Table IV. Molar Extinction Coefficients of PEG and PPG

| Compound | $\varepsilon_1$ (2870 $cm^{-1}$) | $\varepsilon_2$ (2975 $cm^{-1}$) | Degree of polymerization ($m$, or $n'$) | $\varepsilon_1/m$ or $n'$ | $\varepsilon_2/m$ or $n'$ |
|---|---|---|---|---|---|
| PPG 200 | 183.1 | 189.6 | 3.2 | 57.2 | 59.2 |
| 〃 400 | 322.4 | 367.8 | 6.6 | 48.9 | 55.7 |
| 〃 1200 | 1075.9 | 1115.9 | 20.4 | 52.7 | 54.5 |
| 〃 1700 | 1492.0 | 1553.1 | 29.0 | 51.4 | 53.4 |
| 〃 2000 | 1798.7 | 1832.5 | 34.2 | 52.3 | 53.2 |
| PEG 200 | 269.4 | 37.5 | 5.4 | 49.7 | 6.9 |
| 〃 300 | 342.3 | 42.9 | 6.3 | 54.0 | 7.7 |
| 〃 600 | 933.1 | 115.6 | 14.9 | 62.5 | 6.8 |
| 〃 1000 | 1683.1 | 234.5 | 26.9 | 62.7 | 7.7 |
| 〃 1540 | 2408.1 | 260.5 | 36.4 | 66.2 | 7.2 |
| 〃 4000 | 5552.5 | 662.3 | 87.7 | 63.3 | 7.6 |
| 〃 6000 | 8143.0 | 1048.2 | 137.5 | 59.2 | 7.6 |

$\varepsilon_1^P = \varepsilon_1/m$ (average)=52.5 $\varepsilon_1^E = \varepsilon_1/n$ (average)=59.7
$\varepsilon_2^P = \varepsilon_2/m$ (average)=55.2 $\varepsilon_2^E = \varepsilon_2/n$ (average)=7.5

Table V. Influence of the Concentration of Sample in $CCl_4$

| Pluronic | Concentration (W/V %) | log $A_1/A_2$ | Molar percentage of EO by method I |
|---|---|---|---|
| A64 | 1.62 | 0.216 | 43.0 |
| 〃 | 1.51 | 0.224 | 44.5 |
| 〃 | 1.43 | 0.225 | 44.5 |
| 〃 | 1.26 | 0.250 | 50.0 |

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## 11. Noboru Yanaihara and Minoru Sekiya : Synthesis of 1-Glutamic Acid Kallidin.*1,*2

(*Shizuoka College of Pharmacy**3)

The Synthesis of 1-glutamic acid kallidin was performed. The azide and *p*-nitrophenyl ester methods were selectively employed in the coupling stages. The biological activity of this peptide compared to that of synthetic bradykinin is reported.

(Received March 26, 1966)

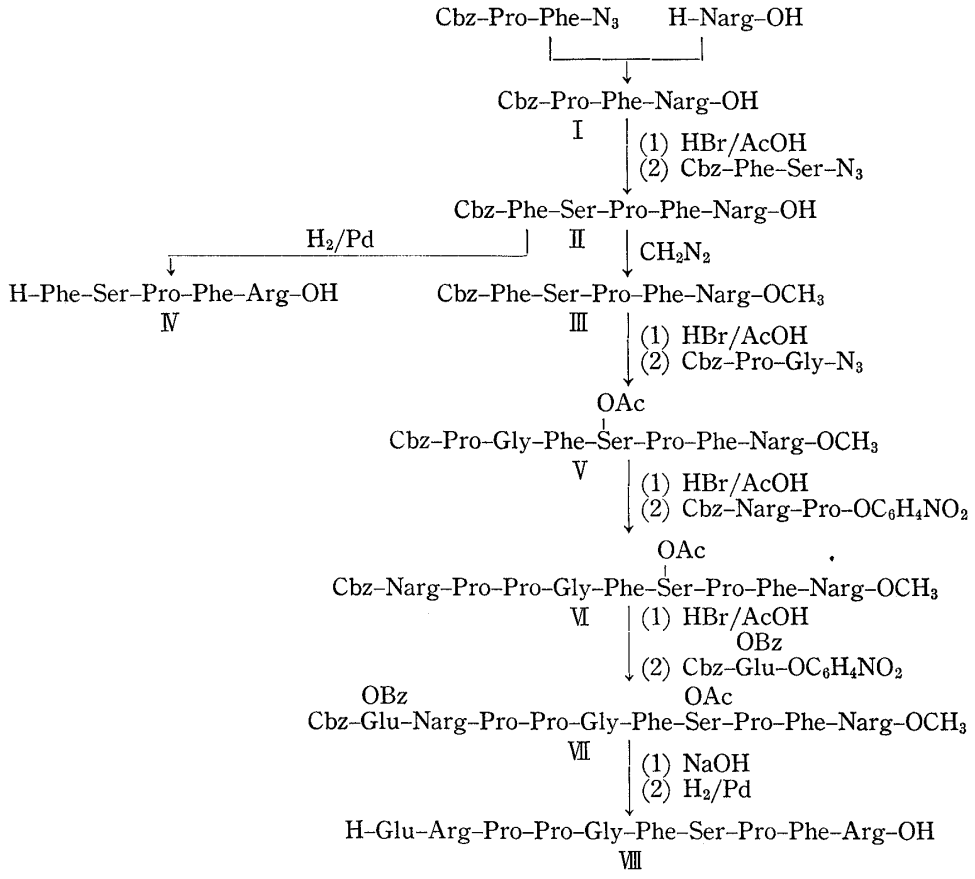
Since amino acid sequences of bradykinin[1] and kallidin[2] were elucidated, a number of their analogs have been synthesized in an attempt to discover relationships between structure and activity.

