[CONTRIBUTION FROM THE U. S. CITRUS PRODUCTS STATION, BUREAU OF CHEMISTRY AND SOILS, U. S. DEPARTMENT OF AGRICULTURE]

Preparation of Rhamnose from Naringin[1]

BY GEORGE N. PULLEY AND HARRY W. VON LOESECKE

Naringin ($C_{27}H_{32}O_{14}\cdot 2H_2O$),[2] the bitter glucoside of grapefruit, was first discovered by DeVry[3] in the flowers of grapefruit trees growing in Java. The glucoside is also found in the fruit, chiefly in the albedo, and is most abundant in immature fruit and decreases as the fruit ripens. It may be extracted from grapefruit canning plant waste (including both flavedo and albedo) by grinding the material, covering the ground mass with water and heating for about ten minutes at approximately 80°. The liquid is then removed and used to treat a fresh batch of peel, in this manner building up the concentration of naringin in the liquor. The liquor is filtered and allowed to stand in a cool place to permit the naringin to precipitate. The precipitated glucoside is then removed by filtration. During the period of standing, the liquid is covered with a thin film of toluene to inhibit the growth of microörganisms. The precipitated naringin is recrystallized from water, the yield amounting to about 0.3% based on the weight of the wet cannery waste.

Another possible source of naringin is the waste liquor from citrus waste drying plants, which contains about 0.5% of the glucoside. At the present time the liquor constitutes a public health nuisance because of its high organic solid content. In Florida, the liquor is ponded, or pumped into tanks to be disposed of by running into swamps or by trucking away in tank trucks. During the period of standing, the liquor ferments and the naringin, mixed with more or less yeast, settles out.

Upon hydrolysis, naringin yields naringenin, glucose and rhamnose according to the equation

$$C_{27}H_{32}O_{14}\cdot 2H_2O + 2H_2O = C_{15}H_{12}O_5 + C_6H_{12}O_6\cdot H_2O + C_6H_{12}O_5\cdot H_2O$$

It was believed, therefore, that naringin might serve as the raw material for the preparation of rhamnose. Canning plant refuse costs nothing but hauling charges (which at present amount to about fifty cents per ton in Florida) and recovery of naringin from the refuse is comparatively simple.

Quercitrin is the most often used source of rhamnose, the commercial products being "Quercitrin Powder," "Quercitrin Extract" and "Flavin." Walton[4] points out that such preparations vary greatly in quercitrin content and considerable difficulty is experienced in preparing a good grade of quercitrin economically. He found "Lemon Flavin" to be the richest in quercitrin and describes the preparation of rhamnose by hydrolysis of the flavin.

In our work we have prepared rhamnose by direct hydrolysis of the glucoside, naringin.

Procedure for Preparing Rhamnose from Naringin.—To every kilogram of naringin are added 15 liters of tap water and 300 cc. of concentrated sulfuric acid. This gives a solution of an acidity of approximately 3.7%. The mixture is then refluxed for about two hours. The hot solution is at first clear, but in about an hour becomes cloudy. As refluxing is continued, a brown, oily liquid separates. This subsequently becomes a flocculent precipitate and finally grainy. The material is naringenin and is only slightly soluble in the hot liquor. As the naringenin precipitates, the mixture bumps violently and this can be partly alleviated by allowing a gentle stream of air to bubble through the solution. Completion of hydrolysis may be judged by the appearance of the precipitate, which is grainy at this point. More accurate check, although not essential, may be ascertained by periodic titrations with Fehling's solution. After hydrolysis of one hour the mixture contained 4.06 g. of glucose per 100 cc.; at one and one-half hours 5.23 g.; at two hours 5.82 g. and remaining constant at this figure. Measuring the optical rotation of samples taken during hydrolysis did not give reliable results.

The hydrolyzed solution is allowed to cool, the naringenin removed by filtration and washed free of acid. The quantity of naringenin obtained amounts to about 42% of the naringin taken, or approximately 88% of the theoretical. The amber colored filtrate and washings are neturalized with calcium carbonate, heated to boiling with decolorizing carbon (1% based on the weight of the solution) and then filtered by suction with a filtering aid (2% based on the weight of the solution). The solution, which is now light amber in color, is concentrated *in vacuo* until the total fermentable sugars amount to from 6 to 8%. This concentration can be ascertained with sufficient accuracy by calculation from the hydrolysis equation given above. During concentration there is gen-

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(2) The formula given in the literature for naringin differs according to the source of the reference. The formula used here is the one most generally accepted and is according to Asahina and Inubuse, *J. Pharm. Soc. Japan*, **49**, 128 (1929); *C. A.*, **23**, 3475 (1929).

