SYNTHESIS OF MULTILABELED HISTIDINE

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SUMMARY

The synthesis of tetralabeled histidine is described. Glycine-^{15}N was converted essentially by literature procedures to 4-chloromethylimidazole-3-^{15}N-2,5-$^{2}H_2$. Alkylation of ethyl acetamidocyanoacetate-2-^{13}C, followed by acid hydrolysis and decarboxylation yielded DL-histidine-α-^{13}C-3-^{15}N-2,5-$^{2}H_2$. The compound is useful as a mass spectrometric standard for the measurement of histidine and metabolites in biological media.

Key Words: Histidine, Mass spectrometry, Multilabel

INTRODUCTION

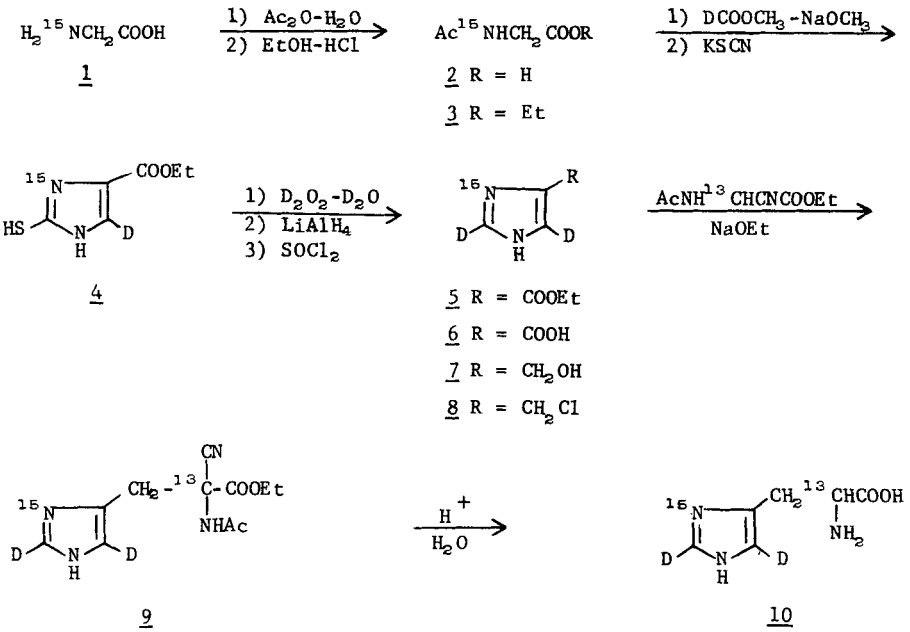
The development of a mass spectrometric method for determination of histidine, histamine and various metabolites in biological fluids or in isolated enzyme assays required the synthesis of histidine increased by four mass units.

Since the histidine was required in some cases to be administered to animals and later assayed, it was preferable to synthesize a mass spectrometric standard with only ^{15}N or ^{13}C isotopic labels to avoid possible loss of label by metabolic action. However, the material expense anticipated for preparation of multigram amounts of the labeled histidine suggested the incorporation of deuterium labels. The synthesis of DL-histidine-α-^{13}C-3-^{15}N-2,5-$^{2}H_2$ (10) is the subject of this paper.

The synthesis of 10, as outlined in Scheme 1, was initiated from glycine-^{15}N (1). Acetylation of 1 by acetic anhydride in aqueous solution[1] afforded ^{15}N-acetylglycine (2), which was converted to its ethyl ester (3) by treatment with 3% ethanolic hydrogen chloride. Condensation of 3 and methyl deuteroformate gave the α-formyl ester, which without isolation, was reacted with aqueous potassium thiocyanate to afford ethyl 2-thio-4-imidazolecarboxylate-3-^{15}N-5-^{2}H (4) in 36% yield.[2] The thiol (4) was oxidized* with deuterium peroxide in D_2O

at room temperature to give the 2,5-dideutero ester (5) in 55% yield. Nmr analysis showed greater than 95% imidazole ring deuteration in 5. If the reaction was allowed to proceed for a prolonged period, the resulting acidic medium caused some hydrolysis to the acid (6), however, reesterification allowed the recovery of valuable labeled material.

