Leucine Enkephalin Analogues Containing a Conformationally Restrained *N*-Terminal Amino Acid Residue

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Abstract □ Three analogues of leucine enkephalin, in which the terminal tyrosine-1 residue has been replaced by conformationally restrained aromatic amino acids, have been synthesized by classical solution methods. Their opiate agonist potencies on electrically stimulated guinea pig ileum and mouse vas deferens preparations were determined and compared with morphine, Met enkephalin, and Leu enkephalin. None of these analogues had analgesic properties when evaluated on the above tissue preparations or when evaluated by the hot-plate test in mice after subcutaneous and intracerebroventricular administration.

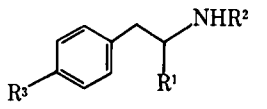
Keyphrases □ Leucine enkephalin—conformationally restrained analogues, synthesis, analgesic activity □ Analgesics—evaluation of conformationally restrained leucine enkephalin derivatives, synthesis

A milestone in neurochemical research during the last decade has been the elucidation of the role of enkephalins as natural endogenous ligands for the opiate receptor (1, 2). Since this discovery, the synthesis of a multitiude of structural analogues of leucine and methionine enkephalins (Ig and Ih, respectively) have been reported (recent review, 3), some of which are more potent analgesics and/or possess greater *in vivo* stability than the natural opiate ligands. These synthetic analogues have resulted from modifications of the parent molecules, such as shortening or lengthening of the pentapeptide chain (4, 5), substitution of individual amino acids by other amino acids (5–11), and chemical modification of individual amino acids (12–14).

Preferred conformations of enkephalin molecules at analgesic receptors have been suggested, based on data from conformational studies involving both spectroscopic measurements and structure–activity considerations (15–33). These studies have attempted to relate the structure of the terminal tyrosine residue in the enkephalin molecule to the tyramine moiety present in the morphine molecule. Since morphine is a conformationally rigid molecule, this implies that the conformationally "loose" tyrosine residue in the enkephalin may interact at opiate receptors in a specific conformation which is related, stereochemically, to the rigid tyramine moiety present in the morphine molecule.

As part of a study designed to evaluate the importance of the tyrosine conformation in the enkephalins on analgesic activity and receptor recognition, we have initiated a preliminary investigation into the synthesis of Leu enkephalin derivatives in which the terminal tyrosine unit has been replaced by a variety of conformationally restrained aromatic amino acids and in which the terminal leucine-5 residue has been esterified to aid passage into the central nervous system. The investigations described in this report are restricted to the preparation of Leu enkephalin analogues containing a nonhydroxylated aromatic amino acid in place of the tyrosine-1 residue in order to initially determine the feasibility of preparing pentapeptides such as IIe–Ve, with a bulky terminal amino acid unit, *via* classical solution methods, before embarking on the preparation of the synthetically more difficult aromatic hydroxy derivatives IIf–Vf, which were regarded as the ultimate target compounds.

We now report the synthesis of the Leu enkephalin analogues IIe–IVe and their evaluation as analgesic agents on isolated guinea pig ileum myenteric plexus muscle, mouse vas deferens tissue, and by the hot-plate test in mice after subcutaneous and intracerebroventricular administration.



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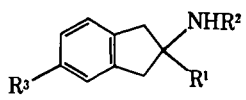

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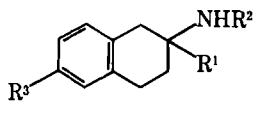

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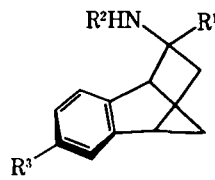
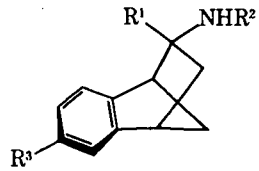


IV



V

a R^1 = COOH, R^2 = R^3 = H.
b R^1,R^2 = CO—NHCO—, R^3 = H.
c R^1 = COOH, R^2 = CO—O—CH_2Ph, R^3 = H.
d R^1 = CONHGlyGlyL—PheL—LeuOCH$_3$,
R^2 = CO—OCH_2Ph, R^3 = H.
e R^1 = CONHGlyGlyL—PheL—LeuOCH$_3$, R^2 = R^3 = H.
f R^1 = CONHGlyGlyL—PheL—LeuOCH$_3$, R^2 = H, R^3 = OH.
g R^1 = CONHGlyGlyL—PheL—LeuOH, R^2 = H, R^3 = OH.
h R^1 = CONHGlyGlyL—PheL—MetOH, R^2 = H, R^3 = OH.

