Analogues of Ornithine as Inhibitors of Ornithine Decarboxylase. New Deductions Concerning the Topography of the Enzyme's Active Site

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Fourteen structural analogues of ornithine were synthesized and evaluated as inhibitors of preparations of the enzyme L-ornithine carboxylyase (ODC) (E.C. 4.1.1.17) obtained from rat liver, rat hepatoma cells in culture, or bull prostate. The synthesis of these compounds was achieved either via a Bucherer type reaction or via alkylation of carbanions derived from ethyl acetamidocyanoacetate, methyl isocyanoacetate, benzyl α-isocyanopropionate, methylbenzaldimine alanate, and the azlactone derivative of ornithuric acid. (+)-α-Methylornithine, which was assigned the L configuration on the basis of rotational criteria, was found to be the most potent reversible inhibitor of ODC among the synthesized compounds. From the degree of inhibition of ODC activity in the presence of the various ornithine analogues, it has been possible to delineate some of the structural features of the substrate L-ornithine which are required for binding to the mammalian ODC active site.

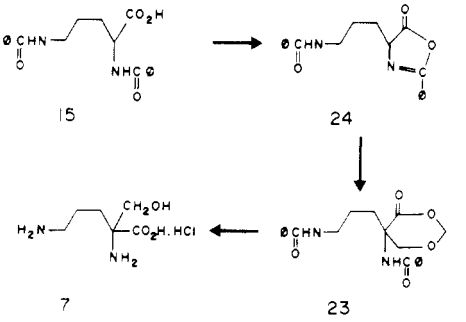
Numerous investigations clearly indicate that the polyamines, spermidine and spermine, and their diamine precursor, putrescine, have essential functions in cellular mechanisms, particularly those involved in cell division.[1] A possible way of elucidating the biological roles of these bioamines is to selectively impair their biosynthesis.[2] In eukaryotic organisms the conversion of ornithine to putrescine catalyzed by ornithine decarboxylase (ODC) (L-ornithine carboxylyase E.C. 4.1.1.17) is believed to be the obligatory and rate-limiting step for their biosynthesis,[3] a concept that has led to the search for specific inhibitors of this enzyme. Thus far, the substrate analogues 2-hydrazino-5-aminopentanoic acid,[4] DL-α-methylornithine,[5] DL-2-hydrazino-2-methyl-5-aminopentanoic acid,[6] *N*-(5′-phosphopyridoxyl)ornithine,[7] and DL-(*E*)-2,5-diamino-3-pentenoic acid[8] have been found to be potent reversible inhibitors of ODC.

We are engaged in a program for the design of specific inhibitors of ODC and report here on some steric, electronic, and conformational features of ornithine analogues essential for their function as inhibitors of ODC. Fourteen structural analogues of ornithine were synthesized and used to probe the specificity of L-ornithine binding sites on ODC. These compounds were selected on the basis of (a) conformational restrictions resulting from ring formation, (b) different substitution patterns on the terminal amine function and/or on the α- and β-carbon atom, (c) modifications of the carboxyl group, and (d) different configurations of the α-carbon atom. The effect of these molecular characteristics has been studied with the crude extract of ODC obtained from rat liver,[9] bull prostate, and rat hepatoma (HTC) cells in culture.[10]

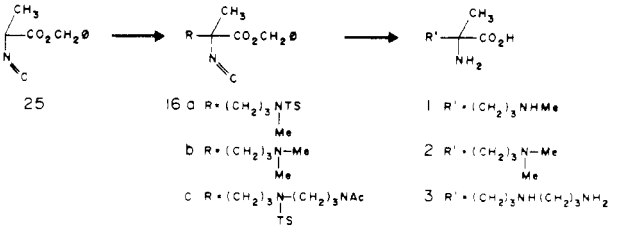
Results and Discussion

Chemistry. The acyclic analogues of ornithine synthesized for this study are listed in Table I. α-Hydroxymethylornithine **7** was prepared in good overall yield from ornithuric acid **15** by the general method described by Kaminski et al.[11] for the synthesis of α-hydroxymethyl-α-amino acids from the corresponding α-amino acids (Scheme I). β-Hydroxyornithine **9**[12] was synthesized by alkylation of the anion derived from ethyl isocyanoacetate[13] with β-phthalimidopropanal,[14] followed by hydrolytic removal of the protective groups, according to the procedure reported by Schöllkopf and Hoppe[15] for the preparation of β-hydroxy-α-amino acids. The synthesis of the α-methylornithine analogues **1–6**, **11**, and **12** relied on the utilization of either the α-isocyanopropionate synthon **25**[16] or the Schiff base methyl alanate synthon **29**.[17] Alkylation of the anion derived from benzyl α-isocyanopropionate (**25**) with (*N*-methyl-*N*-tosyl)-3-iodo-1-propanamine (**26**),[18] *N*,*N*-dimethyl-3-chloro-1-propanamine

Scheme I



Scheme II



(**27**),[19] and [*N*-tosyl-*N*-(3-acetamidopropyl)]-3-chloro-1-propanamine (**28**), in the presence of a catalytic amount of sodium iodide in the two latter cases, gave in good yield the corresponding C-alkylated products **16a–c** (Scheme II). Hydrolytic removal of the ester and amide protective groups from **16a–c**, followed by sodium ammonia reductive cleavage of the *N*-tosyl bond, afforded the corresponding α-methylornithine derivatives **1–3**. The halide **28** was prepared by reacting 3-(*N*-2′-cyanoethyl)amino-1-propanol[20] with 2 equiv of tosyl chloride in pyridine, followed by catalytic reduction of the nitrile in acetic anhydride. The Schiff base methyl alanate synthon **29**, in addition to being more conveniently available than the α-isocyanopropionate synthon **25**, allows a discrimination in the deprotection of the carboxyl and amine functions. This property has been used to advantage for the preparation of the key piperidone intermediate **18** (Scheme III) in the synthesis of DL-α-methylornithine **6** and its enantiomers **4** and **5**. Alkylation of the anion of the Schiff base methyl alanate with *N*-(3-iodopropyl)phthalimide proceeded smoothly to give an excellent yield of **17a**. Mild acidic hydrolysis of **17a** in 1 M HCl for 1 h removed the benzylidene group to give **19** which upon treatment with 1 equiv of hydrazine in the presence of 1 equiv of sodium methylate in methanol afforded quantitatively the piperidone **18** which has been easily resolved via its (+)- and (−)-binaphthylphosphoric salt.[21] Hydrolysis of the enantiomeric piperidone binaphthylphosphoric salt yielded

