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Glycosylation of Smoke-Derived Volatile Phenols in Grapes as a Consequence of Grapevine Exposure to Bushfire Smoke

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The presence of glycosides of smoke-derived volatile phenols in smoke-affected grapes and the resulting wines of Chardonnay and Cabernet Sauvignon was investigated with the aid of high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS). All volatile phenols studied (phenol, *p*-, *m*-, and *o*-cresols, methylguaiacol, syringol, and methylsyringol) could be detected as glycosylated metabolites in smoke-affected grapes in a similar fashion to that previously reported for guaiacol. These phenolic glycosides were found in smoke-affected grapes and wines at significantly elevated levels compared to those in non-smoked control grapes and wines. The extraction of these glycosides from grapes into wine was estimated to be 78% for Chardonnay and 67% for Cabernet Sauvignon. After acid hydrolysis, a large proportion of these phenolic glycosides in grapes (50%) and wine (92%) disappeared but the concentrations of volatile phenols determined by gas chromatography−mass spectrometry (GC−MS) were lower than expected. In the case of wine, the majority of the glycosides of phenol, cresols, guaiacol, and methylguaiacol were decomposed upon acid hydrolysis without releasing their respective aglycones, while syringol and methylsyringol were more effectively released.

KEYWORDS: Volatile phenols; glycosides; smoke exposure; smoke taint; grapes; wine; bushfire; HPLC−MS/MS

INTRODUCTION

In a previous study using a stable isotope tracer technique (*1*), we identified seven different glycoconjugates (glycosides) of guaiacol in grape berries and leaves that had been in direct contact with mixed d_0- and d_3-guaiacol solutions. These guaiacol glycosides were tentatively identified as follows: glucosylglucoside (either gentiobioside or sophoroside, GG), β-D-glucopyranoside (glucoside, MG), the glucoside further substituted with a pentose (diglycoside, DG), such as α-L-arabinosyl-β-D-glucoside, β-D-apiosyl-β-D-glucoside, or β-D-xylosyl-β-D-glucoside, and α-L-rhamnosyl-β-D-glucoside (rutinoside, RG). The study also indicated that there was minimal translocation of guaiacol glycosides between grapevine leaves and berries. The glycosides were present as low-level natural compounds in control leaves and berries, and the glycosides were present in significantly elevated amounts in leaves and berries following exposure of grapevines to smoke derived from actual bushfires.

With regard to smoke taint in grape and wine products, the role of the guaiacol glycosides remains uncertain; however, it is likely that they act as precursors to free guaiacol, contributing to the intensity of smoke taint characters after fermentation and in wine, especially with increasing storage time (*2*, *3*). In addition to guaiacol, smoke contains a variety of other volatile phenols, including phenol, cresols, methylguaiacol, vinylguaiacol, syringol, methylsyringol, and vinylsyringol. Some of these compounds, which have not been considered previously for smoke-affected grape and wine samples, also have distinctive smoke-related sensory characters (*4*, *5*), which may contribute to the overall smoke effect on grapes and wine.

This study was undertaken to investigate the presence of glycosides of smoke-derived volatile phenols in grapes exposed to bushfire smoke and in the wines made from smoke-exposed grapes, using high-performance liquid chromatography−tandem mass spectrometry (HPLC−MS/MS). Acid hydrolysis of glycosides in grape and wine samples was also performed to ascertain the potential extent of volatile phenol release during winemaking and storage.

MATERIALS AND METHODS

Materials. All chromatographic solvents were HPLC-grade. All chemicals were analytical-reagent-grade unless otherwise stated. Water was obtained from a Milli-Q purification system (Millipore, North Ryde, Australia). All prepared solutions were % v/v, with the balance made up with Milli-Q water, unless otherwise specified. Merck solvents were purchased from Rowe Scientific (Lonsdale, Australia). Model wine was made of water/ethanol (87:13) saturated with potassium hydrogen tartrate and adjusted to pH 3.2 with the addition of 10% (w/v) tartaric acid. Guaiacol, 4-methylguaiacol, vinylguaiacol, phenol, *o*-cresol, *m*-cresol, *p*-cresol, syringol and 4-methylsyringol, and d_7-*p*-cresol were purchased

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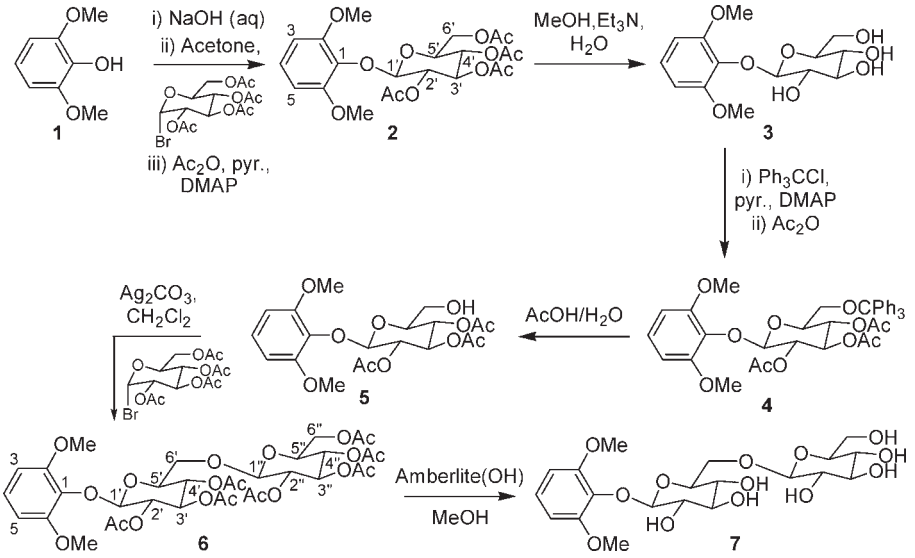

Figure 1. Synthesis of syringol-GG (**7**) via syringol-MG (**3**).

from Sigma-Aldrich (Castle Hill, Australia). d_3-Guaiacol, d_3-4-methylguaiacol, and d_3-guaiacol-β-D-glucopyranoside (d_3-guaiacol-MG) were previously synthesized in house (*1*,*6*). d_3-Syringol was prepared according to the method of Aihara et al. (*7*), and syringol monoglucoside (syringol-MG) and syringol gentiobioside (syringol-GG) were prepared on the basis of modifications to the procedures of Shao et al. (*8*).

Nuclear Magnetic Resonance (NMR) Analysis. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded with Bruker spectrometers operating at 400 or 600 MHz for proton and 100 or 150 MHz for carbon nuclei, respectively. Chemical shifts were recorded as δ values in parts per million (ppm). Spectra were acquired in chloroform-*d* or deuterium oxide (D_2O) 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 HOD (δ 4.79) was used as the internal reference. For ^{13}C NMR spectra, the central peak of the $CDCl_3$ triplet (δ 77.16) or the CH_3 peak of acetonitrile (δ 1.47), added when D_2O was the solvent, 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 or atmospheric pressure chemical ionization (APCI) in negative 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.

2-d_3-Methoxy-6-methoxyphenol (d_3-Syringol) (d_3-1). This compound was prepared as described by Aihara et al. (*7*). Briefly, 3-methoxycatechol (5.00 g, 35.7 mmol) gave the desired 2-benzylated product (2.96 g, 12.84 mmol, 36%) as a colorless oil after purification on silica gel with 10% Et_2O/20% CH_2Cl_2/hexane → 10% Et_2O/40% CH_2Cl_2/hexane (R_f = 0.48) and solvent removal, along with the 1-benzylated compound (12%) and the 1,2-dibenzylated compound (7%). The 2-benzylated material (2.08 g, 9.03 mmol) was d_3-methylated to afford a pale yellow oil (2.22 g, ca. 100%), which was used crude in the next step. Hydrogenolysis of the benzyl group, purification on silica gel with 10% Et_2O/20% CH_2Cl_2/hexane → 10% Et_2O/40% CH_2Cl_2/hexane (R_f = 0.28), and solvent removal afforded title compound **d_3-1** (1.38 g, 8.78 mmol, 98%) as a colorless solid. mp 54.5−55.5 °C [literature mp 55−55.5 °C (*7*)]. The spectroscopic data were in full accordance with those reported by Aihara et al. (*7*).

