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Identification of the Taste Enhancer Alapyridaine in Beef Broth and Evaluation of Its Sensory Impact by Taste Reconstitution Experiments

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An essential compound imparting the sweet taste to beef broth was investigated. Taste activity-guided fractionation of beef broth by ultrafiltration, gel permeation chromatography, and HPLC in combination with the recently developed comparative taste dilution analysis enabled the localization of a fraction possessing sweetness-enhancing activity upon degustation. Comparison of the chromatographic, spectroscopic, and sensory data with those of the synthetic reference compound led to the identification of the sweetness-enhancing *N*-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol inner salt, named alapyridaine, which was recently isolated from heated aqueous solutions of hexoses and L-alanine. After quantification of alapyridaine in beef broth, sensory analysis of synthetic beef taste recombinates spiked with synthetic alapyridaine in its "natural" concentration of 419 μg/L and comparison to the taste quality of a tastant recombinate lacking the alapyridaine revealed a significant increase in sweetness and umami character only when the alapyridaine was present in the recombinate. These data demonstrate for the first time that, in "natural" concentrations, the alapyridaine exhibited a pronounced effect on the overall taste quality of beef broth, in particular, on the sweet and umami character.

KEYWORDS: Alapyridaine; comparative taste dilution analysis; taste enhancer; beef broth; bouillon; sweet taste; umami; Maillard reaction

INTRODUCTION

Due to its desirable flavor, beef broth is frequently used as a base for savory dishes, processed food compositions, or convenience foods. Although the consumer acceptance of such food products is strongly influenced by both the aroma-active volatiles and the taste-active nonvolatile compounds, detailed information on the structures and sensory properties is as yet mainly available on the volatile odor-active compounds. In the past 10 years, the key odorants in beef bouillon (*1*), beef juice (*2*), and roasted beef (*3*, *4*) have been successfully characterized by application of the aroma extract dilution analysis, which is based on the determination of the odor thresholds of volatiles during gas chromatography−olfactometry performed with serial dilutions of an aroma extract.

In contrast to the odorants, the nonvolatile components imparting the unique taste of beef broth have not been determined sufficiently. Most studies addressed only the taste contribution of well-known taste-active food ingredients such as sugars, amino acids, nucleotides, organic acids, and minerals. On the basis of the quantitative analysis in beef broth, aqueous taste recombinates have been prepared consisting of a blend of either 2 amino acids, 2 nucleotides, 2 carbonic acids, 5 inorganic salts, carnosine, anserine, and carnithine (*5*) or 16 amino acids, 4 sugars, 3 nucleotides, 3 carbonic acids, 4 inorganic salts, phosphate, carnosine, creatine, and creatinine (*6*), each in their "natural" concentrations. Sensory evaluation of these biomimetic taste imitations demonstrated that the well-balanced typical taste of the authentic beef broth could not be completely reproduced only through the compounds already identified (*5*, *6*). In addition to the compounds used in the taste recombinates, *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene)alanine was identified as a novel thermally generated taste compound, and it was suggested that this compound imparted the typical brothy taste to beef broth (*6*). Because the proposed key role of that compound in the brothy taste of bouillon was not yet proven by comparative sensory experiments on taste recombinates containing the imidazoline in "natural" concentrations and taste imitates lacking this novel tastant, respectively, it is still unclear whether the *N*-(1-methyl-4-hydroxy-3-imidazolin-2,2-ylidene)alanine actually contributes to the unique taste of beef broth.

In preliminary experiments, we compared the taste quality of an authentic beef broth with the overall taste of a broth taste recombinate and confirmed the data reported earlier (*5*) that

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besides the umami note, in particular, the intensity of the sweet taste quality of beef broth could not be sufficiently covered by the biomimetic imitation. On the basis of these data, it has to be concluded that the attractive and unique taste of beef broth is due to yet unknown taste compounds which might not be present per se in beef meat but are formed from tasteless precursors during thermal treatment.

To identify such thermally generated taste compounds, we recently developed the so-called taste dilution analysis (TDA), which is based on the determination of the detection threshold of taste compounds in serial dilutions of chromatographic fractions (*7*, *8*). This novel bioassay, offering the possibility to rank food components according to their relative taste impact, has proved to be a powerful tool for the identification of key taste compounds. For example, in thermally treated solutions of carbohydrates and amino acids the previously unknown bitter-tasting (*E*)-2-[(2-furyl)methylidene]-7-[(2-furyl)methyl]-3-hydroxymethyl]-1-oxo-1*H*,2*H*,3*H*-indolizinium-6-olate (*7*, *8*) or the cooling-active compounds 5-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one and 3-methyl-2-(1-pyrrolidinyl)-2-cyclopenten-1-one have been successfully identified (*9*).

