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153. Kenji Suzuki and Takashi Abiko: Synthesis of 3-Sarcosine-, 3-Glycine-, and 3-L-Alanine-6-Glycine-Bradykinin and 3-Glycine-Bradykinin.*1

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The three analogs of bradykinin are described in which L-proline residue in 3-position of 6-glycine-bradykinin is substituted for sarcosine, glycine, and L-alanine residue respectively. 3-Glycine-bradykinin and its O-acetyl compound are also described. The biological activity of the five analogs is compared with that of bradykinin on an isolated guinea pig ileum.

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A number of bradykinin analogs showing high bradykinin-like activity have been reported. These analogs are 6-glycine-,[1] 3-L-alanine-,[2] 3-sarcosine-,[3] and 8-*p*-fluoro-L-phenylalanine-bradykinin[4] and these facts have been also reviewed by Schröder, *et al.*[5] compared with the other numerous bradykinin analogs. In 1964, Schröder, *et al.*[6] have reported that bradykinin analog substituted at two positions 3 and 6 for another amino acid residues, namely 3-L-alanine-6-glycine-bradykinin showed high bradykinin-like activity. In the present paper the synthesis of 3-glycine-6-glycine-bradykinin, 3-sarcosine-6-glycine-bradykinin, and 3-glycine-bradykinin is described, because high activity of thece analogs is expected from the results of the study by Schröder, *et al.*[6] as described above. The reasons for the studies on 3-glycine analogs are as follows: the difference on the structual feature between glycine and the other two amino acids, sarcosine and L-alanine is only the existence of the N- or α-methyl group in the latter two amino acid molecules. In addition to these studies, the synthesis of 3-L-alanine-6-glycine-bradykinin is also described, since the experimental details of this bradykinin analog have not been described by Schröder, *et al.*[6] The method employed for the synthesis of the three analogs are closely similar to the method used for the preparation of bradykinin and its analogs by the authors.[7] The synthetic route for 3-sarcosine-6-glycine bradykinin is illustrated in Fig 1. Estericatión of N-benzyloxycarbonyl-L-phenylalanylglycine[8] with *p*-nitrophenol by the N,N'-dicyclohexylcarbodiimide procedure gave N-benzyloxycarbonyl-L-phenylalanylglycine *p*-nitrophenyl ester (I). N-Benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-nitrobenzyl ester[7] was de-benzyloxycarbonylated with a hydrogen bromide-acetic acid solution in the presence of anisole. The resulting product was condensed with I to yield crystalline N-benzyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-

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*1 Nomenclature of bradykinin analogs followed those given in Proc. 2nd Intl. Pharmacol. Meeting, Vol. 10 Oxytocin, Vasopressin, and their Structual Analogues. Ed. J. Rudinger. xi (1964). Czechoslovak Medical Press, Praha. Abbreviations for amino acids and substituents followed those in given in the tentative rules of IUPAC-IUB commission on biochemical nomenclature, Biochemistry, **5**, 2485 (1966).

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1) M. Bodanszky, J. T. Sheehan, M. A. Ondetti, S. Lande: J. Am. Chem. Soc., **85**, 991 (1963). E. Schröder: Ann., **673**, 186 (1964).
2) E. Schröder: Ann., **679**, 207 (1964).
3) N. Yanaihara, M. Sekiya, K. Takagi, H. Kato: The study was presented at the 38 th Annual Meeting of Japanese Biochemical Society, Oct. 17~20, 1965, Fukuoka.
4) E. D. Nicolaides, M. K. Craft, H. A. De Wald: J. Med. Chem., **6**, 524 (1963).
5) E. Schröder, K. Lübke: The Peptides, Vol. II, 65 (1966), Academic Press, N. Y. and London.
6) E. Schröder, R. Hempel: Experientia, **20**, 529 (1964).
7) K. Suzuki, T. Abiko, M. Asaka: This Bulletin, **14**, 217 (1966).
8) O. K. Behrens, D. G. Doherty, M. Bergmann: J. Biol. Chem., **136**, 61 (1940).

