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Sensory-Directed Identification of Taste-Active Ellagitannins in American (*Quercus alba* L.) and European Oak Wood (*Quercus robur* L.) and Quantitative Analysis in Bourbon Whiskey and Oak-Matured Red Wines

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Aimed at increasing our knowledge on the sensory-active nonvolatiles migrating from oak wood into alcoholic beverages upon cooperaging, an aqueous ethanolic extract prepared from oak wood chips (*Quercus alba* L.) was screened for its key taste compounds by application of the taste dilution analysis. Purification of the compounds perceived with the highest sensory impacts, followed by liquid chromatography/mass spectrometry as well as one-dimensional and two-dimensional NMR experiments, revealed the ellagitannins vescalagin, castalagin, and grandinin, the roburins A−E, and 33-deoxy-33-carboxyvescalagin as the key molecules imparting an astringent oral sensation. To the best of our knowledge, 33-deoxy-33-carboxyvescalagin has as yet not been reported as a phytochemical in *Q. alba* L. In addition, the sensory activity of these ellagitannins was determined for the first time on the basis of their human threshold concentrations and dose/reponse functions. Furthermore, the ellagitannins have been quantitatively determined in extracts prepared from *Q. alba* L. and *Quercus robur* L., respectively, as well as in bourbon whiskey and oak-matured red wines, and the sensory contribution of the individual compounds has been evaluated for the first time on the basis of dose/activity considerations.

KEYWORDS: Ellagitannin; oak wood; castalagin; vescalagin; grandinin; roburin; 33-deoxy-33-carboxy-vescalagin; astringency; taste dilution analysis; whiskey; wine

INTRODUCTION

For centuries, spirits such as whiskey and cognac, respectively, or wines are matured in wood barrels to give the beverage a final improvement in aroma, taste, color, and mouthfullness. Although historically numerous types of wood have been used to mature such alcohol-containing products, oak wood and, in particular, American white oak (*Quercus alba* L.) and European oak (*Quercus robur* L.) have emerged as the wood of choice. Several studies have been made to evaluate the oak wood potential for cooperage, focusing on its physical and mechanical properties as well as its chemical composition (*1*, *2*). White oaks normally contain 45−50% cellulose, 22−25% hemicellulose, 23−32% lignins, and 3−10% extractables comprised of acids, carbohydrates, and various phenolic compounds (*3*). Major differences between these oak woods are that European oaks normally contain more extractable solids and more phenol per unit of extractable nonvolatile material than American oaks; however, American oaks are believed to contribute more oak flavor per unit of tannin (*3*).

Although it is believed that some soluble wood constituents migrate into the alcoholic solution and in turn will influence the sensory profile of the spirit, it is still rather unclear which taste-active compounds are released into alcoholic beverages upon cooperaging. Literature studies reported that ethanol extracts of oak wood chips are able to impart oak flavors to wine (*3*). Organoleptic evaluation of young brandy on addition of a combination of soluble wood fractions containing various classes of flavanoid and nonflavanoid phenols was found to reduce the harshness of the product (*4*). Various phenols have been discussed to contribute to the typical taste of ethanolic wood isolates including aromatic aldehydes such as vanillin and aromatic acids such as ferulic acid, vanillic acid, or sinapic acid (*5*), coumarins such as scopoletin and umbelliferone (*6*), lignols such as lyoniresinol (*7*), gallic acid, and hydrolyzable tannins such as castalagin or vescalagin (*8*, *9*). First, sensory evaluation of oak wood tannins tasted in the dry state by two nontrained individuals indicated just a weak oral astringent sensation (*8*). Another study reported the taste of such ellagitannins as being just weakly astringent but as being bitter with high perception thresholds, thus suggesting no impact of ellagitannins to wine astringency (*10*). Although the desirable impact of maturing alcoholic beverages oak wood barrels has been known for several centuries, little work has been documented on the palat attributes of purified single oak-derived components such as castalagin (*8*, *11*). To fully understand the contribution of such

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compounds to the astringent taste and mouthfeel of alcoholic beverages and spirits, further sensory studies on the taste impact of single purified compounds released from the oak into the ethanolic solution upon cooperaging are required (*12*).

To answer the question as to which nonvolatile, key taste compounds are responsible for the typical taste of food products, we have recently developed the so-called taste dilution analysis (TDA) as a powerful screening procedure for taste-active nonvolatiles in foods. This approach, combining instrumental analysis and human bioresponse, led to the discovery of various previously unknown taste compounds such as thermally generated bitter compounds (*13*), cooling compounds in dark malt (*14*), bitter off-tastants in carrot products (*15*), the taste enhancer alapyridaine in beef bouillon (*16*), and astringent key taste compounds in black tea infusions (*17*).

Aimed at elucidating the sensory active nonvolatiles migrating into alcoholic beverages upon cooperaging, the objectives of the present investigation were, therefore, (i) to screen an aqueous ethanolic extract prepared from oak wood chips for its key taste compounds by application of taste dilution (TD) techniques, (ii) to isolate and identify the nonvolatiles inducing the most intense human oral response, (iii) to evaluate their sensory impact on the basis of their human threshold concentrations and dose/reponse functions, and (iv) to quantify these compounds in wine and whiskey.

MATERIALS AND METHODS

Chemicals and Materials. Chips from 2 years air-dried oak wood (*Q. robur* L. and *Q. alba* L.) were obtained from the cooperaging industry (United States). Formic acid was purchased from Grüssing (Filsum, Germany); caffeine, gallic acid, ellagic acid, epigallocatechin 3-gallate (Sigma-Aldrich, Steinheim, Germany), and solvents were of high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany); and deuterated solvents were from Euriso-top (Saarbrücken, Germany). Dionized water used for chromatography was purified by means of a Milli-Q Gradient A10 system (Millipore, Billerica, MA). For sensory analyses, bottled water (Evian) was adjusted to pH 4.5 with trace amounts of formic acid prior to use. The bourbon whiskey (aged for 4 years) was obtained from the food industry. The following red wines were obtained from the food industry: red wine A, Cabernet Sauvignon, 14% vol, 2002, matured in a french oak, which was dried for 2 years and lightly toasted; red wine B, Cabernet Sauvignon, 14% vol, 2002, treated with American lightly toasted oak chips; and red wine C, Cabernet Sauvignon, 13.5% vol, 2004, 70% matured in lightly toasted American oak barrels, 30% matured in steel tanks.

Preparation of an Ethanolic Oak Wood (EOW) Extract. Oak wood chips (500 g) were extracted with ethanol/water (62.5/37.5, v/v; 3 × 1.5 L) for 12 h at 20 °C while stirring. After the ethanol was removed at reduced pressure, the extract obtained was freeze-dried to give the EOW extract (15 g). The EOW extract was kept at −20 °C until used.

Sensory Analyses. *Panel Training.* To familiarize the subjects with the taste language used by our sensory group and to get them trained in recognizing and distinguishing different qualities of oral sensations, 12 assessors with no history of known taste disorders (five women and seven men, ages 24−38 years) participated for at least 2 years in weekly training sessions. For example, the subjects were trained to recognize the taste of aqueous solutions (5 mL each) of the following standard compounds dissolved in bottled water (Evian; low mineralization, 500 mg/L) adjusted to pH 4.5 with aqueous formic acid (0.1%): saccharose (50 mmol/L) for sweet taste, lactic acid (20 mmol/L) for sour taste, NaCl (12 mmol/L) for salty taste, caffeine (1 mmol/L) for bitter taste, and sodium glutamate (3 mmol/L, pH 5.7) for umami taste. For puckering astringency and velvety-like astringency, the panel was trained by using gallustannic acid (0.05%) and quercetin-3-*O*-β-D-galactopyranoside (0.01 mmol/L), respectively, using the half-tongue test (*17*, *18*). Sensory analyses were performed in a sensory panel room at 19−22 °C in three different sessions.

Recognition Thresholds Concentrations. To overcome carry-over effects of astringent compounds, threshold concentrations of astringent compounds were determined in bottled water (pH 4.5) by means of the recently developed half-tongue test (*17*, *18*). 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. At the start of the session and before each trial, the subject rinsed with water and expectorated. An aliquot (1 mL) of the aqueous solution containing the astringent compound was applied with a pipet on one side of the tongue, whereas pure water was applied on the other side of the tongue as the control. The sensory panelists were then asked to move their tongue forward and backward toward the palate for 15 s and to identify the place of astringent sensation by comparison of both sides. After indicating which part of the tongue showed the typical astringent sensation induced by the tastant, the participant rinsed with water and, after 10 min, received another set of one blank and one taste-active sample. To prevent excessive fatigue, tasting began at a concentration level two steps below the threshold concentration that had been determined in a preliminary taste experiment. Whenever the panelist selected incorrectly, the next trial took place at the next higher concentration step. 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 last concentration was calculated and taken as the individual recognition threshold. The threshold value of the sensory group was approximated by averaging the threshold values of the individuals in three independent sessions. Values between individuals and separate sessions differed not more than plus or minus one dilution step; that is, a threshold value of 1.1 μmol/L for castalagin represented a range of 0.55−2.2 μmol/L.

Bitter recognition thresholds, which means the concentrations at which the bitter taste quality of a compound were just detectable, were determined using a whole mouth sip-and-spit approach based on an ascending three-alternative forced-choice method with bottled water (pH 4.5) as the solvent. First, benchtop testing provided some estimate of the approximate threshold range for bitterness. To determine the bitter taste threshold of astringent ellagitannins, appropriate dilutions of the target samples were presented in order of ascending concentrations to the trained panel who was required to choose the bitter stimulus from among a triad containing the target compound and two "control" samples containing the same compound in suprathreshold concentrations for astringency but subthreshold concentrations for bitterness. In detail, the concentrations used for the "controls" in all of the experiments were 400 μmol/L for vescalagin and castalagin, 150 μmol/L for grandinin and roburin E, and 210 μmol/L for 33-deoxy-33-carboxyvescalagin and roburins A−D. The geometric mean of the last incorrect concentration step and the first correct concentration step was calculated and taken as the individual recognition threshold. Threshold values of three sessions were averaged, and values between individuals and separate sessions differed not more than plus or minus two dilution steps.

