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Synthesis of Every Kinds of Peptide Fragments of Bradykinin[1]

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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, inframmatory 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 fairy 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 B[3] and carboxypeptidase N,[4] destroy bradykinin by cleaving the peptide bond of the nonapeptide, producing pharmacologically inert peptides. However, there is a posibility of the appearance of the other bradykinin fragments resulting from unkown 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, phenylalanylserylprolyphenylalanine 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-configration 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)	$[\alpha]_D$ (conc., solvt., temp. °C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N	Rf^1	Rf^2	lit.
Z-Phe-Arg(NO_2)-ONb	84	EtOAc	172—173	−44.1°(1.0, MeOH, 20)	$C_{30}H_{33}O_9N_7$	56.70	5.20	15.40	56.50	5.40	15.60	0.59	0.87	*a, b*
Phe-Arg (monoacetate, hemihydrate)	57	H_2O	135—145	+7.3°(0.7, H_2O, 24)	$C_{15}H_{23}O_3N_5 \cdot CH_3COOH \cdot \frac{1}{2}H_2O$	52.29	7.27	17.94	51.94	7.32	17.43	0.32	0.87	
Z-Pro-Phe-Arg(NO_2)-ONb	70	EtOAc-pet. ether	72—89	−10.4°(1.1, MeOH, 22)	$C_{35}H_{40}O_{10}N_8$	57.40	5.50	15.30	58.10	5.50	14.80	0.70	0.87	*a, b*
Pro-Phe-Arg (monoacetate, dihydrate)	78	H_2O	137—142	−16.4°(0.6, H_2O, 24)	$C_{20}H_{30}O_4N_6 \cdot CH_3COOH \cdot 2H_2O$	51 35	7.44	16.33	50.85	7.17	16.42	0.30	0.45	
Z-Ser-Pro-Phe-Arg(NO_2)-ONb	66	EtOAc-pet. ether	101—106	−50.5°(0.9, AcOH, 18)	$C_{38}H_{45}O_{12}N_9$	55.67	5.53	15.38	55.76	5.38	15.05	0.65	0.87	*a*
Ser-Pro-Phe-Arg (monoacetate, monohydrate)	76	H_2O	139—155	−47.1°(0.2, H_2O, 24)	$C_{23}H_{35}O_6N_7 \cdot CH_3COOH \cdot H_2O$	51.44	7.08	16.08	51.14	7.48	16.41	0.34	0.47	
Z-Phe-Ser-Pro-Phe-Arg(NO_2)-ONb	38	DMF-H_2O	225—226	−36.4°(1.2, AcOH, 14)	$C_{49}H_{56}O_{14}N_{10}$	58.32	5.59	13.88	57.84	5.47	13.68	0.73	0.89	*a*
Phe-Ser-Pro-Phe-Arg (monoacetate, dihydrate)[v]	75	H_2O	143—152	−37.6°(0.3, H_2O, 28)	$C_{32}H_{44}O_7N_8 \cdot CH_3COOH \cdot 2H_2O$	54.53	6.99	14.96	54.40	7.01	15.35	0.48	0.59	*c*
Z-Gly-Phe-Ser(Ac)-Pro-Phe-Arg(NO_2)-ONb	86	acetone-ether	180—182	−35.4°(0.5, AcOH, 19)	$C_{51}H_{59}O_{13}N_{11}$	57.46	5.58	14.45	57.40	5.90	13.84	0.76	0.92	
Gly-Phe-Ser-Pro-Phe-Arg (diacetate, monohydrate)	92	H_2O	153—159	−15.6°(0.7, H_2O, 24)	$C_{35}H_{47}O_8N_9 \cdot 2CH_3COOH \cdot H_2O$	54.47	5.39	14.66	54.54	5.12	14.18	0.47	0.49	
