of benzoquinone methide dimer **13** or trimer **12** in the thermal reaction of products of saligenin alone or in the presence of olefins can lead to a safe conclusion that o-benzoquinone methide is not the intermediate. It is now clear that the C_7 unit of saligenin is added to the olefinic linkage via ionic intermediates. the reactive intermediate actually involved should be electrophilic and simple

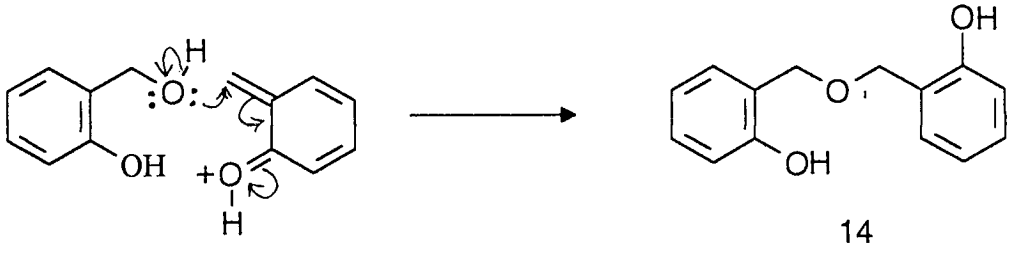
[+] Lasswell and Hufford had obtained the natural 18 in liquid form. Their synthetic product obtained by a different route was also a liquid.

dehydroxylation of **4** would generate reactive intermediate **19** which can react with olefinic linkage as shown in scheme-1.



Scheme - 1

Formation of the 2,2'-dihydroxy dibenzyl ether **14** can be also explained in a straight forward manner (scheme-2).

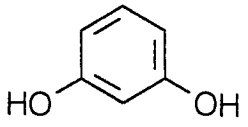


Scheme - 2

The mechanism shown above also explains the formation of **14** by heating saligenin in a sealed tube as reported by Ziegler and Lercher[7].

In view of the several reports on the isolation of o-hydroxy benzylated flavanoids, chalcones, dihydrochalcones from various *Uvaria* species it was of interest to study the thermal reaction of saligenin in presence of reactive aromatic substrates such as resorcinol, phloroglucinol etc.

The results obtained in the thermal reaction of saligenin and resorcinol **20** are descried below.



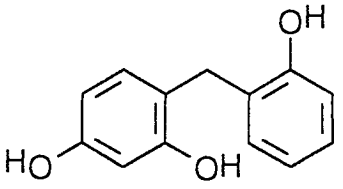
20

Refluxing a equimolar mixture of saligenin **7** and resorcinol **20** in dry xylene for 12 hr and removal of xylene by distillation afforded a solid residue. TLC showed the presence of two major components which could be obtained in pure form by column chromatography over silica gel. The two compounds having melting points 202°C and 193°C are designated as compounds A and B respectively.

Compound A, m.p.202°C analysed for $C_{13}H_{12}O_3$. IR spectrum (KBr) suggested the presence of hydroxyl groups as expected (3220 cm^{-1}). The ^{1}HNMR (fig3.12) was measured in deuterated acetone because of its poor solubility in other solvents. It showed a singlet at δ 3.82 due to a methylene flanked by aromatic rings. The integration showed the presence of seven aromatic hydrogens. In spite of the complex pattern, it was possible to infer the presence of a 1,2,4-trisubstituted benzene

ring. The upfield positions of two protons at δ 6.30 (1H, dd, J=8.0, 2.8 Hz) and δ 6.40 (1H, d, J=2 Hz) indicated that the resorcinol used has undergone alkylation at the expected site. The chemical shift of the third hydrogen of this ring was observed as a doublet (J=8 Hz) at δ 6.96.

The APT (^{13}CNMR) spectrum showed five singlets (δ 119.5,129,155,156 and 158) and seven doublets (δ 103.5,108,116,120.5,128,131and 132). The signal for the methylene carbon appeared to overlap on the signals of the solvent. The compound A is therefore formulated as 2,2',4'-trihydroxy diphenyl methane **21**.

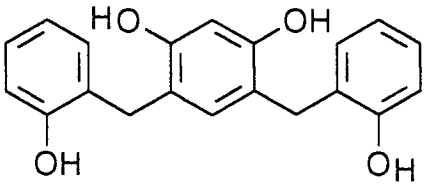


21

Compound B, m.p.193°C, analysed for $C_{20}H_{18}O_4$. Its IR spectrum (KBr) showed striking similarity to that of compound A* and hence must be structurally related to it. The most characteristic difference in ^{1}HNMR was the presence of 1,2,4,5-tetrasubstituted benzene ring. Besides, a complex pattern for 8 hydrogens was observed in the region δ 6.7-7.2. A broad single at δ 8.4 integrated for 4 hydrogens and disappeared on addition of D_2O. Therefore there are four exchangeable hydrogens in compound B. A four proton singlet at δ 3.8 suggested the presence of two Ar-CH_2-

* In both cases a broad band at 2420 $cm^{-1}$was present in IR spectrum. This is most probably due to the molecular association caused due to hydrogen bondings.

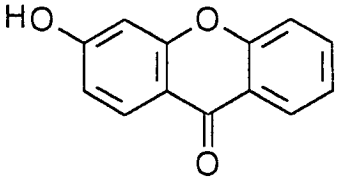
Ar groupings. These features can be easily accommodated in structure **22** for compound B.



22

Further confirmation of the structure **22** including its symmetrical nature came from its ^{13}CNMR spectrum, which showed 4 singlets and 6 doublets all above δ 100. Once again the triplet due to Ar-CH_2-Ar overlapped on the signals of the solvent (deuterated acetone).

We plan to use these compounds as starting materials for the preparation of 3-hydroxy xanthone **23** and benzopyrano xanthone **24**. which would lend further support to the assigned structures.



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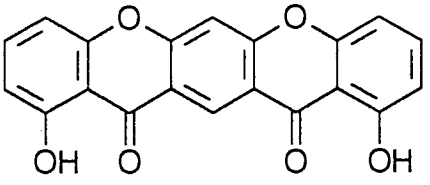


24

3.2 Synthesis of a C_{13} - diosphenol, a constituent of *Ipomea pes caprea*

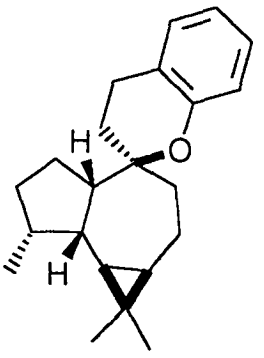
The number of ways in which the enzyme bound isomeric farnesyl pyrophosphate (FPP) precursors gets folded and produce incredibly large numbers of novel sesquiterpene skeletons has attracted the attention of organic chemists all over the world. The observed oxygenation patterns adds further to their novelty and complexity but are rich sources of interesting chemistry. The regular or normal sesquiterpenoids may undergo further transformations e.g. rearrangements which lead to modified or irregular sesquiterpenoids and loss of carbon atoms to produce degraded or [illegible]sesquiterpenoids. The phenomena is not restricted to sesquiterpenoids and characterisation of modified terpenoids* as well as degraded terpenoids has been quite common. In fact steroids (C_{27}) and limonoids (C_{26}) are the well known examples of degraded terpenoids.

In some cases extra carbons are added at some stage in the biosynthesis and such natural products are also considered as modified terpenoids. The terpenoids which have a mixed biogenetic origin are generally regarded as meroterpenoids.

In the previous chapter, synthesis of meroterpenoids (-)-tanzanene **1** and lucidene **6** is described. In this section we report synthesis of a C_{13} - diosphenol **25** and propose that in all probability it is derived biogenetically by the loss of two carbon atoms of the drimane skeleton **26**.

---

* With same number of carbon atoms as present in the bonafied biogenetic precursor



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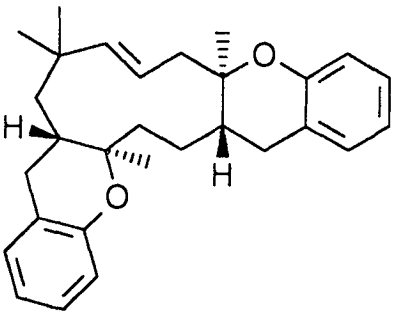

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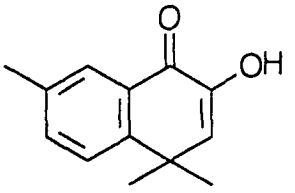

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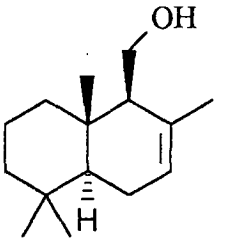
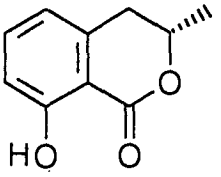


26

Bohlin and coworkers[10] reported that the crude extract of *Ipomea pes caprea* (L) R.Br.(Convolvulaceae) has inhibitory effect on prostaglandin synthesis. Bio-activity guided separation of the extract led to the isolation of four active compounds, C_{13} - diosphenol (2-hydroxy-4,4,7-trimethyl-1(4H)-naphthalenone) **25**, (-)-mellein **27**, eugenol **28**, 4-vinyl guaiacol **29**.



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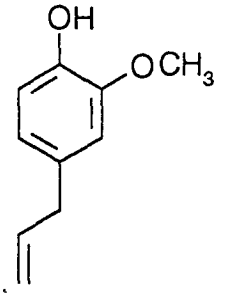

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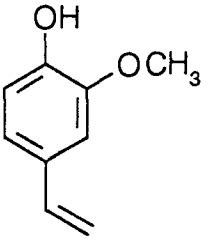
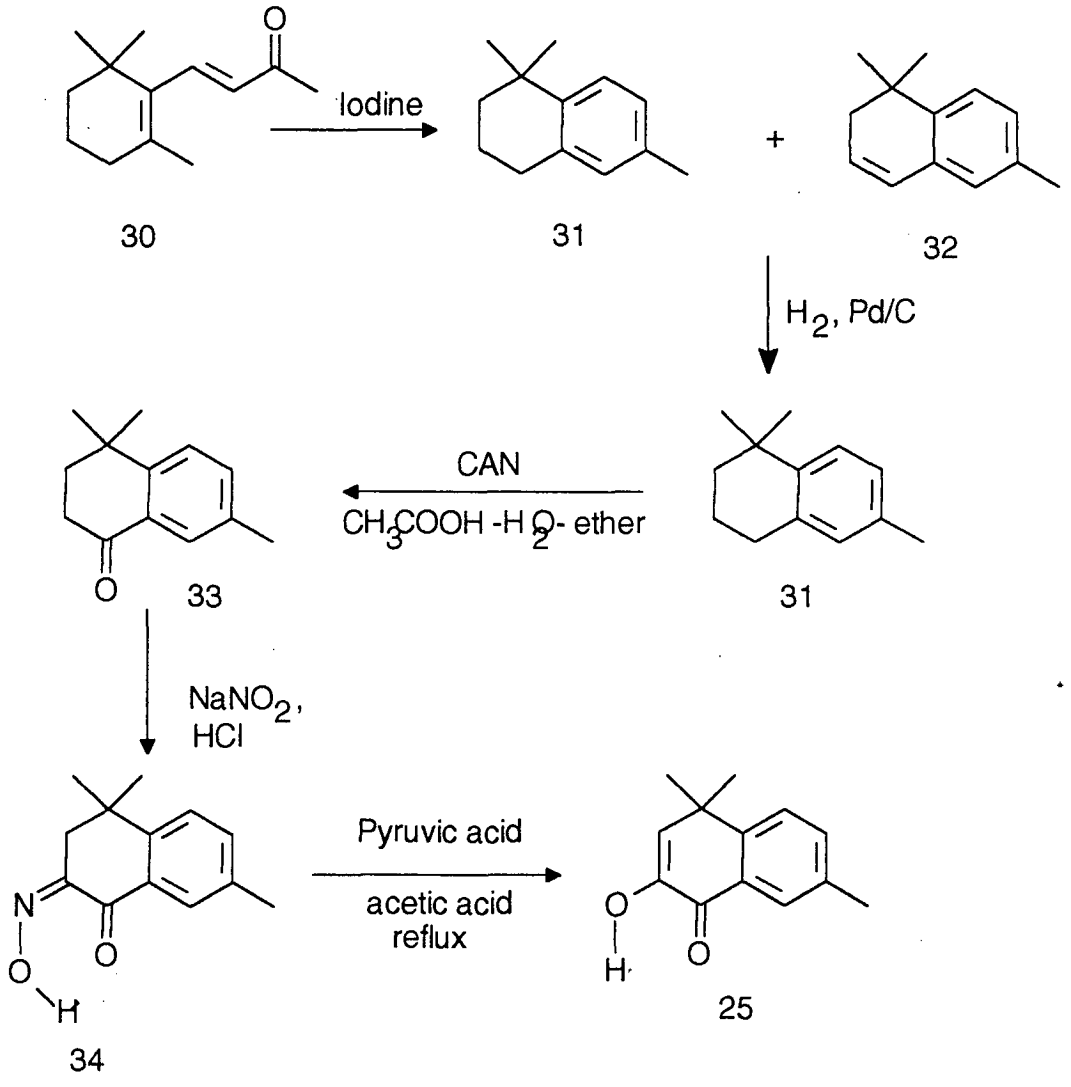


29

Isolation of C_{13} - diosphenol **25** attracted our attention because of our continued interest in the biogenesis and synthesis of modified terpenoids and its reported medicinal properties. We have achieved its synthesis from β-ionone **30** as depicted in scheme-2.



Scheme -2

β-Ionone **30** on distillation in presence of catalytic amount of iodine following the procedure of Bogart and Fourman[11] gave a hydrocarbon fraction in 85% yield. The product was purified by column chromatography over silica gel and distillation over metallic sodium. The IR spectrum showed bands at 1625, 1510 and 832 cm^{-1} due to aromatic ring and was essentially composed of two components (GLC, carbowax, capillary column, 170°C) **31** (77.5%) and **32** (17.1%) having RT 2.89 and 3.29 respectively. Hydrogenation of the hydrocarbon fraction over Pd\C (10%) in ethanol gave pure ionene **31** of more than 90% purity.

Oxidation of ionene **31** with CAN \ acetic acid-water-ether at 35°C for 30 min followed by usual work up and silica gel chromatography gave yellow a oil of 95% purity (GLC). IR bands at 1700, 1620 and 1500 cm^{-1} clearly indicated presence of a conjugated carbonyl group. Its ^{1}HNMR showed three proton singlets at δ 1.44 and 1.45 due to gem dimethyls. Aromatic methyl signal appeared as a singlet at δ 2.34 where as two mutually coupled triplets due to two methylene groups were seen at δ 2.00 and 2.70. Two aromatic protons were observed in the range of δ 7.31-7.34 and third proton at δ 7.82. Identity of the CAN oxidation product with 4,4,7-trimethyl tetralone **33** was confirmed by a direct comparison (co-TLC) and comparison of the spectral data (IR and ^{1}HNMR) recorded on **33** prepared by an alternate route[12*]

The spectral data of the CAN oxidation product was also identical with that reported for 4,4,7-trimethyl tetralone whose synthesis was reported by Liu and Browne employing Diels-Alder reaction[13].

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* Thanks are due to Dr. V. P. Kamat for the reference sample of tetralone **33**.

The present synthesis of tetralone **33** is much shorter and convenient than the previously reported by Kamat and also by Liu.

4,4,7-Trimethyl tetralone **33** on treatment with $NaNO_2$-HCl at 5°C for 2hr. followed by usual work up afforded a white solid mp 177°C (decomp). Its IR spectrum showed bands at 3218,1694,1607 and 1495 cm^{-1}. Its ^{1}HNMR spectrum revealed two three proton singlets at δ 1.38, aromatic methyl at δ 2.39 and methylene proton singlet at δ 3.00. Aromatic protons were seen at δ 7.34, 7.40 and 7.93 where as hydroxyl proton was observed at δ 11.44. Spectroscopic data supported structure **34** for oximino tetralone. Its mass spectral fragmentation did not show a peak due to the molecular ion but showed characteristic fragments (chart-2) confirming the proposed structure.

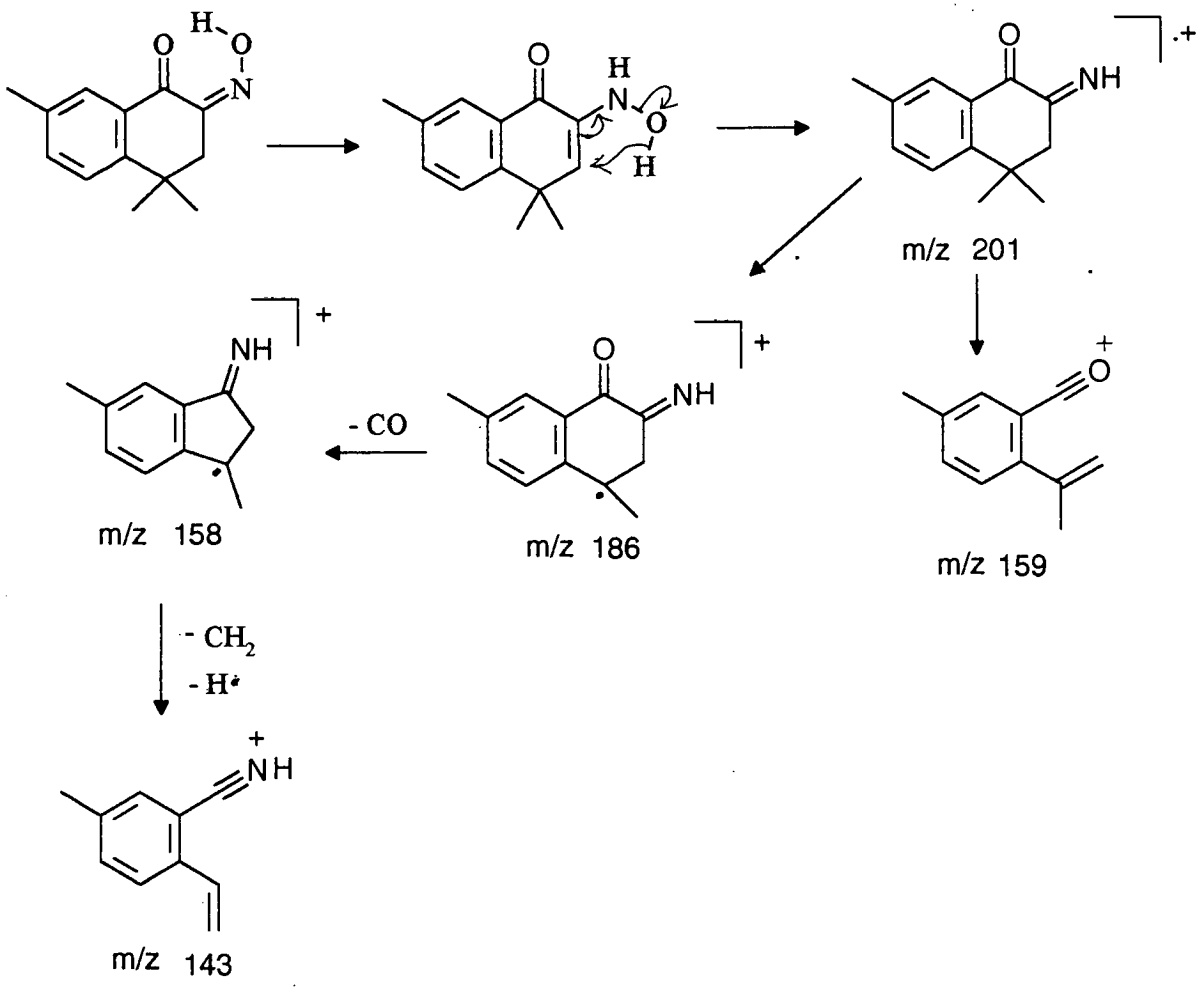


Chart-2

Oximino tetralone **34** on rufluxing with pyruvic acid in acetic acid for 12 hr, followed by usual work up, recrystallisation from benzene and sublimation under vacuum afforded 25 as a white solid mp 117-118^{0}C (lit.[1] 118-118.5^{0}C). IR spectrum showed bands at 3380, 1685, 1618, 1498 cm^{-1}. ^{1}H and ^{13}C NMR spectrums established identity of the synthetic sample with natural as reported by Bohlin and

coworkers[10]. comparison of ^{1}H and ^{13}C NMR are presented in tables 3 and 4 respectively.

Table:3

Carbon	Chemical shift (δ ppm) of natural 0f **25**	Multiplicity	Chemical shift (δ ppm) of synthetic 0f **25**
C_4-CH_3	1.48	s	1.48
C_4-CH_3	1.48	s	1.48
C_7-CH_3	2.42	s	2.42
C_3-H	6.2	s	6.18
C_6-H	7.43	dd	7.4
C_5-H	7.47	d	7.46
C_8-H	8.01	d	8.01
OH	6.41	d	6.41

Table-4

Carbon	Chemical shift (δ ppm) of natural 0f **25**	Chemical shift (δ ppm) of synthetic 0f **25**
C_1	181.5	181.5
C_2	145	145
C_3	126.7	126.6
C_4	36.9	36.8
C_5	126.5	126.5
C_6	134.3	134.2
C_7	136.7	136.6
C_8	127	127
C_9	128.4	128.4
C_{10}	148.6	148.6
C_4-CH_3	30.5	30.4
C_4-CH_3	30.5	30.4
C_7-CH_3	21.9	20.9

The possible mode of major fragmentation and the likely structures for these fragment ions are shown in Chart-3. The EIMS mass spectrum further established the identity.

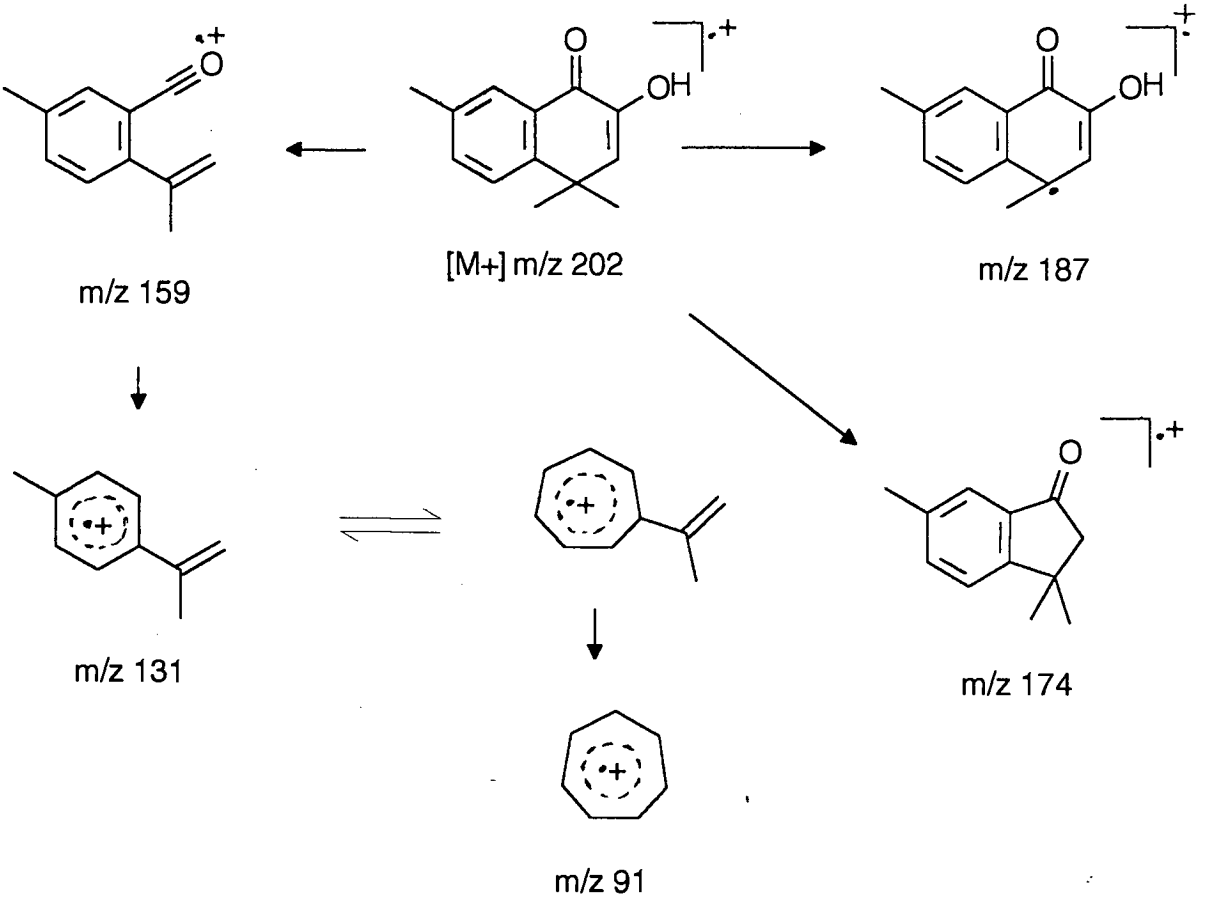
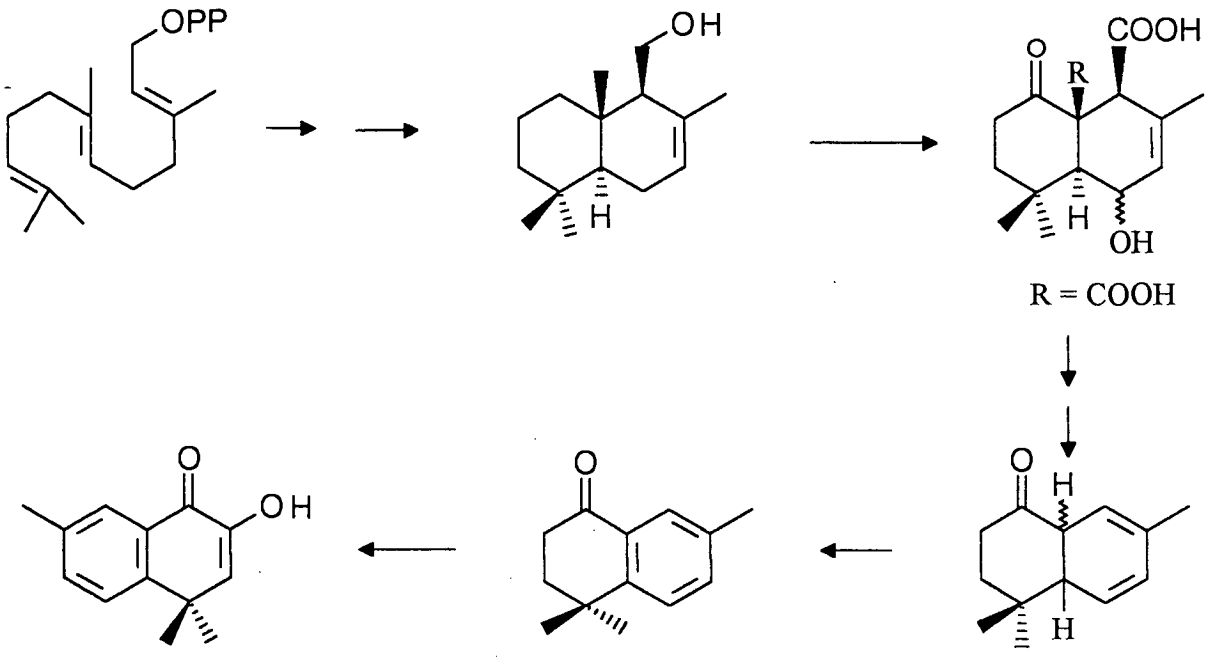


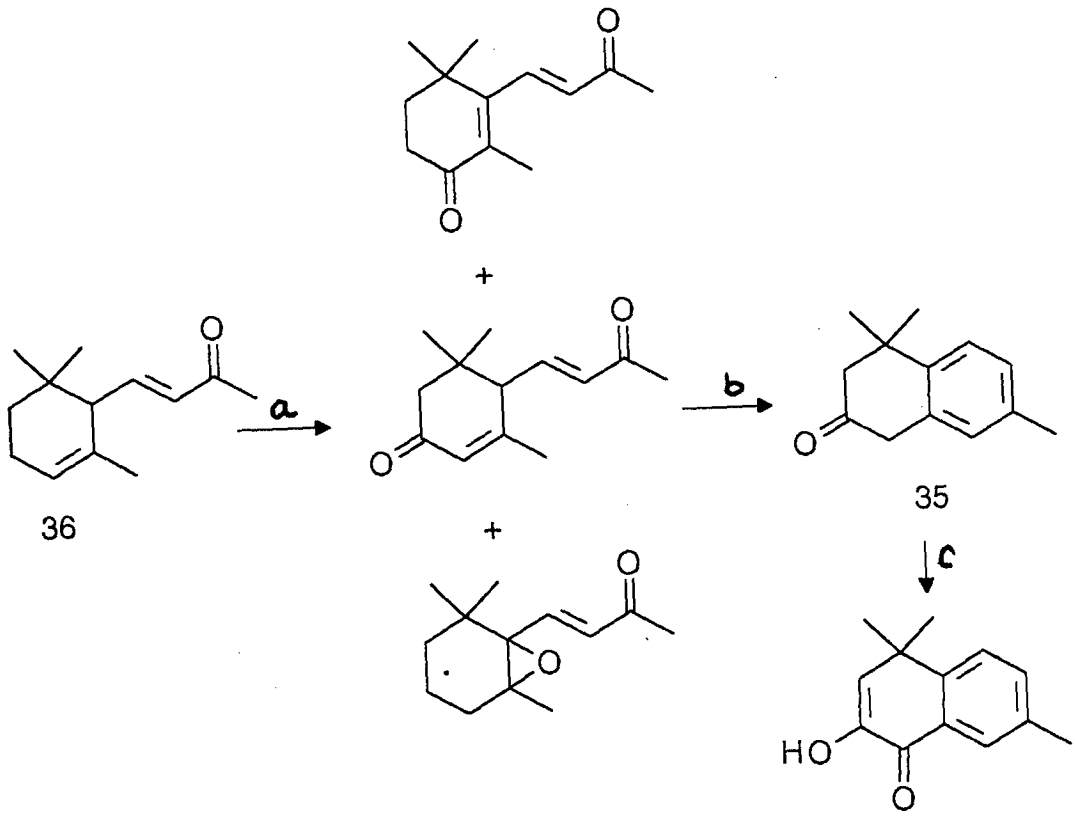
Chart-3

Regarding the biogenesis of diosphenol **26**, we consider it likely that it is derived by loss of two carbon atoms of a drimane skeleton as shown in scheme-3 .



Scheme-3

Incidentally, diosphenol **25** was characterised as an oxidation product of 4,4,7-trimethyl-3,4-dihydro 2(1H) naphthalenone **35** by Davis and co-workers[14] much before its discovery as a natural product. In view of its preparation from α-ionone and β-ionone (present study) it is not easy to rule out its biogenetic origin from a carotenoid precursor. The preparation of **25** reported by Davis and coworkers from α-ionone **36** is presented in scheme-4.

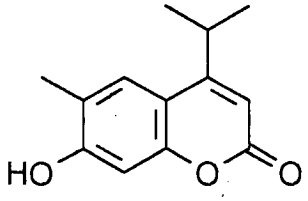


Scheme-4

a) *tert*-butyl chromate oxidation b) 80 % aq. formic acid c) Silver oxide in ethanol

3.3 Synthesis of 7-Hydroxy-4-isopropyl-6-methyl coumarin ; a bis norsesquiterpene.

Although a large number of coumarins having different substitution pattern have been found to occur in nature, there are only three coumarins having a C_4-isopropyl substituent (**37** and **38**) and isopropyl group at C_3 (**39**) and therefore are regarded as a rare group of natural products[15].



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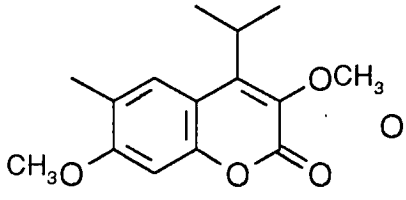

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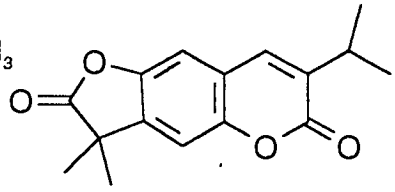
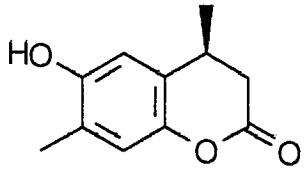


39

Satake and coworkers[16] reported the isolation of **37** and **38** from the fronds of *Macrothelypteris torresiana* CHING var calvata HOLTT. Subsequently pygmaeoherin **39** was isolated from the roots of *Pygmaeopremna herbacea*[17].

Biogenetically, it is appropriate to consider the coumarins **37** and **38** as modified sesquiterpenes. Synthesis of 6-hydroxy-4,7-dimethyl-3,4-dihydrocoumarin **40**[18] has been recently reported from our laboratories and shown to be first well characterised tetranorsesquiterpene. In this section we report the synthesis of **37** and also propose its likely biogenetic pathway.



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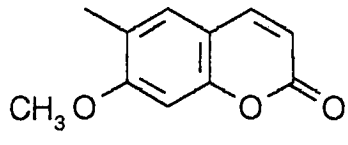
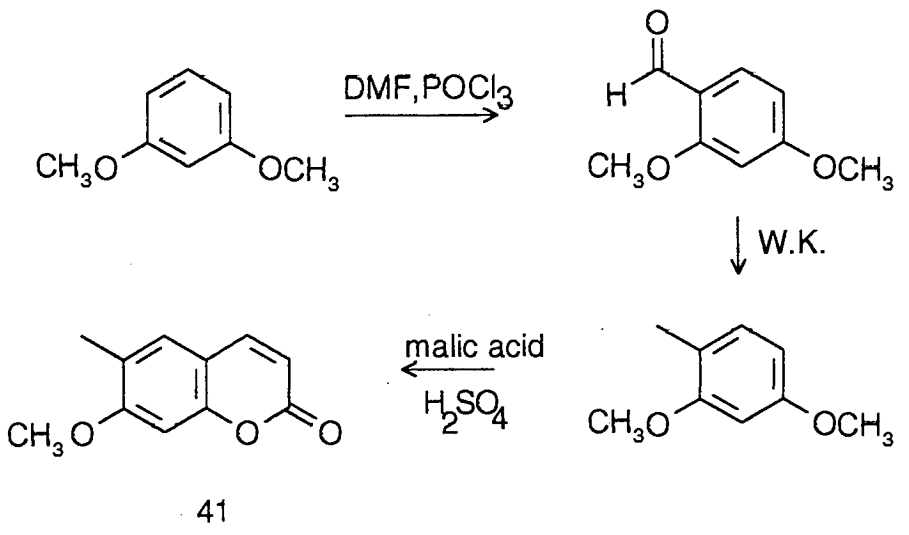


41

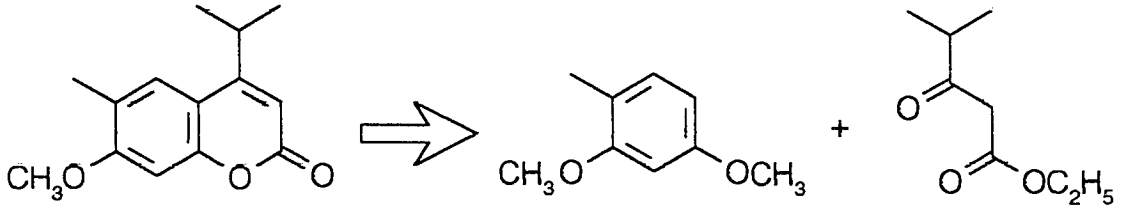
7-Methoxy-6-methylcoumarin **41** has been synthesised from 2,4-dimethoxy toluene in our laboratory[19] (scheme -5).



41

Scheme-5

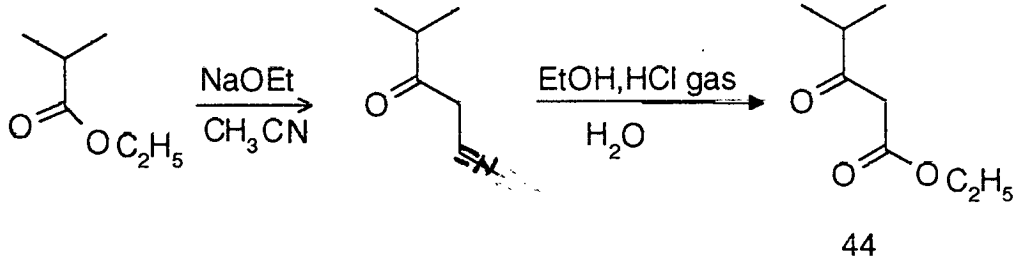
The last step i.e., Pechmann condensation in the above scheme was carried on the methyl ether rather than phenol. Based on this observation we envisaged the synthesis of 4-isopropyl-7-methoxy-6-methylcoumarin **42,** the methyl ether of a naturally occurring coumarin **37** (scheme-6).



42 43 44

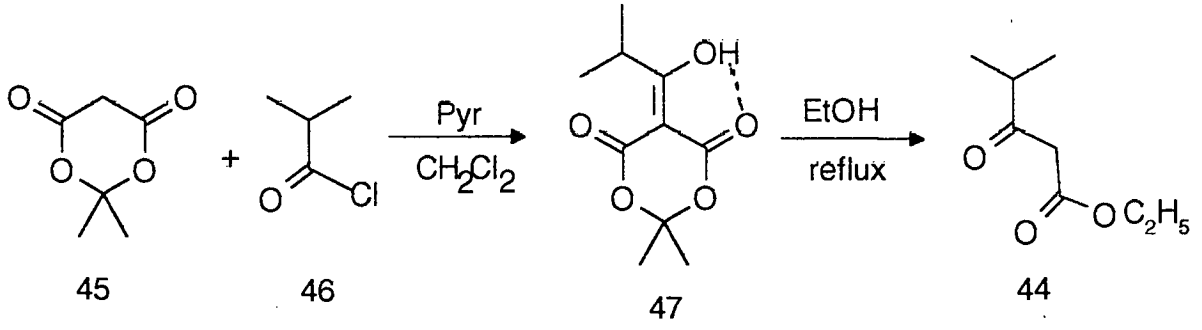
Scheme - 6

Ethyl isobutyryl acetate **44** required for the synthesis of **37** or its methyl ether **42** was prepared earlier by Kroeker and McElvain[20] as shown in scheme-7.



