Chapter – VI

Experimental

Melting points are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in Varian XL-300, 400 spectrometers operating at 75 MHz in the FT model using $CDCl_3$ as solvent and TMS as internal standard (Chemical shifts in δ ppm downfield from TMS). Mass spectra were recorded in TMS-D400 at 70 eV : IR spectra in Beckmann-20 spectrophotometer and optical rotations in Jasco-180 using $CHCl_3$ as solvent. The Chromatography columns were prepared from alumina of mesh-60-20 neutralised by 4ml. of 10% AcOH per 100 g. of alumina and TLC on Chromatoplates of Silica gel G (E - merk) using petrol:benzene (1 : 9) as eluent and the spots were developed in iodine chamber.

Isolation of moretenol from *Sapium sebiferum* Roxb.

Dried and powdered trunk, bark and stem (1kg) of *Sapium sebiferum* Roxb was extracted with benzene in Soxhlet apparatus for 36 hours. 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 by Pradhan as 3,4 di-O-methyl ellagic acid. From the filtrate benzene was distilled off and a gummy residue (18g) was obtained. The gummy residue was dissolved in ether (1 litre) and the ether solution was washed with 10% NaOH solution and water till neutral. The neutral ether solution was dried over anhydrous sodium sulphate. Evaporation of ether furnished gummy residue (16 gm.) which constituted the neutral portion of the extract.

Chromatography of the neutral portion of the extract :

The gummy residue (16g) dissolved in benzene (30ml.) was placed on a column of alumina (640g deactivated with 25.5ml. of 10% aqueous acetic acid.). The Chromatogram was developed with petroleum ether and eluted successively with petroleum ether, petroleum ether: enzene (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 crystallized from a mixture of chloroform and methanol when fine crystals of moretenol separated out m.p. 228 - 30° $[\alpha]_D$ + 25°.

Separation of moretenyl acetate.

Moretenol (0.5g.) isolated was dissolved in pyridine (5ml). 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 and white solid separated out. It was washed with water, filtered through suction and dried.

The dried solid (0.5 g) 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.

Oxidation of moretenyl acetate with NBS in DMSO

Moretenyl acetate (0.5 g) dissolved in $CHCl_3$ (2ml),was mixed with DMSO (20 ml). NBS (0.5g.) was added in portions and the reaction mixture was kept in dark. After 48 hr the mixture was poured in ice cold water when a white solid separated out that was extracted with $CHCl_3$. The $CHCl_3$ layer was thoroughly washed with water and dried over (Na_2SO_4). The solvent was removed by distillation and the residue (0.5g) dissolved in benzene was poured in column of alumina (20g.).The column was then eluted with solvent of increasing polarity as in **Table 1** below :

Table I : Chromatography of oily solid (0.5gm.)

Eluent	Fractions 100 ml. each	Residue of evaporation	Melting point °C
Petroleum ether	1 - 12	White solid ~ (90 mg.)	215 - 8°
Petroleum ether : benzene (9 : 1)	13 - 14	Oil (~25mg.)	-
Petroleum ether : benzene (4 : 1)	15 - 23	White solid (~200mg.)	233 - 7°
Petroleum ether : benzene (3 : 2)	24 - 25	Nil	-
Petroleum ether : benzene (2 : 3)	26 - 36	Solid (~95mg.)	245 - 51°

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

Examination of fractions 1 - 12 (**Table I**) : Isolation of 3β- acetoxy - 30 - bromo - isohop - 22, 29 – ene **87**.

The fractions 1 - 12 (**Table 1**) were combined (90mg) and crystallized thrice from a mixture of chloroform and methanol which afforded fine needle shaped crystals (50mg.), m.p. 222 - 4°. TLC of the compound showed a single round spot. TNM test was positive and so was Beilstein test for halogen.

IR : ν^{nujol}_{max} 1720, 1245 (- 0 - CO - CH_3), 3040, 840 (>C = CH_2) $cm.^{-1}$ (***Fig. 1***)

Mass : Molecular ion peak (M^+) 548, 546, m/z, 488, 487, 486, 473, 471, 411, 407, 400, 269, 248, 205, 189 (Base peak). (***Fig. 2***)

^1H NMR ($\delta CDCl_3$) : Signals at 0.69, 0.76, 0.84, 0.94

1.05 (6s 18H, 6 - C - CH_3)

2.03 (s 3H - O - Co - CH_3)

4.1 (AB q 2H, - CH_2 - Br)

4.45 (t H H - C - O - C - CH_3)

4.9 - 5.9 (2H >C = CH_2) ppm. (***Fig. 3***)

Examination of fractions (15 - 23) **Table I**, Isolation of 3β acetoxy - 29, 29, 30 tribromo isohophane **88** .

