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Identification of Sensory-Active Phytochemicals in Asparagus (*Asparagus officinalis* L.)

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ABSTRACT: Sensory-directed fractionation of extracts prepared from raw and cooked asparagus (*Asparagus officinalis* L.), respectively, followed by LC-TOF-MS, LC-MS/MS, and 1D/2D-NMR experiments revealed the chemical structures of nine bitter tasting mono- and bidesmotic saponins as well as the previously not reported 1,2-dithiolan-4-carboxylic acid 6-D-α/β-glucopyranose ester exhibiting an interesting buttery mouth-coating effect. Sensory studies showed that the orosensation imparted by this sulfur compound was reminiscent to that of melting butter and revealed an orosensory recognition threshold of 276.8 μmol/L.

KEYWORDS: *asparagus, asparagusic acid, steroidal saponin, taste, taste dilution analysis, bitter, mouth-coating*

INTRODUCTION

Raw and cooked spears of white asparagus (*Asparagus officinalis* L.) are highly appreciated by the consumers for its pleasant flavor profile centering around sulfury and buttery notes. Unfortunately, the palatability of asparagus might be strongly decreased by a sporadic bitter taste. This off-taste is often the reason for consumer complaints and, therefore, is causing a major problem for vegetable processors. Although the so-called bottom cuts seem to be rich in bitterness,[1] the knowledge about the key molecules imparting the typical bitter off-taste of white asparagus is rather fragmentary.

After isolation from asparagus storage roots, the furostanol saponins 5β-furostane-3β,22,26 triol-3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside (officinalidin I) and 5β-furostane-3β,22,26 triol-3-*O*-β-D-glucopyranosyl (1→2) [β-D-xylopyranoxyl (1→4)]-β-D-glucopyranoside 26-*O*-β-D-glucopyranoside (officinalisin II), were suggested to contribute to the bitter off-taste of asparagus, although their identification was not based on published NMR data.[2] In addition, 25*S*-furost-5-ene-3β,22,26-triol-3-*O*-(2,4-di-*O*-α-L-rhamnopyranosyl-β-D-glucoyranoside)-26-*O*-β-D-glucopyranoside (ASP-I) was isolated from asparagus bottom cuts and identified as the bitter tasting (25*S*)-configumer of the well-known protodioscin.[1,3] As several studies have demonstrated that the saponin distribution among the organs of a plant varies considerably and that different organs of asparagus do not contain the identical saponins, it is unclear whether these saponins are also the bitter key molecules in the edible spears of asparagus.[2,4] Although treatment with a naringinase preparation comprising naringinin-α-1,2-rhamnosidase and β-glucosidase activity induced the decrease of the bitter taste of the juice prepared from asparagus bottom cuts,[2] the exact structures of the saponins accounting for the bitter off-taste of asparagus spears are still not known. Furthermore, it is unclear as to whether besides the saponins identified,[1,2,5−9] also flavonoids,[10] saccharides,[11−13] polyacetylenes,[14,15] and the sulfur-containing asparagusic acid and its esters,[16−18] respectively, or previously unknown phytochemicals contribute to the perceived bitterness of asparagus spears.

In the past, application of the so-called sensomics approach on various fresh and processed foods enabled the comprehensive mapping of several sensory-active substances such as thermally generated bitter compounds,[19,20] cooling compounds in dark malt,[21] bitter off-tastants in carrot products,[22] the taste enhancer alapyridaine in beef bouillon,[23] and astringent key taste compounds in black tea infusions,[24] roasted cocoa nibs,[25] red current juice,[26] and spinach.[27]

The objective of the present study was to locate the key molecules contributing to the typical taste profile of raw and cooked asparagus by means of a sensomics approach, to determine the chemical structure of the most intense taste-active phytochemicals by means of LC-MS, 1D/2D-NMR, and to evaluate their sensory activity by the determination of human recognition thresholds.

MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially from the sources given in parentheses: formic acid, glucose, hydrochloric acid (Merck, Darmstadt, Germany); sodium chloride, L-glutamic acid monohydrate, rhamnose (Fluka, Taufkirchen, Germany), sodium hydroxide (Riedel-de-Haen, Seelze, Germany). Deuterated solvents were obtained from Euriso-Top (Gif-Sur-Yvette, France). Solvents were of HPLC grade (Merck Darmstadt, Germany). Water for HPLC separation was purified by means of a Milli-Q water advantage A 10 water system (Millipore, Molsheim, France). For sensory analysis, bottled water (Evian, low mineralization: 405 mg/L) was adjusted to pH 5.9 with trace amounts of formic acid prior to use. Freshly harvested, white asparagus spears (~21 cm in length) of the cultivar Grolim were obtained from local producers in Germany, namely, from Quedlinburg (May/June 2005), Münster (June 2007), and Schrobenhausen (May 2008), respectively.

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Preparation of Cooked Asparagus. Freshly peeled, white asparagus spears (2 kg) were divided lengthwise in two equal parts by means of a kitchen knife. One aliquot was used for the analysis of the fresh samples, the other one was cooked for 14 min and 95 °C in water (1.5 L) to simulate domestic processing.

Sequential Solvent Extraction. Cooked and fresh white asparagus spears were cut into 2 cm pieces with a kitchen knife and finely ground in a laboratory blender (Retsch, Haan, Germany) at 3500 U/min for 60 s. Methanol (2.0 L) was added to the puree (~1 kg) of raw and cooked asparagus, respectively, and the mixtures were vigorously stirred for 60 min at room temperature under an atmosphere of argon, followed by filtration (Schleicher & Schuell filter, 24 cm). The filtrates were collected and the residues were extracted three times (2 L each) with methanol/water (70/30, v/v) adjusted to pH 5.9 with aqueous formic acid (1% in water). After filtration, the combined filtrates were separated from methanol in a vacuum at 38 °C, followed by freeze-drying. The lyophilized material obtained from cooked and raw asparagus, respectively, was taken up in water (1 L) and, then, sequentially extracted with *n*-pentane (4 × 0.5 L), dichloromethane (4 × 0.5 L), followed by ethyl acetate (4 × 0.5 L). The corresponding extracts were combined accordingly and separated from solvent in a vacuum at 38 °C, followed by lyophilization to obtain the pentane extractables (fraction I), the dichloromethane extractables (fraction II), the ethyl acetate extractables (fraction III), and the water-solubles (fraction IV), respectively (Table 1). The fully extracted asparagus residues were freeze-dried to give the insoluble materials which did not exhibit any taste activity.

Table 1. Yields of Total Extract and of Fractions I–IV Isolated from Raw and Cooked Peeled White Asparagus, Respectively

	yield[b] (g/100 g) from	
sample[a]	raw white asparagus	cooked white asparagus
total extract	3.95	1.86
fraction I	0.20	0.44
fraction II	0.21	0.06
fraction III	0.09	0.03
fraction IV	3.38	1.22

[a]Total extract obtained by extracting minced asparagus with methanol and methanol/water and individual fractions (I–IV) obtained by sequential solvent extraction of total extract. [b]Yields were determined by weight; based on fresh weight.

Separation of Fraction III by Means of Solid Phase Extraction. An aliquot (600 mg) of lyophilized fraction III isolated from fresh asparagus was dissolved in methanol/water (30/70, v/v; 10 mL) and applied onto the top of a Strata C18-E SPE cartridge (10 g/60 mL; Phenomenex, Aschaffenburg, Germany) preconditioned with methanol (60 mL), methanol/water (50/50, v/v; 100 mL), followed by water (100 mL) by using a vacuum extraction box (J. T. Baker, Philipsburgh, NJ, USA). Separation was performed by flushing the cartridge with a sequence of methanol/water mixtures (100 mL each) to give fraction III-A (0/100, v/v; yield: 57.7%), fraction III-B (30/70, v/v; yield: 5.0%), fraction III-C (70/30, v/v; yield: 6.9%), and finally fraction III-D (100/0, v/v; yield: 10.0%), respectively. Each fraction was collected, separated from solvent in a vacuum, and the residues were taken up in water, freeze-dried twice, and were then kept at −20 °C until used for sensory and chemical analysis, respectively.

