Gas Chromatography——Gas chromatography was performed on a Shimadzu Model GC-1B gas chromatograph equipped with a hydrogen flame ionization detector. The U-shaped stainless steel column (150 cm × 4 mm i.d.) was packed with either 3% neopentyl glycol succinate (NGS) or 2% cyanoethyl methyl silicone polymer (CNSi) on a support of Anakrom (90—100 mesh), or 1.5% methyl phenyl silicone polymer (SE-52) on a support of Gas-Chrom P (80—100 mesh). Nitrogen was used as carrier gas at a flow rate of 90 ml/min. The column operated isothermally at 140°, 160° or 170°, with an injection port temperature of 200° and a detector temperature of 190°. The TFA and the TMS derivatives of alditols and aldonolactones were prepared according to the procedures described by Sweeley, *et al.*[4] and Tamura, *et al.*,[8] respectively.

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Studies on Peptides. XIX.[1,2] Synthesis of a Stereoisomer of α-Melanocyte-stimulating Hormone

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Recently we have reported the synthesis of an enantiomer of an active fragment of α-melanocyte-stimulating hormone (MSH), D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycine (I) and offered the first attempt to examine the physiological properties of an optical antipode of a peptide hormone.[4] We have observed that this pentapeptide (I) inhibited the action of the corresponding pentapeptide of the L-configuration, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine (3.4×10^4 MSH U/g) in the molar ratios of 1 to 1 and the the action of α-MSH (2.0×10^{10} MSH U/g)[5] as well, but in the molar ratios of approximately 1 to 1×10^{-6}. The potency of I as an inhibitor to α-MSH is only about one millionth of that of melatonin,[6,7] which is known as the most potent, but non-specific inhibitor to α-MSH.

We have now prepared a stereoisomeric α-MSH, N^{α}-acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-L-lysyl-L-prolyl-L-valine amide (II) in order to compare the physiological property of such a compound with that of α-MSH.

The synthetic route employed here is essentially the same as described in our synthesis of α-MSH.[8] N^{α}-Benzyloxycarbonyl-D-histidyl-D-phenylalanyl-N^{G}-nitro-D-arginyl-D-tryptophylglycine[4] was condensed with N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide[9] by dicyclohexylcarbodiimide (DCC)[10] and the resulting product was hydrogenated to give D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide (III),

1) The Part XVIII of this series: *Chem. Pharm. Bull.* (Tokyo), **16**, 1342 (1968).
2) Abbreviated designation for amino acids and peptides adopted in this communication are those recommended by IUPAC-IUB commission for biological nomenclature in July 1965 and 1966: *Biochemistry*, **5**, 2485 (1966); *ibid.*, **6**, 362 (1967).
3) Location: *Sakyo-ku, Kyoto*
4) H. Yajima and K. Kubo, *J. Am. Chem. Soc.*, **87**, 2039 (1965).
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9) K. Hofmann, E. Stütz, G. Spühler, H. Yajima, and E.T. Schwatrz, *J. Am. Chem. Soc.*, **82**, 3727 (1960).
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which was then allowed to react with N^{α}-benzyloxycarbonyl-γ-benzyl-L-glutamate p-nitrophenyl ester.[11] The resulting product was hydrogenated to give L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide (IV). This partially protected nonapeptide amide (IV) was coupled with N^{α}-acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionine[8] by the azide procedure to give N^{α}-acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide (V). In order to remove the formyl group from the lysine residue, this peptide (V) was then treated with hydrazine acetate.[8] Thioglycolic acid was used to prevent possible oxidation of the methionine residue during this treatment. The resulting product was purified by ion-exchange chromatography on a column of carboxymethyl cellulose (CM-cellulose) using ammonium acetate buffers. The purified product exhibited a single spot on paper and thin-layer chromatographies and showed the identical *Rf* value with that of the synthetic α-MSH.[8]

