Synthesis and Analgesic Properties of Two Leucine-enkephalin Analogues Containing a Conformationally Restrained N-Terminal Tyrosine Residue

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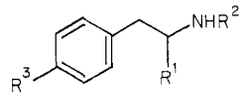
Two analogues of Leu-enkephalin, in which the terminal tyrosine-1 residue has been replaced by a conformationally restrained tyrosine analogue, have been synthesized by classical solution methods, and their opiate agonist potencies on electrically stimulated guinea pig ileum and mouse vas deferens preparations were determined in comparison with Leu-enkephalin. The restriction in the degree of freedom of the tyrosine moiety in [(2-amino-6-hydroxy-2-tetralinyl)carbonyl]glycylglycylphenylalanylleucine methyl ester (**3e**) leads to a 7 to 8 times higher agonist activity at the μ-receptor subtype in guinea pig ileum when compared to Leu-enkephalin, and an almost 30-fold decrease in potency, vs. Leu-enkephalin, on mouse vas deferens preparation. [(2-Amino-5-hydroxy-2-indanyl)carbonyl]-glycylglycylphenylalanylleucine methyl ester (**2e**) was inactive in the above tests. These results demonstrate the differential effect of restricting conformational flexibility on receptor recognition. Neither analogue had any analgesic properties when evaluated by the hot-plate test in mice after sc and icv administration.

The role of enkephalins as natural endogenous ligands for the opiate receptor in the central nervous system is now well established.[1,2] A large number of structural analogues of both leucine- and methionine-enkephalins (**1g** and **1f**, respectively) have been synthesized over the past 5 years,



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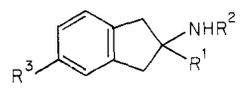

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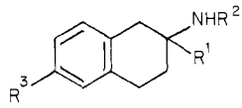

3

Gly-Gly-L-Phe-L-Leu-OCH_3

4

a, R^1 = COOH; R^2 = H; R^3 = OH
b, R^1 = COOH; R^2 = H; R^3 = $OCH_2C_6H_5$
c, R^1 = COOH; R^2 = $COOCH_2C_6H_5$; R^3 = $OCH_2C_6H_5$
d, R^1 = CO-Gly-Gly-L-Phe-L-Leu-OCH_3; R^2 = $COOCH_2C_6H_5$; R^3 = $OCH_2C_6H_5$
e, R^1 = CO-Gly-Gly-L-Phe-L-Leu-OCH_3; R^2 = H; R^3 = OH
f, R^1 = CO-Gly-Gly-L-Phe-L-Leu-OH; R^2 = H; R^3 = OH
g, R^1 = CO-Gly-Gly-L-Phe-L-Met-OH; R^2 = H; R^3 = OH
h, R^1 = CO-D-Ala-Gly-L-Phe-L-Leu-OCH_3; R^2 = H; R^3 = OH

which have been aimed at affording active compounds having greater stability toward metabolizing enzymes and/or to facilitate entry into the central nervous system after oral or peripheral administration.[3] Such structural modifications include shortening or lengthening of the pentapeptide chain, substitution of individual amino acids by other amino acids, and chemical modification of individual amino acids, particularly terminal amino acids. Numerous conformational studies[4] have been carried out that propose particular "active" conformations for enkephalins at the analgesic receptor, and the structural analogy between enkephalins and morphine[5] has been discussed. This analogy emphasizes the close correspondence between the tyramine moiety in the morphine molecule and the phenolic ring and amino group of the terminal tyrosine residue in the enkephalins. Since morphine is a rigid molecule, this infers that the flexible tyrosine moiety in the enkephalins interacts at the analgesic receptor in a specific conformation that is presumably stereochemically compatible with the tyramine unit in the rigid morphine molecule.

Leucine-enkephalin (Leu-enkephalin, **1f**) has been crystallized and its structure determined.[6] X-ray crystallographic data suggest that the orientation of the tyrosine side chain is not unique, the tyrosine aromatic ring experiencing disorder of either a static or dynamic nature. A solid-state NMR investigation of the Leu-enkephalin structure[7] has recently shown that in the polycrystalline state, the aromatic tyrosine ring is undergoing 180° flipping about the C^β–C^γ axis at a rate of approximately 5×10^4 s^{-1} at room temperature.

