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Alleged Salty Taste of L-Ornithyltaurine Monohydrochloride

Tuong Huynh-ba* and Georges Philippossian

Very recently, L-ornithyltaurine hydrochloride (1) was claimed to be salty, with a saltiness equal to that of NaCl. To evaluate its organoleptic properties, peptide 1 was synthesized and rigorously purified: It was found not to be salty. The saltiness claimed earlier probably resulted from NaCl present as an artifact of the method of preparation.

Recently, several dipeptides derived from L-ornithine were described by Tada et al. (1984) to be salty. Among them, L-ornithyltaurine monohydrochloride (1) was claimed to exhibit a clear strong salty taste, equal to that of sodium chloride. Until now, most dipeptides have been reported to taste bitter, sour, sweet, slightly salty, or flat (Schiffman and Engelhard, 1976). This was the first time that a dipeptide was claimed to elicit such a strong salty taste without off-flavors. Compound 1 thus appeared to offer the greatest potential to date as salt substitute for NaCl, the excessive intake of which is considered a causative factor in certain health problems (Fregly and Kare, 1982). Many other salt substitutes have been proposed in different patent disclosures (Japan Organo K.K., 1982; Miles Laboratories Inc., 1978; Morton-Norwick Products Inc., 1974; Nisshin Oil K.K., 1982; Sterling Drug Inc., 1949), but none of them seemed to possess the property of 1. This prompted us to synthesize 1 and to perform a complete organoleptic evaluation, the results of which are here reported.

RESULTS AND DISCUSSION

Compound 1 was prepared according to the synthetic sequence described in the original work (Tada et al., 1984). Condensation of N^{α},N^{δ}-bis(benzyloxycarbonyl)-L-ornithine succinimido ester (2) with taurine (3) led to compound 4. Removal of the protective groups and subsequent acidification with HCl afforded 1 (Scheme I).

Compound 1 was tested at concentrations of 0.5% and 1%, in two separate sessions. A five-member taste panel judged compound 1 to be unsalty. A similar judgment was independently given by a trained 12-member taste panel who tested a 0.5% solution of 1. According to both panels, the off-flavors inherent to 1 were sourness, bitterness, and metallic taste, but their intensities were very low. This result was in complete disagreement with what had been claimed (Tada et al., 1984). The reason could have been the identity of the product, so our compounds 1 and 4 were fully characterized by spectroscopic means (^1H,^{13}C NMR; FAB-MS) as well as by TLC, $[\alpha]_D$ values, and elemental analyses (see the Experimental Section). In comparison, Tada et al. (1984) had assumed the purity and identity of their compounds on the basis of TLC and $[\alpha]_D$ data alone. We suspected that their compound 1 could have been contaminated by Na ion, since its immediate precursor, the sulfonic acid derivative 4, was put in contact with sodium salts (i.e., NaCl, Na_2SO_4) during its workup and would be converted to some extent into its Na salt. This property has been observed with sulfonic acid and attributed to its high acid strength (Fieser and Fieser, 1965).

Specifically to exclude all risk of contamination, we avoided the use of sodium salts in our preparation of compound 4. Compared to the original procedure (see Table I), the ethyl acetate extract containing 4 was neither washed with water saturated by NaCl nor dried over sodium sulfate.

For comparison, compound 1 was also prepared following the procedures previously described and outlined in Table I. Nevertheless, in our hands, a precipitate was formed when the ethyl acetate extract containing 4 was washed with the saturated aqueous NaCl solution and dried over Na_2SO_4. Taking this into account, we slightly modified the workup of 4 (see Table I and the Experi-

