Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 6

NOTE

Synthesis of 1-carboxy-N,N,N-tri-[methyl-^{14}C] methanaminium chloride (betaine [methyl-^{14}C] hydrochloride)†

Stefan Goszczynski and Ronald L. Crawford*

Institute for Molecular and Agricultural Genetic Engineering, and
Center for Hazardous Waste Remediation Research
University of Idaho, Moscow, Idaho 83844-1052

† Idaho Agricultural Experiment Station publication no. 95511

* Author to whom correspondence should be addressed

SUMMARY

Methyl bromoacetate was reacted with trimethylamine-[^{14}C] dissolved in methanol, forming the methyl ester of [^{14}C] labeled betaine hydrobromide. The methyl ester was hydrolyzed in an alkaline medium to (carboxymethyl)trimethylammonium hydroxide inner salt, and then transformed into the hydrochloride by treatment with an equivalent amount of hydrochloric acid, yielding high purity material with a specific activity of 19.2 μCi/mmol and overall yield of 81.7%.

Keywords: [^{14}C] betaine hydrochloride, nucleophilic substitution, [^{14}C] labeled trimethylamine

INTRODUCTION

In biological systems, betaine acts as a methyl donor and participates in a salvage pathway that forms methionine from homocysteine (1). Here we report a novel synthetic procedure for [^{14}C]-labeled betaine, which we required for studies of microbial osmotolerance, another possible physiological role of the compound.

Isotopically labeled betaines containing both ^{14}C and ^{15}N have been prepared by at least two synthetic approaches. The oxidation of choline by permanganate in a slightly acidic medium (2) or as a free base (3) has been described, and the advantages of an enzymatic method of preparation using choline oxidase have been suggested (4-6). An alternative approach is nucleophilic substitution by exhaustive methylation of glycine (7, 8), sarcosine (9) or N,N-dimethylglycine (10) with methyl

CCC 0362–4803/96/060591–04

Received 12 September 1995
Revised 19 December 1995

halogenides or dimethyl sulfate. Nucleophilic substitution of halogenated derivatives of acetic acid (11) by trimethylamine, or of malonic acid (9) by methylamine, gives a satisfactory yield only when an excess of nucleophile is used, a serious disadvantage when a radioactive amine is applied, since the recovery of excess nucleophile is tedious and time-consuming. The application of a chloroacetic ester makes the nucleophilic substitution by trimethylamine easy at low temperature and requires no excessive amine (12). When a bromoacetic ester is used, the reaction with trimethylamine is vigorous even at -10^0 C, and the product yield is high (13). The hydrolysis of the betaine ethyl ester has been studied for developing kinetic methods (14). Starting with methyl bromoacetate and the methanol solution of trimethylamine, the nucleophilic substitution proceeded very smoothly at low temperatures, with no loss of volatile, radioactive amine. Application of silver hydroxide for the preparation of the free trimethylamine from the commercially available form of its hydrochloride, and for the transformation of bromide into ammonium hydroxide, made the hydrolysis of methyl ester very easy and quantitative. The formation of hydrochloride was made possible by acidifying the reaction mixture with an equivalent amount of hydrochloric acid. This procedure allowed the synthesis of betaine hydrochloride of high purity, as confirmed by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) techniques.

EXPERIMENTAL

Materials and Equipment

The following chemicals were used: trimethylamine-^{14}C hydrochloride, 100 μCi , 4.0 mCi/ mmol (Sigma Chemical Co, St. Louis, Mo); trimethylamine hydrochloride, methyl bromoacetate and silver(I) oxide (Aldrich Chemical Co., Milwaukee, Wisc.). All solvents, solutions, and auxiliary chemicals were of the highest grade available.

HPLC assays were performed on a Hewlett Packard 1090 chromatograph equipped with a reverse phase C18 column, combined with UV detection in the range of 190-220 nm. Thin layer chromatographs were run on Merck silica gel F254 plates, developed in solvent systems of methanol and formic acid. The spots were visualized under UV light and in iodine vapor. The R_f value for

betaine hydrochloride (methanol:acetic acid, 5:1) was 0.27. Radioactivity measurements were carried out by standard liquid scintillation techniques. All R_f values for TLC and retention times for HPLC measurements perfectly matched those of authentic samples of the respective unlabeled compounds.

Preparative Procedures

Trimethylamine-^{14}C in methanol. Trimethylamine-^{14}C hydrochloride (100 μCi) and trimethylamine hydrochloride (0.5 g, 5.2 mmoles) were dissolved in methanol (2.0 ml) and the solution was placed in an acetone–dry ice bath (-78°C). Silver(I) oxide (0.65 g, 2.8 mmoles) was added; the reaction mixture was shaken occasionally for 20 min and kept in a freezing bath. The precipitate of silver chloride was separated by filtration on a pre-cooled Buchner funnel and washed with a small amount of cold methanol. The solution was used immediately in the next step.

Methyl ester of betaine hydrobromide. Methyl bromoacetate (0.52 ml, 5.6 mmoles) was introduced into the trimethylamine solution in methanol kept in the acetone–dry ice bath. The reaction vessel was tightly screw-capped, and the contents were shaken until homogeneous. After one hour at room temperature, the reaction vessel was put into a water bath and warmed gradually to 65°C over 30 min. After the vessel cooled to room temperature, the screw cap was removed, and no pressure was observed in the container. No alkaline reaction was detected by indicator paper kept over the reaction solution. After the methanol was evaporated, well-shaped colorless crystals with m.p. 181-182°C were obtained. The yield was 1.08 g (5.1 mmoles), 98% of theoretical amount.

Betaine hydroxide inner salt. Methyl ester of betaine hydrobromide (1.08 g, 5.1 mmoles) was dissolved in 10 ml of water, and silver oxide (0.65 g, 2.8 mmoles) was added in one portion. The suspension was stirred vigorously at room temperature for 15 min. The precipitate of silver bromide was filtered off and washed several times with small portions of water. The filtrate was heated under reflux for 1 hr. The solution was evaporated *in vacuo,* leaving a white crystalline mass. The yield was 0.62 g (4.6 mmoles), 90.2% of the theoretical amount.

Betaine hydrochloride. Betaine hydroxide (0.62 g, 4.6 mmoles) was dissolved in methanol (10 ml), and concentrated hydrochloric acid (0.46 ml, 5.3 mmoles) was added. The solution was

evaporated *in vacuo* and dried in a dessicator over solid KOH, leaving 0.70 g (4.6 mmoles) of a crystalline mass, m.p. 223-227°C. The crude material was dissolved in 10 ml of boiling methanol and cooled slowly. When the temperature dropped to about 30°C and crystallization started, 10 ml of anhydrous diethyl ether was slowly added under swirling. The mixture was refrigerated overnight and the crystals were filtered off, washed with ether, and dried. The yield was 0.65 g (4.25 mmoles), 81.7% of the theoretical amount, the m.p. was 240-242°C (decomp), and the specific activity was 19.2 μCi/mmol.

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