

CHAPTER-V

EXPERIMENTAL

Melting points are uncorrected. The petrol used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution. NMR spectra were determined on a Varian HA-80 spectrophotometer using chloroform -d solution containing tetramethylsilane as internal reference. The IR spectra were recorded in a Beckmann IR-20 spectrophotometer. U.V. absorption spectra were taken in a Beckmann DU-2 spectrophotometer using hexane solution. The mass spectra were determined with an MS-50 mass spectrophotometer, using direct sample introduction into the ion source. Silica gel G for column chromatography was of 60-120 mesh and was activated at 120°. Silver nitrate impregnated Silica Gel was made by the method of Gupta and Sukh Dev²⁷ and activated at 110-20° (12 hours). TLC examinations were carried out on 12% Silver nitrate impregnated silica gel plate. The plates were activated at 110-20° (30 minutes) and then stored in a dessicator. Acetic anhydride-sulphuric acid (9:1) spray followed by heating (120°, 15 minutes) was used for visualisation of TLC spots.

Extraction:

Dried and powdered rhizomes of the fern oleandra nerifolia (2 kg) was extracted with benzene in a soxhlet

apparatus for 20 hours. Benzene was distilled off and the residual gummy material (6 g) was taken up in ether. The ether solution was washed with 10% aqueous sodium hydroxide solution and then with water till neutral and dried (Na_2SO_4). Removal of ether gave a gummy residue (4 g).

Chromatography of the above Gummy Residue:

The above gummy residue (4 g) was dissolved in benzene (12 ml) and was placed on a column of alumina (250 g; deactivated with 10 ml of 10% aqueous acetic acid). The chromatogram was developed with petrol and eluted with the following solvents (Table-II).

Table-II

Eluent	Fractions 100 ml each	Residue on Evaporation	Melting point in °C
Petrol	1-10	Solid with oil (0.8 g)	-
Petrol: Benzene (4:1)	11-15	Trace oil (0.1 g)	-
Petrol: Benzene (3:2)	16-22	Solid (0.5 g)	236-40°
Petrol: Benzene (2:3)	23-32	Solid (1.0 g)	130-34°

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

Rechromatography of Fractions 1-10 (Table-II, Chapter-V):

The oily solid (0.8 g) obtained from fractions 1-10 (Table-II, Chapter-V) was dissolved in benzene (3 ml) and placed on a column of active alumina (50 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-III).

Table-III

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-4	Oil
	5-10	Waxy solid (0.5 g)

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

Examination of Fractions 5-10 (Table-III, Chapter-V):

Isolation of Filicene (22):

Fractions 5-10 (Table-III, Chapter-V) were combined (0.5 g) to furnish a waxy solid which on careful crystallisation from a mixture of chloroform and acetone yielded a solid (0.3 g),

m.p. 226-28°, (α)_D 50°, (TLC-single spot). It was found to be identical (m.m.p, IR and Co-TLC) with an authentic specimen of fillicene¹⁷ (22).

Found :	C, 87.86;	H, 12.12.
Calc. for C ₃₀ H ₅₀ :	C, 87.73;	H, 12.27%

Isolation of the New Triterpene, 29-Ethoxyhopane, C₃₂H₅₆O (35):

The mother liquor from the crystallisation of fillicene (22) on evaporation gave a crude mass (0.1 g), which was found by TLC to be a mixture of three components. Careful fractional crystallisation of this crude mass (0.1 g) from a mixture of chloroform and methanol (3:1) afforded crystals of (35) (10 mg), m.p. 179-80°, (α)_D 27.16° (TLC-single spot).

Found:	C, 84.16;	H, 12.22.
Calc. for C ₃₂ H ₅₆ O:	C, 84.14;	H, 12.36%

<u>Mass spectrum</u> :	M ⁺ 456	<u>Fig-1</u>
<u>IR</u> : \checkmark nujol max	1105 cm ⁻¹	<u>Fig-2</u>

UV : No absorption in the region 200-300 nm.

NMR Spectra (80 MHz) : δ 0.7-0.95 (7 methyl groups)
 δ 2.8-3.6 (multiplet, 4 protons)

Fig-3, Fig-4, Fig-5.

Isolation of Two other unidentified Compounds:

The mother liquor from the crystallisation of 29-ethoxyhopane (35) on evaporation gave a crude mass (50 mg), which was dissolved in benzene (1 ml) and was placed on a column of alumina (5 g) impregnated with 20% silver nitrate. The chromatogram was developed with petrol and eluted with the following solvents (Table-IV).

Table-IV

Eluent	Fractions 25 ml each	Residue on evaporation
Petrol	1-3	Oil
	4-5	Solid (m.p. 180-82°)
	6-7	Solid (m.p. 160-63°)

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

Fractions 4-5 (Table-IV) were combined and on crystallisation from a mixture of chloroform and methanol gave crystals (6 mg), m.p. 182-83°, (TLC-single spot).

