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Quantitative Studies, Taste Reconstitution, and Omission Experiments on the Key Taste Compounds in Morel Mushrooms (*Morchella deliciosa* Fr.)

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Sensory-directed fractionation of an aqueous extract prepared from morel mushrooms led to the identification of γ-aminobutyric acid as the chemical inducer of the mouth-drying and mouth-coating oral sensation imparted by morels. Additionally, L-glutamic acid, L-aspartic acid, succinic acid, and the previously unknown (*S*)-malic acid 1-*O*-β-D-glucopyranoside, coined (*S*)-morelid, were detected as additional important umami-like taste compounds. To further bridge the gap between pure structural chemistry and human taste perception, 33 putative taste compounds were quantified in an aqueous morel extract and then rated for their taste contribution on the basis of dose-over-threshold factors. To confirm these quantitative results, an aqueous taste reconstitute was prepared by blending aqueous solutions of 16 amino acids, 6 organic acids, 3 purines, 4 carbohydrates, 3 minerals, and (*S*)-morelid in their "natural" concentrations. Triangle tests revealed that the taste profile of this biomimetic organoleptic cocktail did not differ significantly from the taste profile of authentic morel extract. To finally narrow down the number of key taste compounds, taste omission experiments were performed demonstrating that (*S*)-morelid together with L-glutamic acid, L-aspartic acid, malic acid, citric acid, acetic acid, and γ-aminobutyric acid are the key organoleptics of morel extract. Moreover, sensory experiments with model solutions showed that (*S*)-morelid not only imparts a sour and umami-like taste but is able to amplify the taste activity of monosodium glutamate, as well as sodium chloride, solutions.

KEYWORDS: (*S*)-Morelid; (*S*)-malic acid 1-*O*-β-D-glucopyranoside; γ-aminobutyric acid; taste enhancer; umami; salt; mushrooms; taste reconstitution

INTRODUCTION

Convenient and tasty, air-dried morel mushrooms (*Morchella* spp.) are widely used to add an intense, attractive flavor and satisfying mouthfeel to savory dishes, including soups, stews, and sauces. In particular, the umami-like taste characteristics and the taste-enhancing activity of the rehydrated, air-dried mushrooms are highly desirable and impart rich mouthfeel, complexity, and palatability to culinary products.

Multiple attempts have been made to correlate the information obtained from sensory studies and the molecules exhibiting the typical taste of edible mushrooms (*1*−*3*). Multiple compounds such as soluble carbohydrates (*4*−*20*), purine-5′-nucleotides (*1*, *9*−*12*, *21*), organic acids (*2*, *9*, *13*), and free amino acids (*1*, *4*, *5*, *9*, *10*, *12*, *14*, *15*) have been proposed as key contributors to the typical mushroom taste, but the data reported so far are contradictory. To answer the puzzling question as to which nonvolatile, key taste compounds are responsible for the typical umami-like and mouth-drying taste of morel mushrooms, we recently analyzed an aqueous morel extract by means of the so-called taste dilution analysis (*22*). Application of this sensory-directed fractionation procedure on freshly prepared morel extracts led to the successful identification of γ-aminobutyric acid as the chemical inducer of the mouth-drying and mouth-coating oral sensation imparted by morel extracts. Also, L-glutamic acid, L-aspartic acid, succinic acid, and the previously not reported (*S*)-malic acid 1-*O*-β-D-glucopyranoside, coined (*S*)-morelid, which is an additional important umami-like taste compound, were identified as predominant taste ingredients.

To evaluate the taste contribution of these individual compounds more precisely, the objectives of the present investigation were to quantify putative taste compounds in a rehydrated, air-dried morel extract, to rate them on the basis of a dose/activity relationship, and, finally, to confirm the taste contribution of selected key compounds by means of taste reconstitution and omission experiments.

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MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: ammonium acetate, ammonium formate, formic acid, amino acids, nucleotides, and carbohydrates (Sigma, Steinheim, Germany). Solvents were of HPLC grade (Merck, Darmstadt, Germany). (*S*)-Malic acid 1-*O*-*β*-D-glucopyranoside, (*S*)-morelid, was synthesized closely following the reported procedure (*22*). Dried wild morel mushrooms (*Morchella deliciosa* Fr.) from the year 2001 were obtained from a local market (Munich, Germany).

Preparation of a Hot Water Extract from Morels. Dried morel mushrooms (100 g) were ground and then soaked in bottled water (1 L) for 12 h at room temperature. The aqueous suspension was heated for 15 min under reflux prior to filtration using a cellulose filter. For sensory experiments, aqueous morel extract was used directly after cooling. For analysis of the taste compounds, the extract was fractionated by means of an ultrafiltration cell (Amicon, Witten, Germany) using sequentially YM 10- and YM 1-type filters (Millipore, Bedford, MA) with cutoffs of 10 and 1 kDa, respectively, at a nitrogen pressure of 3 bar. The low molecular weight (LMW) fraction (MW < 1 kDa) was freeze-dried, and the lyophilysate was submitted to quantitative analysis.