The *in vitro* bioassays were conducted as previously described[12] using frog skins from *Rana pipiens* and *Rana nigromaculate* H. The results were comparable in both specimens. It was found that the partially protected octapeptide amide (III) was a lightening agent. At the molar ratios of 1 to 1, there was partial lightening on skins darkened with L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycyl-N^{ε}-formyl-L-lysyl-L-prolyl-L-valine amide (VI, 2.0×10^{6} MSH U/g).[8] If skins were incubated with III first and then equimolar amount of VI was added, darkening proceeded in normal way. It seems therefore that III is not as strong a lightening agent as VI is a darkening agent. The potency of III is almost a million times less active as an inhibitor to α-MSH than melatonin. This level of potency is about the same as that of I.

The partially protected nonapeptide amide (IV) and its stereoisomeric α-MSH (II) were both shown as skin darkening agents having the potency of 1.5×10^{4} and 3×10^{5} MSH U/g respectively. Since only extremely small quantities of peptides may influence to their bioassays due to their high potencies, the possibility can not be excluded that the darkening activity of the larger peptides compared to the lightening activity of the smaller ones might be due to possible contamination with their L-isomer, existing less than one percent. Microbiological assays of our starting material, for example, D-histidine, could not exclude the possibility of contamination with the L-isomer within one percent.[13] Present synthesis of stereoisomeric peptides encounters always such situation and this makes it difficult to evaluate the physiological activity of synthetic peptides.

On the other hand, there is an indication that the inhibitory power of II and IV, if any, appears to be overwhelmed by the additional MSH activity of the larger subunits, since similar observations were made in the syntheses of 5-methoxytryptamine derivatives.[14] While N-(L-histidyl-L-phenylalanyl-L-arginyl)-5-methoxytryptamine is a lightening agent, N-(N^{α}-acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginly)-5-methoxytryptamine acts as a darkening agent. From these results, it seems very likely that the N-terminal portion of α-MSH has an effect to overcome certain degree of detrimental effect happened within the entire molecule. This effect must be due to the enhancement of the intrinsic hormonal activity of the peptide. For example, the MSH activities of all-L-IV and all-L-II (α-MSH) were 1.4×10^{7} and $2.3\text{—}5.4\times10^{12}$ U/g respectively as reported previously,[8] where tremendous difference was noted between these two figures.

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Full understanding of the mechanism involved in the relationship between elongation of peptide chain and accompanying tremendous increase of activity which is generally known in peptide hormones remains to be achived.

Optical antipodes of oxytocin,[15] bradykinin,[16,17] angiotensin II,[18,19] and the heptapeptide of the C-terminal portion of eledoisin[20] were synthesized and their physiological properties evaluated. Our peptide (II) contains the active fragment with the D-configuration and the rest consists of the L-configuration. Since such attempt has not been made in peptide hormone researches, we offered here the synthesis of a stereoisomer of α-MSH an such an example.

Experimental

The general experimental methods are essentially the same as descrived in Part IV[21] of this series. Rf_1 values refer to the system of *n*-BuOH–AcOH–H_2O (4:1:5) on paper chromatography and Rf_2 values refer to the system of *n*-BuOH–AcOH–pyridine–H_2O (4:1:1:2) on silica gel (Kieselgel G, Merck) thin-layer chromatography.

