α-(1-Acetamidobutyl)-β-naphthol——Was obtained in 70% yield. Recrystallization from EtOH gave prisms, m.p. 223～224°. *Anal.* Calcd. for $C_{16}H_{19}O_2N$: C, 74.68; H, 7.44; N, 5.44. Found : C, 74.38; H, 7.26; N, 5.50. IR λ_{max}^{KBr} cm^{-1} : 1639, 1530 (-CONH-).

α-(1-Acetamidoheptyl)-β-naphthol——Was crystallized by chromatography on a silicagel column using $CHCl_3$ as an eluent. Yield, 41%. Recrystallization from ether gave prisms, m.p. 98～100°. *Anal.* Calcd. for $C_{19}H_{25}O_2N$: C, 76.64; H, 8.68; N, 4.47. Found : C, 76.35; H, 8.29; N, 4.48. IR λ_{max}^{KBr} cm^{-1} : 1627, 1526 (-CONH-).

α-(1-Acetamido-3-phenylpropyl)-β-naphthol——Was obtained in 37% yield. Recrystallization from MeOH gave prisms, m.p. 184～185°. *Anal.* Calcd. for $C_{12}H_{21}O_2N$: C, 78.97; H, 6.68; N, 4.39. Found : C, 78.53; H, 6.72; N, 4.38. IR λ_{max}^{KBr} cm^{-1} : 1639, 1523 (-CONH-).

4-(1-Acetamidoethyl)-2,6-dimethylphenol——Was obtained in 23% yield. Recrystallization from EtOH gave plates, m.p. 177～178°. *Anal.* Calcd. for $C_{12}H_{17}O_2N$: C, 69.54; H, 8.27; N, 6.76. Found : C, 69.33; H, 8.21; N, 6.82. IR λ_{max}^{KBr} cm^{-1} : 1634, 1560 (-CONH-).

4-(1-Acetamidopropyl)-2,6-dimethylphenol——Was crystallized by chromatography on a silicagel column using $CHCl_3$ as an eluent. Yield, 25%. Recrystallization from $CHCl_3$-ether gave prisms, m.p. 131～132°. *Anal.* Calcd. for $C_{13}H_{19}O_2N$: C, 70.55; H, 8.65; N, 6.33. Found : C, 70.45; H, 8.56; N, 6.62. IR λ_{max}^{KBr} cm^{-1} : 1640, 1559 (-CONH-).

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16. Noboru Yanaihara, Minoru Sekiya*¹, Keijiro Takagi, Hitoshi Kato, Masamichi Ichimura, and Taku Nagao*²:

Syntheses of Bradykinin Analogs Containing Sarcosine Residues and Their Biological Activities.*³

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Five sarcosine-containing bradykinin analogs such as 2-, 3-, and 7-sarcosine, 2,3-disarcosine, and 2,3,7-trisarcosine bradykinins were synthesized Biological activities of these analogs were compared with those of synthetic bradykinin.

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Schröder[1] has recently reported the syntheses of bradykinin analogs in which proline residue in position 2, 3, or 7 of the molecule was replaced by alanine, and he described that 3-alanine bradykinin possesses the full activity both on the guinea-pig ileum and on the blood pressure of rabbit, whereas the replacement of proline residue in position 2 or 7 causes a considerable decrease of the biological activity.

On the basis of the evidences[2～6] presently available, including the above observation, it seemed that proline residues in positions 2 and 7 play an important role in

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*³ The peptides and peptide derivatives mentioned in this paper are of the L-configuration.

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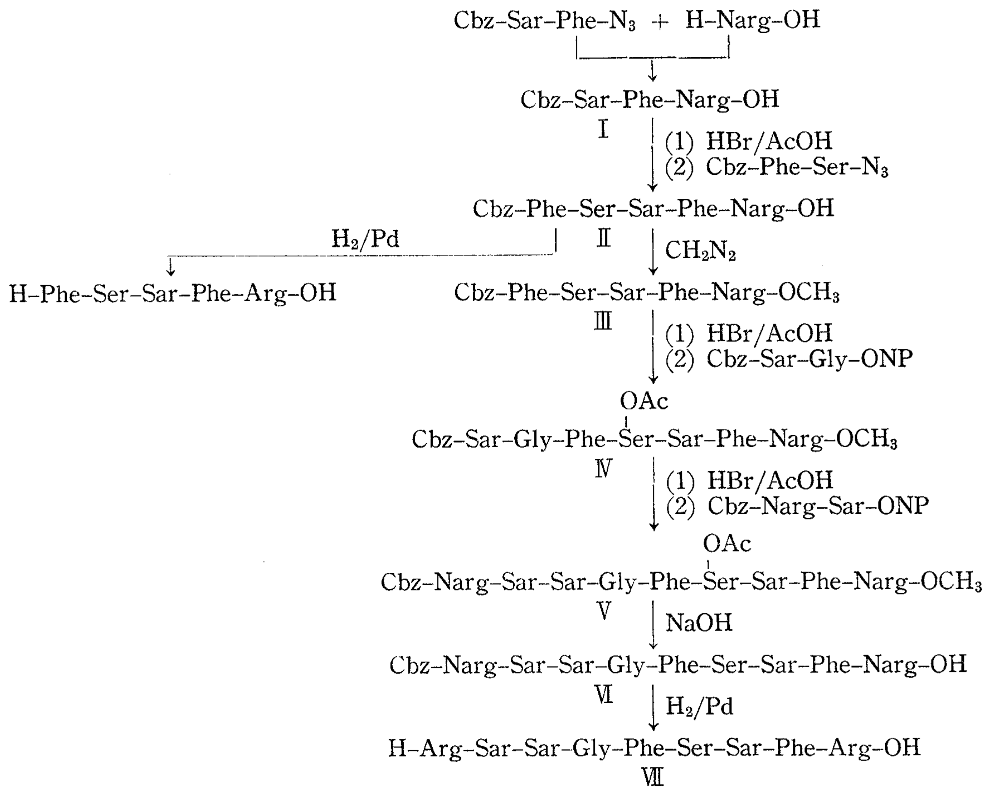
retaining bradykinin-like activity. As proline is a cyclic secondary amino acid having no functional group on its side chain, the above modification on the biological activity of bradykinin may be explainable in steric terms.

We considered that biological evaluation of bradykinin analog in which proline residue is replaced by simple secondary amino acid such as sarcosine may give an information about the requirement of imide linkage provided by proline residue in bradykinin molecule.

