Synthesis of Every Kinds of Peptide Fragments of Bradykinin¹⁾

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(Received February 20, 1969)

Synthesis of every kinds of peptide fragments of bradykinin and results of the biological activities of the synthetic bradykinin fragments on contracting effect of a guinea pig ileum, inflammatory activity on rat's hind paw and vasodilation on rat are described. So far as concerning with the contracting effect, the active site of bradykinin seems to be the phenylalanylserylprolylphenylalanine moiety. The inflammatory activity is fairly high as compared with that of bradykinin.

Bradykinin, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, is released from bradykininogen by the action of kallikreins and brings about smooth muscle stimulation, vasodilation, increases in capillary permeability, accumulation and migration of leucocytes and pain production. They are well known that so-called kininases, for examples catheptic carboxypeptidase B³⁾ and carboxypeptidase N,⁴⁾ destroy bradykinin by cleaving the peptide bond of the nonapeptide, producing pharmacologically inert peptides. However, there is a possibility of the appearance of the other bradykinin fragments resulting from unknown enzymatic hydrolysis of bradykinin in the course of the metabolism, since the details of the metabolism of bradykinin have not been established yet. On the other hand, synthesis of bradykinin fragments and biological activities of the synthetic bradykinin fragments have been reported just a few.⁵⁾ The authors, therefore, have synthesized every kinds of bradykinin fragments, namely thirtyfive kinds from dipeptide to octapeptide, to examine systematically the biological activities of the synthetic bradykinin fragments. Of these synthetic peptides, phenylalanylserylprolylphenylalanine and phenylalanylserylprolylphenylalanylarginine showed bradykinin-like activity on the contracting effect of an isolated guinea pig ileum and fairly high activity in the assay for inflammation of rat's hind paw, as described in a previous communication.⁶⁾ In addition, studies on the structural requirements of bradykinin for binding to antibradykinin antibody with these synthetic bradykinin fragments are now in progress.⁷⁾ In this paper the details

1) Abbreviations of amino acid derivatives and peptides, and naming synthetic modification of the natural peptide used herein are those recommended by IUPAC-IUB commission on Biochemical Nomenclature in July 1965: *Biochemistry*, **5**, 2485 (1966) and *ibid.*, **6**, 362 (1967). Peptide and peptide derivatives mentioned in this paper are the L-configuration except glycine.

2) Location: *Nankozawa, Sendai, 983, Japan.*

3) E.G. Erdös and H.Y.T. Yang, "Hypotensive Peptide," Proceeding of the International Symposium, Oct. 25-29, 1965, Florence, Italy, ed. E.G. Erdös, N. Bach, F. Sicuteri and A.F. Wilde, Springer-Verlag, New York, Inc., 1966, p. 235.

4) L.M. Greenbaum and K. Yamafuji, "Hypotensive Peptide," Proceeding of the International Symposium, Oct. 25-29, 1965, Florence, Italy, ed. E.G. Erdös, N. Bach, F. Sicuteri and A.F. Wilde, Springer-Verlag, New York, Inc., 1966, p. 252.

5) See review article; E. Schröder and K. Lübke, "The Peptides," Vol. II, Academic Press, New York and London, 1966, p. 101.

6) K. Suzuki, T. Abiko, N. Endo, T. Kameyama, K. Sasaki, and J. Nabeshima, *Japan J. Pharmacol.*, submitted for publication.

7) J. Spragg, R.C. Talamo, K. Suzuki, D.M. Appelbaum, K.F. Austen, and E. Haber, *Biochemistry*, **7**, 4086 (1968); J. Fischer, J. Spragg, R.C. Talamo, J.V. Pierce, K. Suzuki, K.F. Austen, and E. Haber, in preparation.

