

## Studies of Bitter Peptides from Casein Hydrolyzate. X.<sup>1)</sup> Synthesis and Bitter Taste of H-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH Corresponding to C-Terminal Portion of $\beta$ -Casein<sup>2)</sup>

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In order to ascertain the validity of the structure of bitter peptide BPIa (H-Arg-Gly-Pro-Pro-Phe-Ile-Val-OH) isolated from casein hydrolyzate by Minamiura *et al.*, the C-terminal octapeptide of  $\beta$ -casein (H-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH) determined by Ribadeau Dumas *et al.*, which is assumed to correspond to BPIa, was synthesized and compared with the synthetic BPIa which was confirmed to be identical with the natural one. The two synthesized peptides, however, did not match on thin-layer chromatography. The result indicates that the structure of BPIa need not be converted to Ribadeau Dumas' formula. The octapeptide possessed an extremely bitter taste, with a threshold value of 0.004 mM (1 M = 1 mol dm<sup>-3</sup>). It is the most bitter peptide.

Many bitter peptides have been found in the proteolytic hydrolyzates of casein. Minamiura *et al.* isolated a bitter heptapeptide named BPIa from cow milk casein hydrolyzate by alkaline proteinase of *Bacillus subtilis*, and determined its amino acid sequence to be H-Arg-Gly-Pro-Pro-Phe-Ile-Val-OH.<sup>3)</sup> In this series of studies of bitter peptides, we previously described the synthetic identification of BPIa.<sup>4)</sup> Synthetic BPIa was indistinguishable from Minamiura's natural one when thin-layer chromatography, paper electrophoresis, and carboxymethylcellulose column chromatography were used. We also found that the synthetic BPIa possessed an extremely bitter taste, whose threshold value was 0.05 mM. Ribadeau Dumas *et al.* determined the whole primary structure of bovine  $\beta$ -casein which contains the partial sequence of -Arg<sup>202</sup>-Gly-Pro-Phe-Pro-Ile-Ile-Val<sup>209</sup>-OH in the C-terminal portion.<sup>5)</sup> Although the sequence of BPIa can not be found in the structure of Ribadeau Dumas' formula, it is similar to the C-terminal of the formula. Aoyagi and Izumiya synthesized a hexapeptide, H-Arg-Gly-Pro-Phe-Pro-Ile-OH (1), corresponding to the C-terminal portion (202–207) of  $\beta$ -casein and suggested that BPIa is to be changed so as to correspond to the C-terminal portion of Ribadeau Dumas' formula from the result that the synthetic hexapeptide possessed a bitter taste, with its threshold value of 0.13–0.25 mM.<sup>6)</sup> They also synthesized a heptapeptide, H-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH (2), corresponding to the C-terminal portion (203–209) and compared it with the natural peptide isolated from tryptic hydrolyzate of casein.<sup>7)</sup> They reported that the two peptides matched each other closely and that the threshold value of bitter taste of the two peptides was 0.17–0.34 mM, and supported Ribadeau Dumas' formula.

In order to ascertain the validity of the structure of BPIa, it becomes necessary to compare BPIa with the C-terminal octapeptide (202–209) of Ribadeau Dumas' formula. In the present paper, we describe the synthesis of the octapeptide, H-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH (3), the measurement of its threshold value of

bitter taste, and the comparison between synthetic BPIa and 3. Further, this paper deals with the synthesis of a heptapeptide, H-Arg-Gly-Pro-Phe-Pro-Ile-Val-OH (4), in which an isoleucine residue of 3 is missing.