Scheme-7

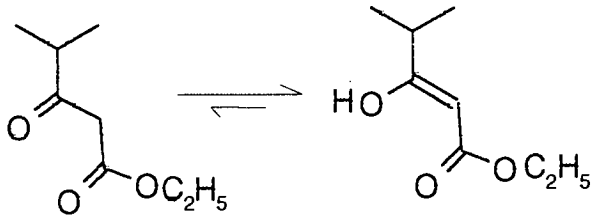
The low yield and tedious experimental procedure involved in the above synthesis of **44** made us to look for an alternate simple synthesis. Following the recent synthesis of β-keto esters reported by Yonemitsu and coworkers[21] using Meldrums acid **45**, ethyl isobutyryl acetate **44** could be obtained in 56% yield as shown in scheme-8.



Scheme-8

The base catalysed acylation of Meldrums acid **45** with isobutyryl chloride **46** resulted in the formation of **47** in almost quantitative yield, which without further purification was refluxed with ethanol for 3 hr. and usual workup afforded **44** as a colourless oil, whose IR spectrum showed bands at 1745 and 1710 cm^{-1} for an ester

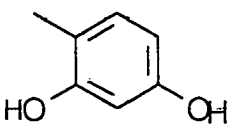
and ketone carbonyls respectively. The ^{1}HNMR spectrum showed doublets at δ 1.13 and 1.16 (J=7 Hz) and a clean septet at δ 2.62 (J=7 Hz) confirmed the presence of an isopropyl group. A three proton triplet at δ 1.26 (J=8 Hz) and a two proton quartet at δ 4.17 (J=8Hz) belong to the ester function. The methylene protons appeared as a clean singlet at δ 3.48. The ^{1}HNMR spectrum also showed the presence of the enol tautomer **44b**.



44a 44b

The present synthesis of **44** is superior to that reported by Kroeker and McElvain[20] because of the simplicity of experimental procedure and better yields.

The Pechmann condensation of ethyl isobutyryl acetate **44** with 2,4-dimethoxy toluene **43** using conc. Sulphuric acid following the standard experimental procedure afforded a complex mixture which on chromatographic separation gave colourless oil as a major product. The spectroscopic analysis of this oil clearly indicated to be an undesired product, the identification of the same is in progress since Pechmann condensation of phenols with β-keto esters normally produces the corresponding coumarins in good yields, we decided to use 2,4-dihydroxy toluene **48** for the Pechmann condensation. Although synthesis of **48** was reported earlier by Clemanson reduction of β–resorcyl aldehyde **49**[22],its preparation from commercially available **43** seemed simple.



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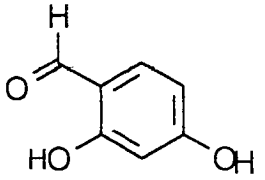


49

Demethylation of **43** using BBr_3 at low temperature[23] was found to be most convenient and yielded **48** (40%) a white solid. Recrystallisation from chloroform afforded needles, m.p. 102°C (lit.[22] 104°C). Its IR spectrum showed a broad band at 3450 cm^{-1} due to hydroxyl functionality. ^{1}HNMR spectrum showed a singlet at δ 2.15 due to aromatic methyl where as aromatic protons were seen at δ 6.34 (2H) and δ 6.98 (1H). A two proton singlets at δ 4.8 disappeared on addition of D_2O and was therefore due to exchangeable hydrogens of the phenolic hydroxyl groups. Thus the ^{1}HNMR spectrum was fully consistent with structure **48** for the demethylated product

The condensation of 4-methyl resorcinol **48** with ethyl isobutyryl acetate **44** using conc. Sulphuric acid at 0°C for 1 hr. and then at room temperature for 16 hr. followed by usual work up afforded yellowish solid which on repeated crystallisation from chloroform-petroleum ether afforded colourless needles of 7-hydroxy-4-isopropyl-6-methylcoumarin **37** in 52 % yield, m.p.197°C (lit[15] 198°C). Comparison of the spectral data (IR, UV, ^{1}HNMR, ^{13}CNMR and MS) measured on the synthetic compound with those recorded on the natural product established their identity beyond doubt. The base peak at m/z 175 in the mass spectrum appears to be derived by loss of CO followed by CH_3 radical as shown in chart-4.

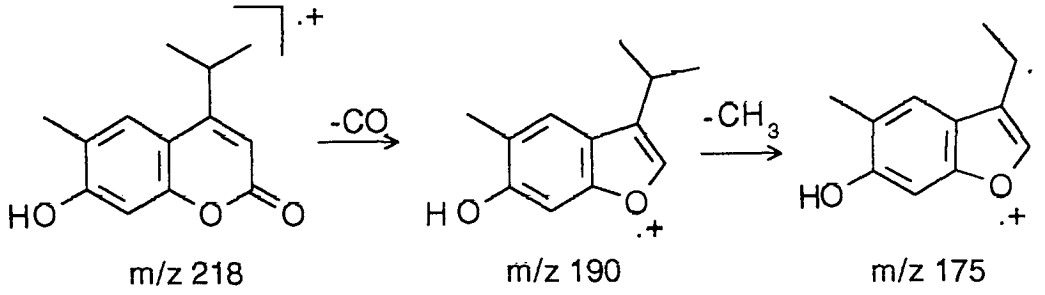
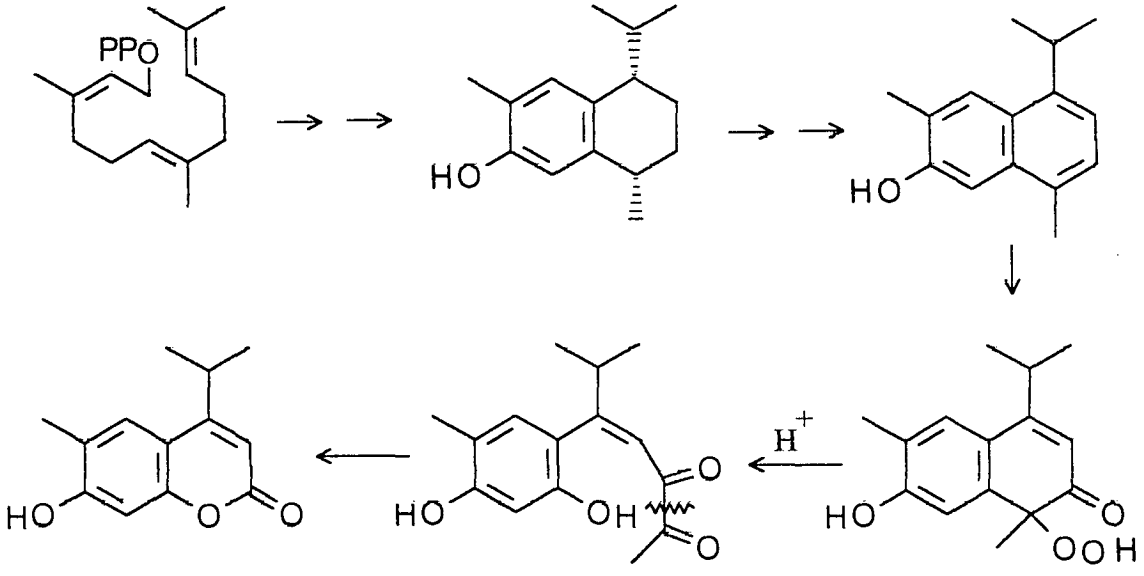


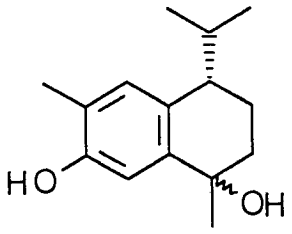
Chart - 4

The biosynthetic origin of 7-hydroxy-4-isopropyl-6-methyl coumarin is interesting. The location of isopropyl, methyl and the hydroxyl substituents suggests that it is derived by loss of two carbon atoms of a sesquiterpene precursor as shown in scheme-9.



Scheme-9

Incidentally 1,7-dihydroxy calamenene **50**[24], 2,7-dihydroxy cadalane,**51**[23], and lancinilene **52**[25],have been isolated as a natural products tends further support to our hypothetical pathway.



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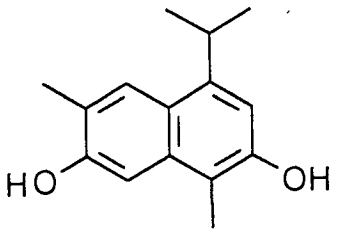

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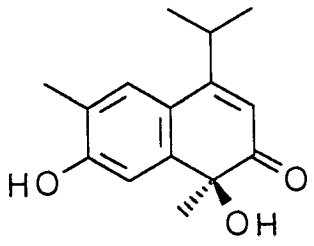
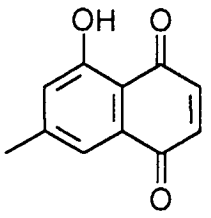


52

3.4 4-Hydroxy-5-methylcoumarin derivatives from *Diospyros kaki* THUNB and *D. kaki* THUNB var. *sylvesyris* MAKINO; structure and synthesis of 11-methylgerberinol

Till 1978 only few naturally occurring 4-hydroxy-5-methylcoumarins and its derivatives were known, but now number has exceeded hundred and most of them have been isolated from Compositae family[26].

Several years ago, Natori and coworkers had isolated naphthaquinones, 7-methyl julgone **53**, diospyrin **54**, isodiospyrin **55**, neodiospyrin **56**, plumbagin **57**, mamegakinone **58**, shinanolone **59**, binaphthyl-1,1`-quinone **60** along with triterpenes lupeol, betulin and betulinic acid from the root extracts of Ebenaceae plants *Diospyros kaki* THUNB and *D. kaki* THUNB var. *sylvesyris* MAKINO[27]. While compounds **53-60** were characterised, two compounds now designated as A and B, were available in minute quantities and could not be identified. We have now fully characterised these compounds and results are presented in this section.



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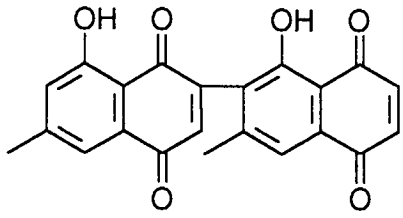

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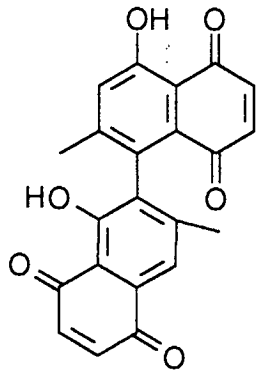

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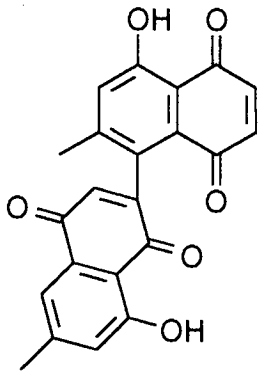

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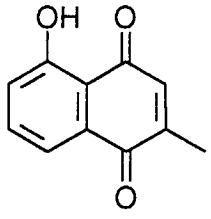

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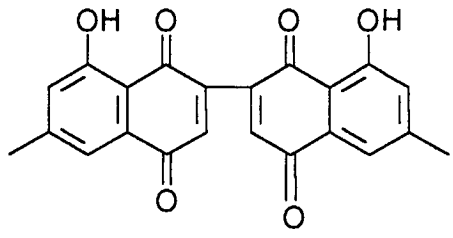

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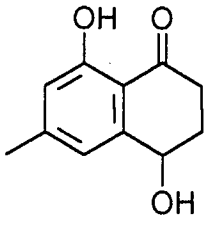

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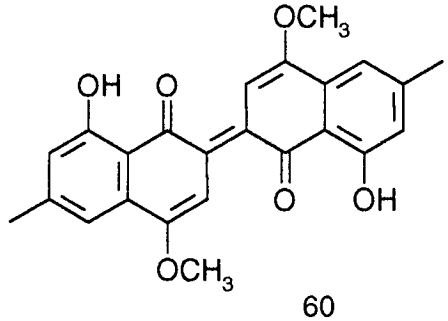
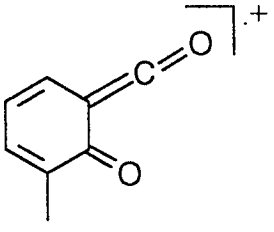


60

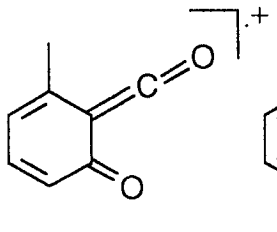
Characterization of compound A

Compound A(unidentified compound from *D.kaki* thunb var *sylvestris* makino)$C_{21}H_{16}O_6$ mp 267-269^{0}C showed UV (λ_{max} 240, 286, 320sh nm) and IR bands (ν_{max} 3030, 1648, 1599 cm^{-1}) typical of a bis-4-hydroxycoumarin[28]. The ^{1}HNMR spectrum showed signels at δ 2.80 (6H, singlet, aromatic methyls), δ 3.77 (2H, s, CH_2 attached to quaternary carbons), δ 6.9-7.5 (6H, m, typical of a 1,2,3-trisubstituted benzene ring) and two exchangeable protons at δ 11.7 (-OH).

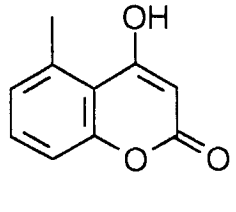
The high resolution EIMS showed a [M+] peak at m/z 364.094 corresponding to the molecular formula $C_{21}H_{16}O_6$. The base peak at m/z 134 which is due to ion **61a** or **61b** suggested that compound A is 4-hydroxy coumarin derivative with a methyl substitution either at C_5 (**62**) or C_8 (**63**) position.



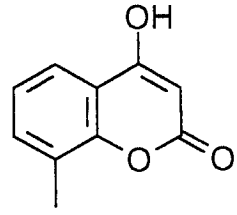
61a



61b

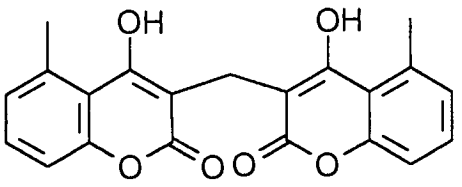


62

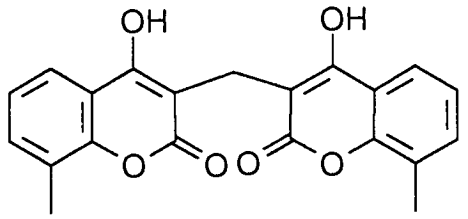


63

Therefore complete structure for compound A must be either **64** or **65**.



64



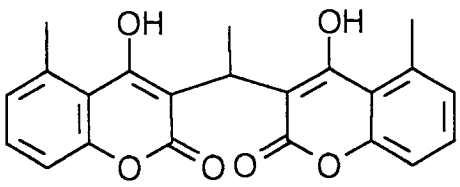
65

A survey of the literature indicated that the spectral data and the mp of compound A corresponded exactly to the data reported for gerberinol **64** (mp 263-65^{0}C), previously isolated from *Gerbera lanuginosa* Benth by Sengupta and coworkers[29]. A direct comparison (IR, UV,^{1}HNMR) unambiguously established the identity*.

Characterisation of compound B

The second compound B (uncharacterised from *D. kaki*), $C_{22}H_{18}O_6$, mp 210-217^{0}C (ethyl acetate), exhibited spectral data UV (λ_{max} 246, 295, 305 and 320 nm); IR (λ_{max} 1660, 1640, 1595 cm^{-1}) characteristic of a bis-4-hydroxycoumarin. The IR bands at 1383 and 1365 cm^{-1} indicated the presence of a methyl groups. The ^{1}HNMR spectrum was strikingly similar to that of compound A (gerberinol **64**), the major difference being the replacement of one of the C_{11} hydrogen by a methyl group (δ 1.85, 3H, d, J= 7 Hz, δ 4.65, 1H, q, J= 7Hz) for aromatic region. Structure **66** was therefore assigned to compound B and named 11-methyl gerberinol.

* We thank Prof. S. Natori for the copies of (IR, UV, MS and NMR) spectra measured on uncharacterised compounds of D. Kaki & Prof. P. sengupta for an authentic sample of gerberinol and the copies of spectra for comparison.



66

Its high resolution EIMS, showed in addition to the [M^+] peak at 378.112 ($C_{22}H_{18}O_6$ requires 378.110), significant peaks at m/z 202, 176, 135(100%), 134(96%), and 106. The observed fragment ions are fully consistent with structure **66** and are presented in chart 5.

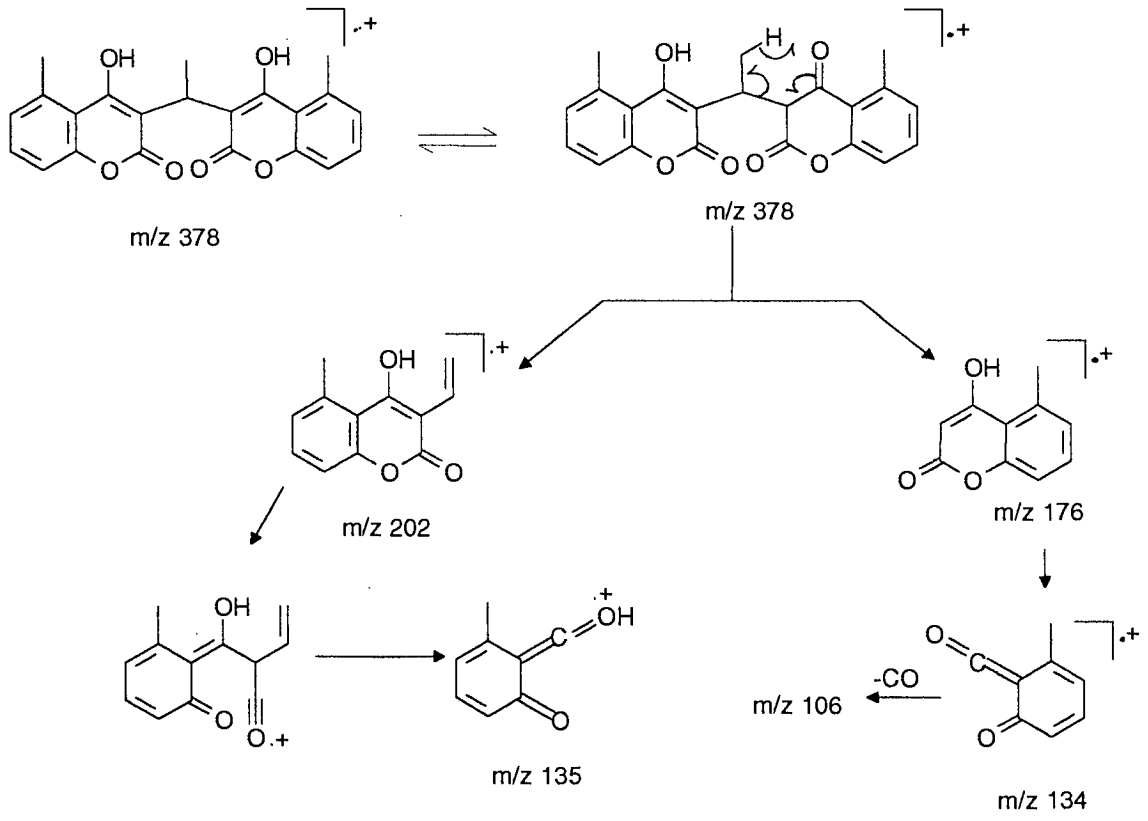
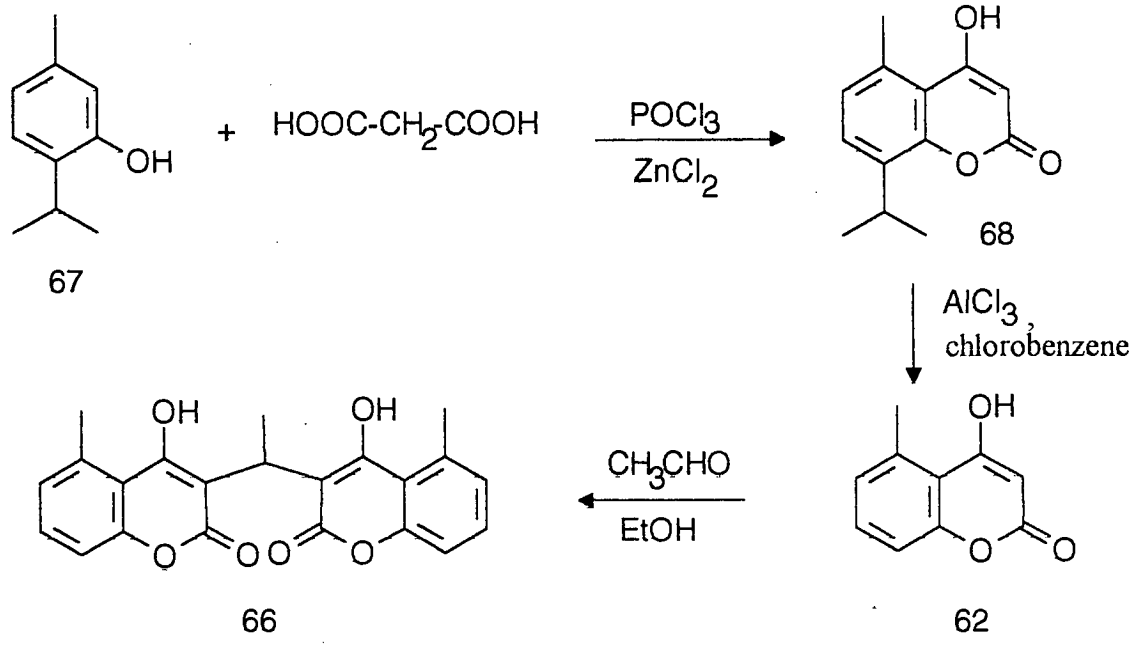


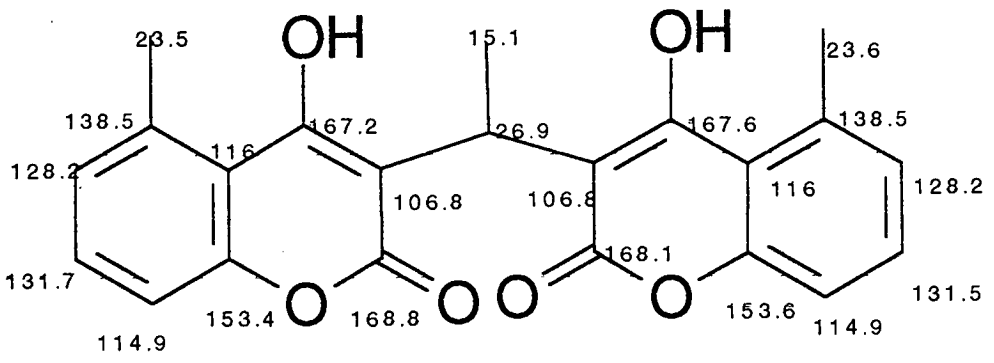
Chart-5: Mass spectral fragmentation of 11-methyl gerberinol **66**

The assigned structure **66** for 11-methyl gerberinol was confirmed by a straight forward synthesis from 4-hydroxy-5-methyl coumarin **62** which was prepared from thymol **67** following a new synthetic route developed by Paknikar and Nadkarni[30]The 4-hydroxy-5-methyl coumarin **62** was then condensed with acetaldehyde by refluxing in ethanol for 5 min. to afford 11-methyl gerberinol **66** in 59% yield as presented in scheme-10.

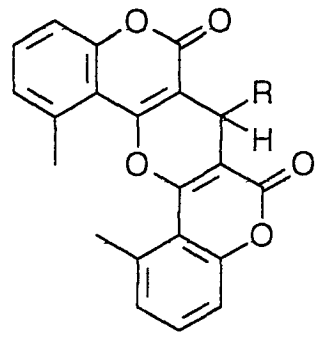


Scheme-10

The synthetic product **66** was identical in all respects to natural **66** (IR, UV, ^{1}HNMR). We have now recorded its ^{13}CNMR spectrum which is fully consistent with the proposed structure. The ^{13}CNMR chemical shifts assignments are shown below.



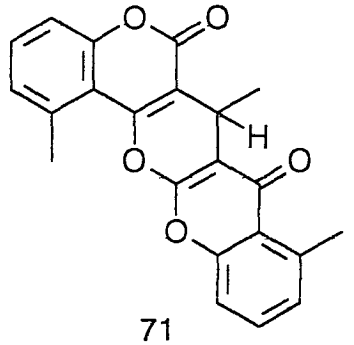
Natural 11-methyl gerberinol **66** on treatment with acetic anhydride and sulphuric acid gave colourless needles, mp 283-284^{0}C (MeOH-$CHCl_3$); $C_{22}H_{16}O_5$, [M^+] 360.098; UV λmax 277, 300 nm; IR νmax 1715, 1670, 1640, 1605, 1410, 1313 and 1260 cm^{-1}. Its EIMS showed in addition to the [M^+], a base peak at m/z 345 [M^+-15] and ^{1}HNMR did not show any signals due to acetate methyls and the molecular formula suggested the formation of an anhydro compound. Interestingly Sengupta and coworkers[29] observed that gerberinol **64** when treated with Ac_2O-pyridine gives an anhydro compound **69** and this structure assignment was based on mechanism and spectral analysis (^{1}H and ^{13}C NMR). Structure **70** was therefore looked likely for anhydro derivative obtained from 11-methyl gerberinol **66**. However ^{1}HNMR and ^{13}CNMR data of anhydro 11-methyl gerberinol were not consistent with structure **70**.



69 R ≡ H

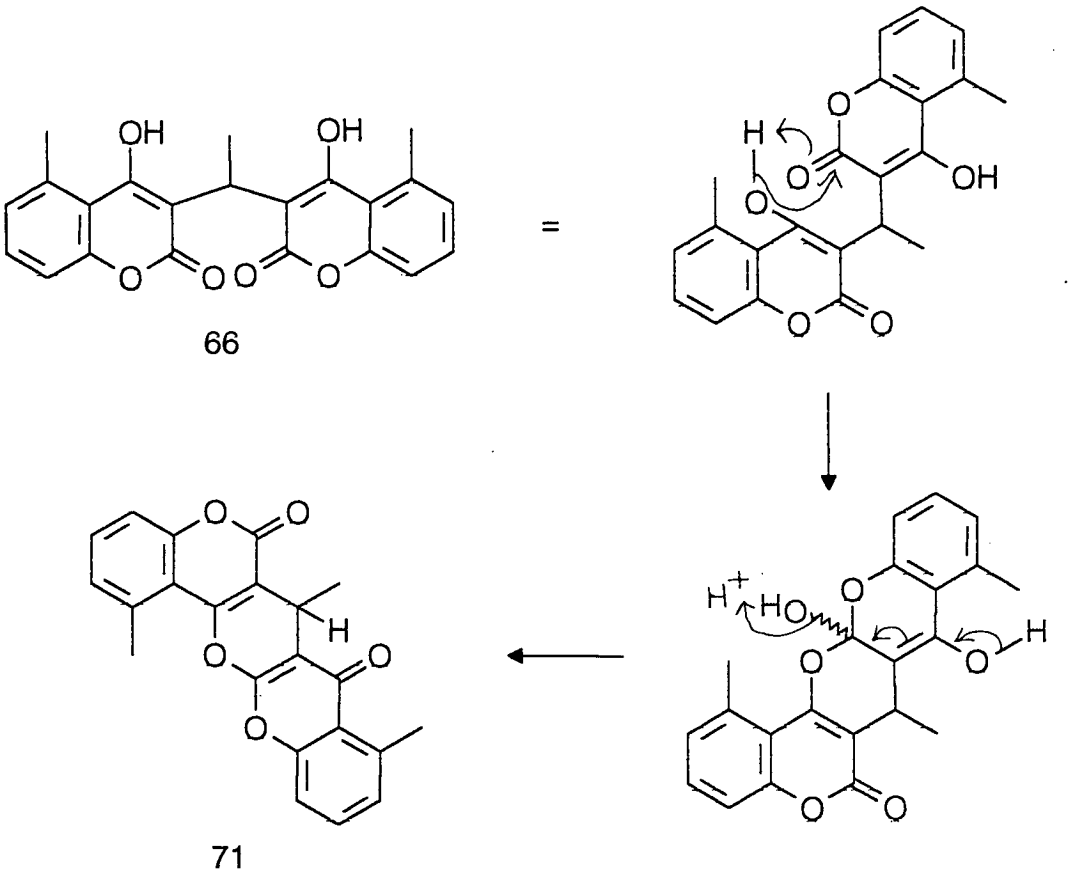
70 R = CH_3

We have now assigned structure **71** to this product, especially after noting two different types of carbonyl groups (singlets at δ 160.4 and 178.7). The assignments of ^{1}H and ^{13}C NMR spectra are given in the experimental section.



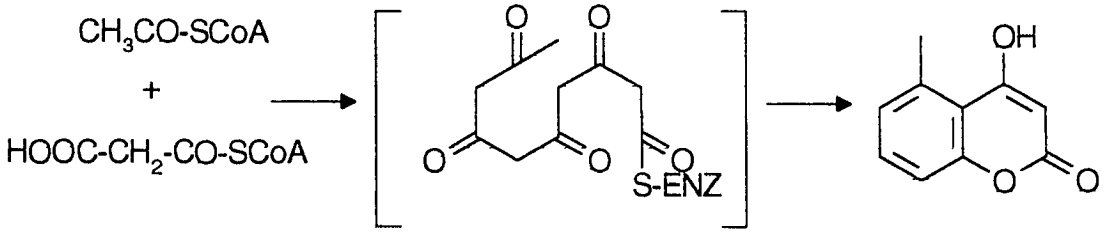
71

The formation of **71** can be accounted by the mechanism shown in scheme-11.



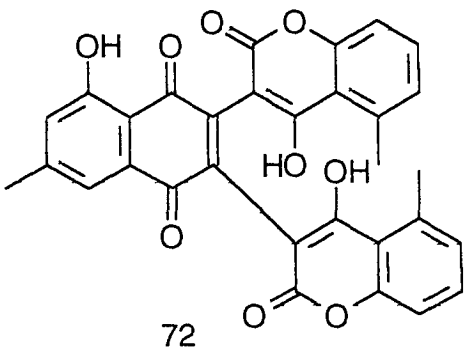
Scheme-11

Toyonaga and co-workers have shown that 4-hydroxy-5-methylcoumarins and their derivatives are biosynthetically derived via a polyketide pathway (scheme-12)[31].



Scheme-12

Similarly it is known that naphthaquinones are derived via polyketides[32]. The co-occurrence of gerberinol **64** and 11-methyl gerberinol **66** with quinones **53-60** in *D. kaki* is therefore understandable. In fact, one can anticipate the presence of 4-hydroxy-5- methylcoumarin and its derivatives in any plant and/or marine source which is known to contain the quinone pigments. Isolation of ismailin **72** and canalicutin **73** from *Diospyros* species [33] both resulting from further reaction/s between 4-hydroxy-5-methylcoumarin and naphthaquinones shows the economy and simplicity used by living systems to produce novel natural products.



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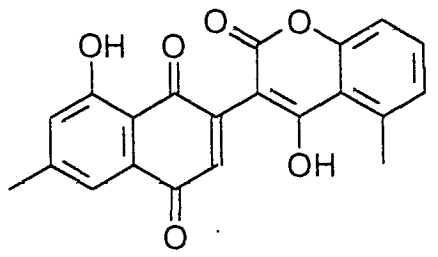

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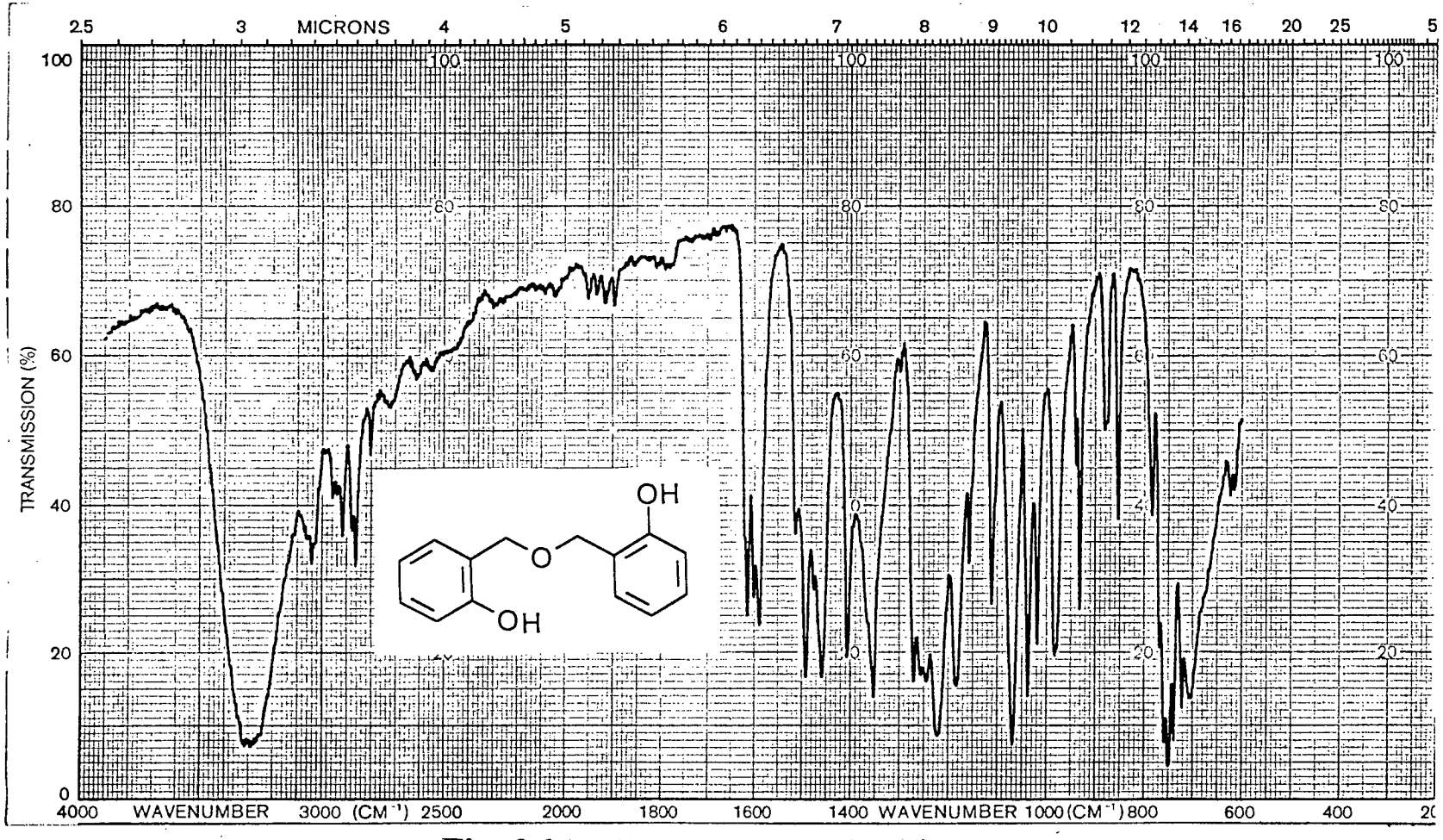

Fig. 3.01 : IR spectrum of 14

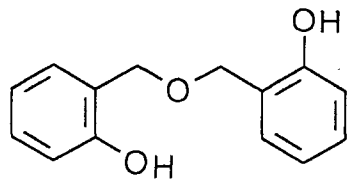

Fig. 3.02 : ^{1}H NMR spectrum of 14

SP 219/93
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EXP2 PULSE SEQUENCE: APT
DATE 12-01-93
SOLVENT CDCL3
FILE APT

