ISOLATION AND SYNTHETIC STUDIES ON NATURAL PRODUCTS

A THESIS SUBMITTED TO THE

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IN

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By

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DONA PAULA - GOA

1990

DEDICATED

TO

MY PARENTS

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 the thesis. The experimental work has been carried out independently and due acknowledgement has been made whenever outside facilities have been availed of.

(S.K. PAKNIKAR)
RESEARCH GUIDE

Jivexa c. Patel
(JIVEXA C. PATEL)
CANDIDATE

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ACKNOWLEDGEMENT

It is indeed a pride for me to have worked under Prof.S.K.Paknikar. I have a deep sense of gratitude for his astute guidance, inspiring thought and constant encouragement during the course of my work.

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Jivexa C. Patel

MAY 1990.

Jivexa C.Patel

GENERAL REMARKS

1. All the figure numbers, chart numbers, scheme numbers, structure numbers and references in a chapter refer to that particular chapter only.

2. Organic extracts were dried over anhydrous Na_2SO_4 unless otherwise stated.

3. Petroleum ether refers to the fraction boiling between the range 60-80°C unless otherwise mentioned.

4. Silica gel used for column chromatography was of 60-120 mesh size and activated at 110°C for 5 hours before use.

5. Thin layer chromatography was done on glass plates (20 x 20 cms.) coated with 0.25 mm. layer of TLC grade silica gel with 13% $CaSO_4$ as binder. Visualization of spots was done by spraying the plate with alcoholic solution of phosphomolybdic acid and warming the plate at 100°C for 10 minutes unless otherwise stated.

6. All melting points and boiling points are uncorrected and recorded in $^{\circ}$C.

7. Spectral data reported for various compounds were mainly obtained through the courtesy of various institutions.

The details regarding the instruments are therefore not given. These has been suitably acknowledged.

8. The chemical shifts parameters in the ~~P~~^{1}HNMR, ^{13}CNMR spectra are expressed in `δ' ppm, with TMS as internal standard.

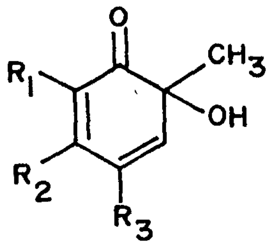
9. All known compounds were identified by direct comparison of the spectral data and physical constants reported in the literature. Molecular formulas of the compounds were assigned on the basis of molecular weight by mass spectroscopy.

CHAPTER-I SECTION-I

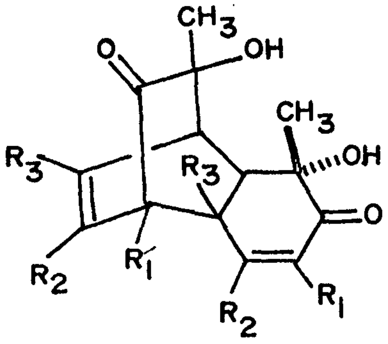
USE OF MONOTERPENE PHENOLS FOR THE SYNTHESIS OF (±)-3, 10-DIHYDROXYDIELMENTHA-5, 11-DIENE-4, 9-DIONE AND RELATED COMPOUNDS

The reaction of a diene with a dienophile to form an adduct was first discovered by Diels and Alder[1]. In this reaction 1,4 addition of a dienophile to a conjugated cisoid diene (a 4 + 2 cycloaddition) results in the formation of an adduct which is always a six membered ring. The reaction which is now called Diels-Alder reaction, is a well-known and useful method for the laboratory preparation of a large number of interesting organic molecules. No attempt is made here to review the literature on the Diels-Alder reaction, as it would be beyond the scope of the present thesis. Moreover, the chemistry, stereochemistry and synthetic applications of this reaction are available in monographs and the review articles[2-13]. However, the literature which has a direct bearing on the present investigation is delineated below to facilitate the understanding and appreciation of the research work done.

Twenty two years prior to the first detailed investigation of the Diels-Alder reaction, Wieland[14] had reported the thermal formation of 1:1 dimerisation products of certain selected dienes. These observations of Wieland have been rationalised as Diels-Alder self dimerisations where one molecule acts as diene and the other as dienophile. Subsequently, it was found[15] that oxidation of 2,4-, 2,5- and 2,6-dimethylphenol as well as mesitol produces the corresponding 6-hydroxy-6-methyl 2,4-cyclohexadienones ("o-quinols",1a-d) which cannot be isolated but undergo self dimerisation to give o-quinol dimers (2a-d). It was further found that o-quinol methyl ethers if produced by oxidation of a phenol in methanol cannot also be isolated and undergo self Diels-Alder dimerisation[16]. Interestingly while the "o-quinols" or "o-quinol methyl ethers" cannot be isolated, o-quinol acetates" eg (3) have been isolated and characterized[15].



1a-d



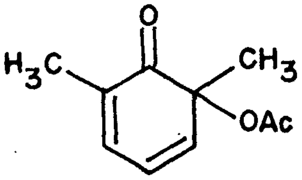
2a-d

1a,2a-$R_3=CH_3;R_1=R_2=H$

1b,2b-$R_2=CH_3;R_1=R_3=H$

1c,2c-$R_1=CH_3;R_2=R_3=H$

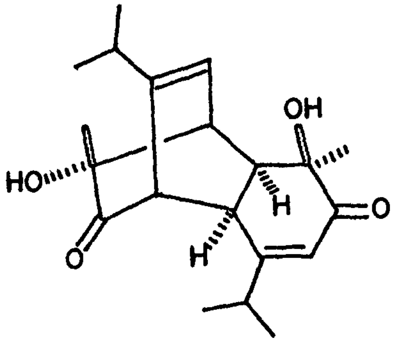
1d,2d-$R_1=R_3=CH_3;\ R_2=H$



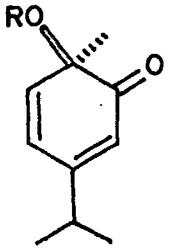
(3)

Though the self dimerization of o-quinols was observed in laboratory first by Wieland in 1906 and the mechanistic aspects were clarified by Diels and Alder in 1928, the natural occurrence of such o-quinol dimers was discovered first in the form of maytenone (36, entry 22, Table-1), a dimeric diterpenoid isolated from the root bark of *Maytenus dispermus*[17]. During the last three decades, a large number of natural products which are presumed to be formed biogenetically by involving of a Diels-Alder reaction, one molecule acting as a diene and the other as a dienophile have been isolated from various terrestrial plants. These are listed in Table-1.

The work reported in this section concerns with the biogenetic type synthesis of (±)3,10-dihydroxy-dielmentha-5,11-diene-4,9-dione (7a) a bismonoterpenoid isolated from the heartwood of *Callitris macleayana*[18]. The structure of this bismonoterpenoid was established by Carman and co-workers on the basis of spectroscopic data, chemical degradation and synthesis[19] of the racemic form. The structure assignment was further confirmed through a single crystal x-ray analysis. The absolute configuration as shown in (7a), was obtained from c.d. measurements.



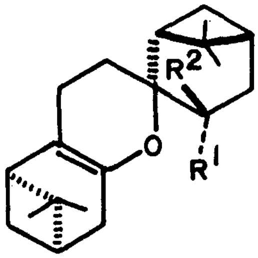
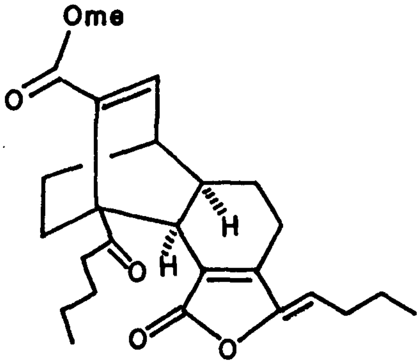
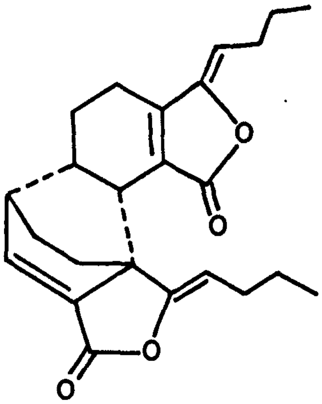
(7a)

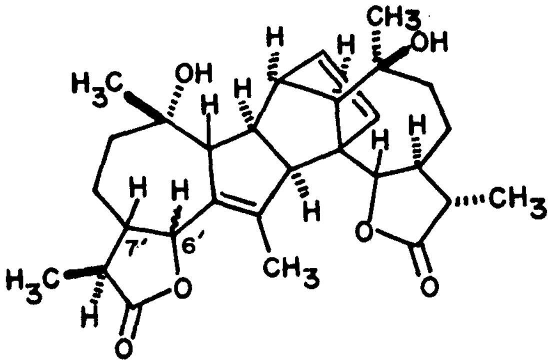
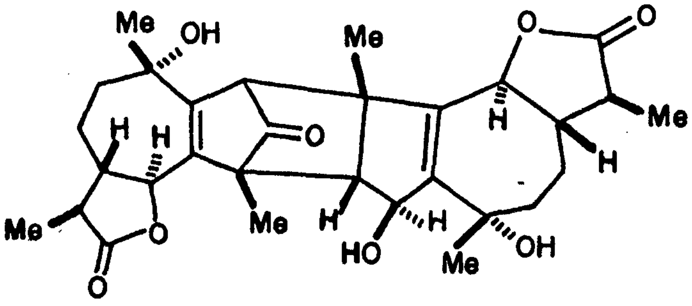
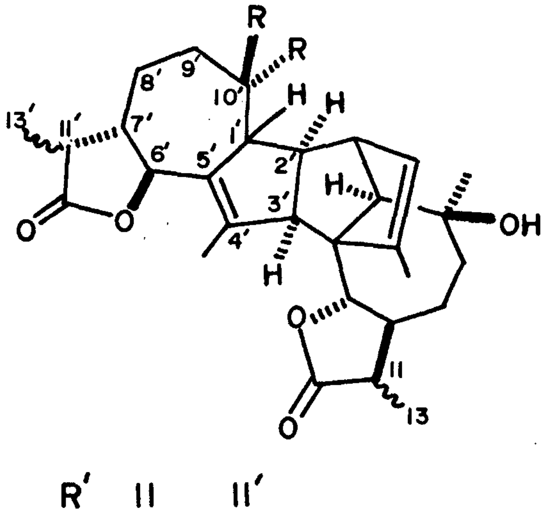


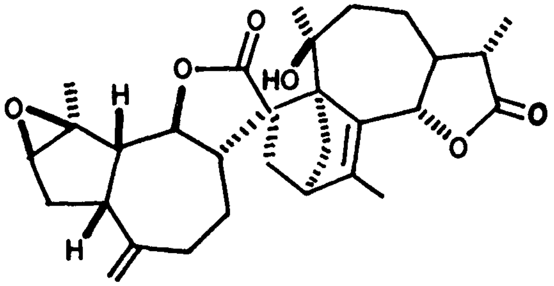
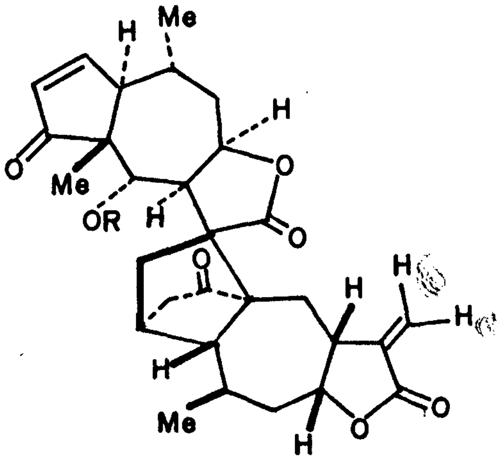
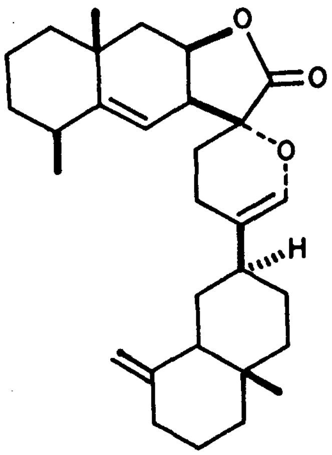
(4), R=H
(5), R=COMe

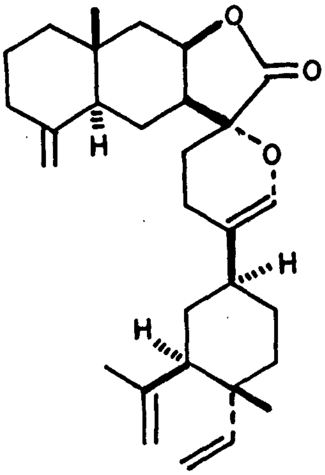
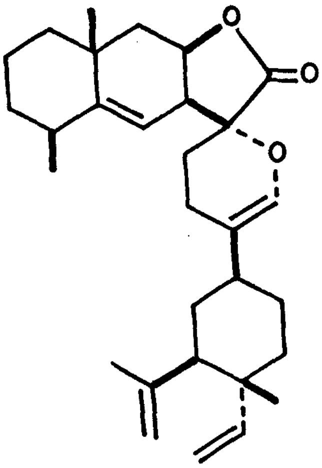
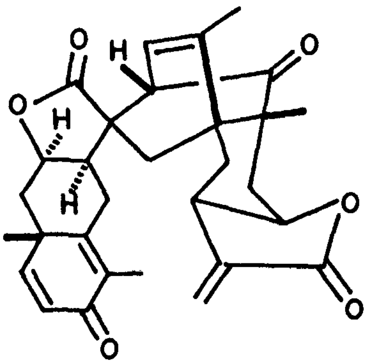
Naturally occuring Diels Alder type dimers

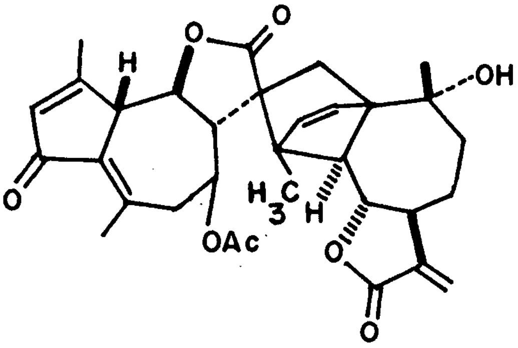
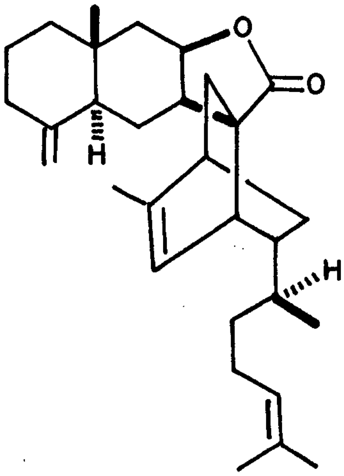
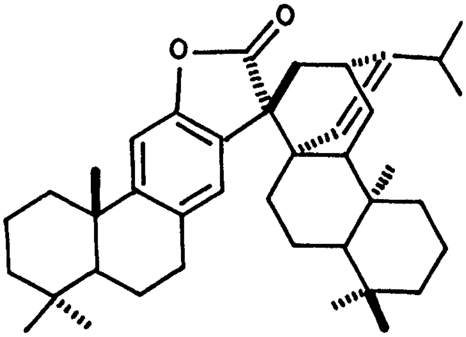
Table -1

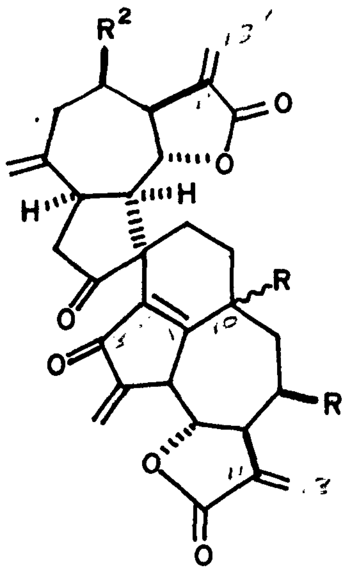
Sl. No.	Structure	Source	Ref.
1	 1 - R1, R2=O	Cedronella canariensis $C_{20}H_{28}O_2$.	33
2	 2 - Wallichilide	Ligusticum wallichi $C_{25}H_{32}O_5$	34
3	 3 - Diliguitilide	Ligusticum wallichi $C_{24}H_{28}O_4$	35

4	 4 α-$H_{6'}$ Absinthin 5 β-$H_{6'}$ Isoabsinthin	Artemisia absinthium $C_{30}H_{40}O_6$	36
5	 6 - Absintholide	Artemisia absinthium $C_{30}H_{38}O_8$ $[\alpha]_D^{25}$ +127° ($CHCl_3$)	37
6	 R R′ 11 11′ 7 Me OH βH αH Absinthin 8 Me OH αH αH 11-Epiabsinthin 9 OH Me βH βH 10′,11′-Epiabsinthin 10 OH Me αH βH -11,10′,11′-Epiabsinthin	Artemisia sieversiana $C_{30}H_{40}O_6$	38

7	 11-Artesieversin	Artemisia sieversiana $C_{30}H_{36}O_5$	38
8	 12 - R=H, Microlenin	Helenium microcephalum $C_{29}H_{34}O_7$ $[\alpha]_D^{22}$ +10° (pyridine)	39
9	 13	Helenium autumnale L. roots $C_{30}H_{42}O_3$	40

10	 14	Helenium autumnale $C_{30}H_{42}O_3$	40
11	 15	Helenium autumnale $C_{30}H_{42}O_3$	40
12	 16 - Fruticolide	Ferreyranthus fruticosus $C_{30}H_{32}O_6$	41

13	 17- Artanomaloide	Artemisia anomala $C_{32}H_{36}O_8$	42
14	 18	Holenium autumnale roots [illegible] $H_{44}O_2$	43
15	 19	Dysoxylum lenticellare [illegible]	44

<table>
<tr><td>16a</td><td>
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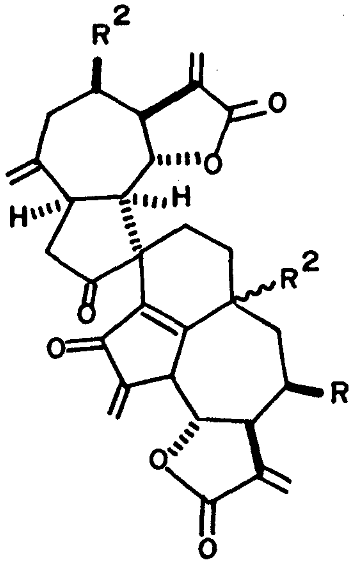
<tr><td></td><td>20</td><td>21</td><td>22</td><td>23</td><td>24</td><td>25</td></tr>
<tr><td>R</td><td>αH</td><td>αH</td><td>αOH</td><td>αOH</td><td>αH</td><td>αH</td></tr>
<tr><td>R¹</td><td>H</td><td>OH</td><td>H</td><td>OH</td><td>H</td><td>H</td></tr>
<tr><td>R²</td><td>H</td><td>H</td><td>H</td><td>H</td><td>H</td><td>H</td></tr>
</table></td><td>Aerial parts of Gochnatia polymorpha
20, [illegible] O6
21, 23, 24 ([illegible] 11,1[illegible] dihydro) C30 H38 O7
23, C30 H3[illegible] O[illegible]
2[illegible] (11,1[illegible] & 11',13' dihydro) C30 H[illegible] [illegible]</td><td>45</td></tr>
<tr><td>16b</td><td>
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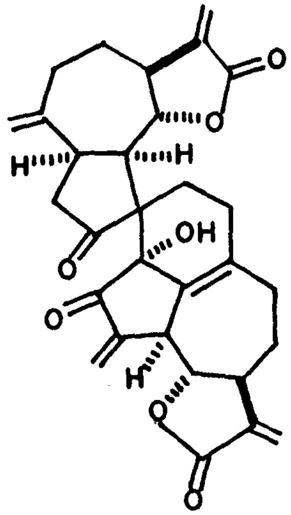
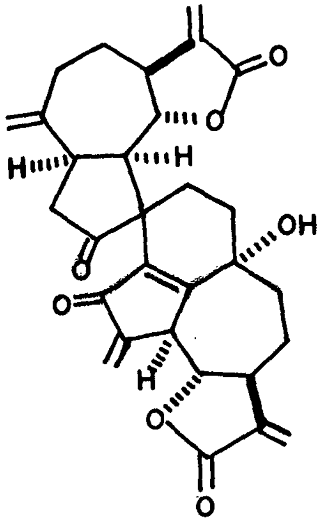
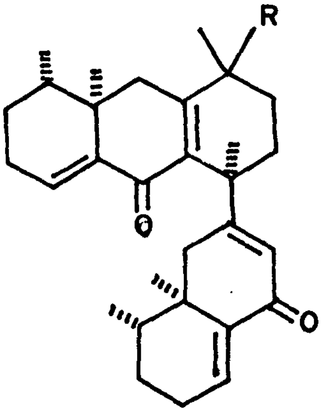
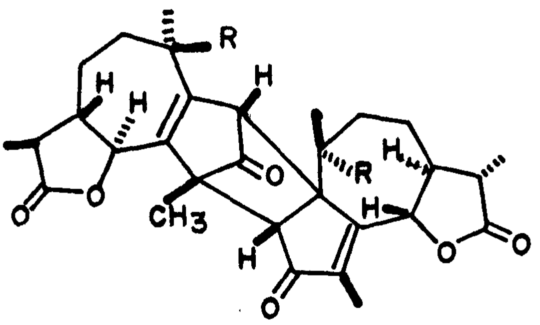
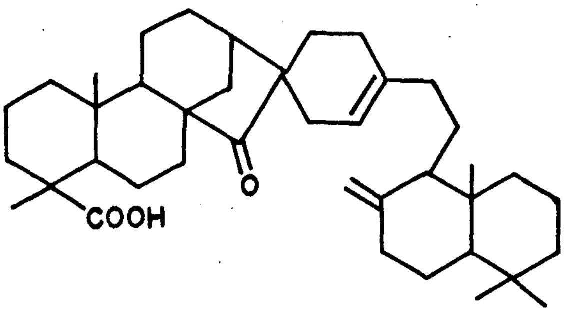
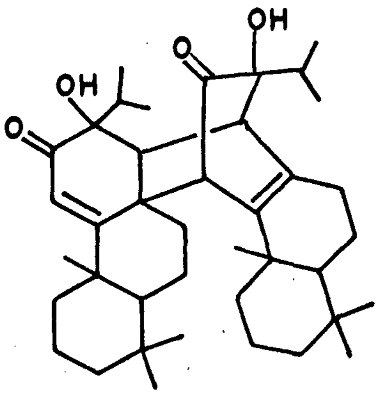
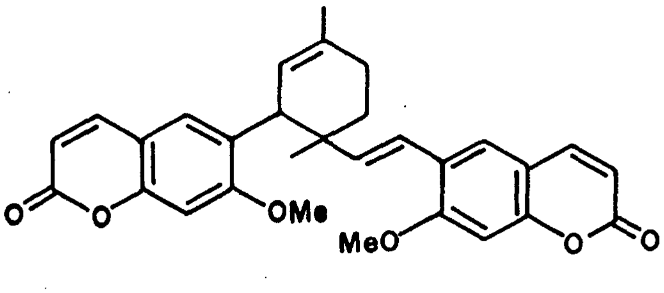
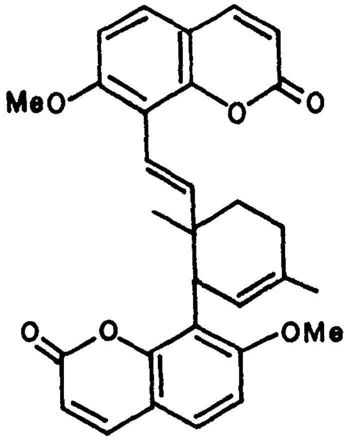
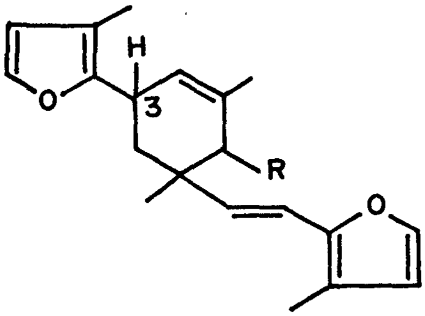
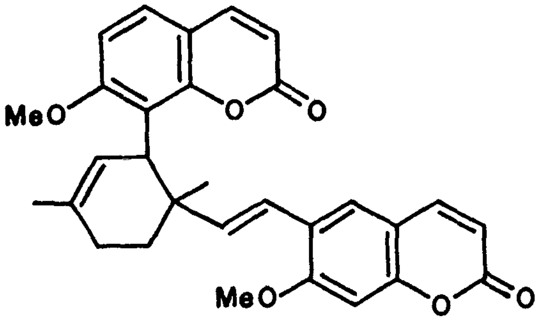
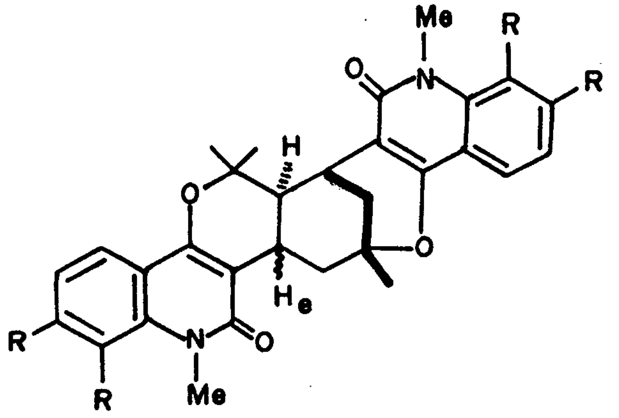
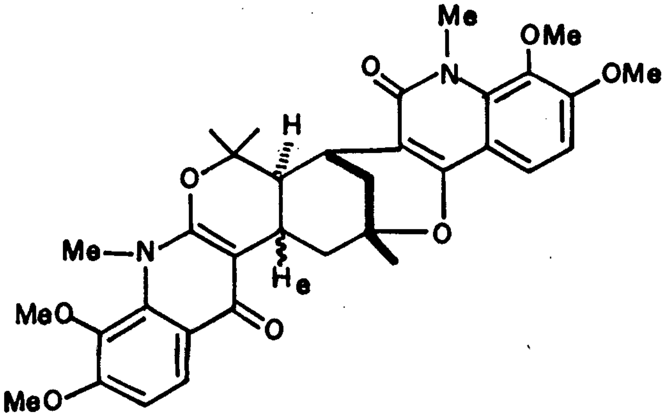
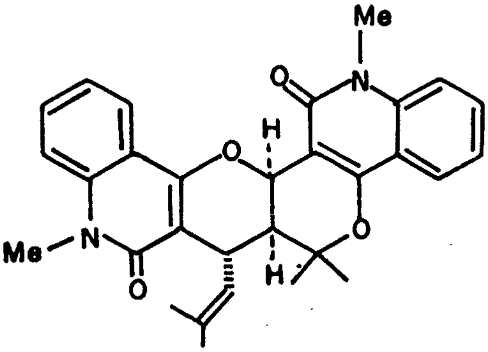
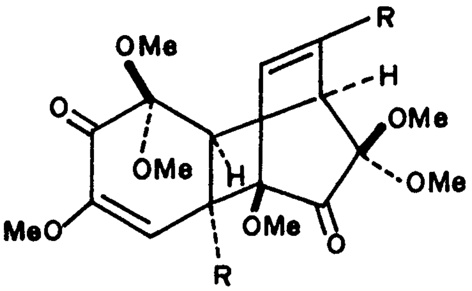
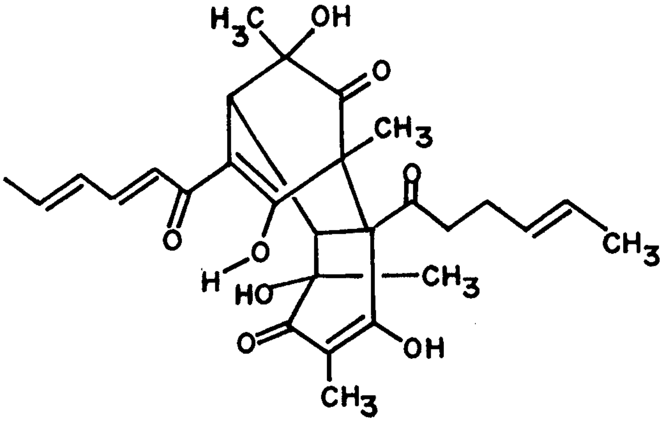
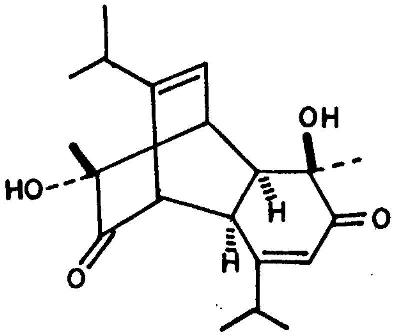
<tr><td>R</td><td>βH</td><td>βH</td><td>βH</td><td>βH</td></tr>
<tr><td>R¹</td><td>H</td><td>H</td><td>OH</td><td>OH</td></tr>
<tr><td>R²</td><td>H</td><td>OH</td><td>H</td><td>OH</td></tr>
</table></td><td>Aerial parts of Gochnatia hypoleuca
29 [illegible] O7</td><td>45</td></tr>
</table>

17	 30- Gochnatiolide B	Gochnatia paniculata $C_{30}H_{30}O_7$	46a 46b
18	 31- Gochnatiolide A	Gochnatia paniculata $C_{30}H_{30}O_7$	46a 46b
19	 32 - R = ∝H 33 - R = ∝OH	Eremophila mitchelli	47

20	 34 - R = OAc	Artemisia absinthium $C_{34}H_{40}O_{10}$ $[\alpha]_D + 40^{\circ}$	48
21	 35 Acutifloric acid	Stem bark of Xylopia acutiflora $C_{40}H_{60}O_3$ $[\alpha]_D - 127^{\circ}$	49
22	 36 - Maytenone	Maytenus dispermus	17
23	 37 - Thamnosin	Thamnosma montana (Aerial part, Bark) $C_{30}H_{28}O_6$	50

24	 38-Phebalin	Phebalium nudum Hook (Bark) $C_{30}H_{28}O_6$	51
25	 39- Diclausenan A 40- Diclausenan B Stereomers at C_3	Clausena willdenovii $C_{20}H_{24}O_2$	52
26	 41- Isothamnosin A	Ruta tene (Aerial part) $C_{30}H_{28}O_6$	53

27	 42 - α H_e , R = Ome Vepridimerin A 43 β H_e , R = Ome Vepridimerin B	Araliopsis tabouensis (Bark)	54
28	 44 - α H_e Vepridimerine C 45 - β H_e Vepridimerine D	Araliopsis tabouensis (Bark)	54
29	 46 - Paraensidimerin D	Euxylophora paraensis $C_{30}H_{30}N_2O_4$	55

30	 $R = CH_2CH{=}CH_2$ 47 - Asatone	Asarum taitonense hayata $C_{24}H_{32}O_8$	27
31	 48 - Bisvertinoquinol	Culture medium of Verticillium intertextum $C_{28}H_{34}O_8$ $[\alpha]_D^{20}$ + 329°	56
32	 49 - 3,10 - Dihydroxydielmentha 5,11 - Diene -4,9 - Dione	Callitris macleayana	18

Once the structure (7a) was established beyond doubt*, Carman and co-workers realised its biogenetic origin through self Diels-Alder type dimerisation of o-quinol (4).

Carman and co-workers considered o-quinol acetate (5) to be the suitable starting material for the synthesis (scheme-1), as it was obtained earlier[20] by lead tetraacetate oxidation of carvacrol (6).

Initially an attempt was made to dimerize the acetate itself, the acetyl derivative (7) of the dimer (7a) then would give the Callitris macleayana dimer on hydrolysis. The o-quinol acetate (5) did not dimerise under a variety of conditions. In view of this, attempts were made to hydrolyse the acetate (5) to o-quinol (4). Both alkaline and acidic conditions led to the rearrangements.

O-quinol acetate when refluxed with ethanolic sulfuric acid gave a dimeric indan (8) and not the desired o-quinol (4) or its self dimerisation product (7a), referred as Carman's dimer throughout this section. The formation of indan (8) has been rationalized as shown in (scheme-2).

A range of alkaline hydrolytic conditions also failed to produce the desired o-quinol (4) but resulted in the

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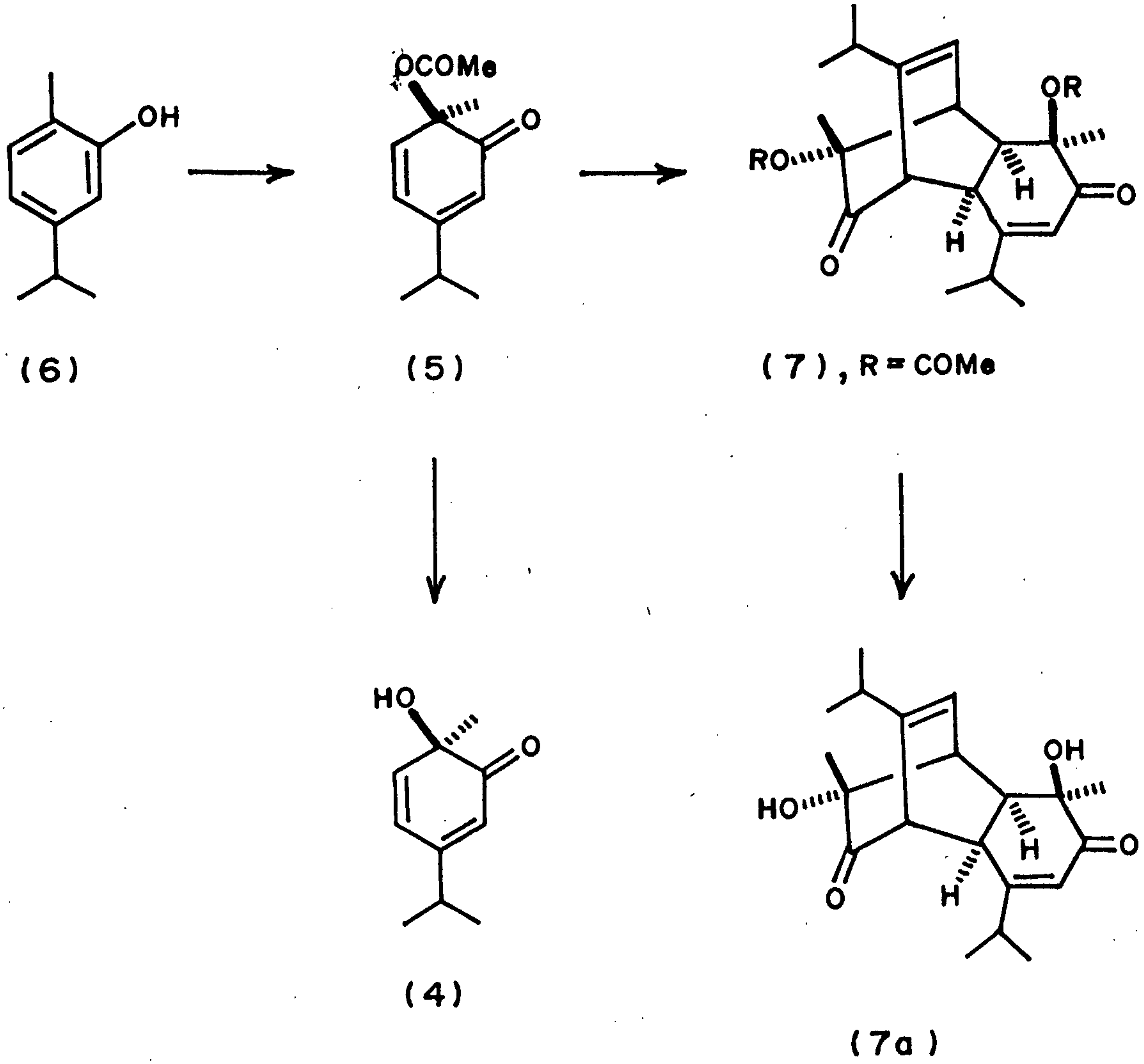
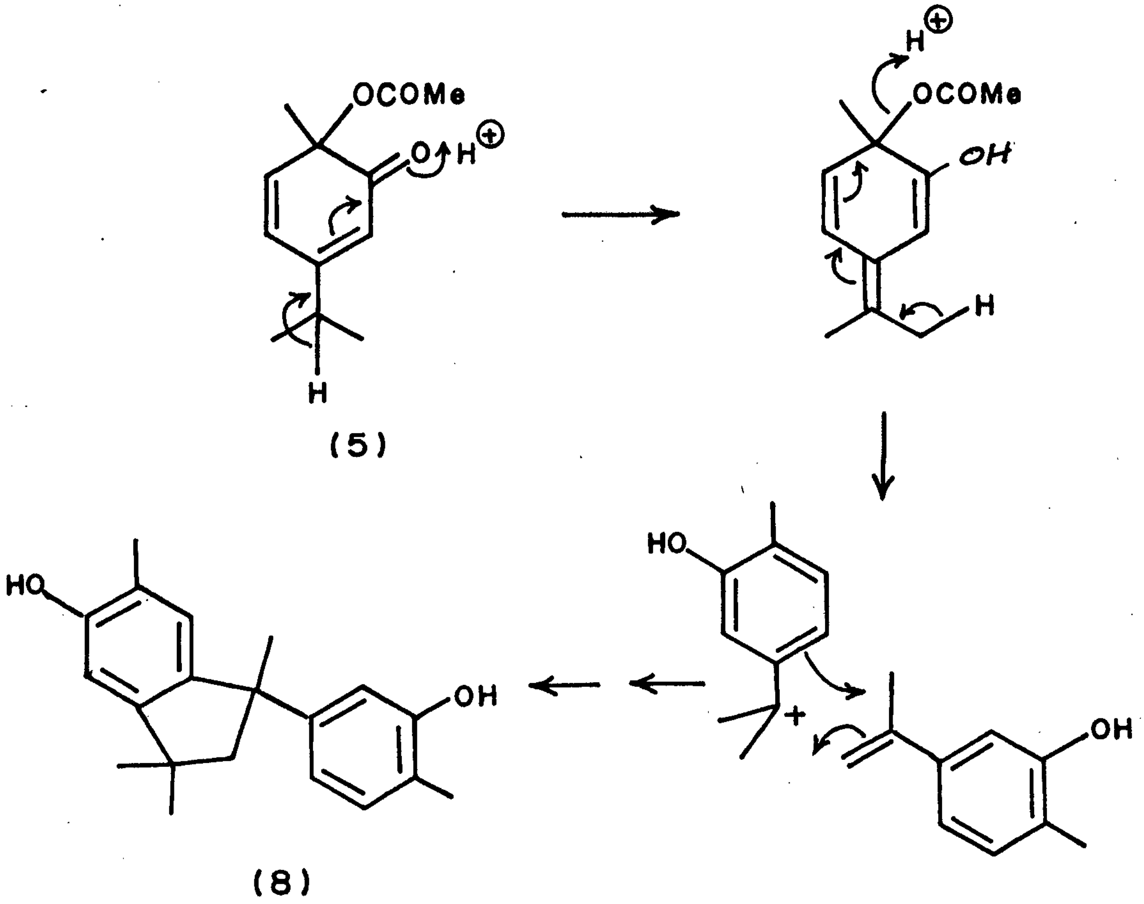
* Initially the natural product was considered to be a diterpenoid.



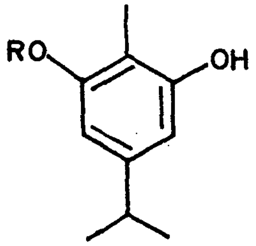
Scheme – 1



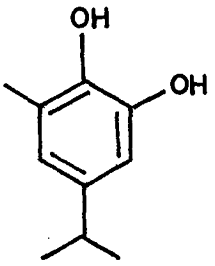
Scheme - 2

formation of two cyclopentene carboxylic acid derivatives (9) and (10) together with the acyclic ester (11). A plausible route for the formation of (9,10,11) from (5) is given in (scheme-3)[20a].

Considerable effort was expended in achieving the Diels-Alder dimerisation (5 → 7). The o-quinol acetate (5) with either tris (p-bromophenyl) ammonium hexachloroantimonate or $HSbCl_6$ at room temperature gave acetoxy migrated acetate (12)[19]. Refluxing with 50% sulfuric acid produced aromatic diols (13) and (14).



(12), R=COMe
(13), R=H



(14)

Finally, refluxing ethanolic sulfuric acid (50 hr) under the conditions of Metlesics and Wessely* afforded

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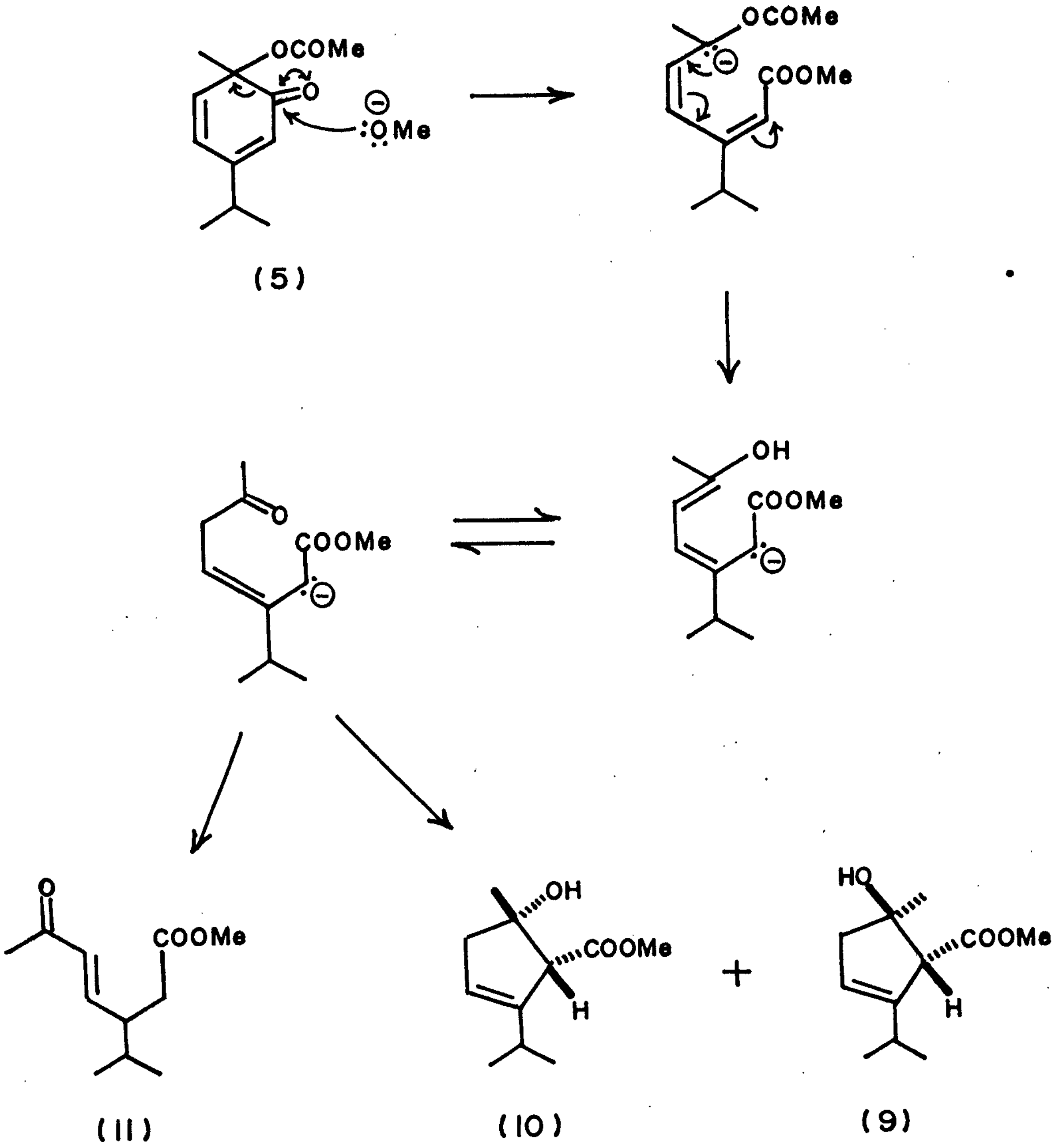
* A mixture of acetate (5 ,4.0 g), ethanol (100 ml) and ethanolic sulfuric acid (5%, 10 ml) was refluxed (50 hr). Usual work-up gave dimer (7a) and the indan (8).


OCOMe
O
⊖
:OMe
(5)
OCOMe
⊖
COOMe
OH
COOMe
⊖
O
COOMe
⊖
O
COOMe
(11)
OH
COOMe
H
(10)
+
HO
COOMe
H
(9)

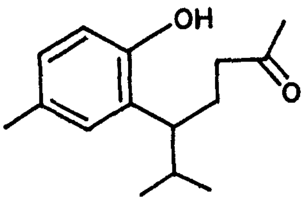
Scheme – 3

dimerisation to give the Carman's dimer (7a $\sim$ 30%) together with indan (8). Carman and co-workers consider that the dimerisation process is acid catalysed and involves non-identical compounds (4), and (5).

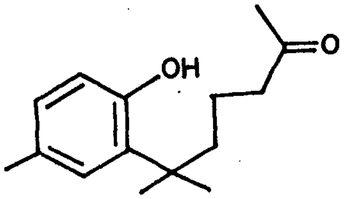
We, however consider that under the hydrolytic conditions of Metlesics and Wessely, o-quinol acetate (5) gets in part hydrolysed to the highly reactive o-quinol (4) which rapidly undergoes Diels-Alder self dimerisation.

Present study:

One of the ongoing research project from this laboratory concerns with the synthesis of modified terpenoids. Biogenetic type syntheses of sesquichamaenol (15), himasecolone (16) have been achieved^{20b}. Few years back

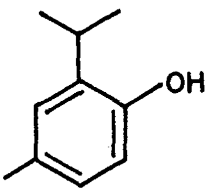


(15)

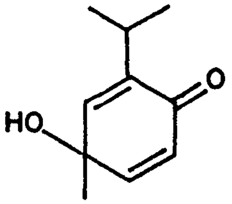


(16)

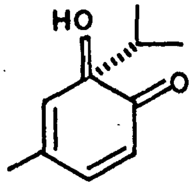
periodic acid oxidation of isothymol (17) was studied as a model compound for the preparation of p-quinol (18). A dimeric compound was obtained in this reaction, but was not investigated further. Since o-quinols are also obtained by oxidation of o-alkyl substituted phenols with periodic acid the dimer was considered to be derived by self-dimerisation of o-quinol (19). This information suggested a biogenetic-type synthesis of Carman's dimer (7a) by oxidation of carvacrol (6) under these set of conditions*. In contrast to the observations of Carman and co-workers concerning the difficulties in effecting the Diels-Alder dimerisation of o-quinol acetate (5), we could get the desired Carman's dimer by oxidation of carvacrol (6) with iodic acid[21]. The oxidation of carvacrol (6), thymol (20),4-methyl thymol (21),



(17)



(18)



(19)

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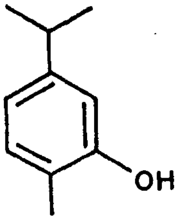
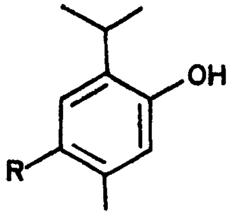
* The synthesis of (7a) was initiated and completed independently prior to our knowledge of the synthesis of (7a) by Carman and co-workers.

4-ethyl thymol (22), has been carried out using iodic acid and sodium metaperiodate as oxidising agents[21,22]. The



(6)



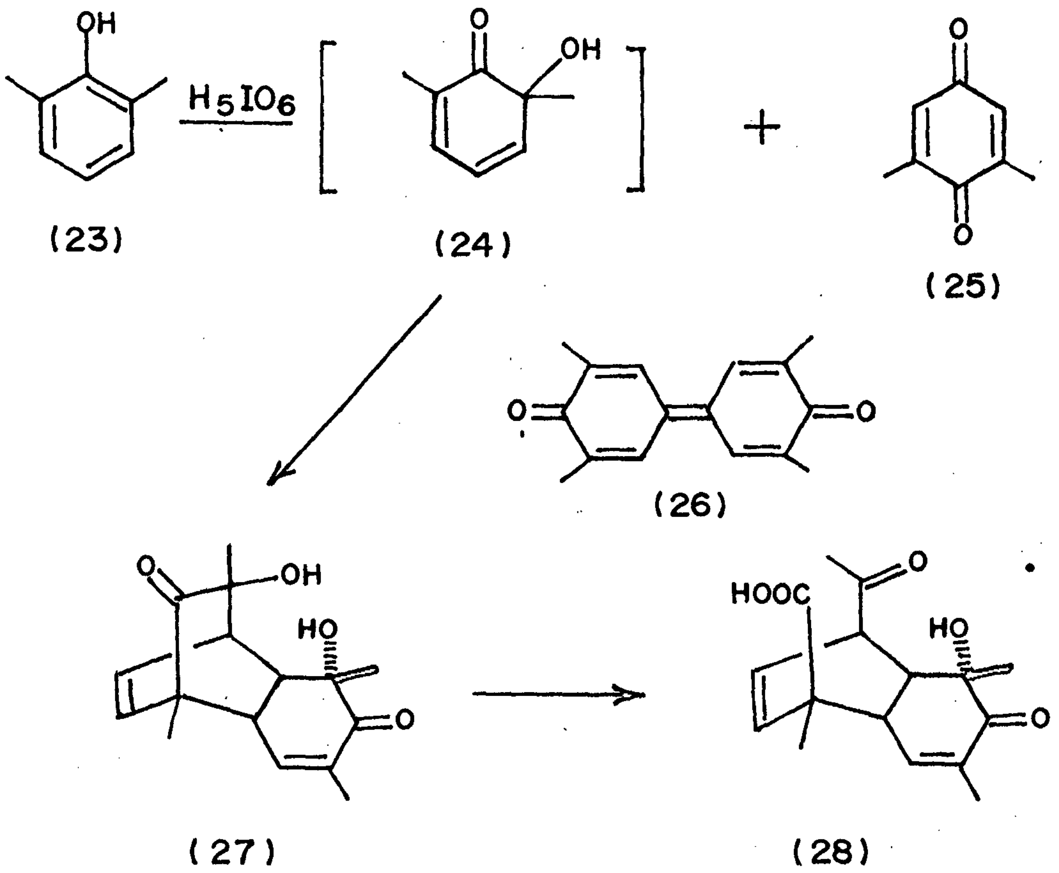
(20), R = H

(21), R = CH_3

(22), R = C_2H_5

structure of the dimer obtained by oxidation of isothymol (17) has been established. It has also been found that these dimers undergo intramolecular ($\pi 2 + \pi 2$) cycloaddition reactions. The details of this investigation are presented herein.

It is known[16] that oxidation of phenols which are unsubstituted at p-position when oxidised with periodic acid gives a complex mixture and separation of individual components becomes tedious, e.g. oxidation of 2,6-dimethyl phenol (23) gives dimer (27) arising by self Diels-Alder dimerisation of initially formed o-quinol (24), 2,6-dimethyl-p-benzoquinone (25), diphenoquinone (26) and keto-acid (28). On the other hand oxidation with iodic acid or sodium metaperiodate results in much cleaner oxidation product. We therefore, decided to follow the experimental procedure used

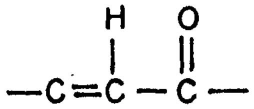


by Adler and Holmberg[21] who reported the formation of dimeric compounds by oxidation of phenols with iodic acid.

The reaction of carvacrol (6) with iodic acid in aqueous ethanol at room temperature provided, after purification by preparative TLC on silica gel, two compounds, A m.p. 156° and B, m.p. 138-9° in yields of 6 and 30 percent respectively. Compound-B, the major product was identified as racemic (7a) by comparison, m.p., IR, ^{1}HNMR spectral data with those of an authentic sample.

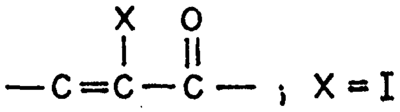
These spectral properties suggested the identity of the major product, compound-B with Carman's dimer (7a). A direct comparison unambiguously established their identity*.

The minor oxidation product, compound-A, m.p. 156° analysed for $C_{20}H_{27}O_4I$. The molecular formula suggested that compound-A is structurally similar to compound-B and one of the hydrogens of the latter is replaced by an iodine. The position of iodine could be fixed at C_5 on the basis of the absence of the α-hydrogen (^{1}HNMR spectrum, Fig.2) of an $\alpha\beta$-unsaturated carbonyl system present in compound-B.



$-C=\overset{H}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-$

Compound – B



$-C=\overset{X}{\overset{|}{C}}-\overset{O}{\overset{\|}{C}}-\;;\; X=I$

Compound – A

Compound-A showed the following spectral data.

Its IR spectrum (Fig. 1) showed bands due to hydroxyl, saturated carbonyl and a conjugated carbonyl (ν max 3460, 1720 and 1670 cm^{-1} respectively) groups. ^{1}HNMR spectrum (Fig. 2) showed the presence of two isopropyl groups (0.86, 0.9, 1.19, 1.34, d, J=7Hz, 3H each), two tertiary methyls geminal to hydroxyls (1.24, s, 6H), two allylic methine protons (1.94 and 3.21, m), four methine protons (3.05, 3.21, 3.41 and 3.57, m, 1H each), one vinyl proton (5.92, m), two

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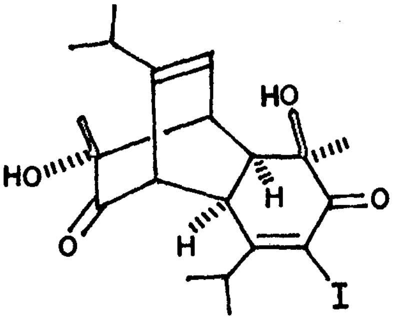
* We thank Dr. R.M. Carman for this comparison.

hydroxyl groups (2.33 and 4.0, sharp singlets exchangeable with D_2O). The ^{1}HNMR spectrum showed a striking similarity with that of (7a) except for the absence of α-hydrogen of the conjugated α,β-unsaturated carbonyl grouping. The structure (32) could therefore be assigned to the compound-A.



(32)

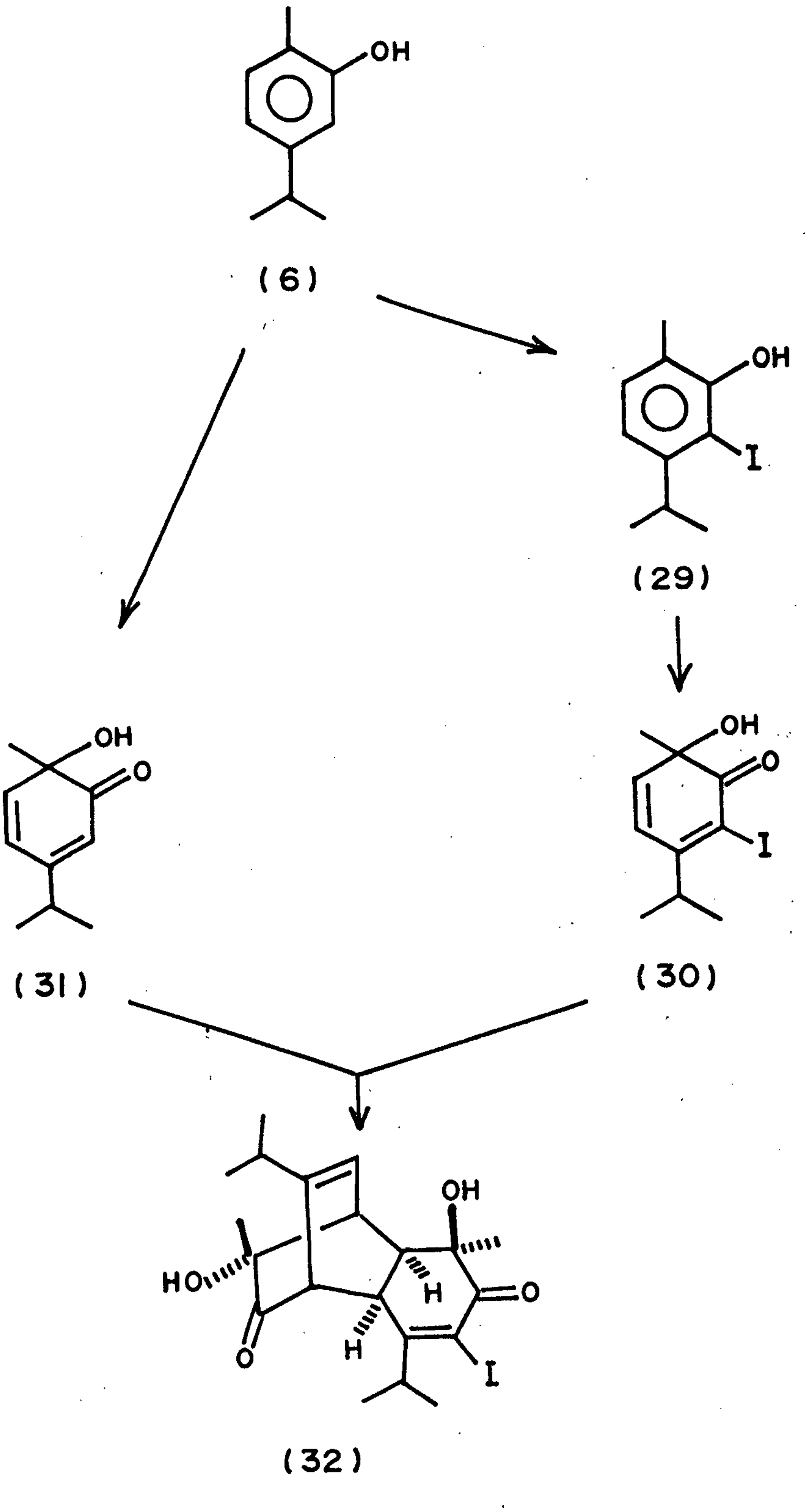
It may be noted that the chemical shifts for the protons C_7, C_{14}, C_{15} and C_{16} were observed downfield when compared to the corresponding protons of Carmans dimer (7a). This deshielding is attributed to the presence of iodine at C_5. Fort et al[23] have reported characteristic long-range ✓ deshielding (five bonds distant from the shifted protons) by chlorine. The data therefore clearly established the location of iodine at C_5 and structure (32) for compound-A.

The formation of the iodinated dimer (32) in the above reaction is not without precedent[24]. The iodine liberated in the oxidation of carvacrol (6) with iodic acid converts part of the carvacrol (6) into o-iodo carvacrol (29). The Diels- ✓

Alder reaction between o-quinol (30) derived by oxidation of (29) with iodic acid and the o-quinol (31) results in the formation of (32). It may be noted that iodo-o-quinol (30) acts as a dienophile and the o-quinol (31) as a diene. A plausible sequence for the formation of (32) is shown in (scheme-4).

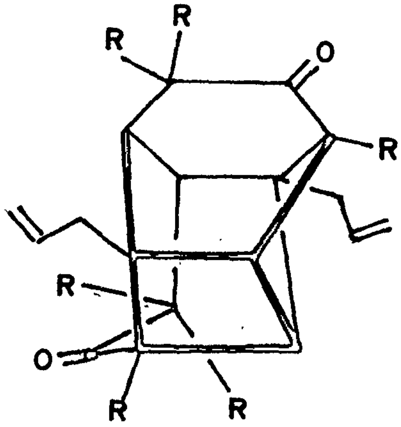
Oxidation of carvacrol (6) with sodium metaperiodate following the experimental conditions reported by Falshaw et al[22] for 4-methyl thymol gave crystalline racemic (7a) in 12 per cent yield. It is noteworthy that oxidation with sodium metaperiodate is a much cleaner reaction and gives racemic (7a) as the only isolable product, though in low yield. We could not detect the formation (32) in this reaction.

