CHAPTER—III

E X P E R I M E N T A L

All melting points are uncorrected. The petroleum used throughout the experiment had the b.p. 60°—80°. Brockmann alumina (S.Merck) deactivated with 5% of 10% AcOH or silica gel (B.D.H.) was used for column chromatography. TLC plates were coated with Silica gel G (acc. to Stahl) having the thickness of about 0.2 mm and the spots located by exposing to iodine vapour. All the optical rotations were determined in chloroform solution. IR Spectra were recorded as nujol mulls and also in KBr disk on a Beckman IR—20 and Perkin Elmer Spectrophotometer respectively. NMR spectra were recorded on Varian A-60 or T-60 or EM-360 and FT-80A (1_H and 13_C) NMR instruments using $CDCl_3$ as solvent containing TMS as internal standard. Mass spectra were recorded by solid probe Cl/CH_4 method.

Treatment of methyl 3 β -acetylalcuritolate 55 with NBS: Formation of 3 β -acetyl olean-15-α -brom-28→13 β -olide 60:

200 mg of methyl 3 β -acetylalcuritolate dissolved in 12 ml dry and distilled $CHCl_3$ was mixed with freshly distilled 6 ml of dimethyl sulfoxide. The reaction mixture was cooled and 200 mg of NBS was added and kept in dark for

12 hours. The reaction mixture was then filtered and the filtrate was extracted with chloroform. The organic layer was washed several times with water and dried over anhydrous Na_2SO_4 . After distilling off chloroform, a gummy residue was obtained. The residue was dissolved in minimum amount of benzene and the solution was chromatographed on a column of alumina (25 gm alumina deactivated with 1 ml 10% acetic acid). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—1

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—7	Nil
Petroleum ether:benzene (3:2)	8—12	Solid m.p. 260°—270°

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

Fraction 8—12 (table—1) were combined and the mixture was crystallised from a mixture of chloroform-methanol to give the product 60, having m.p. 280—82°.

Analysis report:

Found, C, 67.8% ; H, 8.8%

Required for $C_{32}H_{49}O_4Br$ C, 67.1% ; H, 8.6%

UV : No absorption above 220 nm.

IR(nujol): ν_{max} 1730 cm^{-1}, 1250 cm^{-1} (Acetate)
1760 cm^{-1} (γ- lactone) Fig.1

PMR : 0.8 to 1.4 ppm (7 tert. CH_3), 2.04 ppm (s, 3H, O-COC$\underline{H}_3$), 4.3 ppm (dd, 1H, C $\underline{H}$Br, J_{aa} = 14 Hz and J_{ae} = 3Hz), 4.8 ppm (m, 1H, $\underline{H}$-C-O-COCH$_3$) Fig.2

Beilstein test for halogen was positive.

TNM test - Negative.

Treatment of 3 β -acetyl aleuritolic acid 59 with NBS:

150 mg of the acid dissolved in 10 ml chloroform was mixed with 5 ml of dimethyl sulfoxide. Then 150 mg NBS was added to the reaction mixture and it was kept in dark for 24 hours. The chloroform solution was washed several times with water and the organic layer was dried over anhydrous sodium sulphate. After evaporation of chloroform the residue was chromatographed over a column of 10 gms of silica gel. The solid was dissolved in minimum amount of benzene and was

poured on the column. The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—2

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—3	Nil
Petroleum ether:benzene (4:1)	4—7	Nil
Petroleum ether:benzene (3:2)	8—10	Nil
Petroleum ether:benzene (2:3)	11—20	Solid

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

Fractions 11—20 (table—2) were combined and the solid was crystallised from chloroform-methanol mixture. The crystal of m.p. 280—82° was identical with 60 obtained from the previous experiment (m.m.p. 00—1R). Beilstein test - Positive. TNM test - Negative.

