

# Inhibition of Histidine Decarboxylase. Derivatives of Histidine

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Twenty-eight derivatives of histidine were tested as inhibitors of histidine decarboxylase from rat stomach. Potent inhibition was observed with the methyl ester of L-histidine (1) which had a  $K_i = 1.8 \times 10^{-6}$  M. Substitution on 1 at various positions resulted in a decrease or complete loss of inhibition. Substituent requirements for inhibition of histidine decarboxylase are discussed. A simple disposable apparatus for the radiometric assay of histidine decarboxylase is described.

Histamine has been implicated as a causative agent in many physiological processes. The molecule is not only a modulator of normal physiological functions such as dilatation in the cardiovascular system, contraction of smooth muscle, and regulation of gastric acid secretion but is also implicated in the symptomatology of some human disease conditions such as ulcer, allergy, hypersensitivity, atherosclerosis, and the inflammatory response.<sup>1-3</sup>

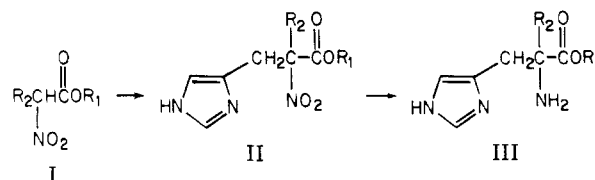
Many drugs have been developed which interfere with the action of histamine by competing for its specific cellular receptor sites. These histaminic receptors have recently been categorized into two types: (1) the  $H_1$ -receptor sites which are blocked by the classical tertiary amine antihistamines<sup>4</sup> and (2) the  $H_2$ -receptor sites which are specifically antagonized by burimamide.<sup>5</sup> An alternative approach to the development of an antihistamine drug would be to develop a compound which alters the de novo biosynthesis of histamine by influencing histidine decarboxylase.<sup>2</sup> Although in vitro decarboxylation of histidine to form histamine can be catalyzed by either a specific histidine decarboxylase (E.C. 4.1.1.22)<sup>6,7</sup> or a nonspecific aromatic amino acid decarboxylase (E.C. 4.1.1.26),<sup>8</sup> animal studies have shown that the specific histidine decarboxylase is responsible for histamine biosynthesis in vivo.<sup>9</sup>

Several potent inhibitors of the specific histidine decarboxylase are known.<sup>1,9</sup> The most active in vitro are the benzyloxyamines,<sup>10-12</sup> of which brocresine (4-bromo-3-hydroxybenzyloxyamine) has been most thoroughly studied. The in vivo action of this compound was reported to be very transient.<sup>13</sup> Furthermore, brocresine is a nonspecific inhibitor since it is a carbonyl reagent and reacts with the cofactor pyridoxal phosphate.<sup>9,14</sup> Consequently, it inhibits the specific histidine decarboxylase and the general aromatic amino acid decarboxylase<sup>1</sup> as well as other pyridoxal phosphate requiring enzymes such as diamine oxidase.<sup>15</sup>

Until Smissman's<sup>16</sup> report on the synthesis of 4-(4-imidazolyl)-3-amino-2-butanone and the subsequent biochemical studies by Taylor et al.<sup>17</sup> which showed that this compound was a potent inhibitor of histidine decarboxylase, the only noncarbonyl reagent inhibitors of histidine decarboxylase which were specific, such as  $\alpha$ -methylhistidine,<sup>18-20</sup> were relatively weak inhibitors.<sup>9,17</sup> A potent and specific inhibitor of histidine decarboxylase appeared to be of interest as a new type of antihistamine as well as a useful research tool. Consequently, we wish to report at this time some of our preliminary work on candidate inhibitors of the specific histidine decarboxylase from rat stomach.

