Synthesis of α-N-Methylated Histidines[1]

VERNON N. REINHOLD, YOSHINORI ISHIKAWA, AND DONALD B. MELVILLE

Department of Biochemistry, University of Vermont College of Medicine, Burlington, Vermont 05401

Received July 5, 1967

Preparative methods have been developed for the synthesis of α-N-methyl-L-histidine, α-N,N-dimethyl-L-histidine, and α-N,N,N-trimethyl-L-histidine (hercynine) from L-histidine. These procedures are suitable for the synthesis of the ^{14}C-labeled compounds.

Studies by Askari and Melville[2] on the synthesis of ergothioneine (2-mercaptohistidine betaine) by *Neurospora crassa* showed that hercynine (histidine betaine) is an intermediate compound in the biosynthesis. To establish the relationship of the monomethyl and dimethyl derivatives of histidine to the synthetic pathway and to investigate the nature of the enzymatic methylation reactions, it was desirable to have methods for the laboratory synthesis of the methylated histidines. The methods which we have developed for the synthesis of the α-N-methylated histidines are applicable for use with ^{14}C-histidine, ^{14}C-formaldehyde, or ^{14}C-methyl iodide for the preparation of the labeled compounds. These compounds should prove of value in studies of other biological reactions of histidine.

Racemic α-N-methylhistidine was first prepared by Fargher and Pyman,[3] by the treatment of α-chloro-β-imidazolepropionic acid with methylamine. Uhle and Harris[4] prepared the DL compound *via* 4(5)-chloromethylimidazole and dimethyl N-methyl-N-acetylaminomalonate. The L isomer was synthesized by du Vigneaud and Behrens[5] from L-histidine by protection of the imidazole nitrogen with a benzyl group and the α-amino nitrogen with a tosyl group, followed by treatment with methyl iodide and then removal of the protecting groups by reduction with sodium in liquid ammonia. In the method reported here, the procedure which Quitt, *et al.*,[6] used with other amino acids was modified and applied to histidine. Reductive alkylation of L-histidine with benzaldehyde yielded α-N-benzylhistidine, which was methylated by the Eschweiler–Clarke[7] modification of the Leuckart method; the benzyl group was removed by catalytic hydrogenolysis to form α-N-methylhistidine.

A method for the preparation of α-N,N-dimethylhistidine has not previously been described, although Ingram[8] showed that histidine solutions which had been subjected to catalytic hydrogenation conditions in the presence of formaldehyde no longer contained appreciable ninhydrin-positive material; also, he assumed the formation of the α-N,N-dimethyl derivative. We have used this method as the basis for a preparative procedure.

The synthesis of hercynine from α-chloro-β-imidazolepropionic acid and trimethylamine has been reported,[9,10] although the final product was characterized only as the platinum salt or the picrate, and the yields appear to be low. The formation of hercynine by the oxidation of ergothioneine with ferric chloride[2,11] is of limited value because of the relatively high cost of L-ergothioneine and the lack of suitable methods for preparing the isotopically labeled compound. We have found that hercynine can be readily synthesized by the alkylation of α-N,N-dimethylhistidine with methyl iodide.

Experimental Section

Optical rotations were determined in a 1-dm tube with a Zeiss precision photoelectric polarimeter at 546 and 578 mμ and were extrapolated to 589 mμ by the use of Drude's equation. Specific rotations were determined in 5 *N* HCl and calculated in terms of the anhydrous free bases. Melting points were obtained with a Thomas–Hoover capillary apparatus calibrated with the compounds supplied with the apparatus. Nitrogen analyses were carried out in a Coleman nitrogen analyzer on samples which had been dried over P_2O_5 for 1 hr at 100° *in vacuo*. Hydrogenations were performed at room temperature in a Parr low-pressure apparatus (H_2 pressure 3.2–3.5 kg/cm², 10% Pd–C). Reaction mixtures were examined qualitatively by ascending chromatography on Whatman No. 1 paper with $(n\text{-Bu})_3N$–EtOH–H_2O (1:8:1 v/v/v) or with 0.1 *M* NaOAc–EtOH (1:3 v/v); compounds were located by spraying with diazotized sulfanilic acid and carbonate[12] or with ninhydrin. The respective R_f values with the two solvents were histidine, 0.20 and 0.32; α-N-benzylhistidine, 0.71 and 0.71; α-N-methylhistidine, 0.29 and 0.50; spinacine, 0.23 and 0.37; α-N,N-dimethylhistidine, 0.41 and 0.57; and hercynine, 0.15 and 0.55. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.3% of the theoretical values.

