CHAPTER -III

EXPERIMENTAL

Melting points are uncorrected. Petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless otherwise stated. NMR spectra were determined on Varian A-60 and HA-100 spectrometers using chloroform - d solution containing tetramethyl silane as reference. IR spectra were recorded in a Perkin Elmer 337 and 221 spectrophotometer. U.V. absorption spectra were taken in a Zeiss VSU-1 spectrophotometer in 95% ethanol solution unless otherwise stated. Thin layer chromatography was done on chromatoplate of Silica gel G (E.Merck) and the spots were developed with sulphuric acid-acetic anhydride (9:1) mixture.

Isolation of taraxerone[61]:

Dried and powdered Stembark of Sapium baccatum Roxb (2 Kg) was extracted with benzene in a soxhlet apparatus for twenty hours. On cooling the benzene extract a yellow insoluble solid precipitated out which was collected by filtration and was kept aside (latter identified as 3,3'-di-o-methyl ellagic acid[66]). From the clear filtrate benzene was distilled off and the residual gummy solid (30 gm) was taken up in ether (2 lit.). A cloudy precipitate which remained in the ether extract was separated out by filtration. The clear ether solution was washed with 10% sodium hydroxide solution (4 x 200 ml)

and then washed with cold water till the washings were neutral and dried over anhydrous sodium sulphate. The solvent was evaporated, when the neutral material (10.6 gm) was obtained as a yellow gummy solid which after chromatography and crystallisation from chloroform-methanol gave shining crystal (1.3 gm) m.p. 238-40° $(\alpha)_D$ +10.8°. Its melting point was not depressed when mixed with an authentic sample of taraxerone. It also showed identical IR throughout the region when compared with that of an authentic specimen of taraxerone.

Other compounds isolated were 1-hexacosanol, taraxerol and another solid of m.p. 210-15°.

Isomerisation of taraxerone[60]: Preparation of β-amyrone 44

To a suspension of taraxerone (600 mg) in glacial acetic acid (140 ml) maintained at 90° was added conc. hydrochloric acid (4 ml) and the reaction mixture was heated on a water bath for twenty minutes during which the solid dissolved in the solvent. The reaction mixture was then cooled and diluted with water. A solid came out which was taken up in ether. The ether layer was washed with water till neutral. On removing the solvent a solid came out (520 mg). The solid on crystallisation from chloroform and methanol mixture afforded fine crystals m.p. 174-6°, $(\alpha)_D$ +105.6° which was found to be identical with an authentic sample of β-amyrone 44 (m.m.p. and rotation).

Autoxidation of β-amyrone 44 : Isolation of Diosphenol 45

β-amyrone (2 gm) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 160 ml of tertiary butanol) was shaken in a stream of oxygen for three hours. The reaction mixture was diluted with water and then 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml), washed with water till neutral and the combined extract was dried (Na_2SO_4). On removal of the solvent under reduced pressure, a yellowish gummy foam was obtained (1.8 gm). The latter on crystallisation from acetone and methanol gave colorless crystals of the diosphenol 45, (1.2 gm), m.p. 200-2°, $(\alpha)_D$ +124.27°. It gave a positive ferric chloride test for diosphenol. TLC of the compound 45 showed two spots on chromatoplate (using benzene as solvent), an upper spot R_f = 0.77 of slightly weaker intensity than the lower spot R_f = 0.75. These were assumed to be due to the tautomeric mixture of the diketone and the diosphenol 45.

Found: C, 79.50, H, 9.83%

Calculated for $C_{30}H_{46}O_2$ $\frac{1}{2}CH_3OH$ C, 79.15; H, 10.62%

UV : λ_{max} 270 mμ(ε , 5104)

IR : ν^{nujol}_{max} 3570, 2960, 1650, 1100cm^{-1}.

Preparation of Diosphenol acetate 46 : Acetylation of Diosphenol 45

Diosphenol 45 (500 mg) was acetylated by treatment with acetic anhydride (10 ml) and pyridine (10 ml) overnight at room temperature. After working up in the usual manner the crude acetate (460 mg) was obtained. This was then chromatographed over a column of alumina (20 gm) deactivated with 0.8 ml of aqueous acetic acid.