In order to explore the effect of a restriction in the degree of conformational freedom of the tyrosine moiety on the biological properties of Leu-enkephalin analogues, we have synthesized two analogues, **2e** and **3e**, of Leu-enkephalin in which the terminal tyrosine unit has been

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Table I. Opioid Activities of Leucine-enkephalin Analogues

compd	guinea pig ileum[a] rel potency (morphine = 1)	guinea pig ileum[a] ID_{50}, nM (95% CL)	mouse vas deferens[a] rel potency (morphine = 1)	mouse vas deferens[a] ID_{50}, nM (95% CL)
2e	0.03		0.02[b]	
3e	1.38	65.3 (32.1-110.2)	2.91[c]	333.4 (261.7-460.1)
1g[d]	1.06	90.1 (31.3-190.5)	55.32	17.3 (6.8-52.4)
1f[e]	0.11	504.2 (297.2-881.2)	89.38	12.1 (5.6-20.9)
1e[f]	no inhibn		8.31	201.2 (142.1-243.1)

[a] Data are from four determinations for each compound. [b] 37% naloxone reversible at 27 μg/mL. [c] 100% naloxone reversible at 27 μg/mL. [d] Methionine-enkephalin. [e] Leucine-enkephalin. [f] Leucine-enkephalin methyl ester.

modified by incorporating it into different ring systems and in which the terminal leucine-5 residue has been esterified to aid passage into the CNS. These analogues have been evaluated for analgesic activity on isolated guinea pig ileum myenteric plexus muscle and mouse vas deferens tissue and by the hot-plate test in mice after icv administration.

Chemistry. 2-Amino-5-hydroxy-2-indancarboxylic acid (**2a**) and 2-amino-6-hydroxy-tetralincarboxylic acid (**3a**) were prepared by literature methods[8,9] and obtained as racemates. The tetrapeptide H-Gly-Gly-Phe-Leu-OCH_3 (4) was synthesized by classical solution methods.[12] Preparation of the pentapeptides **2e** and **3e** were carried out as follows. The racemic tyrosine analogues **2a** and **3a** were N protected with carbobenzoxy chloride in base, O protected with benzyl bromide, and then coupled with tetrapeptide 4 by using *N,N'*-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole. Hydrogenolysis of the fully protected pentapeptides **2d** and **3d** and purification of the resulting deprotected products by gradient buffer elution from carboxymethylcellulose at pH 5.1 afforded the pentapeptide methyl esters **2e** and **3e**. Both pentapeptides were obtained as a mixture of their respective diastereoisomers.

Pharmacology. The opiate agonist potential of the pentapeptide methyl esters **2e** and **3e** were carried out on electrically stimulated guinea pig ileum and in mouse vas deferens preparations and are summarized in Table I. In the guinea pig ileum, the analgesic actions of enkephalins and opiates are mediated by their interaction with the μ receptor, whereas in mouse vas deferens, a different type of receptor, the δ receptor, predominates.[10] This latter receptor type interacts more strongly with opioid peptides than with the classical opiates.

Interestingly, a significant difference in potency was observed between **2e** and **3e** on both guinea pig ileum and mouse vas deferens preparations. The restriction in the degrees of freedom of the tyrosine moiety in **3e** leads to a 7 to 8 times increase in agonist activity at the μ-receptor subtype as compared to Leu-enkephalin, coupled with an almost 30-fold decreased potency, vs. Leu-enkephalin, on the mouse vas deferens preparation. Neither of the two Leu-enkephalin analogues were active as analgesics when examined by the hot-plate test in mice after sc and icv injection.

The results from the isolated tissue experiments demonstrate the differential effect of restricted conformation on receptor recognition and indicate that opiate activity in Leu-enkephalin derivatives can be retained by replacement of the terminal tyrosine unit with a less flexible structural analogue. The difference in potency of almost two orders of magnitude between the indan derivative **2e** and the tetralin derivative **3e** is worthy of note. The drastic decrease in potency of the indan derivative **2e** compared to **3e** suggests a preferred conformation for the tyrosine moiety in Leu-enkephalin at the μ receptor. This finding warrants greater scrutiny. The greater potency of the Leu-enkephalin analogue **3e** at the μ receptor as compared to the δ receptor is in accord with other studies, which indicate that greater structural flexibility is required in analgesic molecules for δ-receptor recognition than for μ-receptor binding. Shaw and Turnbull[4] have reported that the pentapeptide **3h** is about 7 times more potent as an agonist on the guinea pig ileum than on mouse vas deferens preparations. Thus, **3e** appears to resemble morphine, rather than Leu-enkephalin, in its receptor characteristics.

