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Identification of Impact Odorants Contributing to Fresh Mushroom Off-Flavor in Wines: Incidence of Their Reactivity with Nitrogen Compounds on the Decrease of the Olfactory Defect

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Supporting Information

ABSTRACT: Analysis of wines from different grape varieties marked by sometimes intense aromatic nuances of fresh mushroom was performed by gas chromatography coupled with olfactometry. This analysis has led to the identification of several odoriferous zones, which were recalling a fresh mushroom odor. Two trace compounds responsible for these odoriferous zones, 1-nonen-3-one and 1-octen-3-one, have been identified and their content has been determined by using either a multidimensional gas chromatography technique coupled to olfactometry and mass spectrometry detection (in the case of 1-nonen-3-one) or the preparation of the derivative with *O*-2,3,4,5,6-pentafluorobenzylhydroxylamine hydrochloride in the presence of the deuterated form, as the internal standard (in the case of 1-octen-3-one), then gas chromatography coupled to mass spectrometry detection. The assays allowed the quantification of these compounds at concentration levels sometimes well above their detection and recognition olfactory threshold. We show that adding nitrogen compounds to the altered wines, such as an amino acid (glycine) or a tripeptide (glutathione), led to lower concentrations of 1-octen-3-one in wines and diminished smell of fresh mushrooms. The study of the reaction in a model medium, whose composition is close to wine, by liquid chromatography coupled to mass spectrometry demonstrated the formation of adducts between 1-octen-3-one and glycine, and 1-octen-3-one and glutathione characterized by NMR.

KEYWORDS: 1-Nonen-3-one, 1-octen-3-one, 1-octen-3-one-8,8,8-d3, 1-octen-3-ol, *Vitis vinifera*, bunch rot complex, wine, NMR

INTRODUCTION

In enology, experience shows that knowledge of the compounds involved in various nuances of wine aroma makes a major contribution to improving organoleptic quality. This is particularly true for compounds responsible for off-odors, as identification and quantification are the first step in developing processes and methods to minimize their presence in wine.[1]

Some of these off-odors are related to fungal notes, reminiscent of damp earth, camphor, mold, and fresh mushrooms. The presence of these aromatic nuances is sometimes attributed to contact between must or wine and materials (tanks, oak-barrels, and stoppers) that have been polluted during vinification or barrel- or bottle-aging.[1−3] In recent years, several of these aromatic defects have been associated with the use of grapes affected by more or less visible rot, due to *Botrytis cinerea* and various species in the *Penicillium* genus.[4] Among the compounds responsible for the defects, (−)-geosmin and 2-methylisoborneol were identified as producing powerful earthy smells.[4,5] The (−)-geosmin in grapes was found to originate from the *Penicillium* fungus metabolism, particularly *P.expansum*, known to be involved in a bunch rot complex with *B. cinerea*.[6] The *B. cinerea* metabolism itself produces 2-methylisoborneol.[4,7] Fortunately, during alcoholic fermentation and wine aging, 2-methylisoborneol is degraded, unlike (−)-geosmin which gives wines a sustainable earthy off-flavor.[4] However, in recent years, organoleptic defects reminiscent of fresh mushroom have been highlighted in some wines, sometimes at a high-level of intensity. These aromatic nuances are not common in wines but sometimes occur in newly fermented wines that are exposed to light.

Considering the compounds that may be involved in such aromatic nuances, the literature reports that fresh mushroom odors in food products are usually associated with the presence of 8 and 9 carbon-unsaturated aliphatic compounds with an alcohol or carbonyl function, including 1-octen-3-ol, 1-octen-3-one, 1-nonen-3-ol, and 1-nonen-3-one.[8,9] Among this group, the

