CHAPTER 3

EXPERIMENTAL

Melting points in degree Celsius were determined in sulphuric acid bath and are uncorrected. All optical rotations were determined in chloroform. IR spectra were recorded in KBr disc with nujol mulling. NMR spectra were recorded in $CDCl_3$ solution with TMS as an internal standard. All samples for analysis were dried in vacuo for 36 hours. All thin layer chromatography (TLC) experiments were performed on micro plates prepared by dipping the plate in a slurry of silica gel G (Merck) in $CHCl_3$; spots were detected by exposing to iodine vapour. Column chromatography was carried out with BDH silica gel (60-120 mesh) or Merck neutral alumina (activity 1). Each column was developed with petroleum ether. Petroleum ether used had boiling point 60-80° Celsius. Extracts of products in organic solvents were generally washed with water and dried over anhydrous sodium sulphate. All evaporations were carried out under reduced pressure on a water bath. All acetylations (acetic anhydride) and oxidations (chromic oxide) were performed in anhydrous pyridine. After the reactions, the products were diluted with water and extracted with diethyl ether. The ether layer was washed with 2N HCl and

the water repeatedly to remove pyridine, dried and evaporated.

Extraction of Flacourtia sepiaria (Roxb)

Dried and powdered trunk, bark and stem (5 kg) of Flacourtia sepiaria (Roxb) were extracted with benzene in Soxhlet apparatus for 36 hours. The extract was cooled at room temperature and benzene was distilled off and a gummy residue (50 g) was obtained. The gummy residue was dissolved in solvent ether (1.5 litres) and the ether solution was washed with 10% aqueous sodium hydroxide solution (3 x 700 ml) and then with water till neutral. The neutral ethereal solution was dried and the ether was distilled off to yield gummy neutral residue (30 g), which constituted the neutral part of the extract.

The alkali wash portion on acidification with dilute hydrochloric acid (1N) yielded a solid and was extracted with ether. The ethereal solution, containing the acid part, was washed with water till neutral, dried and evaporated to dryness to yield gummy residue (10 g), which constituted the acid part of the extract.

Chromatography of the neutral part of F sepiaria

The neutral part of the extract was dissolved in minimum volume of benzene and placed on a column of alumina (1200 g, deactivated with 48 ml of 10% aqueous acetic acid). The column was eluted with the solvents as shown in Table 7.

Table 7

Chromatography results of the neutral part of Flacourtia sepiaria

Fraction number	Eluent	Nature of residue with different amount of eluents			Melting point of the materials under Column B
		Column A	Column B[X]	Column C	
1	Petroleum ether	5 x 500 ml[Y] Oil (5 g)[Z]	5 x 500 ml[Y] Waxy solid (1 g)[Z]	1 x 500 ml[Y] Nil[Z]	low mp
2	Pet ether:benzene (4:1)	4 x 500 ml[Y] Oil (3 g)[Z]	-	1 x 500 ml[Y] Nil[Z]	-
3	Pet ether:benzene (3:2)	2 x 500 ml[Y] Oil (1 g)[Z]	6 x 500 ml[Y] Solid (5 g)[Z]	1 x 500 ml[Y] Nil[Z]	126-133°
4	Pet ether:benzene (2:4)	2 x 500 ml[Y] Oil (.1 g)[Z]	-	1 x 500 ml[Y] Nil[Z]	-
5	Pet ether:benzene (1:4)	2 x 500 ml[Y] Oil (0.3 g)[Z]	1 x 500 ml[Y] Solid (0.2 g)[Z]	1 x 500 ml[Y] Nil[Z]	209-211°
6	Benzene	2 x 500 ml[Y] Oil (0.5 g)[Z]	1 x 500 ml[Y] Solid (0.4 g)[Z]	1 x 500 ml[Y] Nil[Z]	240-241°
7	Pet ether:solvent ether (9:1)	3 x 500 ml[Y] Oil (1.5 g)[Z]	2 x 500 ml[Y] Solid with oil (0.6 g)[Z]	1 x 500 ml[Y] Nil[Z]	127-130°

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

x Only materials under column B were examined.
Y Amount of eluents.
Z Nature and amount of residue after evaporation of solvents.