Z-Pro-Gly-Phe-Ser(Ac)-Pro-Phe-Arg(NO_2)-ONb	72	AcOH-H_2O	119—125	−48.1°(1.0, AcOH, 27)	$C_{56}H_{66}O_{16}N_{12}$	57.82	5.72	14.44	57.62	5.93	13.90	0.79	0.94	*a*
Pro-Gly-Phe-Ser-Pro-Phe-Arg (diacetate, monohydrate)	41	H_2O	155—165	−35.4°(0.7, H_2O, 24)	$C_{39}H_{54}O_8N_{10} \cdot 2CH_3COOH \cdot H_2O$	54.65	6.83	14.82	54.38	7.25	14.93	0.41	0.43	
Z-Pro-Pro-Gly-Phe-Ser(Ac)-Pro-Phe-Arg(NO_2)-OMe	75	MeOH-EtOAc	150—152	−64.5°(1.0, DMF, 23)	$C_{53}H_{68}O_{14}N_{12}$	58.01	6.25	15.32	57.66	6.13	14.98	0.66	0.76	*d, e*
Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg	55	H_2O	187—191	−115°(1.4, H_2O, 24)	$C_{44}H_{60}O_{10}N_{11} \cdot 2CH_3COOH \cdot H_2O$	55.37	6.78	14.80	55.89	7.01	14.46	0.40	0.64	*f*
Z-Pro-Phe-ONb (monoacetate, monohydrate)	75	EtOAc-pet. ether	88—90	−37.2°(1.5, DMF, 23)	$C_{28}H_{30}O_7N_3 \cdot CH_3COOH \cdot H_2O$	60.97	5.94	6.88	60.50	5.66	7.11	0.76	0.87	*g*
Pro-Phe	41	H_2O-EtOH	264	−41.7°(1.7, 6N HCl, 25)	$C_{14}H_{20}O_3N_2$	59.98	7.19	9.90	59.74	7.16	10.21	0.61	0.63	*h*
Z-Ser-Pro-Phe-OMe	80	EtOAc-pet. ether	40—44	−41.1°(0.8, DMF, 17)	$C_{26}H_{31}O_7N_3 \cdot H_2O$	60.57	6.45	8.15	60.75	6.18	7.55	0.65	0.84	*i*
Z-Ser-Pro-Phe (monodiclycohexylamine, monohydrate)	75	MeOH-ether	152—154	−35.6°(1.2, MeOH, 14)	$C_{25}H_{29}O_7N_3 \cdot C_{12}H_{23}N \cdot H_2O$	65.08	7.97	8.21	64.81	7.98	7.90	0.50	0.55	
Ser-Pro-Phe (di- and hemihydrate)	37	H_2O-EtOH	224	−1.0°(0.2, H_2O, 25)	$C_{17}H_{23}O_5N_3 \cdot 2\frac{1}{2}H_2O$	51.77	7.16	10.66	52.14	7.00	10.76	0.50	0.55	
Z-Phe-Ser-Pro-Phe-OMe (hemihydrate)	41	EtOAc-EtOH	94—96	−18.7°(0.8, DMF, 17)	$C_{35}H_{40}O_8N_4 \cdot \frac{1}{2}H_2O$	64.30	6.32	8.57	64.35	6.48	8.80	0.70	0.89	*i*
Z-Phe-Ser-Pro-Phe (dihydrate)	60	EtOAc-pet. ether	109—116	−29.7°(1.0, DMF, 17)	$C_{34}H_{38}O_8N_4 \cdot 2H_2O$	61.25	6.35	8.40	61.29	6.17	8.24	0.76	0.78	
Phe-Ser-Pro-Phe (tetrahydrate)[w]	87	H_2O	147—150	−15.6°(0.8, H_2O, 24)	$C_{26}H_{36}O_5N_4 \cdot 4H_2O$	56.71	6.96	10.18	56.58	6.77	10.12	0.71	0.86	
Z-Gly-Phe-Ser-Pro-Phe-OMe	77	MeOH-H_2O	112—114	−32.4°(1.0, DMF, 23)	$C_{37}H_{43}O_9N_5$	63.31	6.17	9.98	63.19	6.08	10.13	0.73	0.91	*i*
Z-Gly-Phe-Ser-Pro-Phe	65	acetone-ether	175—180	−28.1°(0.6, DMF, 21)	$C_{36}H_{41}O_{14}N_5$	55.30	7.09	8.96	55.98	5.67	9.04	0.68	0.72	
Gly-Phe-Ser-Pro-Phe (dihydrate)	47	H_2O	190—195	−26.5°(0.3, 1N HCl, 24)	$C_{28}H_{35}O_7N_5 \cdot 2H_2O$	57.03	6.67	11.88	56.64	6.75	11.41	0.68	0.72	
