

CHAPTER III

EXPERIMENTAL

Melting points are uncorrected. The petroleum ether used during investigation had b.p. 60-80°C. Infrared spectra were recorded as nujol mull on Beckman IR-20, Parkin Elmer, Pye Unicam SP 3-300S Spectrophotometers. ¹H NMR spectra were recorded in Bruker WH-400 and Varian - 200 spectrometers using CDCl₃ containing TMS as internal reference. ¹³C NMR spectra were determined on Bruker WH-270 spectrometer using CDCl₃ solution containing TMS as internal reference. The UV-absorption spectra were taken in methanol on Shimadzu UV-240 spectrophotometer and mass spectra were recorded by electron impact method in Varian Mat 711 instrument (70 eV), Varian Mat 12S and JMS-D 300 spectrometers. The column chromatography was performed over silica gel (60-120 mesh, BDH). TLC were performed in chromatoplates prepared on glass strips with silica gel G (E. Merck). All the optical rotations were determined in chloroform solution.

PREPARATION OF LUP-1(2)-EN-3-ONE 1 BY DEHYDROBROMINATION OF 2 α -BROMO LUPANONE.

Described in Part I, page.276.

Lithium aluminium hydride reduction of compound 1 : Preparation of lup-1(2)-ene-3 α -ol 2

A solution of compound 1 (.8g) in dry ether (200 ml) was refluxed with lithium aluminium hydride (0.8g) for 4 hr. Excess LAH was destroyed by adding a saturated solution of Na₂SO₄ at room temperature. The ethereal layer was separated and the aqueous layer was extracted with ether and the two ether solutions were mixed, washed with water and dried (Na₂SO₄). The removal of ether yielded a white residue (.8g). The residue was dissolved in minimum volume of benzene and was chromatographed over silica gel (20g). The column was developed with petroleum ether and eluted with the following solvents (Table 1).

Table 1

Eluents	Fractions 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1-3	Nil	-
Petroleum ether: benzene (4:1)	4-16	White solid	149-52°
Petroleum ether: benzene (3:2)	17-19	Oil	-

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

Examination of fractions 4-16 (Table 1): Isolation oflup-1(2)-ene-3 α -ol.

Fractions 4-16 (Table 1) were combined (0.75g, TLC single spot) and crystallised from chloroform-methanol mixture. The crystallisation afforded white amorphous powder of compound A m.p. 453-55^o, which was identified as lup-1(2)-ene-3 α -ol 2.

Analysis report	%C	%H
Found	84.43	11.80
Calculated for C ₃₀ H ₅₀ O	84.44	11.81

TNM test : Positive

IR : $\int_{\text{max}}^{\text{Nujol}}$ 3200-3450 cm⁻¹ (-OH),
880 cm⁻¹ (= CH₂) (Fig. 1)

Mass : m/z at 426 [M]⁺, 411 [M - CH₃]⁺, 408 [M - H₂O]⁺,
393 [408 - CH₃]⁺, 383 [M - C₃H₇]⁺,
287, 231, 191, 163, 134, 123, 121 (base peak),
109, 95, 81, 169, 69, 55.

(Fig. 2)

¹H NMR(CDCl₃): 0.76-1.06 (8 methyls),

5.95 and 5.87 (2dd, J = 3 and 10 Hz,

HC = CH)

3.86 (t, J = 3 Hz, 3-CH₂-OH)ppm

(Fig. 3)

TREATMENT OF LUP-1(2)-EN-3 α -OL WITH H_2O_2 IN T-BUTANOL--
METHYLENE CHLORIDE CONTAINING p-TOLUENE SULPHONIC ACID.

To a solution of compound 2 (0.7g) in methylene chloride (100 ml) was added 80 ml of oxidising solution which was prepared from 3 ml of 30% H_2O_2 in 80 ml of tertiary butyl alcohol containing 3.0g of p-toluene sulphonic acid at room temperature. The solution was stirred slowly for 72 hours and was then poured into water. The mixture was extracted with methylene chloride and the methylene chloride solution was washed with water, aqueous sodium bicarbonate solution and then again with water. The organic layer was dried over anhydrous Na_2SO_4 and was then evaporated when a solid residue of 0.75 gm, was obtained. The residue was dissolved in minimum volume of benzene and was chromatographed over silica gel (20 gm). The column was developed with petroleum ether and eluted with the following solvents (Table 2).