H_2NGlyGlyL—PheL—LeuOCH$_3$

VI

EXPERIMENTAL

Melting points were determined on a hot-stage microscope[1] 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. ^{1}H-NMR spectra were recorded on a spectrometer[2] and are quoted in ppm on the δ scale. ^{13}C-NMR spectra were recorded on a spectrometer[3]. IR spectra were recorded as KCl disks, nujol mulls, or liquid films

[1] Reichert.
[2] Model HR 220 or SC 300; Varian Instruments.
[3] Model WP 80; Bruker.

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on a spectrophotometer[4]. Mass spectra were determined on a spectrometer[5] operating at a probe temperature of 250°C. TLC separations were carried out on 0.25-mm silica gel[6]. 2-Aminoindan-2-carboxylic acid (34) and (±) 2-aminotetralin-2-carboxylic acid (35) 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; other compounds were visualized in iodine vapor. Hydrogenations were carried out on a hydrogenator[7] operating at atmospheric pressure and room temperature.

Ion-exchange chromatographic separations of *N*-deprotected pentapeptides were carried out on carboxymethylcellulose[8], prewashed with 0.5 M sulfuric acid followed by 0.2 M ammonium acetate buffer adjusted to pH 5.1 with 0.88 M ammonium hydroxide or glacial acetic acid and measured using a pH meter[9] fitted with a combined electrode[10]. Ion-exchange columns were run under slight pressure using a peristaltic pump set for an effluent output of 100 mL/h. Column effluents were monitored by UV spectrophotometry at 280 nm using a spectrophotometer[11] fitted with a 10-mm path length flow-through cell. Columns were eluted with 0.005–0.5 M 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 an amino acid analyzer[12] using ninhydrin as visualizer. The peptides were hydrolyzed in 6 M HCl at 110°C for 24 h in a sealed, evacuated tube.

Synthesis of *endo*-2-Aminobenzonorbornen-2-carboxylic Acid (IVa)—*Spiro[benzonorbornen-2,5′-hydantoin] (IVb)*—A solution of ammonium carbonate (28.6 g, 0.18 mol) in 50% aqueous ethanol (200 mL) was added to benzonorbornen-2-one (36) (11.1 g, 0.07 mol) in 50% aqueous ethanol (40 mL). The mixture was warmed to 50°C and a solution of potassium cyanide (4.6 g, 0.072 mol) in water (40 mL) was added in small portions over a 1-h period. The mixture was stirred at 58–60°C for 5 h. The ethanol was removed under reduced pressure, and the aqueous residue was extracted with ethyl acetate (3 × 200 mL). The combined organic extracts were dried, filtered, and evaporated to dryness, and the residue was triturated with ether to afford white crystals of IVb mp 225–229°C. The aqueous layer was acidified to pH 2 with concentrated sulfuric acid to afford an additional product for a total of 12.07 g 76%). This compound had identical spectrometric properties (IR, NMR) to an authentic sample recently prepared *via* a Strecker synthesis (48).

