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Acid-catalyzed hydrolysis of hesperidin at elevated temperatures☆

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Abstract

Dilute sulfuric acid was used as a catalyst for hydrolysis of hesperidin suspensions in water at temperatures ranging from 25 to 180 °C. Significant acceleration of the reaction was observed at 120 °C and higher temperatures. This increase in the rate of hydrolysis can be attributed to increased solubilization of hesperidin in water at higher temperatures. Partial hydrolysis of hesperidin at 140 °C was used for the preparations of hesperetin-7-glucoside, which has a value in the synthesis of dihydrochalcone sweeteners. Simple separation of hesperetin and hesperetin-7-glucoside by extraction with dry acetone or lower alcohols has been developed.

Keywords: Hesperidin; Hesperetin; Hesperetin-7-glucoside; Rhamnose; Acid hydrolysis

1. Introduction

Hesperidin, (*S*)-7-[α-rhamnopyranosyl-(1→6)-β-D-glucopyranosyloxy]-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one, a tasteless rhamnoglucoside (rutinoside) of hesperetin, is one of the most available flavonoid compounds. It is present in fruit, leaves and in the bark of orange (*Citrus sinensis*) and other citrus species [1]. Its concentration is especially high in immature fruit, which can contain more than 40% hesperidin on a dry-weight basis, but the concentration still exceeds 2% of dry weight of peel, rag and pulp in mature sweet oranges, while the concentration in juice is only 0.02–0.06% [2,3].

The bulk of hesperidin (>90%) is thus present in processing byproducts, and a minor amount is in the juice [2]. The citrus processing industry in Florida alone can potentially supply >10,000 tons of hesperidin on an annual basis, with additional hesperidin available from the processing industry in Brazil and elsewhere. Suspended solids in citrus peel juice and molasses are enriched in hesperidin and other insoluble flavonoids [3], but the bulk of hesperidin would have to be extracted from peel and other solid byproducts [2].

Hesperidin can be a source of valuable chemicals and chemical intermediates. Rhamnose (6-deoxy-L-mannose) is a sweet sugar present in many plants in the form of rhamnosides. It is a precursor for the synthesis of a

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flavoring agent furaneol [4-hydroxy-2,5-dimethyl-3(2*H*)-furanone], an important aroma component of some fruits (e.g., pineapple, raspberry and strawberry) and processed foods [4]. Hesperetin, (*S*)-2,3-dihydro-5,7-dihydroxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one, has potential medicinal applications and can be used for preparation of dyes [2] and sweeteners [5,6]. Hesperetin-7-β-D-glucoside, (*S*)-7-(β-D-glucopyranosyloxy)-2,3-dihydro-5-hydroxy-2-(3-hydroxy-4-methoxy phenyl)-4*H*-1-benzopyran-4-one, an intermediate in the acid-catalyzed hydrolysis of hesperidin, is potentially even more valuable, because it can be converted into an intensely sweet dihydrochalcone by simple hydrogenation in alkaline media, whereas hesperidin dihydrochalcone is tasteless [5,6]. Controlled hydrolysis of hesperidin to produce rhamnose and hesperetin-7-β-D-glucoside could lead to production of valuable chemical intermediates. Such hydrolyses are hampered by the very low solubility of hesperidin in water, which is estimated to be 20 ppm or less [2,7,8] as well as in many organic solvents. The low solubility of hesperidin in water causes an unusual resistance to acid- and enzyme-catalyzed hydrolysis and has led to the development of several hydrolytic mixtures containing acid catalysts in organic solvents [9–12]. Low solubility of hesperidin in hot water is also indicated by the use of this solvent to remove impurities from precipitated hesperidin [13].

Wilson [14] overcame the insolubility of hesperidin in aqueous acids by dissolving hesperidin in aqueous alkali and converting it into a salt of hesperidin chalcone. When this solution was added to a hot aqueous acid at $pH < 1$, hydrolysis occurred faster than reformation and precipitation of hesperidin. A disadvantage of this approach is a high consumption of acid for neutralization of the base and formation of inorganic salts as a byproduct.

Since solubility of a related citrus flavonoid naringin, (*S*)-7-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyloxy]-2,3-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one, in water rapidly increases at temperatures higher than 50 °C [15], we felt that hesperidin may exhibit similar behavior at temperatures higher than 100 °C. We therefore investigated the acid-catalyzed hydrolysis of hesperidin in aqueous suspensions at temperatures from 25 to 180 °C. Results of these investigations are described in this communication.