Dimethyl aniline treatment on 3β -acetyl olean-15α -bromo-28 ⟶ 13β olide 60: Preparation of 3β -acetyloleen-15,16-en-28 ⟶ 13β olide 61:

150 mg of the bromo lactone 60 was refluxed for 4 hr with freshly distilled 30 ml dimethylaniline. The

mixture was poured into water followed by acidification with 6N HCl. The mass was then extracted with ether. The ether solution was washed with water till neutral and dried over anhydrous sodium sulphate. The solvent was removed and the residue (130 mg) was dissolved in minimum amount of benzene and was then chromatographed in a column of 50 gm deactivated alumina. The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—3

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—16	Solid m.p.298°—300°

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

Fractions 9—16 (table—3) were combined and the solid was crystallised from chloroform-methanol mixture. The crystals of the dehydrobromo compound had the m.p. 308—10°.

Analysis report:

Found, C, 77.1% ; H, 9.7%

Calculated for $C_{32}H_{48}O_4$ C, 77.4%; H, 9.7%

UV : No UV absorption above 220 nm

IR(nujol): ν_{max} 1730 cm^{-1}, 1250 cm^{-1} (acetate), 1770 cm^{-1} (lactone), 900 cm^{-1} (cis-disubstituted olefin)

Fig-3

PMR : 0.8 to 1.3 ppm (7 - tert CH_3), 2.04 (s, 3H-O $COC\underline{H}_3$), 5.5 (AB quartet $-\overset{|}{C}-\underline{C}\underline{H}=\underline{C}\underline{H}-\overset{|}{C}-$, JAB = 10 Hz).

4.5 (m, 1H, $\underline{H}-\overset{|}{C}-OCOCH_3$)

Fig.4

Beilstein test - Negative

TNM test - Positive

Hydrogenation of 3β -acetyl olean-15,16 en-28⟶ 13β olide 61:

Preparation of 3β -acetyl-olean-28⟶ 13β -olide 62:

A mixture of the unsaturatedlactone 61 (200 mg), dissolved in 50 ml ethyl acetate and 50 ml of acetic acid was stirredin presence of Adam's catalyst (25 mg) under hydrogen atmosphere in a Pars' hydrogenation apparatus under pressure (40 p.s.i.) till the absorption ceased. The catalyst was removed by filtration and the filtrate was washed with water till neutral. The solvent was dried andremoved under reduced pressure. A solidresidue (180 mg) was obtainedwhich after

crystallisation from chloroform-methanol furnished a solid m.p. 293—94°, $[\alpha]_D$+19° (m.m.p. and CO—IR identical with an authentic sample of 3β -acetyl olean-28⟶13β -olide).

IR : ν_{max} at 1780 cm^{-1} (γ -lactone), 1720, 1240 cm^{-1} (acetate)

Analysis report:

Found,	C, 77.0% ; H, 10.1%
Calculated for $C_{32}H_{50}O_4$	C, 77.1% ; H, 10.1%

TNM test - Negative.

Preparation of authentic 3β -acetyl olean-28⟶13β olide 62:

200 mg pure oleanolic acid was taken in 25 ml chloroform, To the solution a stream of gaseous hydrogen chloride was passed at room temperature. Then the reaction mixture was poured into water. The product was separated in acid and neutral fraction in the usual way. After separation the neutral fraction was heated with 2 ml acetic anhydride and 2 cc pyridine and it was kept over-night. After usual work up followed by crystallisation a product was obtained m.p. 293—94°, $[\alpha]_D$ +19° [Lit. m.p. 293—95°].

Analysis report:

Found, C, 77.2% ; H, 10.2%

Calculated for $C_{32}H_{50}O_4$, C, 77.1% ; H, 10.1%

IR(nujol): ν_{max} 1770 cm^{-1} (γ -lactone), 1720, 1240 cm^{-1} (acetate)

TNM test - Negative.

Lithium aluminium hydride reduction of 3β-acetyl-olean-15, 16-en-28⟶13β-olide 61; Formation of sogloeradiol 58:

50 mg of the lactone was taken in dry ether (100 ml). The solution was refluxed with 100 mg of lithium aluminium hydride for 4 hours. The mixture was cooled, lithium aluminium hydride was decomposed with a saturated solution of Na_2SO_4 and extracted with ether. The ethereal solution was washed with water and then dried over anhydrous sodium sulphate. The residue m.p. 225—28° was dissolved in minimum amount of benzene and was chromatographed over a column of alumina. The chromatogram was developed with petroleum ether and eluted with the following solvents:

Table—4

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil

Table—4 (contd.)

