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Evolution and Occurrence of 1,8-Cineole (Eucalyptol) in Australian Wine

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A new method has been developed for the quantitation of 1,8-cineole in red and white wines using headspace solid-phase microextraction (SPME) combined with stable isotope dilution analysis (SIDA) and gas chromatography−mass spectrometry (GC-MS). An extensive survey of Australian wines (44 white and 146 red) highlighted that only red wines contained significant amounts of 1,8-cineole (up to 20 μg/L). Hydrolytic studies with limonene and α-terpineol, putative precursors to 1,8-cineole, showed a very low conversion into 1,8-cineole (<0.6%) over a 2 year period, which does not account for the difference between white and red wines. 1,8-Cineole was chemically stable in model wine solution over 2 years, and absorption from a Shiraz wine by bottle closures was most evident for a synthetic closure only (14% absorption after 1 year). Two commercial ferments at two different locations were monitored daily to investigate the evolution of 1,8-cineole throughout fermentation. Both ferments showed daily increases in 1,8-cineole concentration while in contact with grape solids, but this accumulation ceased immediately after pressing. This observation is consistent with the extraction of 1,8-cineole into the ferment from the solid portions of the grape berries.

KEYWORDS: Wine aroma; 1,8-cineole; eucalyptol; SPME; SIDA; GC-MS

INTRODUCTION

1,8-Cineole, correctly identified by Jahns in 1884 (*1*), was initially recognized as the major constituent of the essential oil from leaves of *Eucalyptus globulus* by Cloëz, who labeled it eucalyptol (*2*). Eucalyptus essential oil (containing up to 90% 1,8-cineole) has since been used at low concentrations as a flavoring agent in a diverse range of foods and beverages (*3*, *4*), as a constituent in fragrances, cosmetics, and aromatherapy (*3*), and as a therapeutic ingredient with a range of applications (see refs *5*−*7* and citations therein). In fact, the medicinal use of eucalyptus leaves by indigenous Australians dates back many millennia (*7*). 1,8-Cineole is generally recognized as safe (GRAS) and has been used as an additive in cigarettes (see ref *8* and citations therein), evidently to improve flavor properties, reduce throat irritation, or enhance the cooling effects of menthol.

1,8-Cineole has a characteristic aroma described as "eucalyptus", "fresh", "cool", "medicinal", and "camphoraceous" and was first reported in wine by Herve et al. (*9*). That study showed that 1,8-cineole played an important role in the occurrence of "eucalyptus" character in wine. They also determined the difference and recognition thresholds of 1,8-cineole in a California Merlot as 1.1 μg/L and 3.2 μg/L, respectively (*9*). Herve et al. proposed that the "eucalyptus" character in wines occurs due to vineyards being in the vicinity of eucalyptus trees (*9*), but the origin of 1,8-cineole in wine is still unclear.

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To explain the presence of 1,8-cineole in Tannat grapes and wines from Uruguay, Farina et al. suggested that terpene compounds such as α-terpineol and limonene were possible precursors (*10*). Their postulated pathway to the formation of 1,8-cineole involved the hydration of limonene, forming α-terpineol, which was further hydrated to give a mixture of 1,8-terpines, with cyclization of *trans*-1,8-terpine leading to 1,8-cineole. They also put forward other theories involving double-bond epoxidation to explain the formation of minor components arising under their experimental conditions (*10*). Their studies with model wine showed that 1,8-cineole can be produced from limonene and α-terpineol under accelerated aging conditions at wine pH, but they gave only semiquantitative data for the products. Moreover, they found that 1,8-cineole concentrations in their Tannat grape samples at the beginning of ripening were very low, but showed a significant increase throughout ripening, and they determined an odor threshold for 1,8-cineole in the Tannat wine similar to that reported for Merlot (*10*).

Further confounding matters, the results from Farina et al. contrast with the work of Kalua and Boss, who found that 1,8-cineole levels decrease during ripening of Australian Cabernet Sauvignon and Riesling grapes (*11*), whereas other Tannat wines from Uruguay were shown to contain terpenoids but not 1,8-cineole (*12*). It is interesting to note that both Tannat studies involved vineyards in southern Uruguay, which also happens to be an area where eucalyptus plantations are readily encountered (*13*). Nonetheless, the studies relating to 1,8-cineole indicated there are a number of possible explanations for its presence

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in wine, although the relative significance of each is yet to be examined.

This paper describes the development of an accurate analytical method for determining 1,8-cineole in wine using a deuterium - labeled analogue and headspace solid-phase microextraction combined with gas chromatography−mass spectrometry (SPME-GC-MS). The method was applied to 190 commercial Australian wines to determine to what extent 1,8-cineole is present in wine in significant concentrations. Several factors thought to influence the concentration of 1,8-cineole in wine were also investigated, including its evolution during fermentation, formation from potential precursors, and stability during storage.

