mL of EtOH was hydrogenated over 0.9 g of 5% Pd/C at an initial pressure of 45 psig. H_2 uptake was complete in 5 h. The reduction mixture was filtered and the filtrate was evaporated under reduced pressure to give a solid residue, which was recrystallized. See Table I.

Ether Cleavage Reactions. The HCl salt of the methyl ether (0.001 mol) was heated under reflux under N_2 (pot temperature 135 °C) in 11 mL of 48% HBr and 3 mL of AcOH for 2 h. Volatiles were removed under reduced pressure and the residue was recrystallized. See Table I.

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Conformationally Defined Aromatic Amino Acids. Synthesis and Stereochemistry of 2-*endo*- and 2-*exo*-Amino-1,2,3,4-tetrahydro-1,4-ethanonaphthalene-2-carboxylic Acids (2-*endo*- and 2-*exo*-Aminobenzobicyclo[2.2.2]octene-2-carboxylic Acids)

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The synthesis of the title compounds **1a** and **1b** has been accomplished in good yield by conversion of ketone **3** to the corresponding hydantoins **4a** and **4b** via a Bucherer–Bergs reaction, followed by barium hydroxide hydrolysis. The stereochemical assignments of the intermediate hydantoins **4a** and **4b** and the ethyl ester hydrochlorides **5a** and **5b** were determined by ^1H NMR analysis. Attempts toward the synthesis of 2-amino-1,4-dihydro-1,4-ethanonaphthalene-2-carboxylic acid isomers **2a** and **2b** utilizing the pathway discussed for **1a** and **1b** led only to products arising from a retro-Diels–Alder reaction. Preliminary screening of **1a** and **1b** as inhibitors of phenylalanine hydroxylase (PH) and phenylalanine decarboxylase (PAD) is also discussed. The use of the benzobicyclo[2.2.2]octene nucleus for the construction of conformationally defined analogues of important medicinal agents is rationalized, and the title compounds are compared to several other conformationally defined systems. The title compounds represent conformationally defined models of the lower energy conformations of α-methylphenylalanine.

As part of a current project dealing with the synthesis of conformationally defined analogues of aromatic amino acids and adrenergic amines[2] using the benzobicyclo-[2.2.2]octene and -octadiene ring systems, we wish to report the versatile, high-yielding synthesis of 2-amino-1,2,3,4-tetrahydro-1,4-ethanonaphthalene-2-carboxylic acid isomers **1a** and **1b**.[3] The attempted synthesis of 2-amino-1,4-dihydro-1,4-ethanonaphthalene-2-carboxylic acid isomers **2a** and **2b** and preliminary biochemical screening of the title compounds are also discussed. These compounds represent conformationally defined analogues of the lower energy conformations of α-methylphenylalanine.

Chemistry. Synthesis of the two isomeric amino acids **1a** and **1b** in the benzobicyclo[2.2.2]octene series was achieved starting with ketone **3**[4] (Scheme I). Treatment of **3** with potassium cyanide and ammonium carbonate in a Bucherer–Bergs synthesis[5] afforded a mixture of isomeric hydantoins **4a** and **4b** in 92% yield. The nearly equal mixture of isomers has been separated by two methods, either fractional crystallization or column chromatography as described under Experimental Section. The preferred approach involved fractional crystallization to remove **4b**, followed by column chromatography of the mother liquor.