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Scheme I

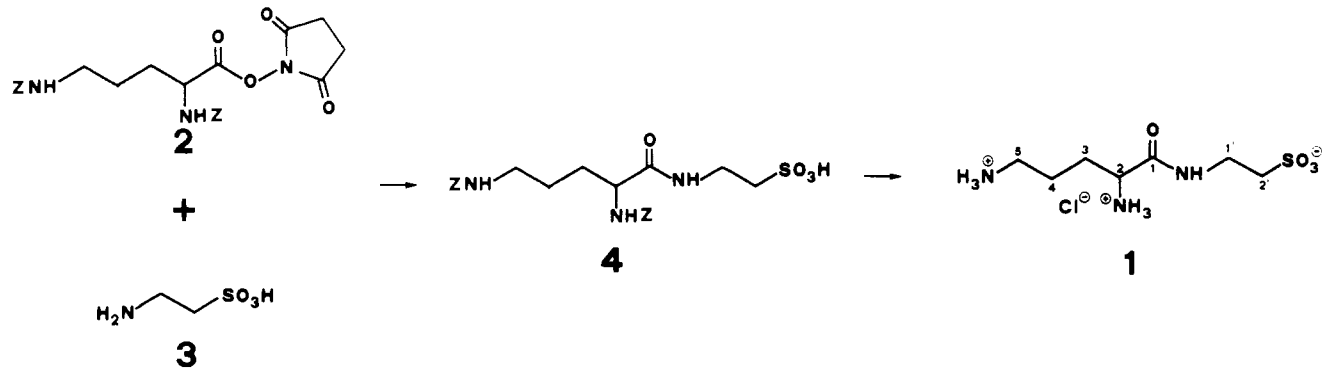


Table I. Different Workup Procedures of 4 and 1 as Used in This Work and In the Literature

compd	procedure used	
	this work	Tada et al. (1984)
4	dissolution of the crude product in H_2O	dissolution of the crude product in H_2O
	acidification with 6 N HCl	acidification with 6 N HCl
	extraction with ethyl acetate	extraction with ethyl acetate
	washing the extract with 2 N HCl	washing the extract with H_2O saturated by NaCl
	evaporation of ethyl acetate from the extract	drying the extract over Na_2SO_4
	azeotropic removal of H_2O by repetitive addition of toluene and evaporation	evaporation of ethyl acetate from the extract
	precipitation of the residue with $MeOH/Et_2O$	precipitation of the residue with Et_2O
1	acidification of the crude product with 1 N HCl	acidification of the crude product with HCl/dioxane
	evaporation of H_2O	precipitation with EtOH
	azeotropic removal of acetic acid by repetitive addition of toluene and evaporation	
	precipitation of the residue with $H_2O/MeOH/EtOH$	

mental Section). According to the judgement of our five-member taste panel, compound 1 tasted as salty as a 0.25% NaCl solution, when tested at the concentration of 1%. Atomic absorption showed it to contain 8.5%, by weight, 1 mol equiv of Na ion, corresponding to 21.7% by weight of NaCl. Sodium ion present in compound 1 apparently originates from the impure 4. Indeed, the analysis of the latter, obtained according to Tada et al. (1984), revealed a mixture of the sulfonic acid and its sodium salt in a ratio of 30:70. These salt impurities change both the melting points and $[\alpha]_D$ values (see the Experimental Section) and would explain the pronounced differences in these properties for 1 and 4 as measured in our laboratories, when compared to those reported by Tada et al. (1984).

In order to elucidate how this contamination occurred, model experiments were carried out with pure sulfonic acid 4. When pure 4 in ethyl acetate was washed with water containing 20% NaCl, the compound was converted to its sodium salt to an extent of 70%. However, surprisingly enough, the sodium salt still remained in the organic phase. This solubility could be accounted for by the large lipophilic groups in the molecule. A similar amount of conversion of compound 4 to its sodium salt was also observed when the compound, in ethyl acetate saturated with water, was dried over sodium sulfate. It then became evident that, following the workup described earlier, the sodium salt, and not the acid, was mainly obtained.

We thus conclude that L-Orn-Tau·HCl is not salty and that its claimed saltiness was the accidental result of an NaCl artifact.

In addition to 1, we also synthesized other dipeptides previously claimed to present some salty character (Tada et al., 1984) namely L-Orn-Gly (5), L-Orn-β-Ala (6), and L-Orn-γ-Abu (L-ornithyl-γ-aminobutyric acid, 7), along with structurally related dipeptides of L-ornithine combined with L-aspartic or L-glutamic acid monoamides or monomethyl esters. They were obtained by condensation of 2 with the corresponding free amino acid, followed by removal of the N protective groups and subsequent acidification with HCl. Their identification was fully provided by elemental analysis and physical and spectroscopic data (see Tables II and III).

