CHAPTER 3

SYSTEMATIC INVESTIGATION ON THE NEUTRAL PART OF GYNOCARDIA ODORATA

Chromatography of the neutral part

The gummy neutral part, isolated from the bark of the plant Gynocardia odorata by extraction with benzene was chromatographed over silica column and the following fractions were collected (Table 6)

Table 6

Chromatography results of the neutral part of G odorata

Fraction number	Eluent	Nature of residue after removal of solvent	Melting point
1	Petroleum ether	oil	-
2	Pet ether:benzene (4:1)	oil	-
3	Pet ether:benzene (3:2)	oil	-
4	Pet ether:benzene (2:3)	solid	128-132°
5	Pet ether:benzene (4:1)	solid	>320°
6	Benzene	solid	>320°

/contd...

Table 6 (contd)

Fraction number	Eluent	Nature of residue after removal of solvent	Melting point
7	Benzene:solvent ether (95:5)	solid	>320°
8	Benzene:solvent ether (9:1)	solid	>320°
9	Benzene:solvent ether (4:1)	solid	285-290°

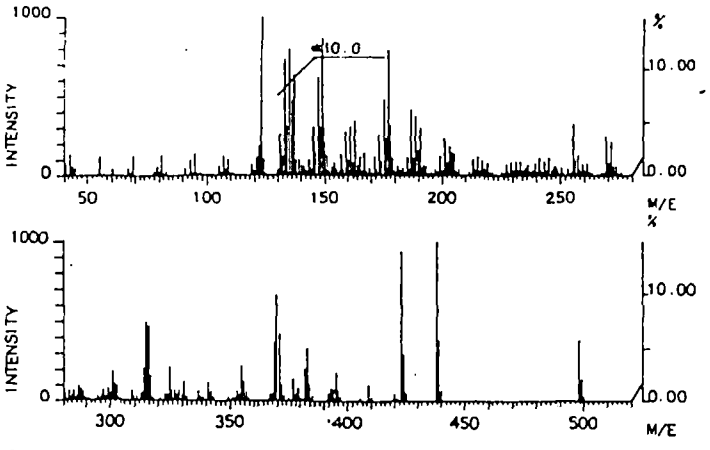
Further elution with more polar solvent did not furnish any solid materials

Examination of fraction 4 (Table 6) : isolation and identification of β-sitosterol

Fraction 4, on repeated crystallization from chloroform-methanol mixture gave flakes, mp 136-137°, $[\alpha]_D$ -34° and was analysed for $C_{29}H_{50}O_3$. The compound gave positive Liebermann-Burchardt 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 6) : isolation and identification of O-acetylodollactone

Fraction 5, on repeated crystallization from chloroform-methanol mixture furnished a solid, mp>320°, $[\alpha]_D$ (MeOH)-19°. Elemental analysis and MS (Fig 11) established the molecular formula, $C_{32}H_{50}O_4$. The IR spectrum (Fig 12) shows the presence an acetoxy group (1730 and 1240 cm^{-1}) and α,γ-lactone moiety (1750 cm^{-1}). The ^1H NMR spectrum (Fig 13) shows the presence of six tertiary and a secondary methyl groups at 0.82(6H), 0.94(3H), 0.97(3H), 0.99(3H), 1.15(3H) and 0.74(3H; d, J = 6.8 Hz) ppm respectively. The



BASE PEAK : M/E 123.0 INT 479.3

M/E	RAW INT	R. INT	SIGMA(%)	
405.0	1.5	3.2	0.88	
409.0	5.0	10.5	2.85	
410.0	1.8	3.8	1.03	
420.0	2.4	5.0	1.36	
421.0	1.5	3.1	0.86	
423.0	45.3	94.6	25.71	
424.0	14.7	30.6	8.33	
425.0	2.7	5.7	1.55	
438.0	51.9	108.2	29.41	
439.0	18.5	38.7	10.51	
440.0	3.6	7.5	2.05	
498.0	18.7	39.0	10.61	
499.0	6.8	14.3	3.90	
500.0	1.5	3.1	0.86	END

Fig 11. MS spectrum of O-acetylodollactone (40)

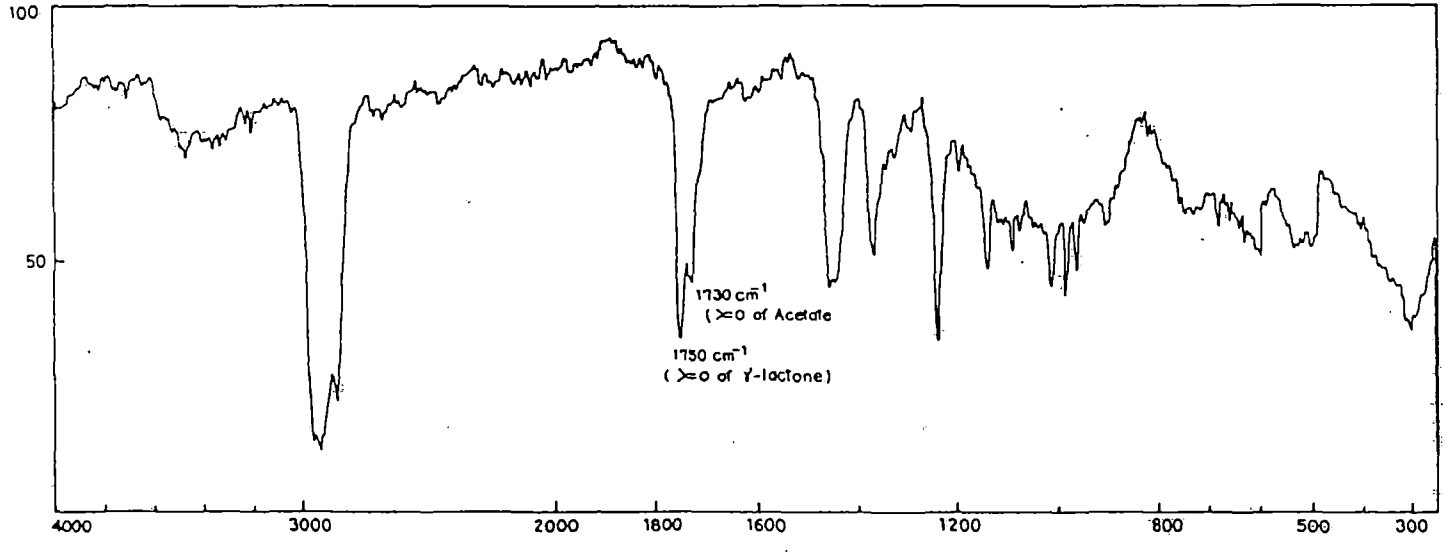


Fig 12. IR spectrum of O-acetylodollactone (40)

