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Sensory-Guided Decomposition of Red Currant Juice (*Ribes rubrum*) and Structure Determination of Key Astringent Compounds

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Sequential application of solvent extraction, gel permeation chromatography, and RP-HPLC in combination with taste dilution analyses, followed by LC-MS and 1D/2D NMR experiments, led to the discovery and structure determination of 25 key astringent compounds of red currant juice. Besides several flavonol glycosides, in particular, 3-carboxymethyl-indole-1-*N*-β-D-glucopyranoside, 3-methylcarboxymethyl-indole-1-*N*-β-D-glucopyranoside, and a family of previously not identified compounds, namely, 2-(4-hydroxybenzoyloxymethyl)-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile, 2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-β-D-glucopyranosyloxy-2(*E*)-butenenitrile, (*E*)-6-[3-hydroxy-4-(*O*-β-D-glucopyranosyl)phenyl]-5-hexen-2-one named dehydrorubrumin, and (*3E*,*5E*)-6-[3-hydroxy-4-(*O*-β-D-glucopyranosyl)phenyl]-3,5-hexadien-2-one named rubrumin, have been identified. Determination of the oral astringency thresholds by means of the half-tongue test revealed that the lowest thresholds of 0.3 and 1.0 nmol/L were found for the nitrogen-containing 3-carboxymethyl-indole-1-*N*-β-D-glucopyranoside and 3-methylcarboxymethyl-indole-1-*N*-β-D-glucopyranoside, which do not belong to the group of plant polyphenols.

KEYWORDS: Red currant; astringency; taste; taste dilution analysis

INTRODUCTION

Red currants (*Ribes rubrum*) have beautiful red, tart berries growing in grape-like clusters and are appreciated for their delicate flavor as a key ingredient in various food products such as fruit juices, fruit soups, purees, jams, and summer puddings. Besides their attractive aroma, the typical sour and astringent taste, in particular, is one of the key parameters determining the sensory quality of red currants and their products. The astringent mouthfeel is perceived as a long-lasting puckering, shrinking, rough, and drying sensation in the oral cavity and can enhance the complexity and palate length of fruit products. Although multiple attempts have been made to find a correlation between the results obtained from sensory panelists and the chemical species imparting the typical astringent sensation of fruits, the data reported in the literature on the key taste components are rather contradictory. In general, polyphenols are believed to be the key contributors to fruit astringency.

Although there is almost no information available on the taste compounds of red currant berries, numerous phenolic compounds have been identified, among them flavan-3-ols, flavon-3-ol glycosides, flavon-3-ol glycoside malonates, hydroxycinnamic acids, hydroxybenzoic acids, and anthocyanins (*1*−*5*). More precisely, catechin, epicatechin, epicatechin gallate, 4→8-linked catechin, and gallocatechin dimers have been reported in red currants (*3*). In addition, a branched quercetin triglycoside was isolated from red currant leaves and was reported to be also present in red currant juice but not in black currants (*5*). It is, however, as yet not known which of these compounds are responsible for the intense astringent perception of red currants or whether some unknown compounds are the key inducers of that oral sensation.

To answer the question as to which nonvolatile, key taste compounds are responsible for the attractive taste of food products, we have recently developed the so-called taste dilution analysis as a powerful screening procedure for taste-active nonvolatiles in foods (*6*). This approach, combining instrumental analysis and human bioresponse, recently led to the discovery of various previously unknown taste compounds such as thermally generated bitter compounds (*6*), cooling compounds in dark malt (*7*), bitter off-tastants in carrot products (*8*), the taste enhancer alapyridaine in beef bouillon (*9*), and astringent key taste compounds in black tea infusions (*10*) and roasted cocoa nibs (*11*).

Aimed at defining the astringent oral sensation induced by red currants on a molecular level, the objectives of the present investigation were, therefore, to screen a red currant puree for its key taste compounds by application of taste dilution techniques, to isolate and determine the chemical structure of the compounds inducing the most intense human taste response, and to evaluate their astringency impact on the basis of their oral recognition threshold concentrations.

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MATERIALS AND METHODS

Chemicals. The following compounds were obtained commercially: 1-(trimethylsilyl)imidazole, glucose, galactose, and rhamnose (Fluka, Neu-Ulm, Germany); (*E*)- and (*Z*)-aconitic acid and pyridine (Sigma, Steinheim, Germany); hydrochloric acid, Na_2CO_3, and hydroxylamine hydrochloride (Merck, Darmstadt, Germany); quercetin-3-*O*-*β*-D-glucopyranoside, quercetin-3-*O*-*β*-D-galactopyranoside, and quercetin-3-O-*β*-rutinoside (Roth, Karlsruhe, Germany); kaempferol-3-*O*-*β*-rutinoside and kaempferol-3-*O*-*β*-glucopyranoside (Extrasynthese, Geney Cedex, France). Solvents were of HPLC grade (Merck). Deuterated solvents were obtained from Euriso-Top (Gif-Sur-Yvette, France). Fresh puree, which was made from red currant fruits harvested in 2003, was obtained from the food industry and kept frozen at −26 °C until used. Bottled water (Evian; low mineralization, 484 mg/L) adjusted to pH 4.5 with aqueous hydrochloric acid (0.1 mol/L) was used for the sensory experiments.

Sensory Analyses. *Training of the Sensory Panel.* Twelve subjects (five women and seven men, aged 25−38 years), who had given informed consent to participate in the sensory tests of the present investigation and had no history of known taste disorders, were trained to evaluate the taste of aqueous solutions (2 mL each) of the following standard taste compounds in bottled water (pH 4.5) by using a triangle test: sucrose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (20 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, and sodium glutamate (3 mmol/L) for umami taste. For the puckering astringency and the velvety astringent, mouth-drying 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 (*10*). The assessors had participated earlier at regular intervals for at least 2 years in sensory experiments and were, therefore, familiar with the techniques applied. Sensory analyses were performed in a sensory panel room at 22−25 °C in three different sessions.

Pretreatment of Fractions. Prior to sensory analysis, the fractions or compounds isolated were suspended in water and, after removal of the volatiles under high vacuum (<5 mPa), were freeze-dried twice. GC-MS and ion chromatographic analysis revealed that food fractions treated by that procedure are essentially free of the solvents and buffer compounds used.

Half-Tongue Test. Taste dilution factors as well as human astringency recognition thresholds were determined by means of the recently developed half-tongue test (*10*, *11*) using bottled water as the solvent. Serial 1:1 dilutions of the samples were presented in order of increasing concentrations to a trained panel of 12 persons in three different sessions using the sip-and-spit method. When the panelist selected correctly, 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 to last concentration was calculated and taken as the individual recognition threshold. The values between individuals and between five separate sessions differed by not more than plus or minus one dilution step.

Sequential Solvent Extraction of Red Currant Puree. A mixture of red currant puree (10 kg) and methanol (40 L) was vigorously stirred for 1 h at room temperature under an atmosphere of argon. After filtration, the residual fruit material was extracted with a mixture (7:3, v/v; 3 × 10 L) of methanol and water adjusted to pH 4.0 with aqueous formic acid (1% in water) for 1 h. After filtration, all of the liquid layers were combined and freed from methanol under vacuum to obtain the methanol extractables. After the addition of water (3 L), the methanol solubles were extracted with ethyl acetate (5 × 1.5 L), and the combined organic layers were freed from solvent under vacuum to give the fraction I, whereas the remaining aqueous phase was freed from solvent under vacuum and freeze-dried to give fraction II. The residual fruit material was then extracted with a mixture (6:4, v/v; 5 × 2 L) of acetone and water adjusted to pH 4.0 with aqueous formic acid (1% in water) for 45 min with stirring. After filtration, the liquid layer was freed from acetone under reduced pressure to give the acetone extractables (fraction III), whereas the remaining fruit material was freeze-dried to give the insoluble residue (fraction IV). The individual fractions were freeze-dried twice to remove trace amounts of solvents, their yields were determined by weight, and their taste profiles were evaluated in aqueous solutions as given in **Table 1**.

Table 1. Yields and Sensorial Evaluation of Fractions I–IV Isolated from Red Currant Puree (RCP)

		intensity[a] perceived for			
sample[b]	yield[c] (g/100 g)	bitterness	sourness	astringency	sweetness
RCP		0.5	5.0	3.0	2.0
fraction I	2.4	2.0	3.2	2.9	1.0
fraction II	85.4	0.3	5.0	3.1	1.6
fraction III	1.3	0	0.2	0.7	0
fraction IV	10.9	<0.5	<0.5	<0.5	<0.5

[a] The taste intensity of aqueous solutions of the "natural" concentrations of the individual fractions in bottled water (0.5 L; pH 4.5) was rated on a scale from 0 (not detectable) to 5.0 (strongly detectable). [b] Individual fractions obtained from RCP by sequential solvent extraction. [c] Yields were determined by weight; based on dry weight.

Absorption Chromatography on Polyamide. An aliquot (100 g) of fraction II isolated from red currant puree was dissolved in water (500 mL) and, then, applied onto the top of a glass column (300 × 60 mm) filled with a slurry of Polyamide MN-SC-6 (Macherey & Nagel, Düren, Germany) conditioned by rinsing with methanol (1.5 L) and finally with water (3 L). Chromatography was performed with water (1 L) to give the PA-fraction I, followed by methanol (4 L), and, finally, a mixture (99:1, v/v; 1 L) of methanol and formic acid. The methanol and methanol/formic acid fractions were combined and freed from solvent under vacuum to give the PA-fraction II. All fractions were freeze-dried twice to remove trace amounts of solvents and were stored at −20 °C until use.

Gel Permeation Chromatography (GPC). An aliquot (~4 g) of fraction I isolated from red currant puree was dissolved in a mixture (40:60, v/v; 20 mL) of methanol and water adjusted to pH 4.0 with aqueous formic acid (1% in water) and then 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 (40:60, v/v; pH 4.0; 0.9 L), followed by methanol/water (50:50, v/v; pH 4.0; 0.9 L), methanol/water (60:40, v/v; pH 4.0; 2.7 L), methanol/water (80:20, v/v; pH 4.0; 0.9 L), and, finally, 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 16 subfractions were collected from fraction I, and the individual fractions were freed from solvent under vacuum and were then freeze-dried twice. The residue of each GPC fraction was used for the taste dilution analysis as well as for chemical analysis.

Using the same GPC equipment, an aliquot (~2 g) of the PA-fraction II was dissolved in a mixture (20:80, v/v; 20 mL) of methanol and water (adjusted to pH 4.0 with formic acid) and was then chromatographically separated into 14 subfractions by sequentially eluting the Sephadex LH-20 column with the following solvent mixtures: methanol/water (20:80, v/v; pH 4.0; 0.9 L), methanol/water (40:60, v/v; pH 4.0; 0.9 L), methanol/water (50:50, v/v; pH 4.0; 0.9 L), methanol/water (60:40, v/v; pH 4.0; 2.7 L), methanol/water (80:20, v/v; pH 4.0; 0.9 L), and, finally, methanol (2.7 L).

Taste Dilution Analysis (TDA). Aliquots of the GPC fractions and the HPLC fractions, respectively, were dissolved in "natural" ratios in exactly 5.0 mL of bottled water (pH 4.5) and, then, sequentially diluted 1:1 with bottled water. 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 (*10*, *11*). 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 (TD) factor (*6*). The TD factors evaluated by three 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.

