Summary

The preparation of the title compounds from the respective methyl 3-acetamido-3-deoxy-α-D-hexopyranosides was described. Introduction of an amino group at C_6 was confirmed by nuclear magnetic resonance spectroscopy and dissociation constants. De-N-acetylation of methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-glucopyranoside by hydrazine was accompanied with reduction of the azido group to give directly methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside. Treatment of methyl 3-acetamido-3-deoxy-2,6-di-O-*p*-tolylsulfonyl-α-D-glucopyranoside with sodium azide afforded, *via* an 2,3-oxazolinium intermediate, methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-mannopyranoside. The isolation of a new compound, methyl 3-amino-3-deoxy-β-L-glucopyranoside from nitromethane condensation products was added.

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123. Kenji Suzuki: Synthesis of Peptides Related to the C-Therminus of B-Peptide of Ox Co-fibrin (Positions 16~21, 15~21, and 13~21).

(*Tohoku College of Pharmacy*[*1])

B-Peptide of ox co-fibrin is one of the peptides which is released from ox fibrinogen by the proteolytic enzyme thromin.[1] The amino acid sequence of B-peptide of ox co-fibrin was elucidated by Folk, *et al.*[2] as a linear peptide, N-acetyl-L-threonyl-L-glutamyl-L-phenylalanyl-L-prolyl-L-asparatyl-O-sulfonyl-L-tyrosyl-L-aspartyl-L-glutamyl-glycyl-L-glutamyl-L-aspartyl-L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine. The marked potentiating effect of B-peptide and hydrolysate by trypsin which splitted the bond between the lysine and the valine of B-peptide upon the bradykinin-induced contraction of isolated rat uterus was observed by Gladner,[3] *et al.*

In this paper, synthesis and biological activities of the nonapeptide, L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅸ) corresponding to the C-terminal portion of B-peptide of ox-cofibrin were described. In addition, the results of biological assay of the two synthetic intermediates, L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅵ), and L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅸ) were reported. The second hexapeptide (Ⅵ) is one of the products of trypsin hydrolysis of B-peptide and the first nonapeptide (Ⅺ) has a partially similar amino acid sequence to bradykinin.[4] The method of peptide synthesis used here was virtually similar with a previous report on the synthesis of bradykinin and its analogs.[5] The synthetic route for the nonapeptide (Ⅺ) is illustrated in Fig. 1.

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*1 Nankozawa, Sendai (鈴木謙次).

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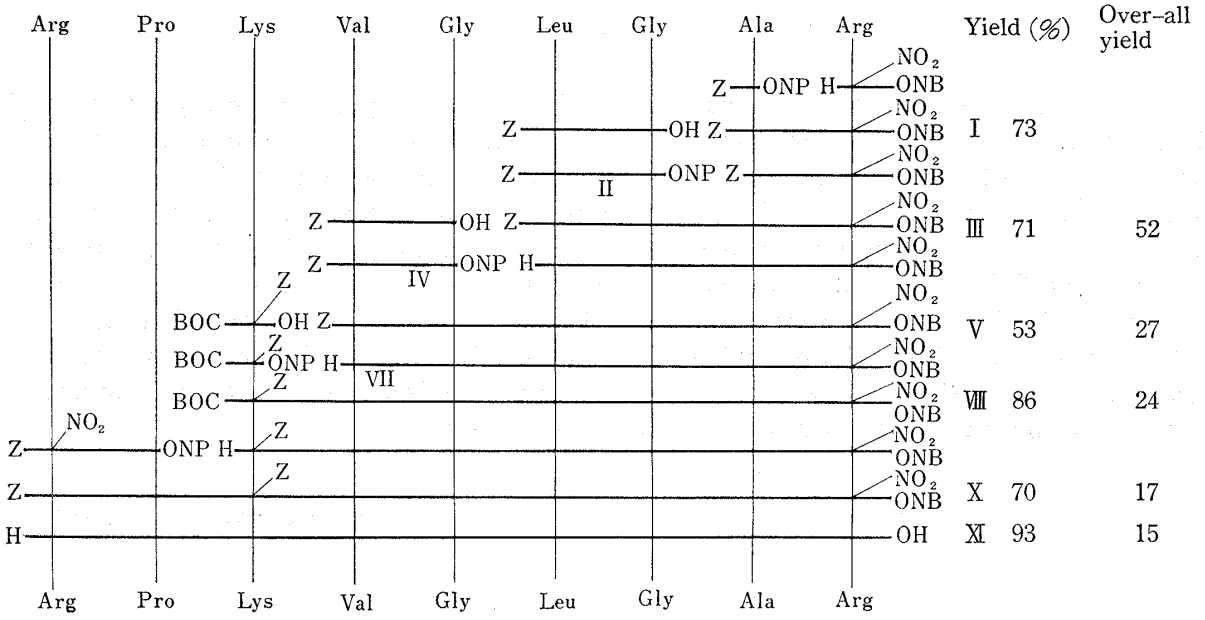

Z=benzyloxycarbonyl, ONB=*p*-nitrobenzyl, ONP=*p*-nitrophenyl, BOC=*tert*-butyloxycarbonyl.

