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193. Kenji Suzuki and Takashi Abiko*1 : Synthesis of 4-L-Valine-6-L-Threonine-, 4-L-Isoleucine-6-L-Threonine-, 4-L-Leucine-6-L-Threonine-, 4-L-Valine-, and 4-L-Isoleucine-bradykinin and Their O-Acetyl Compounds.*2

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The synthesis of the analogs of bradykinin listed in the title are described in which glycine residue in 4-position of bradykinin, 6-L-threonine-bradykinin, and their 6-O-acetyl compound is substituted for another amino acid residues having bulky side chain. The biological activity of the ten analogs is compared with that of bradykinin on an isolated guinea pig ileum.

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In the assay on isolated mouse ileum, the weak anti-bradykinin activity of the synthetic 4-L-leucine- and 4-L-leucine-6-O-acetyl-L-serine bradykinin have been reported in a previous paper.[1] Similary, it was reported by Stewart, *et al.*[2] that 5-L-leucine-6-L-threonine-8-L-leucine-bradykinin, L-prolyl-L-prolylglycyl-L-phenylalanyl-L-threonyl-L-prolyl-L-leucyl-L-arginine, and their esters of the C-terminal carboxyl group showed anti-bradykinin activity at a limited concentration in the assay on isolated rat uterus. Also, it was reported by the same authors[3,4] that 5-O-methyl-L-tyrosine-8-O-methyl-L-tyrosine-bradykinin showed anti-bradykinin activity. These peptides described above are the only few peptides showing some anti-bradykinin activity among the over one hundred analogs which have been reviewed by Schröder, *et al.*[5] In view of these results it was of interest to examine further the biological activity of bradykinin analogs which were substituted for amino acids having bulky and hydrophobic side chains.

In the present paper, the synthesis and the results of biological assay of 4-L-valine (V), 4-L-isoleucine (X), and 4-L-leucine (XV) analogs of 6-L-threonine-bradykinin and 4-L-valine-(XX) and 4-L-isoleucine-bradykinin (XXV) and their O-acetyl analogs of L-threonine and L-serine residues of the peptides are described. The method of peptide synthesis used here was virtually similar with a previous paper on the synthesis of bradykinin and its analogs.[1]

The synthetic route for the nonapeptide (V) is illustrated in Fig. 1. N-Benzyloxycarbonyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester[6] was debenzyloxycarbonylated with a hydrogen bromide-acetic acid solution in the presence of anisole and the resulting pentapeptide ester was condensed

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

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2) J. M. Stewart, D. W. Woolley : Biochemistry, **3**, 700 (1965).
3) *Idem* : International Symposium on Hypotensive Peptide, Florence, Oct. 25~29, Abstracts of papers p. 1399 (1965); Ed. E. G. Erdös, N. Back, F. Sicuteri : Hypotensive Peptides, Proceedings of the International Symposium Oct. 25~29, 1965. Florence, Italy, p. 23 (1966). Springer-Verlag New York Inc.
4) *Idem* : Federation Proceeding, **24**, (2), Part 1, 657 (1965).
5) E. Schröder, K. Lübke : The peptides," Vol. II, 65 (1966). Academic Press, N. Y. and London.
6) K. Suzuki, M. Asaka, T. Abiko : This Bulletin, **14**, 211 (1966).

with N-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester[7] to yield N-benzyloxycarbonyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (I). After the removal of the benzyloxycarbonyl group of I, the resulting hexapeptide ester was condensed with N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester[8] to yield N-benzyloxycarbonyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (II). After the removal of the benzyloxycarbonyl group of II, the resulting heptapeptide ester was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester[9] to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (III). The fully protected nonapeptide (III) was hydrogenated in aqueous acetic acid over 10% palladium on charcoal for 2 days. The hydrogenated product was purified through a carboxymethyl (CM-) cellulose column to obtain 4-L-valine-6-O-acetyl-L-threonine-bradykinin triacetate, L-arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine triacetate (IV). The nonapeptide (IV) so obtained was found to be homogeneous from the result of paper chromatography using two different solvent systems. Determination of the acetyl-ester group by the hydroxamic acid method[10] was 94.0% of the theoretical value and the ratio of amino acids in the acid hydrolysate agreed well with the theoretical value. Saponification of the O-acetyl nonapeptide (IV) with a 1*N* sodium hydroxide solution yielded 4-L-valine-6-L-threonine-bradykinin triacetate (V). The nonapeptide (V) so obtained was found to be homogeneous from the result of paper chromatography using two solvent systems and the ratio of amino acids agreed well with the theoretical value.

