CHAPTER - III

Section - A

Extraction of neutral and acid parts of Caseria Kurzii Clark

Dried and powdered trunk, bark and stem (4 kgs) of Caseria Kurzii was extracted with benzene in soxhlet apparatus for 26 hours. The extract was cooled to room temperature and benzene was distilled off. The gummy residue (50 gm) obtained was dissolved in ether ($\simeq$1.00 litre). The ether solution was washed with 10% aqueous NaOH solution (3 x 700 ml) and then with water till neutral. The neutral ether was dried over anhydrous sodium sulphate and it was evaporated to yield a gummy residue ($\simeq$18 gm), which constituted the neutral part (Part A) of the extract.

The alkali washed portion on acidification with dilute hydrochloric acid ($\simeq$1N) yielded a solid, which was extracted with ether. The ethereal solution containing the acid part was washed with water till neutral and dried. The ether solution was then esterified with diazomethane. The crude methyl ester (7 gm) obtained after evaporation of ether constituted the acid part (Part B) of the extract.

Section - B

Isolation and identification of the compounds from the neutral part (Part A).

The neutral part (Part A) of the extract was dissolved in minimum volume of benzene and placed on a column of alumina (1000 gm, deactivated with 45 ml of 10% aqueous acetic acid). The chromatogram was developed with solvents as shown in Table - 48.

Table - 48

Chromatography of neutral part

Serial No.	Solvent	Fractions 250 ml each	Residue	M.P.
1.	Petroleum ether	1-6	Oil	-
2.	Pet. ether-benzene (4:1)	7-12	Waxy solid	65-67°
3.	Pet. ether-benzene (3:2)	13-17	Oil	-
4.	Pet. ether-benzene (2:3)	18-23	Solid	120-25°

Further elution with more polar solvent did not yield any solid material.

Examination of fraction 7-12 (Table - 48): Isolation of 1-hexacosanol.

Fractions 7-12 (Table - 48) were individually compared in a single t.l.c. plate, which showed a prominent

spot with the same Rf value (0.78 in benzene). These fractions were combined and crystallised several times from acetone which afforded flaky crystals of constant m.p. 78-79°, $[\alpha]_D \pm 0°$. Elemental analysis showed the molecular formula to be $C_{26}H_{54}O$. IR spectrum showed the presence of a hydroxy group at 3350 cm^{-1} (broad). It was transparent in the UV region and did not respond to TMM test. Acetylation of the above alcohol with acetic anhydride-pyridine furnished an acetate, $C_{28}H_{56}O_2$, m.p. 68-69°, $[\alpha]_D$ 0°; IR : 1720, 1240 cm^{-1}. This was found to be indistinguishable with an authentic sample of 1-hexacosanol acetate (IR and m.m.p. comparison). From the above facts it was evident that the original alcohol was 1-hexacosanol.

Examination of fractions 18-23 (Table - 48): Isolation and identification of β -sitosterol.

Each of the fractions No. 18-23 (Table - 48) was compared in a t.l.c. plate when all the fractions were found to contain a prominent spot with the same Rf value (0.4 in benzene as solvent). These fractions were combined and crystallised several times with chloroform-methanol mixture when fine needle-shaped crystals of molecular formula $C_{29}H_{50}O$, m.p. 135-37°, $[\alpha]_D$ - 34° was obtained. Acetylation of the alcohol with Ac_2O - Py furnished an acetate of molecular formula $C_{31}H_{52}O_2$, m.p. 127-28°,

$[\alpha]_D$ - 39. The acetate was compared with an authentic sample of β-sitosterol acetate and was found to be identical with it (m.m.p. co-IR, t.l.c.).

Isolation and identification of the compounds from the acid part (Part B).

The acid part after esterification was dissolved in minimum volume of benzene and was chromatographed over neutral alumina (7 gm).

Table - 49

Chromatography of the esterified product

Serial No.	Eluent	Fractions 100 ml each	Residue	Melting Point
1.	Petroleum ether	1-4	Oil	-
2.	Pet. ether-benzene (4:1)	5-8	Oil	-
3.	Pet. ether-benzene (3:2)	9-15	Solid (≃ 300 mg)	205-10°
4.	Pet. ether-benzene (2:3)	16-22	Solid (≃ 280 mg)	209-12°

Further elution with more polar solvent did not yield any solid material.