**Chemistry.** Synthesis of the  $\alpha$ -substituted histidines was modeled after the work of Robinson and Shepherd<sup>18</sup> and of Slettinger and Pfister<sup>21</sup> as outlined in Scheme I. The sodium salt of the appropriate  $\alpha$ -nitro ester<sup>22</sup> (I) (Scheme I) was alkylated with 4-chloromethylimidazole<sup>18,23</sup> in methanol or dimethylformamide, although with the  $\alpha$ -phenyl analogue (I,  $R_1 = \text{CH}_2\text{CH}_3$ ;  $R_2 = \text{C}_6\text{H}_5$ ) hexa-

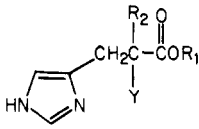
Scheme I



methylphosphoramidate was required to obtain even a low yield of product. Reduction of the nitro esters II was best accomplished by catalytic hydrogenation. Although the reduction required a high catalyst to substrate ratio and extended reaction times, in all but one case the products were obtained in high yield and purity. Attempted reduction of 23 (Table I) failed to give the desired product. In one case (10) the amino ester was hydrolyzed with concentrated hydrochloric acid to give the  $\alpha$ -alkyl amino acid 6. Esterification of 14 and 27<sup>24</sup> catalyzed by thionyl chloride<sup>25</sup> in methanol proceeded smoothly to give 15 and 28. Esterification of histidine, however, with 1-propanol or 1-butanol in the presence of hydrogen chloride<sup>26</sup> or thionyl chloride<sup>25</sup> proceeded sluggishly with only low yield of analytically pure material. The amide 11 was available from DL-histidine methyl ester<sup>27</sup> and methylamine.

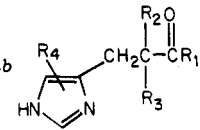
**Enzyme Results.** The inhibition of histidine decarboxylase from rat stomach was measured using a modified literature procedure<sup>28</sup> by following the decarboxylation of L-histidine- $\text{carboxyl-}^{14}\text{C}$ . That  $\alpha$ -methyl-L-histidine (5) was a weak inhibitor was verified<sup>20</sup> (Table II); 5 caused 50% inhibition at 0.32 mM in our test system. When the  $\alpha$ -substituent of 5 was extended to *n*-butyl (6) nearly all inhibition was lost. Excellent inhibition was observed with the methyl ester of L-histidine (1). It caused 50% inhibition at  $4 \times 10^{-6}$  M and had a  $K_i = 1.8 \times 10^{-6}$  M as determined from slopes of a Dixon plot.<sup>29</sup> When the methyl group of 1 was extended to ethyl (2) a threefold loss in inhibitory potency occurred. With the longer chained *n*-propyl (3) and *n*-butyl (4) esters, inhibition was decreased more than 20-fold over 1 indicating a lack of bulk tolerance<sup>30</sup> for alkyl groups larger than ethyl. Since both 1 and 5 were inhibitors, the methyl substituents were combined in a single molecule (7) in hopes that these effects would be additive; however, when a methyl substituent was placed on the  $\alpha$  position of 1 as in 7, nearly complete loss of inhibition occurred. Similarly, none of the  $\alpha$ -alkylamino esters 8, 9, or 10 showed appreciable activity at 0.1 mM. In addition, the importance of the  $\alpha$ -amino group in 1 for binding was evident from the poor inhibition shown by 15. The substituent requirements for inhibition by the methyl ester of L-histidine (1) are quite specific. Within the enzyme-inhibitor complex there is insufficient room for both an  $\alpha$ -alkyl group and the *O*-methyl moiety on the histidine molecule. Substitution on the methyl moiety of ester 1 leads to weaker inhibitors; the need for an  $\alpha$ -amino group is also a requirement for inhibition by 1.

