

NATURAL COUMARINS ISOLATED FROM THE LEAVES OF *CITRUS ACIDA*, ROXB.

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Three natural coumarins, namely isopimpinellin, bergapten and citropten have been isolated from the leaves of *Citrus acida* var. *medica*. This is for the first time that isopimpinellin has been isolated from a *Citrus* species.

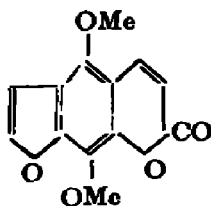
Citrus acida, Roxb., the common *Citrus* species of India, is a variety of *Citrus medica* (*Iconum Botanicarum, Index Londinensis*, 1930, 2, 224, 255). It is highly valued in therapeutics for its antiscorbutic properties and for curing various intestinal complaints and ailments. The leaves yield a carminative oil which possesses a sweet aroma.

The isolation and the identification of three crystalline constituents have been described in the present communication. The method of purification consists of twenty times fractional sublimations of the crude material in high vacuum, followed by fractional crystallisations from suitable solvents and finally by hand-picking. All the three constituents are neutral towards litmus.

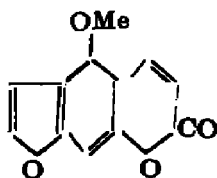
One of the substances melts at 149-50° (yield 0.0095%) and is crystallised from alcohol and ethyl acetate in pale yellow, shining needles. It is readily soluble in ether, fairly in alcohol, ethyl acetate and benzene and insoluble in petroleum ether and water. An alcoholic solution of the substance does not produce any coloration with ferric chloride. It gives an orange-red colour with cold, concentrated sulphuric acid. The substance is insoluble in 1% aqueous alkali but dissolves in alcoholic potassium hydroxide from which the original substance can be precipitated on acidification with mineral acids.

The analytical data of the compound are found to agree with the formula $C_{11}H_4O_3(OCH_3)_2$. The properties of the substance, as also the observed analytical values, suggest its identity with those of isopimpinellin (I) which has been isolated so far from three different sources, namely, *Pimpinella saxifraga* (Späth and Veerhapper, *Ber.*, 1937, 70, 248), *Luranga scandens* (Späth, Bose, Schmid, Dobrovolsky and Mookerjee, *Ber.*, 1940, 73, 1361), *Ferula alluene* (Bose and Chaudhuri, *Ann. Biochem. Expt. Med.*, 1946, 6, 1). A direct comparison has been made between the two substances, an authentic specimen of isopimpinellin was kindly supplied by late Prof. E. Späth of Vienna. The properties of the two compounds are identical and a mixture of the two melted at 150°.

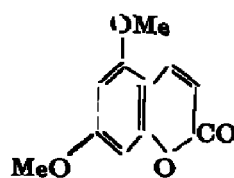
Thus the identity of the first neutral compound with isopimpinellin has been established. No alkaloid could be traced in the leaves.



(I) isoPimpinellin.



(II) Bergapten.



(III) Citropten.

The second constituent forms slender, silky needles, m.p. 187°-88° (yield 0.006%). It develops an orange-red colour with concentrated sulphuric acid. From the properties and behaviour the substance appears to be a coumarin.

The molecular formula of the substance, on analysis, is found to be $C_{12}H_8O_4$. It contains one methoxy group as tested by the method of Zeisel-Pregl-Vieböck (*Ber.*, 1930, 63, 2818, 3207). The molecular formula and the properties indicate its identity with bergapten (II) which could be established by direct comparison with a specimen of bergapten isolated from the leaves of *Skimia lauroleola* (Späth, *Ber.*, 1937, 60, 114).

The third neutral crystalline principle of *C. acida* crystallises from alcohol in heavy, colourless rods, m.p. 150° (yield 0.0175%).

The C—H values show that the compound has the formula $C_{11}H_{10}O_4$. It contains two methoxy groups. The properties of the substance resemble those of citropten (III), 5:7-dimethoxycoumarin with which the molecular formula of the third substance is the same. A direct comparison of the third constituent could, however, be made with an authentic sample of citropten which was obtained from Dr. P. K. Bose to whom the author's best thanks are due.

