Studies of Bitter Peptides from Casein Hydrolyzate. III.[1] Bitter Taste of Synthetic Analogs of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) Containing D-Proline or Glycine in Place of L-Proline[2]

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In order to elucidate the relationship between chemical structure and bitter taste of BPIa, the analogs containing D-proline or glycine in place of the L-proline in the 3- or 4-positions were synthesized. The bitter taste of [D-Pro^{3,4}]-BPIa (**1D-D**), [D-Pro^3]-des-Pro^3-BPIa (**2D**), and [Gly^3]-des-Pro^3-BPIa lacking the L-proline residue was much weaker than that of the peptides containing the L-proline, BPIa (**1L-L**), **1L-D**, **1D-L**, and **2L**. The CD curves of these analogs and BPIa were measured in water. The results suggested that the bitter taste of BPIa is caused by the spatial structure attributed to the L-proline residue.

BPIa is an extremely bitter peptide isolated by Minamiura *et al.* from cow milk casein hydrolyzate by alkaline proteinase of *Bacillus subtilis*. Its amino acid sequence has been determined to be H–Arg–Gly–Pro–Pro–Phe–Ile–Val–OH (**1L-L**).[3] This peptide has been synthesized by the authors and the threshold value of its bitter taste measured.[4] BPIa was found to be one of the most bitter compounds, which include phenylthiourea and quinine; the threshold value of BPIa was 0.05 mM. To study the relationship between chemical structure and bitter taste of BPIa, we prepared a number of fragments and analogs of it, and compared their taste and CD curves with those of BPIa in the previous paper.[1] The report shows that the spatial structure of BPIa might contribute to its bitter taste.

It is of interest to examine the taste variation of BPIa analogs caused by alteration of the spatial structure of BPIa. Among the constituent amino acids of BPIa, the L-proline residue in the 3- or 4-position seems to be very important in the structure. Therefore, we prepared the analogs containing D-proline or glycine residues in place of L-proline residue. The present paper deals with the syntheses and taste of the BPIa diastereomers (**1L-D**, **1D-L**, and **1D-D**), in which L-prolyl-L-proline in the 3- and 4-positions was replaced by L-prolyl-D-proline, D-prolyl-L-proline, and D-prolyl-D-proline respectively. Further, this paper deals with Des-Pro^3-BPIa (**2L**) in which an L-proline residue of BPIa was missing, and with two analogs of BPIa, in which the remaining L-proline residue of **2L** is replaced by D-proline or glycine residues; these are indicated by **2D** and [Gly^3]-des-Pro^3-BPIa(**3**).

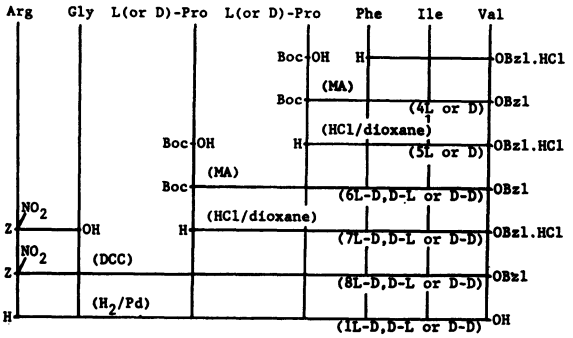

Fig. 1. Syntheses of the diastereomers of BPIa (**1L-D**, **1D-L**, and **1D-D**).

The synthesis of **1L-D** is outlined in Fig. 1. Condensation of Boc–D-Pro–OH with H–Phe–Ile–Val–OBzl·HCl by the mixed anhydride (MA) method gave acyltetrapeptide ester (**4D**). The removal of the Boc group from **4D** with hydrogen chloride in dioxane afforded the corresponding tetrapeptide ester hydrochloride (**5D**). **5D** was coupled with Boc–L-Pro–OH by the MA method to yield acylpentapeptide ester (**6L-D**). It was converted to the corresponding pentapeptide ester hydrochloride (**7L-D**) by the action of hydrogen chloride in dioxane. The protected heptapeptide (**8L-D**) derived from Z–Arg(NO$_2$)–Gly–OH and **7L-D** was hydrogenated in the presence of palladium black to yield the desired product (**1L-D**). A similar procedure was employed for the preparation of **1D-L** and **1D-D**. Des-Pro^3-BPIa (**2L**) and its diastereomer, H–Arg–Gly–D-Pro–Phe–Ile–Val–OH (**2D**), were obtained by hydrogenation of Z–Arg(NO$_2$)–Gly–L(or D)–Pro–Phe–Ile–Val–OBzl (**9L** or **D**) derived from Z–Arg(NO$_2$)–Gly–OH and **5L** or **5D**.