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether;benzene (2;3)	13—16	Nil
Benzene	17—20	Nil
Benzene:ether (4:1)	21—30	Solid m.p.230—231°

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

The compound from flask 21—30 (table—4) were mixed and crystallised from chloroform-methanol mixture. The crystallised product, m.p. 235—36°, was found to be identical with authentic specimen of sapogeradiol (m.m.p. and IR comparison).

Analysis report:

Found, C, 81.7% ; H, 10.9%

Calculated for $C_{30}H_{48}O_2$, C, 81.6% ; H, 11.0%

IR(nujol): ν_{max} at 3360 cm^{-1} (hydroxyl group)

Fig.5

Treatment of 3β -acetyl oleanolate 63 with NBS in DMSO; Formation of 3β -acetyl olean-12α-bromo-28—→ 13β-olide 65:

200 mg of 3β -acetyl oleanolate dissolved in 10 ml of chloroform was mixed with freshly distilled 5 ml of dimethyl

sulphoxide. To the reaction mixture 200 mg NBS was added. The reaction mixture was kept in dark for 24 hr. The chloroform solution was then washed with water and then dried over anhydrous sodium sulphate. The solvent was removed. After removal of solvent an oily residue (180 mg) was obtained which was chromatographed over a column of alumina (25 g alumina deactivated with 1 ml 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—5

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—14	Solid m.p. 200—209°

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

Fractions 9—14 (table—5) were mixed and the mixture was crystallised from chloroform-methanol, m.p. of the crystallised product was found to be 215°—16°. The compound was found to be identical (m.m.p. and CO—IR) with an authentic sample of 12α-bromo-3β-acetyl olean-28⟶ 13-olide.

Analysis report:

Found, C, 67.2% ; H, 8.6%

Required for $C_{32}H_{49}O_4Br$, C, 67.1% ; H, 8.6%

IR : ν_{max} 1770 cm^{-1}, 1180, 1160 cm^{-1} (γ-lactone)
1725 cm^{-1}, 1240 cm^{-1} (acetate) Fig.6

Beilstein test - Positive

TNM test - Negative

Treatment of 3β-acetyl oleanolic acid 64 with NBS; Formation of bromo lactone 65:

200 mg of 3β-acetyl oleanolic acid, dissolved in 10 ml of chloroform, was mixed with 5 ml of dimethyl sulfoxide. To the reaction mixture 200 mg of NBS was added and it was kept in dark for 24 hr. The mixture was diluted with water and extracted with chloroform. The organic layer was washed with water and then the solvent was distilled off. The residue obtained was extracted with ether, stirred with aq. sodium hydroxide (5%) and separated into alkaline and neutral extracts. The alkali extract on acidification and extraction with ether did not furnish any solid residue indicating the absence of any acidic component in the reaction mixture. The neutral ether layer was concentrated after drying over anhydrous sodium sulphate, and afforded a residue (150 mg) which was homogeneous in TLC (single spot). It was crystallised from chloroform-methanol mixture. The m.p. of

the crystallised product was 215°—17° and found identical with 3 β -acetyl-12 α -bromo-oleanan-28⟶ 13β-olide (m.m.p. and CO—IR).

Treatment of methyl 3 β -acetyl betulenate 66 with NBS; Formation of (i) methyl 3 β -acetyl-30-bromobetulenate 68 (ii) a mixture of isomeric bromo compounds 68a ⇌ 68b and (iii) 3 β -acetyl-29-30-dibromo oleanan-18 α-H,28⟶ 19β -olide 69.

