Specificity in Enzyme Inhibition. 3. Synthesis of 5-Substituted 2,2-Dimethyl-4-imidazolidinones as Inhibitors of Tyrosine Decarboxylase and Histidine Decarboxylase

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2,2-Dimethyl-4-imidazolidinone derivatives of the α-amino acids DL-phenylglycine (1), DL-phenylalanine (2), L-tyrosine (3), L-histidine (4), and L-tryptophan (5) were prepared in order to assess their specificity in inhibiting amino acid decarboxylases. Treatment of the α-aminonitriles with acetone in the presence of base and heat or treatment of the α-amino amides with acetone gave the title compounds in 48–85% yield. The compounds afforded moderate ability to inhibit the decarboxylation of L-phenylalanine, L-tyrosine, or L-histidine in vitro, using crude enzymes. 3 was a better inhibitor of tyrosine decarboxylase (*S. faecalis*) than 2. 4 and 5 were comparable to 3 in inhibiting tyrosine decarboxylase. 4 was more selective in inhibiting purified histidine decarboxylase (*Cl. welchii*) than 5, which was inactive. 4 was inactive against fetal rat histidine decarboxylase in vitro.

Previous work from these laboratories has dealt with the preparation of specific inhibitors of histidine decarboxylase.[1,2] This work is a continuation of the design of active-site-directed reversible inhibitors of amino acid decarboxylases. The 4-imidazolidinone derivatives of the α-amino acids DL-phenylalanine, L-tyrosine, L-histidine, and L-tryptophan were prepared in order to assess their specificity in inhibiting decarboxylases utilizing L-phenylalanine, L-tyrosine, and L-histidine as substrates.

On the basis of both enzyme–substrate specificity and the requirement of pyridoxal as a cofactor, the hypothetical receptor site of a decarboxylase enzyme can be considered as having two binding sites and an active site. The binding sites can be designated as a specific site, which differentiates the side chain of the amino acid, and a nonspecific site, which binds the basic amino group. The carbonyl group of the amino acid corresponds to interactions at the active site, where decarboxylation occurs.[1,2]

According to this hypothesis, an active-site-directed inhibitor of an amino acid decarboxylase should be capable of binding at the specific and nonspecific sites of the enzyme, thus specifically blocking the attachment of the normal substrate and rendering it incapable of undergoing decarboxylation at the active site. The synthesis of 5-substituted 2,2-dimethyl-4-imidazolidinones was undertaken to meet these requirements, the amino and carbonyl portions of 4-imidazolidinone corresponding to the nonspecific and active sites, respectively, and substitution at the 5 position of the imidazolidinone conferring specificity for the enzyme.

The title compounds were prepared according to the method of Davis and Levy.[3] Treatment of the corresponding α-aminonitriles[4] with acetone in the presence of base and subsequent rearrangement of the resulting 4-substituted 5-imino-2,2-dimethyloxazolidines **1a** and **2a** in refluxing pyridine afforded the 5-substituted 2,2-dimethyl-4-imidazolidinones **1** and **2**. As was reported[3] for the hydrolysis of **1a**, we found that **2a** is similarly hydrolyzed to phenylalaninamide by shaking in cold water. However, the 4-imidazolidinones are not hydrolyzed by cold water and are only slowly hydrolyzed by boiling water.

† Deceased, 1974. This paper is dedicated to the memory of Professor Smissman.

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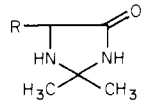
Table I

Compd[a]	Substrate[b]	% inhibn[c]
	Tyrosine Decarboxylase	
1	L-Phenylalanine	9 (7)[d]
2	L-Phenylalanine	17 (13)
2	L-Tyrosine	10
3	L-Phenylalanine	39 (31)
3	L-Tyrosine	38
4	L-Phenylalanine	27 (24)
5	L-Phenylalanine	42 (33)
	Histidine Decarboxylase	
4	L-Histidine	44
5	L-Histidine	2

[a] Inhibitors = 5×10^{-3} *M*. [b] L-Phenylalanine = 5×10^{-3} *M*; L-tyrosine = 8×10^{-4} *M*; L-histidine = 1×10^{-2} *M*. [c] Percent inhibition expressed as average of triplicate runs, obtained by measuring $^{14}CO_2$ as described in the Experimental Section. [d] Numbers in parentheses indicate L-phenylalanine = 1×10^{-2} *M*.