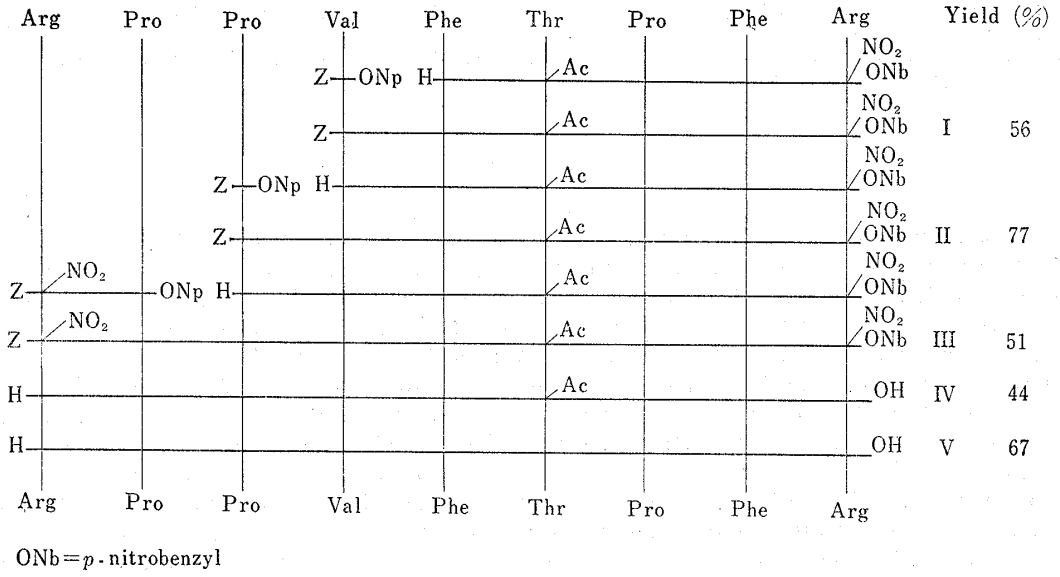

Fig. 1. Synthesis of 4-L-Valine-6-L-threonin-bradykinin

The other four nonapeptides, *i.e.* X, XV, XX, and XXV and their O-acetyl compounds were prepared essentially in a similar approach that had led to the formation of V.

Quantitative examinations were made on the bradykinin-like activity, anti-bradykinin activity, and potentiation of bradykinin activity of the nonapeptides synthesized in the

7) B. Iselin, W. Rittel, P. Sieber, R. Schwyzer: Helv. Chim. Acta, **40**, 373 (1957).
8) M. Bodanszky, V. du Vigneaud: J. Am. Chem. Soc., **81**, 5688 (1959).
9) M. A. Ondetti: J. Med. Chem., **6**, 10 (1963).
10) S. Hestrin: J. Biol. Chem., **180**, 249 (1949).

present work.*3 The result of these biological examinations is given in Table I. All of the synthesized nonapeptides and their O-acetyl compounds showed practically no bradykinin-like activity on an isolated guinea pig ileum as in the case of bradykinin analogs substituted at 4 position of bradykinin for L-alanine,[11] L-proline, sarcosine, and L-serine[12] and showed no anti-bradykinin activity as well. The synthesized 4-L-valine-6-threonin-(V), 4-L-leucine-6-L-threonine-(XV), and 4-L-leucine-6-O-acetyl-L-threonine-bradykinin (XIV), showed an effect of potentiation of bradykinin activity.

