

### 140. *The Constituents of Expressed West Indian Lime Oil.*

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Certain of the non-volatile constituents of expressed West Indian lime oil (from *Citrus aurantiifolia*, Swingle) have been isolated and identified. In addition to limettin (I; R = R<sub>1</sub> = Me) (5 : 7-dimethoxycoumarin), which had been identified earlier, the solid which separates from the oil on standing contains the furocoumarin, isopimpinellin (II; R = OMe, R<sub>1</sub> = Me). These two constituents have also been isolated from the oil, together with 7-methoxy-5-geranoxycoumarin (III), which is present to the extent of 2–2.5%, and traces of bergaptol (II; R = R<sub>1</sub> = H), previously obtained from bergamot oil.

LIME oil is known to commerce in two forms, expressed and distilled. The expressed oil is produced by puncturing the cells in the skin of the fruit and separating the oil and the watery fluid so obtained. The distilled oil is produced either by direct distillation of the fruit rinds or as a by-product in the evaporation of lime juice during the isolation of citric acid.

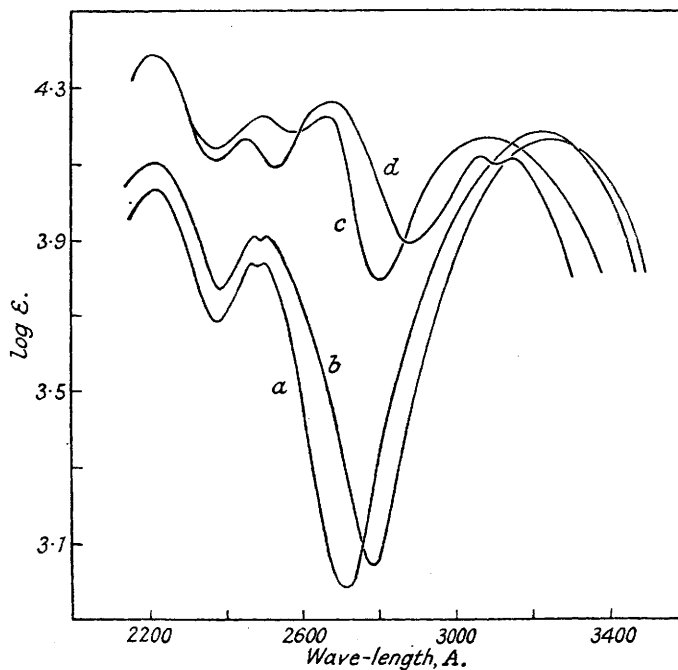
The difference in the origins of the two oils is reflected in differences in their physical and chemical properties. The distilled oil is said to possess a somewhat unpleasant, acrid or turpentine-like smell, in contrast to the pleasant odour of the expressed oil. In general, distilled oils have a lower refractive index, acid number, aldehyde content, and a lower proportion of non-volatile matter than expressed oils, although the figures quoted in the literature vary between rather wide limits.

Until recently, these oils had been but little studied; however, the distilled oil has now been thoroughly investigated by Guenther and Langenau (*J. Amer. Chem. Soc.*, 1943, **65**, 959), who have proved the presence therein of some twenty compounds, including hydrocarbons, alcohols, aldehydes, and esters. The expressed oil has been stated to contain citral, limonene, *l*- $\alpha$ -terpineol, bisabolene, methyl anthranilate, and limettin (citropten; 5 : 7-dimethoxycoumarin) (I; R = R<sub>1</sub> = Me) (Allen's "Commercial Organic Analysis," 5th Ed., 1925, Vol. IV, 204). As part of a detailed study of the expressed oil (West Indian, from *Citrus aurantiifolia*, Swingle; *Citrus medica*, L., var. *acida*, Brandis), the non-volatile constituents have been examined and form the subject of this paper.

