Anal. Calcd. for $C_{58}H_{77}N_{15}O_{16}\cdot 2H_2O$: C, 54.59; H, 6.40; N, 16.46. Found: C, 54.54; H, 6.68; N, 16.40.

L-Citrullyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine. (1-Cit Bradykinin) (VIc).—The nonapeptide Vc (500 mg.) was dissolved in methanol–acetic acid and hydrogenated in the presence of palladium black in the usual manner. The mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was redissolved in water and filtered; the filtrate was shell frozen and lyophilized to yield 300 mg. of a colorless solid, $[\alpha]^{23}D$ −93° (*c* 1, *N* acetic acid), lit.[7] $[\alpha]^{20}D$ −91.2° (*c* 1, *N* acetic acid).

Carbobenzoxy-γ-methyl-L-glutamic Acid *p*-Nitrophenyl Ester.—To a cold (5°) solution of 5 g. (0.017 mole) of carbobenzoxy-γ-methyl-L-glutamic acid in 100 ml. of ethyl acetate was added 2.5 g. of *p*-nitrophenol and 3.6 g. of dicyclohexylcarbodiimide. The mixture was kept 2 hr. at 5°, filtered, evaporated to an oil, the oil was taken up in ether, and cyclohexane was added. The white precipitate was removed, washed with cold ethanol, and was dried; yield, 6 g. (85%), m.p. 103–104°.

Anal. Calcd. for $C_{20}H_{20}N_2O_8$: C, 57.68; H, 4.84; N, 6.73. Found: C, 57.83; H, 4.90; N, 6.88.

Carbobenzoxy-L-glutamyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (Vd).—To a cold (5°) solution of 2.5 g. (0.0024 mole) of the octapeptide methyl ester hydrobromide III in 50 ml. of dimethylformamide was added 1.3 g. of triethylamine. The mixture was filtered and 1 g. (0.0024 mole) of carbobenzoxy-γ-methyl-L-glutamic acid *p*-nitrophenyl ester was added to the filtrate. The solution was stirred 2 days at 30°, evaporated to 10 ml., and ethyl acetate was added. An oil formed which solidified on trituration with ether. The solid was dissolved in 50 ml. of methanol and 5 ml. of 2 *N* NaOH was added. The solution was kept 1 hr. at 25°, diluted with water, and 6 ml. of 2 *N* HCl was added. The precipitate was removed and was reprecipitated twice from methanol with ether as a white solid, m.p. 175–180°, $[\alpha]^{23}D$ −61.6° (*c* 1, methanol), yield, 1.1 g.

Anal. Calcd. for $C_{57}H_{73}N_{13}O_{17}\cdot 2H_2O$: C, 54.84; H, 6.22; N, 14.59. Found: C, 54.73; H, 6.34; N, 14.81.

L-Glutamyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt: 1-Glutamic Acid Bradykinin (VId).—Five hundred milligrams (4.12×10^{-4} mole) of the carbobenzoxynonapeptide Vd in 50 ml. of glacial acetic acid–methanol (3:2) was hydrogenated over palladium black catalyst for 24 hr. as previously described. The mixture was filtered, evaporated to an oil, and the oil was dissolved in 50 ml. of water and freeze-dried, leaving 450 mg. of a cream colored solid, $[\alpha]^{23}D$ −72.8° (*c* 1.03, water).

Anal. Calcd. for $C_{49}H_{68}N_{12}O_{13}\cdot 4H_2O$: C, 53.24; H, 6.93; N, 15.21. Found: C, 52.91; H, 6.62; N, 15.23.

For the paper chromatography of the analogs two different solvent systems were employed: (A) *t*-butyl alcohol–acetic acid–water (2:1:1); (B) isopropyl alcohol–ammonium hydroxide–water (70:5:25). The peptides appeared homogenous after development of the spots with brom phenol blue and Sakaguchi reagents with the following R_f values: 1-Lys (A) 0.71, (B) 0.53; 1-Orn (A) 0.79, (B) 0.51; 1-Desarg (A) 0.74, (B) 0.60; 1-Glu (A) 0.74, (B) 0.66; 1-Cit (A) 0.72, (B) 0.61. Paper eletrophoresis in acetate buffer, pH 5.6, 3 hr. at 30 ma., produced single spots with all of the analogs except the 1-Glu derivative which showed the presence of a minor, faster moving component.

Acknowledgment.—We are indebted to Mr. C. E. Childs and his staff for the microanalyses reported herein and Dr. J. M. Vandenbelt, Mrs. Carola Spurlock, and Mrs. Vivien Lee for the optical rotations.

(7) M. A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963).

The Synthesis of 6-O-Carbamyl-L-Serine, 6-D-Serine, and 6-L-Threonine Bradykinin

H. A. DEWALD, M. K. CRAFT, AND E. D. NICOLAIDES

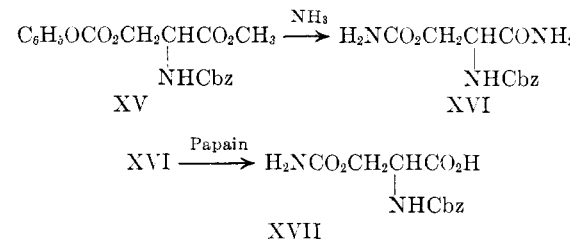
The Research Division, Parke, Davis and Company, Ann Arbor, Michigan

Received May 27, 1963

The synthesis of three analogs of bradykinin is described in which the serine of position 6 has been changed to L-threonine, D-serine, and O-carbamyl-L-serine. The biological activity of the analogs compared to bradykinin is reported.

