Further Study on the Salty Peptide Ornithyl-β-alanine. Some Effects of pH and Additive Ions on the Saltiness

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In 1984, we prepared some salty peptides such as ornithyltaurine and ornithyl-β-alanine. One Swiss research group resynthesized our peptides and alleged that those peptides were not salty. They concluded that the saltiness of those peptides resulted from NaCl present as an artifact of the method of preparation. We, however, reconfirmed that the salty peptides were still salty. We newly found that the pH of the peptide solutions is very important for the salty taste production. We also found that ornithyl-β-alanine and NaCl enhance the saltiness of each other.

In 1984 we reported that a series of basic dipeptides, such as ornithyl-β-alanine hydrochloride (H-Orn-β-Ala-OH·HCl), ornithyltaurine hydrochloride (H-Orn-Tau-OH·HCl), and others, possessed a salty taste (Tada et al., 1984). These salty peptides are expected to have good effects on hypertension, gestosis, diabetes mellitus, and other disease because they contain no sodium ion.

However, in 1987 Huynh-ba and Philippossian reexamined our work and reported our dipeptides not be salty. They concluded that the saltiness of H-Orn-Tau-OH·HCl claimed earlier probably resulted from NaCl present as an artifact of the method of preparation. Is that true? They reported that a part of Z-Orn(Z)-Tau-OH was converted into its sodium salt when a solution of the product in ethyl acetate was dried over sodium sulfate.

The mixture of Z-Orn(Z)-Tau-OH and Z-Orn(Z)-Tau-ONa was in the ratio of 30:70 according to their report. We recalculated the weight ratio of sodium chloride and sodium ion in the final product and found that they made some calculation error. The weight percent of sodium chloride must be 12.28% (they showed 21.7%), and the percent of sodium ion must be 4.83% (they showed 8.5%). According to their report, a 1% solution of the mixture of ornithyltaurine and NaCl (containing 0.123% NaCl by our calculation based on their data) tasted as salty as a 0.25% NaCl solution. They reported a 0.123% NaCl solution produced a saltiness the same as that of a 0.25% NaCl solution. What caused such contradictory results?

We suggest that Huynh-ba and Philippossian refer to the fact that (α,β-diaminopropionyl)taurine hydrochloride (H-Dap-Tau-OH·HCl) and (α,γ-diaminobutyryl)-taurine hydrochloride (H-Dab-Tau-OH·HCl) did not possess a saltiness strength similar to that of H-Orn-Tau-OH·HCl. Again, if the saltiness of H-Orn-Tau-OH·HCl is due to NaCl contaminating the molecule, H-Dap-Tau-OH·HCl and H-Dab-Tau-OH·HCl should produce a saltiness the same as that of H-Orn-Tau-OH·HCl. Because they were prepared in the same manner as H-Orn-Tau-OH·HCl, they must contain an amount of sodium ion equal to that in H-Orn-Tau-OH·HCl.

After the report of Huynh-ba and Philipposian was published, we set out to reconfirm our findings. H-Orn-β-Ala-OH (OBA) was selected as a standard compound because it does not form a sodium salt. They also reported that OBA was unsalty in spite of having no sodium ion. As a part of our reconfirmation, we additionally studied the following: (a) relationship between the saltiness and amount of acid contained in the sample solutions; (b) synthesis of some OBA analogues such as diacetyl-OBA, α-acetyl-OBA, δ-acetyl-OBA, and OBA methyl ester dihydrochloride to investigate the role of each functional group of OBA in producing saltiness; (c) behavior of the saltiness of OBA in the presence of NaCl; (d) preparation of OBA on a large scale using *p*-dimethylsulfoniophenyl (ODSP) ester, which was developed as a new active ester for peptide synthesis in our laboratory (Kouge et al., 1987). In this paper, we discuss an optimal condition for saltiness through the studies showed above.

EXPERIMENTAL SECTION

(1) Sensory Analysis. The tastes of the peptides were organoleptically determined by panel evaluation employing five people. Before the sample was tasted, the mouth was thoroughly rinsed with deionized water. The sample solution was held in the mouth for ca. 10 s and then expectorated, and the taste strengths were determined.