Quantitative Analyses. *(S)-Malic Acid 1-O-D-Glucopyranoside, (S)-Morelid.* Following the procedure reported above, an aqueous extract was prepared from dried morels (10 g), and an aliquot (1 mL) was applied to the top of a solid-phase extraction cartridge filled with Strata SAX anion exchanger (1 g) (Phenomenex, Aschaffenburg, Germany) conditioned with water. The cartridge was then flushed with water (5 mL), followed by aqueous NaOH solution (1 mol/L; 15 mL). The effluent was collected and directly used for hydrophilic liquid interaction chromatography (HILIC)-MS/MS analysis. After identification of the (*S*)-morelid, on the basis of identical LC-MS data and retention time (HILIC) with the synthetic reference, the target compound was quantified by comparing the peak area obtained for the mass transition *m*/*z* 295→115 with those of a defined standard solution of the reference compound in water.

Free Amino Acids. Following the reported procedure (*23*), the LMW fraction was dissolved in aqueous buffer solution (0.1 mol/L) containing sodium acetate (8.2 g/L), methanol (7.5%), formic acid (0.3%), acetic acid (1.5%), and octanoic acid (0.001%), membrane filterered, and then analyzed by means of an LC 3000 amino acid analyzer (Biotronic, Maintal Germany) equipped with a 75 × 6.0 mm i.d. BTC F guard column and a 145 × 3.2 mm i.d. BTC 2410 main column (Eppendorf-Netheler-Hinz, Maintal Germany).

Organic Acids. Organic acids were determined using enzymatic test kits (r-biopharm Boehringer, Mannheim, Germany) closely following the experimental procedures given by the manufacturers.

Soluble Carbohydrates. D-Mannitol and D-glucose were quantified in the LMW fraction by means of high-performance liquid chromatography (HPLC) on an ET 250/4 C-18 Nucleosil 100-5 NH_2 column (Machery-Nagel, Düren, Germany) operating at 30 °C with acetonitrile/water (85:15; v/v) as the mobile phase. The effluent was monitored by means of an ERC-7515A refractive index detector (ERC, Alteglofsheim, Germany), and each carbohydrate was identified by cochromatography with the corresponding reference substance and then quantified using sorbitol as the internal standard. Smaller amounts of fructose, sucrose, and glycerin were quantitatively determined using commercially available enzymatic test kits closely following the protocols of the manufacturers (r-Biopharm Boehringer).

Purine-5′-nucleotides. Nucleotides were quantified in the LMW fraction by HPLC on a 250 × 4.6 mm i.d., 5 *μ*m, phenyl-hexyl column (Phenomenex, Aschaffenburg, Germany) using isocratic elution with an aqueous phosphate buffer (0.01 mol/L; pH 2.8) at a flow rate of 1 mL/min. The effluent was monitored at 254 nm by means of a UV−vis detector, and each 5′-nucleotide was identified by comparing retention times and UV−vis and LC-MS spectra with those of the corresponding reference substances and was then quantified using inosine-5′-monophosphate as the internal standard.

Inorganic Ions. Sodium, potassium, calcium, and magnesium ions were determined by means of atomic absorption spectroscopy using an AA-175 series-type spectrometer (Varian, München, Germany). Phosphate was analyzed by photometry (*24*) and chloride by means of potentiometry (*25*).

Table 1. Taste Qualities, Taste Thresholds, Concentrations, and Dose-over-Threshold (DoT) Factors of Selected Taste Compounds

tastant	threshold (mmol/L)	concn (mmol/L)	DoT factor[a]
group I: umami-like taste compounds			
L-glutamic acid	3.0	6.20	2.1
L-aspartic acid	4.0	2.08	0.5
(*S*)-morelid	6.0	0.45	0.1
adenosine-5′-monophosphate	4.0	0.20	0.1
uridine-5′-monophosphate	17.0	0.36	<0.1
group II: sour and mouth-drying compounds			
γ-aminobutyric acid	0.02	1.29	64.5
malic acid	3.7	7.85	2.1
citric acid	2.6	5.02	1.9
succinic acid[b]	0.9	1.33	1.5
acetic acid	2.0	2.55	1.3
oxalic acid	5.6	0.59	0.1
L-lactic acid	14.0	1.27	0.1
group III: sweet-tasting compounds			
mannitol	20.0	27.20	1.4
L-alanine	8.0	6.72	0.8
glucose	48.0	23.70	0.5
L-serine	30.0	2.61	0.1
L-threonine	40.0	2.51	0.1
ornithine	3.5	0.47	0.1
fructose	5.0	0.53	0.1
glycerin	57.0	2.74	<0.1
glycine	30.0	1.20	<0.1
L-proline	26.0	1.00	<0.1
group IV: bitter-tasting compounds			
L-isoleucine	11.0	1.91	0.2
L-leucine	12.0	2.78	0.2
L-tyrosine	5.0	0.88	0.2
L-tryptophan	5.0	0.38	0.1
L-valine	21.0	2.66	0.1
hypoxanthine	9.0	0.42	<0.1
group V: salty compounds			
ammonia	5.0	4.68	0.9
potassium dihydrogenphosphate	15.0	6.70	0.4
L-cysteine	2.0	0.25	0.1
L-methionine	5.0	0.28	0.1
sodium chloride	7.5	0.63	0.1

[a] DoT factor was calculated as the ratio of the concentration and taste threshold of a compound. [b] Besides its sour taste, this compound exhibits a umami-like sensation at the threshold concentration of 0.7 mmol/L corresponding to a DoT factor of 1.9 for umami taste.

