CHAPTER III

EXPERIMENTAL

Melting points are uncorrected. Petroleum ether used throughout the experiment had b.p. 60-80°. All optical rotations were determined in chloroform solution unless stated otherwise. The mass spectra were determined with an MS-9 mass spectrometer. ^{1}H NMR spectra were determined on Varian A-60 and HA-100 Spectrometer using deuterated chloroform solution containing tetramethyl silane as reference. The IR spectra were recorded in IR-20 spectrophotometer. Silica gel used for column chromatography was of 60-120 mesh (B.D.H) and alumina used for column chromatography was of active basic grade (B.D.H). TLC was done on chromatoplates prepared on glass strips with silica gel using benzene, petroleum ether mixture as solvents and the spots were developed in an iodine chamber.

Isolation of lupeol from Xanthoxylum budrunga

Five kilograms of air dried finely powdered bark of xanthoxylum budrunga[67] was soxhleted for 48 hours with benzene. The extract was cooled and then solvent was distilled off. The residue left was dissolved in minimum volume of benzene, chromatographed over deactivated alumina column and the petroleum-ether eluent collected. Petroleum-ether was distilled off and a solid residue (12 gm) was obtained. The solid on

rechromatography over deactivated alumina afforded lupenone on petroleum-ether elution and lupeol on petroleum-ether : benzene (4:1) elution. Petroleum ether : benzene (4:1) fractions were purified by repeated crystallisations from chloroform-methanol mixture. 9 gm of lupeol, m.p. 215-16°, $[\alpha]_D$ 33°, was obtained and found to be identical with authentic sample of lupeol (CO-TLC, m.m.p. and CO-IR).

Acetylation of Lupeol to lupenyl acetate

To a solution of lupeol (4g) dissolved in pyridine (40 ml) was added acetic anhydride (40 ml) and the mixture kept over water bath for four hours. The mixture was then cooled and poured over ice-cold water when white solid separated out. It was washed with water, filtered through suction and dried.

The dried mass was crystallised several times with $CHCl_3$-MeOH mixture when Lupenyl acetate, m.p. 212-14° was obtained and found to be identical with authentic sample of lupenyl acetate (m.m.p., CO-IR and CO-TLC).

Oxidation of Lupenyl acetate with N-bromo-Succinimide

Lupenyl acetate 71 (1g) was dissolved in 50 ml chloroform. 25 ml dimethyl sulphoxide was added to the chloroform solution of 71. Then NBS (1 gm) was added a little at a time with constant shaking. The mixture was kept in the dark for

about 24 hours. After 24 hours the reaction mixture was poured on ice cold water when a white solid separated out which was then extracted with chloroform. The chloroform layer was washed with water and dried over anhydrous Na_2SO_4. Solvent was removed when an oily residue (900 mg) was left. The residue was dissolved in benzene (10 ml) and poured on a silica gel column. The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 1).

Table 1

Chromatography of the above gummy material (900 mg)

Eluent	Fractions 100 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1	Oil (50 mg)	-
Petroleum ether	2-7	White solid (250 mg)	207-12°
Petroleum ether: benzene (4:1)	8-14	White solid (350 mg)	226-30°
Petroleum ether: benzene (3:2)	15-17	Nil	-
Petroleum ether: benzene (2:3)	18-20	Nil	-
Petroleum ether : benzene (1:4)	21-22	Nil	-
Benzene	23-25	Oil (50 mg)	-
Benzene:chloroform (9:1)	26-32	White solid (200 mg)	245-250°

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

Examination of fractions 2-7 (Table 1)

The fractions 2-7 (Table 1) were combined (250 mg), m.p. 207^{o}-12^{o}. TLC experiment of the compound showed two distinct spots on chromatoplate close to each other, thus existence of at least two compounds was indicative. Hence this was kept apart for further purification. Beilstein test bromine was positive.

Examination of fractions 8-14 (Table 1) : Isolation of 30-bromo-Lup-20(29)-en-3β-yl acetate 72.

The fractions 8-14 (Table 1) were combined (350 mg) and crystallised from a mixture of chloroform and methanol to afford needle shaped crystals (200 mg) 82, m.p. 236-38^{o}, $[\alpha]_D$ + 12^{o}. The compound showed single spot on chromatoplate. TNM test of the compound was positive. Beilstein test for halogen was also positive.

