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ARTICLE

Elucidation of the 1,3-Sulfanylalcohol Oxidation Mechanism: An Unusual Identification of the Disulfide of 3-Sulfanylhexanol in Sauternes Botrytized Wines

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A four-step purification method was developed to isolate a citrus odorant detected by gas chromatography−olfactometry (GC−O), which was apparently specific to Sauternes botrytized wines. A fragmentation pattern of the odorant was obtained by multidimensional gas chromatography−mass spectrometry−olfactometry (MDGC−MS−O). The exact mass measurement was used to determine its elemental formula as $C_6H_{12}OS$. On the basis of these data, the unusual structure of 3-propyl-1,2-oxathiolane was synthesized and characterized for the first time. This confirmed its identification. Its occurrence in Sauternes wine extracts was demonstrated to result from the thermal oxidative degradation of 3-sulfanylhexanol disulfide (3,3′-disulfanediyldihexan-1-ol) in the GC injector. This disulfide was synthesized and then firmly identified for the first time in Sauternes wine. Although the presence of 3-sulfanylhexanol oxidation products had previously been reported in natural extracts (but not wine), the full oxidation pathway from 3-sulfanylhexanol to 3-propyl-γ-sultine via 3,3′-disulfanediyldihexan-1-ol was clearly established for the first time. Because the disulfide has mainly been detected in Sauternes botrytized wines, this finding suggested a singular reactivity of 3-sulfanylhexanol in botrytized wines, thus opening up a wide range of new opportunities in wine chemistry.

KEYWORDS: 3-Sulfanylhexanol; 3-propyl-1,2-oxathiolane; 3,3′-disulfanediyldihexan-1-ol; 3-propyl-γ-sultine; Sauternes wines

INTRODUCTION

Naturally occurring 1,3-sulfanylalcohols are highly volatile compounds with powerful, penetrating aromas, responsible for the sensory characteristics and quality of various foods and beverages. They exhibit both attractive (tropical fruit and box tree) and unpleasant (cat urine, sweat, and onion) odors (*1*−*3*). Indeed, the olfactory descriptors associated with 1,3-sulfanylalcohols depend upon the length and branching of their alkyl chain. The sensory impact of these compounds also depends upon their concentration, varying from favorable to unpleasant with increasing concentrations in the matrix (*1*−*3*). Although 1,3-sulfanylalcohols are generally present at trace levels, they contribute strongly to the overall aroma of natural products, foods, and beverages, especially wine. However, the mechanisms involved in their formation, reactivity, and stability, as well as their particular role and additive effects in complex sensory perception, have not yet been fully elucidated and continue to be investigated.

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Among naturally occurring 1,3-sulfanylalcohols, 3-sulfanylhexanol (**1**) is one of the compounds most studied and analyzed. It is a powerful odorant, reminiscent of citrus and tropical fruit. It was initially isolated from passion fruit and contributes greatly to its characteristic aroma (*4*). It has also been identified as one of the key aroma compounds in Sauvignon blanc wines (*5*) and, more recently, in Riesling, Gewürztraminer (*6*), Petit and Gros Manseng (*6*), Cabernet Sauvignon (*7*, *8*), Merlot (*7*, *8*), and Swiss Petite Arvine (*9*). The major sensory impact of 3-sulfanylhexan-1-ol makes its origin and reactivity of great interest in wine chemistry.

Recent research has reported the major contribution of 3-sulfanylhexanol to the aroma of Sauternes botrytized wines (*6*, *10*). Botrytized sweet wines are produced by a particular wine-making process only encountered in a few areas in the world, which contributes to their uniqueness. Indeed, they are produced from overripe grapes affected by the *Botrytis cinerea* fungus under specific climatic conditions, with alternating foggy mornings and sunny afternoons (*11*). Because of the unusual fungal development, the composition of the grapes is deeply modified and

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requires a customized wine-making process. In addition, botrytized wines are generally aged longer in oak barrels than dry white wines. All of these factors contribute to the development of a complex aromatic profile different from that of dry white wines. This exceptional range of aromas may evoke citrus and dried fruits in young botrytized wines, orange peel in older wines, and honey or waxy nuances in wines that undergo oxidative aging (*12*).

Sauternes, as well as Tokaji and late-harvest Riesling wines from Alsace, Rheingau, and Mosel regions, are some of the most prestigious botrytized wine appellations. Sauternes wines are produced in the region of Bordeaux, France, from two grape varieties (i.e., Sémillon and Sauvignon blanc), also used to make dry white wines. In recent years, the typical aromas of Sauternes wines have been largely studied (*10*, *13*−*18*). Recently, Sarrazin et al. (*14*) demonstrated the contribution of 3(2*H*)-furanones (namely, homofuraneol, furaneol, and norfuraneol) and phenylacetaldehyde to caramel and honey nuances, respectively, as well as the major role of volatile thiols in the citrus aromas of Sauternes wines (*10*). Three new sulfanyl alcohols, 3-sulfanylpentan-1-ol, 3-sulfanylheptan-1-ol, and 2-methyl-3-sulfanylbutan-1-ol, in addition to the two well-known thiols, 3-sulfanylhexanol and 4-methyl-4-sulfanylpentan-2-one, have been identified and characterized. Nevertheless, one citrus odoriferous component detected by gas chromatography−olfactometry (GC−O) was not identified (*14*). This odorant, not detected by GC−O in dry white wines made from the same grape varieties (i.e., Sémillon and Sauvignon blanc), was thus apparently specific to botrytized wines; therefore, an experiment was designed to identify it.

The detection and identification of trace compounds in complex matrices, such as wine, generally require numerous purification steps to suppress major volatile compounds (*19*−*21*). Multidimensional gas chromatography, especially heart-cut bidimensional gas chromatography, now offers an elegant alternative approach for identifying trace odorants. This hyphenated technique, combined with olfactometry, has recently been used to identify off-flavors in wines (*22*, *23*) and other alcoholic beverages (*24*, *25*), thus suggesting new solutions for trace analysis.

In this work, we explain the origin of the specific citrus odorant detected by GC−O in Sauternes wine extracts. Sauternes wine was purified via a four-step method and analyzed using multidimensional gas chromatography combined with mass spectrometry and olfactory detection (MDGC−MS−O). The zone of interest was identified as 3-propyl-1,2-oxathiolane, which was synthesized and fully characterized for the first time. The chemical origin of 3-propyl-1,2-oxthiolane detected by GC−O was then investigated.

MATERIALS AND METHODS

Wine Samples. The botrytized wines analyzed in this study were all from the Bordeaux region and the 2003 vintage. They were produced from Sémillon and Sauvignon blanc grapes, according to standard winemaking procedures.

