Synthesis of Isoornithines and Methylputrescines. An Evaluation of Their Inhibitory Effects on Ornithine Decarboxylase

Gerardo Aizencang,[†] Rosalía B. Frydman,[†] Sergio Giorgieri,[‡] Luis Sambrotta,[‡] Liliana Guerra,[§] and Benjamín Frydman*[,†,‡,∥]

Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina, Programa de Investigaciones Bioorgánicas (CONICET), Buenos Aires, Argentina, and Centro de Investigaciones Médicas Albert Einstein, Buenos Aires, Argentina

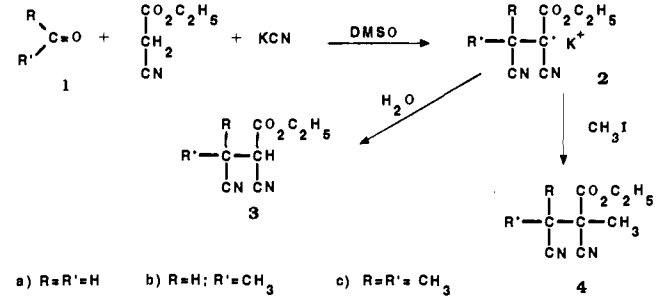
Received March 3, 1995[⊗]

2-(Aminomethyl)-4-aminobutyric acid (isoornithine), 3-methylisoornithine, and 2,3-dimethylisoornithine were not decarboxylated by liver ornithine decarboxylase (ODC, EC 4.1.1.17) of thioacetamide-treated rats but were good competitive inhibitors of the enzyme (K_i ranged from 0.72 to 1.79 mM). When assayed *in vivo* in the treated rats, the above mentioned isoornithines were also found to inhibit liver ODC when administered 1 h before sacrifice. When the methylputrescines formally derived from the decarboxylation of several isoornithines were assayed on rat liver ODC, it was found that only 2,3-dimethylputrescine decreased the enzymatic activity. When assayed *in vivo*, it was found to decrease ODC activity by 60%, when the latter was measured 1 h after administration. The effect was reverted 4 h after administration of the drug. Isoornithines were not taken up by H-35 hepatoma cells; hence they did not affect their ODC activity. 2,3-Dimethylputrescine however, was transported into the cells and significantly decreased its ODC activity.

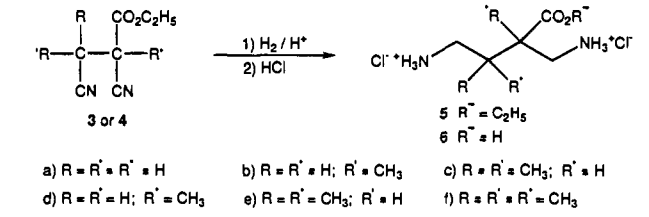
Introduction

Polyamines (putrescine, spermidine, and spermine) as well as their biosynthetic enzymes increase in proliferating tissues and are essential for all growth and cell division processes.[1,2] Ornithine decarboxylase (ODC)—the putrescine forming enzyme—is present in very small amounts in normal nonproliferating tissues and quiescent cells, but its activity is greatly increased at an early stage of carcinogenesis and elevated by a variety of tumor promoters.[3] ODC is a permanent target for studies on the inhibition of proliferative and neoplastic processes. Most successful among its inhibitors are the α-functionalized methylornithine substrate analogs.[4] Product analogs which are competitive inhibitors of ODC are the *N*-alkyl-, 1-alkyl-, and 2-alkylputrescines.[5,6] Among them, 1-methyl and 2-methylputrescines were found to be excellent *in vivo* inhibitors of liver ODC in rats treated with thioacetamide or dexamethasone. They also inhibit *de novo* ODC synthesis in H-35 hepatoma cells.[7] 1,4-Dimethylputrescine isomers (meso, +, and −) were strongly inhibitory of ODC when assayed *in vivo* in rats or in H-35 hepatoma cells[8] although none of the isomers inhibited ODC activity *in vitro*.[7,8] The three isomers were also found to decrease the putrescine pool in rat liver,[8] and they blocked putrescine uptake by certain protozoa.[9] It was therefore of interest to analyze the *in vivo* effects on ODC of ornithine isomers as well as of more heavily methylated putrescine analogs. In the first case a number of 2-(aminomethyl)-4-aminobutyrates isomeric with α-ornithine were prepared, for which we propose the name of isoornithines. Putrescine analogs, formally derived from the decarboxylation of the aforementioned isoornithines, were also obtained by synthesis, and their biological effects were evaluated in order to further define the structural requirements necessary for the inhibition of ODC activity.

