Studies of Bitter Peptides from Casein Hydrolyzate. V.[1] Bitterness of the Synthetic N-Terminal Analogs of des-Gly²-BPIa (Arg-Pro-Pro-Phe-Ile-Val)[2]

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Synopsis. In order to investigate how the basic amino acid residue in the N-terminal contributes to the bitter taste exhibited by BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val), we synthesized several kinds of N-terminal analogs of des-Gly²-BPIa (Arg-Pro-Pro-Phe-Ile-Val). The basic amino acid residue of BPIa was found to play an important role in its bitter taste.

In studies of the relationship between bitterness and chemical structure of BPIa (Arg-Gly-Pro-Pro-Phe-Ile-Val) which was isolated from casein hydrolyzate by Minamiura *et al.*,[3] BPIa and its various analogs and fragments have been synthesized in our laboratory.[1,4-6] In Previous papers,[1,5] we explained that the hydrophobic amino acids moiety in the C-terminal and the L-arginine residue in the N-terminal of BPIa are necessary for an intense bitter taste. However, the participation in the bitter taste of several basic amino acids other than L-arginine in the N-terminal of BPIa was not dealt with in those papers.[1,5] During the synthetic studies of BPIa, we found that des-Gly²-BPIa (Arg-Pro-Pro-Phe-Ile-Val) exhibited a strong bitter taste of the same level as BPIa.[5] We synthesized six kinds of des-Gly²-BPIa analogs, as shown in Table 1, and compared their bitterness with that of des-Gly²-BPIa.

The synthesis of compound **1** was as follows: Z-Lys(ε-Z)-OH·DCHA and H-Pro-Pro-Phe-Ile-Val-OBzl·HCl[4] that is an intermediate in the synthesis of BPIa were coupled by the mixed anhydride method to yield Z-Lys(ε-Z)-Pro-Pro-Phe-Ile-Val-OBzl. The protected hexapeptide was hydrogenated in the presence of palladium black to give H-Lys-Pro-Pro-Phe-Ile-Val-OH (**1**). Other peptides (compounds **2—6**) were prepared by the same procedures mentioned above, employing the corresponding acylamino acids instead of Z-Lys(ε-Z)-OH·DCHA. The purity of synthetic peptides and their intermediates was confirmed by thin-layer examinations on two solvent systems, elemental analyses, and amino acid analyses. The threshold value for bitter taste and the ratio of caffeine ($R_{caf.}$) value of the synthesized peptides were organoleptically determined by panel evaluation with five people, as shown in Table 1.

All the synthesized peptides possessed a bitter taste. However, their bitterness was weaker than that of des-Gly²-BPIa (**7**). By comparing the bitteness of compounds **1—3** and **7** with that of **4—6**, it is obvious that the basic nature in the N-terminal is important for showing the strong bitter taste in compound **7**. The strength of bitterness of compounds **1—3** decreased proportionally to the decreasing of the side chain length of the amino acid residue in the N-terminal. On the other hand, we found an interesting result for the bitter taste of compounds **5** and **6**. The bitter taste of compound **1** where L-lysine residue was located in the N-terminal was stronger than that of compound **2** in

TABLE 1. THE THRESHOLD VALUE FOR BITTER TASTE OF THE SYNTHETIC PEPTIDES

	Compound	T.V./mM	$R_{caf.}$[a]
1	Lys-Pro-Pro-Phe-Ile-Val	0.15	6.67
2	Orn-Pro-Pro-Phe-Ile-Val	0.31	3.33
3	Dab-Pro-Pro-Phe-Ile-Val	0.60	1.67
4	Phe-Pro-Pro-Phe-Ile-Val	0.30	3.33
5	Lys(ε-Ac)-Pro-Pro-Phe-Ile-Val	1.30	0.77
6	Orn(δ-Ac)-Pro-Pro-Phe-Ile-Val	0.60	1.67
7	Arg-Pro-Pro-Phe-Ile-Val[b]	0.08	12.50

a) Ratio of caffeine. b) des-Gly²-BPIa.

