CHAPTER II

Experimental

Melting points are uncorrected. Petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were taken in chloroform solution unlesss otherwise stated. IR spectrum were recorded in a Perkin Elmer 337 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 siliga gel G (E. Merck) and the spots were developed with sulfuric acid-acetic anhydride (9:1) mixture.

Preparation of Δ^{12}-oleanene 2α, 3α diol 149 : Sodium borohydride reduction of diosphenol 109

To a solution of diosphenol 109 (200 mg) in 100 ml of methanol sodium borohydride (100 mg.) was added and the mixture was stirred with a magnetic stirrer for one hour. The reaction mixture was concentrated, diluted and then acidified with dilute hydrochloric acid (6 ml) when a solid precipitated out. The latter was collected by filtration and dried. The solid (200 mg) was dissolved in benzene and was poured on a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with the following solvents.

Table II

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	Nil
Petroleum ether: benzene (3:1)	3-4	Nil
Petroleum ether: benzene (1:1)	5-6	Nil
Petroleum ether: benzene (1:3)	7-8	Nil
Benzene	9-14	Solid (186 mg) m.p. 235-7°

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

The solids from fractions 9-14 (table II) were collected and after crystallisation from methanol gave pure crystalline 2α, 3α-diol m.p. 240-42°, $(\alpha)_D$ + 101.88°.

Found: C, 81.74; H, 11.59%

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

UV (95% ethanol) : no absorption in the region 220-300 mμ

IR spectra : ν_{max} 3420, 2950, 1460, 1275, 1250, 1060, 1025, 998, 835 cm^{-1}.

NMR (100 Mc/s): Peaks at 3.43 (1H, $\underline{H}$-C_3-OH), 3.98 (doublet of a multiplet 1H, $\underline{H}$-C_2-OH), 5.2 (multiplet 1H, vinyl proton) ppm. (fig. 14).

Preparation of Δ^{12}-oleanene 2α, 3α-diacetate 150 : Acetylation of Δ^{12}-oleanene 2α, 3α-diol 149

Δ^{12}-oleanene 2α, 3α-diol 150 (200 mg) was acetylated by heating with pyridine (3 ml) and acetic anhydride (5 ml) on a water bath for three hours. After working up in the usual manner it gave a solid which after several crystallisations from chloroform-methanol mixture afforded pure Δ^{12}-oleanene 2α, 3α-diol diacetate 150 m.p. 221-22°, $(\alpha)_D$ + 83.63°.

Found: 77.07; H, 10.48%

Calc. for $C_{34}H_{54}O_4$: C, 77.56; H, 10.26%

IR spectra : ν_{max} 2960, 1725, 1485, 1455, 1385, 1370, 1255 cm^{-1}

NMR (100 Mc/s) : Peaks at 4.61, 4.65 (doublet 1H, H-C_3-OH), 5.34 (doublet of a multiplet 1H, H-C_2-OH), 5.2 (multiplet 1H, vinyl proton) ppm (fig. 15).

Preparation of acetonide derivative 151 of Δ^{12}-oleanene 2α,3α-diol:

2α, 3α-diol 149 (100 mg) was dissolved in dry acetone (20 ml) and to this a catalytic amount of p-toluene sulfonic acid was added. The reaction mixture was shaken for few minutes and kept overnight. To this reaction mixture 5% sodium bicarbonate solution (2 ml) was added and part of the solvent was removed by distillation and diluted with water. The cloudy precipitate which appeared was extracted with ether. The ethereal layer after being washed with water till neutral was dried (Na_2SO_4). The ether was then removed and the solid residue after several crystallisations from chloroform methanol

mixture afforded the pure acetonide derivative of 101 2α,3α-diol, m.p. 180-82°, $(\alpha)_D$ + 102.56°.

Found: C, 82.74; H, 11.12%

Calc. for $C_{33}H_{54}O_2$: C, 82.15; H, 11.20%

Sodium borohydride reduction of 2-keto-β-amyrin 111 : Isolation of 2α,3α-diol 149

To a solution of 2 keto-α-amyrin 111 (200 mg) in methanol (100 ml) sodium borohydride (75 mg.) was added and the reaction mixture was stirred for one hour. After working up by the above procedure it afforded a pure crystalline solid m.p. 238-40°, $(\alpha)_D$ + 102.52°. The solid was found to be identical with the 2α, 3α-diol m.p. 240-42°, $(\alpha)_D$ + 101.88° described above (m.m.p. and IR comparison).

Found: C, 81.14; H, 11.56%

Calc. for $C_{30}H_{52}O_2$: C, 81.44; H, 11.31%.

The diacetate m.p. 219-21° prepared by acetic anhydride-pyridine method was found to be identical with the acetate of 2α, 3α-diol described earlier (m.m.p.).

