CHEMICAL INVESTIGATION OF INDIAN FRUITS

Part III. A Study of the Characteristic Crystalline Components of Certain Citrus Fruits (Oranges of the Circars)

BY K. C. PATNAYAK, S. RANGASWAMI AND T. R. SESHADRI

(From the Departments of Chemistry and Chemical Technology, Andhra University, Waltair)

Received February 26, 1942

ORANGES of the Northern Circars belong to the three important species of the citrus, (1) *C. aurantium*, (2) *C. medica* and (3) *C. decumana*. They can be characterised in general as the sweet, sour and bitter groups respectively and this is supported by the following analytical results relating to the most important varieties of these three species.

TABLE I

Composition of juice %	*C. aurantium* or sweet group		*C. medica* or sour group		*C. decumana* or bitter group	
	Batavias	*Kamalas*	*Naranja*	*Dabba*	*Shaddock*	*Matheepala*
Acids as citric acid ..	0·85	0·63	3·0	7·4	1·0	6·9
Reducing sugars ..	3·1	3·5	3·8	1·5	3·4	2·3
Sucrose	4·6	3·8	1·2	..	3·7	..
Total sugars	7·7	7·3	5·0	1·5	7·1	2·3
Total solids	10·2	10·1	9·8	9·3	9·6	9·3
Mineral matter ..	0·54	0·52	0·47	0·43	0·48	0·45
Vitamin C (mg. per 100 g. of the juice)	48	43	44	28	38	36
Juice, % fruit	46	48	46	40	39	37

The fruits belonging to the species *aurantium* with sweet juice have high sugar content, the amount of acid being small. The position is just the opposite in the case of the species *medica*. In regard to *decumana*, *Shaddock* is sweeter and *Matheepala* is sourer, but both of them are characterised by bitterness. All the fruits examined are good sources of vitamin C, the sweeter varieties being better than the sourer type.

Other chemical characteristics which are capable of distinguishing between the species were looked for in the peels and rags of the fruits. The following table gives particulars about the chemical composition of the peels expressed as percentage.

TABLE II

	Batavias	*Kamalas*	*Dabba*	*Shaddock*	*Matheepala*
Acids as citric acid ..	0·88	0·72	4·23	1·67	3·84
Crude fat	0·26	0·23	0·27	0·27	0·29
Sugar (as invert sugar) ..	4·22	4·53	0·84	4·54	2·05
Starch (as invert sugar) ..	6·21	6·42	4·83	6·55	5·22
Proteins	2·48	2·23	2·21	1·51	1·82
Crude fibre	2·34	2·48	3·23	3·86	3·42
Total solids	21·24	22·34	23·21	24·12	24·93
Mineral matter	1·94	2·08	1·84	2·08	1·79
Potassium as K_2O ..	0·39	0·42	0·32	0·43	0·28
Phosphorus as P_2O_5 ..	0·22	0·25	0·10	0·20	0·12

It may be noted that even in the peels, the sweeter varieties contain more sugar and less acid as compared with the sourer fruits. Further the former have more potash and phosphoric acid.

In the course of this work special attention was paid to the existence of crystalline compounds in the peels and rags. The fresh rags were directly extracted with alcohol whereas the peels were dried and subjected to a preliminary extraction with petroleum ether or ether before treatment with alcohol. From these alcoholic extracts hesperidin was found to be present in the two varieties of *aurantium* and of *medica*, wheras *naringin* was present in the *decumana* varieties. Thus the bitter taste of the *decumana* species is associated with naringin, and though hesperidin is chemically closely related, its taste is not so markedly bitter. In this connection it may be mentioned that there has been difference of opinion regarding the species to which *Matheepala* belongs. According to Gamble it belongs to *C. decumana*, though some others would place it under *C. medica*. The classification of Gamble is supported by the isolation of naringin from the peels and rags of this fruit, since this bitter principle is characteristic of the *decumana* species. In regard to the petroleum and ether extractable matter it was found to consist usually of essential oils, resins and carotenoids. But from the *Kamalas* a pale yellow crystalline compound has been obtained in a very small yield. When crystallised from aqueous methyl alcohol it sinters at 83° and melts at 125–26° and has the formula $C_{21}H_{22}O_9$, H_2O. It has six methoxyl groups and is a completely methylated compound. On demethylation with hydriodic acid it forms a crystalline substance decomposing above 320°. The corresponding acetate melts at 238–40°. The behaviour of the hydroxy compound is that of a hydroxyflavone. It gives a red colour when reduced with magnesium and hydrochloric acid,[1] forms a brown precipitate with neutral lead acetate and is decomposed in an alkaline solution when exposed to air. In these respects it appears to resemble a flavonol.