Table 2

Eluent	Fraction 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1	Nil	-
Petroleum ether	2-7	white solid	189-90°
Petroleum ether: benzene (4:1)	8-12	White solid	213-14°
Petroleum ether: benzene (3:2)	13-17	Solid	192-93°
Petroleum ether: benzene (2:3)	18-22	Solid	218-19°
Petroleum ether: benzene (1:4)	24-27	White solid	198-200°C
Benzene	28-30	Oil	-

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

Examination of fractions 2-7 (Table 2): Isolation of 3 α -t-butyl-1 α , 2 α -epoxy lupane 3

Fractions 2-7 (Table 2) showed homogeneity on TLC plate were combined (70 mg). The crystallisation of the solid so obtained from a mixture of chloroform and methanol afforded fine needle shaped crystals of compound B m.p. 193-94° and was identified as 3 α -t-butoxy-1 α , 2 α -epoxy lupane 3.

Analysis report	%C	%H
Found	81.89	11.59
Calculated for $C_{34}H_{58}O_2$	81.93	11.65

TNM test : No yellow colouration

IR : No characteristic absorption. (Fig. 4)

1H NMR ($CDCl_3$): 0.76, 0.80, 0.84, 0.95, 0.97, 1.08
 (6s, 18H, 6t- CH_3), 0.79 and 0.85 (2d, 6H,
 $CHMe_2$, $J = 7$ Hz), 1.19 (s, 9H, $-O-\overset{Me}{\underset{Me}{|}{C}}-Me$),
 2.91 (d, 1H, $J = 4$ Hz), 3.55 (dd, 1H,
 $J = 2, 4$ Hz), 4.37 (d, 1H, $J = 2$ Hz)ppm

(Fig. 5)

Mass : m/z at 482 $[M]^+$, 426 $[M - C_4H_8]^+$, 425 $[M - C_4H_9]^+$,
 409 $[M - C_4H_9O]^+$, 408 $[M - C_4H_{10}O]^+$, 393 $[408 -$
 $CH_3]^+$, 342, 315, 286, 233, 219, 216, 205, 154,
 98 (base peak).

(Fig. 6)

Examination of fractions 8-12 (Table 2): Isolation of 3 α -
chloro-1 α , 2 α -epoxy lupane 4

Fractions 8-12 (Table 2) showed single spot on TLC
 plate were combined (75 mg) and crystallised from chloroform-
 methanol mixture. The crystallisation afforded white crystals
 of compound C m.p. 218-19 $^{\circ}$ showed positive Beilstein test for

halogen and was identified as 3 α -chloro-1 α , 2 α -epoxy lupane 4.

Analysis report	%C	%H
Found	77.86	10.78
Calculated for C ₃₀ H ₄₉ OCl	77.92	10.60

Beilstein test : Positive

TNM test : No yellow colouration

IR. : \int Nujol max 880 cm⁻¹ (epoxide ring) (Fig. 7)

¹H NMR (CDCl₃): 0.75, 0.96, 0.99, 1.05, 1.09, 1.12 (6s, 18H, 6t-CH₃), 0.76 and 0.94 (2d, 6H, -CHMe₂, J = 6.5 Hz), 2.91 (d, 1H, J = 3.8 Hz), 3.53 (dd, 1H, J = 2.3 and 3.6 Hz), 4.36 (d, 1H, J = 2.1 Hz) ppm. (Fig. 9)

Mass : m/z at 462, 460 [M]⁺ (1:3), 445 [M - Cl]⁺, 419, 417 [M - CHMe₂]⁺, 274, 259, 231, 191, 163, 123. (Fig. 8)

¹³C NMR (Fig. 11)

Examination of fractions 13-17 (Table 2): Isolation of lup-1(2)-en-3-one 5

Fraction 13-17 (Table 2) showed homogeneity on TLC plate were combined (80 mg) and crystallised from a mixture of chloroform and methanol. The crystallisation afforded fine crystals of compound D m.p. 198-99° which was identified as

lup-1(2)-en-3-one 5 by comparison with an authentic sample.

Analysis report	%C	%H
Found	84.79	11.29
Calculated for $C_{30}H_{48}O$	84.91	11.32
IR : ν_{\max} Nujol	1680 cm^{-1} (α, β -unsaturated ketone).	