The synthetic procedure of **3** is as follows: Condensation of Boc–Gly–Gly–OH (**10**) with H–Phe–Ile–Val–OBzl·HCl by the MA method gave Boc–Gly–Gly–Phe–Ile–Val–OBzl (**11**). It was converted to the corresponding pentapeptide ester hydrochloride (**12**) by the action of hydrogen chloride in dioxane. Z–Arg(NO$_2$)–OH and **12** were condensed by the MA method to yield the protected hexapeptide, Z–Arg(NO$_2$)–Gly–Gly–Phe–Ile–Val–OBzl (**13**). Hydrogenation of **13** gave H–Arg–Gly–Gly–Phe–Ile–Val–OH (**3**). The homogeneity of the final products was confirmed by paper electrophoresis, amino acid analysis, and elemental

Table 1. The threshold values for bitter taste of BPIa and its analogs

Compounds	Threshold value for bitter taste/mM
Arg–Gly–L-Pro–L-Pro–Phe–Ile–Val(**1L-L**, BPIa)	0.05
Arg–Gly–L-Pro–D-Pro–Phe–Ile–Val (**1L-D**)	0.08
Arg–Gly–D-Pro–L-Pro–Phe–Ile–Val (**1D-L**)	0.11
Arg–Gly–D-Pro–D-Pro–Phe–Ile–Val (**1D-D**)	0.20
Arg–Gly–L-Pro–Phe–Ile–Val (**2L**)	0.05
Arg–Gly–D-Pro–Phe–Ile–Val (**2D**)	0.32
Arg–Gly–Gly–Phe–Ile–Val(**3**)	0.80

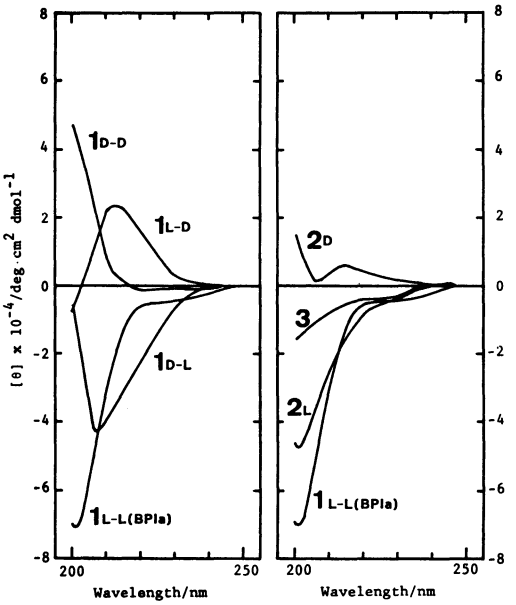

Fig. 2. CD curves of BPIa and its analogs.

analysis.

The taste of the BPIa analogs was organoleptically determined by panel evaluation employing several persons.

The results are listed in the Table. All the synthesized BPIa analogs possessed a bitter taste. The threshold value of BPIa diastereomers shows that the L-proline residue in the 3 position is more necessary for the bitter taste of BPIa than that in the 4-position. The value of **2L** is equal to that of BPIa. However, the bitter taste of **1D-D**, **2D**, and **3** lacking L-proline residue appreciably decreased, compared to that of the peptides containing L-proline residue. This means that L-proline residue is indispensable for the extremely bitter taste of BPIa.

The CD curves of these peptides in water as well as BPIa are shown in Fig. 2. The troughs of **1L-L** and **1D-L** show opposite signs to those of **1D-D** and **1L-D**. **2L** which exhibits the same bitterness as BPIa, has a curve with a negative trough at 202 nm; BPIa possesses a similarly shaped curve. **2D** and **3**, which have much weaker bitterness than BPIa, give different curves. The results of both taste and CD measurements prove that the bitter taste of BPIa is caused by the spatial structure of the molecule attributed to the L-proline residue.