Recording of Human Dose/Response Functions. Serial 1:1 dilutions of the samples in water were prepared starting at the level of 256-fold above the recognition threshold concentration and ending at the concentration level two steps below the individual recognition threshold concentration. To fit the dose/response functions into a five-point intensity scale, first, the taste intensity of the individual compounds was compared at the highest concentration level by means of the recently reported half-tongue tasting method, thus offering a direct comparison of the sensory impact and a reliable evaluation of the gustatory response of different compounds. To achieve this, the solutions of the individual compounds were applied in binary combinations to one side of the tongue and the assessors were asked to determine which side showed the stronger sensation (*19*). On a five-point scale with 0.25 scale subunits, a 10 mmol/L solution of epigallocatechin-3-gallate, used as the reference compound, was evaluated with the highest sensory intensity and set to the maximum score of 5.0. After the sensory intensity of each test compound at its maximum concentration had been rated, the sensory intensities of the other dilutions were determined by using the half-tongue tasting method. To achieve this, first, one dilution

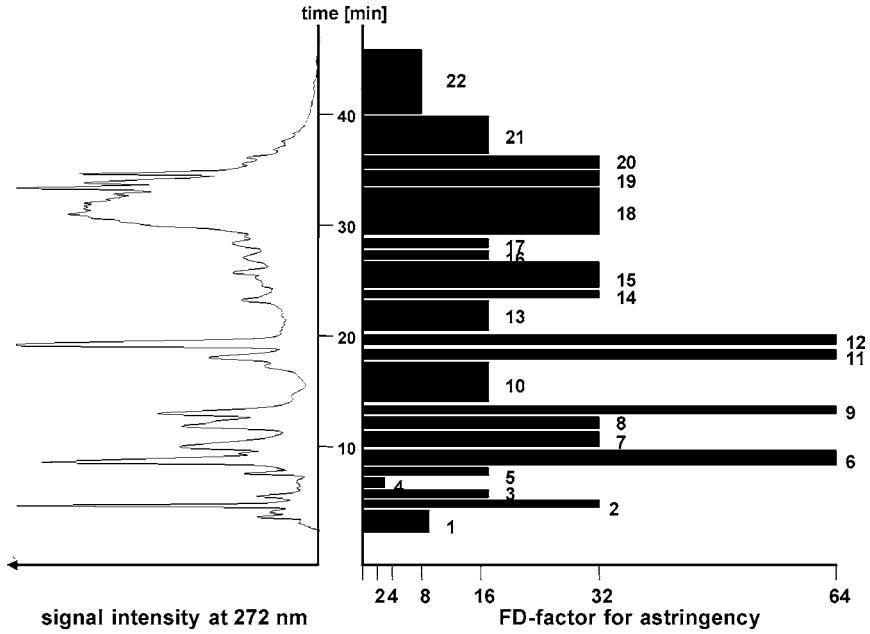

Figure 1. RP-HPLC chromatogram (left side) and TDA (right side) of ethanol–water extract from American oak wood.

of an individual compound was rated against the intensity of the next lower as well as the next higher concentration of the same compound and the intensity of this solution was approximated by comparison to the taste intensity (scores given in brackets) of aqueous solutions containing the reference compound epigallocatechin 3-gallate in concentrations of 0.19 (0.5), 0.38 (1.0), 0.48 (1.5), 0.76 (2.0), 1.05 (2.5), 1.52 (3.0), 1.81 (3.5), 2.47 (4.0), 3.5 (4.5), and 10.0 mmol/L (5.0). Human response functions with dose-over-threshold (DoT) factors on the *x*-axis and taste intensities on the *y*-axis were recorded for each individual subject in triplicate.

HPLC/TDA of EOW Extract. An aliquot (200 mg) of the EOW extract was dissolved in water (2 mL) and membrane filtered, and portions of 200 μL were analyzed by analytical reversed phase (RP)-HPLC. The effluent was separated into 22 fractions, which were individually collected into ice-cooled glass flasks (**Figure 1**). The corresponding fractions obtained from 20 HPLC runs were combined, freed from solvent in a vacuum, and freeze-dried. The 22 fractions obtained were taken up in exactly 10 mL of bottled water (pH 4.5) and then stepwise diluted 1:1 with the same water. The serial dilutions of each fraction were presented in order of increasing concentration to the trained sensory panel, and each dilution was evaluated by means of the half-mouth test (*17*). At the start of the session and before each trial, the subject rinsed with water and expectorated. Whenever the panelist selected incorrectly, the next trial took place at the next higher concentration step. When the panelist selected correctly, the same concentration was presented again besides the blank as a proof for the correctness of the data. The geometric mean of the last and the second last concentration was calculated and taken as the dilution at which a sensory difference between the diluted extract and the blank sample could just be detected. This dilution was defined as the TD factor (*13*). The TD factors evaluated by four different assessors in three different sessions were averaged. The TD factors between individuals and separate sessions did not differ by more than one dilution step.

Adsorption Chromatography (AC). An aliquot (5 g) of the EOW extract was dissolved in methanol/water (40/60, v/v; 50 mL) and placed on the top of a water-cooled 100 cm × 5 cm glass column XK50 (Amersham Pharmacia Biotech, Uppsala, Sweden) filled with a slurry of Sephadex LH 20 material (Amersham Pharmacia Biotech), which was conditioned with a mixture (60/40, v/v) of water (adjusted to pH 4.5 with 0.1% formic acid) and methanol. Chromatography was performed with methanol/water (40/60, v/v; pH 4.5; 2 L), followed by methanol/water (60/40, v/v; pH 4.5; 2 L), methanol/water (80/20, v/v; pH 4.5; 2 L), and, finally, methanol (2 L). The flow rate was kept constant at 3 mL/min by means of a P1 type peristaltic pump (Amersham Pharmacia Biotech). Monitoring the effluent by means of a UV 2070/2075 type UV/vis detector (Jasco, Gross-Umstadt, Germany) operating at 272 nm, 6 min fractions were collected using a LKB Bromma 2070 Ultrarac fraction collector, and the chromatogram was recorded by means of LKB Broma 2210 type two-channel potentiometric recorder. The 6 min fractions collected were recombined to give eight fractions as given in **Figure 2**. The individual fractions were freed from solvent in a vacuum, freeze-dried twice, and then used to isolate the taste-active compounds detected by means of the HPLC/TDA.

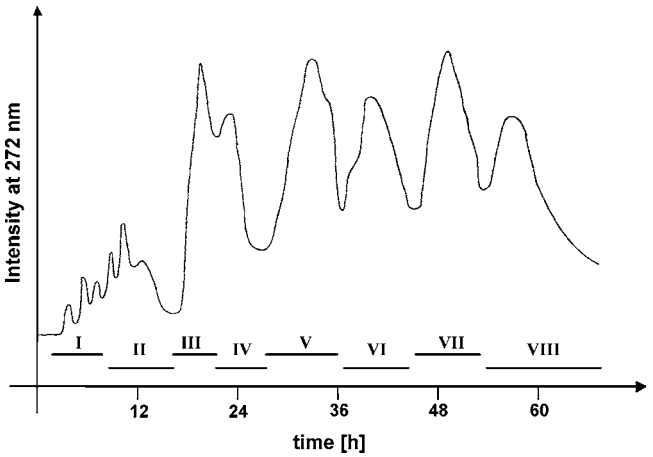

Figure 2. Adsorption chromatogram of an ethanol–water extract of American oak wood.

Isolation and Identification of Taste-Active Ellagitannins. Analytical HPLC analysis of all of the AC fractions revealed that the key taste compounds detected in HPLC fractions no. 6−9, 11, and 12 by means of HPLC/TDA were present in AC fractions V−VIII. Aliquots (200 mg) of the individual AC fractions V−VIII were dissolved in formic acid (0.3% in water; 20 mL), and after membrane filtration, aliquots (2 mL) were applied on a 250 mm × 21.2 mm RP-18 column, ODS-Hypersil, 5 μm (ThermoHypersil, Kleinostheim, Germany). Monitoring the effluent at 272 nm, chromatography was performed with aqueous formic acid (0.3% in water) for 5 min, increasing the acetonitrile content to 5% over 15 min, and then to 15% over another 15 min, and thereafter increasing the acetonitrile content to 60% within 10 min at a flow rate of 18.0 mL/min, and was then held constant for 3 min. After the most active astringent compounds were located by means of HPLC degustation, these target compounds were collected, freed from solvents in a vacuum, and freeze-dried three times, followed by NMR and MS spectroscopic structure determination to give 33-

deoxy-33-carboxy-vescalagin from AC fraction V, roburin B, roburin C, and vescalagin from AC fraction VI, roburin A and castalagin from AC fraction VII, and roburin D from AC fraction VIII as a pale gray, amorphous powder in a purity of >99%.

Castalagin. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 466 (100, [M − 2H]$^{2-}$), 933 (53, [M]$^-$). ^{1}H NMR (400 MHz; D_2O): δ 4.08 [d, 1H, *J* = 12.9 Hz, H−C(6)], 4.86 [d, 1H, *J* = 11.8 Hz, H−C(6)], 5.00 [d, 1H, *J* = 7 Hz, H−C(3)], 5.05 [d, 1H, *J* = 5 Hz, H−C(2)], 5.06 [t, 1H, *J* = 7 Hz, H−C(4)], 5.46 [d, 1H, *J* = 7.3 Hz, H−C(5)], 5.60 [d, 1H, *J* = 4.5 Hz, H−C(1)], 6.68 [s, 1H, H−C(2′)(V)], 6.74 [s, 1H, H−C(2′)(IV)], 6.88 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 65.3 [C(6)], 65.6 [C(1)], 66.1 [C(3)], 68.8 [C(4)], 70.9 [C(5)], 73.7 [C(2)], 107.3 [C(2′)(V)], 108.5 [C(2′)(IV)], 109.4 [C(2′)(III)], 112.1 [C(6′)(II)], 113.4 [C(6′)(I)], 113.5 [C(6′)(III)], 113.7 [C(6′)(V)], 114.9 [C(6′)(IV)], 115.4 [C(2′)(II)], 117.0 [C(2′)(I)], 120.8 [C(1′)(I)], 123.5 [C(1′)(III)], 123.9 [C(1′)(IV)], 125.4 [C(1′)(V)], 126.3 [C(1′)(II)], 134.4 [C(4′)(II)], 135.3 [C(4′)(V)], 136.2 [C(4′)(III)], 136.5 [C(4′)(IV)], 138.0 [C(4′)(I)], 143.3−145.7 [10C, C(3′)(I−V), C(5′)(I−V)], 166.1 [C(7′)-(I)], 166.1 [C(7′)(II)], 167.2 [C(7′)(IV)], 167.5 [C(7′)(III)], 170.0 [C(7′)-(V)].

