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Identification of Novel Orosensory Active Molecules in Cured Vanilla Beans (*Vanilla planifolia*)

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Sequential application of solvent extraction, gel permeation chromatography, and HPLC in combination with taste dilution analyses, followed by LC-MS and 1D/2D NMR experiments, led to the discovery of seven velvety mouth-coating molecules in cured beans of *Vanilla planifolia*. Among these, 5-(4-hydroxybenzyl)vanillin, 4-(4-hydroxybenzyl)-2-methoxyphenol, 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde, (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside, americanin A, and 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde were previously not reported in vanilla beans. Sensory studies revealed human recognition thresholds for the velvety mouth-coating sensation between 1.0 and 5.0 μmol/kg (water). Interestingly, the biphenyl derivatives were found to enhance the perception of creaminess and fatty body of sweetened skim milk, among which 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde showed the lowest threshold level of 5 μmol/kg. Quantitative analysis of these compounds in cured vanilla beans from different origins as well as in noncured beans revealed that, with the exception of americanin A, all of the other taste compounds are not present in the green vanilla beans and are formed during the bean curing process.

KEYWORDS: Vanilla; mouth-coating; taste; taste dilution analysis

INTRODUCTION

Over hundreds of years cured vanilla beans have been used to give their alluring flavor to food products including sweets as well as confectionary, dairy, bakery, and beverages. Among various *Vanilla* species, only *Vanilla planifolia* Andrews (*Vanilla fragrans*), *Vanilla tahitensis* Moore, and *Vanilla pompona* Schiede are cultivated for their economic use (*1*).

It is well-known that the characteristic aroma as well as the typical brown color of vanilla beans develops during the curing process, and multiple enzymatic as well as chemical processes are believed to release various aroma-active volatiles from odor-inactive precursor molecules in the green bean. Among the more than 180 volatile constituents identified so far, the well-known vanillin is accepted as one of the most important odorants of cured vanilla (*2*−*6*).

Besides the aroma-active volatiles, complex tannins and polyphenol resins, as well as free amino acids, have been reported as nonvolatile constituents in cured vanilla, and some structurally nondefined minor constituents are believed to play a significant role in the characteristic mouth-coating sensation induced by authentic vanilla. Among such minor compounds, divanillin was recently identified as a fermentation product in cured vanilla beans and was found to improve the creamy, fatty mouthfeel properties and the body of dairy products when added at low concentration levels of 5−50 ppm (*7*). It is, however, unclear which additional compounds besides the divanillin are responsible for the perceived velvety mouth-coating sensation of products containing extracts from authentic, cured vanilla beans.

To answer the question as to which nonvolatile, key taste compounds are responsible for the attractive taste of food products, the so-called taste dilution analysis was developed as a screening tool to locate taste-active nonvolatiles in complex foods (*8*). This approach, combining instrumental analysis and human bioresponse, recently led to the discovery of various previously unknown taste compounds and taste modulators such as cooling compounds in dark malt (*9*), bitter compounds in carrots (*10*), red wine (*11*), Gouda cheese (*12*), and roasted coffee (*13*, *14*), key astringent molecules in black tea (*15*), roasted cocoa (*16*), whiskey (*17*), and spinach (*18*), as well as taste modulators in beef bouillon (*19*), alkalized cocoa nibs (*20*), and common beans (*21*), respectively.

The objective of the present study was, therefore, to systematically locate the most intense velvety mouth-coating components in cured vanilla beans by application of taste dilution techniques, to isolate the active compounds, to determine their chemical structure, and to evaluate their orosensory impact on the basis of their human recognition threshold concentrations.

MATERIALS AND METHODS

Materials. The following compounds were obtained commercially: pentane, formic acid (Fluka, Neu-Ulm, Germany); ethyl acetate, hydrochloric acid, sodium hydroxide solution, vanillin, divanillin (Sigma, Steinheim, Germany); solvents of HPLC grade (Merck, Germany). Deuterated solvents were

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obtained from Euriso-Top (Gif-Sur-Yvette, France). Bottled water, adjusted to pH 4.5 with aqueous formic acid (0.1 mol/L), was used for the sensory experiments. Traditionally cured *V. planifolia* beans originating from Madagascar (CB-M) and Papua New Guinea (CB-PNG), respectively, were obtained from the food industry and kept frozen at −20 °C until used. A commercially available *V. planifolia* extract (CE-M) from traditionally cured Madagascan beans was obtained from the food industry and kept frozen at −20 °C until used. For the manufacturing of this extract, cured vanilla beans (2005 crop) were chopped and then extracted twice with 50% aqueous ethanol at 60 °C. The combined extracts were concentrated under vacuum below 60 °C to afford the final product in a yield of 19.8% with a solids content of 39.5% and an ethanol content of 0.3%. Green vanilla beans from Papua New Guinea (NCB-PNG), freshly harvested in 2006, were steam-treated to inhibit enzymatic processes and were frozen at −20 °C until used. To achieve this, the fully ripe green *V. planifolia* beans were treated with water steam in a Giusti steam column for 15 min at 0.3 bar (about 107 °C) with cold water condensation. A sample of the steamed beans and, in comparison, a sample of the nonsteamed beans were incubated at 45 °C for 18 h, after which the latter had turned to a dark brown color, whereas the steamed beans remained virtually unchanged, retaining a greenish coloration.

Analytical Sensory Experiments. *General Conditions, Panel Training*. The sensory panel consisted of 12 assessors (5 women and 7 men, ages 25−39 years), who have given informed consent to participate in the sensory tests of the present investigation and have no history of known taste disorders. The individuals participated for at least two years in weekly training sessions with reference solutions to become familiarize with the taste language and to be trained in recognizing and distinguishing different qualities of oral sensations in analytical sensory experiments. In detail, the subjects were trained to evaluate the taste of aqueous solutions (2 mL; pH 4.5) of the following standard taste compounds in bottled water (Evian): NaCl (20 mmol/L) for salty taste, lactose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, and monosodium L-glutamate (3 mmol/L) for umami taste. For training and classification of bitter taste, solutions of $MgSO_4$ (166 mmol/L) representing a short-lasting, metallic bitter taste quality perceived mainly at the anterior part of the tongue, salicin (1.4 mmol/L), imparting a long-lasting bitter taste sensation perceived mainly on the back of the tongue as well as the throat, and caffeine (8.0 mmol/L), providing a long-lasting bitterness perceived throughout the oral cavity, were used as references. For the puckering astringency and the velvety mouth-coating oral sensation, 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 (*15*, *18*). The assessors had participated earlier at regular intervals in sensory experiments and were, therefore, familiar with the techniques applied. Sensory analyses were performed at 22−25 °C in three independent sessions for each test. To prevent cross-modal interactions with odorants, the panelists used noseclips.

Precautions Taken for Sensory Analysis of Food Fractions and Taste Compounds. Prior to sensory analysis, buffer compounds and solvent traces were removed from the freeze-dried fractions isolated from vanilla beans. To achieve this, the individual fractions were dissolved in water, and the remaining volatiles and solvent traces were removed in high vacuum (< 5 mPa, 35 °C), then again taken up in water and freeze-dried twice. HRGC-MS and ion chromatographic analyses revealed that food fractions treated by that procedure are essentially free of the solvents and buffer compounds used. To minimize the uptake of any toxic compound, the sensory analyses were performed by using the sip-and-spit method, which means the test materials were not swallowed but expectorated. Formic acid, which is GRAS listed as a flavoring agent for food and feed applications, was used to adjust the pH value of solutions to be sensorially evaluated, because trace amounts of this acid do not influence the sensory profile of the test solution.

