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ARTICLE

Taste Contribution of (*R*)-Strombine to Dried Scallop

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A broth of dried scallop adductor muscles was prepared. Tasters appreciated the typical seafood, sweet, slightly umami taste of scallop, which is difficult to reproduce with common ingredients. Therefore, the broth was fractionated and, guided by multiple tastings, we isolated a sweet, umami, delicious fraction. This fraction contained glycine, alanine, and (*R*)-strombine ((*R*)-2-(carboxymethylamino)propanoic acid). (*R*) and (*S*)-strombine were prepared, and a sensory analysis with 47 judges demonstrated that the taste thresholds were 0.5 g/L for (*R*)-strombine and 0.7 g/L for (*S*)-strombine. The sensory attributes were described as salty and umami.

KEYWORDS: Opine; (*R*)-2-(carboxymethylamino)propanoic acid; (*R*/*S*)-strombine; dried scallop

INTRODUCTION

Scallops are marine bivalve mollusks of the family Pectinidae. Different kinds of scallops from the world's oceans are exploited for their edible central adductor muscle. In Asia, and especially in Cantonese Chinese cuisine, dried scallops called conpoy are used to prepare soups. The broth obtained from the dried scallop has a nice rounded taste, which is not thoroughly umami nor clearly sweet or salty. Some sensory analyses of seafood have discussed the role of salts, amino acids, and ribotides for the umami taste of seafoods, but all of our attempts to recreate this taste, in the context of dry scallop broth, failed to reproduce the fullness and complexity of the natural dried scallop broth (*1*−*4*).

The discovery of class C G-protein coupled receptors T1R1 and T1R3, amino acid specific taste receptors, has demonstrated the existence in mammals of sensors that respond to most of the 20 standard amino acids (*5*−*7*). In humans, glutamic acid elicits a specific umami taste by activation of the T1R1 and T1R3 dimer, and this receptor has been used by Li and co-workers for high-throughput screening of chemical libraries to discover new umami molecules (*8*, *9*). Similarly, the sweet taste receptor is a T1R2 and T1R3 dimer, and the understanding of taste perception at the cellular level and the development of molecules that enhance taste perception are rapidly growing areas of research (*10*, *11*).

Frérot and co-workers have demonstrated that small peptides and derivatives including Glu-Glu-Leu and lactoyl-Glu, which have been detected in Parmesan cheese, elicited umami taste at isomolar concentrations compared to monosodium glutamate (MSG) (*12*, *13*). Furthermore, alapyridaine, a condensation product of alanine and creatinine (*14*), a Maillard reaction product of alanine and glucose (*15*), and morelid, a malic acid glucopyranoside from morel mushrooms (*16*), have been found to be taste active compounds. A new assay based on T1R1 and T1R3 receptors allowed Tachdjian et al. to screen a large set of molecules (>10000), allowing the identification of oxalamide derivatives that elicited umami taste in the micromolar concentration range (*17*).

It is currently widely accepted that humans possess the ability to distinguish five basic tastes: sweet, sour, bitter, salty, and umami. Some borderline tastes are difficult to describe. An example is the word *kokumi*, which means heavy, thick, strong flavor and has been used to describe the taste of γ-glutamyl peptides (*18*, *19*) or the peptides of mature Gouda cheese (*20*).

This paper discusses the investigation of the dried scallop broth taste, which has been described as fullness. Fractions were prepared and tasted to gain an understanding of the nonvolatile molecules present in dried scallop adductor muscle and to identify the molecule or molecules responsible for the full, sweet, umami taste.

MATERIALS AND METHODS

General. Unless otherwise specified, commercially available reagents and solvents were purchased from Sigma-Aldrich, Germany; Acros Organics, USA; Carlo-Erba, France; and Fluka, Switzerland.

High-Performance Liquid Chromatography (HPLC) for Peak Isolation. An Agilent 1100 HPLC system equipped with a B1312A binary pump was used (Agilent Technologies Inc., Morges, Switzerland).The separations were performed on a 4.6 mm internal diameter × 150 mm, 3 μm particle size Discovery HSF5 column (Supelco, Buchs, Switzerland). The elution solvents were CH_3CN containing 0.01% of formic acid (solvent B) and water containing 0.01% of formic acid (solvent A). The gradient profile was started at 0% CH_3CN, held for 4.5 min, and then gradually increased to 100% CH_3CN over 10 min. The flow rate was 1.2 mL/min. The retention times (t_R) are expressed in minutes. Collections were performed over multiple cycles, and fractions were collected in automatic mode with the Agilent G1364C autocollector. CH_3CN was removed under vacuum, and then the fractions were rediluted in 1−10 mL of water and lyophilized.