Vescalagin. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 466 (100, [M − 2H]$^{2-}$), 933 (93, [M − H]$^-$). ^{1}H NMR (400 MHz; D^2O): δ 4.07 [d, 1H, *J* = 12.8 Hz, H−C(6)], 4.65 [d, 1H, *J* = 7 Hz, H−C(3)], 4.80 [d, 1H, *J* = 13.5 Hz, H−C(6)], 4.90 [s, 1H, H−C(1)], 5.06 [t, 1H, *J* = 6.3 Hz, H−C(4)], 5.28 [s, 1H, H−C(2)], 5.48 [d, 1H, *J* = 6.8 Hz, H−C(5)], 6.63 [s, 1H, H−C(2′)(V)], 6.77 [s, 1H, H−C(2′)-(IV)], 6.85 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 63.7 [C(1)], 65.3 [C(6)], 68.0 [C(3)], 68.8 [C(4)], 70.7 [C(5)], 77.2 [C(2)], 107.0 [C(2′)(V)], 108.8 [C(2′)(IV)], 109.2 [C(2′)(III)], 112.3 [C(6′)-(I)], 113.4 [C(6′)(III)], 113.5 [C(6′)(II)], 114.1 [C(6′)(V)], 115.1 [C(6′)-(IV)], 115.1 [C(2′)(II)], 116.9 [C(2′)(I)], 123.2 [C(1′)(I)], 123.3 [C(1′)(IV)], 123.9 [C(1′)(III)], 125.6 [C(1′)(V)], 126.4 [C(1′)(II)], 134.5 [C(4′)(II)], 135.0 [C(4′)(V)], 136.2 [C(4′)(III)], 136.6 [C(4′)(IV)], 137.8 [C(4′)(I)], 143.7−147.4 [10C, C(3′)(I−V), C(5′)(I−V)], 165.8 [C(7′)-(II)], 166.3 [C(7′)(I)], 167.0 [C(7′)(IV)], 167.4 [C(7′)(III)], 170.0 [C(7′)-(V)].

Roburin A. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 616 (100, [M − 3H]$^{3-}$), 924 (97, [M − 2H]$^{2-}$). ^{1}H NMR (400 MHz; D_2O): δ 2.81 [d, 1H, *J* = 12.9 Hz, H−C(6)], 4.12 [d, 1H, *J* = 12.4 Hz, H−C(6″)], 4.31 [d, 1H, *J* = 12.4 Hz, H−C(6)], 4.60 [d, 1H, *J* = 12.4 Hz, H−C(6″)], 4.75 [d, 1, *J* = 7 Hz, H−C(3)], 4.88 [s, 1H, H−C(1″)], 4.91 [s, 1H, H−C(1)], 5.12 [m, 3H, *J* = 7 Hz, H−C(4), H−C(4″), H−C−(3″)], 5.27 [s, 2H, H−C(2), H−C(2″)], 5.32 [d, 1H, *J* = 5 Hz, H−C(5)], 5.44 [d, 1H, *J* = 5 Hz, H−C(5″)], 6.67 [s, 1H, H−C(2‴)-(V)], 6.85 [s, 1H, H−C(2′)(IV)], 6.86 [s, 1H, H−C(2‴)(IV)], 7.17 [s, 1H, H−C(2‴)(III)], 7.52 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 38.6 [C(1″)], 63.5 [C(1)], 65.3 [C(6)], 65.8 [C(6″)], 68.0 [C(3)], 69.2 [C(4)], 69.3 [C(3″)], 70.5 [C(5″)], 70.9 [C(4″)], 71.3 [C(5)], 77.4 [C(2)], 77.4 [C(2″)], 106.5 [C(2′)(V)], 108.8 [C(2′)(IV)], 108.8 [C(2‴)(IV)], 110.1 [C(2‴)(III)], 110.6 [C(2‴)(III)], 113.3 [C(6′)(IV)], 113.5 [C(6′)(V)], 113.5 [C(6‴)(V)], 113.7 [C(6′)(III)], 113.9 [C(6′)-(IV)], 112.3−114.0 [C(6′)(I, II, I‴, II‴)], 115.1 [C(2′)(II), C(2‴)-(II)], 115.4 [C(6‴)(III)], 115.9 [C(2‴)(I)], 117.2 [C(2′)(I)], 117.6 [C(2′)(V)], 122.8 [C(1′)(I)], 123.0 [C(1‴)(III)], 123.9 [C(1′)(IV)], 124.0 [C(1′)(II), C(1′)(III), C(1′)(V), C(1‴)(II), C(1‴)(IV), C(1‴)(V)], 125.4 [C(1‴)(I)], 134.9 [C(4‴)(V)], 135.9 [C(4′)(V)], 136.0 [C(4′)(IV)], 136.2 [C(4′)(III)], 136.2−137.0 [5C, C(4‴)(IV), C(4′)(I), C(4′)(II), C(4‴)-(I), C(4‴)(II)], 137.1 [C(4‴)(III)], 143.6−145.7 [20C, C(3′)(I−V), C(5′)(I−V), C(3‴)(I−V), C(5‴)(I−V)], 165.9 [C(7′)(II)], 166.1 [C(7‴)-(II)], 166.3 [C(7′)(I)], 166.3 [C(7‴)(I)], 166.5 [C(7‴)(IV)], 167.4 [C(7′)-(IV)], 167.4 [C(7‴)(IV)], 168.5 [C(7′)(III)], 170.2 [C(7′)(V)], 170.2 [C(7‴)(V)].

Roburin D. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 616 (100, [M − 3H]$^{3-}$), 924 (97, [M − 2H]$^{2-}$). ^{1}H NMR (400 MHz; D_2O): δ 2.80 [d, 1H, *J* = 12.6 Hz, H−C(6)], 4.09 [d, 1H, *J* = 12.4 Hz, H−C(6″)], 4.31 [d, 1H, *J* = 11.5 Hz, H−C(6)], 4.59 [d, 1H, *J* = 11.8 Hz, H−C(6″)], 4.90 [s, 1H, H−C(1″)], 5.06 [m, 1H, H−C(2)], 5.08 [m, 2H, *J* = 7 Hz, H−C(3), H−C−(3″)], 5.13 [t, 1H, *J* = 7 Hz, H−C(4″)] 5.15 [t, 1H, *J* = 7 Hz, H−C(4)], 5.26 [s, 1H, H−C(2″)], 5.30 [d, 1H, *J* = 6.4 Hz, H−C(5)], 5.42 [d, 1H, *J* = 7.3 Hz, H−C(5″)], 5.62 [d, 1H, *J* = 4.4 Hz, H−C(1)], 6.67 [s, 1H, H−C(2‴)(V)], 6.80 [s, 1H, H−C(2‴)(IV)], 6.83 [s, 1H, H−C(2′)(IV)], 7.18 [s, 1H, H−C(2‴)(III)], 7.53 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 38.8 [C(1″)], 65.3 [C(6)], 65.6 [C(1)], 65.8 [C(6″)], 66.0 [C(3)], 68.8 [C(4)], 69.3 [C(3″)], 70.6 [C(5″)], 71.2 [C(4″)], 71.6 [C(5)], 73.9 [C(2)], 77.6 [C(2″)], 106.7 [C(2′)(V)], 108.9 [C(2′)(IV)], 109.2 [C(2‴)(IV)], 110.1 [C(2‴)(III)], 110.8 [C(2‴)(III)], 112.5 [C(6‴)(I)], 112.8 [C(2′)(II)], 112.8 [C(2‴)(II)], 113.3 [C(6′)(IV)], 113.6 [C(6‴)-(V)], 113.8 [C(6′)(V)], 114.1 [C(6′)(III)], 114.8 [C(6‴)(IV)], 115.2 [C(6′)(II)], 115.3 [C(6‴)(II)], 115.6 [C(1)(II)], 115.7 [C(6‴)(III)], 115.9 [C(2‴)(I)], 117.2 [C(2′)(I)], 118.0 [C(2′)(V)], 120.6 [C(1′)(I)], 122.2 [C(1‴)(IV)], 123.1 [C(1‴)(III)], 123.9 [C(1‴)(V)], 124.0 [C(1‴)(IV)], 124.1 [C(1′)(III)], 125.0 [C(1‴)(I)], 125.8 [C(1′)(II)], 125.9 [C(1‴)-(V)], 126.6 [C(1‴)(II)], 134.3 [C(4′)(V)], 134.6 [C(4′)(IV)], 134.9 [C(4‴)(V)], 134.9 [C(4′)(III)], 136.1 [C(4‴)(IV)], 136.5 [C(4‴)(III)], 137.0 [C(4′)(I), 137.3 [C(4′)(II), 138.2 [C(4‴)(I), 139.5 [C(4‴)(II)], 143.3−145.9 [20C, C(3′)(I−V), C(5′)(I−V), C(3‴)(I−V), C(5‴)(I−V)], 165.7 [C(7′)(II)], 165.9 [C(7‴)(II)], 166.0 [C(7′)(I)], 166.6 [C(7‴)-(I)], 167.0 [C(7‴)(IV)], 167.4 [C(7‴)(III)], 167.5 [C(7′)(IV)], 168.4 [C(7′)(III)], 169.2 [C(7′)(V)], 170.2 [C(7‴)(V)].

Roburin B. $C_{87}H_{58}O_{55}$, UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 660 (100, [M − 3H]$^{3-}$), 990 (76, [M − 2H]$^{2-}$). ^{1}H NMR (400 MHz; D_2O): δ 2.82 [d, 1H, *J* = 12.0 Hz, H−C(6)], 3.49 [d, 1H, *J* = 12.4 Hz, H−C(1)], 3.69 [m, 1H, H−C(5″)], 3.96 [d, 1H, *J* = 3 Hz, H−C(3″)], 3.99 [m, 1H, *J* = 3 Hz H−C(2″)], 3.86 [m, 1H, H−C(5″)], 4.00 [m, 1H, H−C(4″)], 4.20 [d, 1H, *J* = 12 Hz, H−C(6‴)], 4.30 [d, 1H, *J* = 11 Hz, H−C(6)], 4.60 [m, 1H, *J* = 7 Hz, H−C(3)], 4.75 [d, 1H, *J* = 12 Hz, H−C(6‴)], 4.90 [d, 1H, *J* = 7 Hz, H−C(3)], 4.91 [s, 1H, H−C(1‴)], 5.20 [s, 1H, H−C(2‴)], 5.26 [m, 2H, *J* = 7 Hz, H−C(4), H−C−(4‴)], 5.43 [d, 1H, *J* = 7.0 Hz, H−C(5)], 5.46 [s, 1H, H−C(2)], 5.55 [d, 1H, *J* = 7.3 Hz, H−C(5″)], 6.55 [s, 1H, H−C(2⁗)(V)], 6.74 [s, 1H, H−C(2⁗)(IV)], 7.36 [s, 1H, H−C(7′)-(IV)], 7.41 [s, 1H, H−C(2‴)(III)], 7.48 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (400 MHz; D_2O): δ 39.1 [C(1‴)], 45.4 [C(1)], 62.1 [C(5″)], 64.5 [C(6)], 64.8 [C(6‴)], 69.2 [C(4‴)], 69.2 [C(4″)], 69.3 [C(4)], 70.4 [C(5‴)], 70.4 [C(3)], 70.5 [C(5)], 70.8 [C(3‴)], 71.3 [C(3″)], 71.4 [C(2″)], 71.8 [C(2)], 78.0 [C(2‴)], 100.4 [C(1″)], 106.0 [C(2⁗)(V)], 107.5 [C(2⁗)(IV)], 108.8 [C(2⁗)(III)], 109.4 [C(2′)(IV)], 109.9 [C(2′)-(III)], 112.0 [C(6′)(I)], 113.4 [C(6′)(IV)], 113.6 [C(6⁗)(II)], 113.7 [C(6′)(II)], 113.7 [C(6⁗)(V)], 113.8 [C(6′)(V)], 114.8 [C(6⁗)(IV)], 115.1 [C(2⁗)(II)], 115.3 [C(6′)(III)], 115.3 [C(2′)(II)], 115.3 [C(6⁗)-(I)], 115.4 [C(2′)(I)], 115.4 [C(6⁗)(III)], 115.9 [C(2⁗)(I)], 117.8 [C(2′)(V)], 122.4 [C(1⁗)(IV)], 123.3 [C(1′)(III)], 124.0 [C(1′)(IV)], 125.2 [C(1⁗)(I)], 125.4 [C(1′)(V)], 125.6 [C(1′)(I)], 125.6 [C(1⁗)-(V)], 125.7 [C(1⁗)(III)], 126.2 [C(1′)(II)], 126.4 [C(1⁗)(II)], 134.4 [C(4′)(II)], 134.3 [C(4⁗)(II)], 134.8 [C(4⁗)(V)], 135.2 [C(4′)(V)], 136.0 [C(4′)(IV)], 136.2 [C(4′)(III)], 136.9 [C(4⁗)(III)], 137.3 [C(4′)-(I)], 137.3 [C(4⁗)(IV)], 138.2 [C(4⁗)(I)], 143.6−146.5 [20C, C(3′)-(I−V), C(5′)(I−V), C(3‴)(I−V), C(5‴)(I−V)], 165.2 [C(7′)(II)], 166.3 [C(7⁗)(II)], 165.8 [C(7′)(II)], 166.9 [C(7⁗)(IV)], 167.0 [C(7′)(I)], 167.4 [C(7⁗)(III)], 167.5 [C(7′)(IV)], 168.3 [C(7′)(III)], 170.0 [C(7′)-(V)], 170.1 [C(7‴)(V)].