The objectives of the present investigation were, therefore, (i) to screen a beef broth for taste-enhancing compounds by application of taste dilution analyses, (ii) to identify the key compound contributing to sweet taste, and (iii) to study its sensory impact on the taste quality of beef broth by means of a taste reconstitution experiment.

EXPERIMENTAL PROCEDURES

Chemicals. The following compounds were obtained commercially: D-glucose, L-alanine, lactic acid, NaCl, caffeine, quinine hydrochloride, sucrose, sodium glutamate, tannin (gallustannic acid), ammonium formate (Aldrich, Steinheim, Germany), and trifluoroacetic acid (Merck, Darmstadt, Germany). Solvents were of HPLC grade (Merck, Darmstadt, Germany). *N*-(1-Carboxyethyl)-6-(hydroxymethyl)-pyridinium-3-ol inner salt was synthesized as reported recently (*10*).

Preparation of Beef Broth. Beef meat (1.0 kg, purchased in a local shop) was cut into pieces of 2−3 cm, water (2 L) was added, and the mixture was heated for 3 h at 95 °C. After cooling to 2 °C, fat was discarded and the beef broth was extracted with ethyl acetate (4 × 200 mL) and then filtered.

Beef Broth Taste Recombinate. According to a procedure reported in the literature (*6*), a beef broth taste recombinate was prepared by dissolving the following compounds (mg/L) in tap water: L-threonine (68), L-serine (38), monosodium L-glutamate (64), L-proline (16), glycine (56), L-alanine (192), L-valine (34), L-methionine (20), L-isoleucine (16), L-leucine (28), L-tyrosine (38), L-phenylalanine (26), L-lysine hydrochloride (44), L-histidine (38), L-arginine (32), taurine (248), ribose (4), mannose (8), fructose (20), glucose (40), inosine monophosphate·2Na·5H_2O (424), guanosine monophosphate·2Na·7H_2O (80), adenosine monophosphate (60), pyroglutamic acid (1296), lactic acid (3567), succinic acid (98), NaCl (854), KCl (3940), $MgCl_2$·6H_2O (1104), $CaCl_2$·2H_2O (14), H_3PO_4 (1558), carnosine (912), creatine (1106), and creatinine (572). Finally, the pH was adjusted to 6.0 with aqueous hydrochloric acid (0.1 mol/L).

Sensory Analyses. *Training of the Sensory Panel.* Assessors were trained to evaluate the taste of aqueous solutions (1 mL each) of the following standard taste compounds by using a triangle test as described in the literature (*11*): sucrose (50 mmol/L) for sweet taste; lactic acid (20 mmol/L) for sour taste; NaCl (12 mmol/L) for salty taste; caffeine (1 mmol/L) for bitter taste; sodium glutamate (8 mmol/L, pH 5.7) for umami taste; tannin (gallustannic acid; 0.05%) for astringency. Sensory analyses were performed in a sensory panel room at 22−25 °C in three different sessions.

Taste Profile Analysis. Beef broth and the taste recombinates were presented to the sensory panel, who was asked to score the taste qualities umami, sweet, sour, bitter, salty, and astringent on a scale from 0 (not detectable) to 5 (strong detectable). To achieve this, the samples were swirled around in the mouth briefly and expectorated.

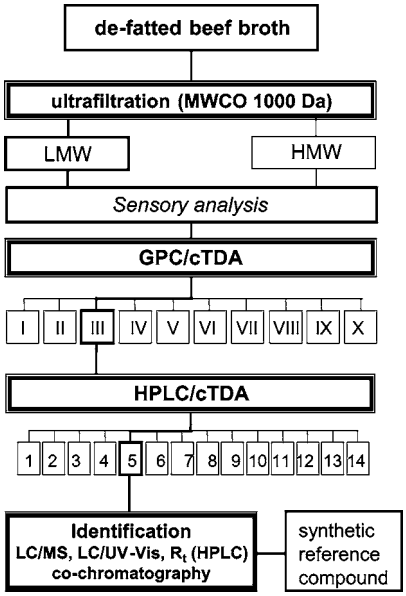

Figure 1. Schematic outline of the activity-guided procedure used for the identification of the sweet-enhancing compound in beef broth.