HPLC Separation of Fraction I, PA-Fraction II, and GPC Fractions, Respectively. Solvent fraction I, the PA-fraction II, and the individual GPC fractions, 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 an 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. The LC program consisted of a linear gradient starting from 5% B to 17% B in 35 min, followed by 17% B for 15 min, a linear gradient from 17% B to 50% B in 20 min, and a linear gradient from 50% B to 100% B in 10 min. Before each injection, the column was equilibrated for 15 min at the starting conditions. The effluent containing a target taste compound was collected from three separate HPLC runs, combined, freed from solvent under vacuum, and freeze-dried twice, and the residues obtained were used for chemical analysis as well as for TDA.

Isolation and Identification of (*Z*)- and (*E*)-Aconitic acid. GPC fraction III, obtained from solvent fraction I, was separated by preparative HPLC using the conditions reported above, and both isomers of aconitic acid were isolated and identified by means of LC-MS and NMR spectroscopy. The spectroscopic data are identical to those obtained for the commercially available reference compounds of (*Z*)-aconitic acid (**1**) and (*E*)-aconitic acid (**2**).

Isolation and Characterization of Polyphenol Glycosides. GPC fractions I−V, obtained from solvent fraction I, were separated by preparative HPLC using the condition reported above. HPLC-MS-MS and NMR analyses led to the unequivocal identification of benzyl-*O*-β-D-glucopyranoside (**3**) in GPC fraction I/II, (*Z*)-*p*-coumaric acid 4-*O*-β-D-glucopyranoside (**4**) and (*E*)-*p*-coumaric acid 4-*O*-β-D-glucopyranoside (**5**) in GPC fraction II/III, and (*E*)-6-[3-hydroxy-4-(*O*-β-D-glucopyranosyl)phenyl]-5-hexen-2-one (**10**) and (*3E,5E*)-6-[3-hydroxy-4-(*O*-β-D-glucopyranosyl)phenyl]-3,5-hexadien-2-one (**11**) in GPC fraction IV/V. In addition, caffeic acid 4-*O*-β-D-glucopyranoside (**12**) was isolated from HPLC fraction 7 of PA-fraction II, and maesopsin-4-*O*-β-D-glucopyranoside (**24**) was obtained from HPLC fraction 12 of PA-fraction II.

*Benzyl-O-β-D-glucopyranoside (**3**):* UV−vis (acetonitrile), λ_{max} 203, 255 nm; LC-MS (ESI^-), *m/z* 269 (100, $[M - H]^-$), 161 (60, $[M - H - 108]^-$); ^1H NMR (400 MHz, MeOD; COSY), δ 3.25 [dd, 1H, *J* = 8.2, 8.2 Hz, H−C(2′)], 3.35 [dd, 1H, *J* = 9.1, 9.1 Hz, H−C(4′)], 3.39 [m, 1H, H−C(5′)], 3.40 [dd, 1H, *J* = 8.8, 8.8 Hz, H−C(3′)], 3.66 [dd, 1H, *J* = 5.9, 11.9 Hz, H−C(6a′)], 3.86 [dd, 1H, *J* = 2.0, 11.9 Hz, H−C(6b′)], 4.47 [d, 1H, *J* = 8.1 Hz, H−C(1′)], 4.67 [d, 1H, *J* = 11.5 Hz, H−C(1a*)], 4.88 [d, 1H, *J* = 11.5 Hz, H−C(1b*)], 7.38 [m, 5H, H−C(2), H−C(3), H−C(4), H−C(5), H−C(6)]; ^{13}C NMR (100 MHz, MeOD; HMQC, HMBC), δ 60.8 [C-(6′)], 69.7 [C-(4′)], 71.4 [C-(1*)], 73.1 [C-(2′)], 75.8 [C-(5′)], 75.8 [C-(3′)], 101.0 [C-(1′)], 128.5 [C-(2), C-(3), C-(4), C-(5), C-(6)], 136.7 [C-(1)].

*(Z)-p-Coumaric acid 4-O-β-D-glucopyranoside (**4**):* UV−vis (acetonitrile), λ_{max} 279 nm; LC-MS (ESI^+), *m/z* 349 (100, $[M + Na]^+$), 187 (54, $[M + Na - glc]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 3.35 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(4′)], 3.42 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(3′)], 3.44 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(2′)], 3.49 [m, 1H, H−C(5′)], 3.60 [dd, 1H, *J* = 5.8, 12.1 Hz, H−C(6a′)], 3.78 [dd, 1H, *J* = 2.3, 12.1 Hz, H−C(6b′)], 5.01 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 5.85 [d, 1H, *J* = 12.6 Hz, H−C(7**)], 7.71 [d, 1H, *J* = 12.6 Hz, H−C(8**)], 6.96 [d, 2H, *J* = 9.0 Hz, H−C(3*), H−C(5*)], 7.56 [d, 2H, *J* = 9.0 Hz, H−C(2*), H−C(6*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 60.6 [C(6′)], 69.3 [C(5′)], 72.9 [C(3′)], 75.7 [C(4′)], 75.8 [C(2′)], 99.9 [C(1′)], 116.0 [C(3*)], 116.0 [C(5*)], 120.9 [C(8**)], 130.5 [C(2*)], 130.5 [C(6*)], 137.7 [C(7**)], 156.5 [C(4*)], 173.3 [C(9**)], 169.1 [C(9*)].

*(E)-p-Coumaric acid 4-O-β-D-glucopyranoside (**5**):* UV−vis (acetonitrile), λ_{max} 234, 295; LC-MS (ESI^+), *m/z* 349 (100, $[M + Na]^+$), 187 (54, $[M + Na - glc]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 3.43 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(4′)], 3.45 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(3′)], 3.50 [dd, 1H, *J* = 8.0, 8.0 Hz, H−C(2′)], 3.50 [m, 1H, H−C(5′)], 3.72 [dd, 1H, *J* = 5.9, 12.3 Hz, H−C(6a′)], 3.92 [dd, 1H, *J* = 2.2, 12.3 Hz, H−C(6b′)], 4.99 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 6.39 [d, 1H, *J* = 15.7 Hz, H−C(8*)], 7.14 [d, 2H, *J* = 9.0 Hz, H−C(3*), H−C(5*)], 7.58 [d, 2H, *J* = 9.0 Hz, H−C(2*), H−C(6*)], 7.65 [d, 1H, *J* = 15.7 Hz, H−C(7*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 61.2 [C(6′)], 69.8 [C(5′)], 71.6 [C(3′)], 73.4 [C(4′)], 76.6 [C(2′)], 100.2 [C(1′)], 116.1 [C(8*)], 116.4 [C(3*)], 116.4 [C(5*)], 128.4 [C(1*)], 129.1 [C(2*)], 129.1 [C(6*)], 144.4 [C(7*)], 159.4 [C(4*)], 169.1 [C(9*)].

*Caffeic acid 4-O-β-D-glucopyranoside (**12**):* UV−vis (acetonitrile) λ_{max} 227, 311; LC-MS (ESI^+), *m/z* 343 (100, $[M + Ha]^+$), 181 (52, $[M + H - glc]^+$); ^1H NMR (400 MHz, D_2O; COSY), δ 3.45 [m, 1H, H−C(3′)], 3.49 [m, 1H, H−C(2′)], 3.58 [m, 1H, H−C(4′)], 3.60 [m, 1H, H−C(5′)], 3.69 [dd, *J* = 6.0, 12.1 Hz, 1H, H−C(6a′)], 3.89 [dd, *J* = 2.0, 12.1 Hz, 1H, H−C(6b′)], 5.09 [d, *J* = 7.6 Hz, 1H, H−C(1′)], 6.32 [d, *J* = 16.0 Hz, 1H, H−C(8*)], 6.94 [d, *J* = 8.1 Hz, 1H, H−C(5*)], 7.21 [dd, *J* = 2.0, 8.1 Hz, 1H, H−C(6*)], 7.35 [d, *J* = 16.0 Hz, 1H, H−C(7*)], 7.36 [d, *J* = 2.0 Hz, 1H, H−C(2*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 61.1 [C(6′)], 69.7 [C(5′)], 71.4 [C(3′)], 73.2 [C(4′)], 76.3 [C(2′)], 99.8 [C(1′)], 115.8 [C(8*)], 116.2 [C(5*)], 112.8 [C(2*)], 119.3 [C(6*)], 127.9 [C(1*)], 144.6 [C(4*)], 144.9 [C(3*)], 145.1 [C(7*)], 171.3 [C(9*)].

*(E)-6-[3-Hydroxy-4-(O-β-D-glucopyranosyl)phenyl]-5-hexen-2-one (**10**):* UV−vis (acetonitrile), λ_{max} = 211, 255 nm; LC-TOF/MS, $C_{18}H_{24}O_8$; LC-MS (ESI^-), *m/z* 367 (100, $[M - H]^-$), 205 (56, $[M - H - glc]^-$); ^1H NMR (400 MHz, MeOD; COSY), δ 2.06 [s, 3H, H−C(1)], 2.30 [m, 2H, H−C(4)], 2.54 [t, 2H, *J* = 7.5 Hz, H−C(3)], 3.31 [m, 1H, H−C(4′)], 3.34 [m, 1H, H−C(5′)], 3.38 [m, 1H, H−C(3′)], 3.40 [m, 1H, H−C(2′)], 3.61 [dd, 1H, *J* = 5.7, 12.1 Hz, H−C(6a′)], 3.83 [dd, 1H, *J* = 2.0, 12.1 Hz, H−C(6b′)], 4.65 [d, 1H, *J* = 7.5 Hz, H−C(1′)], 5.96 [m, 1H, H−C(5)], 6.20 [d, 1H, *J* = 16.3 Hz, H−C(6)], 6.65 [d, 1H, *J* = 8.4 Hz, H−C(2*)], 6.79 [dd, 1H, *J* = 2.0, 8.4 Hz, H−C(6*)], 7.16 [d, 1H, *J* = 2.0 Hz, H−C(5*)]; ^{13}C NMR (100 MHz, MeOD; HMQC, HMBC), δ 60.6 [C(6′)], 26.8 [C(4)], 28.3 [C(1)], 42.3 [C(3)], 61.1 [C(6)], 70.1 [C(4′)], 73.5 [C(3′)], 75.6 [C(2′)], 77.3 [C(5′)], 103.0 [C(1′)], 114.8 [C(5*)], 115.4 [C(2*)], 121.6 [C(6*)], 125.9 [C(5)], 126.2 [C(1*)], 129.8 [C(6)], 144.9 [C(4*)], 146.6 [C(3*)], 209.6 [C(2)].

