CHAPTER IV

Experimental

Isolation of the neutral material

Dried and powdered stem bark of Macaranga denticulata, Muell. Arg. (2 kg.) was extracted with benzene in a Soxhlet apparatus for twenty hours. Benzene was distilled off and the residual gummy solid (20 gm.) was taken up in ether (2 liter). The ether solution was washed with 10% sodium hydroxide solution (2 x 200 ml). The ether layer was washed with cold water till the washings were neutral, dried over anhydrous sodium sulphate and evaporated, when the neutral material (9 gm.) was obtained as a yellow gummy solid.

Chromatography of the above gummy solid

The above gummy material (9 gm) dissolved in benzene (25 ml) was placed on a column of alumina (400 gm.) deactivated with 16 ml. of 10% aqueous acetic acid. The chromatogram was developed with petroleum and eluted with the following solvents (Table I).

Table I

Eluent	Fractions (50 ml each)	Residue on evaporation	Melting point
Petroleum (200 ml)	1-4	Oil (3.gm)	-
Petroleum (300 ml)	5-10	Solid (1.2 g)	230-5°
Petroleum:benzene (9:1) (200 ml)	11-14	Oil (0.6 g.)	-
Petroleum:benzene (4:1) (350 ml)	15-21	Solid (.55 g)	249-55°
Petroleum:benzene (3:2) (250 ml)	22-26	Solid (1.1 g)	128-32°

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

Examination of Fractions 5-10 (Table I) : Isolation of taraxerone 16

The solids from fractions 5-10 (Table I) were combined (1.2 gm), m.p. 230-5° and was rechromatographed over a column of active alumina (60 gm). The solid dissolved in benzene (5 ml) was placed on the column. The chromatogram was developed with petroleum and was eluted with the following solvents (table II).

Table II

Chromatography of the above material (1.2 g.)

Eluent	Fractions (50 ml each)	Residue on evaporation	Melting point
Petroleum	1-4	Trace oil	-
Petroleum:benzene (4:1)	5-10	Crystalline solid	235-7°

Further elution with more polar solvents did not afford any material

The fractions 5-10 (Table II) were combined (0.85 gm) and crystallised from a mixture of chloroform and methanol. After three crystallization it afforded shining crystals (0.5 gm) m.p. 237-9°; $(\alpha)_D$ +9°. Its melting point was not depressed when mixed with an authentic sample of taraxerone. It also showed identical IR spectra when compared with that of authentic specimen of taraxerone.

Found : C, 84.84; H, 11.02%

Calculated for $C_{30}H_{48}O$: C, 84.84; H, 11.39%

U.V. (95% ethanol) : λ max 282 mμ (ϵ 64)

I.R. (KBr disc) : ν max 1705 (carbonyl), 822 (C=C$\langle_H$) cm^{-1}

Colour reaction tests

(a) Tetranitromethane displayed a yellow colour.

(b) Libermann-Burchardt : The compound developed a violet coloration with a mixture of acetic anhydride and conc. sulphuric acid.

(c) Zimmermann colour test was positive.

Lithium aluminium hydride reduction of taraxerone 16 :
Preparation of taraxerol 16a

To the ketone (200 mg) dissolved in dry ether (25 ml) was added LAH (25 mg) and the mixture was refluxed on the water bath for four hours. The reaction mixture was then cooled and to this 15 ml of saturated solution of sodium sulphate was added. The mixture was extracted with ether, washed to neutral with water and dried (Na_2SO_4). Removal of the ether gave a solid (190 mg) which was chromatographed over alumina. A column of alumina (10 gm. deactivated with 0.4 ml of 10% aqueous acetic acid) was developed with petroleum and the above residue dissolved in benzene (4 ml) was added to it. The following solvents were used for elution (Table III).

Table III

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (4:1)	3-4	Nil
Petroleum:benzene (3:2)	5-10	Crystalline solid 170 mg m.p. 268-70°

Further elution with more polar solvents did not yield any material

Fractions 5-10 (table III) were combined and the solid (170 mg) was crystallised from chloroform and methanol mixture when a constant melting solid, m.p. 278-80°, $(\alpha)_D$ + 4.4° was obtained. Its melting point was not depressed when mixed with an authentic specimen of taraxerol.