TABLE 2. YIELD AND ANALYTICAL DATA OF PROTECTED HEXAPEPITDES (Z-X-Pro-Pro-Phe-Ile-Val-OBzl)

X	Yield %	Mp θ_m/°C	$[\alpha]_D^{20}$/° (c 1, methanol)	Formula	Found(Calcd)(%) C	H	N	R_f^1	R_f^2
Lys(ε-Z)	82	74—76	−86.0	$C_{59}H_{75}O_{11}N_7 \cdot 1/2\,H_2O$	66.17 (66.39)	7.34 (7.18)	9.22 (9.19)	0.96	0.66
Orn(δ-Z)	74	78—80	−88.5	$C_{58}H_{73}O_{11}N_7 \cdot 1/2\,H_2O$	65.96 (66.14)	7.31 (7.08)	9.32 (9.31)	0.96	0.73
Dab(γ-Z)	78	84—86	−97.0	$C_{57}H_{71}O_{11}N_7 \cdot 1/2\,H_2O$	66.05 (65.85)	7.06 (6.98)	9.52 (9.44)	0.97	0.73
Phe	69	88—90	−99.5	$C_{54}H_{66}O_9N_7 \cdot 2H_2O$	68.16 (68.32)	7.13 (7.43)	8.85 (8.86)	0.97	0.85
Lys(ε-Ac)	91	78—80	−100.5	$C_{53}H_{71}O_{10}N_7 \cdot H_2O$	64.43 (64.48)	7.35 (7.48)	9.81 (9.96)	0.95	0.60
Orn(δ-Ac)	91	90—92	−101.0	$C_{52}H_{69}O_{10}N_7 \cdot H_2O$	64.65 (64.37)	7.19 (7.38)	9.99 (10.11)	0.93	0.65

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Table 3. Yield and analytical data of final products
(H-X-Pro-Pro-Phe-Ile-Val-OH)

Compd	X	Yield %	Mp θ_m/°C	$[\alpha]_D^{20}$/° (c 1, methanol)	Formula / Amino acid ratios in acid hydrolyzate	Found (Calcd) (%) C	H	N	R_f[1]
1	Lys	71	156—157	−89.0	$C_{36}H_{57}O_7N_7 \cdot CH_3COOH \cdot 3/2\,H_2O$ Lys 0.99, Pro 2.04, Phe 1.00, Ile 1.00, Val 1.04.	57.81 (57.99)	7.91 (8.20)	12.46 (12.46)	0.57
2	Orn	79	155—157	−93.0	$C_{35}H_{55}O_7N_7 \cdot CH_3COOH \cdot 3/2\,H_2O$ Orn 1.03, Pro 1.96, Phe 1.03, Ile 1.00, Val 1.05.	57.26 (57.49)	7.75 (8.08)	12.56 (12.67)	0.56
3	Dab	88	156—157	−86.5	$C_{34}H_{53}O_7N_7 \cdot CH_3COOH \cdot H_2O$ Dab 0.97, Pro 1.95, Phe 1.03, Ile 1.00, Val 1.02.	57.46 (57.66)	7.78 (7.93)	12.90 (13.08)	0.68
4	Phe	97	144—145	−88.0	$C_{39}H_{54}O_7N_6 \cdot H_2O$ Phe 2.10, Pro 2.13, Ile 1.00, Val 0.99.	63.64 (63.56)	7.65 (7.66)	11.35 (11.41)	0.75
5	Lys(ε-Ac)	73	140—142	−84.5	$C_{38}H_{59}O_8N_7 \cdot 1/2\,H_2O$ Lys 1.02, Pro 1.95, Phe 1.03, Ile 1.00, Val 1.00.	60.51 (60.78)	8.10 (8.05)	12.81 (13.06)	0.69
6	Orn(δ-Ac)	79	144—145	−86.0	$C_{37}H_{57}O_8N_7 \cdot 3/2\,H_2O$ Orn 1.01, Pro 1.97, Phe 1.05, Ile 1.00, Val 1.02.	59.10 (59.10)	7.96 (7.64)	12.76 (13.04)	0.68

which L-ornithine replaced L-lysine. However, N^{δ}-acetyl-L-orinithine N-terminal substituted analog (compound **6**) gave a stronger bitterness than N^{ε}-acetyl-L-lysine substituted one (compound **5**), which is due to the acetyl group introduction. From the results described above, the strong bitterness exhibited by BPIa depends on both basicity and side chain length of the amino acid in the N-terminal.

