CHAPTER III

Experimental

Petroleum ether used throughout the investigation had b.p. 60-80°. Melting points are uncorrected. All optical rotations were taken in chloroform solution unless otherwise stated. UV absorption spectra were done in a Zeiss VSU-1 spectrophotometer in ethanol solution. IR spectra were recorded in a Perkin Elmer 337 and 221 spectrophotometer. NMR spectra were obtained on HA-100 spectrophotometer, using chloroform-d solutions containing tetramethylsilane as reference. Thin layer chromatography was done on chromatoplate of silica gel G (E. Merck) and the spots were developed with sulfuric acid-acetic anhydride (1:9) mixture.

β-amyrone from Taraxerone : Isomerisation of Taraxerone

Taraxerone isolated from the bark of Sapium baccatum Roxb[27] was isomerised[26] with acetic acid conc. hydrochloric acid mixture and the product obtained after crystallisation from chloroform methanol gave crystalline solid m.p. 178-9°, $(\alpha)_D$ + 105.6° identical with β-amyrone[49].

Autoxidation of β-amyrone 107 : Isolation of Disophenol 109

β-amyrone (2 gm) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 60 ml of tertiary butanol) was shaken in an stream of oxygen for three hours. The reaction mixture was then diluted with water and then 6N hydro-

chloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml) and the combined extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure. A yellowish gummy foam was first obtained (1.8 gm). Which after crystallisations from acetone-methanol mixture gave colorless needles (1.2 gm) m.p. 200-2°, $(\alpha)_D$ + 124.27°. It gave a positive ferric chloride coloration for diosphenol. Two spots on chromatoplate (using benzene as eluent) were observed; 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 108 and the diosphenol 109.

Found: C, 79.50; H, 9.83%

Calc. for $C_{30}H_{46}O_2$, CH_3OH : C, 79.15; H, 10.62%

UV (ethanol solution) : λ_{max} 270 mμ, ϵ = 7932.

IR : ν_{max}^{nujol} 3570, 2960, 1670, 1650, 1110 cm^{-1}

Preparation of Diosphenol acetate 110 : Acetylation of Diosphenol 109

Diosphenol 109 (200 mg) was acetylated by treatment with acetic anhydride (5 ml) and pyridine (5 ml) overnight at room temperature. After working up in the usual manner the crude acetate (180 mg) was obtained. This was then chromatographed over a column of alumina (10 gm) deactivated with 0.4 ml of aqueous acetic acid.

Table I

Chromatography of the above solid (180 mg)

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	Oil (trace)
Petroleum ether: benzene (9:1)	3-4	Nil
Petroleum ether	5-8	Solid m.p. 165-7°

Further elution with none polar solvents did not afford any solid material.

The solids (160 mg) from fractions 5-7 (table I) were collected which after crystallisation from a mixture of chloroform and methanol afforded needle shaped 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

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

UV (95% ethanol): λmax 236, ϵ = 9915

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

Hydrogenation of Diosphenol 109 : Preparation of 2 keto-8 amyrin 111

Diosphenol 109 (500 mg) dissolved in a mixture of ethyl acetate (150 ml) and absolute ethyl alcohol (20 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 three crystallisations furnished a solid of m.p. 180-82°, $(\alpha)_D$ + 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 $C_{32}H_{48}O_2$: C, 81.81; H, 10.90%

UV (95% ethanol) : λ_{max} 270 mμ, ϵ = 43

IR : ν_{max} 3500 cm^{-1} (hydroxyl), 1720 (carbonyl)

NMR (100 Mc/s) : Peaks at 3.88 (1H, H-C-OH), 3.44 (-C-OH), 2.42, 2.55 ($2H_2$-CO-CH_2-), 5.18 (multiplet, vinyl proton) ppm (fig.7).

Acetylation of 2 keto-β-amyrin 111 : Preparation of 2-keto β-amyrin acetate 112

The hydroxy-ketone 111 (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. 276-7° and was found to be identical with the above acetate. The solid showed a single spot on chromatoplate (R_f 0.35 in benzene).

Found: C, 79.56; H, 10.08%

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

UV (95% ethanol) : λmax 276; ϵ = 281

IR : νmax 1740, 1725, 1235 cm^{-1}.