Ultraperformance Liquid Chromatography−Electrospray Ionization Mass Spectrometry (UPLC-ESI-MS) Identification. The separations were performed on an Acquity UPLC (Waters Inc., Milford, MA) using a 2.1 mm internal diameter × 100 mm, 1.7 μm particle size HSF5 column. The elution solvents were CH_3CN containing 0.01% of formic acid (solvent B) and water containing 0.01% of formic acid

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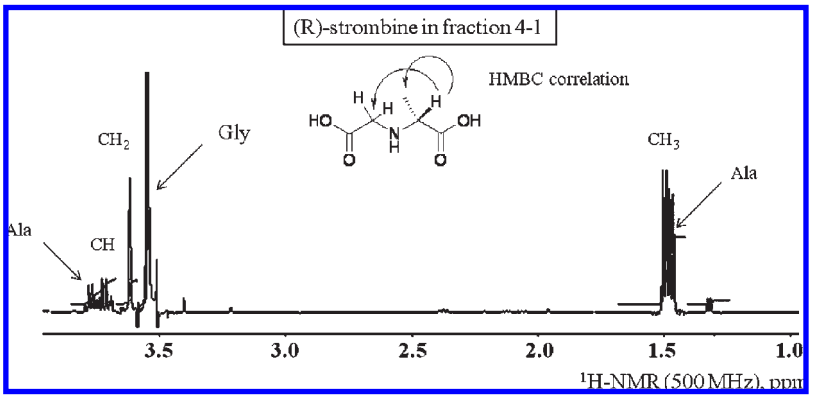

Figure 1. ^{1}H NMR of fraction 4-1 in D_2O.

(solvent A). The gradient profile was 0% B for 1 min and gradually increased to 100% B over 8 min. The flow rate was 0.3 mL/min. The mass spectrometer was a Thermo-Finnigan triple-stage quadrupole (TSQ) quantum ultra AM, with electrospray ion source operated in positive mode (ESI^+). The spray voltage was 3.5 kV. The capillary temperature was 250 °C. The sheath gas was nitrogen at flow rate 50 (Finnigan arbitrary units). The auxiliary gas was also nitrogen at flow rate 40 (Finnigan arbitrary units).

Gas Chromatography−Electron Impact−Mass Spectrometry (GC-EI-MS). An Agilent GC-6890 system connected to an Agilent MSD-5973 quadrupole mass spectrometer was operated at an electron energy of ca. 70 eV. Helium was the carrier gas set at a constant flow rate of 0.7 mL/min. The chiral column was a 25 m × 0.25 mm internal diameter, 0.25 μm fused-silica CP-Chirasil-Dex CB capillary column (Varian, Zug, Switzerland). The oven temperature was programmed from 50 °C for 5 min and then increased to 220 at 2 °C/min. The mass spectra are presented as follows: fragment ions m/z (relative intensity). For quantification, separations were performed on 30 m × 0.25 mm internal diameter, 0.25 μm fused-silica capillary columns coated with SPB-1 (Supelco). The standard oven program was set to 50−220 at 5 °C/min.

^{1}H and ^{13}C NMR Spectra. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 500.13 and 125.76 MHz, respectively. The solvent was D_2O with sodium 3-(trimethylsilyl)tetradeuteriopropionate as the internal standard.

Analysis of Dried Scallops. Dried scallops were purchased from HockHua Tonic (Singapore). The dried scallop muscles (100 g) were cooked in water (1000 g) at 85 °C for 1 h. The broth was filtered to give 736 g of liquid. After lyophilization, 14.7 g of dry powder was obtained. Analysis of free amino acids was performed by UFAG Laboratorium (Sursee, Switzerland). Of the dried matter, total amino acids were 31.7 g/100 g, total free amino acids were 23.5 g/100 g, total nitrogen (N × 6.25, MSDA Kjeldahl) was 62.4 g/100 g, and total minerals were 30.4 g/100 g (MSDA, gravimetry). The composition of free amino acid content (expressed in g/100 g) was as follows: alanine (1.41), arginine (5.40), glutamic acid (0.80), glycine (14.08), isoleucine (0.05), leucine (0.08), lysine (0.11), methionine (0.06), phenylalanine (0.05), proline (0.69), serine (0.07), threonine (0.21), tyrosine (0.02), and valine (0.14). Other free amino acids were not detected. Salts included calcium (88.4 mg/100 g), potassium (4.32 g/100 g), magnesium (236 mg/g), and sodium (8.37 g/100 g).

