A Heterocyclic Analog of Leucine-Enkephalin

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A series of di-, tri-, tetra- and pentapeptide analogs of leucine-enkephalin have been prepared in which the initial tyrosinylglycine fragment has been replaced by the 5,6-dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazineacetic acid moiety.

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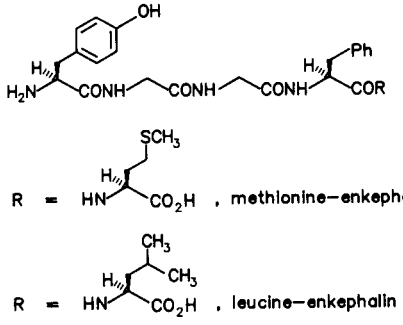
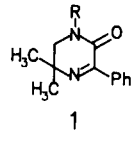
The discovery of the endogenous opioid peptides methionine- and leucine-enkephalin [1,2] has opened an exciting avenue for drug related research and numerous synthetic analogs of these substances have been examined [3,4]. We have been engaged in the syntheses of enkephalin analogs in which various portions of the endogenous peptide have been incorporated into heterocyclic rings



[5-7]. Changes of this nature might be expected to more rigidly define elements of peptide secondary structure as well as confer more resistance to enzymatic degradation [8], the primary mode of biological deactivation of these substances [9]. As part of this project, we have prepared enkephalin analogs in which both the tyrosine unit and the amino function of the initial glycine have been replaced by the 5,6-dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)pyrazine moiety **1**. This has resulted in a series of modified



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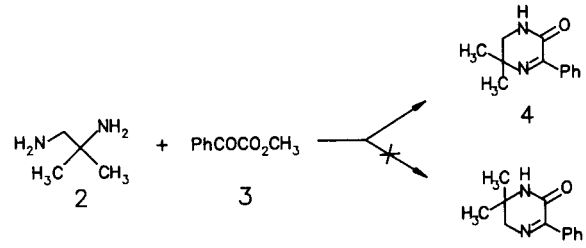
peptides whose chain lengths have been increased in a stepwise fashion [10] ultimately yielding a heterocyclic analog of leucine-enkephalin.

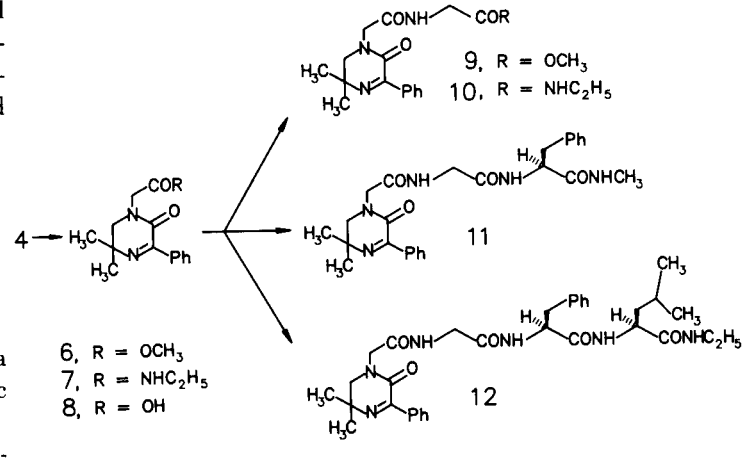
Synthetically, condensation of 1,2-diamino-2-methylpropane (**2**) and methyl phenylglyoxylate (**3**) conceptually could yield two isomeric pyrazine derivatives, **4** and **5**. In practice, however, reaction of **2** and **3** in refluxing ethanol



2 + 3 → 4 + 5

resulted in the isolation of only the 5,5-dimethyl isomer **4**. Differentiation between the two structural possibilities was readily accomplished by ^{1}H nmr. Thus, the methylene protons of **4** appeared as a doublet centered at δ 3.22. Decoupling of the NH resonance at δ 8.05 or exchange with deuterium oxide caused this doublet to collapse to a singlet confirming that the methylene group and the NH were adjacent to one another.