Taste Dilution Analysis (TDA). To determine taste dilution (TD) factors (*8*), aliquots of each isolated HPLC fraction were dissolved in "natural" concentration ratios in exactly 5.0 mL of bottled water (pH 4.5) and, then, sequentially diluted 1:2 with bottled water (pH 4.5). The serial dilutions of each of these fractions were then presented to the sensory panel in order of ascending concentrations, and each dilution was evaluated for astringency by means of the half-tongue test (*15*, *18*). The dilution at which a taste difference between the diluted extract and the blank (control) could just be detected was defined as the taste dilution factor (*8*). The TD factors evaluated by three different assessors randomly selected from the trained panel 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.

Recognition Threshold Concentrations. TD factors as well as human recognition thresholds for velvety mouth-coating compounds were determined by 12 trained assessors using the recently developed half-tongue test (*15*, *18*). Using bottled water as the solvent and an interstimulus interval length of 15 min, serial 1:2 dilutions of the samples were presented in order of increasing concentrations to the trained sensory panel in three different sessions using the sip-and-spit method. In the case of a correct selection by the panelist, the same concentration was presented again along with one blank as a proof for the correctness of the data. The individual threshold concentration of each panelist is calculated as the geometric mean between the last incorrectly identified and the first correctly identified sample solution. The bitter recognition threshold of the panel was calculated from the geometric means of all individual threshold concentrations. The threshold values between individuals and between three separate sessions differed by not more than plus/minus one dilution step; that is, a threshold value of 1.0 μmol/L for divanillin represents a range from 0.5 to 2.0 μmol/L.

The recognition threshold concentration of selected compounds for their activity on creaminess and body of milk was determined in skimmed milk (1.5%) containing sucrose (5%) by means of a triangle test using serial 1:2 dilutions of the samples in order of increasing concentrations in three different sessions. In the case of a correct selection by the panelist, the same concentration was presented again as proof for the correctness of the data. The individual threshold concentration of each panelist was calculated as the geometric mean between the last incorrectly identified and the first correctly identified sample solution, and the panel recognition threshold was determined from the geometric means of all individual threshold concentrations. The threshold values between individuals and between three separate sessions differed by not more than plus/minus two dilution steps.

Sequential Solvent Extraction of Vanilla Extract CE-M. A sample of the Madagascan vanilla extract (500 g, CE-M) was extracted six times with pentane (500 mL each), and the combined organic layers were freed from solvent in vacuum at 40 °C to give the pentane solubles (fraction A). Thereafter, the aqueous residue was extracted six times with ethyl acetate (500 mL each), and the combined organic phase was freed from solvent in vacuum at 40 °C. The organic residue was taken up in water (50 mL) and then freeze-dried to yield the ethyl acetate extractables (fraction B), and the aqueous residue was freeze-dried to give the nonextractable water solubles (fraction C). The yields of fractions A−C were determined by weight, and their taste profiles were evaluated in aqueous solutions (**Table 1**).

Gel Permeation Chromatographic Separation of Vanilla Fraction B. An aliquot (3 g) of vanilla fraction B was dissolved in a mixture (60:40, v/v; 20 mL) of methanol and water adjusted to pH 4.5 with aqueous formic acid (1% in water) and was then

Table 1. Yields and Sensory Evaluation of Fractions A−C Isolated from the Madagascan Vanilla Extract (CE-M)

sample	yield[b] (g/100 g)	intensity[a] perceived for bitterness	sourness	sweetness	velvety, mouth-coating
CE-M		0	0.5	0	4.5
fraction A[c]	2.0	0	0	0	2.0
fraction B[c]	10.1	0	0.5	0	4.0
fraction C[c]	87.9	0	0.5	0	1.0

[a] The taste intensity of aqueous solutions of the "natural" concentrations of the individual fractions in bottled water (0.5 L; pH 4.5) were rated on a scale from 0 (not detectable) to 5.0 (strongly detectable). [b] Yields were determined by weight and are given on a dry weight basis. [c] Individual fractions contain the pentane solubles (A), ethyl acetate extractables (B), and water solubles (C) isolated from the vanilla extract CE-M.

applied onto the top of a water-cooled 400 × 50 mm glass column (Amersham Pharmacia Biotech, Uppsala, Sweden) filled with a slurry of Sephadex LH-20 (Amersham Pharmacia Biotech) conditioned with the same solvent mixture. Chromatography was performed with methanol/water (60:40, v/v; pH 4.5; 2.7 L), followed by methanol/water (80:20, v/v; pH 4.5; 0.9 L) and methanol (2.7 L) with a flow rate of 3 mL/min. Monitoring the effluent by means of an L-7420 type UV−vis detector (Merck Hitachi, Darmstadt, Germany) operating at 272 nm, a total of nine subfractions (I−IX) were collected from fraction B; the individual fractions were freed from solvent in vacuum and were then freeze-dried twice. The residue of each GPC fraction was used for sensory and chemical analysis.

HPLC Separation of Fraction B and GPC Fractions I−IX. An aliquot of fraction B and the individual GPC fractions I−IX, respectively, were dissolved in a mixture of acetonitrile and water (5:95, v/v; 2 mL) and, after membrane filtration, were fractionated by preparative HPLC on an ODS-Hypersil RP-18, 250 × 21.2 mm i.d., 5 μm (ThermoHypersil, Kleinostheim, Germany) using a acetonitrile/aqueous formic acid gradient at a flow rate of 20 mL/min. Solvent A was 1.5% formic acid in water (v/v), and solvent B was acetonitrile. Chromatography was performed by increasing the content of solvent B from 5 to 17% B witin 35 min, then maintaining solvent B constant for 15 min, thereafter increasing solvent B to 50% within 20 min and, finally, to 100% within 10 min. The effluent of the peaks containing a target taste compound was collected from three separate HPLC runs, combined, freed from solvent in vacuum, and freeze-dried, and the residues obtained were used for chemical analysis as well as for TDA.

Isolation and Structure Determination of Taste Compounds. Comparison of spectroscopic (LC-MS, NMR) and sensory data, followed by cochromatography with the reference compound, led to the identification of vanillin (**1**) as a flavor compound in HPLC fraction 11 (**Figure 1**). Preparative RP-HPLC of the individual GPC fractions (**Figure 2**) and rechromatography, followed by degustation as well as LC-MS and 1D/2D NMR experiments, enabled the purification and identification of the following taste compounds (**Table 2**): americanin A (**3**) isolated from GPC fraction VI as the key taste compound in HPLC fraction 22, vanillin-*O*-β-D-glucopyranoside (**2**) from GPC fraction III as the key taste compound in HPLC fraction 5, (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (**4**) from GPC fraction III as the key taste compound in HPLC fraction 23, 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde (**5**) and divanillin (**6**) isolated from GPC fraction VI as the key taste compounds in HPLC fraction 26, and 5-(4-hydroxybenzyl)vanillin (**7**), 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (**8**), and 4-(4-hydroxybenzyl)-2-methoxyphenol (**9**) isolated from GPC fraction VI as the key taste compounds in HPLC fraction 28. The spectroscopic data of divanillin (**6**) were identical to those of the commercially available reference compound.