NMR (100 Mc/s 0): 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. 8).

Preparation of 2-hydroxy-β-amyrone 115 : Potassium tertiary butoxide treatment of 2 keto-β-amyrin 111

A solution of the hydroxy ketone 111 (200 mg) in dry benzene (8 ml) was added to potassium tertiary butoxide (prepared from 50 mg of potassium in 6 ml of dry tertiary butanol). The reaction mixture was then refluxed for three hours in an oil bath (90-95°) in an atmosphere of nitrogen. A portion of the solvent was then removed, cooled, diluted with water water and then acidified with 6N hydrochloric acid. The mixture was extracted with ether. The ethereal layer after washing with water was dried (Na_2SO_4) and the solvent removed. A solid residue (185 mg) was obtained which after crystallisation from chloroform-methanol mixture gave crystals m.p. 256-58°, $(\alpha)_D$ + 76.97°. The compound showed a single spot on a chromatoplate (R_f 0.50 in benzene).

Found: C, 78.96, H, 10.80%

Calc. for $C_{30}H_{48}O_2$, CH_3OH : C, 78.80; H, 11.01%

IR: νmax 1705 (carbonyl), 1650, 3460 (hydroxyl) cm^{-1}.

NMR (100 Mc/s): peaks at 3.88, 3.92, 3.96, 4.00, 4.04, 4.08 (-CO-CH(OH)-CH$_2$-), 3.42 (-C-OH), 5.18 (multiplet, vinyl proton) ppm (fig. 9).

When the above reaction was carried out in the absence of nitrogen a product m.p. 200-2° was obtained, which gave positive ferric chloride coloration and was found to be indentical with the diosphenol 109 (m.m.p. and UV).

Acetylation of 2 hydroxy-ß-amyrone 115

To compound 115 (200 mg) was added acetic anhydride (5 ml) and pyridine (5 ml) and the mixture was kept overnight. After working up in the usual manner and crystallisation from chloroform-methanol mixture it gave a crystalline solid m.p. 275-77°, $(\alpha)_D$ + 126.86°, identical with the previously prepared 2 keto-ß-amyrin acetate 112 (m.m.p. and I.R. comparison).

Hydrogenation of diosphenol acetate 110 : Preparation of 2α-acetoxy-ß-amyrone 117a

To diosphenol acetate 110 (200 mg) dissolved in absolute ethyl alcohol 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 III

Chromatography of the above oily residue (200 mg)

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-3	Oil (trace)
Petroleum: 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 collected and after crystallisation from chloroform-methanol mixture afforded a crystalline solid m.p. 158-60° $(\alpha)_D$ + 108.57° and was found homogeneous on a chromatoplate (R_f 0.38 in benzene).

Found: C, 79.18; H, 10.28%

Calc. for $C_{30}H_{50}O_3$: C, 79.66; H, 10.37%

IR : ν_{max} 1225, 1238, 1730 cm^{-1}

NMR (100 Mc/s): peaks at 2.12 (3H, -O-COC$\underline{H}_3$), 5.19 (1H multiplet, vinyl proton), 5.5, 5.595, 5.6, 5.7 (1H, -CO-C$\underline{H}$ (OAc) -CH_2-) ppm (fig. 10).

Preparation of 2α-acetoxy-3-amyrone 117b, from β-amyrone : Acetoxylation of β-amyrone 107 using lead tetraacetate:

To a solution of β-amyrone (1 gm.) in dry benzene (150 ml) was added lead tetraacetate (1.3 gm) and acetic acid (1 ml) followed by borotrifluoride etherate (4 ml). The reaction mixture was stirred for one and half an hour in an atmosphere of nitrogen. The solution was then poured on ice cold water and the benzene layer was washed first with sodium bicarbonate solution (5%) twice and then with water till neutral. The benzene layer was dried (Na_2SO_4) and the solvent was removed under reduced pressure. A gummy material (1.1 gm) was obtained which was chromatographed over deactivated alumina (25 gm. deactivated with 1 ml of 4% aqueous acetic acid).

Table III

Chromatography of the above gummy material 1.1 gm.