*(3E,5E)-6-[3-Hydroxy-4-(O-β-D-glucopyranosyl)phenyl]-3,5-hexadien-2-one (**11**):* UV−vis (acetonitrile), λ_{max} 251, 351 nm; LC-TOF/MS, $C_{18}H_{22}O_8$; LC-MS (ESI^-), *m/z* 365 (100, $[M - H]^-$), 203 (56, $[M - H - glc]^-$); ^1H NMR (400 MHz, MeOD; COSY), δ 2.20 [s, 3H, H−C(1)], 3.27 [m, 1H, H−C(3′)], 3.38 [m, 1H, H−C(4′)], 3.39 [m, 1H, H−C(5′)], 3.42 [m, 1H, H−C(2′)], 3.60 [dd, 1H, *J* = 6.6, 11.9 Hz, H−C(6a′)], 3.86 [dd, 1H, *J* = 2.2, 11.9 Hz, H−C(6b′)], 4.71 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 6.14 [d, 1H, *J* = 15.7 Hz, H−C(3)], 6.74 [d, 1H, *J* = 8.4 Hz, H−C(5*)], 6.82 [dd, 1H, *J* = 10.2, 15.4 Hz, H−C(5)], 6.86 [d, 1H, *J* = 15.4 Hz, H−C(6)], 7.02 [dd, 1H, *J* = 2.0, 8.4 Hz, H−C(6*)], 7.32 [dd, 1H, *J* = 10.2, 15.7 Hz, H−C(4)], 7.43 [d, 1H, *J* = 2.9 Hz, H−C(2*)]; ^{13}C NMR (100 MHz, MeOD; HMQC, HMBC), δ 25.5 [C(1)], 61.1 [C(6′)], 70.1 [C(3′)], 73.4 [C(2′)], 76.1 [C(5′)], 77.4 [C(4′)], 103.0 [C(1′)], 115.7 [C(2*)], 115.9 [C(5*)], 123.7 [C(1*)], 123.8 [C(6*)], 124.3 [C(5)], 128.5 [C(3)], 141.8 [C(6)], 145.5 [C(4)], 145.5 [C(4*)], 148.3 [C(3*)], 200.1 [C(2)].

*Maesopsin-4-O-β-D-glucopyranoside (**24**):* UV−vis (MeOH), λ_{max} 210, 228, 280 nm; LC-MS (ESI^-), *m/z* 449 (100, $[M - H]^-$), 287 (64, $[M - H - glc]^-$); ^1H NMR (400 MHz, MeOD; COSY), δ 2.98 [s, 2H, H−C(a)], 3.30 [m, 1H, H−C(5′)], 3.30 [m, 1H, H−C(4′)], 3.37 [m, 1H, H−C(3′)], 3.40 [m, 1H, H−C(2′)], 3.57 [dd, 1H, *J* = 6.0, 12.1 Hz, H−C(6a′)], 3.76 [dd, 1H, *J* = 1.7, 12.1 Hz, H−C(6b′)], 4.75 [dd, 1H, *J* = 6.7 Hz, H−C(1′)], 5.83 [m, 1H, H−C(7)], 5.94 [d, 1H, *J* = 1.5 Hz, H−C(5)], 6.46 [dd, 2H, *J* = 5.8, 8.5 Hz, H−C(3*), H−C(5*)], 6.88 [d, 2H, *J* = 8.5 Hz, H−C(2*), H−C(6*)]; ^{13}C NMR (100 MHz, MeOD; HMQC, HMBC): δ 40.6 [C(a)], 60.9 [C(6′)], 69.8 [C(4′)], 72.4 [C(2′)], 76.0 [C(3′)], 76.9 [C(5′)], 91.6 [C(7)], 95.9 [C(5)], 102.2 [C(1′)], 106.1 [C(3a)], 106.3 [C(2)], 114.3 [C(3*)], 114.3 [C(5*)], 124.1 [C(1*)], 130.9 [C(2*)], 130.9 [C(6*)], 155.9 [C(4′)], 156.8 [C(4)], 173.0 [C(7a)], 195.3 [C(3)].

Isolation and Characterization of Astringent Indoles (6, 7) and Nitriles (8, 9). The structure determination of the nitrogen-containing compounds **6**−**9** (**Figure 2**) in HPLC fractions I/8 (**6**), 9 (**7**), 13 (**8**), and 14 (**9**), as well as the details of the NMR, MS, and synthesis demonstrating the chemical identity of those compounds in red currant juice, is published in a companion paper.

Preparative Isolation of Flavonol *O*-Glycosides from Red Currant Leaves. Freshly picked red currant leaves (100 g) were homogenized in methanol/water (70:30, v/v; 3 × 500 mL). After filtration, the extract was concentrated under vacuum, and then applied onto the top of a glass column (300 × 60 mm) filled with a slurry of Polyamide MN-SC-6 (Macherey & Nagel, Düren, Germany) conditioned by rinsing with methanol (1.5 L) and finally with water (3 L). After elution of nonphenolic components with water (1 L), six or three fractions were collected by flushing the column with methanol (100 mL each) or methanol/formic acid (99:1, v/v; 100 mL each), respectively, and were then freed from solvent under vacuum and freeze-dried twice. Aliquots of the second polyamide fraction (500 mg each) were dissolved in a mixture (5:95, v/v) of acetonitrile and water, and, after membrane filtration, aliquots (2 mL) were separated by HPLC on a 250 × 21.2 mm i.d., 5 μm, RP-18 ODS-Hypersil column (ThermoHypersil, Kleinostheim, Germany). Monitoring the effluent at 345 nm, HPLC was performed with mixtures of aqueous formic acid (1.5% in water; solvent A) and acetonitrile (solvent B) using a binary gradient at a flow rate of 20 mL/min. The LC program consisted of a linear gradient increasing solvent B from 5 to 17% within 35 min, then maintained at 17% B for 15 min, then increasing solvent B from 17 to 50% within 20 min, and, finally, increasing the amount of solvent B to 100% within 10 min. Before each injection, the column was equilibrated for 15 min at the starting conditions. HPLC−degustation, HPLC-DAD, HPLC-MS/MS, and NMR analysis led to the detection of six velvety astringent flavonol glycosides, namely, quercetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside (**14**) in HPLC PA-fraction II/7, kaempferol-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside (**13**) in HPLC PA-fraction II/9, myricetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**15**) in HPLC PA-fraction II/15, quercetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**16**) in HPLC PA-fraction II/16, kaempferol-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**18**) in HPLC PA-fraction II/18, and kaempferol-3-*O*-β-D-(6′-malonyl)glucopyranoside (**19**) in HPLC PA-fraction II/23.

*Quercetin-3-O-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-O-β-D-glucopyranoside (**14**):* UV−vis (acetonitrile), λ_{max} 243, 345 nm; LC-TOF/MS, $C_{39}H_{50}O_{25}$; LC-MS (ESI$^-$), *m/z* 917 (100, [M − H]$^-$), 755 (82, [M − H − 162]$^-$), 463 (50, [M − H − 162 − 2 × 146]$^-$), 301 (33, [M − H − 162 − 2 × 146 − 162]$^-$); ^{1}H NMR (400 MHz, D_2O; COSY), δ 0.92 [d, 3H, *J* = 6.2 Hz, H−C(6‴)], 1.04 [d, 3H, *J* = 6.2 Hz, H−C(6″)], 3.17 [dd, 1H, *J* = 9.5, 9.5 Hz, H−C(4‴)], 3.2−3.65 [m, 3H, H−C(3‴), H−C(4‴), H−C(5‴)], 3.22 [m, 1H, H−C(5‴)], 3.26 [m, 1H, H−C(6a′)], 3.28 [m, 1H, H−C(6b′)], 3.37 [m, 1H, H−C(3‴)], 3.40 [m, 1H, H−C(3′)], 3.48 [m, 1H, H−C(4‴)], 3.54 [m, 1H, H−C(2‴)], 3.57 [m, 1H, H−C(4′)], 3.59 [m, 1H, H−C(2‴)], 3.61 [m, 1H, H−C(2′)], 3.69 [m, 1H, H−C(5′)], 3.74 [m, 1H, H−C(6a‴)], 3.93 [m, 1H, H−C(6b‴)], 3.94 [dd, 1H, H−C(3″)], 4.10 [m, 1H, H−C(2″)], 4.26 [m, 1H, H−C(5″)], 4.49 [d, 1H, *J* = 1.5 Hz, H−C(1‴)], 5.12 [d, 1H, *J* = 1.5 Hz, H−C(1″)], 5.17 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 5.25 [d, 1H, *J* = 7.3 Hz, H−C(1‴)], 6.50 [s, 1H, H−C(6)], 6.76 [s, 1H, H−C(8)], 6.97 [d, 1H, *J* = 8.5 Hz, H−C(5*)], 7.57 [dd, 1H, *J* = 2.2, 8.5 Hz, H−C(6*)], 7.63 [d, 1H, *J* = 2.2 Hz, H−C(2*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 16.1 [C(6‴)], 16.1 [C(6″)], 60.8 [C(6‴)], 67.6 [C(5′)], 68.3 [C(5‴)], 69.3 [C(5″)], 70−78 [C(2‴), C(3‴), C(4‴), C(5‴)], 70.0 [C(6′)], 70.0 [C(2′)], 70.0 [C(3‴)], 70.1 [C(2″)], 70.1 [C(3″)], 71.7 [C(4‴)], 72.0 [C(4″)], 74.8 [C(3′)], 76.4 [C(2‴)], 78.4 [C(4′)], 95.4 [C(8)], 99.3 [C(1‴)], 99.4 [C(6)], 99.4 [C(1′)], 100.9 [C(1″)], 101.2 [C(1‴)], 104.6 [C(4a)], 115.6 [C(5*)], 116.7 [C(2*)], 121.9 [C(1*)], 122.9 [C(6*)], 133.2 [C(3)], 143.3 [C(3*)], 147.1 [C(2)], 156.3 [C(4*)], 158.0 [C(8a)], 160.2 [C(5)], 162.2 [C(7)], 178.2 [C(4)].

*Kaempferol-3-O-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-O-β-D-glucopyranoside (**13**):* UV−vis (acetonitrile), λ_{max} 255, 339 nm; LC-TOF/MS, $C_{39}H_{50}O_{24}$; LC-MS (ESI$^-$), *m/z* 901 (100, [M − H]$^-$), 739 (82, [M − H − 162]$^-$), 447 (50, [M − H − 162 − 2 × 146]$^-$), 285 (32, [M − H − 162 − 2 × 146 − 162]$^-$); ^{1}H NMR (400 MHz, D_2O; COSY), δ 0.91 [d, 3H, *J* = 6.2 Hz, H−C(6‴)], 1.10 [d, 3H, *J* = 6.2 Hz, H−C(6″)], 3.17 [dd, 1H, *J* = 9.7, 9.7 Hz, H−C(4‴)], 3.2−3.65 [m, 3H, H−C(3‴), H−C(4‴), H−C(5‴)], 3.24 [m, 1H, H−C(5‴)], 3.26 [m, 1H, H−C(6a′)], 3.28 [m, 1H, H−C(6b′)], 3.36 [m, 1H, H−C(3‴)], 3.41 [m, 1H, H−C(3′)], 3.47 [m, 1H, H−C(4″)], 3.57 [m, 1H, H−C(4′)], 3.57 [m, 1H, H−C(2‴)], 3.58 [m, 1H, H−C(2‴)], 3.58 [m, 1H, H−C(2′)], 3.69 [m, 1H, H−C(5′)], 3.93 [m, 1H, H−C(3″)], 4.10 [m, 1H, H−C(2″)], 4.23 [m, 1H, H−C(5″)], 4.43 [d, 1H, *J* = 1.5 Hz, H−C(1″)], 5.13 [d, 1H, *J* = 7.2 Hz, H−C(1‴)], 5.14 [d, 1H, *J* = 1.5 Hz, H−C(1‴)], 5.18 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 6.44 [d, 1H, *J* = 2.0 Hz, H−C(6)], 6.65 [d, 1H, *J* = 2.0 Hz, H−C(8)], 6.85 [d, 2H, *J* = 8.8 Hz, H−C(3*), H−C(5*)], 7.89 [d, 2H, *J* = 8.8 Hz, H−C(2*), H−C(6*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 16.3 [C(6‴)], 16.3 [C(6″)], 60.8 [C(6‴)], 67.6 [C(5′)], 68.1 [C(5‴)], 69.3 [C(5″)], 70−78 [C(2‴), C(3‴), C(4‴), C(5‴)], 70.0 [C(6′)], 70.1 [C(3″)], 70.2 [C(2′)], 70.2 [C(3‴)], 70.6 [C(2″)], 71.7 [C(4‴)], 72.0 [C(4″)], 74.8 [C(3′)], 76.4 [C(2‴)], 78.4 [C(4′)], 95.5 [C(8)], 99.2 [C(6)], 99.2 [C(1‴)], 99.3 [C(1′)], 100.7 [C(1″)], 101.0 [C(1‴)], 115.0 [C(3*)], 115.0 [C(5*)], 104.6 [C(4a)], 121.7 [C(1*)], 131.2 [C(2*)], 131.2 [C(6*)], 133.2 [C(3)], 143.2 [C(2)], 156.1 [C(4*)], 158.5 [C(8a)], 160.0 [C(5)], 162.2 [C(7)], 178.2 [C(4)].