Analysis report:	%C	%H
Found	70.12	9.29
Calculated for $C_{32}H_{51}O_2Br$	70.20	9.32

IR ν_{max}^{Nujol} 1725, 1255 (-O-CO-CH_3), 3015, 1640 and 880 (>C = CH_2) cm^{-1}

(Fig. 1)

^{1}H NMR ($\delta CDCl_3$) : 2.04 (s, 3H, $-O-COCH_3$), 4.48(m, 1H, $H-\overset{|}{C}-O-\overset{||}{C}-$), 3.99 (s, 2H, $H_2\overset{|}{C}-Br$), peaks at 5.04 and 5.12 (2s, 2H, $>C=CH_2$) peaks at 0.79, 0.84, 0.85, 0.85, 0.95, 1.04 (6s, 18H, $6-\overset{|}{C}-CH_3$) ppm.

(Fig. 2)

Examination of fractions 26-32 (Table 1) : Isolation of oleanan-29, 30-dibromo-19β-hydroxy-3β-yl acetate, 75.

The fractions 26-32 (Table 1) were combined (200 mg) and crystallised from a mixture of chloroform-methanol to afford amorphous white solid (160 mg) 75, m.p. 258-60°. It showed negative TNM test but showed positive Beilstein test for bromine. TLC of the compound showed single spot on a chromatoplate.

Analysis report:

	%C	%H
Found	59.59	8.05
Calculated for $C_{33}H_{52}O_3Br_2$	59.63	8.07

IR : ν_{max}^{Nujol} 3330 (-OH), 1732, 1250 ($-O-CO-CH_3$), 1270-80 ($-CH_2Br$) cm^{-1}

(Fig. 3)

^{1}H NMR (δ $CDCl_3$) : Signals between 3.53 to 3.89 (two AB quartets 4H, $2\underline{H}_2$ C-Br), 4.01 (g, 1H, J_{aa} = 12 Hz, J_{ae} = 6 Hz, $H_2\overset{|}{C}-C\underline{H}OH$), 2.04 (s, 3H, $-O-CO-C\underline{H}_3$), 4.48 (m, 1H, $-H-\overset{|}{C}-O-\overset{O}{\overset{\|}{C}}-$), 0.84, 0.85, 0.87, 0.89, 0.95, 1.06 (6s, 18H, $6-\overset{|}{C}-C\underline{H}_3$).

(Fig. 4)

^{13}C NMR : 171.025 (s, $-O-\underline{C}O-CH_3$), 36.33, 36.87, 37.74, 41.17, 42.80, 44.44, (6s, $6-\overset{|}{\underline{C}}-$), 37.78, 45.54, 49.12, 55.18 (4s, $4-\overset{|}{\underline{C}}-H$), 18.16, 21.14, 23.61, 25.00, 26.36, 28.29, 34.12, 36.54, 37.68, 38.07, 38.29, 43.02 (12s, 12 $-\underline{C}H_2$), 14.40, 15.95, 16.16, 16.50, 18.29, 21.32, 27.91 (7s, $7-\underline{C}H_3$), 80.92 (d, $H-\overset{|}{\underline{C}}-O-CO-CH_3$), 73.06 (d, $-\overset{|}{\underline{C}}H.OH$)ppm.

(Fig. 5)

Mass : Molecular ion peaks at m/z 648, 646, 644, 642 (M^+), 626, 624, 584, 582, 580, 565, 563, 561, 549, 547, 545, 505, 503, 485, 483, 393, 391, 297, 283, 191 (base peak), 189.

(Fig. 7)

Preparation of oleanan-29, 30-dibromo-19 keto3 -acetate 76.

Oleanan 29, 30-dibromo-19 -hydroxy-3β -acetate 75 (50 mg) was oxidised with CrO_3-Py complex prepared from pyridine (0.5 ml) and CrO_3 (50 mg) and was kept at room temperature for 14 hours. The crude product obtained by working up in the usual way was chromatographed over silica gel column. The chromatogram was prepared with petroleum ether and the product dissolved in benzene (1.5 ml) was poured on the column. It was eluted with the following solvents (Table 2).

Table 2

Chromatography of the above crude product ($\approx$40 mg)

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point ^{o}C
Petroleum ether	1-3	Oil	-
Petroleum ether	4	Nil	-
Petroleum ether : benzene (4:1)	5-6	Nil	-
Petroleum ether : benzene (3:2)	7-9	Solid (25 mg)	238-40^{o}

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

Fractions 7-9 (Table 2) were combined (25 mg) and crystallised twice from a mixture of chloroform and methanol to afford fine needle shaped crystals, m.p. 248^{o}. The compound showed single

spot on TLC plate and showed positive Beilstein test but negative TNM test.

