mmol) was added. This mixture was cooled to 0 °C and pyridine (0.81 mL, 10 mmol) was added dropwise. After stirring for 10 min at the same temperature, the mixture was concentrated and diluted with AcOEt. The solution was washed successively with dilute aqueous $NaHCO_3$, 1 N HCl, and brine, dried over $MgSO_4$, and evaporated to give an oil, which was chromatographed on a silica gel column, eluting with CH_2Cl_2–AcOEt (gradient elution), to give 1.69 g (87%) of **19** as an amorphous solid (see Table I).

Acknowledgment. The authors are grateful to Y. Miyazaki for technical assistance, Dr. T. Takaya who provided us some samples of α-(alkoxyimino)acetic acid, and T. Kamimura for biological testing.

Novel Analogues of Enkephalin: Identification of Functional Groups Required for Biological Activity

Fredric A. Gorin, T. M. Balasubramanian, Theodore J. Cicero, John Schwietzer, and Garland R. Marshall*

Departments of Physiology and Biophysics, of Psychiatry, and of Neurobiology, Washington University, St. Louis, Missouri 63110. *Received April 14, 1980*

Novel tri- and tetrapeptide analogues of enkephalin, in conjunction with earlier structure–activity data, confirm that chemical substituents present in the first and fourth residues of enkephalin are required for in vitro biological activity. A class of arylamino and alkylamino derivatized tripeptides also were found to have signficiant in vitro opioid-like activity indistinguishable from [D-Ala^2,D-Leu^5]enkephalin and morphine.

The enkephalins are the smallest members of a family of opioid-like peptides endogenous to the mammalian central nervous system. The in vivo pharmacology of the enkephalins and opiates is complex, and there is growing evidence that these compounds act in vivo at several types of receptors.[1–3] In in vitro systems such as the guinea pig ileum,[4] mouse vas deferens,[5] neuroblastoma glioma NG108-15 cell line,[6] and rat brain binding assays,[7] opiates and the enkephalins produce analogous pharmacologic effects which are reversibly blocked by the opiate antagonist naloxone. These in vitro observations have motivated investigators to compare chemical and conformational similarities between the enkephalins and opiate compounds. Numerous conformational studies have proposed receptor-bound conformations for enkephalins whereby these mammalian pentapeptides and plant opiate alkaloids pharmacologically compete for the same in vitro receptors.[8]

This article describes novel, conformationally constrained analogues of enkephalin which are evaluated pharmacologically in the guinea pig ileum and which displace [^{3}H]naloxone in the rat brain binding assay. There is no assurance that the pharmacologic receptors in these two in vitro assays are equivalent, and recent investigation has suggested the existence of separate high affinity "enkephalin receptors" and "morphine (μ) receptors" present in the rat brain binding assay.[2,3,9] It appears that naloxone binds to the morphine (μ) binding site with 30-fold greater affinity than the enkephalin binding site.[3] The analogues of enkephalins cited in this article have been evaluated in both bioassay systems, and excellent correlation has been found between the ranked potencies of compounds relative to [D-Ala^2,D-Leu^5]enkephalin tested in the two in vitro systems. This article uses the information derived from the structure–activity data of these analogues of enkephalin to deduce requirments about the morphine (μ) receptor.

Experimental Section

Materials. All Boc[10] amino acids, except Boc-Tyr-OH, Boc-Tyr(αMe)-OH, and Boc-Phe(αMe)-OH, were purchased from Bachem. L-Tyr was obtained from Fluka. The preparation of Boc-Phe(αMe)-OH is described elsewhere.[11a] The Boc-Leu-resin was prepared by the method of Gisin[11b] using Lab Systems 1% cross-linked polymer (0.90 mequiv/g). Di-*tert*-butyl dicarbonate was obtained from Fluka. 1-Aminoindan, 2-aminoindan, benzylamine, 1-(aminomethyl)cyclopropane, and 1-(aminomethyl)-cyclobutane were purchased from Aldrich. β-Phenylethylamine was purchased from Sigma. [^{3}H]Naloxone (26 Ci/mmol) was obtained from New England Nuclear, and bacitracin was purchased from P. L. Biochemicals. [Aib^2,Met-NH$_2^5$]Enkephalin was obtained from Peninsula Laboratories.

Analytical Methods. Melting points are uncorrected. DMF was purified according to the procedure described by Stewart and Young.[12] Ascending TLC was performed on 0.25-mm silica gel G plates (Analtech) using the following solvent systems: (I) chloroform–acetone, 15:1; (II) chloroform–acetone, 7:1; (III) chloroform–acetone, 7:2; (IV) chloroform–acetone, 1:1; (V) chlo-

(1) J. N. Jaffe and W. R. Martin, in "The Pharmacological Basis of Therapeutics", L. S. Goodman and A. Gilman, Eds., MacMillan, New York, 1975, p 245.
(2) J. A. Lord, A. A. Waterfield, J. Hughes, and H. W. Kosterlitz, *Nature (London)*, **267**, 495 (1977), and references cited within.
(3) K.-J. Chang and P. Cuatrecasas, *J. Biol. Chem.*, **254**, 2610 (1979).
(4) H. W. Kosterlitz and A. J. Watt, *Br. J. Pharmacol.*, **33**, 266 (1968).
(5) G. Henderson, J. Hughes, and H. W. Kosterlitz, *Br. J. Pharmacol.*, **46**, 764 (1972).
(6) A. L. Lampert, M. Nirenberg, and W. A. Klee, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 3165 (1976).
(7) C. B. Pert and S. H. Snyder, *Mol. Pharmacol.*, 10, 868 (1974).
(8) F. A. Gorin, T. M. Balasubramanian, C. D. Barry, and G. R. Marshall, *J. Supramol. Struct.*, **9**, 27 (1978), and references cited within.
(9) P. E. Gilbert and W. R. Martin, *J. Pharmacol. Exp. Ther.*, **198**, 66 (1976).
(10) Abbreviations used are: Boc, *tert*-butyloxycarbonyl; TLC, thin-layer chromatography; LC, liquid chromatography; DCC, dicyclohexylcarbodiimide; Phe(αMe), α-methylphenylalanine (yl-); DMF, dimethylformamide; TFA, trifluoroacetic acid; Bzl, benzyl; TEA, triethylamine; DPPA, diphenylphosphoryl azide; HOBT, 1-hydroxybenzotriazole; DEPC, diethylphosphoryl cyanidate; Cha, L-cyclohexylalanine; Aib, aminoisobutyric acid.
(11) (a) J. Turk, P. Needleman, and G. R. Marshall, *Mol. Pharmacol.*, 12, 217 (1976); (b) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
(12) J. M. Stewart and J. D. Young, "Solid Phase Peptide Syntheses", W. H. Freeman, San Francisco, 1969.

0022-2623/80/1823-1113$01.00/0 © 1980 American Chemical Society

roform–methanol, 7:1; (VI) chloroform–methanol, 3:1; (VII) chloroform–methanol, 1:1; (VIII) chloroform–methanol, 1:4; (IX) 1-butanol–acetic acid–water, 4:1:1; (X) 1-butanol–ethyl acetate–acetic acid–water, 2:2:1:1. The loads of compounds applied on the TLC plates were 25 μg (in 5 μL of methanol), and the distances of the solvent fronts were usually 14 cm. Plates were developed by spraying with buffered ninhydrin[12] and Chlorox-starch[13] reagents. Purified peptides were analytically chromatographed using high-pressure liquid chromatography (LC) as described in the next section. Using isocratic conditions listed in Table I, the optical isomers of the 1-aminoindan analogues of enkephalin were resolved. All analogues of enkephalin demonstrated impurities of less than 0.1% by analytical high-pressure LC with UV monitoring at 210 ± 8 and 280 ± 8 nM. Optical rotations were measured using a Perkin-Elmer Model 241 polarimeter.

Peptide hydrolyses of protected amino acids were performed in vacuo using concentrated hydrochloric acid–propionic acid, 1:1, at 130 °C for 2 h.[14] Unprotected peptides were hydrolyzed in vacuo using constant-boiling hydrochloric acid for 18 h at 110 °C.[15] Phe(αMe) and Tyr(αMe) content was measured by enzymatic treatment of a portion of the hydrolysate with L-amino acid oxidase as described elsewhere.[11a]

Purification by High-Pressure LC. Deprotected peptides synthesized by solution phase techniques were purified by high-pressure LC on a semipreparative scale (ca. 10 mg) using two Waters 6000 A pumps, an M660 solvent programer, a Rheodyne injector with a 100-μL injection loop, and a Varichrom UV variable detector. Analytical and preparative separations were performed using two 30 cm × 4 mm reverse-phase C18 μBondapak columns (Waters) connected in series. All organic solvents were of UV spectroscopic grade (Burdick and Jackson) and were filtered and degassed prior to use. The eluted peaks were monitored at 210 ± 8 nm.

The deprotected peptide (20 to 30 mg) was dissolved in 2 to 3 mL of UV spectroscopic grade methanol, filtered with a Millipore-Swinney apparatus, and evaporated to a residue with nitrogen. Analytical determination of purification conditions for each peptide was obtained by running a linear, 30-min gradient from 5% acetonitrile–95% 0.01 M ammonium formate, pH 4.00, to 70% acetonitrile–30% 0.01 M ammonium formate as a flow rate of 2.3 mL/min. The desired peptides eluted in the range of 20 to 40% acetonitrile. A semipreparative run was then performed by injecting 100 μL of an 80 to 100 mg/mL solution of peptide in aqueous buffer with a minimal amount of acetonitrile when necessary to clarify the solution. A series of linear, stepwise gradients was programmed with a 20% increase in acetonitrile over 5 min. It was observed that, because of slight sample overloading of the stationary phase, a peak would elute on the semipreparative run at the same retention time as the analytical trial with a solvent composition containing 5 to 8% less acetonitrile. Fractions of 0.8 to 1.0 mL were collected from the desired peaks and evaporated at 25 °C in a Buchler Vortex evaporator. Each fraction was checked on thin-layer chromatography using solvent systems IX and X, and the pertinent fractions were pooled and lyophilized from an excess of glass-distilled water. Using this approach, 8 to 10 mg of peptide could be purified in a single 30- to 40-min high-pressure LC run with 40–50% recovery of purified peptide. The residual ammonium formate was sublimed by continuing to evaporate the fractions under vacuum at 35 °C for 3 to 6 h. Quantitative amino acid analysis demonstrated the purified peptides to have 0 to 60% residual ammonium formate. Table I summarizes the physiochemical properties of these peptides purified by high-pressure LC.

