The α-Hydrazino Analog of Histidine

M. SLETZINGER, R. A. FIRESTONE, D. F. REINHOLD, C. S. ROONEY,

Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, New Jersey

AND W. H. NICHOLSON

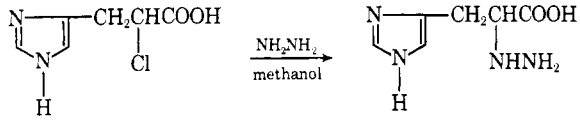
Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., West Point, Pennsylvania

Received September 25, 1967

L-, D-, and DL-2-hydrazino-3-[4(5)-imidazolyl]propionic acids were prepared by the reaction of hydrazine with the α-chloro acids derived from D-, L-, and DL-histidine, respectively. Kinetic data indicated that the hydrazine reaction took place by an SN2 mechanism with inversion of configuration. Deamination of L- and D-histidine with $NaNO_2$ in concentrated HCl therefore proceeded with retention of configuration. Contrary to expectation, deamination of L-histidine methyl ester under these conditions also gave the L-α-chloro acid. The hydrazino acids were prepared for testing as inhibitors of the enzymatic decarboxylation of histidine to histamine.

The high *in vitro* activity of DL-2-hydrazino-2-(3,4-dihydroxybenzyl)propionic acid as an inhibitor of the enzyme dihydroxyphenylalanine (DOPA) decarboxylase has been reported.[1] The preparation of the α-hydrazino analog of histidine, 2-hydrazino-3-[4(5)-imidazolyl]propionic acid (II), was carried out in the hope that this compound would prove an effective inhibitor of the biological decarboxylation of histidine to histamine.[2] The racemate, as well as the optically active D and L isomers, were synthesized for study.

SCHEME I



I II

The method[3] utilized for the preparation of II was the reaction of hydrazine with 2-chloro-3-[4(5)-imidazolyl]propionic acid (I) (Scheme I). The conversion of histidine to I by diazotization has been described by a number of workers.[4] The optical course of the reaction when L-histidine was employed was not clearly indicated, although the work of Fargher and Pyman[4b] suggested that significant racemization occurred. Repetition of the procedure of Edelbacher and von Bidder[4a] starting with L-histidine established that levorotatory 2-chloro-3-[4(5)-imidazolyl]propionic acid of a high degree of optical purity was obtained. When this levorotatory α-chloro acid was allowed to react for 3 weeks with an excess of hydrazine in methanol optically pure (+)-2-hydrazino-3-[4(5)-imidazolyl]propionic acid was obtained in up to 77% yield. The configuration was established by hydrogenolysis to D-histidine using Raney nickel catalyst. Employing (+)- and (±)-I the corresponding (−)- and (±)-2-hydrazino-3-[4(5)-imidazolyl]propionic acids were prepared.

Mechanism and Stereochemistry.—The conversion of an optically active α-amino acid to the α-chloro acid under the conditions employed in this work normally proceeds with retention of configuration.[5] However, the possibility that intramolecular participation of the imidazole ring[6] in the deamination reaction might have altered the usual stereochemical course had to be considered. The reaction of an optically active α-halo acid with hydrazine had been utilized previously by Darapsky[7] for the preparation of optically active D- and L-2-hydrazinophenylacetic acid. The stereochemistry of the reaction was shown to involve Walden inversion. Following completion of our work, Niedrich and Grupe[8] described the preparation of three other optically active α-hydrazino acids, from optically active α-halo acids. Inversion of configuration was proven to have occurred in these hydrazine displacement reactions.

In the present work it was decided that examination of the kinetics of the hydrazine reaction was necessary in order to permit assignment of configurations to the optical enantiomers of I. If participation of either the imidazole nitrogen or carboxylate anion were involved, the rate of reaction should be first order with respect to the α-chloro acid anion and independent of the concentration of hydrazine base. However, if the reaction proceeded by an SN2 mechanism, the rate would depend on the concentrations of both the α-chlorocarboxylate anion and hydrazine base. Testing chloride-release data against SN1 and SN2 mechanisms did not provide an unequivocal answer. However, in another experiment where the concentration of hydrazine base was varied tenfold, the initial rate of appearance of chloride ion also changed by the same factor (Figure 1). This reaction was therefore an SN2 displacement of hydrazine on the α-chloro acid. Since the over-all sequence α-amino acid to α-hydrazino acid occurred with inversion, the diazotization reaction must have proceeded with retention as with simpler amino acids.