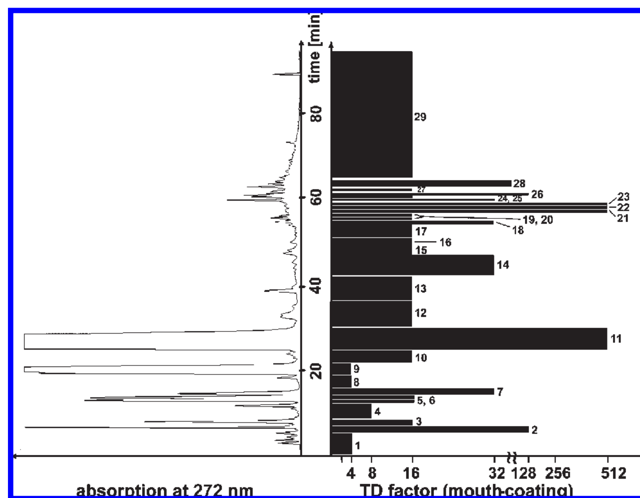

Figure 1. RP-HPLC chromatogram (**A**) and taste dilution (TD) chromatogram of fraction B isolated from an ethanol/water extract (CE-M) of cured vanilla beans from Madagascar.

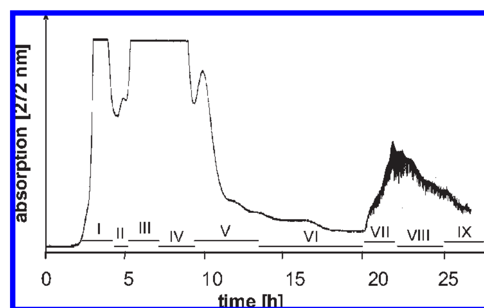

Figure 2. Gel permeation chromatography of fraction B isolated from an ethanol/water extract (CE-M) of cured vanilla beans from Madagascar.

*Vanillin-O-β-D-glucopyranoside, 2 (**Figure 3**):.* LC-MS (ESI$^-$), m/z 313 ([M − H]$^-$), 151 ([M − H − glc]$^-$); ^{1}H NMR (400 MHz, D$_2$O; COSY) δ 3.37 [dd, J = 9.1, 9.1 Hz, 1H, H−C(4′)], 3.47 [dd, J = 9.0, 9.0 Hz, 1H, H−C(3′)], 3.49 [m, 1H, H−C(5′)], 3.51 [dd, J = 9.0, 9.0 Hz, 1H, H−C(2′)], 3.63 [dd, J = 5.6, 12.4 Hz, 1H, H−C(6a′)], 3.79 [dd, J = 2.0 Hz, 12.4 Hz, 1H, H−C(6b′)], 3.82 [s, 3H, H−C(8)], 5.05 [d, J = 7.3 Hz, 1H, H−C(1′)], 7.20 [d, J = 8.3 Hz, 1H, H−C(5)], 7.41 [d, J = 1.8 Hz, 1H, H−C(2)], 7.48 [dd, J = 1,8 Hz, 8.3 Hz, 1H, H−C(6)], 9.69 [s, 1H, H−C(7)]; ^{13}C NMR (100 MHz, D$_2$O; HMQC, HMBC) δ 193.62 [C(7)], 151.74 [C(4)], 149.30 [C(3)], 131.14 [C(1)], 126.57 [C(6)], 114.95 [C(5)], 110.72 [C(2)], 100.01 [C(1')], 76.54 [C(4')], 75.81 [C(3′)], 72.95 [C(2')], 69.51 [C(5′)], 60.70 [C(6)], 55.673 [C(8)].

*Americanin A, 3 (**Figure 3**):.* UV−vis (MeCN) λ_{max} = 245 and 333 nm; LC-MS (ESI$^-$), m/z 327 ([M − H]$^-$), 163 ([M − H − 164]$^-$); ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ 3.51 [dd, J = 4.5, 12.4 Hz, 1H, H−C(γ_a)], 3.71 [dd, J = 2.5, 12.4 Hz, 1H, H−C(γ_b)], 4.10 [m, 1H, H−C(β)], 4.86 [d, J = 8.0 Hz, 1H, H−C(α)], 6.63 [dd, J = 7.8, 15.9 Hz, 1H, H−C(β')], 6.79 [dd, J = 1.8, 8.1 Hz, 1H, H−C(6)], 6.82 [d, J = 8.1 Hz, 1H, H−C(5)], 6.88 [d, J = 1.8 Hz, 1H, H−C(2)], 7.03 [d, J = 8.1 Hz, 1H, H−C(3′)], 7.22 [dd, J = 1.8, 8.1 Hz, 1H, H−C(2′)], 7.25 [d, J = 1,8 Hz, 1H, H−C(6′)], 7.57 [d, J = 15.9 Hz, 1H, H−C(α′)], 9.59 [d, J = 7.8 Hz, 1H, H−C(γ')]; ^{13}C NMR (100 MHz, D$_2$O; HMQC, HMBC) δ 194.5 [C(γ')], 153.6 [C(α′)], 146.2 [C(5′)], 145.7 [C(3)], 145.3 [C(4)], 144.4 [C(4′)], 127.6 [C(1)], 126.6 [C(β')], 126.4 [C(1′)], 122.4 [C(2′)], 119.1 [C(6)], 117.2 [C(3′)], 116.6 [C(6′)], 114.9 [C(5)], 114.1 [C(2)], 79.0 [C(β)], 76.1 [C(α)], 60.0 [C(γ)].

*(1-O-Vanilloyl)-(6-O-feruloyl)-β-D-glucopyranoside, 4 (**Figure 3**):.* UV−vis (MeCN) λ_{max} = 247, 275, 311 nm; LC-MS (ESI$^-$), m/z 489 ([M − H]$^-$), 337 ([M − H − 152]$^-$, 151; LC-TOF-MS, m/z 489.4538 ([M − H]$^-$, measured), m/z 489.4549

Table 2. Human Recognition Threshold Concentrations of Velvety Mouth-Coating Compounds Isolated from Distinct HPLC and GPC Fractions Obtained from Cured Vanilla Beans

compound (no.)[a]	isolated from		TC[d] (μmol/L)
	HPLC fraction[b]	GPC fraction[c]	
vanillin (**1**)	11	III	250
vanillin-*O*-β-D-glucopyranoside (**2**)	5	III	25
5-(4-hydroxybenzyl)vanillin (**7**)	28	VI	5.0 (25.0[e])
americanin A (**3**)	22	VI	4.0
4-(4-hydroxybenzyl)-2-methoxyphenol (**9**)	28	VI	3.0
4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (**8**)	28	VI	2.0 (20.0[e])
(1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (**4**)	23	III	1.5
divanillin (**6**)	26	VI	1.0 (10.0[e])
4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde (**5**)	26	VI	1.0 (5.0[e])

[a] The structures of the individual compounds are given in **Figure 3**. [b] HPLC fraction (cf. **Figure 1**) containing the taste compound. [c] GPC fraction (cf. **Figure 2**), from which the taste compound was isolated preparatively. [d] Recognition threshold concentrations for the velvety mouth-coating sensation were determined in bottled water by means of the half-tongue test (*15*, *18*). [e] Threshold concentrations for the recognition of an enhanced creaminess and fatty body of sweetened skimmed milk (1.5% fat) containing 5% sucrose were determined by means of a triangle test.