Fig. 1. Synthesis of the C-Terminus (Positions 13~21) of B-peptide of Ox Co-fibrin

Debenzyloxycarbonylation of N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester[6] by treatment with hydrobromic acid in acetic in a presence of resorcinol and coupling of the product which was neutralized with triethylamine with N-benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester[7] yielded the crystalline N-benzyloxycarbonyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (I). Esterification of N-benzyloxycarbonyl-L-leucylglycine[8] with *p*-nitrophenol in ethyl acetate by N,N′-dicyclohexylcarbodiimide yielded N-benzyloxycarbonyl-L-leucylglycine *p*-nitrophenyl ester (II). After the removal of the benzyloxycarbonyl group of I, the resulting dipeptide ester was condensed with the protected dipeptide *p*-nitrophenyl ester (II) to yield N-benzyloxycarbonyl-L-leucylglycyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-nitro benzyl ester (III). Esterification of N-benzyloxycarbonyl-L-valylglycine[8] with *p*-nitrophenol by the N,N′-dicyclohexylcarbodiimide method yielded N-benzyloxycarbonyl-L-leucylglycine *p*-nitrophenyl ester (IV). After the removal of the benzyloxycarbonyl group of III, the resulting tetrapeptide ester was condensed with the protected dipeptide *p*-nitrophenyl ester (IV) to yield N-benzyloxycarbonyl-L-valylglycyl-L-leucylglycyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (V). The fully protected hexapeptide (V) was hydrogenated for 40 hr. in the presence of 10% palladium on charcaol in aqueous acetic acid and the hydrogenated product was purified through a carboxymethyl (CM-) cellulose column to obtain L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine di-acetate (VI). The coupling of N^{ε}-benzyloxycarbonyl-L-lysine[9] in 50% dioxane with *tert*-butyl azidoformate[10] in the presence of sodium bicarbonate yielded N^{α}-*tert*-butyloxycarbonol-N^{ε}-benzyloxycarbonyl-L-lysine[11] in oil. The esterification of the N-protected lysine with *p*-nitrophenol by N,N′-dicyclo-hexylcarbodiimide yielded N^{α}-*tert*-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester (VII). After the removal of the benzyloxycarbonyl

6) R. A. Boissonnas, St. Guttmann, P. A. Japuaenoud : Helv. Chim. Acta, **43**, 1349 (1960).
7) M. Goodmann, K. Stueben : J. Am. Chem. Soc., **81**, 3980 (1959).
8) W. Grassmann, E. Wünsch : Ber., **91**, 449 (1958).
9) A. Neuberger, F. Sanger : Biochem. J., **37**, 515 (1943).
10) Org. Synth., **44**, 15 (1964).
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group of V, the resulting hexapeptide ester was condensed with the N-protected L-lysine *p*-nitrophenyl ester (VII) to yield N^{α}-*tert*-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-valylglycyl-L-leucylglycyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (VIII). After the removal of the *tert*-butyloxycarbonyl group of VIII with trifluoroacetic acid, the resultiug N^{ε}-benzyloxycarbonyl heptapeptide ester was hydrogenated in the presence of palladium on charcoal to yield L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine triacetate (IX). After the removal of the *tert*-butyloxycarbonyl group of VIII with trifluoroacetic acid[12] the resulting N^{ε}-benzyloxycarbonyl heptapeptide ester was condensed with N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-proline *p*-nitrophenyl ester[13] to yield N^{α}-benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-nitrobenzyl ester (X). The fully protected nonapeptide (X) was hydrogenated for 40 hr. in the presence of 10% palladium on charcoal in aqueous acetic acid and the hydrogenated product was purified through a CM-cellulose column to obtain L-arginyl-L-prolyl-L-lysyl-L-valylglycyl--L-leucylglycyl-L-alanyl-L-arginine tetraacetate (XI). The nonapeptide (XI) so obtained was found to be a unity from the result of paper chromatography in two different solvent systems and ratio of amino acids in the acid hydrolysate agreed well with the threoretical value.