During our literature survey on the natural products derived by intermolecular Diels-Alder dimerisation, we came across the interesting work of Japanese researchers who reported the isolation of asatone (47, entry 30, Table-1) and isoasatone (33) from <u>Asarum</u> <u>taitonensa</u> Hayata[25-27]. The structures were established by spectral and degradative chemical evidence and partial synthesis[27,28] of isoasatone (33) from asatone (47, entry 30, Table-1). The natural occurrence of asatone and isoasatone (33) in <u>Asarum</u> <u>taitonensa</u> Hayata and the photochemical transformation of (47, entry 30, Table-1) into (33) suggests the possibility of the natural occurrence of (34) in <u>Callitris</u> <u>macleayana</u>

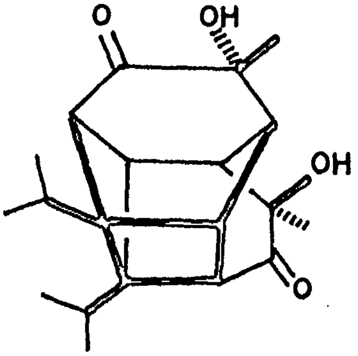


Scheme – 4

heartwood and therefore its preparation from (7a) by photochemical intramolecular ($\pi 2 + \pi 2$) cycloaddition was considered worthwhile. When the dimer (7a) was irradiated



(33), R=OMe



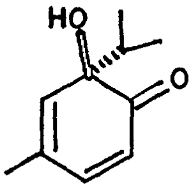
(34)

in hexane solution, a solid material separated out from the solvent. F filteration and crystallisation from ethanol provided the cage compound (34) m.p., 229°, $C_{20}H_{28}O_4$ (M^+ 332, Fig. 5) which was devoid of UV absorption. Its IR spectrum (Fig.3, νmax 3460, 1700 cm^{-1}) showed the presence of hydroxyl and saturated six membered ring carbonyl functionalities. Examination of ^{1}HNMR spectrum (Fig. 4) showed the absence of any vinyl proton, thus confirming the formation of intramolecular cycloaddition product. The ^{1}HNMR spectrum was found to be much simpler and disclosed the presence of two secondary methyl doublets (0.77 and 1.07, J=7Hz) integrating for 12 hydrogens (2 isopropyl groups), a singlet (1.3, 6H) due to 2 tertiary methyl groups, a multiplet centred at 2.04

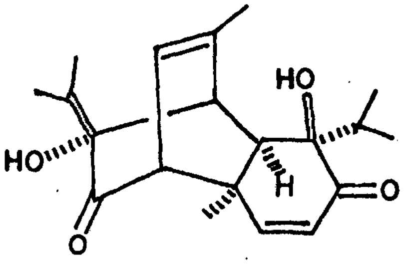
(2H) and a five proton absorption in the 2.7 - 3.0 ppm region. The mass spectrum of (34) (Fig.5) was almost identical with that of (7a) except for the intensities of some peaks, particularly the peak at m/z 159. Clearly the highly strained ring system of (34) undergoes thermal decomposition into (7a) prior to further fragmentation. The spectral data are consistent with the assigned structure (34), the irradiation product of (7a).

Before undertaking the work described above, we had oxidised isothymol (17) with periodic acid in aqueous ethanol at room temperature. The reaction afforded two compounds, C, m.p. 84° and D, m.p. 168° in yields of 22 and 12 percent respectively. The p-quinol structure (18) was assigned to compound-C, m.p. 84° on spectral analysis. Its IR spectrum (Fig. 6) showed the presence of hydroxyl and conjugated carbonyl groups (ν max 3430, 1665, 1632 cm^{-1}). The ^{1}HNMR spectrum (Fig. 7) showed the presence of three vinyl protons (6.08, 1H, d, J=10Hz, C_6-H; 6.56, m, 1H, C_3-H; 6.82, 1H, dd, J=10, 3Hz, C_5-H). The C_3-proton shows a long range W-type coupling with C_5-H and also long range coupling with isopropyl porton (C_7-H). The ^{1}HNMR spectrum also showed signals due to isopropyl group (1.06, 6H, d, J=7Hz) and a tertiary methyl geminal to hydroxyl group (1.47, 3H, s).

Compound-D, $C_{20}H_{28}O_4$ (M$^+$ 332 HRMS, Fig. 11), m.p. 168° was initially considered to be formed by self condensation of the o-quinol intermediate (19) leading to the structure (35).



(19)



(35)

Based on the previous observations the diene system of one molecule was expected to add to the γ-δ double bond of the other molecule*. Its ^{1}HNMR spectrum (Fig. 9), however, was not consistent with structure (35) as it showed the presence of only two vinyl protons (5.85 and 6.0, 1H each) and not three required for structure (35). Furthermore, the ^{1}HNMR spectrum showed striking similarity to that of (7a), particularly in the vinyl proton region, and the appearance of the one proton multiplets. It showed the presence of two isopropyl groups (0.59, 0.89, 0.86 and 0.98, d, J=7Hz, 3H each), two vinyl methyls (1.67, d, J=1.7Hz; 1.97) and six

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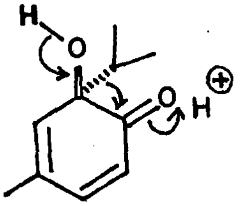
* The experiment was carried out by one of the research colleague, late Miss C.V. Ramannama. The structure assignments as given in this section were not made. The reaction was reinvestigated and the results are presented. The results are published elsewhere[28a].

methine hydrogens (1.60, 1.79, 3.10, 3.18, 3.28 and 3.32 all multiplets). The sharp singlets at 2.1 and 3.8 disappeared on D_2O addition and are attributed to hydroxyl protons. The above data can easily be acomodated if one exchanges the position of methyl and isopropyl groups in structure (7a). Structure (36) is therefore assigned to compound-D. Formation of (36) by periodic acid oxidation of (17) can be explained by the self Diels-Alder condensation of o-quinol (37) which in turn is apparentely derived from (19) by an acid catalysed acyloin rearrangement as shown (see arrows in structure 19).



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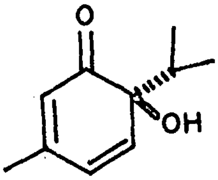
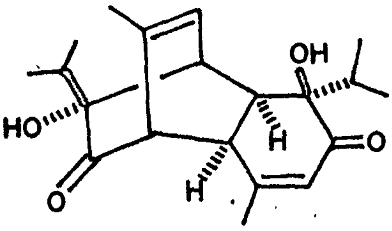


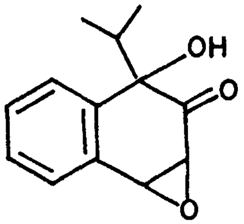
(37)



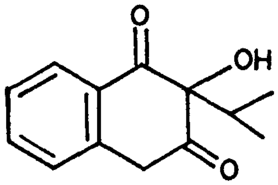
(36)

A precedent for such isopropyl migration in -of the type (19) is known in the literature. Carnduff and Leppard[29] have previously observed that the epoxide (38) undergoes acid catalysed rearrangement to (39).

On the basis of the results of oxidation of carvacrol (vide supra), it was considered that o-quinol (37) can be generated directly by oxidation of thymol with iodic acid or

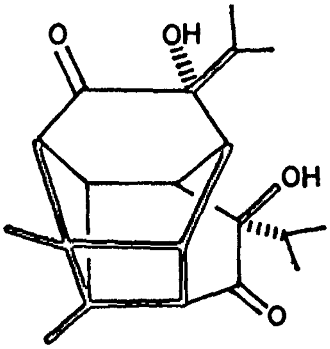


(38)



(39)

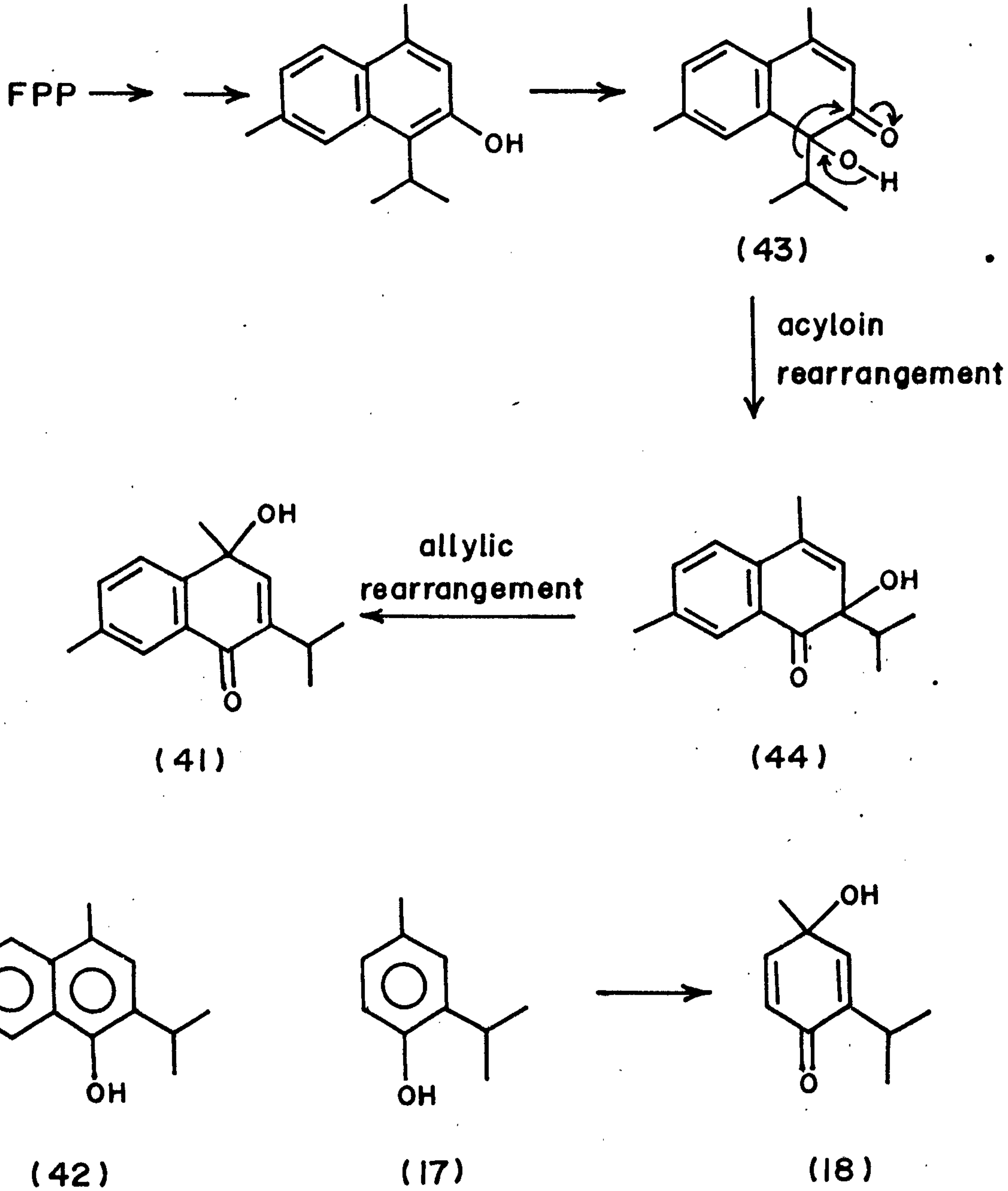
sodium metaperiodate. Therefore (36) should then be available for a direct comparison by oxidation of thymol <u>via</u> the Diels-Alder self condensation of (37). Oxidation of thymol with iodic acid in aqueous ethanol gave two products E, $C_{20}H_{28}O_4$, m.p. 168° and F, $C_{20}H_{27}O_4I$, m.p. 160°. As expected one of the oxidation product (compound-E) was found to be identical in all respect (m.p. mixture m.p. IR, ^{1}HNMR) with (36) obtained earlier from isothymol (17). The stereochemistry of (36) is based on the highly regioselective nature of the self Diels-Alder dimerisation reaction. Further support to the assigned <u>endo</u> stereochemistry came from the irradiation experiment which gave the photocycloaddition product (40) m.p. 155°, $C_{20}H_{28}O_4$ (M^+332 HRMS, Fig. 14) devoid of UV absorption and whose ^{1}HNMR spectrum (Fig. 13) showed the absence of vinyl hydrogens, presence of two isopropyl groups (0.8, d, J=7Hz, 6H; 0.97, d, J=7Hz, 6H), two tertiary methyl (1.16, s, 6H). The pattern above 2.3 ppm was almost identical with that of (34).



(40)

Based on the observed acyloin rearrangement of o-quinol (19) to (37), some comments regarding the biogenesis of isocadalene (41) isolated from the roots of *Heterotheca inuloides*[30] would be in order. It was suggested earlier from this laboratory that the phenol (42) may be the immediate precursor of (41)[31]. Isothymol (17) having structural features similar to (42) was therefore selected as a model compound for its conversion to p-quinol (18) using periodic acid as the oxidising reagent. Successful preparation of p-quinol (18) is reported earlier. It is now suggested that o-quinol (43) having a normal sesquiterpene skeleton can give rise the structure (41) via (44) by acyloin rearrangement followed by an allylic rearrangement. A plausible biogenetic pathway is presented in (Scheme-5).

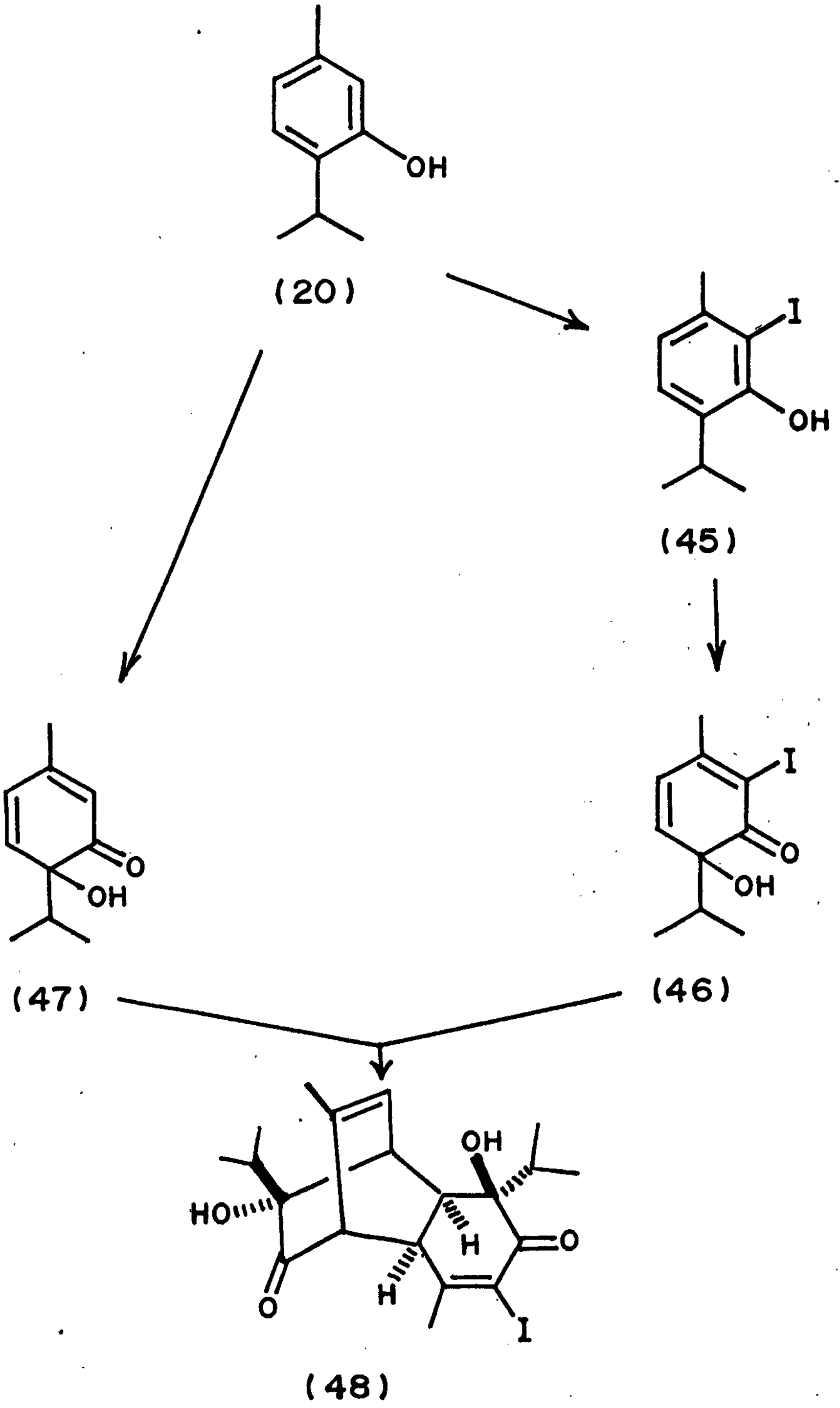
Compound-F, m.p. 160°, the minor compound obtained by oxidation of thymol (20) with iodic acid in aqueous ethanol


FPP
OH
O
O
H
(43)
acyloin rearrangement
OH
allylic rearrangement
OH
O
O
(41)
(44)
OH
OH
OH
O
(42)
(17)
(18)

Scheme – 5

analysed for $C_{20}H_{27}O_4I$. Its IR spectrum (Fig. 15) showed the presence of hydroxyl (ν_{max} 3540) a saturated carbonyl (1725) a conjugated carbonyl (1675) functionalities. The ^{1}HNMR spectrum (Fig. 16) showed the presence of two isopropyl groups (0.59, 0.84, 0.86 and 0.97, d, J=7Hz, 3H each), two vinyl methyls (1.6, d, J=1.7Hz, 3H and 2.2, s, 3H), two isopropyl methines (1.6, m, 1H and 1.78, m, 1H), four methine protons (3.18, 3.28, 3.34, 3.43 all multiplets, 1H each), two hydroxyl protons (two sharp singlets at 2.25, 3.75, 1H each, exchangeable with D_2O), a vinyl proton (5.85, m). The ^{1}HNMR spectrum of compound-F showed striking similarity with the dimer (36), the major and characteristic difference being the absence of the α-proton of the α-β-unsaturated carbonyl grouping present in the dimer (36). The structure (48) was therefore, assigned to compound-F. Its formation obviously involves iodination of thymol at ortho position and iodic acid oxidation of o-iodothymol (45) thus obtained to iodinated o-quinol (46). Diels-Alder reaction between the iodinated o-quinol (46) as dienophile and o-quinol (47) as the diene component then produces compound-F (48),(scheme-6).

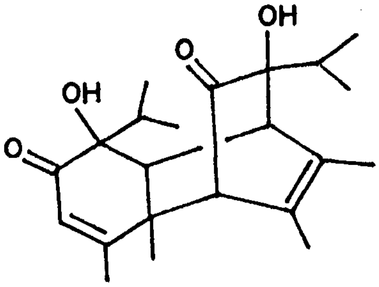
Lastly, it was found that the dimers (7a) and (36) undergo a facile intramolecular ($\pi 2 + \pi 2$) photocycloaddition when their solutions in n-hexane were exposed to sunlight for 1 hr. These observations lend support to the concept that the cage compound (34) may turn out to be a congener of (7a)



Scheme - 6

in Callitris macleayana heartwood. Similarly compounds (18, 36 and 40) will eventually turn out to be naturally occuring substances.

Falshaw et al[22] reported the preparation of a dimeric compound (49) by oxidation of 4-methylthymol with sodium metaperiodate. In the light of the results obtained in the present study, the structure (49) assigned earlier would require modification and the reaction was therefore reinvestigated.



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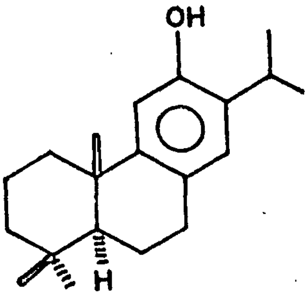

(50)

Reinvestigation on the structure of the dimer obtained by sodium metaperiodate oxidation of 4-methyl thymol (21)

While the structure of maytenone (36, entry 22, Table-1) was established beyond doubt, attempts to synthesise it from ferruginol (50) following the biogenetic pathway were unsuccessful. Johnson and co-workers therefore studied the oxidation of 4-methylthymol* (21) with sodium metaperiodate and obtained a colourless crystalline dimer, m.p. 181-82°. Formation of this dimer was explained by a self Diels-Alder - type dimerisation reaction of o-quinol involving the diene system of one molecule and the γ-δ double bond of the other. Structure (49) was therefore assigned to the dimer.

In principle, four structures can be considered for the Diels-Alder dimer resulting from self dimerisation of a o-quinol. However, in general the adduct with the endo configuration is usually much in agreement to this. Carman and co-workers showed that the hydrolysis of o-quinol acetate (5) results in the formation of single dimer (7a), whose endo stereochemistry was established by x-ray analysis. We have also found that the oxidation of carvacrol (6), thymol (20) and isothymol (17) with iodic acid or sodium metaperiodate gives dimers with endo configuration. This was proved in our case by the preparaion of cage compounds (34,40) by intramolecular $(\pi 2 + \pi 2)$ photocycloaddition. Only dimers with endo configuration can undergo intramolecular photocycloaddition. These results suggested that the dimeric compound obtained previously by Johnson and co-workers by sodium metaperiodate oxidation of 4-methyl thymol in all probability will have structure (51). An unambiguous

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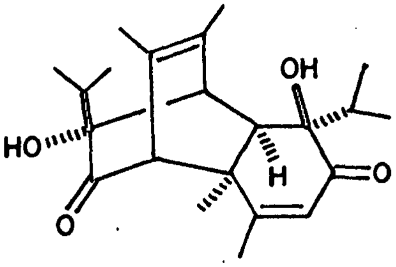
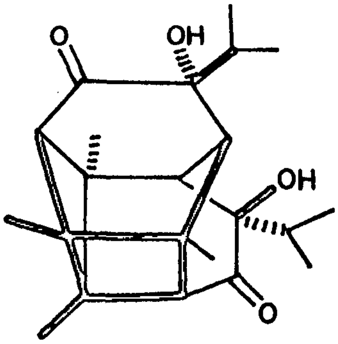
* having identical substitution pattern when compared to the aromatic ring substitution pattern of ferruginol (50).

evidence in favour of structure (51) can be obtained by preparation of cage compound (52) by intramolecular $(\pi 2 + \pi 2)$ photocyclyaddition. We therefore, decided to reinvestigate the oxidation of 4-methyl thymol with sodium metaperiodate.



(51)



(52)

4-methylthymol (21) obtained by the known procedures from thymol (20) on oxidation with sodium metaperiodate following the experimental conditions reported by Falshow et al gave after purification by preparative TLC a crystalline compound m.p. 177-80°, which analysed for $C_{22}H_{32}O_4$. Its IR spectrum (Fig. 17) showed bands at 3470 (OH), 1694 (saturated C=O), 1655 (α-β-unsaturated $>$C=O), 2910, 1610 (C=C) cm^{-1}.

The IR spectral data agreed well with that reported by Falshaw et al for their oxidative dimerisation product of 4-methylthymol. We have now measured its ^{1}HNMR and ^{13}CNMR which shows structure (51) for the dimer. Its ^{1}HNMR spectrum (Fig. 18) shows the presence of four secondary methyls (0.54, 0.83, 0.89, 0.95, d, J=7Hz, 3H each) belonging to two isopropyl groups, a tertiary methyl (1.32, 3H, s) three vinylic methyls (1.43, q, J=1Hz, 3H; 1.58, q, J=1Hz, 3H; 1.86, d, J=1.5Hz, 3H), five methine hydrogen (1.66, m, 2H; 2.74, s, 1H; 2.93, d, J=2Hz, 1H; 3.14,d, J=2Hz,1H), two exchangeable hydrogens (2.4 and 3.7, 1H each, s) and a vinyl proton (5.92, q, J=1.5Hz, 1H). As expected the spectrum is much simpler when compared to the dimer obtained from o-quinol (37). Its ^{13}CNMR spectrum (Fig. 19) showed the presence of eight quartets (15.39, 16.44, 16.73, 17.21, 17.28, 17.50, 19.86 and 24.36), six doublets (33.72, 36.93, 44.14, 46.68, 65.26 and 124.79) and eight singlets (48.6, 76.57, 77.0, 128.94, 133.58, 159.59, 201.0 and 213.30). As expected the spectrum did not show any triplet. The stereochemical assignment of dimer (51) follows from its ^{1}HNMR and ^{13}CNMR data (see Table-2). Conclusive evidence for structure (51) was obtained by its conversion to (52) on exposure of its hexane solution to sunlight for 1 hr. The spectral data (see experimental) on the photochemical$(\pi 2 + \pi 2)$ cycloaddition product of (51), was consistent with its formulation as (52).

It was observed by Falshaw et al that the experimental conditions under which 4-methylthymol underwent oxidative Diels-Alder type dimerisation when used for ferruginol (50) did not lead to maytenone (36, entry 22, Table-1). This appearent failure to obtain maytenone (36, entry 22, Table-1)

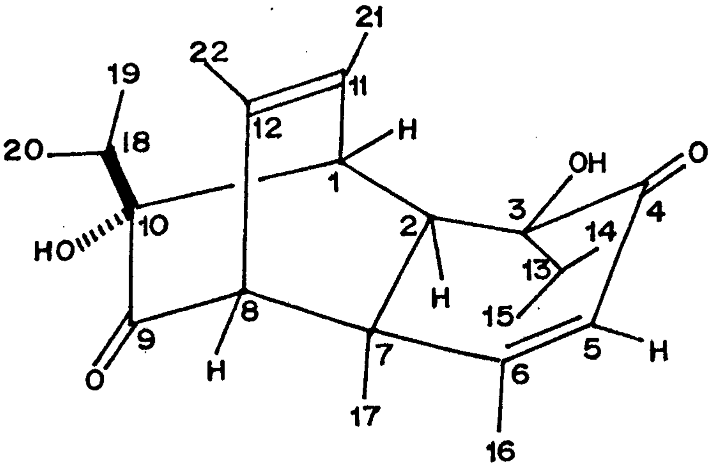


Table - 2

NMR. chemical shifts(^{1}H and ^{13}C) for the dimer **(51)** (derived by oxidation of 4 - methyl thymol) in $CDCl_3$

Hydrogen	δ ^{1}H(Splitting, J in Hz)	Carbon	δ ^{13}C(Splitting)
H1	3.14(d,2.0,1H)	C1	46.68(d)
H2	2.93(d,2.0,1H)	C2	44.14(d)
H3	-	C3	77.00(s)
H4	-	C4	201.00(s)
H5	5.92(q,1.5,1H)	C5	124.79(d)
H6	-	C6	159.59(s)
H7	-	C7	48.60(s)
H8	2.74(s,1H)	C8	65.26(d)
H9	-	C9	213.30(s)
H10	-	C10	76.57(s)
H11	-	C11	133.58(s)

contd.

H12	-	C12	128.94(s)
H13	1.66(m,1H)	C13	36.93(d)
H14	0.83(d,7,3H)	C14	17.50(q)
H15	0.95(d,7,3H)	C15	15.39(q)
H16	1.86(d,1.5,3H)	C16	19.86(q)
H17	1.32(s,3H)	C17	17.21(q)
H18	1.66(m,1H)	C18	33.72(d)
H19	0.54(d,7,3H)	C19	24.36(q)
H20	0.89(d,7,3H)	C20	16.44(q)
H21	1.43(q,1,3H)	C21	16.73(q).
H22	1.58(q,1,3H)	C22	17.28(q)
C 3-OH	2.40 or 3.70	exchangeable with D_2O	
C 10-OH	2.40 or 3.70		

from ferruginol (50) suggested that 4-methylthymol (21) did not serve as the ideal model compound. We therefore considered it worthwhile to study the iodic acid and or sodium metaperiodate oxidation of 4-ethylthymol (22). Oxidation of 4-ethylthymol* (22) with sodium metaperiodate in aqueous ethanol at room temperature gave a mixture from which two compounds G, m.p. 121° and H, m.p. 158° could be isolated by preparative TLC.

The mass spectrum of compound-H, m.p. 158° showed a molecular ion peak at m/z 388 corresponding to molecular formula $C_{24}H_{36}O_4$. Its IR spectrum (Fig. 23) resembled that of (51) and had absorption bands at 3440 (hydroxyl) 1710 (saturated $>C=O$) and 1675 (α-β unsaturated $>C=O$) cm^{-1}. This data clearly showed that the dimer (53) has been obtained. Additional spectral data is being collected.

Biogenesis of Carman's dimer (7a, 3,10-dihydroxy dielmentha-5,11-diene-4,9-dione) poses some intresting questions. While it is clear that self dimerisation of o-quinol (4) is an obligatory step in the biogenesis of (7a) it is not still clear whether the Diels-Alder dimerisation takes place under enzymatic control to produce the optically actaive dimer. Although such a possibility of a Diels-Alder

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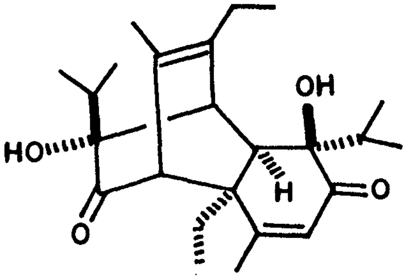
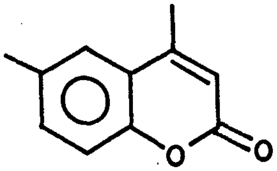
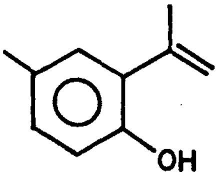
* Obtained by Clemmensen reduction of 4-acetylthymol.



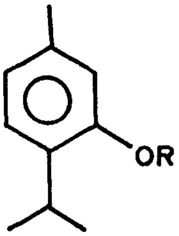
(53)



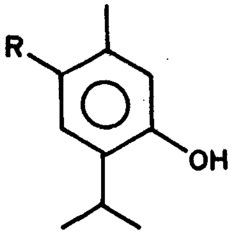
(54)



(55)



(56), R = COMe



(57), R = COMe

(58), R = CHO

dimerisation under enzymatic control cannot be totally ruled out, we consider that carvacrol is not the precursor for the o-quinol (4). We believe that optically active (7a) is formed by Diels-Alder self dimerisation of optically active, o-quinol which inturn is produced from a suitably substituted cyclic monoterpene precursor* (scheme-7).

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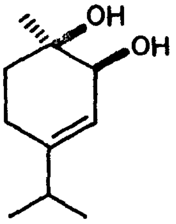
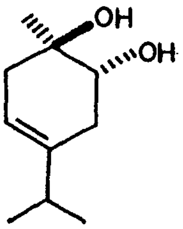
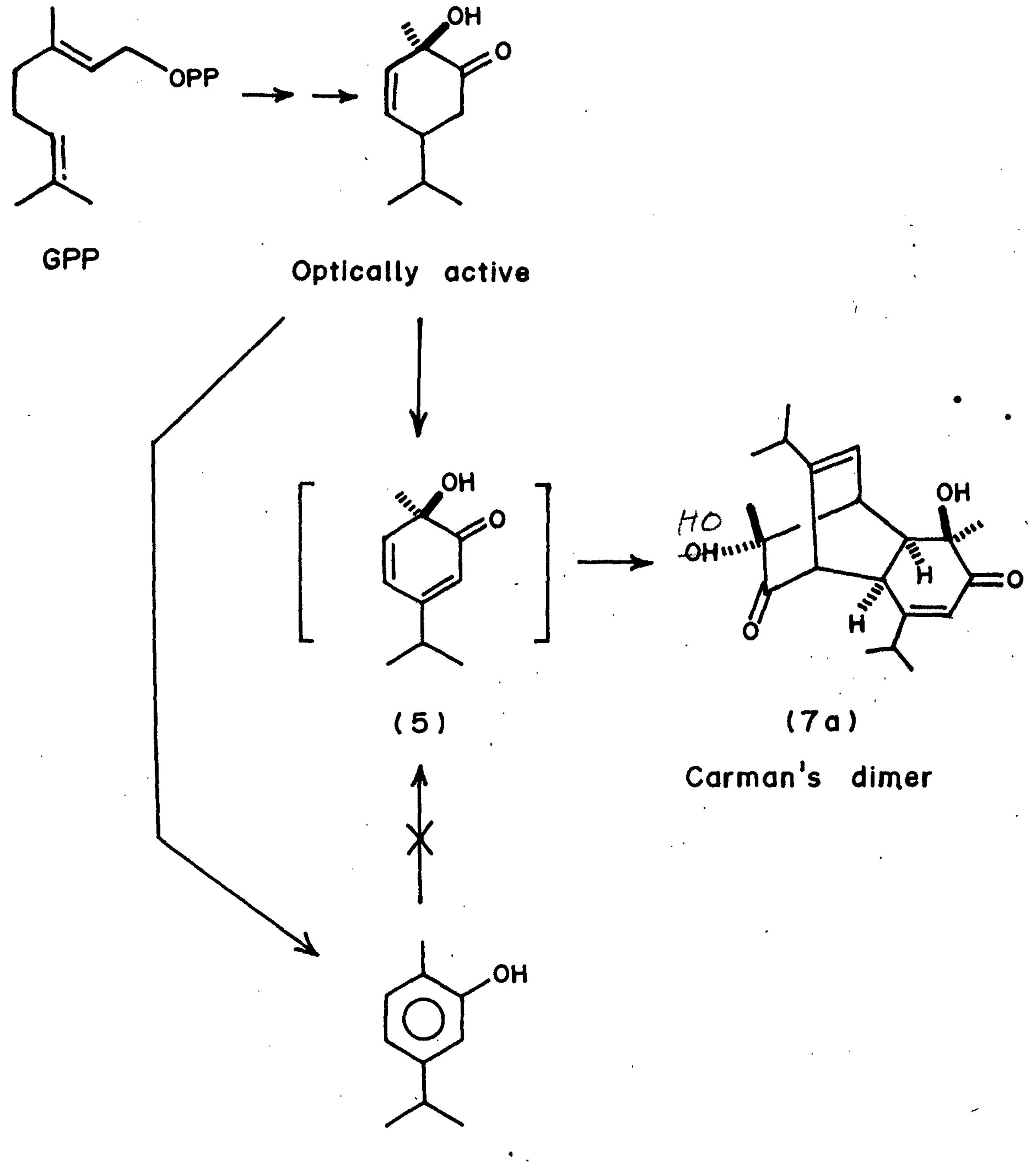
* e.g., Natural occurrence of the optically active monoterpene diols (i & ii) has been recently reported[32].



(i)



(ii)



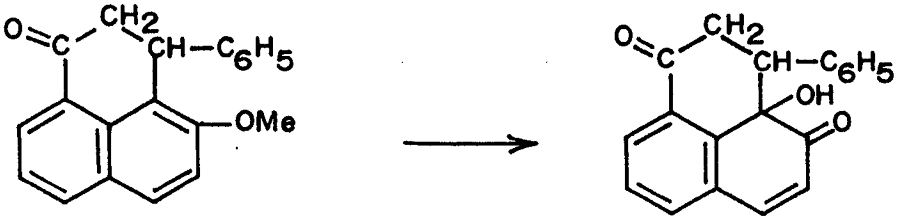
Scheme -7

Proposed biogenetic pathway for (-)-3, 10-dihydroxy-dielmentha-5, 11-diene-4, 9-dione from Callitris macleayana.

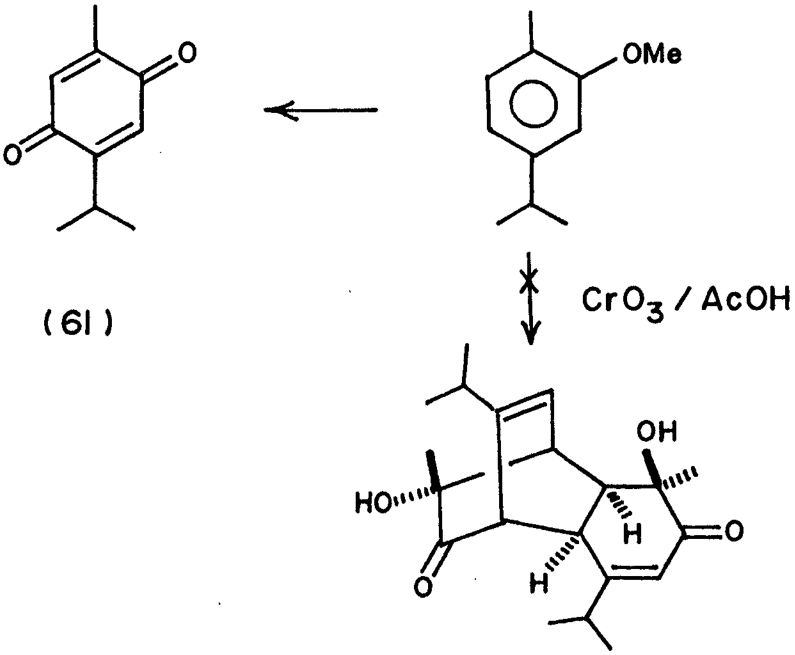
CHAPTER-I SECTION-II

OXIDATION OF METHYL ARYL ETHERS WITH CrO_3 /AcOH

In continuation of our work described in the previous section of this chapter we envisaged a simple synthesis of (±)3,10-dihydroxydielmentha 5,11-diene-4,9-dione (7a) by oxidation of carvacrol methyl ether with CrO_3/AcOH. Based on the report[63] that the aryl methyl ether (59) on oxidation with CrO_3 in dilute AcOH (80%) gives o-quinol (60), it was



(59) (60)



(61)

(7a)

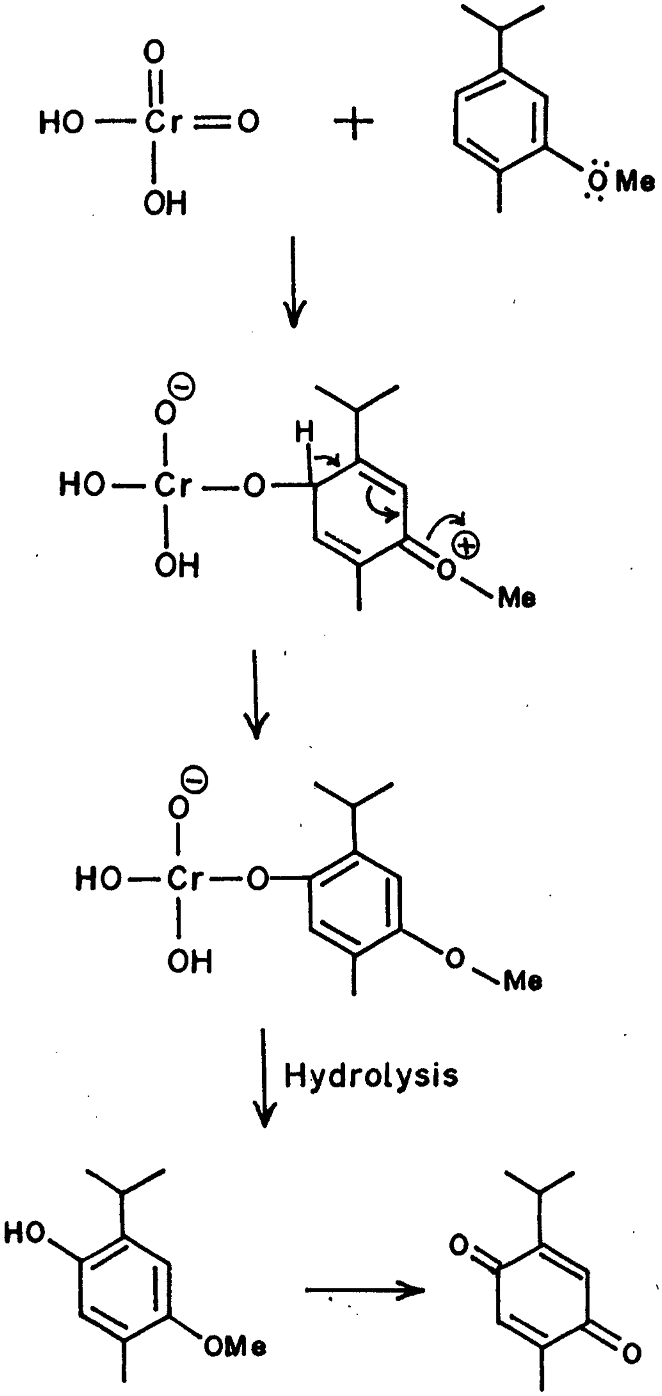
felt that carvacrol methyl ether under these conditions should yield dimer (7a) via the Diels-Alder self condensation of o-quinol (4). However, oxidation of carvacrol methyl ether with CrO_3/AcOH did not furnish the desired dimer (7a) but afforded in good yield (55%) a bright yellow compound - m.p.45°. Its IR spectrum (Fig. 24) showed bands at 1665 and 1620 cm^{-1} due to conjugated carbonyl functionality. Its ^{1}HNMR spectrum (Fig. 25) showed the presence of an isopropyl group 1.17 (6H,d, J=8Hz), an allylic methyl 2.1(3H, d, J=2Hz) an allylic hydrogen 3.1(1H, septet, J=8Hz) and two olefinic hydrogens 6.63(1H, s, and 6.73, 1H, q, J=2Hz). The oxidation product-m.p. 45° was therefore identified as thymoquinone (61). A plausible mechanism for the formation of thymoquinone is depicted in (scheme-8).

A literature search showed that thymoquinone (61) has been prepared previously by different routes as described below.

1. By sulfonation of thymol or carvacrol and subsequent oxidation with MnO_2 or $K_2Cr_2O_7$[64].

2. By oxidation of salts of aminothymol with dichromate, $FeCl_3$ or nascent Br_2[64].

3. By oxidation of 4-aminothymol with HNO_2[64].
4. By electrolytic oxidation of thymol[65].
5. By oxidation of thymol or carvacrol with MCPBA[66].



(61)

Scheme - 8

The above methods can be used for the preparation of p-quinones in general. Though monohydric phenols and aromatic amines can be converted into corresponding p-quinones[67], methyl ethers of monohydric phenols have not been used before for the direct preparation of p-quinones*. The previous report[68] for the conversion of methyl aryl ethers to p-quinones involves introduction of p-amino group (nitration followed by reduction), then oxidation to the p-quinone. The conversion of bergapten (62) to p-quinone (63) (scheme-9) can be cited as a typical example.

It should be possible now to effect such a conversion (62 → 63) in a single step using CrO_3/ AcOH reagent. The formation of thymoquinone (61) by oxidation of carvacrol methyl ether with CrO_3 /AcOH reagent therefore appeared to be of synthetic value and hence oxidation of methyl ethers of other phenols with this reagent was studied (Table-3). As shown in the Table-3, different substituted aryl methyl ethers were chosen for study. It was observed that simple

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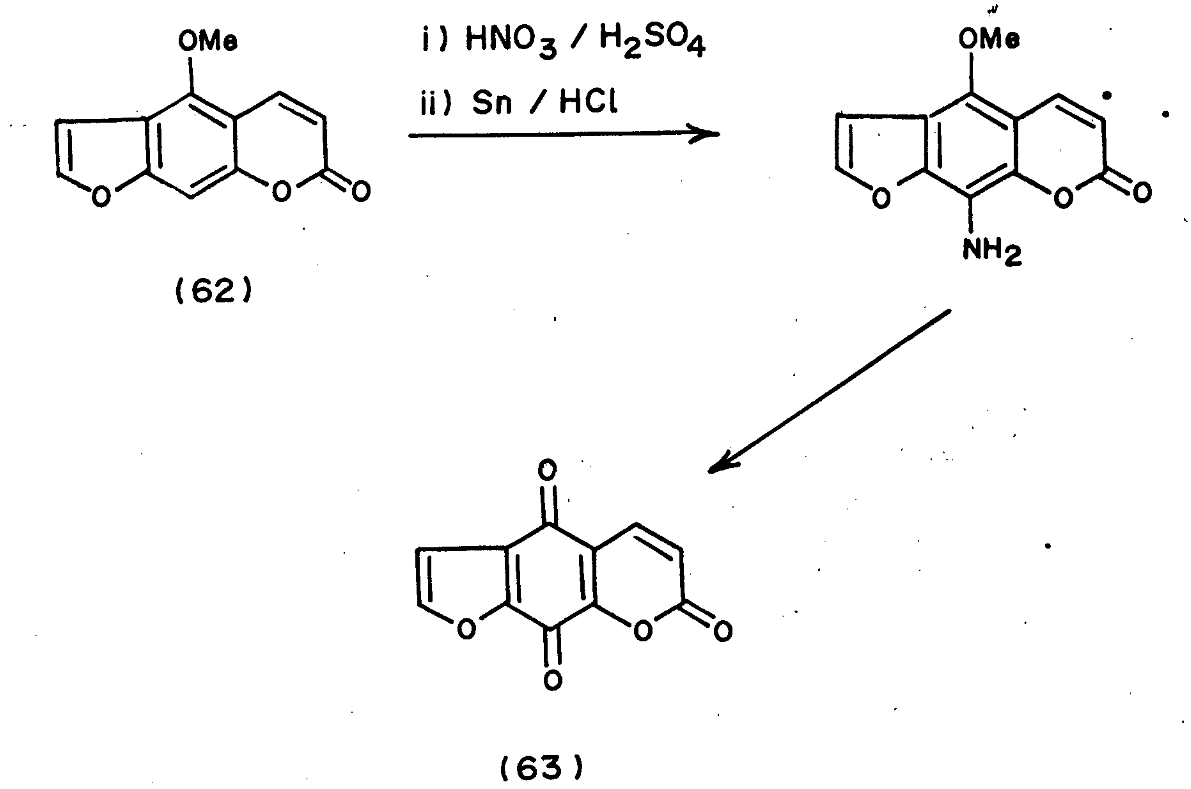
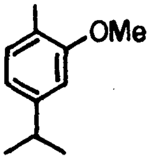
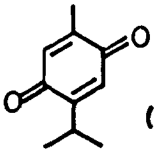
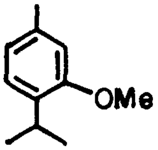
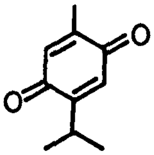
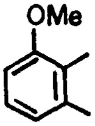
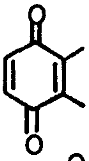
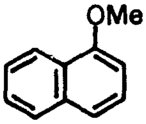
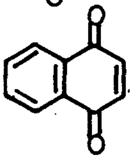
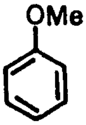
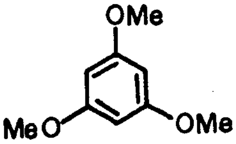
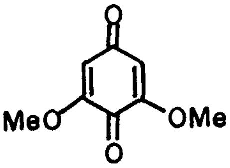
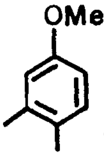
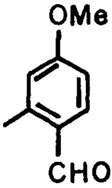
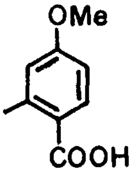
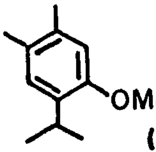
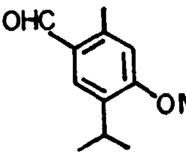
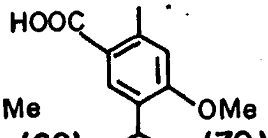
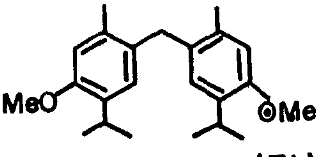
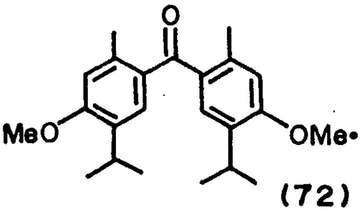
* A literature report[67a] on the preparation of a substituted p-benzoquinone by oxidation of a aryl methylether came to our attention during the Post-IUPAC symposium held at Bangalore, Feb., 1990. We thank Prof. M.V. Sargent for this information.



Scheme -9

Table – 3

Oxidation of methyl aryl ethers with CrO_3 /AcOH (80%)

Sr. No.	Starting material	Products	Yield %	m.p °C
1		 (61)	55	45
2		 (61)	55	45
3			53	55
4		 , Product (A)	15 (A), 11	125 (A), 170
5			4	116
6			66	248–49
7	 (65)	 (66)  (67)	(66), 36 (67), 19	(66), B·P 256 (67), 172–73
8	 (68)	 (69)  (70)	(69), 50 (70), 6	(69), B·P 278 (70), 138
9	 (71)	 (72)	92	132

aryl methyl ethers like anisole , α- naphthyl methyl ether gave poor yield of corresponding p-quinones . α- Naphthyl methyl ether gave expected 1,4 - naphthoquinone but in poor yield. A second product m.p. 168° could be isolated in this reaction which is still uncharacterized. In the case of alkyl substituted aryl methyl ethers it was found that the yields of p-quinones increased considerably. Oxidation of phloroglucinol trimethylether gave quinone (64) in 66% yield*. In all cases when the para position is free only p-quinones were formed. If the para position with respect to - OCH_3 possess an alkyl group, we expected the formation of an orthoquinone. However, oxidation of 3,4-dimethyl phenylmethyl ether (65) gave 2-methyl-4 —methoxy benzaldehyde (66), semicarbazone, m.p. 205° and the corresponding carboxylic acid (67) . We failed to detect any orthoquinone in this reaction. A plausible mechanism for the formation of (66) and (67) is depicted in (scheme-10). In agreement with this observation 3,4-dimethyl-6-isopropyl anisole (68) on oxidation with CrO_3/AcOH gave 4-methyl-5-isopropyl-o-tolualdehyde (69) and the corresponding acid (70). These results led us to believe that oxidation of dimethylether of dithymolylmethane (71) with this reagent would afford the benzophenone derivative (72). As anticipated (72) was

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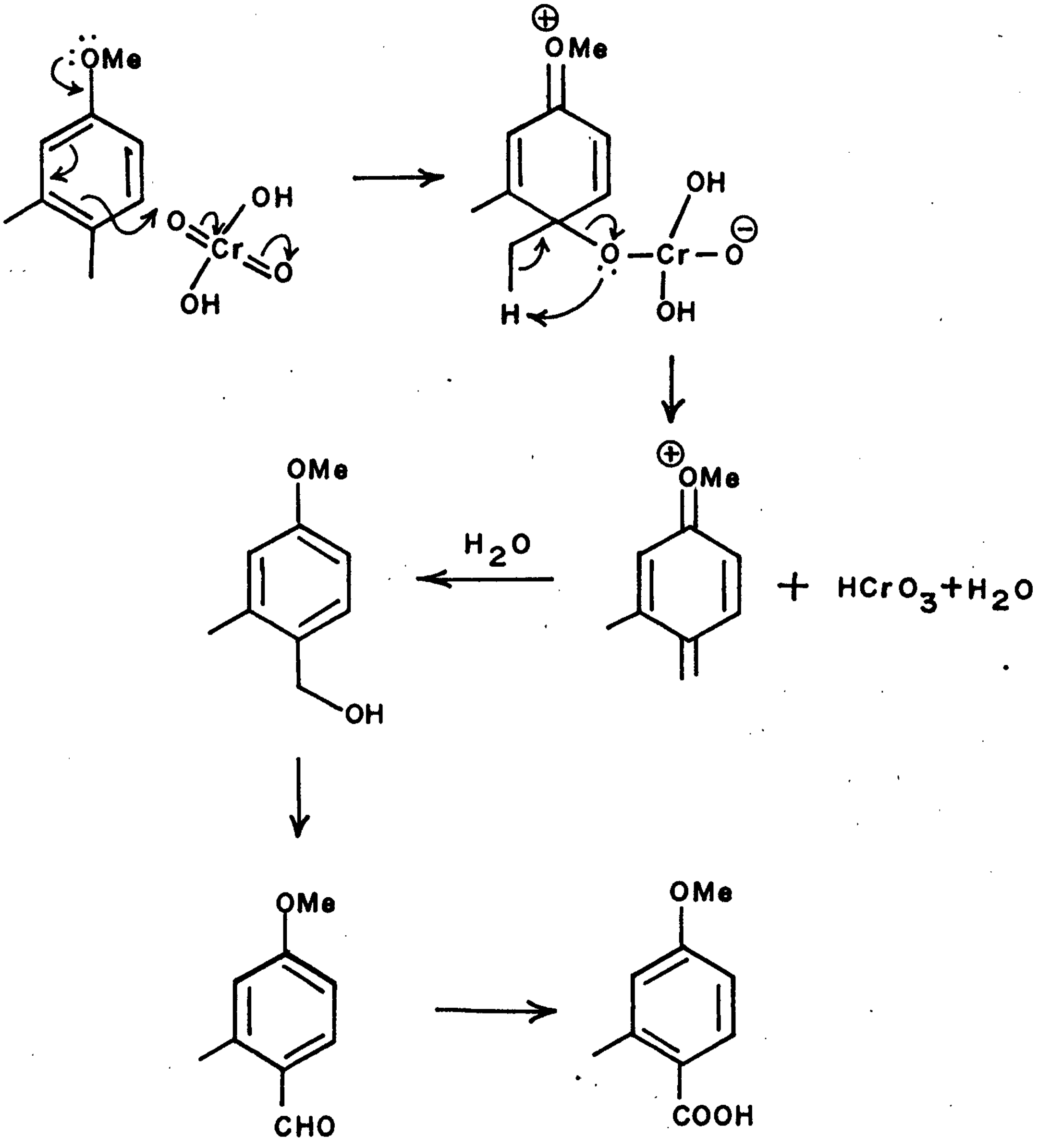
* This experiment was performed by Shri K.K. Nadkarni.


OMe
OH
O
Cr
O
OH
OMe
OH
O
Cr
O
OH
H
OMe
H$_2$O
OMe
+ HCrO$_3$+H$_2$O
OH
OMe
CHO
OMe
COOH

Scheme – 10

obtained in good yield and characterized by its spectral data (see experimental).

From the results presented above it can be seen that aryl methyl ethers having unsubstituted p-position can be converted to p-quinones in a single step and seems to be a general method.

However, it is difficult at this stage to comment on the synthetic utility of this method as only nine compounds were oxidised in the present study. We plan to extend this study to prepare several p-quinones especially in view of the biological activity exhibited by some naturally occurring quinones.

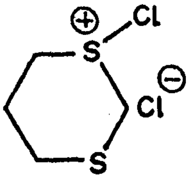
CHAPTER-I SECTION-III

DUFF REACTION OF THYMOL AND CARVACROL : CHARACTERISATION OF THE NON-STEAM VOLATILE PRODUCTS

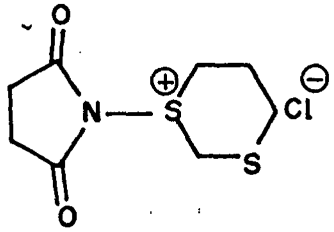
Introduction of a formyl group in o- position with respect to a phenolic group is of wide application and can be used to prepare catechols, coumarins etc. The most well - known method for o- formylation of phenols involves the use of $CHCl_3$ and NaOH and is known as Reimer - Tiemann reaction[83,84]. The incoming group is directed ortho, unless both ortho positions are occupied in which case the substituent gets introduced at para position. It was much used in early work but has now been replaced by more efficient methods, nevertheless it gives good yields in some cases.

A method closely related to the Reimer - Tiemann reaction is known as Duff's reaction[85,86] in which hexamethylene tetraamine is employed usually with glyceroboric acid to convert highly activated aromatic compounds into their o-formyl derivatives. The process is widely used in the conversion of phenols to o-hydroxybenzaldehyde derivatives. The experimental conditions are rather rigorous and the yields are generally low.

In a more recent study Gassman and Amick[87] described a method for the selective o-formylation of phenols using either chlorosulfonium chloride (73) or the salt (74).

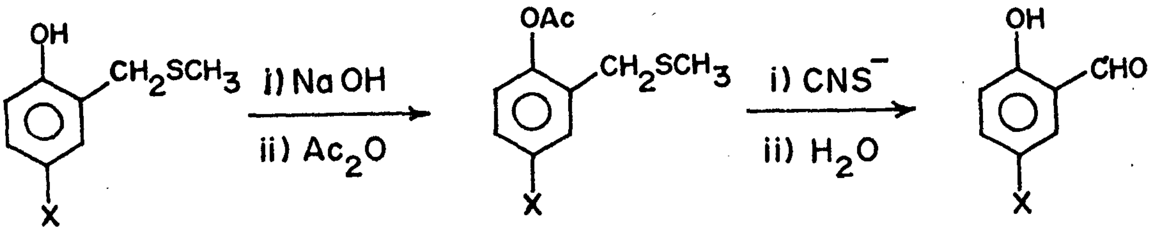


(73)



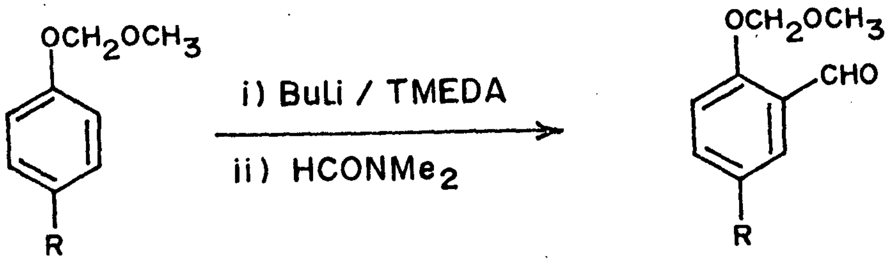
(74)

The yields vary from 20 to 35%. The phenol is ortho, methyl thiomethylated (75) and oxidised with N-chlorosuccinimide followed by hydrolysis to the aldehyde (77). Prior to oxidation, the phenolic hydroxyl group is usually protected by acytylation (76). The overall sequence is as shown below.



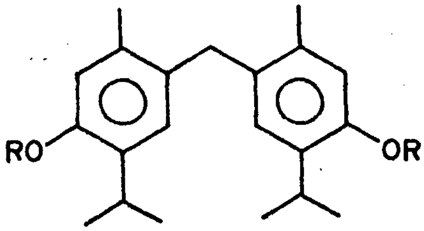
(75) (76) (77)

Titanium tetrachloride has been found[88] to exert profound influence on the direction of electrophilic substitution in o-hydroxycarbonyl compounds such that its use in the formylation of such compounds leads to a large extent to ortho substitution with respect to phenolic hydroxyl group. Very recently, ortho-directed metalation of the methoxy methyl phenolic ethers is reported[89]. This provides synthetic access to ortho-aldehydes via the reaction of ortho-lithiated intermediates with dimethyl formamide, as shown below.

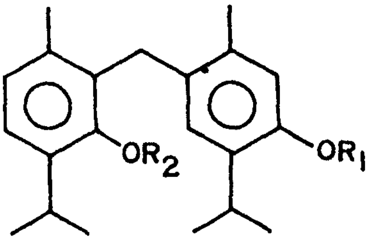


<u>O-Formylation of monoterpene phenols, thymol and carvacrol</u> :

Duff reaction on thymol though studied as early as 1942 was not fully investigated. Only the formation of o-thymoldehyde was unambiguously established. Recently the Duff reaction on thymol was reinvestigated in our laboratory[90]. During this reinvestigation, the non-steam volatile portion of the Duff reaction was subjected to a careful scrutiny. Dithymolyl methane (78) was shown to be one of the components. Another crystalline product of this reaction was tentatively formulated as $C_{21}H_{28}O_2$ on the basis of evidence presented below. The structure (79) was assigned to this product on the basis of spectral data recorded on the acetyl derivative (80).



(78), R = H



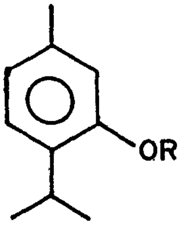
(79), R_1, R_2 = H
(80), R_1, R_2 = COMe
(85), R_1 COMe, R_2 = H

The parent compound shows IR absorption with characteristic peaks for phenolic -OH group at 3460, 1195 and 1045 cm^{-1} further confirming from its solubility in Claisen's alkali and formation of a diacetate , m.p.68-70°, on treatment with acetic anhydride and pyridine. The IR spectrum (Fig. 26) exhibited bands at 1745 and 1210 cm^{-1} due to phenolic acetate grouping. Strong absorption at 820 cm^{-1}

suggested the presence of two adjacent aromatic protons or 1,2,3,4 tetra— substituted benzene and a characteristic splitting at 1380 and 1365 cm^{-1} in the IR spectrum suggested the presence of isopropyl group. The presence of two acetate groups was also evident from its mass spectrum by the appearance of (M^{+}-42) and (M^{+}-84) peaks at m/z 354 (100%) and 312 (98.77%) due to the loss of two -OAc groups successively. Mass spectroscopic analysis indicated a molecular formula of $C_{25}H_{32}O_{4}$ for the diacetate thus confirming the molecular formula $C_{21}H_{28}O_{2}$ for the parent compound. This molecular formula implies the presence of 8 sites of unsaturation which can be attributed to the presence of two aromatic rings in the compound.

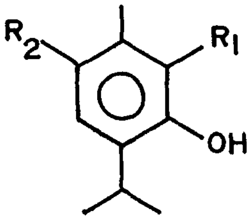
^{1}HN~~P~~MR spectrum of diacetate (Fig. 27) suggested the presence of two non-equivalent isopropyl groups (two pairs of doublets each corresponding to 6H centred at 0.98, (6H, J= 6H_z and 1.2, 6H, J= 6H_z), two benzylic protons (multiple centered at 2.83). It also displayed two sharp singlets at 2.30 (3H) and 2.32 (3H) due to two non-equivalent -CH_3 groups attached to the benzene ring. A singlet at 2.12 corresponding to three protons and a singlet at 2.16 also corresponding to three protons attributed to two non-equivalent acetoxy groups. It also contained a 2H singlet at 3.72 due to benzylic methylene, a pair of doublets each corresponding to 1H at 7.09 and 7.17 (J= 8H_z) the coupling parameters of which indicated that the two protons are ortho- positioned in aromatic nucleus and a pair of singlets at 6.53 and 6.78 due to the para- positioned aromatic protons.The 'HN ~~P~~MR spectrum is consistent with structure (80) for the diacetate. Structure (79), therefore, follows for the parent compound.