Z-Pro-Gly-Phe-Ser-Pro-Phe-OMe	79	acetonitrile-ether	153—155	−39.3°(0.4, DMF, 14)	$C_{42}H_{50}O_{10}N_6$	63.14	6.31	10.32	63.02	6.54	10.39	0.63	0.85	*i*
Z-Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	39	acetone-ether	128—132	−34.0°(0.8, DMF, 14)	$C_{41}H_{48}O_{10}N_6 \cdot 2H_2O$	59.66	6.39	10.24	59.52	6.37	9.73	0.65	0.74	
Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	96	H_2O	164—167	−50.8°(0.6, H_2O, 14)	$C_{33}H_{42}O_8N_6 \cdot 2H_2O$	57.71	6.75	12.24	57.65	6.89	12.48	0.65	0.74	
Z-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OMe(trihydrate)	47	EtOAc-pet. ether	114—120	−34.7°(0.8, DMF, 14)	$C_{47}H_{67}O_{11}N_7 \cdot 3H_2O$	58.78	7.66	10.21	58.98	7.83	10.22	0.72	0.80	
Z-Pro-Pro-Gly-Phe-Ser-Pro-Phe (dihydrate)	55	acetone-ether	131—136	−25.2°(0.8, DMF, 14)	$C_{46}H_{55}O_{11}N_7 \cdot 2H_2O$	60.18	6.48	10.68	60.20	6.60	9.96	0.61	0.73	
Pro-Pro-Gly-Phe-Ser-Pro-Phe (monohydrate)	95	H_2O	163—168	−49.6°(0.4, H_2O, 14)	$C_{38}H_{49}O_9N_7 \cdot H_2O$	59.59	6.71	12.80	59.64	6.38	12.48	0.61	0.73	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OMe (trihydrate)	51	acetone-ether	124—131	−45.5°(0.4, DMF, 14)	$C_{59}H_{68}O_{14}N_{12} \cdot 3H_2O$	55.29	6.48	14.60	54.95	6.61	14.05	0.61	0.83	*i*
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-Pro-Phe (tetrahydrate)	56	MeOH-ether	140—147	−76.4°(0.2, DMF, 14)	$C_{52}H_{66}O_{14}N_{12} \cdot 4H_2O$	53.23	6.53	14.33	53.52	6.25	14.41	0.46	0.61	*i*
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe (monoacetate, dihydrate)	84	H_2O	173—181	−82.0°(0.6, H_2O, 14)	$C_{44}H_{61}O_{10}N_{11} \cdot CH_3COOH \cdot 2H_2O$	55.24	6.96	15.41	55.03	6.89	15.12	0.50	0.62	*i*
Z-Ser-Pro (monohydrate)	26	EtOAc-ether	105—108	−26.7°(0.8, DMF, 21)	$C_{16}H_{20}O_6N_2 \cdot H_2O$	54.23	6.26	7.91	54.68	6.02	8.21	0.26	0.29	
Ser-Pro	99	H_2O-EtOH	153—166	−85.2°(0.9, H_2O, 24)	$C_8H_{14}O_4N_2$	57.52	6.98	13.86	47.49	7.12	13.18	0.18	0.31	*j*
Z-Phe-Ser-Pro	66	EtOAc-ether-pet. ether	75—86	−50.7°(0.7, DMF, 21)	$C_{25}H_{29}O_7N_3$	62.10	6.05	8.69	61.73	6.06	8.50	0.45	0.52	
Phe-Ser-Pro (monohydrate)	99	H_2O	125—133	−24.7°(0.9, H_2O, 24)	$C_{17}H_{23}O_5N_3 \cdot H_2O$	55.57	6.86	11.44	55.30	6.87	11.28	0.45	0.52	
Z-Pro-ONb		EtOAc-pet. ether	oil		$C_{19}H_{18}O_6N_2$							0.55	0.83	
HBr-Pro-ONb		MeOH-ether	171	−21.8°(1.1, DMF, 7)	$C_{12}H_{14}O_4N_2 \cdot HBr$							0.55	0.83	
Z-Phe-Ser-Pro-ONb	77	EtOAc-pet. ether	58—64	−73.0°(0.4, DMF, 21)	$C_{32}H_{34}O_9N_4$	62.12	5.54	9.06	62.76	6.06	9.15	0.73	0.87	