Table I. Derivatives of 3-(4-Imidazolyl)propionate



No.	Y	R <sub>2</sub>	R <sub>1</sub>	Methods	% yield	Mp, °C	Formula <sup>a</sup>
7	NH <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	C	88	120-140 eff <sup>b</sup>	C <sub>8</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub> ·2HCl·0.5H <sub>2</sub> O·2/3MeOH
8	NH <sub>2</sub>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	C <sup>c</sup>	63 <sup>d</sup>	201-203 eff	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> ·2HCl
9	NH <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	C	67	188-189 eff <sup>b</sup>	C <sub>10</sub> H <sub>17</sub> N <sub>3</sub> O <sub>2</sub> ·2HCl
10	NH <sub>2</sub>	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	CH <sub>3</sub>	C	88	178-179 eff <sup>b</sup>	C <sub>11</sub> H <sub>19</sub> N <sub>3</sub> O <sub>2</sub> ·2HCl
15	H	H	CH <sub>3</sub>	D	96	108-110 <sup>e</sup>	C <sub>7</sub> H <sub>10</sub> N <sub>3</sub> O <sub>2</sub> ·HCl
19	NO <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	A	54	170-171 eff <sup>f</sup>	C <sub>8</sub> H <sub>11</sub> N <sub>3</sub> O <sub>4</sub> ·HCl
20	NO <sub>2</sub>	CH <sub>2</sub> CH <sub>3</sub>	CH <sub>2</sub> CH <sub>3</sub>	B	40	181-182 eff <sup>d</sup>	C <sub>10</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> ·HCl
21	NO <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	CH <sub>3</sub>	A	33 <sup>b</sup>	154-155 eff	C <sub>10</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub> ·HCl
22	NO <sub>2</sub>	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	CH <sub>3</sub>	A	40 <sup>b</sup>	133-134 eff	C <sub>11</sub> H <sub>17</sub> N <sub>3</sub> O <sub>4</sub> ·HCl
23	NO <sub>2</sub>	C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> CH <sub>3</sub>	B <sup>g</sup>	15	96-97 <sup>h</sup>	C <sub>14</sub> H <sub>15</sub> N <sub>3</sub> O <sub>4</sub>

<sup>a</sup> All compounds were analyzed for C, H, and N. <sup>b</sup> Recrystallized from MeOH-Et<sub>2</sub>O. <sup>c</sup> Raney nickel in EtOH was used instead of Pd on carbon and 12 N HCl in MeOH. <sup>d</sup> Recrystallized from EtOH-Et<sub>2</sub>O. <sup>e</sup> Recrystallized from 2-ProH. <sup>f</sup> Recrystallized from MeOH-CHCl<sub>3</sub>. <sup>g</sup> HMPA was used as the reaction solvent. <sup>h</sup> Recrystallized from hexanes.

Table II. Inhibition<sup>a</sup> of Histidine Decarboxylase by<sup>b</sup>


No.	R <sub>4</sub>	R <sub>3</sub>	R <sub>2</sub>	R <sub>1</sub>	Concn, mM	% <sup>c</sup> inhibn
1 <sup>d</sup>	H	NH <sub>2</sub>	H	OCH <sub>3</sub>	0.004	50
2 <sup>e</sup>	H	NH <sub>2</sub>	H	OCH <sub>2</sub> CH <sub>3</sub>	0.012	50
3	H	NH <sub>2</sub>	H	O(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	0.10	22
4	H	NH <sub>2</sub>	H	O(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	0.10	62
5 <sup>f</sup>	H	NH <sub>2</sub>	CH <sub>3</sub>	OH	0.10	19
					0.32	50
6	H	NH <sub>2</sub>	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	OH	0.10	3
7	H	NH <sub>2</sub>	CH <sub>3</sub>	OCH <sub>3</sub>	0.10	3
8	H	NH <sub>2</sub>	CH <sub>2</sub> CH <sub>3</sub>	OCH <sub>2</sub> CH <sub>3</sub>	0.10	13
9	H	NH <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	OCH <sub>3</sub>	0.10	2
10	H	NH <sub>2</sub>	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	OCH <sub>3</sub>	0.10	0
11	H	NH <sub>2</sub>	H	NHCH <sub>3</sub>	0.10	0
12 <sup>d</sup>	H	NH <sub>2</sub>	H	NHCH <sub>2</sub> CO <sub>2</sub> H	0.10	0
13 <sup>d</sup>	H	NH <sub>2</sub>	H	NHOH	0.10	14
14 <sup>g</sup>	H	H	H	OH	0.13	9
15	H	H	H	OCH <sub>3</sub>	0.10	10
16 <sup>h</sup>	H	Br	H	OH	0.10	13
17 <sup>g</sup>	H	OH	H	OH	0.13	0
18 <sup>d</sup>	H	NHC(O)CH <sub>3</sub>	H	OH	0.50	0
19	H	NO <sub>2</sub>	CH <sub>3</sub>	OCH <sub>3</sub>	0.10	25
20	H	NO <sub>2</sub>	CH <sub>2</sub> CH <sub>3</sub>	OCH <sub>2</sub> CH <sub>3</sub>	0.10	5
21	H	NO <sub>2</sub>	(CH <sub>2</sub> ) <sub>2</sub> CH <sub>3</sub>	OCH <sub>3</sub>	0.10	32
22	H	NO <sub>2</sub>	(CH <sub>2</sub> ) <sub>3</sub> CH <sub>3</sub>	OCH <sub>3</sub>	0.10	24
23	H	NO <sub>2</sub>	C <sub>6</sub> H <sub>5</sub>	OCH <sub>2</sub> CH <sub>3</sub>	0.10	22
24 <sup>d</sup>	1-CH <sub>3</sub>	NH <sub>2</sub>	H	OH	0.10	0
25 <sup>d</sup>	3-CH <sub>3</sub>	NH <sub>2</sub>	H	OH	0.10	0
26 <sup>i</sup>	2-CH <sub>3</sub>	NH <sub>2</sub>	H	OH	0.10	0
27 <sup>j</sup>	5-NO <sub>2</sub>	NH <sub>2</sub>	H	OH	0.10	0
28	5-NO <sub>2</sub>	NH <sub>2</sub>	H	OCH <sub>3</sub>	0.10	3