It has been reported[91] by Strubell that thymol (81) on treatment with aqueous formalin in the presence of 12% NaOH for 3 days at room temperature gives 2-hydroxymethyl-3-methyl-6-isopropylphenol (82) m.p. 86° as the sole. product.. Since this appeared to be the easiest method to prepare (82), we repeated this reaction. TLC of the reaction product showed the presence of two new compounds besides thymol. Column chromatography (SiO_2) afforded two crystalline solids, compound-A, m.p. 160-61° and compound-B, m.p. 119°. Compound-A was identified as dithymolyl methane (78) on the basis of spectral comparison. Compound-B .was found to be identical with 2,4-dihydroxymethyl thymol (83). Contrary to the observations of Strubell, we were unable to isolate (82) in this reaction.



(81), R = H

(84), R = COMe



(82), $R_1 = CH_2OH, R_2 = H$

(83), $R_1, R_2 = CH_2OH$

(88), $R_1 = H, R_2 = CH_2OH$

The desired o-hydroxymethyl thymol (82) could be obtained by Reimer-Tiemann reaction on thymol followed by reduction with $NaBH_4$. O-Hydroxymethyl thymol (82) thus obtained was purified via its acetonide and regeneration. The compound thus obtained showed spectral properties consistent with its structure but remained viscous liquid as against the previous report claiming it to be a crystalline solid m.p. 86°*.

The assignment of structure (79) of the minor product of the Duff reaction was confirmed by condensation of o-hydroxy methyl thymol (82) with thymyl acetate (84) following the experimental conditions employed by Hufford[92] followed by acetylation of the resulting monoacetate (25). The spectral properties of synthetic (80) and the acetate prepared from compound m.p. 86° were found to be identical. This synthetic

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* We have also found that p-hydroxymethyl thymol (88) is also a viscous liquid and hence Strubell's compound, m.p. 86° obtained by reaction of thymol with CH_2O in the presence of alkali is neither (82) nor (88). Though there is no direct proof, it is tempting to suggest the identity of Strubell's compound, m.p. 86° with the minor product (79), m.p. 86° obtained by us in the Duff reaction on thymol and whose structure assignment is discussed in this section of the thesis.

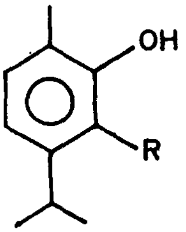
work therefore unambiguously established structure (79) for the minor Duff reaction product of thymol.

Duff reaction of carvacrol (86) has been studied earlier by Duff[86] and recently by Sargent[93]. Formation of o-carvacroldehyde (87) has been reported but once again the non-steam volatile residue was not scrutinised. We have therefore studied the non-steam volatile residue obtained in this reaction.

Column chromatography of the non-steam volatile residue afforded a crystalline solid, $C_{21}H_{28}O_2$, m.p. 177°. Its IR spectrum displayed an intense band at 3500 cm^{-1} due to phenolic-OH group, further confirmed from its solubility in Claisen's alkali, formation of a diacetate m.p. 124°, on treatment with aceticanhydride and pyridine. IR spectrum (Fig. 28) of diacetate derivative is reproduced.

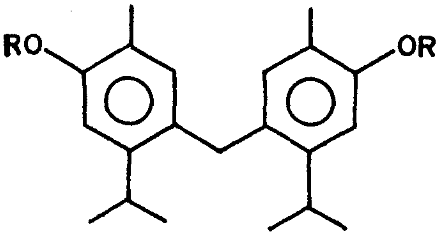
The ^{1}HNMR spectrum (Fig. 29) of diacetate showed a doublet corresponding to 12 protons indicating the presence of two isopropyl groups 1.19(12H, d, J=7.2Hz), two aromatic methyls 2.31(6H, s), a multiplet corresponding to two benzylic protons 3.06(2H, m, J=7.2Hz), a singlet corresponding to two acetoxy groups 2.05(6H, s), presence of benzylic methylene group 3.95(2H, s), two aromatic protons 6.71(2H, s) and aromatic protons ortho to acetoxy groups 6.93(2H,s) indicating aromatic protons are pera substituted.

These results are consistent only with structure (90) for the diacetate. The Duff reaction product, m.p. 177° is therefore, 5,5'dicarvacryl methane (89). To the best of our knowledge, there is no previous report on 5,5'dicarvacryl methane. In order to confirm the structural assignment carvacrol on condensation with formaldehyde in the presence of HCl by following the experimental conditions employed by Strubell[91] for the preparation of dithymolyl methane. The product obtained was found to be identical in all respects with 5,5'dicarvacryl methane (89, m.p., mixed m.p., IR). It gave acetate m.p. 124°.



(86), R=H

(87), R=CHO



(89), R = H

(90), R = $COCH_3$

The non-steam volatile residue is being processed for the characterization of other constituents.

EXPERIMENTAL

Oxidation of monoterpene phenols with iodic acid

General procedure

To a solution of phenol (0.27 m mol) in H_2O-EtOH (4:1, 1600 ml) was added a solution of iodic acid (8.27 g, 0.47 m mol) in H_2O (400 ml) at room temperature. After 24 hr . the mixture was extracted with CH_2Cl_2, washed with $Na_2S_2O_3$ solution, then dried (Na_2SO_4). Purification by column chromatography (silica gel, petroleum ether - EtOAc) followed by preparative TLC (Petroleum ether - EtOAc,, 6:4).

Compound-A, (32): (6%), m.p. 156°.
Found: C, 52.87; H, 6.23; $C_{20}H_{27}O_4I$.
Requires: C, 52.4; H, 5.9%.

IR, ν max, nujol (Fig.1) : 3460, 2900, 1720, 1670, 1565, 1450, 1365, 1260, 1180, 1145, 1125, 1095, 1065, 1025, 995, 940, 900, 850, 835 and 760 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$, δ) (Fig. 2) : 0.86, 0.9, 1.19 and 1.34 (3H each, d, J=7Hz), 1.24 (6H, s), 1.94 (1H, m), 3.21 (1H, m), 3.05 (1H, dd, J=10, 3Hz), 3.21 (1H, m), 3.41 (1H, dd, J=8, 3Hz), 3.57 (1H, dd, J=10, 1.5Hz), 2.33, 4.00 (1H each exchangeable with D_2O), 5.92 (1H, m).

<u>Compound-B</u>: (Carman's dimer, 7a) (30%), m.p. 138-9°.

Identical in all respects (IR, ^{1}HNMR, ^{13}CNMR, m.p.) with natural (7a).

<u>Compound-E</u>(36): m.p. 168°.

Found : C, 72.20; H, 8.65, $C_{20}H_{28}O_4$.

Requires : C, 72.23; H, 8.43%.

IR, ν max, nujol (Fig. 8) : 3500, 2950, 2920, 2850, 1710, 1675, 1630, 1460, 1375, 1335, 1310, 1285, 1230, 1185, 1165, 1130, 1045, 1020, 1000, 930, 870 and 830 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$, δ) (Fig. 9) : 0.59, 0.8, 0.86 and 0.98 (3H each, d, J=7Hz), 1.67 (3H, d, J=1.7Hz), 1.97 (3H, bs), 1.6, 1.79, 3.10, 3.18, 3.28 and 3.32 (1H each, all m), 2.1 and 3.8 (1H each, exchangeable with D_2O), 5.85 (1H, m), 6.0 (1H, bs).

EIMS, 70 ev, m/z (rel. int) (Fig. 11) : [M$^+$] 332 (34), 286 (28), 271 (25), 233 (21), 210 (39) 201 (33) 187 (21), 173 (90), 166 (93), 159 (38), 145 (57), 137 (69), 124 (100).

<u>Compound-F</u> (48): m.p. 160°.

IR, ν max. nujol (Fig. 15) : 3540, 2950, 1725, 1675, 1595, 1465, 1380, 1340, 1175, 1160, 1145, 1120, 1015, 925 and 845 cm^{-1}.

^{1}HNMR (400 MHz, $CDCl_3$, δ) (Fig. 16) : 0.59, 0.84, 0.86 and

0.97 (3H each, d, J=7Hz), 1.6 (3H, d, J=1.7Hz), 2.2 (3H, s), 1.6 (1H, m), 1.78 (1H, m), 3.18 (1H, t, J=2Hz), 3.28 (1H, dd, J=8, 2Hz), 3.34 (1H, dd, J=7, 1.7Hz), 3.43 (1H, dd, J=8, 1.7Hz), 2.25 and 3.75 (1H each exchangeable with D_2O), 5.85 (1H, m).

Oxidation of monoterpene phenols with sodium metaperiodate General procedure:

A solution of phenol (0.13 m mol) in EtOH (125 ml) was added to a solution of sodium metaperiodate (5.75 g, 0.27 m mol) in H_2O (380 ml) and stirred at room temperature for 24 hr. Excess sodium metaperiodate was destroyed by adding ethylene glycol and then extracted with $CHCl_3$ (4 x 50 ml) and then dried (Na_2SO_4). Products were purified by column chromatrography (silica gel and crystallisation from petroleum ether.

Compound-B (Carman's dimer, 7a) : (12%), m.p. 138-9^{o}.
Spectral data as given above.

Compound-E (36) : (12%), m.p. 168^{o}.
Spectral data as given above.

Compound (51) : m.p. 177-80^{o}.

IR, ν max, nujol, (Fig. 17) : 3470, 2910, 2840, 1694, 1655, 1610, 1455, 1445, 1375, 1360, 1315, 1295, 1250, 1240, 1175, 1150, 1130, 1115, 1100, 1035, 1025 and 1005 cm^{-1}.

^{1}HNMR (300 MHz, $CDCl_3$, δ Fig. 18) : 0.54, 0.83, 0.89, 0.95 (3H each, d, J=7Hz), 1.32 (3H, s), 1.43 (3H, d, J=1Hz), 1.58 (3H, d, J=1Hz), 1.86 (3H, d, J=1Hz), 1.66 (2H, m), 2.74 (1H, s), 2.93 (1H, d, J=2Hz), 3.14 (1H, d, J=2Hz), 2.4 and 3.7 (1H each, s, exchangeable with D_2O), 5.92 (1H, q, J=1Hz).

^{13}CNMR (300 MHz, $CDCl_3$, δ) (Fig. 19) : 15.39, 16.44, 16.73, 17.21, 17.28, 17.5, 19.86 and 24.36 (8 quartets), 33.72, 36.93, 44.14, 46.68, 65.26 and 124.79 (6 doublets), 48.6, 76.57, 77.0, 128.94, 133.58, 159.59, 201.0 and 213.3 (8 singlets).

EIMS, 70 ev, (Fig. 20) : [M^+] 360, m/z 349, 314, 313, 299, 272, 271, 261, 243, 228, 221, 200, 183, 180, 179, 168, 150, 138, 137, 136, 120, 109, 108 and 70.

Compound-G: m.p. 121°.

IR, ν max, KBr : 3500, 3460, 2970, 2940, 2880, 1710, 1670, 1640, 1610, 1575, 1450, 1440, 1415, 1380, 1360, 1340, 1315, 1270, 1250, 1190, 1170, 1150, 1130, 1120, 1100, 1075, 1040, 1020 and 950 cm^{-1}.

Elemental Analysis:

Found : C, 74.00; H, 8.70%.

Compound-H (53) : m.p. 158°.

IR, ν max, KBr (Fig. 23) : 3440, 2970, 2880, 1710, 1675, 1630, 1470, 1385, 1365, 1315, 1250, 1190, 1160, 1130, 1060, 1020, 1005, 890 and 870 cm^{-1}.

EIMS, 70 ev : M^+388.

Oxidation of isothymol (18) with periodic acid:

A solution of isothymol (0.4 g), 0.027 m mol) in H_2O-EtOH (4:1, 160 ml) was mixed at room temperature with a solution of periodic acid (0.8 g, 0.035 m mol) in H_2O (40 ml) and stirred for 15 minutes, the unconsumed periodic acid was destryoed by adding ethylene glycol and the mixture extracted with CH_2Cl_2 (5 x 40 ml). The combined extract was washed with saturated aquious $Na_2S_2O_3$, then with H_2O and dried ($CaSO_4$). The residue on column chromatography (silica gel) and elution with EtOAc-petroleum ether (3:1) gave p-quinol (compound-C, 88 mg, 22%), m.p. 84° and compound-D (48 mg, 12%) m.p. 168°.

Compound-C (18) : (88 mg, 22%), m.p. 84°

Found : C, 72.38; H, 8.6; $C_{10}H_{14}O_2$.
Requires : C, 72.28; H, 8.43%.

IR, ν max, nujol (Fig. 6) : 3430, 2950, 1665, 1632, 1620, 1600, 1465, 1390, 1355, 132o, 1290, 1260, 1135, 1060, 1035, 915, 855, and 825. cm^{-1}

^{1}HNMR (90 MHz, $CDCl_3$, δ) (Fig. 7) : 1.06 (6H, d, J=7Hz), 1.47 (3H, s), 2.49 (1H, bs, exchangeable with D_2O), 2.95 (1H, m), 6.08 (1H, d, J-10Hz), 6.56 (1H, m), 6.82 (1H, dd, J=10, 3Hz).

Compound-D (36) : (48 mg, 12%), m.p. 168°

Spectral data is same as given for compound-E (36).

General procedure for photochemical reactions of dimers (7a,36,51)

A solution of the dimer (0.05-0.1%) in n-hexane was subjected to (i) irradiation with a RUL-3000 A° lamp in pyrex tubes under N_2 atmosphere using Raynot Photochemical Reactor Model RRR-208 for six hr, or (ii) exsposed to sunlight in a corning conical flask for one to two hr. After evaporation of solvent, the residue was dissolved in a small amount of $CHCl_3$ and subjected to column chromatography (silica gel) to afford the photocycloaddition products in good yield.

Compound (34) : (47%), m.p. 229°.

IR, ν max, nujol (Fig. 3) : 3460, 2950, 2920, 2840, 1700, 1460, 1380, 1360, 1250, 1190, 1135 and 1095 cm^{-1}.

^{1}HNMR (90MHz, δ, $CDCl_3$) (Fig. 4) : 0.77 (6H, d, J=7Hz), 1.07

(6H, d, J=7Hz), 1.3 (6H, s), 2.05 (2H, m), 2.72 (2H, m), 2.95 (3H, m).

EIMS, 70 ev, m/z (rel. int) (Fig. 5) : [M^+] 332 (20), 286 (25), 271 (27), 261 (17), 253 (6), 243 (62), 229 (31), 215 (21), 201 (74), 191 (40), 173 (26), 166 (69), 159 (100), 149 (75), 137 (53), 123 (97), 109 (34), 95 (14), 91 (22), 71 (13).

Compound (40): (80%), m.p. 155°

IR, ν max, nujol (Fig. 12) : 3490, 2960, 2900, 2860, 1700, 1445, 1380, 1335, 1295, 1150, 1020 and 900 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$, δ) (Fig. 13) : 0.8 (6H, d, J=7Hz), 0.97 (6H, d, J=7Hz), 1.16 (6H, s), 1.2-1.8 (5H, m), 2.56 (2H, m), 2.87 (3H, m).

EIMS, 70 ev, m/z (rel. int.) (Fig. 14) : [M^+] 332 (13), 286 (24), 271 (22), 243 (80), 233 (22), 215 (36), 173 (36), 167 (60), 149 (100), 137 (45).

Compound (52) : (50%), m.p. 190°.

IR, ν max, nujol (Fig. 21) : 3500, 2900, 2840, 1680, 1450, 1370, 1190, 1120, 1065 and 1000 cm^{-1}.

^{1}HNMR (300 MHz, $CDCl_3$,δ) (Fig. 22) : 0.775, 0.935 (6H each, d, J-7Hz), two isopropyl groups), 1.06 (6H, s, two methyls), 1.74 (2H,m, two isopropyl methines), 2.285 (2H, two methine protons), 2.385 (2H, two methine protons), 3.00 (2H, exchangeable with D_2O, two hydroxyl protons).

Preparation of 4,6-dimethyl coumarin (54)

In a 500 ml three necked round bottom flask, equipped with over head stirrer, $CaCl_2$ guard tube and a dropping funnel was added a mixture of P_2O_5 (100 g), orthophosphoric acid (75 ml) and heated and stirred on steam bath for 2 hr while PPA is formed. P-cresol (20 ml) and ethylacetoacetate (26 ml) were added and the reaction mixture was heated on water bath for 2 hr under stirring. After this period the reaction mixture was poured on crushed ice with stirring. The solid obtained was filtered and washed with water. It was crystallised from EtOH to give 4,6-dimethyl coumarin (7 g, m.p. 150-151°, lit m.p. 151°C[57]).

Spectral data given on page 157 .

Preparation of 7,8-dehydroisothymol (55) from (54)[58]

A mixture of 4,6-dimethyl coumarin (4 g,0.023 mol), NaOH (4.33 g, 0.11 mol) and ethyleneglycol (28 ml) was refluxed under N_2 atmosphere for 2 hr. The cooled mixture was diluted with ice water (50 ml) and petroleum ether (80 ml) was added and the solution was carefully acidified with HCl (2N). The petroleum ether layer was separated and the aqueous layer reextracted with petroleum ether (2 x 20 ml). The combined petroleum ether extracts were washed with H_2O and dried. Removal of solvent under vacuo gave 7,8-dehydroisothymol (55, 2.3 g, 65%).

Preparation of Isothymol (17) from (55)

7,8-dehydroisothymol (2 g, 0.014 mol) was dissolved in EtOH (100 ml), to this was added 10% palladium on carbon (0.2 g) and the resulting mixture shaken with hydrogen at atmospheric pressure until no more hydrogen was absorbed (approx. 1 hr). The solution was filtered and concentrated to dryness to give isothymol (2 g).

IR, ν max, neat : 3570, 3030, 1610, 1500, 1455, 1376, 1352, 1325, 1316, 128o, 1255, 1195, 1160, 1111, 1075, 1045, 990, 920, 805, 765 and 700 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$, δ) : 1.25(6H,d, J=7Hz), 2.28(3H, s), 3.13(1H, m, J=7Hz), 4.55(1H, bs), 6.65-7.0(3H, m).

Preparation of Thymyl acetate (56) from (20)

A mixture of thymol (8 g), acetic anhydride (16 ml) and conc. H_2SO_4 (0.14 ml) was refluxed for 10 hr, cooled and poured over crushed ice and extracted with ether (3 x 50 ml). Combined ethereal extracts were was washed with aqueous NaOH (20%) ✓ and then with H_2O. Extract was dried, evaporated and residue was distilled to give thymyl acetate (B.P. 242-43°C).

Preparation of 4-acetyl thymol (57) from (20)[59,60]

To a mixture of thymyl acetate (10 g) in nitrobenzene (10 ml), anhydrous $AlCl_3$ (12.3 g) was added and the reaction mixture stirred at room temperature for 24 hr. Poured over crushed ice containing conc. HCl (12 ml). Nitrobenzene was removed by steam distillation and the non steam volatile portion when left at room temperature for 24 hr, deposited colourless solid which was filtered (8.5 g) and purified by repeated crystallisation from benzene (m.p. 122°C).

IR ν max, nujol: 3500, 3000, 1640, 1610, 1575, 1460, 1370, 1310, 1250, 1185, 1160, 1115, 1095, 1065, 1030, 1010, 940, 890 and 860 cm^{-1}.

^{1}HNMR, (100 MHz, $CDCl_3$, δ): 1.28(6H,d, J=7Hz), 2.5(3H, s), 2.6(3H, s), 3.24(1H, m, J=7Hz), 6.68(1H,s), 7.66(1H,s), 6.3(1H, bs).

Preparation of 4-Ethyl Thymol (22) from (57)[61]

A solution of Zn (16 g), $HgCl_2$ (1.6 g), conc. HCl (2.4 ml) and H_2O (32 ml) was stirred for 5 min. The aqueous solution was decanted and the amalgamated Zn covered with H_2O (12 ml) and conc. HCl (16 ml). To this solution was added 4-acetyl thymol (8 g) and refluxed for 8 hr (more conc. HCl was added after 6 hr of refluxing). The reaction mixture was cooled and saturated NaCl solution was added. The resulting solution was extracted with ether (3 x 50 ml). The combined ethereal extract was dried and concentrated to give crude phenol, which was further purified by distillation under reduced pressure to give 4-ethyl thymol (4 g) as a colourless liquid).

IR ν max, neat: 3500, 2975, 2890, 1625, 1585, 1515, 1460, 1420, 1385, 1365, 1345, 1325, 1285, 1225, 1190, 1075, 1050, 905, 870, 815 and 600 cm^{-1}.

^{1}HNMR (100 MHz, $CDCl_3$, δ) : 1.19(3H, t, J=8Hz), 1.28(6H, d, J=8Hz), 2.26(3H, s), 2.58(2H,q, J=8Hz), 3.17(1H, m, J=8Hz), 4.5(1H, s, not D_2O exchangable), 6.58(1H, s), 6.98(1H, s).

Preparation of p-thymotinic aldehyde (58) from (20)[62]

A 500 ml three necked round bottom flask was fitted with a over head stirrer, a reflux condenser and a wide mouth tube extending nearly to the bottom of the flask. To the inlet was attached a dry HCl generator. To the top of condenser was connected a tube leading to a wash bottle containing H_2SO_4 from this a tube led to a safety bottle and from the latter to the surface of NaOH solution.

In the three neck flask was placed thymol (10 g) in dry benzene (30 ml) and powdered dry $Zn(CN)_2$ (18.5 g, prepared from $ZnCl_2$ and KCN) was then added. The mixture was cooled, dry HCl gas was passed rapidly for 1 hr, under stirring. The stirring was continued while the reaction maintained at 0^{o}. After 1.5 hr the condensor was removed and powdered, dry anhydrous $AlCl_3$ (15 g, 1.5 molar equivalent) was slowly added in one portion. Stirring was continued and HCl gas passed slowly while the mixture was heated at $40-45^{o}$ for 3.5 hr.

The reaction mixture was added to an excess of HCl (10%). The ppte. of imide hydrochloride which separate out was heated to reflux for 0.5 hr. The generated aldehyde was extracted in $CHCl_3$ (3 x 50 ml) and combined $CHCl_3$ extracts were washed with water, dried. Removal of $CHCl_3$ under reduced pressure gave p-thymotinic aldehyde (11 g), m.p. $133^{o}C$, lit m.p. $133^{o}C$[62]).

IR, ν max, nujol: 3250, 2950, 2860, 1675, 1600, 1525, 1435, 1385, 1355, 1305, 1255, 1185, 1155, 1110, 1050, 1045, 915, 895, 865, 845 and 760 cm^{-1}.

4-Methyl thymol (21) by Clemmensen reduction of aldehyde (58)

The aldehyde (58) was subjected to Clemmensen reduction following the procedure described for the conversion of (57 to 22) (page 78).

IR, ν max, neat: 3400, 2875, 2825, 1610, 1575, 1505, 1450, 1400, 1375, 1350, 1330, 1270, 1260, 1220, 1185, 1160, 1095, 1075, 1025, 870, 845, 790 and 735 cm^{-1}.

Preparation of p-quinones

The phenols were converted to the methyl ethers with Me_2SO_4, NaOH by using standard procedure.

General Procedure

Methyl aryl ether (2 g) in AcOH (15 ml) is treated with CrO_3 (4 g) in dil. AcOH (80%). When the vigorous reaction is over, the mixture is poured into H_2O and extracted with ether (3 x 50 ml). The combined ethereal extracts were washed with Na_2CO_3 to remove acidic products. The neutral portion, after washing with H_2O, was dried. Removal of solvent followed by chromatography gave corresponding quinones in pure form.

In case of p-substituted methyl aryl ethers neutral as well as acidic products were recovered. Neutral products were further oxidised by standard procedure to get corresponding acids.

Sl. No.	Name of product	Melting point in °C		Ref
		Observed	Reported	
1.	1,4 Thymoquinone	40-45	43-45	69-71
2.	2,3 Dimethyl-1,4 benzoquinone	55	55	72,73
3.	1,4 Naphthoquinone	120-22	125	74-77
4.	1,4 Benzoquinone	115	116	77,79
5.	2,6 Dimethoxy benzoquinone	248-49	-	80
6.	2-Methyl-p-anisic acid	172-73	176	81
7.	5-Isopropyl-2-methyl p-anisic acid	138	138-39	82
8.	2,2'dimethyl-4,4' dimethoxy-5,5'diisopropyl benzophenone	132	-	-

2-methyl-p-anisic acid (67), m.p. 172-73°

IR, ν max, nujol: 2900, 1685, 1615, 1570, 1500, 1460, 1410, 1380, 1330, 1300, 1250, 1160, 1090, 1050, 1040, 1000, 925, 870, 820 and 775 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$ + D_2O, δ): 2.65(3H,s,Ar-CH_3), 3.86(3H, s, Ar-OCH_3), 6.75(2H,m, C-3,H and C-5,H), 8.078(1H,d, J=9Hz, C-6,H).

5-isopropyl-2-methyl-p-anisic acid (70), m.p.-138°

IR, ν max, nujol: 2925, 1700, 1625, 1575, 1510, 1470, 1375, 1350, 1320, 1300, 1270, 1240, 1180, 1165, 1125, 1100, 1085, 1060, 1035, 990, 930, 850 and 790 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$, δ): 1.21(6H, d, J=7.7Hz,C-5, isopropyl methyls), 2.68(3H, s, Ar-CH_3 at C-2), 3.33(1H, septet, J=7.7Hz, benzylic proton), 3.93(3H,s, Ar-OCH_3 at C-4), 6.81(1H, s, C-3,H) and 8.11(1H, s, C-6,H).

2,2'dimethyl-4,4' dimethoxy-5,5'diisopropyl benzophenone (72), m.p. 132°:

IR, ν max, nujol : 2950, 1650, 1610, 1570, 1505, 1470, 1385, 1355, 1325, 1260, 1250, 1190, 1150, 1110, 1065 and 880 cm^{-1}.

^{1}HNMR (90 MHz, $CDCl_3$, δ) : 1.1 (12H, d, J=7.2Hz, two equivalent isopropyl groups), 2.46 (6H, s, two Ar-CH_3 at C-2 and C-2'), 3.24 (2H, septet, J=7.2Hz, two methine protons), 3.88 (6H, s, two Ar-OCH_3 at C-4 and C-4'), 6.7 (2H, s, C-3, H and C-3', H), 7.18 (2H, s, C-6, H and C-6', H).

Preparation of O-thymotinic aldehyde

A three necked flask was equipped with a condenser, a mechanical stirrer and a thermometer. A warm solution of NaOH (10 g in 10 ml H_2O) was placed in the flask, thymol (5 g, 0.033 mole) was added and the flask was heated till thymol dissolved. The temperature inside the flask was adjusted to

60-65°, so that no solid sodium salt of thymol separates out. $CHCl_3$ (7.3 g, 4.9 ml) was introduced in such rate (by means of dropping funnel fitted into the top of the condenser) to maintain the temperature of the well stirred mixutre at 60-65°. Finally the flask was heated on a boiling water bath for 1 hr to complete the reaction than aqueous solution allowed to cool, acidified and was extracted with $CHCl_3$ (3 x 50 ml), washed with H_2O, dried. Removal of $CHCl_3$ yielded oily residue which contained unreacted thymol and mixture of o and p-thymotinic aldehydes. It was partly purified by column chromatography and unreacted thymol was separated. Remaining fraction was subjected as such to reduction.

Preparation of O-Hydroxy methyl thymol(82)

A three necked flask was equipped with a mechanical stirrer, a thermometer and a dropping funnel, was placed in ice bath. A solution of the mixture of aldehydes from the earlier reaction (1.2 g, 0.007 mole) in MeOH (2 ml) was placed in the flask. A solution of $NaBH_4$ [0.15 g of $NaBH_4$ in 2N NaOH (1.5 ml)] was added dropwise, with occasional cooling to keep the reaction at 20-25°. After 1 hr MeOH was removed under reduced pressure and the residue was neutralised by HCl (2N). The neutralised residue was diluted with H_2O and extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with H_2O, dried and ether was removed on water bath.

The residue containing o and p hydroxymethyl thymol was treated with acetone and few drops of $HClO_4$ (70%) and kept at room temperature for 5 hr. The reaction mixture was then diluted with H_2O and neutralised by $NaHCO_3$ solution. The acetone was removed by concentration in vacuo below 50° and the aqueous residue was extracted with ether (3 x 20 ml). The combined ethereal extracts were washed with H_2O, dried and concentrated. The residue was loaded on column (silica gel), the first fraction eluted in 5% EtOAc in petroleum ether afforded acetonide derivative of o-hydroxy methyl thymol. o-hydroxy methyl thymol was regenerated by treatment with dilute AcOH at room temperature for 3 hr (0.5 g, thick viscous oil).

IR, ν max, neat : 3300, 2940, 1605, 1580, 1485, 1450, 1420, 1375, 1370, 1330, 1275, 1260, 1240, 1190, 1135, 1050, 1035, 960 and 865 cm^{-1}.

Preparation of 2,6' dithymyl methane monoacetate (85)

A solution of o-hydroxymethyl thymol (500 mg, 0.003 mole) and BF_3. Et_2O (2 ml) in dioxane (5 ml) was added over 15 minutes to a solution of thymyl acetate (322 mg, 0.0017 mole) in dioxane (5 ml) at 70°. An additional BF_3. Et_2O (2 ml) in dioxane (1 ml) was added and the mixture was allowed to stand at 70° for 30 minutes. The mixture was then diluted with

H_2O, extracted in $CHCl_3$ (3 x 20 ml), dried. The residue obtained on removal of ether was purified by column chromatography (silica gel) to yield 2,6'-dithymyl methane monoacetate (380 mg, 39%).

2,6'-dithymyl methane diacetate (80) was prepared by standard method (acetic anhydride, pyridine), IR, NMR of diacetate are given below.

IR, ν max, (Fig. 26) : 3000, 2960, 2905, 1780, 1515, 1470, 1350, 1225, 1215, 1180, 1170, 1150, 1110, 1100, 1075, 1045, 990, 920, 825 and 610 cm^{-1}.

^{1}HNMR, ($CDCl_3$,δ) (Fig. 27) : 0.98(6H, d, J=6Hz), 1.2(6H, d, J=6Hz), 2.12(3H, s), 2.16(3H, s), 2.3(3H, s), 2.32(3H, s), 2.83(2H, m, J=6Hz), 3.72(2H,s), 6.53(1H,s), 6.78(1H, s), 7.09(1H, d, J=8Hz) and 7.17(1H, d, J=8Hz).

Attempted preparation of 4-hydroxy methyl thymol (88) from p-thymotinic acid by reduction with $LiAlH_4$

P-thymotinic acid (0.1 g, 0.0005 mole) in dry ether (2 ml) was added dropwise to dry ether (15 ml) containing little excess $LiAlH_4$ (0.011 g) and the solution was refluxed for 2 hr. On usual work-up afforded a product which was characterized as 4-methyl thymol (since we had prepared 4-methyl thymol in our laboratory, direct comparison was done, its IR, co-TLC were identical) indicating p-thymotinic acid was over reduced to give 4-methyl thymol.

Duff reaction on carvacrol

Glycerol (60 g) and boric acid (14 g) were heated at 170^{o}C. At 170^{o} hexamethylene tetraamine (10 g) was added. The mixture was stirred and temperature brought to 160^{o}, carvacrol (10 g) added at once and temperature maintained at $150\text{-}155^{o}$ during 15 minutes. The resulting thick brown liquid was allowed to cool to 110^{o}. A solution of conc. H_2SO_4 (15 ml) in H_2O (50 ml) added and the whole reaction mixture was steam distilled. The steam distillate was extracted with ether (3 x 50 ml), dried and concentrated to give liquid compound. The liquid on distillation under vacuum yielded o-carvacrotinic aldehyde (87, 0.9 g) oil.

The non steam volatile portion was extracted with ether (4 x 50 ml), washed with H_2O, dried. The residue after removal of ether on repeated chromatography (silica gel) could be resolved into two components, one of them was identified on basis of spectral data (IR, ^{1}HNMR). (i) Less polar, viscous, thick oil may be low melting solid compound but did not solidify, 50 mg and requires further purification (ii) more polar, white crystals, 85 mg, m.p. 174^{o} was identified as 5,5'-dicarvacryl methane* (89).

The identity was proved by an independent synthesis.

Found : C=80.9, H=8.83, O=10.27; $C_{21}H_{28}O_2$.
Required : C=80.8, H=8.97, O=10.26

IR, ν max, nujol: 3500, 3350, 3000, 2960, 2900, 1525, 1480, 1420, 1390, 1295, 1270, 1240, 1210, 1175, 1155, 1045, 1030, 910, 895, 885 and 880 cm^{-1}.

Diacetate derivative of 5,5'-dicarvacryl methane (89), m.p.124 was prepared by usual procedure (acetic anhydride and dry pyridine). The IR and ^{1}HNMR spectrum of diacetate derivative (90) is given below:

Found : C=75.51, H=8.1, O=16.39, $C_{25}H_{32}O_4$.
Required : C=75.76, H=8.08, O=16.16.

^{1}HNMR, (360 MHz, $CDCl_3$, δ): 1.19 (12H,d, J=7.2Hz), 2.05 (6H, s), 2.31 (6H, s), 3.06 (2H,m, J=7.2Hz), 3.95 (2H, s, benzylic methylene), 6.71 (2H, s, C-6, H and C-6', H), 6.93 (2H, s, C-3, H and C-3', H).

IR, ν max, nujol: 3000, 1760, 1620, 1580, 1500, 1460, 1410, 1380, 1280, 1220, 1150, 1120, 1100, 1050, 1040, 1020, 950, 930, 880 and 830 cm^{-1}.

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* Numbering is based on p-menthane Skeleton

Preparation of 5,5' dicarvacryl methane

Carvacrol (1 g), formalin (0.43 g, 35%) and Conc.HCl (0.14 ml) were left at room temperature for 3 days and then worked out as usual to yield a solid, which was purified by column chromatography (silica gel) to yield crystalline material m.p. 177°. The product was identical in all respects (m.p., max m.p., IR, ^{1}HNMR) with product obtained from Duff reaction (non steam volatile residue) on carvacrol. 5,5'-dicarvacryl methane was subjected to acetylation with acetic anhydride and pyridine at room temperature. On usual work-up afforded diacetate (90), m.p. 124°.

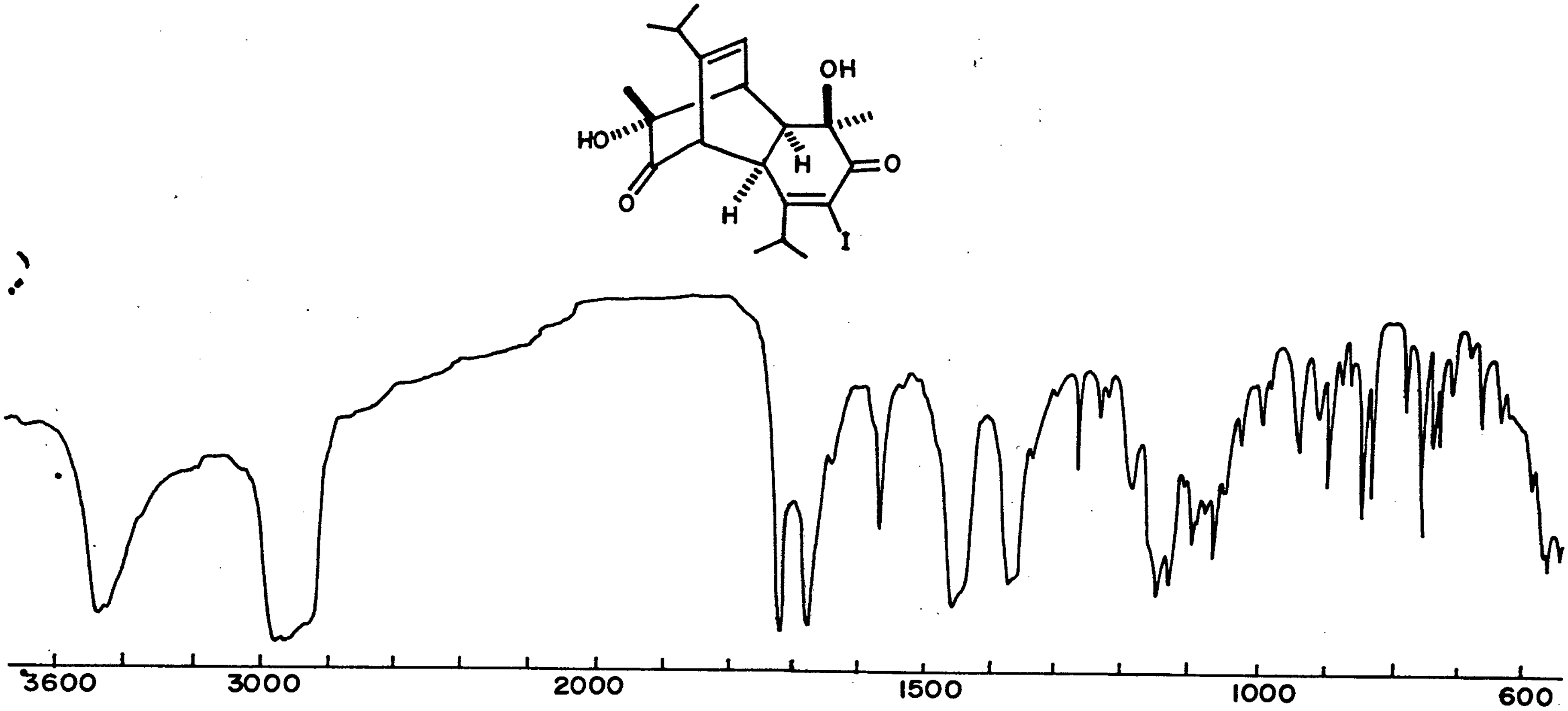

Fig. 1 IR spectrum of (32)

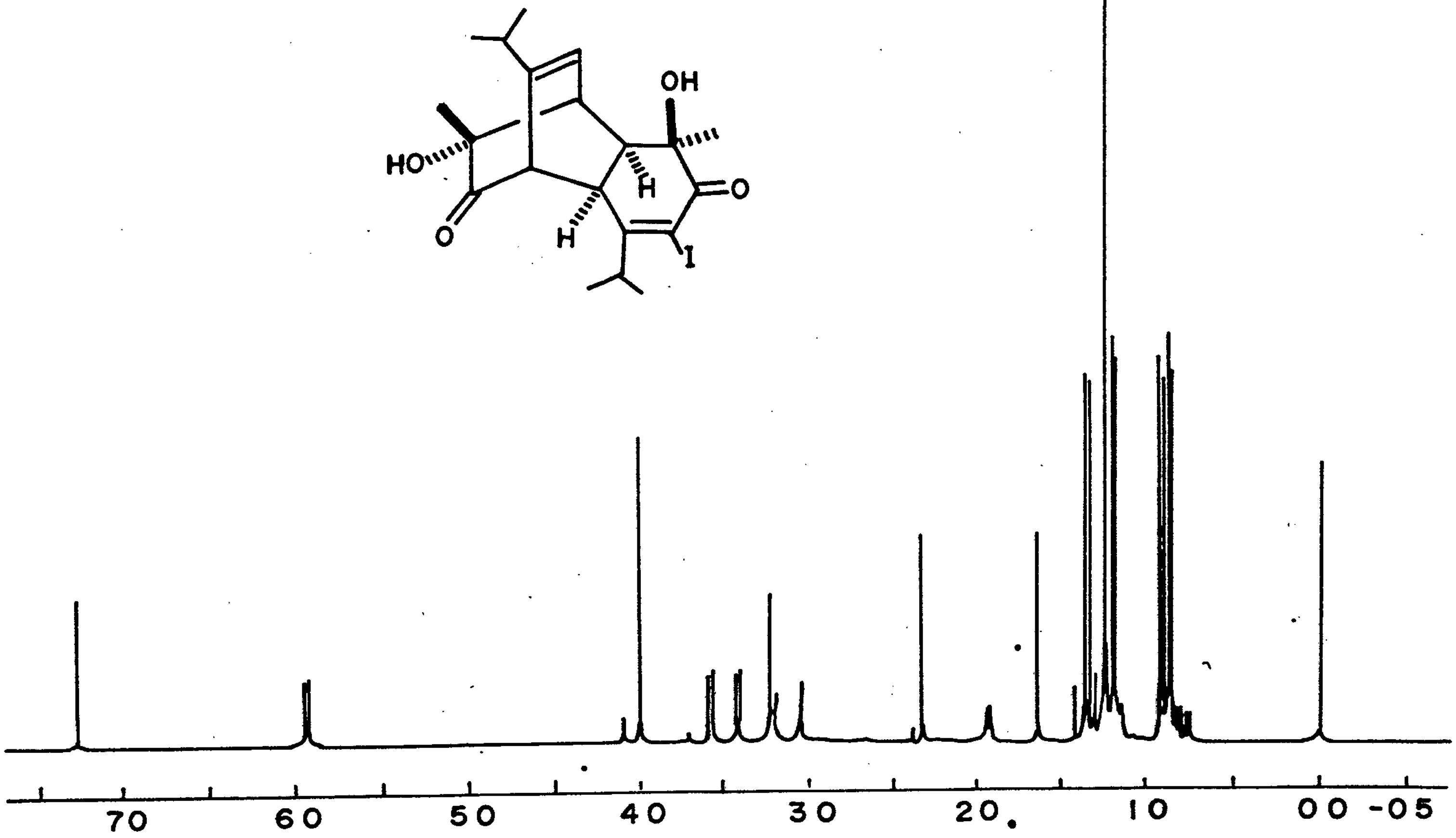

Fig. 2 ^{1}HNMR spectrum of (32)

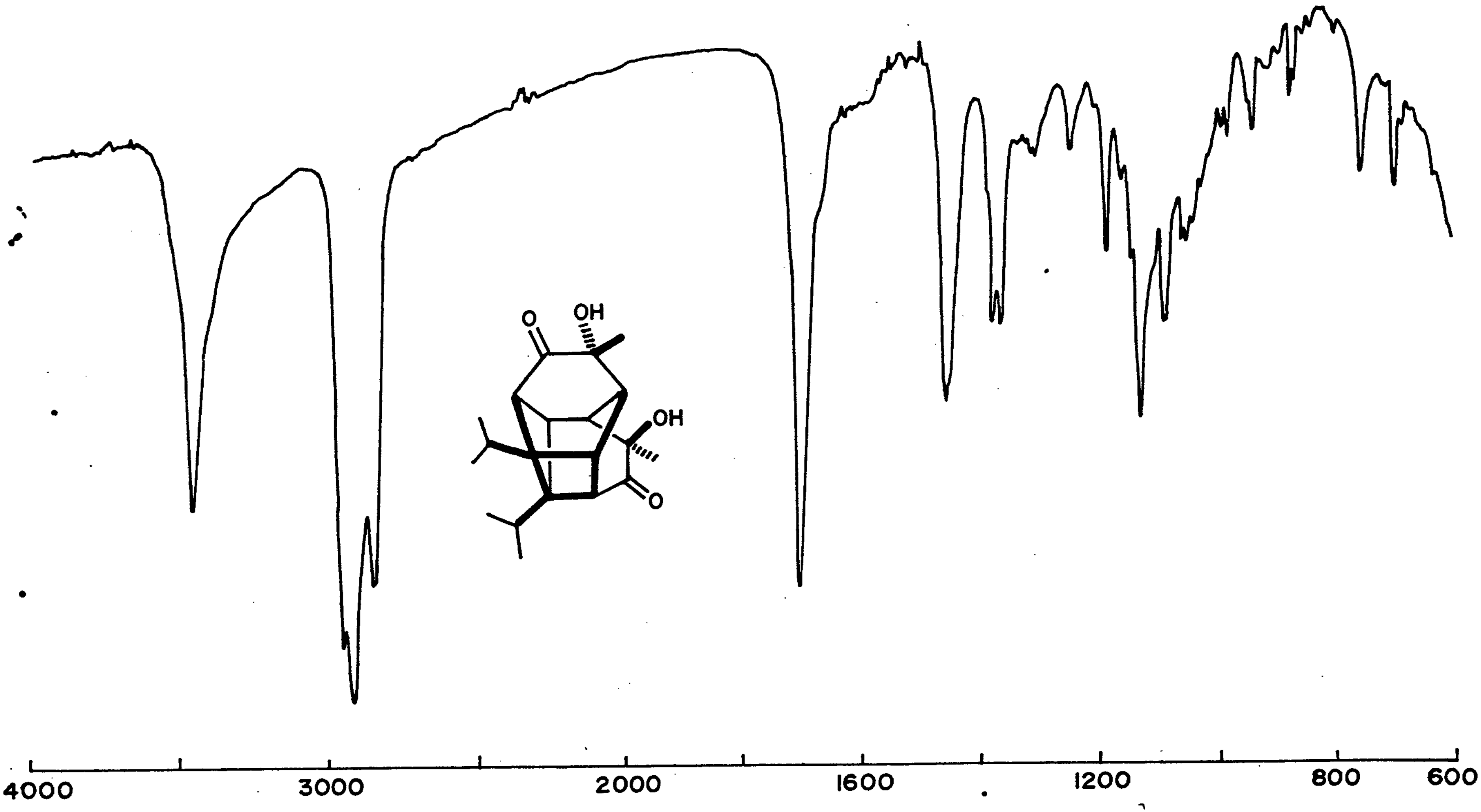

Fig. 3 IR spectrum of (34)

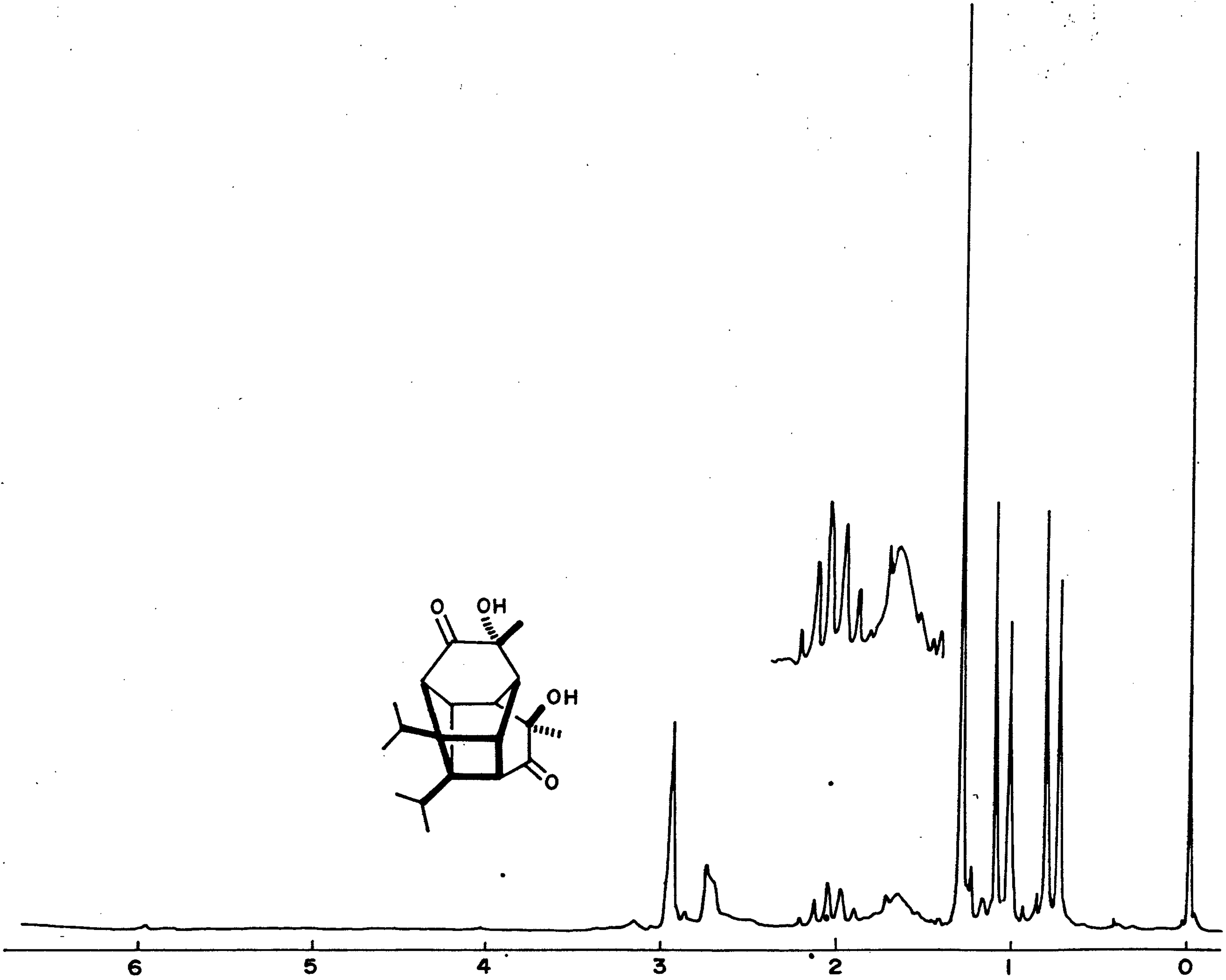

Fig. 4 ^{1}HNMR spectrum of (34)

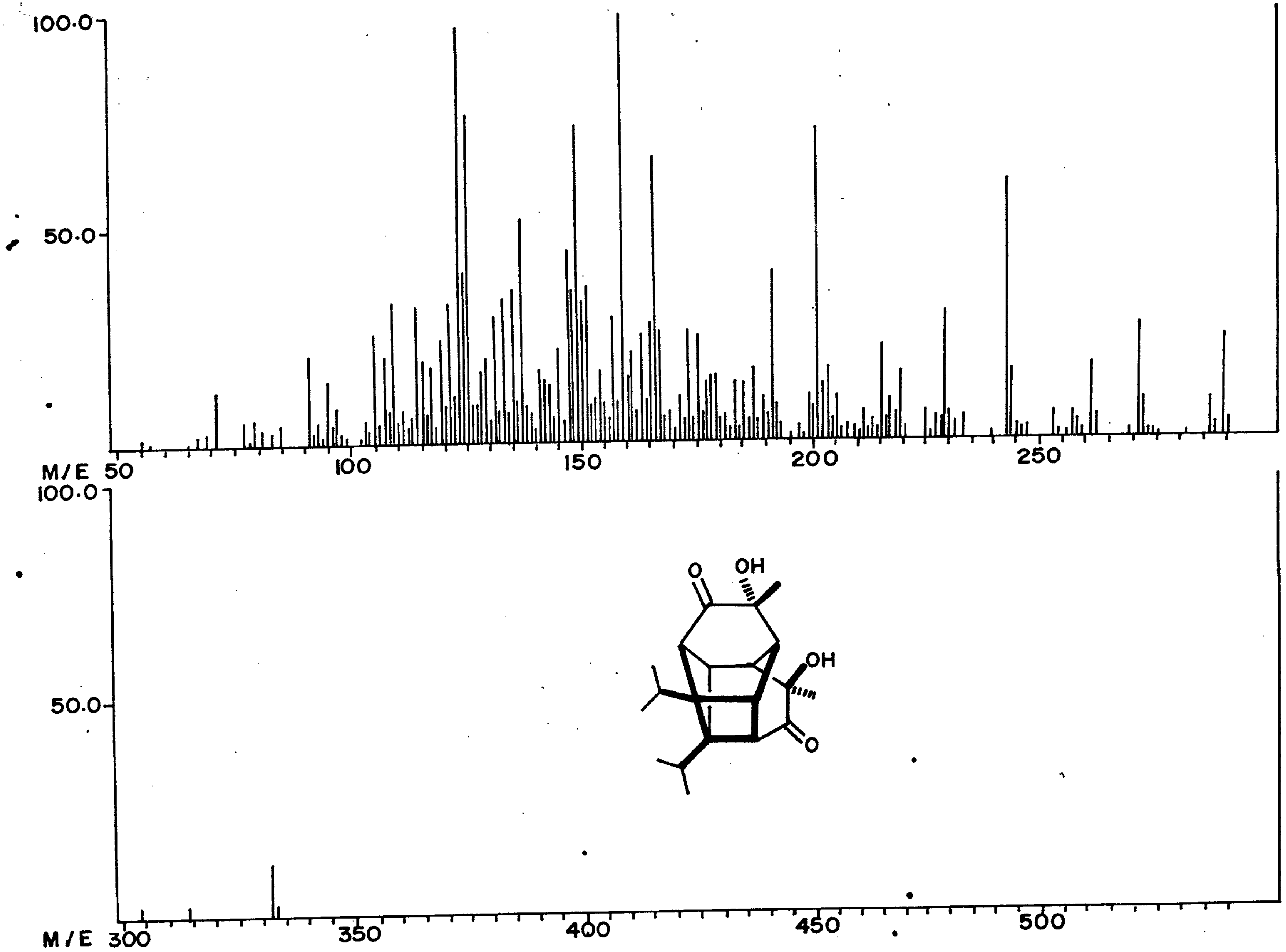

Fig. 5 Mass spectrum of (34)

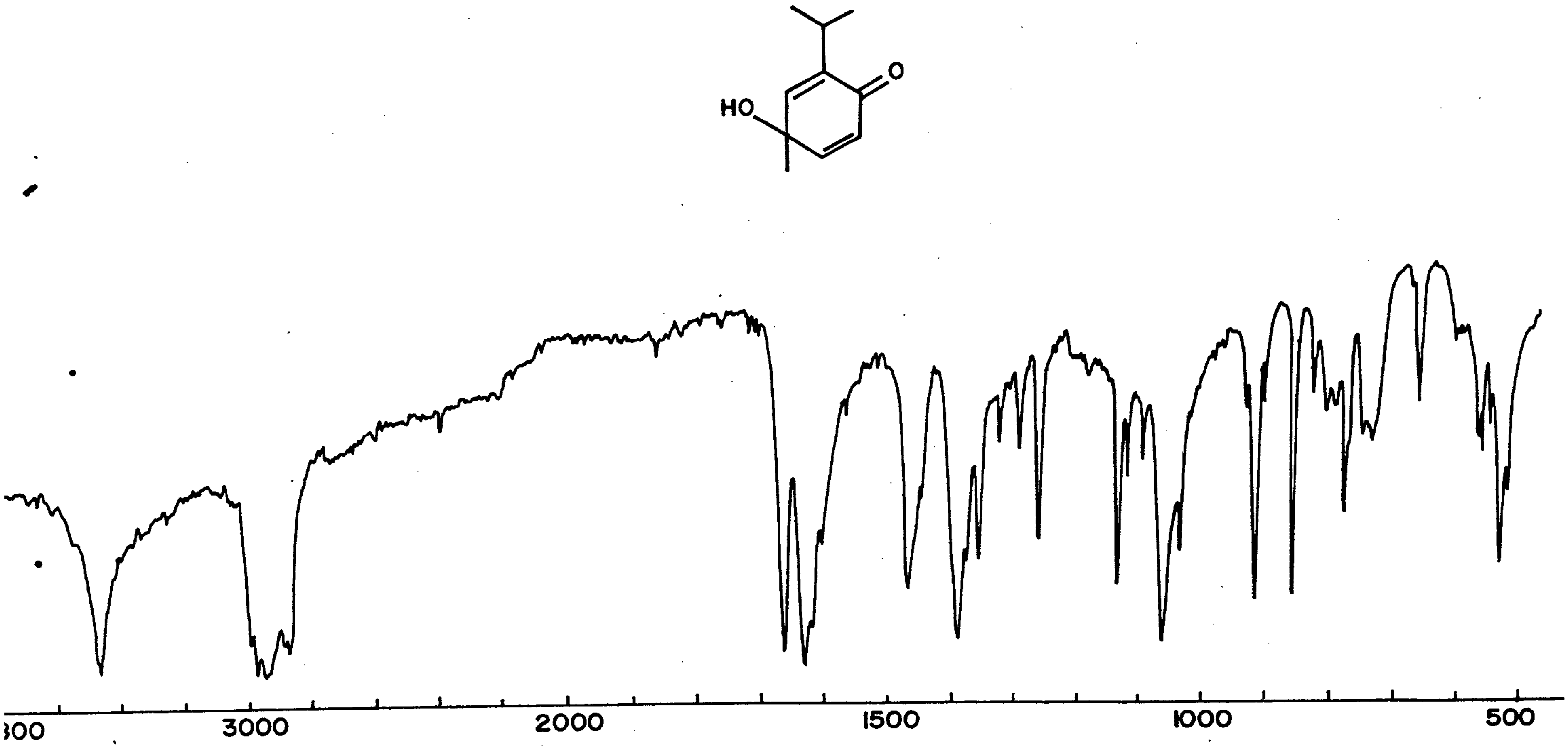

Fig. 6 IR spectrum of (18)

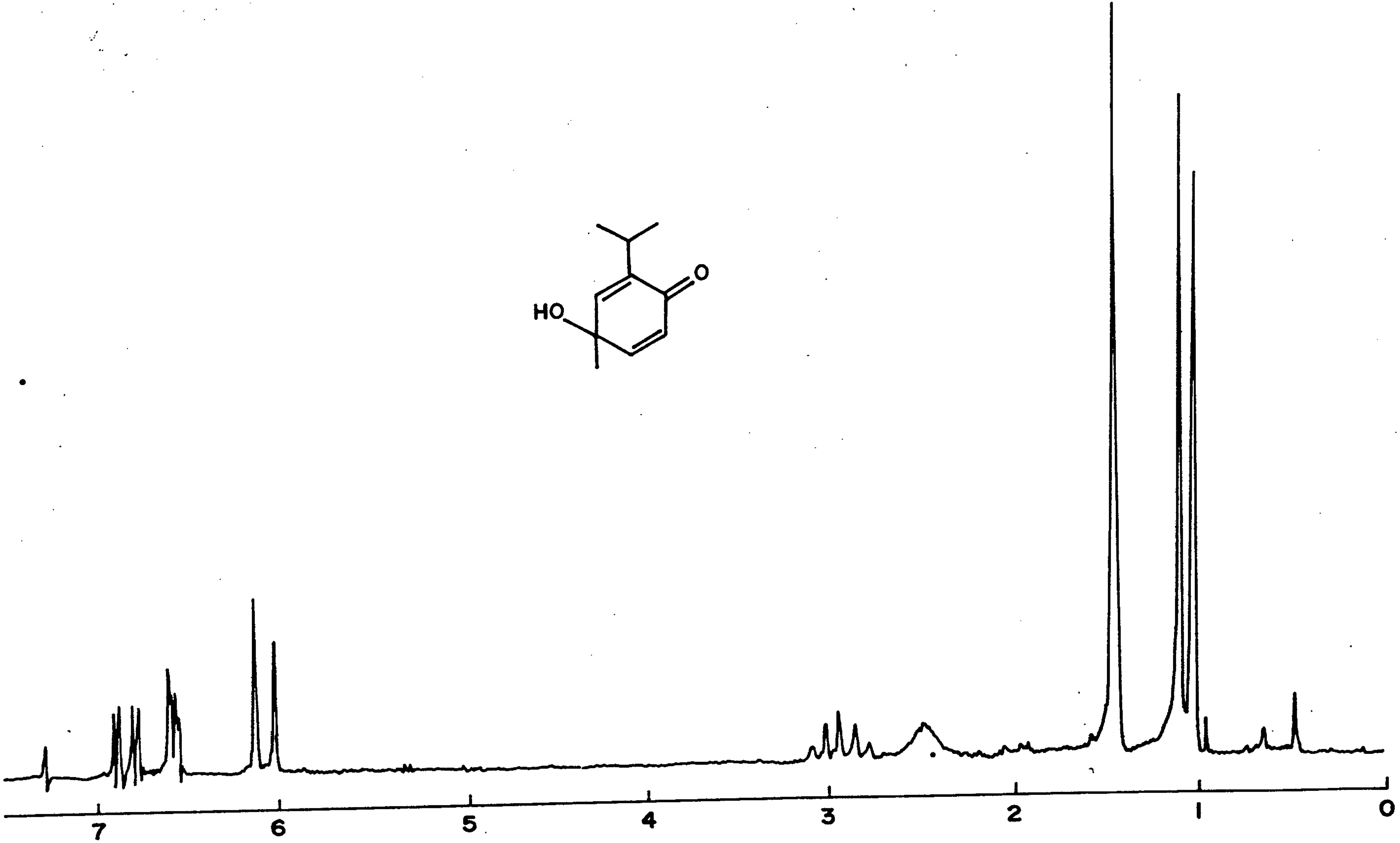

Fig. 7 ^{1}HNMR spectrum of (18)