Sensory Analysis. *Panel Training.* Using triangle tests, 14 assessors (8 males, 6 females, 22−36 years old) were trained to evaluate the taste of solutions (5 mL each) of the following standard compounds: sucrose (50 mmol/L) for sweetness, lactic acid (20 mmol/L) for sourness, NaCl (12 mmol/L) for saltiness, caffeine (1 mmol/L) for bitterness, and monosodium glutamate (MSG) (8 mmol/L, pH 5.7) for umami taste. For mouth-drying and astringency, the panel was trained using tannin (gallustannic acid; 0.01%). Sensory analyses were performed in a sensory panel room at 19−22 °C in three different sessions.

Taste Recognition Threshold Concentrations. Taste threshold concentrations of individual compounds were determined in bottled water (pH 6.5) by means of triangle tests as reported earlier (*22*).

Taste Reconstitution. To prepare an artificial taste imitate of morel extract, the "natural" amounts of the 33 taste compounds, summarized in **Table 1**, were dissolved in bottled water, and the pH value of the solution was then adjusted to 6.5 by the addition of NaOH (0.1 mmol/L). After 10 min of equilibration, the overall taste quality was evaluated by eight trained panelists using nose clips.

Taste Profile Analysis. A freshly prepared morel extract, complete taste recombinants, and partial taste recombinants were presented to the members of the sensory panel, who were asked to score the taste

qualities umami, sour, bitter, sweet, salty, and mouth-drying/astringent on a scale from 0 (not detectable) to 3 (strongly detectable). While the panel members wore nose clips, the samples were briefly swirled around in the mouth and then expectorated.

Omission Experiments. To investigate the taste contribution of the individual taste compounds, partial taste recombinants were prepared one by one by omitting either individual taste groups or single taste compounds from the complete taste reconstitutent. Each of the partial recombinants was presented to the panelists in comparison with the complete taste reconstitutent, using a triangle test. Eight panelists were asked to evaluate whether the solutions were identical in overall taste or not. Those panelists who detected the taste difference correctly were asked to rate the intensity of the given taste descriptors on a scale from 0 to 3.

Isointensity Experiments. Aqueous solutions containing (*S*)-morelid (20 mmol/L) and either NaCl (30 mmol/L) or MSG (10 mmol/L) were adjusted to the pH value of 4.0, 5.5, 6.5, and 8.0, respectively, by adding trace amounts of aqueous NaOH (0.1 mol/L) or formic acid (0.1 mol/L), and a panel consisting of eight assessors evaluated the saltiness or the umami taste of these solutions and compared them to control solutions containing only NaCl (30 mmol/L) or MSG (10 mmol/L) in increasing concentrations from 30 to 100 mmol/L The NaCl or MSG solution showing a taste intensity equal to that of the binary mixture containing (*S*)-morelid was determined to be the isointense concentration.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (BIO-TEK Kontron Instruments, Eching, Germany) consisted of two pumps (type 522), a Rheodyne injector (100 μL loop), and a UV−vis detector (type 535), monitoring the effluent at wavelengths of 254 nm.

Hydrophilic Interaction Liquid Chromatography−Mass Spectrometry (HILIC-MS/MS). For HPLC-ESI-MS/MS analysis, an Agilent 1100 series HPLC was coupled to an API 4000 QTrap mass spectrometer (Applied Biosystems, Darmstadt, Germany) running in the negative electronspray ionization mode. For chromatographic separation, an aliquot of the sample (5 μL) was injected onto a 150 × 2 mm TSKgel Amide-80 column (Tosoh Bioscience, Stuttgart, Germany) operated with a flow rate of 0.2 mL/min. Analysis was performed starting with a mixture (97:3; v/v) of acetonitrile and aqueous formic acid (0.1% in water) and decreasing the acetonitrile content to 50% within 40 min.

RESULTS AND DISCUSSION

To evaluate the taste profile of the morel extract, the trained sensory panel rated the intensity of the taste qualities bitterness, sweetness, sourness, umami, and saltiness as well as the mouth-drying sensation on a scale from 0 (not detectable) to 3 (strongly detectable). By far the highest scores of 2.1 and 1.6 were observed for the intensity of the umami as well as sour taste sensation, respectively, followed by the mouth-drying sensation judged with a somewhat lower intensity of 1.3 (**Figure 1**). In comparison, the taste qualities bitterness, sweetness, and saltiness were rated only with very low intensities.

As reported recently, application of the taste dilution analysis on a freshly prepared aqueous morel extract led to the successful identification of γ-aminobutyric acid as the chemical inducer of a mouth-drying oral sensation and enabled the discovery of the previously unknown (*S*)-morelid as contributing, apart from L-glutamic acid and L-aspartic acid, to the umami-like taste of morels (*21*).

