CHAPTER—III

EXPERIMENTAL

Melting points are uncorrected. The petroleum used throughout had the b.p. 60—80°. Brockmann alumina (S.Merck) and silical gel (BDH) were 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 mull on a Beckmann IR—20 and Perkin-Elmer spectrophotometer and the UV spectra in methanol on a Beckmann DU-2 spectrophotometer. NMR spectra were recorded in Varian A—60, XL—200, XL—300 NMR instruments using $CDCl_3$ as solvent containing TMS as internal standard. Mass spectra were recorded by solid probe CI/CH_4 method.

SECTION—A

Treatment of 3β-acetyl-oleanan-18-α-H-28 ⟶ 13β-olide 67 in ethylenediamine with lithium; isolation of two acids 68 and 69a:

300 mg of the lactone 67 was taken in 100 ml dry ethylenediamine. To the solution 300 mg lithium was added in small portions with stirring in an atmosphere of N_2 gas. After the addition of lithium, the reaction mixture was refluxed for two hours. The reaction mixture was cooled and solid ammonium chloride was added to destroy any excess lithium. Then the mixture was acidified with 6N HCl and extracted with solvent ether. The organic layer was washed with 10% sodium hydroxide. The ether layer was then washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent did not afford any solid material. The alkoli extract was acedified and extracted with ether. The ether layer was washed with water till neutral and the mass obtained after evaporation of solvent was chromatographed over a column of silica gel (40 gms). The following solvents were used for chromatography:

Table—7

Eluent	Fractions 100 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
Petroleum ether:benzene (2:3)	13—20	White solid m.p.260—65°
Petroleum ether:benzene (1:4)	21—25	Nil
Benzene	26—30	Nil
Benzene:solvent ether (4:1)	31—40	White solid m.p. 285—90°

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

Isolation of oleanan-18α-H-28-oic acid <u>68</u>:

Fractions 13—20 (table—7) were mixed. The residue (120 mg) was crystallised from chloroform-methanol to afford crystals of oleanan 18-α-H-28-oic acid <u>68</u>, m.p. 270—1°.

Analysis report:

Found,	C, 81.45% ; H, 11.4%
Calculated for $C_{30}H_{50}O_2$,	C, 81.39% ; H, 11.38%

IR(nujol) : ν_{max} at 1695 cm^{-1} (—COOH function)
Fig.1

PMR : 0.69—0.94 ppm (7-tertiary methyl protons)
Fig.2

Mass : m/e 442 (M^+), 427, 398, 397, 261, 205, 191 (base)
Fig.3

Isolation of 3β-hydroxy-oleanan-18-α-H-28 oic acid <u>69a</u>

Fractions 31—40 (table—7) were combined while the solid residue 120 mg was obtained. It was crystallised from chloroform-methanol mixture. The crystal obtained was identified as 3β-hydroxy-oleanan-18α-H-28-oic acid <u>69a</u>, m.p. 295—8°.

<u>**Analysis report:**</u>

Found,	C, 78.48% ; H, 10.98%
Required for $C_{30}H_{50}O_3$,	C, 78.55% ; H, 10.99%

<u>IR (nujol)</u> : ν_{max} at 3460 cm^{-1} (—OH group)
1690 cm^{-1} (—COOH group)
Fig.4

<u>Mass</u> : m/e 458 (M^+), 443, 440, 425, 414, 413, 250, 235, 206, 203 and 189 Fig.5

<u>Esterification of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:</u>

To 50 mg of the hydroxy acid <u>69a</u> dissolved in dry ether (50 ml) was added a solution of diazomethane in ether (prepared from 50 mg nitrosomethyl urea and 50% NaOH solution) and was kept over-night. On the following day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and was then dried over anhydrous sodium sulphate. Evaporation of solvent gave a solid (40 mg) which was crystallised from a mixture of chloroform and methanol when crystals of $C_{31}H_{52}O_3$, m.p. 198°, $[\alpha]_D$ +17° were obtained.

<u>Analysis report:</u>

Found,	C, 78.69% ; H, 11.08%
Required for $C_{31}H_{52}O_3$,	C, 78.76% ; H, 11.09%

<u>IR (nujol)</u> : ν_{max} at, 3250 cm^{-1} (—OH group)
1725 cm^{-1} (—COOCH$_3$ group)
Fig.6.

<u>PMR</u> : 0.76—0.96 ppm (seven tertiary methyl protons), 3.20 ppm (—C<u>H</u>OH), 3.64 ppm (—COOC<u>H</u>$_3$)
Fig.7

<u>Mass</u> : m/e 472 (M^+), 454, 439, 413, 395, 262, 207, 205, 189
Fig.8

The above physical data of the compound characterised as methyl-3β-hydroxy-oleanan-18αH-28-oate <u>69b</u>.