Z-Gly-Phe-Ser(Ac)-Pro-ONb (monohydrate)	88	EtOAc-pet. ether	60—63	−30.2°(0.6, DMF, 14)	$C_{36}H_{39}O_{11}N_5 \cdot H_2O$	58.77	5.62	9.52	58.37	5.63	9.37	0.69	0.86	
Z-Gly-Phe-Ser-Pro (hemihydrate)	75	EtOAc-pet. ether	50—60	−20.8°(0.6, DMF, 18)	$C_{27}H_{32}O_8N_4 \cdot \frac{1}{2}H_2O$	59.01	6.05	10.20	59.31	5.98	9.89	0.42	0.49	
Gly-Phe-Ser-Pro	99	H_2O	143—153	−42.5°(0.6, H_2O, 18)	$C_{18}H_{26}O_6N_4$	56.14	6.45	13.79	55.88	6.97	13.26	0.45	0.51	
Z-Pro-Gly-Phe-Ser(Ac)-Pro-ONb (hemihydrate)	69	EtOAc-pet. ether	80—92	−32.6°(0.9, DMF, 14)	$C_{41}H_{46}O_{12}N_6 \cdot \frac{1}{2}H_2O$	59.77	5.57	10.20	59.79	5.95	9.77	0.73	0.91	
Z-Pro-Gly-Phe-Ser-Pro (monohyhdrate)	73	THF-pet. ether	54—58	−42.9°(0.6, DMF, 18)	$C_{32}H_{39}O_9N_5 \cdot H_2O$	58.61	6.30	10.68	58.81	6.20	10.01	0.37	0.40	
Pro-Gly-Phe-Ser-Pro (pentahydrate)	84	H_2O	115—120	−60.6°(0.5, H_2O, 18)	$C_{24}H_{33}O_7N_5 \cdot 5H_2O$	48.56	7.30	11.80	48.40	6.94	11.44	0.39	0.49	
Z-Pro-Pro-Gly-Phe-Ser(Ac)-Pro-ONb (monohydrate)	60	EtOAc-pet. ether	98—104	−37.3°(0 4, DMF, 14)	$C_{46}H_{53}O_{13}H_7 \cdot H_2O$	59.41	5.96	10.54	59.17	6.09	10.14	0.73	0.89	

Z-Pro-Pro-Gly-Phe-Ser-Pro (tri- and hemihydrate)	55	EtOAc-pet. ether	82—90	−58.1°(0.5, DMF, 18)	$C_{37}H_{48}O_{10}N_6 \cdot 3\frac{1}{2}H_2O$	55.70	6.70	10.53	55.72	5.98	9.98	0.41	0.50	
Pro-Pro-Gly-Phe-Ser-Pro (pentahydrate)	77	H_2O	154—160	−63.6°(0.5, H_2O, 18)	$C_{29}H_{40}O_8N_6 \cdot 5H_2O$	50.42	7.30	12.17	49.96	6.94	12.66	0.44	0.54	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Ac)-Pro-ONb	48	acetone-ether	114—120	−63.3°(0.5, DMF, 14)	$C_{52}H_{64}O_{16}N_{12} \cdot 3H_2O$	53.51	6.05	14.40	53.63	6.06	14.26	0.53	0.89	
Arg-Pro-Pro-Gly-Phe-Ser-Pro (monoacetate, dihydrate)	90	H_2O	170—184	−75.9°(0.4, H_2O, 14)	$C_{35}H_{52}O_9N_{10} \cdot CH_3COOH \cdot 2H_2O$	52.10	7.09	16.42	52.31	6.65	16.17	0.31	0.39	
Z-Phe-Ser-OMe	95	EtOAc-pet. ether	122—123	−5.7°(2.0, DMF, 23)	$C_{21}H_{24}O_6N_2$	63.00	6.00	7.00	63.10	6.10	7.10	0.58	0.80	*b, d*
Z-Phe-Ser	62	EtOAc-ether	147—149	+44.8°(0.4, DMF, 21)	$C_{20}H_{22}O_6N_2$	62.16	5.74	7.25	61.97	5.78	7.25	0.53	0.51	*k*
Phe-Ser (monohydrate)	51	H_2O-acetone	116—125	+30.1°(0.7, H_2O, 24)	$C_{12}H_{16}O_4N_2 \cdot H_2O$	53.32	6.71	10.37	53.28	6.46	10.19	0.48	0.57	
Z-Gly-Phe-Ser-OMe (hemihydrate)	50	EtOAc-ether	135—138	0.0°(0.8, DMF, 21)	$C_{23}H_{27}O_7N_3 \cdot \frac{1}{2}H_2O$	59.22	6.05	9.01	59.61	5.69	9.04	0.55	0.77	
Z-Gly-Phe-Ser	59	EtOAc-ether	154—155	−15.9°(0.6, DMF, 21)	$C_{22}H_{25}O_7N_3$	59.58	5.68	9.48	59.33	5.55	9.25	0.43	0.57	