The fractions 15 - 23 (**Table I**) were combined (200mg.) and on repeated crystallization 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 to TNM test. TLC of the compound showed single round spot on TLC plate.

IR : ν^{nujol}_{max} 1725, 1245 (-O-CO CH_3) cm^{-1} (***Fig. 4***)

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, 203, 189 (base)

(***Fig.5***)

^1H NMR ($CDCl_3$) : Signals at δ 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 (2 ABq, 4H, 2H_2C - Br) ***(Fig. 6)***

^{13}C NMR δ 15.9, 16.11, 16.17, 16.47, 21.0, 27.9 (7q, 7 - CH_3)
8.23, 20.9, 23.57, 23.66, 23.66, 26.58, 32.83
33.19, 38.36, 38.36, 40.2, 40.8 (12t 12 - CH_2)
45.8, 48.43, 50.17, 54.17, 55.17 (5d 5-C-H)
80.89 (d H - C - O - CO - CH_3)
36.99, 37.76, 41.52, 41.70, 45.36 (5s 5 - C -)
76.05 (s - C - Br)
170.99, (s - O - CO - CH_3) ppm ***(Fig. 8a& 8b)***

Examination of fractions 26 - 36 (**Table 1**) : Isolation of 3β-acetoxy-22-hydroxy-29, 30 dibromo isohopane **89** .

The fractions 26 - 36 (**Table I**) were mixed and crystallised thrice from chloroform and methanol mixture to afford white amorphous solid (95mg.) m.p. 258–9^{0}, $[\alpha]_D$ + 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.

IR : ν^{nujol}_{max} 3360 (-OH), 1730, 1250 (- 0 - $COCH_3$) cm^{-1} ***(Fig. 9)***

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, 465, 427, 425, 409, 407, 269, 267, 249, 189 (base) 137, 121, 119. ***(Fig.10)***.

^{1}H NMR : (δ $CDCl_3$)0.72, 0.84, 0.86, 0.94,

0.98 (6s 18H, 6 -C- $\underline{CH_3}$).

2.06 (s 3H -O-CO-$\underline{CH_3}$)

3.57 - 3.72 (2AB q, 4H, J = 10Hz, 2 - $C\underline{H_2}Br$)

4.45 (m H H-C-O-$COCH_3$) ppm. ***(Fig.11)***

^{13}C NMR : δ15.2, 16.00, 16.4, 16.4, 16.8, 21.0
28.0 (7q, 7 - $\underline{C}H_3$),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 - $\underline{C}H_2$),
38.4, 45.2, 50.2, 52.0, 55.2 (5d, 5 - C – H)
80.8 (d H - C - O - $COCH_3$)
37.6, 37.7, 41.6, 44.4, 44.8 (5s, 5 - C -)
74.2 (s - C - Br)
171.0 (s - C - CO - CH_3) ppm ***(Fig. 12)***

Hydrolysis of3β-acetoxy-22, 29, 30 tribromo-isohopane **88B** over active basic alumina column.

The dried mother liquor of **88** (100mg.) was dissolved in benzene (3ml.) and was poured on a column of dry basic alumina (5g). The compound was kept in the column for seven days followed by addition of 5ml. petroleum ether each day. On 8th day the column was run and eluted with the following solvents.

Table II

Chromatography of the above residue (100mg.)

Eluent	Fractions 50ml	Residue of 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 enter: 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 fractions (3-6) **(Table II)**

The fraction 3-6 **(Table II)** were combined and crystallized 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 **87** (m.m.p, Co-IR and Co-TLC).

Examination of fractions 7-11

The fractions 7-11 (**Table II**) were combined and crystallized from $CHCl_3$- MeOH mixture to give white amorphous solid , m p 258-9°. It showed positive Bielstein test for bromine but did not respond to TNM test. It was found identical with **89** (m. m. p, co-IR, co- TLC).

Examination of fractions 12-17 (**Table II**) : Isolation of 30-bromo-moretenol **90**

The fractions 12-17 (**Table II**) were combined and crystallized 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 chromato plate.