In a previous paper[1] the preparation of three analogs of bradykinin, in which the phenylalanine amino acid in position 8 of the molecule was replaced by D-phenylalanine, *p*-fluoro-L-, and *p*-fluoro-D-phenylalanine, was described. As part of a continuing effort to investigate what effect subtle changes in the bradykinin structure have in relation to its biological activity, this paper describes three analogs which have variations in the serine portion of the molecule; these three new nonapeptides are the 6-O-carbamyl-L-serine, 6-D-serine, and 6-L-threonine bradykinins.

The synthetic method used for the preparation of the 6-O-carbamylserine analog is shown in Scheme I. The required intermediate O-carbamyl-N-carbobenzoxy-L-serine (XVII)[2] was obtained by ammonolysis of the O-phenylcarbonate ester of carbobenzoxy-L-serine methyl ester (XV). The resulting O-carbamyl-N-carbobenzoxy-L-serine amide (XVI) was hydrolyzed enzymatically with papain to yield XVII.



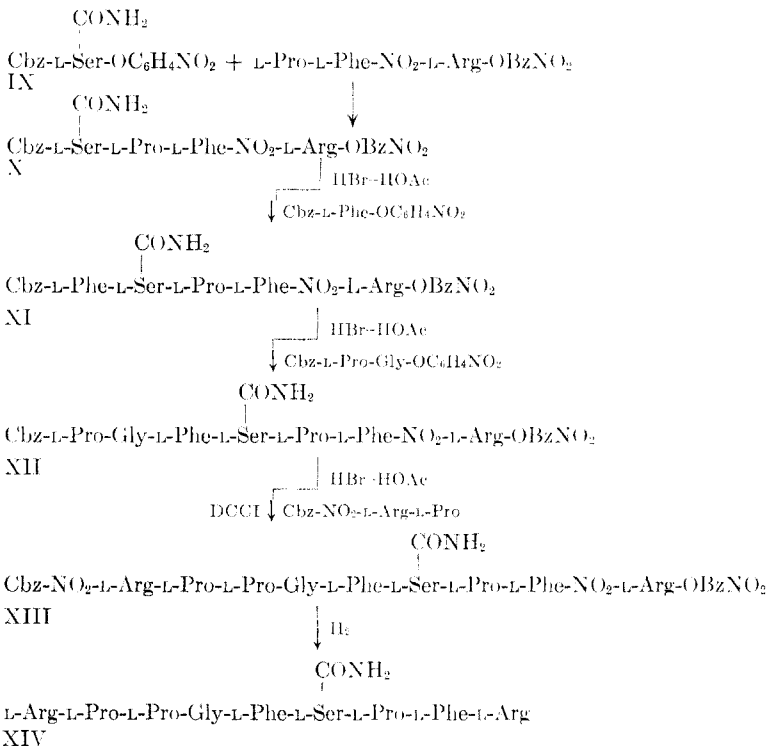
The *p*-nitrophenyl ester of the carbobenzoxy-O-carbamylserine was prepared and subsequent reaction with L-prolyl-L-phenylalanylnitro-L-arginine *p*-nitrobenzyl ester gave the carbobenzoxytetrapeptide X. The *p*-nitrobenzyl ester was utilized, since it was easily removed by hydrogenation and alkaline hydrolysis was to be avoided. The next two steps leading to the carbobenzoxyheptapeptide XII were *p*-nitrophenyl ester reactions and the heptapeptide was obtained in a crystalline state. The fully protected nonapeptide

(1) E. D. Nicolaides, M. K. Craft, and H. A. DeWald, *J. Med. Chem.*, **6**, 524 (1963).

(2) We are indebted to Dr. M. S. Morgan, Mellon Institute, for the use of his unpublished procedure for preparing O-carbamyl-N-carbobenzoxy-L-serine.

Scheme I

Synthetic Scheme for 6-O-Carbamyl-L-serine Bradykinin



XIII was prepared by condensing carbobenzoxynitro-L-arginyl-L-proline with the decarbobenzoxylated heptapeptide in the presence of dicyclohexylcarbodiimide. Hydrogenation of this peptide gave the 6-O-carbamyl-L-serine bradykinin.

The 6-L-threonine and 6-D-serine analogs were prepared by the synthetic scheme indicated in Scheme II. The O-acetyl function was inadvertently introduced at the hexapeptide stage during cleavage of the carbobenzoxypentapeptides with HBr–HOAc.[3] The intermediates in the D-serine series, however, were found to be only partially acetylated. The resulting mixture of products was difficult to purify and the materials were amorphous solids. It is believed that the partial acetylation is due to the inaccessibility of the D-serine hydroxyl function either by folding of the peptide chain or blockage by other groups.

The biological assay of the three analogs is shown in Table I.[4,5] The low order of activity of the 6-O-carbamyl analog is presumably an indication of the importance of the serine hydroxyl function.[6] The 6-D-serine and 6-L-threonine analogs still retain a considerable amount of vasodilatatory activity, but the threonine analogs' ability to cause bronchoconstriction in the guinea pig has been markedly reduced. It is of interest that bronchoconstriction in this case is not antagonized by aspirin. Since antagonism by aspirin is characteristic of kinins,[5] it would appear that the biological action of the threonine analog is not kinin-like, but further work must be done before this can be substantiated.