2,6-Dimethoxyphenyl-1-*O*-β-D-glucopyranoside (Syringol Monoglucoside or Syringol-MG) (3). This compound was prepared as detailed in **Figure 1** based on the method of Shao et al. (*8*). Peracetyl-α-glucopyranosyl bromide (2.54 g, 6.18 mmol) in acetone (22 mL) was added dropwise to a stirred solution of syringol (**1**) (1.00 g, 6.49 mmol) in 0.3 M NaOH (21 mL, 6.30 mmol) at 14 °C. After 5 h, the reaction mixture was concentrated under reduced pressure and dried further under high vacuum, yielding a yellow syrup. Anhydrous pyridine (2.5 mL), acetic anhydride (4 mL), and 4-dimethylaminopyridine (DMAP) (12.4 mg) were added, and the mixture was heated under N_2 at 90 °C for 1 h and then stirred overnight at room temperature. The reaction mixture was cooled with an ice-water bath. Methanol (5 mL) was added, and stirring was continued for 30 min before removal of the volatiles under reduced pressure. The residue was dissolved in CH_2Cl_2 (100 mL) and washed successively with 0.1 M HCl (10 mL), water (10 mL), and brine (3 × 10 mL). The organic phase was dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed on silica gel with CH_2Cl_2 → 4% Et_2O/CH_2Cl_2 (R_f = 0.24 in 10% Et_2O/CH_2Cl_2) to afford, after solvent removal, syringyl acetate (0.613 g, 3.12 mmol, 50%) and desired compound **2** (1.08 g), which was recrystallized from ethanol to yield colorless crystals (0.929 g, 1.92 mmol, 31%; 63% based on recovered syringyl acetate). mp 140−140.5 °C. $[\alpha]_D$ −10.0 (*c* 0.502, MeOH).

^{1}H NMR (ppm, $CDCl_3$) δ: 7.02 (1H, t, J = 8.4 Hz, H_4), 6.56 (2H, d, J = 8.4 Hz, $H_{3,5}$), 5.31 (1H, dd, J = 8.9, 7.4 Hz, $H_{2'}$), 5.27 (1 H, app t, J = 9.2 Hz, $H_{4'}$), 5.25 (1H, app t, J = 9.1 Hz, $H_{3'}$), 5.06 (1H, d, J = 7.4 Hz, $H_{1'}$), 4.25 (1H, dd, J = 12.2, 5.0 Hz, $H_{6a'}$), 4.11 (1H, dd, J = 12.2, 2.6 Hz, $H_{6b'}$), 3.81 (6H, s, 2 × ArOCH_3), 3.68 (1H, ddd, J = 9.4, 5.0, 2.6 Hz, $H_{5'}$), 2.030 (3H, s, COCH_3), 2.020 (3H, s, COCH_3), 2.018 (3H, s, COCH_3), 2.014 (3H, s, COCH_3). ^{13}C NMR (ppm, $CDCl_3$) δ: 170.8 (C═O), 170.6 (C═O), 169.6 (C═O), 169.5 (C═O), 153.3 ($C_{2,6}$), 134.6 (C_1), 124.9 (C_4), 105.6 ($C_{3,5}$), 101.5 ($C_{1'}$), 73.2 ($C_{3'}$), 72.1 ($C_{2'}$), 72.0 ($C_{5'}$), 68.6 ($C_{4'}$), 62.4 ($C_{6'}$), 56.4 (2 × ArO*C*H_3), 20.9 (CO*C*H_3), 20.83 (2 × CO*C*H_3), 20.78 (CO*C*H_3). ESI−HRMS (*m*/*z*): Calcd for $C_{22}H_{28}NaO_{12}^+$ ([M + Na]$^+$), 507.1478; found, 507.1498.

Compound **2** (0.524 g, 1.08 mmol) was dissolved in a mixture of MeOH/Et_3N/H_2O (8:1:1, 10.5 mL) and stirred for 16 h at room temperature before being concentrated to dryness under reduced pressure. Water (2 mL) was added, and the mixture was concentrated to dryness under reduced pressure. The addition of water and then concentration process was repeated 2 more times, yielding a white solid. Recrystallization from ethanol provided monoglucoside **3** as fluffy white crystals (0.307 g, 0.97 mmol, 90%). mp 167.5−168.5 °C. $[\alpha]_D$ −19.1 (*c* 0.366, H_2O).

^{1}H NMR (ppm, D_2O) δ: 7.19 (1H, t, J = 8.5 Hz, H_4), 6.80 (2H, d, J = 8.5 Hz, $H_{3,5}$), 5.03 (1H, d, J = 7.4 Hz, $H_{1'}$), 3.86 (6H, s, 2 × ArOCH_3), 3.80 (1H, dd, J = 12.4, 2.0 Hz, $H_{6b'}$), 3.71 (1H, dd, J = 12.4, 5.2 Hz, $H_{6a'}$), 3.60−3.47 (3H, m, $H_{2',3',4'}$), 3.34 (1H, ddd, J = 9.2, 5.2, 2.0 Hz, $H_{5'}$). ^{13}C NMR (ppm, D_2O) δ: 153.2 ($C_{2,6}$), 134.1 (C_1), 126.3 (C_4), 106.9 ($C_{3,5}$), 103.6 ($C_{1'}$), 77.0 ($C_{5'}$), 76.4 ($C_{3'}$), 74.4 ($C_{2'}$), 70.0 ($C_{4'}$), 61.2 ($C_{6'}$), 56.9 (2 × ArO*C*H_3). ESI−HRMS (*m*/*z*): Calcd for $C_{14}H_{20}NaO_8^+$ ([M + Na]$^+$), 339.1056; found, 339.1053.

2,6-Dimethoxyphenyl-1-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (Syringol Gentiobioside or Syringol-GG) (7). This compound

was prepared as depicted in **Figure 1** based on the methods of Shao et al. (*8*). A stirred solution of compound **3** (0.360 g, 1.14 mmol), anhydrous pyridine (1.2 mL), trityl chloride (0.539 g, 1.93 mmol), and DMAP (14 mg) was heated at 50 °C for 4 h under N_2, stirred overnight at room temperature, and heated for a further 5 h at 50 °C. The mixture was cooled to room temperature. Acetic anhydride (1.1 mL) was added, and stirring was continued for 2.25 h at room temperature. The reaction mixture was cooled with an ice-water bath. Methanol (1 mL) was added, and stirring was continued for 30 min before removal of the volatiles under reduced pressure. Toluene (1 mL) was added, and the mixture was concentrated to dryness under reduced pressure, yielding a white solid. Chromatography on silica gel with CH_2Cl_2 → 3% Et_2O/CH_2Cl_2 (R_f = 0.49 in 5% Et_2O/CH_2Cl_2) followed by solvent removal afforded the desired compound **4** as a white solid (0.505 g, 0.738 mmol, 65%). mp 88.0−91.0 °C. $[\alpha]_D$ +24.3 (*c* 0.474, $CHCl_3$).

^{1}H NMR (ppm, $CDCl_3$) δ: 7.38−7.36 (6H, m, *ortho* C*Ph*$_3$), 7.25−7.23 (6H, m, *meta* C*Ph*$_3$), 7.15 (3H, tt, J = 7.4, 1.2 Hz, *para* C*Ph*$_3$), 7.08 (1H, t, J = 8.4 Hz, H_4), 6.61 (2H, d, J = 8.4 Hz, $H_{3,5}$), 5.38 (1H, dd, J = 8.9, 7.8 Hz, $H_{2'}$), 5.21 (1 H, app t, J = 9.1 Hz, $H_{3'}$), 5.18 (1H, app t, J = 9.4 Hz, $H_{4'}$), 5.16 (1H, d, J = 7.8 Hz, $H_{1'}$), 3.80 (6H, s, 2 × ArOC*H*$_3$), 3.55 (1H, ddd, J = 9.4, 5.4, 2.7 Hz, $H_{5'}$), 3.14 (1H, dd, J = 10.3, 5.4 Hz, $H_{6a'}$), 3.10 (1H, dd, J = 10.3, 2.7 Hz, $H_{6b'}$), 2.08 (3H, s, COC*H*$_3$), 2.00 (3H, s, COC*H*$_3$), 1.69 (3H, s, COC*H*$_3$). ^{13}C NMR (ppm, $CDCl_3$) δ: 170.7 (C═O), 169.7 (C═O), 169.3 (C═O), 153.6 ($C_{2,6}$), 143.7 (*ipso* C*Ph*$_3$), 134.6 (C_1), 128.8 (*ortho* C*Ph*$_3$), 127.9 (*meta* C*Ph*$_3$), 127.0 (*para* C*Ph*$_3$), 124.9 (C_4), 105.8 ($C_{3,5}$), 101.4 ($C_{1'}$), 86.5 (*C*Ph$_3$), 73.6 ($C_{3',5'}$), 72.4 ($C_{2'}$), 69.1 ($C_{4'}$), 61.7 ($C_{6'}$), 56.5 (2 × ArO*C*H$_3$), 21.0 (CO*C*H$_3$), 20.9 (CO*C*H$_3$), 20.6 (CO*C*H$_3$). APCI−HRMS (*m*/*z*): Calcd for $C_{39}H_{39}O_{11}^-$ ($[M - H]^-$), 683.2492; found, 683.2422.