The three synthetic peptide, VI, IX, and XI were tested quantitatively for bradykinin-like activity, antibradykinin activity, and potentiation of bradykinin, on isolated mouse ileum. Result of these biological examination is given in Table I. The nona-

Table I. Biological Activity of Synthesized Three Peptides Related to the C-Terminus of B-Peptide of Ox Co-fibrin[a)]

	Bradykinin-like activity	Bradykinin potentiating activity	Antibradykinin activity[b)]
B-peptide of Co-fibrin			
Positions 13～21 (XI)	1/1000	+[d)]	—
〃 15～21 (IX)	1/1000	—	+[e)]
〃 16～21 (VI)	inactive[c)]	—	+[e)]
Bradykinin (synthetic)	1		

a) Assayed by Magnus method on a mouse ileum (male).
b) Assayed on the inhibition to contract an ileum induced bradykinin 3×10^{-7} g./ml. (in bath).
c) Inactive by 1×10^{-5} g./ml. (in bath).
d) At a concentration of 1×10^{-5} g./ml., caused 50% potentiation of the normal contraction due to 3×10^{-7} g./ml. of bradykinin.
e) At a concentration of 1×10^{-5} g./ml., caused 20% inhibition of the normal contraction due to 3×10^{-7} g./ml. of bradykinin.

peptide (XI) and the heptapeptide (IX) showed a low degree of bradykinin-like activity but the hexapeptide (VI) showed no bradykinin-like activity. The nonapeptide (XI) potentiated the contraction due to bradykinin. The heptapeptide (IX) and the hexapeptide (VI) showed antibradykinin 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^1 values refer to the Partridge system[14] and Rf^2

12) L. A. Caprino : J. Am. Chem. Soc., **79**, 98 (1957); R. Schwyzer, W. Rittel, H. Kappeler, B. Iselin : Angew. Chem., **72**, 915 (1960).
13) M. A. Ondetti : J. Med. Chem., **6**, 10 (1963).
14) S. M. Partridge : Biochem. J., **42**, 238 (1948).

values refer to the system of BuOH–pyridine–AcOH–H_2O (30:20:6:24).[15] The amino acid composition of the acid hydrolystates was determined according to the directions given by Moore, *et al.*[16]

N-Benzyloxycarbonyl-L-alanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester Hemiacetone Solvat (I)——N$^{\alpha}$-Benzyloxycarbonyl-N$^{\omega}$-nitro-L-arginine *p*-nitrobenzyl ester (2.5 g.) and resorcinol (40 mg.) was dissolved in AcOH (7 ml.) and 5.7*N* HBr in AcOH (7 ml.) was added. After 50 min. at room temperature, the reaction mixture was shaken viogorously with dry ether. The precipitate thereby formed was collected, washed with dry ether, and dried over KOH pellets in vacuum. To a solution of this product in dimethylformamide (15 ml.) N-benzyloxycarbonyl-L-alanine *p*-nitrophenyl ester (1.9 g.) was added, followed by $(C_2H_5)_3N$ to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with *N* NH_4OH (3 ml.), stirred for 1 hr, and diluted with EtOAc. The EtOAc solution was washed successively with *N* NH_4OH, H_2O, *N* HCl, and H_2O. The solution was dried over $MgSO_4$ and concentrated to small volume. Petroleum ether was added to the residue and the precipitate was reprecipitated from acetone with ether; yield 2.1 g. (73%), m.p. 69~74°, $[\alpha]_D^{15}$ −23.1° (c=1.1, AcOH), *Anal.* Calcd. for $C_{24}H_{29}O_9N_7 \cdot \frac{1}{2}CH_3COCH_3$: C, 52.03; H, 5.48; N, 16.66. Found: C, 52.46; H, 5.04; N, 17.08. Deblocked peptide ester: Rf^1 0.40, Rf^2 0.83, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-leucylglycine *p*-Nitrophenyl Ester (II)——To a precooled solution of N-benzyloxycarbonyl-L-leucylglycine (5.3 g.) in EtOAc (66 ml.) and dimethylformamide (13 ml.) *p*-nitrophenol (5.2 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (7.3 g.). After 30 min. at 0° and 2 hr. at room temperature, the formed N,N′-dicyclohexylurea was filtered off. The filtrate was washed with *N* $NaHCO_3$ and H_2O and the EtOAc solution was dried over $MgSO_4$. The solvent was evaporated in vacuum and the crystalline residue was recrystallized from EtOH: yield 3.4 g. (47%), m.p. 161°, $[\alpha]_D^{15}$ −24.4° (c=1.1, AcOH), *Anal.* Calcd. for $C_{22}H_{25}O_7N_3$: C, 59.58; H, 5.68; N, 9.48. Found: C, 60.28: H, 5.90; N, 9.14.

