distilled water and the combined percolates were evaporated to dryness under reduced pressure. The pale yellow crystalline residue consisted of essentially pure DL-carnitine (yield 1.05 g., 78%). This material can be recrystallized by dissolving it in 30 volumes of 60:40 acetone–ethanol, filtering from any insoluble material, and adding anhydrous acetone at 0° in successive 60-volume portions (total of 240 volumes). Each fraction is collected after 12–24 hours in the cold room. By this procedure the 1.05-g. sample above gave 0.81 g. of crystalline DL-carnitine (m.p. 194–196°) in 4 fractions (last two collected together). Pure DL-carnitine is a hygroscopic crystalline solid melting at 195–197° dec. (Found: C, 51.78; H, 9.36; N, 8.92.) The chloroaurate melts at 154–156° (Found: C, 16.9; H, 3.15; N, 2.77) and the reineckate melts at 146–147°.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS

Hydrolysis of Some Flavonoid Rhamnoglucosides to Flavonoid Glucosides

BY DANIEL W. FOX, WILLIAM L. SAVAGE AND SIMON H. WENDER

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The rhamnoglucosides rutin, hesperidin and naringin are among the most readily available flavonoid compounds at present. The glucosides corresponding to the above three flavonoids are quercetin-3-glucoside (isoquercitrin), hesperetin-7-glucoside and naringenin-7-glucoside (prunin). These latter three compounds are desired for biological testing, and have not been readily available in the amounts needed. To our knowledge, partial hydrolysis of the rhamnoglucoside to remove the rhamnose and leave the glucose still attached to the flavonoid portion had not been successfully achieved. The present paper reports a method for accomplishing the hydrolyses in significant amount of rutin, hesperidin and naringin to the corresponding flavonoid glucosides. The method appears likely to be a general one for this type of compound.

Formic acid in cyclohexanol has been used for the hydrolysis. The hydrolysate containing a mixture of flavonoids has been separated chromatographically and the identity of each resulting pure flavonoid glucoside has been established.

Experimental

Hydrolysis of Rutin.—Rutin (S. B. Penick and Co., New York) was first purified by treatment with Magnesol[1] from methanol solution, and then by several recrystallizations from methanol. Although the highly purified rutin was used in these experiments, this additional purification is not necessary for routine preparation of isoquercitrin. Ten grams of the pure rutin was dissolved in 225 ml. of boiling cyclohexanol. Seventy-five ml. of formic acid (85–90%) was next added through the reflux condenser, the addition being performed as rapidly as possible without flooding. The mixture was then refluxed, with stirring, for approximately 10 hours at a temperature of 102–107°. Small samples were withdrawn at regular intervals and chromatographed on paper, usually using 15% acetic acid–water as the solvent system. One could thus follow the progress of the hydrolysis and estimate the relative amounts of isoquercitrin, quercetin and unhydrolyzed rutin present (R_f values in the 15% acetic acid are 0.45, 0.09 and 0.62, respectively). When the highly purified rutin is used, and the reaction is followed closely with paper chromatograms, isoquercitrin is detectable before the quercetin.

After about 10 hr. of hydrolysis, distillation of solvent was carried out *in vacuo* until practically no liquid came over. The volume of the mixture was made up to 600 ml. with anhydrous acetone and filtered through a 2.5 × 3 cm. column of Magnesol. The filtrate was then put on a 7 × 26 cm. column of Magnesol packed as an acetone slurry. When all of the flavonoids had been adsorbed, the top half of the column was colored. Several hundred ml. of anhydrous ethyl acetate was then passed through the column to displace the acetone and cyclohexanol. The column was next developed as a liquid chromatogram with wet ethyl acetate. A definite break occurred between the bulk of the quercetin and the glycosides, but sufficient quercetin still remained on the column to make detection of the glycoside zones almost impossible. The eluant was, therefore, collected in 200-ml. fractions. Small portions of each fraction were spotted on paper strips for chromatographic study. At the first appearance of isoquercitrin, the eluted fraction was saved and combined with all following fractions which showed isoquercitrin to be present. The combined solution of all the eluted fractions containing isoquercitrin was concentrated to 750 ml. by distillation and then rechromatographed on a fresh 3.5 × 20 cm. column of Magnesol packed as an anhydrous ethyl acetate slurry, and the column developed with an ethyl acetate–water solution. Four zones were detected on this column in both visible and ultraviolet light as elution proceeded. When the major portion of zone one, containing the quercetin, reached the bottom of the column, a faint zone, containing an unknown glycoside, was noted between this fastest moving pigment (quercetin) and the isoquercitrin which occupied the center portion of the column. Some unhydrolyzed rutin remained at the top of this column, but most of the rutin had been left on the preceding column. The major part of the center zone, containing the isoquercitrin, was eluted and the solvent removed *in vacuo*. Yellow solid isoquercitrin—yield approximately 760 mg.—was obtained.