2. Experimental

Hesperidin (97% pure) and hesperetin (>95% pure) were obtained from Sigma-Aldrich (Milwaukee, WI). An authentic sample of hesperetin-7-β-D-glucoside was prepared by R.M. Horowitz and B. Gentili, USDA, (Pasadena, CA). Other chemicals were of reagent grade and were obtained from Sigma-Aldrich. or Fisher Scientific (Pittsburg, PA).

Acid treatment.—Hesperidin was suspended in deionized water in a 2-L stirred pressure reactor constructed from acid-resistant alloy Carpenter 20 Cb (Parr Instrument Co., Moline, IL). The reactor was equipped with an acid injection device constructed from an acid- and pressure-resistant sample cylinder (Whitney Co., Highland Heights, OH) by adding a valve on each end and a tubing connecting one end of the cylinder to the reactor and the other one to a pressurized nitrogen supply. The diluted acid in the injection device was preheated to a proximity of the desired temperature by an electric tape heater.

The reactor charge was preheated to a desired temperature, the required amount of dilute sulfuric acid was injected by nitrogen overpressure, and the timing of the reaction was initiated. The temperature during the reaction was controlled to ±1 °C, and the reaction was quenched by immersing the reactor in an ice-water bath. A portion of cooled reaction mixture was filtered through a glass fiber filter (1.2 μm pore size) and washed with deionized water for determination of dry weight loss. The combined filtrate and washes were neutralized by adding sodium bicarbonate, and these were used for determination of sugars released by the acid treatment. Samples of solid residues from the reactor were dissolved in Me_2SO and analyzed by thin-layer or reversed-phase liquid chromatography as described below. The solid residues were also

extracted with hot lower aliphatic alcohols or acetone to investigate separation of hesperetin, hesperetin-7-glucoside, and hesperidin.

Analytical procedures.—Rhamnose and glucose were separated and determined by ion-exchange chromatography on a 4×250 mm CarboPac PA-1 anion-exchange column (Dionex Corp., Sunnyvale, CA) using dilute NaOH and NaOH–sodium acetate gradient as eluants [16] and a pulsed amperometric detector (Dionex). 2-Deoxy-D-galactose (Sigma-Aldrich) was used as an internal standard, and the detector response was calibrated for each individual sugar. Hesperidin, hesperetin-7-β-D-glucoside and hesperetin were usually separated by thin-layer chromatography on 20 cm cellulose plates (Sigma-Aldrich, 100 μm thick) using 15% AcOH–water as the solvent system [12]. Plates were dried in a stream of air, developed by exposure to ammonia fumes, and observed under UV light [17] to estimate relative amounts of each compound in various fractions.

Separation and quantitative determination of flavonoids in selected samples was obtained by HPLC using a C_{18} phase bonded to silica gel (Partisil 5ODS3 analytical column, Whatman Lab Sales, Hillsboro, OR) as described previously [3].

Authentic samples of hesperidin, hesperetin-7-β-D-glucoside and hesperetin were used as external standards for identification and quantitative determination of the respective compounds in both chromatographic systems.

Solubility of hesperidin.—Solubility of hesperidin in water at elevated (120, 130 and 140 °C) temperatures and pressures was estimated by placing increasing concentrations of hesperidin powder in water into thick-walled, screw-capped glass vials (Reacti-Vial™, Pierce Chemical Co., Rockford, IL) and heating a set of vials to a desired temperature in a heating block. Even distribution of heat was assured by partially filling the heating block wells with glycerol.