Roburin C. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m/z* 660 (100, [M − 3H]$^{3-}$), 990 (67, [M − 2H]$^{2-}$). ^{1}H NMR (400 MHz; D_2O): δ 2.81 [d, 1H, *J* = 12.6 Hz, H−C(6)], 3.52 [m, 1H, H−C(4″)] 3.53 [s, 1H, H−C(1)], 3.61 [m, 1H, H−C(5″)], 3.70 [d, 1H, *J* = 9 Hz, H−C(3″)], 3.72 [m, 1H, *J* = 9 Hz, H−C(2″)], 3.78 [m, 1H, H−C(5″)], 4.16 [d, 1H, *J* = 12 Hz, H−C(6‴)], 4.30 [d, 1H, *J* = 11 Hz, H−C(6)], 4.62 [d, 1H, *J* = 12 Hz, H−C(6‴)], 4.76 [m, 1H, *J* = 7 Hz, H−C(3)], 4.89 [s, 1H, H−C(1‴)] 5.13 [m, 3H, *J* = 7 Hz, H−C(4), H−C−(3‴) H−C−(4‴)], 5.27 [s, 1H, H−C(2‴)], 5.32 [d, 1H, *J* = 6.4 Hz, H−C(5)], 5.46 [d, 1H, *J* = 7.3 Hz, H−C(5″)], 5.77 [s, 1H, H−C(2)], 6.70 [s, 1H, H−C(2⁗)(V)], 6.82 [s, 1H, H−C(2⁗)(IV)], 6.87 [s, 1H, H−C(2′)(IV)], 7.19 [s, 1H, H−C(2‴)(III)], 7.52 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 38.8 [C(1‴)], 45.6 [C(1)], 62.2 [C(5″)], 65.5 [C(6)], 65.8 [C(6‴)], 69.2 [C(4″)], 69.3 [C(4)], 69.4 [C(3‴)], 70.3 [C(3)], 70.6 [C(5‴)], 70.9 [C(4‴)], 71.1 [C(5)], 71.8 [C(2)], 72.9 [C(3″)], 74.0 [C(2″)], 77.6 [C(2‴)], 99.4 [C(1″)], 106.7 [C(2⁗)(V)], 108.8 [C(2′)(IV)], 108.9 [C(2⁗)(IV)], 110.1 [C(2⁗)(III)], 110.6 [C(2′)-(III)], 112.1 [C(6′)(I)], 113.0 [C(6′)(IV)], 113.6 [C(6⁗)(II)], 113.7 [C(6′)(II)], 113.7 [C(6⁗)(V)], 113.8 [C(6′)(V)], 114.1 [C(6′)(III)], 114.8 [C(6⁗)(IV)], 115.1 [C(2⁗)(II)], 115.2 [C(2′)(II)], 115.3 [C(6⁗)(I)],

115.4 [C(2′)(I)], 115.4 [C(6‴)(III)], 115.9 [C(2‴)(I)], 117.8 [C(2′)(V)], 122.4 [C(1‴)(IV)], 123.4 [C(1′)(V)], 123.7 [C(1′)(III)], 124.1 [C(1′)(IV)], 124.7 [C(1′)(I)], 125.2 [C(1‴)(I)], 125.6 [C(1‴)(V)], 125.7 [C(1‴)(III)], 125.8 [C(1′)(II)], 126.4 [C(1‴)(II)], 134.4 [C(4′)(II)], 134.5 [C(4‴)(II)], 134.7 [C(4‴)(V)], 135.1 [C(4′)(V)], 136.0 [C(4′)(IV)], 136.2 [C(4′)(III)], 136.9 [C(4‴)(III)], 137.2 [C(4′)(I)], 137.3 [C(4‴)(IV)], 138.2 [C(4‴)(I)], 143.6−145.9 [20C, C(3′)(I−V), C(5′)(I−V), C(3‴)(I−V), C(5‴)(I−V)], 165.2 [C(7′)(II)], 166.3 [C(7‴)(II)], 166.3 [C(7′)(I)], 166.9 [C(7‴)(IV)], 167.4 [C(7′)(I)], 167.4 [C(7‴)(III)], 167.5 [C(7′)(IV)], 168.3 [C(7′)(III)], 169.9 [C(7′)(V)], 170.1 [C(7‴)(V)].

33-Deoxy-33-carboxyvescalagin. UV/vis (water) λ_{max} = 229 nm. LC/MS (ESI$^-$): *m*/*z* 430 (100, M − 2H]$^{2-}$), 961 (38, [M − H]$^-$). ^{1}H NMR (400 MHz; D_2O): δ 4.11 [d, 1H, J = 1.4 Hz, H−C(1)], 4.14 [d, 1H, J = 12.8 Hz, H−C(6)], 4.87 [d, 1H, J = 6 Hz, H−C(3)], 4.90 [dd, 1H, J = 2.4;13 Hz, H−C(6)], 5.12 [t, 1H, J = 7 Hz, H−C(4)], 5.32 [d, 1H, J = 7.3 Hz, H−C(5)], 5.76 [s, 1H, H−C(2)], 6.70 [s, 1H, H−C(2′)(V)], 6.81 [s, 1H, H−C(2′)(IV)], 6.92 [s, 1H, H−C(2′)(III)]. ^{13}C NMR (100 MHz; D_2O): δ 46.2 [C(1)], 65.3 [C(6)], 68.8 [C(4)], 70.0 [C(3)], 70.8 [C(5)], 74.2 [C(2)], 107.0 [C(2′)(V)], 108.6 [C(2′)(IV)], 109.1 [C(2′)(III)], 112.4 [C(6′)(I)], 113.5 [C(6′)(II)], 113.3 [C(6′)(III)], 113.5 [C(6′)(V)], 115.0 [C(6′)(IV)], 115.4 [C(2′)(II)], 115.6 [C(2′)(I)], 122.6 [C(1′)(I)], 123.7 [C(1′)(IV)], 123.9 [C(1′)(III)], 125.6 [C(1′)(V)], 126.4 [C(1′)(II)], 134.4 [C(4′)(II)], 135.0 [C(4′)(V)], 136.2 [C(4′)(III)], 136.4 [C(4′)(IV)], 138.0 [C(4′)(I)], 143.6−145.1 [10C, C(3′)(I−V), C(5′)(I−V)], 165.9 [C(7′)(II)], 166.3 [C(7′)(I)], 166.8 [C(7′)(IV)], 167.4 [C(7′)(III)], 170.0 [C(7′)(V)], 174.5 [C(7)].

Quantification of Ellagitannins. Oak wood chips were frozen in liquid nitrogen and powdered in a coffee mill, and an aliquot (30 g) of the oak wood powder was extracted with acetone/water (80/20, v/v; 3 × 200 mL) for 24 h while stirring under an atmosphere of argon. The combined layers were freed from solvent and freeze-dried. An aliquot (10 mg) of the residue obtained was dissolved in water (5 mL), membrane filtered, and then used for HPLC-diode array detection (DAD) and HPLC-MS analysis. Whiskey (500 mL) and the red wines A−C (50 mL) were freeze-dried, and the residues obtained were taken up in water (5 mL), membrane filtered, and then used for HPLC-DAD and HPLC-MS analysis. Verification of the identity of the compounds in the samples was done by HPLC-MS/MS operating in the electrospray ionization (ESI)-negative mode. For each ellagitannin, the mass transition [M − H]$^-$ → [M − 302 − H]$^-$ was measured. Quantification was done by means of HPLC-DAD with a five-point external calibration of each ellagitannin with standard concentrations from 25 to 400 mg/L.

HPLC. The HPLC apparatus (Jasco) consisted of two pumps (PU 2086/2087), a gradient mixer (1000 μL), a Rheodyne injector (200 or 2000 μL loop), and a diode array detector (MD 2010plus, Jasco) monitoring the effluent in a wavelength range between 220 and 500 nm. For preparative separations, aliquots (2 mL) were analyzed on a 250 cm × 21.2 cm RP-18 column, ODS-Hypersil, 5 μm (ThermoHypersil, Kleinostheim, Germany) with solvent mixtures of formic acid (0.3% in water) and acetonitrile at a flow rate of 18 mL/min. For analytical HPLC, aliquots (200 μL) were analyzed on a 250 mm × 10 mm RP-18 column, ODS-Hypersil, 5 μm (Thermo Hypersil, United Kingdom) equipped with a guard column of the same type and using solvent mixtures of formic acid (0.3% in water) and acetonitrile at a flow rate of 3 mL/min.