Experimental

All the melting points are uncorrected. The thin layer chromatography was carried out on Merck silica gel G with the solvent systems: R_f^1, 1-butanol–acetic acid–pyridine–water (4 : 1 : 1 : 2, v/v); R_f^2, chloroform–methanol (5 : 1, v/v). Spots of materials possessing a free amino group on a thin layer plate were detected by spraying ninhydrin, and those of amino group blocked materials by spraying 25% hydrogen bromide in acetic acid and then ninhydrin. The optical rotations were measured on a Union PM-101 polarimeter. Amino acid analyses in acid hydrolyzate with 6 M hydrochloric acid (1 M=1 mol dm^{-3}) at 110 °C for 72 h were performed with a Hitachi amino acid analyzer, KLA-5 type. Molar ratios of amino acids were based on the isoleucine value. Prior to analyses, the compounds were dried over phosphorus pentoxide at 66 °C and 2 mmHg (1 mmHg≈133.322 Pa) for 2 h, except in the case of the peptide ester hydrochlorides.

Boc-L-Pro-Phe-Ile-Val-OBzl (4L). To a solution of Boc-L-Pro-OH (2.15 g, 10 mmol) and NMM (1.1 ml, 10 mmol) in THF (20 ml), ECF (1.0 ml, 10 mmol) was added at −5 °C with stirring. After 10 min, a solution of H-Phe-Ile-Val-OBzl·HCl[4] (5.04 g, 10 mmol) and NMM (1.1 ml, 10 mmol) in DMF (20 ml) was added to it. The reaction mixture was stored in an ice bath for 1 h and then at room temperature overnight. The mixture was evaporated *in vacuo* and dissolved in ethyl acetate. The solution was washed with 4% sodium hydrogencarbonate, 4% citric acid and water successively, and then dried over anhydrous sodium sulfate. The filtrate was evaporated *in vacuo* and the oily residue was crystallized with ether–petroleum ether: yield 6.06 g (91%); mp 111—112 °C; $[\alpha]_D^{20}$ −83° (*c* 1, methanol); R_f^1 0.98 and R_f^2 0.88.

Found: C, 66.54; H, 8.06; N, 8.37%. Calcd for $C_{37}H_{52}O_7N_4$: C, 66.84; H, 7.88; N, 8.43%.

Boc-D-Pro-Phe-Ile-Val-OBzl (4D). This was prepared from Boc-D-Pro-OH (2.15 g, 10 mmol) and H-Phe-Ile-Val-OBzl·HCl[4] (5.04 g, 10 mmol) as described above: yield 6.02 g (91%); mp 145—148 °C; $[\alpha]_D^{20}$ −32° (*c* 1, methanol); R_f^1 0.96 and R_f^2 0.78.

Found: C, 66.71; H, 8.11; N, 8.55%. Calcd for $C_{37}H_{52}O_7N_4$: C, 66.84; H, 7.88; N, 8.43%.

H-L-Pro-Phe-Ile-Val-OBzl·HCl (5L). Compound **4L** (3.32 g, 5 mmol) was dissolved in 4.1 M hydrogen chloride in dioxane (25 ml). The solution was allowed to stand for 1.5 h at room temperature and then evaporated *in vacuo*. The oily residue was solidified by the aid of ether: yield 2.86 g (95%); mp 90 °C (decomp); $[\alpha]_D^{20}$ −66° (*c* 1, methanol); R_f^1 0.88 and R_f^2 0.61.

Found: C, 63.32; H, 7.83; N, 9.11%. Calcd for $C_{32}H_{45}O_5N_4Cl\cdot 1/2\,H_2O$: C, 62.99; H, 7.60; N, 9.18%.

H-D-Pro-Phe-Ile-Val-OBzl·HCl (5D). This was prepared from **4D** (3.22 g, 5 mmol) as described above: yield 2.67 g (89%); mp 92 °C (decomp); $[\alpha]_D^{20}$ −31° (*c* 1, methanol); R_f^1 0.88 and R_f^2 0.59.

Found: C, 62.72; H, 7.74; N, 8.98%. Calcd for $C_{32}H_{45}O_5N_4Cl\cdot 1/2\,H_2O$: C, 62.99; H, 7.60; N, 9.18%.

Boc-L-Pro-D-Pro-Phe-Ile-Val-OBzl (6L-D). Boc-L-Pro-OH (0.65 g, 3 mmol) and **5D** (1.80 g, 3 mmol) were coupled by the same method as described for the preparation of **4L**: yield 2.07 g (91%); mp 107 °C; $[\alpha]_D^{20}$ +2° (*c* 1, methanol); R_f^1 0.97 and R_f^2 0.70.