The oil as originally obtained is a clear, golden-yellow liquid with a faint blue fluorescence, which gradually deposits a pale yellow solid on standing. In the present instance, from some 2 kg. of oil, 12 g. of this solid were separated by filtration. By a tedious purification procedure, including treatment of this solid with alkalis and precipitation with acids, Tilden (*J.*, 1892, **61**, 344) isolated limettin as pale straw-coloured crystals,

m. p. 147-5°, and stated that the original contaminant was some waxy vegetable matter. Limettin was shown to be 5:7-dimethoxycoumarin (I;  $R = R_1 = \text{Me}$ ) (Tilden and Burrows, J., 1902, **81**, 508) and this was confirmed by the synthetic work of Heyes and Robertson (J., 1936, 1831). It has now been found that the resolution of the deposited material into pure constituents can readily be effected by chromatographic analysis on alumina from benzene solution. In this way, pure limettin was isolated in a colourless condition, together with a slightly smaller amount of a yellow crystalline compound, m. p. 147-148°, depressed to about 115° on admixture with an equal amount of limettin. It is noteworthy that Tilden and Beck (J., 1890, **57**, 323) first reported the m. p. of limettin as 121°. The lactonic nature of this yellow compound was soon apparent, the analytical data indicated the molecular formula,  $\text{C}_{13}\text{H}_{10}\text{O}_5$ , and its identity with the furocoumarin, *isopimpinellin* (II;  $R = \text{OMe}$ ,  $R_1 = \text{Me}$ ), described by Wessely and Kallab (*Monatsh.*, 1932, **59**, 161), seemed highly probable. Since a direct comparison was impossible, this identity has been established by the formation of furan-2:3-dicarboxylic acid on oxidation with hydrogen peroxide, and by the preparation of a number of derivatives, the constants of which are compared in the accompanying table with those quoted by the earlier workers.

Compound.	Present work.	Wessely and Kallab ( <i>loc. cit.</i> ).
<i>isoPimpinellin</i>	Yellow needles, m. p. 147-148°	Yellow needles, m. p. 148-151°
Methoxy-acid obtained by fission of the lactone ring	M. p. 157-158-5°	Sintering slightly 143°, strongly 156°, molten 162°
Dihydrohydroxy-acid obtained by sodium-amalgam reduction of <i>isopimpinellin</i>	M. p. 139°, sintering 131°	M. p. 141°, sintering 130°
Dihydro <i>isopimpinellin</i> obtained by heating the above acid	M. p. 93-5-94°	M. p. 95-5°

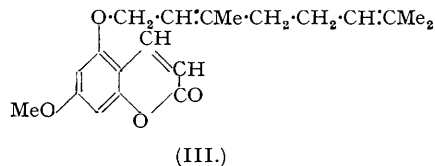
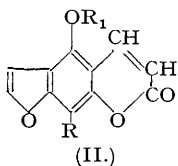
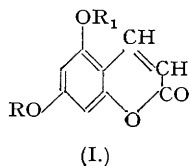


Curve (a): Limettin (I;  $R = R_1 = \text{Me}$ ).  
 (b): 7-Methoxy-5-geranoxycoumarin (III).  
 (c): Me ether of bergaptol (II;  $R = R_1 = \text{H}$ ).  
 (d): *isoPimpinellin* (II;  $R = \text{OMe}$ ,  $R_1 = \text{Me}$ ).

*isoPimpinellin* has previously been isolated from several members of the *Umbelliferae*, viz., *Pimpinella saxifraga*, L. (Wessely and Kallab, *loc. cit.*), *Seseli indicum*, Wall. (Späth, Bose, Matzke, and Guha, *Ber.*, 1939, **72**, 821), and *Heracleum sphondylium*, L. (Späth and Simon, *Monatsh.*, 1936, **67**, 344), but hitherto from only one member of the *Rutaceae* (*Luvunga scandens*, Ham.) (Späth, Bose, Schmidt, Dobrovolny, and Mookerjee, *Ber.*, 1940, **73**, 1361).