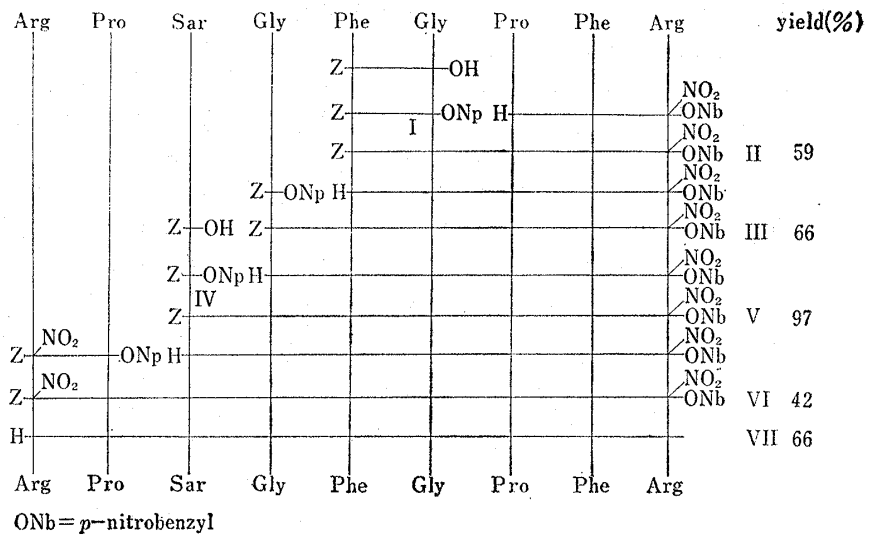

Fig. 1. Synthesis of 3-Sarcocine-6-glycine-bradykinin

N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (II). After the removal of the benzyloxycarbonyl group of II, the resulting pentapeptide ester was condensed with N-benzyloxycarbonylglycine *p*-nitrophenyl ester[9] to yield N-benzyloxycarbonylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (III). Esterification of N-benzyloxycarbonyl sarcosine[10] with *p*-nitrophenol in ethyl acetate by N,N'-dicyclohexylcarbodiimide yielded N-benzyloxycarbonylsarcosine *p*-nitrophenyl ester (IV) as an oil and the ester was used for the next step without further purification. After the removal of the benzyloxycarbonyl group of III, the resulting hexapeptide ester was condensed with IV to yield N-benzyloxycarbonylsarcosylglycyl-L-phenylalanyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (V). After the removal of the benzyloxycarbonyl group of V, the resulting heptapeptide ester was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester[11] to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylsarcosylglycyl-L-phenylalanyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VI). The fully protected nonapeptide (VI) was hydrogenated for 40 hr. over 10% palladium on charcoal in aqueous acetic acid and the hydrogenated product was purified through a carboxymethyl (CM-) cellulose column to obtain L-arginyl-L-prolylsarcosylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine triacetate (VII). The nonapeptide (VII) so obtained was found to be homogeneous from the result of paper chromatography in two different solvent systems and the ratio of amino acids in the acid hydrolysate agreed well with the theoretical value.

For the synthesis of 3-glycine-6-glycine-bradykinin, the following series of reactions were carried out. De-benzyloxycarbonylated II was condensed with N-benzyloxycarbonylglycylglycine *p*-nitrophenyl ester[12] to yield N-benzyloxycarbonylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VIII). After the removal of the benzyloxycarbonyl group of VIII, the resulting heptapeptide ester was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (IX). The fully protected nonapeptide was hydrogenated over palladium on charcoal and the hydrogenated

9) B. Iselin, W. Rittel, P. Sieber, R. Schwyzer: Helv. Chim. Acta, **40**, 373 (1957).
10) D. Ben-Ishai, E. Katchalski: J. Am. Chem. Soc., **74**, 3688 (1952).
11) M. A. Ondetti: J. Med. Chem., **6**, 10 (1963).
12) M. Bodanszky, J. T. Sheehan, M. A. Ondetti, S. Lande: J. Am. Chem. Soc., **85**, 991 (1963).

product was purified through a CM-cellulose column to obtain 3-glycine-6-glycine-bradykinin, L-arginyl-L-prolylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine as triacetate dihydrate (X). The nonapeptide (X) so obtained was found to be homogeneous from the result of paper chromatography in two different solvent systems and the elemental analysis agreed well with the theoretical value.

