JOURNAL OF
AGRICULTURAL AND
FOOD CHEMISTRY

Odorous Impact of Volatile Thiols on the Aroma of Young Botrytized Sweet Wines: Identification and Quantification of New Sulfanyl Alcohols

ELISE SARRAZIN,[†] SVITLANA SHINKARUK,[‡,§] TAKATOSHI TOMINAGA,*[,†]
BERNARD BENNETAU,[‡] ERIC FRÉROT,[#] AND DENIS DUBOURDIEU[†]

Faculté d'Œnologie, Institut des Sciences de la Vigne et du Vin, UMR 1219, INRA, Université Victor Segalen Bordeaux 2, 351 cours de la Libération, 33405 Talence Cedex, France; Laboratoire de Chimie Organique et Organométallique, UMR 5802, CNRS, Université Bordeaux 1, 351 cours de la Libération, 33405 Talence Cedex, France; and Ecole Nationale d'Ingénieurs des Travaux Agricoles de Bordeaux, 1 cours du Général de Gaulle, CS 40201, 33175 Gradignan Cedex, France; and Corporate R&D Division, Analysis and Perception, Firmenich SA, Route des Jeunes 1, P.O. Box 239, CH-1211 Geneva 8, Switzerland

Specific extraction of volatile thiols using sodium *p*-hydroxymercuribenzoate revealed the presence of three new sulfanylalcohols in wines made from *Botrytis*-infected grapes: 3-sulfanylpentan-1-ol (**II**), 3-sulfanylheptan-1-ol (**III**), and 2-methyl-3-sulfanylbutan-1-ol (**IV**). The first two have citrus aromas, whereas the third is reminiscent of raw onion. In addition, 2-methyl-3-sulfanylpentan-1-ol, which has a raw onion odor, was tentatively identified. Like 3-sulfanylhexan-1-ol (**I**), already reported in Sauternes wines, compounds **II**, **III**, and **IV** were absent from must. They were found in wine after alcoholic fermentation, and their concentrations were drastically higher when *Botrytis cinerea* had developed on the grapes. In the commercial botrytized wines analyzed, the mean levels of **II**, **III**, and **IV** were 209, 51, and 103 ng/L, respectively. Despite their low odor activity values, sensory tests showed additive effects among **I**, **II**, and **III**, thus confirming their olfactory impact on the overall aroma of botrytized wines.

KEYWORDS: Botrytized wines; 3-sulfanylpentan-1-ol; 3-sulfanylheptan-1-ol; 2-methyl-3-sulfanylbutan-1-ol; 3-sulfanylhexan-1-ol; 2-methyl-3-sulfanylpentan-1-ol; additive effects

INTRODUCTION

Sweet wines made from *Botrytis*-infected grapes are very famous throughout the world. They are produced from overripe grapes affected by the *Botrytis cinerea* fungus under specific climatic conditions, alternating foggy mornings with sunny afternoons (*1*). Due to the unusual composition of the grapes, these specialty wines are characterized by an exceptional range of aromas, evoking not only citrus but also crystallized and dried fruits, as well as honey. Aroma research aimed at explaining these typical nuances has focused mainly on heterocyclic odoriferous compounds, such as sotolon [4,5-dimethyl-3-hydroxy-2(5*H*)-furanone] (*2*, *3*), γ- and δ-lactones (*4*−*6*), and 3(2*H*)-furanones (*7*). However, these compounds cannot explain the distinctive citrus nuances of young botrytized wines.

Among the volatile compounds found in botrytized wine, two odoriferous molecules have already been identified as contributing to citrus nuances. 3-Sulfanylhexan-1-ol and 4-methyl-4-sulfanylpentan-2-one belong to the chemical family of volatile thiols, among the most powerful odorants of all. In botrytized wines, Tominaga et al. evidenced surprisingly high concentrations of 3-sulfanylhexan-1-ol (*8*, *9*). The contribution of volatile thiols in botrytized wine aroma was recently confirmed by Bailly et al. (*10*), who reported on new polyfunctional thiols, such as 3-methyl-3-sulfanylbutanal. In addition, Sarrazin et al. (*7*) reported the presence of other, not yet identified, volatile thiols that may be involved in the citrus nuances of botrytized wines.

In this paper, we report the identification of three new volatile thiols in wines made from *Botrytis*-infected grapes, as well as their quantification in wines made from grapes with different degrees of botrytization. The contribution of these new sulfanylalcohols to the characteristic aroma of botrytized wines was then examined by sensory analysis.

MATERIALS AND METHODS

Wines Analyzed. The botrytized wines were all made from Semillon and Sauvignon blanc grapes and were from the following appellations of the Bordeaux region: Sauternes (2001, 2002, and 2003), Loupiac (2002 and 2003), and Barsac (2001).

---

* Corresponding author (telephone +33-5 40 00 64 89; fax +33-5 40 00 64 68; e-mail takatoshi.tominaga@oenologie.u-bordeaux2.fr).
[†] Université Victor Segalen Bordeaux 2.
[‡] Université Bordeaux 1.
[§] Ecole Nationale d'Ingénieurs en Travaux Agricoles de Bordeaux.
[#] Firmenich SA.

10.1021/jf062582v CCC: $37.00

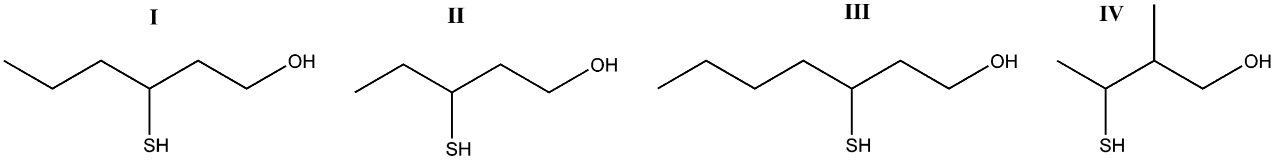


Figure 1. Structures of 3-sulfanylhexan-1-ol (**I**), 3-sulfanylpentan-1-ol (**II**), 3-sulfanylheptan-1-ol (**III**), and 2-methyl-3-sulfanylbutan-1-ol (**IV**).

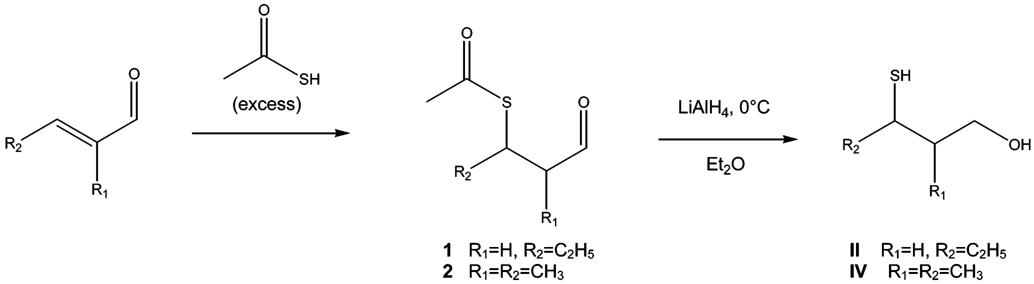


Figure 2. Synthetic pathway from α,β-unsaturated aldehydes to 3-sulfanyl-1-alcohols.

Standard Products. Sodium *p*-hydroxymercuribenzoate, 5,5′-dithiobis(2-nitrobenzoic acid), *trans*-2-pentenal (95%) *trans*-2-methyl-2-butenal (98%), thioacetic acid (96%), and lithium aluminum hydride were purchased from Sigma-Aldrich Chemicals (L'Isle d'Abeau, France). 4-Methoxy-2-methylbutane-2-thiol was purchased from Oxford Chemicals (Grasse, France). 3-Sulfanylhexan-1-ol (**I**) (>95%) was obtained from Lancaster (Bischheim, France). 3-Sulfanylheptan-1-ol (**III**) (>95%) was kindly donated by Firmenich SA (Genève, Switzerland).

