Method C. Reaction of XV with Thionyl Chloride——Into a solution of 5 ml. of ligroin and 5 ml. of $CHCl_3$, 0.5 g. of XV was suspended. To this mixture 0.3 g. of $SOCl_2$ was added and stirred on a water bath (60°) for 5 hr. Water was added and extracted with $CHCl_3$, the extracts were washed with 10% Na_2CO_3 and then with water, dried over anhyd. Na_2SO_4, and $CHCl_3$ was evaporated to obtain brown solid, which was recrystallized from EtOH. m.p. 175~177°. Yield 0.31 g. This compound was confirmed to be identical with a sample obtained by the method of Schuftan, by the mixed melting point determination.

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Summary

As a part of studies on syntheses of pyrazolone derivatives, syntheses of 1-phenyl-2-methyl-3(or 4)-[(5-substituted-2,4,6-trioxohexahydro-5-pyrimidinyl)methyl]-4(or 3)-substituted-3-pyrazolin-5-one were described.

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31. Kenji Suzuki, Mariko Asaka, and Takashi Abiko: Synthesis of 6-L-Leucine, 6-O-Acetyl-L-threonine, and 6-L-Threonine-bradykinin.*1

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A number of reports have appeared on the synthesis of bradykinin homologs substituted with the other amino acids in place of L-serine. The present writers synthesized 6-L-leucine, 6-O-acetyl-L-threonine, and 6-L-threonine-bradykinin, and their biological activity was examined.

During the progress of the present work, de Wald[1] and Stewart[2] reported the synthesis of 6-L-threonine but their method was different from that used in the present work which enables concurrent synthesis of 6-O-acetyl-L-threonine-bradykinin and it becomes possible to examine the biological activity of the O-acetyl compound as well.

The synthetic route for 6-L-leucine-bradykinin is illustrated in Chart 1. N-Benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester[3] is debenzyloxycarbonylated with hydrogen bromide-acetic acid solution and L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester thereby formed was reacted with N-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester[4] to form N-benzyloxycarbonyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrophenyl ester (I). The tetrapeptide ester obtained by the liberation of benzyloxycarbonyl group from I was

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*1 Nomenclature of bradykinin homologs and abbreviation of amino acids 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.

*2 Nankozawa, Sendai (鈴木謙次, 安積茉莉子, 安孫子 敬).

1) H. A. de Wald, M. K. Craft, E. D. Nicolaides: J. Med. Chem., **6**, 741 (1963).
2) J. M. Stewart, D. W. Woolley: Biochemistry, **3**, 700 (1964).
3) K. Suzuki, T. Abiko, M. Asaka: This Bulletin, **14**, 217 (1966).
4) M. Bodanszky, V. Du Vigneaud: J. Am. Chem. Soc., **81**, 5688 (1959).