Sodium borohydride reduction of 2-hydroxy-β-amyrone 115

2-hydroxy β-amyrone 115 (100 mg) was reduced with sodium borohydride (40 mg) by the above method and the crystalline product obtained had m.p. 239-41°, identical with Δ^{12}-oleanene 2α,3α-diol prepared above (m.m.p.).

Preparation of Δ^{12}-oleanene 2α-acetoxy-β-amyrin 152 : Sodium borohydride reduction of 2α-acetoxy-β-amyrone 117a

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 an 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 up 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

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-4	Oil solid small (12 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-14	Solid (210 mg) m.p. 240-45°

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 m.p. 246-48° was obtained.

Acetylation of the above solid m.p. 246-48° 152 : Isolation of 2α, 3β-diacetate 153

The solid (200 mg) m.p. 246-48° 152 was acetylated by heating with pyridine (2 ml) and acetic anhydride (5 ml) on a water bath for five hours. After working up in the usual manner it gave crystals of 2α, 3β diol diacetate 153, m.p. 216-18°, $(\alpha)_D$ + 73.42°.

Found: C, 77.78; H, 10.79%

Calc. for $C_{34}H_{54}O_4$: C, 77.56; H, 10.56%

IR spectra: ν_{max} 2958, 1750, 1480, 1465, 1355 cm^{-1}

NMR (100 MC/s) : Peaks at 1.99, 2.055 (6H, 2-OCO$\underline{CH_3}$), 4.7, 4.8 (2H, $\underline{H}$-C-OCOCH_3), 5.2 (multiplet 1H, vinyl proton) ppm.

Hydrolysis of Δ^{12}-oleanene 2α, 3β-diacetate 153 : Preparation of Δ^{12}-oleanene 2α, 3β-diol 154

To the above diacetate m.p. 216-18° (150 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 then 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 three crystallisations from methanol it afforded the pure 2α, 3β-diol m.p. 202-4°, $(\alpha)_D$ + 60.00°.

Found: C, 81.84; H, 11.62%

Calc. for $C_{30}H_{50}O$: C, 81.44; 11. 11.21%.

UV (95% ethanol) : No absorption in the range 220-300 mμ.

IR spectra : ν max 3360, 2970, 1425, 1375, 1350, 1050, 1030 cm^{-1}

NMR (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 1H, viny proton) ppm (fig. 16).

Preparation of acetonide derivative of Δ^{12}-oleanene 2α,3β-diol

2α,3α-diol 154 (100 mg) m.p. 202-4° was taken in dry acetone (20 ml) and to this catalytic amount of p-toluene sulfornic acid was added. The mixture was shaken for 10 minutes and kept overnight. After usual work up a solid (86 mg) was obtained which after three crystallisations from methanol afforded pure crystals of the acetonide derivative m.p. 173-74°.

Found: C, 82.76; H, 10.83%

Calc. for $C_{33}H_{54}O_2$: C, 82.15; H, 11.20%.

LAH reduction of 2α-acetoxy-3-amyrone 117a

To a solution of 2α-acetoxy-3-amyrone 117a (250 mg) in dry ether (75 ml) was added lithium aluminium hydride (75 mg) and the mixture was refluxed for four hours. The reaction mixture was cooled and then treated successively with moist ether and a saturated solution of sodium sulfate. The mixture was then extracted with ether and the ethereal layer after washing with water was dried (Na_2SO_4). On removing the solvent it gave a solid (200 mg) m.p.220-

30° (two spots on chromatoplate) was chromatographed over a column of alumina (10 gm, deactivated with 0.4 ml of 10% aqueous acetic acid) developed with petroleum ether. The solid dissolving in benzene (6 ml) was poured on the column and eluted with the following solvents.

Table IV

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-2	Nil
Petroleum ether:benzene (3:1)	3-4	Nil
Petroleum ether:benzene (1:1)	5-6	Nil
Petroleum ether:benzene (1:3)	7-8	Nil
Benzene	9-10	Nil
Benzene:ether (9:1)	11-14	Solid (160 mg) m.p. 234-8°
Benzene:ether (4:1)	15-19	Solid (18 mg) m.p. 195-200°

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

The solid fractions from 11-14 (table IV) after crystallisation from methanol gave crystalline solid m.p. 239-40°, identified as the 2α,3α-diol prepared earlier (m.m.p.).

The solid fractions from 15-19 (table IV) after three crystallisations from methanol afforded a crystalline solid m.p. 200-2° which was found to be identical in all respects with the 2α,3β-diol described above (m.m.p. and IR comparison).

