

EXPERIMENTAL

Melting points are uncorrected. The petroleum ether used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. NMR spectra were determined on Varian A-60 and HA-100 spectrometers using chloroform-d solution containing tetramethyl-silane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 and Beckmann I.R - 20 spectrophotometers. UV absorption spectra were taken in Ziess VSU-1 and UV Beckmann DU-2 spectrophotometers in 95% ethanol solution unless otherwise stated.

Isolation of methyl Oleanolate⁵⁸ 134 from Achyranthes aspera Linn.

800 gm of powdered seed of Achyranthes aspera Linn was extracted for 30 hours in a soxhlet apparatus with 95% ethanol. A thick syrup was obtained on concentration and this was shaken with a 200 ml portion of petroleum ether. The petroleum ether layer was decanted and the residual syrup was again shaken with petroleum ether (200 ml). Petroleum ether was decanted and the residue was hydrolysed with a mixture of 95% alcohol (130 ml), conc. hydrochloric acid (95 ml) and water (65 ml) for four hours. The mixture was diluted with water after cooling and then filtered. The solid residue was washed with water till neutral and air dried. The solid was extracted with ether in a soxhlet apparatus for four hours. The resulting ethereal solution was shaken with 50 ml portion of 3% aq. potassium hydroxide. The precipitated potassium salt was collected on a Buchner funnel. The solid

potassium salt was suspended in cold water, acidified with hydrochloric acid (50%) and extracted with ether. The ether extract was washed with water and dried over anhydrous Na_2SO_4 . To the dry ether solution was added a solution of diazomethane in ether prepared from nitroso methyl urea (700 mg) and was kept overnight. Next day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water till neutral and then dried (Na_2SO_4). Evaporation of the ether yielded a solid (2 gm). This crude ester dissolved in 15 ml benzene was placed over a column of alumina (120 gm deactivated with 4.8 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and eluted with following solvents (Table VII).

Table - VII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether (500 ml)	1-10	Solid on digestion with methanol, m.p. $193-4^\circ$

Elution with more polar solvents did not afford any solid material.

The combined solid from fraction 1 to 10 were collected and after crystallisation from chloroform-methanol mixture afforded pure crystals of 134, m.p. 196° , $(\alpha)_D^{25} 73^\circ$ which was found to be identical with an authentic sample of methyl oleanolate (m.m.p. and rotation).

Oxidation of methyl oleanolate 134: Preparation of methyl Olean-
onate 135:

To a solution of methyl oleanolate 134 (200 mg) in pure acetone (30 ml) was added Jones reagent dropwise (5-6 drops) with shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 hour, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried (Na_2SO_4) and the ether evaporated. The residue (150 mg) dissolved in benzene (3 ml) was chromatographed over a column of active alumina (5 gm). The chromatogram was developed with petroleum ether and then eluted with the following solvents. (Table VIII)

Table - VIII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Nil
Petroleum ether: benzene (4:1)	3-6	Solid, m.p. $179-80^{\circ}$ (120 mg)

Further elution with more polar solvent did not yield any solid.

Fractions 3-6 (120 mg) were combined and on crystallisation from chloroform-methanol furnished needle shaped crystals of 135, m.p. $182-4^{\circ}$, $(\alpha)_D^{25} 89^{\circ}$, identical with authentic sample of methyl oleanonate (m.m.p. and I.R. comparison).

Found: C, 79.01; H, 10.02%
Calculated for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32%

Autoxidation of methyl oleanonate 135 : Isolation of methyl 2,3 dioxo-olean-12-en-28-oate (diosphenol) 136:

To a suspension of (2 gm) methyl oleanonate 135 in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm of potassium and 160 ml of tertiary butanol), oxygen was passed for three hours with stirring. The reaction mixture was diluted with water and 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (200 ml) and the combined extract was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure. A yellowish gummy foam was obtained (1 gm), which after crystallisation from methanol gave an amorphous solid, 136, m.p. $130-5^\circ$, $(\alpha)_D^{25} 104^\circ$. It gave a positive ferric chloride colouration for diosphenol. Two spots on chromatoplate, an upper spot at $R_f = 0.79$ of slightly weaker intensity than the lower spot $R_f = 0.76$. These were assumed to be due to tautomeric mixture of the diketone 136A and 136 B.