Standard Products. Dichloromethane (Chromasolv grade), diethyl ether, ethyl acetate, sodium *p*-hydroxymercuribenzoate, Trizma base (99%), and anhydrous ethyl acetate were purchased from Sigma-Aldrich Chemicals (Saint Quentin Fallavier, France). *N*,*O*-Bis(trimethylsilyl)-trifluoroacetamide (BSTFA) in mixture with 1% trimethylchlorosilane (BSTFA−Regisil) was from Regis Technologies (Morton Grove, IL). Anhydrous pyridine was purchased from Interchim (Montluçon, France). Ethanol (Lichrosolv grade) was supplied by Merck (Martillac, France). Pentane (Prolabo Normapur grade) was purchased from VWR (Fontenay-sous-Bois, France) and distilled to improve its quality. 3-Sulfanylhexanol (>95%) and the chemical reagents used for chemical synthesis were obtained from Alfa Aesar (Bischheim, France). Anhydrous sodium sulfate was supplied by VWR (Fontenay-sous-Bois, France).

Identification of 3-Propyl-1,2-oxathiolane (4). *Liquid*−*Liquid Extraction*. Sauternes wine (4.5 L) was extracted with dichloromethane (2 × 600 mL), with magnetic stirring, for 10 min each time. The combined organic phases were centrifuged (4000 rpm) for 15 min and separated in a separating funnel. They were then washed with sodium *p*-hydroxymercuribenzoate solution [1 mM in Trizma base (2-amino-2-(hydroxymethyl)-1,3-propanediol) at 0.2 M] (2 × 120 mL), for 10 min each time. The organic phase obtained was dried over anhydrous sodium sulfate and concentrated to 2 mL using a rotary evaporator.

High-Performance Liquid Chromatography (*HPLC*) *Fractionation*. The concentrate was purified by reverse-phase HPLC, using the method developed by Pineau et al. (*26*). The column used was a 318 mm × 3.9 mm inner diameter, 4 μm, Novapak C18 (Waters, Guyancourt, France). The chromatographic conditions included a flow rate of 0.5 mL/min and an injection volume of 250 μL. The linear program gradient involved phase A, water, and phase B, ethanol, 0% B reaching 100% B in 50 min. A fraction collector (Bio-Rad Laboratories, Marnes-la-Coquette, France) was connected to the end of the column to collect 1 mL eluted solvent every 2 min. The HPLC eluate was recovered in 25 separate fractions, evaluated for their smell.

Fraction Re-extraction. The 25 fractions were again extracted with dichloromethane, as described by Pons et al. (*23*). The alcohol content of the fractions eluted by HPLC was adjusted to 12% (v/v). Then, each fraction was extracted with dichloromethane (3 × 0.5 mL). The solvent extract was concentrated under nitrogen to 200 μL and used for GC−O analysis, as well as MDGC−MS−O analysis.

Extraction and Separation of 3-Sulfanylhexanol Disulfide (6) from Wine. *Liquid*−*Liquid Extraction*. Sauternes wine (4.5 L) was extracted with dichloromethane (300, 150, and 150 mL), with magnetic stirring, for 10 min each time. The combined organic phases were centrifuged (4000 rpm) for 15 min and separated in a separating funnel. The organic phase obtained was dried over anhydrous sodium sulfate and concentrated to 2 mL using a rotary evaporator. The solvent extract was then concentrated under nitrogen to 750 μL.

HPLC Fractionation. The wine extract was purified by normal-phase HPLC. The column used was a 250 mm × 4.6 mm inner diameter, 5 μm, SunFire silica column (Waters, Guyancourt, France). The chromatographic conditions included a flow rate of 1.0 mL/min and an injection volume of 250 μL. The linear program gradient involved phase A, pentane, phase B, ether, and phase C, ethyl acetate: 0−5 min, 100% A; 5−12 min, 100% B; then reaching 80% B and 20% C in 3 min; maintained at 80% B and 20% C for 5 min; then reaching 50% B and 50% C in 3 min; maintained at 50% B and 50% C for 5 min; and finally, reaching 100% C in 10 min. A fraction collector (Bio-Rad Laboratories, Marnes-la-Coquette, France) was connected to the end of the column to collect fractions of the eluted solvent every minute.

Derivatization with BSTFA. Fractions 17−25 were combined, then evaporated to dryness in a vial, and capped under nitrogen flow. The dry residue was resuspended in BSTFA−Regisil solution (20 μL) with anhydrous pyridine (50 μL). The mixture was incubated at 70 °C for 15 min. A total of 2 μL of derivatized sample were analyzed by gas chromatography−mass spectrometry (GC−MS).

Heart-Cut MDGC−MS−O. The MDGC separation was performed on two GC instruments, as described by Pons et al. (*23*). Instrument 1 was a Hewlett-Packard 5890 series II (Agilent Technologies, Massy, France), and Instrument 2 was a 6890 GC coupled with a 5973 quadrupole mass spectrometer (Agilent Technologies, Massy, France). The two instruments were connected with a West 4400 temperature-controlled transfer line set at 230 °C (ILS, Lyon, France). A cryotrap was also placed at the start of the second column for cryofocusing. The column used for pre-separation was a 30 m × 0.25 mm inner diameter, 0.25 μm, SPB1 (Supelco, from Sigma-Aldrich, Saint Quentin Fallavier, France). The second column was a 50 m × 0.25 mm inner diameter, 1.0 μm, BPX5, BPX50, or BPX70 (SGE, Milton Keynes, U.K.). The MDGC system was operated under constant pressure to maintain the balance between the two columns throughout the oven temperature program. The end of the second column was split (1:1) via a crosspiece (Gerstel, from RIC, Lille, France) between the MS detector and the ODP II sniffing port (Gerstel, from RIC, Lille, France). The MS detector functioned in electronic impact (70 eV) or chemical ionization mode (CH_4 reactant). Mass spectra were taken over the m/z 40−300 range.

MDGC Conditions for Wine Extract Analysis. The injector temperature was set at 230 °C. Oven 1 was programmed from 45 °C (1 min) to 250 °C at 3 °C/min, followed by a 40 min isotherm to ensure that all of compounds were eluted. The cut time (28.8−29.8 min) was selected. During this period, the cryogenic trap was maintained at −50 °C. The temperature of oven 2 was initially set at 45 °C for 29.8 min and then raised to 240 °C at 3 °C/min, followed by a 30 min isotherm. Helium 5.3 (Linde Gas, Bassens, France) was used as the carrier gas, with a flow rate of 2 mL/min.

