JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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pubs.acs.org/JAFC

Contribution of Several Volatile Phenols and Their Glycoconjugates to Smoke-Related Sensory Properties of Red Wine

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Supporting Information

ABSTRACT: Guaiacol and 4-methylguaiacol are well-known as contributors to the flavor of wines made from smoke-affected grapes, but there are other volatile phenols commonly found in smoke from forest fires that are also potentially important. The relationships between the concentration of a range of volatile phenols and their glycoconjugates with the sensory characteristics of wines and model wines were investigated. Modeling of the attribute ratings from a sensory descriptive analysis of smoke-affected wines with their chemical composition indicated the concentrations of guaiacol, *o*-cresol, *m*-cresol, and *p*-cresol were related to smoky attributes. The best-estimate odor thresholds of these compounds were determined in red wine, together with the flavor threshold of guaiacol. Guaiacol β-D-glucoside and *m*-cresol β-D-glucoside in model wine were found to give rise to a *smoky/ashy* flavor in-mouth, and the respective free volatiles were released. The study indicated that a combination of volatile phenols and their glycosides produces an undesirable smoke flavor in affected wines. The observation of flavor generation from nonvolatile glycoconjugates in-mouth has potentially important implications.

KEYWORDS: *bushfire, glycosides, grapes, HPLC-MS/MS, partial least-squares regression, sensory descriptive analysis, smoke taint, Vitis vinifera, wine*

INTRODUCTION

During recent years, bushfires and prescribed forest burns have caused significant smoke exposure to Australian winegrape vineyards, ultimately leading to reports from wine producers of 'smoky', 'dirty', and 'burnt' aromas and lingering 'ash' flavor in some of the resultant wines.[1] Although guaiacol and 4-methylguaiacol have been established as important indicators of the smoke effect,[2,3] their presence cannot always explain the smoky sensory attributes observed in wines made from smoke-exposed grapes, with levels of these compounds often at or below levels found in oak-barrel aged wines.[4,5]

A number of volatile phenols have previously been found in smoke,[6] but had not been previously considered in smoke-affected grapes and wine until recently,[7] including *o*-, *m*-, and *p*-cresol, syringol, 4-methylsyringol, 4-vinylguaiacol, 4-allylsyringol, and phenol. These compounds have been implicated in smoke aroma and flavor in other foods and beverages such as whiskey, smoked fish, and meat.[8−12] In addition to the presence of numerous free volatile phenols, smoke-affected grapes and wine can contain elevated levels of volatile phenol glycoconjugates.[7] These glycoconjugates, which hydrolyze during fermentation and wine aging to release free volatile phenols,[7,13,14] have been considered to be a pool of precursors with no direct aroma or flavor properties, as they are nonvolatile. However, model studies have shown that some glycosides are susceptible to hydrolysis in the mouth, through the activity of enzymes derived from oral microflora,[15,16] although their direct sensory significance has not been demonstrated.

The objective of this study was to establish the relationships between the concentration of volatile phenols and the sensory characteristics of wines made from grapes affected by bushfire smoke. The potential for the glycoconjugates of volatile phenols to contribute directly to the flavor of smoke-affected wines was also studied using synthesized pure β-D-glucosides of guaiacol, *m*-cresol, and syringol.

MATERIALS AND METHODS

Materials. All chromatographic solvents were of HPLC grade. All chemicals were of analytical-reagent grade unless otherwise stated. Water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Merck solvents were purchased from Rowe Scientific (Lonsdale, SA, Australia). Guaiacol, 4-methylguaiacol, 4-vinylguaiacol, phenol, *o*-cresol, *m*-cresol, *p*-cresol, syringol, 4-methylsyringol, and d_7-*p*-cresol were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). d_3-Guaiacol, d_3-4-methylguaiacol, d_3-syringol, syringol β-D-glucoside, guaiacol β-D-glucoside, and d_3-guaiacol β-D-glucoside were previously synthesized in house.[7,14,17,18] *m*-Cresol β-D-glucoside was prepared according to modifications of the synthetic methods described by Shao et al.[19] (Figure 1).

Wines. Eighteen 2009 vintage wines were studied, of which 11 were bushfire-smoke affected (from the same fire event during the period February 7−March 14, 2009, in the Yarra Valley, Victoria, Australia), commercially produced wines sourced from industry collaborators. These wines were selected for the study from a larger set of 27 wines by a panel of experienced tasters in an informal blind assessment, based on the lack of winemaking-related off-flavors and to encompass a range of grape varieties and degree of smoke influence. Four further 2009 vintage wines, from frozen Chardonnay, Cabernet Sauvignon, Pinot noir, and Shiraz grapes from Yarra Valley vineyards that had been exposed to bushfire smoke from the same event as the

Received: October 6, 2011
Revised: February 2, 2012
Accepted: February 13, 2012
Published: February 13, 2012

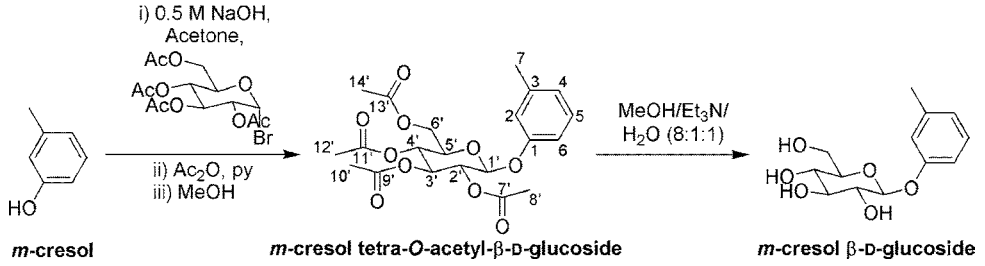

Figure 1. Synthesis of *m*-cresol *β*-D-glucoside.