Bioassay. The compounds were assayed on electrically stimulated, intact, guinea pig ileum using the conditions of Creese and Snyder.[16] Because the native enkephalins have been demonstrated to be proteolytically labile in this system,[17,18] all ana-

Table I. Physical Properties of Enkephalin Analogues

peptide	amino acid ratios					mp, °C[c]	R_f values[b]		HPLC, min from injection[a]	% peptide
	1	2	3	4	5		BEAW, 2:2:1:1	BAW, 4:1:1		
Tyr-D-Ala-Gly-Phe-D-Leu	1.00	0.98	1.00	1.06	0.91	126 (dec)	0.72	0.63	(30%) 5.06	88
Tyr-D-Ala-Gly-Phe(αMe)-Leu	0.92	1.02	1.00	0.98	0.97	127 s, 137 (dec)	0.75	0.65	(30%) 4.42	92
Tyr-D-Ala-Gly-Phe(αMe)	0.92	0.96	1.00	1.00		132 s, 140 (dec)	0.71	0.60	(30%) 2.49	100
Glu(OEt)-D-Ala-Gly-Phe-D-Leu	0.99	1.00	1.00	1.08	0.91	122 s, 126 (dec)	0.71	0.58	(30%) 6.24	100
Tyr(αMe)-D-Ala-Gly-Phe-D-Leu	0.98	1.02	1.00	0.91	1.01	126 s, 143 (dec)	0.76	0.64	(30%) 5.06	78
Tyr-D-Ala-Gly-Phe(αMe)-Val	0.90	1.04	1.00	1.00	0.97	152 (dec)	0.73	0.65	(30%) 3.18	100
Tyr-Gly-Gly-1-aminoindan	1.00		2.00			139 (dec)	0.69	0.56	(20%) 9.26	50
Tyr-Aib-Gly-1-aminoindan	0.95	1.08	1.00			147 (dec)	0.67	0.57	(20%) 11.1, 13.36	100
Tyr-Gly-Aib-1-aminoindan	0.92	1.00	0.93			144 (dec)	0.67	0.57	(20%) 11.04	100
Tyr-D-Ala-Gly-1-aminoindan	0.97	1.03	1.00			120 (dec)	0.72	0.60	(20%) 9.58, 10.33	49
Tyr-D-Ala-Gly-2-aminoindan	1.14	1.04	1.00			141 (dec)	0.72	0.60	(20%) 9.60	30
Tyr-D-Ala-Gly-benzylamine	1.00	0.95	1.00			132.5 (dec)	0.70	0.57	(20%) 5.18	37
Tyr-D-Ala-Gly-phenethylamine	0.93	1.05	1.00			131 (dec)	0.69	0.59	(20%) 7.40	25
Tyr-D-Ala-Gly-(aminomethyl)cyclobutane	1.04	1.03	1.00			133 (dec)	0.67	0.52	(20%) 3.13	23
Tyr-D-Ala-Gly-(aminomethyl)cyclopropane	1.13	1.06	1.00			125.5 (dec)	0.66	0.52	(20%) 3.06	44
Tyr-D-Ala-Gly-OH	1.10	1.02	1.00			114 (dec)	0.62	0.52	(20%) 1.96	72
Tyr-D-Ala-Gly-OBzl	1.00	1.03	1.00			92	0.71	0.68	(20%) 8.31	40
Tyr-D-Ala-Gly-Cha-D-Leu	0.94	1.00	1.00	0.96	0.94	191 (dec)	0.72	0.65	(40%) 10.40	100

[a] High-pressure liquid chromatography: (X%) $CH_3CN/(100 - X)\%$ 0.01 NH_4O_2CH; 1.5 mL/min, 30 cm × 4 mm, C18 μ-Bondapak. [b] BEAW = 1-butanol–ethyl acetate–water; BAW = 1-butanol–acetic acid–water. [c] s, soften.

(13) D. E. Nitecki and J. W. Goodman, *Biochemistry*, **5**, 665 (1966).
(14) J. Scotchler, R. Lozier, and A. B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970).
(15) S. Moore, in "Chemistry and Biology of Peptides", Johannes Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 629.
(16) T. Creese and S. H. Snyder, *J. Pharmacol. Exp. Ther.*, **194**, 205 (1975).

logues of enkephalin were evaluated in the presence and absence of 100 μg/mL bacitracin. This concentration of bacitracin was demonstrated not to affect the dose–response curve of morphine or [D-Ala[2],D-Leu[5]]enkephalin. The ID_{50} used to calculate the potency of an analogue with respect to the reference analogue [D-Ala[2],D-Leu[5]]enkephalin is the mean of these separate determinations. Before and after every complete set of dose–response determinations, the IC_{50} dosage of the reference enkephalin analogue was administered to assure that tissue responsiveness was altered by less than 15%. The naloxone binding assays were carried out essentially as described previously.[19] Briefly, rats were killed by decapitation, and the brains (minus the cerebellum) were obtained. A crude particulate fraction was prepared by homogenizing the brains in 100 volumes of 0.05 M Tris-HCl buffer (pH 7.6). The homogenate was centrifuged at 40000*g* for 10 min, the supernatent fluids were discarded, and the pellet was washed twice in 40 mL of the Tris-HCl buffer and homogenized with seven strokes in a loose-fitting glass homogenizer. This preparation was used in all subsequent incubations. Incubations consisted of the following (all concentrations are final): 100 μL of the washed particulate fraction [^{3}H]naloxone (26 Ci/mmol, 5×10^{-9} M), 1×10^{-7} levorphanol, 5×10^{-5} M bacitracin, and enough buffer to make a total volume of 500 μL. Assays were carried out in the presence and absence of 100 mM NaCl in duplicates or triplicates. The incubations were carried out in an ice–water bath for 2.5 h, with constant agitation, and were terminated by centrifugation (40000*g* for 10 min). The pellet was washed three times with 3 mL of Tris-HCl and the final pellet was dissolved in 200 μL of NCS tissue solubilizer. The dissolved pellets were then transferred to 20-mL plastic scintillation vials, and the contents of the tubes were quantitatively transferred with 3× 1-mL washes with Scintiverse liquid scintillation cocktail. Seven milliliters of Scintiverse was placed in each vial, and 2 drops of glacial acetate acid were added to prevent quenching. The vials were then counted in a liquid scintillation counter. Specific [^{3}H]naloxone binding was determined by subtracting the counts per minute bound in those incubations containing [^{3}H]naloxone and 1×10^{-7} M levorphanol from those containing [^{3}H]naloxone alone. In all of the experiments reported here, approximately 65–70% of the [^{3}H]naloxone bound was specific.

Boc-Tyr-D-Ala-Gly-Phe(αMe)-Leu-resin (1). Boc-Leu-resin (1.84 g, 0.818 mequiv/g) was deprotected with 50% trifluoroacetic acid in toluene for 30 min. This was proceeded by three swelling steps in toluene, three shrinking steps in 95% *tert*-butyl alcohol–5% toluene, and three swelling steps in toluene. A sixfold excess of Boc-Phe(αMe) in toluene was coupled with an equivalent amount of DCC for two successive 4-h periods, with appropriate shrinkage–swelling cycles preceding and following each coupling.[10] A double deprotection cycle of two 30-min periods with appropriate washes preceded the coupling step which used a sixfold excess of Boc-Gly. The remaining synthetic steps were carried out by the customary cycle of operations.[20] In some cases [Boc-Gly and Boc-Tyr(Bzl)], solubility problems required the addition of a small amount of distilled, dry DMF. Fifty percent TFA in toluene was made just prior to each deprotection step.

Tyr-D-Ala-Gly-Phe(αMe)-Leu (2a) and Tyr-D-Ala-Gly-Phe(αMe) (2b). Compound 1 was suspended in 40 mL of anhydrous HBr/TFA in the presence of a 50-fold excess of anisole. The resin was washed three times with 15-mL aliquots of TFA and then concentrated to a residue under vacuum. The peptide was dissolved in 50 mL of 10% HOAc, extracted four times with ethyl ether, and lyophilized. The yield was 350 mg (36%). The peptide was purified by 198 transfers in a countercurrent distribution using solvent system X. Tubes 120–152 (K = 0.69) contained 77.6 mg of the peptide, shown by amino analysis to be **2a** [Tyr, 0.92; D-Ala, 1.02; Gly, 1.00; Phe(αMe), 0.98; Leu, 0.97], while tubes 80–100 (K = 0.45) contained 39.3 mg of **2b** [Tyr, 0.92; D-Ala, 0.96; Gly, 1.00; Phe(αMe), 1.00; Leu, <0.03]. The two peptides were shown to be homogeneous, Pauly positive, ninhydrin positive, and Chlorox–starch positive in the following systems: **2a**, R_f 0.53 (IV), 0.55 (III), 0.73 (II), 0.36 (I); **2b**, R_f 0.57 (IV), 0.45 (III), 0.71 (II), 0.34 (I); $[\alpha]^{25}_D$ +6.9° (**2a**; *c* 1.0, H_2O); $[\alpha]^{25}_D$ +62.0° (**2b**, *c* 1.0, H_2O). The two peptides were also shown to have less than 0.5% impurities by analytical high-pressure LC (Table I) using isocratic conditions.