In the present communication we describe the syntheses of five sarcosine-containing bradykinin analogs such as 2-, 3-, and 7-sarcosine bradykinins, 2,3-disarcosine bradykinin, and 2,3,7-trisarcosine bradykinin and provide their biological properties.

Benzyloxycarbonylsarcosylphenylalanine hydrazide was obtained by the reaction of benzyloxycarbonylsarcosine⁷⁾ with methyl phenylalaninate in the presence of N,N′-dicyclohexylcarbodiimide⁸⁾ followed by exposure of the resulting protected dipeptide methyl ester to methanolic hydrazine. *p*-Nitrophenyl benzyloxycarbonylsarcosylglycinate was prepared by saponification of methyl benzyloxycarbonylsarcosylglycinate and then by the reaction of the ensuing protected dipeptide with *p*-nitrophenol. The preparation of *p*-nitrophenyl benzyloxycarbonyl-N$^{\omega}$-nitroarginylsarcosinate was conducted according to the method similar to that employed for the preparation of *p*-nitrophenyl benzyloxycarbonyl-N$^{\omega}$-nitroarginylprolinate.⁵⁾,⁹⁾

Thus, the five analogs were synthesized by the procedures as shown in Chart 1 in which the case of 2,3,7-trisarcosine bradykinin is schemed as an example.



Cbz = benzyloxycarbonyl; Narg = nitroarginine; ONP = *p*-nitrophenyl ester

Chart 1. Synthesis of 2,3,7-Trisarcosine Bradykinin

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7) D. Ben-Ishai, E. Katchalsky: J. Am. Chem. Soc., **74**, 3688 (1952). D. T. Elmore: J. Chem. Soc., **1959**, 3152.

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N^{ω}-Nitroarginine[10] was employed as the starting material for all of the analogs. The chain elongation was performed in the way essentially similar to that we developed in the preparation of 1-glutamic acid kallidin.[11] The azide[12] or *p*-nitrophenyl ester[13] procedure specifically selected may preclude racemization in the coupling stages. Hydrogenolysis of benzyloxycarbonylpentapeptide (Ⅱ) gave phenylalanylserylsarcosylphenylalanylarginine monoacetate in homogeneous state without further purification. During treatment of the blocked pentapeptide methyl ester (Ⅲ) with HBr-AcOH,[14] O-acetyl function was introduced into the peptide molecule. The NMR spectra of protected heptapeptide methyl esters, methyl benzyloxycarbonylsarcosylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^{ω}-nitroargininate (Ⅳ) and methyl benzyloxycarbonylprolylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^{ω}-nitroargininate (Ⅷ), (measured in trifluoroacetic acid) revealed the presence of O-acetyl group in the molecules by singlet at 7.75τ, as mentioned in the previous paper.[11] The blooked nonapeptide methyl esters, methyl benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^{ω}-nitroargininate (Ⅴ), methyl benzyloxycarbonyl-N^{ω}-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^{ω}-nitroargininate (Ⅸ), methyl benzyloxycarbonyl-N^{ω}-nitroarginylprolylsarcosylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate(XⅢ), methyl benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate (XVI), and methyl benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate (XIX), were respectively treated with $2N$ NaOH to remove O-acetyl and methyl ester functions. The resulting partially deblocked nonapeptides, benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanylserylsarcosylphenylalanyl-N^{ω}-nitroarginine (Ⅵ), benzyloxycarbonyl-N^{ω}-nitroarginylprolylprolylglycylphenylalanylserylsarcosylphenylalanyl-N^{ω}-nitroarginine (X), benzyloxycarbonyl-N^{ω}-nitroarginylprolylsarcosylglycylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroarginine (XIV), benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroarginine (XVⅡ), and benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylprolylglycylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroarginine (XX), were hydrogenated respectively over a palladium catalyst.[15] Sarcosine-containing bradykinin analogs, 2,3,7-trisarcosine bradykinin (Ⅶ), 7-sarcosine bradykinin (Ⅺ), 3-sarcosine bradykinin (XV), 2,3-disarcosine bradykinin (XⅧ), and 2-sarcosine bradykinin (XXI), thus obtained were purified, if necessary, by ion exchange chromatography on carboxymethylcellulose.[16] Each of the final products produced a single ninhydrin- and Sakaguchi-positive spot on paper chromatogram and paper electrophoresis. Quantitative amino acid analyses of acid hydrolysates of the above products gave the expected values. Hydrogenation of sufficiently pure Ⅵ, X, XIV, XVⅡ, and XX over a palladium catalyst yielded Ⅶ, Ⅺ, XV, XⅧ, XXI in homogeneous form without any further purification, respectively.

α-Chymotryptic digestion released 1 equimole of arginine from each of Ⅶ, Ⅺ, XV, XⅧ, and XXI and paper chromatogram of the respective digest showed such a pattern

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10) K. Hofmann, A. Rheiner, W.D. Peckham: J. Am. Chem. Soc., **75**, 6083 (1953); K. Hofmann, W.D. Peckham, A. Rheiner: *Ibid.*, **78**, 238 (1956).
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as expected according to the results of the cases with synthetic and natural17) bradykinins.

The biological activities of the five analogs are shown in Table I.

TABLE I. Biological Activities of Sarcosine-containing Bradykinin Analogs

Peptide	Guinea-pig ileum (isolated)	Guinea-pig blood pressure	Blood flow of dog hind limb
2-Sarcosine Bradykinin (XXI)	1/2.5	1/2	1/3～1/4
3-Sarcosine Bradykinin (XV)	1/1.5	1	1/10～1/20
7-Sarcosine Bradykinin (XI)	1/8	1/3	1/10～1/15
2,3-Disarcosine Bradykinin (XVIII)	1/20	1/3～1/5	1/50
2,3,7-Trisarcosine Bradykinin (VII)	1/100	1/25	1/200
Bradykinin (synthetic)	1	1	1

3-Sacrosine bradykinin XV indicated the almost same order of activity as that of bradykinin itself on the guinea-pig ileum and its blood pressure. The activity of 2-sarcosine analog XXI was slightly lower than that of bradykinin, but higher than that of 7-sarcosine analog XI. 2,3-Disarcosine bradykinin XVIII still retained a considerable amount of bradykinin-like activity. Although 2,3,7-trisarcosine analog VII was lower in biological potency than the other sarcosine-containing analogs prepared in the present work, 1/25 and 1/100 of the bradykinin activity were found on the blood pressure of guinea-pig and the guinea-pig ileum, respectively. On the other hand, on the blood flow of dog hind limb 2-sarcosine bradykinin XXI was the most active and 3- and 7-sarcosine analogs, XV and XI, possessed 1/10 to 1/15 activity of bradykinin.

