PART III

CHEMICAL INVESTIGATION ON FLACOURTIA SEPIARIA (ROXB)

EXTRACTION OF FLACOURTIA SEPIARIA (ROXB)

Dried and powdered trunk, bark and stem of Flacourtia sepiaria (Roxb) were extracted with benzene for 36 hours. The extract was concentrated by distilling off benzene when a gummy residue was obtained. The residue was extracted with ether and the ether extract was washed with aqueous sodium hydroxide solution and then with water till it was neutral. The ether solution was dried over anhydrous sodium sulphate and it was evaporated to yield a gummy residue which constituted the neutral part of the extract.

The alkali washed portion, on acidification with dilute hydrochloric acid, yielded a solid, which was extracted with ether. The ethereal solution containing the acid part was washed with water till neutral and dried. The solvent distilled off to yield a gummy residue which constituted the acid part of the extract.

CHAPTER 1

INVESTIGATION ON THE NEUTRAL PART OF FLACOURTIA SEPIARIA

Chromatography of the neutral part

The neutral part of the benzene extract of powdered trunk, bark and stem of Flacourtia sepiaria was chromatographed over deactivated alumina column and the following fractions were collected (Table 1).

Table 1

Chromatography results of the neutral part of F sepiaria

Fraction number	Eluent	Nature of residue after removal of solvent	Melting point
1	Petroleum ether	oily solid	low mp
2	Pet ether:benzene (4:1)	oil	-
3	Pet ether:benzene (3:2)	solid	126-133°
4	Pet ether:benzene (2:4)	oil	-
5	Pet ether:benzene (1:4)	solid	209-211°
6	Benzene	solid	240-241°
7	Benzene:solvent ether (9:1)	solid with oil	127-130°

Further elution with more polar solvents did not afford any solid materials

Examination of fraction 1 (Table 1) : isolation and identification of 1-hexacosanol

Fraction 1 was purified by repeated crystallization from methanol into waxy solid, $C_{26}H_{54}O$, mp 78-79°, $[\alpha]_D \pm 0°$ and was identified as 1-hexacosanol by direct comparison with an authentic sample and preparing its acetate, mp 68-69°.

Examination of fraction 3 (Table 1) : isolation and identification of β-sitosterol

Fraction 3 on repeated crystallization from chloroform -methanol mixture gave flakes, mp 136-137°, $[\alpha]_D$ -34° and was analysed for $C_{29}H_{50}O$. The compound gave positive Liebermann-Burchardt colour test for sterol and was identified as β-sitosterol by direct comparison with an authentic sample and preparing its acetate, mp 130-132°, $[\alpha]_D$ -40°.

Examination of fraction 5 (Table 1) : isolation and identification of 20-hydroxylupanone

Fraction 5 on repeated crystallization from chloroform -methanol mixture afforded a crystalline solid, mp 213-214°. It gave positive Liebermann-Burchardt test for triterpenoid but gave no colouration with trinitromethane. Elemental analysis indicated its molecular formula as $C_{30}H_{50}O_2$. The IR spectrum (Fig 1) of the compound shows bands at 3450 and 1690 cm^{-1} indicating the presence of a hydroxy group and a carbonyl group, thus accounting for the two oxygen atoms of the molecule $C_{30}H_{50}O_2$. The ^1H NMR spectrum (Fig 2) shows peaks at 0.80(s), 0.92(d, J = 0.80 Hz), 0.94(d, J = 0.80 Hz), 1.01(s), 1.06(s), 1.08(s), 1.10(s) and 1.21(s) ppm for 24 protons indicating the

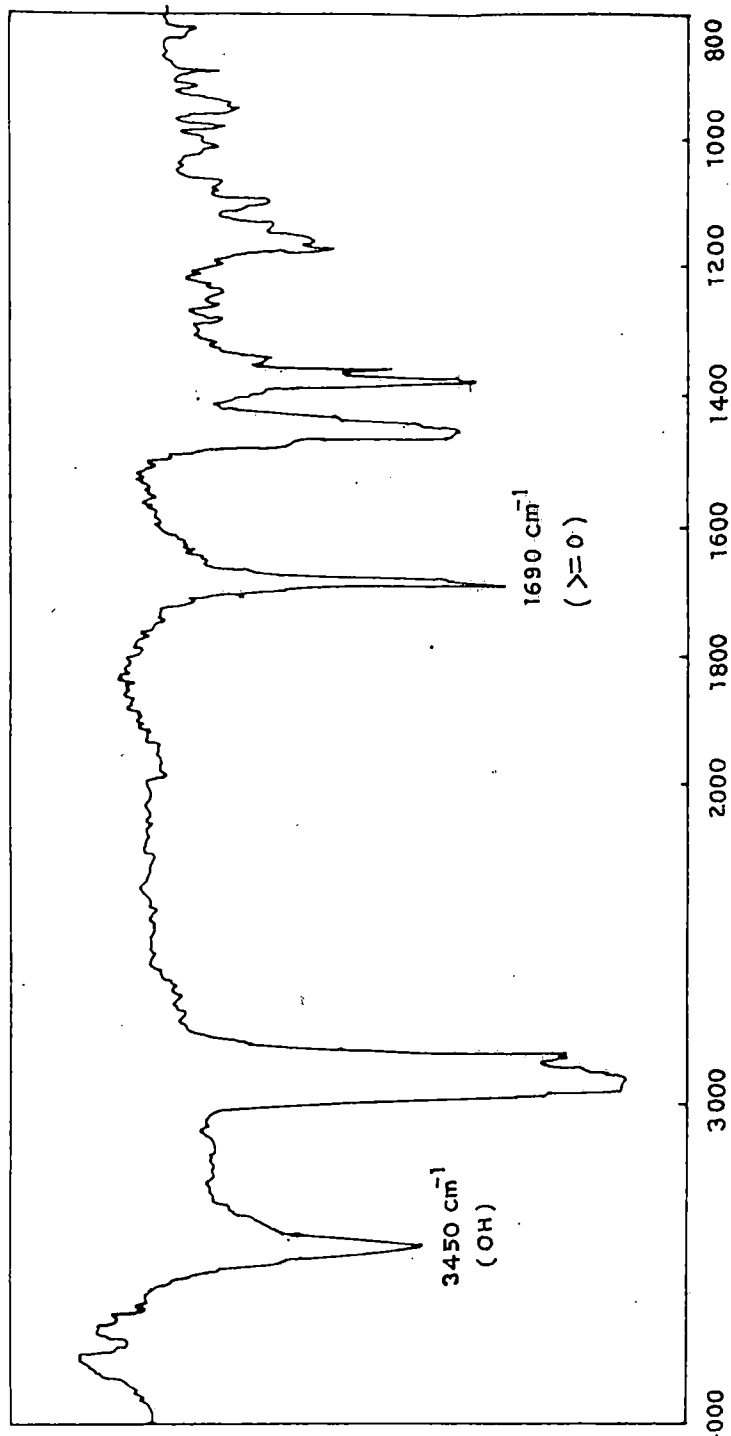


Fig 1. IR spectrum of 20-hydroxylupanone(1)

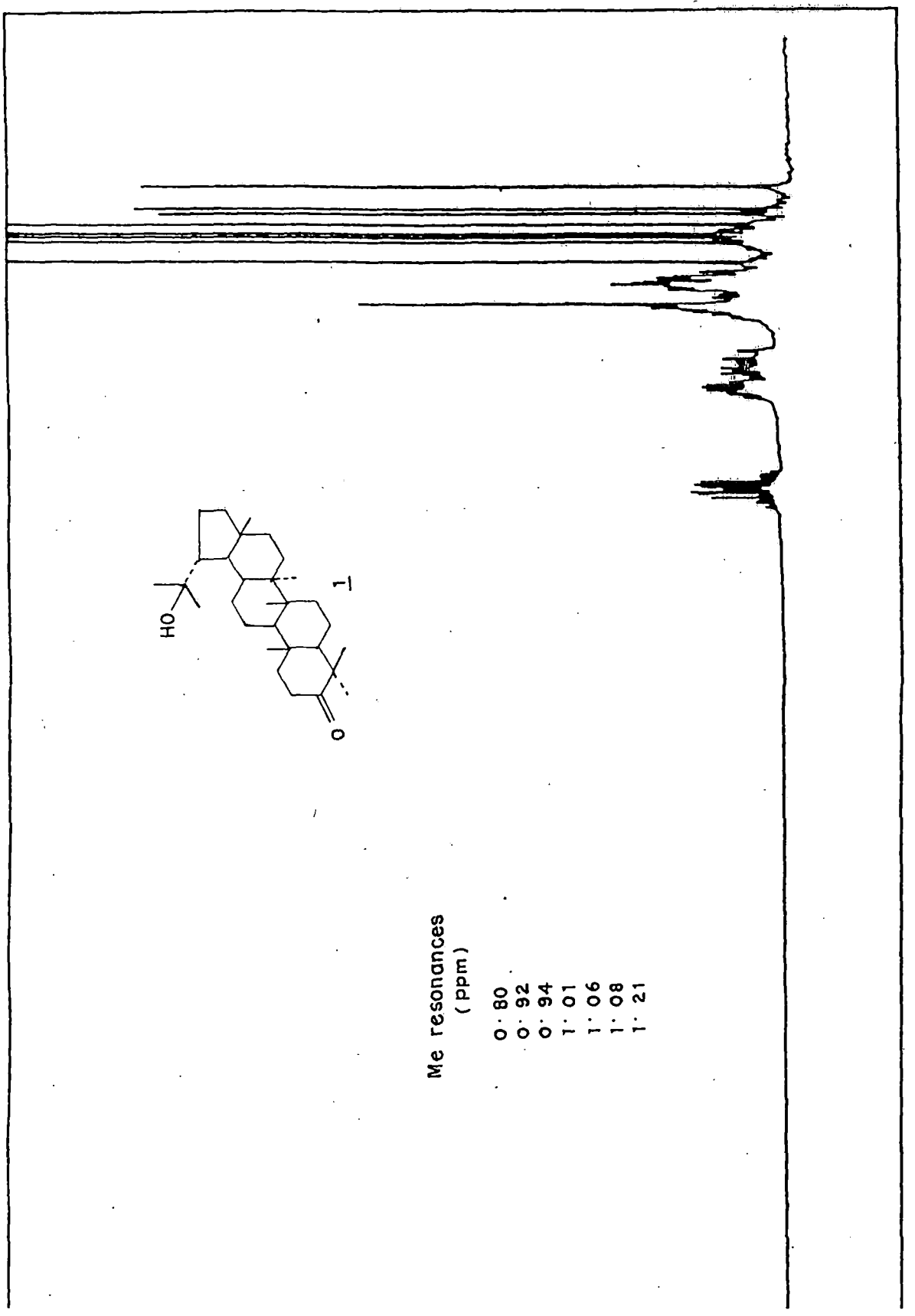


Fig 2. ^1H NMR spectrum of 20-hydroxylupanone (1) at 300 MHz.

presence of eight tertiary methyl groups. The multiplets, centred at 2.47 and 1.88 ppm, represent two protons adjacent and two protons alternate to the carbonyl group, suggesting the presence of the carbonyl group at C-3 of a triterpenoid skeleton. The presence of eight tertiary methyl groups and absence of any peak due to the proton geminal to the hydroxy group indicates that the hydroxy group is attached with a tertiary carbon of the molecule. This is supported by the fact that the hydroxy group is resistant to acetylation.

The ^{13}C NMR spectrum (Fig 3) of the compound displays 29 resolved lines, one at 15.82 ppm being twice the amplitude of the other, confirms a total of 30 carbon atoms in the molecule. The APT[1] spectrum (Fig 4) indicates the nature of the carbon atoms which are shown in Table 2. These functionalities can be accomodated in a molecular

Table 2

Number of different groups and their $^{13}C_{\Lambda}^{chemical}$ shift values (ppm) of the compound found in fraction 5 (Table 1)

Groups	Number	^{13}C shifts
$-CH_3$	8	14.60, 15.82, 15.82, 19.03, 20.86, 24.58, 26.50, 31.46
$-\overset{\vert}{C}H_2$	10	19.51, 21.75, 27.36, 28.50, 28.79, 33.65, 33.94, 35.32, 39.36, 39.96
$-\overset{\vert}{\underset{\vert}{C}}H$	5	37.40, 48.07, 49.43, 49.74, 54.60
$-\overset{\vert}{\underset{\vert}{C}}-$	5	36.58, 41.09, 43.38, 44.41, 47.10
$>C-OH$	1	73.22
$>C=O$	1	213.84