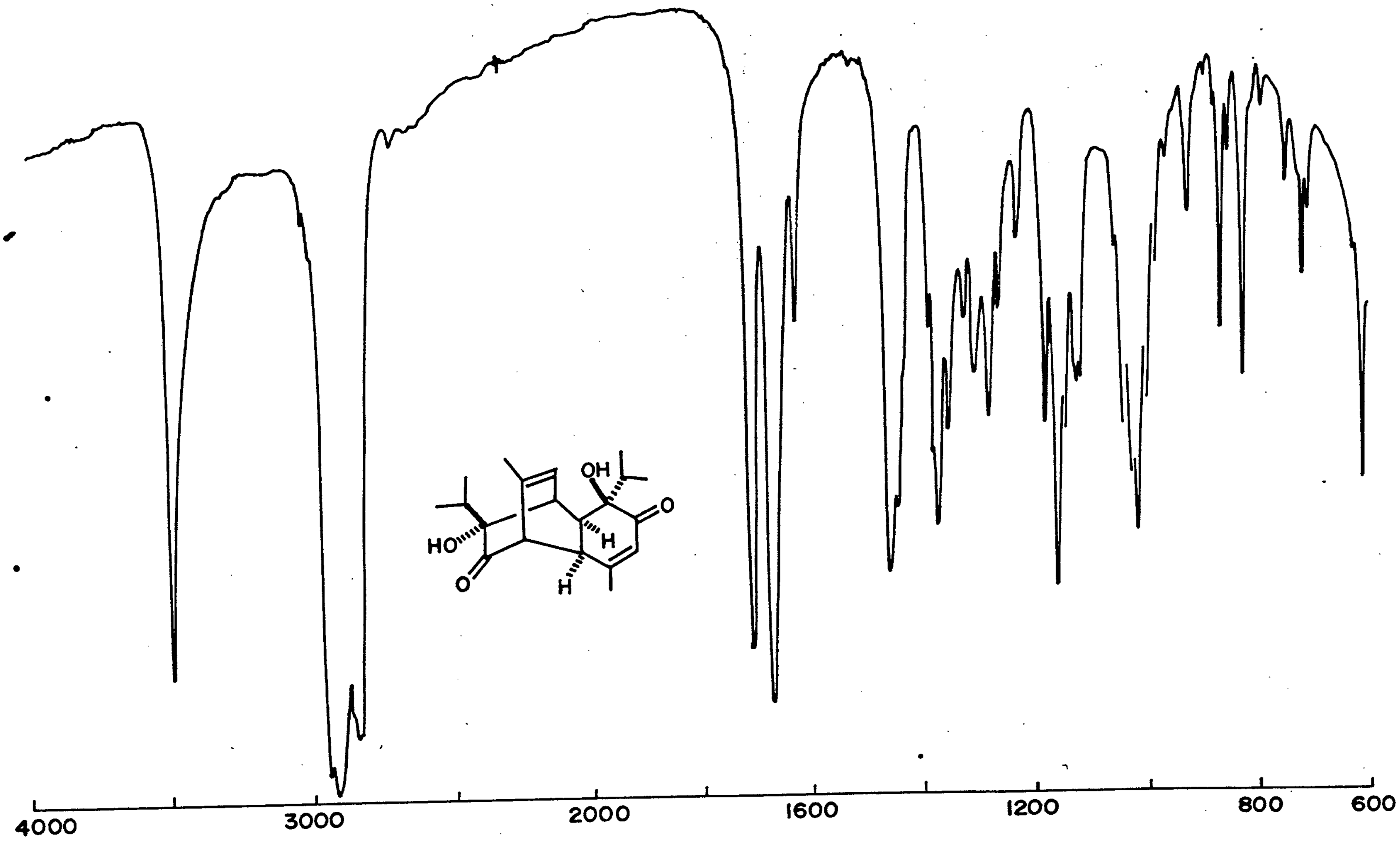

Fig. 8 IR spectrum of (36)

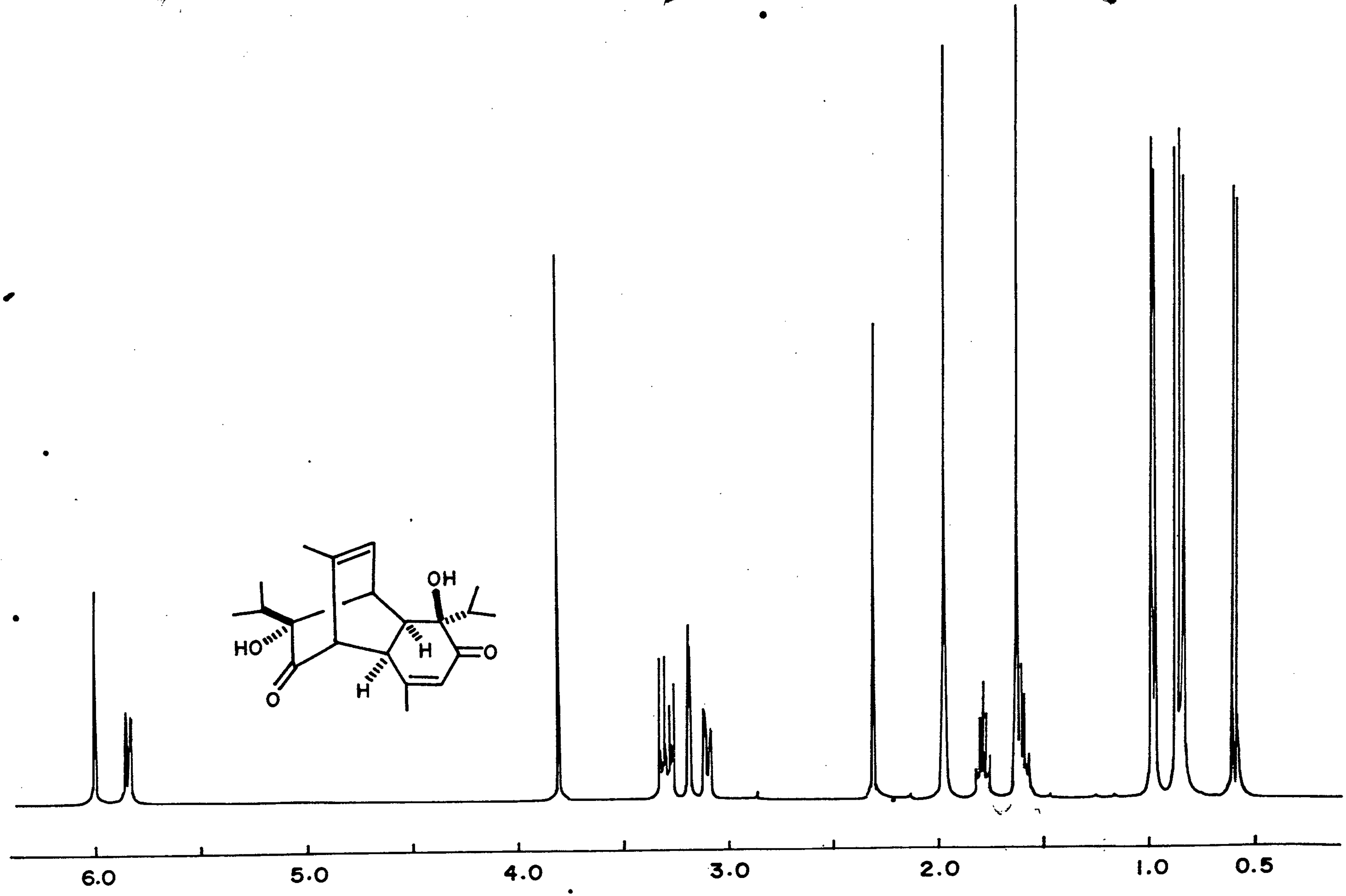

Fig. 9 ^{1}HNMR spectrum of (36)

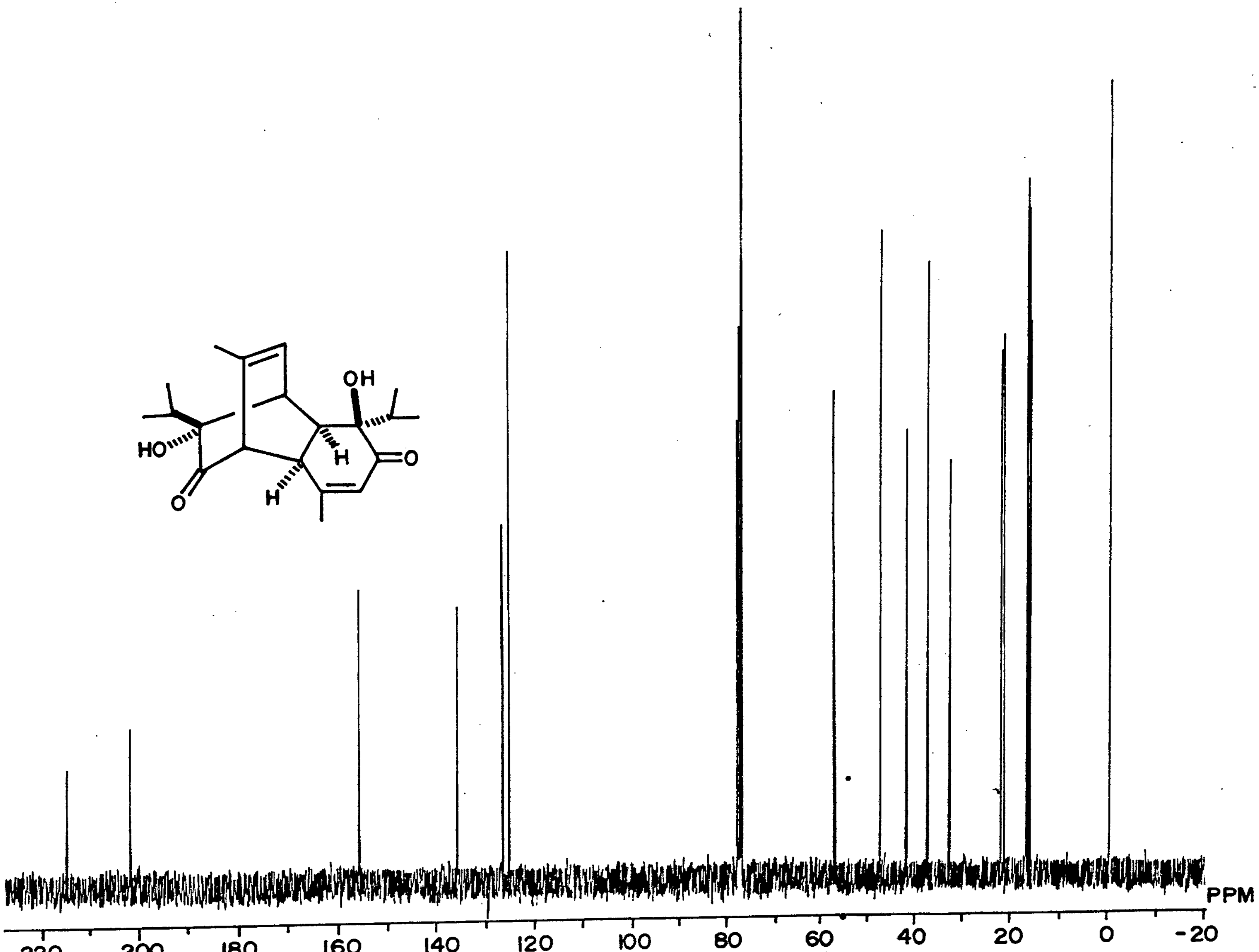

Fig. 10 ^{13}CNMR spectrum of (36)

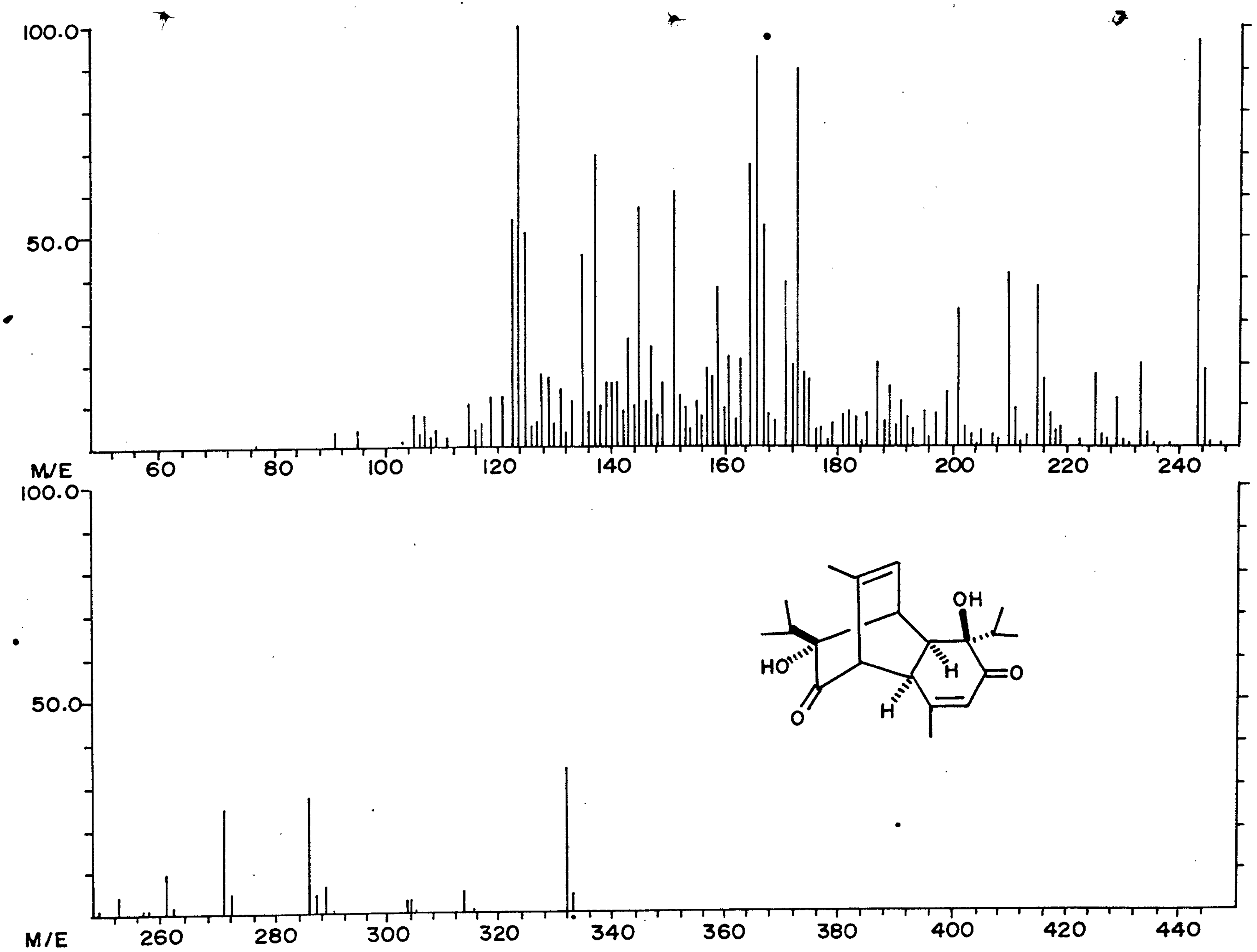

Fig. 11 Mass spectrum of (36)

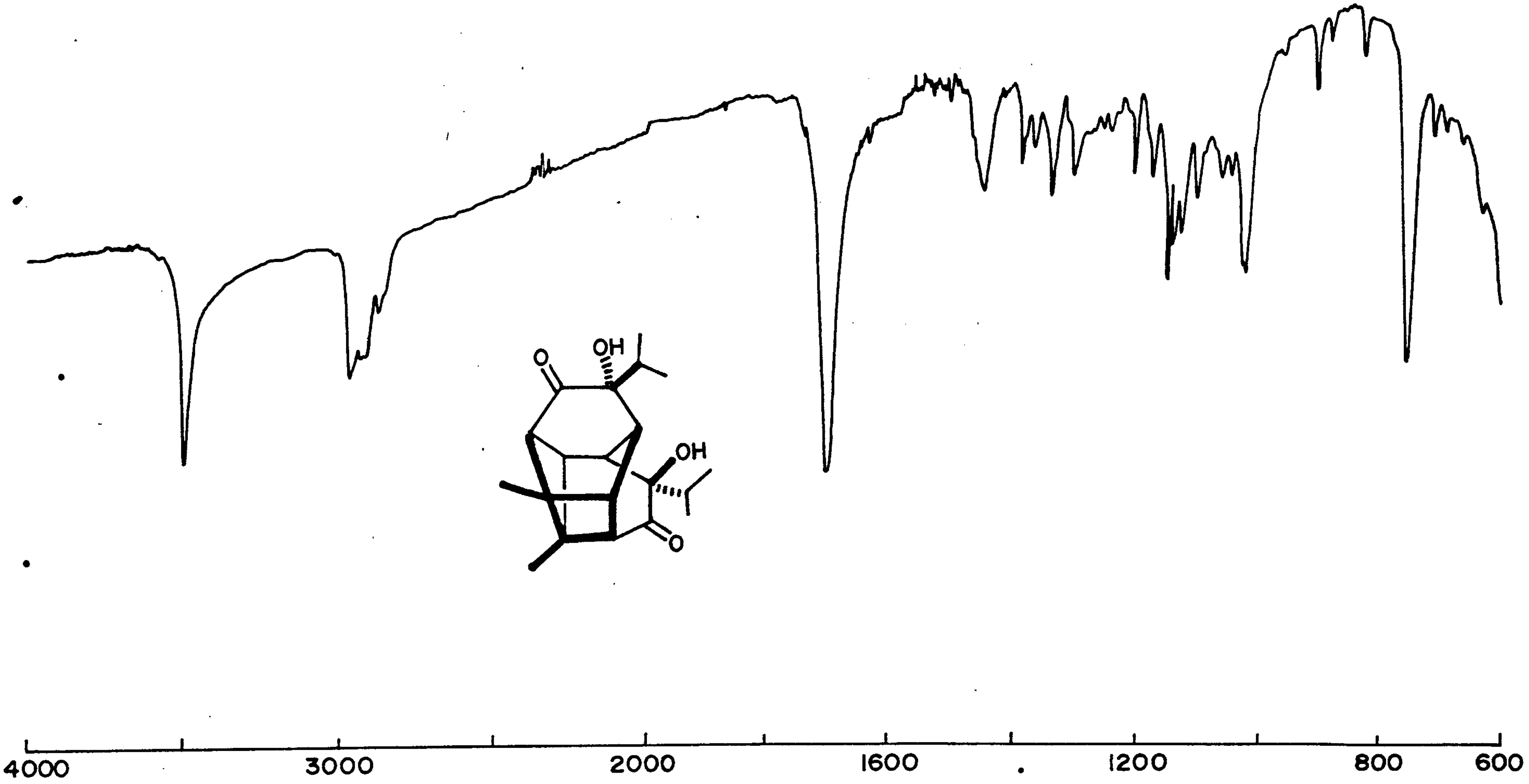

Fig. 12 IR spectrum of (40)

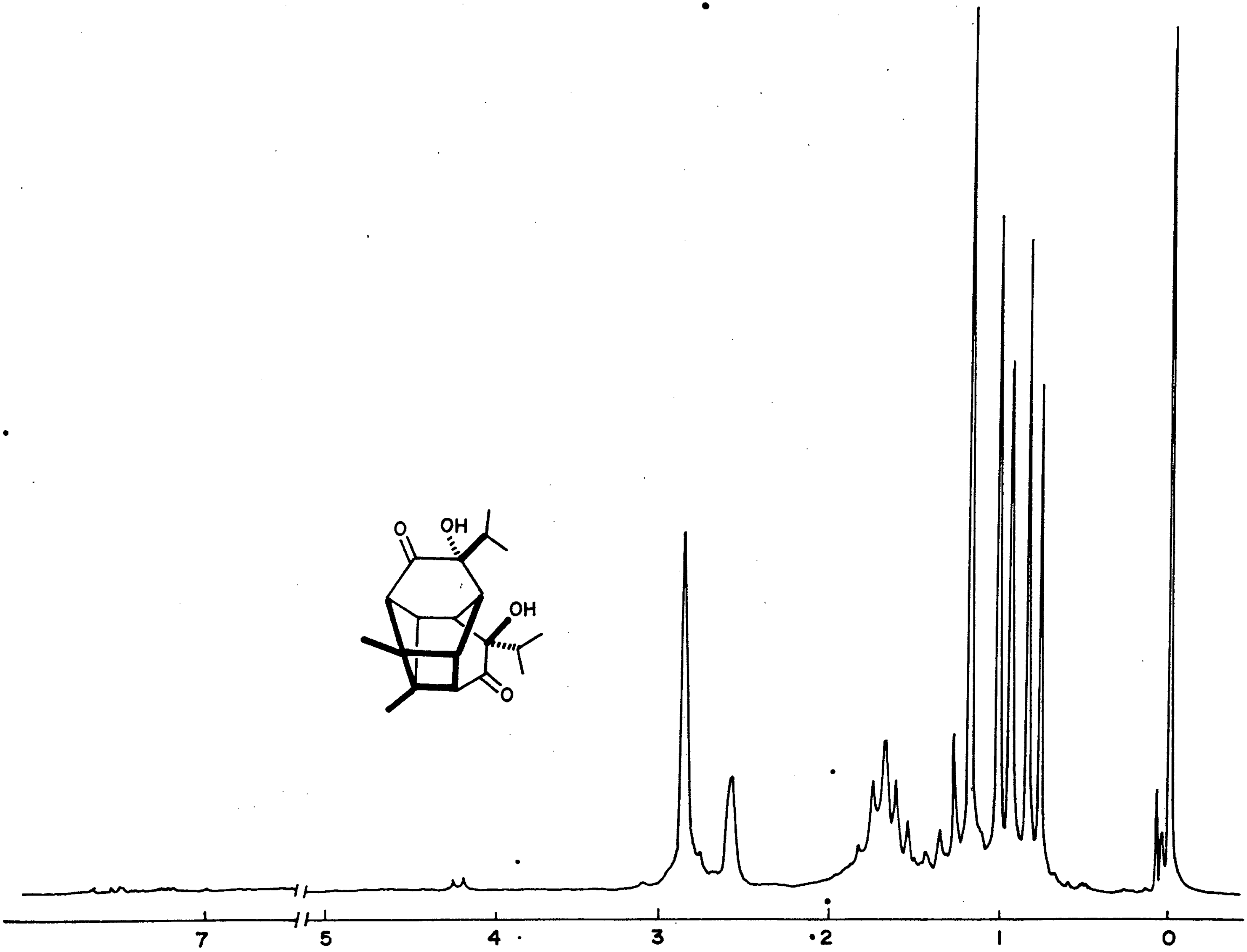

Fig. 13 ^{1}HNMR spectrum of (40)

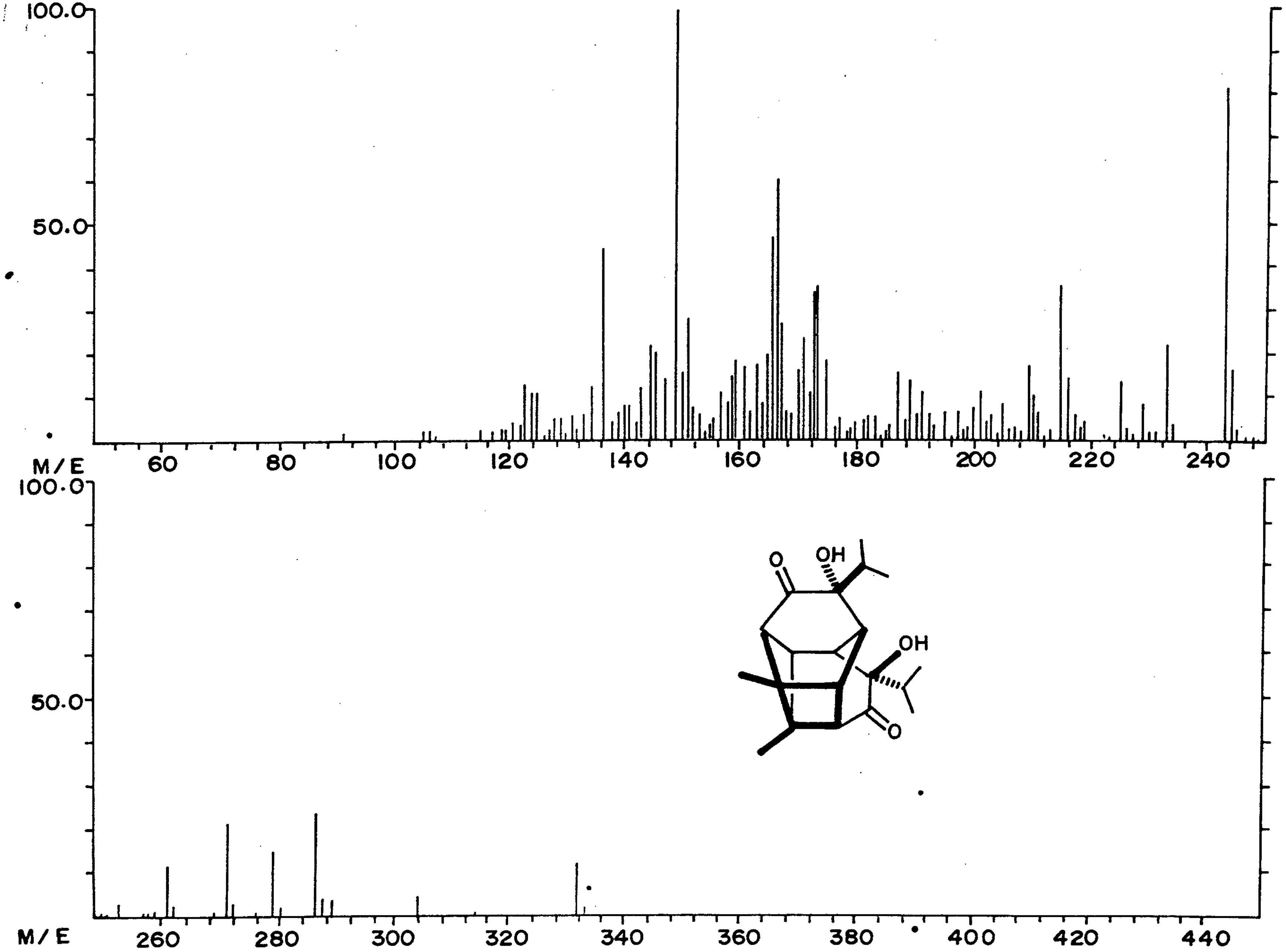

Fig. 14 Mass spectrum of (40)

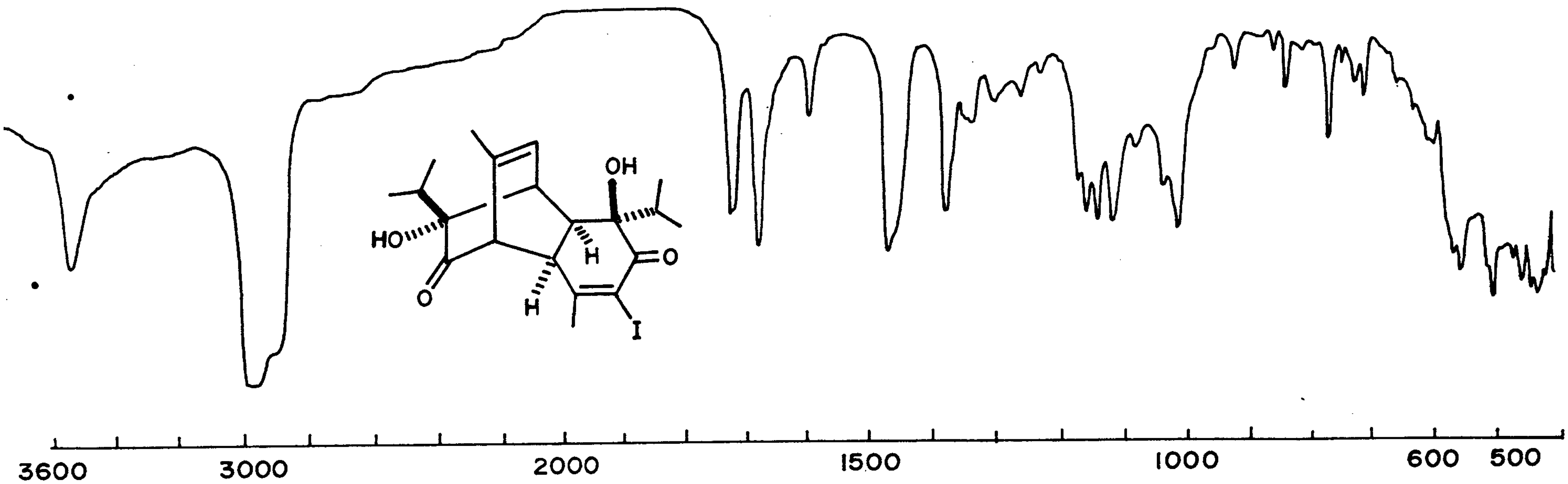

Fig. 15 IR spectrum of (48)

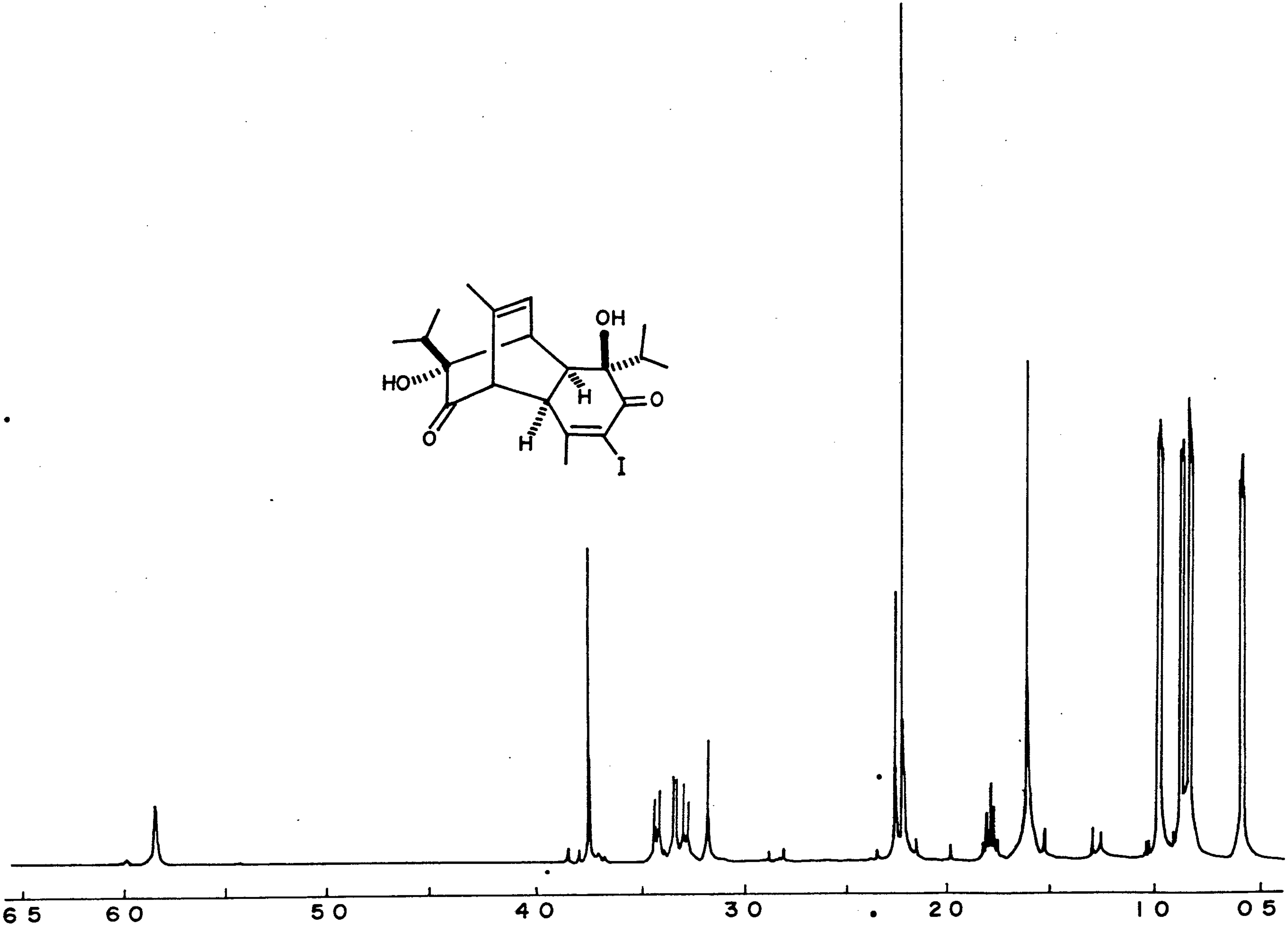

Fig. 16 ^{1}HNMR spectrum of (48)

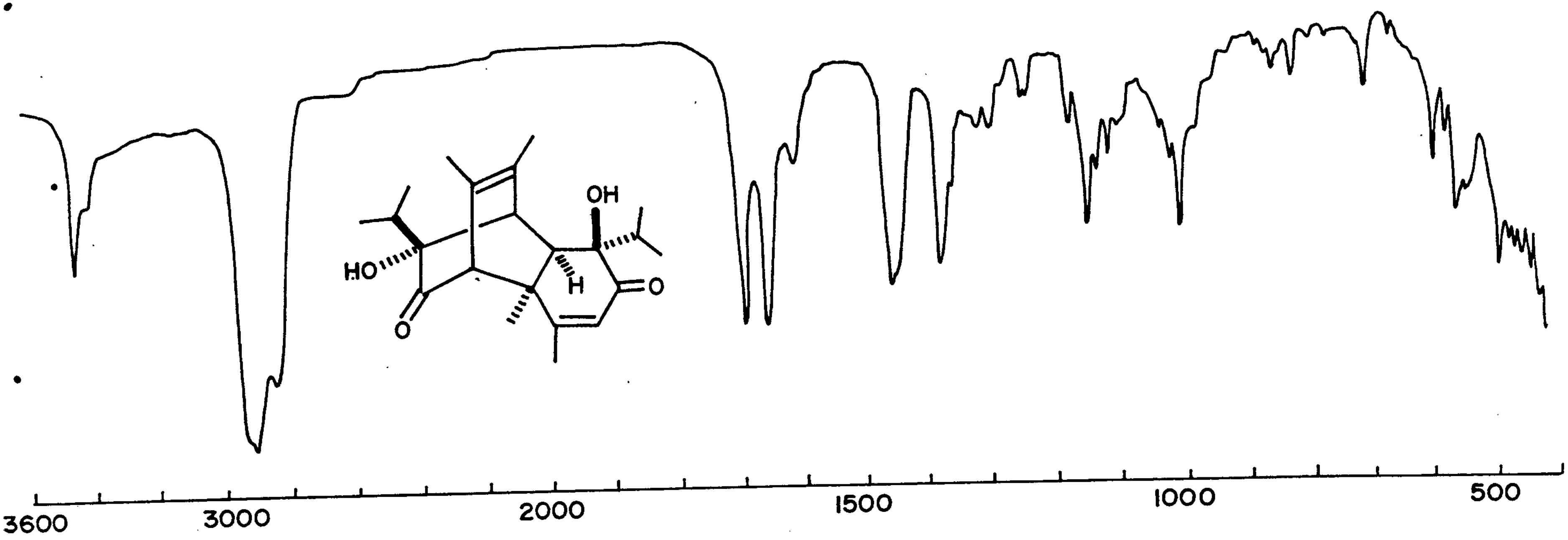

Fig. 17 IR spectrum of (51)

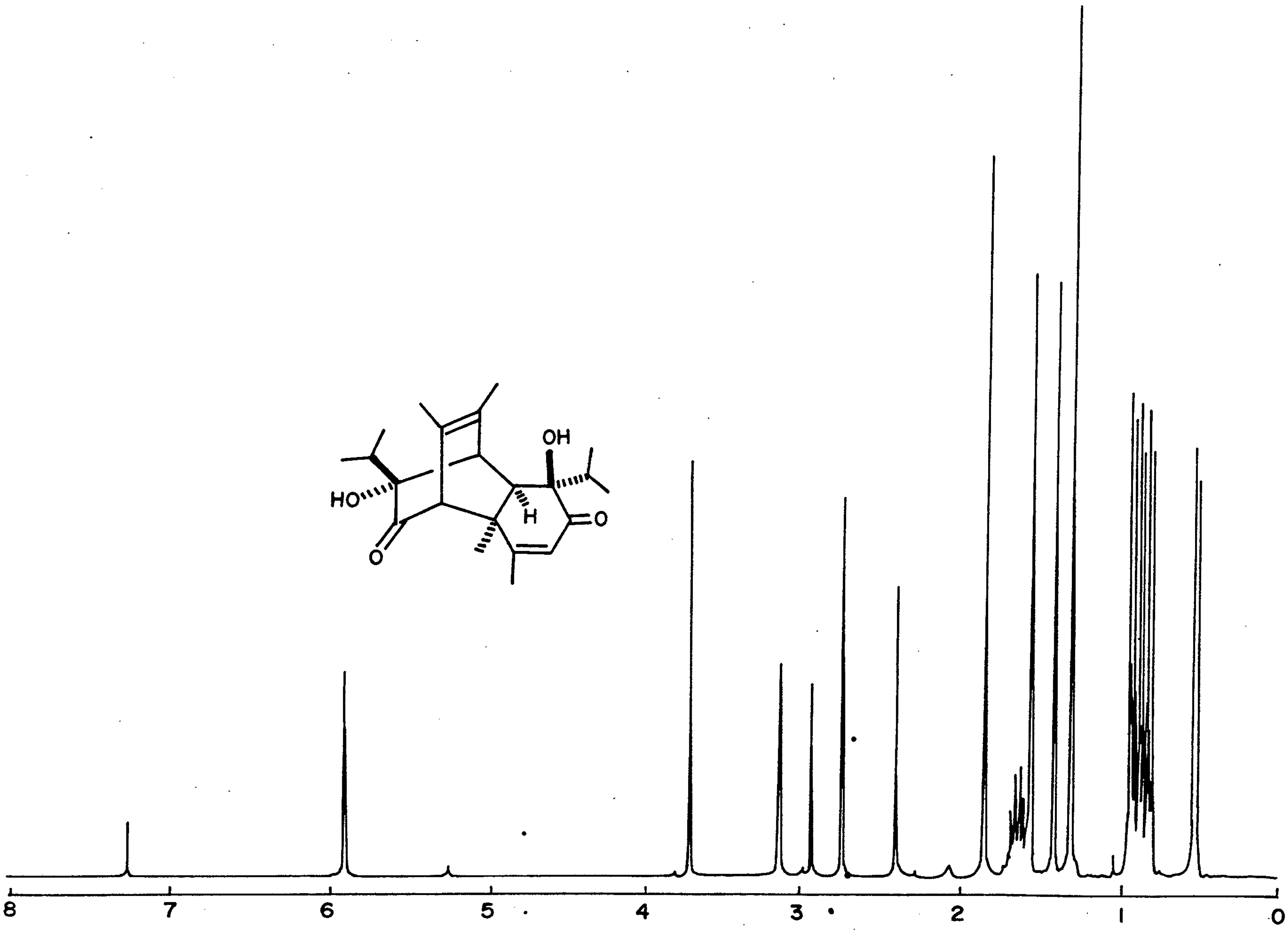

Fig. 18 ^{1}HNMR spectrum of (51)

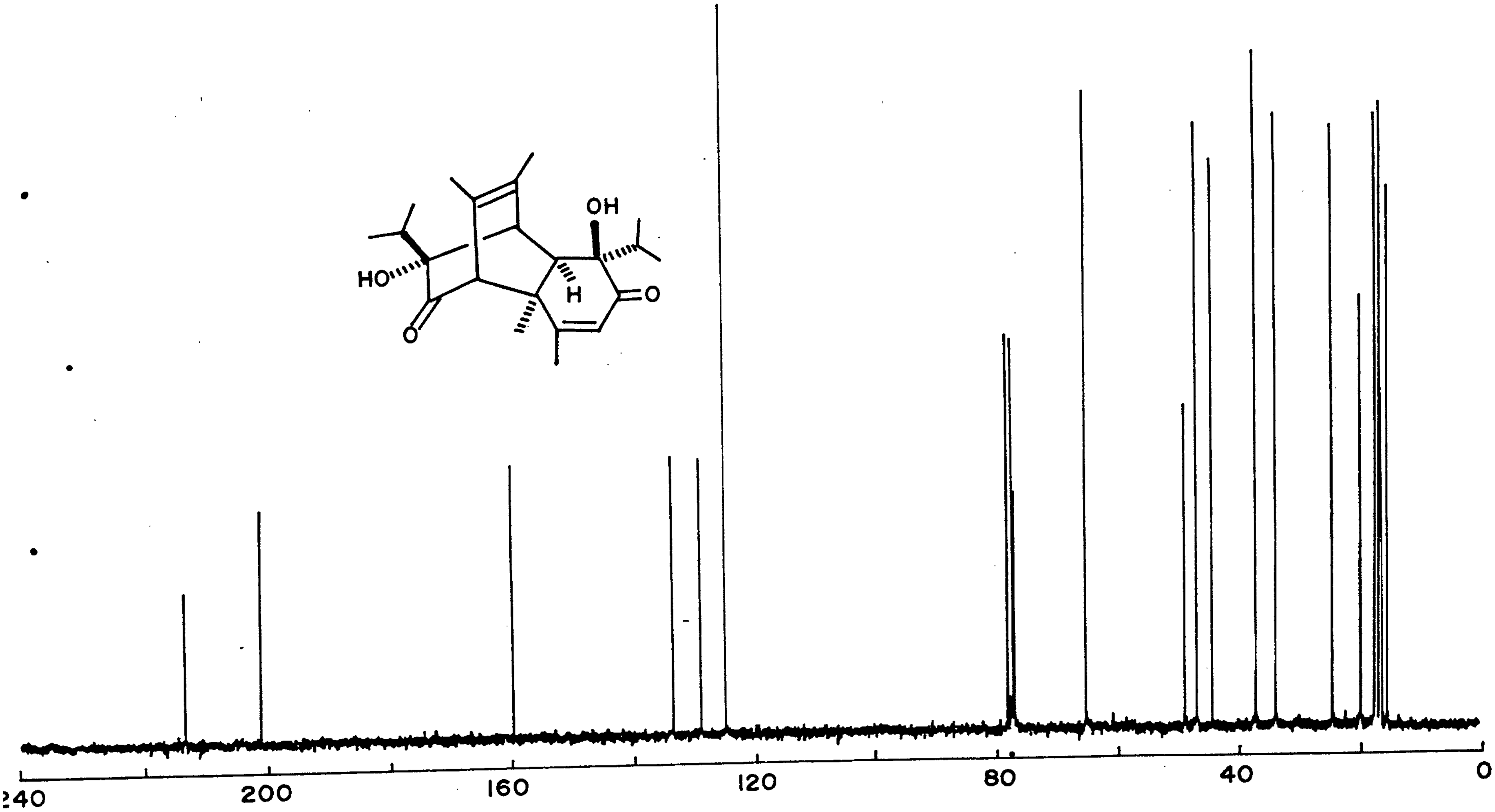

Fig. 19 ^{13}CNMR spectrum of (51)

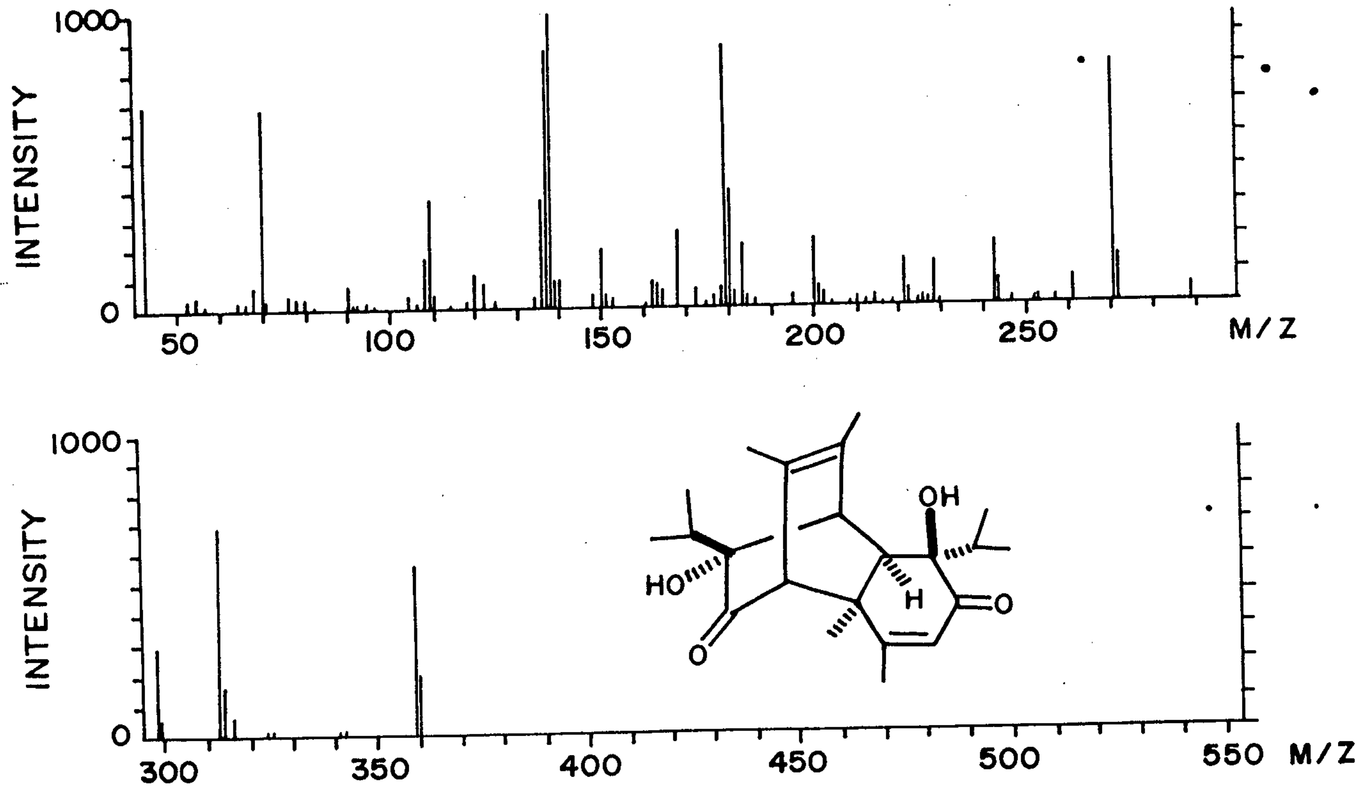

Fig. 20 Mass spectrum of (51)

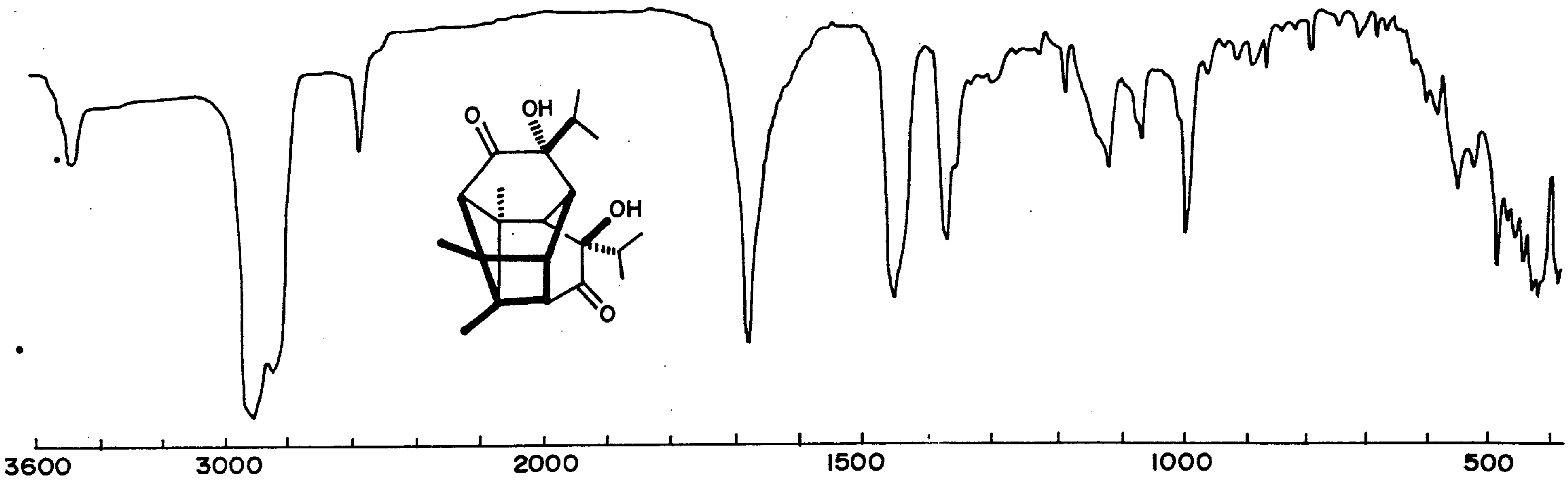

Fig. 21 IR spectrum of (52)

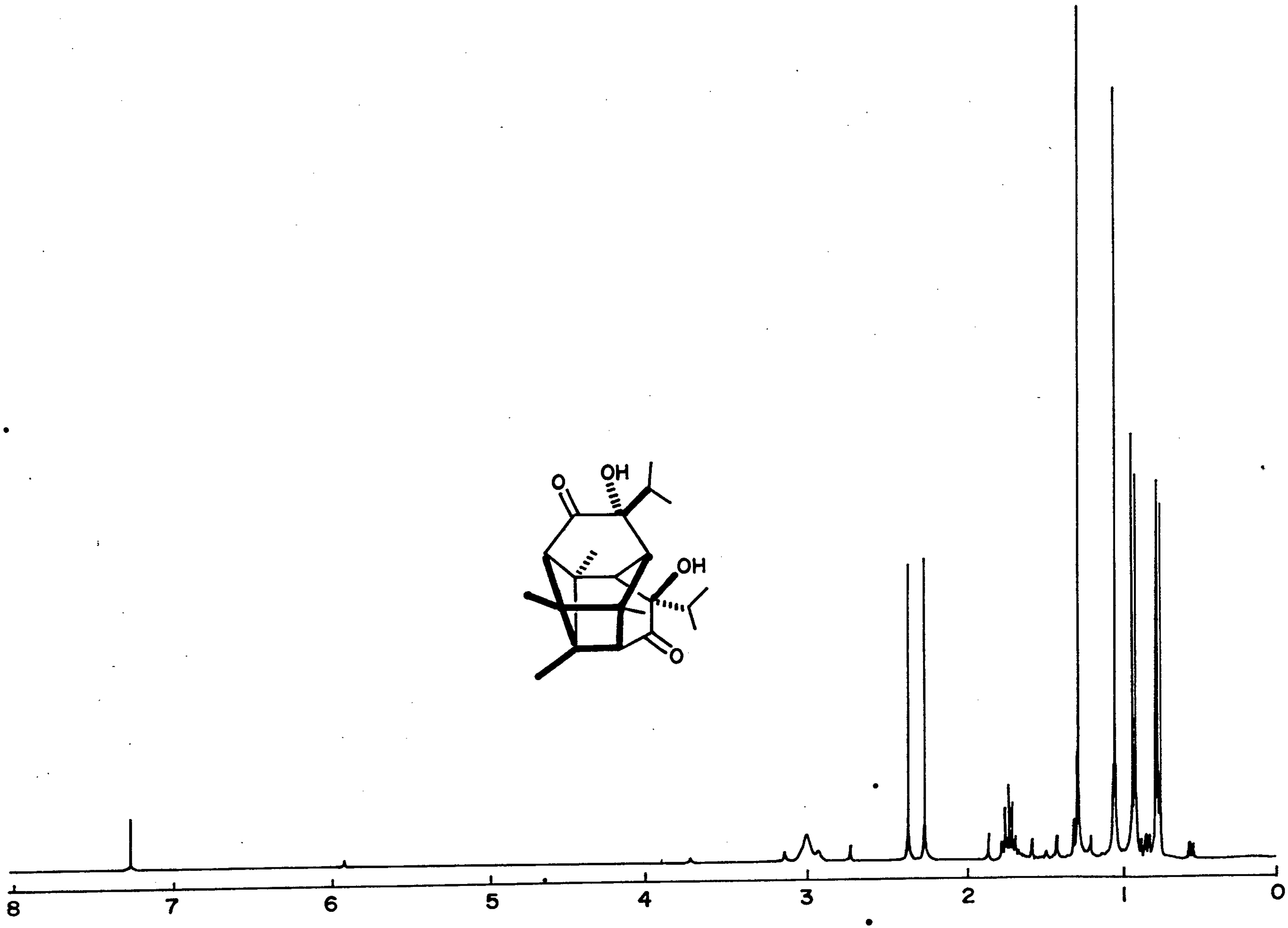

Fig. 22 ^{1}HNMR spectrum of (52).