Liquid Chromatography/Mass Spectrometry (LC/MS). ESI spectra were acquired on a API 4000 Q-Trap LC/MS/MS system (AB Sciex Instruments, Darmstadt, Germany) with direct loop injection of the sample (2−20 μL). The spray voltage was set at −4500 V in the ESI$^-$ mode and at 5500 V in the ESI$^+$ mode. Nitrogen served as the curtain gas (20 psi), and the declustering potential was set at −10 to −30 V in the ESI$^-$ mode and 30 V in the ESI$^+$ mode. The mass spectrometer was operated in the full scan mode monitoring positive or negative ions. Fragmentation of [M − H]$^-$ and [M + H]$^+$ molecular ions into specific product ions was induced by collision with nitrogen (4×10^{-5} Torr) and a collision energy of −40 V.

NMR Spectroscopy. The ^{1}H, ^{13}C, correlation spectroscopy (COSY), heteronuclear multiple quantum correlation (HMQC), and heteronuclear multiple bond correlation (HMBC) spectroscopic experiments were performed on DPX 400 NMR from Bruker (Rheinstetten, Germany). Samples were dissolved in D_2O and placed into NMR tubes (Schott Professional 178 mm × 5 mm) prior to measurement. Data processing was performed by using the NMR Software Mestre-C.

RESULTS AND DISCUSSION

Aimed at increasing our knowledge on the chemical structure as well as the sensory activity of nonvolatiles migrating from the oak wood into spirits and alcoholic beverages, oak wood chips were extracted with ethanol/water for 12 h at room temperature. After filtration, sensory analysis of the EOW extract obtained in a yield of about 3% revealed that the extractables imparted the expected complex mouthfullness and astringent taste sensation. To gain first insight into the chemical species driving this gustatory impact perceived in the oral cavity, the recently developed TDA (*13*) was applied onto the EOW extract.

Screening for Sensory Active Nonvolatiles by Means of TDA. For the application of the TDA, the EOW extract was further analyzed by HPLC on RP-18 material. As outlined in **Figure 1** (left side), the EOW extract consisted of a multiplicity of different substances, of which only a limited number of compounds were expected to contribute significantly to the overall mouth-coating astringent sensation. To focus the identification experiments on these key compounds, it was therefore necessary to sort out the strongly sensory active compounds from the less active or tasteless substances. Aimed at rating the individual compounds in their relative sensory impact, the effluent was separated into 22 fractions, which were freed from solvent and then used for the TDA. To achieve this and to overcome the well-known carry-over effects in astringency perception (*20*), the sensory analyses were performed by means of the recently developed half-mouth test (*17*). As the TD factor obtained for each fraction is proportional to its sensory activity in water, the TD factor rated the 22 HPLC fractions in their relative astringency impact as shown in **Figure 1** (right side). Because of their high TD factor of 64, fraction nos. 6, 9, 11, and 12 were evaluated with the highest taste impacts for astringency, followed by fraction nos. 2, 7, 8, 14, 15, and 18−20 judged with a TD factor of 32 (**Figure 1**). In comparison, the other fractions were evaluated with somewhat lower sensory impacts.

With the exception of fraction nos. 4, 5, 10, and 13, all of these fractions also induced besides the astringent sensation a bitter taste when tasted at higher concentrations levels (data not shown). Because of their high TD factors for the astringent mouthfeel, the following identification experiments were focused on HPLC fraction nos. 6−9, 11, and 12. Further HPLC degustation analysis revealed that fraction nos. 6 and 9, respectively, contained at least three or two strongly astringent compounds. Because the resolution of the HPLC separation was not sufficient to enable the direct isolation of the key compounds in a preparative scale, AC was used in the following to preseparate the complex mixture of oak wood components prior to isolation.

Isolation and Identification of Sensory Active Compounds. The EOW extract was fractionated in a preparative scale by means of AC using LH-20 material as the stationary phase and water/methanol mixtures as the mobile phase. The column effluent monitored at 272 nm was separated into eight fractions (fractions I−VIII, **Figure 2**), which were then analyzed by means of HPLC-DAD in order to locate the key taste compounds detected by means of the HPLC-TDA in HPLC fraction nos. 6−9, 11, and 12. Comparison of the HPLC chromatograms obtained for the individual AC fractions with the results of the

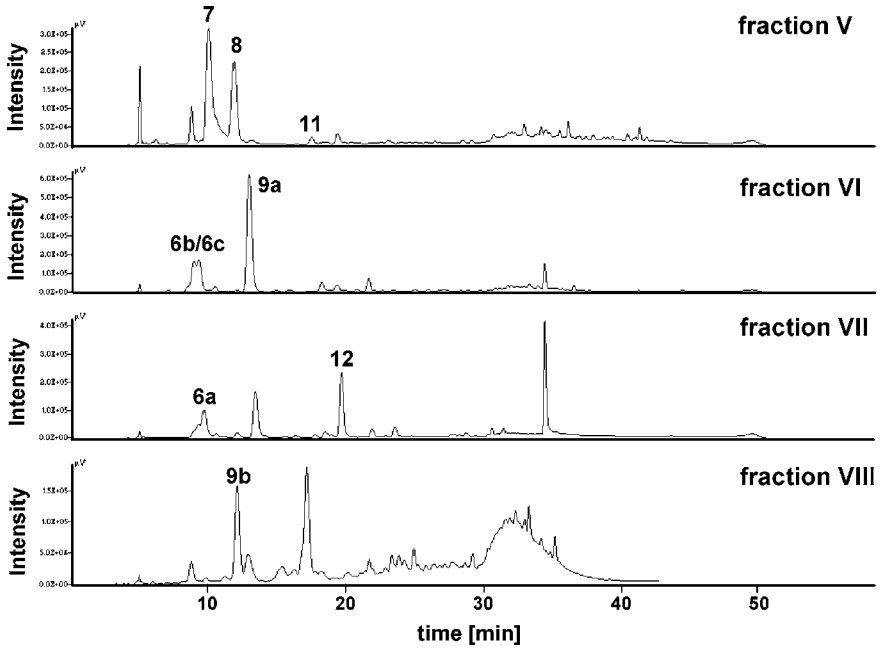

Figure 3. RP-HPLC chromatograms of AC fractions V–VIII from oak wood extract containing main ellagitannins.

HPLC-TDA identified two of the three key taste compounds detected in HPLC fraction no. 6 (**Figure 1**) in AC fraction VII and the third compound in AC fraction VII (**Figure 3**), whereas the two sensory active nonvolatiles detected in HPLC fraction no. 9 (**Figure 1**) were found in AC fractions VI and VIII (**Figures 2** and **3**). In addition, the most intense sensory active compounds detected in HPLC fraction nos. 7, 8, and 11 (**Figure 1**) were eluted in AC fraction V (**Figures 2** and **3**), and the tastant evaluated with high TD factors in HPLC fraction no. 12 (**Figure 1**) was detectable as the main compound in AC fraction VII (**Figures 2** and **3**). After removal of the solvent by means of high vacuum distillation and triple freeze drying, the chemical structure of these taste compounds was determined by LC-MS/MS as well as one-dimensional/two-dimensional NMR spectroscopy.

The astringent compound detected in HPLC fraction no. 12 and isolated from AC fraction VII showed a pseudomolecular ion of *m*/*z* 933 in the mass spectrum obtained in the ESI negative mode and sodium (*m*/*z* 957), potassium (*m*/*z* 973), and ammonium adducts (*m*/*z* 952) in the ESI positive mode, thus suggesting a molecular mass of 934 Da for that taste compound. The ^{1}H NMR as well as the COSY spectrum obtained showed 10 protons, three of them showed chemical shifts at 6.68, 6.74, and 6.88 ppm as expected for aromatic protons, whereas the other seven protons resonated between 4.08 and 5.60 ppm collaborating with the presence of protons of a carbohydrate skeleton. This suggestion was further strengthened by data obtained from the ^{13}C NMR spectrum as well as heteronuclear correlation experiments (HMQC, HMBC) showing signals resonating between 60 and 80 ppm as expected for carbohydrate carbons. In addition, five signals were detected resonating at around 168 ppm and indicating the presence of five galloyl ester groups in the molecule. Considering all of the data obtained from LC/MS and one- and two-dimensional NMR experiments, the chemical structure of the taste compound in HPLC fraction no. 12 could be unequivocally identified as castalagin (**Figure 4**). The NMR data were in agreement with those reported earlier in the literature (*21*).

LC/MS analysis of the second compound in AC fraction VII, which was identified as the taste compound (**6a**) in HPLC fraction no. 6, suggested a molecular mass of 1850 Da collaborating thus suggesting a castalagin dimer. This was further confirmed by NMR spectroscopy demonstrating the presence of 19 protons and 82 carbon atoms in the molecule. Whereas 14 protons of the two glucose cores were detected between 2.81 and 5.44 ppm, the presence of only five aromatic protons and only one hydroxy proton at C(1) indicated that the aromatic ring of the first monomer and the carbon atom C(1) of the second monomer are linked via a C−C bond to give the dimeric ellagitannin. The condensation of carbon atom C(1) of the second subunit with the aromatic proton of ring V in the first unit is well-reflected in the high field signal at 2.81 ppm, assigned as the proton H−C(6) of one subunit, and the carbon resonance at 38.6 ppm, which was assigned to C(1) of the second subunit (*22*). Interestingly, there was no low-field shifted signal for proton H−C(1) as found for castalagin, thus indicating that the dimer does exhibit the inverse stereochemistry as compared to that of castalagin. Comparison of all these spectroscopic data with those reported in the literature (*8*) allowed the identification of compound **6a** among the three taste compounds in HPLC fraction 6 as roburin A (**Figure 4**).

HPLC analysis of AC fraction VI revealed two astringent compounds eluting as a double peak as well as a major peak with retention times of 9 and 13 min, respectively. The later eluting major compound was assigned to the astringent HPLC fraction no. 9, whereas the two compounds in the first eluting double peak were assigned to HPLC fraction no. 6. LC/MS analysis of the later eluting compound revealed a molecular mass of 934 Da, fitting well with the data found above for the castalagin. Also, NMR spectroscopy revealed very similar data as found for castalagin. As an eye-catching difference in the NMR data, the H−C(1) chemical shift of the target compound was observed at 4.90 ppm, whereas the same proton in castalagin showed resonance at 5.60 ppm, thus indicating another stereochemistry for that compound as found for castalagin (no. 12). On the basis of these data and on comparison with data found in the literature (*21*), the key taste compound (no. 9a) detected in HPLC fraction no. 9 was identified as vescalagin (**Figure 4**).