From another broth preparation, ultrafiltration was performed on 3 and 1 kDa Amicon membranes over 9 h; only 4% of the dry weight was lost. Half of the 1 kDa fraction (300 g) was loaded on a 40 cm long Dowex 50WX8-400 (H^+ form) (150 g wet) column, internal diameter = 4 cm. The column was washed with 2 bed volumes of water, and the percolate water was named fraction 1 (after lyophilization, 1.5 g of a powder was obtained), then the column was eluted with an aqueous ammonium gradient, and fractions were collected. Elution with 0.6 mol/L ammonia gave fraction 2 (50 mL, lyophilized: 3 mg), 1.2 mol/L gave fraction 3 (100 mL, lyophilized 11 mg), 1.8 mol/L gave fraction 4 (50 mL, lyophilized 1.6 g), and 7.6 mol/L gave fraction 5 (200 mL, lyophilized 1.7 g). Each fraction was then rediluted in water and adjusted to pH 7 for tasting. Fraction 4 (10 mL) was further purified by HPLC using a Discovery HSF5 column, and five fractions were collected, fractions 4-1 (47.8 mg) (**Figure 1**), 4-2 (35.3 mg), 4-3 (12.4 mg), 4-4 (0.9 mg), and 4-5 (5.2 mg). These fractions were tasted and analyzed by UPLC-ESI-MS and ^{1}H and ^{13}C NMR.

Preparation of Authentic Samples. *Preparation of (R)-2-(Carboxymethylamino)propanoic Acid: (R)-Strombine.* (*R*)-Alanine (15 g, 168 mmol) and glyoxylic acid monohydrate (31 g, 337 mmol) in anhydrous methanol (650 mL) were added to Pd/C 10% (750 mg) suspended in anhydrous ethanol (100 mL). Hydrogenation was performed at atmospheric pressure at 24 °C for 18 h. The crude product was filtered on Celite. The Celite was rinsed with methanol (100 mL). The filtrate, containing impure strombine, was discarded. The Celite was then washed with water (300 mL), and the water was removed by evaporation under reduced pressure to give 30 g of a viscous liquid (30 g). The crude strombine was then rediluted in about 100 mL of water and purified on a Dowex 50WX8-400 H^+ form column (220 g, 45 mm internal diameter). The elution was performed with 200 mL portions of water containing increasing concentrations of 30% NH_4OH as follows: 200 mL of water; 200 mL of water plus 4 mL of NH_4OH 30%; then 200 mL of water with 6 mL of NH_4OH 30%, 2 × 200 mL plus 8 mL of NH_4OH 30% per elution, 200 mL of water plus 10 mL of NH_4OH 30% and 200 mL of water plus 12 mL of NH_4OH 30%. The strombine was in the fraction eluted with 200 mL of 1.2% aqueous NH_4OH. After lyophilization, 13 g of pure strombine was obtained (nonoptimized yield of 52%): ^{1}H NMR δ 1.51 (d, J = 7 Hz, 3H), 3.62 (s, 3H), 3.73 (q, J = 7 Hz, 1H); ^{13}C NMR δ 177.6 (s), 174.2 (s), 60.3 (d), 50.4 (t), 17.5 (q) (*21*); $[\alpha]^{20}_D$ (pH 6, *c* 5) −9.0, $[\alpha]^{20}_D$ (pH 1, *c* 5) −5.6. The optical purity was determined after derivatization of 12 mg of strombine in MeOH/HCl to form the dimethyl ester (1.25 mol/L, 2 mL) by heating for 1 h in a sealed vial at 100 °C. After cooling, ethyl acetate (2 mL) and 0.5 mL of NaOH (1 mol/L) were added, and the organic phase was injected on a chiral GC column [GC (Chirasil-DEX column)]: MS retention time = 21.79 min; MS 116 (100, M+), 100 (2), 88 (10), 56 (50) (98.8%), (*S*)-strombine 21.90 min (1.2%).

The pH of the strombine solutions was 3.4, and it was adjusted to pH 7 with NaOH to obtain monosodium strombine for the tasting.

Preparation of (S)-2-(Carboxymethylamino)propanoic Acid: (S)-Strombine. (*S*)-Strombine was prepared under the same conditions starting from (*S*)-alanine. By chiral GC of the dimethyl ester derivative, the purity was 99.6%: $[\alpha]^{20}_D$ (pH 6, *c* 5) +9.0, $[\alpha]^{20}_D$ (pH 1, *c* 5) +5.4, in agreement with published data (*22*).

Quantification of (*R*)-strombine was performed after derivatization of 10 mg of (*R*)-strombine in four replicates in MeOH/HCl to form the dimethyl ester (1.25 M, 2 mL), heated for 1 h in a sealed vial at 100 °C. The solvent was removed under vacuum, and trichloroacetic anhydride (0.5 mL) was added to acetylate the secondary amine. The sample was heated again for 1 h at 95 °C. The solvent was removed under vacuum, and ethyl acetate (1 mL) was added. The samples were injected on GC-MS in various dilutions (corresponding to 0.125, 0.25, 0.5, and 1 mg/mL) in EtOAc. A calibration curve was established from the total ion current peak area (R^2 = 0.998). The same derivatization was performed on two replicates of 25 and 50 mg of the lyophilized scallop broth. When we compare the peak surface area of the 25 and 50 mg samples, the peak area was double.

Sensory Methodology. *Subjects.* Forty-seven experienced panelists, from Firmenich S.A. Geneva, participated in the study.