While the lack of in vivo activity of **3e** is unlikely to be due to inadequate CNS penetration, metabolic factors may play a significant role in this respect, since it is possible that **3e** could be a substrate for amino peptidase and/or enkephalinase enzymes.

Experimental Section

Melting points were determined on a Reichert hot-stage microscope and are uncorrected. Microanalyses were performed by the Micro-analytical Laboratory, Department of Chemistry, University of Manchester. Analytical results obtained for all compounds were within ±0.4% of the theoretical value unless otherwise stated. ^1H NMR sepctra were recorded on a Varian HR 220 or a Varian SC 300 spectrometer and are quoted on the δ scale. IR spectra were recorded as KCl disks, Nujol mulls, or liquid films on a Perkin-Elmer 237 spectrophotometer. Mass spectra were determined on an A.E.I. MS9 spectrometer operating at a probe temperature of 250 °C. Thin-layer chromatographic separations were carried out on 0.25-mm Polygram silica gel UV_{254}. 2-Amino-5-hydroxy-2-indancarboxylic acid[8] and 2-amino-6-hydroxy-2-tetralincarboxylic acid[9] were prepared by literature procedures. Amino acids and peptides with free amino functions were visualized by spraying with 1% ninhydrin in ethanol. N-Protected amino acids and peptides were visualized with 5% potassium dichromate in concentrated H_2SO_4, and amino acids and peptides possessing a phenolic hydroxy function were visualized with Pauli's reagent.[11] Other compounds were visualized in iodine vapor. Hydrogenations were carried out on a Gallenkampf hydrogenator operating at atmospheric pressure and room temperature.

Ion-exchange chromatographic separations of N-deprotected pentapeptides were carried out on Whatman CMC 52 carboxymethylcellulose, prewashed with 0.5 N aqueous sulfuric acid,

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followed by 0.2 N aqueous ammonium acetate buffer adjusted to pH 5.1 with 0.880 specific gravity ammonia solution or glacial acetic acid and measured with a Pye-Unicam PW 9418 pH meter fitted with a Pye-Unicam 401 combined electrode. Ion-exchange columns were run under slight pressure with a peristaltic pump set for an effluent output of 100 mL/h. Column effluents were monitored by UV spectrophotometry at 280 nm with a Cecil CE 202 spectrophotometer fitted with a 10-mm path-length flow-through cell. Columns were eluted with 0.005–0.5 N gradient ammonium acetate buffer solutions at pH 5.1, and fractions were collected on an automatic fraction collector.

Amino acid analyses of peptides were performed on a Beckmann SPINCO Model 123 amino acid analyzer using ninhydrin as visualizer. The peptides were hydrolyzed in 6 N HCl at 110 °C for 24 h in a sealed, evacuated tube.

2-Amino-5-(benzyloxy)-2-indancarboxylic Acid (2b) and 2-Amino-6-(benzyloxy)-2-tetralincarboxylic Acid (3b). As a general method, the appropriately substituted 2-amino-2-carboxylic acid (0.039 mol) in 2 N NaOH solution (48 mL) was treated with copper sulfate ($CuSO_4 \cdot 5H_2O$, 5.92 g, 0.024 mol) in water (24 mL). The mixture was mechanically stirred at 20–25 °C for 30 min, and methanol (140 mL) and then benzyl bromide (5.90 mL, 0.041 mol) were added. Vigorous stirring was maintained at 22–25 °C for 15 h. The resulting blue copper complex was collected and washed with water (3 × 25 mL), methanol (2 × 25 mL), and diethyl ether (2 × 25 mL), and the air-dried product (11.4 g) was stirred for 30 min at 50–60 °C with concentrated HCl (10 mL) and water (100 mL). While the solution was cooling, a brown colloidal gel formed, which was difficult to filter. This gel was triturated with 2 N hydrochloric acid (100 mL) and centrifuged at 48000 rpm for 10 min. The green supernatant was decanted, and the gel was again stirred at 50–60 °C for 30 min with concentrated hydrochloric acid (10 mL) and water (100 mL). The resulting supernatant was decanted, and the residue was air-dried and crystallized from glacial acetic acid to afford the appropriately substituted 2-amino-2-carboxylic acid.