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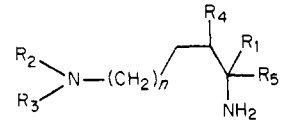
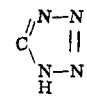
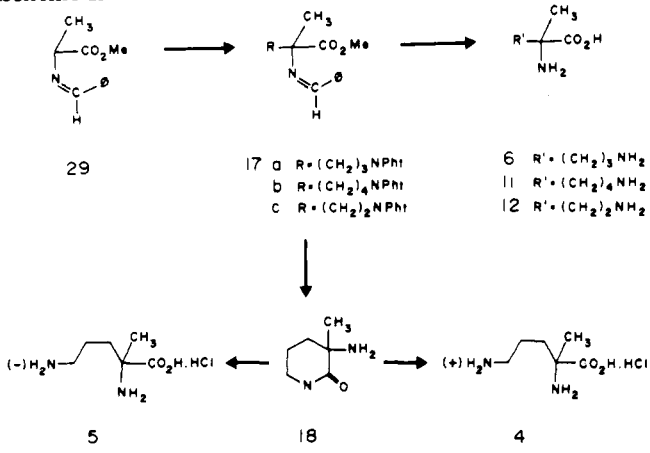
Table I. Analogues of Ornithine Synthesized as Inhibitors of ODC



Compd	R_1	R_2	R_3	R_4	R_5	n	Isomer
1	CH_3	H	CH_3	H	CO_2H	1	±
2	CH_3	CH_3	CH_3	H	CO_2H	1	±
3	CH_3	H	$(CH_2)_3NH_2$	H	CO_2H	1	±
4	CH_3	H	H	H	CO_2H	1	+
5	CH_3	H	H	H	CO_2H	1	−
6	CH_3	H	H	H	CO_2H	1	±
7	CH_2OH	H	H	H	CO_2H	1	±
8	H	H	$(CH_2)_3NH_2$	H	CO_2H	1	±
9	H	H	H	OH	CO_2H	1	±
10	H	H	H	H		1	±
11	CH_3	H	H	H	CO_2H	0	±
12	CH_3	H	H	H	CO_2H	2	±

Scheme III

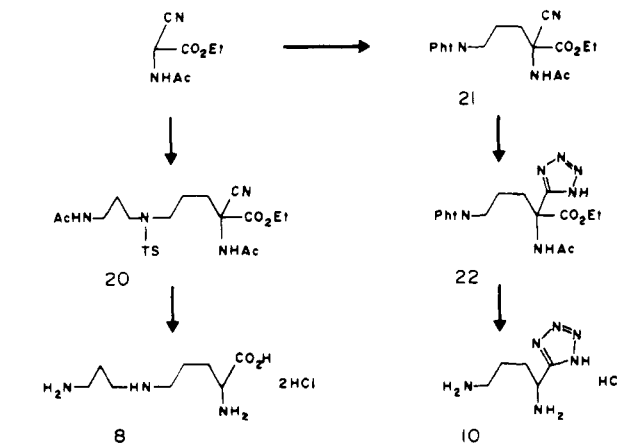


the corresponding α-methylornithine enantiomers. The L configuration was assigned to the (+) enantiomer **4** of α-methylornithine on the basis of the following rotational criteria. (a) The rotatory dispersion curve of **4** is similar to that of L-(+)-ornithine. The rotation of both amino acids becomes strongly positive in the 350-nm region (Table II). (b) The Lutz–Jirgensons rule[22] that L-α-amino acids show a positive shift in rotation upon increasing the acid concentration of the solution, which has been demonstrated by Achiwa et al.[23] to apply also to L-α-methyl-α-amino acids, is obeyed by **4**. The specific rotation difference [α] (6 M HCl) − [α] (0.1 M HCl) of **4** is positive at all wavelengths. The same specific rotation difference measured for L-(+)-ornithine follows a similar pattern (Table II). (c) L-α-Amino acids and L-α-methyl-α-amino acids give hydantoin derivatives with large negative rotation. According to Achiwa et al.,[23] this is the most reliable rule for assigning the absolute configuration of α-methyl-α-amino acids. The hydantoin derivative **30** of (+)-α-methylornithine **4** was synthesized and a negative rotation was measured ($[\alpha]^{20}_D$ −14.5°). The higher and the lower homologues of α-methylornithine, **11** and **12**, were prepared in a similar manner using the higher and the lower homologues of *N*-(3-iodopropyl)phthalimide as alkylating agents. The synthesis of the ornithine analogues **8** and **10** was approached through the alkylation of the ethyl acetamidocyanoacetate synthon[24] with respectively **28** and *N*-(3-iodopropyl)phthalimide. Hydrolytic removal of the protective groups and reductive cleavage of the

Table II. Influence of Wavelength on Optical Rotation and on Shift in Rotation Induced by Acid Concentration on D- and L-Ornithine and on (+)- and (−)-α-Methylornithine

	[α], deg (*c* 2, H_2O)			
λ, nm	**4**	L-Ornithine	**5**	D-Ornithine
589	+8.8	+11.5	−10.2	−11.7
578	+9.4	+12.1	−10.5	−12.4
546	+10.5	+13.9	−11.9	−14.1
436	+18.2	+24.7	−20.5	−25.2
365	+29.0	+42.1	−32.8	−42.8
	[α] (6 M HCl) − [α] (0.1 M HCl), deg (*c* 2)			
λ, nm	**4**	L-Ornithine	**5**	D-Ornithine
589	+2.0	+5.4	−2.0	−3.5
578	+1.8	+5.5	−1.9	−3.4
546	+1.6	+6.5	−2.3	−3.8
436	+3.8	+11.5	−3.6	−7
365	+6.5	+19.4	−5.8	−12.2