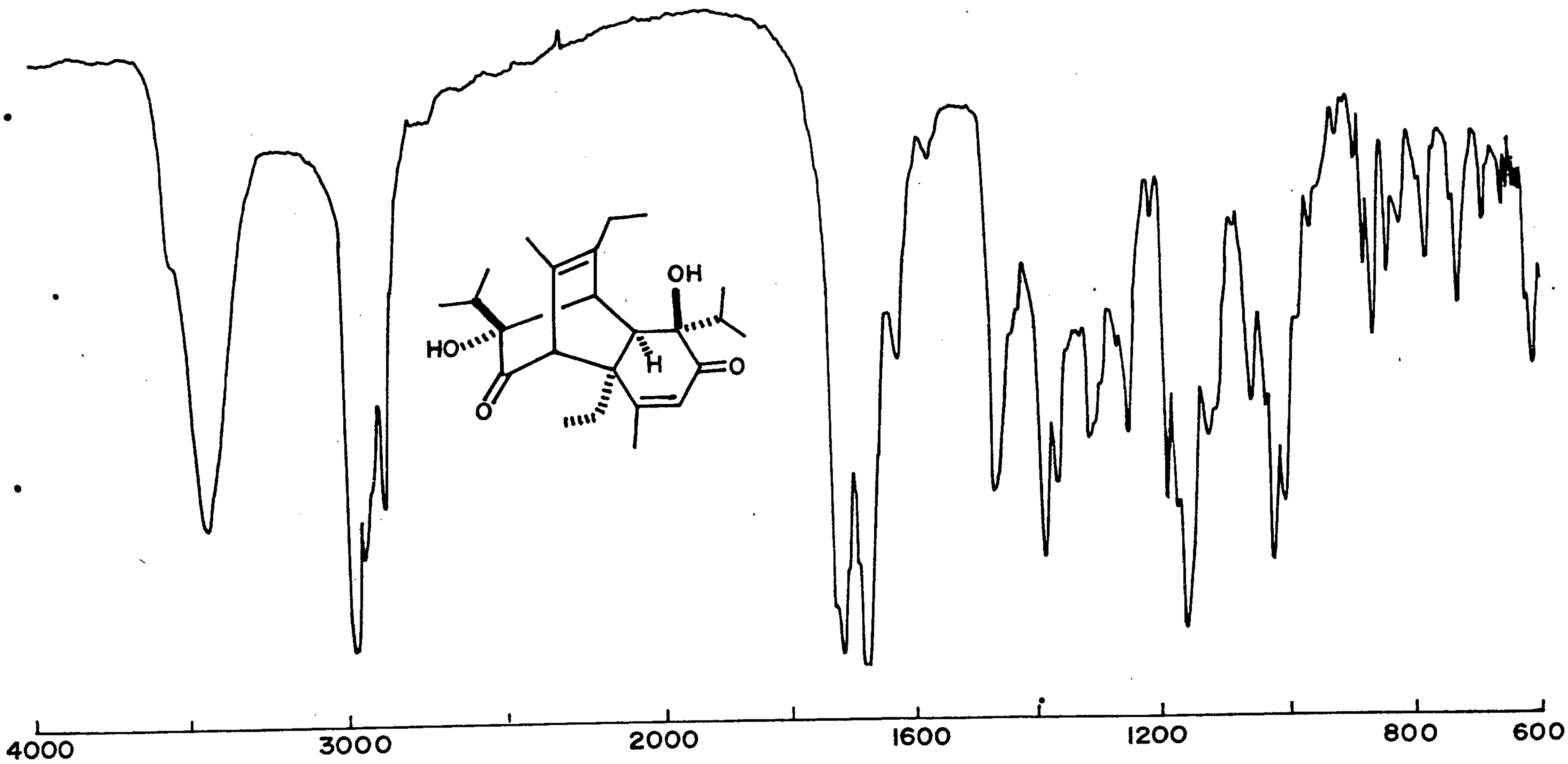

Fig. 23 IR spectrum of (53)

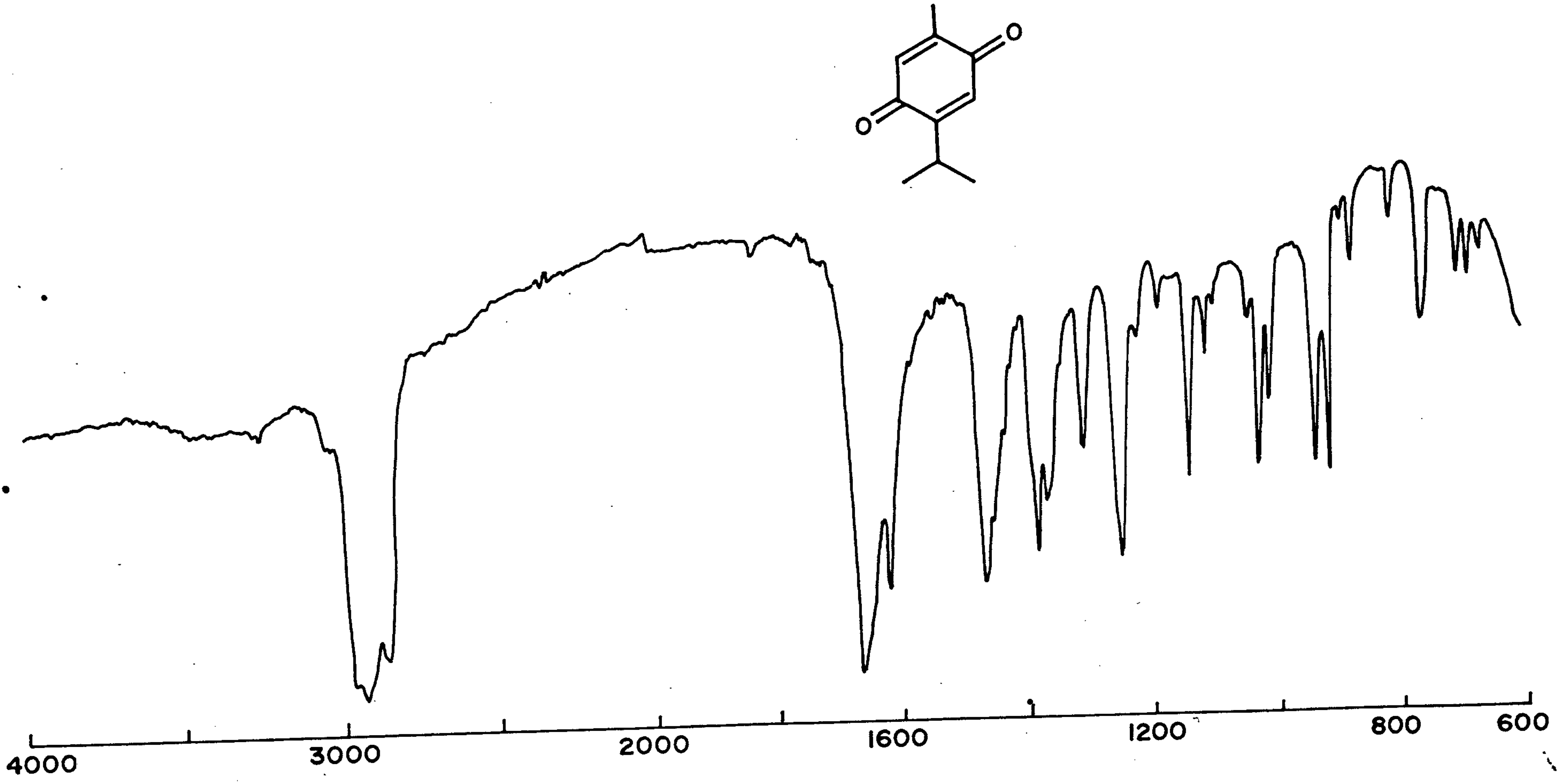

Fig. 24 IR spectrum of (61)

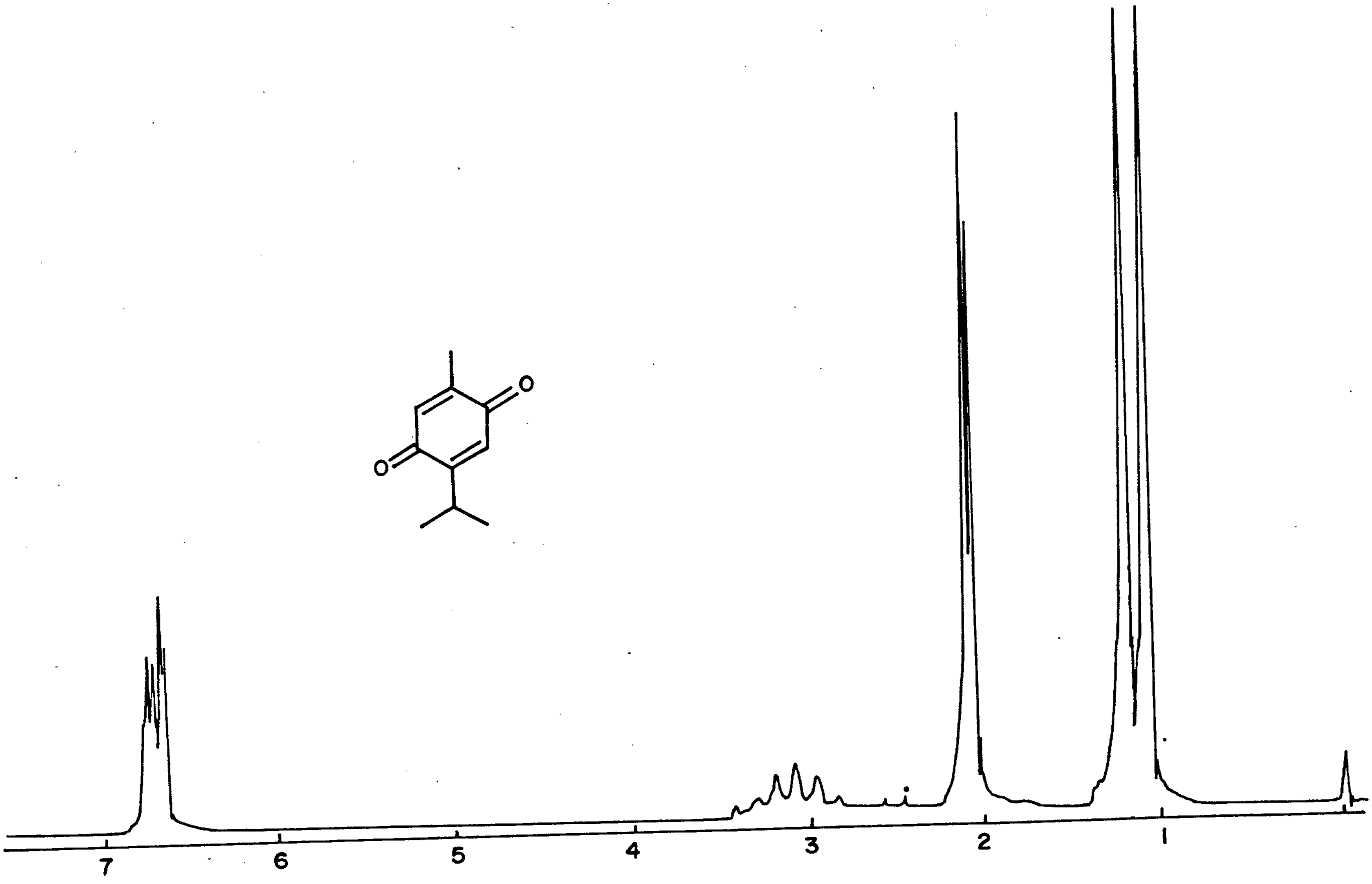

Fig. 25 ^{1}HNMR spectrum of (61)

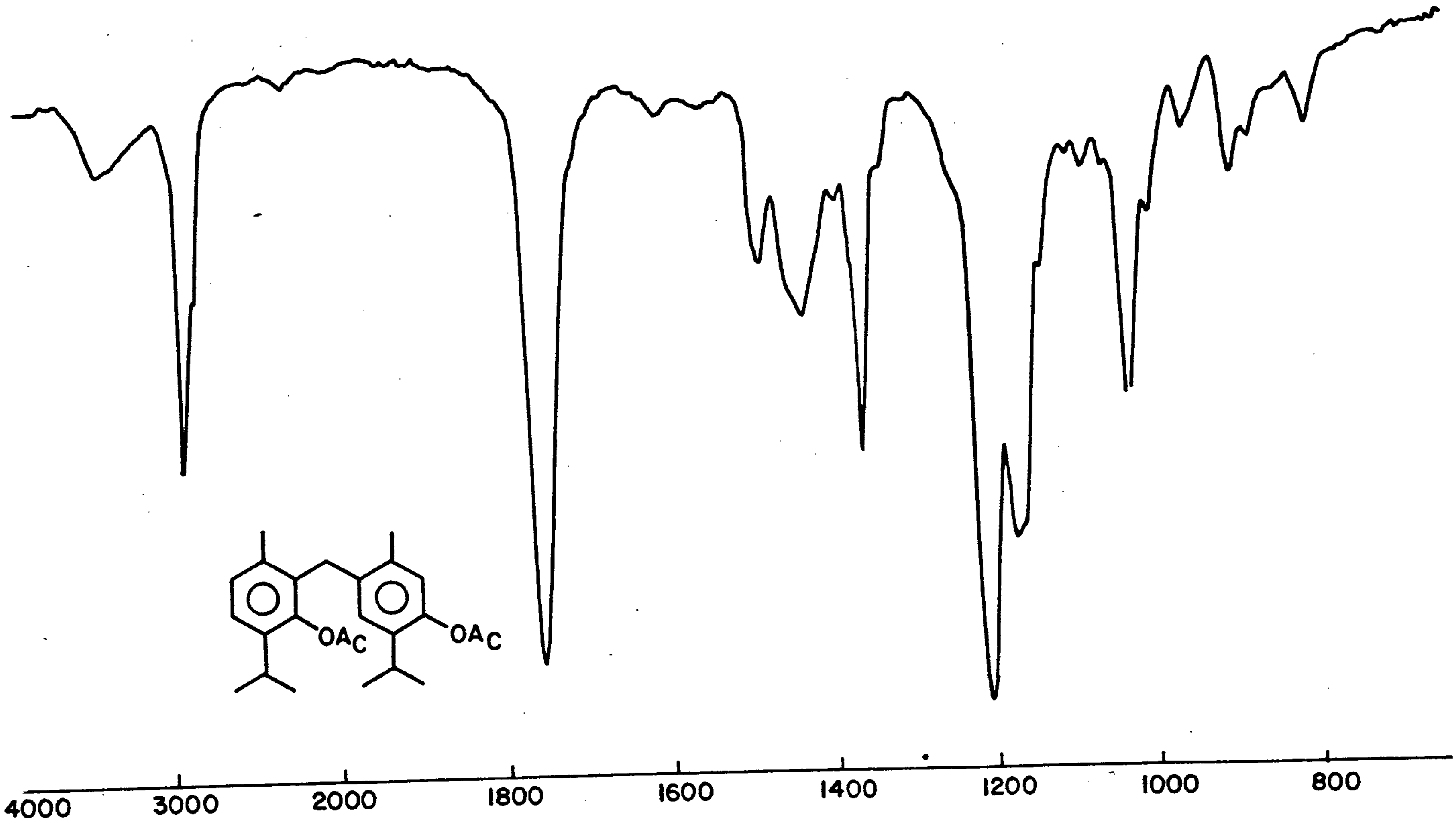

Fig - 26 IR spectrum of diacetate of 2,6′ dithymyl methane

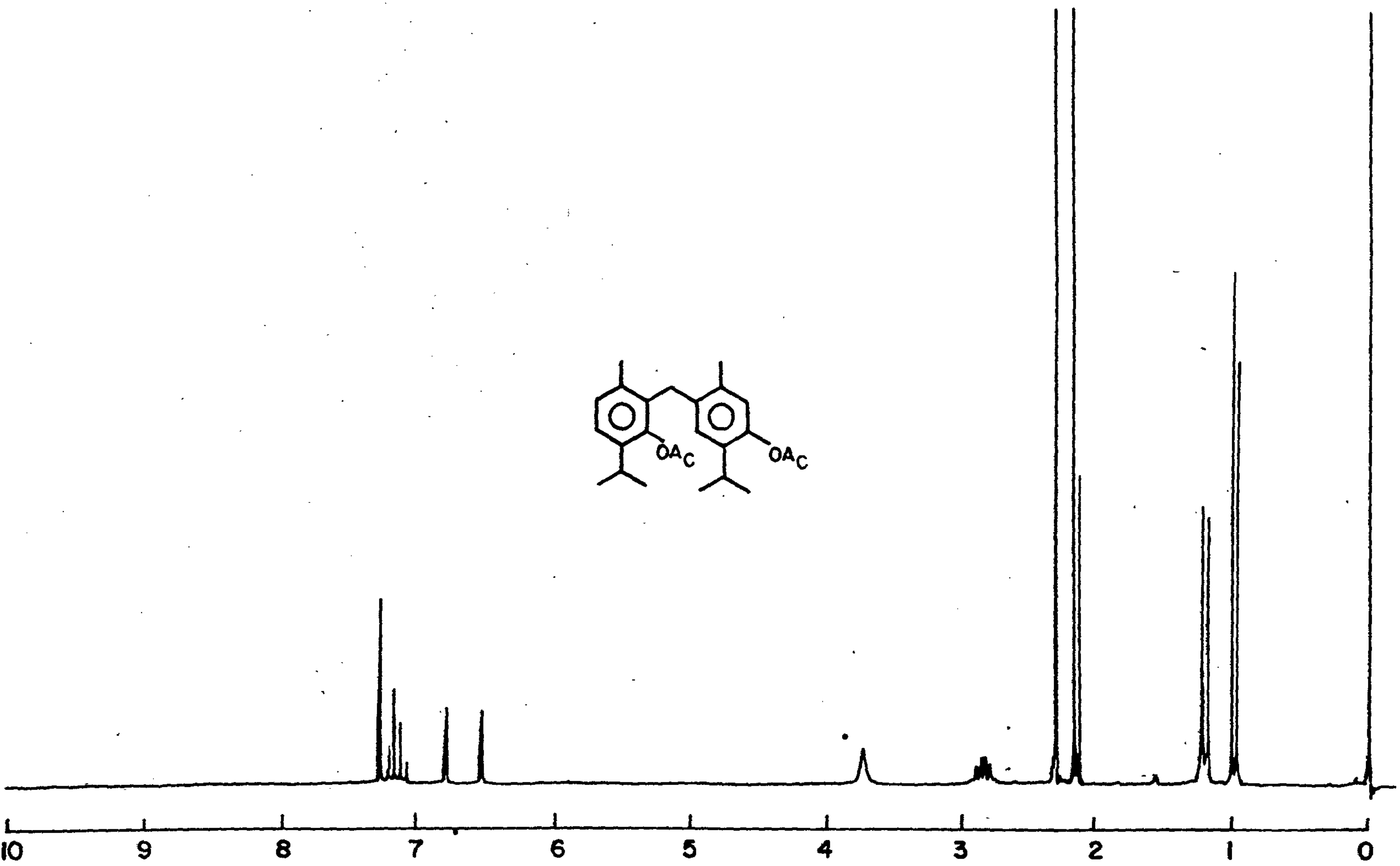

Fig - 27 ^{1}HNMR spectrum of diacetate of 2,6′ dithymyl methane

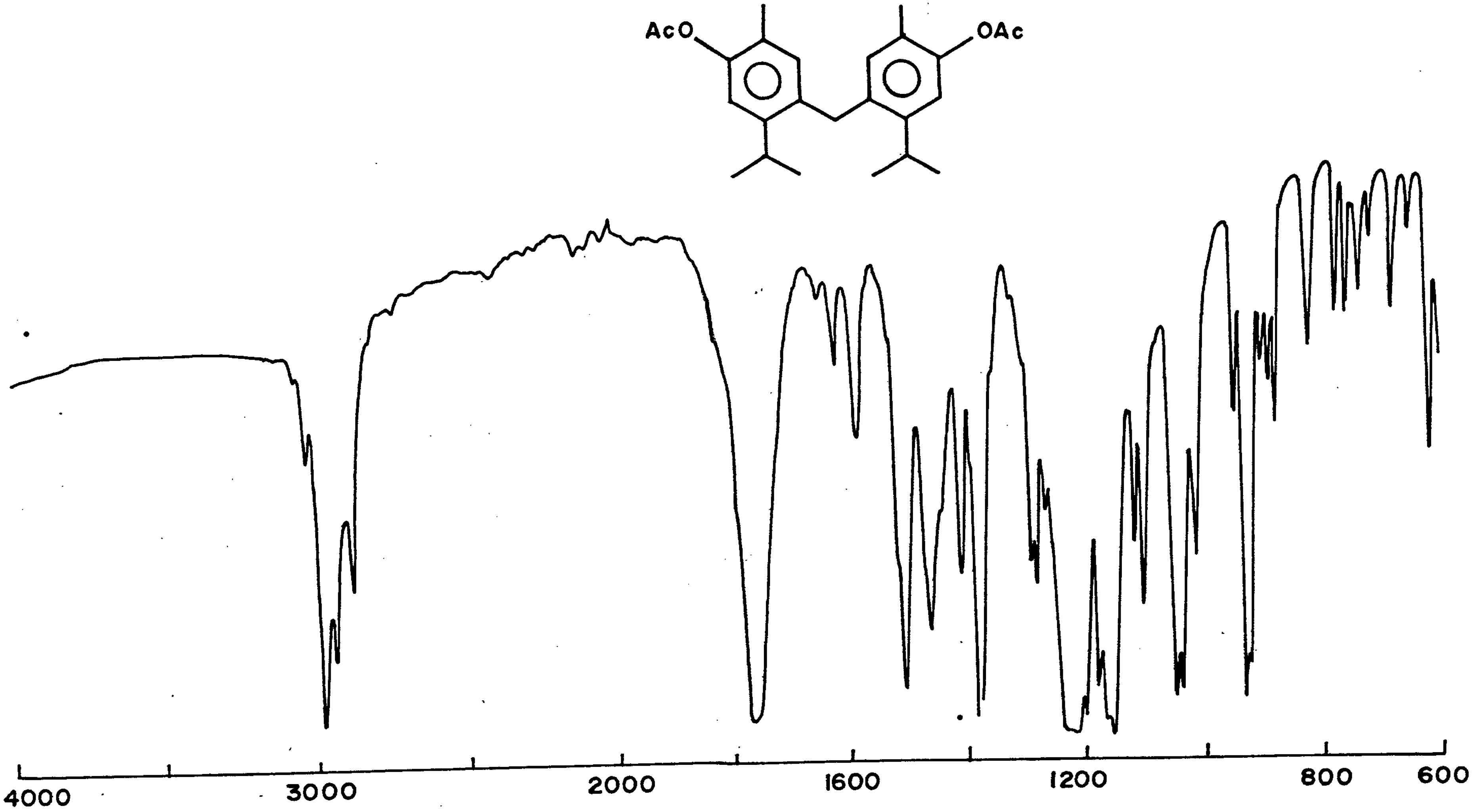

Fig. 28 IR spectrum of (90)

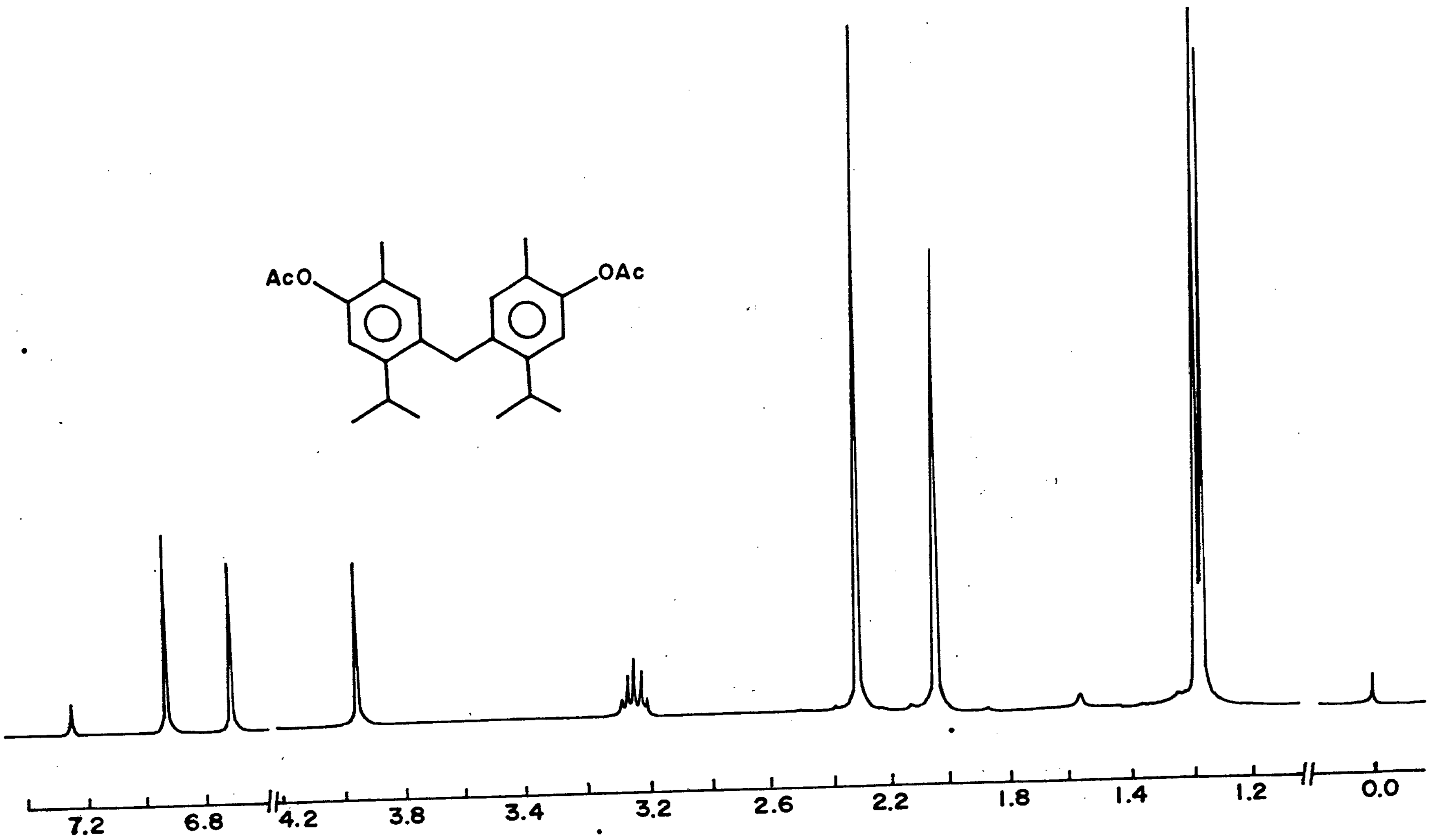

Fig. 29 ^{1}HNMR spectrum of (90)

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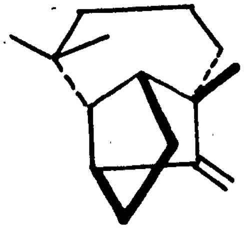
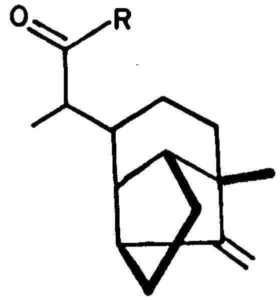
Longifolene (1), the main sesquiterpene of the hydrocarbon fraction obtained from the oleo-resin of Pinus roxburghii Sarg. Syn. P. longifolia Roxb. has been the subject of innumerable investigations during the past seven decades. In contrast to several reports on the chemical transformations on this abundantly available natural product, there was practically no report on its microbial transformation products. In view of this the microbiology department of Goa University initiated a project entitled, 'Microbial Transformation of Longifolene'. Mahtani[1] observed that commercial longifolene on microbial transformation with Pseudomonas alcaligenes T. gives in poor yields an aldehyde which could be characterised as its oxidation product, Sativic acid (2,R=OH). Since the yields were poor, and the commercial longifolene is usually accompanied by small quantities of longipinene, longicyclene, caryophyllene, humulene and bisabolene, it is unsafe to claim that Sativic aldehyde (2,R=H) is a microbial transformation product of longifolene. An analytically pure sample was therefore required to study the growth of Pseudomonas alcaligenes T. and the microbial transformation products.

Purification of Longifolene (1): Known methods

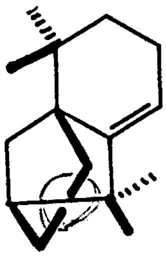
Chromatography over $AgNO_3$ - silica gel was first used by Sukh Dev and co-workers[2] for the purification of longifolene



(1)



R = H ; R = OH
(2)



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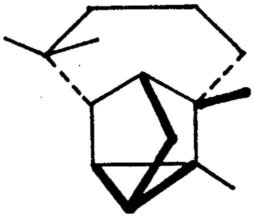

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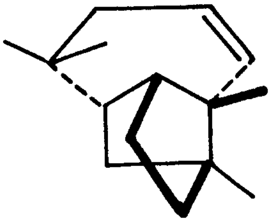

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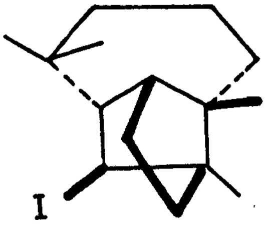

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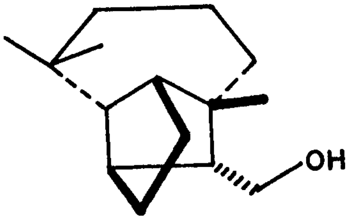

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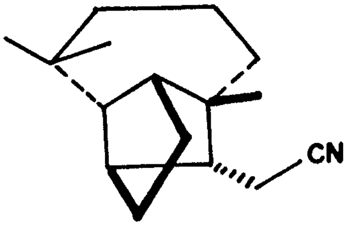
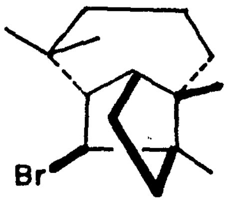
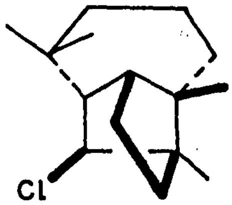
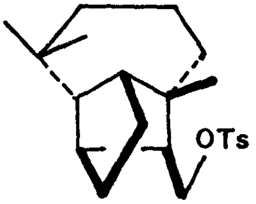
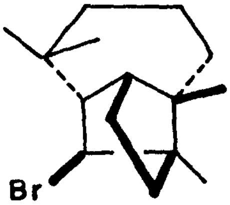
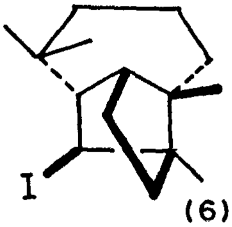


(13)

(1). The procedure is laborious, and expensive too. Mavinkurve and co-workers[3] have reported yet another method which uses microorganisms to remove the impurities present in the commercial longifolene. However, longifolene can be enriched maximum upto 81-83% by this procedure. It was therefore thought that generation of longifolene from one of its crystalline derivatives would be in order. Literature survey showed that there are only a few instances (Table-1) where analytically pure longifolene (99% and above) could be obtained from its derivatives. In all other cases (Table-1) regenerated longifolene (1) was always associated with longicyclene (4) and/or isolongifolene (3). Photolysis of longibornyl iodide (6), however, gives longifolene (1), longicyclene (4) and longiborn-8-ene (5) (entry 5, Table-1). We have now found out that analytically pure longifolene (1) can be obtained from longifolol (7). The details of this work are recorded below.

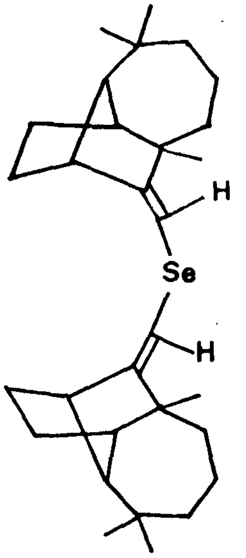
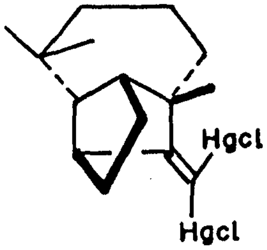
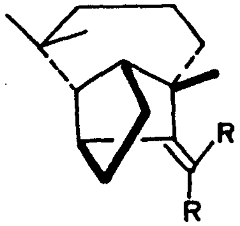
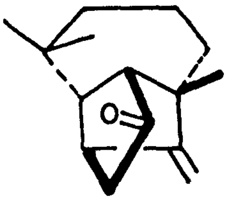
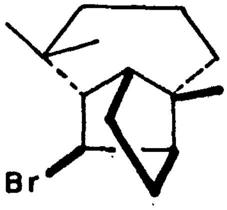
It is reported earlier that exposure of 3-isolongifolyl tosylate (11) to neutral alumina (grade-I) in a chromatography column for 12 hr and then elution with petroleum ether gives a hydrocarbon mixture consisting of longifolene (1, 90%) and isolongifolene (3, 10%). In fact this procedure was used by Sukh Dev and co-workers[4] to prepare longifolene-4,4,5,5-d_4 (9) from 3-isolongifolyl tosylate-$\overset{d}{\cancel{D}}_5$ (8).

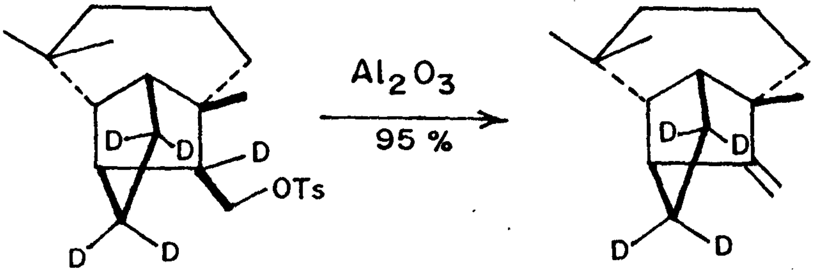
Table - I

Sr. No.		Reagent	Products	Ref. No.
1		KOBu^t – DMSO	(1) + (4) 89 % 11%	5
2	 *	NaOAc – AcOH	(1) + (3) + (4) 80% 12% 8%	6
3		Al_2O_3	(1) + (3) 90% 9 %	4
4		Aq. EtOH – KOH	(1) + (4) 74% 26%	7
5	 (6)	hν – Heptane	(1) + (4) + (5) 40% 44% 4%	8

* The % given requires confirmation.

(Contd.)

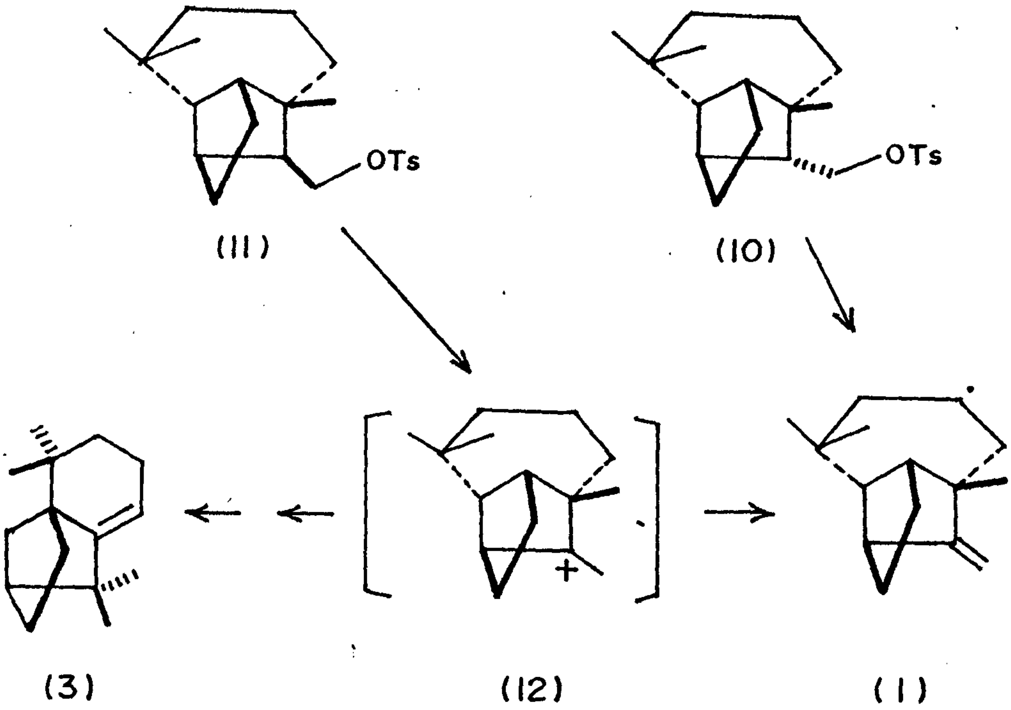
6		Dioxane Raney Ni	(1) 100 %	9
7		$LiAlH_4$	(1) 100 %	10
8	 a) R = Br b) R = I	Na, t - BuOH THF	(1) 100 %	10
9		NH_2——NH_2 KOH	(1) 100 %	11
10		Aq. EtOH $CaCO_3$	(1) 100 %	7



(8) (9)

We could not find any report on the behaviour of 3-longifolyl tosylate (10) on exposure to alumina and therefore considered worthwhile to study this reaction. Interestingly this reaction proceeded very smoothly and afforded longifolene (1) of >99% purity. GLC analysis showed the absence of isolongifolene (3). The difference in the behaviour of 3-longifolyl and 3-isolongifolyl tosylates (10 and 11) on exposure to alumina can be explained by assuming the formation of longifolyl cation (12) as an intermediate in the case of isolongifolyl tosylate (11), while longifolene is generated from 3-longifolyl tosylate (10) without intervention of carbocation (12) (see next page).

We could discover yet another method for the quantitative recovery of longifolene (1) from 3-longifolyl tosylate (10). We were interested in the preparation of nitrile (13) by using the sequence $R - CH_2 - OTs \longrightarrow R-CH_2 - CN$.



However, reaction of 3-longifolyl tosylate with KCN in the presence of DMSO at 90° resulted in the recovery of longifolene (purity 99%). We were unable to detect formation of the desired nitrile (13) in this reaction. It was thus clear that an E_2 elimination of *p*-toluene sulphonic acid takes place in preference to the SN_2 displacement of -OTs by CN^- ions. We therefore anticipated the formation of longifolene (1) by simply heating 3-longifolyl tosylate (10) with DMSO. Indeed, longifolene was the sole product of this reaction.

CHAPTER-II SECTION-II

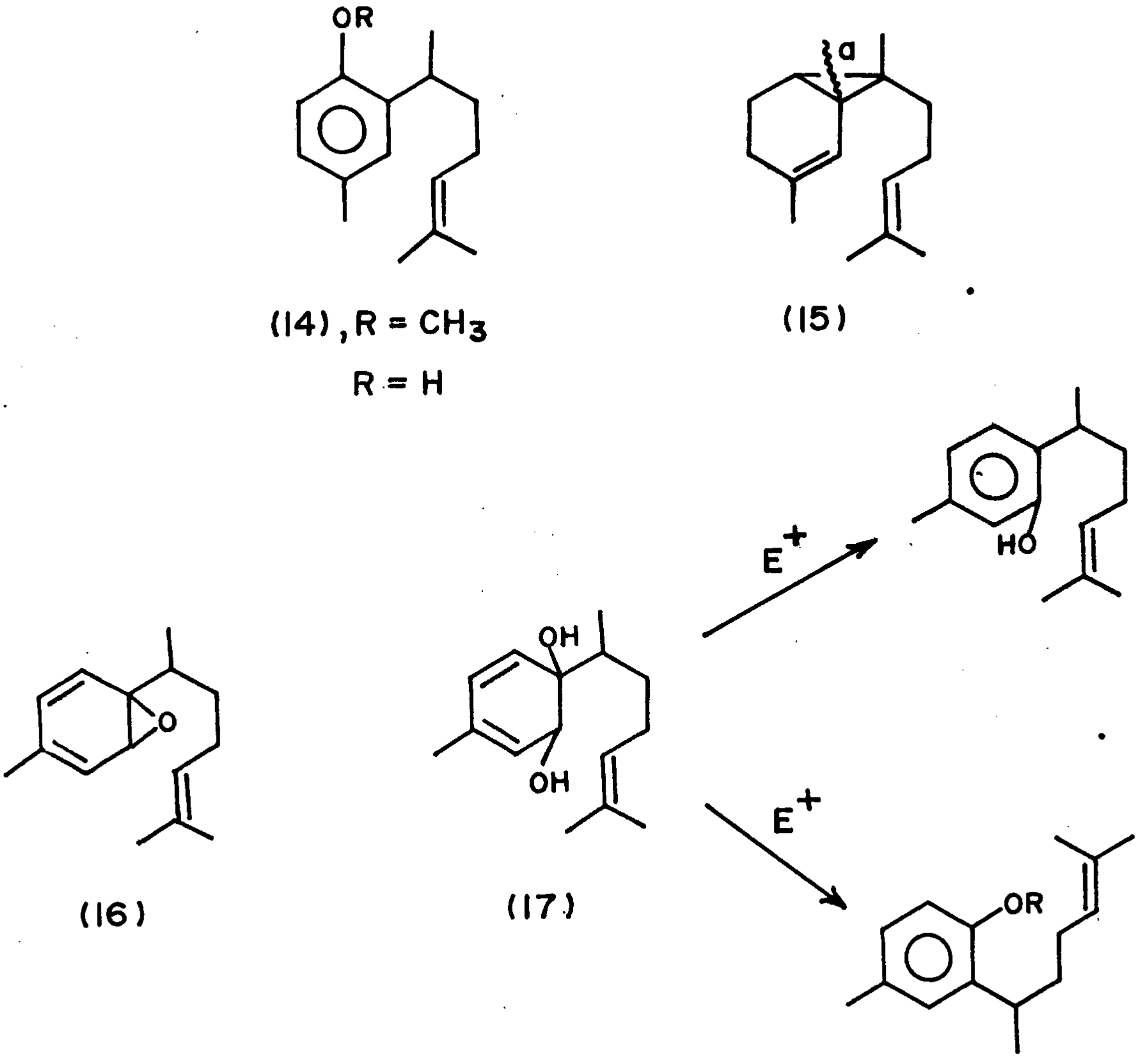
A NEW APPROACH FOR THE SYNTHESIS OF ELVIROL METHYL ETHER

Elvirol (14,R=H), the irregular phenolic sequiterpene was isolated by Bohlmann and co-workers[14] from Elvira biflora (compositae). The structure (14, R=H) was assigned on the basis of spectral data. The novel carbon skeleton of elvirol (14, R=H) is considered to be derived from sesquicarene (15) by oxidative ring fission (bond a) followed by aromatisation[15]. Another attractive proposal has been put forward by Pednekar et al[16]. who considered that the biogenesis of elvirol (14, R=H) involves a pinacolic rearrangement of a glycol (17) derived from an arene oxide (16) (scheme-1). So far there is no experimental support for any of these biogenetic proposals. The novel carbon framework of elvirol (14,R=H) has attracted the attention of organic chemists and several syntheses of this sesquiterpene have been reported[17]. Because of our own interest in the syntheses of modified terpenoids, we have made progress in developing yet another synthetic route to elvirol methyl ether (14, R=CH_3) which will eventually lead us to get in optically active form. Before presentation of our synthetic work, it is considered worthwhile to summarize the various synthetic routes leading to the production of elvirol (14, R=H) or its methyl ether (14, R=CH_3).

These syntheses can be grouped under three headings:

i) Syntheses starting from 4-methylanisole.

ii) Syntheses starting from 2-acetyl-4-methylanisole.



Scheme -1

iii) Syntheses not utilising either 4-methylanisole or 2-acetyl-4-methylanisole.

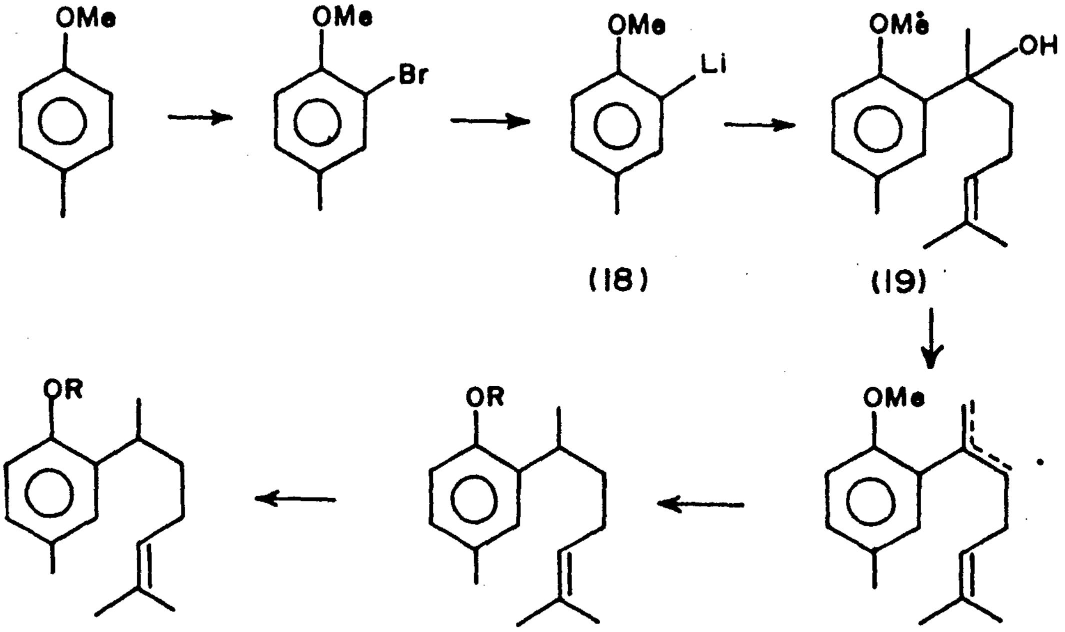
Mirrington and co-workers[18] converted 4-methylanisole into the tertiary alcohol (19), via 3-bromo-4-methoxytoluene. ✓ The bromine-lithium exchange with phenyllithium was the key reaction of this synthesis. The lithio derivative (18) gave (19) on condensation with 6-methylhept-5-en-2-one. Hydrogenolysis of the benzylic tertiary alcohol gave (14, $R=CH_3$), which on demethylation furnished elvirol (14, R=H) (scheme-2). Incidentally John & Rao[15], described the synthesis of elvirol methyl ether (14, $R=CH_3$) using an identical synthetic plan (scheme-2).

Tanaka and Kazuo[19] used a synthetic plan (scheme-3) which is essentially an extension of the Simonsen's synthesis[20] of ar-curcumene. Thus 4-methylanisole on Friedel Crafts acylation with glutaric anhydride gave the ketoacid (20). The tertiary alcohol (21) prepared from (20) in two steps, on dehydration furnished elvirol methyl ether (14, $R=CH_3$). Elvirol (14, R=H) was obtained by demethylation of elvirol methyl ether (14, $R=CH_3$). Kulkarni & Rao[21] reported the synthesis of the tertiary alcohol (21) by an identical route (scheme-3) which was previously transformed into elvirol methyl ether (14, $R=CH_3$).

Vig and co-workers[22,23] transoformed 2-acetyl-4-methylanisole into aldehyde (22) by two different routes

Dennison, Mirrington and Stuart[18]

John and Krishna Rao[15]



(18)

(19)

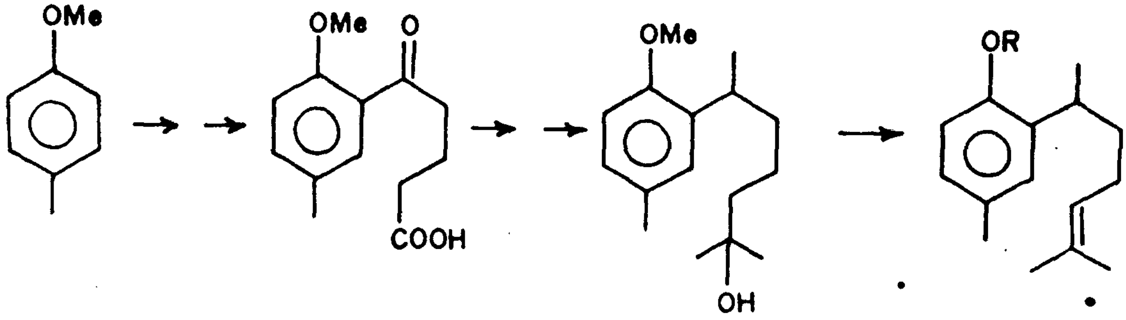
14, R = H

14, R = CH_3

Scheme -2

Tanaka and Kazuo[19]

Kulkarni and Rao[21]

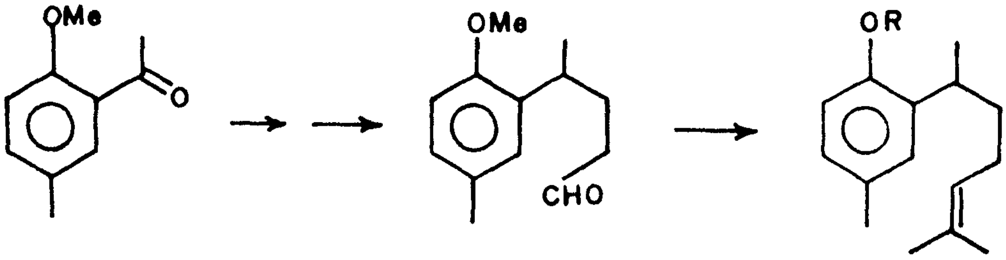


(20) (21) (14), R = CH_3

Scheme - 3

Vig, Vig, Puri and Ahuja[23]

Vig, Sharma, Kumar and Handa[22]

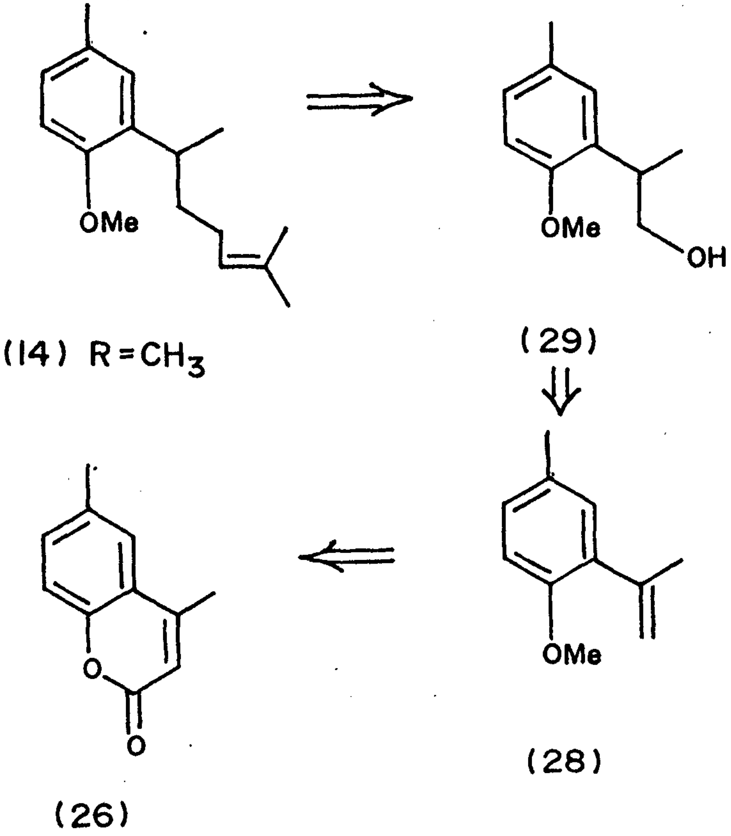


(22) (14), R = CH_3

Scheme - 4

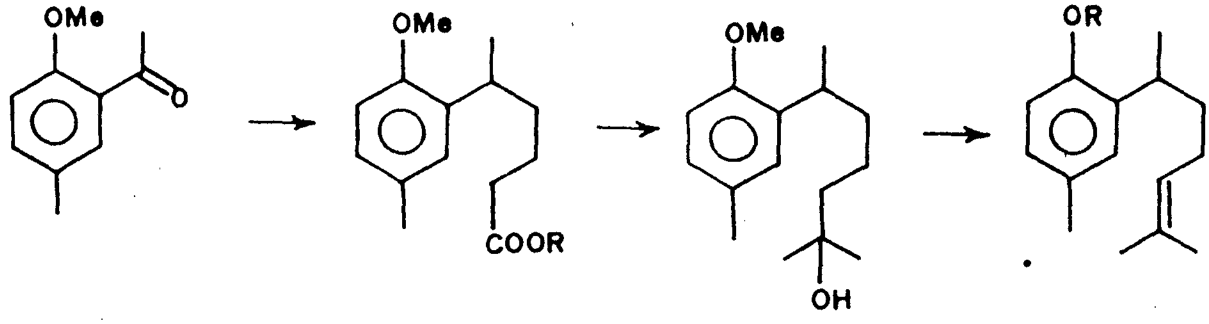
which finally gave elvirol methyl ether on (14, $R=CH_3$) Wittig reaction (scheme-4). ✓

Pednekar et al[16]. converted 2-acetyl-4-methylanisole into acid (23, R=H) by reaction with crotonic acid in the presence of lithium naphthalenide followed by hydrogenation. Alcohol (21) prepared from the ethyl ester (23, R=Et) on dehydration with P-toluenesulfonic acid in benzene afforded (14, $R=CH_3$) (scheme-5). ✓



Scheme - 7

Pednekar, Chakravarti and Paknikar[16]



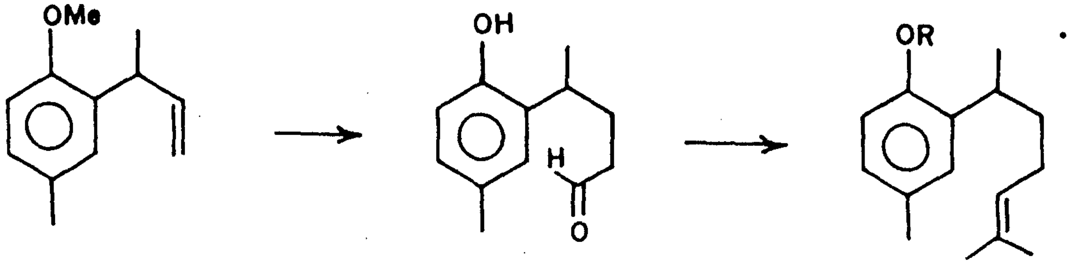
(23), a) R = H
b) R = Et

(21)

(14), $R = CH_3$

Scheme-5

Bohlmann and Koerrig[24]



(24)

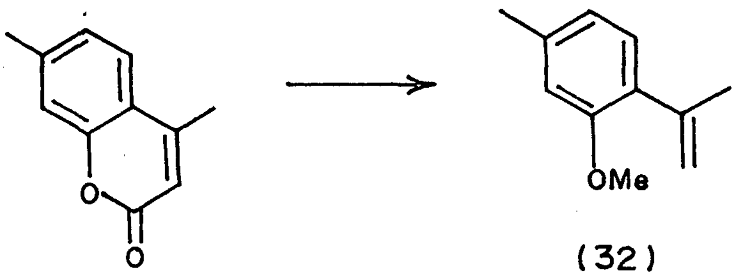
(25)

(14), R = H

Scheme-6

Bohlmann and Koerning[24] prepared the vinyl ether (24) by a routine procedure and transformed it to the phenolic aldehyde (25). Wittig reaction then furnished elvirol (14,R=H)* (scheme-6).

The retrosynthetic plan used in the present synthesis of elvirol methyl ether is depicted in (scheme-7, see previous page). Though it should be possible to prepare 2-(2'methoxy- ✓ 5'-methylphenyl)-1-propene (28) from 2-acetyl-4-methyl-anisole, we prefered its preparation from 4,6-dimethyl- ✓ coumarin (26). The reason for this was the observation of Rao and co-workers[25] who recently reported a convenient transformation of 4,7-dimethyl coumarin (31) into 2-(2'-methoxy-4'-methylphenyl)-1-propene (32) and claimed it to be superior to the conventional method. The styrene derivative (28) which can be made available from (26) would give the primary alcohol (29) by hydroboration oxidation. The final stage of the synthesis requires prenylation of the C_{10} intermediate.



(31)

(32)

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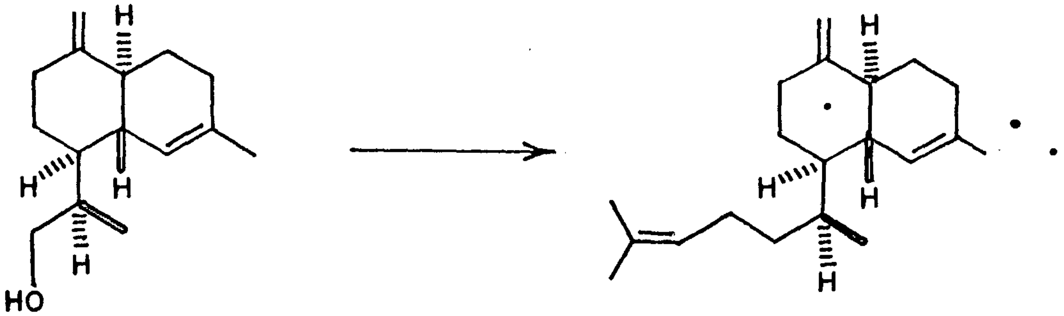
* This is the first synthesis of elvirol. The presentation of literature reports is not in chronological order.

Literature survey showed that the most efficient method of converting $R-CH_2-OH$ to $R-CH_2-CH_2-CH{=}C(CH_3)_2$ involves tosylation of the primary alcohol and then copper mediated coupling with prenyl magnesium chloride[26]. Recently this method was used for the synthesis of (-) bifloratriene (34) from (-) khusol (33)[27].



(33) (34)

The progress made towards the synthesis of elvirol methyl ether (14, $R=CH_3$) following our synthetic plan (scheme-8) is presented.

4,6-Dimethyl coumarin (26) was prepared by condensation of p-cresol and ethyl acetoacetate in the presence of PPA*. ✓ Saponification of (26) with aqueous NaOH and subsequent methylation of the resulting coumaric acid with Me_2SO_4 furnished (z)-3-(2'-methoxy-5'-methylphenyl) but-2-enoic acid ✓

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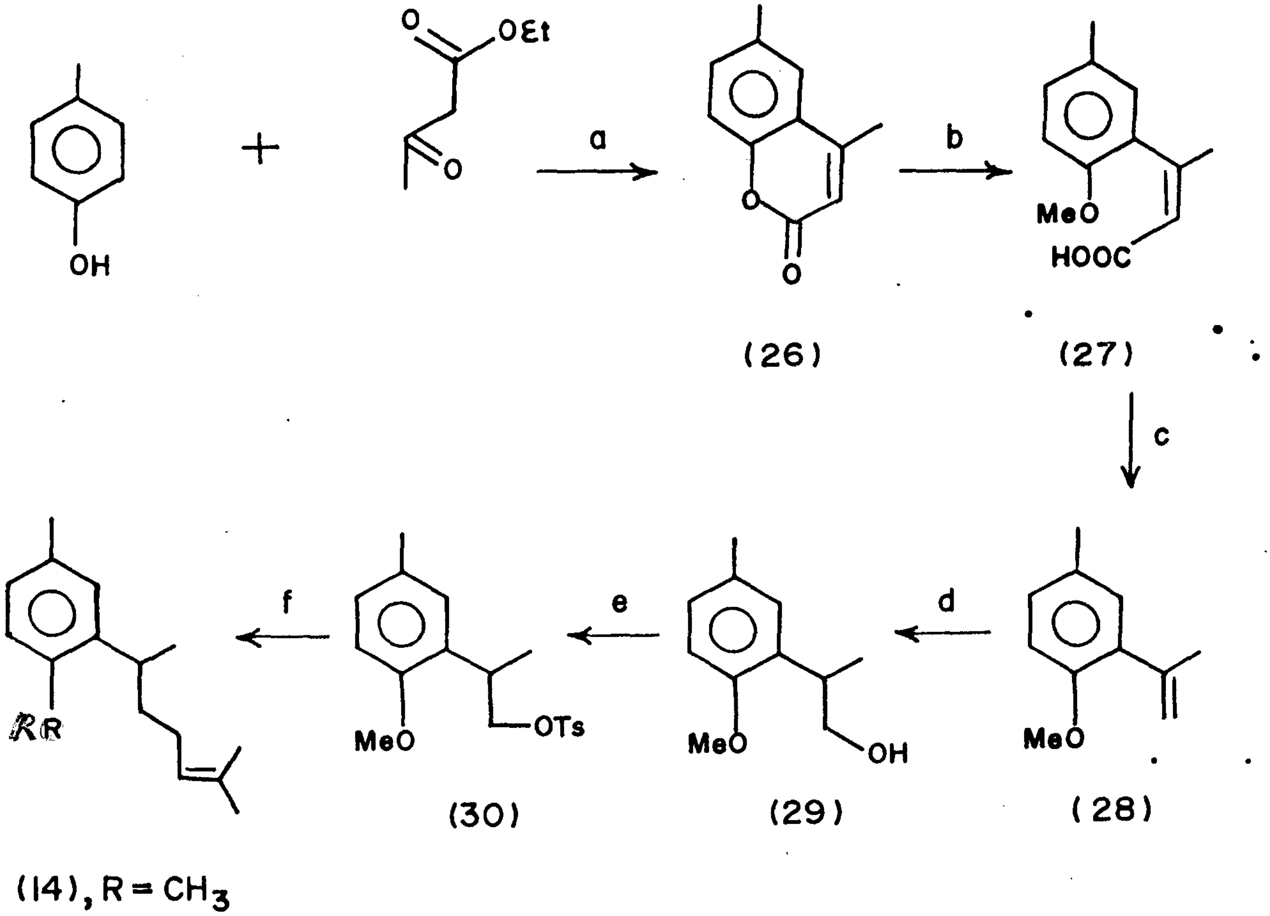
* In one of the experiments, we obtained besides 4,6-dimethyl coumarin (26) another crystalline compound m.p. 78° which was identified as 2, 6-dimethyl chromone.



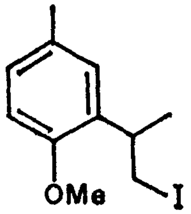
a – PPA ; b – NaOH , Me_2SO_4 ; C – Δ, 210 – 230° C ;

d – B_2H_6 , NaOH , H_2O_2; e – Pyridine , p – TsCl ;

f – $\succ\!\!=\!\!\diagup\!\!\diagdown$MgCl , $C_3H_7C{\equiv}CCu$, H^+.

Scheme – 8

(27). Its IR spectrum is reproduced in (Fig. 6). Pyrolysis of (27) proceeded smoothly giving 2-(2'-methoxy-5'-methylphenyl)-1-propene (28). Its IR spectrum (Fig. 7) showed bands at 3110, 1635 and 895 cm^{-1} due to a terminal methylene group. The IR spectrum also exhibited bands at 1610 ~~&~~ and 810 cm^{-1} for the aromatic ring. The styrene derivative (28) was subjected to <u>in situ</u>, hydroboration-oxidation in THF to give the primary alcohol (29) in good yield. The IR spectrum (Fig. 8) of (29) showed the presence of hydroxyl group (bands at 3400, 1030 cm^{-1}) and absence of the bands due to the terminal methylene group. Its ^{1}HNMR spectrum (Fig. 9) showed the signals due to a secondary methyl (1.16, 3H,d, J=7.2H$_z$), and aromatic methyl (2.18, 3H, s), a benzylic hydrogen (3.32, 1H, m), a primary alcohol (3.62, 2H, m and 2.0, 1H,bs, exchangable) and a methoxyl group (3.7, 3H, s). Besides these signals, the spectrum also showed three aromatic protons. The alcohol (29) was tosylated using p-toluenesulfonyl chloride in pyridine at 0^{o} till addition of compound is over and then at room temperature. The crude tosylate (30) was then immediately subjected to prenylation with prenyl magnesium chloride in the presence of catalytic amount of 1-pentynyl copper under inert atmosphere. The usual work-up afforded a mixture which contained a less polar hydrocarbon fraction and more polar material which was proved to be mainly the alcohol (29). Results were not encouraging even when the prenylation reaction was tried on the iodide (30a). Since the prenylation sequence has been used successfully in several other cases, the reason for failure in the present case is not clear. We, however, feel that it is a matter of experimentation and we will succeed in finding out the right experimental conditions and efforts are on in this direction.



(30 a)

Although elvirol (14, R=H) contains a chiral centre, no reference was made to any optical activity in the natural compound. This may perhaps be due to the extremely small quantity available for study. However, elvirol (14, R=H) in principle may be present in three forms i) (+), elvirol, ii) (-) elvirol, iii) (±) elvirol. We considered it worthwhile to prepare elvirol (14, R=H) in one or both optically active forms and then make configurational assignment* by correlation studies. This looked particularly attractive because 4,6-dimethyl coumarin (26)used as a starting material in the present synthesis of (±) elvirol methylether has been previously converted into (+)-(3S)-3(2'-methoxy-5'-methylphenyl) butanoic acid (35) and (-)-(3R)-3(2'-methoxy-

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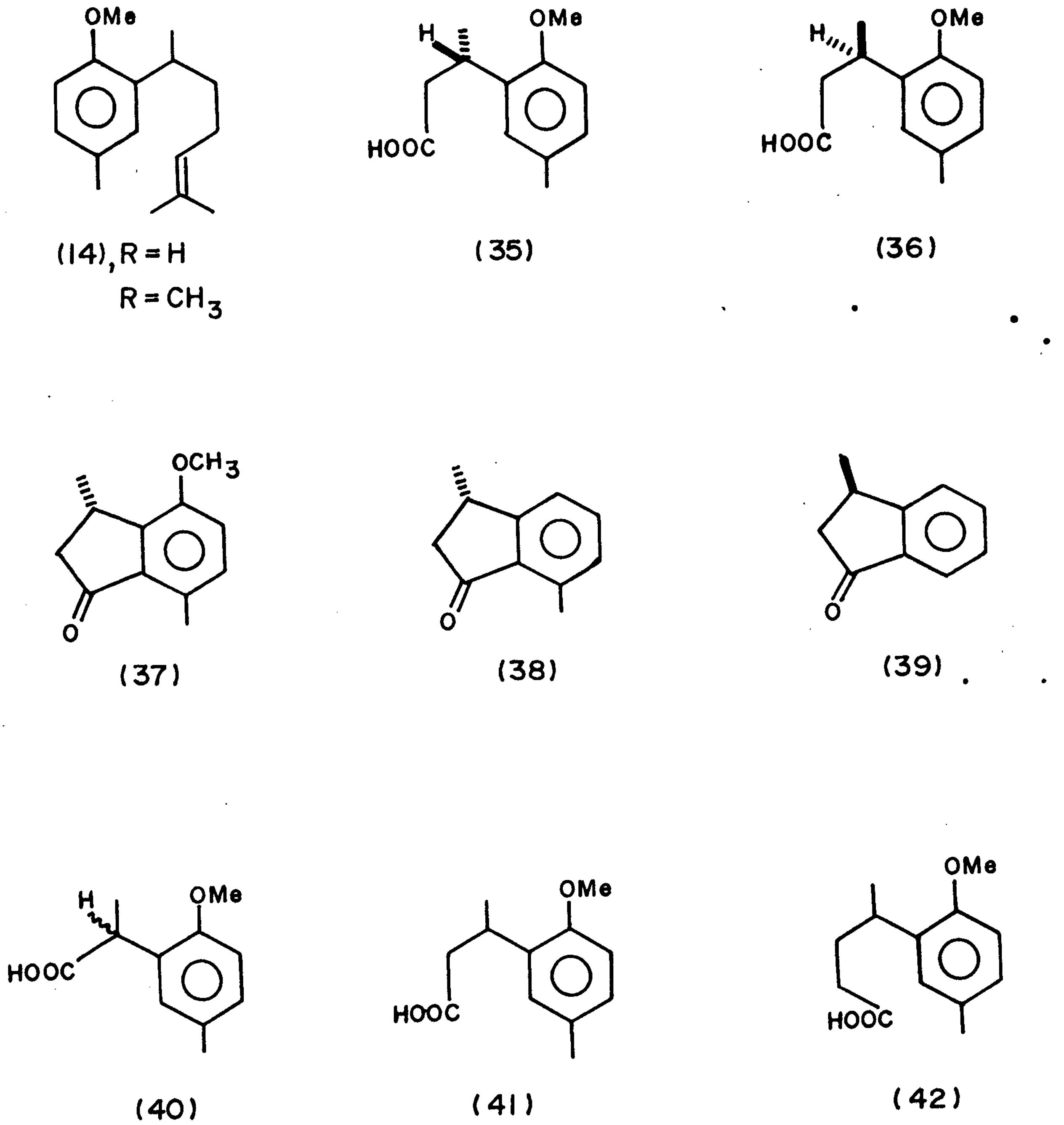
* Using R-S configurational nomenclature.