To confirm the importance of these compounds as key taste compounds of morels and to evaluate the taste contribution of additional putative taste compounds such as organic acids, carbohydrates, nucleotides, and minerals, we aimed at preparing a biomimetic taste imitate containing these taste compounds in their natural concentrations and to compare the taste profile of this tastant cocktail to that of authentic aqueous morel extract.

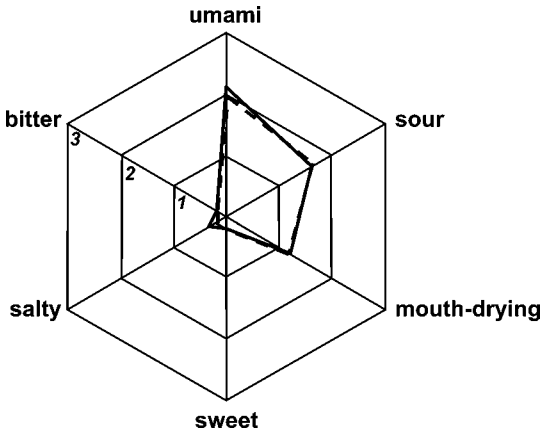

Figure 1. Taste profile of a freshly prepared morel extract (solid line) and the morel extract taste recombinant containing 33 compounds (dotted line).

To achieve this, all of the individual taste compounds needed to be quantified in freshly prepared morel extract and the taste recognition thresholds determined.

Quantification and Calculation of Dose-over-Threshold (DoT) Factors. Aimed at the evaluation of the taste contribution of the individual taste compounds, (*S*)-morelid, 16 amino acids, 6 organic acids, 4 soluble carbohydrates, 3 inorganic salts, and 3 purine-5′-nucleotides were quantitatively determined in the aqueous morel extract. The taste quality, as well as the taste recognition threshold, of each substance was evaluated by the sensory panel. Because we aimed to elucidate the key contributors for each individual taste quality, the single taste compounds were grouped into five classes differing in their taste qualities (**Table 1**).

Tastant group I, imparting an umami-like taste sensation, contained L-glutamic acid, L-aspartic acid, two 5′-nucleotides, and (*S*)-morelid (**Table 1**). By means of the triangle test, the human sensory recognition thresholds of these compounds were determined (*22*). All compounds exhibited an umami taste, differing in threshold concentrations from 3.0 mmol/L for glutamic acid to 17.0 mmol/L for uridine-5′-monophosphate. In this group, glutamic acid was found in the highest concentration; 6.2 mmol/L was present in the morel extract. In comparison, the morel extract contained only low amounts of 5′-nucleotides, with a maximum concentration of 0.36 mmol/L for uridine-5′-monophosphate (**Table 1**). Quantitation of (*S*)-morelid by means of HILIC-MS/MS analysis revealed a concentration of 0.45 mmol/L. To gain first insights into the taste impact of these compounds, they were rated on the basis of their DoT factors, defined as the ratio of the concentration and the taste recognition threshold of a compound (*26*). Calculation of the DoT factors revealed that, exclusively, the concentration of L-glutamic acid in the morel extract exceeded its taste threshold concentration by a factor of 2.1 (**Table 1**). In contrast, the concentration of all other substances in group I was found to be below their taste threshold concentration. The concentration of L-aspartic acid was 2-fold and the concentrations of adenosine 5′-monophosphate and (*S*)-morelid were 10-fold below their taste recognition threshold, whereas a DoT factor of <0.1 was found for uridine-5′-monophosphate (**Table 1**).

Group II, the compounds imparting a sour and/or mouth-drying sensation to the oral cavity, contained six organic acids and the amino acid γ-aminobutyric acid (**Table 1**). Sensory experiments demonstrated that aqueous solutions of γ-aminobutyric acid exhibited a slightly sour taste besides the typical

mouth-drying sensation above the recognition threshold concentration of 0.02 mmol/L. Compared to this amino acid, the organic acids were found to induce a sour taste at higher threshold levels, spanning from 0.9 to 14 mmol/L (**Table 1**). Besides its sour taste, succinic acid exhibited a umami-type taste sensation above the threshold of 0.7 mmol/L (*22*). Quantitative analysis of these compounds revealed by far the highest concentrations for malic acid; for example, 7.9 mmol/L has been detected in morel extract. In comparison, all other taste compounds summarized in group II ranged from 5 mmol/L for citric acid to 0.6 mmol/L for oxalic acid (**Table 1**). Calculation of the DoT factors of these compounds revealed a rather high value of 64.5 for γ-aminobutyric acid. Additionally, also malic acid, citric acid, succinic acid, and acetic acid gave DoT factors above 1, whereas the concentrations of oxalic acid and lactic acid were below their taste threshold concentrations and, therefore, could be excluded as important taste contributors (**Table 1**).