A stirred solution of compound **4** (0.406 g, 0.593 mmol) in 80% acetic acid (2.6 mL) was heated at 60 °C for 4 h. The mixture was cooled to room temperature and concentrated under reduced pressure. Toluene (1 mL) was added, and the mixture was again concentrated under reduced pressure. The addition of toluene and then concentration process was repeated 1 more time, yielding a white solid. Chromatography on silica gel with CH_2Cl_2 → 30% Et_2O/CH_2Cl_2 (R_f = 0.39) followed by solvent removal afforded the desired compound **5** as a white solid (0.218 g, 0.493 mmol, 83%). mp 169.5−170.0 °C. $[\alpha]_D$ −11.3 (*c* 0.178, CH_2Cl_2).

^{1}H NMR (ppm, $CDCl_3$) δ: 7.03 (1H, t, J = 8.4 Hz, H_4), 6.57 (2H, d, J = 8.4 Hz, $H_{3,5}$), 5.33 (1H, app t, J = 8.3 Hz, $H_{2'}$), 5.27 (1 H, app t, J = 9.0 Hz, $H_{3'}$), 5.22 (1H, app t, J = 9.2 Hz, $H_{4'}$), 5.09 (1H, d, J = 7.0 Hz, $H_{1'}$), 3.82 (6H, s, 2 × ArOC*H*$_3$), 3.70−3.66 (1H, m, $H_{6a'}$), 3.65−3.62 (1H, m, $H_{6b'}$), 3.61−3.58 (1H, m, $H_{5'}$), 2.62 (1H, app t, J = 6.8 Hz, OH), 2.04 (3H, s, COC*H*$_3$), 2.034 (3H, s, COC*H*$_3$), 2.031 (3H, s, COC*H*$_3$). ^{13}C NMR (ppm, $CDCl_3$) δ: 170.6 (C═O), 169.9 (C═O), 169.5 (C═O), 153.2 ($C_{2,6}$), 134.7 (C_1), 125.1 (C_4), 105.6 ($C_{3,5}$), 101.7 ($C_{1'}$), 74.5 ($C_{5'}$), 73.0 ($C_{3'}$), 72.2 ($C_{2'}$), 68.8 ($C_{4'}$), 61.7 ($C_{6'}$), 56.4 (2 × ArO*C*H$_3$), 20.9 (CO*C*H$_3$), 20.86 (CO*C*H$_3$), 20.82 (CO*C*H$_3$). ESI−HRMS (*m*/*z*): Calcd for $C_{20}H_{26}NaO_{11}^+$ ($[M + Na]^+$), 465.1373; found, 465.1375.

This procedure was adapted from the method of Kartha et al. (*9*). Peracetyl-α-glucopyranosyl bromide (0.302 g, 0.734 mmol) in CH_2Cl_2 (2 mL + 0.5 mL rinse) was added dropwise to a stirred mixture of compound **5** (0.108 g, 0.244 mmol), Ag_2CO_3 (0.215 g, 0.779 mmol), and powdered 4 Å molecular sieves (0.56 g) in CH_2Cl_2 (2.5 mL) under N_2 at 0 °C. The mixture was stirred for 3 h at room temperature and filtered through celite, and the filtrate was concentrated under reduced pressure. Chromatography on silica gel with 1% EtOH/15% EtOAc/$CHCl_3$ (R_f = 0.26) followed by solvent removal afforded the desired compound **6** as a white solid (0.356 g), which was a mixture of polyacetylated coupled products, starting material compound **5**, and acetylated glucose, by ^{1}H NMR and HPLC−MS analyses. The mixture was used in the subsequent step without further purification. ESI−HRMS (*m*/*z*): Calcd for $C_{34}H_{44}NaO_{20}^+$ ($[M + Na]^+$), 795.2324; found, 795.2338.

This procedure was adapted from the method of Pathak (*10*). Amberlite IRA-400(Cl) resin was exchanged with 1 M NaOH to IRA-400(OH) resin. A solution of compound **6** (0.244 g, 0.316 mmol) containing dry IRA-400(OH) (5.3 g) in MeOH (16 mL) was gently stirred for 24 h at room temperature. The mixture was filtered, and the filtrate was concentrated under reduced pressure to yield a white solid, which was purified by semi-preparative HPLC to yield the title gentiobioside **7** as a fluffy white solid (0.016 g, 0.033 mmol, 10%) after solvent removal and freeze-drying. mp 195−200 °C (began decomposing from 170 °C). $[\alpha]_D$ −29.1 (*c* 0.216, H_2O).

^{1}H NMR (ppm, D_2O) δ: 7.19 (1H, t, J = 8.5 Hz, H_4), 6.81 (2H, d, J = 8.5 Hz, $H_{3,5}$), 5.11 (1H, d, J = 7.5 Hz, $H_{1'}$), 4.31 (1H, d, J = 7.9 Hz, $H_{1''}$), 4.06 (1H, dd, J = 12.2, 1.4 Hz, $H_{6a'}$), 3.88−3.84 (m, 2H, $H_{6b',6a''}$), 3.86 (6H, s, 2 × ArOC*H*$_3$), 3.67 (1H, dd, J = 12.2, 6.0 Hz, $H_{6b''}$), 3.58−3.46 (4H, m, $H_{2',3',4',5'}$), 3.31 (1H, app t, J = 9.4 Hz, $H_{4''}$), 3.25 (1H, app t, J = 9.2 Hz, $H_{3''}$), 3.23 (1H, ddd, J = 9.4, 6.0, 2.1 Hz, $H_{5''}$), 3.15 (1H, dd, J = 9.2, 7.9 Hz, $H_{2''}$). ^{13}C NMR (ppm, D_2O) δ: 153.3 ($C_{2,6}$), 133.6 (C_1), 126.2 (C_4), 106.9 ($C_{3,5}$), 102.83 ($C_{1'}$), 102.77 ($C_{1''}$), 76.9 ($C_{5'}$), 76.5 ($C_{5''}$), 76.3 ($C_{3'}$), 76.1 ($C_{3''}$), 74.2 ($C_{2'}$), 73.6 ($C_{2''}$), 70.2 ($C_{4''}$), 69.9 ($C_{4'}$), 68.1 ($C_{6'}$), 61.3 ($C_{6''}$), 56.8 (2 × ArO*C*H$_3$). ESI−HRMS (*m*/*z*): Calcd for $C_{20}H_{30}NaO_{13}^+$ ($[M + Na]^+$), 501.1584; found, 501.1588.

Semi-preparative HPLC Purification of Compound 7. An Agilent 1100 HPLC (Agilent, Forest Hill, Australia) equipped with a quaternary pump and diode array detector (DAD) was used. The column was a 250 × 10 mm inner diameter, 4 μm, 80 Å, Synergi Hydro-RP operated at 25 °C and protected by a guard column of the same material (Phenomenex, Lane Cove, Australia). The solvents were water (solvent A) and acetonitrile (sovent B), with a flow rate of 2.00 mL/min. The linear gradient for solvent B was as follows: 0 min, 15%; 15 min, 30%; 17 min, 80%; 18 min, 15%; and 25 min, 15%. A 100 μL injection volume was used. DAD signals were recorded at 270 and 280 nm, and spectra were stored between 220 and 600 nm. Fractions were collected manually on the basis of retention time and detector response. Data acquisition and processing were performed using Agilent ChemStation software (revision B.03.01).

Smoke Analysis. *Smoke Sampling*. Smoke was collected from a prescribed burnoff in the Adelaide Hills in South Australia, Australia, in June 2009. Active air sampling was carried out using a Gerstel thermal desorption unit (TDU) desorption tube containing Tenax TA as an adsorbent (Lasersan, Robina, Australia), connected to a pocket pump (SKC, Eighty Four, PA). Smoke in the prescribed burnoff site was introduced into the TDU tube at a rate of 200 mL/min for 40 min. The TDU tube was then kept at 4 °C until analysis.

