glacial acetic acid these derivatives show maximum absorption at 430 and 433 $\mu\mu$, respectively.

Summary

Isatin has been condensed with phenyl, *o*-, *m*-, and *p*-tolyl-, ψ-cumidyl-, *o*- and *p*-anisidyl-, 4-*m*- and 2-*p*-xylidyl-, α- and β-naphthyl-rhodanic acids to yield the corresponding 3-aryl-rhodanal-$\Delta^{5,3'}$-oxindoles.

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NOTES

Glucosides of the Navel Orange.[1]—Hesperidin has long been known to be a constituent of many varieties of citrus fruits. Chemically, it is a member of the large group of glucosides built around phenolic nuclei. As far as we are aware, no satisfactory explanation has yet been offered as to the physiological functions of members of this group.

The relative proportion of hesperidin present in unripe oranges is greater than in ripe fruit, but O. Tunmann[2] points out that it forms in the young plant and persists throughout its life. The total quantity of hesperidin in a given orange probably does not decrease upon ripening of the fruit, although its percentage decreases due to the increase in the weight of the fruit.

W. Pfeffer[3] reported the occurrence of hesperidin in practically all parts of *Citrus Aurantium* Risso except the seeds and oil cells. He mounted sections in alcohol, whereupon the hesperidin crystals became visible. He states that hesperidin occurs *in solution in the living cell* but raises the question as to the conditions under which this is possible, since the glucoside is nearly insoluble in water and dilute acids.

It is the purpose of this note to present certain evidence bearing on the mode of occurrence of hesperidin in the plant, and its possible physiological function.

Precipitation of Hesperidin in Frozen Oranges.—Navel and Valenica oranges, within a few days after freezing on the tree, develop small, white crystalline aggregates imbedded in the endocarp or section covering tissue. These have been called hesperidin.[4] Further, in the case of navel oranges, these aggregates disappear usually within three months after freezing. In Valencia oranges we have found them eight months after freezing.

[1] The following observations were made in the course of related work on certain constituents of the Navel Orange. Their incomplete nature is fully recognized. However, the problems presented appear to be of sufficient biochemical interest to warrant the publication of this note with a view to opening a field whose further investigation may prove inviting. Other demands on the writer's time prevent his following the work further at present.

[2] Tunmann, "Pflanzenmicrochemie, Gebrüder Borntraeger chemie," Berlin, 1913.

[3] Pfeffer, *Bot. Z.*, **32**, 529 (1874).

[4] Univ. California Agr. Expt. Sta., *Bull.*, **304**, 257 (1919).

Three dozen specimens of navel oranges, artificially frozen on the tree, were kindly provided by Mr. E. M. Chace of the United States Laboratory of Fruit and Vegetable Chemistry. The endocarp was separated from this fruit and the crystalline aggregates were carefully removed under a lens. This very tedious operation yielded 0.107 g. of crystals to which a very small quantity of tissue adhered.

The material was treated with 20 cc. of 5% alcohol containing 1% of sodium hydroxide. The crystals dissolved, forming a light yellow solution. A current of carbon dioxide was then passed into the filtered solution until it was colorless and a minute precipitate appeared. This precipitate, examined under the microscope, appeared to consist entirely of the slender, pointed needles of hesperidin.

A small quantity of the crystals suspended in water was reduced with sodium amalgam; the yellow solution was filtered and acidified with concd. hydrochloric acid, when it developed the fuchsin-red coloration given by hesperidin. Two melting-point determinations gave 249–250°, uncorrected. The melting point of hesperidin is 251°.

There is no doubt that the crystals on the endocarp of frozen navel oranges consist of hesperidin. It will be of interest to determine whether the similiar crystals on frozen Valencias, lemons, grapefruit and tangerines consist of the same substance.

Precipitation of Hesperidin from Filtered Navel Orange Juice.—The expressed juice of navel oranges was filtered brilliantly clear through paper pulp and Filter-Cel. This juice, containing 0.2% of sodium benzoate as a preservative, continued to deposit a white precipitate for weeks, upon standing. Several grams of this substance was collected and examined. Its color reactions were those of hesperidin, and it melted sharply at 251° without further purification.