Table I. Biological Activities of Synthetic Nonapeptides[a]

	Bradykinin-like activity	Bradykinin potentiating activity	Anti-bradykinin activity
Bradykinin	100		
4-L-Valine-6-O-acetyl-L-threonine-bradykinin (IV)	0.2	—	—
4-L-Valine-6-threonine-bradykinin (V)	0.2	+[b]	—
4-L-Isoleucine-6-O-acetyl-L-threonine-bradykinin (IX)	0.1	—	—
4-L-Isoleucine-6-L-threonine-bradykinin (X)	0.1～0.3	—	—
4-L-Leucine-6-O-acetyl-L-threonine-bradykinin (XV)	0	+[c]	—
4-L-Leucine-6-L-threonine-bradykinin (XIV)	0.4	+[d]	—
4-L-Valine-6-O-acetyl-L-serine-bradykinin (XIX)	0	—	—
4-L-Valine-bradykinin (XX)	0.2	—	—
4-L-Isoleucine-6-O-acetyl-L-serine-bradykinin (XXIV)	0.3	—	—
4-L-Isoleucine-bradykinin (XXV)	0.1	—	—

a) Assayed by Magnus method on a guinea pig ileum (male).
b) At a concentration of 1×10^{-6} g./ml. (in bath), caused 60% potentiation of the normal contraction due to 1×10^{-8} g./ml. of bradykinin.
c) At a concentration of 1×10^{-8} g./ml., caused 50% potentiation of normal contraction due to 1×10^{-8} g./ml. of bradykinin.
d) At a concentration of 1×10^{-6} g./ml. caused 100% potentiation of the normal contration due to 1×10^{-8} g./ml. of bradykinin.

Experimental

Melting points are uncorrected. For paper chromatography, the protected peptides were deblocked with HBr in AcOH unless otherwise mentioned and the resulting hydrobromides were chromatographed on a fiter paper, Toyo Roshi No. 51, at room temperature. Rf^1 value refer to the Partridge system,[13] and Rf^2 value refer to the system of BuOH–pyridine–AcOH–H_2O (30:20:6:24).[14] The amino acid composition of the acid hydrolysates was determined according to the directions given by Moore, *et al.*[15]

N-Benzyloxycarbonyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (I)——N-Benzyloxycarbonyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N$^{\omega}$-nitro-L-arginine *p*-nitrobenzyl ester (661 mg.) was dissolved in AcOH (2 ml.), anisole (0.2 ml.), and 4.8*N* HBr in AcOH (2 ml.). After 50 min. at room temperature, the reaction mixture was shaken vigorously with dry ether. The precipitate thereby formed was washed with dry ether and dried over KOH pellets in vacuum. To a solution of the resulting peptide ester HBr salt in dimethylformamide (7 ml.) was added, N-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester (254 mg.) followed by addition of Et_3N to keep the solution slightly alkaline. After 24 hr. at room temperture, the reaction mixuture was diluted with 1*N* NH_4OH (3 ml.), stirred for 1 hr., and mixed with EtOAc (90 ml.). The EtOAc solution was washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O, and dried over $MgSO_4$. The solution was concentrated to a small volume and petroleum ether was added to the residue. The precipitate thereby formed was reprecipitated from acetone with ether; yield 406 mg. (56%), m.p. 115～121°, $[\alpha]_D^{15}$ −33.6°(c=0.7, AcOH). *Anal.* Calcd.

*3 The details of the biological assays will be reported in separate paper by Prof. T. Kameyama, *et al.* of this college.

11) E. Schröder, H. S. Petras, E. Klieger: Ann., **679**, 221 (1964).
12) E. Sehröder, R. Hompel: Experientia, **20**, 529 (1964).
13) S. M. Partridge: Biochem. J., **42**, 238 (1948).
14) S. G. Waley, G. Watson: *Ibid.*, **55**, 328 (1953).
15) S. Moore, D. H. Spackman, W. H. Stein: Anal. Chem., **30**, 1185 (1958).