structure if the hydroxy group is placed at C-20 of lupane

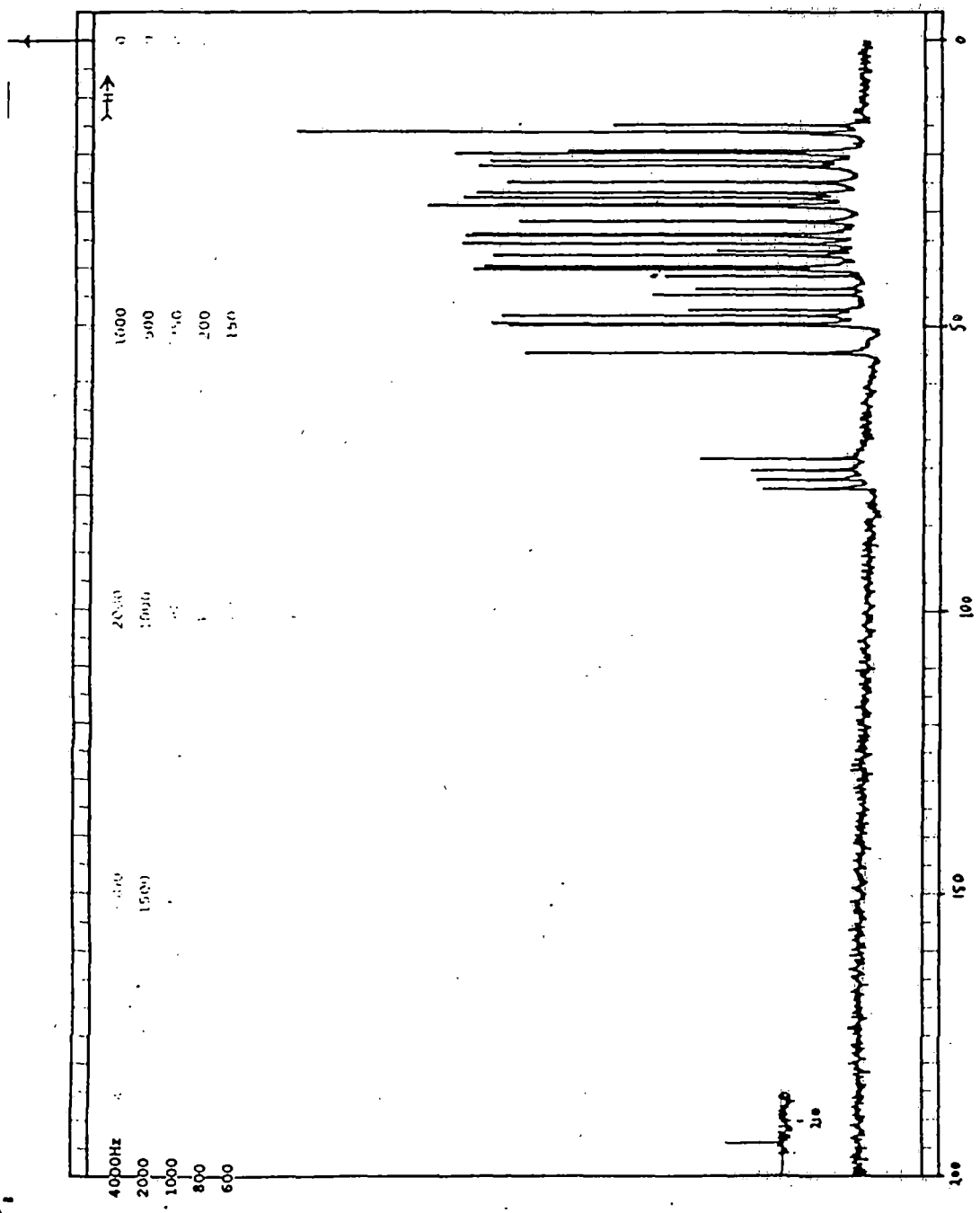


Fig 3. ^{13}C NMR spectrum of 20-hydroxylupanone (1) at 20 MHz

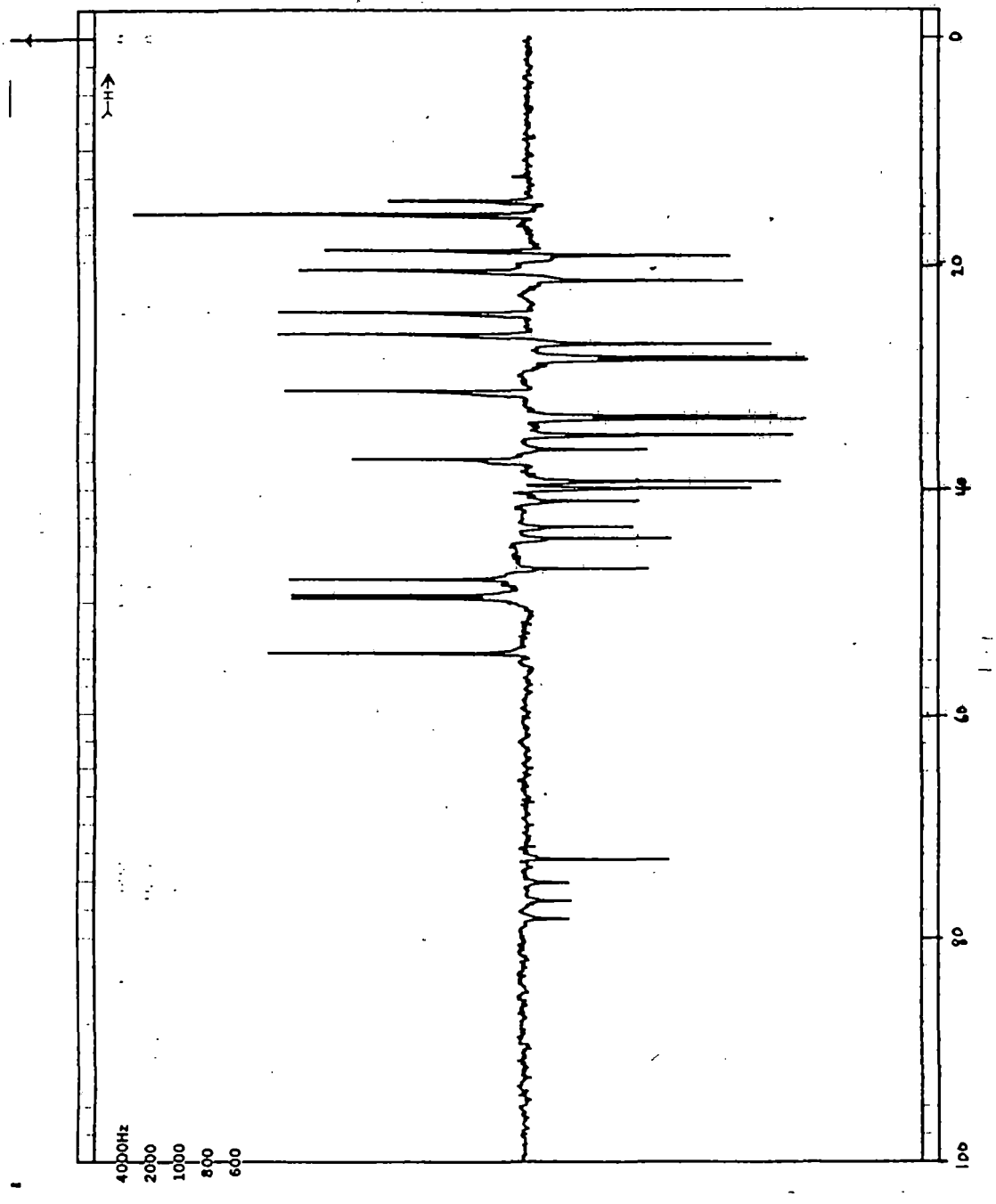


Fig 4. APT spectrum of 20-hydroxylupanone (1) at 75 MHz

or at C-28 of hopane (or isohopane) skeleton, the keto group being placed at C-3 position.

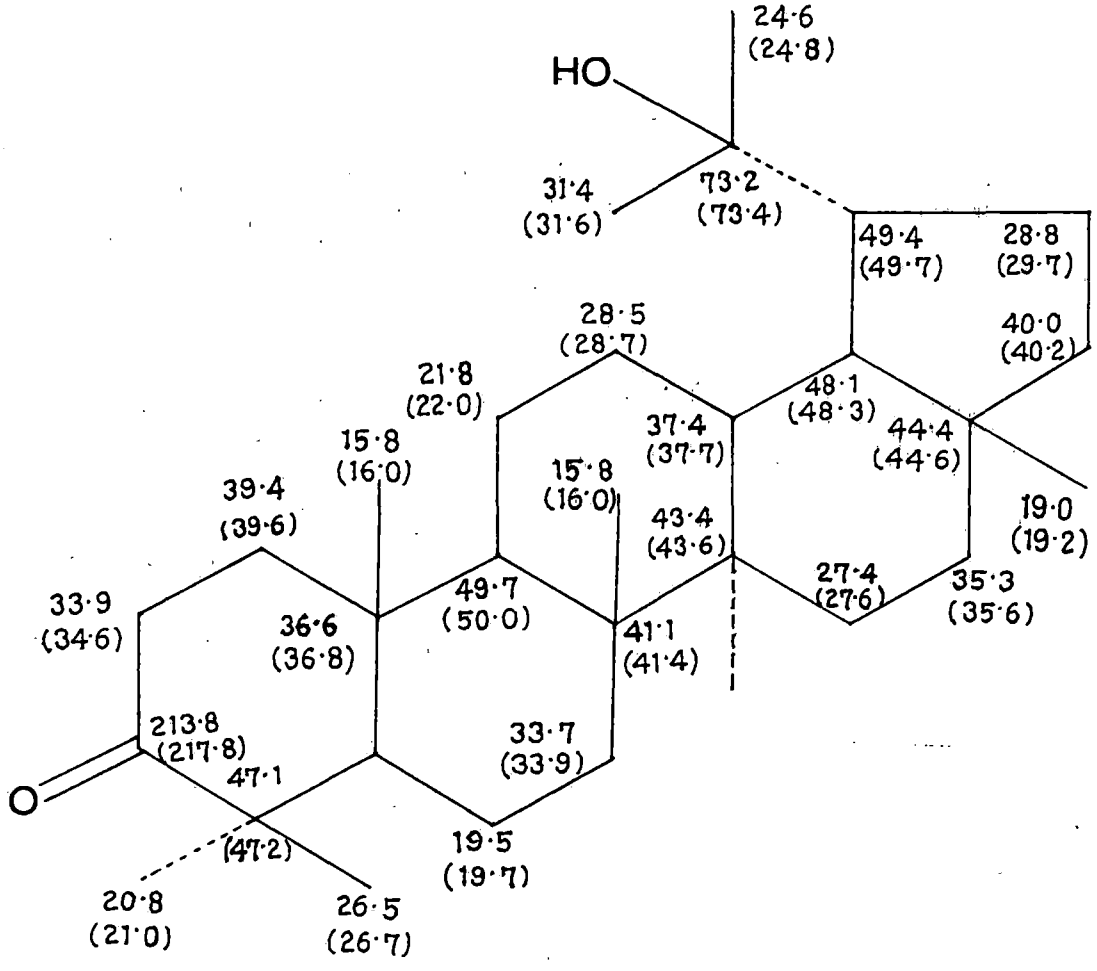
A comparison of the ^{13}C NMR data of the compound with those of previously reported triterpenoid hydroxy compounds reveals that it has strikingly similar values with that of 20-hydroxylupanone[2] with a few exceptions which are indicated in structure 1. A similar comparison of methyl resonance of the compound with those of 20-hydroxylupanone[3] (Table 3) also indicates the compound to be 20-hydroxylupanone (1).

Table 3

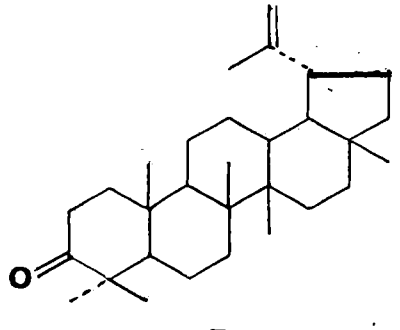
Methyl resonances (ppm) of 20-hydroxylupanone (1) and the compound in fraction 5 (Table 1)

Methyl carbons	Methyl shifts of 20-hydroxylupanone (1)	Methyl resonances of the compound in fraction 3
C-23	1.10	1.08
C-24	1.02	1.01
C-25	0.93	0.92
C-26	1.09	1.06
C-27	0.97	0.94
C-28	0.80	0.80
C-29/C-30	1.12/1.22	1.10/1.21

That the compound is 20-hydroxylupanone is further supported by converting it to lupenone (2) on heating the compound with acetic anhydride-pyridine containing few drops of boron trifluoride-etherate over boiling water bath for 10 hours. The product on usual work-up and purification by chromatography and crystallization furnished a compound of mp 169-171°, $[\alpha]_D$ + 57°. The IR spectrum (Fig 5) shows the presence of bands at 3060, 1695, 1640 and 880 cm^{-1} indicating the introduction of a methylene double bond with the loss of hydroxy group.


HO
24·6
(24·8)
31·4
(31·6)
73·2
(73·4)
49·4
(49·7)
28·8
(29·7)
40·0
(40·2)
28·5
(28·7)
21·8
(22·0)
48·1
(48·3)
37·4
(37·7)
44·4
(44·6)
15·8
(16·0)
15·8
(16·0)
39·4
(39·6)
43·4
(43·6)
19·0
(19·2)
33·9
(34·6)
49·7
(50·0)
27·4
(27·6)
35·3
(35·6)
36·6
(36·8)
41·1
(41·4)
213·8
(217·8)
33·7
(33·9)
O
47·1
(47·2)
19·5
(19·7)
20·8
(21·0)
26·5
(26·7)

1


O

2

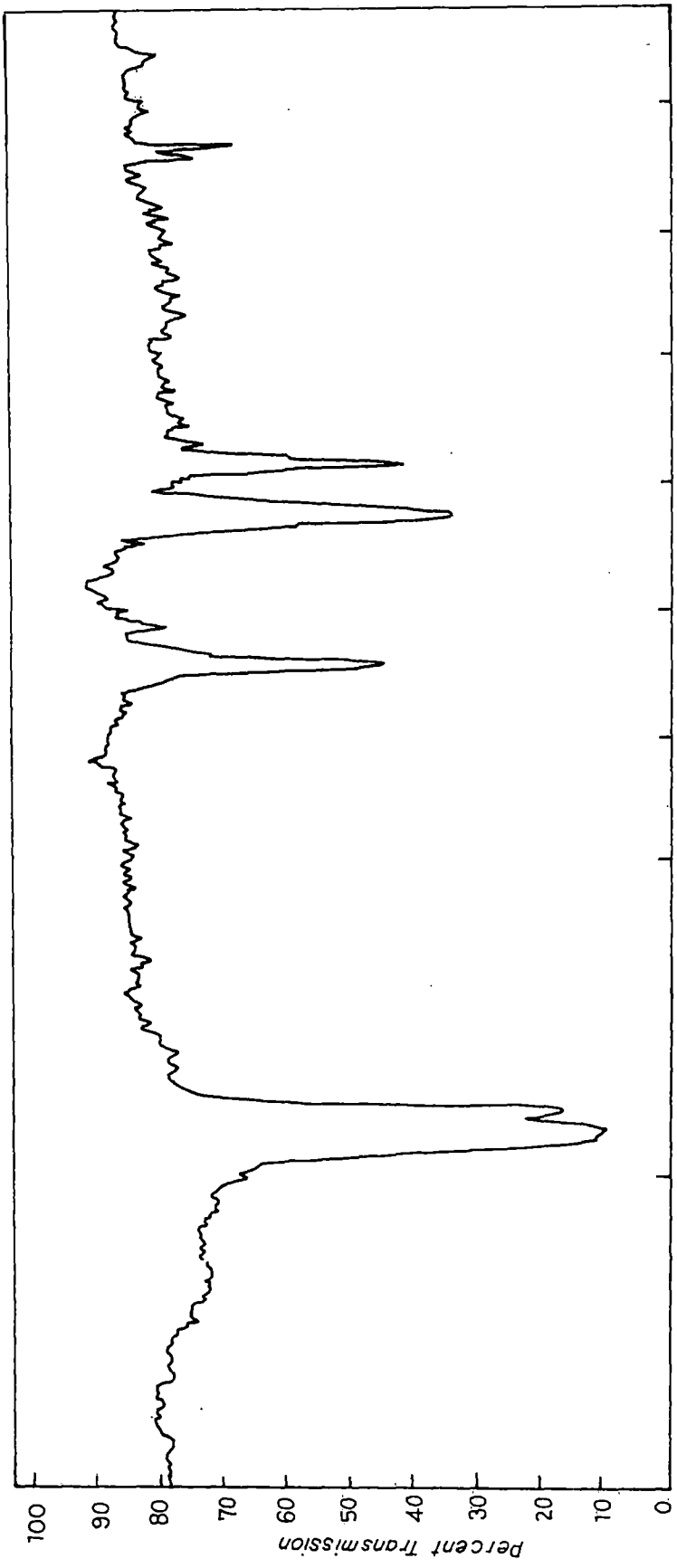


Fig 5. IR spectrum of lupenone (2)

The band at 1700 cm^{-1} shows that the carbonyl group is isolated from the newly introduced double bond. The compound thus prepared is identified as lupenone (2) by direct comparison with an authentic sample (mixed mp, co-TLC and co-IR).

Examination of fraction 6 (Table 1) : isolation and identification of 3β, 20-dihydroxylupane

The fraction 6 on repeated crystallization from chloroform-methanol mixture furnished solid, mp 243-244°, $[\alpha]_D$ + 26.7°. It responded to Liebermann-Burchardt test for triperpenoid but developed no colouration with trinitromethane indicating the absence of unsaturation. Elemental analysis showed the molecular formula to be $C_{30}H_{52}O_2$. The IR spectrum (Fig 6) of the compound shows absorption at 3480 and 3580 cm^{-1} which indicates the compound to be dihydroxy one. On treatment with acetic anhydride-pyridine at room temperature, it gave a mono-acetate, mp 253-254°, $[\alpha]_D$ + 20.6. The IR spectrum (Fig 7) of the acetate shows the presence of a hydroxy group at 3490 cm^{-1} and an acetate group at 1710 and 1260 cm^{-1}. Attempted acetylation of the second hydroxy group at higher temperature furnished a solid, mp 216-218°, $[\alpha]_D$ + 47.5° which was analysed for $C_{32}H_{52}O_2$. The IR (Fig 8) absorption bands of the product at 3040, 1640, 1730, 1260 and 888 cm^{-1} indicates the introduction of a methylene double bond with the loss of the hydroxy group.