<sup>a</sup> The enzyme from rat stomach was assayed with 0.24 mM L-histidine-carboxyl-<sup>14</sup>C as described in the Experimental Section. <sup>b</sup> The compounds were tested as DL mixtures except for 1, 2, 5, 12, 13, 17, 18, 24, 25, 27, and 28 which were the L isomer. <sup>c</sup> Values were obtained in triplicate and were within 5% of one another. <sup>d</sup> Commercially available from Sigma Chemical Co. as the L isomer. <sup>e</sup> Commercially available from Pfaltz and Bauer as the L isomer. <sup>f</sup> Commercially available from Grand Island Biological as the L isomer. <sup>g</sup> Commercially available from Calbiochem. <sup>h</sup> Commercially available from Pierce Chemical Co. as the DL isomer. <sup>i</sup> Commercially available from Cyclo Biochemicals as the DL isomer. <sup>j</sup> Prepared by the method in ref 24.

A few additional structural modifications of the methyl ester 1 and of the substrate, L-histidine, were examined. When the ester oxygen of 1 was replaced with NH as in 11, no inhibition was observed as was also the case for the dipeptide 12. The weak activity of the hydroxamic acid 13 was confirmed.<sup>31</sup> When the α-amino group of the substrate was replaced with hydrogen (14), bromo (16),

hydroxyl (17), or acetylamino (18), little or no inhibition was observed. This observation supports the supposition of Smissman and co-workers<sup>16,31</sup> that the α-amino group is a binding site for the histidine molecule.

Several of the α-nitro esters, synthetic intermediates to the α-amino esters, were also tested. Surprisingly, 19, 21, 22, and 23 showed significant amounts of inhibition at 0.1

mM. This was most unexpected since the  $\alpha$ -amino counterparts (7, 9, and 10) showed essentially no inhibition at comparable concentrations. When a methyl group was introduced into the 1, 2, or 3 position of the imidazole ring (see 24, 25, and 26) of histidine, no inhibition of the enzyme was observed. Introduction of a 5-nitro group into 1 was also detrimental to inhibition as was evident with 28. These observations indicate that the imidazole ring of histidine must be unsubstituted for it to serve as a substrate or inhibitor of histidine decarboxylase. The lack of activity by 28 could also be ascribed to the change in  $pK_a$  of the imidazole ring by the strongly electron-withdrawing nitro substituent.

In conclusion, these results show that histidine decarboxylase can be inhibited by esters of histidine although the structural requirements are quite specific. The methyl ester of histidine (1), which has a  $K_i = 1.8 \times 10^{-6}$  M, is approximately tenfold more active than 4-(4-imidazolyl)-3-amino-2-butanone reported by Taylor et al.,<sup>17</sup> who employed assay conditions similar to those in the present study. These workers found 4-(4-imidazolyl)-3-amino-2-butanone to be 30-fold less inhibitory (based on molar  $I_{50}$  values) than brocresine as an inhibitor of histidine decarboxylase. Thus 1 may have an inhibitory potency in the same range as that of the carbonyl reagent brocresine. 1 was subjected to a variety of pharmacological tests, but no activity of interest was found. This lack of *in vivo* activity may be due to hydrolytic cleavage of the ester to histidine by endogenous esterases.

## Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on TLC. The analytical samples gave combustion values for C, H, and N within 0.4% of theoretical values. All compounds synthesized for enzyme studies were analytically pure.