IR : ν^{nujol}_{max} 3280- 3320 (-OH), 3040 - 60 (>CH_2), 1620 Cm^{-1} ***(Fig. 13)***

Mass : Molecular ion peaks (M^+) 506, 504 (1 : 1) *m/z* 491, 489, 426, 425, 358, 269, 207 (base) 189 ***(Fig. 14).***

Examination of fractions 18 - 23 (**Table II**)

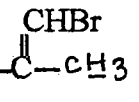
Isolation of 29 bromo - moretenol, **91**

The fractions 18 - 23 (**Table II**) were combined and on careful crystallization from $CHCl_3$ – MeOH, it afforded amorphous solid m.p. 206 - 8°. It showed green coloration 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 :

Mass : Molecular ion peaks (M^+) 506, 504 (1 : 1) *m/z* 491, 489, 426, 425, 358, 269, 267, 207, (base 189) (***Fig. 15***).

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

1.68 - 1.70 (s, 3H, )

3.0 (m H, -C- H)

3.15 (m H, -CH-OH) ***(Fig. 16).***

Extraction of diosgenin from Dioscorea tubers and conversion to corresponding diosgenin acetate.

Extraction of diosgenin from ***Dioscorea*** tubers

Matured ***Dioscorea*** tubers were washed with water and chopped. The chopped material was dried in sun and hydrolyzed with 2.5(N) HCl. To the hydrolyzed mass sodium bicarbonate was added and washed. The acid free hydrolyzed mass was then again dried in sun and then in hot air oven. The dried mass was powdered and extracted with hexane using Soxhlet apparatus. The extract was chilled overnight and filtered. The residue was collected as diosgenin.

Conversion of diosgenin to its acetate.

Diosgenin (1 gm.) was dissolved in 7.5ml. pyridine. To the solution 7.5 ml. acetic anhydride was added and the mixture was kept over water bath for five hours. The mixture was then cooled and poured over ice-cold water and white solid was separated out which was washed and filtered through suction and dried. The dried solid (0.6g.) was crystallized from chloroform - methanol mixture which afforded crystals of m.p.193 ^{0}C. It was found to be identical with an authentic sample of diosgenin acetate.

Treatment of diosgenin acetate with N-bromosuccinimide (NBS) in dimethyl sulphoxide (DMSO)

Diosgenin acetate (1 g.) dissolved in $CHCl_3$ (5ml.) was mixed with DMSO (50 ml.) and the solution was cooled to 20°C. NBS (1g.) was added in small lots so as not to allow the temperature to rise above 25°C and the solution was kept in dark for 14 days. The solution was then extracted with $CHCl_3$ and washed with water several times and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue (1 g.) was chromatographed over silica gel. Elution of the column with solvents of increasing polarity furnished four different fractions designated as **A**, **B**, **C**, **D**.

The following fractions were collected as eluents from various solvents mixture as in **Table III**.s

Table III

Eluent	Fracxtions 100 ml. each	Residue	Melting poing after crystallisation
Petroleum ether	(1 - 8) **A**	White solid	230°C
Petroleum ether : benzene (1 : 4)	(9 - 15) **B**	White solid	220°C
Petroleum ether : benzene (2 : 3)	(16 - 20) **C**	White solid	188°C
Chloroform : methanol (3 : 2)	(20 - 26) **D**	White oily non-crystallisable.	Oily

Examination of fractions (1 - 8) (**Table III**) : Isolation of 6α-bromo -5β - hydroxy diosgenin -3β - acetate **93**

The fractions (1 - 8) **A** (**Table III**) were combined and crystallized twice from $CHCl_3$ - MeOH which afforded fine needle shaped crystals (0 - 5g) of 6α-bromo-5β-hydroxy diosgenin-3β-acetate **93**, m.p. 230°C.

Analysis report :

Beilstein test : +ve

TNM test : -ve

IR : 3350 cm^{-1}, 1710 and 1240 cm^{-1} ***(Fig. 19).***

Mass : Molecular ion peak M^+ (554).

m/z 537, 525, 519, 455, 395, 377

345, 298, 281, 267, 139 (base peak)

105, 95, 69, 67 ***(Fig 20)***

^1H NMR : δ 0.81 ppm. (d 3H - sec - CH_3, J = 6.5Hz.)

0.92 (d 3H, sec - CH_3, J = 6.5Hz.)