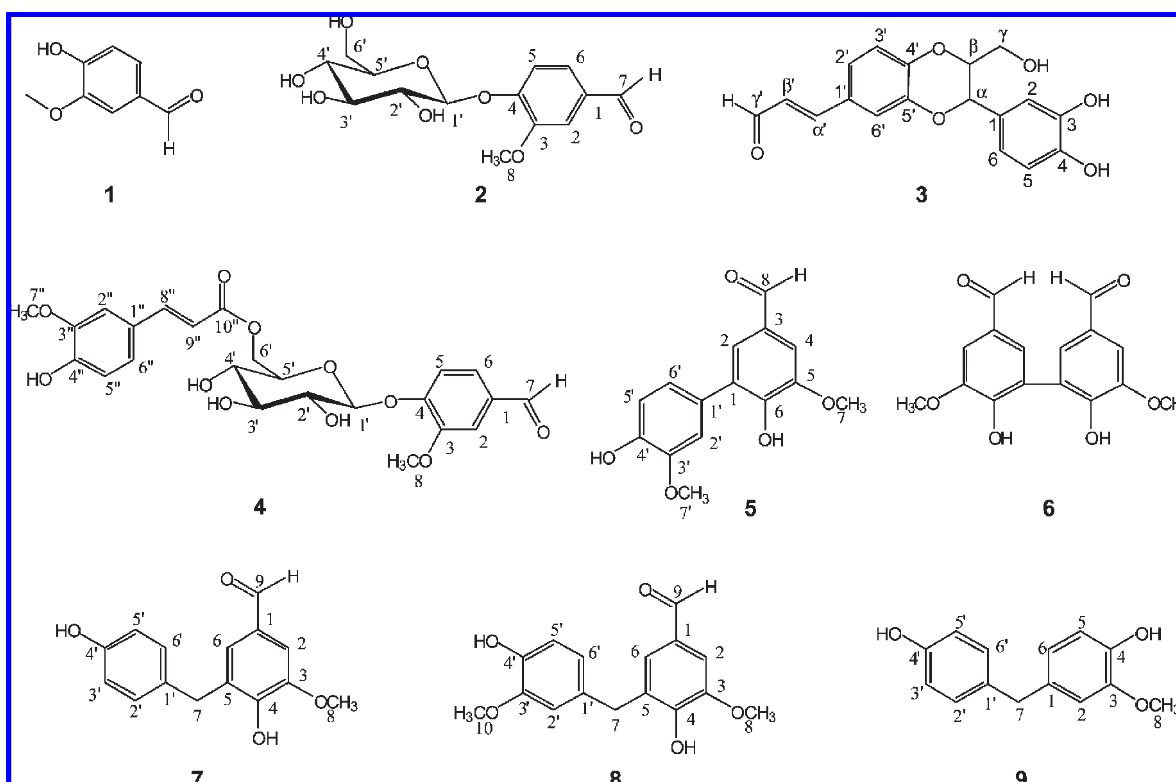

Figure 3. Chemical structures of velvety mouth-coating compounds **1**−**9** isolated from cured vanilla beans. The arbitrary numbering of the carbon atoms refers to the assignment of the NMR data.

([M − H]$^-$, calcd for $C_{24}H_{15}O_{11}$); ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ 9.69 [s, 1H, H−C(7)], 7.42 [d, 1H, J = 15,9 Hz, H−C(8″)], 7.36 [m, 2H, H−C(2), H−C(6)], 7.22 [m, 2H, H−C(2″), H−C(6″)], 7.03 [dd, 1H, J = 8.3, 1.8 Hz, H−C(5)], 6.72 [d, 1H, J = 8.6 Hz, H−C(5″)], 6.38 [d, 1H, J = 15.9 Hz, H−C(9″)], 5.31 [m, 3H, HO−(C2′), HO−C(3′), HO−C(4′)], 5.07 [d, 1H, J = 7.5 Hz, H−C(1′)], 4.28 [m, 2H, HO−C(4″), H−C(6a′)], 4.13 [dd, 1H, J = 12.2, 5.3 Hz, H−C(6b′)], 3.75 [m, 7H, H−C(8), H−C(5′), H−C(7″)], 3.67 [m, 3H, H−C(2′), H−C(3′), H−C(4′)]; ^{13}C NMR (100 MHz, DMSO-d_6, HMQC, HMBC) δ 192.7 [C(7)], 166.8 [C(10″)], 152.4 [C(4)], 149.7 [C(3″)], 148.3 [C(3)], 145.5 [C(8″)], 144.9 [C(4″)], 130.8 [C(1)], 128.8 [C(1″)], 125.4 [C(6)], 123.7 [C(5)], 115.6 [C(5″)], 115.1 [C(6″)], 114.8 [C(9″)], 111.4 [C(2″)], 111.1 [C(2)], 99.6 [C(1′)], 73.9 [C(3′)], 71.5−71.7 [C(2′), C(5′), C(4′), C(6′)], 56.1 [C(7″), C(8)].

*4′,6′-Dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde, **5** (**Figure 3**):.* UV−vis (MeCN) λ_{max} 231, 261, 285 nm; LC-MS (ESI$^-$), *m/z* 273 ([M − H]$^-$), 215 ([M − H − 58]$^-$); LC-TOF-MS, *m/z* 273.2640 ([M − H]$^-$, measured), *m/z* 273.2647 ([M − H]$^-$, calcd for $C_{15}H_{13}O_5$); ^{1}H NMR (400 MHz, MeOD-d_4; COSY) δ 9.71 [s, 1H, H−(C 8)], 7.43 [d, 1H, J = 1.8 Hz, H−C(2)], 7.21 [d, 1H, J = 1.8 Hz, H−C(4)], 7.13 [d, J = 1.8 Hz, 1H, H−C(2′)], 6.96 [dd, 1H, J = 8.0, 2.0 Hz, H−C(6′)], 6.76 [d, 1H, J = 8.0 Hz, H−C(5′)], 3.90 [s, 1H, H−C(7)], 3.80 [s, 1H, H−C(7′)]; ^{13}C NMR (100 MHz, MeOD-d_4, HMQC, HMBC) δ 192.3 [C(8)], 149.6 [C(6)], 148.6 [C(5)], 147.3 [C(3′)], 146.4 [C(4′)], 128.5−127.5 [C(1), C(1′), C(2), C(3)], 121.6 [C(6′)], 114.6 [C(5′)], 112.7 [C(2′)], 107.5 [C(4)], 55.3 [C(7)], 55.1 [C(7′)].