Chemicals.—L-Histidine hydrochloride hydrate was obtained from Cyclo Chemical Corp. and from General Biochemicals Corp. CH_2O was obtained from Fisher Scientific Co. as an approximately 37% aqueous solution, with and without MeOH as a stabilizer. Catalyst (10% Pd–C) was from Nutritional Biochemicals Corp. The $C_6H_5CH_2NMe_2$ and 97% HCO_2H were from Eastman Kodak Co. The IRA-410, ion-exchange resin was from Rohm and Haas.

α-N-Benzyl-L-histidine.—To 21.0 g (0.1 mole) of L-histidine·HCl·H_2O in 120 ml of H_2O were added 120 ml of MeOH, 40 ml of benzaldehyde, and 20 ml of $C_6H_5CH_2NMe_2$. The clear solution was hydrogenated (5 g of catalyst) until the rate of H_2 uptake decreased markedly (1–1.5 hr). The catalyst was separated on a sintered-glass funnel and washed with H_2O, and the combined filtrates were concentrated to a white solid in a rotary evaporator. The residue was dissolved in 100 ml of H_2O, and crystallization was effected by the addition of 500 ml of Me_2CO. After storage overnight at 5°, the bulky precipitate was collected, washed with Me_2CO, air dried, and recrystallized as before; yield 21.1 g

(1) This investigation was supported by NSF Research Grant GB-1201, PHS Research Grant AM-08638 from the National Institute of Arthritis and Metabolic Diseases, and PHS Training Grant GM-757 from the National Institute of General Medical Sciences.

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(12) Ten volumes of 0.2% sulfanilic acid in 1% HCl mixed with 1 vol of 8% $NaNO_2$; color developed by spraying with 7% Na_2CO_3.

(86%), mp 198–200°, $[\alpha]^{22}D$ +28.9° (*c* 1.0, 5 *N* HCl). *Anal.* ($C_{13}H_{15}N_3O_2$) N.

A 2.45-g sample of the product was debenzylated by catalytic hydrogenation in 50% HOAc. The reduction product was acidified with HCl and crystallized from H_2O–EtOH to yield 1.76 g (84%) of L-histidine·HCl·H_2O, mp 253–254° dec, $[\alpha]^{22}D$ +14.1° (*c* 1.0, 5 *N* HCl). The histidine·HCl·H_2O used for the synthesis of the benzyl derivative had mp 253–254° dec, $[\alpha]^{22}D$ +14.0° (*c* 1.0, 5 *N* HCl) after crystallization from H_2O–EtOH.

α-N-Methyl-L-histidine.—To 24.5 g (0.1 mole) of α-N-benzyl-L-histidine in 300 ml of HCO_2H was added 8.4 ml (0.11 mole) of 36.8% CH_2O solution. The solution was heated rapidly to reflux temperature, maintained under reflux for 15 min, and cooled under running water to room temperature. The solution was concentrated to a thick syrup in a rotary evaporator, and the residue was dissolved in 100 ml of H_2O and reconcentrated. This last step was repeated, the residue was dissolved in 100 ml of H_2O, and 100 ml of HOAc was added. The solution was hydrogenated (2 g of catalyst) for 30 min. The catalyst was filtered off and washed with H_2O. To the combined filtrates was added 8.0 ml of concentrated HCl (0.1 mole), and the solution was concentrated to a thick syrup in a rotary evaporator. The evaporation was repeated twice after dissolving the residue in 100-ml portions of H_2O, the last evaporation being continued until a dry glass was obtained. The residue was dissolved, with heating, in 20 ml of H_2O, the solution was mixed with 100 ml of DMSO, and 800 ml of EtOH was added. Crystallization commenced within a few minutes and was completed during storage overnight at 5°.

