Quantitative Determination of Sulfur Containing Wine Odorants at Sub-ppb Levels. 1. Synthesis of the Deuterated Analogues

Yorgos Kotseridis,* Jean-Loïc Ray, Christian Augier, and Raymond Baumes

Unité Biopolymères Arômes, INRA-IPV, 2 Place Viala, 34060 Montpellier, Cedex 01, France

[$^2H_{10}$]-4-Sulfanyl-4-methylpentan-2-one (d_{10}-SMP), [$^2H_2$]-3-sulfanylhexan-1-ol (d_2-3SH), and [$^2H_5$]-3-sulfanylhexyl acetate (d_5-3SHAc), the labeled analogues of impact odorants of wines and other foods, were synthesized to be used for the quantitative determination of the natural compounds in white and red wines by stable isotope dilution assay. The sulfidation was achieved by Michael addition, on mesityl oxide or ethyl hex-2-enoate, respectively, of the sulfhydryl anion generated in situ from triphenylsilanethiol and potassium fluoride under phase transfer conditions. The labeling of 4-sulfanyl-4-methylpentan-2-one (SMP) was obtained from the commercial starting material, [$^2H_6$]-acetone, so that this method could be used to synthesize ^{13}C-labeled SMP from ^{13}C-labeled acetone. The labeling of 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHAc) was obtained from reduction with lithium aluminum deuteride of the Michael adduct ethyl 3-sulfanylhexanoate and [$^2H_3$]-acetylation. During the synthesis, 3SH and 3SHAc were partially oxidized to their disulfide, which were reduced back to the thiols by an additional reduction step; the tertiary thiol SMP was less sensitive to this oxidation.

Keywords: *Wine aroma; 4-sulfanyl-4-methylpentan-2-one; 3-sulfanylhexan-1-ol; 3-sulfanylhexyl acetate; labeling; deuterium; disulfide*

INTRODUCTION

Sulfur containing compounds exhibit, in general, intense smelling properties due to their extremely low odor thresholds. Depending on their levels in beverages and foods they contribute either favorably to the aroma or to off-flavor. Thus 4-sulfanyl-4-methylpentan-2-one (SMP) was described to have a pleasant box tree and blackcurrant bud odor at trace levels in Sauvignon wines but an unpleasant catty urine odor at higher levels (Darriet, 1993). Its occurrence in Chenin blanc and Colombar wines was hypothesized in 1981 (Du Plessis and Augustyn, 1981), but it was positively identified in Sauvignon and Scheurebe wines only recently (Darriet, 1993; Darriet et al., 1995; Guth, 1997).

3-Sulfanylhexan-1-ol (3SH), first identified in yellow passion fruits (Engel and Tressl, 1991; Weber et al., 1994; Werkhoff et al., 1998) has an odor reminiscent of grape fruit and passion fruit. It was recently reported in Sauvignon blanc wines (Tominaga et al., 1998a) and in red Bordeaux wines (Merlot, Cabernet Sauvignon cultivars) (Bouchilloux et al., 1998; Kotseridis and Baumes, 2000). Its acetate, 3-sulfanylhexyl acetate (3SHAc), reminiscent of exotic fruits, was also first identified in yellow passion fruits (Engel and Tressl, 1991; Werkhoff et al., 1998; Weber et al., 1995) and later in wines (Tominaga et al., 1996; Bouchilloux et al., 1998). These compounds have extremely low odor thresholds (Tominaga et al., 1996; Tominaga et al., 1998a; Tominaga et al., 1998b; Bouchilloux et al., 1998) and are impact odorants of Sauvignon, Merlot, Cabernet Sauvignon (Tominaga et al., 1996; Tominaga et al., 1998a; Tominaga et al., 1998b; Bouchilloux et al., 1998), and probably of wines of other varieties.

The levels of these powerful odorant compounds were reported in the sub-ppb range in white and red wines (Bouchilloux et al., 1996; Bouchilloux et al., 1998; Tominaga et al., 1998b). As discussed previously the use of an internal standard whose chemical structure is different from that of the analyte does not allow an accurate quantification (Guichard 1988), especially in the case of thiols which are very reactive species (Hofmann et al., 1996). Thus a stable isotope dilution assay (SIDA) was the most accurate method for the quantitative determination of these trace odorants. Hence, we reported in this paper the synthesis of [$^2H_{10}$]-4-sulfanyl-4-methylpentan-2-one (d_{10}-SMP), of [$^2H_2$]-3-sulfanylhexan-1-ol (d_2-3SH), and of [$^2H_5$]-3-sulfanylhexyl acetate (d_5-3SHAc) which will be used as labeled standards in the quantitative determination of their natural analogues in wines, as reported in the following paper (Kotseridis et al., submitted to *J. Agric. Food Chem.*).

