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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-,* 3-L-alanine-,* 3-sarcosine-,* and 8-p-fluoro-3-phenylalanine-bradykinin* and these facts have been also reviewed by Schröder, et al.* 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 these analogs is expected from the results of the study by Schröder, et al.* as described above. The reasons for the studies on 3-glycine analogs are as follows: the difference on the structural feature between glycine and the other two amino acids, sarcosine and L-alanine is only the existence of the N- or a-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.* 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.* The synthetic route for 3-sarcosine-6-glycine bradykinin is illustrated in Fig 1. Esterification of N-benzoyloxy carbonyl-L-phenylalanyl glycine* with p-nitrophenol by the N,N'-dicyclohexylcarbodiimide procedure gave N-benzoyloxycarbonyl-L-phenylalanylglycine p-nitrophenyl ester (I). N-Benzoyloxy carbonyl-L-prolyl-L-phenylalanyl-N-"nitro-L-arginine p-nitrobenzyl ester* was de-benzoyloxy carbonylated with a hydrogen bromide-acetic acid solution in the presence of anisole. The resulting product was condensed with I to yield crystalline N-benzoyloxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-
**Fig. 1. Synthesis of 3-Sarcocine-6-glycine-bradykinin**

N\(^\text{N}\)-nitro-l-arginine \( p \)-nitrobenzyl ester (II). After the removal of the benzoyloxy carbonyl group of II, the resulting pentapeptide ester was condensed with N-benzyloxy carbonyl glycine \( p \)-nitrophenyl ester\(^9\) to yield N-benzyloxy carbonylglycyl-\( l \)-phenylalanlyglycyl-\( l \)-prolyl-\( l \)-phenylalanly-N\(^\text{N}\)-nitro-\( l \)-arginine \( p \)-nitrobenzyl ester (III). Esterification of N-benzyloxy carbonyl sarcosine \( p \)-nitrophenol in ethyl acetate by \( N_N \)-dicyclohexylcarbodiimide yielded N-benzyloxy carbonylsarcosine \( p \)-nitrophenyl ester (IV) as an oil and the ester was used for the next step without further purification. After the removal of the benzoyloxy carbonyl group of III, the resulting hexapeptide ester was condensed with IV to yield N-benzyloxy carbonylsarcosylglycyl-\( l \)-phenylalanly-\( l \)-prolyl-\( l \)-phenylalanly-N\(^\text{N}\)-nitro-\( l \)-arginine \( p \)-nitrobenzyl ester (V). After the removal of the benzoyloxy carbonyl group of V, the resulting heptapeptide ester was condensed with N\(^\text{N}\)-benzyloxy carbonyl-N\(^\text{N}\)-nitro-\( l \)-arginyl-\( l \)-proline \( p \)-nitrophenyl ester\(^{10}\) to yield N\(^\text{N}\)-benzyloxy carbonyl-N\(^\text{N}\)-nitro-\( l \)-arginyl-\( l \)-prolylsarcosylglycyl-\( l \)-phenylalanly-\( l \)-prolyl-\( l \)-phenylalanly-N\(^\text{N}\)-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-\( l \)-phenylalanlyglycyl-\( l \)-prolyl-\( l \)-phenylalanly-\( l \)-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-benzoyloxy carbonylated II was condensed with N-benzyloxy carbonylglycylglycine \( p \)-nitrophenyl ester\(^{10}\) to yield N-benzyloxy carbonylglycylglycyl-\( l \)-phenylalanlyglycyl-\( l \)-prolyl-\( l \)-phenylalanly-N\(^\text{N}\)-nitro-\( l \)-arginine \( p \)-nitrobenzyl ester (VII). After the removal of the benzyloxy carbonyl group of VII, the resulting heptapeptide ester was condensed with N\(^\text{N}\)-benzyloxy carbonyl-N\(^\text{N}\)-nitro-\( l \)-arginyl-\( l \)-proline \( p \)-nitrophenyl ester to yield N\(^\text{N}\)-benzyloxy carbonyl-N\(^\text{N}\)-nitro-\( l \)-arginyl-\( l \)-prolylglycylglycyl-\( l \)-phenylalanlyglycyl-\( l \)-prolyl-\( l \)-phenylalanly-N\(^\text{N}\)-nitro-\( l \)-arginine \( p \)-nitrobenzyl ester (IX). The fully protected nonapeptide was hydrogenated over palladium on charcoal and the hydrogenated