Soluble carbohydrates and sweet-tasting L-amino acids were classified in group III representing compounds imparting sweetness (**Table 1**). Quantitative analysis revealed mannitol and D-glucose as the predominating sweet compounds occurring in morel extract at concentrations of 27 and 24 mmol/L, respectively. In contrast, the concentrations of the sweet-tasting amino acids were much lower, for example, 6.7 and 2.6 mmol/L for L-alanine and L-serine, respectively. Relating these concentrations to the taste recognition thresholds of these sweet compounds revealed that, exclusively, mannitol showed a DoT factor of >1.0, thus demonstrating mannitol as a potential taste contributor. In contrast, the concentration of none of the amino acids exceeded the corresponding sweet threshold concentrations.

Finally, bitter-tasting amino acids and hypoxanthine were grouped into the bitter-tasting group IV, and salts and sulfury-tasting amino acids were summarized in the salty-tasting group V (**Table 1**). Quantitative analysis and sensory studies revealed that the natural concentration of each of these compounds in morel extract was always below the corresponding recognition threshold concentration. Taking these data into consideration, the bitter- as well as the salty-tasting compounds may not be of major importance for the typical morel taste.

Biomimetic Reconstitution of Morel Taste. To confirm the results of the quantitative analysis and to check whether the compounds already identified can create the typical taste of the morel mushroom, we prepared an aqueous biomimetic taste reconstitute containing the natural concentrations of the 33 compounds given in **Table 1** and compared its taste profile with that of the authentic, freshly prepared morel extract. All of the compounds summarized in taste groups I−V were dissolved in bottled water in their natural concentrations, and the pH value as well as the color tone was adjusted to those of the authentic morel extract by adding some trace amounts of NaOH as well as sugar couleur. The sensory panel then evaluated the taste profile of these samples, scoring the taste descriptors, given in **Figure 1**, on a scale from 0 (not detectable) to 3 (strongly detectable). Sensory evaluation of this complete taste recombinant, as well as authentic morel extract, revealed the highest intensities for the umami and sour sensations, followed by a mouth-coating and mouth-drying sensation. Bitterness, sweetness, and saltiness were detectable only with a very low intensity. As shown in **Figure 1**, the taste profile of the recombinant was very close to that of the authentic morel extract. The intensities of the sweet, sour, salty, and mouth-drying notes were identical to those of the original, and the umami taste was evaluated with just slightly lower intensity compared to authentic morel extract. This experiment confirmed that all key taste compounds of the morel extract have been successfully identified and quantified.

Table 2. Taste Quality of Taste Groups Compared to Bottled Water (Control) by Means of a Triangle Test

tastant group	n^a	significance[b]
group I: umami-like	8	***
group II: sour/mouth-drying	8	***
group III: sweet	1	
group IV: bitter	0	
group V: salty	0	

[a] Number of eight panelists detecting a taste difference by means of a triangle test. [b] Significance of results: ***, very highly significant ($p < 0.001$); statistics were done with the computer program SPSS (version 8.0, SPSS GmbH Software, Munich, Germany).

Evaluation of the Taste Contribution by Omission Experiments. Following the above experiments, investigations to confirm the taste contribution of the five taste groups and individual compounds, respectively, by means of so-called omission experiments were conducted.

To understand the influence of the taste groups, partial recombinants of the single taste groups were prepared in their natural concentrations and tested by means of a triangle test using two samples of bottled water as the control (**Table 2**). The umami-like-tasting group I and the sour-tasting group II were significantly identified by all panelists as tasting umami-like and sour, respectively. In contrast, groups III, IV, and V could not be differentiated from water (control). Only by increasing the concentration of the corresponding ingredient 10-fold was a sweet, bitter, or salty taste observed (data not shown).

In an additional experiment, individual taste recombinants lacking in either one or more taste compounds, or lacking in a taste group, were evaluated by means of triangle tests using two samples of the complete taste recombinant as the control. Those panelists who detected the taste difference correctly were asked to describe it. In a first set of experiments, the complete taste group I, containing all umami-like-tasting substances, was omitted from the taste recombinant. As given in **Table 3**, all panelists were able to detect this sample in a triangle test with two samples of the complete recombinant as the reference. The partial recombinant was significantly less intense in umami taste and mouthfeel than the total recombinant. To investigate the taste contribution of the individual taste classes in taste group I, additional partial recombinants were prepared lacking either both the amino acids, the nucleotides, or the (*S*)-morelid. All panelists successfully detected the omission of the amino acids, L-aspartic acid and L-glutamic acid, and described this partial recombinant as being less umami-like when compared to the total recombinant (**Table 3**). Consequently, these amino acids contribute to the umami-like taste of mushrooms as earlier suggested (*1*, *4*, *5*, *9*, *10*, *12*, *14*, *15*). Additionally, six of eight panelists noted the lack of (*S*)-morelid and judged the reduced recombinant with a significantly reduced umami taste intensity and less mouthfullness when compared to the complete recombinant. As the DoT factor of (*S*)-morelid was found to be only 0.1, these data pinpoint (*S*)-morelid as a taste-enhancing molecule significantly contributing to the morel taste. Significantly, the lack of nucleotides could not be detected, thus excluding these compounds as important taste contributors.

