result could not be simply explained but promoted our research on the solvent dependency of the chiral coumarin. The precise discussion on this matter will be published elsewhere in the near future.

Experimental[7]

Isolation of R-(+)-Peucedanol Methyl Ether (1)——The dried and powdered whole plant (1.5 kg) collected at Marmara Island (Turkey) in May 1971 was extracted with $CHCl_3$. After evaporation, the residue was chromatographed on SiO_2. The fraction eluted with $CHCl_3$ yielded a crude product (1.2 g) which was purified using preparative TLC ($CHCl_3$: EtOH=9: 1 (v/v), Rf=0.32) followed by recrystallization from EtOH to give colourless fine needles, mp 139—140° (lit.[4b] 136—138°). High resolution mass spectrum: 278.1163 (M^+, 29.5%) ($C_{15}H_{18}O_5$ requires: 278.1155), 220.0732 (M^+-58, 45.6%) ($C_{12}H_{12}O_4$ requires: 220.0733). IR ν_{max}^{KBr} cm^{-1}: 3600—3100 (OH), 1733 (CO). NMR ($CDCl_3$) δ: 1.29 and 1.38 (each 3H, s, C-Me), 2.06 and 2.24 (each 1H, br. s, OH, disappeared on addition of D_2O), 2.53 (1H, d.d, J=10.5 and 14.0 Hz, -CH-C$\underline{H}_A$H$_B$-Ar), 3.03 (1H, d.d, J=14.0 and 2.0 Hz, -CH-CH$_A$$\underline{H}_B$-Ar), 3.63 (1H, d.d, J=10.5 and 2.0 Hz, O-C$\underline{H}$-CH$_2$-), 3.91 (3H, s, OMe), 6.22 and 7.62 (each 1H, d, J=10.0 Hz, C_3 and C_4-H), 6.80 and 7.30 (each 1H, s, arom. H). $[\alpha]_{589}^{22.5°}$ +65.1 (c=4.99×10^{-2}, MeOH).

7) All melting points were taken on micro melting point hot stage (Yanagimoto) and uncorrected. IR, NMR, and high resolution mass spectrum were obtained with Hitachi EPI-G3, JEOL JMN-MH-100, and JEOL JMS-01SG-2 spectrometer, respectively.

[Chem. Pharm. Bull. 27(4)1050—1054(1979)]

UDC 547.466.1.04 : 547.269.3.04

Synthesis of Two Peptides corresponding to α-Endorphin and γ-Endorphin by the Methanesulfonic Acid Deprotecting Procedure[1]

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(Received November 24, 1978)

Methanesulfonic acid was employed as a deprotecting reagent for the syntheses of the hexadecapeptide and the heptadecapeptide corresponding to α-endorphin and γ-endorphin. Their analgesic potencies were 0.021 and 0.013 relative to that of morphin respectively.

Keywords——methanesulfonic acid deprotection; α-endorphin; γ-endorphin; β-human endorphin; analgesic effect

In 1976, Ling *et al.*[3] reported the structural elucidation and the solid phase synthesis of two morphinomimetic peptides, α-endorphin (I) and γ-endorphin (II), isolated from a crude extract of porcine hypothalamus-neurohypophysis.

We wish to report alternative syntheses of the hexadecapeptide and heptadecapeptide corresponding to these two peptides, which were performed in a conventional manner, using

1) Amino acids and peptides are of the L-configuration. Following abbreviations were used: Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, DCC=dicyclohexylcarbodiimide, HOBT=1-hydroxybenzotriazole, DMF=dimethylformamide, DMSO=dimethylsulfoxide, THF=tetrahydrofuran, TFA=trifluoroacetic acid.

2) Location: *a) Minamifunabori-cho, Edogawa-ku, Tokyo, 132, Japan*; *b) Sakyo-ku, Kyoto, 606, Japan.*

3) N. Ling, R. Burgus and R. Guillemin, *Proc. Natl. Acad. Sci. U.S.A.*, 73, 3942 (1976); N. Ling, *Biochem. Biophys. Res. Commun.*, 74, 248 (1977).

