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Contribution of Benzenemethanethiol to Smoky Aroma of Certain *Vitis vinifera* L. Wines

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Benzenemethanethiol, a volatile thiol with a strong empyreumatic aroma reminiscent of smoke, has been identified in boxwood (*Buxus sempervirens* L.) as well as in red and white *Vitis vinifera* L. wines. The perception threshold in a model hydroalcoholic solution is ∼0.3 ng/L. All of the wines analyzed for this study contained this compound in concentrations of several dozen nanograms per liter. The Chardonnay wines had 30−40 ng/L. Sensory discrimination between a wine containing 7 ng/L benzenemethanethiol and the same wine with an additional 4 ng/L is very significant; the difference in smell is described as "empyreumatic". This compound can therefore significantly contribute to the aroma of certain wines (Sauvignon Blanc, Semillon, Chardonnay, etc.) containing concentrations as high as 30−100 times higher than their perception threshold.

KEYWORDS: Benzenemethanethiol; boxwood; Sauvignon Blanc; Chardonnay; Semillon; smoky aroma

INTRODUCTION

Empyreumatic aromas, reminiscent of smoke and burnt wood, often feature in descriptions used by wine tasters. The following sulfur compounds with such odors have been identified and measured in wines at levels higher than their perception threshold: methylthiopropionic acid (*1*), 2-mercaptoethyl and 3-mercaptopropyl acetates (*2*), and furanmethanethiol (*3*).

Sauvignon Blanc wine aromas often have overtones of minerals and smoke, often referred to as *pierre à fusil* or gun flint. This characteristic led to the grape variety's other name, Blanc Fumé, literally "smoky white" (*4*). Sauvignon Blanc is often described as having a boxwood aroma. The presence of 4-mercapto-4-methylpentan-2-one, with a strong "feline" (cat urine) smell, in both boxwood (*5*) and Sauvignon Blanc (*6*), only partly explains the similar aromas. The gun flint aroma, present in several varieties of boxwood as well as Sauvignon Blanc wines, had never previously been analyzed or interpreted.

This study describes the identification and measurement of benzenemethanethiol, which has a strong smoky aroma, but the presence of which had not previously been reported in plants.

MATERIALS AND METHODS

Chemicals. Benzenemethanethiol, benzaldehyde, and benzyl alcohol were supplied by Sigma-Aldrich. Thier purity was >99%.

Wines Analyzed. Sauvignon Blanc (1999 and 2000 vintages) wines were from the Graves and Pessac-Léognan appellations (Bordeaux), as well as Sancerre and Pouilly-Fumé (Loire region). The Semillon wines (2000 vintage) came from the same Bordeaux appellations. The Chardonnay wines (1999 vintage) were from Burgundy (Puligny-Montrachet, Chassagne-Montrachet, and Chablis) as well as the south of France (Limoux appellation). The red Bordeaux wines were from St. Julien (1998, 1989, and 1978 vintages) and Pauillac (1998, 1990, and 1982 vintages).

Specific Extraction of Volatile Thiols from Boxwood. Boxwood (*Buxus sempervirens* L.) leaves (50 g fresh weight) were collected during May and macerated at room temperature in 200 mL of dichloromethane for 18 h. An organic extract was obtained; the plant matter was washed with 50 mL of the same solvent. The two dichloromethane extracts were combined, decanted, and filtered through glass wool. The volatile thiols contained in the organic phase were extracted using a *p*-hydroxymercuribenzoate (*p*-HMB) solution and purified by percolation through a Dowex 1 column, according to the method described by Tominaga et al. (*3*, *7*).

Specific Extraction of Volatile Thiols in Wine. The specific extraction of volatile thiols in wine was carried out according to the method described by Tominaga et al. (*3*, *7*) using *p*-HMB. This was modified by using ethyl acetate instead of dichloromethane to extract all of the wine's volatile compounds.