Alkylation of the preformed anion of **4** with methyl bromoacetate gave the ester **6** which was converted to the corresponding ethyl amide **7** by stirring with anhydrous ethylamine [14]. Hydrolysis of **6** gave carboxylic acid **8** which was coupled to the appropriate amino acid fragments of the leucine-enkephalin sequence. In this manner,



coupling of **8** and glycine, methyl ester using 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline [15] as a coupling agent gave methyl ester **9** which was further converted to

ethyl amide **10**. On the other hand, coupling of **8** and glycyl-*N*-methyl-L-phenylalaninamide [16] using the mixed anhydride coupling method [17] gave the heterocyclic tetrapeptide **11** in 42% yield. Finally, coupling of **8** and glycyl-L-phenylalanyl-*N*-ethyl-L-leucinamide afforded, in 62% yield, the pentapeptide **12** which represent a heterocyclic analog of the endogenous leucine-enkephalin.

EXPERIMENTAL

Melting points were determined in open capillaries on a Thomas Hoover apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on Varian FT80A, EM390, and XL300 spectrometers. The chemical shifts are given in parts per million from tetramethylsilane as the internal standard. Mass spectra were obtained on a Finnigan 4600 mass spectrometer.

5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazine (**4**).

A solution of methyl phenylglyoxylate (24.0 g, 0.147 mole), 1,2-diamino-2-methylpropane (15.4 ml, 0.147 mole), and absolute ethanol (500 ml) was heated at reflux for 18 hours. The reaction was concentrated to approximately 250 ml and hot hexane (450 ml) was added. After several hours a beige precipitate (0.15 g) was removed by filtration and the filtrate was placed in the freezer. After 17 hours, 21.8 g (73%) of **4** was isolated as large colorless spars, mp 126-128°; ^{1}H nmr (deuteriochloroform): δ 1.33 (s, 6, geminal CH_3s), 3.22 (d, 2, CH_2, J = 3.8 Hz), 7.2-7.5 (m, 3, aromatic), 7.7-7.9 (m, 2, aromatic), 8.05 (broad s, 1, NH).

Anal. Calcd. for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.32; H, 6.87; N, 13.98.

5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazineacetic Acid, Methyl Ester (**6**).

To a stirred, room temperature solution of **4** (10.1 g, 0.050 mole) and dry tetrahydrofuran (80 ml) was added portionwise sodium hydride, 61% oil dispersion (1.32 g, 0.055 mole). The reaction was stirred 1 hour before methyl bromoacetate (4.6 ml, 0.055 mole) was added slowly *via* syringe. The reaction was stirred for 17 hours before being poured into water. The aqueous mixture was extracted several times with ether. The ethereal extracts were combined, washed with saturated aqueous sodium chloride, and dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure affording an oil. Kugelrohr distillation at 165° (0.05 mm) gave 6.6 g (48%) of **6** as colorless prisms, mp 58-60°; ^{1}H nmr (deuteriochloroform): δ 1.37 (s, 6, geminal CH_3s), 3.41 (s, 2, ring CH_2), 3.72 (s, 3, ester CH_3), 4.20 (s, 2, glycine CH_2), 7.2-7.5 (m, 3, aromatic), 7.7-8.0 (m, 2, aromatic).

Anal. Calcd. for $C_{15}H_{18}N_2O_3$: C, 65.68; H, 6.61; N, 10.21. Found: C, 65.81; H, 6.63; N, 10.18.

N-Ethyl-5,6-dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazineacetamide (**7**).

Methyl ester **6** (5.5 g, 0.020 mole) and anhydrous ethylamine (22.8 g, 0.506 mole) were stirred at room temperature in a sealed flask for 17 hours. The excess ethylamine was evaporated at reduced pressure leaving an oil which when triturated with ether gave a solid. Crystallization from toluene-hexane afforded 4.1 g (71%) of **7** as colorless needles, mp 113-115°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.04 (t, 3, ethyl CH_3, J = 7.5 Hz), 1.28 (s, 6, geminal CH_3s), 3.12 (dq, 2, ethyl CH_2, J = 5.1 and 7.5 Hz), 3.47 (s, 2, ring CH_2), 4.06 (s, 2, glycine CH_2), 7.3-7.5 (m, 3, aromatic), 7.7-7.8 (m, 2, aromatic), 8.05 (t, 1, NH, J = 5.1 Hz).

Anal. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 67.01; H, 7.32; N, 14.63.