Gly-Phe-Ser (hemihydrate)	73	H_2O-acetone	145—155	+28.1°(0.4, H_2O, 24)	$C_{14}H_{19}O_5N_3 \cdot \frac{1}{2}H_2O$	52.82	6.33	13.20	52.36	6.19	13.13	0.38	0.44	
Z-Pro-Gly-Phe-Ser-OMe	58	EtOAc-ether-pet. ether	85—95	−39.2°(0.7, DMF, 21)	$C_{26}H_{34}O_8N_4$	60.64	6.18	10.10	60.76	5.90	10.17	0.61	0.72	*l*
Z-Pro-Gly-Phe-Ser	41	EtOAc-ether	162—168	−40.3°(0.8, DMF, 24)	$C_{27}H_{32}O_8N_4$	59.99	5.97	10.37	60.39	6.03	10.50	0.40	0.45	
Pro-Gly-Phe-Ser (monohydrate)	63	H_2O-acetone	170—185	−22.4°(0.9, H_2O, 24)	$C_{19}H_{25}O_6N_4 \cdot H_2O$	53.89	6.43	13.23	54.18	6.65	13.38	0.48	0.53	
Z-Pro-Pro-Gly-Phe-Ser-OMe	46	EtOAc-pet. ether	65—74	−29.2°(0.7, DMF, 21)	$C_{33}H_{41}O_9N_5$	60.81	6.34	10.75	60.80	6.72	10.09	0.61	0.74	
Z-Pro-Pro-Gly-Phe-Ser (sesquihydrate)	66	EtOAc-ether	101—113	−18.8°(0.4, DMF, 21)	$C_{32}H_{39}O_9N_5 \cdot 1\frac{1}{2}H_2O$	57.82	6.37	10.54	58.18	6.53	9.90	0.41	0.51	
Pro-Pro-Gly-Phe-Ser (trihydrate)	98	H_2O	165—174	−29.7°(0.6, H_2O, 24)	$C_{24}H_{33}O_7N_5 \cdot 3H_2O$	51.69	7.05	12.56	51.23	6.64	12.92	0.40	0.51	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser-OMe	42	EtOAc-ether	110—118	−28.4°(0.4, DMF, 21)	$C_{39}H_{52}O_{12}N_{10}$	54.92	6.15	16.42	55.27	6.40	16.24	0.47	0.72	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser	70	Acetone-ether	115—122	−31.3°(0.6, DMF, 21)	$C_{38}H_{50}O_{12}N_{10}$	54.41	6.01	16.70	54.65	6.41	16.87	0.50	0.55	
Arg-Pro-Pro-Gly-Phe-Ser (diacetate, dihydrate)	84	H_2O	158—173	−49.4°(0.9, H_2O, 24)	$C_{30}H_{45}O_8N_9 \cdot 2CH_3COOH \cdot 2H_2O$	50.05	7.04	15.45	50.50	7.37	14.99	0.34	0.42	
Z-Gly-Phe-OMe	82	EtOAc-pet. ether	oil	−2.1°(0.7, DMF, 22)	$C_{20}H_{22}O_5N_2$							0.60	0.80	
Z-Gly-Phe	87	EtOAc-pet. ether	125—126	−30.8°(1.3, DMF, 23)	$C_{19}H_{20}O_5N_2$	64.03	5.66	7.86	63.99	5.61	7.65	0.50	0.50	*m, n, o*
Gly-Phe	88	H_2O-EtOH	223—224	+3.3°(0.7, H_2O, 23)	$C_{11}H_{14}O_3N_2$	59.43	6.35	12.61	59.16	6.22	12.72	0.42	0.45	*m*
Z-Pro-Gly-Phe-OMe	87	EtOAc-pet. ether	92—93	−7.0°(1.8, DMF, 21)	$C_{25}H_{29}O_6N_3$	64.22	6.14	8.99	63.97	6.25	8.69	0.65	0.74	*p*
Z-Pro-Gly-Phe	77	EtOAc-pet. ether	160	−9.8°(2.1, DMF, 21)	$C_{24}H_{27}O_6N_3$	63.56	6.00	9.26	63.73	6.22	9.11	0.30	0.53	*p, q*
Pro-Gly-Phe	84	H_2O-EtOH	237—238	+1.1°(0.8, H_2O, 22)	$C_{16}H_{21}O_4N_3$	60.17	6.63	13.16	59.92	6.45	13.09	0.47	0.52	*p, q*
Z-Pro-Pro-Gly-Phe-OMe (hexahydrate)	68	EtOAc-pet. ether	53—67	−30.9°(0.7, DMF, 23)	$C_{30}H_{36}O_8N_4 \cdot 6H_2O$	52.31	7.03	8.14	52.48	6.40	8.81	0.57	0.74	
