The Synthesis of an MSH-active Tetrapeptide, L-Histidyl-L-phenylalanyl-L-arginyl-L-tryptophan

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Hofmann and Yajima[1] and Pickering and Li[2] concluded that the key to the melanocyte-stimulating activity resided in the His-Phe-Arg-Try-Gly sequence (positions 6 to 10) in the α-MSH molecule. However, the requirement for the glycine is not yet clear.

We have now synthesized a tetrapeptide, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophan (I), which exhibits the same level of MSH potency as do the pentapetides, L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine[3] and its D-phenylalanine analog.[4] From this observation it may be concluded that the glycine is not essential for the biological activity, and that the tetrapeptide is the smallest peptide fragment exhibiting MSH activity found so far. The outline of the synthetic route is:

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3) K. Hofmann, M. E. Woolner, H, Yajima, G. Spühler, T. A. Thompson and E. T. Schwarz, *J. Am. Chem. Soc.*, **80**, 6458 (1958); R. Schwyzer and C. H. Li, *Nature*, **182**, 1669 (1958).
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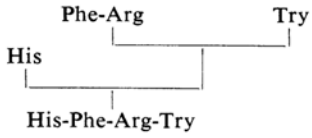

N^G-Tosyl-L-arginine methyl ester (crystalline free base, m. p. 98~98.5°C, $[\alpha]_D^{25}=+14.8°$ (MeOH)) and *t*-butyloxycarbonyl-L-phenylalanine (prepared from the dicyclohexylamine salt; m. p. 210~212°C decomp., $[\alpha]_D^{25.5}=+28.9°$ (MeOH)) were coupled by the *N*, *N'*-dicyclohexylcarbodiimide (DCCI) method[5] to give *t*-butyloxycarbonyl-L-phenylalanyl-N^G-tosyl-L-arginine methyl ester (II); amorph., $[\alpha]_D^{25}=-5.9°$ (MeOH). Found: C, 56.8; H, 7.0; N, 11.8; S, 5.6. Calcd. for $C_{28}H_{39}O_7N_5S$: C, 57.1; H, 6.7; N, 11.9; S, 5.4%. Compound II was converted into the corresponding acid (III) (amorph., $[\alpha]_D^{24}=+1.0°$ (MeOH). Found: C, 56.0; H, 6.8; N, 11.4; S, 5.8. Calcd. for $C_{27}H_{37}O_7N_5S$: C, 56.3; H, 6.5; N, 12.2; S, 5.6%.) by saponification or into the hydrazide (IV) (crystalline, m. p. 110~114°C, $[\alpha]_D^{24}=-6.3°$ (MeOH)). Found: C, 55.2; H, 7.2; N, 16.6; S, 5.5. Calcd. for $C_{27}H_{39}O_6N_7S$: C, 55.0; H, 6.7; N, 16.6; S, 5.4%. The coupling of the azide (derived from compound IV) with L-tryptophan benzyl ester (m. p. 71°C, $[\alpha]_D^{26.5}=+12.8°$ (MeOH); lit.[6] m. p. 71°C) gave a tripeptide, *t*-butyloxycarbonyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophan benzyl ester (V); amorph., purified on a silica gel column, $[\alpha]_D^{24.5}=-6.6°$ (MeOH). Found: C, 63.3; H, 6.4; N, 11.1; S, 3.7. Calcd. for $C_{45}H_{53}O_8N_7S$: C, 63.5; H, 6.3; N, 11.5; S, 3.8%. Compound V was, after the removal of the *t*-butyloxycarbonyl group by trifluoroacetic acid treatment, condensed by the DCCI method with N^{α}, N^{Im}-dicarbobenzoxy-L-histidine[7] to afford a tetrapeptide derivative, N^{α}, N^{Im}-dicarbobenzoxy-L-histidyl-L-phenylalanyl-N^G-tosyl-L-arginyl-L-tryptophan benzyl ester (VI); amorph., purified on silica gel, $[\alpha]_D^{24.5}=-10.9°$ (MeOH). Found: C, 64.3; H, 5.9; N, 11.9; S, 3.0 Calcd. for $C_{62}H_{64}O_{11}N_{10}S$: C, 64.4; H, 5.6; N, 12.1; S, 2.8%. The protecting groups of compound VI could be removed by treatment with sodium in liquid ammonia to give peptide I, which was, after purification on a carboxymethyl cellulose column, homogeneous with ninhydrin, Pauly, Ehrlich, and Sakaguchi reagents on paper chromatography ($R_f=0.55$ in the system of *n*-butanol/acetic acid/water (4/1/2)) and on paper electrophoresis at pH 3.8, 6.6, and 11.1. $\lambda_{max}^{HCl}=280.5$ mμ (ε, 5100), $[\alpha]_D^{24.5}=-5.4°$ (N HCl). Found: C, 55.3; H, 6.7; N, 18.0. Calcd. for $C_{32}H_{40}O_5N_{10}\cdot CH_3COOH\cdot 2H_2O$: C, 55.1; H, 6.5; N, 18.9%. The amino acid ratios[8] were $His_{1.00}$ $Phe_{1.00}$ $Arg_{1.03}$ ($Try_{0.79}$) (recovery 92.2%) in acid hydrolysate and $His_{1.00}$ $Phe_{1.03}$ $Arg_{0.97}$ $Try_{0.98}$ (recovery 92.5%) in LAP[9] digest. The MSH activity, as estimated by the method of Shizume and Lerner,[10] was 3.6×10^4 units per gram.[11]

The synthesis of compound V is the first instance in which the azide method could be successfully employed in the synthesis of arginyl peptide. When the Arg-Try bond was formed by means of the DCCI method, by the route shown above, the tetrapeptide obtained was contaminated by at least 10 to 15% of the racemate, but it exhibited the same hormonal potency.

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11) We wish to express our sincere thanks to Prof. C. H. Li, Hormone Research Laboratory, University of California, for giving us a standard preparation of α-MSH (1×10^9 units per gram).