MATERIALS AND METHODS

Materials. Nondeuterated standards including 1,8-cineole, (*S*)-(−)-limonene and α-terpineol were purchased from Sigma-Aldrich (Castle Hill, NSW, Australia). Stock solutions of standards were prepared volumetrically in redistilled ethanol and stored at −20 °C, and working solutions were stored at 4 °C until required. All chemicals were of analytical reagent grade unless otherwise stated, and water was obtained from a Milli-Q purification system (Millipore, North Ryde, NSW, Australia). Merck solvents, sodium chloride (NaCl), and L-(+)-tartaric acid were purchased from Rowe Scientific (Lonsdale, SA, Australia), and other chemicals were obtained from either Sigma-Aldrich or BDH (Kilsyth, VIC, Australia). Supelco SPME fibers (Sigma-Aldrich) were polydimethylsiloxane/divinylbenzene (PDMS/DVB) 65 μm, carboxen/polydimethylsiloxane (CAR/PDMS) 75 μm, polyacrylate coating (PA) 85 μm, polydimethylsiloxane (PDMS) 100 μm, and both a 1 cm and a 2 cm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) 50/30 μm.

Wine and Juice Samples. A range of bottled commercial white wines (44 in total) comprising 12 Riesling, 10 Sauvignon blanc, 10 Semillon, and 12 Chardonnays and red wines (146 in total) comprising 43 Shiraz, 45 Cabernet Sauvignon, 25 Merlot, 17 Pinot noir, 10 blends of Cabernet Sauvignon and Merlot, and 6 Durif wines were obtained from retail outlets. An additional seven commercial Shiraz wines of differing vintages were all produced from a single vineyard in the Padthaway region of southeastern Australia. Shiraz juice and fermentation samples were supplied by Australian producers from fruit obtained from a single vineyard in the Padthaway region and a single vineyard in the McLaren Vale region for the fermentation experiments.

NMR Analysis. Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded with Bruker spectrometers operating at 400 or 600 MHz for proton and at 100 or 150 MHz for carbon nuclei, respectively. Chemical shifts were recorded as δ values in parts per million (ppm). Spectra were acquired in $CDCl_3$ at ambient temperature, and resonances were assigned by routine 2D correlation experiments. For ^{1}H NMR spectra, the peak due to residual $CHCl_3$ (δ 7.26) was used as the internal reference. For ^{13}C NMR spectra, the central peak of the $CDCl_3$ triplet (δ 77.16) was used as the internal reference.

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

Preparation of d_6-1,8-Cineole (6). The synthetic route to d_6-1,8-cineole (**6**) is shown in **Figure 1**. Ethyl 4-methylcyclohex-3-ene-1-carboxylate (**3**) was prepared according to the method of Inukai et al. (*14*) from isoprene (**1**) and ethyl acrylate (**2**) (*15*) on a multigram scale. Spectroscopic data for ester **3** were in full accord with those reported by Fringuelli et al. (*16*).

To magnesium turnings (0.867 g, 35.7 mmol) and iodine (ca. several crystals) in dry Et_2O (20 mL) under N_2 was added d_3-methyl iodide (5.17 g, 2.22 mL, 35.7 mmol) in dry Et_2O (20 mL) dropwise at reflux. After complete addition of the iodide, the mixture was heated for 30 min, ester **3** (2.02 g, 12.0 mmol) in dry Et_2O (10 mL) was added, and heating was continued for a further 1 h. The solution was chilled in an ice bath and quenched with a saturated solution of NH_4Cl. The organic layer was concentrated in vacuo to yield d_6-α-terpineol (**4**) (1.85 g, 11.5 mmol, 96%) as a pale yellow oil. Spectroscopic data were in full accord with those reported for the unlabeled compound (*17*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum. Compound **4** was used without further purification in the next step.

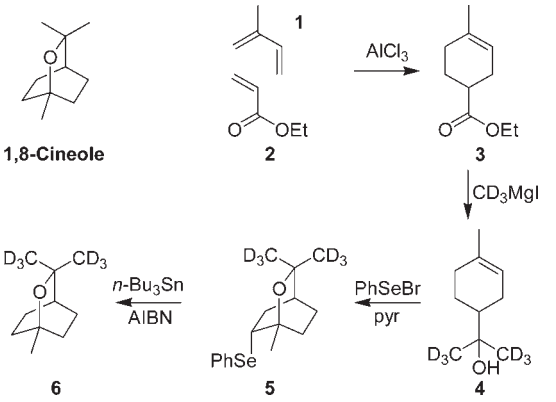

Figure 1. Structure of 1,8-cineole and synthetic route to d_6-1,8-cineole (**6**) used as an internal standard for GC-MS analysis.