2-Amino-5-(benzyloxy)-2-indancarboxylic acid hydrochloride (2b) was prepared as described above from **2a** as white crystals (5.0 g, 40%): mp 220–225 °C; IR (Nujol) 3350, 3145, 3090, 3000, 1728, 1612, 1588 cm^{-1}; NMR (Me_2SO-d_6, 60 MHz) 3.35 (m, 4 H, C-1 and C-3 protons), 5.03 (s, 2 H, benzyl CH_2), 6.68–7.56 (m, 8 H, aromatic protons), 8.95 (br s, 4 H, replaceable with D_2O, NH_3 and COOH). Anal. ($C_{17}H_{17}NO_3 \cdot HCl$) C, H, N.

2-Amino-6-(benzyloxy)-2-tetralincarboxylic acid hydrochloride (3b) was prepared as described above from **3a** as white crystals (5.6 g, 43%): mp 212–215 °C; IR (KCl) 3370, 3030, 2890, 2650, 2550, 1720, 1605, 1573 cm^{-1}; NMR (TFA, 60 MHz) 2.55 (m, 2 H, C-3 protons), 3.07 (m, 2 H, C-4 protons), 3.42 (m, 2 H, C-1 protons), 5.19 (s, 2 H, benzylic CH_2), 6.70–7.65 (m, 8 H, aromatic protons). Anal. ($C_{18}H_{19}NO_3 \cdot HCl$) C, H, N.

Preparation of *N*-Benzyloxycarbonyl-Protected Amino Acids. As a general method, the appropriate amino acid (0.011 mmol) suspended in 2 N NaOH solution (1000 mL) was heated on a steam bath to dissolve the amino acid and then cooled in an ice bath to 0–5 °C, which caused the sodium salt of the amino acid to precipitate out. Carbobenzyloxy chloride (2.11 g, 0.012 mol) was added dropwise to the cooled, stirred mixture, over 40 min. The ice bath was then removed, and the mixture was stirred at room temperature for 30 h. The reaction mixture was cooled to 0–5 °C, and a further 2.11 g (0.012 mol) of carbobenzyloxy chloride was added dropwise. After a total reaction time of 60 h, the mixture was carefully adjusted to pH 1 with 5 N HCl and extracted with diethyl ether (2 × 100 mL). The combined organic extracts were dried, filtered, and evaporated to dryness, and the residue was crystallized from diethyl ether/petroleum ether (bp 30–40 °C) to afford the appropriate *N*-benzyloxycarbonyl-protected amino acid.

5-(Benzyloxy)-2-[(benzyloxy)carbamido]-2-indancarboxylic acid (2c) was prepared as described above from **2b** in 41% yield: mp 115.5–121 °C; IR (KCl) 3320, 2995, 2970, 2870, 1738, 1705, 1650, 1604, 1580, 1534 cm^{-1}; NMR ($CDCl_3$, 60 MHz) 3.37 (m, 4 H, C-1 and C-3 protons), 4.96 (s, 2 H, benzyl CH_2), 5.03 (s, 2 H, benzloxy CH_2), 6.60–7.95 (m, 14 H, reducing to 13 H on addition of D_2O, NH and aromatic protons), 9.78 (br s, 1 H, replaceable with D_2O, COOH); mass spectrum, m/e 417 (M^+). Anal. ($C_{25}H_{23}NO_5$) C, H, N.

6-(Benzyloxy)-2-[(benzyloxy)carbamido]-2-tetralincarboxylic acid (3c) was prepared as described above from **3b** in 52% yield: mp 150–156 °C; IR (KCl) 3380, 3255, 3050, 3020, 2945, 2915, 2840, 1718, 1710, 1685, 1605, 1545, 1500 cm^{-1}; NMR ($CDCl_3$, 60 MHz) 2.28 (m, 2 H, C-3 protons), 2.82 (m, 2 H, C-4 protons), 3.12 (m, 2 H, C-1 protons), 4.98 (s, 2 H, benzyl CH_2), 5.05 (s, 2 H, benzyloxy CH_2), 6.55–7.50 (m, 14 H, reducing to 13 H on addition of D_2O, NH and aromatic protons), 9.00 (br s, 1 H, replaceable with D_2O, COOH). Anal. ($C_{26}H_{25}NO_5$) C, H, N.