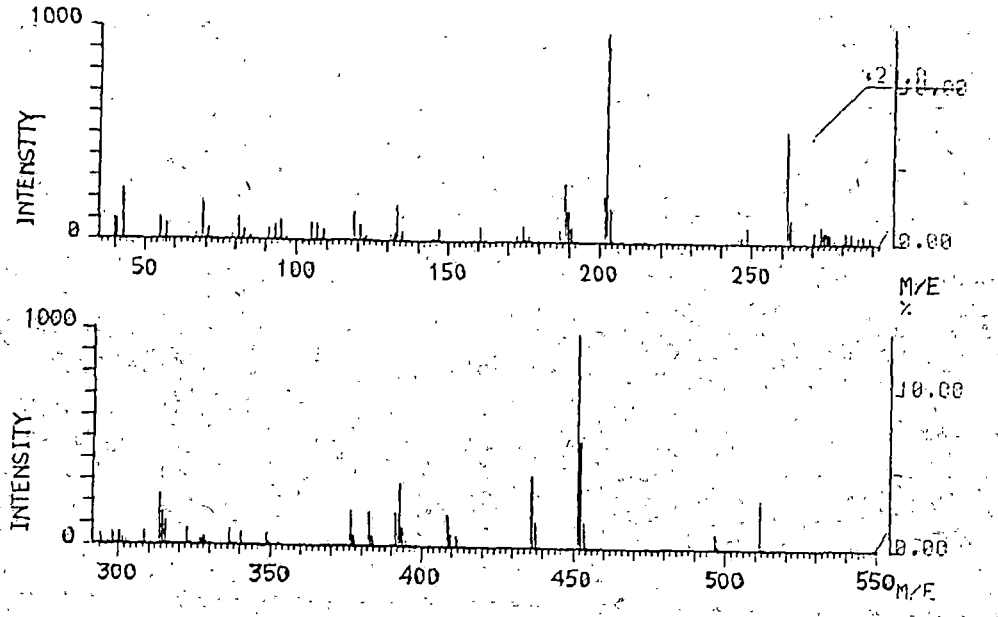


Fig.70 Mass Spectrum of acetyl methyl oleanolate, 251a.

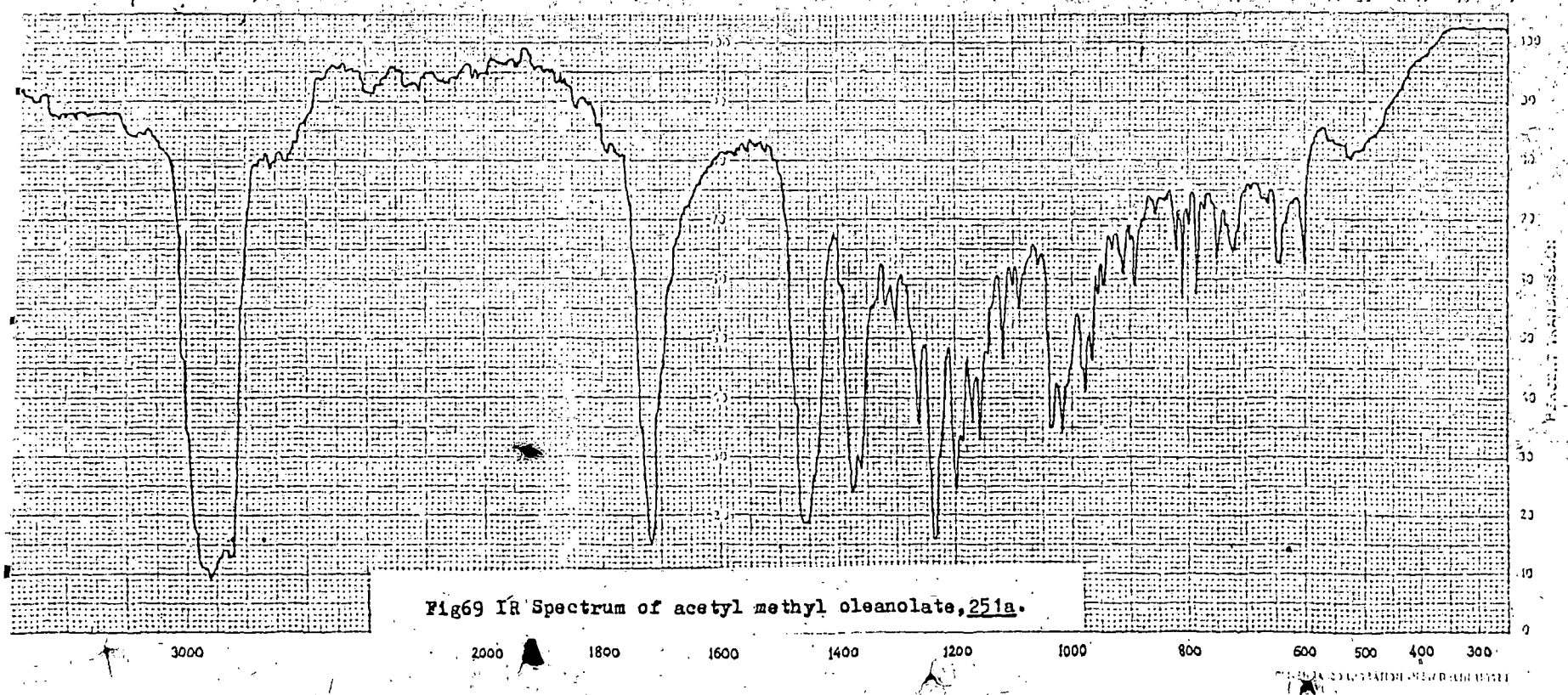
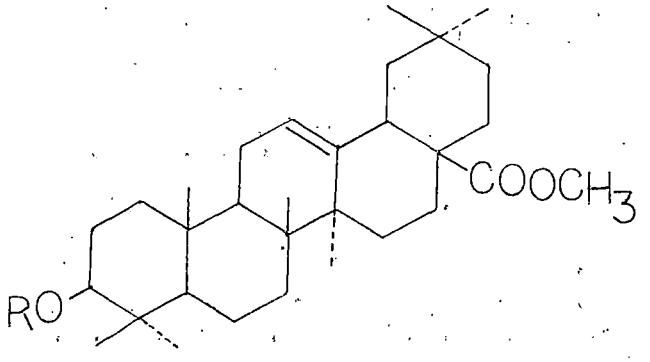