5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazineacetic Acid (**8**).

A mixture of methyl ester **6** (6.1 g, 0.022 mole), 1 molar aqueous lithium hydroxide (24 ml, 0.024 mole), and methanol (50 ml) was stirred at room temperature for 2 hours. Most of the methanol was evaporated at reduced pressure and the aqueous concentrate was acidified by the addition of 1 molar hydrochloric acid (50 ml, 0.050 mole). The acidified mixture was extracted several times with ethyl acetate. The organic extracts were combined, washed with saturated aqueous sodium chloride, and dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure leaving a solid. Crystallization from ethyl acetate-hexane gave 4.0 g (69%) of **8** as colorless irregular prisms, mp 159-167°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.29 (s, 6, geminal CH_3s), 3.48 (s, 2, ring CH_2), 4.16 (s, 2, glycine CH_2), 7.2-7.5 (m, 3, aromatic), 7.6-7.9 (m, 2, aromatic).

Anal. Calcd. for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.42; H, 6.17; N, 10.57.

N-[(5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazinyl)acetyl]glycine, Methyl Ester (**9**).

Carboxylic acid **8** (6.8 g, 0.026 mole), glycine, methyl ester, hydrochloride (3.3 g, 0.026 mole), and 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (6.5 g, 0.026 mole) were stirred in dichloromethane (100 ml) for 17 hours. The reaction mixture was washed with 0.5 molar hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride before being dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure leaving an oil which slowly solidified. Crystallization from ethyl acetate-hexane afforded 5.4 g (62%) of **9** as colorless needles, mp 99-101°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.27 (s, 6, geminal CH_3s), 3.43 (s, 2, ring CH_2), 3.59 (s, 3, ester CH_3), 3.87 (d, 2, 3-glycine CH_2 [18], J = 6.0 Hz), 4.12 (s, 2, 2-glycine CH_2), 7.2-7.5 (m, 3, aromatic), 7.6-7.9 (m, 2, aromatic), 8.45 (t, 1, NH, J = 6.0 Hz).

Anal. Calcd. for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.55; H, 6.39; N, 12.56.

N-[(5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazinyl)acetyl]-*N*-ethylglycinamide (**10**).

Methyl ester **9** (3.4 g, 0.010 mole) and anhydrous ethylamine (17.5 g, 0.388 mole) were stirred at room temperature in a sealed flask for 7 hours. The excess ethylamine was evaporated at reduced pressure leaving a solid. Two crystallizations from ethyl acetate gave 1.9 g (54%) of **10** as colorless plates, mp 158-162°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.01 (t, 3, ethyl CH_3, J = 7.5 Hz), 1.28 (s, 6, geminal CH_3s), 3.10 (dq, 2, ethyl CH_2, J = 5.3 and 7.5 Hz), 3.49 (s, 2, ring CH_2), 3.71 (d, 2, 3-glycine CH_2, J = 5.8 Hz), 4.15 (s, 2, 2-glycine CH_2), 7.3-7.5 (m, 3, aromatic), 7.7-7.8 (m, 2, aromatic), 7.84 (t, 1, ethyl amide NH, J = 5.3 Hz), 8.38 (t, 1, 3-glycine NH, J = 5.8 Hz).

Anal. Calcd. for $C_{18}H_{24}N_4O_3$: C, 62.77; H, 7.02; N, 16.27. Found: C, 62.46; H, 7.07; N, 16.24.

N-[(5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazinyl)acetyl]glycyl-*N*-methyl-L-phenylalaninamide (**11**).

To a stirred, −10° solution of carboxylic acid **8** (2.6 g, 0.010 mole) and dry tetrahydrofuran (30 ml) was added in turn triethylamine (1.5 ml, 0.011 mole) and isobutyl chloroformate (1.4 ml, 0.011 mole). After 12 minutes, a freshly neutralized solution of glycyl-*N*-methyl-L-phenylalaninamide, hydrochloride (3.0 g, 0.011 mole), triethylamine (2.8 ml, 0.020 mole), tetrahydrofuran (30 ml), and water (15 ml) was added in one portion. The cooling bath was removed and the reaction warmed to room temperature. After 17 hours, the reaction was poured into a separatory funnel containing 0.5 molar hydrochloric acid. The aqueous mixture was extracted two times with ether and two times with dichloromethane. The ethereal extracts were combined and washed with both saturated aqueous sodium bicarbonate and saturated aqueous sodium chloride. The dichloromethane extracts were combined and washed similarly. The ethereal and dichloromethane extracts were combined and dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure leaving a solid. Crystallization from isopropanol gave 2.0 g (42%) of **11** as colorless irregular prisms, mp 194-204°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 1.28 (s, 6, geminal