The taste strengths of the sample solutions were averaged after several examinations by the panelists with given peptides. The salty quality of peptides was compared with that of a standard NaCl solution. It was evaluated on a score of 0–5, where 5 was judged to be equivalent in salty taste to a reference 0.5% NaCl solution. Scores of 4, 3, 2, and 1 indicated a descending degree of saltiness, compared with the reference solutions. Score 3 means that the saltiness of the sample solution was the same as that of a 0.25% NaCl solution while score 1 was equated to the saltiness of a 0.1% NaCl solution. Scores 4 and 2 show that their taste strengths were between 5 and 3 and 3 and 1, respectively. Score 0 indicates tastelessness or other tastes (see Table II). For determination of the threshold value, the sensory analysis was carried out following the procedure of Ishibashi et al. (1987).

(2) Preparation of Sample Solutions. (*a*) *Preparation of OBA·nHCl Solutions* ($n = 0–1.3$). Following each condition of the sensory analysis, OBA·*n*HCl solutions, which had optional pH values or variable HCl concentrations, were prepared by adding HCl solution to HCl-free OBA solutions. See Table II.

(*b*) *Preparation of OBA Solutions with 1.3 equiv of Acid Component.* OBA solutions with 1.3 equiv of acid component were prepared by adding acid component to HCl-free OBA solutions. The result was shown in Table III.

(*c*) *Preparation of Mixed Solutions of OBA·1.3HCl and NaCl.* Mixed solutions were prepared by adding HCl and NaCl to HCl-free OBA solutions. The results are listed in Table VII.

(*d*) *Preparation of Mixed Solutions of OBA·1.3HCl and NaCl from OBA·2HCl.* The pHs of the sample solutions were adjusted at 4.20 by adding NaOH to OBA·2HCl. Additional NaCl was added to the solutions following the conditions listed in Table IX.

(*e*) *Preparation of OBA Analogue Solutions.* Sample solu-

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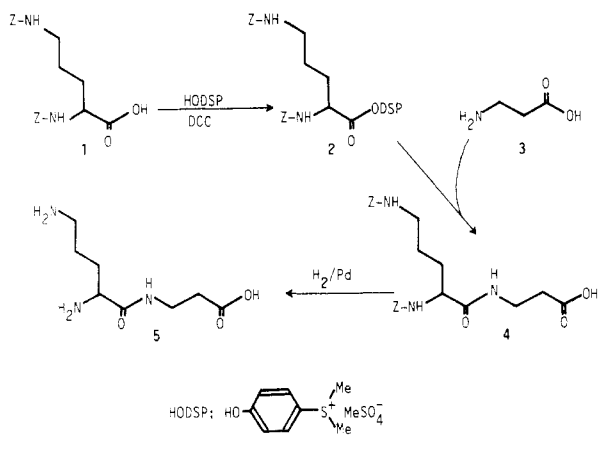
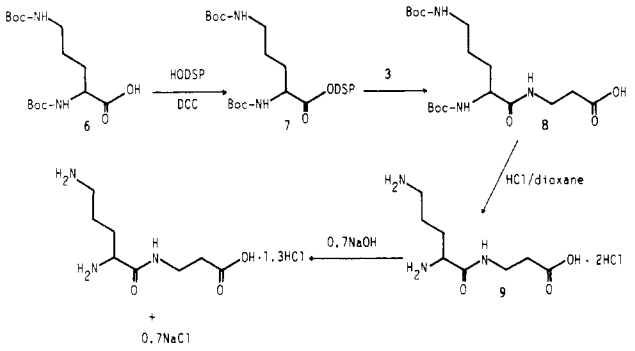
Table I. Physical Constants of the Intermediates and Final Products of OBA Analogues