(3) DeVry, *Jahresber. Pharmacol.*, 132 (1886).

(4) Walton, THIS JOURNAL, **43**, 127 (1921).

erally a further precipitation of calcium salts and these are removed by filtration before proceeding with the next step of the process, namely, fermentation of the glucose.

To the cooled (about 30°) concentrated solution, ammonium phosphate is added at the rate of 0.75 g. per liter. A precipitate of calcium phosphate is sometimes formed. It is not necessary to remove this precipitate. The solution is then inoculated with baker's yeast[5] and incubated at approximately 30° Baker's yeast is used to ferment the glucose because this yeast does not attack rhamnose while, as is well known, it ferments glucose.

The solution is allowed to ferment until evolution of gas has ceased and the supernatant liquor begins to clear. This generally requires about seventy-two hours. The fermented liquor is then filtered by means of a filtering aid, the cake washed free of sugar and the filtrate and washings concentrated *in vacuo* until a sample withdrawn and cooled to room temperature shows graining. Caution should be exercised during concentration because of foaming. Sufficient hot 95% ethyl alcohol is added to the sirup to precipitate inorganic impurities and gummy matter still present. The precipitate is filtered off, washed with alcohol and a small amount of acetic acid added to combined filtrate and washings to obtain small crystals. This facilitates subsequent washing of the crystals. The filtrate and washings are again concentrated *in vacuo* until graining starts.

The crystals are filtered by suction to free them from mother liquor and washed three times with cold 95% ethyl alcohol, three times with equal volumes of cold 95% alcohol and ether, and finally twice with ether. The mother liquor is filtered and concentrated *in vacuo* to yield a further crop of crystals. The yield amounts to approximately 20% of the naringin taken, or about 62% of the theoretical.

The product obtained is pure white and may be recrystallized by dissolving in three parts of 80% alcohol by gently warming, filtering through decolorizing carbon and diluting the clear, water-white filtrate with about an equal volume of water. The solution is then concentrated in a vacuum to a thick sirup (about 50% total solids). The cooled sirup quickly grains, especially in the ice box. The sugar is filtered off by suction, washed with a small amount of ice water and the washings and drained mother liquor added to the mother liquor from the first crystallization. The washed crystals are dried at 45 to 50°.

Rhamnose, once crystallized, had an initial reading after three and one-half minutes (4.162 g. in 100 cc. read in a 200-mm. tube) of −1.1°V. with a final rotation $[\alpha]^{20}D$ of +7.5°.[6]

We wish to acknowledge the kindness of the Exchange Orange Products Co., of Ontario, California, who furnished part of the naringin used in this investigation.

Summary

A method is described for preparing rhamnose from naringin, the bitter glucoside of grapefruit. The yield of sugar amounts to about 20% of the naringin taken, or approximately 62% of the theoretical.

(5) Yeast from an ordinary cake of Fleischmann's yeast is satisfactory.

(6) We are indebted to Dr. E. Yanovsky of the Carbohydrate Division for his kindness in making this determination.

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The Nitrogen Products Formed by Chlorination of Isothioureas

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The correct interpretation of the mechanism of the reaction occurring when an isothiourea is allowed to interact with chlorine in aqueous solution calls for a knowledge of the structures of the characteristic final reaction products that are formed. It has been the experience of the authors that it is a characteristic behavior of most of the isothiourea salts thus far examined to interact with chlorine in aqueous solution at ordinary temperature, to form an alkyl sulfonyl chloride II. This type of reaction product, however, does not account for all the sulfur of the isothiourea, as a part of this element is oxidized in some cases to the sulfate ion. Also, in some isothiourea structures examined, in which the sulfur is attached to groupings as $(CH_3)_3C$—, $C_2H_5OCH_2$—, $HOOCCH_2$—, $C_2H_5OOCCH_2$— and $C_4H_3OCH_2$— (furfuryl), it was impossible to obtain the corresponding sulfonyl chlorides by chlorination. The nitrogen of a simple isothiourea I can be accounted for completely by the formation of the hydrochloride of cyanamide III. These changes in the case of S-ethylisothiourea hydrochloride are expressed as follows[2]

$$\underset{\text{I}}{C_2H_5SC(NH_2)=NH\cdot HCl} + Cl_2 + H_2O = \underset{\text{II}}{C_2H_5SO_2Cl} + \underset{\text{III}}{NH_2CN\cdot 2HCl}$$

(1) Sterling Professorship of Chemistry Research Assistant, 1936–1937.

(2) Johnson and Sprague, THIS JOURNAL, **58**, 1348 (1936), Sprague and Johnson, *ibid.*, **59**, 1837 (1937); **59**, 2439 (1937).