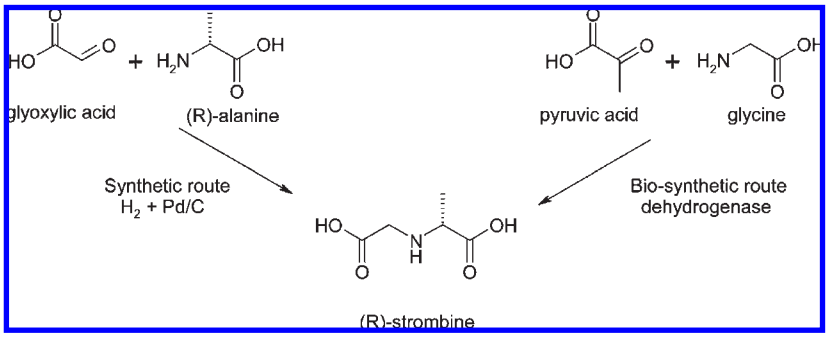

Figure 2. Synthetic preparation and biological pathways of (*R*)-strombine.

Stimuli. For the threshold determination of (*R*) and (*S*)-strombine, a stock solution was prepared at a concentration of 4 g/L in mineral water, and the pH was adjusted to neutral with 1 M aqueous NaOH. Six solutions were obtained by successive dilution of the stock solution. Therefore, for both replicates, the seven concentrations of (*R*) and (*S*)-strombine that were evaluated were 4, 2, 1, 0.5, 0.25, 0.125, and 0.625 g/L.

Tasting Protocol for All Sensory Evaluations. Subjects received a 20 mL sample in a cup (blind test). They were asked to take the entire sample into their mouths. They spit out the sample after 5 s and then completed the questionnaire.

Threshold Determination. According to the ASTM E679 Ascending Concentration Series Method of Limits, seven different concentrations of (*R*)-strombine were chosen for each of three alternative forced-choice (3-AFC) tests [one sample containing (*R*)-strombine and two others mineral water as references]. The subjects started with the weakest concentration of (*R*)- or (*S*)-strombine and continued to the strongest (ascending order). The ratio between the two successive concentrations of (*R*)- and (*S*)-strombine were steps of 2-fold concentrations. The presentation order of each triangle test was randomized. Individual thresholds were determined from the ASTM procedure. The geometric mean was used to determine the threshold average for the panel.

Sensory characterization was realized together with threshold measurement in one session, with choices of sensory attribute after each triangle. The panelists were asked to choose among taste descriptors such as sweet, salty, bitter, umami, acid, fullness, metallic and random choice. Free comments were also requested under the option "others". Two repetitions were done for both (*R*)- and (*S*)-strombine.

Quality Attributes—R-Strombine in Dried Scallop Reconstitution. A reconstituted sample, without (*R*)-strombine, was used as a reference for the three-alternative forced-choice (3-AFC test). The subjects started with the weakest concentration of (*R*)-strombine and continued to the strongest (ascending order). The presentation order in each 3-AFC test was randomized.

The panelists were asked to evaluate the different samples, after the 3-AFC test, but only if the panelist had chosen the correct answer. Choices of sweet, salty, bitter, umami, acid, fullness, metallic, and random choice were proposed. Free comments were also solicited under the option "others".

RESULTS

The dried scallop muscle broth was fractionated by ultrafiltration and tasted at each stage of the process. The fullness remained in the fraction that contained compounds with a molecular mass of < 1000 Da. The analysis of amino acids present in the scallop broth showed a concentration of 3.11 g/L of glycine, the most abundant amino acid, followed by 1.19 g/L arginine, 0.31 g/L alanine, and 0.18 g/L glutamic acid. The concentrations of sodium and potassium were also determined, and sodium chloride was estimated to be around 4.70 g/L, whereas the concentration of potassium chloride was 1.81 g/L. These values were used to design a formula for a first broth reconstitution. The reconstituted broth was tasted at pH 7 with nose clips, and the conclusion of the tasting was that we were far from the umami, sweet fullness of the broth ultrafiltrate. Therefore, we decided to investigate further to understand the origin of this typical fullness. The ultrafiltered fraction was loaded on a cation-exchange chromatography column and eluted with a gradient of ammonia. The sweet, umami, salty, full taste was located in only one fraction. This was a complex fraction, which was further purified by preparative HPLC and separated into five fractions. The first fraction elicited the sweet, umami fullness of interest, whereas the fifth fraction had a harsh, common umami taste according to flavorists' comments. The analysis of the fifth fraction by ^{1}H and ^{13}C NMR showed a mixture of two major compounds. One of these compounds had a singlet in the ^{1}H NMR spectrum at 3.3 ppm, whereas the other compound showed signals at 8.5, 8.2, 6.18, 4.7, 4.5, 4.4, and 4.1 ppm, corresponding to adenosyl monophosphate (AMP). The ^{13}C NMR and LC-MS-ESI^{+} confirmed the presence of AMP. In the ^{13}C NMR spectrum a quadruplet at 62.2 ppm as well as a mass obtained by LC-MS-ESI^{+} of 76.06 suggested the presence of trimethylammonium oxide (TMAO) (C_3H_9NO, mw 75) in the fraction. These propositions were confirmed by UPLC-MS-ESI^{+} injections of commercially available TMAO and AMP. On the basis of the integrated signals, it was reasonably possible to estimate that the ratio of TMAO/AMP was 2.4.