TABLE I. Physical Constants and Analytical Data of Synthesized Peptides and Intermediates, and Main Starting Materials

Compounds	Yield (%)	Recryst. solvt.	mp (°C)	[α] _D (conc., solvt., temp. °C)	Formula	Analysis (%)						R _f ¹	R _f ²	lit.
						Calcd.			Found					
						C	H	N	C	H	N			
Z-Phe-Arg(NO ₂)-ONb	84	EtOAc	172-173	-44.1°(1.0, MeOH, 20)	C ₃₀ H ₃₈ O ₁₀ N ₇	56.70	5.20	15.40	56.50	5.40	15.60	0.59		a, b
Phe-Arg (monoacetate, hemihydrate)	57	H ₂ O	135-145	+7.3°(0.7, H ₂ O, 24)	C ₂₄ H ₃₀ O ₈ N ₅ · CH ₃ COOH · ½H ₂ O	52.29	7.27	17.94	51.94	7.32	17.43	0.37		a, b
Z-Pro-Phe-Arg(NO ₂)-ONb	70	EtOAc-pet. ether	72-89	-10.4°(1.1, MeOH, 22)	C ₃₄ H ₄₀ O ₁₀ N ₈	57.40	5.50	15.30	58.10	5.50	14.80	0.70		a, b
Pro-Phe-Arg (monoacetate, dihydrate)	78	H ₂ O	137-142	-16.4°(0.6, H ₂ O, 24)	C ₃₄ H ₄₀ O ₁₀ N ₈ · 2H ₂ O	51.35	7.44	16.33	50.85	7.17	16.42	0.30		a
Z-Ser-Pro-Phe-Arg(NO ₂)-ONb	66	EtOAc-pet. ether	101-106	-50.5°(0.9, AcOH, 18)	C ₃₈ H ₄₆ O ₁₂ N ₉	55.67	5.53	15.38	55.76	5.38	15.05	0.65		a
Ser-Pro-Phe-Arg (monoacetate, monohydrate)	76	H ₂ O	139-155	-47.1°(0.2, H ₂ O, 24)	C ₃₈ H ₄₆ O ₁₂ N ₉ · H ₂ O	51.44	7.08	16.08	51.14	7.48	16.41	0.34		a
Z-Phe-Ser-Pro-Phe-Arg(NO ₂)-ONb	38	DMF-H ₂ O	225-226	-36.4°(1.2, AcOH, 14)	C ₄₂ H ₅₀ O ₁₄ N ₁₀	58.32	5.59	13.88	57.84	5.47	13.68	0.73		a
Phe-Ser-Pro-Phe-Arg (monoacetate, dihydrate) ⁹	75	H ₂ O	143-152	-37.8°(0.3, H ₂ O, 28)	C ₄₂ H ₅₀ O ₁₄ N ₁₀ · 2H ₂ O	54.53	6.99	14.96	54.40	7.01	15.35	0.48		c
Z-Gly-Phe-Ser-Pro-Phe-Arg(NO ₂)-ONb	86	acetone-ether	180-182	-35.4°(0.5, AcOH, 19)	C ₄₂ H ₅₀ O ₁₄ N ₁₀	57.46	5.58	14.45	57.40	5.90	13.84	0.76		
Gly-Phe-Ser-Pro-Phe-Arg (diacetate, monohydrate)	92	H ₂ O	153-159	-15.6°(0.7, H ₂ O, 24)	C ₄₈ H ₅₈ O ₁₆ N ₁₂ · 2CH ₃ COOH · H ₂ O	54.47	5.39	14.66	54.54	5.12	14.18	0.47		
Z-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO ₂)-ONb	72	AcOH-H ₂ O	119-125	-48.1°(1.0, AcOH, 27)	C ₄₈ H ₅₈ O ₁₆ N ₁₂	57.82	5.72	14.44	57.62	5.93	13.90	0.79		a
Pro-Gly-Phe-Ser-Pro-Phe-Arg (diacetate, monohydrate)	41	H ₂ O	155-165	-35.4°(0.7, H ₂ O, 24)	C ₄₈ H ₅₈ O ₁₆ N ₁₂ · 2CH ₃ COOH · H ₂ O	54.65	6.83	14.82	54.38	7.13	14.93	0.41		
Z-Pro-Gly-Phe-Ser-Pro-Phe-Arg(NO ₂)-OMe	75	MeOH-EtOAc	150-152	-64.5°(1.0, DMF, 23)	C ₄₈ H ₅₈ O ₁₆ N ₁₂	58.01	6.25	15.32	57.66	6.13	14.98	0.66		d, e
Pro-Phe-Ser-Pro-Phe-Arg	55	H ₂ O	187-191	-115°(1.4, H ₂ O, 24)	C ₄₄ H ₅₀ O ₁₄ N ₁₀ · 2CH ₃ COOH · H ₂ O	55.37	6.78	14.80	55.89	7.01	14.46	0.40		f