According to our taste panel, the compounds in Table II also elicit no salty taste at a concentration of 1%. Unlike the case of 1, the possibility of NaCl contamination of the dipeptides 5–7 as prepared by Tada et al. (1984) can be excluded, since these authors carefully purified and characterized the immediate precursors, the N,N′-diprotected benzyl esters, and used no sodium salt in the last step of the synthesis. Therefore, no obvious explanation can be given for the differences in taste perception of these dipeptides observed by the Japanese workers and ourselves.

EXPERIMENTAL SECTION

Qualitative Analysis. TLC was carried out on Merck silica gel (G60, F254) plates eluted with 1-BuOH/AcOH/Py/H_2O (4:1:1:2). The compound spots were visualized under UV and/or revealed by ninhydrin. Polarimetric data were measured with a Perkin-Elmer 241 polarimeter. NMR spectra were taken with a Varian-FT-80A spectrometer (^1H NMR at 80 MHz, ^{13}C NMR at 20 MHz). Fast atom bombardment (FAB) MS were obtained with a Kratos MS-30 spectrometer equipped with an in-house designed FAB source [matrix, glycerol; fast atoms, Xe (7 kV)]. Determination of Na was performed with a Perkin-Elmer 603 atomic absorption spectrophotometer. Elemental analysis and Karl Fischer determination of water were performed by E. Thommen, Institut für Organische Chemie der Universität Basel.

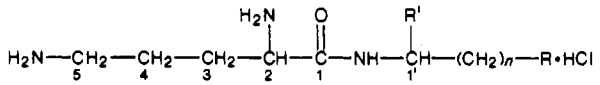
Organoleptic Evaluation. The compounds to be tested were diluted in mineral water, and their concentrations were expressed in weight/volume of added water. A solution of 1 (0.5% or 1%) and a 0.25% solution of NaCl were presented in random order to a five-member taste

Table II. Analytical Data of Synthetic Non Sulfonic Acid Containing Dipeptides[a]

no.	compd	mp, °C	$[\alpha]_D$, deg (c 1, H_2O)	formula[b]	% calcd (% found) C	H	N	Cl	H_2O
5	H-Orn-Gly-OH·HCl	hygroscopic	+46.2	$C_7H_{16}ClN_3O_3 \cdot 1.24H_2O$	33.87 (33.76)	7.51 (7.75)	16.93 (16.80)	14.30 (14.46)	9.07 (9.00)
6	H-Orn-β-Ala-OH·HCl	hygroscopic	+3.3	$C_8H_{18}ClN_3O_3 \cdot 0.80H_2O$	37.81 (37.96)	7.71 (7.89)	16.54 (16.51)	13.96 (13.76)	5.67 (5.78)
7	H-Orn-γ-Abu-OH·HCl	hygroscopic	+17.3	$C_9H_{20}ClN_3O_3 \cdot 0.84H_2O$	40.17 (40.44)	8.13 (8.23)	15.62 (15.60)	13.19 (13.37)	5.69 (5.65)
8	H-Orn-Asp-NH_2·HCl	138–146[c]	+32.2	$C_9H_{19}ClN_4O_4 \cdot 1.00H_2O$	35.94 (36.09)	7.04 (7.13)	18.63 (18.64)	11.79 (11.49)	5.99 (5.95)
9	H-Orn-Asp-OCH_3·HCl	175–179	+18.3	$C_{10}H_{20}ClN_3O_5$	40.34 (40.00)	6.77 (6.66)	14.11 (14.21)	11.91 (11.65)	0 (0)
10	H-Orn-Asp(OCH_3)-OH·HCl	149–153	+14.5	$C_{10}H_{20}ClN_3O_5 \cdot 0.60H_2O$	38.92 (38.94)	6.92 (7.04)	13.62 (13.52)	11.50 (11.06)	3.50 (3.11)
11	H-Orn-Glu-NH_2·HCl	158–163[c]	+27.8	$C_{10}H_{21}ClN_4O_4 \cdot 1.20H_2O$	37.72 (37.78)	7.41 (7.36)	17.59 (17.25)	11.15 (11.03)	6.79 (6.41)
12	H-Orn-Glu-OCH_3·HCl	119–128[c]	+2.3	$C_{11}H_{22}ClN_3O_5 \cdot 1.00H_2O$	40.06 (40.11)	7.33 (7.52)	12.74 (12.71)	10.76 (10.65)	5.46 (5.54)
13	H-Orn-Glu(OCH_3)-OH·HCl	172–174	+18.3	$C_{11}H_{22}ClN_3O_5$	42.37 (42.34)	7.11 (7.11)	13.48 (13.38)	11.38 (11.25)	0 (0)