For the synthesis of 3-L-alanine-6-glycine-bradykinin, the following series of reactions were carried out. Esterification of N-benzyloxycarbonyl-L-alanylglycine[13] with *p*-nitrophenol by N,N'-dicyclohexylcarbodiimide yielded N-benzyloxycarbonyl-L-alanylglycine *p*-nitrophenyl ester (XI). De-benzyloxycarbonylated II was condensed with XI to yield N-benzyloxycarbonyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (XII). After the removal of the benzyloxycarbonyl group of XII, the resulting heptapeptide ester was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (XIII). The fully protected nonapeptide (XIII) was hydrogenated over 10% palladium on charcoal and the hydrogenated product was purified through a CM-cellulose column to obtain 3-L-alanine-6-glycine-bradykinin, L-arginyl-L-prolyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine as triacetate (XIV). The nonapeptide (XIV) so obtained was found to be homogeneous from the result of paper chromatography in two different solvent systems and the ratio of amino acids in the acid hydrolysate agreed well with theoretical value.

For the synthesis of 3-glycine-bradykinin, the following series of reactions were carried out. After the removal of the benzyloxycarbonyl group of N-benzyloxycarbonyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester,[7] the resulting pentapeptide ester was condensed with N-benzyloxycarbonylglycylglycine *p*-nitrophenyl ester to yield N-benzyloxycarbonylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (XV). De-benzyloxycarbonylated XV was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (XVI). The fully protected nonapeptide was hydrogenated over palladium on charcoal and the hydrogenated product was purified through a CM-cellulose column to obtain 3-glycine-6-O-acetyl-L-serine-bradykinin, L-arginyl-L-prolylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine triacetate (XVII). The nonapeptide (XVII) so obtained was found to be homogeneous from the results of a similar analysis for VI. Saponification of XVII with 1*N* sodium hydroxide gave 3-glycine-bradykinin, L-arginyl-L-prolylglycylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine triacetate (XVIII). The nonapeptide so obtained was found to be homogeneous from the results of the similar analysis for VI.

Quantitative examinations were made on the bradykinin-like activity, anti-bradykinin activity, and potentiation of bradykinin activity of the nonapeptide synthesized in the present work.*3 Results of these biological examinations are given in Table I. Bradykinin-like activity of 3-sarcosine-6-glycine-bradykinin (VII) was lower than that of 3-sarcosine-bradykinin[4] and 6-glycine-bradykinin.[2] Bradykinin-like activity of 3-L-alanine-6-glycine-bradykinin (XIV), as reported also by Schröder, *et al.*,[6] was lower than that of 3-alanine-bradykinin and 6-glycine-bradykinin.[2] Bradykinin-like activity of 3-glycine-bradykinin XVIII and its O-acetyl compound (XVII) was fairly high, but the

*3 The detail of the biological assays will be reported in separate paper by Prof. Kameyama of this college.

13) M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider, H. Schleich: J. Biol. Chem., **109**, 325 (1935).

activity of 3-glycine-6-glycine-bradykinin (X) was markedly lower than that of XVIII and 6-glycine-bradykinin. From these results of the biological assay, it is assumed that the biological activity of bradykinin analog is affected not only by the substituted amino acid residues but also by the comformation of bradykinin analog. The four analogs, VII, X, XVII, and XVIII showed no anti-bradykinin activity and no potentiation of bradykinin activity. 3-L-Alanine-6-glycine-bradykinin (XIV) showed potentiating activity.