Glucosides of the Endocarp.—A large quantity of the dried endocarp of ripe navel oranges was prepared by carefully removing it from the sections, avoiding as much as possible the crushing of juice sacs. The thin tissue was then dried in a current of warm air until brittle (three hours) and then dried under a pressure of about 20 mm. at 60°. The fresh tissue was found to contain only 25% of moisture. The dried material was roughly crushed and preserved for use in air-tight containers.

A 25 g. quantity of the dried and crushed tissue was extracted with 200 cc. of C. P. methyl alcohol in a Soxhlet apparatus for six hours. The subsequent procedure is indicated in Table I. The reddish-brown extract was concentrated to 75 cc. and allowed to cool slowly, when a fine, white precipitate formed which was apparently crystalline while in the beaker. However, when filtered off by suction, it almost immediately changed to a brown gum upon exposure to the air.

The filtrate was found to contain more of the substance, and this was

TABLE I

<table>
<tr><td colspan="4">I DRIED ENDOCARP OF NAVEL ORANGES—Extracted with methyl alcohol residue discarded.</td></tr>
<tr><td colspan="4">EXTRACT—Added ether.</td></tr>
<tr><td colspan="3">II WHITE PRECIPITATE—Exposed to air.</td><td rowspan="2">III FILTRATE Concentrated</td></tr>
<tr><td colspan="3">IV BROWN GUM—Dissolved in water and allowed to stand.</td></tr>
<tr><td colspan="2">VI WHITE PRECIPITATE—Boiled with ethyl alcohol.</td><td rowspan="3">VII FILTRATE, GLUCOSE by Osazone</td><td rowspan="3">V CRYSTALS M. p. 253–254°</td></tr>
<tr><td rowspan="2">VIII RESIDUE, HESPERIDIN M. p. 247–248.5°</td><td>SOLUTION Concentrated.</td></tr>
<tr><td>IX CRYSTALS M. p. 236–237°</td></tr>
</table>

obtained by adding ether until no further precipitate appeared. It came down as a fluffy, white mass which soon became a brown gum upon exposure to the air.

This brown gum dissolved in water very readily, forming a clear, light brown solution. In a few minutes a white precipitate appeared that gradually increased in quantity on standing overnight. This precipitate was filtered off and boiled with ethyl alcohol, part dissolving.

From the alcoholic solution, two small fractions of blunt, microscopic needles were obtained which melted at (I) 236–237°, (II) 234–235° (uncorr.). These needles had not the appearance of hesperidin, being blunt, rather than pointed. They gave a similiar reaction to the latter when reduced with sodium amalgam and treated with concd. hydrochloric acid. They dissolved in dil. sodium hydroxide solution to form a yellow solution. Hesperidin melts at 251°; this substance is probably not hesperidin, but closely related to it.

The residue, which was insoluble in ethyl alcohol, was dissolved in 5% sodium hydroxide and reprecipitated with carbon dioxide. This precipitate consisted of the typical needles of hesperidin, gave the fuchsin red with sodium amalgam and hydrochloric acid, gave a yellow solution in sodium hydroxide, was soluble in pyridine and melted at 246–248.5°. This was doubtless hesperidin.

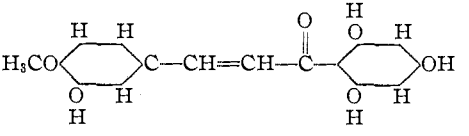
The filtrate from the aqueous solution of the gum from which the substances discussed above were obtained, was sweet to the taste and reduced Fehling's solution. From it a good yield of osazone was prepared which was recrystallized from alcohol. Three fractions melted at 203–204°, 204–205° and 203–204°, respectively.

The melting point of the glucosazone is 204–205°. The sugar present was therefore glucose. No quantitative relations were determined, but it was evident that there was a much greater bulk of sugar than of the glucosides.