**Method A. Methyl DL- $\alpha$ -Methyl- $\alpha$ -nitro- $\beta$ -(4-imidazolyl)propionate Hydrochloride (19).** To a stirred solution of 2.16 g (40.0 mmol) of sodium methoxide in 50 mL of MeOH cooled to 0 °C was added a solution of 2.66 g (20.0 mmol) of methyl  $\alpha$ -nitropropionate<sup>22</sup> in 10 mL of MeOH. The ice bath was removed and the mixture was stirred at ambient temperature for 1 h. The reaction was again cooled on an ice bath and a solution of 3.06 g (20.0 mmol) of 4-chloromethylimidazole hydrochloride<sup>18,23</sup> in 15 mL of MeOH was added dropwise. After 2 h at ambient temperature the reaction mixture was spin evaporated in vacuo, the residue was partitioned between 100 mL of H<sub>2</sub>O and 100 mL of Et<sub>2</sub>O, the layers were separated, and the aqueous phase was extracted with three 30-mL portions of Et<sub>2</sub>O. The combined extracts were washed with 50 mL of H<sub>2</sub>O and 50 mL of brine, dried over MgSO<sub>4</sub>, and evaporated to give a clear oil. The product was purified by dissolving it in a minimum of acetone, to which was added 2.0 g (22.0 mmol) of oxalic acid in a minimum of acetone. Upon cooling, 3.47 g (68%) of the crude salt precipitated, mp 145–147 °C. This was converted to the HCl salt by dispersing the solid in H<sub>2</sub>O, basifying with 5% aqueous NaHCO<sub>3</sub>, and then extracting with CHCl<sub>3</sub>. The CHCl<sub>3</sub> extracts were washed with H<sub>2</sub>O and brine, dried with Na<sub>2</sub>SO<sub>4</sub>, and evaporated to a colorless oil. This oil was dissolved in MeOH, diluted with a solution of MeOH previously saturated with HCl, and evaporated to a solid which was collected and washed with Et<sub>2</sub>O: yield, 2.70 g (54%); mp 169–170 °C eff. Recrystallization from MeOH–CHCl<sub>3</sub> afforded the analytical sample, mp 170–171 °C eff. Anal. (C<sub>8</sub>H<sub>12</sub>ClN<sub>3</sub>O<sub>4</sub>) C, H, N.

**Method B. Ethyl DL- $\alpha$ -Nitro- $\alpha$ -(4-imidazolylmethyl)butanoate Hydrochloride (20).** To a stirred, ice bath cooled dispersion of 1.77 g (44.5 mmol) of NaH (60% dispersion in mineral oil which had been previously washed with hexanes) in 40 mL of DMF was added a solution of 3.22 g (20.0 mmol) of ethyl  $\alpha$ -nitrobutyrate<sup>22</sup> dropwise over 60 min. The ice bath was removed and the reaction stirred for 3 h at ambient temperature when effervescence was complete. The reaction solution was cooled

and 3.06 g (20.0 mmol) of 4-chloromethylimidazole hydrochloride<sup>18,23</sup> in 10 mL of DMF was added and then stirred for 20 h at ambient temperature. The reaction was poured over 300 mL of ice water layered over with 400 mL of Et<sub>2</sub>O. The layers were separated and the aqueous fraction was extracted with two 100-mL portions of Et<sub>2</sub>O. The combined ethereal fractions were washed with three 100-mL portions of H<sub>2</sub>O and brine and then dried with MgSO<sub>4</sub>. Spin evaporation in vacuo afforded an oil which was dissolved in 100 mL of Et<sub>2</sub>O and diluted with 10 mL of EtOH previously saturated with HCl. The resultant precipitate was collected, washed with Et<sub>2</sub>O, and dried: yield, 2.24 g (40%); mp 172–175 °C eff. Several recrystallizations from EtOH–Et<sub>2</sub>O afforded the analytical sample, mp 181–182 °C eff. Anal. (C<sub>10</sub>H<sub>16</sub>ClN<sub>3</sub>O<sub>4</sub>) C, H, N.