Table I. Biological Activities of Synthetic Nonapeptides[a)]

	Bradykinin-like activity	Bradykinin potentiating activity	Anti-bradykinin activity
Bradykinin	100		
3-Sarcosine-6-glycine-bradykinin (VII)	21.3	—	—
3-Glycine-6-glycine-bradykinin (X)	1.2	—	—
3-L-Alanine-6-glycine-bradykinin (XIV)	24.8 (20.0[6)])	+[b)]	—
3-Glycine-6-O-acetyl-L-serine-bradykinin (XVIII)	18.7	—	—
3-Glycine-bradykinin (XIX)	14.6	—	—
3-L-Alanine-bradykinin	100[2)]		
3-Sarcosine-bradykinin	67.0[3)]		
6-Glycine-bradykinin	33.3～100[1)]		

a) Assayed by Magnus method on an isolated guinea pig ileum (male). +: active; −: inactive.
b) At a concentration of 1×10^{-9} g./ml., caused 35% potentiation of the normal contration due to 1×10^{-8} g./ml. of bradykinin.

Experimental

Melting points are uncorrected. For paper chromatography, the protected peptides were deblocked with HBr in AcOH unless otherwise mentioned and the resulting hydrobromides were chromatographed on a filter paper, Toyo Roshi No. 51, at room temperature, Rf^1 value refer to the Partridge system,[14)] and Rf^2 value refer to the system of BuOH-pyridine-AcOH-H_2O (30:20:6:24).[15)] The amino acid composition of the acid hydrolysates was determined according to the directions given by Moore, *et al.*[16)]

N-Benzyloxycarbonyl-L-phenylalanylglycine *p*-Nitrophenyl Ester (I)——To a pre-cooled solution of N-benzyloxycarbonyl-L-phenylalanylglycine (1.25 g.) in EtOAc (15 ml.) and dimethylformamide (2 ml.) *p*-nitrophenol (0.55 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (0.80 g.). After 30 min. at 0° and 2 hr. at room temperature, a few drops of AcOH was added to the reaction mixture, and the reaction mixture was stirred for 20 min. The formed N,N′-dicyclohexylurea was filtered off and the filtrate was washed with 1*N* $NaHCO_3$ and H_2O. The EtOAc solution was dried over $MgSO_4$, evaporated to dryness, and the residue was recrystallized from EtOH. The precipitate was recrystallized from acetone (25 ml.), yield 0.90 g. (72%) of crystals, m.p. 175～179°, $[\alpha]_D^{19}$ −97.0° (c=0.5 dimethylformamide), *Anal.* Calcd. for $C_{25}H_{23}O_7N_3$: C, 62.89; H, 4.86; N, 8.80. Found: C, 62.84; H, 4.74; N, 8.63.

N-Benzyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (II)——N-Benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-nitrobenzyl ester (730 mg.) was dissolved in AcOH (2.5 ml.), anisole (0.2 ml.), and 5.7*N* HBr in AcOH (2.5 ml.). After 50 min. at room temperature, the reaction mixture was shaken vigorously with dry ether. The precipitate thereby formed was washed with dry ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (10 ml.) N-benzyloxycarbonyl-L-phenylalanylglycine *p*-nitrophenyl ester (550 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr., the reaction mixture was diluted with 1*N* NH_4OH (4 ml.), stirred for 1 hr., and diluted with EtOAc (100 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The EtOAc solution was dried over $MgSO_4$ and concentrated to small volume in vacuum. Petroleum ether was added to the residue and the precipitate thereby formed was reprecipitated from AcOH, H_2O, and a few drops of 50% NH_4OAc, yield 550 mg. (59%) of crystals, m.p. 94～100°, $[\alpha]_D^{15}$ −2.7° (c=0.6, AcOH), *Anal.* Calcd. for $C_{46}H_{52}O_{12}N_{10}$: C, 58.96; H, 5.59;

14) S. M. Partridge: Biochem. J., **42**, 238 (1948).
15) S. G. Waley, G. Watson: *Ibid.*, **55**, 328 (1953).
16) S. Moore, D. H. Spackman, W. H. Stein: Anal. Chem., **30**, 1185 (1958).

N, 14.95. Found: C, 59.01; H, 5.77; N, 14.59. Deblocked peptide ester: Rf^1 0.71, Rf^2 0.93, single ninhydrin positive spot.