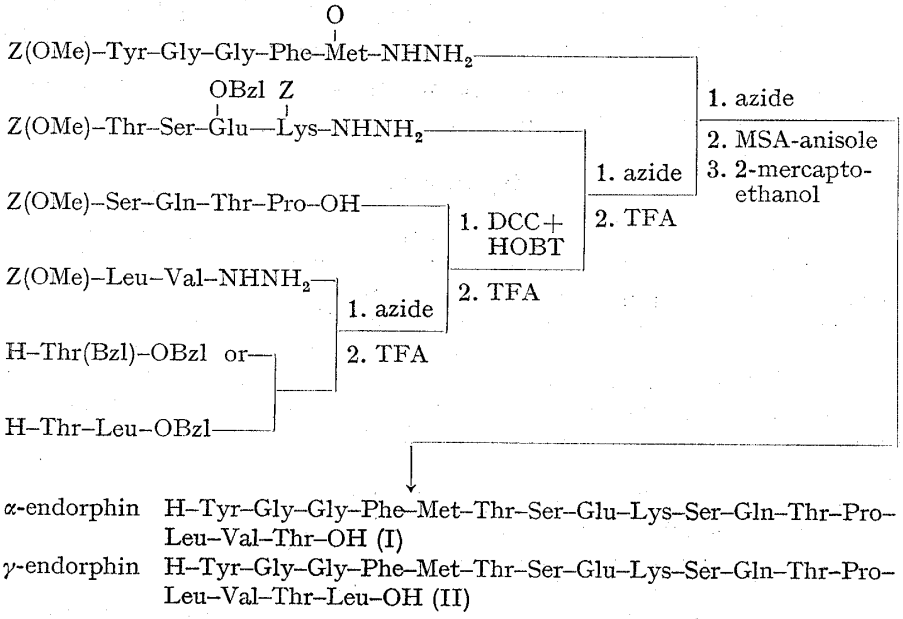

Fig. 1. Synthetic Routes to α- and γ-Endorphins

methanesulfonic acid (MSA) as a deprotecting reagent[4] as shown in Fig. 1. Starting with either H-Thr(Bzl)-OBzl or H-Thr-Leu-OBzl, available four peptide fragments used for our previous synthesis[5] of human β-endorphin; [6] *i.e.*, Z(OMe)-Leu-Val-$NHNH_2$, Z(OMe)-Ser-Gln-Thr-Pro-OH, Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-$NHNH_2$ and Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-$NHNH_2$, were successively condensed by either the azide[7] or the DCC plus HOBT procedure,[8] while the N^{α}-protecting Z(OMe) group was cleaved by the usual TFA treatment[9] prior to each condensation reaction. All protected intermediates and the protected α-endorphin and γ-endorphin, *i.e.*, Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr(Bzl)-OBzl and Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OBzl, were purified by the batchwise washing procedure followed by precipitation from appropriate organic solvents.

At the final steps, protecting groups employed, *i.e.*, Z(OMe), Z and Bzl, were removed from the protected α- and γ-endorphins by MSA respectively, as performed in our previous synthesis of β-endorphin.[5] The resulting peptides were converted to the corresponding acetate by Dowex 1×4 (acetate form), treated with dilute ammonia for establishment of a reversible N→O shift at the Ser and Thr residues[10] and then partially purified by column chromatography on Sephadex G-15. [5-Met(O)]-α-endorphin and [5-Met(O)]-γ-endorphin thus obtained were then reduced by incubation with 2-mercaptoethanol respectively and the reduced products were purified by partition chromatography[11] on Sephadex G-25 with the solvent system of *n*-BuOH–AcOH–H_2O (4: 1: 5). After elution of a small front peak, the desired fractions were obtained as a main symmetrical peak in each case. Homogeneities of synthetic peptides thus isolated in more than 50% yield respectively were assessed by thin-layer chromatography in four different solvent systems, acid hydrolysis and enzymatic digest.

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5) M. Kubota, T. Hirayama, O. Nagase and H. Yajima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2139 (1978).
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Bioassay was conducted by Professor H. Takagi of Faculty of Pharmaceutical Sciences, Kyoto University. Potency ratios of our synthetic α-endorphin and γ-endorphin to morphin (taken as 1) were 0.021 and 0.013 respectively, when assayed, by intracisternal injection, the analgesic effect on tail pinch method in mice.