400 mg of 3β -acetyl betulenate dissolved in 20 ml chloroform was mixed with 10 ml dimethyl sulphoxide. To the reaction mixture 800 mg NBS was added in lots of 100 mg each and the solution was kept in dark for 24 hr. The mixture was diluted with water. The organic layer was washed with water and dried over anhydrous sodium sulphate and after removal of solvent a residue (0.35 g) was obtained. It was dissolved in minimum amount of benzene and chromatographed over a column of silica gel (400 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents;

Table—6

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Solid m.p. 215—220°
Petroleum ether:benzene (4:1)	10—12	Solid
Petroleum ether:benzene (3:2)	14—16	Nil
Petroleum ether:benzene (2:3)	17—21	Solid m.p. 270°

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

The fractions 5—8 (table—6) were mixed and crystallised from chloroform-methanol. The m.p. of the crystallised product 68 was 235°—36° $[\alpha]_D$ +42.55°.

Analysis report:

Found, C, 65.2%; H, 8.6%, Br, 13.5%

Calculated for $C_{33}H_{51}O_4Br$, C, 65.3%; H, 8.6%, Br, 13.5%

IR(nujol): ν_{max} 1735 cm^{-1} ($-OCOCH_3$), 1725, 1240 ($-OCOCH_3$), 1260 ($-CH_2Br$), 1660, 876 cm^{-1} ($=CH_2$) Fig.8

NMR : 0.87—1.02 (15H, 5 tert-CH_3), 2.03 (s, 3H, $-OCOCH_3$) 4.9 (m, 2H, $=CH_2$), 4.5 (m, 1H, CH-O-$COCH_3$) ppm.

The fractions 10—12 (table—6) were combined and crystallised from $CHCl_3$—MeOH, m.p. 228—30°, $[\alpha]_D$ +50°.

IR(nujol): 1735 ($COOCH_3$), 1725, 1240 ($O-COCH_3$) 1265, 900 cm^{-1}.

PMR : 0.78—1.0 ppm ($C-t-CH_3$) , 1.7, 1.78 (2s, 3H, $C=C-CH_3$), 2.05 (s, 3H, $OCOCH_3$), 3.7 (s, 3H, $COOCH_3$), 3.9 (s, CH_2, Br), 4.48 (m, 1H, $H-C-O-COCH_3$), 5.1 (d, $C=CH_2$), 5.76 and 5.97 (2s, cis trans $C=C<^{Br}_{H}$) Fig.9

The compound was identified as 68a $\rightleftharpoons$ 68b

Compound obtained in the petroleum ether:benzene: 2:3 elution was white solid which was crystallised from $CHCl_3$—MeOH to give the dibromolactone, 69 m.p. 303°—304°, $[\alpha]_D$ +47.22°; CD (n-hexane) 218 nm, (ϵ -0.99).

Analysis report:

Found, C, 58.5% ; H, 7.3%

$C_{32}H_{48}O_4Br$ requires, C, 58.6% ; H, 7.4%

IR(nujol): ν_{max} at 1780 cm^{-1} (γ-lactone), 1720, 1240($-OCOCH_3$), 1260 cm^{-1} ($-CH_2Br$) Fig.10

PMR : 0.87—0.92 (15H, 5 tert CH_3), 2.03 (s, 3H,$-OCOCH_3$), 3.44 (m), 3.55 (m); 3.73 (d) and 3.73 (d) (4H, $C-29-H_2Br$ and $C_{30}-H_2Br$, 4.34 (s, 1H, $HC-OO$) and 4.5 (m, 1H, $H-C-OCOCH_3$) ppm Fig.11

CMR : 178.48 (s, lactone -O-C=O), 171 (s, -O-$\underline{C}$O-CH_3), 80.77 (d, $H\underline{C}$-O-C=O), 80.95 (d, $H\underline{C}$-O-C=O) ppm

Fig.12

Mass peaks: m/e at 659, 657, 655, 599, 597, 595, 503, 501, 407, 517, 515, 489, 487, 437, 435, 249, 191 (base), 189

Fig.13

Treatment of 3β-acetyl betulinic acid 67 with N-bromosuccinimide in presence of dimethyl sulfoxide:

To a solution of the acid 67 (0.2 gm) in chloroform (10 ml) containing DMSO (5 ml), N-bromosuccinimide (0.2 g) was added and the reaction mixture was kept in dark for 24 hours. The mixture was diluted with water and then extracted with chloroform. The organic layer was washed with water and then dried over anhydrous sodium sulfate. After removal of solvent a gummy mass was obtained which was chromatographed over a column of silica gel (30 gm). The column was eluted with the following solvents:

Table—7

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil

Table—7 (contd.)