The sequence of reactions employed for the synthesis of 3 is shown in Fig. 1. The syntheses of the intermediates (5, 6, 15, and 16) had been performed and was described in the previous paper.<sup>4)</sup> Compound 6 was coupled with Boc-Ile-OH by the mixed anhydride (MA) method to yield Boc-Ile-Ile-Val-OBzl (7). Removal of Boc group from 7 with hydrogen chloride in dioxane afforded the corresponding tripeptide ester hydrochloride (8). Compound 8 was coupled with Boc-Pro-OH by MA method to yield Boc-Pro-Ile-Ile-Val-OBzl (9). This was converted to the corresponding tetrapeptide ester hydrochloride (10) by the action of hydrogen chloride in dioxane. Compound 10 was coupled with Boc-Phe-OH by MA method to form Boc-Phe-Pro-Ile-Ile-Val-OBzl (11). After the treatment of 11 with hydrogen chloride in dioxane, the resulting pentapeptide ester hydrochloride (12) was coupled with Boc-Pro-OH by MA method to yield Boc-Pro-Phe-Pro-Ile-Ile-Val-OBzl (13). It was converted to the corresponding hexapeptide ester hydrochloride (14) by the action of hydrogen chloride in dioxane. Then compound 14 was coupled with Z-Arg(NO<sub>2</sub>)-Gly-OH (16) by the dicyclohexylcarbodiimide (DCC) method to yield the protected octapeptide (17). Catalytic hydrogenation of 17 gave the desired product (3).

The synthetic route for heptapeptide 4 is shown in Fig. 2. MA and DCC methods were used for the coupling reactions. The removal of Boc groups of the intermediates was performed by the action of hydrogen chloride in dioxane. The protected heptapeptide (24) derived from Z-Arg(NO<sub>2</sub>)-Gly-OH (16) and H-Pro-Phe-Pro-Ile-Val-OBzl·HCl (23) was hydrogenated in the presence of palladium black to yield the heptapeptide 4. The homogeneity of final products was confirmed by thin-layer chromatography, paper electrophoresis, amino acid analysis, and elemental analysis.

The taste of 3 and 4 was organoleptically determined by a panel evaluation employing four persons. The

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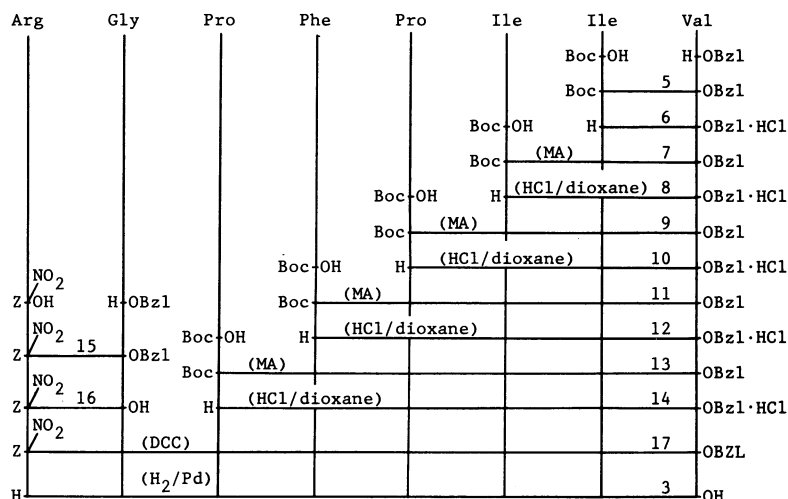


Fig. 1. Synthesis of the octapeptide 3.

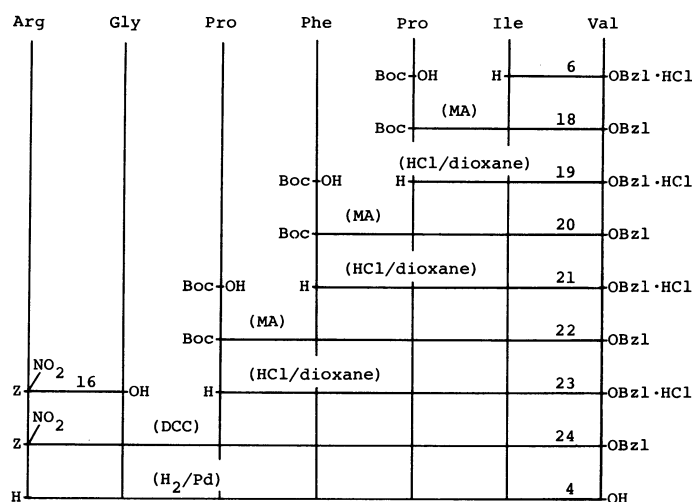


Fig. 2. Synthesis of the heptapeptide 4.