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carbonyls (1-octen-3-one and 1-nonen-3-one) have the most powerful odor.[8] 1-Octen-3-one may be formed by chemical autooxidation of unsaturated fatty acids in many foods,[10−13] particularly under the action of light,[10,14] or via the reaction of peroxides in the skin with ferrous ions.[15] 1-Octen-3-ol and 1-octen-3-one are also metabolites of many fungal species from basidiomycetes[16] and ascomycetes phyla.[17−19] These compounds are also produced by a species of orchids, *Dracula chestertonii*.[20] 1-Nonen-3-one was first identified by GC-MS-MS in yogurt,[21] following a tentative identification in thermally oxidized polyethylene,[22] strawberry,[23] soy lecithin,[24] cola,[25] and wine.[26] However, unlike 1-octen-3-one and 1-octen-3-ol, the microbiological origin of this compound has not been demonstrated. Note also that some thionolactones,[27] as well as some gamma-lactones with unsaturated alkyl chains, also present aromatic nuances of fresh mushrooms.[28]

In grapes and wine, 1-octen-3-ol is a well-known compound associated with rotten grapes, particularly due to *B. cinerea*.[29,30] 1-Octen-3-one has also been detected by GC-olfactometry and identified by GC-MS in grapes and grape juices contaminated by various Ascomycetes fungi, particularly *Erysiphe necator* (*Uncinula necator*),[31] *B. cinerea*, and *P. brevicompactum*[4] reaching concentrations up to 130 ng/L, i.e., above its perception threshold. Fortunately, during alcoholic fermentation, 1-octen-3-one may be enzymatically reduced by *Saccharomyces cerevisiae* to 3-octanone, a much less odoriferous compound, resulting in the decreased intensity or disappearance of the odoriferous zone corresponding to this compound, as detected by gas chromatography-olfactometry (GC-O).[31] However, 1-octen-3-one may be detected by GC-olfactometry (GC-O) in wine extracts, including those made from healthy grapes, unaffected by rot, but concentrations do not exceed 65 ng/L, the olfactory perception threshold for this compound.[32]

This publication reports both the identification and assay of 1-nonen-3-one in wines and the assay of 1-octen-3-one, at concentrations indicating that these compounds can contribute to fresh mushroom aromas in wine. Then, the chemical mechanisms implicating glycine and a tripeptide, glutathione, with 1-octen-3-one, were studied by HPLC-MS and NMR in wine and wine-like solution.

MATERIALS AND METHODS

Chemicals and Biological Compounds. Anhydrous sodium sulfate, 3-octanol, 3-decanone, *O*-2,3,4,5,6-pentafluorobenzylhydroxylamine hydrochloride (PFBHA), glycine, glutathione, tartaric acid, and diethyl ether were from Sigma-Aldrich-Fluka (Saint Quentin Fallavier, France). Pentane (Normapur, Prolabo) was distilled in order to improve its purity. The degree of purity of odorous compounds was determined by GC-MS analysis of an alcoholic solution of each volatile. 1-Octen-3-ol (93% purity) was from Sigma-Aldrich (Saint Louis, USA), and 1-octen-3-one (97% purity) was provided by Alfa Aesar (Karlsruhe, Germany).

1-Nonen-3-one Synthesis. *Chemicals and Experimental Equipment.* 1-Nonen-3-ol and dichloromethane (Chromasolv grade) were purchased from Sigma-Aldrich Chemicals (Saint Quentin Fallavier, France). Dess-Martin periodinane was supplied from Alfa Aesar (Bischheim, France). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC-300 FT spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz). Chemical shifts (δ) and coupling constants (J) are expressed in ppm and Hz, respectively. 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 $KMnO_4$ (0.05% in water).

Experimental Protocol. A solution of 1-nonen-3-ol (1.42 g, 1.7 mL, 10 mmol) in dichloromethane (5 mL) was added dropwise to an ice-cooled solution of Dess-Martin periodinane (4.66 g, 11 mmol, 1.1 equiv.) in dichloromethane (10 mL).[33] The mixture was stirred for 15 min at 0 °C and then allowed to warm to room temperature for 1 h. The reaction was monitored by TLC, using pentane/diethyl ether (80:20, v/v) as eluent. Once the reaction was complete, the mixture was filtered and concentrated under reduced pressure. The residue was purified by flash silica gel chromatography, using pentane/diethyl ether (10:90, v/v) as eluent, to afford the target compound as a colorless liquid (1.38 g, 98.6%). Its purity was determined by GC-MS at 89.3%.

