Chapter II

Isolation of Betulic acid as acidic constituent and epi-friedelanol acetate, friedelin and β-sitosterol as neutral constituents from benzene extract of the bark of Bischofia javanica Blume.

Section A : Extraction

Dried and powdered bark of Bischofia javanica was extracted with benzene in a Soxhlet apparatus. Distillation of the solvent gave a gummy residue, which was taken up in ether. The ether solution was treated with aqueous sodium hydroxide solution and the alkali layer separated. The ether solution was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether furnished a gummy residue.

Section B : Chromatography of the neutral part :

(Table I)

The above gummy neutral part was chromatographed over alumina and the following fractions were collected.

Fractions no.	Eluent	Eluate	M.p. of the residue
1	Petroleum ether	Solid with oil	280-285°
2	Petroleum ether: benzene (4:1)	Solid	252-4°
3	" (3:2)	Nil	[illegible]°
4	" (2:3)	Solid	128-33°

Section C : Examination of Fraction No. 1 and isolation of epi-friedelanol acetate 1

Fraction 1 (Table I) on rechromatography over active alumina followed by several crystallization from chloroform and methanol mixture furnished crystals of 1, m.p. 289-92°, $(\alpha)_D$ + 40°. Elemental analysis and mass spectrum established the molecular formula of the compound as $C_{32}H_{54}O_2$ (M^+ 470). It gave no coloration with tetra nitromethane indicating that it was a saturated compound. I.R. spectrum showed absorption peaks at 1736 and 1239 cm^{-1} (acetate). The acetate on alkaline hydrolysis and subsequent chromatography of the reaction product afforded a crystalline solid 2, m.p. 277-9°, $(\alpha)_D$+9°. Elemental analysis corresponded to the molecular formula $C_{30}H_{52}O$. In I.R. spectrum it showed absorption peak at 3420 cm^{-1} (OH). UV spectrum of the compound did not show any absorption in the region 215 to 300 mμ. The above physical and chemical data of the acetate 1 and alcohol 2 closely corresponded to that of epi-friedelanol acetate and epi-friedelanol respectively[4-6]. The acetate 1 was found to be identical with an authentic sample of epi-friedelanol acetate by m.m.p. determination and I.R. comparison.

Section D : Examination of the Fraction No. 2 and isolation of Friedelin 3

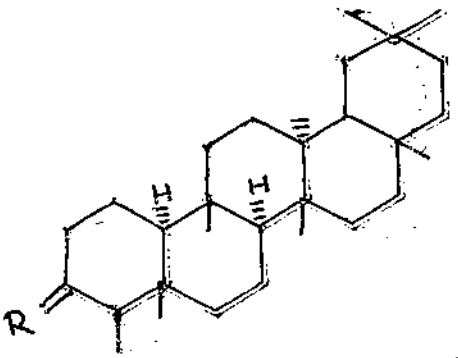
Fraction 2 (Table 1) on rechromatography over alumina followe by several crystallization from a mixture of chloroform and methano furnished fine needle shaped crystals m.p. 256-8°, $(\alpha)_D$ - 32°.

Elemental analysis and mass spectrometric determination showed the molecular formula of the compound to be $C_{30}H_{50}O$ (M^+ 426). It developed no coloration with tetranitromethane indicating that it was a saturated compound. It gave a violet coloration in Libermann Burchardt reaction and a positive Liebermann colour test indicating that the compound is a triterpene ketone, the keto group being at the customary C-3 position[7].

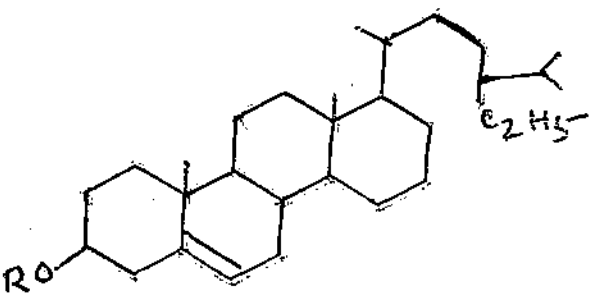
The compound 3 gave an oxime $C_{30}H_{51}NO$, m.p. 294-6°, showing that oxygen atom was present as a carbonyl group. The I.R. spectrum of the compound showed a peak at 1705 cm^{-1} (six membered ring ketone). The compound exhibited an UV absorption at λmax 255 mμ (ϵ=71). The above chemical and physical data closely corresponded to that of friedelin and the compound 3 was found to be identical with an authentic sample of friedelin by m.m.p. determination and I.R. comparison.