*Myricetin-3-O-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**15**):* UV−vis (acetonitrile), λ_{max} 249, 345 nm; LC-TOF/MS, $C_{33}H_{40}O_{21}$; LC-MS (ESI$^+$), *m/z* 773 (100, [M + H]$^+$), 627 (81, [M + H − 146]$^-$), 481 (62, [M + H − 2 × 146]$^-$), 319 (41, [M + H − 2 × 146 − 162]$^-$); ^{1}H NMR (400 MHz, D_2O; COSY), δ 1.05 [d, 3H, *J* = 6.0 Hz, H−C(6‴)], 1.10 [d, 3H, *J* = 6.0 Hz, H−C(6″)], 3.17 [dd, 1H, *J* = 9.4, 9.4 Hz, H−C(4‴)], 3.22 [m, 1H, H−C(5‴)], 3.26 [m, 1H, H−C(6a′)], 3.28 [m, 1H, H−C(6b′)], 3.37 [m, 1H, H−C(3‴)], 3.40 [m, 1H, H−C(3′)], 3.48 [m, 1H, H−C(4‴)], 3.57 [m, 1H, H−C(4′)], 3.59 [m, 1H, H−C(2‴)], 3.61 [m, 1H, H−C(2′)], 3.69 [m, 1H, H−C(5′)], 3.94 [m, 1H, H−C(3‴)], 4.10 [m , 1H, H−C(2″)], 4.26 [m, 1H, H−C(5″)], 4.53 [d, 1H, *J* = 1.5 Hz, H−C(1″)], 5.23 [d, 1H, *J* = 1.5 Hz, H−C(1‴)], 5.58 [d, 1H, *J* = 7.8 Hz, H−C(1′)], 6.20 [d, 1H, *J* = 2.3 Hz, H−C(6)], 6.39 [d, 1H, *J* = 2.3 Hz, H−C(8)], 7.25 [s, 2H, H−C(2*, 6*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 16.0 [C(6‴)], 16.3 [C(6″)], 67.6 [C(5′)], 68.3 [C(5‴)], 69.3 [C(5″)], 70.0 [C(6′)], 70.0 [C(2′)], 70.0 [C(3‴)], 70.1 [C(2″)], 70.1 [C(3″)], 71.7 [C(4‴)], 72.0 [C(4″)], 74.8 [C(3′)], 76.4 [C(2‴)], 78.4 [C(4′)], 93.1 [C(8)], 98.3 [C(6)], 99.1 [C(1′)], 100.7 [C(1″)], 101.3 [C(1‴)], 104.9 [C(4a)], 108.8 [C(2*, 6*)], 121.0 [C(1*)], 133.2 [C(3)], 136.7 [C(4*)], 145.1 [C(3*, 5*)], 157.7 [C(2)], 157.8 [C(8a)], 162.3 [C(5)], 165.3 [C(7)], 179.3 [C(4)].

*Quercetin-3-O-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**16**):* UV−vis (acetonitrile), λ_{max} 243, 345 nm; LC-MS (ESI$^+$), *m/z* 779 (100, [M + Na]$^+$), 633 (81, [M + Na − 146]$^+$), 477 (61, [M + Na − 2 × 146]$^+$, [M + Na − 302]$^+$), 331 (40, [M + Na − 302 − 146]$^+$, 185 (24, [M + Na − 2 × 146 − 302]$^+$); ^{1}H NMR (400 MHz, D_2O; COSY), δ 0.94 [d, 3H, *J* = 6.3 Hz, H−C(6‴)], 1.25 [d, 3H, *J* = 6.0 Hz, H−C(6″)], 3.17 [dd, 1H, *J* = 9.6 Hz, H−C(4‴)], 3.22 [m, 1H, H−C(5‴)], 3.26 [m, 1H, H−C(6a′)], 3.28 [m, 1H, H−C(6b′)], 3.37 [m, 1H, H−C(3‴)], 3.40 [m, 1H, H−C(3′)], 3.48 [dd, 1H, *J* = 9.6, 9.6 Hz, H−C(4″)], 3.57 [dd, 1H, *J* = 9.3, 9.3 Hz, H−C(4′)], 3.59 [m, 1H, H−C(2‴)], 3.61 [dd, 1H, *J* = 9.3, 9.3 Hz, H−C(2′)], 3.69 [m, 1H, H−C(5′)], 3.94 [dd, 1H, *J* = 3.1, 9.8 Hz, H−C(3″)], 4.10 [m, 1H, H−C(2″)], 4.26 [m, 1H, H−C(5″)], 4.42 [d, 1H, *J* = 1.5 Hz, H−C(1‴)], 5.14 [d, 1H, *J* = 7.3 Hz, H−C(1′)], 5.16 [d, 1H, *J* = 1.5 Hz, H−C(1‴)], 5.96 [s, 1H, H−C(6)], 6.13 [s, 1H, H−C(8)], 6.74 [d, 1H, *J* = 8.5 Hz, H−C(5*)], 7.23 [dd, 1H, *J* = 2.0, 8.5 Hz, H−C(6*)], 7.39 [d, 1H, *J* = 2.0 Hz, H−C(2*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 16.3 [C(6‴)], 16.6 [C(6″)], 67.6 [C(5′)], 68.3 [C(5‴)], 69.3 [C(5″)], 70.0 [C(6′)], 70.0 [C(2′)], 70.0 [C(3‴)], 70.1 [C(2″)], 70.1 [C(3‴)], 71.7 [C(4‴)], 72.0 [C(4″)], 74.8 [C(3′)], 76.4 [C(2‴)], 78.4 [C(4′)], 94.1 [C(8)], 98.8 [C(6)], 99.4 [C(1′)], 100.2 [C(1″)], 100.2 [C(1‴)], 104.6 [C(4a)], 115.3 [C(5*)], 116.6 [C(2*)], 121.9 [C(1*)], 122.7 [C(6*)], 133.2 [C(3)], 143.4 [C(3*)], 147.0 [C(2)], 156.1 [C(4*)], 158.1 [C(8a)], 160.0 [C(5)], 162.4 [C(7)], 177.4 [C(4)].

*Kaempferol-3-O-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**18**):* UV−vis (acetonitrile), λ_{max} 255, 339 nm; LC-MS (ESI$^+$), *m/z* 763 (100, [M + Na]$^+$), 617 (81, [M + Na − 146]$^+$), 477 (63, [M + Na − 286]$^+$), 331 (43, [M + Na − 286 − 146]$^+$, 185 (24, [M + Na − 2 × 146 − 286]$^+$); ^{1}H NMR (400 MHz, D_2O; COSY), δ 0.93 [d, 3H, *J* = 6.0 Hz, H−C(6‴)], 1.22 [d, 3H, *J* = 6.3 Hz, H−C(6″)], 3.17 [dd, 1H, *J* = 9.6, 9.6 Hz, H−C(4‴)], 3.24 [m, 1H, H−C(5‴)], 3.26 [m, 1H, H−C(6a′)], 3.28 [m, 1H, H−C(6b′)], 3.36 [m, 1H, H−C(3‴)],

3.41 [m, 1H, H−C(C(3′)], 3.47 [dd, 1H, J = 9.6, 9.6 Hz, H−C(4″)], 3.57 [m, 1H, H−C(4′)], 3.57 [m, 1H, H−C(2‴)], 3.58 [m, 1H, H−C(2‴)], 3.58 [m, 1H, H−C(2′)], 3.69 [m, 1H, H−C(5′)], 3.93 [dd, 1H, J = 3.3, 3.3 Hz, H−C(3″)], 4.10 [dd, 1H, J = 1.5, 1.5 Hz, H−C(2″)], 4.23 [m, 1H, H−C(5″)], 4.41 [d, 1H, J = 1.2 Hz, H−C(1″)], 5.14 [d, 1H, J = 7.5 Hz, H−C(1′)], 5.15 [d, 1H, J = 1.2 Hz, H−C(1′)], 6.00 [s, 1H, H−C(6)], 6.15 [s, 1H, H−C(8)], 6.75 [d, 2H, J = 8.8 Hz, H−C(3*), H−C(5*)], 7.71 [d, 2H, J = 8.8 Hz, H−C(2*), H−C(6*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 16.3 [C(6‴)], 16.6 [C(6″)], 67.6 [C(5′)], 68.1 [C(5‴)], 69.3 [C(5″)], 70.0 [C(6′)], 70.1 [C(3″)], 70.2 [C(3‴)], 70.2 [C(2′)], 70.6 [C(2″)], 71.7 [C(4‴)], 72.0 [C(4″)], 74.8 [C(3′)], 76.4 [C(2‴)], 78.4 [C(4′)], 94.2 [C(8)], 98.8 [C(6)], 100.7 [C(1‴)], 100.9 [C(1′)], 100.9 [C(1″)], 104.6 [C(4a)], 115.0 [C(3*)], 115.0 [C(5*)], 121.5 [C(1*)], 131.0 [C(2*)], 131.0 [C(6*)], 133.0 [C(3)], 143.1 [C(2)], 156.1 [C(4*)], 158.3 [C(8a)], 160.5 [C(5)], 162.4 [C(7)], 177.4 [C(4)].

*Kaempferol-3-O-β-D-(6′-malonyl)glucopyranoside (**19**):* UV−vis (acetonitrile), λ_{max} 255, 339 nm; LC-MS (ESI$^+$), *m*/*z* 533 (100, [M − H]$^-$), 489 (92, [M − H − 44]$^-$), 285 (53, [M − H − 204]$^-$); ^{1}H NMR (400 MHz, MeOD; COSY), δ 3.56 [m, 1H, H−C(3′)], 3.70 [m, 1H, H−C(5′)], 3.80 [m, 1H, H−C(4′)], 3.80 [m, 1H, H−C(2′)], 4.17 [m, 2H, H−C(6′)], 5.05 [d, 1H, J = 7.7 Hz, H−C(1′)], 6.24 [s, 1H, H−C(6)], 6.45 [s, 1H, H−C(8)], 6.90 [d, 2H, J = 8.8 Hz, H−C(3*, 5*)], 8.10 [d, 2H, J = 8.8 Hz, H−C(2*, 6*)]; ^{13}C NMR (100 MHz, D_2O; HMQC, HMBC), δ 64.9 [C(6′)], 69.9 [C(4′)], 72.5 [C(2′)], 74.1 [C(5′)], 74.6 [C(3′)], 94.9 [C(8)], 100.0 [C(6)], 105.6 [C(1′)], 105.6 [C(4a)], 116.1 [C(3*, 5*)], 122.7 [C(1*)], 132.5 [C(2*, 6*)], 135.7 [C(3)], 158.6 [C(2)], 159.5 [C(8a)], 161.7 [C(4*)], 163.1 [C(5)], 166.2 [C(7)], 168.4 [C(1″)], 170.2 [C(3″)], 179.7 [C(4)].