Identification and Measurement of Benzenemethanethiol. The volatile thiol was identified by GC-MS according to the spectrum obtained in scan mode and the linear retention index (LRI) determined relative to the series of *n*-alkanes compared with those of the reference compound. Quantification was carried out in SIM mode (see below).

GC-FPD, GC-MS. The chromatographic conditions were identical to those described by Tominaga et al. (*7*), using a BPX-35 column (SGE, 50 m × 0.22 mm, 0.25 μm) instead of a BP-20. Benzenemethanethiol was measured in SIM mode, selecting the ions *m*/*z* 91 and 124. Benzaldehyde and benzyl alcohol were assayed using a BP-20 column (SGE, 50 m × 0.22 mm, 0.25 μm) in SIM mode, selecting ions as follows: *m*/*z* 106 for benzaldehyde and *m*/*z* 108 for benzyl alcohol (*8*).

Sensory Analysis. The benzenemethanethiol perception threshold was determined using the method described by Boidron et al. (*9*). This includes a triangular tasting by a jury of 62 experienced tasters. The perception threshold corresponded to the minimum concentration

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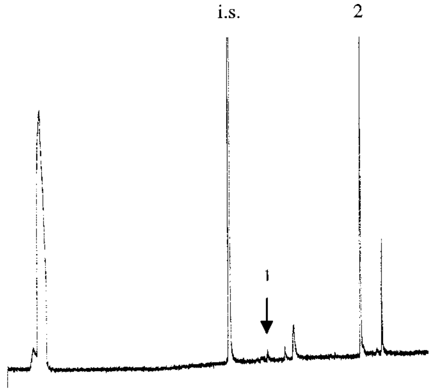

Figure 1. Analysis by GC-FPD of the volatile thiols extracted from boxwood leaves (*B. sempervirens* L.). Peaks: 1, 4-mercapto-4-methylpentan-2-one; 2, benzenemethanethiol; i.s., internal standard, 4-methoxy-2-methyl-2-mercaptobutane.

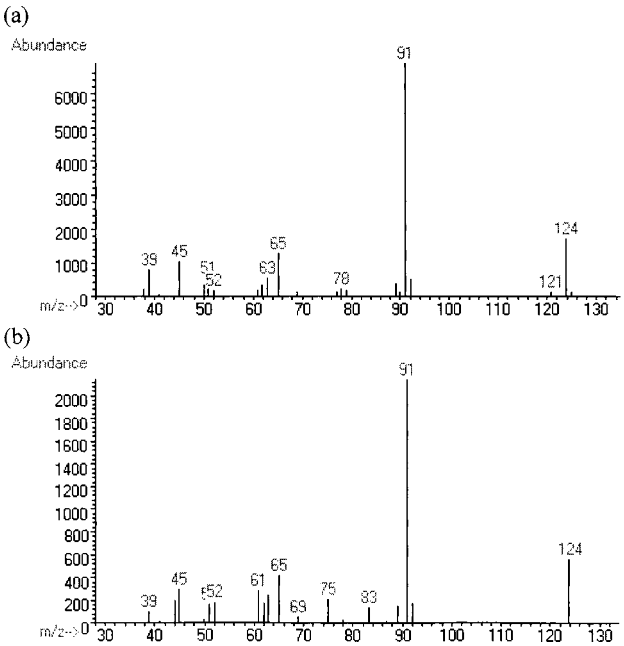

Figure 2. Mass spectrum of benzenemethanethiol obtained from leaf extract of the boxwood (a) and from a Chardonnay wine (b).

recognized by 50% of the tasters. The olfactory discrimination between a white Bordeaux wine (blend of Sauvignon Blanc and Semillon) containing 7 ng/L benzenemethanethiol and the same wine with additional levels of this compound (11 or 14 ng/L) was studied in the following way: both wines were submitted to a two of five test (*10*) carried out by a jury of 65 experienced tasters, who were asked to choose from a list of five characteristics (floral, fruity, empyreumatic, spicy, and mineral) to describe the difference in aroma resulting from the supplementations.