Fraction 1 gave a heterogeneous UPLC-MS-ESI^{+} peak formed by masses (M + 1) 76.0, 90.0, and 147.9, possibly corresponding to glycine $C_2H_5NO_2$ (MW 75), alanine $C_3H_7NO_2$ (MW 89), and glutamic acid $C_5H_9NO_4$ (MW 147). Fraction 1 reconstitutions consisting of glycine, alanine, and MSG, with the appropriate concentrations of salts, were prepared and evaluated by tasting but did not have the same rich, full taste as the scallop broth. Lyophilized fraction 1 was analyzed by ^{1}H NMR in D_2O (**Figure 1**). The major products were glycine, estimated to represent 70% of the fraction on the basis of proton NMR integration of the singlet at 3.04 ppm, alanine (15%) as determined from the doublet at 1.48 ppm and a corresponding triplet at 3.77 ppm, and an unknown compound (15%). The ^{1}H NMR spectrum of the unknown contained a doublet at 1.5 ppm, which was assigned to a methyl group that was coupled to a quadruplet located at 3.73 ppm and a singlet at 3.62 ppm, most likely located on a carbon bonded to a nitrogen atom. The ^{1}H and ^{13}C NMR spectra of the unknown compound were identical to data reported in the literature for 2-(carboxymethylamino)propanoic acid, with the trivial name strombine ($C_5H_9NO_4$, MW 147) (*21*). The occurrence of (*R*)-strombine in sea organisms is well-known as a result of the reduction of the Schiff base between pyruvic acid and glycine under anaerobic conditions (*23*−*25*) (**Figure 2**).

(*R*) and (*S*)-strombine were prepared by reductive amination starting from (*R*)- or (*S*)-alanine and glyoxylic acid. Both enantiomers and the lyophilisate of the scallop broth were treated with HCl/MeOH to esterify the acid functionalities, and they were injected on a GC-MS equipped with a chiral column, which

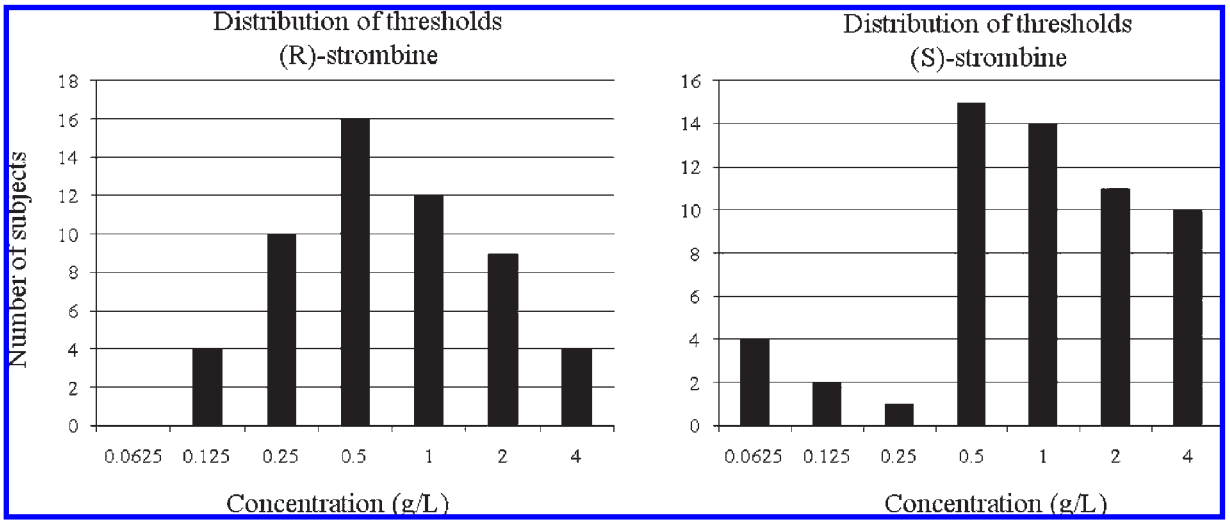

Figure 3. Taste threshold determination of (*R*)- and (*S*)-strombine in water (averaged results of two testing sessions). Individual thresholds were not calculated.