N-Benzyloxycarbonylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^\omega$-nitro-L-arginine *p*-Nitrobenzyl Ester (III)——The benzyloxycarbonyl group was removed from N-benzyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^\omega$-nitro-L-arginine *p*-nitrobenzyl ester (600 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (6 ml.) N-benzyloxycarbonylglycine *p*-nitrophenyl ester (220 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperture, the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then mixed with EtOAc (60 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The EtOAc solution was dried over $MgSO_4$ and concentrated to small volume in vacuum. A concentrated solution containing some precipitate was added petroleum ether. The precipitate thereby formed was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc, yield 417 mg. (66%) of crystals, m.p. 96~103°, $[\alpha]_D^{15}$ −15.0°(c=0.5, AcOH), *Anal.* Calcd. for $C_{48}H_{55}O_{13}N_{11}$: C, 58.00; H, 5.58; N, 15.50. Found: C, 57.79; H, 5.45; N, 15.31. Deblocked peptide ester: Rf^1 0.55, Rf^2 0.84, single ninhydrin positive spot.

N-Benzyloxycarbonylsarcosine *p*-Nitrophenyl Ester (IV)——To a pre-cooled solution of N-benzyloxycarbonylsarcosine (3.6 g.) in EtOAc (40 ml.), *p*-nitrophenol (2.4 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (3.6 g.). After 30 min. at 0° and 2 hr. at room temperature, the formed N,N′-dicyclohexylurea was filtered off. The filtrate was evaporated to dryness in vacuum, and the residue was reprecipitated five times from EtOH; light yellow sticky oil; yield 3.1 g. (55%).

N-Benzyloxycarbonylsarcosylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^\omega$-nitro-L-arginine *p*-Nitrobenzyl Ester Hemihydrate (V)——The benzyloxycarbonyl group was removed from the fully protected hexapeptide (III) (335 mg.) as described above. To a solution of this resulting HBr salt in dimethylformamide (4 ml.), N-benzyloxycarbonylsarcosine *p*-nitrophenyl ester (140 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then diluted with EtOAc (40 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The solution was dried over $MgSO_4$ and evaporated to dryness in vacuum. The residue was reprecipitated from acetone with ether. The precipitate thereby formed was reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 347 mg. (97%), m.p. 103~108°, $[\alpha]_D^{15}$ 0.0°(c=0.7, AcOH). *Anal.* Calcd. for $C_{51}H_{60}O_{14}N_{12}\cdot\frac{1}{2}H_2O$: C, 57.03; H, 5.73; N, 15.65. Found: C, 57.03; H, 5.85; N, 15.50. Deblocked peptide ester: Rf^1 0.63, Rf^2 0.83, single ninhydrin positive spot.

N$^\alpha$-Benzyloxycarbonyl-N$^\omega$-nitro-L-arginyl-L-prolylsarcosylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^\omega$-nitro-L-arginine *p*-Nitrobenzyl Ester Monohydrate (VI)——The benzyloxycarbonyl group was removed from the fully protected heptapeptide (V) (255 mg.) as deseribed above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.), N$^\alpha$-benzyloxycarbonyl-N$^\omega$-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (164 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 2 days at room temperature the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then diluted with EtOAc (40 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The solution was dried over $MgSO_4$ and evaporated to dryness in vacuum. The residue was reprecipitated from acetone with ether. The precipitate thereby formed was reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 137 mg. (42%), m.p. 123~128°, $[\alpha]_D^{17}$ −17.7°(c=0.3, AcOH). *Anal.* Calcd. for $C_{62}H_{78}O_{18}N_{18}\cdot H_2O$: C, 53.90; H, 5.84; N, 18.25. Found: C, 54.10; H, 5.88; N, 18.33. Deblocked peptide ester: Rf^1 0.55, Rf^2 0.77, single ninhydrin positive spot.