*1 The peptides and peptide derivatives mentioned in this paper are of the L-configuration.
*2 1-Glutamic acid kallidin=Glutamylarginylprolylprolylglycylphenylalanylserylprolylphenylalanylarginine.
*3 160 Oshika, Shizuoka (矢内原 昇, 関屋 実).

1) D. F. Elliott, G. P. Lewis, E. W. Horton : Biochem. Biophys. Res. Commun., **3**, 87 (1960).
2) E. Werle, I. Trautschold, G. Leysath : "Hoppe-Seyler's Z., **326**, 174 (1961); J. V. Pierce, M. E. Webster: Biochem. Biophys. Res. Commun., **5**, 353 (1961).

Kallidin, decapeptide, possesses one-third of bradykinin activity in the guinea-pig ileum, while on the blood pressure of the rabbit it is twice as active as bradykinin.[3] The replacement of N-terminal lysine residue of kallidin by neutral or basic amino acid other than lysine has been known to lead to in general a slight decrease of the activity.[4] As an effort to investigate the effect of complementarily charged amino acid in the kallidin molecule on its biological activity, we felt it was of interest to synthesize kallidin analog with acidic amino acid in place of the lysine residue and to evaluate the physiological effects.

This paper describes the synthesis and biological evaluation of l-glutamic acid kallidin. The method used for the preparation is shown in Chart 1.




Cbz=benzyloxycarbonyl; OBz=$\gamma$-benzyl ester; Narg=$N^{\omega}$-nitroarginine

Chart 1.

The chain lengthening was performed by starting from the carboxyl end, $N^{\omega}$-nitroarginine,[5] essentially according to azide[6] and *p*-nitrophenyl ester[7] methods. Every blocked intermediate employed in the present work was extensively purified.

Benzyloxycarbonylprolylphenylalanine azide prepared from the corresponding hydrazide[8] was allowed to react with the triethylammonium salt of $N^{\omega}$-nitroarginine

3) E. Sturmer, B. Berde : J. Pharmacol. Exp. Therap., **139**, 38 (1963); J. Pless, E. Sturmer, St. Guttmann, R. A. Boissonnas : Helv. Chim. Acta, **45**, 394 (1962).
4) E. Schröder, R. Hempel : Experientia, **20**, 529 (1964).
5) K. Hofmann, A. Rheiner, W. D. Peckham : J. Am. Chem. Soc., **75**, 6083 (1953); K. Hofmann, W. D. Peckham, A. Rheiner : *Ibid.*, **78**, 238 (1956).
6) T. Curtius : J. Prakt. Chem., **70**, 57 (1902); *Idem* : Ber., **35**, 3226 (1902).
7) M. Bodanszky : Nature, **175**, 685 (1955).
8) E. Schröder, H. Gibian : Ann., **673**, 176 (1964).