RESULTS AND DISCUSSION

Identification of Benzenemethanethiol in Boxwood. An organic extract of boxwood containing volatile thiols was purified by the method described above and analyzed by GC-FPD and GC-MS. The FPD chromatogram (**Figure 1**), in a BPX-35 column, shows only a few peaks. Peak 1 corresponds to 4-mercapto-4-methylpentan-2-one already identified in this plant (*5*). The compound corresponding to peak 2 was identified as benzenemethanethiol by GC-MS using a moderately polar column (BPX-35). The mass spectrum obtained (**Figure 2a**) was completely matched up to that of the reference compound as well as the linear retention indice (1210, BPX-35 column).

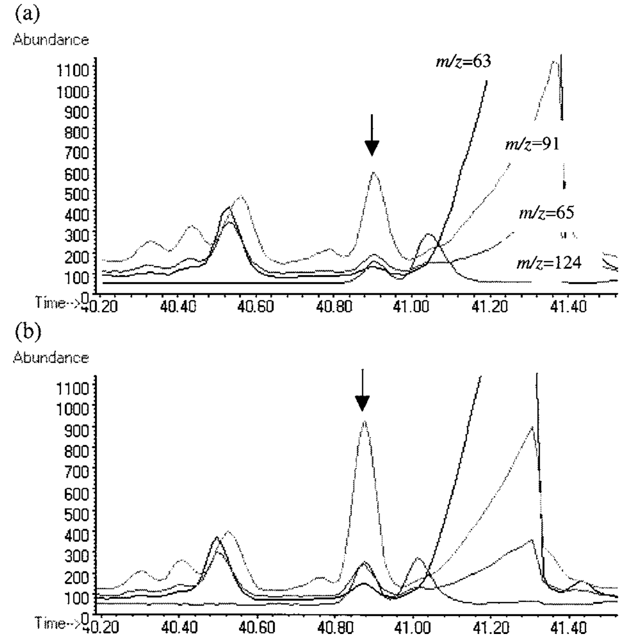

Figure 3. (a) Detection of benzenemethanethiol (arrow) from a Sauvignon Blanc wine (Sancerre appellation, 1999 vintage) in SIM mode by overlapping the ions selected, *m*/*z* 63, 65, 91, and 124, at the retention time of the reference compound. (b) Verification of the peak purity by co-injection of a comparable quantity of the reference compound. No change of the peak shape was obtained.

Up until now, this volatile thiol had not been characterized other than in the flesh of carp and in beef broth (*11*, *12*). It is also known to be an exogenous enhancer of the empyreumatic aroma in certain food products (*13*). This is the first time, to our knowledge, that this compoud has been reported to be found in a plant.

Identification of Benzenemethanethiol in White and Red Wines. Volatile thiols were selectively extracted from wines using *p*-HMB and analyzed by GC-MS in scan mode. According to the mass spectrum obtained (**Figure 2b**) from wines (Chardonnay) at the linear retention index (1210, BPX-35 column) of the reference compound of benzenemethanethiol, the volatile thiol was identified in wines. The existence of benzenemethanethiol in wines having low concentrations of the thiol was comfirmed by overlapping selecting ions *m*/*z* 63, 65, 91, and 124 in SIM mode at the linear retention index of the reference compound (**Figure 3a**). Furthermore, the peak shape formed by the selected ions has not been modified by co-injection of a comparable quantity of the reference compound (**Figure 3b**). This is also the first time that the volatile thiol was reported in wines.

Measurement of Benzenemethanethiol in Boxwood and in White and Red Wines. The standard curve for benzenemethanethiol assay was prepared on a BPX-35 column. In the concentration range (10−80 ng/L), the regression equation was linear, [benzenemethanethiol, ng/L] $= 273.1H/H_{i.s.} - 1.04$, $r^2 = 0.986$ (H = height of the thiol peak; $H_{i.s.}$ = height of internal standard peak), and the coefficient of variation was 4.9%.