Identification of Key Taste Compounds in Fraction III-B. Fraction III-B was dissolved in acetonitrile/water (10/90, v/v; 2 mL/50 mg) and, after membrane filtration, was injected onto a 250 × 21.2 mm i.d., 5 μm, Microsorb-MV C18 column (Varian, Darmstadt, Germany) equipped with a guard column of the same type operated with a flow rate of 18 mL/min. Using 0.1% formic acid in water (v/v) as solvent A and 0.1% formic acid in acetonitrile (v/v) as solvent B, chromatography was performed with the following gradient: 0 min, 0% B; 5 min, 0% B; 10 min, 10% B; 27 min, 10% B; 40 min, 100% B; 50 min, 100% B; 55 min, 0% B; 68 min, 0% B. The effluent was separated into 19 subfractions, namely, III-B-1 up to II-B-19, which were collected individually in several runs. After addition of water (10 mL), the solvent was removed in a vacuum. Each fraction was finally separated from buffer by means of solid phase extraction using C-18 E cartridges (1 g/6 mL; Phenomenex, Aschaffenburg, Germany) which were preconditionated with methanol (3 × 10 mL), followed by water (3 × 10 mL). After sample application, the cartridges were rinsed with water (6 mL), followed by methanol (10 mL) to obtain the target compounds, which were separated from solvent in a vacuum at 38 °C, followed by lyophilization. Fraction III-B-12 and III-B-14, judged with the highest sensory impact, were further purified by rechromatography by means of semipreparative RP-HPLC using a 0.5 mL sample loop and a 250 × 10 mm i.d., 5 μm, HyperClone ODS C18 column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Using 1% formic acid in water (v/v) as solvent A and 1% formic acid in acetonitrile (v/v) as solvent B, the following solvent gradient (3 mL/min) was used for chromatography: 0 min, 0% B; 5 min, 0% B; 10 min, 10% B; 15 min, 10% B; 25 min, 100% B; 30 min, 100% B; 35 min, 0% B; 40 min, 0% B. Before each injection the column was equilibrated for 10 min at the starting conditions. The effluents of the chromatographic separation of fractions III-B-12 and III-B-14 were collected individually in several runs and the corresponding fractions were combined. After removing the solvent in vacuum, each HPLC fraction was again separated from buffer by means of solid phase extraction as described above. After freeze-drying twice, the structures of taste compound **1** (III-B-12) and **2** (III-B-14) were determined as 1,2-dithiolan-4-carboxylic acid 6-D-α-glucopyranoseester and 1,2-dithiolan-4-carboxylic acid 6-D-β-glucopyranoseester, respectively, by means of UV–vis, LC-MS/MS, TOF-MS, 1D/2D NMR, and hydrolytic degradation experiments.

*1,2-Dithiolan-4-Carboxylic acid 6-D-α/ß-Glucopyranoseester, **1**/**2**, Figure 1.* LC-MS (ESI^-): *m/z* (%) 357.7 (100, $[M + HCOO]^-$), 311.0 (21, $[M - H]^-$), 347.6 (15, $[M + Cl]^-$); LC-MS/MS (DP = −60 V): *m/z* (%) 311 (6), 144 (5), 131 (100), 112 (22), 104 (10); LC-MS-TOF: *m/z* 311.0273 (measured), *m/z* 311.0254 (calcd. for $[C_{10}H_{16}O_7S_2-H^+]^-$); ^1H and ^{13}C NMR data are given in Table 2.

Alkaline Hydrolysis of 1 and 2 and Identification of Asparagusic Acid. Aliquots (~0.5 mg) of compounds **1** and **2**, respectively, were dissolved in aqueous sodium hydroxide (2 mol/L; 1 mL) and heated at 110 °C for 120 min in a closed vial. After cooling, water (50 mL) was added and the pH value was adjusted to 5.0 with an aqueous hydrogen chloride solution (4 mol/L). High-performance ion chromatographic analysis of a minor aliquot (1 mL) of this hydrolysate and cochromatography with reference carbohydrates revealed glucose as the carbohydrate moiety in **1** and **2**, respectively. The major aliquot (50 mL) of the hydrolysate was extracted three times with ethyl acetate (30 mL), and the pooled organic extracts were separated from solvent in vacuum to afford asparagusic acid (**3**) as a pale yellow powder in a purity of >98% (HPLC/ELSD).

*Asparagusic Acid, **3**, Figure 1.* LC-MS (ESI^-): *m/z* (%) 149 (100, $[M - H]^-$), 299 (87, $[2M - H]^-$), 195 (80, $[M + HCOO]^-$), 185 (14, $[M + Cl]^-$); LC-MS/MS (DP = −60 V): *m/z* (%) 104 (20), 63 (100); LC-MS-TOF: *m/z* 148.9744 (measured), *m/z* 148.9731 (calcd. for $[C_4H_5O_2-H^+]^-$); ^1H NMR (500 MHz, DMSO-d_6; COSY): δ/ppm: 3.30 [dd, 2H, *J* = 4.2 Hz, *J* = 11.4 Hz, H–C(3a, 5a)]; 3.40 [dd, 2H, *J* = 6.1 Hz, 11.4 Hz, H–C(3b, 5b)]; 3.49–3.54 [m, 1H, H–C(4)]; 12.8 [s, 1H, COOH]; ^{13}C NMR (125 MHz, DMSO-d_6; HMQC, HMBC): 41.5 [C(3, 5)]; 50.7 [C(4)]; 173.4 [C(6)].

Identification of Key Taste Compounds in Fraction III-C. A solution (100 mg/10 mL) of an aliquot of fraction III-C in methanol/water (50/50, v/v) was separated by preparative HPLC using a 1 mL injection loop and a 250 × 21.2 mm i.d., 5 μm, HyperClone ODS 120 column (Phenomenex, Aschaffenburg, Germany) with a binary gradient using 0.1% formic acid in water as solvent A and 0.1% formic acid in acetonitrile as solvent B (flow rate 20.0 mL/min): 0 min, 5% B; 5 min, 5% B; 10 min, 30% B; 25 min, 30% B; 30 min, 35% B; 40 min, 40% B; 45 min, 40% B; 45 min, 100% B; 50 min, 100% B; 55 min, 5% B; 60 min, 5% B. Prior to injection, the column was equilibrated for 10 min using the starting conditions. The effluent was