Z-Pro-Phe-ONb (monoacetate, monohydrate)	75	EtOAc-pet. ether	88-90	-37.2°(1.5, DMF, 23)	C ₃₈ H ₄₆ O ₁₂ N ₉ · CH ₃ COOH · H ₂ O	60.97	5.94	6.88	60.50	5.66	7.11	0.76		g
Pro-Phe	41	H ₂ O-EtOH	264	-41.7°(1.7, 6N HCl, 25)	C ₉ H ₁₀ O ₂ N ₂	59.98	7.19	9.90	59.74	7.16	10.21	0.61		h
Z-Ser-Pro-Phe-OMe	80	EtOAc-pet. ether	40-44	-41.1°(0.8, DMF, 17)	C ₂₈ H ₃₄ O ₈ N ₅ · H ₂ O	60.57	6.45	8.15	60.75	6.18	7.55	0.65		i
Z-Ser-Pro-Phe	75	MeOH-ether	152-154	-35.6°(1.2, MeOH, 14)	C ₂₈ H ₃₄ O ₈ N ₅ · C ₁₂ H ₂₂ N ₃ · H ₂ O	65.08	7.97	8.21	64.81	7.98	7.90	0.50		
Ser-Pro-Phe (di- and hemihydrate)	37	H ₂ O-EtOH	224	-1.0°(0.2, H ₂ O, 25)	C ₁₇ H ₂₂ O ₆ N ₃ · 2½H ₂ O	51.77	7.16	10.66	52.14	7.00	10.76	0.50		
Z-Phe-Ser-Pro-Phe-OMe (hemihydrate)	41	EtOAc-EtOH	94-96	-18.7°(0.8, DMF, 17)	C ₃₈ H ₄₆ O ₁₂ N ₉ · ½H ₂ O	64.30	6.32	8.57	64.35	6.48	8.80	0.70		i
Z-Phe-Ser-Pro-Phe (dihydrate)	60	EtOAc-pet. ether	109-116	-29.7°(1.0, DMF, 17)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	61.25	6.35	8.40	61.29	6.17	8.24	0.76		
Phe-Ser-Pro-Phe (tetrahydrate) ¹⁰	87	H ₂ O	147-150	-15.6°(0.8, H ₂ O, 24)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 4H ₂ O	56.71	6.96	10.18	56.58	6.77	10.12	0.71		
Z-Gly-Phe-Ser-Pro-Phe-OMe	77	MeOH-H ₂ O	112-114	-32.4°(1.0, DMF, 23)	C ₃₃ H ₄₀ O ₁₀ N ₇	63.31	6.17	9.98	63.19	6.08	10.13	0.73		i
Z-Gly-Phe-Ser-Pro-Phe	65	acetone-ether	175-180	-28.1°(0.6, DMF, 21)	C ₃₃ H ₄₀ O ₁₀ N ₇	57.03	6.67	9.98	56.98	5.67	9.04	0.68		
Gly-Phe-Ser-Pro-Phe (dihydrate)	47	H ₂ O	190-195	-26.5°(0.3, 1N HCl, 24)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	57.03	6.67	11.88	56.64	6.75	11.41	0.68		
Z-Pro-Gly-Phe-Ser-Pro-Phe-OMe	79	acetonitrile-ether	153-155	-39.3°(0.4, DMF, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	63.14	6.31	10.32	63.02	6.54	10.39	0.63		i
Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	39	acetone-ether	128-132	-34.0°(0.8, DMF, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	59.66	6.39	10.24	59.52	6.37	9.73	0.65		
Z-Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	96	H ₂ O	164-167	-50.8°(0.6, H ₂ O, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	57.71	6.75	12.24	57.65	6.89	12.48	0.65		
Z-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OMe(trihydrate)	47	EtOAc-pet. ether	114-120	-34.7°(0.8, DMF, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 3H ₂ O	58.78	7.66	10.21	58.98	7.83	10.22	0.72		
Z-Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	55	acetone-ether	131-138	-25.2°(0.8, DMF, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · 2H ₂ O	60.18	6.48	10.68	60.20	6.60	9.96	0.61		
