nonhygroscopic salt: mp 279 °C dec; IR (liquid film, amine base) 3380, 3310 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 6.02 (m, 2, C=CH), 2.87 (m, 3, OH, NH_2, exchangeable), 2.72 (m, 4, H-1, H-2, H-4, C*H*OH), 1.97 (m, 2, CH_2NH_2); mass spectrum, *m/e* 153 (M^+). Anal. [$C_9H_{16}NO(SO_4)_{0.5}\cdot 0.5\ H_2O$] C, H, N.

1-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]hept-2-ene (11). To 1.30 g (34 mmol) of $LiAlH_4$ in 100 mL of anhydrous Et_2O was added 3.60 g (13.3 mmol) of **20**. The amine was recovered as a yellowish oil (2.02 g, 81% from **19**). This oil was precipitated as the hydrochloride salt from Et_2O and recrystallized from EtOAc to yield 1.26 g (59%) of a nonhygroscopic salt: mp 238 °C dec; IR (liquid film, amine base) 3380, 3300 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 5.98 (m, 2, C=CH), 3.80 (m, 1, C*H*OH), 3.37 (m, 3, OH, NH_2, exchangeable), 2.80 (m, 3, H-4, CH_2NH_2); mass spectrum, *m/e* 153 (M^+). Anal. ($C_9H_{16}NOCl$) C, H, N.

General Procedure for Synthesis of 12–14. The method of Augustine was used.[12] The catalyst (5% Pd on carbon, 0.1 g per 1 g of olefin) was suspended in 10% HOAc–90% EtOH in a N_2-flushed flask. The olefin was dissolved in the same solvent and added to the flask. Hydrogenation was accomplished on either the Parr low pressure or atmospheric hydrogenation apparatus. After 1 equiv of H_2 was consumed, the catalyst was filtered and the solvent removed in vacuo. The residue was dissolved in water and extracted with Et_2O, then the pH was adjusted to 12, and the residue was again extracted with Et_2O. The second Et_2O extract was washed with saturated brine and dried (Na_2CO_3). The solvent was removed in vacuo to yield the amine as a clear oil. The amine was stored as either the sulfate or hydrochloride salt.

***exo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (12).** To a Parr reaction vessel was added 5.81 g (37.9 mmol) of **9** in 200 mL of solvent. The hydrogenation was accomplished at 46 psi for 16 h. The clear oily product (3.79 g, 65%) was precipitated as the sulfate salt from absolute EtOH and recrystallized from 95% EtOH to yield 3.95 g (51%) of a nonhygroscopic salt: mp 299 °C dec; IR (liquid film, amine base) 3380, 3310, 3190 cm^{-1} (NH, OH); NMR ($CDCl_3$, amine base) δ 3.10 (m, 1, C*H*OH), 2.40 (m, 1, H-2), 2.25 (m, 3, NH_2, OH, exchangeable), 2.23 (m, 1, H-1), 2.00 (m, 1, H-4); mass spectrum, *m/e* 155 (M^+). Anal. [$C_9H_{18}NO(SO_4)_{0.5}$] C, H, N.

***endo*-2-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (13).** To a Parr reaction vessel was added 3.01 g (20 mmol) of **10** in 200 mL of solvent. The hydrogenation was accomplished at 36 psi. The clear oily product (2.84 g, 93%) was precipitated as the sulfate salt from EtOAc and recrystallized from 95% EtOH–EtOAc to yield 2.27 g (57%) of a nonhygroscopic salt: mp 275 °C dec; IR (liquid film, amine base) 3380, 3310 cm^{-1} (OH, NH); NMR ($CDCl_3$, amine base) δ 3.32 (m, 1, C*H*OH), 2.7–2.4 (m, 2, CH_2NH_2), 2.55 (s, 3, OH, NH_2, exchangeable), 2.17 (m, 2, H-1, H-4); mass spectrum, *m/e* 155 (M^+). Anal. [$C_9H_{18}NO(SO_4)_{0.5}\cdot 0.5H_2O$] C, H, N.

(12) Augustine, R. L. "Catalytic Hydrogenation"; Marcel Dekker: New York, 1965; p 57.

1-(1-Hydroxy-2-aminoethyl)bicyclo[2.2.1]heptane (14). To a round-bottom flask was added 1.00 g (6.5 mmol) of **11** in 25 mL of solvent. Hydrogenation was accomplished at atmospheric pressure for 70 min. The clear oily product (0.79 g, 78%) was precipitated as the hydrochloride salt from Et_2O and recrystallized from EtOAc to yield 0.73 g (75%) of a nonhygroscopic salt: mp 314 °C dec; IR (liquid film, amine base) 3370, 3300 cm^{-1} (OH, NH); NMR ($CDCl_3$, amine base) δ 3.62 (d of d, 1, C*H*OH), 2.57 (m, 2, CH_2NH_2), 2.43 (s, 3, OH, NH_2, exchangeable), 2.13 (m, 1, H-4); mass spectrum, *m/e* 155 (M^+). Anal. ($C_9H_{18}NOCl$) C, H, N.