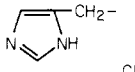
Hot concentrated alkali or hot dilute acid are necessary for complete hydrolysis, and compound **1** is reportedly stable in 2 *N* HCl.[3] Condensation of acetone with the corresponding L-α-amino amides gave compounds **3**, **4**, and **5**. Work is continuing on the synthesis of other amino acid analogs in this series.

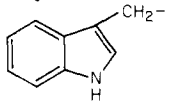


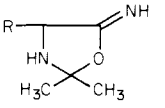
1, R = C_6H_5-

2, R = $C_6H_5CH_2$-

3, R = *p*-$HOC_6H_4CH_2$-

4, R= 

5, R= 



1a, **2a**

Biological Results. Studies of the L-histidine analog **4** on the inhibition of fetal rat histidine decarboxylase in vitro[5] were performed. Compound **4** was reported to be inactive at 10^{-3} *M*.[6] In a preliminary screen of histamine antagonism in vitro, using an isolated guinea pig ileum assay,[7,8] **4** proved ineffective at decreasing the histamine response, affording 57% inhibition of the response at only 10^{-3} *M*.[9]

Compounds **1–5** were tested for their ability to inhibit

the decarboxylation of L-phenylalanine, L-tyrosine, and L-histidine in vitro, using crude enzymes. The results are summarized in Table I. Tyrosine decarboxylase (*S. faecalis*)[10] effectively utilized L-phenylalanine as a substrate, although to a lesser extent than L-tyrosine, and was used in the assay for phenylalanine decarboxylase activity. The L-tyrosine analog **3** showed a greater inhibition of tyrosine decarboxylase than the DL-phenylalanine analog **2** when either L-phenylalanine or L-tyrosine were the substrates. Compounds **4** and **5** were comparable to **3** in inhibiting tyrosine decarboxylase. The hydrolysis product of **2a**, DL-phenylalaninamide, slightly increased (5%) the rate of L-phenylalanine decarboxylation with tyrosine decarboxylase and had no effect on the decarboxylation of L-tyrosine. The L-histidine analog **4** was more selective in inhibiting histidine decarboxylase (*Cl. welchii*) than the L-tryptophan analog **5**, which was inactive.

The relatively low inhibitory activities expressed by the 2,2-dimethyl-4-imidazolidinones in these biological systems indicate that these compounds are not effective in reaching the active sites of the enzymes. The apparently low affinities afforded by these analogs may be due to the steric bulk of the methyl groups at the 2 position of the imidazolidinone ring or to an unfavorable conformation of the imidazolidinone ring which might prevent the necessary binding to the enzymes. Other biological systems utilizing these compounds are presently under investigation.

Experimental Section

Melting points were determined on a Thomas-Hoover Uni-Melt and are uncorrected. Ir data were recorded on a Beckman DB spectrophotometer, and NMR spectra were obtained on a Varian T60 spectrometer. All compounds were consistent with their ir and NMR spectra, the latter of which were determined in $CDCl_3$, D_2O, or Me_2SO-d_6. Microanalyses were performed on a F & M CHN analyzer Model 185 in this department. Where analyses are indicated only by symbols of the elements, the analytical results were within ±0.3% of the theoretical values. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. L-Phenylalanine-*1*-^{14}C and L-tyrosine-*1*-^{14}C were purchased from New England Nuclear and L-histidine-*1*-^{14}C from Biochemical and Nuclear Corp. The tyrosine decarboxylase (Worthington) was a crude acetone powder from *S. faecalis*, and histidine decarboxylase was a purified powder (type II, Sigma) from *Cl. welchii*. Radioactivity was measured on a Beckman scintillation counter, using Packard II scintillation cocktail and Hyamine 10-X hydroxide (Packard) to absorb $^{14}CO_2$.

