CHAPTER IV

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 on Varian A-60 and HA-100 Spectrometers using deuterated chloroform solution containing tetramethyl silane as reference. The IR spectra were recorded in Beckman IR 20 Spectrophotometer. Silica gel used for column chromatography was of 60-120 mesh (B.D.H). TLC was done on chromatoplates prepared on glass strips with silica gel G using benzene, petroleum ether and ethylacetate as solvents and the spots were developed in an iodine chamber.

Extraction

Dried and powdered root of Gynocardia Odorata R. Br. (5 kg) was extracted with benzene in soxhlet apparatus for 36 hours. The extract was cooled to room temperature. The clear brown coloured extract was concentrated by distilling off benzene when a gummy residue (16 gm) was obtained. The residue was extracted with ether. The ether extract was shaken with 10% NaOH solution in a separatory funnel. The alkali layer was separated from the ethereal extract. The ether solution was washed with water till neutral and dried over anhydrous sodium sulphate.

Evaporation of ether furnished a gummy residue (10 gm) which constituted the neutral portion of the extract.

Chromatography of the above gummy material (10 gm)

The gummy residue (10.0 gm) was dissolved in benzene and poured on a column of silica gel (150 gm) developed with petroleum ether and eluted with the following solvents (Table 1).

Table 1

Chromatography of the above gummy material

Eluent	Fractions 100 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-4	Oil (3 gm)	-
Petroleum ether: benzene (4:1)	5-7	Solid (.2 gm)	299-300°
Petroleum ether : benzene (3:2)	8-10	Oil (2.5 gm)	-
Benzene: petroleum ether (3:2)	11-13	Solid(.5 gm)	132-33°
Benzene : Petroleum ether (4:1)	14-16	Solid(.1 gm)	126-28°
Benzene	17-19	Oil(1.5 gm)	-
Benzene : Ethyl acetate (4:1)	20-26	Solid(2.2 gm)	212-15°

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

Examination of fractions 5-7 (Table 1) : Isolation of Odolactone

The fractions 5-7 (Table 1) were combined (.2 g) and crystallised carefully from a mixture of chloroform and methanol to afford fine needle shaped crystal m.p. 304-5°. It gave negative TNM test but responded Libbermann-Burchard test. It was found identical with an authentic specimen of Odolactone[44] (m.m.p., CO-IR and CO-TLC).

Examination of fractions 11-13 (Table 1): Isolation of β-sitosterol

The fractions 11-13 (Table 1) were combined (.5 g) and crystallised from chloroform and methanol to afford crystals m.p. 136-7°. It was identified as β-sitosterol by direct comparison with authentic specimen of β-sitosterol (m.m.p., CO-IR and CO-TLC).

Examination of fractions 14-16 (Table 1) : Isolation of two new diterpene lactones Odolide (I_a) and Iso Odolide (I_b) as isomeric mixture.

The fractions 14-16 (Table 1) were combined (.1 g) and crystallised thrice from acetone to give fine plate like crystals, m.p. 131-2°, $[\alpha]_D$ -72°. The compound showed single round spot on chromatoplate. With TNM the compound showed characteristic yellow colouration. GLC showed that the fractions 14-16 (Table 1) are isomeric mixture in the ratio 80:20.

GLC : Isomeric mixture with 80:20 compositions (Fig. 12)

Analysis report:

	%C	%H
Found	79.93	9.28
Calculated for $C_{20}H_{28}O_2$	80	9.33

IR : ν_{max}^{Nujol} 1725, 1150 (-C=O, δ-lactone), 3010, 1600, 880, 810 (C = CH_2) cm^{-1}

(Fig. 9)

^1H NMR for Odolide (I_a) (δ, $CDCl_3$) : 0.904 (s, 3H, -C - CH_3),
2.078 (q, 2H, -CH_2)
3.967-4.316 (AB_q, 2H, -C(=O)-CH_2)

For Iso Odolide (I_b) : 0.904(s, 3H, -C - CH_3)
1.703-1.720 (s, 3H, -C - CH_3)
3.9-4.3 (AB_q, 2H)
5.015 (s, H, -CH = C -) ppm

(Figs. 10,11)

Mass spectra : m/z 300 (M^+ 100%), 272(67.6), 257(53.6), 224(21.2), 211(42.1), 183(20.5), 157(11.9)

(Fig. 13)

^{13}C NMR (For Odolide (I_a) : 19.77, 20.74, 21.51, 22.56, 30.98,
37.34, 39.28, 40.97, 48.23
(9t, 9 - $\underline{C}H_2$)
76.71 (t, O = C - O - $\underline{C}H_2$)
102.5 (t, -C = $\underline{C}H_2$)
33.13, 48.97/48.27,
43.9 (3s, 3-C-)
156.18(s, -$\underline{C}$ = CH_2)
175.15/174.62 (s, -$\underline{C}$(=O) - O - CH_2)
44.73, 53.05, 50.09 (3d, 3 - $\underline{C}H$)
23.79 (q, -C-$\underline{C}H_3$)