LC/MS analysis of the early eluting double peak in AC fraction VI revealed a molecular mass of 1982 Da for both compounds, thus indicating the presence of two isomers. The

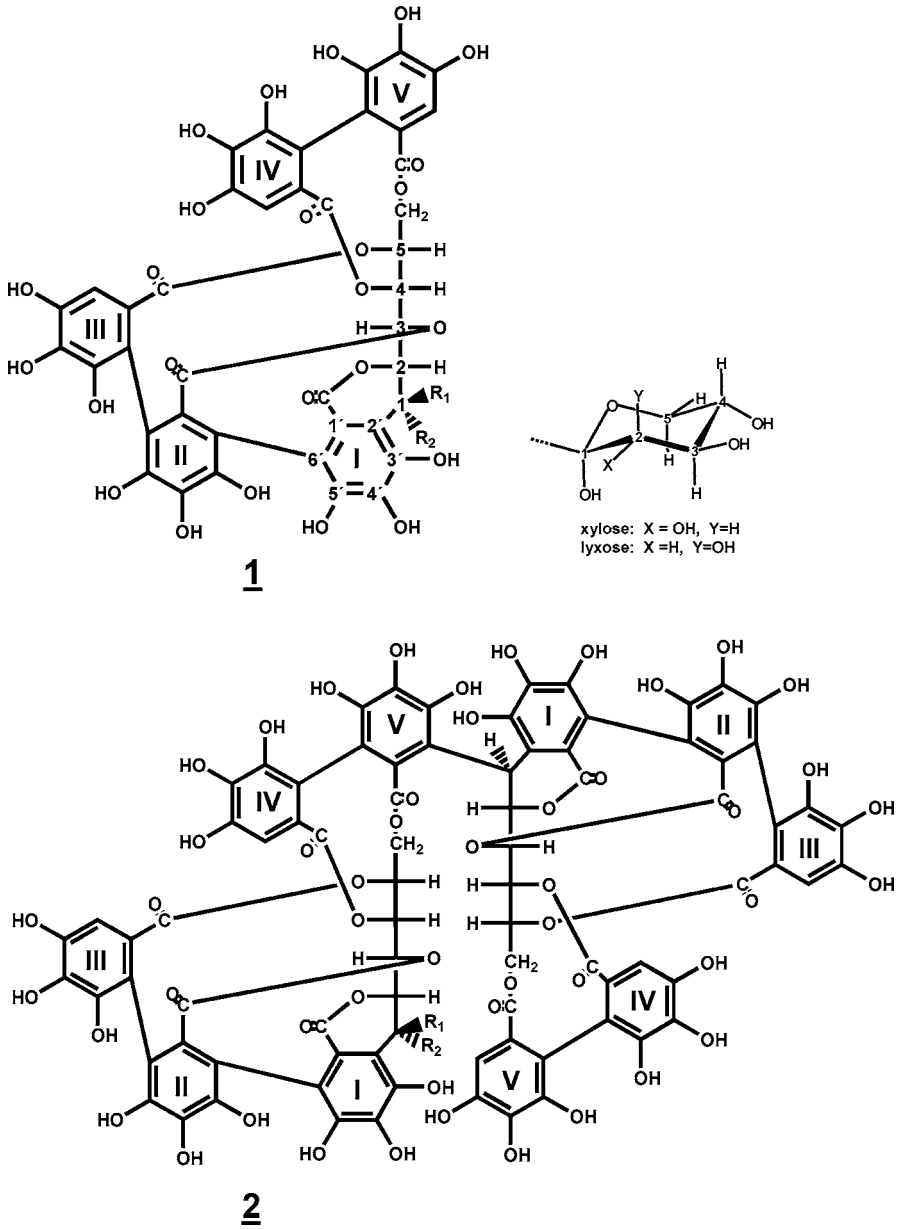

Figure 4. Structures of monomeric ellagitannins castalagin (I, R_1═H, R_2═OH), vescalagin (**1**, R_1═OH, R_2═H), grandinin (**1**, R_1 = lyxose, R_2═H), roburin E (**1**, R_1 = xylose, R_2═H), and 33-deoxy-33-carboxyvescalagin (**1**, R_1═COOH, R_2═H) and dimeric ellagitannins roburin A (**2**, R_1═OH, R_2═H), roburin D (**2**, R_1═H, R_2═OH), roburin B (**2**, R_1 = lyxose, R_2═H), and roburin C (**2**, R_1 = xylose, R_2═H).

molecular mass difference of 132 Da to roburin A suggested the presence of a pentose moiety in these dimeric molecules. As compared to the NMR data found for roburin A, the NMR spectra of these compounds exhibited five additional proton signals resonating between 3.60 and 3.78 ppm, thus confirming the proposed pentose moiety. In addition, the ^{13}C NMR spectrum showed a carbon signal at 99.4 ppm as expected for the anomeric carbon atom of *C*-glycosides (*8*). Careful assignment of the $J_{2,3}$ and $J_{3,4}$ coupling constants furthermore demonstrated a lyxose configuration for the earlier eluting isomer (no. 6a) and a xylose configuration for the later eluting isomer (no. 6b), thus confirming the structure of the *C*-pentosides roburin B (**Figure 4**) and roburin C (**Figure 4**) reported earlier in the literature (*8*).

HPLC analysis of AC fraction V revealed two major and a minor sensory active compound, which were assigned to HPLC fraction nos. 7, 8, and 11 (**Figure 3**). LC/MS analysis using the ESI negative mode revealed a pseudomolecular ion with *m*/*z* 961 for the later eluting minor compound peak. Furthermore, MS/MS experiments demonstrated the formation of the fragment ion *m*/*z* 917 upon cleavage of 44 amu collaborating well with the loss of carbon dioxide and showing some evidence for the presence of a carboxyl group in the target molecule. Also, the mass difference of 28 to the molecular mass of vescalagin and castalagin suggested that a hydroxyl group of these ellagitannins might be substituted by a carboxyl function. This was further strengthened by the ^1H NMR spectrum exhibiting a high-field shift of H−C(1) from 4.90 to 4.11 ppm and a low field shift of H−C(2) from 5.28 to 5.76 ppm when compared to the NMR data found for vescalagin (**Figure 5**). Besides the five carbonyl signals from galloyl ester linkages, an additional low-field carbon atom was detectable resonating at 174.5 ppm. As given in **Figure 5**, HMBC experiments revealed heteronuclear long-range couplings between the protons H−C(1) and H−C(2) and this carboxyl atom, thus enabling the identification of the compound in HPLC fraction no. 11 as 33-deoxy-33-carboxyvescalagin (**Figures 4** and **5**). Although this compound was previously reported as a constituent in *Quercus mongolica* (*23*),

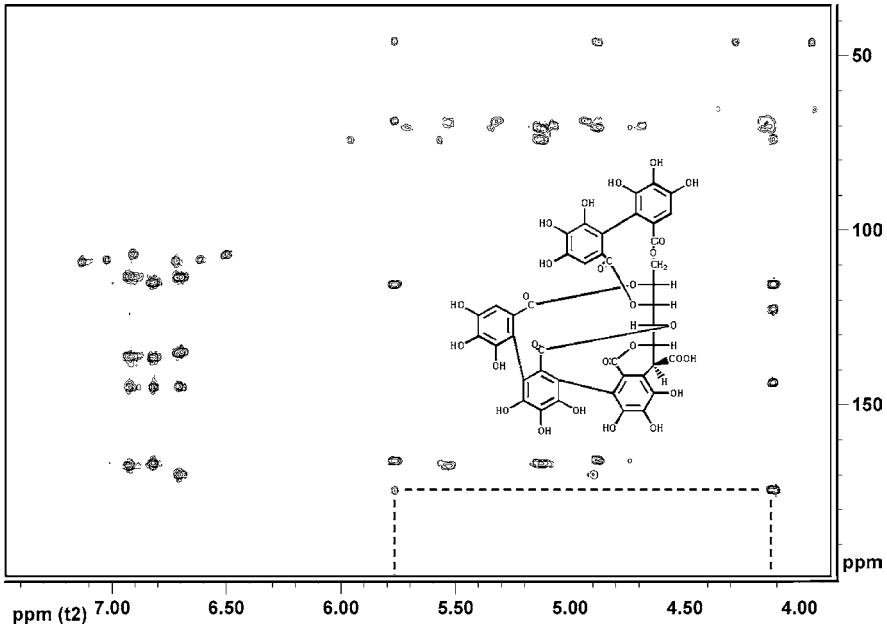

Figure 5. HMBC spectrum (400 MHz, D_2O) and chemical structure of 33-deoxy-33-carboxyvescalagin.

it is the first time that this ellagitannin has been identified in *Q. alba* L. and *Q. robur* L., respectively.

Both of the earlier eluting major compounds detected in AC fraction V showed a molecular mass of 1066. Again, the mass difference of 132 to vescalagin and castalagin suggested the structures of these compounds as monomeric ellagitannin *C*-pentosides. Comparison of chromatographic, spectroscopic, and sensory data with those obtained for the reference compounds isolated as reported earlier (*18*) led to the identification of the earlier eluting compound as the astringent *C*-lyxoside grandinin in HPLC fraction no. 7 and the astringent *C*-xyloside roburin E in HPLC fraction no. 8 (**Figure 4**).

HPLC analysis of AC fraction VIII showed another previously not identified compound, which based on its retention time was assigned to HPLC fraction no. 9 evaluated with a high TD factor of 64 for astringency. LC/MS analysis of that compound revealed a pseudomolecular ion with *m*/*z* 1849 in negative ionization mode, thus fitting well with the molecular mass found for roburin A. Similar to the NMR data obtained for roburin A, 19 proton signals and 82 carbon atoms were detected for that compound indicating the presence of an isomer. Contrary to roburin A, the protons H−C(1) and H−C(2) were shifted to 5.66 and 5.14 ppm, respectively, being very similar to the resonances observed for castalagin. Also, the resonance of carbon atom C(2) of 74.0 ppm matched the chemical shift of the corresponding carbon atom of castalagin, thus demonstrating the presence of one castalagin subunit in the dimer. In contrast, the second subunit showed chemical shifts for the protons H−C(1) and H−C(2) at 4.89 and 5.26 ppm indicating a vescalagin-like stereochemistry. The vescalagin configuration of the second subunit was further supported by the resonance of the carbon atom C(2) detected at 77.0 ppm, thus enabling the unequivocal identification of the taste compound **9a** in HPLC fraction no. 9 as roburin D (**Figure 4**). Although the spectroscopic data were reported earlier in the literature (*8*), the sensory activity of that compound was yet unclear.

Concentrations of Ellagitannins in Oak Wood. To gain insight into the influence of the oak source on the concentrations of these taste-active ellagitannins, the compounds were quantified in aqueous ethanol extracts prepared from American oak (*Q. alba* L.) and French oak (*Q. robur* L.). After confirming the identify of the individual compounds by means of HPLC-MS/MS, the ellagitannins were quantitatively determined by means of HPLC coupled to a diode array detector. As given in **Table 2**, *Q. robur* L. contained higher amounts of the individual ellagitannins as *Q. alba* L., thus fitting well with earlier reports (*2*). Among the individual ellagitannins, independent from the source, the castalagin and vescalagin were present in the highest amounts; for example, 3400 and 1600 mg/kg of these monomers were present in *Q. robur* L. The *C*-glycosides grandinin and roburin E were present in somewhat lower amounts of 1300 and 1250 mg/kg, whereas the dimers roburin A−D were present just in concentrations between 350 and 700 mg/kg in *Q. robur* L. As compared to these ellagitannins, the concentration of 33-deoxy-33-carboxyvescalagin was rather low with 150 mg/kg.