Preparation of Δ^{12}-oleanane 2β,3β-diol 155 : Meerwin Pondorff reduction of 2 keto-β-amyrin 111

A mixture of 2-keto-β-amyrin 111 (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% sulfuric 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 V

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

Table V (Contd.)

Eluent	Fractions 50 ml each	Residue
Petroleum ether benzene (1:3)	10-12	Nil
Benzene	13-15	Nil
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 V) were combined which after crystallisation from methanol afforded the crystalline 2β,3β-diol 155 (350 mg) m.p. 278-80°, $(\alpha)_D$ + 71.28°.

Found: C, 81.40; H, 11.08%

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

UV (in 95% ethanol) : No absorption in the region 220-300mμ

IR spectra: ν max 3420, 2960, 1450, 1370, 1350, 1040 cm^{-1}

NMR (100 Mc/S) : Peaks at 3.2 (broad 1H, H-C_3-OH), 4.06, 4.1 doublet 1H, H-C_2-OH), 5.2 (multiplet 1H, vinyl) ppm (fig. 17).

The solids from fractions 22-25 (table V) after crystallisation from methanol afforded a crystalline solid (28 mg) m.p. 200-2°, identical with the 2α,3β-diol prepared earlier.

Acetylation of Δ^{12}-oleanene 2β,3β-diol 155 : Preparation of Δ^{12}-oleanene 2β,3β diol diacetate 156

^{12}-oleanene 2β,3β-diol 155 (200 mg) was acetylated by heating with pyridine (5 ml) and acetic anhydride (5 ml) on a water bath for four hours. After working up in the usual manner, it gave a solid which after crystallisation from chloroform-methanol mixture afforded pure crystals of 2β,3β diol diacetate 156 m.p. 280-82°, $(\alpha)_D$ + 40.77°.

Found: C, 77.49; H, 10.13%

Calc. for $C_{34}H_{54}O_4$: C, 77.56; H, 10.26%

IR spectra : ν_{max} 1720, 1438, 1362, 1340, 1225 cm^{-1}.

NMR (100 Mc/s) : Peaks at 3.94, 3.98 (doublet 1H, H-C_3-O-$COCH_3$), 5.26, 5.28, 5.3, 5.34 (quartet of a doublet 1H, H-C_2-OCO CH_3), 5.18 (multiplet 1H, vinyl proton) ppm. (fig. 18).

Preparation of acetonide derivative of Δ^{12}-oleanene 2β,3β-diol 155

Δ^{12}-oleanene 2β, 3β-diol (100 mg) was converted to the acetonide derivative by the method described earlier. The acetonide derivative after crystallisation from chloroform-methanol mixture had m.p. 199-200°, $(\alpha)_D$ + 97.73°.

Found: C, 82.63; H, 11.02%

Calc. for $C_{33}H_{54}O_2$: C, 82.15; H, 11.20%.

Meerwin Pondorff reduction of β-amyrone : Preparation of epi-β-amyrin

A mixture of β-amyrone (1.00 mg), Al-isopropoxide (1.3 gm) in absolute isopropanol (12.5 ml) was distilled slowly with the addition

of isopropanol to maintain a constant volume. After five hours the distillate no longer contained acetone and the solution was concentrated to a small volume. The product isolated in the usual way with ether was dissolved in benzene (10 ml) and poured on a column of alumina (60 gm. deactivated with 2.2 ml of 10% aqueous acetic acid) developed with petroleum ether. The following solvents were used for elution.

Table VI

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Nil
Petroleum ether: benzene (9:1)	4-7	Nil
Petroleum ether: benzene (4:1)	8-14	Solid (300 mg), m.p. 214-7°
Petroleum ether: benzene (3:2)	15-20	Solid (630 mg) m.p. 190-3°

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

Fractions 8-14 (table VI) were combined and crystallised from methanol to give crystals of epi-β-amyrin m.p. 222-24° which did not depress the melting point when mixed with authentic sample of epi-β-amyrin.

Fractions 15-20 (table VI) were combined and crystallised from chloroform-methanol mixture to give β-amyrin m.p. 199-200°, identical with an authentic sample of β-amyrin.

Preparation of $\Delta^{2,12}$-olean diene (β-amyrilene II) 157 : $POCl_3$ pyridine dehydration of epi-β-amyrin

To an ice cold solution of epi-β-amyrin (600 mg) in pyridine (10 ml), phosphorus oxychloride (8 ml) was added and the mixture was kept overnight. It was then poured in an crushed ice cautiously and extracted with ether. The ether extract was washed with water, dried (Na_2SO_4) and the solvent was removed. A solid residue (580 mg) was obtained which was dissolved in benzene (5 ml) and poured on a column of active alumina (30 gm) developed with petroleum ether. The following solvents were used for elution.

