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

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Recent work (1, 3) on the synthesis of peptides composed of amino acid sequences that appear in both the adrenocorticotropins and the melanotropins 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-terminal 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-tosyl-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-arginyll-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 ratios for glycine-histidine-phenylalanine-arginine-tryptophan of 1:1:1:0.8:0.9:1.0. Chymotryptic digestion of the peptide 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 NaNO₂. The pH of this 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 Na₂S₀₄, 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, 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 0.55 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°.

**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 NaNO₂. The pH of this 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 0.55 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°.

CasH₁₂N₄O₃ (507.53)

Calculated: C 61.52, H 5.76, N 13.80

Found: C 61.27, H 5.57, N 13.49

[a]₂⁰ = 11.0° (c = 2, in methanol)

¹ The former solvent system will be abbreviated throughout as BAW, the latter, SBA.
Carbobenzoxyglycyl-L-histidyl-L-phenylalanine—The above ester, in the amount of 1.95 g, was dissolved in 60 ml of a 1:1 mixture of ethyl alcohol and chloroform and kept at room temperature for 1.5 hours after the addition of 7 ml of 1 M NaOH. The pH was adjusted to 5.3 by the addition of 1 M HCl, and the solvent was then removed under reduced pressure. The residue left after evaporation was dissolved in methanol, and water was added to this solution, whereupon a sirup precipitated. The sirup crystallized after titration with acetone. The material was recrystallized from pyridine-methanol in a yield of 1.2 g (64%); the melting point was 180–182° with decomposition.

C9H11NO4 (453.51)
Calculated: C 58.77, H 6.04, N 15.51
Found: C 58.69, H 5.80, N 14.79

\[ \alpha \] ~18.2° (c = 1, in pyridine)

In paper chromatography, \( R_F \text{BA} = 0.23 \) and \( R_F \text{BAW} = 0.55 \).

Carbobenzoxyglycyl-L-histidyl-L-phenylalanyl-G-tosyl-L-arginyl-L-tryptophyl-glycine Methyl Ester—Carbobenzoxyglycyl-L-histidyl-L-phenylalanine (1165 mg) and the methyl ester of G-tosyl-L-arginyl-L-tryptophyl-glycine (1430 gm) (5) were dissolved in 50 ml of dimethylformamide. After the solution was cooled to 0°, 525 mg of dicyclohexylcarbodiimide (15) were added and the mixture was kept in the refrigerator for a week. The urea (430 mg) was removed by filtration and the di-tosyl-L-arginyl-L-tryptophyl-glycine (1430 gm) (5) were dissolved in 180 ml of liquid ammonia and small pieces of metallic sodium were added to this solution until the blue color persisted for 10 minutes. The excess sodium was destroyed by addition of ammonium acetate and the solvent was evaporated in a vacuum. The residue was dried over POCl3 and dissolved in 0.5 ml of ethyl acetate and the solvents were removed by distillation in a vacuum. The residue was washed well with much water and then dried over POCl3, to give a product which melted at 158-162°.

C13H14NO4 (268.23)
Calculated: C 56.38, H 6.11, N 22.15
Found: C 56.40, H 5.99, N 21.91

\[ \alpha \] ~18.3° (c = 1, in methanol)

In paper chromatography, \( R_F \text{BA} = 0.83 \).

Carbobenzoxyglycyl-L-histidyl-L-phenylalanyl-G-tosyl-L-arginyl-L-tryptophyl-glycine—The blocked hexapeptide ester (1.0 g) was dissolved in 60 ml of methanol, 3 ml of 1 M NaOH were added, and the solution was kept at room temperature for 90 minutes. The pH was then adjusted to 5.3 to 5.4 by the addition of 3 ml of 1 M HCl and the solvents were removed by distillation in a vacuum. The residue was washed well with much water and then dried over P2O5, to give a product which melted at 158–162°. After reprecipitation from methanol, the product, obtained in a yield of 1.1 g (70%), melted at 158–158° with sintering from 178°.

C15H16NO6 (324.32)
Calculated: C 53.20, H 6.48, N 20.1
Found: C 53.30, H 6.00, N 15.72

\[ \alpha \] ~13.8° (c = 2, in methanol)

In paper chromatography, \( R_F \text{BA} = 0.30 \).

**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.

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