compound	yield, %	mp, °C	$[\alpha]^{25}_D$ (concn, solvent)	formula	calcd C	calcd H	calcd N	found C	found H	found N	FD-MS calcd	FD-MS $(M+H)^+$
Boc-Orn(Cbz)-β-Ala-OBzl	98	81–82	−8 (c 1.0, MeOH)	$C_{28}H_{37}N_2O_7$	63.74	7.07	7.96	63.59	7.23	7.89		
H-Orn(Cbz)-β-Ala-OBzl·HCl	95	143–144	+9 (c 1.0, MeOH)	$C_{23}H_{30}N_3O_5Cl$	59.54	6.52	9.06	59.47	6.59	8.98		
Ac-Orn(Cbz)-β-Ala-OBzl	85	112–113	−13 (c 1.0, MeOH)	$C_{25}H_{31}N_3O_6 \cdot {}^1/_{10}H_2O$	63.71	6.67	8.92	63.76	6.77	8.88		
Ac-Orn-β-Ala-OH (10)	88	hygroscopic	−15 (c 1.0, MeOH)	$C_{10}H_{19}N_3O_4$	48.97	7.81	17.13				245.28	246
Cbz-Orn(Boc)-β-Ala-OBzl	96	84–85	−6 (c 1.0, MeOH)	$C_{28}H_{37}N_3O_7$	63.74	7.07	7.96	63.75	7.29	8.04		
Cbz-orn-β-Ala-OBzl·HCl	98	hygroscopic	−10 (c 1.0, MeOH)	$C_{23}H_{30}N_3O_5Cl$	59.54	6.52	9.06					
Cbz-Orn(Ac)-β-Ala-OBzl	89	133–134	−8 (c 1.0, MeOH)	$C_{25}H_{31}N_3O_6 \cdot {}^1/_5H_2O$	63.46	6.69	8.88	63.55	6.71	8.80		
H-Orn(Ac)-β-Ala-OH (11)	88	hygroscopic	+1 (c 1.0, MeOH)	$C_{10}H_{19}N_3O_4$	48.97	7.81	17.13				245.28	246
Cbz-Orn(Cbz)-β-Ala-OMe	61	128–129	+6 (c 1.0, DMF)	$C_{25}H_{31}N_3O_7$	61.84	6.44	8.65	62.00	6.70	8.70		
H-Orn-β-Ala-OMe (12)	90	hygroscopic	+29 (c 1.0, MeOH)	$C_9H_{21}N_3O_3Cl_2$	37.25	7.29	1.45				217.27	218

Scheme I



Scheme II



tions of OBA analogues were prepared following the procedure of Ishibashi et al. (1987).

(3) Synthesis of Peptides. *(a) General Procedures.* All the melting points are uncorrected. Thin-layer chromatography was carried out on Merck silica gel G with two solvent systems: (1) 1-butanol–acetic acid–pyridine–water (4:1:1:2, v/v); (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. NMR spectra were recorded on a JEOL JNM-GX270 spectrometer. FD mass spectra were measured on a Hitachi M-80B. Prior to analyses, the compounds were dried over phosphorus pentoxide at 66 °C (2 mmHg) (1 mmHg = 133 Pa) for 4 h.

(b) Syntheses of H-Orn-β-Ala-OH (5) and Cbz-Orn(Cbz)-β-Ala-OH (4). Cbz-Orn(Cbz)-OH·DCHA (11.6 g, 20 mmol) was dissolved in ethyl acetate (40 mL), and 1 M sulfuric acid (40 mL) was added to the mixture with stirring. The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated. The oily residue and HODSP (Kouge et al., 1987; 5.3 g, 20 mmol) was dissolved in acetonitrile (40 mL). DCC (4.1 g, 20 mmol) was added to the solution, and the mixture was stirred at 0 °C for 2 h and then at room temperature overnight. Resultant *N,N'*-dicyclohexylurea was filtered off, and the filtrate was evaporated. The oily residue was washed with ether by the decantation and dried under reduced pressure, giving 11.7 g (90%) of **2**: $[\alpha]^{25}_D$ +6.3° (c 0.8, MeOH); $R_f(1)$ 0.54, $R_f(2)$ 0.44.

To a solution of **2** (11.7 g, 18 mmol) in acetonitrile (60 mL) was added a solution of H-β-Ala-OH (**3**; 2.0 g, 22 mmol) and triethylamine (3.1 mL, 22 mmol) in water (60 mL). The mixture was stirred at room temperature overnight and evaporated to remove the organic solvent. The aqueous layer was washed with ether and acidified with 6 M HCl. The precipitated product of **4** was collected, washed with 0.5 N HCl and ether, and dried under reduced pressure: yield 7.5 g (88%); mp 163–167 °C; $[\alpha]^{25}_D$ +3.6° (c 1, DMF); $R_f(1)$ 0.89, $R_f(2)$ 0.50; FD-MS, $(M+H)^+$ 472, calcd 471.51.