Table VII

Elution	Fractions 50 ml each	Residue
Petroleum ether	1-3	Solid (530 mg) m.p. 137-8°

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

The solids from fractions 1-3 (table VII) were combined and crystallised from chloroform-methanol mixture to afford β-amyrilene II 157 m.p. 141-5°.

Osmium tetroxide oxidation of β-amyrilene II

A solution of β-amyrilene II 157 (1.0 gm) and osmium tetroxide (650 mg) in pyridine (10 ml) and dry ether (5 ml) was stirred for twelve hours at room temperature and then kept in the dark for eight days. After this period the solvents were removed under reduced pressure and a black residue was obtained. It was dissolved in benzene (25 ml) and 95% ethanol (25 ml) and refluxed[15] for six hours after the addition of a solution of mannitol (5.6 gm) and KOH (5.6 gm) in ethanol (25 ml) and water (12.5 ml). The reaction mixture was diluted with water and extracted with ether. The organic layer was washed with water, dried (Na_2SO_4) and evaporated to yield a crude solid (960 mg) which was chromatographed over a column of alumina (50 gm, deactivated with 2 ml of 10% aqueous acetic acid) developed with petroleum ether. The residue dissolved in benzene (8 ml) was poured on the column and eluted with the following solvents.

Table VIII

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Oil
Petroleum ether:benzene (3:1)	4-6	Oil
" " (1:1)	7-9	Nil
" " (1:3)	10-12	Nil
Benzene	13-14	Oil
Benzene:ether (4:1)	15-21	Solid (510 mg) m.p. 258-64°

Further elution with more polar solvents did not give any material

The solids (510 mg) from fractions 15-21 (table VIII) were collected and crystallised from methanol to furnish a solid m.p. 262-8°. This solid showed two spots (very close R_f values) on a chromatoplate and could not be separated even after repeated column chromatography. However crystallisation from methanol at first deposited a homogeneous solid (TLC) (460 mg) m.p. 278-80°, $(\alpha)_D$ + 69.23°. The IR spectrum of this solid was found to be identical throughout the entire region with the diol 155 prepared by Meerwin Pondorff reduction of 2-keto-β-amyrin 111. It also showed no depression in mixed melting point when mixed with the diol 155. From the mother liquor a second diol m.p. 239-40° (40 mg) was isolated and was found to be identical with 2α, 3α- diol described earlier (m.m.p. and IR comparison).

The diacetate of the diol above m.p. 278-80° prepared in the usual manner had m.p. 280-81° and was found to be indistinguishable from 2β,3β-diacetate prepared from the diol obtained by Meerwin Pondorff procedure described earlier.

Preparation of Δ^{12}-oleanene 2β,3α-diol 158

To a solution of hydrocarbon 157 (200 mg) in hexane (10 ml) taken in an erlenmeyer flask was added formic acid (98-100%, 50 ml) water (4 ml) and hydrogen peroxide (0.5 ml) and the mixture was stirred for eight hours at 55-60°. The reaction mixture was then kept at room temperature for sixteen hours. The solvents were removed under reduced pressure and the residue was extracted with ethyl acetate. The ethyl acetate layer was washed with water, dried

(Na_2SO_4) and the solvent was removed. The residue (180 mg) was then heated on a water bath for half an hour by adding to a 20% sodium hydroxide solution (20 ml). The reaction mixture was then cooled, acidified with dil. hydrochloric acid and extracted with ether. The ethereal layer after being washed with water was dried (Na_2SO_4) and the solvent was removed. The residue (130 mg) was dissolved in benzene and poured on a column of alumina (15 gm, deactivated with 0.6 ml of 10% aqueous acetic acid) developed with petroleum ether. The chromatogram was eluted with the following solvents.

Table IX

Eluent	Fractions 50 ml each	Residue
Petroleum ether	1-3	Oil
Petroleum ether: benzene (3:1)	4-6	Nil
" (1:1)	7-10	Nil
" (1:3)	11-13	Nil
Benzene	14-18	Solid (55 mg) m.p. 248-52°

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

The solids (55 mg) from fractions 14–18 (table IX) were collected and after crystallisation from methanol it afforded the crystalline 2β,3α-diol m.p. 248-50°, $(\alpha)_D$ + 120°.

Found: C, 80.96; H, 11.23%

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

UV (95% ethanol) : No absorption in the region 220-300 mμ

IR spectra : ν_{max} 3440, 2970, 1475, 1375, 1044, 1008 cm^{-1}.

The above diol 158 m.p. 248-50° was acetylated with acetic anhydride-pyridine and after working up in the usual manner and crystallisation gave a crystalline 2β,3α diol diacetate m.p. 161-63°.

Acetonide derivative of 2β,3α-diol could not be prepared for want of sufficient material. Preparation of this will be reported latter.

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