for $C_{55}H_{67}O_{15}N_{11}$: C, 58.86; H, 6.02; N, 13.73. Found: C, 58.65; H, 6.54; N, 13.60. The deblocked peptide ester: Rf^1 0.81, Rf^2 0.94; single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Dihydrate (II)——This protected peptide (II) was prepared essentially in the same manner as described above by using I (350 mg.) and N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (127 mg.). The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 293 mg. (77%), m.p. 106~112°, $[\alpha]_D^{15}$ −36.5°(c=1.0, AcOH). *Anal.* Calcd. for $C_{60}H_{74}O_{16}N_{12}\cdot 2H_2O$: C, 57.40; H, 6.26; N, 13.39. Found: C, 57.43; H, 6.37; N, 13.62. The deblocked peptide ester: Rf^1 0.73, Rf^2 0.88; single ninhydrin positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Monohydrate (III)——This compound was prepared from II (250 mg.) and N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (140 mg.) essentially in the same manner as described in the preparation of I. The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 148 mg. (51%), m.p. 115~120°, $[\alpha]_D^{15}$ −25.6°(c=0.2, AcOH). *Anal.* Calcd. for $C_{71}H_{92}O_{20}N_{18}\cdot H_2O$: C, 55.53; H, 6.17; N, 16.42. Found: C, 55.67; H, 6.61; N, 16.32. The deblocked peptide ester: Rf^1 0.63, Rf^2 0.84; single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (IV)——The fully protected nonapeptide (III) (107 mg.) was hydrogenated in 2:1 mixture of AcOH and H_2O (15 ml.) for 48 hr. over 10% Pd-C (40 mg.). The catalyst was removed by the aide of Cellite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH pelletes in vacuum. The solution of the hydrogenated product in H_2O (10 ml.) was subjected to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient method from H_2O (300 ml.) in a mixing chamber to a 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. The agrinine-containing peptide was located in the eluate by Sakaguchi reaction. The eluate in tubes No. 25 to 36 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. Analysis by paper chromatography revealed the presence of two ninhydrin and Sakaguchi positive spots with Rf^1 0.33 (major) and 0.58 (minor). The solution of the crude product in the solvent of Partridge system (10 ml.) was added to a Toyo Roshi cellulose powder (200 to 300 mesh) column 2×28 cm. which was eluted with the same solvent. Fractions of 13 ml. each were collected at a flow rate 0.7 ml./min. with an automatic fraction collector and arginine-containing peptide was located in the eluate showing positive Sakaguchi reaction. The eluates in tubes No. 5 to 9 containing the nonapeptide were pooled, evaporated to dryness, and lyophilized; yield 44 mg. (44%), $[\alpha]_D^{15}$ −40.3°(c=0.3, H_2O). Rf^1 0.43, Rf^2 0.53, single ninhydrin and Sakaguchi positive spot. The content of the acetyl ester group determined according to the method of Hestrin 10, was 94.0% of the theory; amino acid ratios in the acid hydrolysate: Arg 1.81, Pro 2.93, Val 0.99, Phe 2.00, Thr 0.87.

L-Arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (V)——4-L-Valine-6-O-acetyl-L-threonine-bradykinin (IV) (25.0 mg.) in H_2O (0.2 ml.) was saponified with 1*N* NaOH (0.3 ml.) for 1 hr. at room temperature. The solution neutralized with 1*N* AcOH, was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted as described above. The eluates in tubes No. 34 to 43 were pooled, evaporated to dryness in vacuum, and lyophilized; yield 16.1 mg. (67%), $[\alpha]_D^{15}$ −78.7°(c=0.4, H_2O), Rf^1 0.30, Rf^2 0.57, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate; Arg 1.91, Pro 3.01, Val 0.98, Phe 2.00, Thr 0.91.