Anal. Calcd for $C_{24}H_{29}N_3O_7$: C, 61.14; H, 6.20; N, 8.91. Found: C, 61.20; H, 6.30; N, 8.80.

H-Orn-β-Ala-OH (OBA, 5). Compound **4** (0.47 g, 1 mmol) was dissolved in MeOH (10 mL) and the resultant mixture hydrogenated at room temperature for 7 h using palladium black as a catalyst. After the catalyst was removed, the filtrate was evaporated and the product was obtained as a very hygroscopic powder: yield 0.83 g (90%); $[\alpha]^{25}_D$ −4° (c 2.0, MeOH); $R_f(1)$ 0.30; ^{1}H NMR (D_2O) δ 1.68–1.77 (m, 2 H, Orn CγH), 1.88–1.96 (m, 2 H, Orn CβH), 2.62 (t, 2 H, J = 6.3 Hz, β-Ala CαH), 3.02 (t, 2 H, J = 7.3 Hz, Orn CδH), 3.40–3.63 (m, 2 H, β-Ala CβH), 3.98 (t, 1 H, J = 6.4 Hz, Orn CαH); ^{13}C NMR data (D_2O) δ 25.3, 31.3, 39.1, 39.2, 41.5, 57.9, 175.8, 178.1; FD-MS, $(M+H)^+$ 204, calcd 203.24.

(c) Synthesis of H-Orn-β-Ala-OH·2HCl via Boc_2 Derivative. Boc-Orn(Boc)-β-Ala-OH. Boc-Orn(Boc)-OH (**6**; 1.7 g, 5 mmol) was converted to the ODSP-active ester **7** in the same manner as described for the preparation of Cbz-Orn(Cbz)-ODSP in quantitative yield: $[\alpha]^{25}_D$ −4° (c 1.0, MeOH); $R_f(1)$ 0.69, $R_f(3)$ 0.21.

To a solution of **7** (2.2 g, 4.5 mmol) in acetonitrile (20 mL) was added a solution of **3** (0.53 g, 6.0 mmol) and triethylamine (0.84 mL, 6.0 mmol) in water (20 mL). The mixture was stirred at room temperature overnight and evaporated to remove the organic solvent. The aqueous layer was washed with ether, acidified with 10% citric acid, and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. Compound **8** was solidified from ether–petroleum ether: yield 1.42 g (70%); $[\alpha]^{25}_D$ −12° (c 1.0, EtOAc); $R_f(Z)$ 0.79.

Table II. Influence of HCl on the Salty Taste of OBA[a]

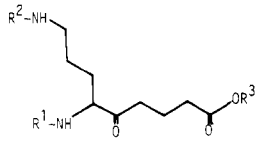
HCl, equiv	pH	score	sourness
0	8.9	0	
0.27	8.0	0	
0.79	7.0	0	
0.97	6.0	+1	
1.00	5.5	+2	
1.10	4.7	+3	±
1.20	4.3	+3.5	+
1.30	4.2	+4	++

[a] Concentration of OBA was 30 mM.

Anal. Calcd for $C_{18}H_{33}N_3O_7$: C, 53.58; H, 8.24; N, 10.41. Found: C, 53.72; H, 8.01; N, 10.44.

H-Orn-β-Ala-OH·2HCl (**9**). To a solution of 8 (1.2 g, 3 mmol) in dioxane (3 mL) was added 4 N HCl in dioxane (20 mL). The solution was allowed to stand at room temperature for 1 h and evaporated. An oil of the product was obtained: yield 0.8 g (100%); R_f(1) 0.30; FD-MS $(M + H)^+$ 204, calcd 203.24.

(*d*) *Synthesis of OBA Analogues.* Boc-Orn(Cbz)-OH was condensed with H-β-Ala-OBzl. The resultant protected dipeptide was treated with HCl to remove the Boc protecting group and acetylated. Ac-Orn(Cbz)-β-Ala-OBzl was hydrogenated to give Ac-Orn-β-Ala-OH (**10**). H-Orn(Ac)-β-Ala-OH (**11**) was prepared by a procedure similar to that for **10**. Cbz-Orn(Boc)-OH was used as a starting material instead of Boc-Orn(Cbz)-OH. Cbz-Orn(Cbz)-OH was condensed with H-β-Ala-OMe, and the resultant dipeptide was hydrogenated to give H-Orn-β-Ala-OMe·2HCl (**12**). See Table I for details of the physical constants of those intermediates and final products.