Gas Chromatography−Mass Spectrometry (GC−MS) Analysis. Analysis was carried out with a Hewlett-Packard (HP) 6890 gas chromatograph and HP 5973 mass selective detector (Agilent Technologies, Forest Hill, Australia) fitted with a Gerstel autosampler (MPS 2XL), TDU, and programmed temperature vaporization inlet (CIS-4) (Lasersan). A TDU tube containing the smoke sample was thermally desorbed by increasing the TDU temperature from 30 to 280 °C at a rate of 500 °C/min and holding for 3 min at 280 °C. The thermally desorbed compounds were trapped in the CIS-4 at −20 °C. Immediately following completion of desorption, the temperature of the CIS-4 was sharply elevated to 280 °C at 12 °C/s to introduce the desorbed compounds onto a 30 m × 0.25 mm DB-Wax with a film thickness of 0.25 μm fused silica capillary column (Agilent Technologies). The GC oven temperature was started at 50 °C, held at this temperature for 1 min, ramped to 220 °C at a rate of 10 °C/min, held at this temperature for 5 min, then ramped to 240 °C at a rate of 20 °C/min, and held for 6 min. Helium was used as a carrier gas at 1.2 mL/min in constant flow mode. The transfer line was maintained at 240 °C, and positive electron impact ion spectra at 70 eV were recorded in the mass range of *m*/*z* 35−350 in 1 s. Volatile phenols were identified according to their retention times and mass spectra by a comparison to those of the respective reference compounds.

Smoke-Affected and Control Grapes. *Smoke-Affected Grapes*. *Vitis vinifera* L. cv. Chardonnay and Cabernet Sauvignon grapes were collected from closely located vineyards in Victoria (Australia) in March 2009. The vineyards had been affected by smoke from a series of bushfires that occurred in the period of February 7−March 14, 2009.

Control Grapes. Three samples each of non-smoked Chardonnay and Cabernet Sauvignon grapes were collected from various regions of South Australia.

Winemaking. *Smoke-Affected Wines*. Wine was made from smoke-affected Cabernet Sauvignon and Chardonnay grapes that had been frozen for approximately 6 months at −20 °C prior to winemaking. Grapes were randomized, thawed at 4 °C overnight, then crushed, and destemmed. A 5 kg sample of grapes was used for winemaking. Potassium metabisulfite and tartaric acid were added to the must to achieve a free sulfur dioxide content of approximately 15 mg/L and pH 3.00−3.10 for Chardonnay and pH 3.50−3.60 for Cabernet Sauvignon. The musts were then inoculated with yeast strain AWRI 796 at 20 °C and fermented on skins for both red and white wines at 25 °C. Ferments were drained and

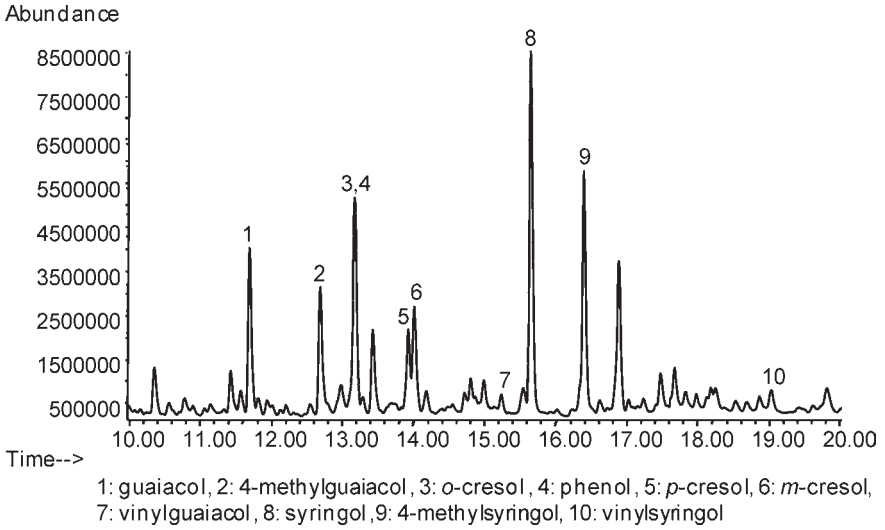

Figure 2. GC−MS analysis of smoke from a prescribed burnoff.

pressed after 7 days and fermented to dryness (< 1 g/L residual sugar, confirmed via Clinitest and then enzymatic analysis). Once dry, wines were racked off gross lees into appropriately sized glass storage vessels, and potassium metabisulfite was added to achieve a free SO_2 level of 30−35 mg/L for Chardonnay and approximately 80 mg/L total for Cabernet Sauvignon. The wines were cold settled at 4 °C for 3 weeks, carefully bottled into 375 mL screw cap bottles using dry ice, and stored at 4 °C.

Control Wines. Three each of commercial Chardonnay and Cabernet Sauvignon wines were obtained and used as controls. These wines were made from grapes grown in regions unaffected by bushfire.

HPLC−MS/MS Analysis for Glycosides of Volatile Phenols. *Sample Preparation*. Berry extracts were prepared according to the method described by Hayasaka et al. (*1*), with a minor modification. Briefly, a 5 g aliquot of the grape homogenate containing 0.5 mg/kg of d_3-guaiacol-MG as internal standard was centrifuged at 4000 rpm for 5 min to collect the supernatant. A 2 mL aliquot of the supernatant (or juice hydrolysate for the hydrolysis experiment described later) was applied to a preconditioned Extract Clean C18-HF SPE 500 mg/4 mL cartridge (Grace Davison Discovery Sciences, Australia) to obtain the methanol extract. After removal of methanol, the residue (extract) was reconstituted with 0.5 mL water, filtered (0.45 μm), and transferred to a vial ready for analysis.

Wine. A 1 mL aliquot of wine containing 1 mg/L of d_3-guaiacol-MG as internal standard was filtered (0.45 μm) and transferred to a vial ready for analysis. For acid hydrolysis experiments, the hydrolysate was adjusted to pH 3−4 with 5 M NaOH, then spiked with the internal standard, and transferred to a vial ready for analysis.

HPLC−MS/MS Analysis. HPLC−MS/MS with APCI was carried out according to the method previously described (*1*). The tandem mass spectrometry (MS/MS) parameters were set at −18 V for collision potential, −5 V for collision cell exit potential, and high for collision gas pressure. For HPLC−MS/MS in selected reaction monitoring (HPLC−SRM), the mass transitions from $[M - H + CH_3COOH]^-$ ions of the respective glycosides to the common fragments m/z 323 for glucosylglucoside (GG), m/z 161 for monoglucoside (MG), m/z 293 for diglycoside (DG), and m/z 307 for rutinoside (RG) were monitored with a dwell time of 50 ms (Supplementary Table 1 in the Supporting Information). For HPLC−SRM combined with in-source fragmentation, the declustering potential was increased from −40 to −80 V and the mass transitions from $[M - H + CH_3COOH]^-$ and $[M - H]^-$ ions of the respective glycosides to the common fragment ions for GG, MG, DG, and RG were simultaneously monitored (Supplementary Table 1 in the Supporting Information).

Quantitative Analysis of the Glycosides. Concentrations of the glycosides were determined using d_3-guaiacol-MG (monitoring the mass transition from m/z 348 → 161) as internal standard using the same response factor for the internal standard and the respective glycosides.

Acid Hydrolysis. Acid hydrolysates were prepared from smoke-affected Chardonnay and Cabernet Sauvignon grapes and their corresponding small-lot wines, as well as from control grape and wine samples of the same grape varieties. A 10 mL aliquot of wine or supernatant obtained from the grape homogenate was acidified to pH 1.0 with concentrated sulfuric acid and heated at 100 °C for 1 h (*3*). Samples were subjected to GC−MS for the analysis of volatile phenols and HPLC−SRM for the analysis of volatile phenol glycosides, before and after hydrolysis.

GC−MS Analysis of Volatile Phenols. *Sample Preparation*. A 5 mL aliquot of grape juice from the homogenate, wine, or hydrolysate sample was transferred to a 10 mL glass vial with a foil-lined screw cap and spiked with 100 μL of the labeled internal standard solution detailed below. After the addition of 2 mL of freshly prepared pentane/ethyl acetate (1:1), the sample vial was manually shaken for 15 s and left until the sample mixture separated into two layers. The organic layer was transferred to a vial ready for analysis.