Scheme I

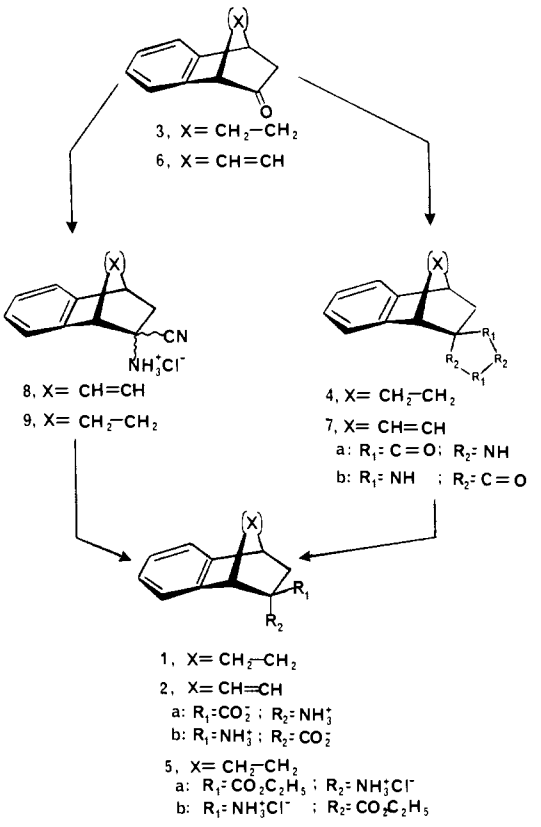

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Table I. Method of Preparation and Yields of Intermediates and Amino Acids with a Benzobicyclo[2.2.2]octene or Benzobicyclo[2.2.2]octadiene Ring Nucleus

compd	method	octene	yield, %	octadiene	yield, %
hydantoins	Bucherer-Berg	4	92	7	71
aminonitrile	Strecker	9[b]	38	8	26
amino acids	70% H_2SO_4	1		2	*a, c*
	$Ba(OH)_2$	1	75[f]	2	*a*
	3 N NaOH	1		2	*a, d*
	conc HCl	1	41[e]	2	*a, e*

[a] Hydrolysis resulted in a retro-Diels–Alder reaction, yielding naphthalene in essentially quantitative yield. [b] Isolated as the hydrochloride. [c] Aminonitrile hydrochloride was heated at a reflux with 70% sulfuric acid for 18 h. [d] The hydantoin was heated at reflux with 3 N sodium hydroxide for 80 h. [e] Aminonitrile hydrochloride was left to stand overnight with concentrated HCl; water was then added and the solution was heated at a reflux for 24 h. [f] See Experimental Section.

Table II. ^{1}H NMR Chemical Shifts and Stereochemical Assignments

hydantoin	NMR, δ, amide (NH)	ester	NMR, δ (CH_2CH_3)	stereochem assignment[b]
4a	7.05	5a	1.32	endo
4b	8.45	5b	0.95	exo
7a	6.95	2a	*a*	endo
7b	7.80	2b	*a*	exo

[a] See text. [b] See ref 3.

Hydrolysis of the hydantoins **4a** and **4b** afforded the respective amino acids **1a** and **1b** in good yields. Best yields were obtained with barium hydroxide hydrolysis (Table I). Amino acids **1a** and **1b** have also been prepared from the corresponding Strecker[6] aminonitrile hydrochloride **9**, which upon hydrolysis gave a nearly equal mixture of amino acids **1a** and **1b** as shown by ^{1}H NMR analysis of their ethyl esters **5a** and **5b**. In a number of alicyclic systems[7-10] it has been shown that the Strecker synthesis afforded a clear preference of one isomer over the other, but this was not observed in the present case, and, since the overall yields in the Bucherer–Bergs synthesis were superior, the Strecker synthesis was not investigated further in this system.

While hydantoins **7a** and **7b** could be prepared from ketone **6**[4] by a Bucherer–Bergs synthesis in 71% yield, attempted synthesis of amino acids **2a** and **2b** in the benzobicyclo[2.2.2]octadiene series proved unsuccessful (Scheme I). Due to the temperature required for hydrolysis of the hydantoins, only products arising from a retro-Diels–Alder reaction were isolated (Table I).[11] Similarly, by treating ketone **6** in a Strecker-type synthesis[6] with potassium cyanide and ammonium chloride the aminonitrile hydrochloride **8** was obtained in 26% yield; however, attempted hydrolysis of **8** resulted only in retro-Diels–Alder products (Table I).