TABLE 1. THE THRESHOLD VALUES FOR BITTER TASTE OF SYNTHESIZED PEPTIDES AND TYPICAL BITTER SUBSTANCES

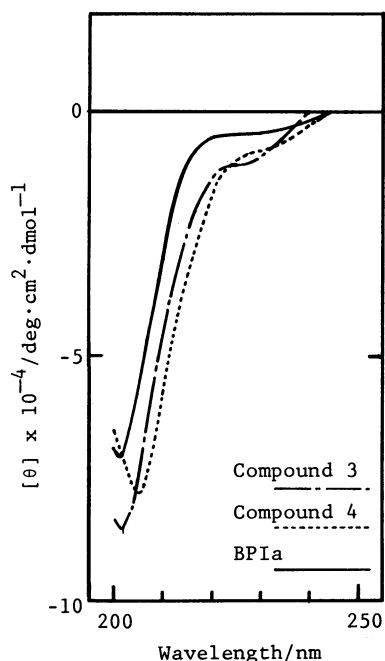
| Compound                            | Threshold value for bitter taste/mM |
|-------------------------------------|-------------------------------------|
| Arg-Gly-Pro-Phe-Pro-Ile (1)         | 0.13—0.25                           |
| Gly-Pro-Phe-Pro-Ile-Ile-Val (2)     | 0.17—0.34                           |
| Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val (3) | 0.004                               |
| Arg-Gly-Pro-Phe-Pro-Ile-Val (4)     | 0.11                                |
| Arg-Gly-Pro-Pro-Phe-Ile-Val (BPIa)  | 0.05                                |
| Caffeine                            | 1.0                                 |
| Strychnine                          | 0.003                               |
| Brucine                             | 0.0008                              |

results are listed in Table 1. The synthesized peptides possessed a strong bitter taste. The threshold value of **3** was 0.004 mM; this is the most bitter among the peptides as far as we know. By comparison with some alkaloids known to have strong bitterness, the potency of **3** corresponds to 250 times that of caffeine and is nearly equal to that of strychnine. In this connection, the threshold value of brucine, which is an alkaloid and the most bitter substance known, is 0.0008 mM. The cause of the strong bitterness of this peptide can be shown by comparing the bitterness of **3** with that of **1**

and **4**. It is obvious that **3** became the most bitter one due to the increase of the number of hydrophobic amino acids in the C-terminal moiety. Further, the difference in potency between **2** and **3** indicates that N-terminal arginine residue is indispensable for the extremely bitter taste observed in **3**. These findings agree with those obtained in our previous investigations of bitterness in BPIa.

The CD curves of **3**, **4**, and BPIa are presented in Fig. 3. These peptides showed similarly shaped curves. It seems that the whole molecular shape is also important for showing the strong bitter taste in peptides which are related to BPIa, in addition to the presence of both basic and hydrophobic amino acids.

In addition, electrophoretic tests of the octapeptide **3**, the heptapeptide **4**, and the synthetic BPIa, which was confirmed to be identical with the natural one,<sup>4)</sup> were carried out. The mobilities of both **3** and **4** were indistinguishable from that of BPIa on paper electrophoresis. However, on thin-layer chromatography, by using the solvent system, 1-butanol-acetic acid-pyridine-water (4:1:1:2, v/v), the *R<sub>f</sub>* value of BPIa was not identical with that of **3**, but nearly equal to that of **4**. These results indicate that BPIa does not correspond to the C-terminal portion of  $\beta$ -casein

Fig. 3. CD curves of **3**, **4**, and BPIa.

determined by Ribadeau Dumas *et al.* One possible explanation for our finding is that there are other kinds of variants in  $\beta$ -casein, perhaps due to differences between the cattle species throughout the world, a possibility of gene mutation among the same species, and other factors besides those known at the present.