Ultrafiltration. According to the procedure given in **Figure 1**, the aqueous phase of the beef broth obtained after solvent extraction was separated by ultrafiltration through a 63.5 mm i.d., YM1 filter (Millipore, Bedford, MA) with a molecular weight cutoff of 1000 Da. The low molecular weight fraction, matching the taste quality of the total beef broth, was collected and freeze-dried, yielding a nonvolatile residue (15.6 g) that was used for sensory analysis as well as for gel permeation chromatography.

Gel Permeation Chromatography/Comparative Taste Dilution Analysis (GPC/cTDA). Aliquots (1.0 g) of the low molecular weight material (<1000 Da) obtained by ultrafiltration of two broth batches were applied onto the top of a water-cooled 400 × 55 mm glass column (Amersham Pharmacia Biotech, Freiburg, Germany) filled with a slurry of Sephadex G-15 (Amersham Pharmacia Biotech) in an aqueous acetic acid solution (0.1 mol/L). Chromatographic separation was monitored by means of a model UV/VIS-151 UV−vis detector (Gilson, Germany) operating at 254 nm; the effluent was collected into 15 mL fractions and then combined to give 10 fractions (fractions I−X) (**Figure 2**). The fractions obtained were divided into two equal aliquots, and each independently freeze-dried and used for the cTDA. The residues obtained from the 10 fractions isolated from the first GPC run (separation A) were dissolved in tap water (1 mL), whereas the residues obtained from the second run (separation B) were dissolved in an aqueous solution of sucrose (50 mmol/L, 1 mL). For both separations, the individual GPC fractions were then diluted stepwise 1:1 with pure water. The serial dilutions of each of these fractions were presented to the sensory panel in order of increasing concentrations, and, while wearing nose clamps, the panel sensorially evaluated each dilution for sweetness in a triangle test. The dilution at which a sweet taste difference between the diluted fraction and two blanks (water) could just be detected was defined as the taste dilution (TD) factor. The fractions obtained from separation A did not show any sweetness, whereas the fractions of the GPC separation B showed sweetness with varying TD factors (**Table 1**). The TD factors evaluated by five different assessors in three different sessions were averaged. The TD factors between individuals and separate sessions differed by not more than one dilution step.

High-Performance Liquid Chromatography/Comparative Taste Dilution Analysis (HPLC/cTDA) of GPC Fraction III. The freeze-dried material obtained from GPC fraction III was divided into two equal portions and separately dissolved in water and membrane-filtered, and aliquots (20 × 100 μL) were analyzed by RP-HPLC (**Figure 3**). The residues obtained from the 14 fractions isolated from the first HPLC separation (separation A) were dissolved in tap water (1 mL), whereas

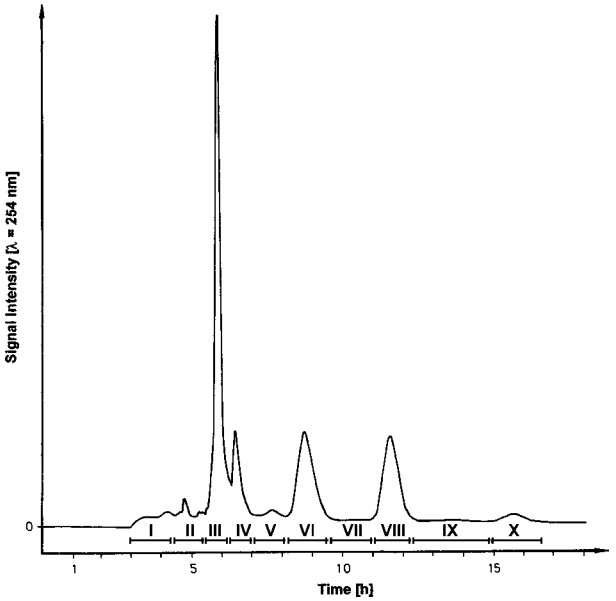

Figure 2. GPC chromatogram of the low molecular weight fraction (MW < 1 kDa) isolated from defatted beef broth by means of ultrafiltration. The effluent was collected in 10 separate fractions I–X.