Solution Phase Synth[illegible]. Boc-D-Ala-Gly-OBzl (3). To a stirred solution of 1.93 g (9.7 mmol) of Gly-OBzl·HCl in 15 mL of DMF was added 3.0 mL (22 mmol) of TEA at 0 °C. To this solution, 1.9 g (10 mmol) of Boc-D-Ala and 3.3 g (12 mmol) DPPA were added successively.[21] The reaction mixture was stirred for 4 h at 0 °C and 48 h at 25 °C. After the coupling reaction in DMF was completed, the reaction mixture was filtered, concentrated, diluted with ethyl acetate (3[illegible]L), [illegible] washed successively with 50 mL of 1 N sodium bisulfate (three times), 50 mL of water (three times), 50 mL of 1 N sodium bicarbonate (three times), and 50 mL of water (three times). The process is designated as "worked up in a standard fashion". Drying the extract followed by evaporation gave an oil, which was crystallized from cyclohexane to give compound **3** (2.38 g, 88%); mp 85–86 °C; $[\alpha]^{25}_D$ +28.4° (*c* 1.0, MeOH); R_f 0.50 (III), 0.57 (VIII). Amino acid analysis: Gly, 1.00; D-Ala, 1.01. Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.76; H, 7.20; N, 8.33. Found: C, 60.99; H, 7.44; N, 8.17.

D-Ala-Gly-OBzl·HCl (4). Compound **3** (3.36 g, 10 mmol) was deprotected in 25 mL of 4 N HCl in dioxane and stirred for 3 h at 25 °C. The reaction mixture was taken to dryness in vacuo. The oily residue obtained was dried over potassium pentoxide at 0.5 mmHg for several hours. The oily peptide obtained (2.7 g, quantitative) was used in situ for the next step: R_f 0.44 (IX), 0.45 (VIII).

Boc-Tyr(Bzl)-D-Ala-Gly-OBzl (5). Compound **4** (2.7 g, 10 mmol) was dissolved in 20 mL of DMF and 1.5 mL (11 mmol) of TEA was stirred into this solution at 0 °C. To this solution, 3.71 g (10 mmol) of Boc-Tyr(Bzl)-OH, 1.35 g (10 mmol) of HOBT, and 2.06 g (10 mmol) of DCC were added successively. After a reaction period of 4 h at 0 °C and 120 h at 25 °C, the mixture was worked up in a standard fashion. Drying the organic extracts followed by evaporation gave a crude, white peptide, which was purified by passage down a 2 × 100 cm silica gel column in solvent system I. The ninhydrin-positive fractions yielded compound **5** (2.7 g, 46%) as a white powder: mp 143–145 °C; $[\alpha]^{25}_D$ +32.7° (*c* 1.0, MeOH); R_f 0.72 (V), 0.85 (VI). Amino acid analysis: Gly, 1.02; Ala, 1.00; Tyr, 0.95. Anal. Calcd for $C_{33}H_{39}N_3O_7$: C, 67.23; H, 6.62; N, 7.13. Found: C, 67.40; H, 6.85; N, 6.99.

Boc-Tyr-D-Ala-Gly-OH (6). Hydrogenation of 2.7 g (4.5 mmol) of compound **5** in a 100 mL solution of methanol–acetic acid–water (10:1:1) was carried out overnight with 2 g of 10% palladium on charcoal in a Parr low-pressure hydrogenation apparatus at 40 psi. The solution was filtered, and the filtrate was evaporated to dryness in vacuo. The oily peptide was purified by passage down a 2 × 100 cm silica gel column using solvent system III. The peptide was obtained as a white crystalline product from methanol–water (1.7 g, 4.15 mmol): mp 168–170 °C (dec); $[\alpha]^{25}_D$ +30.45° (*c* 1.0, MeOH); R_f 0.52 (VII), 0.57 (VIII). Amino acid analysis: Gly, 1.02; Ala, 1.00; Tyr, 0.95. Anal. Calcd for $C_{19}H_{27}N_3O_7{\cdot}H_2O$: C, 53.39; H, 6.79; H, 9.84. Found: C, 53.50; H, 7.09; N, 9.56.

Syntheses of Tetrapeptides. Boc-Tyr-D-Ala-Gly-X. A solution of the amino component (fourth residue) was prepared by dissolving 2 mmol of the appropriate compound in about 10 mL of DMF. To this solution were added compound **4** (0.5 mmol), DCC (2 mmol), and HOBT (2 mmol) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C and 48 h at 25 °C and worked up in a standard fashion. The residue obtained was purified by passage down a 2 × 25 cm silica gel column using solvent system III, to remove all the ninhydrin-negative impurities, and then with solvent system IV, to elute the peptide. The peptides were obtained as powdery products after the evaporation of appropriate fractions and crystallization (Table II).

Boc-Phe(αMe)-Val-OBzl (7). To a stirred, ice-cold solution of 486 mg (2 mmol) of H-Val-OBzl·HCl in DMF (5 mL) was added

(17) C. R. Beddell, R. B. Clark, L. A. Lowe, S. Wilkinson, and K.-J. Chang, *Br. J. Pharmacol.*, **61**, 351 (1977).
(18) R. J. Miller, K.-J. Chang, and P. Cuatrecasas, *Biochem. Biophys. Res. Commun.*, **74**, 1311 (1977).
(19) T. J. Cicero, C. E. Wilcox, R. D. Bell, and E. R. Meyer, *J. Pharmacol. Exp. Ther.*, **212**, 573 (1980).
(20) G. R. Marshall and R. B. Merrifield, *Biochemistry*, **4**, 2394 (1965).
(21) T. Shiori and S. Yamada, *Chem. Pharm. Bull.*, **22**, 855 (1965).

Table II. Physicochemical Properties of Tetrapeptide Analogues

Boc-Tyr(OH)-D-Ala-Gly-X

X	% yield	R_f (solv system)[a]	amino acid ratios	mp, °C	$[\alpha]^{25}_D$, deg (c 1.0, MeOH)	formula	anal.	C	H	N
1-aminoindan	70	0.54 (V), 0.71 (VI)	Tyr, 0.98 Ala, 1.01 Gly, 1.00	123–124 (dec)	+18.9	$C_{28}H_{36}N_4O_6$	calcd: found:	64.12 64.24	6.87 7.12	10.67 10.75
2-aminoindan	70	0.52 (V), 0.72 (VI)	Tyr, 0.96 Ala, 0.99 Gly, 1.00	108–110 (dec)	+29.8	$C_{28}H_{36}N_4O_6$	calcd: found:	64.12 64.37	6.87 7.08	10.67 10.75
2-phenethylamine	50	0.52 (V), 0.70 (VI)	Tyr, 0.94 Ala, 1.00 Gly, 1.00	100–105 (dec)	+27.6	$C_{27}H_{36}N_4O_6$	calcd: found:	63.28 62.97	7.03 7.38	10.94 10.60
benzylamine	54	0.67 (V), 0.71 (VIII)	Tyr, 0.97 Ala, 0.99 Gly, 1.00	105–108 (dec)	+25.1	$C_{26}H_{34}N_4O_6$	calcd: found:	62.65 62.42	6.83 7.04	11.24 11.13
1-(aminomethyl)cyclopropane	49	0.35 (V), 0.65 (VI)	Tyr, 0.95 Ala, 0.97 Gly, 1.00	100–103 (dec)	+27.3	$C_{23}H_{34}N_4O_6 \cdot CH_3OH$	calcd: found:	58.30 58.30	7.69 7.69	11.34 11.39
1-(aminomethyl)cyclobutane	71	0.36 (V), 0.66 (VI)	Tyr, 1.02 Ala, 0.99 Gly, 1.00	93–95 (dec)	+32.1	$C_{24}H_{36}N_4O_6 \cdot 0.5CH_3COOC_2H_5$	calcd: found:	60.00 60.09	7.69 7.92	10.77 10.74

[a] Solvent systems: V, chloroform–methanol, 7:1; VI, chloroform–methanol, 3:1.

0.56 mL (4 mmol) of TEA, followed by the addition of 600 mg (2 mmol) of Boc-Phe(αMe)-OH and 325 mg (2 mmol) of DEPC.[22] The reaction proceeded for 4 h at 0 °C and 48 h at 25 °C and was worked up in a standard fashion. The crude protein was obtained after drying and evaporating the ethyl acetate extract. Compound 7 was crystallized from ethyl acetate and petroleum ether (700 mg, 75%): mp 141–142 °C; $[\alpha]^{25}_D$ −62.1° (c 1.0, MeOH); R_f 0.68 (I), 0.67 (III). Amino acid analysis: Phe(αMe), 0.97; Val, 1.00. Anal. Calcd for $C_{27}H_{36}N_2O_5$: C, 69.23; H, 7.69; N, 5.98. Found: C, 69.03; H, 7.81; N, 5.80.

Boc-Tyr-D-Ala-Gly-Phe(αMe)-Val-OH (8). Compound 7 (235 mg, 0.5 mmol) was deprotected with 20 mL of 4 N HCl in dioxane in the same fashion as compound 4, and an oily residue was obtained after the evaporation of dioxane with drying over potassium pentoxide at 0.5 mmHg for several hours.

To a stirred solution of 200 mg (0.5 mmol) of the hydrochloride of compound 7 in DMF (10 mL) was added 0.1 mL (0.7 mmol) of TEA, followed by the addition of 205 mg (0.5 mmol) of compound 6, 270 mg (2 mmol) of HOBT, and 412 mg (2 mmol) of DCC. The reaction proceeded for 4 h at 0 °C and for 100 h at 25 °C, after which it was worked up in a standard fashion. The crude peptide was obtained as an oil after drying and evaporating the solvent and was purified by passage down a 2 × 25 cm silica gel column using solvent system III. The desired peptide was eluted from the column with solvent system IV. The oily peptide was then hydrogenated overnight in methanol (50 mL) with 400 mg of 10% Pd/C at 40 psi. A white, powdery peptide was obtained after filtering the catalyst and evaporating the solvent from the hydrogenated mixture and later crystallized from methanol (110 mg, 30%): mp 130 °C (softens) and 165 °C (dec); $[\alpha]^{25}_D$ −16.7° (c 1.0, MeOH); R_f 0.73 (X), 0.65 (IX). Amino acid analysis: Tyr, 1.00; Gly, 1.07; Ala, 1.07; Phe(Me), 0.95; Val, 0.97. Anal. Calcd for $C_{34}H_{47}N_5O_8 \cdot 2CH_3OH$: C, 56.61; H, 7.20; N, 9.17. Found: C, 56.44; H, 7.19; N, 9.13.