<u>Acetylation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a; preparation of 3β-acetyl-oleanan-18α-H-28-oic acid 69c:</u>

50 mg of the hydroxy acid <u>69a</u> was dissolved in 1 ml pyridine. This was treated with 1 ml of acetic-anhydride. The reaction mixture was kept on water bath for 4 hours. It was cooled and poured into ice cold water

and extracted with solvent ether. The ethereal layer was washed several times with water and then dried over anhydrous sodium sulphate. Removal of solvent afforded a gummy mass (50 mg) which was chromatographed over a silica gel (10 gms) column. The following solvents were used for elution.

Table—8

Eluent	Fraction collected 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
Petroleum ether:benzene (2:3)	13—18	White solid

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

Fractions obtained from 13—18 (table—8) were combined and the residue (40 mg) was crystallised from chloroform-methanol mixture, when crystals of m.p.290-91°, $[\alpha]_D$ +15° were obtained.

Analysis report:

Found, C, 76.77% ; H, 10.47%

Required for $C_{32}H_{52}O_4$, C, 76.75% ; H, 10.47%

IR (nujol) : ν_{max} at, 1695 cm^{-1} (—COOH)
1725 and 1245 cm^{-1} (acetate)
Fig.9

PMR : 0.81—0.94 ppm (methyl groups),
1.96 ppm (s, —$OCOC\underline{H}_3$), 4.3 ppm (t, -$\underline{H}$-CO-$COCH_3$)
Fig.10

Mass : m/e 500 (M^+), 455, 440, 425, 250, 249,
236, 205 Fig.11

The compound was identified as 3β-acetyl-oleanan-18α-H-28-oic acid 69c.

Li-ethylenediamine reaction on 3-oxo-oleanan-18α-H-28 ⟶ 13β-olide 70; isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:

200 mg of the lactone 70 was taken in 75 ml of dry ethylenediamine. To the solution 200 mg of lithium metal was added in small pieces with stirring in an atmosphere of nitrogen gas. After addition the reaction mixture was refluxed for a period of two hours. Then it was cooled and solid ammonium chloride was added till excess lithium

was destroyed. The mixture was acidified with 6N HCl and then extracted with solvent ether. After washing with water the ether layer was separated into neutral and acid fractions in the usual way by treatment with 10% sodium-hydroxide. The neutral fraction did not afford any solid material. The acid fraction was chromatographed over a column of silical gel (40 gms). The following solvents were used for elution:

Table—9

Eluent	Fractions 100 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—9	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—16	Nil
Petroleum ether:benzene (1:4)	17—20	Nil
Benzene	21—24	Nil
Benzene:ether (4:1)	25—32	White solid

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

Fractions 25—32 (table—9) were combined and the residue (170 mg) obtained was crystallised from a mixture of chloroform and methanol. The crystals of m.p. 295—6°, was identified as 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, when compared with authentic specimen (m.m.p. and CO—IR).

Analysis report:

Found,	C, 78.54% ; H, 10.8%
Calculated for $C_{30}H_{50}O_3$	C, 78.55% ; H, 10.99%

Esterification of the above acid:

50 mg of the acid was dissolved in dry ether (50 ml) and a solution of diazomethane was added to it and kept for over-night. On the following day usual work up followed by crystallisation from chloroform-methanol mixture afforded crystals of m.p. 198°.

Analysis report:

Found,	C, 78.75% ; H, 11.07%
Calculated for $C_{31}H_{52}O_3$	C, 78.76% ; H, 11.08%

The compound was identified as methyl-3β-hydroxy-oleanan-18α-H-28-oate when compared with an authentic specimen (m.m.p. and CO—IR comparison).

Preparation of 3β-acetyl-olean-18α-H-28→19β-olide 71:

Acetyl betulenic acid (1 gm) was dissolved in benzene (10 cc) and a mixture of acetic acid (80 cc) and sulphuric acid (15 gm, d 1.84) was added with shaking.

After 2 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 crystallised from chloroform-methanol mixture. The crystals of m.p. > 350° (lit. m.p. > 350°) was identified as 3β-acetyl-oleanan-18α-H-28→19β-olide.

Analysis report:

Found, C, 77.09%; H, 10.05%

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

IR (nujol) : ν_{max} at 1765 cm^{-1} (γ-lactone)
1725 and 1240 cm^{-1} (acetate).

Treatment of 3β-acetyl-oleanan-18α-H-28$\rightarrow$19β-olide 71, in ethylenediamine with lithium; isolation of (i) olean-18α-H-28-oic acid 68, (ii) 3β-hydroxy-oleanan-18α-H-28-oic acid 69a (iii) oleanan-18α-H-3β, 19β, 28-triol 72a:

The lactone 71 (200 mg) was taken in dry ethylenediamine (100 ml). To the solution 300 mg of metallic lithium was added in small pieces with stirring in an atmosphere of nitrogen gas. Then the mixture was refluxed for a period of two hours. After cooling solid ammonium chloride was added until excess lithium was destroyed. The mixture was acidified with 6N HCl and then extracted with solvent ether. The ether layer was separated into acid and neutral fractions in the usual way by treatment with 10% sodium hydroxide.