Stereochemical assignments in the hydantoin series were based upon the chemical shift of the amide NH proton (Table II). The shielding effect of the aromatic ring shifted the endo-amide NH absorption to higher field than that of the corresponding exo-amide proton.[12-14] Confirmation of the stereochemical assignments were obtained by ^{1}H NMR analysis of the ethyl ester hydrochlorides **5a** and **5b** of the amino acids **1a** and **1b**. The ethyl ester protons in isomer **5b** appear at higher chemical shift than those in **5a** due to the anisotropic shielding effect of the aromatic ring in the former (Table II). This analysis is in complete agreement with data reported in other related systems, i.e., *endo*- and *exo*-2,3-bis(methoxycarbonyl)-benzobicyclo[2.2.2]octene,[15] *endo*- and *exo*-2-acetoxybenzobicyclo[2.2.2]octene,[16] *endo*- and *exo*-2-(methoxycarbonyl)bicyclo[2.2.1]hept-5-ene,[17] *endo*- and *exo*-*N*-(*o*-tolyl)benzobicyclo[2.2.2]octene-2,3-dicarboxylic acid imide,[18] 5-benzoyl-8-*endo*-9-*endo*-bis(methoxycarbonyl)-2,6-*exo*- and -*endo*-3-oxa-4-azatricyclo[5.2.2.$0^{2,6}$]undec-4-ene,[19] and the 1-amino-2-phenylcyclohexanecarboxylic acids.[20] Thus, the assignment of the stereochemistry based on the chemical shifts of the ethyl groups in esters **5a** and **5b** confirms the assigned stereochemistry of the hydantoins **4a** and **4b**. Also, the shift in the infrared to lower energy in the carbonyl of ethyl ester **5b** (endo carbonyl) compared to **5a** (exo carbonyl) is in agreement with that reported in related systems, i.e., 1-methoxy-*anti*-8-methylbicyclo-[2.2.2]oct-5-en-*endo*- and -*exo*-2-carboxamide,[21] as well as with the assigned stereochemistry.

Pharmacology. Compounds **1a** and **1b** were tested for their ability to inhibit the decarboxylation of L-phenylalanine using a crude enzyme preparation of phenylalanine decarboxylase (PAD) from *Streptococcus faecalis*.[22] The maximum concentration that could be tested, due to limitations of solubility, was 1.76 mM. The percent inhibition of PAD was determined in triplicate by measuring the $^{14}CO_2$ evolution in the presence of 10 mM L-phenylalanine. The results obtained showed limited inhibition: DL-**1a**, 7%; DL-**1b**, 8%; compare to DL-α-Me-Dopa (8%) at 1.76 mM. Thus, both **1a** and **1b** inhibit the enzyme to the same degree as does α-Me-Dopa at this concentration.

Amino acids **1a** and **1b** were also screened as inhibitors of phenylalanine hydroxylase (PH) prepared from guinea

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pig liver according to the procedure developed by Ayling and co-workers.[23] This assay involves measuring the phenylalanine-dependent change in absorbance at 330 nm of the tetrahydropteridine cofactor, 2-amino-4-hydroxy-6,7-dimethyl-4,6,7,8-tetrahydropteridine ($DMPH_4$), as it is oxidized to the dihydro form. The degree of inhibition brought about by a 1 mM concentration of test compound at a 1 mM concentration of L-phenylalanine was determined. DL-**1a** showed 24.2% and DL-**1b** showed 25.0% inhibition. This moderate degree of inhibition was comparable to that reported[29] for DL-*p*-chlorophenylalanine (24%). Again, both isomers inhibited the enzyme to the same degree.

Discussion

We have previously shown[24] that the benzobicyclo-[2.2.2]octene nucleus offers many advantages for the construction of conformationally defined analogues of methamphetamine. It appears to offer the same attractive advantages for a study of conformational preferences in aromatic amino acids; i.e., normal bond angles and bond lengths of the parent (flexible) α-methylphenylalanine are retained with minimal strain added by the conformational restraint, a minimal number of extra atoms are added to produce the conformational definition, and the physical properties of the amino acid analogues **1a** and **1b** resemble those of the flexible parent.