Isolation and Identification of Flavon-3-ol *O*-Glycosides. Flavon-3-ol *O*-glycosides were isolated from red currant leaves following the same isolation procedure as described for the flavon-3-ol *O*-glycosides. In addition, HPLC-DAD, HPLC-MS/MS, and comparison of chromatographic, spectroscopic, and sensory data with those obtained for the corresponding reference compounds led to the unequivocal identification of quercetin-3-*O*-β-D-glucopyranoside (**21**) in HPLC fraction 19 of PA-fraction II, quercetin-3-*O*-β-D-galactopyranoside (**20**) in HPLC fraction 20 of PA-fraction II, quercetin-3-*O*-β-rutinoside (**17**) in HPLC fraction 18 of PA-fraction II, and kaempferol-3-*O*-β-D-glucopyranoside (**23**) as well as kaempferol-3-*O*-β-D-rutinoside (**22**) in HPLC fraction 22 of PA-fraction II.

*Quercetin-3-O-β-rutinoside (**17**):* UV−vis (acetonitrile), λ_{max} 243, 345 nm; LC-MS (ESI$^+$), *m*/*z* 611 (100; [M + H]$^+$), 465 (10; [M + H − rha]$^+$), 303 (14, [M + H − rha − glc]$^+$); ^{1}H and ^{13}C NMR data were identical with those measured for the reference compound.

*Quercetin-3-O-β-D-galactopyranoside (**20**):* UV−vis (acetonitrile), λ_{max} 243, 345 nm; LC-MS (ESI$^+$), *m*/*z* 465 (100; [M + H]$^+$), 303 (53 [M + H − gal]$^+$); ^{1}H and ^{13}C NMR data were identical with those measured for the reference compound.

*Quercetin-3-O-β-D-glucopyranoside (**21**):* UV−vis (acetonitrile), λ_{max} 243, 345 nm; LC-MS (ESI$^+$), *m*/*z* 465 (100; [M + H]$^+$), 303 (53 [M + H − glc]$^+$); ^{1}H and ^{13}C NMR data were identical with those measured for the reference compound.

*Kaempferol-3-O-β-rutinoside (**22**):* UV−vis (acetonitrile), λ_{max} 255, 339 nm; LC-MS (ESI$^+$), *m*/*z* 595 (100; [M + H]$^+$), 449 (75 [M + H − rha]$^+$), 287 (48, [M + H − rha − glc]$^+$); ^{1}H and ^{13}C NMR data were identical with those measured for the reference compound.

*Kaempferol-3-O-β-D-glucopyranoside (**23**):* UV−vis (acetonitrile), λ_{max} 255, 339 nm; LC-MS (ESI$^+$), *m*/*z* 449 (100; [M + H]$^+$), 287 (47 [M + H − glc]$^+$); ^{1}H and ^{13}C NMR data were identical with those measured for the reference compound.

Analysis of Glycosidically Bound Carbohydrates. An aliquot (∼2 mg) of the target compound, dissolved in aqueous hydrochloric acid (2 mol/L; 1 mL), was placed in a closed glass vial and then heated at 110 °C for 120 min. After cooling, an aqueous solution of Na_2CO_3 (4 mol/L; 300 μL), followed by pyridine (50 μL), was added, and the solution was freeze-dried. The water-free residue was dissolved in a solution (100 μL) of 1% hydroxylamine hydrochloride in water-free pyridine and was then heated at 70 °C for 30 min. After cooling, 1-(trimethylsilyl)imidazole (100 μL) was added, and the solution was heated for an additional 30 min at 70 °C, and, after cooling, was directly injected into the HRGC-MS system.

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) either in analytical (250 × 4.6 mm i.d., flow rate = 1.0 mL/min), in semipreparative scale (250 × 10 mm i.d., flow rate = 3.5 mL/min), or in preparative scale (250 × 21.2 mm i.d., flow rate = 20 mL/min).

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

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

High-Resolution Gas Chromatography−Mass Spectrometry (HRGC-MS). Electron impact (EI) GC-MS data were acquired on an HP 6890 series gas chromatograph and an HP 5973 mass spectrometer (Hewlett-Packard/Agilent, Böblingen, Germany). Data acquisition was carried out with ChemStation software (Agilent). Chromatographic separation was performed on a 60 m × 0.25 mm, 0.25 μm fused silica J&W Scientific DB-1 capillary (Agilent), using 0.6 mL/min helium as carrier gas. The injector temperature was set at 250 °C, and the injection volume was 1 μL with split injection (1: 50). The initial oven temperature was set to 140 °C and then raised at a rate of 4 °C/min to 210 °C, thereafter at a rate of 8 °C/min to 300 °C, and, finally, held isothermally for 10 min at 300 °C. Heating the transfer line at 300 °C, the mass spectrometer was operated in the electron impact mode (EI; 70 eV electron energy) with a source temperature of 230 °C and the quadrupole heated at 150 °C. Mass spectra were acquired in the full-scan mode ranging from *m*/*z* 40 to 800 with a scan rate of 2.0 scans/s.

Nuclear Magnetic Resonance Spectroscopy (NMR). ^{1}H, ^{13}C, and 2D 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) as well as Mestre-C (Mestrelab Research, A Coruña, Spain).

RESULTS AND DISCUSSION

The juice obtained from red currant puree upon centrifugation imparted the typical sour and astringent taste and was used for taste profile analysis. To achieve this, a trained sensory panel was asked to rate the intensity of the taste qualities bitter, sour, sweet, salty, umami, and astringency on a scale from 0 (not detectable) to 5 (intensely detectable). A high score of 5.0 was found for the intensity of sourness, followed by the typical astringency sensation (3.0) and some sweetness (2.0) (**Table 1**). In addition, a faint bitter taste judged with an intensity of 0.5 was perceived, whereas saltiness and umami tastes were not detectable at all. To gain first insight into the hydrophobicity of the compounds imparting the typical astringent oral sensation, the red currant puree were extracted sequentially with solvents of different polarities.

Solvent Fractionation of Red Currants. The red currant puree was extracted with methanol and methanol/water to obtain the methanol extractables after removal of solvents under vacuum. Extraction of the methanol solubles with ethyl acetate afforded an organic layer, which was freed from solvent under

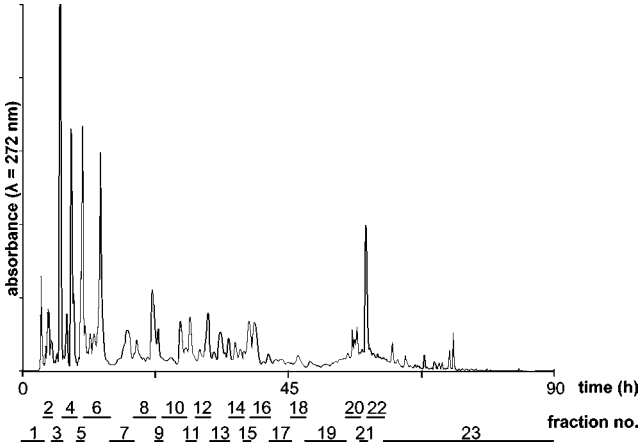

Figure 1. RP-HPLC chromatogram of fraction I isolated from red currants.

vacuum to give fraction I, and an aqueous phase yielding fraction II after lyophilization. The residual fruit material was then extracted with acetone/water to give the soluble fraction III after removal of the solvents under vacuum. The remaining fruit material was freeze-dried to give the insoluble fraction IV. After freeze-drying, the yields of the individual fractions were determined by weight. The highest yields were obtained for fractions II and IV, accounting for >96% of the dry mass of the red currant puree (**Table 1**). A comparatively low yield of 2.4% was found for fraction I, whereas fraction III was isolated in small amounts with yields of <2%.

Sensory evaluation of aqueous mixtures of the individual solvent-free fractions by means of taste profile analysis demonstrated that the nonsoluble red currant (fraction IV) was nearly tasteless, thus indicating that the key taste compounds were fully isolated by the solvent extraction (**Table 1**). Besides some faint astringency (0.7), the compounds present in fraction III also did not exhibit any significant taste quality. The highest scores for sourness (5.0), astringency (3.1), and sweetness (1.6) were found for fraction II, followed by the ethyl acetate extractables (fraction I) evaluated with somewhat lower taste intensities for sourness (3.2), astringency (2.9), and sweetness (1.0) (**Table 1**). In addition, the bitter taste of the hydrophobic fraction I was judged with an intensity of 2.0. The following investigations were focused on the identification of intensely tasting astringent compounds in fractions I and II, whereas data on the sour- and sweet-tasting compounds will be published separately.

Sensory-Guided Decomposition of Fraction I. To sort out the strongly taste-active compounds from the bulk of less taste-active or tasteless substances, first, fraction I was separated by means of preparative RP-HPLC. Monitoring the effluent at 272 nm (**Figure 1**), 23 HPLC subfractions were collected, freed from solvent in vacuum, taken up in water, and, in order to evaluate their taste impact, analyzed by means of the TDA using the recently developed half-tongue test (*10*, *11*).

As given in **Table 2**, the highest TD factor of 2048 was found for the extremely puckering astringent taste of fraction 8, followed by fractions 9, 13, and 15, which still showed astringent taste after dilutions of 1:256 and 1:128, respectively. In addition, HPLC fractions 1, 2, 3, 7, 14, and 16 exhibited a puckering astringent sensation evaluated with TD factors of 32 or 64. Besides astringency, fractions 7 and 20 also showed some bitterness detected with relatively small TD factors. Furthermore, sour taste was detectable in fraction 1, judged with a TD factor of 64, whereas for fraction 2 sourness was perceived at a dilution of 1:64; fractions 3 and 4 perceived sourness only at dilutions of equal to or less than 1:8 (**Table 2**).

Table 2. Taste Qualities and Taste Dilution (TD) Factors of HPLC Fractions Isolated from Solvent Fraction I of RCP

fraction[a]	taste quality[b]	TD factor	taste compound no.[c]
1	sour	64	**1**
	astringent	64	
2	sour	8	**2**
	astringent	64	
3	sour	4	
	astringent	32	
4	sour	1	
	astringent	4	
5	astringent	2	
6	astringent	4	
7	astringent	32	**3**
	bitter	1	
8	puckering astringent	2048	**4**–**6**
9	puckering astringent	256	**7**
10	astringent	8	
11	astringent	16	
12	astringent	16	
13	puckering astringent	128	**9**
14	puckering astringent	32	**8**
15	puckering, velvety astringent	128	**10**
16	astringent	64	**11**
17	astringent	8	
	bitter	2	
18	astringent	2	
19	nd	<1	
20	astringent	2	
	bitter	1	
21	astringent	4	
22	astringent	4	
23	astringent	4	

[a] Number of HPLC fraction referring to **Figure 1**. [b] Taste quality and TD factor were determined by using the half-tongue test. [c] Structures of compounds given as numbers are displayed in **Figure 2**.

To gain more detailed insight into the compounds imparting the astringent taste sensation perceived for those fractions evaluated with high TD factors, each individual HPLC fraction was further purified by preparative RP-HPLC and evaluated by means of the LC tasting approach. HPLC-DAD and HPLC-MS analysis and sensory experiments on the key taste compounds in HPLC fractions 1 and 2, followed by cochromatography with the corresponding reference materials, led to the unequivocal identification of (*Z*)-(**1**) and (*E*)-aconitic acid (**2**) (**Figure 2**) as the key astringent compounds in these early eluting fractions (**Table 2**).