Found: C, 65.92; H, 8.10; N, 9.01%. Calcd for $C_{42}H_{59}O_8N_5$: C, 66.20; H, 7.81; N, 9.19%.

Boc-D-Pro-L-Pro-Phe-Ile-Val-OBzl (6D-L). Boc-D-Pro-OH (0.65 g, 3 mmol) and **5L** (1.80 g, 3 mmol) were coupled by the same method as described for the preparation of **4L**: yield 1.82 g (80%); mp 68 °C; $[\alpha]_D^{20}$ −50° (*c* 1, methanol); R_f^1 0.94 and R_f^2 0.76.

Found: C, 66.06; H, 8.03; N, 8.98%. Calcd for $C_{45}H_{59}O_8N_5$: C, 66.20; H, 7.81; N, 9.19%.

Boc-D-Pro-D-Pro-Phe-Ile-Val-OBzl (6D-D). Boc-D-Pro-OH (0.65 g, 3 mmol) and **5D** (1.80 g, 3 mmol) were coupled by the same method as described for the preparation of **4L**: yield 1.60 g (73%); mp 82—84 °C; $[\alpha]_D^{20}$ +10° (*c* 1, methanol); R_f^1 0.97 and R_f^2 0.77.

Found: C, 66.40; H, 7.98; N, 8.83%. Calcd for $C_{42}H_{59}O_8N_5$: C, 66.20; H, 7.81; N, 9.19%.

H-L-Pro-D-Pro-Phe-Ile-Val-OBzl·HCl (7L-D). Compound **6L-D** (1.52 g, 2 mmol) was treated with hydrogen chloride as described for **5L**: yield 1.33 g (95%); mp 103 °C (decomp); $[\alpha]_D^{20}$ −85° (*c* 1, methanol); R_f^1 0.80 and R_f^2 0.45.

Found: C, 62.74; H, 7.75; N, 9.72%. Calcd for $C_{37}H_{52}O_6N_5Cl \cdot 1/2\ H_2O$: C, 62.82; H, 7.55; N, 9.90%.

*H–D-Pro–L-Pro–Phe–Ile–Val–OBzl·HCl (**7D-L**).* Compound **6D-L** (1.52 g, 2 mmol) was treated with hydrogen chloride as described for **5L**: yield 1.16 g (83%); mp 98 °C (decomp); $[\alpha]_D^{20}$ −73° (*c* 1, methanol); R_f^1 0.81 and R_f^2 0.59.

Found: C, 62.40; H, 7.55; N, 9.51%. Calcd for $C_{37}H_{52}O_6N_5Cl \cdot H_2O$: C, 62.04; H, 7.60; N, 9.78%.

*H–D-Pro–D-Pro–Phe–Ile–Val–OBzl·HCl (**7D-D**).* Compound **6D-D** (1.52 g, 2 mmol) was treated with hydrogen chloride as described for **5L**: yield 1.34 g (96%); mp 115 °C (decomp); $[\alpha]_D^{20}$ −35° (*c* 1, methanol); R_f^1 0.82 and R_f^2 0.62.

Found: C, 62.42; H, 7.60; N, 9.42%. Calcd for $C_{37}H_{52}O_6N_5Cl \cdot H_2O$: C, 62.04; H, 7.60; N, 9.78%.

*Z–Arg(NO2)–Gly–L-Pro–D-Pro–Phe–Ile–Val–OBzl (**8L-D**).* To a solution of Z–Arg(NO2)–Gly–OH[4] (0.62 g, 1.5 mmol), **7L-D** (1.05 g, 1.5 mmol), and NMM (0.17 ml, 1.5 mmol) in DMF (6 ml), DCC (0.34 g, 1.65 mmol) was added at 0 °C with stirring. The reaction mixture was stirred for 3 h at 0 °C and then at room temperature overnight. DCUrea was filtered off and the filtrate was diluted with ethyl acetate. The solution was washed with 4% sodium hydrogencarbonate, 0.5 M hydrochloric acid, and water successively and then dried over anhydrous sodium sulfate. The filtrate was evaporated *in vacuo* and the residue was collected by the aid of ether. It was recrystallized from methanol–ether: yield 1.22 g (77%); mp 108 °C (decomp); $[\alpha]_D^{20}$ −35° (*c* 1, methanol); R_f^1 0.84 and R_f^2 0.68.