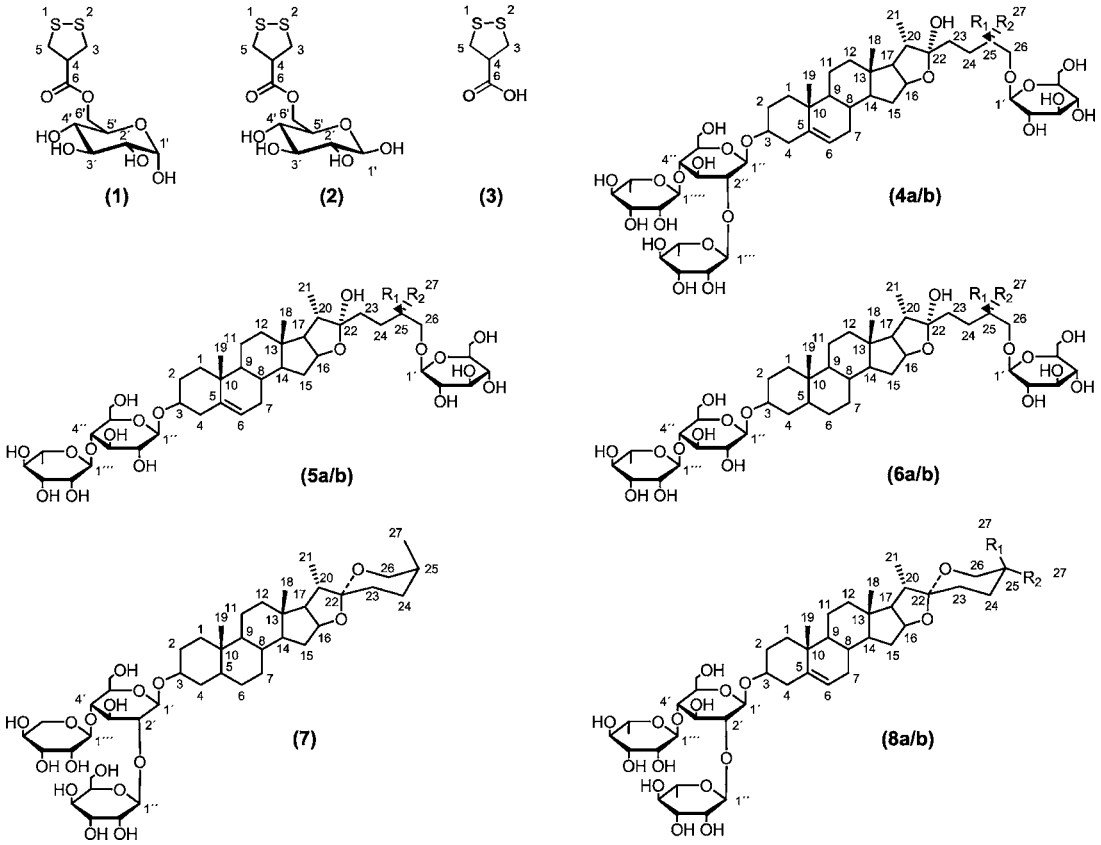

Figure 1. Chemical structures of 1,2-dithiolan-4-carboxylic acid 6-D-α-glucopyranose ester (**1**), 1,2-dithiolan-4-carboxylic acid 6-D-β-glucopyranose ester (**2**), asparagusic acid (**3**), 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol (**4a**; R_1 = CH_3, R_2 = H), 3-O-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol (**4b**; R_1 = H, R_2 = CH_3), (25*R*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**5a**; R_1 = CH_3, R_2 = H), (25*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**5b**; R_1 = H, R_2 = CH_3), (25*R*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6a**; R_1 = CH_3, R_2 = H), (25*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6b**; R_1 = H, R_2 = CH_3), and 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ole (**7**), 3-O-[{α-L-rhamnopyranosyl-(1→2)}{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-(25*S*)-spirost-5-ene-3β-ol (**8a**; R_1 = CH_3, R_2 = H), and 3-O-[{α-L-rhamnopyranosyl-(1→2)}{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-(25*R*)-spirost-5-ene-3β-ol (**8b**; R_1 = H, R_2 = CH_3).

Table 2. Assignment of ^{1}H NMR (400 MHz, DMSO-d_6/D_2O; 95/5, v/v) and ^{13}C NMR Signals (100 MHz, DMSO-d_6/D_2O; 95/5, v/v) of 1,2-Dithiolan-4-Carboxylic Acid 6-D-α-Glucopyranose Ester (1) and 1,2-Dithiolan-4-Carboxylic acid 6-D-β-Glucopyranose Ester (2)

	1 (α)[a,b]				2 (β)[a,b]			
position	δ_C / ppm	HSQC	δ_H / ppm	M [*J* Hz]	δ_C / ppm	HSQC	δ_H / ppm	M [*J* Hz]
3	41.5	[CH_2]	3.27−3.34	m	41.5	[CH_2]	3.27−3.34	m
			3.35−3.45	m			3.35−3.45	m
4	50.2	[CH	3.56−3.64	m	50.2	[CH]	3.56−3.64	m
5	41.5	[CH_2]	3.27−3.34	m	41.5	[CH_2]	3.27−3.34	m
			3.35−3.45	m			3.35−3.45	m
6	171.9	[C]			171.9	[C]		
1′	92.5	[CH]	4.87	d [*J* = 3.6]	97.2	[CH]	4.27	d [*J* = 7.9]
2′	72.4	[CH]	3.02−3.14	m	74.9	[CH]	2.89	t [*J* = 8.4]
3′	73.5	[CH]	3.35−3.43	m	76.5	[CH]	3.02−3.14	m
4′	70.7	[CH]	3.02−3.14	m	70.2	[CH]	3.02−3.14	m
5′	69.3	[CH]	3.73−3.77	m	73.0	[CH]	3.27−3.45	m
6′	65.0	[CH_2]	4.02−4.10	m	65.0	[CH_2]	4.02−4.10	m
			4.25−4.32	m			4.25−4.32	m

[a]Arbitrary numbering according to structures **1** and **2** in Figure 5. [b]Assignments confirmed by HSQC, HMBC, DEPT, and COSY experiments.

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separated into 16 subfractions, namely, III-C-1 to III-C-16, which were collected individually in several runs. After combining the corresponding fractions, the fractions were separated from solvent in vacuum, freeze-dried twice, and then dissolved in water containing 2% ethanol in their "natural" concentration ratios to be used for taste dilution analysis. The bitter tasting fractions III-C-5, III-C-6, and III-C-7 were further separated by means of semipreparative RP-HPLC using a 0.5 mL sample loop and a 250 × 10 mm i.d., 5 μm, Hyperclone ODS 120 column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type. Chromatography was performed using the following gradient at a flow rate of 3.5 mL/min: 0 min, 60% B; 20 min, 60% B; 25 min, 100% B; 30 min, 100% B; 40 min, 60% B; 65 min, 60% B. Prior to injection, the column was equilibrated for 10 min using the starting conditions. The peaks were collected individually in several runs, the corresponding fractions were combined, and the purity (≥98%) of each fraction was checked by means of analytical HPLC/ELSD as well as HPLC-MS. After separating the solvent in vacuum, the isolated materials were freeze-dried twice and used for sensory analysis as well as structure determination by means of LC-MS/MS, UPLC-TOF-MS, 1D/2D NMR, and hydrolysis experiments. The bitter compounds **4a/b**–**6a/b** (Figure 1) could be successfully identified in the following HPLC fractions: 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol (**4a**) and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol (**4b**) in fraction III-C-5, (25*R*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**5a**) and (25*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**5b**) in fraction III-C-6, and (25*R*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6a**) and (25*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6b**) in fraction III-C-7. The analytical details of the structure determination, including the comprehensive set of MS and 1D/2D-NMR data are published in a companion paper.[28]

Identification of Key Taste Compounds from Fraction III-D. Aliquots (50 mg) of SPE fraction III-D were separated by preparative HPLC using a 2.0 mL sample loop and a 250 × 21.2 mm i.d., 5 μm, Microsorb-MV 100-5 C18 column (Varian, Darmstadt, Germany) with gradient elution at a flow rate of 20 mL/min. Using 0.1% formic acid in water (v/v) as solvent A and 0.1% formic acid in acetonitrile (v/v) as solvent B, chromatography was performed as follows: 0 min, 20% B; 5 min, 50% B; 10 min, 50% B; 20 min, 65% B; 25 min, 100% B; 30 min, 100% B; 35 min 20% B; 40 min, 20% B. Prior to injection, the column was equilibrated for 10 min using the starting conditions. The effluent containing the target taste compound was collected from several separate HPLC runs, combined, freed from solvent in a vacuum, freeze-dried twice, and the residues obtained were used for sensory and instrumental analysis. HPLC-MS/MS and 1D/2D-NMR analysis led to the unequivocal identification of 3-*O*-[{β-D-glucopyranosyl-(1→2)}{β-D-xylopyranosyl-(1→4)} β-D-glucopyranosyl] (25*S*),5β-spirostan-3β-ol (**7**, Figure 1) as well as 3-*O*-[{α-L-rhamnopyranosyl-(1→2)}{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-(25-*S*)-spirost-5-ene-3β-ol (**8a**, Figure 1) and 3-*O*-[{α-L-rhamnopyranosyl-(1→2)}{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-(25*R*)-spirost-5-ene-3β-ol (**8b**, Figure 1) in fraction III-D-4. The details of the isolation and full spectroscopic structure determination demonstrating the chemical identity of these bitter saponins **4a/b**–**8a/b** are published separately in a companion paper.[28]

High-Performance Ion Chromatography. Anion exchange chromatography was performed using an ICS-2500 ion chromatography system (Dionex, Idstein, Germany) consisting of a GS 50 gradient pump, an AS 50 autosampler, an AS 50 thermal compartment, and an ED 50 electrochemical detector operating in pulsed amperometric detection mode. The detector was equipped with a gold working electrode operating with a standard carbohydrate quadrupole waveform supplied by manufacturer. Data acquisition and instrumental control was completed with the Chromeleon software (version 6.80, Dionex). Chromatographic separation was performed at 30 °C on a 150 × 3 mm CarboPac PA-20 column (Dionex) connected with a 30 × 3 mm CarboPac PA-20 guard column (Dionex) using an isocratic gradient of sodium hydroxide solution (2.5 mM) for 20 min. After each sample, the column was washed with a sodium hydroxide solution (200 mM) and equilibrated with sodium hydroxide solution (2.5 mM) for 10 min prior to injection. Chromatography was performed with an injection volume of 10 μL and a flow rate of 0.5 mL/min. For qualitative analysis, glycosidically bound carbohydrates were identified by comparison of retention times and cochromatography with reference monosaccharides.