Scheme 1



Scheme 2



Chemistry

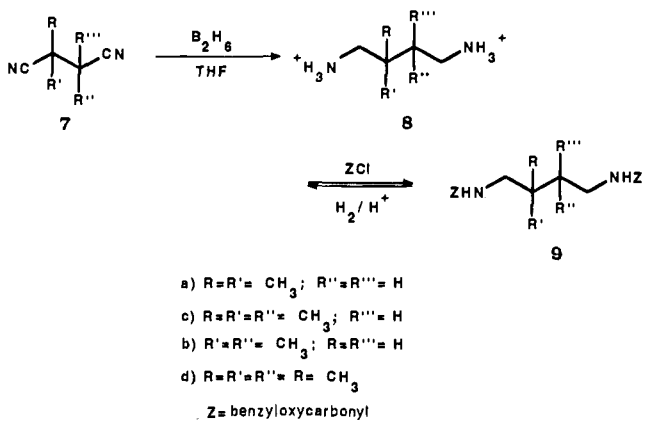
Isoornithines **6** were obtained following the outline of a one-pot synthesis of butanedinitriles.[10] By condensation of either formaldehyde, acetaldehyde, or acetone with ethyl cyanoacetate and KCN, the potassium salt **2** was obtained (Scheme 1). The latter was either protonated with water to **3** or alkylated with methyl iodide to give **4**. Either **3** or **4** were reduced with hydrogen over PtO_2 in acid medium to give the ethyl esters **5**, which were then hydrolyzed with 3 N HCl to afford the isoornithine dihydrochlorides **6** (Scheme 2).

Methylated putrescines **8** were obtained by diborane reduction of the corresponding butanedinitriles **7** (Scheme

† Facultad de Farmacia y Bioquímica.
‡ Programa de Investigaciones Bioorgánicas.
§ Centro de Investigaciones Médicas Albert Einstein.
∥ Current address: School of Pharmacy, University of Wisconsin, 425 N Charter St., Madison, WI 53706.
⊗ Abstract published in *Advance ACS Abstracts*, September 15, 1995.

0022-2623/95/1838-4337$09.00/0

Scheme 3



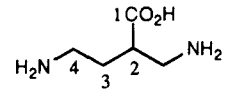
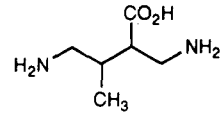
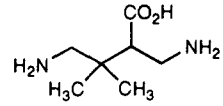
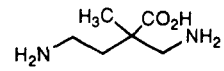
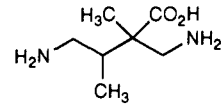
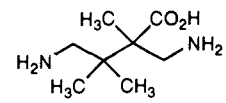
3). Since the hydrochlorides of **8** were very hygroscopic, their more stable bis(benzyloxycarbonyl) derivatives **9** were prepared to use as stock derivatives. The dihydrochlorides **8** were recovered from **9** by hydrogenolysis in acid medium. This procedure had been successfully applied for the synthesis of analogous alkylputrescines.[11,12] The chiral mixtures of isomers were not resolved.

Biological Results and Discussion

Effect of Isoornithines on the *in Vitro* and *in Vivo* Activity of ODC. L-α-ornithine has a K_m = 0.6 mM for liver ODC in thioacetamide-treated rats. Isoornithine **6a** is not a substrate of the enzyme, but is a competitive inhibitor with a K_i = 1.79 mM. A methyl residue at C-3 (as in **6b**) increases the inhibitory effect, while two methyl residues at C-2 and C-3 (as in **6e**) also afford a good competitive inhibitor of ODC (Table 1). 2-(Aminomethyl)-2-methyl-4-aminobutyrate **6d**, **6c**, and **6f**, were very weak inhibitors of ODC. The isoornithines were not decarboxylated by the enzyme. When isoornithines **6a–f** were examined as a function of concentration, the 3-methyl derivative **6b** was again found to be the best competitive inhibitor of ODC. When assayed *in vivo* in thioacetamide-treated rats, the isoornithines (50 μmol/100 g of body weight) were administered 1, 2, and 4 h before sacrifice. Only isoornithine **6a** and its 3-methyl analog **6b** showed an inhibitory effect on ODC. The latter inhibited 40% of the enzymatic activity 1 h after administration, an effect which was reverted after 2 h. Isoornithines **6a**, **6b**, and **6e** decreased by ca. 20% the endogenous levels of putrescine in the liver, but did not affect the endogenous levels of spermidine and spermine.

