CHAPTER-IV

EXPERIMENTAL

Melting points are uncorrected. Petroleum ether used had b.p. 60-80°. All optical rotations were determined in chloroform unless stated otherwise. NMR spectra were taken in Varian-60 spectrometers. IR spectra recorded were taken in Perkin-Elmer spectrometer 337. UV spectra were observed in Zeiss VSU-1 spectrophotometer.

Extraction

Dried and powdered trunk bark and stem (1.0 kg.) of Sapium sebiferum Roxb., was extracted with benzene in Soxhlet apparatus for 36 hrs. During the extraction a yellow insoluble solid separated out. The extract was cooled to room temperature and the yellow insoluble solid was collected on a Buchner funnel, the solid was washed with benzene, dried and kept as benzene insoluble portion C (0.6 g.), m.p. 280-300°. From the clear filtrate, benzene was distilled off and a gummy residue (18.0 g.) was obtained. The gummy residue was dissolved in ether (1 litre) and the ether solution was washed with 10% aqueous NaOH solution (3 x 300 ml.) and water till neutral. The neutral ether solution was dried (Na_2SO_4) and the ether evaporated. The gummy residue (16.0 g.) was kept as neutral portion B.

The aqueous alkaline layer was extracted with ether to remove any neutral material that might be present. This was then acidified with cold and dilute 10% HCl (1 litre) when some insoluble solids separated out. The acidified solid solution was then extracted with ether and the ethereal solution was washed with water till neutral and then dried (Na_2SO_4). Ether was removed when a gummy residue (1.0 g.) was obtained. To the latter dissolved in ether (200 ml.) was added a solution of diazomethane in ether prepared from

nitrosomethyl urea (0.7 g.). 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 (0.5 g.) - acid portion A.

Chromatography of the above gummy material (0.5 g.) : Isolation of methyl sebiferate 19b.

The above esterified gummy material (0.5 g.) dissolved in benzene (5 ml.) was placed over a column of alumina (30.0 g., deactivated with 1.2 ml. of 10% aqueous acetic acid). The chromatogram was developed with petroleum ether and was eluted with following solvents (Table I).

TABLE I

Chromatography of the above gummy material (0.5 g.)

Eluent	Fractions, 50 ml. each	Residue on evaporation
Petroleum ether (200 ml.)	1-4	Semi solid, (0.4 g.) (m.p. 80-120°) on digestion with methanol
Further elution with more polar solvents did not yield any material		

Fractions 1-4 (Table I) were combined (0.4 g.) and crystallised twice from methanol and the melting point, 100-120° could not be improved by further crystallisation. It was further purified by the following method:

The above methyl ester was hydrolysed with 10% methanolic KOH solution (20.0 ml.) by refluxing for four hours. Methanol was removed from the reaction mixture and the residue was diluted with cold water and extracted thrice with

ether to remove any neutral material that might be present. The aqueous alkaline layer which contained the sparingly soluble solids was acidified with cold 10% HCl when white flocculent solids separated out. The solids were extracted with ether and the ether layer was washed several times with water till neutral and dried (Na_2SO_4). Evaporation of ether gave solid residue (0.35 g.). The latter was esterified with diazomethane as usual and the gummy ester (0.35 g.) obtained thereby was dissolved in benzene (3 ml.) and placed over a column of alumina (20 g., deactivated with 0.8 ml. of 10% aqueous acetic acid). The chromatogram was developed in petroleum ether and was eluted with the following solvents (Table II).

TABLE II

Chromatography of the above residue (0.35 g.)

Eluent	Fractions, 50 ml. each	Residue on evaporation
Petroleum ether (150 ml.)	1-3	Semi solid, (0.32 g.) formed solid (m.p. 130-134°) with methanol
Further elution with more polar solvents did not yield any material		

The above fractions 1-3 (Table II) were combined and crystallised from a mixture of chloroform and methanol, when a crystalline solid m.p. 134-6°, $[\alpha]_D$ +24.4° was obtained. The melting point could not be raised by further crystallisations from methanol.

Found	:	C,81.58; H,11.31%.
$C_{31}H_{50}O_2$ requires	:	C,81.93; H,11.01%.
IR(KBr disc)	:	ν_{max} 1737 ($-COOCH_3$), 1640, 895, 875 ($C=CH_2$) cm^{-1}.

NMR (60 Mc)	:	6.33 ($-COOCH_3$, 3H, s); 5.14, 5.30 ($2=CH_2$, 4H, m); 8.28, 8.32 ($2=C-CH_3$, 6H, s); 8.57 ($-CH_2COOCH_3$, 2H, s), and 8.97, 9.03, 9.17, 9.30 (4 tert.-CH_3 groups, 12H, s) τ (Fig.1).
UV (in 95% ethanol)	:	No absorption in the region 220-300 mμ.