Scheme IV



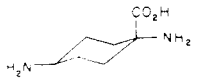
N-tosyl bond of the C-alkylated product **20** afforded the aminopropylornithine derivative **8**, whereas treatment of the C-alkylated product **21** with sodium azide in dimethylformamide gave the tetrazolyl derivative **22** which upon boiling in concentrated HCl and chromatography on ion-exchange resin yielded the tetrazolylornithine analogue **10** (Scheme IV). The cyclic analogues **13** and **14** were

Table III. Kinetic Constants K_I (mM) for ODC Inhibition by Ornithine Analogues

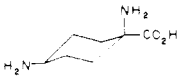
Compd	Rat liver	HTC cell	Bull prostate
L-Ornithine	0.087[a]	0.09[a]	0.072[a]
1	0.5[b]		0.74
2	No inhibn at 1 mM		
3		No inhibn	4.4
4	0.019	0.02	0.02
5	1.3	1.05	1.0
6	0.04	0.04	
7	No inhibn at 1 mM		
8			5.5
9		No inhibn at 1 mM	
10	0.12	0.14[b]	0.07
11	No inhibn at 4 mM		
12	No inhibn at 4 mM		
13	0.07	0.07	
14	No inhibn at 0.25 mM		

[a] Corresponds to the K_M. [b] These values were calculated from the ID_{50} measured at an ornithine concentration equal to the K_M. Under these conditions, for a competitive inhibitor $ID_{50} = 2 \times K_I$ (ref 36).

obtained via a route essentially identical with the one described by Christensen and Cullen[25] except that 4-acetamidocyclohexanone instead of 4-*p*-toluenesulfonamidocyclohexanone was used as starting material. The stereochemistry of the 4-amino group in compounds **13** and



13



14

14 was established by treating the isomers separately at 300 °C for 1 min in a sealed tube. The *trans*-1,4-diaminocyclohexane-1-carboxylic acid **13** gave the bicyclic lactam 4-amino-2-azabicyclo[2.2.2]octan-3-one, whereas the cis isomer **14** remained unchanged.

Enzyme Inhibitory Activity. The structural analogues of ornithine synthesized as part of this study were tested as competitive inhibitors of ODC. Rat liver, hepatoma cells in culture, and bull prostate were used as the source of enzyme. All three enzyme preparations had the same $K_M \simeq 0.08$ mM for L-ornithine. An apparent inhibition at high concentration of the substrate was observed with bull prostate ODC. Such an inhibition has already been reported for rat prostate ODC[4] and for mouse liver ODC.[26]

The degree of inhibition of ODC activity by the structural analogues of ornithine expressed by their K_I is shown in Table III. (+)-α-Methylornithine **4** was the most effective inhibitor with a $K_I = 0.02$ mM for the three different ODC preparations. Its inhibitory activity was twice that of the racemic mixture **6**. Its antipode **5** displayed a very low inhibitory activity ($^1/_{70}$th of that of **4**). This finding is an agreement with the results of Ono et al.[9] who showed that D-ornithine is a very weak inhibitor of rat prostate ODC (25% inhibition at a concentration of 10 mM) and of Inoue et al.[27] who demonstrated that rat prostate and liver ODC have much less affinity for D-2-hydrazino-5-aminopentanoic acid than for L-2-hydrazino-5-aminopentanoic acid and, therefore, confirms the preferred L configuration of the ligand for binding to the enzyme's active site.

A primary terminal amino group in the ligand appears to be important for enzyme inhibition, since the δ-*N*-monomethyl- and the δ-*N*,*N*-dimethyl-α-methylornithine analogues **1** and **2** showed little or no inhibitory activity on rat liver ODC. Furthermore, the δ-*N*-aminopropyl derivatives of ornithine and of α-methylornithine **8** and **3**, which incorporate in the same molecule the spermidine and the ornithine or α-methylornithine skeletons, proved also to have a very low affinity for bull prostate ODC (K_I = 5.5 and 4.4 mM), despite the fact that spermidine was shown to be a weak competitive inhibitor of bull prostate ODC with a $K_I = 3$ mM, comparable to the value (K_I = 2.7 mM) already reported for rat prostate ODC.[28] This result suggests that spermidine and ornithine compete for the same binding site of prostate ODC and also that the enzyme cannot accommodate ligands with bulky groups on the terminal amino group.

Of the two cyclic ornithine analogues, only the *trans*-1,4-diaminocyclohexane-1-carboxylic acid (**13**) is a potent inhibitor of ODC. Its $K_I = 0.07$ mM is 3.5 times higher than that of (+)-α-methylornithine **4**. The cis isomer **14** is devoid of activity. These findings which indicate that the distance between the two nitrogen atoms of the ornithine analogues is of major importance for inhibition are consistent with the fact that neither the lower and the higher homologues of ornithine[9] nor their corresponding α-methyl analogues **11** and **12** are substrates or inhibitors of ODC. This suggests that ornithine adopts a fully stretched conformation in the enzyme's active site with the two nitrogen atoms as far away as possible, which according to the Dreiding model corresponds to a distance of about 6 Å. Most probably hydrophobic interactions with residues of the active site favor this stretched conformation. This assumption is corroborated by the high affinity for ODC displayed by DL-(*E*)-2,5-diamino-3-pentenoic acid. The presence of the trans double bond results in a restricted number of possible conformers, most of them displaying the favorable distance of about 6 Å between the two nitrogen atoms, and also in an enhanced polarization when compared to the saturated analogue, thus leading to a higher interaction energy with the residues of the enzyme's active site via hydrophobic bonding. Relyea and Rando[8] reported for this inhibitor a K_I of 4.4 $\times 10^{-6}$ M for rat liver ODC, that is to say an affinity for the enzyme 30 times higher than that of DL-ornithine. Introduction of a hydrophilic substituent such as a hydroxyl function on the β-carbon atom, analogue **9**, or on the α-methyl group, analogue **7**, completely abolished the affinity for the enzyme's active site. The major contribution to this loss of affinity cannot be steric hindrance to binding, since the β-methyl-[29] and the α-ethylornithine[30] analogues have been reported to retain some inhibitory activity, but is rather due to a disruption by the hydrophilic function of the hydrophobic interactions between the residues of the enzyme's active site and the inhibitors.