5'-methylphenyl) butanoic acid (36)[28]. The absolute configurations of these acids were deduced from the comparison of optical property of indanones (37) and (38) , prepared from (+) acid (35) with that of well established (-) -(3R)-3-methyl-1-indanone (39)[29]. The ORD curves of (37) and (38) are almost mirror images to that of (39)[28]. The acid (35) therefore was shown to possess `S' configuration at the only asymmetric carbon.

The primary alcohol (29) desired for the synthesis of optically active elvirol methyl ether (14, R=CH_3) can be prepared in two ways, (i) via resolution of the (±) acid (40), conversion of resolved acid to its methyl ester and then reduction with $LiAlH_4$ and (ii) by degradation of acids (41) or (42) and functional group transformation by routine reactions.

Since the (±) alcohol (29) was already with us, it was decided to follow the first procedure for its preparation in optically active form. The assignment of the absolute configuration at its chiral centre can then be done by correlation (scheme-9) with optically active acids (41) or (42) with known absolute sterochemistry.

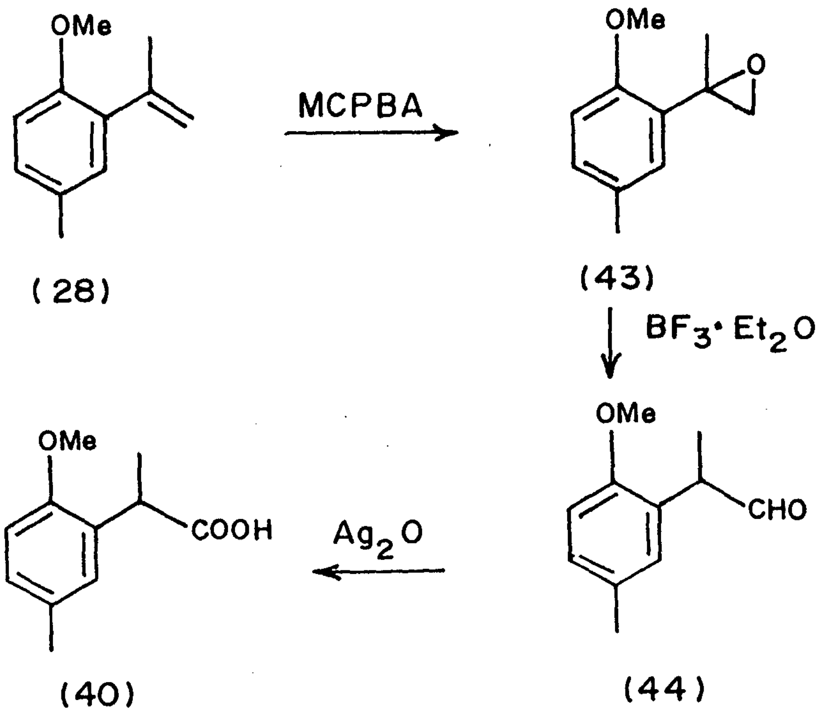
Since the resolution of a racemic alcohol (29) is much more difficult than the corresponding acid, we made efforts to prepare (40) from (29). It is well known that oxidation


OMe
(14), R = H
R = CH3
H
OMe
HOOC
(35)
H
OMe
HOOC
(36)
OCH3
O
(37)
O
(38)
O
(39)
H
OMe
HOOC
(40)
OMe
HOOC
(41)
OMe
HOOC
(42)

Scheme - 9

of a primary alcohol with excess Jones reagent in acetone usually gives good yields of the acid. The primary alcohol (29) however, under these experimental conditions afforded the desired acid (40) IR (Fig.10),m.p.118°, only in 10% yield*.

Since the yields of (40) were poor, we attempted its preparation by another route (scheme-10). This sequence was



Scheme-10

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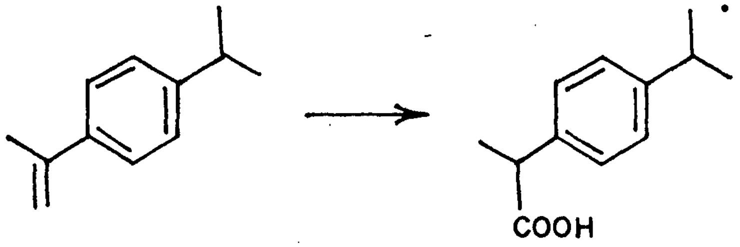
* Average yield based on several batches. The low yield may be due to the formation of other oxidation products. We have observed that CrO_3/AcOH oxidation of 3,4-dimethyl anisole gives 2-methyl-p-anisic acid.

used earlier by Welch and co-workers[30] for conversion of (45) into (46). The reported yields were almost quantitative. The procedure when applied to the styrene derivative (28), we obtained an acid m.p 115-20°C, distinctly different from the acid (40) obtained by Jones' oxidation of alcohol (29). The IR spectra of these acids differed considerably establishing their nonidentity. The structure of the acid m.p. 115-20°C remains to be established.



(45) (46)

Recently Asakawa and co-workers[31] reported* oxidation of the aromatic ring with m-chloperbenzoic acid. It therefore, looks likely that besides the side chain conversion in the desired manner the aromatic ring must have undergone oxidation resulting in the formation of different acid then (40).

The resolution of the (±)-acid (40) with optically

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* See page 52 of this thesis

active bases was then studied. We found resolution with (-) strychnine to give low yields of (-) form. Ephedrine was found by Roger[32] to give both forms of mandelic acid in high yield. Similar observations were made by Neilson & Peter[33] who reported resolution of -methoxy phenyl acetic acid with ephedrine in high yields. We, therefore, used (+)-ephedrine for resolution of (±) acid (**40**) using aqueous ethanol as solvent. After 24 hr at room temperature, the precipitated salt was removed by filtration. Recrystallisation of the salt from aqueous ethanol and decomposition of the salt with 20% H_2SO_4 gave (+) acid, m.p. 120-21°C, $[\alpha]_D^{24}$ + 43.5° (c,0.5,$CHCl_3$). The IR spectrum (Fig. 10) of the resolved acid was identical with the one recorded on the racemic acid (**40**).

The racemic and resolved acid (+ form) were converted into the corresponding methyl esters by reaction with diazomethane. The ^{1}HNMR spectrum of the methyl ester (**47a**) (Fig. 11) showed the presence of a secondary methyl group (1.43, 3H,d,J=7Hz), an aromatic methyl (2.28, 3H, s), a carbomethoxy (3.64,3H,s), a methoxy (3.78, 3H,s), benzylic hydrogen (4.02, q, J=7Hz), two ortho substituted aromatic protons (6.77,1H,d,J=8Hz) and 7.0, 1H dd, J=8,2Hz) and another aromatic hydrogen (7.01, 1H,d, J=2Hz). The ^{1}HNMR spectrum of the methyl ester (**47b**) of resolved acid was indistinguishable from that recorded on racemic (47a).

Addition of the shift reagent Eu(fod)$_3$ and measurement of the ^{1}HNMR spectrum (Fig. 12) caused shifting of the signals of the secondary methyl, benzylic hydrogen and the carbomethoxy group. The signals for each enantiomer can clearly be seen upon addition of Eu(fod)$_3$. The signals of the carbomethoxy group which were of equal intensities in the ^{1}HNMR spectra (Fig. 12) were then used for finding the optical purity of the resolved acid. The ^{1}HNMR spectrum (Fig. 13) of the methyl ester (47b) derived from the resolved acid showed two signals (singlets of relative ratio 83:17) and the integration ratio showed the presence of 66% ee.

It is thus clear that further purification is desirable to obtain optically pure (100% ee) methyl ester (47b). Efforts are being made in this direction.

The assignment of the `S' configuration of the chiral centre in the resolved acid - $[\alpha]_D^{24}$ + 43.5° was established through correlation studies. The resolved ester (47b) was converted to the acid (41) by employing the sequence $R\text{-}COOMe \longrightarrow R\text{-}CH_2OH \longrightarrow R\text{-}CH_2\,OTs \longrightarrow R\text{-}CH_2\text{-}CN \longrightarrow R\text{-}CH_2COOH^*$. The IR spectrum (Fig. 14) established its identity with the acid (41) prepared from 4,6-dimethyl,3,4-dihydrocoumarin. The

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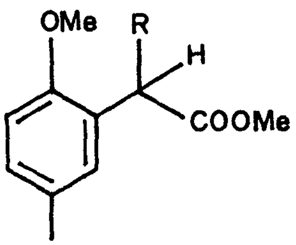
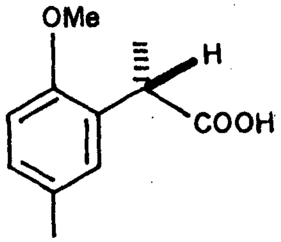
* Homologation by Arndt-Estert method was tried but we failed to get the acid (41).

magnitude and sign of the optical rotation $[\alpha]_D^{24}$ -18°* (c 1%, $CHCl_3$) showed its identity with (-)-(3R)-3(2'methoxy-5' methylphenyl) butanoic acid (36). The absolute stereochemistry at the chiral centre in (+)acid (41), obtained by homologation is therefore, ‘R' with methyl having B-configuration. The absolute stereochemistry at the chiral centre of the resolved (+)acid, therefore, ‘S' as shown in structure (48). Efforts are being made to obtain optically pure (+) acid (48) which in turn would be used for the preparation of optically active elvirol methylether (14, $R=CH_3$).



(47) a) $R = CH_3$
b) $R = \alpha CH_3$



(48)

** Subsequent batches failed to give acid with rotation + 43.5° and correlation studies were done with acid having rotation + 28° which gave on homologation acid (41) having rotation - 18°.

EXPERIMENTAL

Preparation of 3-longifolol (7) from (1)

Commercial longifolene (1,10 g) was dissolved in dry and peroxide-free THF (250 ml). A slow stream of N_2 carrying diborane [generated by adding a solution of $NaBH_4$ (5 g) in anhydrous diglyme (100 ml) to a solution of $BF_3.Et_2O$ (100 ml)] was passed through an ice-cold solution of the hydrocarbon during 3 hr, after which the dissolved diborane gas was released by heating the generation flask. The reaction mixture was then left overnight at room temp., cooled and made alkaline by dropwise addition of cold aqueous KOH (15%). After the addition of a few pieces of ice, excess of H_2O_2 (150 ml,30%) was added and the mixture left overnight, extracted with ether , washed thoroughly with H_2O, dried and the solvent removed. The product (11 g) was chromatographed on a silica gel column and eluted with petroleum ether followed by ethyl acetate in petroleum ether (20%). The 3-longifolol fraction was rechromatographed when pure 3-longifolol, m.p.77^{o}C (Lit m.p. 70^{o}C[6], 75-80^{o}C[12] 83-84^{o}C[13]) was obtained.

IR,νmax, nujol, (Fig.1): 3300, 2900, 1465, 1385, 1125, 1045, 1020, 1000 and 975.cm^{-1}.

3-Longifolyl tosylate (10) from 3-longifolol (7)

3-Longifolol (7,10 g) in dry pyridine (120 ml) was cooled to 0^{o} and p-toluenesulphonyl chloride (16 g) added in one lot. After one hr at 5^{o} and 12 hr at room temp. the mixture was diluted with ice-water , the product extracted with benzene, washed with HCl (1N) and then with H_2O and dried to furnish 3-longifolyl tosylate (10,16 g), m.p. 96^{o}C. ✓ ✓%

IR, ν max., nujol, (Fig. 2): 2960, 1490, 1465, 1365, 1185, 1170, 1090, 950, 905, 860, 820 and 775. cm^{-1}.

Regeneration of longifolene (1) from 3-longifolyl tosylate(10)

Preparation of neutral alumina (activity I)

Commercial chromatographic alumina was repeatedly washed with boiling distilled water till washings were neutral and then activated at 450^{o}-500^{o} for 6 hr before use.

3-Longifolyl tosylate (10,1 g, m.p.96^{o}C) in petroleum ether was loaded on dry packed column (20 cm x 4 cm) of alumina and left for 12 hr after which the product was eluted with petroleum ether (150 ml), removal of solvent followed by distillation yielded longifolene (1, 0.36 g, 66%), GLC analysis (FFAP, 96^{o}C) 99.1% (Fig. 4).

IR, ν max, neat (Fig. 3): 3100, 2950, 1670, 1470, 1385, 1372, 1305, 1240, 1230, 1215, 1185, 1170, 1160, 1130, 1100, 990, 980, 965, 950, 910, 880, 855, 825, 820 and 795. cm^{-1}

Attempted preparation of nitrile (13) from (10)

The tosylate (10, 2 g, 0.005 mole) was dissolved in anhydrous DMSO (25 ml), KCN (0.4 g, 0.006 mole) was added to the above solution and the mixture was stirred at 90° for 5 hr in an atmosphere of N_2. The reaction mixture was poured into H_2O containing NH_4Cl and extracted with CH_2Cl_2 (3 x 50 ml). The combined extract was washed well with H_2O, dried and evaporated to give crude product (0.5 g). The crude product was chromatographed (silica gel) and eluted with petroleum ether. On concentrating the petroleum ether fraction yielded pure (GLC 99%, FFAP, 96°) (Fig .5) longifolene (1, 0.22 g).

Similarly, above experiment was repeated without addition of KCN ,when 1 g 3-longifolyl tosylate (10) yielded (0.37 g, 68%) of pure longifolene (1,GLC 99%, FFAP, 96°).

Preparation of 4,6-dimethyl coumarin (26)

4,6-dimethyl coumarin was preapred by Pechmann reaction as described on page .

IR, ν max, nujol: 2920, 2840, 1710, 1610, 1605, 1570, 1490, 1460, 1420, 1380, 1320, 1280, 1270, 1250, 1200, 1180, 1070, 1030, 1015, 940, 910, 880, 860 and 830. cm^{-1}

^{1}HNMR (80 MHz, $CDCl_3$, δ): 2.43(3H, d, J=1.5Hz),2.44(3H, s), 6.25(1H, d, J=1.5Hz), 7.2-7.4(3H,m).

Preparation of (z)-3-(2'-methoxy-5'-methylphenyl)but-2-enoic acid (27) from (26)

A mixture of 4,6-dimethylcoumarin (26, 25 g, 0.14 mole) and aqueous NaOH [prepared by dissolving NaOH (143.7 g, 3.6 mole) in H_2O (359 ml)] was warmed on a steam bath with stirring till all the coumarin dissolved. Me_2SO_4 (241.4 g, 1.9 mole) was added during the course of 3.5 hr, care being taken to maintain the temperature of the reaction mixture below 50°. The reaction mixture was stirred for 1 hr at 50° after the completion of addition of Me_2SO_4, cooled to 5° and acidified with HCl (2N). The precipitated acid (27) was filtered, washed with H_2O, dried (26 g, 89%). Part of the acid (27) was recrystallised from benzene, m.p. 108°C.

IR, ν max, nujol, (Fig. 6): 2950, 2880, 1700, 1650, 1510, 1470, 1385, 1300, 1220, 1200, 1170, 1150, 1075, 1030, 940, 865 and 810. cm^{-1}

Preparation of 2-(2'-methoxy-5'-methylphenyl)-1-propene (28) from (27)

The acid (27, 25 g, 0.12 mole) was taken in a distillation flask. The distillation flask was heated in a sand bath. When the temperature of the molten acid reached 210°, there was vigorous evolution of CO_2 and the pyrolysed product started distilling over. The contents of the flask were maintained at 210-240°C till completion of distillation.

The styrene derivative (28) was obtained (10 g, 50%).

IR, ν max, neat, (Fig. 7) : 3110, 2980, 2850, 1765, 1635, 1610, 1505, 1465, 1295, 1275, 1245, 1180, 1150, 1100, 1035, 895, 810, 760 and 745. cm^{-1}

Preparation of 2-(2'-methoxy-5'-methylphenyl)-1-propanol (29) from (28)

To a stirred mixture of $NaBH_4$ (1.24 g, 0.032 mole), dry and peroxide free THF (35 ml) and 2-(2'-methoxy-5'-methylphenyl)-1-propene (28, 10g, 0.062 mole) was added dropwise freshly distilled BF_3/Et_2O (5 ml, 0.04 mole), under dry N_2 atmosphere during 20 minutes. The mixture was stirred for another 1 hr and the excess hydride was decomposed by dropwise addition of H_2O (8 ml). Then NaOH (3N, 16 ml) was added, followed by H_2O_2 (30%, 8 ml) maintaining the temperature throughout at 35°C, after an additional stirring for 1 hr, the reaction mixture was saturated with NaCl (2 g) to cause the THF solution of the alcohol (29) to separate. The aqueous layer was extracted with ether (3 x 50 ml). The combined organic extracts were washed with H_2O, dried and the solvent removed. The crude product was purified by column chromatography (silica gel), to yield (29,8.5 g, 75%).

IR, ν max, neat, (Fig. 8): 3400, 2960, 1610, 1500, 1465, 1285, 1245, 1175, 1135, 1030, 875, 800, 735 and 705. cm^{-1}

^{1}HNMR (90 MHz, $CDCl_3$, δ) (Fig. 9): 1.16(3H, d, J=7.2Hz), 2.0(1H, bs), 2.18(3H,s), 3.32(1H, m), 3.62(2H,m), 3.7(3H,s), 6.7(1H, d, J=10Hz), 6.7-6.94(2H, m).

Preparation of 2-(2'-methoxy-5'-methylphenyl)-1-propanoic acid (40) from (29)

To a vigorously stirred solution of alcohol (29, 8 g, 0.044 mole) in acetone (160 ml) cooled between 0-5°C, was added Jones* chromic acid reagent (8N) dropwise, till a brown colour persisted. After addition of reagent, the stirring was continued for 1 hr and the excess reagent destroyed with methanol.

The reaction mixture was diluted with H_2O (250 ml) and extracted with $CHCl_3$ (3 x 50 ml). The combined $CHCl_3$ extracts were then extracted with saturated $NaHCO_3$ solution (4 x 40 ml) and the $CHCl_3$ layer rejected. The combined $NaHCO_3$ extracts were cooled to 5°C and neutralised with HCl (10%). The acidified mixture was extracted with $CHCl_3$ (3 x 30 ml), washed with H_2O, dried and evaporated. This afforded acid (40, 0.8g, 9%), m.p. 118°C.

IR ν max, nujol, (Fig. 10): 2900, 1700, 1610, 1585, 1505, 1460, 1410, 1372, 1330, 1310, 1295, 1250, 1240, 1172, 1150, 1135, 1080, 1070, 1025, 990, 910, 880, 840, 810, 775, 735 and 700. cm^{-1}

Preparation of epoxide (43) from styrene derivative (28)

2-(2'-methoxy-5'-methylphenyl)-1-propene (**28**, 3.58 g), 0.022 mole) in CH_2Cl_2 (65 ml) was stirred and to this MCPBA (7.5 g, 0.04 mole, of 55% assay) was added at such a rate to maintain temperature at 25°C. After stirring for 3 hr at 25°C, excess of MCPBA was destroyed by sodium hydrosulphite solution (10%). The reaction mixture was then transferred to a separatory funnel and the organic layer was washed with Na_2CO_3 solution (10%) in order to remove m-chlorobenzoic acid, followed by washing with H_2O. The organic extracts on drying and concentrating yielded (4.7 g) of epoxide (**43**).

Preparation of aldehyde (44) from epoxide (43):

To epoxide (**43**, 4.7 g) in dry benzene (30 ml) was added BF_3.Et_2O (4 ml) and the contents were stirred at room temperature for 10 minutes. On usual work-up afforded aldehyde (**44**, 3.1 g).

Preparation of acid from aldehyde (44)

Ag_2O was prepared by mixing $AgNO_3$ (5.7 g, 0.034 mole in 15 ml H_2O) and NaOH (2.9 g, 0.074 mole in 20 ml H_2O). To the precipitated brown silver oxide, was added aldehyde (**44**, 3 g, 0.017 mole) and the contents were stirred for 1 hr. At the end of 1 hr. black ppte of silver were filtered off, filtrate was acidified and extracted in $CHCl_3$ (3 x 50 ml). The combined $CHCl_3$ extracts were again extracted in $NaHCO_3$ (10%) which on usual work up afforded acid, m.p. 115-120°C.

IR, ν max, nujol : 2950, 1715, 1610, 1590, 1520, 1485, 1395, 1315, 1275, 1190, 1150, 1085, 1040, 910, 860, 815, 760 and 725. cm^{-1}

Resolution of acid (40) with (-)-strychnine

✓

2-(2'-methoxy-5'-methylphenyl)-1-propanoic acid (40, 1 g) was dissolved in minimum amount of $CHCl_3$ and then EtOH (0.5 ml) was added. To this solution strychnine (2.58 g) was added, warmed and kept at room temperature for two days. The precipitated salt was filtered and recrystallised twice from EtOH. Decomposition of the salt with 2N HCl gave (-) acid, m.p. 117-118°C (0.036 g), $[\alpha]_D^{25}$ -10° (CHCl$_3$). ✓

c — ,

Resolution of acid (40) with (+)-ephedrine

✓

Ephedrine (2.5 g) and 2-(2'-methoxy-5'-methylphenyl)-1-propanoic acid (2.5 g) were dissolved in warm EtOH (10 ml) and left for 24 hr at room temperature. The ephedrine salt, which was precipitated, was filtered off and crystalised twice from EtOH. Decomposition of this salt with 20% H_2SO_4 gave, (+) acid (0.2 g), which after one more recrystallization from benzene-Petroleum ether afforded (+) acid (m.p. 121°C), $[\alpha]_D^{24}$ + 43.5° (c 0.5, $CHCl_3$).

Preparation of Methyl ester (47b) from (+) acid (48)

Ethereal solution of diazomethane (20 ml) was added ethereal solution of (+) acid (48, 0.13 g), cooled at 0°, until the reaction mixture attained a little yellow colour. The reaction mixture was kept for 1 hr at 0° and 24 hr at room temperature. Then ether was evaporated to give methyl ester (47b),as an oil (0.14 g), $[\alpha]_D^{24}$ + 49° (c 0.5, $CHCl_3$).

Similarly methyl ester of racemic acid (40) and resolved (+) acid (48) having rotation +28° were prepared.

^{1}HNMR (300 MHz, $CDCl_3$, δ), (Fig. 11) : 1.43(3H,d, J=7Hz), 2.28(3H,s), 3.64(3H,s), 3.78(3H, s), 4.02(1H, q, J=7Hz), 6.77(1H, d, J=8Hz), 7.0(1H,dd,J=8,2Hz), 7.01(1H,d,J=2Hz).

Preparation of alcohol (29) from methyl ester (47b)

To dry ether (25 ml), excess $LiAlH_4$ (0.05 g) was added. The methyl ester (47b, 0.14 g) in dry ether (10 ml) was added dropwise to it, at such a rate that the ether gently refluxed. The contents were stirred during addition. After addition is over the flask was kept at room temperature with stirring for 24 hr, after that refluxed for 2 days (monitored by TLC). The excess $LiAlH_4$ was then destroyed by adding EtOAC, filtered and filtrate on drying and concentrating afforded alcohol (29, 0.118 g).

Preparation of tosylate (30) from alcohol (29)

The alcohol (29, 0.11 g) in dry pyridine (5 ml) was cooled to 0° and p-toluenesulphonyl chloride (0.2 g) added in one lot. After 1 hr at 5° and 18 hr at room temperature, the mixture was diluted with ice water, the product extracted with ether (2 x 20 ml), washed with HCl (1N), H_2O and dried to furnish tosylate (30, 0.1 g). The tosylate was immediately used for the next reaction.

Preparation of 3-(2'-methoxy-5'-methylphenyl)-butyronitrile from tosylate (30)

The tosylate (30, 0.11 g) was dissolved in anhydrous DMSO (5 ml). NaCN (0.03 g) was added to the solution and the mixture was stirred at 90° for 5 hr in an atmosphere of N_2. The reaction mixture was poured into H_2O containing NH_4Cl and extracted with CH_2Cl_2 (3 x 10 ml). The combined CH_2Cl_2 extracts were washed well with H_2O, dried and evaporated to give (0.05 g) crude 3-(2'-methoxy-5'-methylphenyl)-butyronitrile.

Attempted preparation of Elvirol methyl ether (14, R=CH_3 from Tosylate (30)

Prenylchloride (1.06 g) in THF (5 ml) was added over 1 hr to Mg turnings (1.22 g) in THF (10 ml) with vigorous

G

stirring at room temperature under N_2 atmosphere. After 3 hrs of stirring, the grignard reagent was syringed out and added to a two neck flask containing pentynylcopper (0.211 g) under N_2 atmosphere. To this mixature was added at 0°C, a solution of tosylate (30, 0.167 g) in THF (5 ml) in one lot and stirring continued at 0° for 3 hrs and thereafter at room temperature overnight. The resulting black mixture was poured into saturated aqueous NH_4Cl-ether which was then stirred for 1 hr. The crude product was isolated by usual work-up.

TLC and IR showed it to be mixture of alcohol (29) and a less polar hydrocarbon fraction.

<u>Preparation of iodide (30a) from tosylate (30)</u>

To the tosylate (30, 0.334 g) in dry acetone (10 ml) was added NaI (0.64 g, 3 molar equivalent) and refluxed overnight. After that most of the acetone was removed under reduced pressure, reaction mixture diluted with H_2O (10 ml) and extracted with ether (3 x 20 ml). Ethereal layer washed with sodium thiosulphate solution, H_2O, brine and dried . Removal of ether yielded iodide (30a, ~ 0.33 g).

<u>Attempted preparation of Elvirol methyl ether (14, R=CH_3) from Iodide (30a)</u>

The crude iodide (0.33 g) was dissolved in THF (10 ml) and added over to a stirred mixature of pentynylcopper

(0.240 g) and prenyl magnesium chloride [prepared from prenyl chloride (2.12 g), Mg turnings (2.44 g) in THF (15 ml)] in THF (5 ml) at 0°C under N_2 atmosphere. After stirring for 2 hr at 0°C, reaction mixture was stirred overnight at room temperature. The resulting black solution was poured over aqueous NH_4Cl-ether solution. The ether layer was separated, washed with H_2O, brine, dried. The IR of crude product did not show presence of elvirol methyl ether (14, $R=CH_3$).

Preparation of acid (36) from 3-(2'-methoxy-5'-methylphenyl)-butyronitrile

3-(2'-methoxy-5'-methylphenyl)-butyronitrile (0.05 g) was refluxed with 40% alcoholic KOH (5 ml), until NH_3 ceases to be evolved (2 hr). The reaction mixture was diluted with H_2O, acidified with HCl (2N) and extracted in $CHCl_3$ (2 x 10 ml). The combined $CHCl_3$ extracts were shaken with $NaHCO_3$ (10%). The bicarbonate extract on usual work up afforded pure acid (36) as a thick, viscous oil,(0.02 g) $[\alpha]_D^{24}$ -18°. The IR spectrum of the acid (36) was identical with the acid prepared from 4,6-dimethyl-3,4-dihydrocoumarin.

IR, ν max, nujol, (Fig. 14): 2950, 1715, 1615, 1505, 1465, 1415, 1380, 1340, 1295, 1250, 1185, 1160, 1140, 1105, 1090, 1030, 940, 875, 805 and 745. cm^{-1}.

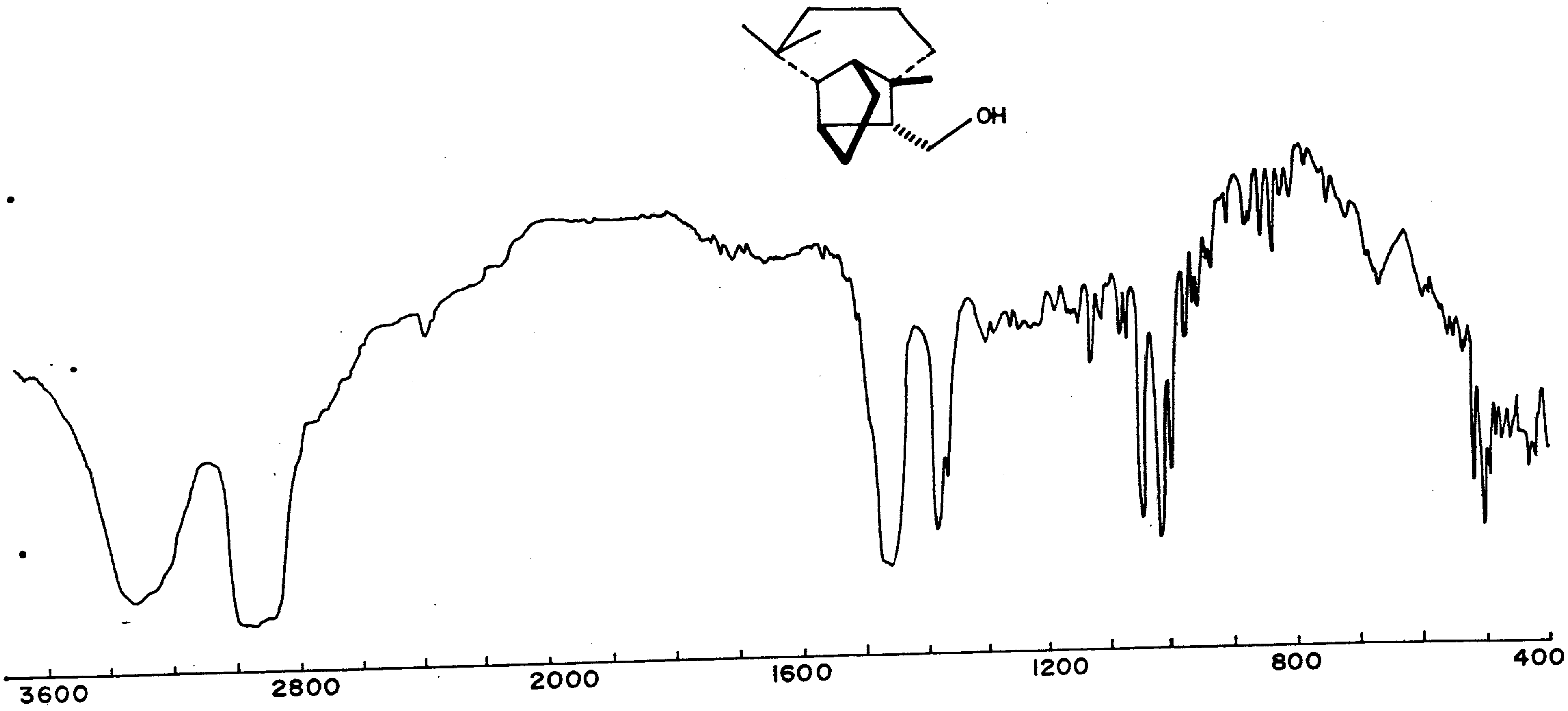


Fig. 1 IR Spectrum of (7)

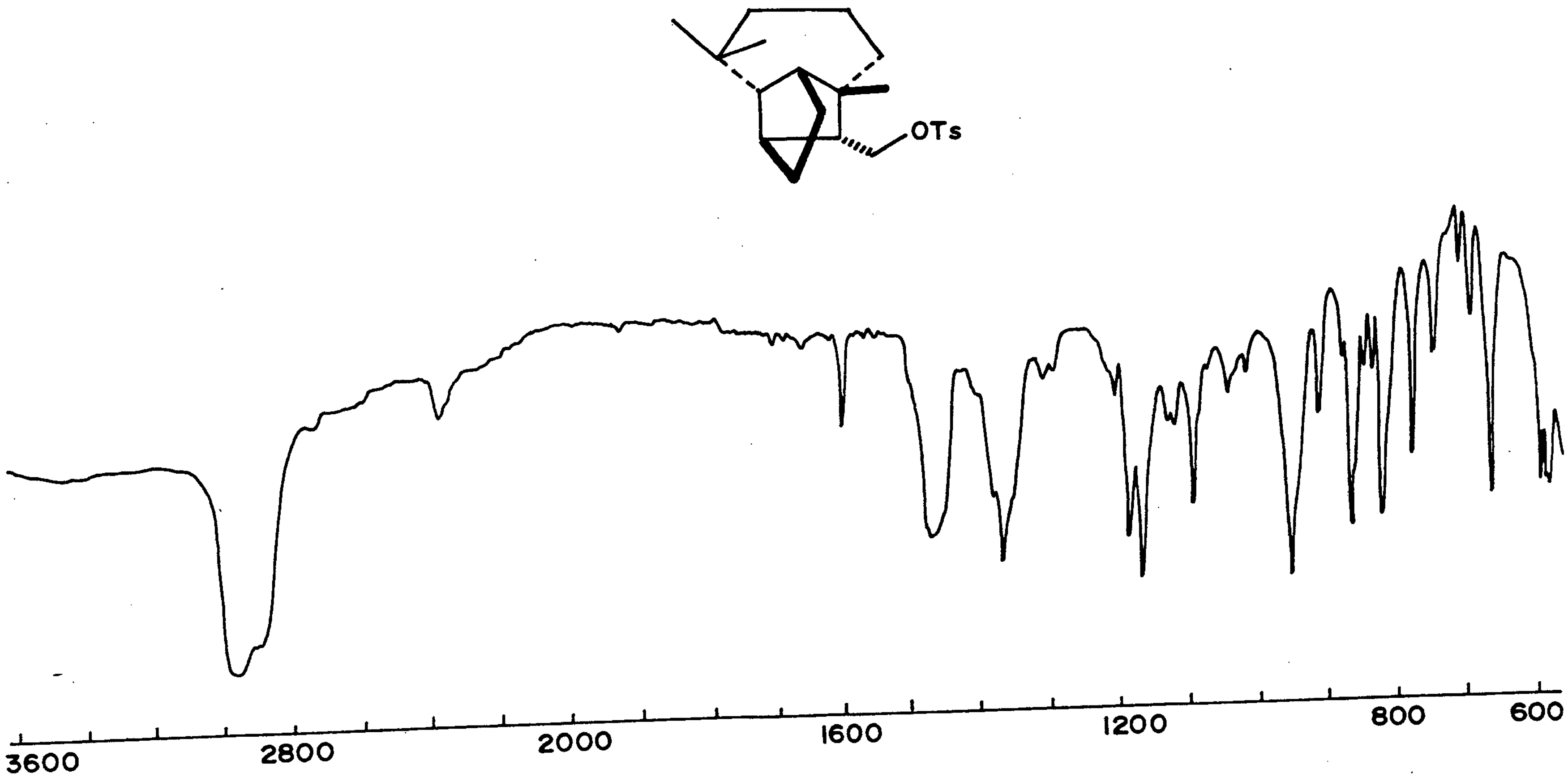


Fig. 2 IR Spectrum of (10)

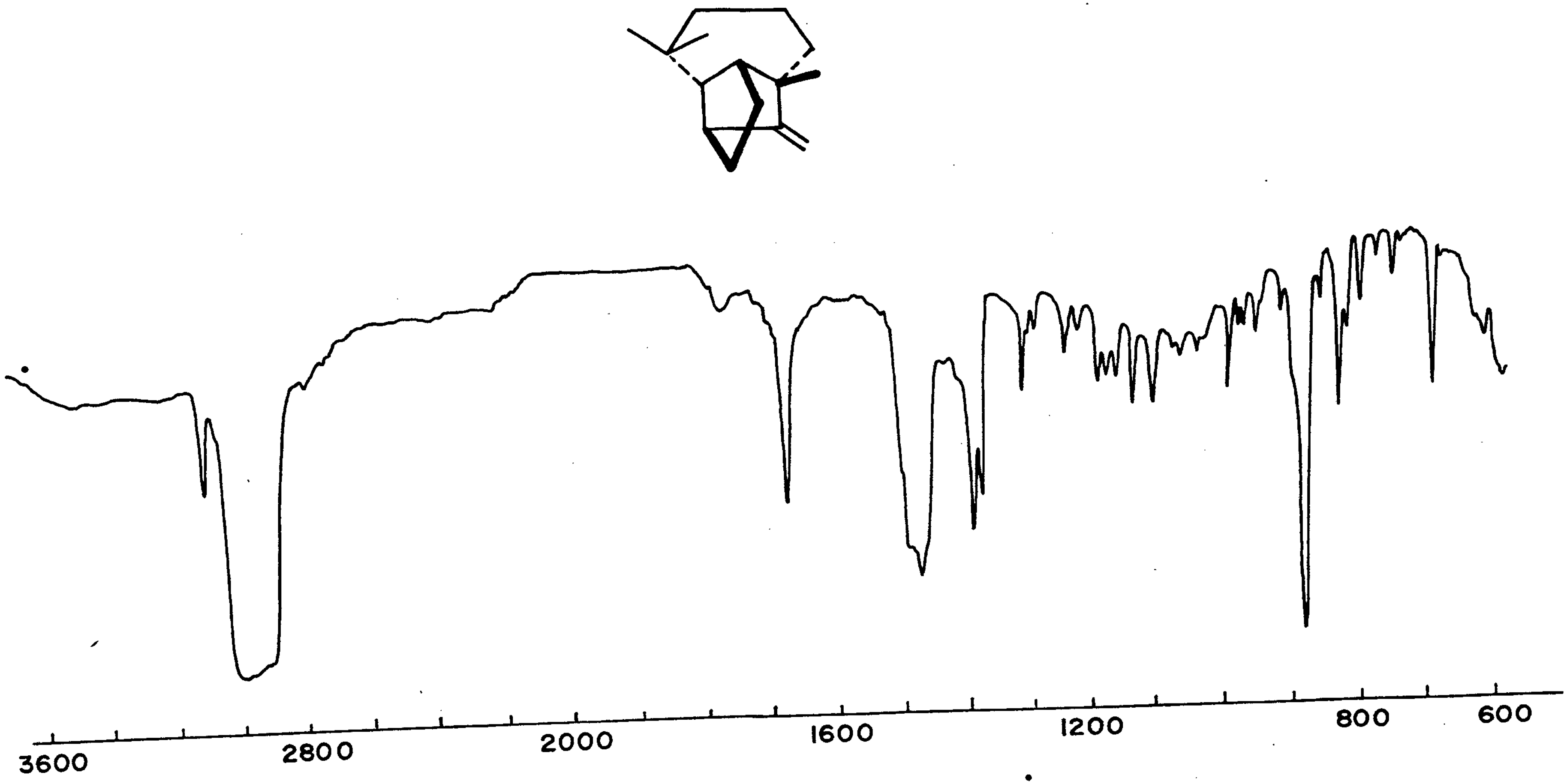


Fig. 3 IR Spectrum of (I)

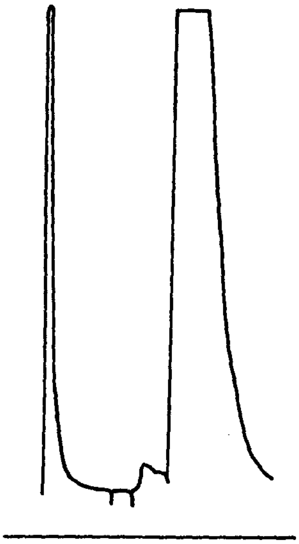


Fig. 4 GLC of (1)(Al_2O_3)

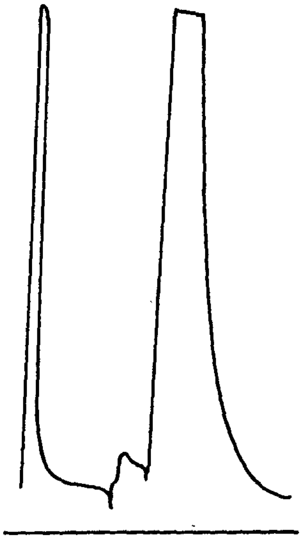


Fig. 5 GLC of (1) (DMSO)

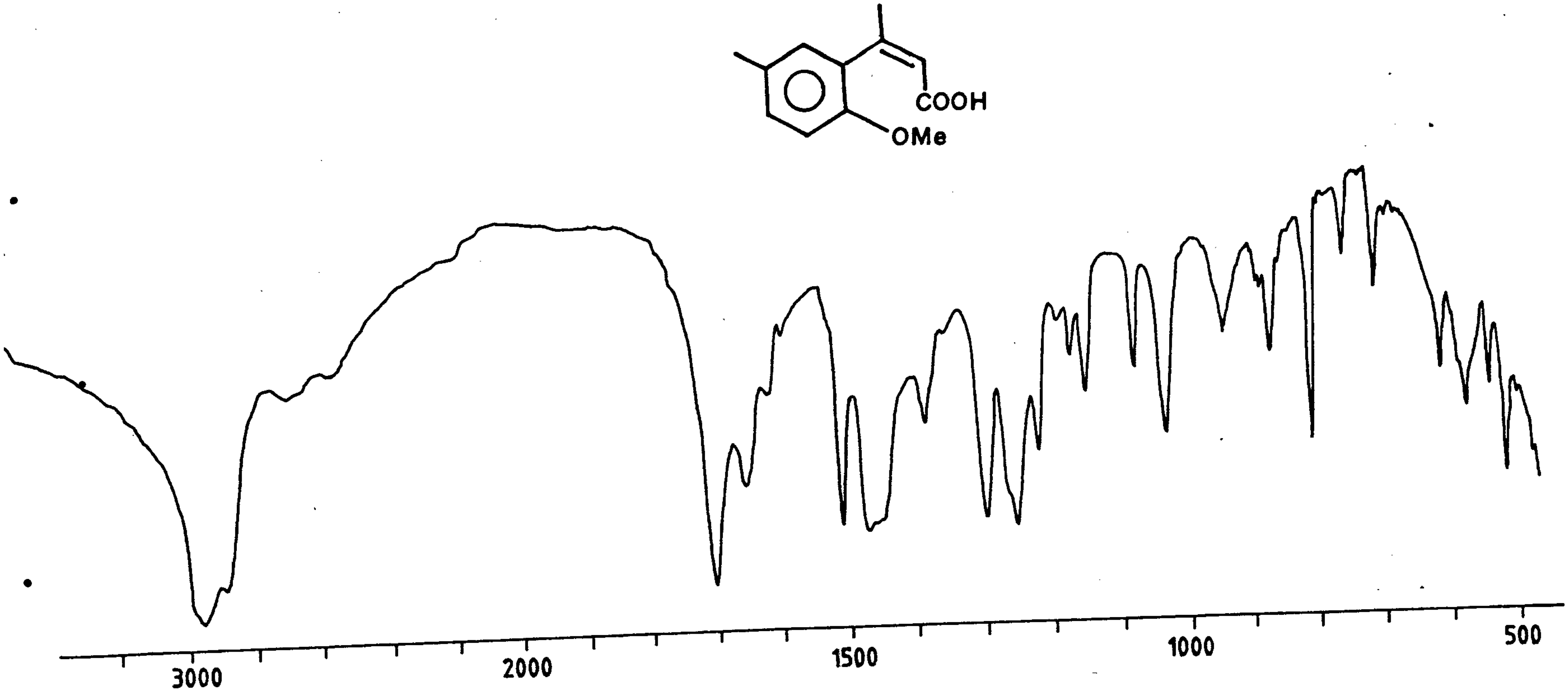


Fig. 6 IR spectrum of (27)

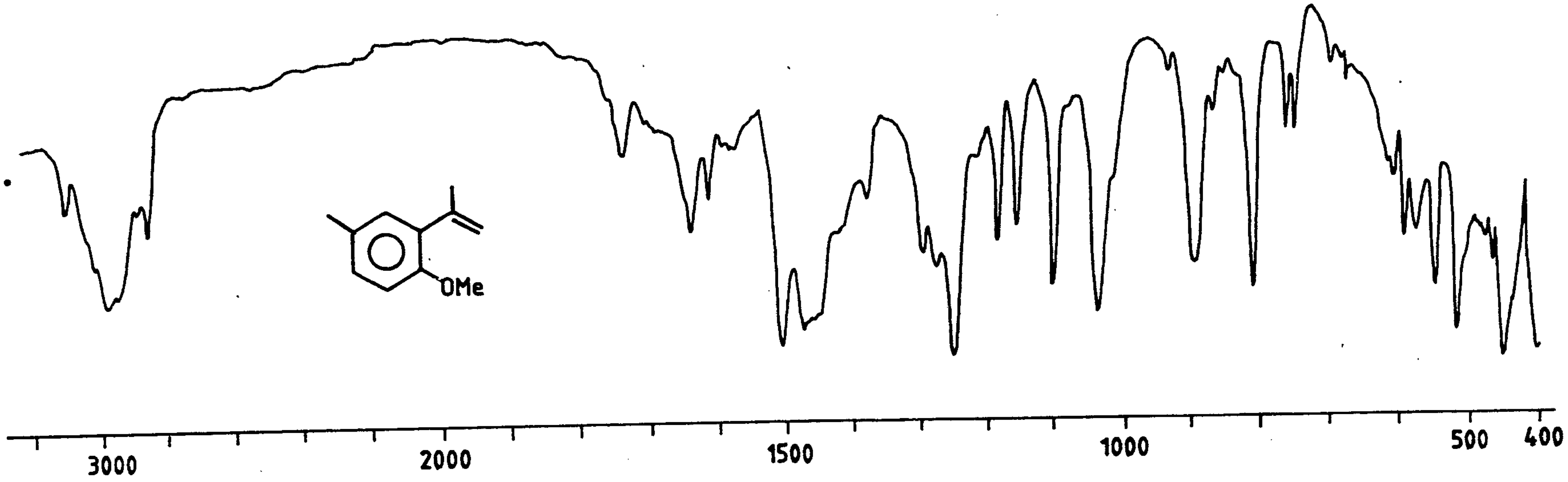


Fig. 7 IR spectrum of (28)

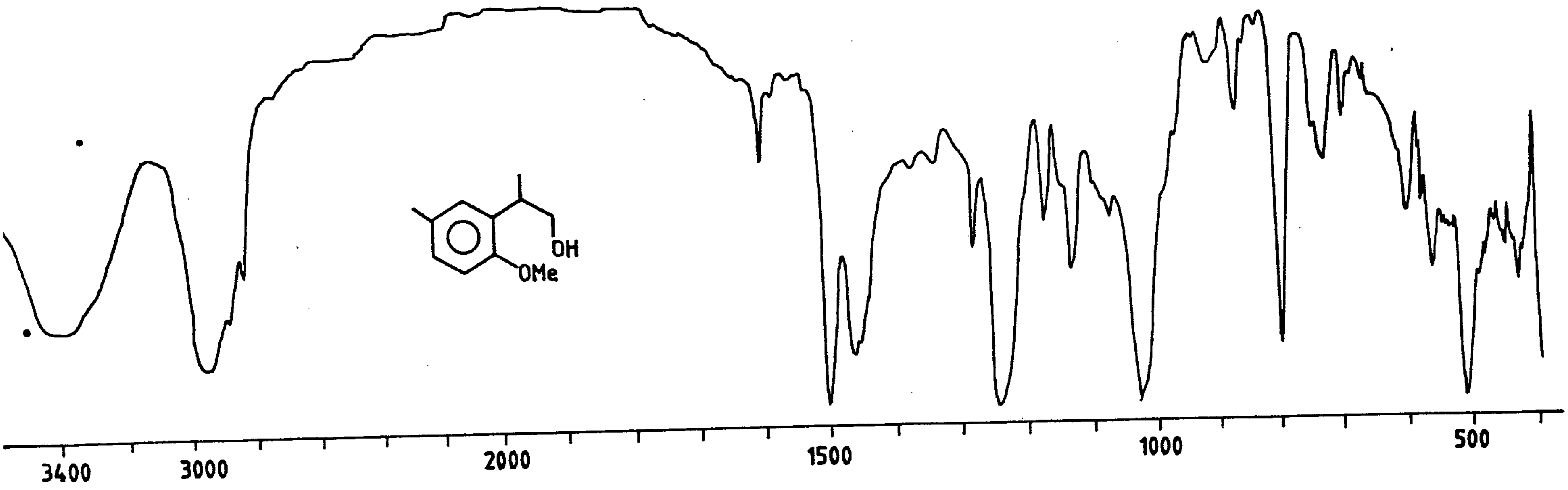


Fig. 8 IR spectrum of (29)

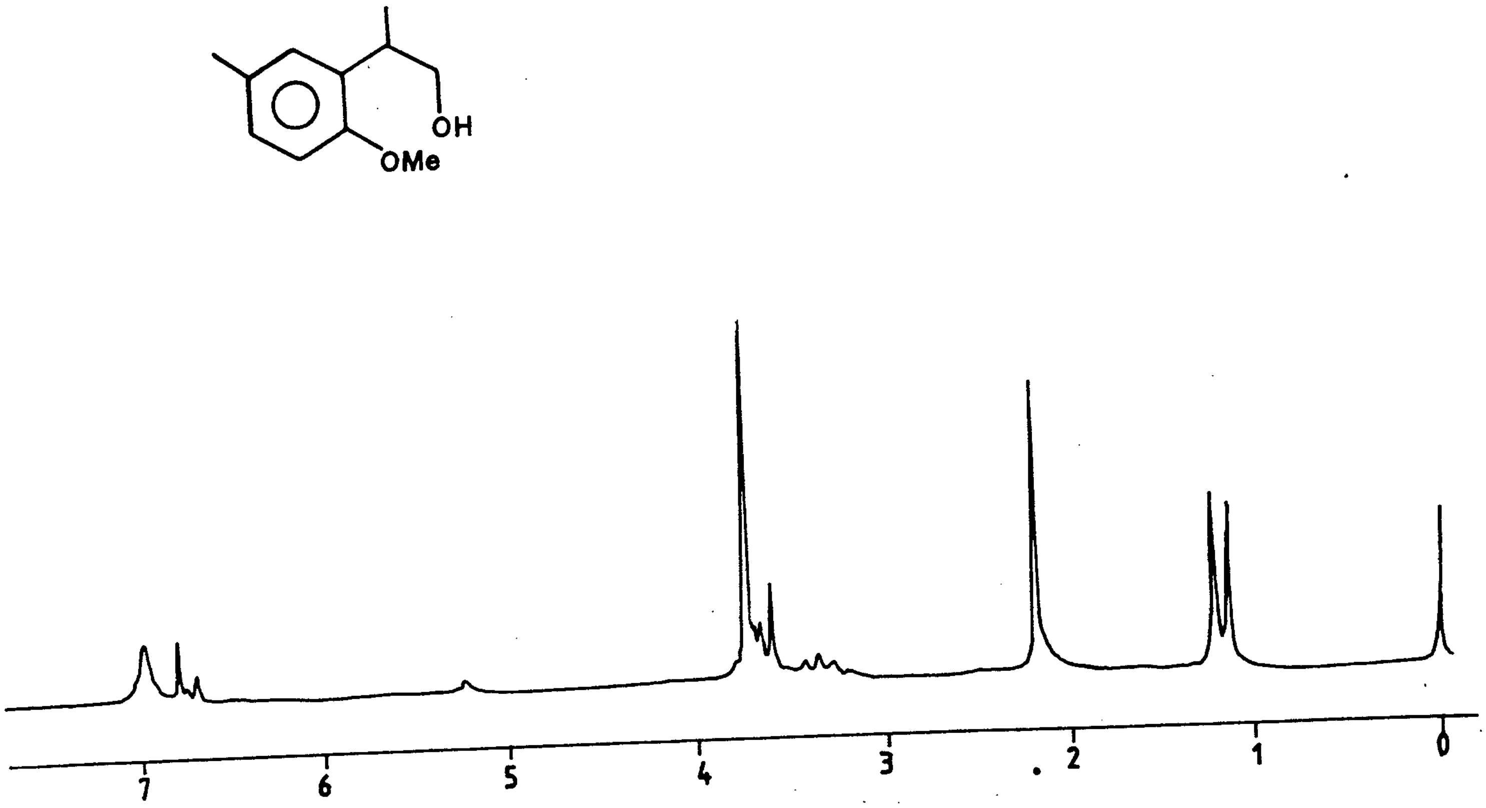


Fig. 9 ^{1}HNMR spectrum of (29)

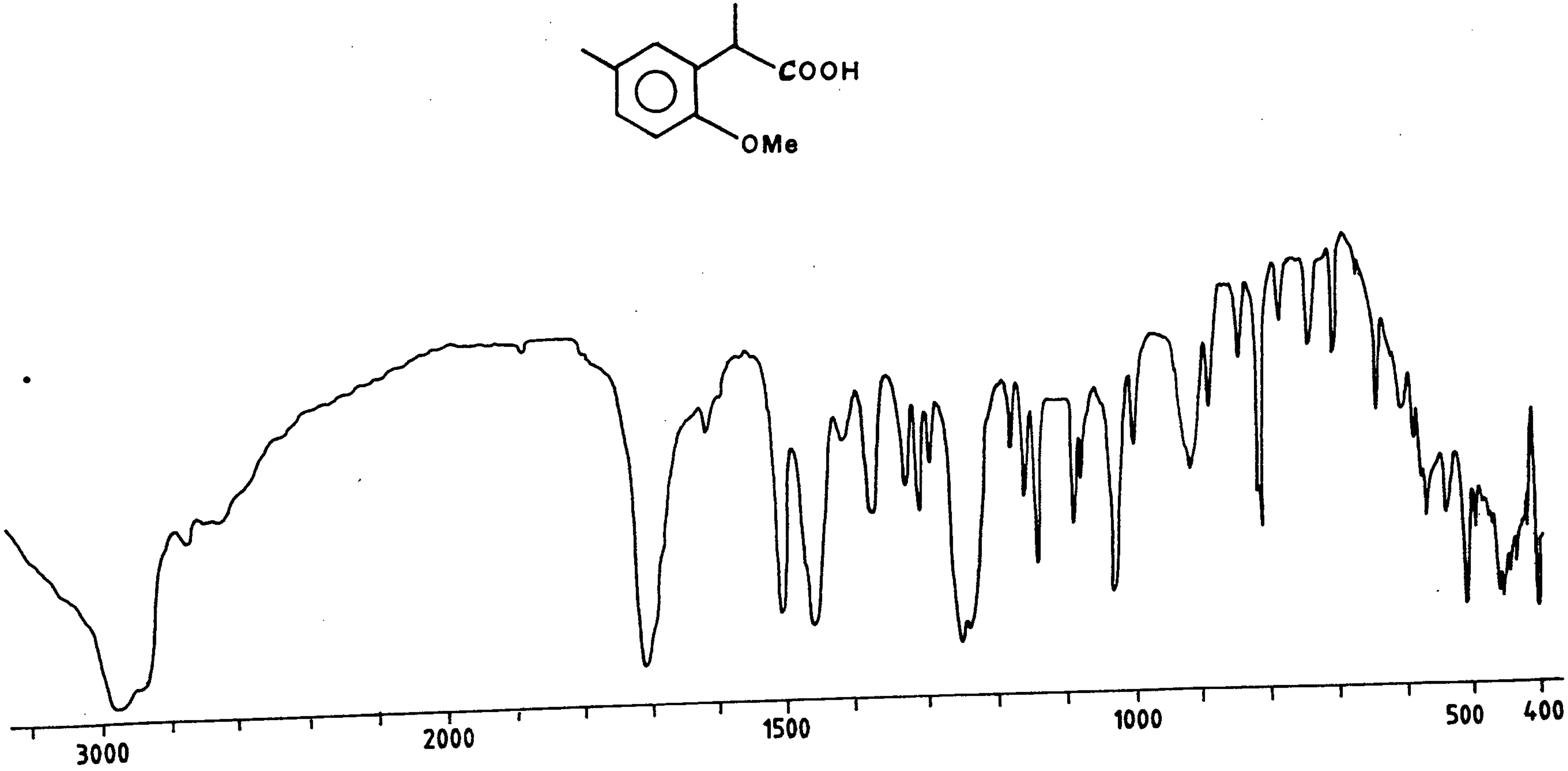


Fig. 10 IR spectrum of (40)

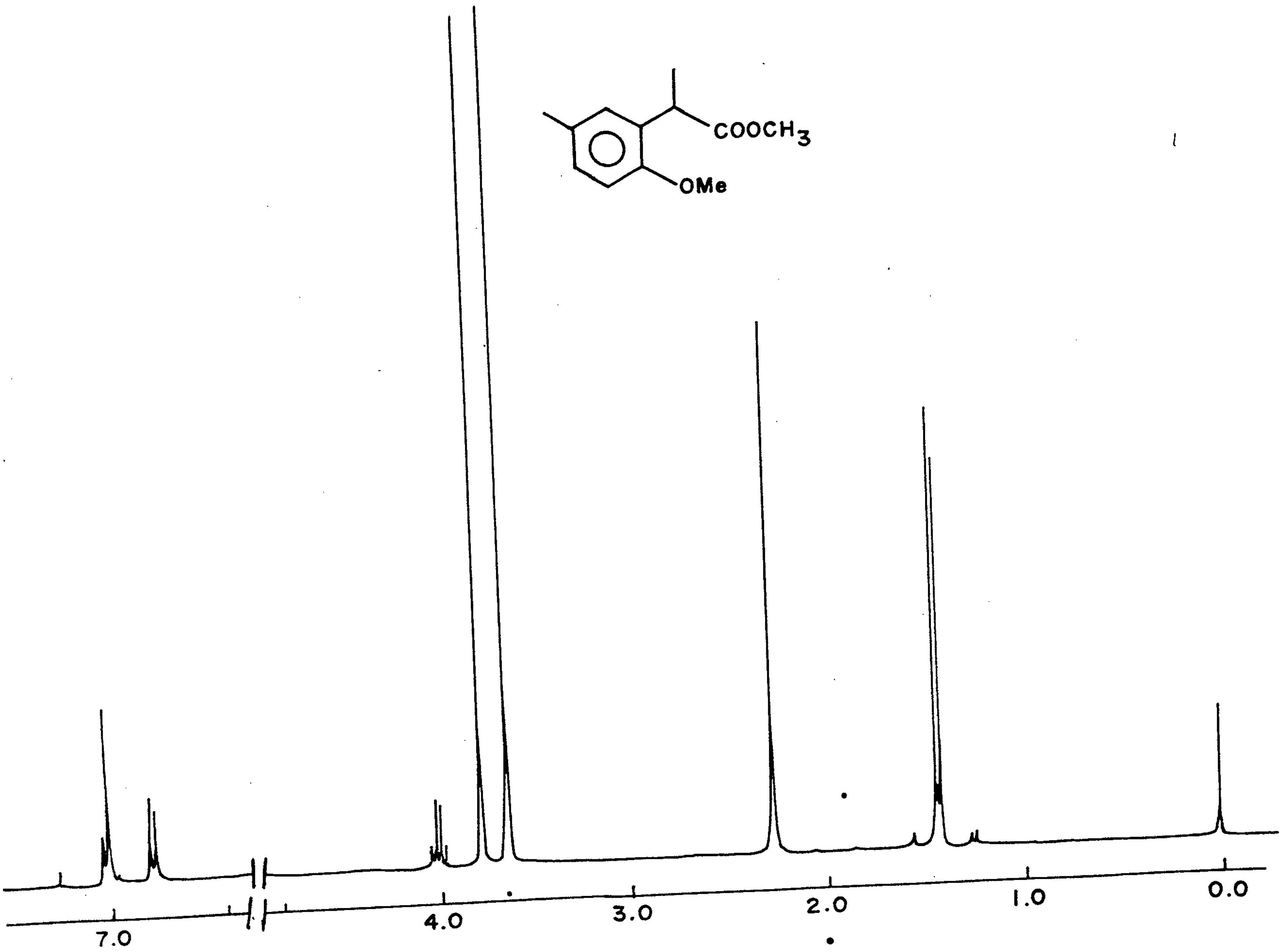


Fig. 11 ^{1}HNMR Spectrum of (47a).