LC-MS analysis of the key taste compound detected in HPLC fraction I/7, exhibiting astringency and some bitterness, showed a molecular mass of 270 Da. MS/MS(ESI^-) analysis of the ion peak at *m*/*z* 269 revealed a loss of 108 amu, consistent with the cleavage of a benzyl alcohol moiety, thus generating the ion peak at *m*/*z* 161 as expected for a hexose moiety. Acidic hydrolysis of an aliquot of the isolated compound and derivatization of the liberated sugar to give the persilylated aldoxime prior to HRGC-MS analysis unequivocally identified glucose as the carbohydrate moiety present in the tastant. To further confirm these findings, the glycoside was preparatively isolated from GPC-fraction I/II of solvent fraction I by RP-HPLC, and 1D and 2D NMR measurements were performed. All of the coupling constants of the sugar moiety in the molecule, and, in particular, the coupling constant of 8.1 Hz observed for the anomeric proton H−C(1′) indicated that glucose was attached to the remainder of the molecule via a β-linkage. Considering all of the spectroscopic data obtained, the chemical structure of the taste compound was unequivocally identified as the

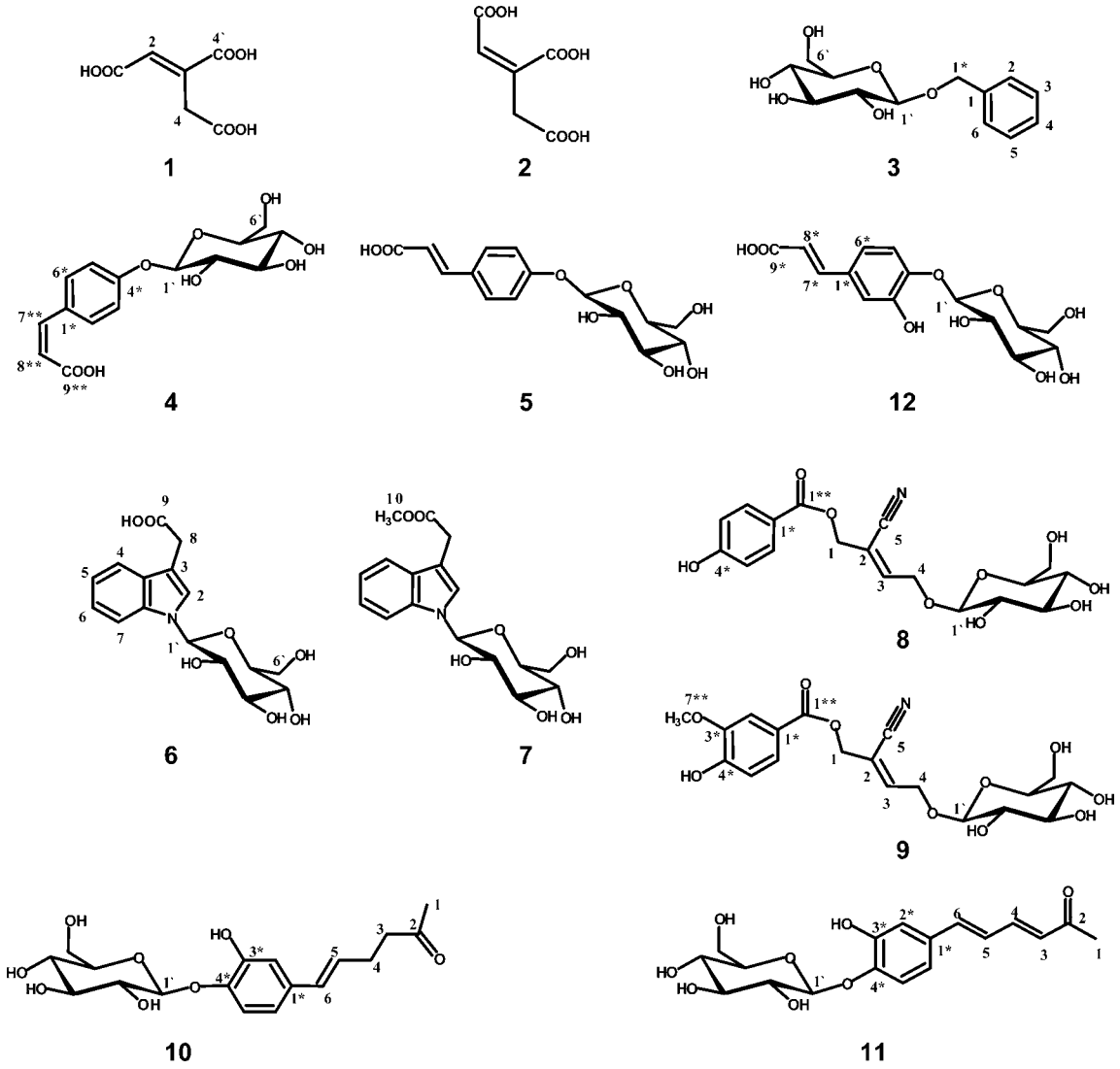

Figure 2. Chemical structures of astringent compounds **1**–**12** isolated from red currants.

benzyl-*O*-*β*-D-glucopyranoside (**3**; **Figure 2**). The bitter taste of that benzyl glycoside is well in agreement with data reported in the literature (*12*).

Rechromatography of HPLC fraction I/8 led to the isolation of three intensely astringent compounds. LC-MS analysis of two of these compounds, exhibiting an absorption maximum at 311 nm, revealed the pseudomolecular ion $[M + Na]^+$ with *m*/*z* 349. Additional MS/MS investigations using the ESI^+ mode indicated a daughter ion with *m*/*z* 187 upon loss of 162 amu, consistent with the cleavage of one molecule of a hexose. After acid hydrolysis and derivatization, GC-MS analysis confirmed the hexose as *β*-glucose. 1D and 2D NMR experiments revealed a coupling constant of 7.3 Hz for the anomeric proton H−C(1′), thus indicating that the glucose is *β*-glycosidically linked to the aglycone of both the target compounds. In addition, two olefinic protons resonating at 5.85 and 6.71 ppm and showing a coupling constant of 12.6 Hz suggested a *Z*-configured double bond in the aglycone of one compound. In contrast, the ^1H NMR signals at 6.39 and 7.65 ppm with a coupling constant of 15.7 Hz indicated the *E*-configuration of the second isomer. The observation of a heteronuclear coupling between the proton H−C(1′) and the carbon signal C(4*) resonating at 156.5 or 159.4 ppm, respectively, undoubtedly identified the *β*-D-glucose unit to be linked with the C(4*) position of (*Z*)- and (*E*)-*p*-coumaric acid. Coinciding well with literature data (*13*, *14*), the structure of the target compound was identified as (*Z*)-(**4**) and (*E*)-coumaric acid 4-*O*-*β*-D-glucopyranoside (**5**, **Figure 2**). As the *E*-configured derivative **5** was shown to be converted into the corresponding *Z*-isomer **4**, the isolation and purification of these compounds were repeated very carefully with cooling and in the absence of light to prevent any artifact formation. Even under these conditions, both isomers were found in similar concentrations, thus indicating that both isomers are naturally occurring in red currant.

The third compound isolated from HPLC fraction I/8 showed an extraordinarily strong astringent taste. Comparison of spectroscopic (LC-MS, NMR, UV−vis), chromatographic, and sensory data with those of the synthetic reference compound led to the identification of 3-carboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**6**, **Figure 2**) as the key astringent compound in HPLC fraction I/8. The corresponding methyl ester, namely, 3-methylcarboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**7**, **Figure 2**), was identified as the key astringent tastant in HPLC fraction I/9 evaluated with a TD factor of 256. Moreover, structure determination of the compounds eluting in HPLC fractions I/13 and I/14, evaluated with TD factors of 128 and 32, respectively, led to the discovery of the previously not reported 2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-*β*-D-glucopyranosyloxy-2(*E*)-butenenitrile (**8**, **Figure 2**) and 2-(4-hydroxybenzoyloxymethyl)-4-*β*-D-glucopyranosyloxy-2(*E*)-

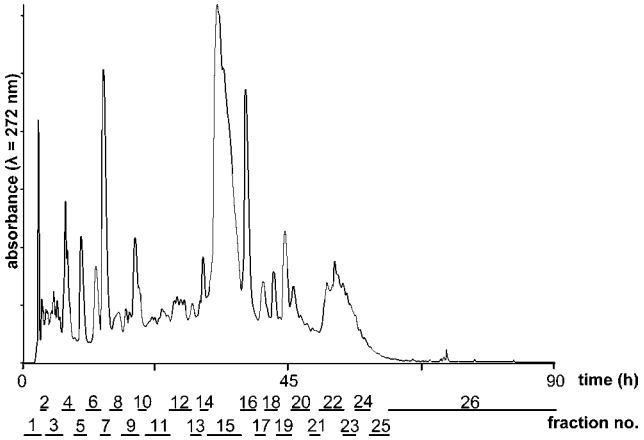

Figure 3. RP-HPLC chromatogram of PA-fraction II isolated from red currants.

butenenitrile (**9**), named nigrumin-5-(4-hydroxybenzoate) and nigrumin-5-(4-hydroxy-3-methoxybenzoate), respectively, as astringent taste compounds. The details of the isolation, structure determination, and synthesis demonstrating the chemical identity of these previously not reported, nitrogen-containing astringent compounds **6**−**9** are published in a companion paper.

LC-MS/MS (ESI$^-$) analysis of the taste compound **10** in the astringent fraction I/15, rated with a TD factor of 128, revealed the pseudomolecular ion $[M - H]^-$ with *m*/*z* 367 and a daughter ion with *m*/*z* 205. Acid hydrolysis, oximation/silylation, followed by HRGC-MS analysis of an aliquot of the compound as well as ^{1}H and ^{13}C NMR spectroscopy of compound **10** demonstrated again the presence of a β-D-glucopyranosyl unit. ^{13}C NMR, HMQC, and HMBC experiments identified 12 carbon signals for the aglycone containing a keto carbon atom resonating at 209.6 ppm. Two olefinic protons with chemical shifts at 5.96 and 6.20 ppm showed a coupling constant of 16.3 Hz, thus indicating an *E*-configured double bond in the molecule. In addition, two methylene carbons at 26.4 and 42.3 ppm and three methyl protons resonating at 2.06 ppm were found. Moreover, three olefinic ^{1}H proton signals were observed at 6.65, 6.79, and 7.16 ppm. Careful assignment of homo- and heteronuclear correlations revealed the structure of the aglycone as (*E*)-6-(3,4-dihydroxyphenyl)-5-hexen-2-one. Furthermore, the coupling of 7.5 Hz between the anomeric proton resonating at 4.65 ppm in the ^{1}H dimension and the aromatic carbon atom C(4*) detected at 144.9 ppm in the ^{13}C dimension identified the hydroxyl group at C(4*) of the aglycon as the glucosylation site. Taking all of these data into consideration, the chemical structure of the astringent compound **10** was determined as the previously not reported (*E*)-6-(3-hydroxy-4-β-D-glucopyranosyloxy)phenyl-5-hexen-2-one (**10**, **Figure 2**), named dehydrorubrumin.