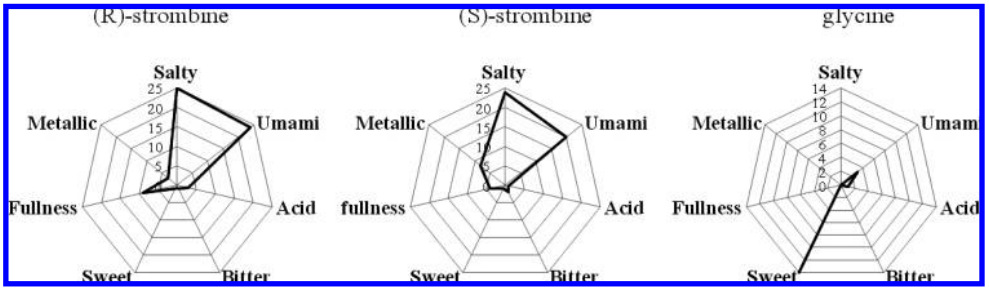

Figure 4. Taste profiles of (*R*)-strombine, (*S*)-strombine, and glycine at concentrations of 2 g/L in water at pH 7.

allowed us to confirm that (*R*)-strombine is the natural enantiomer in scallop broth. No epimerization occurred during the reductive amination or the derivatization.

2-(Carboxyalkylamino)propanoic acid is usually analyzed by HPLC methods (*25*−*28*). Because MSG and (*R*)-strombine have the same retention times on Aquity HSF5 columns and similar MS fragmentation patterns, the direct quantitation of strombine by UPLC-MS-ESI$^+$ in single reaction monitoring ion mode was not possible. It was possible to separate (*R*)-strombine from MSG on a QS Uptisphere-5-SX 250 mm column using an ammonium formate gradient, but for practical reasons, the quantitative estimation of (*R*)-strombine in scallop broth was performed on GC-MS in total ion current mode, after derivatization. A synthetic sample of strombine was methylated at the acid functionalities, and the amine function was acetylated. This derivatized sample served as an external standard. A sample of the lyophilized scallop broth was derivatized in the same way. By GC-MS using the derivatized external standard to establish a calibration curve, the concentration of (*R*)-strombine in the broth was estimated to be around 180 (±10) mg/L.

Sensory analysis was performed with the help of 47 panelists in two sessions, 13 of whom participated in both sessions. Sixty responses were obtained, 55 of which were used to calculate the threshold values of (*R*)- and (*S*)-strombine. For the other 5 responses, the individual thresholds were above 4 g/L. The detection threshold value of (*R*)-strombine was 0.5 g/L (geometric mean of each individual threshold for the two repetitions), whereas the detection threshold value of (*S*)-strombine was 0.74 g/L (geometric mean of each individual threshold for the two repetitions) (**Figure 3**). The distribution threshold of (*S*)-strombine (**Figure 3**) was unexpected, and we cannot explain why, at 0.25 g/L, only one right answer was obtained. This is an indication that taste evaluation of umami and saltiness at low concentration levels is not straightforward. The qualitative sensory descriptions and the free comments of panelists who gave the correct answer to the 3-AFC test were recorded. Saltiness and umami were the most often used descriptors (**Figure 4**).

To understand the impact of (*R*)-strombine in the broth, it was added to a reconstitution made of glycine (1.4 g/L), MSG (0.08 g/L), alanine (0.14 g/L), NaCl (2.1 g/L), and KCl (0.8 g/L). The ratio of constituents was based on the analytical results, but the concentration was divided by a factor of 2 to better evaluate the effect of (*R*)-strombine. A 3-AFC test was performed using the diluted reconstitution without (*R*)-strombine as a blank solution. When the concentration of (*R*)-strombine was 0.15 g/L, 20 correct answers from 53 tastings were obtained (not significant, risk = 0.293) but when 0.30 g/L (*R*)-strombine was added to the reconstituted solution, the number of correct answers increased to 26, to give a significant statistical result (risk = 0.0128). The panelists who were able to find the difference gave similar comments. When (*R*)-strombine was added, the reconstituted sample had more saltiness, umami, and full character.

DISCUSSION

The presence of opines in the adductor muscles of many marine organisms is well-known. Opines are a class of compounds resulting from the natural reductive amination of pyruvic acid and amino acids. Most studies of opines have focused on the oxidoreductase activities of opine dehydrogenase during anaerobiosis (*23*−*25*). The muscles of marine organisms have high free

amino acid contents, and amino acids such as glycine, alanine, and arginine have important roles as kosmotropes (*11*). The most well-studied opines include strombine, alanopine, and octopine, as well as tauropine, which is derived from taurine (*29*−*32*). Although opines are well described, the taste properties of (*R*)-strombine has never been described. Seasonal variation induces significant taste differences in scallops, and Komata (*1*) reported that glutamic acid, alanine, glycine, and glycine betaine play a central role in taste quality. The same author reported that nondocumented studies have also suggested that amines such as betaines, TMAO, and octopine in squid and octopus are associated with taste. This is the only documented reference to the role of opines in taste that we have found (*1*).