Sensory Analyses. *Training of the Sensory Panel.* Seven female and five male panelists (25–40 years in age), who had given the informed consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, participated in this study. Each panelist took place in a weekly training session for at least two years in order to get familiarized with the taste language and methodologies used. Aqueous solutions (2.0 mL; pH 5.9) of the following reference compounds were used for sensory training: sucrose (50 mmol/L) for sweet taste, L-lactic acid (20 mmol/L) for sour taste, NaCl (20 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, monosodium L-glutamate (3 mmol/L) for umami taste. For astringency the panel was trained by using tannic acid (0.05%) and quercetin-3-*O*-β-D-glucopyranoside (0.01 mmol/L), respectively, using the so-called half-tongue test.[24,25] Sensory analyses were performed by means of the sip-and-spit method in an air-conditioned room at 22–25 °C in three independent sessions. To prevent cross-model interactions with odorants reported in asparagus,[16,17] nose clips were used by the assessors.

Precaution Taken for Sensory Analysis of Food Fractions and Taste Compounds. To remove solvent traces and buffer compounds from all fractions and compounds isolated from asparagus, the individual fractions were suspended in water, and, after the volatiles were removed in high vacuum (<5 mPa), were freeze-dried twice. ^{1}H NMR chromatographic, GC/MS, and high-performance ion chromatographic analysis of an aliquot revealed that fractions treated by that procedure are essentially free of solvents and buffer compounds used.

Taste Profile Analysis. Aliquots of the puree prepared from cooked or fresh white asparagus spears, respectively, were pressed through a tea towel and, then, filtered (Schleicher & Schuell filter, 24 cm) to obtain a juice which was judged by the trained sensory panelists in the intensity of sweet, sour, umami, salty, bitter, astringent, and buttery mouth-coating on a linear scale from 0 (not detectable) up to 5 (very intense).

Comparative Taste Profile Analysis. Aliquots of the lyophilized fractions isolated from raw and cooked asparagus, respectively, were dissolved in their "natural" concentrations (corresponding to 25 g of fresh asparagus) in bottled water (Evian, low mineralization: 405 mg/L) containing 2% ethanol and were then adjusted to pH 5.9 with trace amounts of aqueous formic acid. These solutions were then presented to the sensory panel, which was asked to rate the intensity of the individual taste qualities on a linear scale from 0 to 5 in comparison to the raw or cooked asparagus juice, respectively, as the control.

Taste Dilution Analysis (TDA). Aliquots of the HPLC fractions were dissolved in "natural" ratios in 5.0 mL of bottled water (pH 5.9, 1% ethanol) and, then, sequentially diluted 1:1 with bottled water (pH 5.9, 1% ethanol). The serial dilutions of each of these fractions were then presented in order of increasing concentration to the trained sensory panel, which was asked to evaluate each dilution step by means of the recently developed half-tongue test.[24,25] As previously reported, the dilution at which a taste difference between the diluted extract and the blank (control) could just be detected was defined as taste dilution (TD) factor.[18] The TD factors for each HPLC-fraction evaluated by four different assessors in three different sessions were averaged. The TD factors between individuals and separate sessions did not differ by more than plus/minus one dilution step.

Half-Tongue Test. Taste dilution factors and oral recognition thresholds for astringent and buttery mouth-coating fractions and compounds, respectively, were determined by means of the recently developed half-tongue test[24,25] in order to overcome carry-over effects.

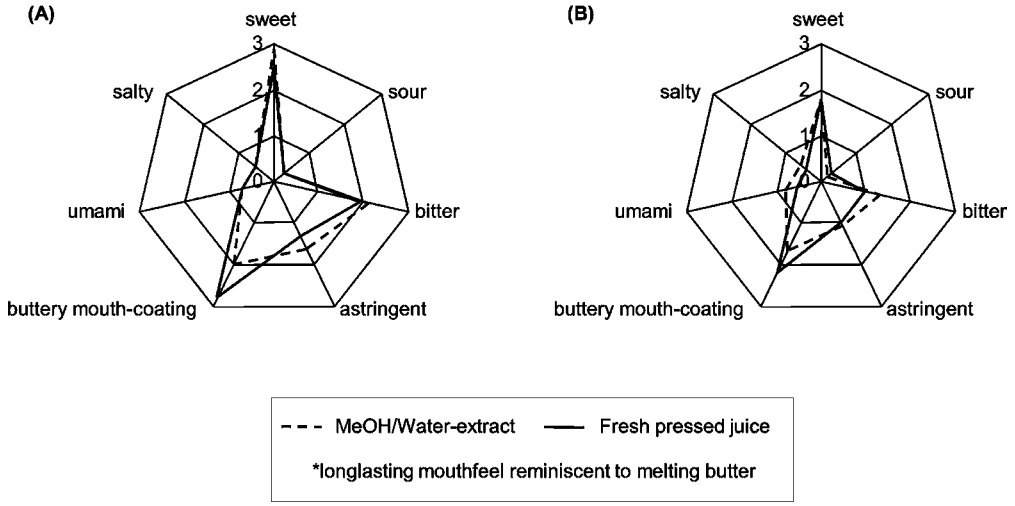

Figure 2. Taste profile analysis of fresh pressed juice (——) and methanol extractables (- - -) of (A) raw and (B) cooked white asparagus (14 min at 95 °C).

Therefore, serial 1:1 dilutions of the samples using bottled water adjusted to pH 5.9 with trace amounts of aqueous formic acid (1% in water) as the solvent were presented in order of increasing concentrations to a trained panel of 12 subjects in three different sessions using the sip-and-spit method and an interstimulus interval length of 2 min. In case of a correct selection by the panelist, the same concentration was presented again besides one blank as a proof for the correctness of the data. The geometric mean of the last and the second last concentration was calculated and taken as the individual recognition threshold. The values between individuals and between three separate sessions differed by not more than plus or minus one dilution step, which means a threshold value of 276.8 μmol/L for the 1,2-dithiolan-4-carboxylic acid 6-D-α/β-glucopyranoseesters represents a range from 138.4 to 553.6 μmol/L.

High Performance Liquid Chromatography (HPLC). Preparative analyses of fraction III-B was performed on a HPLC apparatus consisting of two S 1122 pumps (Sykam, Eresing, Germany), a Sedex LT-ELSD detector Model 85 (Sedere, Alfortville, France), and a Rh 7725i type Rheodyne injection valve (Rheodyne, Bensheim, Germany). Chromatography was done using a preparative 250 × 21.2 mm i.d., 5 μm, Microsorb column (Varian, Darmstadt, Germany) operated with a flow rate of 18.0 mL/min, respectively. The split ratio was set to 1.0 mL/min for the ELSD detector. Data acquisition was done by means of Chromstar V. 6.2 (SCPA, Weyhe, Germany).

Semipreparative analyses of fraction III-B was performed on a HPLC apparatus (Shimadzu, Duisburg, Germany) consisting of a LC-20AT pump, a SIL-20A autosampler, a DGU-20A3 degaser, and a Sedex LT-ELSD detector Model 75 (Sedere, Alfortville, France). Chromatography was done using a semipreparative 250 × 10 mm i.d., 5 μm, HyperClone ODS 120 column (Phenomenex, Aschaffenburg, Germany) operated with a flow rate of 3.5 mL/min, respectively. The split ratio was set to 0.8 mL/min for the ELSD detector. Data acquisition was done by means of LabSolutions LCsolutions V. 1.21 (Shimadzu, Duisburg, Germany).

Analytical and (semi)preparative analyses of fractions III-C and III-D were performed on a HPLC apparatus (Jasco, Groß-Umstadt, Germany) consisting of two PU-2087 Plus pumps, an AS-2055 Plus autosampler, a Sedex LT-ELSD detector Model 85 (Sedere, Alfortville, France), and a Rh 7725i type Rheodyne injection valve (Rheodyne, Bensheim, Germany). Chromatography was done using an analytical 250 × 4.6 mm i.d., a semipreparative 250 × 10 mm i.d., and a preparative 250 × 21.2 mm i.d., 5 μm, HyperClone ODS 120 column (Phenomenex, Aschaffenburg, Germany) operated with a flow rate of 1.0 mL/min, 3.5 mL/min, and 20.0 mL/min, respectively. The split ratio was set to 1.0 mL/min for the ELSD detector. Data acquisition was done by means of Chrompass 1.8.6.1 (Jasco, Groß-Umstadt, Germany).