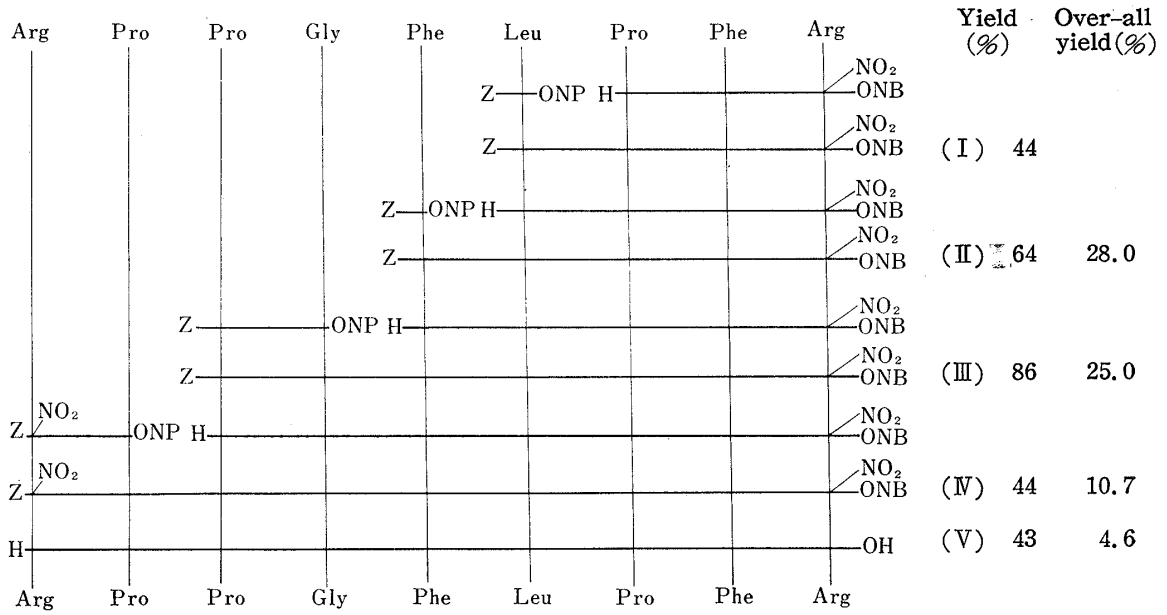

Chart 1. Synthesis of 6-L-Leucine-bradykinin

condensed with N-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester[5] to obtain N-benzyloxycarbonyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (II). The pentapeptide ester obtained by the liberation of benzyloxycarbonyl group from II was condensed with N-benzyloxycarbonyl-L-prolylglycine *p*-nitrophenyl ester[6] to form N-benzyloxycarbonyl-L-prolylglycyl-L-phenylalanyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (III). The heptapeptide ester obtained by liberation of benzyloxycarbonyl group from III was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester[6] to obtain N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (IV). The protected nonapeptide (IV) was reduced over 10% palladium-carbon in acetic acid solution during 48 hours and the reduction product was purified through column chromatography using carboxymethyl (CM)-cellulose to obtain 6-L-leucine-bradykinin, *i.e.*, L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-L-arginine triacetate (V). The nonapeptide (V) so obtained was found to be a unity from the result of paper chromatography using two different solvent systems. The ratio of amino acid in the acid hydrolysate of V agreed with theoretical value.

For the synthesis of 6-L-threonine-bradykinin, the following series of reactions was carried out. The benzyloxycarbonyl group in N-benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester was removed by treatment with hydrogen bromide-acetic acid solution and the tripeptide ester thereby formed was condensed with N-benzyloxycarbonyl-L-threonine[7] in methylene chloride by the N,N'-dicyclohexylcarbodiimide method[8] to form N-benzyloxycarbonyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VI). The benzyloxycarbonyl group in VI was removed by treatment with hydrogen bromide-acetic acid solution and

5) M. Bodanszky, V. Du Vigneaud: J. Am. Chem. Soc., **81**, 6072 (1959).
6) M. A. Ondetti: J. Med. Chem., **6**, 10 (1963).
7) J. P. Greenstein, M. Winitz: "Chemistry of the Amino Acids," **11**, 895 (1961). John Wiley & Sons, Inc., New York.
8) J. C. Sheehan, C. P. Hess: J. Am. Chem. Soc., **77**, 1067 (1955).

O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester so formed was condensed with N-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester to form N-benzyloxycarbonyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VII). The pentapeptide ester formed by liberation of benzyloxycarbonyl group from VII was condensed with N-benzyloxycarbonyl-L-prolylglycine *p*-nitrophenyl ester[6] to obtain N-benzyloxycarbonyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VIII). The benzyloxycarbonyl group in VIII was removed and the heptapeptide ester so obtained was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester[6] to obtain N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (IX). The protected nonapeptide (IX) was submitted to catalytic reduction over 10% palladium carbon in acetic acid solution during 48 hours and the reduction product was purified through CM-cellulose column to obtain 6-O-acetyl-L-threonine-bradykinin triacetate (X). The nonapeptide (X) so obtained was found to be a unity from the result of paper chromatography using two different solvent systems. Determination of the acetyl-ester group by the hydroxamic acid method[9] was 46.3% of the theoretical value and ratio of amino acids in the acid hydrolysate agreed well with the theoretical value.