Table 1. Taste Threshold Concentrations of Ellagitannins and Selected Reference Compounds

	threshold concentration for			
	astringency[a]		bitterness[b]	
compound	μmol/L	mg/L	μmol/L	mg/L
grandinin	0.2	0.21	615	655.6
roburin E	0.2	0.21	615	437.1
vescalagin	1.1	1.03	1690	1578.5
castalagin	1.1	1.03	1690	1578.5
33-deoxy-33-carboxyvescalagin	2.6	2.50	666	640.1
roburin A	2.9	5.37	742	1535.5
roburin D	3.0	5.55	768	1372.7
roburin B	6.1	12.09	585	1159.5
roburin C	6.3	12.49	605	1199.1
1,2,3,4,6-pentagalloyl-β-d-glucose	1.8	1.69	ND[c]	ND[c]
ellagic acid	6.6	1.99	ND[d]	ND[d]
gallic acid	292.0	44.97	ND[e]	ND[e]
epigallocatechin 3-gallate	190.0	87.00	190.0	87.00
caffeine	ND[f]	ND[f]	500	81.00

[a] Group taste threshold concentrations for astringency were determined by means of the half-mouth test in bottled water (pH 4.5). [b] Bitter taste threshold concentrations were determined by means of a three-alternative forced-choice test in bottled water (pH 4.5). [c] Not detectable up to the concentration of 900 μmol/L. [d] Not detectable up to the concentration of 50 μmol/L. [e] Not detectable up to the concentration of 900 μmol/L. [f] Not detectable up to the concentration of 1000 μmol/L.

Sensory Evaluation of Ellagitannins. Prior to sensory analysis, the purity of all of the compounds was checked by

Table 2. Concentrations of Ellagitannins in American (*Q. alba* L.) and French Oak (*Q. robur* L.)

compound	concentration (mg/kg) in *Q. robur* L.	concentration (mg/kg) in *Q. alba* L.
vescalagin	1600	720
castalagin	3400	1500
grandinin	1300	510
roburin E	1250	420
roburin A	700	270
roburin D	400	150
roburin B	650	210
roburin C	350	120
33-deoxy-33 carboxyvescalagin	150	50

LC/MS as well as ^{1}H NMR spectroscopy. To evaluate the sensory quality and sensory impact of these ellagitannins, the oral recognition threshold concentrations were determined in water (pH 4.5) using the half-mouth test for astringency and an ascending three-alternative forced-choice test for bitterness (**Table 1**). The oral sensation imparted by these ellagitannins was described as astringent detectable already at relatively low threshold concentrations spanning from 0.2 to 6.3 μmol/L, whereas, strongly depending on the ellagitannin structure, a bitter taste was perceived at threshold concentrations between 410 and 1650 μmol/L.

The lowest group threshold concentrations for the lingering astringent mouthfeel were observed for the monomeric *C*-pentosides grandinin and roburin E; for example, these compounds elicit an astringent sensation at the low concentration level of 0.2 μmol/L (**Table 1**). In contrast, the monomeric ellagitannins vescalagin and castalagin lacking the pentose moiety were evaluated with a 5-fold higher threshold concentration of 1.1 μmol/L, thus indicating that the *C*-glycation of the ellagitannin monomers is favoring the astringent sensation. This observation is well in line with recent findings showing that the glycosylation is strongly favoring the astringent sensation of flavon-3-ols (*17*). In contrast to the C-glycosidic moiety, the introduction of a carboxy function at C(1) was not beneficial for the sensory activity; for example, a threshold level of 2.6 μmol/L was determined for 33-deoxy-33-carboxyvescalagin. As compared to the ellagitannin monomers, the dimers were found with higher threshold concentrations for astringency ranging between 2.9 and 6.3 μmol/L (**Table 1**). In contrast to the observations made for the monomers, the *C*-pentosides of the dimers do not have lower thresholds as compared to the nonglycated homologues. One reason for that phenomenon might be that the interaction of the glycosidic moiety with proline-rich saliva proteins or other receptive biomolecules in the oral cavity is disfavored by a steric shielding induced by the second subunit of the dimer. In addition, it is interesting to note that the stereochemistry does not have any influence on the sensory activity of these ellagitannins; for example, the diastereomeric pairs vescalagin/castalagin, grandinin/roburin E, roburin A/D, and roburin B/C, respectively, were found to have identical threshold concentrations (**Table 1**).

To gain some insight into the essential structural elements required for the astringent sensation of these complex molecules, we also determined the group threshold concentrations of the smaller subunit gallic acid, the gallic acid dimer ellagic acid, 1,2,3,4,6-pentagalloyl-D-glucose, which is well-accepted in the literature as a key intermediate in ellagitannin biosynthesis (*24*), as well as the astringent green tea phenol epigallocatechin-3-gallate. As given in **Table 1**, gallic acid and epigallocatechin-3-gallate, bearing a gallic acid ester moiety in the molecule, were evaluated with comparatively high taste threshold concentrations of 190 and 292 μmol/L, respectively. Surprisingly, the ellagic acid, a dimerized gallic acid dilactone, was found with rather low thresholds of 6.6 μmol/L, which is very close to the threshold found for roburin C. Moreover, 1,2,3,4,6-pentagalloyl glucose, exhibiting a C_6-carbohydrate skeleton as well as five galloyl moieties as present in the ellagitannins, exhibited astringency at the threshold concentration of 1.8 μmol/L, which is very similar to the threshold found for castalagin and vescalagin. These data clearly show that the recognition threshold level of an astringent hydrolyzable tannin type compound is neither solely dependent on the molecular mass, nor on the rigidy of its molecular shape.

In addition, we determined the group recognition thresholds for the bitter taste of the ellagitannins using an ascending three-alternative forced-choice method with two "control" samples containing the test compound in suprathreshold concentrations for astringency but subthreshold concentrations for bitterness. As given in **Table 1**, castalagin and vescalagin showed the highest bitter threshold concentrations of 1690 μmol/L. In contrast, the dimers exhibited significantly lower bitter taste thresholds between 605 and 742 μmol/L fitting well with the bitter taste threshold (500 μmol/L) determined for the bitter reference compound caffeine.

Human Dose/Response Functions. As independent from their sensory training status, panelists have difficulty in memorizing the intensity of a taste compound for a longer period of time, and they are known to give different ratings for the same solution of the test compound tasted at different time intervals (*25*). Consequently, recording dose/response functions with standard sensory methodologies usually leads to unreliable curves with very high error margins. To overcome the carry-over problem mentioned above, we, therefore, applied the recently reported half-tongue testing (*17*), thus offering the possibility of a direct comparison of the sensory impact of two samples. On a five-point numerical scale with 0.25 scale subunits, human dose/response functions were determined for each individual subject for the taste compounds 1,2,3,4,6-pentagalloyl-β-D-glucose, vescalagin, and roburins A, C, and E using standard solutions of epigallocatechin 3-gallate as the reference to define the astringent intensity represented by the individual scores (**Figure 6**). After the taste intensity of each compound at its maximum solubility had been rated, the taste intensities of the other dilutions were determined by using the half-tongue tasting method so that one dilution of an individual compound was rated against the intensity of another dilution of the same compound and the intensity of this solution was approximated by comparison to the taste intensity of aqueous solutions containing the reference compound epigallocatechin 3-gallate in defined concentrations. Human response functions with DoT factors on the *x*-axis and taste intensities on the *y*-axis were recorded for each individual subject in triplicates. The intensity values between trained individuals and separate sessions did not differ more than plus or minus 0.4 units (**Figure 6**).

As the above studies already demonstrated that the sensory activities of the individual diastereomers of an ellagitannin do not differ, we recorded human dose/response functions for vescalagin as the representative for the ellagitannin monomers, roburin E as the representative for a *C*-pentosylated monomer, roburin A as the representative for a vescalagin dimer, and roburin C as the representative for a roburin E dimer. In addition, 1,2,3,4,6-pentagalloyl-β-D-glucose was included in these sensory experiments. The results, outlined in **Figure 6**, clearly demon-

Table 3. Concentrations and DoT Factors of Taste-Active Ellagitannins in Whiskey and Red Wines

compound	concentration (mg/L) (DoT factors[a]) in bourbon whiskey	red wine A	red wine B	red wine C
vescalagin	0.30 (0.3[a])	1.3 (1.3[a])	1.29 (1.3[a])	0.26 (0.3[a])
castalagin	0.43 (0.4[a])	3.7 (3.6[a])	2.12 (2.1[a])	0.51 (0.3[a])
grandinin	0.11 (0.5[a])	1.80 (8.6[a])	0.77 (3.7[a])	0.34 (1.4[a])
roburin E	0.08 (0.4[a])	1.33 (6.3[a])	0.90 (4.3[a])	0.73 (3.7[a])
roburin A	0.12 (<0.1[a])	0.17 (<0.1[a])	0.17 (<0.1[a])	0.15 (<0.1[a])
roburin D	0.05 (<0.1[a])	0.34 (0.1[a])	0.20 (<0.1[a])	0.21 (<0.1[a])
roburin B	0.05 (<0.1[a])	0.29 (<0.1[a])	0.13 (<0.1[a])	0.1 (<0.1[a])
roburin C	0.05 (<0.1[a])	0.47 (<0.1[a])	0.16 (<0.1[a])	0.1 (<0.1[a])
33-deoxy-33-carboxyvescalagin	<0.01 (<0.1[a])	<0.01 (<0.1[a])	<0.01 (<0.1[a])	<0.01 (<0.1[a])

[a] DoT factor calculated as the ratio of the concentration and the taste threshold concentration (cf. **Table 1**).