### Experimental

All the melting points are uncorrected. 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 at 110°C for 72 h were performed with a Hitachi amino acid analyzer, KLA-5 type. Prior to analyses, the compounds were dried over phosphorus pentoxide at 66°C and 2 mmHg (1 mmHg  $\approx$  133.332 Pa) for 2 h, except in the case of compound **22**.

**Boc-Ile-Ile-Val-OBzl (7).** Boc-Ile-OH  $\cdot$  DCHA (9.08 g, 22 mmol) was dissolved in ethyl acetate (150 ml), and 1 M sulfuric acid (50 ml) was added to the mixture with stirring. The organic layer was washed with water and dried over anhydrous sodium sulfate. The solution was concentrated to dryness *in vacuo*, and the oily residue was dissolved in THF (40 ml) and NMM (2.2 ml, 20 mmol). ECF (2.0 ml, 20 mmol) was added to the mixture at  $-5^\circ\text{C}$  with stirring. After 10 min, a precooled solution of H-Ile-Val-OBzl  $\cdot$  HCl<sup>4</sup> (7.14 g, 20 mmol) and NMM (2.2 ml, 20 mmol) in chloroform (40 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 oily residue was dissolved in ethyl acetate. The solution was washed successively with 4% sodium hydrogen carbonate, 4% citric acid, and water, and then dried over anhydrous sodium sulfate. The filtrate was evaporated *in vacuo* and the oily residue was crystallized with ether-

petroleum ether: yield 8.86 g (83%); mp 173–175°C (lit.<sup>7</sup> 173–174°C);  $[\alpha]_D^{20} -75^\circ$  ( $c$  1, ethanol) (lit.<sup>7</sup>  $-77^\circ$  ( $c$  2.2, ethanol));  $R_f^1$  0.98 and  $R_f^2$  0.72.

Found: C, 65.30; H, 8.98; N, 7.75%. Calcd for  $\text{C}_{29}\text{H}_{47}\text{O}_6\text{N}_3$ : C, 65.26; H, 8.88; N, 7.87%.

**H-Ile-Ile-Val-OBzl  $\cdot$  HCl (8).** Compound **7** (5.34 g, 10 mmol) was dissolved in 4.1 M hydrogen chloride in dioxane (50 ml). The solution was allowed to stand for 2 h at room temperature and then evaporated *in vacuo*. The oily residue was solidified by the aid of ether: yield 4.52 g (96%); mp 240°C;  $[\alpha]_D^{20} -39^\circ$  ( $c$  1, methanol);  $R_f^1$  0.92 and  $R_f^2$  0.64.

Found: C, 61.13; H, 8.67; N, 9.05%. Calcd for  $\text{C}_{24}\text{H}_{40}\text{O}_4\text{N}_3\text{Cl}$ : C, 61.32; H, 8.58; N, 8.94%.

**Boc-Pro-Ile-Ile-Val-OBzl (9).** To a chilled solution of Boc-Pro-OH (1.05 g, 5 mmol) and NMM (0.55 ml, 5 mmol) in THF (10 ml), ECF (0.5 ml, 5 mmol) was added at  $-5^\circ\text{C}$  with stirring. After 10 min, a precooled solution of **8** (2.35 g, 5 mmol) and NMM (0.55 ml, 5 mmol) in DMF (10 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 diluted with ethyl acetate and the solution was washed successively with 4% sodium hydrogen carbonate, 4% citric acid, and water, and then dried over anhydrous sodium sulfate. The filtrate was evaporated *in vacuo* and the residue was crystallized with ether-petroleum ether: yield 2.74 g (87%); mp 192–194°C (lit.<sup>7</sup> 191–193°C);  $[\alpha]_D^{20} -100^\circ$  ( $c$  1, ethanol) (lit.<sup>7</sup>  $-82^\circ$  ( $c$  1.1, ethanol));  $R_f^1$  0.98 and  $R_f^2$  0.78.