^{1}H NMR (δ, $CDCl_3$) : Signals in the region between 0.6 to 0.95 (6s, 18H, 6-$\underset{|}{\overset{|}{C}}$-$CH_3$), 1.95 (s, 3H, -O-CO-$CH_3$), 2.44 (d, 1H, J = 2.5 Hz, H$C(=O)$-), 3.46(d, 1H, J = 3 Hz), 3.68 (d, 1H, J = 2.5 Hz), 3.81 (d, 2H, J = 2.7 Hz), 4.38 (m, 1H, H-$\underset{|}{C}$-O-$\underset{\|}{C}$(=O)-CH_3).

(Fig. 9)

Mass : Molecular ion peak m/z M^+ (644-H_2O), 584, 582, 580, 569, 567, 565, 503, 502, 501, 475, 474, 473, 393, 391, 191, 189.

(Fig. 8)

Preparation of 29-bromo-3β-, 30β -diacetoxy olean-18(19)-en, 77.

To a solution of oleanan 29, 30 dibromo-19 hydroxy 3β -acetate 75 (50 mg) dissolved in pyridine (1.0 ml) was added acetic anhydride (1.0 ml) and the mixture was kept over water bath for four hours. After usual work up a solid was obtained which showed single round spot on TLC plate. It was therefore crystallised from chloroform-methanol mixture. Fine needle shaped crystals separated out which was collected (40 mg), m.p. 175-8°. The crystals on repeated crystallisation afforded very vine crystals, 86, m.p. 180-1°.

TNM test was positive and so was Beilstein test for bromine.

IR : ν_{max}^{Nujol} 1740, 1250 ($-O-CO-CH_3$), 3015, 1640 and 810 ($-\overset{|}{C} = CH$) cm^{-1}

(Fig. 10)

Mass : Molecular ion peaks at m/z 606, 604 (M^+), 546, 544, 466, 465, 406, 405, 189.

(Fig. 11)

^1H NMR (δ,$CDCl_3$) : Signals in the region 0.7 to 0.9 (6s, 18H, $6-\overset{|}{C}-CH_3$), 1.95, 2.02 (2s, 6H, $2-O-CO-CH_3$), 3.3 and 3.45 (AB_q , 2H, $-CH_2-Br$), 4.38 (m, 1H, -CH-), 4.67 (AB quartet, 2H, $-CH_2-O-COCH_3$), 6.1 (s, 1H, $-CH = C$).

(Fig. 12)

Rechromatography of fractions 2-7 (Table 1) on alumina column

The fractions 2-7 (Table 1) were mixed (250 mg), dissolved in benzene (3 ml) and placed on active alumina column (20 gm) developed with petroleum ether. It was allowed to stand for 24 hours and then eluted with the following solvents (Table 3).

Table 3

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-3	Solid (≈60 mg)	180-85°
Petroleum ether: benzene (4:1)	4-7	Solid (≈50 mg)	228-30°
Petroleum ether: benzene (3:2)	8-10	Nil	-
Benzene : Petroleum ether (4:1)	14-16	Nil	-
Benzene	17-23	Solid (≈100 mg)	240-42°
Benzene : ether (4:1)	24-27	Solid (≈30 mg)	220-22°

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

Examination of fractions 1-3 (Table 3) : Isolation of 29-bromo-lup-20(29)-en-3β-yl acetate 73.

The fractions 1-3 (Table 3) were mixed (≈60 mg) and crystallised from chloroform-methanol to afford white amorphous solid, m.p. 186-88°, $[\alpha]_D$ + 32°. It gave positive TNM test and showed positive Beilstein test for bromine.

Analysis report:

	%C	%H
Found	70.12	9.29
Calculated for $C_{32}H_{51}O_2Br$	70.20	9.32

IR : ν_{max}^{Nujol} 1735, 1240 ($-O-CO-CH_3$), 1265 ($=CHBr$) cm^{-1}

(Fig. 13)

Mass : Molecular ion peaks at m/z 548, 546 (M^+), 467, 407, 203, 189, 109 (base).

(Fig. 15)

^1H NMR (δ, $CDCl_3$): 0.78-1.04 (7s, 21H, 7-$\overset{|}{C}-CH_3$), 2.05 (s, 3H, $-O-\overset{O}{\overset{\|}{C}}-CH_3$), 1.729 (s, H, $H-C-O-\overset{|}{C}-$), 5.72, 5.90 (2s, $-\overset{|}{C}=CH_2$) ppm.