Eluent	Fractions 5 ml each	Residue
Petroleum ether	1-4	Oil (400 mg)
Petroleum ether:benzene	5-11	Solid m.p. 152-4° (460 mg)

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

The oil from fractions 1-4 (table III) was not characterised further. The solids from fractions 5-11 (table III) were collected and after crystallisation from acetone-methanol mixture afforded crystalline solids m.p. 158-60°, $(\alpha)_D$ + 105.07°. (TLC - homogeneous).

Found: C, 79.28; H, 10.10%

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

IR : ν_{max} 1225, 1238, 1730, 1750 cm^{-1}

NMR (10 Mc/s): Peaks at 2.12 (3H, -O-COC<u>H</u>$_3$), 5.18 (multiplet 1H, vinyl proton), 5.50, 5.54, 5.565, 5.62, 5.7 (1H, -CO-C<u>H</u> (OAc)-CH_2-) ppm. (fig. 11).

<u>Isomerisation of 2α-acetoxy-β-amyrone 117a to 2-keto-β-amyrin acetate 112</u>

2-acetoxy-ketone 117a (100 mg) was dissolved in a small volume of benzene (3 ml) and was adsorbed on a column packed with basic alumina (Brockman) and left overnight. Next day it was eluted with benzene and the solid obtained was crystallised from chloroform and methanol mixture. The crystallised solid m.p. 276-8°, $(\alpha)_D$ + 126.8° was identical with 2 keto-β-amyrin acetate <u>112</u> (m.m.p. and IR comparison). Similar attempts to induce isomerization using acid washed alumina failed, the starting material being recovered unchanged.

<u>Acid hydrolysis 2α-acetoxy-β-amyrone 117a</u>: To a solution of 2α-acetoxy-β-amyrone (150 mg) in absolute ethyl alcohol (15 ml) was added 15 ml of ethanolic hydrochloric acid (13.5 ml of ethanol and 1.5 ml of conc. hydrochloric acid) and the mixture was refluxed for

three hours in an atmosphere of nitrogen. The reaction mixture was concentrated, then cooled, diluted with cold water and then extracted with ether. The ethereal extract after being washed successively with $NaHCO_3$ solution (5%) and water was dried (Na_2SO_4). The solvent was removed and a gummy residue (140 mg) was obtained. It was then chromatographed over silica gel. Petroleum ether : benzene (4:1) eluted a solid which after crystallisation from ethanol gave an amorphous solid melting at 212-8°. The solid showed two spots on chromatoplate (R_f 0.44 and 0.51 in benzene) presumably due to the presence of a mixture of 2 hydroxy-β-amyrone 115 and 2-keto-β-amyrin 111. The separation of this two constituents were attempted by fractional crystallisation and column chromatography but no fruitful results were obtained.

However, acetylation of the above solid m.p. 212-180 with acetic anhydride-pyridine afforded a crystalline solid m.p. 276-8° (homogeneous on a chromatoplate) which was found to be identical with 2 keto-β-amyrin acetate.

2 keto-β-amyrin acetate was also hydrolysed by the above method and the same results were obtained.

Hydrolysis of both 2 keto-β-amyrin acetate 112 and 2 α-acetoxy-β-amyrone 117a under alkaline conditions were attempted but in each case an intractable gum was obtained.

Isolation of lactol 123

The mother liquors left after the isolation the diosphenol 109 were concentrated when a gummy residue (600mg) was obtained. The latter was chromatographed over a column of alumina (25 gm) deactivated with 2 ml of 4% aqueous acetic acid. The gum dissolved in benzene (5 ml) was placed on the column of alumina. The chromatogram was developed in petroleum ether and was eluted with the following solvents (Table IV).

Table IV

Chromatography of the above gummy material (600 mg)

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Oil (trace)
Petroleum ether: benzene (4:1)	4-6	Nil
Petroleum ether: benzene (1:1)	7-9	Nil
Petroleum ether: benzene (1:3)	10-12	Nil
Benzene	13-15	Nil
Benzene:chloroform (4:1)	16-22	Solid 380 mg m.p. 248-52°

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

The solids (380 mg) from fractions 16-22 (table IV) were collected which after crystallisation from chloroform-methanol mixture afforded the crystalline lactol 123 m.p. 262-65°, $(\alpha)_D$ + 66.66°.