For final purification, the isoquercitrin was recrystallized at least four times from boiling alcohol–water. The supernatant liquid was removed by decantation after centrifugation. A trace of an oily material could not be removed if regular filtration were used. The recrystallized isoquercitrin was dried at 110° for 1 hr. and gave a m.p. of 228°, uncor. On paper chromatograms, it showed no trace of quercetin or any other impurity, and its R_f values corresponded to those of authentic isoquercitrin isolated from plant sources.[2] The 3′,4′,5,7-tetramethoxy-3-hydroxyflavone was obtained according to the method of Shimokoriyama,[3] m.p. 195°. Hydrolysis of the prepared isoquercitrin yielded one sugar, glucose, which was identified by paper chromatography, and quercetin, which was identified by its R_f values, its m.p. 314–315°, and its pentaacetate, m.p. 194–195°.[4]

Hydrolysis of Hesperidin.—Five grams of hesperidin was dissolved in 250 ml. of boiling cyclohexanol (minimum solvent requirement) and 125 ml. of formic acid (85–90%) was added as rapidly as possible through the reflux condenser. The hydrolysis mixture was refluxed approximately 20 hr. and then distilled until the temperature reached 135°. Samples were withdrawn at 3–4-hr. intervals, and chromatographed on paper, using 15% acetic acid–water as the solvent system. R_f values in this system were 0.80 for hesperidin, 0.70 for hesperetin-7-glucoside, and 0.43 for hesperetin. From the paper chromatograms, no apparent improvement of yield resulted after about 15 hr., but the amount of aglycone increased, and the amount of hesperidin decreased.

The entire hydrolysis mixture was filtered through a 2.5 × 5 cm. column of Magnesol to remove decomposition material. On washing this column with dry acetone, the very dark material which had been adsorbed was removed. Only the initial filtrate was saved. A rough separation was effected on a 7.5 × 25 cm. column of Magnesol. The cyclohexanol solution was put on an acetone–Magnesol column; washed with dry ethyl acetate to displace the cyclohexanol; and finally developed as a liquid chromatogram with wet ethyl acetate. The entire column was white in ultraviolet light, but a slight break was detectable between the aglycone and the hesperetin-7-glucoside after considerable elution. The hesperidin moved very slowly and remained near the top of the column. The 7-glucoside fraction was concen-

(1) C. H. Ice and S. H. Wender, *Anal. Chem.*, **24**, 1616 (1952).

(2) B. L. Williams, C. H. Ice and S. H. Wender, THIS JOURNAL, **74**, 4566 (1952).

(3) M. Shimokoriyama, *Acta Phytochim. (Japan)*, **15**, 63 (1949).

(4) St. v. Kostanecki, V. Lampe and J. Tambor, *Ber.*, **37**, 1405 (1904).

trated by distillation to 300 ml., and rechromatographed on a 3.7 × 25 cm. column of Magnesol packed from dry ethyl acetate. All three compounds, hesperidin, hesperetin and the hesperetin-7-glucoside were present on this column, but a relatively clean separation was possible. The central portion of the middle zone was collected as a separate fraction and taken to dryness under reduced pressure. The resulting solid—yield about 0.35 g.—was purified by four recrystallizations from alcohol. This sample had a m.p. of 206–207° uncor., and an optical rotation $[\alpha]^{27}D$ −51.8° in pyridine, which agree well with those reported for hesperetin-7-glucoside by Zemplén, *et al.*[5] On hydrolysis, the sugar was identified as glucose, with no trace of rhamnose detectable.

Hydrolysis of Naringin.—Five grams of naringin, 100 ml. of cyclohexanol and 50 ml. of formic acid were refluxed 20 hr. and then the solvent distilled off until the temperature reached 135°. The first detectable trace of naringenin-7-glucoside was found after 3 hr., and continued to increase relatively for about 15–20 hr. R_f values in 15% acetic acid were 0.80 for naringin; 0.75 for naringenin-7-glucoside; and 0.47 for naringenin. After hydrolysis, the volume of the remaining solution was doubled with acetone and filtered through a 1.5 × 3 cm. column of Magnesol. Filtration removed dark, flocculent material. The solution was then chromatographed on a 7 × 25 cm. column of Magnesol packed as an acetone slurry. When all of the reaction mixture had been adsorbed, a column volume of dry ethyl acetate was passed through to displace the cyclohexanol and then wet ethyl acetate was used to elute the liquid chromatogram. The column was too overloaded to permit detection of zones, but some fractionation was possible. The eluate was collected in fractions and microliter portions were spotted on paper. The first fractions contained mainly naringenin and were discarded. Elution was continued, and fractions containing naringenin-7-glucoside now appeared, and were collected, combined, concentrated to 200 ml. *in vacuo*, and rechromatographed on a 4.5 × 20 cm. column of Magnesol, packed this time with an ethyl acetate slurry. Some separation of the zones was detected under ultraviolet light, but a clear-cut separation was not achieved. The naringenin-7-glucoside fraction from this column was concentrated and again rechromatographed on a fresh 2.5 × 15 cm. column of Magnesol. A good separation between the leading naringenin and the central naringenin-7-glucoside was obtained in this case. A dark, narrow zone appeared at both the top and bottom of the central naringenin-7-glucoside zone. The dark zones were collected separately, and the center of the middle zone was taken as the naringenin-7-glucoside. After evaporation at reduced pressure, the resulting solid—yield about 0.40 g.—was recrystallized several times from methanol–water. The m.p. of the naringenin-7-glucoside was 225–226°, uncor., and no lowering occurred on admixture with a sample of authentic prunin. Mixed paper chromatograms of the prepared naringenin-7-glucoside with prunin showed no separation. R_f values for both were 0.84 in 60% acetic acid–water and 0.92 in the butyl alcohol–acetic acid–water system (4-1-5). The naringenin-7-glucoside and the prunin from the cherry tree[6] are thus identical. After hydrolysis, paper chromatography showed the presence of glucose, but no rhamnose, in the neutralized filtrate.

