SYNTHESIS OF DEUTERIUM ENRICHED L-GLUTAMINE AND 4-AMINOBUTANAMIDE FROM PYRIDAZINONES

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SUMMARY

A convenient method is presented for the preparation of the amino acid glutamine and its decarboxylation product 4-aminobutanamide (GABAMIDE) enriched with deuterium. In a single step, 6-carboxy-3(2H)-pyridazinone was reduced with deuterium gas to give racemic glutamine-2,3,4-$^2H_3$. L-Glutamine-2,3,4-$^2H_3$ was prepared by the stereospecific deacetylation of N^2-acetylglutamine-2,3,4-$^2H_3$ with acylase I. 4-Aminobutanamide-2,3,4-$^2H_3$ was synthesized from 3(2H)-pyridazinone in two steps. Reduction of 3(2H)-pyridazinone with deuterium gas gave the intermediate, 1,4,5,6-tetrahydro-3(2H)-pyridazinone-4,5,6-$^2H_3$, which was further reduced with Raney nickel and hydrogen to yield 4-aminobutanamide-2,3,4-$^2H_3$.

Key Words: DL-Glutamine-$^2H_3$, L-Glutamine-$^2H_3$, 4-Aminobutanamide-$^2H_3$, Pyridazinone reduction

INTRODUCTION

L-Glutamine is one of the most important amino acids known in mammalian systems. As part of a program to study the metabolic fate of glutamine (1) and its decarboxylated derivative 4-aminobutanamide (GABAMIDE) (2), specifically deuterated compounds were required. Although the synthesis of L-glutamine-2,3,3,4,4-$^2H_5$ has been achieved by Blomquist, *et al.*[1] in twelve steps, the

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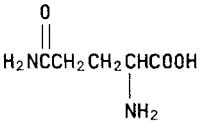
process is expensive and time consuming. Unlabeled GABAMIDE has been synthesized in low yield (14 percent crude yield) from the ethyl ester of 4-aminobutanoic acid and ammonia.[2]

The observation of Kline and Cox[3] that substituted dihydropyridazinones can be reduced catalytically to glutamine analogues in a single step led us to utilize this synthetic pathway for the convenient preparation of deuterium labeled compounds.



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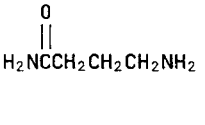

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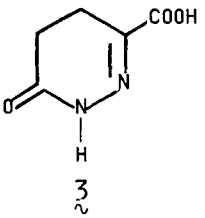

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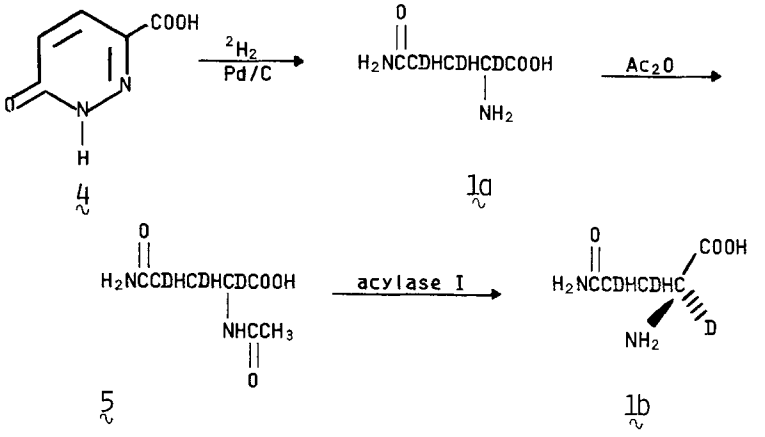
DISCUSSION

A method for the incorporation of one deuterium atom into glutamine arises directly from the substitution of deuterium gas for hydrogen in the catalytic reduction of 4,5-dihydro-6-carboxy-3(2H)-pyridazinone[3] (3). Since the labeled compounds needed for our experiments required at least two deuterium atoms per molecule, the further unsaturated 6-carboxy-3(2H)-pyridazinone (4) was chosen as starting material. Reduction of compound 4 provides for a total of three incorporated deuterium atoms. While the pyridazine ring is generally resistant to hydrogenation,[4] pyridazinone compounds appear to reduce in a manner similar to the reactivity of unsaturated ketones. The reaction sequence is shown in Scheme 1. Compound 4, prepared from 2-ketoglutaric acid by the method of Evans and Wislogle,[5] was reduced with $^2H_2$ to form racemic glutamine-2,3,4-$^2H_3$ (1a) in 56 percent yield. Although the most abundant product was 1a containing three deuterium atoms, a significant amount of 1a-$^2H_4$ was