Examination of acid fraction:

The alkaline solution was acedified with dilute HCl and then extracted with ether. The organic layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a residue which was chromatographed over a column of silica gel (30 gms). The chromatogram was developed with petroleum ether and the following solvents were used for elution of the column.

Table—10

Eluent	Fractions collected 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
Petroleum ether:benzene (2:3)	13—20	White solid
Petroleum ether:benzene (1:4)	21—25	Nil
Benzene	26—30	Nil
Benzene:ether (4:1)	31—40	White solid

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

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Isolation of oleanan-18α-H-28-oic acid:

Fractions 13—20 (table—10) were combined and the residue obtained (90 mg) was crystallised from chloroform-methanol mixture. The crystal of m.p. 270—1° was found to be identical with oleanan-18α-H-28-oic acid 68, when compared with an authentic specimen (m.m.p. and CO—IR).

Analysis report:

Found,	C, 81.37% ; H, 11.4%
Calculated for $C_{30}H_{50}O_2$	C, 81.39% ; H, 11.39%

IR (nujol) : ν_{max} at, 1690 cm^{-1} (—COOH group)

Isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:

Fractions 31—40 (table—10) were combined and the residue obtained (105 mg) was crystallised from a mixture of chloroform and methanol. The crystallised product, m.p. 295—6°, was found to be identical with 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, when compared with an authentic sample (m.m.p. and CO—IR).

Preparation of methyl ester of the above acid 69a:

To the ethereal solution of the acid (50 mg), ethereal solution of diazomethane was added and the mixture was kept over-night. On the following day the mixture was acidified with acetic acid to destroy any excess diazomethane present in the mixture. Then the ether layer was washed with water and then dried over anhydrous sodium sulphate. Removal of solvent afforded a compound which was crystallised from chloroform and methanol. The crystals of m.p. 197—98° was identified as methyl-3β-hydroxy-oleanen-18α-H-28-oate 89b, when compared with an authentic specimen (by m.m.p. and IR comparison).

Analysis report:

Found,	C, 78.77% ; H, 11.08%
Calculated for $C_{31}H_{52}O_3$	C, 78.76% ; H, 11.09%

IR (nujol): ν_{max} at 1735 cm^{-1} ($-COOCH_3$),
3450 cm^{-1} ($-OH$).

Preparation of acetyl derivative of the hydroxy acid 89a:

50 mg of the hydroxy acid 89a was dissolved in 1 ml pyridine and to the solution 1 ml of acetic anhydride was added and the reaction mixture was kept on water bath for four hours. Then it was cooled and poured on ice cold water and extracted with solvent ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Removal of solvent gave a compound which was crystallised from chloroform-methanol mixture. The crystals

of m.p. 290—1° was found to be identical with 3β-acetyl-oleanan-18α-H-28-oic acid 69c, when compared with an authentic specimen (m.m.p. and IR comparison).

Analysis report:

Found,	C, 76.75% ; H, 10.5%
Calculated for $C_{32}H_{52}O_4$	C, 76.75% ; H, 10.47%

IR (nujol): ν_{max} at 1695 cm^{-1} (—COOH),
1725 and 1240 cm^{-1} (—OCOCH$_3$).

Examination of neutral fraction:

The neutral fraction obtained (50 mg) on removal of solvent was chromatographed over a column of alumina (20 gms) deactivated with 10% acetic acid. The chromatogram was developed with petroleum ether. The following solvents were used for elution of the column:

Table—11

Eluent	Fractions collected in 50 cc	Residue on evaporation
Petroleum ether	1—4	Nil
Petroleum ether:benzene (4:1)	5—8	Nil
Petroleum ether:benzene (3:2)	9—12	Nil
Petroleum ether:benzene (2:3)	13—16	Nil
Benzene	17—20	Nil
Benzene:ether (4:1)	21—30	White solid

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

Isolation of oleanan-18α-H-3β, 19β, 28-triol 72a:

Fractions 21—30 (table—11) were combined and the residue (30 mg) crystallised from chloroform-methanol mixture. The crystals had m.p. 290—92°, $[\alpha]_D$ +23°.

Analysis report:

Found, C, 78.52% ; H, 10.96%

Required for $C_{30}H_{52}O_3$ C, 78.55% ; H, 10.99%

IR(nujol): ν_{max} at 3350-3400 cm^{-1} (hydroxyl groups)

Fig.12

PMR : 0.8—1.3 ppm (methyl groups), 3.5 ppm ($-C\underline{H}_2OH$), 4.13 ppm (m, $-C\underline{H}OH$), 4.26 ppm($-C_{18}-\underline{H}-OH$)

Fig.13

Mass : m/e 442 (M^+-H_2O), 424, 409, 399, 394, 381 (base peak), 203, 189.