Purification of Volatile Thiols. The volatile thiols were specifically extracted by reversible combination of the thiols with sodium *p*-hydroxymercuribenzoate (*p*-HMB) as described by Tominaga et al. (*11*). A 500 mL sample of wine, containing 2.5 nmol of 4-methoxy-2-methylbutane-2-thiol as internal standard, was extracted with two successive additions of 100 mL of dichloromethane in a 1 L flask with magnetic stirring for 10 min each time. The combined organic phases were centrifuged at 4000*g* for 15 min to break the emulsion and separated in a separating funnel. The organic phase obtained was then extracted with two successive additions of 20 mL of *p*-HMB solution [1 mM in Trizma base [2-amino-2-(hydroxymethyl)-1,3-propanediol] at 0.2 M] for 10 min each time. The two aqueous phases were combined and then loaded on a strongly basic anion-exchange column (1.5 × 3 cm) (Dowex 1-1 × 2-100). The column was then washed with 50 mL of sodium acetate buffer (0.1 M, pH 7). The volatile thiols were released from the thiol−*p*-HMB complex fixed on the column by percolating with 60 mL of cysteine solution (10 g/L) adjusted to pH 7 with NaOH (10 M). The eluate containing the volatile thiols was collected in a 100 mL flask, and 0.5 mL of ethyl acetate was added. The eluate was extracted twice with dichloromethane (4 and 3 mL, respectively) for 10 min each time, under magnetic stirring. The two organic phases were combined, dried over anhydrous sodium sulfate, and then concentrated under nitrogen flow in a 10 mL graduated tube to approximately 200 μL. The concentrate was then transferred to a 1 mL vial and concentrated to 25 μL.

Gas Chromatography Coupled Simultaneously with Olfactometry (GC-O) and Flame Photometric Detection (GC-FPD). Olfactory analyses were carried out using a Hewlett-Packard 5890 gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a flame photometric detector (from Agilent Technologies) and a sniffing-port (ODO-1 from SGE, Ringbow, Australia). Three microliters of each concentrated extract was injected by a splitless injector (230 °C; purge time = 1 min, purge flow = 50 mL/min) at oven temperature (45 °C) onto a type BP20 capillary column [SGE, 50 m, 0.22 mm internal diameter (i.d.), 0.25 μm film thickness] or a type BPX5 fused silica capillary column (SGE, 50 m, 0.25 mm i.d., 1.0 μm film thickness). For all analyses, the temperature program was as follows: 45 °C for 10 min, raised at 3 °C/min to 230 °C (BP20 column) and 250 °C (BPX5 column), followed by a 20 min isotherm. The flame photometric detector was held at 230 °C. The carrier gas was hydrogen with a column-head pressure of 22 psi and a flow rate of 1 mL/min. Each GC-O analysis was performed by three experienced judges.

Identification and Quantification by Gas Chromatography−Mass Spectrometry (GC-MS). A 3 μL sample of each concentrated extract was analyzed on a 6890N gas chromatograph (Agilent Technologies) under the conditions described above. The detector was a mass spectrometer (MS 5973, Agilent Technologies), functioning in EI mode (70 eV), and was connected to the GC with a transfer line heated to 250 °C. Mass spectra were taken over the *m*/*z* 40−300 range. Volatile thiols were identified on the basis of their linear retention indexes and a comparison of MS fragmentation patterns obtained in SCAN mode on two capillaries (BP20 and BPX5) with those of reference compounds previously reported.

The three new volatile thiols (**II**−**IV**, **Figure 1**), as well as 3-sulfanylhexan-1-ol (**I**), were quantified in SIM mode, selecting the following ions: *m*/*z* 134, 116, and 100 for **I**, *m*/*z* 120, 102, and 86 for **II** and **IV**, and *m*/*z* 148, 114, and 96 for **III**. They were quantified using *m*/*z* 134 for **I**, *m*/*z* 120 for **II** and **IV**, and *m*/*z* 148 for **III**. The internal standard, 4-methoxy-2-methylbutane-2-thiol, was detected with the *m*/*z* 134 and 100 ions. Calibration curves were determined using a dry white wine supplemented with dilute alcohol solutions containing **I**, **II**, **III**, and **IV**, at final concentrations ranging from 0 to 9000 ng/L for **I**, from 0 to 400 ng/L for **II**, from 0 to 200 ng/L for **III**, and from 0 to 200 ng/L for **IV**. The concentrations of volatile thiol standards were previously determined according to Ellman's method (*12*) using 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). For each concentration, the volatile thiols were extracted from the wine according to the process described above. Repeatability of the measuring system was assessed over a series of five extractions. The quantification limit was calculated as the minimum concentration that generated a peak signal 10 times higher than the signal from background noise.

Synthesis. 3-Sulfanylpentan-1-ol (**II**) and 2-methyl-3-sulfanylbutan-1-ol (**IV**) were synthesized in two steps by the Michael-type addition of thioacetic acid to corresponding α,β-unsaturated aldehydes, followed by reducing the carbonyl group to alcohol (**Figure 2**). Our approach was similar to combinatorial synthesis previously described by Vermeulen et al. (*13*).

Experimental Equipment. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC-300 FT (^{1}H, 300 MHz; ^{13}C, 75 MHz), using TMS as an internal standard. Chemical shifts (δ) and coupling constants (J) are expressed in parts per million and hertz, respectively. IR spectra were recorded with a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. Thin-layer chromatography (TLC) was performed on SDS TLC plates: thickness = 0.25 mm, particle size = 15 μm, pore size = 60 Å. Merck silica gel 60 (70−230 mesh and 0.063−0.200 mm) was used for flash chromatography. Spots were revealed with UV as well as with $KMnO_4$ (0.05% in water). Diethyl ether was dried by refluxing a solution containing sodium wires and benzophenone under nitrogen and distilled immediately before use. The thioacetic acid was distilled immediately before use. All moisture-sensitive reactions were carried out in an argon atmosphere in oven- or flame-dried glassware.

3-Sulfanylpentan-1-ol. *Thioacetic Acid S-(1-Ethyl-3-oxopropyl) Ester (**1**).* *trans*-2-Pentenal (3.35 g, 3.9 mL, 40 mmol) was added dropwise to ice-cooled freshly distilled thioacetic acid (4.27 g, 4 mL, 56 mmol) under an argon atmosphere. The ice bath was then removed and the reaction mixture stirred at room temperature for 16 h. Thioacetic acid excess was removed under reduced pressure. The crude product was purified by silica gel chromatography (petroleum ether/diethyl ether 70:30, $R_f = 0.44$), resulting in a colorless oil (4.9 g, 77%).

TLC (petroleum ether/diethyl ether 80:20), $R_f = 0.28$; MS (EI, 70 eV), *m*/*z* (%) 160 (M^+, 4), 132 (2), 117 (35), 100 (9), 89 (13), 85 (10), 75 (9), 56 (15), 55 (19), 43 (100); ^{1}H NMR ($CDCl_3$) δ 0.93 (dd, J = 7.9 Hz, J = 6.8 Hz, 3H, $-C\mathbf{H_3}$), 1.62 (m, 1H, 4-H_a, $-CH-C\mathbf{H_2}-CH_3$), 1.69 (m, 1H, 4-H_b, $CH-C\mathbf{H_2}-CH_3$), 2.28 (s, 3H, $COC\mathbf{H_3}$), 2.69 (m, 2H, $-CH-C\mathbf{H_2}-CHO$), 3.84 (tt, $J_{2-3} = J_{3-4} = 6.8$ Hz, 1H, $-CH_2-C\mathbf{H}-CH_2-$), 9.64 (dd, 1H, J = 3.0 Hz, J = 1.9 Hz, $C\mathbf{H}O$); ^{13}C NMR ($CDCl_3$) δ 11.3 ($-\mathbf{CH_3}$), 27.5 ($-CH-\mathbf{CH_2}-CH_3$), 30.6 ($CO\mathbf{CH_3}$), 39.9 ($-CH_2-\mathbf{CH}-CH_2-$), 48.3 ($-CH-\mathbf{CH_2}-CHO$), 195.1 ($CH_3\mathbf{C}O$), 200.0 ($-\mathbf{C}HO$); IR, ν 952, 1114, 1354, 1425, 1690, 1724, 2922, 2966 cm^{-1}.