LC/Time-of-Flight Mass Spectrometry (LC/TOF-MS). High-resolution mass spectra were measured on a Bruker Micro-TOF-Q mass spectrometer (Bruker Daltronics, Bremen, Germany) with flow injection referenced on sodium formate (5 mmol). Data acquisition was performed by using Daltonics DataAnalysis software (version 3.4; Bruker).

High-Performance Liquid Chromatography/Tandem Mass Spectrometry (HPLC-MS/MS). For compound identification, mass and product ion spectra were acquired on an API 4000 Q Trap triple quadrupole/linear ion trap mass spectrometer (ABSciex, Darmstadt, Germany). The isolated fractions were dissolved in a mixture of methanol/water (50/50, v/v) and directly introduced into the mass spectrometer by flow infusion using a syringe pump. For electrospray ionization, the ion spray voltage was set at −4500 V in the negative mode and at 5500 V in the positive mode. Both quadrupoles operated at unit mass resolution and nitrogen served as curtain gas (25 psi) and as turbo gas (425 °C). Fragmentation of the pseudo molecular ions $[M + H]^+$ or $[M - H]^-$ into specific product ions was induced by collision with nitrogen (4.5×10^{-5} Torr). Data acquisition and instrumental control was performed with Analyst 1.4.2 software (ABSciex, Darmstadt, Germany).

HPLC-MS/MS analysis of the bitter compounds **4a/b**−**8a/b** in asparagus fractions was performed on a 150 × 2.1 mm i.d., 5 μm, Zorbax Eclipse XDB-C 18 column (Agilent, Waldbronn, Germany) connected to the mass spectrometer operating in the multiple reaction monitoring (MRM) mode with negative electrospray ionization (spray voltage: −4500 V). The quadrupoles operated at unit mass resolution and nitrogen was served as curtain gas (20 psi), nebulizer gas (50 psi), and as turbo gas (425 °C). Fragmentation of the pseudo molecular ions $[M - H]^-$ into specific product ions was induced by collision with nitrogen (4×10^{-5} Torr).

The following mass transitions were recorded for the taste compounds using the declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) given in parentheses: **4a/b**, m/z 1047.5 → 901.6 (DP/CE/CXP: −180/−60/−25) and m/z 1047.7 → 755.4 (DP/CE/CXP: −180/−70/−27); **5a/b**, m/z 903.6 → 757.5 (DP/CE/CXP: −195/−58/−21) and m/z 903.6 → 59.0 (DP/CE/CXP: −195/−128/−7); **6a/b**, m/z 901.6 → 755.5 (DP/CE/CXP: −190/−52/−21) and m/z 901.6 → 101.0 (DP/CE/CXP: −185/−82/−15); **7**, m/z 871.5 → 739.6 (DP/CE/CXP: −195/−52/−11); **8a/b**, m/z 867.5 → 721.4 (DP/CE/CXP: −200/−46/−43) and m/z 867.5 → 59.0 (DP/CE/CXP: −160/−110/−1). Using acetonitrile containing 0.1% formic acid as solvent A and 0.1% formic acid in water as solvent B, chromatography was performed using the following gradient at a flow rate of 0.2 mL/min: 0 min, 5% A; 5 min, 5% A; 10 min, 25% A; 25 min, 30% A; 30 min, 0% A; 38 min, 0% A; 42 min, 5% A; 50 min, 5% A.

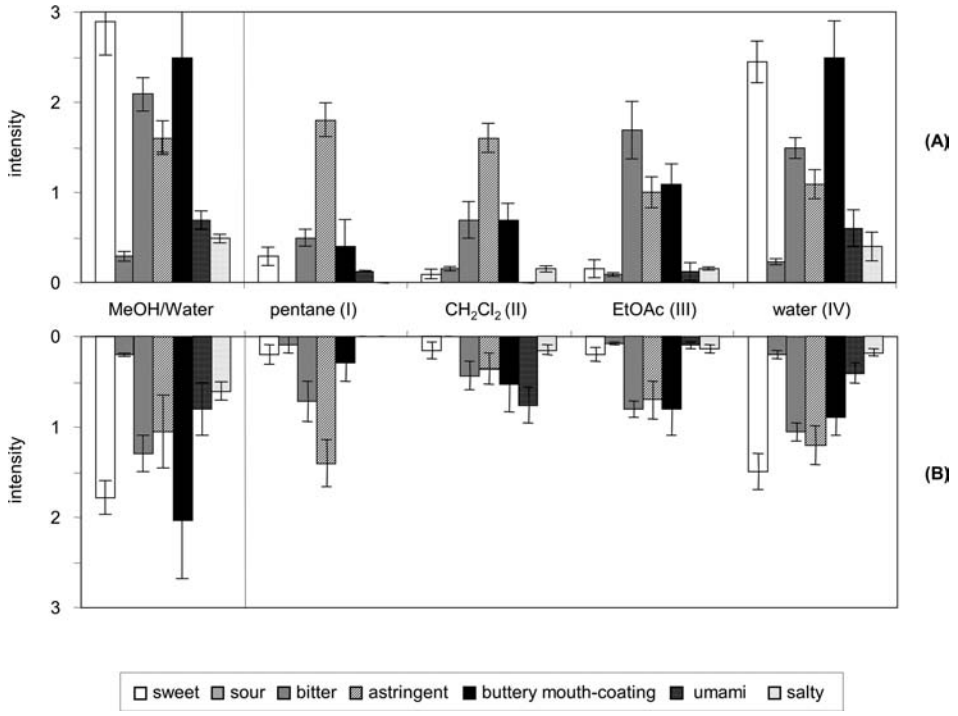

Figure 3. Taste profile of fractions I–IV isolated from (A) raw and (B) cooked white asparagus (14 min at 95 °C). Sensory data are given as the means of triplicates; error bars indicate the 95% confidence interval of the arithmetical mean.

Nuclear Magnetic Resonance Spectroscopy (NMR). One- and two-dimensional ^{1}H and ^{13}C NMR spectra were acquired on a 500 MHz Bruker Avance III and a 400 MHz DRX spectrometer (Bruker, Rheinstetten, Germany). Samples were analyzed in a mixture (95/5, v/v) of DMSO-d_6 and D_2O. Chemical shifts are reported in parts per million relative to the solvent signal (DMSO-d_6: ^{1}H: 2.49 ppm; ^{13}C: 39.7 ppm). For structure elucidation and NMR signal assignment 2-D NMR experiments, like COSY-, *J*-RESOLVE-, HMQC/HSQC-, DEPT-, and HMBC-spectroscopy were carried out using the pulse sequences taken from the Bruker software library. Data processing was performed by using XWin-NMR software (version 3.5; Bruker, Rheinstetten, Germany) and (Mestrelab Research, La Coruña, Spain), respectively.

RESULTS AND DISCUSSION

Preliminary sensory analysis of the fresh juice prepared from white asparagus by mincing and pressing revealed a taste profile centering around sweet, bitter, and astringent taste qualities and was complemented by another orosensation described by the trained panel as a typical white asparagus-like, buttery mouth-coating effect, reminiscent to melting butter on the tongue.

Taste Profile Analysis of Raw and Cooked Asparagus. In order to gain a first insight into the influence of cooking on the taste profile of white asparagus, juices prepared from raw and cooked peeled spears, respectively, were presented to the trained sensory panel who were asked to rate the intensity of the taste modalities astringent, bitter, sour, salty, sweet, umami, and buttery mouth-coating on a linear 5-point intensity scale. The asparagus-like, buttery mouth-coating sensation was evaluated with highest scores of 2.8 and 2.2 in the raw and cooked sample, respectively, and was judged as a lingering and long-lasting orosensation (Figure 2), followed by sweetness (2.5/1.8) and bitterness (1.9/1.0) with a somewhat lower taste impact. In comparison, umami taste (0.7/0.5), saltiness (0.5/0.4), and sourness (both 0.3) were rated with rather low intensities. It is interesting to notice that the individual descriptors were evaluated with lower scores in the cooked asparagus as in the fresh sample.