N-Benzyloxycarbonyl-L-leucylglycyl-L-alanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (III)——The protected dipeptide ester (I) (2.8 g.) was dissolved in AcOH (10 ml.) and 5.7*N* HBr in AcOH (10 ml.) was added. After 50 min. at room temperature, the solvent was evaporated to about half volume and the concentrated solution was shaken vigorously with dry ether. The precipitate thereby formed was collected, washed with dry ether and dried over KOH pellets in vacuum. To a solution of this product in dimethylformamide (25 ml.) N-benzyloxycarbonyl-L-leucylglycine *p*-nitrophenyl ester (2.4 g.) was added, follwed by $(C_2H_5)_3N$ to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with *N* NH_4OH (5 ml.), stirred for 1 hr., and the solution was diluted with EtOAc. The EtOAc solution was washed successively with *N* NH_4OH, H_2O, *N* HCl, and H_2O. The EtOAc solution was dried over $MgSO_4$ and concentrated to small volume. Petroleum ether was added to the residue and the precipitate was reprecipitated from dioxane with ether. Some sticky precipitate was turned into crystalline in 1 hr. in a refregerator; yield 2.6 g. (71%), m.p. 128~129°, $[\alpha]_D^{15}$ 0.0° (c=0.7, AcOH), *Anal.* Calcd. for $C_{32}H_{43}O_{11}N_9$: C, 52.67; H, 5.94; N, 17.28. Found: C, 52.63; H, 5.53; N, 16.81. Deblocked peptide ester: Rf^1 0.59, Rf^2 0.87, single ninhydrin positive spot.

N-Benzyloxycarbonyl-L-valylglycine *p*-Nitrophenyl Ester (IV)——To an ice-cooled solution of N-benzyloxycarbonyl-L-valylglycine (1.25 g.) in EtOAc (20 ml.) and dimethylformamide (4 ml.) *p*-nitrophehol (0.67 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (1.00 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 *N* $NaHCO_3$, and H_2O. The EtOAc solution was dried over $MgSO_4$, evaporated to dryness, and the residue was recrystallized from EtOH; yield 1.0 g. (57%) of needles, m.p. 204~205°, $[\alpha]_D^{15}$ −40.7° (c=0.86, AcOH), *Anal.* Calcd. for $C_{21}H_{23}O_7N_3$: C, 58.73: H, 5.40; N, 9.79. Found: C, 59.04: H, 5.08: N, 9.26.

N-Benzyloxycarbonyl-L-valylglycyl-L-leucylglycyl-L-alanyl-N$^{\omega}$-nitro-L-arginine *p*-Nitrobenzyl Ester (V)——The protected tetrapeptide ester (III) (1.4 g.) was dissolved in AcOH (7 ml.) and 5.7*N* HBr in AcOH (7 ml.) was added. After 50 min. at room temperature, the solvent was evaporated to about half volume and the concentrated solution 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 this product in dimethylformamide (14 ml.) N-benzyloxycarbonyl-L-valylglycine *p*-nitrophenyl ester (0.95 g.) was added, followed by $(C_2H_5)_3N$ to keep the solution slightly alkaline. After 24 hr. at room temperature, the reaction mixture was diluted with *N* NH_4OH (2 ml.), stirred for 1 hr., and the solution was mixed with EtOAc. The EtOAc solution was washed successively with *N* NH_4OH, H_2O, *N* HCl, and H_2O. The EtOAc solution was dried over $MgSO_4$ and concentrated to small volume. Petroleum ether was added to the residue and the precipitate therby formed was collected. The finely powdered precipitate suspended in acetone (50 ml.), was refluxed for 1 hr. and kept in refrigerator overnight. The precipitate was collected and washed with acetone; yield 0.9 g. (53%), m.p. 193~196°. For analysis a sample was reprecipitated from AcOH, H_2O, and 50% NH_4Ac. m.p. 151~153°, $[\alpha]_D^{15}$ −17.7° (c=0.7, AcOH), *Anal.* Calcd. for $C_{39}H_{55}O_{13}N_{11}$: C, 52.87: H, 6.26; N, 17.39. Found: C, 52.22: H, 6.48: N, 17.20. Deblocked peptide ester: Rf^1 0.68, Rf^2 0.89, single ninhydrin positive spot.