*3-Sulfanylpentan-1-ol (**II**).* The purified compound **1** (1.83 g, 11.4 mmol) in 10 mL of dry diethyl ether was slowly added to an ice-cold suspension of lithium aluminum hydride (0.87 g, 22.8 mmol) in 30 mL of dry diethyl ether under an argon atmosphere. The reaction mixture was allowed to warm to room temperature for 3 h and then cooled again (ice bath). The suspension was carefully treated with cold saturated aqueous ammonium chloride solution (100 mL), and the pH was adjusted to pH 2−3 with 1 M hydrochloric acid. The organic phase was separated, and the aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic phases were washed with saturated sodium chloride solution (2 × 50 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give the title compound as a colorless oil (1.15 g, 90%). The purity of the compound obtained, determined by GC-MS and ^{1}H NMR, was >95%. This compound was thus used without further purification.

TLC (petroleum ether/diethyl ether 60:40), $R_f = 0.35$; MS (EI, 70 eV), *m*/*z* (%) 120 (M^+, 34), 102 (6), 86 (100), 75 (25), 73 (47), 69 (84), 61 (53), 57 (96), 47 (24), 45 (25); ^{1}H NMR ($CDCl_3$) δ 0.97 (t, J = 7.3 Hz, 3H, $-C\mathbf{H_3}$), 1.36 (d, J = 7.5 Hz, 1H, $S\mathbf{H}$), 1.50 (m, 1H, 4-H_a, $CH-C\mathbf{H_2}-CH_3$), 1.65 (m, 1H, 4-H_b, $-CH-C\mathbf{H_2}-CH_3$), 1.70 (m, 1H, 2-H_a, $-CH-C\mathbf{H_2}-$), 1.90 (m, 1H, 2-H_b, $-CH-C\mathbf{H_2}-CH_2$-OH), 2.83 (m, 1H, $-CH_2-C\mathbf{H}-CH_2-$), 3.02 (br s, 1H, $O\mathbf{H}$), 3.74 (m, 2H, $-C\mathbf{H_2}OH$); ^{13}C NMR ($CDCl_3$) δ 11.4 ($-\mathbf{C}H_3$), 32.1 ($-CH-\mathbf{CH_2}-CH_3$), 39.5 ($-CH_2-\mathbf{CH}-CH_2-$), 40.9 ($-CH-\mathbf{CH_2}-CH_2OH$), 60.3 ($-\mathbf{C}H_2OH$); IR, ν 1053, 1461, 2590, 3340 cm^{-1}.

2-Methyl-3-sulfanylbutan-1-ol. *(l, u)-Thioacetic Acid S-(1,2-Dimethyl-3-oxopropyl) Ester) (**2**).* *trans*-2-Methyl-2-butenal (3.35 g, 3.9 mL, 40 mmol) was treated with thioacetic acid under the conditions described for thioacetic acid *S*-(1-ethyl-3-oxopropyl) ester to produce a colorless oil (4.3 g, 67%). The two diastereoisomers [*like* (RR+SS) and *unlike* (SR+RS)] cannot be separated by standard silica gel chromatography, but have different ^{1}H and ^{13}C NMR spectra. Chemical shift assignment and the ratio of *l*:*u* diastereoisomer pairs was determined by NMR (^{1}H, ^{13}C, confirmed by 2D NMR analysis) and compared with literature data (*14*). The *l*:*u* ratio was 2:1.

TLC (petroleum ether/diethyl ether 70:30), $R_f = 0.51$; MS (EI, 70 eV), *m*/*z* (%) 160 (M^+, 1), 132 (8), 117 (46), 100 (9), 89 (29), 82 (10), 77 (9), 56 (44), 55 (27), 43 (100); IR, ν 955, 1114, 1354, 1380, 1452, 1691, 1727, 2920, 2972 cm^{-1}.

(l)-Thioacetic Acid S-(1,2-Dimethyl-3-oxopropyl) Ester. ^{1}H NMR ($CDCl_3$) δ 1.11 [d, J = 7.17 Hz, 3H, $-CH(C\mathbf{H_3})-CH(CH_3)-S-$], 1.34 [d, J = 7.53 Hz, 3H, $-CH(CH_3)-CH(C\mathbf{H_3})-S-$], 2.29 (s, 3H, $COC\mathbf{H_3}$), 2.60 [m, 1H, $-C\mathbf{H}(CH_3)-CH(CH_3)-S-$], 4.00 [qd, J = 7.53 Hz, J = 4.89 Hz, 1H, $-CH(CH_3)-C\mathbf{H}(CH_3)-S-$], 9.60 (s, 1H, $C\mathbf{H}O$); ^{13}C NMR ($CDCl_3$) δ 10.4 [$-CH(\mathbf{CH_3})-CH(CH_3)-S-$], 19.2 [$-CH(CH_3)-CH(\mathbf{CH_3})-S-$], 30.7 ($CO\mathbf{CH_3}$), 39.2 [$-CH(CH_3)-\mathbf{CH}(CH_3)-S-$], 51.0 [$-\mathbf{CH}(CH_3)-CH(CH_3)-S-$], 194.7 ($CH_3\mathbf{C}O$), 202.6 ($-\mathbf{C}HO$).

(u)-Thioacetic Acid S-(1,2-Dimethyl-3-oxopropyl) Ester. ^{1}H NMR ($CDCl_3$) δ 1.12 [d, J = 7.17 Hz, 3H, $-CH(C\mathbf{H_3})-CH(CH_3)-S-$], 1.25 [d, J = 7.53 Hz, 3H, $-CH(CH_3)-CH(C\mathbf{H_3})-S-$], 2.30 (s, 3H, $COC\mathbf{H_3}$), 2.60 [m, 1H, $-C\mathbf{H}(CH_3)-CH(CH_3)-S-$], 3.96 [qd, J = 7.53 Hz, J = 4.89 Hz, 1H, $-CH(CH_3)-C\mathbf{H}(CH_3)-S-$], 9.59 (s, 1H, $C\mathbf{H}O$); ^{13}C NMR ($CDCl_3$) δ 10.4 [$-CH(\mathbf{CH_3})-CH(CH_3)-S-$], 17.8 [$-CH(CH_3)-CH(\mathbf{CH_3})-S-$], 30.6 ($CO\mathbf{CH_3}$), 38.6 [$-CH(CH_3)-\mathbf{CH}(CH_3)-S-$], 50.9 [$-\mathbf{CH}(CH_3)-CH(CH_3)-S-$], 195.0 ($CH_3\mathbf{C}O$), 202.3 ($-\mathbf{C}HO$).

*(l,u)-2-Methyl-3-sulfanylbutan-1-ol (**IV**).* The purified compound (*l*,*u*)-**2** enriched in *like* enantiomers (*l*:*u* = 2:1) (1.2 g, 7.5 mmol) was treated with lithium aluminum hydride (0.57 g, 15 mmol) under the same conditions as described for **II**. The silica gel chromatography (petroleum ether/diethyl ether 70:30) of the crude product gave the title compound as a colorless oil (0.76 g, 85%) with purity >95% (GC-MS and ^{1}H NMR). The two diastereoisomers [*like* (*RR*+*SS*) and *unlike* (*SR*+*RS*)] cannot be separated by standard silica gel chromatography, but do have different ^{1}H and ^{13}C NMR spectra. The chemical shift assignment as well as the *l*:*u* ratio of diastereoisomer pairs was determined by NMR (^{1}H, ^{13}C, confirmed by 2D NMR analysis) and compared with literature data (*14*). The *l*:*u* ratio was the same as in the starting material, 2:1.