0.72 ppm. (s, 3H, t- CH_3)

1.0 ppm (s, t-CH_3)

2.06 ppm (s, 3H , OCO CH_3)

3.00 ppm (s, OH)

4.51 ppm (d, 1H, J = 14Hz and 5Hz CH_2- $CH_{(a)}$-Br)

5.3 ppm (s , 1H, J at ½ h = 5Hz, C- $3H_{(e)}$- OAc)

(Fig.21A & Fig. 21 B)

^{13}CNMR :δ 21.5, 17.1, 14.4, 16.7, 16.3 (5q, 5 - CH_3)

66.8, 28.7, 31.3, 31.7, 40.7, 21.5

39.6, 39.0, 26.5, 31.7 (10 t, 10 - CH_2)

30.2, 41.6, 61.9, 80.5, 55.7, 43.0

36.6, 61.9, 70.3 (9d, 9 - C - H)

169.4, 109.2, 40.3, 43.2

75.0 (5s, 5 - C -) ***(Fig. 22A and 22B)***

Examination of fractions (9-15) (**Table III**) : Isolation of 7, 7, dibromo-5α- hydroxy-6 keto diosgenin - 3β acetate **94**.

The fractions (9 - 15) **B** which was obtained by elution from petroleum ether : benzene (1 : 4) were combined and crystallized thrice from $CHCl_3$ - MeOH which afforded fine crystals of 7, 7. dibromo-5α- hydroxy-6 keto- diosgenin-3β acetate **94** m.p. 220°C

Analysis report :

Beilstein test : +ve

IR: 3350 cm^{-1}(OH), 1700 cm^{-1} (C = O), 1710 cm^{-1} and 1240 cm^{-1}(-$OCOCH_3$) ***(Fig. 23)***

Mass : Molecular in peak, M^+ 630,*m/z* 615, 568, 566, 531, 488, 473, 458, 374, 341, 296, 139 (base peak), 115, 69, 55. ***(Fig. 24)***

^{1}H NMR : δ 3.35 ppm (t, eq H at C - 26)

4.41 ppm (q axial H)

3.47 ppm (m, 1H, axial H, C-16 H_a)

2.76 ppm (t, 1 H,axial H, C-8 H_a)

5.03 ppm (hep, 1H,axial H,C-3 H_a)

2.01 ppm (s, 3H, $OCOCH_3$)

2.27 ppm (s, 1 H, t C-5-OH) ***(Fig. 25A and 25B)***

Examination of fractions (16 - 20) **C (Table III)** : Isolation of diosgenin-5α-bromo-3β-hydroxy-6-one **95.**

Fractions (16 - 20) **C** (0.4g.) which was obtained by elution from benzene : ethyl acetate (2 : 3) afforded fine crystals of m.p. 188°C.

Analysis report :

Beilstein test : +ve

IR : 3400 - 3650 cm. $^{-1}$(6,OH),1705cm^{-1}(s, Six membered ring ketone)

Mass spectrum :M^+ 509, *m/z* 451, 430, 415, 413, 402, 400, 371, 359, 316, 287, 140, 139.***(Fig 26)***

^{1}H NMR : δ 0.77 (d ,3H, J= 6.5Hz, sec C<u>H</u>$_3$)
0.95 (d ,3H, J = 6.5Hz, sec C<u>H</u>$_3$)
3.76 ppm (broad hump, J = 10Hz), C-3 α <u>H</u> geminal to <u>OH</u>
2.75 ppm , <u>t</u>, 1H, C-8 <u>H$_a$</u>
4.46 ppm , <u>q</u>, 1H, -O-C-26-<u>H$_a$</u>
2.69 ppm and 2.73 ppm (dd, J=10 and 10 Hz, 10 and 3 Hz), 1H+1H, C-7 <u>H$_a$</u> <u>H$_e$</u> ***(Fig. 27)***

Examination of fractions (20 - 26) D **(Table III)**

Elution of the column with chloroform : methanol (3 : 2) furnished the most polar fraction D (20 - 26) (Table III) which could not be crystallized. Fraction D was thus acetylated by (Py - AC_2O). After work up in the usual way and crystallization from $CHCl_3$ - MeOH it afforded fine needle shaped crystals of diosgenin- 7,7-. dibromo 3β, 5α, 6α- triacetate **96** with m.p. 205 - 6°C

Analysis report :

Beilstein test : +ve

TNM Test : -ve

IR : 1710, 1230 cm^{-1}(-O-CO-CH_3)

^{1}H NMR :δ 2.06 ppm (3H OCOCH_3)

2.095 ppm (3H, OCOCH_3)

2.11 ppm (3H, OCOCH_3)

5.45 ppm (hep, C-3 α H)

3.07 ppm (1H, C-7 α H) ***(Fig. 28)***

Mass :*m /z* 674, 660, 658, 656, 632, 618, 616, 614, 596, 594, 578, 576, 556, 554, 537, 535, 527, 525, 522, 476, 454, 412 (base peak), 397, 298, 139, 115

(Fig.29)

Treatment of **Dimedone** with NBS in DMSO.