The ^1H NMR spectrum (Fig 9) of the dehydrated product of mono-hydroxy-mono-acetate shows the presence of seven methyl groups. The peak at 2.02 ppm represents the methyl group of the acetate. The multiplets centred at 4.60 and 4.70 ppm are due to the two terminal methylene protons coupled to the methyl at 1.25 ppm which confirms the

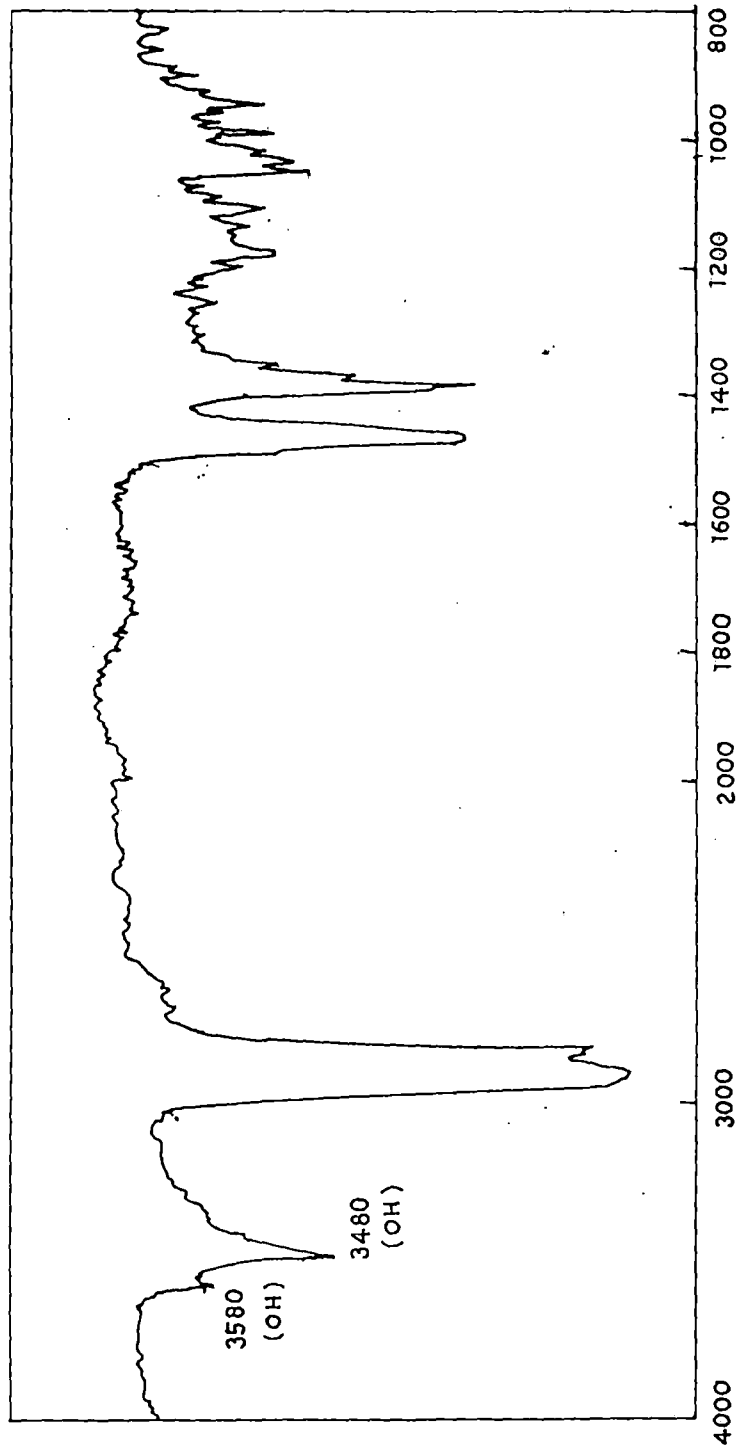


Fig.6. IR spectrum of 3β,20-dihydroxylupan (3)

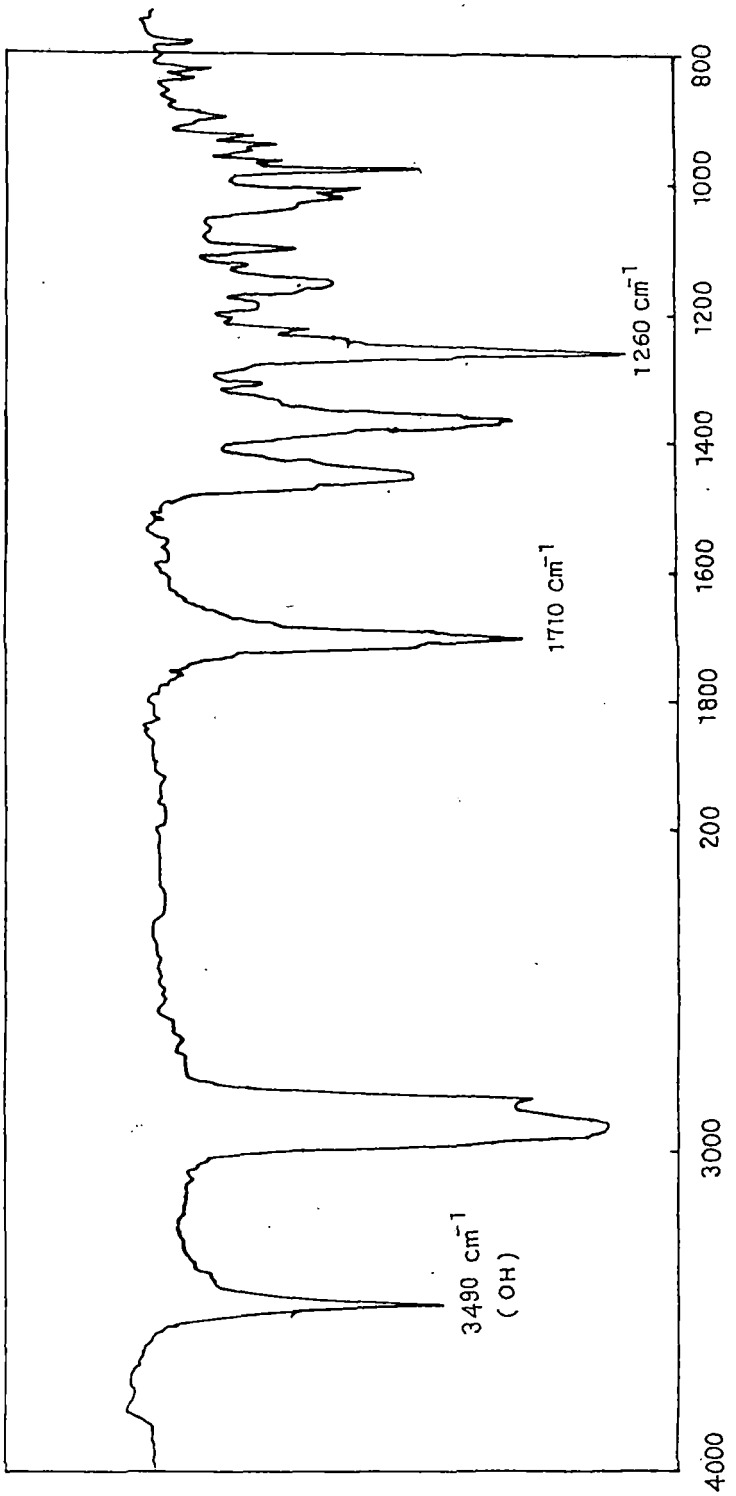


Fig 7. IR spectrum of 20 hydroxy lupan-3β-acetate (4)

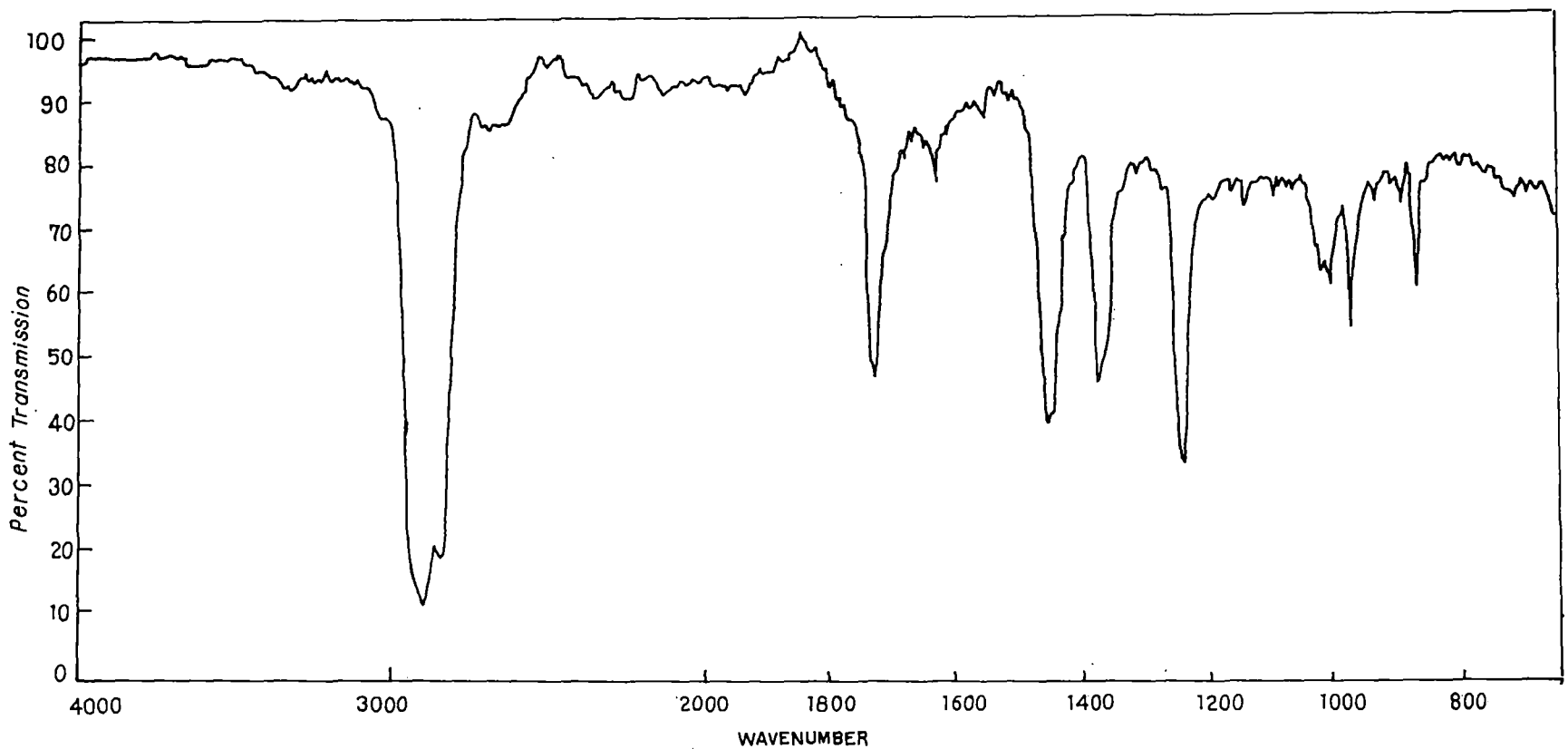


Fig 8. IR spectrum of lupenyl acetate (5)

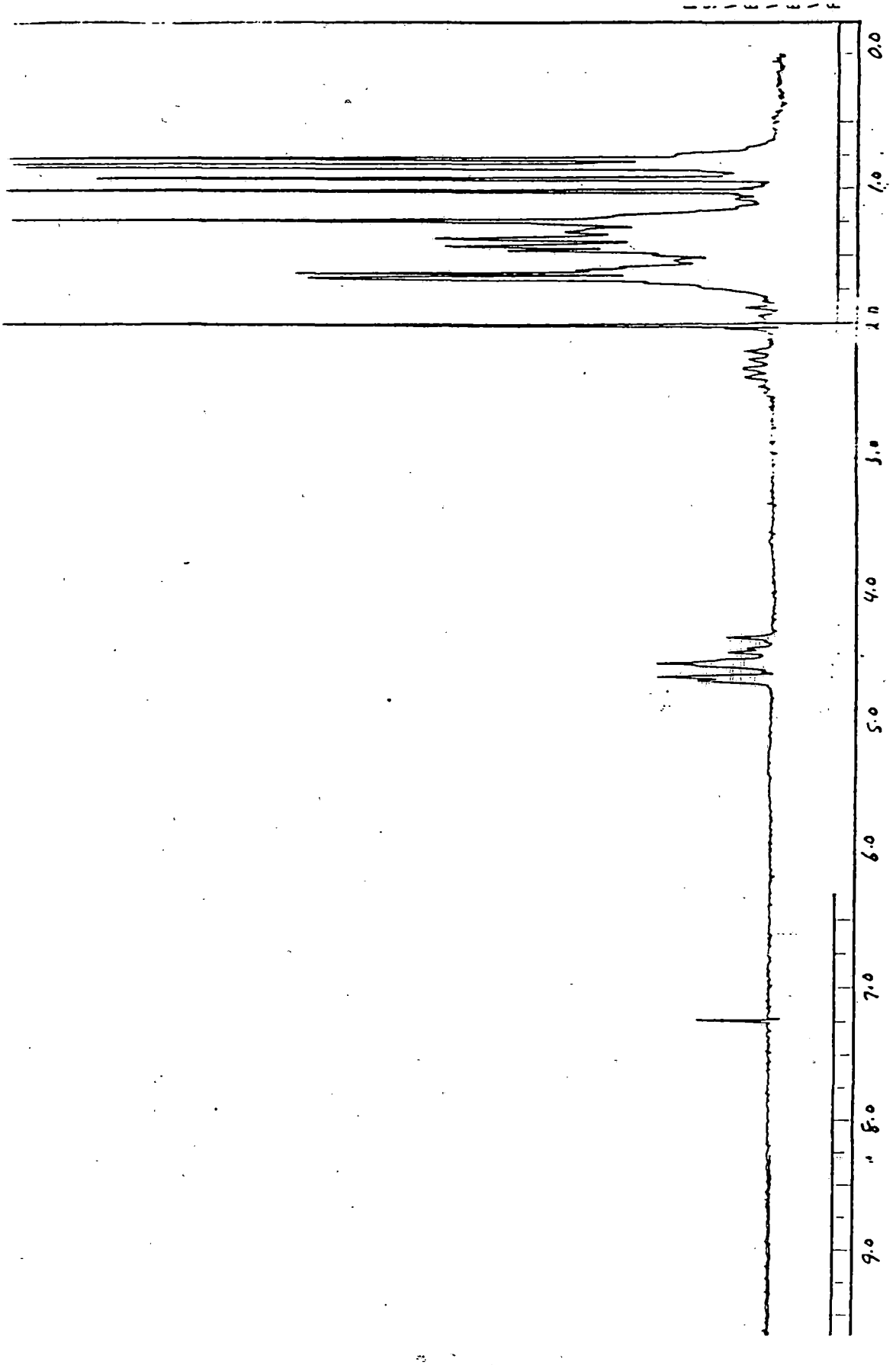
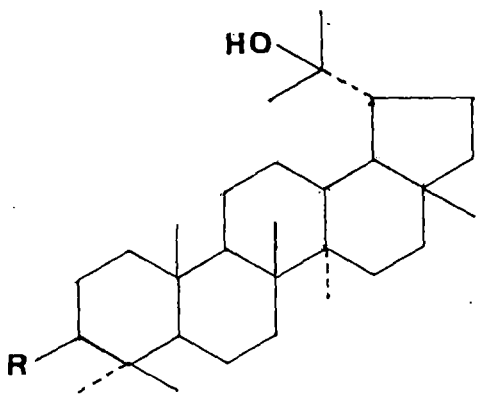
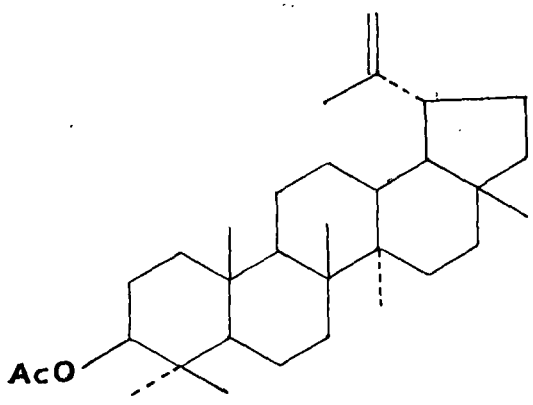


Fig 9. ^1H NMR of lupenyl acetate (5) at 200 MHz.

presence of isopropylidine moiety. The multiplet centred at 4.50 ppm has arisen from the proton geminal to the acetoxy group at C-3 with β-orientation.