Fairly high activities exhibited by the sarcosine-containing bradykinin analogs would suggest that imide linkages between positions 1 and 2 and between positions 6 and 7 are a structural requirement somewhat necessary for the bradykinin-like activity.

Experimental[*4]

Methyl Benzyloxycarbonylsarcosylphenylalaninate——N,N'-Dicyclohexylcarbodiimide (4.1 g.) was added to an ice-cold solution of benzyloxycarbonylsarcosine6) (4.5 g.), methyl phenylalaninate hydrochloride (4.3 g.), and triethylamine (2.8 ml.) in methylene chloride (60 ml.). The mixture was kept at 4° for 30 min. and then at room temperature for 12 hr. The N,N'-dicyclohexylurea formed was removed by filtration and washed with methylene chloride, and the filtrate and washings were combined and evaporated. The residue was taken up in AcOEt (100 ml.), and the solution was washed successively with *N* HCl, saturated Na_2HCO_3, and saturated NaCl and dried over Na_2SO_4. The solvent was evaporated to give oily residue, which was crystallized from petroleum ether; yield 7.3 g. (95%), m.p. 66～67°. A sample for analysis was recrystallized from ether-petroleum ether (1:5 v/v); 71～72°, $[\alpha]_D^{28}$ +3.1° (c=1.1, MeOH). *Anal.* Calcd. for $C_{21}H_{24}O_5N_2$: C, 65.61; H, 6.29; N, 7.29. Found: C, 65.92; H, 6.53; N, 7.54.

Benzyloxycarbonylsarcosylphenylalanine Hydrazide——Hydrazine hydrate (2.5 ml.) was added to a solution of the foregoing dipeptide methyl ester (3.5 g.) in MeOH (20 ml.) and the mixture was kept at room temperature for 6 hr. when the solvent was removed. Ether was added to give crystalline product, which

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[*4] All melting points are uncorrected. Optical rotations were measured with a Rex Model NEP-2 photoelectric polarimeter. The amino acid composition of acid hydrolysates was determined with a Hitachi Model KLA-2 amino acid analyzer. The NMR spectra were measured with a Japan Electron Optics Laboratory 3H-60 spectrometer. Paper chromatography was performed on Toyo No. 51 filter paper with the solvent system of *n*-BuOH-AcOH-H_2O (4:1:5) by descending technique (Rf^1) and with the system of *n*-BuOH-pyridine-AcOH-H_2O (30:20:6:24) by ascending technique (Rf^2). Paper electrophoresis was carried out in ammonium formate buffer at pH 3.5 using a constant current of 40 milliamp. for 1 hr. The following abbreviations are used: arg=arginine; pro=proline; sar=sarcosine; phe=phenylalanine; ser=serine; gly=glycine; DMF=dimethylformamide. The amino acids used were obtained from Ajinomoto Co., Inc., Tokyo.

was recrystallized from EtOH; yield 2.6 g. (74%), m.p. 157~158°, $[\alpha]_D^{28}$ +7.1° (c=1.1, DMF). *Anal.* Calcd. for $C_{20}H_{24}O_4N_4$: C, 62.48; H, 6.29; N, 14.57. Found : C, 62.41; H, 6.49; N, 14.35.

Methyl Benzyloxycarbonylsarcosylglycinate——N,N′-Dicyclohexylcarbodiimide (2.1 g.) was added to an ice-cold methylene chloride solution (20 ml.) containing benzyloxycarbonylsarcosine6) (2.3 g.), methyl glycinate hydrochloride (1.3 g.), and triethylamine (1.4 ml.). The mixture was stirred for 30 min. in an ice bath and for further 1 hr. at room temperature. The N,N′-dicyclohexylurea formed was removed by filtration and washed with methylene chloride, the combined filtrate and washings were evaporated to give solid residue. This was recrystallized from AcOEt-ether to give needles; yield 2.3 g. (84%), m.p. 86~87°. *Anal.* Calcd. for $C_{14}H_{18}O_5N_2$: C, 57.13; H, 6.16; N, 9.52. Found : C, 57.20; H, 6.25; N, 9.77.

Benzyloxycarbonylsarcosylglycine——The above protected dipeptide methyl ester (2.0 g.) was dissolved in MeOH (15 ml.), 2*N* NaOH (5 ml.) was added and the mixture was kept at room temperature for 1 hr. The bulk of the MeOH was removed by evaporation. The residual solution was acidified by the addition of 2*N* HCl and extracted with AcOEt, and the organic phase was washed with *N* HCl and then with saturated NaCl and dried over Na_2SO_4. Evaporation of the solvent gave viscous oily residue, which was crystallized by the addition of ether. Recrystallization from AcOEt-Et_2O (1:5 v/v) gave needles; yield 1.5 g. (79%), m.p. 123~124°. *Anal.* Calcd. for $C_{13}H_{16}O_5N_2$: C, 55.70; H, 5.75; N, 9.99. Found : C, 55.80; H, 5.72; N, 9.99.

***p*-Nitrophenyl Benzyloxycarbonylsarcosylglycinate**——N,N′-Dicyclohexylcarbodiimide (1.7 g.) was added to an ice-cold solution of benzyloxycarbonylsarcosylglycine (2.2 g.) and *p*-nitrophenol (1.2 g.) in AcOEt (40 ml.) The mixture was stirred for 30 min. in an ice bath and then for 1 hr. at room temperature. The solvent was evaporated to give crystalline residue, which was recrystallized from EtOH to yield pale yellow prisms; yield 2.5 g. (79%), m.p. 98~100°. *Anal.* Calcd. for $C_{19}H_{19}O_7N_3$: C, 56.85; H, 4.77; N, 10.46. Found: C, 56.56; H, 5.02; N, 10.42.