Isolation and crystallization of 1-hexacosanol

The material under column B (Table 7) of fraction 1 was crystallized from methanol. The process of crystallization was carried out for four times which afforded white flakes, mp 78-79°, $[\alpha]_D$ 0°.

Elemental analysis : Found : C 81.32, H 13.86%
Calculated for $C_{26}H_{54}O$: C 81.69, H 14.13%

Preparation of 1-hexacosanyl acetate

The above solid (0.2 g) was dissolved in 2 ml pyridine and 2 ml acetic anhydride was added to it. The mixture was kept overnight at room temperature. After usual work-up, the solid obtained on crystallization from methanol had mp 68-69°. It was found to be identical with an authentic sample of 1-hexacosanyl acetate (mixed mp, co-TLC) and co-IR).

Isolation and crystallization of β-sitosterol

The material under column B (Table 7) of fraction 3 on repeated crystallization from chloroform-methanol mixture yielded silky solid, mp 136-137°, $[\alpha]_D$ - 34°. Mixed melting point with authentic sample of -sitosterol showed no depression.

Elemental analysis : Found : C 83.34, H 11.62%
Calculated for $C_{29}H_{50}O$: C 83.98, H 12.15%

Preparation of β-sitosterol acetate

-sitosterol (0.3 g) was acetylated with acetic anhydride-pyridine in the usual method. The acetate on crystallization from chloroform-methanol had mp 130-132°, $[\alpha]_D$ - 40°. Mixed melting point of the acetate with an authentic sample showed no depression.

Elemental analysis : Found : C 78.68, H 11.05%
Calculated for $C_{31}H_{52}O_3$: C 78.81, H 11.01%

Isolation of 20-hydroxylupanone (1)

Compound from fraction 5 under column B (Table 7) was crystallized several times from chloroform-methanol to afford needle shaped crystals of 20-hydroxylupanone (1), mp 213-214°.

Elemental analysis : Found : C 81.21, H 11.48%
Calculated for $C_{30}H_{50}O_2$: C 81.39, H 11.38%
IR spectrum : Fig 1
^1H NMR spectrum : Fig 2
^{13}C NMR spectrum : Fig 4

Attempted acelylation of 20-hydroxylupanone (1): conversion of 20-hydroxylupanone to lupenone (2)

0.1 g of 20-hydroxylupanone (1) was dissolved in pyridine (1 ml) followed by the addition of acetic anhydride (1 ml) and boron trifluoride etherate (few drops) to it. The reaction mixture was kept on water bath for 10 hours. The product on usual work-up afforded an oily substance, which was dissolved in minimum volume of benzene and poured on a column of alumina, deactivated with 0.2 ml of

10% aqueous acetic acid. Elution of the column with petroleum ether afforded a solid substance. The solid on crystallization from chloroform-methanol furnished crystals of lupenone (2), mp 169-171°, $[\alpha]_D$ + 57°. Mixed melting point of the compound with an authentic sample showed no depression.

Isolation of 3β, 20-dihydroxylupane (3)

Compound from fraction 6 under column B (Table 7) on repeated crystallization from chloroform-methanol furnished solid, mp 243-244°, $[\alpha]_D$ + 26.7°. It was found to be 3β, 20-dihydroxylupane (3).

Elemental analysis : Found : C 80.92, H 11.84%
Calculated for $C_{30}H_{52}O_2$: C 81.02, H 11.79%
IR spectrum : Fig 6

Acetylation of 3β, 20-dihydroxylupane (3) : preparation of 20-hydroxylupane-3β-acetate (4)

To a solution of 3β, 20-dihydroxylupane 3 (0.3 g) in pyridine (3 ml) was added acetic anhydride (3 ml) and left overnight at room temperature. After usual work-up the compound obtained was purified by crystallization from chloroform-methanol to afford needle shaped crystals of 20-hydroxylupan-3β-acetate (4), mp 253-254°, $[\alpha]_D$ + 20.6°.