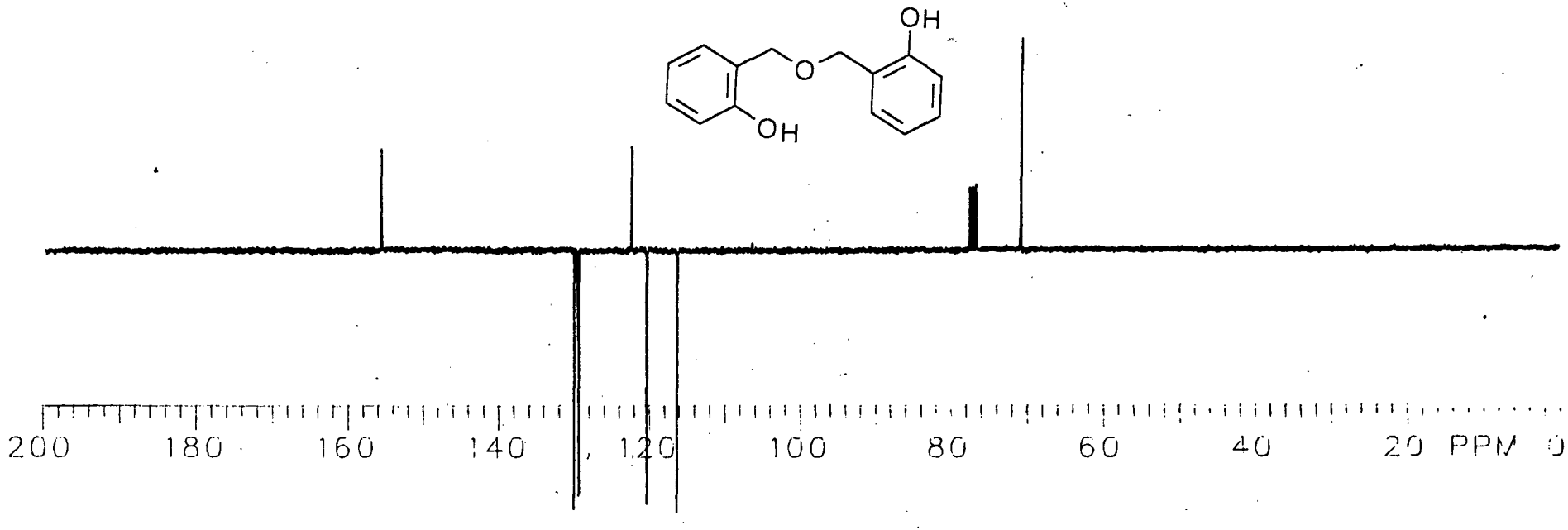


Fig. 3.03 : ^{13}C NMR spectrum of 14

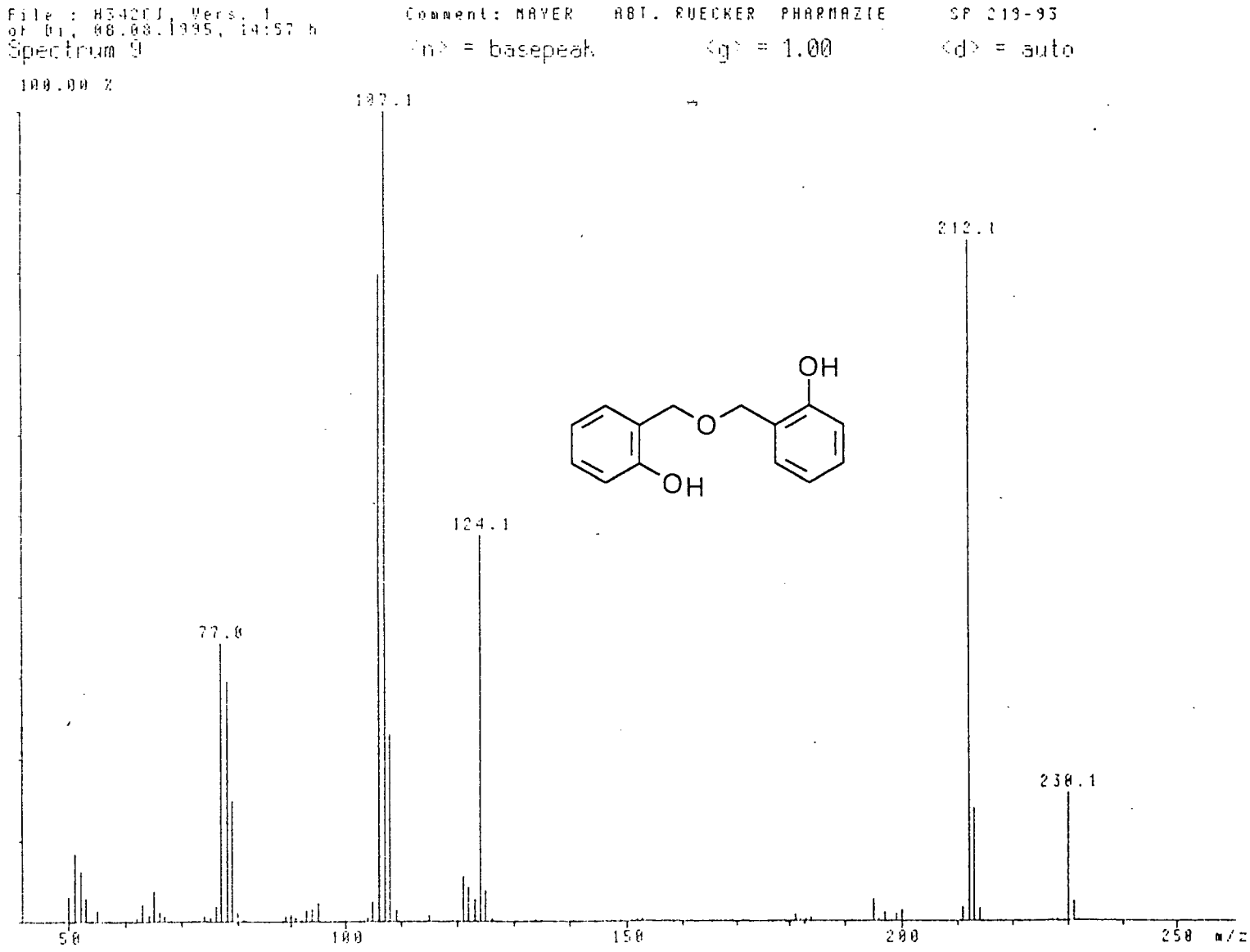


Fig. 3.04 : Mass spectrum of 14

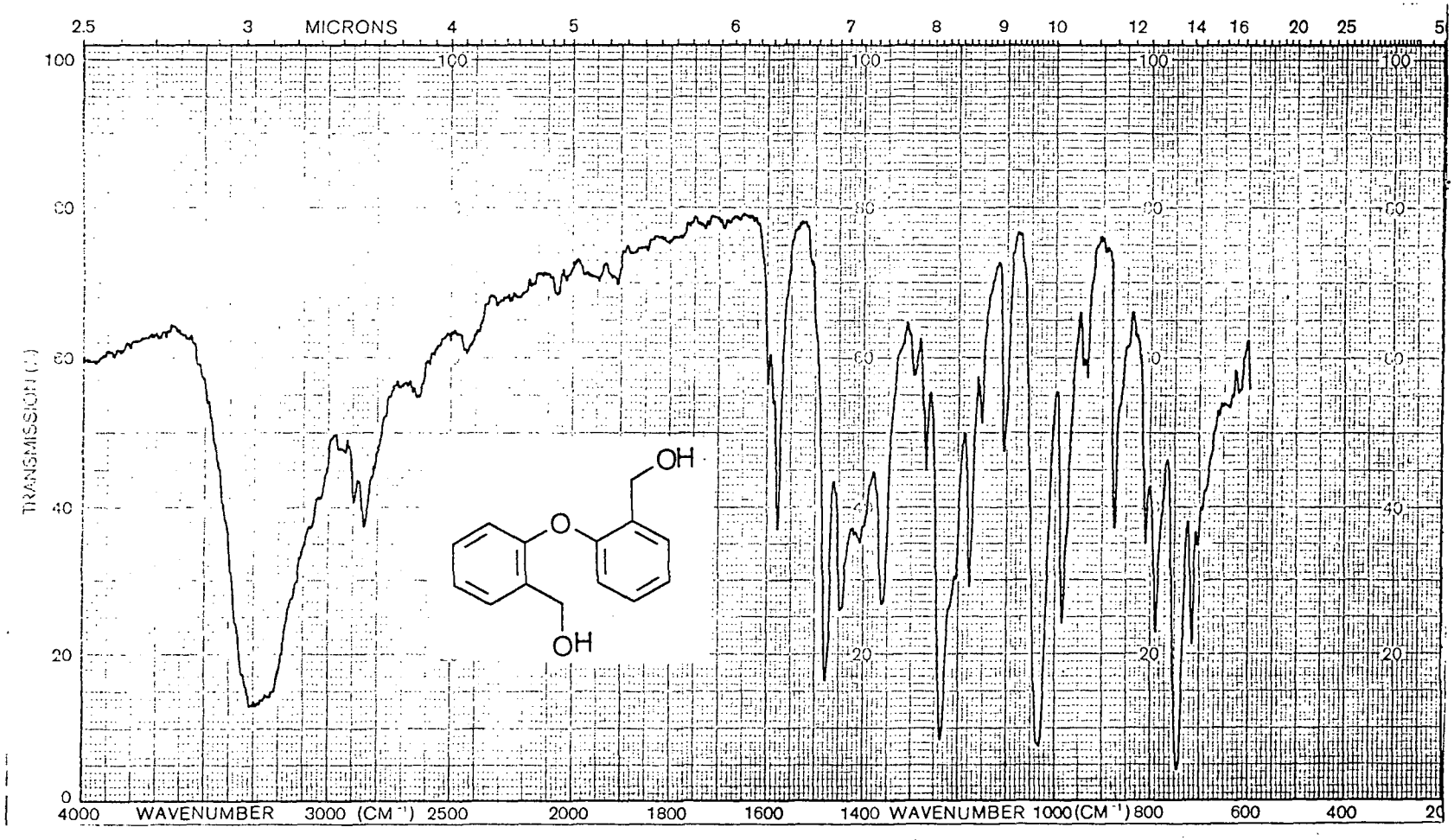


Fig. 3.05 : IR spectrum of 15

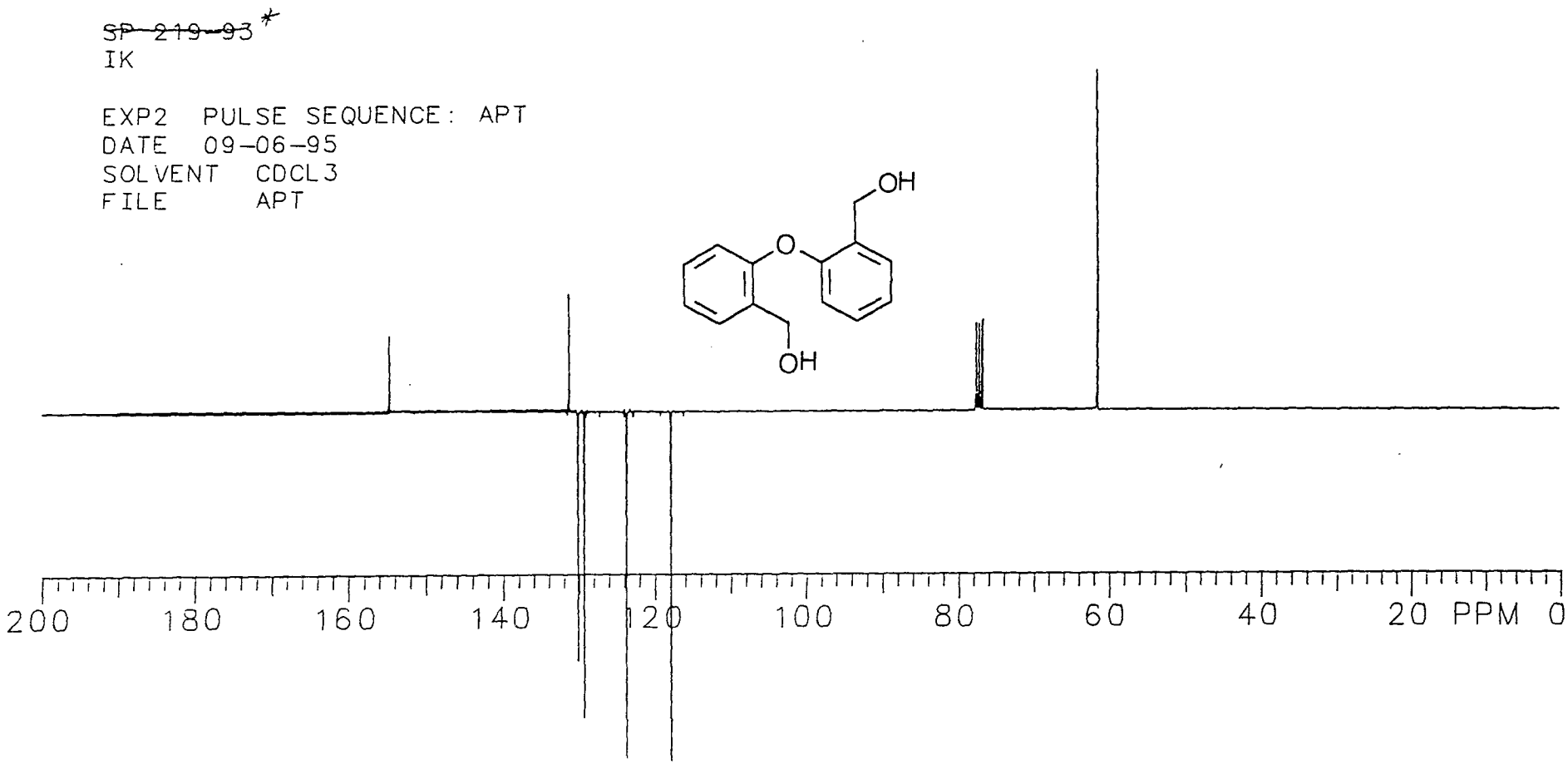


Fig. 3.06 : ^{13}C NMR spectrum of 15

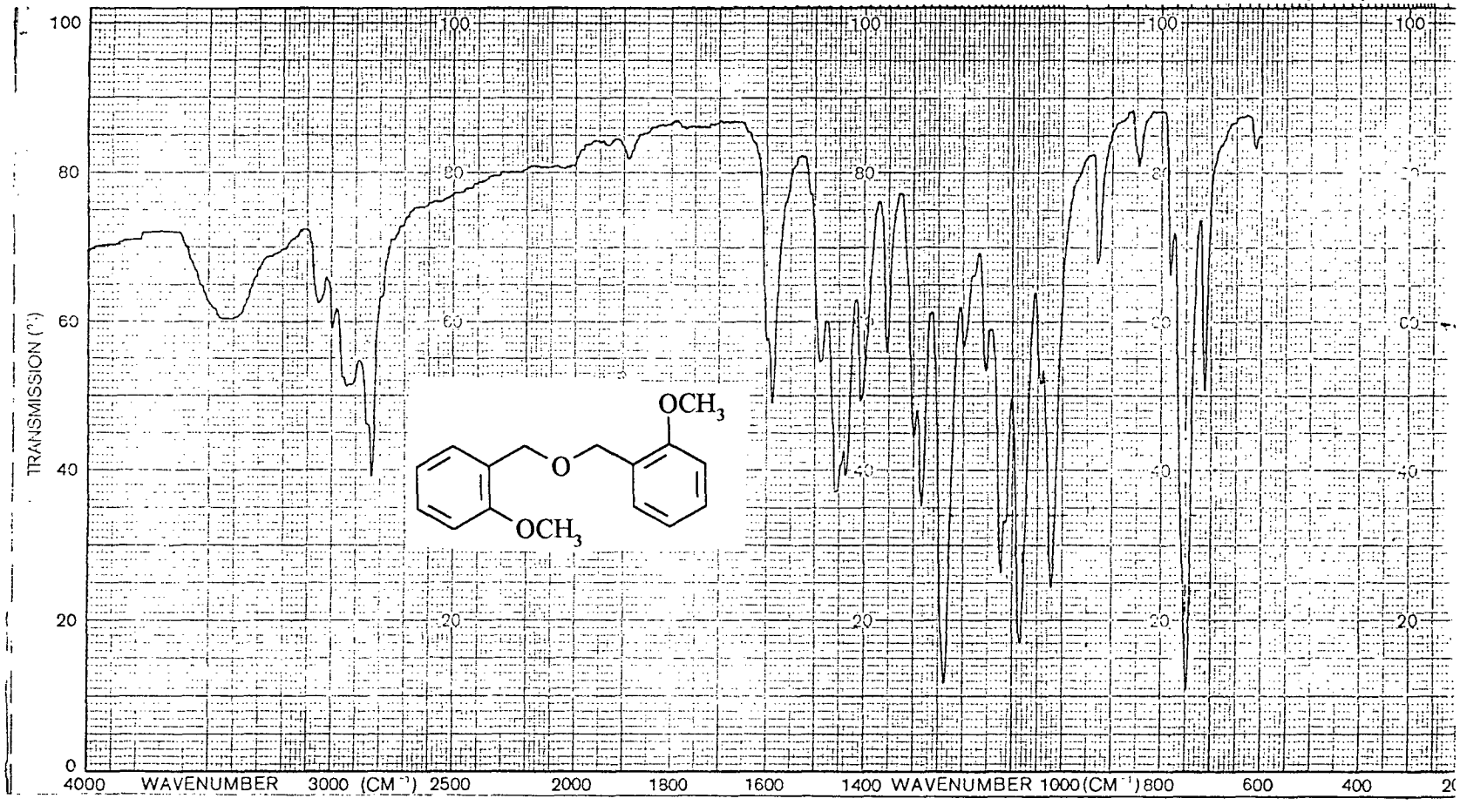


Fig. 3.07 : IR spectrum of 18

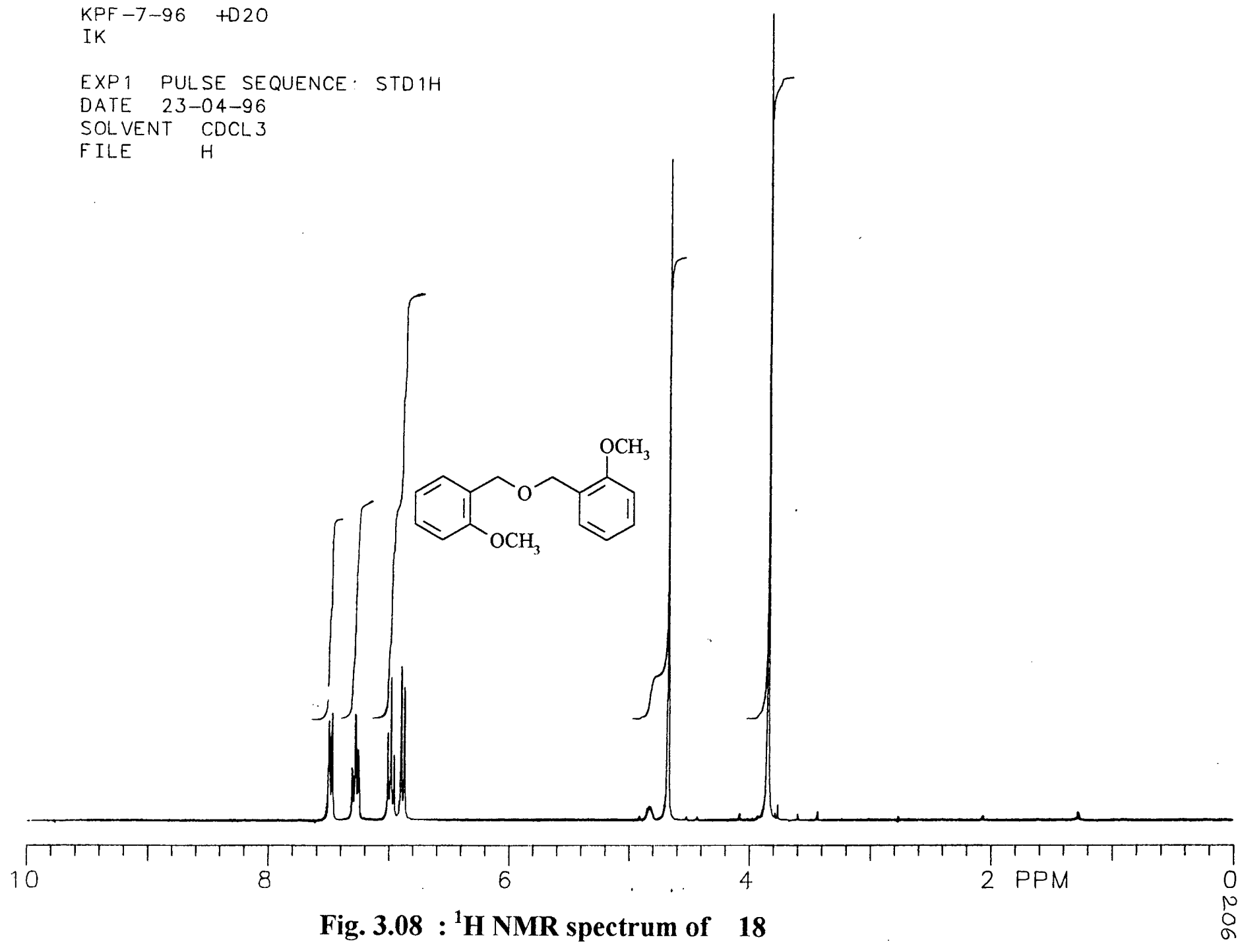


Fig. 3.08 : ^{1}H NMR spectrum of 18

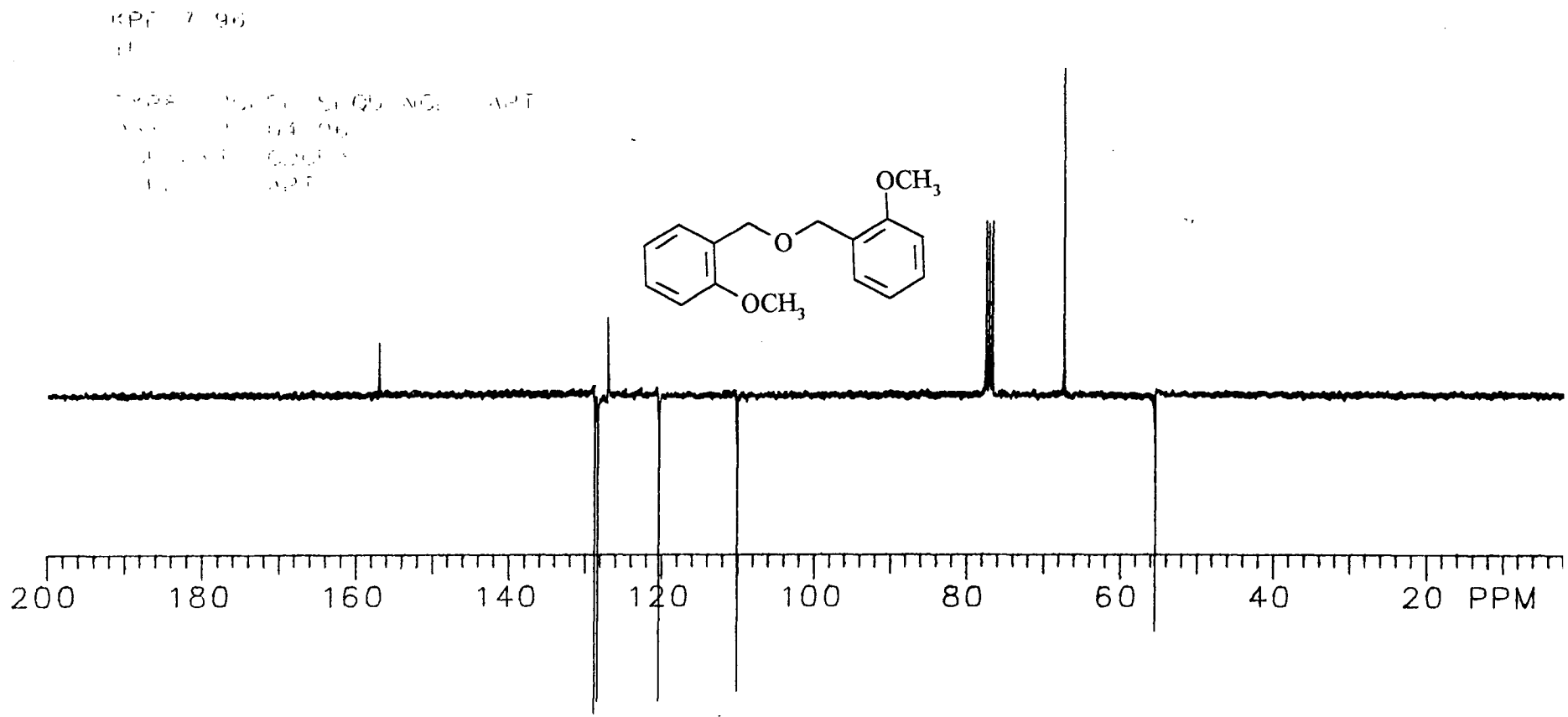


Fig. 3.09 : ^{13}C NMR spectrum of 18

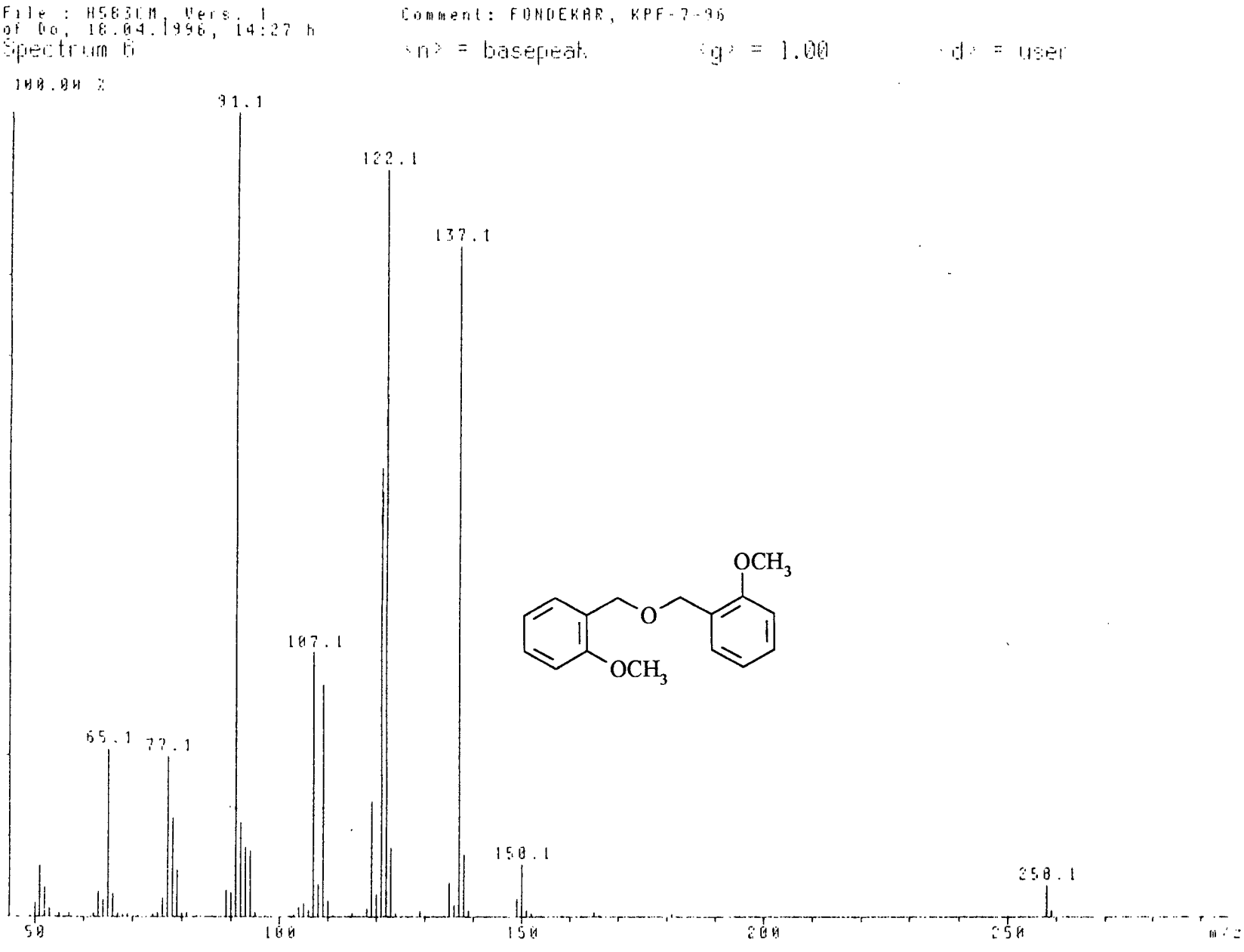


Fig. 3.10 : Mass spectrum of 18

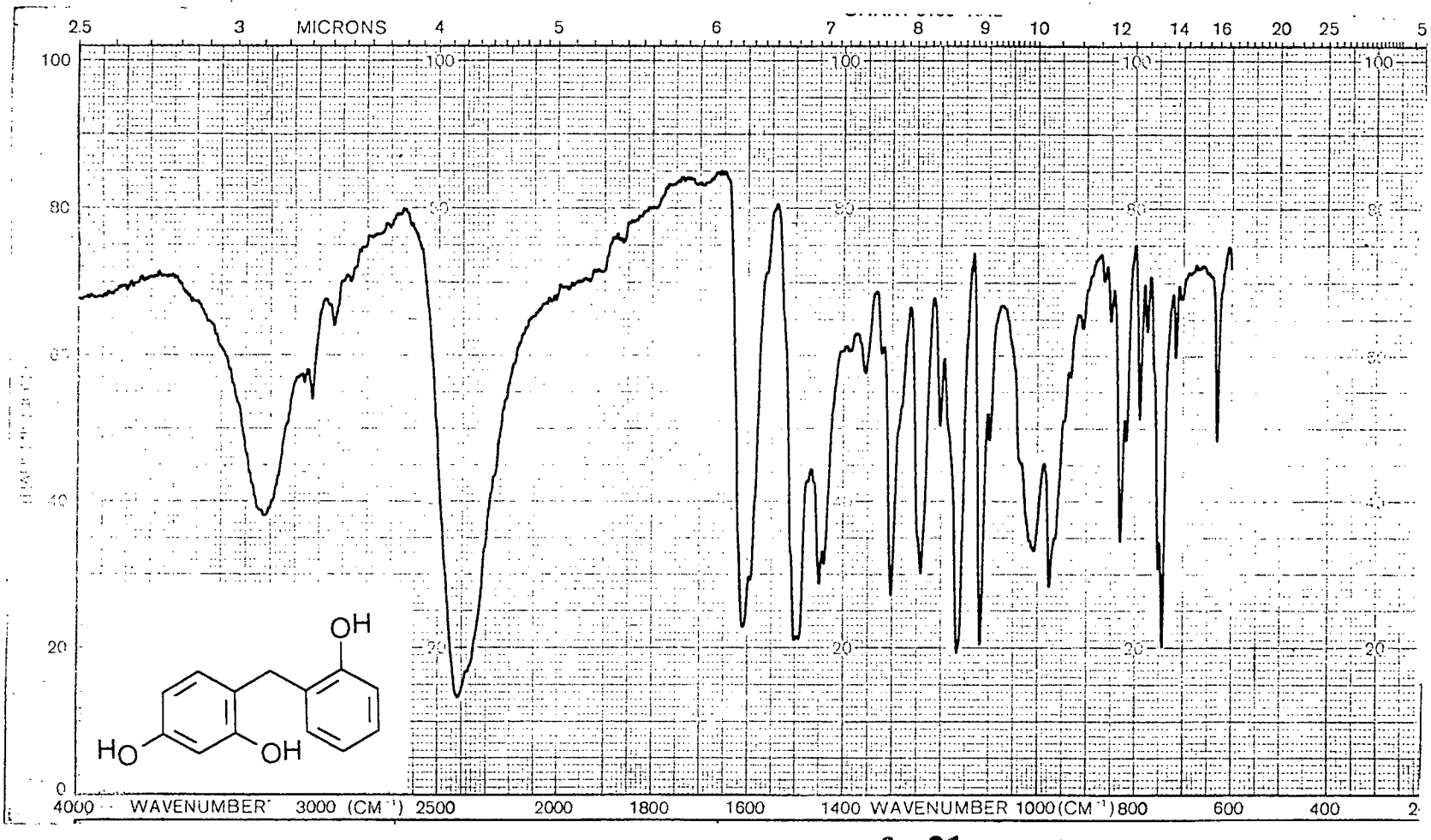


Fig. 3.11 : IR spectrum of 21

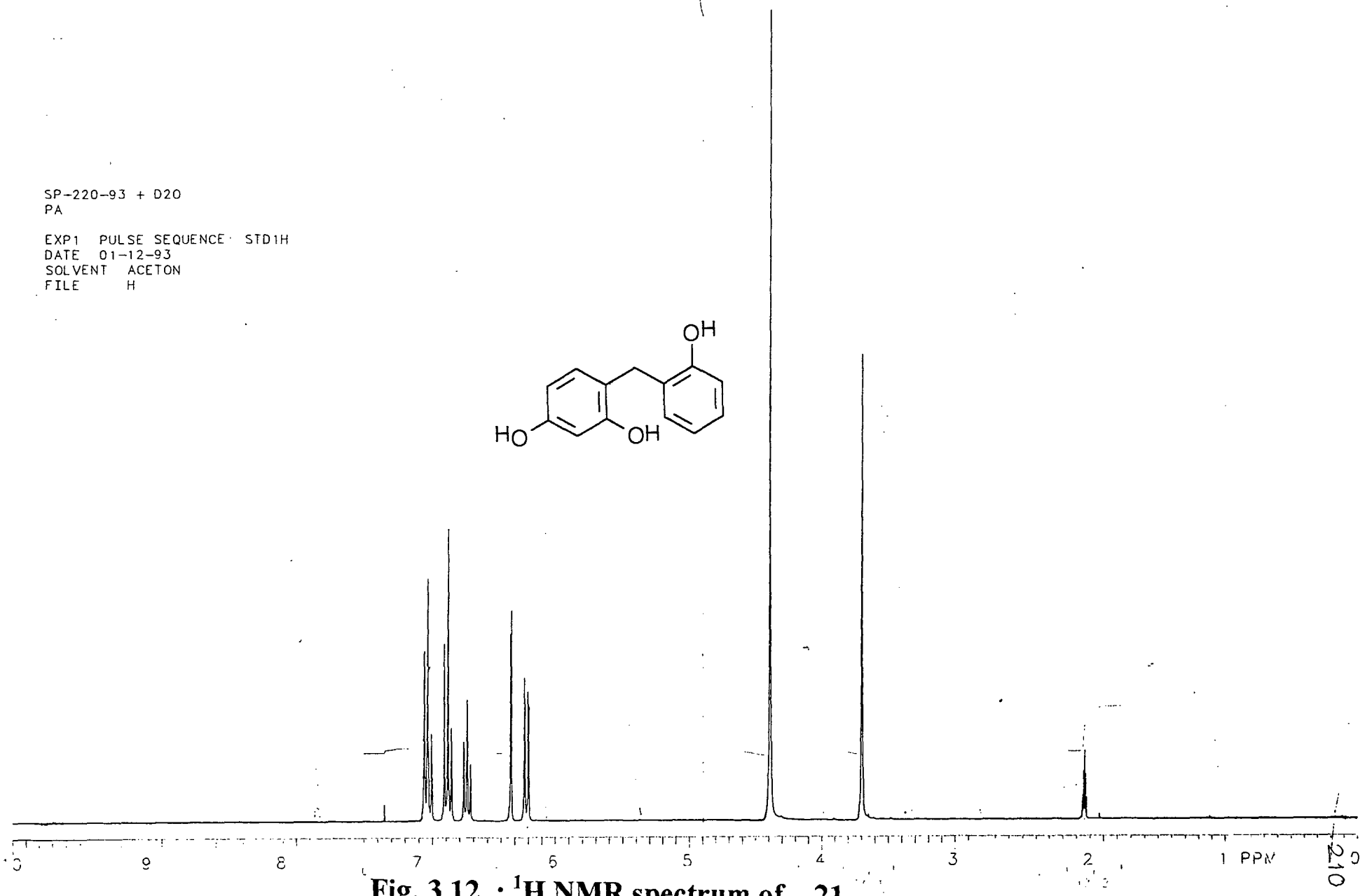


Fig. 3.12 : ^{1}H NMR spectrum of 21

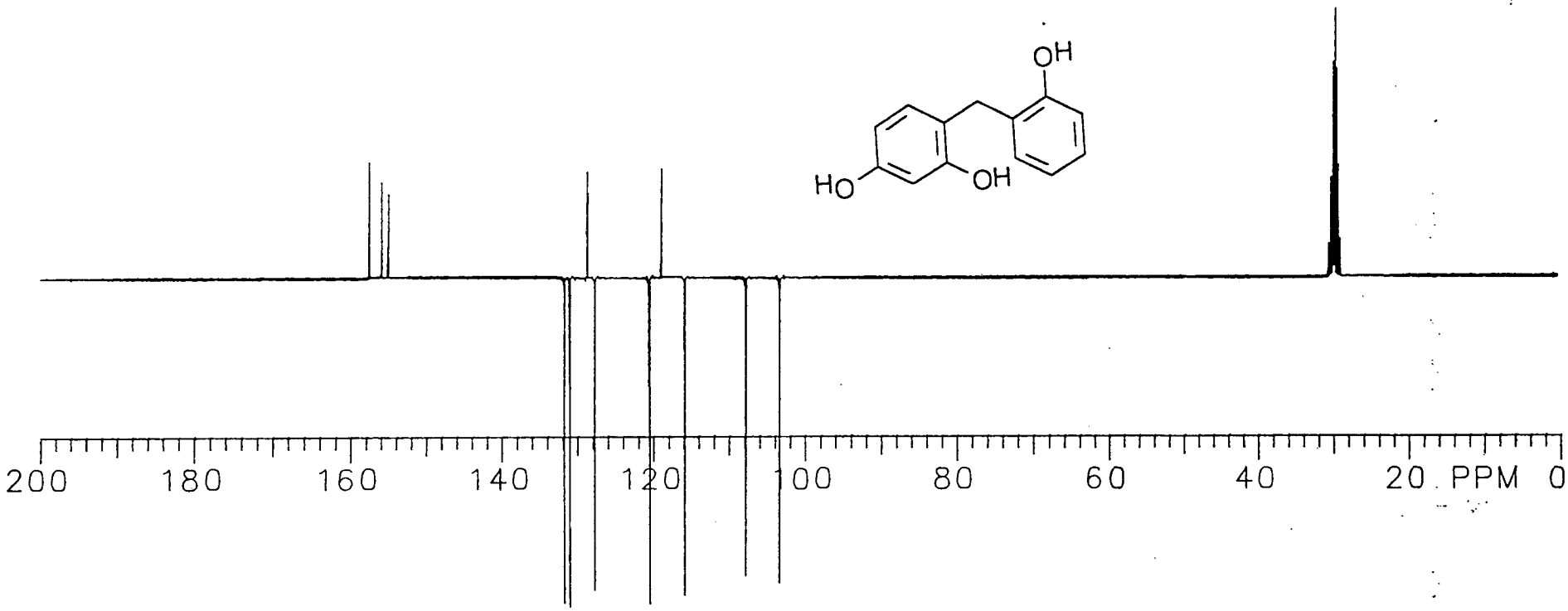


Fig. 3.13 : ^{13}C NMR spectrum of 21

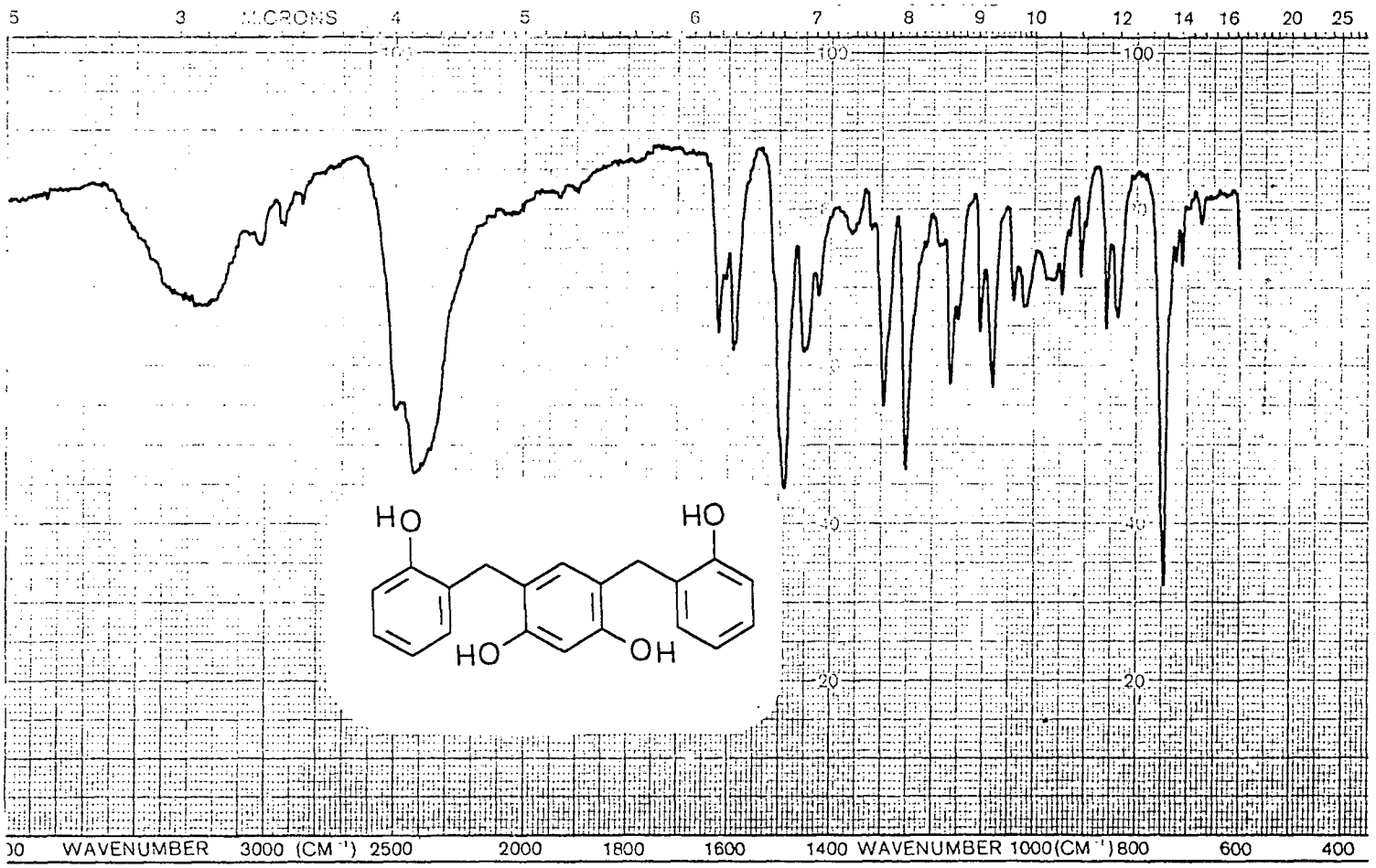


Fig. 3.14 : IR spectrum of 22

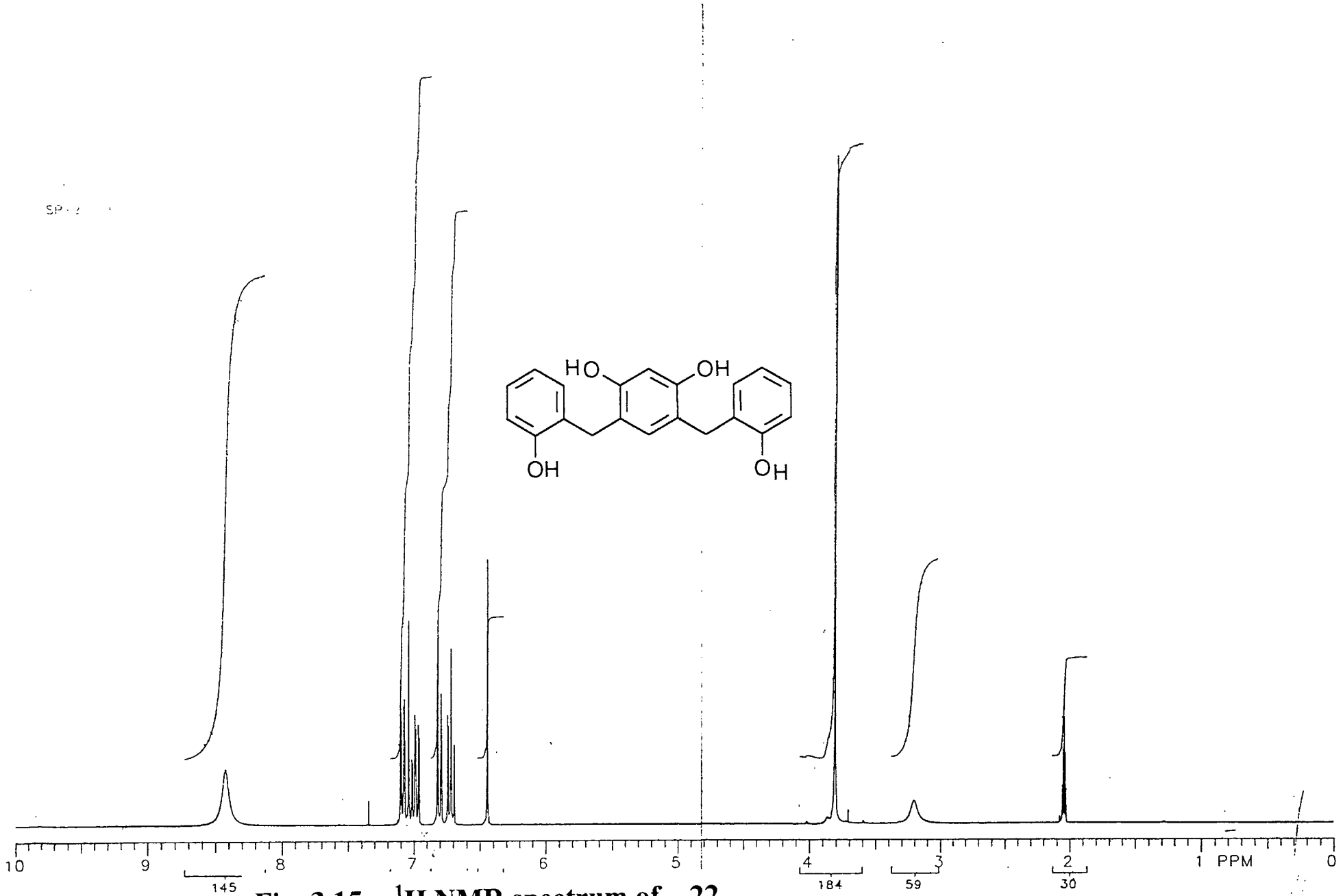


Fig. 3.15 : ^{1}H NMR spectrum of 22