Methyl Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosinate——A solution of benzyloxycarbonyl-N^{ω}-nitroarginine10) (3.5 g.), methyl sarcosinate hydrochloride (1.4 g.), and triethylamine (1.4 ml.) in DMF (5 ml.) and methylene chloride (40 ml.) was cooled in an ice bath and N,N′-dicyclohexylcarbodiimide (2.4 g.) was added thereto with stirring. The mixture was stirred for 30 min. in an ice bath and then for 15 hr. at room temperature. The resultant N,N′-dicyclohexylurea was removed by filtration and washed with methylene chloride, and the filtrate and washings were combined and evaporated to dryness. The residue was dissolved in AcOEt (100 ml.), and the solution was washed successively with *N* HCl, saturated $NaHCO_3$, and saturated NaCl and dried over Na_2SO_4. The solvent was evaporated and the residue was recrystallized from EtOH to give prisms; yield 3.6 g. (82%), m.p. 96~102°, $[\alpha]_D^{28}$ −7.3° (c=1.23, DMF). *Anal.* Calcd. for $C_{18}H_{26}O_7N_6$: C, 49.30; H, 5.97; N, 19.17. Found : C, 48.91; H, 5.97; N, 18.73.

Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosine—— The foregoing dipeptide methyl ester (3.0 g.) was dissolved in MeOH (10 ml.) and dioxane (10 ml.), and 2*N* NaOH (5 ml.) was added thereto. The mixture was kept at room temperature for 1 hr. The bulk of the solvents was evaporated, and H_2O (20 ml.) was added. The solution was acidified to Congo red with 2*N* HCl and extracted with AcOEt, the organic phase was successively washed with *N* HCl and saturated NaCl and dried over Na_2SO_4. Evaporation of the solvent gave crystalline residue, which was recrystallized from EtOH to give needles; yield 2.6 g. (89%), m.p. 119~120°, $[\alpha]_D^{28}$ −8.8° (c=1.1, DMF). *Anal.* Calcd. for $C_{17}H_{24}O_7N_6$: C, 48.00; H, 5.69; N, 19.80. Found: C, 47.88; H, 5.78; N, 19.64.

***p*-Nitrophenyl Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosinate**—— N, N′-Dicyclohexylcarbodiimide (1.1 g.) was added to an ice-cold solution of benzyloxycarbonyl-N^{ω}-nitroarginylsarcosine (2.0 g.) and *p*-nitrophenol (0.7 g.) in DMF (5 ml.) and methylene chloride (10 ml.). The mixture was stirred for 30 min. in an ice bath and then for 14 hr. at room temperature. After the resulting N,N′-dicyclohexylurea was removed, the solvent was evaporated. The residue was dissolved in AcOEt (50 ml.) and the solution was washed with saturated $NaHCO_3$ and then with saturated NaCl and dried over Na_2SO_4. The solvent was evaporated to dryness, and the residue was dissolved in hot EtOH and cooled. The resulting viscous solution was treated with ether to give solid; yield 1.4 g. (54%), m.p. 78~87°, $[\alpha]_D^{28}$ −8.2° (c=1.2, DMF). *Anal.* Calcd. for $C_{23}H_{27}O_9N_7$: C, 50.63; H, 4.99; N, 17.97. Found : C, 50.18; H, 5.19; N, 17.55.

Benzyloxycarbonylsarcosylphenylalanyl-N^{ω}-nitroarginine (I)——Sodium nitrite (0.35 g.) dissolved in ice-cold H_2O (3 ml.) was added to an ice-cold solution of benzyloxycarbonylsarcosylphenylalanine hydrazide (1.9 g.) in *N* HCl (10 ml.) and tetrahydrofuran (15 ml.). The mixture was stirred for 5 min. in an ice bath and adjusted to pH 7.5 by the addition of triethylamine. This solution containing the azide was added to an ice-cold solution of N^{ω}-nitroarginine10) (1.1 g.) and triethylamine (0.7 ml.) in DMF (15 ml.) and H_2O (30 ml.). The mixture was stirred for 15 hr. at 4°. The bulk of tetrahydrofuran was evaporated and *N* NH_4OH (40 ml.) was added. The solution was washed with AcOEt, cooled in an ice bath, acidified with 2*N* HCl, and extracted with AcOEt. The extracts were washed with saturated NaCl and dried over Na_2SO_4. The solvent was removed by evaporation. The product was dissolved in EtOH and precipitated by the addition of ether yield; 1.6 g. (56%), m.p. 131~138°, $[\alpha]_D^{28}$ −6.1° (c=1.3, DMF). *Anal.* Calcd. for $C_{26}H_{33}O_8N_7$: C, 54.63; H, 5.81; N, 17.15. Found : C, 54.28; H, 5.98; N, 16.84.

Sarcosylphenylalanyl-N^{ω}-nitroarginine Hydrobromide——The above protected tripeptide I (2.0 g.) was dissolved in glacial AcOH (3 ml.) and 28% HBr in AcOH (10 ml.) was added thereto. The mixture was kept at room temperature for 1 hr. and ether (30 ml.) was added. The resultant precipitate was collected by filtration and washed several times with ether and dried over KOH in a vacuum desiccator. The yield was quantitative.

Benzyloxycarbonylphenylalanylserylsarcosylphenylalanyl-N$^{\omega}$-nitroarginine (II)——Sodium nitrite (0.24 g.) dissolved in cold H_2O (3 ml.) was added to an ice-cold solution of benzyloxycarbonylphenylalanylserine hydrazide$^{18\sim20)}$ (1.4 g.) in *N* HCl (7 ml.) and tetrahydrofuran (10 ml.). The mixture was stirred for 5 min. in an ice bath and adjusted to pH 7.5 by the addition of triethylamine. This solution containing the azide was added to an ice-cold solution of sarcosylphenylalanyl-N$^{\omega}$-nitroarginine hydrobromide prepared from I (1.0 g.) in DMF (5 ml.) containing triethylamine (0.8 ml.). The mixture was stirred for 30 hr. at 4°. The bulk of the solvents was evaporated and *N* NH_4OH (50 ml.) was added. The aqueous solution was washed with AcOEt, cooled in an ice bath, acidified with 2*N* HCl, and then extracted with AcOEt. The extracts were washed with saturated NaCl and dried over Na_2SO_4 and the solvent was removed by evaporation. The residue was dissolved in EtOH and the addition of ether gave precipitate of the desired product; yield 0.75 g. (53%), m.p. 151~155° (sintering at 129°), $[\alpha]_{\mathrm{D}}^{28}$ −13.8° (c=0.8, DMF). *Anal.* Calcd. for $C_{38}H_{47}O_{11}N_9$: C, 56.63; H, 5.88; N, 15.64. Found : C, 56.26; H, 6.20; N, 15.38.