Found : C, 84.14; H, 11.69%

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

Preparation of taraxeryl acetate 16b

To the alcohol 17a (200 mg.) dissolved in pyridine (5 ml) was added acetic anhydride (5 ml) and the solution was heated on a water bath for five hours. The solution was cooled and then poured into ice cold water when a crystalline solid separated out. The solid collected by filtration was recrystallised from a mixture of chloroform and methanol to afford crystals of taraxeryl acetate 16b, (120 mg) m.p. 294-6°, $(\alpha)_D$ + 9.16°.

Found : C, 81.72; H, 11.52%

Calculated for $C_{32}H_{52}O_2$: C, 82.05; H, 11.11%

Examination of fractions 15-21 (Table I) : Isolation of epi-taraxerol 17

The fractions 15-21 (Table I) were combined (0.55 gm.), m.p. 249-55° and was rechromatographed over a column of active alumina (30 g.). The solid dissolved in benzene (6 ml) was placed on the column. The chromatogram was developed in petroleum and was eluted

with the following solvents (Table IV).

Table IV

Eluent	Fractions 50 ml each	Residue
Petroleum	1-3	Oil
Petroleum:benzene (4:1)	4-6	Nil
Petroleum:benzene (3:2)	7-10	Solid (0.45 g) m.p. 257-9°

More polar solvent did not elute any further material

Fractions 7-10 (Table IV) were combined and crystallised from chloroform-methanol mixture to afford needle shaped crystals of 3-epi-taraxerol 17 m.p. 261-2°, $(\alpha)_D$ - 22.6°.

Found : C, 84.60; H, 11.93%

$C_{30}H_{50}O$ requires : C, 84.44; H, 11.87%

UV (ethanol) : No absorption within the range 220-300 mμ

IR (KBr) : ν_{max} 3420 (hydroxyl), 825 cm^{-1} (C=C$\diagdown_H$)

NMR (60 Mc) : δ.85 to δ1.05 (24H, 8 CH_3), 5.3 δ (1H, multiplet) C=C$\underset{H}{\diagdown}$), 3.45 δ (1H, W 1/2 7HZ)

Mass : m/e 426, 302, 284, 269, 204, 189

Acetylation of 3-epitaraxerol : Preparation of 3-epi-taraxeryl acetate 17a:

3-epi-taraxerol (0.2 g) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The solution was then poured on ice cold water. The crude acetate was crystallised from a mixture of chloroform and methanol to afford a pure sample of 3-epi-taraxeryl acetate 17a, m.p. 161-2°, $(\alpha)_D$ - 41°.

Found :		C, 81.68; H, 11.34%
$C_{32}H_{52}O_2$ requires :		C, 82.05; H, 11.11%
IR (Nujol)	:	1730, 1240 (acetate), 830 (C = C$\swarrow_H$) cm^{-1}
NMR (60 Mc)	:	0.82-1.08 δ (24H, 8 CH_3), 2.07 δ (3H, singlet, $C\underline{H}_3$-C-O-), 4.64 δ (1H, multiplet $\underline{H}$-C-OAc), 5.3 δ (C=C$\swarrow\underline{H}$, 1H, multiplet)
Mass	:	m/e 468, 344, 284, 269, 204, 189

Preparation of taraxerone 16

3-epi-taraxerol (0.2 g) was oxidised with CrO_3-Py complex prepared from pyridine (2 ml) and CrO_3 (0.2 g) at 15°C. The crude product (0.12 g) obtained by working up in the usual manner was chromatographed over a column of active alumina (6 g). The chromatogram was prepared with petroleum and the product dissolved in benzene (4 ml) was poured on the column. It was eluted with the following solvents (Table V).

Table V

Eluent	Fractions 50 ml each	Residue
Petroleum	1-2	Nil
Petroleum:benzene (9:1)	3-4	Nil
Petroleum:benzene (4:1)	5-6	Solid m.p. 236-8° (0.1 g)

Further elution with more polar solvents did not yield any material

Fractions 5-6 (Table V, 0.1 g) on recrystallisation from a mixture of chloroform and methanol furnished pure crystals of taraxerone 16, m.p. 237-9°, $(\alpha)_D$ + 10°, which was found to be identical with authentic sample of taraxerone (m.m.p. and I.R. comparison).

