CHAPTER III

Experimental

Extraction : Isolation of the neutral fraction

Dried and powdered trunk bark of Bischofia javanica (2 kg) was extracted with benzene in a Soxhlet apparatus for twenty hours. Benzene was distilled off and the gummy residue (9 gm.) was taken up in ether (1 liter). The ether solution was washed with 10% aqueous sodium hydroxide solution (3 x 300 ml) and then with water till neutral. The ether solution was dried over anhydrous sodium sulphate and the ether evaporated, when a gummy material (4.6 gm.) was obtained.

Chromatography of the above gummy material

The above gummy material (4.6 gm) was dissolved in benzene (6 ml) and was placed on a column of alumina (300 gm) deactivated with 6 ml of 10% aqueous acetic acid. The chromatogram was developed with petroleum and was eluted with the following solvents (table II).

Table II

Chromatography of the above gummy material

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (300 ml)	1-6	Solid contaminated with ~~with~~ oil m.p. 280-285° (0.750 gm.)
Petroleum:benzene (4:1) (400 ml)	7-14	Solid (1.2 gm.) m.p. 252-4°
Petroleum:benzene (3:2) (200 ml)	15-18	Nil
Petroleum:benzene (2:3) (300 ml)	19-24	Solid m.p. 128-33° (2 gm.)

Further elution with more polar solvent did not afford any crystalline material

Isolation of epi-friedelinol acetate 1

Fractions 1-6 (table II) were combined (0.750 gm), dissolved in 3 ml of benzene and was rechromatographed over a column of active alumina (40 gm). The chromatogram was developed with petroleum (Table III).

Table III

Chromatography of the residue of fractions 1-8 (Table II)

Eluent	Fractions 50 50 ml each	Residue on evaporation
Petroleum (150 ml)	1-3	Oil
Petroleum (200 ml)	4-7	Solid (0.2 g.) m.p. 282-6°

Further elution with more polar solvents did not afford any crystalline material.

The above solid (fractions 4-7, Table III) were combined (0.2 g) and was crystallised from a mixture of chloroform and methanol to afford crystals of 1, m.p. 289-92°, $(\alpha)_D$ + 40°. Its melting point was not depressed when mixed with an authentic sample of epi-friedelanol acetate. I.R. spectra of the two were also superimposable.

Found :	C, 81.37; H, 11.25%
Calculated for $C_{32}H_{54}O_2$:	C, 81.70; H, 11.48%
I.R. (Nujol) :	1736 and 1239 cm^{-1}
U.V. (95% ethanol) :	No absorption in the region 220-300 mμ.

Hydrolysis of the acetate 1 : Preparation of epi-friedelanol 2

Epi-friedelanol acetate (0.1 g.) was dissolved in benzene (5 ml) and was refluxed with methanolic potassium hydroxide solution (10 ml, 10%) for 3 hours. The reaction mixture was concentrated on water

bath and then diluted with water. The precipitated solid (0.08 gr.) was collected by filtration and crystallised from a mixture of chloroform and methanol to afford crystals of 2, m.p. 277-9°, $(\alpha)_D$ + 9°. Its melting point was not depressed when mixed with an authentic sample of epi-friedelanol. I.R. spectra of the two compounds were also superimposable.

Found : C, 84.32; H, 12.06%

$C_{30}H_{52}O$ requires : C, 84.11; H, 12.14%

I.R. (Nujol) : 3420 cm^{-1} (-OH)

U.V. (95% ethanol): No absorption in the region 215-300 mμ.

Isolation of friedelin 3

Fractions 7-14 (Table II) were combined (1.2 gm), dissolved in benzene (5 ml) and was chromatographed over a column of active alumina (60 gm.). The chromatogram was developed with petroleum (Table IV).

Table IV

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum 100 ml	1-2	Oil
Petroleum (300 ml)	3-8	Solid (.9 gr.), m.p. 255-7°

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

The fractions 3-8 (Table IV) were combined and crystallised from a mixture of chloroform and methanol to afford fine needles shaped crystals 3, m.p. 256-8°, $(\alpha)_D$ - 32°. Its melting point was not depressed when mixed with an authentic specimen of friedelin. I.R. spectra of the two compounds were superimposable.