Z-Pro-Pro-Gly-Phe (dihydrate)	87	EtOAc-pet. ether	78—105	−31.3°(0.8, DMF, 22)	$C_{29}H_{31}O_6N_4 \cdot 2H_2O$	61.25	6.38	9.85	61.97	6.59	9.32	0.46	0.52	
Pro-Pro-Gly-Phe (trihydrate)	71	H_2O-EtOH	164—170	−48.9°(0.76, H_2O, 24)	$C_{21}H_{28}O_5N_4 \cdot 3H_2O$	53.60	7.28	12.17	52.97	7.23	12.12	0.56	0.64	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe-OMe (sesquihydrate)	72	EtOAc-pet. ether	84—100	0.0°(0.4, DMF, 21)	$C_{38}H_{47}O_{10}N_9 \cdot 1\frac{1}{2}H_2O$	54.56	6.31	15.91	54.28	6.37	16.78	0.40	0.68	
Z-Arg(NO_2)-Pro-Pro-Gly-Phe (monoacetone)	66	acetone-ether	95—130	−44.1°(0.8, DMF, 21)	$C_{35}H_{45}O_{10}N_9 \cdot CH_3COCH_3$	55.13	6.45	15.23	55.07	6.09	15.42	0.39	0.58	
Arg-Pro-Pro-Gly-Phe (monoacetate, dihydrate)	87	H_2O	158—176	−52.5°(0.6, H_2O, 15)	$C_{27}H_{40}O_6N_8 \cdot CH_3COOH \cdot 2H_2O$	52.08	7.24	16.76	51.87	7.16	16.53	0.29	0.42	
Z-Pro-Gly-OEt	64	EtOAc-pet. ether	oil		$C_{17}H_{22}O_5N_2$							0.61	0.62	*m, r*
Z-Pro-Gly	85	EtOAc-pet. ether	122—123	−63.2°(5.0, MeOH, 21)	$C_{15}H_{18}O_5N_2$	58.81	5.92	9.15	58.66	5.97	9.25	0.61	0.62	*m, r*
Pro-Gly	88	H_2O-EtOH	233—235	−22.5°(2.0, H_2O, 19)	$C_7H_{12}O_2N_3$	44.20	7.37	14.75	44.48	7.27	14.81	0.24	0.32	*m, r*
Z-Pro-Pro-Gly-OEt	74	EtOAc-pet. ether	oil	−98.8°(0.9, DMF, 21)	$C_{22}H_{29}O_6N_3$							0.49	0.62	*s*
Z-Pro-Pro-Gly	89	EtOAc-pet. ether	oil	−105°(0.9, DMF, 21)	$C_{20}H_{25}O_6N_3$							0.39	0.39	*s*
Pro-Pro-Gly (monohydrate)	95	EtOH-ether	109—111	−51.7°(0.6, H_2O, 22)	$C_{12}H_{18}O_4N_3 \cdot H_2O$	50.20	7.40	14.60	49.90	8.20	14.90	0.35	0.44	*j, s*
Z-Arg(NO_2)-Pro-Pro-Gly-ONb (monoacetone, dihydrate)	51	acetone-ether	88—98	−42.8°(0.7, DMF, 21)	$C_{33}H_{41}O_{11}N_9 \cdot CH_3COCH_3 \cdot 2H_2O$	51.85	6.17	15.12	52.30	6.17	15.01	0.45	0.70	
Arg-Pro-Pro-Gly (monoacetate, dihydrate)	97	H_2O	130—152	−104.3°(0.6, H_2O, 15)	$C_{19}H_{31}O_6N_7 \cdot CH_3COOH \cdot 2H_2O$	46.05	7.54	18.80	46.72	7.67	18.88	0.21	0.27	
Z-Pro-Pro-ONb	67	EtOAc-pet. ether	90	−21.6°(0.5, DMF, 29)	$C_{25}H_{27}O_7N_3$	62.36	56.50	8.73	62.60	5.50	9.25	0.60	0.73	
Pro-Pro	51	EtOAc-ether	110—122	−160.2°(1.0, H_2O, 24)	$C_{10}H_{16}O_3N_2$	56.60	7.60	13.20	56.80	7.20	13.20	0.39	0.30	*t*
Z-Arg(NO_2)-Pro-Pro-ONb	45	EtOAc-pet. ether	87—95	−50.2°(0.7, DMF, 21)	$C_{24}H_{33}O_8N_7$	54.54	5.61	16.42	54.68	5.27	16.13	0.63	0.79	
Arg-Pro-Pro (monoacetate, sesquihydrate)	45	H_2O	125	−81.8°(0.38, H_2O, 24)	$C_{16}H_{29}O_4N_8 \cdot CH_3COOH \cdot 1\frac{1}{2}H_2O$	47.46	7.75	18.45	47.71	7.58	17.72	0.22	0.38	