The optical rotatory dispersion curves for L-histidine, D-2-hydrazino-3-[4(5)-imidazolyl]propionic acid, and

(1) M. Sletzinger, J. M. Chemerda, and F. W. Bollinger, *J. Med. Chem.*, **6**, 101 (1963).

(2) For a description of the biological activities of D-2-hydrazino-3-[4(5)-imidazolyl]propionic acid see R. J. Levine, T. L. Sato, and A. Sjoerdsma, *Biochem. Pharmacol.*, **14**, 139 (1965).

(3) For references to the preparation of α-hydrazino acids by this procedure see G. Pollak, H. Yellin, and A. Carmi, *J. Med. Chem.*, **7**, 220 (1964); A. Carmi, G. Pollak, and H. Yellin, *J. Org. Chem.*, **25**, 44 (1960), and references therein.

(4)(a) S. Edelbacher and H. von Bidder, *Z. Physiol. Chem.*, **276**, 126 (1942); (b) R. G. Fargher and F. L. Pyman, *J. Chem. Soc.*, 734 (1921); (c) O. Gerngross, *Ber.*, **42**, 404 (1909); (d) A. Windaus and W. Vogt, *Beitr. Chem. Physiol. Pathol.*, **11**, 406 (1908).

(5)(a) P. Brewster, F. Hiron, E. D. Hughes, C. K. Ingold, and P. A. D. Rao, *Nature*, **166**, 178 (1950); (b) C. K. Ingold, "Structure and Mechanism in Organic Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p 397; (c) *ibid.*, p 383.

(6) For reactions involving intramolecular participation of imidazole see T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. I, W. A. Benjamin, New York, N. Y., 1966, p 133.

(7) A. Darapsky, *J. Prakt. Chem.*, **99**, 179 (1919).

(8) H. Niedrich and R. Grupe, *ibid.*, **27**, 108 (1965).

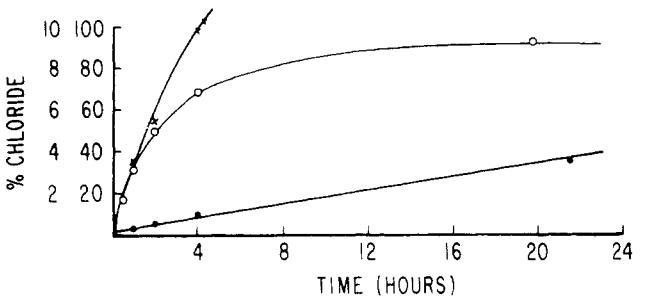


Figure 1.—Rate of chloride liberation in reaction of L-2-chloro-3-[4(5)-imidazolyl]propionic acid and hydrazine in refluxing methanol: O–O, molar ratio of hydrazine to α-chloro acid 6:1, scale of % ionic chloride 0–100%; ●–●, molar ratio of hydrazine to α-chloro acid 1.5:1, scale of % ionic chloride 0–100%; X–X molar ratio of hydrazine to α-chloro acid 1.5:1, scale of % ionic chloride 0–10%.

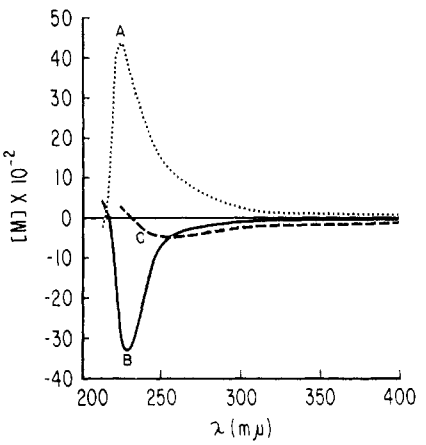


Figure 2.—Rotatory dispersion curves in 1 *N* HCl of L-histidine (curve A, 6.24 mg/100 ml), D-2-hydrazino-3-[4(5)-imidazolyl]propionic acid hydrochloride (curve B, 6.15 mg/100 ml), and L-2-chloro-3-[4(5)-imidazolyl]propionic acid (curve C, 6.10 mg/100 ml) determined on the Cary Model 60 spectropolarimeter. For rotatory dispersion curves of L-histidine at different pH values, see D. W. Urry and H. Eyring, *J. Am. Chem. Soc.*, **86**, 4574 (1964).