None of the isoornithines (1 mM of each was added to the media 1 h before harvest) affected ODC activity when assayed in H-35 hepatoma cells. Since poor cell-uptake could be the reason for these negative results, the uptake of [1-^{14}C]-L-ornithine by H-35 cells was measured. Uptake was found to level-off after 20 min when it amounted to ca. 0.15% of the label present in the incubation medium. Neither **6a** nor **6b** affected the uptake of ornithine. They were not detected in the intracellular medium when a TLC analysis of the dansylated amino acids was carried out. It was evident that the synthetic isoornithines were not transported into the cells, at variance with the highly enhanced uptake of the α-alkyl ornithine derivatives.[13]

Table 1. Inhibition Constants of Isoornithines for ODC from Rat Liver

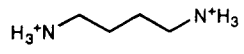
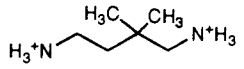
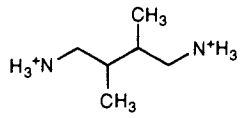
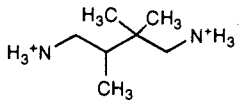
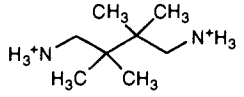
Inhibitor		Ki (mM)[a]
	**6a**	1.79
	**6b**	0.72
	**6c**	> 11.0
	**6d**	> 11.0
	**6e**	1.65
	**6f**	> 11.0

[a] The rat liver enzyme had a specific activity of 4.7 ± 0.3 units/mg of protein. The K_i constants were calculated from the data obtained by replotting the K_m^{app} values vs concentrations of inhibitors and also by utilizing a nonlinear regression program (Enzfitter 1.05). Both sets of data were coincident. K_m^{app} values were determined by measuring enzymatic rates vs ornithine concentrations (0.2–5 mM) at different concentrations of the inhibitor **6** (0–15 mM). Due to solubility problems, concentrations above 15 mM could not be used; therefore the K_i's above 10 mM are approximate.

Effect of Methylputrescines on the *in Vitro* and *in Vivo* Activity of ODC. Since isoornithines **6** are not decarboxylated by ODC to their putrescine derivatives, several of the latter were prepared by synthesis and assayed on ODC activity. Putrescine is a weak inhibitor of ODC (K_i = 3.5 mM for the liver enzyme; K_i = 2.4 mM for the H-35 enzyme). While monomethylation at the butane backbone enhanced the inhibitory effect (see Introduction), more extensive methylation resulted in weaker inhibitors (Table 2). The most heavily methylated putrescine, **8d**, was the weakest inhibitor of the rat liver enzyme. When assayed *in vivo*, 2,3-dimethylputrescine **8b** (50 μmol/100 g of body weight) inhibited ODC activity by 60% 1 h after administration; the effect was reverted after 4 h (Figure 1). It also decreased the endogenous levels of putrescine, a decrease which paralleled the rate of ODC inhibition. Both effects might reflect the metabolic fate of 2,3-dimethylputrescine **8b**; 1 h after administration its hepatic levels indicated an uptake of 12% of the injected total; 4 h later only 0.5% remained in the liver. It did not decrease the liver pools of spermidine or spermine.

When the methylputrescines **8** (1 mM was added to the culture media 1 h before harvest) were assayed with

Table 2. Inhibition Constants of Methylputrescines for ODC from Rat Liver

Inhibitor		Ki (mM)[a]
	putrescine	3.5 ± 0.50[b]
	8a	> 10.0
	8b	6.1 ± 0.80[b]
	8c	>10.5
	8d	> 11.0

[a] The experimental conditions were those given in Table 1. Putrescine and its analogs were used in the 0–15 mM concentration range. Due to solubility problems, concentrations above 15 mM could not be used; therefore the K_i's above 10 mM are approximate. [b] These values correct the former ones given in ref 8.

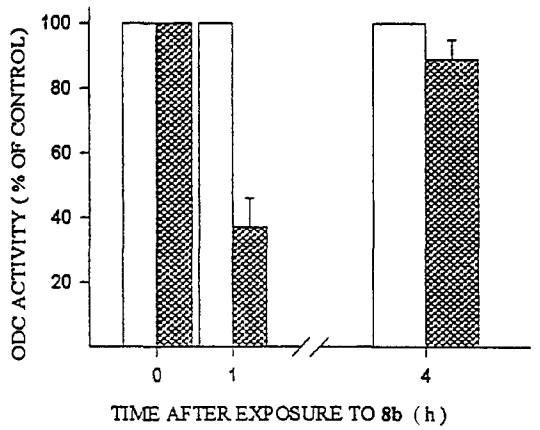


Figure 1. Time response of rat liver ODC activity in thioacetamide-treated rats injected with 2,3-dimethylputrescine. ODC activity is given as percent of the ODC liver activity in thioacetamide-treated rats which is taken as 100%. 2,3-Dimethylputrescine **8b** (50mmol/100 g rat weight) was injected at the times shown before sacrifice: (unfilled) control, (hatched) **8b**. The results are the mean ±SD of three experiments with three rats each.