Z-Arg(NO_2)-Pro-OBzl	56	MeOH	147—148.5	−42°(1.0, DMF, 20)	$C_{16}H_{32}O_7N_6$	57.77	5.92	15.55	57.69	5.90	15.67	0.61	0.83	*e*
Arg-Pro (diacetate, monohydrate)	51	H_2O	122	−69.6°(0.23, H_2O, 24)	$C_{11}H_{21}O_3N_5 \cdot 2CH_3COOH \cdot H_2O$	44.00	7.63	17.11	44.10	7.75	18.07	0.17	0.28	*u*

a) ref. 8 *b*) R.A. Boissonnas, St. Gutttmann and P.A. Jaquenoud, *Helv. Chim. Acta*, **43**, 1349 (1960) *c*) N. Yanaihara and M. Sekiya, *Chem. Pharm. Bull.* (Tokyo), **15**, 82 (1967) *d*) E.D. Nicolaides and H.A. DeWald, *J. Org. Chem.*, **26**, 3872 (1961)
e) M.A. Ondetti, *J. Med. Chem.*, **6**, 10 (1963) *f*) E.D. Nicolaides, H.A. DeWald and M. K. Craft, *Ann. N.Y. Acad. Sci.*, **104**, 15 (1963). *Idem, J. Med. Chem.*, **6**, 739 (1963) *g*) H. Schwarz and K. Arakawa, *J. Am. Chem. Soc.*, **81**, 5691 (1959)
h) *H. Schwarz, F.M. Bumpus and I.H. Page, J. Am. Chem. Soc.*, **79**, 5697 (1957) *i*) H.A. DeWald and E.D. Nicolaides, *J. Med. Chem.*, **7**, 50 (1964) *j*) E. Wünsch, H.G. Heidrich and W. Grassmann, *Chem. Ber.*, **97**, 1818 (1964)
k) M. Bodanszky, J.T. Sheehan, M.A. Ondetti and S. Lande, *J. Am. Chem. Soc.*, **85**, 991 (1963) *l*) K. Vogler, R.O. Studer and W. Lergier, *Helv. Chim. Acta*, **44**, 1495 (1961) *m*) W. Grassmann, E. Wünsch and A. Rieder, *Chem. Ber.*, **91**, 449 (1958)
n) S. Goldschmidt and K.K. Gupta, *Chem. Ber.*, **98**, 2831 (1965) *o*) G.W. Anderson and F.M. Callahan, *J. Am. Chem. Soc.*, **82**, 3359 (1960) *p*) W. Grassmann, E. Wünsch and A. Riedel, *Chem. Ber.*, **91**, 455 (1958)
q) I.Z. Siemion, *Roczniki Chem.*, **38** (5), 811 (1964) *r*) H.N. Rydon and P.W.G. Smith, *J. Chem. Soc.*, 1956, 3642 *s*) ref. 12 *t*) N.C. Devis and E.L. Smith, *J. Biol. Chem.*, **200**, 373 (1953)
u) P.M. Scopes, K.B. Walshaw, M. Welford and G.T. Young, *J. Chem. Soc.*, 1965,728. *v*) Amino acid analysis: Phe 2.06, Ser 1.05, Pro 1.02, Arg 1.00 *w*) Amino acid analysis: Phe 2.01, Ser 1.00, Pro 1.00

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.

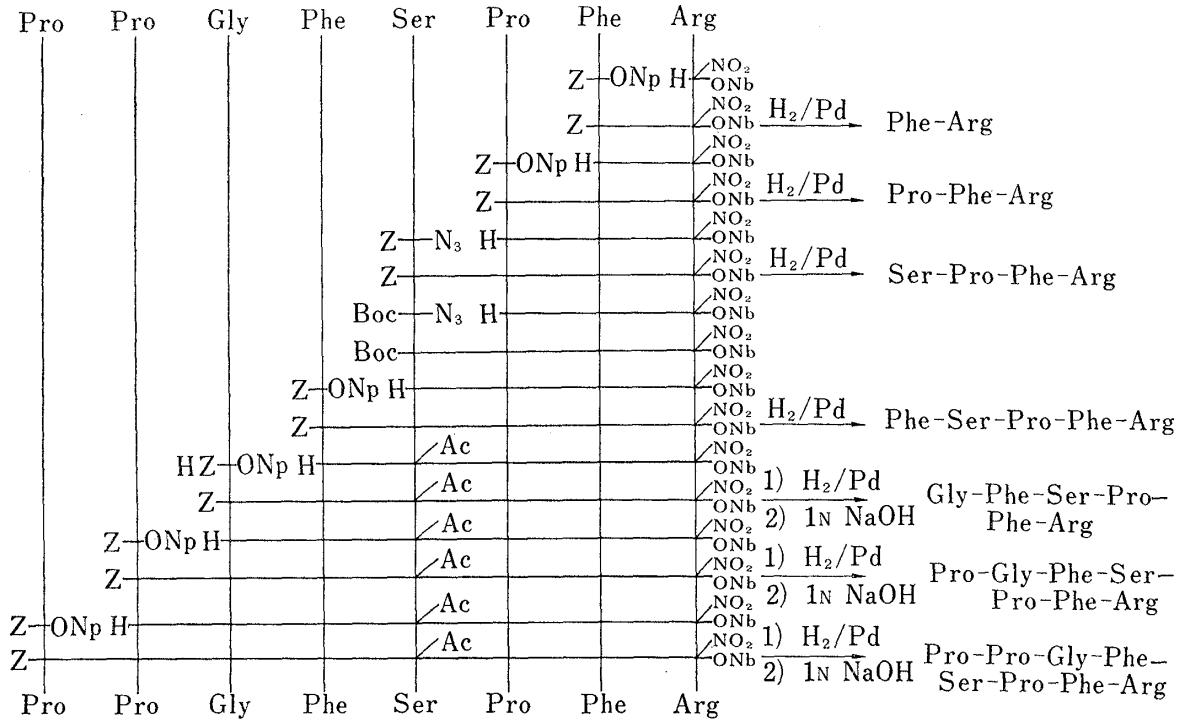

Fig. 1. Synthesis of Prolylprolylglycylphenylalanylserylprolylphenylalanylarginine and Its Fragments, as an Example of Synthetic Approach starting with Amino Acid *p*-Nitrobenzyl Esters

In Table I, the compounds are arranged in the order as follows: main starting materials, blocked peptides, and deblocked peptides which were synthesized strating 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).