Preparation of N-Protected Pentapeptides. As a general method, a solution of the appropriate racemic *N*-benzyloxycarbonyl-protected amino acid (1.9 mmol), glycylglycyl-L-phenylalanyl-L-leucine methyl ester (4;[12] 1.9 mmol), and triethylamine (2.2 mmol) in CH_2Cl_2 (50 mL) was cooled in an ice bath, and 1-hydroxybenzotriazole (3.9 mol) in CH_2Cl_2 (10 mL) was added dropwise. The mixture was stirred for 5 min before adding *N*,*N*′-dicyclohexylcarbodiimide (2.1 mol) in CH_2Cl_2 (20 mL) in one portion. The ice bath was removed, the reaction was stirred at room temperature for 56 h, the precipitated dicyclohexylurea was removed by filtration, and the filtrate was evaporated to a volume of 10 mL and then refiltered. The resulting filtrate was evaporated to dryness, dissolved in ethyl acetate (50 mL), and washed with 1 N HCl (50 mL), saturated $NaHCO_3$ solution (50 mL), and finally water (50 mL). The organic layer was then dried and filtered, and the filtrate was evaporated to dryness. The resulting gummy residue was triturated with diethyl ether to afford the appropriate N-protected pentapeptide as a buff-colored, amorphous solid, consisting of a mixture of diastereoisomers.

[[5-(Benzyloxy)-2-[(benzyloxy)carbamido]-2-indanyl]carbonyl]glycylglycyl-L-phenylalanyl-L-leucine methyl ester (2d) was prepared as above from **2c** in 74% yield: mp 75–78 °C; TLC (silica) R_f (ethyl acetate/methanol, 40:60) 0.64; IR (KCl) 3175, 3050, 3020, 2945, 2920, 2860, 1718, 1682, 1670, 1645, 1635, 1535, 1525, 1518 cm^{-1}; NMR ($CHCl_3$, 300 MHz) 0.86 [d, 6 H, leucine δ-$(CH_3)_2$], 1.57 (m, 2 H, leucine β-CH_2), 1.98 (m, 1 H, leucine γ-CH_2), 3.06 (d of d, 4 H, indan 1- and 3-protons), 3.29 (m, 2 H, phenylalanine β-CH_2), 3.56 and 3.61 (2 s, 3 H, leucine OCH_3), 3.72 (m, 2 H, one of glycine α-CH_2's), 3.85 (m, 2 H, one of glycine α-CH_2's), 4.54 (m, 1 H, leucine α-CH), 4.68 (m, 1 H, phenylalanine α-CH), 5.01 (d, 4 H, 2 benzyloxy CH_2's), 6.72–7.45 (m, 18 H, aromatic protons), 6.82 (d, 1 H, replaceable with D_2O, NH), 7.20 (m, 2 H, replaceable with D_2O, 2 NH), 7.72 (m, 1 H, replaceable with D_2O, NH), 8.30 (m, 1 H, replaceable with D_2O, NH). Anal. ($C_{45}H_{51}N_5O_9$) C, N; H: calcd, 6.4; found, 6.9.

[[6-(Benzyloxy)-2-[(benzyloxy)carbamido]-2-tetralinyl]carbonyl]glycylglycyl-L-phenylalanyl-L-leucine methyl ester (3d) was prepared as above from **3c** in 89% yield: mp 78–81 °C; TLC (silica) R_f (chloroform/methanol/acetic acid, 120:90:5) 0.68; IR (KCl) 3265, 3060, 3020, 2950, 2920, 2855, 1758, 1688, 1675, 1655, 1545, 1525 cm^{-1}; NMR ($CDCl_3$, 300 MHz) δ 0.88 [d, 6 H, leucine δ-$(CH_3)_2$], 1.29 (m, 2 H, leucine β-CH_2), 1.56 (m, 1 H, leucine γ-CH_2), 1.94 (m, 2 H, tetralin 3-protons), 3.12 (m, 4 H, tetralin 1- and 4-protons), 3.34 (m, 2 H, phenylalanine β-CH_2), (s, 3 H, leucine OCH_3), 3.87 (m, 2 H, one of glycine α-CH_2's), 3.93 (m, 2 H, one of glycine α-CH_2's), 4.51 (m, 1 H, leucine α-CH), 4.72 (m, 1 H, phenylalanine α-CH), 5.02 (m, 4 H, benzyloxy CH_2's), 6.32 (m, 1 H, replaceable with D_2O, NH), 6.74–7.60 (m, 18 H, aromatic protons), 7.12 (m, 2 H, replaceable with D_2O, 2 NH), 7.80 (m, 1 H, replaceable with D_2O, NH), 8.31 (m, 1 H, replaceable with D_2O, NH). Anal. ($C_{46}H_{53}N_5O_9$) C, H, N.