Table 1. Wines Included in the Study: Codes and Basic Composition

sample code[a]	variety	alcohol (% v/v)	glucose + fructose (g/L)	pH	titratable acidity (g/L)	volatile acidity (g/L)	free SO_2 (mg/L)	total SO_2 (mg/L)
control a	Cabernet Sauvignon	14.8	0.2	3.50	7.5	0.50	31	54
control b	Pinot noir	14.2	1.3	3.55	5.4	0.52	28	57
control c	Pinot noir	13.2	<0.27	3.72	5.4	0.37	26	73
1	Pinot noir	12.6	3.5	3.69	4.7	0.49	54	91
2	Pinot noir	11.8	0.1	3.84	5.4	0.61	22	38
3	Pinot noir	13.7	0.6	3.68	5.4	0.57	25	42
4	Shiraz	13.6	0.4	3.64	5.8	0.38	41	70
5	Pinot noir	12.8	0.2	3.48	6.9	0.38	27	37
6	Pinot noir	14.9	0.8	3.70	6.6	0.72	31	90
7	Pinot noir	11.8	0.5	3.52	6.2	0.45	19	45
8	Pinot noir	13.1	0.5	3.70	5.6	0.58	26	41
9	Pinot noir	11.8	0.6	3.53	6.2	0.45	24	49
10	Cabernet Sauvignon	12.2	0.7	3.52	6.4	0.18	28	63
11	Shiraz	13.3	0.1	3.68	5.7	0.56	28	38
12	Chardonnay	13.3	<0.27	3.02	8.2	0.25	16	56
13	Pinot noir	13.2	<0.27	3.44	5.6	0.30	23	54
14	Cabernet Sauvignon	11.2	0.2	3.39	5.9	0.19	21	49
15	Shiraz	13.1	0.7	3.40	8.0	0.55	26	92
base wine	Merlot	13.1	<0.27	3.50	5.7	0.21	27	69

[a]All wines were characterized in the sensory descriptive analysis study except the base wine, which was used for threshold testing and for reference training standards. The three control wines, a–c, were not smoke affected, sourced from Coonawarra (South Australia), Yarra Valley (Victoria), and Adelaide Hills (South Australia), respectively. Samples 1–15 were smoke-affected wines from the Yarra Valley, Victoria, with samples 12–15 made using small lot research winemaking methods.

commercially produced wines, were made under small-scale winemaking conditions, with triplicate 5 kg standardized fermentations carried out with skin contact, including the batch of Chardonnay grapes, as described previously.[7] The individual fermentation replicates of these wines were assessed in an informal preliminary tasting, and a single replicate was selected to be assessed by the sensory panel. In addition, three control wines made from non-smoke-affected grapes were sourced. The wines and their basic composition are detailed in Table 1. All wines had been through malolactic fermentation except for the small-scale wines 12–15. These small-scale wines had malic acid levels from 1.07 to 1.41 g/L, with the malic acid concentration of all other wines being <0.06 g/L.

Table 1 also shows the basic composition of a 2009 Merlot bag-in-box commercial wine used as the base wine for threshold testing and for the sensory descriptive analysis reference standards as described below. The base wine was selected on the basis of having low levels of volatile phenols, with undetectable levels of the *o*-, *p*- and *m*-cresols (<1 μg/L), and 2 μg/L of guaiacol.

Nuclear Magnetic Resonance (NMR) Analysis. Proton (^{1}H) and carbon (^{13}C) NMR spectra for the synthesized compounds were recorded with a 600 MHz Bruker spectrometer. Chemical shifts were recorded as *δ* values in parts per million (ppm). Spectra were acquired in chloroform-*d* or methanol-d_4 at ambient temperature, and resonances were assigned by routine 2D correlation experiments. For ^{1}H NMR spectra, the peak as a result of residual $CHCl_3$ (*δ* 7.26) or CH_3OH (*δ* 3.31) was used as the internal reference. For ^{13}C NMR spectra, the central peak of the $CDCl_3$ triplet (*δ* 77.16) or the CD_3OD septet (*δ* 49.00) was used as the internal reference.

High-Resolution Mass Spectrometry (HRMS). Spectra were obtained on a Bruker microTOF-Q II with electrospray ionization (ESI) in positive mode. Samples dissolved in water or methanol at concentrations of approximately 1–2 mg/L were analyzed by flow injection.

Optical Rotations. Specific rotations were recorded with a PolAAr 21 polarimeter, referenced to the sodium D line (589 nm) at 20 °C, using the spectroscopic grade solvents specified and at the concentrations (*c*, g/100 mL) indicated. The measurements were carried out in a cell with a 1 dm path length.

Melting Points. A Buchi Melting Point B-540 unit was used, and melting points were uncorrected.

3-Methylphenyl 1-*O*-2′,3′,4′,6′-Tetra-*O*-acetyl-*β*-D-glucopyranoside (*m*-Cresol Tetra-*O*-acetyl-*β*-D-glucoside). A solution of acetobromo-*α*-D-glucose (2.06 g, 5.0 mmol) in acetone (30 mL) was added dropwise to a stirred solution of *m*-cresol (540 mg, 5.0 mmol) in 0.5 M NaOH (13 mL, 6.5 mmol) at 0 °C. The reaction was stirred at room temperature for 6 h in the dark and then concentrated under reduced pressure. The syrup was acetylated with acetic anhydride (6 mL, 64 mmol) in pyridine (8 mL, 99 mmol) at room temperature overnight. The flask was cooled on ice, and methanol (20 mL) was added; the mixture was stirred for a further 30 min before being concentrated under reduced pressure. The resulting oil was purified by silica column chromatography (CH_2Cl_2→10% Et_2O/CH_2Cl_2; R_f = 0.59 in 10% Et_2O/CH_2Cl_2) and recrystallized from EtOH to give a

dx.doi.org/10.1021/jf2040548 | J. Agric. Food Chem. 2012, 60, 2629–2637

Table 2. Sensory Attributes and Composition of the Reference Standards for the Sensory Descriptive Analysis Study