In order to isolate the nonvolatile taste compounds, raw and cooked asparagus spears were comprehensively extracted with methanol and methanol/water. After separating the solvent in vacuum and freeze-drying, the extracts prepared from both asparagus samples were solubilized in bottled water in concentrations of 3.95 (raw) and 1.86 g/100 mL (cooked) to match the concentration in asparagus. After pH adjustment (pH 5.9) with trace amounts of formic acid, the solutions were presented to the sensory panelists, who were asked to evaluate the intensities of given taste descriptors on a linear 5-point scale (Figure 2). The methanol extractables from raw (+0.2) and cooked asparagus (+0.3) showed somewhat higher bitter intensities, but a decrease in the buttery mouth-coating sensation (−0.8/−0.5). The lower intensities of this buttery mouth-coating sensation might be explained by the instability of the respective chemosensates during sample workup or by mixture suppression due to the increased intensity of the perceived bitterness.[29−34] The following experiments were focused on the identification of the molecules imparting the bitterness and the typical buttery asparagus-like orosensation.

Solvent Fractionation of Raw and Cooked Asparagus. To gain first insight into the hydrophobicity of the taste-active molecules, the freeze-dried methanol extractables isolated from fresh and cooked asparagus, respectively, were taken up in water and separated by sequential solvent extraction affording the pentane extractables (fraction I), the dichloromethane extractables (fraction II), the ethyl acetate extractables (fraction III), and the remaining water-solubles (fraction IV), respectively. The highest yields were found for fraction IV accounting for 86 and 66% of the fresh weight of raw and cooked asparagus (Table 1). Fractions I, II, and III were obtained in comparatively low yields of less than 2%.

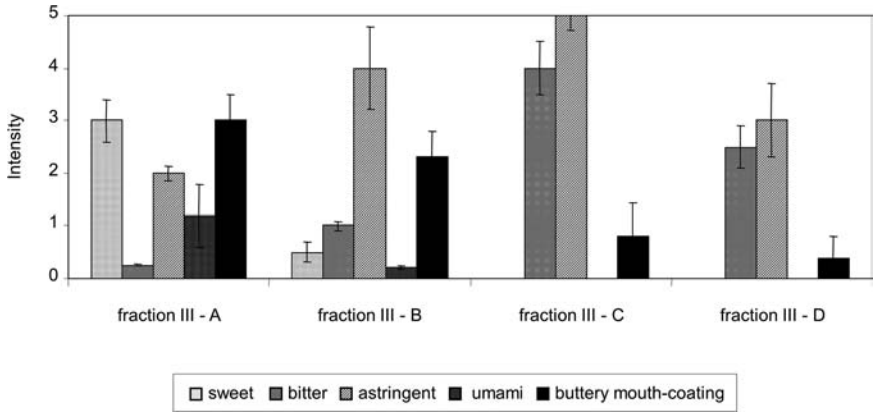

Figure 4. Taste profile of fractions III-A to III-D isolated from raw white asparagus. Sensory data are given as the means of triplicates; error bars indicate the 95% confidence interval of the arithmetical mean.

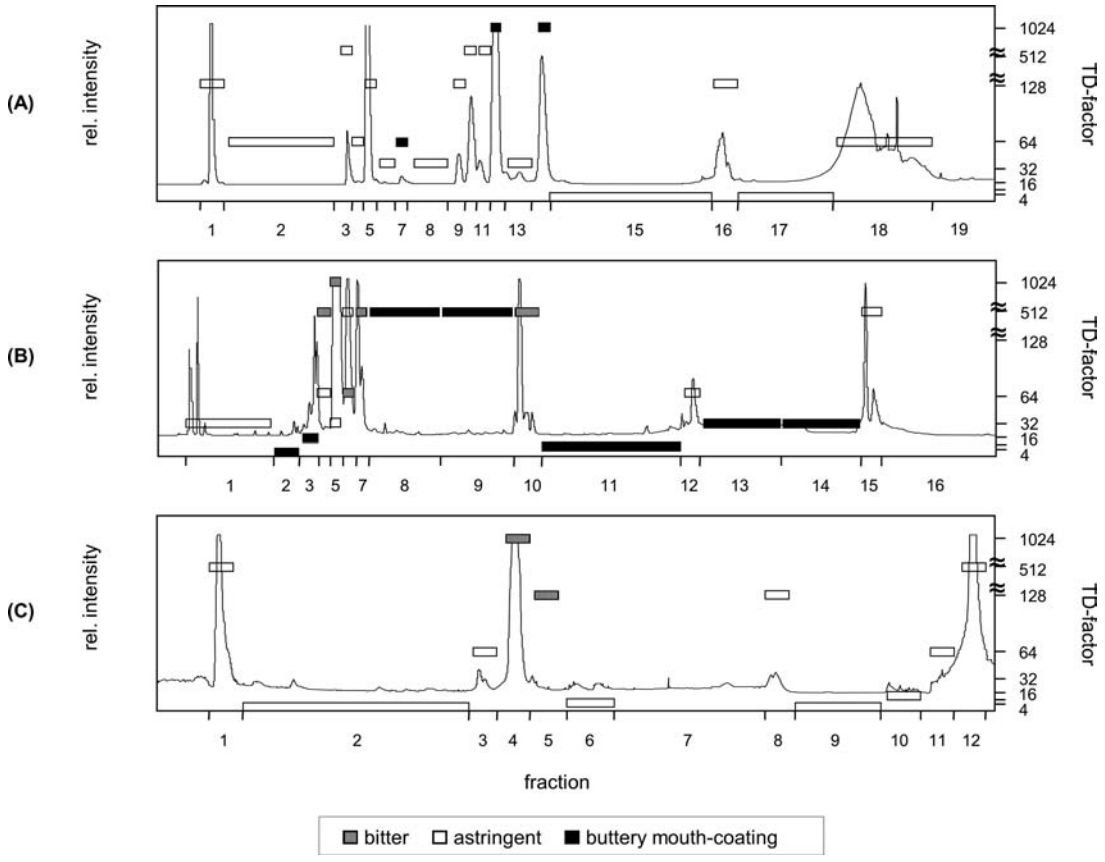

Figure 5. RP-HPLC chromatograms and taste dilution (TD)-factors of SPE-fractions III-B (A), III-C (B), and III-D (C) prepared from white asparagus.

To evaluate their taste impact, fractions I–IV isolated from fresh and cooked asparagus, respectively, were taken up in water (pH 5.9) containing 2% aqueous ethanol and were then comparatively analyzed by means of taste profile analysis. Sensory evaluation of the individual fractions revealed higher intensities of the individual taste descriptors in raw than in cooked asparagus (Figure 3). Independent on the thermal treatment of asparagus, the typical buttery, asparagus-like orosensation as well as the bitterness could be located primarily in fractions III and IV, e.g., fractions III and IV exhibited bitterness with an intensity of 1.7/0.8 and 1.5/1.1 for raw/cooked asparagus while fractions I and II exhibited just a very low bitterness (<0.7). Therefore the following experiments were focused on the identification of the bitter and buttery mouth-coating compounds in the taste-active fractions III and IV isolated from raw asparagus.

Sensory-Directed Separation of Raw Asparagus Fraction III. To sort out the bitter and mouth-coating compounds from the bulk of less taste-active or tasteless substances present in fraction III, the ethyl acetate extractables isolated from raw asparagus were separated by means of solid phase extraction on RP-18 material using methanol/water mixtures to give fraction III-A (0/100, v/v), fraction III-B (30/70, v/v), fraction III-C (70/30, v/v) and fraction III-D (100/0, v/v), respectively.