In a second experiment, the sour and mouth-drying taste group II, containing the organic acids as well as the amino acid

Table 3. Influence of Taste Groups or Individual Taste Compounds on the Taste Profile of the Taste Recombinant

tastant omitted	n^a	description of tast difference	S^b
total group I	8	less umami-like, less mouthfeel	***
L-aspartic acid, L-glutamic acid	8	less umami-like	***
5′-nucleotides	2	nd	
(*S*)-morelid	6	less umami-like taste, less mouthfeelness, complexity	*
total group II	7	less sour and mouth-drying, less mouthfeel	**
organic acids	7	less sour, less umami-like	**
γ-aminobutyric acid	6	less mouth-drying, slightly less umami and mouthfeel, slightly increased bitterness	*
total group III	7	reduced overall taste intensity	**
carbohydrates	0	nd	
sweet amino acids	2	nd	
total group IV	8	slightly reduced taste intensity	***
total group V	6	slightly reduced complexity	*

[a] Number of eight panelists detecting a taste difference by means of a triangle test. [b] Significance: ***, very high significant ($p < 0.001$); **, highly significant ($p < 0.01$); *, significant ($p < 0.05$); nd, not detectable. Statistics were done with the computer program SPSS (version 8.0, SPSS GmbH Software, Munich, Germany).

γ-aminobutyric acid, was omitted from the complete taste recombinant. As shown in **Table 3**, seven panelists were able to detect this sample by means of a triangle test using two samples of the complete recombinant as the control. The panelists rated this partial recombinant as being significantly less sour and less mouth-drying and having less mouthfeel than the total recombinant, thus fitting well with the high DoT factors determined for most of the organic acids. To elucidate the individual tastant classes in group II, partial recombinants solely lacking either the organic acids or γ-aminobutyric acid were compared to the complete recombinant by means of triangle tests. Organic acid omission gave significant reduction to the sour and umami-like taste evaluated with the same ratings for the recombinant lacking the total taste group II (**Table 3**). Note that the omission of organic acids also resulted in a significant reduction of umami-like taste, most likely due to the omission of succinic acid. We also prepared a partial recombinant lacking only γ-aminobutyric acid. Six of the panelists were able to sensorially detect the omission of that amino acid from the taste recombinant and characterized it as being less sour and mouth-drying. Thus, γ-aminobutyric acid is the key inducer of the mouth-drying sensation in morels. In addition, the panelists evaluated this partial recombinant with slightly lower ratings for umami-like taste and mouthfeel and a slightly higher rating for bitterness. These data imply that the γ-aminobutyric acid has some modulatory activity in the perception of umami taste and bitterness.

To investigate the importance of sweet-tasting compounds, group III, consisting of carbohydrates and sweet-tasting amino acids, was omitted from the complete taste recombinant (**Table 3**). Seven of the eight panelists detected a taste difference. The panelists described the partial recombinant as being slightly less intense and having less overall taste compared to the total taste recombinant, thus indicating that group III does not influence sweetness but does to some extent modulate the overall morel taste. In contrast, omission of the sweet-tasting amino acids and the carbohydrates alone from the taste recombinant was not detectable by the sensory panelists. By considering their DoT factors, a contribution of the soluble carbohydrates mannitol and glucose to sweetness was observed (**Table 1**). However, the omission experiment clearly demonstrated that the joint interplay between carbohydrates and sweet amino acids contributes to morel taste.

Finally, partial recombinants have been prepared lacking either the bitter-tasting group IV or the salty-tasting group V. As shown in **Table 3**, all eight panelists detected a taste difference between these partial recombinants and the complete taste recombinant. As observed for taste group III, the reduced recombinant was not lacking the expected bitter or salty taste, respectively, but the reduced recombinant was described as tasting slightly less complex and less intense. Consequently, the bitter-tasting amino acids and the salty-tasting compounds might make a minor contribution to morel extract taste, although the DoT factors did not exceed the threshold.

Taking all of these findings into account, it can be concluded that umami-like L-glutamic acid, L-aspartic acid, (*S*)-morelid, the organic acids, and γ-aminobutyric acid are the key taste compounds of morel extract, whereas soluble carbohydrates, the residual amino acids, and salts have only a minor taste impact.

Influence of (*S*)-Morelid on the Taste Intensity of Basic Taste Solutions. Although the calculated DoT factor ruled out any taste contribution of the (*S*)-morelid, the omission of this glycoside from the complete taste recombinant led to a significant reduction of the umami-like taste intensity as well as the mouthfeelness (cf. **Table 3**). To investigate the modulatory activity of (*S*)-morelid on the taste intensity of selected basic taste compounds, aqueous solutions of umami-tasting MSG (10 mmol/L), salty-tasting sodium chloride (30 mmol/L), sweet-tasting sucrose (40 mmol/L), bitter-tasting caffeine (4 mmol/L), or sour-tasting citric acid (6 mmol/L) were sensorially evaluated in the absence or presence of (*S*)-morelid (20 mmol/L). Preliminary sensory analysis of these solutions revealed that just the taste intensity of MSG and NaCl solutions was intensified when evaluated in the presence of (*S*)-morelid. The sensory perception of all other taste compounds did not seem to be influenced by (*S*)-morelid (data not shown).