Several analogues of phenylalanine with various degrees of conformational mobility have been prepared.[20,25-29,32-58] In general, these amino acids have a greater degree of conformational mobility (less conformational restriction) than do **1a** or **1b** and have more extra atoms, thus interfering with interpretations of the biological data in terms of steric fit and hydrophobic interactions of α-methylphenylalanine with its active site on PH and PAD. In general, none of the analogues, including **1a** and **1b**, is as water soluble as phenylalanine itself, thus limiting the concentrations that can be tested. This problem, coupled with the apparent lack of any conformational preference for either **1a** or **1b** in the two enzymes studied, makes interpretation of the results tenuous. It is clear, however, that both **1a** and **1b** are inhibitors of PH and PAD, comparable to other known inhibitors as noted above. The lack of conformational preference suggests that either conformation can bind adequately to the inhibitor binding sites of the enzymes to block normal processing of the substrate, phenylalanine. It remains to be established that **1a** and **1b** serve as substrates for either enzyme, a situation in which conformational preferences might be more clearly shown.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared spectra were obtained on Beckman IR-8, IR-10, IR-33, and Perkin-Elmer 727 spectrophotometers. ^1H NMR spectra were determined at 60 MHz with Varian Models A-60A, T-60, and

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EM-360 spectrometers and at 100 MHz with a Varian Model HA-100 spectrometer. The chemical-shift values are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard. TFA refers to trifluoroacetic acid. High-resolution mass spectra were recorded on a Varian CH-5 spectrometer. Microanalyses were performed either on an F&M Model 185 or a Hewlett-Packard Model 185B CHN analyzer in this department. Elemental analyses on the amino acids were obtained after recrystallization from water and drying over P_2O_5 at 80 °C and 1 mm pressure for 48 h. Column chromatography was performed using Brinkmann silica gel (70–230 mesh). R_f values reported were determined using Brinkman precoated silica gel (F-254 0.25 × 50 × 100 mm) plates and 30% hexane–ethyl acetate as solvent. Skelly B refers to petroleum ether, boiling range 60–68 °C. L-[1-^{14}C]Phenylalanine was purchased from New England Nuclear. L-Phenylalanine and DL-α-Me-Dopa [DL-2-methyl-3-(3,4-dihydroxyphenyl)alanine] were purchased from Sigma. Tyrosine decarboxylase (Sigma) was a crude acetone powder from *S. feacalis*. $DMPH_4$ (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine) was purchased from Calbiochem. Buffers were prepared according to the procedure of Gomori.[30] Radioactivity was measured on a Beckman LS-3155P scintillation counter, using Packard II scintillation cocktail and Hyamine 10-X hydroxide (Packard) to absorb $^{14}CO_2$.

Hydantoin 4. Ketone **3**[4] (4.0 g, 23.0 mmol), potassium cyanide (3.8 g, 58.4 mmol), ammonium carbonate (11.2 g, 0.117 mol), and 50% aqueous methanol (75 mL) were magnetically stirred under reflux for 6 h. The reaction mixture was diluted with water (20 mL) and left overnight in the refrigerator. The product was filtered, washed with water, and dried to afford 5.2 g (92%) of white crystals. Recrystallization from 95% ethanol afforded 4.7 g (84%) of **4**: mp 218–222 °C; IR (KBr) 3500–2800 (br, multiple bands), 1780 (C=O), 1720 (C=O), 1410, 1260, 1225, 1190, 1041, 750 cm^{-1}; MS *m/e* (relative intensity) 242 (M^+, 6), 130 (100), 129 (40), 128 (15), 115 (14), 51 (3). Anal. ($C_{14}H_{14}N_2O_2$) C, H, N.