Replacement of the carboxyl group of the substrate by a tetrazolyl group did not decrease the affinity for ODC, as shown by the $K_I = 0.07$ mM measured for the (±)-tetrazolyl analogue **10** vs. a $K_M = 0.07$ mM for L-ornithine for bull prostate ODC. If we assume that only one enantiomer of the tetrazolyl analogue **10** is accepted by the enzyme's active site, as suggested by the inhibitory activities of (+)- and (−)-α-methylornithine, the above-mentioned replacement results in fact in a slightly increased affinity for the binding sites. The pK_a values of the various functions of the tetrazolyl analogue **10** were

Table IV. Comparison of the Stability Constants K_d for the Formation of the Schiff Base with PLP and the K_I of Some ODC Inhibitors

	L-Ornithine	1	3	4	Spermidine	Putrescine
K_d, mM	1.25	5.36	0.9	2.35	0.7	0.8
K_I, mM	0.07	0.74	4.4	0.02	3	

found to be in the same range as those of the corresponding functions of ornithine (2.95, 7.80, and 10.25, respectively, for the tetrazolyl, the α-amino, and the terminal amino group of **10** vs. 2.8, 8.75, and 10.4 for the corresponding functions of L-ornithine). One interpretation of these data is that the carboxyl group is an attachment point via electrostatic binding for the ligand and that the area on the enzyme which accommodates the carboxyl group is not sensitive to changes in the steric bulk of the ligand. An alternative explanation suggested by the poor affinity of putrescine for the enzyme's active site is that the binding of the ligand is highly dependent on the basicity of the α-amino group and that the carboxyl or the tetrazolyl group would not participate directly in the binding but would act by decreasing the pK_a of the α-amino group.

It was of importance to ascertain that the affinity of the ligand for the enzyme was not dependent on its affinity for the cofactor. For this purpose, the dissociation or stability constants K_d for the formation of the Schiff base with pyridoxal phosphate (PLP) of some ornithine analogues and of spermidine and putrescine were determined and compared to the K_I of the same analogues for ODC. The results summarized in Table IV undoubtedly show that there is no correlation between the K_d and the K_I and therefore rule out any important role for the ligand affinity for the PLP in its binding to the enzyme.

Conclusion

The delineation of the structural features of L-ornithine which are required for binding to mammalian ODC was approached by measuring the degree of inhibition of ODC activity in the presence of various structural analogues of ornithine. The requirement for an L configuration of the ligand for binding has been confirmed. It has also been demonstrated that (1) a distance between the two nitrogen atoms of the ligand of about 6 Å is crucial for binding; (2) the area of the enzyme which accommodates the terminal amino group of the substrate is very sensitive to steric hindrance, whereas that which accommodates the carboxyl group is not; (3) hydrophobic interactions with the side chain of the ligand are an important feature for the binding, since hydrophilic functions attached on the α- and the β-carbon atoms abolish all affinity for the enzyme; and (4) the affinity of the ligand for the enzyme is not directly dependent on its affinity for the PLP cofactor.

Experimental Section

Enzyme Purification. Bull Prostate ODC. The enzyme was purified about fourfold according to the procedure of Jänne and Williams-Ashman[31] with some modifications. A fresh bull prostate was rapidly dissected free of surrounding connective tissue, weighed, minced, and homogenized in 2 vol of homogenization medium (Tris–HCl buffer, pH 7.2, 10 mM; sucrose, 25 mM; tetrasodium EDTA, 0.1 mM; dithiothreitol, 5 mM; PLP, 0.1 mM) with a Waring blendor and then with a glass homogenizer. All operations were carried out at 4 °C. The homogenate was centrifuged at 20 000g for 30 min at 2 °C. The resulting supernatant fraction was passed through a cheesecloth in order to remove floating lipids. This fraction was centrifuged at 105 000g for 1 h. The supernatant fluid was fractionated with solid $(NH_4)_2SO_4$. The fraction precipitating between 20 and 50% of saturation was dissolved in 6 mL of conservation medium (homogenization medium minus sucrose) and dialyzed against 100 vol of the same buffer for 16 h, the dialyzing buffer being changed after the first 6 h. The dialyzed $(NH_4)_2SO_4$ fraction was divided into small aliquots and stored at −20 °C.

Rat Liver ODC. This was prepared from the livers of rats which had been injected with thioacetamide 18 h before sacrifice and was purified about tenfold by acid treatment at pH 4.6 as described by Ono et al.[9]

HTC Cell ODC. The preparation was obtained as described by McCann et al.[10]

Assay of Ornithine Decarboxylase Activity. The reactions were followed by measurement of release of $^{14}CO_2$ from DL-[1-^{14}C]ornithine by the method of Jänne and Williams-Ashman.[31] The standard incubation mixture contained in a final volume of 0.95 mL: 30 μmol of Tris–HCl, pH 7.2 (bull prostate ODC), or sodium phosphate buffer, pH 7.1 (HTC and rat liver ODC), 0.1 μmol of pyridoxal 5-phosphate, 5 μmol of dithiothreitol, 0.043 μmol of DL-[1-^{14}C]ornithine (58 Ci/mol), and 0.8 μmol of L-ornithine. The reaction was initiated by addition of 50 μL of enzyme solution. Release of $^{14}CO_2$ was measured after 1 h of incubation at 37 °C. Under these conditions, the activity of ODC preparations from bull prostate, rat liver, and HTC cells was respectively 4.8, 7.2, and 7.5 mmol of CO_2/h per mg of protein.

Data Processing. Kinetic constants were calculated from Lineweaver–Burk plots (K_M)[32] or from Dixon plots (K_I).[33] Dissociation constants (K_d) of PLP adduct with ornithine analogues were determined at 25 °C in a phosphate buffer (30 mM, pH 7.1) by the method of Lucas et al.[34] All inhibition constants were obtained from a least-squares fit of the data points with a Hewlett-Packard 9820 A calculator (accuracy better than 20%).