TLC (petroleum ether/diethyl ether 60/40), $R_f = 0.40$; MS (EI, 70 eV), *m*/*z* (%) 120 (M^+, 34), 102 (6), 89 (22), 86 (100), 71 (76), 69 (39), 61 (76), 60 (81), 55 (55), 45 (46); IR, 1036, 1379, 1449, 2559, 2876, 2926, 2964, 3358 cm^{-1}.

(l)-2-Methyl-3-sulfanylbutan-1-ol. ^{1}H NMR (CDCl3) δ 0.86 [d, J = 6.8 Hz, 3H, $-CH(C\mathbf{H_3})-CH(CH_3)SH$], 1.25 (d, J = 7.9 Hz, 1H, $S\mathbf{H}$), 1.32 [d, J = 7.2 Hz, 3H, $-CH(CH_3)-CH(C\mathbf{H_3})SH$], 1.80 [m, 1H, $-C\mathbf{H}(CH_3)-CH(CH_3)SH$], 2.63 (br s, $O\mathbf{H}$), 3.23 [apparent quintet d, $J_{CH-CH3} = 7.9$ Hz, $J_{CH-SH} = 7.2$ Hz, $J_{CH-CH} = 3.8$ Hz, 1H, $-CH(CH_3)-C\mathbf{H}(CH_3)SH$], 3.48 (dd, $^2J_{CHH} = 10.6$ Hz, $^3J_{CH2-CH} = 6.0$ Hz, 1H, 1H_a, $-C\mathbf{H_2}OH$), 3.57 (dd, $^2J_{CHH} = 10.6$ Hz, $^3J_{CH2-CH} = 7.9$ Hz, 1H, 1H_b, $-C\mathbf{H_2}OH$); ^{13}C NMR ($CDCl_3$) δ 10.9 [$-CH(\mathbf{CH_3})-CH(CH_3)$-SH], 23.6 [$-CH(CH_3)-CH(\mathbf{CH_3})SH$], 36.9 [$-CH(CH_3)-\mathbf{CH}(CH_3)$-SH], 41.6 [$-\mathbf{CH}(CH_3)-CH(CH_3)SH$], 65.8 ($-\mathbf{CH_2}OH$).

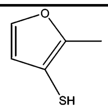
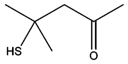
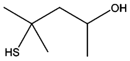
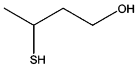
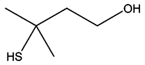
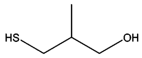
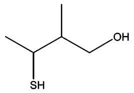
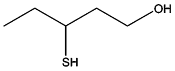
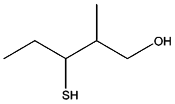
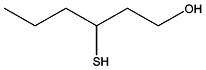
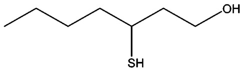
(u)-2-Methyl-3-sulfanylbutan-1-ol. ^{1}H NMR ($CDCl_3$) δ 0.96 [d, J = 7.1 Hz, 3H, $-CH(C\mathbf{H_3})-CH(CH_3)SH$], 1.32 [d, J = 7.1 Hz, 3H, $-CH(CH_3)-CH(C\mathbf{H_3})SH$], 1.49 (d, J = 7.1 Hz, 1H, $S\mathbf{H}$), 1.75 [m, 1H, $-C\mathbf{H}(CH_3)-CH(CH_3)SH$], 2.63 (br s, $O\mathbf{H}$), 3.10 [apparent sextuplets, $J_{CH-CH3} \approx J_{CH-SH} \approx J_{CH-CH} \approx 7.1$ Hz, 1H, $-CH(CH_3)-C\mathbf{H}(CH_3)SH$], 3.58 (d, J = 10.5 Hz, 2H, $-C\mathbf{H_2}OH$); ^{13}C NMR ($CDCl_3$) δ 13.5 [$-CH(\mathbf{CH_3})-CH(CH_3)SH$], 21.7 [$-CH(CH_3)-CH(\mathbf{CH_3})SH$], 37.3 [$-CH(CH_3)-\mathbf{CH}(CH_3)SH$], 43.1 [$-\mathbf{CH}(CH_3)-CH(CH_3)SH$], 65.6 ($-\mathbf{CH_2}OH$).

Microvinification. *Must Preparation.* Eight kilograms of Semillon and Sauvignon blanc grapes were picked in the same vineyard (Château d’Yquem, Sauternes, France, 2005) at four stages in *B. cinerea* development: *healthy* (grapes not infected by *B. cinerea*), *pourri plein* (grapes entirely botrytized but not desiccated, picked 2 weeks after healthy grapes), *pourri rôti* (grapes botrytized and desiccated, picked 2 weeks after full-rotten grapes), and *late pourri rôti* (shriveled grapes left for a further 10 days before picking). Grapes were crushed in a pneumatic press under a CO_2 atmosphere and left to settle with 50 mg/L SO_2 for 24 h at 12 °C. The mean grape volume was determined by measuring the must volume from 1000 grapes. Sugar concentrations varied from 217 to 400 g/L, depending on the stage of *B. cinerea* development. The assimilable nitrogen content was estimated by using the Sörensen method (*15*) and corrected to 190 mg/L in all must samples by adding Thiazote (Laffort Œnologie, Bordeaux, France) before alcoholic fermentation (*16*).

Fermentation. Must was inoculated with *Saccharomyces cerevisiae* (strain Zymaflore ST-Laffort Œnologie, Bordeaux, France) precultured for 24 h (200 mg/L) (*17*) and fermented in 750 mL sterile bottles (650 mL must per bottle). Yeast strain establishment was assessed by comparing the initial industrial yeast karyotype with the biomass, using pulsed field electrophoresis (*18*). Fermentation took place in a temperature-controlled environment at 23 °C and was monitored by CO_2 release (*16*, *17*). Every experiment was carried out in triplicate. When the required alcoholic concentration, that is, 13% vol, was reached, fermentation was stopped by adding sulfur dioxide solution (300 mg/L).

Sensory Tests. *Determining Odor Thresholds.* Perception thresholds of synthesized **II** and **III** were assessed by directional triangular tests of five increasing concentrations in ultrapure water (Milli-Q, Millipore,

Table 1. Odorous Zones (OZ) Corresponding to Volatile Thiols Detected in Specific Extracts of Botrytized Wines

	LRI[a]				
OZ	BP20	BPX5	Descriptors	Compound	Formula
1	1341	879	meaty	2-methylfuran-3-thiol[b]	
2	1389	968	box tree	4-methyl-4-sulfanylpentan-2-one[b,c]	
3	1568	1055	grapefruit	4-methyl-4-sulfanylpentan-2-ol[b,c]	
4	1670	957	onion, leek	3-sulfanylbutan-1-ol[b,c]	
5	1677	1009	onion, leek	3-methyl-3-sulfanylbutan-1-ol[b,c]	
6	1708	983	onion, leek	2-methyl-3-sulfanylpropan-1-ol[b,c]	
7	1745	1060	onion, leek	2-methyl-3-sulfanylbutan-1-ol[b,c,d]	
8	1772	1076	grapefruit	3-sulfanylpentan-1-ol[b,c]	
9	1833	1158	onion, leek	2-methyl-3-sulfanylpentan-1-ol[b]	
10	1857	1174	grapefruit	3-sulfanylhexan-1-ol[b,c]	
11	1956	1286	grapefruit	3-sulfanylheptan-1-ol[b,c]	

[a] LRI, linear retention index calculated on both BP20 and BPX5 capillarie. [b] Coincidence of LRI and odors on two capillary columns (BP20 and BPX5). [c] Mass spectrum in agreement with spectra of synthesized substances on two columns (BP20 and BPX5). [d] The two pairs of diastereoisomers (*like* and *unlike*) have the same LRI.