GC−MS Analysis with Exact Mass Measurement Used To Identify 3-Propyl-1,2-oxathiolane (4). Exact mass measurement was kindly performed by Dr. Estelle Delort (Firmenich SA, Geneva, Switzerland). Analyses were carried out using a 7890 GC (Agilent Technologies) coupled to a GCT Premier time of flight mass spectrometer (Waters, Milford, MA). The column used was 30 m × 0.25 mm inner diameter, 0.25 μm, SPB1 (Supelco from Sigma-Aldrich, Saint Quentin Fallavier, France). A total of 1 μL extract was injected using a split injector (250 °C, split ratio of 1:5). The carrier gas was helium, with a flow rate of 1.0 mL/min. For all analyses, the oven temperature program was as follows: 60 °C for 5 min and then a 5 °C/min gradient to 250 °C, followed by a 60 min isotherm. The acquisition time was set to 0.50 s, with an interscan delay of 0.01 s, over a mass range of 1−350 Da. Spectra were recorded in electron impact (EI) mode, using an electron energy of 70 eV, emission current of 608 μA, trap current of 200 μA, and source temperature of 200 °C. Calibration was performed using heptacosa (perfluorotributylamine, mass spectrometry grade, Apollo Scientific Ltd., Bradbury, U.K.). Calibration data were collected for 1 min in centroid mode. A total of 60 spectra were summed to generate a 24-point calibration curve from *m*/*z* 69 to 614. The curve was fitted to a second-order polynomial such that the standard deviation of the residuals was 0.001 amu or lower. Heptacosa was continuously introduced into the ion source, and the ion *m*/*z* 218.9856 was used as a lock mass. Mass spectra and molecular formula were obtained using MassLynx software (Waters). The difference, *d*, between the exact mass calculated from the molecular formula and that measured is calculated by the software and expressed in parts per million (ppm) ($d = (M_{meas.} - M_{calc.})/M_{calc.} \times 10^6$).

GC−O. Analyses were carried out using a Hewlett-Packard 5890 GC, equipped with a flame ionization detector (from Agilent Technologies, Massy, France) and a ODO-1 sniffing port (SGE, Milton Keynes, U.K.). The column used was a 50 m × 0.25 mm inner diameter, 1.0 μm, BPX5 (SGE, Milton Keynes, U.K.). A total of 2 μL of extract was injected using a splitless injector (230 °C; purge time, 1 min; purge flow, 50 mL/min) or an on-column injector, at oven temperature (50 °C). For all analyses, the oven temperature program was as follows: 50 °C for 1 min and then a 3 °C/min gradient to 250 °C, followed by a 15 min isotherm. The carrier gas was hydrogen (Linde Gas, Bassens, France), with a flow rate of 1.0 mL/min.

GC−MS. Analyses were carried out using a 6890−5973 GC−MS (Agilent Technologies, Massy, France). The column used was a 50 m × 0.25 mm inner diameter, 1.0 μm, BPX5 (SGE, Milton Keynes, U.K.). A total of 2 μL of extract was injected using a splitless injector (230 °C; purge time, 1 min; purge flow, 50 mL/min) or an on-column injector, at oven temperature (50 °C). For all analyses, the oven temperature program was as follows: 50 °C for 1 min and then a 3 °C/min gradient to 250 °C, followed by a 15 min isotherm. The carrier gas was hydrogen (Linde Gas, Bassens, France), with a flow rate of 1.0 mL/min. The 5973 MS detector, functioning in EI mode (70 eV), was connected to the GC via a transfer line heated to 250 °C. Mass spectra were taken in the SCAN mode over the *m*/*z* 40−300 range.

GC−MS Analysis with Exact Mass Measurement Was Used To Identify 3-Sulfanylhexanol Disulfide (6) in Wine. Analyses were carried out using a 7890 GC (Agilent Technologies, Massy, France) coupled to an AccuTOF JMS-T100GC time of flight mass spectrometer (JEOL Europe, Croissy sur Seine, France). The column used was 60 m × 0.25 mm inner diameter, 0.25 μm, DB1 (J&W, from Agilent Technologies, Massy, France). A total of 2 μL extract was injected using a splitless injector (240 °C; purge time, 1 min; purge flow, 50 mL/min). The carrier gas was helium, with a flow rate of 1.0 mL/min. For all analyses, the temperature program was as follows: 45 °C for 1 min, then a 5 °C/min gradient to 200 °C, 3 °C/min to 240 °C, and then 5 °C/min to 280 °C, followed by a 15 min isotherm. The JEOL detector, functioning in EI mode (70 eV), was connected to the GC via a transfer line heated to 250 °C.

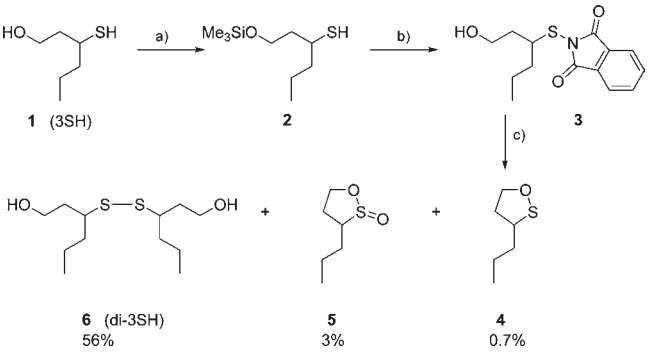

Figure 1. Synthesis of of 3-propyl-1,2-oxathiolane (**4**), 3-propyl-γ-sultine (**5**), and 3,3′-dithiobishexan-1-ol (**6**). Reagents and conditions: (a) Me_3SiCl, Et_3N, and THF; (b) *N*-bromophthalimide and Py; and (c) pyrolysis *in vacuo*.

Mass spectra were recorded over the *m*/*z* 30−550 range, with 5 ppm sensitivity.

Synthesis. *Experimental Equipment.* ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra, as well as 2D NMR experiments, were recorded with a Bruker AC-300 FT (^1H, 300 MHz; ^{13}C, 75 MHz). Chemical shifts (δ) and coupling constants (*J*) are expressed in ppm and Hz, respectively. No internal standard was present in NMR samples. Spectra were referenced using the frequency of the deuterated solvent (the lock frequency). Standard Bruker pulse sequences were used for homo- and heteronuclear correlation experiments [correlation spectroscopy (COSY), heteronuclear multiple-quantum coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC)]. Infrared (IR) spectra were recorded with a Perkin−Elmer Paragon 1000 FTIR spectrophotometer. Thin-layer chromatography (TLC) was performed on TLC plates: thickness, 0.25 mm; particle size, 15 μm; pore size, 60 Å (SDS, Peypin, France). Merck silica gel 60 (70−230 mesh and 0.063−0.200 mm) was used for flash chromatography. Spots were revealed with ultraviolet (UV) as well as $KMnO_4$ (0.05% in water). Tetrahydrofuran (THF) was dried by refluxing a solution containing sodium wires and benzophenone under nitrogen and distilled immediately before use. All moisture-sensitive reactions were carried out under an argon atmosphere in oven- or flame-dried glassware.