2,2-Dimethyl-5-phenyl-4-imidazolidinone (**1**). This compound was prepared from 2,2-dimethyl-5-imino-4-phenyloxazolidine (**1a**) in 74% yield, mp 154–155°, as previously described.[3] Anal. ($C_{11}H_{14}N_2O$) C, H, N.

2,2-Dimethyl-5-benzyl-4-imidazolidinone (**2**). 2,2-Dimethyl-5-imino-4-benzyloxazolidine (**2a**) was prepared from α-amino-α-benzylacetonitrile in dry acetone as described for **1a** above.[3] White crystals were obtained in 60% yield: mp 98–100°. Anal. ($C_{12}H_{16}N_2O$) C, H, N.

Compound **2a** (4 g, 0.02 mol) was refluxed in pyridine (10 ml) for 1 hr. After removal of the solvent in vacuo, a single recrystallization from EtOAc (20 ml) yielded **2** as needles (1.9 g, 48%): mp 87–89°. Anal. ($C_{12}H_{16}N_2O$) C, H, N.

(−)-2,2-Dimethyl-5-(*S*)-(*p*-hydroxybenzyl)-4-imidazolidinone (**3**). L-Tyrosinamide (10 g, 0.055 mol) was refluxed 30 min in a 4:1 dry acetone–benzene solution. After concentration in vacuo to a yellow oil, chilling in a dry ice–acetone bath afforded fine, white crystals which were collected and washed with acetone, yielding 7.0 g (58%) of **3**: mp 183–184°; $[\alpha]^{26}D$ −71.0° (*c* 0.283, MeOH–1% HOAc). Anal. ($C_{12}H_{16}N_2O_2$) C, H, N.

(−)-2,2-Dimethyl-5-(*S*)-(4-imidazolylmethyl)-4-imidazolidinone (**4**). L-Histidinamide (10 g, 0.066 mol) was dissolved in MeOH (50 ml). Acetone (50 ml) and 2,2-dimethoxypropane (50 ml) were added and the solution was refluxed 5 hr. Removal of solvent in vacuo left **4** as a hygroscopic brown oil (11.1 g, 85%). The dihydrochloride salt of **4** was obtained by addition of Et_2O–HCl to an EtOH–$CHCl_3$ solution of the free base followed by filtration of the resulting precipitate. **4** was obtained in a nonhygroscopic form as the dihydrochloride monohydrate by addition of acetone to an aqueous methanolic solution of the salt. The precipitate was collected, washed with Et_2O, and dried in vacuo at 70° for 20 hr: mp 245–246°; $[\alpha]^{26}D$ −68.7° (*c* 0.91, MeOH). Anal. ($C_9H_{14}N_4O \cdot 2HCl \cdot H_2O$) C, H, N.

(−)-2,2-Dimethyl-5-(*S*)-(3-indolylmethyl)-4-imidazolidinone (**5**). L-Tryptophanamide was refluxed for 30 min in 40 ml of dry acetone–benzene (4:1). Compound **5** was obtained as a brown oil after removal of solvent in vacuo. The camsylate salt of **5** was obtained by adding *d*-10-camphorsulfonic acid (CSA) in acetone to an equimolar portion of **5**. Concentration of the solution afforded white needles which were recrystallized from MeOH–acetone: mp 180° (dec 280°); $[\alpha]^{26}D$ −56.6° (*c* 1.44, DMF–1% HOAc). Anal. Calcd for $C_{24}H_{35}N_3O_5S \cdot H_2O$: C, 58.42; H, 7.10; N, 8.52. Found: C, 57.78; H, 7.21; N, 8.38.