**Method C. Methyl DL- $\alpha$ -Amino- $\alpha$ -(4-imidazolylmethyl)hexanoate Dihydrochloride (10).** A mixture of 0.60 g (2.0 mmol) of 22 oxalate, 25 mL of MeOH, 0.5 mL of 12 N HCl, and 400 mg of 10% Pd on carbon was shaken in the presence of H<sub>2</sub> at 2–3 atm for 18 h at which time reduction was complete. The filtered solution was spin evaporated in vacuo and the residue was triturated with acetone to give a crystalline product: yield, 0.530 g (88%); mp 174–175 °C eff. Recrystallization from MeOH–Et<sub>2</sub>O afforded the analytical sample, mp 178–179 °C eff. Anal. (C<sub>11</sub>H<sub>21</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**Method D. Methyl L- $\alpha$ -Amino- $\beta$ -(4-nitro-5-imidazolyl)propionate Hydrochloride Methanolate (28).** To a stirred mixture of 3.46 g (15.9 mmol) of 27<sup>24</sup> and 33 mL of MeOH was added 3 mL of SOCl<sub>2</sub> dropwise. A clear solution soon formed which was stirred overnight at ambient temperature and then spin evaporated in vacuo. The residual foam was dissolved in a minimum of MeOH and diluted with Et<sub>2</sub>O to afford a granular solid: yield, 4.37 g (98%); mp 181–190 °C eff. Two recrystallizations from MeOH–Et<sub>2</sub>O afforded analytically pure material: yield, 2.03 g (45%); mp 198–200 °C dec. The presence of methanol was confirmed by NMR in D<sub>2</sub>O. Anal. (C<sub>7</sub>H<sub>15</sub>ClN<sub>4</sub>O<sub>5</sub>) C, H, N.

**DL- $\alpha$ -Amino- $\alpha$ -(4-imidazolylmethyl)hexanoic Acid Dihydrochloride (6).** A solution of 0.93 g (3.1 mmol) of 10 and 25 mL of 12 N HCl was heated on a steam bath for 42 h. The resultant solution was evaporated to a clear oil which crystallized under acetone: yield 0.66 g (75%); mp 225–227 °C eff. Recrystallization from EtOH–Et<sub>2</sub>O afforded the analytical material, mp 175–210 °C eff. Anal. (C<sub>10</sub>H<sub>18</sub>ClN<sub>3</sub>O<sub>2</sub>) C, H, N.

***n*-Propyl DL- $\beta$ -(4-Imidazolyl)propionate (3).** A mixture of 11.0 g (57.6 mmol) of DL-histidine and 200 mL of *n*-PrOH was refluxed with stirring for 2 h during which time HCl gas was bubbled through the reaction. The mixture was filtered to remove the unreacted histidine and diluted with 1 L of Et<sub>2</sub>O. The solids were collected and recrystallized from *n*-PrOH–C<sub>6</sub>H<sub>6</sub>: yield, 4.39 g (35%); melting point, broad effervescence beginning about 98 °C. An additional recrystallization afforded the analytical sample of unchanged melting point. Anal. (C<sub>9</sub>H<sub>17</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

***n*-Butyl DL- $\beta$ -(4-Imidazolyl)propionate Dihydrochloride (4).** A mixture of 1.94 g (10.1 mmol) of DL-histidine, 30 mL of *n*-BuOH, and 2 mL of SOCl<sub>2</sub> was heated on an oil bath (105 °C) for 48 h. The cooled reaction was diluted with Et<sub>2</sub>O. The solids, which appeared to be a mixture of ester and acid, were dispersed in 200 mL of CHCl<sub>3</sub> and washed with several portions of 5% NaHCO<sub>3</sub> and brine, dried with MgSO<sub>4</sub>, and then saturated with HCl for 5 min. This solution was evaporated to a syrup which was crystallized several times from *n*-BuOH–C<sub>6</sub>H<sub>6</sub>: yield, 0.075 g (2%); mp 122–123 °C. Anal. (C<sub>10</sub>H<sub>19</sub>Cl<sub>2</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**N-Methyl DL- $\beta$ -(4-Imidazolyl)propionamide Dihydrochloride (11).** A solution of 0.97 g (4.0 mmol) of DL-histidine methyl ester<sup>27</sup> in 25 mL of MeOH was saturated with MeNH<sub>2</sub> at 0 °C and then heated in a stainless steel bomb at 50 °C for 18 h. The solvent was evaporated; the residue was dissolved in about 50 mL of H<sub>2</sub>O and then stirred with excess ion-exchange resin [Rexyn 201(OH)] until a negative AgNO<sub>3</sub> test was obtained. The mixture was filtered and the filtrates were spin evaporated in vacuo to give a clear oil. The oil was dissolved in a minimum of EtOH, acidified with EtOH previously saturated with HCl, and again evaporated. The residual solid was collected and washed with Et<sub>2</sub>O: yield, 0.52 g (54%); mp 223–225 °C dec. Recrystallization from EtOH afforded the analytical sample of unchanged melting point. Anal. (C<sub>7</sub>H<sub>14</sub>Cl<sub>2</sub>N<sub>4</sub>O) C, H, N.