The ester (5) was reduced with lithium aluminum hydride in ether[3] to give the alcohol (7), isolated as the picrate salt. The picrate was exchanged with Dowex 2($Cl^{\ominus}$) resin and the resulting hydrochloride salt was converted to the chloromethyl imidazole (8) by the action of thionyl chloride.[4] The chloride (8) was used to alkylate ethyl acetamidocyanoacetate-2-^{13}C according to the procedure of Albertson and Archer[5] to afford the intermediate cyano ester (9). Acid hydrolysis of 9 with concomittant decarboxylation yielded DL-histidine-α-^{13}C-3-^{15}N-2,5-$^{2}H_2$ (10).



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* The oxidation of thiol by hydrogen peroxide was reported by Backer[7] and applied to thioimidazoles in a synthesis of pilocarpine.[8]

EXPERIMENTAL

^{15}N-ACETYLGLYCINE (2)

To a solution of 7.0 g (82 mmole) of glycine-^{15}N (Koch Isotopes, 99% ^{15}N) in 30 ml of water was added 10.0 ml (0.106 mole) of acetic anhydride in one portion. The mixture was stirred for 20 minutes at ambient temperature and chilled for 20 hours. The precipitate was collected, washed with 5 ml of water and dried to yield 6.6 g of white crystals, mp 203-204° (lit.[1] 206-208°). A second crop of 0.9 g was obtained by concentration of the mother liquor for a total of 7.5 g (69%).

ETHYL ^{15}N-ACETYLGLYCINATE (3)

To 300 ml of ice cold 3% hydrogen chloride in ethanol was added 15.3 g of 2. The mixture was stirred for 2 hours at ambient temperature followed by refrigeration for 20 hours. The solvent was removed *in vacuo* and a 50-ml portion of ethanol and two 50-ml portions of benzene were successively added and evaporated. The residue was dissolved in 300 ml of dichloromethane and stirred with 15 g of anhydrous potassium carbonate for 1 hour. After filtration and removal of solvent, the residue was distilled at 0.25 mm to afford 15.9 g (84%) of crystalline distillate at bp 100-105°. NMR ($CDCl_3$) 6.17 (NH), 4.23 (2H, q, CH_2O), 4.05 (2H, d, $^{15}NCH_2$), 2.07 (3H, s, CH_3CO), 1.28 (3H, t, CH_3CH_2).

ETHYL 2-THIO-4-IMIDAZOLECARBOXYLATE-3-^{15}N-5-^{2}H (4)

To a stirred mixture of 15.9 g (0.109 mole) of 3, 20 g (0.33 mole) of methyl formate-1-d and 5 ml of benzene cooled to 6° was added 6.6 g (0.12 mole) of sodium methoxide as a slurry in 25 ml of benzene. The addition was made in 6 portions over 30 minutes with maintenance of the temperature below 13°. The yellow-orange enolate mixture was refrigerated for 18 hours and then treated with 55 ml of ice water to dissolve all solid material. The aqueous phase was separated, chilled in an ice bath and treated with 20.5 ml (0.25 mole) of 12N hydrochloric acid followed by 11.5 g (0.12 mole) of potassium thiocyanate. The solution was heated for 2 hours on the steam bath, cooled to room temperature and then chilled for 4 hours. The precipitate was collected, washed with 10 ml of water and dried to leave 5.0 g of yellow crystals, mp 182-183° (lit.[2] 184°).

The filtrate was adjusted to pH 3 and chilled for 20 hours to afford another 1.7 g for a total of 6.7 g (36%). The nmr spectrum showed proton signals only for the ethyl group of the ester.

ETHYL 4-IMIDAZOLECARBOXYLATE-3-^{15}N-2,5-^{2}H (5)

A suspension of 6.7 g (38.5 mmole) of the thiol ester (4) in 250 ml of D_2O was warmed into solution on the steam bath over 30 minutes and kept at room temperature 15 hours. The solution was cooled in a cold water bath and treated with 15.0 ml (0.125 mole) of 30% D_2O_2 (Crescent) over a 5 minute period. The mixture was stirred at ambient temperature for 70 hours and adjusted to pH 8 with solid sodium carbonate. After extraction with eight 50-ml portions of dichloromethane and drying over magnesium sulfate, the solvent was removed to leave 3.0 g (55%) of white crystals; the nmr spectrum showed protons for the ester group, but the imidazole region was free of significant proton peaks (less than 5% by integration). An unlabeled run was similarly performed to afford 5 in 72% yield, mp 155-157° (lit.[2] mp 157-158°); labeled and unlabeled esters were identical by tlc.