1-(Hydroxymethyl)bicyclo[2.2.1]hept-2-ene (18). To a 500-mL three-neck flask fitted with a reflux condenser and N_2 bubbler was added 2.08 g (55 mmol) of $LiAlH_4$ and 200 mL of anhydrous Et_2O. This suspension was stirred for 20 min under N_2, and then 8.22 g (54.7 mmol) of **17** was added dropwise at a rate that maintained a gentle reflux. After the addition was complete (15 min), the mixture was heated at reflux for 1 h. The mixture was cooled in an ice bath, and the excess $LiAlH_4$ was destroyed by the method described by Fieser.[11] The resultant slurry was filtered under vacuum, and the filter cake was washed (3 × 30 mL) with Et_2O. The combined filtrates were dried (Na_2SO_4). The solvent was removed in vacuo to yield 6.39 g (94%) of a clear oil: IR (liquid film) 3370 (OH), 3080 cm^{-1} (olefin); NMR ($CDCl_3$) δ 6.02 (m, 2, C=CH), 3.87 (s, 2, CH_2OH), 3.38 (s, 1, OH), 2.83 (m, 1, H-4); mass spectrum, *m/e* 124 (M^+).

1-Formylbicyclo[2.2.1]hept-2-ene (19). To a 240-mL round-bottom flask fitted with a dropping funnel and N_2 bubbler was added 16.59 g (77 mmol) of dry pyridinium chlorochromate (PCC) and 150 mL of CH_2Cl_2. This slurry was stirred under N_2 for 10 min, and then 6.38 g (51.5 mmol) of **18** dissolved in 50 mL of CH_2Cl_2 was added dropwise via the addition funnel over a period of 10 min. The black solution was stirred 2 h at 25 °C and then poured into 200 mL of Et_2O containing Celite (filter aid). The resultant suspension was filtered, and the filter cake was washed with Et_2O (3 × 20 mL). The solvent volume was reduced to 25% of the original volume in vacuo and filtered again to remove chromium salts. The greenish-brown filtrate was washed with 1% aqueous EDTA (4 × 50 mL). The clear Et_2O solution was washed with saturated brine and dried (Na_2SO_4).

The solvent was removed in vacuo to yield 4.40 g (71%) of a clear oil with a strong odor resembling heptanal: IR (liquid film) 3080 (olefin), 2830, 2730 (CHO), 1725 cm^{-1} (C=O); NMR ($CDCl_3$) δ 9.95 (d, 1, C*H*O), 6.18 (m, 2, C=CH), 2.97 (m, 1, H-4).

Acknowledgment. This research was supported by NIH Training Grant GM1341, a grant-in-aid from the American Heart Association (Kansas Affiliate, Inc.), the University of Kansas General Research Fund, and the National Institutes of Health (Research Grant HL 21887). The technical assistance of Richard Lampe (PNMT assays) and assistance in preparation of this manuscript by Michael F. Rafferty are gratefully acknowledged.

α-Ethynyl and α-Vinyl Analogues of Ornithine as Enzyme-Activated Inhibitors of Mammalian Ornithine Decarboxylase

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α-Ethynyl- and α-vinylornithine were designed and synthesized as potential enzyme-activated inhibitors of mammalian ornithine decarboxylase. These two new inhibitors produce both immediate and time-dependent inhibition of rat liver ornithine decarboxylase in vitro. The inhibitions exhibit saturation kinetics. The apparent dissociation constants (K_I) are 10 and 810 μM, and the times of half-inactivation at infinite concentration of inhibitor ($t_{1/2}$) are 8.5 and 27 min, respectively, for α-ethynyl- and α-vinylornithine. In rats, α-ethynylornithine causes a rapid dose-dependent decrease of ornithine decarboxylase activity in prostate and, to a lesser extent, in thymus and testis.

The pyridoxal phosphate (PLP) dependent enzyme L-ornithine carboxy-lyase (EC 4.1.1.17, ODC), which catalyzes the decarboxylation of ornithine to putrescine, can be rate limiting in the biosynthesis of the polyamines

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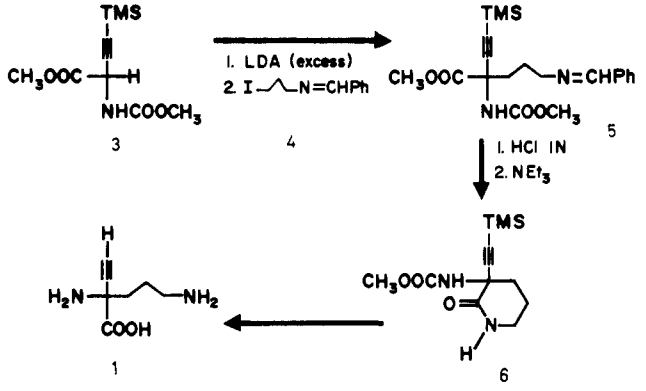
Scheme I



