[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Synthesis of S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine, a Pentapeptide Derivative Related to Vasopressin[1]

BY PANAYOTIS G. KATSOYANNIS AND VINCENT DU VIGNEAUD

RECEIVED APRIL 12, 1956

This protected pentapeptide has been prepared by two routes, one involving the coupling of S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine with L-phenylalanyl-L-glutaminyl-L-arsparagine and the other, the coupling of S-benzyl-N-carbobenzoxy-L-cysteine with L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine, by the mixed anhydride method. Both synthetic products possessed the same melting point and optical rotation, thus indicating that no racemization had occurred in the course of the synthesis.

Degradation studies on purified preparations of arginine vasopressin, the principal pressor and antidiuretic hormone of the beef posterior pituitary gland, revealed the sequence[2] of the amino acids in the molecule and enabled the postulation of its structure.[3,4] In this structure the sequence—cystinyl - tyrosyl - phenylalanyl - glutaminyl - asparaginyl—is present. It has also been shown that lysine vasopressin, the pressor and antidiuretic principle of the hog posterior pituitary gland, has the same composition as arginine vasopressin with the exception that it contains lysine instead of arginine,[5] and a similar structure has been suggested for this hormone.[3]

In connection with the synthetic approach to arginine vasopressin, the pentapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine was desired, and its preparation is described herein.[6] This synthesis has been accomplished by two routes in order to check on the optical purity of the peptide. In both syntheses the key intermediate was the tripeptide L-phenylalanyl-L-glutaminyl-L-asparagine, which was prepared by Popenoe and du Vigneaud[7] by reduction of its N-tosyl-(*p*-toluenesulfonyl) derivative with sodium in liquid ammonia. This tosyl tripeptide has now been prepared by a slightly modified procedure, namely, by dissolving the tosyl-L-phenylalanyl chloride in dioxane and then coupling it with an aqueous suspension of L-glutaminyl-L-asparagine[8] in the presence of magnesium oxide. This procedure facilitated the handling of the reaction and a higher yield was obtained.

For preparation of the protected pentapeptide, the mixed anhydride between S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine[9] and isobutylcarbonic acid, which was formed[10] in tetrahydrofuran solution at −10°, was allowed to react with an aqueous solution of L-phenylalanyl-L-glutaminyl-L-asparagine in the presence of one equivalent of triethylamine. S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine was obtained. The product crystallized as long needles, which melted at 214°, $[\alpha]^{24}D$ −29° (*c* 1, dimethylformamide).

By the alternate route, a solution of O,N-dicarbobenzoxy-L-tyrosine[11] in tetrahydrofuran was caused to react, by the mixed anhydride method[10] using isobutyl chlorocarbonate, with an aqueous solution of L-phenylalanyl-L-glutaminyl-L-asparagine in the presence of one equivalent of triethylamine, to give O,N-dicarbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine in a yield of over 70%. The same protected tetrapeptide was also prepared in 30% yield by the coupling of O,N-dicarbobenzoxy-L-tyrosyl chloride[11] with an aqueous suspension of L-phenylalanyl-L-glutaminyl-L-asparagine in the presence of magnesium oxide. The removal of the carbobenzoxy groups was effected by treatment with hydrogen bromide in acetic acid,[12] the hydrobromide of L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine being obtained in a yield of over 90%. This compound was dissolved in water in the presence of two equivalents of triethylamine and allowed to react with the mixed anhydride between N-carbobenzoxy-S-benzyl-L-cysteine[13] and isobutylcarbonic acid in tetrahydrofuran solution, to give S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine. The crystalline pentapeptide melted at 214°, $[\alpha]^{24}D$ −29° (*c* 1, dimethylformamide).

Experimental[14,15]

Tosyl-L-phenylalanyl-L-glutaminyl-L-asparagine.—A solution of 7.5 g. of tosyl-L-phenylalanyl chloride[7] in 35 ml. of purified dioxane was added in portions, with shaking, over a period of 1 hr. to an ice-cold suspension of 6.2 g. of L-glutaminyl-L-asparagine[8] and 2.6 g. of magnesium oxide in 50

(1) This work was supported in part by grants from the National Heart Institute, Public Health Service, Grant H-1675, and Lederle Laboratories Division, American Cyanamid Co.

(2) E. A. Popenoe and V. du Vigneaud, *J. Biol. Chem.*, **206**, 353 (1954).

(3) V. du Vigneaud, H. C. Lawler and E. A. Popenoe, THIS JOURNAL, **75**, 4880 (1953).