A yellow astringent compound showing absorption maxima at 251 and 351 nm was isolated from HPLC fraction I/16. The MS/MS (ESI$^-$) analysis gave a pseudomolecular ion $[M - 1]^-$ with *m*/*z* 365 and a daughter ion with *m*/*z* 203, thus suggesting an oxidized derivative of dehydrorubumin. This was further confirmed by 1D/2D NMR spectroscopy showing similar signal patterns as observed for compound **10**. The most striking difference was the presence of a second olefinic double bond as part of a conjugated *E*,*E*-configured diene. The coupling pattern of the four olefinic protons fitted well with those found for the pungent compound piperine (*15*). Consequently, the structure of the astringent compound **11** was identified as the previously not reported (3*E*,5*E*)-6-(3-hydroxy-4-β-D-glucopyranosyloxy)phenyl-3,5-hexadien-2-one (**11**, **Figure 2**), named rubrumin.

Table 3. Taste Qualities and Taste Dilution (TD) Factors of HPLC Fractions Isolated from PA-Fraction II of RCP

fraction[a]	taste quality[b]	TD factor	taste compound no.[c]
1	sour	64	
2	astringent	32	**1**, **2**
3	sour	1	
	astringent	4	
4	astringent	2	
5	astringent	4	
6	astringent	8	
7	astringent	32	**12**, **14**
8	astringent	8	
9	puckering astringent	128	**4**, **5**, **6**, **13**
10	astringent	4	
11	astringent	4	**3**
	bitter	2	
12	astringent	64	**7**, **24**
13	astringent	32	**7**
14	astringent	8	
15	sour	2	**15**
	velvety astringent	8	
16	velvety astringent	64	**16**
17	astringent	8	
18	velvety astringent	64	**17**, **18**
19	velvety astringent	64	**20**
20	velvety astringent	32	**21**
21	astringent	4	
	bitter	1	
22	astringent	64	**22**, **23**
	bitter	1	
23	astringent	32	**19**
24	astringent	16	
25	astringent	4	
26	astringent	2	

[a] Number of HPLC fraction referring to **Figure 3**. [b] Taste quality and TD factor were determined by using the half-tongue test. [c] Structures of compounds given as numbers are displayed in **Figures 2** and **4**.

Sensory-Guided Decomposition of Fraction II. To enrich the astringent-tasting compounds in fraction II, carbohydrates and organic acids were removed by polyamide absorption chromatography. Sensory analysis revealed that the astringent compounds could be successfully retained on the polyamide material when water (PA-fraction I) was used as the effluent, whereas the following elution with methanol revealed the intensely astringent-tasting PA-fraction II.

Monitoring the effluent at 272 nm, PA-fraction II was then separated by means of RP-HPLC into 26 subfractions as given in **Figure 3**. After freeze-drying, these 26 fractions were used for the TDA using the half-tongue test (*10*, *11*). The highest TD factor of 128 was found for the puckering astringent taste of fraction 9 followed by fractions 12, 16, 18, 19, and 22, which were rated with TD factors of 64 (**Table 3**). With a somewhat lower astringent taste impact, fractions 2, 7, 13, 20, and 23 were evaluated with TD factor of 32, and fraction 1, exhibiting a sour taste, was judged with a TD factor of 64.

Rechromatography of the astringent HPLC PA-fraction II/7 revealed two astringent-tasting compounds, **12** and **14**. LC-MS/MS analysis of compound **12**, exhibiting an absorption maximum at 311 nm, showed a pseudomolecular ion $[M + H]^+$ with *m*/*z* 343 and a daughter ion peak with *m*/*z* 181, thus indicating the cleavage of a hexose moiety from a caffeic acid aglycone. After confirmation of the glucose moiety in an acidic hydrolysate by means of HRGC/MS analysis, 1D and 2D NMR spectroscopy of the glucoside revealed an anomeric proton signal resonating

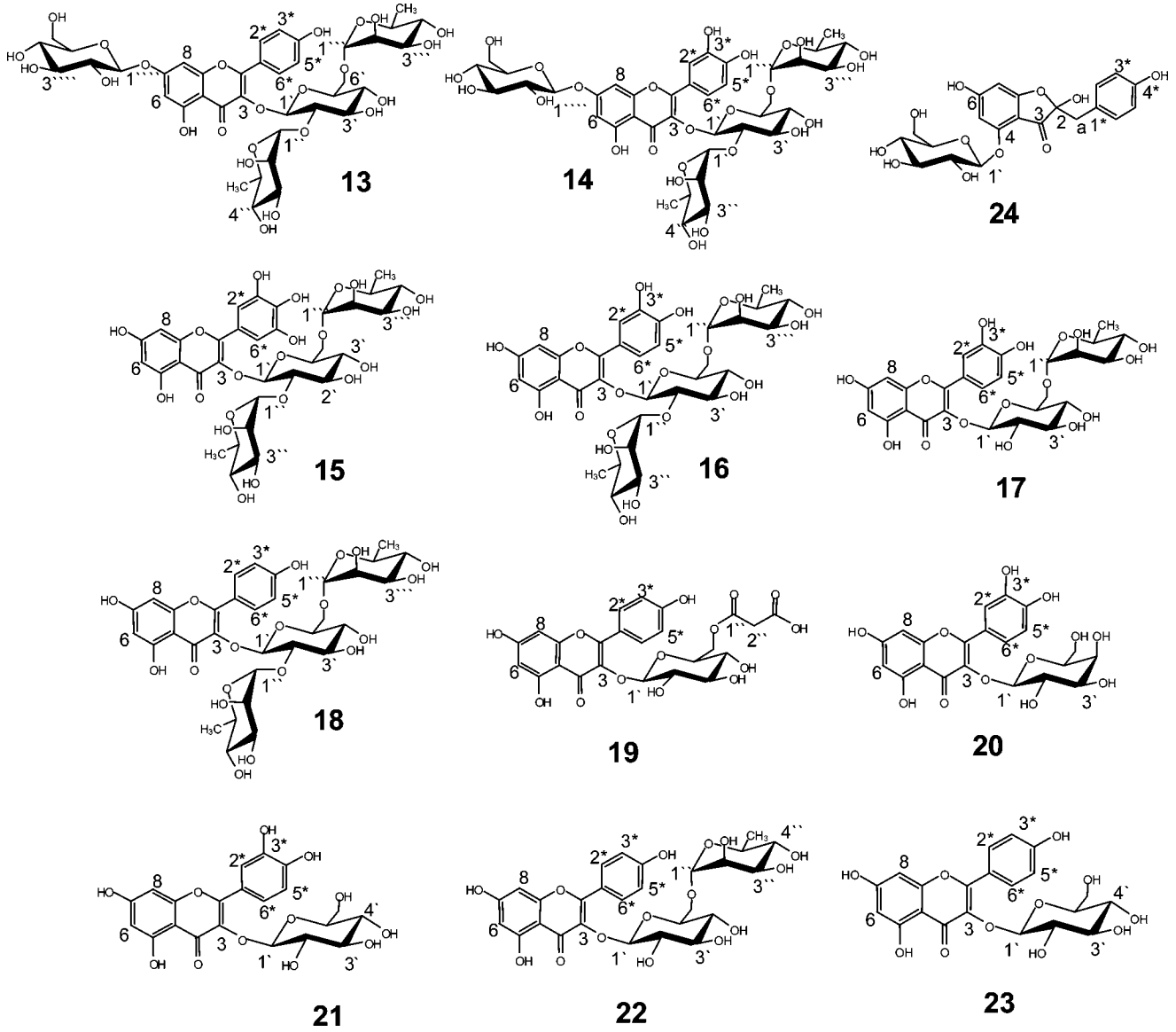

Figure 4. Chemical structures of astringent compounds **13**–**24** isolated from red currants.

at 5.09 ppm and showing a large coupling constant of 7.6 Hz, thus indicating a β-configuration of the glucose moiety. Two olefinic protons resonating at 6.32 and 7.35 ppm and showing a coupling constant of 16 Hz indicated an *E*-configured double bond in the caffeic acid moiety. On the basis of the heteronuclear long-range correlation, observed between the anomeric proton at 5.09 ppm and the aromatic carbon atom C(4*) of the aglycone by means of an HMBC experiment, the structure of the target compound was unequivocally assigned as (*E*)-caffeic acid 4-*O*-β-D-glucopyranoside (**12**, **Figure 2**), thus confirming previous literature reports (*16*).

LC-MS analysis of the second compound, **14**, isolated from HPLC PA-fraction II/7 showed a pseudomolecular ion $[M - H]^-$ with *m*/*z* 917. Additional MS/MS experiments in the ESI^- mode led to the identification of the daughter ions *m*/*z* 755 $[M - H - 162]^-$, 463 $[M - H - 162 - 2 \times 146]^-$ and 301 $[M - H - 162 - 2 \times 146 - 162]^-$, thus indicating the presence of two glucose and two rhamnose moieties in the taste compound. This finding was further confirmed by the identification of rhamnose and glucose in an acidic hydrolysate by means of oximation/silylation and HRGC-MS analysis. The UV−vis absorption maximum observed at 243 and 345 nm indicated a flavonol aglycone in the molecule (*10*). As comparison of the spectroscopic (MS, UV−vis), chromatographic (retention times), and sensory data revealed that the target compound is present in much higher concentrations in red currant leaves than in the fruit juice, the taste compound **14** was preparatively isolated from fresh leaves. The ^1H NMR spectrum of **14** displayed five aromatic protons as expected for a quercetin moiety and, in addition, the protons of two hexosyl and two methyl pentose moieties. Four anomeric protons were observed; the anomeric proton of the two hexose moieties resonated at 5.17 and 5.25 ppm and showed coupling constants of 7.3 Hz each, thus indicating a β-configuration. The anomeric protons of the two rhamnosyl moieties showed resonance at 4.49 and 5.12 ppm and showed a coupling constant of 1.5 Hz, thus indicating an α-configuration. On the basis of the careful interpretation of heteronuclear connectivities observed in an HMBC experiment, the structure of the astringent taste compound **14** was identified as the previously unknown quercetin *O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside (**Figure 4**).

Rechromatography of HPLC PA-fraction II/9 revealed four intensely astringent compounds. LC-MS/MS analysis of compound **13** showed a pseudomolecular ion $[M - H]^-$ with *m*/*z* 901 and daughter ions with *m*/*z* 739 $[M - H - 162]^-$, 447

$[M - H - 162 - 2 \times 146]^-$, and 285 $[M - H - 162 - 2 \times 146 - 162]^-$). As the loss of 162 amu is characteristic for a hexose unit and the loss of 146 amu is expected for a desoxyhexose moiety, two rhamnose and two glucose moieties were expected as part of the molecule. Furthermore, the UV−vis spectrum of **13** exhibiting absorption maxima at 255 and 339 nm indicated kaempferol as the aglycone. As comparison of the spectroscopic (MS, UV−vis), chromatographic (retention times), and sensory data revealed that the target compound is present in much higher concentrations in red currant leaves than in the fruit juice, the taste compound **13** was preparatively isolated from fresh leaves. Careful interpretation of the spectroscopic data obtained from 1D and 2D NMR experiments led to the identification of the chemical structure of the taste compound **13** (**Figure 4**) as kaempferol-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside)-7-*O*-β-D-glucopyranoside, which was previously not reported. By comparison of spectroscopic (MS, UV−vis), chromatographic (retention time on RP-18), and sensory data with those found for the reference compounds isolated already from solvent fraction I, the other three taste compounds in that fraction were identified as (*Z*)-*p*-coumaric acid 4-O-β-D-glucopyranoside (**4**), (*E*)-*p*-coumaric acid 4-O-β-D-glucopyranoside (**5**), and 3-carboxymethyl-indole-1-*N*-β-D-glucopyranoside (**6**, **Figure 2**).