Found: C, 78.94; H, 10.32%

Calc. for $C_{29}H_{46}O_3$: C, 78.73; H, 10.40%

UV: No absorption between 220-300 mμ

IR: ν_{max} 3350, 1770 cm^{-1}.

NMR: peaks at 5.18 (vinyl proton) 5.24 (1H; -O-CH(OH)-C-) ppm.

Preparation of lactol acetate 124

Lactol 123 (300 mg) was treated with acetic anhydride (10 ml) and pyridine (5 ml) and the mixture was kept overnight of room temperature. The reaction mixture was poured in ice cold water and worked up in the usual manner. The semi solid mass (260 mg) obtained after three crystallisations gave a solid m.p. 186-8°, $(\alpha)_D$ + 117.64°.

Found: C, 76.28; H, 9.63%

Calc. for $C_{31}H_{48}O_4$: C, 76.85; H, 9.91%

UV : No absorption between 220-300 mμ

IR: ν_{max} 1740, 1752, 1462, 1458, 1380, 1360 cm^{-1}

NMR (100 mc/s) : peaks at 5.16 (multiplet, vinyl proton), 6.25 (1H, O-O-CH (OAc)-C-) ppm (fig. 13).

Autoxidation of maretanone : Isolation of Diosphenol and preparation of its acetate

Moretanone (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 an stream of oxygen for three hours. The reaction mixture after working up by the method described earlier afforded crystalline solid 190-92°. The solid showed two spots on chromatoplate and gave positive ferric chloride coloration.

Found: C, 81.62; H, 10.56%

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

UV (95% ethanol) : λ max 269 mμ; ϵ = 5104.

The above solid on acetylation with acetic anhydride-pyridine afforded a highly viscuous oil which could not be crystallised. The compound showed a single spot on a chromatoplate and did not give positive ferric chloride coloration.

UV (95% ethanol) : λ max 236 mμ; ϵ = 6514.

Hydrogenation of diosphenol : preparation 2-keto-moretanol

Diosphenol (200 mg) dissolved in absolute ethyl alcohol (80 ml) was stirred in presence of 10% palladium-on-charcoal catalyst (25 mg) in an atmosphere of hydrogen till the absorption ceased. After working up in the usual manner and crystallisation from chloroform methanol mixture it afforded a crystalline solid m.p. 181-83°.

Found: C, 81.02; H, 11.62%

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

UV (95% ethanol) : λ_{max} 278; ϵ = 78.

Acetylation of 2 keto-moretanol : Preparation of 2 keto-moretanyl acetate

2 keto-moretanol (200 mg) was acetylated by keeping it with acetic anhydride-pyridine mixture at room temperature overnight. After working up in the usual manner and crystallisations from chlorofoform-methanol mixture it afforded crystalline solid m.p. 264-67°.

Found: C, 79.62; H, 10.36%

Calc. for $C_{32}H_{52}O_3$: C, 79.33; H, 10.74%.

Hydrogenation of diosphenol acetate : Preparation of 2α-acetoxy moretanone

To diosphenol acetate (200 mg) dissolved in absolute ethanol was added 10% palladium-on-charcoal catalyst (25 mg) and the mixture was then shaken in an atmosphere of hydrogen till the absorption ceased. The reaction mixture after working up in the usual manner and crystallising from chloroform-methanol mixture afforded crystals m.p. 179-81°.

Found: C, 79.59; H, 10.62%

Calc. for $C_{32}H_{52}O_3$: C, 79.33; H, 10.74%

UV (95% ethanol) : λ_{max} 276 mμ, ϵ = 82.

Isomerisation of 2α-acetoxy-moretanone to 2-ketomoretanyl acetate

2α-acetoxy-ketone (100 mg) was dissolved in benzene (3 ml) and was adsorbed on a basic column of alumina and left overnight. Next day after eluating with benzene a solid was obtained, which after crystallisation from chloroform-methanol mixture afforded crystalline solid m.p. 264-6°, identical with the 2-keto-moretanyl acetate prepared earlier (m.m.p.).

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