Found: C, 60.17; H, 6.84; N, 14.40%. Calcd for $C_{53}H_{71}O_{12}N_{11}$: C, 60.38; H, 6.79; N, 14.62%.

*Z–Arg(NO2)–Gly–D-Pro–L-Pro–Phe–Ile–Val–OBzl (**8D-L**).* This was prepared from Z–Arg(NO2)–Gly–OH[4] (0.41 g, 1 mmol) and **7D-L** (0.70 g, 1 mmol) as described above: yield 0.72 g (68%); mp 118 °C (decomp); $[\alpha]_D^{20}$ −62° (*c* 1, methanol), R_f^1 0.87 and R_f^2 0.69.

Found: C, 60.08; H, 6.81; N, 14.48%. Calcd for $C_{53}H_{71}O_{12}N_{11}$: C, 60.38; H, 6.79; N, 14.62%.

*Z–Arg(NO2)–Gly–D-Pro–D-Pro–Phe–Ile–Val–OBzl (**8D-D**).* This was prepared from Z–Arg(NO2)–Gly–OH[4] (0.62 g, 1.5 mmol) and **7D-D** (1.05 g, 1.5 mmol) as described above: yield 1.10 g (70%); mp 118 °C (decomp); $[\alpha]_D^{20}$ +16° (*c* 1, methanol); R_f^1 0.86 and R_f^2 0.64.

Found: C, 60.09; H, 6.79; N, 14.34%. Calcd for $C_{53}H_{71}O_{12}N_{11}$: C, 60.38; H, 6.79; N, 14.62%.

*H–Arg–Gly–L-Pro–D-Pro–Phe–Ile–Val–OH·AcOH (**1L-D**).* Compound **8L-D** (0.32 g, 0.3 mmol) was dissolved in a mixture of methanol (3 ml) and acetic acid (3 ml) and hydrogenated in the presence of palladium black for 24 h at room temperature. The filtrate from catalyst was evaporated *in vacuo* and the residual oil was solidified by the aid of acetone. The product was recrystallized from methanol–ether: yield 0.20 g (79%); $[\alpha]_D^{20}$ −16° (*c* 1, H_2O); R_f^1 0.68 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 0.96, Gly 1.00, Pro 2.06, Phe 1.03, Ile 1.00, Val 0.99.

Found: C, 56.35; H, 7.79; N, 16.13%. Calcd for $C_{38}H_{60}O_8N_{10} \cdot CH_3COOH \cdot 1/2\ H_2O$: C, 56.25; H, 7.75; N, 16.40%.

*H–Arg–Gly–D-Pro–L-Pro–Phe–Ile–Val–OH·AcOH (**1D-L**).* Compound **8D-L** (0.32 g, 0.3 mmol) was hydrogenated as described above: yield 0.19 g (73%); $[\alpha]_D^{20}$ −24° (*c* 1, H_2O); R_f^1 0.69 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 1.02, Gly 1.16, Pro 2.31, Phe 1.21 Ile 1.00, Val 0.98.

Found: C, 55.17; H, 7.70; N, 15.96%. Calcd for $C_{38}H_{60}O_8N_{10} \cdot CH_3COOH \cdot 3/2\ H_2O$: C, 55.09; H, 7.74; N, 16.06%,

*H–Arg–Gly–D-Pro–L-Pro–Phe–Ile–Val–OH·AcOH (**1D-D**).* Compound **8D-D** (0.32 g, 0.3 mmol) was hydrogenated as described above: yield 0.16 g (62%); $[\alpha]_D^{20}$ +78° (*c* 1, H_2O); R_f^1 0.62 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 0.86, Gly 1.09, Pro 2.18, Phe 1.20, Ile 1.00, Val 0.97.

Found: C, 55.80; H, 7.74; N, 15.90%. Calcd for $C_{38}H_{60}O_8N_{10} \cdot CH_3COOH \cdot H_2O$: C, 55.67; H, 7.71; N, 16.23%.

