Chapter III

Investigation of Macaranga Denticulata, Muell Arg.

Isolation of taraxerone, β-sitosterol and a new triterpene, 3-epitaraxerol from the stem-bark of M. Denticulata.

Section A : Extraction

Dried and powdered stem bark ~~and~~ of M. Denticulata was extracted with benzene. The benzene solution was concentrated by distilling off benzene when a gummy residue was obtained. The residue was extracted with ether, washed with aqueous NaOH solution and then with water till neutral. The ether solution was dried over sodium sulphate and ether evaporated when a gummy residue was obtained. The gummy residue, thus obtained was chromatographed and the following fractions were isolated.

Section B : Chromatography of the neutral part

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

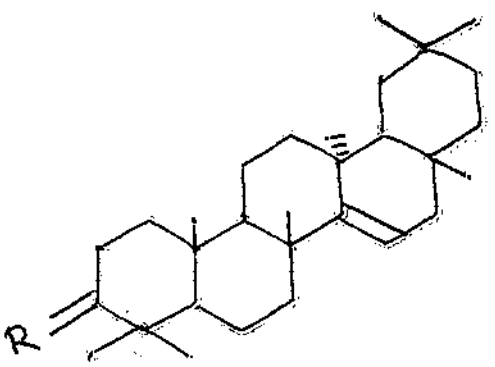
Fraction No.	Eluent	Eluate	M.p. of the residue on evaporation
1	Petroleum ether	Solid with oil	230-35°
2	Petroleum ether: benzene (4:1)	Solid	249-55°
3	Petroleum ether: benzene (3:2)	Solid	128-32°

Section C : Examination of fraction No. 1 and isolation of taraxerone 16

Fraction No. 1 on rechromatography over alumina and several crystallization from chloroform-methanol mixture furnished crystals having constant m.p., 237-39°, $(\alpha)_D$ + 9°. Elemental analysis coupled with mass spectrometric determination of molecular weight established the molecular formula of the compound to be $C_{30}H_{48}O$ (M^+ 424). It developed a yellow colour with tetranitro methane indicating unsaturation in the compound. It gave a violet coloration in Liebermann-Burchard reaction and gave a positive test in Zimmermann colour reaction showing the compound is a triterpene ketone, the keto group being at C-3 position. The I.R. spectrum of the compound showed absorption peaks at 1705 (6 membered ring ketone) and 822 cm^{-1} (trisubstituted double bond, $c = c\langle_H$). The compound showed UV absorption at λ_{max}^{EtOH} 282 mμ (ϵ 64) showing that the keto group and the double bond were unconjugated.

On LAH reduction it gave an alcohol 16a, $C_{30}H_{50}O$ m.p. 278-80°, $(\alpha)_D$ + 4.4°, which on acetylation furnished an acetate 16b, $C_{32}H_{52}O_2$, m.p. 294-6°, $(\alpha)_D$ + 9.16°. The physical constants of the ketone 16, the derived alcohol 16a and its acetate 16b are very close to the reported[28] physical constants of triterpene ketone taraxerone, m.p. 240°, $(\alpha)_D$ + 12°, taraxerol, m.p. 282-3°, $(\alpha)_D$ + 0° and taraxeryl acetate, m.p. 304-5°, $(\alpha)_D$ + 9° respectively.

The identity of the isolated ketone with taraxerone 16 was confirmed by m.m.p. determination and I.R. comparison with an authentic sample of taraxerone.



16, R = O

16a, R = H (OHβ)

16b, R = H (OAcβ)

Section D : Examination of fraction No. 2 and isolation of the new triterpene - 3 epi-taraxerol

Fraction No. 2 on rechromatography over alumina and several crystallization from a mixture of chloroform and methanol furnished needle shaped crystals 17 having a constant melting point, 261-2°, $(\alpha)_D$ - 22.6°. Elemental analysis coupled with mass spectroscopic determination established the molecular formula to be $C_{30}H_{50}O$ (M^+ 426). I.R. spectrum of the compound (Fig. 33) showed absorption peaks at 3420 (hydroxyl) and 825 cm^{-1} (trisubstituted double bond, $C = C\langle_H$). The compound was transparent in the UV region 220 to 300 mμ. The NMR spectrum besides 8 methyl groups in the region δ0.85 to δ1.05 , showed peaks at δ5.38 (1H, multiplet) attributed to vinylic proton. A signal located at δ3.45 (width at half wave, $W_{1/2}$ 7Hz) showed the proton at C-3 to be equatorial.