Quantitation. Calibration functions were prepared for the quantitation of guaiacol, 4-methylguaiacol, phenol, three cresol isomers, syringol, and 4-methylsyringol. The labeled internal standards were d_3-guaiacol for guaiacol, d_3-4-methylguaiacol for 4-methylguaiacol, d_7-*p*-cresol for phenol and cresol isomers, and d_3-syringol for syringol and 4-methylsyringol. A mixed labeled internal standard solution of approximately 10 mg/L was prepared in ethanol. The respective volatile phenol standards, prepared as a mixture in ethanol and spiked from 0 to 200 μg/L (and up to 1000 μg/L for syringol only), and 100 μL of labeled internal standard mixture were added to 5 mL of model wine. The calibration samples underwent sample preparation and GC−MS analysis as described.

GC−MS Analysis. Analysis was carried out with a ThermoQuest Trace GC 2000 gas chromatograph combined with a TSQ mass spectrometer (Thermo Fisher Scientific, Scoresby, Australia). The GC was equipped with the same capillary column described under Smoke Analysis. Helium was used as carrier gas at a flow rate of 1.5 mL/min. The injector was in splitless mode, and the split vent was opened after 0.5 min, with an injected sample volume of 2 μL. Temperatures of the injector and transfer line were 200 and 250 °C, respectively. The GC column was maintained at 50 °C for 1 min, ramped at a rate of 8 °C/min to 255 °C, and held for 10 min. Mass spectra (EI at 70 eV) were recorded in selected ion monitoring (SIM) mode (Supplementary Table 2 in the Supporting Information). The respective target ions were used for quantitation of the respective volatile phenols (by peak area).

RESULTS AND DISCUSSION

Smoke Analysis. To investigate major smoke-derived volatile organic compounds (VOCs), smoke generated from a prescribed burnoff was trapped in Tenax tubes and analyzed by GC−MS. In addition to guaiacol, volatile phenols, including syringol, methylsyringol, *o*-, *p*-, and *m*-cresols, phenol, and methylguaiacol, were found to be major components of smoke-derived VOCs (**Figure 2**). Vinylguaiacol and vinylsyringol were also present as

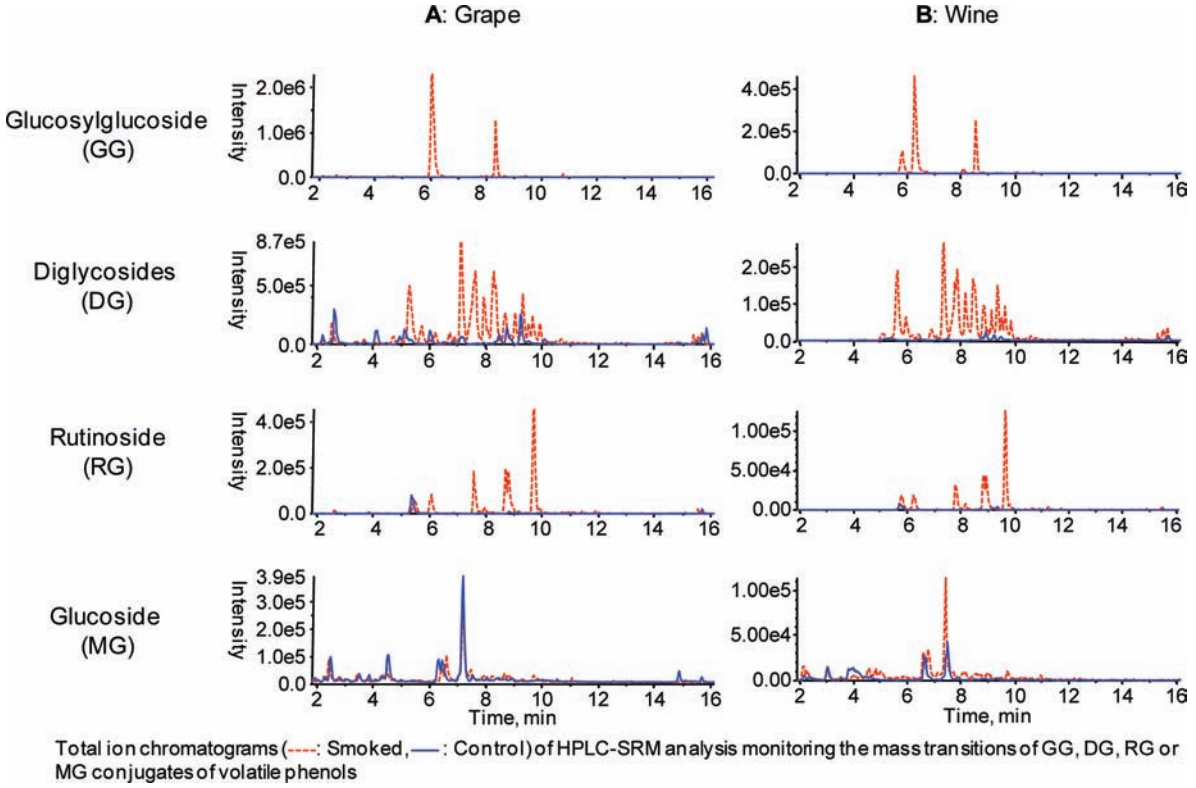

Figure 3. Total ion chromatograms obtained by HPLC−SRM analysis of smoke-affected and control Chardonnay (A) grapes and (B) wines for screening GG, DG, RG, and MG conjugates of volatile phenols.

minor components. Being derived by the thermal oxidative decomposition of lignin (*5*, *11*), these volatile phenols have been commonly found as major components of liquid smoke flavorings (*12*, *13*) and smoke generated from wood stoves (*14*). The concentration of the individual volatile phenols can vary significantly because of the variation of the fuel source, moisture content, combustion temperature, and availability of oxygen. Nevertheless, this experiment demonstrated the possibility that grapevines can be severely exposed to a number of volatile phenols from bushfire events in the vicinity of vineyards, as seen in the case of guaiacol (*1*, *15*, *16*).

Glycosides of Volatile Phenols. As first observed in previous studies (*1*,*3*), the glycosides of guaiacol were consistently detected as their acetic acid adduct ions $[M - H + CH_3COOH]^-$ under the HPLC−MS conditions used. Product ion spectra of the adduct ions commonly exhibited two distinctive fragment ions, which were the deprotonated molecular ions $[M - H]^-$ resulting from the neutral loss of 60 Da and glycoside ions representing the mass of the sugar moiety of guaiacol glycosides, i.e., *m*/*z* 161 for monoglucoside (MG), *m*/*z* 323 for glucosylglucoside (GG), *m*/*z* 293 for diglycoside (DG), and *m*/*z* 307 for rutinoside (RG) (*1*). Because of the structural similarity of guaiacol to the other volatile phenols found from the smoke analysis, any volatile phenol glycosides present were expected to fragment the same as the guaiacol glycosides. On the basis of this assumption, a screening experiment was conducted to detect glycosides of individual volatile phenols by HPLC−SRM mode, monitoring the mass transitions from the expected $[M - H + CH_3COOH]^-$ ions to the respective glycoside ions of MG, GG, DG, or RG (Supplementary Table 1 in the Supporting Information).

Smoke-affected and control samples of Chardonnay and Cabernet Sauvignon grapes and wines were analyzed to confirm whether peaks derived from the putative volatile phenol glycosides increased as a result of smoke exposure. Both grape and wine analyses showed that the smoke-affected samples had substantial increases in the intensity and number of peaks derived from volatile phenols conjugated to GG, DG, or RG compared to those from the control samples (**Figure 3**). In contrast, there was no obvious effect on the intensity of MG conjugates in grapes and the resulting wine from smoke exposure, and MG conjugates were excluded from further investigation.

In the control grape and wine, only small peaks were recorded (**Figure 3**), which were likely to represent volatile phenol glycosides naturally present in grapes, as seen in the case of guaiacol (*1*). The presence of precursors to volatile phenols in various grape varieties has been reported for phenol, guaiacol, 4-vinylguaiacol, 4-vinylphenol (*17*), cresols, 4-ethylguaiacol, eugenol, 4-ethylphenol, syringol, and 4-methylsyringol (*18*). Both studies identified volatile phenols in enzyme and/or acid hydrolysates, indicating that trace amounts of phenolic glycosides were natural components of the grapes.