(Fig. 14)

Examination of fractions 4-7 (Table 3) : Isolation of 30-bromo-lup-20(29)-en-3 β-yl acetate 72.

The fractions 4-7 (Table 3) were combined (60 mg) and crystallised from chloroform-methanol to give shining crystals, m.p. 236-37°. TNM test was negative but showed positive Beilstein test for bromine.

It was characterised as 30-bromo-lup-20(29)-en-3 β-yl acetate 82 by comparison of m.p. and IR with an authentic sample.

Examination of fractions 17-23 (Table 3): Isolation of 30-hydroxy-lup-20(29)-en-3β-yl acetate 78.

The fractions 17-23 (Table 3) were combined and the solid ($\simeq$100 mg) was crystallised from chloroform and methanol mixture when a constant melting needle shaped crystals m.p. 248-49^{o} obtained. TLC of the compound in a mixture of benzene: ethyl acetate (9:1) showed a single spot in TLC plate. It did not respond to Beilstein test for bromine but responded to positive TNM test, $[\alpha]_D = \pm 0^{o}$.

Analysis report:

	%C	%H
Found	79.31	10.75
Calculated for $C_{32}H_{52}O_3$	79.29	10.81

IR : ν_{max}^{Nujol} 3500 (-OH), 1715, 1250 ($-O-CO-CH_3$), 3100, 1640, 890 ($>C=CH_2$) cm^{-1}.

(Fig. 16)

^1H NMR (δ, $CDCl_3$) : 0.78, 0.83, 0.84, 0.85, 0.94, 1.03 (6s, 18H, 6-$\underset{|}{C}-CH_3$), 2.04 (s, 3H, $-O-CO-CH_3$), 4.15 (q, J_{AB} = 6 Hz, 2H, $-CH_2-OH$), 4.48 (m, 1H, $H-\underset{|}{C}-O-\underset{\|}{C}-CH_3$), 4.94 (m, 2H $>C=CH_2$).

(Fig. 17)

Mass : m/z 483 (M^+-1), 465, 425, 424, 423, 408, 380, 356, 248, 233, 220, 203, 189 (base peak), 175.

(Fig. 18)

Hydrolysis of 30-hydroxy-lup-20(29)-en-3β-acetate 78 :

Isolation of 3β-30 lupenyl diol 79.

30-hydroxy-lup-20(29)-en-3β-acetate 78 (50 mg) was refluxed with 10% methanolic potassium hydroxide solution (5 ml) for 4 hours. The solution was cooled, acidified with cold 10% HCl (10 ml) and extracted with ether. The ethereal layer was washed with water till neutral and then dried over anhydrous Na_2SO_4. The solvent was distilled off and the solid residue was crystallised from methanol. Repeated crystallisation (3 times) from methanol gave a crystalline m.p. 226-28°. It gave yellow colour with TNM but no green colour flame in Beilstein test for halogen.

Analysis report:

	%C	%H
Found	81.21	11.36
Calculated for $C_{30}H_{50}O_2$	81.39	11.38

IR : ν_{max}^{Nujol} Peak between 3300-3400 (OH), 3100, 1640, 880 ($>C=CH_2$) cm^{-1}

(Fig. 19)

Mass : m/z 442 (M^+), 441, 423, 408, 383, 380, 314, 233, 220, 207, 203, 189 (base peak), 175.

(Fig. 20)

Acetylation of 30-hydroxy-lup-20(29)-en-3β -acetate 78 : Isolation of 3β-, 30-Lupenyl diacetate 80.

To a solution of 30-hydroxy Lup-20(29)-en-3 -acetate 78 (50 mg) in pyridine (2.5 ml) was added acetic anhydride and the mixture was kept over water bath for four hours. After usual work up, a solid residue was obtained. It was crystallised from chloroform-methanol mixture. Fine needle shaped crystals (40 mg) separated out, m.p. 153-58^{o} which was collected. The crystals on careful crystallisation from chloroform-methanol (three times) yielded very fine needle shaped crystals of constant melting point 163-64^{o}, $[\alpha]_D$ + 8^{o}. TLC of the compound showed single spot on the chromatoplate in a mixture of solvent benzene : petroleum ether 3:2.