(3) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **28**, 1926, (1963).

(4) We wish to thank Dr. H. O. J. Collier, Miss P. G. Shorley, and Miss R. A. Hamilton for the biological test reported.

(5) H. O. J. Collier, J. A. Holgate, M. Schachter, and P. G. Shorley, *Brit. J. Pharmacol.*, **15**, 290 (1960).

(6) However, it should be noted that M. Bodanszky, M. A. Ondetti, J. T. Sheehan, and S. Lande, *Ann. N. Y. Acad. Sci.*, **104**, 24 (1963), concluded from their results with the 6-glycine bradykinin that the serine hydroxyl function is not important for activity.

Table I

Biological Activity of Bradykinin Analogs

Nonapeptide	Bronchoconstriction (guinea pig) Antagonized by aspirin		Vasodilation (guinea pig)
6-O-Carbamyl-L-serine bradykinin	<1/500	+	1/75
6-D-Serine bradykinin	1/40	+	1/2.5
6-L-Threonine bradykinin	<1/500	−	1/2
Bradykinin	1	+	1

Experimental[7]

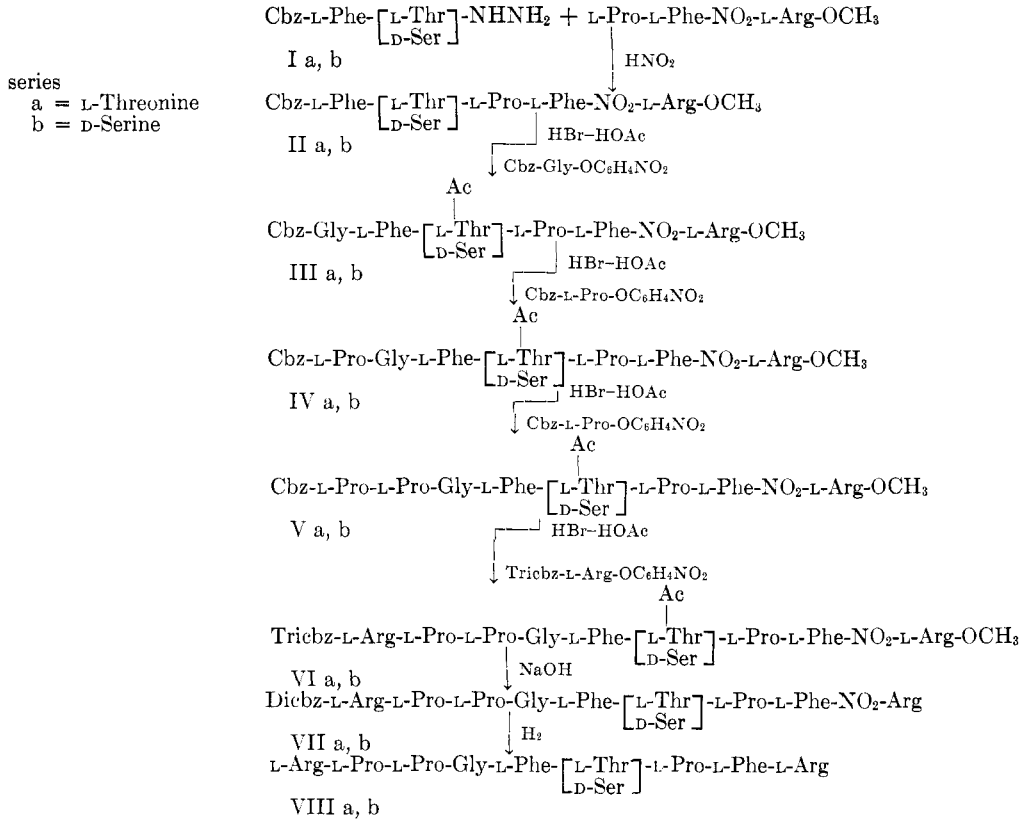
Carbobenzoxy-L-phenylalanyl-L-threonine Methyl Ester.—To a stirred, cold (−20°) solution of 26.4 g. (0.088 mole) of a carbobenzoxy-L-phenylalanine in 200 ml. of methylene dichloride was added 8.8 g. (0.088 mole) of triethylamine followed by 9.4 g. (0.088 mole) of ethyl chloroformate. After stirring the solution for 35 min. at −15°, a cold mixture of 15 g. (0.088 mole) of L-threonine methyl ester hydrochloride and 8.8 g. (0.088 mole) of triethylamine was added. The solution was stirred at 0° for 3 hr. and at room temperature overnight. It was washed with water, aqueous saturated $NaHCO_3$, water, dilute HCl, and was dried over $MgSO_4$. The ethyl acetate layer was evaporated to an oil which gradually solidified on the addition of petroleum ether. The product was recrystallized from ethyl acetate–petroleum ether, yield, 22 g. (61%), m.p. 117–119°, $[\alpha]^{23}D$ −20° (*c* 1, methanol).

Anal. Calcd. for $C_{22}H_{26}N_2O_6$: C, 63.76; H, 6.33; N, 6.76. Found: C, 64.00; H, 6.32; N, 6.93.