MS/MS Experiments To Confirm the Presence of Individual Volatile Phenol Glycosides. Volatile phenol glycosides gave prominent $[M - H + CH_3COOH]^-$ and $[M - H]^-$ ions by in-source fragmentation with a higher declustering potential, and both pseudo-molecular ions were dissociated to the same glycoside fragment ion, *m*/*z* 323, 297, and 307 for GG, DG, and RG, respectively. Therefore, the putative glycosides of volatile phenols (Supplementary Table 1 in the Supporting Information) were screened by simultaneously monitoring the mass transitions from both pseudo-molecular ions to the respective glycoside ions using HPLC−SRM combined with in-source fragmentation (**Figure 4**). Analysis of the smoke-affected Chardonnay grape sample clearly indicated a single peak derived from syringol-GG, methylsyringol-GG, guaiacol-RG, or methylguaiacol-RG (panels **A**, **B**, **E**, and **F** of **Figure 4**). Phenol-DG and cresol-DG (panels **C** and **D** of **Figure 4**) gave multiple peaks derived from the expected mass transitions because of the presence of multiple DG and cresol

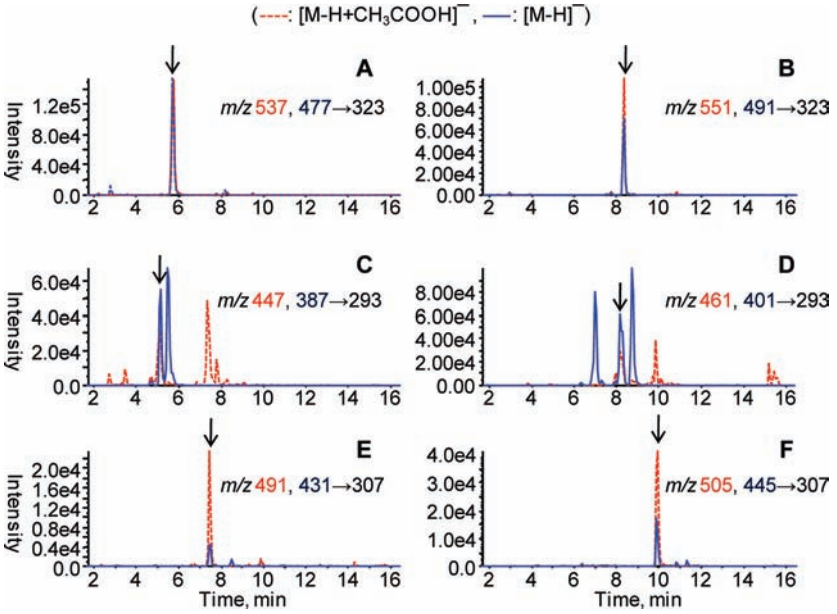

Figure 4. HPLC−SRM analysis combined with in-source fragmentation of smoke-affected Chardonnay grapes to confirm the presence of (A) syringol-GG, (B) methylsyringol-GG, (C) phenol-DG, (D) cresol-DG, (E) guaiacol-RG, and (F) methylguaiacol-RG.

isomers. However, some peaks were characterized by only one mass transition; therefore, those peaks were ruled out as phenol-DG or cresol-DG. Furthermore, the product ion spectra of the respective $[M - H + CH_3COOH]^-$ ions of the target phenolic glycoside peaks (↓ in **Figure 4**) were obtained by HPLC−MS/MS (Supplementary Figure 1 in the Supporting Information). All of the spectra showed good agreement with the expected fragment ions (Supplementary Table 1 in the Supporting Information); therefore, the identification of these glycosides was further supported. In summary, a broad range of glycosides of volatile phenols was found in the smoke-affected grape and wine samples of Chardonnay and Cabernet Sauvignon (Supplementary Table 3 in the Supporting Information). These glycosides were typically found in both sample types (grape or wine) for both grape varieties.

For additional confirmation of the presence of syringol glycosides and because syringol was one of the most dominant volatile phenols (**Figure 2**), syringol-GG was synthesized via syringol-MG using a selective protection and coupling regime, as shown in **Figure 1**. Pure 1→6-linked, β,β-configured syringol-GG was obtained after semi-preparative HPLC and fully characterized. The chromatographic and mass spectrometric properties of the reference compound were confirmed to be identical to those of the tentatively identified syringol-GG found in the smoke-affected grapes by HPLC−MS/MS and HPLC−SRM with co-injection experiments. Therefore, syringol-GG detected during this study was determined to be a gentiobioside.

Quantitative Distribution of Glycosides of Volatile Phenols. The quantitative analysis of the individual glycosides in the grape and wine samples was carried out by HPLC−SRM, and concentrations were expressed as d_3-guaiacol-MG equivalents (**Figure 5**). The smoke-affected grape and wine samples contained the glycosides at greatly elevated concentrations compared to those of the respective control samples. The concentration of total glycosides for Chardonnay (sum of all phenolic glycosides quantified, but MG was excluded) was 0.28/21.15 mg/kg in grapes (control/smoke-affected) and 0.20/16.42 mg/L in wine; for Cabernet Sauvignon, the concentration was 0.19/21.02 mg/kg in grapes and 0.21/14.17 mg/L in wine. In both varieties, the distribution pattern of phenolic glycosides in the smoke-affected grape samples was similar to that in the resulting wines (**Figure 5**), but the concentration of the total glycosides decreased from around 21 mg/kg in the grapes to 14−16 mg/L in the wines. Accordingly, the apparent extraction rate in the wine was calculated to be 78% for Chardonnay and 67% for Cabernet Sauvignon.

It should be noted that winemaking was undertaken using grapes that had been frozen and the Chardonnay wine was made with the same winemaking procedures used for the Cabernet Sauvignon wine. Localization of the glycosides in the berries that we used may be different from that in fresh grapes as a result of freezing, while the skin contact time for the experimental Chardonnay wine was considerably longer than employed during usual winemaking practice for white wine. Such factors could conceivably have influenced the extraction rate of the glycosides, particularly for the Chardonnay wine, although plant secondary metabolites, such as glycosides, are usually highly water-soluble and should be readily extracted into musts and ferments. Furthermore, because a loss of glycosides resulting from chemical and enzymatic hydrolysis during fermentation could also have contributed to a reduction in the concentration of glycosides in the wines, the overall extraction was likely higher than the observed value.

Upon examination of the glycosylation pattern, it appeared that only syringol and methylsyringol were dominantly present as GG conjugates (**Figure 5**). In fact, syringol-GG was the most abundant glycoside in all smoke-affected samples. Other GG conjugates of volatile phenols were either not detected or detected only in trace amounts. Multiple isomers of DG conjugates were the most common glycosides of volatile phenols, including phenol, cresols, guaiacol, methylguaiacol, syringol, and methylsyringol. RG conjugates were also commonly found, with the exception of syringol and methylsyringol. Additionally, vinylguaiacol and vinylsyringol glycosides were present in the smoke-affected samples in very low amounts (**Figure 5**).

Acid Hydrolysis and Fermentation. Acid hydrolysis of smoke-affected grapes and the resulting wines along with control grapes and wines was carried out to investigate the susceptibility of the phenolic glycosides to acid. The free volatile phenols were determined by GC−MS, and the glycosides were determined by HPLC−SRM, before and after acid hydrolysis. The concentrations

