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SHORT COMMUNICATION

THE OCCURRENCE OF ERGOSTEROL IN CITRUS

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Abstract—A $\Delta^{5,7,22}$ sterol has been isolated from the peel of the citrus Rangpur lime and identified as ergosterol.

DURING the course of investigation of the carotenoid pigments of the citrus Rangpur lime, the unsaponifiable fraction (in hexane) of the acetone extractives of the peel was allowed to stand in the cold at *ca.* −15°. A TLC of the precipitated material showed a prominent component that corresponded with authentic ergosterol. Subsequently, extraction of a larger sample of peels of Rangpur lime yielded crystalline ergosterol identified by its u.v. and i.r. spectra, and the properties of its crystalline acetate and benzoate.

In earlier reports[2] citrostadienol, β-sitosterol, stigmasterol, campesterol, cycloeucalenol, 24-methylene lophenol, cycloartenol, 24-methylene cycloartenol, and friedelin had been isolated from citrus peel. The $\Delta^{5,7,22}$ sterol, ergosterol, had not previously been detected in citrus.

Rangpur lime is considered as being more closely related to the mandarin orange (*Citrus reticulata*) than to the lime (*C. aurantifolia*). It is evidently to be considered either as a hybrid of the lime with the mandarin orange or as a sour mandarin orange.[3]

EXPERIMENTAL[4]

In the initial study the unsaponifiable fraction (in hexane) from the extraction of a sample of peels of Rangpur lime[5] was left overnight in the cold at *ca.* −15°. The precipitated material was examined by TLC on kieselguhr G plates.[6] The plates were sprayed with a 20 per cent ethanolic solution of phosphomolybdic acid and then heated about 5–10 min at 90° and revealed a spot corresponding to authentic ergosterol.

A larger sample of 2 kg of freeze-dried peels of Rangpur lime was extracted with acetone to yield an oily residue. This was saponified with 1 l. of ethanolic KOH (5 per cent) on the steam bath. The solution was

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[2] A. WEIZMANN, A. MEISELS and Y. MAZUR, *J. Org. Chem.* **20**, 1173 (1955); A. WEIZMANN and Y. MAZUR, *J. Org. Chem.* **23**, 832 (1958); Y. MAZUR, A. WEIZMANN and F. SONDHEIMER, *J. Am. Chem. Soc.* **80**, 6293 (1958); B. L. WILLIAMS, L. J. GOAD and T. W. GOODWIN, *Phytochem.* **6**, 1137 (1967).

[3] W. T. SWINGLE, In *The Citrus Industry* (edited by H. J. WEBBER and L. D. BATCHELOR). Vol. 1, p. 626, University of California Press, Berkeley and Los Angeles (1943).

[4] All m.p. determinations were carried out in capillary tubes on an Electrothermal melting point apparatus and are uncorrected. Ultra-violet spectra were measured with a Cary Model 14 spectrophotometer. Infrared spectra were run on a Perkin-Elmer Model 521 spectrophotometer. Analyses were provided by L. M. White.

[5] H. YOKOYAMA and M. J. WHITE, *Phytochem.* **5**, 1159 (1966).

[6] J. W. C. PEEREBOOM and W. H. BEEKES, *J. Chromatogr.* **9**, 316 (1962).

concentrated at reduced pressure to about 200 ml, equal volume of 20 per cent NaCl solution added, and the unsaponifiable part extracted with ether. The ether extract was washed free of alkali, dried ($MgSO_4$), and evaporated. The dark, unsaponifiable residue was dissolved in hot methanol and left in the cold overnight. The precipitated material obtained was dissolved in light petroleum and chromatographed on a column of neutral alumina (Woelm) with light petroleum-benzene mixtures starting with 4:1, and proceeding through benzene to a final eluant of benzene-ether 1:1. Intermediate fractions yielded ergosterol, λ_{max}^{EtOH} 271 ($\epsilon \times 10^{-3}$ 11·5), 282 ($\epsilon \times 10^{-3}$ 12·0), and 293 nm ($\epsilon \times 10^{-3}$ 6·9), $[\alpha]_D$ $-134°$. The i.r. spectrum of the isolated sterol (in CCl_4 and KBr) was identical with that of an authentic sample; the two samples had the same chromatographic movement on a thin layer of kieselguhr G.

Reaction of the isolated sterol with acetic anhydride in pyridine afforded the acetate derivative, m.p. 179–181° (m.p. of authentic sample 179–181°). (Found: C, 83·8; H, 9·48. Calc. for $C_{30}H_{46}O_2$: C, 83·95; H, 9·66 per cent) $[\alpha]_D -90°$. The i.r. spectrum of the acetate derivative of the isolated sterol was superimposable on that of the authentic sample. The benzoate derivative was prepared in the usual manner, m.p. 168–171° (m.p. of authentic sample 169–171°); $[\alpha]_D -88°$.

The fruit used in these experiments were collected in mid-March, 1965. Examination of the fruit of late 1967 crop also revealed the presence of ergosterol in the peels.

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