(Fig. 13)

UV : λ_{\max}^{MeOH} 228 nm ($\epsilon = 18,000$)

(Fig. 14)

1H NMR ($CDCl_3$): 0.79, 0.94, 1.07, 1.08, 1.11,

1.14 (6s, 18H, 6t- CH_3)

0.77 and 0.865 (2d, 6H, $CHMe_2$, $J = 8$ Hz)

5.80 (d, 1H, $-C = \underline{CH}$, $J = 10$ Hz)

7.11 (d, 1H, $\underline{HC} = C-$, $J = 10$ Hz) ppm

(Fig. 16)

Mass: m/z at 424 [M] $^+$, 381 [$M - CHMe_2$] $^+$, 288, 287,
231, 150 (base peak), 137, 95, 69, 55.

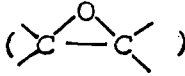
(Fig. 15)

Examination of fractions 18-22 (Table 2): Isolation of lupan-
1 α , 2 α -epoxy-3-one 6

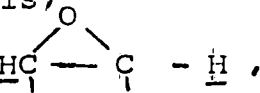
Fractions 18-22 (Table 2) were combined (100 mg) and crystallised from chloroform-methanol mixture. The crystallisation furnished white crystals of compound E m.p. 223-24 $^{\circ}$, $[\alpha]_D + 66.7^{\circ}$ which was identified as lupan-1 α , 2 α -epoxy-3-one 6 by comparison with an authentic sample (Lit²¹ m.p. 223-25 $^{\circ}$, $[\alpha]_D + 67.52^{\circ}$).

Analysis report	%C	%H
Found	81.57	11.03
Calculated for $C_{30}H_{48}O_2$	81.76	10.98

TNM test : No yellow colouration.

IR : $\int_{\text{max}}^{\text{Nujol}}$ 1705 cm^{-1} (CO) and 880 cm^{-1} 

(Fig. 17)

^1H NMR (CDCl_3): 0.76 - 1.10 ppm (8-Methyls)
3.36 and 3.57 (2d, 2H, )
J = 5 Hz) Ppm

(Fig. 19)

Mass : m/z 440 $[\text{M}]^+$, 425 $[\text{M} - \text{CH}_3]^+$, 397 $[\text{M} - \text{CHMe}_2]^+$,
328, 206, 191, 163, 149, 123 (base peak),
109, 95, 81, 55.

(Fig. 18)

Examination of fractions 24-27 (Table 2): Isolation of lupan-1 α , 2 α -epoxy-3 α -ol 7

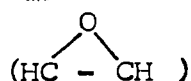
The fractions 24-27 (Table 2) showed homogeneity on TLC plate were combined (200 mg) and crystallised from CHCl_3 -MeOH mixture. The crystallisation afforded colourless crystals of compound F, m.p. 203-4 $^\circ$. It was identified as lupan-1 α , 2 α -epoxy-3 α -ol 7.

Analysis report	%C	%H
Found	81.12	11.21
Calculated for $C_{30}H_{50}O_2$	81.45	11.31

TNM test : No yellow colouration

Beilstein test : Negative

IR : $\int_{\text{max}}^{\text{Nujol}}$ 3530 cm^{-1} (OH), 880 cm^{-1}



(Fig. 20)

^1H NMR (CDCl_3): 0.75-1.17 (8-methyls)

2.31 and 3.05 (2d, 2H, $J = 3.5$ Hz)

3.53 (dd, 1H, $J = 3.5$ and 4.47 Hz)

3.64 (dd, 1H, $J = 6.04$ and 8.7 Hz) ppm

(Fig. 22)

Mass : m/z at 442 $[\text{M}]^+$, 427 $[\text{M} - \text{Me}]^+$, 424 $[\text{M} - \text{H}_2\text{O}]^+$,
409 $[\text{42} - \text{H}_2\text{O}]^+$, 406 $[\text{424} - \text{H}_2\text{O}]^+$, 399 $[\text{M} - \text{CHMe}_2]^+$,
381 $[\text{399} - \text{H}_2\text{O}]^+$, 331, 272, 231,
217, 191, 169, 163, 149, 55 (base peak).

(Fig. 21)

^{13}C NMR

(Fig. 26)

R E F E R E N C E S

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