Experimental

The azide was prepared according to Honzl and Rudinger.[7] Under cooling with ice–NaCl, isoamyl nitrite (1 equiv.) was added to a stirred solution of the hydrazide (1 equiv.) in DMF and HCl–DMF (2 equiv.). After 15 min, when the hydrazine test became negative, the solution was neutralized with Et_3N (2 equiv.) and then combined with a solution of the amino component. Thin-layer chromatography was performed on silica gel (Kieselgel G, Merck). *Rf* values refer to the following solvent systems: Rf_1 $CHCl_3$–MeOH–H_2O (8:3:1), Rf_2 $CHCl_3$–MeOH–AcOH (95:5:3), Rf_3 *n*-BuOH–AcOH–AcOEt–H_2O (1:1:1:1), Rf_4 *n*-BuOH–AcOH–pyridine–H_2O (30:6:20:24), Rf_5 *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2), Rf_6 *n*-BuOH–AcOH–H_2O (4:1:5). For amino acid analysis, peptides and their derivatives were hydrolyzed with 3 N *p*-toluenesulfonic acid.[12]

1. Synthesis of the Peptide corresponding to Porcine α-Endorphin

Z(OMe)-Leu-Val-Thr(Bzl)-OBzl——The azide (prepared from 3.27 g of Z(OMe)-Leu-Val-$NHNH_2$) in DMF (10 ml) and Et_3N (1.12 ml) were added to a solution of H-Thr(Bzl)-OBzl (prepared from 2.75 g of the oxalate with 1.12 ml of Et_3N) in DMF (15 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was extracted with AcOEt. The organic phase was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O–NaCl, dried over Na_2SO_4 and then condensed. Trituration of the residue with ether afforded a powder, which was recrystallized from AcOEt and ether; yield 3.11 g (58%), mp 120—123°, $[\alpha]_D^{24}$ −4.8° (c=0.6, DMF), Rf_2 0.73. *Anal.* Calcd. for $C_{38}H_{49}N_3O_8$: C, 67.53; H, 7.31; N, 6.22. Found: C, 67.45; H, 7.43; N, 6.61.

Z(OMe)-Ser-Gln-Thr-Pro-Leu-Val-Thr(Bzl)-OBzl——Z(OMe)-Leu-Val-Thr(Bzl)-OBzl (1.35 g) was treated with TFA (1.5 ml) in the presence of anisole (0.9 ml) in an ice-bath for 60 min and 3.87 N HCl–DMF (2.07 ml) was added. Dry ether was then added and the resulting powder, after drying over KOH pellets *in vacuo* for 3 hr, was dissolved in DMF (14 ml) together with Et_3N (0.28 ml), Z(OMe)-Ser-Gln-Thr-Pro-OH (1.19 g) and HOBT (0.54 g). After addition of DCC (0.50 g), the solution was stirred at room temperature for 48 hr, filtered and then condensed. The residue was treated with AcOEt and the resulting powder was purified by batchwise washing with 5% citric acid, 5% $NaHCO_3$ and H_2O followed by precipitation from DMF with MeOH; yield 1.29 g (59%), mp 181—185,° $[\alpha]_D^{24}$ −45.9° (c=0.3, DMF), Rf_1 0.56. Amino acid analysis: Ser 1.02, Glu 1.01, Thr 1.99, Pro 1.00, Leu 1.00, Val 0.93 (average recovery 92%). *Anal.* Calcd. for $C_{55}H_{76}N_8O_{15}$: C, 60.64; H, 7.03; N, 10.29. Found: C, 60.48; H, 7.10; N, 10.24.

Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr(Bzl)-OBzl——The above protected heptapeptide ester (762 mg) was treated with TFA (1 ml)–anisole (0.3 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (4 ml) containing Et_3N (0.1 ml). To this ice-chilled solution were added the azide (prepared from 606 mg of Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-$NHNH_2$) in DMF (1.5 ml) and Et_3N (0.1 ml). The mixture was stirred at 4° for 48 hr and the solvent was evaporated. Treatment of the residue with AcOEt afforded a powder, which was washed batchwise as mentioned above and then precipitated from DMF with MeOH; yield 704 mg (57%), mp 203—207,° $[\alpha]_D^{24}$ −26.3° (c=0.4, DMF), Rf_1 0.54. Amino acid analysis: Thr 2.95, Ser 1.93, Glu 2.00, Lys 1.02, Pro 0.99, Leu 1.00, Val 0.93 (average recovery 87%). *Anal.* Calcd. for $C_{88}H_{119}N_{13}O_{25}$: C, 60.09; H, 6.82; N, 10.35. Found: C, 60.25; H, 6.99; N, 10.51.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr(Bzl)-OBzl——The above protected undecapeptide ester (598 mg) was treated with TFA (0.5 ml)–anisole (0.4 ml) and the N^{α}-deprotected peptide isolated as mentioned above was dissolved in DMF–DMSO (3 ml–1 ml) containing Et_3N (0.05 ml). To this ice-chilled solution were added the azide (prepared from 260 mg of Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-$NHNH_2$) in DMF (2 ml) and Et_3N (0.05 ml). After stirring at 4° for 48 hr, the solution was condensed and the residue was treated with AcOEt. The resulting powder was purified by batchwise washing as mentioned above followed by precipitation from DMF with MeOH; yield 455 mg (57%). mp 213—217°, $[\alpha]_D^{24}$ −26.4° (c=0.3, DMF), Rf_1 0.50. Amino acid analysis: Tyr 0.80; Gly 1.83, Phe 0.87, Met+Met(O) 0.89, Thr 2.71; Ser 2.17, Glu 2.08, Lys 1.04, Pro 1.04, Leu 1.00, Val 0.91 (average recovery 85%). *Anal.* Calcd. for $C_{115}H_{152}N_{18}O_{32}S$: C, 59.26; H, 6.57; N, 10.82. Found: C, 59.07; H, 6.77; N, 11.13.