Methyl Benzyloxycarbonylphenylalanylserylsarcosylphenylalanyl-N$^{\omega}$-nitroargininate (III)——Ethereal diazomethane was added to an ice-cold solution of the benzyloxycarbonylpentapeptide II (0.7 g.) in MeOH (25 ml.) until the yellow color remained. After a few drops of glacial AcOH were added, the solvents were removed by evaporation. The residue was dissolved in a small amount of EtOH and ether was added to precipitate the desired product. Repeated precipitation gave white powders; yield 0.55 g. (77%), m.p. 121~123° (sintering at 103°), $[\alpha]_{\mathrm{D}}^{28}$ −25.6° (c=0.64, DMF). *Anal.* Calcd. for $C_{39}H_{49}O_{11}N_9$: C, 57.12; H, 6.02; N, 15.37. Found : C, 57.55; H, 6.07; N, 15.33.

Phenylalanylserylsarcosylphenylalanylarginine Monoacetate Dihydrate——The benzyloxycarbonylpentapeptide II (150 mg.) dissolved in 50% aqueous AcOH was hydrogenated over Pd for 15 hr. in the usual manner, and the catalyst was removed by filtration. The filtrate was evaporated to a small volume and repeatedly lyophilized to give white powders; yield 125 mg. (93%), $[\alpha]_{\mathrm{D}}^{28}$ +1.7° (c=0.5, H_2O), Rf^1 0.51, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $\mathrm{phe}_{2.07}\mathrm{ser}_{0.78}\mathrm{sar}_{1.00}\mathrm{arg}_{0.97}$. *Anal.* Calcd. for $C_{32}H_{46}O_9N_8 \cdot 2H_2O$: C, 53.17; H, 6.97; N, 16.31. Found : C, 52.99; H, 6.84; N, 16.28.

Methyl Benzyloxycarbonylsarcosylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N$^{\omega}$-nitroargininate (IV)——The foregoing protected pentapeptide methyl ester III (0.55 g.) was dissolved in AcOH (1 ml.) and 28% HBr in AcOH (5 ml.) was added. The mixture was kept at room temperature for 1 hr. Ether (20 ml.) was added, and the resulting precipitate was collected by filtration, washed with ether, and dried over KOH *in vacuo*. This deblocked material was dissolved in DMF (4 ml.) containing triethylamine (0.2 ml.). The solution was cooled in an ice bath and filtered. To the filtrate was added benzyloxycarbonylsarcosylglycine *p*-nitrophenyl ester (0.28 g.). The mixture was kept at room temperature for 2 days, when AcOEt (100 ml.) containing *n*-BuOH (20 ml.) was added. The solution was successively washed with 2*N* HCl, 2*N* NH_4OH, and H_2O. Evaporation of the solvents gave oily residue which was solidified by the addition of ether. The product was recrystallized from EtOH-ether; yield 0.41 g. (66%), m.p. 122~129°, $[\alpha]_{\mathrm{D}}^{28}$ −10.2° (c=0.5, DMF). *Anal.* Calcd. for $C_{46}H_{59}O_{14}N_{11}$: C, 55.80; H, 6.00; N, 15.56. Found : C, 55.98; H, 6.19; N, 15.56.

Methyl Benzyloxycarbonyl-N$^{\omega}$-nitroarginylsarcosylsarcosylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N$^{\omega}$-nitroargininate Dihydrate (**V**)——The benzyloxycarbonylheptapeptide methyl ester IV (0.27 g.) was dissolved in AcOH (1 ml.) and 28% HBr in AcOH (5 ml.) was added. After 1 hr., the solution was diluted with ether (20 ml.). The ensuing precipitate was collected by filtration, washed several times with ether, and dried over KOH *in vacuo*. This crude product was dissolved in DMF (4 ml.), triethylamine (0.1 ml.) was added thereto, and the solution was filtered. To the filtrate was added benzyloxycarbonyl-N$^{\omega}$-nitroarginylsarcosine *p*-nitrophenyl ester (0.16 g.) and the solution was kept at room temperature for 3 days. The solvent was removed by evaporation, and AcOEt (30 ml.) was added. The precipitate was collected by filtration, washed with 5% AcOH, 5% NH_4OH, and then H_2O, and dried over P_2O_5 *in vacuo*. This was recrystallized twice from EtOH; yield 0.19 g. (54%), m.p. 138~145° (sintering at 129°), $[\alpha]_{\mathrm{D}}^{28}$ −26.7° (c=0.5, DMF). *Anal.* Calcd. for $C_{55}H_{75}O_{18}N_{17} \cdot 2H_2O$: C, 50.87; H, 6.13; N, 18.34. Found : C, 50.59; H, 6.38; N, 18.72.

Benzyloxycarbonyl-N$^{\omega}$-nitroarginylsarcosylsarcosylglycylphenylalanylserylsarcosylphenylalanyl-N$^{\omega}$-nitroarginine Dihydrate (VI)——The above nonapeptide methyl ester V (150 mg.) was dissolved in MeOH (1.5 ml.), and 2*N* NaOH (0.18 ml.) was added. The mixture was kept at room temperature for 1 hr., when H_2O (10 ml.) was added. The solution was filtered and acidified with *N* HCl to Congo red. The cloudy solution was kept in a refrigerator for 2 hr., and the resulting precipitate was collected by filtration, washed with H_2O, and dried. This solid was recrystallized from EtOH; yield 95 mg. (66%), m.p. 158~169°, $[\alpha]_{\mathrm{D}}^{28}$ −27.5° (c=0.3, DMF). *Anal.* Calcd. for $C_{52}H_{71}O_{17}N_{17} \cdot 2H_2O$: C, 50.27; H, 6.08; N, 19.16. Found: C, 49.84; H, 6.04; N, 19.04.