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product was purified through a CM-cellulose column to obtain 3-glycine-6-glycine-
bradykinin, L-arginyl-L-prolylglycylglycyl-L-phenylalanlyglycyl-L-prolyl-L-phenylalanly-
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-benzoxycarbonyl-L-alanylglucose with
p-nitrophenol by N,N'-dicyclohexylcarbodiimide yielded N-benzyloxycarbonyl-L-alanyl-
glycine p-nitrophenyl ester (XI). De-benzyloxycarbonylated II was condensed with XI to
yield N-benzyloxycarbonyl-L-alanylglucyl-L-phenylalanlyglucyl-L-prolyl-L-phenylalanly-
N'-nitro-L-arginine p-nitrobenzyl ester (XII). After the removal of the benzyloxycar-
boneyl group of XII, the resulting heptapeptide ester was condensed with N'-benzyloxycar-
boneyl-N'-nitro-L-arginyl-L-proline p-nitrophenol ester to yield N'-benzyloxycarbonyl-
N'-nitro-L-arginyl-L-prolyl-L-alanylglucyl-L-phenylalanlyglycyl-L-prolyl-L-phenylalanly-
N'-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-alanylglucyl-L-phenylalanlyglycyl-L-prolyl-L-phenylalanly-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-phenylalanly-L-seryl-L-prolyl-L-phenylalanly-N'-nitro-L-arginine p-nitrobenzyl ester,
the resulting pentapeptide ester was condensed with N-benzyloxycarbonylglycylglycine
p-nitrophenyl ester to yield N-benzyloxycarbonylglycylglycyl-L-phenylalanly-O-acety-
L-seryl-L-prolyl-L-phenylalanly-N'-nitro-L-arginine p-nitrobenzyl ester (XV). De-ben-
zylloxycarbonylated XV was condensed with N'-benzyloxycarbonyl-N'-nitro-L-arginy-
L-proline p-nitrophenyl ester to yield N'-benzyloxycarbonyl-N'-nitro-L-arginy-
L-prolylglycylglycyl-L-phenylalanly-O-acetyl-L-seryl-L-prolyl-L-phenylalanly-N'-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-cellu-
lose column to obtain 3-glycine-6-O-acetyl-L-serine-bradykinin, L-arginy-
L-prolylglycylglycyl-L-phenylalanly-O-acetyl-L-seryl-L-prolyl-L-phenylalanly-O-acetyl-L-seryl-L-prolyl-
L-phenylalanly-L-arginine triacetate (XVII). The nonapeptide (XVII) so obtained
was found to be homogeneous from the results of a similar analysis for XIV. Saponification
of XVII with 1N sodium hydroxide gave 3-glycine-bradykinin, L-arginy-
L-prolylglycylglycyl-L-phenylalanly-L-seryl-L-prolyl-L-phenylalanly-L-arginine triacetate (X XVIII).
The nonapeptide so obtained was found to be homogeneous from the results of the similar
analysis for XIV.

Quantitative examinations were made on the bradykinin-like activity, anti-brady-
kinin activity, and potentiation of bradykinin activity of the nonapeptide synthesized
in the present work. 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 and 6-glycine-bradykinin. Bradykinin-like activity of 3-L-alanine-6-glycine-bradykinin (XIV), as reported also by Schröder, et al., was lower
than that of 3-alanine-bradykinin and 6-glycine-bradykinin. Bradykinin-like activity of 3-glycine-bradykinin XVIII and its O-acetyl compound (XVII) was fairly high, but the

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83 The detail of the biological assays will be reported in separate paper by Prof. Kameyama of this college.
activity of 3-glycine-6-glycine-bradykinin (X) was markedly lower than that of XVII 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 conformation 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.