Assay of Phenylalanine Decarboxylase. Phenylalanine decarboxylase activity was determined[11] by measuring the $^{14}CO_2$ produced from L-phenylalanine-*1*-^{14}C in the presence of tyrosine decarboxylase.[10] The reactions were performed in 10-ml Kontes reaction flasks with a side arm and a plastic center well containing Hyamine 10-X hydroxide to absorb the $^{14}CO_2$. The standard reaction mixture (total volume, 0.5 ml) contained 0.5 m*M* pyridoxal 5-phosphate in 0.2 *M* $NaPO_4$ buffer (pH 5.5), 5 or 10 m*M* L-phenylalanine-*1*-^{14}C (10^{-2} μCi/μmol) in 0.2 *M* $NaPO_4$ buffer (pH 5.5), and 0.5 mg of tyrosine decarboxylase in 0.075 *M* citrate–0.15 *M* phosphate buffer (pH 5.5). Inhibitor concentrations were 5 m*M* in citrate–phosphate buffer (pH 5.5). Reactions were incubated in a shaking water bath at 37° (30 min for 5 m*M* and 20 min for 10 m*M*), and the reactions were stopped by injection of 0.1 ml of 50% trichloroacetic acid. $^{14}CO_2$ was absorbed for 30 min, whereupon the plastic center wells were transferred to scintillation vials. The radioactivity was counted and compared with controls containing no inhibitor.

Assay of Tyrosine Decarboxylase. Tyrosine decarboxylase activity was determined[11] as described for phenylalanine decarboxylase using L-tyrosine-*1*-^{14}C as the substrate. The reaction mixtures (total volume, 0.5 ml) contained 0.5 m*M* pyridoxal 5-phosphate, 0.8 m*M* L-tyrosine-*1*-^{14}C (2.85×10^{-1} μCi/μmol), and 8×10^{-3} mg of tyrosine decarboxylase in 0.075 *M* citrate–0.15 *M* phosphate buffer (pH 5.5). Inhibitor concentrations were 5 m*M*. Incubation time (37°) was 20 min. $^{14}CO_2$ absorption time was 20 min, and the results were compared as previously described.

Assay of Histidine Decarboxylase. Histidine decarboxylase activity was determined[12] as previously described for phenylalanine decarboxylase by measuring the $^{14}CO_2$ produced from L-histidine-*1*-^{14}C. The standard reaction mixture (0.5 ml) contained 20 μg/ml of bovine serum albumin,[13] 10 m*M* L-histidine-*1*-^{14}C (2.5×10^{-1} μCi/μmol), and 0.1 mg of histidine decarboxylase in 0.2 *M* sodium acetate buffer (pH 4.8). Inhibitor concentrations were 5 m*M*. The incubation and absorption times were 20 min each, and the results were compared as described above.

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References and Notes

(1) E. E. Smissman and J. A. Weis, *J. Med. Chem.*, 14, 945 (1971).
(2) E. E. Smissman and V. D. Warner, *J. Med. Chem.*, 15, 681 (1972).
(3) A. C. Davis and A. L. Levy, *J. Chem. Soc.*, 3479 (1951).
(4) R. E. Steiger, *Org. Synth.*, 3, 84 (1955).
(5) F. J. Leinweber, *Mol. Pharmacol.*, 4, 337 (1968).
(6) R. J. Taylor, private communication.
(7) M. C. Gerald, O. P. Sethi, Z. Muhi-Eldeen, N. Mahishi, and D. T. Witiak, *Arch. Int. Pharmacodyn. Ther.*, 192, 78 (1971).

(8) R. A. Turner, Ed., "Screening Methods in Pharmacology", Academic Press, New York, N.Y., 1965, Chapter 4.
(9) M. C. Gerald and T. K. Gupta, private communication.
(10) "Worthington Enzyme Manual", Worthington Biochemical Corp., Freehold, N.J., 1972.
(11) E. Roberts and D. G. Simonsen, *Biochem. Pharmacol.* 12, 113 (1963).
(12) D. Russell and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **60**, 1420 (1968).
(13) G. W. Chang and E. E. Snell, *Biochemistry*, 7, 2005 (1968).

Synthesis and Evaluation of Brain Catecholamine Depletion by *N*-Alkyl Derivatives of 6-Aminodopamine†,[1]

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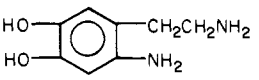
Three analogs of 6-aminodopamine were synthesized and tested for their ability to deplete the central nervous system of norepinephrine and dopamine. The compounds were analogs in which the aliphatic nitrogen of the ethyl side chain was substituted with dimethyl, isopropyl, and methyl groups. The first two compounds showed only very weak depletion of norepinephrine stores, while having no effect on dopamine levels. The third compound was not tested due to its instability.