attribute	description (reference standard[a])
aroma	
overall fruit	intensity of the overall fruit aroma: includes red fruit, red berry, dark berry, strawberry, raspberry, cherry, apple
cold ash	burnt aroma associated with ashes: includes ashtray, earthy, muddy, tarry (approx 0.5 g of cigarette ash)
smoke	perception of any type of smoke aroma: includes charry, smoked meat, bacon (four drops of Tone's liquid smoke)
medicinal	aromatic characteristic of bandages or disinfectant: includes cleaning product, disinfectant, phenols, Band-Aid, doctor's waiting room (syringol, 115 μg/L; *o*- and *p*-cresol, 50 and 100 μg/L, respectively)
solvent	volatile aroma associated with solvents: includes varnish, shoe polish
by mouth	
overall fruit flavor	overall level of fruit flavor
smoky flavor	smoke flavor: includes bacon and smoked-meat flavor
sour	sour/acid taste of tartaric acid
metallic	'tinny' canned flavor associated with metals
bitter	bitter taste and aftertaste, taste of quinine sulfate solution
ashy aftertaste	length of flavor associated with residue of ashtray flavor after expectorating: includes coal ash, ashtray, tarry, acrid
drying	drying, puckering mouthfeel after expectoration of the wine

[a]In 30 mL of neutral Merlot base wine.

white crystalline material (451 mg, 21%): ^{1}H NMR ($CDCl_3$), δ 7.17 (1H, t, J = 7.8 Hz, H_5), 6.88 (1H, dt, J = 7.8, 0.8 Hz, H_4), 6.80 (1H, t, J = 2.3 Hz, H_2), 6.78 (1H, dd, J = 7.8, 2.3 Hz, H_6), 5.29 (1H, dd, J = 9.1, 8.9 Hz, $H_{3'}$), 5.26 (1H, dd, J = 9.1, 7.5 Hz, $H_{2'}$), 5.16 (1H, dd, J = 10.1, 8.9 Hz, $H_{4'}$), 5.07 (1H, d, J = 7.5 Hz, $H_{1'}$), 4.27 (1H, dd, J = 12.2, 5.5 Hz, $H_{6a'}$), 4.17 (1H, dd, J = 12.2, 2.5 Hz, $H_{6b'}$), 3.86 (1H, ddd, J = 10.1, 5.5, 2.5 Hz, $H_{5'}$), 2.32 (3H, s, H_7), 2.08 (3H, s, $H_{14'}$), 2.05 (3H, s, $H_{8'}$), 2.04 (3H, s, $H_{12'}$), 2.03 (3H, s, $H_{10'}$); ^{13}C NMR ($CDCl_3$), δ 170.73 ($C_{13'}$), 170.39 ($C_{9'}$), 169.55 ($C_{11'}$), 169.45 ($C_{7'}$), 156.97 (C_1), 139.86 (C_3), 129.40 (C_5), 124.22 (C_4), 117.82 (C_2), 113.83 (C_6), 99.16 ($C_{1'}$), 72.85 ($C_{5'}$), 72.08 ($C_{3'}$), 71.27 ($C_{2'}$), 68.44 ($C_{4'}$), 62.15 ($C_{6'}$), 21.60 (C_7), 20.83 ($C_{14'}$), 20.79 ($C_{10'}$), 20.76 ($C_{8'}$), 20.74 ($C_{12'}$); HR-MS calcd for $C_{21}H_{26}NaO_{10}^+$ ($[M + Na]^+$) 461.1424, found 461.1432; $[\alpha]_D$, −20.1 (*c* 0.5, $CHCl_3$) [lit. −20 [*c* 1, $CHCl_3$][20]; mp 110−111 °C [lit. mp 111−112 °C].[21]

3-Methylphenyl 1-*O*-β-D-Glucopyranoside (*m*-Cresol β-D-Glucoside). *m*-Cresol tetra-*O*-acetylglucoside (451 mg, 1.0 mmol) was stirred overnight at room temperature in methanol/water/triethylamine (8:1:1 v/v, 9 mL). The resultant clear solution was concentrated in vacuo, water was added, and the solution was concentrated again. This procedure was repeated several times until the solid residue reached a constant weight. The solid was recrystallized from ethanol to give a white powder (192 mg, 69%; R_f = 0.41 in $CH_2Cl_2/CH_3OH/CH_3COOH$ 79.5:20:0.5 v/v), which was >99% pure by HPLC-DAD at 280 nm: ^{1}H NMR (CD_3OD), δ7.14 (1H, t, J = 7.8 Hz, H_5), 6.92 (1H, s, H_2), 6.86 (1H, dd, J = 7.8, 2.3 Hz, H_6), 6.80 (1H, d, J = 7.8 Hz, H_4), 4.88 (1H, d, J = 7.5 Hz, $H_{1'}$), 3.86 (1H, dd, J = 12.2, 2.5 Hz, $H_{6b'}$), 3.67 (1H, dd, J = 12.2, 5.5 Hz, $H_{6a'}$), 3.47 (1H, dd, J = 9.1, 8.9 Hz, $H_{3'}$), 3.46 (1H, dd, J = 9.1, 7.5 Hz, $H_{2'}$), 3.43 (1H, ddd, J = 10.1, 5.5, 2.5 Hz, $H_{5'}$), 3.39 (1H, t, J = 8.9 Hz, $H_{4'}$), 2.31 (s, 3H, H_7); ^{13}C NMR (CD_3OD), δ 159.12 (C_1), 140.53 (C_3), 130.14 (C_5), 124.08 (C_4), 118.41 (C_2), 114.66 (C_6), 102.26 ($C_{1'}$), 78.07 ($C_{5'}$), 77.94 ($C_{3'}$), 74.90 ($C_{2'}$), 71.36 ($C_{4'}$), 62.48 ($C_{6'}$), 21.52 (C_7); HR-MS calcd for $C_{13}H_{18}NaO_6^+$ ($[M + Na]^+$) 293.1001, found 293.1005; $[\alpha]_D$, −64.1 (*c* 0.5, H_2O) [lit. −69.7 (H_2O)];[21] mp 178−179 °C [lit. 183−184 °C].[21]

Chemical Analysis. The basic chemical composition of all wines was determined by the AWRI Commercial Services as detailed in Iland et al.[22] The titratable acidity, volatile acidity, and alcohol were measured using FTIR WineScan (FOSS, Hillerød, Denmark).

