

However, the marked change in their absorption spectrum by the addition of borate could not be observed and the complex could not be isolated in the similar way.

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Kenji Suzuki and Takashi Abiko: Synthesis of 3-L-Lysine-  
bradykinin and Its O-Acetyl Compound.\*<sup>1</sup>

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The potentiating effect of L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (I) corresponding to positions 13 to 21 of B-peptide of ox co-fibrin upon the bradykinin-induced contraction of isolated mouse ileum have been reported in a previous paper.<sup>1)</sup> In a comparison between the amino acid sequence of I and bradykinin, L-arginyl-L-prolyl-L-prolylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine (II), as shown in Fig. 1, it is noticed that L-lysine residue of 3-position of I has a basic amino group in the side chain, L-serine residue of 6-position of II has a hydrophylic hydroxyl group, and the other side chains between the N- and the C-terminal arginine residue of I and II have hydrophobic groups. The synthetic 6-L-leucine bradykinin<sup>2)</sup> in which the hydrophylic hydroxymethyl group in 6-position of II, was substituted for hydrophobic isobutyl group showed no potentiating effect upon the bradykinin-induced contraction of isolated mouse ileum and practically no bradykinin-like activity.

In the present paper, the synthesis of 3-L-lysine bradykinin is reported to elucidate whether the substituted L-lysine residue in 3-position of bradykinin contributes toward potentiating effect upon bradykinin-induced contraction of isolated guinea pig ileum. The method employed for the synthesis of 3-L-lysine bradykinin is essentially the same as described in the preparation of bradykinin analogs,<sup>3)</sup> and ox co-fibrin peptide fragment.<sup>1)</sup> N-Benzyloxycarbonylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N<sup>ω</sup>-nitro-L-arginine *p*-nitrobenzyl ester<sup>4)</sup> was de-benzyloxycarbonylated with hydrogen bromide-acetic acid solution in the presence of anisole and the resulting pentapeptide ester was condensed with N<sup>α</sup>-*tert*-butyloxycarbonyl-N<sup>ω</sup>-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester<sup>1)</sup> to yield N<sup>α</sup>-*tert*-butyloxycarbonyl-N<sup>ω</sup>-benzyloxycarbonyl-L-lysylglycyl-L-phenylalanyl-L-prolyl-L-phenylalanyl-N<sup>ω</sup>-nitro-L-arginine *p*-nitrobenzyl

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

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1) K. Suzuki: This Bulletin, 14, 909 (1966).

2) K. Suzuki, M. Asaka, T. Abiko: *Ibid.*, 14, 211 (1966).

3) K. Suzuki, T. Abiko, M. Asaka: *Ibid.*, 14, 217 (1966).

4) K. Suzuki, T. Abiko: *Ibid.*, in press. Synthesis of 4-L-valine-6-L-threonine-, 4-L-isoleucine-6-L-threonine-, 4-L-leucine-6-L-threonine-, 4-L-valine-, 4-L-isoleucine-bradykinin and their O-acetyl compounds.