(4) R. Acher and J. Chauvet, *Biochem. Biophys. Acta*, **12**, 487 (1953).

(5) E. A. Popenoe, H. C. Lawler and V. du Vigneaud, THIS JOURNAL, **74**, 3713 (1952).

(6) A preliminary report of this work has already appeared [V. du Vigneaud, D. T. Gish and P. G. Katsoyannis, *ibid.*, **76**, 4751 (1954)].

(7) E. A. Popenoe and V. du Vigneaud, *ibid.*, **76**, 6202 (1954).

(8) J. M. Swan and V. du Vigneaud, *ibid.*, **76**, 3110 (1954).

(9) C. R. Harington and R. V. Pitt Rivers, *Biochem. J.*, **38**, 417 (1944); C. W. Roberts and V. du Vigneaud, *J. Biol. Chem.*, **204**, 871 (1953).

(10) J. R. Vaughan, Jr., and J. A. Eichler, THIS JOURNAL, **75**, 5556 (1953).

(11) E. Abderhalden and A. Bahn, *Z. physiol. Chem.*, **219**, 72 (1933).

(12) G. W. Anderson, J. Blodinger and A. D. Welcher, THIS JOURNAL, **74**, 5309 (1952); D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952); I. Schumann and R. A. Boissonnas, *Helv. Chim. Acta*, **35**, 2237 (1952).

(13) C. R. Harington and T. H. Mead, *Biochem. J.*, **30**, 1598 (1936).

(14) Capillary melting points were determined for all compounds and are corrected.

(15) The authors are indebted to Mr. Joseph Albert for carrying out the analyses.

ml. of water. Care was taken to keep the *p*H of the mixture above 8 by the addition of more magnesium oxide when necessary. The reaction mixture was shaken for another 20 minutes, diluted with 100 ml. of water and acidified with dilute HCl to *p*H 2. The product was separated by filtration, washed with water and dried. After trituration with ethyl acetate, 9.8 g. was obtained. Purification by precipitation from dilute $KHCO_3$ with HCl gave 8.6 g. (70%); m.p. 193–195°, literature[7] 193–195°.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine.—A solution of 1 g. (0.002 mole) of S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosine[9] in 6 ml. of purified tetrahydrofuran and 0.2 g. (0.002 mole) of triethylamine was cooled to −10°, and 0.28 g. (0.002 mole) of isobutyl chlorocarbonate was added with stirring. After 8 minutes at this temperature a cooled solution of 0.85 g. (5% excess) of L-phenylalanyl-L-glutaminyl-L-asparagine[7] and 0.22 g. of triethylamine in 5 ml. of water was added. The mixture was then allowed to come to room temperature over a period of 20 minutes and the triethylamine salt of the product was precipitated with ether. The precipitate was filtered off, washed with ether, suspended in 100 ml. of water and acidified with HCl. The product was separated by filtration, washed with water, dried and triturated with ethyl acetate; wt. 1.1 g. (64%); m.p. 207–209°. The pentapeptide crystallized from 80% aqueous tetrahydrofuran in the form of needles, m.p. 214°, $[\alpha]^{24}D$ −29° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{45}H_{51}O_{11}N_7S$: C, 60.2; H, 5.72; N, 10.9. Found: C, 60.2; H, 5.99; N, 10.6.

O,N-Dicarbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine. A. Mixed Anhydride Method.—A solution of 1.35 g. (0.003 mole) of O,N-dicarbobenzoxy-L-tyrosine[11] and 0.30 g. (0.003 mole) of triethylamine in 10 ml. of purified tetrahydrofuran was cooled to −10° and 0.41 g. (0.003 mole) of isobutyl chlorocarbonate was added with stirring. After 10 minutes at this temperature a cooled solution of 1.28 g. (5% excess) of L-phenylalanyl-L-glutaminyl-L-asparagine and 0.32 g. of triethylamine in 7 ml. of water was added. The reaction mixture was allowed to come to room temperature over a 20-minute period, and the triethylamine salt of the tetrapeptide was precipitated with ether as a heavy oil, which solidified after standing in the refrigerator for a short time. The product was then filtered off, washed with ether, suspended in 150 ml. of water and acidified with HCl. The tetrapeptide thus obtained was separated by filtration, washed with water, dried, triturated with ethyl acetate and purified by precipitation from 50% aqueous formic acid; wt. 1.78 g. (70.8%); m.p. 219–220°; $[\alpha]^{21}D$ −21° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{43}H_{46}N_6O_{12}$: C, 61.6; H, 5.52; N, 10.0. Found: C, 61.5; H, 5.50; N, 9.50.

