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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 histidylphenylalanylglycinyltryptophylglycine 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-L-tryptophylglycine. The MSH assays were performed according to the method of Shizume, et al., 6 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 values refer to the Partridge system; RP values refer to the sec-ButOH-NH4OH system and were expressed as a multiple of distance traveled by Phe under identical conditions.

D-Histidyl-L-phenylalanyl-L-arginyl-D-tryptophylglycine —— (α)20D = −16.5° (c = 0.5, 1N HCl), RF = 0.50, RP = 1.0. Amino acid ratios in acid hydrolysate, His1.0, Phe1.0, Arg1.0, Gly 1.0 (average recovery 90%). Try was destroyed during the hydrolysis.

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*3 Part VI : This Bulletin, 13, 1326 (1965).
5) K. Shizume, A.B. Lerner, T.B. Fitzpatrick : Endocrinol., 54, 553 (1954). The authors wish to express their appreciation to Dr. S. Lande, School of Medicine, Yale University, for these biological assays.
Treatment of this peptide (2.88 μmoles) with leucine amino peptidase (LAP) gave a negligible amount of His (less than 0.025 μmole). Anal. Calcd. for C₉₃H₁₀₂O₉N₁₁·CH₃COOH·5H₂O: 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 changing activity (approximately 10⁻⁶ of that of melatonin) against the action of α-MSH.

D-Histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine —— [α]₆° —— 49.2° (c=0.8, 1N HCl), Rp² 0.45, Rp¹ 1.0. Amino acid ratios in acid hydrolysate, His₆, Phe₁₀, Arg₁₀, Gly₁₀, (average recovery 99%). α-Chymotryptic digestion of this peptide was performed in 0.1M "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–NH₄OH system by ninhydrin test. Anal. Calcd. for C₉₁H₀₉O₉N₁₁·CH₃COOH·4H₂O: 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 5.5×10⁴ MSH U/g. Previously we have observed that the histidine residue in histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine must possess L-configuration in order for the pentapeptide to exert MSH activity. 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 —— [α]₆° —— -20.9° (c=0.6, 1N HCl) (lit.¹¹) [α]₆° —— -20° in 0.1M NH₄OH), Rp² 0.45, Rp¹ 1.0. Amino acid ratios in acid hydrolysate, His₆, Phe₁₀, Arg₁₀, Gly₁₀, (average recovery 99%). Digestion of this peptide with α-chymotrypsin 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–NH₄OH system. Anal. Calcd. for C₉₃H₁₀₂O₉N₁₁·CH₃COOH·5H₂O: 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. who reported that their synthetic compound exhibited the characteristic prolonged MSH activity in vitro. Our preparation did not show any significant MSH activity in vitro when it was compared to that of alkali treated α-MSH.

It exhibited the activity, 1×10⁴ MSH U/g. Our observation that this peptide has much higher activity than that of l-histidyl-D-phenylalanyl-L-arginyl-L-tryptophylglycine is in close agreement with the finding of Li, et al. who reported the activity of 1×10⁴ MSH U/g for this compound.

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