Procedure for preparing hesperetin-7-glucoside from hesperidin.—Hesperidin (50 g) was suspended in 850 mL of deionized water, and the slurry was poured into a pressure-resistant reactor. The reactor was closed and heated under stirring to 140 °C. Preheated 0.5% (v/v) H_2SO_4 (100 mL) was then injected, hesperidin was hydrolyzed for 1 h at 140 ± 1 °C and the reaction was stopped by immersing the reactor in an ice-water bath. The reactor and its contents were stored in the cold room at 2 °C for 2–12 h to allow precipitation of hesperetin and hesperetin glycosides from the aq phase. The clear aq layer could then be poured off, with only minor (0.2–0.3 g) losses of total hesperetin derivatives. Resinous material which coated vessel walls and other interior surfaces was dissolved in 500 mL of warm (40–45 °C) acetone containing 50 mL of deionized water. The acetone solution was cooled to rt, filtered and evaporated to dryness under reduced pressure. Approximately 26–28 g of beige powdery residue was recovered and dried overnight in the vacuum oven at 40 °C. Dry solids were broken into small pieces and stirred with 100 mL of dry, warm (40 ± 5 °C) acetone for 2–3 h. A light beige powdery residue was filtered off, washed twice with 50 mL of dry acetone, dried in the stream of air and finally dried in the vacuum oven. Approximately 8–13 g of essentially pure (95%) hesperetin-7-glucoside were obtained in this manner. An additional 2–3 g of hesperitin-7-glucoside were obtained by repeating the procedure described above, using 50 mL of warm dry acetone to dissolve the bulk of the dry residue from the first filtrate, filtering the insoluble solids and washing the filter with 50 mL of dry acetone. Hesperetin-7-glucoside was thus obtained in approximately 26–42% of theoretical yield. Additional removal of hesperetin can be accomplished by suspending dry hesperetin-7-glucoside in 100 mL of dry, hot (60–80 °C) 1-butanol or 1-propanol and filtering the slurry. Hesperetin-7-glucoside is practically insoluble in these solvents, but hesperetin forms approximately a 3 and 5% solution in 1-propanol at 65 and 80 °C, respectively. Purified hesperetin-7-glucoside was sometimes contaminated by charred particles from the reactor. In these cases hesperetin-7-glucoside was further purified by dissolving it in DMF to 10–20% concentration. Black particles were removed by centrifugation, hesperetin-7-glu-

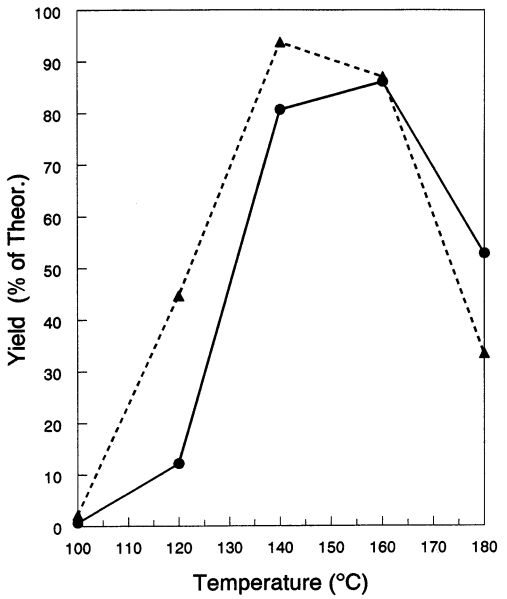

Fig. 1. Yield of glucose and rhamnose during hydrolysis of 0.5% hesperidin slurry in 0.5% (v/v) sulfuric acid for 10 min at 100–180 °C. –●– glc, --▲-- rha.

coside was precipitated by diluting the DMF solution with 4–9 volumes of water with recovery of the material by filtration or centrifugation. The resulting white precipitate was washed with deionized water and dried at rt.

3. Results and discussion

An initial set of experiments designed to test the hypothesis that the acid-catalyzed hydrolysis of hesperidin at elevated temperatures should proceed at much higher rates is summarized in Fig. 1. Using a low concentration of hesperidin (0.5 wt.%), we observed a rapid production of rhamnose and glucose at 120 °C and higher temperatures. A sizable decrease in the yields of rhamnose and glucose at 160–180 °C can be attributed to the acid-catalyzed decomposition of monosaccharides at elevated temperatures [18]. The hydrolyses at 160 and 180 °C must have been very rapid, because another hydrolysis at 140 °C for 5 min produced rhamnose in 92.3% and glucose in 51.5% yields, respectively. Unusually large effects of elevated temperatures on the rate of hesperidin hydrolysis were confirmed by several reactions performed at 60–100 °C and longer reaction times. Results of these reactions, summarized in Table 1, show that the rate of hesperidin hydrolysis decreases dramatically at 100 °C and lower temperatures. The yield of rhamnose was only 54.3% after 10 h of reaction at 100 °C. Hesperidin is also quite resistant to acid-catalyzed hydrolysis at room temperature, because no rhamnose or glucose was released by treatment of 0.5% hesperidin slurry with 0.5% H_2SO_4 for 5 days at room temperature.

