

CHAPTER-III

EXPERIMENTAL

The melting points are uncorrected. The optical rotations were taken in chloroform unless stated otherwise. Petroleum ether used throughout the investigation had b.p. 60-80°. The UV spectra were taken in Ziess VSU-1 spectrophotometer in ethanol solution unless stated otherwise. The IR spectra were recorded in Perkin-Elmer spectrophotometer 337.

Dried and powdered whole plant (960 g., collected in winter season) was extracted with benzene for 30 hours. Benzene was distilled off and the residual gummy mass was taken up in ether. The ether solution was washed with 10% aqueous NaOH solution (2 x 200 ml.) and then with water till neutral. The neutral ether solution dried (Na_2SO_4) and evaporated when a gummy residue (16 g.) was obtained. It was dissolved in benzene (25 ml.) and poured on a column of alumina (650 g., deactivated with 26 ml. of 10% aqueous acetic acid), developed with petroleum ether. It was eluted with the following solvents.

TABLE-I

Eluent	Fractions, 100 ml.each	Fraction No.	Residue on evaporation
Petroleum ether	1 - 8		Oil (6 g.)
Petroleum ether	10 - 19	I	Solid(1 g.),m.p. 220-28°
Petroleum ether:benzene(4:1)	22 - 30	II	Solid(1.3 g.),m.p.80-85°
Petroleum ether:benzene(3:2)	36- 47	III	Solid(2 g.), m.p.125-32°

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

EXAMINATION OF FRACTION NO.I : ISOLATION OF GLUT-5-EN-3-ONE

Fraction No.1 (Fractions 10-19, Table-I) were combined and on crystallisation from chloroform and methanol mixture afforded crystals, m.p. 238-40°, $[\alpha]_D^{25} +39^\circ$, identical with an authentic specimen of glut-5-en-3-one³ (m.m.p. and IR comparison).

Found	: C,84.74; H,11.31%.
Calculated for C ₃₀ H ₄₈ O	: C,84.84; H,11.39%.
IR (KBr disc)	: ν_{\max} 1707 cm ⁻¹ .

LAH reduction of Glut-5-en-3-one : Preparation of Glut-5-en-3-ol

Glut-5-en-3-one (0.1 g.) was refluxed with LAH (0.075 g.) in dry ether (25 ml.) for 4 hours. The reaction mixture was cooled and a saturated solution of Na₂SO₄ was added and extracted with ether. The ether extract was washed with water, dried (Na₂SO₄) and evaporated to afford solid m.p. 190-92°. The solid on crystallisation from chloroform and methanol afforded fine crystals, m.p. 196-7°, $[\alpha]_D^{25} +57^\circ$.

Found : C,84.36; H,11.76%.
 Calculated for $C_{30}H_{50}O$: C,84.44; H,11.81%.

Preparation of Glut-5-en-3 α -yl acetate

Glut-5-en-3 α -ol (0.1 g.) was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) by keeping over water bath for 4 hours. The product obtained by working up in the usual manner had m.p. 225-7 $^{\circ}$ which on crystallisation from chloroform and methanol furnished crystals, m.p. 231-3 $^{\circ}$, $[\alpha]_D^{25} +46^{\circ}$. This was found to be identical with an authentic specimen of glut-5-en-3 α -yl acetate³ (m.m.p. and IR).

Found : C,81.82; H,11.15%.
 Calculated for $C_{32}H_{52}O_2$: C,81.99; H,11.18%.

EXAMINATION OF FRACTION NO.II (Fractions 22-30 - Table-I)

Fraction II (1.3 g.) dissolved in benzene (8 ml.) was placed on a column of alumina (100 g., deactivated with 4 ml. of 10% aqueous acetic acid) developed with petroleum ether. It was eluted with the following solvents (Table-II).

TABLE-II

Eluent	Fractions, 50 ml.each	Residue
Petroleum ether	1 - 6	Oil (0.2 g.)
Petroleum ether:benzene(4:1)	8 - 15	Solid (1.0 g.), m.p. 85-7 $^{\circ}$

Further elution with more polar solvents did not yield any material

Fractions 8-15 (Table-II) were combined (1.0 g.) and crystallised from methanol several times when fine needle shaped crystals of compound A, m.p. $95-6^{\circ}$, $[\alpha]_D^{25} +58.6^{\circ}$, was obtained.

Found : C, 84.60; H, 11.78%.
Calculated for $C_{31}H_{52}O$: C, 84.50; H, 11.82% (M^+ 440).
IR ($CHCl_3$) : ν_{max} 3600 cm^{-1}

Acetylation of compound A

Compound A (0.1 g.) was acetylated with pyridine (1 ml.) and acetic anhydride (1 ml.) in the usual way. The solid obtained on crystallisation from chloroform and methanol furnished crystals m.p. $107-8^{\circ}$, $[\alpha]_D^{25} +50^{\circ}$.

Found : C, 82.10; H, 11.11%.
Calculated for $C_{33}H_{54}O_2$: C, 82.16; H, 11.20%.

Benzoylation of compound A

Compound A (0.1 g.) was benzoylated with pyridine (1 ml.) and benzoyl chloride (1 ml.) in the usual way. The solid obtained on crystallisation from chloroform and methanol furnished fine needle shaped crystals, m.p. $274-5^{\circ}$.

Found : C, 83.74; H, 10.12%.
 $C_{38}H_{56}O_2$ requires : C, 83.82; H, 10.29%.