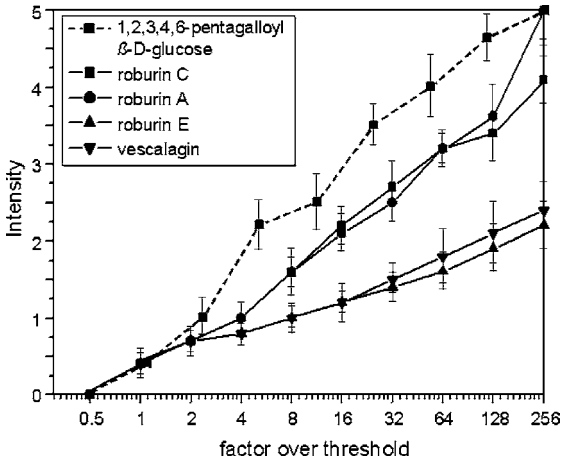

Figure 6. Individual dose/response functions recorded for vescalagin, roburin A, roburin C, roburin E, and 1,2,3,4,6-pentagalloyl-β-D-glucose; aqueous solutions of epigallocatechin 3-gallate were used as references representing the astringent intensity perceived at a score of 0.5 (0.19 mmol/L), 1.0 (0.38 mmol/L), 1.5 (0.48 mmol/L), 2.0 (0.76 mmol/L), 2.5 (1.05 mmol/L), 3.0 (1.52 mmol/L), 3.5 (1.81 mmol/L), 4.0 (2.47 mmol/L), 4.5 (3.5 mmol/L), and 5.0 (10 mmol/L).

strated that the gustatory response for the different ellagitannins follows rather different dose/response functions. In particular, the perception of 1,2,3,4,6-pentagalloyl-β-D-glucose, closely followed by the dimeric roburins A and C, is reflected in rather high slopes and high sensory intensities at higher concentration levels. The highest intensity of 5.0 was found for an aqueous solution of 1,2,3,4,6-pentagalloyl-β-D-glucose and roburin A exceeding the threshold concentration by 256-fold. Also, roburin C reached a high maximum bioresponse with a score of 4.0, whereas the monomeric ellagitannins vescalagin and roburin E did not reach the same taste intensity as found for both of the ellagitannin dimers and were just perceived with an intensity score of 2.2 or 2.3 in 256-fold threshold concentration.

Concentrations and DoT Factors of Ellagitannins in Whiskey and Red Wines. To answer the question as to whether these ellagitannins might contribute to the astringent mouthcoating of oak-matured spirits and wine, the taste compounds were quantified in a bourbon whiskey as well as in three red wines matured in either American or French oak, respectively. As given in **Table 3**, castalagin and vescalagin were the predominating ellagitannins in whiskey with concentrations of 0.43 and 0.30 mg/L. The *C*-glycosides grandinin and roburin E were detectable in comparatively low concentrations of 0.11 and 0.08 mg/L. In particular, the dimeric ellagitannins roburin B−D were detectable just in trace amounts. To gain first insights into the taste contribution of these compounds, these were rated in their sensory impact based on the ratio of the concentration and the taste recognition threshold of a compound (*19*). Calculation of these DoT factors revealed that none of these ellagitannins contribute to the taste of the bourbon whiskey since the concentration of the sensory active nonvolatiles was present below their individual threshold concentrations.

Quantitative analysis and calculation of the DoT factors for the ellagitannins in red wines revealed that wines A and B, matured in French and American oak, respectively, contained rather high concentrations of castalagin, vescalagin, grandinin, and roburin E and demonstrated DoT factors above 1 for these monomeric ellagitannins. The highest DoT factors of 8.6 and 6.3 were found for grandinin and roburin E indicating that these compounds exceed their threshold concentrations by a factor of 8.6 or 6.3 in red wine A. Also, in wine B, these two *C*-glycosides exhibited the highest DoT factors, followed by castalagin and vescalagin; however, the concentrations of the dimeric ellagitannins roburin A−D were more than 10 times below their threshold concentration, thus excluding any taste contribution. In contrast, in red wine C, which was only partially matured in American oak, just grandinin and roburin E showed DoT factors above one, whereas vescalagin and castalagin did not exceed their taste threshold. In summary, these data show evidence that the *C*-glycosides grandinin and roburin E and for some wines also vescalagin and castalagin might contribute to some extent to the astringent mouthfeel of the oak-matured red wines. These findings in collaboration with data from earlier studies indicate that the concentrations of oak-derived polyphenols in barrel-aged wines are close to the sensory thresholds of the phenols and that their contribution to the taste of wine is a subtle one strongly depending on the training and sensory acuity of an individual (*26*). For the Bourbon whiskey, which is typically matured for a longer time in toasted and/or charred oak barrels, the concentration of the ellagitannins is below the threshold concentration. As compared to the red wine, these low concentrations found in whiskey suggest that the oak wood toasting during cooperaging induces severe chemical changes of the ellagitannins. Studies on the thermal transformation of such ellagitannins upon oak toasting are currently under progress and will be published separately.

LITERATURE CITED

(1) Miller, D. P.; Howell, G. S.; Michaelis, C. S.; Dickamnn, D. I. The content of phenolic acid and aldehyde flavor compounds of white oak as affected by site and species. *Am. J. Enol. Vitic.* **1992**, *43*, 333−338.

(2) Chatonnet, P.; Durbourdieu, D. Comparative study of the characteristics of Americam white oak (*Quercus alba*) and European oak (*Quercus petraea* and *Q. robur*) for production of barrels used in barrel aging of wines. *Am. J. Enol. Vitic.* **1998**, *49*, 79−85.

(3) Singleton, V. L. Some aspects of the wooden container as a factor in wine maturation. In *Chemistry of Wine Making*; Webb, A. D., Ed.; ACS Symposium Series 137; American Chemical Society: Washington, DC, 1974; pp 254−277.
(4) Venkataramu, K.; Patel, J. D.; Subba Rao, M. S. Fractionation of wood phenolics and their use in brandy. *J. Food Sci. Technol.* **1983**, *20*, 16−18.
(5) Maga, J. A. Flavor contribution of wood in alcoholic beverages. In *Progress in Flavour Research*; Adda, J., Ed.; Elsevier Science Publishers B. V.: Amsterdam, Netherlands, 1984; pp 409−416.
(6) Salagoity-Auguste, M.-H.; Tricard, Ch.; Sudraud, P. Simultaneous determination of aromatic aldehydes and coumarins by high-performance liquid chromatography. Appication to wines and brandies stored in oak barrels (in French). *J. Chromatogr.* **1987**, *392*, 379−387.
(7) Nabeta, K.; Yonekubo, J.; Miyake, M. Phenolic compounds from the heartwood of European oak (*Quercus robur* L.) and brandy. *Mokuzai Gakkaishi* **1987**, *33*, 408−415.
(8) Herve du Penhoat, C.; Michon, V.; Peng, S.; Viriot, C.; Scalbert, A.; Gage, D. Structural elucidation of new dimeric ellagiannins from *Quercus robur* L. roburines A−E. *J. Chem. Soc.* **1991**, 1653−1660.
(9) Mayer, W.; Gabler, W.; Riester, A.; Korger, H. On tannins in chestnut and oak wood, II, The isolation of castalagin, vescalagin, castalin und vescalin, *Liebigs Ann. Chem.* **1967**, *707*, 177−181.
(10) Puech, J.-L.; Feuillat, F.; Mosedale, J. R. The tannins of oak heartwood: Structure, properties, and their influence on wine flavour. *Am. J. Enol. Vitic.* **1999**, *50*, 469−478.
(11) Masson, G.; Moutounet, M.; Puech, J.-L. Ellagitannin content of oak wood as a function of species and sampling position in the tree. *Am. J. Enol. Vitic.* **1995**, *46*, 262−268.
(12) Quinn, M. K.; Singelton, V. L. Isolation and identification of ellagitannins from white oak wood and an estimation of their roles in wine. *Am. J. Enol. Vitic.* **1985**, *36*, 148−155.
(13) Frank, O.; Ottinger, H.; Hofmann, T. Characterization of an intense bitter-tasting 1*H*,4*H*-quinolizinium-7-olate by application of the taste dilution analysis, a novel bioassay for the screening and identification of taste-active compounds in foods. *J. Agric. Food Chem.* **2001**, *49*, 231−238.
(14) Ottinger, H.; Bareth, A.; Hofmann, T. Characterization of natural "cooling" compounds formed from glucose and L-proline in dark malt by application of taste dilution analysis. *J. Agric. Food Chem.* **2001**, *49*, 1336−1344.
(15) Czepa, A.; Hofmann, T. Structural and sensory characterization of compounds contributing to the bitter off-taste of carrots (*Daucus carota* L.) and carrot puree. *J. Agric. Food Chem.* **2003**, *51*, 3865−3873.
(16) Ottinger, H.; Hofmann, T. Identification of the taste enhancer alapyridaine in beef broth and evaluation of its sensory impact by taste reconstitution experiments. *J. Agric. Food Chem.* **2003**, *51*, 6791−6796.
(17) Scharbert, S.; Holzmann, N.; Hofmann, T. Identification of the astringent taste compounds in black tea by combining instrumental analysis and human bioresponse. *J. Agric. Food Chem.* **2004**, *52*, 3498−3508.
(18) Scharbert, S.; Jezussek, M.; Hofmann, T. Evaluation of the taste contribution of theaflavins in black tea infusions using the taste activity concept. *Eur. Food Res. Technol.* **2004**, *218*, 442−447.
(19) Stark, T.; Hofmann, T. Structures, sensory activity, and dose/response functions of 2,5-diketopiperazines in roasted cocoa nibs (*Theobroma cacao*). *J. Agric. Food Chem.* **2005**, *53*, 5419−5428.
(20) Noble, A. C. In *Chemistry of Taste−Mechanism, Behaviours, and Mimics*; Given, P., Paredes, D., Eds.; ACS Symposium Series 825; American Chemical Society: Washington, DC, 2002; pp 192−201.
(21) Tang, H. R.; Hancock, R. A. Studies of vegetable tannins. Complete structural determination of two chestnut tannins-vecalagin and castalagin using nuclear magnetic resonance spectroscopic methods. *J. Soc. Leather Technol. Chem.* **1995**, *79*, 181−187.
(22) Herve du Penhoat, C.; Michon, V.; Ohassan, A.; Peng, S.; Scalbert, A.; Gage, D. Roburin A, a dimeric ellagitannin from the heartwodd of *Quercus robur*. *Phytochemistry* **1991**, *30*, 329−332.
(23) Ishimaru, K.; Ishimatsu, M.; Nonaka, G.; Mihashi, K.; Iwase, Y.; Nishioka, I. Tannins and related compounds LXXII. Isolation and characterization of mongolicanin (procyanidino-ellagitannin), mongolinin A, acutissimin C and vescalagin carboxylic acid, novel tannins from *Quercus mongolica* var. grosseserrata. *Chem. Pharm. Bull.* **1988**, *36*, 3319−3327.
(24) Niemetz, R.; Gross, G. G. Ellagitannin biosynthesis: Oxidation of pentagalloylglucose to tellimagrandin II by a phenol oxidase from *Tellima grandiflora* leaves. *Phytochemistry* **2003**, *62*, 301−306.
(25) Galopin, C.; Tigani, L.; Furrer, S. Structure property relationship of chemosensates. In *State-of-the-Art in Flavour Chemistry and Biology*; Proceedings of the 7th Wartburg Symposium; Hofmann, T., Rothe, M., Schieberle, P., Eds.; 2005; pp 61−67.
(26) Pocock, K. F.; Sefton, M. A.; Williams, P. J. Taste thresholds of phenolic extracts of French and American oakwood: The influence of oak phenols on wine flavor. *Am. J. Enol. Vitic.* **1994**, *45*, 429−434.

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