15) S. G. Waley, G. Watson: *Ibid.*, **55**, 328 (1953).
16) S. Moore, D. H. Spackmann, W. H. Stein: Anal. Chem., **30**, 1185 (1958).

L-Valylglycyl-L-leucylglycyl-L-alanyl-L-arginine Diacetate (VI)——The protected hexapeptide ester (110 mg.) (V) was hydrogenated in 10:5 mixture of AcOH and H_2O (15 ml.) for 40 hr. in the presence of 10% Pd-C. The catalyst was removed by filtration with the aid of Cellite. The solution was evaporated to dryness in vacuum under 30° of bath temperature and the residue was dried over KOH pellets 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 0.03*M* NH_4Ac 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. Arginine-containing peptide was located in the eluate with Sakaguchi reaction. The eluates in tubes No. 28 to 38 containing the hexapeptide was pooled, evaporated to dryness in vacuum under 30° of bath temperature, and lyophilized. NH_4Ac was removed by repeated lyophilization to constant weight. Colorless fluffy material; yield 80.0 mg. (95%), $[\alpha]_D^{23}$ −15.6°(c=1.7, H_2O), Rf^1 0.29, Rf^2 0.46, single ninhydrin and Sakaguchi positive spot.

N^{α}-*tert*-Butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine *p*-Nitrophenyl Ester (VII)——Finely powdered N^{ε}-benzyloxycarbonyl-L-lysine (4.0 g.) and $NaHCO_3$ (4.6 g.) was suspended in H_2O (120 ml.) and dioxane (120 ml.). To the suspension, *tert*-butyl azidformate (prepared from 3.6 g. of the hydrazide) was added, stirred for 40 hr. at 40~50°, and dioxane was evaporated in vacuum. Some insolubles were filtered off and washed with H_2O. The combined filtrate was washed with EtOAc three times. The aqueous solution was cooled in an ice bath, acidified with solid citric acid, and extracted with EtOAc. The EtOAc solution was washed with H_2O and dried over $MgSO_4$. The solvent was evaporated in vacuum to give a light brown oil (3.5 g.). To an ice-cooled EtOAc solution (40 ml.) of the oily N^{α}-*tert*-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine, *p*-nitrophenol (1.4 g.) was added, followed by N,N′-dicyclohexylcarbodiimide (2.1 g.). After 30 min. at 0° and 2 hr. at room temperature, a few drops of AcOH was added, and the mixture was stirred for 15 min., and the N,N′-dicyclohexylurea thereby formed was filtered off. The filtrate was evaporated in vacuum and the residue was recrystallized from EtOH and petroleum ether; yield 2.3 g. (32%), needles. m.p. 70~77°, $[\alpha]_D^{21}$ 0.0°(c=0.85, AcOH), *Anal.* Calcd. for $C_{25}H_{31}O_8N_3$: C, 59.87; H, 6.23; N, 8.38. Found: C, 60.35: H, 6.50: N, 8.13.

N^{α}-*tert*-Butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-N^{ω}-nitro-L-arginine *p*-Nitrobenzyl Ester (VIII)——The protected heptapeptide ester (V) (177 mg.) was dissolved in AcOH (1.0 ml.) and 5.7*N* HBr in AcOH (1.0 ml.) was added. After 50 min. at room temperature, the solution 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 this product in dimethylformamide (4 ml.) N^{α}-*tert*-butyloxycarbonyl-N^{ε}-benzyloxycarbonyl-L-lysine *p*-nitrophenyl ester (VII) (110 mg.) was added, followed by $(C_2H_5)_3N$ to keep the solution slightly alkaline. After 2 days at room temperature, the reaction mixture was diluted *N* NH_4OH (1 ml.), stirred for 1 hr., and the solution was mixed with EtOAc. The solution was washed successively with *N* NH_4OH, H_2O, *N* citric acid, and H_2O. The EtOAc solution was dried over $MgSO_4$ and evaporated to dryness. The residue was recrystalized from MeOH and ether; yield 190 mg. (86%), m.p. 175°, $[\alpha]_D^{24}$ −10.9°(c=0.9, AcOH), *Anal.* Calcd. for $C_{50}H_{75}O_{16}N_{13}$: C, 53.90; H, 6.79; N, 16.34. Found: C, 54.24; H, 7.23; N, 16.67. For paper chromatography *tert*-butyloxycarbonyl group of the protected peptide ester was deblocked with trifluoroacetic acid: Rf^1 0.87, Rf^2 0.91, single ninhydrin positive spot.