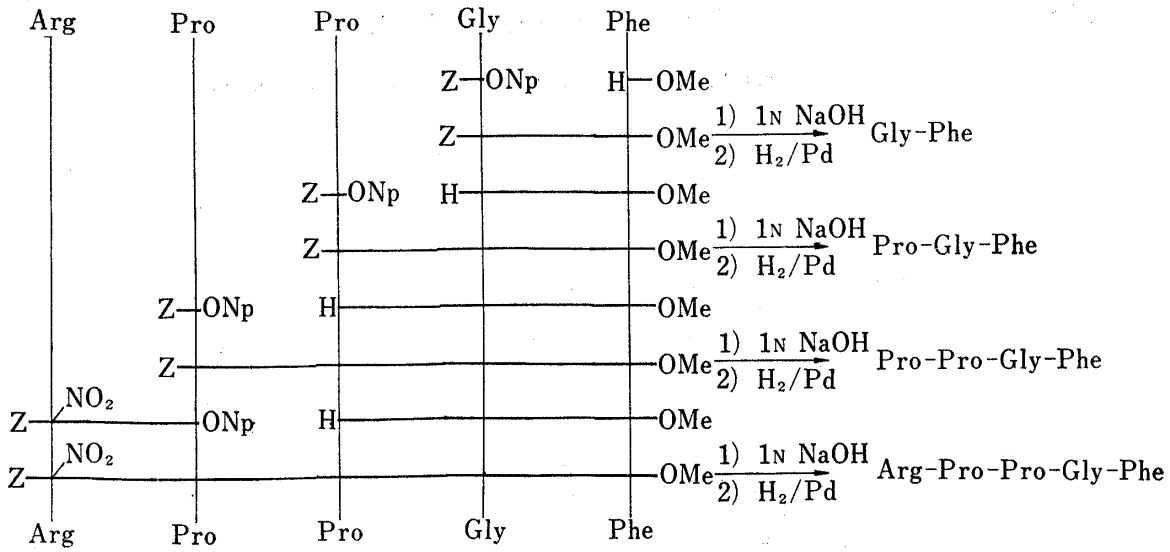

Fig. 2. Synthesis of Arginylprolylprolylglycylphenylalanine and Its Fragments, as an Example of Synthetic Approach starting with Amino Acid Methyl Esters

group in the C-terminus were treated with hydrobromicacid 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 ninhdyrin 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}–nitroarginylprolylprolylglycyl-phenylalanylserylproline *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. Rf^1 was 0.27 and 0.49, and Rf^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. Rf^1 0.4 9and Rf^2 0.62 corresponded to that of prolylprolyl-glycine 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 Gut-tmann.[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 paw[14] and the assay for vasodilation with few bradykinin fragments were made. Results of these biological examinations are given in Table II.

Table II. Biological Acitvities 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 μg 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 contratcion 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. Guttmann, 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 acid[17] 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 impotrance of the phenylalanine residues in the bradykinin molecule so far as concernig 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 phenylalanylseryprolylphenylalanine 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 paw[14] 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 inframmatory tissues have been demonstrated in the course of Arthus-type inframmation 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 labolatory 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 biossay of synthetic bradykinin analogues by the different reseach 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-Benzyloxycarbonyl 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-benzyloxycarbonyl amino acid *p*-nitrophenyl ester (0.011 mole) followed by addition of Et_3N to keep the solution slightly alkaline and stirred overnight. To the reaction mixture 1N NH_4OH (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 H_2O, 1N NH_4OH, H_2O, 1N HCl, and H_2O. 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 preciptate 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^{α}-benzyloxycarbonyl-N^{ω}-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 lyophylisation 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.

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