CH_3s), 2.57 (d, 3, amide CH_3, J = 4.4 Hz), 2.77 (dd, 1, phenylalanine CH_2, J_{vic} = 10.4 Hz, J_{gem} = 13.8 Hz), 3.00 (dd, 1, phenylalanine CH_2, J_{vic} = 4.8 Hz, J_{gem} = 13.8 Hz), 3.47 (s, 2, ring CH_2), 3.65 (dd, 1, 3-glycine CH_2, J_{vic} = 6.3 Hz, J_{gem} = 16.6 Hz), 3.82 (dd, 1, 3-glycine CH_2, J_{vic} = 6.0 Hz, J_{gem} = 16.6 Hz), 4.14 (s, 2, 2-glycine CH_2), 4.44 (m, 1, phenylalanine CH), 7.1-7.8 (m, 10, aromatic), 7.92 (q, 1, methyl amide NH, J = 4.4 Hz), 8.18 (d, 1, phenylalanine NH, J = 8.1 Hz), 8.35 (t, 1, 3-glycine NH, J = 6.2 Hz); ms: 477 (M^+, 15).

Anal. Calcd. for $C_{26}H_{31}N_5O_4$: C, 65.39; H, 6.54; N, 14.66. Found: C, 65.30; H, 6.59; N, 14.39.

N-[(5,6-Dihydro-5,5-dimethyl-2-oxo-3-phenyl-1(2*H*)-pyrazinyl)acetyl]glycyl-L-phenylalanyl-*N*-ethyl-L-leucinamide (**12**).

To a stirred, room temperature mixture of carboxylic acid **8** (5.2 g, 0.020 mole, glycyl-L-phenylalanyl-*N*-ethyl-L-leucinamide, hydrochloride, hydrate (8.0 g, 0.020 mole), 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (5.0 g, 0.020 mole), and dichloromethane (300 ml) was added triethylamine (2.8 ml, 0.020 mole). After 17 hours, the reaction mixture was transferred to a separatory funnel where it was washed with 0.5 molar hydrochloric acid, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. After drying over anhydrous sodium sulfate, the dichloromethane was evaporated at reduced presure leaving a foam which upon trituration with ether afforded a solid. Crystallization from isopropanol gave 7.5 g (62%) of **12** as colorless irregular prisms, mp 210-213°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 0.82 (d, 3, leucine CH_3, J = 6.3 Hz), 0.87 (d, 3, leucine CH_3, J = 6.6 Hz), 1.00 (t, 3, ethyl CH_3, J = 6.8 Hz), 1.27 (s, 6, geminal CH_3s), 1.4-1.6 (m, 3, leucine CH, CH_2), 2.79 (dd, 1, phenylalanine CH_2, J_{vic} = 10.1 Hz, J_{gem} = 14.0 Hz), 3.0-3.1 (m, 3, phenylalanine CH_2, ethyl CH_2), 3.45 (s, 2, ring CH_2), 3.64 (dd, 1, 3-glycine CH_2, J_{vic} = 5.4 Hz, J_{gem} = 16.4 Hz), 3.81 (dd, 1, 3-glycine CH_2, J_{vic} = 5.4 Hz, J_{gem} = 16.4 Hz), 4.13 (s, 2, 2-glycine CH_2), 4.23 (m, 1, leucine CH), 4.54 (m, 1, phenylalanine CH), 7.1-7.5 (m, 10, aromatic), 7.71 (t, 1, ethyl amide NH, J = 4.8 Hz), 8.04 (d, 1, leucine NH, J = 8.7 Hz), 8.12 (d, 1, phenylalanine NH, J = 7.8 Hz), 8.40 (t, 1, 3-glycine NH, J = 5.4 Hz); ms: 604 (m^+, 1).