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Arginylsarcosylsarcosylglycylphenylalanylserylsarcosylphenylalanylarginine Diacetate Pentahydrate (2,3,7-Trisarcosine Bradykinin) (VII)——The protected nonapeptide VI (45 mg.) dissolved in 50% aqueous AcOH (15 ml.) was hydrogenated over Pd for 15 hr. in the usual manner. The catalyst was removed by filtration and the solution was concentrated to a small volume and lyophilized. The crude product obtained was dissolved in H_2O (80 ml.) and the solution was applied to a CMC column (1.5 × 7 cm.) which was eluted successively with the following pH 5 pyridinium acetate buffers: 0.025*M* (100 ml), 0.05*M* (100 ml.), 0.1*M* (100 ml.), and 0.125*M* (200 ml.). Spot test with Sakaguchi reagent served to locate the peptide in the various eluates. The 0.125*M* eluates containing the desired material were pooled, the bulk of the solvent was removed *in vacuo*, and the residue was lyophilized; yield 32 mg. (74%), $[\alpha{}^{28}_{D}$ $-13.6°$ (c=0.22, H_2O), Rf^1 0.48, Rf^2 0.46, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $arg_{2.00}sar_{2.86}gly_{1.07}phe_{2.00}ser_{0.64}$. *Anal.* Calcd. for $C_{48}H_{75}O_{15}N_{15} \cdot 5H_2O$: C, 48.35; H, 7.19; N, 17.62. Found: C, 48.04; H, 7.25; N, 17.32. Incubation with α-chymotrypsin released 1 equimole of arginine from this peptide. Paper chromatogram of the digest in the system of *n*-BuOH-AcOH-H_2O revealed two ninhydrin- and Sakaguchi-positive spots, Rf^1 0.21 (arginine) and Rf^1 0.42 (presumably arginylsarcosylsarcosylglycylphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, Rf^1 0.47 (presumably serylsarcosylphenylalanine), and no spot corresponding to VII was detected.

Methyl Benzyloxycarbonylprolylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^ω^-nitroargininate (VIII)——The benzyloxycarbonyl group of III (0.65 g.) was removed by HBr-AcOH treatment as described in the preparation of IV and the resulting deblocked peptide hydrobromide was dissolved in DMF (5 ml.) and the solution was cooled in an ice bath, to which triethylamine (0.22 ml.) was added. The mixture was filtered. To the filtrate was added benzyloxycarbonylprolylglycine *p*-nitrophenyl ester[9] (0.34 g.), and the mixture was allowed to stand at room temperature for 3 days. The solution was diluted with AcOEt (100 ml.), washed successively with *N* HCl, *N* NH_4OH, and saturated NaCl, and dried over Na_2SO_4. The solvents were evaporated and the residue was dissolved in hot EtOH and cooled. The resultant gummy oily was separated by decantation and triturated with ether; yield 0.49 g. (61%), m.p. 136~140° (sintering at 125°), $[\alpha]^{28}_{D}$ $-56.7°$ (c=0.4, DMF). *Anal.* Calcd. for $C_{48}H_{61}O_{14}N_{11}$: C, 56.73; H, 6.05; N, 15.16. Found: C, 56.48; H, 5.99; N, 15.03.

Methyl Benzyloxycarbonyl-N^{ω}-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylsarcosylphenylalanyl-N^{ω}-nitroargininate Monohydrate (IX)——The benzyloxycarbonyl group was removed from the forgoing heptapeptide methyl ester VIII (0.25 g.) by HBr-AcOH treatment, and the hydrobromide thus obtained was allowed to react with benzyloxycarbonyl-N^{ω}-nitroarginylproline *p*-nitrophenyl ester[5,9] (0.17 g.) for 3 days. The reaction mixture was processed in the same manner as described for the preparation of V. The crude product was twice recrystallized from EtOH; yield 0.17 g. (52%), m.p. 148~155° (sintering at 137°), $[\alpha]^{28}_{D}$ $-46.8°$ (c=1.0, DMF). *Anal.* Calcd. for $C_{59}H_{79}O_{18}N_{17} \cdot H_2O$: C, 53.18; H, 6.12; N, 17.87. Found: C, 52.70; H, 6.18; N, 17.73.

Benzyloxycarbonyl-N^{ω}-nitroarginylprolylprolylglycylphenylalanylserylsarcosylphenylalanyl-N^{ω}-nitroarginine Dihydrate (X)——To a solution of the blocked nonapeptide methyl ester IX (0.25 g.) in MeOH (3 ml.) was added 2*N* NaOH (0.27 ml.). After the mixture was allowed to stand at room temperature for 45 min., H_2O (10 ml.) was added. The clear solution was cooled in an ice bath and acidified with 2*N* HCl to Congo red. The resulting cloudy solution was stored in a refrigerator overnight and the precipitate formed was collected by filtration, washed with cold H_2O, and dried, which was then recrystallized from EtOH; yield 0.17 g. (70%), m.p. 165~174° (sintering at 154°), $[\alpha]^{28}_{D}$ $-50.8°$ (c=0.5, DMF). *Anal.* Calcd. for $C_{56}H_{75}O_{17}N_{17} \cdot 2H_2O$: C, 51.95; H, 6.15; N, 18.39. Found: C, 52.21; H, 6.11; N, 18.72.

Arginylprolylprolylglycylphenylalanylserylsarcosylphenylalanylarginine Diacetate Heptahydrate (7-Sarcosine Bradykinin) (XI)—— The benzyloxycarbonylnonapeptide X (70 mg.) dissolved in 50% aqueous AcOH (15 ml.) was hydrogenated over Pb for 15 hr. in the usual manner. The catalyst was removed by filtration and the solution was concentrated to a small volume and lyophilized; yield 61 mg. (88%), $[\alpha]^{28}_{D}$ $-57.8°$ (c=0.3, H_2O). Rf^1 0.52, Rf^2 0.51, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $arg_{2.09}pro_{2.07}gly_{1.11}phe_{2.08}ser_{0.70}sar_{1.00}$. *Anal.* Calcd. for $C_{52}H_{79}O_{15}N_{15} \cdot 7H_2O$: C, 48.77; H, 7.32; N, 16.41. Found: C, 48.30; H, 7.72; N, 16.61. α-Chymotryptic digest of this peptide contained 1 equimole of arginine. Paper chromatogram of the digest in the system of *n*-BuOH-AcOH-H_2O gave two ninhydrin- and Sakaguchi-positive spots, Rf^1 0.21 (arginine) and Rf^1 0.48 (arginylprolylprolylglycylphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, Rf^1 0.47 (presumably serylsarcosylphenylalanine), and a negligible ninhydrin- and Sakaguchi-positive spot, Rf^1 0.59, was detected.

Methyl Benzyloxycarbonylsarcosylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate (XII)——The benzyloxycarbonyl group of methyl benzyloxycarbonylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroargininate[11,18,19] (1.58 g.) was removed by HBr-AcOH treatment as described in the preparation of IV, and the resulting partially deblocked pentapeptide hydrobromide was dissolved in DMF (10 ml.). To the solution was added triethylamine (0.56 ml.) and the mixture was filtered. To the filtrate was added benzyloxycarbonylsarcosylglycine *p*-nitrophenyl ester (0.80 g.). The mixture was kept at room temperature for 2 days and AcOEt (100 ml.) was added. The resulting organic solution was washed with *N* HCl, *N* NH_4OH, and then H_2O and the solvents were evaporated. The residue was recrystallized from 70% aqueous

EtOH; yield 1.56 g. (81%), m.p. 209~210°, $[\alpha]_D^{28}$ −65.8° (c=0.6, DMF). *Anal.* Calcd. for $C_{48}H_{61}O_{14}N_{11}$; C, 56.73; H, 6.05; N, 15.16. Found: C, 56.22; H, 6.12; N, 15.54.

Methyl Benzyloxycarbonyl-N^{ω}-nitroarginylprolylsarcosylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate Monohydrate (XIII)——The removal of the benzyloxycarbonyl group of the above heptapeptide methyl ester XII (0.50 g.) was carried out in the usual manner using HBr-AcOH. The resulting hydrobromide was dissolved in DMF (5 ml.), triethylamine (0.14 ml.) was added thereto, and the mixture was filtered. To the filtrate was added benzyloxycarbonyl-N^{ω}-nitroarginylproline *p*-nitrophenyl ester[5,9] (0.29 g.). The mixture was kept for 3 days at room temperature and then processed in the manner as described above for the preparation of V. The crude product thus obtained was recrystallized twice from EtOH; yield 0.33 g. (50%), m.p. 145~149° (sintering at 136°), $[\alpha]_D^{28}$ −51.2° (c=0.6, DMF). *Anal.* Calcd. for $C_{59}H_{79}O_{18}N_{17} \cdot H_2O$: C, 53.18; H, 6.12; N, 17.87. Found: C, 52.80; H, 6.29; N, 17.45.

Benzyloxycarbonyl-N^{ω}-nitroarginylprolylsarcosylglycylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroarginine Dihydrate (XIV)——To a solution of the blocked nonapeptide methyl ester XIII (0.14 g.) in MeOH (2 ml.) was added 2*N* NaOH (0.2 ml.). The mixture was allowed to stand for 45 min., when H_2O (5 ml.) was added. The clear solution was cooled in an ice bath and acidified with 2*N* HCl to Congo red. The crude desired product was isolated in the manner as described for the preparation of VI and recrystallized from EtOH; yield 0.11 g. (81%), m.p. 168~172°, $[\alpha]_D^{29}$ −60.0° (c=0.4, DMF). *Anal.* Calcd. for $C_{56}H_{75}O_{17}N_{17} \cdot 2H_2O$: C, 51.95; H, 6.15; N, 18.39. Found: C, 51.52; H, 6.28; N, 18.12.

Arginylprolylsarcosylglycylphenylalanylserylprolylphenylalanylarginine Diacetate Hexahydrate (3-**Sarcosine Bradykinin**) **(XV)**——The above blocked nonapeptide XIV (40 mg.) dissolved in 50% aqueous AcOH (15 ml.) was hydrogenated over Pd for 15 hr. in the usual manner. The catalyst was removed by filtration and the solution was concentrated to a small volume and lyophilized; yield 35 mg. (89%), $[\alpha]_D^{29}$ −50.3°, (c=0.3, H_2O), Rf^1 0.51, Rf^2 0.50, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $arg_{1.82}pro_{2.17}sar_{0.97}gly_{1.00}phe_{2.11}ser_{0.94}$. *Anal.* Calcd. for $C_{52}H_{79}O_{15}N_{15} \cdot 6H_2O$: C, 49.48; H, 7.26: N, 16.64. Found: C, 49.15; H, 6.99; N, 16.57. Incubation of this material with α-chymotrypsin yielded 1 equimole of arginine. Paper chromatogram of the digest in the system of *n*-BuOH-AcOH-H_2O revealed two ninhydrin- and Sakaguchi-positive spots, Rf^1 0.21 (arginine) and Rf^1 0.44 (presumably arginylprolylsarcosylglycylphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, Rf^1 6.64 (serylprolylphenylalanine), and a very faint ninhydrin- and Sakaguchi-positive spot, Rf^1 0.59, was also detected.

Methyl Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate Monohydrate (XVI)——The deblocked heptapeptide hydrobromode (prepared from the blocked peptide XII (0.40 g.)) was dissolved in DMF (5 ml.), triethylamine (0.11 ml). was added thereto, and the mixture was filtered. To the filtrate was added benzyloxycarbonyl-N^{ω}-nitroarginylsarcosine *p*-nitrophenyl ester (0.22 g.). The mixture was allowed to stand at room temperature for 3 days. The crude desired product was isolated in the same manner as described above for the preparation of V and recrystallized from EtOH; yield 0.27 g. (53%), m.p. 146~151° (sintering at 141°), $[\alpha]_D^{28}$ −34.2° (c=0.7, DMF). *Anal.* Calcd. for $C_{57}H_{77}O_{18}N_{17} \cdot H_2O$: C, 52.40; H, 6.09; N, 18.23. Found: C, 52.07; H, 6.25; N, 17.92.

Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylsarcosylglycylphenylalanylserylprolylphenylalanyl-N^{ω}-nitroarginine Dihydrate (XVII)——The above blocked nonapeptide methyl ester XVI (0.20 g.) was dissolved in MeOH (2.5 ml.) and 2*N* NaOH (0.25 ml.) was added thereto. The mixture was allowed to stand at room temperature for 45 min., when H_2O (5 ml.) was added. The resulting clear solution was cooled in an ice bath and acidified with 2*N* HCl to Congo red. The crude desired product obtained in the same manner as described for the preparation of VI was recrystallized from EtOH; yield 0.14 g. (72%), m.p. 158~165° (sintering at 145°), $[\alpha]_D^{28}$ −46.3° (c=0.4, DMF). *Anal.* Calcd. for $C_{54}H_{73}O_{17}N_{17} \cdot 2H_2O$: C, 51.14; H, 6.12; N, 18.77. Found: C, 50.90; H, 6.30; N, 18.24.