Removal of some 85% of the filtered oil by distillation under reduced pressure left a residual pale brown oil with a bluish fluorescence from which solid material separated on standing at 0° in methyl-alcoholic solution. Fractional crystallisation of this solid effected no appreciable purification, but chromatographic analysis on alumina from benzene solution resulted in a sharp separation into three fractions, consisting of limettin, *isopimpinellin*, and a new colourless compound A, m. p. 86-87°, of composition  $\text{C}_{20}\text{H}_{24}\text{O}_4$  (one methoxyl group). Its marked fluorescence and its light-absorption properties (see Fig.) are strongly reminiscent of those of limettin,

and in view of its relatively low melting point, and fission to a *dimethoxy-acid*,  $C_{21}H_{28}O_5$ , on treatment with alkali and methyl sulphate, it appeared possible that it was a methoxygeranoxycoumarin. The geranyl ether grouping is not common amongst naturally occurring compounds, but Späth and Kainrath (*Ber.*, 1937, **70**, 2272) have shown that the bergamottin (m. p. 59—61°) of bergamot oil is the geranyl or neryl ether (II;  $R = H$ ,  $R_1 = \text{geranyl}$ ) of bergaptol.



Ozonolysis of compound A gave acetone and lævulic aldehyde in good yields, indicating the presence of a geranyl residue in the molecule, and on treatment with acetic acid containing a little sulphuric acid a phenolic compound,  $C_{10}H_8O_4$  (one methoxyl group), m. p. 228—229°, was produced. Since limettin was formed on methylation of this phenol with diazomethane it must be represented by either (I;  $R = \text{Me}$ ,  $R_1 = H$ ) or (I;  $R = H$ ,  $R_1 = \text{Me}$ ). Of these, the latter is already described by Howell and Robertson (*J.*, 1937, 293) as having m. p. 246° and giving no colour with ferric chloride. The present phenol, which gives a pale green coloration with ferric chloride and a positive indophenol reaction indicating a free *p*-position to the hydroxyl group (cf. Gibbs, *J. Biol. Chem.*, 1927, **72**, 649), must be 5-hydroxy-7-methoxycoumarin, and compound A is therefore 7-methoxy-5-geranoxycoumarin (III). The presence of this compound in the oil in such relatively large amount (2.0—2.5%) doubtless accounts for the characteristic blue fluorescence of the expressed oil. This fluorescence has usually been attributed to the presence in the oil of methyl anthranilate (*e.g.*, Schimmel and Co.'s *Report*, 1904, Vol. II, 54), but the present work does not support this view, in that no nitrogenous material could be detected in the less volatile portions of the oil, and methyl anthranilate must be present in very small amount, if at all.

The viscous oil remaining after removal of the substances described above was distilled in a vacuum, finally at  $10^{-4}$  mm. From the portion distilling at 100—125° (bath temp.) there was isolated a very small amount of a phenolic compound, m. p. 275—278° which gave a methyl ether, m. p. 182—185°, but there was insufficient material available for complete purification. The light absorption (see Fig.) of the methyl ether is characteristic of the furocoumarin structure, and it is highly probable that this material is identical with the bergaptol (II;  $R = R_1 = H$ ) (m. p.\* 280—282°; methyl ether, m. p.\* 190—191°) isolated by Späth and Socias (*Ber.*, 1934, **67**, 59) from bergamot oil (from *Citrus bergamia* Risso—*Rutaceæ*).

#### EXPERIMENTAL.

(Light-absorption data were determined in alcoholic solutions.)