ESI-HRMS, *m*/*z* calcd for $C_{10}H_{11}D_6^+$ ([M + H$^+$ − H_2O]), 143.1701; found, 143.1710.

EI-MS, *m*/*z* (%) 160 (M$^+$, 0.1), 142 (68), 124 (61), 93 (66), 92 (25), 81 (41), 79 (11), 67 (15), 65 (100), 46 (23).

d_6-2-Phenylseleno-1,8-cineole (**5**) was prepared according to the method of Bugarčić et al. for the unlabeled compound (*18*). Briefly, reaction of d_6-α-terpineol (**4**) (0.479 g, 2.99 mmol), pyridine (0.237 g, 240 μL, 2.99 mmol), and phenylselenyl bromide (0.776 g, 3.29 mmol) afforded phenylselenoether **5** (0.700 g, 2.22 mmol, 74%) as a colorless oil after purification on silica gel with CH_2Cl_2 followed by solvent removal. Spectroscopic data were in full accord with those reported for the unlabeled compound (*19*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum.

Reduction of selenide **5** was performed according to the procedure of Nicolaou et al. (*20*). Accordingly, compound **5** (0.700 g, 2.22 mmol), tri-*n*-butyltin hydride (0.938 g, 867 μL, 3.22 mmol), and azobisisobutyronitrile (2.22 mL, 0.02 M in toluene, 0.044 mmol) gave title compound **6** (0.271 g, 1.69 mmol, 76%) as a colorless oil after purification on silica gel with CH_2Cl_2 followed by solvent removal. Spectroscopic data were in full accord with those reported for the unlabeled compound (*21*), apart from the absence of signals corresponding to the labeled positions in the ^{1}H NMR spectrum.

ESI-HRMS, *m*/*z* calcd for $C_{10}H_{11}D_6^+$ ([M + H$^+$ − H_2O]), 143.1701; found, 143.1692.

EI-MS, *m*/*z* (%) 160 (M$^+$, 100), 142 (79), 132 (12), 131 (19), 114 (78), 113 (94), 96 (48), 90 (85), 89 (38), 81 (98), 75 (46), 72 (89), 59 (30), 55 (26), 46 (57), 43 (84).

Method Optimization. "Bag-in-box" wine (200 mL) was spiked with 1,8-cineole at a concentration of 0, 5, or 100 μg/L, and the mixtures were shaken. Aliquots (10 mL) were transferred into 22 mL amber screw-cap vials for headspace SPME-GC-MS analysis. Various preconditioned SPME fibers were trialed on these samples. The fibers investigated were PDMS/DVB, CAR/PDMS, PA, PDMS, and DVB/CAR/PDMS at the recommended operating temperatures for each fiber. Once the best fiber was determined, different sampling parameters were investigated individually. The parameters were no dilution, no salt, and no mixing; diluting the sample by 10 and 50% with Milli-Q water (v/v); salting the sample with either 1 or 2 g of NaCl; and inclusion of stirring (500 rpm) or agitation (400 rpm, agitation on 99 s and off 1 s) during fiber extraction.

GC-MS Instrumentation. Samples were analyzed with an Agilent 6890N gas chromatograph (Santa Clara, CA) fitted with a Gerstel MPS2 autosampler (Lasersan Australasia Pty Ltd., Robina, QLD, Australia) and coupled to an Agilent 5973N mass spectrometer. The gas chromatograph was fitted with either a 30 or 60 m J&W DB-Wax fused silica capillary column (0.25 mm i.d., 0.25 μm film thickness) during method development. The carrier gas was helium (BOC gases, ultrahigh purity), and the flow rate was 1.7 mL/min. The oven temperature started at 50 °C, was held at this temperature for 4 min, then increased at 10 °C/min to 125 °C, then increased at 30 °C/min to 240 °C, and held at this temperature for 10 min. The injector was held at 240 °C throughout the run. Positive ion

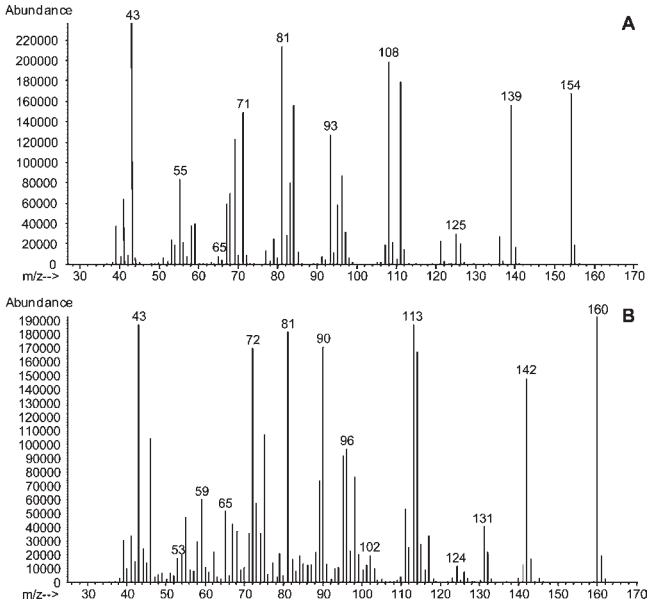

Figure 2. Electron ionization mass spectra of (**A**) unlabeled 1,8-cineole and (**B**) d_6-1,8-cineole (**6**).

electron impact spectra at 70 eV were recorded in the range m/z 35−350 for scan runs.