Elemental analysis : Found : C 79.09, H 11.28%
Calculated for $C_{32}H_{54}O_3$: C 78.96, H 11.18%
IR spectrum : Fig 7

Attempted acetylation of 20-hydroxylupan-3 β -acetate (4) : preparation of lupenyl acetate (5)

0.1 g of 20-hydroxylupan-3 β -acetate (4) was dissolved in 1 ml pyridine and 1 ml acetic anhydride was added. The reaction mixture was kept at 80° for 24 hours. After usual work-up, a solid was obtained which on crystallization from chloroform-methanol afforded crystalline solid of lupenyl acetate (5), mp 216-218°, $[\alpha]_D$ + 47.5°.

Elemental analysis : Found : C 81.65, H 11.39%
Calculated for $C_{32}H_{52}O_2$: C 81.99, H 11.18%
IR spectrum : Fig 8
^1H NMR spectrum : Fig 9

Oxidation of 3 β , 20-dihydroxylupana (4) to 20-hydroxylupanone (1)

To a solution of the alcohol 4 (0.2 g) dissolved in pyridine (5 ml) was added chromic oxide and the mixture was kept at room temperature for 2 hours. The crude product obtained after work-up in the usual manner was crystallized from chloroform-methanol. The melting point of the crystals was found to be 210-212° and the compound was found identical with 20-hydroxy-lupanone 1 (mixed mp, co-TLC and co-IR).

Isolation of sepesteonol (7)

The oily mass (0.6 g) from fraction 7 under column B (Table 7) was dissolved in minimum volume of benzene and poured over silica gel (15 g) column. The column was eluted with the solvents as shown in Table 8.

The fractions 11-12 (Table 8) were rejected because they mainly contained oily mass. The fractions 13-15 were mixed and crystallized from chloroform-methanol to yield solid, named sepesteonol (7), mp 132-133°, $[\alpha]_D$ - 98°.

Table 8

Rechromatography of sepesteonol

Eluent	Fractions collected (50 ml each)	Residue after evaporation of solvent	Melting point
Petroleum ether	1 - 2	oil	-
Pet ether : benzene (1:4)	3 - 6	oil	-
Benzene	7 - 10	oil	-
Benzene:solvent ether (9:1)	11 - 16	solid	129-132°

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

Elemental analysis : Found : C 81.02, H 10.90%

Calculated for $C_{29}H_{48}O_2$: C 81.30, H 11.21%

Mass spectrum : Fig 10a

IR spectrum : Fig 10b

Acetylation of sepesteonol (7)

0.4 g sepesteonol (7) was dissolved in 4 ml pyridine and 4 ml acetic anhydride was added to it. The reaction mixture was kept overnight at room temperature. After usual work-up, the solid obtained on crystallization from chloroform-methanol resulted crystalline sepesteonyl acetate (8), mp 169-170°, $[\alpha]_D$ - 92°.

Elemental analysis : Found	: C 78.89, H 10.02%
Calculated for $C_{31}H_{50}O_3$	: C 79.15, H 10.64%
Mass spectrum	: Fig 11a
IR spectrum	: Fig 11b
UV spectrum	: Fig 12
^1H NMR spectrum	: Fig 13
^{13}C NMR spectrum	: Fig 16

Preparation of sepesteonyl acetate (8) from β-sitosterol acetate

6 g β-sitosterol acetate was dissolved in 250 ml benzene and the solution was refluxed for 15 minutes. A solution of sodium dichromate (6 g) in glacial acetic acid (180°), maintaining the temperature at about 70° was added slowly. When the addition was complete, refluxing of the solution was continued for 4 hours. The reaction mixture was cooled at room temperature and 25 ml rectified spirit was added and then the solution was concentrated to one-third of the volume. It was then poured on ice-cold water when an insoluble solid seperated out. It was extracted with ether, washed with water and dried. The solvent ether was removed under reduced pressure when a solid (6 g) was obtained. The solid was dissolved in minimum volume of benzene and poured on a silica gel (150 g) column. The column was eluted with the solvents as shown in Table 9.

Examination of fractions 3-6 (Table 9)

The fractions 3-6 (Table 9) were mixed and crystallized from chloroform-methanol to yield a solid, mp 130-131°. It was found to be identical with β-sitosterol acetate (mixed mp and co-TLC).

