The Synthesis of Glycyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine and Its Melanocyte-stimulating Activity

Eugen Schnabel and Choh Hao Li

From The Hormone Research Laboratory, University of California, Berkeley, California

(Received for publication, March 7, 1960)

Recent work (1-3) on the synthesis of peptides composed of amino acid sequences that appear in both the adenocorticotropic and the melanotropic peptides has revealed that melanocyte-stimulating activity is enhanced as the length of the peptide chain increases (4). It was shown that the hexapeptide L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine possesses an activity almost 10 times higher than that of the pentapeptide from which the N-terminal glutamyl residue is absent (2). The question arose whether this increase in biological activity is due specifically to the presence of the glutamyl residue itself or merely to the added length of peptide chain. We decided, therefore, to synthesize the hexapeptide with glycine at the N-terminus instead of glutamic acid. It will be seen herein that this substitution results in no alteration of melanocyte-stimulating activity.

The synthesis of glycyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine was accomplished by coupling the tripeptide derivative, G-toxy-L-arginyl-L-tryptophyl-glycine methyl ester (5), with another tripeptide derivative, carbobenzoxyglycyl-L-histidyl-L-phenylalanine. The latter was obtained from the reaction of carbobenzoxyglycyl-L-histidine azide (6) with the methyl ester of L-phenylalanine (7). The azide coupling was not satisfactory because of poor solubility, and the yield was low; however, when the tripeptide ester was saponified, the derivative desired for the final coupling was obtained. The blocked hexapeptide did not crystallize; saponification yielded an amorphous product, carbobenzoxyglycyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine, which was then subjected to reduction with sodium in liquid ammonia (8). The free hexapeptide was desalted on an Amberlite IRC-50 (XE-64) ion exchange column, as previously described (9), and lyophilized.

The content of tryptophan in the peptide was estimated spectrophotometrically (10), and the other amino acids were determined by the method of Levy (11); these analyses gave molar values, relative to the position of glycine on the chromatograms, for the various fragments as follows: Gly. His. Phe, 1.45; Arg. Try, 1.58; Gly. His. Phe. Arg, 0.4; and Try. Gly, 2.8.

Identification of the hydrolytic products were achieved in the same manner as in previously reported studies (12). The hexapeptide was assayed for melanocyte-stimulating activity by the frog skin method in vitro (13), and on the basis of change in the melanophore index in hypophysectomized frogs (14). The results of these bioassays, summarized in Table I, indicate that the melanotropic activity of Gly-L-His-L-Phe-L-Arg-L-Try-Gly is not different from that exhibited by the glutamyl analogue. It is evident that glutamic acid can be replaced by glycine in the hexapeptide without alteration of the melanocyte-stimulating potency. These data clearly show that an increase in the length of the peptide chain from a pentapeptide to a hexapeptide results in enhancement of melanotropic potency.

**EXPERIMENTAL PROCEDURE**

All melting points are uncorrected. Elementary analyses were performed by the Microchemical Laboratory of the Department of Chemistry of this University. Paper chromatography was carried out on Whatman No. 1 filter paper at room temperature; the solvents used were n-butanol-acetic acid-water in a ratio of 4:1:1 and sec-butanol-10% NH₃ in a ratio of 85:15.²

Carbobenzoxyglycyl-L-histidyl-L-phenylalanine Methyl Ester—Carbobenzoxyglycyl-L-histidine hydrazide (6) in the amount of 4 g was dissolved in a mixture of 3 ml of concentrated hydrochloric acid and 30 ml of water and converted to the azide form by means of reaction with a saturated aqueous solution consisting of 300 mg of NaN₃. The pH of this mixture was then adjusted to 3.5 by the addition of 5 M K₂CO₃, and the oily azide was extracted with four 80 ml portions each of ethyl acetate and chloroform. The combined extracts were washed with 1 M NaHCO₃ and water, the washed extracts were dried with anhydrous Na₂SO₄, and a solution of L-phenylalanine methyl ester (7) in ethyl acetate was added. The reaction mixture was then adjusted to 9.5 by the addition of 5 M K₂CO₃, and the oily azide was extracted with four 80 ml portions each of ethyl acetate and chloroform. The combined extracts were washed with 1 M NaHCO₃ and water, the washed extracts were dried with anhydrous Na₂SO₄, and a solution of L-phenylalanine methyl ester (7) in ethyl acetate was added. The reaction mixture was kept in the refrigerator for 24 hours and at room temperature for an additional 24 hours. The resulting colorless crystals in a yield of 1.55 g were then filtered off and washed with NaHCO₃ and water. An additional crop of 0.35 g of crystals was obtained when the mother liquor was processed. The crystals were combined and recrystallized several times from methanol in a yield of 1.9 g (33%); the melting point was 194–195°.

C₂₉H₃₂N₄O₁₀ (507.53)

**Calculated:** C 61.52, H 5.76, N 13.80

**Found:** C 61.27, H 5.57, N 13.49

[α]₂⁰° = −11.0° (c = 2, in methanol)

¹ In paper chromatography carried out for 60 hours in the solvent system consisting of n-butanol-acetic acid-water (4:1:1), the Rₐ values, relative to the position of glycine on the chromatograms, for these various fragments were as follows: Gly. His. Phe, 1.45; Arg. Try, 1.58; Gly. His. Phe. Arg, 0.4; and Try. Gly, 2.8.

² The former solvent system will be abbreviated throughout as BAW, the latter, SBA.
**Table I**

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Frog skin</th>
<th>Hypophysectomized frogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-His-L-Pho-L-Arg-L-Try-Gly</td>
<td>3.1 x 10⁴</td>
<td>10</td>
</tr>
<tr>
<td>L-Glu-L-His-L-Pho-L-Arg-L-Try-Gly</td>
<td>2.2 x 10⁴</td>
<td>2</td>
</tr>
<tr>
<td>Gly-L-His-L-Pho-L-Arg-L-Try-Gly</td>
<td>2.3 x 10⁴</td>
<td>2</td>
</tr>
</tbody>
</table>

* An average based on at least 3 assays; each assay was performed on 4 to 6 pieces of frog skin.
* A single dose caused a change in melanophore index from 1+ to 3+ within 1 hour in hypophysectomized Rana pipiens.

**Summary**

Glycyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine has been synthesized and its melanocyte-stimulating potency estimated by two assay procedures. It was found that the melanotropic activity of this hexapeptide is almost identical to that exhibited by L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophyl-glycine.

**Acknowledgments**—This study was undertaken as the result of a discussion with Dr. R. Schwzyzer. The authors wish to thank the Conference Board of the Associated Research Councils for a Fulbright travel grant.
REFERENCES

3. HOFMANN, K., Abstracts of papers of the 139th Meeting of the American Chemical Society, Washington, 1959, p. 21e.
The Synthesis of Glycyl-l-histidyl-l-phenylalanyll-arginyll-l-tryptophyl-glycine and Its Melanocyte-stimulating Activity
Eugen Schnabel and Choh Hao Li


Access the most updated version of this article at
http://www.jbc.org/content/235/7/2010.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/235/7/2010.citation.full.html#ref-list-1