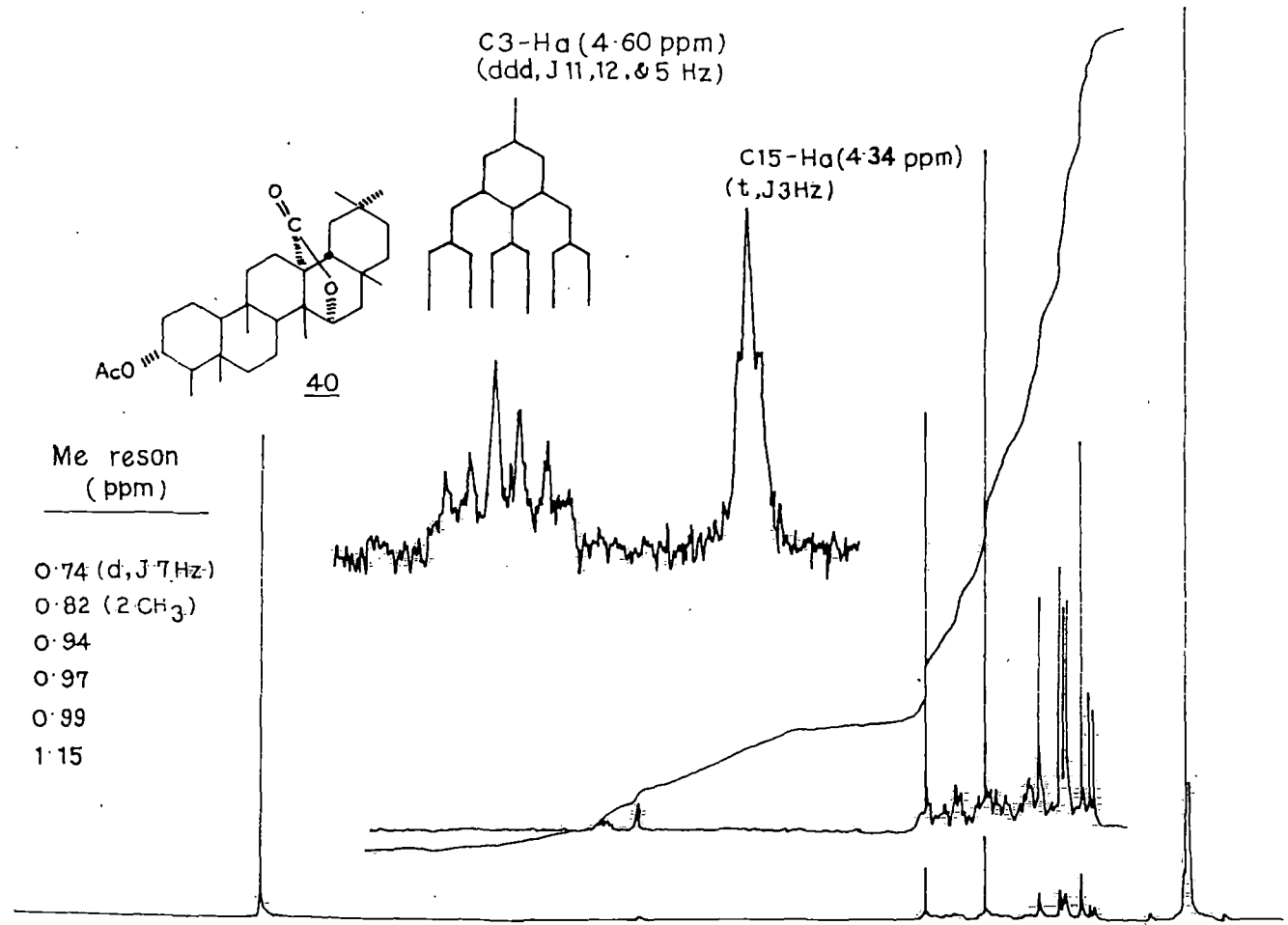


Fig. 13. ^{1}H NMR of 0- acetylodollactone (40) at 200 MHz

peak at 2.02 ppm is due to the acetoxy methyl group. The sextet centred at 4.60 ppm is due to a proton, geminal to the acetoxy group. The splitting pattern of the proton (shown in Fig 13) suggests that the proton is axial at C-3; the acetoxy group being the equatorial at C-3. This axial proton at C-3 is coupled with two axial protons at C-2 and C-4 with J = 11 Hz to give a triplet which is further coupled with an equatorial proton at C-2 with J = 5 Hz; thus resulting a sextet. The peak centred at 4.34 ppm is due to a proton, geminal to the lactone oxygen. The triplet nature of the peak suggests the presence of two protons neighbouring to it and the small coupling constant (J= 3 Hz) suggests that the lactone oxygen is axial.

The compound, on hydrolysis by 10% methanolic potassium hydroxide, furnished the corresponding alcohol, mp>320°, $[\alpha]_D$ -12.14°. The alcohol, on oxidation with CrO_3-pyridine complex, gave odolactone. Thus the compound is 3α-acetoxyfriedelan-27→15α-olide (40), named O-acetyl-odollactone.

Examination of fraction 6 (Table 6) : isolation and identification of odolactone

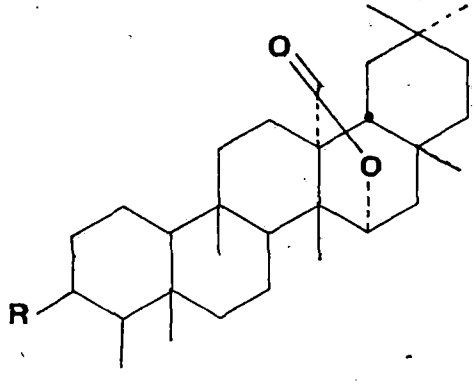
Fraction 6, on repeated crystallization from chloroform-methanol mixture furnished a amorphous solid, mp>320°. The presence of a keto group (1710 cm^{-1}) and a γ-lactone moiety (1755 cm^{-1}) is evident from IR. The compound is found to be identical with odolactone (co-IR and co-TLC).