Synthesis of 3-Propyl-1,2-oxathiolane, 3-Propyl-γ-sultine, and 3,3′-Dithiobishexan-1-ol. The compounds were synthesized from 3-sulfanylhexanol (**1**) using an optimized procedure, similar to that proposed by Davis and Whitham (*27*) (**Figure 1**). Freshly distilled chlorotrimethylsilane (6.32 mL, 49.5 mmol, 1.1 equiv) was added dropwise to a solution of 3-sulfanylhexanol (6.03 g, 6.2 mL, 45 mmol) and freshly distilled triethylamine (5 g, 6.9 mL, 49.5 mmol, 1.1 equiv) in anhydrous THF (50 mL), at room temperature, under an argon atmosphere. The mixture was stirred for 16 h. The white precipitate that formed was filtered, and the solvent evaporated under reduced pressure. The colorless, viscous liquid containing 1-*O*-trimethylsilyl-3-hexanthiol (**2**) (9.2 g, 99% purity by GC−MS and NMR) was immediately dissolved in anhydrous pyridine (10 mL, 8 equiv) under an argon atmosphere and cooled to 0 °C. *N*-Bromophthalimide (12.21 g, 54 mmol, 1.2 equiv) was added in one portion. The resulting mixture was stirred at 0 °C for 1 h, then allowed to warm to room temperature overnight, and filtered. The filtrate was washed with 30% citric acid aqueous solution (180 mL). The aqueous layer was extracted with diethyl ether (2 × 100 mL). Combined organic layers were washed with brine (100 mL), dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give 2-(1-hydroxyhexan-3-ylthio)isoindoline-1,3-dione (**3**) as an orange oil (9 g, 62%). A Kugelrohr short-path vacuum distillation apparatus was used for the thermal decomposition of **3** and the fractionation of the resulting compounds, by smooth heating *in vacuo* as follows: at 60 °C and 9 mmHg for 5 min, 80 °C and 9 mmHg for 10 min, 80 °C and 1 mmHg for 1 min, and finally, 100 °C and 1 mmHg for 2 h. Three fractions were collected and characterized by NMR (^1H, ^{13}C, HMBC, HMQC, and COSY), IR, and GC−MS. The first fraction, collected in a cold trap (liquid nitrogen), gave 3-propyl-1,2-oxathiolane (**4**) as a colorless liquid (40 mg, 0.7%, ∼85% purity by GC−MS and NMR). Compound **4** was highly unstable and oxidized rapidly to 3-propyl-γ-sultine (3-propyl-1,2-oxathiolane-2-oxide) (**5**). The distillate collected in the first receiving bulb contained compound **5**. This fraction

was further purified by silica gel chromatography, using petroleum ether/diethyl ether (50:50), to afford compound **5** as a colorless liquid (0.2 g, 3%, +95% purity by GC−MS and NMR). NMR and IR data were similar to those already reported (*28*, *29*), indicating that 3-propyl-γ-sultine consisted of a mixture of the two pairs of diastereoisomers [form *trans* corresponds to the unlike (2*S*3*R* + 2*R*3*S*) mixture of stereoisomers, and form *cis* corresponds to the like (2*R*3*R* + 2*S*3*S*) mixture of stereoisomers]. In our case, the compound **5** was obtained in 75:25 *trans*/*cis* ratio and cannot be separated by standard silica gel chromatography. The anisotropic effect of the sulfinyl functional group on nearby nuclei produced the deshielding of H-3 and H-6 signals in *trans* and *cis* pairs, respectively. Together with 2D NMR analysis, it allowed the full ^{1}H and ^{13}C chemical-shift assignment for both *trans* and *cis* products.

The last fraction, obtained from the distilling flask (pot flask) and the first collecting flask, contained the disulfide (**6**). It was purified by silica gel chromatography, using petroleum ether/diethyl ether (50:50), to give compound **6** as a colorless liquid (6.7 g, 56%, >99% purity by GC−MS and NMR). The MS data were identical to those previously published (*30*).

1-O-Trimethylsilyl-3-hexanthiol (*2*). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.13 (s, 9H, $Si(CH_3)_3$), 0.91 (t, *J* = 6.6 Hz, 3H, CH_3-6), 1.34 (d, *J* = 7.15, 1H, SH), 1.37−1.69 (m, 5H, CH_2-2a, CH_2-4, CH_2-5), 1.91 (m, 1H, CH_2-2b), 2.95 (m, 1H, CH-3), 3.75 (m, 2H, CH_2-1). MS (70 eV), *m*/*z* (%): 206 (1) [M^+], 191 (4), 157 (39), 143 (8), 129 (55), 121 (29), 116 (67), 103 (34), 91 (56), 88 (68), 83 (55), 73 (100), 55 (43), 47 (9), 45 (14).

2-(1-Hydroxyhexan-3-ylthio)isoindoline-1,3-dione (*3*). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (t, *J* = 7.2 Hz, 3H, CH_3-6), 1.33−1.90 (m, 6H, CH_2-2, CH_2-4, CH_2-5), 2.86 (m, 1H, CH-3), 2.92 (br s, 1H, OH), 3.65−3.85 (m, 2H, CH_2-1), 7.78 (d, *J* = 8.6, 2H, CH_{ar}), 7.91 (d, *J* = 8.6, 2H, CH_{ar}).

3-Propyl-1,2-oxathiolane (*4*). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (t, *J* = 7.2 Hz, 3H, CH_3-8), 1.32−1.60 (m, 2H, CH_2-7), 1.35−1.78 (m, 2H, CH_2-6), 1.81−2.05 (m, 1H, CH-4b), 2.15−2.33 (m, 1H, CH-4a), 3.16−3.29 (m, 1H, CH-3), 3.33−3.85 (m, 2H, CH_2-5). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0 (CH_3-8), 22.5 (CH_2-7), 37.0 (CH_2-4), 38.5 (CH_2-6), 53.5 (CH-3), 75.1 (CH_2-5). COSY data: H-8 → H-7; H-7 → H-6, H-8; H-6 → H-3, H-7; H-4b → H-3, H-4a, H-5; H-4a → H-3, H-4b, H-5; H-3 → H-4a, H-4b, H-6; H-5 → H-4a, H-4b. IR, ν (cm^{-1}): 480, 733, 1055, 1464, 3500. MS (70 eV), *m*/*z* (%): 132 (40) [M^+], 103 (4), 89 (65), 83 (19), 77 (14), 67 (7), 61 (15), 55 (100), 45 (16), 43 (12). HR-MS (EI, 70 eV), *m*/*z*: 132.0644 for $[M]^+$ (calcd for $[C_6H_{12}OS]^+$, 132.0609), 89.0054 for $[C_3H_5OS]^+$ (calcd, 89.0061).