Analysis report:

	%C	%H
Found	77.39	10.29
Calculated for $C_{34}H_{54}O_4$	77.52	10.33

IR : ν_{max}^{Nujol} 1750, 1730, 1265, 1250 (-O-CO-CH_3), 3080, 1640, 840 ($\rangle C = CH_2$) cm^{-1}.

Examination of fractions 24-27 (Table 3) : Isolation of 3β-, 30-Lupenyl diol 79.

The fractions 24-27 (Table 3) were mixed (≈30 mg) and crystallised from a mixture of chloroform-methanol to furnish crystals of 3β-, 30 Lupenyl diol m.p. 226-28°, $[\alpha]_D$ + 4°. It gave negative test for bromine in Beilstein test.

The compound was found identical with 3 , 30 Lupenyl diol prepared by hydrolysis of 30-hydroxy 3β -lupenyl acetate from m.m.p. and IR data comparison.

Oxidation of a mixture of 29-bromo and 30-bromo Lupenyl acetate in chloroform with DMSO and water.

To a solution of a mixture of 29-bromo and 30-bromo lupenyl acetate (.5g) in chloroform (25 ml) was added NBS (.5g) in portions followed by addition of dimethyl sulphoxide (12.5 ml) and water (2 ml). The reaction mixture was kept in dark for 15 days. After 15 days the reaction mixture was poured on ice cold water when a white solid separated out which was then extracted with chloroform. The chloroform layer was washed with water and dried over anhydrous Na_2SO_4. Solvent was removed when an oily residue (425 mg) was left. The residue was dissolved in benzene (5 ml) and poured on a column of silica gel. The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 4).

Table 4

Chromatography of the above residue (425 mg)

Eluent	Fractions 100 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-3	Oil	-
Petroleum ether: benzene (4:1)	4-7	Nil	-
Petroleum ether : benzene (3:2)	8-17	White solid (≈200 mg)	165-70°
Petroleum ether : benzene (2:3)	18-19	Nil	-
Petroleum ether: benzene (1:4)	20-21	Nil	-
Benzene	22-28	Solid (≈125 mg)	226-31°

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

Examination of fractions 8-17 (Table 4) : Isolation of 29-bromo-lup-20(29)-en-3β-ol, 81 (A_1).

The fractions 8-17 (Table 4) were combined (≈200 mg) and crystallised from a mixture of chloroform and methanol which afforded amorphous solid, m.p. 173-5°. TLC of the compound

showed a single round spot on TLC plate. TNM test was positive and so was Beilstein test for bromine.

Analysis report:

	%C	%H
Found	71.23	9.79
Calculated for $C_{30}H_{49}OBr$	71.15	9.83

IR : ν_{max}^{Nujol} 3580-3200 (-OH), 1690 (>C = CH_2) cm^{-1}

(Fig. 21)

Mass : Molecular ion peaks m/z (M^+) 506, 504 (for isotopic bromine), 425, 408, 407, 207, 203, 189, 135, 109 (base) 107, 95, 81, 79, 69, 67, 55.

(Fig. 22)

^1H NMR (δ,$CDCl_3$) : Signals at 0.75-1.01 (6s, 18H, 6-C-CH_3),

3.16-3.2 (q , H, -C(H)-OH),

5.89 (d, H, =C(H)-Br).

(Fig. 23)

Examination of fractions 22-28 (Table 4) : Isolation of 30-aldehyde-lup-20(29)-en-3β-ol, 82 (B_1)

The fractions 22-28 (Table 4) were combined (≈125 mg), m.p. 226-31° and crystallised thrice from chloroform and methanol to give fine crystals, m.p. 235-6°. It responded positive TNM test, but did not respond Beilstein test for halogen.

Analysisis report:

	%C	%H
Found	81.67	10.89
Calculated for $C_{30}H_{48}O_2$	81.81	10.90

UV (methanol) : λ_{max} 226 nm

(Fig. 25)

IR : ν_{max}^{Nujol} 3560-3100 (-OH), 1640, 810 ($=CH_2$), 1680 ($-\overset{H}{\underset{|}{C}}=O$)$cm^{-1}$

(Fig. 24)

Mass : Molecular ion peak (M^+) 440, m/z 422, 407, 207, 203, 190, 189, 135, 107, 95, 93, 81, 69, 67, 55 (base).