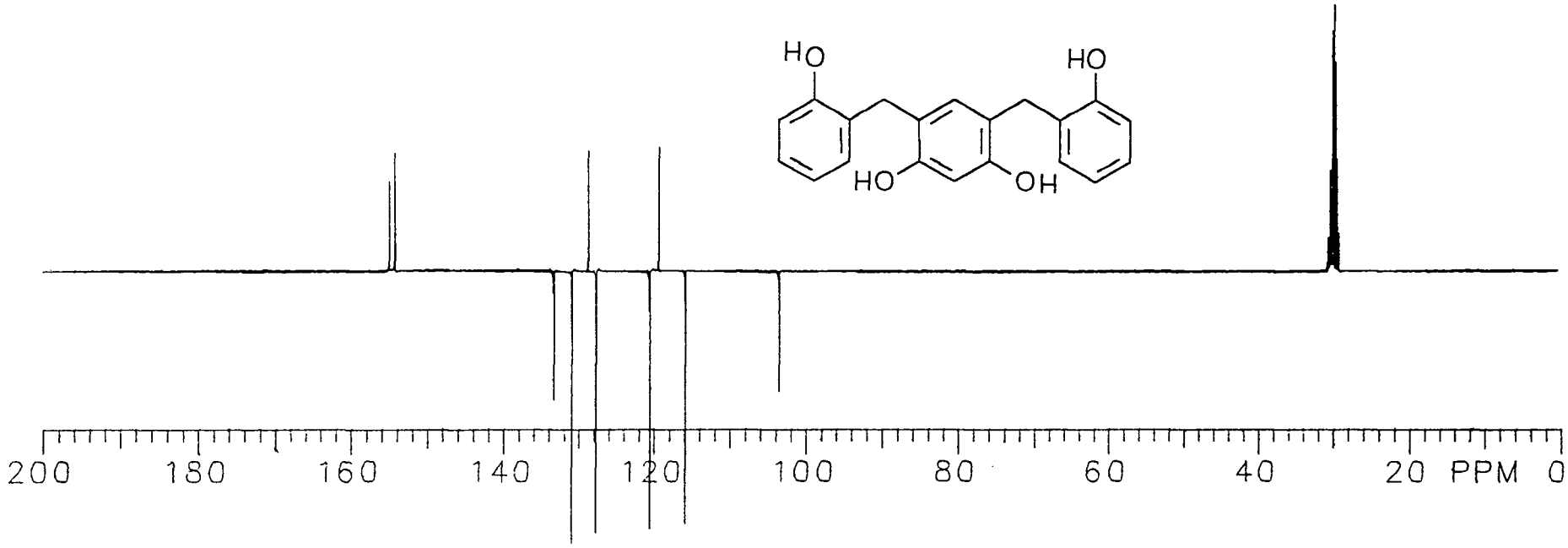


Fig. 3.16 : ^{13}C NMR spectrum of 22

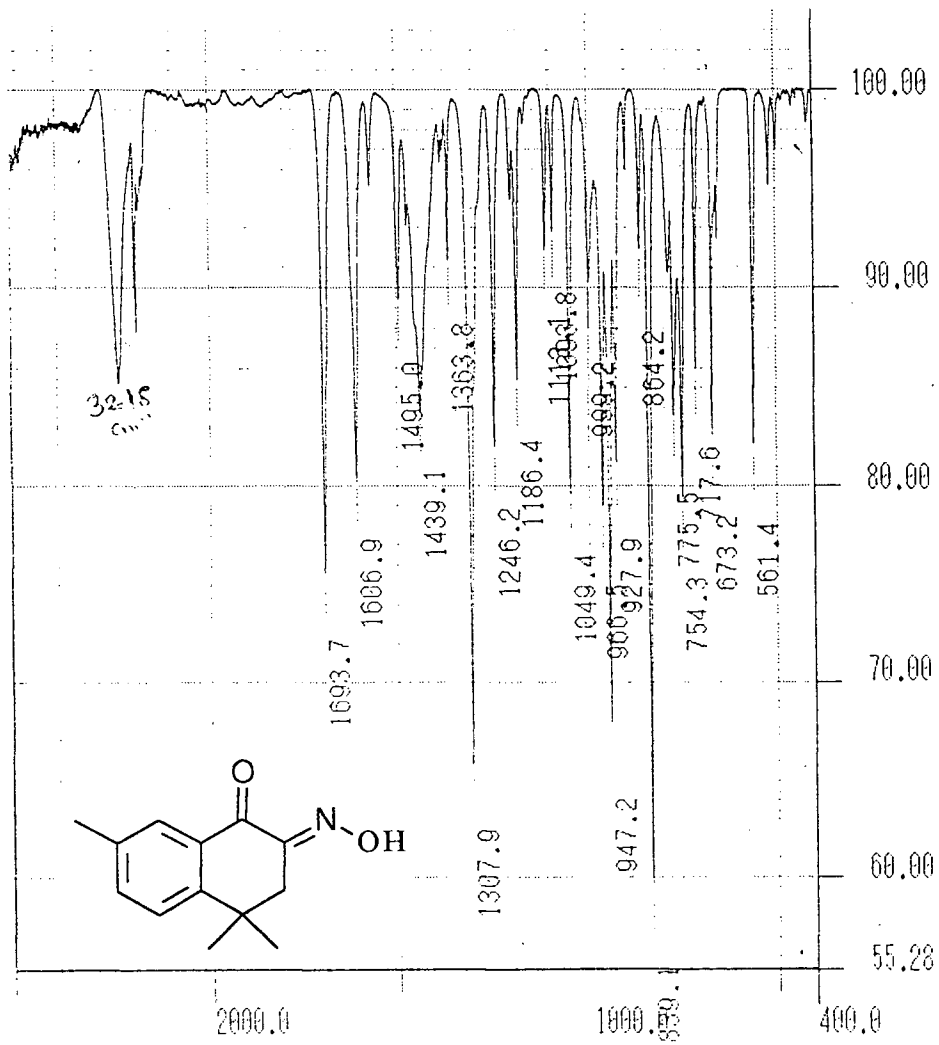


Fig. 3.17 : IR spectrum of 34

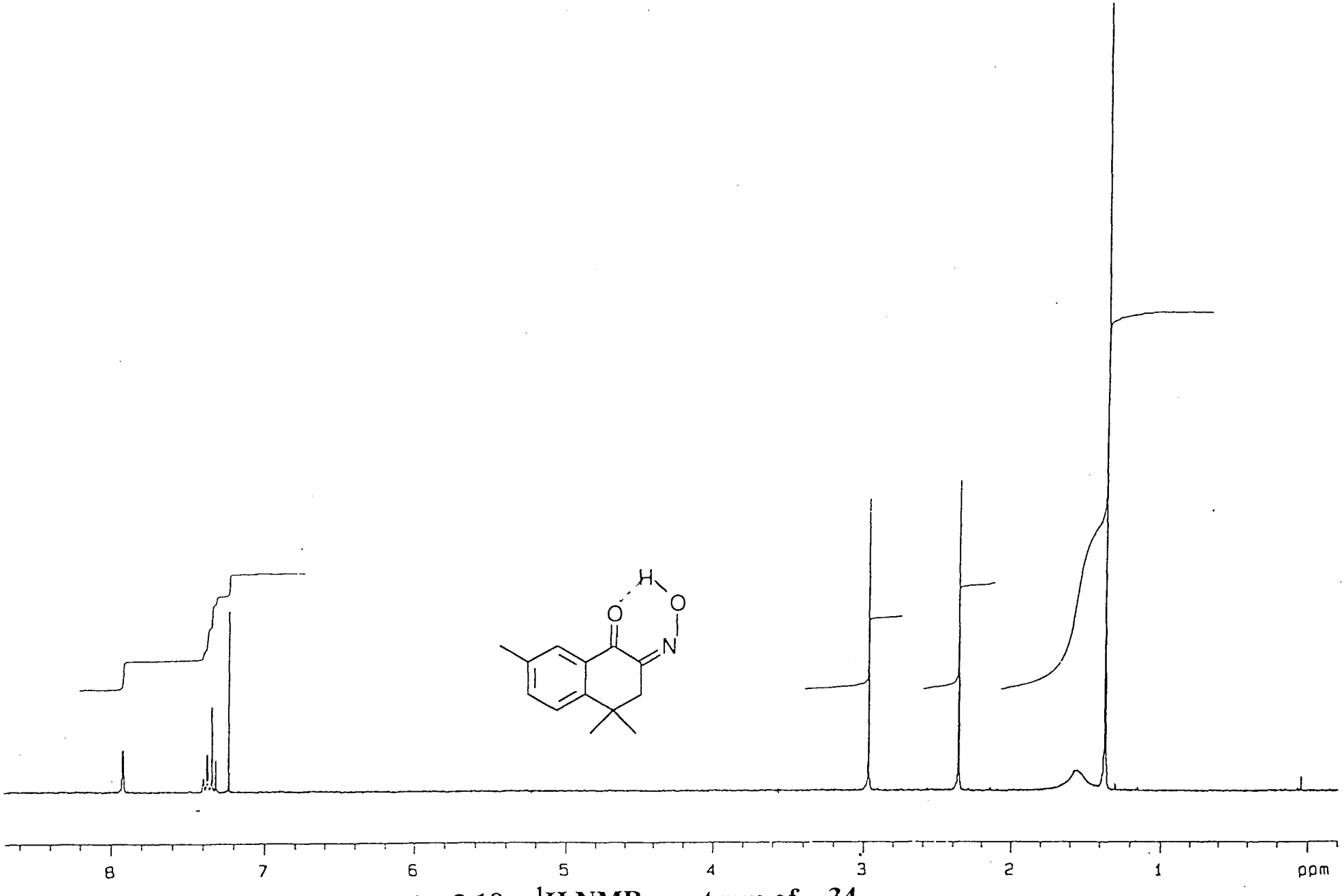


Fig. 3.18 : ^{1}H NMR spectrum of 34

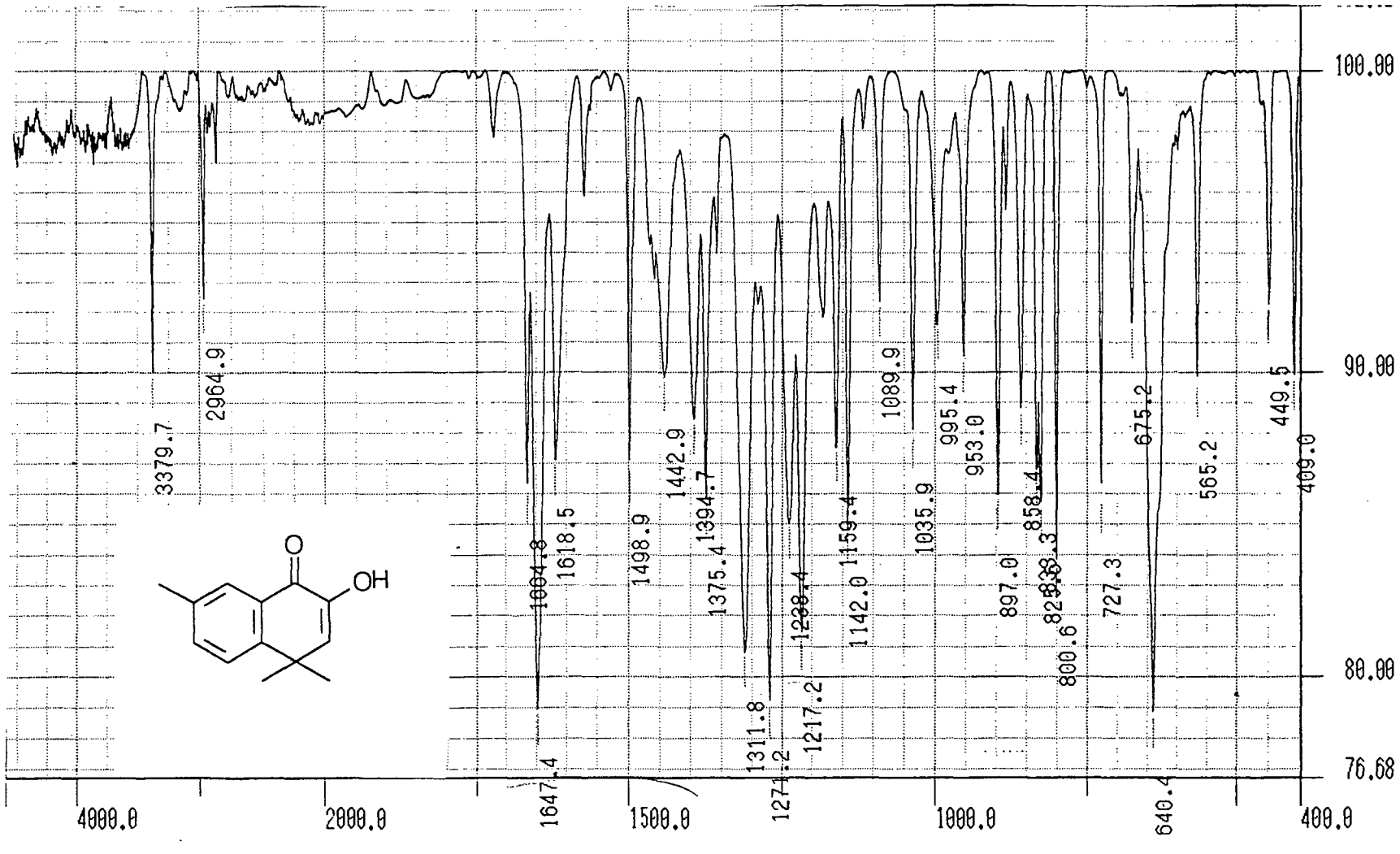


Fig. 3.19 : IR spectrum of 25

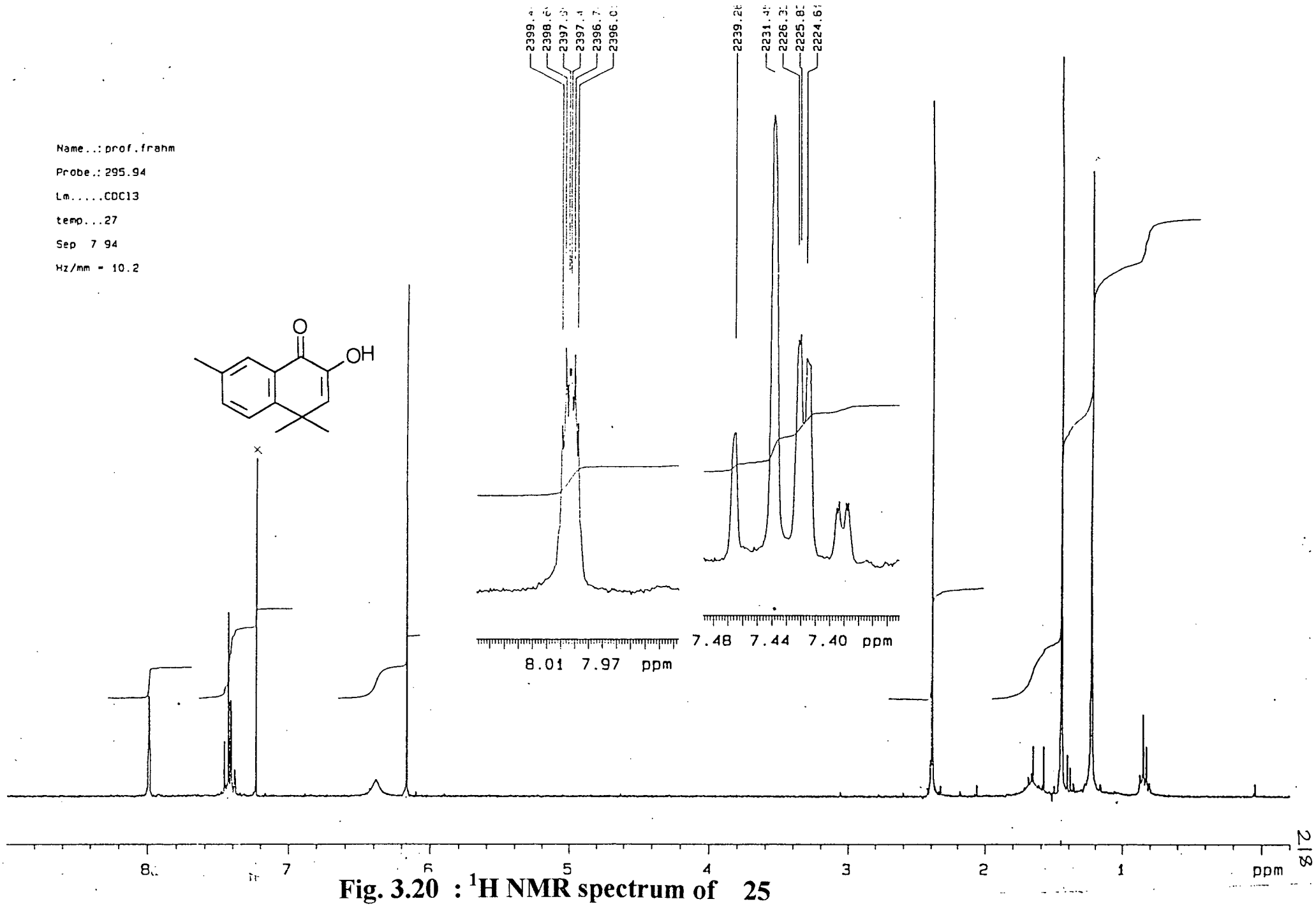


Fig. 3.20 : ^{1}H NMR spectrum of 25

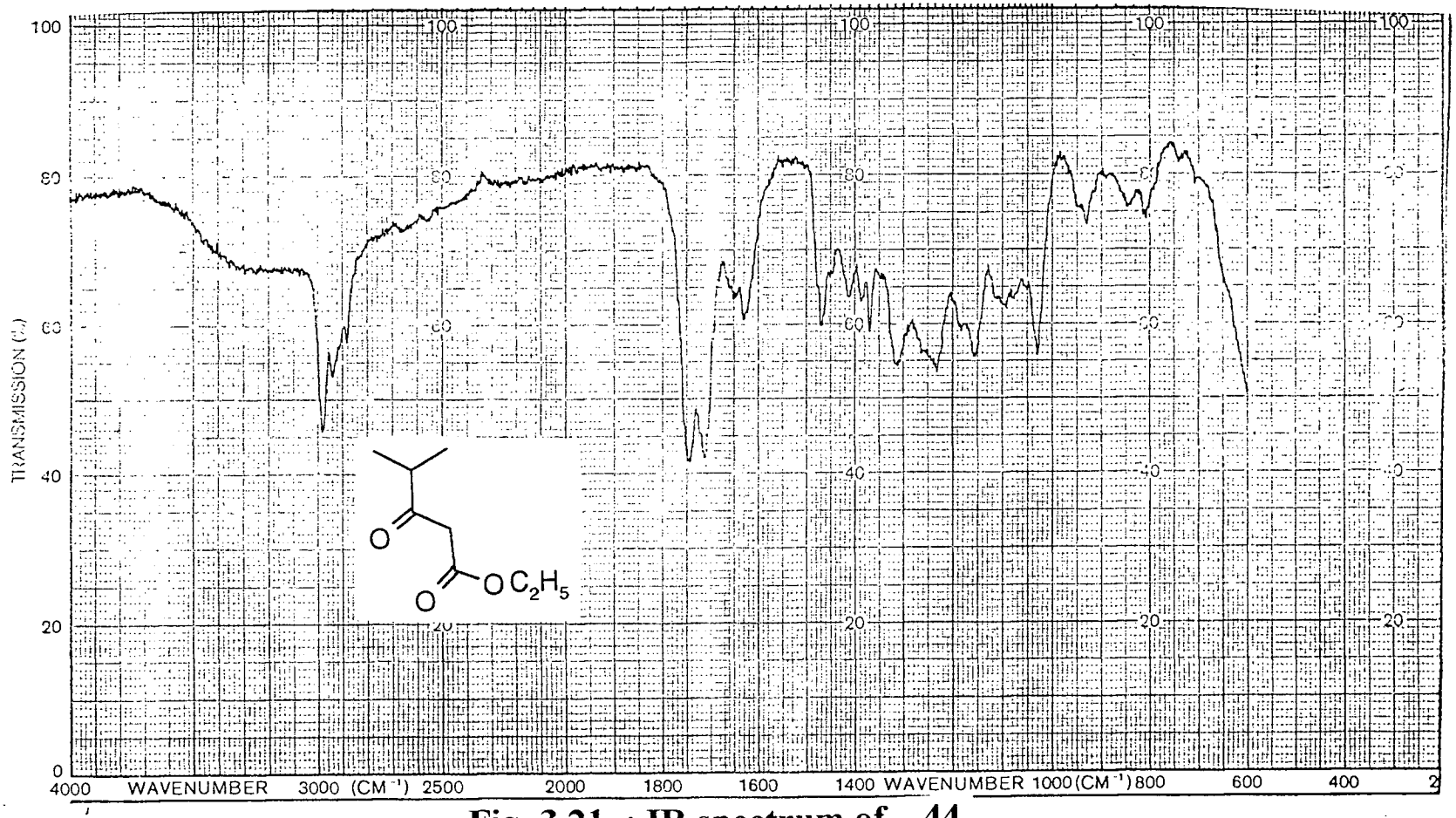


Fig. 3.21 : IR spectrum of 44

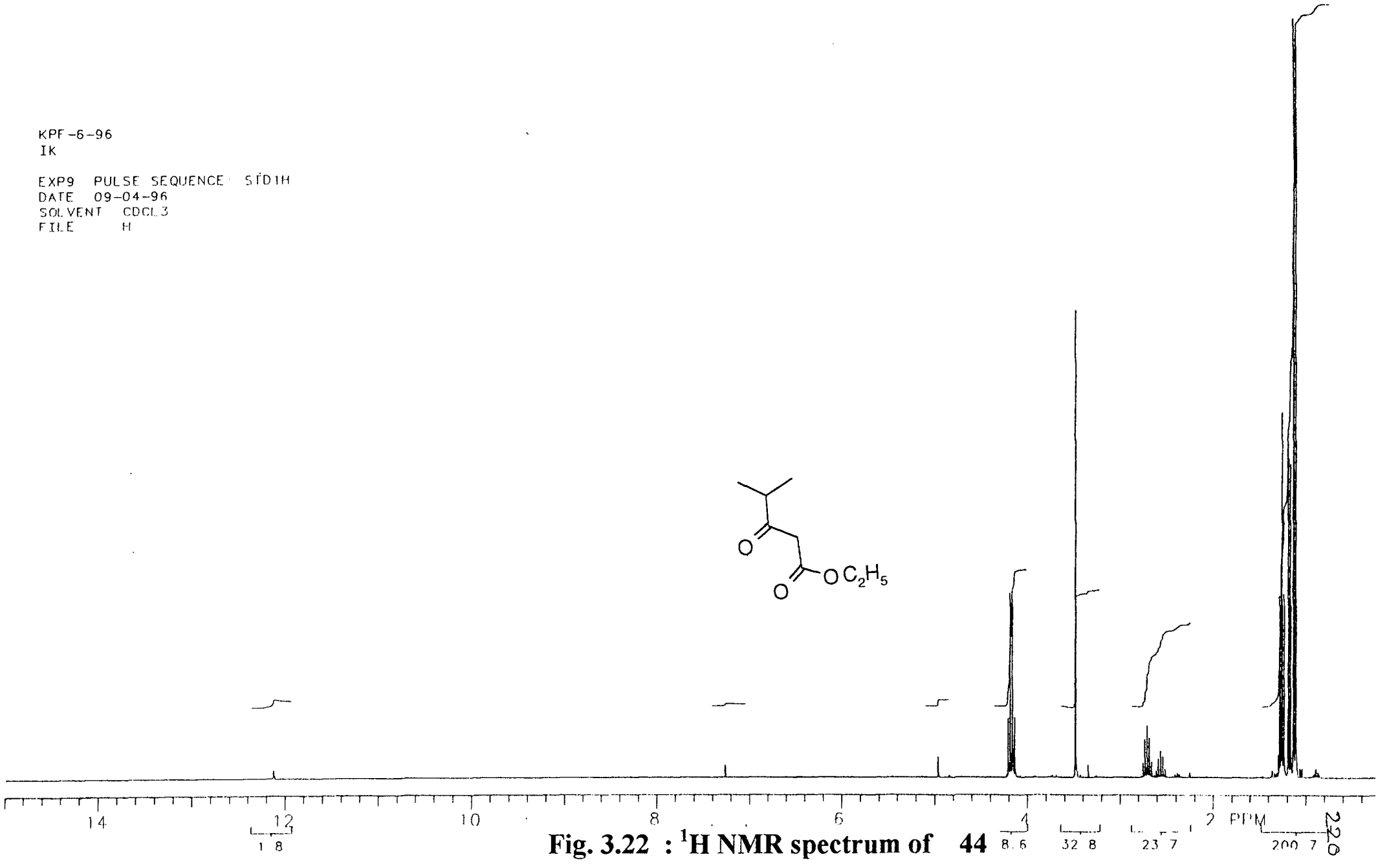


Fig. 3.22 : ^{1}H NMR spectrum of 44

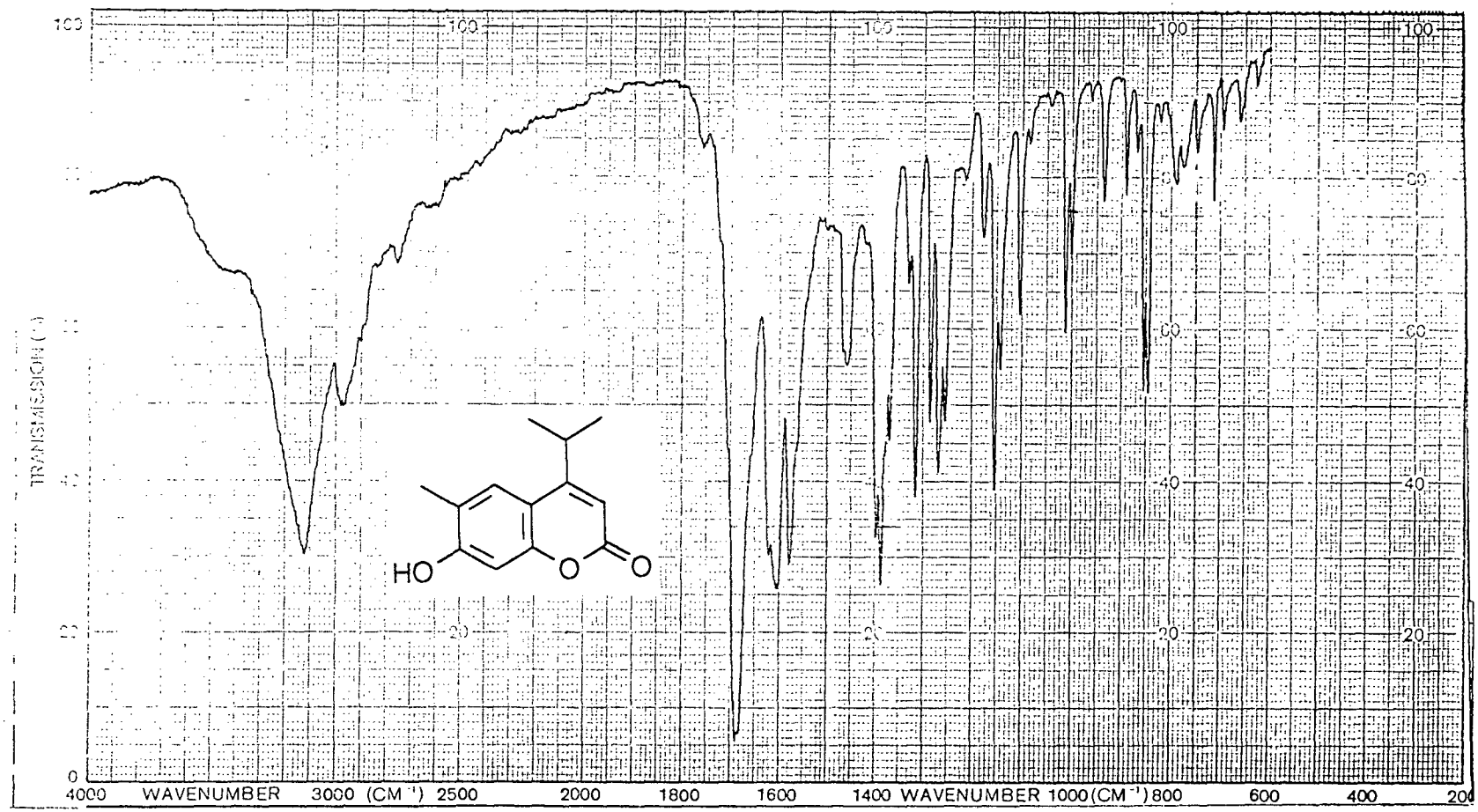


Fig. 3.23 : IR spectrum of 37

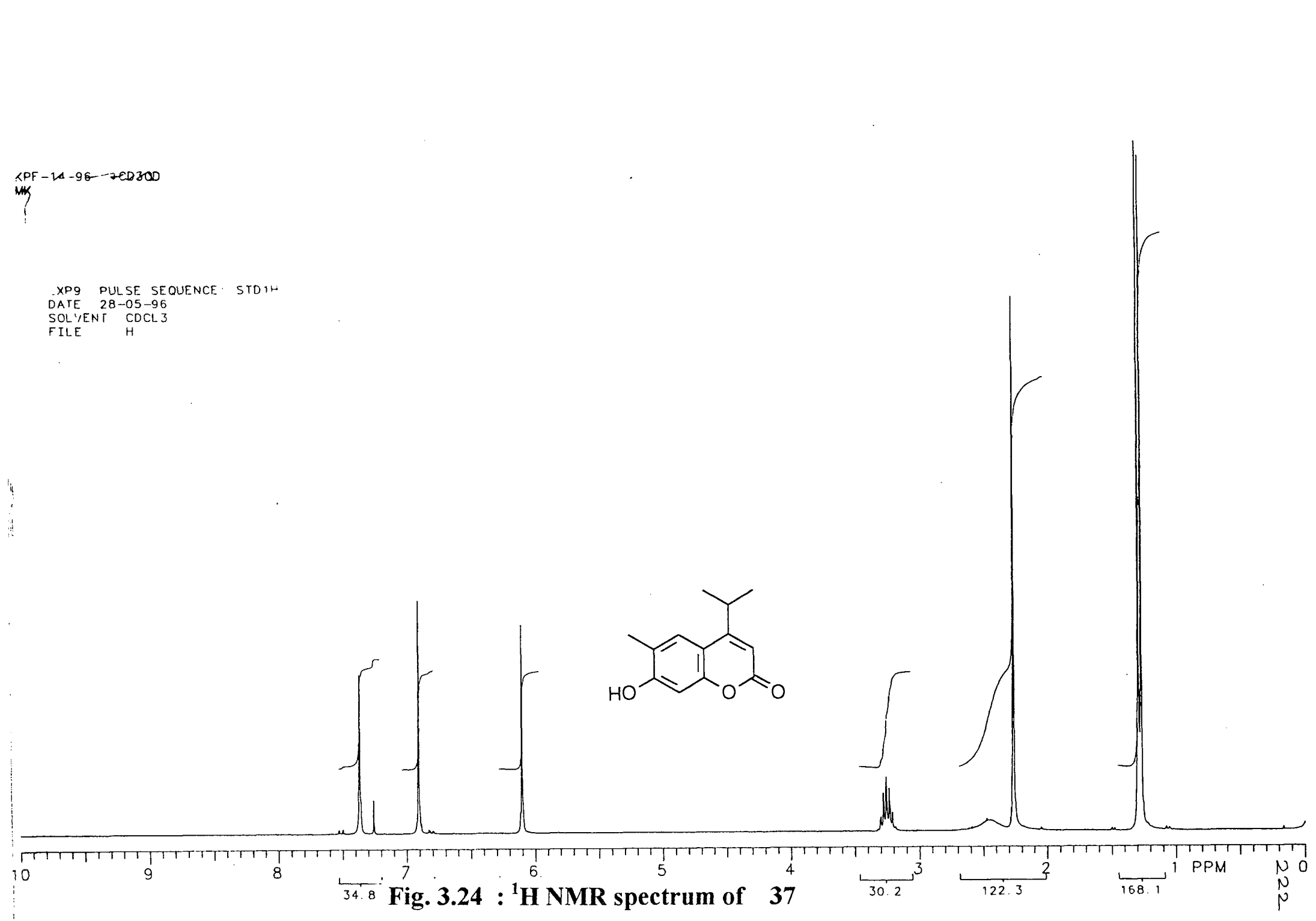


Fig. 3.24 : ^{1}H NMR spectrum of 37

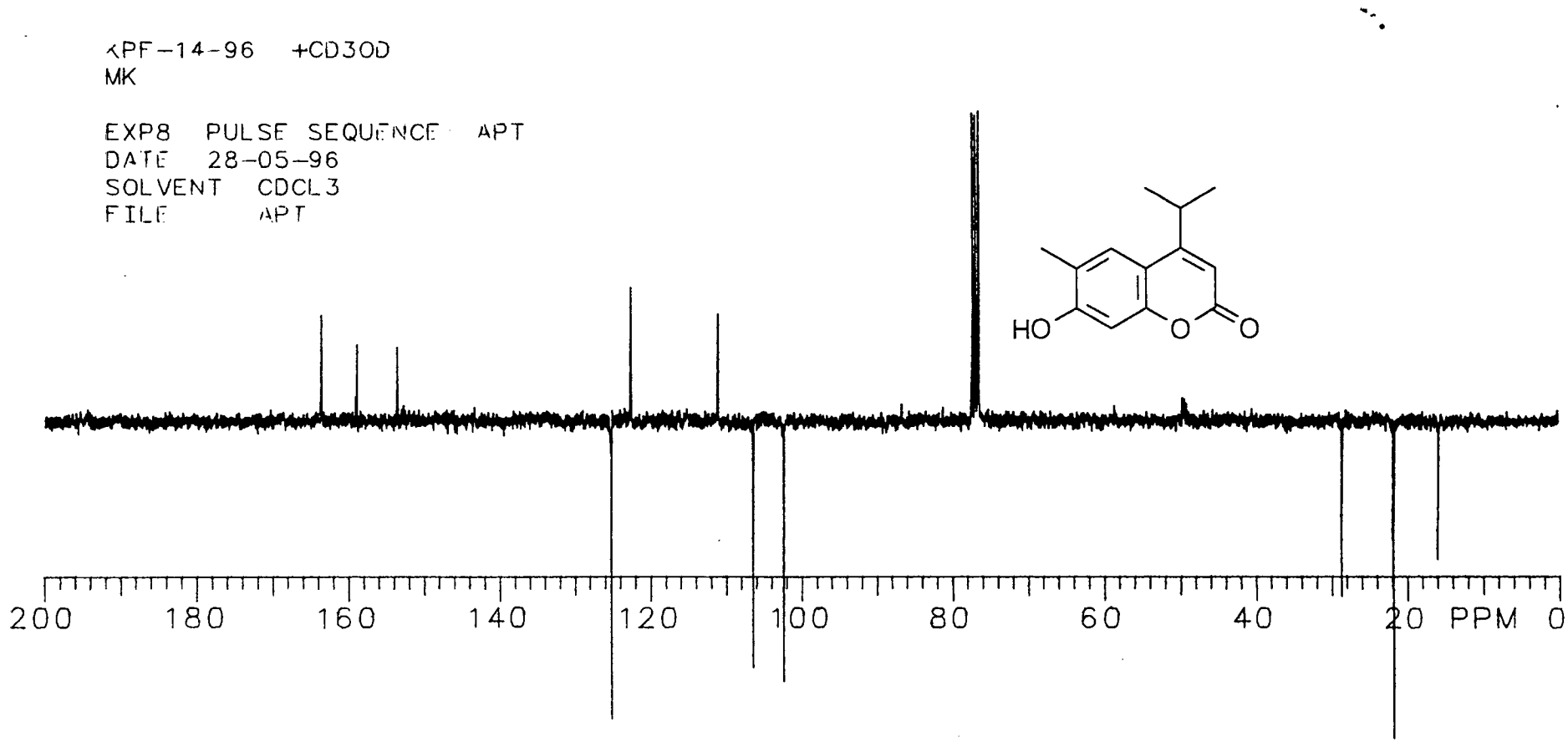


Fig. 3.25 : ^{13}C NMR spectrum of 37

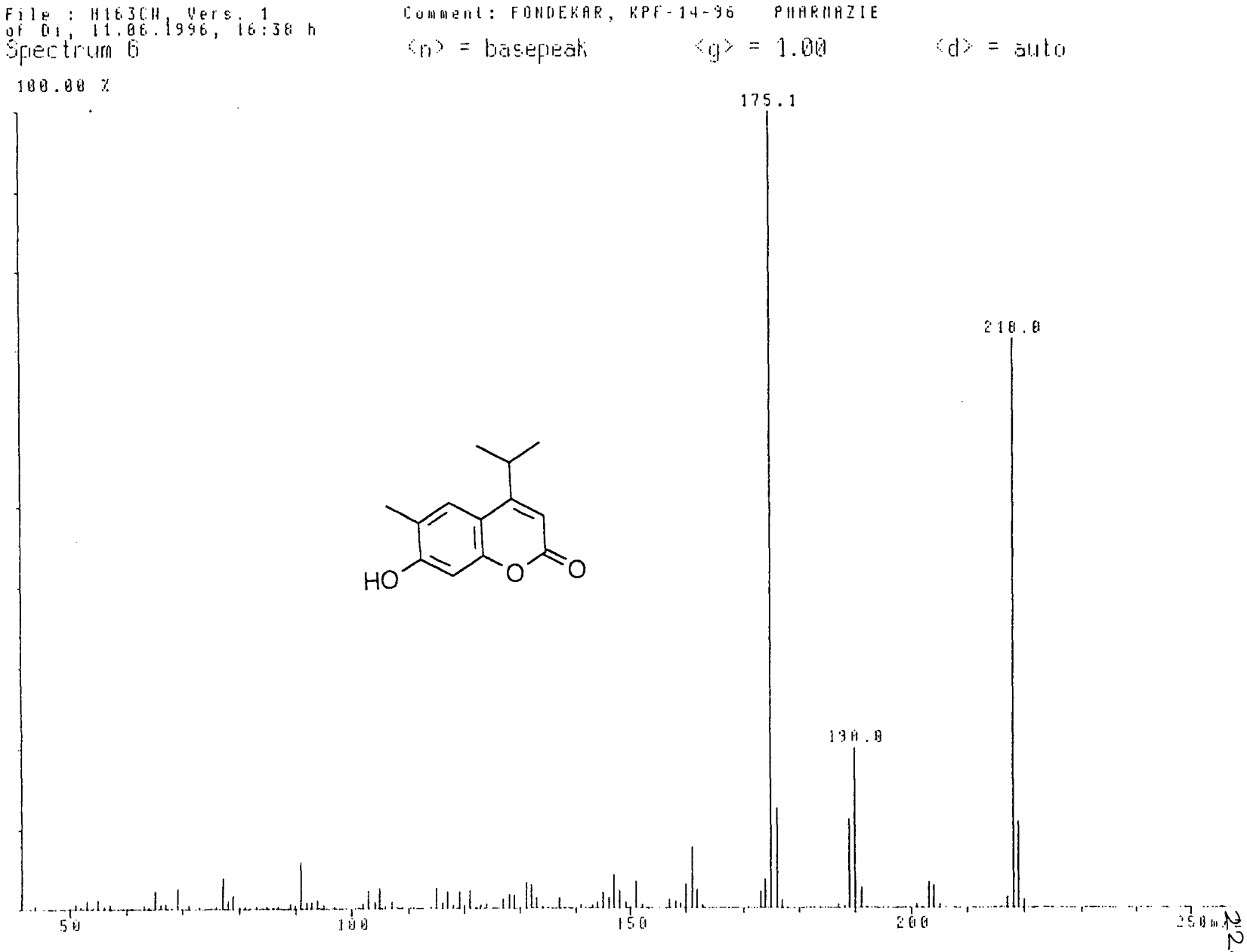


Fig. 3.26 : Mass spectrum of 37

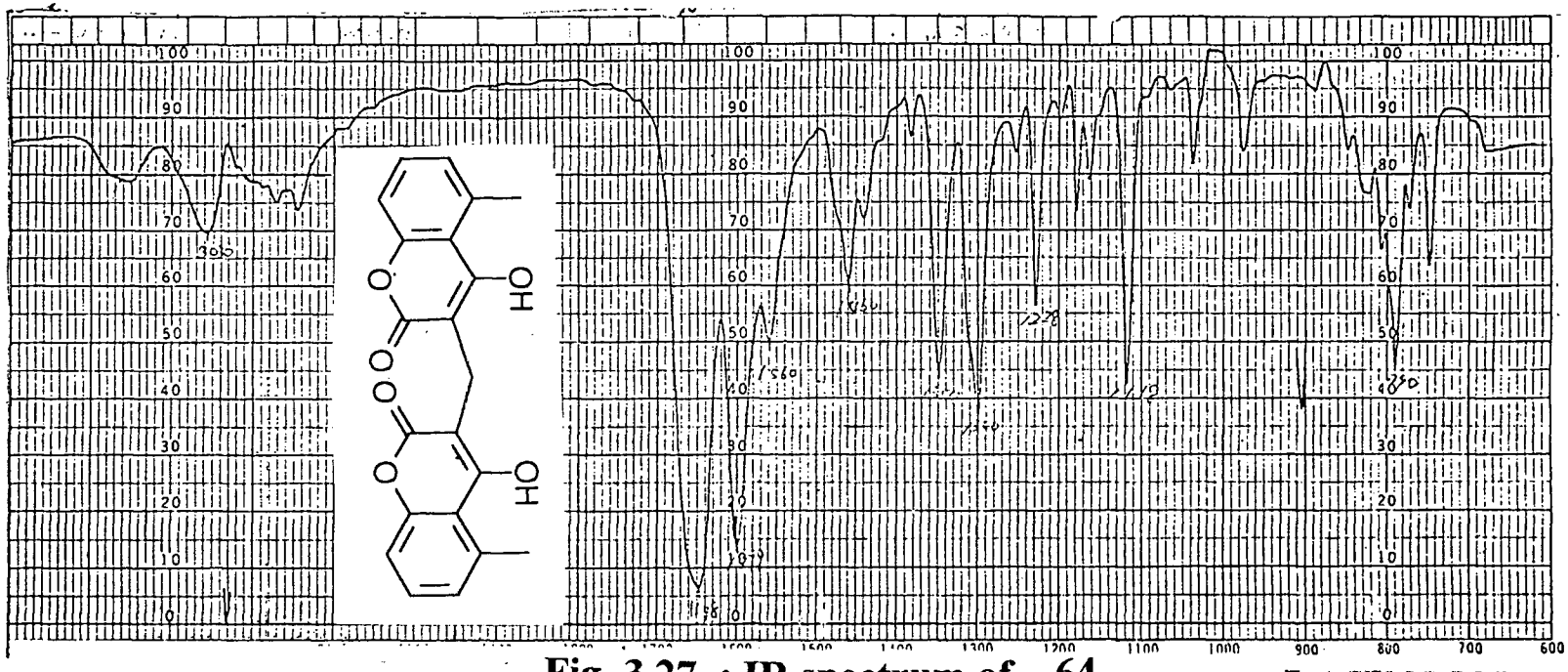


Fig. 3.27 : IR spectrum of 64

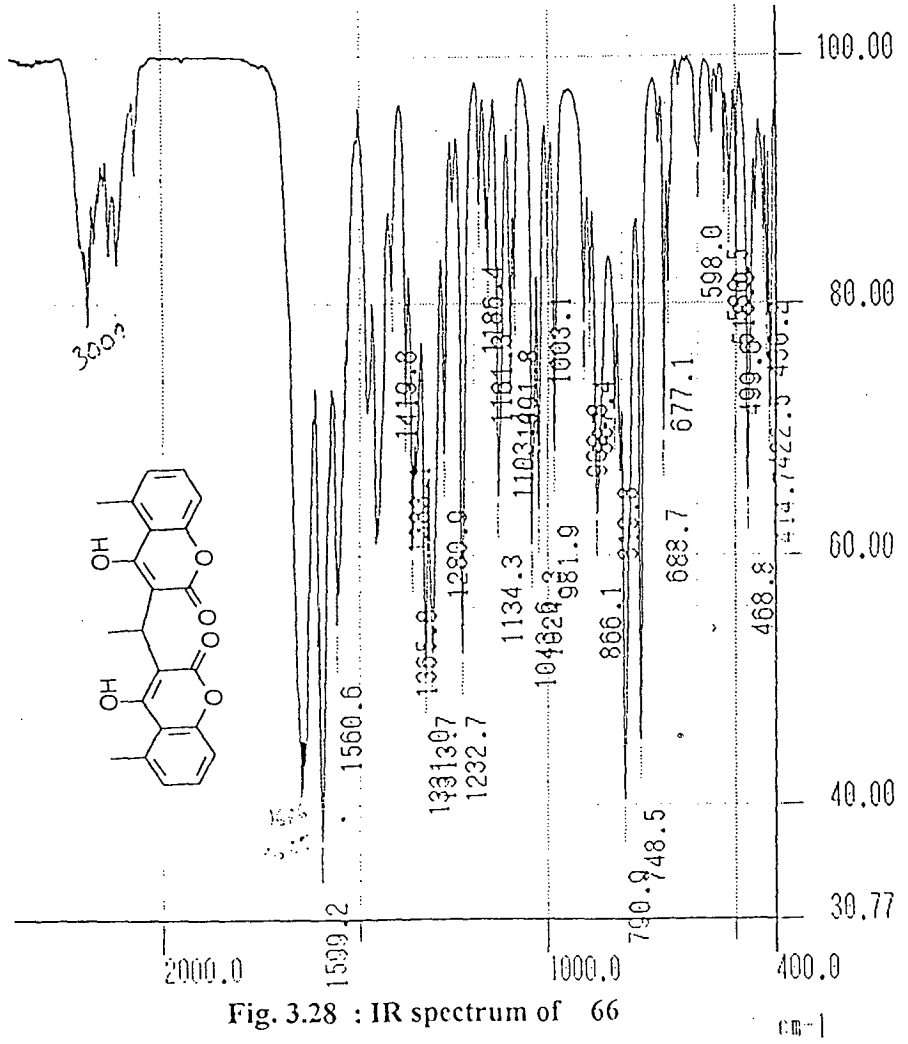