3-Propyl-γ-sultine (*5*). IR, ν (cm^{-1}): 732, 903, 1125, 1465.

MS (70 eV), *m*/*z* (%): 148 (3) [M^+], 83 (21), 78 (5), 69 (5), 56 (10), 55 (100), 43 (10), 42 (10).

cis Isomer. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.99 (t, *J* = 7.2 Hz, 3H, CH_3-8), 1.45−1.60 (m, 2H, CH_2-7), 1.66−1.95 (m, 2H, CH_2-6), 2.15−2.32 (m, 1H, CH_2-4), 2.90−3.04 (m, 1H, CH-3), 4.19−4.38 (m, 1H, CH_2-5a), 4.73−4.88 (m, 1H, CH_2-5b). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.0 (CH_3-8), 21.7 (CH_2-7), 28.0 (CH_2-4), 28.8 (CH_2-6), 68.7 (CH-3), 75.1 (CH_2-5). COSY data: H-8 → H-7; H-7 → H-6, H-8; H-6 → H-3, H-7; H-4 → H-3, H-5a, H-5b; H-3 → H-4, H-6; H-5b → H-4, H-5a; H-5a → H-4, H-5b; HMQC data: H-8 → C-8, H-7 →C-7, H-6 → C-6, H-4 → C-4, H-3 → C-3, H-5b → C-5, H-5a → C-5; HMBC data: H-8 → C-6, C-7; H-7 → C-3, C-6, C-8; H-6 → C-3, C-4, C-7, C-8; H-4 → C-3, C-6; H-3 → C-4, C-6; H-5b → C-4; H-5a → C-3, C-4.

trans Isomer. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.97 (t, *J* = 7.2 Hz, 3H, CH_3-8), 1.32−1.45 (m, 1H, CH_2-6a), 1.45−1.60 (m, 2H, CH_2-7), 1.60−1.70 (m, 1H, CH_2-6b), 1.78−1.95 (m, 2H, CH_2-4a), 2.58−2.70 (m, 1H, CH_2-4b), 3.15−3.28 (m, 1H, CH-3), 4.45−4.52 (m, 1H, CH_2-5a), 4.73−4.86 (m, 1H, CH_2-5b). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.8 (CH_3-8), 21.2 (CH_2-7), 29.1 (CH_2-4), 31.2 (CH_2-6), 73.3 (CH-3), 74.7 (CH_2-5). COSY data: H-8 → H-7; H-6b → H-3, H-6a, H-7; H-7 → H-6a, H-6b, H-8; H-6a → H-3, H-6b, H-7; H-4b → H-3, H-4a, H-5a, H-5b; H-4a → H-3, H-4b, H-5a, H-5b; H-3 → H-4a, H-4b, H-6a, H-6b; H-5b → H-4a, H-4b, H-5a; H-5a → H-4a, H-4b, H-5b; HMQC data: H-8 → C-8, H-7 →C-7, H-6b → C-6, H-6a → C-6, H-4b → C-4, H-4a → C-4, H-3 → C-3, H-5b → C-5, H-5a → C-5. HMBC data: H-8 → C-6, C-7; H-6b → C-3, C-4, C-7, C-8; H-7 → C-3, C-6, C-8; H-6a → C-3, C-4, C-7, C-8; H-4b → C-3, C-6; H-4a → C-3, C-6; H-3 → C-5, C-6; H-5b → C-3, C-4; H-5a → C-3, C-4.

3,3′-Disulfanediyldihexan-1-ol (*6*). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.92 (t, *J* = 7.3 Hz, 6H, CH_3-6, CH_3-6′), 1.35−1.65 (m, 8H, CH_2-4, CH_2-4′, CH_2-5, CH_2-5′), 1.83−1.93 (m, 4H, CH_2-2, CH_2-2′), 2.85 (apparent p, *J* = 6.4 Hz, 2H, CH-3, CH-3′), 3.10 (br s, 2H, OH), 3.70−3.90 (m, 4H, CH_2-1, CH_2-1′). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 13.7 and 13.9 (CH_3-6 and CH_3-6′), 20.0 and 20.05 (CH_2-5 and CH_2-5′), 36.9, 37.0, 37.1, and 37.15 (CH_2-2, CH_2-2′, CH_2-4, and CH_2-4′), 59.9 and 60.0 (CH_2-1 and CH_2-1′), 48.7 and 49.6 (CH-3 and CH-3′). COSY, HMQC and HMBC data (not shown) confirmed the signal attribution. IR, ν (cm^{-1}): 549, 734, 1054, 1465, 1727, 3373. MS (70 eV), *m*/*z* (%): 266 (16) [M^+], 148 (23), 115 (9), 101 (10), 84 (5), 83 (83), 57 (6), 55 (100), 45 (6), 43 (14).

1,2-Bis(1-O-trimethylsilylhexan-3-yl)disulfide (*7*). The compound **6** was silylated as already described for the synthesis of **2**.

^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.13 (s, 18H, $Si(CH_3)_3$), 0.93 (t, *J* = 7.3 Hz, 6H, CH_3-6, CH_3-6′), 1.39−1.51 (m, 4H, CH_2-5, CH_2-5′), 1.54−1.68 (m, 4H, CH_2-4, CH_2-4′), 1.83 (apparent q, *J* = 6.4 Hz, 4H, CH_2-2, CH_2-2′), 2.79 (apparent p, *J* = 6.4 Hz, 2H, CH-3, CH-3′), 3.66−3.80 (m, 4H, CH_2-1, CH_2-1′). ^{13}C NMR (75 MHz, $CDCl_3$) δ: −0.47 (Si-CH_3), 13.9 (CH_3-6 and CH_3-6′), 19.9 and 20.0 (CH_2-5 and CH_2-5′), 36.5, 36.6, 37.0, and 37.1 (CH_2-2, CH_2-2′, CH_2-4, and CH_2-4′), 48.3 and 48.4 (CH-3 and CH-3′), 59.9 and 60.0 (CH_2-1 and CH_2-1′). COSY, HMQC and HMBC data (not shown) confirmed the signal attribution. MS (70 eV), *m*/*z* (%): 410 (10) [M^+], 310 (3), 173 (14), 157 (3), 148 (12), 129 (3), 122 (5), 115 (8), 103 (61), 91 (9), 83 (100), 75 (14), 73 (63), 55 (28). HR-MS (EI, 70 eV), *m*/*z*: 410.2183 $[M]^+$ (calcd for $[C_{18}H_{42}O_2S_2Si_2]^+$, 410.2165).