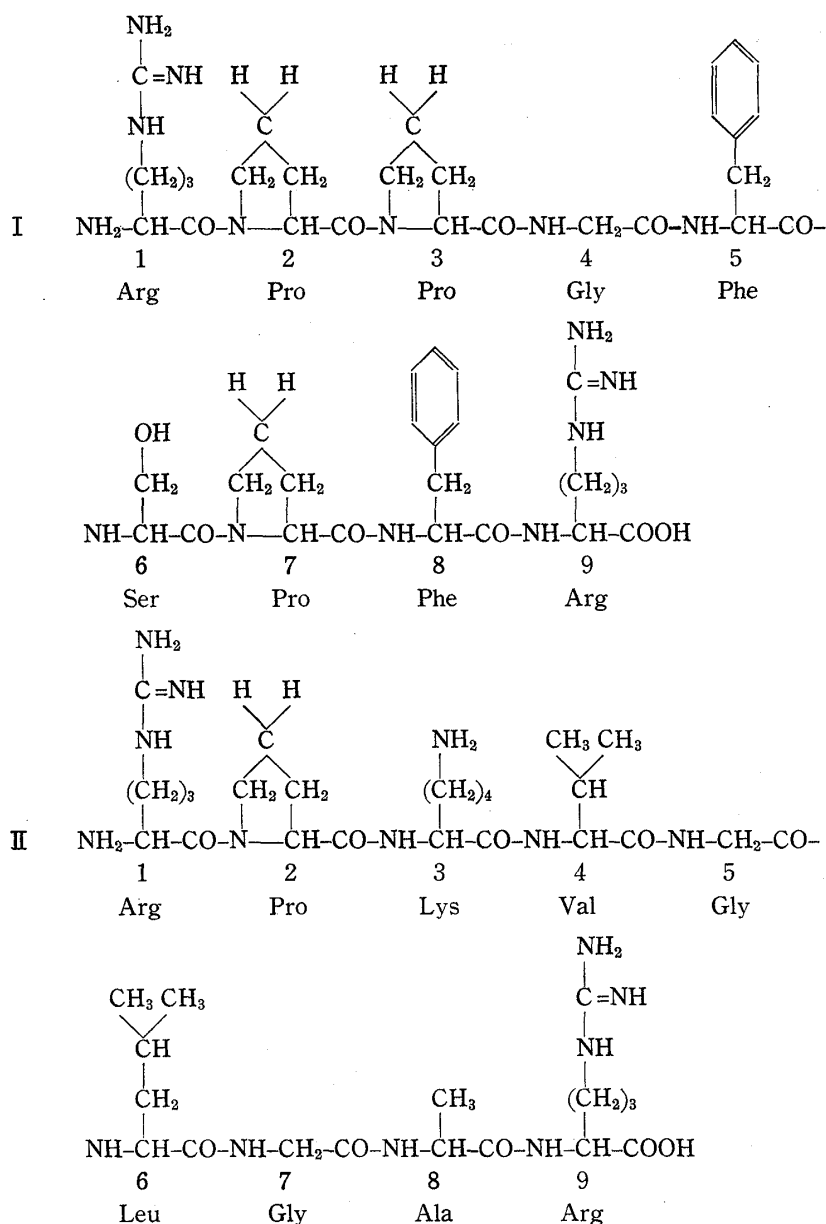


Fig. 1. Structure of Bradykinin (I) and C-Terminal Peptide of B-Peptide of Ox Co-fibrin (II) (Positions 13~21).

ester (III). After the removal of the benzyloxycarbonyl group of III, the resulting heptapeptide ester was condensed with  $N^\alpha$ -benzyloxycarbonyl- $N^\omega$ -nitro-L-arginyl-L-proline *p*-nitrophenyl ester<sup>5)</sup> to yield  $N^\alpha$ -benzyloxycarbonyl- $N^\omega$ -nitro-L-arginyl-L-prolyl- $N^\epsilon$ -benzyloxycarbonyl-L-lysylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl- $N^\omega$ -nitro-L-arginine *p*-nitrobenzyl ester (IV). The fully protected nonapeptide (IV) was hydrogenated in aqueous acetic acid in the presence of 10% palladium on charcoal for 2 days. The hydrogenated product was purified through carboxymethyl (CM-) cellulose column to obtain 3-L-lysine-6-O-acetyl-L-serine-bradykinin, L-arginyl-L-prolyl-L-lysylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine tetraacetate (V). The nonapeptide so obtained was found to be a unity from the result of paper chromatography using two different solvent systems. Determination of the acetylesther group by the hydroxamic acid method<sup>6)</sup> was 65.4% of the theoretical value and the

5) M. A. Ondetti : J. Med. Chem., **6**, 10 (1963).

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

ratio of amino acids in the acid hydrolysate agreed well with the theoretical value. Saponification of the O-acetyl nonapeptide (V) with 1N sodium hydroxide solution yielded 3-L-lysine-bradykinin, L-arginyl-L-prolyl-L-lysylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine tetraacetate (VI). The nonapeptide (VI) so 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.

The two synthetic peptides, V and VI were tested quantitatively for bradykinin-like activity, anti-bradykinin activity, and potentiation of bradykinin on the isolated guinea pig ileum by Magnus method.\*<sup>3</sup> The two peptides, V and VI showed 3/1000 and 1/1000 of the activity of bradykinin respectively. The two peptides showed no potentiating effect and anti-bradykinin activity.