MS (EI, 70 eV), m/z (%) 70 (100), 55 (83.8), 43 (29.7), 97 (16.2), 83 (15.3), 111 (15.3), 84 (10.8), 71 (9.9), 140 (M^+, 2.7), 125 (1.8).

^{1}H NMR ($CDCl_3$) δ 0.89 (t, 3H, J = 6.8 Hz, CH_3-9), 1.30 (m, 6H, CH_2-6,7,8), 1.62 (pentuplet, 2H, J = 7.1 Hz, CH_2-5), 2.59 (t, 2H, J = 7.3 Hz, CH_2-4), 5.82 (dd, 1H, J_{vic} = 10.2 Hz, J_{gem} = 1.3, CH-1a), 6.22 (dd, 1H, J_{vic} = 17.7 Hz, J_{gem} = 1.3, CH-1b), 6.36 (dd, 1H, J_{vic} = 10.2 and 17.7 Hz, CH-2).

^{13}C NMR ($CDCl_3$) δ 14.0 (CH_3-9), 22.5 (CH_2-8), 24.0 (CH_2-5), 28.9 (CH_2-6), 31.6 (CH_2-7), 39.6 (CH_2-4), 127.9 (CH_2-1), 136.6 (CH-2), 201.2 (C═O).

Volatile Compound Extraction from Grape Juice, Wine, and Assays. *1-Octen-3-one Extraction and Assay.* Three hundred fifty milliliters of grape juice or wine was filled in a 1-L flask and supplemented with 150 μL of 1-octen-3-one-8,8,8-d3 (1.7 mg/L) as internal standard. 1-Octen-3-one-8,8,8-d3 (75% purity determined by GC-MS) was kindly provided by Dr. Robert Henry (INSA Lyon). 3-Decanone (150 μL at 2 mg/L) could be used as the second internal standard. After 5 min of stirring at 150 rpm, 120 mg of PFBHA was added, and the mixture was stirred again until complete dissolution during 45 min (150 rpm). Then, the grape juice or wine was extracted by three successive extractions with distilled pentane (15, 10, and 5 mL, respectively) with magnetic stirring (650 rpm) for 10 min each time. The combined organic phases were then dried using anhydrous sodium sulfate and concentrated to 150 μL under nitrogen flow (100 mL/min) before analysis on an HP6890 gas chromatograph (Agilent) coupled to an HP5973 mass spectrometer (Agilent, Palo Alto, CA).

In a range of concentrations from 20 ng/L to 250 ng/L in a supplemented white wine, the regression equation was linear ([1-octen-3-one, ng/L] = 439.38 × −1.686 (x = ratio of peak surface of the derivatized 1-octen-3-one on the peak surface of the derivatized internal standard); R^2 = 0.994). The repeatability for 1-octen-3-one (determined 6 times on the same white wine containing 106 ng/L of 1-octen-3-one) was 5.2%. Limit of detection (LOD) was 5 ng/L and limit of quantification (LOQ) was 15 ng/L. Using 3-decanone as the complementary internal standard, in the range of concentrations from 20 ng/L to 250 ng/L, measurements were also linear (R^2 = 0.995). Calibration curves were performed in the wine matrix before each important series of analyses.

1-Nonen-3-one Extraction and Assay. Three hundred fifty milliliters of grape juice or wine was filled in a 1-L flask and supplemented with 150 μL of 3-octanol (10 mg/L) as internal standard. Wine was extracted in 1-L flasks by three successive extractions of diethyl ether and pentane blend (1/2; V/V) (15 then 15 and 10 mL successively) with 5 min of magnetic stirring each time. The combined organic phases were then dried with anhydrous sodium sulfate and concentrated to 50 μL under a nitrogen stream before analysis by multidimensional gas chromatography coupled with mass spectrometry (see Multidimensional GC coupled with Mass Spectrometry). In a range of concentrations from 15 ng/L to 150 ng/L in a supplemented white wine, measurements were linear: [1-nonen-3-one, ng/L] = 0.0188x + 0.0191 (x = ratio of peak surface of 1-nonen-3-one on the peak surface of the internal standard);

R^2: 0.995). Limit of detection was 5 ng/L, and limit of quantification was 15 ng/L.