Table 1. Comparative Taste Dilution Analysis of GPC Fractions Obtained from the Low Molecular Weight Fraction (MW < 1 kDa) of Defatted Beef Broth

		TD factor[a]	
fraction[b]	taste quality[c]	I	II
1	salty	64	32
	sweet	<1	2
2	salty	2048	2048
	sweet	<1	2
3	umami	64	32
	salty	32	32
	sour	4	2
	sweet	***<1***	***16***
4	umami	16	8
	salty	8	8
	sweet	<1	4
5	salty	16	8
	umami	2	2
	sweet	<1	1
6	salty	8	8
	sour	8	4
	sweet	<1	2
7	salty	2	2
	sweet	<1	2
8	salty	8	4
	umami	8	4
	sweet	<1	2
9	salty	4	4
	sweet	<1	1
10	sweet	<1	2

[a] The freeze-dried fractions were taken up in water (I) or in an aqueous sucrose solution (50 mmol/L; II) and were then evaluated by the TDA. Data are given as the mean of the TD factors evaluated by five different assessors in three different sessions. [b] Number of GPC fraction refers to **Figure 2**. [c] Taste quality was determined by a trained sensory panel.

the residues obtained from the second run (separation B) was dissolved in an aqueous solution of sucrose (50 mmol/L, 1 mL). For both separations, the 20 pooled HPLC fractions were then separately diluted stepwise 1:1 with pure water and then rated for their taste impact using the TDA as detailed above. System A did not show any sweetness in any HPLC fraction, whereas HPLC fractions ofystem B showed sweetness with varying TD factors (**Table 2**). The TD factors evaluated by three different assessors in three different sessions were averaged.

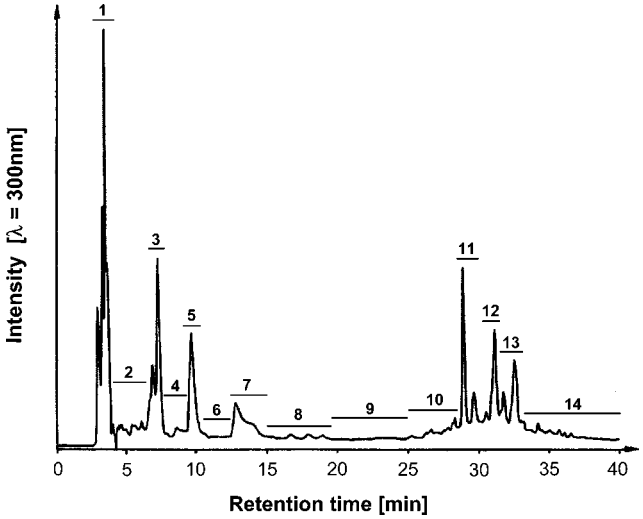

Figure 3. RP-HPLC chromatogram of GPC fraction III. The effluent was collected in 14 separate fractions 1–14.

Table 2. Comparative Taste Dilution Analysis of HPLC Fractions Isolated from GPC Fraction III

		TD factor[a]	
fraction[b]	taste quality[c]	I	II
1	umami	128	128
	salty	128	64
	sweet	<1	2
2	sour	32	16
	sweet	<1	2
3	bitter	2	2
	sweet	<1	1
4	sour	2	2
	sweet	<1	2
5	***sweet***	***2***	***8***
6	umami	16	16
	sweet	<1	2
7	umami	16	8
	sweet	<1	1
8	umami	1	1
	sweet	<1	2
9	sweet	<1	2
10	sweet	<1	2
11	sweet	<1	2
12	sweet	<1	2
13	capsaicin-like	1	2
	sweet	<1	1
14	sweet	<1	2

[a] The freeze-dried fractions were taken up in water (I) or in an aqueous sucrose solution (50 mmol/L; II) and were then evaluated by the TDA. Data are given as the mean of the TD factors evaluated by five different assessors in three different sessions. [b] Number of HPLC fraction refers to **Figure 3**. [c] Taste quality was determined by a trained sensory panel.

The TD factors between individuals and separate sessions differed not more than one dilution step.