Fig. 3.28 : IR spectrum of 66

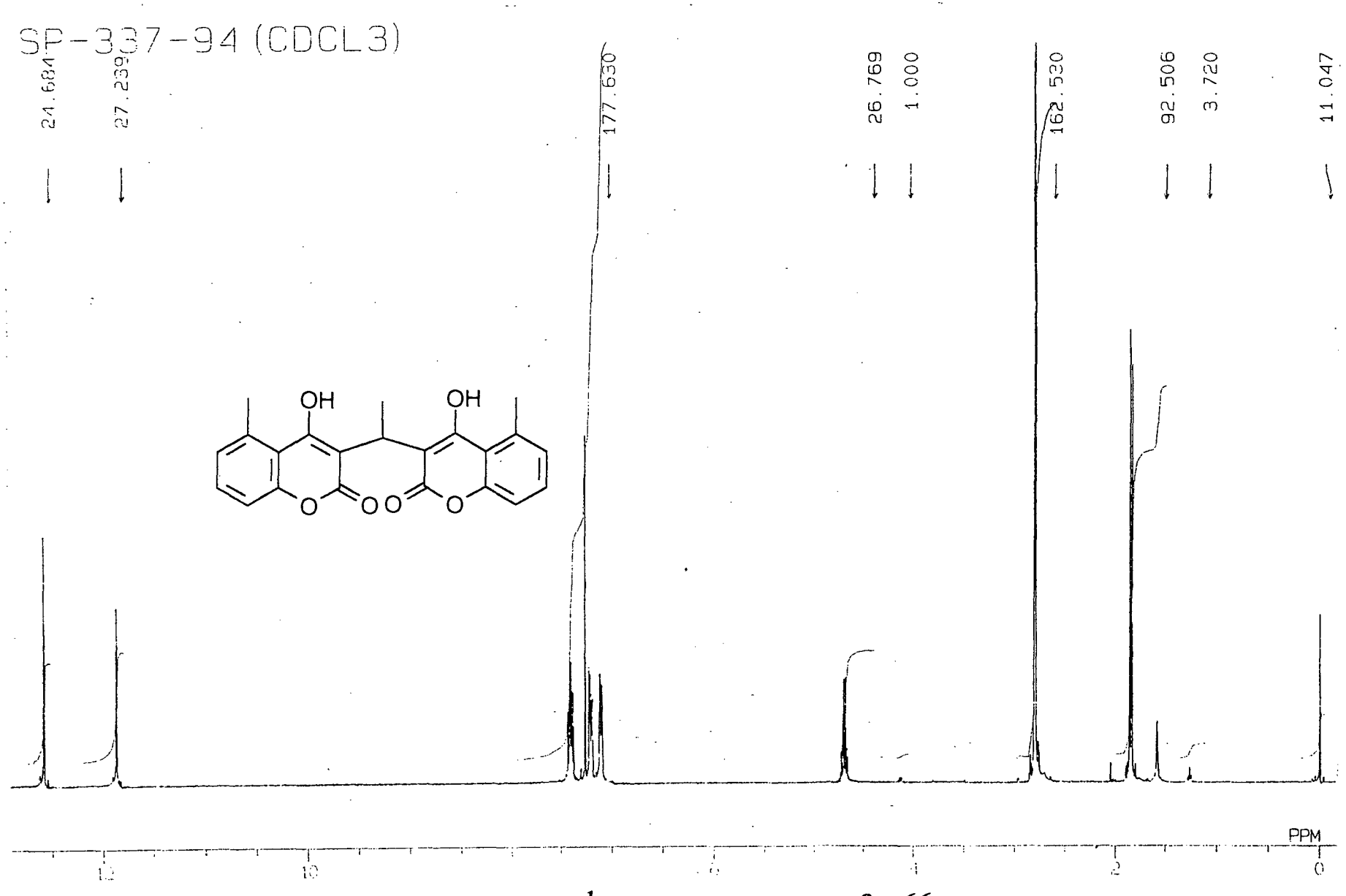


Fig. 3.29 : ^{1}H NMR spectrum of 66

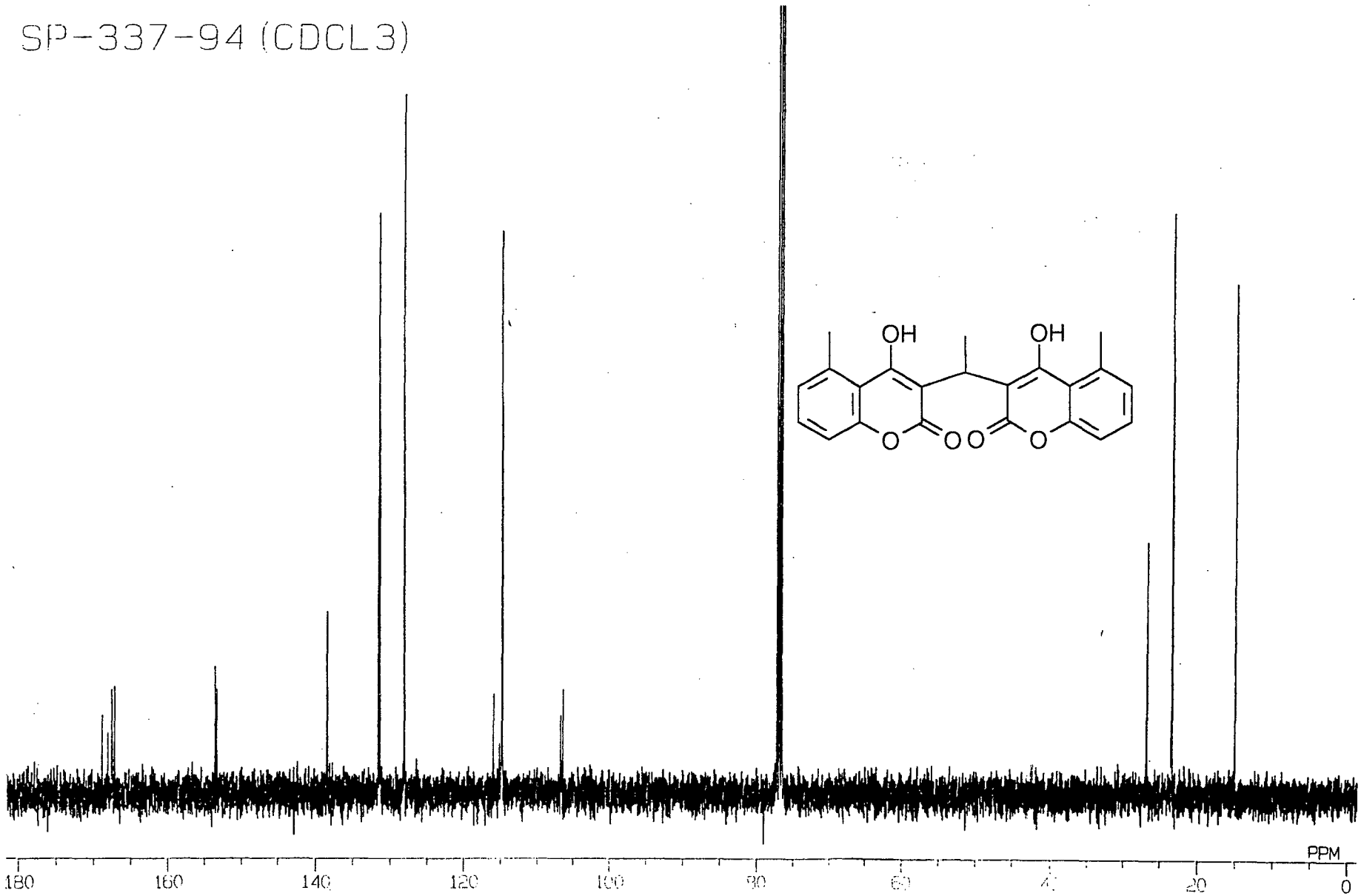


Fig. 3.30 : ^{13}C NMR spectrum of 66

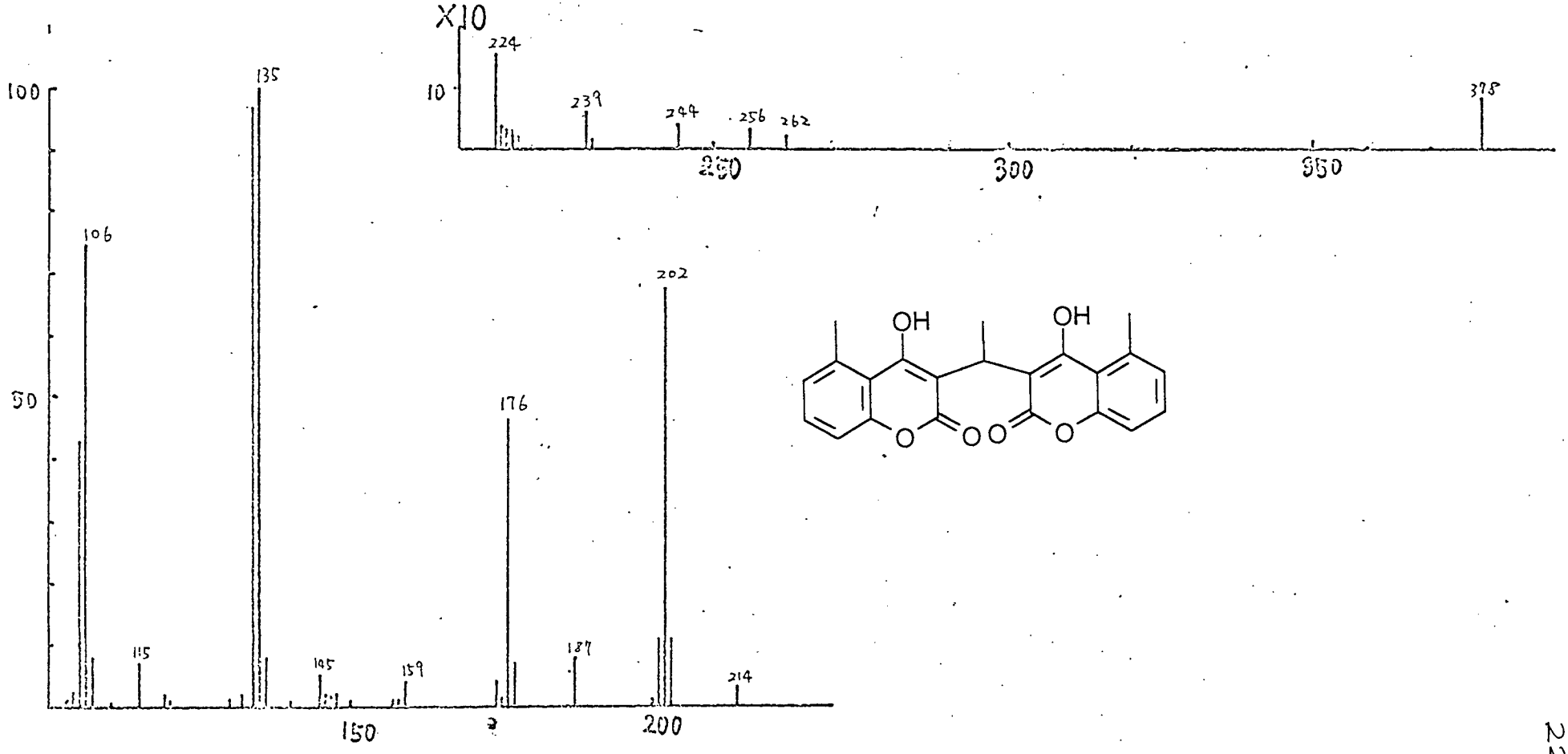


Fig. 3.31 : Mass spectrum of 66

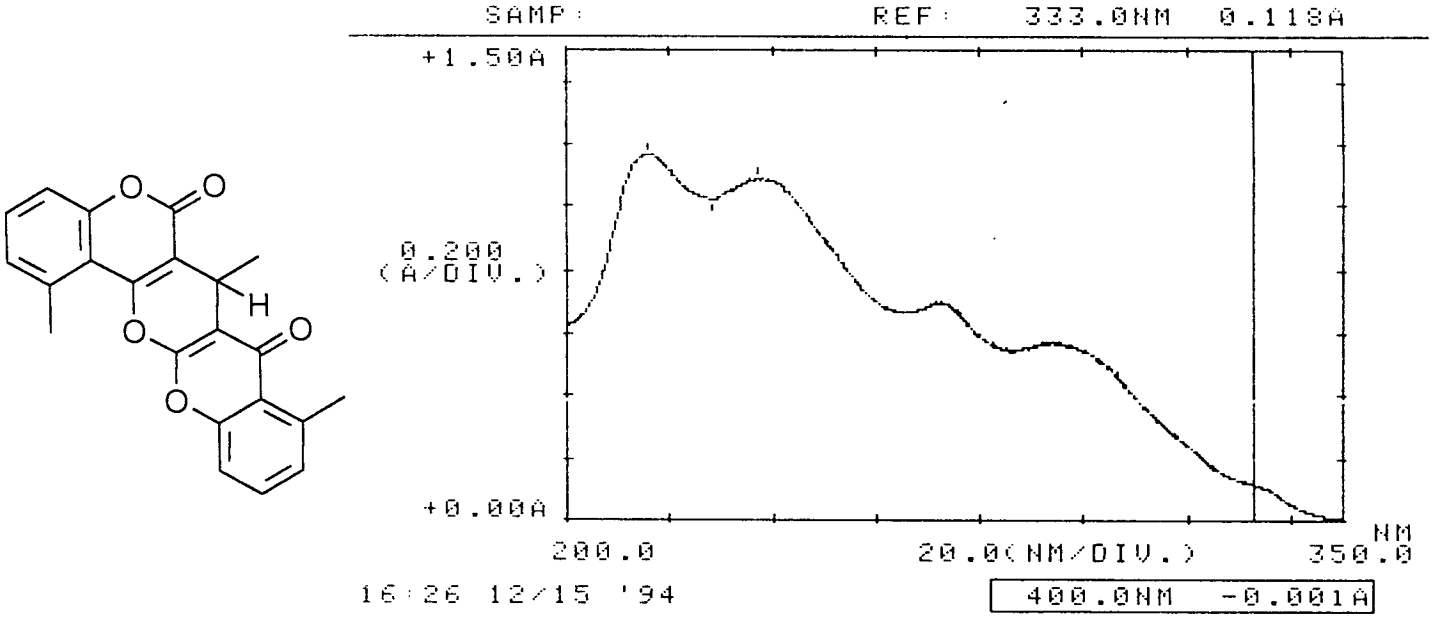


Fig. 3.32 : UV spectrum of 71

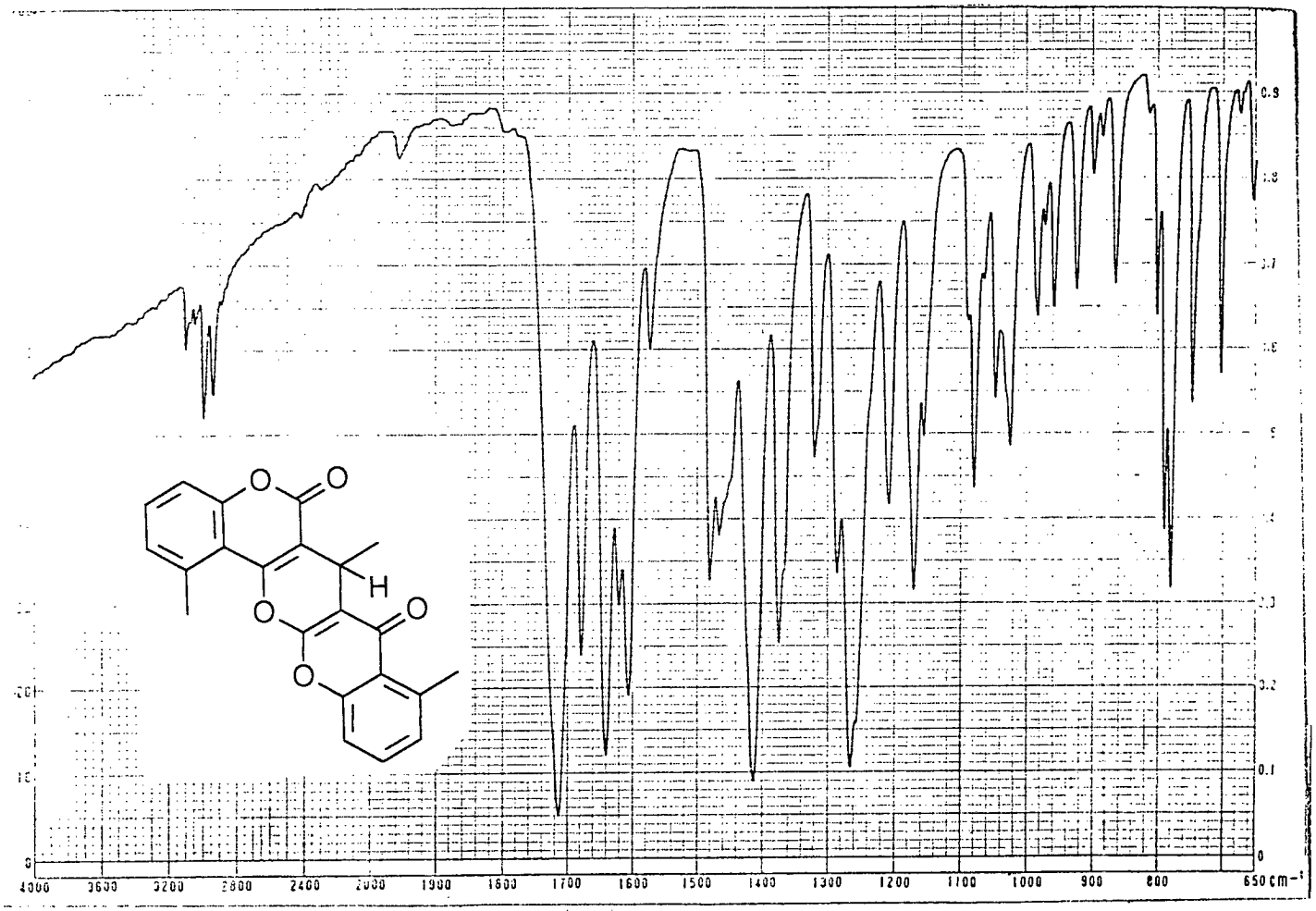


Fig. 3.33 : IR spectrum of 71

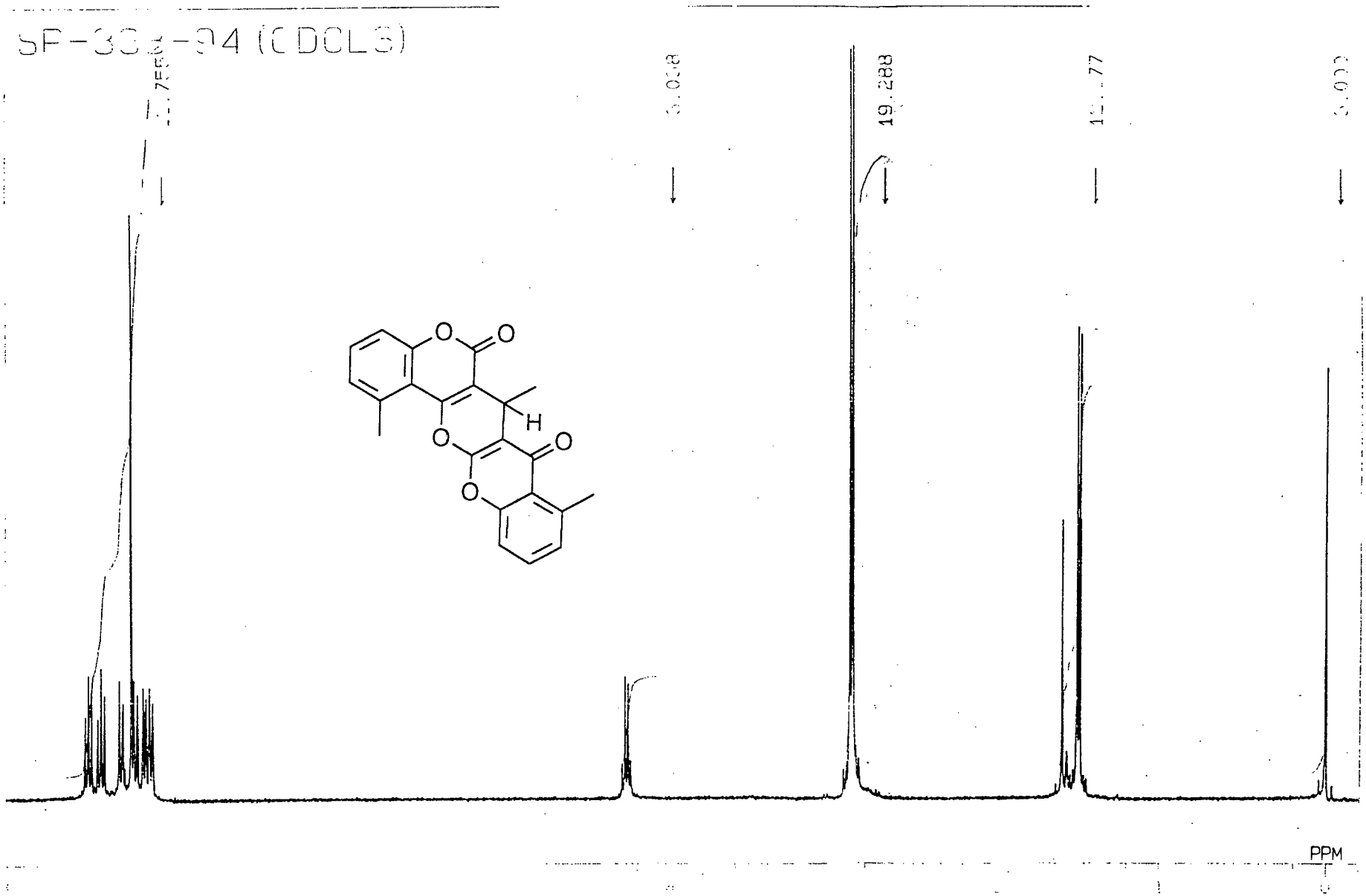


Fig. 3.34 : ^{1}H NMR spectrum of 71

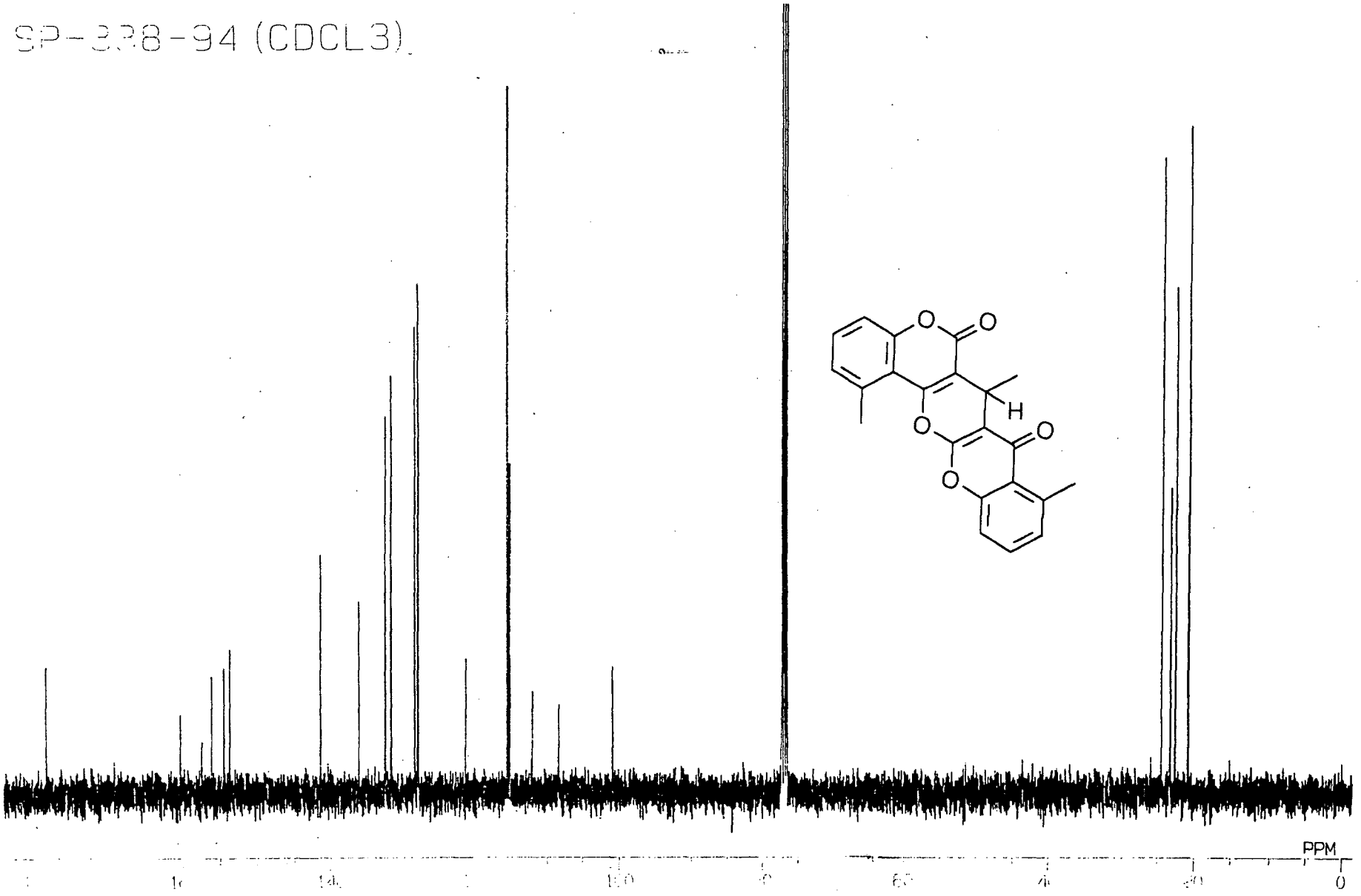


Fig. 3.35 : ^{13}C NMR spectrum of 71

Experimental :

2,2'-Dihydroxy bibenzyl ether **14**

A solution of o-hydroxy benzyl alcohol **4** (0.300 gm, 2.4 mmole) in dry xylene (5 mL) was refluxed for 10 hr. Xylene was distilled off under reduced pressure, residue chromatographed on silica gel and eluted with ethyl acetate-pet.ether (2:8) to afford 2,2'-dihydroxy bibenzyl ether **14** as a white solid. Recrystallised from benzene-pet.ether (0.117gm, 42%) m.p. 122°C .