^{13}C NMR (For Iso Odolide) : 19.14, 20.79, 21.51, 22.42,
30.98, 39.48, 40.80, 41.99
(8t, 8-$\underline{C}H_2$)
76.71 (t, -$\underline{C}$(=O) - O - $\underline{C}H_2$)
33.13, 43.9, 48.27/48.97, 144.0
(4s, 4 - $\underline{C}$ -)
174.62/175-15 (s, -$\underline{C}$(=O) - O - CH_2)
45.70, 50.02, 53.05 (3d, 3 - $\underline{C}H$)
132.6 (d, C = $\underline{C}H$)
15.2, 23.79 (2q, 2-C-$\underline{C}H_3$)

(Fig. 14)

Examination of fractions 20-26 (Table 1) : Isolation of a new diterpene hydroxy Odolide (I_c)

The fractions 20-26 (Table 1) were combined (2.2g) and crystallised thrice from a mixture of ethyl acetate and methanol to afford fine needle shaped crystals, m.p. 218°, $[\alpha]_D$ - 72°. TLC of the compound in a mixture of solvents benzene and ethyl acetate (4:1) showed single round spot on TLC plate. GLC of the compound also showed homogeneity. With TNM the compound did not develop characteristic yellow colouration, indicating thereby the absence of unsaturation.

Analysis report:

	%C	%H
Found	75.40	9.37
Calculated for $C_{20}H_{30}O_3$	75.47	9.43

IR : ν_{max}^{Nujol} 3330-3240 (-OH), 1730, 1720, 1140 ($-\overset{O}{\overset{\|}{C}}=O$, δ-lactone) cm^{-1}

(Fig. 1)

Mass spectra : m/z 318(M^+), 300 ($C_{20}H_{28}O_2$), 285 ($C_{19}H_{25}O_2$), 272 ($C_{18}H_{24}O_2$), 260 ($C_{17}H_{24}O_2$), 257 ($C_{17}H_{21}O_2$)

(Fig. 4)

^{1}H NMR (δ_{CDCl_3}) : 0.87, 1.33 (2s, 6H, 2-C-CH_3),
2.09-2.13 (ABq, 2H, -CH_2)
4.01-4.12 (d, 2H, -O-CH_2) ppm
J = 14 Hz

(Fig. 2)

^{13}C NMR : 18.77, 20.54, 22.48, 23.90, 35.24, 39.10,
39.66, 40.57, 56.66 (9t, 9-CH_2)
76.51 (t, -C(=O)-O-CH_2)
32.84, 44.66, 47.65 (3s, 3-$\overset{|}{C}$-)
79.25 (s, HO - C-)
174.78 (s, -C(=O) - O - CH_2)
23.58, 24.30 (2q, 2-CH_3)
53.14, 49.19, 49.66 (3d, 3 - CH)

(Fig. 3)

Attempted preparation of 2,4 D.N.P. derivative

2,4-Dinitrophenyl hydrazine (0.3g) dissolved in rectified spirit (5 ml) and a few drops of conc. H_2SO_4 was added to the solution of compound I_c in $CHCl_3$. The reaction mixture was concentrated but no characteristic orange red crystals separated. This indicated the absence of ketonic or aldehydic carbonyl.

Attempted preparation of oxime derivative

To hydroxy odolide (I_c) dissolved in pyridine was added hydroxylamine hydrochloride and absolute alcohol and the mixture was refluxed for 3 hours. After the reaction was over the alcohol was evaporated off and diluted with cold water. The precipitated solid was collected, washed with methanol and dried under suction. The solid m.p. 212-14° crystallised from $CHCl_3$-MeOH to afford the starting material I_c, m.p. 217-18°.

Dehydration of hydroxy Odolide (I_c) by $POCl_3$/Pyridine

200 mg of hydroxy Odolide (I_c) was added to a solution of 2 ml $POCl_3$ in 4 ml pyridine and the mixture was kept on water bath for 6 hours. The reaction mixture was then diluted (very cautiously as the reaction is vigorous) with water, extracted with ether. Then washed successively with saturated solution of $NaHCO_3$, dil. HCl and finally with water till neutral. Dried with Na_2SO_4 (anhydrous). The gummy solid obtained (150 mg) after evaporation of solvent was chromatographed over silica gel (5 gm). The chromatogram was developed in petroleum ether and was eluted with the following solvents (Table 2).

Table 2

Chromatography of the above gummy solid (150 mg)

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-3	Oil (70 mg)	-
Petroleum ether : benzene (4:1)	4-5	Nil	-
Petroleum ether: benzene (3:2)	6-7	Nil	-
Benzene : Petroleum ether (3:2)	8-10	Nil	-
Benzene : Petroleum ether (4:1)	11-14	Solid (80 mg)	125-128°
Benzene	15-16	Nil	-

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

The fractions 11-14 (Table 2) were combined and the solid (75 mg) was crystallised from chloroform methanol mixture when fine crystals of constant m.p. 131-2° was obtained. TLC of the compound showed single spot on a chromatoplate. It gave yellow colouration with TNM. It was found identical with $C_{20}H_{28}O_2$ [fractions 14-16 (Table 1)] by comparison with m.m.p., CO-TLC and CO-IR.