On acetylation with acetic anhydride and pyridine the compound afforded an acetate 17a, m.p., 161-2°, $(\alpha)_D$ - 41°. Elemental analysis coupled with mass spectrometric determination established its molecular formula to be $C_{32}H_{52}O_2$ (M^+ 468). IR spectrum (Fig.34) showed absorption peaks at 1730 and 1240 (acetate), and 830 ($C=C\langle_H$) $cm.^{-1}$. The NMR spectrum besides signals for 8 methyl groups at saturated carbon in the region 0.82 to 1.08 δ, showed peaks at 2.07δ (3H, singlet, -O.CO.$C\underline{H}_3$), 4.64 δ (1H, multiplet, proton of the carbon, bearing the acetoxy group, $\underline{H}$-C-O.$COCH_3$) and 5.3 δ (C = $C\langle_{\underline{H}}$). The downfield shift of the C-3 proton is consistent with its equatorial orientation and hence the axial orientation of C-3 acetoxy group. The similarity of the NMR spectra of the alcohol and its derived acetate 17a to that of taraxerol and its acetate suggested that the alcohol 17 might be 3-epimer of taraxerol.

Chromium trioxide-pyridine oxidation on the alcohol gave a ketone $C_{30}H_{48}O$, m.p. 238-40°, $(\alpha)_D$ + 10° which was found to be identical with taraxerone 16, by m.m.p. and comparison of IR spectra with an authentic sample. Since the 3β-alcohol 16a, taraxerol, prepared by LAH reduction of taraxerone was found to be different in every respect from the alcohol 17, the hydroxyl group at C-3 must be α-oriented. Thus the new triterpene was established as 3-epi-taraxerol 17.