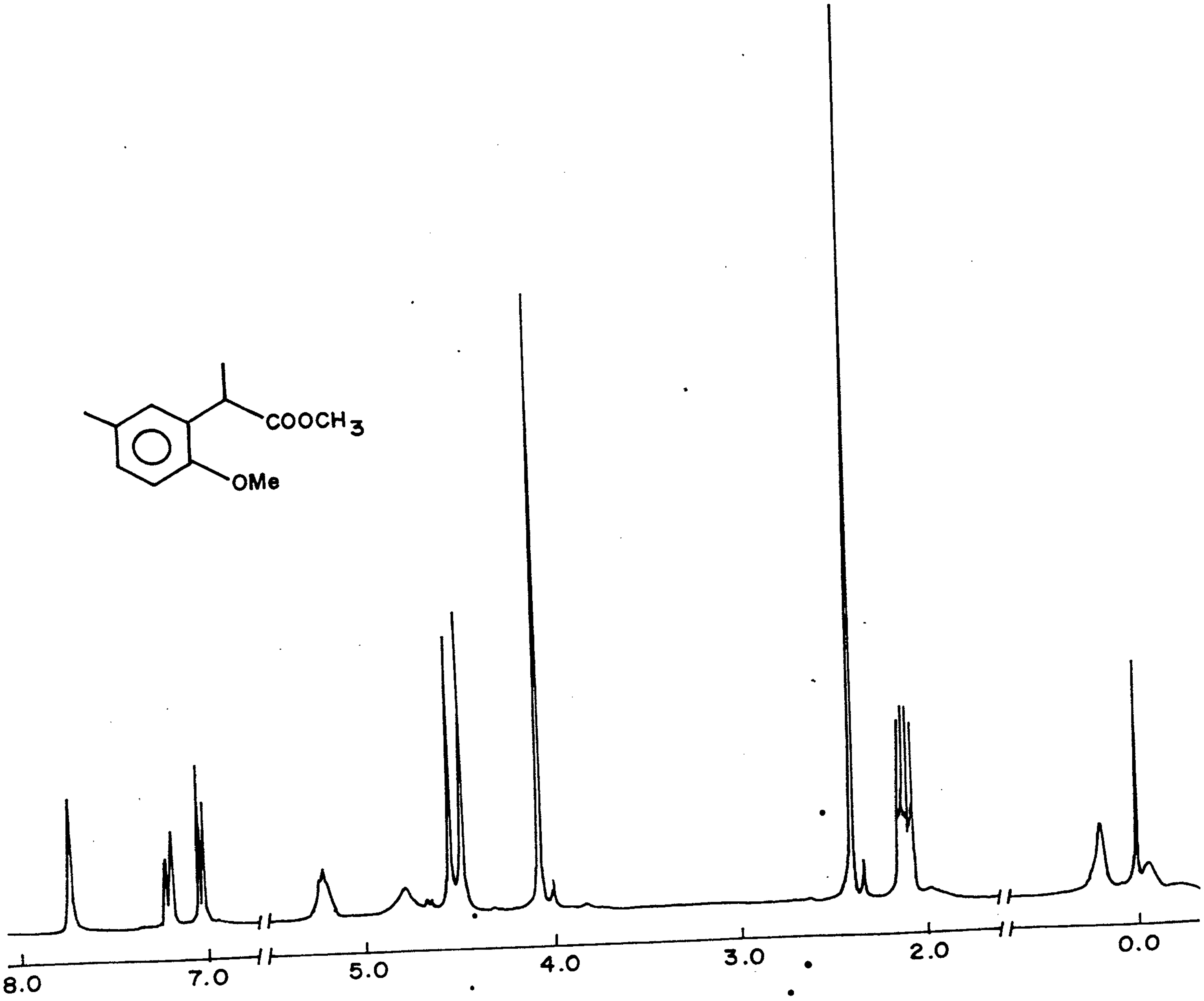


Fig. 12 ^{1}HNMR Spectrum of (47a) with Eu(fod)$_3$

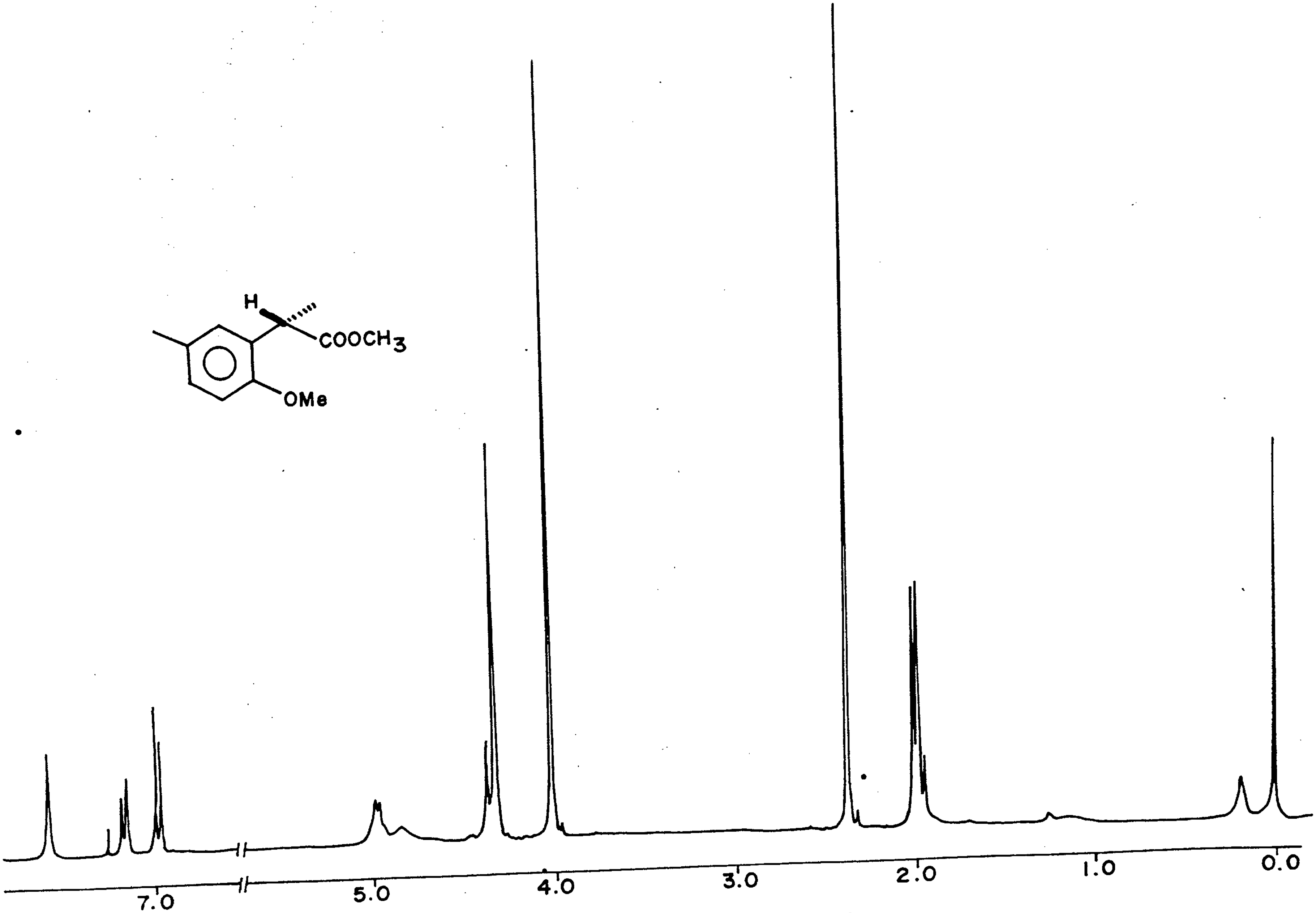


Fig. 13 ^{1}HNMR Spectrum of (47b) with Eu (fod)$_3$

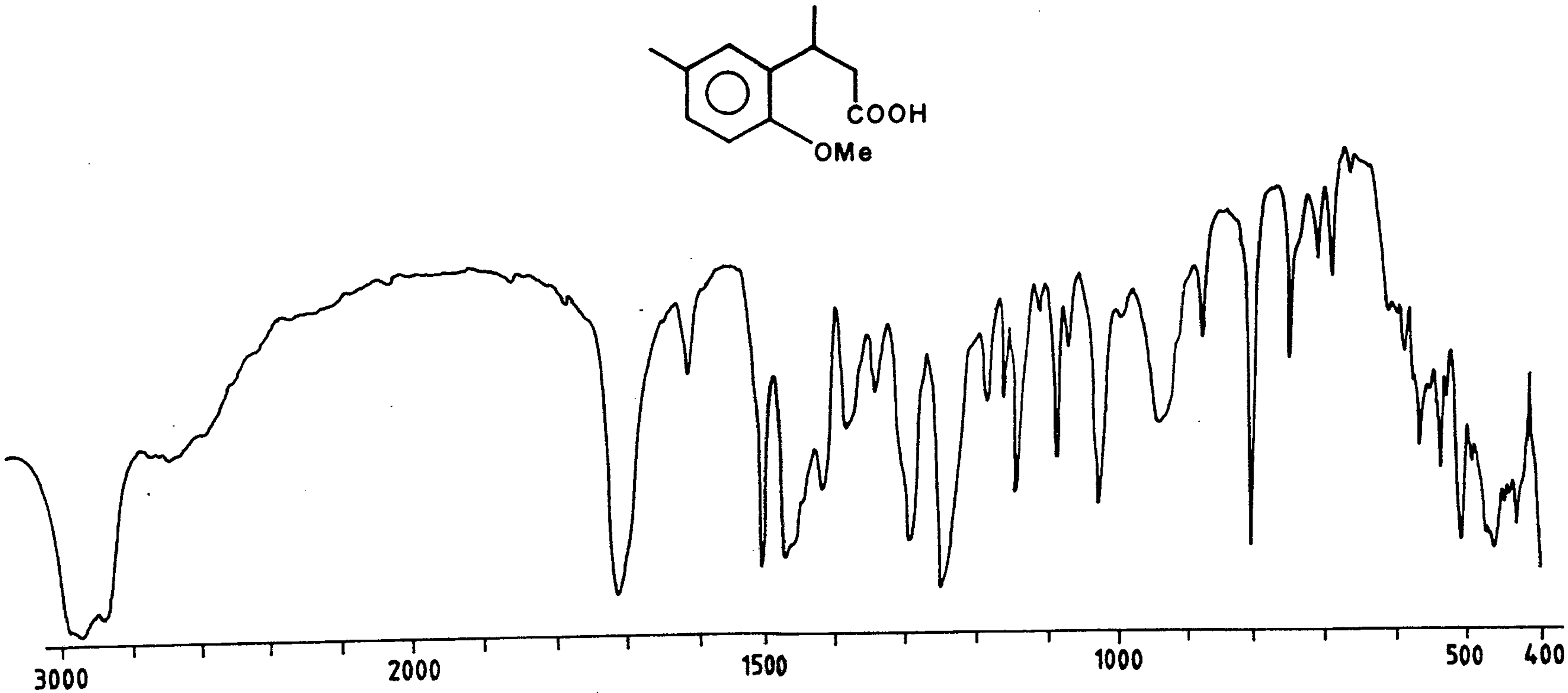


Fig. 14 I·R spectrum of (41)

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CHAPTER-III SECTION-I

BIOACTIVE COMPOUNDS FROM THE PODS OF *ACANTHUS ILLICIFOLIUS* LINN.

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Certain shrubs and trees of the families Rhizophoraceae, Verbenaceae, Sonneratiaceae and Arecaceae (Palmae) that grow in dense thickets or forests along tidal estuaries, in salt marshes, on muddy coasts and characteristically have prop roots are grouped under the general name mangroves. These are further subdivided in two classes, red and black mangroves. Some of the common mangrove plants are listed below:

Plant	Family
Rhizophora mangle	Rhizophoraceae
Rhizophora mucronata	"
Avicennia marina (A. nitada)	Verbenaceae
Sonneratia caseolaris	Sonneratiaceae
Nypa fruticans	Arecaceae
Acanthus illicifolius	Acanthaceae

The most characteristic property of mangroves is their high rate of primary productivity. Mangroves provide a substantial input of detrital material in the surrounding ecosystem. This detrital material is an important source of particulate and soluble organics. The information about the nature of organics arising from mangroves is available through the work of Wannigrama et al[1] who reported composition of the lipid portion of leaves and pneumatophores of Avicennia marina. It has been shown that mangroves are rich sources of fatty acids. Hedges et al[2,3] have shown the presence of p-coumaric acid in higher concentrations in Avicennia germminans. Recently, Hogg and Gilan[4] have studied the composition of fresh leaves of eleven species of mangrove and reported the characterisation of sterols and fatty acids. Ghosh and co-workers[5] have reported the isolation and characterisation of triterpenoids and steroids from seven mangrove species found in Sunderban mangrove forest*.

The family Acanthaceae consists of 240 genera and about 2000 species. Of these, plant species belonging to only 24 genera are listed under medicinal plants. One of them is Acanthus illicifolius[6,7,8] which is an evergreen spiny herb found on the sea coasts of India, Ceylon, Malaysia, Philippines, Australia, South Africa. The plant is medicinally useful and is reported to be effective in treatment of paralysis and asthama[6,9]. The leaves of this plant are recorded to be used for fomentation in rheumatism and neuralgia[6]. Due to these reported medicinal properties, different parts of this mangrove plant have been subjected to chemical investigation. Incidentally, all the publications on the chemical constituents of Acanthus illicifolius have appeared after 1980. This plant occurs extensively, fringing

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* West Bengal, India

the tidal creeks of the Mandovi-Zuari estuaries in Goa. The previous findings on A. illicifolius are summerised in Table 1. While some useful information is available on the chemical constituents of the leaves, roots and the whole plant, the pods of the A. illicifolius were not subjected to chemical investigations.

During our search for the biologically active compounds from marine sources, we had an occasion to test the crude methanolic extract of the pods of A. illicifolius for its biological activity. The extract exhibited significant analgesic activity as well as anti-inflammatory activity in carrageenin induced oedema in rats[10]. Further efforts led us to locate the analgesic activity in chloroform soluble and antiinflammatory activity in both chloroform and water soluble fractions*

Chloroform soluble and water soluble fractions were therefore subjected to chromatography over silica gel. Elution with petroleum ether and progressively increasing the polarity with ethyl acetate yielded a crystalline solid, designated as compound-A, m.p. 138°. Compound-A was identified as benzoxazolin-2-one (1) on the basis of evidence presented below.

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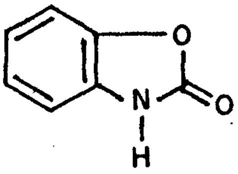
* details of the testing are given in the experimental part.

TABLE-1

CHEMICAL CONSTITUENTS OF *ACANTHUS ILLICIFOLIUS LINN*

Sl No.	Part of the Plant	Chemical constituents	Ref
1.	Whole plant	Acanthicifoline	30
2.	Whole plant	Oleanolic acid, β-sitosterol, Lupeol, Quercetin, Quercetin-3-O-β-D-glucopyranoside, Trigonelline.	31
3.	Roots	α-L-arabinofuranosyl-(1→4)-β-D-glucuronopyranosyl (1→3)]-3β-hydroxy-lup-20 (29)-ene.	32
4.	Roots	Octacosyl alcohol, stigmasterol, Benzoxazoline-2-one, stigmasteryl-β-D-glucopyranoside	15
5.	Leaves	2-Benzoxazolinone	14
6.	Leaves	Fatty acids, sterols, Hydrocarbons	4
7.	Leaves	Cholesterol, campesterol, stigmasterol, sitosterol, stigmast-7-en-3β-ol, α & β amyrin, Lupeol, Oleanolic acid, Ursolic acid.	5
8.	Leaves	Methylapigenin 7-O-β-D-glucopyranuronate, Apigenin 7-O-glucuronide.	33

The IR spectrum of compound-A (Fig.1) showed bands at 3560 (-NH), 1750 ($>$C=0), 1275(C-O-C stretching) and 1480, 1375 and 1015 cm^{-1} (characteristic of oxazole ring system). Its ^{1}HNMR spectrum (Fig.2) showed a 4H multiplet at 7.1-7.2 due to aromatic hydrogens and broad one proton signal at 10.1 due to oxazole hydrogen. Its EI-mass spectrum (Fig.3) showed molecular ion at m/z 135 which is also the base peak. Other intense peaks are observed at m/z 91, m/z 79 and m/z 64[11]. All the spectral data suggested that compound-A must be benzoxazolin-2-one (1) commonly known as BOA. We have measured its ^{13}CNMR spectrum and since no earlier information is available on this , it is reproduced in (Fig. 4). The assignments are as follows:



(I)

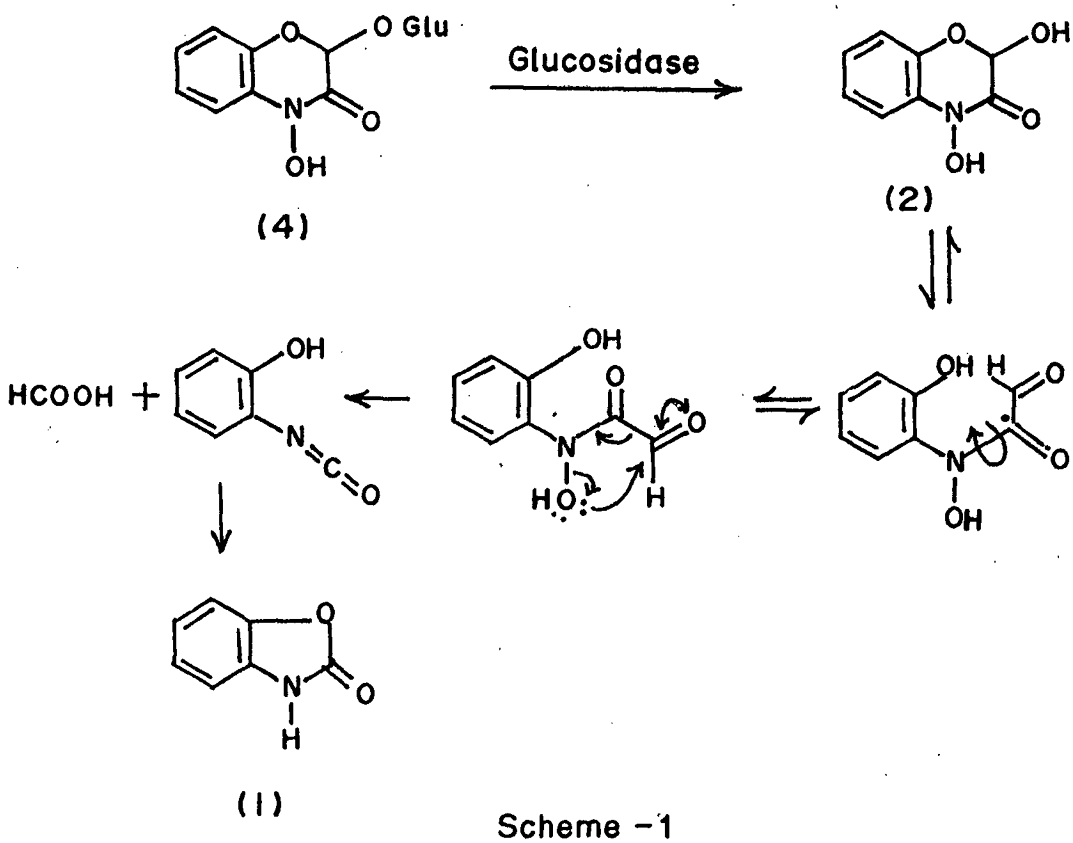
Carbon No.	Chemical shift
C-2	157.0
C-4	110.7*
C-5	110.5*
C-6	123.0**
C-7	124.0**
C-8	145.0
C-9	131.0

*,** - assignments interchangeable

The identity of compound-A with BOA (1) was further established by the preparation of latter by the known synthetic procedure[12,13]. Direct comparison established their identity.

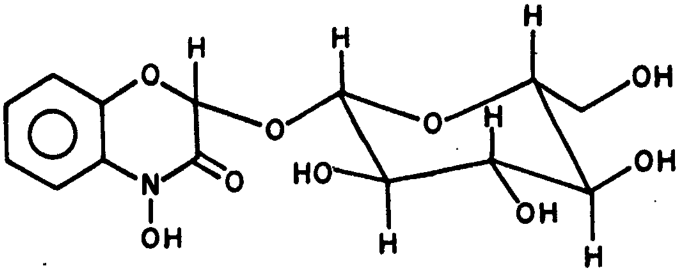
Prior to this report, BOA (1) has been isolated from the leaves[14], roots[15] of A. illicifolius. There have been reports of isolation of BOA (1) and its derivatives from other sources also[16]. It was first discovered in nature by Virtanen and Hietala[17] as an anti-fusarium factor in rye-seedlings (Secale cereale). BOA (1) has been extensively investigated for medicinal value[18] as CNS depressant, analgesic, antipyretic, anticonvulsant, hypnotic and muscle relaxant activities and antiinflammatory activity[19]. Furthermore there are reports[18-20] that ribose derivative of BOA (1) is active as anti-cancer and anti-viral agent. In view of the medicinal properties refered above isolation of BOA (1) from the pods of A. illicifolius is of special significance. It may be noted that the concentration of BOA (1) in the pods of A.illicifolius is higher than leaves.

It has been shown earlier that BOA (1) is formed when 2,4-dihydroxy-1,4-benzoxazin-3-one (2,DIBOA) is heated and BOA (1) and DIBOA (2) invariably occur together[21-23]. Moreover, DIBOA (2) is derived by enzymatic hydrolysis of the glucoside (4). These conversions which take place during the process of isolation are summarized in scheme-1.



Scheme -1

Although, BOA (1) was isolated before from the leaves and roots of A. illicifolius, there was no report on the isolation of DIBOA (2) or the glucoside (4) from the source. The reported co-occurrence of BOA (1), DIBOA (2), DIBOA-Glu (4) in the plants of the family Gramineae[24-28] and the mangrove plant Acanthus mollis of the family Acanthaceae[29] suggested that the various organs of A. illicifolius should also contain DIBOA (2), DIBOA-Glu (4) and related ompounds. The chloroform soluble extract of the pods of A. illicifolius did not show the presence of DIBOA (2), DIBOA-Glu (4) and hence efforts were made to detect their presence in the water



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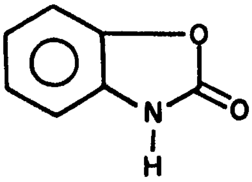
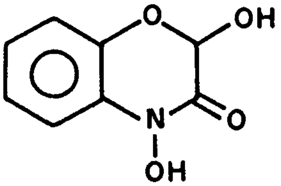
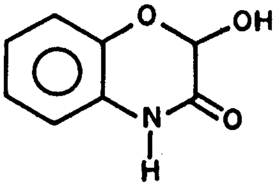


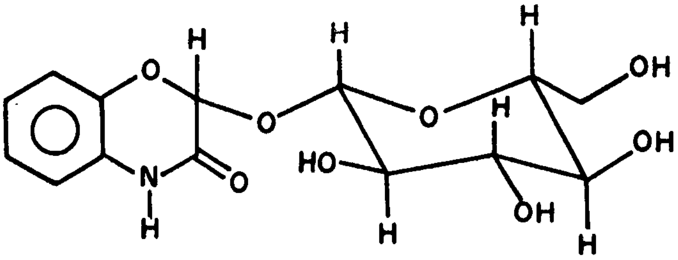
(1)



(2)



(3)



(5)

soluble fraction. Indeed, we succeeded in confirming their presence in this fraction by actual isolation of DIBOA-Glu (4) and its reduction product HBOA-Glu (5). Chromatography of the active water soluble fraction over silica gel and elution with chloroform: methanol (80:20) afforded a solid fraction, m.p. 214^{o} which appearead to be homogeneous on T.L.C. This solid fraction refered to as fraction-B was found to be heat sensitive and hence subjected to desorption chemical ionisation (DCI) mass spectrometry which is an useful tool for molecular weight determinations of non-volatile and thermally labile compounds. DCI spectra were recorded by using NH_3, ND_3 and methane as the reagent gas.

Ammonia desorption chemical ionisation (DCI) mass spectra are known to give useful information because intense $(M + NH_4)^+$ ions are formed in most cases. NH_3 - DCI mass spectrum of fraction-B showed it to be a mixture as it showed two intense ions at m/z 345 $(M_1 + NH_4)^+$ and m/z 361 $(M_2 + NH_4)^+$. Therefore fraction-B which contains compounds with molecular weights 327 (M_1) and 343 (M_2) are now named as B_1 and B_2 respectively. The NH_3-DCI mass spectrum of fraction-B is shown in (Fig. 5). The peak observed at m/z 198 is characteristic of the fragment $(C_6H_{12}O_6 + NH_4)^+$ and suggested that compounds B_1 and B_2 are glucosides. This possibility was further substantiated by the presence of peaks at m/z 180 $(C_6H_{10}O_5 + NH_4)^+$ and 162 $(C_6H_8O_4 + NH_4)^+$ resulting due to consecutive eliminations of water molecules from the carbohydrate moiety. Further support came from the ND_3-DCI mass spectrum (Fig. 6) of fraction-B, wherein the peaks at m/z 345 and 361 (present in NH_3-DCI spectrum) were now observed at m/z 349 and 365 corresponding to $(M_1 + ND_4)^+$ and $M_2 + ND_4)^+$ respectively. Besides cluster of ions were observed from m/z 349 to 354 and m/z 365 to 370 revealing the presence of five acidic hydrogens in B_1 and B_2. The acidic hydrogens are known to get exchanged with deuterium in the ion source when deuterated ammonia (ND_3) is employed as the reagent gas.

Methane-DCI mass spectra usually show two molecular ions which are due to $(M + H)^+$ and $(M + C_2H_5)^+$ ions. CH_4-DCI mass spectrum (Fig. 7) of fraction-B while confirmed the presence of B_1 and B_2 with molecular weights 327 and 343 [molecular ions m/z 328 $(M_1 + H)^+$, 356 $(M_1 + C_2H_5)^+$ and m/z 344 $(M_2 + H)^+$, 372 $(M_2 + C_2H_5)^+$] respectively. Curious features of this spectrum were intense peaks at m/z 166 and 182. The origin of these peaks (166 & 182) is still obscure but these could be molecular ions, $(M_3 + H)^+$ and $(M_4 + H)^+$ suggesting the presence of two more compounds B_3 and B_4 with molecular weights 165 & 181 in fraction-B. The aglucones (2 & 3) respectively of DIBOA-Glu (4), and HBOA-Glu (5) if present in the fraction-B can account for these peaks*.

Final proof for the presence of HBOA-Glu (5) and DIBOA-Glu (4) in fraction-B was obtained by actual isolation of compounds B_1, m.p. 158-60°C and B_2, m.p. 200° in pure form and establishing their identity with HBOA-Glu (5) and DIBOA-Glu (4).

Fraction-B which was obtained by chromatography of water soluble portion of the pods was subjected to careful chromatography over silica gel. Elution of the column with $MeOH-CHCl_3$ and increasing the concentration of MeOH, afforded two compounds B_1 (less polar) and B_2 (more polar) in pure form.

Compound-B_1, m.p. 158-60°: It showed uv absorption at λ max 255 with sh. at 286 nm. Its IR (Fig. 8) showed an intense absorption at 860 cm^{-1} indicative of β linkage between the aglycone and sugar moiety. The presence of three absorption at 1000, 1025, 1060 suggest pyranose structure of glucose. Its ^{1}HNMR (Fig. 9) showed a multiplet in the region 3.1-3.9 for 6H of glucose. A doublet at 4.8 with a coupling constant of 7.7Hz was assigned to the anomeric proton. This

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* $(M_3 + C_2H_5)$ and $(M_4 + C_2H_5)^+$ are not clearly seen and hence presence of aglucones (2 & 3) in fraction-B could not be infered with certainity.

coupling constant (J=7.7Hz) requires that $C_{1'}$ and $C_{2'}$ protons of the hexose to be trans diaxial suggesting a β-glucopyranoside. ^{1}HNMR also showed singlet at 5.9 assigned to the CH methine proton at C-2 and a broad multiplet at 7.1 integrated for 3H alongwith a broad singlet for 1H at 7.4 were attributed to the aromatic protons. A sharp singlet at 4.6 is due to HDO*.

Its ^{13}C NMR spectrum (Fig.10) showed signals which were assigned (Table-2) to different carbon in B_1 on the basis of co-relation with the reported values for related compounds. The additional signal observed at 171.7 has been assigned to C-3 of the tautomeric form of B_1.

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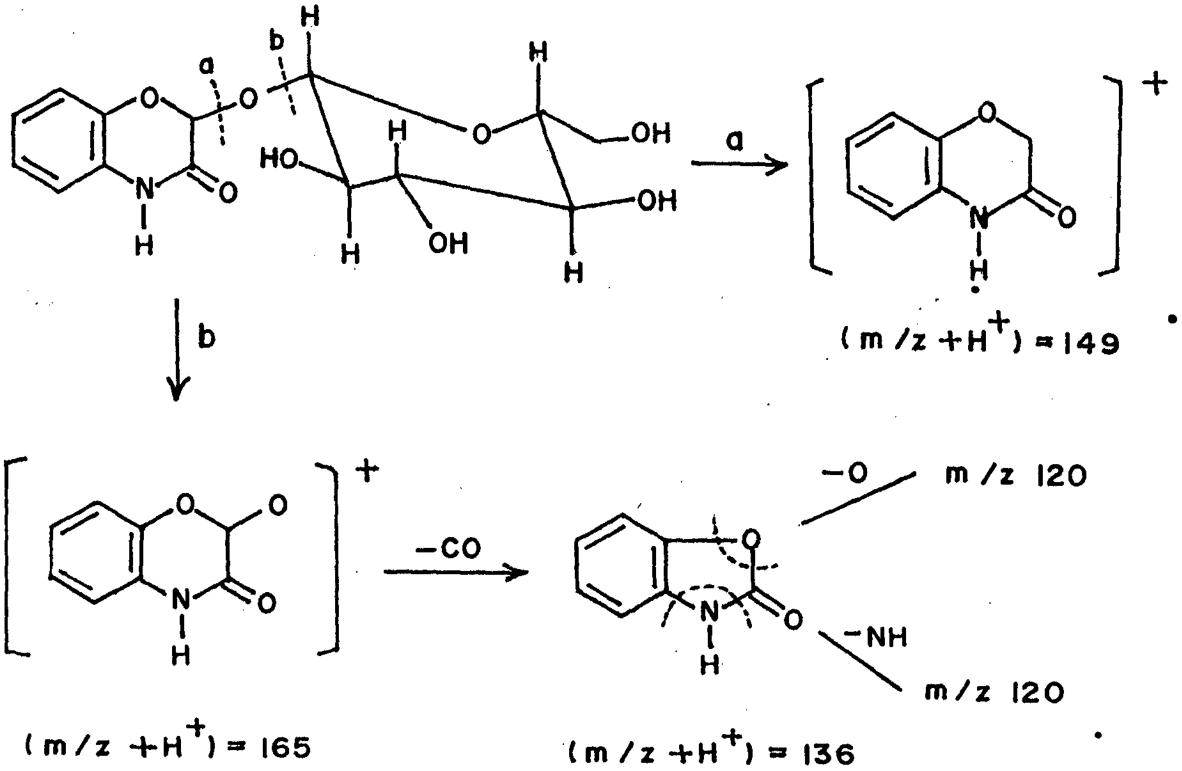
* D_2O was used as a solvent.

Table-2

^{13}CNMR chemical shifts of compound-B_1 in D_2O

C	ppm
2	164.75
3	173.99
5	119.18
6	127.61
7	126.54
8	104.59
9	120.42
10	142.50
1'	97.95
2'	75.54
3'	79.01
4'	71.88
5'	78.18
6'	63.28

The EI (electron impact) mass spectrum of compound-B_1 is characterized by a molecular ion peak at m/z 327 and a number of fragments. The probable genesis of some of the major fragments is shown in scheme-2.



Scheme - 2

<u>Compound</u>-B$_2$, m.p. 200°C: Its uv spectrum (λ max, MeOH) 255, 280 nm sh. showed striking similarity to that of compound-B$_1$. Its IR (Fig. 11) showed absorption at 830 cm^{-1} indicative of B-linkage between the aglycone and sugar moiety. The three absorptions at 980, 1020 and 1070 cm^{-1} were indicative of pyranose structure of glucose. Its IR spectrum also showed peaks of variable intensities at 1660 , 1600, 1455, 1375, 1300, 1270, 1210, 1150, 1130, 880 and 840 cm^{-1}. Its ^{1}HNMR

(Fig. 12) showed a multiplet in the region 3.0 - 3.8 assigned to glucose protons. The anomeric proton was evident from a doublet at 4.55(J=7.7Hz). The signal at 5.83 was attributed to methine H-2 proton. The aromatic protons were evident from the broad singlet at 7.3 and multiplet at 6.95.

Its (EI-MS) mass spectrum showed M^+ 343 besides fragments at m/z 327, 181, 165, 152, 149, 148, 136, 135, 109, 108, 80, 79 and 73. It is evident from the spectral data that compounds B_1 and B_2 are structurally very closely related. Their molecular weight differs by 16 mass units. Compound-B_2 must therefore contain -OH grouping. To locate the position of the -OH group, uv spectrum was measured after the addition of 0.1N NaOH and 0.1N HCl. It neither showed bathochromic nor hypsochromic shift. The hydroxyl is not phenolic , and therefore must be attached to nitrogen of the basic HBOA-Glu (5) structure. It was therefore possible to assign structure (4) for compound-B_2 and hence compound-B_2 must be identical with DIBOA-Glu (4), isolated earlier from rye seedling. The spectral data recorded on compound-B_2 showed excellent agreement with those recorded on DIBOA-Glu (4). Due to non-availability of the copies of the spectral data, it was not possible to make a direct comparison. It has not been possible so far to isolate the aglucones (2 & 3) from the chloroform soluble or water soluble fractions but efforts are being made to locate and isolate these compounds.

CHAPTER-III SECTION-II

5α-CHOLESTANE-3,6-DIONE, AN ANTIBACTERIAL SUBSTANCE FROM THE RED ALGA *ACANTHOPHORA SPICIFERA*

Substances from marine sources especially seaweeds have been used in folk medicine all over the world since ancient times. However, the development of modern chemical, pharmacological and engineering technology has facilitated the investigations and exploitation of hitherto untapped drug resource of the oceans. During last three decades, several bioactive compounds have been isolated from marine sources which include a wide spectrum of chemical metabolites e.g. organic acids, carbohydrates, proteins, amino acids, steroids, lipids, and enzymes.

Notable among these are prostaglandins, alginates, kainic acids, cephalosporin and fish toxins like tetradotoxin and saxitoxin. The potential use of many of these compounds as drugs has acted as an incentive for the intensification of research into these compounds and their synthetic analogues.

A great deal of chemical work has been done on Indian seaweeds during the last twenty five years. However, this work was confined mostly to the information on the mineral constituents, carbohydrates with special reference to alginates, agar and carrageenin and other organic chemicals[37]. However, very little is known about the biomedical potential of Indian Ocean.

In order to obtain bioactive compounds from these marine sources a project was initiated at N.I.O. in collaboration with C.D.R.I.*, Lucknow. In connection with this work, we have screened methanolic extracts of a number of marine organisms including the red alga Acanthophora spicifera belonging to the order Ceramiales and family Rhodomelaceae, for different pharmacological activities. This red alga has been reported to possess *in vitro* antimicrobial activity against *S. aureus, C.albicans* and *myobacterium smeqmates*[38]. *Acanthophora spicifera* (Vahl) Boergeesen is generally found in tropical and subtropical oceans. It was collected from the intertidal zone of Anjuna beach (Goa, west coast of India) during premonsoon period washed, air dried and powdered. About 3 kg of this powder was extracted thrice with 90% aqueous methanol. The combined extract was concentrated at reduced pressure to yield crude methanolic extract which was sent to C.D.R.I for screening.

Acanthophora spicifera has been extensively investigated for its mineral content[39-41] but the literature on the isolation of specific organic constituents is scanty[42]. There being some studies on its evaluation for polysaccharides, proteins, amino acids[43-45], fatty acids, lipids[45-47] content. Tocopherol content has been reported by Jayasree et al[48] and its ascorbic acid and dehydro ascorbic acid by Qasim and Barkati[49]. The screening results indicated that the crude extract exhibited promising (100%) -

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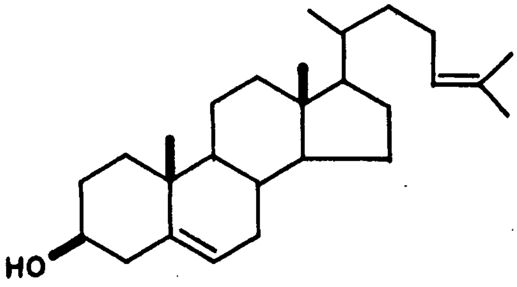
* Central Drugs Research Institute

antifertility activity[50]. Follow-up study resulted in the location of the activity in petroleum ether and chloroform soluble fractions. In this section, we wish to report the results concerning the isolation and identification of a steroid, 5α-cholestane-3,6-dione (6a) from the active chloroform soluble fraction.

The marine sterols of the majority of red algae are C_{27} compounds, cholesterol (7) being the major component of most of them although in a few species desmosterol (8) has been reported to be present in substantial amount and may even be the major steroid[51-53].

Red algae also contains, though in minor amounts, C_{26},C_{28} and C_{29} sterols and in some species the presence of Δ^4 Cholestene-3-one (9) has been reported[54,55].

Since the active principle of <u>Acanthophora spicifera</u> was located in petroleum ether and $CHCl_3$ soluble fractions, air dried seaweed (7 Kg) was extracted with $CHCl_3$ and this extract was chromatographed over silica gel and the column eluted successively with solvents of increasing polarity. From the EtOAc-petroleum ether (10:90) eluents, a crystalline compound, m.p. 168-170° was isolated which appeared to be pure (TLC. single spot, Golden yellow colour with 2,4-dinitrophenyl hydrazine as spray reagent). Its ketonic nature was evident from the strong IR (Fig. 14) absorption at



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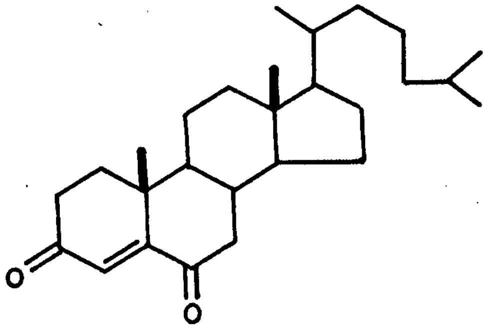

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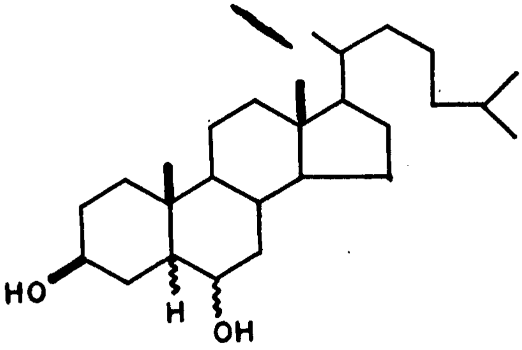

(15a) ; $C_5 = \alpha$H, $C_6 = \alpha$OH

(15b) ; $C_5 = \beta$H, $C_6 = \beta$OH

γ max 1700 cm^{-1}, there was no ^{1}HNMR evidence for an aldehyde proton and no IR band for an hydroxyl group. It also showed in the IR spectrum the presence of $-\overset{O}{\overset{\|}{C}}-CH_2$ functionality (γ max 1410 cm^{-1}).

The ^{1}HNMR (Fig. 15) spectrum of the compound revealed that it was a steroid but it gave no reaction with the Lieberman-Burchard test thus indicating the absence of a 3β-OH group. It displayed signals at 0.68(3H,H_{18}) and 0.98 (3H, H_{19}) for the two tertiary methyls, a signal at 0.7 was attributed to the secondary methyl at C_{21} and a signal for six protons at 0.85 was assigned to the secondary methyls situated at the end of side chain. Moreover, the spectrum demonstrated signals for seven protons resonating between 2.1-2.6 probably alpha to the $>C=O$ group. Signals due to olefinic hydrogen were absent in the ^{1}HNMR spectrum. The presence of two ketonic groups was evident from its ^{13}CNMR (Fig. 16) spectrum which displayed signals for this functionality at 208 and 210. In view of this data, it was concluded that the compound is a saturated keto-steroid.

The mass spectrum exhibited a base peak molecular ion at m/z 400 $(M)^{+}$ corresponding to molecular formula $C_{27}H_{44}O_2$ further confirming the saturated nature of the compound and the tetracyclic carbon skeleton with two ketonic groups. The mass spectrum also showed prominent peaks at m/z 385, 371, 287, 260, 246, 244, 231, 149, 137, 123, 109, 94, 79, 69 and

55. The peaks at m/z 287 (loss of side chain); 260, 246, (ring D cleavage); 109 (ring C cleavage); the peaks at 149 associated with fragments at 137 and 123 and 55 were indicative of a steroid belonging to the cholestane series with the two carbonyl groups in rings A and B. Since cholesterol is a precursor of this type of compound, one of the carbonyl group was placed at C_3 and the other would be either at C_6 or C_7. As the melting point (168-70°) of the natural product agreed well with that reported[56] for 5α-cholestan-3,6-dione (6a) (m.p. 171-72°), and since the mass spectrum did not show peaks at m/z 135 and 178 which are characteristic of 7-ketones[57], the second carbonyl was placed at C_6. The sterochemistry at C_5 was infered as α from the presence of a prominent peak at m/z 371(M^+-29)[58]. 3,6-diketo-5β-steroids are known to show peaks at (M^+-70) and (M^+-96). No fragments corresponding to m/z 330 (M^+-70) and 304 (M^+-96) were observed in the mass spectrum of the diketosteroid from <u>A. spicifera</u>. The natural product was, therefore identified as 3,6-diketocholestane (6). The genesis of various fragments in the mass spectrum of 5α-cholestane-3,6-dione (6a) are shown in chart-1.

A definite proof of the assigned structure came finally from its synthesis by a known procedure[59]. Cholesterol (7) was subjected to hydroboration oxidation to yield a mixture of 5α-cholestane-3β, 6α-diol (15a, 70%) and 5β-cholestane-3β,6β-diol (15b, 16%). Column chromatography over silica

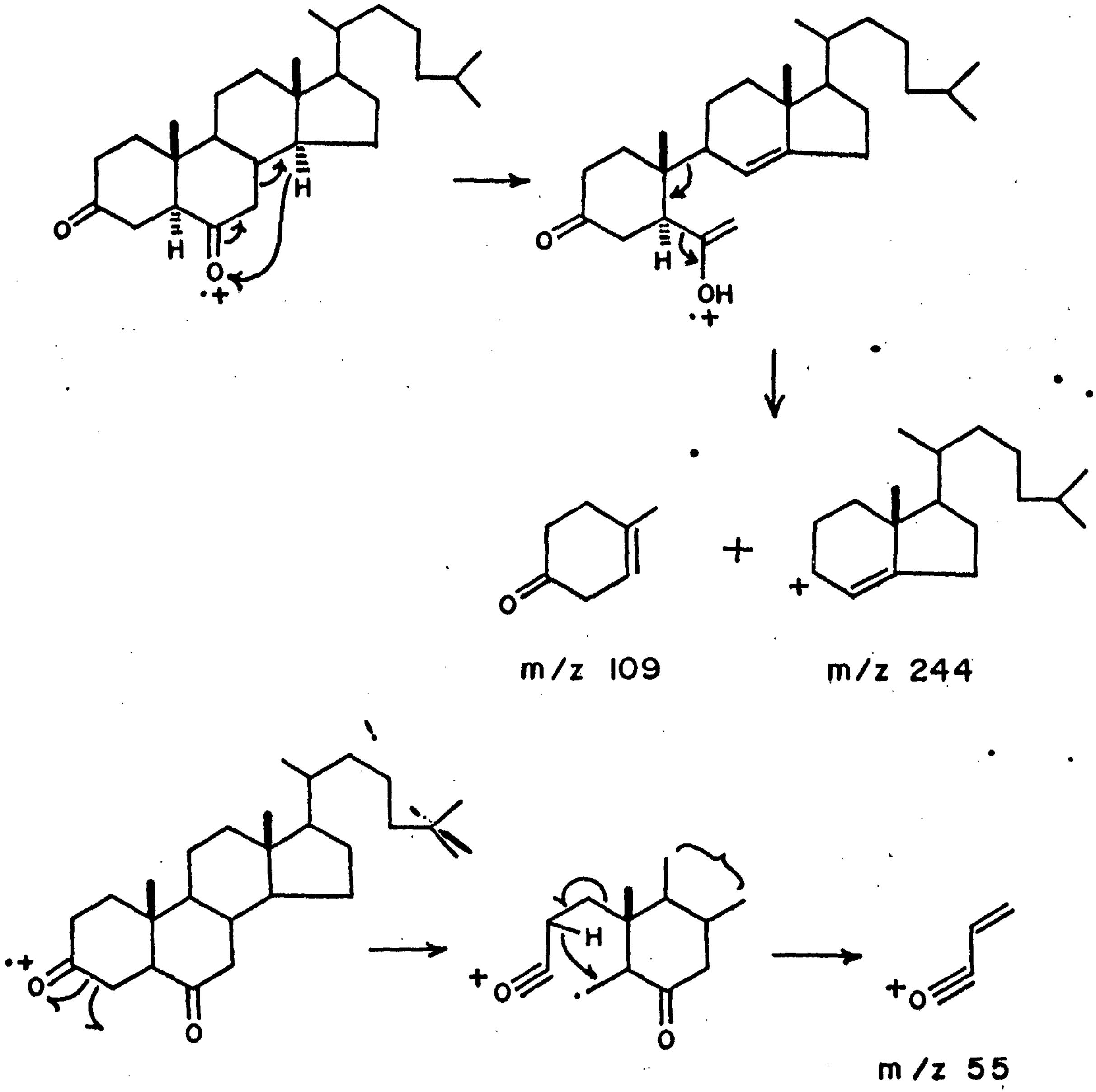

Chart -1

gel gave 5α-cholestane-3β,6α-diol, (15a), m.p. 213° which on oxidation with Sarett's reagent afforded 5α-cholestane-3,6-dione (6a), m.p. 171°, identical in all respects (Co TLC, IR, m.p., mix m.p. and ^{1}HNMR) with the natural diketone isolated from the red alga <u>Acanthophora</u> <u>spicifera</u>. Cholesterol (7) was subjected to dichromate oxidation by known method[60] to furnish Δ^4-cholestene-3,6-dione (14), which was reduced by zinc and acetic acid to give 5α-cholestan-3,6-dione (6a) and its 5β isomer (6b). A pure sample of 5α-cholestan-3,6-dione (6a) could be obtained by careful chromatography.

An interesting feature which has been observed here is the relatively low concentrations of cholesterol present in the alga. This can be explained by it being utilized in the biosynthesis of the keto steroid. Also the presence of oxygenated sterols is known to inhibit cholesterol synthesis[61]. It may be pointed out that this is the first report of the isolation of 5α-cholestane-3,6-dione (6a) from <u>Acanthophora</u> <u>spicifera</u>. To the best of our knowledge this substance has not been isolated before from any other source.

Besides the diketone mentioned above cholesterol (7), cholest-4-en-3-one (9), 11α-hydroxy-5α-cholestane-3,6-dione (10)[62] and aurantiamide (11), its acetate (12) and diaaurantiamide acetate (13)[63] have also been isolated from the same source*.

Characterisation of trace constituents of the chloroform soluble fraction is in progress.

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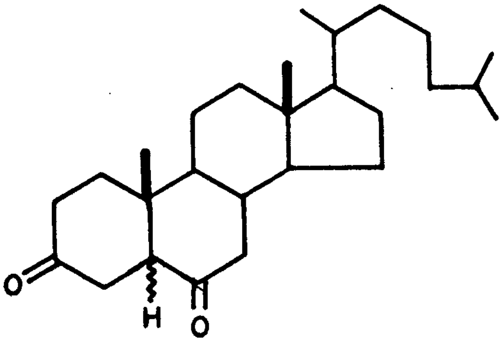
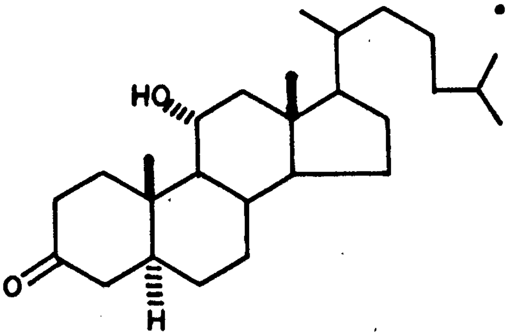
* Isolation and characterisation of these constituents has been carried out by a research colleague and hence the details are not given.



(6a): $C_5 = \alpha H$

(6b); $C_5 = \beta H$



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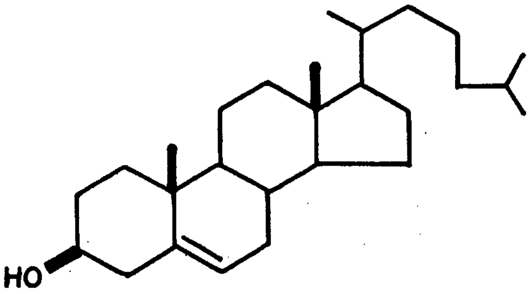

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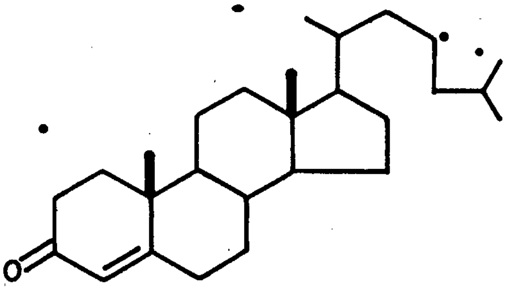

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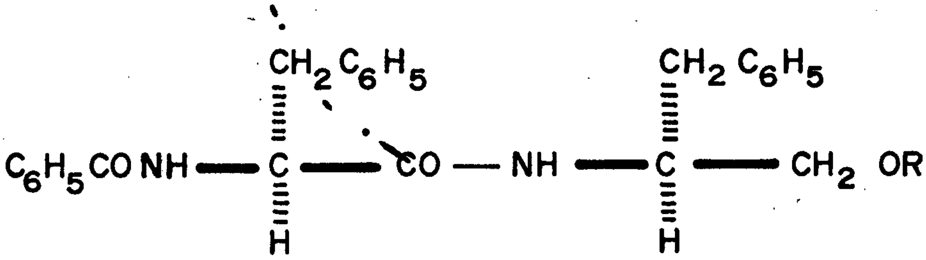
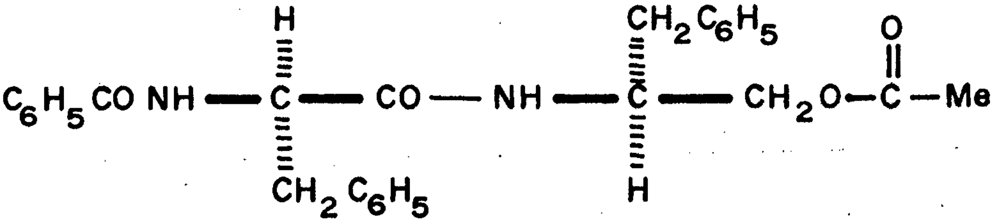


$C_6H_5CONH-\underset{H}{\overset{CH_2C_6H_5}{C}}-CO-NH-\underset{H}{\overset{CH_2C_6H_5}{C}}-CH_2OR$

(12), R = COMe

(11), R = H



$C_6H_5CONH-\underset{CH_2C_6H_5}{\overset{H}{C}}-CO-NH-\underset{H}{\overset{CH_2C_6H_5}{C}}-CH_2O-\overset{O}{\overset{\|}{C}}-Me$

(13)

Constituents of red alga *Acanthophora spicifera*

EXPERIMENTAL

Preparation of the extract

Fresh *Acanthus illicifolius* pods (5 kg) were collected from the banks of Cumbarjua canal connecting the two estuaries of the river Mandovi and Zuari during the month of May. As such the pods were soaked in 90% aq. methanol at room temp. for 72 hrs. This process was repeated thrice and the combined extracts were concentrated to dryness under reduced pressure at 50°C. The concentrated extract was kept in a vacuum descicator for final drying, before it was tested for pharmacological activity.

Analgesic activity

This activity was studied in albino mice (Haffkine strain) of either sex weighing 25-30 g. Six mice fasted overnight were used per group.

Tail clip method

An artery clip with the jams covered with thin rubber tubing was applied to the base of the tail of the mouse. Only those animals which made repeated attempts to remove the clip within 15 seconds were used. Absence of any attempt to remove the clip within 30 seconds was taken as criterion of analgesia. Pethidine hydrochloride was used as a standard for comparison. Different doses of the extract or pethidine were administered intraperitoneally (IP) 30 min before application of the clip.

Writhing method

An aqueous solution (v/v) of hydrochloric acid 0.5% was administered IP in the dose of 0.2 ml per mouse. The animals were observed continuously for 20 minutes for writhing syndrome. Sodium salicyclate was used as a standard for comparison. Different doses of the extract or sodium salicylate were administered IP 30 minutes before the injection of HCl and the percent protective effect was noted.

Anti-inflammatory activity

This activity of the extract was studied in group of 6 albino rats (Haffkine strain) of either sex weighing 150 - 200 g. Oedema was induced by injection of 0.1 ml of 1% carrageenin into the hind paws of the rats according to the method of Winter et al[34,35]. The paw volume was measured before and 3 hr after the injection of carrageenin by the plethysmographic method of Buttle et al[36]. Phenylbutazone was used as a standard and saline was used as a control.

Fractionation of the methanolic extract

The crude methanolic extract was fractionated into petroleum ether, chloroform, n-butanol and water soluble fractions. The follow-up investigations led us to locate the analgesic activity in chloroform soluble fraction and water soluble fraction.

Isolation of BOA (1)

The chloroform fraction dissolved in a minimum amount of chloroform adsorbed over silica gel, was placed on a chromatographic column containing silica in petroleum ether and eluted with progressively increasing polar mixture of petroleum ether - EtOAc. Fractions of 20 ml each were collected. Elution of the column with petroleum ether: EtOAc (90:10) yielded a slightly brownish crystalline compound which on repeated chromatography afforded colourless crystals of BOA (1), (688 mg, m.p. 138°C).

IR, γ max, Nujol, (Fig. 1): 3560, 2965, 1750, 1480, 1375, 1325, 1275, 1158, 1015, 960, 900, 850, 765, 750 and 710. cm^{-1}

^{1}HNMR: (200 MHz, δ, $CDCl_3$) (Fig.2) : 7.1-7.2(4H,m), 10.1(1H,s)

Mass spectrum : EI-MS, 70 ev (Fig.3)-M^+ 135 m/z 91, 79 and 64.

^{13}CNMR (200 MHz, $CDCl_3$) : 110.1, 110.2, 122.7, 124.2, 129.52, 143.99, and 156.2.

Synthesis of BOA (1)

O-aminophenol (1 g) and urea (0.6 g) were refluxed for 14 hr in dry pyridine. Usual work-up, afforded after chromatography BOA (1, 0.98 g, m.p. 138^{o}), identical in all respect with the natural product.

Isolation of glucosides

The aqueous fraction was chromatographed over silica gel filled in $CHCl_3$ and eluted with $CHCl_3$, MeOH: $CHCl_3$ and H_2O:MeOH. The MeOH:$CHCl_3$ eluent was rechromatographed, being eluted with increasing concentration of MeOH in $CHCl_3$. The fraction eluted in MeOH: $CHCl_3$ (20:80), gave a crystalline solid (B, 1.567 g, m.p. 214^{o}C)

TLC solvent system MeOH:$CHCl_3$ (40:60). Spots were visualized with iodine vapour and vanilline: H_2SO_4 . Surprisingly when a fluorescent TLC plate was used an additional compound was obtained which could be visualized only with fluorescent TLC plate and under short wavelength (254 nm) uv radiation, as purplish spot on greenish back ground.

The solid B on DCI indicated it to be a mixture of two major compounds. Separated by careful column chromatography over silica gel using gradually increasing concentration of MeOH: $CHCl_3$. The less polar was designated as B_1 (m.p. 158-60^{o}C, with decomp.) and the more polar one as B_2 (m.p. 200^{o}C)

Compound-B_1, (5)

UV MeOH, λmax 255, 286 nm.

IR, ν max, Nujol (Fig. 8): 3600, 2900, 1640, 1610, 1510, 1440, 1320, 1290, 1230, 1060, 1025, 1000 and 860. cm^{-1}

^{1}HNMR (200 MHz, D_2O, δ) (Fig. 9): 7.4(1H,bs), 7.1(3H,m), 5.9(1H,s), 4.8(1H,d,J=7.7Hz), 3.1-3.9(6H,m).

Mass spectrum: (EI-MS, 70 ev) : M^+327 m/z 166, 165, 150, 149, 148, 135, 136, 119, 120, 109, 108, 85, 80, 73, 57, 43 and 31.

^{13}CNMR: (200 MHz, D_2O) (Fig. 10): 174, 164.6, 142.5, 127.6, 126.5, 120.4, 119.2, 104.6, 98, 79.01, 78.18, 75.5, 71.9 and 63.3.

Compound-B_2, (4)

UV meOH, λ max 255, 280 nm.

IR, ν max, nujol (Fig. 11) : 1660, 1680, 1600, 1455, 1375, 1300, 1270, 1210, 1150, 1130, 1070, 1020, 980, 880, 840 and 830. cm^{-1}

^{1}HNMR (200 MHz, D_2O, δ) (Fig 12) 7.3 (1H,bs), 6.95(3H,m), 5.83(1H,s), 4.55(1H,d,J=7.7Hz), 3.0-3.8(6H,m).

Mass spectrum: (EI-MS, 70 ev) M^+ 343, m/z :327, 181, 165, 152, 149, 148, 136, 135, 109, 108, 79, 80 and 73.

^{13}CNMR (200 MHz, D_2O) (Fig. 13) : 143. 110, 128.1, 126.4, 119.962, 116.764, 104.518, 79.021, 78.140, 75.519, 71.875, 63.291 and 63.130.

Preparation of Δ^4-cholestene-3,6-dione (14) from (7)

Commercial cholesterol (7, 12.5 g, 0.0325 mol) was dissolved in benzene (112.5 ml) by warming the solution and AcOH (112.5 ml) was added. The solution was cooled to 20°C. To this solution was added a cold dichromate solution [(112.5 ml), prepared by dissolving $Na_2Cr_2O_7$ (32 g) in AcOH] where upon thick orange paste of cholesteryl chromate $(C_{27}H_{45}O_2)$ CrO_2 separates. It was kept in a refrigerator for 40-48 hr, the temperature soon dropped to 0°C and the dichromate dissolved in a few hr hours.

The resulting brown solution was diluted with H_2O (250 ml) and extracted ~~in~~ with petroleum ether (4 x 50 ml) and was washed well with H_2O. The combined organic layer was extracted with Claisen's alkali (4 x 50 ml) (Claisen's alkali extracts enedione as yellow enolate). The aqueous extract was neutralized by HCl (36%) in cold. The neutral solution was then extracted in ether (4 x 50 ml). The combined ethereal solutions ~~was~~ were washed with Na_2CO_3 solution (5%, 2 x 50 ml) followed by saturated NaCl solution (2 x 20 ml), dried and evaporated to dryness, to give yellow solid (6.5 g, 40%). This was recrystallised in boiling MeOH (50 ml) when Δ^4-

cholestene-3,6-dione separates as thin yellow plates (m.p. 124-125°C, lit. 125°C[60]).

Preparation of 5α-cholestane-3,6-dione (6a) from (14)

A mixture of Δ^4-cholestene-3,6-dione (14, 3 g) and Zn dust (30 g) in glacial AcOH (350 ml) was refluxed for 18 hr. The mixture was diluted with benzene and filtered, the Zn was repeatedly washed with benzene. The filtrate was washed with H_2O, dried and evaporated in vacuo. The product was analysed by TLC and was found to consist of a 3:1 mixture of 5α-cholestane-3,6-dione (6a) and 5β-cholestane-3,6-dione (6b). The products were separated by column chromatography (silica gel). Yield

Preparation of 5α cholestane,3β,6α,diol (15a) from (7)

Diborane was generated by adding a solution of $NaBH_4$ (1.8 g, 47.7 mmol) in diglyme (90 cc) to a solution of $BF_3 \cdot Et_2O$ (15 g, 105.6 mmol) in diglyme (60 cc), under inert atmosphere (N_2). The diborane gas was passed into a solution of commercial cholesterol (7, 3 g) in dry THF (120 cc) during 3 hr by means of slow stream of N_2. After an additional hr at room temperature, H_2O (20 cc) was added to destroy excess diborane. To the alkyl borane thus generated was added NaOH (3N, 25 ml) followed by dropwise addition of H_2O_2 (30%, 25 ml) during 30 min maintaining the temperature throughout at 35°C. After an additional stirring for 1 hr, the reaction mixture was saturated with NaCl (2 g) and the organic layer separated. The aqueous layer was extracted with benzene (3 x 25 ml). The combined organic layer was washed with H_2O and dried, solvent evaporated in vacuo. The product was purified on column chromatography (silica gel), yielded 5α-cholestane-3β, 6α-diol (15a, m.p. 215-217°C lit. m.p. 213-15°[59],70%) and 5β-cholestane-3β, 6β-diol(15b, m.p. 197-199°C, lit 198-200°C[59], 16%).