L-Lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine Triacetate Salt (IX)——The protected heptapeptide ester (VIII) (115 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 filtered and dried over KOH pellets in vacuum. The heptapeptide ester trifluoroacetate (90 mg.) in 1:1 mixture of AcOH and H_2O (14 ml.) was hydrogenated in the presence of 10% Pd-C (10 mg.) for 40 hr. The catalyst was removed by filtration with the aid of Cellite. The solvent was evaporated to dryness in vacuum, and the residue was dried over KOH pellets in vacuum. The solution of the crude product in H_2O (10 ml.) was applied 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 0.2*M* pyridinium acetate buffer (pH 5.1) (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. Arginine-containing peptide was located in the eluate with Sakaguchi reaction. The eluates in tubes No. 13 to 24 were pooled, evaporated to dryness in vacuum, and lyophilized; yield 57 mg. (63%), $[\alpha]_D^{27}$ −26.9°(c=0.5, H_2O), Rf^1 0.20, Rf^2 0.37, single ninhydrin and Sakaguchi positive spot.

N^{α}-Benzyloxycarbonyl-N^{ω}-nitro-L-arginyl-L-prolyl-N^{ε}-benzyloxycarbonyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-N^{g}-nitro-L-arginine *p*-Nitrobenzyl Ester (X)——The protected heptapeptide ester (VIII) (130 mg.) was dissolved in anhydrous trifluoroacetic acid (0.7 ml.) and the solution was kept at room temperature for 20 min. when dry ether was added. The precipitate thereby formed was collected and dried over KOH pellets 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 (75 mg.) was added, followed by $(C_2H_5)_3N$ to keep the solution slightly alkaline. After 2 days at room temperature the reaction mixture was diluted with *N* NH_4OH (1 ml.), stirred for 1 hr., and the solution was diluted with EtOAc. The EtOAc solution was washed with *N* NH_4OH, and H_2O. A few percent of EtOH to the EtOAc solution was added, and the solution was washed with *N* HCl, and H_2O. The EtOAc solution was dried over $MgSO_4$ and evaporated to dryness. The residue

was reprecipitated from MeOH and ether; yield, wt. 118 mg. (70%) of crystals, m.p. 124~131°, $[\alpha]_D^{24}$ −53.1° (c=0.5, AcOH), *Anal.* Calcd. for $C_{64}H_{91}O_{20}N_{19}$: C, 53.14; H, 6.34; N, 18.40. Found: C, 53.09; H, 6.46; N, 18.37. Deblocked peptide ester: Rf^1 0.41, Rf^2 0.75, single ninhydrin positive spot.

L-Arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine Tetraacetate (XI)—The fully protected nonapeptide (X) (80 mg.) was hydrogenated in 10:5 mixture of AcOH and H_2O (15 ml.) for 40 hr. in the presence of 10% Pd-C. The catalyst was removed by filtration with the aid of Cellite. The solution was evaporated to dryness in vacuum under 30° of bath temperature and the residue was dried over KOH pellets in vacuum. The aqueous solution (10 ml.) of the crude product was added to a (2.0×6.0 cm.) CM-cellulose column which was eluted with a linear gradient elution from H_2O (300 ml.) in a mixing chamber to 0.2*M* NH_4Ac 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. Arginine-containing peptide was located in the eluate by the Sakaguchi reaction. The eluates in tubes No. 25~36 containing the nonapeptide were pooled, evaporated to dryness in vacuum, and lyophilized. NH_4Ac was removed by repeated lyophilization to constant weight; yield, 61 mg. (93%) of colorless fluffy material, $[\alpha]_D^{23}$ −48.8° (c=1.2, H_2O), Rf^1 0.21, Rf^2 0.36, single ninhydrin and Sakaguchi positive spot, amino acid ratios in the acid hydrolysate: Arg 1.8, Lys 1.0, Pro 0.9, Val 0.9, Gly 2.0, Leu 1.1, Ala 1.0.

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Summary

The synthesis of three peptides related to the C-terminus of B-peptide of ox cofibrin (positions 16~21, 15~21, and 13~21) is described. The biological activity of the peptides on isolated mouse ileum is reported.

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