Carbobenzoxy-L-phenylalanyl-D-serine Methyl Ester.—To a cold (0°) solution of 5.7 g. (0.036 mole) of D-serine methyl ester hydrochloride in 50 ml. of dimethylformamide was added 4 g. of triethylamine. After a few minutes, the mixture was filtered and 15 g. (0.036 mole) of carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester was added to the filtrate. The yellow solution was stirred overnight at room temperature, evaporated to half volume, and ethyl acetate was added. The precipitate which formed was removed, washed with ether, and recrystallized from methanol–ether as white needles, m.p. 149–150°, $[\alpha]^{23}D$ −18.8° (*c* 2, methanol), yield, 11 g. (77%).

(7) Melting points were taken using a Thomas–Hoover capillary melting point apparatus and are corrected.

SCHEME II
SYNTHETIC SCHEME FOR 6-L-THREONINE AND 6-D-SERINE BRADYKININ



Anal. Calcd. for $C_{21}H_{24}N_2O_6$: C, 62.98; H, 6.04; N, 7.00. Found: C, 62.92; H, 5.94; N, 7.04.

Carbobenzoxy-L-phenylalanyl-L-threonine Hydrazide (Ia).—The carbobenzoxy-L-phenylalanyl-L-threonine methyl ester (20 g., 0.051 mole), was dissolved in 150 ml. of methanol, and 1.7 g. (0.053 mole) of anhydrous hydrazine was added. The solution was kept at room temperature for 32 hr. and, after the addition of ether, the white precipitate was removed by filtration, washed with ether, and dried; yield, 17.7 g. (84%), m.p. 202–204°, $[\alpha]^{23}D$ −8.9° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{21}H_{26}N_4O_5$: C, 60.85; H, 6.33; N, 13.51. Found: C, 61.11; H, 6.35; N, 13.45.

Carbobenzoxy-L-phenylalanyl-D-serine Hydrazide (Ib).—Treatment of 10 g. (0.025 mole) of carbobenzoxy-L-phenylalanyl-D-serine methyl ester with 1 g. of anhydrous hydrazine in 100 ml. of methanol gave 9.5 g. (95%) of the crystalline hydrazide, m.p. 208–209°, $[\alpha]^{23}D$ +7.2° (*c* 1, *N* HCl).

Anal. Calcd. for $C_{20}H_{24}N_4O_5$: C, 60.00; H, 6.04; N, 13.99. Found: C, 60.16; H, 6.19; N, 13.95.

Carbobenzoxy-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IIa).—The carbobenzoxydipeptide hydrazide (Ia) (14.9 g., 0.036 mole) was added to a mixture of 150 ml. of glacial acetic acid and 21 ml. of 2 *N* hydrochloric acid. The compound was dissolved with slight warming, cooled to 5°, and 4.8 g. (0.037 mole) of sodium nitrite in 20 ml. of water was added in portions. After 5 min., the solution was diluted with 1 l. of ice–water and the gum which was formed was extracted with three 400-ml. portions of cold ethyl acetate. The ethyl acetate solution was quickly washed twice with ice–water and then with cold, saturated aqueous sodium bicarbonate until the wash water was basic. The organic layer was dried over $MgSO_4$ at 0°. To a previously freshly prepared solution of 18.5 g. (0.034 mole) L-prolyl-L-phenylalanylnitro-L-arginine methyl ester hydrobromide[8] in 150 ml. of dimethylformamide at 5° was added 9.3 g. (0.093 mole) of triethylamine. The mixture was filtered and the filtrate was added to the ethyl acetate solution containing the dipeptide azide. The solution was allowed to stand at 5° for 2 days and was then washed with water, aqueous $NaHCO_3$ solution, water, and dilute HCl. It was dried, and evaporated to a white solid which was recrystallized from ethyl acetate-petroleum ether; yield, 15.5 g. (53%), m.p. 145–155°, $[\alpha]^{23}D$ −34.2° (*c* 1, dimethylformamide).

(8) E. D. Nicolaides and H. A. DeWald, *J. Org. Chem.*, **26**, 3872 (1961).

Anal. Calcd. for $C_{42}H_{53}N_9O_{11}$: C, 58.65; H, 6.21; N, 14.66. Found: C, 58.74; H, 6.02; N, 14.48.

Carbobenzoxy-L-phenylalanyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IIb).—Twelve grams (0.032 mole) of carbobenzoxy-L-phenylalanyl-L-serine hydrazide was converted to the azide and allowed to react with 16.5 g. (0.03 mole) of L-prolyl-L-phenylalanylnitro-L-arginine methyl ester hydrobromide as previously described. The product was obtained as an oil which solidified with ether treatment, m.p. 90–100°, $[\alpha]^{23}D$ −44.2° (*c* 1.3, dimethylformamide), yield, 17 g. (68%).

Anal. Calcd. for $C_{41}H_{51}N_9O_{10}$: C, 58.21; H, 6.08; N, 14.90. Found: C, 58.25; H, 6.03; N, 14.41.

Carbobenzoxyglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IIIa).—Into a cooled (10°) solution of 14 g. (0.0163 mole) of the carbobenzoxypentapeptide methyl ester IIa in 150 ml. of glacial acetic acid was bubbled 20 g. (0.25 mole) of dry hydrogen bromide. The solution was kept at room temperature for 1.5 hr. with occasional swirling, and then poured rapidly into 1 l. of vigorously stirred, anhydrous ether. The white precipitate which formed was allowed to settle, the ether was decanted, and the solid was washed several times with ether. The solid was collected on a sintered glass filter, washed with ether, and vacuum dried for 2 days, giving 17.8 g. (theor. 11.8 g.) of a white solid. The crude product was dissolved in 150 ml. of dimethylformamide and cooled along with 8 g. (0.08 mole) of triethylamine. The solutions were combined and the precipitate which was formed was removed by filtration. To the filtrate was added 6.1 g. (0.018 mole) of carbobenzoxyglycine *p*-nitrophenyl ester. The solution was kept 3 days at room temperature and evaporated to an oil. Ethyl acetate was added to the oil and a gum formed which was washed with water, dilute NH_4OH, water, and dilute HCl. The gum was taken up in ethyl acetate–ethanol and it gradually solidified upon the addition of petroleum ether. The off-white product was crystallized from methanol–water, yield, 10 g. (67%), m.p. 115–125°, $[\alpha]^{23}D$ −33.7° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{46}H_{58}N_{10}O_{13}\cdot H_2O$: C, 56.55; H, 6.19; N, 14.34; OAc, 4.48. Found: C, 56.52; H, 6.20; N, 14.63; OAc, 2.72.

Carbobenzoxyglycyl-L-phenylalanyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IIIb).—A similar reaction to the one just described was carried out using 12 g. of the carbo-

benzoxy-D-serinepentapeptide IIb and yielded 9 g. (65%) of a cream colored solid, m.p. 170–175°, $[\alpha]^{23}D$ −33.8° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{43}H_{54}N_{10}O_{12}$: C, 57.20; H, 6.02; N, 15.52; OAc, 4.55. Found: C, 57.14; H, 6.16; N, 15.54; OAc, 1.46.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IVa).—The carbobenzoxy group was removed from 9 g. (0.0098 mole) of the carbobenzoxyhexapeptide IIIa with HBr–HOAc, as previously described, yielding 10.1 g. of cream colored solid. The product was dissolved in 100 ml. of dimethylformamide, cooled to 0°, and 4.0 g. (0.04 mole) of triethylamine was added. The mixture was filtered and 4.4 g. (0.012 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester was added to the filtrate. The solution was allowed to stand for 2.5 days and evaporated to 50 ml. A gummy solid formed upon the addition of ethyl acetate–ether. The gum was washed several times with ether and ethyl acetate and was recrystallized from methanol–water as a cream colored solid; yield, 8.5 g. (86%), m.p. 127–137°, $[\alpha]^{23}D$ −45.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{51}H_{65}N_{11}O_{14}$: C, 57.96; H, 6.19; N, 14.58; OAc, 4.07. Found: C, 57.66; H, 6.04; N, 14.67; OAc, 2.96.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-acetyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (IVb).—Removal of the carbobenzoxy group from 12 g. of the carbobenzoxy-D-serinehexapeptide IIIb was accomplished with HBr–HOAc and the product was allowed to react with 5.2 g. of carbobenzoxy-L-proline *p*-nitrophenyl ester. A yellowish solid was obtained which could not be crystallized, m.p. 130–135°, $[\alpha]^{23}D$ −46.5° (*c* 1, methanol), yield, 8 g. (60%).

Anal. Calcd. for $C_{50}H_{63}N_{11}O_{14}$: C, 57.63; H, 6.05; N, 14.79; OAc, 4.13. Found: C, 57.00; H, 6.24; N, 14.59; OAc, 3.19.

Carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (Va).—Decarbobenzoxylation of the heptapeptide methyl ester IVa (7 g., 0.0069 mole) was carried out with HBr in glacial acetic acid and the crude hydrobromide was isolated as before. The dried product was dissolved in 100 ml. of dimethylformamide, cooled to 5°, 3 g. (0.03 mole) of triethylamine added, and the solution was filtered after 5 min. Carbobenzoxy-L-proline *p*-nitrophenyl ester (2.7 g., 0.0074 mole) was added to the filtrate and the solution was stirred at room temperature for 5 days. The solution was evaporated to a small volume and a tan precipitate formed upon the addition of ether and ethyl acetate. The solid was recrystallized 3 times from methanol–water; yield, 4.5 g. (59%), m.p. 138–145°, $[\alpha]^{23}D$ −51° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{56}H_{72}N_{12}O_{15}$: C, 58.32; H, 6.29; N, 14.58; OAc, 3.73. Found: C, 57.77; H, 6.16; N, 14.56; OAc, 2.93.

Carbobenzoxy-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (Vb).—A *p*-nitrophenyl ester reaction involving 3.5 g. (0.0095 mole) of carbobenzoxy-L-proline *p*-nitrophenyl ester and 8 g. (0.008 mole) of the carbobenzoxy-D-serine heptapeptide IVb gave 4 g. (46%) of a cream colored solid, m.p. 130–135°, $[\alpha]^{23}D$ −60° (*c* 1, methanol).

Anal. Calcd. for $C_{55}H_{70}N_{12}O_{15}\cdot 2H_2O$: C, 56.21; H, 6.00; N, 14.31; OAc, 3.77. Found: C, 56.44; H, 6.28; N, 14.03; OAc, 2.00.

Tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (VIa).—Two grams (0.0018 mole) of the carbobenzoxyoctapeptide methyl ester Va was treated with 10 g. (0.125 mole) of dry hydrogen bromide in a cooled (10°) solution of 100 ml. of glacial acetic acid. The solution was allowed to remain at room temperature for 2 hr. and was poured into 1 l. of vigorously stirred dry ether. The solid was filtered, washed thoroughly with dry ether, and dried *in vacuo* overnight, giving 3.1 g. (theor. 1.9 g.) of a white solid. The crude product was dissolved in 75 ml. of dimethylformamide, cooled to 0°, and 2 g. (0.02 mole) of triethylamine was added. The precipitate was removed and 2 g. (0.0029 mole) of tricarbobenzoxy-L-arginine *p*-nitrophenyl ester was added. The yellow solution was kept at room temperature for 3 days, evaporated to a gum which was washed several times with ethyl acetate and ether, during which time it gradually formed a solid. The solid was recrystallized twice from methanol–water and twice from methanol–ether; yield, 2.3 g. (85%) of light cream colored solid, m.p. 125–135°, $[\alpha]^{23}D$ −47° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{78}H_{96}N_{16}O_{20}$: C, 59.41; H, 6.14; N, 14.21; OAc, 2.73. Found: C, 59.20; H, 6.18; N, 1434; OAc, 2.74.

Tricarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine Methyl Ester (VIb).—The carbobenzoxy group was removed from 2 g. (0.00182 mole) of the octapeptide Vb with HBr–HOAc. The crude hydrobromide was allowed to react with 1.3 g. (0.0018 mole) of tricarbobenzoxy-L-arginine *p*-nitrophenyl ester and a yellow solid was isolated (1.5 g.) which formed a gummy solid on attempted crystallization.

Dicarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanylnitro-L-arginine (VIIa).—To a solution of 2.2 g. (0.0014 mole) of the tricarbobenzoxynonapeptide methyl ester VIa dissolved in 50 ml. of methanol at room temperature was added 1.5 ml. of 2 *N* NaOH. The solution was stirred for 1 hr. and remained clear upon the addition of water. Upon the addition of 2 ml. of 2 *N* HCl a tan gum precipitated which solidified upon standing. The solid was crystallized from methanol–ether; yield, 1.5 g. (78%), m.p. 172–177°, $[\alpha]^{23}D$ −52.5 (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{87}H_{86}N_{16}O_{17}\cdot H_2O$: C, 57.27; H, 6.31; N, 15.95. Found: C, 57.19; H, 6.37; N, 15.86.

Dicarbobenzoxy-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-D-seryl-L-prolyl-L-phenylalanylnitro-L-arginine (VIIb).—Hydrolysis of the crude tricarbobenzoxy-D-serine nonapeptide (VIb, 1.5 g.) with 2 *N* NaOH yielded 900 mg. of a light cream colored solid after crystallization from methanol–ethanol; m.p. 185–190°, $[\alpha]^{23}D$ −77.5° (*c* 1, methanol).

Anal. Calcd. for $C_{86}H_{84}N_{16}O_{17}\cdot 2H_2O$: C, 56.21; H, 6.01; N, 15.90. Found: C, 55.62; H, 5.97; N, 15.67.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (VIIIa).—The dicarbobenzoxynonapeptide (VIIa) (500 mg., 3.5×10^{-4} mole) was dissolved in 30 ml. of glacial acetic acid, then 200 mg. of palladium black catalyst and 20 ml. of methanol were added. The mixture was hydrogenated for 24 hr. at room temperature and atmospheric pressure. The catalyst was removed by filtration and was washed with 10 ml. of glacial acetic acid. The filtrate was evaporated *in vacuo* to an oil. The residue was dissolved in 100 ml. of water, the solution was filtered, shell frozen, and lyophilized, leaving 380 mg. of white powder. The product melted at 138–145°, $[\alpha]^{23}D$ −85.5° (*c* 1.34, water).

Anal. Calcd. for $C_{71}H_{97}N_{15}O_{17}\cdot H_2O$: C, 53.03; H, 7.05; N, 16.51. Found: C, 53.28; H, 7.08; N, 16.60.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-D-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (VIIIb).—Hydrogenation of 200 mg. of the protected 6-D-serine nonapeptide VIIb using the same conditions as with the 6-threonine analog gave, after lyophilization, 100 mg. of white solid; $[\alpha]^{23}D$ −68° (*c* 1.03, water).

Anal. Calcd. for $C_{64}H_{85}N_{15}O_{17}\cdot 3H_2O$: C, 51.96; H, 7.09; N, 16.24. Found: C, 50.92; H, 7.02; N, 16.37.

O-Carbamyl-N-carbobenzoxy-L-serine Amide.—To a cold (5°) solution of 63.5 g. (0.25 mole) of carbobenzoxy-L-serine methyl ester in 400 ml. of pyridine was added 41 g. (0.26 mole) of phenyl chloroformate with stirring. The mixture was allowed to stand overnight at room temperature, was poured into 2 l. of water, and the oil was extracted into chloroform. The chloroform extract was washed with dilute hydrochloric acid, dried over anhydrous magnesium sulfate, and evaporated to an oil which weighed 84 g. The crude phenylcarbonate ester of carbobenzoxy-L-serine methyl ester (84 g., 0.227 mole) was dissolved in 120 ml. of methanol and was added with stirring to *ca.* 600 ml. of liquid ammonia. The excess ammonia was allowed to evaporate overnight. The residue was diluted with 500 ml. of ether, cooled, filtered, and the filter cake was washed with ether. The yield of O-carbamyl-N-carbobenzoxy-L-serine amide was 26.4 g. (38%), m.p. 170–172°. An analytical sample from methanol melted at 173–174°, $[\alpha]^{23}D$ −10° (*c* 0.7, dimethylformamide).

