

entirely in organic combination as an ethereal sulphate. The rôle of this ethereal sulphate in the plant is not known.

The anomalous carbohydrate metabolism of *Irideae laminarioides* is difficult to explain, in view of the fact that no free sugars were found to be present, but instead a sugar alcohol, dulcitol. Perhaps sugars are formed in this plant, but their rate of formation is conditioned by the same rate of utilization and, therefore, they are not detectable; this is a question which requires further study. The formation of dulcitol and its rôle in metabolism of the plant are also obscure. It is possible to assume that galactose is formed as the first product of photosynthesis and then immediately transformed partly into galactan and partly reduced to dulcitol. Since starch is also found to be present in the plant, glucose might perhaps be the first product of photosynthesis and there is a mechanism in operation which permits the transformation of glucose into galactose.

These, however, are mere assumptions and illustrate the great complexity of carbohydrate metabolism in the plant.—W. Z. HASSID, *University of California, Berkeley, California*.

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COLORIMETRIC DETERMINATION OF NARINGIN

The writers have made numerous quantitative determinations of naringin in grapefruit rinds by the method which depends upon the reducing power of the hydrolyzed glucoside. The method was an adaptation of that used for phloridzin (1, 2, 3). When this method was employed in conjunction with regular sugar analyses it was satisfactory, but when, as in the present

case, it was desired to follow the changes in naringin content alone it was necessary to develop a more simple and rapid method.

In the search for a method it was found that the vinaceous red color resulting from the interaction of naringin and ferric chloride could be used for quantitative colorimetric determinations. The new colorimetric method is rapid and apparently more accurate than that previously employed. The naringin content of one or more samples of fresh tissue can be determined within less than one hour. Naringin may, if necessary, be determined from samples of fresh tissue as small as 0.1 gm. The most serious objection to the method lies in the fact that the color, after formation, continues slowly to deepen. To overcome this behavior it is necessary to develop uniform manipulation, especially in the timing. Furthermore, the tissue extracts must be relatively free of pigment to allow satisfactory comparison with the pure standards. The flavedo, but not the albedo, portion of grapefruit rind, offers some difficulty in this respect.

The colorimeter used by the writers is the Duboseq type with 50 mm. tubes and translucent scales marked with 1 mm. graduations. In place of the usual mirror mount there is installed a daylight colorimetric lamp.

The standard solution is made from a 0.2 per cent. naringin¹ stock solution in 95 per cent. ethanol. One ml. of this solution diluted to 25 ml. with water becomes the standard solution. Immediately before using, one drop of a 50 per cent. ferric chloride solution is added to the standard solution which is then transferred to the right hand colorimetric tube, and the vernier set at 50 mm.

The general procedure for the preparation of grapefruit rind extract is as follows: Separate a quantity of albedo from the flavedo and grind it in a food chopper through the nut-butter attachment. Mix this ground tissue and weight out 10 gm. Transfer the 10-gm. sample to an 800-ml. beaker and cover with 500 ml. of hot water. Boil for 10 minutes and strain through cheese cloth into a 500-ml. volumetric flask. Cool the solution and dilute to 500 ml. by adding water. This extract may be used in the left hand colorimetric tube with or without further dilution, depending upon the approximate percentage of naringin in the tissue. The amount of dilution may be determined from the following general statements: (a) For tissue containing less than 1 per cent. of naringin by fresh weight no further dilution is necessary. Immediately before transferring the material to the colorimetric tube add 1 drop of 50 per cent. ferric chloride solution for each 25 ml. of extract. (b) For tissue containing 1 to 2.5 per cent. of naringin dilute 10 ml. of the extract to 25 ml. with water and add one drop of 50 per cent. ferric chloride solution. (c) For tissue containing 2 to 4 per cent. of naringin dilute 10 ml.

¹ Naringin can be purchased from the California Fruit Growers Exchange, Ontario, California. This material should be dissolved in alcohol, filtered and recrystallized before using.

of the extract to 50 ml. with water and add two drops of 50 per cent. ferric chloride solution.

The calculation of naringin from the colorimeter scale readings is simple. The standard solution contains 8 mg. of naringin per 100 ml. The extract is always diluted so that it contains somewhat more naringin than the standard solution, thus all readings for the unknown are less than 50 mm. Assume that for a certain dilution of rind extract the reading is 40 mm.

Then the amount of naringin in such a dilution will be $\frac{50}{40}$ of 8 mg., or 10 mg. per 100 ml. The writers have made a curve of reference for each dilution of extract so that any scale reading can be translated directly and quickly into percentage of naringin in the original fresh tissue. For percentage of naringin on a dry weight basis reference must, of course, be made to the results from corresponding samples taken for dry weight determinations.

As an aid in avoiding the gradual deepening of the color after formation, the standard solution in the colorimeter tube is renewed frequently and easily by making up a number of 25-ml. portions of the standard solution before beginning a series of determinations. Each of these portions is ready for the colorimeter except for the addition of one drop of 50 per cent. ferric chloride solution which should be added, in each case, just before the material is placed in the colorimeter.

Temperature, within a wide range, has practically no effect upon the quality and intensity of the initial color reaction, but it has a noticeable effect upon the rate of deepening of that color afterward. It has been observed also that the 0.2 per cent. naringin stock solution after standing at room temperature undergoes a slow change which is equivalent to about 0.005 per cent. naringin per day if calculated on the basis of the fresh weight of grapefruit rind. These facts suggest that the colorimetric procedure should be carried out under uniform room temperature, and that the naringin stock solution should be made up in quantities which can be used within one or two days.—E. M. HARVEY and G. L. RYGG, *U. S. Department of Agriculture, Division of Fruit and Vegetable Crops and Diseases, Pomona, California.*

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