Optimized Method for Preparation of Juice and Wine Samples for Analysis. An aliquot (50 μL) of an ethanol solution containing d_6-1,8-cineole (**6**) (5.12 μg/mL) was added to white or red wine (10 mL) in a 22 mL glass screw-cap amber SPME vial. For red wine, 5 mL of the sample was removed and 5 mL of Milli-Q water was added to the vial. The sample was mixed, 2 g of NaCl was added, and the contents were shaken by hand and then sealed prior to GC-MS analysis.

Quantitative GC-MS Analysis of 1,8-Cineole. Quantitation was carried out using the GC-MS system with a 60 m DB-Wax column as described above. The autosampler was fitted with a 2 cm, 50/30 μm DVB/CAR/PDMS SPME fiber. The sample headspace was extracted at 50 °C for 40 min with agitation at 400 rpm (99 s on, 1 s off) and desorbed in the inlet for 15 min. The splitter, at 42:1, was opened after 36 s. Injection was done in pulsed/splitless mode with an inlet pressure of 45.0 psi maintained until splitting. The injection liner was a Supelco injection sleeve made of deactivated borosilicate glass, 0.75 mm i.d. The oven temperature started at 50 °C, was held at this temperature for 2 min, then increased at 5 °C/min to 150 °C, then increased at 20 °C/min to 240 °C, and held at this temperature for 10 min. For quantitation, mass spectra were recorded in selected ion monitoring (SIM) mode. **Figure 2** displays the full-scan mass spectrum of each compound. The ions monitored in SIM runs were m/z 113, 114, 117, 132, 142, and 160 for d_6-1,8-cineole and m/z 108, 111, 126, 139, and 154 for 1,8-cineole. Selected fragment ions were monitored for 20 ms each. The underlined ion for each compound was the ion typically used for quantitation, having the best signal-to-noise ratio and the least interference from other wine components, whereas the other ions were used as qualifiers.

Analytical Method Validation. The analytical method was validated by a series of duplicate standard additions of 1,8-cineole (0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, 50, and 100 μg/L) to a commercial young dry white "bag-in-box" wine (9.5% ethanol, pH 2.98) and a commercial young dry red "bag-in-box" wine (12.5% ethanol, pH 3.16). To determine the precision of the analysis, seven replicate samples were spiked with 1,8-cineole at two different concentrations (2 and 25 μg/L). For quantifying the analyte in batches of unknown samples, duplicate sets of standards were prepared at the same time as the juice and wine samples, by adding d_6-1,8-cineole standard solution (50 μL) to 10 mL of model wine (10% aqueous ethanol, saturated with potassium hydrogen tartrate, pH adjusted to 3.2 with tartaric acid) spiked with 1,8-cineole at concentrations of 0, 2, 10, 25, 50, and 100 μg/L (total of 12 standards). To ensure that the accuracy of the analysis was maintained, duplicate control wine samples, spiked with 1,8-cineole at concentrations of 0, 2, and 25 μg/L (total of six control wines), were included with every set of samples to be quantified. All validation samples were prepared and analyzed according to the optimized method.

Hydrolytic and Stability Studies. Model wines at pH 3 and 3.4 (10% ethanol, saturated with potassium hydrogen tartrate, adjusted to the required pH with tartaric acid) were used in each case. For the hydrolytic study, limonene and α-terpineol were separately spiked at 500 μg/L, and for the degradation study, 1,8-cineole was spiked at 50 μg/L (giving six spiked solutions in total). The solutions were divided into 25 mL glass ampules (54 for each, containing approximately 20 mL), sparged with nitrogen, and sealed. Thirty ampules of each spiked solution were stored at 25 °C, and the remaining 24 ampules of each were stored in an incubator at 45 °C (accelerated aging). Samples stored at 25 °C were analyzed for 1,8-cineole after 0, 4, 8, 16, 52, and 104 weeks, and those stored at 45 °C were analyzed for 1,8-cineole after 0, 1, 4, 8, and 16 weeks. Triplicate samples were analyzed for 1,8-cineole at each time point according to the optimized method.