H-35 hepatoma cells, is was found that only **8b** decreased significantly the specific activity of ODC: from 13.7 nmol CO_2/mg of protein/h in the controls to 9.8 nmol CO_2/mg of protein/h in the **8b**-treated cells. It also decreased the cell levels of putrescine. To establish if this decrease was related only to the inhibition of ODC or to a simultaneous inhibition of putrescine uptake, [1,4-^{14}C]putrescine uptake by H-35 cells was measured. It was found to reach a maximum after a 20 min incubation period. The cytoplasmic label was then measured when either unlabeled putrescine or **8b** was added to the medium. Putrescine itself was found to decrease endogenous label by 95% and **8b** by 54%. The latter was taken up by the cells; the uptake amounted to ca. 2% of the total added to the incubation medium.

It could be concluded that, on enhancing the hydrophobic character of the putrescine analogs, they become less inhibitory of ODC activity. Also, by displacing the methyl residues from C_1 and C_4 (as in 1,4-dimethylputrescine) toward the center of the butane backbone (as in **8**) the inhibitory activity toward ODC is lost. This loss could be attributed to the oxidation of **8** by the cell diamine oxidases. We had shown[15] that the latter oxidize β-alkylputrescines more efficiently than α-alkylputrescines. Also, 1,4-dimethylputrescine is a strong inhibitor of ODC[7,8,14] and is not a substrate of diamino oxidases. The search for substituted putrescines with inhibitory effects on ODC should probably take into account their interaction with the cell oxidase systems.

Experimental Section

Melting points were determined on a Kofler melting point apparatus and are uncorrected. ^{13}C NMR spectra were recorded on a FT-80A spectrometer. Mass spectra were obtained with a Varian CH-7 spectrometer. Microanalyses were performed by UMYMFOR (Buenos Aires). Analytical results were within ±0.4% of the theoretical value. TLC was performed either on silica gel F-254 plates (Merck, 0.25 mm layer thickness) or on precoated cellulose plates (Merck, 0.1 mm layer thickness). Diamines and amino acids were spotted by spraying with a ninhydrin solution (0.5% ninhydrin, 0.4% acetic acid, 5% 2,6-lutidine in acetone) followed by heat (100 °C).

Ethyl 2,3-Dicyanopropionates (3) and Ethyl 2,3-Dicyanobutyrates (4). General Procedure. The aldehyde or ketone **1** (0.15 mol) was slowly added to a stirred solution of KCN (0.16 mol, 10.3 g) and ethyl cyanoacetate (0.15 mol, 16 mL) in 150 mL of dimethyl sulfoxide while the reaction mixture was kept at 5 °C. The solution was then kept with constant stirring at 20 °C during 15 h, after which it was cooled again to 5 °C and 0.16 mol of methyl iodide was slowly added. This last step was omitted when **3** was prepared. The resulting solution was then poured over 600 mL of ice–water, and the aqueous mixture was extracted with ethyl ether (3 × 200 mL). The pooled extracts were washed with water (2 × 50 mL), dried (Na_2SO_4), and evaporated to dryness. The oily residue was either distilled (100 °C/0.050 Torr) or purified by column chromatography using silica gel 60 (Merck).

Ethyl 2,3-dicyanopropionate (3a): yield 80% after purification by column chromatography using 4% methanol in methylene chloride as eluant; ^{13}C NMR ($CDCl_3$) δ 162.7 (CO), 115.3 (2-CN), 114.0 (3-CN), 63.2 (CH_2O), 33.0 (C_2), 17.3 (C_3), 12.8 (CH_3). Anal. ($C_7H_8N_2O_2$) C, H, N.

Ethyl 2,3-dicyanobutyrate (3b) was obtained as the diastereoisomeric mixture: yield 72%; bp 105 °C/0.050 Torr; ^{13}C NMR ($CDCl_3$) δ 162.3 (CO); 118.3, 118.1 (2-CN); 113.1 (3-CN); 63.0 (CH_2O); 40.3, 39.6 (C_2); 25.7, 25.1 (C_3); 15.0, 14.1 (C_4); 12.8 (CH_3CH_2). Anal. ($C_8H_{10}N_2O_2$) C, H, N.

Ethyl 2-methyl-2,3-dicyanopropionate (4a): yield 72% after purification by column chromatography using methylene chloride as eluant; ^{13}C NMR ($CDCl_3$) δ 166.3 (CO), 117.5 (2-CN), 115.0 (3-CN), 63.7 (CH_2O), 41.0 (C_2), 25.7 (C_3), 22.5 (CH_3), 13.4 (CH_3CH_2). Anal. ($C_8H_{10}N_2O_2$) C, H, N.