Found: C, 76.62; H, 9.56%
Calculated for $C_{31}H_{48}O_4$: C, 76.82; H, 9.95%
UV : λ_{max} 269.5 m μ (ϵ , 5700)
IR : ν_{max}^{KBr} 3420, 2960, 1730, 1670, 1650 cm $^{-1}$ Fig. 5

Hydrogenation of methyl 2,3-dioxo-olean-12-en-28-oate 136: Preparation of methyl-3 β hydroxy-2-keto-olean-12-en-28-oate 137 :

Diosphenol 136 (500 mg) dissolved in absolute ethyl alcohol (50 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 evaporated to dryness under reduced pressure. A solid residue (460 mg) was obtained which after crystallisation from methanol furnished a solid 137, m.p. 129-31°, (α)_D 109.09°. This solid did not give ferric chloride colouration and showed one single spot on chromatoplate (R_f = 0.42 in benzene).

Found: C, 76.54; H, 10.28%

Calculated for C₃₁H₅₀O₄ : C, 76.50; H, 10.31%

UV : λ_{max} 270 m μ (ϵ , 43)

IR : ν_{max} nujol 3450 (-OH), 1710 (-C = O), 1730 (-COOMe) cm⁻¹.

Fig. 6.

Acetylation of methyl 3 β -hydroxy-2-keto-olean-12-en-28-oate 137:

Preparation of methyl 3 β -acetoxy-2-keto-olean-12-en-28-oate 138:

The hydroxy ketone 137 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and heated on a water bath for 5 hours. After usual work up it gave a crystalline solid 138, m.p. 182-4°, (α)_D 84.85°.

Found: C, 74.36; H, 9.52%
Calculated for $C_{33}H_{52}O_5$: C, 74.76; H, 9.91%
UV: λ_{max} 275 m μ (ϵ , 80)
IR: ν_{max} KBr 1725, 1740, 1235 cm $^{-1}$ Fig. 7

Preparation of methyl 2 α , 3 α -dihydroxy-olean-12-en-28-oate 139:

Meerwein-Ponndorff reduction of 137:

A mixture of methyl 3 β -hydroxy-2-keto-olean-12-en-28-oate 137 (500 mg) aluminium isopropoxide (650 mg) in dry isopropanol (12.5 ml) was distilled slowly with the addition of isopropanol to maintain constant volume. After 6 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 (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 as eluent. (Table IX)

Table - IX

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

Contd..

Table - IX (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. 285-6°

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

The solid from fraction 16-21 (Table IX) were combined which after crystallisation from methanol afforded methyl 2α , 3α -dihydroxy-olean-12-en-28-oate 139, m.p. 286-7°, $(\alpha)_D^{25} +11.11^\circ$ identical with an authentic sample of methyl 2α , 3α -dihydroxy-olean-12-en-28-oate (m.m.p. and Co-TLC).

Found: C, 75.40; H, 10.31%

Calculated for $C_{31}H_{50}O_4$: C, 76.50; H, 10.40%

UV : No absorption in the region 220-300 m μ .

IR : ν_{max} 3340 (-OH), 1725 (-COOMe) cm $^{-1}$

Acetylation of methyl 2 α , 3 α -dihydroxy-Olean-12-en-28-oate 139:

Preparation of methyl 2 α , 3 α -diacetoxy-olean-12-en-28-oate 140:

The solid 139 (200 mg) was acetylated by heating with acetic anhydride (2 ml) and pyridine (2 ml) on a water bath for four hours. After working up in the usual manner it gave \ddagger crystals of methyl 2 α , 3 α -diacetoxy olean-12-en-28-oate 140, m.p. 226-8°, (α)_D 95.20°.

Found: C, 73.20; H, 9.40%

Calculated for C₃₅H₅₄O₆ : C, 73.60; H, 9.50%

UV : No absorption above 200 m μ .

