CHAPTER - III

EXPERIMENTAL

Melting points are uncorrected. The petroleum 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, HA-100 and XL-200 spectrophotometer using chloroform-d solution containing tetramethysilane as reference. The IR spectra were recorded in Perkin-Elmer 337 and 221 spectrophotometers in ~~95% ethanol~~ Nujol mull solution unless stated otherwise. TLC was done on chromatoplate of silica gel G and the spots were developed in iodine chamber. UV Beckmann DU-2 spectrophotometers in 95% ethanol unless stated otherwise. Mass spectra on Jeol D-300 mass spectrometer (70 eV, direct inlet).

Extraction of Biscofia javanica Blume : Isolation of betulinic acid 27:

Described ~~on~~ in Part I, page 82 .

Esterification of betulinic acid 27 : Preparation of methyl betulinate 28:

Described ~~on~~ in Part I, page 83

Hydrogenation of methyl betulinate 28 : Preparation of methyl dihydro betulinate 9a:

Methyl betulinate 28 (8 gm) dissolved in ethyl acetate (400 ml) was shaken in an atmosphere of hydrogen in presence of Paladium in charcoal catalyst (500 mg) for three hours until

absorption of hydrogen ceased. Ethyl acetate was removed by distillation after ~~filtered~~ filtering off the catalyst. The solution was diluted with water whereby a white solid (7.5 gm) separated out which was collected by filtration. Crystallisation from a mixture of ~~chloroform~~ chloroform and methanol furnished colourless needles of 9a m.p. 236-38°. This compound 9a was found to be identical with an authentic sample of methyl dihydrobetulinate (m.m.p and IR).

Found	: C 78.92 H 11.23%
Calculated for $C_{31}H_{52}O_3$	: C 78.76 H 11.09%
UV	: transparent in the region 220 to 300 nm.
IR (Nujol)	: 3540(OH), 1705 cm^{-1} (CO_2Me) (Fig. 2)
NMR (100 MHz)	: δ3.65 (3H, S, $-COOCH_3$), δ3.20 (1H, m, -CHOH) δ.75-1.00 (for seven saturated methyl groups) (Fig. 3)

Jones oxidation of methyl dihydrobetulinate 9a : Preparation of methyl dihydrobetulonate 9b:

To a solution of methyl dihydrobetulinate 9a (7 gm) in pure acetone (1 litre) was added Jones reagent dropwise 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 with water, dried (Na_2SO_4) and the ether evaporated. The residue

(5.9 gm) dissolved in benzene (15 ml) was chromatographed over a column of silica gel (120 gm). The chromatogram was developed with petroleum and then eluted with the following solvents (Table II) .

Table - II

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-4	Nil
Petroleum ether: Benzene (4:1)	5-20	Solid (5.7 gm) m.p. 197-99°

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

Fractions 5-20 (5.7 gm) were combined and on crystallisation from methanol furnished needle shaped crystals of 9b m.p. 202-4°, $[\alpha]_D$ + 8°, identical with an authentic sample of methyl dihydrobetulonate (m.m.p and IR comparison).

Found	: C 79.32	H 10.86%
Calculated for $C_{31}H_{50}O_3$	: C 79.10	H 10.71%
IR (Nujol)	: 1730 (CO_2Me), 1708 cm^{-1} (CO)	

(Fig. 4)

Autoxidation of methyl dihydrobetulonate 9b : Preparation of diosphenol 29b:

Methyl dihydrobetulonate 9b (2 gm) suspended in potassium tertiary butoxide in tertiary butanol (prepared from 6 gm potassium and 160 ml of tertiary butanol) was shaken in a steam of oxygen for 75 minutes. The reaction mixture was diluted with water and then 6N hydrochloric acid was added till the solution was acidic. It was then extracted with chloroform (150 ml), washed with ether till neutral and dried (Na_2SO_4). On removal of the solvent under reduced pressure, a yellowish gummy residue (1.8 gm) was obtained. This residue dissolved in benzene (10 ml) was chromatographed on a column of alumina (120 gm, deactivated with 4.8 ml of 10% aqueous acetic acid) developed with petroleum and was eluted with the following solvents (Table - III).

Table - III

Eluent	Fractions 50 ml each	Residue on evaporation
Petroleum ether	1-4	Nil
Petroleum ether: benzene (4:1)	5-15	Solid (1.6 gm) m.p. 129-132°

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

Fractions 5-15 (1.6 gm) were combined and on crystallisation from a mixture of chloroform and methanol furnished colourless needles of 29b m.p. 131-133°, $[\alpha]_D$ - 1.96°. It gave a positive ferric chloride colouration for diosphenol and showed two spots on chromatoplate. These were assumed to be due to tautomeric forms of the diketone 29a and diosphenol 29b.