N-Benzyloxycarbonyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (VI)——This compound was prepared from N-benzyloxycarbonyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (600 mg.) and N-benzyloxycarbonyl-L-isoleucine *p*-nitrophenyl ester[8] (250 mg.) essentially in the same manner as described in the preparation of I. The product was reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 430 mg. (65%) of crystals, m.p. 104~124°, $[\alpha]_D^{28}$ −6.2°(c=1.1, dimethylformamide). *Anal.* Calcd. for $C_{56}H_{69}O_{15}N_{11}$: C, 59.19; H, 6.12; N, 13.56. Found: C, 58.95; H, 6.36; N, 13.73. The deblocked peptide ester Rf^1 0.78, Rf^2 0.92, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitro-benzyl Ester Monohydrate (VII)——The compound was prepared from VI (256 mg.) and N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (91 mg.) as described above. The product was reprecipitated from acetone with ether; yield 191 mg. (69%) of crystals, m.p. 108~115°, $[\alpha]_D^{28}$ −43.4° (c=0.3, dimethylformamide). *Anal.* Calcd. for $C_{61}H_{76}O_{16}N_{12}\cdot H_2O$: C, 58.55; H, 6.28; N, 13.43. Found: C, 58.18; H, 6.06; N, 13.91. The deblocked peptide ester: Rf^1 0.79, Rf^2 0.92, single ninhydrin positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Dihydrate (VIII)——The compound was prepared from VII (160 mg.) and N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (89 mg.) as described above. In this case the time for the coupling reaction was for 2 days. The product was reprecipitated from MeOH and ether; yield 127 mg. (64%) of crystals, m.p. 124~133°, $[\alpha]_D^{28}$

$-48.0°$(c=0.3, dimethylformamide). *Anal.* Calcd. for $C_{72}H_{94}O_{20}N_{18}\cdot 2H_2O$; C, 55.16; H, 6.30; N, 16.08. Found: C, 55.02; H, 6.64; N, 16.46. The deblocked peptide ester: Rf^1 0.75, Rf^2 0.97, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (IX)——This compound was prepared from Ⅷ (90 mg.) essentially in the same manner as described in the preparation of Ⅳ; yield 37.0 mg. (46%), $[\alpha]_D^{28}$ $-54.6°$(c=0.3, H_2O), Rf^1 0.39, Rf^2 0.52, single ninhydrin and Sakaguchi positive spot. Acetyl ester group was 50.0% of the theory; amino acid ratios in the acid hydrolysate: Arg 1.76, Pro 2.87, Ile 1.00, Phe 1.91, Thr 0.82.

L-Arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (X)——The compound was prepared from Ⅸ (20 mg.) essentially in the same manner as described in the preparation of Ⅴ; yield 16.7 mg. (86%), $[\alpha]_D^{28}$ $-89.6°$(c=0.4, H_2O), Rf^1 0.39, Rf^2 0.52, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolyste: Arg 1.82, Pro 2.93, Ile 0.96, Phe 2.00, Thr 0.78.

N-Benzyloxycarbonyl-L-leucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-Nitrobenzyl Ester (XI)——The compound was prepared from N-benzyloxycarbonyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-nitrobenzyl ester (650 mg.) and N-benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester[8] (270 mg.) essentially in the same manner as described in the preparation of I. The reaction mixture was diluted with 1*N* NH_4OH (2 ml.), stirred for 1 hr., and then poured into cold 1*N* NH_4OH (40 ml.) with stirring. To the suspension, 50% NH_4OAc (2 ml.) was added with stirring and the precipitate was filtered and washed successively with 1*N* NH_4OH, H_2O, 1*N* HCl, and H_2O. The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 479 mg. (66%) of crystals, m.p. 105~115°, $[\alpha]_D^{28}$ $-22.6°$(c=0.7, AcOH). *Anal.* Calcd. for $C_{56}H_{69}O_{15}N_{11}$: C, 59.19; H, 6.12; N, 13.56. Found: C, 59.19; H, 6.37; N, 13.05. The deblocked peptide ester: Rf^1 0.80, Rf^2 0.98, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-prolyl-L-leucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-Nitrobenzyl Ester Monohydrate (XII)——The compound was prepared from Ⅺ (435 mg.) and N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (156 mg.) as described above. The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 288 mg. (61%), m.p. 104~115°, $[\alpha]_D^{15}$ $-41.8°$(c=0.7, AcOH). *Anal.* Calcd. for $C_{61}H_{76}O_{16}N_{12}\cdot H_2O$: C, 58.55; H, 6.28; N, 13.43. Found: C, 58.30; H, 6.30; N, 13.32. The deblocked peptide ester: Rf^1 0.81, Rf^2 0.95, single ninhydrin positive spot.