*Z–Arg(NO2)–Gly–L-Pro–Phe–Ile–Val–OBzl (**9L**).* To a solution of Z–Arg(NO2)–Gly–OH[4] (0.41 g, 1 mmol) and NMM (0.11 ml, 1 mmol) in THF (2 ml), ECF (0.1 ml, 1 mmol) was added at −5 °C with stirring. After 10 min, a solution of **5L** (0.60 g, 1 mmol) and NMM (0.11 ml, 1 mmol) in THF (2 ml) was added to it. The reaction mixture was stored in an ice bath for 1 h and then at room temperature overnight. The mixture was evaporated *in vacuo* and the residue was solidified with water. It was filtered, washed with 4% sodium hydrogencarbonate, 0.5 M hydrochloric acid, and water successively, and dried. The product was dissolved in methanol and collected by the aid of ethyl acetate: yield 0.72 g (75%); mp 175—180 °C; $[\alpha]_D^{20}$ −24° (*c* 1, DMF); R_f^1 0.88 and R_f^2 0.74.

Found: C, 60.12; H, 6.74; N, 14.52%. Calcd for $C_{48}H_{64}O_{11}N_{10}$: C, 60.23; H, 6.74; N, 14.64%.

*Z–Arg(NO2)–Gly–D-Pro–Phe–Ile–Val–OBzl (**9D**).* This was prepared from Z–Arg(NO2)–Gly–OH[4] (0.62 g, 1.5 mmol) and **5D** (0.90 g, 1.5 mmol) as described for **8L-D**: yield 1.10 g (77%); mp 175 °C (decomp); $[\alpha]_D^{20}$ +3° (*c* 1, DMF); R_f^1 0.96 and R_f^2 0.78.

Found: C, 59.78; H, 6.84; N, 14.89%. Calcd for $C_{48}H_{64}O_{11}N_{10}$: C, 60.23; H, 6.74; N, 14.64%.

*H–Arg–Gly–L-Pro–Phe–Ile–Val–OH·AcOH (**2L**).* Compound **9L** (0.52 g, 0.5 mmol) was hydrogenated as described for **1L-D**: yield 0.27 g (69%); $[\alpha]_D^{20}$ −114° (*c* 1, H_2O); R_f^1 0.74 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 0.91, Gly 1.02, Pro 0.98, Phe 1.00, Ile 1.00, Val 1.13.

Found: C, 55.21; H, 7.95; N, 16.81%. Calcd for $C_{33}H_{53}O_7N_9 \cdot CH_3COOH \cdot 1/2\ H_2O$: C, 55.53; H, 7.72; N, 16.66%.

*H–Arg–Gly–D-Pro–Phe–Ile–Val–OH·AcOH (**2D**).* Compound **9D** (0.29 g, 0.3 mmol) was hydrogenated as described for **1L-D**: yield 0.16 g (69%); $[\alpha]_D^{20}$ +19° (*c* 1, H_2O); R_f^1 0.68 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 0.90, Gly 1.19, Pro 1.03, Phe 1.11, Ile 1.00, Val 1.00.

Found: C, 55.94; H, 8.00; N, 16.69%. Calcd for $C_{33}H_{53}O_7N_9 \cdot CH_3COOH$: C, 56.21; H, 7.68; N, 16.86%.

*Boc–Gly–Gly–OH (**10**).* A solution of Boc–Gly–Gly–OBzl[5] (3.22 g, 10 mmol) in methanol (30 ml) was hydrogenated in the presence of palladium black for 10 h. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. The residue was collected by the aid of ether. It was recrystallized from ethanol–ether: yield 2.21 g (95%); mp 136 °C; R_f^1 0.75 and R_f^2 0.40.

Found: C, 46.75; H, 6.95; N, 12.00%. Calcd for $C_9H_{16}O_5N_2$: C, 46.54; H, 6.94; N, 12.06%.

*Boc–Gly–Gly–Phe–Ile–Val–OBzl (**11**).* This was prepared from **10** (0.70 g, 3 mmol) and H–Phe–Ile–Val–OBzl·HCl[4] (1.51 g, 3 mmol) as described for the preparation of **4L**. The product was recrystallized from ethanol–ether: yield 1.79 g (87%); mp 209 °C; $[\alpha]_D^{20}$ −14° (*c* 1, DMF); R_f^1 0.96 and R_f^2 0.65.

Found: C, 63.13; H, 7.56; N, 10.27%. Calcd for $C_{36}H_{51}O_8N_5$: C, 63.41; H, 7.54; N, 10.27%.