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<td>6-Glycine-bradykinin</td>
<td>33.3~100c)</td>
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a) Assayed by Magnus method on an isolated guinea pig ileum (male).
+ : active; - : inactive.
b) At a concentration of 1x10^8 g/mL, caused 35% potentiation of the normal contraction due to 1x10^6 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 value refers to the Partridge system,14) and Rf value refers to the system of BuOH-pyridine-ACOH-H2O (90:20:6:24).15) The amino acid composition of the acid hydrolysates was determined according to the directions given by Moore, et al.16)

N-Benzoxycarbonyl-L-phenylalanylglycine p-Nitrophenyl Ester (I) — To a pre-cooled solution of N-benzoxycarbonyl-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 AcOEt were 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 1N NaHCO3 and H2O. The EtOAc solution was dried over MgSO4, 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°, [α]20° +97.6° (c=0.5 dimethylformamide), Anal. Calcd. for C38H36O2N3: C, 62.89; H, 4.86; N, 8.80. Found: C, 62.84; H, 4.74; N, 8.63.

N-Benzoxycarbonyl-L-phenylalanylglycyl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-Nitrobenzyl Ester (II) — N-Benzoxycarbonyl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-nitrobenzyl ester (730 mg) was dissolved in AcOH (2.5 mL), anisole (1.2 mL), and 5.7N 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 to over KOH in vacuum. To a solution of this product in dimethylformamide (10 mL) N-benzoxycarbonyl-L-phenylalanylglycine p-nitrophenyl ester (550 mg) was added, followed by Et3N to keep the solution slightly alkaline. After 24 hr., the reaction mixture was diluted with 1N NH4OH (4 mL), stirred for 1 hr., and diluted with EtOAc (100 mL). The EtOAc solution was washed successively with 1N NH4OH, H2O, 1N HCl, and H2O. The EtOAc solution was dried over MgSO4 and concentrated to small volume in vacuum. Petroleum ether was added to the residue and the precipitate thereby formed was reprecipitated from AcOH, H2O, and a few drops of 50% NH4OAc, yield 55 mg. (53%) of crystals, m.p. 94~105°, [α]20° +2.7° (c=0.6, AcOH), Anal. Calcd. for C41H39O12N10: C, 58.96; H, 5.59;

N.-Benzyloxy carbonyl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-nitro-L-arginine p-Nitrobenzyi Ester (III)—The benzyloxy carbonyl group was removed from N-benzyloxy carbonyl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-nitro-L-arginine p-nitrobenzyi ester (600 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (6 ml.) N-benzyloxy carbonyl glycline p-nitrophenyl ester (220 mg.) was added, followed by Et₂N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH₄OH (2 ml.), stirred for 1 hr., and then mixed with EtOAc (60 ml.). The EtOAc solution was washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O. The EtOAc solution was dried over MgSO₄ 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 H₂O and a few drops of 50% NH₄OAc, yield 417 mg. (66%) of crystals, m.p. 96~103°C, [α]D ~ -15.0° (c=0.5, AcOH). Anal. Calcd. for C₃₅H₃₉NO₁₄: C, 58.00; H, 5.58; N, 15.50. Found: C, 57.79; H, 5.45; N, 15.31. Deblocked peptide ester : Rp 0.55, Rp 0.84, single ninhydrin positive spot.

N.-Benzyloxy carbonylsarcosine p-Nitrophenyl Ester (IV)—To a pre-cooled solution of N-benzyloxy carbonylsarcosine (3.6 g.) in EtOAc (40 ml.), p-nitrophenol (2.4 g.) was added, followed by N,N'-dicyclohexyl carbodiimide (3.6 g.). After 30 min. at 0°C 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 EtOAC; light yellow sticky oil; yield 3.1 g. (55%).

N.-Benzyloxy carbonylsarcosylglycl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-nitro-L-arginine p-Nitrobenzyi Ester Hemihydrate (V)—The benzyloxy carbonyl group was removed from the fully protected hexapeptide (II) (335 mg.) as described above. To a solution of this resulting HBr salt in dimethylformamide (4 ml.), N-benzyloxy carbonylsarcosine p-nitrophenyl ester (140 mg.) was added, followed by Et₂N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH₄OH (2 ml.), stirred for 1 hr., and then diluted with EtOAc (40 ml.). The EtOAc solution was washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O. The solution was dried over MgSO₄ and evaporated to dryness in vacuum. The residue was reprecipitated from acetone with ether. The precipitate thereby formed was reprecipitated from AcOH with H₂O and 50% NH₄OAc; yield 347 mg. (97%), m.p. 105~108°C, [α]D ~ 0.0° (c=0.7, AcOH). Anal. Calcd. for C₃₅H₃₉NO₁₄·½H₂O: C, 57.03; H, 5.73; N, 15.85. Found: C, 57.03; H, 5.85; N, 15.50. Deblocked peptide ester : Rp 0.63, Rp 0.83, single ninhydrin positive spot.