Fig.14

The compound was identified as oleanan-18-α-H-3β, 19β, 28-triol 72a. (lit. m.p. 296—298°. $[\alpha]_D$ +24°)

Preparation of the acetyl derivative of the above triol 72a:

20 mg of the triol 72a was taken in 1 cc pyridine and 1 cc acetic anhydride was added to the mixture. It was kept on water bath for four hours. Then it was cooled and poured into ice cold water and then extracted with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Removal of solvent afforded a compound which was crystallised from $CHCl_3$—MeOH. The crystals had m.p. 211—12°.

Analysis report:

Found,	C, 73.68% ; H, 9.87%
Required for $C_{36}H_{58}O_6$	C, 73.7% ; H, 9.89%

IR (nujol): ν_{max} 1720—1750 (b), 1240—1250 cm^{-1} (b).

The compound was identified as oleanan-18α-H-3β, 19β, 28-triacetate 72b (lit, m.p. 213—16°).

Treatment of friedelan-26 → 12β-olide 74a with Li-ethylenediamine; isolation of (i) 3-deoxy-trichadenic acid 75a, (ii) friedelan-12β, 26-diol 76.

150 mg of friedelan-26 → 12β-olide[33] 74a was taken 50 ml of dry ethylenediamine and to the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for two hours. After two hours it was cooled and on usual work up afforded a mass which was separated into acid and neutral fractions by treatment with 10% sodium hydroxide solution.

Examination of the acid fraction:

The alkali extract was acidified, extracted with ether. The ether layer was washed with water and dried over anhydrous sodium sulphate. Removal of solvent afforded a residue (120 mg) which was dissolved in minimum amount of benzene and poured into a column of silica gel (20 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—12

Eluent	Fractions collected in 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
Petroleum ether:benzene (2:3)	13—20	White solid

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

Isolation of 3-deoxy-trichadenic acid 75a:

Fractions 13—20 (table—12) were combined and the residue (113 mg) was crystallised from chloroform-methanol mixture. The crystals of m.p. 290—91°, $[\alpha]_D$ +28.57°, was identified as 3-deoxy-trichadenic acid 75a [lit, m.p. 291—92°, $[\alpha]_D$ +4.2°].

Analysis report:

Found, C, 81.35% ; H, 11.37%

Required for $C_{30}H_{50}O_2$ C, 81.39% ; H, 11.38%

IR(nujol): ν_{max} 1680 cm^{-1} (—COOH function)

Fig.15

Mass : m/e 442 (M^+), 427, 397, 371, 291, 290, 274, 273, 260, 259, 257, 231, 217, 205, 191, 149 (base peak)

Fig.16

Esterification of the above acid; preparation of 3-deoxy-methyl trichadenate 75b:

50 mg of the acid 75b was dissolved in 200 cc ether and a solution of diazomethane in ether (prepared from 50 mg nitrosomethyl urea and sodium hydroxide) was

added to it. The reaction mixture was kept for overnight. On the following day acetic acid was added to destroy any excess diazomethane present in the mixture. The ethereal solution was then washed with water and $NaHCO_3$. Then the organic layer was dried over anhydrous sodium sulphate. Removal of solvent gave a compound which was crystallised from chloroform-methanol mixture. The crystals of m.p. 180°, $[\alpha]_D$ +30.3° was identified as 3-deoxy-methyl trichadenate 75b. (lit, m.p. 180°, $[\alpha]_D$ -21.0°).

Analysis report:

Found,	C, 81.54% ; H, 11.4%
Calculated for $C_{31}H_{52}O_2$	C, 81.57% ; H, 11.4%

IR (nujol): ν_{max} 1735 cm^{-1} (—$COOCH_3$)

PMR : 0.76—1.22 ppm (tertiary methyls),
3.72 ppm (s, —$COOCH_3$) Fig.17

Mass : m/e 456 (M^+), 425, 424, 409, 397, 396,
317, 306, 305 (base) Fig.18

Examination of the neutral part:

The neutral fraction obtained, on removal of solvent, was chromatographed over a column of alumina (20 gms), deactivated with 10% acetic acid. The chromatogram was developed with petrdeum-ether and was eluted with the following solvents:

Table—13

Eluent	Fraction collected 20 ml each	Residue on evaporation
Petroleum ether	1—4	Nil
Benzene:petroleum ether (1:4)	5—8	Nil
Benzene:petroleum ether (2:3)	9—12	Nil
Benzene:petroleum ether (3:2)	13—16	Nil
Benzene:petroleum ether (4:1)	17—20	Nil
Benzene	21—25	Nil
Benzene:solvent ether (4:1)	26—40	White solid

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

Isolation of friedelan 12β, 26 diol:

Fractions 26—30 (table—13) were combined and the residue (8 mg) was crystallised from $CHCl_3$—MeOH mixture. The crystals of $C_{30}H_{52}O_2$, had m.p. 240—1°.

