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γ-SITOSTEROL FROM THE LEAVES OF *AEGLE MARMELOS* CORREA

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The sterol from the leaves of *Aegle marmelos* Correa (N.O. Rutaceae) has been identified as γ-sitosterol by preparation of the acetate and the benzoate.

In connection with our work on the conversion of naturally occurring steroid bodies into the steroid hormones (Barua, Mrs. Chakravarti and Chakravarti, *Bull. Nat. Inst. Sci. India*, No. 4, p. 15, 1955 ; Chakravarti and Mitra, *Nature*, 1957, **179**, 1188) we were interested in finding out the structure of aegelin, $C_{18}H_{16}O_4$, isolated from the leaves of *Aegle marmelos* (Family *Rutaceae*) and described as a sterol by Chatterjee and Bose (this *Journal*, 1952, **29**, 425). After a critical study of the various derivatives and degradation products of aegelin (Chakravarti and Dasgupta, *Chem. & Ind.*, 1955, 1632) it was found to be a neutral nitrogenous product having the molecular formula, $C_{18}H_{19}O_3N$, and structure (I).

(I) p-MeO.C_6H_4.CH(OH).CH_2 NH.CO.CH : CH.C_6H_5.

As aegelin was isolated by extraction of the dry leaves with ether and filtration of the separated product, which is not the usual method for isolation of the sterols, it has been thought desirable to find out the real sterols of the leaves of this plant by the procedure usually adopted for the purpose (Chakravarti *et al.*, this *Journal*, 1952, **29**, 374 ; *J. Sci. Ind. Res.*, 1956, **15C**, 86, 89) which consists in saponification of the petroleum ether extract of the plant material, followed by steam-distillation to remove volatile matters. The non-volatile, non-saponifiable fraction, obtained by extraction of the liquor left after steam-distillation with a suitable solvent (ether). contains the sterols, if any, of the plant material. From this fraction the pure sterols may be isolated by repeated crystallisation of the corresponding acetates and benzoates, and/or chromatography over aluminium oxide.

The method used by Chatterjee and Bose (*loc. cit.*) in this respect is quite an arbitrary one, as any product, which is insoluble in dilute hydrochloric acid and sparingly soluble in ether, may be obtained under these conditions.

The sterol isolated from the leaves of *Aegle marmelos* by the procedure described above and purified by chromatography over aluminium oxide, has been identified as γ-sitosterol by the preparation of the acetate and the benzoate and comparing their percentage compositions, melting points and rotations with those for known sitosterols. Unlike aegelin, the free sterol isolated develops a purple to blue, and then green colour in the Liebermann-Burchard reaction of the sterols. A distinct bluish shade in the colour is a characteristic feature of this test. The yellow coloration recorded by Chatterjee and Bose (*loc. cit.*) in the Liebermann-Burchard test of aegelin is really a negative response, indicating that aegelin is not a sterol.

EXPERIMENTAL

Isolation of Sterol Fraction.—Air-dried powdered leaves (2.5 kg.) of *Aegle marmelos* were exhaustively extracted with a large excess of 90% alcohol at the room temperature in a glass percolator till the colour of the extract was light green (about 5-6 drainings necessary, covering 10 to 12 days). On distillation of the solvent, the alcohol-free extract was obtained as a thick liquid with a dark green colour. It was intimately mixed up with sufficient filter paper pulp, and dried at 80°.

In one experiment, the dried mass was extracted exhaustively with petroleum ether (b.p. 60-80°) in a Soxhlet extractor for about 30-35 hours. The oily product obtained after evaporation of the petroleum ether was hydrolysed by refluxing on a water-bath for 10 to 12 hours with a large excess of 15-20% aqueous alcoholic potassium hydroxide.

In a second experiment, the dried mass was extracted with ether in an all-glass Soxhlet extractor for about 80 hours. The ether extract was then concentrated to about 500 c.c. and kept in the ice-chest for about a week. The separated crystalline product, aegelin, was removed by filtration and the ethereal extract was distilled to remove the ether completely. The residual viscous liquid (about 100 g.) was then hydrolysed by refluxing with 20% aqueous alcoholic solution of KOH (120 g.) on a water-bath for 10 to 12 hours. After hydrolysis, the product was diluted with a little water and the alcohol was evaporated off on a water-bath with addition of a corresponding amount of water from time to time. The alcohol-free alkaline solution was extracted several times with ether. The total ethereal extract after washing with water was distilled, and the brown semi-solid residue was distilled in steam to remove volatile essential oils. The residue was again refluxed with 15-20% aqueous alcoholic KOH for 10 to 12 hours to hydrolyse any traces of unchanged fatty oil. The non-saponifiable portion after removal of alcohol was extracted with ether. The extract was washed with water, dried by $CaCl_2$ and the solvent distilled when the crude sterol fraction was obtained. It was completely dried in a vacuum desiccator.

Purification of the Sterol.—The crude sterol fraction was purified by chromatography of the product over Brockmann alumina using petroleum ether (b.p. 40-60°), petroleum ether-benzene mixture (1 : 1), benzene and ether successively as eluents. The petroleum ether-benzene fraction was found to contain the sterol in a crystalline form. The product was crystallised several times from alcohol when it was obtained as colorless shining plates, m.p. 138-39°, yield 1.3 g. (0.05%).

Sterol Acetate.—The sterol (0.5 g.) was heated under reflux with 2.5 c.c. of acetic anhydride at 140° for 2 hours in an oil-bath. The reaction product was cooled and filtered from the separated plates which were dried over solid caustic potash in a vacuum desiccator. The *sterol acetate* crystallised from alcohol in shining plates, m.p. 140-41°, $[\alpha]_D^{16}$ − 47° ($CHCl_3$). [Found: C, 80.97; H, 10.93. Calc. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48%].

Hydrolysis of the Sterol Acetate.—Sterol acetate (0.1 g.) was hydrolysed with 5 c.c. of alcohol, 1 g. of KOH and a few drops of water by heating on a water-bath under reflux for 6 hours. After hydrolysis alcohol was removed, the residue diluted

with water and extracted with ether. On removal of the ether the *sterol* crystallised from alcohol in shining plates, m.p. 144-45°, $[\alpha]_D^{18}$ − 40° ($CHCl_3$). [Found: C, 83.30; H, 11.9. Calc. for $C_{20}H_{50}O$: C, 83.99; H, 12.15%]. With the Liebermann-Burchard reagent the sterol developed a purple, then blue to green colour.

Sterol Benzoate.—Benzoyl chloride (0.25 c.c.) was added to a solution of the sterol (0.1 g.) in dry pyridine (2 c.c.), cooled in ice. The mixture was kept overnight at room temperature, decomposed with crushed ice, acidified with HCl (conc.) to Congo red and extracted with ether. The ethereal extract was washed successively with dilute acid, sodium bicarbonate solution, water and then dried over a piece of fused calcium chloride. On removal of ether the *sterol benzoate* crystallised from alcohol in plates, m.p. 150-51°, $[\alpha]_D^{18}$ − 17° ($CHCl_3$). [Found: C, 83.69; H, 10.19. Calc. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49%].

Identification of the Sterol.—From a comparison of the analyses, melting points and rotations of the sterol, the acetate and the benzoate, with those for known sterols, the sterol was identified as γ-sitosterol. As pointed out by Fieser and Fieser ("Natural Products Related to Phenanthrene", Reinhold Pub. Corp., New York), determination of the mixed melting point with authentic specimens is not much helpful for identifying the sterols, particularly in the case of sitosterols.

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