to give benzyloxycarbonylprolylphenylalanyl-N$^{\omega}$-nitroarginine (I), which was partially deblocked by HBr-AcOH treatment[9] with formation of prolylphenylalanyl-N$^{\omega}$-nitroarginine hydrobromide. This hydrobromide was then coupled with benzyloxycarbonylphenylalanylserine azide prepared from the corresponding hydrazide[8,10,11] to yield benzyloxycarbonylphenylalanylserylprolylphenylalanyl-N$^{\omega}$-nitroarginine (II). For assessment of homogeneity, this compound was hydrogenated over palladium[12]. After lyophilization the free pentapeptide (IV) obtained in the form of monoacetate dihydrate showed a single spot on paper chromatogram. The amino acid ratios in an acid hydrolysate were identical with those predicted by theory. Methylation of the protected pentapeptide (II) with diazomethane resulted in the formation of the methyl ester (III), whose physical constants were identical with those reported in the literatures.[8,10] After removal of the benzyloxycarbonyl group, the resulting pentapeptide ester hydrobromide was coupled with benzyloxycarbonylprolylglycine azide prepared from the corresponding hydrazide to give methyl benzyloxycarbonylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (V).[13] Acetylation of the hydroxyl group of serine residue by HBr-AcOH treatment has been well known.[14] The NMR spectrum of the heptapeptide (V) (measured in trifluoroacetic acid) showed two singlets at 7.75 and 6.08$\tau$, indicating the presence of mono-O-acetyl function and monomethyl ester in the molecule. This compound was treated with HBr-AcOH to remove the benzyloxycarbonyl group. The ensuing hydrobromide was then allowed to react with *p*-nitrophenyl benzyloxycarbonyl-N$^{\omega}$-nitroarginylprolinate[13,15] to give methyl benzyloxycarbonyl-N$^{\omega}$-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (VI) which was deblocked again by HBr in glacial AcOH. The nonapeptide hydrobromide thus obtained was coupled with $\alpha$-*p*-nitrophenyl $\gamma$-benzyl benzyloxycarbonylglutamate[16] to yield methyl benzyloxycarbonyl-$\gamma$-benzylglutamyl-N$^{\omega}$-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (VII). This material was saponified and complete removal of the remaining protecting groups was achieved by hydrogenation over palladium in 50% aqueous AcOH.

The crude decapeptide, glutamylarginylprolylprolylglycylphenylalanylserylprolylphenylalanylarginine (VIII), was purified by ion exchange chromatography on carboxymethylcellulose.[17] The purified material produced a single sharp spot on paper chromtogram and its acid hydrolysate contained the constituent amino acids in the ratios expected by theory.

The $\alpha$-chymotryptic*4 digest of VIII was examined by paper chromatography comparing with that of synthetic bradykinin. Three spots corresponding to arginine, seryl-prolylphenylalanine, and presumably glutamylarginylprolylprolylglycylphenylalanine were detected.

*4 This enzyme was obtained from Sigma Chemical Company, No. 113B-0310. The authors express their appreciation to Prof. T. Suzuki of Institute for Protein Research of University of Osaka for his kind supply of $\alpha$-chymotrypsin for the preliminary experiment.

*5 The authors wish to express their gratitude to Prof. K. Takagi and Dr. H. Kato of Faculty of Pharmaceutical Sciences, University of Tokyo for the biological assay.

9) G. W. Anderson, J. Blodinger, A. D. Welcher : J. Am. Chem. Soc., **74**, 5309 (1952).
10) E. D. Nicolaides, H. A. DeWald : J. Org. Chem., **26**, 3872 (1961).
11) R. A. Boissonnas, St. Guttmann, P.-A. Jaquenoud : Helv. Chim. Acta, **43**, 1349 (1960).
12) M. Bergman, L. Zervas : Ber., **65**, 1192 (1932).
13) M. A. Ondetti : J. Med. Chem., **6**, 10 (1963).
14) E. D. Nicolaides, H. A. DeWald : J. Org. Chem., **28**, 1926 (1963).
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16) M. Goodman, K. C. Stueben : J. Am. Chem. Soc., **81**, 3980 (1959).
17) E. A. Peterson, H. A. Sober : *Ibid*, **78**, 751 (1956).

Result of the biological assay*5 is shown in Table I.

Table I. Biological Activity

| Peptide | Guinea-pig ileum (isolated) | Guinea-pig blood pressure |
|---|---|---|
| l-Glutamic acid kallidin | 1/3 | 1～1/2 |
| Bradykinin (synthetic) | 1 | 1 |

Thus, l-glutamic acid kallidin showed one-third activity of bradykinin on the guinea-pig ileum and nearly equal on the blood pressure of guinea-pig. The replacement of N-terminal residue of kallidin molecule with glutamic acid led to no remarkable change in the biological activity.