Analysis report:

Found,	C, 81.05% ; H, 11.75%
Required for $C_{30}H_{52}O_2$	C, 81.02% ; H, 11.79%

IR (nujol): ν max at 3250—3300 cm^{-1}(b) (hydroxyl group) Fig.19

PMR : 0.724 ppm (d), 0.7976, 0.899, 0.9677, 0.9836, 1.002 and 1.244 ppm (s) (Methyl groups), 4.0 ppm (A-B q, for C-26-H_2-OH) 3.65 ppm (m, C-12-H-OH) Fig.20

Mass : m/e at 426, 395, 302, 301, 220, 205, 189, 177 (base peak) Fig.21

The compound was identified as friedelan-12β-26-diol 76.

Lithium aluminium hydride reduction of friedelan-26 → 12β-olide 74a; preparation of friedelan-26, 12β-diol 76:

50 mg of friedelan-26 → 12β-olide was taken in 100 cc of dry ether and 50 mg of LAH was added to it.

The reaction mixture was stirred at 0°C for two hours. After two hours a saturated solution of Na_2SO_4 was added to it which separated the organic layer. The organic layer was washed with water and then dried over anhydrous Na_2SO_4. Removal of solvent afforded a compound which was crystallised from chloroform-methanol mixture. The crystals of m.p. 240—1° was found to be identical with friedelan-28, 12β-diol 76.

Treatment of friedelan-28⟶11β-olide 74b in ethylenediamine with lithium; isolation of (i) friedelan-11β-28-diol 75a (ii) 3-deoxy-trichadenic acid 75a:

150 mg of friedelan-28⟶ 11β olide 74b was dissolved in 50 ml of dry ethylenediamine. To the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2 gas. Then the mixture was refluxed for two hours. The mixture on usual work up was separated into neutral and acid fractions by treatment with 10% aqueous sodium hydroxide solution.

Examination of the neutral part:

The neutral part was dissolved in minimum amount of benzene and was poured into a column of alumina (30 gms) deactivated with 10% acetic acid. The chromatogram was developed with petroleum-ether and the following solvents were used for elution:

Table—14

Eluent	Fractions collected 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
Petroleum ether:benzene (2:3)	13—16	Nil
Petroleum ether:benzene (1:4)	17—20	Nil
Benzene	21—24	Nil
Benzene:ether (4:1)	25—32	White solid

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

Isolation of friedelan - 11β-26-diol 76a:

Fractions 25—32 (table—14) were combined and the residue (112.5 mg) was crystallised from chloroform-methanol mixture and the crystal of $C_{30}H_{52}O_2$, m.p. $>$ 350°, was identified as friedelan - 11β -28-diol 76a.

Analysis report:

Found,	C, 81.05% ; H, 11.77%
Required for $C_{30}H_{52}O_2$	C, 81.02% ; H, 11.79%

IR(nujol): ν_{max} at 3200—3500 cm^{-1} (b) (for hydroxyl group)

Fig.22

PMR : 0.8 (d), 0.9—1.28 ppm(s) (for seven methyls), 3.9 ppm (m, C—28-H_2-OH), 4.35 ppm (m, C—11-H-OH)

Fig.23

Mass : m/e at 426 (M^+—H_2O), 395, 302, 301, 189 and 177 (base peak).

Examination of the acid fraction:

The acid fraction was dissolved in minimum amount of benzene and poured into a column of silica gel (20 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—15

Eluent	Fractions collected 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
Petroleum ether:benzene (2:3)	13—20	White solid

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

Fractions 13—20 (table—15) were combined and the residue obtained (7.6 mg) was crystallised from chloroform-methanol mixture. The crystals of $C_{30}H_{50}O_2$, m.p. 293—94° were obtained. The compound was identified as 3-deoxy-trichadenic acid (compared with an authentic specimen by m.m.p. & CO—IR).

Esterification of the above acid:

20 mg of the above acid was taken in 100 ml of solvent ether and an ethereal solution of diazomethane was added to it at 0°C. After keeping over-night at low temperature the reaction mixture was worked up in the usual way. After work up the solid obtained was crystallised from $CHCl_3$—MeOH mixture when the crystal was found to be identical with methyl-3-deoxy-trichadenate 75b, when compared with an authentic specimen (m.m.p. & CO—IR).