Chemistry. Melting points were obtained on a Mettler FP5 or a Büchi SMP-20. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyzer. Unless otherwise stated, the IR, NMR, and UV data were consistent with the assigned structure. IR data were recorded on a Beckman IR-577 or Beckman IR 257 spectrophotometer, NMR data on a Varian Associates Model T-60 spectrophotometer (Me_4Si), and UV data on a Perkin-Elmer 402 spectrophotometer.

5-Benzoylamino-5-(3-benzoylaminopropyl)-1,3-dioxan-4-one (23). A mixture of 2-phenyl-4-(3-benzoylaminopropyl)-4,5-dihydro-1,3-oxazol-5-one (**24**) (25 g, 0.0775 mol) (prepared by treatment of ornithuric acid with acetic anhydride at 90 °C for 0.5 h), pyridine (17 mL), and formaldehyde (57 mL of a 37% aqueous solution) was stirred at ambient temperature for 20 h. The reaction mixture was then quenched with water (50 mL). The white solid which precipitated was filtered, washed with water and then with petroleum ether, and dried over P_2O_5 to give a white powder, 24.18 g (81%), mp 190–193 °C, which could be used without further purification. The analytical sample was recrystallized from acetone–petroleum ether, mp 196–197 °C. Anal. ($C_{21}H_{22}N_2O_5$) C, H, N.

α-Hydroxymethylornithine Monohydrochloride (7). To a suspension of **23** (24 g, 0.063 mol) in anhydrous MeOH (100 mL) was added a catalytic amount of NaOMe. After stirring for 0.25 h, the reaction mixture which had become homogeneous was neutralized with concentrated HCl. The solvent was evaporated under reduced pressure and the residue extracted with CH_2Cl_2. The organic phase was washed with brine and then concentrated in vacuo to leave an oily residue (24.12 g) which was treated in 6 M HCl (150 mL) at reflux for 48 h. The benzoic acid which precipitated on cooling was separated and the filtrate was extracted with ether (4 × 100 mL). The aqueous phase was concentrated under reduced pressure. The residue was dissolved in absolute ethanol (180 mL) and the pH of the solution was adjusted to 4 by adding a solution of triethylamine in ethanol (1 M) (56 mL). The solid which formed was collected by filtration, washed with ethanol, and recrystallized (H_2O–EtOH) to yield 10.4 g (87%) of **7**, mp 246 °C. Anal. ($C_6H_{15}N_2O_3Cl$) C, H, N.

Methyl 2-Methyl-2-benzylideneamino-5-phthalimidopentanoate (17a). To a solution of lithium diisopropylamine (0.01 mol) in anhydrous THF (15 mL) [prepared in situ from a solution of diisopropylamine (1 M) in THF (0.01 mol) and a solution of butyllithium (2 M) in hexane (0.01 mol)] cooled to

−78 °C was added slowly under nitrogen a solution of methylbenzaldimine alanate (**29**) (1.91 g, 0.01 mol) in anhydrous THF. The reaction mixture turned red immediately. The temperature was allowed to rise to −20 °C and then a solution of *N*-(3-iodopropyl)phthalimide (3.94 g, 0.0125 mol) in anhydrous THF (30 mL) was added. Stirring was continued for 4 h while the temperature was allowed to rise to 20 °C. The reaction mixture was quenched with water. The organic phase was separated and the aqueous phase was extracted with ether (2 × 100 mL). The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to yield an oily residue of crude **17a** (3.6 g).

Methyl 2-Methyl-2-benzylideneamino-6-phthalimidohexanoate (17b). Crude **17b** was obtained in a manner similar to that described for **17a**. *N*-(4-Iodobutyl)phthalimide was used in place of *N*-(3-iodopropyl)phthalimide.

Methyl 2-Methyl-2-benzylideneamino-4-phthalimidobutanoate (17c). Crude **17c** was obtained in a manner similar to that described for **17a** except that HMPA was added as a cosolvent. *N*-(2-Iodoethyl)phthalimide was used in place of *N*-(3-iodopropyl)phthalimide.

DL-α-**Methylornithine Hydrochloride Monohydrate (6).** Crude **17a** (3.6 g) was suspended in 6 M HCl (50 mL) and heated at reflux for 4 days. The phthalic acid which precipitated on cooling was filtered off and the filtrate was extracted with $CHCl_3$. The aqueous phase was concentrated under reduced pressure. The remaining phthalic acid which precipitated was eliminated by filtration. The residue was then dissolved in absolute ethanol and the pH of the solution adjusted to 4 with a solution of triethylamine in ethanol (1 M). The solid which formed was collected by filtration, washed with $CHCl_3$, and recrystallized (H_2O–EtOH) to yield 1.16 g of analytically pure α-methylornithine hydrochloride monohydrate, mp 255 °C. Anal. ($C_6H_{17}O_3N_2Cl$) C, H, N.

DL-α-**Methyllysine Hydrochloride Monohydrate (11).** Treatment of crude **17b** in a manner similar to that described for **17a** afforded **11**, mp 266 °C. Anal. ($C_7H_{19}N_2O_3Cl$) C, H, N.

DL-**2-Methyl-2,4-diaminobutyric Acid Hydrochloride (12).** Treatment of crude **17c** in a manner similar to that described for **17a** afforded **12**, mp 224 °C. Anal. ($C_5H_{13}N_2O_2Cl$) C, H, N.

δ-(***N*-Phthaloyl**)-α-**methylornithine Methyl Ester Hydrochloride (19).** **17a** (700 g) was stirred vigorously for 3 h in aqueous 1 M HCl (4 L). The oily phase which separated was eliminated by decantation and the aqueous phase was washed with petroleum ether (3 × 1.5 L) and then concentrated in vacuo. The solid which precipitated was collected by filtration, washed with petroleum ether and CCl_4, and then dried in vacuo to yield 479 g of **19**. The analytical sample was obtained by recrystallization from H_2O: mp 243 °C. Anal. ($C_{15}H_{19}N_2O_4Cl$) C, H, N.

