

A SIMPLE SYNTHESIS OF (RS)-[2-²H] GLYCINE BY THE REDUCTIVE AMINATION OF GLYOXYLIC ACID

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SUMMARY

The synthesis of (RS)-[2-²H] glycine by the reduction of a dilute solution of glyoxylic acid in aqueous ammonia by sodium borodeuteride is described. A reaction condition is presented under which the principal side product of the reduction, iminodiacetic acid, is minimized.

Key Words: Deuterium, Schiff Base Reduction, Glycine, Sodium Borodeuteride.

INTRODUCTION

Numerous enzymatic and chemical methods have been reported for the synthesis of (R)- and (S)-[2-²H] labeled glycines. These syntheses involve either detailed chemical transformations of chiral starting materials, i.e., L-serine (1), or they involve lengthy chemical sequences in which one or two of the steps are mediated by enzymes (2,3,4,5,6). Straightforward enzymatic syntheses involving the cleavage of serine by serine hydroxymethyltransferase (7) and enzymatic transamination (8) between glyoxylate and aspartate have also been described. In general, all of these procedures are quite involved and were not really suitable for the preparation of (RS)-[2-²H] glycine which was required for a study directed at understanding how [2-²H₂] glycine can arise metabolically from deuterated serine. Therefore, a more practical chemical synthesis which leads directly to a racemic product was designed and is reported herein.

One procedure described in the literature for the production of α -labeled amino acids is the incorporation of deuterium from deuterated water during the decarboxylation of substituted aminomalonates (9). This procedure readily leads to the monodeuterated amino acids. However, with glycine, some dideuteration would be expected due to the rapid exchange of the aminomalononic acid C-2 proton with the solvent prior to decarboxylation.

A more direct route for the preparation would be the reduction of the Shiff base formed between glyoxylic acid and ammonia. The reduction of Shiff bases by sodium cyanohydroborate (10), sulfurated borohydrides (11) and sodium borodeuteride (12) has been reported, but only the latter have been used to prepare amino acids. The recent synthesis of 3-fluoroalanine by the sodium borodeuteride reduction of the Shiff base produced by the reaction of 3-fluoropyruvate with ammonia demonstrates the feasibility of using this type of reaction to produce other α -deuterated amino acids (12).

RESULTS AND DISCUSSION

Preliminary reactions have shown that glyoxylic acid is readily reduced by sodium borohydride in aqueous ammonia to give glycine. However, depending on the reaction condition, a second and sometimes major product was also formed. This product was identified by its *n*-butyl trifluoroacetyl derivative to be iminodiacetic acid (M^+ m/z , 341). As expected, when the reduction was performed with sodium borodeuteride the mass of the glycine derivative increased by 1 m/z and the mass of iminodiacetic acid derivative increased by 2 m/z .

In order to reduce the yields of the iminodiacetic acid, the reaction was run under different conditions of temperature and reagent concentrations and, as can be seen in Table I, higher temperatures and lower concentrations of glyoxylic acid favored the formation of glycine.

The increased yield of glycine at higher temperatures and lower concentrations results from the faster rate of reduction of the Shiff base and reduced Shiff base formation between glycine and glyoxylic acid which leads to the production of iminodiacetic acid after a second reduction.

TABLE I. Effect of reaction conditions on the ratio of glycine to iminodiacetic acid

<u>Temp(°C)</u>	<u>Ratio of glycine to iminodiacetic acid</u>	
	<u>0.027 M glyoxylic acid</u>	<u>0.27 M glyoxylic acid</u>
23	4.37	0.59
55	6.97	0.92

Reactions were run in 15 M aqueous NH₃ containing 0.13 M sodium borohydride in sealed vials.

Since the 55°C reaction produced seven times more glycine than iminodiacetic acid (which was shown to be completely removed by crystallization) it was chosen as the standard reaction condition. Even higher yields of glycine should be possible by further optimization of the reaction conditions.

The synthetic material produced by the synthesis outlined in the experimental section showed 96.6% deuterium. Since the starting material was 98% deuterated, a small exchange of the deuterium with the solvent must have occurred. This small degree of scrambling was previously reported for other sodium borodeuteride reductions in aqueous ammonia (11).

EXPERIMENTAL

Instruments: A Varian MAT 112 gas chromatograph-mass spectrometer modified with a single-stage glass separator was used to establish the identity of the amino acids produced in the reduction and to analyze the deuterium content of the reaction products.

Reagents: Sodium borodeuteride 98 atom % ²H₂ was obtained from Aldrich Chemical Company. Glyoxylic acid was obtained from Sigma Chemical Company.

Derivative formation: Samples (~1 mg) were converted into N-trifluoroacetyl n-butyl esters as previously described (13). The resulting derivatives were separated by gas chromatography on a 6 ft x 1/8 in. column packed with 3% OV-17 on Gas-Chrom A (Applied Sciences, Inc.).

Preparation of (RS)-[2-²H] glycine: Glyoxylic acid (200 mg) was dissolved in 0.5 ml of water and the resulting solution was added to 100 ml of 15 M ammonia in water. The solution was then warmed to 55°C and 100 mg of sodium borodeuteride, which was dissolved in 5 ml of the same ammonia solution, was added in one portion with stirring. After 2 hr, the ammonia and water was removed in vacuum and the residue dissolved in 0.1 M hydrochloric acid to remove excess borodeuteride. The resulting solution was applied to a Dowex 50 H⁺ column which was washed with water and the glycine was eluted with 3 M ammonia. After drying, the resulting solid material was crystallized from water/ethanol to yield 160 mg (79%) of (RS)-[2-²H] glycine containing 96.6 atom % ²H₁. The mother liquor still contained a substantial amount of glycine in addition to the iminodiacetic acid produced in the reaction.

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