5; R^1=H, R^2=H, R^3=H
10; R^1=Ac, R^2=H, R^3=H
11; R^1=H, R^2=Ac, R^3=H
12; R^1=H, R^2=H, R^3=Me

RESULTS AND DISCUSSION

(1) Action of HCl for Saltiness on Ornithyl-β-alanine. There has been a problem getting a constant saltiness from OBA. At this point, we understand the argument of Huynh-ba and Philippossian. However, we found that when the pH of the sample solution was high, the saltiness was weak. The pH of the solution must be a key to the saltiness of OBA. We therefore, established an optimal amount of HCl for producing the strongest saltiness of H-Orn-β-Ala-OH. Solutions of 30 mM OBA containing 0–1.3 equiv of HCl were prepared by adding HCl to an HCl-free OBA solution. The result of the sensory analysis is shown in Table II. We found a relationship between OBA and HCl. When OBA contained HCl less than 0.97 equiv, it did not produce the salty taste. OBA containing 0.97 equiv of HCl produced a very weak salty taste, and the saltiness strength of OBA became stronger as the amount of HCl was increased. However, sourness originating from HCl became evident when more than 1.1 equiv of HCl was added. We obtained the strongest saltiness by adding 1.3 equiv of HCl to the OBA solution, which produced a weak sour taste.

We then studied salty taste production using other acids. As shown in Table III, five series of 30 mM OBA salts were prepared. Hard-acid salts such as hydrochloride and sulfate were found to produce an NaCl-like saltiness with a little sourness while organic acid salts produced a weak saltiness. The acidity of the acid seems to be a very important factor for strong taste production. We prepared solutions of OBA·1.3HCl in several concentrations and also prepared NaCl solutions that produced

Table III. Influence of the Anion Component on the Salty Taste of OBA

OBA + acid (30 mM)	pH	score	sourness
OBA·1.3HCl	4.3	+3	+
OBA·0.65H_2SO_4	3.9	+3	+
OBA·1.3AcOH	4.7	+2	+
OBA·1.3Ac-Tau-OH	4.2	+2	±
OBA·0.65Mal[a]	4.3	+2	++

[a] Malate.

Table IV. Strength of the Salty Taste of OBA

OBA·1.3HCl soln, mM	score	NaCl soln, mM	score
60 (1.44%, pH 4.1)	+5	85.6 (0.5%)	+5
45 (1.08%, pH 4.2)	+4	64.2 (0.375%)	+4
30 (0.72%, pH 4.2)	+3	42.8 (0.25%)	+3
15 (0.36%, pH 4.3)	+1	21.4 (0.1%)	+1

Table V. Sensory Analysis of OBA Analogues

compound	pH	TV,[a] mM	taste
H-Orn-β-Ala-OH·1.3HCl	4.3	1.57	salty > sour
Ac-Orn-β-Ala-OH	5.1	4.95	sweet > sour
Ac-Orn-β-Ala-OH·0.3HCl	4.3	3.16	sweet > sour
H-Orn(Ac)-β-Ala-OH	5.2	6.85	sweet > sour
H-Orn(Ac)-β-Ala-OH·0.3HCl	4.3	2.76	sweet > sour
Ac-Orn(Ac)-β-Ala-OH	3.0	1.23	sour
H-Orn-β-Ala-OMe·0.5HCl			no taste
H-Orn-β-Ala-OMe·1.0HCl			no taste
H-Orn-β-Ala-OMe·2.0HCl			salty > sour

[a] Threshold value.

the same saltiness in each concentration. As shown in Table IV, OBA needs about 3 times the weight percentage or 70% mol concentration to produce the same saltiness as NaCl.

(2) Role of Each Ionic Group of OBA in the Production of Salty Taste. In order to make clear the role of each ionic group of OBA for saltiness, we synthesized OBA analogues (**10–12**) that were blocked by an acetyl or methyl group. As shown in Table V, only OBA-OMe·2HCl (12·2HCl) produced saltiness although the saltiness was much weaker than that of OBA·1.3HCl. An α- and δ-acetyl-OBA analogue (**10, 11**) produced sweetness instead. From the results shown in Table V, it is obvious that all of the ionic groups are essential for saltiness.