Fermentation Study. Fermentations were followed every day from berry crush to the end of fermentation with two separate, commercially harvested Shiraz grape parcels at two independent wineries. Fruit from the McLaren Vale region, South Australia (SA), was fermented in an open fermentor (10 tonne), and fruit from the Padthaway region, SA, was fermented in a closed fermentor (19.33 tonne in a 20 tonne fermentor). Samples (100 mL) were collected in triplicate each day, spiked with 500 μL an ethanolic solution of d_6-1,8-cineole (5.12 μg/mL) immediately after collection, and then shaken by hand, sealed, and transported to the laboratory on ice. An aliquot of each sample (5 mL) was placed into a 22 mL amber screw-cap vial and diluted with 5 mL of Milli-Q water, and 2 g of NaCl was added. The samples were heated in a water bath at 67 °C for 15 min to terminate fermentation and then analyzed according to the optimized method.

Scalping Study. Sixty liters of Shiraz wine (14.1% ethanol, pH 3.15, titratable acidity = 7.4 g/L, SO_2 (free) = 27 mg/L, SO_2 (total) = 87 mg/L) were spiked with 1,8-cineole at approximately 100 μg/L. The wine was passed through a Z6 grade pad (nonsterile, 0.8 μm nominal pore size) and transferred into either 750 mL flint glass bottles or glass ampules. Bottles (24 of each) were sealed with Reference 2 natural cork (cork mouth bottle finish), Nomacorc synthetic closure (cork mouth bottle finish), and Stelvin screw cap (BVS bottle finish), and 48 ampules (50 mL) and 24 ampules (25 mL) were also sealed at the time of bottling. Bottles and ampules were stored in a climate-controlled cellar (between 18 and 20 °C) until analysis. Triplicate samples were analyzed for 1,8-cineole after 0, 3, 6, and 12 months according to the optimized method.

Statistical Analysis. The results reported for the calibration of the method were derived from the average of two replicate measurements for each concentration of analyte (and seven replicates for repeatability samples). The limit of detection (LOD) and limit of quantitation (LOQ) for 1,8-cineole were determined by multiplying the standard error of the y-intercept by 3.3 (for LOD) and 10 (for LOQ) and dividing these values by the slope of the calibration curve for each standard. Statistical analyses were performed with Microsoft Excel 2003, with the LINEST function used to obtain calibration function slopes and intercepts and their associated standard errors.

RESULTS AND DISCUSSION

Method Development and Optimization. For reliable determinations by headspace SPME-GC-MS, a deuterated analogue of 1,8-cineole was prepared for use as an internal standard. **Figure 1** depicts the synthetic route, which relied on a Lewis acid catalyzed Diels−Alder reaction (*14*) to form ester **3**, followed by Grignard addition to incorporate the deuterium labels, furnishing d_6-α-terpineol (**4**). Phenylselenoetherification in the presence of pyridine (*18*) afforded bicyclic ether **5**, and reduction of the selenide (*20*) gave several hundred milligrams of d_6-1,8-cineole (**6**), with an overall yield of 54% from ester **3**. Recently, Horst and Rychlik prepared small quantities of d_3-1,8-cineole with a comparable yield using a similar strategy (*22*). Ions used in the GC-MS method for quantitation and qualification were selected from the full-scan mass spectra of labeled and unlabeled 1,8-cineole (**Figure 2**).

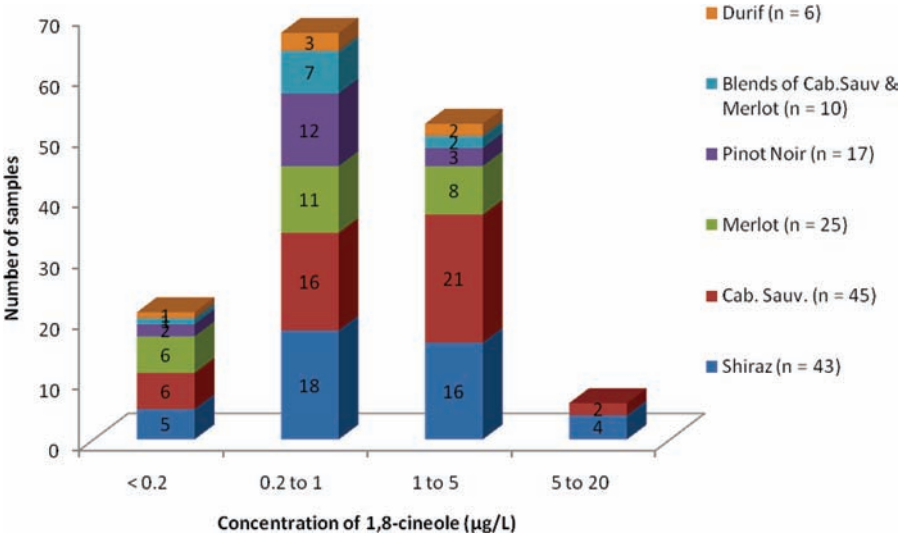

Figure 3. Concentration of 1,8-cineole (μg/L) in 146 commercially available Australian red wines of different vintages and varieties (analyzed in May 2007).