The physical data of the dehydrated product are found to be identical with lupenyl acetate (5) and the mono-hydroxy-mono-acetate is found to be identical with an





3 R = OH
4 R = OAc

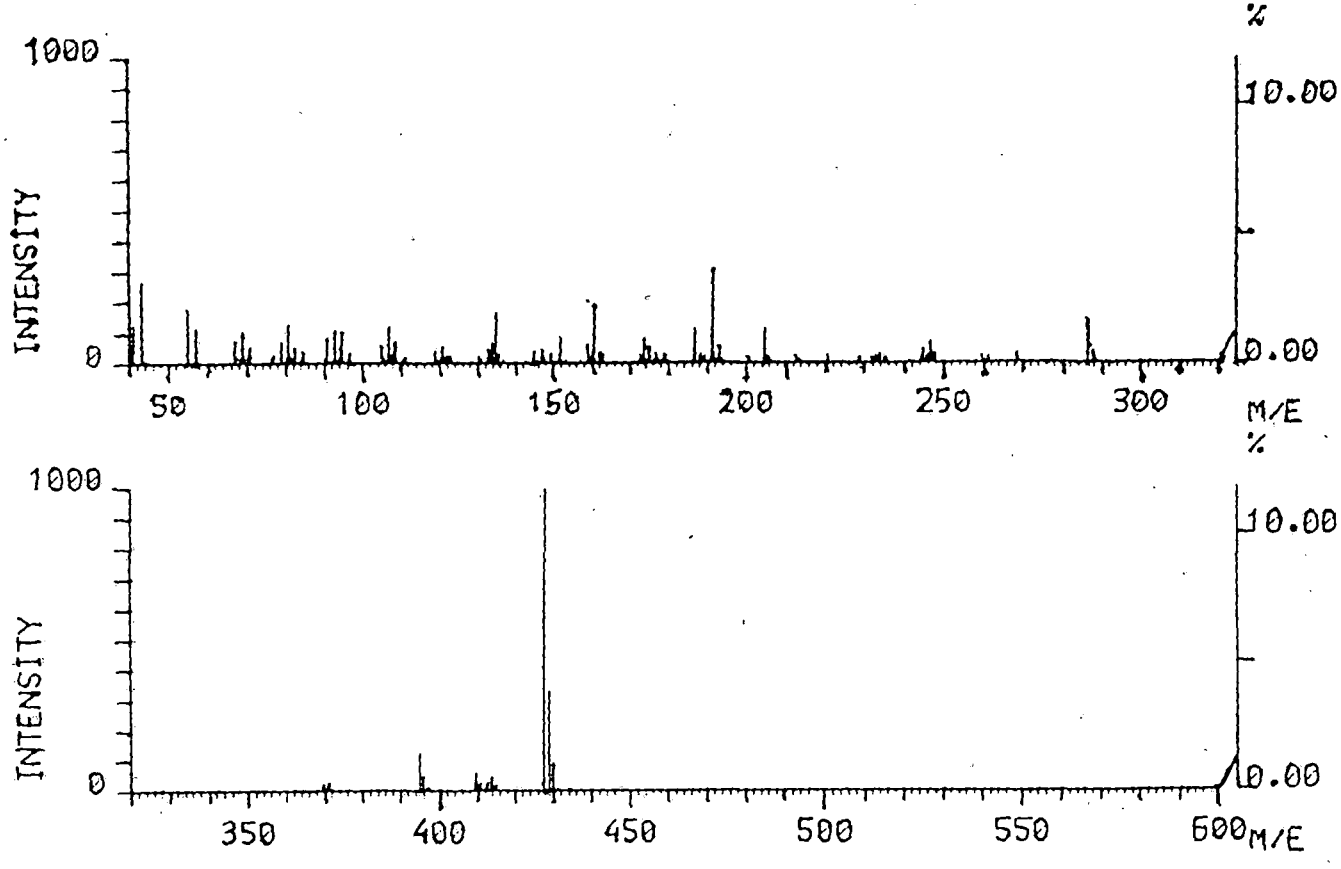
5

authentic sample of 20-hydroxylupan-3β-acetate (4). Thus the diol is confirmed as 3β, 20-dihydroxylupane (3).

Examination of fraction 7 (Table 1) : Isolation and structure elucidation of a new sterol, sepestenol

The fraction 7 was purified by rechromatography over silica gel column and crystallized from chloroform-methanol to yield crystalline solid, mp 132-133°, $[\alpha]_D$ - 98°. The elemental analysis and MS (Fig 10a) showed that the compound to have molecular formula, $C_{29}H_{48}O_2$. The IR (Fig 10b) absorption bands at 3520, 1655 and 1620 cm^{-1} indicated that the compound contains one hydroxy group and an α,β-unsaturated carbonyl moiety. It gave positive Liebermann-Burchardt test for sterol and the compound is designated as sepesteonol.

BASE PEAK : M/E 428.0 INT. 667.3



M/E	RAW INT.	R.INT.	SIGMA(%)	
41.0	86.0	128.8	3.21	
43.0	178.0	266.8	6.65	
55.0	122.3	183.3	4.57	
57.0	86.9	130.2	3.24	
69.0	75.5	113.2	2.82	
81.0	91.3	136.9	3.41	
93.0	77.3	115.9	2.89	
95.0	72.6	108.9	2.71	
107.0	80.4	120.5	3.00	
135.0	112.4	168.5	4.20	
152.0	62.6	93.9	2.34	
161.0	131.2	196.7	4.90	
187.0	78.9	118.2	2.94	
192.0	205.6	308.1	7.68	
205.0	78.2	117.2	2.92	
287.0	98.4	147.4	3.67	
395.0	89.1	133.5	3.33	
428.0	667.3	1000.0	24.93	
429.0	220.2	330.1	8.23	
430.0	61.2	91.7	2.28	END

Fig 10a. M S spectrum of sepesteonol (I)

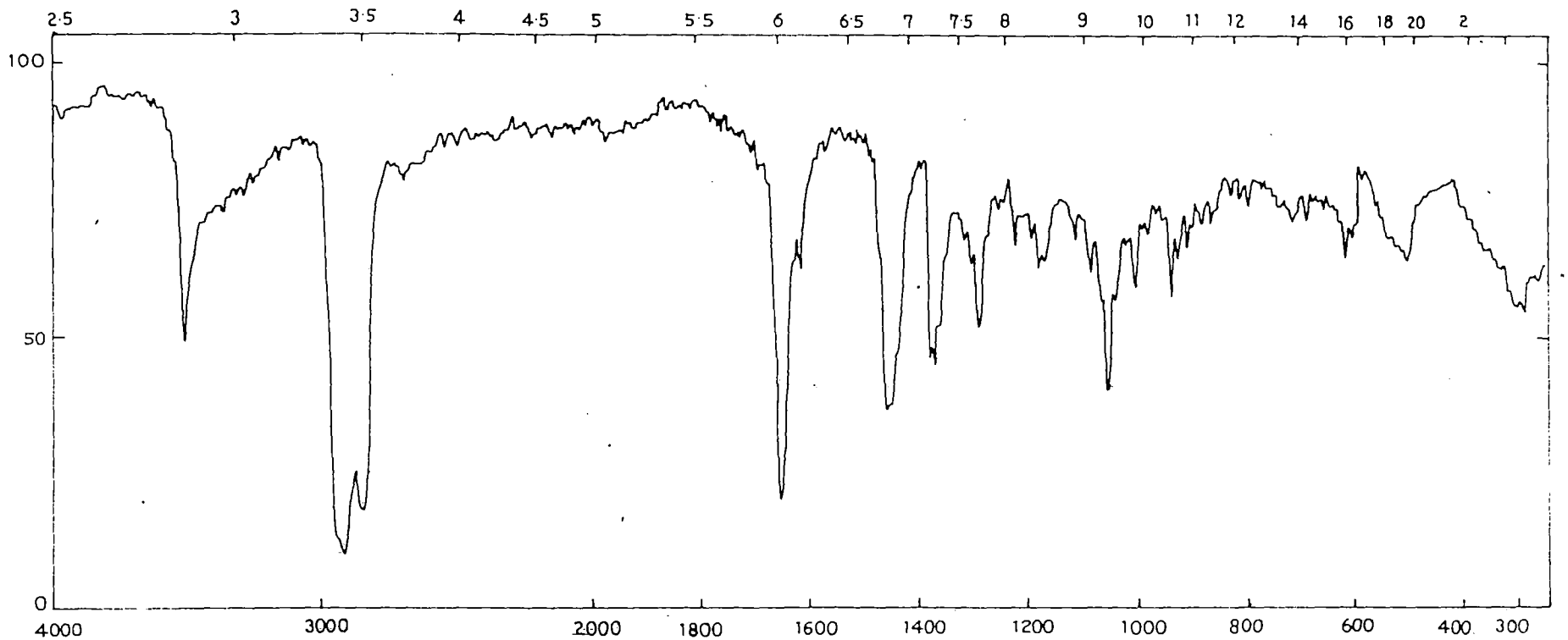
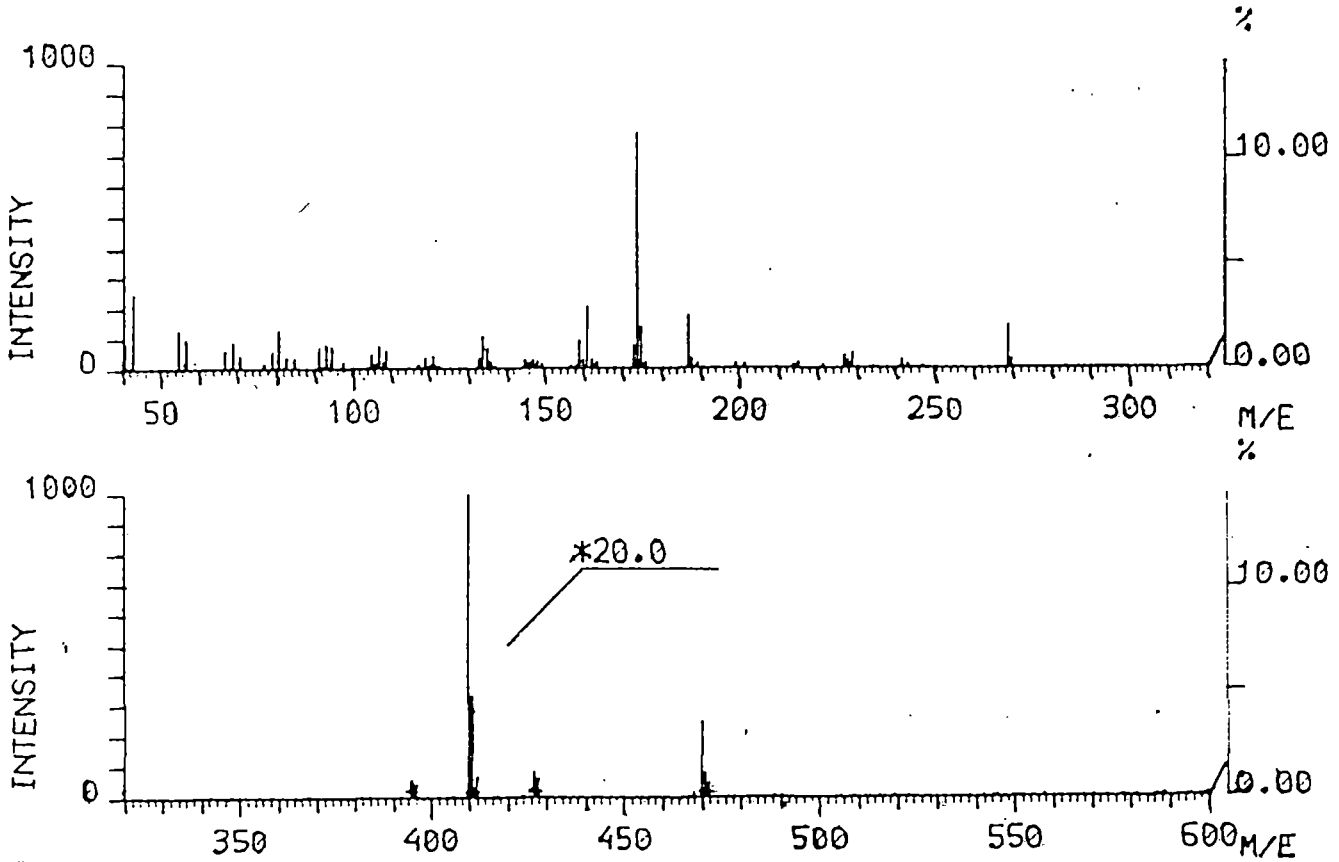


Fig 10b. IR Spectrum of sepesteonol (7)

Sepesteonol on acetylation with acetic anhydride-pyridine gave sepesteonyl acetate, $C_{31}H_{50}O_3$ (M^+ 470; see Fig 11a), mp 169-170°, $[\alpha]_D$ - 92°. The IR (Fig 11b) bands of sepesteonyl acetate at 1724 and 1260 cm^{-1} show the presence of an acetoxy group and the bands at 1665 and 1240 cm^{-1} for an α,β-unsaturated carbonyl moiety. The α,β-unsaturated carbonyl moiety was further supported by the peak at λ_{max} 235 nm in UV spectrum (Fig 12).

The ^1H NMR spectrum (Fig 13) of sepesteonyl acetate shows the presence of six methyl groups within the range 0.67-1.19 ppm, an acetoxy methyl group at 2.01 ppm, a proton on the carbon bearing the acetoxy group at 4.70 ppm as a multiplet and a vinyl proton at 5.70 ppm as a doublet with a small coupling constant J = 2 Hz. The integration curve over the methyl region shows that the peaks at 0.67 and 1.19 ppm represent two singlet methyls while the peaks in the region 0.78-0.92 ppm are due to four methyl groups which are splitted. The assignment of these four methyl group is made by the use of a 2-dimensional NMR experiment, called HOM2DJ (homonuclear 2-dimensional J-spectroscopy)[4] which plots chemical shifts of the methyls along one axis and spin-spin couplings along an axis at right angles to the first. This is shown in Fig 14. From this plot it is very clear that the C-26 (0.78 ppm) and C-27 (0.80 ppm) methyl groups are non-equivalent (peaks a,a and b,b) and split into doublets with J=7.5 Hz by a proton on C-25, while the three peaks at 0.77, 0.81 and 0.85 ppm (marked as C, C, C in Fig 14) are the members of a triplet of C-29 methyl (0.81 ppm) and thereby prove the presence of a C-ethyl moiety. The peaks d,d are the members of a doublet due to C-21 methyl (0.89 ppm).