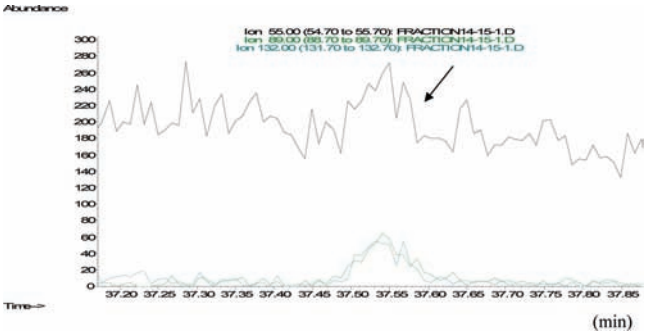

Figure 2. Partial GC chromatogram of wine fractions 14−15 obtained with three selected ions (*m*/*z* 55, 89, and 132). The retention time of the peak indicated by an arrow corresponds to 3-propyl-1,2-oxathiolane.

RESULTS AND DISCUSSION

Identification of 3-Propyl-1,2-oxathiolane. The volatile compounds from a total of 4.5 L Sauternes wine were isolated by liquid−liquid extraction with dichloromethane. Acidic compounds, as well as volatile thiols, were removed by washing the organic extract with an alkaline mercury solution. Because the citrus compound could not be accurately identified from this extract by MDGC−MS−O, the extract was further purified to avoid coelution, using C_{18} reverse-phase HPLC, according to the method developed by Ferreira et al. (*31*) and adapted by Pineau et al. (*26*). This separation afforded 25 fractions that were extracted again and analyzed by GC−O on a BPX5 capillary column. A citrus odorant was detected in fractions 14−15 at the target linear retention index (1105) (*14*). The two fractions were pooled and analyzed by MDGC−MS−O, using a SPB1 column in the first oven and a BPX5 column in the second oven. Mass spectrometry combined with olfactory detection produced a fragmentation pattern corresponding to the odorant (**Figures 2** and **3A**). Repeat MDGC−MS−O analysis, using the same fractions but changing the column in the second oven (using either BPX70 or BPX50 columns), confirmed this result. Linear retention indices on these two capillaries were 1592 and 1236, respectively.

Chemical ionization mode was performed to obtain the molecular weight of the citrus compound (M, 132; $[M + H]^+$, 133) (data not shown). Subsequently, the exact mass measurement was used to determine the elemental formula as $C_6H_{12}OS$, revealing one degree of unsaturation in the structure. The MS pattern, presenting only a few fragmentations, was indicative of a cyclic structure.

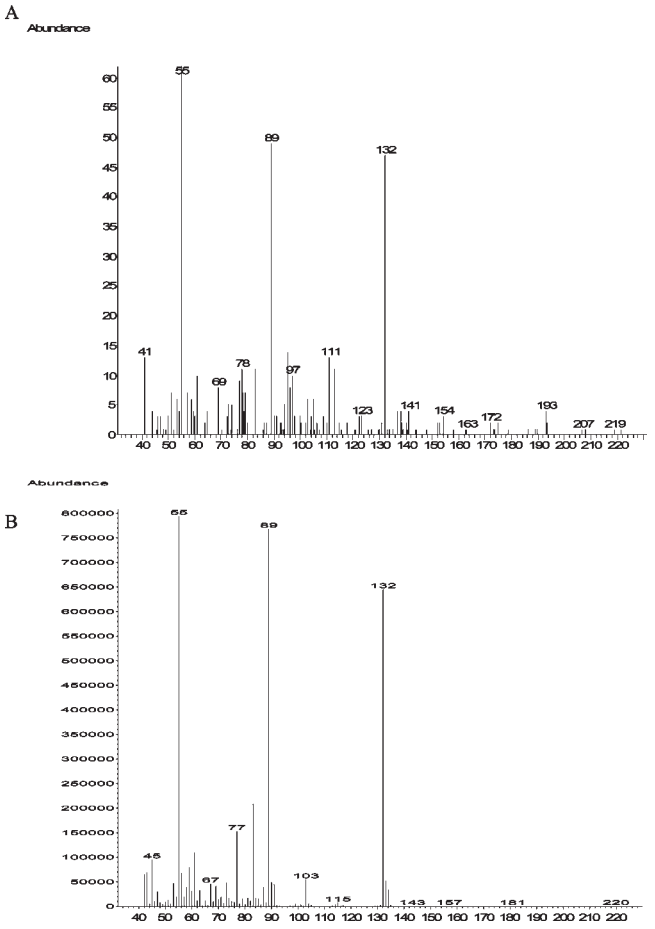

Figure 3. Mass spectrum of 3-propyl-1,2-oxathiolane detected in the wine: (A) fractions 14−15 and (B) synthesized.

Moreover, the loss of m/z 43 corresponded to the loss of a propyl radical. An initial hypothesis concerning the unusual structure of 3-propyl-1,2-oxathiolane was formulated on the basis of these data. To our knowledge, this compound had never previously been reported. To confirm its structure, it was synthesized by a pathway similar to that proposed by Davis and Whitham (*27*) for synthesizing 1,2-oxathiolane. The synthesis consisted of three steps (**Figure 1**). First, the hydroxy group of 3-sulfanylhexanol (**1**) was protected via silylation, and then *N*-bromophtalimide derivative (**3**) was formed and pyrolyzed to give three oxidation products: 3-propyl-1,2-oxathiolane (**4**), 3-propyl-γ-sultine (**5**), and disulfide (**6**). Synthetic 3-propyl-1,2-oxathiolane was reminiscent of citrus and grapefruit, in agreement with the olfactory descriptors used for the target odorant. Its mass spectrum and linear retention indices, identical to data obtained from wine fractions, confirmed its identification (**Figure 3B**). To the best of our knowledge, this was the first time that 3-propyl-1,2-oxathiolane was synthesized and fully characterized (^{1}H and ^{13}C NMR, 2D NMR, and IR).

Unfortunately, 3-propyl-1,2-oxathiolane proved to be very unstable and quickly oxidized to 3-propyl-γ-sultine. Furthermore, the citrus odor was detected by GC−O using a high-temperature injection mode but not when the Sauternes wine extract was injected at room temperature, using an on-column injector. The origin of 3-propyl-1,2-oxathiolane thus required further clarification.