Pro-Pro-Gly-Phe-Ser-Pro-Phe (monohydrate)	95	H ₂ O	163-168	-49.6°(0.4, H ₂ O, 14)	C ₃₈ H ₄₆ O ₁₂ N ₉ · H ₂ O	59.59	6.71	12.80	59.64	6.38	12.48	0.61		
Z-Arg(NO ₂)-Pro-Gly-Phe-Ser-Pro-Phe-OMe (trihydrate)	51	acetone-ether	124-131	-45.5°(0.4, DMF, 14)	C ₅₀ H ₅₈ O ₁₆ N ₁₃ · 3H ₂ O	55.29	6.48	14.60	54.95	6.61	14.05	0.61		i
Z-Arg(NO ₂)-Pro-Gly-Phe-Ser-Pro-Phe (tetrahydrate)	56	MeOH-ether	140-147	-76.4°(0.2, DMF, 14)	C ₅₂ H ₆₀ O ₁₆ N ₁₃ · 4H ₂ O	53.23	6.53	14.33	53.52	6.25	14.41	0.46		i
Arg-Pro-Gly-Phe-Ser-Pro-Phe (monoacetate, dihydrate)	84	H ₂ O	173-181	-82.0°(0.6, H ₂ O, 14)	C ₄₄ H ₅₀ O ₁₄ N ₁₀ · CH ₃ COOH · 2H ₂ O	55.24	6.96	15.41	55.03	6.89	15.12	0.50		i
Z-Ser-Pro (monohydrate)	26	EtOAc-ether	105-108	-26.7°(0.8, DMF, 21)	C ₁₆ H ₂₀ O ₄ N ₂ · H ₂ O	54.23	6.26	7.91	54.68	6.02	8.21	0.26		
Ser-Pro	39	H ₂ O-EtOH	153-166	-85.2°(0.9, H ₂ O, 24)	C ₁₆ H ₂₀ O ₄ N ₂	57.52	6.98	13.86	47.49	7.12	13.18	0.18		j
Z-Phe-Ser-Pro	66	EtOAc-ether-pet. ether	75-86	-50.7°(0.7, DMF, 21)	C ₂₈ H ₃₄ O ₈ N ₅	62.10	6.05	8.69	61.73	6.06	8.50	0.45		
Phe-Ser-Pro (monohydrate)	99	H ₂ O	125-133	-24.7°(0.9, H ₂ O, 24)	C ₂₈ H ₃₄ O ₈ N ₅ · H ₂ O	55.57	6.86	11.44	55.30	6.87	11.28	0.45		
Z-Pro-ONb		oil												
HBr-Pro-ONb		MeOH-ether	171	-21.8°(1.1, DMF, 7)	C ₁₆ H ₂₀ O ₄ N ₂ · HBr									
Z-Phe-Ser-Pro-ONb	77	EtOAc-pet. ether	58-64	-73.0°(0.4, DMF, 21)	C ₂₄ H ₃₀ O ₈ N ₅	62.12	5.54	9.06	62.76	6.06	9.15	0.73		
Z-Gly-Phe-Ser-Pro-ONb (monohydrate)	88	EtOAc-pet. ether	60-63	-30.2°(0.6, DMF, 14)	C ₂₈ H ₃₄ O ₈ N ₅ · H ₂ O	58.77	5.62	9.52	58.37	5.63	9.37	0.69		
Z-Gly-Phe-Ser-Pro (hemihydrate)	75	EtOAc-pet. ether	50-60	-20.8°(0.6, DMF, 18)	C ₂₇ H ₃₃ O ₈ N ₅ · ½H ₂ O	59.01	6.05	10.20	59.31	5.98	9.89	0.42		
Gly-Phe-Ser-Pro	99	H ₂ O	143-153	-42.5°(0.6, H ₂ O, 18)	C ₁₆ H ₂₀ O ₄ N ₂	56.14	6.45	13.79	55.88	6.97	13.26	0.45		
Z-Pro-Gly-Phe-Ser-Pro-ONb (hemihydrate)	69	EtOAc-pet. ether	80-92	-32.6°(0.9, DMF, 14)	C ₄₁ H ₄₈ O ₁₃ N ₉ · ½H ₂ O	59.77	5.57	10.20	59.79	5.95	9.77	0.73		
Z-Pro-Gly-Phe-Ser-Pro (monohydrate)	73	THF-pet. ether	54-58	-42.9°(0.6, DMF, 18)	C ₃₃ H ₄₀ O ₁₀ N ₇ · H ₂ O	58.61	6.30	10.68	58.81	6.20	10.01	0.37		
Pro-Gly-Phe-Ser-Pro (pentahydrate)	84	H ₂ O	115-120	-60.6°(0.5, H ₂ O, 18)	C ₃₃ H ₄₀ O ₁₀ N ₇ · 5H ₂ O	48.56	7.30	11.80	48.40	6.94	11.44	0.39		
Z-Pro-Gly-Phe-Ser-Pro-ONb (monohydrate)	60	EtOAc-pet. ether	98-104	-37.3°(0.4, DMF, 14)	C ₄₆ H ₅₄ O ₁₄ N ₁₀ · H ₂ O	59.41	5.96	10.54	59.17	6.09	10.14	0.73		