The APT[1] spectrum (Fig 15) of sepesteonyl acetate shows that there are 10 CH_2, 9 CH, 7 CH_3 (signal at 11.78



BASE PEAK : M/E 410.0 INT. 413.5

M/E	RAW INT.	R.INT.	SIGMA(%)	
41.0	39.5	95.6	2.24	
43.0	106.8	258.2	6.06	
55.0	58.2	140.7	3.30	
57.0	43.5	105.3	2.47	
69.0	37.4	90.6	2.12	
81.0	54.6	132.0	3.10	
91.0	30.5	73.8	1.73	
93.0	37.3	90.2	2.11	
95.0	32.4	78.5	1.84	
107.0	34.1	82.4	1.93	
134.0	44.4	107.4	2.52	
159.0	42.1	101.9	2.39	
161.0	86.2	208.4	4.89	
173.0	36.0	87.2	2.04	
174.0	321.0	776.2	18.22	
175.0	59.3	143.4	3.36	
187.0	77.6	187.7	4.40	
269.0	66.8	161.6	3.79	
410.0	413.5	1000.0	23.47	
411.0	139.5	337.5	7.92	END

Fig 11a. MS spectrum of sepesteonyl acetate (8)

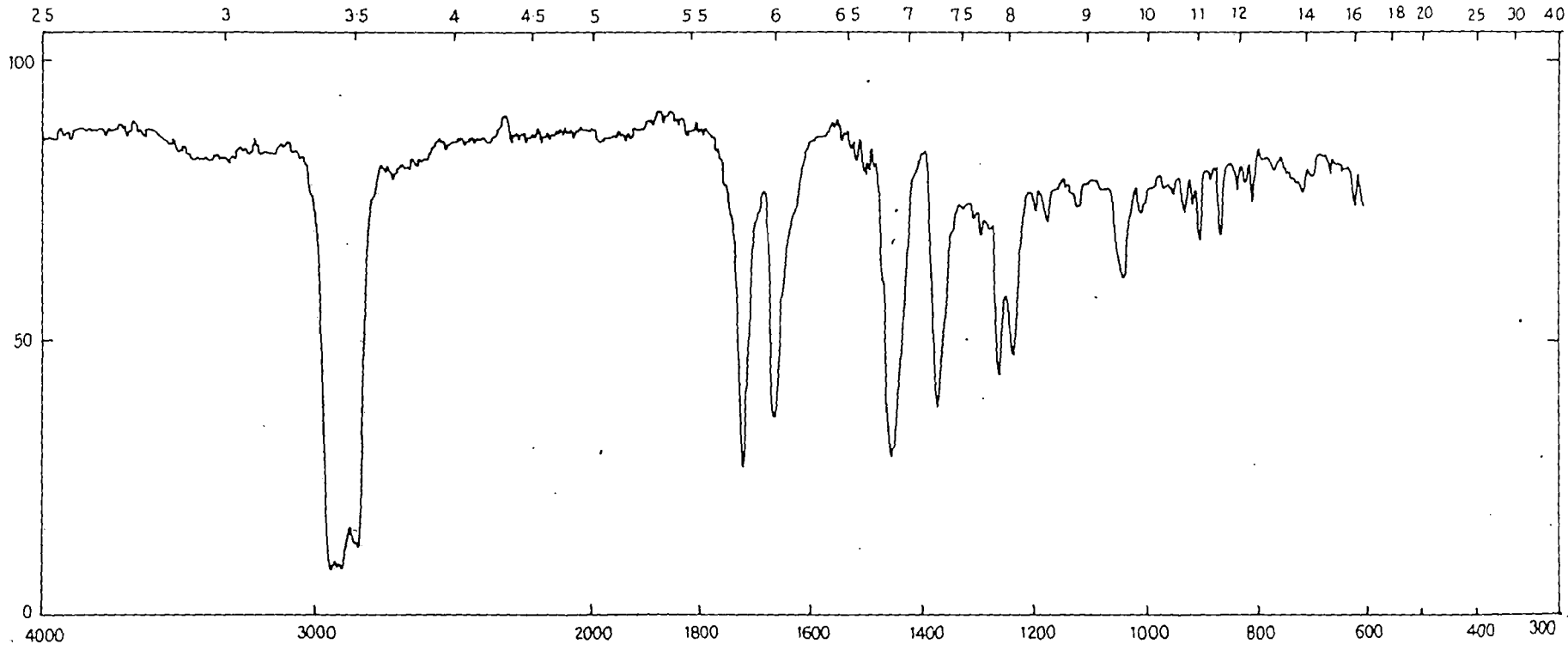


Fig 11b. IR spectrum of sepesteonyl acetate (8)

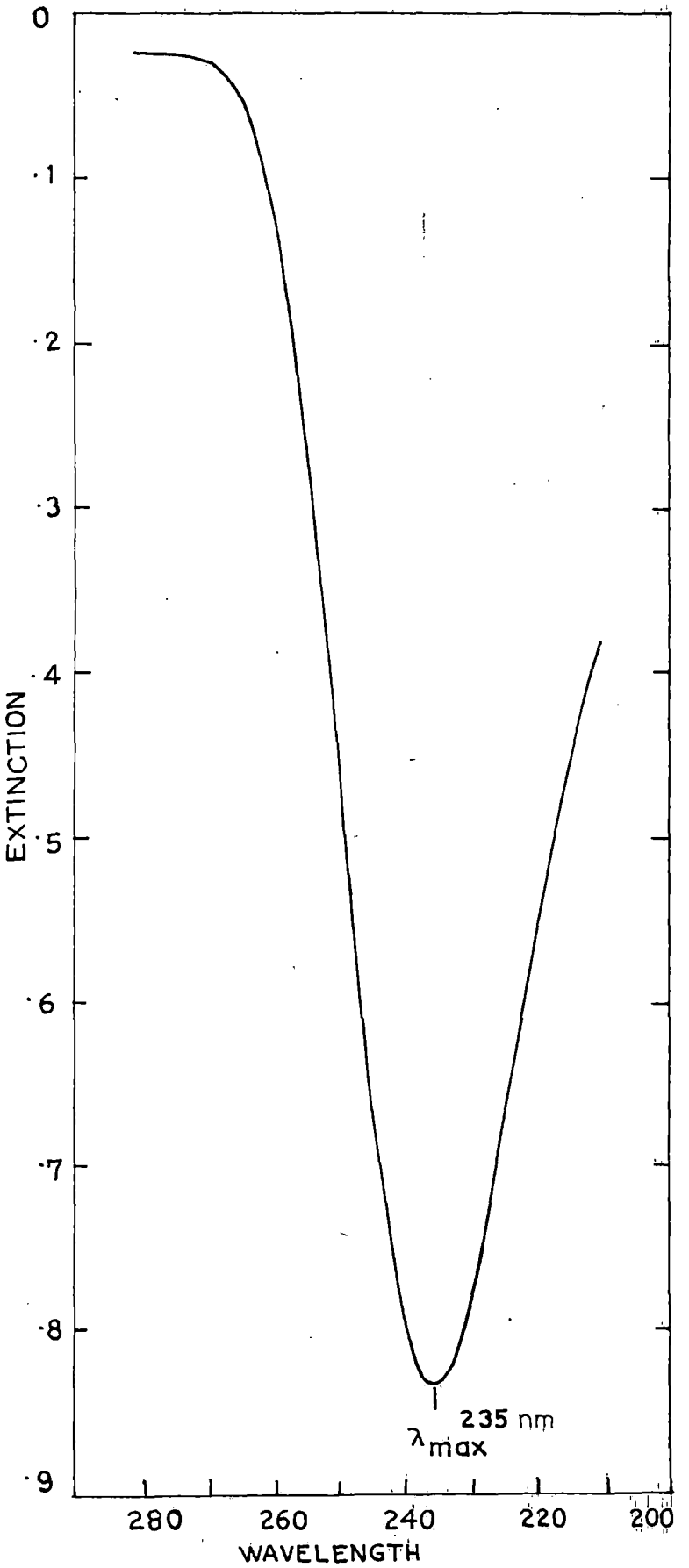


Fig.12. UV spectra of sepesteonyl acetate (8)

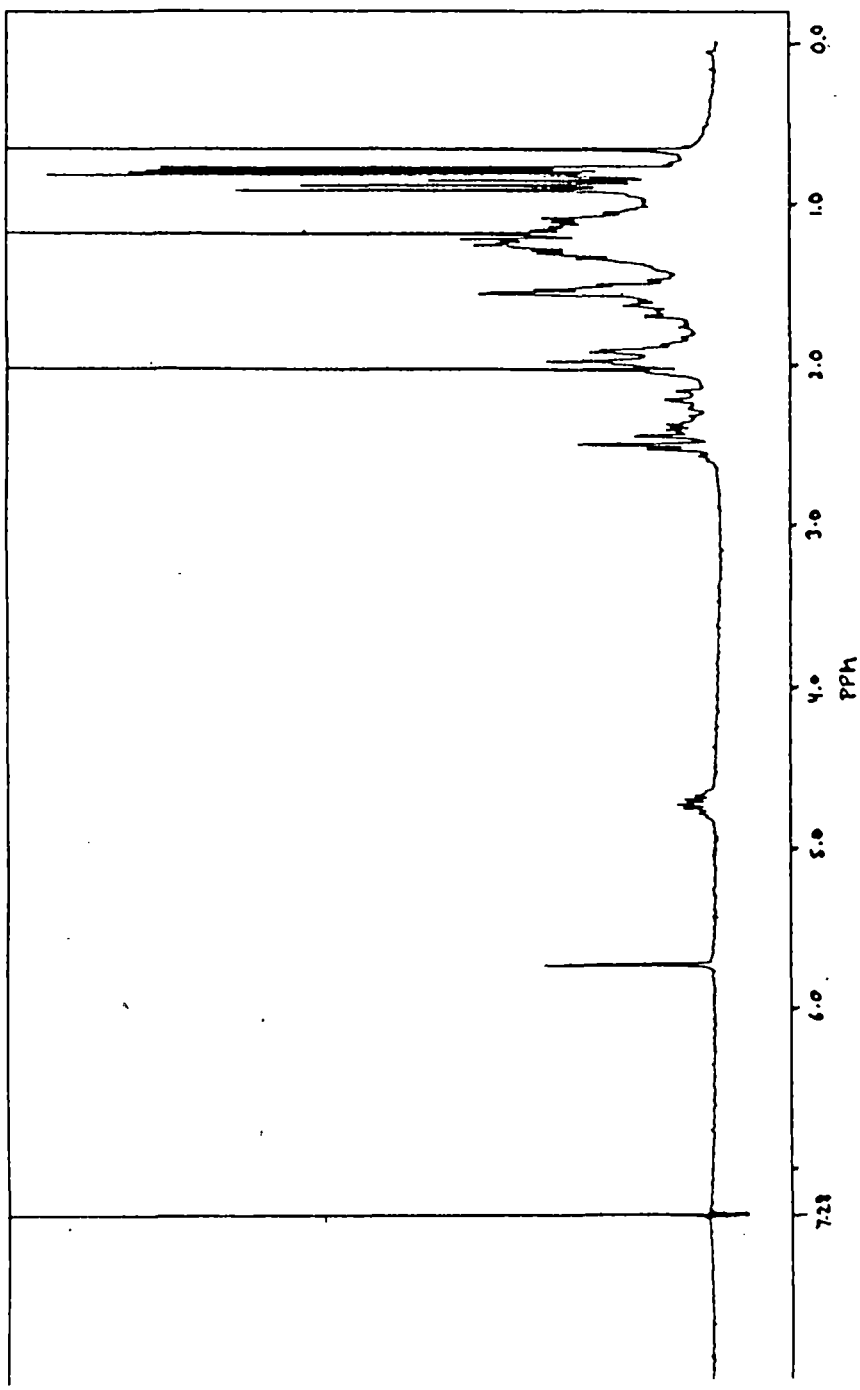


Fig 13. ^1H NMR spectrum of sepesteonyl acetate (8) at 300 MHz

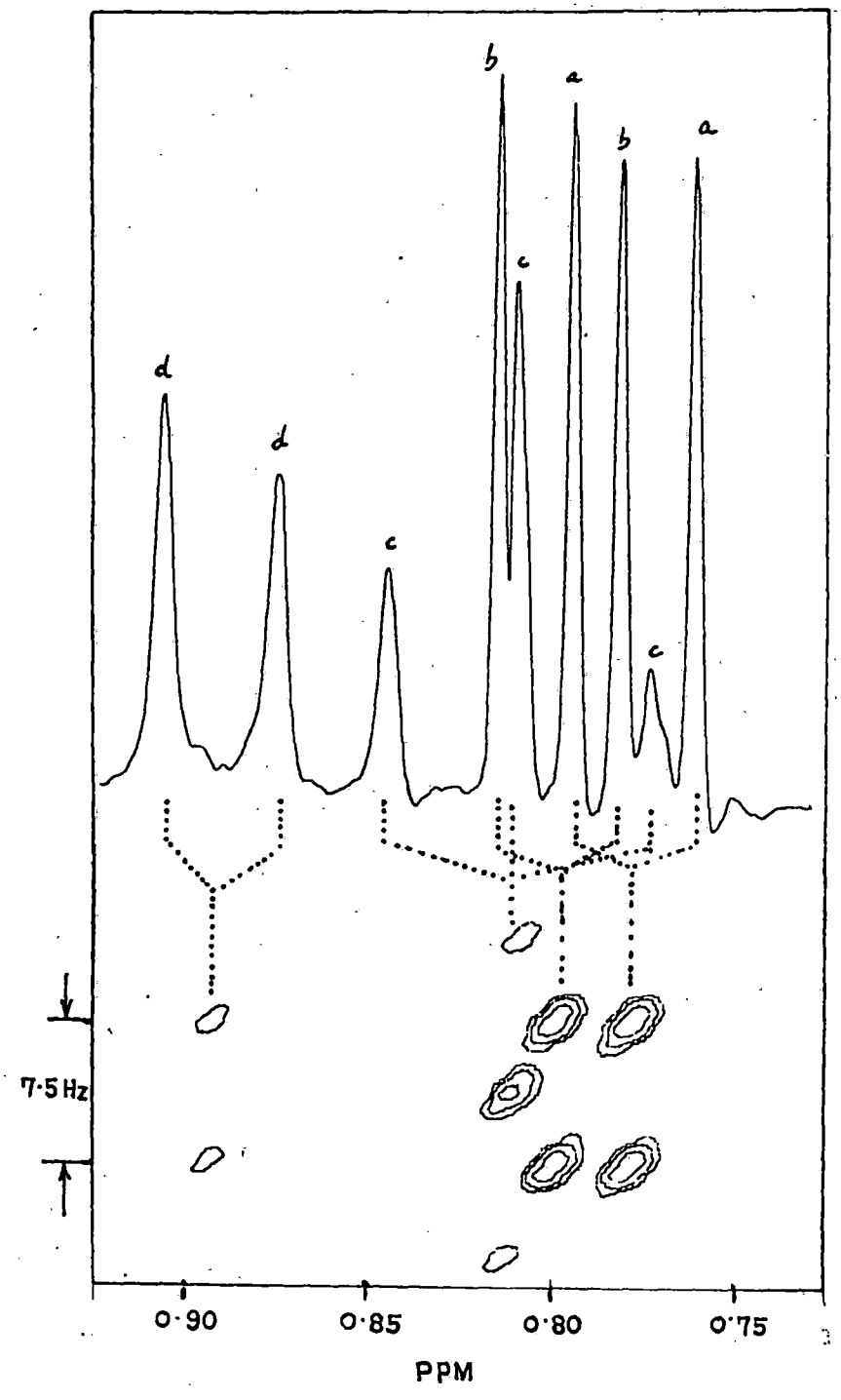


Fig 14. HOM2D J spectrum of sepesteonyl acetate (8)