## Experimental*6

**Benzyloxycarbonylprolylphenylalanine Hydrazide**——*p*-Nitrophenyl benzyloxycarbonylprolinate[16] (11.1 g.) was added to a solution of methyl phenylalaninate hydrochloride (6.4 g.) and triethylamine (4.2 ml.) in $CHCl_3$ (60 ml.). The mixture was allowed to stand for 20 hr. at room temperature. Then the solvent was evaporated. The residue was taken up in AcOEt and the solution was washed successively with *N* HCl, 2*N* $NH_4OH$, and saturated NaCl and dried over $Na_2SO_4$. Evaporation of the solvent gave an oily residue (11.7 g.). This crude methyl benzyloxycarbonylprolylphenylalaninate was dissolved in MeOH (50 ml.) and hydrazine hydrate (6.0 ml.) was added. The mixture was kept at room temperature for 6 hr. The solvent was removed and ether (30 ml.) was added. The resulting crystalline product was collected by filtration and recrystallized from EtOH–ether; yield 9.3 g. (79%), m.p. 148～149°, $[\alpha]_D^{28}$ −60.4° (c=1.0, DMF), $[\alpha]_D^{28}$ −69.0° (c=1.1, MeOH) (lit.[8] m.p. 142～144, $[\alpha]_D^{22}$ −68.6° (MeOH)). *Anal.* Calcd. for $C_{22}H_{26}O_4N_4$: C, 64.37; H, 6.38; N, 13.62. Found: C, 64.72; H, 6.37; N, 13.83.

**Benzyloxycarbonylprolylglycine Hydrazide**——N,N′-Dicyclohexylcarbodiimide[18] (2.1 g.) was added with stirring to an ice-cold solution of benzyloxycarbonylproline[19] (2.5 g.), methyl glycinate hydrochloride (1.3 g.), and triethylamine (1.4 ml.) in methylenechloride (15 ml.) and dimethylformamide (DMF) (5 ml.). The mixture was stirred for 1 hr. in an ice-cold bath and for further 14 hr. at room temperature. N,N′-Dicyclohexylurea was removed by filtration and the filtrate was diluted with AcOEt. The solution was washed successively with *N* HCl, saturated $NaHCO_3$, and saturated NaCl and dried over $Na_2SO_4$. Evaporation of the solvents gave oily residue (3.1 g.). This crude product was dissolved in MeOH (15 ml.), and hydrazine hydrate (1.2 ml.) was added. The mixture was kept for 15 hr. at room temperature and the bulk of the solution was evaporated. Ether (20 ml.) was added to the residue and the mixture was stored in a refrigerator. The resulting crystalline product was collected and recrystallized from EtOH–ether to give needles; yield 2.4 g. (77%), m.p. 144～145°, $[\alpha]_D^{28}$ −47.3° (c=1.5, MeOH). *Anal.* Calcd. for $C_{15}H_{20}O_4N_4$: C, 56.23; H, 6.29; N, 17.49. Found: C, 56.26; H, 6.40; N, 17.55.

**Benzyloxycarbonylprolylphenylalanyl-$N^{\omega}$-nitroarginine (I)**——A solution of $NaNO_2$ (0.7 g.) in cold $H_2O$ (3 ml.) was added dropwise to an ice-cold solution of benzyloxycarbonylprolylphenylalanine hydrazide (4.1 g.) in tetrahydrofuran (30 ml.) and *N* HCl (20 ml.). After 5 min., the mixture was adjusted with triethylamine to pH 7.5. This azide solution was added to an ice-cold solution of $N^{\omega}$-nitroarginine[5] (2.2 g.) and triethylamine (1.4 ml.) in $H_2O$ (50 ml.) and DMF (30 ml.). The mixture was stirred for 15 hr. at 4°. The solvents were evaporated to a half volume and 2*N* $NH_4OH$ (70 ml.) was added thereto. The solution was washed with AcOEt, cooled in an ice bath, acidified with cold 4*N* HCl, and extracted twice with AcOEt. The