N^α-Benzyloxycarbonyl-N^ω-nitro-L-arginyl-L-prolyl-L-prolyl-L-leucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-Nitrobenzyl Ester Dihydrate (XIII)——The compound was prepared from Ⅻ (250 mg.) and N^α-benzyloxycarbonyl-N^ω-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (139 mg.) as described above. The product was reprecipitated from AcOH with H_2O and a few drops of 50% NH_4OAc; yield 179 mg. (53%), m.p. 117~124°, $[\alpha]_D^{15}$ $-78.7°$ (c=0.5, AcOH). *Anal.* Calcd. for $C_{72}H_{94}O_{20}N_{18}\cdot 2H_2O$: C, 55.16; H, 6.30; N, 16.08. Found: C, 55.21; H, 6.53; N, 15.73. The deblocked peptide ester: Rf^1 0.64, Rf^2 0.91, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-L-leucyl-L-phenylalanyl-O-acetyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (XIV)——The compound was prepared from XIII (110 mg.) essentially in the same manner as described in the preparation of Ⅳ; yield 61 mg. (68%), $[\alpha]_D^{15}$ $-83.8°$(c=0.7, H_2O), Rf^1 0.45, Rf^2 0.59, single ninhydrin and Sakaguchi positive spot. Acetyl ester group was 93.3% of the theory; amino acid ratios in the acid hydrolysate: Arg 1.84, Pro 2.89, Leu 1.00, Phe 1.95, Thr 0.82.

L-Arginyl-L-prolyl-L-prolyl-L-leucyl-L-phenylalanyl-L-threonyl-L-prolyl-L-phenylalanyl-L-arginine Triacetate Salt (XV)——The compound was prepared from XIV (30.4 mg.) essentially in the same manner as described in the preparation of Ⅴ; yield 25.0 mg. (85%), $[\alpha]_D^{15}$ $-98.4°$(c=0.6, H_2O), Rf^1 0.37, Rf^2 0.53, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the hydrolysate: Arg 1.92, Pro 2.95, Leu 1.00, Phe 1.83, Thr 0.79.

N-Benzyloxycarbonyl-L-valyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-Nitrobenzyl Ester (XVI)——This compound was prerared from N-benzyloxycarbonyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-nitrobenzyl ester (500 mg.) and N-benzyloxycarbonyl-L-valine *p*-nitrophenyl ester (204 mg.) essentially in the same manner as described in the preparation of I. The product was reprecipitated from acetone with ether; yield 317 mg. (57%) of crystals, m.p. 103~109°, $[\alpha]_D^{28}$ $-30.0°$ (c=1.0, dimethylformamide). *Anal.* Calcd. for $C_{54}H_{65}O_{15}N_{11}$: C, 58.53; H, 5.91; N, 13.91. Found: C, 58.38; H, 6.15, N, 14.24. The deblocked peptide ester: Rf^1 0.74, Rf^2 0.96, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^ω-nitro-L-arginine *p*-Nitrobenzyl Ester (XVII)——The compound was prepared from XVI (250 mg.) and N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (92 mg.) as described above. The product was reprecipitated from acetone with ether; yield 177 mg. (65%) of crystals, m.p. 104~109°, $[\alpha]_D^{28}$ $-26.6°$(c=0.4, dimethylformamide). *Anal.* Calcd. for $C_{59}H_{72}O_{16}N_{12}$: C, 58.79; H, 6.02; N, 13.95. Found: C, 58.36; H, 6.13; N, 13.50. The deblocked peptide ester: Rf^1 0.76, Rf^2 0.88, single ninhydrin positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (XVIII)——The compound was prepared from XVII (160 mg.) and N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (19 mg.) as described above. In this case the time for the coupling reaction was for 2 days. The product was reprecipitated from AcOH with H_2O and 50% NH_4OAc; yield 160 mg. (65%) of crystals, m.p. 112~118°, $[\alpha]_D^{23}$ −33.7° (c=0.4, dimethylformamide). *Anal.* Calcd. for $C_{70}H_{90}O_{20}N_{18}$: C, 55.91; H, 6.03; N, 16.77. Found: C, 56.00; H, 5.99; N, 16.52. The deblocked peptide ester: Rf^1 0.62, Rf^2 0.77, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XIX)——The compound was prepared from XVIII (80 mg.) essentially in the same manner as described in the preparation of IV; yield 40 mg. (56%), $[\alpha]_D^{23}$ −59.7° (c=0.3, H_2O), Rf^1 0.24, Rf^2 0.48, single ninhydrin and Sakaguchi positive spot. Acetyl ester group was 65.4% of the theory, amino acid ratios in the acid hydrolysate: Arg 1.80, Pro 2.82, Val 1.00, Phe 1.81, Ser 0.72.