B. **Acid Chloride Method.**—A solution of 0.56 g. (0.0012 mole) of O,N-dicarbobenzoxy-L-tyrosyl chloride[11] in 4 ml. of purified dioxane was added in portions with shaking over a period of 20 minutes to an ice-cold suspension of 0.5 g. (0.0012 mole) of L-phenylalanyl-L-glutaminyl-L-asparagine and 0.1 g. of magnesium oxide in 6 ml. of water. Care was taken to keep the *p*H of the mixture above 8 by the addition of more magnesium oxide when necessary. After all of the acid chloride had been added, 10 ml. of water was added and the mixture was acidified with HCl. The product was filtered off, washed with water, dried and triturated with ethyl acetate; wt. 0.31 g. (30%); m.p. 219–220°.

L-Tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine Hydrobromide.—O,N-Dicarbobenzoxy-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine (1 g.) was suspended in 20 ml. of 2 *N* hydrogen bromide in glacial acetic acid, and the mixture was warmed for 15 minutes at 65° to give a homogeneous solution. After 10 minutes at room temperature the product was precipitated with ether as a white solid which was filtered off, washed several times with ether and purified by reprecipitation from ethanol–ether; wt. 0.695 g. (90%); m.p. indefinite, $[\alpha]^{21}D$ +6.3° (*c* 1, H_2O).

Anal. Calcd. for $C_{27}H_{35}O_8N_6Br$: N, 12.9; Br, 12.3. Found: N, 12.6; Br, 11.9.

S-Benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine.—A solution of 360 mg. (1.04 mmoles) of S-benzyl-N-carbobenzoxy-L-cysteine[13] and 105 mg. (1.04 mmoles) of triethylamine in 4 ml. of purified tetrahydrofuran was cooled to −10° and 143 mg. (1.04 mmoles) of isobutyl chlorocarbonate added with stirring. After 10 minutes at this temperature a cooled solution of 677 mg. (1.04 mmoles) of L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine hydrobromide and 210 mg. (2.08 mmoles) of triethylamine in 3 ml. of water was added. The reaction mixture was allowed to come to room temperature over a period of 20 minutes and then the triethylamine salt of the product was precipitated with ether. The precipitate was filtered off, washed with ether, suspended in 75 ml. of water and acidified with HCl. The product was separated by filtration, washed with water, dried and triturated with ethyl acetate; wt. 0.8 g. (86.9%); m.p. 205–207°. The pentapeptide crystallized from 80% aqueous tetrahydrofuran, as needles, m.p. 214°, $[\alpha]^{24}D$ −29° (*c* 1, dimethylformamide).

NEW YORK 21, NEW YORK

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE]

Poly-L-cysteine

BY ARIEH BERGER, JUNZO NOGUCHI[1] AND EPHRAIM KATCHALSKI

RECEIVED APRIL 2, 1956

S-Carbobenzoxy-N-carboxy-L-cysteine anhydride (III) was prepared from N,S-dicarbobenzoxy-L-cysteine (I) or from S-carbobenzoxy-L-cysteine (II). Poly-S-carbobenzoxy-L-cysteine (IV), obtained by the polymerization of III, yielded on reduction with sodium in liquid ammonia poly-L-cysteine (V). The reactivity of the thiol groups of V toward various SH-reagents was investigated. V yielded poly-S-carboxymethylcysteine (VI) on treatment with iodoacetic acid. VI was also obtained by the polymerization of S-carbomethoxymethyl-N-carboxycysteine anhydride (XI), followed by de-esterification. Poly-L-cysteic acid (VIII) was derived from IV by oxidation with performic acid.

The sulfhydryl groups of proteins have been extensively investigated, because of their importance in biological processes, their high chemical reactivity and the ease with which they may be detected and estimated. Native proteins have been found to contain thiol groups of three grades of reactivity[2]: (a) freely reacting –SH groups, reacting readily with nitroprusside and with mild oxidizing agents, (b) sluggish –SH groups, which do not give the nitroprusside reaction but may react with iodine and mercaptide forming compounds, and (c) masked –SH groups which can be detected only after the denaturation of the native protein.

Because of the complexity of the protein molecule, however, this different reactivity of the various types of sulfhydryl groups has as yet not been satisfactorily explained. The study of the chemical behavior of the –SH groups of polypeptides containing cysteine residues may shed new light on this prob-

(1) Weizmann Fellow, 1955–1956; on leave of absence from Kanazawa University, Kanazawa, Japan.

(2) E. S. G. Barron in "Advances in Enzymology," Vol. 11, Interscience Publishers, New York, N. Y., 1951, p. 219.