SCHEME 1

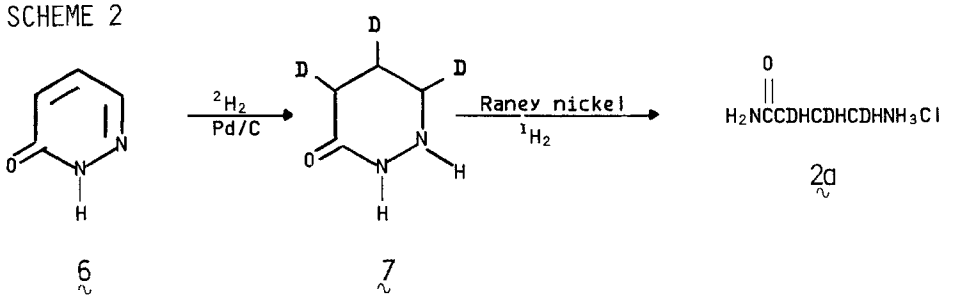


observed. This excess incorporation can best be explained by an exchange process with the solvent during the catalytic reduction.[6] The position of the exchanged proton was found to be evenly distributed between C-3 and C-4 based on the integration of the NMR spectrum of 1a. When the reduction was carried out in water, low incorporation of label resulted and attempts to use aprotic solvents for the reaction were unsuccessful.

Resolution of DL-glutamine was accomplished by taking advantage of the stereospecificity of renal acylase.[1,7] Racemic labeled glutamine was first converted to N^2-acetylglutamine-2,3,4-$^2H_3$ (5) with acetic anhydride. Incubation of 5 with acylase I yielded pure L-glutamine-2,3,4-$^2H_3$ (1b) with no apparent loss of label.

Reaction Scheme 1 was extended to provide the decarboxylated analogue of glutamine, 4-aminobutanamide-2,3,4-$^2H_3$ (2a). The main product of the reaction was found to be the heterocyclic tetrahydropyridazinone 7. Ring

SCHEME 2



6 7 2a

opening to the desired 4-aminobutanamide-2,3,4-$^2H_3$ (2a) was accomplished with Raney nickel and hydrogen. Although the overall yield for the production of 2 by this method is less than by the procedure of Farquharson and MacLean[2], reduction of the pyridazinone allows for the convenient introduction of deuterium in positions on the molecule not readily back exchanged. Furthermore, in our hands, the synthetic method of Farquharson and MacLean did not provide 2. The main product of the reaction by NMR analysis was determined to be the cyclized compound, 2-pyrrolidinone.

EXPERIMENTAL

Melting points were determined using a Thomas-Hoover melting point apparatus and are uncorrected. Electron impact mass spectra were obtained with a DuPont 21-490 mass spectrometer operating at 70 eV and interfaced with a DuPont 21-094 data system. Gas chromatography conditions were as described previously.[8] Evaporation of volatile solvents was carried out on a rotary evaporator at a bath temperature of 40°-60°C. Optical rotations were measured with a Perkin-Elmer Model 141 Polarimeter. Proton NMR spectra were recorded on a Varian T-60A NMR spectrometer using deuterium oxide as solvent and sodium 2,2-dimethyl-2-silapentane-5- sulfonate (SDSS) as internal standard.

Deuterium gas (99.5%) was obtained from Merck Isotopes, deuterium oxide (99.8%) from Aldrich Chemical Co., and Raney nickel (water suspension) from Fluka. Renal acylase I was obtained from Sigma Chemical Co. All other chemicals were reagent grade.

Chemical purity of products was verified by high voltage electrophoresis. All electrophoresis was performed with a water cooled Camag High Voltage Electrophoresis System using paper (20 x 40 cm) as support. The buffer consisted of pyridine, acetic acid, citric acid, and water (10:7.5:3:230). Ninety minutes at 225 mA with voltage between 775-840 volts was used. Spots were visualized with ninhydrin or I_2.

Glutamine-2,3,4-$^2H_3$ (1a). A solution of 6-carboxy-3(2H)-pyridazinone[5] (2.0 g, 14.4 mmol) in ^2H_2O (40 mL) was shaken with 5% Pd/C (0.5 g) and $^2H_2$ (3 atm) overnight. The mixture was filtered and acetone was added to the filtrate until cloudy. The precipitate which formed on cooling was collected to yield 1.19 g (56%) of 1a: mp 182-185°C (lit.[9] mp for unlabeled racemic glutamine 185-186°C). Isotopic incorporation of 1a (with readily exchangeable positions back-exchanged with H_2O) was 37% $^2H_4$, 43% $^2H_3$, 13% $^2H_2$, 4% $^2H_1$, 3% $^2H_0$.