Found:	C, 84.16;	H, 11.64
Calc. for $C_{30}H_{50}O$:	C, 84.44;	H, 11.81%

Fractions 6-7 (Table-IV) were combined to yield a solid which on crystallisation from a mixture of chloroform and methanol afforded yet another solid (4 mg), m.p. 163-64°, (TLC-single spot).

Found:	C, 87.61;	H, 12.16
Calc. for $C_{30}H_{50}$:	C, 87.73;	H, 12.27%.

The structure elucidations of these two compounds were not possible because of their very poor yield. Further work is in progress to isolate them in quantity to enable us to investigate their structures.

Examination of Fractions 16-22 (Table-II, Chapter-V): Isolation of Nerifoliol (17a):

The solid (0.5 g) obtained from fractions 16-22 (Table-II, Chapter-V) was dissolved in benzene (5 ml) and placed on a column of active alumina (30 g). The chromatogram was developed

with petrol and eluted with the following solvents (Table-V).

Table-V

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-4	Oil
Petrol: Benzene (4:1)	5-6	Nil
Petrol: Benzene (3:2)	7-8	Nil
Petrol: Benzene (2:3)	9-14	Solid, m.p. 241-43° (0.4 g)

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

The solid (0.4 g) from fractions 9-14 (Table-V) on crystallisation from a mixture of chloroform and methanol afforded fine crystals of (17a), m.p. 242-44°, (α)_D 35° (TLC-single spot).

Found:	C, 83.91;	H, 12.34
Calc. for $C_{30}H_{52}O$:	C, 84.04;	H, 12.22%

IR ν_{max} ^{nujol} 3320 cm^{-1} (OH)

Mass spectrum: M^+ 428.

The physical and chemical data of the compound (17a) showed that this was identical with nerifoliol (17a) isolated by Pandey and Mitra⁶ from the same plant O. nerifolia.

Acetylation of Nerifoliol (17a): Preparation of Nerifoliol Acetate(17b):

A solution of the compound (17a; 200 mg) in pyridine (2 ml) and acetic anhydride (2 ml) was heated on a water bath for 4 hours. After working up in the usual way it gave a solid residue (200 mg), which was dissolved in benzene (4 ml) and placed over a column of active alumina (10 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-VI).

Table-VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-5	Solid (180 mg), m.p. 194-96°

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

The solid (180 mg) from fractions 1-5 (Table-VI) on crystallisation from a mixture of chloroform and methanol afforded crystals of (17b), m.p. 195-96°, (α)_D 20°.

Found: C, 81.48; H, 11.48.
Calc. for C₃₂H₅₄O₂ : C, 81.64; H, 11.56%

IR ν _{max} nujol : 1730, 1225 cm⁻¹ (OCOCH₃)

Mass spectrum: M⁺470.

The physical and chemical data of the compound showed that it was identical with nerifoliol acetate (17b) prepared by Pandey and Mitra⁶.

Examination of Fractions 23-32 (Table-II, Chapter-V): Isolation and Identification of β -Sitosterol:

The solid (1.0 g) obtained from the fractions 23-32 (Table-II, Chapter-V) was rechromatographed over active alumina (60 g). Elution with a mixture of petrol and benzene (1:4) gave a solid which on crystallisation from a mixture of chloroform and methanol furnished fine needle shaped crystals of an alcohol, m.p. 136-37°, $(\alpha)_D -32^\circ$.

Found:	C, 83.56;	H, 11.76
Calc. for $C_{29}H_{50}O$:	C, 83.98;	H, 12.15%.

The alcohol (200 mg) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The product on crystallisation from a mixture of chloroform and methanol furnished crystals of the acetate, m.p. 127-29°, $(\alpha)_D -40^\circ$.

Found:	C, 81.23;	H, 11.32.
Calc. for $C_{31}H_{52}O_2$:	C, 81.52;	H, 11.48%.

The alcohol and the acetate were identified as β -sitosterol and β -sitosteryl acetate respectively by direct comparison (m.m.p, IR and Co-TLC) with their respective authentic specimens.

Potassium Metal-Ethyl Iodide Treatment^{22,23} of Nerifoliol (17a):
Partial Synthesis of the New Triterpene, 29-Ethoxy hopane (35):

To a solution of nerifoliol (17a) (300 mg) in dry benzene (25 ml) was added potassium metal (400 mg) and the mixture was refluxed under anhydrous condition for 3 hours. The reaction mixture was then allowed to cool to room temperature. Freshly prepared dry ethyl iodide (25 ml) was added dropwise to the reaction mixture. The reaction mixture was again refluxed for 3 hours and then allowed to cool to room temperature. Excess potassium metal was decomposed by cautious addition of ethanol. The reaction mixture was diluted with water and the organic layer extracted with petrol. The petrol benzene layer was washed with saturated sodium chloride solution till neutral and dried (Na_2SO_4). Removal of solvent gave a gummy residue (300 mg), which was dissolved in benzene (5 ml) and placed on a column of active alumina (30 g). The chromatogram was developed in petrol and eluted with the following solvents (Table-VII).