Volatile phenol compounds were quantified by gas chromatography−mass spectrometry (GC-MS), using deuterium-labeled guaiacol and 4-methylguaiacol as internal standards as previously described.[7] For the determination of the smoke-affected wine samples at the time of the sensory analysis, single replicates were analyzed; a subsequent analysis of the wines a month later in triplicate showed that the relative standard deviation was generally <15% except for the compound phenol, which was 40%. The glycosidically bound forms of the volatile phenols were analyzed by high-performance liquid chromatography−tandem mass spectrometry (HPLC-MS/MS).[7]

Sensory Analysis. Unless otherwise specified, all sensory assessments were conducted in isolated well-ventilated sensory booths. Wines (30 mL) were presented in a randomized order at 22−24 °C in three-digit-coded, covered, ISO standard glasses. All sensory data were obtained in compliance with institutional procedures for sensory evaluation, involving risk assessment and informed consent, and all samples were expectorated. The data were collected using Fizz software (Biosystemes, France, version 2.2).

Sensory Descriptive Analysis. The wines were profiled by a trained sensory descriptive panel of four males and nine females, including wine research staff and wine science postgraduate students. All panelists except one had previous experience on wine sensory descriptive panels. The sensory panel generated descriptive terms for the wines over four training sessions during which wines from the set were presented, plus reference standards when necessary, until panelists decided upon a consensus list of attributes to rate and their definitions. As the wines possessed diverse aroma properties, and the primary aim of the study was to assess smoke-related attributes, the panel agreed to use the general terms *overall fruit* aroma and flavor rather than more specific fruit-related attributes. Training was followed by one practice rating session during which judges rated a subset of the wines in duplicate under the same conditions applied in the subsequent formal sessions, except in a constant presentation order. The final list of attributes consisted of five aroma and seven by-mouth attributes (Table 2).

The descriptive study was performed in November 2009 under sodium lights to mask any color differences that could bias the assessments, with samples evaluated in triplicate. Eight to ten samples were assessed per session over six sessions held over a two week period.

The intensity of the sensory attributes was rated on a 15 cm unstructured line scale marked with indented anchor points of "low" and "high" placed at 10 and 90% of the scale, respectively. Panelists had a forced rest of 45 s between samples when they rinsed their mouths with aqueous citrus pectin solution (1 g/L, Fluka) followed by water.

Sensory Threshold Tests. A three-alternative forced choice (3-AFC) test was carried out on the basis of ASTM standard method E 679-04.[23] Compounds under evaluation were checked for purity by GC-MS−olfactometry. Ethanol (1.0 mL) was added to each liter of base wine used as control so spiked and control samples had the same alcohol level. A set of reference samples including a control (base wine) and the base wine with additions of the test compound labeled as moderate (80 μg/L for guaiacol and 160 μg/L for the cresols) and high (200 μg/L for guaiacol and 400 μg/L for the cresols) were assessed by the panelists before entering the sensory booths for

Table 3. Concentrations (Micrograms per Liter) of Free Volatile Phenols in Wines and Reported Sensory Detection Threshold Literature Values, Determined in Model Systems

sample code	guaiacol	syringol	4-allylsyringol	4-methylsyringol	4-vinylguaiacol	4-methyguaiacol	phenol	*o*-cresol	*p*-cresol	*m*-cresol
control a	3	4	nd[a]	nd	nd	nd	2	nd	nd	1
control b	6	15	18	4	nd	6	6	6	2	4
control c	6	10	7	2	nd	1	nd	2	nd	1
1	12	6	7	3	1	3	17	8	5	6
2	18	21	11	8	2	6	18	10	5	8
3	8	11	11	3	1	4	17	8	5	5
4	36	20	17	12	9	9	26	6	3	3
5	15	18	4	4	3	3	15	6	4	7
6	23	22	5	5	14	3	52	11	6	9
7	10	16	20	3	11	1	22	5	4	5
8	16	18	13	7	3	2	33	11	6	8
9	7	14	18	4	5	2	1	6	4	3
10	16	23	6	5	4	5	29	6	3	7
11	35	23	6	3	5	4	17	6	2	2
12	7	10	5	4	12	4	13	6	4	6
13	55	26	7	9	4	10	44	26	6	13
14	31	16	2	10	0	5	40	9	4	8
15	27	15	3	3	3	1	43	3	1	2
detection threshold	9.5[b]	570[b]	1200[b]	10000[c]	40[b]	21[d]	7100[e]	31[e]	3.9,[d] 10[e]	15,[d] 68[e]

[a]Not detected: for all compounds except phenol, <1 μg/L; phenol, <2 μg/L. [b]Odor detection threshold in aqueous 10% alcohol at pH 3.2.[25] [c]Taste detection threshold in water.[26] [d]Odor detection threshold in water.[27] [e]Odor detection threshold in aqueous 10% alcohol.[12]

familiarization. Panelists were asked to assess these reference samples and consider sensory differences among them. Assessors were not informed of the nature of the compound tested. Samples were prepared freshly each day.

For each threshold test, six 3-AFC tests were presented to the judges in increasing order of concentration, with the concentration steps increasing by a factor of 2 (from 2.5 to 80 μg/L for guaiacol and from 5.0 to 160 μg/L for the cresols). These concentrations were confirmed by GC-MS analysis. In the cases when an individual panelist's threshold did not fall in the original concentration range, this person was retested at concentrations that were two steps lower or higher, so that judges were presented with three sets of 3-AFC tests, including the concentration that overlapped with the original set. The best-estimate threshold (BET) for each assessor was calculated using the geometric mean of the concentration at which the panelist had the last incorrect response and the first concentrations at which the panelist had consecutive correct responses. The panel threshold was then calculated using the geometric mean of each panelist's BET.