Found :	C, 84.18; H, 11.76%
Calculated for $C_{30}H_{50}O$:	C, 84.44; H, 11.81%
I.R. (Nujol) :	1705 cm^{-1} (six membered ring ketone)
U.V. (95% ethanol) :	λmax 255 mμ. (ϵ = 71).

Isolation of β-sitosterol 4

The solid fractions 19-24 (Table II) were combined (2 gm) and crystallised from chloroform and methanol mixture when fine needle shaped crystals of β-sitosterol 4 were obtained m.p. 136-7°, $(\alpha)_D$ - 36°.

Found :	C, 83.34; H, 11.62%
Calculated for $C_{29}H_{50}O$:	C, 89.98; H, 12.15%

Preparation of β-sitosterol acetate 5

β-sitosterol 4 (0.5 g.) was acetylated with pyridine (5 ml) and acetic anhydride (5 ml) in the usual way. The solid (0.45 g), thus obtained, was crystallised from chloroform and methanol mixture when crystals of the acetate 5, m.p. 126-7°, $(\alpha)_D$ - 37° were obtained. It was identified as β-sitosterol acetate by comparing with an authentic specimen of β-sitosterol acetate (m.m.p. and I.R. romparison).

Found : C, 81.15; H, 11.35%

Calculated for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48%

Isolation of Methyl betulinate 7

The aqueous alkaline layer was throughly shaken with ether to remove any neutral material that might be present. The aqueous layer was acidified with cold and dilute 10% hydrochloric acid (1 litre) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water till neutral and then dried (Na_2SO_4). Ether was removed when a gummy residue (2 gm) was obtained. To the latter dissolved in ether (250 ml) was added a solution of diazomethane in ether prepared from nitrosomethyl urea (1.4 g.) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and then dried (Na_2SO_4). Evaporation of the ether yielded a gummy residue (1.5 g.)

Chromatography of the above gummy material (1.5 g) : Isolation of methyl betulinate 7

The above crude ester (1.5 g.) dissolved in benzene (12 ml) was placed over a column of alumina (100 gm. deactivated with 4 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum and was eluted with following solvents (Table V).

Table V

Chromatography of the above gummy material (1.5 g.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum (200 ml)	1-4	Oil
Petroleum:benzene (4:1) (200 ml)	5-8	Nil
Petroleum:benzene (3:2) (300 ml)	9-14	Solid (1.2 g), m.p. 216-8°

Further elution with more polar solvents did not yield any crystalline solid

Solids obtained from the fractions 9-14, m.p. 216-18° (Table V) were combined (1.2 g), and crystallised from a mixture of chloroform and methanol to afford colourless needles of methyl betulinate 7, m.p. 222-4°, $(\alpha)_D$ + 5°, identical with authentic sample (m.m.p. and I.R.).

Found : C, 78.79; H, 10.52;

Calculated for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71;

UV : No absorption in the region 220-300 mμ.

I.R. (Nujol): 3520 (-OH), 1735 ($-COOCH_3$), 1660 and 876 cm^{-1} (= CH_2)

NMR (60 Mc) : 4.8-4.9 δ (two doublets; = CH_2), 3.75 δ (singlet, $-COOCH_3$), 2.01 δ (singlet, -CHOH), 1.75 δ (sharp singlet $H_2C=C-$) and 1.00 δ (a tall singlet accounting for 15 protons, 5 CH_3).

Preparation of acetyl methyl betulinate 8

Methyl betulinate 7 (200 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The crude acetate (160 mg) thus obtained was crystallised from a mixture of chloroform and methanol to give crystals of acetyl methyl betulinate 8, m.p. 200-1°, found to be identical with an authentic sample of acetyl methyl betulinate (m.m.p.).

Found : C, 77.31; H, 10.38%

Calculated for $C_{33}H_{52}O_4$: C, 77.34; H, 10.15%

Preparation of betulinic acid 6

To a normal solution of potassium tertiary butoxide in tertiary butanol (10 ml), a solution of methyl betulinate 7 (150 mg) in dimethyl sulfoxide (10 ml) was added and the reaction mixture was heated at 100° for 4 hours in an oil bath. After working up in the usual manner it afforded betulinic acid, m.p. 299-302°, identical with an authentic sample of betulinic acid (m.m.p. and I.R. comparison).

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