Found: C, 84.69; H, 11.08%

Calculated for $C_{30}H_{48}O$: C, 84.84; H, 11.23%

Meerwein Pondorf reduction of taraxerone : Preparation of 3-epi-taraxerol 17 and taraxerol 16a

A mixture of taraxerone (1.0 g) and Al-isopropoxide (1.3 g) in absolute isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 5 hours the distillate no longer contained acetone and the solution was evaporated to dryness. The product isolated in the usual way with ether was dissolved in benzene (10 ml) and poured on a column of

alumina (60 g., deactivated with 2.2 ml of 10% aqueous acetic acid) developed with petroleum. The following solvents were used for elution (Table VI).

Table VI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-4	Nil
Petroleum:benzene (9:1)	5-8	Nil
Petroleum:benzene (4:1)	9-16	Solid (0.38), m.p. 256-8°
Petroleum:benzene (3:2)	20-28	Solid (0.6 g) m.p. 275-7°
Further elution with more polar solvents did not yield any material		

Fractions 9-16 (Table VI) were combined and crystallised from chloroform-methanol mixture to give 3-epi-taraxerol, m.p. 260-2° which did not depress the melting point when mixed with an authentic sample of 3-epi-taraxerol. The acetate prepared in the usual manner had melting point 161-2°, identical with 3-epi-taraxeryl acetate (m.m.p.).

Fractions 20-28 (Table VI) were combined and crystallised from a mixture of chloroform and methanol to give crystals, m.p. 277-8°, acetylation of which afforded an acetate 295-6°, identical with an authentic specimen of taraxeryl acetate (m.m.p.).

Sodium and isoamyl alcohol reduction of taraxerone : Preparation 3-epi-taraxerol 17 and taraxerol 16a

Sodium (2 gm) was added slowly to a refluxing solution of taraxerone (500 mg) in isoamyl alcohol (25 ml) and refluxing continued until all the sodium had dissolved. After steam distillation the solid precipitate was collected by filtration. The crude solid (450 mg) was chromatographed. It was dissolved in benzene (6 ml) and poured on a column of alumina (30 g, deactivated by 1.1 ml of 10% aqueous acetic acid). The chromatogram was developed in petroleum and was eluted with the following solvents (Table VII).

Table VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-2	Nil
Petroleum:benzene (9:1)	3-4	Nil
Petroleum:benzene (4:1)	5-7	Solid (0.13 g) m.p. 256-7°
Petroleum:benzene (3:2)	9-12	Solid (0.28 g) m.p. 276-8°

Further elution with more polar solvents did not afford any material

Fractions 5-7 (Table VII) were combined and crystallised from chloroform-methanol mixture to afford needle shaped crystals of 3-epi-taraxerol, m.p. 260-1° which did not depress the melting point when mixed with an authentic samples of 3-epi-taraxerol. The

acetate prepared in the usual way had melting point 160-2°, identical with 3-epi-taraxeryl acetate (m.m.p.).

Fractions 9-12 (Table VII) were combined and crystallised from a mixture of chloroform and methanol to afford crystals m.p. 277-9°, which on acetylation afforded an acetate m.p. 296-7°, identical (m.m.p.) with an authentic specimen of taraxeryl acetate.

Examination of Fractions 22-26 (Table I) : Isolation of β-sitosterol

Fractions 22-26 (table I) were combined and the solid crystallised from chloroform and methanol mixture when fine needle shaped crystals of β-sitosterol was obtained, m.p. 136-7°, $(\alpha)_D$ -32°.

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

β-sitosterol (0.2 g) was acetylated with pyridine (2 ml) and acetic anhydride (2 ml) in the usual manner. The product, isolated in the usual way with ether was crystallised from chloroform and methanol mixture when crystals of the acetate m.p. 127-8°, $(\alpha)_D$-40° were obtained, identified as β-sitosterol acetate by comparing with an authentic specimen of β-sitosterol acetate (m.m.p. and I.R. comparison).

Found : C, 81.15; H, 11.35%

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

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