(spermidine and spermine) in mammalian cells.[1,2] Previous reports described the synthesis and evaluation of enzyme-activated irreversible inhibitors of this enzyme.[3,4] Considerable knowledge on the physiological role of polyamines has been accumulated during the last few years owing to such inhibitors[5] which combine, by their mechanism of action, potency and selectivity.[6,7] The present report describes the synthesis of α-ethynyl- and α-vinylornithine and their evaluation as new potential irreversible inhibitors of rat ODC in vitro and in vivo. The rationale for the design of such compounds was based on mechanisms already proposed to explain the partially irreversible inhibition of Dopa decarboxylase, another PLP-dependent enzyme, by α-ethynyl- and α-vinyl-Dopa.[8,9]

Chemistry. The synthesis of α-ethynylornithine (1) is depicted in Scheme I. The regioselective propargylic alkylation of the dianion derived from **3**, the synthon of α-ethynyl α-amino acids,[10] with 1-iodo-3-benzaldiminopropane[3] gave the nonpurified intermediary imine **5**, and no allenic products were detected by IR and NMR. Mild acidic hydrolysis of the imine and cyclization afforded the lactam **6**. The free amino acid was obtained by cleavage of the carbamate with Me_3SiI,[11] followed by alkaline hydrolysis of the trimethylsilyl group and of the lactam ring. Purification by ion-exchange chromatography and recrystallization in $EtOH/H_2O$ gave the α-ethynylornithine (1).

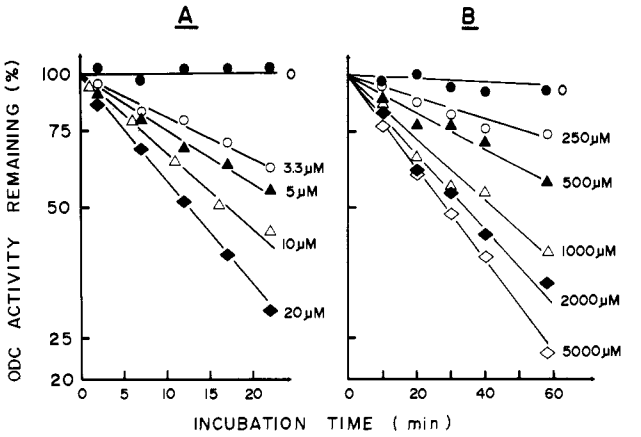


Figure 1. Time-dependent inhibition of ODC by α-ethynylornithine (A) and by α-vinylornithine (B). The enzyme preparation was incubated at 37 °C in 30 mM, pH 7.1, sodium phosphate buffer, 5 mM dithiothreitol, and 0.1 mM pyridoxal phosphate, with different concentrations of inhibitor. At given time intervals, aliquots were withdrawn and assayed for remaining enzyme activity using DL-[1-^{14}C]ornithine according to a published procedure.[3]

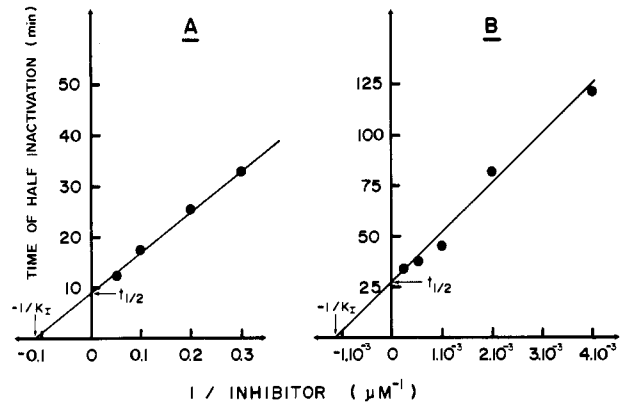


Figure 2. Effect of inhibitor concentration (A, α-ethynylornithine; B, α-vinylornithine) on the time of half-inactivation of ODC.