Anal. Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.38; N, 14.94. Found: C, 51.57; H, 5.13; N, 14.89.

O-Carbamyl-N-carbobenzoxy-L-serine.—A papain solution was prepared from 16 g. of papain and 2 g. of L-cysteine hydrochloride in 1 l. of water. The pH of the mixture was adjusted to 5.5 and the mixture was filtered. To the filtrate was added 28 g. (0.1 mole) of O-carbamyl-N-carbobenzoxy-L-serine amide and the mixture was incubated at 37° for 48 hr. The solution was evaporated *in vacuo* and the residue was extracted with three 400-ml. portions of hot methanol. The methanol extracts were acidified to congo red with hydrochloric acid and evaporated *in vacuo*. The oil was taken up in chloroform and extracted with excess aqueous sodium bicarbonate solution. The aqueous extracts

were cooled and acidified to precipitate 16.9 g. of solid as colorless needles; m.p. 143–145°, $[\alpha]^{23}D$ −12.4° (*c* 1.2, dimethylformamide).

Anal. Calcd. for $C_{12}H_{14}N_2O_6$: C, 51.06; H, 5.00; N, 9.93. Found: C, 51.25; H, 5.17; N, 10.08.

O-Carbamyl-N-carbobenzoxy-L-serine *p*-Nitrophenyl Ester (IX).—To a cold (5°) solution of 14 g. (0.05 mole) of O-carbamyl-N-carbobenzoxy-L-serine and 7.5 g. (0.055 mole) of *p*-nitrophenol in 100 ml. of dimethylformamide was added 11 g. (0.05 mole) of dicyclohexylcarbodiimide. The mixture was kept overnight at 5° and then filtered. The filtrate was diluted with 250 ml. of ethyl acetate, and the solution was washed with water, saturated aqueous sodium bicarbonate, and saturated aqueous sodium chloride. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was crystallized from 150 ml. of warm ethyl acetate by the addition of 600 ml. of ether. The yield of colorless solid was 13 g. (65%), m.p. 144–146°, $[\alpha]^{23}D$ −27.3° (*c* 1.1, dimethylformamide).

Anal. Calcd. for $C_{18}H_{17}N_3O_8$: C, 53.60; H, 4.25; N, 10.42. Found: C, 53.90; H, 4.53; N, 10.57.

O-Carbamyl-N-carbobenzoxy-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-Nitrobenzyl Ester (X).—Carbobenzoxy-L-prolyl-L-phenylalanylnitro-L-arginine *p*-nitrobenzyl ester[9] (19 g., 0.026 mole) was dissolved in 200 ml. of glacial acetic acid containing 60 g. of dry hydrogen bromide. After 30 min. at room temperature the mixture was poured into 1.5 l. of cold dry ether. The precipitate was removed, washed well with ether, and dried *in vacuo*. The yield of crude product was 18 g. The solid was dissolved in 70 ml. of dimethylformamide, cooled to 0°, and 4.5 ml. of triethylamine was added. The precipitate was removed, and to the filtrate was added 10.5 g. (0.026 mole) of O-carbamyl-N-carbobenzoxy-L-serine *p*-nitrophenyl ester. The solution was kept 3 days at room temperature, then diluted with 300 ml. of ethyl acetate. It was washed with water, aqueous potassium carbonate, and saturated aqueous sodium chloride. The ethyl acetate solution was evaporated *in vacuo*. The residue was crystallized from methanol–water, 7.0 g. (32%), m.p. 135–138°, $[\alpha]^{23}D$ −41.9° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{39}H_{46}N_{10}O_{18}$: C, 54.29; H, 5.37; N, 16.23. Found: C, 54.12; H, 5.23; N, 16.64.

Carbobenzoxy-L-phenylalanyl-O-carbamyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-Nitrobenzyl Ester (XI).—O-Carbamyl-N-carbobenzoxy-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-nitrobenzyl ester (6.75 g., 0.0078 mole) was dissolved in 70 ml. of glacial acetic acid containing 30 g. of anhydrous hydrogen bromide. After 1 hr. at room temperature, the solution was poured into 800 ml. of dry ether. The hygroscopic solid was collected, washed with ether, and dried *in vacuo*. The solid (6.4 g.) was dissolved in 50 ml. of dimethylformamide and 3.5 g. (0.008 mole) of carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester was added. The solution was stirred as 2.8 ml. of triethylamine in 10 ml. of dimethylformamide was added very slowly. After standing 3 days, the mixture was filtered, diluted with ethyl acetate, and washed with water, aqueous potassium carbonate, and saturated aqueous sodium chloride solution. A colorless solid separated, 2.8 g. (36%), m.p. 208–211°. An analytical sample was recrystallized from dimethylformamide, m.p. 215–217°, $[\alpha]^{23}D$ −50.5° (*c* 1, dimethylformamide).

Anal. Calcd. for $C_{48}H_{55}N_{11}O_{14}$: C, 57.08; H, 5.49; N, 15.26. Found: C, 56.77; H, 5.33; N, 15.19.