IR : ν _{max} 1750, 1740 (-OCOCH₃), 1725 (-COOMe) cm⁻¹.

Preparation of acetonide derivative 141 of methyl 2 α , 3 α -dihydroxy-Olean-12-en-28-oate 139:

2 α , 3 α -diol 139 (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 a few minutes and kept overnight. To the reaction mixture 5% sodium bicarbonate solution was added and part of the solvent was removed by distillation and then diluted with water. The cloudy precipitate that appeared was extracted with ether. The ethereal layer after being washed with water till neutral was dried (Na₂SO₄). The ether was then removed and the solid residue after several crystallisation from chloroform-methanol mixture afforded the pure acetonide derivative 141, m.p. 236-8°.

Found: C, 77.42; H, 10.28%
Calculated for $C_{34}H_{54}O_4$: C, 77.50; H, 10.30%

Preparation of methyl 2β , 3β -dihydroxy-olean-12-en-28-oate 142:

Sodium borohydride reduction of diosphenol 136:

To a solution of diosphenol 136 (200 mg) in methanol (100 ml) sodium borohydride (100 mg) was added and the mixture was stirred for one hour. The reaction mixture was concentrated, diluted with water 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 following solvents. (Table X)

Table - X

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 (190 mg) m.p. 267-71°

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

The solid from fractions 9-14 (Table X) ^{was} collected and after crystallisation from methanol gave pure needle-shaped crystals of 142, m.p. 269-72°, $(\alpha)_D^{25}$ 88.88°.

Found: C, 76.48; H, 10.39%

Calculated for $C_{31}H_{50}O_4$: C, 75.50; H, 10.40%

UV: No absorption in the region 220-300 m μ .

NMR (60 MC/S): Peaks at 3.15 (multiplet) and 4.4 (multiplet)
5.15 ppm. Fig. 8

Acetylation of methyl 2 β , 3 β -dihydroxy-olean-12-en-28-oate 142:

Isolation of methyl 2 β , 3 β -diacetoxy-olean-12-en-28-oate 143:

The diol 142 (200 mg) was acetylated by heating with pyridine (4 ml) and acetic anhydride (4 ml) on a water bath for four hours. After working up in the usual manner it gave a solid which after several crystallisation from chloroform-methanol afforded pure methyl 2 β , 3 β -diacetoxy-olean-12-en-28-oate 143, m.p. 220-222°, $(\alpha)_D^{25}$ 86.20°.

Found: C, 73.87; H, 9.54%

Calculated for $C_{33}H_{54}O_6$: C, 73.68; H, 9.50%

UV: No absorption in the region 220-300 m μ .

IR: ν_{max} 1258, 1720, 1745 cm $^{-1}$

NMR (60 MC/S): Peaks at 3.65 (singlet, COOMe)

2.06 (singlet, 6H), 4.6, 5.04 ppm Fig. 9

Preparation of acetonide 144 of methyl 2β , 3β -dihydroxy-olean-12-en-28-oate 142:

To 2β , 3β -diol 142 (100 mg) dissolved in dry acetone (20 ml) was added a few crystals of p-toluene sulfonic acid and the mixture was shaken for 10 minutes and then kept overnight. After usual work up a solid (80 mg) was obtained which after crystallisation from methanol afforded pure crystals of the acetonide derivative 144, m.p. $75-80^\circ$.

Found: C, 77.31; H, 10.25%

Calculated for $C_{34}H_{54}O_4$: C, 77.50; H, 10.30%

Synthesis of methyl crategolate 148 : (methyl 2α , 3β -dihydroxy-olean-12-en-28-oate).