[a] When tested at a concentration of 1%, the dipeptides were judged not to be salty by a five-member taste panel. [b] Compounds 9 and 13 were purified by crystallization from organic solvents; the other compounds were first crystallized from organic solvents and then freeze-dried from water to eliminate trace amounts of residual organic solvents. [c] Larger melting points ranges are due to the larger residual water contents.

Table III. ^{13}C NMR Spectra[a] of Non Sulfonic Acid Containing Dipeptides



compd	R	R′	n[b]	C-1	C-2	C-3	C-4	C-5	C-1′	C-2′	C-3′	C=O(R)	C=O(R′)	OCH_3(R)	OCH_3(R′)
5	COOH	H	0	169.2	52.8	27.8	22.4	38.8	43.4			176.1			
6	COOH	H	1	169.7	53.5	28.6	23.2	39.6	37.7*	37.2*		180.4			
7	COOH	H	2	169.4	53.2	28.3	22.9	39.2*	39.7*	25.2	34.1	181.4			
8	COOH	$CONH_2$	1	169.4	52.8	28.0	22.5	39.0	51.9	39.0		177.5	175.7		
9	COOH	$COOCH_3$	1	169.4	52.8	28.1	22.5	39.1	50.9	38.2		177.5	173.4		53.4
10	$COOCH_3$	COOH	1	169.1	52.8*	28.2	22.5	39.1	52.1*	36.7		174.0	176.5	52.9	
11	COOH	$CONH_2$	2	169.8	53.0	28.4	22.8	39.3	54.0	28.0	33.4	181.1	176.2		
12	COOH	$COOCH_3$	2	170.0	53.1*	28.5	22.8	39.4	53.5*	27.3	33.6	181.3	174.2		53.7*
13	$COOCH_3$	COOH	2	169.0	52.8	28.1	22.4	39.0	52.6	26.8	30.7	176.2	177.8	55.1	

[a] Spectra were recorded in D_2O. Chemical shifts are expressed in ppm; internal standard was dioxane (67.4 ppm). When asterisked, the assignments might be inverted. [b] For n = 1 or 2, the additional C atoms are numbered respectively C-2′, C-3′ following C-1′.

panel, asked to describe whether the solutions were salty and to characterize off-flavors. The 12-member taste panel received two solutions, 0.5% of 1 and 0.5% of NaCl. They were not informed which taste to expect but were asked to identify any tastes they perceived and to record the intensities of those tastes.

Material Synthesis. *N^{α},N^{δ}-Bis(benzyloxycarbonyl)-L-ornithyltaurine (4).* To a stirred solution of 5 g (10 mmol) of **2** in 30 mL of THF was added a solution of 1.64 g (13 mmol) of **3** and 1.32 g (13 mmol) of triethylamine in 30 mL of H_2O. After 1 h, the reaction mixture was left overnight and then concentrated to dryness. The residue was dissolved in 30 mL of H_2O, acidified to pH 0.1 with 17 mL of 6 N HCl, and extracted with 80 mL of ethyl acetate. The organic extract was washed twice with 10 mL of 2 N HCl and the solvent evaporated under vacuum at room temperature. The residue was triturated with 30 mL of toluene, which was then removed under vacuum. The treatment with toluene was repeated until removal of H_2O was complete. The crude product, dissolved in 8 mL of MeOH and precipitated by 80 mL of Et_2O, yielded 4.35 g (86%) of $4 \cdot 2.5H_2O$: mp 85–88 °C; R_f 0.65; $[\alpha]^{25}_D$ −13.5° (c 1, H_2O); ^1H NMR (Me_2SO-d_6) δ 7.33 (s, 10 aromatic H), 5.7–6.5 (m, 3 NH and SO_3H), 5.03 (s, 2 H, C-Ar), 5.01 (s, 2 H, C-Ar), 3.92 [m, 1 H C(2)], 3.39 [pseudo t, 2 H, C(2′)], 3.00 [m, 2 H, C(5)], 2.66 [m, 2 H C(1′)], 1.53 [m, 2 H, C(3); 2 H, C(4)]. Anal. Calcd for $C_{23}H_{29}N_3O_8S \cdot 2.5H_2O$ (552.61): C, 49.99; H, 6.20; N, 7.61; S, 5.80; H_2O, 8.15. Found: C, 50.27; H, 6.21; N, 7.59; S, 5.89; H_2O, 8.12.