1-Octen-3-ol Extraction and Assay. 1-Octen-3-ol was analyzed in wines as previously described.[4]

Gas Chromatography-Olfactometry (GC-O). The analysis was performed as described previously on BP20, BPX5, and BP1 capillaries (SGE, Ringwood, Australia).[4]

Gas Chromatography−Mass Spectrometry (GC-MS). The analysis was performed by electronic impact (EI) 70 eV mode. The mass spectrometer interface temperature was at 250 °C. The carrier gas was Helium N60 (Air Liquide, Floirac, France). Two microliters of organic extract was injected in splitless mode (injector temperature, 250 °C; column head pressure, 140 kPa; purge flow, 50 mL/min) on a BPX5 capillary (SGE; 50 m, 0.22 internal diameter; 1 μm film thickness). The temperature program was as follows for 1-octen-3-one analysis: 40 °C during 5 min, then the temperature increased to 260 at 3 °C/min, and a final increase at 350 °C (rate 10 °C/min) and isothermal during 15 min.

The analyses for quantification were performed in SIM mode (selected ion monitoring) after evidencing the retention time of quantified compounds with that of the standards in full scan mode. 1-Octen-3-one was quantified using the ratio of the areas of their main characteristic ions (m/z 140) for Z and E isomers of the oxime with those of 1-octen-3-one-8,8,8-d3 (m/z 143) and (m/z 267) for 3-decanone. For 1-octen-3-ol, measurements were done with ions (m/z 72; 57) and ions (m/z 83; 59) for the internal standard (3-octanol).

Multidimensional GC Coupled with Mass Spectrometry and with Olfactometry Detection (MDGC-MS-O). This analysis was carried out, as described by La Guerche et al.[4] and Pons et al.,[34] on a Hewlett-Packard 5890 gas chromatograph connected to an Agilent 6890 gas chromatograph coupled with an Agilent 5973 mass spectrometer. The two chromatographs were connected with a temperature-controlled transfer line (West 4400, ILS, Lyon, France) at 230 °C during GC analysis. A Gerstel multicolumn switching system (Gerstel, Mülheim, Germany) was used for multidimensional analysis inside the HP5890 oven. At first, a precolumn (BP20: 30 m, 0.25 mm i.d., 0.22 μm) was installed on a splitless injector port (230 °C). This column was connected at its outlet to a column switching device, where the compounds eluting could be eliminated or transferred directly, through the transfer line, into the analytical column (Varian VF-5ms: 50 m, 0.25 mm i.d., 0.25 μm) installed in the Agilent 6890 chromatograph. At the column switching device level, the precolumn could also be connected via a deactivated fused silica capillary to the flame ionization detector or to the ODO1 sniffing port (SGE). At the exit of the analytical column (VF-5ms) in the Agilent 6890 chromatograph, there was a split (1:1) with another switching device between a sniffing port (ODP2, Gerstel) and the mass spectrometer.

At the level of the injector port in the HP5890 gas chromatograph, the column head pressure was programmed to maintain the same helium flow (close to 1.1 mL/min) during capillary analysis on the precolumn and the analytical column [i.e., 357 kPa at 45 °C for the (30 + 50) meter column, then 1.8 kPa/min increase during the temperature program (45 °C initial temperature), then 3 °C/min increase up to 230 °C, and isothermal during 20 min before cooling]. At a desired retention time, there was a heartcut, and the compounds eluted from the precolumn were transferred on the VF-5ms column and trapped at −50 °C with liquid nitrogen until the program was accomplished. Then, the column was quickly warmed up to 150 °C (temperature increase: 50 °C/min) and subjected to another temperature program from 45 to 230 °C at 3 °C/min.

The quantification was performed in SIM mode (selected ion monitoring) after evidencing the retention time of the quantified compound with that of the standards in FULL SCAN mode. For the classical assay, 2 μL of concentrated wine extract was injected by splitless mode. 1-Nonen-3-one was quantified using the ratio of the areas of their main characteristic ions (m/z 140; 111; 97) with those of 3-octanol (m/z 83; 59).