From the astringent-tasting HPLC PA-fraction II/12 two taste compounds were isolated. One of these compounds was identified as the 3-methylcarboxymethyl-indole-1-*N*-β-D-glucopyranoside (**7**), which was already isolated above from HPLC fraction I/9. The second compound (**24**) showed a pseudomolecular ion $[M - H]^-$ with *m*/*z* 449 and a daughter ion with *m*/*z* 287 upon cleavage of a hexose moiety. LC-MS and 1D and 2D NMR spectroscopic data obtained for the purified compound fitted very well with those reported for 2,4,6-trihydroxy-2-[(4′-hydroxyphenyl) methyl]-3(2*H*)-benzofuranone-4-yl-β-D-glucopyranoside, known as maesopsin 4-O-β-D-glucopyranoside (**24**, **Figure 4**) in the literature (*17*).

In HPLC PA-fractions II/15 and II/16, two velvety astringent compounds (**15** and **16**) were detected, which were preparatively isolated from red currant leaves. Compound **15**, exhibiting UV−vis absorption maxima at 249 and 345 nm, showed a pseudomolecular ion $[M + H]^+$ with *m*/*z* 773 and the daughter ions *m*/*z* 627 $[M + H - 146]^+$, 481 $[M + H - 2 \times 146]^+$, and 319 $[M + H - 2 \times 146 - 162]^+$ and indicated the presence of a myricetin aglycone linked to two rhamnose and one hexose moiety. In comparison, compound **16**, exhibiting UV−vis absorption maxima at 243 and 345 nm, showed a pseudomolecular ion $[M + Na]^+$ with *m*/*z* 779 and the daughter ions *m*/*z* 633 $[M + Na - 146]^+$, 477 ($[M + Na - 2 \times 146]^+$ and $[M + Na - 302]^+$), 331 $[M + Na - 302 - 146]^+$, and 185 $[M + Na - 2 \times 146 - 302]^+$, thus indicating the presence of quercetin aglycone linked to a hexose and two rhamnose moieties. On the basis of 1D and 2D NMR experiments, these velvety astringent taste compounds were identified as myricetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**15**), which was previously not reported in literature, and quercetin-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**16**, **Figure 4**). The NMR data of compound **16** were in agreement with those reported earlier (*5*).

Rechromatography of HPLC PA-fraction II/18 revealed two astringent taste compounds. By comparison of spectroscopic, chromatographic, and sensory data with those obtained for the reference compound, compound **17** was identified as quercetin-3-*O*-β-rutinoside (**17**). The second compound, which was isolated in preparative amounts from red currant leaves, showed UV−vis absorption maxima at 255 and 339 nm as expected for flavon-3-ol glycosides (*10*). LC-MS/MS analysis in the ESI^+ mode revealed a pseudomolecular ion $[M + Na]^+$ with *m*/*z* 763 and daughter ions with *m*/*z* 739, 447, and 285. The spectroscopic data obtained from 1D and 2D NMR data were well in line with those reported for kaempferol-3-*O*-(2,6-α-L-dirhamnopyranosyl-β-D-glucopyranoside) (**18**, **Figure 4**) reported earlier as a natural product isolated from *Lysimachia christinae* (*18*).

The astringent compound obtained from HPLC PA-fraction II/23 was isolated in preparative amounts from red currant leaves and was analyzed by means of LC-MS in the ESI^- mode showing a pseudomolecular ion $[M - H]^-$ with *m*/*z* 533 and the daughter ions *m*/*z* 489 and 285, which indicate the cleavage of a molecule of carbon dioxide. ^1H and ^{13}C NMR spectra revealed the signals expected for a kaempferol aglycone, a glucose moiety, and a malonate moiety. As expected, the proton signals of the methylene protons in the malonate moiety disappeared upon addition of D_2O. The HMBC spectrum showed connectivities between the anomeric glucose proton resonating at 5.05 ppm and the carbon atom C(3) of the kaempferol resonating at 135.7 ppm. In addition, the HMBC experiment revealed connectivity between the glucose methylene protons H−C(6′) resonating at 4.17 ppm and the carbon atom C(1″) of the malonate moiety resonating at 168.4 ppm. Taking all of the spectroscopic data into consideration, the taste compound **19** was identified as kaempferol-3-*O*-β-D-(6′-malonyl)glucopyranoside (**Figure 4**).

In addition, by comparison of spectroscopic (MS, UV−vis), chromatographic (retention time on RP-18), and sensory data with those found for reference compounds as well as by

Table 4. Human Taste Recognition Thresholds of Astringent Compounds Isolated from Red Currant

compound (no.[a])	taste threshold (μmol/L) for astringency[b]
benzyl-*O*-β-D-glc*p*[c] (**3**)	50.0
kaempferol-3-*O*-(2,6-α-L-dirha*p*-β-D-glc*p*)-7-*O*-β-D-glc*p* (**13**)	17.0
quercetin-3-*O*-(2,6-α-L-dirha*p*-β-D-glc*p*) (**16**)	16.0
kaempferol-3-*O*-(2,6-α-L-dirha*p*-β-D-glc*p*) (**18**)	11.0
myricetin-3-*O*-(2,6-α-L-dirha*p*-β-D-glc*p*) (**15**)	10.0
(*Z*)-*p*-coumaric acid-4-*O*-β-D-glc*p* (**4**)	9.3
2-(4-hydroxybenzoyloxymethyl)-4-β-D-glc*p*-2(*E*)-butene-nitrile (**9**)	5.9
(*E*)-6-[3-hydroxy-4-(*O*-β-D-glc*p*)phenyl]-5-hexen-2-one (**10**)	4.3
(*3E*,*5E*)-6-[3-hydroxy-4-(*O*-β-D-glc*p*)phenyl]-3,5-hexa-dien-2-one (**11**)	4.0
quercetin-3-*O*-(2,6-α-L-dirha*p*-β-D-glc*p*)-7-*O*-β-D-glc*p* (**14**)	3.5
(*E*)-*p*-coumaric acid-4-*O*-β-D-glc*p* (**5**)	3.5
(*E*)-caffeic acid-4-*O*-β-D-glc*p* (**12**)	3.2
maesopsin-4-*O*-β-D-glc*p* (**24**)	2.1
2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-β-D-glc*p*-2(*E*)-butenenitrile (**8**)	1.2
kaempferol-3-*O*-β-D-(6′-malonyl)glc*p* (**19**)	0.8
quercetin-3-*O*-β-D-glc*p* (**21**)	0.7
kaempferol-3-*O*-β-D-glc*p* (**23**)	0.7
quercetin-3-*O*-β-D-gal*p* (**20**)	0.4
kaempferol-3-*O*-β-rutinoside (**22**)	0.3
(*E*)-aconitic acid (**1**)	0.3
(*Z*)-aconitic acid (**2**)	0.3
quercetin-3-*O*-β-rutinoside (**17**)	0.0015
3-methylcarboxymethyl-indole-1-*N*-β-D-glc*p* (**7**)	0.0010
3-carboxymethyl-indole-1-*N*-β-D-glc*p* (**6**)	0.0003

[a] Structures of numbered compounds are given in **Figures 2** and **4**. [b] Taste recognition threshold concentrations were determined by means of a half-tongue test in bottled water.. [c] The compound was perceived as bitter tasting above a threshold concentration of 150 μmol/L. *p* is used as an abbreviation for the pyranoside configuration of the carbohydrate moiety.

cochromatography with the reference compounds, (*Z*)- and (*E*)-aconitic acid (**1** and **2**) were identified as the sour and astringent compounds in HPLC PA-fraction II/1 and II/2, benzyl-*O*-*β*-D-glucopyranoside (**3**) was identified as the compound inducing the astringent and bitter taste sensation of HPLC PA-fraction II/11, quercetin-3-*O*-*β*-D-galactopyranoside (**20**) and quercetin-3-*O*-*β*-D-glucopyranoside (**21**) (**Figure 4**) were undoubtedly identified as the most intense astringent compounds in HPLC PA-fraction II/19 and II/20, and kaempferol-3-*O*-*β*-rutinoside (**22**) and kaempferol-3-*O*-*β*-D-glucopyranoside (**23**) were found as key astringent compounds in HPLC PA-fraction II/22.

Oral Threshold Concentrations of Astringent Compounds. Prior to sensory analysis, the purity of all compounds was checked by HPLC-MS as well as ^{1}H NMR spectroscopy to be >99%. To determine the human threshold concentrations for the astringent oral sensation, aqueous solutions of the target compound were evaluated by means of the half-tongue test (*10*). To evaluate bitter taste thresholds, a triangle test was used.

As given in **Table 4**, the human oral astringency threshold concentration was strongly dependent on the structure of the compound and ranged from 50.0 *μ*mol/L for benzyl-*O*-*β*-D-glucopyranoside (**3**) to 0.0003 *μ*mol/L for 3-carboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**6**). It is interesting to note that the lowest thresholds of 0.3 and 1.0 nmol/L were found for the nitrogen-containing 3-carboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**6**) and 3-methylcarboxymethyl-indole-1-*N*-*β*-D-glucopyranoside (**7**), which do not belong to the group of plant polyphenols. Also, the nonphenolic isomers of aconitic acid (**1** and **2**) were evaluated with an astringent sensation at a low threshold concentration of 0.3 *μ*mol/L, whereas sour taste was induced at much higher concentrations of about 0.5 mmol/L (data not shown).

The previously not reported compounds 2-(4-hydroxy-3-methoxybenzoyloxymethyl)-4-*β*-D-glc*p*-2(*E*)-butenenitrile (**8**), 2-(4-hydroxybenzoyloxymethyl)-4-*β*-D-glc*p*-2(*E*)-butenenitrile (**9**), (*E*)-6-[3-hydroxy-4-(*O*-*β*-D-glc*p*)phenyl]-5-hexen-2-one (**10**), and (*3E*,*5E*)-6-[3-hydroxy-4-(*O*-*β*-D-glc*p*)phenyl]-3,5-hexadien-2-one (**11**) showed astringency thresholds ranging between 1.2 and 5.9 *μ*mol/L.

In addition, quercetin-3-*O*-*β*-D-rutinoside (**17**) was found to induce oral astringency at extremely low threshold concentrations of 1.5 nmol/L (**Table 4**). The low thresholds found for quercetin-3-*O*-*β*-D-rutinoside, as well as the somewhat higher thresholds of 0.4 and 0.7 *μ*mol/L determined for quercetin-3-*O*-*β*-D-galactopyranoside (**20**) and quercetin-3-*O*-*β*-D-glucopyranoside (**21**), are consistent with findings in our previous studies (*10*). In addition, the tri- and tetraglycosylated flavon-3-ols such as compounds **16** and **14** exhibited significantly higher thresholds of 16.0 and 3.5 *μ*mol/L, thus demonstrating that the astringency of flavon-3-ols is strongly dependent on their glycosylation pattern.

In summary, the sensory data obtained for the compounds identified clearly demonstrate that oral thresholds of astringent compounds cannot be predicted from chemical structures, but have to be investigated on the basis of systematic sensory studies with purified reference compounds. Aimed at demonstrating their contribution to the taste of red currant juice, quantitative studies, followed by taste reconstitution and omission experiments using these compounds in their "natural" concentrations as well as the recording of human dose/response functions, will be published elsewhere.

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