Ethyl 2-methyl-2,3-dicyanobutyrate (4b) was obtained as a diastereoisomeric mixture: yield 76% after purification by column chromatography using benzene as eluant; ^{13}C NMR ($CDCl_3$) δ 166.2, 166.0 (CO); 118.3, 118.0 (2-CN); 116.9, 116.6 (3-CN); 63.5, 63.34 (CH_2O); 46.7, 46.47 (C_2); 29.3, 28.7 (C_3); 32.7, 32.2 (2-CH_3); 21.8, 20.4 (C_4); 14.4, 13.3 (CH_3CH_2). Anal. ($C_9H_{12}N_2O_2$) C, H, N.

Ethyl 3-methyl-2,3-dicyanobutyrate (3c): yield 67% after a purification step as described for **4b**; ^{13}C NMR ($CDCl_3$) δ 162.3 (CO); 120.4 (2-CN); 112.9 (3-CN); 63.0 (CH_2O); 45.6 (C_2); 33.5 (C_3); 24.8, 24.0 (CH_3); 13.3 (CH_3CH_2). Anal. ($C_9H_{12}N_2O_2$) C, H, N.

Ethyl 2,3-dimethyl-2,3-dicyanobutyrate (4c): yield 45% after purification as described for **4b**; ^{13}C NMR ($CDCl_3$) δ 162.1 (CO); 120.3 (2-CN); 112.9 (3-CN); 62.7 (CH_2O); 45.3 (C_2); 33.3 (C_3); 24.3, 23.7 (CH_3); 13.0 (CH_3CH_2). Anal. ($C_{10}H_{14}N_2O_2$) C, H, N.

Ethyl 2-(Aminomethyl)-4-aminobutyrate Dihydrochlorides. General Procedure. 2,3-Dicyano esters **3** or **4** (3 mmol) dissolved in 30 mL of methanol containing 1.5 mL of concentrated HCl were reduced with hydrogen over 0.1 g of PtO_2 at 50 psi during 2 h. The catalyst was then filtered, the solvent evaporated to dryness in vacuum, and the resulting dihydrochloride was crystallized from methanol–ethyl ether.

Ethyl 2-(aminomethyl)-4-aminobutyrate dihydrochloride (5a): yield 77%; mp 100–102 °C (hygroscopic); ^{13}C NMR (D_2O) δ 174.2 (CO), 64.1 (CH_2O), 41.3 (CH_2NH_2), 38.7 (C_2), 28.0 (C_3), 15.1 (CH_3). Anal. ($C_7H_{18}N_2O_2Cl_2$) C, H, N.

Ethyl 2-(aminomethyl)-3-methyl-4-aminobutyrate dihydrochloride (5b) was obtained as a mixture of diastereoisomers: yield 75%; mp 98–100 °C; ^{13}C NMR (D_2O) δ 173.8, 173.0 (CO); 64.0, 63.9 (CH_2O); 46.5, 46.0 (CH_2NH_2); 44.6, 43.6 (C_4); 40.0, 38.5 (C_2); 33.3, 33.2 (C_3); 15.0 (CH_3CH_2); 14.3, 14.1 (CH_3). Anal. ($C_8H_{20}N_2O_2Cl_2$) C, H, N.

Ethyl 2-(aminomethyl)-2-methyl-4-aminobutyrate dihydrochloride (5d): yield 80%; mp 109–111 °C; ^{13}C NMR (D_2O) δ 176.0 (CO), 64.3 (CH_2), 46.3 (CH_2NH_2), 44.3 (C_4), 36.7 (C_2), 34.5 (C_3), 20.7 (CH_3), 14.8 (CH_3CH_2). Anal. ($C_8H_{20}N_2O_2Cl_2$) C, H, N.

Ethyl 2-aminomethyl-2,3-dimethyl-4-aminobutyrate dihydrochloride (5e) was obtained as a mixture of diastereoisomers: yield 78%; mp 90–93 °C; ^{13}C NMR (D_2O) δ 175.9, 175.5 (CO); 64.1 (CH_2O); 48.0 (CH_2NH_2); 44.1, 43.9 (C_4); 42.3, 42.1 (C_2); 38.0, 37.6 (C_3); 18.1, 17.4 (CH_3(2)); 14.3 (CH_3CH_2); 12.8, 12.8 (CH_3(3)). Anal. ($C_9H_{22}N_2O_2Cl_2$) C, H, N.

Ethyl 2-(aminomethyl)-3,3-dimethyl-4-aminobutyrate dihydrochloride (5c): yield 76%; mp 110 °C; ^{13}C NMR (D_2O) δ 73.1 (CO); 64.1 (CH_2O); 51.0 (CH_2NH_2); 48.8 (C_4); 38.5 (C_2); 36.1 (C_3); 23.5, 22.8 (CH_3); 14.6 (CH_3CH_2). Anal. ($C_9H_{22}N_2O_2Cl_2$) C, H, N.