endo-*2-Aminobenzonorbornen-2-carboxylic Acid (IVa)*—A mixture of spiro[benzonorbornen-2,5′-hydantoin] (IVb) (6.63 g, 0.029 mol), barium hydroxide [$Ba(OH)_2{\cdot}8H_2O$, 17.6 g, 0.056 mol], and water (100 mL) was heated under reflux, in an atmosphere of nitrogen, for 70 h and then filtered while hot. The collected solid was washed with an equal volume of water, and the combined filtrate and washings were saturated with carbon dioxide, heated to boiling point, and refiltered. The filtrate (on cooling) afforded white crystals of IVb (140 mg, 2% recovery). The mother liquors were evaporated to ~50 mL to afford white crystals of IVa (4.69 g, 79%), mp 227.0–229.5°C [lit. (48) mp 227.5–229.5°C]. The hydrochloride salt was prepared from a portion of the product and had mp 228–231°C (dec.). ^{13}C-NMR (CH_3OH): 173.8 (s, CO_2H), 149.5 and 141.8 (2 × s, C-4a and C-8a), 129.5, 128.0, 125.2, and 123.1 (4 × d, ArC), 65.1 (s, C-2), 53.9 (d, C-4), 51.1 (d, C-1), 44.9 (t, C-9), and 39.8 (t, C-3); *m/z* 203 (M^+).

Preparation of *N*-Benzyloxycarbonyl Amino Acids—As a general method, the appropriate amino acid (0.011 mol) suspended in 2 M NaOH solution (100 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. Carbobenzyloxy chloride (2.11 g, 0.012 mol) was added in a dropwise manner 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 mixture was cooled to 0–5°C, and a further 2.11 g (0.012 mol) of carbobenzyloxy chloride added in a dropwise manner. After 60 h the mixture was carefully adjusted to pH 1 with 5 M HCl and extracted with ether (2 × 100 mL). The combined organic extracts were dried, filtered, and evaporated to dryness, and the residue was crystallized from ether–petroleum ether (bp 30–40°C) to afford the appropriate *N*-benzyloxycarbonyl amino acid.

2-Benzyloxycarbamidoindan-2-carboxylic acid (IIc)—This compound was prepared as described above from IIa in 32% yield, mp 46.5–53.0°C. IR (Nujol): 1748, 1663, 1545, and 1532 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 3.48 (m, 4, C-1 and C-3 H), 5.04 (s, 2, benzyloxy CH_2), 7.05–7.45 (m, 9, ArH), 7.88 (s, 1, exchangeable with D_2O, NH), and 9.10 ppm (br s, 1, exchangeable with D_2O, COOH); *m/z* 311 (M^+).

Anal.—Calc. for $C_{18}H_{17}NO_4$: C, 69.5; H, 5.5; N, 4.5. Found: C, 69.9; H, 5.8; N, 4.2.

2-Benzyloxycarbamidotetralin-2-carboxylic Acid (IIIc)—Compound IIIc was prepared as described above from IIIa in 19% yield, mp 131.0–137.5°C. IR (KCl): 1772, 1728, 1688, 1680, 1580, and 1526 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.24 (m, 2, C-3 H), 2.80 (m, 2, C-4 H), 3.18 (m, 2, C-1 H), 5.05 (s, 2, benzyloxy CH_2), 5.30 (br s, 1, exchangeable with D_2O, NH), 6.90–7.50 (m, 9, ArH), and 10.32 (br s, 1, exchangeable with D_2O, COOH); *m/z* 325 (M^+).

Anal.—Calc. for $C_{19}H_{19}NO_4$: C, 70.2; H, 5.8; N, 4.3. Found: C, 70.1, H, 5.9, N, 4.0.

endo-*2-Benzyloxycarbamidobenzonorbornene-2-carboxylic Acid (IVc)*—This compound was prepared as described above from IVa in 27% yield, mp 90–93°C. IR (KCl): 1742, 1686, 1641, 1600, and 1582 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.28 (d of d, 1, J = 3 and 12 Hz, C-3 *endo*-H), 1.91 and 2.26 (2 × d, AB, J = 9 Hz, C-9 H), 2.95 (d of d, 1, J = 4 and 12 Hz, C-3 *exo*-H), 3.37 (m, 1, C-4 H), 3.84 (m, 1, C-1 H), 4.80 (br s, 1, exchangeable with D_2O, NH), 5.00 (d, 2, J = 4 Hz, benzyloxy CH_2), 6.95–7.50 (m, 9, ArH), and 11.05 ppm (s, 1, exchangeable with D_2O, COOH).