Fig69 IR Spectrum of acetyl methyl oleanolate, 251a.

Examination of fractions 9-15 (Table - 49): Isolation of acetyl methyl oleanolate 251a:

Fractions 9-15 were first compared in a t.l.c. plate and combined to a single lot. It was repeatedly crystallised from chloroform and methanol. Needle shaped crystals had m.p. 213-14°, $[\alpha]_D$ 56°; IR spectrum (Fig. 69) showed bands at 1720, 1240, 810 cm^{-1}. Elemental analysis of the compound showed the molecular formula to be $C_{33}H_{52}O_4$. Mass spectrum (Fig. 70) of the compound showed peaks at m/e 512 (M^+), 497, 452, 437, 393, 340, 282, 203 (base peak) 189. The fragmentation pattern of this compound showed the presence of a double bond at 12-13 position, typical of oleanane skeleton. The above compound was hydrolysed with 5% methanolic KOH by refluxing for four hours. The product after usual work up had the m.p. 192-94°, IR spectrum showed the presence of a hydroxy group at 3530 and ester group at 1735 and a trisubstituted double bond at 820 cm^{-1}. Elemental analysis showed the molecular formula to be $C_{31}H_{50}O_3$. The compound was characterised as methyl oleanolate by comparison (t.l.c., Co-IR and m.m.p) with an authentic sample. Thus the acid present in the plant was acetyl oleanolic acid 251b.



251. a R=Ac
b R=H

Examination of fraction 16-22 (Table - 49): Isolation of methyl betulinate 252a.

Fractions 16-22 (Table -49) were found to contain the same compound from t.l.c. experiment, hence, were combined. The solid was crystallised from chloroform-methanol, which afforded crystalline solid m.p. 219-20°, $[\alpha]_D$ +5°. IR spectrum (Fig. 71) showed the presence of a free hydroxy group at 3520 cm^{-1} and an ester group at 1710 cm^{-1}. The presence of an exocyclic methylene group was evident from the presence of peaks at 3030, 1640 and 880 cm^{-1}. Elemental analysis showed the molecular formula to be $C_{31}H_{50}O_3$. On acetylation of the above compound with acetic anhydride -pyridine an acetate of m.p. 200-2° was formed. The acetate was found to be identical with an authentic sample of acetyl methyl betulinate by