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of the synthesis of the every kinds of bradykinin fragments and results of the bioassay with the synthetic bradykinin fragments are described.

The method of the peptide synthesis used here is virtually similar with a previous paper on the synthesis of bradykinin and its analogues,⁸⁾ in which the *p*-nitrophenyl ester method, a stepwise elongation procedure, and the azide procedure only for the introducing seryl residue is used, and the protecting group of C-terminal arginine residue is a *p*-nitrobenzyl ester group. Also, the protecting group of N-terminus was a benzyloxycarbonyl group except a *t*-butyloxycarbonyl group for serine. In the present investigation, the *p*-nitrobenzyl ester group for the protection of the carboxyl groups of proline (position 7) and arginine (position 9) is used, since proline *p*-nitrobenzyl ester hydrobromide forms fine needles and N^ω-nitroarginine *p*-nitrobenzyl ester has been used in a previous paper.⁸⁾ A methyl or ethyl ester group for the others is used, since the benzyloxycarbonyl group of benzyloxycarbonylpeptide methyl or ethyl ester containing a serine residue is removed by catalytic hydrogenolysis without acetylation of a hydroxyl group of serine residue which causes during the treatment with hydrobromic acid-acetic acid.⁹⁾ The synthetic routes of the two series of peptides starting with N^ω-nitroarginine *p*-nitrobenzyl ester (position 9) and phenylalanine methyl ester (position 5) are illustrated in Fig. 1 and 2 respectively as examples.

Fig. 1. Synthesis of Prolylprolylglycylphenylalanylserylprolylphenylalanylarginine and Its Fragments, as an Example of Synthetic Approach starting with Amino Acid *p*-Nitrobenzyl Esters

In Table I, the compounds are arranged in the order as follows: main starting materials, blocked peptides, and deblocked peptides which were synthesized starting with arginine (position 9), phenylalanine (position 8), proline (position 7) and so on. In Table I, even known compounds prepared by another approach in the literature, have been synthesized in this laboratory by the method described above, but benzyloxycarbonylprolylprolylglycine ethyl ester have been synthesized by the dicyclohexylcarbodiimide procedure for the reason described below. For paper chromatography, the protected peptides having *p*-nitrobenzyl

8) K. Suzuki, T. Abiko and M. Asaka, *Chem. Pharm. Bull.* (Tokyo), **14**, 217 (1966).

9) St. Guttmann and R.A. Boissonnas, *Helv. Chim. Acta*, **42**, 1257 (1959).

Fig. 2. Synthesis of Arginylprolylprolylglycylphenylalanine and Its Fragments, as an Example of Synthetic Approach starting with Amino Acid Methyl Esters

group in the C-terminus were treated with hydrobromic acid in acetic acid and the protected peptides having a methyl or ethyl group in the C-terminus were hydrogenated in the presence of 10 percent palladium on charcoal for the removal of the benzyloxycarbonyl group, and the amino group free peptides were chromatographed on a filter paper, Toyo Roshi No. 51, at room temperature. Rf^1 values refer to the Partridge system,¹⁰ and Rf^2 values refer to the system of *n*-butanol-pyridine-acetic acid-water (30:20:6:24).¹¹ The chromatograms were stained with ninhydrin and with Sakaguchi reagent for the arginine-containing peptides.

In a series of peptides synthesized starting with proline *p*-nitrobenzyl ester (position 7), the fully protected peptides except N^α-benzyloxycarbonyl-N^ω-nitroarginylprolylprolylglucylphenylalanylserylproline *p*-nitrobenzyl ester were hydrogenated in the presence of 10 percent palladium on charcoal and the hydrogenated products were put on a column of Dowex 50 × 8 to purify, which was eluted with ammonium acetate buffer solution. Elemental analysis of these peptides gave about 50 percent of ash being probably due to solubles of the resin. Therefore, the fully protected peptides were saponified to remove the *p*-nitrobenzyl group followed by hydrogenolysis to yield chemically pure peptides.