Preparation of 3β-acetyl-12α-bromo-oleanan-28$\rightarrow$13β-olide 77; Treatment of 3β-acetyl oleanolic acid with NBS:

400 mg of 3β-acetyl oleanolic acid was taken in dry chloroform (25 ml), containing dimethyl sulfoxide (10 ml). To the solution 400 mg of NBS was added in one lot and the mixture kept in dark for 24-hours. The mixture was diluted with water, extracted with chloroform and the organic layer was dried over anhydrous sodium sulphate. The solvent was removed and the residue was again extracted with ether, stirred with aq. sodium hydroxide (5%) and separated into alkaline and neutral extracts. The alkali extract on acidification and extraction with water did not furnish any solid material. The neutral ethereal

portion was concentrated to give a residue (300 mg) which was homogeneous in TLC (single spot). It was crystallised from chloroform-methanol, m.p. 215—17° found identical with 3β-acetyl-12α-bromo-oleanan-28$\longrightarrow$13β-olide 77.

Treatment of 3β-acetyl-12α-bromo-oleanan-28$\longrightarrow$13β-olide 77 in ethylenediamine with lithium; isolation of 3-deoxy-oleanolic acid 78a and oleanolic acid 78c:

300 mg of 3β-acetyl-12α-bromo-oleanan-28$\longrightarrow$13β-olide 77, was taken in 100 ml of dry ethylenediamine and to the solution 300 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for two hours and then on usual work up the mass obtained was separated into acid and neutral fractions by treatment with 10% aqueous sodium hydroxide solution. The neutral fraction did not furnish any solid material. The acidic fraction was chromatographed over a column of silica gel (40 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—16

Eluent	Fractions collected 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
Petroleum ether:benzene (2:3)	13—18	White solid
Petroleum ether:benzene (1:4)	19—22	Nil
Benzene	23—26	Nil
Benzene:solvent ether (4:1)	27—34	White solid

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

Isolation of 3-deoxy-oleanolic acid:

Fractions 13—18 (table—16) were mixed and crystallised from methanol when the crystal of $C_{30}H_{48}O_2$, m.p. 265—66°, was identified as 3-deoxy-oleanolic acid 78a.

Analysis report:

Found, C, 81.76% ; H, 10.8%

Required for $C_{30}H_{48}O_2$ C, 81.8% ; H, 10.8%

Esterification of the above acid:

50 mg of the acid was dissolved in 100 ml of solvent ether and an ethereal solution of diazomethane (prepared from 50 mg of nitrosomethyl urea) was added to it. On the following day it was worked up and crystallised from chloroform-methanol mixture. The crystals had m.p. 169° was identified as methyl-3-deoxy-oleanolate (lit, m.p. 170°) 78b.

Analysis report:

Found, C, 81.95% ; H, 11.00%

Required for $C_{31}H_{50}O_2$ C, 81.93% ; H, 11.01%

IR(nujol): ν_{max} at 1730 cm^{-1} ($-COOCH_3$)

Fig.24a

Isolation of oleanolic acid 78c:

Fractions 27—34 (table—16) were combined and the residue (105 mg) obtained was crystallised from methanol. The crystals of $C_{30}H_{48}O_3$, m.p. 303—4°, were identified as oleanolic acid.

IR (nujol): ν_{max} at 3450 cm^{-1} (—OH),
1690 cm^{-1} (—COOH)

Preparation of methyl ester of the above acid; isolation of methyl oleanolate 78d:

50 mg of the above acid was dissolved in ether (50 ml), added a solution of diazomethane in ether prepared from nitrosomethyl urea (50 mg) and KOH and was kept over-night at low temperature. On the following day excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and was then dried with anhydrous sodium sulphate. Evaporation of solvent gave a solid (40 mg) which was crystallised from a mixture of chloroform-methanol, when crystals of m.p. 198—9° were obtained. This was found to be identical with methyl oleanolate (m.m.p. CO—IR and CO—TLC comparison with authentic specimen).

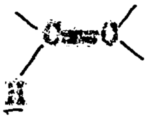
TNM test : Yellow colouration.

Analysis report:

Found,	C, 79.08% ; H, 10.67%
Calculated for $C_{31}H_{50}O_3$	C, 79.10% ; H, 10.71%

IR (nujol): ν max at 3280 (—OH) and 1730 cm^{-1} (—COOCH$_3$)

Fig.24

PMR : 0.58—0.96 ppm (methyl groups), 3.5 ppm (s, —COOC$\underline{H}_3$), 3.8 ppm (m, —C$\underline{H}$—OH), 5.10 ppm (t, ) Fig.25

Mass : m/e at 470 (M$^+$), 452, 455, 411, 232, 207, 203, 189

Fig.26

Preparation of 3β-acetyl-29, 30-dibromo-oleanan-18-α-H-28→19β-olide 79; Treatment of 3β-acetyl betulenic acid with NBS:

To a solution of 3 gms of 3β-acetyl betulenic acid in 150 ml of chloroform containing 80 ml of dry dimethylsulfoxide, 6 gms of crystal NBS were added in lots of 300 mg each and the solution kept in dark for 24 hrs. The mixture was diluted with water and the organic layer was separated and washed several times with water. The chloroform layer was dried over anhydrous sodium sulphate and evaporation of solvent afforded a gummy mass which was taken in ether and washed with 10% aq. sodium hydroxide solution to separate it into acid and neutral parts. The neutral part was washed with water and evaporation of solvent afforded a mass which after crystallisation from chloroform-methanol mixture to give the crystals of $C_{33}H_{51}O_4Br_2$, m.p. 235–6° $[\alpha]_D$ +42.55°. It was identified as 3β-acetyl-29, 30-dibromo-oleanan-18α-H-28⟶ 19β-olide <u>79</u>, by comparison with an authentic specimen (mmp and Co–IR comparison). The yield was 300 mg.

<u>Treatment of Li-ethylenediamine on 3β-acetyl-29,30-dibromo-oleanan-18αH-28⟶ 19β-olide 79; isolation of compounds (i) oleanan-18α-H-28-oic acid 68, (ii) 3β-hydroxyoleanan-18α-H-28-oic acid 69a:</u>

250 mg of 3β-acetyl-29,30-dibromo-oleanan-18α-H-28⟶ 19β-olide <u>79</u>, was taken in dry ethylene-diamine (100 ml). To the solution 250 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. Then the reaction mixture was refluxed for two hours. The reaction mixture was cooled and on usual work up the mass obtained was separated into acid and neutral fractions by treatment with 10% sodium hydroxide. The neutral fraction did not afford any solid material. The acid fraction was dissolved in minimum amount of benzene and poured into a column of silica gel (40 gms). The chromatogram was developed with petroleum ether and was eluted with the following solvents:

Table—17

Eluent	Fractions collected 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
Petroleum ether:benzene (2:3)	13—20	White solid
Petroleum ether:benzene (1:4)	21—24	Nil
Benzene	25—28	Nil
Benzene:solvent ether (4:1)	29—40	White solid

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

Isolation of oleanan-18αH-28-oic acid 68:

Fraction 13—20 (table—17) were combined and the residue (100 mg) was crystallised from chloroform-methanol mixture, while the crystal of m.p. 270—1°, was found to be identical with oleanan-18α-H-28-oic acid (comparison with authentic sample by mmp. CO—IR). Beilstein test negative.

Isolation of 3β-hydroxy-oleanan-18α-H-28-oic acid 69a:

Fractions 29—40 (table—17) were combined and the residue (100 mg) obtained was crystallised from $CHCl_3$—MeOH mixture. The crystal of $C_{30}H_{50}O_3$, m.p. 295—6°, was identified as 3β-hydroxy-oleanan-18α-H-28-oic acid 69a, when compared with an authentic specimen (mmp and Co—IR comparison). Beilstein test negative.

SECTION—B

Treatment of lupanone 80a in ethylenediamine with lithium;
Isolation of lupanol 80b:

150 mg of lupanone 80a, was taken in dry ethylenediamine (50 ml). To the solution 150 mg of metallic lithium was added in small pieces with stirring in an atmosphere of N_2. The reaction mixture was refluxed for a period of 2 hrs. Then it was cooled and on usual work up followed by crystallisation a compound, which was not identical with the starting material (TLC & mmp), was obtained. The crystal, $C_{30}H_{52}O$, m.p. 206°, $[\alpha]_D$ -17.8°, was found to be identical with lupanol 80b (by mmp and Co—IR comparison with authentic specimen). The yield was 120 mg.

Analysis report:

Found,	C, 84.1%; H, 12.20%
Calculated for $C_{30}H_{52}O$	C, 84.04%; H, 12.22%

IR(nujol): ν_{max} at 3330 cm^{-1} (—OH)

Fig.27

Li-ethylenediamine treatment on moretanone 81a; isolation of moretanol 81b:

150 mg of moretanone 81a was taken in ethylenediamine (50 ml). To the solution lithium metal (150 mg) was added as in the earlier experiments in nitrogen atmosphere. After refluxing the reaction mixture for two hours and then usual work up a compound was isolated. The compound was found to be not identical with the starting material (comparison of TLC). The compound was crystallised from chloroform-methanol mixture when the crystal of m.p. 223—24° was obtained. It was found to be identical with moretanol 81b (compared with authentic specimen by mmp, CO--IR and TLC).