EXPERIMENTAL PROCEDURES

Chemicals. Mesityl oxide (98 wt %), tetrahydrofuran, anhydrous (99.9 wt %), deuterium oxide (D_2O, 99.8 atom % D), deuterated sulfuric acid (D_2SO_4, 98 wt %, solution in D_2O), [$^2H_6$]acetone (d_6-acetone, 99.8 atom % D), [$^2H_3$]acetic acid (99.9 + atom % D), [$^2H_3$]acetyl chloride (99 + atom % D), lithium aluminum deuteride (98 atom % D), 18-crown-6 ether (99.5 + wt %), triphenylsilanethiol (98 wt %), potassium fluoride anhydrous powder (99.99 + wt %), magnesium oxide (98 wt %), sodium hydrosulfide hydrate ($NaSH \times H_2O$), cyclohexane (99+ %), (*E*)-hex-2-enal (98 wt %), carbon tetrachloride anhydrous (99.9 + wt %) and lithium diisopropylamide mono-(tetrahydrofuran) complex solution (LDA, 1.5 M in cyclohexane) were all purchased from Aldrich Chemical Co. Inc.

Concentrated sulfuric acid (95−97%), chlorhydric acid (32%), acetone (GR for analysis), acetic acid (glacial), diethyl ether,

* Author to whom correspondence should be addressed (telephone 30 31 936 616; fax 0030 31 902 132; e-mail yorgos@erevnon.com). Permanent address: Erevnon − Food Technology, Poliviou str. 81-83, GR-54351 Thessaloniki, Greece.

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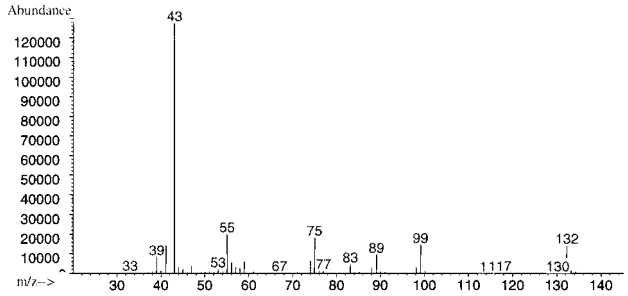

Figure 1. Mass spectrum of 4-mercapto-4-methylpentan-2-one.

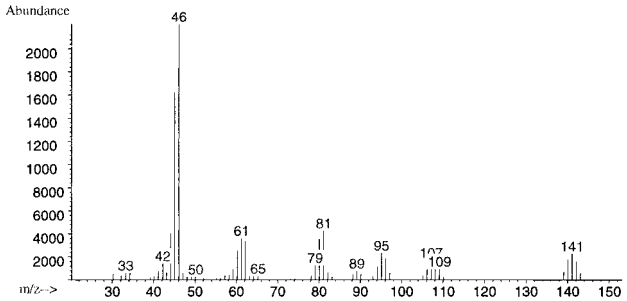

Figure 2. Mass spectrum of [$^2H_{10}$]-4-mercapto-4-methylpentan-2-one.

pentane, dichloromethane (ultrapure grade), molecular sieve (4 Å), and silica gel 60 (230−400 mesh ASTM) were all obtained from Merck, 64271 Darmstadt Germany. 2,6-Di-*tert*-butyl-*p*-cresol (>99 wt %) and zinc (purum powder) were purchased from Fluka Chemie AG, CH-9470 Buchs.

3-Sulfanylhexan-1-ol (97 wt %), 3-sulfanylhexyl acetate (97 wt %), and ethyl (*E*)-hex-2-enoate (96 wt %) were purchased from Interchim, Montlucon (France).

All glassware were cleaned by washing with acetone, alcohol, and dichloromethane, followed by oven baking at 100 °C prior to use.

Instrumental Analysis. GC/EIMS analysis was carried out using a Hewlett-Packard HP gas chromatograph 5890 series II fitted with a 30 m fused-silica column (0.32 mm i.d. and 0.5 μm film thickness), coated with DB WAX (J&W Scientific). The injection of the extracts (3 μL) was on-column at 35 °C; then the temperature of the injector was increased 180 °C/min to 250 °C, till the end of the run. The carrier gas was Helium 6.0 (Linde gaz, Marseille), with a flow-rate of 1.35 mL/min. The oven temperature program was 35 °C (for 3 min), then increased at 3 °C/min to 245 °C, and held at this temperature for a further 20 min. The GC instrument was coupled to a 5989A mass selective detector and a MS chemstation (HP-UX). The electron impact (EI) energy was 70 eV, and the quadrupole temperature was set at 250 °C.

GC-CIMS analysis was carried out using a Varian CP 3800 gas chromatograph fitted with the same column as above and coupled to a Varian Saturn 2000. The GC conditions were the same as above except that the initial temperatures of the injector and of the oven were respectively 20 °C and 60 °C. The temperatures of the transfer line, the manifold, and the trap were respectively 250, 40, and 170 °C. The reactant gas was methane, the emission current was 20 μA, and the maximum ionization and reaction times were respectively 2 ms and 60 ms.