Bedford, MA) or in model dilute alcohol solution (5 g/L tartaric acid, 12%v/v ethanol, pH 3.5). The solutions were presented in glasses corresponding to AFNOR (Association Française des Normes) standards. The odor perception threshold was the minimum concentration below which at least 50% of 45 tasters statistically failed to distinguish the sample from the control (*19*).

Study of Additive Effects among Volatile Thiols. To assess additive effects among the volatile thiols identified, triangular tests were carried out in model solution, as follows. A control solution containing 5000 ng/L of **I** was compared with three test solutions containing 5000 ng/L of **I**, together with 200 ng/L of **II** (test A) or 50 ng/L of **III** (test B), or both at the same time (test C). The solutions were presented in glasses corresponding to AFNOR standards and coded with three-digit numbers (*20*). The panel consisted of 49 tasters, and the significance of the results was determined according to the binomial law.

RESULTS AND DISCUSSION

Identification of Three New 3-Sulfanylalcohols in Botrytized Wines. Volatile thiols were extracted selectively from a commercial botrytized wine, using *p*-HMB. The extract was analyzed using GC-O, GC-FPD, and GC-MS. As shown in **Table 1**, 11 odoriferous zones (OZ), corresponding to sulfur-containing compounds, were detected. Seven of them (OZ 1, 2, 3, 4, 5, 6, and 10) had already been identified in wines, including five (OZ 1, 2, 3, 5, and 10) previously reported in botrytized wines (*7*−*10*). OZ 1, smelling of cooked meat, corresponds to 2-methyl-3-furanthiol (*21*). OZ 2, with a strong box-tree odor, corresponds to 4-methyl-4-sulfanylpentan-2-one (*22*). OZ 3, reminiscent of citrus and grapefruit, corresponds to 4-methyl-4-sulfanylpentan-2-ol (*23*). OZ 4 and 5, reminiscent of cooked leeks, correspond to 3-sulfanylbutan-1-ol (*24*) and 3-methyl-3-sulfanylbutan-1-ol (*23*), respectively. OZ 6, smelling of broth, corresponds to 2-methyl-3-sulfanylpropan-1-ol (*25*). Finally, OZ 10, with a grapefruit aroma, corresponds to 3-sulfanylhexan-1-ol (**I**) (*23*). However, under our conditions we did not detect the two sulfanylaldehydes (3-methyl-3-sulfanylbutanal and 3-sulfanylheptanal) recently reported by Bailly et al. (*10*).

Among the four unknown odoriferous zones (OZ 7, 8, 9, and 11), two were reminiscent of citrus zest and grapefruit (OZ 8 and 11), whereas the other two had onion and sweat odors (OZ 5 and 7). GC-MS on two capillary columns with different polarities was used to obtain three mass spectra corresponding to OZ 7, 8, and 11. The three fragmentation patterns strongly indicated the presence of a thiol and a hydroxyl group, due to the loss of *m*/*z* 34 and *m*/*z* 18, respectively (**Figure 3**). Therefore, the volatile compounds associated with OZ 7, 8, and 11 were assumed to be sulfanyl alcohols. Moreover, the compound corresponding to OZ 7 was characterized by a loss of the *m*/*z* 15 ion, due to a methyl substitution. On the basis of their mass spectra and linear retention indices compared with those of synthetic substances (*13*, *26*), these three compounds were identified as 2-methyl-3-sulfanylbutan-1-ol (OZ 7, **IV**), 3-sulfanylpentan-1-ol (OZ 8, **II**), and 3-sulfanylheptan-1-ol (OZ 11, **III**). Like 3-sulfanylhexan-1-ol (**I**), 4-methyl-4-sulfanylpentan-2-one, and 4-methyl-4-sulfanylpentan-2-ol, these three new volatile thiols are strong odorants, with the O and S functions in the 1,3-position. To our knowledge, none of these three

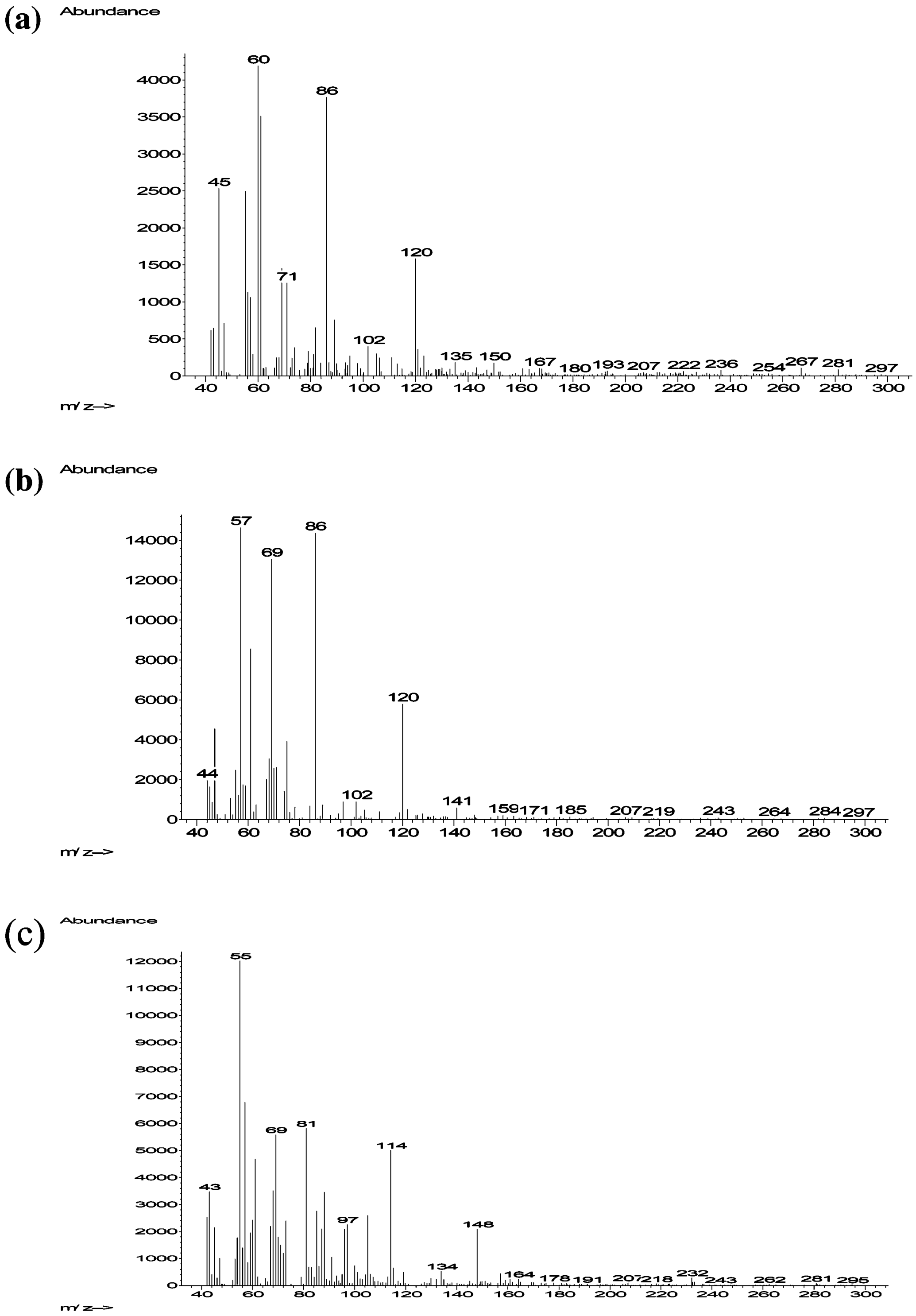


Figure 3. Mass spectra of 3-sulfanyl-2-methylbutan-1-ol (OZ 4, **IV**) (**a**), 3-sulfanylpentan-1-ol (OZ 5, **II**) (**b**), and 3-sulfanylheptan-1-ol (**c**) (OZ 8, **III**) (isolated from wine).

volatile thiols had ever been described in wine before. Compounds **II** and **IV** were previously reported as odorous compounds in human axillary sweat (*27, 28*), whereas **III** was recently identified in *Ruta chalepensis* (*29*). In addition, a position isomer of **IV**, 3-methyl-2-sulfanylbutan-1-ol, was recently reported in lager beer aroma (*30*).