L-Arginyl-L-prolylsarcosylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (VII)——The fully protected nonapeptide (VI) (70 mg.) was hydrogenated in 1:1 mixture of AcOH and H_2O (15 ml.) for 2 days over 10% Pd-C. The catalyst was removed by the aid of Cellite and the filtrate was evaporated to dryness in vacuum. The hydrogenated product was dried over KOH pellets in vacuum. The solution of the product in H_2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H_2O (300 ml.) in a mixing chamber to 0.15*M* pyridinium acetate buffer (pH 5.1) in a reservoir. Fractions of 14 ml. each were collected at a flow rate 3 to 4 ml./min. with an automatic fraction collector. Arginine-containing peptide was located in the eluate with Sakaguchi reaction. The eluate in tubes No. 29 to 41 were pooled, evaporated in vacuum, and lyophilized; yield 40 mg. (66%), $[\alpha]_D^{15}$ −21.2°(c=0.7, H_2O), Rf^1 0.31, Rf^2 0.45, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.90, Pro 1.97, Sar 0.96, Phe 2.00, Gly 2.03.

N-Benzyloxycarbonylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N$^\omega$-nitro-L-arginine L-Nitrobenzyl Ester (VIII)——The benzyloxycarbonyl group was removed from the fully protected pentapeptide (II) (735 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (7 ml.), N-benzyloxycarbonylglycylglycine *p*-nitrophenyl ester (334 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1*N* NH_4OH (4 ml.), stirred for 1 hr., and then diluted with EtOAc (60 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The solution was dried over $MgSO_4$, evaporated to small volume in vacuum, and petroleum ether was added to the residue. The precipitate thereby formed was collected by filtration and reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 643 mg. (78%), m.p. 100~110°,

$[\alpha]_D^{20}$ 0.0°(c=0.5, AcOH). *Anal.* Calcd. for $C_{50}H_{58}O_{14}N_{12}$: C, 57.13; H, 5.56; N, 15.99. Found : C, 57.64; H, 6.02; N, 15.84. Deblocked peptide ester : Rf^1 0.70, Rf^2 0.87, single ninhydrin positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Trihydrate (IX)——The benzyloxycarbonyl group was removed from the fully protected heptapeptide (Ⅷ) (300 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.), N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (196 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 2 days at room temperature, the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then poured into 1*N* NH_4OH (50 ml.) with stirring. The precipitate thereby formed was collected by filtration, and washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The precipitate was reprecipitated from AcOH with H_2O and 50% NH_4OAc, yield 241 mg. (62%), m.p. 124~129°, $[\alpha]_D^{20}$ −20.0°(c=0.4, AcOH). *Anal.* Calcd. for $C_{61}H_{76}O_{18}N_{18}\cdot 3H_2O$: C, 52.20; H, 5.89; N, 17.97. Found : C, 52.46; H, 5.82; N, 18.13. Deblocked peptide ester : Rf^1 0.59, Rf^2 0.84, single ninhydrin positive spot.

L-Arginyl-L-prolylglycylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Dihydrate (X)——The fully protected nonapeptide (Ⅸ) (72 mg.) was hydrogenated over 10% Pd-C as described above. The hydrogenated product was dried over KOH pellets in vacuum. The solution of the product in H_2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H_2O (300 ml.) in a mixing chamber to 0.15*M* pyridinium acetate buffer (pH 5.1) (300 ml.) in a reservoir. Fractions of 13 ml. each were collected at a flow rate 3 to 4 ml./min. with an automatic fraction collector. Arginine-containing peptide was located in the eluate with Sakaguchi reaction. The eluate in tubes No. 27 to 38 were pooled, evaporated to dryness, and lyophilized; yield 41 mg. (66%), $[\alpha]_D^{20}$ −43.6° (c=0.8, H_2O), Rf^1 0.30, Rf^2 0.50, single ninhydrin and Sakaguchi positive spot. *Anal.* Calcd. for $C_{46}H_{67}O_{10}N_{15}\cdot 3CH_3COOH\cdot 2H_2O$: C, 51.77; H, 6.94; N, 17.42. Found : C, 51.59; H, 6.83; N, 17.12.