**Extract Preparation.** Sprague-Dawley rats (males, about 200 g, not fasted) were sacrificed by decapitation; their stomachs were removed, washed quickly in ice-cold isotonic saline, and frozen on dry ice. Subsequent procedures were performed at 0–4 °C. The stomachs were homogenized in a Waring Blendor in a 2.5-fold wet tissue weight to volume ratio of 0.1 M potassium phosphate–0.1 mM pyridoxal phosphate, pH 6.9. The homogenate was centrifuged at 39 000g for 30 min, and then the supernatant was centrifuged at 100 000g for 60 min. Saturated ammonium sulfate was added to this supernatant to 20% saturation. After centrifugation at 39 000g for 30 min, the supernatant was brought to 45% saturation with ammonium sulfate and centrifuged again. The pellet from this centrifugation was resuspended in a small volume of the above phosphate-pyridoxal phosphate buffer (about 2 mL per dozen rats) and stored at –67 °C. This extract gave a maximum velocity of 0.2 nmol/min per milligram of protein.

**Histidine Decarboxylase Assay.** The decarboxylation of L-histidine-carboxyl-<sup>14</sup>C (New England Nuclear Corp.) was followed. This substrate was adjusted with unlabeled L-histidine (Mann Research Laboratories) to give a stock solution with a specific radioactivity of 1.7 mCi/mmol and a concentration of 6 mM. Assays were performed in triplicate at pH 6.9 in a total volume of 0.25 mL of 0.05 M potassium phosphate buffer, which contained 25 µL of enzyme extract (0.2–0.3 mg of protein), 0.03 mM pyridoxal phosphate, and the above substrate at a final concentration of 0.24 mM. This concentration of histidine is about one-half the  $K_m$  value. Blanks which contained either no enzyme or boiled enzyme gave similar low activities. Inhibitors were dissolved within 1 h before use in glass distilled water and were generally present at 0.1 mM in assays.

Assay mixtures were incubated at 37 °C in disposable glass culture tubes (15 × 85 mm, Becton-Dickinson no. 7818) topped with Parafilm-covered corks, each of which was equipped with a small cup-hook. From each cup-hook was suspended a 4 × 0.5 cm strip of Whatman No. 2 filter paper, which was impregnated with 30 µL of Hyamine hydroxide. After 30 min of incubation at 37 °C, the tubes were placed in an ice water bath, 0.1 mL of 0.1 N HCl was carefully pipetted into the bottom of each tube, and the cork immediately replaced. The tubes were incubated 1 h longer at 37 °C to allow [<sup>14</sup>C]-CO<sub>2</sub> to be trapped by the Hyamine. Filter strips were then placed in scintillation vials containing 15 mL of a scintillation mixture made by dissolving 4 g of Omnifluor (New England Nuclear Corp.) in 1 L of toluene and adding 40 mL of Triton X-100. Samples were allowed to remain in the scintillation counter for at least 2 h before counting, in order to allow for the decay of chemiluminescence caused by Hyamine. CPM obtained in triplicate samples were within 5% of one another. After blank values were subtracted, percent inhibition was calculated using the equation

$$\%I = 1 - \left[ \frac{\text{CPM (+ inhibitor)}}{\text{CPM (– inhibitor)}} \right] \times 100$$

The  $K_i$  value for the methyl ester of L-histidine (1) was obtained by the Dixon method,<sup>29</sup> in which the reciprocal of enzyme activity was plotted against inhibitor concentration. The inhibitor was used in duplicate assays at four different concentrations ranging from 1 to 50 µM. The intercept of the straight lines obtained using two different substrate concentrations (0.25 and 0.50 mM) gave the  $K_i$  value.

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## References and Notes

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