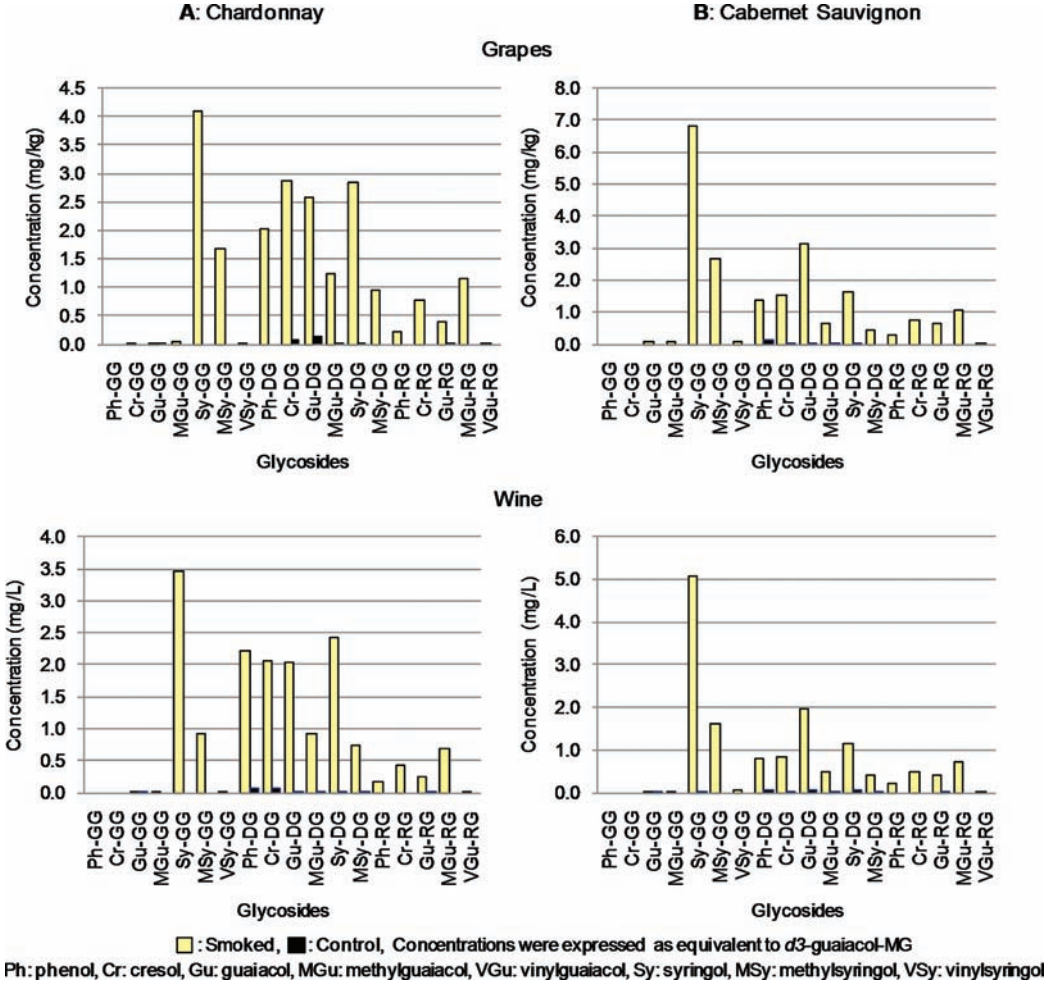

Figure 5. Quantitation of glycosides of volatile phenols in smoke-affected and control grapes and wines of (A) Chardonnay and (B) Cabernet Sauvignon.

of free volatile phenols in the control grape and wine samples were unchanged or slightly elevated by acid hydrolysis (data not shown), further supporting that only small amounts of the phenolic glycosides were present as natural components of grapes and wine (**Figures 3** and **5**). In smoke-affected grapes, the concentration of GG conjugates decreased markedly after hydrolysis but RG conjugates remained apparently unchanged (**Figure 6A**). The intensity of DG conjugate peaks also declined but to a lesser extent than GG conjugates. In the wine samples, however, all of the glycosides disappeared almost completely after acid treatment (**Figure 6B**).

After hydrolysis, approximately 50% (grape) and 92% (wine) of the total phenolic glycosides (MG conjugates were excluded) had been eliminated from samples of both varieties (**Table 1**). In the grape samples in particular, syringol and methylsyringol glycosides were hydrolyzed to the greatest extent (up to 77%) compared to the other phenols (up to 49%). Under the hydrolysis conditions employed, syringol glycosides in wine were cleaved to the highest extent (up to 98%), although hydrolysis of the other phenols was also high (up to 94%) (**Table 1**). The differences between grapes and wine were likely due to the much higher sugar content of the grape samples, where juice sugar levels may have interfered with hydrolysis.

The greater hydrolysis of phenolic glycosides in the wine samples was supported by the observation that the concentrations of total free volatile phenols in the hydrolysates of both grape varieties were around 1.2 times higher in the wines than in the grapes (**Table 2**). The higher release in the wine samples varied between individual volatile phenols and differed somewhat between white and red varieties. Syringol and methylsyringol were found in the highest amounts in grape and wine hydrolysates. The syringol concentration was over 600 and 700 μg/L (grape juice and wine, respectively), and the methylsyringol concentration was up to 177 and 239 μg/L. Of the remaining phenols, guaiacol was present in concentrations up to 44 and 72 μg/L in the Cabernet Sauvignon hydrolysates, while phenol, cresols, and methylguaiacol were in the order of 20 and 50 μg/L or less (**Table 2**). In general, the red grape and wine hydrolysates had higher levels of phenols, although on the whole, the results for red and white varieties were comparable.

Free volatile phenols in wine can be also released by enzymatic activity during fermentation. The release of smoke-derived phenols through fermentation has been studied by Kennison et al. (*2*), using Merlot grapes after experimental exposure of the grapevines to smoke generated by the combustion of dry straw. In that study, the concentrations of guaiacol and methylguaiacol were reported to increase from trace levels in free run juice to 388 and 93 μg/L, respectively, in the finished wine. In the present study, a significant proportion (up to 73%, depending upon the analyte and grape variety) of volatile phenols in the wine hydrolysates was already present before acid treatment, with the exception of syringol and methylsyringol (around 2−3%) (**Table 2**). These pre-existing phenols in the wines were therefore considered to be released during fermentation. Although the smoke-affected grapes contained the highest concentrations of the glycosides (total glycosides, 21 mg/kg for both varieties;

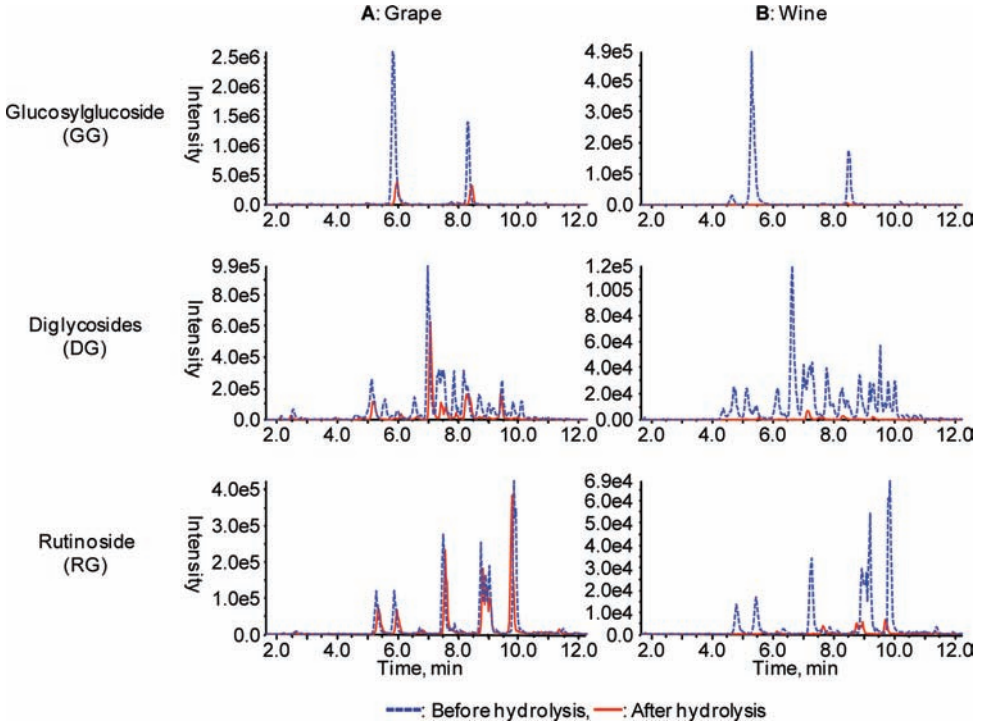

Figure 6. Total ion chromatograms of glycosides (GG, DG, and RG) of volatile phenols obtained by HPLC−SRM analysis of smoke-affected Cabernet Sauvignon (A) grapes and (B) wines before and after acid hydrolysis.