In some runs, the acidic medium caused partial hydrolysis of 5 to the acid (6). Evaporation of the aqueous mother liquor, after extraction of 5, followed by refluxing the residue with saturated ethanolic hydrogen chloride, regenerated the ester.

4-HYDROXYMETHYLIMIDAZOLE-3-^{15}N-2,5-$^{2}H_2$ (7)

A mixture of 1.20 g (32 mmole) of lithium aluminum hydride and 35 ml of ether was cooled in an ice bath with stirring, followed by the addition of 2.90 g (20 mmole) of the ester (5) over 15 minutes. Stirring was continued for 20 hours at ambient temperature. The mixture was cooled in ice, diluted with 5 ml of tetrahydrofuran and treated successively with 1.2 ml of ice water, 1.2 ml of 15% sodium hydroxide and 3.6 ml of water. The mixture was filtered and the cake washed with tetrahydrofuran. The cake was then suspended in hot methanol, saturated with carbon dioxide and filtered. This process was twice repeated and the methanol filtrates were combined with the original filtrate and evaporated *in vacuo*. The residue was dissolved in 40 ml of water and the solution adjusted to pH 6-7 with a little acetic acid. A solution of 4.3 g (19 mmoles) of picric

acid in 100 ml of warm water was then added to give a yellow precipitate. The material was collected, washed with water and dried to leave 4.30 g (45%) of the picrate of 1, mp 200-202° (lit.[6] mp 203.5°).

A mixture of the picrate, 20 g of Dowex 2 (Cl^-) resin, and 100 ml of water was warmed on the steam bath for 15 minutes and stirred for 20 hours. The resin was removed and the filtrate evaporated *in vacuo* to leave 2.1 g (100%) of the crystalline hydrochloride hydrate of 7.

α-CYANO-N-ACETYLHISTIDINE ETHYL ESTER-α-^{13}C-3-^{15}N-2,5-$^{2}H_2$ (9)

A mixture of 2.1 g of 7 and 5 ml of thionyl chloride was stirred at reflux for 45 minutes, a modification of the procedure of Turner, et al:[4] Evaporation of the reagent followed by addition and evaporation of two 20-ml portions of carbon tetrachloride yielded 2.2 g of the crude chloromethylimidazole hydrochloride (8). To a solution of sodium ethoxide (from 0.62 g, 26.8 mg atom sodium) in 23 ml of ethanol was added 2.29 g (13.4 mmole) of ethyl acetamidocyanoacetate-2-^{13}C (Koch Isotopes). The solution was chilled to 0-5°, rapidly added to the chloromethyl compound (8) and the mixture was stirred at room temperature for 15 hours. The solvent was removed *in vacuo* and the product crystallized from 7 ml of ice water to afford 2.63 g (80%) of white crystals, mp 85° (lit.[5] mp 100-103°). The material was considered sufficiently pure for conversion to histidine.

DL-HISTIDINE-α-^{13}C-3-^{15}N-2,5-$^{2}H_2$ (10)

A solution of 2.06 g of the cyano ester (9) in 16 ml of 3N sulfuric acid was heated 18 hr at 100°, cooled to room temperature and adjusted to pH 8-9 with saturated barium hydroxide. Barium sul-ate was removed by filtration through Celite and the filtrate evaporated *in vacuo*. The residue was dissolved in 15 ml of water and the solution saturated with carbon dioxide and filtered. The filtrate was evaporated to dryness *in vacuo* and crystallized from 10 ml of ethanol to afford 1.00 g (59%) of white crystals; paper chromatography (BuOH-HOAc-H_2O, 4:1:5), R_f 0.15, single ninhydrin positive spot, identical with natural histidine; nmr (proton) showed less than 5% imidazole H and nmr (^{13}C) showed only one enriched carbon (55.48 ppm); GC-MS (trimethylsilylation) m/e 360 (tri-

N-TMS-CH_3), 80% M+4 and 20% M+3.

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