Origin of 3-Propyl-1,2-oxathiolane. One key odorant in Sauternes wine is 3-sulfanylhexanol (**1**) (*6*, *10*). This compound, as well as its oxidative dimerization product, the disulfide (**6**), are also found in yellow passion fruit (*30*). 3-Sulfanylhexanol was

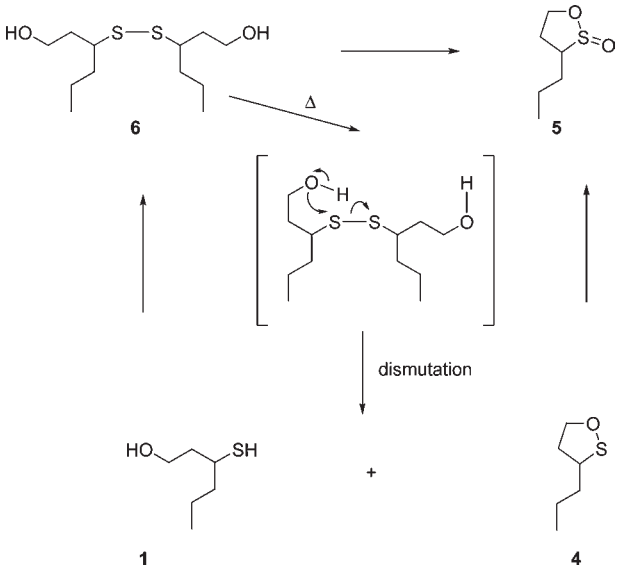

Figure 4. Oxidation pathway of 3-sulfanylhexanol (**1**) demonstrated by the thermal degradation of compound **6** in the GC port and chemical oxidation experiments.

reported to undergo chemical oxidation using sodium metaperiodate (*32*). Dependent upon the oxidizing agent amount and reaction time, the nature and ratio of final products varied and led to either the disulfide (**6**) or 3-propyl-γ-sultine (**5**) (*32*). Afterward, the compound **5**, the final oxidation product of 3-sulfanylhexanol, was identified in yellow passion fruit (*28*). Therefore, the disulfide (**6**) was hypothesized to oxidize to the corresponding γ-sultine (**5**) via a dismutation mechanism, with simultaneous formation of 3-sulfanylhexanol (**1**) (*28*). Because the new compound, 3-propyl-1,2-oxathiolane (**4**), was identified, a more complete mechanism was suggested on the basis of its sulfur oxidation state (**Figure 4**). This mechanism is in agreement with the oxidation pathways already proposed for sulfur-containing organic compounds and based on a high affinity of neighboring hydroxyl groups for electrophilic sulfur atom (*28*, *33*, *34*). 3-Propyl-1,2-oxathiolane (**4**) was hypothesized to be an intermediate oxidizing product between compounds **5** and **6**, resulting from the dismutation of the disulfide (**6**). To validate this hypothesis, synthetic compound **6** and compound **5** were analyzed by GC−MS, using either a 50 or 230 °C injector. GC−MS analysis indicated that compound **4** was detected when synthetic compound **6** was injected at 230 °C but not at 50 °C. In addition, compound **4** was not formed when compound **5** was injected at either temperature. Consequently, the formation of compound **4** was demonstrated to result from the thermal degradation of the disulfide (**6**). To confirm these results, synthetic compound **6** was injected at varying GC injector temperatures and the products obtained were analyzed. The total ion current (TIC) area percentage of compound **6** was shown to decrease as the GC injector temperature increased (**Table 1**). At the same time, the TIC area percentages of compounds **1**, **4**, and **5** increased, confirming that they were formed in the GC injector. The disulfide (**6**) undergoes oxidative cleavage to compounds **1** and **4**, in accordance with internal Cannizzaro-like rearrangement mechanism. Because the parts of the GC contain only oxygen-free carrier gas, the oxygen needed for further oxidation of compound **4** to compound **5** must already be present in trace concentrations in the sample preparation before injection. Therefore, because compound **4** was formed via the thermal dismutation of compound **6** and oxidized quickly to compound **5**, it was revealed to be the intermediate oxidizing product between compounds **6** and **5**.

Table 1. Influence of the GC Injector Temperature on the Degradation of 3-Sulfanylhexan-1-ol Disulfide (**6**)[a]

		GC injector temperature (°C)			
	retention time (min)	200	230	250	300
3-propyl-1,2-oxathiolane (**4**)	35.3	<0.1%	0.3%	0.3%	0.1%
3-sulfanylhexanol (**1**)	38.7	0.1%	0.3%	0.4%	0.7%
3-propyl-γ-sultine (isomer 1) (**5**)	45.8	<0.1%	<0.1%	0.1%	0.2%
3-propyl-γ-sultine (isomer 2) (**5**)	46.6	0.1%	0.1%	0.1%	0.1%
disulfide (**6**)	83.9	99.7%	99.3%	99.1%	98.9%

[a] TIC area percentage of the products detected (analysis performed on a BPX5 capillary column).

In addition, GC−MS analysis of the mixture obtained following thermolysis of compound **6** at 200 °C in a sealed ampule for 17 h revealed the quasi-total decomposition of compound **6**, with the formation of compounds **1**, **4**, **5**, and other degradation products (data not shown). This oxidation pathway was further confirmed by the reaction between compound **6** and *N*-bromosuccinimide (NBS), a soft oxidative reagent (*35*). Treating compound **6** with 1 equiv of NBS in dichloromethane at room temperature for 1 h gave compound **5** as the principal compound (90% yield). GC−MS analysis of the crude revealed the presence of compounds **1** (3%) and **4** (2%), but the latter disappeared rapidly as it oxidized to compound **5**. All of these experiments clearly confirmed that the oxidation of the disulfide (**6**) led to the formation of both compound **1** and the corresponding γ-sultine (**5**) via 3-propyl-1,2-oxathiolane (**4**), the molecule identified and characterized in this work. To our knowledge, this was the first time that this oxidation pathway was demonstrated experimentally. Consequently, the identification of compound **4** in Sauternes wines by GC−MS analysis provided indirect evidence of the presence of the disulfide (**6**) and not of compound **5**.

Identifying 3-Sulfanylhexanol Disulfide (6). To validate this observation, we developed a purification procedure for isolating the disulfide (**6**) from Sauternes wine, using normal-phase HPLC. First, a botrytized wine extract supplemented with synthetic compound **6** was used to optimize the elution conditions as well as to determine the retention time of the disulfide, which was found in fractions 17−25. The method was then applied to a botrytized wine extract. Fractions 17−25 were assembled, concentrated, and analyzed by GC−MS. Using an apolar column, the retention time of the disulfide (**6**) was 83.9 min, whereas the retention times of 3-sulfanylhexanol and 3-propyl-γ-sultine were 38.7 and 45.8−46.6 min, respectively. Compound **6** eluted at the maximal oven temperature (250 °C) and was thus poorly volatile. This result was in agreement with the work of Werkhoff et al., who only described a linear retention index above 2600 on a DB-Wax column for compound **6** (*30*). Because compound **1** is detected in wine at trace concentrations (generally under 10 ppb), the levels of the disulfide (**6**) were supposed to be in the same concentration range in wine. Consequently, we derivatized compound **6** to increase its volatility and improve its detection. The Sauternes-concentrated fraction pool was derivatized using BSTFA and analyzed immediately by GC−MS. We obtained a signal corresponding to the MS pattern of the silylated disulfide (**7**) at the same retention time as the extract supplemented with the synthetic disulfide (**Figure 5**). Identification was then confirmed by analyzing the same concentrated sample using a time of flight high-resolution mass spectrometer (**Figure 6**). As far as we know, this was the first time that the disulfide (**6**) had been identified in wine.