Table 1. Concentrations of Glycosides of Volatile Phenols in Smoke-Affected Grapes and Wines before and after Acid Hydrolysis

	Chardonnay				Cabernet Sauvignon			
	acid hydrolysis		loss		acid hydrolysis		loss	
glycosides	before[a]	after[a]	amount[a]	%	before	after	amount	%
			Grapes (mg/kg)					
phenol	2.32	1.88	0.45	19	1.67	0.86	0.82	49
cresols	3.76	2.37	1.39	37	2.27	1.68	0.59	26
guaiacol	3.03	2.00	1.03	34	3.82	2.44	1.38	36
methylguaiacol	2.41	1.61	0.80	33	1.71	1.42	0.29	17
syringol	7.02	2.36	4.65	66	8.46	1.95	6.51	77
methylsyringol	2.62	0.70	1.92	73	3.09	0.70	2.38	77
total glycosides	21.16	10.92	10.24	48	21.02	9.05	11.97	57
			Wines (mg/L)					
phenol	2.40	0.14	2.26	94	0.94	0.05	0.88	94
cresols	2.49	0.45	2.04	82	1.35	0.26	1.08	80
guaiacol	2.32	0.30	2.02	87	2.37	0.31	2.06	87
methylguaiacol	1.63	0.35	1.28	78	1.23	0.25	0.97	79
syringol	5.89	0.15	5.74	97	6.25	0.09	6.16	98
methylsyringol	1.70	0.05	1.65	97	2.05	0.06	1.99	97
total glycosides	16.43	1.44	14.99	91	14.19	1.02	13.14	93

[a] The sum of GG, DG, and RG was expressed as d_3-guaiacol-MG equivalents.

Table 1), the concentration of free volatile phenols in the resulting wines was considerably lower (total phenols, 0.064 mg/L for Chardonnay and 0.118 mg/L for Cabernet Sauvignon; **Table 2**). Accordingly, the release of free volatile phenols from glycosylated precursors (mainly diglycosides) during the winemaking process applied was estimated to be low relative to the amounts of grape phenolic glycosides available.

Considering the substantial loss of the glycosides observed in wines after acid treatment (**Table 1**), the amounts of free phenols released were comparatively low (**Table 2**). Recovery of the individual volatile phenols from the corresponding glycosides following acid treatment of wine was calculated by comparing an increase of the free phenol to a decrease of the glycosides on a molar basis (**Table 3**). The recovery of free phenol, cresols, guaiacol, and methylguaiacol was estimated to be 3−8%; accordingly, more than 90% of the eliminated glycosides were decomposed without releasing aglycones. In contrast, syringol and methylsyringol were more effectively released, with a recovery of around 30% (**Table 3**). It should be emphasized that the concentrations of the glycosides were expressed as d_3-guaiacol glucoside equivalents for comparison purposes and were not absolute amounts. Therefore, these recovery values were also considered to be used for comparison purposes. Sefton (*17*) reported that phenol, guaiacol, 4-vinylguaiacol, and 4-vinylphenol were mostly not detectable in acid hydrolysates (pH 3.2 and 45 °C for 4 weeks) of Merlot or Cabernet Sauvignon grapes but were found in significantly elevated concentrations in enzyme hydrolysates using Rohapect C. Loscos et al. (*18*) also reported that volatile phenols in the extracts of juice from seven different grape varieties were much more efficiently released by enzymatic hydrolysis using AR 2000 pectinase enzyme than acid hydrolysis conducted at pH 2.5 and 100 °C for 1 h. Interestingly, the only exception to this observation was syringol, which was found in comparatively similar concentrations in both hydrolysates. This difference between syringol and other phenols was in accordance with results from fermentation versus acid hydrolysis in the present study.

Unlike enzymatic hydrolysis releasing mainly an intact aglycone, acid-catalyzed hydrolysis is less specific and cleaves either the glycosidic linkage (*O*-sugar moiety) to release aglycone or the ether (*O*-aglycone moiety) to yield the carbocation of the aglycone. The carbocation would be further decomposed and/or readily reacted with some components of grapes (*17*). Syringol and methylsyringol glycosides may have a weaker glycosidic linkage, which makes the release of an aglycone more favorable.

Table 2. Concentrations of Volatile Phenols in Smoke-Affected Grapes and Wines before and after Acid Hydrolysis

volatile phenols	phenols		cresols		guaiacol		methylguaiacol		syringol		methylsyringol		total phenols	
hydrolysis	before	after	before	after	before	after	before	after	before	after	before	after	before	after
						Chardonnay								
grape juice (μg/L)	nd[a]	19	5	18	3	22	nd	8	nd	613	nd	136	8	816
wine (μg/L)	18	44	15	42	7	36	2	15	20	722	2	170	64	1029
released by fermentation (%)	40.9[b]		23.8		11.1		13.3		2.8		1.2		5.4	
released by acid (%)	59.1[c]		64.3		80.6		86.7		97.2		98.8		93.8	
						Cabernet Sauvignon								
grape juice (μg/L)	nd	12	4	20	8	44	nd	9	nd	634	nd	177	12	896
wine (μg/L)	35	48	22	42	26	72	4	16	25	710	6	239	118	1127
released by fermentation (%)	72.9		42.9		25.0		25.0		3.5		2.5		9.4	
released by acid (%)	27.1		47.6		63.9		75.0		96.5		97.5		89.5	

[a] Not detected. [b] % = (wine concentration − juice concentration)/wine hydrolysate concentration × 100. [c] % = (wine hydrolysate concentration − wine concentration)/wine hydrolysate concentration × 100.

Table 3. Recovery of Free Phenols from Glycosides in Wine following Acid Hydrolysis

	loss of glycosides (μM)[a]	gain of free phenols (μM)	conversion (%)
		Chardonnay	
phenol	5.610	0.277	4.9
cresols	4.890	0.250	5.1
guaiacol	4.670	0.234	5.0
methylguaiacol	2.857	0.094	3.3
syringol	12.405	4.558	36.7
methylsyringol	3.467	1.000	28.8
		Cabernet Sauvignon	
phenol	2.192	0.138	6.3
cresols	2.605	0.185	7.1
guaiacol	4.763	0.371	7.8
methylguaiacol	2.181	0.087	4.0
syringol	13.303	4.448	33.4
methylsyringol	4.181	1.387	33.2

[a] Average molecular weight of glycosides (GG, DG, and RG) was 403, 417, 433, 447, 463, and 477 for phenol, cresols, guaiacol, methylguaiacol, syringol, and methylsyringol, respectively.

All together, we demonstrated that volatile phenols from bushfire smoke, including phenol, cresols, methylguaiacol, syringol, and methylsyringol, can be metabolized to glycoconjugate forms within grapes in a similar fashion to that shown previously for guaiacol. Using extensive HPLC−MS/MS experiments, these phenolic glycosides were found in smoke-affected grapes and wines at significantly elevated levels compared to concentrations in the non-smoked control grapes and wines. Synthesis and co-injection of syringol-GG confirmed the presence of this compound in smoke-affected samples. The apparent extraction rate of these glycosides from grapes to wine was estimated to be 78% for Chardonnay and 67% for Cabernet Sauvignon. A large proportion of the total glycosides in grapes (50%) and wine (92%) disappeared after acid treatment, but the amounts of free phenol volatiles released were comparatively low. In the case of wine, more than 90% of the eliminated glycosides of phenol, cresols, guaiacol, and methylguaiacol were decomposed without yielding their known aglycones, but syringol and methylsyringol were more effectively released (around 30%) by acid hydrolysis. Nonetheless, these results provide useful information about the relative contributions of fermentation and chemical hydrolysis to volatile phenol release into wine. We have shown that, from a pool of approximately 20 mg/L phenolic glycosides in smoke-affected grapes, there is the potential to release over 100 μg/L in total of volatile phenols during fermentation and around 1000 μg/L by strong acid hydrolysis, which may mimic wine storage conditions to some extent. This knowledge is important to estimate the effects of winemaking and storage on wine volatile phenol concentrations resulting from phenolic glycosides present in grapes following grapevine exposure to bushfire smoke.

ABBREVIATIONS USED

DMAP, 4-dimethylaminopyridine; TDU, thermal desorption unit; SRM, selected reaction monitoring; VOC, volatile organic compound.

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Supporting Information Available: Tables of precursor and product ions of volatile phenol glycosides used for HPLC−MS/MS analyses, target and qualifier ions of volatile phenols used for quantitative GC−MS analysis, and retention times of volatile phenol glycosides detected by HPLC−SRM analysis of smoke-affected samples and figure displaying product ion spectra of volatile phenol glycosides obtained from HPLC−MS/MS analysis of smoke-affected Chardonnay grapes. This material is available free of charge via the Internet at http://pubs.acs.org.

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