Carbobenzoxy-L-prolylglycine *p*-Nitrophenyl Ester.—Carbobenzoxy-L-prolylglycine (1.5 g., 0.005 mole) and 0.8 g. of *p*-nitrophenyl were dissolved in 10 ml. of dimethylformamide. The solution was cooled to 4° and 1.1 g. (0.005 mole) of dicyclohexylcarbodiimide was added. The mixture was allowed to stand at 4° overnight. It was filtered and then evaporated *in vacuo*. The residue crystallized from ether, 1.6 g. (75%), m.p. 142–144°, $[\alpha]^{23}D$ −60° (*c* 1, dimethylformamide); lit.[10] m.p. 143.5–145°, $[\alpha]^{22}D$ −63° (*c* 1, dimethylformamide).

(9) R. A. Boissonnnas, St. Guttmann, and P. A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960).

(10) M. A. Ondetti. *J. Med. Chem.*, **6**, 10 (1963).

Anal. Calcd. for $C_{21}H_{21}N_3O_7$: C, 59.02; H, 4.95; N, 9.83. Found: C, 59.27; H, 5.21; N, 9.71.

Carbobenzoxy-L-prolylglycyl-L-phenylalanyl-O-carbamyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-Nitrobenzyl Ester (XII).—Carbobenzoxy-L-phenylalanyl-O-carbamyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-nitrobenzyl ester (2.65 g.) was dissolved in 30 ml. of glacial acetic acid containing 12 g. of dry hydrogen bromide. After standing 1 hr. the solution was diluted with 400 ml. of dry ether to precipitate 2.9 g. of hygroscopic solid. The solid was dissolved in 25 ml. of dimethylformamide, cooled to 4°, and 0.8 ml. of triethylamine was added. The mixture was filtered and 1.2 g. of carbobenzoxy-L-prolylglycine *p*-nitrophenyl ester was added. The mixture was allowed to stand at room temperature for 3 days. The solution was diluted with ethyl acetate and washed successively with water, aqueous potassium carbonate, and saturated sodium chloride solutions. It was concentrated on the steam bath to yield 1.45 g. (53%) of colorless microcrystals; m.p. 167–170°, $[\alpha]^{23}D$ −53.9° (*c* 0.5, dimethylformamide).

Anal. Calcd. for $C_{56}H_{55}N_{13}O_{16}$: C, 56.75; H, 5.63; N, 15.64. Found: C, 56.79; H, 5.63; N, 15.76.

Carbobenzoxynitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-carbamyl-L-seryl-L-prolyl-L-phenylalanylnitro-L-arginine *p*-Nitrobenzyl Ester (XIII).—The carbobenzoxyheptapeptide ester XII (1.4 g.) was dissolved in a mixture of 20 ml. of glacial acetic acid and 10 g. anhydrous hydrogen bromide. After standing for 45 min., the solution was poured into 400 ml. of dry ether. The solid was collected and dried *in vacuo*. The hydrobromide (1.3 g.) was dissolved in 10 ml. of dimethylformamide, the solution was cooled to 0°, and triethylamine (0.35 ml.) was added. The mixture was filtered after 15 min., and 0.56 g. of carbobenzoxynitroarginylproline[9] and 0.25 g. of dicyclohexylcarbodiimide were added. The mixture was kept at 0° for 2 days, filtered, and evaporated *in vacuo*. The residue was triturated with ether. The solid was crystallized from methanol–water to yield 1.4 g. (80%) of a colorless solid, m.p. 152–155°, $[\alpha]^{23}D$ −48° (*c* 0.5, dimethylformamide).

Anal. Calcd. for $C_{66}H_{83}N_{19}O_{20}$: C, 54.19; H, 5.72; N, 18.20. Found: C, 53.73; H, 5.41; N, 18.07.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-carbamyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (XIV).—A solution of 800 mg. of the protected nonapeptide XIII in 50 ml. of glacial acetic acid–methanol (3:2) was hydrogenated over 200 mg. of palladium black catalyst for 24 hr. The reaction mixture was filtered and evaporated *in vacuo*. The residue was taken up in 50 ml. of water, shell frozen, and lyophilized leaving 650 mg. (91%) of hard, tan crystals. The product was dried at 110° for 18 hr., $[\alpha]^{23}D$ −63° (*c* 1.04 water).

Anal. Calcd. for $C_{57}H_{86}N_{16}O_{18}$: C, 53.34; H, 6.76; N, 17.46. Found: C, 53.81; H, 7.00; N, 17.59.

Paper chromatography of the three analogs was carried out using two different solvent systems; (A) *t*-butyl alcohol–acetic acid–water (2:1:1); (B) isopropyl alcohol–concentrated NH_4OH–water (70:5:25). The spots were detected with brom phenol blue and the products appeared homogeneous. The R_f values obtained were: 6-L-threonine bradykinin (A) 0.78, (B) 0.45; 6-D-serine bradykinin (A) 0.72, (B) 0.52; 6-O-carbamyl-L-serine bradykinin (A) 0.77, (B) 0.56.

Paper electrophoresis of the nonapeptides was done in 0.05 *M* acetate buffer, at pH 5.6, using a constant current of 30 ma. for 3 hr. Single spots were obtained for the 6-D-serine and 6-L-threonine analogs which migrated towards the cathode a distance of 5.5 and 5 cm., respectively, compared to 6 cm. for synthetic bradykinin. Two spots were obtained for the 6-O-carbamyl analog, the major spot moving 4.5 cm. from the origin and a minor one at 1.5 cm.

Acknowledgment.—We wish to thank Dr. J. M. Vandenbelt, Mrs. Carola Spurlock, and Mrs. Vivien Lee for the optical rotations and Mr. C. E. Childs and staff for the microanalyses reported herein.