Concerning the last odorous zone, reminiscent of raw onion and sweat (OZ 9), no signal (peak) or spectrum was obtained by GC-FPD or GC-MS. Nevertheless, retention indices on two capillary columns and olfactory descriptors correlated well with those of 2-methyl-3-sulfanylpentan-1-ol (*13*), already identified in raw onion (*31*). A comparison of retention indices and sensory descriptors of this odorous zone with those of synthesized 2-methyl-3-sulfanylpentan-1-ol confirmed the presence of this volatile thiol.

According to our previous olfactometric study (*7*), using GC-AEDA with three experienced judges, **II**, **III**, and **IV** were among the most potent odorants in young botrytized wines. Therefore, they were thought to contribute to botrytized wine aroma and had to be examined. Compound **I**, already known to make a significant contribution to botrytized wine aroma (*7*–*10*), was also studied.

Table 2. Calibration Line Parameters

compd	repeatability ($n = 5$) (%)	calibration line	R^2	linear range (ng/L)	quantification limit (ng/L)	validation range (ng/L)
I	3	$y = 68.737x$	0.9939	0–9000	3.1	3.1–9000
II	6	$y = 113.73x$	0.9996	0–400	2.8	2.8–400
III	6	$y = 145.69x$	0.9941	0–200	3.7	3.7–200
IV	6	$y = 203.88x$	0.9982	0–200	4.8	4.8–200

Standard Curves, Sensitivity, and Repeatability. Table 2 shows the calibration lines, R^2, repeatability, and quantification limits obtained for each of the four compounds. The recovery rate for the volatile thiols was calculated according to the method described by Tominaga et al. (*32*) and was >70%, irrespective of the quantity added.

Impact of *B. cinerea* Development on Grapes on Concentrations of I−IV in Wine. *B. cinerea* development on grapes is well-known to deeply modify grape composition and, thus, the volatile compounds present in the corresponding wines. Semillon and Sauvignon blanc grapes were picked on the same plots at different stages in botrytization (*healthy*, *pourri plein*, *pourri rôti*, and *late pourri rôti*). They were crushed and microfermented. Specific extraction of volatile thiols was carried out on must and wine from each grape sample to assay the three new compounds (**II**−**IV**), as well as 3-sulfanylhexan-1-ol (**I**). As shown in **Table 3**, wines made from *healthy* grapes contained only trace levels of **II**, **III**, and **IV** and a few hundred nanograms per liter of **I**. On the contrary, the three new volatile thiols were found in every wine made from both varieties of rotten grapes, whereas concentrations of **I** were considerably higher. Furthermore, the levels of the four volatile thiols increased as *B. cinerea* developed on the grapes. Considering the thiol to grape volume ratio between the *healthy* and *pourri plein* stages, the increase in the four volatile thiols could not be explained simply by the decrease in grape volume. Then, between the *pourri plein* and the *pourri rôti* stages, grapes were severely desiccated and decreased significantly in volume. The increase in volatile thiols in the wine was, therefore, mainly explained by desiccation. Finally, due to rainy weather between the *pourri rôti* and the *late pourri rôti* stages, the grapes absorbed water and increased slightly in volume. This led to a dilution of the grape content that partially masked the increase in volatile thiol levels.

These results demonstrated the predominant role of the *B. cinerea* metabolism on the sulfanyl alcohol contents in wine. Specific extraction of volatile thiols from botrytized must showed that compounds **II**, **III**, and **IV** were absent before alcoholic fermentation, like **I** (data not shown). Therefore, this demonstrated that these three new volatile thiols were formed during fermentation. Previous studies showed that **I**, present in a cysteinylated conjugate precursor form in must, was released by yeast during alcoholic fermentation (*33*). As compounds **II**, **III**, and **IV** have chemical structures similar to that of **I**, they are likely to be formed by the same pathway. Furthermore, **II** and **IV** have been recently shown to be released through the cleavage of cysteine precursors by a C−S lyase produced by axillary bacteria (*27*, *34*, *35*). However, Schneider et al. (*36*) described an alternative pathway to explain the genesis of 3-sulfanylhexan-1-ol and 4-methyl-4-sulfanylpentan-2-one in wine, starting from the corresponding conjugated carbonyl compounds. Further research is now needed to understand the origin of these new volatile compounds in wines made from *Botrytis*-infected grapes. Moreover, the role of the *B. cinerea* metabolism in their formation requires further study.

Sensory Impact of Compounds II and III on Botrytized Wine Aroma. The olfactory impact of these new sulfanyl alcohols on the overall aroma of botrytized wines was examined by determining their odor thresholds, in water and model dilute alcohol solution, and their mean levels in commercial wines. **Table 4** lists olfactory descriptions of these three new compounds and their perception thresholds. The aroma of compound **II** is somewhat reminiscent of grapefruit, but its perception threshold is much higher than that of compound **I** (620 ng/L in water, 950 ng/L in model dilute alcohol solution). Compound **III** has a citrus odor and a relatively low odor threshold (10 ng/L in water, 35 ng/L in model dilute alcohol solution). No sensory tests were possible for compound **IV**. Due to the presence of two asymmetric centers, significant olfactory differences have already been described between its *like* and *unlike* pairs: the odor perception threshold of the *like* pair is 100-fold higher than that of the *unlike* pair (*14*). The same drastic sensory differences were found for 3-mercapto-2-methylpentan-1-ol (*37*), illustrating the predominant role of chirality in the olfactory perception of molecules with two asymmetric centers. As no information concerning the configuration of **IV** in botrytized wines was obtained under our GC conditions, no sensory tests could be performed.

In a second stage, seven commercial botrytized wines (vintages from 2001−2003) from the Bordeaux region were analyzed to assay the mean levels of these new sulfanyl alcohols. As shown in **Table 5**, the three new volatile thiols were consistently found in botrytized wines, and the mean levels obtained for **II**, **III**, and **IV** were 209, 51, and 103 ng/L,

Table 3. Quantitative Assays (Nanograms per Liter) of the Three New Volatile Thiols (**II**–**IV**) and 3-Sulfanylhexan-1-ol (**I**), at Different Stages in Botrytization [Comparison with Decrease in Mean Grape Volume (Milliliters per Grape)]

variety	*Botrytis* stage	mean grape volume[a]	variation of mean grape volume (%)	**I**	**II**	**III**	**IV**
Semillon	*healthy*[b]	0.85	100	195[c] ± 58[d]	tr[e]	tr	tr
	pourri plein	0.68	80	2326 ± 419	93 ± 14	34 ± 5	67 ± 9
	pourri rôti	0.37	44	3678 ± 1765	124 ± 54	50 ± 26	118 ± 61
	late pourri rôti	0.38	45	6334 ± 1267	291 ± 128	118 ± 13	134 ± 55
Sauvignon	*healthy*	0.78	100	161 ± 27	tr	tr	tr
	pourri plein	0.52	67	3003 ± 300	141 ± 8	95 ± 39	50 ± 5
	pourri rôti	0.21	27	9648 ± 1544	348 ± 42	263 ± 92	185 ± 20
	late pourri rôti	0.29	37	9319 ± 2050	375 ± 71	258 ± 44	185 ± 18

[a] Based on the volume obtained from crushing 1000 grapes (mL/grape). [b] *healthy*, grapes not infected by *B. cinerea*; *pourri plein*, grapes entirely botrytized but not desiccated, picked 2 weeks after healthy grapes; *pourri rôti*, grapes botrytized and desiccated, picked 2 weeks after *pourri plein* grapes; *late pourri rôti*, shriveled grapes left a further 10 days before picking. [c] Mean value ($n = 3$). [d] Standard deviation s ($n = 3$). [e] Traces.