The dense, powdery product was collected and washed with EtOH. The air-dried material (13.8 g, mp 252–254°) contained spinacine (**2**) as indicated by paper chromatography. The product was purified by two crystallizations from H_2O–DMSO, in each case by heating with 1 ml of H_2O/g of solid and adding 5 vol of DMSO. This procedure, which removed spinacine, was followed by two crystallizations from aqueous EtOH, by heating with 5 ml of H_2O/g of solid and adding 5 vol of EtOH. The final product, 8.2 g (40%) of small hexagonal plates, was chromatographically pure; mp 266–267° dec, $[\alpha]^{22}D$ +39.3° (*c* 1.0, 5 *N* HCl). Methylhistidine·HCl which we prepared from L-histidine by the method of du Vigneaud and Behrens[5] showed mp 266–267°, $[\alpha]^{22}D$ +39.5° (*c* 1.0, 5 *N* HCl). *Anal.* ($C_7H_{12}ClN_3O_2$) N.

A sample of the methylhistidine·HCl was converted to the free base by treatment with the hydroxide form of IRA-410 ion-exchange resin and crystallization from H_2O–EtOH. The product had mp 256–257°, $[\alpha]^{22}D$ −15.3° (*c* 1.0, H_2O), compared to the literature[5] values of mp 266°, $[\alpha]^{20}D$ −13.5° (*c* 1, H_2O).

A 2.06-g sample of the methylhistidine·HCl was converted to the dimethyl derivative by catalytic hydrogenation in the presence of CH_2O under the conditions used for the conversion of histidine to the dimethyl derivative as described below. From the reduction mixture, 2.33 g (98%) of crystalline product, mp 112–115°, was obtained, which on recrystallization from H_2O–EtOH yielded 1.72 g (72%) of α-N,N-dimethyl-L-histidine·HCl·H_2O, mp 114–115°, $[\alpha]^{22}D$ +58.5° (*c* 1.0, 5 *N* HCl).

α-N,N-Dimethyl-L-histidine.—To 21.0 g (0.1 mole) of L-histidine·HCl·H_2O in 300 ml of H_2O was added 17 ml of 36.8% aqueous CH_2O solution and 5 g of catalyst; the mixture was hydrogenated for 1.25 hr. The catalyst was separated by filtration and washed with H_2O, and the combined filtrates were concentrated to a thick syrup; this was dissolved in 200 ml of MeOH, and then 500 ml of Et_2O was added portionwise. Crystallization of the oil which separated was aided by trituration. After storage at 5° overnight the crystals were collected and washed with Et_2O. The somewhat hygroscopic material was dissolved in 20 ml of H_2O with heating, and 200 ml of EtOH was added. The crystals which separated from the solution during storage overnight at 5° were collected and washed with cold EtOH. The air-dried α-N,N-dimethyl-L-histidine·HCl·H_2O weighed 19.4 g (82%), mp 114–115°, $[\alpha]^{22}D$ +58.9° (*c* 1.0, 5 *N* HCl). On drying for analysis it showed a weight loss corresponding to that expected for a monohydrate. *Anal.* ($C_8H_{14}ClN_3O_2$) N.

Hercynine.—A solution of 23.8 g (0.1 mole) of α-N,N-dimethyl-L-histidine·HCl·H_2O in 500 ml of MeOH was adjusted to give a reading of 9 on a pH meter by the addition of concentrated NH_4OH (*ca.* 42 ml). MeI (10 ml) was added, and the solution was kept at room temperature overnight. Evaporation of the solution at reduced pressure gave a white, solid residue which, in the minimum amount of H_2O, was passed through an ion-exchange resin column prepared by converting 240 g of IRA-410 chloride to the hydroxide form with 2 *N* NaOH and washing with H_2O. The sample was washed through the column with H_2O. The first 500 ml of effluent was collected and concentrated under reduced pressure to a solid residue. This was dissolved in 150 ml of warm MeOH, and Et_2O (350 ml) was added to turbidity. Storage at 5° overnight yielded 18.2 g of crystals which were recrystallized from 150 ml of MeOH and 360 ml of Et_2O. The Et_2O-washed air-dried product weighed 17.5 g (89%), mp 237–238° dec, $[\alpha]^{22}D$ +44.5° (*c* 1.0, 5 *N* HCl). *Anal.* ($C_9H_{15}N_3O_2$) N.