Benzenemethanethiol in boxwood leaves was measured. The concentration of the volatile thiol reached 25 ng/g in fresh leaves. Considering the very low perception threshold (see below), it seems certain that the thiol contributes to the characteristic aroma of the plant.

The volatile thiol was also quantified in various white wines made from Sauvignon Blanc, Semillon, and Chardonnay grape

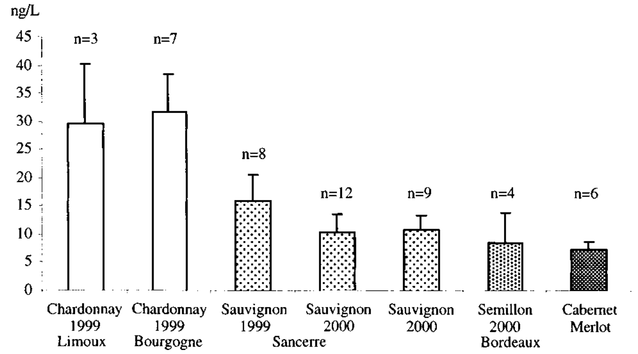

Figure 4. Concentrations (nanograms per liter) of benzenemethanethiol in some white and red wines made from different *V. vinifera* grape varieties.

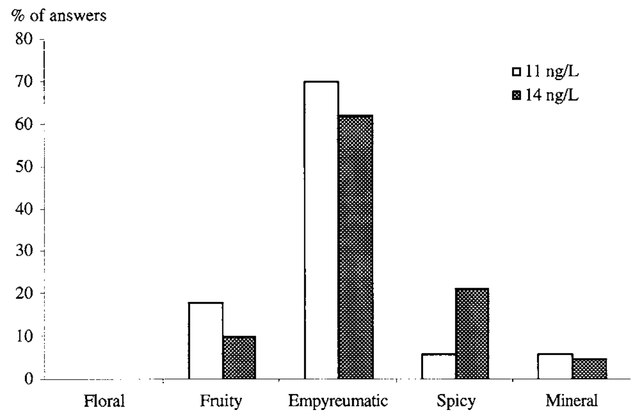

Figure 5. Sensory qualifications of white wines with added benzenemethanethiol leading to a final concentration of 11 or 14 ng/L.

varieties from several French appellations, as well as in several Bordeaux red wines made from Cabernet Sauvignon and Merlot grape varieties. Irrespective of their origin, Chardonnay wines contained 2−3 times as much benzenemethanethiol as the other grape varieties studied (**Figure 4**). Sauvignon Blanc wines from Loire and Bordeaux and the Semillon from Bordeaux had roughly the same concentrations of benzenemethanethiol (10−15 ng/L). The red Bordeaux wines, from different vintages and appellations, had levels of benzenemethanethiol close to 10 ng/L.

Sensory Analysis. A jury of 62 experienced tasters took part in triangular tests of model hydroalcoholic solution “close to wine” [12% (v/v) ethanol, 5 g/L tartaric acid, pH 3.5] with increasing concentrations of benzenemethanethiol. The perception threshold under these conditions was 0.3 ng/L. Benzenemethanethiol is therefore extremely odorous, as much as 2-furanmethanethiol (0.4 ng/L) (*3*) and much more than 4-mercapto-4-methylpentan-2-one (0.8 ng/L) (*6*), 3-mercaptohexyl acetate (4.2 ng/L) (*14*), and other strongly aromatic thiols already known to be present in wine.

Sensory discrimination between a wine containing 7 ng/L benzenemethanethiol and the same wine with an additional 4 and 7 ng/L was very significant. Over 70 and 90% of tasters gave the correct answers in the “two of five” differentiation tests for the comparison between 7 and 11 or and 14 ng/L, respectively. Among tasters capable of making this distinction, a large majority (70%) described the difference in odor as “empyreumatic” (**Figure 5**). The most appropriate description for this aroma is probably “gun flint”. Therefore, the contribution of volatile thiol to the empyreumatic aroma of Chardonnay and some Sauvignon Blanc and Semillon wines is undeniable, because the majority of wines analyzed contained this volatile thiol in quantities >10 ng/L. It is also quite feasible that, in view of its extremely low perception threshold, benzenemethanethiol contributes to the empyreumatic character of red wines to a similar extent.