Examination of fraction 7 (Table 6) : isolation and identification of epi-odollactone

Fraction 7, on repeated crystallization from chloroform-methanol furnished a pure compound, mp>320°, $[\alpha]_D$

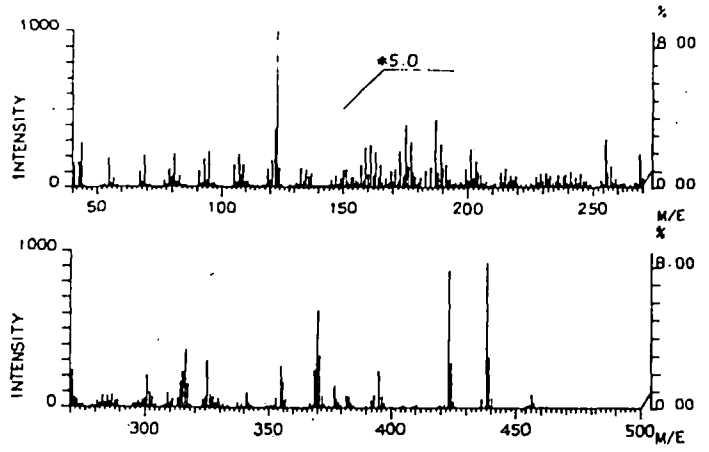
-2.48°. Elemental analysis and MS (Fig 14) established its molecular formula, $C_{30}H_{48}O_3$. The IR spectrum (Fig 15) shows the presence of a hydroxy group (3520 cm^{-1}) and a γ-lactone moiety (1740 cm^{-1}). The ^1H NMR spectrum (Fig 16) shows the presence of six tertiary and a secondary methyl groups at 0.84(3H), 0.93(3H), 0.95(6H), 0.98(3H) 1.13(3H) and 0.91(3H, d, J = 6.5 Hz) ppm respectively. The peak centred at 3.73 ppm ($W_{\frac{1}{2}}$ = 8 Hz) is due to an equatorial proton at C-3 geminal to the hydroxy group. The triplet centred at 4.34 ppm (J = 3 Hz) is due to an equatorial proton on the carbon bearing the lactone oxygen which must have axial orientation.

The compound, on acetylation, gave the corresponding acetyl compound (45), $C_{32}H_{50}O_4$, mp>320°. The IR spectrum (Fig 17) shows the disappearance of the hydroxy peak and the appearance of the peaks 1735 cm^{-1} and 1240 cm^{-1} for an acetoxy group. The peak at 1750 cm^{-1} is due a γ-lactone moiety. The ^1H NMR spectrum (Fig 18) of the acetoxy compound shows the presence of six tertiary and a secondary methyl groups at 0.86, 0.93, 0.94, 0.96, 0.99, 1.15 and 0.81 (d, J = 6.5 Hz) respectively. The peak at 2.02 ppm



44 R = OH

45 R = OAc



BASE PEAK . M/E 123.0 INT 129.3

M/E	RAW INT	R INT	SIGMA(%)	
423.0	23.0	178.0	32.84	
424.0	7.6	59.1	10.91	
425.0	1.2	9.9	1.82	
436.0	1.4	11.5	2.13	
438.0	24.0	186.0	34.31	
439.0	8.5	66.0	12.17	
440.0	1.6	12.9	2.39	
456.0	2.3	18.3	3.39	END

Fig 14. MS spectrum of epi-odollactone (44)

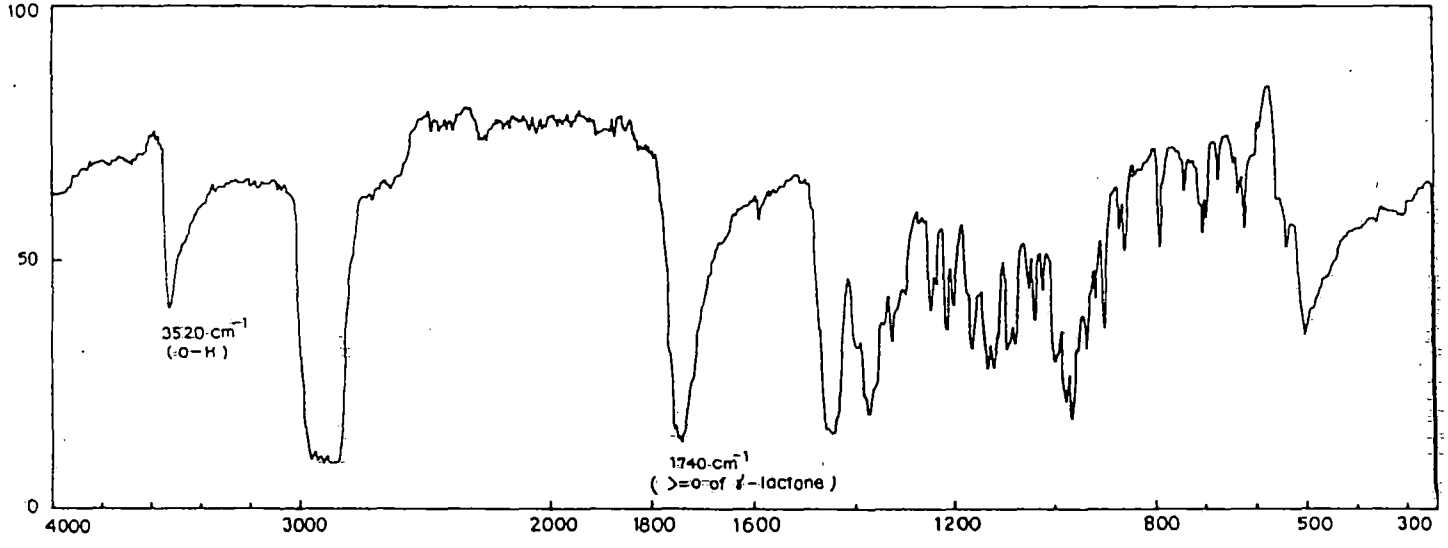


Fig 15. IR spectrum of epi-odollactone (44)