Table I

Chromatography of the above solid (460 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether: benzene (9:1)	3-4	Nil
Petroleum ether: benzene (4:2)	5-9	Solid m.p. 165-7° (320 mg)

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

The solids (320 mg) from fractions 5-9 (table I) were collected which after crystallisation from a mixture of chloroform and methanol afforded crystals m.p. 172-3°, $(\alpha)_D$ 107.69°. It showed a single round spot on a chromatoplate.

Found: C, 80.17; H, 9.78%.

Calculated for $C_{32}H_{48}O_3$: C, 80.00; H, 10.00%

UV: λ_{max} 236 mμ, (ϵ, 9915)

IR: ν_{max}^{Nujol} 2950, 1720, 1685, 1205 cm^{-1}.

Hydrogenation of diosphenol acetate 46 : Preparation of 2α-acetoxy β-amyrone 47:

To diosphenol acetate 46 (200 mg) dissolved in absolute ethanol was added 10% palladium-on-charcoal catalyst (50 mg) and the mixture was shaken in an atmosphere of hydrogen till the absorption of hydrogen ceased (absorption of one mole equivalent of hydrogen within one hour). The solution was filtered and after removing the solvent from the filtrate an oily residue (200 mg) was obtained which was chromatographed over silica gel (20 gm).

Table II

Chromatography of the above oily residue (200 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-3	Oil (trace)
Petroleum ether: benzene (9:1)	4-8	Solid m.p. 155-7° (120 mg)

On elution with more polar solvents did not afford any crystalline solid.

The solids from fractions 4-8 (table II) were combined and after crystallisation from chloroform and methanol mixture afforded a crystalline solid m.p. 158-60°, $(\alpha)_D$ +108.57° and was found homogenous on a chromatoplate (R_f 0.38 in benzene).

Found: C, 79.18; H, 10.28%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.44%

IR: ν^{KBr}_{max} 1730, 1238, 1225 cm^{-1}.

NMR (100 Me/S): Peaks at 2.12 (3H, -O-COC<u>H</u>$_3$), 5.19 (1 H multiplet, vinyl proton), 5.5, 5.595, 5.6, 5.7 (1H, -Co-C<u>H</u>(OAc)-CH_2) ppm (Fig.16)

Hydrogenation[62] of Diosphenol 45 : Preparation of 2 Keto-β-amyrin 48:

Diosphenol 45 (500 mg) dissolved in a mixture of absolute ethyl alcohol (20 ml) and ethyl acetate (150 ml) was stirred in presence of 10% palladium-on-charcoal catalyst (50 mg) in an atmosphere of hydrogen till the absorption ceased. The catalyst was removed by filtration and the solvent was distilled off under reduced pressure from the filtrate. A solid residue (460 mg) was obtained which after crystallisation from chloroform-methanol furnished a solid of m.p. 180-182°, $(\alpha)_D$ 101.70°. The solid did not give ferric chloride coloration and showed one single spot on chromatoplate (R_f 0.44 in benzene).

Found: C, 82.29; H, 10.94%

Calculated for $C_{30}H_{48}O_2$: C, 81.81; H, 10.90%

UV: λ_{max} 270 mμ (ε, 43).

IR : ν^{nujol}_{max} 3500 cm^{-1} (hydroxyl), 1720 cm^{-1}(carbonyl).

NMR (100 Mc/S): Peaks at 3.88 (1H, H-C-OH), 3.44 (-C-OH), 2.42, 2.55 (2H, CO-CH_2), 5.18 (multiplet, vinyl proton) ppm.

Fig. 19.

Acetylation of 2 Keto-β-amyrin 48 : Preparation of 2-keto-β-amyrin acetate 49

The hydroxy ketone 48 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and kept overnight at room temperature. Next day a crystalline solid separated out from the

solvent mixture, which was collected by filtration. The latter after crystallisation from chloroform-methanol mixture gave crystals m.p. 276-8° $(\alpha)_D$ 127.08°. The filtrate on dilution with ice cold water precipitated from above a solid which after usual working up and crystallisation from chloroform methanol mixture afforded crystals m.p. 276-8° and was found to be identical with the above acetate. The solid 49 showed a single spot on chromatoplate (R_f 0.35 in benzene).