Found	: C 76.48	H 10.01%	
Calculated for $C_{31}H_{48}O_4$	: C 76.82	H 8.98%	
UV	: λ_{max} 270 mμ (ϵ = 7932) (in MeOH)		
	λ_{max} 310 mμ (in KOH + MeOH)		
			(Fig. 5)
IR	: 3460 (OH), 1730 (CO_2Me), 1670, 1650, 860 cm^{-1}		
$FeCl_3$ colour test	: Positive		

Acetylation of diosphenol 29b: Preparation of diosphenol acetate 30 :

Diosphenol 29b (1 gm) was treated with acetic anhydride (10 ml) and pyridine (10 ml) and kept overnight at room temperature. After working up in the usual manner the crude acetate (0.9 gm) was obtained. This was chromatographed over a column of silica gel (20 gm). The compound was dissolved in benzene (7 ml) and the chromatogram was developed with petroleum. Fractions obtained in petroleum ether were combined and crystallisation from chloroform and methanol mixture afforded crystals of 30 m.p. 194-96°, $[\alpha]_D$ + 9.43°. It showed a single spot on a chromatoplate and did not

respond to the colour test with ferric chloride solution for diosphenol.

Found	: C 75.52	H 9.73%	
Calculated for $C_{33}H_{50}O_5$	: C 75.25	H 9.57%	
UV	: λ_{max}^{EtOH} 237 m (ϵ, 9968)		

Hydrogenation of diosphenol 29b: Diosphenol reduced product 31

To diosphenol 29b (1 gm) dissolved in ethyl acetate was added 10% palladium-on-charcoal catalyst (100 mg) and the mixture was shaken in an atmosphere of hydrogen till the absorption of hydrogen ceased. The solution was filtered and after removing the solvent from the filtrate a solid residue was obtained which after crystallisation from methanol and chloroform mixtured afforded 31 m.p. 124-26°.

Found	: C 76.24	H 10.58	
Calculated for $C_{31}H_{50}O_4$	: C 76.54	H 10.41%	
UV	: λ_{max}^{EtOH} 270 m (ϵ = 43)		(Fig. 6)
IR	: 3500 (OH), 1740 cm^{-1} (a band combined for easter and ketone).		(Fig. 7)
PMR (100MHz)	: δ ·78-1·1 (7 Saturated methyls), 5·1 (1H, m, H-C(2)-OH), 3·62 (3H, S, -COOCH3)		

Meerwein-Ponndorff reduction of 31 : Preparation of methyl 2α, 3α-dihydroxy-lupan 28-oate 9e:

A mixture of compound 31 (1 gm), Al-isopropoxide (1.5 gm) in dry isopropanol (25 ml) was distilled slowly with the addition of isopropanol to maintain constant volume[45]. After 5 hours the distillate no longer contained acetone and the solution was concentrated to a small volume. The reaction mixture was diluted with water followed by 10% sulfuric acid solution (20 ml) and then extracted with chloroform. The product obtained after removal of chloroform was dissolved in benzene and chromatographed on a column of silica gel (30 gm) developed with petroleum ether and eluted with the following solvents (Table - IV).

Table - IV

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)	9-12	Nil
Petroleum ether: Benzene (2:3)	13-16	Nil
Petroleum ether : Benzene (1:4)	17-20	Nil
Benzene	21-24	Nil
Benzene : chloroform (4:1)	25-28	Solid m.p. 210-14°

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

Fractions 25-28 (700 mg) were combined and on crystallisation from a mixture of chloroform and methanol furnished crystals of 9e m.p. 215°.

Found	: C 76.81%	H 11.01%
Calculated for $C_{31}H_{52}O_4$	: C 76.22%	H 10.65%
IR (Nujol)	: 3420 (OH), 1730 cm^{-1} ($COOCH_3$)	

(Fig. 8)

NMR (XL 200): (Without D_2O Treatment)

δ .69-1.01 (7 saturated methyls),
δ 3.39 (1H, d, J = 3 Hz, H-C_3-OH)
δ 3.66 ($COOCH_3$),
δ 3.95 (1H, ddd, J = 12, 4,3 Hz, H-C_2-OH)

Acetylation of methyl 2α, 3α dihydroxy-Lupan 28-oate 9e : Preparation of methyl 2α, 3α diacetoxy-lupan 28-oate 32

The diol 9e (500 mg) was acetylated with acetic anhydride (15 ml) and pyridine (15 ml). The reaction mixture was kept on water bath for 7 hours. The solid obtained by usual work up was dissolved in benzene (20 ml) and chromatographed on a column of silica gel (20 gm) and eluted with the following solvents (Table - V).

Table - V

Eluent	Fractions of 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	Solid m.p. 196-98°

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

Fractions (9-12) (400 mg) were combined and on crystallisation from a mixture of chloroform and methanol furnished needle shaped crystals of 32, m.p. 198°.