Saponification of 6-O-acetyl-L-threonine-bradykinin (X) with 1*N* sodium hydroxide solution afforded 6-L-threonine-bradykinin[1,2] (XI). The nonapeptide here obtained was found to be a unity from the result of paper chromatography using two different solvent systems and the ratio of amino acids in the acid hydrolysate agreed well with the theoretical value.

Quantitative examinations were made on the bradykinin-like activity, antibradykinin action, and potenciation of bradykinin activity of the nonapeptides synthesized in the present work.*[8] Result of these biological examination is given in Table I.

Table I. Biological Activities of Synthesized Nonapeptides[a]

	Bradykinin-like activity	Bradykinin potentiating activity	Antibradykinin activity
Bradykinin	1		
6-O-Acetyl-L-threonine-bradykinin (V)	4.5/10	—	—
6-L-Threonine-bradykinin (VI)	7/10	—	—
6-L-Leucine-bradykinin (XI)	1/1000	—	—

a) Assayed by Magnus method on a mouse ileum (male).

The bradykinin-like activity of 6-L-threonine (XI) and 6-O-acetyl-L-threonine-bradykinin (X) is lower than bradykinin but the activity is fairly apparent. The fact that the activity of the O-acetyl derivative (X) is lower than that of 6-L-threonine-bradykinin (XI) with free hydroxyl is similar to the relationship between bradykinin and 6-O-acetyl-L-serine-bradykinin reported in the previous paper. The bradykinin-like activity was markedly low in 6-L-leucine-bradykinin (V) in which L-serine, which does not take the α-helix structure, was substituted with L-leucine,[10] which does take the α-helix structure and which has a bulky side chain. None of the three bradykinin homologs showed antibradykinin activity or potentiation of bradykinin activity.

* Details of the biological assay will be reported in a separate paper by Dr. Tsutomu Kameyama

9) S. Hestrin: J. Biol. Chem., **180**, 249 (1949).

10) E.R. Blout: "Polyamino Acids, Polypeptides and Proteins," Ed. M. Stahmann, 275 (1962). University of Wisconsin Press, U.S.A.

Experimental

All melting points are uncorrected. For paper chromatography, the protected amino acids and peptides were deblocked with HBr in AcOH and the resulting hydrobromides were chromatographed on filter paper, Toyo Roshi No. 51, at room temperature. Rf^1 values refer to the Partridge system[11] and Rf^2 values to the system of BuOH–pyridine–AcOH–H_2O (30:20:6:24).[12] All the benzyloxycarbonylamino acids for the intermediates, with the exception of N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginine due to its low yield, were prepared by the use of $NaHCO_3$ instead of NaOH in the Schotten-Baumann reaction.[7] The amino acid composition of the acid hydrolysates was determined according to the directions given by Moore, *et al.*[13]

N-Benzyloxycarbonyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (I)——N-Benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (2.40 g.) was dissolved in AcOH (4 ml.) and 5.7*N* HBr in AcOH (4 ml.). After 45 min. at room temperature, the solvent was evaporated to small volume and the residue was shaken vigorously with dehyd. ether. The precipitate thereby formed was collected, washed with dehyd. ether, and dried over KOH in vacuum. To a solution of this product in dimethylformamide (17 ml.), N-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester (1.40 g.) 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 (5 ml.), stirred for 1 hr., and mixed with AcOEt (150 ml.). The AcOEt 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 concentrated to a small volume. The residue was kept in a refrigerator and the precipitate thereby formed was collected by filtration and recrystallized from AcOEt to 1.2 g. (44%) of crystals, m.p. 111~113°, $[\alpha]_D^{18}$ −46.0° (c=1.39, AcOH). *Anal.* Calcd. for $C_{41}H_{51}O_{11}N_9$: C, 58.21; H, 6.08; N, 14.90. Found: C, 58.09; H, 6.19; N, 14.48. Deblocked peptide ester: Rf^1 0.76, Rf^2 0.92; single ninhydrin-positive spot.