IR : ν max (KBr) (fig. 3.01) : 3300,1610, 1600, 1590, 1490, 1455,1405, 1350, 1270, 1220, 1185, 1110, 1070, 1040, 1015, 980, 930, 850, 760, 750, 740 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) (fig. 3.02) : 4.77 (4H, s, -CH_2-), 6.89 (2H, ddd, J=7.5 and 1.2 Hz, C_4-H and $C_{4'}$-H), 6.91 (2H, d, J=7.5 Hz, C_2-H and $C_{2'}$-H), 7.13 (2H, dd, J=7.5 and 1.8 Hz, C_3-H and $C_{3'}$-H), 7.23 (2H, ddd, J= 9.0, 7.5 and 1.8 Hz, C_3-H and $C_{3'}$-H) 7.65 (2H, s, exchanges with D_2O, -OH).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 3.03): 155.5 (s, C_1 and $C_{1'}$), 129.9 (d, C_3 and $C_{3'}$), 129.3(d, C_5 and $C5'$),122.1 (s, C_6 and $C_{6'}$), 120.3 (d, C_4 and $C_{4'}$), 116.2 (d, C_2 and $C_{2'}$), 70.6 (t, C_7 and $C_{7'}$).

HRMS (EI) (fig. 3.04) : m/z 230 (M^+), 212, 124, 107 (100%), 106, 77.

2,2'-Dimethoxy bibenzyl ether **18:**

To a stirred solution of 2,2'-Dihydroxy bibenzyl ether **14** (0.200 gm, 0.87 mmole), NaOH (0.04 gms) in water (2 mL), dimethyl sulphate (0.4 mL) was added over period

of 10 min at 0-5°C. After attaining room temp. the reaction mixture was refluxed for 2 hr., then quenched with ice-water and diluted with EtOAc. The organic phase was separated, washed with H_2SO_4 (10%), water and dried over anhydrous sodium sulphate. Removal of solvent yielded yellow oil. Chromatography over silica gel and elution with ethyl acetate-pet. ether (1:9) yielded 2,2'-Dimethoxy bibenzyl ether **18** as white solid (0.055 gms, 23%) m.p. 59°C (lit. oil).

IR : ν max (KBr) (fig. 3.07): 1590, 1490, 1455, 1438, 1407, 1355, 1300, 1285, 1238, 1125, 1085, 1020, 750, 720 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) (fig. 3.08): 3.84 (6H, s, $-OCH_3$), 4.67 (4H, s, $-CH_2-$), 6.88 (2H, dd, J=8.0 and 1.0 Hz, C_2-H and $C_{2'}$-H), 6.98 (2H, ddd, J=7.5, 7.5 and 1.2 Hz, C_4-H and $C_{4'}$-H), 7.28 (2H, ddd, J=8.2,7.5 and 1.8 Hz, C_3-H and $C_{3'}$-H), 7.49 (2H, ddt, J=7.5,1.8 and1.0 Hz, C_5-H and $C_{5'}$-H)

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 3.09) : 156.9 (s, C_1 and $C_{1'}$), 128.7 (d, C_3 and $C_{3'}$), 128.3 (d, C_5 and $C_{5'}$), 127.0 (s, C_6 and $C_{6'}$), 120.3 (d, C_4 and $C_{4'}$), 110 (d, C_2 and $C_{2'}$), 67.3 (t, C_7 and $C_{7'}$), 55.3 (q, OCH_3).

HRMS (EI) (fig. 3.10): m/z 258 (M^+), 150, 137, 122, 91(100%), 77, 69.

2,2'-Diacetyl bibenzyl ether **17 :**

Acetic anhydride (2 mL) was added to a solution of 2,2'-dihydroxy bibenzyl ether **2** (0.100 gm, 6.43 mmole) in dry pyridine (2 mL). After stirring for 16 hr at room temp.,the reaction mixture was diluted with CH_2Cl_2,washed with HCl (10%), and dried over anhydrous sodium sulphate. Concentration followed by column

chromatography using EtOAc- pet.ether (1:1) yielded 2,2'-diacetyl bibenzyl ether **4** (0.063 gm, 52%) as colourless oil.

IR : ν max (film):1756, 1610, 1586, 1489, 1453, 1369, 1206, 1112, 1079, 1011, 754 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,60 MHz) : 2.19 (6H, s, $OCOCH_3$), 4.44 (4H, s, $-CH_2-$), 7.0-7.6 (8H,m, Ar-H).

Reaction of resorcinol **20** with saligenin **4** :

An equimolar mixture saligenin **4** (0.44 gms, 4.0 mmole) and resorcinol **20** (0.496 gms, 4.0 mmole) in dry xylene (5 mL) was refluxed for 14 hrs. At the end of this period the TLC pattern remained unchanged. Solvent was removed under reduced pressure, residue chromatographed over silica gel and eluted with EtOAc- pet.ether (2:8) to afforded 2,2',4'-trihydroxy diphenyl methane **21** as a white solid which was recrystallised from EtOAc, m.p. 202°C (84 mgs,33 %).

IR : ν_{max} (KBr) (fig. 3.11): 3220, 2420, 1605, 1500, 1300,1245,1070,1015, 745 cm^{-1}.

^{1}H NMR (δ ppm, d_6-acetone,300 MHz) (fig. 3.12): 3.82 (2H, s, Ar-CH_2-Ar), 6.30 (1H, dd, J=8.0,2.8 Hz, C_6-H), 6.40 (1H, d, J=2.0 Hz, $C_{3'}$-H), 6.74 (1H, ddd, J=8.0, 8.0, 2.5 Hz, C_4-H), 6.83 (1H, dd, J=8.0, 2.0 Hz, C_3-H), 6.96 (1H, d, J=8.0 Hz, $C_{5'}$-H), 7.01 (1H, ddd, J=8.0, 8.0, 2.0 Hz, C_5-H), 8.0-8.4 (3H, br.d, exchanges with D_2O, -OH).

^{13}C NMR (δ ppm, d_6-acetone, 75 MHz, APT) (fig. 3.13): 158 (s, C_2), 156 (s, $C_{2'}$), 155 (s, $C_{4'}$), 132 (d, $C_{6'}$), 131 (d, C_4), 129 (s, C_1), 128 (d, C_6), 120.5 (d, C_5), 119.5 (s,

$C_{1'}$), 116 (d, C_3), 108 (d, $C_{5'}$), 103.5 (d, $C_{3'}$), 30.0 (t, C_7).

Further elution with the same solvent afforded 3,5-di (o-hydroxy benzyl) resorcinol **22** as a white solid recrystallised from benzene m.p.193°C (81 mgs,11 %).

IR : ν_{max} (KBr) (fig. 3.14): 3200, 2420, 1595, 1490, 1295, 1250, 740 cm^{-1}.

^{1}H NMR (δ ppm, d_6-acetone,300 MHz) (fig. 3.15): 3.8 (4H, s), 6.44 (1H, s), 6.72 (1H, ddd, J=8.0, 8.0, 1.5 Hz), 6.80 (1H, dd, J=8.0, 1.5 Hz), 6.98 (1H, ddd, J=8.0, 8.0, 2.0 Hz), 7.04 (1H, s), 7.09 (1H, dd, J=8.0, 2.0 Hz), 8.4 (4H, br.s, exchanges with D_2O).

^{13}C NMR (δ ppm, d_6-acetone, 75 MHz, APT) (fig. 3.16): 153 (s), 152 (s), 133 (d), 131 (d), 129 (s), 128 (d), 120.5 (d), 119 (s), 118 (d), 103 (d), 30 (t).

Cyclodehydration of β-ionone : Preparation of a mixture of **31** and **32**

β-*I*onone **30**(19.2 gms, 0.1 mole) was distilled over catalytic amount of iodine using magnetically stirred oil bath. To the distillate, powdered sodium thiosulphate was added, filtered through silica gel column and eluted with pet.ether to yield hydrocarbon fraction (15.8 gms, 91%) which was further purified by distilling over metallic sodium to give mixture of **31**(77%) and **32**(17%) by GLC.

Catalytic hydrogenation of **31** and **32** :

Hydrocarbon fraction (2.4 gms) was hydrogenated over 10% Pd-C (0.200 gms) in ethanol (15 mL) for 3 hr. The reaction product was filtered, residue washed with ethanol, concentrated and purified by column chromatography using pet.ether as eluent to yield **31**(89% purity by GLC)

IR : ν_{max} (film) : 2970,1510,1460, 830 cm^{-1}.

4,4,7-Trimethyl - 1- tetralone **33:**

To a stirred solution of CAN (28.6 gms, 51.6 mmole) in (1:1:1) mixture of glacial acetic acid, ether and water (525 mL) was added ionene **31**(1.5 gms, 8.6 mmole).After heating at 35°C on a water bath for 30 min., reaction mixture was allowed to cool and extracted with ether (3 x 100 mL). The combined organic extracts were washed with sat. $NaHCO_3$ soluton, water and dried over anhydrous sodium sulphate. Evoporation of the solvent yielded yellow oil which was purified by column chromatography using benzene - pet.ether (3:7) to give 4,4,7-trimethyl - 1- tetralone **33**(0.575 gms, 36%) as colourless oil.

IR : ν_{max} (film) : 2970, 1700, 1620, 1500, 1290, 1190, 820 cm^{-1}.

^{1}H NMR (δ ppm,$CDCl_3$,300 MHz) : 1.44 (3H, s, C_4 - CH_3), 1.45 (3H, s, C_4 - CH_3), 2.00 (2H, t, J=7.0 Hz, C_3 - H), 2.34 (3H, s, Ar - CH_3), 2.70 (2H, t, J=7.0 Hz, C_2 - H), 7.31 - 7.34 (2H, m, C_5- H and C_6 - H), 7.82 (1H, s, C_8 - H).

4,4,7-Trimethyl - 2 - oximino - 1- tetralone **34:**

To a well cooled solution of 4,4,7-trimethyl - 1- tetralone **33**(0.328 gms, 2 mmole) in ethanol (20 mL) was added conc. HCl (2 mL) and a solution of $NaNO_2$ (1 gms) in water (5 mL).After stirring at 5°C for 2 hr., ethanol was distilled off and reaction mixture was diluted with water, neutralised with saturated solution of $NaHCO_3$ and extracted with ether (3 x 50 mL). The combined organic extracts were washed with

water, dried over anhydrous sodium sulphate and concentrated to yield yellow oil. Chromatography over silica gel using benzene gave unreacted 4,4,7-trimethyl - 1-tetralone **33**(0.137 gms). Further elution with EtOAc - benzene(1:4) yielded white solid which was recrystallised from benzene - pet.ether to afford 4,4,7-trimethyl - 2-oximino-1- tetralone **34**(60 mgs, 48% based on recovered **33** m.p. 177°C (dec).

IR ν_{max}(KBr) (fig. 3.17):3218, 1694, 1607, 1495, 1307, 947,840 cm^{-1}.

^{1}HNMR (δ ppm, $CDCl_3$, 300MHz) (fig. 3.18): 1.38 (6H, s, C_4-CH_3), 2.39 (3H, s, Ar-CH_3), 3.00 (2H, s, C_3-H), 7.34 (1H, d, J=8Hz, C_5-CH_3), 7.40 (1H, dd, J=8.0, 2.44 Hz, C_6-H), 7.93 (1H, d, J=2.44 Hz, C_8-H), 11.44 (1H, -OH).

GCMS: m/z 201,186 (100%), 159, 158, 143 and 115.

2-Hydroxy-4,4,7-trimethyl -1-(4H)-naphthalenone **25:**

A mixture of 4,4,7-trimethyl - 2-oximino-1- tetralone **34**(30 mg, 0.14 mmole), glacial acetic acid (2mL), pyruvic acid (0.1 mL) and water (0.5 mL) was refluxed for 12 hr. The reaction mixture was then decomposed on ice and extracted with ether (3x10 mL). The combined organic extracts were washed with sat. $NaHCO_3$ soln., water and dried over anhydrous $MgSO_4$. Evaporation of the solvent yielded crude **25**which on recrystallisation (benzene) followed by sublimation afforded white needles of 2-hydroxy-4,4,7-trimethyl -1-(4H)-naphthalenone **25**(15 mg, 54%), m.p. 117-118°C (lit[9]. 118°C).

IR ν_{max}(KBr) (fig. 3.19): 3380,2965,1685,1645,1618,1498,1312,1271,1288, 829 cm^{-1}.

^{1}HNMR (δ ppm, $CDCl_3$, 300MHz) (fig. 3.20): 1.48 (6H, s, C_4-CH_3), 2.42 (3H, s, Ar-CH_3), 6.18 (1H, s, C_3-H), 6.41 (1H, br.s, C_2-OH), 7.40 (1H, dd, J=8.2, 2.1 Hz, C_6-H), 7.46 (1H, J=8.1 Hz, C_5-H), 8.01 (1H, d, J=2.0Hz, C_8-H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz) : 181.5 (C_1), 148.6 (C_{10}), 145.0 (C_2), 136.6 (C_7), 134.2 (C_6), 128.4 (C_9), 127.0 (C_8), 126.6 (C_3), 126.5 (C_5), 36.8 (C_4), 30.4 (C_4-CH_3), 30.4 (C_4-CH_3), 20.9 (C_7-CH_3).

GCMS :m/z 202 (M+), 187, 174, 173, 159 (100%), 115, 91.

2-Isobutyril-Meldrums acid **47 :**

To a stirred solution of Meldrums acid **45** (1.44 gms, 0.01 mole) in dry CH_2Cl_2 (10mL) at 0°C, dry pyridine (1.6 mL, 0.02 mole) was added under nitrogen atmosphere, followed by addition of isobutyryl chloride **46** (1.2 mL, 0.011 mole). After stirring the reaction mixture for 1 hr at 0°C and 2 hr at room temp, it was decomposed on ice containing 2N HCl and diluted with CH_2Cl_2 (30 mL). The organic layer was then separated, washed with dil HCl (2 x 20 mL), water and dried over anhydrous sodium sulphate. Evoporation of the solvent gave 2-isobutyril-Meldrums acid **47** as yellow oil in quantitative yield which was used in the following experiment without further purification.

Isobutyryl acetic ester **44 :**

A solution of 2-isobutyril Meldrums acid **47** (1.93 gms, 0.01 mole) in ethanol (20 mL) was refluxed for 3 hr and then ethanol was distilled out under reduced pressure.

Concentrated product was chromatographed over silica gel and eluted with EtOAc - pet ether (1:9) to yield isobutyryl acetic ester **44** (0.880 gms, 56%) as colourless oil.

IR : ν_{max} (film) (fig. 3.21): 2990, 1745, 1710,1620, 1470, 1310, 1220, 1055 cm-1.

^{1}H NMR (δ ppm, $CDCl_3$,300 MHz) (fig. 3.22) : 1.13 (3H, d, J=7.0 Hz, C_2 - CH_3), 1.16 (3H, d, J=7.0 Hz, C_2 - CH_3), 1.26 (3H, t, J= 8 Hz, C7 - CH_3), 2.62 (1H, s, C_2 - H), 3.48 (2H, septet, C_5 - H), 4.17 (2H, d, J= 8 Hz, C_7 - H).

2,4-Dihydroxy toluene **48** :

To a stirred solution of 2,4-dimethoxy toluene **43** (1.0 gm, 6.5 mmole) under nitrogen atmosphere in dry CH_2Cl_2 (25 mL) was added BBr_3 (4 mL, 26 mmole) in dry CH_2Cl_2 (45 mL) over a period of 45 minutes. After stirring for 15 hr at room temp, the reaction mixture was refluxed for 30 min, decomposed with moist EtOAc, ice and extracted with 2N NaOH. The aqueous layer was neutralised with dil HCl and extracted with ethyl acetate (3 x 25 mL). The combined organic extracts were washed with brine, water and dried over anhydrous sodium sulphate. Removal of the solvent yielded dark oil which was chromatographed over silica gel and eluted with EtOAc - pet ether (4:6) to give white solid. Recrystallisation from $CHCl_3$ afforded 2,4-dihydroxy toluene **48** (0.325 gms, 40%), m.p. 102°C (lit[22]. 104°C).

IR : ν_{max} (KBr): 3420, 1620, 1520, 1470, 1300, 1245, 1150, 1110, 1000, 960, 840, 790 and 620 cm-1.

^{1}H NMR (δ ppm, CDCl3,300 MHz) : 2.15 (3H, s, C_1 - CH_3), 4.75 (2H, br s, both -OH), 6.37 (2H, m, C_3- H and C_6 - H), 6.88 (1H, d, J= 6 Hz, C_5 - H).

7-Hydroxy-4-isopropyl-8-methylcoumarin **37 :**

To vigorously stirred conc. H_2SO_4 (1.0 mL) at 0°C, solution of 2,4-dihydroxy toluene **48** (0.124 gms, 0.1mmole) and isobutyryl acetic ester **44** (0.158 gms, 0.1 mmole) was added slowly. After stirring for 1 hr at room temperature, the reaction mixture was diluted with cold water and extracted with EtOAc (3 x 20 mL). The combined organic extracts were washed with sat. $NaHCO_3$ solution, brine, water and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded crude yellow solid which was recrystallised from $CHCl_3$ - pet ether to give colourless needles of 7-hydroxy-4-isopropyl-8-methylcoumarin **37** (0.113 gms, 52%), m.p.194°C(lit[16]. 198°C).

UV : λ_{max} (CH_3OH) :330, 220, 204 nm.,

UV : λ_{max} (CH_3OH + NaOAc) :375, 220 nm.

IR : ν_{max} (KBr) (fig. 3.23) : 3120, 1680, 1608, 1530, 1400, 1390, 1315, 1285, 1270, 1155, 1105 cm^{-1}.

^{1}H NMR (δ ppm, $CDCl_3$,300 MHz) (fig. 3.24) : 1.27 (6H, d, J=7 Hz, both C_9 - CH_3), 2.27 (3H, s, C_6 - CH_3), 2.45 (1H, br s, exchanges with D_2O, C_7 - OH), 3.25 (1H, septet, C_9 - H), 6.10 (1H, s, C_3 - H), 6.90 (1H, s, C_8 - H), 7.38 (1H, s, C_5 - H),

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 3.25) : 16.0 (q, C_6 - CH_3), 21.9 (s each, both C_9 - CH3), 28.6 (d, C_9), 102.3 (d, C_8), 106.4 (d, C_3), 111.1 (s, C_{4a}), 122.6 (s, C_6),125.2 (d, C_5), 153.5 (s, C_{8a}), 158.9 (s, C_4),163.5 (s, C_7),163.5 (s, C_2).

HRMS :m/z 218 (M^+), 190, 175(100%).

4-Hydroxy-8-isopropyl-5-methylcoumarin **68** :

A mixture of thymol **67** (25 gms, 0.17 mole), malonic acid (17 gms, 0.17 mole), anhydrous $ZnCl_2$ (68 gms, 0.5 mole) and $POCl_3$ (45 mL, 3.5 mole) was stirred at 60-65°C for 35 hr. The reaction mixture was cooled to room temp. and decomposed on ice. The solid obtained was filtered and dissolved in 10% Na_2CO_3 solution, acidified with dil. HCl and extracted with ethyl acetate (3x 100 mL). The combined organic extracts were washed with brine, water and dried over anhydrous sodium sulphate. Evaporation of the solvent yielded a black solid which was chromatographed on silica gel and eluted with ethyl acetate -pet.ether (1:1) to yield a solid. Recrystallisation from EtOH afforded 4-hydroxy-8-isopropyl-5-methylcoumarin **68** (11.4 gms, 32%), m.p. 223°C (lit[30]223-224°C).

4-Hydroxy-5-methylcoumarin **62 :**

To a stirred solution of 4-hydroxy-8-isopropyl-5-methylcoumarin **68** (0.400 gms, 0.92 mmole) in chlorobenzene (13 mL), finely powdered anhydrous $AlCl_3$ (1.3 gms, 10 mmole) was added in portions over a period of 30 min. After heating at 95°C for 1 hr, the reaction mixture was cooled, poured on crushed ice containing some dil HCl and diluted with EtOAc. The organic layer was separated and extracted with saturated solution of $NaHCO_3$ (3x50 mL). Aqueous layer was then neutralised with dil HCl to give white gelatinous precipitate. which was filtered , washed with water and dried to yield 4-hydroxy-5-methylcoumarin **62** (0.210 gms, 65%) m.p. 235°C (lit.[30]233-234°C).

11-Methyl gerberinol **66** :

Acetaldehyde (5mL) was added to a boiling solution of 4-hydroxy-5-methylcoumarin **62** (75 mgs, 0.43 mmole) in aqueous ethanol (3 mL). After refluxing the reaction mixture for 5 min excess of acetaldehyde and ethanol was distilled off to afford a solid which on repeated crystallisation from methanol gave white crystalline 11-methyl gerberinol **66** (48 mgs, 59%) m.p. 218°C (lit.[27] 210-217°C).

IR : ν_{max} (KBr) (fig. 3.28): 3009, 1686, 1655, 1599, 1560, 1409, 1383, 1365, 1331, 1280, 1232, 1134, 981, 866, 790 cm^{-1}.

^{1}H NMR (δ ppm, $CDCl_3$,400 MHz) (fig. 3.29) : 1.85 (3H, d, J=7.55 Hz, C_{11}- CH_3), 2.80 (3H, s, C_5-CH_3), 2.81 (3H, s, $C_{5'}$- CH_3), 4.70 (1H, q, J=7.55 Hz, C_{11}-H), 7.10 (1H, dd, J=8.3 and 2.0 Hz, C_8-H), 7.12 (1H, dd, J=7.6 and 1.99 Hz, $C_{8'}$-H), 7.20 (1H, d, J=8.3 Hz, C_6-H), 7.22 (1H, d, J=7.95 Hz, $C_{6'}$-H), 7.39 (1H, dd, J=7.95 and 4.37 Hz, C_7-H), 7.43 (1H, dd, J=7.94 and 4.37 Hz, $C_{7'}$-H), 11.86 (1H, br.s, exchanges with D_2O, -OH), 12.58 (1H, br.s, exchanges with D_2O, -OH).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, DEPT) (fig. 3.30) :15.1 (q, C_{11}- CH_3), 23.5 and 23.6 (q, C_5- CH3 and q, $C_{5'}$- CH_3), 26.9(d, C_{11}), 106.5 and 106.8 (s, C_3 and s, $C_{3'}$), 114.9 (d, C_8 and d, $C_{8'}$), 116.0 (s, C_{10} and s, $C_{10'}$), 128.2 (d, C_6 and d, $C_{6'}$), 131.5 and 131.7(d, C_7 and d, $C_{7'}$), 138.5 (s, C_5 and s, $C_{5'}$), 153.4 and 153.6 (s, C_9 and s, $C_{9'}$), 167.2 and 167.6 (s, C_4 and s, $C_{4'}$), 168.1 and 168.8 (s, C_2 and s, $C_{2'}$).

HRMS :m/z 378(M+), 202, 176, 135(100%), 134, 106.

Anhydro-11-methyl gerberinol **71 :**

To a solution of 11- methyl gerberinol **66** (50 mgs, 0.13 mmole) in acetic anhydride (4 mL), a few drops of conc H_2SO_4 were added and warmed to give a clear solution, it was then left aside at room temperature for 24 hr. The white solid separated, was filtered and crystallised from CH_3OH-$CHCl_3$ (46 mgs, 98%), m.p. 283°C.

UV : λ_{max} (CH3OH) (fig. 3.32) : 277, 300, 333 nm.

IR : ν_{max} (KBr) (fig. 3.33): 1795, 1680, 1640, 1610, 1480, 1415, 1375, 1320, 1265, 1170, 1080, 1025, 790 and 780 cm^{-1}.

^{1}H NMR (δ ppm, $CDCl_3$,400 MHz) (fig. 3.34) : 1.49 (3H, d, J= 6.4 Hz, C_{11}-CH_3), 2.87 (3H, s, C_5-CH_3), 2.89 (3H, s, C_{16}- CH_3), 4.26 (1H, q, J=6.8 Hz, C_{11}-H), 7.14 (1H, d, J=7.6 Hz, C_8-H), 7.18 (1H, d, J=7.6 Hz, C_{19}-H), 7.24 (1H, d, J=8.4 Hz, C_6-H), 7.33 (1H, d, J=8.4 Hz, C_{17}-H), 7.44 (1H, dd, J=8.0 and 8.0 Hz, C_7-H), 7.52 (1H, dd, J=8.0 and 8.0 Hz, C_{18}-H).

^{13}C NMR (δ ppm, $CDCl_3$, 100 MHz, DEPT) (fig. 3.35): 20.8 (q, C_{11} -CH_3), 22.6 (q, C_5 -CH_3), 23.3 (q, C_{16}- CH_3), 24.5 (d, C_{11}), 100.9 (s, C_{14}), 108.4 (s, C_3), 112.1 (s, C_{21}), 115.3 (d, C_8), 115.6 (d, C_{19}), 128.1 (d, C_6), 128.5 (d, C_{17}), 131.7 (d, C_7), 132.6 (d, C_{18}), 136.3 (s, C_5), 141.4 (s, C_{16}), 153.7 (s, C_9), 154.5 (s, C_{20}), 156.2 (s, C_4), 157.5 (s, C_{13}), 160.4 (s, C_2), 178.7 (s, C_{15}).

References :

1. Weenen H, Nkunya M H H, Mgani Q A, Posthumus M A, Waibel R, Achenbach H, J. Org.Chem, **56**, 5865 (1991)
2. Paknikar S K, Pai Fondekar K P, Mayer R, Nat. Prod. Lett, **8**, 253 (1996)
3. Cavitt S B, Sarrafizadeh H R, Gardner P D, J. Org. Chem, **27**, 1211 (1962)
4. Turner A B, Quat. Rev, **18**, 347 (1964)
5. Yang Z, Wong H N C, Hon P M, Chang H M, Lee C M, J. Org. Chem, **57**, 4033 (1992)
6. Shapiro R, Slobodin D, J. Org. Chem. **34**, 1165 (1969)
7. Ziegler E, Lercher K, Ber. Dtsch. Chem. Ges, **74B**, 841 (1941)
8. Yeddanapalli L M, Francis J D, Makromol. Chem, **55**, 74 (1962)
9. Lasswell W L, Hufford C D, Phytochemistry, **16**, 1439 (1977)
10. Pongprayoon U, Baeckstrom P, Jacobson U, Lindstorm M, Bohlin L, Planta Med., **57**, 515 (1991).
11. Bogert M, Fourman V, J. Am. Chem. Soc., **55**, 4670 (1933).
12. Kamat V P, Ph.D. Thesis, University of Bombay, (1984).
13. Liu H, Browne E, Can. J. Chem., **57**, 377 (1979).
14. Davis D, Stevens K, Jurd L, J. Agric. Food Chem., **24**, 187 (1976).
15. Murray R D H, Nat. Prod. Rep. **12**, 477 (1995).
16. Hori K, Satake T, Saiki Y, Murakami T, Chen C, Yakugaku Zasshi **107**, 491 (1987).
17. Meng Q, Chen W, Planta Med. **54**, 88, (1988).

18. Nadkarni K K, Kamat S P, Paknikar S K, Ind. J. Chem. **33B**, 432 (1994).

19. Malik B L, Ph. D. Thesis, Goa University (1995).

20. Koeker E M, McElvain J M, J. Am. Chem. Soc. **56**, 1171 (1934).

21. Oikawa y, Sugano K, Yonemitsu O, J. Org. Chem. **43**, 2087 (1978).

22. Yanagita M, Chem. Ber. **71**, 2269 (1938).

23. McOmie J F W, Watts M L, West D E, Tetrahedron **24**, 2289 (1968).

24. Stipanovic R D, Greenblatt G A, Beier R C, Bell A L, Phytochemistry. **20**,729 (1981).

25. Jeffs P W, Cynn D G, J. Org. Chem. **40**, 2058 (1975).

26. Murray R D H, Forstchr.Chem.Org.Naturst. **58**, 83 (1991) .

27. Tezuka M, Kuroyanagi M, Yoshihira K, Natori S, Chem.Pharm.Bull, **20**, 2029 (1972).

28. Murray R D H, Mendez J, Brown S A, The Natural Coumarins, John Wiley and sons ltd, chichster, (1982).

29. Sengupta P, Sen M, Kauri P, Wenkert E, Halls T D J, J. Ind. Chem. Soc., **63**, 916 (1985).

30. Paknikar S K, Nadkarni K K, Unpublished work.

31. Inoue T, Toyonaga T, Nagumo S, Nagai M, Phytochemistry, **28**, 2329 (1989).

32. Manito P, Biosynthesis of Natural Products, Ellis Horwood Ltd, 193 (1981).

33. Jeffreys J A D, Zakaria M B, Waterman P G, Zhong S, Tet. Lett., **24**, 1085 (1983).

4.1 Oxidative Modifications of Santonic acid with Ceric ammonium nitrate

Ceric ammonium nitrate, $Ce(NH_4)_2(NO3)_6$ (CAN) has been found to be a good oxidising agent for several decades but its synthetic utility has been realised only recently. This can be seen by the appearance of two excellent review articles[1,2] and large number of publications since then.

A number of solvents such as acetonitrile, ethanol, aqueous acetic acid are used while carrying out oxidations with CAN. Some of the common functional groups oxidised by CAN are indicated in Table-1.

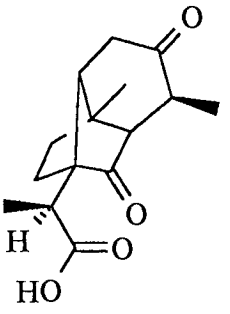
Table - 1

Functional groups	Product / Products
Primary alcohols	Aldehydes
Secondary alcohols	Ketones and /or Products derived by fragmentation
Aldehydes	Carboxylic acid
Ketones (unhindered)	Nitro carboxylic acid, lactones / esters*
Carboxylic acids	Normally resistant to decarboxylation**
Carboxylic acid hydrazides	Hydrolysis to carboxylic acids
Hydroquinones	Quinones
Hydrocarbons : benzylic methyl, methylene	Aryl aldehydes / aryl ketones
Diaryl sulfides	Diaryl sulfones
Cyclic epoxides	Dicarbonyl compounds

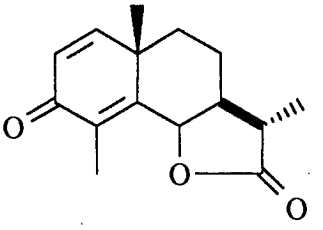
* The products are generally opposite to those obtained by Bayer-Villiger oxidation.
** Oxalic and malonic acid readily undergo decarboxylation

In addition to the synthetic utility (Table -1) CAN promoted oxidative addition of carbonyl compounds to alkenes is worth mentioning.

Santonic acid **1**, the diketocarboxylic acid obtained from santonin **2** on digestion with aq. alkali has been used for chemical transformations and like its precursor has unravelled interesting chemistry. Oxidative modifications of **1** were first reported by Wadekind and Jackh much before the correct structure of santonic acid was established.[3] Subsequently, Hortmann and Daniel reported the reaction of santonic acid **1** with alkaline hydroperoxide.[4]

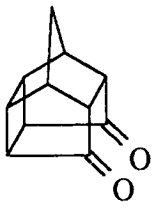


1



2

In 1976, Mehta and Pandey reported that the oxidation of pentacyclic diketone **3** with ceric ion proceeds regiospecifically and yields lactone **4** in excellent yield.[5] Recently, Singh and Raju extended the scope of the reaction by using spirocage diketones **5a** and **5b** and obtained substituted succinic anhydrides **6a**, **6b** and **7a**, **7b** besides ketolactones **8a** and **8b** respectively.[6]



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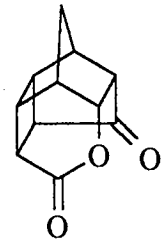

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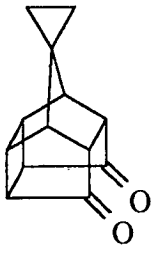
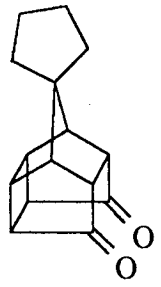
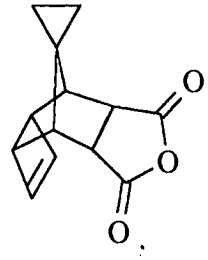


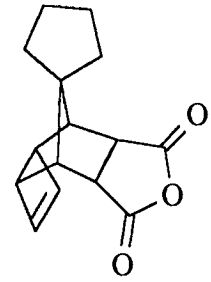
5a



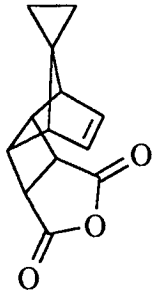
5b



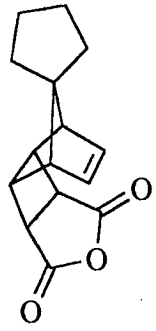
6a



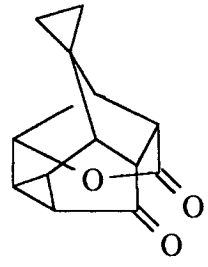
6b



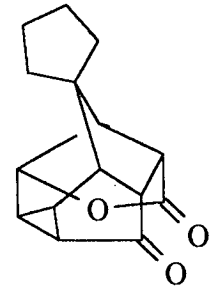
7a



7b



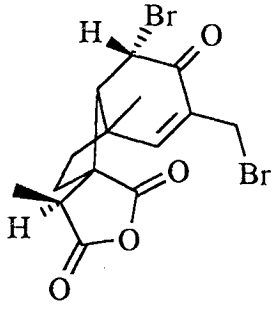
8a



8b

The conformational formula of santonic acid **1** reveals an interesting relationship with the 1,4 - diketone system of **3** and hence we thought of oxidative modifications of the former with CAN. Besides the 1,4 - diketone grouping, santonic acid also contains a carboxyl group. However, literature survey showed that carboxyl groups are usually stable to CAN except the decarboxylations observed in the case of oxalic and malonic acids. If the oxidation is initiated at the ketonic carbonyl group, we

envisaged the cleavage of C_5-C_6 bond and participation of the carboxyl group to produce substituted succinic anhydride derivative/s. Earlier we had observed the cleavage of C_5-C_6 bond and obtained succinic anhydride derivatives **9** and **10** when santonic acid was subjected to treatment with bromine in moist chloroform[7] and Zn-HCl-ether respectively[8]. Contrary to our expectation, oxidation of santonic acid **1** with CAN produced products having both the carbonyl groups intact. The oxidation products are based on the initiation of the reaction at the carboxyl group. The results are certainly surprising as carboxylic acids other than oxalic and malonic acids are known to be stable under CAN oxidation conditions. The characterization of products are presented in this section.