N-Benzyloxycarbonyl-L-alanylglycine *p*-Nitrophenyl Ester (XI)——To a cooled solution of N-benzyloxycarbonyl-L-alanylglycine (1.60 g.) in EtOAc (18 ml.) and tetrahydrofuran (5 ml.) *p*-nitrophenol (0.88 g.) was added, followed by N,N′-dicyclohexycarbodiimide (1.30 g.). After 30 min. at 0° and 2 hr. at room temperature, a few drops of AcOH was added to the reaction mixture, and the reactiom mixture was stirred for 20 min. The formed N,N′-dicyclohexylurea was filtered off and the filtrate was washed with 1*N* $NaHCO_3$ and H_2O. The EtOAc solution was dried over $MgSO_4$, evaporated to dryness and the residue was recrystallized from EtOH; yield 1.15 g. (50%) of needles, m.p. 179°, $[\alpha]_D^{15}$ −33.7 (c=0.7, AcOH), *Anal.* Calcd. for $C_{19}H_{19}O_7N_3$: C, 56.86; H, 4.77; N, 10.47. Found : C, 56.86; H, 4.17; N, 10.75.

N-Benzyloxycarbonyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (XII)——The benzyloxycarbonyl group was removed from Ⅱ (204 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.), Ⅺ (91 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1*N* NH_4OH (0.5 ml.), stirred for 1 hr., and then poured into cold 1*N* NH_4OH (30 ml.) with stirring. To the suspension 50% NH_4OAc (2 ml.) was added with stirring and precipitate thereby formed was collected by filtration, washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The product was reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 144 mg. (64%), m.p. 112~118°, $[\alpha]_D^{20}$ −17.4° (c=0.2, AcOH), *Anal.* Calcd. for $C_{51}H_{60}O_{14}N_{12}$: C, 57.51; H, 5.68; N, 15.78. Found : C, 57.22; H, 5.48; N, 15.49. Deblocked peptide ester : Rf^1 0.66, Rf^2 0.87; single ninhydrin postive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (XIII)——The benzyloxycarbonyl group was removed from Ⅻ (144 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (4 ml.), N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (80 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1*N* NH_4OH, stirred for 1 hr., and poured into 1*N* NH_4OH (50 ml.). The precipitate thereby formed was collected on filter and washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O; yield 92 mg. (49%). For analysis a sample was reprecipitated from AcOH with H_2O and 50% NH_4OAc, m.p. 128~134°, $[\alpha]_D^{15}$ −71.4°(c=0.04, AcOH). *Anal.* Calcd. for $C_{62}H_{78}O_{18}N_{18}$: C, 54.62; H, 5.77; N, 18.49. Found : C, 54.30; H, 5.34; N, 18.67. Deblocked peptide ester : Rf^1 0.70, Rf^2 0.82; single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-alanylglycyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XIV)——The fully protected nonapeptide (XIII) (62 mg.) was hydrogenated as described above. The hydrogenated product in H_2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H_2O (300 ml.) in a mixing chamber to 0.1*M* NH_4OAc (pH 6.50) (300 ml.) in a reservoir. Fractions of 13 ml. each were collected at a flow rate of 3 to 4 ml./min. with an automatic fraction collector and the absorbancy of each fraction was determined at 230 mμ. The eluate in tubes No. 24 to 33 containing the nonapeptide were pooled, evaporated to dryness, and lyophilized. NH_4OAc was removed by repeated lyophilization to constant weight; colorless fluffy material, yield 31 mg. (57%), $[\alpha]_D^{20}$ −54.5°(c=0.6, H_2O), Rf^1 0.27, Rf^2 0.40, single ninhydrin and Sakaguchi positive spot, amino acid ratios in the acid hydrolysate; Arg 2.01, Pro 1.95, Ala 1.00, Gly 2.10, Phe 1.95.

N-Benzyloxycarbonylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (XV)——The benzyloxycarbonyl group was removed from N-benzyloxycarbonyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-nitrobenzyl ester (361 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.) N-benzyloxycarbonylglycylglycine *p*-nitrophenyl ester (159 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr., at room temperature the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then poured into cold 1*N* NH_4OH (40 ml.) with stirring. To the suspension, 50% NH_4OAc (2 ml.) was added with stirring and the precipitate was filtered and washed with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The product was reprecipitated from AcOH, H_2O and a few drops of 50% NH_4OAc; yield 282 mg. (67%) of crystals, m.p. 104~112°, $[\alpha]_D^{15}$ 0.0° (c=1.1, AcOH), *Anal.* Calcd. for $C_{53}H_{62}O_{16}N_{12}$: C, 56.22; H, 5.52; N, 14.85. Found : C, 56.62; H, 5.81; N, 14.77. Deblocked peptide ester : Rf^1 0.63, Rf^2 0.85, single ninhydrin positive spot.