Separation of the Isomers 4a and 4b. Method A. Fractional crystallization from 95% ethanol resulted in one of the two isomers in pure form. The isomer **4b** with mp 324–326 °C (R_f 0.35) was found to crystallize out first. Three fractional crystallizations of the recovered mixture of isomers from the supernatant yielded isomer **4a**.

Method B. A mixture of hydantoin isomers (4.1 g) was dissolved in hot methanol (50 mL). Silica gel (6 g) was added to the solution and the methanol was removed in vacuo to obtain a uniformly coated silica gel. This was placed on a 400-g slurry packed silica gel column (2.5 × 80 cm) and slowly eluted with 30% hexane–ethyl acetate. The early fractions contained 1.6 g of pure isomer **4a** [mp 225–226 °C; TLC R_f 0.50; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 10.40 (br, 1 H, NH), 7.23 (s, 4 H, aromatic), 7.05 (br, 1 H, NH), 3.15 (m, 1 H, bridgehead), 2.98 (m, 1 H, bridgehead), 2.60–0.90 (m, 6 H, C-3, C-9, and C-10 CH_2)], the midfractions contained a mixture (1.3 g) of the two isomers and the late fractions contained 0.8 g of pure isomer **4b** [mp 324–326 °C; TLC R_f 0.35; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) δ 10.35 (br, 1 H, NH), 8.45 (br, 1 H, NH), 7.50 (s, 4 H, aromatic), 3.12 (m, 1 H, bridgehead), 2.92 (m, 1 H, bridgehead), 2.63–0.90 (m, 6 H, C-3, C-9, and C-10 CH_2)].

2-Amino-1,2,3,4-tetrahydro-1,4-ethanonaphthalene-2-carboxylic Acid (1). A mixture of isomers of hydantoin **4** (1.0 g, 4.0 mmol) and barium hydroxide (5.04 g, 16.0 mmol) in 50 mL of water was heated at reflux for 6 days. The reaction mixture was cooled, acidified with 6 N sulfuric acid, and filtered through Celite. The filtrate was made basic with concentrated ammonium hydroxide and concentrated in vacuo until the first signs of a precipitate appeared. The concentrate was chilled overnight, and the resulting precipitate was collected by filtration to yield the desired product as white crystals: mp 254–256 °C; MS *m/e* (relative intensity) 217 (M^+, 11), 144 (8), 130 (100), 129 (50), 128 (23), 115 (24), 89 (42). M_r calcd for $C_{13}H_{15}NO_2$: 217.11019. Found: 217.11052.

Hydrolysis of the pure hydantoin isomer **4a** afforded product **1a** as a white powder: mp 251–252 °C (H_2O); IR (KBr) 3690–3340 (sh), 3290, 3250–2290 (br), 2030, 1985, 1635–1595 (COO^-), 1565 (aromatic), 1495, 1475, 1455, 1355, 1335 (sh), 1285, 1225, 1145, 1125, 1080, 1030, 950, 895, 865, 770, 745 (sh), 735 (aromatic) cm^{-1}; ^{1}H NMR (TFA) δ 7.30 (s, 4 H, aromatic), 6.77 (br, 3 H, NH_3^+), 3.52 (m, 1 H, bridgehead), 3.32 (m, 1 H, bridgehead), 3.17–1.23 (m, 6 H, C-3, C-9, and C-10 CH_2). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

Hydrolysis of the pure hydantoin isomer **4b** afforded the product **1b** as a white powder: mp 286–287 °C (H_2O); IR (KBr) 3680–3360 (sh), 3220–2520 (br), 2220–2020 (br), 1640 (COO^-), 1595 (aromatic), 1535, 1465, 1380, 1275, 890, 750 (aromatic) cm^{-1}; ^{1}H NMR (TFA) δ 7.47 (br, 3 H, NH_3^+), 7.18 (s, 4 H, aromatic), 3.50 (m, 1 H, bridgehead), 3.27 (m, 1 H, bridgehead), 3.07–1.40 (m, 6 H, C-3, C-9, and C-10 CH_2). Anal. ($C_{13}H_{15}NO_2$) C, H, N.