Table I. Effects of Preincubation with Different Effectors on the Time of Half-inactivation of Ornithine Decarboxylase by α-Ethynylornithine

additions to incubation media	time of half-inactivation of ornithine decarboxylase, min
10 μM α-ethynylornithine	18 ± 1
10 μM α-ethynylornithine + 1 mM L-ornithine	81 ± 8
10 μM α-ethynylornithine + 1 mM L-2-methylornithine	270 ± 70
10 μM α-ethynylornithine + 1 mM D-2-methylornithine	26 ± 3

The α-vinyl derivative of ornithine was synthesized as shown in Scheme II. Regioselective α-alkylation of the anion derived from methyl crotonate with 1-iodo-3-chloropropane at −78 °C gave the nonconjugated intermediary product,[12] which was isomerized into the corresponding conjugated product **9** by leaving it at −30 °C overnight. A second anion generated from **9** afforded, by acylation at the α position, the methyl ketone **10**. A phthalimido group was introduced by a Gabriel synthesis

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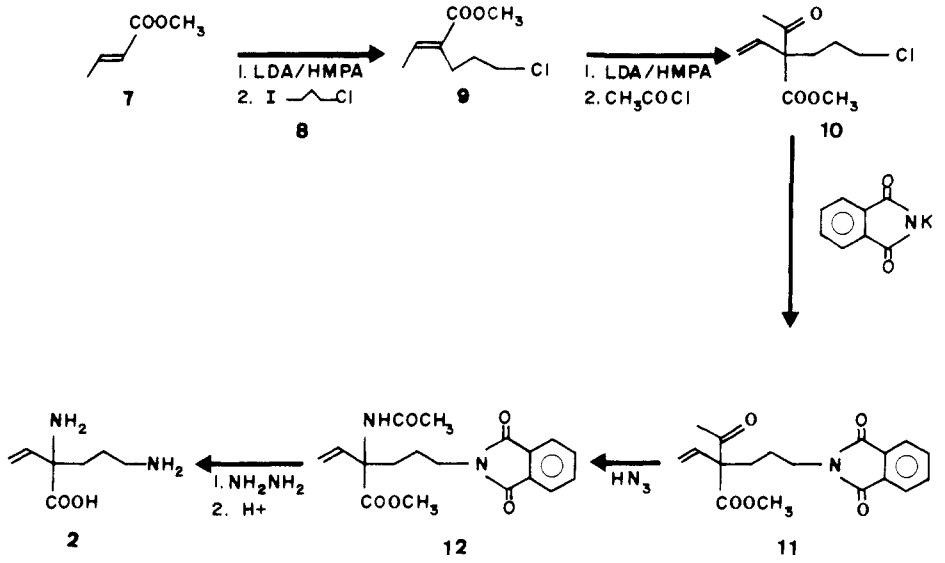
Scheme II



to yield 11. Then the protected form of α-vinylornithine, 12, was obtained by a Schmidt reaction. The removal of the protective groups by treatment with hydrazine, followed by acid hydrolysis, afforded 2, which was purified by ion-exchange chromatography and recrystallization in $EtOH/H_2O$.

Enzyme Inhibitory Activity. Both the α-ethynyl and α-vinyl analogues of ornithine were tested as time-dependent irreversible inhibitors of ODC. Livers from thioacetamide-treated rats were used as the source of the enzyme for the in vitro experiments (see Experimental Section). Incubation of the enzyme preparation with 1 and 2 resulted, in both cases, in a time-dependent loss of enzyme activity which followed pseudo-first-order kinetics for at least 2 half-lives (Figure 1). Loss of activity was related to the concentration of inhibitor. By plotting the time of half-inactivation ($t_{1/2}$) as a function of the reciprocal of the inhibitor concentration ($1/I$) according to Kitz and Wilson,[13] straight lines were obtained (Figure 2). These lines did not pass through the origin but intercepted the positive y axis, demonstrating saturation effects which involve the enzyme's active site in the inhibitory process. That the inhibition of ODC is active-site directed is confirmed by the protective effects of the natural substrate L-ornithine and of a potent competitive inhibitor L-2-methylornithine.[14] Moreover, D-2-methylornithine, which is practically devoid of affinity toward ODC,[14] does not protect this enzyme against inactivation by α-ethynylornithine (Table I). The presence of dithiothreitol (5 mM) in the preincubation medium and the absence of a lag time before the onset of inhibition rule out the possibility of inhibition via affinity labeling by a diffusible alkylating species.[15] Incubation with 1, the most efficient inhibitor, at 0.2 mM concentration resulted in 98% inactivation of ODC after 1 h. Prolonged (24 h) dialysis of this inactivated ODC against a buffer solution containing phosphate (30 mM), PLP (0.1 mM), and dithiothreitol (5 mM) (conditions where the native enzyme is stable) led to partial regeneration of enzyme activity, from 2% of control before dialysis to 16% of control after dialysis. A similar recovery has been observed after dialysis of Dopa decarboxylase previously incubated and inhibited by α-ethynyl-Dopa.[8,9]

