

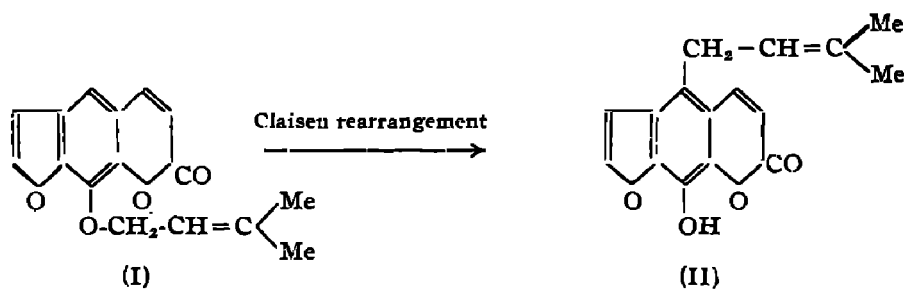
# ISOLATION OF ALLO-IMPERATORIN AND $\beta$ -SITOSTEROL FROM THE FRUITS OF *AEGLE MARMELOS* CORREA

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The fruits of *Aegle marmelos* Correa contain allo-imperatorin and  $\beta$ -sitosterol, besides imperatorin, a  $\gamma\gamma$ -dimethylallyloxyfurocoumarin.

The fruit of *Aegle marmelos* Correa (N. O. *Rutaceae*), commonly known as *Bael*, possesses distinct medicinal values. It has been the subject of investigation by several workers (Dikshit and Dutt, this *Journal*, 1939, 7, 579; 1932, 9, 271) and the latest researches on it have revealed the presence of a furocoumarin, identified as imperatorin (I) (Späth, Bose, Guha and Grüber, *Ber.*, 1939, 70, 1021).

The present authors have observed that the fruit pulp of *A. marmelos* contains allo-imperatorin [5-( $\gamma\gamma$ -dimethylallyl)-8-hydroxyfurocoumarin], (II), besides imperatorin (I) and  $\beta$ -sitosterol. A benzene solution of a concentrated alcoholic extract of the fruit pulp of *A. marmelos* yielded allo-imperatorin ( $C_{16}H_{14}O_4$ ) in 0.003% yield. From its mother-liquor by chromatographic resolution (Chatterjee and Choudhury, *Naturwiss.*, 1955, 42, 335) imperatorin ( $C_{18}H_{14}O_4$ ) and  $\beta$ -sitosterol ( $C_{28}H_{48}O$ ) were obtained in 0.006% and 0.00125% yield respectively.



Allo-imperatorin dissolves in alcoholic caustic potash with a blue-violet fluorescence and a yellow solution which remains clear on dilution with water and reprecipitates the original compound unchanged on acidification with mineral acids. This furocoumarin produces a green coloration with alcoholic ferric chloride. With diazomethane it forms a monomethyl ether,  $C_{16}H_{13}O_3(OCH_3)$ . Identity of this furocoumarin with allo-imperatorin has been established from their mixed m.p. determination and mixed chromatogram (an authentic sample of allo-imperatorin being prepared from imperatorin by Claisen rearrangement), and also by the negative depression in mixed m.p. of their monomethyl ether.

$\beta$ -Sitosterol has been characterised from the studies of its elementary analyses, characteristic colour reactions with sterol reagents, mixed melting point determination and mixed chromatogram with an authentic sample of  $\beta$ -sitosterol.

### EXPERIMENTAL

*Isolation of Allo-imperatorin.*—The fruit pulp of *A. marmelos* (4.0 kg.) was crushed in a Waring blender in alcohol medium and kept for a month. It was then freed from alcohol under reduced pressure and soxhletted with benzene. The benzene layer after washing successively with 2*N*-HCl (4 × 50 c.c.) and water (2 × 50 c.c.), was dried over anhydrous sodium sulphate, concentrated to 50 c.c. and kept in a refrigerator for one week. A solid, m.p. 220–24° (decomp.), separated (A). Upon repeated crystallisation from 95% ethanol, the m.p. 226–27° (decomp.) of (A) did not change any more. It is fairly soluble in methanol, ethanol, benzene, but insoluble in ether and water. Mixed m.p. with an authentic sample of allo-imperatorin showed no depression. (Found: C, 71.35; H, 5.46. Calc. for  $C_{16}H_{14}O_4$ : C, 71.11; H, 5.18%).

*Methylation of Allo-imperatorin.*—Allo-imperatorin (0.05 g.), dissolved in dry methanol (5 c.c.), was treated with an excess of diazomethane at 0° and kept overnight. The solvent was removed and the residual solid crystallised from a mixture of benzene and petroleum ether (b. p. 40°–60°) in colorless crystals, m. p. 113°. (Found: -OMe, 10.98.  $C_{16}H_{13}O_3$ .OMe requires OMe, 10.90%).

*Conversion of Imperatorin into Allo-imperatorin.*—Imperatorin (0.1 g.) was taken in a distillation bulb and heated for 1 hour at 200–205°/3 mm. It was then sublimed at 200–10°/0.4 mm. The sublimate (0.095 g.) crystallised from 95% ethanol in plates, m. p. 226–27° (decomp.). Upon recrystallisation from the same solvent the m.p. did not change. (Found: C, 71.15; H, 5.58. Calc. for  $C_{16}H_{14}O_4$ : C, 71.11; H, 5.18%).

*Isolation of Imperatorin and  $\beta$ -Sitosterol.*—The mother-liquor left after the separation of allo-imperatorin (*vide supra*) was chromatographed over a column of Brockmann alumina (12 cm × 1 cm).

Elution with benzene (collected in fractions of 25 c.c. each) yielded from the first four fractions 0.25 g. of a material, which on three crystallisations from a mixture of petroleum ether (b.p. 40°–60°) and benzene (1:4) furnished colorless and fine needle-shaped crystals with a constant m.p. 101° (B). Mixed m.p. with an authentic sample of imperatorin did not show any depression. (Found: C, 71.05; H, 5.31. Calc. for  $C_{16}H_{14}O_4$ : C, 71.11; H, 5.18%).

On further elution with the same solvent, the fifth and the sixth fractions yielded 0.05 g. of a material which on repeated crystallisations from dry methanol furnished colorless flakes with constant m.p. 136–37° (C). The product, which was fairly soluble in benzene, methanol and ethanol and readily soluble in ether and ethyl acetate, did not depress the m.p. of an authentic sample of  $\beta$ -sitosterol. (Found: C, 84.02; H, 12.09. Calc. for  $C_{27}H_{46}O$ : C, 83.93; H, 12.15%).

*Mixed Chromatogram of the Solid (A) and an authentic sample of Allo-imperatorin.*—The solid A (0.02 g.), mixed with an equal quantity of an authentic sample

of allo-imperatorin, was taken in a minimum quantity of benzene and chromatographed over a column of alumina (9 cm  $\times$  0.5 cm.). Elution with benzene yielded from the first two fractions 0.035 g. of a material which on crystallisation from ethanol furnished pure allo-imperatorin with a constant m.p. 226-27°.

*Mixed Chromatogram of the Solid (C) and an authentic sample of  $\beta$ -Sitosterol.*—The solid C (0.03 g.) with an equal quantity of an authentic sample of  $\beta$ -sitosterol was taken in minimum quantity of benzene and chromatographed over a column of Brockmann alumina (12 cm  $\times$  0.5 cm). Elution with benzene yielded from the first three fractions 0.05 g. of a material, which on repeated crystallisation from dry methanol furnished colorless flakes with constant m.p. 137°.

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