light yellow oil which solidified on cooling to large almost colorless rectangular plates, b.p. 161–164° (0.25 mm.), m.p. 51–53°. For analysis a sample was crystallized from hexane, m.p. 54–55° (lit. 56°).[9]

Ethyl 5-Benzyloxyindole-2-carboxylate.—4-Benzyloxyaniline (116 g., 0.583 mole) was dissolved in 250 ml. of boiling ethanol. The hydrochloride was precipitated in a finely divided form by the rapid addition with vigorous stirring of a solution of 233 ml. of concentrated hydrochloric acid in 360 ml. of water followed by the addition of 1 kg. of ice. A solution of 47.7 g. (0.68 mole) of sodium nitrite in 110 ml. of water was run in below the surface of the cream-colored slurry and stirring was continued until a clear solution resulted (*ca.* 20 min.).

Ethyl methylacetoacetate (92 g., 0.64 mole) was dissolved in 600 ml. of ethanol. To this solution was added 100 g. of potassium hydroxide dissolved in 100 ml. of water followed by 1 kg. of ice. The diazonium solution was then added in one portion with stirring. The red oil which separated was taken up in benzene, the benzene extracts dried superficially by filtration through anhydrous potassium carbonate, and the solvent removed under vacuum on the steam-bath. The crude red liquid phenylhydrazone was dissolved in 450 ml. of absolute ethanol and a rapid stream of dry hydrogen chloride was passed into the solution until a precipitate formed (*ca.* 20 min.). The introduction of hydrogen chloride was then continued for 10 min. longer. When the spontaneous exothermic reaction subsided (*ca.* 15 min.) the mixture was allowed to stand overnight in the refrigerator. The product was filtered off, washed with several small portions of ice-cold ethanol and then with water until the washings were substantially free of chloride ion. The air-dried yellow prisms weighed 110–117.5 g. (46–49%), m.p. 161–163°. A sample recrystallized from carbon tetrachloride for analysis melted at 162–164°.

Anal. Calcd. for $C_{18}H_{17}NO_3$: N, 4.74. Found: N, 4.69, 4.75.

5-Benzyloxyindole-2-carboxylic Acid.—Ethyl 5-benzyloxyindole-2-carboxylate (117.5 g., 0.4 mole) was dissolved in 3 l. of boiling ethanol. With stirring a solution of 237 g. (3 moles) of potassium hydroxide in 400 ml. of water was added rapidly. Crystallization of the potassium salt began in a few minutes. The suspension was refluxed 1 hr., acidified with acetic acid, and poured into 10 l. of cold water. The solid was filtered off, washed well with water, and crystallized from aqueous acetic acid. The yield of light tan crystals was 89–101 g. (84–95%), m.p. 194.5–195.5° (dec.) (lit. m.p. 193–194°[10]).

5-Benzyloxyindole.—5-Benzyloxyindole-2-carboxylic acid (45.0 g., 0.169 mole) was heated for 1.5 hr. in a Claisen flask at a bath temperature of 210–220°. The dark brown melt was distilled in vacuum yielding 27.5 g. of an almost colorless liquid which solidified on cooling, b.p. 176–190° (0.1 mm.). Redistillation of the crude product (26.0 g., b.p. 182–188° (0.1 mm.), m.p. 81–86°) and crystallization from toluene–hexane gave 24.3 g. (65%) of fine colorless needles, m.p. 94–96° (lit. m.p. 96–97°[6]).

(9) P. Jacobson, *Ann.*, **287**, 182 (1895); L. Spiegel and S. Sabbath, *Ber.*, **34**, 1944 (1901).

(10) F. Bergel and A. L. Morrison, *J. Chem. Soc.*, 49 (1943).