*Isolation of Limettin* (I;  $R = R_1 = \text{Me}$ ) and *isoPimpinellin* (II;  $R = \text{OMe}$ ,  $R_1 = \text{Me}$ ).—The sediment which had formed in the oil (*ca.* 2 kg.) was filtered off and washed with light petroleum (b. p. 40—60°), giving a pale yellow solid (12 g.). A small portion had m. p. 114—116° after one crystallisation from methyl alcohol. The crude solid was dissolved in benzene (300 c.c.) and adsorbed on a  $40 \times 3$  cm. column of "Birlec" alumina, forming a yellow upper band and a colourless lower band showing a strong blue fluorescence in ultra-violet light. Elution with benzene yielded initially a small amount of gummy matter, followed by limettin (5.7 g.), crystallising from methyl alcohol in prismatic needles, m. p. 145—146° (Found: C, 63.8; H, 5.05. Calc. for  $C_{11}H_{10}O_4$ : C, 64.05; H, 4.9%). *Light absorption*: Maxima, 2220, 2470, 2505, 3240 Å.;  $\log \epsilon = 4.03, 3.84, 3.84, 4.18$ .

Subsequent elution with a mixture of benzene and chloroform (5 : 1) gave a pale yellow solid which, on crystallisation from methyl alcohol, yielded *isopimpinellin* (4.8 g.) in long, fine, golden-yellow needles, m. p. 147—148° (Found: C, 63.7, 63.7; H, 4.15, 3.95;  $\text{OCH}_3$ , 23.4, 25.2, 18.9, 20.1. Calc. for  $C_{13}H_{10}O_5$ : C, 63.4; H, 4.1;  $2\text{OCH}_3$ , 25.2%). *Light absorption*: Maxima, 2220, 2450, 2690, 3080, 3160 Å.;  $\log \epsilon = 4.39, 4.17, 4.26, 4.11, 4.11$ .

*Fission of Lactone Ring in isoPimpinellin*.—*isoPimpinellin* (200 mg.) was treated with potassium hydroxide and methyl sulphate according to Wessely and Kallab (*loc. cit.*). The acid (180 mg.) was obtained as small prismatic needles, m. p. 157—158.5°, after two recrystallisations from benzene—light petroleum (b. p. 60—80°) (charcoal). [In one similar experiment the acid sintered at 148° and melted at 156—158°; the m. p. did not improve on recrystallisation (cf. the m. p. reported by Wessely and Kallab).] (Found: C, 60.55, 60.7; H, 5.05, 5.25;  $\text{OCH}_3$ , 30.0. Calc. for  $C_{14}H_{14}O_6$ : C, 60.45; H, 5.05;  $3\text{OCH}_3$ , 32.7%).

*Oxidation of isoPimpinellin with Hydrogen Peroxide*.—*isoPimpinellin* (200 mg.) was dissolved in sodium hydroxide solution (16 c.c.; 5%), and hydrogen peroxide (6 c.c.; 9%) added. The mixture was set aside at room temperature for 17 hours, and then heated to 70—80° for 6 hours. After acidification with dilute sulphuric acid, the solution was evaporated to dryness under reduced pressure, the residue extracted with alcohol, and the dark tarry residue remaining on evaporation of the extract was distilled at  $10^{-4}$  mm. The solid distilling at 90—100° (bath temp.) had m. p. 212—215° (decomp.), and after a further sublimation at  $10^{-4}$  mm. it had m. p. 215—217° (decomp.) (lit., m. p. of furan-2 : 3-dicarboxylic acid, 221°).

*Reduction of isoPimpinellin with Sodium Amalgam*.—*isoPimpinellin* (200 mg.) was treated with sodium amalgam (20 g.; 5%) by the method of Wessely and Kallab (*loc. cit.*). The dihydro-acid (190 mg.) formed long needles, m. p. 139°, softening at 131°, on crystallisation from benzene—light petroleum (b. p. 60—80°) (charcoal).

*Preparation of Dihydroisopimpinellin*.—The acid obtained as above (20 mg.) was heated at 140—150° for 2 hours, and the product distilled at 80° (bath temp.)/ $10^{-4}$  mm. The sublimate, which had m. p. 90—92°, crystallised from benzene—light petroleum (b. p. 60—80°) in long, fine needles, m. p. 93.5—94°. *Light absorption*: Maxima, 2200, 2550 Å.;  $\log \epsilon = 4.44, 4.16$ .