L-Ornithyltaurine Hydrochloride (1). A solution of 3 g (5.91 mmol) of **4** dissolved in 25 mL of acetic acid was hydrogenated during 3 h in the presence of 0.1 g of 10% palladized charcoal. The reaction mixture was filtered and concentrated to dryness. The residue was dissolved in 10 mL of H_2O, treated with 10 mL of 1 N HCl. H_2O was evaporated under vacuum at room temperature, and the trace amount of acetic acid was removed azeotropically with toluene. The crude product was dissolved in 2 mL of H_2O and 10 mL of MeOH, and **1** was precipitated by adding 70 mL of ethanol; 1.4 g (86%). For analytical and testing purposes, freeze-drying of the solution of 1.4 g of **1** dissolved in 200 mL of H_2O yielded $1 \cdot H_2O$: mp 125–130 °C; R_f 0.15; $[\alpha]^{25}_D$ +8.5° (c 1, H_2O); ^1H NMR (D_2O) δ 4.04 [pseudo t, $J_{AX} + J_{BX}$ = 11.9 Hz, H, C(2)], 3.67 [m, 2 H, C(2′)], 3.10 [m, 2 H, C(1′); 2 H, C(5)], 1.89 [m, 2 H, C(3); 2 H, C(4)]; ^{13}C NMR (D_2O) δ 69.5 [C(1)], 53.3 [C(2)], 50.0 [C(2′)], 39.3 [C(5)], 35.9 [C(1′)], 28.3 [C(3)], 22.9 [C(4)]; FAB-MS, 240 $[M-Cl]^+$. Anal. Calcd for $C_7H_{18}N_3O_4SCl \cdot H_2O$ (293.81): C, 28.62; H, 6.86; N, 14.30; S, 10.91; Cl, 12.08; H_2O, 6.13. Found: C, 28.83; H, 7.09; N, 14.33; S, 11.07; Cl, 12.18; H_2O, 6.31.