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-OH (α-Endorphin)——The above protected hexadecapeptide ester (200 mg) was treated with MSA (3 ml) in the presence of anisole (1 ml) in an ice-bath for 15 min and at room temperature for 60 min and dry ether was added. The resulting gummy precipitate was washed with ether and then dissolved in H_2O (10 ml). The solution was treated with Dowex 1×4 (acetate form, approximately 3 g) for 30 min, then filtered and lyophilized to give a powder (170 mg).

12) T.Y. Liu and Y.H. Chang, *J. Biol. Chem.*, **246**, 2842 (1971).

This powder was dissolved in 2 N NH_4OH (3 ml) and the solution, after 30 min in an ice-bath, was lyophilized. The powder obtained was then dissolved in 0.5 N AcOH (1 ml) and the solution was applied to a column of Sephadex G-15 (3×115 cm), which was eluted with the same solvent. Individual fractions (4.2 ml, each) were collected and the absorbancy at 275 mμ was determined. Fractions corresponding to the front main peak (tube No. 58—70) were collected and the solvent was removed by lyophilization; yield 120 mg (79%), [5-Met(O)]-α-endorphin thus obtained was dissolved in H_2O (5 ml) and reduced with 2-mercaptoethanol (0.3 ml) at 50° for 24 hr. After lyophilization, the reduced product was dissolved in the upper phase (1 ml) of the solvent system of *n*-BuOH–AcOH–H_2O (4: 1: 5) and the solution was applied to a column of Sephadex G-25 (3×60 cm) equilibrated previously with the lower phase of the above solvent system. Individual fractions (5 ml each) were collected and absorbancy was determined as stated above. The desired fractions (tube No. 53—69) were combined, the solvent was removed by evaporation and the residue was lyophilized to give a fluffy white powder; yield 62 mg (52%). $[\alpha]_D^{24}$ −77.7° (*c*=0.2, 0.25 N AcOH), (lit.[3] −76.5° in 1% AcOH), Rf_3 0.85, Rf_4 0.74, Rf_5 0.72, Rf_6 0.55. Amino acid ratios in acid hydrolysate: Tyr 1.06, Gly 2.13, Phe 1.05, Met 0.99, Thr 3.17, Ser 2.10, Glu 2.09, Lys 1.08, Pro 1.08, Leu 1.05, Val 1.00 (average recovery 89%). Amino acid ratios in aminopeptidase (AP-M)[13] digest: Tyr 0.86, Gly 1.76, Phe 0.90, Met 0.81, Thr+Gln 3.42 (calcd. as Thr), Ser 1.85, Glu 0.97, Lys 0.99, Pro 0.87, Lsu 1.00, Val 1.00 (average recovery 82%).

2. Synthesis of the Peptide corresponding to Porcine γ-Endorphin

Z(OMe)-Thr-Leu-OBzl——The azide (prepared from 8.92 g of Z(OMe)-Thr-$NHNH_2$) in DMF (60 ml) and Et_3N (4.19 ml) were added to an ice-chilled solution of H-Leu-OBzl (prepared from 11.80 g of the tosylate with 4.19 ml of Et_3N) in DMF (50 ml) and the mixture was stirred at 4° for 48 hr. The solvent was evaporated and the residue was dissolved in AcOEt. The extract was washed with 5% citric acid, 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and then condensed. The residue was triturated with ether and recrystallized from AcOEt and ether; yield 11.85 g (81%), mp 59—61°, $[\alpha]_D^{24}$ −12.0° (*c*=0.5, DMF), Rf_1 0.92. *Anal.* Calcd. for $C_{26}H_{34}N_2O_7$: C, 64.18; H, 7.04; N, 5.76. Found: C, 63.99; H, 6.90; N, 5.70.