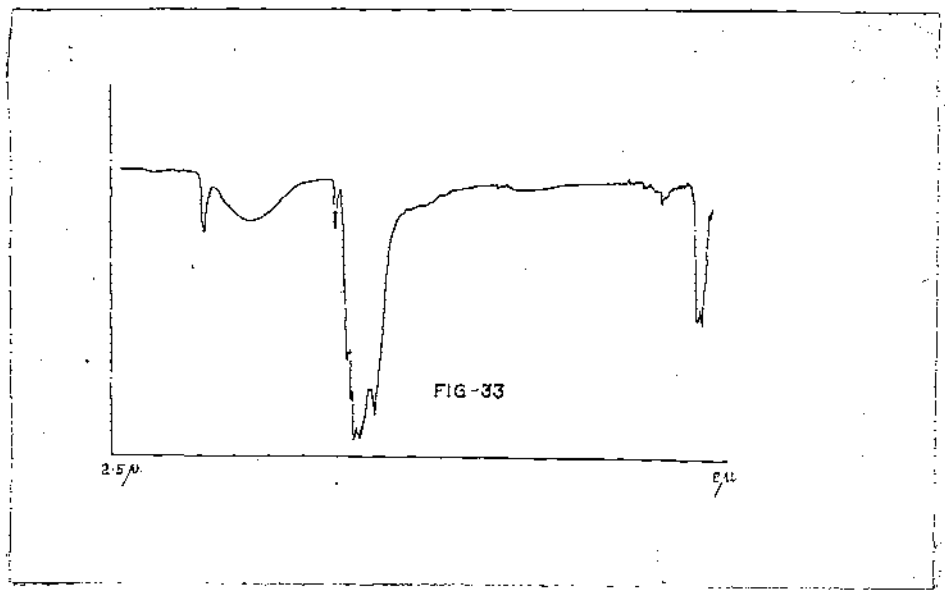


FIG-33

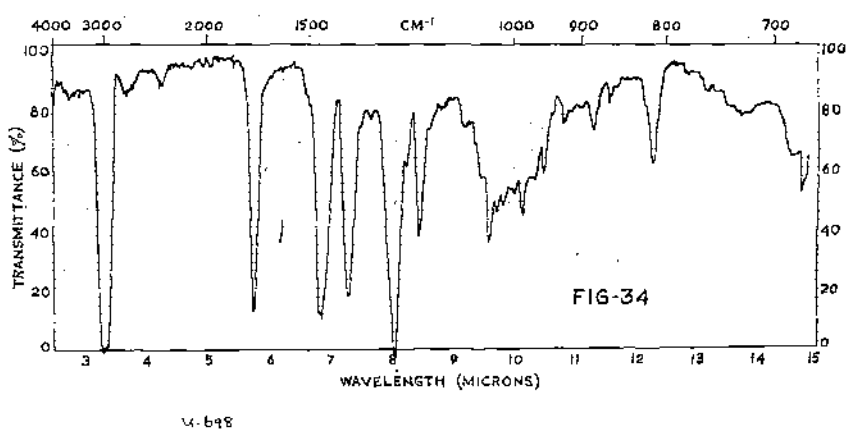
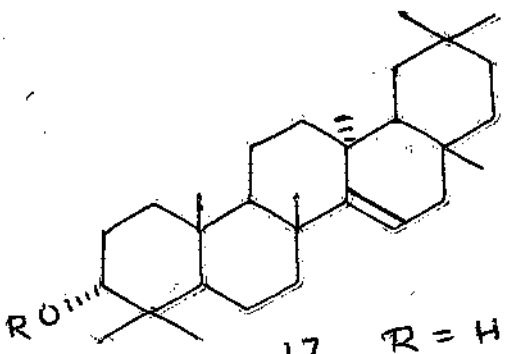