Ethyl 2-(aminomethyl)-2,3,3-trimethyl-4-aminobutyrate dihydrochloride (5f): yield 71%; mp 105–107 °C; ^{13}C NMR (D_2O) δ 73.2 (CO); 64.1 (CH_2O); 51.1 (C_1); 49.0 (C_4); 38.7 (C_2); 36.2 (C_3); 23.6, 23.0 (CH_3); 14.7 (CH_3CH_2). Anal. ($C_{10}H_{24}N_2O_2Cl_2$) C, H, N.

2-(Aminomethyl)-4-aminobutyric Acid Dihydrochlorides (6). General Procedure. Ethyl diaminobutyrate dihydrochlorides **5** (0.5 g) were dissolved in 15 mL of 3 N HCl, and the solution was heated under reflux during 3 h. It was then evaporated to dryness, and the residue was recrystallized from methanol–dry ethyl ether. The resulting dihydrochlorides were white hygroscopic solids.

2-(Aminomethyl)-4-aminobutyric acid (isoornithine) dihydrochloride (6a): yield 88%; mp 207–210 °C; ^{13}C NMR (D_2O) δ 175.4 (CO), 40.8 ($CH_2NH_3^+$), 40.5 (C_4), 38.2 (C_2), 27.4 (C_3). Anal. ($C_5H_{14}N_2O_2Cl_2$) C, H, N.

2-(Aminomethyl)-3-methyl-4-aminobutyric acid dihydrochloride (6b) was obtained as a mixture of diastereoisomers: yield 89%; mp 200–203 °C; ^{13}C NMR (D_2O) δ 175.5, 174.8 (CO); 46.1, 45.8 ($CH_2NH_3^+$); 44.3, 43.6 (C_4); 39.8, 38.3 (C_2); 32.9 (C_3); 14.5, 13.8 (CH_3). Anal. ($C_6H_{16}Cl_2N_2O_2$) C, H, N.

2-(Aminomethyl)-2-methyl-4-aminobutyric acid dihydrochloride (6d): yield 83%; mp 220–221 °C; ^{13}C NMR (D_2O) δ 177.8 (CO), 46.1 ($CH_2NH_3^+$), 44.1 (C_4), 36.4 (C_2), 34.3 (C_3), 20.4 (CH_3). Anal. ($C_6H_{16}N_2O_2Cl_2$) C, H, N.

2-(Aminomethyl)-2,3-dimethyl-4-aminobutyric acid dihydrochloride (6e) was obtained as a mixture of diastereoisomers: yield 84%; mp 200–206 °C; ^{13}C NMR (D_2O) δ 177.8, 177.6 (CO); 47.8 ($CH_2NH_3^+$); 44.0, 42.4, 42.2 (C_4); 37.9, 37.4 (C_3); 18.4, 17.3 (CH_3 (2)); 12.9, 12.6 (CH_3(3)). Anal. ($C_7H_{18}N_2O_2Cl_2$) C, H, N.

2-(Aminomethyl)-3,3-dimethyl-4-aminobutyric acid dihydrochloride (6c): yield 87%; mp 220–222 °C; ^{13}C NMR (D_2O) δ 174.5 (CO); 50.6 ($CH_2NH_3^+$); 48.6 (C_4); 38.4 (C_2); 35.3 (C_3); 23.3, 22.4 (CH_3). Anal. ($C_7H_{18}N_2O_2Cl_2$) C, H, N.

2-(Aminomethyl)-2,3,3-trimethyl-4-aminobutyric acid dihydrochloride (6f): yield 82%; mp 215–217 °C; ^{13}C NMR (D_2O) δ 177.7 (CO), 51.3 ($CH_2NH_3^+$), 49.0 (C_4), 38.8 (C_2), 35.7 (C_3), 23.8, 22.9 (CH_3). Anal. ($C_8H_{20}N_2O_2Cl_2$) C, H, N.

2,2-Dimethylbutanedinitrile (7a). **7a** was prepared following the described procedure[10] from 8.7 g of acetone, 16 mL of ethyl cyanoacetate, and 10 g of potassium cyanide. The reaction product was purified by distillation at 60 °C/0.1 Torr; 7.2 g (63% yield); mp 59–60 °C; ^{13}C NMR ($CDCl_3$) δ 122.5, 116.1 (CN); 30.8 (C_2); 29.0 (C_3); 25.6 (CH_3). Anal. ($C_6H_8N_2$) C, H, N.