*5-(4-Hydroxybenzyl)vanillin, **7** (**Figure 3**):.* UV−vis (MeCN) λ_{max} 231, 283 nm; LC-MS (ESI$^-$), *m/z* 257 ([M − H]$^-$), 214 ([M − H − 43]$^-$), 149 ([M − H − 43 − 65]$^-$); LC-TOF-MS, *m/z* 257.2647 ([M − H]$^-$, measured), *m/z* 257.2653 ([M − H]$^-$, calcd for $C_{15}H_{13}O_4$); ^{1}H NMR (400 MHz, MeOD-d_4; COSY) δ 9.56 [s, 1H, H−C(9)], 7.22 [d, 1H, J = 2.0 Hz, H−C

(2)], 7.14 [d, 1H, J = 2.0 Hz, H−C(6)], 6.95 [dd, 2H, J = 8.2, 2.0 Hz, H−C(2′), H−C(6′)], 6.58 [dd, 2H, J = 8.2, 2.0 Hz, H−C(3′), H−C(5′)], 3.83 [s, 3H, H−C(8)], 3.81 [s, 3H, H−C(7)]; ^{13}C NMR (100 MHz, MeOD-d_4, HMQC, HMBC) δ 191.7 [C(9)], 155.5 [C (4′)], 150.9 [C (3)], 150.7 [C(4)], 131.6−129.5 [C(1′), C(5), C(6′), C(2′)], 127.6 [C(1)], 127.1 [C(6)], 114.6 [C(5′), C(3′)], 107.5 [C(2)], 55.1 [C(8)], 33.9 [C(7)].

*4-Hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde, **8** (**Figure 3**):.* UV−vis (MeCN) λ_{max} 231 and 283 nm; LC-MS (ESI$^-$), m/z 287 ([M − H]$^-$), 272 ([M − H − 15]$^-$), 257 ([M − H − 30]$^-$); LC-TOF-MS, m/z 287.2911 ([M − H]$^-$, measured), m/z 287.2915 ([M − H]$^-$, calcd for $C_{16}H_{15}O_5$); ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ 9.70 [s, 1H, H−C(9)], 7.28 [d, J = 1.8 Hz, 1H, H−C(2)], 7.25 [d, J = 1.8 Hz, 1H, H−C (6)], 6.89 [d, J = 1.6 Hz, 1H, H−C(2′)], 6.64 [d, J = 7.9 Hz, 1H, H−C(5′)], 6.56 [dd, J = 1.6, 7.9 Hz, 1H, H−C(6′)], 3.86 [s, 3H, H−C(8)], 3.81 [s, 2H, H−C(7)], 3.69 [s, 3H, H−C(10)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC) δ 191.4 [C(9)], 147.9 [C (3,4′)], 147.7 [C(3′)], 145.5 [C(4)], 131.2 [C(1′)], 129.5 [C(5)], 128.2 [C(1)], 126.9 [C(6)], 121.2 [C(6′)], 115.7 [C(5′)], 113.6 [C (2′)], 109.1 [C(2)], 56.4 [C(8)], 56.1 [C(10)], 34.7 [C(7)].

*4-(4-Hydroxybenzyl)-2-methoxyphenol, **9** (**Figure 3**):.* UV−vis (MeCN) λ_{max} 223, 231, and 277 nm; LC-MS (ESI$^-$), m/z 229 ([M − H]$^-$), 93 ([M − H − 136]$^-$); LC-TOF-MS, m/z 229.2542 ([M − H]$^-$, measured), m/z 229.2549 ([M − H]$^-$, calcd for $C_{14}H_{13}O_3$); ^{1}H NMR (400 MHz, DMSO-d_6; COSY) δ 3.69 [s, 2H, H−C(7)], 3.71 [s, 3H, H−C(8)], 6.54 [dd, J = 2.0, 8.0 Hz, 1H, H−C(6)], 6.65 [m, 2H, H−C(3′,5′)], 6.65 [d, J = 8.0 Hz, 1H, H−C(5)], 6.74 [d, J = 2.0 Hz, 1H, H−C(2)], 6.98 [m, 2H, H−C (2′,6′)], 8.67 [s, 1H, HO−C(4)], 9.13 [s, 1H, HO-C(4′)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC) δ 155.9 [C(4′)], 147.9 [C(3)], 145.3 [C(4)], 133.8 [C(1)], 130.6 [C(1′)], 129.8 [C(2′,6′)], 121.1 [C (6)], 115.4 [C(3′,5′,5)], 113.3 [C(2)], 55.9 [C(8)], 40.4 [C(7)].

Quantitative Analysis of Compounds 3−9 in Vanilla Bean Samples. The vanilla bean samples CB-M, CB-PNG, and NCB-PNG were frozen in liquid nitrogen and ground in a mill, and an aliquot (5.0 g) of the powdered material was extracted with methanol/water (70:30, v/v; 5 × 100 mL) with stirring. After centrifugation, the liquid layer was freed from methanol under reduced pressure at 40 °C, and the aqueous extract was adjusted to 1000 mL with methanol/water (30:70, v/v). After membrane filtration, aliquots (5−10 μL) were analyzed by means of LC-MS/MS, which was equipped with a 150 × 2 mm i.d., 4 μm, RP-18 Synergi Fusion column (Phenomenex) operated with a flow rate of 0.25 mL/min. Chromatography was performed starting with a mixture (13:87, v/v) of acetonitrile and aqueous formic acid (1.0% in water) for 5 min; the acetonitrile content was increased to 25% within 10 min, held for 5 min, then increased to 38% within 15 min, again held for 5 min, and, finally, raised to 100% within 15 min. Using the purified compounds **3**−**9** as external standards, the taste compounds **3** (m/z 326.9→162.9), **4** (m/z 489.1→150.9), **5** (m/z 273.0→214.9), **6** (m/z 301.0→240.1), **7** (m/z 257.0→149.1), **8** (m/z 287.0→256.8), and **9** (m/z 229.0→93.0) were quantitatively determined by means of the multiple reaction monitoring (MRM) mode measuring the mass transitions given in parentheses. Calibration mixtures were used containing analyte concentrations between 0.1 and 3.0 mg/L.

High-Performance Liquid Chromatography (HPLC). The HPLC apparatus (Jasco, Gross-Umstadt, Germany) consisted of an MD-2010 plus photodiode array detector and two PU 2087 pumps. Chromatographic separations were performed on stainless steel columns packed with ODS-Hypersil, 5 μm, RP-18 material (ThermoHypersil, Kleinostheim, Germany) in either a 250 × 4.6 mm i.d. analytical scale (flow rate = 1.0 mL/min), a 250 × 10 mm i.d. semipreparative scale (flow rate = 3.5 mL/min), or a 250 × 21.2 mm i.d. preparative scale (flow rate = 20 mL/min).

Liquid Chromatography−Mass Spectrometry (LC-MS). Electrospray ionization (ESI) mass and product ion spectra were acquired on an API 4000 QTRAP and an API 3200 mass spectrometer (Applied Biosystems, Darmstadt, Germany), respectively, using direct flow infusion. For ESI, the ion spray voltage was set at −4500 V in the negative mode and at 5500/5000 V in the positive mode. The mass spectrometer was operated in the full-scan mode detecting positive or negative ions.

LC−Time-of-Flight Mass Spectrometry (LC-TOF-MS). High-resolution mass spectra of the compounds were measured on a Bruker Micro-TOF mass spectrometer (Bruker Daltronics, Bremen, Germany).