N.-Benzyloxy carbonyl-N'-nitro-L-arginyl-L-prolyl-N-l-sarcosylglycl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-nitro-L-arginine p-Nitrobenzyi Ester Monohydrate (VI)—The benzyloxy carbonyl group was removed from the fully protected heptapeptide (V) (255 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.), N,N'-benzyloxy carbonyl-N'-nitro-L-arginyl-L-proline p-nitrophenyl ester (164 mg.) was added, followed by Et₂N to keep the solution slightly alkaline. After 2 days at room temperature the reaction mixture was diluted with 1N NH₄OH (2 ml.), stirred for 1 hr., and then diluted with EtOAc (40 ml.). The EtOAc solution was washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O. The solution was dried over MgSO₄ and evaporated to dryness in vacuum. The residue was reprecipitated from acetone with ether. The precipitate thereby formed was reprecipitated from AcOH with H₂O and 50% NH₄OAc; yield 137 mg. (42%), m.p. 123~128°C, [α]D ~ -17.7° (c=0.3, AcOH). Anal. Calcd. for C₃₅H₃₉NO₁₄·H₂O: C, 53.90; H, 5.84; N, 18.35. Found: C, 54.10; H, 5.88; N, 18.33. Deblocked peptide ester : Rp 0.55, Rp 0.77, single ninhydrin positive spot.

L-Arginyl-L-prolyl-N-sarcosylglycl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-arginine Triacetate Salt (VII)—The fully protected nonapeptide (VII) (70 mg.) was hydrogenated in 1:1 mixture of AcOH and H₂O (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 H₂O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H₂O (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%), [α]D ~ -21.2° (c=0.7, H₂O). Rp 0.31, Rp 0.45, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate : Arg 1.90, Pro 1.97, Ser 0.96, Phe 2.00, Gly 2.03.

N.-Benzyloxy carbonylglycl-L-phenylalanine glycl-L-prolyl-L-phenylalanine N'-nitro-L-arginine L-Nitrobenzyi Ester (VIII)—The benzyloxy carbonyl 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-benzyloxy carbonyl glycline p-nitrophenyl ester (334 mg.) was added, followed by Et₂N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH₄OH (4 ml.), stirred for 1 hr., and then diluted with EtOAc (63 ml.). The EtOAc solution was washed successively with 1N NH₄OH, H₂O, 1N HCl, and H₂O. The solution was dried over MgSO₄, 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 H₂O and 50% NH₄OAc; yield 643 mg. (78%), m.p. 100~110°C.
[α]B 0.0° (c=0.5, AcOH). Anal. Calcd. for C9H8O4N3: C, 57.13; H, 5.56; N, 15.99. Found: C, 57.64; H, 6.02; N, 15.84. Deblocked peptide ester: Rf 0.70, Rf 0.87, single ninhydrin positive spot.

N'-Benzyloxy carbonyl-N'-nitro-L-arginyl-L-prolylglucyclglycyl-L-phenylalanlyglycyl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-Nitrobenzyl Ester Trihydrate (IX)—The benzyloxy carbonyl group was removed from the fully protected heptapeptide (VII) (300 mg) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 mL), N'-benzyloxy carbonyl-N'-nitro-L-arginyl-L-proline p-nitrophenyl ester (196 mg) was added, followed by Et3N to keep the solution slightly alkaline. After 2 days at room temperature, the reaction mixture was diluted with 1N NH4OH (2 mL), stirred for 1 hr, and then poured into 1N NH4H2O (50 mL) with stirring. The precipitate thereby formed was collected by filtration, and washed successively with 1N NH4OH, H2O, 1N HCl, and H2O. The precipitate was recrystallized from AcOH with H2O and 50% NH4OAc yield 241 mg (62%), m.p. 124~129°, [α]B 20.0° (c=0.4, AcOH). Anal. Calcd. for C9H8O4N3.H2O: C, 52.20; H, 5.89; N, 17.97. Found: C, 52.46; H, 5.82; N, 18.13. Deblocked peptide ester: Rf 0.59, Rf 0.84, single ninhydrin positive spot.