D-Histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N$^{\varepsilon}$-formyl-L-lysyl-L-prolyl-L-valine Amide Diacetate (III)——DCC (1.24 g) was added to a solution of N$^{\alpha}$-benzyloxycarbonyl-D-histidyl-D-phenylalanyl-N^{G}-nitro-D-arginyl-D-tryptophylglycine[4] (1.85 g) and N$^{\varepsilon}$-formyl-L-lysyl-L-prolyl-L-valine amide (prepared from 1.30 g of the hydrochloride[9] and 0.42 ml of triethylamine) in dimethylformamide (DMF) (30 ml). After the solution was stirred at room temperature overnight, the solvent was evaporated and the residue was treated with AcOEt to give a solid, which was washed with H_2O. This crude prodiuct in 50% AcOH (150 ml) was hydrogenated in the usual manner, the solution was filtered and the filtrate was evaporarted. The residue, after drying over KOH pellets *in vacuo*, was dissolved in H_2O (500 ml) and the solution was applied to a column of CM-cellulose (3×35 cm), which was then eluted successively with the pH 6.9 ammonium acetate buffers, 0.04 M (2500 ml) and 0.05 M (300 ml) and finally with 20% AcOH. Individual fractions (17 ml each) were collected and absorbancy at 280 mμ located the desired compound in 0.05 M eluates, which were pooled. The solvent was evaporated and the residue was repeatedly lyophilized to constant weight to give a fluffy powder; yield 0.71 g (30%); $[\alpha]_D^{30}$ −39.4° (*c*=0.7, 10% AcOH); Rf_1 0.53, Rf_2 0.51, single spot positive to ninhydrin, Pauly, Sakaguchi and Ehrlich tests; amino acid ratios in an acid hydrolysate $His_{1.00}Phe_{0.89}Arg_{0.93}Gly_{1.00}Lys_{0.98}Pro_{0.89}Val_{0.94}$ (average recovery 91%). *Anal.* Calcd. for $C_{51}H_{72}O_9N_{16}\cdot 2CH_3COOH$ (dried at 100°): C, 56.3; H, 6.9; N, 19.1. Fonud: C, 56.3; H, 6.9; N, 19.3.

L-Glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N$^{\varepsilon}$-formyl-L-lysyl-L-proyl-L-valine Amide Acetate (IV)——A solution of D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N$^{\varepsilon}$-formyl-L-lysyl-L-prolyl-L-valine amide (0.36 g) in H_2O (6 ml) containing 1 N HCl (0.9 ml) was lypohilized. The resulting hydrochloride and N$^{\alpha}$-benzyloxycarbonyl-γ-benzyl-L-glutamate *p*-nitrophenyl ester[11] (0.45 g) were dissolved in DMF (20 ml) containing triethylamine (0.08 ml). After the solution was stirred at room temperature for 24 hr, the solvent was evaporated and the residue was treated with AcOEt. The resulting solid was hydrogenated in 50% AcOH for 8 hr. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was dried over KOH pellets and then dissolved in H_2O (300 ml). The solution was applied to a CM-cellulose column (3×13 cm), which was eluted successively with the following pH 6.9 ammonium acetate buffers; 0.015 M (1000 ml), 0.02 M (500 ml), 0.03 M (1500 ml). Absorbancy at 280 mμ was determined in various chromatographic fractions, in which the desired compound was present in 0.03 M eluates. These fractions were collected and the solvent was removed first by evaporation and finally by lyophilization to constant weight. A fluffy colorless powder was obtained; yield 0.29 g. (80%); $[\alpha]_D^{20}$ −26.2° (*c*=0.5, 10% AcOH); Rf_1 0.44, Rf_2 0.55, single spot positive to ninhydrin, Pauly, Sakaguchi and Ehrlich tests; amino acid ratios in an acid hydrolysate $Glu_{1.00}His_{1.00}Phe_{0.96}Arg_{1.02}Gly_{1.00}Lys_{1.02}Pro_{1.02}Val_{0.97}$ (average recovery 97%). *Anal.* Calcd. for $C_{56}H_{79}O_{12}N_{17}\cdot CH_3COOH$ (dried at 100°): C, 56.1; H, 6.7; N, 19.2. Found: C, 56.9; H, 6.7; N, 18.5.