A Varian 500 MHz NMR spectrometer was used to record the ^{1}H- and ^{13}C NMR spectra. Sample spectra were from deuterated chloroform solutions; TMS (0 ppm δ) and $CDCl_3$ (77.0 ppm δ) were used as reference.

Synthesis of 4-Sulfanyl-4-methylpentan-2-one. In a conical flask 98 mg of mesityl oxide (1 mmol), 584 mg of triphenylsilanethiol (2 mmol), 264 mg of 18-crown-6 ether (1 mmol), 116 mg of potassium fluoride (2 mmol), and 10 mg of 2, 6-di-*tert*-butyl-*p*-cresol (0.045 mmol) were added to 5 mL of dry tetrahydrofuran (THF) and stirred at room temperature under nitrogen atmosphere for 2 h. Then the dark green reaction mixture was acidified, filtered on glasswool, and concentrated under vacuum. The residue was taken up in 2 mL of pentane, and the solution was further purified by flash chromatography on silica gel using pentane (200 mL) and then pentane/diethyl ether (5/95, 200 mL) as eluent. SMP (92.4 mg, 70%) was obtained as a colorless oil; ^{13}C NMR ($CDCl_3$) δ 31.8 (C-1), 32.5 (C-5, C-5′), 41.4 (C-4), 57.7 (C-3), 206.7 (C-2). ^{1}H NMR ($CDCl_3$) δ 1.40 (s, C-4-$(CH_3)_2$), 2.09 (s, (H-1)$_3$), 2.35 (s, SH), 2.70 (s, (H-3)$_2$). EI-MS, see Figure 1.

Synthesis of [$^2H_{10}$]-4-Sulfanyl-4-methylpentan-2-one. *[$^2H_{10}$]Mesityl Oxide.* The porous thimble of a Soxhlet extraction apparatus was filled with a mixture of magnesium oxide and of molecular sieve (4 Å). The apparatus was fitted to a flask containing 4 mL (54.4 mmol) of [$^2H_6$]acetone and 16 mL of cyclohexane which were boiled gently for 72 h. The reaction mixture was then cooled to room temperature, and the solvent was concentrated under vacuum at 35 °C: 2.35 g (21.8 mmol; 40%) of deuterated mesityl oxide were obtained; EI-MS (70 eV), *m*/*z* (%) 90(100)-62(82)-89(65)-61(51)-107(35)-108(33)-46(32)-42(26).

[$^2H_{10}$]-4-Sulfanyl-4-methylpentan-2-one. In a flame dried conical flask 108 mg of [$^2H_{10}$]mesityl oxide (1 mmol), 584 mg of triphenylsilanethiol (2 mmol), 264 mg of 18-crown-6 ether (1 mmol), 116 mg of potassium fluoride (2 mmol), and 10 mg of 2,6-di-*tert*-butyl-*p*-cresol (0.045 mmol) were added to 5 mL of dry tetrahydrofuran (THF) and stirred at room temperature under nitrogen atmosphere for 2 h. The dark green reaction mixture was acidified, filtered on glasswool, and concentrated under vacuum at 35 °C, and the residue was taken up in 2 mL of pentane. This solution was further purified by flash chromatography on silica gel as described above. d_{10}-SMP (67 mg, 47%) was obtained as a colorless oil. Its chemical purity was 85% as determined by GC-EIMS. As the byproducts, triphenylsilane and the crown ether had no impact in its use in SIDA; it was not further purified. CI-MS (methane): *m*/*z* 140−143 (M + H$^+$; 1−1.5%), 46 (CD3CO$^+$, 100%); EI-MS, see Figure 2.

Synthesis of [$^2H_2$]-3-Sulfanylhexan-1-ol. *Ethyl 3-Sulfanylhexanoate*: In a conical flask containing 10 mL of anhydrous tetrahydrofuran (THF), 573 mg of ethyl (*E*)-hex-2-enoate (4 mmol), 2.41 g of triphenylsilanethiol (8 mmol), 1.057 g of 18-crown-6 ether (4 mmol), 465 mg of potassium fluoride (8 mmol), and 20 mg of 2, 6-di-*tert*-butyl-*p*-cresol (0.09 mmol) were added and stirred at room temperature, under nitrogen atmosphere for 2 h. Then the dark green reaction mixture was acidified, filtered on glasswool, and concentrated at 35 °C under vacuum. The residue was taken up in 5 mL of glacial acetic acid, added with 1.2 g of powdered zinc (18.2 mmol) and refluxed under nitrogen atmosphere for 4 h. Then the reaction mixture was concentrated at 50 °C under vacuum. The residue was taken up in 2 mL of pentane, and the solution was further purified by flash chromatography on silica gel with pure pentane (200 mL) and then with pentane/diethyl ether (95/5, v/v, 200 mL) as eluent. Ethyl 3-sulfanylhexanoate (450 mg, 2.56 mmol, 64%) was obtained as a colorless oil; EI-MS, see Figure 3a.