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H, ^{13}C, and 2-D NMR data were acquired on a Bruker DPX-400 (Bruker BioSpin, Rheinstetten, Germany). DMSO-d_6, MeOH-d_4, or D_2O was used as solvent, and chemical shifts were referenced to the solvent signal. For structural elucidation and NMR signal assignment, COSY, HMQC, and HMBC experiments 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) as well as Mestre-C (Mestrelab Research, A Coruña, Spain).

RESULTS AND DISCUSSION

As the Madagascan vanilla extract (CE-M) imparted the typical velvety mouth-coating orosensory sensation even at the low concentration of 1 in water, a taste profile of that aqueous dilution of the vanilla extract was recorded. To achieve this, a trained sensory panel was asked to rate the intensity of the taste qualities bitter, sour, sweet, salty, umami, as well as the velvety, mouth-coating sensation, on a scale from 0 (not detectable) to 5 (intensely detectable). A high score of 4.5 was found for the intensity of the velvety mouth-coating sensation, and only a faint sour taste judged with an intensity of 0.5 was perceived in addition (**Table 1**). Sweetness, saltiness, and umami taste were not detectable at all. To gain first insight into the hydrophobicity of the compounds imparting the typical velvety mouth-coating oral sensation, the vanilla extract was sequentially extracted with solvents of increasing polarity.

Solvent Fractionation of Vanilla Extract CE-M. An aliquot of the vanilla sample CE-M was extracted with pentane to obtain the pentane extractables (fraction A) after removal of solvents in vacuum. The extraction of the aqueous phase with ethyl acetate afforded an organic layer, which was freed from solvent in vacuum to give fraction B, and an aqueous phase yielding fraction C after lyophilization. After freeze-drying, the yields of the individual fractions were determined by weight. The highest yields were obtained for fractions B and C, respectively, accounting for 10.1 and 87.9% of the dry mass of the total vanilla extract (**Table 1**). A comparatively low yield of 2.0% was found for fraction A.

Sensory evaluation of aqueous solutions of fractions A, B, and C, respectively, by means of taste profile analysis demonstrated that the ethyl acetate isolate (fraction B) was evaluated with the highest scores for the velvety, mouth-coating oral sensation (4.0). In comparison, fractions A and C were judged with significantly lower intensities of 2.0 and 1.0, respectively (**Table 1**). On the basis of these data, the following investigations were focused on the identification of velvety mouth-coating compounds in fraction B.

Sensory-Guided Separation of Fraction B. To sort out the strongly taste-active compounds from the bulk of less taste-active or tasteless substances, first, fraction B isolated from sample CE-M was separated by means of preparative RP-HPLC. By monitoring the absorption at 272 nm (**Figure 1**), a total of 29 HPLC subfractions were collected, separated from solvent using vacuum, taken up in water, and analyzed by means of the TDA using the recently developed

half-tongue test (*15*, *18*) to evaluate their taste impact. The highest TD factor of 512 was found for the velvety mouth-coating taste of fractions 11, 21, 22, and 23, followed by fractions 2, 7, 14, 18, 25, 26, and 28, which still showed velvety mouth-coating taste after dilutions of 1:128 and 1:64, respectively (**Table 2**).

Comparison of spectroscopic (LC-MS, NMR) and sensory data, followed by cochromatography with the reference compound, led to the identification of vanillin (**1**) as flavor compound in HPLC fraction 11 (**Table 2**). To obtain suitable amounts of the compounds imparting the velvety mouth-coating taste sensation perceived for the additional fractions evaluated with high TD factors, an aliquot of vanilla fraction B was separated by means of preparative gel permeation chromatography (GPC) using Sephadex LH-20 as the stationary phase and methanol/water mixtures as the mobile phase (**Figure 2**). Preparative RP-HPLC of the individual GPC fractions, followed by degustation as well as LC-MS and 1D/2D NMR experiments enabled the localization and identification of compound **3** isolated from GPC fraction VI as the key taste compound in HPLC fraction 22, compound **2** from GPC fraction III as the key taste compound from HPLC fraction 5, compound **4** from GPC fraction III as the key taste compound from HPLC fraction 23, compounds **5** and **6** isolated from GPC fraction VI as the key taste compounds in HPLC fraction 26, and compounds **7**−**9** isolated from GPC fraction VI as the key taste compounds in HPLC fraction 28 (**Table 2**).

LC-MS/MS (ESI^-) analysis of the velvety mouth-coating compound **2**, eluting in HPLC fraction 5, revealed *m*/*z* 313 as the pseudomolecular ion $[M - -H]^-$ and *m*/*z* 151 as the fragment ion fitting well with the $[M - H]^-$ ion of vanillin. In addition, the loss of 162 amu corresponded with the cleavage of a hexose moiety and indicated the presence of a vanillin-*O*-hexoside. Upon comparison of the LC-MS and NMR data with those reported earlier in the literature (*22*), vanillin-*O*-*β*-D-glucopyranoside (**2**; **Figure 3**) was identified as the taste compound in HPLC fraction 5.

LC-MS/MS (ESI^-) analysis of the velvety mouth-coating compound in HPLC fraction 22, judged with a high TD factor of 512, revealed the pseudomolecular ion *m*/*z* 327 ($[M - H]^-$) and the daughter ion *m*/*z* 163. The ^1H NMR spectrum showed two doublets resonating at 9.59 or 7.57 ppm and showing a coupling constant of 7.8 or 15.9 Hz, respectively. In addition, a doublet of a doublet was observed at 6.63 ppm, exhibiting coupling constants of 7.8 and 15.9 Hz, thus indicating the presence of an acrolein moiety in the target molecules. By means of homonuclear (COSY) and heteronuclear chemical shift correlation experiments (HMQC, HMBC), two sets of three aromatic protons showing the same coupling pattern were successfully assigned and, upon comparison with literature data (*23*), led to the unequivocal identification of the taste compounds as americanin A, **3** (**Figure 3**). Although this compound was isolated earlier as a natural product from American pokeweed (*Phytolacca americana*) (*23*), the orosensory activity of this lignan was not previously reported.

The UV−vis spectrum of compound **4** isolated from HPLC fraction 23 exhibited absorption maxima at 247, 275, and 311 nm. LC-MS/MS (ESI^-) analysis of this compound revealed the pseudomolecular ion *m*/*z* 489 ($[M - H]^-$) and the fragment ions *m*/*z* 337 and 151, respectively, thus indicating the presence of a vanillin moiety in the molecule. This was further confirmed by the resonance signals detected in the ^{13}C NMR spectrum showing the eight carbon signals expected for the vanillin moiety with the carbon atom of the aldehyde function resonating at 192.7 ppm. In addition, homo- and heteronuclear chemical shift correlation experiments demonstrated the presence of a *β*-D-glucopyranosyl unit in the molecule, and the two olefinic protons detected at 6.38 and 7.42 ppm showing a coupling constant of 15.9 Hz allowed the assignment of an *E*-configured double bond in a ferulic acid moiety. Heteronuclear correlations were observed between the anomeric proton H−C(1′) of the glucose and the aromatic carbon atom C(4) of the vanillin moiety as well as between the methylene protons H−C(6′) of the glucose and the carboxyl carbon C(10) of the ferulic acid moiety, respectively. These findings undoubtedly demonstrate that the hydroxyl group at the carbon C(4) of vanillin is glycosidically bound to the anomeric carbon atom C(1′) of the *β*-D-glucopyranose and that the primary hydroxyl group at C(6′) of the hexose is esterified with carbon atom C(10) of ferulic acid. When all of these data were taken into consideration, the chemical structure of the velvety mouth-coating compound in HPLC fraction 23 was determined as (1-*O*-vanilloyl)-(6-*O*-feruloyl)-*β*-D-glucopyranoside, **4** (**Figure 3**), which was previously not reported in the literature.