N$^{\alpha}$-Acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-N$^{\varepsilon}$-formyl-L-lysyl-L-prolyl-L-valine Amide Acetate Decahydrate (V)——N$^{\alpha}$-Acetyl-L-seryl-L-tyrosyl-L-seryl-L-methionine azide (prepared from 58 mg of the hydrazide[8] as previously described) was

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added to an ice-cooled solution of IV (50 mg) in 80% aqueous pyridine (3.5 ml) containing 1% triethylamine (0.56 ml). The solution was stirred at 4° for 40 hr and then the solution was lyophilized. The residue was dissolved in H_2O (200 ml), which was applied to a column of CM-cellulose (1.5×8 cm). It was first eluted with H_2O (400 ml) and then the following ammonium acetate buffers (pH 6.9): 0.01 M (500 ml) and 0.02 M (750 ml). Individual fractions (10 ml each) were collected and absorbancy at 280 mμ was determined. The desired fraction in the 0.01 M eluates was collected and the solvent was evaporated *in vacuo*. The residue was lyophilized to constant weight; yield 45 mg (58%), $[\alpha]_D^{22}$ −37.9° (c=0.3, 30% AcOH); Rf_1 0.57, Rf_2 0.66; amino acid ratios in an acid hydrolysate $Ser_{2.01}Tyr_{1.06}Met_{1.06}Glu_{0.97}His_{1.07}Phe_{1.00}Arg_{1.05}Gly_{1.03}Lys_{1.03}Pro_{1.00}$-$Val_{0.91}$ (average recovery 87%). *Anal.* Calcd. for $C_{78}H_{109}O_{20}N_{21}S\cdot CH_3COOH\cdot 10H_2O$: C, 49.7; H, 6.9; N, 15.2. Found: C, 49.7; H, 6.2; N, 14.8.

N$^{\alpha}$-Acetyl-L-seryl-L-tyrosyl-L-methionyl-L-glutamyl-D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycyl-L-lysyl-L-prolyl-L-valine Amide Diacetate Tridecahydrate (II)——To a solution of V (45 mg) in H_2O (5 ml), 80% hydrazine hydrate (0.18 ml) and thioglycolic acid (0.02 ml) were added and the solution, after adjusting the pH to 6 with AcOH, was incubated at 100° for 3 hr. The solvent was lyophilized and the residue was applied to a column of CM-cellulose (1.5×16 cm), which was first eluted with H_2O (390 ml) and then the following ammonium acetate buffers; 0.01 M (650 ml), 0.025 M (600 ml) and 0.05 M (550 ml). Individual fractions (15 ml each) were collected and measurement of absorbancy at 280 mμ served to locate the desired fraction in 0.025 M eluate, which was pooled and the solvent was evaporated. The residue was lyophilized to constant weight; yield 28 mg (61%), $[\alpha]_D^{29}$ −23.3° (c=0.3, 10% AcOH), Rf_1 0.39, Rf_2 0.49; amino acid ratios in an acid hydrolysate $Ser_{2.10}Tyr_{1.10}Met_{1.08}Glu_{1.10}His_{1.03}Phe_{1.00}Arg_{1.04}Gly_{1.06}Lys_{1.01}Pro_{0.97}$-$Val_{1.00}$ (average recovery 97%). *Anal.* Calcd. for $C_{77}H_{109}O_{19}N_{21}S\cdot 2CH_3COOH\cdot 13H_2O$: C, 48.2; H, 6.3; N, 14.6. Found: C, 48.2; H, 6.3; N, 14.6.

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Syntheses and Properties of Several 4-Alkyl- or Arylsulfonylquinoline 1-Oxides

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It is now well known that 4-nitroquinoline 1-oxide (4-NQO) is one of the most potent carcinogenic and mutagenic agents.[2-5] In 1955, the mutagenic mechanism of this compound was proposed to involve a nucleophilic displacement *in vivo* of the nitro group by sulfhydril group of cystein residue of celluler protein. An alternative could not be excluded that the nitrous acid liberated in the displacement reaction process may play a substantial role in carcinogenesis or mutagenesis of the compound.[6]

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