Increased solubilization of hesperidin in water at elevated temperatures was confirmed by a separate set of experiments described in Section 2. The solubility of hesperidin in water is approximately 0.1, 0.15 and 0.25 wt.% at 120, 130, 140 °C, respectively. These solubilities compare very favorably with the extremely low (0.002%) solubility of hesperidin in water at 60 °C [8] and are a probable reason for accelerated acid-catalyzed hydrolysis of hesperidin at high temperatures. We could not determine the solubility of hesperidin at temperatures higher than 140 °C, due to increased pressures and equipment limitations, but even higher solubility can be expected at these temperatures.

Since concentration of the acid catalyst affects the relative rates of hydrolysis of individual glycosidic bonds, we performed several investigations of hesperidin hydrolysis at 120 and 140 °C using different concentrations of sulfuric acid. The results are summarized in Figs. 2 and 3. The results summarized for hydrolysis with 0.5% sulfuric acid at 120 and

Table 1
Hydrolysis of hesperidin at 60–100 °C

Temperature (°C)	60	80	100			
Reaction time (min)	60	60	10	60	300	600
Rhamnose yield (% theor.)	0	0.1	2.2	6.9	32.7	54.3
Glucose yield (% theor.)	0	0	0.7	2.2	18.3	37.3

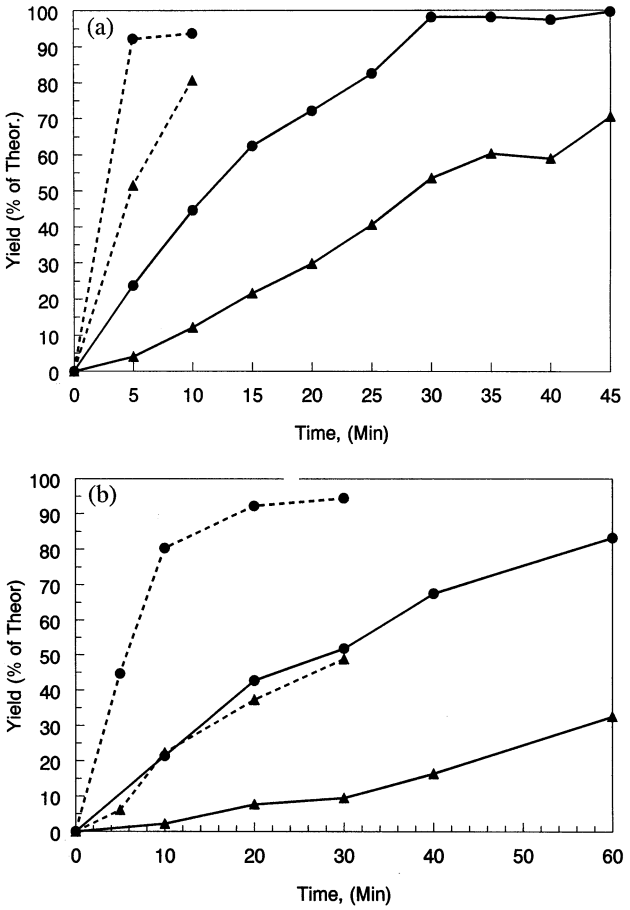

Fig. 2. (a) Production of glucose and rhamnose during hydrolysis of 0.5% hesperidin slurry in 0.5% (v/v) sulfuric acid at 120 and 140 °C. –▲– glc 120 °C, –●– rha 120 °C, –▲– glc 140 °C, –●– rha 140 °C. (b) Production of glucose and rhamnose during hydrolysis of 0.5% hesperidin slurry in 0.01 and 0.05% (v/v) sulfuric acid at 140 °C. –▲– glc 0.01% H_2SO_4, –●– rha 0.01% H_2SO_4, –▲– glc 0.05% H_2SO_4, –●– rha 0.05% H_2SO_4.

140 °C (Fig. 2(a)) support rapid increase in the rate of hydrolysis of both glycosidic bonds with increasing temperature. A tenfold decrease in acid concentration led to only fourfold increase in the time required for complete hydrolysis of rhamnosyl groups (Fig. 2(b)), but an additional decrease in acid concentration to 0.01% considerably decreased the reaction rate.

The selectivity (i.e., ratio of rhamnose to glucose produced) of acid-catalyzed hydrolysis is illustrated in Fig. 3 for data from Fig. 2. The selectivity was high during initial stages of reaction, when concentration of hesperidin was high, but it steadily decreased with increasing degree of conversion and all data converged at an approximate rha:glc ratio of 2:1 when almost complete release of rhamnose had been achieved. The molar ratio of hesperetin-7-glucoside to hesperetin is approximately 1:1 at this point with no remaining hesperidin (data not shown). Additional hydrolysis of hesperidin-7-glucoside will produce additional glucose and hesperetin, so the ratio of rhamnose to glucose will ultimately become 1:1.