Anal.—Calc. for $C_{20}H_{25}NO_5$: C, 71.2; H, 5.6; N, 4.2. Found: C, 71.6; H, 5.8; N, 4.0.

Preparation of *N*-Protected Pentapeptides—As a general method, a solution of the appropriate *N*-benzyloxycarbonyl amino acid (1.9 mmol), glycylglycyl-L-phenylalanyl-L-leucine methyl ester (VI) (37) (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 in a dropwise manner. 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 M HCl (50 mL), saturated $NaHCO_3$ solution (50 mL), and finally water (50 mL). The organic layer was then dried, filtered, and evaporated to dryness. The resulting gummy residue was triturated with ether to afford the appropriate *N*-protected pentapeptide as a buff-colored, amorphous solid.

{[(*2 - Benzyloxycarbamido) -2- indanyl]carbonyl}glycylglycyl-L-phenylalanyl-L-leucine Methyl Ester (IId)*—Compound IId was prepared as above from IIc in 61% yield, mp 134–138°C. TLC (silica, ethyl acetate–methanol, 40:60): R_f 0.60; IR (KCl): 1726, 1675, 1665, 1650, 1595, 1532, and 1520 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.81 [d, 6, leucine δ-$(CH_3)_2$], 1.51 (m, 2, leucine β-CH_2), 1.78 (m, 1, leucine γ-CH), 3.07 (d of d, 4, indane 1- and 3-H), 3.09 (m, 2, phenylalanine β-CH_2), 3.55 and 3.57 (2 × s, 3, conformational forms of leucine OCH_3), 3.72 (m, 2, one of glycine α-CH_2's), 3.92 (m, 2, one of glycine α-CH_2's), 4.49 (m, 1, leucine α-CH), 4.71 (m, 1, phenylalanine α-CH), 4.99 (d, 2, $C_6H_5OCH_2$—O), 6.28 (d, 1, exchangeable with D_2O, NH), 7.03 (t, 2, exchangeable with D_2O, 2 × NH), 7.08–7.35 (m, 14, ArH), and 7.66 ppm (m, 2, exchangeable with D_2O, 2 × NH).

Anal.—Calc. for $C_{38}H_{45}N_5O_8$: C, 65.2; H, 6.2; N, 10.0. Found: C, 65.3; H, 6.2; N, 10.0.

{[(*2-Benzyloxycarbamido)-2-tetralyl]carbonyl} glycylglycyl-L-phenylalanyl-L-leucine Methyl Ester (IIId)*—This compound was prepared as above from IIIc in 91% yield, mp 86–90°C. TLC (silica, chloroform–methanol–acetic acid, 120:90:5): R_f 0.62; IR (KCl): 1740, 1725, 1704, 1690, 1675, 1660, 1564, and 1530 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.87 [d, 6, leucine δ-$CH_3)_2$], 1.55 (m, 2, leucine β-CH_2), 2.22 (m, 1, leucine γ-CH), 2.41 (m, 2, tetralin 3-H), 2.60–3.80 (m, 4, tetralin 1- and 4-H), 3.32 (m, 2, phenylalanine β-CH_2), 3.52 and 3.54 (2 × s, 3, leucine OCH_3's), 3.80 (m, 2, one of glycine α-CH_2's), 3.91 (m, 2, one of glycine α-CH_2's), 4.54 (m, 1, leucine α-CH), 4.73 (m, 1, phenylalanine α-CH), 5.02 (d, 2, benzyloxy CH_2), 6.78–7.70 (m, 14, ArH), 6.83 (d, 1, exchangeable with D_2O, NH), 7.16 (t, 2, exchangeable with D_2O, 2 × NH), 7.78 (m, 1, exchangeable with D_2O, NH), and 7.89 ppm (m, 1, exchangeable with D_2O, NH).

Anal.—Calc. for $C_{39}H_{47}N_5O_8$: C, 65.6; H, 6.6; N, 9.8. Found: C, 65.2; H, 6.7; N, 10.1.