Assessment was carried out by aroma only for all compounds, and additionally a by-mouth only test was performed for guaiacol.

A total pool of 30 judges participated in the threshold tests. Judges were selected on the basis of their previous ability and performance in difference testing. Six of the judges had previously participated in the descriptive analysis study on tainted wines. A minimum of 22 judges was used for each compound tested depending on availability. Assessments took place from January 28 to March 2, 2010.

The samples (20 mL for aroma and 30 mL for flavor threshold) were presented in two trays of three sets of 3-AFC tests, with each set consisting of two control samples and one spiked sample. For each set, judges were instructed to identify the sample with the added compound.

Volatile Phenol β-D-Glucoside Rating Test. For a preliminary sensory assessment, 0.50 and 5.0 mg/L aqueous solutions of guaiacol β-D-glucoside were prepared. An informal sensory assessment was conducted with 10 mL aliquots, assessed blind with a control, in silence, in a constant presentation order across assessors, in an open sensory evaluation room. Eight assessors evaluated the samples, comprising research staff experienced in evaluations of volatile phenol and smoke taint samples. The 5 mg/L sample was expectorated and frozen at −20 °C for subsequent analysis, although the sample from one assessor was not obtained. Following written free-choice comments regarding the perceptions of the samples, the samples were discussed.

Four samples were prepared for a formal rating test, consisting of guaiacol β-D-glucoside, syringol β-D-glucoside, and *m*-cresol β-D-glucoside in model wine (10% food grade ethanol in saturated potassium hydrogen tartrate solution, pH 3.55) at 0.5 mg/L, and a control model wine with no addition.

The concentration of the free volatile phenols for all samples, including the control model wine with no addition, was found to be below 1 μg/L. The three glucoside samples plus the control were assessed by a sensory panel of 30 tasters with moderate to very extensive experience in wine sensory evaluation. Five of these tasters had participated in the sensory descriptive test previously described. The panel rated *smoky* and *medicinal* aroma and *smoky/ashy* and *medicinal* flavor for the four samples, in duplicate, with a forced rest of 2 min between samples and a longer break between replicates. Prior to the test, separate tasting standards of guaiacol and *m*-cresol (both 100 μg/L) additions in model wine were presented to the tasters labeled *smoky* and *medicinal*, respectively. Judges also received *smoky* and *medicinal* standards (Table 2) for aroma assessment.

The concentration of volatile phenols in the glucoside solutions was determined in duplicate in the presence of saliva. Model wine solution (4 mL), with or without the phenol-glucosides as assessed by the sensory panel, was added to a 2 mL aliquot of saliva from a single subject in a 7 mL screw cap sealed vial and incubated, after shaking, at 40 °C for 30 min. The free volatile phenols were extracted and analyzed as previously described.[7]

A follow-up test was performed with six of the same assessors who participated in the glucoside rating test, comprising three sensitive tasters and three assessors who were unable to perceive aroma differences among the previously tested glycoside samples, to further investigate the in-mouth release from guaiacol-glycoconjugates in the mouth. Two samples (10 mL, guaiacol β-D-glucoside added to model wine and control model wine with no additions) were evaluated in triplicate by the six panelists, who rated the intensity of *smoky* and *medicinal* aroma and *smoky/ashy* and *medicinal* flavor.

Data Analysis. Analysis of variance (ANOVA) was conducted on the descriptive analysis and rating test data for each attribute testing for the main effects of wine, judge, and replicate and their interactions,

treating judges as a random effect. Principal component analysis (PCA) was performed on the correlation matrix of mean attribute ratings across the wines for all attributes that showed significant differences according to the ANOVA. Partial least-squares regression (PLS2) was used to relate each of the smoke-related sensory attributes (y-data) with the volatile phenol concentration (x-data) to assess whether any particular compound or group of compounds was important to the smoke-related sensory attributes of smoke-affected wines. All data were standardized prior to analysis, with full cross-validation employed, and the residual validation variance values examined to determine the appropriate number of factors to include in the model. The statistical significance of the chemical components to the model were evaluated using Marten's uncertainty test.[24]

Statistical analyses were performed with Fizz (Biosystemes, France, version 2.2), JMP (SAS Institute, USA, version 5.0.1a), and The Unscrambler (version 9.5, CAMO Process AS, Norway).

RESULTS AND DISCUSSION

Volatile Phenol Sensory Thresholds and Their Concentrations in Smoke-Affected Wines. The volatile phenol concentration of each of the wines studied is shown in Table 3, together with reported estimates of sensory detection threshold values determined in 10% v/v aqueous ethanol or in water. The control wines generally had low or undetectable concentrations of the compounds of interest. The concentration of guaiacol was above its reported odor detection threshold value in many of the smoke-affected wines, with the only other compound measured above the reported threshold being *p*-cresol. The *o*- and *m*- cresols were present at a level approaching threshold. Wines 4, 11, 13, 14, and 15 had the highest levels of guaiacol, whereas wine 13 was highest in the cresols, together with wines 2, 6, and 8.

To better assess the sensory significance of the cresols, the odor thresholds of guaiacol and the cresols in a red wine matrix were determined, together with the flavor thresholds of guaiacol in the same wine. Table 4 provides the best-estimate panel aroma threshold for the compounds.