CrO₃-Py oxidation of compound A

Compound A (0.1 g.) dissolved in pyridine was added to a complex prepared from CrO₃ (0.1 g.) and pyridine (1 ml.) at 10° and was allowed to come to room temperature overnight. On working up in the usual manner

a solid product was obtained which was chromatographed over active alumina. On elution with petroleum ether:benzene (3:2) a solid was obtained which on crystallisation from chloroform and methanol furnished crystals, m.p. 103-4°, $[\alpha]_D +27^\circ$.

Found : C,84.84; H,11.31%.

Calculated for $C_{31}H_{50}O$: C,84.93; H,11.44%.

ISOLATION OF BUTYROSPERMOL

The mother liquors of the compound A were combined and dried (0.3 g.) and then acetylated as usual with pyridine (3 ml.) and acetic anhydride (3 ml.). The solid obtained on working up of the reaction product was crystallised several times from methanol. The mother liquors on standing for three days furnished long needle shaped crystals, m.p. 141-3°, $[\alpha]_D +14.2^\circ$ which was identical (m.m.p. and IR comparison) with an authentic specimen of butyrospermyl acetate⁴.

Found : C,81.72; H,10.90%.

Calculated for $C_{32}H_{52}O_2$: C,81.99; H,11.18%.

Hydrolysis of butyrospermyl acetate : Preparation of butyrospermol

To the above acetate (0.1 g.) dissolved in benzene (3 ml.) was added 5% methanolic KOH (25 ml.) and the mixture was refluxed for 4 hours. This was worked up as usual and the product obtained was chromatographed over alumina (10 g., deactivated with 0.4 ml. of 10% aqueous acetic acid). Petroleum ether:benzene (3:2) eluted a solid which on crystallisation from methanol gave crystals, m.p. 110-11°, $[\alpha]_D -11.5^\circ$.

Found : C,84.34; H,11.76%.

Calculated for $C_{30}H_{50}O$: C,84.44; H,11.81%.

Benzoylation of butyrospermol : Preparation of butyrospermyl benzoate

To butyrospermol (0.1 g.) dissolved in pyridine (1 ml.) was added benzoyl chloride (1 ml.) and the mixture heated over water bath for 4 hours. After working up in the usual manner the crude benzoate (0.1 g.) was chromatographed over alumina (10 g., deactivated with 0.4 ml. of 10% aqueous acetic acid). Petroleum ether eluted a solid which on crystallisation from a mixture of chloroform and methanol furnished crystals, m.p. 130-32°, $[\alpha]_D^{25} +32.6^\circ$. It was identical (m.m.p. and IR comparison) with an authentic specimen of butyrospermyl benzoate kindly supplied by Prof. Barton.

Found : C, 83.65; H, 10.16%.

Calculated for $C_{37}H_{54}O_2$: C, 83.72; H, 10.25%.

Isolation of 1-hexacosanol

Dried and powdered whole plant (960 g.) of E. Sikkimensis Boiss (collected during rainy season) was extracted with benzene for 30 hours and worked up as described above. The neutral gummy residue (10 g.) obtained was chromatographed over alumina (400 g., deactivated with 16 ml. of 10% aqueous acetic acid). It was eluted with the following solvents (Table-III).

TABLE-III

Eluent	Fractions, 50 ml. each	Residue
Petroleum ether	1 - 16	Solid (4 g.), m.p. 60-70°
Petroleum ether:benzene(4:1)	18 - 20	Nil
Petroleum ether:benzene(3:2)	21 - 32	Solid (2.6 g.), m.p. 130-32°

Further elution with more polar solvents did not yield any material

EXAMINATION OF FRACTIONS 1-16 (TABLE-III) : ISOLATION OF 1-HEXACOSANOL

Fractions 1-16 (Table-III) were combined and on crystallisation several times from methanol afforded flaky crystals m.p. 79-80°, $[\alpha]_D^{20}$.

Found : C, 81.64; H, 14.11%. Mol.wt. (Rast) 376.
Calculated for $C_{26}H_{54}O$: C, 81.65; H, 14.13%. Mol.wt. 382.
IR ($CHCl_3$) : $\nu_{max}^{CHCl_3}$ 3350 cm^{-1} .

Acetylation of 1-hexacosanol

1-Hexacosanol (0.2 g.) was acetylated by pyridine (2 ml.) and acetic anhydride (2 ml.) in the usual manner. The solid obtained on crystallisation from methanol had m.p. 68-69°, $[\alpha]_D^{20}$ identical in all respects with 1-hexacosanyl acetate⁵.

EXAMINATION OF FRACTION NO. III (FRACTIONS 36-47, Table-I) AND FRACTIONS 21-32 (Table-III) : ISOLATION OF β -SITOSTEROL

Fractions 36-47 (Table-I) and fractions 21-32 (Table-III) were crystallised separately from chloroform and methanol mixture when crystals of m.p. 132-34° were obtained. Both the two solids were found to be identical with each other (m.m.p.). It was identified as β -sitosterol by m.m.p. with an authentic sample.

Preparation of β -sitosterol acetate

β -sitosterol (0.3 g.) isolated above was acetylated with pyridine (3 ml.) and acetic anhydride (3 ml.) in the usual method. The acetate on crystallisation had the m.p. 124-6°, $[\alpha]_D^{20}$ -34°, which was identical with an authentic specimen of β -sitosterol acetate.

REFERENCES

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