CHAPTER - IV

Section - A

Extraction of neutral and acid parts of Caseria graveolens.

Dried and powdered bark (5 kgs) of caseria graveolens was extracted with benzene in Soxhlet apparatus for 36 hours. The filtrate was cooled to room temperature and then benzene was distilled off. The gummy residue (35 gms) obtained was dissolved in ether (≃1.00 litre). The ether solution was washed with 10% aqueous NaOH solution (3 x 700 ml) and then with water till neutral. The neutral ether was dried over anhydrous sodium sulphate and it was evaporated to yield a gummy residue (11 gm) which constituted the neutral part of the extract (Part A).

The alkali washed portion on acidification with dilute hydrochloric acid (≃ 1 N) yielded a solid, which was extracted with ether. The ethereal solution containing the acid part was washed with water till neutral and dried. The ether solution was then esterified with diazomethane. The crude methyl ester (7.5 gm) obtained after evaporation of ether constituted the acid part (Part B) of the extract.

Section - B

Isolation and identification of the compounds from the neutral part.

The neutral part (Part A) of the extract was dissolved in minimum volume of benzene and placed on a column of alumina (700 gm deactivated with 30 ml of 10% aqueous acetic acid). The chromatogram was developed with solvents as shown in Table -50.

Table - 50

Chromatography of neutral part

Serial No.	Solvent	Fractions 100 ml each	Residue	Melting point
1.	Petroleum ether	1-5	Oil	-
2.	Pet. ether-benzene (4:1)	6-12	Waxy solid	63-67°
3.	Pet. ether-benzene (3:2)	13-17	Oil	-
4.	Pet. ether-benzene (2:3)	18-23	Solid	120-24°
5.	Pet. ether-benzene (1:4)	23-27	Nil	-

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

Examination of fraction 6-12 (Table - 50) : Isolation of 1 hexacosanol.

Fractions 6-12 (Table - 50) were individually compared in a single t.l.c. plate, which showed a prominent spot with the same Rf value. These fractions were combined and crystallised several times from acetone which afforded flaky crystals of constant m.p. 78-79°, $[\alpha]_D \pm 0^\circ$. On the basis of spectral data and elemental analysis the compund was identified as 1-hexacosanol.

Examination of fractions 18-23 (Table - 50) : Isolation of β-sitosterol.

Each of the fractions No. 18-23 (Table - 50) was compared in a t.l.c. plate when all the fractions were found to contain a prominent spot with the same Rf value (0.40 in benzene as solvent). These fractions were combined and crystallised several times with chloroform-methanol mixture when fine needle shaped crystals of molecular formula $C_{29}H_{50}O$, m.p. 135-37°, $[\alpha]_D$ - 34° was obtained. The acetyl derivative of the alcohol was found to be identical with an authentic sample of β-sitosterol acetate (m.m.p., Co-IR, t.l.c.).

Isolation and identification of the compounds from acid part (Part B)

The acid part after esterification was dissolved in minimum volume of benzene and was chromatographed over neutral alumina (450gm).

Table - 51

Chromatography of the esterified product

Serial No.	Eluent	Fraction 100 ml each	Residue	Melting Point
1.	Petroleum ether	1-4	Oil	-
2.	Pet. ether-benze (4:1)	5-8	Oil	-
3.	Pet. ether-benzene (3:2)	9-15	Oil	-
4.	Pet. ether benzene (2:3)	16-25	Solid (450 mg)	-
5.	Pet. ether-benzene (1:4)	26-30	Nil	-

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

Examination of fraction 16-25 (Table - 51): Isolation of methyl betulinate 252a:

Fractions 16-25 (Table - 51) were found to contain the same compound from t.l.c. experiment. The fractions were, therefore, combined. The solid compound was crystallised from chloroform-methanol, which afforded crystalli- solid m.p. 219-20°, $[\alpha]_D^+ 5^0$; infrared spectrum showed the presence of free hydroxy group at 3520 cm^{-1} ester gro-

at 1710 cm^{-1}. The presence of an exocyclic methylene group was evident from the presence of peaks at 3030, 1640 and 880 cm^{-1}. Elemental analysis showed the molecular formula to be $C_{31}H_{50}O_3$. On acetylation of the above compound with acetic anhydride and pyridine an acetate of m.p. 200-1°, $[\alpha]_D$ 1.5° was formed. The acetate was found to be identical with an authentic sample of acetyl methyl betulinate 252b by comparison (t1.c., m.m.p. and Co-IR). Thus the acid fraction of the plant contained betulinic acid.

Section - C

Experimental:

Melting points are uncorrected. Petroleum ether used had b.p. 60-80°. Optical rotations were determined in chloroform. IR spectra were recorded in Beckman IR-20 Spectrophotometer.

Extraction of the plant has been described earlier (page 276 of this chapter).

Isolation and identification of 1-hexacosanol.

Fractions 6-12 (Table -30) were individually compared in a single t.l.c. plate, which showed a prominent spot with the same R_f value. These fractions were combined and crystallised several times from acetone, which afforded flaky crystals, m.p. 78-79°, $[\alpha]_D \pm 0°$. Elemental analysis, found C 81.48, H 13.96%; Calculated C 81.69, H 14.13%. IR : ν_{max}^{nujol} 3350 cm^{-1}, UV : no absorption above 220 nm.

Acetylation of 1-hexacosanol.

Acetylation of the compound (Ac_2O - Py, 1:1) in the usual manner and purification of the compound by crystallisation from methanol afforded crystals of m.p. 68-69°, $[\alpha]_D \pm 0°$. It was found to be identical in t.l.c., m.p. and i.r. with an authentic sample of 1-hexacosanol acetate. Elemental analysis, found C 78.96, H 13.56%, calculated for $C_{28}H_{56}O_2$ C 79.12, H 13.28%.

Isolation and identification of β -sitosterol.

Fractions (18-23) (Table - 50) were combined and on repeated crystallisation from chloroform-methanol mixture yielded silky solid, m.p. 135-36°, $[\alpha]_D$ -36°. Elemental analysis, found C 83.68, H 11.88%; calculated for $C_{29}H_{50}O$, C 83.98, H 12.15%. Mixed m.p. with authentic sample showed no depression.

The compound (0.40 gm) was acetylated with Py - Ac_2O (1:1) in the usual way. The acetate on crystallisation had m.p. 124-26°, $[\alpha]_D$ -34°. Mixed m.p. with authentic sample showed no depression. Co-IR with an authentic sample was in complete agreement. Elemental analysis, found C 81.18, H 11.22%; calculated for $C_{31}H_{52}O_2$, C 81.52, H 11.48% .

Isolation and identification of methyl betulinate 252a

Solids obtained from fractions 16-25 (Table - 51) were combined and on repeated crystallisation from a mixture of chloroform and methanol afforded shining, colourless, needle shaped crystals of methyl betulinate 252b, m.p. 220-22°, $[\alpha]_D$ +1.4° . Elemental analysis, found C 78.88, H 10.80%; calculated for $C_{31}H_{50}O_3$, C 79.40, H 11.71%. UV : no absorption in the region 220-300 nm. IR : ν_{max}^{nujol} 3520, 3030, 1710, 1640, 880 cm^{-1}. Co-IR with an authentic sample of methyl betulinate was in

complete agreement.

Acetylation of methyl betulinate 252a: Isolation of acetyl methyl betulinate 252b.

Methyl betulinate 252a (100 mg) was acetylated with pyridine (1 ml) and acetic anhydride (1 ml) in usual manner. The solid obtained was purified by crystallisation from chloroform-methanol to afford fine crystals of m.p. 200-2°. Found C 77.37, H 10.13%; calculated for $C_{33}H_{52}O_4$ C 77.34, H 10.15% .