A SIMPLE SYNTHESIS OF L-[^{35}S]CYSTEINE SULFINIC ACID

Ronald M. Spears and David L. Martin
Department of Chemistry
University of Maryland
College Park, Maryland 20742 U.S.A.

SUMMARY

The synthesis of L-[^{35}S]cysteine sulfinic acid (L-2-amino-3-[^{35}S]sulfinopropanoic acid) in 65% yield from S-[^{35}S]cystine is described. The procedure was designed for use with milligram quantities of starting material and requires no purification of intermediates. L-[^{35}S]Cystine was converted first to its thiosulfonate. Subsequent reaction of the thiosulfonate with ammonium hydroxide generated L-[^{35}S]cysteine sulfinic acid and L-[^{35}S]cystine as the major products. The L-[^{35}S]cystine was recovered and reprocessed thereby increasing the yield.

Key Words: cysteine sulfinic acid

INTRODUCTION

L-cysteine sulfinic acid has been shown to be an intermediate in the biosynthetic pathway of taurine (2-aminoethanesulfonic acid) in the mammalian brain (1,2). The enzyme, cysteine sulfinic acid decarboxylase (EC 4.1.1.29), converts cysteine sulfinic acid to hypotaurine (2-aminoethanesulfinic acid) and carbon dioxide. Labeling cysteine sulfinic acid with ^{35}S allows the use of a radioenzymatic assay technique for studies of cysteine sulfinic acid decarboxylase.

Cysteine sulfinic acid has been synthesized by a number of methods (3-6) most of which were multistep procedures and required the isolation of the intermediate products. DeMarco *et al.* (6) reported the production of cysteine sulfinic acid in one step by treatment of cystine with dilute sodium hydroxide and catalytic amounts of Cu^{+2}. Although the yields were low (about 10%), this method has been used to prepare DL-[1-^{14}C]cysteine sulfinic acid (7).

Emiliozzi and Pichat (5) reported a macroscale synthesis of L-cysteine sulfinic acid from L-cystine by the following route:

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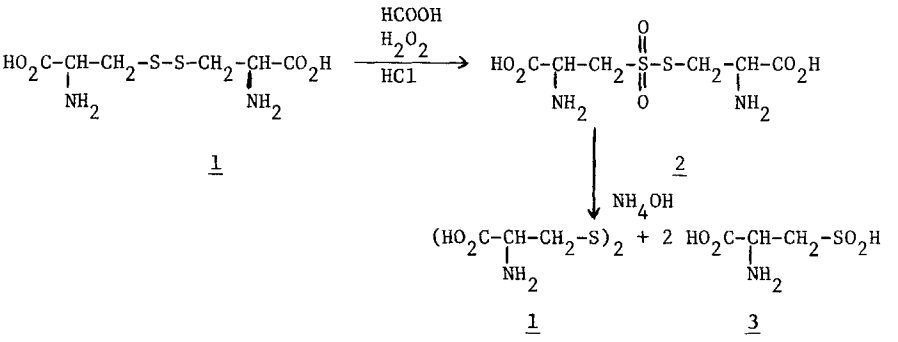

The L-cystine (1) was first converted into the thiosulfonate intermediate (2) (also referred to incorrectly as cystine disulfoxide) by reaction with a prepared mixture of formic acid, hydrogen peroxide and concentrated hydrochloric acid. The thiosulfonate was isolated by crystallization and subsequently treated with ammonium hydroxide to yield L-cysteine sulfinic acid (3) and the starting material, L-cystine, in a 2 to 1 molar ratio.

Our microscale synthesis of L-[^{35}S]cysteine sulfinic acid was based on this work. The same synthetic route was adopted but was redesigned for use with milligram quantities (1-5 mg) of cystine and to eliminate intermediate purification steps which resulted in major losses of the small quantities of isotopically labeled compounds involved. Our procedure yields L-[^{35}S]cysteine sulfinic acid in 50-55% yield. Application of this same procedure to the reclaimed L-[^{35}S]-cystine afforded an increase in the overall yield of L-[^{35}S]cysteine sulfinic acid to 65-70%.

EXPERIMENTAL

One milligram of L-[^{35}S]cystine (obtained from Amersham Corporation) was placed in the bottom of a conical vial or tube and mixed with 0.0252 ml of a reagent mixture composed of 2.32 ml of 88% formic acid, 0.093 ml of concentrated hydrochloric acid, and 0.104 ml of 30% hydrogen peroxide (this reagent mixture must be prepared immediately before use or its effectiveness is lost). The ratio of reagent mixture to cystine must not be exceeded as excess reagent will cause further oxidation of the thiosulfonate to cysteic acid. The reaction was allowed to proceed for 1.5 hr at room temperature after which mixture was evaporated to dryness under a stream of nitrogen at 37°C. The products were taken directly to the next step without purification.

The thiosulfonate was mixed with 1 ml of 7 M ammonium hydroxide (in the vessel in which it was prepared), covered, and left for 1 hour. This mixture was then reduced to approximately 0.25 ml under a stream of nitrogen and streaked on a 20 x 20 cm thin-layer plate which had been coated with a 250 μm layer of

cellulose. Cysteine sulfinic acid was separated from cysteic acid, cystine and other unidentified reaction products by developing the plate to the top with a mixture of 2-propanol/2-butanone/1 M HCl, 12/3/5 by volume. The plate was developed a second time with 1-butanol/acetic acid/water, 12/3/5 by volume, until the solvent front has moved 3-4 cm above the origin. This second development consolidated the cystine into a well-defined band but the solvent did not reach the cysteic acid or cysteine sulfinic acid bands and, therefore, left them unaffected. Labelled procuts were visualized by autoradiography with Kodak Royal X-OMAT XRP-5 X-ray film and amino acids were detected with ninhydrin. Products were identified by comparing the R_f values of the reaction products with those of standards on cellulose thin-layer plates. Ninhydrin-positive components of the product mixture had identical R_f values with standards and autoradiograms of the chromatograms showed spots of exposure which coincided with ninhydrin-positive spots of authentic samples of the products. After the first development, the R_f values of L-[^{35}S]cysteine sulfinic acid and L-[^{35}S]cysteic acid were 0.5-0.6 and 0.3-0.4, respectively. Although the R_f values of these compounds varied slightly among experiments, the distance between the two products was constant. After the second development, the R_f value (with respect to the first solvent front) of L-[^{35}S]cystine was 0.17-0.23.

L-[^{35}S]Cysteine sulfinic acid and L-[^{35}S]cystine were eluted from the cellulose with hot (>90°C) water. L-[^{35}S]cysteine sulfinic acid was rechromatographed in the first solvent system to fully remove any impurities. This repurification yielded a single band (as visualized by autoradiography) of L-[^{35}S]cystine sulfinich acid, which matched the R_f of the standard on the same plates, in 50-55% yield.

The reclaimed starting material (L-[^{35}S]cystine), which comprised 25-40% of the initial cystine (about 0.25-0.45 mg) was used to prepare additional L-[^{35}S]-cystine by the procedure described above. The masses of reclaimed cystine (needed to determine the correct amount of performic acid reagent) and the products were determined from the radioactivity of the materials (as determined by liquid scintillation counting) and the specific radioactivity of the L-[^{35}S]-cystine.

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