Synthesis of Unprotected Pentapeptides. As a general method, the appropriate N-protected pentapeptide (16 nmol) was dissolved in absolute ethanol (100 mL), 5% palladium on charcoal catalyst (0.5 g) was added, and the mixture was hydrogenated, with stirring, at room temperature and atmospheric pressure for 20 h. The catalyst was then removed by filtration, the filtrate was evaporated to dryness, and the residue was dissolved in ethyl acetate (50 mL). The organic solution was extracted with 1 N hydrochloric acid (2 × 50 mL), followed by water (1 × 30 mL). The combined aqueous extracts were basified to pH 9 with solid sodium bicarbonate and back extracted with ethyl acetate (2 × 50 mL). The combined ethyl acetate extracts were dried and filtered, the filtrate was evaporated to dryness, and the residue was purified on a carboxymethylcellulose column, eluting with ammonium acetate buffer, as previously described, to afford the

appropriate N-deprotected pentapeptide in buffer solution. The water and buffer salts were removed by freeze-drying, redissolving the residue in water (200 mL), and redrying on an Edwards EF03 freeze-drying apparatus as 10^{-1} to 10^{-2} torr.

[(2-Amino-5-hydroxy-2-indanyl)carbonyl]glycylglycyl-L-phenylalanyl-L-leucine methyl ester (2e) was prepared as above from **2d** in 13% yield: mp 173-176 °C; TLC (silica) R_f (chloroform/methanol/acetic acid, 120:90:5) 0.68; IR (KCl) 3395, 3358, 3315, 3280, 3080, 3035, 2960, 2925, 2860, 1750, 1690, 1652, 1635, 1585, 1558, 1540, 1525, 1508 cm^{-1}; NMR ($CDCl_3$, 220 MHz) 0.82 [m, 6 H, leucine δ-$(CH_3)_2$], 1.50 (m, 2 H, leucine β-CH_2), 1.95. (m, 1 H, leucine γ-CH), 2.62 (m, 2 H, phenylalanine β-CH_2), 2.96 (m, 4 H, indan 1- and 3-protons), 3.62 (s, 3 H, leucine OCH_3), 3.65 (br s, replaceable with D_2O, NH_2 and OH), 3.69 (m, 2 H, one of glycine α-CH_2's), 3.75 (m, 2 H, one of glycine α-CH_2's), 4.24 (m, 1 H, leucine α-CH), 4.60 (m, 1 H, phenylalanine α-CH), 6.50-7.16 (m, 8 H, aromatic protons), 8.03 (m, 2 H, replaceable with D_2O, 2 NH), 8.34 (m, 1 H, replaceable with D_2O, NH) 8.42 (m, 1 H, replaceable with D_2O, NH). Anal. ($C_{30}H_{39}N_5O_7$) C, H, N; amino acid (after acidic hydrolysis): **2a**, 0.94; Gly, 2.03; Phe, 1.01; Leu, 1.00.