(Fig. 26)

^1H NMR (δ, $CDCl_3$) : Signals at 0.75-1.01 (6s, 18H, $6-\overset{|}{\underset{|}{C}}-CH_3$)

3.16-3.19 (q, H, $-\overset{H}{\underset{|}{C}}-OH$),

5.91 and 6.28 (2d, 2H, $-\overset{|}{C}=CH_2$),

9.5 (s, H, $-\underset{H}{\underset{|}{C}}=O$).

(Fig. 27)

Isolation of moretenol from Sapium Sebiferum Roxb.

Extraction

Dried and powdered trunk bark and stem (1.0 kg) of Sapium Sebiferum Roxb[68] was extracted with benzene in soxhlet apparatus for 36 hours. During the extraction a yellow insoluble solid separated out. The extract was cooled to room temperature and the yellow insoluble solid was collected by filtration. It was identified previously as 3, 4-Di-O-methyl ellagic acid by Pradhan[69]. From the clear filtrate benzene was distilled off and a gummy residue (18 gm) was obtained. The gummy residue was dissolved in ether (1 litre) and the ether solution was washed with 10% NaOH solution (3 x 300 ml) and water till neutral. The neutral ether solution was dried over anhydrous sodium sulphate. Evaporation of ether furnished a gummy residue (16.0 gm) which constituted the neutral portion of the extract.

Chromatography of the neutral portion of the extract.

The gummy residue (16.0 g) dissolved in benzene (30.0 ml) was placed on a column of alumina (640.0 gm, deactivated with 25.5 ml of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and eluted with petroleum ether, petroleum ether : benzene (9:1), petroleum ether : benzene (4:1), petroleum ether : benzene (3:2), petroleum ether : benzene (2:3) and benzene solvents.

The petroleum ether : benzene (3:2) eluents were combined and crystallised from a mixture of chloroform and methanol when fine crystals of moretenol separated out, m.p. 228-30°, $[\alpha]_D$ + 25°.

Preparation of Moretenyl acetate.

Moretenol (.5g) isolated above was dissolved in pyridine (5 ml). To this solution was added acetic anhydride (5 ml) and the mixture kept over water bath for four hours. The mixture was then cooled and poured over ice-cold water when white solid separated out. It was washed with water, filtered through suction and dried.

The dried solid (0.5g) was crystallised thrice from chloroform-methanol mixture to afford crystals, m.p. 277-8°, $[\alpha]_D$ + 28°. It was found identical with an authentic specimen of moretenyl acetate (m.m.p., CO-IR and CO-TLC).

Oxidation of moretenyl acetate with N-bromo Succinimide.

To a solution of moretenyl acetate 83 (.5g) in chloforom (25 ml) was added NBS (.5g) in portionsfollowed by addition of dimethyl sulphoxide (12.5 ml) and the reaction mixture was kept in the dark for about 24 hours. The reaction mixture was then poured on ice-cold water when a white solid separated out which was extracted with chloroform. The chloroform layer was washed

with water and dried over anhydrous Na_2SO_4. Removal of chloroform gave an oily solid (.45g). TLC of the compound showed three distinct spots indicating the presence of at least three compounds in it. The residue was dissolved in benzene (5 ml) and poured on a column of silica gel developed with petroleum ether. The column was eluted with the following solvents (Table 5).

Table 5

Chromatography of the oily solid (.45 gm)

Eluent	Fractions 100 ml each	Residue on evaporation	Melting point ^oC
Petroleum ether	1-12	White solid (≃85 mg)	215-8°
Petroleum ether: benzene (9:1)	13-14	Oil (≃25 mg)	-
Petroleum ether: benzene (9:1)	15-23	White solid (≃185 mg)	233-7°
Petroleum ether : benzene (4:1)	24-25	Nil	-
Petroleum ether : benzene (3:2)	26-36	Solid (≃130 mg)	245-51°

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

Examination of fractions 1-12 (Table 5) : Isolation of 3β-acetoxy-30-bromo-isohop-22, 29-en, 84 (A).

The fractions 1-12 (Table 5) were combined (85 mg) and crystallised thrice from a mixture of chloroform and methanol which afforded fine needle shaped crystals (50 mg), m.p. 222-4°. TLC of the compound showed a single round spot. TNM test was positive and so was Beilstein test for halogen. Analysis report:

	%C	%H
Found	70.12	9.28
Calculated for $C_{32}H_{51}O_2Br$	70.32	9.34

IR : ν_{max}^{Nujol} 1720, 1245 ($-O-CO-CH_3$), 3040, 840 ($>C=CH_2$) cm^{-1}.