[$^2H_2$]-3-Sulfanylhexan-1-ol. In a flame dried three-necked flask, fitted with a dropping funnel and a nitrogen inlet, 152 mg of lithium aluminum deuteride ($LiAlD_4$, 3.6 mmol) was carefully added to 10 mL of dry diethyl ether (freshly distilled from $CaCl_2$), and the mixture was stirred for further 10 min. After the formation of a milky suspension, a solution of ethyl 3-sulfanylhexanoate (528 mg, 3 mmol) in 3 mL of dry diethyl ether was added carefully via the dropping funnel. Stirring was continued for further 3 h, and then 2 mL of diethyl ether saturated with water was added carefully to the reaction mixture. The ether layer was separated, and the aqueous phase was acidified to pH 1 with sulfuric acid (2 mol/L), then extracted with pentane, and concentrated at 30 °C under vacuum. The residue was taken up in acetic acid and treated with powdered zinc as above for ethyl 3-sulfanylhexanoate to yield 202 mg (1.48 mmol, 49%) of crude [$^2H_2$]-3-sulfanylhexan-1-ol. As its chemical purity determined by GC-EIMS was 95%,

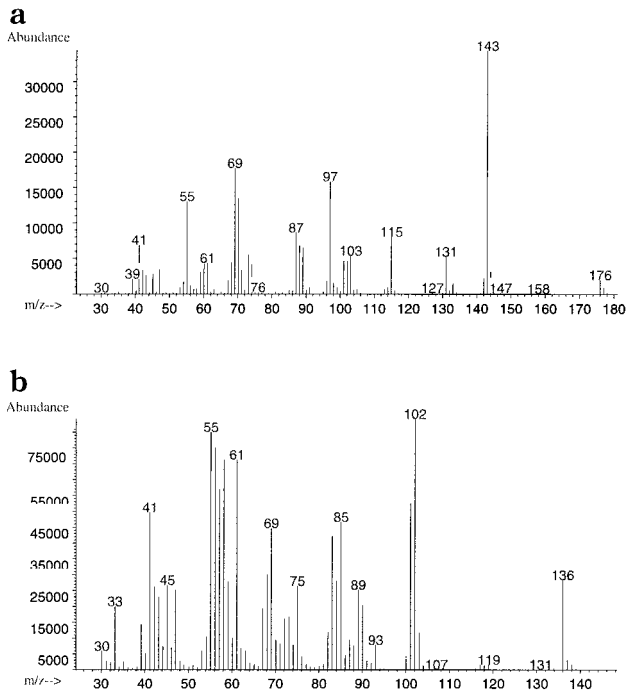

Figure 3. Mass spectra of (a) ethyl 3-sulfanylhexanoate and (b) [$^2H_2$]-3-sulfanylhexan-1-ol.

it was used in SIDA without further purification; ^{1}H NMR ($CDCL_3$) 0.85 (t, J = 7 Hz, (H-6)$_3$), 1.32 (d, J = 7.9 Hz, SH), 1.34−1.50 (m, (H-4)$_2$, (H-5)$_2$), 1.56 (br dd, J = 14.3 Hz, J = 9.5 Hz, H-2), 1.88 ((br dd, J = 14 Hz, J = 3.7 Hz, H-2′), 2.88 (m, H-3); the integration of the signal for the residual H-1 relative to that of the signal for H-3 showed an isotopic purity of 98%, consistent with that of the deuteride used. CI-MS (methane): m/z 119 (M + H^+ − H_2O; 40%), 85 (M + H^+ − H_2O − H_2S; 100%); EI-MS, see Figure 3b.

Synthesis of [$^2H_5$]-3-Sulfanylhexyl Acetate. [$^2H_5$]-3-Sulfanylhexyl acetate was prepared using the synthetic route reported previously for the unlabeled compound (Heusinger and Mosandl, 1984). Under strictly anhydrous conditions and under nitrogen, [$^2H_2$]-3-sulfanylhexan-1-ol (80 mg, 0.6 mmol) was added to 3 mL of anhydrous carbon tetrachloride (CCl_4), and the solution was stirred at room temperature for 10 min. [$^2H_3$]Acetyl chloride (128 μL, 1.8 mmol) was added, and the reaction mixture (monitored by GC/MS) was further stirred for 5 h at room temperature, under nitrogen, and then concentrated at 35 °C under vacuum. The residue was taken up in [$^2H_3$]acetic acid and treated with powdered zinc as above for ethyl 3-sulfanylhexanoate, to yield 56 mg (0.3 mmol, 50%) of crude [$^2H_5$]-3-sulfanylhexyl acetate used in SIDA without further purification (chemical purity 95% as determined by GC/EIMS). ^{1}H NMR ($CDCL_3$) δ 0.86 (t, J = 7 Hz, (H-6)$_3$), 1.31 (d, J = 7.3 Hz, SH), 1.33−1.60 (m, (H-4)$_2$, (H-5)$_2$), 1.65 (br dd, J = 14.1 Hz, J = 9.2 Hz, H-2), 1.94 (br dd, J = 14 Hz, J = 4.3 Hz, H-2′), 2.81 (m, H-3); the integration of the signal for the residual H-1 relative to that of the signal for H-3 showed an isotopic purity of 98% for those positions, while the signal for the acetate group was too small to be accuretely integrated, consistent with the high isotopic purity of the [$^2H_3$]acetyl chloride used. CI-MS (methane): m/z 164 (M + H^+ − H_2O; 60%), 119 (M + H^+ − d3AcOH; 65%), 46 ($CD3CO^+$, 100%); EI-MS, see in Figure 4.