N$^{\alpha}$-Benzyloxycarbonyl-N$^{\omega}$-nitro-L-arginyl-L-prolylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (XVI)——The benzyloxycarbonyl group of the protected heptapeptide ester (XV) (282 mg.) was removed as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.), N$^{\alpha}$-benzyloxycarbonyl-N$^{\omega}$-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (156 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 2 days at room temerature, the reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then poured into cold 1*N* NH_4OH (40 ml.) with stirring. To the suspension, 50% NH_4OAc (2 ml.) was added with stirring and the precipitate was filtered and washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 160 mg. (45%) of crystals, m.p. 124~132°, $[\alpha]_D^{15}$ 0.0° (c=1.0, AcOH), *Anal.* Calcd. for $C_{64}H_{80}O_{20}N_{18}$: C, 54.08; H, 5.67; N, 17.74. Found : C, 53.69; H, 5.27; N, 17.93. Deblocked peptide ester : Rf^1 0.64, Rf^2 0.81, single ninhydrin positive spot.

L-Arginyl-L-prolylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XVII)——The fully protected nonapeptide (XVI) (70 mg.) was hydrogenated in 1:1 mixture of AcOH and H_2O (15 ml.) for 48 hr. over 10% Pd-C (20 mg.). Fresh catalyst was added during the hydorgenation. The catalyst was removed by the aid of Cellite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH in vacuum. The solution of the product in H_2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H_2O (300 ml.) in a mixing chamber to 0.1*M* NH_4OAc buffer (pH 6.50) (300 ml.) in a reservoir. Fractions of 13 ml. each were collected at a flow rate of 3 to 4 ml./min. with an automatic fraction collector and the absorbancy of each fraction was determined at 230 mμ. The eluate in tubes No. 26 to 32 containing the nonapeptide were pooled, evaporated to dryness in vacuum and lyophilized. NH_4OAc was removed by repeated lyophilization to constant weight; colorless fluffy material, yield 43 mg. (69%), $[\alpha]_D^{15}$ −30.0°(c=0.4, H_2O), Rf^1 0.32, Rf^2 0.49, single ninhydrin and Sakaguchi positive spot. The content of the acetyl ester group was 96.2% of the theory; amino acid ratios in the acid hydrolysate : Arg 1.96, Pro 1.98, Gly 2.03, Phe 2.00, Ser 0.88.

L-Arginyl-L-prolylglycylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XVIII)——3-Glycine-6-O-acetyl-L-serine-bradykinin (XVII) (20 mg.) in H_2O (0.3 ml.) was saponified with 1*N* NaOH (0.2 ml.) for 1 hr. The solution neutralized with 1*N* AcOH was added to a column (2.0×6.0 cm.) of CM-cellulose which was eluted with a linear gradient method from H_2O (360 ml.) in a mixing chamber to 0.1*M* NH_4OAc buffer (pH 6.50) (300 ml.) in a reservoir. Fractions of 13 ml. each were collected at a flow rate of 3 to 4 ml./min. with an automatic fraction collector and the absorbancy of each fraction was determined at 230 mμ. The eluate in tubes No. 28 to 34 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. NH_4OAc was removed by repeated lyophilization to constant weight, colorless fluffy material; yield 14 mg. (75%), $[\alpha]_D^{15}$ −41.2°(c=0.3, H_2O), Rf^1 0.21, Rf^2 0.42, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate : Arg 1.95, Pro 1.92, Gly 2.00, Phe 1.96, Ser 0.91.

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Added in Proof.-After this manuscript had been submitted for publication, we received a paper (N. Yanaihara, M. Sekiya, K. Takagi, H. Kato, M. Ichimura, T. Nagao : This Bulletin, **15**, 110 (1967)) in which the work concerning with the foot note 3) has been described in detail.