The authors extend their gratitude to Mr. H. Matsui for the infrared spectral measurements, and to Miss Y. Indo and Mr. M. Shido for the microanalyses.

(Received October 29, 1965)

[Chem. Pharm. Bull. 14(7) 775~776 (1966)]

UDC 547.964.4.07 : 612.398.145

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Studies on Peptides. VII.[*3] Synthesis of Three Stereoisomeric Pentapeptides of Histidylphenylalanylarginyltryptophylglycine and Their Melanocyte-Stimulating Activities *in vitro*.

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In our recent communications,[*3,1~4] we have reported the syntheses and the physiological properties of stereoisomeric pentapeptides related to histidylphenylalanylarginyltryptophylglycine which corresponds to positions 6 to 10 in α-melanocyte-stimulating hormone (α-MSH). Of particular interest was the finding that the all-D-pentapeptide, D-histidyl-D-phenylalanyl-D-arginyl-D-tryptophylglycine[1,2] possessed an inhibitory action toward the physiological activity of the corresponding pentapeptide of all L-form.

We wish to record here the MSH activities of other three pentapeptide isomers which we have further synthesized. The method employed for the synthesis of these isomers are essentially the same as described in the preparation of L-histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine.[3,4] The MSH assays were performed according to the method of Shizume, *et al.*[5] using isolated pieces of frog-skins of *Rana pipiens*.

Experimental

The amino acid compositions of the acid and enzymatic hydrolysates were determined with a Hitachi amino acid analyzer, Model KLA-2 according to the method of Moore, *et al.*[6] The following abbreviations for the constituent amino acids, His=histidine, Phe=phenylalanine, Arg=arginine, Try=tryptophan, and Gly=glycine were used. Rf^1 values refer to the Partridge system[7]; Rf^2 values refer to the *sec*-BuOH-NH_4OH system[8] and were expressed as a multiple of distance traveled by Phe under identical conditions.

D-Histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine——$[\alpha]_D^{30}$ −16.5° (c=0.5, 1*N* HCl), Rf^1 0.50, Rf^2 1.0. Amino acid ratios in acid hydrolysate, $His_{1.00}Phe_{1.00}Arg_{1.00}Gly_{0.94}$ (average recovery 89%, Try was destroyed during the hydrolysis).

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Treatment of this peptide (2.88 μmoles) with leucine amino peptidase (LAP)[9] gave a neglisible amount of His (less than 0.025 μmole). *Anal.* Calcd. for $C_{34}H_{43}O_6N_{11}\cdot CH_3COOH\cdot 5H_2O$: C, 50.7; H, 6.7; N, 18.1. Found: C, 50.3; H, 6.6; N, 18.2.

The assay result indicated that it has no MSH activity, but exhibits a color lightening activity (approximately 10^{-6} of that of melatonin[10]) against the action of α-MSH.

D-Histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine——$[\alpha]_D^{25}$ −49.2°(c=0.8, 1*N* HCl), Rf^1 0.45, Rf^2 1.0. Amino acid ratios in acid hydrolysate, $His_{1.05}Phe_{1.00}Arg_{1.00}Gly_{1.00}$ (average recovery 99%). α-Chymotryptic digestion*4 of this peptide was performed in 0.1*M* "tris" buffer at pH 8.0 with an enzyme-substrate ratio of 1/28 (w/w) at 37° for 18 hr. The hydrolysate was examined by paper chromatography. No extra spot besides the original pentapeptide was detected on paper chromatogram in both Partridge and *sec*-BuOH-NH_4OH system by ninhydrin test. *Anal.* Calcd. for $C_{34}H_{43}O_6N_{11}\cdot CH_3COOH\cdot 4H_2O$: C, 51.9; H, 6.7; N, 18.5. Found: C, 52.1; H, 6.6; N, 18.2.

It was found that this peptide exhibited the darkening activity equivalent to 5.5×10^4 MSH U/g. Previously we have observed that the histidine residue in histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine must possess L-configuration in order for the pentapeptide to exert MSH activity.[3] The present result offered an example of possible steric requirements for the pentapeptide possessing a D-histidine residue to exert MSH activity.

L-Histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine——$[\alpha]_D^{25}$ −20.9°(c=0.6, 1*N* HCl) (lit.[11] $[\alpha]_D^{27}$ −20° in 0.1*M* NH_4OH), Rf^1 0.45, Rf^2 1.0. Amino acid ratios in acid hydrolysate, $His_{1.00}Phe_{1.00}Arg_{0.97}Gly_{1.00}$ (average recovery 99%). Digestion of this peptide with α-chymotrypsin*4 and examination of the hydrolysate by paper chromatography were conducted as described above. A single ninhydrin positive spot corresponding to the original peptide was detected on paper chromatogram in both Partridge and *sec*-BuOH-NH_4OH system. *Anal.* Calcd. for $C_{34}H_{43}O_6N_{11}\cdot CH_3COOH\cdot 5H_2O$: C, 50.7; H, 6.7; N, 18.1. Found: C, 51.2; H, 6.7; N, 17.4.

This pentapeptide was prepared by the different method from that of Li, *et al.*[11] who reported that their synthetic compound exhibited the characteristic prolonged MSH activity *in vitro*. Our preparation did not show any significant prolonged MSH activity *in vitro* when it was compared to that of alkali treated α-MSH.[12]

It exhibited the activity, 1×10^6 MSH U/g. Our observation that this peptide has much higher activity than that of L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophylglycine is in close agreement with the finding of Li, *et al.*[11] who reported the activity of 1×10^5 MSH U/g. for this compound.

This work has been supported in part by a grant from Tanabe Amino Acid Research Foundation. The authors express their gratitude to Prof. S. Uyeo for his encouragement during the course of this investigation.

(Received November 9, 1965)

*4 α-Chymotrypsin was purchased from Worthington Biochem. Corp. Lot. No. H29809.

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