Stability of d_{10}-SMP at Wine pH. In a 0.5 L Erlenmeyer 0.328 mg of d_{10}-SMP (in 1 mL pentane) and 0.4 g of tartaric acid were added to 100 mL of an ethanol/water solution (12/88 v/v), and the pH of the mixture was adjusted to 2.8 with potassium bicarbonate and stirred at room temperature for 24 h. Then the flask was placed in an ice bath under nitrogen: 30 mL of pentane/dichloromethane (2/1 v/v) was added, and the mixture was stirred during 15 min at 700 rpm. The mixture was supplemented with 20 mL of dichloromethane, and stirring was continued for 15 min. The organic phase was separated in a separatory funnel, centrifuged for 5 min at 9000g (4 °C), dried over sodium sulfate, then concentrated to about 1 mL under vacuum at 30 °C, and analyzed by GC/MS (full scan mode) to check if any exchange had occurred.

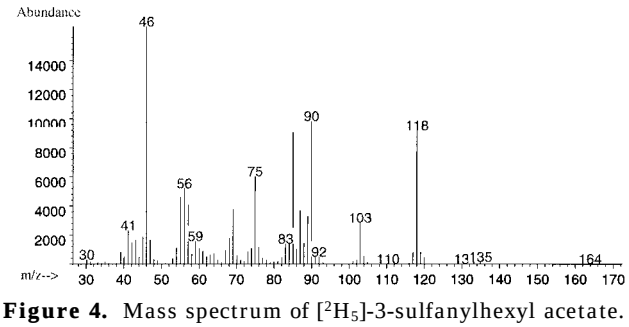

Figure 4. Mass spectrum of [$^2H_5$]-3-sulfanylhexyl acetate.

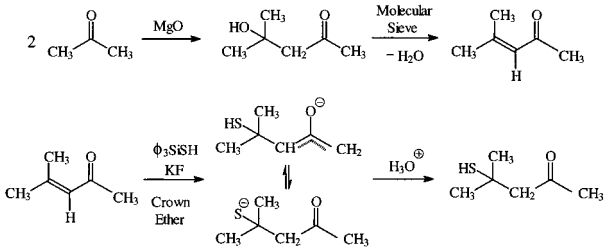

Figure 5. 4-Sulfanyl-4-methylpentan-2-one synthesis.

RESULTS AND DISCUSSION

The key step of the synthesis of SMP, 3SH, and 3SHAc was the Michael addition of the sulfhydryl anion generated in situ from triphenylsilanethiol and potassium fluoride under phase transfer conditions. Triphenylsilanethiol was reported previously as a solid hydrogen sulfide equivalent in the ring opening of epoxides (Brittain and Gareau, 1993). The labeling was obtained either in the beginning of the synthesis for SMP (deuterated starting material, [$^2H_6$]acetone) or in the end for 3SH and 3SHAc (reduction with lithium aluminum deuteride and [$^2H_3$]-acetylation.

4-Sulfanyl-4-methylpentan-2-one Synthesis. Synthesis of SMP was attempted following the synthetic route proposed by Guth (1997). In our hands, the yield of the first step of this synthesis, the acid catalyzed condensation of acetone, determined using GC/MS, was very low (mesityl oxide yield 4%), and the final yield of SMP was lower than 1%. Modifications were attempted to improve the mesityl oxide yield. Ten times more sulfuric acid and use of cyclohexane as a solvent at 50 °C yielded up to 40% mesityl oxide. However, the same conditions, using D_2SO_4 and acetone-d_6, yielded only 13% of deuterated mesityl oxide. Basic conditions by preforming the enolate at low temperature and then adding acetone (Kleschick et al., 1977) yielded up to 33% of diacetone alcohol and about the same yield for mesityl oxide, but the same conditions using acetone-d_6, yielded only 12% of deuterated mesityl oxide.