L-Arginyl-L-prolylglucyclglycyl-L-phenylalanlyglycyl-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 solution of the product in H2O (10 mL) was added to a (2.0×6.0 cm) CM-cellulose column which was eluted with a linear gradient method from H2O (300 mL) in a mixing chamber to 0.15M pyridinium acetate buffer (pH 5.1) (300 mL) in a reservoir. Fractions of 13 mL each were collected at a flow rate of 3 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%), [α]B 43.6° (c=0.8, H2O), Rf 0.30, Rf 0.50, single ninhydrin and Sakaguchi positive spot. Anal. Calcd. for C9H8O4N3.6CH3COOH.2H2O: C, 51.77; H, 6.94; N, 17.42. Found: C, 51.59; H, 6.83; N, 17.12.

N-Benzoxycarbonyl-L-alaninyglycine p-Nitrophenyl Ester (XI)—To a cooled solution of N-benzyloxy carbonyl-L-alaninyglycine (1.60 g) in EtOAc (18 mL) and tetrahydrofuran (5 mL) p-nitrophenol (0.88 g) was added, followed by N,N'-dicyclohexyl carbodiimide (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 reaction mixture was stirred for 20 min. The formed N,N'-dicyclohexylurea was filtered off and the filtrate was washed with 1N NaHCO3 and H2O. The EtOAc solution was dried over MgSO4, evaporated to dryness and the residue was recrystallized from EtOH; yield 11.5 g (50%) of needles, m.p. 179°, [α]B 33.7° (c=0.7, AcOH). Anal. Calcd. for C9H8O4N3: C, 56.86; H, 4.77; N, 10.47. Found: C, 56.86; H, 4.17; N, 10.75.

N-Benzoxycarbonyl-L-alaninyglycine p-Nitrophenyl Ester (XII)—The benzyloxy carbonyl group was removed from (VIII) (204 mg) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 mL), (X) (91 mg) was added, followed by Et3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH4H2O (0.5 mL), stirred for 1 hr., and then poured into cold 1N NH4H2O (30 mL) with stirring. To the suspension 50% NH4OAc (2 mL) was added with stirring and precipitate thereby formed was collected by filtration, washed successively with 1N NH4OH, H2O, 1N HCl, and H2O. The product was recrystallized from AcOH with H2O and 50% NH4OAc yield 144 mg (64%), m.p. 112~118°, [α]B 17.4° (c=0.2, AcOH). Anal. Calcd. for C9H8O4N3: C, 57.51; H, 5.68; N, 15.78. Found: C, 57.22; H, 5.48; N, 15.49. Deblocked peptide ester: Rf 0.66, Rf 0.87; single ninhydrin positive spot.

N'-Benzyloxy carbonyl-N'-nitro-L-arginyl-L-prolyl-L-alaninyglycine p-Nitrophenyl Ester (XIII)—The benzyloxy carbonyl group was removed from VIII (144 mg) as described above. To a solution of the resulting HBr salt in dimethylformamide (4 mL), N'-benzyloxy carbonyl-N'-nitro-L-arginyl-L-proline p-nitrophenyl ester (80 mg) was added, followed by Et3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH4H2O, stirred for 1 hr., and then poured into 1N NH4H2O (50 mL). The precipitate thereby formed was collected on filter and washed successively with 1N NH4H2O, H2O, 1N HCl, and H2O; yield 92 mg (49%). For analysis a sample was recrystallized from AcOH with H2O and 50% NH4OAc, m.p. 128~134°, [α]B 71.4° (c=0.04, AcOH). Anal. Calcd. for C9H8O4N3: C, 54.62; H, 5.77; N, 18.49. Found: C, 54.30; H, 5.34; N, 18.67. Deblocked peptide ester: Rf 0.70, Rf 0.82; single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-alaninyglycine p-Nitrophenyl Ester (XIV)—The fully protected nonapeptide (XIII) (62 mg) was hydrogenated as described above. The hydrogenated product in H2O (10 mL) was added to a (2.0×6.0 cm) CM-cellulose column which was eluted with a linear gradient method from H2O (300 mL) in a mixing chamber to 0.1M NH4OAc (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 mp. The eluate in tubes No. 24 to 33 containing the nonapeptide were pooled, evaporated to dryness, and lyophilized. NH4OAc was removed by repeated lyophilization to constant weight; colorless fluffy material, yield 31 mg (57%), [α]B 54.5° (c= 0.6, H2O), Rf 0.27, Rf 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-Benzylxocarboxylglycylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-Nitrobenzyl Ester (XV) —— The benzylxocarboxyl group was removed from N-benzylxocarboxyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-nitrobenzyl ester (361 mg.) as described above. To a solution of the resulting HBr salt in dimethylformamide (3 ml.) N-benzylxocarboxylglycylglycyl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-nitrophenyl ester (159 mg.) was added, followed by Et3N to keep the solution slightly alkaline. After 24 hr., at room temperature the reaction mixture was diluted with 1N NH4OH (2 ml.), stirred for 1 hr., and then poured into cold 1N NH4OH (40 ml.) with stirring. To the suspension, 50% NH4OAc (2 ml.) was added with stirring and the precipitate was filtered and washed with 1N NH4OH, H2O, 1N HCl, and H2O. The product was reprecipitated from AcOH, H2O and a few drops of 50% NH4OAc; yield 282 mg., (67%) of crystals, m.p. 104~112°. [α]D 0.9° (c=1.1, AcOH), Anal. Calcd. for C35H39O13N13: C, 56.22; H, 5.52; N, 14.85. Found: C, 56.62; H, 5.81; N, 14.77. Deplobled peptide ester: Rf 0.63, Rf 0.85, single ninhydrin positive spot.