Analysis report:

Found, C, 84.02% ; H, 12.21%

Calculated for $C_{30}H_{52}O$, C, 84.04% ; H, 12.22%

IR(nujol): ν_{max} at 3320 cm^{-1} (free hydroxyl group)

Fig.28

Li-ethylenediamine reaction on methyl betulonate 82a; isolation of dihydro betulonic acid 82b:

300 mg of methyl betulonate 82a was taken in 100 cc of dry ethylenediamine and lithium (300 mg) metal was added to the solution in small pieces with stirring in an atmosphere

of N_2. The reaction mixture was refluxed in N_2 atmosphere for 2 hrs. It was cooled and solid NH_4Cl was added to destroy excess lithium present in the mixture. It was acidified with 6N HCl and extracted with ether. The ether layer was washed with water and then treated with 10% sodium hydroxide. The neutral ether layer was evaporated. Absence of residue showed no neutral component was formed. The alkaline extract was acidified and extracted with ether. The ethereal solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of solvent gave a compound (TLC single spot) which was crystallised from chloroform-methanol mixture. The crystal, $C_{30}H_{50}O_3$, m.p. 323—24°, $[\alpha]_D$ +28.0° , was found to be identical with dihydrobetulenic acid. Yield of the acid was 240 mg. TNM test was negative.

<u>Analysis report:</u>

Found, C, 78.53% ; H, 10.99%

Calculated for $C_{30}H_{50}O_3$, C, 78.55% ; H, 10.99%

<u>IR(nujol):</u> ν_{max} at 3440 cm^{-1} (—OH group), 1680 cm^{-1} (—COOH group). Fig.29

<u>Mass</u> : m/e at 458 (M^+), 443, 415, 413, 189 (base peak) Fig.30

Acetylation of dihydro betulenic acid 82b; preparation of 3β-acetyl dihydrobetulenic acid 82c:

100 mg of the above prepared dihydrobetulenic acid was dissolved in 1 cc pyridine and 1cc acetic anhydride was added to the solution. The reaction mixture was kept on water bath for 4 hours. Then it was cooled and poured into ice cold water when a solid separated out which was filtered. The residue obtained was crystallised from chloroform-methanol mixture and the crystals of $C_{32}H_{52}O_4$ had m.p. 310—11°, $[\alpha]_D$ -11.5°. It was found to be identical with 3β-acetyl-dihydro-betulenic acid 82c, when compared with an authentic specimen (mmp and Co—IR comparison).

Analysis report:

Found,	C, 76.74% ; H, 10.45%
Required for $C_{32}H_{52}O_4$,	C, 76.75% ; H, 10.47%

IR(nujol): ν_{max} at 1735 and 1240 cm^{-1} (acetate)
1690 cm^{-1} (—COOH) Fig.31

PMR : 0.66—0.93 ppm (methyl groups), 2.03 ppm (s, —OCO$C\underline{H}_3$), 4.5 ppm (m, $-\underline{H}-C-O-\overset{O}{\overset{\|}{C}}-CH_3$)
Fig.32

Mass : m/e at 500 (M^+), 455, 440, 249, 189 (base peak)
Fig.33

Li-ethylenediamine reaction on methyl oleanonate 78e; isolation of oleanolic acid 78c:

200 mg of methyl oleanonate 78e was taken in 70 ml of dry ethylenediamine and 200 mg lithium was added as in the previous cases. It was refluxed under nitrogen atmosphere for two hours. After work up and seperation of acid and neutral fractions a compound was isolated from the acid part. It was crystallised from methanol while the crystal of $C_{30}H_{48}O_3$ had m.p. 301—32° $[\alpha]_D$+75.3°. It was found to be identical with oleanolic acid 78c, when compared with an authentic specimen (mmp and Co—IR comparison). It showed positive TNM test. The yield was 160 mg.

Analysis report:

Found,	C, 78.85% ; H, 10.58%
Calculated for $C_{30}H_{48}O_3$	C, 78.9% ; H, 10.59%

IR(nujol) : ν_{max} at 3400 cm^{-1} (—OH group),
1685 cm^{-1} (—COOH group).

Fig.34

Treatment of methyl trichadenate A 83a, in ethylenediamine with lithium; isolation of trichadenic acid A 83b:

200 mg of methyl trichadenate was taken in 50 ml of dry ethylenediamine and 200 mg of lithium metal was added with stirring in an atmosphereof N_2 gas. Then the reaction mixture was refluxed for about 2 hrs. It was then cooled and solid NH_4Cl was added and then acidified with 6N HCl. After extraction with ether the organic layer was washed with water and then dried over anhydrous sodium sulphate. Evaporation of solvent afforded a compound which was crystallised from chloroform-methanol mixture. The crystals had m.p. 330—32° $[\alpha]_D$ +36° was identified as trichadenic acid A, when compared with an authentic specimen. Yield was 100 mg.

Analysis report:

Found,	C, 78.53% ; H, 10.96%
Calculated for $C_{30}H_{50}O_3$.	C, 78.55% ; H, 10.99%

IR(nujol): ν_{max} at 3440 cm^{-1} (—OH),

1690 cm^{-1} (—COOH).

Fig.36.

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