In an attempt of the synthesis of benzyloxycarbonylprolylprolylglycine ethyl ester, a coupling product of benzyloxycarbonylproline *p*-nitrophenyl ester and prolylglycine ethyl ester hydrochloride in the presence of equivalent triethylamine was hydrogenated to remove the benzyloxycarbonyl group, and the hydrogenated product gave two spots on paper chromatograms using two different solvent systems. R_f^1 was 0.27 and 0.49, and R_f^2 was 0.33 and 0.62 respectively. The color of the two spots stained with ninhydrin was yellow and the area of the two spots was about fifty-fifty. R_f^1 0.49 and R_f^2 0.62 corresponded to that of prolylprolylglycine ethyl ester hydrochloride derived from the benzyloxycarbonylprolylprolylglycine ethyl ester which was synthesized by coupling benzyloxycarbonylprolylproline and glycine ethyl ester with the dicyclohexylcarbodiimide procedure according to the direction given by Guttmann.¹²⁾ Identification of the by-product is remained to be done.

Quantitative examinations were made on the contracting effect of an isolated guinea pig ileum, antibradykinin activity, and potentiation of bradykinin on the contracting effect with the bradykinin fragments synthesized in the present work.¹³⁾ The assay for inflammation in

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

11) S.G. Waley and G. Watson, *Biochem. J.*, **55**, 328 (1953).

the rat's hind paw¹⁴⁾ and the assay for vasodilation with few bradykinin fragments were made. Results of these biological examinations are given in Table II.

TABLE II. Biological Activities of Synthetic Bradykinin Fragments

Peptides ^{a)}	Guinea Pig Ileum ^{b)}			Rat Edema ^{c)}	Vasodepression in Rat
	Contraction	Potentiation	Inhibition		
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	100.000	—	—	100.00	100.000
Phe-Arg	0.016	—	—	56.07	0.000
Pro-Phe-Arg	0.004	—	—	112.42	0.061
Ser-Pro-Phe-Arg	0.014	+ ^{d)}	—	39.39	0.205
Phe-Ser-Pro-Phe-Arg	2.915	—	—	46.06	0.007
Gly-Phe-Ser-Pro-Phe-Arg	0.000	—	—	59.01	
Pro-Gly-Phe-Ser-Pro-Phe-Arg	0.214	—	—	71.56	0.008
Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	0.029	—	—	72.10	
Pro-Phe	0.001	—	—	20.43	
Ser-Pro-Phe	0.003	—	—	30.97	0.045
Phe-Ser-Pro-Phe	0.026	—	—	35.25	0.002
Gly-Phe-Ser-Pro-Phe	0.000	—	—	64.62	
Pro-Gly-Phe-Ser-Pro-Phe	0.000	—	—	86.78	
Pro-Pro-Gly-Phe-Ser-Pro-Phe	0.000	—	—	91.46	0.151
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe	0.000	—	—	93.32	
Ser-Pro	0.002	—	—	27.64	
Phe-Ser-Pro	0.000	—	—	30.97	
Gly-Phe-Ser-Pro	0.000	—	—	21.30	
Pro-Gly-Phe-Ser-Pro	0.000	—	—	10.14	
Pro-Pro-Gly-Phe-Ser-Pro	0.000	—	—	16.73	
Arg-Pro-Pro-Gly-Phe-Ser-Pro	0.000	—	—	24.34	
Phe-Ser	0.000	—	—	73.56	0.000
Gly-Phe-Ser	0.000	—	—	42.19	
Pro-Gly-Phe-Ser	0.000	—	—	32.04	
Pro-Pro-Gly-Phe-Ser	0.004	—	—	40.45	
Arg-Pro-Pro-Gly-Phe-Ser	0.000	—	—	75.03	0.000
Gly-Phe	0.001	—	—	51.94	0.027
Pro-Gly-Phe	0.001	—	—	39.25	
Pro-Pro-Gly-Phe	0.001	—	—	16.42	
Arg-Pro-Pro-Gly-Phe	0.005	—	—	46.04	0.094
Pro-Gly	0.001	+ ^{e)}	—	3.64	0.000
Pro-Pro-Gly	0.000	—	—	6.64	
Arg-Pro-Pro-Gly	0.000	—	—	39.79	0.052
Pro-Pro	0.000	—	—	26.30	0.025
Arg-Pro-Pro	0.000	—	—	24.43	0.202
Arg-Pro	0.098	—	—	22.29	0.002

a) Water of crystallization for acetate is not expressed.