Acetylation of diosphenol 136: Preparation of diosphenol acetate 145:

Diosphenol 136 (200 mg) was treated with acetic anhydride (5 ml) and pyridine (5 ml) and kept overnight at room temperature. After working up in the usual manner the crude acetate (180 mg) was obtained. This was chromatographed over a column of alumina (10 gm) deactivated with 0.4 ml of 10% aqueous acetic acid. The compound was dissolved in benzene and the chromatogram was developed with petroleum ether. The following solvents were used as eluent. (Table XI)

Table - XI

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-2	Oil (trace)
Petroleum ether: benzene (4:1)	3-8	Solid m.p. 165-7° (160 mg)

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

The solid (160 mg) from fractions 3-8 (Table XI) ^{as} were collected which after crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 145, m.p. 168-70°, (α)_D 93.33°. It showed a single round spot on a chromatoplate and did not give ferric chloride coloration for diosphenol.

Found: C, 77.50; H, 9.78%

Calculated for C₃₃H₅₀O₄: C, 77.60; H, 9.87%

UV: λ_{max} 237 m μ (ϵ , 8500)

IR: ν_{max} nujol 1205, 1685, 1720, 1738 cm⁻¹ Fig. 10

Hydrogenation of diosphenol acetate 145: Preparation of methyl 3 Keto-2 α -acetoxy olean-12-en-28-oate 146:

To diosphenol acetate 145 (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 a semisolid residue (200 mg) was obtained which after crystallisation from methanol afforded 146, m.p. 208-9°, $(\alpha)_D^{25}$ 52°.

Found: C, 77.20; H, 10.02%

Calculated for $C_{33}H_{52}O_4$: C, 77.30; H, 10.22%

UV : 272 m μ (ϵ , 84)

IR : $\nu_{\text{Max}}^{\text{nujol}}$ 1225, 1730, 1750 cm $^{-1}$

Preparation of methyl 2α -acetoxy- 3β -hydroxy-Olean-12-en-28-oate

147: Sodium borohydride reduction of 146:

To methyl 3-keto- 2α -acetoxy-olean-12-en-28-oate 146 (200 mg) dissolved in dry dioxan (20 ml), was added, with cooling a slurry of sodium borohydride (200 mg) prepared in an NH_4Cl-NH_4OH buffer (PH = 8, 3 ml) and the mixture was stirred at room temperature for three 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 (150 mg) which was chromatographed over a column of alumina (25 mg, deactivated with 1 ml of 10% aqueous acetic acid). The residue was dissolved

in benzene, poured on the column and was eluted with the following solvents. (Table XII)

Table- XII

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum	1-4	Oil (15 mg)
Petroleum ether: benzene (4:1)	5-7	Nil
Petroleum ether: benzene (3:2)	8-12	Solid (100 mg) m.p. 199-200°

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

The solid from fractions 8 to 12 (Table XII) were combined and crystallized from methanol. After several crystallisation pure methyl 2α -acetoxy- 3β -hydroxy-olean-12-en-28-oate 147, m.p. 199-204°, $(\alpha)_D^{27.9}$ was obtained.

Found: C, 74.8; H, 9.8%

Calculated for $C_{33}H_{52}O_5$: C, 75.0; H, 9.9%

Hydrolysis of methyl 2α -acetoxy- 3β -hydroxy-Olean-12-en-28-oate

147: Preparation of methyl 2α , 3β -~~di~~hydroxy-olean-12-en-28-oate
(methyl crategolate) 148:

To a solution of 2α -acetoxy- 3β -hydroxy olean-12-en-28-oate 147 (100 mg) in benzene (5 ml) was added 10% sodium hydroxide solution (15 ml) and the reaction mixture was refluxed for 3 hours on a water bath. The reaction mixture, after removal of solvent, was diluted with water and then extracted with ether. The ethereal layer was washed with water till neutral and dried (Na_2SO_4). On removal of ether, a solid was obtained, which on crystallisation from methanol gave crystals of methyl crategolate 148 m.p. $220-22^{\circ}$, $(\alpha)_D^{25} 36^{\circ}$, which was found to be identical with an authentic sample (m.m.p. and Co-TLC, rotation comparison).

Found: C, 76.49; H, 10.28%

Calculated for $\text{C}_{31}\text{H}_{50}\text{O}_4$: C, 76.50; H, 10.40%

UV: No absorption in the region $220-300 \text{ m}\mu$.