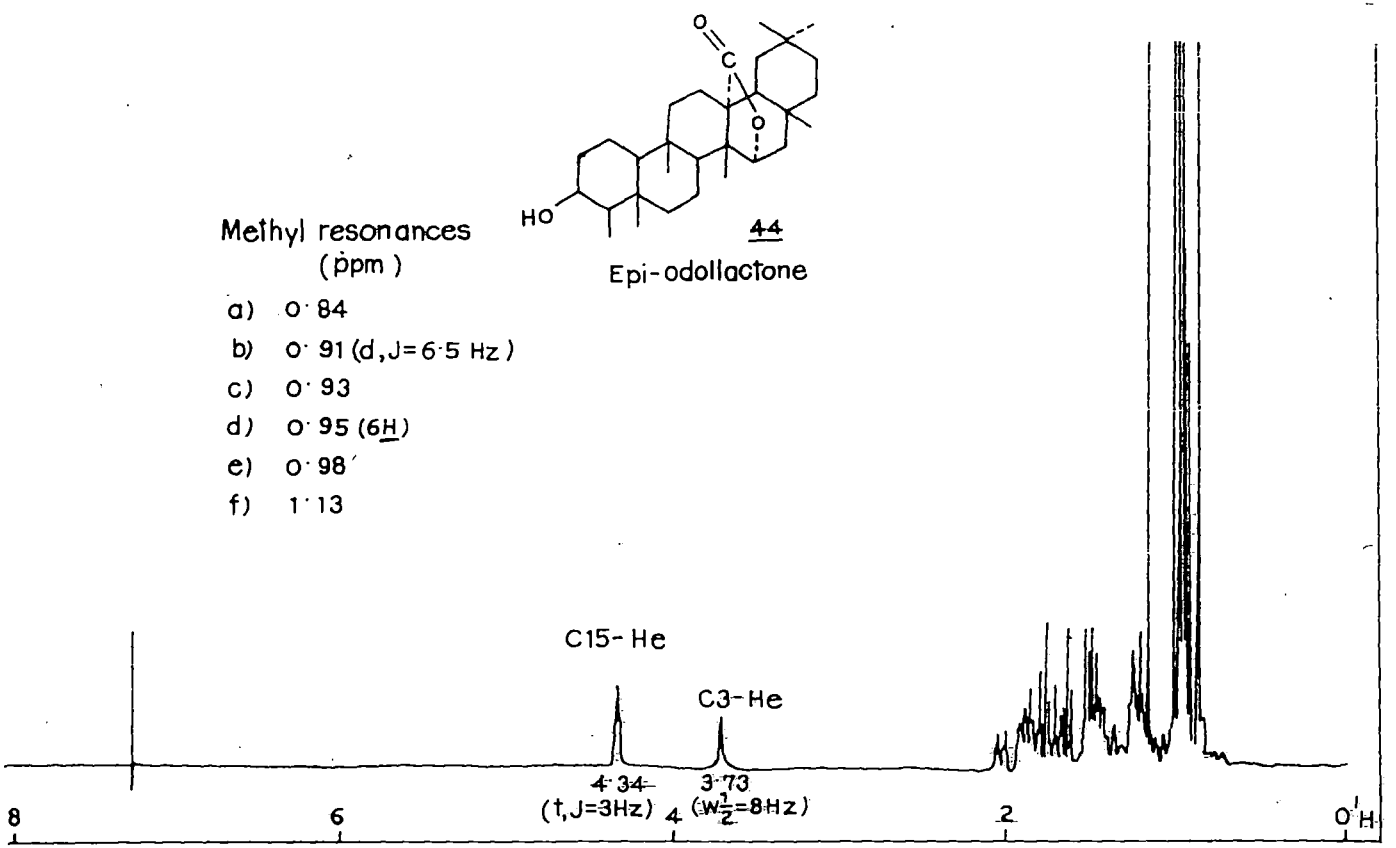


Fig. 16. ^{1}H NMR spectrum of epi-odollactone (44) at 300 MHz

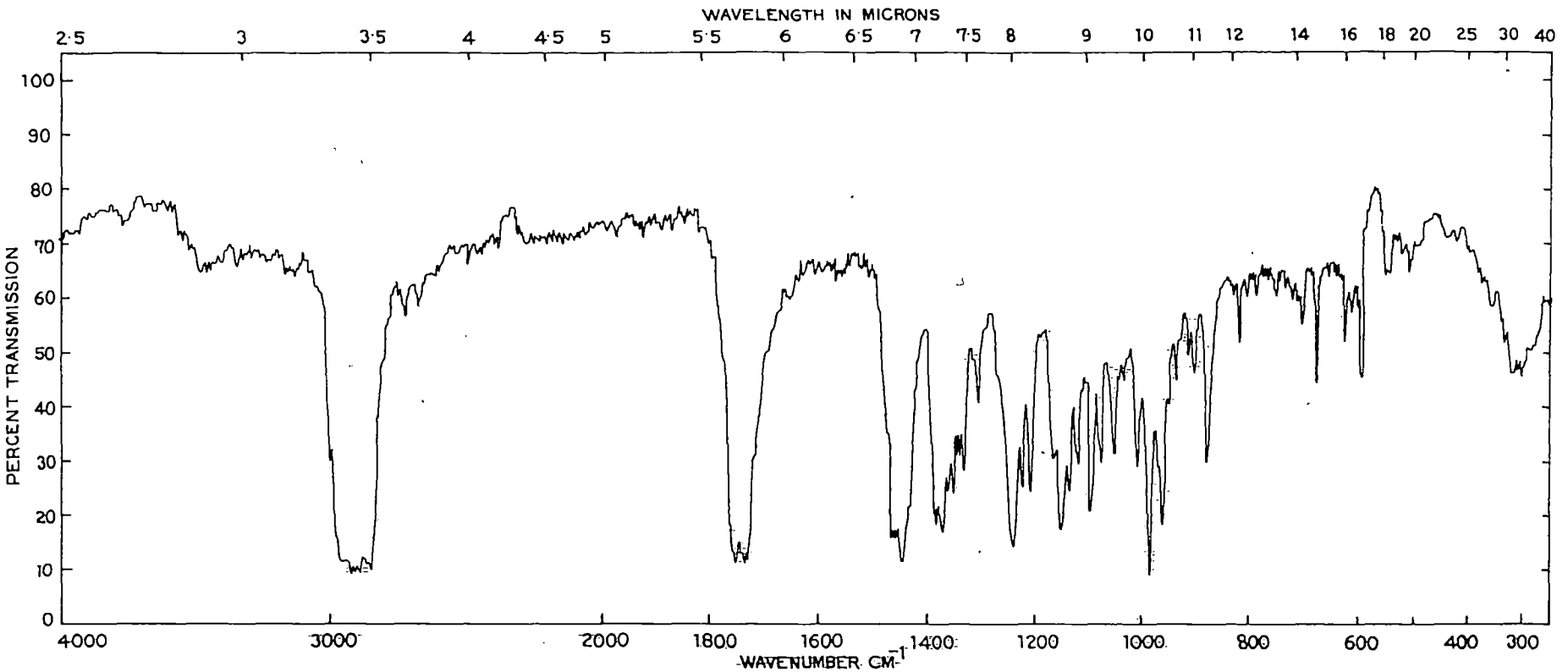


Fig. 17. IR spectrum of O-acetyl epi-odollactone (45)