### Experimental

Melting points are uncorrected. For paper chromatography, the protected peptides were de-benzyloxycarbonylated with HBr in AcOH unless otherwise mentioned and the resulting hydrobromides were chromatographed on paper, Toyo Roshi No. 51 at room temperature, Rf<sup>1</sup> values refer to the Partridge system<sup>7)</sup> and Rf<sup>2</sup> values refer to the system of BuOH-pyridine-AcOH-H<sub>2</sub>O (30:20:6:24).<sup>8)</sup> The amino acid composition of the acid hydrolysates was determined according to the directions given by Moore, *et al.*<sup>9)</sup>

**N<sup>α</sup>-tert-Butyloxycarbonyl-N<sup>ω</sup>-benzyloxycarbonyl-L-lysylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N<sup>ω</sup>-nitro-L-arginine p-Nitrobenzyl Ester Monohydrate (III)**—N-Benzyloxycarbonylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-N<sup>ω</sup>-nitro-L-arginine p-nitrobenzyl ester<sup>4)</sup> (300 mg.) was dissolved in AcOH (1 ml.), anisole (0.1 ml.), and 4.8N HBr in AcOH (1 ml.). After 50 min. at room temperature, dry ether was added and the mixture was shaken vigorously. The precipitate thereby formed was collected and washed with dry ether and dried over KOH in vacuum. To a solution of this product in dimethylformamide (3 ml.) N<sup>α</sup>-tert-butyloxycarbonyl-N<sup>ω</sup>-benzyloxycarbonyl-L-lysine p-nitrophenyl ester (156 mg.) was added, followed by Et<sub>3</sub>N to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with 1N NH<sub>4</sub>OH (1.5 ml.), stirred for 1 hr., and mixed with EtOAc (40 ml.). The EtOAc solution was washed successively with 1N NH<sub>4</sub>OH, H<sub>2</sub>O, 1N citric acid, and H<sub>2</sub>O. The solution was dried over MgSO<sub>4</sub> and concentrated to a small volume. Petroleum ether was added to the residue and the precipitate thereby formed was re-precipitated from acetone and ether; yield 260 mg. (71%) of crystals, m.p. 98~104°, [α]<sub>D</sub><sup>25</sup> -29.3°(c=0.6, dimethylformamide). *Anal.* Calcd. for C<sub>62</sub>H<sub>70</sub>O<sub>18</sub>N<sub>13</sub>·H<sub>2</sub>O: C, 56.25; H, 6.21; N, 13.88. Found: C, 56.57; H, 5.99; N, 14.03. For paper chromatography the tert-butyloxycarbonyl group of the fully protected peptide was deblocked with trifluoroacetic acid: Rf<sup>1</sup> 0.88, Rf<sup>2</sup> 0.96, single ninhydrin positive spot.

**N<sup>α</sup>-Benzyloxycarbonyl-N<sup>ω</sup>-nitro-L-arginyl-L-prolyl-N<sup>ε</sup>-benzyloxycarbonyl-L-lysylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-N<sup>ω</sup>-nitro-L-arginine p-Nitrobenzyl Ester Dihydrate (IV)**—The protected heptapeptide ester (III) (210 mg.) was dissolved in anhydrous trifluoroacetic acid (1 ml.) and the solution was kept at room temperature for 30 min. when dry ether was added. The precipitate thereby formed was collected, washed with dry ether, and dried over KOH in vacuum. To a solution of this product in dimethylformamide (3 ml.), N<sup>α</sup>-benzyloxycarbonyl-N<sup>ω</sup>-nitro-L-arginyl-L-proline p-nitrophenyl ester (112 mg.) was added, followed by Et<sub>3</sub>N to keep the solution slightly alkaline. After 2 days at room temperature, the reaction mixture was diluted 1N NH<sub>4</sub>OH (0.5 ml.), stirred for 1 hr., and added EtOAc (30 ml.). The EtOAc solution was washed successively with 1N NH<sub>4</sub>OH and H<sub>2</sub>O. AcOH was added to the EtOAc solution to prevent some precipitation and the mixture was washed successively with 1N HCl and H<sub>2</sub>O. The EtOAc solution was dried over MgSO<sub>4</sub> and concentrated to a small volume. Petroleum ether was added to the residue and the precipitate was recrystallized from AcOH with H<sub>2</sub>O and 50% NH<sub>4</sub>OAc; yield 181 mg. (67%) of crystals, m.p. 110~117°, [α]<sub>D</sub><sup>25</sup> -7.6°(c=0.7, dimethylformamide). *Anal.* Calcd. for C<sub>76</sub>H<sub>95</sub>O<sub>22</sub>N<sub>19</sub>·2H<sub>2</sub>O: C, 54.90; H, 6.00; N, 16.01. Found: C, 54.99; H, 5.97; N, 15.97. Deblocked peptide ester: Rf<sup>1</sup> 0.63, Rf<sup>2</sup> 0.71: single ninhydrin positive spot.