Found: C, 79.56; H, 10.05%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.37%

UV: λ_{max} 276 mμ (ε, 81)

IR: ν_{max}^{KBr} 1725 and 1740 cm^{-1}

NMR (100 Me/S): Peaks at 4.95 (1H, H-C-OCOCH$_3$), 2.49, 2.37 (2H, -CO-CH$_2$), 2.16(3H, -OCOCH$_3$), 5.2 (multiplet 1H, vinyl proton)ppm (Fig. 20).

Sodium borohydride reduction of 2α-acetoxy-β-amyrone 47: Preparation of Δ^{12}-Oleanene 2α-acetoxy-β-amyrin 50:

To 2α-acetoxy-β-amyrone (300 mg) dissolved in dry dioxan (25 ml) was added, with cooling a slurry of sodium borohydride (300 mg) prepared in a NH_4Cl-NH_4OH buffer (P_H = 8, 4 ml). The mixture was stirred at room temperature for two hours. A portion of the solvent was removed by distillation, cooled and acidified with dilute hydrochloric acid and then extracted with ether. The ethereal

layer was washed with water till neutral and dried (Na_2SO_4). Removal of ether gave a solid residue (250 mg) which was chromatographed over a column of alumina (30 gm, deactivated with 1.2 ml of 10% aqueous acetic acid) developed with petroleum ether. The residue was dissolved in benzene, poured on the column and was eluted with the following solvents.

Table III

Chromatography of the above solid 250 mg

Eluent	Fractions 50 ml each	Residue on evaporation.
Petroleum ether	1-4	Oil small amount (12 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-14	Solid (210 mg) m.p. 240-5°

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

The solid from fractions 8-14 (Table III) were collected and crystallised from chloroform-methanol mixture. After two crystallisation pure 2α-acetoxy-β-amyrin 50 m.p. 246-8° was obtained.

Hydrolysis of Δ^{12}-Oleanene-2α-acetoxy-β-amyrin 50 : Preparation of Δ^{12} Oleanene 2α, 3β-diol 51:

To the Δ^{12} oleanene 2α-acetoxy-β-amyrin 50 (200 mg) in dioxan (40 ml) was added 10% sodium hydroxide solution (10 ml) and the mixture was heated under reflux for three hours. The reaction mixture was then cooled, diluted with water and extracted with ether. The ethereal layer after washing with water till neutral was dried (Na_2SO_4). The solvent was removed and a solid (190 mg) m.p. 196-8° was obtained. After crystallisation from methanol it afforded pure 2α, 3β diol 51, m.p. 202-4° $(\alpha)_D$ +60.00°. This was found to be identical with an authentic sample of 2α, 3β dihydroxy - Δ^{12}-oleanene.

Found: C, 81.84; H, 11.62%

Calculated for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%

UV: No absorption in the range 220-300 mμ.

I.R. spectra: ν_{max} 3360, 2970, 1425, 1375, 1350, 1050, 1030 cm^{-1}

NMR spectra (100 Mc/S): Peaks at 2.94, 3.14 (1H, H-C_3-OH), 3.74 (quartet of a doublet, H-C_2-OH), 5.18 (multiplet 1 H, vinyl proton) ppm Fig. 22.

Preparation of Δ^{12}-Oleanene 2α, 3α-diol 52 : Meerwein Pondorff reduction of 2 keto-β-amyrin 48:

A mixture of 2 keto-β amyrin 48 (500 mg), Al-isopropoxide (650 mg) in dry 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 concentrated to a small volume. The reaction mixture was diluted with water followed by 10% sulphuric acid solution (20 ml) and then extracted with ether. The product obtained after removal of ether was dissolved in benzene (6 ml) and poured on a column of alumina (25 gm deactivated with 1 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table IV

Chromatography of the above product (450 mg)

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Nil
Petroleum ether: benzene (3:1)	4-6	Nil
Petroleum ether: benzene (1:1)	7-9	Nil
Petroleum ether: benzene (1:3)	10-12	Nil
Benzene	13-15	Nil

Contd...