Rechromatography of fraction 26 by means of RP-HPLC led to the isolation of two intense velvety mouth-coating compounds. The first compound showed UV absorption maxima at 231, 261, and 285 nm, and LC-MS/MS (ESI^-) analysis revealed a pseudomolecular ion ($[M - 1]^-$) with *m*/*z* 273 as well as a daughter ion with *m*/*z* 215. ^1H and ^{13}C NMR spectroscopy revealed signal patterns as typically detected for a vanillin as well as a 2-methoxyphenol moiety. On the basis of heteronuclear *C*,*H*-correlation experiments (HMQC, HMBC), the taste compound in the first subfraction isolated from HPLC fraction 26 was identified as the previously unreported 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde, **5** (**Figure 3**). In addition, the taste compound in the second subfraction of HPLC fraction 26 induced a strong velvety mouth-coating sensation. Comparison of spectroscopic (LC-MS, NMR, UV−vis), chromatographic (RP-HPLC), and sensory data with those of the synthetic reference compound led to the identification of divanillin (**6**) in that subfraction, thus confirming previous literature reports (*7*).

From the velvety, mouth-coating HPLC fraction 28 were isolated a total of three taste-active compounds purified by rechromatography using RP-HPLC. LC-MS/MS analysis of the first compound (**7**), exhibiting UV absorption maxima at 231 and 283 nm, revealed the pseudomolecular ion $[M - H]^-$ with *m*/*z* 257 and the two daughter ions *m*/*z* 214 and 149, respectively. ^{13}C NMR, HMQC, and HMBC experiments identified a total of 15 carbon signals for the target compound, among which the carbon atom resonating at 191.7 ppm was assigned as an aldehyde group. The three protons H−C(8) resonating at 3.83 ppm could be assigned to the methoxy group of a vanillin moiety, and the heteronuclear ^3J coupling between the methylene protons H−C(7) and the quaternary carbons C(1′), C(5), and C(4) undoubtedly identified a 4-hydroxybenzyl unit to be linked with the C(5) position of vanillin. When all these spectroscopic data were taken into consideration, the structure of the first taste compound isolated from HPLC fraction 28 was identified as the previously unreported 5-(4-hydroxybenzyl)vanillin, **7** (**Figure 3**).

LC-MS analysis of the second compound (**8**) isolated from HPLC fraction 28 showed a pseudomolecular ion $[M - H]^-$ with *m*/*z* 287 and two main daughter ions with *m*/*z* 272

and 257, respectively. ^{13}C NMR spectroscopy and HMQC, as well as HMBC, experiments led to the assignment of 16 carbon signals, among which an aldehyde carbon atom was found to resonate at 191.4 ppm. In addition, the protons H−C(8) and H−C(10), resonating at 3.69 and 3.86 ppm, were assigned to the methoxy group of a vanillin as well as a guaiacol moiety, respectively. The observation of a heteronuclear ^3J coupling between the methylene protons H−C(7) and the quaternary carbon atoms C(1′) and C(5), respectively, undoubtedly demonstrated that the 4-hydroxy-3-methoxybenzyl unit is linked with the C(5) position of vanillin. When all of these spectroscopic data were taken into account, the chemical structure of the second velvety mouth-coating compound isolated from HPLC fraction 28 was determined as 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde, **8** (**Figure 3**). To the best of our knowledge, compound **8** has not been previously reported in the literature.

Also, the third compound isolated from HPLC fraction 28 induced a strong velvety mouth-coating sensation. LC-MS/MS analysis of the target compound, exhibiting UV absorption maxima at 223, 231, and 277 nm, showed a pseudomolecular ion ([M − H]$^-$) with *m*/*z* 229 and a daughter ion with *m*/*z* 93. ^{13}C NMR spectroscopy as well as heteronuclear chemical shift correlation experiments (HMQC, HMBC) detected a total of 14 carbons, among which the 4 protons H−C(2′), H−C(3′), H−C(5′), and H−C(6′), resonating at 6.65 and 6.98 ppm, respectively, could be assigned to a *p*-cresol moiety in the molecule. In addition, the protons H−C(2), H−C(5), and H−C(6), showing chemical shifts of 6.54, 6.65, and 6.74 ppm, were assigned to be part of a guaiacol moiety in the target compound. The observation of heteronuclear ^3J couplings between the protons of the methylene group H−C(7) and the quaternary carbon atoms C(1) and C(1′), respectively, undoubtedly identified the structure of the taste compound isolated from fraction 28 as 4-(4-hydroxybenzyl)-2-methoxyphenol, **9** (**Figure 3**). Although compound **9** has been already synthesized as a lignin-type diphenylmethane dimer (*24*), its occurrence in nature as well as its sensory activity has not been previously reported.

Orosensory Activity of Taste-Active Compounds. Prior to sensory analysis, the purity of all compounds was confirmed by HPLC-MS as well as ^{1}H NMR spectroscopy to be >99%. To determine the human threshold concentrations for the velvety mouth-coating oral sensation, aqueous solutions of the target compound were evaluated by means of the recently developed half-tongue test (*15*, *18*).

The human oral threshold concentration for the velvety mouth-coating sensation was strongly dependent on the structure of the compound and ranged from 250 μmol/L for vanillin (**1**) to 1.0 μmol/L found for the vanillin derivatives divanillin (**6**) and 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde (**5**) (**Table 2**). Interestingly, glucosylation seems to have an influence on the perception of the velvety mouth-coating sensation; for example, attachment of a glucose to vanillin (250.0 μmol/L) decreased the threshold by a factor of 10 to give 25 μmol/L for 1-*O*-vanilloyl-β-D-glucopyranoside (**2**) (**Table 2**). These data clearly confirm earlier observations made for flavon-3-ol *O*-glycosides (*15*), flavan-3-ol *C*-glycosides (*20*), ellagitannin *C*-glycosides (*17*), and indole-3-acetic acid glucosides (*25*) showing that the oral threshold of the corresponding aglycon is significantly lowered when glycosylated. It is interesting to note that additional feruloylation of this glucoside induced another decrease of the sensory threshold from 25.0 to 1.5 μmol/L as found for (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (**4**) (**Table 2**).

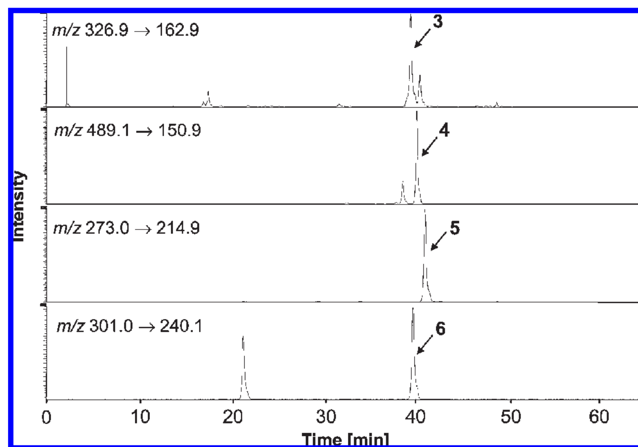

Figure 4. HPLC-MS/MS analysis of selected compounds in cured vanilla by means of LC-MS using the MRM mode.