Boc-Glu(OEt)-OH (9). Boc-Glu-OBzl (4.0 g, 11.9 mmol) in 15 mL of 85% EtOH–H_2O was neutralized to pH 7.0 with $CsHCO_3$. After repeated evaporation to dryness with benzene, the product was dried overnight with P_2O_5. The white solid was dissolved in anhydrous DMF (8 mL) and 3.84 g (24.6 mmol) of ethyl iodide was added dropwise. The reaction mixture was stirred at 50 °C for 10 h, the CsI was filtered, and the solvent was evaporated. The residue was dissolved in 150 mL of ethyl acetate and extracted with 1 M Na_2CO_3 four times, with 1 M $NaHSO_4$ four times, and with water once. After drying over Na_2SO_4 and evaporating the ethyl acetate, 3.77 g (87%) of a clear oil was obtained: ninhydrin positive, bromocresol negative, R_f 0.73 (chloroform–methanol, 4:1). The oil was hydrogenated overnight in a MeOH–H_2O (9:1) solution with 3.00 g of 10% Pd/C in a Parr low-pressure apparatus at 38 psi. The material was purified on a 2 × 100 mm silica column using chloroform–methanol, 15:1, and followed by chloroform–methanol, 7:1. A clear oil (2.57 g, 59%) was obtained which was bromocresol positive and ninhydrin positive with heat: R_f 0.51 (VI), 0.45 (III). Amino acid analysis was quantitative for Glu. IR demonstrated new absorbances at 1720 and 1260 cm^{-1}, indicating the γ-ester linkage. NMR indicated the appearance of five methylene protons: δ 4.2 (d, 2, CH_2-O), 1.3 (t, 3, CH_3-C).

Boc-Tyr(αMe)-OH (10). To a stirred solution of 1 g (5 mmol) of L-Tyr(αMe), 200 mg (5 mmol) of sodium hydroxide in 1 mL of water, and 1 mL of *tert*-butyl alcohol was added dropwise 2.18 g (10 mmol) of di-*tert*-butyl dicarbonate within an hour. The reaction was brought to completion after the addition of 1 mL of *tert*-butyl alcohol and was stirred overnight. The turbid solution was diluted with 10 mL of water and extracted three times with 100 mL of pentane. The aqueous phase was acidified to pH 2–3 by addition of sodium hydrogen sulfate in cold and extracted with four 100-mL portions of ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and filtered. The solvent was removed at 35–40 °C under reduced pressure, and the white residue finally dried at 0.5 mm to constant weight (520 mg, 35%): R_f 0.49 (VII), 0.30 (VI). Anal. Calcd for $C_{15}H_{21}N_{65}$: C, 61.01; H, 7.12; N, 4.74. Found: C, 60.75; H, 7.30; N, 4.98.

(22) S. Yamada, N. Ikota, and T. Shiori, *J. Am. Chem. Soc.*, **97**, 1233 (1975).

Boc-Phe-D-Leu-OBzl (11). To an ice-cold solution of 1.3 g (5 mmol) of H-D-Leu-OBzl·HCl, in 10 mL of DMF, 1.5 mL (11 mol) of TEA was added with stirring. To this solution, 1.56 g (5 mmol) of Boc-Phe and 1.65 g (6 mmol) of DPPA were added and stirred for a period of 4 h at 0 °C and 20 h at 25 °C. The reaction mixture was worked up in a standard fashion and yielded a pale yellow peptide after drying and evaporating the organic layer. The peptide was crystallized from methanol (2.35 g, 90%): mp 78 °C; $[\alpha]^{25}{}_D$ +19.3° (*c* 1.0, MeOH); R_f 0.68 (V), 0.58 (III). Amino acid analysis: Phe, 0.96; Leu, 1.00. Anal. Calcd for $C_{27}H_{36}N_2O_5$·5CH$_3$OH: C, 68.18; H, 7.85; N, 5.78. Found: C, 68.48; H, 7.57; N, 5.58.

Boc-D-Ala-Gly-OH (12). Hydrogenation of 1 g (3 mmol) of compound **3** in 80 mL of methanol was carried out overnight with 1 g of 10% Pd/C in a Parr low-pressure hydrogenation apparatus at 40 psi. The solution was then filtered and evaporated to dryness in vacuo. The crystalline residue was dried over potassium hydroxide in vacuo to give compound **12** (730 mg, quantitative), which was used in situ for the next reaction.

Boc-D-Ala-Gly-Phe-D-Leu-OBzl (13). Compound 11 (1 g, 2.14 mmol) was deprotected and worked up in the same manner as compound 7. The oily peptide (860 mg, quantitative) was then dissolved in an ice-cold solution of 5 mL of DMF and 1 mL (0.7 mmol) of TEA. This solution was added to 730 mg (3 mmol) of compound **12** and 1.15 g (4.2 mmol) of DPPA. The reaction proceeded for 4 h at 0 °C and for 48 h at 25 °C and was then worked up in a standard fashion. After drying and evaporating the organic phase, a white crystalline peptide was obtained. It was recrystallized from methanol: yield 420 mg (34%); mp 160–162 °C; $[\alpha]^{25}{}_D$ +28.0° (*c* 1.0, MeOH); R_f 0.83 (VI), 0.88 (VII). Amino acid analysis: Ala, 1.01; Gly, 1.00; Phe, 1.06; Leu, 0.96. Anal. Calcd for $C_{32}H_{44}N_4O_7$·5CH$_3$OH; C, 63.73; H, 7.52; N, 9.15. Found: C, 63.68; H, 7.43; N, 9.40.

Compound **13** was deprotected in 4 N HCl in dioxane and worked up in the same manner as compound **7**. The peptide hydrochloride was used directly for the ensuing coupling reactions.

Boc-AA$_1$-D-Ala-Gly-Phe-D-Leu-OH [AA$_1$ = Glu(OEt) (14); Tyr(αMe) (15)]. To an ice-cold solution of 870 mg (1.45 mmol) of H-D-Ala-Gly-Phe-D-Leu-OBzl·HCl in 10 mL of DMF was added 0.21 mL (1.5 mmol) of TEA with stirring. This was followed by the addition of 1.5 mmol of Boc-Glu(OEt)-OH or Boc-Tyr-(αMe)-OH, 270 mg (2 mmol) of HOBT, and 412 mg (2 mmol) of DCC. The reaction proceeded for 4 h at 0 °C and 72 h at 25 °C, after which it was worked up in a standard fashion. The crude oily peptides were obtained after drying and evaporating the organic extracts. The peptides were then hydrogenated in 80 mL of methanol at 40 psi using 1.5 g of 5% Pd/C for 24 h. After filtering the hydrogenated mixtures, the filtrates were evaporated to dryness to give white solid peptide. These peptides were then purified over 2.5 × 25 cm silica gel columns using solvent system III and then using solvent system VII. Compound **14**: yield 43%; mp 100 °C dec; $[\alpha]^{25}{}_D$ −6.0° (*c* 2.0, MeOH); R_f 0.35 (VII), 0.63 (VIII). Amino acid analysis: Glu, 1.10; D-Ala, 1.02; Gly, 1.00; Phe, 0.99; D-Leu, 1.02. Anal. Calcd for $C_{32}H_{49}N_5O_{10}$: C, 58.00; H, 7.25; N, 10.57. Found: C, 57.92; H, 7.39; N, 10.55. Compound **15**: yield 78%; mp 115–118 °C; $[\alpha]^{25}{}_D$ −24.6° (*c* 1.0, MeOH); R_f 0.15 (VI), 0.68 (XII). Amino acid analysis: Tyr(αMe), 1.13; D-Ala, 1.00; Gly, 1.00; Phe, 1.01; D-Leu, 0.96. Anal. Calcd for $C_{35}H_{49}N_5O_9$·2CH$_3$OH: C, 58.09; H, 7.63; N, 9.37. Found: C, 57.93; H, 7.68; N, 9.54.

Boc-Cha-D-Leu-OBzl (16). To an ice-cold solution of 530 mg (2.5 mmol) of H-D-Leu-OBzl·HCl in 15 mL of DMF was added 0.4 mL (2.7 mmol) of TEA, 338 mg (2.5 mmol) of HOBT, 515 mg (2.5 mmol) of DCC, and 675 mg (2.5 mmol) of Boc-Cha-OH successively. After warming to 35 °C (4 h), the reaction was stirred overnight. The reaction was worked up in a standard fashion, and after drying and evaporating the organic phase yielded an oily peptide, which was purified over a silica gel column (2 × 100 cm) in solvent system I. The peptide was crystallized out of petroleum ether–ether (958 mg, 81%): mp 91–92 °C; $[\alpha]^{25}{}_D$ +1.0° (*c* 1.0, MeOH); R_f 0.66 (I), 0.84 (VI). Amino acid analysis: Cha, 1.03; D-Leu, 1.00.

Boc-Tyr-D-Ala-Gly-Cha-D-Leu-OH (17). To an ice-cold solution of 390 mg (1 mmol) of H-Cha-D-Leu-OBzl·HCl in 10 mL of DMF was added 0.14 mL (1 mmol) of TEA with stirring. To this solution, 410 mg (1 mmol) of compound **6**, 270 mg (2 mmol) of HOBT, and 412 mg (2 mmol) of DCC were added. The reaction proceeded with stirring for 4 h at 25 °C and for approximately 120 h at 0 °C and was worked up in a standard fashion. Drying and evaporating the organic phase yielded an oily yellow peptide (525 mg, 68%). Hydrogenation of a methanolic solution of the peptide (525 mg in 100 mL) with 1 g of 10% Pd/C for a period of 24 h yielded a crude, powdery peptide. The peptide was purified by passage down a 2 × 25 cm silica gel column using solvent system III. Compound **17** was obtained as a white amorphous powder (460 mg, 68%): mp 122–125 °C (dec); $[\alpha]^{25}{}_D$ +2.3° (*c* 1.0, MeOH); R_f 0.69 (XII), 0.74 (IX). Amino acid analysis: Tyr, 0.96; D-Ala, 1.01; Gly, 1.00; Cha, 0.93; D-Leu, 1.02. Anal. Calcd for $C_{34}H_{53}N_5O_9$: C, 60.44; H, 7.85; N, 10.35. Found: C, 60.65; H, 8.03; N, 10.11.