N^2-Acetylglutamine-2,3,4-$^2H_3$ (5). - Acetic anhydride (0.7 mL) was added dropwise over a 5 min period to a solution of glutamine-2,3,4-$^2H_3$ (1.74 g, 11.7 mmol) in 4 mL of 2 *N* NaOH cooled in an ice bath. The pH of the reaction mixture was maintained at pH 7-9 with 12 *N* NaOH. The mixture was stirred for 30 min at room temperature, cooled in an ice bath and then acidified to pH 2 with conc. HCl. The crystals which formed in the cooled solution over a four day period were collected to give 1.31 g (59%) of product: mp 191-194° (lit.[7] mp for unlabeled N^2-acetylglutamine 197°).

L-Glutamine-2,3,4-$^2H_3$ (1b). - A mixture of 5 (300 mg, 1.6 mmol) and H_2O (4 mL) was adjusted to pH 8 with 1 *N* NaOH and treated with acylase I (10 mg). After shaking for 2 hr at 37°C, the mixture was acidified to pH 5.5 with 1 *N* HCl and filtered. Acetone was added to the filtrate to induce

crystallization of crude product: 119 mg, mp 170-178°. The solid was dissolved in H_2O (5 mL) and acidified to pH 2 with 1 *N* HCl. The solution was filtered, and applied to a cation exchange column (AG 50 W-X8, 50-100 mesh, H^+, 2 g). A H_2O (10 mL) wash of the column was discarded. Pure 1b was obtained by eluting with 1 *N* NH_4OH (15 mL) followed by evaporation under reduced pressure: 62 mg (53%); mp 180-183°C (lit.[10] mp for unlabeled L-glutamine 185-186°C); $[\alpha]_D^{28}$ + 29.6° (c=2, 1 *N* HCl), (lit.[1] L-glutamine-2,3,3,4,4-$^2H_5$ $[\alpha]_D^{25}$ + 29.5°); isotopic incorporation 38% $^2H_4$, 44% $^2H_3$, 14% $^2H_2$, 2% $^2H_1$, 2% $^2H_0$.

1,4,5,6-Tetrahydro-3(2H)-pyridazinone-4,5,6-$^2H_3$ (7). 3(2H)-Pyridazinone (4.0 g, 42 mmol) was dissolved in ^2H_2O (30 mL) and reduced with $^2H_2$ (3 atm) over 5% Pd/C (2 g) at room temperature for 18 hr. At that time, analysis by NMR indicated the reaction was about 40% complete. More catalyst (1 g) was added and the reaction carried out for an additional 24 hr. The catalyst was filtered and the solvent evaporated. The residue was fractionally distilled to obtain pure product: 1.7 g (39%) bp 96°/0.05 mm Hg (lit.[11] 127-130°/1 mm Hg). Isotopic incorporation was determined from the *N,N'*-ditrifluoroacetyl derivative by GCMS and found to be 30% $^2H_4$, 52% $^2H_3$, 18% $^2H_2$.

4-Aminobutanamide-2,3,4-$^2H_3$ Hydrochloride (2a). - A solution of 7 (1.7 g, 16.5 mmol) in 95% ethanol (50 mL) was shaken with hydrogen (3 atm) over Raney nickel (6 g) for 24 hr at room temperture. The mixture was then filtered and the pH of the filtrate adjusted to 6.5 with 5 *N* HCl. The resulting green solution was treated with charcoal, filtered and the solvents evaporated. The residue was dried under vacuum for one hour then stirred with CH_3CN (100 mL) overnight. The white solid which formed was collected to give 230 mg (13.5%) of 2a: mp 131-133°C (lit.[2] mp 126-129°C). The CH_3CN soluble fraction was primarily starting material. Isotopic incorporation of 2a was determined by GCMS of the derivative, *N*-trifluoroacetyl-2-pyrrolidinone-3,4,5-$^2H_3$, which was formed by melting 2a and dissolving in trifluoroacetic anhydride.[8]

Isotopic incorporation was 28% $^{2}H_{4}$, 58% $^{2}H_{3}$, 12% $^{2}H_{2}$, 2% $^{2}H_{1}$.

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