Ethyl 2-Amino-1,2,3,4-tetrahydro-1,4-ethanonaphthalene-2-carboxylate Hydrochloride (5a and 5b). Amino acid **1a** (0.2 g, 0.900 mmol) was heated at reflux with absolute ethanol (20 mL), benzene (50 mL), and concentrated sulfuric acid (3 mL) for 12 h using a Dean–Stark trap to remove water. The solution was concentrated and made basic with 10% aqueous sodium carbonate, extracted with ether, dried (Na_2SO_4), and evaporated to afford 0.2 g of **5a** as a crude oil. The oil was dissolved in acetone (15 mL) and the hydrochloride prepared by passing dry HCl gas through the solution. The solvent was removed in vacuo, affording a white powder. The powder was dissolved in hot chloroform, filtered, and cooled to room temperature. The salt was precipitated from the solution by the addition of cyclohexane. After the solution was left standing overnight in the refrigerator, the solid was removed by filtration and dried for 72 h over P_2O_5 at 60 °C under high vacuum: mp 176–178 °C dec; IR (KBr) 3700–3180 (br), 3120–2400 (NH_3^+), 1745 (C=O), 1625 (NH_3^+), 1515 (NH_3^+), 1455, 1195, 1140, 1000, 750 (aromatic) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.43–5.47 (br, 3 H, NH_3^+), 7.12 (s, 4 H, aromatic), 4.19 (q, 2 H, CH_2CH_3), 3.40 (m, 1 H, bridgehead), 3.08 (m, 1 H, bridgehead), 2.93–0.63 (m, 6 H, C-3, C-9, and C-10 CH_2), 1.32 (t, 3 H, CH_2CH_3). Anal. ($C_{15}H_{20}NO_2Cl$) C, H, N.

5b was prepared from **1b** using the same procedure as above: mp 217–218 °C dec; IR (KBr) 3420 (br), 3150–2600 (NH_3^+), 1725 (C=O), 1575 (NH_3^+), 1505 (NH_3^+), 1455, 1365, 1305, 1230, 1200, 1125, 1060, 995, 735 (aromatic) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.17 (br m, 3 H, NH_3^+), 7.07 (s, 4 H, aromatic), 3.89 (q, 2 H, CH_2CH_3), 3.52 (m, 1 H, bridgehead), 3.13 (br, 1 H, bridgehead), 2.77–1.23 (m, 6 H, C-3, C-9, and C-10 CH_2), 0.95 (t, 3 H, CH_2CH_3). Anal. ($C_{15}H_{20}NO_2Cl$) C, H, N.

Hydantoin 7 was prepared in an analogous manner to that for **4** from ketone **6**. From 2.7 g (16 mmol) of **6** was obtained 2.7 g (71%) of **7**: mp 320 °C (95% ethanol); IR (KBr) 3400–3000 (br), 1775 (C=O), 1725 (C=O), 1420 (br), 1280, 1180, 1120, 1040, 760, 600 (sh) cm^{-1}. Mixture **7**, 1.3 g, was separated by method B to give 0.5 g of **7a**, 0.45 g of **7b**, and 0.25 g of unresolved mixture: ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) for **7a**, 7.22 (br, 1 H, NH), 7.05 (m, 4 H, aromatic), 6.90 (s, 1 H, NH), 6.43 (m, 2 H, olefinic), 3.97 (m, 1 H, bridgehead), 3.82 (m, 1 H, bridgehead), 2.28–1.30 (m, 2 H, C-3 CH_2); for **7b**, δ 7.87 (m, 2 H, NH), 7.37, (m, 4 H, aromatic), 6.62 (m, 2 H, olefinic), 3.98 (m, 2 H, bridgehead), 2.52–1.67 (m, 2 H, C-3 CH_2); MS *m/e* (relative intensity) 128 (100), 112 (36), 64 (10). Anal. ($C_{14}H_{12}N_2O_2$) C, H, N.