The original substance thus belongs to the group of completely methylated compounds of the type of tangeretin[2] and nobiletin,[3] isolated from other species of the citrus. Since the new compound possesses properties different from them it is here named ' aurantin ' and the corresponding hydroxy-compound ' nor-aurantin '. When subjected to decomposition with concentrated soda, aurantin yields an acid whose nature is not yet clearly understood.

Experimental

Examination of the rags.—As the rags contained very little of oily matter they were directly extracted with alcohol. They were well crushed in a mortar and boiled with enough methylated spirit for three hours under reflux. The extract was decanted off and the process repeated twice. The combined alcoholic solution was filtered to remove suspended impurities and concentrated under reduced pressure to the consistency of a thin syrup. The residue was filtered with suction while hot and the filtrate was left in the ice-chest for crystallisation. The crystalline solid that separated after a few days was filtered off and washed on the filter with a few drops of dilute alcohol. It was then crystallised from the appropriate solvent—hot absolute alcohol and acetic acid in the case of *Batavia*, *Kamala*, *Naranja* and *Dabba*, and hot water in the case of *Shaddock* and *Matheepala*. The first four yielded hesperidin melting at 251–52° and the last two naringin, the air-dried sample melting at 83°, and when dried at 110° melting at 172°. The identities were confirmed by direct comparison with authentic specimens.

Examination of the peels.—Of the different methods tried, the following gave the best yield of crystalline bitter principles. The dried and powdered peels were extracted with petroleum ether in a Soxhlet until all resinous and oily matter was removed. The residue was dried and extracted twice with boiling methylated spirit, refluxing for two hours each time. The combined extract was filtered and treated with a slight excess of neutral lead acetate in alcohol. After removal of the lead precipitate by filtration, the alcoholic filtrate was heated to boiling and saturated with hydrogen sulphide gas until the excess of the lead salt was precipitated. The lead-free alcoholic solution was then concentrated under reduced pressure until a syrupy residue was obtained. When left in the ice-chest for a few days this residue deposited crystalline material which was subsequently purified by recrystallisations. The same bitter principles were obtained in this experiment as from the rags.

Isolation of aurantin.—The dried and powdered peels of *Kamala* oranges (2·5 kg.) were extracted in lots of half a kilo each with ligroin by heating on

a steam-bath for about 10 hours. The extract which was pale yellow in colour was run off completely and the residue was re-extracted twice with ligroin in the above manner. The combined extract was concentrated by distilling off the solvent from an oil-bath at about 110°. The residue which was highly coloured (yellowish green) due to the presence of carotene and other pigments was boiled with a large excess of methyl alcohol (200 c.c.) and the hot solution filtered. The methyl alcohol insoluble portion was discarded and the alcoholic filtrate concentrated on a water-bath. During this process some resinous matter separated out slowly. The clear alcoholic solution was decanted into another flask and the concentration continued until a volume of about 25 c.c. was obtained, insoluble matter being removed now and then as described above. The residual alcoholic concentrate was diluted with an equal volume of water, heated to boiling, cooled and again filtered from insoluble material. After again concentrating to about 35 c.c. the residue was cooled ; on scratching the sides of the flask with a glass rod an amorphous solid slowly separated. This was filtered off (yield, about 1 g.). It was slightly sticky to the touch and was purified by dissolving in a slight excess of cold methyl alcohol and filtering through a fluted filter to remove colloidal inpurities. After dilution with half its volume of water it was set aside for crystallisation overnight in a loosely covered flask. Fine pale yellow needles separated which appeared quite pure and homogeneous when examined under the microscope. The solid sintered at 83° and melted at 125–26°.

The compound was sparingly soluble in water, moderately soluble in methyl and ethyl alcohol and very easily in acetic acid. It was insoluble in cold dilute alkali and gave no colour with alcoholic ferric chloride. It dissolved readily in cold concentrated sulphuric acid to give a pale yellow solution which did not exhibit any fluorescence. It was only sparingly soluble in concentrated hydrochloric acid in the cold, but dissolved on heating to produce a yellow solution from which a crystalline solid slowly separated out. [Found : C, 59·7 ; H, 6·0 ; MeO, 44·4; $C_{21}H_{22}O_8$, H_2O requires C, 60·0 ; H, 5·7; MeO, 44·3%.]