*H–Gly–Gly–Phe–Ile–Val–OBzl·HCl (**12**).* Compound **11** (1.36 g, 2 mmol) was treated with hydrogen chloride as described for the preparation of **5L**: yield 1.31 g (98%); mp 227 °C; $[\alpha]_D^{20}$ −23° (*c* 1, DMF); R_f^1 0.76 and R_f^2 0.17.

Found: C, 59.06; H, 7.19; N, 11.03%. Calcd for $C_{31}H_{44}O_6N_5Cl \cdot 1/2\ H_2O$: C, 59.36; H, 7.23; N, 11.17%.

*Z–Arg(NO2)–Gly–Gly–Phe–Ile–Val–OBzl (**13**).* This was prepared from Z–Arg(NO2)–OH (0.71 g, 2 mmol) and **12** (1.0 g, 1.6 mmol) as described for the preparation of **4L**. The product was recrystallized from ethanol–ether: yield 1.10 g

(75%); mp 139—140 °C; $[\alpha]_D^{20}$ −11° (*c* 1, DMF); R_f^1 0.98 and R_f^2 0.51.

Found: C, 58.65; H, 6.63; N, 15.04%. Calcd for $C_{45}H_{60}O_{11}N_{10}$: C, 58.94; H, 6.60; N, 15.04%.

H–Arg–Gly–Gly–Phe–Ile–Val–OH·AcOH (3). Compound **13** (0.92 g, 1.02 mmol) was hydrogenated as described for **1L-D**: yield 0.64 g (91%); $[\alpha]_D^{20}$ −19° (*c* 1, H_2O); R_f^1 0.74 and R_f^2 0.00. Amino acid ratios in acid hydrolyzate: Arg 0.85, Gly 2.25, Phe 0.92, Ile 1.00, Val 1.03.

Found: C, 53.85; H, 7.85; N, 17.95%. Calcd for $C_{30}H_{49}O_7N_9 \cdot CH_3COOH \cdot 1/2\ H_2O$: C, 53.85; H, 7.59; N, 17.95%.

Paper Electrophoresis. This was carried out under the following conditions: paper, Toyo Roshi No. 51A chromatography paper; solvent, pyridine–acetic acid–water (10 : 0.4 : 90, v/v) (pH 6.4); voltage gradient, 15 V/cm; charge period, 2.5 h. Electrophoretic mobilities were recorded as R_{Arg}, the ratio of the distance the compounds moved to that which a standard arginine spot moved on the same electrophoreogram. The compounds (**1**, **2**, and **3**) migrated toward the cathode and revealed a single spot by spraying Sakaguchi reagent; ninhydrin gave the same result. The R_{Arg} value of each compound was 0.72.

CD Measurements. This was performed with a JASCO J-20 A. A cell of path length 0.2 mm was used. Patterns in water are shown in Fig. 2.

Sensory Test. Taste of the peptides was organoleptically determined by panel evaluation employing four people. A series of solutions of decreasing concentration, each half as strong as the proceeding one, were prepared. Before tasting the sample, the mouth was thoroughly rinsed with deionized water. The sample solution was held in the mouth for *ca.* 10 s and then spit out and the threshold value was determined. The results are listed in the Table.

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References

1) Part II. K. Otagiri, I. Miyake, N. Ishibashi, H. Fukui, H. Kanehisa, and H. Okai, *Bull. Chem. Soc. Jpn.*, **56**, 1116 (1983).

2) The abbreviations recommended by IUPAC-IUB commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used. Amino acid symbols except glycine denoted the L-configuration unless otherwise noted. Additional abbreviations: AcOH, acetic acid; DCC, dicyclohexylcarbodiimide; DCUrea, *N,N'*-dicyclohexylurea; DMF, *N,N*-dimethylformamide; ECF, ethyl chloroformate; NMM, *N*-methylmorpholine; THF, tetrahydrofuran.

3) N. Minamiura, Y. Matsumura, J. Fukumoto, and T. Yamamoto, *Agric. Biol. Chem.*, **36**, 588 (1972).

4) Part I. H. Fukui, H. Kanehisa, N, Ishibashi, I. Miyake, and H. Okai, *Bull. Chem. Soc. Jpn.*, **56**, 766 (1983).

5) V. M. Kozhukhouskaya, S. D. Luova, and R. P. Evstigneeva, *Khim. Prir. Soedin.*, **6**, 599 (1970).