{[(endo-*2-Benzyloxycarbamido) -2- benzonorbornenyl]carbonyl}glycylglycyl-L-phenylalanyl-L-leucine Methyl Ester (IVd)*—Compound IVd was prepared as above from IVc in 43% yield, mp 118–121°C. TLC (silica, chloroform–methanol–acetic acid, 120:90:5): R_f 0.72; IR (KCl): 1740, 1705, 1690, 1660, 1632, 1565, 1545, and 1520 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.84 [d, 6, leucine δ-$(CH_3)_2$], 1.30 (m, 1, benzonorbornenyl C-3 *exo*-H), 1.53 (m, 2, leucine β-CH_2), 1.94 (m, 1, leucine γ-CH), 1.98 (m, 2, benzonorbornenyl C-9 H), 2.64 (m, 1, benzonorbornenyl C-3 *endo*-H), 3.09 (m, 2, phenylalanine

[4] Model 237; Perkin-Elmer.
[5] A.E.I. model M59.
[6] Polygram silica gel UV_{254}.
[7] Gallenkampf.
[8] Whatman CMC 52.
[9] Model PW 9418; Pye Unicam.
[10] Pye Unicam 401.
[11] Cecil CE 202.
[12] *Model 123*; Beckmann SPINCO.

β-CH_2), 3.62 (s, 3, leucine OCH_3), 3.63 (m, 2, benzonorbornenyl C-1 and C-4 H), 3.72 (m, 2, one of glycine α-CH_2's), 3.94 (m, 2, one of glycine α-CH_2's), 4.52 (m, 1, leucine α-CH), 4.69 (m, 1, phenylalanine α-CH), 5.17 (d, 2, benzyloxy CH_2), 6.38 (m, 1, exchangeable with D_2O, NH), 6.70–7.55 (m, 16, ArH and 2 exchangeable × NH), 7.60 (m, 1, exchangeable with D_2O, NH), and 7.70 ppm (m, 1, exchangeable with D_2O, NH).

Anal.—Calc. for $C_{40}H_{47}N_5O_8$: C, 66.2; H, 6.5, N, 9.2. Found: C, 65.8; H, 6.6; N, 8.9.

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 evaporated to dryness, and the residue dissolved in ethyl acetate (50 mL). The organic solution was extracted with 1 M HCl (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, filtered, and 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 a freeze-drying apparatus[13] at 10^{-1}–10^{-2} torr.

[*(2-Aminoindanyl)carbonyl*]*glycylglycyl -L- phenylalanyl -L- leucine Methyl Ester (IIe)*—This compound was prepared as above from IId in 17% yield, mp 93–95°C. TLC (silica, chloroform–methanol–acetic acid, 120:90:5): R_f 0.69; IR (KCl): 1740, 1686, 1672, 1650, 1580, 1562, 1545, and 1525 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.85 and 0.93 [t and d, 6, leucine δ-$(CH_3)_2$], 1.60 (m, 2, leucine β-CH_2), 2.12 (m, 1, leucine γ-CH), 2.82 (d of d, 4, indane 1- and 3-H), 3.08, (m, 2, phenylalanine β-CH_2), 3.65 and 3.70 (2 × s, 3, leucine OCH_3), 3.81 (s, 2, exchangeable with D_2O, NH_2), 3.95 (d of d, 2, one of glycine α-CH_2's), 4.06 (d of d, 2, one of glycine α-CH_2's), 4.48 (m, 1, leucine α-CH), 4.92 (t, 1, phenylalanine α-CH), 7.00–7.31 (m, 9, ArH), 7.26 (d, 1, exchangeable with D_2O, NH), 7.49 (d, 1, exchangeable with D_2O, NH), 7.63 (m, 1, exchangeable with D_2O, NH), and 8.45 ppm (m, 1, exchangeable with D_2O, NH); amino acid analysis (after acidic hydrolysis): 2d 0.95, Gly 1.97, Phe 1.00, and Leu 1.01.