**L-Arginyl-L-prolyl-L-lysylglycyl-L-phenylalanyl-O-acetyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Tetraacetate (V)**—The fully protected nonapeptide (IV) (100 mg.) was hydrogenated in 1:1 mixture of

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

7) S. M. Partridge: *Biochem. J.*, **42**, 238 (1948).

8) S. G. Waley, G. Watson: *Ibid.*, **55**, 328 (1953).

9) S. Moore, D. H. Spackmann, W. H. Stein: *Anal. Chem.*, **30**, 1185 (1958).

AcOH and H<sub>2</sub>O (15 ml.) for 48 hr. in the presence of 10% Pd-C (20 mg.). The catalyst was removed with the aid of Cellite. The solution was evaporated to dryness in vacuum and the residue was dried over KOH in vacuum. A solution of the hydrogenated product in H<sub>2</sub>O (10 ml.) was added to a column (2.0 × 6.0 cm.) of CM-cellulose which was eluted with a linear gradient method from H<sub>2</sub>O (300 ml.) in a mixing chamber to 0.3M pyridinium acetate buffer solution (pH 5.1) (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. Arginine-containing peptide was located in the eluate by the Sakaguchi reaction. The eluate in tubes No. 24 to 51 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. The solution of the residue (70 mg.) in BuOH-pyridine-AcOH-H<sub>2</sub>O (30:20:6:24) (5 ml.) was added to a (2.0 × 44 cm.) cellulose powder (200 to 300 mesh.) column which was eluted with the same solvent. Fractions of 5 ml. each were collected at a flow rate of 5 ml./8 min. with an automatic fraction collector. Arginine-containing peptide was located in the eluate by the Sakaguchi reaction. The eluates in tubes No. 18 to 32 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized; yield 47.0 mg. (58%) of crystals,  $[\alpha]_D^{25} -23.5^\circ$  (c=0.3, H<sub>2</sub>O), Rf<sup>1</sup> 0.19, Rf<sup>2</sup> 0.40: single ninhydrin and Sakaguchi positive spot. Acetyl ester group was 65.4% of theory; amino acid ratios in the acid hydrolysate: Arg 1.90, Pro 1.91, Lys 1.00, Gly 1.05, Phe 2.04, Ser 0.80.

**L-Arginyl-L-prolyl-L-lysylglycyl-L-phenylalanyl-L-seryl-L-prolyl-L-phenylalanyl-L-arginine Tetraacetate (VI)**—3-L-Lysine-6-O-acetyl-L-serine-bradykinin (V) (20.0 mg.) in H<sub>2</sub>O (0.2 ml.) was saponified with 1N NaOH (0.27 ml.) for 1 hr. at room temperature. 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 H<sub>2</sub>O (300 ml.) in a mixing chamber to 0.3M pyridinium acetate buffer solution (pH 5.1) (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. Arginine-containing peptide was located in the eluate by the Sakaguchi reaction. The eluate in tubes No. 30 to 44 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized; yield 17.6 mg. (91%) of crystals,  $[\alpha]_D^{25} -18.8^\circ$  (c=0.3, H<sub>2</sub>O), Rf<sup>1</sup> 0.23, Rf<sup>2</sup> 0.42: single ninhydrin and Sakaguchi positive spot; amino acid ratios in the acid hydrolysate: Arg 1.90, Pro 1.95, Lys 1.00, Gly 1.00, Phe 2.01, Ser 0.81.

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