*Isolation of 7-Methoxy-5-geranoxycoumarin* (III) (*Compound A*).—The lime oil (525 g.), from which the solid deposit

\* These m. p.'s are the highest of a number of values quoted in the literature.

had been separated, was distilled under reduced pressure from a Claisen flask, all the material with b. p. up to  $88^{\circ}/4$  mm. (bath temp. not greater than  $110^{\circ}$ ) being removed. The residual pale brown, greenish-blue-fluorescent oil (73 g.) was dissolved in methyl alcohol (*ca.* 50 c.c.) and set aside at  $0^{\circ}$  for several days. The almost white solid which separated was filtered off and washed, first with methyl alcohol and then with light petroleum (b. p.  $40-60^{\circ}$ ). The solid (16.6 g.), dissolved in benzene (300 c.c.), was adsorbed on a  $38 \times 3$  cm. column of "Birlec" alumina, and three well-defined bands were then visible under an ultra-violet lamp, *viz.*, (a) a large lower band with a bright blue fluorescence, (b) a small middle band showing an intense greenish-blue fluorescence, and (c) a small yellow band at the top showing no fluorescence. The large band (a) was cut out and extracted with ether, evaporation of which gave 7-methoxy-5-geranoxy-coumarin (11.8 g.) which crystallised from light petroleum (b. p.  $60-80^{\circ}$ ) in small shining plates, m. p.  $86-87^{\circ}$  [Found: C, 72.85; H, 7.6;  $\text{OCH}_3$ , 8.0, 7.4, 9.1; *M* (Rast), 336.  $\text{C}_{20}\text{H}_{24}\text{O}_4$  requires C, 73.15; H, 7.35;  $\text{IOCH}_3$ , 9.4%; *M*, 328]. *Light absorption*: Maxima, 2220, 2480, 2510, 3250  $\text{\AA}$ .,  $\log \epsilon = 4.10, 3.91, 3.91, 4.16$ .

The other bands (b) and (c) were cut out of the column and extracted with ether, yielding limettin (1.2 g.) and *isopimpinellin* (2.2 g.), respectively, both identified by mixed m. p. with the specimens obtained earlier.

*Fission of Lactone Ring in Compound A*.—Compound A (1.0 g.) was treated with potassium hydroxide and methyl sulphate in a manner similar to that used for *isopimpinellin*. The 4 : 6-dimethoxy-2-geranoxy-cinnamic acid (900 mg.) crystallised from light petroleum (b. p.  $60-80^{\circ}$ ) or aqueous methyl alcohol in small shiny needles, m. p.  $98-99^{\circ}$  (Found: C, 69.75, 69.65; H, 7.8, 7.75;  $\text{OCH}_3$ , 13.8.  $\text{C}_{21}\text{H}_{28}\text{O}_5$  requires C, 69.95; H, 7.85;  $2\text{OCH}_3$ , 16.9%).

*Ozonolysis of Compound A*.—Ozonised oxygen was passed into a solution of compound A (500 mg.) in "AnalaR" acetic acid (20 c.c.) until oxidation was complete (5 hours). Water (30 c.c.) was then added, and the reaction mixture warmed on the steam-bath until it became clear. More water (70 c.c.) was added and the solution was distilled in steam, the steam-distillate being collected in fractions, to each of which an aqueous solution of 2 : 4-dinitrophenyl-hydrazine sulphate was added. The first 10 c.c. of distillate yielded a bulky orange precipitate, which dissolved almost completely in hot methyl alcohol, the filtered solution on cooling depositing orange plates (250 mg.; 70% of theory) of acetone-2 : 4-dinitrophenylhydrazone, m. p. and mixed m. p.  $123-124^{\circ}$ . Later fractions of the steam-distillate gave a bright yellow derivative, separating from nitrobenzene-ethyl alcohol as a powder, m. p.  $232-233^{\circ}$ , identified as *lævulic aldehyde* by comparison with an authentic specimen.

