

Rebuttal on L-Ornithyltaurine, a New Salty Peptide

Sir: Thank you very much for sending us a copy of the letter from Drs. Huynh-Ba and Philipposian. We would like to argue our findings and to point out some problems in their paper again.

Dr. Huynh-Ba and Philipposian reported that they found 8.5% w/w Na ion in the OT·HCl (ornithyltaurine hydrochloride) prepared according to our method. In their paper, we found that they obtained a mixture of Z-Orn(Z)-Tau-OH and Z-Orn(Z)-Tau-ONa in a ratio of 30:70. If their data were correct, the 30:70 mixture of Z-Orn(Z)-Tau-OH and Z-Orn(Z)-Tau-ONa would give 8.5% w/w Na ion content. Therefore, we tried to calculate Na ion content starting from their mixture of Z-Orn(Z)-Tau-OH and Z-Orn(Z)-Tau-ONa. First, we did not know whether 30:70 meant weight or molar ratio. We recalculated the mole and weight ratio of the acid and its sodium salt and found that both mole and weight ratio gave the same result (30:70 mol/mol or 30:70 w/w). After the hydrogenation in acetic acid, the mixture should have, of course, given H-Orn-Tau-OH·AcOH and H-Orn-Tau-ONa·2AcOH in the ratio 30:70 (mol/mol). To convert 100 mmol of the mixture of H-Orn-Tau-OH·AcOH and H-Orn-Tau-ONa·2AcOH (30:70 mol/mol) into H-Orn-Tau-OH·HCl, 170 mmol (30 mL + 70 mL × 2) of HCl (1.7 equiv) is necessary. However, they added only 1 equiv of HCl to the solution to the mixture. They converted 100 mmol of the mixture of H-Orn-Tau-OH·AcOH and H-Orn-Tau-ONa·2AcOH (30:70 mol/mol) into 8.52 g (30 mmol) of H-Orn-Tau-OH·HCl, 20.68 g (70 mmol) of H-Orn-Tau-OH·AcOH, and 4.04 g (70 mmol) of NaCl using 100 mmol of HCl. Therefore, 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%). If we believe their result of atomic absorption spectroscopy, the mixture of Z-Orn(Z)-Tau-OH and Z-Orn(Z)-Tau-ONa gave Na ion almost twice the amount the intermediate should have given. Even if 100% of the Z-Orn(Z)-Tau-OH was converted into the Na salt (of course, this is very unlikely), Na content in the final product must be only 18%. OT·HCl cannot contain as much as 21.7% NaCl in the final product. This is simple mathematics. Where did the extra Na ion come from? Is there any possibility that one molecule of Z-Orn(Z)-Tau-OH can form a salt with more than two Na ions? If so, their result of atomic absorption spectroscopy would be correct. If Z-Orn(Z)-Tau-OH can form a salt with only one Na ion and if their result of atomic absorption spectroscopy is correct, the extra Na ion must have appeared without interaction between the Na ion and the sulfonyl group of Tau.

When we reexamined their synthetic procedure for H-Orn-Tau-OH·HCl carefully, we discovered that they washed Na₂SO₄ with methanol to recover precipitated Z-Orn(Z)-Tau-OH after the organic solvent dried over Na₂SO₄. It is well-known that a large quantity of Na⁺ becomes mixed in the organic solvent by such a procedure. Apparently, most of the Z-Orn(Z)-Tau-ONa was formed at that time. H-Orn-Tau-OH·HCl must be synthesized carefully to prevent its being mixed with NaCl. The above

shows that the total NaCl mixed with H-Orn-Tau-OH·HCl is under the threshold value if it is synthesized carefully. Of course, we did not do such a thing, although they reported they traced our experimental procedure.

We also discovered that they azeotropically removed the H₂O from the mixed solutions by codistillation with toluene several times as the last step in the coupling procedure. They should have known that even a small amount of toluene, which may not be detected by NMR, could have had influences on the taste of the sample at the concentration required for sensory analysis. They reported that the compounds prepared by using toluene codistillation produced sourness, bitterness, and a metallic taste. Bitterness and the metallic taste were produced by a small amount of toluene which was not removed completely from the samples. Toluene should mask the saltiness of the peptides. Compounds that are going to be tasted (especially in the case of compounds possessing very weak taste intensity like salty peptides), with the exception of acetic acid, should not be prepared with solvents having boiling points higher than water.

We understand that Na ion has a chance to contaminate in the intermediate. However, if we carried out the synthesis very carefully, the contamination of the Na ion content could control less than its threshold value. Even if the synthesis procedure was very rough (as Drs. Huynh-Ba and Philipposian's was), 70% of Z-Orn(Z)-Tau-OH was converted to the Na salt, which could not produce the saltiness in a normal concentration solution.

Since Drs. Huynh-Ba and Philipposian reported the saltiness was due to Na ion contaminated in the peptide, we had to show our peptide produced the saltiness without Na ion. Therefore, we selected ornithyl-β-alanine (they reported this compound did not produce the saltiness either) as a model compound because it should not form Na salt during the preparation procedure. We know we still need to clarify the saltiness of OT·HCl. Now we are studying a preparation method of Na-free OT·HCl without using toluene. We will publish a new paper elsewhere shortly.

LITERATURE CITED

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