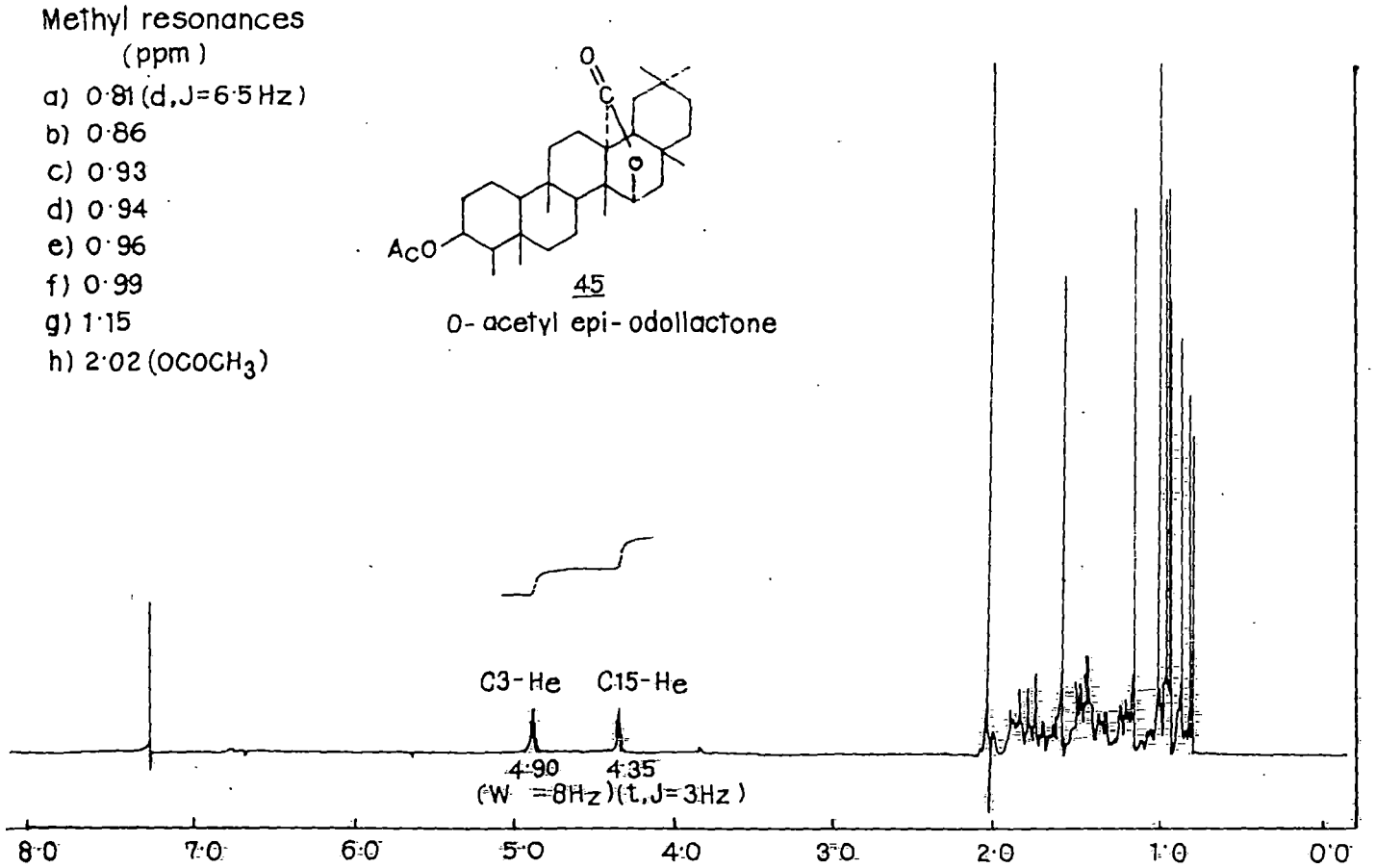


Fig 18. ^{1}H NMR spectrum of O-acetyl epi-odollactone (45) at 300 MHz

is due to the acetoxy methyl group. The multiplet centred at 4.90 ppm with $W_{\frac{1}{2}}$ = 8 Hz is due an equatorial proton, geminal to the acetyl group. The proton geminal to the lactone oxygen appears at 4.35 ppm as triplet with J = 3 Hz.

The hydroxy compound, on oxidation with CrO_3-pyridine complex, gave odolactone. Therefore, the hydroxy compound of fraction 7 must be 3β-hydroxyfriedelan-27→15α-olide (44), named epi-odollactone.

Examination of fraction 8 : isolation and identification of odollactone

Fraction 8, on repeated crystallization from chloroform-methanol furnished a crystalline solid, $C_{30}H_{48}O_3$, mp>320°, $[\alpha]_D$-12.14°. The IR spectrum (Fig 19) shows the presence of a hydroxy group (3480 cm^{-1}) and α,γ-lactone moiety (1758 cm^{-1}). The ^1H NMR spectrum (Fig 20) shows the presence of six tertiary and a secondary methyl groups at 0.74, 0.79, 0.92, 0.94, 0.96, 1.12 and 0.85 (d, J = 6.5 Hz) ppm respectively. The multiplet centred at 3.30 ppm with $W_{\frac{1}{2}}$ = 18 Hz is due to an axial proton geminal to the hydroxy group, ie the hydroxy group has equatorial orientation. The triplet centred at 4.34 ppm (J = 3 Hz) is due to an equatorial proton on the carbon bearing the lactone oxygen.