L-2-chloro-3-[4(5)-imidazolyl]propionic acid are plotted in Figure 2. The close similarity in the curves for the amino and hydrazino acids, plus the reversal of the Cotton effect with the latter, supported the assignment of the D configuration to this hydrazino acid enantiomer. Sufficient ORD data were not available from analogous α-halo acids to permit the use of this method for assignment of configuration to the α-chloro acid.

The extent of racemization when the hydrazine reaction was run at room temperature appeared to be very small. When carried out in refluxing methanol, the reaction was complete within 24 hr, with considerable racemization occurring. The rate of racemization of the L-α-chloro acid under these conditions was found to be more rapid than that of the product.

Diazotization of L-histidine methyl ester using the conditions employed for the free amino acid gave a 48% yield of L-α-chloro acid. Retention of configuration under these conditions was contrary to expectation.[5b] A control experiment demonstrated that the ester survived the diazotization conditions in the absence of sodium nitrite. It is not clear whether hydrolysis occurred during the diazotization step or during subsequent work-up. However, if an α-lactone mechanism[5c] operated here, then facile demethylation might be expected on this intermediate.[9]

Biological Activity.—D-2-Hydrazino-3-[4(5)-imidazolyl]propionic acid has been shown by Sjoerdsma and co-workers[2] to be a potent inhibitor of both specific and nonspecific histidine decarboxylase *in vitro*. These workers also demonstrated its effectiveness in decreasing histamine levels *in vivo* in female rats. The results of biological studies with the L enantiomer and the racemate, carried out by our colleagues in the Merck Institute for Therapeutic Research, will be submitted for publication shortly.

Experimental Section[10]

L-, D-, and DL-2-Chloro-3-[4(5)-imidazolyl]propionic Acid (I).—The reaction of L-histidine hydrochloride with $NaNO_2$ and HCl, as described by Edelbacher and von Bidder,[4a] gave L-2-chloro-3-[4(5)-imidazolyl]propionic acid in 58% yield, mp 193° dec, $[\alpha]_{578}$ −14.7°, $[\alpha]_{546}$ −17.5°, $[\alpha]_{436}$ −30.1°, $[\alpha]_{405}$ −36.2°, $[\alpha]_{365}$ −48.3° (*c* 4%, 1 *N* HCl). *Anal.* ($C_6H_7ClN_2O_2$) C, H, Cl, N.

Employing D-histidine, D-2-chloro-3-[4(5)-imidazolyl]propionic acid, mp 188° dec, was obtained in 67% yield; $[\alpha]_{578}$ +14.7°, $[\alpha]_{546}$ +16.8°, $[\alpha]_{436}$ +29.2°, $[\alpha]_{405}$ +35.2°, $[\alpha]_{365}$ +47.1° (*c* 4%, 1 *N* HCl). DL-Histidine gave the DL-α-chloro acid, mp 197–198° dec (lit.[4b] 201°).

D-, L-, and DL-2-Hydrazino-3-[4(5)-imidazolyl]propionic Acid Hydrochloride (II).—A solution containing 17.45 g (0.1 mole) of L-2-chloro-3-[4(5)-imidazolyl]propionic acid, 35.5 ml (0.6 mole) of 85% hydrazine hydrate, and 150 ml of MeOH was allowed to stand at room temperature for 3 weeks. Following evaporation to dryness, concentrated HCl (100 ml) was added. Hydraine dihydrochloride was removed by filtration and the filtrate was evaporated to dryness. H_2O was added and the solution was evaporated to dryness. After thorough drying *in vacuo*, MeOH (150 ml) was added. The resulting mixture was filtered and a solution of $(n\text{-Bu})_3N$ (18.5 g, 0.1 mole) in MeOH was added slowly to the filtrate. The solids after filtration and washing with MeOH, weighed 16.0 g (77%), mp 187° dec. The analytical sample, obtained by evaporative crystallization from MeOH, of the D isomer had mp 187° dec. Phase-solubility analysis in MeOH showed slope = 1%, $[\alpha]_{578}$ +22.8°, $[\alpha]_{546}$ +26.2°, $[\alpha]_{436}$ +41.1°, $[\alpha]_{405}$ +46.9° (*c* 4%, H_2O).

Anal. ($C_6H_{10}N_4O_2 \cdot HCl$) C, H, Cl, N.

L-2-Hydrazino-3-[4(5)-imidazolyl]propionic acid hydrochloride, prepared in a similar manner from the D-2-chloro acid, had mp 184° dec. Phase-solubility analysis (MeOH) showed a slope = 5 ± 0.5%, $[\alpha]_{578}$ −22.4°, $[\alpha]_{546}$ −25.1°, $[\alpha]_{436}$ −39.3°, $[\alpha]_{405}$ −45.3° (*c* 4%, H_2O).