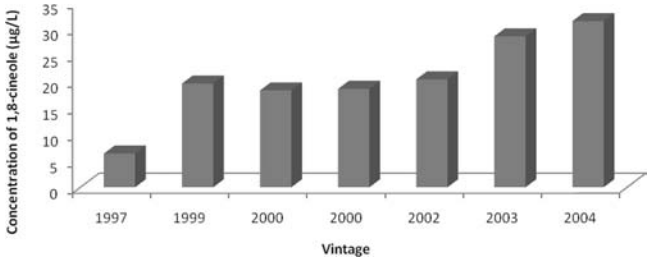

Figure 4. Concentration of 1,8-cineole (μg/L) in Australian commercial Shiraz wine produced from the same vineyard over different vintages (analyzed in July 2007).

Of the various fibers investigated for the extraction of 1,8-cineole from wine, the 2 cm DVB/CAR/PDMS fiber gave the strongest recovery (Supplementary Figure 1 in the Supporting Information). The PA fiber was the least effective at absorbing 1,8-cineole from wine. Evaluation of the various parameters trialed for white wine with the chosen fiber showed the addition of 2 g of salt and agitation gave the best extraction efficiency (Supplementary Figure 2 in the Supporting Information). Dilution of the samples to 50% with water had no effect on the white wine (9.5% alc/vol) (Supporting Information, Supplementary Figure 2) but increased the sensitivity by approximately 17% for a higher alcohol content red wine (12.5% alc/vol) (Supplementary Figure 3 in the Supporting Information). Method sensitivity relative to ethanol content when using headspace SPME has been demonstrated before (*23*, *24*), and the importance of diluting higher alcohol wines prior to analysis has been shown previously for red wine when using headspace SPME (*25*). Initially, using a 30 m DB-Wax column gave adequate sensitivity, but there was another peak coincident with 1,8-cineole. Changing to a 60 m column of the same phase with a slower temperature ramp separated the analyte from the coeluter. The method was then validated with the optimized sampling and chromatographic conditions.

Method Validation. The standard addition curves obtained were linear throughout the concentration range, with a coefficient of determination (R^2) of 0.999 for a white wine and 1.000 for a red wine. The method sensitivity was excellent, with calculated LOQs of 0.29 and 0.20 μg/L for the white and red wines, respectively, and calculated LODs of 0.09 and 0.07 μg/L for the white and red wines, respectively. The precision of the analysis was determined for seven replicate samples containing internal standard at two concentrations of 1,8-cineole. Spikes at 2 and 25 μg/L gave respective standard deviations of 0.07 and 0.48 μg/L for the white wine and 0.04 and 0.36 μg/L for the red wine. This equates to relative standard deviations of $< 5\%$ in all cases. Furthermore, red, white, and model wines all gave identical calibration slopes, showing the quantitative analysis was not dependent on the matrix (data not shown).

Evaluation of 1,8-Cineole in Commercial Australian Wine. The method was applied to a survey of 190 commercially available bottled Australian wine samples. The wines were chosen randomly from different parts of Australia and comprised 146 red wines incorporating Shiraz (43), Cabernet Sauvignon (45), Merlot (25), Pinot noir (17), Durif (6), and red wine blends (10), along with 44 white wines made up of Riesling (12), Sauvignon blanc (10), Semillon (10), and Chardonnay (12). The results from the red wines are summarized in **Figure 3**. Of the red wines analyzed, 40% contained 1,8-cineole above the reported detection threshold, and several wines were substantially higher. Incidentally, the wine in this survey that contained the highest amount of 1,8-cineole (19.6 μg/L) was a Shiraz produced from a vineyard that had eucalyptus trees within a few meters of the nearest row of vines. In contrast to the situation for red wines, 1,8-cineole was not detected above 0.8 μg/L in any of the 44 white wines analyzed (data not shown). These results provided the basis for additional investigation into the occurrence and evolution of 1,8-cineole, which seemed to be important in red wine only.

Scalping and Stability Studies. Further examination of a number of commercial wine vintages produced over a number of years from a single Shiraz vineyard that had eucalyptus trees within several meters of the nearest row of vines showed various levels of 1,8-cineole and indicated an apparent trend toward increased 1,8-cineole concentrations in younger wines (**Figure 4**). This invoked a number of possibilities for the differences, such as the age of the vines, changes to winemaking practices, the instability of 1,8-cineole, or scalping of the compound by closures. The most feasible studies were to address the issues of stability and scalping. To this end we examined a Shiraz wine, spiked with 1,8-cineole, at various time points. Over a 12 month period no significant scalping was observed for wine stored under natural cork or Stelvin closure relative to the wine stored in glass ampules; the only closure that showed a moderate reduction (14%) was a synthetic closure (data not shown). The latter result is not surprising considering that 1,8-cineole is relatively nonpolar