FIG-34

M-698



17 , R = H

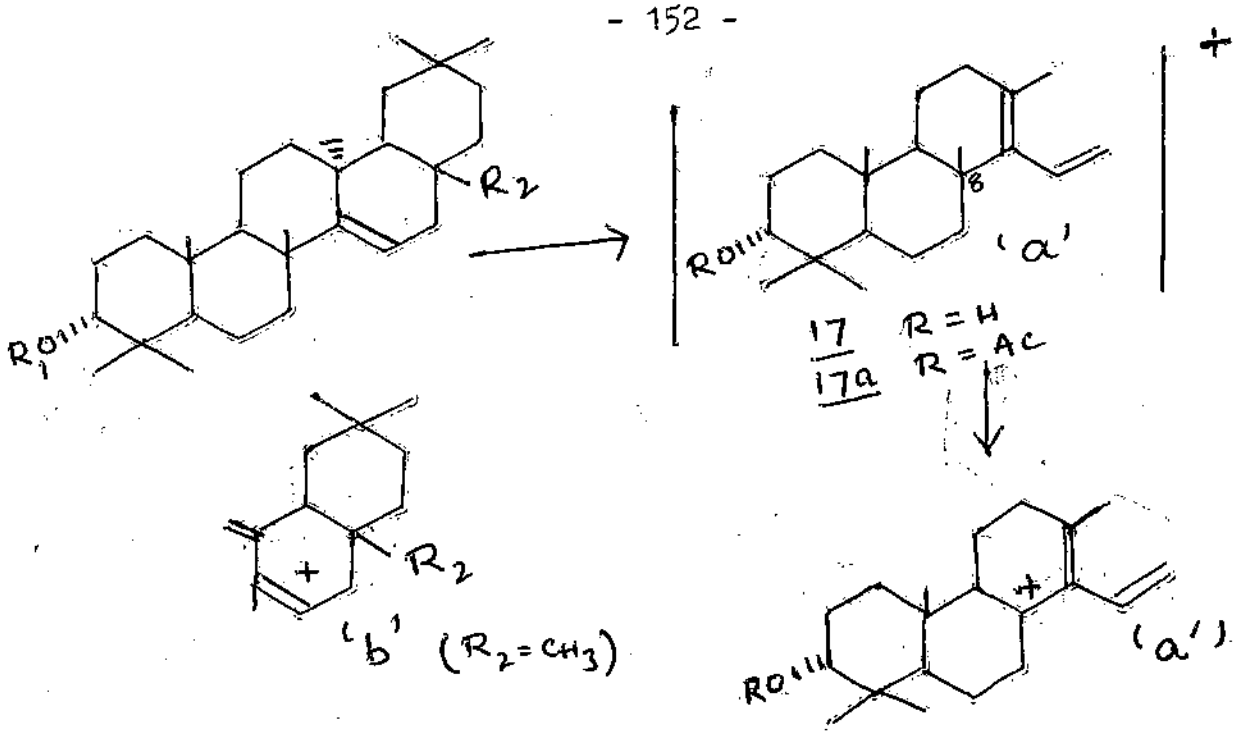
17a , R = Ac

Application of Mass spectrometry

Djerassi and his coworkers[29] measured the mass spectrometric fragmentations of taraxerol and taraxeryl acetate. The mass spectrometric fragmentations of epi-taraxerol 17 and its acetate 17a are in complete agreement with that of taraxerol and its acetate. This clearly demonstrated that carbon skeleton of both the alcohols must be same.

In these molecules a similar retro-Diels-Alder decomposition was observed as was in the case of Δ^{12}-unsaturated derivative, except that the collapse of ring D rather than ring C occurred. The resulting fragment 'a' exhibits a mass of m/e 302 for 17 and 344 for 17a depending on the C_3-substituent 'a'. Ion 'a' is accompanied by a satellite peak 15 mass unit lower, which is formed by the loss of methyl group, probably the allylically activated one at C-8(α').

The spectrum of 17 exhibited additional peaks at 284, 269 m/e due to the loss of H_2O and (H_2O + CH_3) respectively from the species 'a', while that of 17a showed ('a'-CH_3COOH) and ('a'-CH_3COOH) ion peaks at 284 and 269 m/e.



In addition to species 'a' and its further decomposition products, the spectra of 17 and 17a showed a very abundant fragment at m/e 204 ('b'). This arises out of the cleavage of ring C and comprises ring D and E. Furthermore, the fragment 'b' losses the substituent at C-17 giving rise to a fragment 'b'-CH_3 in both cases. Nothing specifically could be said regarding the formation of the species 'b', as no experimental proof was available.

The structure 17 was further confirmed by its partial synthesis from taraxerone 16 by Meerwein-Pondorf reduction according to the method of Paton et al.[30] when 3-epi-taraxerol and taraxerol were produced and separated by chromatography.

Takeda and his coworkers[10,11] carried out reduction of taraxerone with sodium and iso-amyl alcohol and reported the isolation of an alcohol m.p. 267-69°, $(\alpha)_D$ + 11.9°, acetate m.p. 205-7°, $(\alpha)_D$ - 21.8°. The alcohol, thus obtained was thought to be the 3'-

epimer of taraxerol and was named iso-taraxerol. But the physical constants recorded by them are not in agreement with those of our compound 3 epi-taraxerol and its acetate. To clarify this sodium and iso amyl alcohol reduction of taraxerone was repeated. Two products were obtained and were separated by chromatography. The less polar material eluted in petroleum:benzene (4:1) was found to be identical with epi-taraxerol, isolated from the plant as well as with the one synthesised from taraxerone by Paton's method, in all respects. The more polar material, eluted in petroleum:benzene (3:2) was found to be identical with taraxerol. This is also in conformity with the chromatographic behaviour of the equatorial and axial isomers of triterpene 3-alcohols.

Section E:

Examination of fraction 3 : Isolation and identification of β-sitosterol

Fraction 3 on crystallization 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, $C_{31}H_{52}O_2$, m.p. 127-8°, $(\alpha)_D$ -40°. 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.