DL-2-Hydrazino-3-[4(5)-imidazolyl]propionic acid hydrochloride prepared by the same procedure in 68% yield showed slight melting at 143° (apparently a phase transition) with mp 184° dec, mmp (DL- and D-) 174° dec. The ir spectrum (Nujol mull) differed from that of the D and L enantiomers, indicative of a DL compound.

Hydrazine Reaction in Refluxing Methanol.—When 4.36 g of L-2-chloro-3-[4(5)-imidazolyl]propionic acid, 8.8 ml of 85% hydrazine hydrate and 50 ml of MeOH were allowed to react at reflux for 23 hr and worked up as described previously, a 59% yield of D-hydrazino acid of 92% optical purity was obtained. In a repeat experiment the rate of reaction was determined by titration of Cl^-. The data are plotted in Figure 1. Changes in rotation of the reaction mixture were determined at four wavelengths from 20 to 44.33 hr. The decrease in rotation over this period was approximately 1%/hour.

(9) S. Kabuss, *Angew. Chem. Intern. Ed. Engl.*, **5**, 675 (1966).

(10) Melting points were determined with a Thomas–Hoover melting point apparatus or with a Kofler micro hot stage. Specific rotations were measured at room temperature. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.3% of the theoretical values.

TABLE I

λ, mμ	Recrystd product from hydrogenolysis, [α], deg	L-Histidine, [α], deg
589	−13.5	+12.7
578	−14.0	+13.5
546	−16.5	+16.2
436	−32.5	+31.1

In another experiment a mixture of 4.36 g of L-2-chloro-3-[4(5)-imidazolyl]propionc acid, 3.0 ml of H_2O, 2.20 ml of 85% hydrazine hydrate, and 50 ml of MeOH was refluxed and the rate of liberation of Cl^- was determined (see Figure 1). Assuming that 1 mole of hydrazine was utilized in converting the α-chloro acid to the anion, the concentration of free hydrazine present at the start of this reaction was one-tenth that of the mixture with the standard excess of hydrazine.

An attempt was made to determine the rate of racemization of α-chloro acid under the refluxing MeOH conditions. L-2-Chloro-3-[4(5)-imidazolyl]propionic acid (4.36 g), 8.8 ml of hydrazine hydrate, and 40 ml of MeOH were refluxed 1 hr (30% completion based on liberated Cl^-). An aliquot (1% of mixture) was evaporated to dryness. The residue was chromatographed on a column of 50 g of silica gel using 4:1 MeOH–concentrated NH_4OH. The chlorine-containing fraction was partially evaporated and then flushed with MeOH. The partly crystalline residue, after washing with MeOH, gave 0.16 g (36%) of L-2-chloro acid, mp 192° dec, of about 96% optical purity. The mother liquor fraction on chromatography on 40 g of silica gel using 4:1 MeOH–H_2O gave 0.10 g (23%) of 2-chloro acid, mp 192° dec, of 35% optical purity. This material gave a single spot on tlc on a silica gel plate using 4:1 MeOH–H_2O. The average optical purity of the recovered α-chloro acid was 72%.

Diazotization of L-Histidine Methyl Ester.—To a mixture of L-histidine methyl ester dihydrochloride (2.57 g, 0.01 mole) and 14.3 ml of concentrated HCl at −3 to +3° was added over 6 min a solution of 1.92 g of $NaNO_2$ in 3.75 ml of water. The mixture was kept at about −5° for 45 min and then allowed to warm to room temperature over 1.5 hr. Following filtration to remove NaCl the solution was evaporated to dryness. After flushing twice with H_2O and twice with *t*-BuOH the residue was dissolved in 10 ml of H_2O and the solution was neutralized to pH 4.2. The product (0.84 g, 48%) was identical with the L-2-chloro-3-[4(5)-imidazolyl]propionic acid prepared from L-histidine. In a blank experiment in which the $NaNO_2$ was omitted, starting ester was recovered in 90.7% yield.