Identification and Quantification of Alapyridaine. The freeze-dried residue of GPC fraction III was dissolved in water, membrane-filtered, and then analyzed by analytical RP-HPLC using a mixture (99.9:0.1, v/v) of aqueous trifluoroacetic acid (0.1% in water) and methanol (99.9:0.1, v/v) as the solvent and monitoring the effluent by means of a diode array detector or a mass spectrometric detector, respectively. A compound was detected showing an absorption maximum at 298 nm and a molecular weight of 197 Da: UV−vis (water) λ_{max} = 251, 328 nm (pH 8.2), λ_{max} = 298 nm (pH 3.5); LC-MS (ESI^+), *m*/*z* 198 (100, $[M + 1]^+$), 220 (57, $[M + Na]^+$), 395 (19, $[2M + 1]^+$), 417 (29, $[2M + Na]^+$); LC-MS (ESI^-), *m*/*z* 197 (100, $[M]^-$). On the basis of identical LC-MS data, retention time (RP-HPLC), and sensory attributes with the synthetic reference compound, the

compound imparting the sweetness to GPC fraction III was identified as *N*-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol inner salt, recently isolated from a heated glucose/L-alanine solution (*10*). This was further confirmed by cochromatography of the GPC fraction III, with synthetic alapyridaine. Quantitative analysis, performed by comparing the peak area obtained at $\lambda = 298$ nm with those of a defined standard solution of the reference compound in water, revealed that alapyridaine is present in beef broth in concentrations of 419 μg/L.

High Performance Liquid Chromatography. The HPLC apparatus (Bio-Tek Kontron Instruments, Eching, Germany) consisted of two pumps (type 522), a Rheodyne injector (100 μL loop), and a diode array detector (DAD type 540), monitoring the effluent in a wavelength range between 220 and 500 nm. Separations were performed on a stainless steel column packed with RP-18, ODS-Hypersil, 5 μm, 10 nm (Shandon, Frankfurt, Germany), in either a 250 × 4.6 mm i.d. analytical scale (0.8 mL/min) or a 250 × 10 mm i.d. semipreparative scale (1.6 mL/min). After injection of the sample (20−100 μL), analysis was performed using an isocratic solvent mixture with an aqueous solution of ammonium formate (10 mmol/L; pH 8.2) or an aqueous solution of trifluoroacetic acid (0.1%; pH 2.2) and methanol (99.9:0.1, v/v).

Liquid Chromatography—Mass Spectrometry. A Nucleosil 100-5C18 analytical HPLC column (Macherey and Nagel, Dürren, Germany) was coupled to an LCQ-MS (Finnigan MAT GmbH, Bremen, Germany) using electrospray ionization. After injection of the sample (2−20 μL), analysis was performed using isocratic solvent mixtures (1:99, v/v) of methanol and either an aqueous ammonium formate solution (10 mmol/L; pH 8.2) or an aqueous trifluoroacetic acid solution (0.1%; pH 2.2).

RESULTS AND DISCUSSION

To locate hydrophilic, taste-enhancing compounds formed during the cooking of meat, a beef broth was freshly prepared and then defatted by fat crystallization at 2 °C, followed by solvent extraction. After removal of trace amounts of solvent in vacuo, a trained sensory panel described the overall taste of the defatted beef broth as rich and complex, centering around umami, saltiness, sweetness, and sourness. To map the broth tastants and to locate potential compounds contributing to the sweetness of the beef broth, the aqueous solution was fractionated following the activity-guided procedure outlined in **Figure 1**.

Mapping of Compounds Contributing to Sweet Taste. With the aim of removing proteins and high molecular weight, melanoidin-type material (HMW compounds) from the low molecular weight (LMW) taste compounds, the defatted beef broth was separated by means of ultrafiltration with a molecular weight cutoff of 1000 Da (**Figure 1**). Sensory analysis demonstrated that the LMW fraction exhibited the typical complex, broth-like taste including the sweetness, whereas the HMW fraction showed a comparatively poor overall taste.

To analyze for taste compounds in beef broth, the LMW fraction was further fractionated by GPC using a Sephadex G-15 as the stationary phase and aqueous acetic acid as the eluent. Monitoring the effluent at 254 nm, the GPC chromatogram displayed in **Figure 2** was recorded, and 10 fractions (fractions I−X) were collected separately. These fractions were separately freeze-dried and the odorless residues dissolved in the same amount of water. The aqueous solution of each fraction was then presented to the sensory panel to judge the taste qualities and intensities by application of the TDA. To achieve this, each solution was stepwise (1:1) diluted with water and the dilutions were then presented in order of increasing concentrations to trained sensory panelists, who were asked to evaluate the taste quality and to determine the dilution at which a taste difference between the diluted fraction and two blanks (tap water) could just be detected. As this so-called TD factor, obtained for each fraction, is related to its taste activity in water, the 10 GPC fractions were rated according to their relative taste intensity (I in **Table 1**). Fraction II exhibited a pure salty taste quality and, due to its high TD factor of 2048, was evaluated with by far the highest taste impact (I in **Table 1**). In contrast, fraction III showed a very complex taste covering umami, salty, and sour notes. The taste dilution technique, however, succeeded in rating these four taste qualities in their taste impact, for example, the umami and the salty notes were judged with the highest TD factors of 64 and 32, closely followed by a sour sensation coming up when the original fraction was diluted to less than 1:4. However, none of the GPC fractions showed a significant sweet note.