Table IV (Contd)

Eluent	Fractions 50 ml each	Residue
Benzene: ether (4:1)	16-21	Solid (400 mg) m.p. 276-8°
Benzene: ether (4:1)	22-25	Solid (48 mg) m.p. 196-8°

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

The solid from fraction 16-21 (Table IV) were combined which after crystallisation from chloroform-methanol afforded the crystalline 2α, 3α-diol 52 (350 mg), m.p. 278-80°, $(\alpha)_D$ 71.28°.

Found: C, 81.40; H, 11.08%

Calculated for $C_{30}H_{50}O_2$: C, 81.44; H, 11.31%

U.V. : No absorption in the region 220-300 mμ

IR spectra: ν_{max}^{nujol} 3420, 2960, 1450, 1040, 835 cm^{-1}

NMR (100 Mc/S): Peaks at 3.2 (broad 1H, $\underline{H}$-C_3-OH), 4.06, 4.1 (doublet 1H, $\underline{H}$-C_2-OH), 5.2 (multiplet 1H, vinyl) ppm. (Fig. 23)

The solids from fractions 22-25 (table IV) after crystallisation from methanol afforded a crystalline solid (28 mg) m.p. 200-2° identical with 2α, 3β-diol 51 described on page 75.

Partial acetylation of Δ^{12}-Oleanene-2α, 3α-diol 52 : Preparation of 2α-acetoxy -3α-hydroxy β-amyrin 53:

200 mg of the 2α, 3α-diol 52 was treated with pyridine (12 ml) and acetic anhydride (0.5 ml, 1.1 mole) and the reaction mixture was allowed to stand at 0°C for 12 hours. The reaction mixture was then poured on ice-water and extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). After removal of ether a solid (150 mg) was obtained. The solid was dissolved in benzene (2 ml) and poured on a column of alumina (12 gm, deactivated with 0.5 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table V

Eluent	Fractions 50 ml each	Residue on evaporation
1. Petroleum ether	1-3	Oil (trace)
2. Petroleum ether: Benzene (4:1)	4-8	Solid (120 mg) m.p. 193-4°

Elution with more polar solvent did not give any solid material.

The solids from fractions 4-8 (table V) were combined which after crystallisation from methanol afforded crystalline mono-acetate 53 m.p. 195-7°, $(\alpha)_D$ 89.53°.

This compound showed a single spot on a chromatoplate (R_f 0.409) in benzene.

Found: C, 79.40; H, 10.61%

Calculated for $C_{32}H_{52}O_3$: C, 79.29; H, 10.81%

I.R. : ν_{max}^{nujol} 3450 (-OH), 1750 ($-OCOCH_3$) 1720(ketonic carbonyl), 1280 cm^{-1} (Fig. 24).

Oxidation[67] of 2α-acetoxy-3α-hydroxy β-amyrin 53 : Preparation of 2α-acetoxyβ-amyrone 54:

40 mg of chromium trioxide was added to a solution of pyridine (5 ml) in methylene chloride (35 ml) and the mixture stirred for 15 minutes. 200 mg of 2α-acetoxy-3α-hydroxy β-amyrin dissolved in methylene chloride (10 ml) was added to the stirred solution and the stirring was continued for another 15 minutes. The solution was then decanted and taken in ether. The ethereal layer was washed with 5% sodium hydroxide (5 ml portions twice) and then with water till neutral and dried over Na_2SO_4. On removal of ether a solid was obtained which on crystallisation from chloroform-methanol gave crystalline ~~the~~ solid 54 m.p. 158-9° $(\alpha)_D$ 108.57° which was indistinguisable from 47 described in page 71 (no depression of m.m.p. with 47).

It gave a single spot on a chromatoplate (R_f 0.38 in benzene) even Co TLC with 47 gave the same R_f value.

Found: C, 79.28; H, 10.30%

Calculated for $C_{32}H_{50}O_3$: C, 79.66; H, 10.37%

I.R. : ν_{max}^{KBr} 1730, 1238, 1225 cm^{-1} .