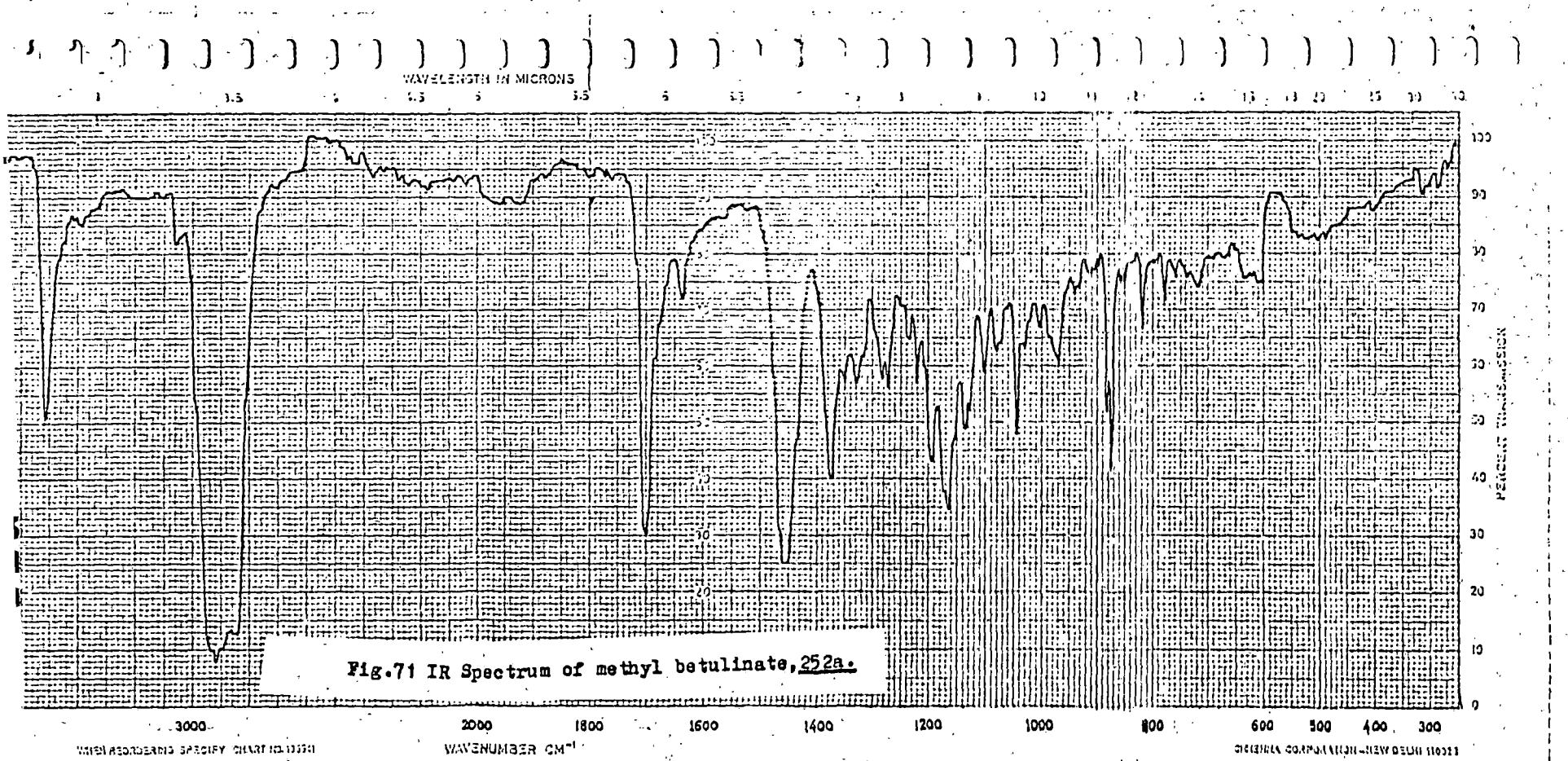
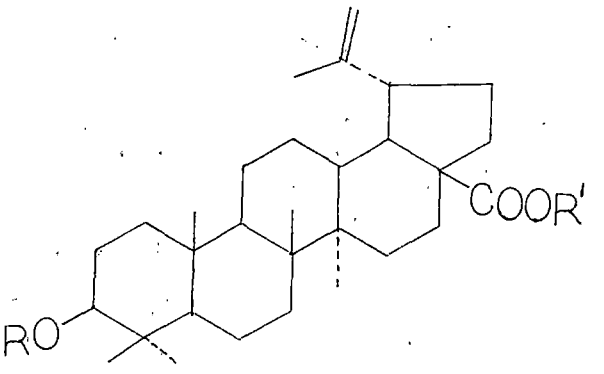


Fig.71 IR Spectrum of methyl betulinate, 252a.

comparison (t.l.c., m.m.p.). Thus it was found that the plant contained betulinic acid 252c.