Table II. Effect of Single Doses of α-Ethynylornithine, Given Intraperitoneally 5 h before Sacrifice of Animals, on ODC Activities in Ventral Prostate, Testis, and Thymus of Rat (See Experimental Section)

dose of α-ethynylornithine, mg/kg	ODC act. remaining,[a] % of control		
	ventral prostate	testis	thymus
0 (Saline)	100 ± 11	100 ± 6	100 ± 10
12.5	59 ± 9*	ND	ND
25	56 ± 4**	ND	ND
50	40 ± 2**	ND	ND
100	19 ± 2***	ND	ND
200	18 ± 1***	80 ± 4*	54 ± 7*

[a] ODC activities are expressed as percent of control. Each value is the mean ± SEM of five animals. ODC activities of control animals were 376 ± 40, 25 ± 2, and 38 ± 4 nmol of CO_2 h^{-1} (g of wet tissue)$^{-1}$ in prostate, testis, and thymus, respectively. The significance of the differences between controls and treated animals was calculated by Student's t test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; ND = not determined.

However, this does not rule out a covalent linkage of the activated inhibitor to the enzyme active site but suggests that this bond, if it exists, can be slowly hydrolyzed.[16]

Kinetic constants for the time-dependent inhibition of rat liver ODC can be extrapolated from Figure 2.[13] The apparent dissociation constants (K_I) for α-ethynyl- and α-vinylornithine hydrochloride are 10 and 810 μM, respectively, and the times of half-inactivation at infinite concentration of inhibitor ($t_{1/2}$) are 8.5 and 27 min, respectively. The great difference between the kinetic constants of the two compounds may be due either to differences of steric hindrance between the α-substituents, as previously reported by Abdel-Monem et al. for competitive inhibitors of ODC,[17] or (and) to differences of the pK_a values of the α-amino group.[18]

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(17) M. M. Abdel-Monem, N. E. Newton, B. C. Ho, and C. E. Weeks, *J. Med. Chem.*, 18, 600 (1975).
(18) P. Vitali and J. Wagner, unpublished results. The pK_a values of the various functions of α-ethynylornithine are 2.2, 7.5, and 10.5, respectively, for the carboxyl, the α-amino, and the terminal amino group, and 1.6, 8.6, and 10.8 for the corresponding functions of α-vinylornithine.

Because of the high potency of α-ethynylornithine as an ODC inhibitor in vitro, we have tested this compound ex vivo. As with other known enzyme-activated inhibitors of ODC, it caused a preferential decrease of prostatic ODC[4,19] (Table II). In addition, this compound caused a significant decrease of ODC in thymus and testis.

Conclusion

α-Ethynyl- and α-vinylornithine hydrochloride are time-dependent inhibitors of mammalian ODC, and our data are consistent with these compounds being effective enzyme-activated inhibitors (or K_{cat} inhibitors[6] or suicide inactivators[7]). Our results concerning ODC inhibition and results concerning Dopa decarboxylase inhibition[8,9] allow one to formulate the general concept that amino acids α-substituted by a vinyl or an acetylenic group are enzyme-activated inhibitors of their respective decarboxylases.

The efficiency of α-ethynylornithine as an inhibitor of ODC in vitro and in vivo is comparable to that of the best known inhibitors of this enzyme.[3,4,19,20] This compound is a new tool which may be useful for assessing the biological role of polyamines.

Experimental Section

Melting points were obtained on a Büchi SMP-20. Microanalyses were conducted on a Perkin-Elmer 240 CHN analyzer. IR data were recorded on a Beckman IR-577 or IR 257 spectrophotometer. NMR data were obtained with a Varian Associates Model T-60 spectrophotometer with Me_4Si as external standard. IR data, NMR data, and analyses were consistent with the assigned structure.

Methyl 1-[(Trimethylsilyl)ethynyl]-*N*-carbomethoxyglycinate (3). To a cooled (0 °C) solution of methyl α-chloro-*N*-carbomethoxyglycinate[21] (91 g, 0.5 mol) and bis(trimethylsilyl)acetylene (95 g, 0.56 mol) in freshly distilled CH_2Cl_2 (500 mL) was added $AlCl_3$ (75 g, 0.56 mol) in small portions. After the addition was completed, the reaction mixture was maintained at 20 °C overnight. The solution was washed with water (3 × 200 mL), dried, and distilled to give **3** (55 g, 45%), bp 90 °C (0.15 mm). Anal. ($C_{10}H_{17}NO_4Si$) C, H, N.

1-Iodo-3-benzaldiminopropane (4). To a solution of 1,3-bromopropylamine (22.5 g, 0.1 mol) and benzaldehyde (10.6 g, 0.1 mol) in CH_2Cl_2 (150 mL) was added NEt_3 (10.1 g). After standing for 2 h at room temperature, the solution was washed with water (2 × 100 mL), dried over $MgSO_4$, and yielded by distillation 1-bromo-3-benzaldiminopropane (18.5 g, 82%), bp 90 °C (0.05 mm). Anal. ($C_{10}H_{12}BrN$) C, H, N.