Found: C, 64.55; H, 8.80; N, 8.88%. Calcd for  $\text{C}_{34}\text{H}_{54}\text{O}_7\text{N}_4$ : C, 64.73; H, 8.63; N, 8.88%.

**H-Pro-Ile-Ile-Val-OBzl  $\cdot$  HCl (10).** Compound **5** (3.15 g, 5 mmol) was treated with hydrogen chloride as described for the preparation of **8**: yield 2.69 g (95%); mp 240°C;  $[\alpha]_D^{20} -91^\circ$  ( $c$  1, methanol);  $R_f^1$  0.90 and  $R_f^2$  0.55.

Found: C, 61.13; H, 8.34; N, 10.03%. Calcd for  $\text{C}_{29}\text{H}_{47}\text{O}_5\text{N}_4\text{Cl}$ : C, 61.41; H, 8.35; N, 9.88%.

**Boc-Phe-Pro-Ile-Ile-Val-OBzl (11).** This was prepared from Boc-Phe-OH  $\cdot$  DCHA (1.56 g, 3.5 mmol) and **10** (1.70 g, 3 mmol) in the same way as described for the preparation of **7**: yield 2.06 g (88%); mp 163–165°C (lit.<sup>7</sup> 165–166°C);  $[\alpha]_D^{20} -84^\circ$  ( $c$  1, ethanol) (lit.<sup>7</sup>  $-81^\circ$  ( $c$  2.0, ethanol));  $R_f^1$  0.98 and  $R_f^2$  0.71.

Found: C, 66.54; H, 8.02; N, 8.89%. Calcd for  $\text{C}_{43}\text{H}_{63}\text{O}_8\text{N}_5$ : C, 66.38; H, 8.16; N, 9.00%.

**H-Phe-Pro-Ile-Ile-Val-OBzl  $\cdot$  HCl (12).** Compound **11** (2.34 g, 3 mmol) was treated with hydrogen chloride, as described for the preparation of **8**: yield 2.09 g (97%); mp 160–162°C;  $[\alpha]_D^{20} -80^\circ$  ( $c$  1, methanol);  $R_f^1$  0.90 and  $R_f^2$  0.64.

Found: C, 63.25; H, 7.77; N, 9.81%. Calcd for  $\text{C}_{38}\text{H}_{56}\text{O}_6\text{N}_5 \cdot 1/2\text{H}_2\text{O}$ : C, 63.10; H, 7.94; N, 9.68%.

**Boc-Pro-Phe-Pro-Ile-Ile-Val-OBzl (13).** This was prepared from Boc-Pro-OH (0.65 g, 3 mmol) and **12** (2.14 g, 3 mmol), as described for the preparation of **9**: yield 2.33 g (89%); mp 131–134°C;  $[\alpha]_D^{20} -114^\circ$  ( $c$  1, methanol);  $R_f^1$  0.97 and  $R_f^2$  0.80.

Found: C, 65.59; H, 8.36; N, 9.50%. Calcd for  $\text{C}_{48}\text{H}_{70}\text{O}_9\text{N}_6$ : C, 65.88; H, 8.06; N, 9.60%.

**H-Pro-Phe-Pro-Ile-Ile-Val-OBzl  $\cdot$  HCl (14).** Compound **13** (1.65 g, 2 mmol) was treated with hydrogen chloride, as described for the preparation of **8**: yield 1.49 g (92%); mp 157–159°C;  $[\alpha]_D^{20} -95^\circ$  ( $c$  1, methanol);  $R_f^1$  0.87 and  $R_f^2$  0.60.

Found: C, 63.44; H, 7.91; N, 10.79%. Calcd for  $\text{C}_{43}\text{H}_{63}\text{O}_7\text{N}_6\text{Cl}$ : C, 63.64; H, 7.83; N, 10.36%.