The best yield of deuterated mesityl oxide was obtained in the one-pot basic condensation of acetone with magnesium oxide and dehydration of the intermediate deuterated 4-hydroxy-4-methylpentan-2-one (diacetone alcohol) (Figure 5). These reactions were carried out in a Soxhlet extraction apparatus using cyclohexane as a solvent, which azeotropically distilled with water and acetone, and mixing in the porous thimble the base

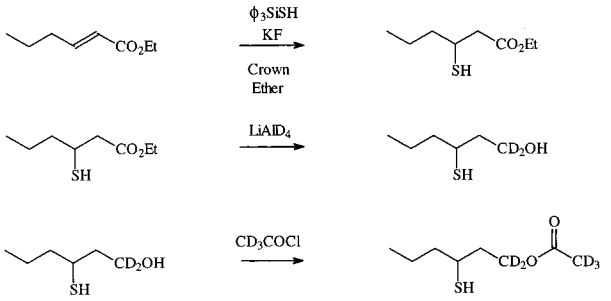

Figure 6. Synthesis of [$^2H_2$]-3-sulfanylhexan-1-ol and of [$^2H_5$]-3-sulfanylhexyl acetate.

necessary for the condensation and a molecular sieve to trap the water formed from the dehydration. Thus the reaction equilibrium was displaced toward the deuterated mesityl oxide, which was then drained back to the flask by the solvent where it remained due to its higher boiling point. The solvent allowed the use of small amounts of the labeled acetone to prevent further condensation of mesityl oxide. The mass spectrum of the deuterated mesityl oxide showed a molecular ion at *m*/*z* 108 and fragment ions at *m*/*z* 62 ($C_4D_7^+$) and 46 ($COCD_3^+$), which was consistent with the incorporation of 10 deuterium atoms in the molecule.

The second step was the addition of the sulfhydryl anion to mesityl oxide using triphenylsilanethiol and potassium fluoride under phase transfer catalysis conditions (Figure 5). The conditions of this reaction, determined for the unlabeled SMP, allowed for the obtaining of SMP in 70% yield. Its structure was confirmed by ^{1}H- and ^{13}C NMR (Experimental Procedures) and MS (Figure 1). When the starting material was the deuterated mesityl oxide synthesized above, the yield in deuterated SMP was 47%. As triphenylsilanethiol contained a SH group and as the workup was made with water, two protons were incorporated in the interchangeable positions of the molecule (Figure 5). That was consistent with the molecular ion at *m*/*z* 142 and the fragment ion at *m*/*z* 109 ($M^+ - SH$) in its mass spectrum, showing the occurrence of 10 deuterium atoms in the molecule (Figure 2). In fact, the clusters of molecular ions in the EI- and CI-MS of the product obtained showed that it was a mixture of isotopomers containing on average 10 deuterium atoms, each of them retaining the deuterated gem-dimethyl moiety.

As the labeling of the synthesized SMP was obtained from the starting material, [$^2H_6$]acetone, this synthesis could be used to obtain the [$^{13}C_6$]-labeled SMP from the much more expensive [$^{13}C_3$]acetone.

[$^2H_2$]-3-Sulfanylhexan-1-ol synthesis. The synthesis of 3-sulfanylhexan-1-ol starting from (*E*)-hex-2-enal and hydrogen sulfide was reported previously (Winter et al., 1976; Engel and Tressl, 1991). As the use of hydrogen sulfide was an unpleasant operation, its anion was generated as above from triphenylsilanethiol under phase transfer conditions, but the yield obtained was not satisfactory (25%), probably due to the instability of the unsaturated aldehyde in these conditions.

However, these conditions allowed for obtaining in good yield ethyl 3-sulfanylhexanoate starting from ethyl (*E*)-hex-2-enoate (Figure 6). Despite the care being taken to prevent oxidation of the sulfanyl group, the corresponding disulfide (Figure 7a) was obtained as a secondary product (about 30% as assessed by GC/MS). Thus a reduction step was carried out to obtain a crude product containing mainly ethyl 3-sulfanylhexanoate,