The experiments described above indicate that optimal conditions for the preparation of rhamnose and hesperetin-7-glucoside by hydrolysis of hesperidin in aqueous sulfuric acid occur at 0.05–0.5% concentration of sulfuric acid and 140–180 °C. A temperature range of 140–160 °C may be preferable, because we observed significant darkening of the reaction mixture hydrolyzed at 180 °C. Since several publications describe purification and recovery of rhamnose from hydrolyzates of hesperidin and naringin [19–21], we did not investigate recovery of this sugar. However, only chromatographic purification of hesperetin-7-glucoside has been investigated [12], which would make preparation of this compound tedious and expensive. Therefore, we have investigated purification of hesperetin-7-glucoside by a simpler solvent extraction. Tests of solubility of hesperetin revealed that

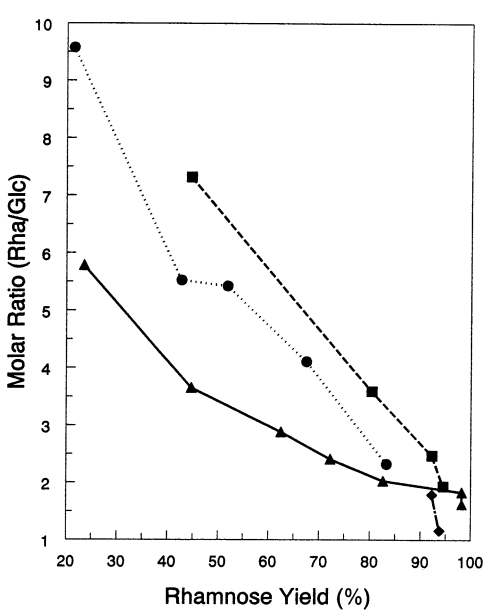

Fig. 3. Molar ratio of rhamnose to glucose as a function of rhamnose yield during hydrolysis of 0.5% hesperidin slurry in 0.01–0.5% (v/v) sulfuric acid at 120 and 140 °C. –▲– 120 °C, 0.05% H_2SO_4; ···●··· 140 °C, 0.01% H_2SO_4; –■– 140 °C. 0.05% H_2SO_4; ·–◆–· 140 °C, 0.5% H_2SO_4.

it is quite soluble in dry acetone, forming approximately 8% (w/v) solution at 25 °C, 11% at 40 °C and 13% at 50 °C, respectively. Hesperetin is also soluble in methanol, forming a 2% solution at 30 °C and in hot lower alcohols, forming a 4% solution in methanol at 55, ethanol at 65, 1-propanol at 70 and 2-propanol at 75 °C, respectively. It is less soluble in 2-butanol, and glacial acetic acid, forming 2% solution at 65 and 80 °C, respectively, and ethyl acetate which produces approximately 1.5% solution at 50 °C. The solubility of hesperetin-7-glucoside in dry acetone is approximately 0.1% at room temperature; therefore, use of this solvent allows efficient separation of relatively insoluble hesperetin-7-glucoside from hesperetin. Hesperidin is practically insoluble in all solvents listed above.

Hesperetin-7-glucoside and hesperetin can be separated from hesperidin by dissolving the first two compounds in aqueous acetone. Hesperetin-7-glucoside forms approximately 2% solution in 60 and 80% acetone–water mixtures at room temperature and 5% solution in both solvents at 50 °C. Solubility of hesperidin in both solvents is less than 0.1% in the temperature range of 25–50 °C, but hesperetin is very soluble in 80% acetone, forming approximately 19 and 24% solutions at room temperature and 50 °C, respectively. Hesperetin is less soluble in 60% acetone, forming 4 and 8% solutions at room temperature and 50 °C, respectively. Of the solvent systems we investigated, acetone, either dry or diluted with a small amount of water, was the most efficient solvent for the separation of hesperetin, hesperetin-7-glucoside and hesperidin.

Hydrolysis of hesperidin in aqueous sulfuric acid provides several advantages over previous methods utilizing acid catalysts in organic solvents, namely shorter reaction time, simple separation of sugars from hesperetin derivatives, lower solvent costs, and decreased safety and environmental problems.

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