Table 4. Best-Estimate Threshold (BET) for Odor and Standard Error (SE) of the Mean Values Determined for Four Volatile Phenol Compounds in Red Wine, as well as the Flavor Threshold for Guaiacol[a]

compound	BET (μg/L)	SE
m-cresol (n = 23)	20	0.6
guaiacol (n = 23)	23	0.8
guaiacol (flavor, n = 22)	27	0.6
p-cresol (n = 22)	64	0.5
o-cresol (n = 22)	62	0.8

[a]The number of assessors is also provided.

m-Cresol, somewhat unexpectedly given the previously reported threshold in aqueous ethanol,[12] had the lowest aroma threshold value, comparable to that of guaiacol. A recent paper[28] describing thresholds in air found that substitution at the meta position lowered thresholds substantially for a range of alkyl phenols. The flavor threshold for guaiacol was 27 μg/L, which was similar to the aroma threshold for this compound. Each of the thresholds, not surprisingly, was higher than those reported in aqueous ethanol or water. The thresholds of these compounds studied would suggest that only guaiacol and *m*-cresol are likely to be important contributors to the aroma of the smoke-affected wines studied (Table 3). There may, however, be a possible additive contribution of the volatile phenols, as previously suggested for below -threshold volatile compounds with a similar structure and sensory properties (see ref 29 and references cited therein).

Relating Sensory Properties to Volatile Phenol Concentration. Descriptive sensory analysis was used to characterize differences in the aroma and flavor attributes of the wines. The wines differed significantly in all sensory attributes rated (Table 1) except for *metallic*. The smoke-related attributes *smoke* aroma, *medicinal*, *cold ash*, *ashy* aftertaste, and *smoky* flavor were highly significantly different among the samples at $p < 0.001$. Figure 2 shows the first two principal components (PCs) from a PC analysis performed on the mean data averaged over judges and replicates.

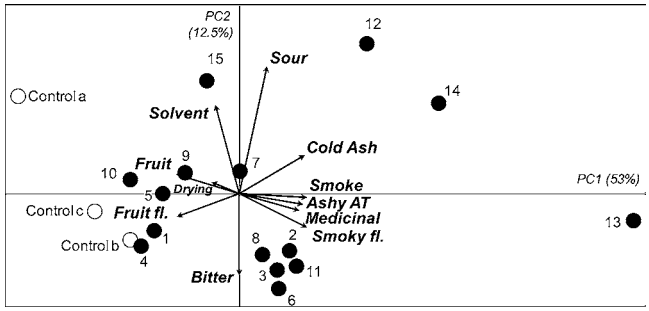

Figure 2. Principal component analysis biplot of the sensory attribute mean values for each of the wines. Control wines (open symbols) were produced from grapes not affected by bushfire smoke. fl: flavor.

The PCA biplot shown in Figure 2 gives an overview of the variation among the samples in their sensory attribute ratings. The first two PCs explain 65.5% of the variance in the data. The smoke-related attributes *cold ash* aroma, *smoke* aroma, *medicinal* aroma, *ashy* aftertaste, and *smoky* flavor were positively correlated with one another, as indicated by the small angle between the vectors ($r \geq 0.63$, $p < 0.01$). The first dimension, which explained 53% of the total variance in the sensory data, contrasts wines rated relatively highly in *fruit* aroma and flavor (including control wines) with those rated highly in smoke-related attributes, located on the right of the figure. Wines 12–14, all made using small-scale winemaking methods, were rated highest in smoke-related attributes and relatively low in fruit, and wines 2, 3, 6, 8, and 11 were moderately high in smoke attributes. There was no evident pattern related to grape variety or alcohol level.

Separation of the samples along PC2 was on the basis of *sourness*, which was not related to the smoke sensory attributes. PC3 (not shown) explained a further 12% of the variance and separated samples on the basis of the *bitter* attribute, but there was no pattern related to smoke influence.

For evaluation of the relationships among the sensory data and the volatile phenol results, a PLS approach was used. From an initial PLS analysis, it was found that sample 4 was a strong outlier, being rated low in smoke-related sensory attributes but high in guaiacol and other volatile phenols (Table 4). This wine was considered an outlier and was accordingly removed from the data set. In the model with the remaining 17 wines, two optimal factors were used, with the model explaining 73% of the variance in the sensory data. Figure 3 shows the chemical data regression coefficients for a subset of the smoke-related attributes from the PLS regression analysis. The coefficients of the other smoke-related attributes were similar to those of

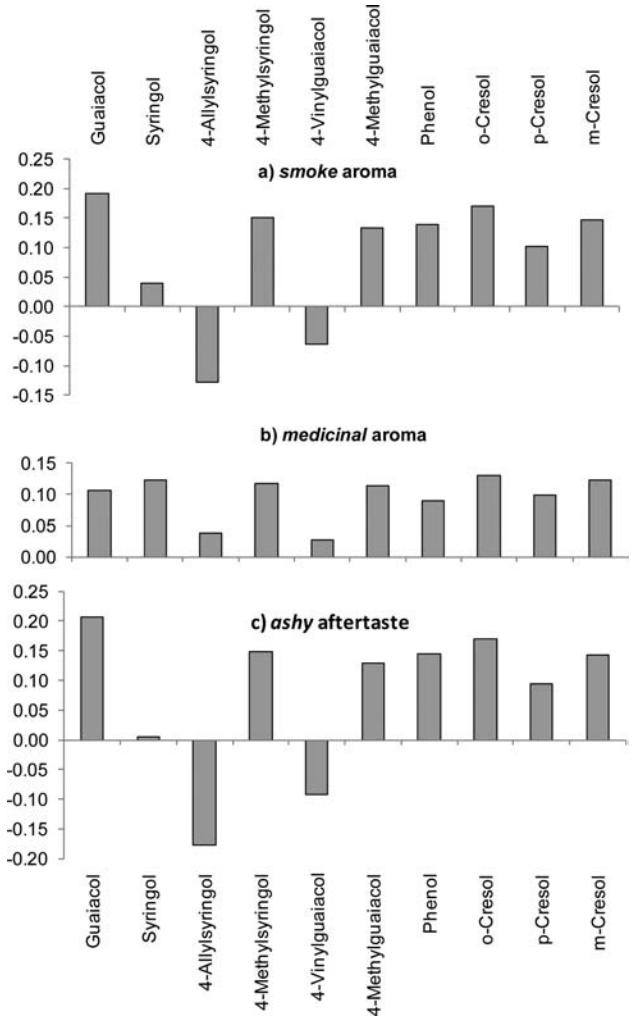

Figure 3. Regression coefficients from the partial least-squares model for the volatile phenols associated with (a) *smoke* aroma, (b) *medicinal* aroma, and (c) *ashy* aftertaste.

smoky aroma and *ashy* aftertaste. Longer bars represent the compounds most important to the model.