EXPERIMENTAL

Extraction of C. Acida leaves.—Crushed leaves (air-dried, 2 kg.) of *C. acida* were extracted with ether (4 litres) for 48 hours in a Soxhlet apparatus. The deep green ethereal extract was concentrated to 250 c.c. and kept in a fridaire for four weeks when a pale yellow solid (A : 0.89g., m.p. 60°-108°) separated. It was collected, washed with a mixture of ether and petroleum ether and dried. The mother-liquor (M) was carefully freed from the solvent and worked up further for coumarins by the method of Späth and Socias, thus :

The oil was cooled to about 15° and to it were added 100 c.c. of 20% methyl alcoholic potassium hydroxide with constant shaking. The mixture was allowed to stand for 45 minutes at the same temperature and then poured into 500 c.c. of ice-water. The mixture was then extracted with ether (250 c.c., in 5 instalments). The red aqueous alkaline solution was acidified with hydrochloric acid (congo red) and allowed to stand overnight. Next morning the greater portion of methyl alcohol was removed under reduced pressure, then cooled and thrice extracted with ether. The yellow ethereal digest was dried over anhydrous sodium sulphate and distilled. The residue was kept in the frigidaire for a week. The residue solidified to a pale yellow solid (B: yield 1.5 g., m.p. 50°-110°). It was washed with petroleum ether to remove adherent oil.

Isolation of isoPimpinellin from Fractions (A) and (B).—The fractions A and B (*vide supra*) were mixed together and crystallised successively from ethyl acetate, methyl alcohol and acetone when pale yellow needles (P), m.p. 147-49° separated. The residue obtained from the mother-liquor on crystallisation from various solvents gave a product of m.p. 115-160°. This was subjected to fractional sublimation in high vacuum twenty times when three sublimates in a fairly pure state could be obtained. Fraction (C) subliming at 115-120°/0.1 mm., fraction (D) at 150-55°/0.1 mm., and fraction (E) at 165-170°/0.1 mm.

Isolation of isoPimpinellin from the Fractions P and C.—The fractions P and C were crystallised from ethyl acetate when a yellow globular mass, mixed with colourless needles of m.p. 174-76°, separated. The colourless needles (F) were separated from the yellow globular mass by hand-picking. The yellow substance (0.0955 g.) was crystallised thrice from methyl alcohol when glistening yellow needles, m.p. 149-50°, were obtained. Recrystallisation from alcohol and benzene did not raise the m.p. [Found in a sample dried in *vacuo* over P₂O₅ for 4 hours at 100-105°: C, 62.97; H, 3.98; OMe, 24.9. C₁₁H₄O₃(OCH₃) requires C, 63.4; H, 4.05; OMe, 25.2 per cent.]. Mixed with a specimen of pure *isopimpinellin* it showed no depression in m.p.

Isolation of Bergapten from the Fractions D and F.—The fractions D and F (*vide supra*) were mixed and crystallised from methyl alcohol. Colourless needles (0.05 g.) separated along with a slimy magma. The slimy matters being lighter could be readily removed from the solution, the needles collecting at the bottom of the crystallising vessel. On recrystallisation from methyl alcohol and ethyl acetate the needles could be obtained pure. The silky, colourless crystals melted at 186-88°. Further crystallisations did not change the m.p. [Found in a sample dried in *vacuo* over P₂O₅ for 4 hours at 100°: C, 66.48; H, 3.67; OMe, 14.5. C₁₁H₄O₃(OCH₃) requires C, 66.66; H, 3.70; OMe, 14.35 per cent.]. The substance showed no change in melting point when mixed with an authentic sample of bergapten having m.p. 189°.

Isolation of Citropten from the Fraction (E).—The fraction E (*vide supra*), m.p. 145-50°, yield, 0.175 g.) was subjected to repeated crystallisations from methyl

alcohol when heavy, colourless rods of m.p. 150° separated out. On further crystallisations the m.p. did not change. [Found in a sample dried in *vacuo* over P_2O_5 for 4 hours at 100°: C, 64.4; H, 4.8; OMe, 29.8. $C_9H_4O_2(OCH_3)_2$ requires C, 64.07; H, 4.8; OMe, 30.09 per cent] When mixed with citropten, m.p. 150°, the sample showed no depression in m.p.

In conclusion the author expresses his thanks to Dr. (Mrs) A. Chatterjee for his keen interest and to Mr N. Ghosh for microanalysis.

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