**Z-Arg(NO<sub>2</sub>)-Gly-Pro-Phe-Pro-Ile-Ile-Val-OBzl (17).** To a solution of Z-Arg(NO<sub>2</sub>)-Gly-OH<sup>4</sup> (0.68 g, 1.65 mmol) in DMF (3 ml), DCC (0.34 g, 1.65 mmol) was added at 0°C with stirring. After 20 min, a precooled solution of **14** (1.22 g, 1.5 mmol) and NMM (0.17 ml, 1.5 mmol) in chloroform (3 ml) was added to it. 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 successively with 4% sodium hydrogencarbonate, 0.5 M hydrochloric acid, and water, 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 1.36 g (78%); mp 129–132°C;  $[\alpha]_D^{20}$  –103° (*c* 1, methanol);  $R_f$  0.95 and  $R_f$  0.59.

Found: C, 60.22; H, 7.33; N, 13.90%. Calcd for  $C_{59}H_{82}O_{13}N_{12} \cdot 1/2H_2O$ : C, 60.23; H, 7.11; N, 14.29%.

*H-Arg-Gly-Pro-Phe-Pro-Ile-Ile-Val-OH·HCl* (3).

Compound **17** (0.47 g, 0.4 mmol) was dissolved in a mixture of methanol (3 ml) and acetic acid (3 ml) and hydrogenated in the presence of palladium black for 23 h at room temperature. The filtrate from catalyst was evaporated *in vacuo* and the residual oil was solidified by the aid of ether to give 0.31 g (81%) of **3** as a hygroscopic acetate form. It was dissolved in methanol (3 ml) and 4.1 M hydrogen chloride in dioxane (1 ml) was added to it. The solution was evaporated *in vacuo* and the residue was crystallized from acetone and ether: yield 0.29 g (76%);  $[\alpha]_D^{20}$  –108° (*c* 0.5,  $H_2O$ );  $R_f$  0.65 and  $R_f$  0.00. Amino acid ratios in acid hydrolyzate: Arg 0.88, Gly 1.02, Pro 1.97, Phe 1.01, Ile 1.89, Val 1.00.

Found: C, 52.62; H, 7.80; N, 14.92%. Calcd for  $C_{44}H_{72}O_9N_{11}Cl \cdot 4H_2O$ : C, 52.50; H, 8.01; N, 15.31%.

*Boc-Pro-Ile-Val-OBzl* (18).

This was prepared from Boc-Pro-OH (2.15 g, 10 mmol) and H-Ile-Val-OBzl·HCl<sup>9</sup> (3.57 g, 10 mmol), as described for the preparation of **9**: yield 4.47 g (86%); mp 102–103°C;  $[\alpha]_D^{20}$  –94° (*c* 1, methanol);  $R_f$  0.94 and  $R_f$  0.69.

Found: C, 64.78; H, 8.50; N, 8.23%. Calcd for  $C_{28}H_{43}O_6N_3$ : C, 64.96; H, 8.37; N, 8.12%.

*H-Pro-Ile-Val-OBzl·HCl* (19).

Compound **18** (1.04 g, 2 mmol) was dissolved in 4.1 M hydrogen chloride in dioxane (10 ml). The solution was allowed to stand for 1 h at room temperature and then evaporated *in vacuo*. The residual syrup was washed three times with ether by decantation, and dried in a vacuum desiccator: yield 0.91 g (100%);  $R_f$  0.82 and  $R_f$  0.48.

*Boc-Phe-Pro-Ile-Val-OBzl* (20).

This was prepared from Boc-Phe-OH·DCHA (0.98 g, 2.2 mmol) and **19** (0.91 g, 2 mmol), as described for the preparation of **7**. The product was obtained as an oily form: yield 1.12 g (84%);  $R_f$  0.98 and  $R_f$  0.70.

*H-Phe-Pro-Ile-Val-OBzl·HCl* (21).