Eluent	Fraction 50 ml each	Residue on evaporation
Petroleum ether:benzene (2:3)	13—20	White solid m.p. 290—300°
Petroleum ether:benzene (4:1)	21—25	Nil
Benzene	26—30	Nil
Benzene:ether (4:1)	31—35	Solid

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

The fractions obtained from 15—20 (table—7) were combined and crystallised from chloroform-methanol and the product m.p. 303—4° was identified as 3β -acetyl 29,30-dibromo olean-18α-H, 28⟶ 19β -olide 68 (compared with the authentic sample). Fractions obtained from benzene:ether (4:1) polarity was identified as its ester. Esterification of the compound by ether solution of diazomethane furnished the compound 68 i.e. 3β -acetyl-30-bromo betulonate (compared with an authentic specimen).

Attempted dehydrobromination of 3β -acetyl-29,30-dibromo-18α-H oleanan-28⟶ 19β olide, 69 by dimethyl aniline:

150 mg of the dibromolactone was refluxed for 4 hours with freshly distilled dimethyl aniline (30 ml). The mixture was poured into water, acidified with 6N HCl and extracted

with ether. The ether layer was washed with water till neutral and dried over anhydrous sodium sulphate. The solvent was removed and the compound obtained was found to be identical with the starting material.

Debromination of 3 β -acetyl-30-bromo betulenate 68; Preparation of 3 β -acetyl-betulenate 66:

200 mg of the compound was dissolved in 40 cc glacial acetic acid. 40 mg of Zn dust was added to the mixture and it was refluxed for 8 hours. After work up in the usual way it afforded a solid which on crystallisation from chloroform-methanol gave crystals, m.p. 201—2°, identified as 3β -acetyl betulinate (m.m.p. and IR comparison). Beilstein test — negative.

Debromation of lactone 69; Preparation of 3 β -acetyl oleanan-28—→ 19β olide 70:

200 mg of the lactone 69 was dissolved in 50 ml of rectified spirit and then 5 gm of Raney Nickel was added to the reaction mixture and the reaction mixture was refluxed for 8 hours. The mixture was filtered and the filtrate on concentration furnished 140 mg of 3 β -acetyl-olean-28—→ 19β -olide m.p. $>$ 360°, $[\alpha]_D$ +59°. It was compared with an authentic specimen of 3 β -acetyl oleanan-28—→ 19β olide (m.m.p. and IR comparison).

Preparation of authentic 3β -acetyl oleanan 28⟶ 19β olide 70:

Acetyl betulenic acid (1 g) was dissolved in benzene (10 cc) and a mixture of acetic acid (60 cc) and sulphuric acid (18 g, d, 1.84) was added with shaking. After two days at 20°, the reaction mixture was diluted with water and taken in benzene. The benzene layer was washed several times with water till neutral and dried over anhydrous sodium sulphate. Removal of solvent gave a mass which was taken in solvent ether. The ether layer was washed with 5% cold sodium hydroxide solution and the washings were discarded. The ether layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a mass which was crystallized from chloroform-methanol mixture. The crystals of m.p. $>$ 350° (lit. m.p. $>$ 360°) were identified as 3β -acetyl-oleanan-13α-H-28⟶ 19β -olide.

Analysis report:

Found,	C, 77.03% ; H, 10.06%
Calculated for $C_{32}H_{50}O_4$	C, 77.1% ; H, 10.04%

IR(nujol): ν_{max} at 1765 cm^{-1} (γ -lactone)
1735 and 1240 cm^{-1} (acetate).