A sample of hercynine prepared from natural L-ergothioneine by oxidation with $FeCl_3$[2] showed $[\alpha]^{22}D$ +44.7° (*c* 1.0, 5 *N* HCl) and possessed an infrared spectrum identical with that of the synthetic hercynine.

Discussion

The procedures reported here provide convenient methods for the preparation of the α-N-methylated histidines in good to excellent yields from the readily available L-histidine. The optical purity of the synthetic hercynine is established by comparison with L-hercynine prepared by the oxidative desulfurization of natural ergothioneine. The identical specific rotations of the two preparations also provide confirmatory evidence for the L configuration of natural ergothioneine, which heretofore has been based on a partly racemic preparation of synthetic ergothioneine.[13] The optical purities of the monomethyl- and dimethylhistidines are established by the conversion of these compounds to L-hercynine, and in the case of the monomethylhistidine by comparison with the same compound prepared by the method of du Vigneaud and Behrens.[5]

The major difficulties encountered in developing these methods were associated with the synthesis of methylhistidine. It seems likely that other workers have also experienced difficulties, for although Quitt, *et al.*,[6] described the synthesis of several methylamino acids including α-N-methyl-L-lysine and α-N-methyl-L-arginine, they did not describe studies with histidine. More recently, Ebata, Takahashi, and Otsuka[14] extended the method of Quitt, *et al.*,[6] to other amino acids, but stated that they experienced difficulties with histidine, and reported that the reaction product showed a high, negative optical rotation. In our studies, it became evident that it was necessary to control carefully the conditions used both for the synthesis of α-N-benzylhistidine and for its subsequent methylation in order to avoid or minimize the formation of bicyclic compounds in each step.

The formation of α-N-benzylhistidine by the reductive benzylation of histidine with benzaldehyde and hydrogen is subject to two competing reactions: the base-catalyzed cyclization of the intermediate azomethine or carbinolamine, and the acid-catalyzed hydrogenolysis of the reduction product, benzylhistidine. In the method of Quitt, *et al.*,[6] in which NaOH is used as the base, we found that the reaction mixture contained, in addition to benzylhistidine, appreciable amounts of histidine and a Pauly-positive compound which was isolated as a crystalline dihydrate and which appears to be 4-phenylspinacine (**1**, Scheme I). This was readily formed from a solution of histidine and benzaldehyde in 1 *N* NaOH at room temperature;

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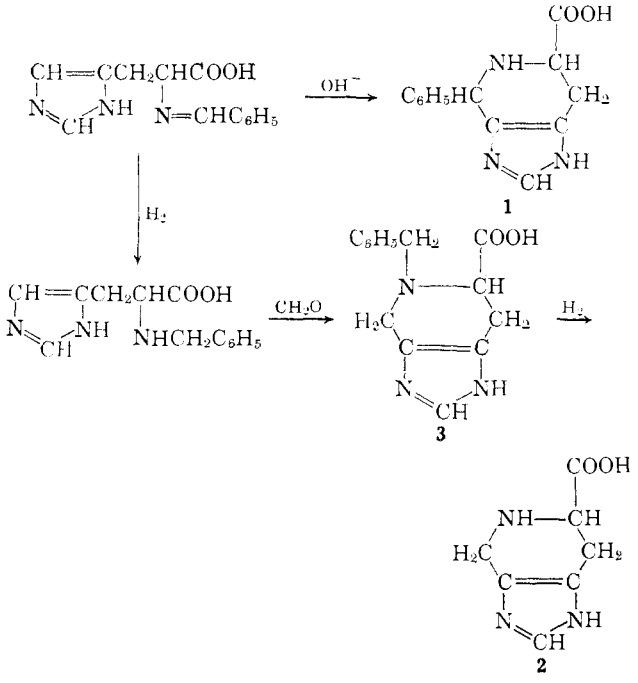
Scheme I



quantitative conversion to 4-phenylspinacine occurred when the solution was heated at 100° for 1 hr. The assigned structure (**1**) is in agreement with a nitrogen analysis and with the further finding that on catalytic reduction in H_2O–AcOH, a ninhydrin-positive substance was formed which was not identical with 1(3)-benzylhistidine[5] and which gave a yellow Pauly test in contrast to the red or orange colors given by histidine and its derivatives which have no substituents on the 4(5) carbon atom. The reduction product is presumably 4(5)-benzylhistidine. An alternative structure for the cyclization product, formed from nucleophilic attack by the nitrogen atom of the imidazole ring on the azomethine carbon atom, is less likely because 1(3)-substituted histidines generally give negative Pauly tests, and the expected ninhydrin-positive reduction product would be either 1(3)-benzylhistidine or histidine.