3-Amino-3-methyl-2-piperidone (18). To a suspension of **19** (469 g, 1.44 mol) in anhydrous MeOH (1 L) was added under N_2 a solution of NaOMe (33 g of Na, 1.44 g-atoms) in MeOH (1 L), followed by a solution of hydrazine hydrate (72 g, 1.44 mol) in MeOH (0.5 L). The reaction mixture was heated at reflux for 7 h. The solid which formed was separated by filtration and the filtrate was concentrated in vacuo. The residue was treated with hot $CHCl_3$ and the insoluble part (22 g) was discarded. Evaporation of the solvent afforded 178 g of **18** (97.5%), mp 142 °C. Anal. ($C_6H_{12}N_2O$) C, H, N.

(+)-α-Methylornithine Hydrochloride (4). To a solution of **18** (3.07 g, 0.0236 mol) in ethanol (50 mL) maintained at 50 °C was added a boiling solution of (−)-binaphthylphosphoric acid (8.2 g, 0.0236 mol) in ethanol (250 mL). The reaction mixture was maintained at 60 °C until crystallization started. Thereafter, it was allowed to cool slowly to room temperature and then maintained at 4 °C for 1.5 h. The crystals were collected by filtration and washed with ethanol (50 mL) and ether (2 × 100 mL) to give 5.47 g of the (−)-binaphthylphosphoric salt (95%) [mp 336 °C dec; $[\alpha]^{20}_D$ −432° (*c* 0.9, MeOH). Anal. ($C_{26}H_{25}N_2O_5P$) C, H, N], which was then heated at reflux temperature in 6 M HCl (40 mL) for 2 h. The solid which separated was filtered and washed with water (3 × 10 mL), and the filtrate was concentrated in vacuo. The crystalline residue was dissolved in water (~10 mL) and the pH of the solution was adjusted to 3.5 by adding triethylamine. Concentration under reduced pressure yielded a solid which was treated with hot $CHCl_3$ (3 × 10 mL). The insoluble residue was then crystallized (H_2O–EtOH) to yield 1.7 g of **4** (80%): mp 218 °C; $[\alpha]^{20}_D$ +8.4° (*c* 0.5, H_2O). Anal. ($C_6H_{15}N_2O_2Cl$) C, H, N.

(−)-α-Methylornithine hydrochloride (5) was obtained in a manner similar to that described for **4**. (+)-Binaphthylphosphoric acid was used in the place of the (−) enantiomer: mp 214 °C; $[\alpha]^{20}_D$ −10° (*c* 1, H_2O). Anal. ($C_6H_{15}N_2O_2Cl$) C, H, N.

Ethyl 2-Cyano-2-acetamido-5-(*N*-tosyl-*N*-3-acetamidopropyl)aminopentanoate (20). To a suspension of sodium hydride (0.557 g of a 55% oil suspension, 1.16×10^{-2} mol) in anhydrous DMF (5 mL) cooled to 0 °C was added under N_2 a solution of ethyl acetamidocyanoacetate (1.972 g, 1.16×10^{-2} mol) in anhydrous DMF (15 mL). Stirring was continued at 0 °C for 1 h; then a solution of *N*-(3-chloropropyl)-*N*-tosyl-*N'*-acetyl-1,3-propanediamine (**28**) (4 g, 1.16×10^{-2} mol) in anhydrous DMF containing a catalytic amount of NaI was added over a period of 0.5 h. The reaction mixture was stirred overnight at room temperature and then heated at 100 °C for 1 h. The solvent was eliminated under reduced pressure and the residue dissolved in $CHCl_3$. The organic phase was washed with water and brine, dried over $MgSO_4$, and then concentrated under reduced pressure. Crystallization from AcOEt–EtOH yielded 2.4 g of **20** (45%): mp 94 °C. Anal. ($C_{22}H_{32}O_6N_4S$) C, H, N.

Ethyl 2-cyano-2-acetamido-5-phthalimidopentanoate (21) was obtained by the same procedure as above. *N*-(3-Iodopropyl)phthalimide was used in place of *N*-(3-chloropropyl)-*N*-tosyl-*N'*-acetyl-1,3-propanediamine: overall yield from ethyl acetamidocyanoacetate, 62%; mp 206 °C. Anal. ($C_{18}H_{19}O_5N_3$) C, H, N.

1-(5-Tetrazolyl)-1,4-butanediamine Hydrochloride (10). A suspension of **21** (3.99 g, 1.15×10^{-2} mol), sodium azide (0.81 g, 1.25×10^{-2} mol), and ammonium chloride (0.69 g, 1.3×10^{-2} mol) in DMF (11 mL) was heated at 90 °C for 20 h. The solvent was eliminated under reduced pressure and the residue taken up in aqueous 0.5 M HCl (30 mL). The white precipitate which formed was collected by filtration, washed with water, and dried under vacuo over P_2O_5 to give 4.58 g of the tetrazolyl derivative **22** as an amorphous powder which was treated in concentrated HCl (40 mL) at reflux temperature for 72 h. The phthalic acid which precipitated on cooling was filtered off and the filtrate was concentrated under reduced pressure. The residue was purified by ion-exchange chromatography (Amberlite IR 120 H^+ form, elution with 2 M NH_4OH). The fractions giving a positive test to ninhydrin were collected and concentrated to yield 1.17 g of the free base of **10**. The monohydrochloride was prepared by adjusting the pH of an aqueous solution of the free diamine to 3.3 with 0.1 M HCl. Concentration of this solution and recrystallization (H_2O–EtOH–AcOEt) yielded analytically pure **10**, mp 275 °C. Anal. ($C_5H_{13}N_6Cl$) C, H, N.