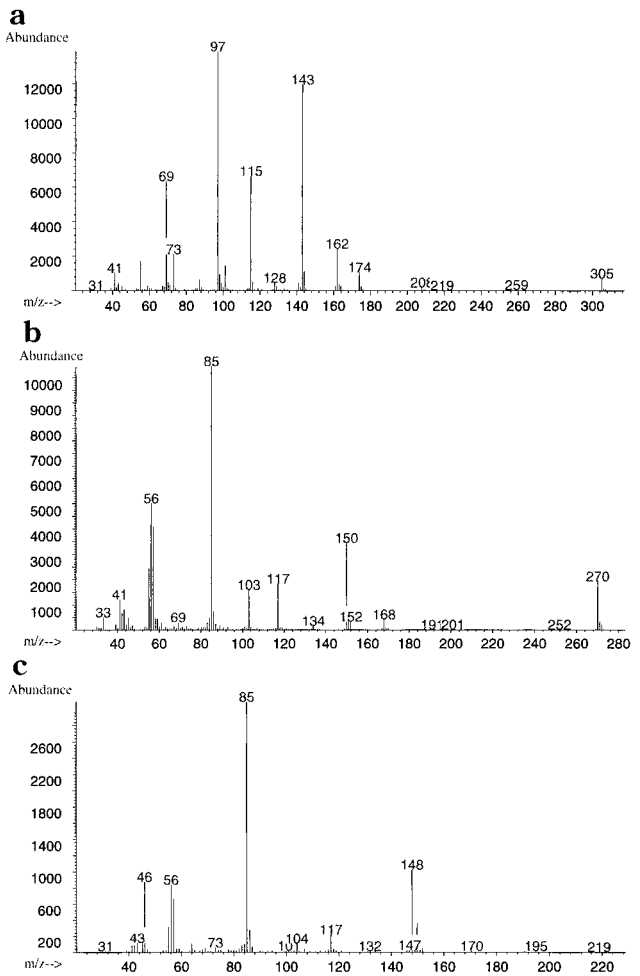

Figure 7. Mass spectra of (a) bis-(1′-carbethoxypent-2′-yl)-disulfide, (b) [$^2H_4$]bis-(1′-hydroxyhex-3′-yl)disulfide, and (c) [$^2H_{10}$]bis-(1′-acetoxyhex-3′-yl) disulfide.

that was further purified by flash chromatography on silica gel. Although the following step should have reduced the disulfide, this additional step made easier the flash chromatography purification.

The second step was the reduction with lithium aluminum deuteride of the ethyl ester compound to the corresponding alcohol, [$^2H_2$]-3-sulfanylhexan-1-ol, labeled on the first carbon of the aliphatic chain. During the workup of the basic reaction mixture, the product was partially oxidized to the corresponding disulfide (mass spectrum see in Figure 7b); therefore a reduction step under acidic conditions was used again to obtain a crude product containing only [$^2H_2$]-3-sulfanylhexan-1-ol with an estimated total yield of 31%. The ^{1}H NMR showed the disappearance of the signal for H-1 and H-1′, reported at 3.74 ppm in the unlabeled compound (Kleschick et al., 1977) and the collapse of the multiplets for H-2 and H-2′ to two doublets of doublet with broad lines due to the residual couplings with D-1 and D-1′. The mass spectrum was consistent with this labeling (Figure 3).

[$^2H_5$]-3-Sulfanylhexyl Acetate Synthesis. The synthesis of [$^2H_5$]-3-sulfanylhexyl acetate was achieved using the synthetic route proposed by Heusinger and Mosandl (1984), slightly modified. Thus the acetylation of the synthesized [$^2H_2$]-3-sulfanylhexan-1-ol was carried out with [$^2H_3$]acetyl chloride in carbon tetrachloride, affording [$^2H_5$]-3-sulfanylhexyl acetate with an estimated yield of 50% (Figure 6). The reaction was

monitored by GC/MS and stopped when the hydroxyl function was acetylated in order to avoid the slower acetylation of the sulfanyl group. As a small amount of the corresponding disulfide was detected by GC/MS (mass spectrum see in Figure 7c) an additional reduction step, using [$^2H_3$]acetic acid and powdered zinc, was used. The ^1H NMR was similar to that of 3SH; its comparison with the spectrum reported for the unlabeled compound (Engel and Tressl, 1991) confirmed the labeling of the acetate moiety and of the methylene in position 1. The mass spectrum was consistent with this labeling (Figure 4).

Stability of d_{10}-SMP at Wine pH. Contrary to d_2-3SH and d_5-3SHAc, d_{10}-SMP contained enolizable deuterium atoms that could be back exchanged during the analysis process (Kotseridis et al., submitted to *J. Agric. Food Chem*.). Thus d_{10}-SMP was treated in a model wine (pH 2.8) for 24 h at room temperature. Using GC/MS in the full scan mode, no proton-deuterium exchange in d_{10}-SMP was observed. This experiment allowed its use as labeled standard in the conditions used to determine quantitatively SMP in wines process (Kotseridis et al., submitted to *J. Agric. Food Chem*.). However, should a labeled SMP be used in conditions favoring any D/H exchange, ^{13}C-labeled SMP could be synthesized by the method described above.

CONCLUSIONS

The methods reported in this paper to synthesize d_{10}-SMP, d_2-3SH, and d_5-3SHAc were relatively easy and presented fairly good yields. The use of a solid hydrogen sulfide equivalent (Brittain and Gareau, 1993) rendered the preparation of these thiols a not unduly unpleasant operation. Furthermore these methods seemed of quite general application to be used in the preparation of some other deuterated thiols and of ^{13}C-labeled SMP.

We also observed that these sulfanyl compounds were easily oxidized to their disulfides, especially when basic conditions were used, so that additional reduction steps were necessary. This observation was in agreement with the oxidative instability of some thiols occurring in food flavor (Hofmann et al., 1996), but SMP, which was a tertiary thiol, was found to be less sensitive to this oxidation. Moreover, no formation of its disulfide was observed during the experiment to test its stability under acidic conditions.