The most important volatile phenols positively associated with both *ashy* aftertaste and *smoke* aroma were guaiacol, 4-methylsyringol, 4-methylguaiacol, phenol, and *o*- and *m*-cresol. For the *medicinal* attribute, all of the volatile phenols had positive coefficients, and syringol was one of the largest contributors to the model, together with *o*-cresol and methylsyringol. According to Marten's uncertainty test,[24] 4-methylsyringol, *m*-cresol, and phenol were indicated as significant contributors for *smoke* aroma, whereas only 4-methylsyringol was significant for *medicinal* aroma, and guaiacol, 4-ethylguaiacol, and *m*-cresol were indicated as significant for *ashy* aftertaste. The model indicated that the smoke-related sensory attributes were well predicted by the volatile phenol data, with calibration coefficients of determination between measured and predicted values for *smoky* aroma, *medicinal* aroma, and *ashy* aftertaste being 0.83, 0.57, and 0.82, respectively. Given the very high sensory thresholds of 4-methylsyringol and syringol, these compounds are unlikely to be contributing flavor, although there could be an additive effect. These compounds may be considered good markers of smoke exposure of grapes and persist in wine, but are likely to contribute only very little to smoke-related aroma or flavor in wine. A model including only the compounds guaiacol, methylguaiacol, vinylguaiacol, and *o*- and *m*-cresol gave a slightly improved predictive ability, with the two cresols being indicated as particularly important to the model.

The coefficients of determination between predicted and measured using simple regressions between the individual volatile phenol data and the smoke-related sensory attribute scores indicated that the predictive ability of measuring only guaiacol, although reasonable, was poorer than the PLS model including multiple volatile phenols. For guaiacol only, coefficients of determination for predicted versus measured for *smoky* aroma, *medicinal* aroma, and *ashy* aftertaste were 0.72, 0.45, and 0.49, respectively. The low predictive ability of the guaiacol concentration for the *ashy* aftertaste attribute is of particular interest, as this sensory attribute of the smoke-affected wines is considered to be a distinct difference between oak-matured wines and wines made from grapes exposed to forest fire smoke. A multiple linear regression stepwise approach indicated that the best model for *ashy* aftertaste included only *m*-cresol and guaiacol, with the R^2_{adj} for this model being 0.74. The cresols have previously been suggested as important to red wine flavor,[30,31] and it is noteworthy that *m*-cresol, which was determined to have the lowest sensory detection threshold in red wine, was consistently indicated as important to the models produced.

The wines had been analyzed for individual glycoconjugates of volatile phenols (Supplementary Table 1 in the Supporting Information), with data similar to those shown previously.[7] Individual monosaccharide and disaccharide glycosides of phenols were found up to 2.4 mg/L, with total phenol glycoside concentrations of up to 11 mg/L. For completeness, a final PLS model was generated for all the smoke-related sensory data using all available phenol data, including those of the glycoconjugates. Whereas the regression coefficients of the glycosides for the model were all below 0.1, many of the glycosides were considered to be significant contributors to the model by the Martens uncertainty test, and the model statistics improved slightly with the inclusion of the glycosides. The coefficient of determination for predicted versus measured values for *ashy* aftertaste was 0.86 compared to 0.82 with the volatile phenols only. The gentiobioside glycosides of phenol, 4-methylguaiacol, and syringol, together with the rutinosides of the cresols, guaiacol, and methylguaiacol, were indicated as significant contributors to *ashy* aftertaste.

Sensory Attribute Rating of Volatile Phenol Glucosides. From the results of both the formal sensory-compositional study and an informal reconstitution sensory assessment, together with the information from the PLS analysis, there was considered to be a possibility that the glycoconjugate component of smoke-affected wines might have an influence on smoky flavor, through possible release of aglycones in-mouth.

A further informal sensory assessment was conducted using 10 mL aliquots of guaiacol β-D-glucoside in water (0.5 and 5 mg/L), assessed blind with a control. This concentration was selected on the basis of previous studies[7,14] in which, in particular, guaiacol β-D-glucoside was found at 0.4 mg/L in smoke-affected wine. Informal sensory evaluations indicated that nearly all panelists could perceive a 'smoky' aftertaste from a 0.5 mg/L solution of each glycoside. Of the eight tasters, all except one reported that they could easily perceive a smoke-like flavor and lingering aftertaste in the glucoside samples, but no smoky aroma by orthonasal evaluation. The concentration of

free guaiacol in the expectorated 5.0 mg/L sample for each taster was determined following frozen storage. No guaiacol was detected in the sample before tasting or in the blank control (<1 μg/L). The range of guaiacol values for the assessors who indicated they could perceive a smoky flavor was 13−148 μg/L.

Subsequently, a replicated sensory study was conducted with a panel of 30 assessors, rating the aroma and flavor intensity of smoke-related attributes for model wine solutions of guaiacol β-D-glucoside, syringol β-D-glucoside, and *m*-cresol β-D-glucoside, all at 0.5 mg/L.

From the ANOVA of the sensory data there was a highly significant ($p < 0.001$) difference among the samples for the attribute *smoky/ashy* flavor. Mean values are shown in Table 5.