Hydrolysis of methyl sebiferate 19b : Isolation of Sebiferic acid 19a

Methyl sebiferate 19b (0.15 g.) was refluxed with 10% methanolic KOH solution (10 ml.) for 4 hrs. The solution was cooled, acidified with cold 10% HCl (20 ml.) and extracted with ether. The ethereal layer was washed with water till neutral, dried (Na_2SO_4), and ether distilled off. The residue was crystallised from methanol when an amorphous solid crystallised out, m.p. 178-80°, $[\alpha]_D$ +28.57°.

Found	:	C,81.45; H,10.62%.
Calculated for $C_{30}H_{48}O_2$	:	C,81.75; H,11.00%.
IR (KBr disc)	:	3070, 1707 (-COOH), 1640, 895, 875 ($=CH_2$) cm^{-1}.
UV (in 95% ethanol)	:	No absorption in the region 220-300 mμ.

Preparation of tetrahydromethyl sebiferate 22

Methyl sebiferate 19b (0.2 g.) was hydrogenated in ethanol at room temperature and atmospheric pressure in presence of palladium-on-charcoal catalyst (0.05 g.). Two molar equivalents of hydrogen were consumed during one hour. The hydrogenated product was filtered and on concentration of the filtrate, fine needle shaped crystals m.p. 110° separated out. The crystals were recrystallised for analytical sample, m.p. 118-19°, $[\alpha]_D$ + 7.5°

Found : C,80.98; H,11.47%.

Calculated for $C_{31}H_{54}O_2$: C,81.22; H,11.79%.

IR (KBr disc) : ν_{max} 1737 ($-COOCH_3$) cm^{-1}.

NMR (60 Mc) : 6.33 ($-COOC\underline{H}_3$, 3H, s); 8.57 ($-C\underline{H}_2-CO-$, 2H, s); 9.07-9.35 τ(8 $-C\underline{H}_3$, 24H) (Fig.2).

Estimation of double bonds : Perbenzoic acid titration of methyl sebiferate

Methyl sebiferate (0.9 g.) was taken in a 25 ml. volumetric flask and dissolved in chloroform (10 ml.). A solution of perbenzoic acid in chloroform (5 ml.) was pipetted out and added to the solution and the volume made up to 25 ml. by adding chloroform. A blank solution of perbenzoic acid (5 ml.) was similarly prepared in a 25 ml. volumetric flask and the volume made up to 25 ml. with chloroform. 5 ml. aliquot portions were titrated from each of the above solutions against standard sodium thiosulphate solution.

Strength of sodium thiosulphate solution = 0.093 $\frac{N}{10}$.

Results

Time interval	Blank	Thio.req. ml. (a)	Reaction mixture	Thio.req. ml. (b)	Diff. ml. (a-b)	No.of double bonds
30 min.	Blank(5 ml.) + 2% KI(10 ml.) + AcOH (2 ml.) + starch	15.5	Aliquot(5 ml.) + 2% KI(10 ml.) + AcOH(2 ml.) + starch	9.8	5.7	1.34
1 hr.	"	15.5	"	8.5	7.0	1.64
2 hrs.	"	15.5	"	7.9	7.6	1.79
3 hrs.	"	15.5	"	7.0	8.5	2.00
4 hrs.	"	15.5	"	7.0	8.5	2.00
12 hrs.	"	15.5	"	7.0	8.5	2.00
24 hrs.	"	15.5	"	7.0	8.5	2.00

It was found that two moles of perbenzoic acid was consumed within three hours showing thereby that methyl sebiferate 19b contained two active double bonds.

Base Titration

Sebiferic acid (0.116 g.) was taken in a 50 ml. volumetric flask and made up the volume to 50 ml. with methanol. A standard $\frac{N}{100}$ methanolic sodium hydroxide solution was prepared. 20 ml. of the sebiferic acid solution was titrated with the standard sodium hydroxide solution, using phenolphthalein as the indicator.

Strength of sodium hydroxide solution = 0.002N.

20 ml. of the acid solution was equivalent to 5 ml. of the alkali solution.

Calculation showed that the acid was mono basic and the neutral equivalent was 434.

Preparation of moretenone oxime 26

To moretenone 25 (1.0 g.) dissolved in pyridine (10 ml.) was added hydroxylamine hydrochloride (0.6 g.) and absolute alcohol (10 ml.) and the mixture was refluxed for three 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 (0.95 g.), m.p. 247° was crystallised twice from chloroform to afford moretenone oxime 26, m.p. 274-6°.

Found	:	C,81.62; H,10.86%.
Calculated for $C_{30}H_{49}NO$	:	C,81.90; H,11.20%.