2-Amino-2-cyano-1,4-dihydro-1,4-ethanonaphthalene Hydrochloride (8). Ketone **6**[4] (4.0 g, 23.5 mmol) was stirred with ammonium chloride (1.30 g, 24.3 mmol) and potassium cyanide (1.6 g, 24.3 mmol) in 50% aqueous methanol (50 mL) at room temperature for 10 days. The solution was diluted with water (20 mL) and extracted with ether (3 × 50 mL). The ether was washed with water, dried ($MgSO_4$), concentrated to 50 mL, and saturated with dry HCl gas. The precipitate obtained was filtered and recrystallized (95% ethanol saturated with HCl) to afford 1.4 g (26%) of **8**: mp 193–194 °C dec; IR (KBr) 3200–2500 (NH_3^+), 2040 (C≡N), 1620 (C=C), 1560–1500 (NH_3^+), 1430 (C–N), 1380, 1200, 1100, 850, 780 (sh), 750 (aromatic), 700 (C=C) cm^{-1}; ^{1}H NMR (Me_2SO-d_6/$CDCl_3$) (mixture of isomers) δ 8.25 (m, 1.5 H, NH_3^+), 7.38 (m, 1.5 H, NH_3^+), 7.22 (m, 4 H, aromatic), 6.87 (m, 1 H, olefinic), 6.45 (m, ^{1}H, olefinic), 4.77 (m, 0.5 H, bridgehead), 4.68 (m, 0.5 H, bridgehead), 4.18 (m, 1 H, bridgehead), 2.15 (m, 2 H, C-3 CH_2); MS *m/e* (relative intensity) 129 (13), 128 (100), 115 (1), 102 (5), 77 (1.5), 51 (3), 36 (30). Anal. ($C_{13}H_{13}N_2Cl$) C, H, N.

2-Amino-2-cyano-1,2,3,4-tetrahydro-1,4-ethanonaphthalene Hydrochloride (9). **9** was prepared in an analogous manner to that for **8** from ketone **3**. From 4.0 g (23.2 mmol) of **3** was obtained 1.4 g (26%) of **9**: mp 172–173 °C (95% ethanol–ether); IR (KBr)

3460 (br), 3200–2500 (NH_3^+), 2050 (C≡N), 1580 (aromatic), 1530–1460 (NH_3^+), 1335, 1150, 1110, 1065, 1040 (sh), 750 (aromatic) cm^{-1}; ^1H NMR (Me_2SO-d_6/$CHCl_3$) (mixture of isomers) δ 10.33–8.67 (br, 1.5 H, NH_3^+), 7.33 (s, 4 H, aromatic), 7.25 (m, 1.5 H, NH_3^+), 3.88 (m, 0.5 H, bridgehead), 3.75 (m, 0.5 H, bridgehead), 3.23 (m, 1 H, bridgehead), 2.48–0.75 (m, 6 H, C-3, C-9, and C-10 CH_2). Anal. ($C_{13}H_{15}N_2Cl \cdot 1H_2O$) C, H, N.