Compound **20** (1.12 g, 1.68 mmol) was treated with hydrogen chloride, as described for the preparation of **8**. The product was obtained as a hygroscopic solid: yield 0.93 g (92%);  $R_f$  0.87 and  $R_f$  0.63.

*Boc-Pro-Phe-Pro-Ile-Val-OBzl* (22).

This was prepared from Boc-Pro-OH (0.33 g, 1.55 mmol) and **21** (0.93 g, 1.55 mmol), as described for the preparation of **9**: yield 0.84 g (71%); mp 61–63°C;  $[\alpha]_D^{20}$  –113° (*c* 1, methanol);  $R_f$  0.93 and  $R_f$  0.69.

Found: C, 65.84; H, 7.90; N, 8.93%. Calcd for  $C_{42}H_{59}O_8N_5$ : C, 66.20; H, 7.81; N, 9.19%.

*H-Pro-Phe-Pro-Ile-Val-OBzl·HCl* (23).

This was prepared from **22** (0.76 g, 1 mmol), as described for the preparation of **8**. The product was obtained as a hygroscopic solid: yield 0.57 g (81%);  $R_f$  0.79 and  $R_f$  0.55.

*Z-Arg(NO<sub>2</sub>)-Gly-Pro-Phe-Pro-Ile-Val-OBzl* (24).

To a solution of Z-Arg(NO<sub>2</sub>)-Gly-OH<sup>4</sup> (0.41 g, 1 mmol) and **23** (0.59 g, 0.81 mmol) and NMM (0.09 ml, 0.81 mmol) in DMF (4 ml), DCC (0.21 g, 1 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 successively with 4% sodium hydrogencarbonate, 0.5 M hydrochloric acid, and water, and then dried over anhydrous sodium sulfate. The filtrate was evaporated *in vacuo* and the residue was solidified with ether. It was

recrystallized from methanol-ether: yield 0.67 g (79%); mp 93–94°C;  $[\alpha]_D^{20}$  –90° (*c* 1, methanol);  $R_f$  0.83 and  $R_f$  0.66.

Found: C, 59.48; H, 6.98; N, 14.46%. Calcd for  $C_{53}H_{71}O_{12}N_{11} \cdot H_2O$ : C, 59.37; H, 6.86; N, 14.37%.

*H-Arg-Gly-Pro-Phe-Pro-Ile-Val-OH·AcOH* (4).

A solution of **24** (0.32 g, 0.3 mmol) in methanol-acetic acid (1:1, 6 ml) was hydrogenated in the presence of palladium black for 23 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated *in vacuo*. The residue was crystallized by the aid of acetone. It was recrystallized from methanol-ether: yield 0.17 g (67%);  $[\alpha]_D^{20}$  –110° (*c* 0.5,  $H_2O$ );  $R_f$  0.57 and  $R_f$  0.00. Amino acid ratios in acid hydrolyzate: Arg 0.91, Gly 1.01, Pro 1.88, Phe 1.09, Ile 1.00, Val, 0.98.

Found: C, 56.05; H, 7.84; N, 16.30%. Calcd for  $C_{38}H_{60}O_8N_{10} \cdot CH_3COOH \cdot 1/2H_2O$ : C, 56.35; H, 7.75; N, 16.40%.

*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 (**3** and **4**) and BPIa migrated toward the cathode and revealed a single spot after being sprayed with Sakaguchi reagent; ninhydrin gave the same result. The  $R_{Arg}$  value of both **3** and **4** was 0.72; BPIa gave the same  $R_{Arg}$  value.

*CD Measurement.*

This was performed with a JASCO J-20A. A cell of path length 0.2 mm was used and runs were made at ambient temperature. Patterns in water are shown in Fig. 3.

*Sensory Test.*

Taste of the synthesized peptides was organoleptically determined by panel evaluation employing four persons. A series of solutions of decreasing concentration, each half as strong as the preceding 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 then the threshold value was determined. The results are listed in the Table.

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## References

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