A solution of the bromoimine (22.5 g, 0.1 mol) and NaI (15 g, 0.1 mol) in anhydrous THF (200 mL) was heated under reflux overnight. The reaction mixture was cooled to room temperature, and the solid formed was filtered off.

The iodo compound **4** was used without further purification in the next step. However, a sample was distilled for analytical reasons with some decomposition: bp 85 °C (0.07 mm); yield 13 g (48%). Anal. ($C_{10}H_{12}IN$) C, H, N.

3-[(Trimethylsilyl)ethynyl]-3-(*N*-carbomethoxyamino)-2-piperidinone (6). To a cooled (−78 °C) solution of LDA (0.02 mol) in THF/HMPA (100 mL, 9:1) was added a solution of **3** (2.45 g, 10 mmol) in THF (5 mL) under N_2. After 1 h at −78 °C, a solution of **4** (2.75 g, 10 mmol) in THF (5 mL) was added. The reaction mixture was stirred at −78 °C for 2.5 h and then the solution was hydrolyzed with 1 N AcOH (100 mL). After extraction with ether, the intermediary imine **5** was hydrolyzed with 1 N HCl (100 mL). The benzaldehyde was extracted with ether, and the aqueous phase was evaporated and dried in vacuo. The residue was dissolved in MeOH (100 mL), and triethylamine was added (1.5 mL, 10 mmol). After 12 h at room temperature, MeOH was evaporated and ether (100 mL) was added. The solution was washed with water (3 × 100 mL) and dried over $MgSO_4$. After evaporation of the solvent, the residue was chromatographed on a silica gel column (50 g). Elution with ether afforded 1.2 g (45%) of **6**, mp 146 °C. Anal. ($C_{12}H_{20}N_2O_3Si$) C, H, N.

α-Ethynylornithine Hydrochloride (1). A solution of **6** (1.42 g, 5.3 mmol) and Me_3SiI (1.5 mL, 10 mmol) in $CHCl_3$ (5 mL) was heated under reflux for 1 h and then 1 mL of MeOH was added slowly. After evaporation, the residue was hydrolyzed with 2 N NaOH (7.5 mL) overnight. The aqueous solution was diluted with water (100 mL), washed with $CHCl_3$ (2 × 50 mL), acidified with 1 N HCl, and washed with $CHCl_3$ (2 × 50 mL). After concentration, the aqueous phase, adjusted to pH 6 with 1 N NH_4OH solution, was passed through an Amberlite IR 120, H^+ form, resin column. The ninhydrin-positive fractions eluted with 1 N NH_4OH were collected and evaporated to give 0.8 g of crude α-ethynylornithine (**1**). The solid was dissolved in 1 N HCl (10 mL), and the residue obtained after evaporation was dissolved in EtOH (10 mL). The monochlorhydrate was obtained by addition of NEt_3 and recrystallization in EtOH/H_2O (9:1) (600 mg, 59%), mp 171 °C. Anal. ($C_7H_{13}N_2O_2Cl$) C, H, N.

1-Iodo-3-chloropropane (8). A solution of 1-bromo-3-chloropropane (100 g, 0.64 mol) in acetone (500 mL) was treated with NaI (96 g, 0.64 mol) at room temperature overnight, then filtered, concentrated, and distilled to afford an orange-red liquid (94.2 g, 60%), bp 56–58 °C (water pump).

6-Chloro-3-(methoxycarbonyl)-2-hexene (9). Redistilled methyl crotonate (36.2 g, 0.36 mol) was added to a solution of lithium diisopropylamide and HMPA at −78 °C prepared as follows: *n*-BuLi (200 mL of a 2 M solution, 0.4 mol) was added to diisopropylamine (40.4 g, 56.0 mL, 0.4 mol) in THF (400 mL) at −78 °C. HMPA (74 mL, 0.425 mol) was then added and the solution stirred for 30 min at −78 °C, during which time a precipitate appeared.

After the addition of methyl crotonate, the solution was stirred for 10 min at −78 °C, and the iodide (81.6 g, 0.4 mol) was added. The solution was maintained at −78 °C for 4 h, when an aliquot was removed with a syringe. The NMR spectrum showed the presence of the nonconjugated alkylated ester and the starting iodide. The solution was then placed at −30 °C overnight. Saturated aqueous NH_4Cl was then added, and the product was isolated by ether extraction. The ether solution was washed several times with brine and then dried, evaporated, and distilled: fraction bp 64 °C (0.1 mm); yield 37.2 g (58%); NMR, conjugated isomer, δ 6.78 (1 H, vinylic quadruple, J = 7 Hz). Anal. ($C_8H_{13}O_2Cl$) C, H.