Table 4. Olfactory Descriptions and Perception Thresholds of the New Volatile Thiols (**I**–**IV**) Identified in Botrytized Wines (Nanograms per Liter) in Water and Model Solution (5 g/L Tartaric Acid, 12% v/v Ethanol, pH 3.5)

		olfactory perception threshold	
ref compd	olfactory description	water	model solution
I	grapefruit	17[a]	60[a]
II	citrus, sulfur	620	950
III	grapefruit	10	35
IV	raw onion, sweat	nd[b]	nd

[a] Tominaga et al. (*23*). [b] Not determined.

Table 5. Quantitative Assays (Nanograms per Liter) and Odor Activity Values of Some Volatile Thiols (**I**–**IV**) in Young Botrytized Wines from Various Appellations of the Bordeaux Region

	appellation	vintage	**I**	**II**	**III**	**IV**
Doisy Daëne	Sauternes	2001	7033	299	63	149
Tour Blanche	Sauternes	2001	5606	217	59	87
Cantegrille	Barsac	2001	5034	223	44	120
Doisy Daëne	Sauternes	2002	4765	203	48	83
Dauphiné Rondillon	Loupiac	2002	4749	235	72	105
Doisy Daëne	Sauternes	2003	5386	199	44	96
Dauphiné Rondillon	Loupiac	2003	2450	91	26	84
mean level			5003 (83)[a]	209 (0.2)	51 (1.5)	103 (nd[b])

[a] (number): odor activity value. [b] Not determined.

Table 6. Triangular Tests Involving 49 Tasters

test	test solution	control solution	correct answers/ total answers	*p*
A	5000 ng/L **I** + 200 ng/L **II**	5000 ng/L **I**	17/49	NS[a]
B	5000 ng/L **I** + 50 ng/L **III**	5000 ng/L **I**	13/49	NS
C	5000 ng/L **I** + 200 ng/L **II** + 50 ng/L **III**	5000 ng/L **I**	29/49	0.001

[a] Nonsignificant.

respectively. Compound **I** was also quantified, and its mean level was 5003 ng/L. This result agreed with the high concentrations of **I** found in botrytized wines by Tominaga et al. (*8*, *9*).

The mean values of compounds **II** and **III** were compared with their olfactory thresholds in model dilute alcohol solution to determine their odor activity values (OAVs). These values corresponded to the ratio between the mean concentration of each thiol in botrytized wine and its perception threshold in model dilute alcohol solution (*38*). In botrytized wines, compounds **II** and **III** were shown to have low OAVs (0.2 and 1.5, respectively). The OAV of **I** was calculated according to the perception threshold determined as 60 ng/L by Tominaga et al. (*23*). As compounds **I**, **II**, and **III** are all reminiscent of citrus, possible additive effects were studied by triangular tests, as shown in **Table 6**. A control model dilute alcohol solution containing 5000 ng/L **I** was compared with three test solutions containing 5000 ng/L **I**, together with 200 ng/L **II** (test A) or 50 ng/L **III** (test B), or both (test C). These concentrations corresponded to the mean levels of each thiol in botrytized wines. There was no significant difference when only **II** (test A, 17/49) or **III** (test B, 13/49) was added. As compound **II** was present at concentrations far below its threshold, its impact was thought to be insufficient for tasters to distinguish it from the solution containing only compound **I**. The content of **III** was above its perception threshold, but its aroma was probably masked by that of **I**. On the contrary, there was a significant difference with a risk of 0.1% between the control solution and the solution containing both **II** and **III** (test C, 29/49). According to Guadagni et al. (*39*), as **I**, **II,** and **III** belong to the same class of compounds and have the same odors, these results demonstrated additive effects. This kind of molecular interaction among volatile thiols has also recently been described between 3-methyl-3-sulfanylbutanal and 2-methylfuran-3-thiol, giving a strong bacon−petroleum odor (*10*).

Therefore, this demonstrated that compounds **II** and **III** had a considerable impact on the overall aroma in the presence of other volatile thiols, such as **I**, although they had low individual OAVs. As previously described (*39*−*40*), the aromatic profile of complex matrices, such as wine, cannot solely be explained by the odorants with the highest OAVs. Due to molecular interactions, volatile compounds with lower OAVs may also contribute to the overall aroma through additive effects. Although these compounds have odors different from that of wine, they may play a predominant role. Further work is now required, particularly on compound **IV**, to improve our understanding of the character and complexity of botrytized wine aromas.

ABBREVIATIONS USED

I, 3-sulfanylhexan-1-ol; **II**, 3-sulfanylpentan-1-ol; **III**, 3-sulfanylheptan-1-ol; **IV**, 2-methyl-3-sulfanylbutan-1-ol; GC-O, gas chromatography−olfactometry; GC-FPD, gas chromatography−flame photometry detection; GC-MS, gas chromatography−mass spectrometry; OAV, odor activity value; OZ, odorous zone; *p*-HMB, sodium *p*-hydroxymercuribenzoate.

ACKNOWLEDGMENT

We thank Dr. Philippe Darriet in the Faculté d'Oenologie (Talence, France) and Dr. Sandrine Garbaye in the Château d'Yquem (Sauternes, France) for their help.

LITERATURE CITED

(1) Ribéreau-Gayon, P.; Dubourdieu, D.; Donêche, B.; Lonvaud, A. Botrytized sweet wines (Sauternes and Tokay). In *Handbook of Enology—Vol. 1: The Microbiology of Wine and Vinifications*; Wiley: New York, 1998; pp 410−419.

(2) Masuda, M.; Okawa, E.; Nishimura, K.; Yurome, H. Identification of 4,5-dimethyl-3-hydroxy-2(5*H*)-furanone (sotolon) and ethyl 9-hydroxynonanoate in botrytised wine and evaluation of the roles of compounds characteristics on it. *Agric. Biol. Chem.* **1984**, *48*, 2707−2711.

(3) Sponholz, W. R.; Hühn, T. 4,5-Dimethyl-3-hydroxy-2,5-furanon, sotolon, an indicator of *Botrytis* infection. *Wein-Wissenschaft, Wiesbaden* **1994**, *49*, 37−39.

(4) Miklósy, É.; Kerényi, Z. Comparison of the volatile aroma components in noble rotted grape berries from two different locations of the Tokaj wine district in Hungary. *Anal. Chim. Acta* **2004**, *513*, 177−181.

(5) Miklósy, É.; Kalmar, Z.; Pölös, V. Z. K. Study of the volatile aroma components in young Tokaji Aszu wines by GC-MS. *Chromatogr. Suppl.* **2000**, *51*, 305−308.

(6) Miklósy, É.; Kalmar, Z.; Kerényi, Z. Identification of some characteristic aroma compounds in noble rotted grape berries and Aszu wines from Tokaj by GC-MS. *Acta Aliment.* **2004**, *33*, 215−226.

