[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, TRACERLAB, INC.]

An Improved Synthesis of Citric Acid-1,5-C^{14} [1]

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The synthesis of citric acid labeled symmetrically in the 1- and 5-positions with carbon-14 has been accomplished in relatively high yield. A new procedure for the extraction of citric acid from the reaction mixture simplifies the isolation of pure material.

Recent interest in carbon-14 labeled citric acid as a tool in the study of carbohydrate metabolism makes it desirable to present a well-defined synthesis of this compound. Early reports[2,3,4] on the preparation of citric acid are vague as to the experimental details and yields, while a recent paper[5] on the preparation of asymmetrically labeled citric acid reports too low a yield for a general synthetic approach to labeled citric acid.

We have examined the method of Grimaux and Adam in detail and have succeeded in defining the conditions required for the preparation of citric acid-1,5-C^{14} in 35–40% yield by the reaction of labeled cyanide with potassium bis-chloromethylglycolate. The citric acid was isolated as trimethyl citrate prepared by addition of an excess of an ethereal solution of diazomethane to the dry hydrolysis mixture. This method of isolation is superior to the usual method which employs the calcium salt, inasmuch as trimethyl citrate is a crystalline solid with a sharp melting point and is easily purified prior to its conversion to citric acid.

During the course of this work two new compounds were characterized, bis-chloromethylglycolamide (Ia) and methyl bis-chloromethylglycolate (Ib).

$$(ClCH_2)_2C(OH)CO\text{—} \qquad \begin{array}{l} Ia = \text{—}NH_2 \\ Ib = \text{—}OCH_3 \end{array}$$

Experimental[6]

Bis-chloromethylglycolic Acid.—A twofold excess of hydrogen cyanide was added rapidly to a slurry of 105 g. of symmetrical dichloroacetone in 10 ml. ethanol at 0°. About 100 mg. of sodium cyanide was added and the mixture was stirred at 0 to 10° for two hours and then at 60° for two hours. After cooling, 250 ml. of cold concd. hydrochloric acid was cautiously added with stirring. The solution was kept at 0° overnight and finally boiled under reflux for 24 hours. The hydrochloric acid was evaporated, water was added, and the mixture extracted with ether. A total of 117 g. (82%) of product was isolated, m.p. 93–94°; reported 91–92°.[1]

Bis-chloromethylglycolamide.—When the hydrochloric acid hydrolysis of the nitrile was conducted at 100° for one hour, bis-chloromethylglycolamide was the principal product, m.p. 93–94°.

Anal. Calcd. for $C_4H_7O_2NCl_2$: C, 27.93; H, 4.10; N, 8.14. Found: C, 28.3; H, 4.2; N, 8.1.

Methyl Bis-chloromethylglycolate.—Esterification of the free acid with excess diazomethane in ether solution yielded the methyl ester in 80% yield, m.p. 44–46°. The analytical sample was isolated by sublimation at 35° in high vacuum, m.p. 45.5–46.5°.

Anal. Calcd. for $C_5H_8O_3Cl_2$: C, 32.11; H, 4.31. Found: C, 32.0; H, 4.3.

Trimethyl Citrate-1,5-C^{14}.—To a solution of 3.91 g. of potassium cyanide-C^{14} (sp. act. 0.5 mc. per mmole) in 10 ml. of water maintained at 75°, a solution of potassium bis-chloromethylglycolate prepared from 5.17 g. of the acid and 2.07 g. of potassium carbonate in 7 ml. of water was added over a ten-minute period. Heating was continued for 15 minutes. The solution, cooled and transferred to a 500 ml. 3-neck r.b. flask, was acidified with hydrochloric acid and evaporated to dryness *in vacuo*. The residue, in 50 ml. of concd. hydrochloric acid, was boiled overnight and then evaporated to dryness *in vacuo*. Removal of the residual hydrochloric acid was achieved by distilling from the product several portions of water. To the residue, cooled in ice-water, a solution of diazomethane prepared from 30 g. of nitrosomethylurea in 450 ml. of ether was added with stirring. After several hours the ether solution was filtered and dried. Distillation of the solvent left a dark oil which readily afforded almost colorless crystals of trimethyl citrate when taken up in several ml. of isopropyl ether; yield 3.11 g. (44%); m.p. 76–77°, reported[7] 78.5–79.0°.

Citric Acid-1,5-C^{14}.—Trimethyl citrate, 1.99 g., was boiled in 20 ml. of concd. hydrochloric acid for several hours. After removal of the hydrochloric acid by distillation, the residue was dissolved in water, decolorized with Norit A, and evaporated to dryness. A solution of the residue in 5 ml. of acetone to which 10 ml. of benzene was added was heated to remove acetone until deposition of crystals began. There was obtained 1.48 g. of citric acid-1-C^{14} (sp. act. 1 mc. per mmole), m.p. 148–150°. An additional 80 mg. was obtained from the filtrate bringing the total yield to 96%.

Analyses were performed on non-labeled citric acid prepared by the above method.

Anal. Calcd. for $C_6H_8O_7$: C, 37.51; H, 4.20; neut. equiv., 64.0. Found: C, 37.5; H, 4.3; neut. equiv., 63.8.

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