The isostere of 6-hydroxydopamine, 6-aminodopamine (**1**), was reported by Stone and coworkers[2a] to be an effective noradrenaline depletor in the dog heart. Similar results have also been obtained in the mouse brain after intracerebral injection of 6-aminodopamine.[2b] Subsequent studies[3,4] have shown that 6-aminodopamine is a neurotoxic agent, capable of producing a degeneration of catecholamine neurons, similar to 6-hydroxydopamine.[5]

For 6-aminodopamine to exert its neurotoxic action a critical amount of 6-aminodopamine must be taken up by the neuron. Modification of the structure of 6-aminodopamine, so as to alter its uptake, could lead to a more selective neurotoxic agent for the noradrenergic or dopaminergic neurons of the central nervous system. This report is concerned with the modification of the nitrogen of the ethylamine side chain of **1**. In order to examine the effect of alkyl substituents on the ethylamine nitrogen compounds **2**–**4** were prepared and examined for their ability to act as catecholamine depletors in the central nervous system.



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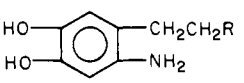
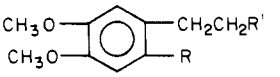


2, R = $N(CH_3)_2$
3, R = $NHCH(CH_3)_2$
4, R = $NHCH_3$

The synthesis of **2** was initiated by nitration of **5** to yield **6**, which was subsequently allowed to react under Eschweiler–Clarke conditions to yield the dimethyl derivative **7**. Reduction of the nitro group of **7** afforded the diamine **8** which was treated with 48% HBr to remove the methoxy-protecting groups.

Reductive alkylation of **5** with acetone afforded the *N*-isopropyl compound **9**. Nitration of **9** afforded **10**, which was reduced and the methoxy-protecting groups were removed as before to yield **3**.

Nitration of **11** with nitric acid afforded **12**. Catalytic reduction of **12** with platinum oxide yielded 1-(2-amino-4,5-dimethoxyphenyl)-2-(methylamino)ethane which was isolated as its diacetamide **13**. The acetate and methoxy-protecting groups were removed with 48% HBr to yield **4** as its dihydrobromide salt.



5, R = H; R' = NH_2
6, R = NO_2; R' = NH_2
7, R = NO_2; R' = $N(CH_3)_2$
8, R = NH_2; R' = $N(CH_3)_2$
9, R = H; R' = $NHCH(CH_3)_2$
10, R = NO_2; R' = $NHCH(CH_3)_2$
11, R = H; R' = $NHCH_3$
12, R = NO_2; R' = $NHCH_3$
13, R = $NHC(=O)CH_3$; R' = $NCH_3C(=O)CH_3$

Pharmacology and Discussion. Compounds **1**–**3** were evaluated for their ability to deplete the central nervous system of norepinephrine and dopamine. Compound **4** was so unstable that it could not be tested for biological activity. The compounds were dissolved in 0.9% saline solution containing 1 mg/ml of ascorbic acid. In a series of four experiments, white mice (15–30 g) were injected intracranially with 50 μg (calculated as free base) of test compound at intervals of 24 hr on three successive days. The animals were sacrificed 24 hr after the final injection and the brains removed. The catecholamine content of the brain was assayed according to the procedure of Shellenberger and Gordon.[6] The catecholamine content of the treated mouse brains is given in Table I and is expressed as a percentage of that found in control brains.

As can be seen from Table I, compounds **2** and **3** caused no reduction in the dopamine levels and only a very slight decrease in the levels of norepinephrine in the central nervous system. The inability of these compounds to cause a reduction in the catecholamine content of the central nervous system may be attributed to at least two reasons. It is possible these analogs were ineffective because of the inability of the uptake system to transport these compounds to the site of action within the neuron. It has been previously shown[7] that increasing bulk on the nitrogen of the ethyl side chain decreases a compound's ability to participate in the uptake process. In addition, the 6-aminodopamine analogs may be ineffective because of their ability to undergo facile cyclization and rearrangement to indoles.[8] In essence this reaction removes the ethylamine side chain from participating in the uptake process.

† Submitted in memory of Dr. Edward E. Smissman.

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