Found	: C 73.21% H 10.10%	
Calculated for $C_{35}H_{56}O_6$	: C 73.42% H 9.72%	
Mass	: M^+ 572, 512 (M^+-60), 469 (M^+ - 103) m/z	(Fig. 9)
IR (Nujol)	: 1740, 1260 cm^{-1} (acetate), 1730 (-COOCH3)	(Fig. 10)
NMR (XL 200)	: δ.78-.91 (7 saturated methyls) δ1.89, δ2.04 (two sharp singlet for -COCH3 at the position 2 and 3) δ3.58 (3H, S, -COOCH3) δ4.94 (1H, d, J = 3Hz, H-C3-OAc) and δ5.19 (1H, ddd, J = 11, 4, 3 Hz, H-C2-OAc)	(Fig. 11)

Chromatography on a silica gel column of the autoxidation of dihydro methyl betulonate 9b. Isolation of the hydroxy acid 33.

The autoxidation product of dihydro methyl betulonate 9b (1 gm) obtained during prolonged period of reaction (3 hrs), was dissolved in benzene (20 ml) and chromatographed on a column of silica gel (20 gm) developed with petroleum ether and eluted with the following solvents (Table - VI).

Table - VI

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)	9-12	Nil
Petroleum ether: Benzene (2:8)	13-16	Nil
Petroleum ether: Benzene (1:4)	17-20	Nil
Benzene	25-28	Nil
Benzene:chloroform (4:1)	29-32	Nil
Benzene:chloroform (3:2)	33-36	Nil
Benzene:chloroform (2:3)	37-40	Nil
Benzene:chloroform (1:4)	41-44	Solid

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

Fractions 41-44 (700 mg) were combined and crystallised from a mixture of chloroform and petroleum ether furnished the compound 33, m.p. 255-56°.

Found	: C 73.78%	H 10.21%	
Calculated for $C_{31}H_{50}O_5$	: C 74.1%	H 9.96%	
IR (Nujol)	: 3420 cm^{-1} (OH, broad peak), 1720 cm^{-1} (-COOH and -$COOCH_3$)		(Fig. 12)
UV	: No absorption in the range 220-300 mμ.	λ_{max} 285 (6,55)	(Fig. 13)

Esterification of the compound 33 : Preparation of the ester 34.

To the compound 33 (500 mg) dissolved in ether (100 ml) was added a solution of diazomethane in ether prepared from nitrosomethyl urea (300 mg) and was kept overnight. Next day excess diazomethane was destroyed with acetic acid (.5 ml). The ether solution was washed with water, 10% sodium bicarbonate solution and again with water till neutral and then dried (Na_2SO_4). Evaporation of the ether yielded a gummy residue (400 mg) was dissolved in benzene (10 ml) and was chromatographed over a column of silica gel (40 gm) developed with petroleum ether and was eluted with the following solvents (Table - VII).

Table - VII

Eluent	Fractions of 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	Solid m.p. 148-52°

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

Fractions 9-12 (300 mg) were combined and crystallised from a mixture of chloroform and methanol furnished needle shape crystals of 34, m.p. 155-56°.

Found	:C 74.01%	H 10.22%
Calculated for $C_{32}H_{52}O_5$	:C 74.41%	H 10.07%

Mass : M^+ 516, 473 (M^+ - 43)
445, 384, ~~360~~ 372 m/z (Fig. 14)

IR (Nujol) : 3400 (OH), 1730, ~~1240~~ cm^{-1} (COOMe)
(Fig. 15)

UV : No absorption ~~on~~ in the range 220-300 mμ.
(Fig. 16)

Oxidation of the compound 33 by PbO_2 : Preparation of methyl decarboxydehydrodihydroceanothate 35 :

α-hydroxy carboxylic acid 33 (1 gm) and lead dioxide (1 gm) were suspended in glacial acetic acid (7 ml). The mixture was heated under mild reflux for 3 hours with occasional stirring. The excess of lead dioxide was filtered and the clear straw coloured liquid was diluted with water. This aqueous portion was extracted with ether, washed with 10% sodium bicarbonate solution, washed with water till neutral and then dried (Na_2SO_4). On removal of the solvent a solid residue (0.75 gm) was obtained which on crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals of 35, m.p. 179-80°, $[\alpha]_D$ 75.4°. This compound was found to be identical in all respect with the sample prepared by de Mayo and Starratt[46].

Found	C 78.96	H 10.48%
Calculated for $C_{30}H_{48}O_3$	C 78.90	H 10.59%
IR (Nujol)	1735 cm^{-1} (unresolved band for cyclopentanone and ester)	
	On expansion : Two peaks at 1734 (cyclopentanone) and 1728 cm^{-1} (COOMe).	

R E F E R E N C E S

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