XXIII.—*Some Crystalline Substances obtained from the Fruits of various species of* Citrus. PART I.

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THE starting point of this investigation was the examination of a solid substance deposited from the écuelled essence of limes manufactured in the island of Montserrat, West Indies, for which we are indebted to the kindness of Mr. F. Watts, F.C.S., chemist to the Montserrat Company.

This substance was found to be most easily purified from the accompanying essential oil, resin, and vegetable *débris*, by dissolution in strong alcohol, and subsequent crystallisation from alcohol, to which a little caustic potash (about 1 per cent.) was added. The preparations thus obtained consisted of tufts of small prisms of a pale-yellow colour, which was not removed by repeated crystallisation from alcohol containing a larger proportion of potash.

As it will be necessary to distinguish this substance from several others derived from a similar source, we propose to call it *limettin*, the botanical name of the lime being *Citrus limetta.*

Limettin melts at 121—122°. It loses nothing when dried in the steam bath or heated to fusion. It dissolves very sparingly in water, much more freely in alcohol. It also dissolves without change in alcoholic potash or soda, and from strong solutions is immediately reprecipitated by acids; it is, however, neutral to test paper, and possesses no acid properties. It has a bitterish, styptic taste. An alkaline solution does not reduce Fehling's solution, or only to a very slight extent after long boiling. Boiled with dilute sulphuric acid, it gives no indication of the formation of a glucose, and the substance filtered from the liquid on cooling has the original melting point. Limettin gives no special coloration with ferric chloride. It contains no nitrogen.

Submitted to combustion, it gave the following percentages of carbon and hydrogen:—

	I.	II.	III.	IV.	Mean.
C	62·92	63·07	63·05	63·52	63·14
H	4·90	5·13	4·62	4·78	4·86

Three combustions made for us by Mr. A. L. Stern, B.Sc., gave the following numbers:—

	I.	II.	III.	Mean.
C	63·36	63·38	63·12	63·29
H	4·80	4·67	4·68	4·71

From these results it appears that the formula of limettin is $C_{16}H_{14}O_6$, as shown by the following comparison:—

		Experiment. T. and B.	Experiment. Stern.	Calculated. $C_{16}H_{14}O_6$.
C_{16}......	192	63·14	63·29	63·57
H_{14}	14	4·86	4·71	4·63
O_6	96	32·09	32·00	31·80
	302			

A bromo-substitution compound can be obtained by dissolving the substance in alcohol, or much better in glacial acetic acid, and adding an excess of bromine dissolved in a portion of the same solvent. It crystallises in colourless scales which gave 43·56 per cent. of bromine. It therefore contains 3 atoms of bromine; $C_{16}H_{11}Br_3O_6$ corresponds to 44·52 per cent. of bromine. Limettin is not attacked by acetyl chloride when heated with it for about four hours, in a sealed tube, at the temperature of the water-bath; it therefore seems to contain no hydroxyl. When heated to about 160° with concentrated hydrochloric acid, limettin is converted into a red resinous substance, but no gaseous or volatile alkyl chloride is produced. Dissolved in strong acetic acid and digested on the water-bath with excess of phenylhydrazine for some hours, there is no perceptible reaction, and no hydrazone is produced.

When the substance is heated above its melting point, the colour deepens, and at about 200° it boils. On distillation it yields a black charred residue, and a yellow distillate which solidifies on cooling. After dissolution of the latter in alcohol, it crystallises in pale-yellow, silky needles, somewhat different in appearance from the original substance, and melting at 140°. Submitted to combustion, it gave—

	I.	II.	III.	Mean.
C..........	64·87	64·21	64·34	64·47
H..........	5·30	5·14	5·03	5·16

This substance requires further investigation.

Evidence regarding the relationships of limettin was obtained by fusing it with potash; 30 grams of limettin were boiled for about an hour in a flask with 100 grams of potash and 100 grams of water, until very concentrated. The substance dissolved very slowly, and no hydrogen or vapour was evolved. When cold, the solid brown mass was dissolved in water, and acidified with hydrochloric acid, which caused copious effervescence of carbon dioxide, and the formation of a brown precipitate of resinous matter. The filtered liquid was extracted with ether, and the watery solution submitted to distillation. The ethereal extract yielded crystals of phloroglucol; this was identified by its sweet taste, by the violet coloration with ferric chloride, by staining deal wood, by the formation of the characteristic red azobenzene-compound, and by a determination of the water of crystallisation. The air-dried crystals lost at 100° 22·2 per cent. of water; $C_6H_6O_3 2H_2O$ corresponds to 22·2 per cent. The melting point was about 210°.

Acetic acid and a small quantity of formic acid are contained in the aqueous distillate. Other fatty acids were carefully sought for, but were not detected. When caustic potash without addition of water was employed, and the fusion effected at a higher temperature, gas was evolved, and acetic acid the only isolable product.*

When limettin is boiled for some time with a strong solution of caustic potash or soda, without allowing the liquid to become concentrated by evaporation, it slowly dissolves, and on acidifying it is precipitated to all appearance unchanged. The recovered substance, however, melts at 147°, and by combustion gave—

C....................	64·40	per cent.
H....................	4·60	,,

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* We found that phloroglucol alone fused with potash yields acetic acid; probably thus: $C_6H_6O_3 + 3KHO = 3C_2H_3KO_2$.