Section E : Examination of fraction No. 4 : Isolation and identification of β-sitosterol 4

Fraction 4 (Table I) on crystallisation from chloroform and methanol mixture had m.p. 136-7°, $(\alpha)_D$ - 32°. Elemental analysis showed the molecular formula as $C_{29}H_{50}O$. On treatment with acetic anhydride and pyridine it afforded an acetate 5, $C_{31}H_{52}O_2$, m.p. 126-7°, $(\alpha)_D$ - 37°. The acetate was identified as β-sitosterol acetate by direct comparison with an authentic specimen of β-sitosterol acetate. Hence the parent alcohol was identified as β-sitosterol.



1 , R = H(OAcα)
2 ; R = H(OHα)
3 , R = O



4 , R = H
5 , R = Ac

Section F : Examination of Acid fraction : Isolation and identification of betulinic acid 6

The alkali washed portion of the benzene extract, on acidification with dilute hydrochloric acid yielded a solid which was extracted with ether. The ethereal solution containing the acid fraction was esterified with diazomethane. The crude methyl ester obtained after evaporation of ether was chromatographed over deactivated alumina to afford a solid 7, which after crystallisation from $CHCl_3$-MeOH mixture had m.p. 222-4°, $(\alpha)_D$ + 5°. Elemental analysis showed the molecular formula to be $C_{31}H_{50}O_3$. It did not show any absorption in UV spectrum in the region 220-300 mμ, I.R. spectrum showed peaks at 3520 (-OH), 1735 ($-COOCH_3$), 1660 and 876 cm^{-1} ($=CH_2$). NMR spectrum of the compound showed signals:

(a) Olefinic proton signals for = $C\underline{H}_2$; two doublets in the region 4.8-4.9 ppm.

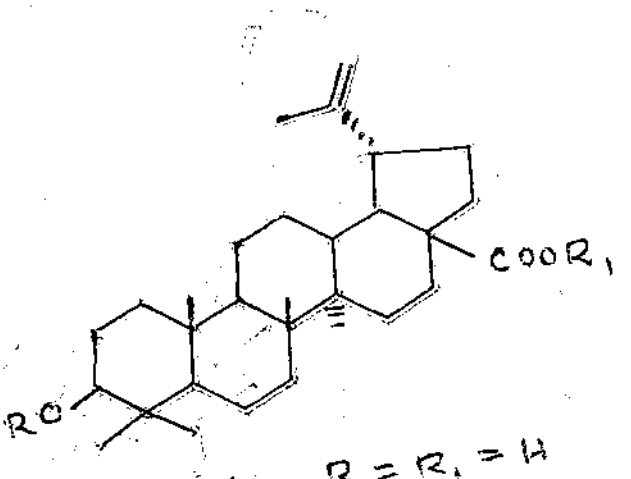
(b) An intense signal at 3.75 ppm for -COOC$\underline{H}_3$ group.

(c) A singlet for -C$\underline{H}$OH at 2.01 ppm.

(d) A sharp peak for -CH_3 occurring as -CH=C(-$\underline{CH}_3$)-C at 1.75 ppm.

(e) A tall singlet corresponding to 5-CH_3 group at 1.00 ppm.

The physical constants of the compound 7 are very close to that of methyl betulinate[8] and was found to be identical with an authentic sample of methyl betulinate by m.m.p. determination and I.R. comparison. The characterization was further confirmed by the preparation of its acetyl derivative when acetyl-methyl betulinate 8, $C_{33}H_{52}O_4$, m.p. 200-1°, $(\alpha)_D$ + 4° was obtained. It was identical with an authentic sample of acetyl methyl betulinate. Hydrolysis of the methyl betulinate 7 with potassium tertiary butoxide in dimethyl sulfoxide gave betulic acid 6, $C_{30}H_{48}O_3$ m.p. 299-302° identical with an authentic sample (IR comparison).



6, R = R, = H

7, R = H, R, = CH3

8, R = AC, R, = CH3