(7) Sarrazin, E.; Dubourdieu, D.; Darriet, P. Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. *Food Chem.* **2007**, in press.

(8) Tominaga, T.; Baltenweck-Guyot, R.; Peyrot des Gachons, C.; Dubourdieu, D. Contribution of volatile thiols to the aromas of white wines made from several *Vitis vinifera* grape varieties. *Am. J. Enol. Vitic.* **2000**, *51*, 178−181.

(9) Tominaga, T.; Niclass, Y.; Frérot, E.; Dubourdieu, D. Stereoisomeric distribution of 3-mercaptohexan-1-ol and 3-mecraptohexyl acetate in dry and sweet white wines made from *Vitis vinifera* (var. Sauvignon blanc and Semillon). *J. Agric. Food Chem.* **2006**, *54*, 7251−7255.

(10) Bailly, S.; Jerkovic, V.; Marchand-Brynaert, J.; Collin, S. Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. *J. Agric. Food Chem.* **2006**, *54*, 7227−7234.

(11) Tominaga, T.; Murat, M.-L.; Dubourdieu, D. Development of a method for analyzing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. *J. Agric. Food Chem.* **1998**, *46*, 1044−1048.

(12) Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70−77.

(13) Vermeulen, C.; Guyot-Declerck, C.; Collin, S. Combinatorial synthesis and sensorial properties of mercapto primary alcohols and analogues. *J. Agric. Food Chem.* **2003**, *51*, 3623−3628.

(14) Acuna, G.; Gautschi, M.; Kumli, F.; Schmid, J.; Zsindely, J. Mercapto-alkanol flavor compounds, Givaudan SA. U.S. Patent 6,610,346B1, 2003.

(15) Masneuf, I.; Dubourdieu, D. Assimilable nitrogen: analysis by formol titration and study of cultivar dependent changes of must nitrogen contents during berry ripening. *Rev. Fr. Oenol.* **1999**, *93*, 31−32.

(16) Bely, M.; Rinaldi, A.; Dubourdieu, D. Influence of assimilable nitrogen on volatile acidity production by Saccharomyces cerevisiae during high sugar fermentation. *J. Biosci. Bioeng.* **2003**, *96*, 507−512.

(17) Bely, M.; Masneuf-Pomarède, I.; Dubourdieu, D. Influence of physiological state of inoculum on volatile acidity production by *Saccharomyces cerevisiae* during high sugar fermentation. *J. Int. Sci. Vigne Vin* **2005**, *39*, 191−197.

(18) Frezier, V.; Dubourdieu, D. Ecology of yeast strain *Saccharomyces cerevisiae* during spontaneous fermentation in a Bordeaux winery. *Am. J. Enol. Vitic.* **1992**, *43*, 375−380.

(19) Boidron, J. N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines substances odorantes du vin. *Connaiss. Vigne Vin* **1988**, *22*, 275−294.

(20) AFNOR V 09-013. In *Contrôle de la Qualité des Produits Alimentaires*; AFNOR: Paris, France, 1995.

(21) Bouchilloux, P.; Darriet, P.; Dubourdieu, D. Identification d'un thiol fortement odorant, le 2-méthyl-3-furanthiol, dans les vins. *Vitis* **1998**, *37*, 177−180.

(22) Darriet, P.; Tominaga, T.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-sulfanyl-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385−392.

(23) Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J.* **1998**, *13*, 159−162.

(24) Tominaga, T. Recherches sur l'arôme variétal des vins de *Vitis vinifera* L. cv. Sauvignon blanc et sa genèse à partir des précurseurs inodores du vin. Ph.D. thesis, University of Bordeaux II, France, 1998.

(25) Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful odorous thiols in Bordeaux red wine varieties. *J. Agric. Food Chem.* **1998**, *46*, 3095−3099.

(26) Vermeulen, C. Synthèse et caractérisation organoleptique de thiols polyfonctionnels: recherche de leur présence dans la bière. Ph.D. Thesis, Université catholique de Louvain, Belgium, 2005.

(27) Natsch, A.; Schmid, J.; Flachsmann, F. Identification of odoriferous sulfanylalkanols in human axilla secretions and their formation through cleavage of cysteine precursors by a C-S lyase isolated from axilla bacteria. *Chem. Biodiversity* **2004**, *1*, 1058−1072. Hasegawa, Y.; Yabuki, M.; Matsukane, M. Identification of new odoriferous compounds in human axillary sweat. *Chem. Biodiversity* **2004**, *1*, 2042−2050.

(28) Escher, S.; Niclass, Y.; van de Waal, M.; Starkenmann, C. Combinatorial synthesis by nature: volatile organic sulfur-containing constituents in *Ruta chalepensis* L. *Chem. Biodiversity* **2006**, *3*, 943−957.

(29) Vermeulen, C.; Lejeune, I.; Tran, T. T. H.; Collin, S. Ocurrence of polyfunctional thiols in lager beers. *J. Agric. Food Chem.* **2006**, *54*, 5061−5068.

(30) Widder, S.; Sabater Lüntzel, C.; Dittner, T.; Pichenhagen, W. 3-Sulfanyl-2-methylpentan-1-ol, a new powerful aroma compound. *J. Agric. Food Chem.* **2000**, *48*, 418−423.

(31) Tominaga, T.; Dubourdieu, D. A novel method for quantification of 2-methyl-3-furanthiol and 2-furanmethanethiol in wines made from *Vitis vinifera* grape varieties. *J. Agric. Food Chem.* **2006**, *54*, 29−33.

(32) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: *S*-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215−5219.

(33) Troccaz, M.; Starkenmann, C.; Niclass, Y.; van de Waal, M.; Clark, A. J. 3-Methyl-3-sulfanylhexan-1-ol as a major descriptor for the human axilla-sweat odour profile. *Chem. Biodiversity* **2004**, *1*, 1022−1035.

(34) Starkenmann, C.; Niclass, Y.; Troccaz, M.; Clark, A. J. Identification of the precursor of (*S*)-3-methyl-3-sulfanylhexan-1-ol, the sulfury malodour of human axilla sweat. *Chem. Biodiversity* **2005**, *2*, 705−716.

(35) Schneider, R.; Charrier, F.; Razungles, A.; Baumes, R. Evidence of an alternative biogenetic pathway leading to 3-mercaptohexanol and 4-mercapto-4-methylpentan-2-one in wines. *Anal. Chim. Acta* **2006**, *563*, 58−64.

(36) Sabater Lüntzel, C.; Widder, S.; Vössing, T.; Pickenhagen, W. Enantioselective syntheses and sensory properties of the 3-mercapto-2-methylpentanols. *J. Agric. Food Chem.* **2000**, *48*, 424−427.

(37) van Ruth, S. Methods for gas chromatography−olfactometry: a review. *Biomol. Eng.* **2001**, *17*, 121−128.

(38) Guadagni, D. G.; Buttery, R. G.; Okano, S.; Burr, H. K. Additive effect of sub-threshold concentrations of some organic compounds associated with food aromas. *Nature* **1963**, *200*, 1288−1289.

(39) Ferreira, V.; Ortin, N.; Escudero, A.; Lopez, R.; Cacho, J. Chemical characterization of the aroma of Grenache rosé wines: aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem.* **2002**, *50*, 4048−4054.

(40) Escudero, A.; Gogorza, B.; Melus, A.; Ortin, N.; Cacho, J.; Ferreira, V. Characterization of the aroma of a wine from Maccabeo. Key role played by compounds with low odor activity values. *J. Agric. Food Chem.* **2004**, *52*.

Received for review September 8, 2006. Revised manuscript received December 4, 2006. Accepted December 17, 2006. We thank the CIVB (Conseil Interprofessionnel des Vins de Bordeaux) and the Conseil Régional d'Aquitaine for their financial support for this research.

JF062582V