

CHAPTER III

Experimental

Melting points are uncorrected. The petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were measured in chloroform solution. NMR spectra were determined on Varian A-60 spectrometer. The I.R. spectra were recorded in Beckman I.R. -20 spectrophotometer.

Isolation of the petroleum ether  
soluble material:

Dried and powdered bark of *Pinlaysonia Obovata* Wall (2 kg) was extracted with petroleum ether in a soxhlet apparatus for thirty hours. The petroleum ether extract was concentrated and the mass obtained (6 gm) was subjected to chromatography over a column of 350 gm alumina deactivated with 14 ml 10% aqueous acetic acid and the following fractions were collected.

Table III

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Fraction No.	Eluent	Fractions collected (250 ml each)	Residue on evaporation
1	Petroleum ether	1-3	Trace oil
2	Petroleum ether	4-20	Solid

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

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Fraction No. 2 (table III) on crystallisation from chloroform-methanol mixture yielded crystals of  $C_{32}H_{52}O_2$  Lupool acetate m.p. 218-220°.

Hydrolysis of Lupool acetate with methanolic alkali

200 mg of the organic compound was refluxed for 4 hours with 10% methanolic potassium hydroxide. The mass was concentrated and was then poured into cold water when a solid was obtained. It was separated by filtration and was washed to remove free alkali.

The mass on crystallisation from acetone-methanol mixture gave lupeol m.p. 212-14°, ( $\alpha$ )<sub>D</sub> 27° (mp undepressed).

Acetylation of the alcohol, Lupeol:

200 mg of the alcohol was dissolved in 2 ml pyridine, 2 ml of acetic anhydride was added and the mixture was heated on a waterbath for about 4 hours. After usual work up a solid was obtained which on crystallisation from chloroform-methanol gave back the original acetate, Lupeol acetate (mp undepressed).

Benzoylation of the alcohol:

200 mg of the alcohol was benzoylated using benzoyl chloride and pyridine. After usual work up, a solid of m.p. 273-74° was obtained.

Isolation and identification of  $\beta$ -sitosterol and Ursolic acid from the benzene extract of the bark of *Finlaysonia Obovata* Wall:

The mass left after the extraction with petroleum ether was extracted with benzene in a soxhlet apparatus for twentyfour hours. The benzene extract was subjected to distillation under reduced pressure when benzene was distilled off. The residue was taken up in ether, washed with alkali (4 x 200 ml) and then with

water till the ethereal solution was found to be neutral. The neutral ether layer was then dried with anhydrous sodium sulphate and then the ether was distilled off when the neutral part of the benzene extract was left. This was subjected to chromatography using deactivated alumina.

The alkali washing of the benzene extract was acidified with dilute hydrochloric acid and was then extracted with ether. The ether solution was washed with water to make it neutral and was then dried over anhydrous sodium sulphate. The solvent was then removed when a residual mass was obtained.

Chromatography of the neutral part of the Benzene extract:

The mass obtained (3 gm) was chromatographed in a column of 100 gm alumina deactivated with 4.3 ml 10% aqueous acetic acid and the following fractions were collected.

Table IV

Fraction No.	Eluent	Fraction collected (100 ml each)	Residue on evaporation
1	Petroleum ether	1-3	Trace oil
2	Petroleum ether: Benzene (4:1)	4-6	Nil

Contd...

Table IV

Fraction No.	Eluent	Fraction collected (100 ml each)	Residue on evaporation
3	Petroleum ether: Benzene (3:2)	7-16	Solid (m.p. 128-132°)
4	Petroleum ether: Benzene (2:3)	17-20	Trace oil

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

Fractions 7-16 (table IV) were combined and the mixture was crystallised from chloroform-methanol mixture when crystals of  $\beta$ -sitosterol were obtained m.p. 136-7°,  $(\alpha)_D -34^\circ$ .

Acetylation of  $\beta$ -sitosterol:

200 mg of the compound was taken in 2 ml pyridine. Then 2 ml acetic anhydride was added and the mixture was heated on a waterbath for 4 hours. After usual work up and crystallisation,

the mass obtained was found to be identical with  $\beta$ -sitosterol acetate (amp comparison) m.p. 129-30°, ( $\alpha$ )<sub>D</sub> -29°.

Treatment of the acid part from the Benzene extract:

The acid part (4.8 gm) was acetylated by the usual procedure using acetic anhydride and pyridine and the mass (7 gm) was chromatographed using 420 gm alumina deactivated with 17 ml of 10% aqueous acetic acid. The following fractions were collected.

Table V

Fraction No.	Eluent	Fraction collected (250 ml each)	Residue on evaporation
1	Petroleum Ether	1-3	Trace oil
2	Petroleum Ether: Benzene (4:1)	4-6	Nil
3	Petroleum Ether: Benzene (3:2)	7-9	Nil

Contd..

Table V

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Fraction No.	Eluent	Fraction collected (250 ml each)	Residue on evaporation
4	Petroleum Ether: Benzene (2:3)	10-12	Nil
5	Petroleum Ether: Benzene (1:4)	13-15	Nil
6	Benzene	16-18	Nil
7	Benzene: Ether (4:1)	19-31	Solid mp 287-290°

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Further elution with more polar solvents did not give any solid material.

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The solid obtained (fractions 19-31, Table V) was crystallised from chloroform-methanol mixture when crystals of  $C_{32}H_{50}O_4$  were obtained m.p. 294-5°.