DL-δ-***N*-3-Aminopropylornithine Dihydrochloride (8).** A suspension of **20** (2 g, 0.42×10^{-2} mol) in concentrated HCl was heated at reflux temperature for 2 days. The residue obtained after evaporation of the solvent was passed through an Amberlite IR 120 H^+ form column. Elution with 2 M NH_4OH and concentration of the fraction giving a positive test to ninhydrin afforded 0.79 g of a mixture of δ-*N*-3-aminopropylornithine and of its δ-N-tosylated analogue. This mixture was dissolved in liquid ammonia (150 mL) and sodium was added until a blue color persisted for at least 2 min. Ammonium chloride was added to discharge the color and ammonia was stripped off under a stream of N_2. The residue was passed through an Amberlite IR 120 H^+ form resin column. The ninhydrin-positive fractions eluted with 2 M NH_4OH were collected and concentrated to give 0.47 g of crude δ-*N*-3-aminopropylornithine which was purified through recrystallization of its dipicrate salt. The dipicrate salt (1.19 g) was triturated with 1 M HCl (25 mL) for 0.5 h. The precipitate was collected by filtration and the filtrate was extracted with ether (6 × 20 mL) and then concentrated under reduced pressure. The residue was passed through an Amberlite IR 120 H^+ form resin column. Elution with 2 M NH_4OH yield 230 mg of δ-*N*-3-aminopropylornithine as an oil. The dihydrochloride was obtained by addition of HCl until a pH 4 was reached and was recrystallized from H_2O–EtOH (245 mg) (23%): mp 241 °C. Anal. ($C_8H_{21}N_3O_2Cl_2$) C, H, N.

DL-δ-*N*-3-Aminopropyl-α-methylornithine Dihydrochloride (3). Treatment of **16c** (1.55 g, 0.325×10^{-2} mol) in a manner similar to that described for **20** afforded **3** (390 mg, 45%), mp 245 °C. Anal. ($C_9H_{23}N_3O_2Cl_2$) C, H, N.

DL-δ-*N*-Methyl-α-methylornithine Hydrochloride Monohydrate (1). Treatment of **16a** in a manner similar to that described for **20** afforded **1** (45%), mp 239 °C. Anal. ($C_7H_{19}N_2O_3Cl$) C, H, N.

DL-α-Methyl-δ-*N*,*N*-dimethylornithine Dihydrochloride (2). A solution of **16b** (2.1 g) in 6 M HCl was treated at 90 °C for 15 h. The solvent was concentrated under reduced pressure and the residue crystallized from ethanol to give 1.6 g of **2** (90%), mp 246 °C. Anal. ($C_8H_{20}O_2N_2Cl_2$) C, H, N.

N-(3-Chloropropyl)-*N*-tosyl-*N'*-acetyl-1,3-propanediamine (28). A solution of *N*-(2-cyanoethyl)-*N*-tosyl-3-chloro-1-propanamine (3.87 g, 0.129×10^{-2} mol) in acetic anhydride (120 mL) was hydrogenated over Platin Adams (400 mg) at atmospheric pressure. After the theoretical quantity of H_2 has been absorbed, the catalyst was filtered off, and the filtrate was concentrated under reduced pressure. The residue was chromatographed on a silica gel column (200 g). Elution with AcOEt afforded 2.09 g of **28** (50%), oil. Anal. ($C_{15}H_{23}N_2O_3SCl$) C, H, N.

N-(2-Cyanoethyl)-*N*-tosyl-3-chloro-1-propanamine. To a solution of 3-(*N*-2'-cyanoethyl)amino-1-propanol[20] (6.83 g, 5.3×10^{-2} mol) in pyridine (10 mL) was added slowly 2 equiv of TsCl (22.4 g, 11.7×10^{-2} mol). The stirring was continued for 24 h at room temperature. Recrystallization (Et_2O–pentane) of the residue obtained after evaporation of the solvent gave 12.2 g of *N*-(2'-cyanoethyl)-*N*-tosyl-3-chloro-1-propanamine (74%), mp 37 °C. Anal. ($C_{14}H_{19}N_2SO_2Cl$) C, H, N.

Benzyl α-isocyanopropionate (25) was prepared from *N*-formylalanine benzyl ester according to the method described by Walborsky[35] (65%): bp 94–96 °C (0.1 mmHg). Anal. ($C_{11}H_{10}O_2N$) C, H, N.

Benzyl 2-Isocyano-2-methyl-5-(*N*,*N*-dimethylamino)-pentanoate (16b). A suspension of NaH (0.02 mol) in THF (7.5 mL) was added dropwise under N_2 to a well-stirred solution of **25** (3.74 g, 0.02 mol), *N*,*N*-dimethyl-3-chloro-1-propanamine (2.57 g, 0.02 mol), and NaI (catalytic amount) in a mixture of anhydrous Me_2SO (10 mL) and THF (10 mL). After the evolution of H_2 had stopped, the reaction mixture was treated at reflux temperature for 0.5 h, allowed to cool to room temperature, and then poured into ice water. Extraction with CH_2Cl_2 (4 × 60 mL) gave, after evaporation of the solvent, an oily residue (4.5 g) which was chromatographed on a silica gel column (250 g). Elution with AcOEt–EtOH (3:1) afforded 2.4 g of **16b** (44%): oil; IR (film) 2140 (N≡C), 1750 cm^{-1} (CO_2CH_2Ph); NMR ($CDCl_3$) 1.66 (3 H, s, CCH_3), 2.18 [6 H, s, $N(CH_3)_2$], 5.25 (2 H, s, $-OCH_2Ph$), 7.4 (5 H, s, H aromatic); no analysis.

Benzyl 2-isocyano-2-methyl-5-(*N*-methyl-*N*-tosyl)-aminopentanoate (16a) was obtained from *N*-methyl-*N*-tosyl-3-iodo-1-propanamine[18] in a manner similar to that described for **16b** (74%), an oil. Anal. ($C_{22}H_{26}N_2O_4S$) C, H, N.

Benzyl 2-isocyano-2-methyl-5-(*N*-tosyl-*N*-3-acetamidopropyl)aminopentanoate (16c) was obtained from **28** in a manner similar to that described for **16b** (30%): an oil; no analysis.