The formation of 4-phenylspinacine in the reductive benzylation reaction was effectively eliminated or markedly reduced by replacing the NaOH with an organic base. When Et_3N was used, phenylspinacine was not formed, but benzylation was incomplete and appreciable amounts of histidine were present in the reaction mixture. This result may be ascribed to the fact that an appreciable increase in pH occurred during the reduction. Complete benzylation could be accomplished by adding the benzaldehyde in two equal portions, with catalytic reduction after each addition. When $C_6H_5CH_2NMe_2$ was used in the reductive benzylation, no phenylspinacine was detected and essentially quantitative conversion to benzylhistidine occurred.

The reductive methylation of α-N-benzylhistidine with CH_2O and HCO_2H likewise required careful control of conditions to minimize the formation of 5-benzylspinacine (**3**). This substance was readily formed when a mixture of benzylhistidine and CH_2O was heated in DMSO or DMF. The crystalline product which was isolated gave a nitrogen analysis corresponding to 5-benzylspinacine hydrate and, after catalytic reduction in H_2O–HOAc, yielded a compound which was isolated as the crystalline hydrochloride. This reduction product analyzed correctly for the hydrochloride of spinacine (**2**) and gave a yellow color in the Pauly reaction. This compound was identical, in rotation, chromatographic behavior, and response to the Pauly test, with spinacine prepared by the treatment of L-histidine in concentrated HCl with CH_2O.[15] However, the specific rotation of the spinacine, isolated after hydrogenation of the Leuckart reaction mixture, varied with the time of heating. Spinacine hydrochloride which was isolated after refluxing periods of 5–10 min showed $[\alpha]^{22}D$ −115° (*c* 1.0, 5 *N* HCl), while the spinacine hydrochloride isolated after a refluxing period of 1 hr showed $[\alpha]^{22}D$ −15.0°, compared to $[\alpha]^{22}D$ −115° (*c* 1.0, 5 *N* HCl) for spinacine hydrochloride prepared according to Neuberger.[15] We were not able to devise conditions for the Leuckart reaction which would completely eliminate the formation of 5-benzylspinacine. Optimum yields of methylhistidine and more ready purification of the product were experienced when the refluxing time was limited to 10–20 min. Although it was possible to isolate, in crystalline form, the intermediate α-N-benzyl-α-N-methylhistidine as the dihydrochloride, this substance crystallized only with difficulty and the final yield of α-N-methylhistidine was higher when the synthetic procedure was carried out without isolation of the intermediate.

The reductive methylation of histidine with CH_2O and H_2 resulted in quantitative conversion to α-N,N-dimethylhistidine, as indicated by chromatographic analysis of the reaction mixture. Similarly, the conversion of dimethylhistidine to hercynine by methylation with MeI was essentially quantitative. This latter finding is in contrast to the difficulties which are experienced in attempts to synthesize hercynine by the direct methylation of histidine, in which methylation of the imidazole ring occurs and a pentamethyl derivative can be formed.[9] Under the conditions which we used for the methylation of dimethylhistidine, histidine itself is not readily methylated. In contrast, the α-nitrogen atom of α-N,N-dimethylhistidine is apparently a sufficiently strong nucleophile that it is readily methylated under these conditions, while neither the imidazole ring nor the NH_4OH which is used as the base in the reaction is methylated to any significant extent.

All three synthetic procedures have been successfully applied on a milligram scale to the synthesis of the ^{14}C-labeled, methylated histidines, using labeled histidine or CH_2O.

Acknowledgment.—We express our appreciation to Miss Jeanne Choquette and Mrs. Laura Gonyeau for carrying out the nitrogen analyses.

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