To analyze for putative taste modifiers enhancing the sweetness of the sugars in beef broth, the fractions of another aliquot of the GPC fractionation were dissolved in the same amount of an aqueous 2-fold hyperthreshold sucrose solution, each solution was stepwise diluted 1:1 with water, and their TD factors were then determined by the sensory panel as described above. The TDA revealed a high TD factor of 16 for sweetness in fraction III (II in **Table 1**). As this fraction showed no sweetness in the absence of sucrose (I in **Table 1**) and the sucrose concentration present was 2-fold above the sweetness threshold, this fraction was assumed to contain a reaction product enhancing the sweetness of the sucrose solution by a factor of 8. Because sweetness-enhancing compounds were not yet reported in beef broth, the following identification experiments were focused on the sapid taste modifier present in GPC fraction III (**Figure 1**).

Identification of the Sweetness Enhancer in GPC Fraction III. To further resolve fraction III into distinct sensory active compounds and to rate them according to their relative taste impacts, this fraction was then separated by RP-HPLC (**Figure 3**) into 14 subfractions which, after freeze-drying, were used for the comparative TDA using water as the solvent (I in **Table 2**) or sucrose as the basic tastant (II in **Table 2**).

The highest TD factor was found for fraction III-1, in which umami and salty notes were perceived even when the original fraction was diluted 1:128 (**Table 2**). Fraction III-2, exhibiting a sour taste, and fractions III-6 to III-8, all of which tasted umami-like, were evaluated with somewhat lower TD factors. In addition, all fractions showed sweetness with TD factors of 1 or 2 when sucrose was present with the exception of fraction III-5, which imparted a sweet sensation with a TD factor of 8 (II in **Table 2**). These data clearly pointed out that the sweetness-enhancing compound was present in fraction III-5.

To gain further insights into the chemical structure of the compound causing the sweet taste, fraction III-5 was separately collected and analyzed by RP-HPLC connected to either a diode array detector or a mass spectrometer. The compound exhibiting sweet taste activity upon degustation showed a molecular mass of 197 Da and exhibited two UV−vis absorption maxima at 251 and 328 nm when measured at pH 8.2 or a sole maximum at 298 nm when measured at pH 3.5. As the sweet compound present in fraction III-5 could not be detected in noncooked beef juice (data not shown), we suggested that it might be formed upon thermal processing from nontasting precursors in the beef meat, for example, by Maillard reactions involving amino acids and reducing carbohydrates. Because L-alanine and hexoses are the quantitatively predominating Maillard precursors in beef juice, we compared the LC-MS and UV−vis data, retention time (RP-HPLC), and sensory activity with those of a sweet-enhancing Maillard reaction product that was very recently isolated from a thermally treated aqueous glucose/L-alanine solution (*10*). On the basis of identical spectroscopic,

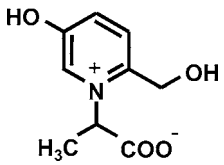

Figure 4. Structure of *N*-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol inner salt (alapyridaine).