Hydrogenolysis of D-2-Hydrazino-3-[4(5)-imidazolyl]propionic Acid Hydrochloride.—Raney Ni catalyst (0.5 teaspoon), neutralized by storage under EtOAc, was added to a solution of D-2-hydrazino-3-[4(5)-imidazolyl]propionic acid hydrochloride (2.0 g) in 40 ml of MeOH and 50 ml of H_2O. The mixture was hydrogenated at 2.8 kg/cm² and 50° for 5 hr. Two-thirds of the theoretical quantity of H_2 was consumed. Following removal of the catalyst, the filtrate was evaporated to dryness. MeOH (20 ml) was added and the solution was evaporated to dryness. After repeating this step several times a crystalline product was isolated (0.55 g, mp 263–266°). A second fraction (0.25 g, softened at 207°, mp 240°) was isolated on evaporation of the mother liquors. Recrystallization of the first fraction from water gave D-histidine, mp 272–280°. Amino acid analysis (Spinco) showed 96.8% hisitidine. *Anal.* ($C_6H_9N_3O_2$) C, H, N. The ir spectrum was identical with that of L-histidine. The specific rotations (2% in 6 *N* HCl) compared to L-histidine are listed in Table I.

Acknowledgment.—The authors wish to express their appreciation to Mr. R. N. Boos and Mr. K. B. Streeter and their associates for the microanalytical data, to Dr. N. R. Trenner and associates for the phase-solubility analyses, and to Dr. J. J. Wittick for rotatory dispersion measurements.

Syntheses and Biological Activities of Some Cycloalkenealanines

THOMAS H. PORTER,[1a] ROBERT M. GIPSON,[1b] AND WILLIAM SHIVE

The Clayton Foundation Biochemical Institute and the Department of Chemistry, The University of Texas, Austin, Texas 78712

Received November 3, 1967

The alicyclic amino acids, DL-3-cyclopentene-1-alanine, DL-2-cyclopentene-1-alanine, DL-2-cyclohexene-1-alanine, and DL-1-cycloheptene-1-alanine were synthesized, and their effects upon the growth of several microorganisms were determined. DL-3-Cyclopentene-1-alanine inhibits the growth of three *Escherichia coli* strains and the lactic acid bacteria, *Lactobacillus plantarum* 8014; the growth inhibition of *E. coli* 10856 is reversed in a competitivelike manner by leucine only in the presence of a small supplement of methionine in a manner suggesting that the analog inhibits utilization of leucine and the biosynthesis of methionine. DL-2-Cyclohexene-1-alanine and DL-2-cyclopentene-1-alanine inhibit the growth of *Leuconostoc dextranicum* 8086, and the toxicities of both analogs were reversed in a competitivelike manner over a 10–20-fold range by increasing concentrations of leucine. DL-1-Cycloheptene-1-alanine prevented the growth of *Leuconostoc dextranicum* 8086 only at very high levels; no reversal of toxicity could be demonstrated with phenylalanine.

Several alicylic amino acids have been reported to have rather specific antimetabolite activity, and the structural relationships between the natural metabolites and the active analogs have demonstrated the importance of steric factors in the biological activity of these compounds. For example, 1-cyclopentene-1-alanine and 1-cyclohexene-1-alanine have been found to be competitive antagonists of phenylalanine in contrast to the corresponding saturated derivatives, cyclopentanealanine and cyclohexanealanine, the first of which has slight activity only as a leucine antagonist and the second of which has no demonstrable antimetabolite activity in the microorganisms studied. The emanation of the first carbon of the side chain in the plane of the 1-carbon and adjacent carbons of the alicyclic ring apparently is important for phenylalanine antagonism.[2] Other unsaturated alicyclic amino acid analogs which have been found to be active include 2-cyclopentene-1-glycine, 3-cyclopentene-1-glycine, 2-cyclohexene-1-glycine, and 3-cyclohexene-1-glycine, all of which are antagonists of isoleucine;[3,4] however, 2-cyclopentene-1-glycine also inhibits assimilation of

(1) (a) NSF Predoctoral Cooperative Graduate Fellow, 1965–1967; (b) Rosalie B. Hite Predoctoral Fellow, 1962–1965.

(2) (a) J. Edelson, P. R. Pal, C. G. Skinner, and W. Shive, *J. Am. Chem. Soc.*, **79**, 5209 (1957); (b) P. R. Pal, C. G. Skinner, R. L. Dennis, and W. Shive, *ibid.*, **78**, 5116 (1956).

(3) J. Edelson, J. D. Fissekis, C. G. Skinner, and W. Shive, *ibid.*, **80**, 2698 (1958).

(4) R. M. Gipson, C. G. Skinner, and W. Shive, *Arch. Biochem. Biophys.*, **111**, 264 (1965).