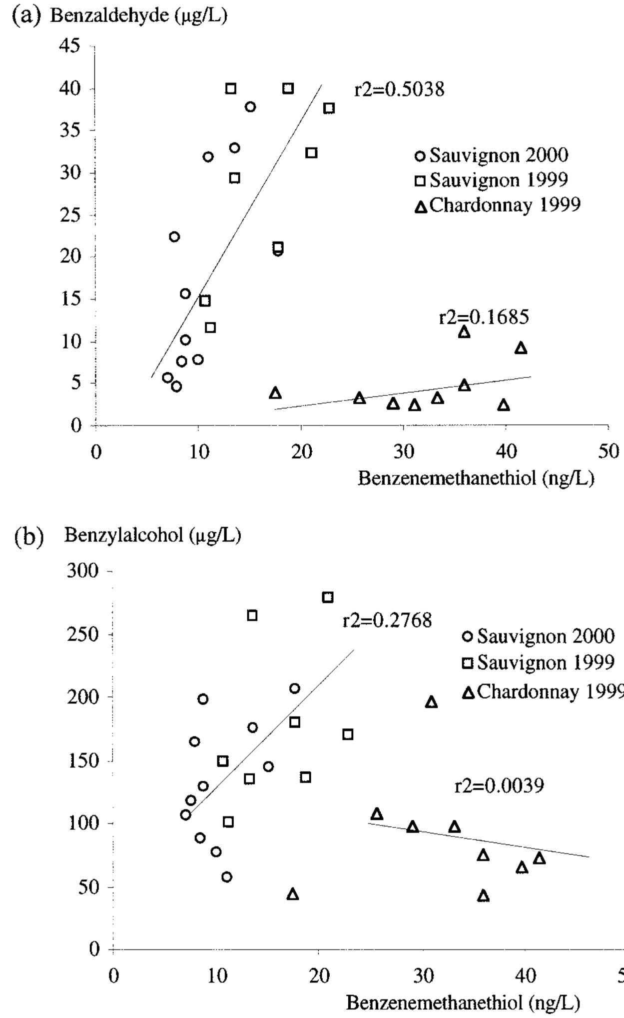

Figure 6. Relationship between benzenemethanthiol and benzaldehyde (a) or benzyl alcohol (b) in wines.

Relationship between Benzenemethanthiol and Benzaldehyde or Benzyl Alcohol. Blanchard et al. recently (*15*) proved the formation of furanemethanethiol in white wine from the furfural released by oak barrels during alcoholic fermentation. It is thus entirely possible that benzenemethanethiol is produced from the corresponding aldehyde, benzaldehyde. There is, in fact, a linear correlation between the levels of benzaldehyde and benzenemethanethiol in Sauvignon Blanc wines from Sancerre ($r^2 = 0.5038$) (**Figure 6a**), whereas the correlation between benzenemethanethiol and benzyl alcohol is weak ($r^2 = 0.2768$) (**Figure 6b**). On the other hand, the Chardonnay wines, with a greater concentration of benzenemethanethiol than Sauvignon Blanc, contained less benzaldehyde and benzyl alcohol. No link was established between these two compounds and benzenemethanethiol (**Figure 6**). Furthermore, benzenemethanethiol was formed in only trace amounts during fermentation in a model medium to which 300 μg/L of benzaldehyde was added (a concentration close to that found in grape must) (result not shown). Consequently, the formation of benzenemethanethiol from benzaldehyde during alcoholic fermentation has not yet been conclusively demonstrated.

The chemical or biological mechanisms causing the formation of this volatile thiol have yet to be determined.

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