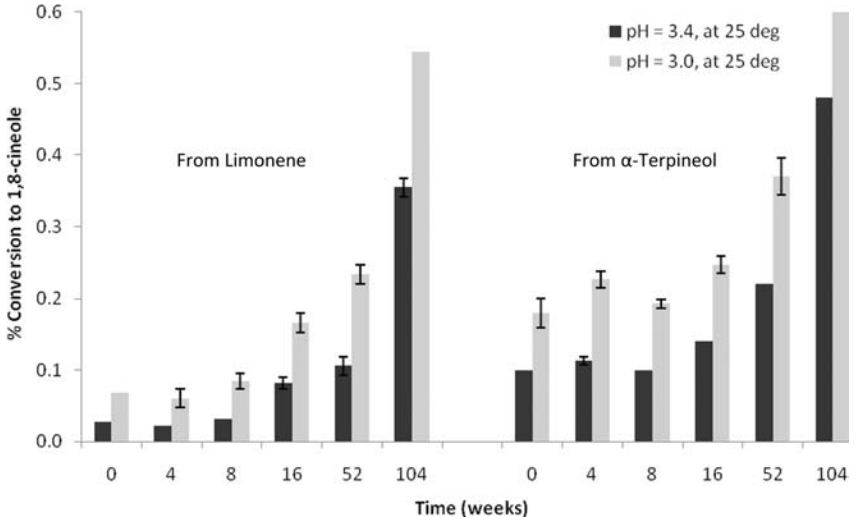

Figure 5. Hydrolytic study assessing percent molar conversion to 1,8-cineole of limonene and α-terpineol in model wine at pH 3.0 and 3.4 stored at 25 °C. Model wines were spiked separately with 500 μg/L of terpene precursors and assessed for 1,8-cineole at each time point. Error bars represent the standard deviation of three replicates. Where no error bars are shown, the standard deviation was zero.

and could be prone to scalping, particularly by synthetic closures (*26*). With regard to stability, 1,8-cineole was found to be very persistent in model wine (10% ethanol, saturated with potassium hydrogen tartrate, adjusted to the required pH with tartaric acid) when stored at different pH and temperatures. For samples stored at pH 3.0 or 3.4 and 25 °C, there was no discernible degradation of 1,8-cineole at either pH even after 2 years (data not shown). Additionally, samples stored at pH 3.0 and 3.4 under accelerated aging conditions (45 °C) showed no diminution of 1,8-cineole concentration after 16 weeks (data not shown), highlighting the stability of the compound under wine-like conditions. We can conclude from these scalping and stability experiments that 1,8-cineole is unlikely to suffer any substantial decline in concentration during aging of wine under ordinary storage conditions. Therefore, it appears that drivers of 1,8-cineole concentration in red wines may be associated with environmental factors or winemaking and viticultural practices.

Hydrolytic Studies. Farina et al. have suggested that significant quantities of 1,8-cineole could be generated from limonene and α-terpineol (*10*). To obtain precise data related to conversion of terpenoid precursors, experiments were carried out to determine if it was possible to generate significant quantities of 1,8-cineole from limonene and α-terpineol as suggested by Farina et al. (*10*). Monoterpenes such as these are more commonly associated with white grape varieties, yet the white wines we analyzed contained levels of 1,8-cineole well below its aroma detection threshold. Farina et al. proposed a mechanism for the formation of 1,8-cineole from either limonene or α-terpineol, which proceeds via the *trans*-isomer (*10*). However, it is unlikely that such a pathway would produce the product; the only arrangement likely to do so must arise from the *cis*-isomer adopting a boat conformation. Furthermore, their mechanism requires α-terpineol as an intermediate forming from hydration of limonene. However, contrary to expectation, if this was indeed true, their reported levels of cineole produced were 3-fold higher when limonene was the sole spiked compound than when α-terpineol was the sole spiked compound (*10*).

We therefore conducted precise hydrolytic experiments and analyzed for the production of 1,8-cineole in model wines spiked separately with limonene and α-terpineol. Samples were treated in the same way as the stability studies and examined over a period of time. The results were expressed as percent conversion to 1,8-cineole on a molar basis at both 25 °C (**Figure 5**) and 45 °C (data not shown). It was our observation, when the low levels of 1,8-cineole already present in the samples at $t = 0$ were subtracted from the total, that the amounts of additional 1,8-cineole generated were similar for both substrates. As expected, the production of 1,8-cineole at higher temperature (45 °C) was 2−4 times greater than the corresponding time points at room temperature (25 °C), with 16 weeks at 45 °C being similar to 2 years at 25 °C. Production of 1,8-cineole was also higher at the lower pH, consistent with the acid-catalyzed nature of the conversions. Overall, the amount of 1,8-cineole produced was low, however; even after 104 weeks at 25 °C, there was, at most, around 0.6% conversion (**Figure 5**), giving concentrations of 1,8-cineole close to its aroma detection threshold and about 10 times lower than those reported by Farina et al. (*10*). Furthermore, the results must be considered in the context of the high spiking levels of terpenoid precursors (500 μg/L). Under normal circumstances their conversion to 1,8-cineole would appear to be relatively unimportant to wines that are a few years old but might contribute to 1,8-cineole in older wines, that is, 10 years or older.