*6 All melting points are uncorrected. Optical rotations were measured with a Rex Model NEP-2 photoelectric polarimeter. The amino acid composition of acid hydrolysates was determined with a Hitachi Model KLA-2 amino acid analyzer. The NMR spectra were measured with a Japan Electron Optics Laboratory 3H-60 spectrometer. Paper chromatography was performed on Toyo No. 51 filter paper with the solvent system of *n*-BuOH–AcOH–$H_2O$ (4:1:5) by descending technique ($Rf^1$) and with the system of *n*-BuOH–pyridine–AcOH–$H_2O$ (30:20:6:24) by ascending technique ($Rf^2$). Paper electrophoresis was carried out in ammonium formate buffer at pH 3.5 using a constant current of 40 milliamp. for 1 hr. The following abbreviations are used: arg=arginine; pro=proline; gly=glycine; ser=serine; ala=alanine; phe=phenylalanine; glu=glutamic acid; DMF=dimethylformamide. The amino acids used were obtained from Ajinomoto Co., Inc., Tokyo.

18) J. T. Sheehan, G. P. Hess: J. Am. Chem. Soc., **77**, 1067 (1955).
19) A. Berger, J. Kurtz, E. Katchalsky: *Ibid.*, **76**, 5552 (1954).

organic layers were washed once with *N* HCl and three times with saturated NaCl and dried over $Na_2SO_4$. Evaporation of the solvent gave oily viscous residue, which was dissolved in a minimum volume of EtOH. The product desired was precipitated by addition of ether; yield 3.6 g. (60%), m.p. 126~131°, $[\alpha]_D^{28}$ −38.3° (c=1.1, DMF), $[\alpha]_D^{28}$ −47.8° (c=1.1, MeOH). *Anal.* Calcd. for $C_{28}H_{35}O_8N_7$: C, 56.26; H, 5.90; N, 16.41. Found: C, 56.58; H, 6.13; N, 16.49.

**Benzyloxycarbonylphenylalanylserylprolylphenylalanyl-N$^{\omega}$-nitroarginine (II)**——To a solution of I (3.0 g.) in glacial AcOH (5 ml.) was added 28% HBr solution in AcOH (15 ml.). The solution was kept for 1 hr. at room temperature and anhyd. ether (50 ml.) was added. The precipitate was collected, washed with ether and dried over KOH under reduced pressure. This tripeptide hydrobromide was dissolved in DMF (15 ml.), and triethylamine (2.0 ml.) was added. The solution was kept for 5 min. in an ice bath and then filtered. To the filtrate cooled in an ice bath was added an ice-cold solution of benzyloxycarbonylphenylalanylserine azide prepared from benzyloxycarbonylphenylalanylserine hydrazide[8,10,11] (2.8 g.) and $NaNO_2$ (0.5 g.) in *N* HCl (14 ml.) and tetrahydrofuran (15 ml.) in the manner described for the preparation of I. The mixture was stirred for 15 hr. at 4°. The bulk of the solution was evaporated and AcOEt (70 ml.) was added. The resulting solution was extracted twice with *N* $NH_4OH$. The combined aqueous layers were cooled in an ice bath, acidified with 4*N* HCl, and the oily product separated was extracted twice with AcOEt. The extracts were washed once with *N* HCl and three times with saturated NaCl and dried over $Na_2SO_4$. The solvent was evaporated and ether was added to the residue to bring about crystallization. The product was recrystallized from EtOH–ether; yield 2.7 g. (65%), m.p. 125~137°, $[\alpha]_D^{28}$ −44.9° (c=1.1, DMF). *Anal.* Calcd. for $C_{40}H_{49}O_{11}N_9$: C, 57.74; H, 5.93; N, 15.15. Found: C, 57.31; H, 6.07; N, 14.83.

**Methyl Benzyloxycarbonylphenylalanylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (III)**——Ethereal diazomethane was added to an ice-cold solution of the benzyloxycarbonylpentapeptide II (2.7 g.) in MeOH (100 ml.) until the yellow color remained. The solution was kept for 5 min. when a few drops of glacial AcOH were added, and the solvents were removed. The residue was crystallized from EtOH–ether; yield 2.5 g. (91%), m.p. 147~150°, $[\alpha]_D^{28}$ −43.0° (c=1.1, DMF) (lit.[8] m.p. 150~152°, $[\alpha]_D^{21}$ −42.8° (DMF); lit.[10] m.p. 152~153°, $[\alpha]_D^{23}$ −42.3° (DMF)). *Anal.* Calcd. for $C_{41}H_{51}O_{11}N_9$: C, 58.21; H, 6.08; N, 14.90. Found: C, 57.91; H, 6.05; N, 14.69.

