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Improved Synthesis of DL-Carnitine Hydrochloride[1]

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We have recently been interested in studying the radiation sensitivity of DL-carnitine hydrochloride, $[(CH_3)_3NCH_2CH(OH)CH_2CO_2H]^+Cl^-$. The first synthetic route to carnitine, involving a Gabriel-type synthesis, was described by Tomita[3]—however, the yields were poor. Another synthesis, based on an oxazolidine intermediate of Bergmann's,[4] has been described by Carter.[5] A final procedure, recently published by Strack, Röhnert and Lorenz[6] involves the preparation of the mononitrile from $ClCH_2CH(OH)CH_2Cl$, followed by treating the chloronitrile with trimethylamine, and finally hydrolyzing the cyano group to carboxyl. This latter procedure was of little interest to us as the over-all yields were also low and because we wished to introduce C^{14} into the carnitine molecule, via $C^{14}H_3I$, at the last step of a reaction sequence. We therefore turned our attention to the Bergmann-Carter synthesis. It is carried out in six steps: (1) epichlorohydrin is treated with benzaldehyde and ammonia to give 5-chloromethyl-2-phenyloxazolidine; (2) the product is treated successively with benzoyl chloride and concentrated HCl to form 3-benzoylamino-1-chloro-2-hydroxypropane; (3) the chloro group is converted to cyano by reaction with KCN to give the corresponding benzoylaminonitrile; (4) the nitrile is hydrolyzed and esterified to the benzoylamino ethyl ester; (5) the ester and benzoylamino groups are simultaneously hydrolyzed to give the free amino acid; and (6) carnitine is formed by quaternization with methyl iodide and KOH.

We have found that this reaction sequence can be simplified by going directly from the benzoylamino-nitrile to the amino acid in a single step.[7] The final step in the sequence, the methylation of the amino acid, is very troublesome. The best methylation procedure now available[5] is hard to apply, particularly for a small-scale preparation with C^{14}, because it involves successive extractions with phenol, countercurrent washings with water, and final washings of aqueous extracts with ether. Our experiments have shown that the use of barium hydroxide as the base in the methylation, removal of barium with H_2SO_4, and exchange of other anions for hydroxide on an ion exchange column, leads to an 88% yield of recrystallized DL-carnitine hydrochloride from the amino acid. The experimental conditions for the improved synthesis are described below. (All melting points are uncorrected.)

EXPERIMENTAL

5-Chloromethyl-2-phenyloxazolidine. The directions given by Carter[5] were followed. It is desirable to use freshly purified benzaldehyde and epichlorohydrin, and to add the epichlorohydrin very slowly (to decrease polymerization). We have obtained a yield of 85% of impure 5-chloromethyl-2-phenyloxazolidine by this procedure and, after crystallization, the analytically pure compound was obtained in 76% yield; m.p. 71°.

3-Benzoylamino-1-chloro-2-hydroxypropane. This is a modification of the procedure given by Bergmann, Randt and Brand.[8] To a solution of 40 g. (0.2 mole) of the pure oxazolidine in 150 ml. of chloroform was added 16 g. (0.2 mole) of pyridine. The solution was cooled to −40° in a dry ice-isopropyl alcohol bath, removed from the bath, and 28 g. (0.2 mole) of benzoyl chloride was added dropwise, with stirring. During this addition the temperature of the reaction mixture reached a maximum of 0°. The mixture was then left overnight at room temperature; however, another experiment indicated that the overnight standing was unnecessary.

Concentrated HCl (200 ml.) was then added and the mixture stirred for 5 min. Finally, 500 ml. of water and 500 ml. of petroleum ether (b.p. 60–70°) were added and the flask was placed in a refrigerator. Crystals soon appeared in the upper (pet. ether) phase and the crystallization was complete in 1–2 hours. The yield of crystallized benzoylamino chlorohydroxypropane was 26.8 g. (yield 79%); m.p. 108°.

3-Benzoylamino-1-cyano-2-hydroxypropane. Ten grams (0.047 mole) of crystallized benzoylaminochlorohydroxypropane was dissolved in 60 ml. of 67% ethanol and 5 g. (0.077 mole) of KCN and 50 mg. of KI were added. The solution was left at room temperature for 72 hr. The alcohol and water were removed by evaporation at reduced pressure, and the crystalline residue was washed with ice water, filtered, re-washed with ice water, and dried. It was recrystallized from acetone-petroleum ether giving 7.7 g. (yield 80%) of pure nitrile, m.p. 126°.

γ-Amino-β-hydroxybutyric acid. The cyano group was hydrolyzed to carboxyl, and the benzoyl group was simultaneously removed, as follows:

To 1.60 g. (7.8 mmoles) of the pure benzoylaminonitrile was added 10 ml. of reagent-grade 48% aqueous HBr and the solution was refluxed for 45 min.; shorter (10, 20 or 30 min.) and longer (1 or 3 hr.) times led to lesser yields. The benzoic acid freed by the hydrolysis was filtered off, washed

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with a few ml. of water which was added to the reaction mixture, and the remainder of the benzoic acid in solution was extracted by ether. Gaseous ethylene oxide was then passed into the solution until a final pH of about 6 was reached. Two-thirds of the water was removed under reduced pressure and 25 ml. of ethanol was added to give an ethanol:-water ratio of about 3:1. A few drops of conc. NH_4OH were added to bring the pH to 7; this causes crystallization of the amino acid. The product was washed with 30 ml. of 3:1 ethanol-water and recrystallized from the same solvent mixture. The yield was 0.70 g. (75%) of amino acid with a melting point of 212°, dec.

Anal. Calcd. for $C_4H_9NO_3$: C, 40.33; H, 7.62. Found: C, 40.11; H, 7.64.

DL-*Carnitine hydrochloride.* To a suspension of 6.6 g. (70 meq.) of $Ba(OH)_2.H_2O$ in 20 ml. of water was added 1.0 g. (8.4 mmoles) of recrystallized amino acid. Ten g. (70 mmoles) of methyl iodide was added and brought into the solution with the addition of 100 ml. of methanol. The reaction flask was tightly stoppered and stirred overnight at room temperature. Seventy meq. of H_2SO_4 (11.6 ml. of 6*N*) was added and the barium sulfate removed by centrifugation. The supernatant liquid and washings, containing carnitine, iodide, and sulfate ions, was then passed into 50 ml. of Dowex 1-X anion exchange resin in the hydroxide form. The column was washed with about 25 ml. of distilled water (until aliquot portions of the effluent gave, after removal of the methanol, negative reineckate[9] tests for carnitine) and the effluent was then made acid with a slight excess of dilute HCl. This solution was evaporated to dryness and the carnitine hydrochloride was recrystallized from methanol-ether solution to give 1.46 g. (yield 88%). The over-all yield from epichlorohydrin to carnitine hydrochloride was 32%.

Anal. Calcd. for $C_7H_{16}ClNO$: C, 42.51; H, 8.16. Found: C, 42.60; H, 8.01.

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