Anal. Calcd. for $C_{33}H_{44}N_6O_5$: C, 65.54; H, 7.33; N, 13.90. Found: C, 65.46; H, 7.20; N, 13.58.

N-(*t*-Butoxycaronyl)glycyl-L-phenylalanyl-*N*-ethyl-L-leucinamide (**13**).

N(*t*-Butoxycarbonyl)glycyl-L-phenylalanyl-L-leucine, methyl ester [19] (20.6 g, 0.046 mole) and anhydrous ethylamine (60.0 g, 1.33 mole) were stirred at room temperature in a sealed flask. After 15 hours, the excess ethylamine was evaporated at reduced pressure leaving a foam which solidified when triturated with ether. Crystallization from ethyl acetate afforded 12.6 g (59%) of **13** as colorless irregular prisms, mp 183-185°; ^{1}H nmr (dimethylsulfoxide-d_6): δ 0.83 (d, 3, leucine CH_3, J = 5.4 Hz), 0.88 (d, 3, leucine CH_3, J = 6.9 Hz), 1.00 (t, 3, ethyl CH_3, J = 7.3 Hz), 1.36 (s, 9, t-boc CH_3s), 1.4-1.6 (m, 3, leucine CH, CH_2), 2.81 (dd, 1, phenylalanine CH_2, J_{vic} = 9.3 Hz, J_{gem} = 14.1 Hz), 2.9-3.1 (m, 3, phenylalanine CH_2, ethyl CH_2), 3.42 (dd, 1, glycine CH_2, J_{vic} = 5.7 Hz, J_{gem} = 16.7 Hz), 3.57 (dd, 1, glycine CH_2, J_{vic} = 5.7 Hz, J_{gem} = 16.7 Hz), 4.23 (dt, 1, leucine CH, J = 7.2 and 8.3 Hz), 4.52 (m, 1, phenylalanine CH), 7.01 (t, 1, glycine NH, J = 5.7 Hz), 7.1-7.3 (m, 5, aromatic), 7.71 (t, 1, ethyl amide NH, J = 5.4 Hz), 7.92 (d, 1, phenylalanine NH, J = 7.8 Hz), 8.03 (d, 1, leucine NH, J = 8.1 Hz).

Anal. Calcd. for $C_{24}H_{38}N_4O_5$: C, 62.32; H, 8.28; N, 12.11. Found: C, 62.00; H, 8.17; N, 12.13.

Glycyl-L-phenylalanyl-*N*-ethyl-L-leucinamide, Hydrochloride, Hydrate (**14**).

A stirred solution of protected tripeptide **13** (1.5 g, 0.0032 mole) and methanl (20 ml) was cooled to 0° in an ice bath. Gaseous hydrogen chloride was then bubbled through the solution for 30 minutes. The reaction was stirred an additional 30 minutes before being filtered. Evaporation of the filtrate and drying at high vacuum gave 1.2 g (92%) of **14** as a colorless powder, mp >210° dec; ^{1}H nmr (dimethylsulfoxide-d_6): δ 0.84 (d, 3, leucine CH_3, J = 5.7 Hz), 0.89 (d, 3, leucine CH_3, J = 5.4 Hz), 1.01 (t, 3, ethyl CH_3, J = 7.4 Hz), 1.3-1.7 (m, 3, leucine CH, CH_2), 2.78 (dd, 1, phenylalanine CH_2, J_{vic} = 8.7 Hz, J_{gem} = 13.7 Hz), 3.1 (m, 3, phenylalanine CH_2, ethyl CH_2), 3.4-3.6 (m, 2, glycine CH_2), 4.26 (m, 1, leucine CH), 4.62 (m, 1, phenylalanine CH), 7.1-7.4 (m, 5, aromatic), 7.98 (t, 1, ethyl amide NH, J = 5.1 Hz), 8.21 (broad s, 2, glycine NH_2), 8.43 (d, 1, leucine NH, J = 8.4 Hz), 8.74 (d, 1, phenylalanine NH, J = 8.4 Hz).

Anal. Calcd. for $C_{19}H_{30}N_4O_3 \cdot HCl \cdot 0.75\ H_2O$: C, 55.33; H, 7.94; N, 13.58. Found: C, 55.39; H, 7.61; N, 13.64.

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