Z(OMe)-Leu-Val-Thr-Leu-OBzl——Z(OMe)-Thr-Leu-OBzl (3.74 g) was treated with TFA–anisole (6 ml–4.2 ml) as usual and the excess TFA was removed by evaporation. The residue was washed with *n*-hexane, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (6 ml) containing Et_3N (1.07 ml). To this ice-chilled solution were added the azide (prepared from 3.15 g of Z(OMe)-Leu-Val-$NHNH_2$) in DMF (10 ml) and Et_3N (1.07 ml) and the mixture was stirred at 4° for 48 hr. After evaporation of the solvent, the residue was treated with ether and the resulting powder was purified by batchwise washing with 5% citric acid and H_2O followed by precipitation from DMF with ether; yield 2.80 g (52%), mp 175—177,° $[\alpha]_D^{24}$ −20.0° (*c*=0.4, DMF), Rf_1 0.84, *Anal.* Calcd. for $C_{37}H_{54}N_4O_9$: C, 63.59; H, 7.79; N, 8.02. Found: C, 63.21; H, 7.70; N, 7.94.

Z(OMe)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OBzl——Z(OMe)-Leu-Val-Thr-Leu-OBzl (1.40 g) was treated with TFA–anisole (2.3 ml–1.1 ml) as usual and 2.49 N HCl–THF (3 ml) was added. The hydrochloride was precipitated by ether, dried over KOH pellets *in vacuo* for 2 hr and then dissolved in DMF (7 ml) together with Et_3N (0.28 ml), Z(OMe)-Ser-Gln-Thr-Pro-OH (1.20 g), HOBT (0.54 g) and DCC (0.50 g). After stirring at room temperature for 48 hr, the mixture was filtered and the filtrate was condensed. Treatment of the residue with AcOEt afforded a powder, which was purified by batchwise washing with 5% citric acid, 5% $NaHCO_3$ and H_2O followed by precipitation from DMF with MeOH; yield 1.52 g (68%), mp 205—208°, $[\alpha]_D^{24}$ −42.7° (*c*=0.4, DMF), Rf_1 0.57. Amino acid ratios in acid hydrolysate: Thr 2.15, Ser 0.97, Glu 0.98, Pro 1.05, Val 1.00, Leu 2.24 (average recovery 84%). *Anal.* Calcd. for $C_{54}H_{81}N_9O_{16}$: C, 58.31; H, 7.34; N, 11.33. Found: C, 58.24; H, 7.27; N, 11.29.

Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OBzl——The above protected octapeptide ester (0.69 g) was treated with TFA–anisole (1 ml–0.7 ml) as usual and dry ether was added. The resulting powder was collected by filtration, dried over KOH pellets *in vacuo* for 3 hr and then dissolved in DMF (1 ml) containing Et_3N (0.09 ml). To this ice-chilled solution were added the azide (prepared from 0.65 g of Z(OMe)-Thr-Ser-Glu(OBzl)-Lys(Z)-$NHNH_2$) in DMF (3.0 ml) and Et_3N (0.12 ml). After stirring at 4° for 48 hr, the mixture was condensed and the residue was treated with AcOEt. The resulting powder was purified by batchwise washing with 5% citric acid, 5% $NaHCO_3$ and H_2O followed by precipitation from DMF with MeOH; yield 0.82 g (75%), mp 219—224°, $[\alpha]_D^{24}$ −36.2° (*c*=0.2, DMF), Rf_1 0.50. Amino acid ratios in acid hydrolysate: Lys 1.07, Thr 3.19, Ser 2.12, Glu 2.16, Pro 1.03, Val 1.00, Leu 2.24 (average recovery 89%). *Anal.* Calcd. for $C_{87}H_{124}N_{14}O_{26}$: C, 58.64; H, 7.01; N, 11.01. Found: C, 58.49; H, 7.10; N, 11.03.

Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-Thr-Ser-Glu(OBzl)-Lys(Z)-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OBzl——The above protected dodecapeptide ester (0.54 g) was treated with TFA–anisole (0.5 ml–0.3 ml) as usual and dry ether was added. The resulting powder was collected as stated above and then dissolved in DMF (1 ml) together with Et_3N (0.04 ml). To this ice-chilled solution were added the azide (prepared from 0.28 g of Z(OMe)-Tyr-Gly-Gly-Phe-Met(O)-$NHNH_2$) in DMF (3 ml) and Et_3N (0.06 ml). After stirring at 4° for 48 hr, the mixture was condensed and the residue was treated with AcOEt. The resulting powder was purified by batchwise washing with 5% citric acid, 5% $NaHCO_3$ and H_2O followed by precipitation from DMF

13) G. Pfleiderer and G.P. Celliers, *Biochem. Z.*, **339**, 186 (1963). The enzyme was purchased from The Protein Research Foundation, Osaka, Lot. No. 210520.

with MeOH; yield 0.46 g (65%), mp 206—211°, $[\alpha]_D^{24}$ −37.5° (c=0.3, DMF), Rf_1 0.44. Amino acid ratios in acid hydrolysate: Lys 1.00, Thr 2.95, Ser 1.83, Glu 1.95, Pro 1.06, Gly 2.10, Val 1.00, Leu 2.14, Tyr 0.71, Phe 0.98 (average recovery 92%). *Anal.* Calcd. for $C_{114}H_{157}N_{19}O_{33}S \cdot H_2O$: C, 57.73; H, 6.76; N, 11.22. Found: C, 57.54; H, 6.76; N, 11.51.

H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH (γ-Endorphin)—— The protected heptadecapeptide ester (200 mg) was treated with MSA (3 ml) in the presence of anisole (1 ml) in an ice-bath for 15 min and then at room temperature for 60 min and dry ether was added. The resulting gummy precipitate was dissolved in H_2O (10 ml) and the aqueous solution was treated with Dowex 1×4 (acetate form, approximately 3 g) for 30 min and then filtered. The filtrate was lyophilized to give a powder (168 mg). The powder was dissolved in H_2O (9 ml) and 2 N NH_4OH (3 ml) was added and the solution, after standing in an ice-bath for 30 min, was lyophilized. The powder obtained was dissolved in 0.5 N AcOH (2 ml) and the solution was applied to a column of Sephadex G-15 (3×115 cm), which was eluted with the same solvent system. Individual fractions (4.2 ml each) were collected and the absorbancy at 275 mμ was determined. Fractions corresponding to the front main peak (tube No. 56—67) were collected and the solvent was removed by lyophilization to give a fluffy powder (132 mg). [5-Met(O)]-γ-endorphin thus obtained was dissolved in H_2O (5 ml) and reduced by 2-mercaptoethanol (0.3 ml) at 50° for 24 hr. The solution was evaporated and the residue was applied to a column of Sephadex G-25 (3×60 cm) previously equilibrated with the lower phase of *n*-BuOH–AcOH–H_2O (4:1:5) and the column was then developed with the upper phase of the above solvent system. Individual fractions (5 ml each) were collected and the absorbancy was determined as stated above. Fractions of the main peak (tube No. 32—43) were collected, the solvent was evaporated and the residue was lyophilized to give a fluffy white powder; yield 76 mg (63%), $[\alpha]_D^{24}$ −84.5° (c=0.2, 0.25 N AcOH), (lit.[3] −80.5° in 1% AcOH). Rf_3 0.86, Rf_4 0.80, Rf_5 0.76, Rf_6 0.64. Amino acid ratios in acid hydrolysate: Tyr 0.99, Gly 2.15, Phe 1.01, Met 0.94, Thr 3.06, Ser 1.96, Glu 2.02, Lys 1.06, Pro 1.01, Leu 2.09, Val 1.00 (average recovery 83%). Amino acid ratios in AP-M digest: Tyr 0.90, Gly 1.72, Phe 0.92, Met 0.82, Thr+Gln 3.42 (calcd. as Thr), Ser 1.73, Glu 1.00, Lys 0.97, Pro 0.84, Leu 1.82, Val 1.00 (average recovery 79%).

Acknowledgement The authors express their sincere appreciation to Dr. Yoshio Morita, the vice director of the Research Institute, Daiichi Seiyaku Co. Ltd. for his encouragement throughout this work and to Professor H. Takagi, Faculty of Pharmaceutical Sciences, Kyoto University, for biological assay.