(Fig. 28)

Mass : Molecular ion peak (M^+) 548, 546, m/z 488, 487, 486, 473, 471, 411, 407, 400, 269, 267, 248, 205, 189 (base).

(Fig. 29)

^1H NMR (δ, $CDCl_3$) : Signals at 0.69, 0.76, 0.84, 0.94, 0.97,
1.25 (6s, 18H, 6-$\underset{|}{C}-CH_3$),
2.03 (s, 3H, $-O-CO-CH_3$),
4.1 (AB_q, 2H, $-CH_2-Br$),
4.45 (t, H, $H-\underset{|}{C}-O-\underset{\underset{O}{\|}}{C}-CH_3$),
4.9-5.9 (2H, $>C=CH_2$) ppm.

(Fig. 30)

Examination of fractions 15-23 (Table 5) : Isolation of 3β-acetoxy-22, 29, 30-tribromo-isohopane, 85 (B).

The fractions 15-23 (Table 5) were combined (185 mg) and on repeated crystallisations from a mixture of chloroform and methanol furnished white needle shaped crystals, m.p. 241-2°, $[\alpha]_D$ + 23.14°. It showed positive Beilstein test for bromine but did not respond TNM test. TLC of the compound showed single round spot on TLC plate.

Analysis report:

	%C	%H
Found	54.01	7.11
Calculated for $C_{32}H_{51}O_2Br_3$	54.09	7.18

IR : ν_{max}^{Nujol} 1725, 1245 (-O-COCH$_3$) cm^{-1}.

(Fig. 31)

Mass : Molecular ion peaks (M^+) 704, 706, 708, 710 in the ratio (1:3:3:1),
m/z 692, 690, 647, 645, 632, 628, 547, 545, 429, 427, 349, 347, 269, 249, 203, 189 (base).

(Fig. 32)

^{1}H NMR ($CDCl_3$) : Signals at 0.75, 0.84, 0.85, 0.95, 1.00, 1.27 (6s, 18H, 6-C(CH$_3$)-), 2.05 (s, 3H, -O-CO-CH$_3$), 3.8-4.2 (2AB$_q$, 4H, 2H$_2$C-Br)

(Fig. 33)

^{13}C NMR : 15.9, 16.11, 16.17, 16.47, 16.75, 21.0, 27.9 (7q, 7-CH_3), 18.23, 20.9, 23.57, 23.66, 23.66, 26.58, 32.83, 33.19, 38.36, 38.86, 40.2, 40.8 (12t, 12 -$\underline{C}H_2$) 45.8, 48.43, 50.17, 54.17, 55.17 (5d, 5-$\underline{C}H$) 80.89 (d, H-$\underline{C}$-O-$COCH_3$) 36.99, 37.76, 41.52, 41.70, 45.36 (5s, 5-$\underline{C}$-) 76.05 (s, -$\underline{C}$-Br); 170.99 (s, -O-$\underline{C}$O-CH_3) ppm

(Fig. 36)

Examination of fractions 26-36 (Table 5): Isolation of 3β-acetoxy-22-hydroxy-29, 30-dibromo isohopane, 86 (C)

The fractions 26-36 (Table 5) were mixed and crystallised thrice from chloroform and methanol mixture to afford white amorphous solid (≃100 mg), m.p. 258-9°, $[\alpha]_D$ + 25°. It showed positive Beilstein test for halogen. It did not develop characteristic yellow colour with TNM. TLC of the compound showed a single round spot on TLC plate.

Analysis report:

	%C	%H
Found	59.40	8.03
Calculated for $C_{32}H_{52}O_3Br_2$	59.53	8.06

IR : ν_{max}^{Nujol} 3360 (-OH), 1730, 1250 ($-O-COCH_3$)cm^{-1}

(Fig. 37)

Mass : Molecular ion peaks (M^+) 645, 643, 641 (1:2:1),
m/z 585, 583, 581, 570, 568, 566, 565, 547, 545, 505,
503, 472, 470, 466, 465, 427, 425, 409, 407, 269, 267,
249, 189 (base), 137, 121, 119.

(Fig. 38)

^1H NMR (δ,$CDCl_3$) : 0.72, 0.84, 0.85, 0.86, 0.94,
0.98 (6s, 18H, 6-$\overset{|}{C}$-CH_3),
2.06 (s, 3H, $-O-CO-CH_3$),
3.57-3.72 (2 AB_q, 4H, J = 10 Hz, $2-CH_2Br$),
4.45 (m, H, H-$\overset{|}{C}$-$O-COCH_3$)ppm.