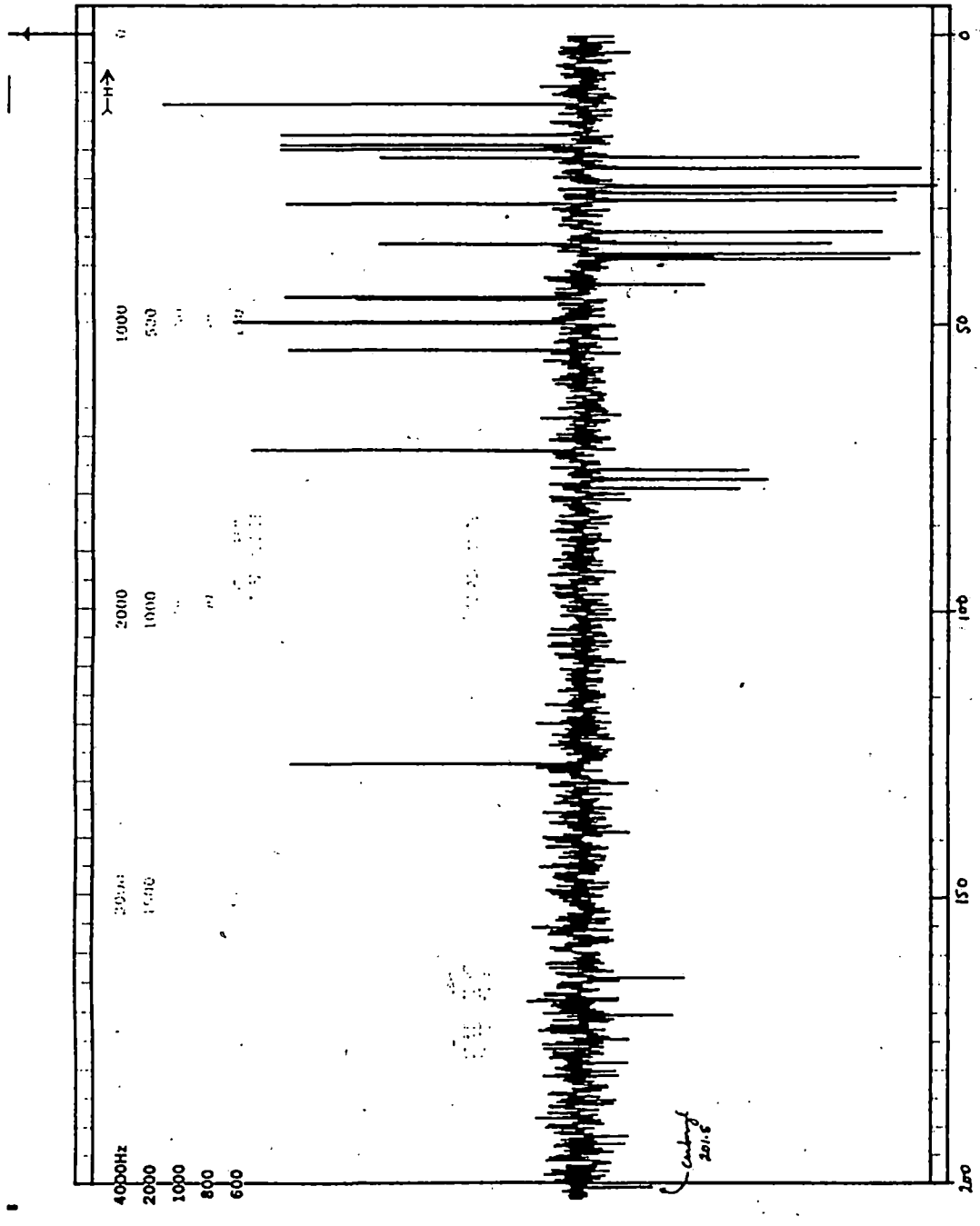
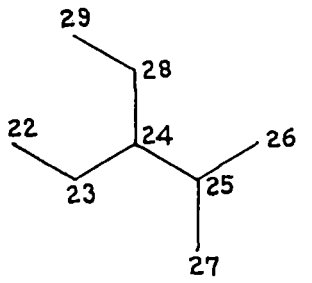


Fig 15. APT spectrum of sepesteonyl acetate (8) at 20 MHz

ppm represents two CH_3) and 5 non-protonated carbons. The ^{13}C shift values of different type of carbon atoms are represented in Table 4.



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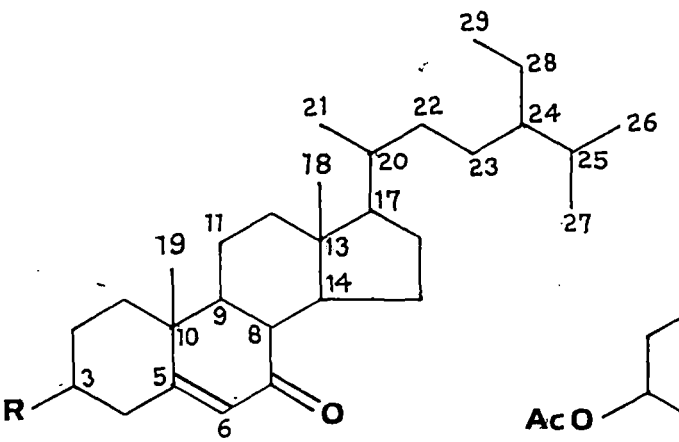
(Model compound)



7 R = OH
8 R = OAc

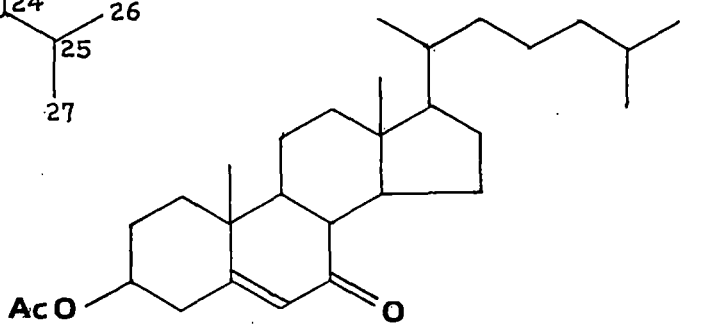


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Table 4

Carbon-13 chemical shifts of different type of carbons of sepesteonyl acetate

Groups	Number of groups	Carbon-13 chemical shifts (ppm)
$-CH_3$	7	11.78(26), 17.07, 18.74, 18.85, 19.60, 21.07

/contd ...

Table 4 (contd)

$-\overset{\vert}{C}H_2$		10	20.97, 22.86, 25.92, 26.12, 27.17, 28.36, 33.76, 35.82, 37.56, 38.47
$-\overset{\vert}{\underset{\vert}{C}}H$		7	28.96, 35.91, 45.24, 45.64, 49.64, 49.78, 54.51
$O-\overset{\vert}{\underset{\vert}{C}}H$	(Diagnosed from shift value)	1	72.03
$=\overset{\vert}{C}H$	(Diagnosed from shift value)	1	126.53
$-\overset{\vert}{\underset{\vert}{C}}-$		2	38.13, 42.93
$=\overset{\vert}{C}-$	(Diagnosed from shift value)	1	163.53
$-\overset{\vert}{C}=O$		2	170.63 (acetate carbonyl), 201.50 (keto carbonyl)

Total number of carbons = 31, protons = 50, oxygens = 3

The ^{13}C shifts of sepesteonyl acetate as structure 8 agree well with those for the model compound (6). If we number the carbon atoms with the same numbering system in both the steroid (8) and the model (6), we get the following comparison of shifts (Table 5).

Table 5

^{13}C shifts (ppm) comparison for the side chain of sepesteonyl acetate (8) with the model compound (6).

Carbon atoms	Model compound (6)[a]	Sepesteonyl acetate (8)[b]
22	11.80	33.76
23	22.60	25.92
24	47.60	45.64
25	29.10	28.96
26	19.00	18.85
27	19.00	19.60
28	22.60	22.86
29	11.80	11.78

[a]Values are obtained by applying Lindeman-Adams' additivity rules[5]

[b]Values are obtained experimentally

Carbon atoms 22 and 23 show the expected shift in accordance with Lindeman-Adams' additivity rules[5], in going from the model hydrocarbon (6) to the steroid, sepesteonyl acetate (8), since C-22 adds an alpha, two beta and two gamma carbons which would increase the ^{13}C chemical shift by about 21 ppm, while C-23 gains a beta and two gamma carbons which would be expected to increase the shift by 3 ppm. The rest of the side chain carbons are quite similar to the model compound (6). Any other position of C-ethyl except at C-24 would change most of the ^{13}C shifts significantly, so that the observed match constitutes very strong evidence for the side chain as shown in the proposed structure 8 for sepesteonyl acetate. The ^{13}C shifts of the rest of the carbon atoms of sepesteonyl acetate (8) are found to be very close to those of 3 β-acetoxy-cholest-5-en-7-one (9)[6] as shown in Fig 16. This observed match of ^{13}C chemical shifts of sepesteonyl acetate (8) with 3 β-acetoxy-cholest-5-en-7-

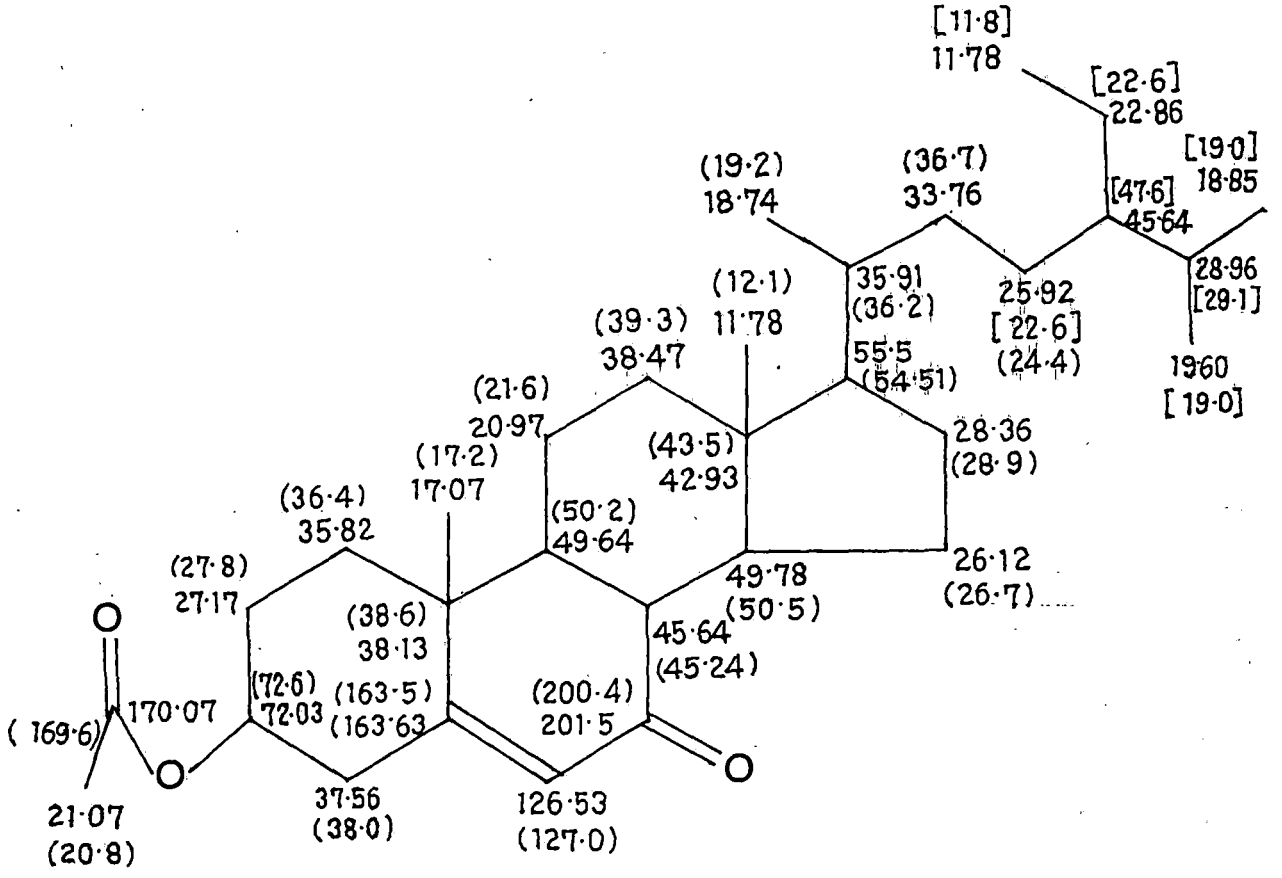
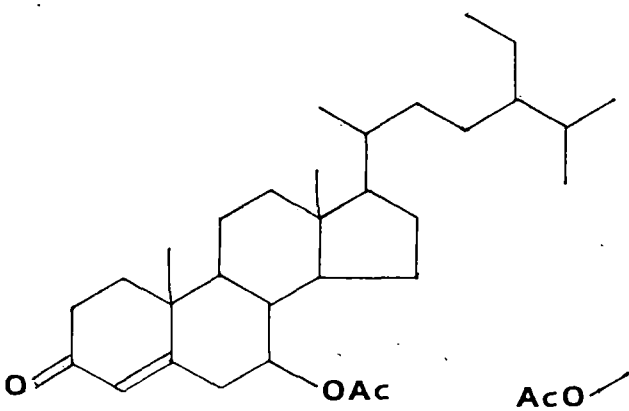


Fig 16. Numerical figures are the ^{13}C chemical shifts of sepesteonyl acetate. Data in parentheses are the ^{13}C shifts of 9 . Beyond C-22 in the side chain, the shifts in square brackets represent the shifts from the model hydrocarbon (6).

one (9) provides strong evidence in favour of the proposed structure, 3β-acetoxystigmast-5-en-7-one (8) for sepesteonyl acetate.