To investigate the modulatory activity of the glycoside in more detail, aqueous solutions of MSG (10 mmol/L) or sodium chloride (30 mmol/L), respectively, were evaluated in the absence or presence of (*S*)-morelid (20 mmol/L) at pH 4.0, 5.5, 6.5, and 8.0 in their taste intensity on a 5-point scale. The taste intensity of a 75 mmol/L MSG or a 100 mmol/L NaCl solution was set to the score of 5.0 (**Figure 2**). The data given in **Figure 2A** demonstrate that the addition of (*S*)-morelid increased the umami intensity of the MSG solution in slightly acidic medium; for example, the umami taste intensity of the MSG solution as pH 4.0, 5.5, and 6.0 was increased by a factor of 2. The highest taste intensity was observed for the MSG/(*S*)-morelid mixture adjusted to pH 6.5. In contrast, at pH 8.0 there was no significant influence of (*S*)-morelid on MSG taste. Comparison of a dilution series of aqueous MSG solutions with the taste intensity of the binary mixture of MSG (10 mmol/L) and (*S*)-morelid (20 mmol/L) mixtures further revealed that the tastant mixture adjusted

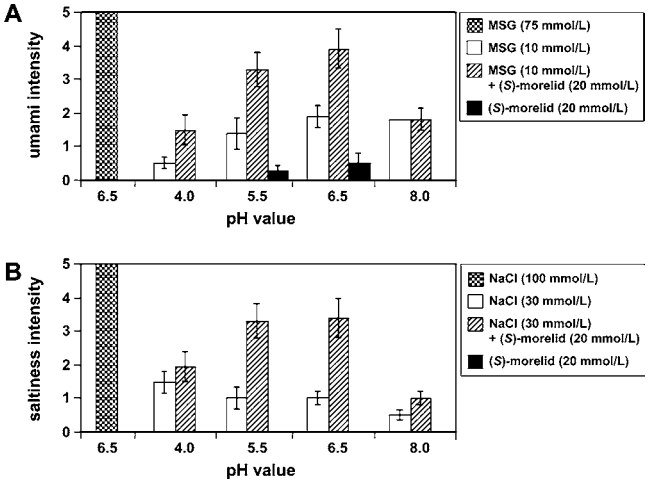

Figure 2. Influence of (*S*)-morelid (20 mmol/L) on the taste intensity of aqueous solutions of (**A**) MSG (10 mmol/L) and (**B**) NaCl (30 mmol/L) solutions differing in pH value. The score of 5.0 was defined as the taste intensity of a 100 mmol/L NaCl and a 75 mmol/L MSG solution, respectively.

to pH 6.5 showed isointensity of the umami taste with a 50 mmol/L MSG solution, thus demonstrating that the (*S*)-morelid amplifies the umami taste of MSG.

Sensory analysis of the NaCl/(*S*)-morelid solution revealed that the glycoside, which did not exhibit any salt taste on its own, intensified the salty taste of NaCl solutions (**Figure 2B**). Similar to the experiment with MSG, the most pronounced effect was detectable at pH 6.5; for example, addition of 20 mmol/L (*S*)-morelid to an aqueous solution (pH 6.5) of NaCl (30 mmol/L) increased the taste intensity from 1.0 to 3.5. This mixture showed isointensity for saltiness with an aqueous 62 mmol/L NaCl solution, demonstrating that (*S*)-morelid is able to intensify the saltiness of NaCl solutions.

In conclusion, (*S*)-morelid and the umami-tasting L-glutamic acid, L-aspartic acid, and succinic acid, as well as the sour-tasting malic acid, citric acid, acetic acid, and γ-aminobutyric acid, have been successfully identified as the key taste compounds of the morel mushroom by means of quantitative studies and taste recombination experiments as well as taste omission studies. Moreover, it was demonstrated that (*S*)-morelid not only imparts a sour and umami-like taste to the oral cavity but also contributes to the umami-like taste and mouthfeelness of morel extracts by amplifying the taste activity of MSG present in the mushroom.

LITERATURE CITED

(1) Kasuga, A.; Fujihara, S.; Aoyagi, Y. The relationship between the varieties of dried shiitake mushrooms and chemical composition. The effect of the varieties of dried shiitake mushrooms on their taste. Part I. *J. Jpn. Soc. Food Sci. Technol*. **1999**, *46*, 692−703.

(2) Muresan, S.; Wilkinson, C.; Ponne, C. Changes in flavour profiles of mushrooms during cooking. *Czech J. Food Sci.* **2000**, *18*, 36−38.

(3) Dijkstra, F. Y.; Wiken, T. O. Studies on mushroom flavours 1. Organoleptic significance of constituents of the cultivated mushroom (*Agaricus bisporus*). *Z. Lebensm. Unters.-Forsch*. **1976**, *160*, 255−262.

(4) Stijve, T.; de A. Amazonas, M. A. L.; Giller, V. Flavour and taste components of *Agaricus blazei* ss. Heinem.—a new gourmet and medicinal mushroom. *Dtsch. Lebensm.-Rundsch.* **2002**, *89*, 448−453.