*5-Hydroxy-7-methoxycoumarin* (I; R = Me,  $\text{R}_1 = \text{H}$ ).—Compound A (500 mg.) was set aside at room temperature for 19 hours with glacial acetic acid (7 c.c.) and concentrated sulphuric acid (2 drops). The solution was then dark brown and a pale yellow precipitate had formed. This solid (230 mg.; 80% of theory), m. p.  $227-229^{\circ}$ , was filtered off and on crystallisation from ethyl acetate gave small colourless needles of 5-hydroxy-7-methoxycoumarin, m. p.  $228-229^{\circ}$ . It was readily soluble in 2*N*-sodium hydroxide and gave a pale green colour with ferric chloride solution (Found: C, 62.45; H, 4.1,  $\text{OCH}_3$ , 13.7.  $\text{C}_{16}\text{H}_{18}\text{O}_4$  requires C, 62.5; H, 4.2;  $\text{IOCH}_3$ , 16.1%). *Light absorption*: Maxima, 2520, 2560, 3240  $\text{\AA}$ .;  $\log \epsilon = 3.84, 3.84, 4.17$ .

*Methylation*. The phenol (15 mg.) was dissolved in ether and a little methyl alcohol and treated with an excess of an ethereal solution of diazomethane. After standing for 30 minutes, the ether was evaporated, and the residue was sublimed at  $70^{\circ}$  (bath temp.)/ $10^{-4}$  mm. Crystallisation of the sublimate from aqueous methyl alcohol gave needles of limettin, m. p.  $144-145^{\circ}$ , undepressed on admixture with an authentic specimen.

*Isolation of Bergaptol* (II; R =  $\text{R}_1 = \text{H}$ ).—The methyl-alcoholic solution of the oil remaining after removal of compound A was evaporated under reduced pressure, and the residue distilled at  $10^{-2}$  mm. The distillate (14.5 g.), collected up to b. p.  $105^{\circ}$ , was a pale-green oil (the colour doubtless due to the azulenic compound known to be present in the oil). The dark, viscous residue in the flask was distilled from an electrically heated retort at  $10^{-4}$  mm. The earlier fractions (distilling up to bath temp.  $100^{\circ}$ ) consisted of small amounts of the solids already isolated along with some colourless and blue-green oily substances. At  $100-125^{\circ}$  (bath temp.) some pale yellow solid distilled along with an oil which set to a glass on cooling. The solid (0.3 g.) was separated by trituration with methyl alcohol and melted at *ca.*  $200-240^{\circ}$ . After a further sublimation at  $100^{\circ}$  (bath temp.)/ $10^{-4}$  mm. the now colourless solid had m. p.  $255-270^{\circ}$ . After three crystallisations from aqueous methyl alcohol and aqueous acetone and a sublimation at  $110^{\circ}$  (bath temp.)/ $10^{-4}$  mm., a small amount (20 mg.) of bergaptol, m. p.  $275-278^{\circ}$ , was obtained. The compound was readily soluble in dilute alkalis and gave a pale green colour with ferric chloride solution (Found: C, 64.4; H, 3.1. Calc. for  $\text{C}_{11}\text{H}_8\text{O}_4$ : C, 65.35; H, 3.0%). Methylation of the phenol (8 mg.) with diazomethane gave the ether, m. p.  $182-185^{\circ}$  after a sublimation at  $60^{\circ}$  (bath temp.)/ $10^{-4}$  mm. and crystallisation from aqueous methyl alcohol. *Light absorption*: Maxima, 2200, 2500, 2675, 3070  $\text{\AA}$ .;  $\log \epsilon = 4.39, 4.23, 4.23, 4.16$ .

The authors thank Professor I. M. Heilbron, D.S.O., F.R.S., and Professor J. L. Simonsen, F.R.S., for their interest in this work, and the Colonial Products Research Council both for the lime oil and for the financial support which rendered this investigation possible.