The possibility still remained that a 7-acetoxy-4-en-3-one (10) might satisfy the spectral data. A second 2-dimensional experiment (2D COSY)[7] provided further evidence for 3-acetoxy-5-en-7-one (8) structure. This is shown in Fig 17 as a contour plot (looking down). The lower right corner is the chemical shift of TMS and the



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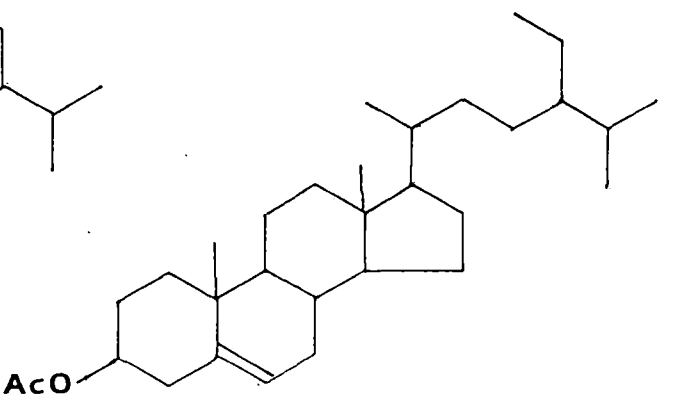

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spectral peaks appear along the diagonal of the plot.* The off-diagonal elements of the plot represent spin-spin coupling between the protons whose signals on the diagonal have the same x- and y- coordinates. Thus, the dotted lines show that the doublet at 5.70 ppm (proton on C-6) is spin-spin coupled to the group of signals at 470 Hz (~2.50 ppm) and no other protons. The signals

*There is a small shift of frequency between 2D COSY plot (Fig 17) and the proton spectrum (Fig 13) due to running the spectra with different instrument settings. For example, the multiplet at 4.70 ppm (940 Hz relative to TMS) is found near 900 Hz on the 2D plot.

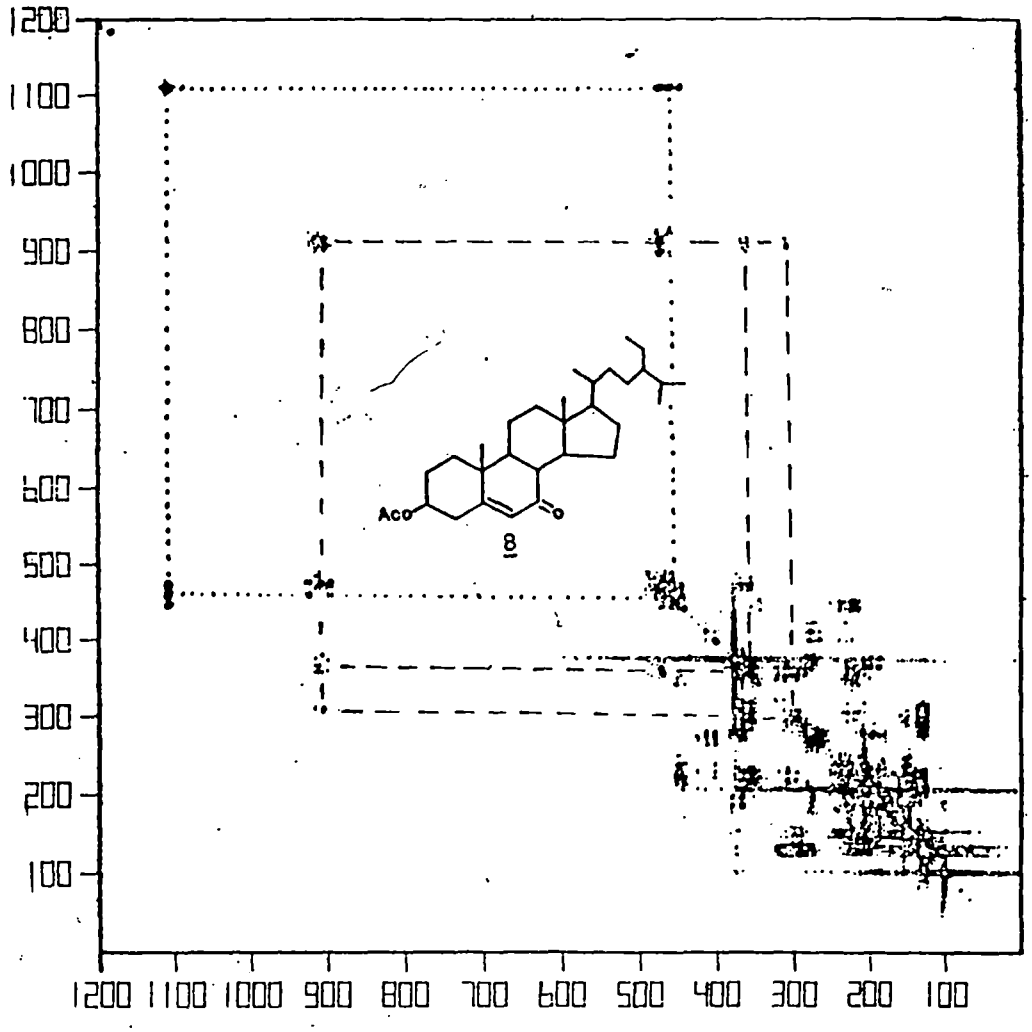


FIG 17. 2D HOMONUCLEAR CORRELATION SPECTRUM OF 8

at 2.50 ppm would be expected to arise from the protons on C-4, which would couple to the proton on C-6 with an allylic coupling constant (J = 2 Hz). The proton at 4.70 ppm (proton on C-3, ie the proton geminal to the acetoxy group), on the other hand, shows coupling (shown by the dashed lines in Fig 17) to the protons on C-4 (2.50 ppm), but also to two other protons, namely the axial (1.70 ppm) and equatorial (2 ppm) protons on C-2. Consequently, it must have four neighbouring protons, and hence cannot arise from 7-acetoxy-4-en-3-one structure (10) which (proton at 4.70 ppm) would have only three neighbours.

It is possible to preject the peaks, represented by the spots and circles in the contour plot. These projections look like partial spectra in which only the peaks, coupled to the proton on the diagonal, show up. Fig 18 illustrates this technique and locates the 2a and 2e protons a bit more precisely.

The above discussion confirms the structure 8 for sepesteonyl acetate. Therefore, sepesteonol is 3β-hydroxy-stigmast-5-en-7-one (7).

Partial synthesis of sepesteonyl acetate from β-sitosteryl acetate

β-sitosteryl acetate (11) in benzene was refluxed with a solution of sodium dichromate in glacial acetic acid for 4 hours. The excess sodium dichromate was destroyed by the addition of methanol and the bulk of the whole mass was reduced to one-third of the original volume and then poured into ice-cold water when a solid material separates out. After usual work-up, the product was purified by column chromatography and repeated crystallization from chloroform-methanol mixture. The crystalline

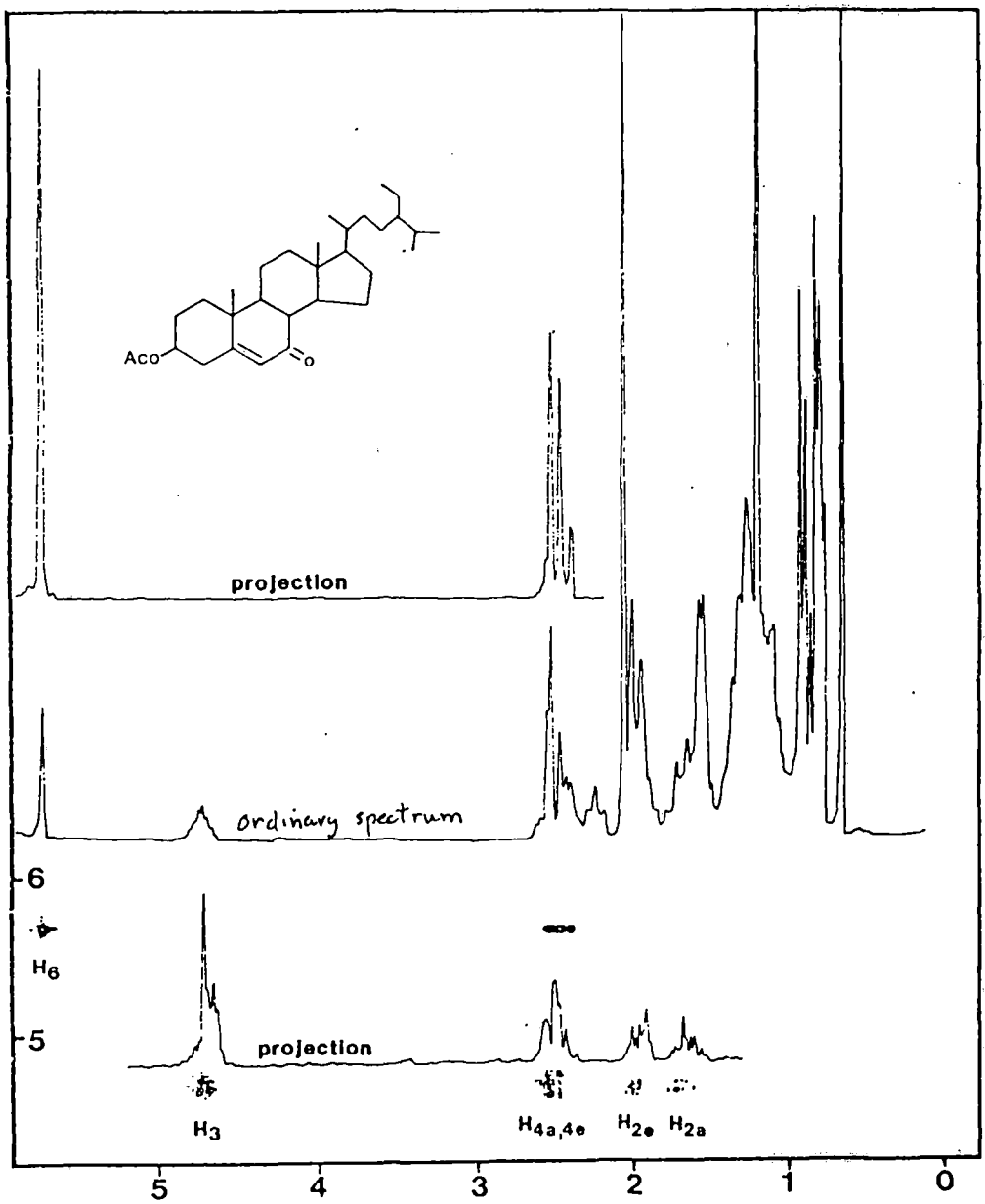


Fig 18. Partial projection of ^1H spectrum of sepesteonyl acetate (8

solid, mp 168-169°, $[\alpha]_D$ - 90° was found to be identical with sepesteonyl acetate 8 (mixed mp, co-TLC and co-IR).

Attempted hydrolysis of sepesteonyl acetate

Sepesteonyl acetate (8) was refluxed with 10% methanolic potassium hydroxide for 3 hours. The product, after usual work-up was chromatographed and crystallized from acetone to yield crystalline solid, mp 111-112° $[\alpha]_D$ - 290°. The same product was obtained when sepesteonyl acetate was refluxed with methanol in presence of p-toluenesulphonic acid.

Elemental analysis showed that the molecular formula of the compound to be $C_{29}H_{46}O$, which was supported by the MS (Fig 22) that exhibited the molecular ion peak at m/z 410. The IR spectrum (Fig 19) of the product shows the absence of hydroxy or the acetoxy carbonyl bands, but exhibits the existence of an α,β-unsaturated carbonyl band at 1650 cm^{-1} and carbon-carbon double bond bands at 1620 and 1600 cm^{-1}. The UV spectrum (Fig 20) shows absorption maximum at λ_{max} 280 nm and the difference of λ_{max} (45 nm) from that of sepesteonyl acetate (8) indicates the introduction of a double bond (between C-3 and C-4) in conjugation to the enone system of 8. Therefore, the ^1H NMR spectrum (Fig 21) of the product showed six methyl groups within the range 0.73 to 1.13 ppm, three olefinic protons at 5.62, 6.11 and 6.20 ppm which are consistent with the structure 12 for the product. The singlet peaks at 1.13 and 0.73 ppm are due to two tertiary methyls (C-18 and C-19 methyls), doublet at 0.95 ppm (J = 6.5 Hz) is due to a secondary methyl (C-21 methyl), and the peaks at 0.88, 0.86, 0.84 and 0.82 ppm are due to two secondary (C-26 and C-27 methyls) and one primary methyl (C-29 methyl) groups.

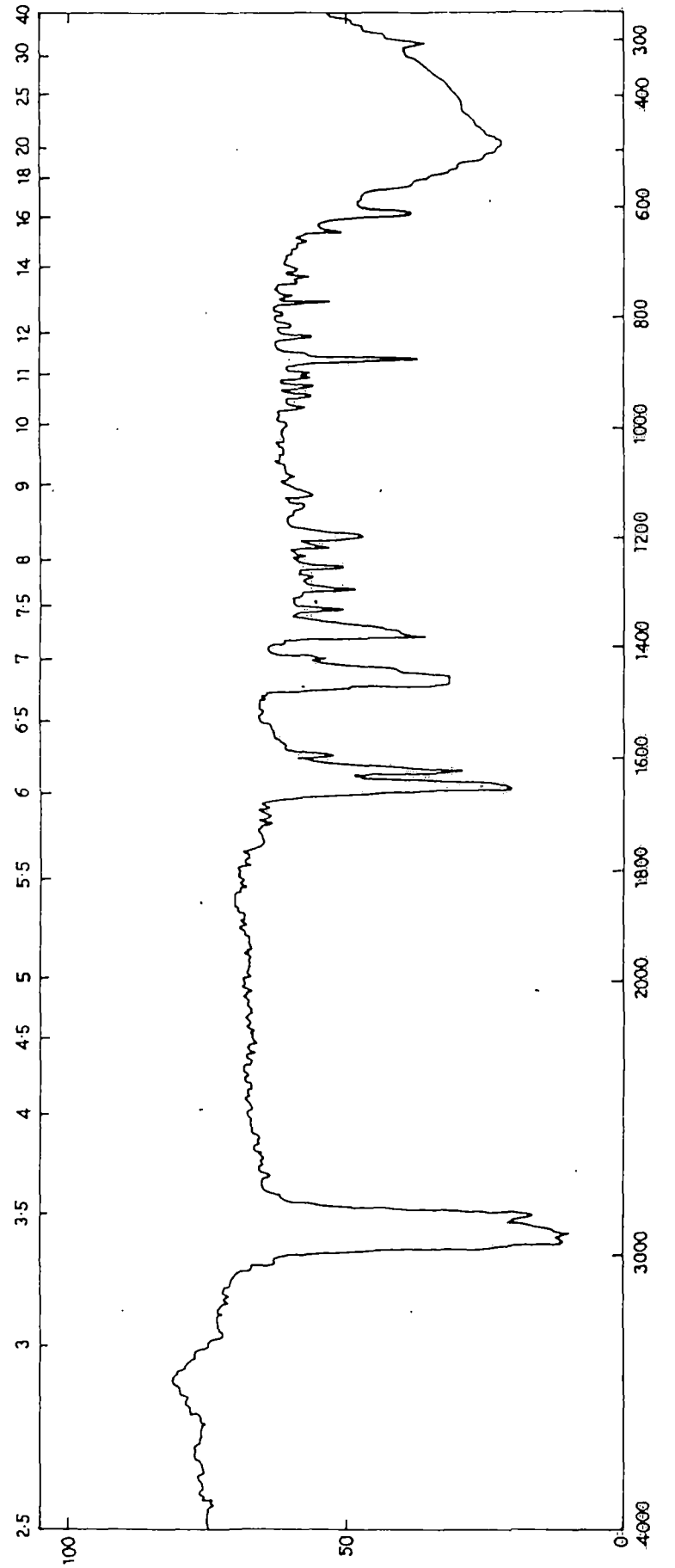


Fig 19. IR spectrum of tremulone (12)