^{1}H NMR spectral analysis in deuterium oxide of those compounds were carried out to find whether the major structural changes occurred by masking the functional groups (see Table VI). Protons of the β-carbon of β-alanine of OBA gave a very broad multiplet. This probably shows that the free rotation of the peptide chain is limited on that carbon. On the other hand, the signal of the same proton of H-Orn(Ac)-β-Ala-OH gave the triplet. The sweet peptide (**11**) seems to have free rotation along the peptide chain. Such a difference might be a reason for the taste changing. For further study, ^{13}C NMR analysis of those compound is under way and we are going to discuss why acetyl-OBA analogues produced the sweetness instead of the saltiness elsewhere.

(3) Behavior of a Solution of OBA·1.3HCl and NaCl. We studied the possibility of OBA as an NaCl substitute. Since foods are composed of many ionic compounds, we attempted to make a simple sample model using OBA·1.3HCl and NaCl. We prepared solutions of OBA·1.3HCl and of NaCl and mixed them in several ratios as shown in Table VII. All mixed solutions of each series possessed the same saltinessstrength. This shows that

Table VI. ^{1}H NMR Data of OBA and Analogues

compd	Orn α	Orn β	Orn γ	Orn δ	β-Ala α	β-Ala β	α-Ac	δ-Ac	OMe
5	3.98 (t, J = 6.4)	1.88–1.96 (m)	1.68–1.77 (m)	3.02 (t, J = 7.3)	2.62 (t, J = 6.3)	3.40–3.63 (m)			
10	4.21 (t, J = 6.3)	1.68–1.78 (m)		2.98 (t, J = 6.8)	2.36 (t, J = 6.8)	3.34–3.43 (m)	2.01		
11	3.90 (t, J = 6.8)	1.78–1.86 (m)	1.47–1.56 (m)	3.17 (t, J = 6.8)	2.41 (t, J = 3.4)	3.43 (t, J = 6.8)		1.96	
12	3.97 (t, J = 6.4)	1.88–1.94 (m)	1.72–1.77 (m)	3.02 (t, J = 7.8)	2.63 (t, J = 6.4)	3.47–3.56 (m)			3.69

[a] Signals of β- and γ-protons of Orn were not separated.

Table VII. Saltiness of OBA·1.3HCl by Adding NaCl

combinations: OBA·1.3HCl	combinations: NaCl	pH	saltiness
60 mM (1.44%)	0.0% (0 mM)	4.20	+5, contains sour taste
45 mM (1.08%)	0.125% (21.4 mM)	4.38	+5, contains weak sour taste
30 mM (0.72%)	0.25% (42.8 mM)	4.29	+5, contains very weak sour taste
15 mM (0.36%)	0.375% (64.2 mM)	4.37	+5
0 mM (0%)	0.5% (85.6 mM)	6.31	+5
30 mM (0.72%)	0.0% (0 mM)	4.28	+3, contains sour taste
22.5 mM (0.54%)	0.063% (10.7 mM)	4.33	+3, contains weak sour taste
15 mM (0.36%)	0.125% (21.4 mM)	4.35	+3, contains very weak taste
7.5 mM (0.18%)	0.188% (32.1 mM)	4.42	+3
0 mM (0%)	0.25% (42.8 mM)	6.31	+3

the saltiness of OBA·1.3HCl and NaCl was produced independently.

We found an interesting phenomenon during this study. The sourness arising from excess HCl was weakened by adding NaCl even though the pH was still acidic. It shows that the mechanism of sourness cannot be simply applied to H^+ concentrations. In order to decrease the sourness of the solution, we prepared mixed solutions of NaCl and OBA containing several amounts of HCl. We found that OBA·HCl produced a saltiness equal to OBA·1.3HCl when NaCl was added (see Table VIII). Although OBA containing just 1 equiv of HCl produced a weak saltiness (score was +2), it produced the same saltiness as that of OBA·1.3HCl in the presence of NaCl. This result showed that OBA·HCl and NaCl enhanced the saltiness of each other.