Several amino acids taste sweet, bitter, insipid, or delicious (umami) to humans (*33*, *34*). Other compounds that are closely related to amino acids also increase the fullness and complexity of taste, which has been described as a kokumi taste (*18*, *19*, *35*, *36*). In our experiments the tasters were not trained to use the taste descriptor "kokumi",but **Figure 4** shows that whereas glycine is clearly described as sweet, (*R*)-strombine is described as both umami and salty.

Some D-amino acids, such as leucine, isoleucine, and valine, taste sweet, but their L-form is bitter. Only in the cases of alanine, threonine, and serine are both the D- and L-enantiomers sweet (*34*). In the case of (*R*)- and (*S*)-strombine, the stereochemistry did not influence taste perception to a great extent.

In conclusion, (*R*)-strombine is an important contributor to the taste of scallop adductor muscle and was shown to modify the sweetness of glycine. The taste-modifying properties of strombine may be more important in fresh scallop muscle as it has been reported to occur at a concentration of 800 mg/kg (*37*). Other opines have also been identified in fresh scallop meat (*37*), and their impact on the taste is unknown.

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LITERATURE CITED

(1) Komata, Y. Umami taste of seafoods. *Food Rev. Int.* **1990**, *6*, 457–487.

(2) Wongso, S.; Yamanaka, H. Extractive components of the adductor muscle of Japanese baking scallop and change during refrigerated storage. *J. Food Sci.* **1998**, *63*, 1–5.

(3) Hayashi, T.; Yamaguchi, K.; Konosu, S. Sensory analysis of taste-active components in extract of boiled snow crab meat. *J. Food Sci.* **1981**, *46*, 479–493.

(4) Yoneda, C.; Okubo, K.; Kasai, M.; Hatae, K. Extractive components of boiled-dried scallop adductor muscle and effect on the taste of soup after mixing with chicken leg meat. *J. Sci. Food Agric.* **2005**, *85*, 809–816.

(5) Nelson, G.; Chandrashekar, J.; Hoon, M. A.; Feng, L.; Zhao, G.; Ryba, N. J.; Zuker, C. S. An amino-acid taste receptor. *Nature* **2002**, *416*, 199.

(6) Li, X.; Staszewski, L.; Xu, H.; Durick, K.; Zoller, M.; Adler, E. Human receptors for sweet and umami taste. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4692–4696.

(7) Xu, H.; Staszewski, L.; Tang, H.; Adler, E.; Zoller, M.; Li, X. Different functional roles of T1R subunits in the heteromeric taste receptors. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14258–14263.

(8) Li, X.; Staszewski, L.; Xu, H.; Li, Q. Senomyx. PCT Int. Appl. WO 2005/015158 (*Chem. Abstr.* **2005**, *142*, 237172).

(9) Li, X.; Zhang, F.; Xu, H.; Li, Q. Senomyx. PCT Int. Appl. WO 2007/047988 (*Chem. Abstr.* **2007**, *146*, 456423).

(10) Chandrasshekar, J.; Hoon, M. A.; Ryba, N. J. P.; Zucker, C. S. The receptors and cells for mammalian taste. *Nature* **2006**, *444*, 288–294.

(11) Winkel, C.; de Klerk, A.; Visser, J.; de Rijke, E.; Bakker, J.; Koenig, T.; Renes, H. New developments in umami (enhancing) molecules. *Chem. Biodivers.* **2008**, *5*, 1195–1203.

(12) Frérot, E.; Escher, S. D.; Näf, F. The contribution of peptides to savory flavors. *ACS Symposium, Chicago, IL*, **1995**; Abstract 216.

(13) Frerot, E.; Benzi, F. Firmenich. PCT Int. Appl. WO 2004/075633 (*Chem. Abstr.* **2004**, *141*, 259707).

(14) Shima, K.; Yamada, N.; Suzuki, E.; Harada, T. Novel brothy taste modifier isolated from beef broth. *J. Agric. Food Chem.* **1998**, *46*, 1465–1468.

(15) Ottinger, H.; Hofmann, T. Identification of the taste enhancer alapyridaine in beef broth and evaluation of its sensory impact by taste reconstitution experiments. *J. Agric. Food Chem.* **2003**, *51*, 6791–6796.

(16) Rotzoll, N.; Dunkel, A.; Hofmann, T. Quantitative studies, taste reconstitution and omission experiments on the key taste compounds in morel mushrooms (*Morchella dliciosa* Fr.). *J. Agric. Food Chem.* **2006**, *54*, 2705–2711.

(17) Tachdjian, C.; Patron, A. P.; Qi, M.; Adaminski-Werner, S.; Tang, X. Q.; Quing, C.; Darmohusodo, M.; Lebl-Rinnova, M.; Priest, C. Senomyx. PCT Int. Appl. WO 2006/084246 (*Chem. Abstr.* **2006**, *144*, 273163).