Boc-Gly-Gly-OH (18). To a stirred, ice-cold solution of 2.01 g (10 mmol) of H-Gly-OBzl·HCl in DMF (25 mL) was added 1.4 mL (10 mmol) of TEA, followed by the addition of 1.75 g (10 mmol) of Boc-Gly-OH, 1.35 g (10 mmol) of HOBT, and 2.06 g (10 mmol) of DCC. The reaction proceeded for 4 h at 0 °C and 48 h at 25 °C, after which it was worked up in a standard fashion. After drying and evaporating the organic phase, the petide was obtained as an oil (2.0 g, 62%). The oily peptide was then hydrogenated overnight in a methanolic solution (2.0 g in 100 mL) with 1 g of 10% Pd/C at 40 psi. After filtering and evaporating the hydrogenated mixture, the peptide was obtained as a white powder. The peptide was purified by passage down a 2 × 50 cm silica gel column using solvent system III: yield 1.2 g (52%); mp 131–132 °C; R_f 0.32 (VII), 0.56 (IX). Anal. Calcd for $C_9H_{16}N_2O_5$: C, 46.55; H, 6.90; N, 12.07. Found: C, 46.51; H, 7.16; N, 11.81.

Boc-Gly-Gly-1-aminoindan (19). To a stirred, ice-cold mixture of 665 mg (5 mmol) of 1-aminoindan and 1.16 g (5 mmol) of compound 18 in DMF (15 mL) was added 0.84 mL (6 mmol) of TEA, followed by the addition of 1.65 g (6 mmol) of DPPA. The reaction proceeded for 4 h at 0 °C and for 100 h at 25 °C, after which it was worked up in a standard fashion. Drying the organic extract, followed by evaporation, gave a white peptide, which was crystallized from ethyl ether (1.38 g, 80%): mp 149 °C; R_f 0.32 (VII), 0.56 (IX). Anal. Calcd for $C_{18}H_{25}N_3O_4$: C, 62.25; H, 7.20; N, 12.10. Found: C, 62.39; H, 7.28; N, 11.91.

Boc-Tyr-Gly-Gly-1-aminoindan (20). Compound **19** (700 mg, 2 mmol) was converted to its hydrochloride derivative in the same manner as compound **7** and used in situ for the next coupling step. To a stirred, ice-cold solution of 565 mg (2 mmol) of H-Gly-Gly-1-aminoindan hydrochloride was added 0.56 mL (4 mmol) of TEA, followed by the addition of 560 mg (2 mmol) of Boc-Tyr-OH in DMF (10 mL) and 825 mg (3 mmol) of DPPA. The mixture was stirred for 4 h at 0 °C and 24 h at 25 °C. The reaction mixture was then diluted with ethyl acetate (300 mL) and washed as usual. Drying the organic extract followed by evaporation to dryness gave an oily peptide. Addition of ethyl ether with subsequent crystallization gave a pale yellow powdery peptide (500 mg, 50%); R_f 0.70 (VI), 0.79 (VII); mp 115 °C (dec). Amino acid analysis: Tyr, 1.00; Gly, 2.00. Anal. Calcd for $C_{27}H_{34}N_4O_6$: C, 63.53; H, 6.67; N, 10.98. Found: C, 63.19; H, 6.75; N, 10.62.

Boc-Gly-Aib-OH (21). To a stirred, ice-cold solution of 1.15 g (5 mmol) of H-Aib-OBzl·HCl in DMF (20 mL) was added 0.7 mL (5 mmol) of TEA, followed by the addition of 0.88 g (5 mmol) of Boc-Gly-OH, 0.68 g (5 mmol) of HOBT, and 1.03 g (5 mmol) of DCC. The reaction proceeded for 4 h at 0 °C and for 48 h at 25 °C, after which it was worked up in a standard fashion. After drying and evaporating the organic extract, the peptide was obtained as an oil (1.14 g, 65%). The oily peptide was then directly hydrogenated at 40 psi overnight in methanolic solution (1.14 g in 100 mL) with 600 mg of 10% Pd/C. After filtering and evaporating the hydrogenation mixture, the peptide was purified by passage down a 2 × 50 cm silica gel column using solvent system III: yield 780 mg (60%); mp 135–137 °C; R_f 0.48 (VII), 0.67 (VIII). Amino acid analysis: Gly, 1.00; Aib, 0.93. Anal. Calcd for $C_{11}H_{20}N_2O_5$: C, 50.77; H, 7.69; N, 10.77. Found: C, 50.52; H, 7.99; N, 10.42.

Boc-Gly-Aib-1-aminoindan (22). To a stirred, ice-cold mixture of 665 mg (5 mmol) of 1-aminoindan and 780 mg (3 mmol) of compound **21** in DMF (10 mL) was added 0.84 mL (6 mmol) of TEA, followed by the addition of 1.65 g (6 mmol) of DPPA. The mixture was stirred for 4 h at 0 °C and for 48 h at 25 °C, after which it was worked up in a standard fashion. Drying the organic extract followed by evaporation yielded a pale yellow

peptide, which was crystallized from ethyl ether (570 mg, 51%): mp 168 °C; R_f 0.78 (VII), 0.73 (VIII). Amino acid analysis: Gly, 1.00; Aib, 0.99. Anal. Calcd for $C_{20}H_{29}N_3O_4$: C, 64.00; H, 7.73; N, 11.20. Found: C, 63.85; H, 7.86; N, 10.90.

Boc-Tyr-Gly-Aib-1-aminoindan (23). Compound **22** (375 mg, 1 mmol) was converted to its hydrochloride derivative in the same manner as compound **7** and used in situ for the next coupling reaction. To a stirred, ice-cold solution of 310 mg (1 mmol) of Gly-Aib-1-aminoindan hydrochloride was added 0.28 mL (2 mmol) of TEA, followed by the addition of 281 mg (1 mmol) Boc-Tyr in DMF (10 mL) and 550 mg (2 mmol) of DPPA. The mixture was stirred for 4 h at 0 °C and for 48 h at 25 °C. The reaction mixture was then worked up in a standard fashion. Drying and evaporation of the organic extract gave an oily peptide. Addition of ethyl ether with subsequent evaporation of solvent yielded a pale yellow powdery peptide (350 mg, 65%): mp 110–112 °C (dec); $[\alpha]^{25}{}_D$ +4.3° (*c* 1.0, MeOH); R_f 0.73 (VI), 0.79 (VII). Amino acid analysis: Tyr, 1.00; Gly, 0.98; Aib, 0.95. Anal. Calcd for $C_{29}H_{38}H_4O_6$: C, 64.60; H, 7.06; N, 10.41. Found: C, 64.39; H, 7.36; N, 10.18.

Boc-Gly-1-aminoindan (24). To a stirred mixture of 670 mg (5 mmol) of 1-aminoindan and 880 mg (5 mmol) of Boc-Gly in DMF (10 mL) was added 0.7 mL (5 mmol) of TEA, followed by the addition of 1.4 g (5 mmol) of DPPA. The reaction proceeded for 48 h at 25 °C, after which it was worked up in a standard fashion. Drying and evaporation of the organic extract gave an oily peptide which resisted several attempts of crystallization (1.4 g, 96%). Anal. Calcd for $C_{16}H_{22}N_2O_3$: C, 65.28; H, 7.74; N, 9.30. Found: C, 64.90; H, 7.58; N, 8.99.

Boc-Tyr-Aib-OH (25). To a stirred, ice-cold solution of 1.15 g (5 mmol) of H-Aib-OBzl·HCl in DMF (20 mL) was added 1.4 mL (10 mmol) of TEA, followed by the addition of 1.4 g (5 mmol) of Boc-Tyr-OH and 1.4 g (5 mmol) of DPPA. The mixture was stirred for 4 h at 0 °C and for 48 h at 25 °C, after which it was worked up in a standard fashion. Drying and evaporation of the organic extract gave an oily peptide (1.26 g, 56%), which was used directly for the subsequent hydrogenation step. The dipeptide was hydrogenated overnight in methanolic solution (1.2 g in 80 mL) with 1 g of 10% Pd/C at 35 psi. The hydrogenated mixture was then filtered and evaporated in vacuo to give a white product. The peptide was purified by passage down a 2 × 25 cm silica gel column using solvent system V. The product was crystallized from methanol–water: yield 950 mg (52%); mp 186–187 °C; $[\alpha]^{25}{}_D$ +1.0° (*c* 2.0, MeOH); R_f 0.48 (VII), 0.58 (XII). Amino acid analysis: Tyr, 1.00; Aib, 0.95. Anal. Calcd for $C_{18}H_{26}N_2O_6 \cdot 1.5H_2O$: C, 58.53; H, 7.85; N, 7.58. Found: C, 58.60; H, 7.48; N, 7.33.

Boc-Tyr-Aib-Gly-1-aminoindan (26). The deprotection of compound **24** (1.0 g, 3.4 mmol) was performed in 30 mL of 4 N HCl in dioxane in the same manner as compound **7**. The hydrochloride (770 mg, quantitative) was used in situ for the next step.

To a stirred, ice-cold solution of 770 mg (3.4 mmol) of H-Gly-1-aminoindan hydrochloride in DMF (15 mL) was added 1 mL (7 mmol) of TEA, followed by the addition of 732 mg (2 mmol) of Boc-Tyr-Aib-OH and 825 mg (3 mmol) of DPPA. The reaction proceeded for 4 h at 0 °C and for 100 h at 25 °C, after which it was worked up in a standard fashion. Drying and evaporating the organic extract gave a pale yellow product, which was further purified by passage down a 2.5 × 5 cm silica gel column using solvent system III (700 mg, 65%): $[\alpha]^{25}{}_D$ +13° (*c* 1.0, MeOH); R_f 0.79 (VII), 0.76 (VIII); mp 126 °C (dec) (softens at 110 °C). Amino acid analysis: Tyr, 1.00; Aib, 0.94; Gly, 0.93. Anal. Calcd for $C_{29}H_{38}N_4O_6$: C, 64.60; H, 7.06; N, 10.41. Found: C, 64.87; H, 7.20; N, 10.12.