(4) Preparation of OBA on a Large Scale. If OBA is to be utilized practically, a large supply will be required. Therefore, we established a synthetic method using *tert*-butoxycarbonyl (Boc) groups for amino group protections instead of benzyloxycarbonyl (Cbz) groups. After the deprotection of Boc groups by HCl, OBA was obtained as a dihydrochloride. Excess HCl was neutralized by adding NaOH to give a mixture of OBA·1.3HCl and NaCl. The results of sensory analysis are listed in Table IX. We determined that its saltiness was the same as that of OBA·1.3HCl and NaCl prepared by the conventional method. OBA can be prepared on a large scale with use of Boc-protecting groups.

Table VIII. Enhanced Effect of Saltiness of OBA by Adding NaCl

combination: OBA, mM	combination: HCl, equiv	combination: NaCl, %	pH	saltiness
30	1.3	0.25	4.28	+5, contains sour taste
30	1.0	0.25	5.29	+5
30	0.5	0.25	7.57	+4
30	0	0.25	8.86	+3
15	1.3	0.125	4.31	+3, contains sour taste
15	1.0	0.125	5.35	+3
15	0.5	0.125	7.61	+2.5
15	0	0.125	8.90	+2

Table IX. Sensory Analysis of the Mixture of OBA·1.3HCl and NaCl Prepared from Boc_2-OBA

combinations: OBA, mM	combinations: amt NaOH added, mM	pH	concn of NaCl, %	saltiness
60	37.5	4.20	0.219	+5, contains sour taste
45	30.0	4.20	0.175	+5, contains weak sour taste
30	20.5	4.20	0.123	+3, contains very weak sour taste
15	10.8	4.20	0.063	+1, contains very weak sour taste
30[a]			0.250	+5
15[a]			0.375	+5
0[a]			0.5	+5

[a] OBA·1.3HCl prepared by the conventional method and mixed with NaCl.

CONCLUSIONS

We have discussed (a) the action of HCl for the saltiness of H-Orn-β-Ala-OH, (b) the role of each ionic group of OBA for saltiness, (c) the behavior of a solution of OBA·1.3HCl and NaCl, and (d) preparation of OBA on a large scale. We showed that a slight excess of HCl enhanced the saltiness of OBA and that OBA produced the strongest saltiness in the presence of 1.3 equiv of HCl. Additionally, we showed that OBA can be added to NaCl without any changing of the quality of the saltiness. The amount of sodium ion can be decreased as low as one-third of the conventional use by adding OBA according to our results. We believe that those results are a big step toward producing an NaCl substitute for patients of hypertension, gestosis, diabetes mellitus, and other diseases.

We also obtained some new results on the relationship between saltiness and ornithyltaurine. However, development of the usage and synthetic method are still under way. We will report these results on this journal after the work is done.

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Registry No. 1, 2274-58-0; 1·DCHA, 119853-36-0; 3, 107-95-9; 3 (benzyl ester), 14529-00-1; 3 (methyl ester), 4138-35-6; 4, 122663-58-5; 4 (methyl ester), 123486-25-9; 5, 94588-27-9; 6, 57133-29-6; 7, 123486-20-4; 8, 123486-19-1; 9, 123486-18-0; 10, 123486-23-7; 11, 123486-24-8; 12, 123486-26-0; HODSP, 45797-54-4; Boc-Orn(Cbz)-OH, 2480-93-5; Boc-Orn(Cbz)-β-Ala-OBzl, 123486-21-5; Ac-Orn(Cbz)-β-Ala-O-Bzl, 123486-22-6; Cbz-Orn(Boc)-OH, 7733-29-1; Cbz-Orn(Boc)-β-Ala-OBzl, 123486-27-1; H-Orn(Cbz)-β-Ala-OBzl·HCl, 123486-28-2; Cbz-Orn-β-Ala-OBzl·HCl, 123486-29-3; Cbz-Orn(Ac)-β-Ala-OBzl, 123505-62-4; OBA·1.3HCl, 123486-30-6; OBA·0.65HCl, 123486-31-7; OBA·1.3AcOH, 123486-32-8; OBA·1.3Ac-Tau-OH, 123486-33-9; OBA·0.65mal, 123486-34-0; Ac-Orn-β-Ala-OH·0.3HCl, 123486-35-1; H-Orn(Ac)-β-Ala-OH·0.3HCl, 123486-36-2; Ac-Orn(Ac)-β-Ala-OH, 123486-37-3; H-Orn-β-Ala-OMe·0.5HCl, 123486-38-4; H-Orn-β-Ala-OMe·1.0HCl, 123486-26-0; NaCl, 7647-14-5.