This corresponds to $C_{14}H_{12}O_5$, which requires—

C....................	64·61 per cent.
H	4·61 „

A small quantity of acetic acid is found in the liquid. This substance is evidently the result of a process of saponification; it contains the elements of limettin in which an acetyl group has been displaced by hydrogen.

$$C_{14}H_{11}(O{\cdot}C_2H_3O)O_4 + NaHO = C_{14}H_{11}(OH)O_4 + NaO{\cdot}C_2H_3O.$$

The product of destructive distillation already described is possibly the same substance; the hydrogen found in that compound was, however, ½ per cent. higher.

Treated with nitric acid, limettin yields chiefly oxalic acid, with a small quantity of an undetermined nitro-acid.

The facts related can be explained by the hypothesis that limettin is an acetic derivative of a kind of anhydride, which by hydrolysis yields phloroglucol.

This substance evidently does not agree in any of its properties with either of the substances hitherto obtained from the fruits of the different species of *Citrus*, and described under the names aurantiin, hesperidin, and limonin respectively. A brief synopsis of the sources and characters of these substances will be given at the end.

The greasy deposits which are well known to form in essence of bergamot and of lemon have been shown to contain crystalline constituents (Hanbury and Flückiger "Pharmacographia"), but these have never been closely examined. Under the impression that the crystalline constituent in the deposit from lemon oil would probably be identical with that obtained from limes, we applied to Mr. C. Umney, F.C.S., of the firm of Wright, Layman, and Umney, for a supply of the raw material, and he was good enough to collect a few ounces of the deposit from essence of lemon for us.

The greasy mass was mixed with about an equal volume of alcohol, and strained through calico. The pasty residue was then boiled with strong alcohol containing about 2 per cent. caustic soda, and filtered hot from the brown mud, consisting of fruit pulp and dirt. On cooling, the solution solidified to a jelly, which was broken up, and the liquid drained off as completely as possible. The brown alcoholic solution was then heated to boiling, acidified with hydrochloric acid, and mixed with hot water till slight turbidity was produced. On cooling, a copious crystallisation of small, flat prisms was obtained. These were purified by repeated crystallisation from strong alcohol.

The substance thus prepared forms yellow, lustrous needles. We refrain for the present from giving this substance a name, although it is manifestly different from limettin. After drying at 100°, the crystals melt at 115—116°. They contain no water of crystallisation.

By combustion they gave the following percentages of carbon and hydrogen :—

	I.	II.	III.	Mean.
C	60·40	60·19	60·52	60·37
H	5·77	5·51	5·75	5·67

These percentages correspond to the formula $C_{14}H_{14}O_6$, which represents a compound containing 2 atoms of carbon less than limettin.

	Calculated.	Per cent.	Experiment.
C_{14}	168	60·43	60·37
H_{14}	14	5·03	5·67
O_6	96	—	—
	278		

$C_{14}H_{16}O_6$ requires C, 60·00; H, 5·70 per cent.

This compound is now undergoing investigation. In the meantime a small quantity of the deposit from bergamot oil was purified in a similar manner, and was found to contain a crystalline substance entirely distinct from limettin, and from the compound procured from lemon oil. It forms small, *colourless*, prismatic crystals which melt at 270—271°. The quantity of this substance in our possession was too small to make a combustion, but we hope shortly to receive a supply from Messina sufficient for a complete investigation of its constitution.

Mulder, in 1837, and Ohme, in 1839, gave concordant analyses of a substance they obtained from bergamot oil, to which the name bergaptene was given. Hanbury and Flückiger ("Pharmacographia") state that they have obtained this substance in colourless acicular crystals, but evidently further inquiry is necessary.

Up to the present time, therefore, six distinct crystalline substances have been obtained from the fruits of various species of *Citrus*, and to these may be added the glucoside obtained by De Vrij from the flowers of *Murraya exotica*, which belongs to the same natural order.

Of these only hesperidin has received careful attention. This substance has been shown by Tiemann and Will (*Ber.*, **14**, 946) to be a glucoside which splits without assumption of water into hesperetin and glucose. Hesperetin is an "ester" of phloroglucol and an acid (hesperetic acid) which they have shown to be identical with isoferulic acid—

$$COOH{\cdot}C_2H_2{\cdot}C_6H_3(OH){\cdot}OCH_3.$$

Name.	Source.	General character.	M. p.	Formula.
I. Murrayin	*Murraya exotica*	Glucoside ..	170°	$C_{18}H_{22}O_{10}$.
II. Aurantiin (Hesperidin, De Vrij.) ..	Flowers and other parts of *Citrus decumana*	Glucoside ..	171° (anhydrous)	$C_{23}H_{26}O_{12},4H_2O$.
III. Hesperidin ...	*C. aurantium*, *C. limonum*, *C. medica*, others ?	Glucoside ..	251°	$C_{22}H_{26}O_{12}$.
IV. Limonin	Seeds of orange and lemon	Glucoside ?	275°	$C_{22}H_{26}O_7$.
V. Limettin	Epicarp of *C. limetta*	Not a glucoside	122°	$C_{16}H_{14}O_6$.
VI. Substance from	Lemon epicarp	?	116°	$C_{14}H_{14}O_6$.
VII. Bergaptene ...	Epicarp of bergamot	?	270—71°	$C_9H_6O_3$? (Mulder and Ohme).

Limettin has just been shown to be a derivative of phloroglucol, and we believe that that compound will be found to play an important part in the physiology of the orange and lemon fruits. Its sweet taste suggested that it might possibly replace part of the sugar in the ripening fruit, and the isolation of the sweet principles of orange juice will accordingly be attempted by one of us.

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