Table 9

Eluents	Fractions collected (250 ml each)	Residue after evaporation of solvent	Melting point
Petroleum ether	1 - 2	oil	-
Pet ether : benzene (9:1)	3 - 6	solid (2 g)	129-130°
Pet ether : benzene (4:1)	7 - 8	oil	-
Pet ether : benzene (3:2)	9 - 14	solid	165-169°

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

Examination of fractions 9-14 (Table 9) : isolation of sepesteonyl acetate (8)

The fractions 9-14 (Table 9) were mixed and crystallized from chloroform-methanol repeatedly unless it gave a solid with constant mp 169-170°. It showed no depression of melting point when mixed with sepesteonyl acetate obtained by acetylating the naturally occuring sepesteonol.

Attempted alkali hydrolysis of sepesteonyl acetate (8) : preparation of tremulone (12)

A mixture of sepesteonyl acetate 8 (0.3 g) and 5% methanolic potassium hydroxide (1 ml) was refluxed for 3 hours and then water was added. The resulting mixture was acidified with dilute hydrochloric acid and then extracted with ether. The extract was washed with water, dried and evaporated to dryness. The residue was dissolved in minimum volume of petroleum ether and chromatographed over

silica gel (8 g); petroleum eluates of which gave a solid, crystallized from acetone to yield a crystalline solid, mp 111-112°, $[\alpha]_D$ - 290°. It was characterized as tremulone (12).

Elemental analysis	: Found	: C 84.52, H 11.01%
	Calculated for $C_{29}H_{46}O$	: C 84.88, H 11.22%
	IR spectrum	: Fig 19
	UV spectrum	: Fig 20
	^1H NMR spectrum	: Fig 21
	Mass spectrum	: Fig 22

Attempted acid hydrolysis of sepesteonyl acetate (8) : preparation of tremulone (12)

0.2 g sepesteonyl acetate was dissolved in 15 ml dioxane and a few drops of concentrate sulphuric acid was added to it and then refluxed for 3 hours. After usual work-up, the solid obtained was chromatographed over silica gel column and was crystallized from acetone to yield a solid, mp 113-114°. It was found to be identical with tremulone 12 (mixed mp, co-TLC and co-IR).

Chromatography of the acid part of F sepiaria

The acid part of extract was dissolved in minimum volume of benzene and poured over a column of silica gel (250 g). The column was eluted with the solvents as shown in Table 10.

Table 10

Chromatography results of the acid part of Flacourtia sepiaria

Eluent	Fractions collected (250 ml each)	Nature of residue after removal of solvent	Melting point
Petroleum ether	1 - 4	waxy solid (2 g)	80-82°
Pet ether : benzene (4:1)	5 - 10	oil (5 g)	-
Pet ether : benzene (3:2)	11 - 12	solid (0.5 g)	117-120°

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

Isolation of tetracosanoic acid

The fractions 1-4 (Table 10) were mixed and crystallized from methanol to yield white flakes, mp 86-87°, $[\alpha]_D$ 0°. It was identified as tetracosanoic acid.

Elemental analysis : Found : C 78.02, H 13.01%

Calculated for $C_{24}H_{48}O_2$: C 78.53, H 13.09%

Preparation of methyltetracosanate

Tetracosanoic acid (1 g) was dissolved in ether (200 ml) and cooled at 0°. In this solution, a solution of diazomethane in ether at 0°, prepared from nitrosomethyl urea (0.7 g) by the decomposition with potassium hydroxide, was added. The mixture was kept for 8 hours maintaining the temperature below 5° and then kept at room temperature. The excess of diazomethane was destroyed with acetic acid. Then the ether solution was washed with water, 10% sodium

bicarbonate solution and again with water till neutral, and dried. Evaporation of ether yielded the solid substance (1 g) which on repeated crystallization from methanol furnished waxy solid of methyltetracosanate, mp 60-61°, $[\alpha]_D$ 0°.

Elemental analysis	: Found	: C 78.08, H 12.98%
	Calculated for $C_{25}H_{50}O_2$	: C 78.53, H 13.09%
	IR spectrum	: Fig 24
	^1H NMR spectrum	: Fig 25
	^{13}C NMR spectrum	: Fig 26

Isolation of benzoic acid

The fractions 11-12 (Table 10) were mixed and crystallized from hot water to yield shiny crystalline solid, mp 121°. It was found to be identical with an authentic sample of benzoic acid (mixed mp, co-TLC and co-IR).

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