Results

The biological activities of 19 analogues of enkephalin in the guinea pig ileum and rat brain binding assay are summarized in Table III. The IC_{50} is the concentration of compound necessary to inhibit the amplitude of the electrically stimulated guine pig ileum by 50%. The EC_{50} is the effective concentration of a compound necessary to displace 50% of the specifically bound [^{3}H]naloxone from a rat brain homogenate. None of the analogues of enkephalin antagonize the ID_{50} dosage of the agonist [D-Ala^2,D-Leu^5]enkephalin in the guinea pig ileum system. Simantov et al.[42] have shown that in the presence of 100 mM sodium chloride the binding activity to rate brain homogenate of opiate antagonists is slightly augmented, while the binding affinity of opiate agonists is decreased. The "sodium shift" is defined as the ratio of the ED_{50} for binding of an opiate compound in the presence of sodium divided by the EC_{50} obtained from binding of the same compound in the absence of sodium. A large sodium shift is seen for opiate compounds which are strong agonists.[7] A sodium shift has been observed with peptide analogues of enkephalin, but the shift correlates poorly with the degree of agonism or antagonism of the analogue of enkephalin.[23] Table III, column 6, demonstrates a range of sodium shift values from 0.9- to 20-fold for the different analogues of enkephalin. These sodium shift values do not correlate with the observed biological agonism of these analogues in either the guinea pig ileum or rat brain binding assays.

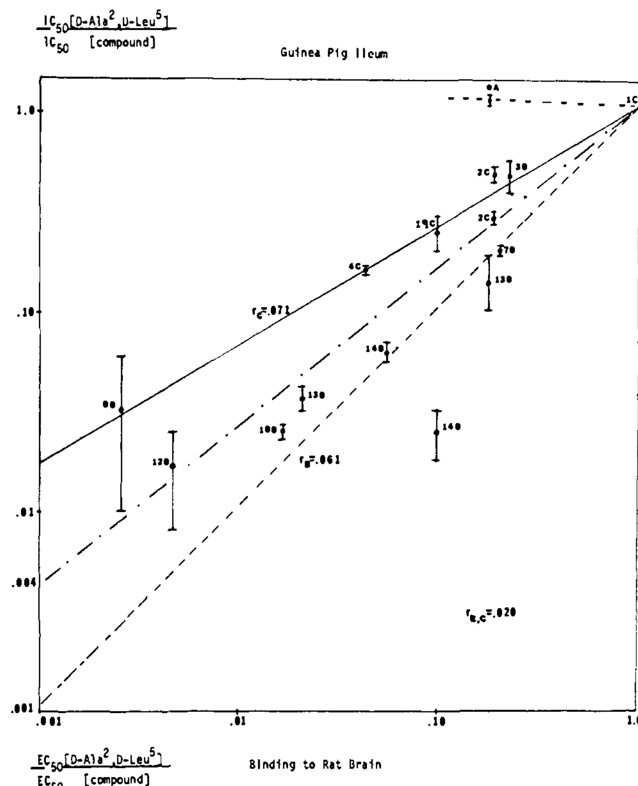

Figure 1. The correlation between the relative potencies of opioid-like peptides in Table III in the rat brain binding assay (ordinate) and guinea pig ileum (abcissa) is shown. Excluding [Aib^2,Met-NH$_2$^{5}]enkephalin (6A), the correlation coefficient is $r_{B,C}$ = 0.92 (dotted–dashed line). Peptides with a free carbonyl terminus (C) are 18 times more potent in the ileum assay than in the brain binding assay (solid line, r_C = 0.97). Descarboxyl analogues (B) are nearly equipotent in the two assay systems (dashed line, r_B = 0.96). See text for further details.

The relative potency of a compound in the guinea pig ileum assay is reported as the ratio of the ID_{50} of the reference compound [D-Ala^2,D-Leu^5]enkephalin divided by

(23) C. B. Pert, D. L. Bowie, A. Pert, J. L. Morell, and E. Gross, *Nature (London)*, **269**, 73 (1977).
(24) A. R. Day, M. Lujan, W. L. Dewey, L. S. Harris, J. A. Radding, and R. J. Freer, *Res. Commun. Pathol. Pharmacol.*, **14**, 597 (1976).
(25) A. Goldstein, J. Goldstein, and B. M. Cox, *Life Sci.*, **17**, 1643 (1975).
(26) H. H. Büscher, R. C. Hill, D. Römer, F. Cardinaux, A. Closse, D. Hauser, and J. Pless, *Nature (London)*, **261**, 423 (1976).

Table III. Biological Activities of Enkephalin Analogues

	guinea pig ileum			rat brain assay		
compd	IC_{50} (no. of expts)	IC_{50} of [D-Ala[2],D-Leu[5]]enkephalin[a] / IC_{50} of compd	EC_{50},[b] +NaCl (100 mM)	EC_{50}, −NaCl	sodium[c] shift	−NaCl: EC_{50} of [D-Ala[2],D-Leu[5]]enkephalin / EC_{50} of compd
		Reference Compound				
Tyr-D-Ala-Gly-Phe-D-Leu	$1.7 \pm 1.2 \times 10^{-8}$ (20)	1.00 (20)	4.6×10^{-7}	8.7×10^{-8}	5.3	1.00
		α-Methyl Amino Acid Analogues				
Tyr-D-Ala-Gly-Phe(αMe)-Leu	$4.2 \pm 2.5 \times 10^{-8}$ (3)	0.48 ± 0.03 (3)	3.5×10^{-6}	4.6×10^{-7}	7.6	0.19
Tyr-D-Ala-Gly-Phe(αMe)-Val	$12.5 \pm 9.3 \times 10^{-8}$ (3)	0.29 ± 0.01 (3)	6.2×10^{-6}	4.5×10^{-7}	13.8	0.19
Tyr-D-Ala-Gly-Phe(αMe)	$7.5 \pm 2.7 \times 10^{-8}$ (3)	0.16 ± 0.01 (3)	1.2×10^{-5}	2.0×10^{-6}	6.0	0.044
L-Tyr(αMe)-D-Ala-Gly-Phe-D-Leu	inact at 1.7×10^{-6} (3)	<0.0075 (3)	$>2.0 \times 10^{-4}$	$>6.5 \times 10^{-4}$		<0.0001
Tyr-Aib-Gly-Phe-Met-NH_2[d]	$1.6 \pm 0.1 \times 10^{-8}$ (2)	1.17 ± 0.05 (2)	3.2×10^{-6}	4.8×10^{-7}	6.7	0.18
		Descarboxyl Analogues				
Tyr-D-Ala-Gly-phenethylamine	$9.8 \pm 3.7 \times 10^{-8}$ (3)	0.19 ± 0.01 (3)	4.13×10^{-6}	4.00×10^{-7}	8.6	0.21
Tyr-D-Ala-Gly-1-aminoindan	$4.0 \pm 4.4 \times 10^{-8}$ (3)	0.47 ± 0.08 (3)	2.55×10^{-6}	3.72×10^{-7}	6.9	0.23
Tyr-Gly-Gly-1-aminoindan	$2.6 \pm 3.5 \times 10^{-6}$ (3)	0.033 ± 0.03 (3)	3.25×10^{-5}	3.35×10^{-5}	0.9	0.0026
Tyr-Aib-Gly-1-aminoindan	$1.4 \pm 1.0 \times 10^{-6}$ (2)	0.025 ± 0.005 (2)	2.5×10^{-5}	5.00×10^{-6}	5.0	0.017
Tyr-Gly-Aib-1-aminoindan	inact at 3.2×10^{-6} (2), IC_{50} 9.03×10^{-6} (1)	<0.008 (2), <0.0015 (1)	$\sim 5.2 \times 10^{-4}$	$\sim 1.4 \times 10^{-4}$	3.7	~0.00062
Tyr-D-Ala-Gly-2-aminoindan	inact at 2.71×10^{-6} (3), IC_{50} 9.01×10^{-6} (1)	<0.009 (3), 0.017 (1)	5.10×10^{-5}	1.86×10^{-5}	2.7	0.0047
Tyr-D-Ala-Gly-benzylamine	$1.4 \pm 0.8 \times 10^{-7}$ (3)	0.14 ± 0.04 (3)	4.17×10^{-6}	4.76×10^{-7}	8.8	0.18
Tyr-D-Ala-Gly-(aminomethyl)cyclobutane	$3.2 \pm 2.3 \times 10^{-7}$ (3)	0.063 ± 0.006 (3)	3.11×10^{-5}	1.54×10^{-6}	20.2	0.056
Tyr-D-Ala-Gly-(aminomethyl)cyclopropane	$4.8 \pm 2.1 \times 10^{-7}$ (3)	0.037 ± 0.006 (3)	2.51×10^{-5}	1.96×10^{-6}	6.3	0.02
Tyr-D-Ala-Gly-benzyl ester	$2.2 \pm 1.6 \times 10^{-7}$ (2)	0.025 ± 0.007 (2)	1.48×10^{-5}	8.80×10^{-7}	16.8	0.099
		Opiate–Enkephalin Modeling				
p-tyramine	inact at 2.19×10^{-4} (5)	<0.00007 (5)	$>1.0 \times 10^{-3}$	$>1.0 \times 10^{-3}$		<0.000087
Tyr-D-Ala-Gly	inact at 2.44×10^{-5} (5)	<0.0008 (5)	$\sim 1.9 \times 10^{-3}$	$\sim 7.0 \times 10^{-4}$	2.7	~0.0001
Tyr-D-Ala-Gly-L-Cha-D-Leu	$9.0 \pm 3.5 \times 10^{-9}$ (3)	0.25 ± 0.05 (3)	7.8×10^{-6}	9.2×10^{-7}	8.5	0.095
Glu(OEt)-D-Ala-Gly-Phe-D-Leu	inact at 7.33×10^{-6} (2)	<0.0006 (2)	$\sim 1.3 \times 10^{-3}$	$\sim 7.6 \times 10^{-4}$		0.0001
morphine sulfate	$1.6 \pm 1.4 \times 10^{-9}$ (2)	0.62 ± 0.04 (2)	1.05×10^{-7}	5.5×10^{-8}	19.1	1.6