(–)-5-(3-Aminopropyl)-5-methyl-2,4-imidazolinedione (30). A suspension of (+)-α-*N*-carbamoylbenzyloxycarbonyl-α-methylornithine (150 mg) [$[\alpha]^{20}_D$ +3.8° (*c* 0.45, MeOH), prepared by treating (+)-δ-*N*-benzyloxycarbonyl-α-methylornithine with an excess of KNCO in 1 M HCl (5 mL)] was heated at 90 °C for 4 h. The homogeneous reaction mixture was then concentrated in vacuo. The amorphous residue was dissolved in EtOH (5 mL) and the solution was stirred under an H_2 atmosphere for 3 h in the presence of 5% Pd/C (25 mg). Elimination of the catalyst by filtration and recrystallization from absolute EtOH (three times) yielded 22 mg of **30**: mp 208–209 °C; $[\alpha]^{20}_D$ –14.5° (*c* 0.42, MeOH). Anal. ($C_7H_{13}N_3O_2$) C, H, N.

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References and Notes

(1) A. Raina and J. Jänne, *Med. Biol.*, **53**, 121 (1975).
(2) For a recent review, see H. G. Williams-Ashman, A. Corti, and B. Tadolini, *Ital. J. Biochem.*, **25**, 5 (1976), and references cited therein.
(3) (a) D. H. Russell and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **60**, 1420 (1968); (b) J. Jänne and A. Raina, *Acta Chem. Scand.*, **22**, 1349 (1968); (c) H. G. Williams-Ashman, A. E. Pegg, and D. H. Lockwood, *Adv. Enzyme Regul.*, **7**, 291 (1969).
(4) S. I. Harik and S. H. Snyder, *Biochem. Biophys. Acta*, **327**, 501 (1973).
(5) (a) D. H. Russell in "Polyamines in Normal and Neoplastic Growth", D. H. Russell, Ed., Raven Press, New York, N.Y., 1973 p 5; (b) M. M. Abdel-Monem, N. E. Newton, and C. E. Weeks, *J. Med. Chem.*, **17**, 447 (1974).
(6) M. M. Abdel-Monem, N. E. Newton, and C. E. Weeks, *J. Med. Chem.*, **18**, 945 (1975).
(7) J. S. Heller, E. S. Canellakis, D. L. Bussolotti, and J. K. Coward, *Biochem. Biophys. Acta*, **403**, 197 (1975).
(8) N. Relyea and R. Rando, *Biochem. Biophys. Res. Commun.*, **67**, 392 (1975).
(9) M. Ono, H. Inoue, F. Suzuki, and Y. Yakeda, *Biochem. Biophys. Acta*, **284**, 285 (1972).
(10) P. P. McCann, C. Tardif, P. S. Mamont, and F. Schuber, *Biochem. Biophys. Res. Commun.*, **64**, 336 (1975).
(11) Z. J. Kaminski, M. T. Leplawy, and J. Zabrocki, *Synthesis*, 792 (1973).
(12) H. Inoue, Y. Kato, M. Takigawa, K. Adachi, and Y. Takeda, *J. Biochem.*, **77**, 879 (1975), reported the testing of *threo*- and *erythro*-DL-β-hydroxyornithine as an inhibitor of ODC. The synthesis was not described.
(13) I. Ugi, *Agnew. Chem., Int. Ed. Engl.*, 472 (1965).
(14) K. Bott, *Chem. Ber.*, **106**, 2513 (1973).
(15) V. Schöllkopf and D. Hoppe, *Justus Liebigs Ann. Chem.*, **763**, 1 (1972).
(16) V. Schöllkopf, D. Hoppe, and R. Jentsch, *Angew. Chem., Int. Ed. Engl.*, **10**, 331 (1971).
(17) P. Bey and J. P. Vevert, *Tetrahedron Lett.*, 1455 (1977).
(18) U.S. Patent 3 549 703; *Chem. Abstr.*, **74**, 141404Z (1971).
(19) A. P. Gray, *J. Am. Chem. Soc.*, **77**, 3533 (1955).
(20) H. A. Bruson, *Org. React.*, **5**, 85 (1949).
(21) J. Jacques, C. Fouquet, and R. Viterbo, *Tetrahedron Lett.*, 4617 (1974).
(22) O. Lutz and B. Jirgensons, *Chem. Ber.*, **63**, 448 (1930); **64**, 1221 (1931).
(23) K. Achiwa, S. Terashima, H. Mizuno, N. Takamuro, T. Kitagawa, K. Ishikawa, and S. Yamada, *Chem. Pharm. Bull.*, **18**, 61 (1970).
(24) N. L. Albertson and B. F. Tullar, *J. Am. Chem. Soc.*, **67**, 502 (1945).
(25) H. N. Christensen and A. M. Cullen, *Biochem. Biophys. Acta*, **298**, 932 (1973).
(26) C. G. D. Morley and H. Ho, *Biochem. Biophys. Acta*, **438**, 551 (1976).
(27) H. Inoue, Y. Kato, M. Takigawa, K. Adachi, and Y. Takeda, *J. Biochem.*, **77**, 879 (1975).
(28) A. E. Pegg and H. G. Williams-Ashman, *Biochem. J.*, **108**, 533 (1968).
(29) W. A. Skinner and J. G. Johansson, *J. Med. Chem.*, **15**, 427 (1972).
(30) M. M. Abdel-Monem, N. E. Newton, B. C. Ho, and C. E. Weeks, *J. Med. Chem.*, **18**, 600 (1975).
(31) J. Jänne and H. G. Williams-Ashman, *J. Biol. Chem.*, **246**, 1725 (1971).
(32) H. Lineweaver and D. Burk, *J. Am. Chem. Soc.*, **56**, 658 (1934).
(33) M. Dixon and E. C. Webb, "Enzymes", 2nd ed, Longmans, Green and Co., London, 1964, p 329.
(34) N. Lucas, H. K. King, and S. J. Brown, *Biochem. J.*, **84**, 118 (1962).
(35) H. M. Walborsky and G. E. Niznik, *J. Org. Chem.*, **37**, 187 (1972).
(36) T. C. Chou, *Mol. Pharmacol.*, **10**, 235 (1974).