The compound, on acetylation with acetic anhydride -pyridine, gave O-acetylodollactone (40). It gave odolactone on oxidation with CrO_3-pyridine complex. Therefore the alcohol from fraction 8 is 3α-hydroxyfriedelan-27→15 α-olide (41), named odollactone.

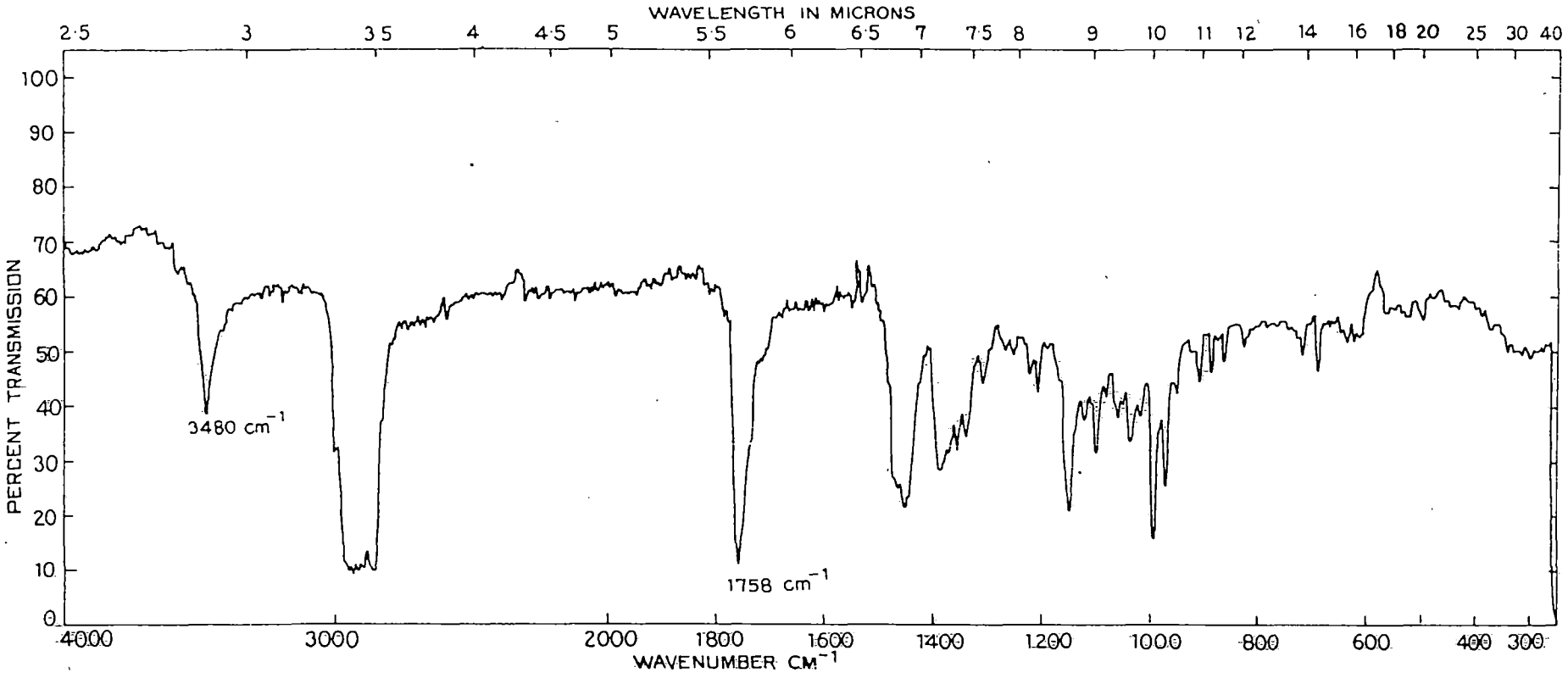


Fig 19. IR spectrum of odoriactone (41)

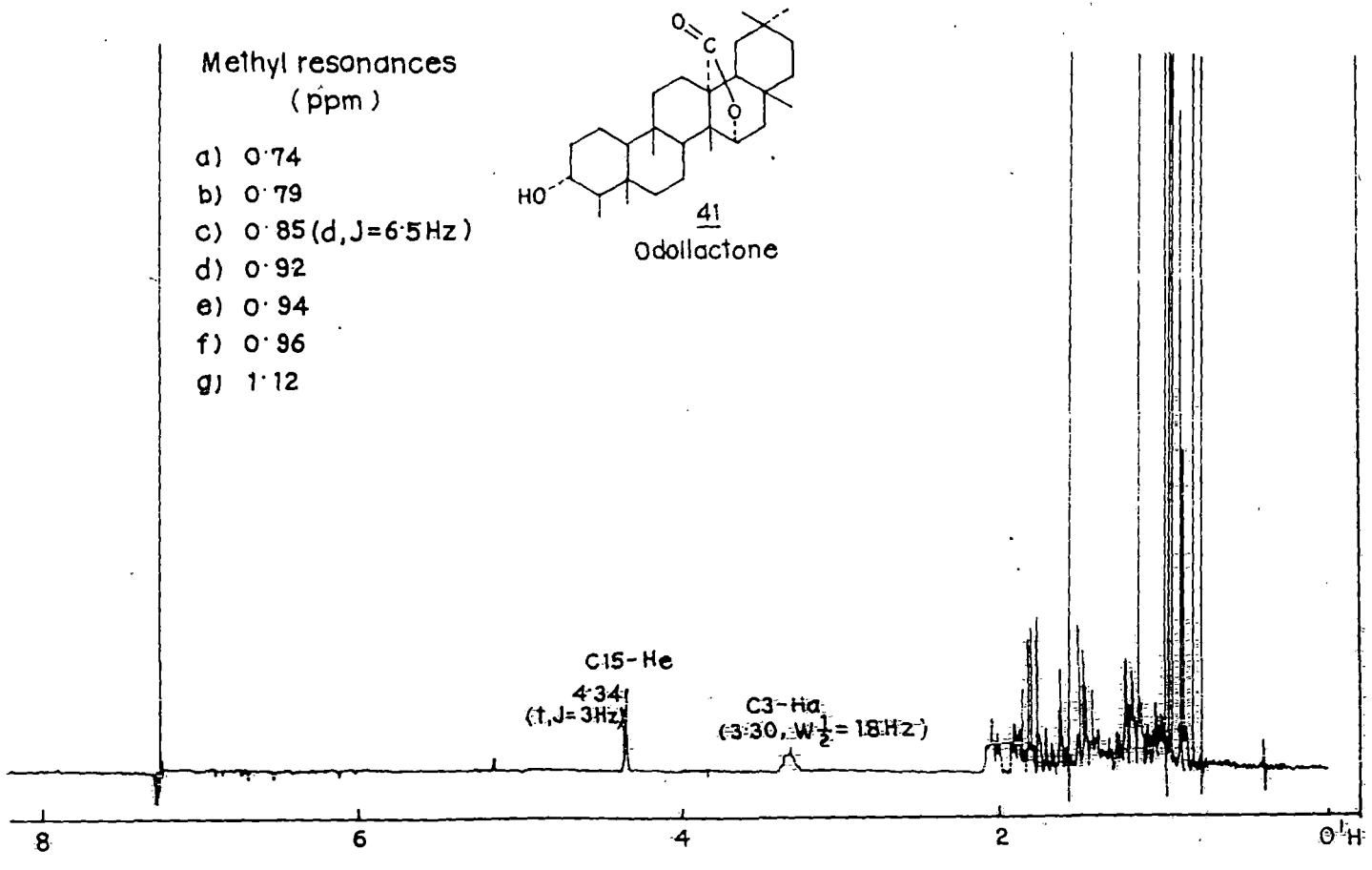
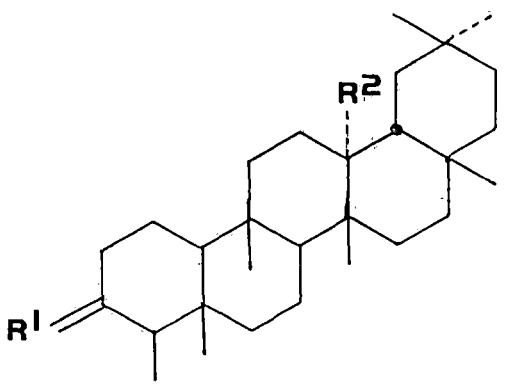