3-(Methoxycarbonyl)-3-vinyl-6-chloro-2-hexanone (10). The ester (42.5 g, 0.24 mol) was added at −78 °C to LDA/HMPA prepared as described previously from DIA (25.3 g, 35 mL, 0.25 mol), *n*-BuLi (125 mL of a 2 M solution, 0.25 mol), and HMPA (45 mL) in THF (250 mL). After 10 min, acetyl chloride (19.5 g, 17.8 mL, 0.25 mol) was added and the solution was quenched at −78 °C after a further 15 min. The product was isolated by ether extraction and distilled, a fraction of 36.7 g, bp 80–118 °C (0.1 mm), being collected. This was further purified by chromatography on silica gel (900 g), the pure product (26.4 g, 33.5%) being eluted with 20% petroleum ether. A sample was distilled for analysis (Kugelrohr, 120 °C, 0.1 mm). Anal. ($C_{10}H_{15}ClO_3$) C, H.

3-(Methoxycarbonyl)-3-vinyl-6-phthalimido-2-hexanone (11). A solution of the chloride (26.4 g, 0.12 mol), potassium phthalimide (22.5 g, 0.12 mol), and sodium iodide (2 g) in DMF (200 mL) was heated for 3 h at 100 °C. The DMF was then removed by distillation under reduced pressure (12 mm), and the residue was taken up in ether, washed with bisulfite and water, and then dried ($MgSO_4$) and evaporated to afford an oil (35.6 g, 97%), which was sufficiently pure to use in the next step. A sample was purified for analysis by column chromatography on silica gel (elution with 50% ether–petroleum ether), followed by recrystallization from petroleum ether, mp 65 °C. Anal. ($C_{18}H_{19}NO_5$) C, H, N.

(19) C. Danzin, M. J. Jung, B. W. Metcalf, J. Grove, and P. Casara, *Biochem. Pharmacol.*, **28**, 627 (1979).

(20) For instance, the corresponding kinetic constants of α-(difluoromethyl)ornithine, another enzyme-activated inhibitor of ODC, are K_I = 39 μM and $t_{1/2}$ = 3.1 min (see ref 3) and, in rats, the ODC activity remaining 5 h after a single dose (200 mg/kg) of this compound injected ip was 12, 52, and 61% of control in prostate, testis, and thymus, respectively (see ref 4).

(21) Z. Bernstein and D. Ben-Ishai, *Tetrahedron*, **33**, 881 (1977).

3-(Methoxycarbonyl)-3-acetamido-6-phthalimido-1-hexene (12). A solution of HN_3 in $CHCl_3$ (51 mL of a 2.04 M solution, 105 mmol) was added to the ketone (34.4 g, 105 mmol) in $CHCl_3$. This solution was then added to a mixture of concentrated H_2SO_4 (50 mL) and $CHCl_3$ (200 mL) under N_2 at room temperature. On completion of the addition the mixture was stirred at room temperature for 15 min and then poured on ice. The mixture was extracted with $CHCl_3$, washed with $NaHCO_3$, dried, and evaporated. The residue was then chromatographed on silica gel (300 g), and the product (25.3 g, 70%) eluted with 7% methanol/$CHCl_3$ as an oil.

A sample was distilled (Kugelrohr, 250 °C, 0.02 mm) for analysis and was found to solidify on standing. Anal. ($C_{18}H_{20}N_2O_5$) C, H, N.

α-Vinylornithine Hydrochloride (2). A solution of the amide 12 (26.3 g, 0.076 mol) and hydrazine hydrate (4.2 g, 0.084 mol) in ethanol (500 mL) was heated under reflux overnight. When the solution cooled, a white precipitate was filtered off and the filtrate was evaporated. The residue was then heated under reflux with 6 N HCl (150 mL) for 2 h and then filtered, washed with CH_2Cl_2, and evaporated to yield an oil (15.6 g). This dihydrochloride was dissolved in ethanol (250 mL) and treated with Et_3N (6.8 g, 0.67 mmol, 9.4 mL). The resulting precipitate was filtered off and recrystallized from EtOH/water (9:1) to afford a colorless solid (8.2 g, 55%), mp 242 °C. Anal. ($C_7H_{15}ClN_2O_2$) C, H, N.

Enzyme Preparation. Rat liver ODC was prepared from the livers of rats which had been injected with thioacetamide (150 mg/kg of body weight) 18 h before sacrifice and was purified about tenfold by acid treatment at pH 4.6 as described by Ono et al.[22] The specific activity of this preparation was 0.2 nmol of CO_2 min^{-1} (mg of protein)$^{-1}$.