b) Assayed by Magnus method on a guinea pig ileum (male).

c) Ten μ g of the peptides were injected into rat paw.

d) At a concentration of 1.5×10^{-5} M in both, caused 36% potentiation of the normal contraction due to 1.8×10^{-9} M of bradykinin.

e) At a concentration of 6.4×10^{-5} M, caused 37% potentiation of the normal contraction due to 3.0×10^{-9} M of bradykinin.

The activity, except inflammatory activity, is calculated on molar concentration per kilogram of body weight. Of these synthetic peptides, arginylproline, prolylprolylglycylphenylalanylserylprolylphenylalanylarginine, prolylglycylphenylalanylserylprolylphenylala-

12) St. Guttman, J. Pless and R.A. Boissonnas, *Helv. Chim. Acta*, **45**, 170 (1962).

13) The details of the biological assays will be reported in separate paper by Dr. Tsutomu Kameyama of this college.

14) E. Stürmer and A. Cerletti, *Helv. Physiol. Acta*, Suppl. **XI**, C32 (1961).

nylarginine, phenylalanylserylprolylphenylalanylarginine, and phenylalanylserylprolylphenylalanine showed relatively high activity as compared with that of the other synthetic bradykinin fragments in the assay on contracting effect of an isolated guinea pig ileum. The dose response curves for phenylalanylserylprolylphenylalanylarginine and phenylalanylserylprolylphenylalanine containing two phenylalanine residues of bradykinin were exactly in parallel with that of bradykinin, but that of the other three synthetic peptides were not. As described in a previous communication,⁶⁾ these facts suggest the similarity of the mode of action of phenylalanylserylprolylphenylalanylarginine, phenylalanylserylprolylphenylalanine, and bradykinin. The importance of the aromatic ring in the bradykinin molecule for the biological activity has been suggested from the results of bioassay of synthetic bradykinin analogues.¹⁵⁾ In addition, as described in a review by Stewart,¹⁶⁾ bradykinin analogues thus far studied indicate that phenylalanine residues can be replaced by other one or both of aromatic amino acids (*e.g.*, tyrosine, tyrosine methyl ether, *p*-fluorophenylalanine) and even by L-phenyllactic acid¹⁷⁾ with retention of the significant activity, while incorporation of aliphatic residues (glycine, alanine, leucine) at these position causes serious loss of the activity. The authors have presented the further evidences of the importance of the phenylalanine residues in the bradykinin molecule so far as concerning with the contracting effect on an isolated guinea pig ileum. The phenylalanylserylprolylphenylalanine moiety of bradykinin seems to be the active site (or active area) of the bradykinin molecule so far as concerning with the contracting effect. However, the activity of the other synthetic bradykinin fragments containing the phenylalanylserylprolylphenylalanine moiety was very low or practically inactive as shown in Table II. This discrepancy is presumably due to differences of the conformation in the neighborhood of the phenylalanine residues of the peptides. In the assay for inflammation in the rat's hind paw¹⁴⁾ as shown in Table II, most of the synthetic small peptides showed fairly high activity. In connection with the inflammatory activity of these small peptides, it is of interest that the increase of proteolytic activity in inflammatory tissues have been demonstrated in the course of Arthus-type inflammation by Hayashi.¹⁸⁾ As may be seen in Table II, the vasodepressor activity of the synthetic bradykinin fragments in rat have not been investigated systematically as yet, however, it is evident thus far studied in this laboratory that the contracting effect on an isolated guinea pig ileum with the synthetic bradykinin analogues and the vasodepressor activity is not in parallel as have been shown from the results of the bioassay of synthetic bradykinin analogues by the different research groups.⁵⁾ These evidences seem to suggest the differences of active site of the two biological activity.

Experimental

Melting points are uncorrected. The amino acid composition of acid hydrolysates was determined according to the directions given by Moore, *et al.*¹⁹⁾ All of the synthesized compounds are listed in Table I.