Olfactory Threshold Determination. The olfactory perception threshold (detection threshold) and recognition threshold of 1-octen-3-one and 1-nonen-3-one were determined by directional triangular test at five increasing concentrations in distilled water, in a model solution similar to wine [12% ethanol and 5 g/L tartaric acid, pH 3.5[1]], in neutral white and red wines. For detection threshold measurement, a 37-person jury smelled the solutions presented in the glasses. For recognition threshold tests, the jury consisted of 12 persons. The olfactory detection threshold and olfactory recognition threshold correspond to the minimum concentration recognized by 50% of the tasters.

Analysis by HPLC-MS and NMR. HPLC/ESI/MS, was performed on a API Q-Star (Applied Biosystem) instrument using an electrospray ionization source in positive-ion mode. The capillary temperature was selected at 275 °C, and the source temperature was selected at 400 °C. The column was a reverse-phase Interchim C18 column (5 μm, 250 mm, 4.7 mm i.d.) protected with a guard column of the same material; solvent A, water with TFA (0,1%); solvent B, acetonitrile. The column was placed at ambient temperature (294 K). The elution program was performed at a constant flow of 1 mL/min using the HPLC inlet and splitter outlet connection permitting the reduction of the flow at 0.5 mL/min, passing from 20% to 100% of B in 30 min, and then rising to 100% of B in 5 min, followed by washing and re-equilibration of the column during 15 min with solvent A. The injection volume was 10 μL.

For the analysis by HPLC-MS, the model medium close to wine[4] was prepared with various modalities: 1-octen-3-one only at 50 mg/L, 1-octen-3-one at 50 mg/L with glutathione at 1 g/L, and 1-octen-3-one at 50 mg/L with glycine at 1 g/L. These solution were kept at 20 °C during 10 days, and then ethanol was evaporated with a rotavapor (Büchi, Switzerland), and the residue was frozen at −20 °C and then lyophilized on a freeze-dryer (Christ L1, Osterode, Denmark). The dried powder was then dissolved in water and reinjected by HPLC-MS.

NMR spectra were recorded on a Bruker Avance 600 spectrometer (600 MHz for ^{1}H and 151 MHz for ^{13}C experiments) at 300 K, in the solvent mixture deuterated water 90% and methanol-d_4 10%. Spectra are referenced to the signal of methanol-d_4 at δ_H 3.31 and δ_C 49.1.

RESULTS AND DISCUSSION

Identification and Assay of Compounds with Mushroom Odor in Altered Wines. Pinot meunier, Pinot gris, and Sauvignon blanc from vintages 2006, 2007, and 2005, respectively, were selected for their strong fresh mushroom flavor, evidenced by wine-makers and enologists, several months after the end of the vinifications. These wines had been elaborated with at least partly rotten grapes, due to the deterioration of climatic conditions before or during the harvest period. Therefore, the grapes were affected by *Botrytis cinerea* or by bunch rot complexes implicating *B. cinerea* with other saprophytic fungi.[35] The wines mentioned above were submitted, after liquid−liquid extraction and concentration, to gas chromatography analysis coupled with olfactometric detection. Doing so, it was possible for us to detect in wine extracts at least one odoriferous zone, sometimes two or three which were expressing this aromatic note. The use of standard compounds made it possible to evidence that the odoriferous zones corresponded on three capillaries (BP20, BPX5, and BP1), with the retention time (determined as linear retention indices, LRI) of 1-octen-3-one (LRI 1315, 992, and 950), 1-nonen-3-one (LRI 1402, 1105, and 1047), and 1-octen-3-ol (LRI 1458, 992, and 950), respectively. Surprisingly, these odoriferous zones could be detected also in red Pinot noir wines, which were marked by off-odors related to camphoreous and earthy notes. In order to clarify the contribution of the