Assay of Time-Dependent Inhibition of Ornithine Decarboxylase (in Vitro). Assay and measurement of the kinetic constants of the inhibition were performed essentially as described previously.[3]

Measurement of Ornithine Decarboxylase Activity (ex Vivo). Male rats of the Sprague–Dawley strain (200–220 g of body weight), purchased from Charles-River, France, were given food and water ad libitum under a constant 12 h light–12 h dark lighting schedule. Animals were killed by decapitation at about the same time of day to minimize effects due to diurnal fluctuations. α-Ethynylornithine hydrochloride, dissolved in 0.9% saline, was injected intraperitoneally. Rats given saline served as controls. Immediately after sacrifice the ventral prostate, testis, and thymus were excised and homogenized, and the corresponding ODC activities were measured according to a published procedure.[4]

Data Processing. Kinetic constants were calculated by the method of Kitz and Wilson[13] using a least-squares fit of the data points with a Hewlett-Packard 9820 calculator (accuracy better than 20%). Ex vivo values of ODC activity were the mean ± SEM of five animals. The significance of the differences between control and treated animals was calculated by Student's t test with the above-described calculator.

(22) M. Ono, H. Inoue, F. Suzuki, and Y. Takeda, *Biochim. Biophys. Acta*, 284, 285 (1972).

Novel Peptidoaminobenzophenones, Terminal N-Substituted Peptidoaminobenzophenones, and *N*-(Acylglycyl)aminobenzophenones as Open-Ring Derivatives of Benzodiazepines[1,2]

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Peptidoaminobenzophenones (1), terminal N-substituted peptidoaminobenzophenones (14), and acylglycylaminobenzophenones (16) were prepared as a novel series of ring-opened derivatives of 1,4-benzodiazepine. Z-Gly- and Z-Ala-*N*-methylaminobenzophenones (4) were treated with HBr–HOAc to give Gly- and Ala-*N*-methylaminobenzophenone hydrobromides (8). Reaction of 8 with chloroacetyl chloride in dimethylformamide (DMF) or hexamethylphosphoramide (HMPA) gave chloracetamide (13), which was allowed to react with various amines to afford a number of terminal N-substituted derivatives (14). Reaction of 8 with various acyl halides in HMPA or DMF gave a number of acylglycyl-*N*-methylaminobenzophenones (16). Peptidoaminobenzophenones (1) were also prepared by several convenient methods. Many of these compounds exhibited high CNS activity in animals when given orally. In antianxiety activity the potency of some compounds is equal to or higher than that of diazepam.

In the previous paper,[3] we reported a series of peptidoaminobenzophenones (1) as a novel class of ring-opened derivatives of 1,4-benzodiazepine. Hassall et al. have also reported peptidoaminobenzophenones as being novel latentiated 1,4-benzodiazepines.[4] The interesting pharmacological and physicochemical properties of peptidoaminobenzophenones prompted us to investigate various types of these and related compounds. Here we report the synthesis and pharmacological activities of some peptidoaminobenzophenones, terminal nitrogen substituted peptidoaminobenzophenones (14), and acylglycyl-*N*-methylaminobenzophenones (16).

Chemistry. According to our method reported in the preceding paper,[1] protected amino acids were coupled with aminobenzophenones (**2a–d**) by $SOCl_2$ in hexamethylphosphoramide (HMPA) to obtain Z-Gly-[5] and Z-Ala-aminobenzophenones (**3a–e**) in high yields (Scheme I). The amides (**3a–e**) were readily N-methylated via sodio derivatives to give the *N*-methyl derivatives (**4a–d**). The coupling reaction of Z-amino acids with *N*-methylaminobenzophenones (**2e**) also gave the *N*-methylamides (**4a**).

(1) This paper is Part 4 of a series on "Benzophenone Related Compounds". Part 3: Hirai, K.; Ishiba, T.; Fujishita, T.; Sugimoto, H. *Heterocycles* **1980**, *14*, 635.

(2) A part of this paper was presented at the ACS/CSJ Chemical Congress, a joint meeting of the American Chemical Society and the Chemical Society of Japan. See K. Hirai, T. Ishiba, H. Sugimoto, T. Fujishita, Y. Tsukinoki, and K. Hirose, In "Abstracts of Papers", ACS/CSJ Chemical Congress, Honolulu, HI, Apr 1–6, 1979; American Chemical Society: Washington, D.C., 1979; Abstr MEDI 6.

(3) Hirai, K.; Ishiba, T.; Sugimoto, H.; Sasakura, K.; Fujishita, T.; Toyoda, T.; Tsukinoki, Y.; Joyama, H.; Hatakeyama, H.; Hirose, K. *J. Med. Chem.* **1980**, *23*, 764.

(4) Hassall, C. H.; Holmes, S. H.; Johnson, W. H.; Kröhn, A.; Smithen, C. E.; Thomas, W. *Experientia* **1977**, *33*, 1492.

(5) Abbreviations used are those recommended by the IUPAC–IUB Commission of Biochemical Nomenclature Symbols for Amino Acid Derivatives and Peptides [*J. Biol. Chem.* **1972**, *247*, 977]. Additional abbreviations used are: Z, benzyloxycarbonyl; Pht, phthalyl.

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