N-Benzyloxycarbonyl-L-phenylanyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (II)——The protected tetrapeptide ester (I) (1.20 g.) was dissolved in AcOH (8 ml.) and 5.7*N* HBr in AcOH (8 ml.). After 50 min. at room temperature, the solvent was evaporated to a small volume and the residue was shaken vigorously with dehyd. ether. The precipitate thereby formed was collected, washed with dehyd. ether, and dried over KOH in vacuum. To a solution of this product in dimethylformamide (15 ml.), N-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (0.70 g.) was added, followed by Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted 1*N* NH_4OH (3 ml.), stirred for 1 hr., and mixed with AcOEt (150 ml.). The AcOEt 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 concentrated to a small volume. Petroleum ether was added to the residue and the precipitate was recrystallized from AcOEt to 0.9 g. (64%) of crystals, m.p. 132~134°, $[\alpha]_D^{19}$ −39.0° (c=0.71, AcOH). *Anal.* Calcd. for $C_{50}H_{60}O_{12}N_{10}$: C, 60.47; H, 6.09; N, 14.11. Found: C, 60.38; H, 6.07; N, 13.91. Deblocked peptide ester: Rf^1 0.81, Rf^2 0.98; single ninhydrin-positive spot.

N-Benzyloxycarbonyl-L-prolylglycyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (III)——The protected pentapeptide ester (II) (0.90 g.) was dissolved in AcOH (5 ml.) and 5.7*N* HBr in AcOH (5 ml.). After 1 hr. at room temperature, the solvent was evaporated to a small volume and the residue was shaken vigorously with dehyd. ether. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (10 ml.), N-benzyloxycarbonyl-L-prolylglycine *p*-nitrophenyl ester (0.46 g.) 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 mixed with AcOEt (150 ml.). The AcOEt 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 concentrated to a small volume. Addition of petroleum ether to this residue resulted in precipitation. Yield, 0.9 g. (86%) of crystals, m.p. 109~112°, $[\alpha]_D^{19}$ −12.9° (c=0.9, AcOH). *Anal.* Calcd. for $C_{57}H_{70}O_{14}N_{12} \cdot H_2O$: C, 58.75; H, 6.23; N, 14.43. Found: C, 58.83; H, 6.44; N, 14.64. Deblocked peptide ester: Rf^1 0.77, Rf^2 0.95; single ninhydrin-positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (IV)——The protected heptapeptide ester (III) (230 mg.) was dissolved in AcOH (2 ml.) and 5.7*N* HBr in AcOH (2 ml.). After 50 min. at room temperature, dehyd. ether was added and the residue was shaken vigorously. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (3 ml.) N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (115 mg.) was added, followed by Et_3N to keep the solution slightly alkaline. After 2 days, at room temperature,

11) S. M. Partridge: Biochem. J., **42**, 238 (1948).
12) S. G. Waley, G. Watson: *Ibid.*, **55**, 328 (1953).
13) S. Moore, D. H. Spakman, W. H. Stein: Anal. Chem., **30**, 1185 (1958).

the reaction mixture was diluted with 1*N* NH_4OH (1 ml.), stirred for 1 hr., and diluted with AcOEt. The AcOEt solution was washed successively with 1*N* NH_4OH and H_2O. AcOH was added to the AcOEt solution to prevent some precipitate and the mixture was washed with 1*N* HCl and H_2O. The AcOEt solution was dried over $MgSO_4$ and concentrated to a small volume. Petroleum ether was added to the residue and the precipitate was recrystallized from AcOH, H_2O, and 50% $AcONH_4$. Yield, 135 mg. (44%) of crystals, m.p. 128~131°, $[\alpha]_D^{21}$ −38.1° (c=0.64, AcOH). *Anal.* Calcd. for $C_{68}H_{88}O_{18}N_{18}$: C, 56.50; H, 6.14; N, 17.44. Found: C, 56.84; H, 6.40; N, 16.43. Deblocked peptide ester: Rf^1 0.64, Rf^2 0.88; single ninhydrin-positive spot.