N'-Benzylxocarboxyl-N'-nitro-L-arginyi-L-prolylglcylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanin-L-arginine p-Nitrobenzyl Ester (XVI) —— The benzylxocarboxyl 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'-benzy1xocarboxyl-L'-nitro-L-arginyl-L-prolyl-L-phenylalanyl-N'-nitro-L-arginine p-nitrophenyl ester (156 mg.) was added, followed by Et3N to keep the solution slightly alkaline. After 2 days at room temperature, the reaction mixture was diluted with 1N NH4OH (2 ml.), stirred for 1 hr., and then poured into cold 1N NH4OH (40 ml.) with stirring. To the suspension, 50% NH4OAc (2 ml.) was added with stirring and the precipitate was filtered and washed successively with 1N NH4OH, H2O, 1N HCl, and H2O. The product was reprecipitated from AcOH with H2O and a few drops of 50% NH4OAc; yield 160 mg. (45%) of crystals, m.p. 124~132°, [α]D 0.6° (c=1.0, AcOH), Anal. Calcd. for C34H38O13N13: C, 54.08; H, 5.67; N, 17.74. Found: C, 53.69; H, 5.27; N, 17.93. Deplobled peptide ester: Rf 0.84, Rf 0.81, single ninhydrin positive spot.

L-Arginyl-L-prolylglcylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanin-L-arginine Triacetate (XVII) —— The fully protected nonapeptide (XVI) (70 mg.) was hydrogenated in 1:1 mixture of AcOH and H2O (15 ml.) for 48 hr. over 10% Pd-C (30 mg.). Fresh catalyst was added during the hydorgenation. The catalyst was removed by the aid of CelIite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH in vacuum. The solution of the product in H2O (10 ml.) was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H2O (330 ml.) in a mixing chamber to 0.1M NH4OAc 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 mp. The eluate in tubes No. 26 to 32 containing the nonapeptide were pooled, evaporated to dryness in vacuum and lyophilized. NH4OAc was removed by repeated lyophilization to constant weight; colorless fluffy material, yield 43 mg. (69%), [α]D 30.0° (c=0.4, H2O), Rf 0.32, Rf 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-prolylglcylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanin-L-arginine Triacetate (XVIII) —— 3-Glycine-6-O-acetyl-L-serine-bradykinin (XVII) (20 mg.) in H2O (0.3 ml.) was saponified with 1N NaOH (0.2 ml.) for 1 hr. The solution neutralized with 1N AcOH was added to a column (2.0×6.0 cm.) of CM-cellulose which was eluted with a linear gradient method from H2O (360 ml.) in a mixing chamber to 0.1M NH4OAc 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 mp. The eluate in tubes No. 28 to 34 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. NH4OAc was removed by repeated lyophilization to constant weight; colorless fluffy material; yield 14 mg. (75%), [α]D 41.2° (c=0.3, H2O), Rf 0.21, Rf 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.