Fermentation Study. The results of our survey of commercial Australian wines (**Figure 3**) indicated that only in red wines was the concentration sufficiently high to have a possible sensory impact, as indicated by threshold data (*9*, *10*). This led us to examine the hypothesis that the compound accumulates in grape solids (skins, stalks, etc.) and is only extracted through maceration during winemaking. Therefore, two different commercial fermentations were sampled on a daily basis from crush to the end of fermentation with Shiraz grapes from two different commercial producers. Samples were heated after preparation to terminate the fermentation process prior to analysis. With both ferments there was a steady increase in 1,8-cineole concentration during fermentation on skins, which ceased after pressing (**Figures 6** and **7**). Also noteworthy is the difference in the concentrations of 1,8-cineole extracted during winemaking with these two parcels of fruit, and the variability in concentration of the replicates for the first 3 days (**Figure 7**) due to less homogeneous mixing during cold soaking. The minor decrease of 1,8-cineole observed after pressing is unexplained but might be due to loss of the compound during transfer between tanks. Nonetheless, these results strongly indicated that 1,8-cineole was extracted from grape solids with increases in ethanol as fermentation

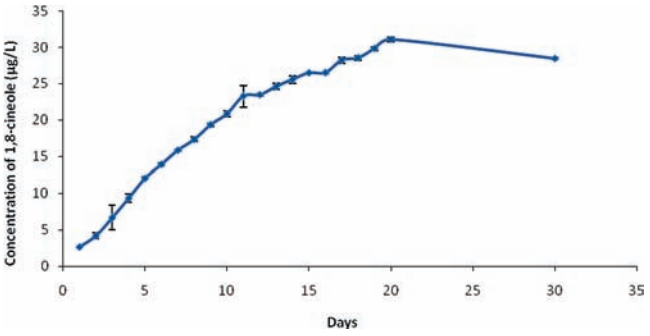

Figure 6. Concentration of 1,8-cineole (μg/L) extracted during fermentation with Padthaway fruit in a closed fermentor. The wine was pressed off skins on day 20. Error bars represent the standard deviation of three replicates.

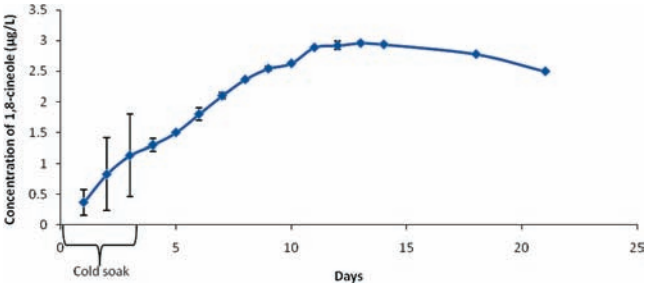

Figure 7. Concentration of 1,8-cineole (μg/L) extracted during fermentation with McLaren Vale fruit in an open fermentor. The wine was pressed off skins on day 11. Error bars represent the standard deviation of three replicates.

progressed, although at this point it cannot be ruled out that matter other than grapes (MOG) in the ferments (e.g., eucalyptus leaves) has also played a role.

Questions remain about whether 1,8-cineole is present in wines due to being biosynthesized in the grapevine or absorbed from the environment due to vineyard proximity to eucalyptus trees. We have provided further insight into the origin/occurrence of 1,8-cineole in wine by showing that it is a phenomenon chiefly associated with red wine, that the compound is stable during storage and barely scalped by closures, and that it is extracted during red winemaking in the presence of solids only. We have also discounted terpenoid precursors as being substantial contributors to 1,8-cineole concentrations in younger wine. Future work will focus closely on the effect of eucalyptus trees in an attempt to resolve the origin surrounding 1,8-cineole in wines.

ABBREVIATIONS USED

SIDA, stable isotope dilution analysis; SPME, solid-phase microextraction; GC-MS, gas chromatography−mass spectrometry; SIM, selected ion monitoring; LOD, limit of detection; LOQ, limit of quantitation; MOG, matter other than grapes.

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Supporting Information Available: Figures displaying SPME fiber performance and evaluation of parameters for 1,8-cineole extraction from white and red wine. This material is available free of charge via the Internet at http://pubs.acs.org.

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