Jone's Oxidation of hydroxy Odolide (I_c)

To a solution of hydroxy Odolide I_c (.1 gm) in pure acetone 15 ml was added Jone's reagent dropwise with shaking until a faint orange colour persist. The mixture was kept at room temperature for 1 hour, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried over anhydrous Na_2SO_4 and the ether evaporated. It gave a single spot on TLC plate. Hence it was twice crystallised from chloroform-methanol mixture to yield needle shaped crystals, m.p. 217-18°. It showed no depression in melting point when mixed with pure specimen of hydroxy odolide (I_c). Therefore Jone's oxidation product of hydroxy odolide is the starting material hydroxy odolide itself.

Reduction of hydroxy odolide (I_c) by Li-EDA.

The hydroxy odolide (0.2 gm) was dissolved in ethylene diamine and to the solution lithium metal (0.2 gm) was added in portions. The mixture was then refluxed for 2 hours under nitrogen atmosphere. Excess lithium was destroyed by adding ammonium chloride and then acidified with dilute HCl. It was extracted with ether. The ether solution was treated with dil. NaOH to separate neutral and acid part. The alkali layer on acidification with HCl gave white solid. It was filtered, washed with water and dried. The dried residue (.170 gm) was dissolved

in minimum amount of benzene and placed on a column of silica gel (2.5 gm). The chromatogram was developed with petroleum ether and was eluted with the following solvents (Table 3).

Table 3

Chromatography of the above residue (.170 gm)

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-2	Oil	-
Petroleum ether: benzene (4:1)	3-4	Nil	-
Petroleum ether: benzene (3:2)	5-6	Nil	-
Petroleum ether: benzene (2:3)	7-8	Nil	-
Petroleum ether: benzene (1:4)	9-10	Nil	-
Benzene	11-16	White solid (0.14 gm)	275-78°

Further elution with more polar solvents did not afford any material

Fractions 11-16 (Table 3) were combined (0.14 gm) and crystallised twice from chloroform and methanol to give amorphous solid m.p. 281-2°, $[\alpha]_D$ - 92° TLC of the compound gave single spot

on TLC plate. It was analysed for $C_{20}H_{32}O_3$ and was found identical with 16α-hydroxy-(-)kauran-19-oic acid (m.p. and IR data comparison).

Analysis report:	%C	%H
Found	74.88	9.95
Calculated for $C_{20}H_{32}O_3$	75.0	10.0

Esterification of hydroxy acid, $C_{20}H_{32}O_3$ [Fractions 11-16 (Table 3)]

To 100 mg of hydroxy acid [fractions 11-16 (Table 3)] dissolved in ether (50 ml) was added a solution of diazomethane in ether prepared from nitrosomethyl urea (200 mg). The mixture was kept overnight. Next day, the excess of diazomethane was destroyed with acetic acid. The ether solution was washed with water, 10% $NaHCO_3$ solution and again with water till neutral and was dried (Na_2SO_4). Evaporation of ether yielded a gummy residue (90 mg).

Chromatography of the above gummy residue (90 mg): Isolation of methyl ester of 16α-hydroxy-(-)-kauran-19-oic acid (I_d)

The above esterified gummy material (90 mg) dissolved in benzene (2 ml) was placed over a column of silica gel (1.5 gm). The chromatogram was developed with petroleum ether and was eluted with the following solvents (Table 4).

Table 4

Chromatography of the above gummy residue (90 mg)

Eluent	Fractions 50 ml each	Residue on evaporation	Melting point °C
Petroleum ether	1-2	Oil	-
Petroleum ether: benzene (4:1)	3-4	Nil	-
Petroleum ether: benzene (3:2)	5-7	White solid (60 mg)	150-53°

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

The above fractions 5-7 (Table 4) were combined (60 mg) and crystallised thrice from a mixture of chloroform and methanol, when fine needle shaped crystals m.p. 155-7° was obtained. TLC gave single spot on TLC plate.

Analysis report:

	%C	%H
Found	75.31	10.10
Calculated for $C_{21}H_{34}O_3$	75.43	10.18

IR : ν_{max}^{Nujol} 3340-3240 (-OH), 1720 ($-\overset{O}{\overset{\|}{C}}-OCH_3$) cm^{-1}

(Fig. 6)

^{1}H NMR ($\delta CDCl_3$) : 0.74, 0.896, 1.360 (3s, 9H, 3-C-CH_3)
2.23-2.30 (ABq, 2H, -CH_2)
2.61-2.64 (d, 2H, -CH_2)
3.60 (s, 3H, $-\overset{O}{\overset{\|}{C}}-O-CH_3$)ppm

(Fig. 7)

Mass spectra : m/z 334 (M^+), 316, 301, 276, 257.

(Fig. 8)

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