Phenylalanine Decarboxylase Assay. L-Phenylalanine decarboxylase (PAD, EC 4.1.1.53) activity was determined[22] by measuring the $^{14}CO_2$ produced from L-[1-^{14}C]phenylalanine in the presence of tyrosine decarboxylase.[31] The reactions were performed in 10-mL Kontes reaction flasks with a side arm and a plastic center well containing Hyamine 10-X hydroxide to absorb the $^{14}CO_2$. The standard reaction mixture (total volume 0.5 mL) consisted of 5×10^{-4} M pyridoxal 5-phosphate in 0.2 M Na_2HPO_4 buffer (pH 5.5), 1×10^{-2} M L-[1-^{14}C]phenylalanine (10^{-2} μCi/μmol) in 0.2 M Na_2HPO_4 buffer (pH 5.5), and 0.5 mg of tyrosine decarboxylase (crude powder also contains L-phenylalanine decarboxylase activity) in 75 mM citrate–0.15 M phosphate buffer (pH 5.5). Inhibitor concentrations were 1.76 mM in citrate–phosphate buffer (pH 5.5), unless otherwise noted. Reactions were incubated for 20 min in a shaking water bath at 37 °C, and the reactions were stopped by injection of 0.1 mL of 50% trichloroacetic acid. $^{14}CO_2$ was absorbed for 30 min, whereupon the plastic center wells were transferred to scintillation vials. The radioactivity was counted and compared with controls containing no inhibitor.

Phenylalanine Hydroxylase Assay. Phenylalanine hydroxylase (PH, EC 1.14.3.1) activity was determined by measuring the phenylalanine-dependent change in absorbance of the tetrahydropteridine cofactor, 2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine ($DMPH_4$), as it is oxidized to the dihydro form.[23] The reaction mixture (total volume 1.0 mL) consisted of 0.17 mM $DMPH_4$, 1 mM substrate (L-phenylalanine or test compound) plus or minus 1 mM inhibitor, phenylalanine hydroxylase [2 mg of protein, 45% $(NH_4)_2SO_4$ fraction from guinea pig liver] in 0.1 M Tris-HCl, pH 7.4, at 30 °C. L-Phenylalanine, phenylalanine hydroxylase, and the compound to be tested were preincubated together for 2.5 min at 30 °C before initiation of the reaction by the addition of the cofactor. The change of absorbance at 330 nm was monitored, and the inhibition was measured as the decrease in absorbance in the presence of the compound relative to the control incubation.

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Bradykinin Analogues Containing N^{α}-Methyl Amino Acids

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Analogues of bradykinin were synthesized containing single substitutions by N^{α}-methyl amino acids in the 1, 4, 5, 8, and 9 positions. [MeArg^1]Bradykinin possessed 60% of the muscle-contracting activity of the parent compound in a guinea pig ileum assay. The other analogues were very weak agonists (<2%) and, disappointingly, failed to show blocking activity except at very high doses.

Bradykinin (1) was one of the first peptides to be investigated in what might be called the modern era of peptide synthesis, i.e., since du Vigneaud's preparation of oxytocin in 1953.[1] In fact, the structure of the isolated substance was reported incorrectly,[2] and the correct structure was first obtained by synthesis based on what must have been an inspired hunch by Boissonnas.[3-5]

H-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH
1

The pharmacological effects of bradykinin are numerous, while its physiological role is still poorly understood. A major stumbling block to elucidation of the latter has been the lack, despite the preparation of a hundred or two analogues, of an effective in vivo inhibitor. Thus, finding a potent bradykinin blocker is still, after nearly 20 years, a challenging problem in medicinal chemistry. The biological activities of bradykinin and the need for an inhibitor have been reviewed in an excellent fashion by Marshall[6] and by Regoli.[7]

Our approach to the search for a bradykinin blocker was to prepare a series of analogues in which each of the six amino acids bearing an α-NH_2 group was successively replaced by an N-methyl amino acid. Although the N-methyl group certainly profoundly influences the conformation of the peptide backbone, its effect on receptor binding really cannot be predicted, since the latter could depend largely on the various side groups attached to the main chain. In a small linear peptide such as bradykinin, there is great uncertainty about the effects of N-methylation because the parent compound has a random conformation in solution.[8] N-Methylation of both angiotensin[9] and enkephalin[10] analogues has given compounds with improved pharmacological properties, such as enhanced potency and duration of action. It was assumed this was due to resistance to proteolysis[11] and, in fact, [Sar^1]angiotensin II has been shown to be completely stable in the presence of angiotensinase.[12] Also, in an

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