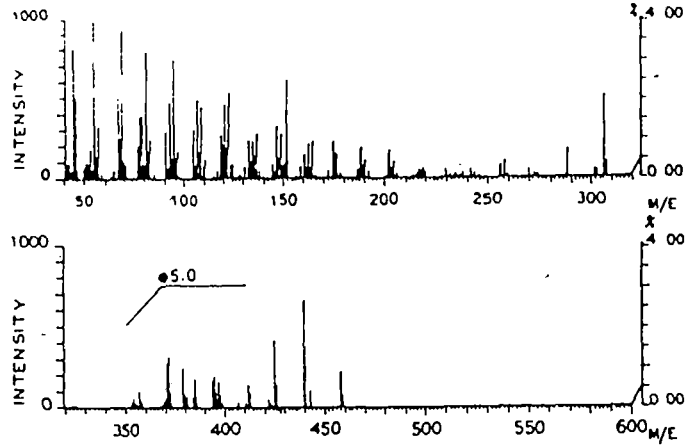
Fig 20. ^{1}H NMR spectrum of Odollactone (41) at 300 MHz

Examination of fraction 9 (Table 6) : isolation and identification of trichadenic acid A

Fraction 9, on repeated crystallization from chloroform-methanol gave a white shiny needles, mp 292-293°, $[\alpha]_D$ +25.0°. Elemental analysis and MS (Fig 21) established its molecular formula $C_{30}H_{50}O_3$. The IR spectrum (Fig 22) shows the presence of a hydroxy group (3300 cm^{-1}) and a carboxylic acid group (1685 cm^{-1}). It gives the corresponding methyl ester, $C_{31}H_{52}O_3$, mp 200-201°, $[\alpha]_D$ +95.5°, with diazomethane. The ^1H spectrum (Fig 23) of the ester shows seven tertiary and a secondary methyl groups at 0.76, 0.89, 0.93, 0.98, 1.11, 1.20, 3.67($COOCH_3$) and 0.85 (d, J = 6 Hz) ppm respectively. The multiplet centred at 3.34 ppm with $W_{\frac{1}{2}}$ = 20 Hz is due an axial proton at C-3, geminal to the hydroxy group. The physical properties of the hydroxy acid and its methyl ester are similar to trichadenic acid A (isolated from T zeylanica[33]) and methyl trichadenate A respectively. Therefore, the compound isolated from fraction 9 is identified as trichadenic acid A, the structure of which is revised as 43 in chapter 2, section D. Thus the structure of methyl trichadenate A is 46.



46 R^1 = α-OH, β-H, R^2 = $COOCH_3$
47 R^1 = O , R^2 = COOH
48 R^1 = O , R^2 = $COOCH_3$



BASE PEAK M/E 55 0 INT 234 9

M/E	RAW INT	R INT	SIGMA(%)	
371 0	13.1	56.1	8.15	
372 0	15.0	64 1	9.32	
379 0	11 7	50.0	7 25	
385.0	8.4	35.9	5.23	
394 0	7.9	33.8	4.92	
395.0	9.5	40.9	5 94	
397.0	8.0	34.2	4 98	
412.0	6 8	29.3	4 26	
425 0	20.0	85.3	12 40	
426.0	6 7	28 8	4 19	
440.0	31 4	139 8	19 46	
441 0	11 5	49 0	7 19	
458.0	10 8	45.9	6 68	END

Fig. 21. MS spectrum of trichadenic acid A (43)