However, this oxidative instability of thiols showed the necessity of SIDA for their quantification, particularly in wines in which they occurred at trace levels. Development and application of such a method will be reported in the following paper (Kotseridis et al., submitted to *J. Agric. Food Chem*.).

LITERATURE CITED

Bouchilloux, P.; Darriet, P.; Dubourdieu, D. Mise au point d'une méthode de dosage de la 4-mercapto-4-méthylpentan-2-one dans les vins de Sauvignon. *J. Int. Sci. Vigne Vin* **1996**, *30*, 23−29.

Bouchilloux, P.; Darriet, P.; Henry, R.; Lavigne-Cruège, V.; Dubourdieu, D. Identification of volatile and powerful odorous Thiols in Bordeaux wines. *J. Agric. Food Chem.* **1998**, *46*, 3095−3099.

Brittain J.; Gareau, Y. Triphenylsilanthiol: a solide H_2S equivalent in the ring opening of epoxides. *Tetrahedron Lett.* **1993**, *34*, 3363−3366.

Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis Vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385−392.

Darriet, P. Recherches sur l'arôme et les précurseurs d'arômes du Sauvignon, Thèse doctorat de l'Université de Bordeaux II, 1993.

Du Plessis, C. S.; Augustyn, O. P. H. Initial study on the guava aroma of Chenin blanc and Colombar wines. *S. Afr. J. Enol. Vitic.* **1981**, *2*, 101−103.

Engel, K. H.; Tressl, R. Identification of new sulfur-containing volatiles in yellow passion fruits (*Passiflora edulis* f.flavicarpa). *J. Agric. Food Chem.* **1991**, *39*, 2249−2252.

Guichard, E. Quantification of some volatile aromatic compounds of apricot by adding standards. *J. Food Sci.* **1988**, *53*, 1902−1904.

Guth, H. Identification of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3022−3026.

Heusinger, G.; Mosandl, A. Chirale, schwefelhaltige aromastoffe der gelben passionfrucht (*Passiflora edulis* f.flavicarpa). Darstellung der enantiomeren und absolute konfiguration. *Tetrahedron Lett.* **1984**, *25*, 507−510.

Hofmann, T.; Schieberle, P.; Grosch, W. Model studies on the oxidative stability of odor-active thiols occurring in food flavors. *J. Agric. Food Chem.* **1996**, *44*, 251−255.

Kleschick, W. A.; Buse, C. T.; Heathcock, C. H. Stereoselection in the Aldol condensation. *J. Am. Chem. Soc.* **1977**, *99*, 247−248.

Kotseridis, Y.; Baumes, R. Identification of impact odorants in Bordeaux red grape juice, in the commercial yeast used for its fermentation and in the produced wine. *J. Agric. Food Chem.* **2000**, *48*, 400−406.

Kotseridis, Y.; Ray, J. L.; Lepoutre, J. P. L.; Baumes, R. Quantitative determination of Sulfur containing wine odorants at sub-ppb levels. Part II: Development and application of a Stable Isotope Dilution Assay. Submitted to *J. Agric. Food Chem*.

Pearce, T. J. P.; Peacock, J. M.; Aylward, F.; Haisman, D. R. Catty odours in food: reactions between Hydrogen Sulphide and unsaturated ketones. *Chem. Ind. (London)* **1967**, 1562−1563.

Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acétate de 3-mercaptohexanol, composé a forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis* **1996**, *35*, 207−210.

Tominaga, T.; Furrer, A.; Henry, R.; Dubourdieu, D. Identification of new volatile thiols in the aroma of *Vitis Vinifera* L.var. Sauvignon. *Flavour Fragnance J.* **1998**, *13*, 159−162.

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

Weber, B.; Maas, B.; Marx, A.; Olk, J.; Mosandl, A. Enantiomeric distribution of the chiral sulphur-containing alcohols in yellow and purple passion fruits. *Z. Lebensm. Unters. Forsch.* **1994**, *199*, 48−50.

Weber, B.; Maas, B.; Mosandl, A. Stereoisomeric distribution of some chiral sulphur-containing trace components of yellow passion fruits *J. Agric. Food Chem.* **1995**, *43*, 2438−2441.

Werkhoff, P.; Guntert, M.; Krammer, G.; Sommer, H.; Kaulen, J. Vacuum headspace method in aroma research: flavor chemistry of yellow passion fruits. *J. Agric. Food Chem.* **1998**, *46*, 1076−1093.

Winter, M.; Furrer, A.; Willham, B.; Thommen, W. Identification and synthesis of two new organic sulfur compounds from the yellow passion fruit (*Passiflora edulis* f. flavicarpa). *Helv. Chim. Acta* **1976**, *59*, 1613−1620.

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