**Phenylalanylserylprolylphenylalanylarginine Monoacetate Dihydrate (IV)**——The blocked pentapeptide II (0.15 g.) disssolved in 50% aqueous AcOH (40 ml.) was hydrogenated over Pd in the usual manner. The catalyst was removed by filtration and the filtrate was evaporated. The residue was dissolved in a small amount of $H_2O$ and the solution was lyophilized to give white powder; yield 0.12 g. (91%), $[\alpha]_D^{28}$ −37.6° (c=0.3, $H_2O$), $Rf^1$ 0.52, $Rf^2$ 0.55, single ninhydrin- and Sakaguchi-positive spot, amino acid ratios in acid hydrolysate $phe_{2.08}ser_{0.78}pro_{0.91}arg_{1.00}$. *Anal.* Calcd. for $C_{34}H_{48}O_9N_8 \cdot 2H_2O$: C, 54.53; H, 6.99; N, 14.96. Found: C, 54.40; H, 7.01; N, 15.36.

**Methyl Benzyloxycarbonylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (V)**——$NaNO_2$ (0.07 g.) dissolved in ice-cold $H_2O$ (2 ml.) was added to an ice-cold solution of benzyloxycarbonylprolylglycine hydrazide (0.32 g.) in *N* HCl (2 ml.) and tetrahydrofuran (5 ml.). The mixture was stirred for 5 min. in an ice bath and then adjusted to pH 7.5 by the addition of triethylamine. This solution containing the azide was added with stirring to an ice-cold solution of the pentapeptide methyl ester hydrobromide, prepared from the benzyloxycarbonylpentapeptide methyl ester III (0.80 g.) by treatment with HBr–AcOH, in DMF (5 ml.) containing triethylamine (0.28 ml.). The mixture was stored in a refrigerator for 48 hr. when AcOEt (50 ml.) was added. The solution was washed with *N* HCl and then with saturated NaCl and dried over $Na_2SO_4$. Evaporation of the solvents gave white solid, which was recrystallized from 95% EtOH; yield 0.52 g. (53%), m.p. 190~192°, $[\alpha]_D^{28}$ −64.7° (c=0.63, DMF), (lit.[13] m.p. 193~196°, $[\alpha]_D^{20}$ −59° (DMF)). *Anal.* Calcd. for $C_{50}H_{63}O_{14}N_{11}$: C, 57.63; H, 6.05; N, 14.79. Found: C, 57.53; H, 6.23; N, 14.91.

**Methyl Benzyloxycarbonyl-N$^{\alpha}$-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate Monohydrate (VI)**——A solution of 28% HBr in AcOH (6 ml.) was added to a solution of the benzyloxycarbonylheptapeptide methyl ester V (1.10 g.) in AcOH (1.5 ml.). The mixture was kept for 1 hr. at room temperature and ether (20 ml.) was added. The ensuing precipitate was collected and dried over KOH. This was dissolved in DMF (5 ml.) containing triethylamine (0.25 ml.), and the solution was cooled and filtered. To the filtrate was added benzyloxycarbonyl-N$^{\omega}$-nitroarginylproline *p*-nitrophenyl ester[11,13] (0.39 g.). The mixture was kept at room temperature for 3 days when the solvent was evaporated under reduced pressure. To the residue, AcOEt (30 ml.) was added. The resulting prepicitate was collected by filtration, washed successively with AcOEt, 5% AcOH, 5% $NH_4OH$, and $H_2O$, and dried over $P_2O_5$ under reduced pressure. Twice recrystallization from EtOH gave white solid; yield 0.76 g. (53%), m.p. 152~161°, $[\alpha]_D^{28}$ −57.6° (c=0.62, DMF). *Anal.* Calcd. for $C_{61}H_{81}O_{18}N_{17} \cdot H_2O$: C, 53.93; H, 6.15; N, 17.53. Found: C, 53.70; H, 6.09; N, 17.58.