[a] Both IC_{50} values obtained from the same tissue. [b] Duplicate values used to obtain EC_{50}; each EC_{50} value represents a separate experiment. [c] Soluble shift − EC_{50} (+100 mM NaCl)/LC_{50} (−NaCl). [d] This compound was purchased from Peninsula Laboratories.

the IC_{50} obtained for that compound from the same tissue preparation. This technique corrects for the variability in pharmacologic responsiveness among the different ileum preparations (Table III, columns 2 and 3). Figure 1 compares the relative potencies of the 14 biologically active peptides listed in Table III with respect to [D-Ala^2,D-Leu^5]enkephalin in the guinea pig ileum and rat brain binding assays. [D-Ala^2,D-Leu^5]enkephalin is approximately 5.5 times more potent in the guinea pig ileum than in the brain binding assay in the absence of sodium chloride. The relative potencies of the different enkephalin analogues with respect to [D-Ala^2,D-Leu^5]enkephalin in the two bioassay systems correlate well (Figure 1, $r_{B,C}$ = 0.92), excluding [Aib^2,Met-NH_2]enkephalin (Peninsula Laboratories). If one separately examines relative potencies of the C-terminal carboxylated peptide analogues and C-terminal *des*carboxylated peptide analogues with respect to [D-Ala^2,D-Leu^5]enkephalin, one finds improved correlation (r_c = 0.97 and r_B = 0.96, respectively). Peptide analogues with a carboxylated terminus are more potent in the guinea pig ileum than in the rat brain binding assay (Figure 1, solid line), while the *des*carboxylate analogues are essentially equipotent in the two bioassay systems (Figure 1, dashed line).

The C-terminal amide compound, [Aib^2,Met-NH_2^{5}]-enkephalin, confirms the importance of the chemical status of the C terminus by having 30 times the biological potency in the ileal assay as compared to the rat brain binding assay as noted by Lord et al.[2]

Discussion

Early structure–activity data demonstrated that (1) the phenolic group of Tyr^1 is important for activity[24,25] and (2) [*des*amino-Tyr^1]methionine-enkephalin is devoid of biological activity.[26] These first two observations prompted comparisons of the tyramine (hydroxyphenethylamine) portion of morphine and the morphine classes of opiates with the phenolic side chain of the enkephalins and the amino terminus of Tyr^1.[27] However, *p*-tyramine (4′-hydroxyphenethylamine) is inactive in millimolar concentrations in the rat brain binding and guinea pig ileum assays (Table III). *p*-Tyramine possesses sufficient conformational freedom to adopt the appropriate spatial conformation available to the phenolic ring and quaternary nitrogen present in the phenanthracene nucleus of the opiate alkaloids and tyrosine-1 of the enkephalins. These results suggest that the tyramine moiety provides two sites which are necessary but not significant requirements for opiate and enkephalin recognition and binding. Other chemical substituents in these compounds must play an essential role for receptor recognition and activation.

Demonstration of a Third Site. It had been observed previously that the tripeptide fragment Tyr-Gly-Gly is inactive, while the tetrapeptide fragment Tyr-Gly-Gly-Phe has 1 to 3% of the activity of methionine-enkephalin.[28] To corroborate these observations and exclude the possibilty that biological inactivity is due to in situ proteolysis, the tripeptide Tyr-D-Ala-Gly was synthesized and found to be inactive (Table III, compound **18**). The D-alanine substitution in the second residue has been shown by other investigators to inhibit hydrolysis.[17,29] Significantly, the benzyl ester of Tyr-D-Ala-Gly is biologically active in both

(27) A. S. Horn and J. R. Rodgers, *J. Pharm. Pharmacol.*, **29**, 257 (1976).
(28) L. Terenius, A. Wahlstrom, G. Lindeberg, S. Karlsson, and V. Ragnarsson, *Biochem. Biophys. Res. Commun.*, **71**, 175 (1976).
(29) C. B. Pert, A. Pert, J.-K. Chang, and B. T W. Fong, *Science*, **194**, 332 (1976).

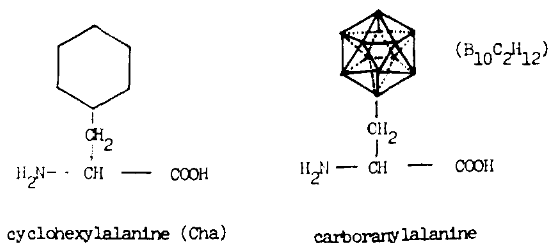

Figure 2. Nonaromatic L-amino acid analogues of L-phenylalanine.

the ileal and brain assays (Table III, compound **16**). These two tripeptide analogues confirm earlier proposals[30,31] that, in addition to the phenolic ring and amino terminus in the first residue, a third site is required in enkephalin which is essential for biological activity and that some portion of the peptide other than the first three residues provides this site. The synthesis of the proteolytically resistant tetrapeptide analogues Tyr-D-Ala-Gly-Phe(αMe) and Tyr-D-Ala-Gly-phenethylamine with significant biological activity (Table III, compounds 4 and 7) also confirm that the fourth residue provides the third site and that the fifth residue is not essential for biological activity.

Characterization of the Third Site. Several investigators have related the aromatic side chain of Phe^4 with the aromatic 19-phenylethyl substituent (F ring) of the opiate compound 7-(1-phenyl-3-hydroxybutyl)-3-*endo*-ethenotetrahydrothebaine (PET).[32,39] This proposed structural homology does not explain the necessity of the fourth residue in the enkephalins, since many of the morphine, morphinan, and oripavine compounds active at the μ receptor lack this phenethyl side group but provide nonaromatized chemical substituents in their C ring which can fulfill the binding requirements of a third site.[38] This is in contradistinction to the analogy drawn between the benzene ring of Phe^4 of enkephalin and the C19 phenethyl substituent proposed for compounds binding to the "enkephalin receptor".[3]

L-Cyclohexylalanine (L-Cha) is a nonaromatic analogue of L-phenylalanine (Figure 2), and there are conflicting reports about the biological activity of L-Cha analogues of enkephalin.[33,34] Tyr-D-Ala-Gly-L-Cha-D-Leu was synthesized in our laboratory, and this analogue demonstrates significant biological activity in both the guinea pig ileum and rat brain binding assays (Table III, compound **19**). The report[35] of a biologically active analogue of enkephalin

(30) A. P. Feinberg, I. Creese, and S. H. Snyder, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 4215 (1976).
(31) F. A. Gorin and G. R. Marshall, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 5179 (1977).
(32) A. F. Bradbury, D. G. Smyth, and C. R. Snell, *Nature (London)*, **260**, 165 (1976).
(33) A. S. Dutta, J. J. Gormley, C. F. Hayward, J. S. Morley, J. S. Shaw, G. J. Stacey, and M. T. Turnbull, *Life Sci.*, **21**, 559 (1977).
(34) R. C. Frederickson, *Life Sci.*, **21**, 23 (1977).
(35) A. Eberle, O. Leukart, P. Schiller, J.-L. Fauchere, and R. Schwyzer, *FEBS Lett.*, **82**, 325 (1977).
(36) C. F. G. Smith, in "Peptides", M. Goodman and J. Meienhofer, Eds., Wiley, New York, 1977, p 111.
(37) G. R. Marshall and F. A. Gorin, in "Peptides", M. Goodman and J. Meienhofer, Eds., Wiley, New York, 1977, p 84.
(38) G. R. Marshall and H. E. Bosshard, *Circ. Res.*, 31(**II**), 30, 43 (1972).
(39) H. E. Boshard, C. D. Barry, J. M. Fritsch, R. A. Ellis, and G. R. Marshall, *Proc. 1972 Summer Simulation Conf.*, 1, 58 (1972).

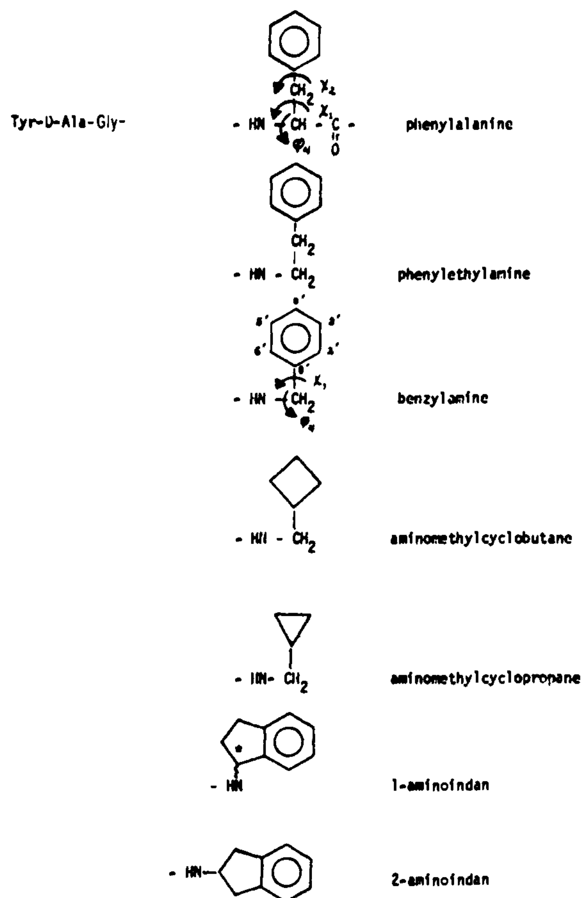

Figure 3. Fourth-position analogues of Tyr-D-Ala-Gly having opioid-like in vitro activity. See text for further details.