(18) Ueda, Y.; Tsubuku, T.; Miyajima, R. Composition of sulfur-containing components in onion and their flavor characters. *Biosci., Biotechnol., Biochem.* **1994**, *58*, 108–110.

(19) Ueda, Y.; Yonemitsu, M.; Tsubuku, T.; Sakaguchi, M.; Miyajima, R. Flavor characteristics of glutathione in raw and cooked foodstuffs. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 1977–1980.

(20) Toelstede, S.; Dunkel, A.; Hofmann, T. A Series of kokumi peptides impart the long-lasting mouthfulness of maturated gouda cheese. *J. Agric. Food Chem.* **2009**, *57*, 1440–1448.

(21) Garrigues, B. Synthese d'aminoacides. *Tetrahedron* **1984**, *40*, 1151–1156.

(22) Miyazawa, T. Studies of unsual amino acids and their peptides. The chemistry of N-(carboxymethyl)amino acids. The preparation, properties and characterization of *N*-(carboxymethyl)amino acids and their esters. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2555–2565.

(23) Thompson, J.; Donkersloot, J. A. *N*-(Carboxyalkyl)amino acids: occurrence, synthesis, and functions. *Annu. Rev. Biochem.* **1992**, *61*, 517–557.

(24) Gäde, G.; Grieshaber, M. K. Pyruvate reductases catalyze the formation of lactate and opines in anaerobic invertebrates. *Comp. Biochem. Physiol. B* **1986**, *83*, 255–272.

(25) Siegmund, B.; Grieshaber, M. K. Determination of *meso*-alanopine and D-strombine by high pressure liquid chromatography in extracts from marine invertebrates. *Hoppe-Seyler's Z. Physiol. Chem.* **1983**, *364*, 807–812.

(26) Sato, M.; Suzuki, S.; Yasuda, Y.; Kawauchi, H.; Kanno, N.; Sato, Y. Quantitative HPLC analysis of opines by phenylthiocarbamyl derivatization. *Anal. Biochem.* **1988**, *174*, 623–627.

(27) Sandee, B.; Schipper, C. A.; Eertman, R. H. M. High-performance liquid chromatographic determination of the imino acids (opines) *meso*-alanopine and D-strombine in muscle extract of invertebrate. *J. Chromatogr., B* **1996**, *685*, 176–180.

(28) Haque, M. E.; Fujisawa, T.; Yamamoto, M.; Ohmori, S. Improved HPLC determination of acidic opines by phanylisothiocyanate derivatization and its application to marin animals. *Acta. Med. Okayama* **2000**, *54*, 1–8.

(29) Yancey, P. H.; Clark, M. E.; Hand, S. C.; Bowlus, R. D.; Somero, G. N. Living with water stress: evolution of osmolyte systems. *Science* **1982**, *217*, 1214–1222.

(30) Fields, J. H.; Eng, A. K.; Ramsden, W. D.; Hochachka, P. W.; Weinstein, B. Alanopine and strombine are novel imino acids produced by a dehydrogenase found in the adductor muscle of the oyster, *Crassostrea gigas*. *Arch. Biochem. Biophys.* **1980**, *201*, 110–114.

(31) Gäde, G. Energy metabolism during anoxia and recovery in shell adductor and foot muscle of the gastropod mollusc *Haliotis lamellosa*: formation of the novel anaerobic end product tauropine. *Biol. Bull*. **1988**, *175*, 122–131.
(32) Fiore, G. B.; Nicchitta, C. V.; Elligton, W. R. High-performance liquid chromatography separation and quantification of alanopine and strombine in crude tissue extracts. *Anal. Biochem*. **1984**, *139*, 413–417.
(33) Yamaguchi, S. Chapter 2. Fundamental properties of umami in human taste sensations. *Umami: A Basic Taste: Physiology, Biochemistry, Nutrition, Food Science*; Kawamura, Y., Kare, M. R., Tannenbaum, S. R., Eds.; Dekker: New York, 1987; pp 42−78.
(34) Haefeli, R. J.; Glaser, D. Taste responses and thresholds obtained with the primary amino acids in humans. *Lebensm. Wiss. Technol*. **1990**, *23*, 523–527.
(35) Ueda, Y.; Sakaguchi, M.; Hirayama, K.; Miyajima, R.; Kimizuka, A. Characteristic flavor constituents in water extract of garlic. *Agric. Biol. Chem*. **1990**, *54*, 163–169.
(36) Dunkel, A.; Koster, J.; Hofmann, T. Molecular and sensory characterization of γ-glutamyl peptide as key contributors to the kokumi taste of edible beans (*Phaseolus vulgaris* L.). *J. Agric. Food Chem*. **2007**, *55*, 6712–6719.
(37) Wongso, S.; Ushio, H.; Ohshima, T.; Yamanaka, H. Changes in the content of octopine, acidic opines, related amino acids and phosphoarginine in the adductor muscle of three species of scallop during storage. *J. Food Biochem*. **1998**, *22*, 65–81.

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