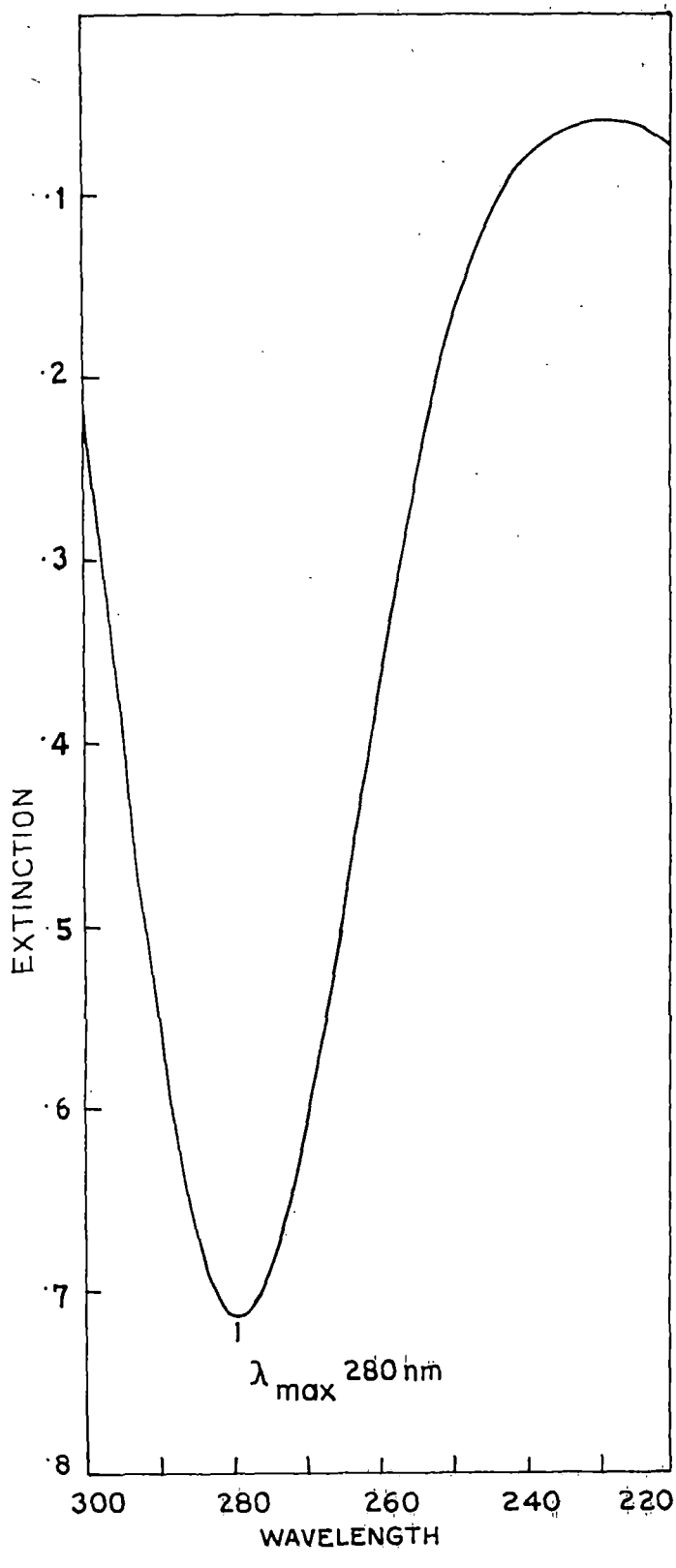


Fig 20. UV spectrum of tremulone (12)

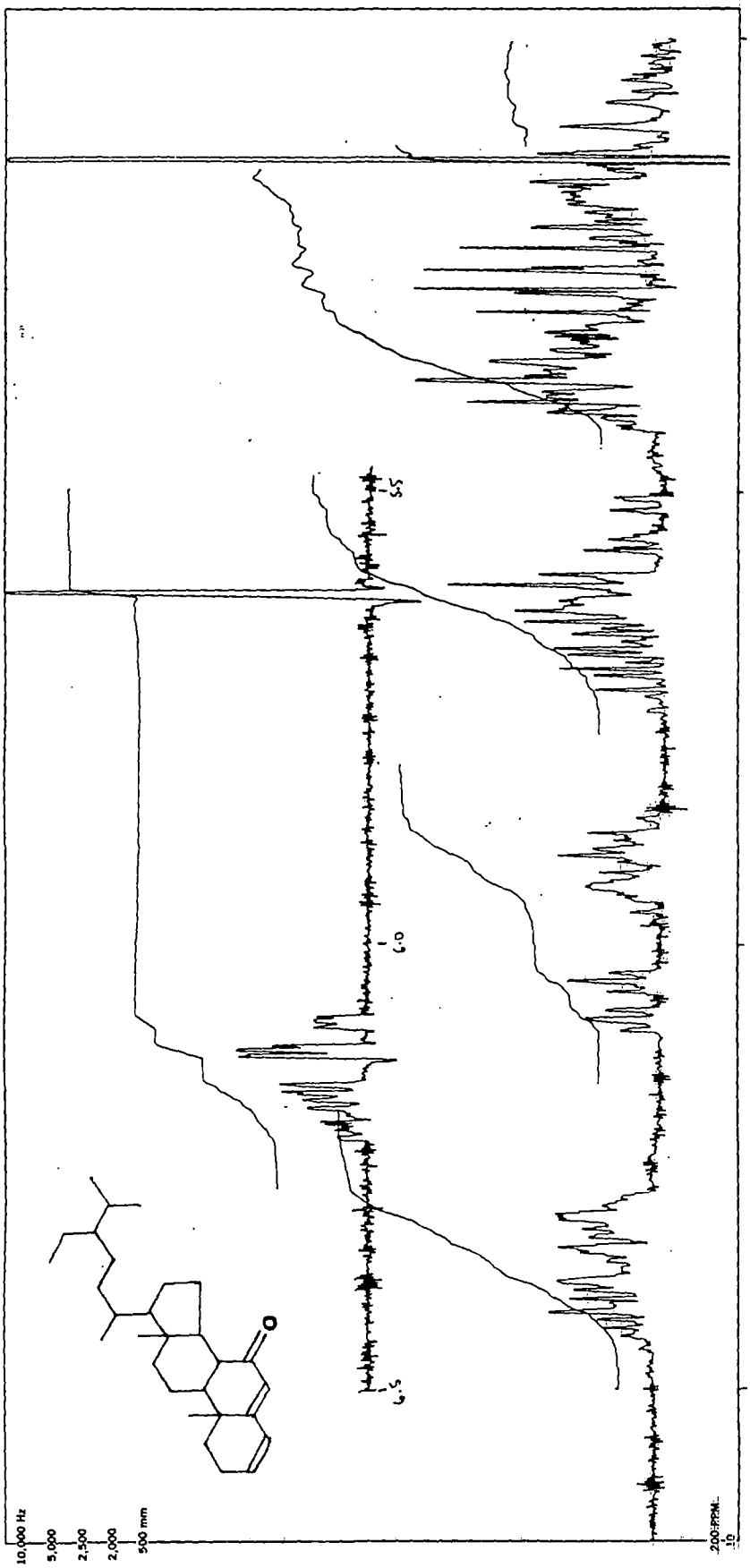


Fig 21. ^1H NMR spectrum of tremulone (12) at 300 MHz

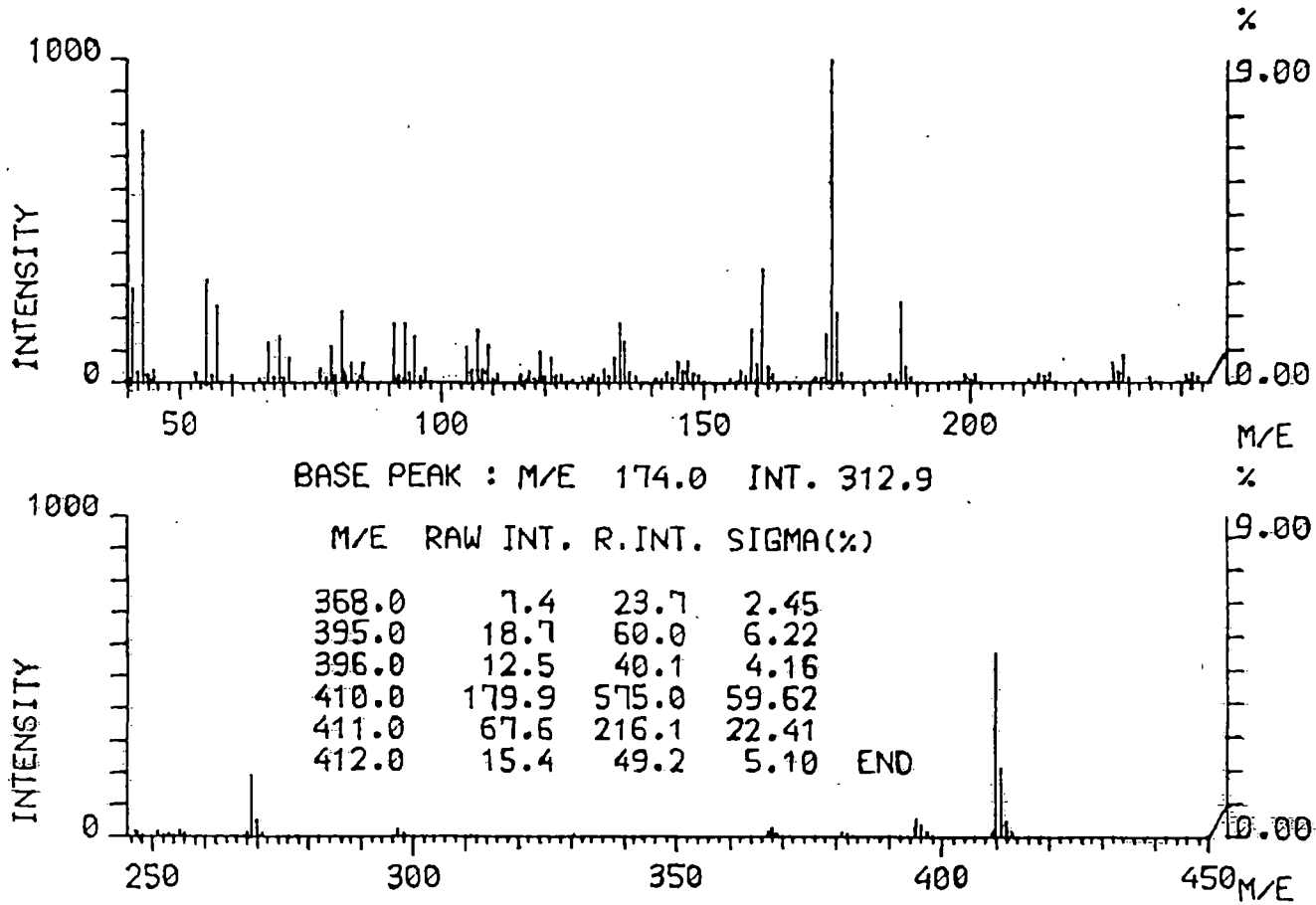
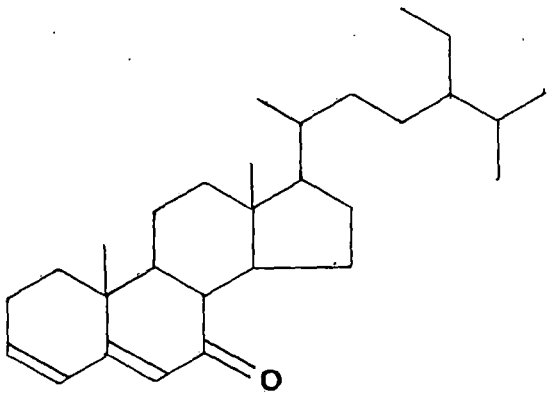


Fig 22. MS spectrum of tremulone (12)

The three olefinic protons are attributed for the protons on C-3 (5.20 ppm), C-4 (6.10 ppm) and C-6 (5.62 ppm).



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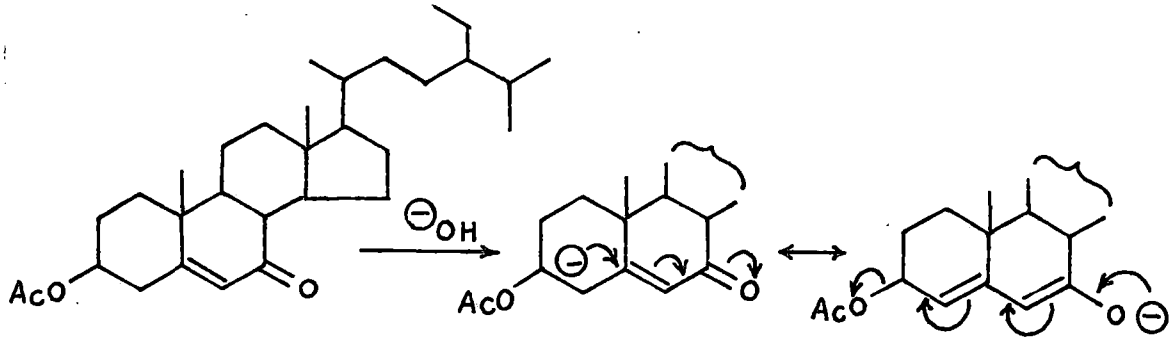
The structure of the product, 12 is further supported by the fact that the mass fragmentation peaks (Fig 22) of 12 are strikingly identical[8] with that of tremulone[9] and its formation is followed by very wellknown pathways (scheme 1).

Scheme-1

Under alkaline condition



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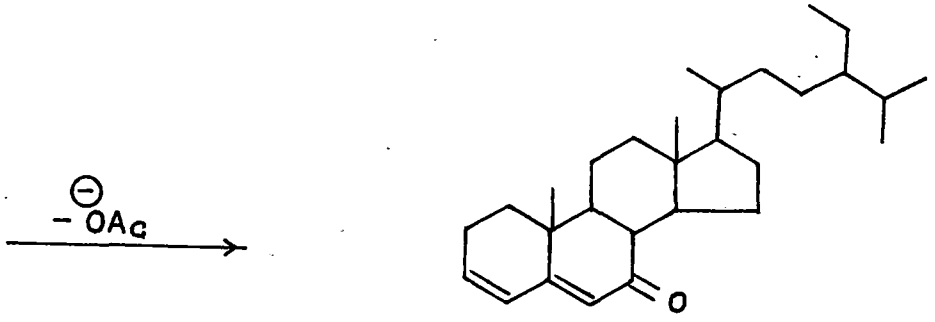
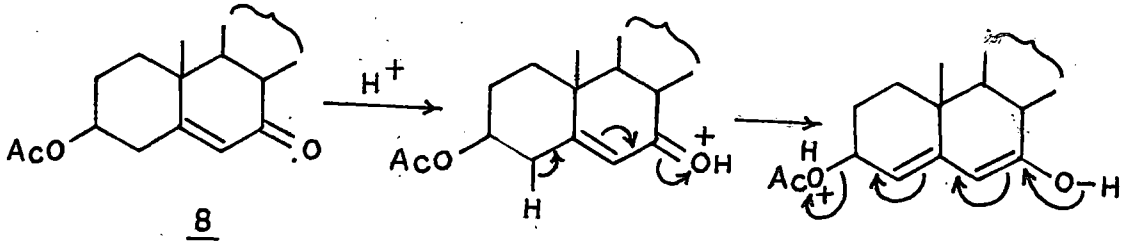


12

Under acidic condition



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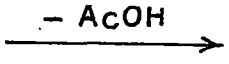

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