In preparing a sample of naringenin from naringin, using sulfuric acid, according to the method of Will,[7] paper chromatograms at the end of 2 hr. revealed the presence of some naringenin-7-glucoside. Thus, for naringin hydrolysis, there may be a possibility for using sulfuric instead of formic acid.

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(5) G. Zemplén and R. Bognár, *Ber.*, **75B**, 1043 (1942).
(6) M. Hasegawa and T. Sherato, THIS JOURNAL, **74**, 6114 (1952).
(7) W. Will, *Ber.*, **18**, 1316 (1885).

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CHEMISTRY DEPARTMENT
UNIVERSITY OF OKLAHOMA
NORMAN, OKLAHOMA

The Synthesis and Alkaline Decomposition of γ-Aminopropylsulfuric Acid

BY HAROLD W. HEINE, RICHARD W. GREINER, MARJORIE A. BOOTE AND BETSY A. BROWN

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The preparation of γ-aminopropylsulfuric acid was undertaken in an effort to convert it into trimethylenimine. The sulfuric ester was first synthesized by Gabriel and Lauer[1] in unreported yields by treating γ-bromopropylamine with silver sulfate. Yields of 90% crude γ-aminopropylsulfuric acid were obtained in this Laboratory by the treatment of γ-aminopropanol with sulfuric acid.

The γ-aminopropylsulfuric acid was subjected to a flash distillation with excess base to give a small yield of impure trimethylenimine.

Experimental

γ-Aminopropylsulfuric Acid.—In a one-liter flask equipped with a stirring motor, thermometer and dropping funnel were placed 50 g. of water and 105 ml. of concentrated sulfuric acid. To this was added dropwise and with cooling a solution containing 150 g. (2 moles) of redistilled γ-aminopropanol and 75 g. of water. The dropping funnel was replaced by a condenser set for downward distillation and the mixture was distilled under slightly reduced pressure until the temperature of the reaction reached 190°. Approximately 140 ml. of water was collected. The mixture was allowed to cool and crystallization was induced by scratching the walls of the flask with a glass rod. The solid cake was then softened with 300 ml. of 95% ethanol, removed from the flask, ground with an additional 400 ml. of ethanol, filtered and dried. The crude γ-aminopropylsulfuric acid (278 g.) was then recrystallized from water. The once recrystallized product melted at 219–220°. Gabriel[1] reported a melting point of 221°. Further recrystallization gave a product which melted at 227–228°.

Anal.[2] Calcd. for $C_3H_9C_4NS$: N, 9.02; S, 20.66. Found: N, 8.84; S, 20.54.

Trimethylenimine.—The γ-aminopropylsulfuric acid (155 g.) was decomposed in an analogous fashion employed by Reeves[3] for the decomposition of β-aminoethylsulfuric acid. A fraction (1 g.) distilling at 66–69° was obtained. This corresponds to a 1.7% yield of trimethylenimine. The picrate of this fraction melted at 166° and the chloroplatinate at 201°. Gabriel[4] reported melting points for the picrate and chloroplatinate of trimethylenimine at 165° and 200–203°, respectively. The refractive index, however, of the 66–69° fraction was n^{25} 1.3450 which did not correspond to the value observed by Ruzicka[5] for trimethylenimine, *viz.*, $n^{25}D$ 1.4287. The product was considered moist and redried over anhydrous sodium sulfate and redistilled. The refractive index did not change. Further attempts of drying over solid potassium hydroxide and redistillation still gave the same value for the refractive index. A low-boiling fraction 47–64° and a high-boiling fraction 110–191° were also obtained but were not characterized.

(1) S. Gabriel and W. E. Lauer, *Ber.*, **23**, 87 (1890).
(2) Analyses were made by Schwarzkopf Microanalytical Laboratory, Middle Village, N. Y.
(3) W. A. Reeves, G. L. Drake and C. Hoffpaur, THIS JOURNAL, **73**, 3522 (1951).
(4) S. Gabriel and J. Weiner, *Ber.*, **21**, 2676 (1888).
(5) L. Ruzicka, G. Salomon and K. E. Meyer, *Helv. Chim. Acta*, **20**, 109 (1937).