Also, the diphenylmethane derivatives 5-(4-hydroxybenzyl) vanillin (**7**), 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (**8**), and 4-(4-hydroxybenzyl)-2-methoxyphenol (**9**) were found to induce the velvety mouth-coating sensation at low threshold concentrations between 2.0 and 5.0 μmol/L (**Table 2**).

Among the compounds identified, divanillin was recently reported to improve the creamy, fatty mouthfeel properties and the body of dairy products (*7*). As preliminary sensory studies showed that, besides the divanillin (**6**), also the previously not reported vanillin derivatives **5**, **7**, and **8** induced a creamy mouthfeel and fatty body when added to sweetened milk, the recognition threshold concentrations for this activity of compounds **5**−**8** were determined in sweetened skim milk by means of a triangle test. The threshold concentrations determined were rather similar for compounds **5**−**8** and ranged between 5.0 and 25.0 μmol/L (**Table 2**). The lowest threshold of 5.0 μmol/L was found for the previously not reported compound **5**, followed by divanillin (**6**), evaluated with a recognition threshold of 10.0 μmol/L. The methylene-bridged vanillin derivatives **8** and **7** showed somewhat higher thresholds of 20.0 and 25.0 μmol/L, respectively.

Concentrations and DoT Factors of Taste Compounds in Vanilla Beans. To gain a first insight into the contribution of the identified compounds to the velvety mouth-coating sensation induced by cured vanilla extracts, the taste compounds **3**−**9** were quantitatively determined by HPLC-MS/MS in the commercial Madagascan *V. planifolia* extract (CE-M) and in cured Madagascan (CB-M), as well as in cured Papua New Guinea vanilla beans (CB-PNG), respectively. In addition, compounds **3**−**9** were analyzed in noncured, green vanilla beans from Papua New Guinea (NCB-PNG) to determine the influence of the curing process on the concentrations of these taste compounds in vanilla beans. To achieve this, the vanilla bean samples were frozen with liquid nitrogen, ground, and exhaustively extracted with methanol/water, whereas the extract was used directly. After concentration, a sample of each extract was analyzed by analytical RP-HPLC-MS/MS operating in the MRM mode (**Figure 4**).

Independent of the origin of the cured vanilla beans, the concentrations of compounds **3**−**8** ranged between 16.3 and 52 μmol/kg, whereas 4-(4-hydroxybenzyl)-2-methoxyphenol (**9**) was present in just trace amounts (**Table 3**). The commercially available Madagascan extract (CE-M)

Table 3. Concentrations of Compounds **3**−**9** in a Commercial Madagascan *V. planifolia* Extract (CE-M), in Cured Beans from Madagascar (CB-M), and in Cured Beans from Papua New Guinea (CB-PNG) as well as in Green, Noncured Beans from Papua New Guinea (NCB-PNG), Respectively

compound (no.)[c]	concentration[a] (μmol/kg) [DoT factor[b]] in			
	CE-M	CB-M	CB-PNG	NCB-PNG
americanin A (**3**)	30.5 [76]	28.0 [70]	52.1 [130]	14.3 [36]
(1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glc*p* (**4**)	35.1 [234]	18.4 [123]	16.3 [109]	<0.1 [<1]
4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde (**5**)	26.6 [266]	33.2 [332]	30.3 [303]	nd[d]
divanillin (**6**)	18.1 [281]	32.1 [321]	26.5 [265]	nd
5-(4-hydroxybenzyl)vanillin (**7**)	24.8 [50]	42.2 [84]	26.7 [53]	nd
4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (**8**)	26.3 [132]	21.4 [107]	24.6 [123]	nd
4-(4-hydroxybenzyl)-2-methoxyphenol (**9**)	<1 [<1]	<1 [<1]	<1 [<1]	nd

[a] Concentrations are determined as the mean of triplicates and are given on a bean fresh weight basis. [b] Dose-over-threshold (DoT) factor is calculated as the ratio of the concentration and the taste threshold concentration of a compound. [c] The structures of the individual compounds are given in **Figure 3**. [d] Not detectable.

contained (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (**4**) in the highest concentration of 35.1 μmol/kg, but comparatively low concentrations of divanillin (**6**). In the cured Madagascan beans (CB-M), the highest concentration of 42.4 μmol/kg was found for 5-(4-hydroxybenzyl) vanillin (**7**), and americanin A (**3**) predominated in the cured beans from Papua New Guinea (CB-PNG), whereas (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoside (**4**) was present in both samples in comparatively low concentrations of 18.4 and 16.3 μmol/kg, respectively (**Table 3**). In contrast, 4′,6′-dihydroxy-3′,5-dimethoxy-[1,1′-biphenyl]-3-carboxaldehyde (**5**) and 4-hydroxy-3-(4-hydroxy-3-methoxybenzyl)-5-methoxybenzaldehyde (**8**) were found to be present in rather similar concentrations in the different cured bean samples.

Quantitative analysis of the same taste compounds in noncured, green Papua New Guinea beans (NCB-PNG) revealed that only americanin A was present in significant amounts of 14.3 μmol/kg and, in addition, (1-*O*-vanilloyl)-(6-*O*-feruloyl)-β-D-glucopyranoide (**4**) was detectable in this sample, whereas compounds **5**−**9** were found not even in trace amounts (**Table 3**). Considering water contents of 70 and 25% of the noncured and fermented beans, respectively, these data clearly demonstrate that these compounds are formed from precursor molecules upon curing of vanilla beans.

To answer the question as to whether these compounds contribute to the mouth-coating sensation of vanilla beans, dose-over-threshold (DoT) factors were determined from the ratio of the concentration and the taste recognition threshold of each compound (*26*). The data revealed that, with the exception of compound **9**, all of the other taste compounds showed high DoT factors between 50 and 332 in the cured bean sample and, therefore, should contribute to the typical mouth-coating sensation induced by vanilla beans (**Table 3**). In comparison, americanin A (**3**) was found as the only compound exceeding the taste threshold in green vanilla beans prior to curing. This finding clearly indicates that the curing process is important not only for the release of the odor-active vanillin from nonvolatile precursors but also for the generation of orosensory active nonvolatiles, among which compounds **5**−**8** were found to enhance the creaminess and fatty body of sweetened skim milk at threshold concentrations of 5−25 μmol/L.

The quantitative monitoring of these compounds as analytical markers in different steps of the curing process as well as the elucidation of their formation pathways from precursors might be helpful to design innovative, natural vanilla products by a science-based optimization of postharvest technologies. This might be achieved either by tailoring the curing process toward increased concentrations of such chemosensates or by separation of the volatile aroma fraction from an odorless, crude vanilla isolate exhibiting mouth-coating and/or creaminess-enhancing activity of foods such as confectionary and ice cream, respectively.

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