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Improvements in the Synthesis of DL-Carnitine[1]

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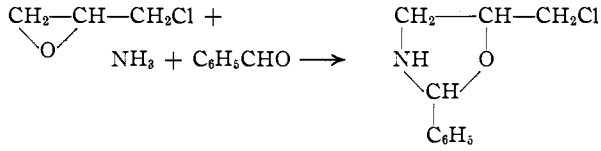
In the course of studies on the biochemistry of (−)-carnitine (β-hydroxy-γ-butyrobetaine) we had occasion to synthesize a quantity of the racemic form. Several crystalline salts of DL-carnitine have been prepared but the substance itself has been obtained only as a hygroscopic gum. The present note describes the preparation of crystalline DL-carnitine by a modified procedure giving considerably better yields than those obtained by Bergmann, *et al.*[2] It was discovered that a key intermediate—2 - phenyl - 5 - chloromethyloxazolidine—could be obtained in one step (instead of three) by the direct condensation of epichlorohydrin, ammonia and benzaldehyde (yield 70–80%).



Subsequent reactions leading to β-hydroxy-γ-aminobutyric acid were carried out as previously described.[2]

Methylation of β-hydroxy-γ-aminobutyric acid was effected by a slight modification of the procedure of Tomita[3] and the product was purified by extraction into phenol from an aqueous solution. This extraction procedure has been used to advantage in the isolation of (−)-carnitine from natural sources.[4] The yield of crystalline DL-carnitine in this step was 75–78% and the over-all yield was 20–25% (compared to the 5–7% yield obtained according to methods previously described). In view of the recent identification of (−)-carnitine as an essential growth factor for *Tenebrio molitor*[5] it seemed worthwhile to make available these modifications in the synthesis of DL-carnitine.

Experimental

2-Phenyl-5-chloromethyloxazolidine.—To a solution of 212 g. of benzaldehyde in 1000 ml. of ethanol was added with stirring 200 ml. of concentrated aqueous ammonium hydroxide (29% NH_3). This solution was stirred continually while 185 g. of epichlorhydrin was added in a thin stream. The reaction mixture warmed spontaneously to 40–45° over a 2-hour period. It was allowed to stand overnight at room temperature and was then heated on the steam-bath for 20 minutes to complete the reaction. The alcohol and ammonia were removed on the water pump and the residual yellow sirup was poured into 200 ml. of ice-water. The mixture was cooled in an ice-bath and stirred from time to time. Over a 4-hour period the entire mass solidified. The solid residue was filtered and sucked dry on the filter giving 345 g. of crude material. Recrystallization of this material from 4800 ml. of hot hexane gave 271 g. (69% yield) of pure 2-phenyl-5-chloromethyloxazolidine melting at 81–83°. The mother liquor was cooled overnight at −11° giving 42 g. of less pure material.

Conversion of β-Hydroxy-γ-aminobutyric Acid to DL-Carnitine.—A solution of 7 g. of methyl iodide in 50 ml. of methanol was added to a solution of 1.0 g. of β-hydroxy-γ-aminobutyric acid and 2.0 g. of potassium hydroxide in 5 ml. of water. The mixture was refluxed slowly under an efficient condenser for 36 hours (*p*H of solution about 5.5). The reaction mixture was evaporated to dryness on the water pump and the residue was dissolved in 50 ml. of water. The solution was extracted with three 50-ml. portions of phenol saturated with water. The three phenol extracts were washed countercurrently with two 50-ml. portions of water. The phenol extracts were then combined and poured into 450 ml. of ether in a separatory funnel. The aqueous layer was separated and the ether–phenol layer was washed with three 50-ml. portions of water. The combined aqueous extracts were washed with 450 ml. of ether and passed through a column containing 100 ml. of Amberlite IRA 45 in the hydroxyl phase. The column was washed with 500 ml. of

(1) The authors are happy to acknowledge a research grant from Merck and Co., Inc., in support of this work.

(2) M. Bergmann, E. Brand and F. Weinmann, *Z. physiol. Chem.*, **131**, 1 (1923).

(3) M. Tomita, *ibid.*, **124**, 253 (1922–1923).

(4) G. Fraenkel, *Arch. Biochem. Biophys.*, **34**, 468 (1951).

(5) H. E. Carter, P. K. Bhattacharyya, K. R. Weidman and G. Fraenkel, *ibid.*, **38**, 405 (1952).

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°.

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Hydrolysis of Some Flavonoid Rhamnoglucosides to Flavonoid Glucosides

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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).