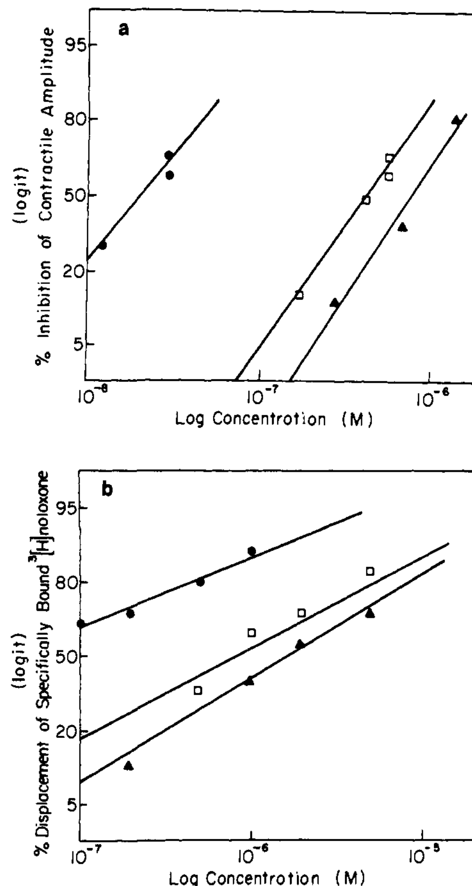

Figure 4. Dose–response curves for [D-Ala^2,D-Leu^5]enkephalin (O), Tyr-D-Ala-Gly-phenethylamine (□), and Tyr-D-Ala-Gly-benzylamine (Δ) determined for (a) a single guinea pig ileum preparation and (b) a common, pooled, rat brain homogenate.

with carboranylalanine in the fourth residue further supports the contention that the third site can be nonaromatic (Figure 2).

In order to identify which of these substituents are absolutely essential, descarboxyl, tetrapeptide analogues of enkephalin were synthesized by the condensation of a number of commerically available alkylamines and arylamines with the tripeptide fragment Tyr-D-Ala-Gly. It was hoped that these analogues would help define the structural requirements of the fourth residue of [D-Ala^2,D-Leu^5]enkephalin. Tyr-D-Ala-Gly-phenethylamine is a potent compound in both guinea pig ileum and rat brain binding assays (Table III, compound **7**). This is in agreement with an earlier report by Morgan et al.,[36] who evaluated the same analogue in the guinea pig ileum and mouse vas deferens assays. The analogue Tyr-D-Ala-Gly-benzylamine lacks a methylene group in the fourth residue corresponding to the β-methylene group of the side chain of the phenethylamine analogue (Figure 3). This analogue is essentially equipotent with Tyr-D-Ala-Gly-phenethylamine (Table III, compound **13**). Several analogues closely related to this benzylamine analogue were synthesized, Tyr-D-Ala-Gly-(aminomethyl)cyclopropane and Tyr-D-Ala-Gly-(aminomethyl)cyclobutane (Figure 3). The (aminomethyl)cyclobutyl and (aminomethyl)cyclopropyl analogues were found to be approximately 38 and 15% as active as the benzylamine and phenethylamine analogue in both assay systems (Table III, compound **14** and **15**).

If the first three residues of these derivatized tripeptides adopt a common backbone conformation when bound to a common receptor, then one can utilize the well-defined ring geometries of cyclopropane, cyclobutane, and benzene to identify the common chemical substituents shared by the (aminomethyl)cyclopropyl, (aminomethyl)cyclobutyl, and benzylamino derivatives of Tyr-D-Ala-Gly. By varying ϕ_4 and χ_1, only the atomic coordinates of Cα and Cβ carbons of the different side chains of the fourth residue can be superimposed (Figure 3). Of course, structural definition of the necessary hydrophobic site could be a region defined by the more approximate geometrical overlap of the benzyl, cyclopropyl, and cyclobutyl rings. This would correspond to the lower portion of the phenyl ring, carbon atoms 1′, 2′, 6′, of the benzylamino analogue (Figure 3).

Alternatively, these structurally truncated analogues might adopt different backbone conformations when bound to the morphine (μ) receptor. These novel, opioid-like tripeptides are competitively antagonized by naloxone in the rat brain binding and guinea pig ileum assays and produce parallel dose–response curves in both assay systems (Figure 4).

Aminoindan Analogues of Enkephalin. Additional derivatized tripeptide analogues were synthesized which further restricted the conformational freedom of Tyr-D-Ala-Gly-benzylamine without the further elimination of chemical groups. 2-Aminoindan is a symmetrically bridged analogue of phenethylamine. 1-Aminoindan has an optically active center at Cα and can be thought of as an analogue of benzylamine with a three-carbon bridge (Figure 3).

Interestingly, Tyr-D-Ala-Gly-2-aminoindan is almost without biological activity, while Tyr-D-Ala-Gly-1-aminoindan is at least as potent as either of the phenethylamine or benzylamine analogues (Table III, compounds **12** and **8**). Resolution by analytical high-pressure liquid chromatography (Table I) suggests that Tyr-D-Ala-Gly-1-

aminoindan is present as a racemic mixture, so that it is conceivable that the active component in this compound could be twice the measured agonistic potency. Sufficient quantities of the separated racemate still must be obtained for isomeric assignments. The α-methyl amino acid, aminobutyric acid (Aib), has been substituted successively into both the second and third residues of the 1-aminoindan analogue. Tyr-Aib-Gly-1-aminoindan is approximately 6% as active as the D-Ala[2]-1-aminoindan analogue, while Tyr-Gly-Aib-1-aminoindan is almost entirely without biological activity (Table III, compounds **10** and **11**).

The aminoindan analogues of enkephalin almost completely eliminate the torsional freedom of both χ_1 and χ_2 corresponding to the side chain torsional bonds of Phe[4] in enkephalin. Thus, there are a total of eight torsional bonds for Tyr-D-Ala-Gly-aminoindan instead of the ten rotational parameters required for the tetrapeptide fragment Tyr-D-Ala-Gly-Phe.[37] The aminoindan analogues of enkephalin containing α-methyl amino acids should also prove to be useful conformation probes of the opiate–enkephalin receptors. Marshall et al.[38] have shown that the α-methyl substituent severely restricts local peeptide backbone flexibility in a quantifiable fashion. The predicted torsional values are consistent with experimental ϕ_i, ψ_i torsional values obtained from the crystallographic determinations of peptides containing α-methyl amino acids.[39] The analogues of aminoindan containing Aib therefore have only six freely rotatable torsional bonds because of the severe conformational constraints imposed by the α-methyl amino acid. Conformational analyses of these conformationally hindered and yet biologically active 1-aminoindan analogues of enkephalin using analytical as well as the more traditional, iterative algorithms should aid in the characterization of the μ receptor found in the guinea pig ileal and rat brain binding assays.

Conclusion

These novel analogues of enkephalin, in conjunction with earlier structure–activity data, confirm that chemical substituents present in the first and fourth residues of enkephalin fulfill a three-site requirement for biological activity. These substituents correspond to the phenolic ring and amino terminus of tyrosine-1 and substituents present in phenylalanine-4 which fulfill the requirements of a required hydrophobic third site. Our earlier three-site model[31,37] resulted in a proposed conformation for receptor-bound enkephalin, which is consistent with structure–activity data obtained from the guinea pig ileal and rat brain binding assays for pentapeptide analogues of enkephalin containing *N*-methyl amino acids, D-amino acids, and α-methyl amino acids. However, a class of derivatized tripeptides, of which Tyr-D-Ala-Gly-benzylamine is representative, must adopt a receptor-bound conformation different from that of the native enkephalins and their pentapeptide analogues in order to fit the three-site model. Interestingly, some of the flexible classes of opiates such as the meperidine family do not have a readily identifiable tyramine[40] moiety like that of morphine, and it has been proposed by Portoghese[40] and more recently by Galt[41] that these compounds bind in a different fashion than the rigid opiates. Further synthesis and conformational analysis of the 1-aminoindan family of peptide analogues should aid in characterizing the chemical and conformational in vitro requirements of these derivatized tripeptides with opioid-like activity.

Acknowledgment. This work was supported in part by NIH Grant GM 24483 and NIDA Grant DA 00259. Fredric Gorin was supported by NIH Training Grant 5T32 GM07200. Dr. T. J. Cicero is a recipient of a Research Scientist Development award from NIAAA (AA70180). We thank Dr. Philip Needleman for the use of his facilities in the Department of Pharmacology.

(40) P. S. Portoghese, *J. Med. Chem.*, **8**, 609 (1965).
(41) R. H. B. Galt, *J. Pharm. Pharmacol.*, **29**, 711 (1977).
(42) R. Simantov, A. M. Snowman, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 977 (1976).

β-Adrenergic Blocking Agents. 20.[1] (3-Hydroxyprop-1-enyl)-Substituted 1-(Aryloxy)-3-(alkylamino)propan-2-ols

H. Tucker

Imperial Chemical Industries Limited, Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, England.
Received January 23, 1980

The synthesis of a series of (3-hydroxyprop-1-enyl)-substituted 1-(aryloxy)-3-(alkylamino)propan-2-ols is described. These compounds were investigated for their β-adrenoreceptor blocking properties and their selectivity of action. Among the *o*-(hydroxypropenyl)-substituted derivatives we have found some potent noncardioselective β-adrenoreceptor blocking agents which have a greater blocking action on the β_2 receptor, thus resembling propranolol. The *p*-(hydroxypropenyl)-substituted analogues were generally less potent and tended to be cardioselective. The structure–activity relationships are discussed in the light of the hypothesis that the cardioselectivity of *p*-amido-substituted (aryloxy)propanolamines is attributable, in part, to binding of the amide group to some additional site on the β receptor; our findings argue against a similar interaction for the allylic hydroxyl group.

The factors which influence the potency and the cardioselectivity of the β-adrenoreceptor blocking actions of substituted (aryloxy)propanolamines have been extensively studied. There is evidence that various substituents in the aryl ring para to the oxypropanolamine side chain tend to give compounds which are relatively more cardioselective than the corresponding ortho isomers, although the potency is also markedly reduced.[2,3] It has also been stated that para amidic substituents in the aryl ring confer both potency and cardioselectivity and that this is possibly due to binding between the para-amidic group and some ad-

(1) For Part 19, see M. S. Large and L. H. Smith, *J. Med. Chem.*, **23**, 112 (1980).
(2) L. H. Smith, *J. Appl. Chem. Biotechnol.*, **28**, 201–212 (1978).
(3) B. Åblad, M. Brogård, E. Carlsson, and L. Ek, *Eur. J. Pharmacol.*, **13**, 59–64 (1970).

 © 1980 American Chemical Society