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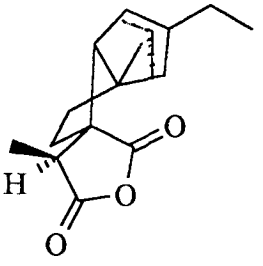
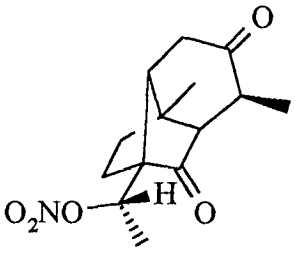


10

The oxidation of santonic acid was carried out in aqueous acetonitrile using 5 mole of CAN per mole of **1**. The reaction mixture was separated into neutral and acidic fractions. The acidic fraction was found to be a complex mixture and not processed further. TLC of the neutral portion showed clearly four closely spaced spots and could be resolved by a combination of column chromatography and preparative

TLC. The compounds obtained in order of elution from the column are designated as **A**, m.p 179°; **B**, m.p.104°; **C**, m.p.192° and **D**, 182°. Characterization of these products is mainly based on NMR spectral analysis as only small quantities were available.

The most characteristic feature of the ^{1}HNMR spectrum of compound **A**, m.p. 179° is the one proton quartet (J = 6.4 H z) at δ 5.29 due to C_{11}- H. This downfield shift is certainly due to replacement of carboxyl group by -ONO_2 group. The total proton count showed the presence of 19 hydrogens and indirectly confirmed the presence of one nitrogen atom. The chemical shifts and the splitting pattern of the all the protons (for assignments see experimental) unambiguously showed that the tricyclic skeleton and the two ketonic carbonyls have remained intact. Based on this data structure **11**[*] could be assigned to compound A.



11

Compound **B**, though obtained as solid did not show a sharp m.p. (104°) but appeared as a single compound (TLC). The ^{1}HNMR spectrum showed it to be a mixture of two compounds (60:40) and revealed strong similarities to the spectrum of

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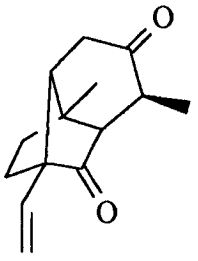
[*] Footnote has appeared on next page

11 but also some diagnostic differences. Signals for only two methyl groups (C_4-CH_3 and C_{10}-CH_3) could be seen in the region δ 1.0 - 2.0 ppm. The olefinic region contained a four line pattern at δ 5.70 (1H, dd, J= 17.3, 11.0 Hz) and AB part of ABX system at δ 5.31 (1H, d, J=11.0 Hz) and δ 5.42 (1H, d, J=17.3 Hz) characteristic of a vinyl group attached to a quaternary carbon.[9] The ratio of the integration under the olefinic proton region and the methyl groups showed the major component (60%) contains a vinyl group and must have structure **12**. The structure **13** is assigned to the other component (40%) on the basis of a sharp signal at δ 2.3 due to a methyl keto grouping. Interestingly Naik had prepared **12** by lead tetra acetate oxidation of santonic acid.[10] A fresh sample prepared following the reported procedure showed identical behavior on TLC. Efforts to resolve compound **B** into pure compounds **12** and **13** were unsuccessful.



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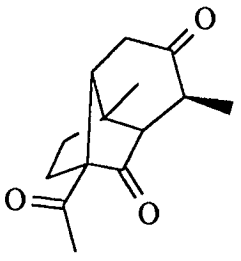

13

Compound **C**, m.p. 192° showed ^{1}HNMR data very similar to those of **11** (compound **A)** and was consistent with the replacement of the carboxyl of the

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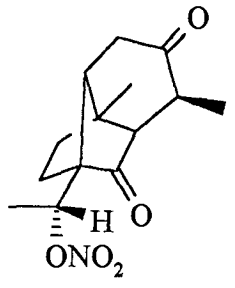
* Stereochemical assignment at C_{11} is based on comparison of chemical shifts of C_{11}-CH_3 in compounds **A** and **C**

santonic acid **1** by -ONO_2 group. The main difference being the upfield shift of C_{11}-CH_3 (from δ 1.45 for compound **A** to 1.35 for compound **C**). The C_{11}-H appeared as a quartet (J= 6.6 Hz) at δ 5.35 once again confirming the presence of -ONO_2 at C_{11}. Further the signal for C_1-H was not resolved as in the case of compound **A** while C_5-H was found to be a doublet (J=4.2 Hz) in place of dd in **11** (compound **A**). The chemical shifts of remaining protons showed an identical pattern when compared to **11**. Compounds **A** and **C** are therefore isomeric and differ in the configuration at C_{11}. The upfield chemical shift of C_{11}-CH_3 shows that it is not shielded by C_6 carbonyl group. Stereostructure **14** is therefore assigned to compound **C**.

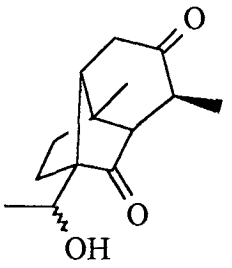


14

^{13}CNMR data could be measured only on compound C and is presented in experimental section.

Compound **D** was obtained as a crystalline solid, m.p. 182°. The ^{1}HNMR spectrum displayed a sharp singlet at δ 1.45 (3H, C_{10}-CH_3) and two secondary methyls at δ 1.27 (d, J=6.3 Hz, C_{11}-CH_3) and δ 1.09 (d, J= 6.8 Hz, C_4-CH_3). The C_{11}-H appeared as a quartet at δ 4.01 (J= 6.3 Hz). The upfield shift of this methine compared to the corresponding methines of diketonitro-oxy compounds **11** and **14** can be easily

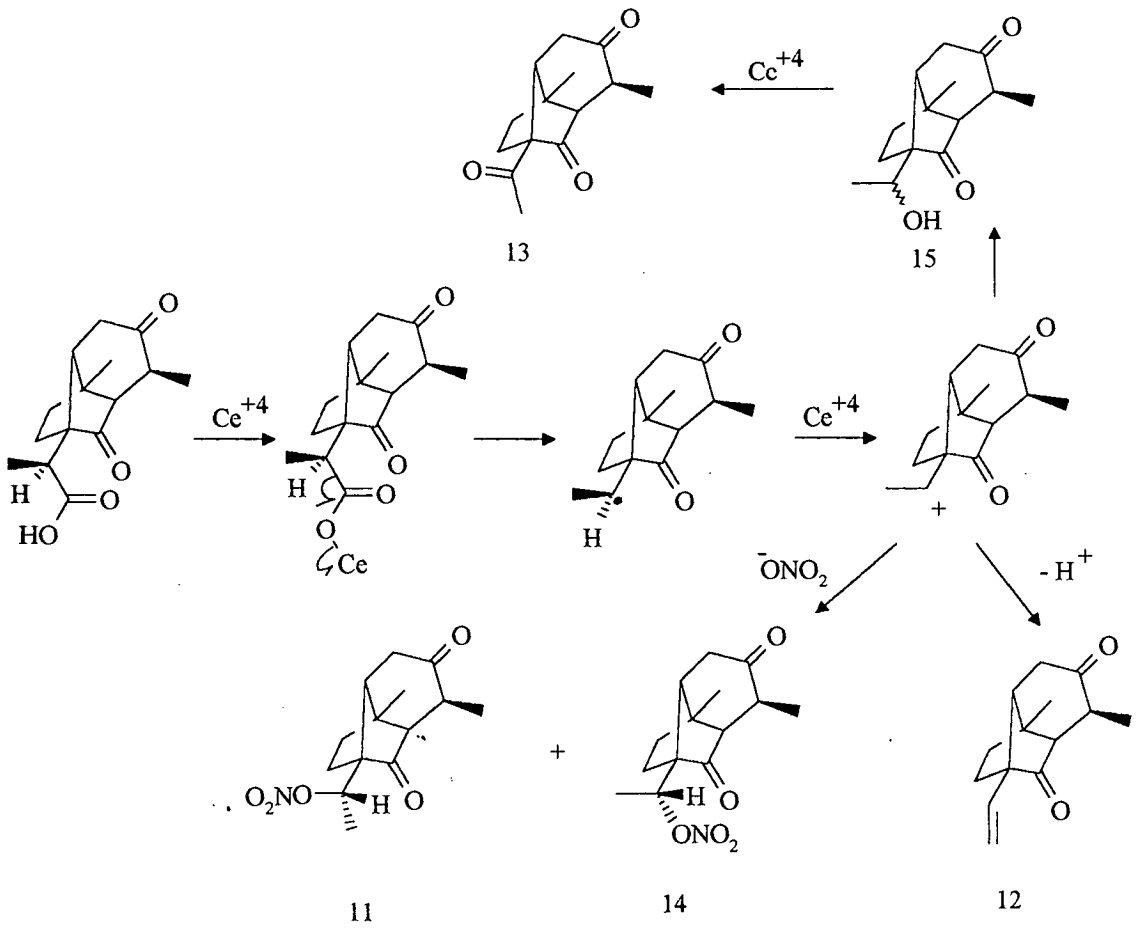
accounted for by the presence of hydroxy function at C_{11}. Structure **15** is therefore assigned to compound **D**.



15

Thus we have characterised five oxidation products obtained by oxidation of santonic acid **1** with CAN. All the products are derived by oxidation by Ce^{+4} at the carboxyl group. This observation is particularly interesting because carboxylic acids with the exception of oxalic and malonic acids are reported to be quite stable under the Ce^{+4} oxidation conditions. Moreover the two ketonic carbonyls of **1** remain unaffected.

A rational mechanism which can account for all the product formed is presented in scheme-1.



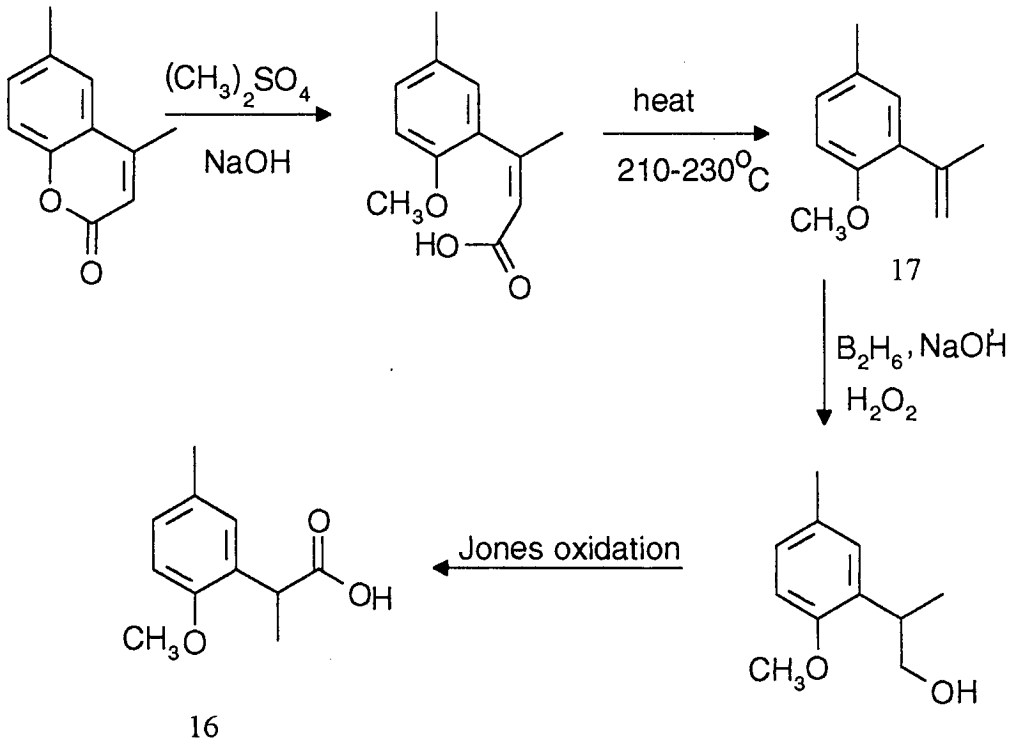
Scheme - 1

The proposed mechanism has some similarity to the Hunsdiecker reaction. The radical generated on loss of CO_2 further undergoes oxidation with Ce^{+4} to give a carbocation which then gets converted to the observed products **11** to **15**.

Further investigations of the decarboxylative oxidation with CAN would prove generality or otherwise of the results obtained by us.

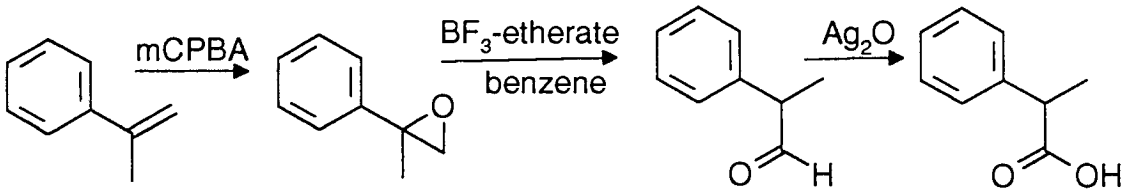
4.2 Formation of 2,5-dimethyl benzofuran by skeletal rearrangement of 2-(2'-methoxy-5'methylphenyl)-1,2-epoxypropane

In connection with the synthesis of a modified sesquiterpene, substantial quantity of acid **16** was required. We envisaged the preparation of **16** from 2-isopropenyl-4-methyl anisole **17**, a readily available intermediate from 4,6-dimethyl coumarin[11]. The desired acid **16** could be obtained by following the sequence shown (scheme-2).However, the yields were very low.



Scheme - 2

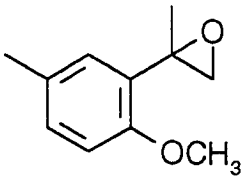
In view of the high yields reported for the conversion of the 2-phenyl propene **18** in to acid **19** via aldehyde(scheme-3), we attempted the preparation of acid **16** following the procedure adopted by Welch and co-workers[12].



18 19

Scheme - 3

The key reaction in this sequence is the high yield epoxide-aldehyde rearrangement in the presence of BF_3-etherate. Interestingly, the epoxide **20** when treated with BF_3-etherate yielded a complex mixture* from which only one pure compound, m.p.140°C could be isolated by repeated column and preparative TLC. This compound has been unambiguously identified as 2,5-dimethyl benzofuran **21**. The spectral data and mechanism of its formation are presented in this section.



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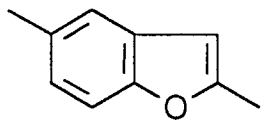


21

* Obtained earlier by Dr. Mrs. Jivexa Bhattacharjee (nee Jivexa Patel) in an attempted synthesis of elvirol methyl ether (Ph.D. thesis, Goa University,1990). We thank her for the sample. Repetition of the experiments gave identical results. Presence of aldehyde could be seen as a minor component (sharp singlet at δ 9.7 in the ^{1}HNMR spectrum).

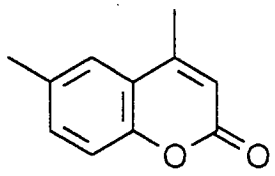
2-(2'methoxy-5'-methylphenyl) propene **17** was prepared from 4,6-dimethylcoumarin **22** following the literature procedure[11]. reaction of **2** with mCPBA in CH_2Cl_2 gave the epoxide **20** which without further purification was treated with BF_3-etherate in dry benzene at 25°C for 10 min. The IR spectrum of the reaction product obtained in 66% yield showed the presence of carbonyl band at 1725 cm^{-1} bat no absorption around 2700 cm^{-1} characteristic (C-H stretching) of aldehyde. purification of this product yielded a solid m.p.140°C. Its ^1H and ^{13}C NMR data (for assignments see table-1 and table-2) unambiguously established it as 2,5-dimethyl benzofuran **21**. The alternate structure viz. 3,5-dimethyl benzofuran **23** was ruled out by ^1H NMR chemical shift of the furan hydrogen at δ 6.27 which clearly shows the location of methyl group at C_2 and further confirmed by appearance of singlets at δ152.21 and 151.64 (C_2 and C_{7a}) in its ^{13}C NMR spectrum. Moreover, comparison of the chemical shifts of furan hydrogen and the methyl groups of **21** and those recorded for the 3-methyl substituted naturally occurring benzofuran, farfugin-A[13] **24** further supported the assignment.

Table-1 : ^1H NMR assignments of **21**

Protons	Chemical shift δ (ppm)	multiplicity	Coupling constants J (Hz)
3	6.27	q	1
4	7.37	s br	W1/2 2
6	7.33	dd	8.3 & 1.7
7	7.23	d	8.3
2-CH_3	2.42	d	1
5-CH_3	2.43	s	-

Table-2 : ^{13}C NMR assignments of **21**

Carbons	Chemical shifts δ (ppm)	multiplicity
2	152.21	s
3	115.04	d
4	124.4	d
5	133.78	s
6	132.67	d
7	1116.79	d
7a	151.64	s
3a	119.66	s
C_2-CH_3	20.94	q
C_2-CH_3	18.59	q



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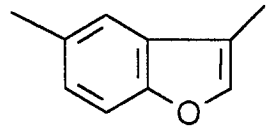

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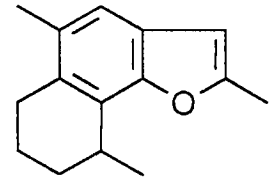
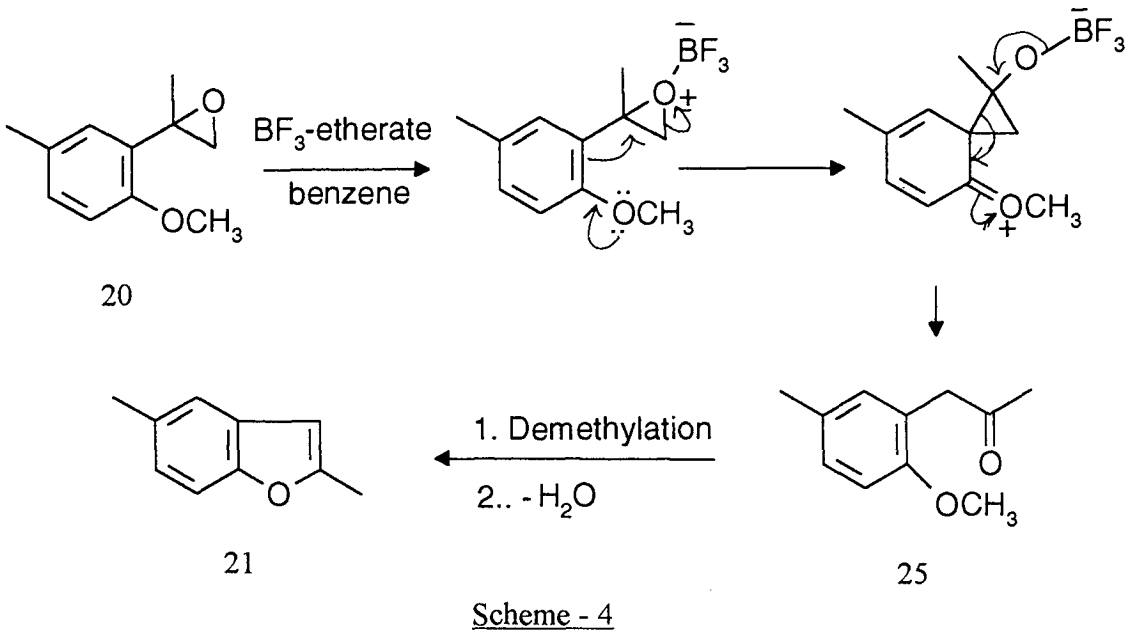


24

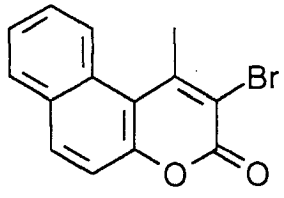
Formation of 2,5-dimethyl benzofuran **21** from **20** can be explained by a mechanism depicted in scheme-4 which involves a three membered ring intermediate formed via participation of 2'-methoxyphenyl substituent as shown. 2,5-dimethyl benzofuran **21** is then derived from **25** by demethylation and cyclisation of the resulting γ-ketophenol[14].



20 21 25

Scheme - 4

Formation of a structurally similar γ-ketophenol was observed by Carnduff and Marks[15] in the alkalline hydrolysis of 1-methyl-2-bromonaphtho [2,1-b] pyran-3-one **26** which on treatment with methanolic HCl gave 2-methylnaptho [2,1-b] furan **27**.



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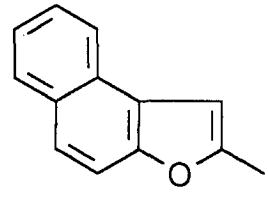
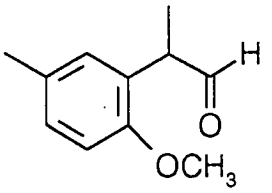


27

It may be noted that Divakar and Rao[1] observed the formation of 1-(2'-methoxy-5'methylphenyl)-2-propanone **25** by oxidative rearrangement of **17** with lead tetra acetate. Though we have not characterised **28** the presence of a carbonyl band at 1725 cm^{-1} in the crude reaction mixture suggests its presence.



28

Though there are several reports[16-18] on the preparation of 2,5-dimethyl benzofuran **21** to our knowledge, this is the first report of its preparation by a molecular rearrangement. This procedure may find use in the preparation of other 2-methyl substituted benzofurans.