Anal.—Calc. for $C_{30}H_{39}N_5O_6 \cdot H_2O$: C, 61.7; H, 7.1; N, 12.0. Found: C, 61.7; H, 6.8; N, 11.6.

[*(2-Aminotetralyl)carbonyl*] *glycylglycyl -L- phenylalanyl -L- leucine Methyl Ester (IIIe)*—Compound IIIe was prepared as above from IIId in 11% yield, mp 165–170°C. TLC (silica, chloroform–methanol–acetic acid, 120:90:5): R_f 0.61; IR (KCl): 1740, 1690, 1678, 1655, 1550, and 1522 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.92 [m, 6, leucine δ-$(CH_3)_2$], 1.39 (m, 2, leucine β-CH_2), 1.58 (m, 2, tetralin 3-H), 1.82 (br s, 2, exchangeable with D_2O, NH_2), 1.92 (m, 1, leucine γ-CH), 3.01 (m, 4, tetralin 1- and 4-H), 3.38 (m, 2, phenylalanine β-CH_2), 3.70 and 3.72 (2 × s, 3, leucine OCH_3), 3.98 (m, 2, one of glycine α-CH_2's), 4.05 (m, 2, one of glycine α-CH_2's), 4.46 (m, 1, leucine α-CH), 4.82 (m, 1, phenylalanine α-CH), 6.88 (m, 1, exchangeable with D_2O, NH), 6.90–7.45 (m, 9, ArH), 7.54 (m, 1, exchangeable with D_2O, NH), 7.64 (m, 1, exchangeable with D_2O, NH), and 8.64 ppm (m, 1, exchangeable with D_2O, NH); amino acid analysis (after acidic hydrolysis): 3d 0.92, Gly 2.02, Phe 1.00, and Leu 1.12.

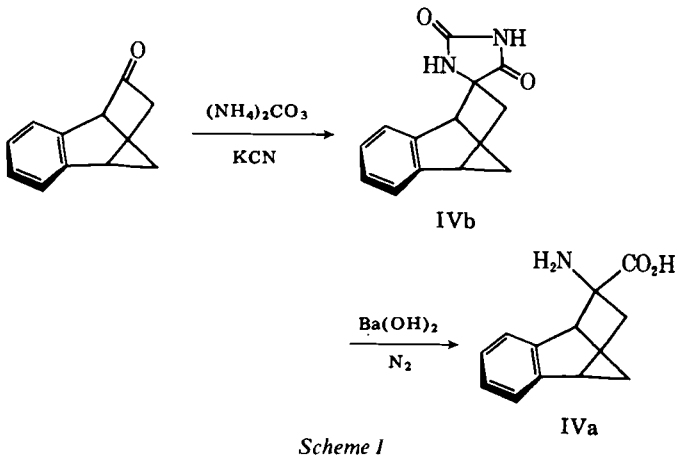
Anal.—Calc. for $C_{31}H_{41}N_5O_6 \cdot H_2O$: C, 62.3; H, 7.2; N, 11.7. Found: C, 62.8; H, 6.7; N, 11.2.

[(endo-*2-Aminobenzonorbornenyl)carbonyl*] *glycylglycyl -L- phenylalanyl-L-leucine Methyl Ester (IVe)*—This compound was prepared as above from IVd in 31% yield, mp 113–116°C. TLC (silica, chloroform–methanol–acetic acid, 120:90:5): R_f 0.78; IR (KCl): 1742, 1703, 1688, 1657, 1560, 1545, and 1520 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.85 [m, 6, leucine δ-$(CH_3)_2$], 1.32 (m, 2, leucine β-CH_2), 1.52 (d of d, 1, benzonorbornene C-3 *endo*-H), 1.60 (m, 1, leucine γ-CH), 1.70 (m, 2, benzonorbornene C-9 H), 1.92 (m, 2, benzonorbornene C-3 *exo*-H), 2.47 (m, 2, benzonorbornene 1- and 4-H), 3.08 (m, 2, phenylalanine β-CH_2), 3.34 (br s, 2, exchangeable with D_2O, NH_2), 3.65 (m, 3, leucine OCH_3), 3.90 (m, 2, one of glycine α-CH_2's), 4.04 (m, 2, one of glycine α-CH_2's), 4.52 (m, 1, leucine α-CH), 4.76 (m, 1, phenylalanine α-CH), 6.80–7.56 (m, 9, ArH), 7.02 (m, 1, exchangeable with D_2O, NH), 7.63 (m, 1, exchangeable with D_2O, NH), 7.77 (m, 1, exchangeable with D_2O, NH), and 8.70 ppm (m, 1, exchangeable with D_2O, NH); amino acid analysis (after acidic hydrolysis): 4d 0.91, Gly 2.4, Phe 1.00, and Leu 1.01.