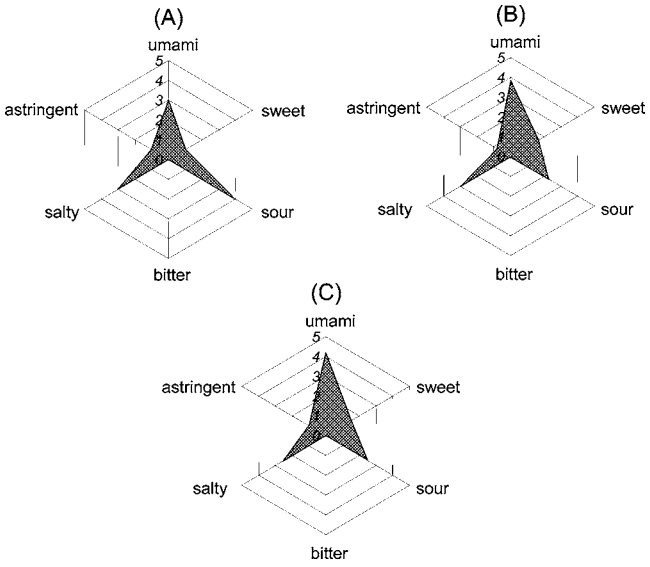

Figure 5. Taste profiles of (**A**) beef taste recombinate, (**B**) beef taste recombinate plus "natural" concentration of alapyridaine (419 μg/L), and (**C**) authentic beef broth.

chromatographic, and sensory data, the tastant imparting the sweetness to fraction III-5 was identified as *N*-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol inner salt (**Figure 4**) and, finally, confirmed by cochromatography of an aliquot of fraction III-5 and the synthetic reference compound (*10*). The identification of this compound, named alapyridaine, verifies for the first time the natural occurrence of taste-enhancing Maillard-type pyridinium betaines in thermally processed foods.

Taste Reconstitution Experiments. To investigate the contribution of alapyridaine to the overall taste of a beef broth, first, the natural amount of the tastant was determined quantitatively in an authentic beef broth. Quantitative HPLC analysis was, therefore, performed on GPC fraction **III** by comparing the peak area obtained at $\lambda = 298$ nm with that of a defined standard solution of the reference compound in water. As the mean of triplicates, the concentration of alapyridaine in the beef broth was found to be 419 μg/L.

To prove the results of the chemical analysis and to check whether the Maillard product contributes to the typical taste of beef broth, we investigated whether the "natural" amount of alapyridaine in beef broth is sufficient to impart a sensory effect to a synthetic blend of taste chemicals present in their authentic concentrations. To achieve this, we first prepared an aqueous taste recombinate consisting of 16 amino acids, 4 sugars, 3 5′-nucleotides, 3 organic acids, 3 salts, phosphate, carnosine, creatine, and creatinine, each in their natural concentrations present in beef broth, and asked a trained sensory panel to score the taste descriptors given in **Figure 5** on a scale from 0 (not detectable) to 5 (strong detectable). Sensory evaluation of this beef taste recombinate revealed the highest intensity for the sour note (4.0), closely followed by a salty (3.0) and umami-like taste quality (3.0) (**Figure 5A**). In contrast, sweetness was judged with an intensity of 1.0 only. The overall taste of that blend was described as not well balanced. The panelists concluded that the typical taste of an authentic beef broth, which showed increased intensity in the umami (4.2) and sweet characters (1.6), and less intensity in sourness (2.4), cannot be completely reconstituted only by the blend of compounds already identified.

To check the influence of alapyridaine on the overall taste of the beef recombinate, synthetic alapyridaine was added in its "natural" concentration of 419 μg/L to the taste recombinate, and the overall taste of that solution was compared with that of the taste recombinate lacking in the alapyridaine. As given in **Figure 5B**, the sensory panel perceived an increase in sweetness (1.7) and umami character (3.8) and, in parallel, a slight decrease in the sour note in the recombinate, thus demonstrating that, in authentic concentrations, the alapyridaine exhibited a pronounced effect on the overall taste quality of beef broth. These data fit very well with our recent finding that alapyridaine is able to enhance both sweet and umami taste tonalities in solutions of sugars and/or monosodium glutamate (*12*), but demonstrate for the first time that the alapyridaine is also active in modulating sweet and umami tastes of real beef broth when present in "natural" concentrations. These findings give strong evidence that naturally occurring levels of alapyridaine may contribute to the typical taste of some thermally processed foods.

Comparing the taste profiles of the blend of taste chemicals including the alapyridaine (**Figure 5B**) with that of an authentic beef broth (**Figure 5C**) demonstrated furthermore that the individual taste qualities could be mimicked quite well in their intensities, thus demonstrating the naturally occurring alapyridaine to be a novel key contributor to the desirable taste of beef broth.

The data indicate that the application of comparative taste dilution techniques, combining instrumental analysis and human taste perception, on fractions isolated from foods opens the possibility of mapping and identifying tasteless but taste-enhancing compounds in foods. Information obtained by such investigations will help to unravel the complex mechanisms involved in thermally induced taste development on a molecular level and will open the possibility to control the formation of desirable flavor compounds more efficiently.

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