Nor-aurantin.—Aurantin (250 mg.) was dissolved in phenol (5 g.) and treated with hydriodic acid (d. 1·7, 10 c.c.). The mixture was heated in an oil-bath maintained at 140–50° with a gentle current of carbon dioxide passing through the system. The progress of the demethylation which occupied about half an hour could be easily followed by the marked smell of methyl iodide in the exit tube of the system. After heating for about 2 hours the mixture was allowed to cool. On dilution with water (70 c.c.) a yellow powder was precipitated. The mixture was decolorised by passing

sulphur dioxide through it and subsequently heated to boiling, when the solid dissolved, leaving behind a small amount of a heavy slimy material. The clear solution was carefully filtered in the hot and allowed to cool. The yellow powder that separated was filtered and washed with water to remove traces of phenol. Crystallisation was effected using a mixture of alcohol and acetic acid. A second crystallisation using acetic acid alone gave the nor-aurantin as yellow rectangular rods which when heated turned brown at about 300° and decomposed above 320°. There was no definite melting point or decomposition point; the tube was too dark for any observation above 320°. [Found : C, 56·5 ; H, 3·4 ; $C_{15}H_{10}O_8$ requires C, 56·6 ; H, 3·1%.]

Nor-aurantin was a yellow solid easily soluble in cold dilute alkali, forming a yellow solution and giving with alcoholic ferric chloride a dark olive green colour and with neutral lead acetate a brown precipitate. It was sparingly soluble in alcohol and only moderately in glacial acetic acid. On reduction with magnesium powder and hydrochloric acid in alcoholic solution it gave a red colour. Its solutions in concentrated acids were yellow. An aqueous suspension turned greenish blue on boiling. On adding sodium amalgam to a solution of the substance in absolute alcohol (Bargellini's test) a brown flocculent precipitate was obtained. When treated with 50% aqueous potash and shaken with air it first formed a dark red solution which slowly turned yellowish brown. After 24 hours the original compound was not recovered by acidification. It gave the following colour reactions with buffer solutions:—

p_H 8·0: Dissolves slowly forming a clear yellow solution after shaking for a minute. Turns greenish yellow and becomes deep green in 3 minutes. In 10 minutes pale greenish blue. After 20 minutes slowly changes and becomes pale yellow in 3 hours which is then stable even after 24 hours.

p_H 9·2: Dissolves more rapidly to form a yellow solution. Develops a green tinge in the course of a minute; the colour is stable for 15 minutes and then slowly fades to pale yellow which is stable.

p_H 10·4: Dissolves immediately to golden yellow which persists for an hour. Then it slowly diminishes in intensity (3 hours) and assumes a permanent pale yellow.

p_H 11·6: Immediate yellow solution changing to orange and red within 20 seconds. This colour is fairly stable and in the course of 2 hours changes to a deeper yellow which persists after 24 hours.

p_H 12·8: The red colour is deeper and is formed more quickly. It also changes more rapidly to yellow.

The hexa-acetate of nor-aurantin was prepared by boiling with sodium acetate and acetic anhydride for 4 hours. The solid that separated on pouring the mixture into excess of cold water and leaving overnight was filtered, washed with water and crystallised from alcohol-acetic acid mixture. A second crystallisation from acetic acid gave the acetate as fine needles melting at 238–40° with slight sintering at 231°. [Found : C, 57·0 ; H, 4·0 ; $C_{27}H_{22}O_{14}$ requires C, 56·8; H, 3·9%.]

Summary

The important citrus fruits (oranges) of the Northern Circars belong to three species : (1) *C. aurantium*, (2) *C. medica*, (3) *C. decumana*. Their chemical compositions support their classification as the sweet, the sour and the bitter types. In the peels and rags of the first two types hesperidin is present whereas naringin occurs as the characteristic crystalline bitter principle of the varieties of the *decumana*. From the peels of *Kamala* (*C. aurantium*) a new substance has been obtained by extraction with ligroin and named aurantin. It is a completely methylated compound of the type of tangeretin and nobiletin. Its properties and reactions along with those of nor-aurantin are described.

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