LITERATURE CITED

Huynh-ba, T.; Philippossian, G. Alleged Salty Taste of L-Ornithyltaurine Monohydrochloride. *J. Agric. Food Chem.* **1987**, *35*, 165.

Ishibashi, N.; Arita, Y.; Kanehisa, H.; Kouge, K.; Okai, H.; Fukui, S. Studies on Flavored Peptide. I. Bitterness of Leucine-containing Peptides. *Agric. Biol. Chem.* **1987**, *51*, 2389.

Kouge, K.; Koizumi, T.; Okai, H.; Kato, T. Peptide Synthesis in Aqueous Solution. I. Application of *p*-Dialkylsulfoniophenols as a Water Soluble Coupling Reagents. *Bull.Chem. Soc. Jpn.* **1987**, *60*, 2409.

Tada, M.; Shinoda, I.; Okai, H. L-Ornithyltaurine, a New Salty Peptide. *J. Agric. Food Chem.* **1984**, *32*, 992.

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Physicochemical Basis for Hardseededness in Mung Bean (*Vigna radiata* (L.) Wilczek)

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Hard seeds ranged from 0 to 3.8% in four varieties and four seed lots of mung bean from commercial sources. Uncooked normal and hard seeds were indistinguishable although, after boiling for 30 min, the hard seeds remained uncooked and were hard, brownish, and wrinkled. The hardness and seed coat thickness of hard seeds were twice those of normal seeds. Hard and normal seeds had similar chemical proximate composition except for fiber content, which was 9–25% greater in the hard seeds. The seed coats of hard seeds had 12% higher fiber content, 7 times more lignin, and 23% higher silica than the normal. The amino acid composition and pectic substances content of the two types of mung bean were similar. Histochemical analysis and scanning electron microscopy revealed a more rigid and highly structured palisade layer in the hard seed than in the normal seeds.

The hard seed (hard coat, hard shell) phenomenon has been reported for several leguminous species including soybean (Saio, 1976), cowpea (Sefa-Dedeh et al., 1979; Sefa-Dedeh and Stanley, 1979), and black beans (Molina et al., 1976; Varriano-Marston and Jackson, 1981; Jackson and Varriano-Marston, 1981) as well as for several species of Papilionaceae, like yellow peas (Werker et al., 1979). Various studies have sought to explain the causes of this phenomenon. The impermeability to water of *Pisum elatius*, *Pisum fulvum*, and *Pisum humile* is said to be due to the continuous, very hard pectinaceous layer of the caps of the palisade cells as well as the presence of quinones in a continuous layer of cells around the seed both in the lumen and the cell wall (Werker et al., 1979). Hard soybeans have higher amounts of crude fiber and calcium than normal soybeans (Saio, 1976). Lignification and presence of pectate could be possible causes of the hard-coat phenomenon resulting in descreased cookability (Bourne, 1967; Molina et al., 1976). The cross-linking of hydroxyprolyl residues in proteins to lignin has been suggested as an initial step in lignification of the cell wall (Whitmore, 1978). This phenomenon has also been established to occur after prolonged storage under unfavorable conditions of high temperature and high humidity (Molina et al., 1976).

Hard seeds, locally termed "patol", have also been observed in mung bean, a popular legume in the Philippines and in Southeast Asia. These hard seeds remain raw even after the normal seeds are fully cooked. To avoid the presence of these hard seeds in viands of mung bean, some crush the seeds before cooking. Others preboil the mung bean and then remove the uncooked seeds before fully cooking the rest of the seeds.

The mung bean hard seeds occur from 2 to 5% in newly harvested seeds during the dry season and occur to a greater extent among the yellow variety (Mendoza et al., 1988). This study is aimed at providing some basic information on the physicochemical characteristics of the mung bean hard seeds, which could explain this phenomenon and aid in the search for methods to preserve its cooking quality and acceptability.

EXPERIMENTAL SECTION

Materials. Samples of known varieties of mung bean seeds were obtained from the Legume Division of the Institute of Plant Breeding through the courtesy of Rudy S. Navarro. Samples

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