(5) Harada, A.; Gisusi, S.; Yoneyama, S.; Aoyama, M. Effects of strain and cultivation medium on the chemical composition of the taste components in fruit-body of *Hypsizygus marmoreus*. *Food Chem.* **2004**, *84*, 265−270.

(6) Holtz, B. Qualitative and quantitative analysis of free neutral carbohydrates in mushroom tissue by GLC and AS. *J. Agric. Food Chem*. **1971**, *19*, 1272−1273.

(7) Zakhary, J. W.; Abo-Bakr, T. M.; El-Mahdy, A. R.; El-Tabey, S. A. M. Chemical composition of wild mushrooms collected from Alexandria, Egypt. *Food Chem.* **1983**, *11*, 31−41.

(8) Lizarraga-Guerra, R.; Lopez, M. G. Monosaccharide and alditol contents of huitlacoche (*Ustilago maydis*). *J. Food Compos. Anal.* **1998**, *11*, 333−339.

(9) Tsai, M.-J.; Tsai, S.-J. Taste compounds of the frozen mushroom concentrates. *Food Sci. China* **1987**, *14*, 143−153.

(10) Mau, J.-L.; Lin, Y.-P.; Chen, P.-T.; Wu, Y.-H.; Peng, J.-T. Flavor compounds in King Oyster mushrooms *Pleurotus eryngii*. *J. Agric. Food Chem*. **1998**, *46*, 4587−4591.

(11) Kurkela, R.; Koivurinta, J.; Kuusinen, R. Non-protein nitrogen compounds in the higher fungi—a review. *Food Chem*. **1980**, *5*, 109−130.

(12) Hanssen, H.-P.; Klingenberg, A. Determination of some important flavour compounds in commercial mushroom concentrates. *Z. Lebensm. Unters. Forsch.* **1983**, *177*, 333−335.

(13) Souci, S. W.; Fachmann, W.; Kraut, H. *Food Composition and Nutrition Tables* (in German); Scherz, H., Senser, F., Eds.; Medpharm Scientific Publishers: Stuttgart, Germany, 2000.

(14) Sasaki, H.; Nakamura, N.; Aoyagi, Y.; Sugahara, T. The changes of free amino acids during rehydration of dried shiitake mushrooms. *J. Jpn. Soc. Food Sci Technol.* **1988**, *35*, 90−97.

(15) Lizarraga-Guerra, R.; Lopez, M. G. Content of free amino acids in huitlacoche (*Ustilago maydis*). *J. Agric. Food Chem.* **1996**, *44*, 2556−2559.

(16) Ikeda, K. On a new seasoning (in Japanese). *J. Tokyo Chem. Soc.* **1909**, *30*, 820−826.

(17) Arai, S.; Yamashita, M.; Fujimaki, M. Glutamyl oligopeptides as factors responsible for tastes of a proteinase-modified soybean protein. *Agric. Biol. Chem.* **1972**, *36*, 1253−1256.

(18) Nogushi, M.; Arai, S.; Yamashita, M.; Kato, H.; Fujimaki, M. Isolation and identification of acidic oligopeptides occurring in a flavor potentiating fraction from a fish hydrolysate. *J. Agric. Food Chem.* **1975**, *23*, 49−53.

(19) Matsushita, I.; Ozaki, S. Purification and sequence determination of tasty tetrapeptide (Asp-Asp-Asp-Asp) from beer yeast seasoning and its enzymatic synthesis. *Pept. Chem*. **1994**, *32*, 249−251.

(20) van den Oord, A. H.; van Wassemar, P. D. Umami peptides: assessment of their alleged properties. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 125−130.

(21) Heyland, S.; Moll, H. Determination of flavor-enhancing nucleotides using high-pressure chromatography on ion exchangers (in German). *Mitt. Geb. Lebensm. Unters. Hyg.* **1977**, *68*, 72−77.

(22) Rotzoll, N.; Dunkel, A.; Hofmann, T. Activity-guided identification of (*S*)-malic acid 1-*O*-D-glucopyranoside (morelid) and γ-aminobutyric acid as contributors to umami taste and mouth-drying oral sensation of morel mushrooms (*Morchella deliciosa* Fr.). *J. Agric. Food Chem.* **2005**, *53*, 4149−4156.

(23) Wieser, H.; Mödl, A.; Seilmeier, W.; Belitz, H.-D. High-performance liquid chromatography of gliadins from different wheat varieties: amino acid composition and N-terminal amino acid sequence of components. *Z. Lebensm. Unter. Forsch.* **1987**, *185* (5), 371−378.

(24) Winkler, O. On the photometric analysis of phosphate for the determination of the egg content in foods. *Z. Lebensm. Unters. Forsch.* **1955**, *102*, 111−114.

(25) Kolthoff, I. M.; van Berk, L. H. The accuracy of the halide and thiocyanate titration according to Fajans and according to the usual methods compared to the results of potentiometric determinations. *Z. Anal. Chem.* **1927**, *70*, 369−394.

(26) Scharbert, S.; Hofmann, T. Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *J. Agric. Food Chem.* **2005**, *53*, 5377−5384.

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