L-Arginyl-L-prolylglycyl-L-phenylalanyl-L-leucyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (V)——The fully protected nonapeptide (IV) (100 mg.) was hydrogenated in 10:5 mixture of AcOH and H_2O (15 ml.) for 48 hr. in the presence of 10% Pd-C (30 mg.). Fresh catalyst was added during the hydrogenation. 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 crude 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 mixing chamber to 0.1*M* $AcONH_4$ buffer (pH 6.50) (300 ml.) in 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. 27~36 containing the nonapeptide were pooled, evaporated to dryness in vacuum and lyophilized. $AcONH_4$ was removed by repeated lyophilization to constant weight. Colorless fluffy material; yield, 40.5 mg. (43%) of crystals, $[\alpha]_D^{21}$ −85.6° (c=1.0, H_2O). Rf^1 0.53, Rf^2 0.68; single ninhydrin and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate: Arg 2.05, Pro 2.90, Gly 0.95, Phe 2.05, Leu 1.10 (average recorvery, 91%).

N-Benzyloxycarbonyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (VI)——N-Benzyloxycarbonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (2.00 g.) was dissolved in AcOH (5 ml.) and 5.7*N* HBr in AcOH (5 ml.). After 40 min. at room temperature, the solvent was evaporated to a small volume and the residue was shaken vigorously with dehyd. ether. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (3 ml.) and CH_2Cl_2 (10 ml.), Et_3N was added to keep the solution slightly alkaline. After 30 min., the solution was cooled and N-benzyloxycarbonyl-L-threonine (0.75 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (0.65 g.). The reaction mixture was stirred in the cold overnight and the formed N,N′-dicyclohexylurea was filtered off. The filtrate was concentrated in vacuum and the residue was diluted with AcOEt (80 ml.). The AcOEt solution was washed successively with 1*N* HCl, H_2O, 1*N* NH_4OH, and H_2O. The solution was dried over $MgSO_4$ and concentrated to a small volume and petroleum ether was added to the residue. The precipitate was reprecipitated from MeOH and ether. Yield, 1.6 g. (68%) of crystals, m.p. 105~110°, $[\alpha]_D^{18}$ −46.8° (c=1.0, AcOH), *Anal.* Calcd. for $C_{39}H_{47}O_{12}N_9$: C, 56.17; H, 5.68; N, 15.12. Found: C, 55.63; H, 5.42; N, 15.02. Deblocked peptide ester: Rf^1 0.67, Rf^2 0.86, single ninhydrin-positive spot.

N-Benzyloxycarbonyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (VII)——The protected tetrapeptide (VI) (1.40 g.) was dissolved in AcOH (3 ml.) and 5.7*N* HBr in AcOH (3 ml.). After 40 min. at room temperature, the solvent was evaporated to a small volume and the residue was shaken vigorously with dehyd. ether. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (10 ml.) N-benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester (0.75 g.) 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 mixed with AcOEt (100 ml.). The AcOEt 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 concentrated to a small volume and ether was added to the residue. Yield, 1.10 g. (72%) of crystals, m.p. 100° (sint. 106~110°), $[\alpha]_D^{27}$ −46.8° (c=0.64, AcOH). For analysis, a sample was reprecipitated from AcOH, H_2O, and a few drops of conc. $AcONH_4$. *Anal.* Calcd. for $C_{50}H_{58}O_{14}N_{10}$: C, 58.70; H, 5.73; N, 13.69. Found: C, 58.65; H, 5.91; N, 14.29. Deblocked peptide ester: Rf^1 0.76, Rf^2 0.85; single ninhydrin-positive spot.

N-Benzyloxycarbonyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (VIII)——The protected pentapeptide ester (VII) (0.90 g.) was dissolved in AcOH (2 ml.) and 5.7*N* HBr in AcOH (2 ml.). After 1 hr. at room temperature, dehyd. ether (40 ml.) was added and the residue was shaken vigorously. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (9 ml.) N-benzyloxycarbonyl-L-prolylglycine *p*-nitrophenyl ester (0.44 g.) 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 (3 ml.), stirred for 1 hr., and mixed with AcOEt (70 ml.). The AcOEt solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The AcOEt solution was dried over $MgSO_4$ and concentrated to a small volume and petroleum ether was added. Yield, 0.79 g. (77%) of crystals, m.p. 144~150°. Deblocked peptide ester: Rf^1 0.80, Rf^2 0.88; single ninhydrin-positive spot. For analysis