As previously reported, the odoriferous zone corresponding to 3-propyl-1,2-oxathiolane (**4**) was detected by GC−O in botrytized Sauternes wines but not in dry white wines made from the same grape varieties (*14*). This indicated that the disulfide (**6**) was preferentially present in botrytized wines. To validate this observation, a dry white Bordeaux wine was extracted, derivatized using BSTFA, and then analyzed by GC−MS TOF. A signal corresponding to the silylated disulfide (**7**) was obtained, but its intensity was near the detection limit. This proved the formation of the disulfide (**6**) during wine aging thanks to the unique composition of the botrytized wine. Various studies have demonstrated a decrease in volatile thiol levels in the presence of oxygen during wine aging and after bottling (*36*−*38*). Indeed, volatile thiols have been established to react with polymeric phenolic compounds in wine in the presence of oxygen, via nucleophilic-acid-catalyzed substitutions. More recently, compound **1** and other wine volatile thiols have also been shown

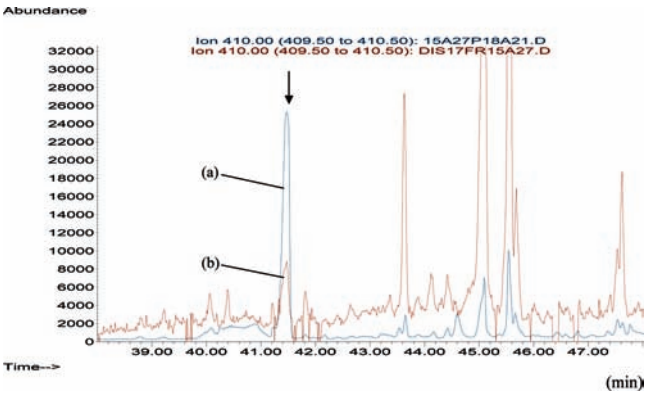

Figure 5. Partial GC chromatogram of the BSTFA-derivatized wine fraction (a) supplemented or (b) not with synthetic disulfide (**6**). Chromatogram obtained with the selected ion *m*/*z* 410. The retention time of the peak indicated by an arrow corresponds to BSTFA-derivatized disulfide (**7**).

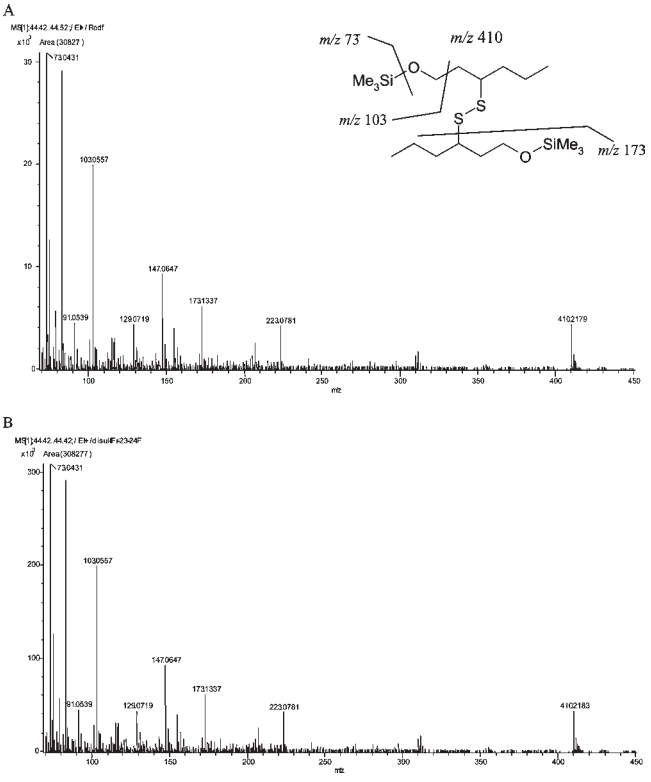

Figure 6. High-resolution mass spectrum of silylated 3-sulfanylhexan-1-ol disulfide (**7**) detected in the wine: (A) fractions 17−25 and (B) synthesized.

to react readily with two main flavonoids [(−)-epicatechin and (+)-catechin] via an iron-catalyzed mechanism (*39*). However, the broad enzymatic pool of *B. cinerea* induces significant changes in the chemical composition of the grapes used in botrytized wines (*11*). Among the fungal activities of *B. cinerea*, the laccase (benzendiol oxygen oxidoreductase) oxidizes a wide range of grape phenolic substrates, leading to the formation of quinones, followed by their polymerization and precipitation (*40*−*42*). The phenolic composition of botrytized grapes and wines is thus deeply modified. This may contribute to the explanation of the singular reactivity of compound **1** in botrytized wines in comparison to dry white wines, thus bringing new insights into the evolution of the wine key aroma compound **1**.

1,3-Sulfanylalcohols, especially compound **1**, play a major role in the aroma of various food products. It is thus challenging to extend our knowledge of their reactivity and stability in natural matrices. Our work focused on Sauternes wine aroma and identified a new citrus odorant, compound **4**, detected by GC−O in Sauternes wine extracts. This unusual compound was shown to result from the thermal oxidative degradation of the disulfide (**6**) in the GC injector, which was then firmly identified in Sauternes wine extracts. Although the presence of 3-sulfanylhexanol oxidation products had previously been reported in natural extracts (but not wine), the full oxidation pathway from compounds **1** to **5** via a disulfide intermediate (**6**) was clearly established in this research for the first time. This finding may be generalized to other 1,3-sulfanylalcohols, thus bringing new insights into the sensory stability of natural substances. In particular, because compound **1** has a high olfactory impact in various wines, further work is now required to investigate the presence of the disulfide (**6**) in other botrytized and non-botrytized wines, as well as to explore the presence of the sultine (**5**) in Sauternes botrytized wines.

ACKNOWLEDGMENT

This paper is dedicated to the memory of Dr. Takatoshi Tominaga, who suddenly passed away on June 8, 2008. Takatoshi devoted his life to understand the mysteries of wine aroma. His passion, knowledge, and friendship are deeply missed.

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