Arginylsarcosylsarcosylglycylphenylalanylserylprolylphenylalanylarginine Diacetate Pentahydrate (2,3-**Disarcosine Bradykinin**) **(XVIII)**——The above blocked nonapeptide XVII (80 mg.) dissolved in 50% aqueous AcOH (20 ml.) was hydrogenated over Pd for 15 hr. in the usual manner. The catalyst was removed by filtration and the solution was concentrated to a small volume and lyophilized; yield 66 mg. (86%), $[\alpha]_D^{28}$ −35.8° (c=0.6, H_2O), Rf^1 0.49, Rf^2 0.48 single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $arg_{1.90}sar_{1.90}gly_{1.04}phe_{2.00}ser_{1.00}pro_{1.02}$ *Anal.* Calcd. for $C_{50}H_{77}O_{15}N_{15} \cdot 5H_2O$: C, 49.28; H, 7.19; N, 17.24. Found: C, 49.44; H, 7.00; N, 17.47. Incubation of this peptide with α-chymotrypsin yielded 1 equimole of arginine. Paper chromatogram by means of the solvent system of *n*-BuOH-AcOH-H_2O revealed two ninhydrin- and Sakaguchi-positive spots, Rf^1 0.21 (arginine) and Rf^1 0.42 (presumably arginylsarcosylsarcosylglyclyphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, Rf^1 0.64 (serylprolylphenylalanine), and a negligible ninhydrin- and Sakaguchi-positive spot was also detected.

Methyl Benzyloxycarbonyl-N^{ω}-nitroarginylsarcosylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate Monohydrate (XIX)——The deblocked heptapeptide hydrobromide (prepared from methyl benzyloxycarbonylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N^{ω}-nitroargininate[9,11] (0.40 g.) by the treatment with HBr-AcOH) dissolved in DMF (5 ml.), triethylamine (0.11 ml.) was added, and the mixture was filtered. To the filtrate was added benzyloxycarbonyl-N^{ω}-nitroarginylsarcosine *p*-nitrophenyl ester (0.22 g.). The mixture was allowed to stand at room temperature for 3 days and then processed in the manner described for the preparation of V. The crude product thus obtained was recrystallized from

EtOH; yield 0.32 g. (63%), m.p. 146~152° (sintering at 135°), $[\alpha]_D^{28}$ −57.0° (c=0.8, DMF). *Anal.* Calcd. for $C_{59}H_{79}O_{18}N_{17}\cdot H_2O$: C, 53.18; H, 6.12; N, 17.87. Found : C, 52.74; H, 6.31; N, 17.52.

Benzyloxycarbonyl-N$^{\omega}$-nitroarginylsarcosylprolylglycylphenylalanylserylprolylphenylalanyl-N$^{\omega}$-nitroarginine Dihydrate (XX)——The blocked nonapeptide methyl ester XIX (0.23 g.) was dissolved in MeOH (2.5 ml.) and 2*N* NaOH (0.25 ml.) was added. The mixture was kept at room temperature for 45 min. and H_2O (5 ml.) was added. The clear solution was acidified with *N* HCl. The crude desired material was isolated in the manner as described for the preparation of Ⅵ and crystallized from EtOH; yield 0.20 g. (89%), m.p. 155~159° (sintering at 146°), $[\alpha]_D^{28}$ −60.9° (c=0.5, DMF). *Anal.* Calcd. for $C_{56}H_{75}O_{17}N_{17}\cdot 2H_2O$: C, 51.95; H, 6.15; N, 18.39. Found : C, 51.85; H, 6.30; N, 18.23.

Arginylsarcosylprolylglycylphenylalanylserylprolylphenylalanylarginine Diacetate Heptahydrate (2-Sarcosine Bradykinin) (XXI)——The above blocked nonapeptide XX (0.12 g.) dissolved in 50% aqueous AcOH (30 ml.) was hydrogenated over Pd for 15 hr. in the usual manner. The catalyst was removed by filtration and the solution was concentrated to a small volume and lyophilized to give white powders; yield 0.10 g. (84%), $[\alpha]_D^{28}$ −60.3° (c=0.4, H_2O), Rf^1 0.51, Rf^2 0.50, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $arg_{1.82}sar_{1.00}pro_{2.06}gly_{1.00}phe_{2.11}ser_{0.89}$. *Anal.* Calcd. for $C_{52}H_{79}O_{15}N_{15}\cdot 7H_2O$: C, 48.77; H, 7.32; N, 16.41. Found : C, 48.89; H, 7.25; N, 16.80. α-Chymotryptic digest of this peptide contained 1 equimole of arginine. Paper chromatogram of the digest in the system of *n*-BuOH-AcOH-H_2O showed two ninhydrin- and Sakaguchi-positive spots, Rf^1 0.21 (arginine) and Rf^1 0.45 (presumably arginylsarcosylprolylglycylphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, Rf^1 0.64 (serylprolylphenylalanine), and a faint ninhydrin- and Sakaguchi-positive spot, Rf^1 0.59, was also detected.

Enzymatic Experiment——The peptide (1 mg.) in 0.1*M* ammonium bicarbonate buffer pH 8.0 (0.5 ml.) was mixed with 0.1 w/v% α-chymotrypsin (Sigma Chemical Co., No. 113B-0310) solution in the same buffer (0.6 ml.). The mixture was incubated at 40° for 24 hr., and the solution was then acidified with glacial AcOH and evaporated to dryness in a vacuum desiccator over P_2O_5. The residue was subjected to analysis.

Biological Assay——Ileum segments isolated from the male guinea-pig (470~550 g. body weight) were mounted in 35 ml. bath containing Tyrode's solution at 37°.

The activity on the blood pressure of anesthetized male guinea-pig (sodium pentobarbital 40 mg./kg., i.p.) was obtained through a transducer connected to the common carotid artery.

The activity on the blood flow of dog hind limb was measured using an electromagnetic flowmeter placed on the femoral artery of anesthetized male mongrel dog (sodium pentobarbital 35 mg./kg., i.v.).

Each peptide was freshly dissolved in distilled water for each experiment. The test solution within 0.4 ml. was injected into the artery in a period of 10 sec. No influence of dose used on the systemic blood pressure was observed.

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