(Fig. 39)

^{13}C NMR : 15.2, 16.00, 16.4, 16.4, 16.8, 21.0,
28.0 (7q, 7-CH_3),
18.5, 20.0, 20.9, 23.6, 23.6, 23.6, 24.08, 32.6,
33.2, 39.08, 39.2, 40.4 (12t, 12 -$\overset{|}{C}H_2$),
38.4, 45.2, 50.2, 52.0, 55.2 (5d, 5-$\overset{|}{C}$-H),
80.8 (d, H-$\overset{|}{C}$-$O-COCH_3$),
37.6, 37.7, 41.6, 44.4, 44.8 (5s, 5-$\overset{|}{C}$-),
74.2 (s, -$\overset{|}{C}$-Br),
171.0 (s, $-O-\underline{C}O-CH_3$) ppm.

(Fig. 40)

Hydrolysis of 3β -acetoxy-22, 29, 30-tribromo-isohopane, 85(B) over active basic alumina column.

The dried mother liquor of 85 (≃100 mg) was dissolved in benzene (3 ml) and poured on a column of dry basic alumina (5g). The compound was kept in the column for seven days followed by addition of 5 ml petroleum ether each day. After seven days, the column was run and eluted with the following solvents.

Table 6

Chromatography of the above residue (≃100 mg)

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-2	Oil	-
Petroleum ether	3-6	Solid	218-20°
Petroleum ether: benzene (4:1)	7-11	Solid	250-2°
Petroleum ether : benzene (3:2)	12-17	Solid	231-4°
Benzene : petroleum ether (3:2)	18-23	Solid	201-5°

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

Examination of the fractions 3-6 (Table 6)

The fractions 3-6 (Table 6)were combined and crystallised from mixture of chloroform and methanol to afford white needle shaped crystals, m.p. 222-4°. It showed single spot on TLC plate and responded positive TNM and Beilstein test. It was found identical with 84 (m.m.p., CO-IR and CO-TLC).

Examination of fractions 7-11 (Table 6)

The fractions 7-11 (Table 6) were combined and crystallised from $CHCl_3$-MeOH mixture to give white amorphous solid, m.p. 258-9°. It showed positive Beilstein test for bromine but did not respond TNM test. It was found identical with 86 (m.m.p., CO-IR, CO-TLC).

Examination of fractions 12-17 (Table 6): Isolation of 30-bromo moretenol, 87.

The fractions 12-17 (Table 6) were combined and crystallised carefully from chloroform-methanol mixture to afford fine crystals, m.p. 238-9°. It showed positive TNM test and positive Beilstein test. TLC of the compound showed a single round spot on a chromatoplate.

Analysis report:

	%C	%H
Found	71.02	9.55
Calculated for $C_{30}H_{49}OBr$	71.15	9.67

IR : ν_{max}^{Nujol} 3280-3320 (-OH), 3040-60 ($>CH_2$), 1620 cm^{-1}

(Fig. 41)

Mass : Molecular ion peaks (M^+) 506, 504 (1:1),

m/z 491, 489, 426, 425, 358, 269, 207 (base), 189.

(Fig. 42)

Examination of fractions 18-23 (Table 6): Isolation of 29-bromo moretenol, 88.

The fractions 18-23 (Table 6) were combined and on careful crystallisation from $CHCl_3$-MeOH it afforded amorphous solid m.p. 206-8°. It showed green colouration for bromine in Beilstein test. It developed yellow colour with TNM. TLC of the compound showed a single round spot on a chromatoplate.

Analysis report:

	%C	%H
Found	71.10	9.62
Calculated for $C_{30}H_{49}OBr$	71.15	9.67

Mass : Molecular ion peaks (M^+) 506, 504 (1:1),

m/z 491, 489, 426, 425, 358, 269, 267, 207 (base, 189.

(Fig. 43)

^1H NMR (δ, $CDCl_3$) : 0.64, 0.74, 0.79, 0.88, 0.95, 1.52 (6s, 18H, 6-$\overset{|}{C}$-CH_3), $\overset{||}{C}HBr$

1.68-1.70 (s, 3H, -$\overset{||}{C}$-CH_3)

3.0 (m, H, -$\overset{|}{C}$-H),

3.15 (m, H, -$\overset{|}{C}$H-OH).

(Fig. 44)