General Procedure for the Synthesis of the Peptides starting with Amino Acid *p*-Nitrobenzyl Ester—N-Benzylloxycarbonyl amino acid *p*-nitrobenzyl ester (0.01 mole) was treated with 3N HBr in AcOH (50 ml) for 30 min to 1 hr in the presence of anisole (1 ml), when dry ether was added. The resulting HBr salt of amino acid *p*-nitrobenzyl ester in dimethylformamide (DMF) (20 ml) was added 10% excess of N-benzylloxycarbonyl amino acid *p*-nitrophenyl ester (0.011 mole) followed by addition of Et₃N to keep the solution slightly alkaline and stirred overnight. To the reaction mixture 1N NH₄OH (about 10 ml) was added to saponify the unchanged *p*-nitrophenyl ester and stirred for 1 hr. The reaction mixture was treated in two manner. When the product was soluble in EtOAc, the mixture was diluted with EtOAc and the EtOAc solution was washed successively with H₂O, 1N NH₄OH, H₂O, 1N HCl, and H₂O. The EtOAc layer was

15) E. Schröder and R. Hempel, *Experientia*, **20**, 529 (1964).

16) J.M. Stewart, *Federation Proc.*, **27**, 63 (1968).

17) G.A. Ravdel, M.P. Filatova and L.A. Shchukina, *J. Med. Chem.*, **10**, 242 (1967).

18) H. Hayashi, K. Udaka, H. Miyoshi and S. Kudo, *Lab. Invest.*, **14**, 665 (1965).

19) S. Moore, D.H. Spackman and W.H. Stein, *Anal. Chem.*, **30**, 1185 (1958).

dried over MgSO_4 , concentrated to small volume, and added petroleum ether to give the product which was reprecipitated or recrystallized from appropriate solvents listed in Table I. When the product was insoluble in EtOAc, the reaction mixture was poured into an ice cold 1N NH_4OH (about 200 ml) with stirring. The precipitate thereby formed was washed successively with 1N NH_4OH , H_2O , 1N HCl , and H_2O and purified as described above. The coupling of the amino acid remained to be elongated to the protected peptide was carried out in the same manner as described above, except fragment condensation using N-benzyloxycarbonylprolylglycine *p*-nitrophenyl ester or N $^{\alpha}$ -benzyloxycarbonyl-N $^{\omega}$ -nitroarginylproline *p*-nitrophenyl ester and the azide procedure for introducing seryl residue. The fully protected peptide so obtained were deblocked by catalytic hydrogenolysis as usual manner to yield the final peptide, but in a series of peptides synthesized starting with proline *p*-nitrobenzyl ester (position 7), as described in the text, the fully protected peptides were saponified with 1N NaOH followed by catalytic hydrogenolysis to yield the desired peptides. Arginine-containing peptides were purified through carboxymethyl cellulose column which was eluted with a linear gradient method from H_2O in a mixing chamber to a 0.06M pyridinium acetate buffer (pH 5.1) in a reservoir and the arginine-containing peptide was located in the eluates by Sakaguchi reaction, followed by lyophilization to yield the desired peptides.

General Procedure for Synthesis of Peptides starting with Amino Acid Methyl or Ethyl Esters—Amino acid methyl or ethyl ester hydrochloride (0.01 mole) in DMF (40 ml) was added 10% excess of N-benzyloxycarbonyl amino acid *p*-nitrophenyl ester (0.011 mole) followed by addition of Et_3N to keep the solution slightly alkaline and treated for purification as described above. After the removal of the benzyloxycarbonyl group by catalytic hydrogenolysis, the coupling of the amino acid remained to be elongated to the protected peptide was carried out as described above. The fully protected peptides (0.01 mole) so obtained were saponified with 1N NaOH (11 ml) in MeOH (35 ml) and the resulting carboxyl free peptides were hydrogenated as usual manner to yield the desired peptides.

Acknowledgement The authors wish to thank Mmes. Y. Abiko (née Ito) and N. Wakayama (née Ito) for the preparation of a part of the starting materials, Prof. T. Kameyama, Messrs. K. Sasaki and J. Nabeshima for biological assay, and the staff of Central Analysis Laboratory, Department of Chemistry, Faculty of Sciences, University of Tohoku for elemental analysis.