Further Treatment of the Methyl Alcoholic Extract.—The filtrate from the amorphous precipitate was distilled to remove ether. Upon concentrating to about 50 cc., a fluffy, white precipitate appeared in some quantity. This was completely soluble in methyl alcohol, from which three fractions were crystallized, all of which melted at 253–254° and were obtained as fine microscopic prisms whose form was quite distinct from that of hesperidin. They dissolved in dil. aqueous sodium hydroxide to form a yellow solution. When reduced with sodium amalgam and acidified with hydrochloric acid as in the hesperidin test, it developed a reddish tint, but not so pronounced as with hesperidin.

Discussion.—It appears highly probable that the first amorphous precipitate consisted of two substances in combination with glucose. One of these was hesperidin, the other related to it.

Hesperidin contains in its molecule:[5] 2 hesperetin + 2 glucose + 1 rhamnose. Hesperetin, according to Power and Tutin,[6] probably has the following structure.



It is difficult to conceive of free hesperidin with less than three phenolic hydroxyl groups unsatisfied with sugar molecules. The results recorded above indicate that they are satisfied in the navel orange by glucose, forming a glucose-hesperidin complex. A similar compound with another glucoside accompanies the glucose-hesperidin complex, fulfilling an analogous function.

It would seem that the original white precipitate consisted of these two glucose—glucoside complexes which underwent partial hydrolysis upon exposure to the air. Solution in water and standing completed the hydrolysis, yielding glucose, hesperidin and a related compound.

The final substance, melting at 253–254°, could not be further examined, although the elucidation of its structure should be very interesting. We feel that it is another of the hesperidin group.

It is seen that many of the phenomena described earlier in this paper are explicable on the basis of the existence of hesperidin in the plant in the form of a soluble glucose combination. For example, the slow deposition of hesperidin from filtered juice may be due to the hydrolysis of this compound present in the juice. The appearance of the "crystals" on the endocarp of frozen fruit is always accompanied by ruptured juice sacs. Again we may postulate the hydrolysis of this compound.

[5] Van Rijn, "Die Glykoside," Gebrüder Borntraeger, Berlin, 1900.

[6] Power & Tutin, *J. Chem. Soc.*, **91**, 887 (1907).

Dr. W. J. Robbins,[7] of the University of Missouri, states that when thin sections of fruit tissue are mounted in alcohol and left for hours, the hesperidin appears to be highly concentrated in the immediate vicinity of the fibrovascular bundles, and to be almost entirely absent from the more remote portions. This can hardly be due to the slow infiltration of the alcohol because of the time of immersion, but indicates that the glucoside is really concentrated around the bundles.

These observations lead to the interesting idea that hesperidin and perhaps other phenolic glucosides, may serve as a medium for translocation of the glucose synthesized in the chlorophyllous tissue. The glucose is in combination with the glucoside, forming a soluble, easily hydrolyzable compound, and is thus temporarily withdrawn from metabolism until brought to that portion of the plant where it is to be stored or utilized.

Conclusions.—1. Indications have been found of the existence of a soluble compound of glucose and hesperidin in the endocarp of mature navel oranges. This compound is probably accompanied by another similar substance.

2. Another substance, related to hesperidin but probably not in combination with glucose, also exists in the endocarp.

3. An hypothesis is suggested to explain the possible function in plants of hesperidin and phenolic glucosides with free hydroxyl groups.

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Preparation of Diacetonamine.—There is only one method given in the literature for the preparation of diacetonamine which gives satisfactory yields. This method is given by Everest.[1] The other methods are not numerous and are very unsatisfactory, being laborious and giving low yields. There is one great advantage in using mesityl oxide instead of acetone for the preparation of diacetonamine, namely, the elimination of the possibility of the formation of such compounds as triacetonamine, triacetondiamine and resinous products.

Since it is relatively easy to obtain pure mesityl oxide in large quantities using the method of Roger Adams,[2] the preparation of diacetonamine from mesityl oxide and ammonia was tried. Only the results of experiments with aqueous ammonia are given since the yields from anhydrous ammonia were very poor.

Experiments.—Two hundred g. of mesityl oxide and 280 cc. of aqueous

[7] Private communication.

[1] Everest, *J. Chem. Soc.*, **115**, 588 (1919).

[2] "Organic Syntheses," Vol. I, Wiley and Sons, **1921.**