Beckmann Rearrangement of moretenone oxime 26 : Preparation of the nitrile 27 and the lactam 28

To moretenone oxime 26 (1.0 g.) dissolved in pyridine (40 ml.) was added p-toluene sulphonyl chloride (1.0 g.) and the reaction mixture kept at room temperature for 16 hours. A few drops of water was added and the mixture was set aside for a further period of 30 minutes. After addition of dil.HCl (1:1) the product was isolated with ether. A part of the solid remained in the aqueous layer. The ethereal part was washed with water till neutral, and dried (Na_2SO_4). Removal of ether gave a solid residue (0.2 g.) which was chromatographed over alumina (12 g., deactivated with 0.4 ml. of 10% aqueous AcOH). The chromatogram was developed with petroleum ether.

TABLE IV

Chromatography of the above ether soluble part (0.2 g.)

Eluent	Fractions, 50 ml. each	Residue obtained on evaporation
Petroleum ether	1	Oil
" "	2-4	Solid with methanol, m.p. 142°

Further elution with more polar solvents did not give any material

The fractions 2-4 (Table IV) were combined and the solid crystallised twice from methanol to afford fine needle shaped crystals of 3-cyano-3,4-seco-hopa-4(23),28-diene, m.p. 141-42°, $[\alpha]_D$ +34.66°.

Found : C,85.05; H,10.91%.

Calculated for $C_{30}H_{47}N$: C,85.40; H,11.20%.

IR (KBr disc) : ν_{max} 2240(C≡N), 1640, 895, 875(=CH_2) cm^{-1}.

The aqueous layer containing some ether insoluble solid was extracted with chloroform. The chloroform extract was washed with water, dried (Na_2SO_4) and the solvent removed. The residue (0.5 g.) was then dissolved in benzene (2 ml.) and chromatographed over alumina (36 g., deactivated with 1.3 ml. of 10% aqueous AcOH). The chromatogram was developed in petroleum ether and eluted with the following solvents (Table V).

TABLE V

Chromatography of the above chloroform soluble part (0.5 g.)

Eluent	Fractions, 50 ml. each	Residue on evaporation
Petroleum ether	1-3	Nil
Petroleum ether:benzene (9:1)	4-6	Nil
Petroleum ether:benzene (4:1)	7-9	Nil
Petroleum ether:benzene (7:3)	10-15	Solid with yellow colour, m.p. 282-3°

Further elution with more polar solvents did not elute any material

The fractions 10-15 (Table V) were combined and decolourised by boiling with charcoal in methanol for five minutes and then filtered. The clear filtrate on concentration afforded the crystalline lactam 28 (0.4 g.), m.p. 283-4°. The m.p. rose to 288-9° on further crystallisation from methanol, $[\alpha]_D$ +39.28°.

Found : C,81.56; H,11.00%.

Calculated for $C_{30}H_{49}NO$: C,81.90; H,11.20%.

IR (KBr disc) : ν_{max} 3220 (NH), 1667 (-CONH-), 1640, 875 ($=CH_2$) cm^{-1}.

Hydrolysis of 3-cyano-3,4-seco-hopa-4(23),28-diene 27 : Preparation of sebiferic acid 19a

The above nitrile 27 (0.1 g.) was heated under reflux with 10% methanolic KOH (20 ml.) for four hours. The reaction mixture was cooled and diluted with water, acidified with cold 10% HCl and extracted with ether. The ether solution washed with water, dried (Na_2SO_4). Removal of ether yielded a solid, m.p. 176°. The latter on crystallisation twice from methanol afforded an amorphous solid, m.p. 179-80°, $[\alpha]_D$ +27.12°. It showed no depression in m.p. when mixed with sebiferic acid 19a. Its IR was superimposable over that of sebiferic acid.

Found : C,81.34; H,10.56%.

Calculated for $C_{30}H_{48}O_2$: C,81,75; H,11.00%.

Esterification of the above acid : Preparation of methyl sebiferate 19b

To the above acid (0.1 g.) dissolved in ether (10 ml.) was added a solution of diazomethane in ether in cold and kept for 2 hours. Excess diazomethane was destroyed with acetic acid and the solution was washed with water, sodium biocarbonate solution and then with water till neutral. The ether solution dried (Na_2SO_4) and evaporated. Crystallisation of the residue from methanol afforded flaky crystals, m.p. 135-6°, $[\alpha]_D$ +24.40°. This ester showed no depression in melting point when mixed with methyl sebiferate 19b. The IR spectra was identical with the methyl sebiferate isolated from the plant.

Found : C,81.63; H,10.81%.

Calculated for $C_{31}H_{50}O_2$: C,81.93; H,11.01%.

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