IR, ν max, nujol(15a): 3350, 2960, 2910, 1475, 1385, 1175, 1065, 1050 and 965. cm^{-1}

<u>Preparation of 5α-cholestane-3,6-dione (6a)from (15a)</u>

CrO_3 (4 g) was added to dry pyridine (100 ml) slowly and with stirring till a solid complex was obtained. Then the compound (15a, 2 g) in pyridine (20 ml) was added dropwise while stirring and the mixture left overnight, poured in cold H_2O, filtered and extracted with benzene (3 x 50 ml). The combined extracts were washed with dil HCl and then with H_2O. Dried and removal of the solvent furnished 5α-cholestane-3,6-dione (6a, m.p. 170°C, lit m.p. 173°C[23]), yield (1.5 g).

IR, ν max, KBr (Fig. 14): 3000, 1730, 1485, 1445, 1405, 1395, 1360, 1350, 1320, 1270, 1250, 1175, 1130, 1100, 1035 and 1000. cm^{-1}

^{1}HNMR (90 MHz, $CDCl_3$, δ) (Fig. 15) : 0.68(3H, C_{18}), 0.7(3H, C_{21}), 0.85(6H, d), 0.98(3H,C_{19}), 2.1-2.6(7H).

^{13}CNMR (100 MHz, $CDCl_3$) (Fig. 16): 211, 209, 57.6, 56.7, 56, 53.6, 46.6, 43, 41, 39, 38.1, 38, 37, 36.9, 36.1, 35.7, 28, 24, 23.8, 22.7, 22.5, 21.7, 18.7, 12.5 and 12.

Mass spectrum : (EI-MS, 70 ev) : $(M)^+$ 400, m/z, 385, 371, 287, 260, 246, 244, 231, 149, 137, 123, 109, 94, 79, 69 and 55.

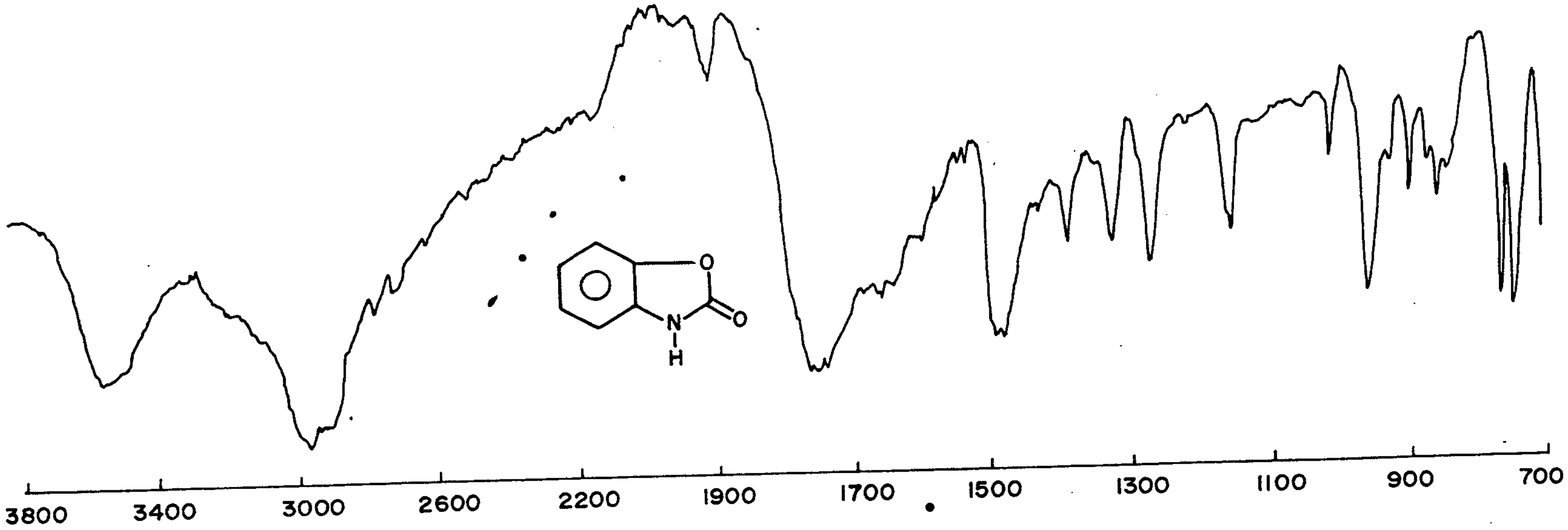


Fig. 1 IR Spectrum of BOA (I)

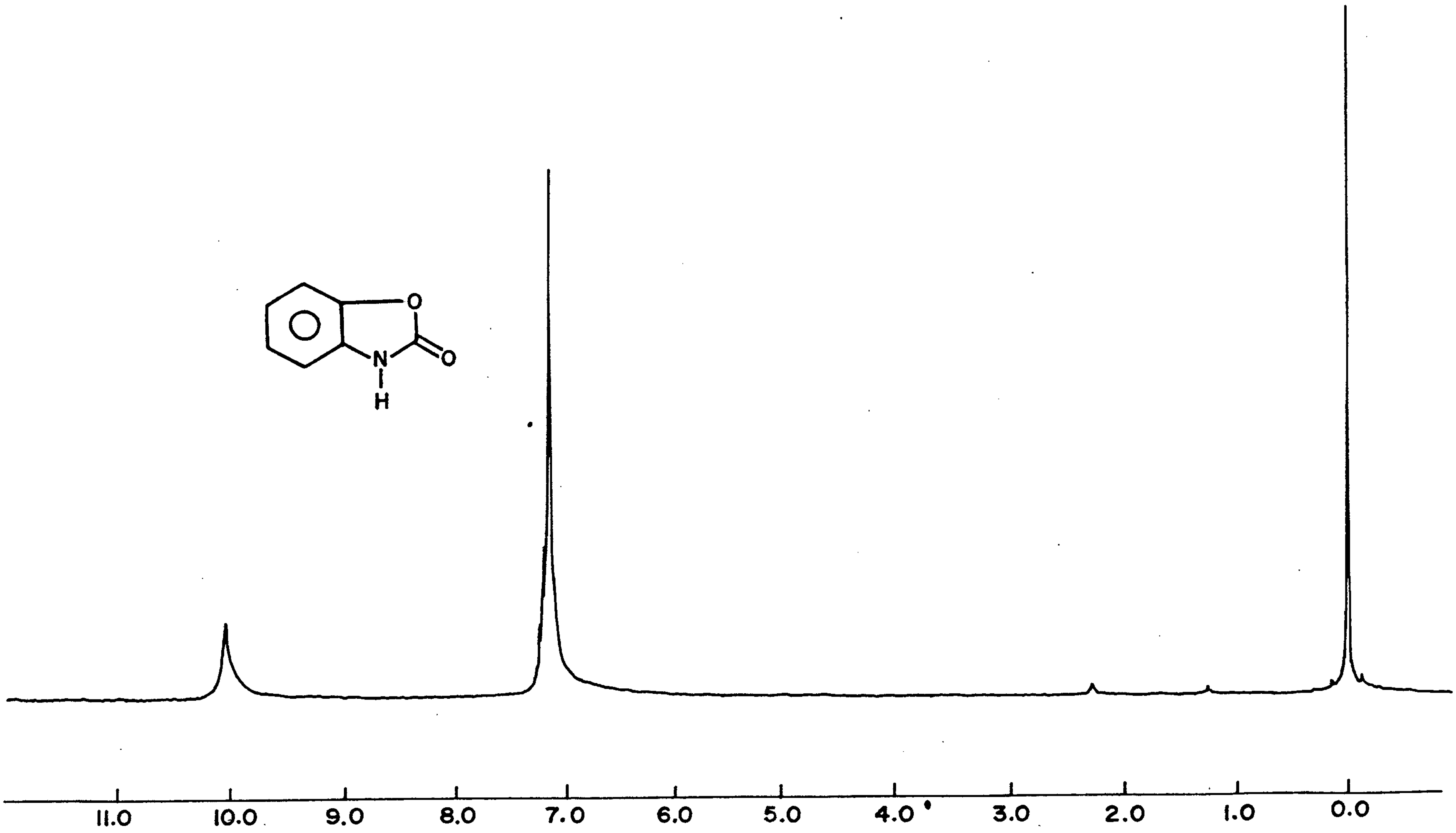


Fig. 2 ^{1}HNMR spectrum of BOA (I)

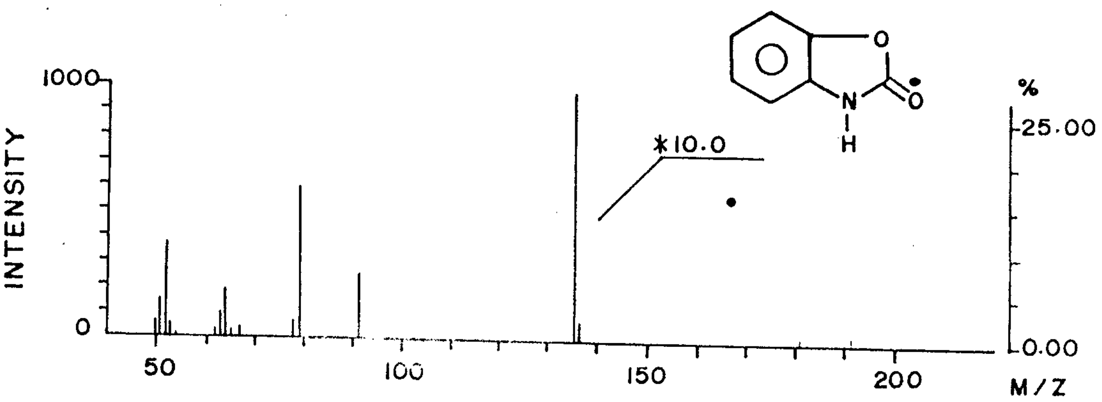


Fig. 3 Mass Spectrum of BOA (1)

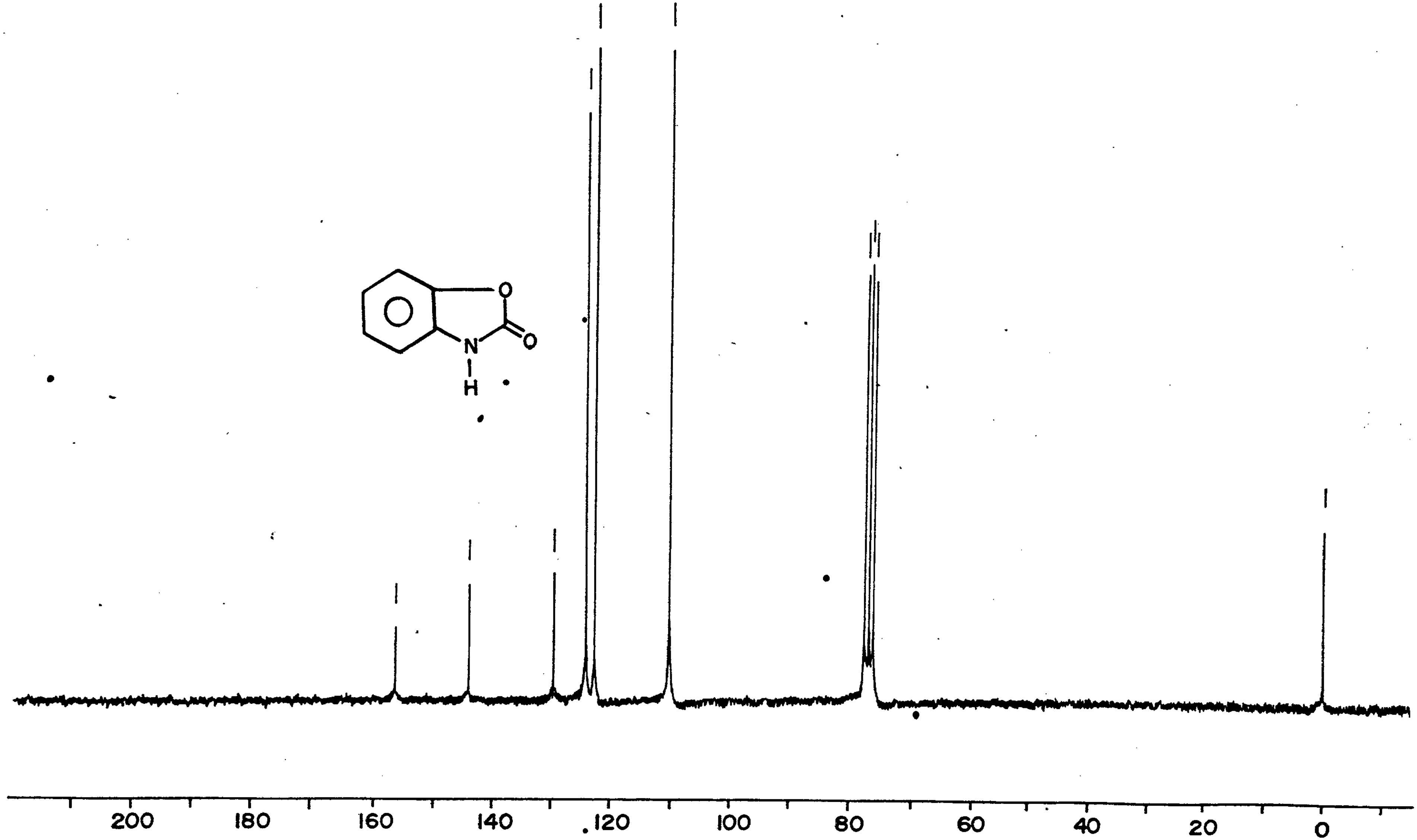


Fig. 4 ^{13}CNMR Spectrum of BOA (I)

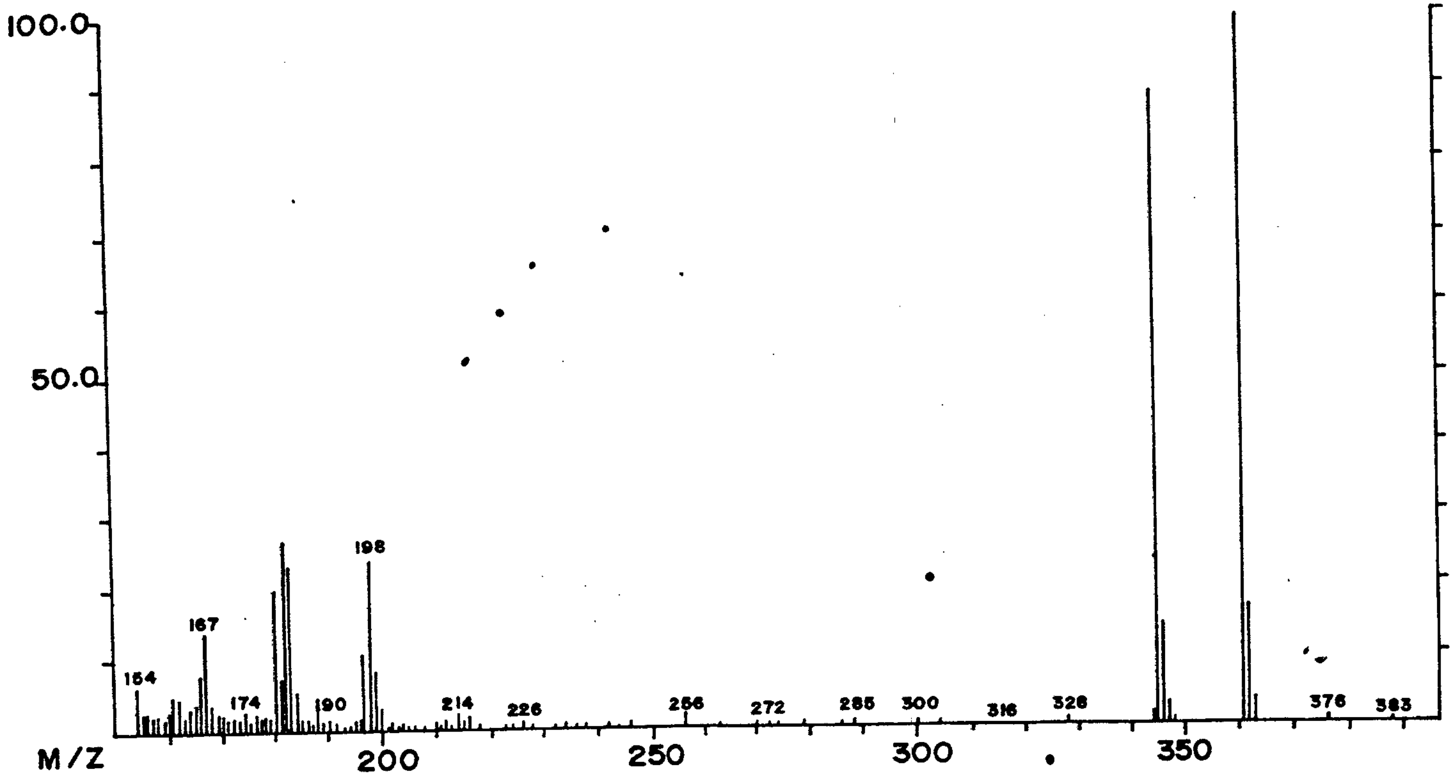


Fig. 5 NH_3—DCI mass spectrum of fraction-B

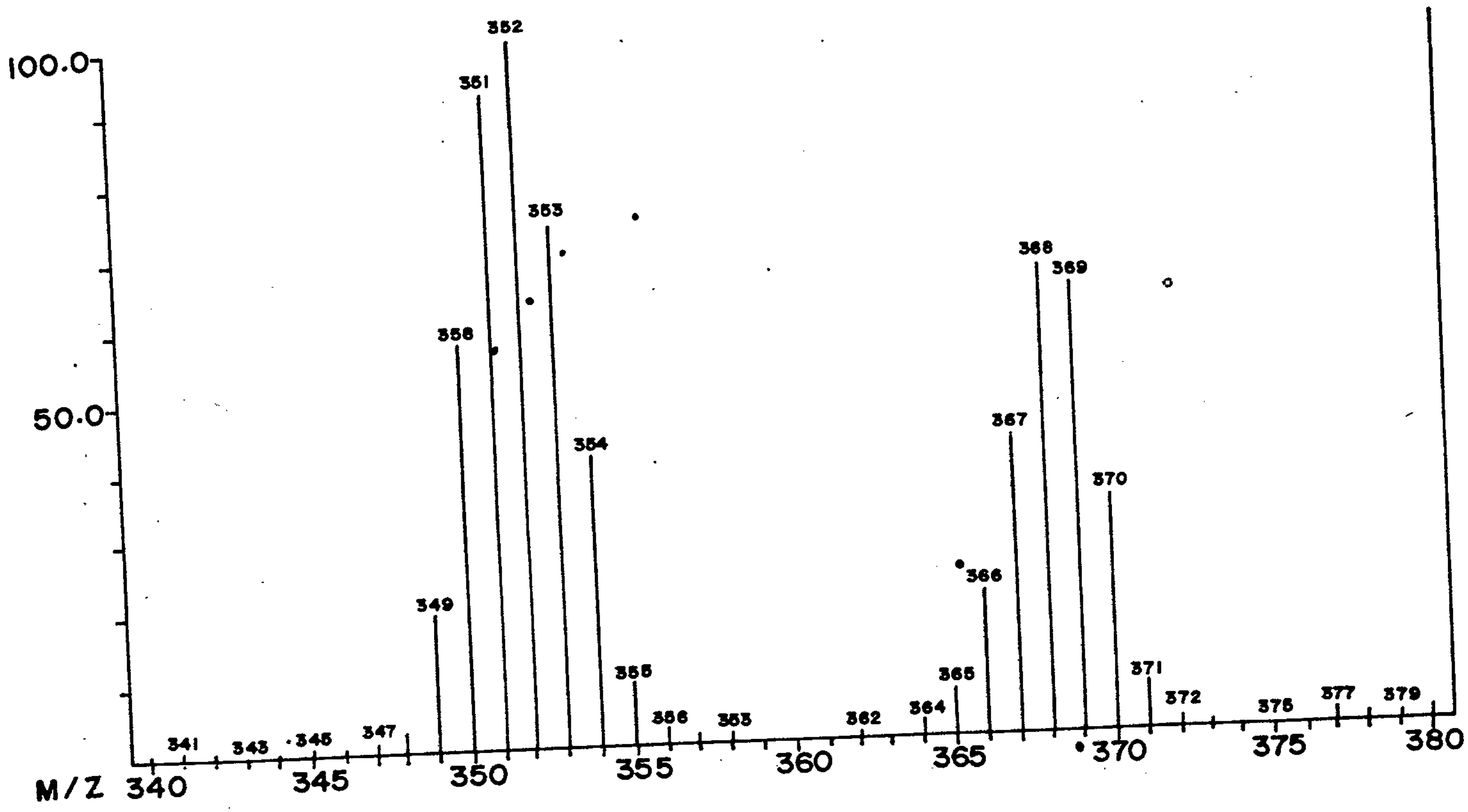


Fig. 6 ND_3-DCI mass spectrum of fraction - B

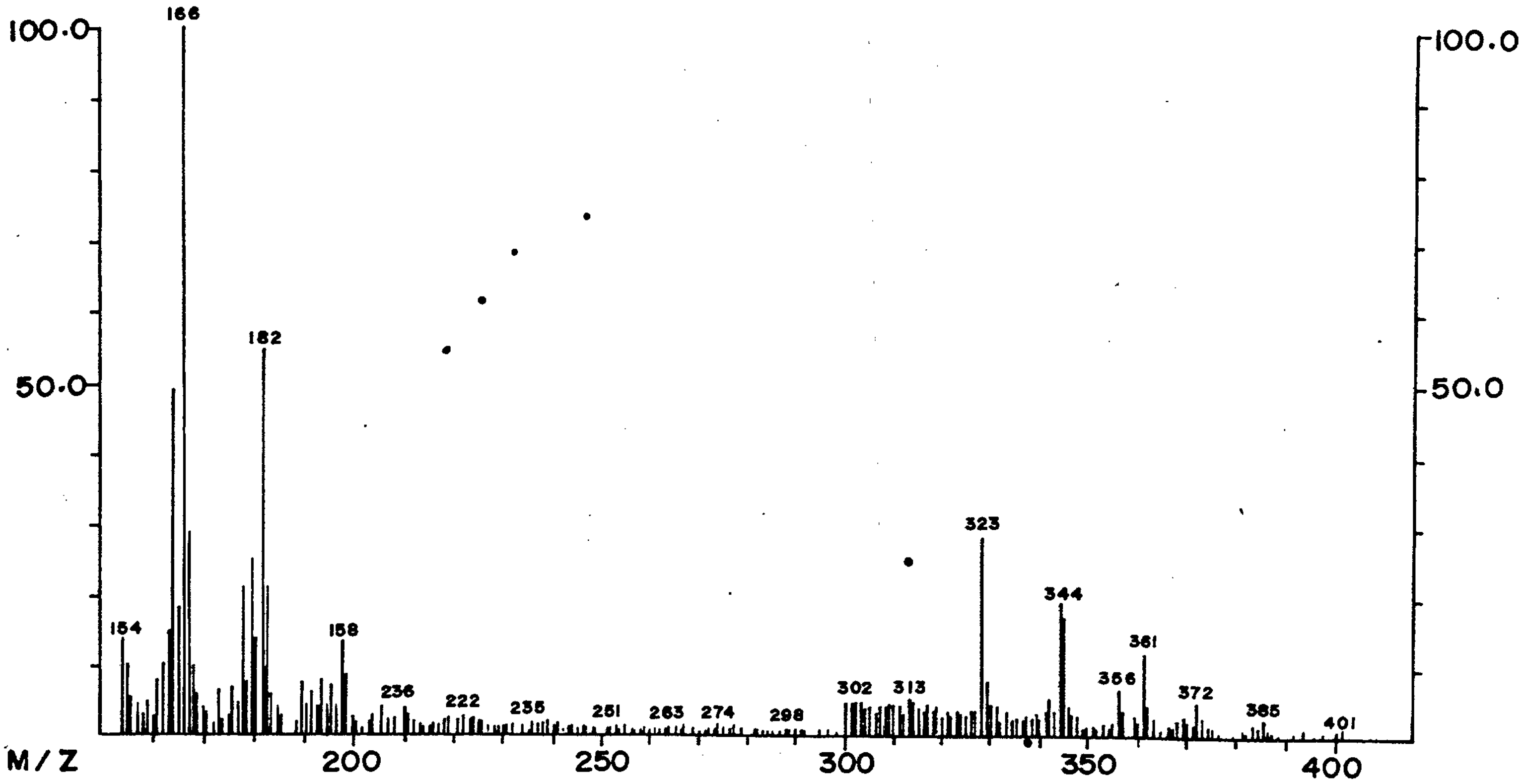


Fig. 7 CH_4—DCI mass spectrum of fraction-B

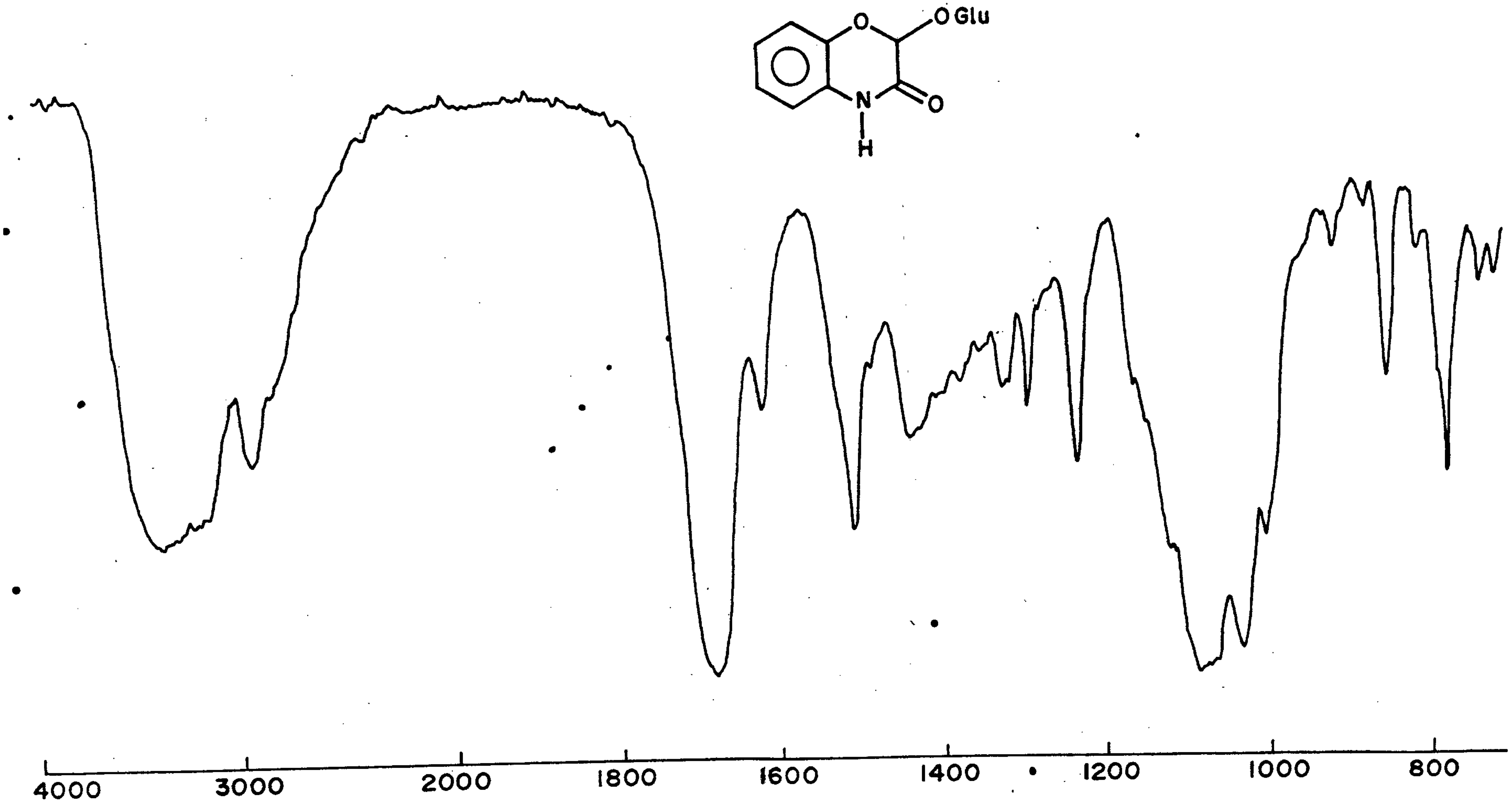


Fig. 8 IR Spectrum of (5)

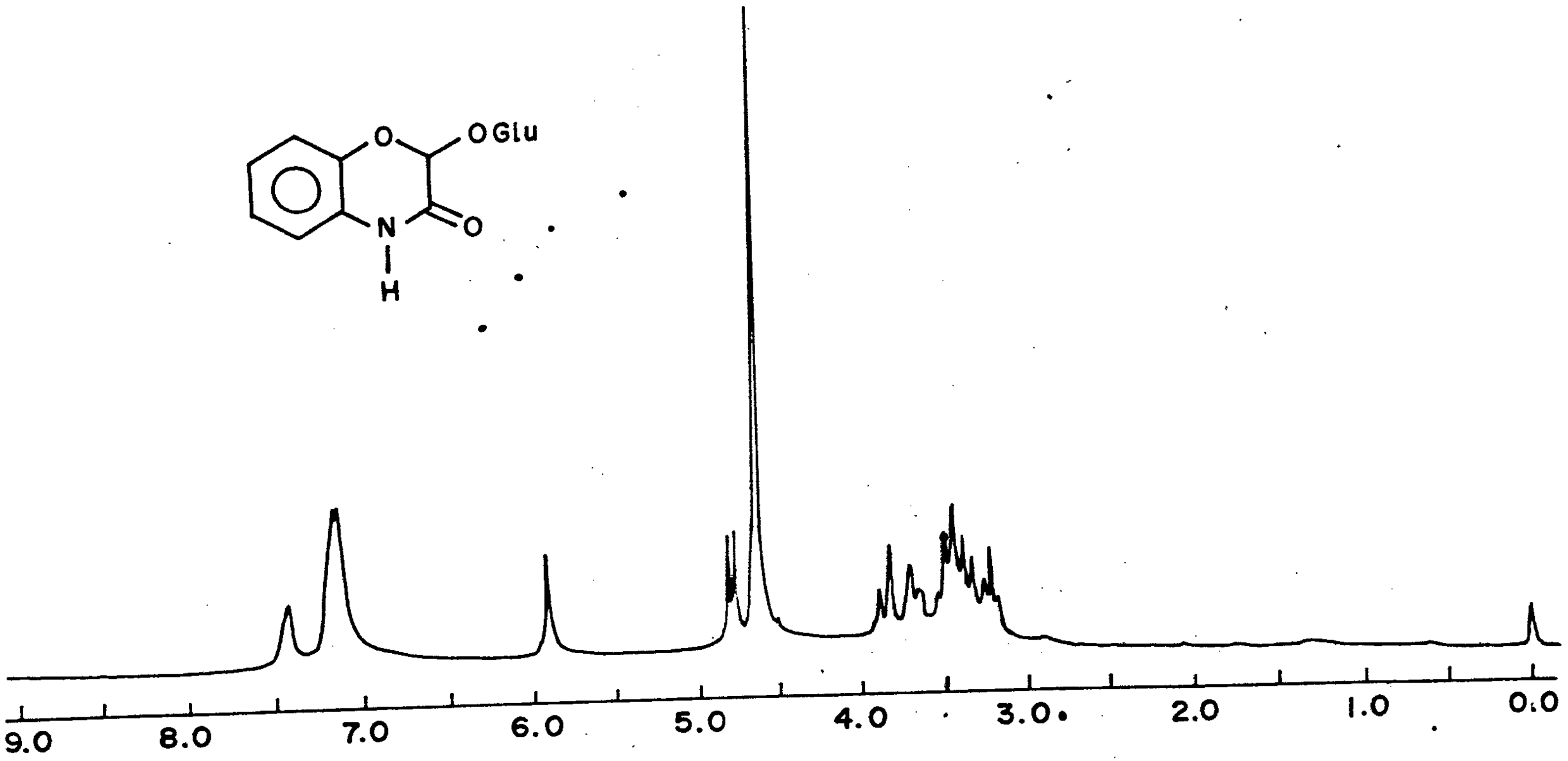


Fig. 9 ^{1}HNMR Spectrum of (5)

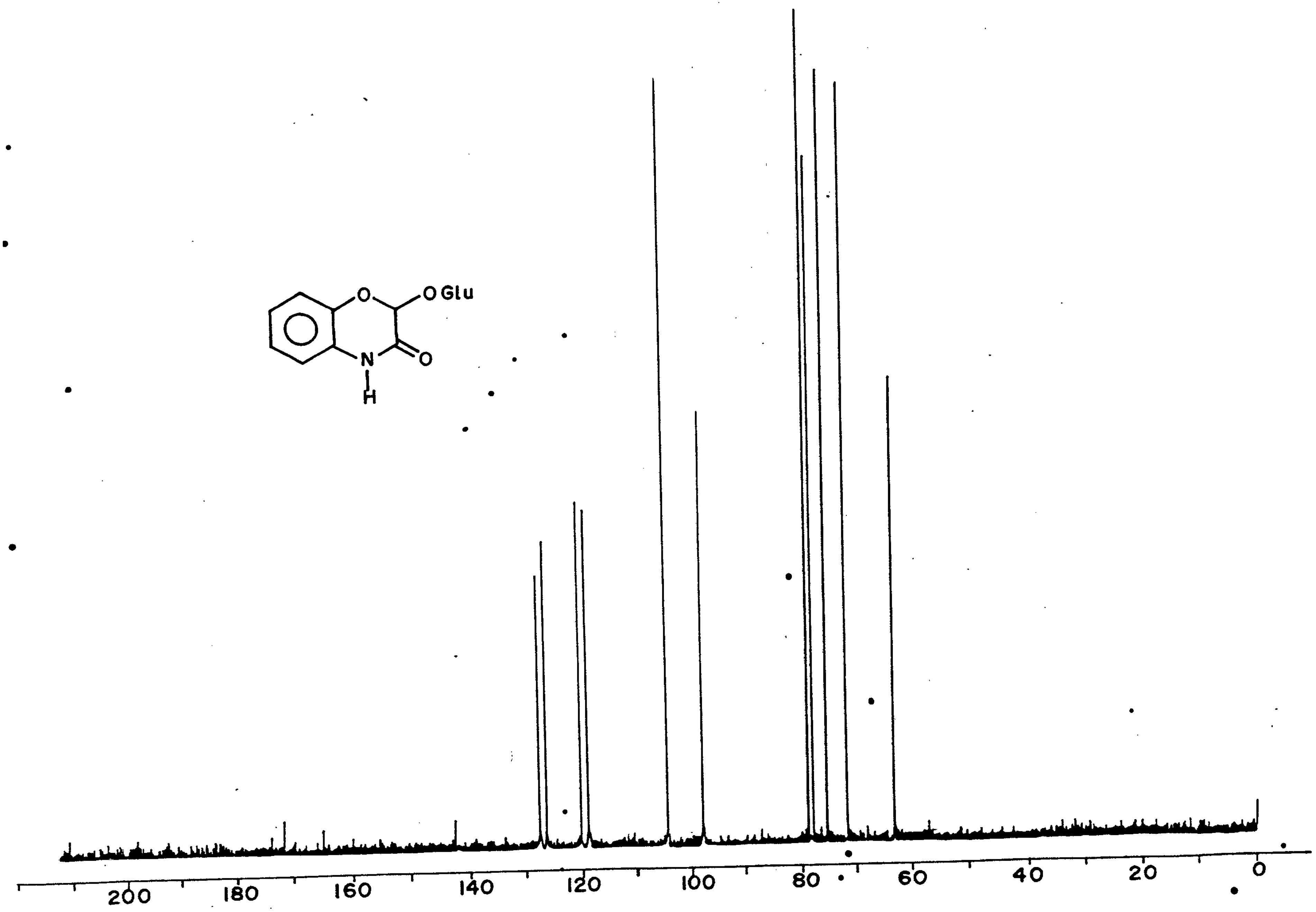


Fig. 10 ^{13}CNMR Spectrum of (5)

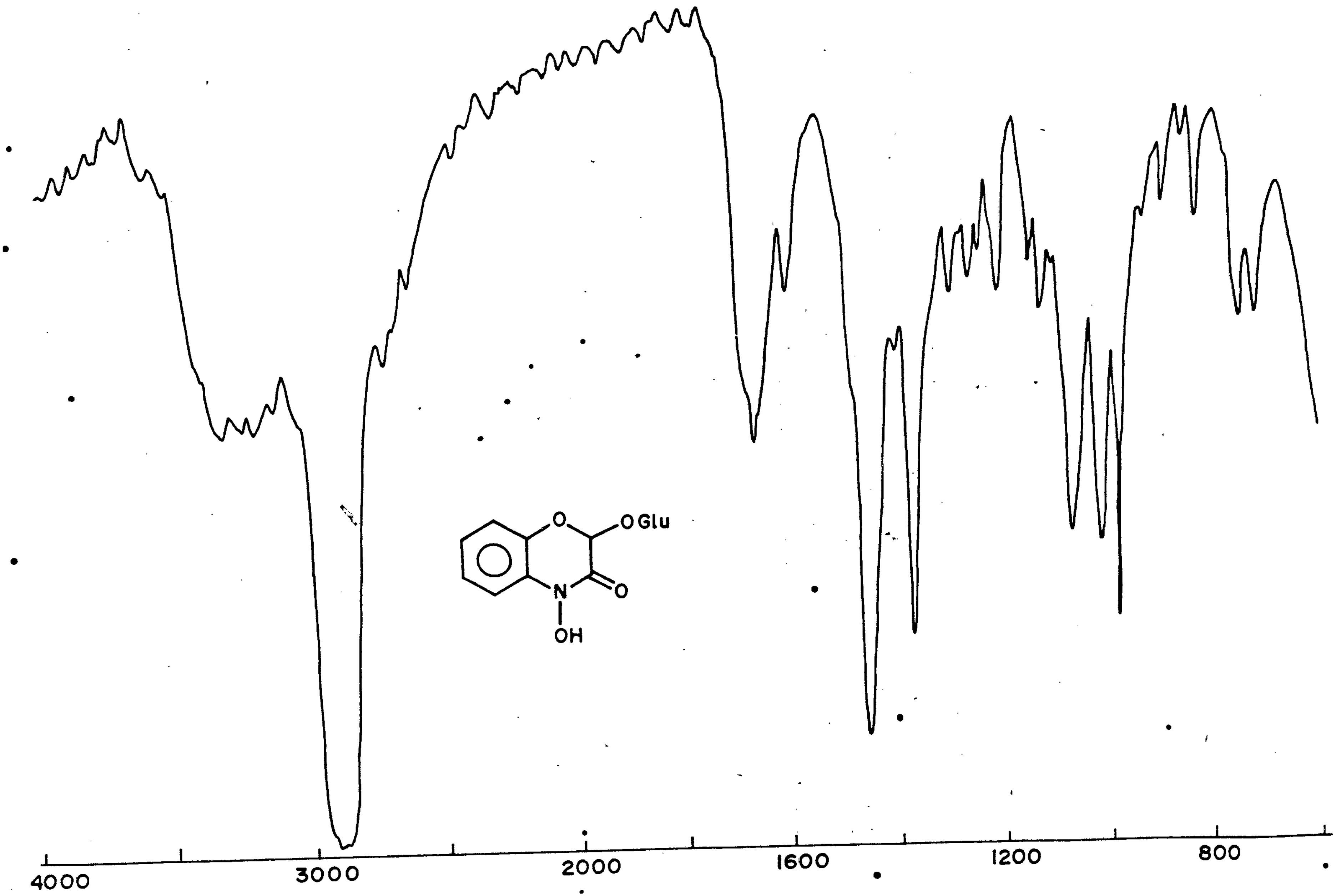


Fig. 11 IR Spectrum of (4)

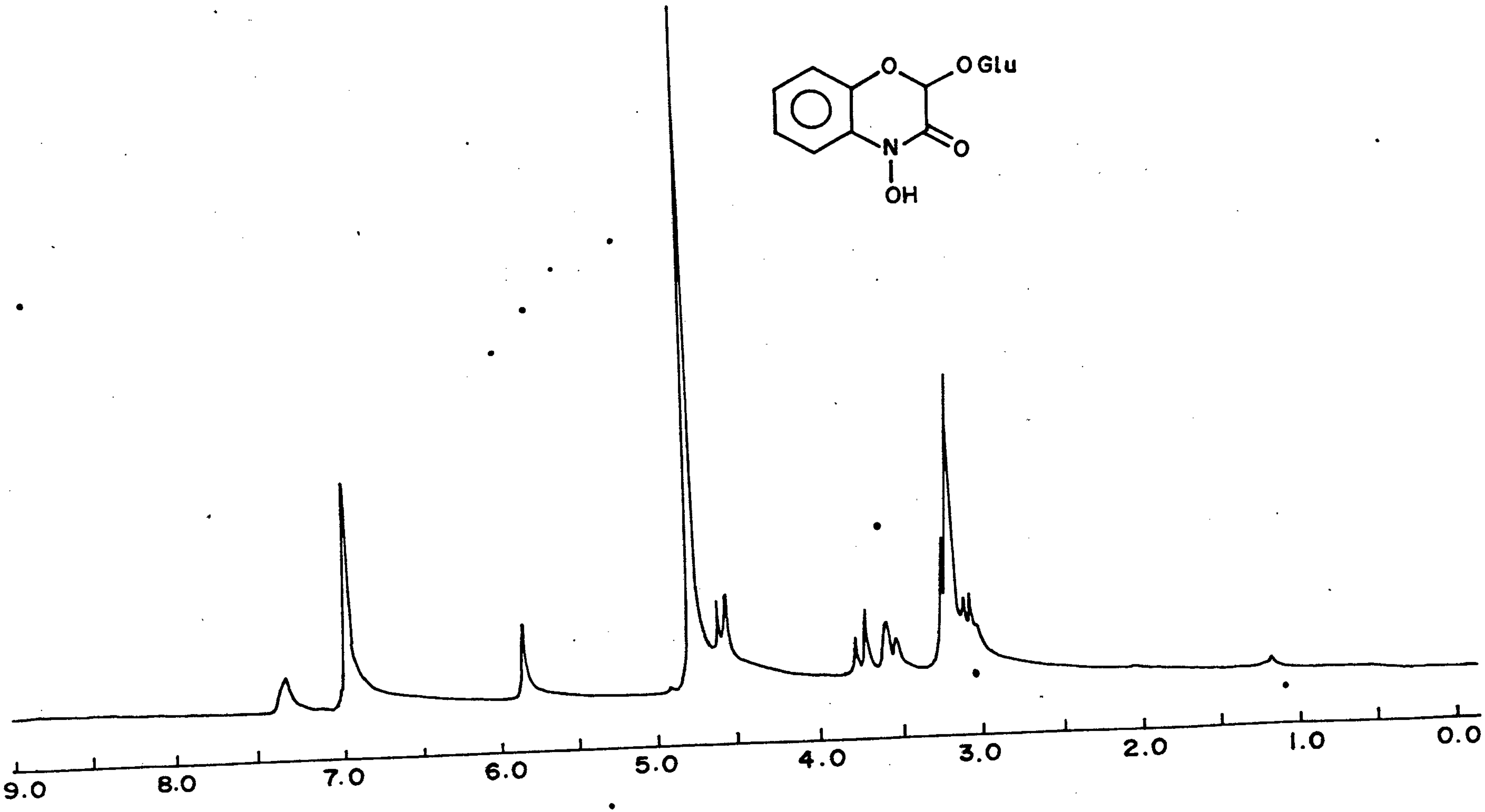


Fig.-12 ^{1}HNMR Spectrum of (4)

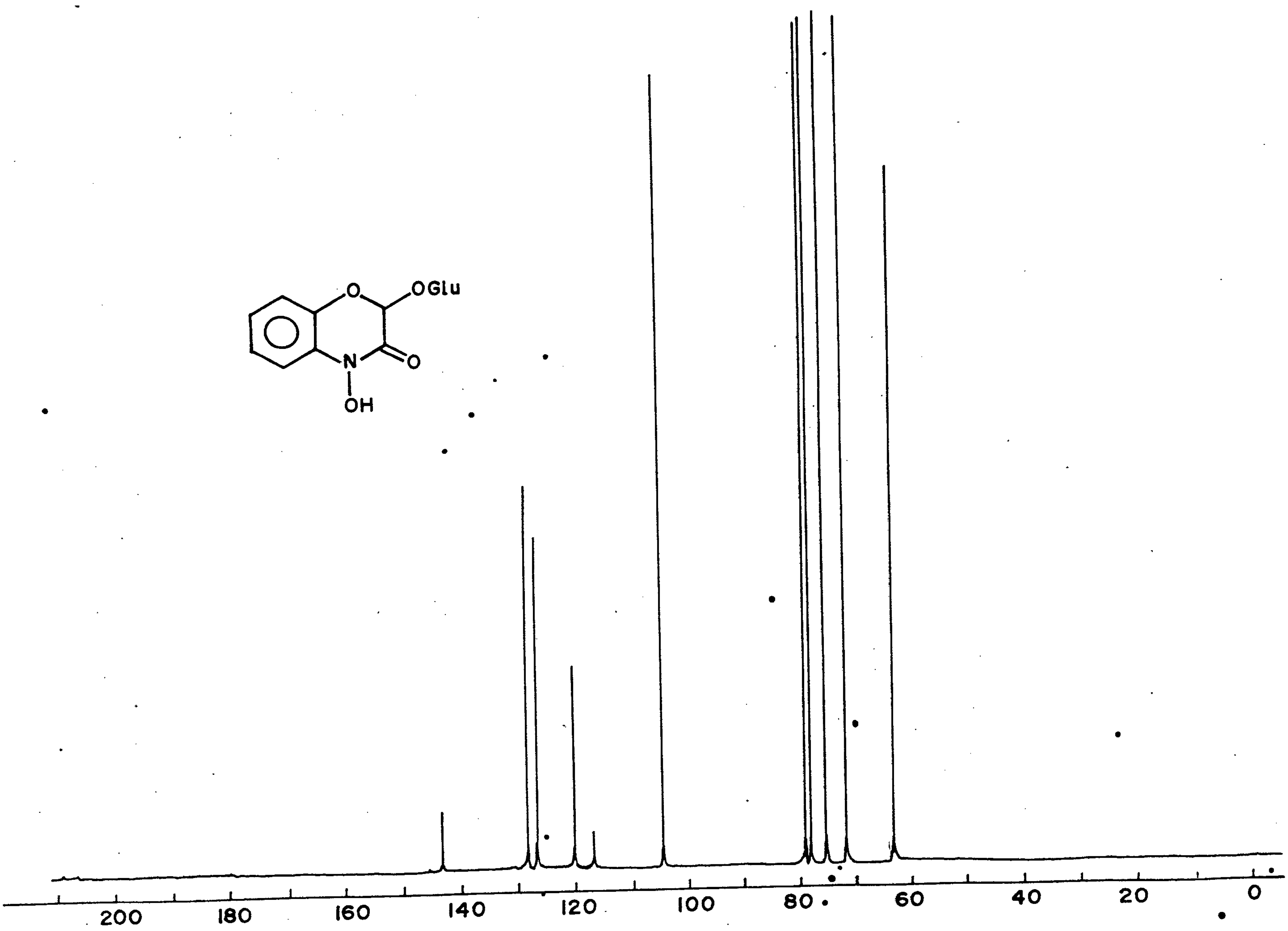


Fig. 13 ^{13}CNMR Spectrum of (4)

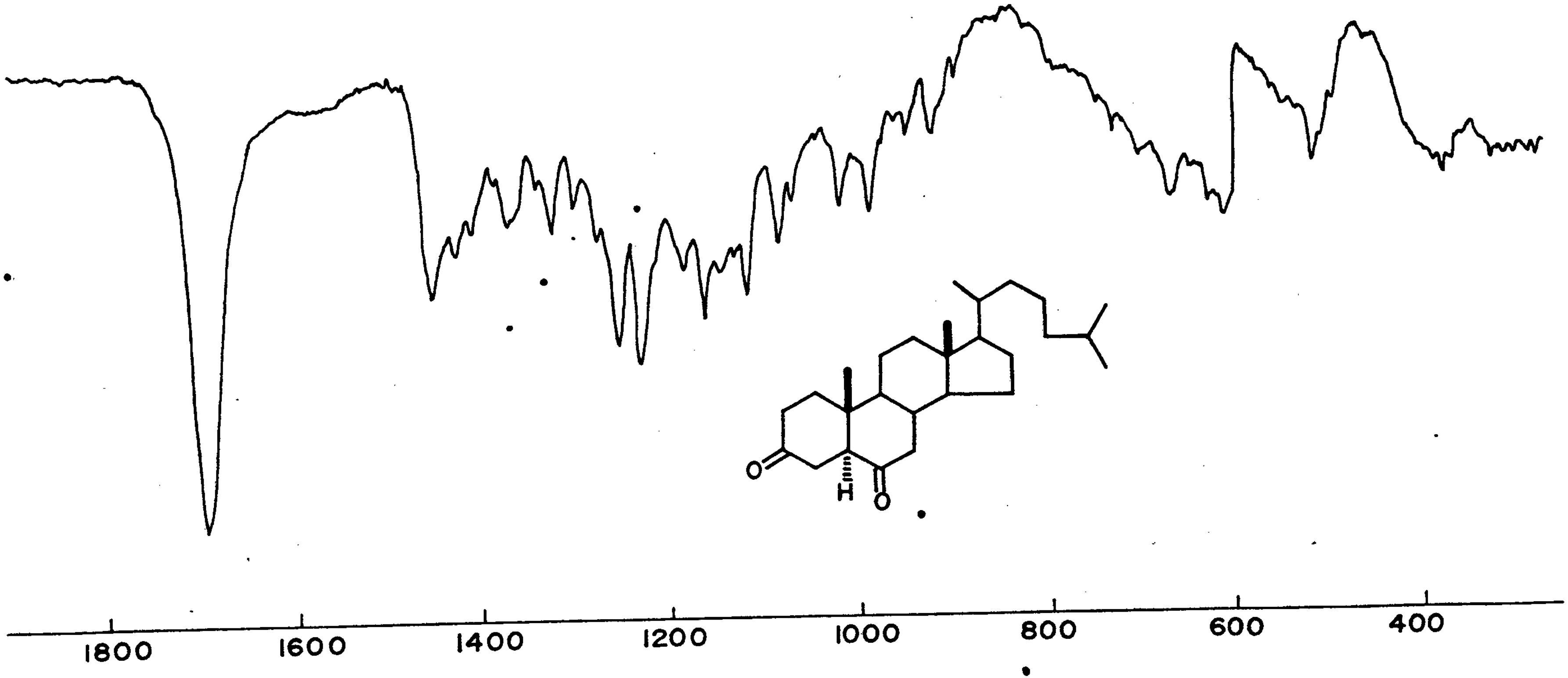


Fig. 14 IR Spectrum of (6a)

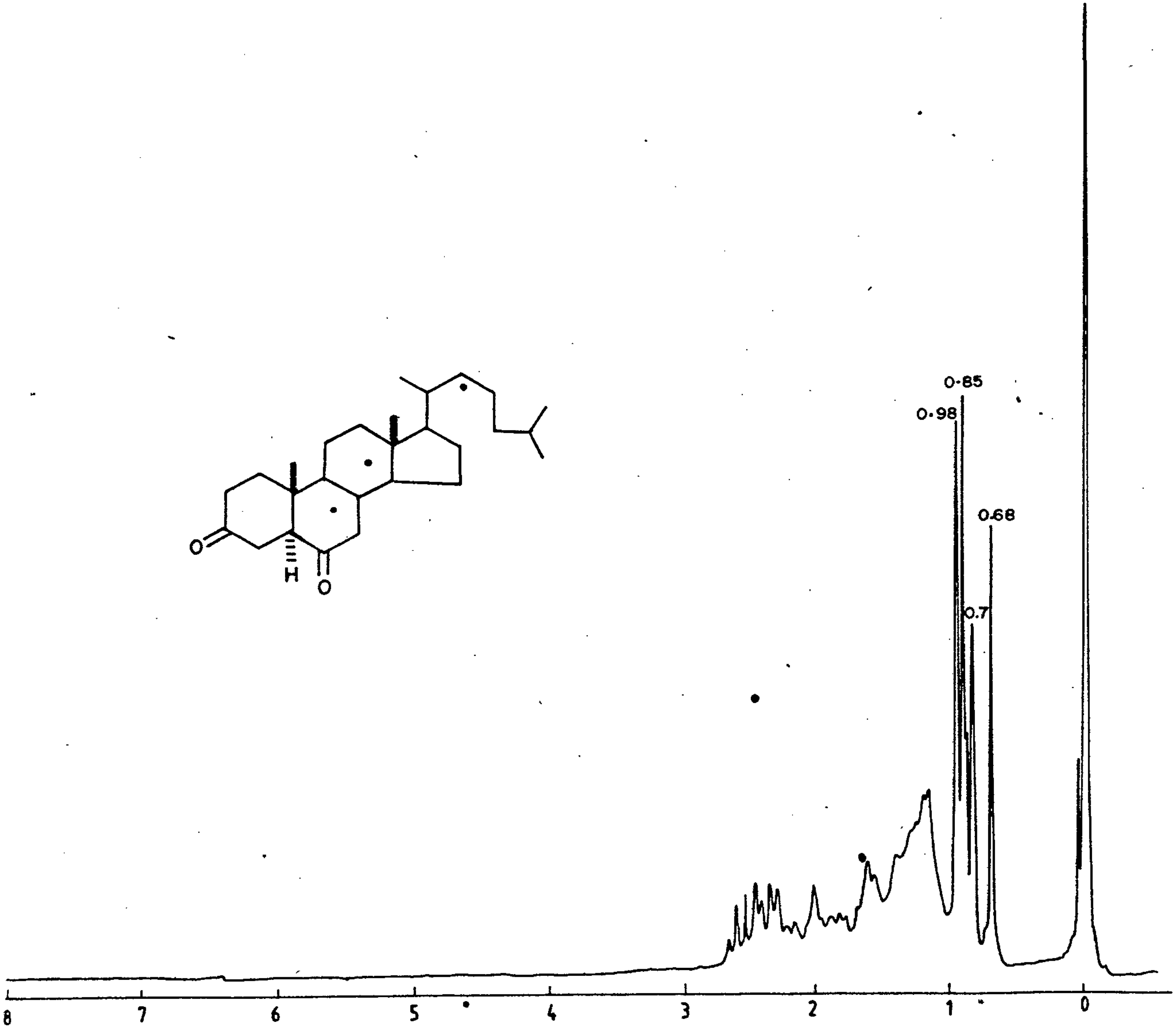


Fig. 15 ^{1}HNMR spectrum of (6a)

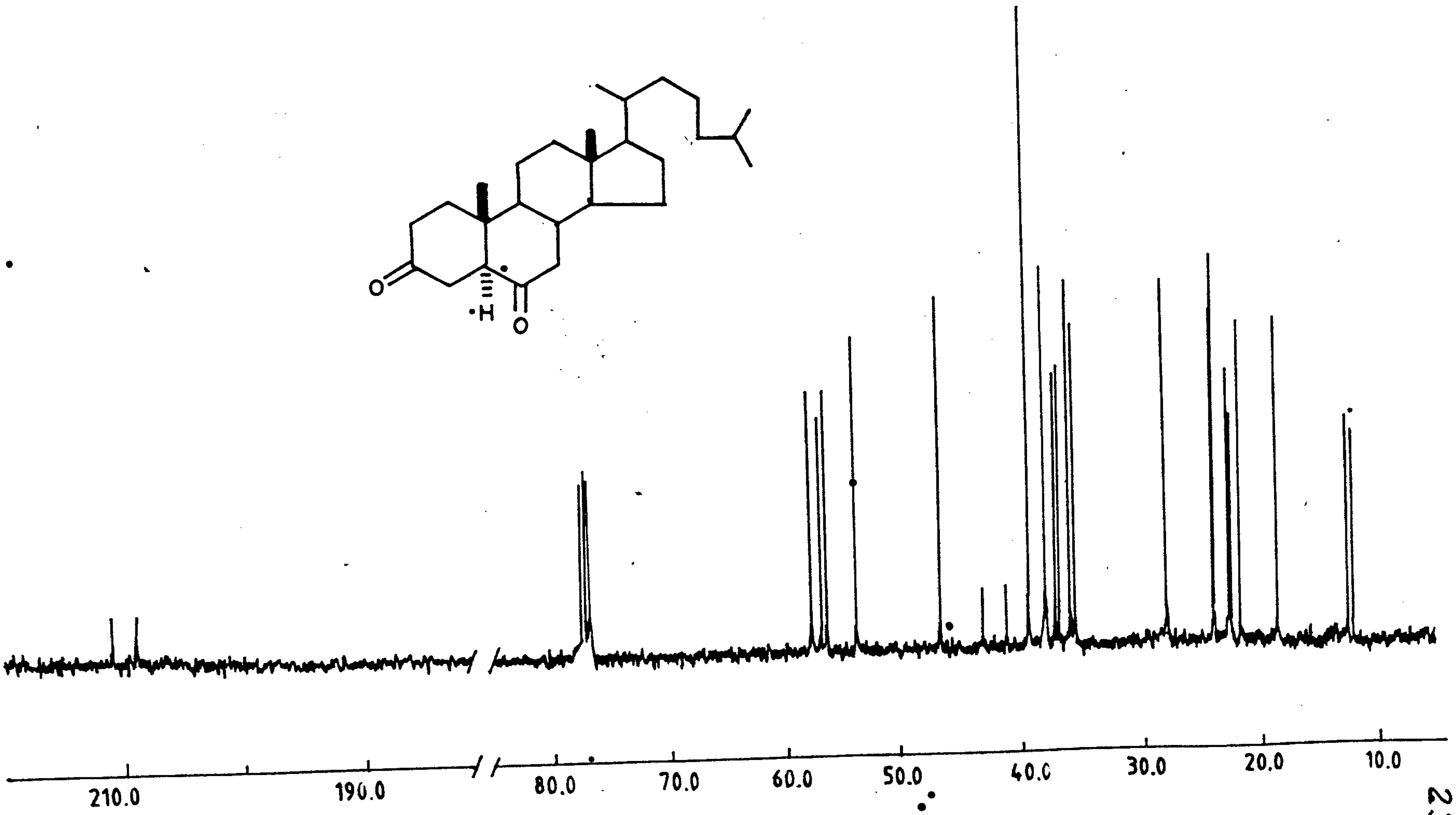


Fig. 16 ^{13}CNMR spectrum of (6a)

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SUMMARY

This thesis deals with isolation and synthetic studies on natural products.

It is divided into three chapters.

<u>CHAPTER</u> - I - This chapter has three sections.

SECTION - I

Deals with the Diels-Alder type self dimerization of *o*-quinols obtained from different monoterpene phenols and their derivatives, such as carvacrol, thymol, isothymol, 4-methyl thymol, 4-ethyl thymol by oxidation with $NaIO_4$ and HIO_3. The dimer (±)-3,10-dihydroxy – dielmentha-5,11-diene-4,9-dione, obtained from carvacrol is a natural product isolated from the heartwood of *Callitris mecleayana*. These dimers undergo intramolecular $(\pi 2 + \pi 2)$ cyclo addition reaction to give cage compounds.

SECTION - II

Describes the oxidation of various methyl aryl ethers with $CrO_3/AcOH$. 1,4-quinones were obtained in good yields and it was observed that better yields were obtained from more substituted phenols.

SECTION - III

Deals with the structures of the side products formed during the Duff reaction on thymol and carvacrol. These side products were confirmed by comparison with products obtained from unambigous syntheses.

CHAPTER - II - is divided in two sections.

SECTION - I

Deals with some reactions of 3-longifolyl tosylate. It was found that analytically pure longifolene (99% and above) could be obtained by reaction of 3-longifolyl tosylate with (i) neutral alumina and (ii) heating with DMSO.

SECTION - II

Describes a new approach for the synthesis of elvirol methyl ether a irregular phenolic sesquiterpene, occuring in *Elvira biflora*.

CHAPTER - III - is divided into two sections.

SECTION - I

Deals with the isolation of 2-benzoxazolone and hydroxamic acids from the extracts of the pods of the mangrove plant *Acanthus illicifolius*.

SECTION - II

Describes the isolation of 5β-cholestane-3,6-dione from the red sea weed Acanthophora spicifera. This is the first report of the natural occurrence of this compound. The structure was confirmed by synthesis.