Experimental

All the melting points are uncorrected. Thin-layer chromatographies were performed on Merck silica gel G. Developing solvents commonly used were (1) 1-butanol-acetic acid-pyridine-water (4:1:1:2, v/v) and (2) chloroform-methanol (5:1, v/v). Materials possessing a free amino group on a thin-layer plate were detected by spraying with ninhydrin. Compounds with blocked amino groups were detected by spraying with 25% hydrogen bromide in acetic acid and then with ninhydrin. The optical rotations were measured on a Union PM-101 polarimeter. Amino acid analyses of peptides were carried out on samples that had been hydrolyzated with constant boiling hydrochloric acid for 65 h in evacuated, sealed tubes at 110 °C, and were recorded on a Hitachi amino acid analyzer KLA-5 type.

Syntheses of Protected Hexapeptides. Z-Lys(ε-Z)-Pro-Pro-Phe-Ile-Val-OBzl: A solution of Z-Lys(ε-Z)-OH·DCHA (0.89 g, 1.5 mmol) and *N*-methylmorpholine (0.16 ml, 1.5 mmol) in tetrahydrofuran (1.5 ml) was chilled to −5 °C. Ethyl chloroformate (0.15 ml, 1.5 mmol) was added, and then the mixture was kept for 15 min at −5 °C. A solution of H-Pro-Pro-Phe-Ile-Val-OBzl·HCl[4] (1.05 g, 1.5 mmol) and *N*-methylmorpholine (0.16 ml, 1.5 mmol) in *N,N*-dimethylformamide (10 ml) was added to the mixture and stirred for 1 h, then at room temperature overnight. The mixture was evaporated *in vacuo*, and the oily residue was dissolved in ethy acetate. The solution was washed with water, 0.5 M (1 M=1 mol dm^{-3}) hydrochloric acid, 4% sodium hydrogencarbonate, and water successively, then dried over anhydrous sodium sulfate. Sodium sulfate was removed by filtration, and the filtrate was evaporated *in vacuo*. The oily residue was crystallized with ether-petroleum ether; yield, physical constants, and elemetal analysis of this compound is given in Table 2. Other protected hexapeptides were prepared from the corresponding acylamino acids and H-Pro-Pro-Phe-Ile-Val-OBzl·HCl by the same method. Their yields and analytical data are also presented in Table 2.

Syntheses of Final Products. H-Lys-Pro-Pro-Phe-Ile-Val-OH (1): Z-Lys(ε-Z)-Pro-Pro-Phe-Ile-Val-OBzl (1.05 g, 1 mmol) was hydrogenated in methanol (2 ml) and acetic acid (2 ml) for 20 h by the aid of palladium black. The catalyst was filtered off, and the filtrate was evaporated *in vacuo*. The oily residue was crystallized from acetone-ether. It was recrystallized from methanol-acetone-ether; Compounds **2—6** were prepared from the corresponding hexapeptides by hydrogenation. Yields and analytical data of final products are given in Table 3.

Sensory Test. Taste of the peptides was organoleptically determined by the same manner as described in the previous papers.[1,4-6]

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2) The abbreviations recommended by the IUPAC-IUB commission of Biochemical Nomenclature (*J. Biol. Chem.*, **247**, 977 (1972)) have been used. Amino acid symbols except glycine denote the L-configuration. Additional abbreviations: Ac, acetyl; Dab, 2,4-diaminobutyric acid; DCHA, dicyclohexylamine.

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