a sample was reprecipitated from MeOH, H_2O, and a few drops of conc. $AcONH_4$. m.p. 118~122°, $[\alpha]_D^{19}$ −40.0°(c=1.0, AcOH). *Anal.* Calcd. for $C_{57}H_{68}O_{16}N_{12}$: C, 58.15; H, 5.82; N, 14.28. Found : C, 57.53; H, 5.74; N, 14.37.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (IX)——The protected heptapeptide ester (Ⅷ)(215 mg.) was dissolved in AcOH (0.7 ml.) and 5.7*N* HBr in AcOH (0.7 ml.). After 40 min. at room temperature, dehyd. ether was added and the residue was shaken vigorously. The precipitate was collected and washed with dehyd. ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (6 ml.), N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (120 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 (1 ml.), stirred for 1 hr., and mixed with AcOEt (80 ml.). The AcOEt solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The AcOEt solution was dried over $MgSO_4$ and concentrated to a small volume. The crystalline residue was reprecipitated from dimethylformamide, H_2O, and a few drops of conc. $AcONH_4$. Yield, 155 mg.(57%) of crystals, m.p. 116~128°, $[\alpha]_D^{16}$ −57.0°(c=0.89, AcOH). *Anal.* Calcd. for $C_{68}H_{86}O_{20}N_{18}$: C, 55.35; H, 5.88; N, 17.09. Found : C, 55.47; H, 5.87; N, 15.99. Deblocked peptide ester : Rf^1 0.61, Rf^2 0.73; single ninhydrin-positive spot.

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (X)——The fully protected nonapeptide (Ⅸ)(120 mg.) was hydrogenated in 2:1 mixture of AcOH and H_2O (15 ml.) for 48 hr. in the presence of 10% Pd-C (20 mg.). Fresh catalyst was added during the hydrogenation. 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. Analysis by paper chromatography revealed the presence of major ninhydrin-positive spot at Rf^1 0.28 and Rf^2 0.44, and minor spots at Rf^1 0.12, 0.39, 0.69, 0.89, and Rf^2 0.23, 0.58, 0.97. The spots at Rf^1 0.28, 0.69, and Rf^2 0.44, 0.58 were Sakaguchi-positive. A solution of the crude product in H_2O (10 ml.) was added to a column (2.0×6.0 cm.) of CM-cellulose which was eluted with a linear gradient elution from 0.01*M* $AcONH_4$ buffer (pH 6.50) (300 ml.) to 0.1*M* $AcONH_4$ buffer (pH 6.50)(300 ml.). Fractions of 10 ml. each were collected at a flow rate of 3 to 4 ml./min. with an automatic fraction collector and absorbancy of each fraction was determined at 230 mμ. The eluate in tubes No. 29~39 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. $AcONH_4$ was removed by repeated lyophilization to constant weight; Colorless fluffy material; yield, 54 mg. (52%) of crystals, m.p. 172~182°, $[\alpha]_D^{19}$ −76.0° (c=0.93, H_2O). Rf^1 0.36, Rf^2 0.41 ; single ninhydrin and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate : Arg 2.00, Pro 2.90, Gly 1.00, Phe 2.01, Thr 0.90 (average recorvery, 91%).

L-Arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XI)——6-O-Acetyl-L-threonine-bradykinin (X) (25 mg.) in H_2O (0.2 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 (300 ml.) in a mixing chamber to 0.1*M* $AcONH_4$ buffer (pH 6.50)(300 ml.) in the 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 elute in tubes No. 31~38 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. $AcONH_4$ was removed by repeated lyophilization to constant weight. Colorless fluffy material; yield, 17.0 mg.(70%) of crystals, $[\alpha]_D^{22}$ −78.2° (c=0.7, H_2O), (reported[1] $[\alpha]_D^{23}$ −85.5°(c=1.34, H_2O)). Rf^1 0.23, Rf^2 0.47; single ninhydrin and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate : Arg 2.10, Pro 2.91, Gly 1.00, Phe 2.00, Thr 0.92 (average recorvery, 93%).

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Summary

The three analogs of bradykinin is described in which the L-serine 6-position has been substituted with L-leucine, O-acetyl-L-threonine, and L-threonine. The biological activity of these analogs were compared with that of bradykinin.

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