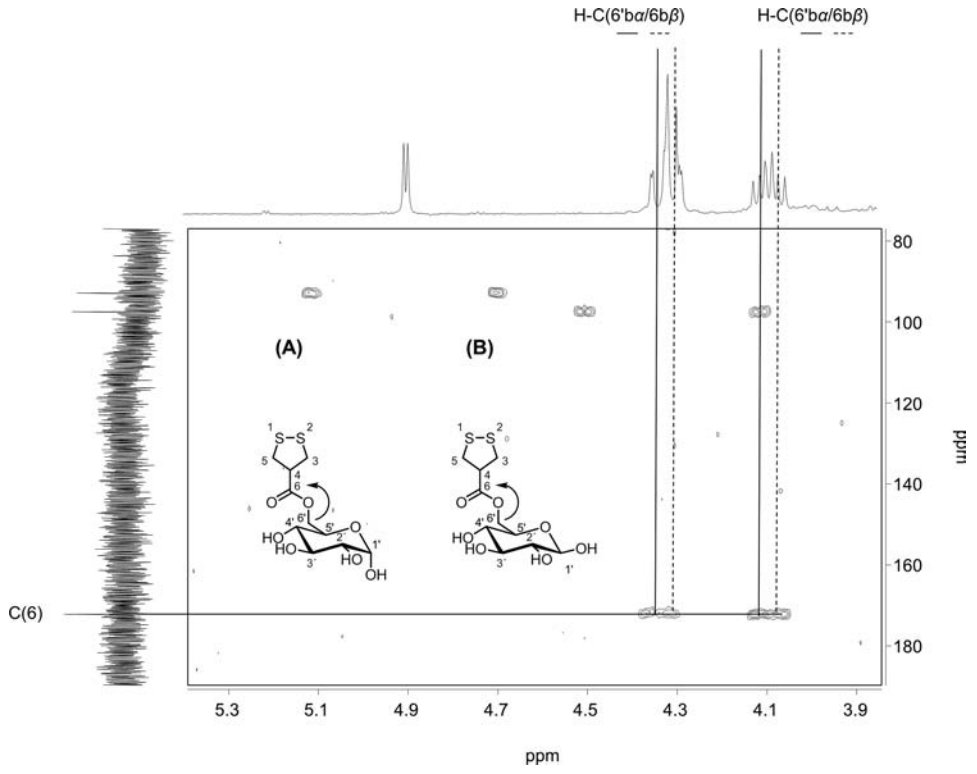

Figure 6. Excerpt of HMBC spectrum (400 MHz, DMSO) of 1,2-dithiolan-4-carboxylic acid 6-D-α-glucopyranose ester (**1**, A) and 1,2-dithiolan-4-carboxylic acid 6-D-β-glucopyranose ester (**2**, B).

After separating the solvent in a vacuum, the individual fractions were taken up in water in their natural concentration ratios and were presented to the trained sensory panel which was asked to rate the intensity of different taste modalities astringent, bitter, sour, salty, sweet, umami and buttery mouth-coating on a linear 5-point intensity scale (Figure 4). Fractions III-C and III-D showed the highest bitter intensities of 4.0 and 2.5, respectively, whereas fractions III-A and III-B exhibited just a rather faint bitterness. In comparison, the buttery mouth-coating orosensation was detectable in all the fractions III-A to III-D, among which the early eluting fractions III-A and III-B were judged with the highest scores between 3.0 and 2.2 (Figure 4). As preliminary HPLC analysis revealed fractions III-A and III-B to be substantially equivalent with higher levels of polar carbohydrates in III-A, the following investigations were focused on the less complex fraction III-B.

Taste Dilution Analysis of Fraction III-B. To trace the compounds imparting the buttery mouth-coating effect, a taste dilution analysis was applied on fraction III-B. To achieve this, fraction III-B was separated by means of RP-HPLC coupled to an evaporative light scattering detector (ELSD) to give a total of 19 HPLC fractions, which were collected individually and freeze-dried (Figure 5A). The lyophilizates of these fractions were taken up with the same amount of bottled water (pH 5.9), stepwise diluted 1:1 with water (pH 5.9), and then presented in order of ascending concentrations to the trained sensory panelists who were asked to determine their taste dilution (TD)-factors by means of an half-tongue procedure. Fractions III-B-12 and III-B-14 were identified with the highest TD-factor of 1024 and were, therefore, considered as key contributors to the buttery mouth-coating oral sensation of white asparagus.

After purifying the taste-active fractions III-B-12 and III-B-14 by means of semipreparative RP-HPLC, the chemical structures of the respective chemosensates **1** and **2** were determined by means of LC-MS/MS and 1D/2D-NMR experiments. It is interesting to notice that isolation of the target compound **1** or **2** always led to a mixture of compounds **1** and **2** eluting in fraction III-B-12 and III-B-14, thus indicating the existence of two isomers. LC-TOF-MS analysis confirmed the target compounds **1** and **2** to have the same molecular formula of $C_{10}H_{16}O_7S_2$. The ^{1}H NMR spectrum of this isomeric mixture displayed two anomeric protons of a glycoside resonating at 4.27 and 4.87 ppm. On the basis of the coupling constants between each anomeric and vicinal protons, one of the isomers was identified as the α-anomer (4.87 ppm; 3.8 Hz), while the other sugar indicated a β-configuration (4.27 ppm) with a larger coupling constant of 7.9 Hz. The assignment of the sugar moiety as well as the linkage of the sugar to the aglycone was performed by COSY- and HMBC-experiments. For example, the HMBC spectrum allowed the determination of the linkage of the low-field shift protons H−C(6′) resonating at 4.02−4.10 ppm and 4.25−3.32 ppm to the carboxy carbon C(6) of asparagusic acid by a long-range coupling (Figure 6). The unequivocal identification of glucose as the glycosidically bound carbohydrate was further confirmed by high-performance ion chromatography after alkaline hydrolysis. Ethyl acetate extraction of the alkaline hydrolysate of **1** and **2**, followed by solvent removal in vacuum revealed asparagusic acid (**3**; Figure 1) as the aglycone in glucosides **1** and **2** by means of LC-MS and NMR analysis. LC-TOF-MS exhibiting m/z 148.974427 $[C_4H_5O_2{-}H^+]^-$ as the pseudomolecular ion ($[M + H]^-$) and ^{1}H/^{13}C NMR spectra showing a total of five protons and four carbon atoms was well in line with the elemental formula of $C_4H_6O_2S_2$ for the aglycone. 1D/2D NMR data unequivocally validated the structure of the aglycone as asparagusic acid (**3**), which has been previously reported as a phytochemical in

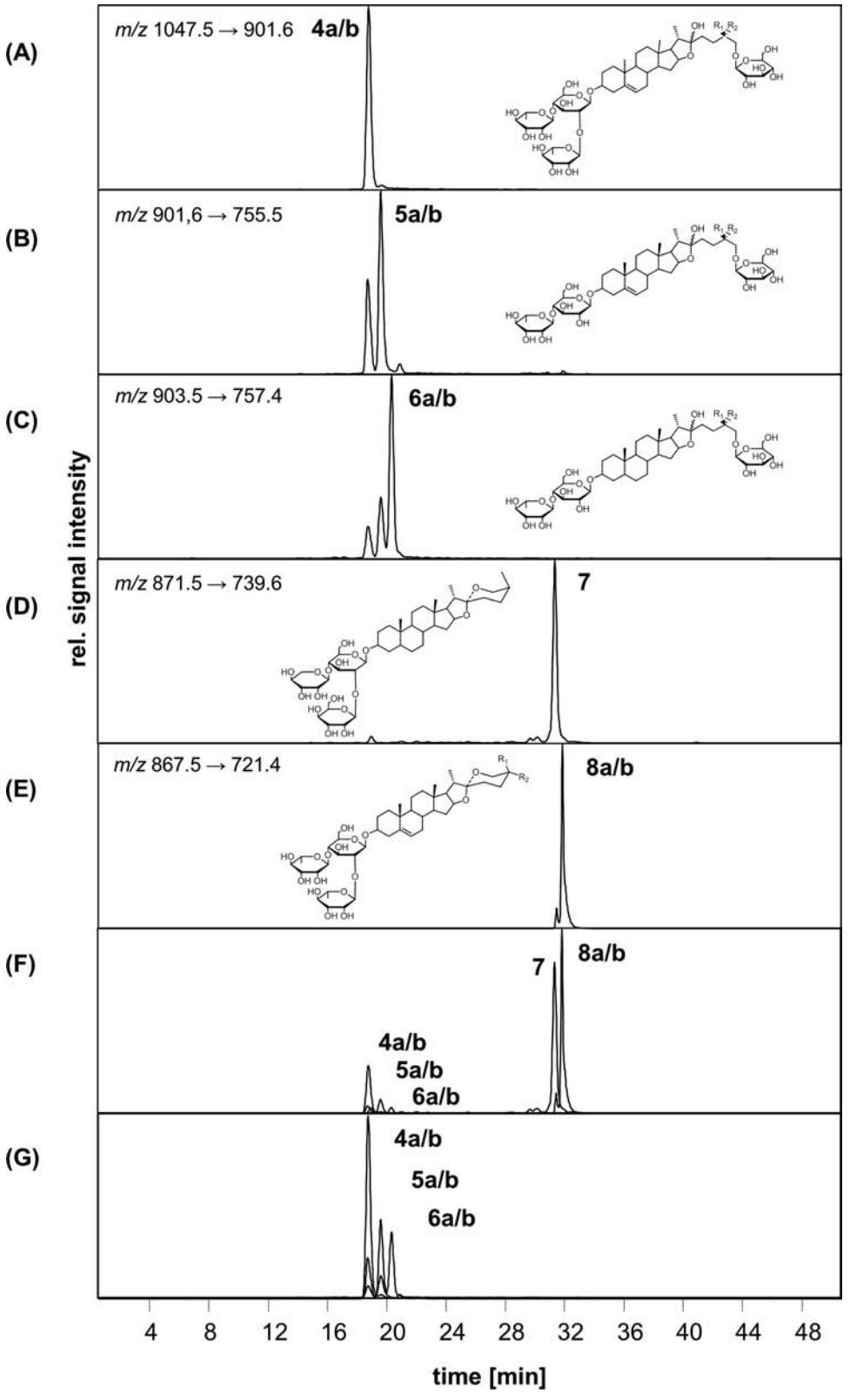

Figure 7. RP-HPLC-MS/MS (ESI$^-$) analysis of purified saponin references **4a/b** (A), **5a/b** (B), **6a/b** (C) and **7** (D), **8a/b** (E), and fractions III (F) and IV (G) isolated from asparagus.