Hydrolysis of the acetate  $C_{32}H_{50}O_4$

500 mg of the acetate was refluxed with 10% methanolic KOH on a waterbath for about 8 hours. The solvent was removed. The mass was then poured into water, a solid was obtained which was taken up in ether. It was washed to remove free alkali, dried, crystallised from methanol when 300 mg of the alcohol  $C_{30}H_{48}O_3$  m.p. 267-68° ( $\alpha$ )<sub>D</sub> 72° was obtained.

Analysis Report:

Found:	C, 79.00; H, 10.52%
Calc. for $C_{30}H_{48}O_3$ :	C, 78.90; H, 10.59%

Oxidation of the alcohol  $C_{30}H_{48}O_3$

A solution of the alcohol (200 mg) dissolved in pyridine (5 ml) was added to a chromium trioxide-pyridine complex prepared from pyridine (2 ml) and chromium trioxide (200 mg) and the mixture was kept at room temperature for fifteen hours. The crude product obtained by working up in the usual manner was crystallised when .15 gm of  $C_{30}H_{46}O_3$  m.p. 277-78° was obtained.



Analysis Report:

Found:	C, 79.81; H, 10.32%
Calc. for $C_{30}H_{46}O_3$ :	C, 79.25; H, 10.20%

$LiAlH_4$  reduction of the acetylated compound  $C_{32}H_{50}O_4$

The acetylated compound  $C_{32}H_{50}O_4$  (150 mg) dissolved in dry THF (25 ml) was added lithium aluminium hydride (100 mg) and the reaction mixture was heated on a waterbath for four hours. When the reaction was complete, excess of lithium aluminium hydride was decomposed carefully with moist ether and then a saturated solution of sodium sulphate was added. The ethereal solution was washed with water and was dried over anhydrous sodium sulphate. After removal of the solvent a solid residue (140 mg) was obtained, which was chromatographed over alumina (10 gm) deactivated with 0.4 ml of 10% aqueous acetic acid.

Table VI

Fraction No.	Eluent	Fraction collected (50 ml each)	Residue on evaporation
1	Petroleum Ether	1-2	Nil
2	Petroleum Ether: Benzene (4:1)	3-4	Nil

Contd..

Table VI (Contd..)

Fraction No.	Eluent	Fraction collected (50 ml each)	Residue on evaporation
3	Petroleum Ether: Benzene (3:2)	5-6	Nil
4	Petroleum Ether: Benzene (2:3)	7-8	Nil
5	Petroleum Ether: Benzene (1:4)	9-10	Nil
6	Benzene	11-16	Solid mp 220-5°

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

Fractions 11-16 (table VI) were combined and the residue was crystallised from methanol when crystals of  $C_{30}H_{50}O_2$  mp 230-31°,  $(\alpha)_D^{20}$  70° were obtained.

Analysis Report:

Found:	C, 81.20; H, 11.22%
Calc. for $C_{30}H_{50}O_2$ :	C, 81.39; H, 11.33%

The LAH reduction product was found to be identical with Uvaol<sup>4</sup> (no mp depression).

Acetylation of Uvaol,  $C_{30}H_{50}O_2$

200 mg of the compound was dissolved in 2 ml pyridine. This was treated with 5 ml acetic anhydride and the mixture was heated on a waterbath for 5 hours. Working up in the usual manner followed by crystallisation from a mixture of chloroform and methanol yielded crystals of diacetate  $C_{34}H_{54}O_4$ , mp 167-68° ( $\alpha$ )<sub>D</sub> 60°.

Analysis Report:

Found:	C, 77.45; H, 10.21%
Calc. for $C_{34}H_{54}O_4$	C, 77.52; H, 10.33%

Perbenzoic Acid titration with Uvaol diacetate:

To a solution of the compound (0.1501 gm) in chloroform in a 25 ml volumetric flask was added a solution of perbenzoic acid (5 ml) and the volume was made upto 25 ml with chloroform. A similar blank solution of perbenzoic acid (5 ml) was prepared in a 25 ml volumetric flask. Aliquots (5 ml) of the above two solutions were titrated separately at regular intervals with standard  $\frac{N}{100}$  sodium thiosulphate solution. No difference in titre value was

observed even after twentyfour hours.

Attempted hydrogenation on the diacetate:

To uvaol diacetate (200 mg) dissolved in ethyl acetate (40 ml) was added 10% palladium on charcoal catalyst (100 mg) and the mixture was stirred at room temperature in an atmosphere of hydrogen. No absorption of hydrogen took place even after six hours. It was then filtered and the filtrate was evaporated to dryness. The residue mp 155-6° (190 mg), on crystallisation from chloroform and methanol gave fine crystals mp 157-58° and was found to be unchanged in its mp with uvaol diacetate.

Esterification of the acetylated compound  $C_{32}H_{30}O_4$ :

1.0 gm compound was dissolved in ether (150 ml), added a solution of diazomethane in ether prepared from nitrosomethyl urea (800 mg) and was kept overnight. On the following 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 was then dried with anhydrous sodium sulphate. Evaporation of solvent gave a solid (600 mg) which was crystallised from a mixture of chloroform and methanol when crystals

of  $C_{33}H_{52}O_4$  m.p.  $240-1^\circ$ , ( $\alpha$ )<sub>D</sub>  $40^\circ$  were obtained. This was found to be identical with methyl ursolate acetate<sup>3</sup> (no mmp depression).

Analysis Report:

Found:	C, 77.59; H, 10.43%
Calc. for $C_{33}H_{52}O_4$ :	C, 77.30; H, 10.22%