1 Prepared according to Tada et al. (1984). The reaction mixture of 8.3 g (16.7 mmol) of **2**, 2.8 g (22.2 mmol)

of **3**, and 2.3 g (22.4 mmol) of triethylamine in 30 mL of THF and 30 mL of H_2O was concentrated to dryness after standing overnight. The residue was dissolved in 30 mL of H_2O, acidified with 20 mL of 6 N HCl, and extracted with 100 mL of ethyl acetate. The organic extract was washed with 50 mL of H_2O containing 20% of NaCl and dried over sodium sulfate. A voluminous precipitate was formed and then filtered. To the filtered solid was added 50 mL of MeOH. After removal of sodium sulfate, MeOH was evaporated. The residue was dissolved in 25 mL of MeOH, filtered again, and precipitated with 100 mL of Et_2O: 7.3 g; mp 158–182 °C; R_f 0.65; $[\alpha]^{25}_D$ −11° (*c* 1, H_2O); FAB-MS, 552 $[M(4\text{-}Na) + Na]^+$, 530 $[M(4\text{-}Na) + H]^+$. The atomic absorption of Na (found: 4.18% by weight), the elemental analysis (found: C, 49.77; H, 5.18; N, 7.47; S, 6.03; Cl, 1.62), and the water determination (found: 2.01% by weight) would correspond to a mixture of **4** and its Na salt in an approximate ratio of 30:70, contaminated with 0.25 mol equiv of NaCl and 0.04 mol equiv of $NaHSO_4$ and hydrated with 0.6 mol equiv of H_2O. A solution of 5.07 g of that compound in 80 mL of acetic acid was hydrogenated during 4 h in the presence of 0.2 g of 10% palladized charcoal. The reaction mixture was filtered; acetic acid was evaporated. The residue was treated with 2 mL (10.8 mmol) of 5.4 M HCl/dioxane and precipitated with 30 mL of ethanol, yielding 2.4 g of very hydroscopic white solid: R_f 0.15; $[\alpha]^{25}_D$ +7° (*c* 1, H_2O); ^1H NMR (D_2O) δ 4.13 [pseudo t, $J_{AX} + J_{BX}$ = 12 Hz, 1 H, C(2)], 3.73 [m, 2 H, C(2′)], 3.20 [m, 2 H, C(1′); 2 H, C(5)], 1.99 [m, 2 H, C(3); 2 H, C(2′); 2 H, C(4)]; ^{13}C NMR (D_2O) δ 169.4 [C(1)], 53.2 [C(2)], 50.0 [C(2′)], 39.3 [C(5)], 35.9 [C(1′)], 28.2 [C(3)], 22.9 [C(4)]; Na content, 8.5% by weight, corresponding to 1 mol equiv.

Sodium Exchange between 4 and NaCl. The reaction mixture of 5 g (10 mmol) of **2**, 1.64 g (13 mmol) of **3**, and 1.32 g (13 mmol) of triethylamine in 30 mL of THF and 30 mL of H_2O was evaporated after standing overnight, then dissolved in 30 mL of H_2O, acidified with 17 mL of 6 N HCl, and extracted with 80 mL of ethyl acetate. The organic extract was washed with 60 mL of H_2O containing 20% of NaCl and concentrated to dryness. The residue was triturated with 30 mL of toluene, which was then removed under vacuum. The treatment with toluene was repeated until complete removal of H_2O. The crude product was dissolved in 20 mL of MeOH and precipitated with 40 mL of Et_2O: 4.45 g; mp 156–174 °C; R_f 0.65; FAB-MS, 552 $[M(4\text{-}Na) + Na]^+$, 530 $[M(4\text{-}Na) + H]^+$. The atomic absorption of Na (found: 3.55% by weight), the elemental analysis (found: C, 50.68; H, 5.55; N, 7.57; S, 5.68; Cl, 0.97), and the water determination (found: 2.20% by weight) would correspond to a mixture of **4** and its Na salt in an approximate ratio of 30:70, contaminated with 0.15 mol equiv of NaCl and hydrated with 0.7 mol equiv of H_2O.

Sodium Exchange between 4 and Sodium Sulfate. To a solution of 0.5 g (1 mmol) of **4** dissolved in 25 mL of ethyl acetate and 1 mL of H_2O, was added 2.5 g of anhydrous sodium sulfate (Merck). After 30 min of stirring, the voluminous forming precipitate was filtered. To this was added 20 mL of MeOH, and sodium sulfate was removed by filtration. From the filtrate was evaporated MeOH, and the residue was dissolved in 5 mL of MeOH, filtered again, and precipitated by 20 mL of Et_2O: 0.4 g; mp 153–157 °C; R_f 0.65. The atomic absorption of Na (found: 3.44% by weight), the elemental analysis (found: C, 49.12; H, 5.55; N, 7.56; S, 6.68), and the water determination (found: 3.17% by weight) would correspond to a mixture of **4** and its Na salt in an approximate ratio of 30:70, contaminated with 0.17 mol equiv of $NaHSO_4$ and hydrated with 1 mol equiv of H_2O.

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Registry No. **1**, 90970-64-2; **2**, 90970-61-9; **3**, 107-35-7; **4**, 90990-60-6; **5**, 90970-33-5; **6**, 90970-63-1; **7**, 90970-34-6; **8**, 106252-19-1; **9**, 106252-20-4; **10**, 106252-21-5; **11**, 106252-22-6; **12**, 106252-23-7; **13**, 106252-24-8; Na, 7440-23-5.

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