2,2,3-Trimethylbutanedinitrile (7c). **7c** was prepared following the described procedure[10] from 8.7 g of acetone, 16 mL of ethyl cyanoacetate, 10 g of KCN and 23 g of methyl iodide. The dinitrile was purified by distillation at 40 °C/0.1 Torr; 8.4 g (46% yield); mp 38–39 °C; ^{13}C NMR ($CDCl_3$) δ 122.4, 116.0 (CN); 34.9 (C_2); 29.0 (C_3); 25.7 (CH_3 (2)); 4.0 (CH_3 (3)). Anal. ($C_7H_{10}N_2$) C, H, N.

Preparation of Methylputrescines (8). General Procedure. To a solution of 0.1 mol of butanedinitrile **7** in dry tetrahydrofuran (10 mL) was added 10 mL of dry tetrahydrofuran saturated with diborane. The solution was stirred during 18 h at 20 °C, the excess diborane was then destroyed by addition of dry methanol, and the mixture was adjusted to pH 2 with hydrogen chloride. It was then heated under reflux for 3 h, the solvent was evaporated in a vacuum, the residue was taken up in 20 mL of water, the solution was adjusted to pH 10 with a concentrated NaOH solution, and the diamine **8** was extracted with CH_2Cl_2 (3 × 10 mL). The pooled extracts were dried (Na_2SO_4) and evaporated to dryness in a vacuum, and the crude **8** was dissolved in a mixture of 50 mL of 10% NaOH and 10 mL of $CHCl_3$. Benzyl chloroformate (15 mL) was added to the stirred mixture over 30 min, the mixture was further stirred for 1 h, the $CHCl_3$ layer was then separated, and the aqueous layer was extracted with $CHCl_3$ (4 × 10 mL). The extracts were pooled, dried (Na_2SO_4), evaporated to dryness, and the bis-benzyloxycarbonyl derivatives **9** were purified by flash chromatography on silica gel using $CHCl_3$ as an eluant. They were crystallized from benzene–cyclohexane. The crystalline carbamates were then dissolved in 100 mL of ethanol containing 1 mL of concentrated HCl and were reduced with H_2 at 50 psi during 8 h over 200 mg of PtO_2. The catalyst was filtered, and on evaporation of the solution to dryness it left behind the dihydrochlorides **8** which were crystallized from methanol–ethyl ether as white hygroscopic solids.

2,3-Dimethyl-1,4-bis[(benzyloxycarbonyl)amino]butane (9b) was obtained from 2,3-dimethylbutanedinitrile **7b**:[10] yield 73%; mp 93–96 °C. Anal. ($C_{22}H_{28}N_2O_4$) C, H, N. Hydrogenolysis afforded the dihydrochloride of **8b**: yield 85%; MS (*m/z*) 116 (M^+ · 14); ^{13}C NMR (D_2O) δ 43.8, 42.8 (C_1); 34.3, 33.0 (C_2); 14.4, 11.9 (CH_3).

2,2-Dimethyl-1,4-bis[(benzyloxycarbonyl)amino]butane (9a) was obtained from 2,2-dimethylbutanedinitrile **7a**: yield 81%; mp 130–132 °C. Anal. ($C_{22}H_{28}N_2O_4$) C, H, N. Hydrogenolysis afforded the dihydrochloride of **8a**; yield 90%; MS (*m/z*) 116 (M^+, 10); ^{13}C NMR (D_2O) δ 45.9 (C_1); 32.9 (C_4); 32.5 (C_2); 28.8 (C_3); 20.0 (CH_3).

2,2,3-Trimethyl-1,4-bis[(benzyloxycarbonyl)amino]butane (9c) was obtained from butanenitrile **7c**: yield 75%; mp 90–91 °C. Anal. ($C_{23}H_{30}N_2O_4$) C, H, N. Hydrogenolysis afforded the dihydrochloride of **8c**; yield 90%; MS (*m/z*) 130 (M^+, 13); ^{13}C NMR (D_2O) δ 46.2 (C_1); 43.9 (C_4); 36.5 (C_2); 32.8 (C_3); 20.0 (CH_3 (2)); 16.1 (CH_3)).

2,2,3,3-Tetramethyl-1,4-bis[(benzyloxycarbonyl)amino]-butane (9d) was obtained from the tetramethylbutane dinitrile **7d**:[16] yield 88%; mp 120–122 °C. Anal. ($C_{24}H_{32}N_2O_4$) C, H, N. Hydrogenolysis afforded the dihydrochloride of **8d**; yield 80%; MS (*m/z*): 144 (M^+, 15); ^{13}C NMR (D_2O) δ 46.5 (C_1); 38.0 (C_2); 19.4 (CH_3).