Anal.—Calc. for $C_{32}H_{41}N_5O_6 \cdot H_2O$: C, 63.1; H, 7.1; N, 11.5. Found: C, 62.9; H, 7.4; N, 11.5.

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 electronically stimulated contractions of the guinea pig ileum myenteric plexus muscle using the method of Kosterlitz and Watt (38) and by measuring the inhibition of mouse vas deferens tissue after stimulation with twin rectilinear pulses 10 ms apart (39). *In vivo* evaluation was carried out using the mouse hot-plate test (40).

[13] Model EF03; Edwards.



Scheme I

RESULTS

Chemistry—The amino acids IIa and IIIa were prepared by literature methods (34, 35). Synthetic routes to IVa and Va were designed based on the stereochemistry of the products that have been obtained from Strecker and Bucherer reactions on norbornane-2-one and related compounds (41–47). Reaction of benzonorbornen-2-one with ammonium carbonate and KCN afforded exclusively the expected spirohydantoin, IVb, which could be hydrolyzed with barium hydroxide to the *endo*-amino acid, IVa (Scheme I). The unequivocal structural assignment of IVa was determined from data obtained from ^{1}H- and ^{13}C-NMR spin-spin coupling data and from two-dimensional ^{1}H-NMR studies, both of which are reported by us elsewhere (48). Attempted synthesis of Va from benzonorbornen-2-one *via* a modified Strecker synthesis resulted in formation of only the *endo*-amino acid IVa (48).

The amino acids IIa, IIIa, and IVa were each *N*-protected with carbobenzoxy chloride in base and coupled with tetrapeptide VI (37) using the *N,N'*-dicyclohexylcarbodiimide-1-hydroxybenztriazole method. Hydrogenolysis of the protected pentapeptides IId, IIId, and IVd and purification of the resulting deprotected products by gradient buffer elution from carboxymethylcellulose at pH 5.1 afforded the pentapeptide methyl esters IIe, IIIe, and IVe.

Pharmacology—Compound IIe exhibited only weak analgesic properties when evaluated on electrically stimulated guinea pig ileum, showing an ID_{50} of 3.8 μm (ID_{50} values for morphine, Met enkephalin, and Leu enkephalin on the same preparation are 90.5, 86.8, and 450 nM, respectively), whereas compounds IIIe and IVe were inactive. None of the pentapeptides were active analgesics in the mouse vas deferens preparation or in the hot-plate test in mice after subcutaneous and intracerebroventricular injection.

CONCLUSIONS

This study has developed synthetic routes, based on classical solution techniques, which can be used to prepare Leu enkephalin derivatives in which the terminal tyrosine-1 residue has been replaced by a variety of conformationally restrained amino acid moieties, as represented in structures IIe, IIIe, and IVe. None of these pentapeptides possess any significant analgesic activity, both *in vitro* and *in vivo*, which is consistent with the observation that enkephalins lacking an aromatic *para*-hydroxy group in the tyrosine-1 moiety are inactive as analgesics (4, 7). The synthetic procedures developed in this study should be of value in the preparation of Leu enkephalin derivatives incorporating aromatic hydroxylated derivatives of IIa, IIIa, and IVa in place of the tyrosine-1 moiety.

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