**Methyl Benzyloxycarbonyl-γ-benzylglutamyl-N$^{\omega}$-nitroarginylprolylprolylglycylphenylalanyl-O-acetylserylprolylphenylalanyl-N$^{\omega}$-nitroargininate (VII)**——The blocked nonapeptide methyl ester VI (22 mg.) in AcOH (2 ml.) was treated with 28% HBr in AcOH (5 ml.) for 30 min. at room temperature. The resultant partially deblocked product was dissolved in DMF (5 ml.) containing triethylamine (0.05 ml.), and benzyloxycarbonyl-γ-benzylglutamic acid *p*-nitrophenyl ester[19] (81 mg.) was added thereto. The mixture was allowed

to stand at room temperature for 4 days. The solvent was evaporated *in vacuo*, the residue was dissolved in *n*-BuOH, and the solution was successively washed with 3% AcOH, 3% $NH_4OH$, and $H_2O$ and evaporated. The residue was taken up in tetrahydrofuran, and the solution was filtered. To the filtrate was added AcOEt. The resulting precipitate was collected by filtration and recrystallized from 95% EtOH; yield 145 mg. (57%), m.p. 140~142°, $[\alpha]_D^{28}$ −67.2° (c=0.6, DMF). *Anal.* Calcd. for $C_{73}H_{94}O_{21}N_{18}$: C, 56.21; H, 6.07; N, 16.17. Found: C, 55.83; H, 6.34; N, 16.34.

**Glutamylarginylprolylprolylglycylphenylalanylserylprolylphenylalanylarginine Diacetate Octahydrate (VIII)**——To a solution of the foreging benzyloxycarbonyldecapeptide methyl ester VII (100 mg.) in MeOH (3 ml.) was added *N* NaOH (0.15 ml.). The mixture was allowed to stand for 1 hr. at room temperature and $H_2O$ (10 ml.) was then added. The clear solution was acidified with *N* HCl to Congo red. The resulting precipitate was collected by filtration and washed with $H_2O$. This (81 mg.) dissolved in aqueous 50% AcOH (15 ml.) was subjected to hydrogenation over Pd for 24 hr., and the catalyst was removed by filtration. The filtrate was lyophilized. Paper chromatogram of the crude material revealed the presence of one major ($Rf^1$ 0.28, ninhydrin and Sakaguchi positive) and one minor ($Rf^1$ 0.39, ninhydrin negative and Sakaguchi positive) components. This crude material was dissolved in $H_2O$ (80 ml.), and the solution was applied to a CMC column (1.5×25 cm.) which was eluted successively with the following pH 6.5 ammonium acetate solutions: 0.005*M* (250 ml.), 0.0075*M* (250 ml.), 0.01*M* (200 ml.), 0.02*M* (200 ml.), 0.025*M* (250 ml.), and 0.03*M* (200 ml.). Individual fractions of 10 ml. each were collected and examined by Sakaguchi reagent. The desired peptide was located in the 0.025*M* eluates. These fractions were pooled, concentrated to a small volume *in vacuo*, and lyophilized several times; colorless powder, yield 41 mg. (44%), $[\alpha]_D^{28}$ −70.6° (c=0.2, $H_2O$), $Rf^1$ 0.28, $Rf^2$ 0.20, ninhydrin and Sakaguchi positive spot, single ninhydrin positive component on paper electrophoresis, amino acid ratios in acid hydrolysate $glu_{1.00}arg_{2.01}pro_{3.10}gly_{1.03}ser_{0.89}phe_{1.89}$. *Anal.* Calcd. for $C_{59}H_{88}O_{18}N_{16}\cdot 8H_2O$: C, 48.75; H, 7.21; N, 15.42. Found: C, 48.36; H, 7.12; N, 15.37. Paper chromatogram of the α-chymotryptic digest in the system of *n*-BuOH–AcOH–$H_2O$ revealed two ninhydrin- and Sakaguchi-positive spots, $Rf^1$ 0.20 (arginine) and 0.38 (presumably glutamylarginylprolylprolylglycylphenylalanine), and one ninhydrin-positive and Sakaguchi-negative spot, $Rf^1$ 0.66 (serylprolylphenylalanine), and no spot corresponding to VIII was detected.

**Enzymatic Experiment**——The peptide (1 mg.) in 0.1*M* ammonium bicarbonate buffer pH 8.0 (0.5 ml.) was mixed with 0.1% (w/v) α-chymotrypsin solution in the same buffer (0.6 ml.). The mixture was incubated for 24 hr. at 40°, when the solution was acidified with glacial AcOH and evaporated to dryness in a vacuum desiccator over $P_2O_5$. The residue was dissolved in $H_2O$ and the solution was used for paper chromatographic analysis.

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