Biological Methods. ODC was obtained from the livers of rats which had been injected with thioacetamide (100 mg/kg of body weight) and assayed as described elsewhere.[7,8] The specific activity of the preparation was 0.2 nmol/min/mg of protein. Reuber H-35 hepatoma cells were cultured as

described.[7] ODC activity was induced with insulin and assayed in the cells as described.[7] Polyamine analyses of both the livers and the cells were done on perchloric extracts as reported elsewhere.[7,8] The dansyl derivatives were separated on silica gel TLC using chloroform–triethylamine (9:1; v:v) for the separation of the isoornithine derivatives, and *n*-hexane–ethyl acetate (1:1; v:v) for the putrescine derivatives. The individual dansyl derivatives were then eluted from the plates (ethyl acetate), and the fluorescence of the eluates was determined by exciting at 365 nm and reading at 510 nm.

Acknowledgment. This work was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) of Argentina.

References

(1) Tabor, C. W.; Tabor, H. Polyamines. *Ann. Rev. Biochem.* **1984**, *53*, 749–790.
(2) Pegg, A. E,; Mc Cann, P. P. Polyamine Metabolism and Function. *Am. J. Physiol.* **1982**, *234*, C212-C221.
(3) Gilmour, S. K.; Robertson, F. M.; Megosh, L.; O'Connell, S. M.; Mitchell, J.; O'Brien, T. G. Induction of Ornithine Decarboxylase in Specific Subpopulations of Murine Epidermal Cells Following Multiple Exposures to 12-*O*-Tetradecanoylphorbol-13-acetate, Merzerein and Ethyl Phenylpropiolate. *Carcinogenesis* **1992**, *13*, 51-56.
(4) McCann, P. P.; Pegg, A. E. Ornithine Decarboxylase: an Enzyme Target for Therapy. *Pharmacol. Ther.* **1992**, *54*, 195-215.
(5) Ruiz, O.; Alonso Garrido, D. O.; Buldain, G.; Frydman, R. B. Effect of *N*-Alkyl and *C*-Alkylputrescines on the Activity of Ornithine Decarboxylase from Rat Liver and *E. coli*. *Biochim. Biophys. Acta* **1986**, *873*, 53–61.
(6) Ruiz, O.; Buldain, G.; Alonso Garrido, D. O.; Frydman, R. B. Interaction of Alkylputrescines with Ornithine Decarboxylase from Rat Liver and *E. coli*: An *In Vitro* and *In Vivo* Study. *Biochim. Biophys. Acta* **1988**, *454*, 114–125.
(7) Frydman, J.; Ruiz, O.; Robetto, E.; Dellacha, J. M.; Frydman, R. B. Modulation of Insulin Induced Ornithine Decarboxylase by Putrescine and Methylputrescines in H-35 Hepatoma Cells. *Mol. Cell. Biochem.* **1991**, *100*, 9–23.
(8) Moyano, N.; Frydman, B.; Buldain, G.; Ruiz, O.; Frydman, R. B. Inhibition of Ornithine Decarboxylase by the Isomers of 1,4-Dimethylputrescine. *J. Med. Chem.* **1990**, *33*, 1969-1974.
(9) González, N. S.; Algranati, I. D. Regulation of Putrescine Uptake in *Leischmania mexicana* Promastigotes. *Cell. Mol. Biol.* **1994**, *40*, 907–914.
(10) Whiteley, R. V.; Marianelli, R. S. The Synthesis of 2,3-Disubstituted Succinodinitriles. *Synthesis* **1978**, 392–394.
(11) Alonso Garrido, D. O.; Buldain, G.; Ojea, M. I.; Frydman, B. Synthesis of 2-Alkylputrescines from 3-Alkylpyrroles. *J. Org. Chem.* **1988**, *53*, 403–407.
(12) Alonso Garrido, D. O.; Buldain, G.; Frydman, B. 1,4-Diaminoalkanes from Pyrroles. A New Synthetic Approach to Substituted Putrescines. *J. Org. Chem.* **1984**, *49*, 2619–2623.
(13) Alhonen-Hongisto, L.; Seppanen, P.; Janne, J. Intracellular Putrescine and Spermidine Deprivation Induces Increased Uptake of the Natural Polyamines and Methylglyoxal bis-(Guanylhydrazone). *Biochem. J.* **1980**, *192*, 941–945.
(14) Holm, I.; Persson, L.; Heby, O.; Seiler, N. Feedback Regulation of Polyamine Synthesis in Ehrlich ascites Tumor Cells. *Biochim. Biophys. Acta* **1988**, *972*, 239–248.
(15) Frydman, R. B.; Ruiz, O.; Kreisel, M.; Bachrach, U. Oxidation of N-Alkyl and C-Alkylputrescines by Diamine Oxidases. *FEBS Lett.* **1987**, *219*, 380–386.
(16) Thiele, J.; Heuser, K. *Ann. Chem.* **1896**, *290*, 1–43.

JM9501590