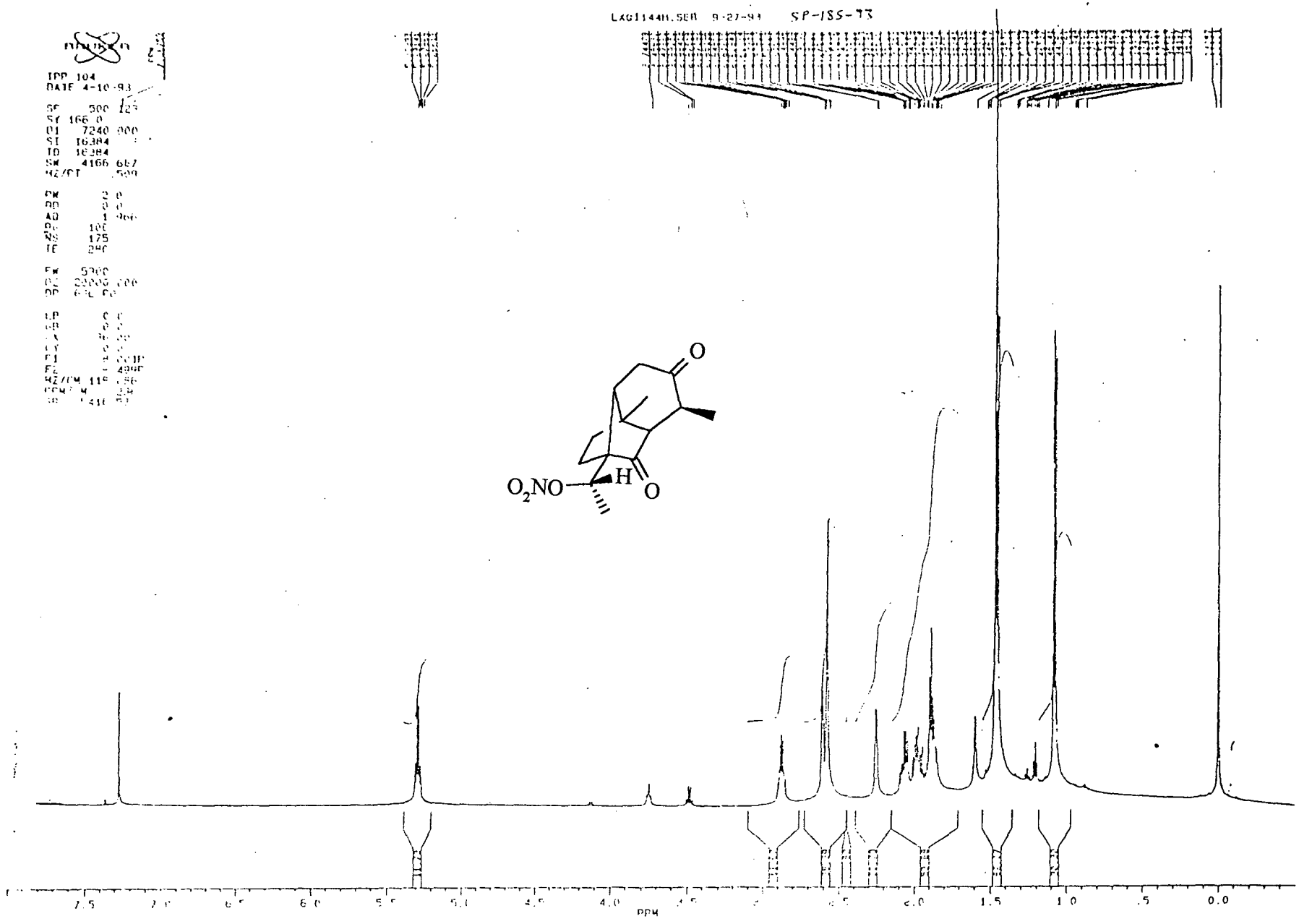


Fig. 4.01 : ^{1}H NMR spectrum of 11

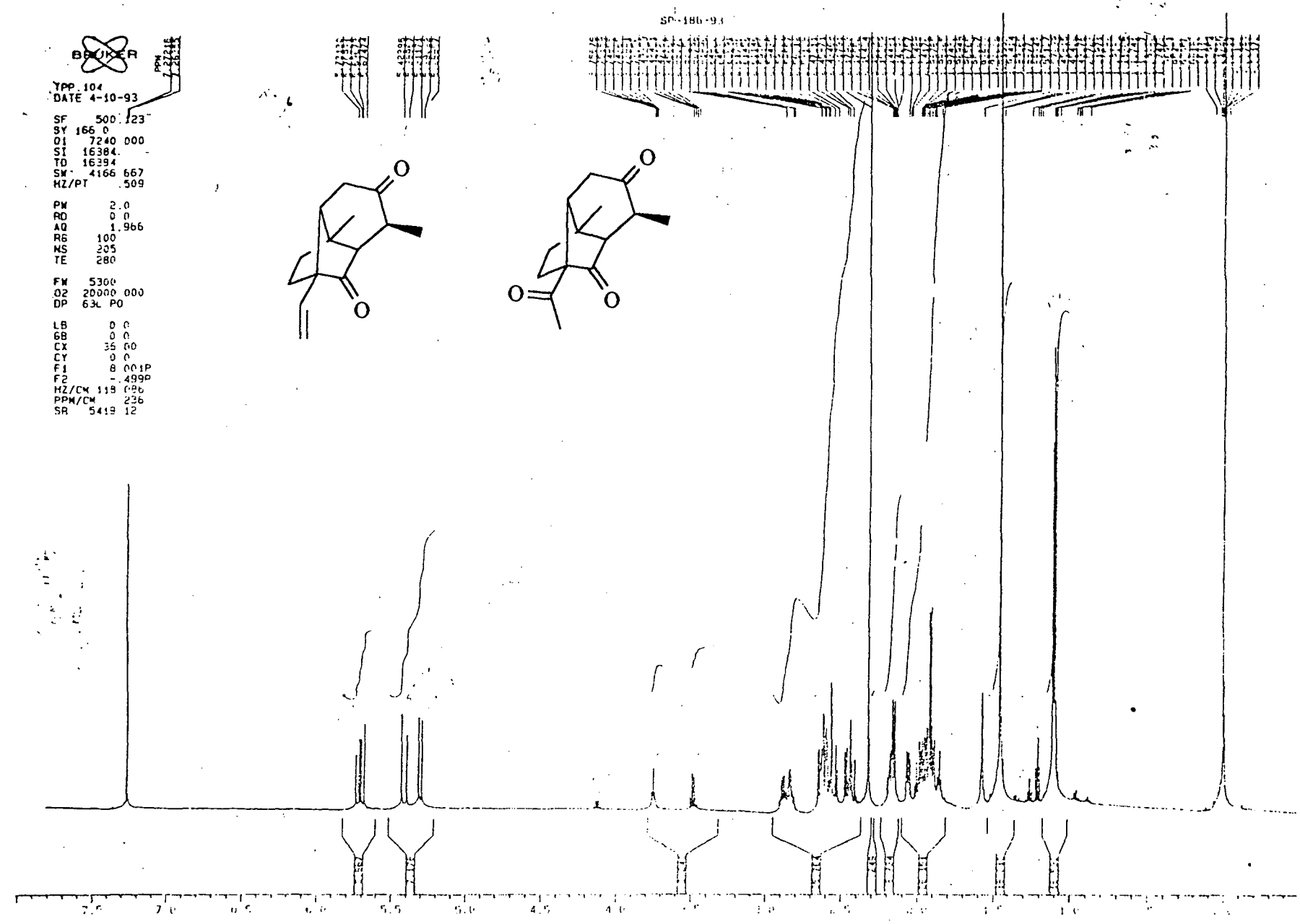


Fig. 4.02 : ^{1}H NMR spectrum of 12 + 13

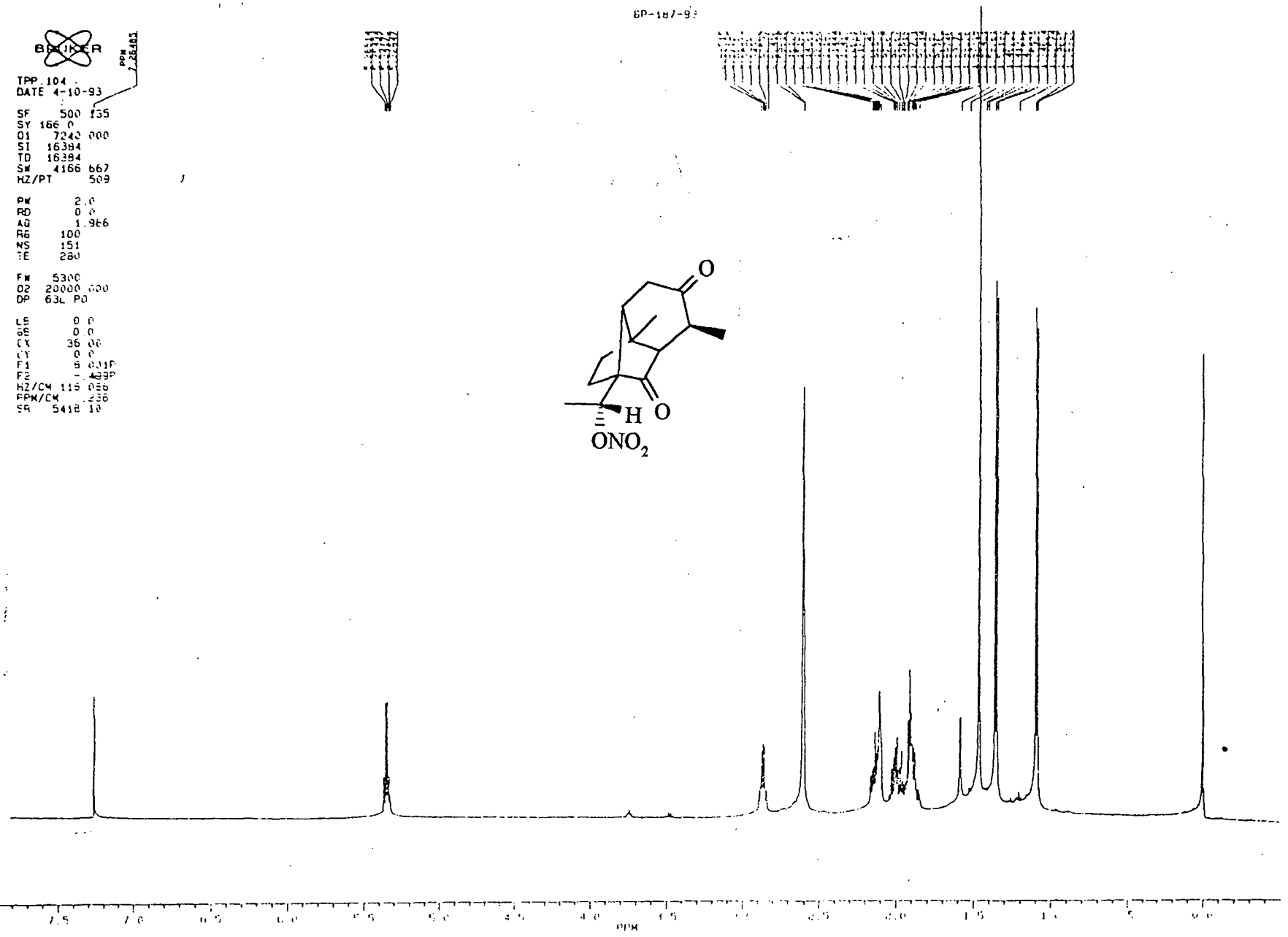


Fig. 4.03 : ^{1}H NMR spectrum of 14

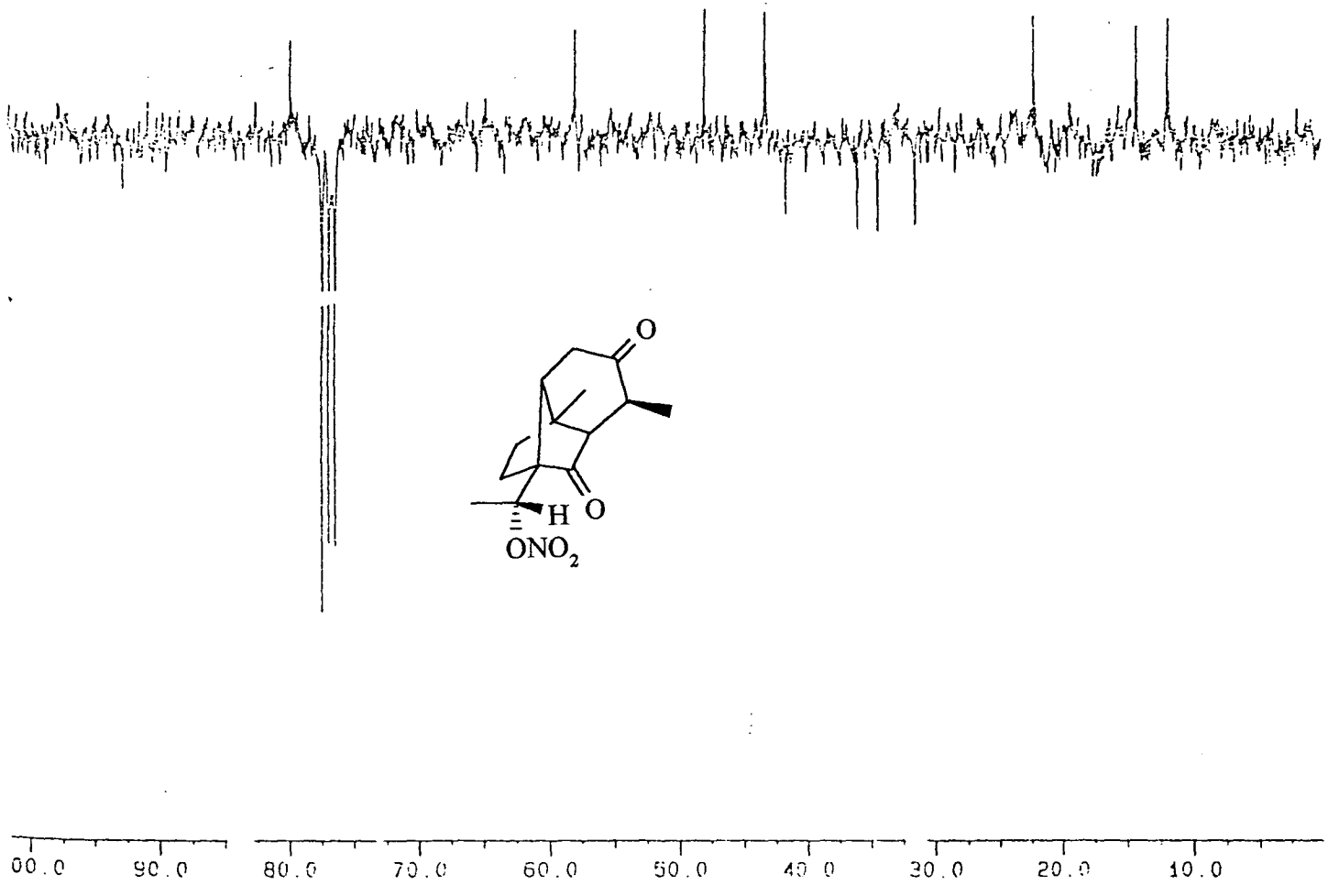


Fig. 4.04 : ^{13}C NMR spectrum of 14

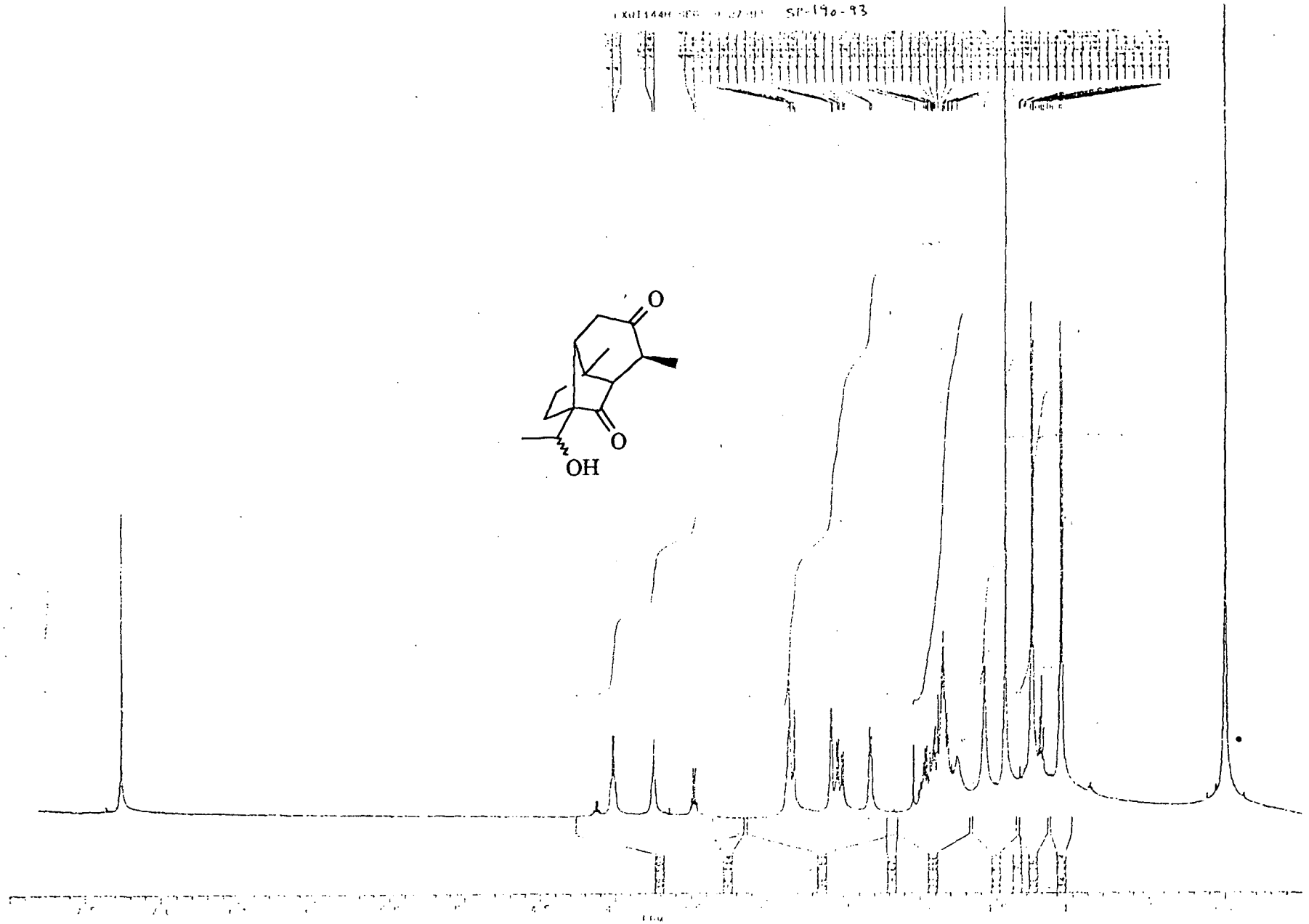
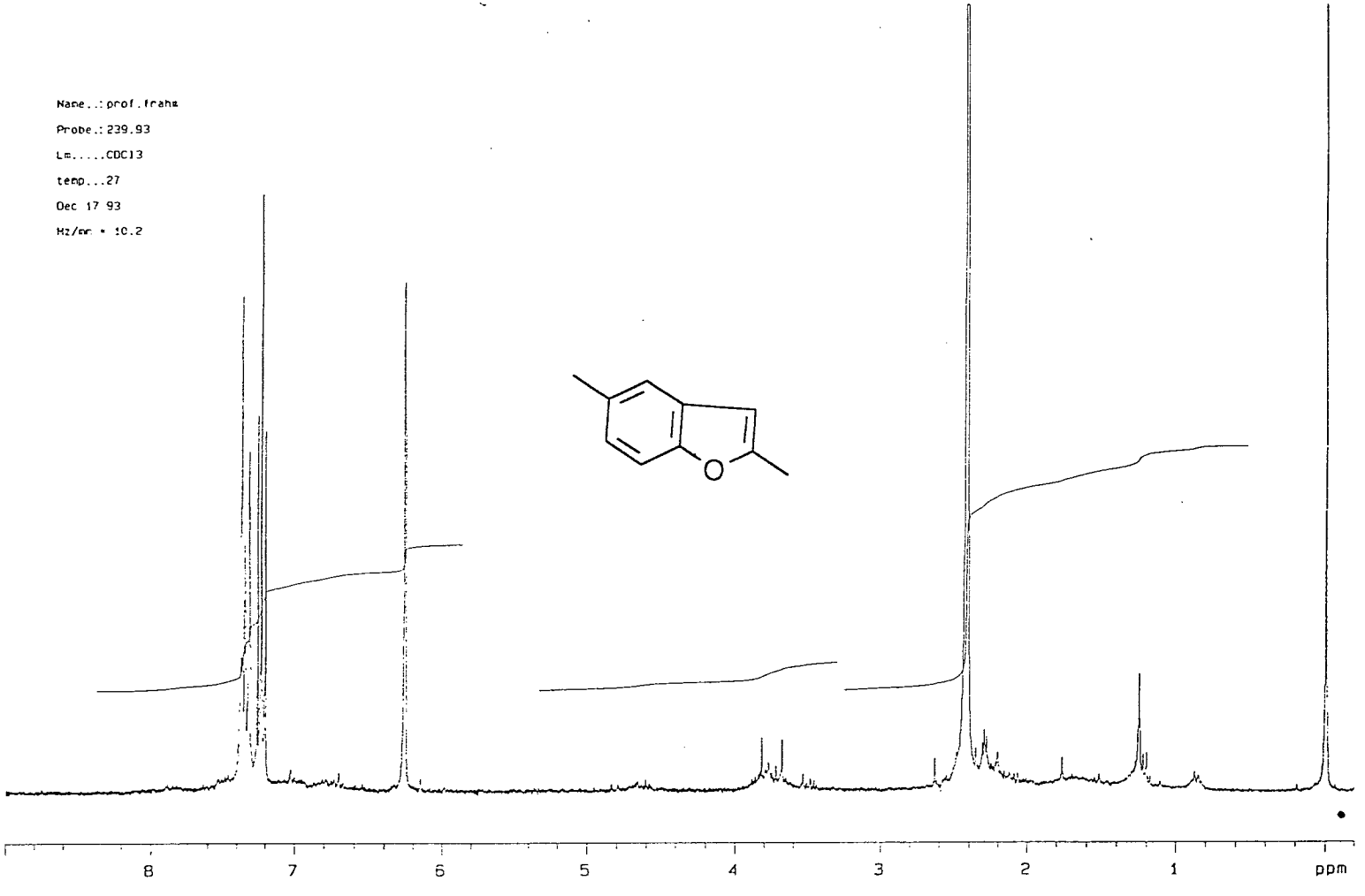
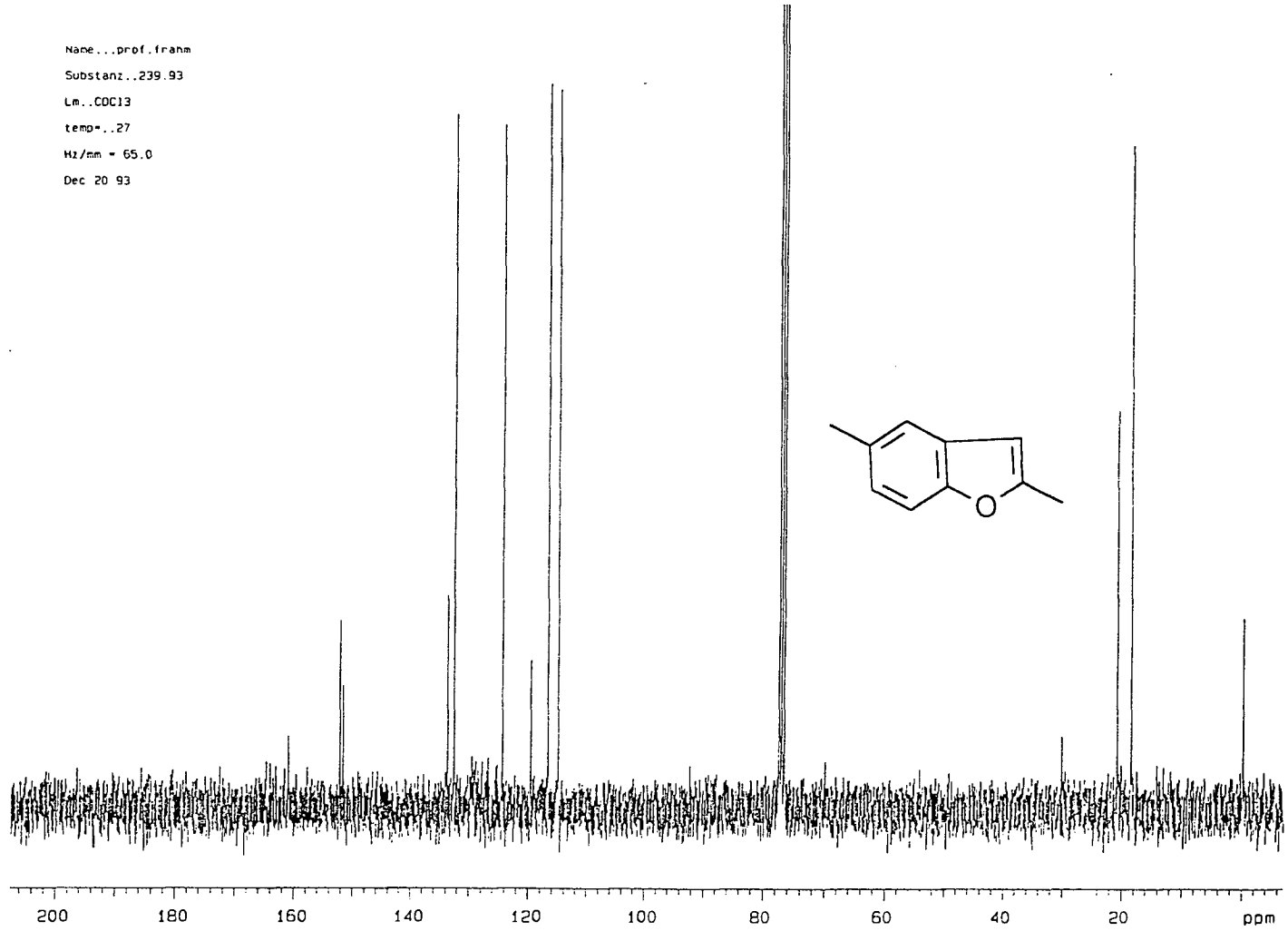


Fig. 4.05 : ^{1}H NMR spectrum of 15



Varian U-300

Fig. 4.06 : ^{1}H NMR spectrum of 21



Varian U-300

Fig. 4.07 : ^{13}C NMR spectrum of 21

Experimental :

Santonic acid **1** :

Santonic acid **1** was prepared from (-) - α -santonin **2** (5 gms, 0.02 mole) following the literature procedure[19] to furnish yellowish crystals (2.53 gms, 50%) m.p. 164°C (lit[19] 165°C).

Reaction of santonic acid 1 with CAN :

A solution of santonic acid **1** (0.5 gms, 0.002 mole) in acetonitrile (20 mL) was added to a solution of CAN (5.5 gms, 0.01 mole) in water (50 mL). After stirring the reaction mixture for 90 min. at room temp, it was extracted with CH_2Cl_2 (3 x 40 mL).The combined organic extracts were washed with saturated solution of $NaHCO_3$,water and dried over anhydrous sodium sulphate. The neutral fraction on concentration yielded a viscous mass (0.223 gms) which was chromatographed on silica gel. Elution with pet.ether - benzene (1:1) afforded white crystalline solid, recrystallised from ethanol to give **11** (35 mgs, 7%) m.p. 179°C.

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) (fig. 4.01) : 1.08 (3H, d, J=6.7 Hz, C4 - CH_3), 1.45 (3H, d, J= 6.4 Hz, C_{11} - CH_3), 1.46 (3H, s, C_{10} - CH_3), 1.85 - 1.93 (2H, m, C_9 - H),1.98 (1H, ddd, J=14.1, 11.2, 7.3 Hz, C_8 - H), 2.07 (1H, ddd, J=14.1, 8.7, 5.4 Hz, C_8 - H), 2.25 (1H, td, J= 3.9, 2.0 Hz, C_1 - H), 2.57 (2H, d, J=3.9 Hz, C_2- H), 2.61 (1H, dd, J=4.5, 2.0 Hz, C_5 - H), 2.87 (1H, qd, J=6.7,4.5 Hz, C_4 - H), 5.29 (1H, q, J=6.4 Hz, C_{11} - H).

Further elution with benzene gave another white solid, recrystallised from benzene - pet.ether to afford a mixture (4:6) of **13** and **12** (42 mgs) m.p. 104°C.

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) (fig. 4.02) : 1.11 (3H, d, J=6.7 Hz, C_4 - CH_3), 1.8 - 2.0 (4H, m, C_8 - H, C_9 - H), 2.06 (1H, td, J= 6.8, 1.8 Hz, C_1 - H), 2.33 (3H, s, C_{11} - CH_3), 2.45 (1H, dd, J=17.3, 6.8 Hz, C_2 - H_a), 2.83 (1H, qd, J=6.8,4.4 Hz, C_4 - H), 5.31 (1H, d, J=11.0 Hz, C_{12} - H), 5.42 (1H, d, J=17.3 Hz, C_{12} - H), 5.70 (1H, dd, J=17.3, 11.0 Hz, C_{11} - H).

Continuing elution with $CHCl_3$ - benzene (1:9) afforded third solid, recrystallised from benzene - pet.ether yielded **14** (38 mgs, 7%) m.p. 192°C.

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) (fig. 4.03) : 1.09 (3H, d, J=6.8 Hz, C_4 - CH_3), 1.35 (3H, d, J=6.6 Hz, C_{11} - CH_3), 1.46 (3H, s, C_{10} - CH_3), 1.85 - 1.95 (2H, m, C_9 - H), 2.00 (1H, ddd, J=14.4, 12.3, 6.6 Hz, C_8 - H), 2.60 (2H, d, J=4.0 Hz, C_2- H), 2.60 (1H, d, J=4.2 Hz, C_5- H), 2.86 (1H, qd, J=6.8,4.2 Hz, C_4 - H), 5.35 (1H, q, J=6.6 Hz, C_{11} - H).

^{13}C NMR (δ ppm, $CDCl_3$, 75 MHz, APT) (fig. 4.04): 11.8 (q, C4 - CH_3), 14.2 (q, C_{11} - CH_3), 22.2 (q, C_{10} - CH_3), 31.5 (t, C_2), 34.5 (t, C_8), 36.1(t, C_9), 41.7 (s, C_{10}), 43.3 (d, C_4), 48.0 (d, C_5), 58.0 (d, C_1), 80.0 (d, C_{11}).

Further elution with EtOAc gave another white solid, recrystallised from benzene to afford **15** (27 mg, 6%) m.p. 182°C

^{1}H NMR (δ ppm,$CDCl_3$,500 MHz) (fig. 4.05): 1.08 (3H, d, J=6.8 Hz, C_4 - CH_3), 1.27 (3H, d, J=6.3 Hz, C_{11} - CH_3), 1.45 (3H, s, C_{10} - CH_3), 1.8 - 2.0 (4H, m, C_8 - H, C_9 - H), 2.33 (1H, td, J= 6.3,1.8 Hz, C_1 - H), 2.52 (1H, dd, J=16.6, 6.3 Hz, C_2 - Hb),

2.58 (1H, dd, J=4.2, 2.1 Hz, C_5- H), 2.84 (1H, dd, J=16.6, 1.5 Hz, C_2 - Ha), 2.86 (1H, qd, J=6.8,4.2 Hz, C_4 - H), 4.01 (1H, q, J=6.3 Hz, C_{11} - H).

Lead tetra-acetate decarboxylation of santonic acid 1 :

Santonic acid **1** (1.7 gms,) was added to the mixture of cupric acetate (0.25 gms), pyridine (2 mL), lead tetra-acetate (4 gms) and dry benzene (100 mL). The reaction mixture was stirred under nitrogen for a period of 45 min. and then refluxed under nitrogen for further 90 min. Excess of lead tetra-acetate was decomposed by addition of ethylene glycol (15 mL) and the reaction mixture was taken up in benzene. It was then extracted with 5% aq. Sodium bicarbonate solution to remove unreacted santonic acid **1**. The neutral portion gave a semi solid residue (1.3 gms) which showed single spot on TLC. Elution of the compound through column using benzene as a solvent gave a white solid m.p. 50° which was identified as **12** on the basis of co-TLC and spectral data.

References :

1. Richardson W H, in : Oxidation in Organic Chemistry, Wiberg K B, Ed. Part A, Academic press, Ch. 4, (1965).
2. Ho T L, Synthesis, 347 (1973).
3. Wedekind E, Jackh I, J. Prakt. Chem, **139**, 129 (1934).
4. Hortmann A G, Daniel D S, J. Org. Chem, **37**, 4446 (1972).
5. Mehta G, Pandey P N, Ho T L, J. Org. Chem, **41**, 953 (1976).
6. Singh V, Raju B N S, Ind. J. Chem. **31B**, 725 (1992).
7. Naik U S, Paknikar S K, Bates R B, Comou F A, Tet. Lett. **28**, 5641 (1987)
8. Malik B L, Ph. D. Thesis, Goa University (1995).
9. Gopichand Y, Schmitz F J, Tet. Lett., 3641 (1978).
10. Naik U S, Ph. D. Thesis, University of Bombay (1987).
11. Divakar K J, Rao A S, Ind. J. Chem, **14B**, 704 (1976)
12. Welch S C, Hagan C P, Kim J H, Chu P S, J. Org. Chem, **42**, 2879 (1977)
13. Tada M, Moriyama Y, Tanahashhi Y, Takahashi T, Tet. Lett, 5251 (1972)
14. For cyclisation of γ-keto phenol to benzofuran, see Kalyanasundaram M, Rajagopalan K, Swaminathan S, Tet. Lett, **21**, 4391 (1980)
15. Carnduff J, Marks R B, Tet. Lett, 4073 (1975)
16. Alemagna a, Baldoli C, Buttero P D, Licandro E, Maiorana S, Synthesis, 192 (1987)
17. Andreev N A, Bunina-Krivorukova L I, Levashova V T, J. Org. Chem. USSR (Engl. Transl) 345 (1986)

18. Adams R, J. Am. Chem. Soc, **41**, 662 (1919)

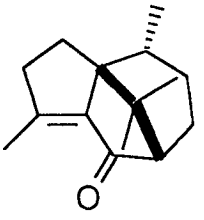
19. Woodward R B, Brutschy F J, Baer H, J. Am. Chem. Soc, **70**, 4216 (1948).

Summary

The thesis is divided in to four chapters which are further subdivided in to sections.

Chapter 1 is subdivided into three sections.

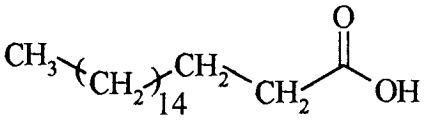
1.1 Several plant species belonging to the genus *Uvaria* (Annonaceae) have been subjected to chemical investigations during the past 20 years due to their medicinal properties. Some of the constituents of the genus *Uvaria* have now been shown to have various biological activities, e.g. uvaretin has been shown to possess inhibitory activity against P-388 lymphocytic leukemia[1]. *Uvaria narum* is abundantly available in the coastal regions of southern parts of Goa, Karnataka and Kerala. Though some work was reported on *uvaria narum* we chose this plant for chemical investigation because of its reported medicinal properties[2] and also due to the report on presence of a sesquiterpene ketone narcinone having a novel carbon skeleton. It was of interest to look for further sesquiterpenes sharing the carbon skeleton of narcinone. When our investigation was in progress, narcinone was shown to be identical with patchoulenone **1**[3]. A brief survey depicting the characteristic structural features of constituents of the genus *Uvaria* is presented.



1

1.2 This section describes the results obtained during the chemical examination of the leaves extract of *Uvaria narum.* A mixture of saturated alkanes

($C_{22}H_{46}$ to $C_{33}H_{68}$), steric acid **2**, steryl alcohol **3**, β-sitosterol **4** and stigmasterol **5** and benzoic acid have been shown to be the constituents.



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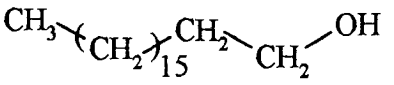

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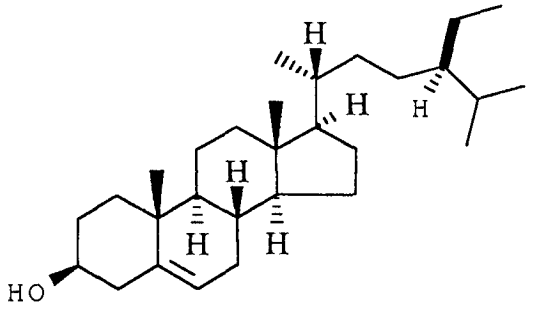

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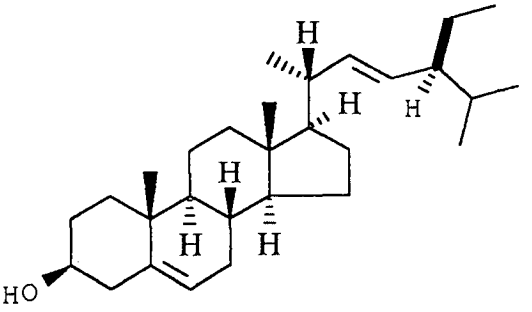
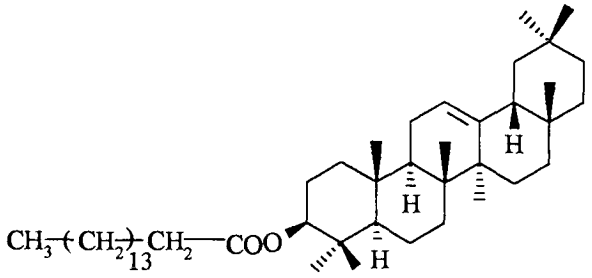


5

1.3 Chemical examination of the methanolic extract of the root bark of *Uvaria narum* resulted in the characterisation of β- amyrin palmitate **6**, germancyl palmitate **7**, methyl oleate **8**, methyl stearate **9**, glutinone **10**, patchoulenone **1**, glutinol **11**, taraxerol **12**, benzoic acid and β-sitosterol **4** and stigmasterol **5.** The methanol extract of the stem bark of *Uvaria narum* has been shown to contain lupeol based triterpenes. Twelve new terpenoids have been tentatively identified in the essential oil obtained from the root bark of *U.narum*. The details are presented in this section.



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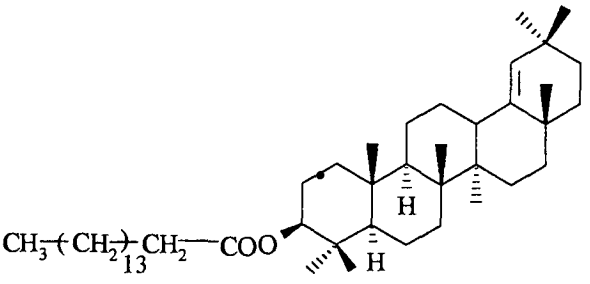

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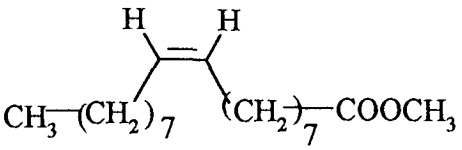

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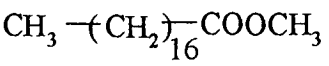

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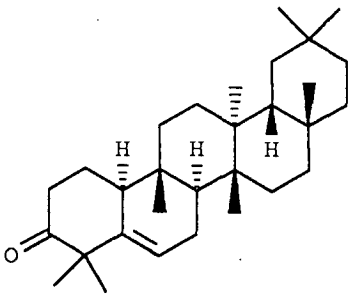

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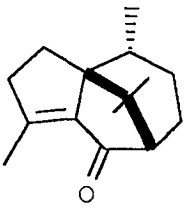

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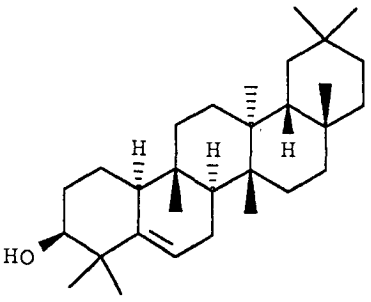

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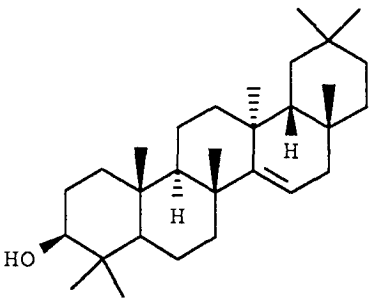
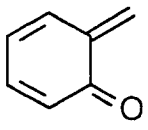


12

Chapter 2 is subdivided into three sections.

2.1 Benzopyranyl sesquiterpenes have been isolated from different species of genus *Uvaria.* These are considered to be biogenetically derived by 4+2 cycloaddition of o-benzoquinone methide **13** to the olefinic linkage of the corresponding sesquiterpenes. Alternatively these could be considered as the outcome of Michael type addition of olefins to o-benzoquinone methide **13** followed by ring closure. The sesquiterpenes, (-) tanzanene **14** and lucidene **15** were considered to be interesting target molecules for a biomimetic type synthesis.

A general method for the synthesis of benzopyranyl terpenoids has been developed during the present study. The new method is a simple, one pot reaction and yields the novel terpenoids in fair to good yields. Saligenin (o-hydroxy benzyl alcohol) **16** on heating with the unsaturated compounds in dry xylene at reflux temperature produces the benzopyranyl compounds. The synthesis of non-natural benzopyranyl terpenoids by using (-) limonene **17**, (-)-α-phellandrene **18**, camphene **19**, (-) zingiberene **20**, (-) caryophyllene **21** has been achieved. The product derived by thermal reaction of saligenin **16** with anethole **22** has also been well characterised. The structures of these synthetic products have been established by detailed spectroscopic analysis. It appears that under our experimental conditions, the conjugate acid of o-benzoquinone methide **23** is the reacting species rather than of o-benzoquinone methide **13** itself.



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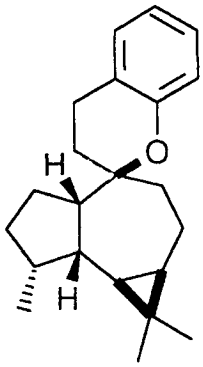

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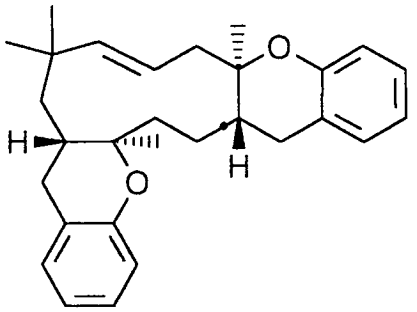

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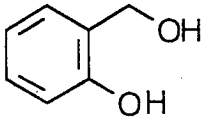

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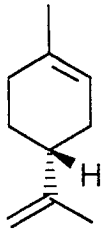

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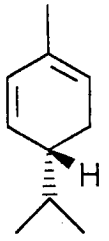

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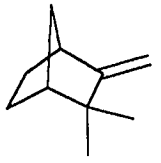

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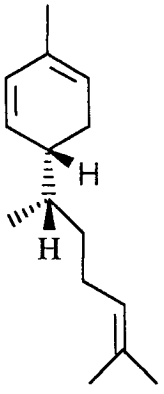

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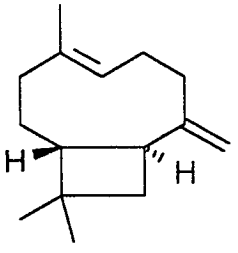

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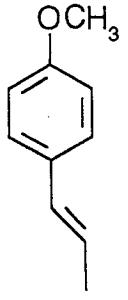

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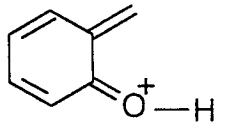
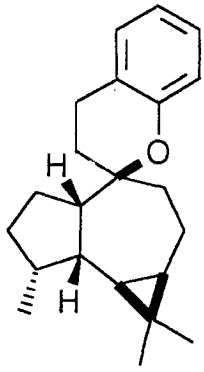


23

2.2 (-) Tanzanene **14**, a spirobenzopyranyl sesquiterpene isolated from *uvaria tanzania* Verde [4] looked to be an attractive target molecule for a biomimetic synthesis and checking the generality of the one pot synthetic procedure descried in the previous

section. Moreover, the absolute configuration was not established by Weenen et. al. There was no report on the synthesis of (±)-tanzanene or its optically active form. Heating (-)-alloaromadendrene **24** of known absolute configuration with saligenin in dry xylene at reflux temperature for 12 hrs. produced (-)-tanzanene in 45% yield. Identity of the synthetic product with natural product was established by direct spectral comparison, mixture melting point and co-TLC. The present study established the absolute configuration as shown in **14**.



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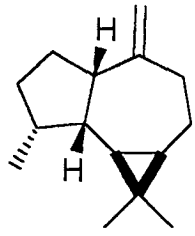
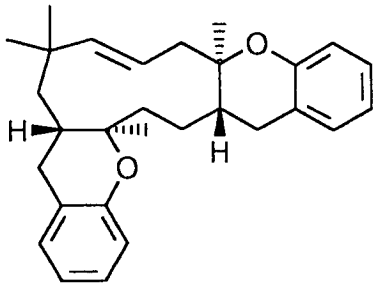


24

2.3 Lucidene **15**, a bis (benzopyranyl) sesquiterpene isolated from root bark of *uvaria lucida* ssp lucida [5]. The structure assigned is based on detailed spectroscopic and X-ray analysis. It is evident that lucidene is derived by addition of two C_7 units to humulene **25**. In the present study lucidene has been synthesised. It appears that reaction is stepwise as we could isolate and fully characterise the intermediate **26** which we have named semilucidene. Some interesting observations have been made as the X-ray analysis confirmed the structure of synthetic lucidene and

showed that the synthetic compound used for X-ray analysis is a single enantiomer as against the racemic nature of natural lucidene. The details are presented in this section.



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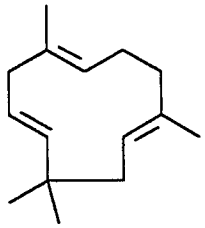

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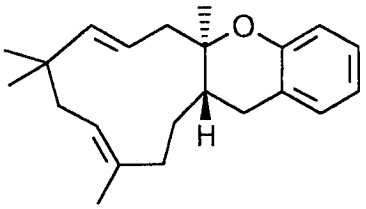
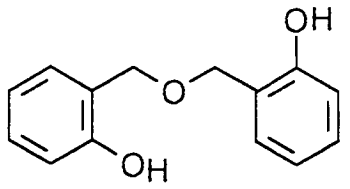


26

Chapter 3 This chapter is divided in to four sections and deals with the synthesis of four natural products.

3.1 Heating of saligenin with olefins results in the formation of benzopyranyl compounds or C-ortho hyrdoxy benzylated derivatives. The reaction product always contain a product derived from two molecules of saligenin. This was confirmed by refluxing saligenin alone in dry xylene and isolating the product. The product has been found to be a bis benzyl derivative **27**. Methylation of **27** with DMS/NaOH gave dimethyl ether **28**, a constituent of *uvaria chamae*[6]. This section also describes the characterisation of a product obtained by heating saligenin and resorcinol in dry xylene at reflux temperature.



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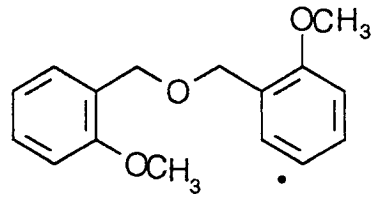
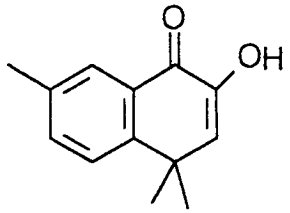


28

3.2 Bohlin and co-workers[7] reported the isolation of a biologically active C_{13}-diosphenol **29** from *Ipomoea-pes-caprae* (L) R.Br. (convolvulaceae). The structure **29** assigned to the diosphenol clearly shows that it is a degraded sesquiterpene derived by the loss of two carbon atoms of the drimane skeleton. Now we have synthesised this compound in five steps from commercially available β-ionone **30**. The details of this synthetic work along with the biogenesis will be presented in this section.



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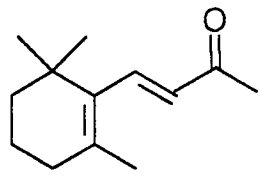

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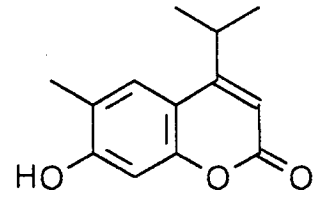
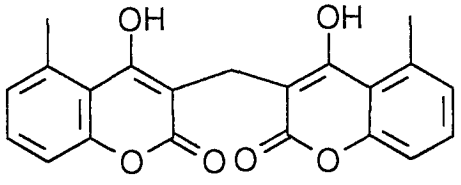


31

3.3 There are only three naturally occurring coumarins which contains an isopropyl group at C_4 position. A cursory look at the proposed structures showed that these are degraded sesquiterpenes. Synthesis of 7-hydroxy- 4 -isopropyl- 6-methyl coumarin **31** a natural product isolated by Satake and coworkers[8] from

macrothelpteris torresiana CHING var calvatta HOLTT has been achieved and the details will be described in this section.

3.4 Two minor and unidentified compounds previously isolated by Natori and co-workers[9] from *Diospyros kaki* THUNB and *Diospyros kaki* THUNB *var sylvestris* MAKINO have been now shown to be derivatives of 4-hydroxy-5-methyl coumarins. One of these has been shown to be identical with gerberinol **32** and the structure **33** has been assigned to the other compound. The assigned structure is confirmed by synthesis.



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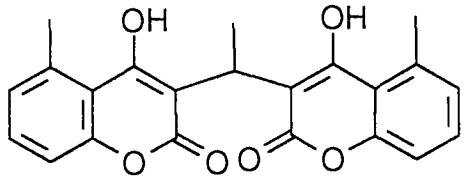
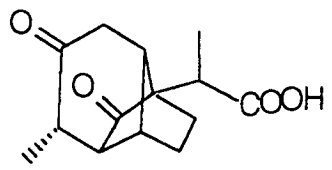


33

Chapter 4 This chapter is divided in to two sections and deals with the characterisation of compounds derived by oxidation of santonic acid **34** with ceric ammonium nitrate (CAN) structures **35**, **36**, **37**, **38** and **39** structure assigned to a product previously obtained in our laboratory by reaction of epoxide **40** with BF_3-etherate has been shown to be 2,5-dimethyl benzofuran **41** . A rational mechanism involving a molecular rearrangement is proposed to explain the formation of 2,5-dimethyl benzofuran **41** from epoxide **40**.



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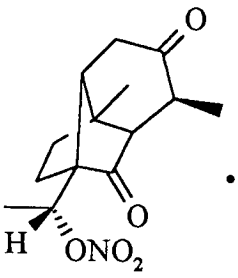

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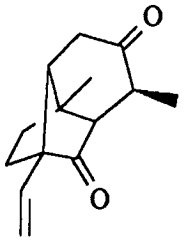

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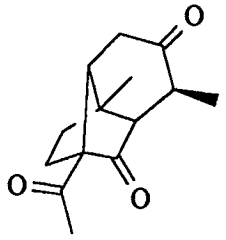

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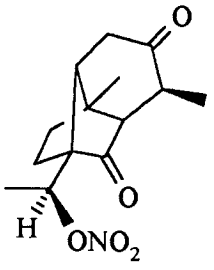

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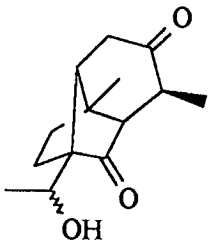

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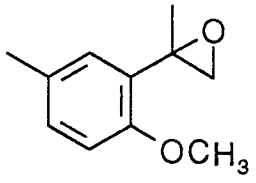

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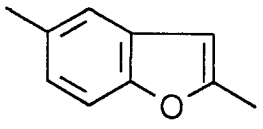

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References :

1. Cole J R, Torrance S J, Wiedhoph R M, Arora S K, Bates R B, J. Org. Chem., **41**(10), 1852, (1976).
2. Nanda M, Narayana C S, Kulkarni D R, Iyengar M A, Indian Drugs, **23**(12), 647, (1986).
3. Hisham A, Wray V, Pieters L, Claeys M, Dommisse R, Vlietinck A, Mag. Res. Chem., **30**(4), 295, (1992).
4. Weenen H, Nkunya M H H, Mgani Q A, Posthumus M A, Waibel R, Achenbach H, J. Org. Chem., **56**(20), 5865, (1991).
5. Weenen H, Nkunya M H H, El-Fadl A A, Harkema S, Zwanenburg B, J. Org. Chem. 55(17), 5107, (1990).
6. Lasswell W L, Hufford C D, Phytochem., **16**, 1439, (1977).
7. Pongprayoon U, Baeckstrom P, Jacobson U, Lindstorm M, Bohlin L, Planta Med., **57**, 515, (1991).
8. Hori K, Satake T, Saiki Y, Murakami T, Chen C M, Yakugaku Zasshi, **107**(7), 491, (1987).
9. Tezuka M, Kuruyanagi M, Yoshihira K, Natori S, Chem. Pharm. Bull., **20**(9), 2029, (1972).