asparagus[16,17,35−37] (Figure 1). Taking all these data into consideration, the taste active compounds **1** and **2** were unequivocally identified as 1,2-dithiolan-4-carboxylic acid 6-D-α-glucopyranoseester and 1,2-dithiolan-4-carboxylic acid 6-D-β-glucopyranoseester (Figure 1), which to the best of our knowledge have not been previously reported.

Taste Dilution Analysis of Fractions III-C and III-D. To locate the major bitter compounds in raw asparagus fraction III, the SPE fraction III-C and III-D were further separated by

means of RP-HPLC/ELSD to give a total of 16 and 12 subfractions from III-C and III-D, respectively, which were used for taste dilution analysis (Figure 5B/C). The highest TD-factor of 1024 was found for the bitter fractions III-C-5 and III-D-4, followed by fractions III-C-4, III-C-7, and III-C-10 exhibiting bitterness up to a dilution of 1:512. Next to their bitter taste, fractions III-C-4 to III-C-6 exhibited oral astringency with TD-factors between 32 and 512. Aimed at discovering the molecules imparting the typical bitter taste of white asparagus, the following identification experiments were focused on the taste compounds located in fractions III-C-5 to III-C-7 and III-D-4.

HPLC-MS/MS and 1D/2D-NMR analysis of the bitter compounds in fraction III-C-5 led to the identification of an epimeric mixture as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*R*)-22-hydroxyfurost-5-ene-3β,26-diol (**4a**, Figure 1), earlier reported as protodioscin in asparagus seed,[3] and 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-(25*S*)-22-hydroxyfurost-5-ene-3β,26-diol (**4b**, Figure 1), which has been coined protoneodioscin, AS-P2-I, or ASP-I, respectively, and has been reported in the bottom cuts as well as in the edible shoots of *Asparagus officinalis* L.[1,3,38] Both saponins, protodioscin (**4a**) and protoneodiscin (**4b**), were earlier isolated also from the fresh stems of *Dracaena cochinchinensis*.[39]

LC-MS and NMR analysis of the key bitter compounds in fraction III-C-6, evaluated with a TD factor of 64, led to the identification of the epimeric mixture of the bidesmosidic saponin (25*R*/*S*)-furost-5-en-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**5a**/**b**) (Figure 1). Although the (25*R*)-epimer **5a** was reported earlier to be released from protodioscin upon treatment with an enzyme from *Curvularia lunata*, to the best of our knowledge has this saponin not yet been reported as a bitter compound in asparagus. In contrast, its (25*S*)-epimer (**5b**) was previously postulated in asparagus.[1,40] Moreover, the bitter compounds **6a** and **6b** isolated from HPLC fraction III-C-7 could be identified as (25*R*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6a**) and (25*S*)-furostane-3β,22,26-triol-3-*O*-[α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside]-26-*O*-β-D-glucopyranoside (**6b**, Figure 1).

In addition to the saponins **4a**/**b**−**6a**/**b**, the bitter taste of the comparatively hydrophobic fraction III-D-4 was found to be due to 3-*O*-[β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl]-(25*S*)-5β-spirostan-3β-ole (AS-1) (**7**, Figure 1) and the epimeric mixture of 3-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl]-(25*R*/*S*)-spirost-5-en-3β-ol (**8a**/**b**, Figure 1). While compound **7** was previously isolated from asparagus bottom cuts,[38,41] to the best of our knowledge this saponin has not been reported as a bitter compound in asparagus spears. Although **8a** was recently isolated from asparagus bottom cuts,[12] the epimeric dioscin (**8b**) has just been reported in other plant families such as, e.g., *Dioscorea*.[38] The details of the isolation and full spectroscopic structure determination demonstrating the chemical identity of these bitter saponins **4a**/**b**−**8a**/**b** are published separately in a companion paper.[28]

LC-MS/MS Screening of Bitter Saponins 4a/b−8a/b in Asparagus Fractions. In order to answer the question as to whether the saponins **4a**/**b**−**8a**/**b** identified in fraction III are also present in the bitter tasting aqueous fraction IV (Figure 3), fractions prepared from raw and cooked asparagus, respectively, were screened for the bitter compounds **4a**/**b**−**8a**/**b** by means of RP-HPLC-MS/MS (ESI^-) operating in the multiple reaction monitoring (MRM) mode. As postfragmentation in the ion source of compound **4a**/**b** was found to deliver the same daughter ions as analytes **5a**/**b** and **6a**/**b** (Figure 7), HPLC conditions were optimized to achieve full baseline separation of the saponins **4a**/**b**−**8a**/**b**. This helped also to overcome problems regarding the spectral-overlap of the mass transitions 901.6 → 755.5 and 903.5 → 757.4 of the precursor ions of **5a**/**b** and **6a**/**b**, respectively, showing only a 2 Da difference in mass/charge ratio. Comparison of retention times and characteristic mass transitions, followed by cochromatography with the purified compounds isolated from fraction III led to the unequivocal identification of the polar saponins **4a**/**b**−**6a**/**b** in the bitter fraction IV of asparagus, while the less polar, monodesmotic saponins **7** and **8a**/**b** could not be detected (Figure 7). To the best of our knowledge, the saponins **4a**/**b**−**8a**/**b** have not been previously reported as key bitter taste compounds in white asparagus spears.

Oral Threshold Concentrations of Asparagusic Acid Derivatives. Prior to sensory analysis, the purity of the test compounds was checked by HPLC-ELSD as well as HPLC-MS to be more than 98%. To determine the recognition threshold concentration for the buttery mouth-coating and astringent oral sensation of the glucosides **1**/**2** and the aglycone asparagusic acid (**3**), aqueous solutions (pH 5.9) of the target compounds were evaluated by means of the half-tongue test.[24,25] The mixture of 1,2-dithiolan-4-carboxylic acid 6-D-glucopyranose esters **1** and **2** in bottled water was found to exhibit a low recognition threshold of 276.8 μmol/L (Table 3) and imparted a lingering orosensation with described as a buttery, typical asparagus-like mouth-coating effect, reminiscent of the perception of melting butter on the tongue. Interestingly, a solution of asparagusic acid (**3**) in bottled water (pH 5.9) was described as astringent only with a recognition threshold concentration of 132.9 μmol/L, implying the prime importance of the glucosylation of aspargusic acid for the buttery mouth-coating activity of **1** and **2**.

Table 3. Human Recognition Taste Thresholds of Compounds 1−3

	threshold concentrations [μmol/L] of compound[a]	
	1/2	3
buttery mouth-coating	276.8[b]	
astringent		132.9[b]

[a]Structures of compounds are given in Figure 1. [b]Taste threshold concentrations were determined in aqueous solution.

In conclusion, sensory-guided fractionation of asparagus extracts led to the identification of two previously not reported 1,2-dithiolan-4-carboxylic acid 6-D-glucopyranose esters **1** and **2** as chemosensates imparting a buttery mouth-coating orosensation. Furthermore a series of saponins (**4a**/**b**−**8a**/**b**) were found to impart the bitter taste of white asparagus. Aimed at demonstrating their contribution to the taste of raw and cooked asparagus, quantitative studies are currently in progress and will be published elsewhere.

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Notes

The authors declare no competing financial interest.

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