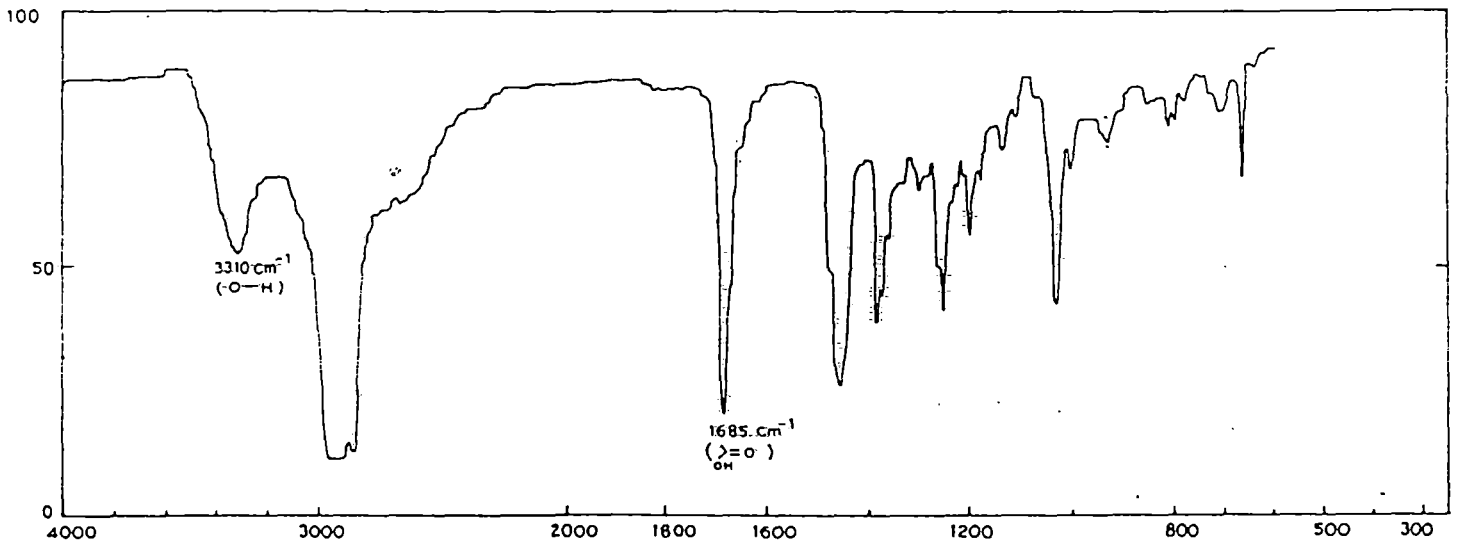


Fig 22. IR Spectrum of trichadenic acid A (43)

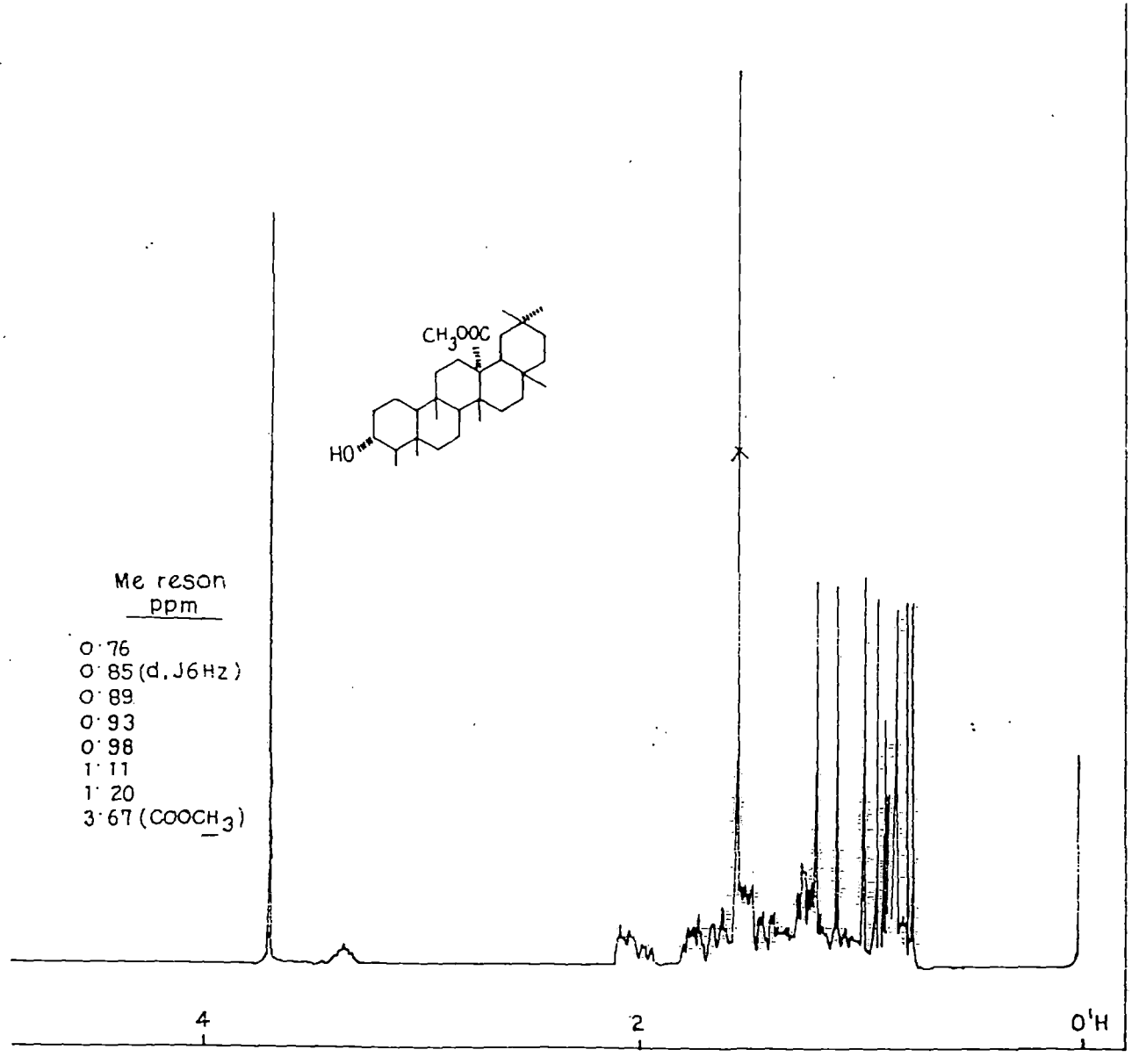


Fig 23. ^1H NMR spectrum of methyltrichdenate A (46) at 300 MHz.

The acid gives O-acetyltrichadenic acid A (43a), mp 250-251°, $[\alpha]_D$ + 28.34, on acetylation with Ac_2O-pyridine and trichadonic acid (47), mp 243-244°, $[\alpha]_D$ + 2.69°, on oxidation with CrO_3-pyridine complex. On esterification with diazomethane, trichadonic acid (47) forms methyl trichadonate (48), mp 180-181°, $[\alpha]_D$ + 5.0°. The physical properties of these derivatives are very similar with those of reported values[33] which are strongly supporting the identification of the compound from fraction 9 (Table 6) as trichadenic acid A (43).