L-Arginyl-L-prolyl-L-prolyl-L-valyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XX)——The compound was prepared from XIX (20 mg.) essentially in the same manner as described in the preparation of V; yield 16 mg. (84%), $[\alpha]_D^{23}$ −87.5° (c=0.5, H_2O), Rf^1 0.38, Rf^2 0.54, single ninhydrin and Sakaguchi positive spot, amino acid ratios in the acid hydrolysate: Arg 1.73, Pro 2.96, Val 1.00, Phe 1.93, Ser 0.75.

N-Benzyloxycarbonyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (XXI)——The compound was prepared from N-benzyloxycarbonyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (600 mg.) and N-benzyloxycarbonyl-L-isoleucine *p*-nitrophenyl ester (264 mg.) essentially in the same manner as described in the preparation of I. The product was reprecipitated from MeOH with ether; yield 346 mg. (50%) of crystals, m.p. 138~144°, $[\alpha]_D^{25}$ −4.7° (c=0.4, dimethylformamide). *Anal.* Calcd. for $C_{55}H_{67}O_{15}N_{11}$: C, 58.86; H, 6.02; N, 13.73. Found: C, 58.58; H, 5.96; N, 13.88. The deblocked peptide ester: Rf^1 0.77, Rf^2 0.97, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Monohydrate (XXII)——The compound was prepared from XXI (290 mg.) and N-benzyloxycarbonyl-L-proline *p*-nitrophenyl ester (101 mg.) as described above. The product was reprecipitated from acetone with ether; yield 205 mg. (63%) of crystals, m.p. 125~134°, $[\alpha]_D^{25}$ −19.3° (c=0.7, dimethylformamide). *Anal.* Calcd. for $C_{60}H_{74}O_{16}N_{12}\cdot H_2O$: C, 58.24; H, 6.19; N, 13.59. Found: C, 57.98; H, 6.24; N, 13.06. The deblocked peptide ester: Rf^1 0.59, Rf^2 0.96, single ninhydrin positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester Dihydrate (XXIII)——The compound was prepared from XXII (173 mg.) and N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester (101 mg.) as described above. The product was reprecipitated from acetone with ether; yield 120 mg. (54%) of crystals, m.p. 123~130°, $[\alpha]_D^{25}$ −7.5° (c=0.2, dimethylformamide). *Anal.* Calcd. for $C_{71}H_{92}O_{20}N_{18}\cdot 2H_2O$: C, 54.89; H, 6.23; N, 16.23. Found: C, 54.42; H, 6.29; N, 16.10. The deblocked peptide ester: Rf^1 0.70, Rf^2 0.92, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XXIV)——The compound was prepared from XXIII (72 mg.) essentially in the same manner as described in the preparation of IV; yield 37 mg. (59%), $[\alpha]_D^{19}$ −90.0° (c=0.54, H_2O), Rf^1 0.29, Rf^2 0.53, single ninhydrin and Sakaguchi positive spot. Analysis of the acetyl group was 87.0% of the theory; amino acid ratios in the acid hydrolysate: Arg 1.90, Pro 2.84, Ile 1.00, Phe 1.89, Ser 0.73.

L-Arginyl-L-prolyl-L-prolyl-L-isoleucyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Triacetate (XXV)——The compound was prepared from XXIV (20 mg.) essentially in the same manner as described in the preparation of V; yield 16 mg. (83.0%), $[\alpha]_D^{19}$ −93.0° (c=0.53, H_2O), Rf^1 0.32, Rf^2 0.59, single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.84, Pro 2.79, Ile 1.00, Phe 1.90, Ser 0.75.

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