Table 5. Mean Sensory Scores (n = 30 Judges × 2 Replicates) for Smoke Attributes of Volatile Phenol Glucosides Added to a Model Wine at 0.5 mg/L[a]

	mean sensory score			
sample	*smoky* aroma	*medicinal* aroma	*smoky/ashy* flavor	*medicinal* flavor
control model wine	1.29	2.02	2.08	2.63
guaiacol β-D-glucoside	1.76	2.24	3.40	2.91
m-cresol β-D-glucoside	1.59	2.00	2.77	3.24
syringol β-D-glucoside	1.59	2.29	2.21	2.87
LSD	ns	ns	0.68	ns

[a]The least significant difference (LSD, $p = 0.05$) is also shown.

There was a fairly high rating for the control model wine, presumably due to a false-positive effect, probably due to limited training of the panelists who were generally not familiar with assessing model wine samples. The guaiacol and *m*-cresol β-D-glucoside samples were rated significantly higher in *smoky/ashy* flavor compared to the control model wine and imparted no significant difference in the aroma. There was no significant sensory effect of the syringol β-D-glucoside, which may be related to the high aroma threshold of the free compound (Table 3). Whereas *medicinal* flavor was not significant overall, there were nine people who, from a one-way analysis of variance, rated *medicinal* flavor significantly higher in the sample with added *m*-cresol β-D-glucoside. This may relate to idiosyncratic use of the attributes, higher individual sensitivity to this compound, or greater ability to release the free compound from its glycosidic form. Further studies are needed to address these questions. It is possible that the glycosides might have a taste effect, with the assessors giving a false response due to a category dumping effect. This is not likely given the assessors were experienced in sensory evaluation and were encouraged to note any other flavor or taste attribute that might be perceived.

Chemical analysis of saliva with added model wine solution, with saliva collected from a single assessor, confirmed that the respective free volatiles had been released from the glycoside samples, with between 15 and 20 μg/L of the particular volatile phenol detected. The model wine with no added glucoside with or without saliva gave no detectable volatile phenols, as did the glucoside sample with no added saliva. The release of volatile phenols was approximately 10−18% of the total, which is similar to that reported previously.[7]

These results were confirmed by a test conducted in triplicate with six assessors, including three of the highest raters from the previous test and three who could not perceive any flavor due to the glucosides. As found previously, the presence of guaiacol glucoside gave rise to a significantly higher *smoky/ashy* flavor rating compared to the control model wine for the three sensitive assessors, and no significant difference between control and the model wine with guaiacol β-D-glucoside addition was found in the aroma attributes. Two assessors rated the *smoky* flavor attribute in the guaiacol β-D-glucoside sample and one taster rated the character as *medicinal*, whereas the remaining three assessors, as expected, did not rate the guaiacol β-D-glucoside sample differently from the control for any attribute (data not shown).

Together, these data suggest strongly that glycosides of volatile phenols have a role in contributing to the in-mouth flavor and aftertaste of smoke-affected wines. A previous paper[15] provided evidence that bacterial microflora, or alternatively the presence of epithelial cells, were the source of β-glucosidases and that activity could vary greatly among individuals, as observed in the present study. These authors showed that only monoglucosides of the flavonoids they studied could be substantially hydrolyzed rapidly in the mouth.

A separate study on the effect of saliva on volatile composition of wine[32] showed that enhancements of some volatiles can occur, but fewer influences were evident in a red wine compared to a white, suggesting an effect of phenolic compounds inhibiting enzymatic activity. This work did not provide evidence that glycoside hydrolases were active in saliva. Studies have previously shown that salivary enzyme activity can be highly variable and have complex effects on specific volatiles.[33,34] To our knowledge the present study is the first to show that in-mouth release of aglycones has a sensory effect. It is noteworthy that the effect occurs in tasting a model wine at low pH, and not necessarily at optimal pH for most glycosidase enzymes, but further work is required to determine if such an effect occurs during wine consumption.

As forest fires that affect vineyards are becoming more frequent in Australia and elsewhere, it will be essential for wine producers to know at what concentration smoke-related compounds affect the sensory properties of a particular wine. Overall, the results from the present study allow a determination of the sensory significance of volatile phenols when measured in smoke-affected wines. The compounds shown to be most important in this study were the cresols, especially *m*-cresol, guaiacol, and their glycosides.

The likelihood that release of glycosides in-mouth contributes a lingering flavor is of notable interest. Given that there is a large pool of glycosides that have been shown to act as precursors to a wide range of fruity flavor-active volatiles,[35] in-mouth release has potentially highly significant importance to other flavors in wines, and indeed other beverages, foods, and fruit-based products in which glycosides are present. The persistence of flavors after swallowing is considered an important part of food and beverage quality, and glycosides may be key contributors to this phenomenon. Given that β-glucosidase enzymes are inhibited strongly by glucose,[36] although there are a number of relatively glucose tolerant β-glucosidases, it is likely that release of flavor by microflora or endogenous enzymes in the mouth would predominantly occur for low sugar products and that tasting fruits would be unlikely to result in substantial hydrolysis of the precursor compounds, and thus no flavor from this source would be perceived. Accordingly, tasting grape berries to assess *smoky/ashy* flavor

related to glycosides would not likely be a reliable indication of how affected a resulting wine might be.

Although the compounds quantified in this study were strongly related to *smoky* or *medicinal* aroma and flavor, further study is required to investigate the flavor-release potential of the various glycoconjugates present in smoke-affected wines, most of which are not monoglucosides, and to assess the relative contribution of the glycoconjugates compared to the free volatiles.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplementary Table 1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

The Australian Wine Research Institute (AWRI), a member of the Wine Innovation Cluster in Adelaide, is supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian government.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dr. James Kennedy, Adrian Coulter, Randell Taylor, and Con Simos of the Australian Wine Research Institute (AWRI) for valuable discussions and for assistance in sourcing smoke-exposed grapes and wines. We also thank the many Australian wine companies for their supply of samples and valuable discussions, in particular Vanessa Stockdale and Treasury Wine Estates. We acknowledge Dr. Simon Odell and Dr. Renata Ristic for their contribution in conducting winemaking and assisting with the sensory study and the sensory panelists as well as Belinda Bramley and Dr. Kerry Wilkinson for their contribution to the sensory work.

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