Dimedone (500 mg.) was dissolved in $CHCl_3$ and mixed with DMSO (50 c.c.), 1 gm. NBS was added in small portions and the mixture was kept in dark for 12 days. The mixture was then extracted with $CHCl_3$ and the extract was thoroughly washed with water and dried over Na_2SO_4. The solvent was removed under reduced pressure. The residue (0.5g.) was chromatographed over silica gel. The column was eluted with solvents of increasing polarity as in **Table (IV).**

Table IV

Eluent	Fraction	Residue	Melting point
benzene : Pet (20 : 80)	---	Nil	---
benzene : Pet (40 : 60)	---	Nil	---
benzene : Pet (80 : 20)	---	Nil	---
Benzene (100%)	---	Nil	---
Benzene : $CHCl_3$ (90 : 10)	---	Nil	---
Benzene : $CHCl_3$ (80 : 20)	---	Nil	---
Benzene : $CHCl_3$ (70 : 30)	(1 - 10)	White residue	---
Benzene : $CHCl_3$ (40 : 60)	---	Nil	---
Benzene : $CHCl_3$ (20 : 80)	---	Nil	---
Chloroform (100%)	---	Nil	---

Examination of fraction **J** : Isolation of 1, 1dimethyl- 4-bromo cyclohex 2,4 diene- 3, 5 hypobromite **98**

Fractions (1 - 10) J (**Table IV**) eluted from benzene : $CHCl_3$ (70 : 30) was crystallized from $CHCl_3$ - MeOH mixture to afford crystals of 1,1- dimethyl 4- bromo cyclohex- 2, 4 diene. 3, 5 hypobromite **98**

Analysis report :

Beilstein Test : +ve

TNM test : +ve

^{1}H NMR :δ1.11 ppm (6H, 2t , CH_3)

2.48 ppm (d, 2H J = 10HZ - CH_2)

5.99 ppm (1 H, isolated olefinic H) ***(Fig. 30)***

^{13}C NMR : δ 28.03 ppm (1q , two-CH_3 attached at C - 1)

32.0 ppm (1s, C - 6)

101 ppm (1 s. C - 4 olefinic carbon containing bromine) ***(Fig31)***

Mass :M^+ 380, *m/z* 299, 218, 255, 203, 164 (base peak) 122, 83, 56, 54 ***(Fig. 32)***

UV: 284 nm ***(Fig. 33)***

Action of N-bromosuccinimide in dimethyl sulphoxide on Benzoyl acetone.

A solution of benzoyl acetone (2 g.) in chloform (5ml.) was mixed with dimethyl sulphoxide (20 ml.). N- bromosuccinimide (5g.) was added to the solution in small lots and kept in dark for 30 days. The reaction mixture was extracted with ether and washed several times with water. The extract was dried over Na_2SO_4 and chromatographed over silica gel. Different fractions were obtained as in **Table V.**

Table V

Eluent	Fraction	Residue	m.p.
Petrol	(1 - 9) K	White solid	120°
benzene : MeOH 9:1	(10 - 15) L	White solid	119 - 120°

Examination of fraction (1 - 9) (**Table V**) : Isolation of tribromo benzoyl ethylene **100**

The fractions (1 - 9) **K** (**Table V**) that was eluted with 100% Petrol was recrystallised from $CHCl_3$ - MeOH which afforded fine crystals of m.p. 120°C.

Analysis report :

Beilstein test : +ve

TNM test : +ve

IR : 1690 cm^{-1}

^1H NMR :δ 8.4 ppm (2 dd, 2H, ortho protons of benzene ring)

7.62 ppm (m, 1H, Para protons of benzene ring)

7.48 ppm (2dd , 2H, meta protons of benzene ring) ***(Fig35)***

Mass :M^+ 367, *m/z* 262, 234, 217, 188,

173, 172, 159, 146, 145, 122,

106, 105 (base peak), 91, 77, 69, 65. ***(Fig. 36)***

Examination of fraction (10 - 15) **L** (**Table V**) : Isolation of benzoic acid.

The fraction (10 - 15) L (**Table V**) was benzoic acid as evident by its melting point and I.R. spectra.