[(2-Amino-6-hydroxy-2-tetralinyl)carbonyl]glycylglycyl-L-phenylalanyl-L-leucine methyl ester (3e) was prepared as above from **3d** in 34% yield: mp 100-104 °C; TLC (silica) R_f (chloroform/methanol/acetic acid, 120:90:5) 0.70; IR (KCl)) 3270, 3055, 3020, 2950, 2930, 2860, 1740, 1690, 1675, 1645, 1565, 1546, 1500 cm^{-1}; NMR ($CDCl_3$, 300 MHz) 0.80 and 0.85 [2 d, 6 H, leucine δ-$(CH_3)_2$], 1.33 (m, 2 H, leucine β-CH_2), 1.51 (m, 1 H, leucine γ-CH), 1.56 (m, 2 H, tetralin 3-protons), 3.06 (m, 2 H, phenylalanine β-CH_2), 3.07 (m, 4 H, tetralin 1- and 4-protons), 3.65 (s, 3 H, leucine OCH_3), 3.82 (m, 2H, one of glycine α-CH_2's), 3.93 (m, 2 H, one of glycine α-CH_2's), 4.30 (br s, 3 H, replaceable with D_2O, NH_2 and OH), 4.47 (m, 1 H, leucine α-CH), 4.70 (m, 1 H, phenylalanine α-CH), 6.56-7.40 (m, 8 H, aromatic protons), 7.00 (m, 2 H, replaceable with D_2O, 2 NH), 7.42 (m, 1 H, replaceable with D_2O, NH), 8.43 (m, 1 H, replaceable with D_2O, NH). Anal. ($C_{31}H_{41}N_5O_7$) C, H, N; amino acid (after acidic hydrolysis): **3a** no color reaction with ninhydrin; Gly, 2.08; Phe, 1.00; Leu, 1.00.

Pharmacology. Compounds were evaluated for analgesic properties in albino mice (Tuck, TFW strain) by the following procedures: in vitro testing was carried out by measuring the inhibition of electrically stimulated contractions of the guinea pig ileum myenteric plexus muscle using the method of Kosterlitz and Watt[13] and by measuring the inhibition of mouse vas deferens tissue after stimulation wth twin rectilinear pulses 10-ms apart.[14] In vivo evaluation was carried out by using the mouse hot-plate test.[15]

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Registry No. **2a**, 33709-81-8; **2b**·HCl, 84802-81-3; **2c**, 84802-82-4; **2d**, 84802-83-5; **2e**, 73309-72-5; **3a**, 84809-70-1; **3b**·HCl, 84802-84-6; **3c**, 84802-85-7; **3d**, 84802-86-8; **3e**, 73301-07-2; 4, 68709-94-4; benzyl bromide, 100-39-0; carbobenzyloxy chloride, 501-53-1.

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Antidepressant Activity of 5-Aryl-2,3,5,6-tetrahydroimidazo[2,1-*a*]isoquinolin-5-ols

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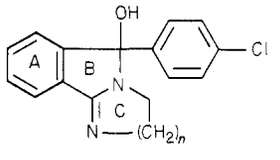
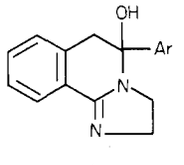
A series of 5-aryl-2,3,5,6-tetrahydroimidazo[2,1-*a*]isoquinolin-5-ols was prepared and evaluated for potential antidepressant activity in the reserpine-induced hypothermia model and selected central nervous system and autonomic activity tests. Several members of the series, notably the 4-chloro- and 4-fluorophenyl analogues, demonstrated pharmacological activity in the range of imipramine. Both compounds provided a marked potentiation of the 5-hydroxytryptophan-facilitated monosynaptic spike in the spinal cat preparation.

During the development of the anorectic agent mazindol (1, Sanorex, Teronac), it was found that the compound produced in animals a profile of CNS activity between that



1, n = 1
2, n = 2
3, n = 3



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observed with the centrally acting sympathomimetics and the antidepressant agents amitriptyline and imipramine.[1] Evidence for the latter activity was noted from antagonism of reserpine-induced lowering of body temperature in mice and antagonism of tetrabenazine-induced catalepsy in rats. The six- and seven-member ring C homologues **2** and **3** possess very weak anorectic activity[2] and and appear to be devoid of CNS activity.[3] In an attempt to determine the effect of modifying the size of ring B of mazindol (1), we prepared and evaluated 5-(*p*-chlorophenyl)-2,3,5,6-tetrahydroimidazo[2,1-*a*]isoquinolin-5-ol (4, Ar = 4-ClC_6H_4) for anorectic and antidepressant activity. The substance showed minimum anorectic activity in rats but was a potent antagonist of reserpine-induced hypothermia in mice. This finding prompted us to prepare a number of analogues of 4 and evaluate them for potential antidepressant activity.

Chemistry. The synthesis of 4 was carried out by reaction of the recently reported[4] dilithium reagent of 2-

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