252 a R=H, R'=CH_3
b R=Ac, R'=CH_3
c R=H, R'=H

Section - C

Experimental:

Melting points were uncorrected. Petroleum ether used had b.p. 60-80°. Optical rotations were determined in chloroform. IR spectra were recorded in Beckman IR-20 Spectrophotometer. ^1H NMR spectra were recorded in $CDCl_3$ with tetramethyl silane as an internal standard.

Extraction of the plant has been described on page 265 of this chapter.

Isolation and identification of 1-hexacosanol.

Fractions 7-12 (Table -48) were mixed up and crystallised several times from acetone, which yielded flaky crystals, m.p. 78-79°, $[\alpha]_D \pm 0°$. Elemental analysis found C 81.44, H 13.13%; calculated for $C_{26}H_{54}O$ C 81.69, H 14.13%. IR : ν_{max}^{nujol} 3350.

UV : No absorption above 220 nm.

Acetylation of 1-hexacosanol.

Acetylation of the compound in the usual manner and after purification by crystallisation from methanol afforded crystals of m.p. 68-69°, $[\alpha]_D \pm 0°$. It was found to be identical in t.l.c., m.p. and i.r. with an authentic sample of 1-hexacosanol acetate. Elemental analysis, found, C 78.83, H 13.52, calculated for $C_{28}H_{56}O_2$, C 79.24, H 13.26% .

Isolation and identification of β-sitosterol:

Fractions 18-23 (Table-48) were combined and on repeated crystallisation from chloroform-methanol mixture yielded silky solid, m.p. 135-36°, $[\alpha]_D$ -36°. Elemental analysis, found C 83.56, H 11.76%; calculated for $C_{29}H_{50}O$, C 83.98, H 12.15%. Mixed m.p. with authentic sample showed no depression.

The compound (0.5 gm) was acetylated with Py-Ac_2O in the usual method. The acetate on crystallisation had m.p. 124-26°, $[\alpha]_D$ -34°. Mixed m.p. with authentic sample showed no depression. Co-IR with authentic sample was in complete agreement. Elemental analysis, found C 81.26, H 11.32%; calculated for $C_{31}H_{52}O_2$, C 81.52, H 11.48%.

Isolation and identification of acetyl methyl oleanolate 251a.

Fractions 9-15 (Table - 49) were combined and on repeated crystallisation from chloroform-methanol mixture afforded needle shaped crystals of m.p. 213-14°, $[\alpha]_D$ +56°. Elemental analysis, found C 77.92, H 11.08%; calculated for $C_{33}H_{52}O_4$ C 77.30, H 10.22%. IR: ν_{max}^{nujol} 1730, 1720, 1240, 810 cm^{-1} (Fig. 69). Mass spectrum of the compound showed peaks at m/e 512 (M^+), 497, 452, 437, 393, 340, 262, 203 (base peak) 189. (Fig. 70).

Hydrolysis of acetyl methyl oleanolate : Isolation of methyl oleanolate 251b:

Acetyl methyl oleanolate (100 mg) dissolved in benzene (2 ml) was refluxed in 10% methanolic KOH (15 ml) for 4 hours. The mixture was cooled, acidified with dil. HCl (20 ml) and extracted with ether. Removal of the solvent afforded a solid, which on repeated crystallisation from chloroform-methanol mixture furnished methyl oleanolate 251b, m.p. 192-94°. Found C 79.63, H 10.21%; calculated for $C_{31}H_{50}O_3$ C 79.10, H 10.71%. IR : ν_{max}^{nujol} 3530, 1735, 820 cm^{-1}.

Isolation and identification of methyl betulinate 252a:

Solids obtained from fraction 16-22 (Table - 49) were combined and on repeated crystallisation from a mixture of chloroform and methanol afforded shining colourless needle-shaped crystals of methyl-betulinate 252a, m.p. 219-20°, $[\alpha]_D$ +5°. Elemental analysis found C 73.91, H 10.80%; calculated for $C_{31}H_{50}O_3$, C 79.10, H 10.71%.

UV : no absorption in the region 220-300 nm.

IR ν_{max}^{nujol} : 3520, 3030, 1710, 1640, 880 cm^{-1}

(Fig. 71)

Acetylation of methyl betulinate : Isolation of acetyl methyl betulinate 252b:

Methyl betulinate 251a (100 mg) was acetylated with pyridine (1 ml) and acetic anhydride (1 ml) in the usual way. The solid obtained was purified by crystallisation from chloroform-methanol which afforded crystals of acetyl methyl betulinate 252b, m.p. 200-2°. Elemental analysis, found C 77.37, H 10.13%; calculated for $C_{33}H_{52}O_4$ C 77.24, H 10.02%.