Table-VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-2	Oil
	3-8	Solid (100 mg), m.p. 176-78°

Contd....

Table-VII (Contd)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (4:1)	9-11	Nil
Petrol: Benzene (3:2)	12-14	Nil
Petrol: Benzene (2:3)	15-22	Solid (160 mg), m.p. 240-42°

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

The solid (100 mg) from ^{fractions} 3-8 (Table-VII) on crystallisation from a mixture of chloroform and methanol furnished a crystalline solid, m.p. 179-80°, $(\alpha)_D^{20}$ 27.16° which was found to be identical (m.m.p, Co-TLC and Co-IR, Fig. 6) with 29-ethoxyhopane (35) isolated from the plant Oleandra nerifolia as described above.

Found: C, 84.36; H, 12.53.

Calc. for C₃₂H₅₆O: C, 84.14; H, 12.36%.

IR ν_{max} nujol 1105 cm⁻¹

Co IR Fig. 6

The solid (160 mg) from fractions 15-22 (Table-VII) on crystallisation from a mixture of chloroform and methanol afforded crystals, m.p. 242-44° which were found to be identical (m.m.p, Co-TLC and IR) with the starting material nerifoliol (17a).

Potassium Metal-Ethyl Iodide Treatment of Dryocrassol (12a):

Preparation of Dryocrassol Ethyl Ether (30-Ethoxyhopane) (34):

Dryocrassol^{12,24,25} (12a; 100 mg) dissolved in dry benzene (10 ml) was similarly treated with potassium metal (180 mg) and ethyl iodide (10 ml). The gummy residue (100 mg) obtained after working up the reaction mixture in the same manner was dissolved in benzene (2 ml) and was placed over a column of active alumina (15 g). The chromatogram was developed with petrol and eluted with the following solvents (Table-VIII).

Table-VIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-3	Oil
	4-5	Solid (30 mg), m.p. 146-49°
Petrol: Benzene (4:1)	6-7	Nil
Petrol: Benzene (3:2)	8-9	Nil

Contd...

Table-VIII (Contd.)

Eluent	Fractions 50 ml each	Residue on evaporation
Petrol: Benzene (2:3)	10-12	Solid (50 mg), m.p. 243-46°

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

The solid (30 mg) from fractions 4-5 (Table-VIII) on crystallisation from a mixture of chloroform and methanol afforded crystals of (34), m.p. 148-50°. This compound (34) showed depression of melting point on admixture with the natural product (35) and its IR spectra was distinctly different from that of 29-ethoxyhopane (35) isolated from the natural source. \angle Co IR of Synthetic dryocrassol Ether (34) and natural 29-ethoxyhopane (35) -Fig.7 7.

Found: C, 84.28; H, 12.47.
Calc. for $C_{32}H_{56}O$: C, 84.14; H, 12.36%.
IR $\nu_{\max}^{\text{nujol}}$ 1110 cm^{-1}

Co IR of Synthetic Dryocrassol Ether and 29-ethoxyhopane
(natural)- Fig. 7

Fractions 10-12 (Table-VIII) were combined (50 mg) and on crystallisation from a mixture of chloroform and methanol afforded a solid, m.p. 244-47° which was found to be identical (m.m.p., IR and Co-TLC) with the starting material dryocrassol (12a).

Anhydrous Ferric Chloride-Acetic Anhydride-Ethyl acetate

Treatment of 29-Ethoxyhopane (35): Preparation of Nerifoliol Acetate (17b):

The triterpene 29-ethoxyhopane (35; 5 mg) was dissolved in a mixture of dry acetic anhydride and ethyl acetate mixture (1:1; 5 ml) with gentle heating. After cooling to room temperature, anhydrous ferric chloride (1.25 mg) was added and the reaction mixture kept at 80° for 24 hours. The reaction mixture was cooled, poured into water and extracted with hexane. The organic layer was washed with water, 5% aqueous NaHCO₃ solution and again with water till neutral and then dried (Na₂SO₄). Removal of the solvent gave a residue (3 mg), which on crystallisation from a mixture of chloroform and methanol afforded nerifoliol acetate (17b; 2 mg), m.p. 195-96° which was found to be identical (no m.m.p. depression, Co-TLC and superimposable IR spectra - Fig. 8) with an authentic specimen.