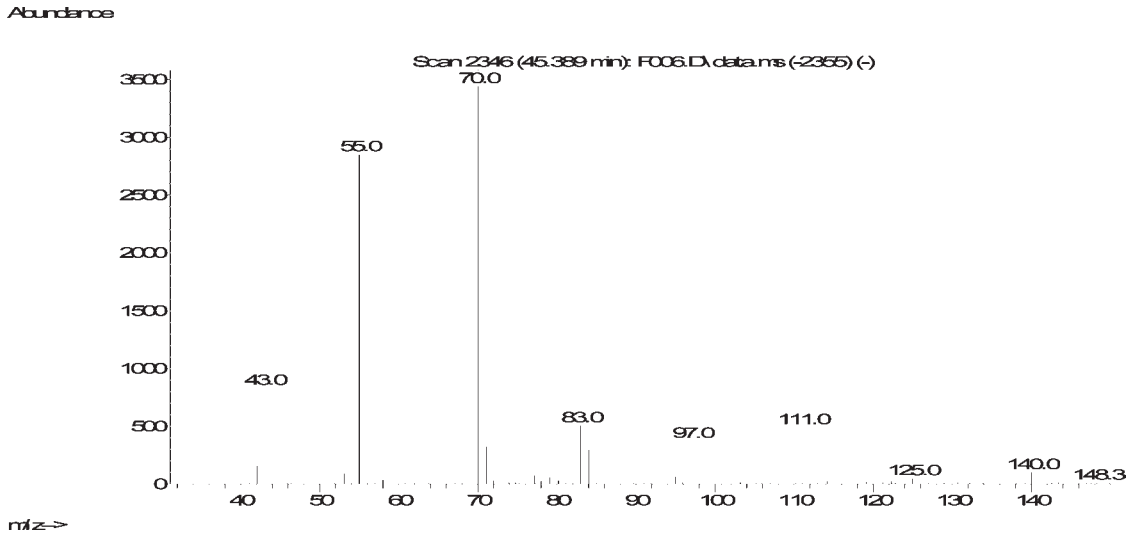

Figure 1. Mass spectra of 1-nonen-3-one obtained by the MDGC-MS-O analysis of an altered wine extract.

above-mentioned compounds to the wines' off-flavors, their identification and quantification was considered in various wines marked or not by the mushroom off-flavor.

Identification and Quantification of 1-Nonen-3-one in Red and White Wines. The 1-nonen-3-one mass spectrum does not present specific ions that could permit one to identify and quantify easily this trace compound in a nonpurified wine extract by one-dimensional gas chromatography (see Materials and Methods). Therefore, multidimensional gas chromatography coupled to mass spectrometry and olfactometry (MDGC-MS-O) was considered as a pertinent tool for the identification and assay of this compound in wines. At close linear retention indices of 1-nonen-3-one on the first capillary (BP20), a heartcut (duration 1.5 min) was made in order to transfer the eluting compounds on a less polar capillary (VF-5ms) coupled both to a mass spectrometer and to an olfactory port (see Materials and Methods). At the retention time of this compound on the VF-5ms column (LRI 1085), it was possible to detect a mass spectrum whose ions abundance and retention time were in accordance with those of the pure compound, thus evidencing the identification of this compound in wine (Figure 1). For the quantification of 1-nonen-3-one in wine, 3-octanol was used as the internal standard. In fact, on the precolumn capillary (BP20) the linear retention indices of 1-nonen-3-one (LRI 1420) was quite close to that of 3-octanol (LRI 1390), and thus, this compound could be transferred during the same heartcut on the analytical column. Quantification of 1-nonen-3-one was done by MDGC-MS after the constitution of a calibration curve with increasing concentrations of this compound supplemented in white wine. Concentrations were determined at levels up to 127 ng/L in Pinot noir wine (Tables 1 and 3). This compound could be quantified in wines from various grape varieties as Pinot noir, Pinot meunier, Pinot gris, Sémillon, and Chardonnay, all elaborated with at least rotten grapes due to gray rot and complex rot (all varieties) or noble rot for Sémillon wines. These concentrations could be in some wines over the olfactory perception threshold of this compound determined in various media (respectively, 1 ng/L in water, 8 ng/L in model media close to wine, 30 ng/L in red wine) and sometimes over the recognition threshold estimated at 30−35 ng/L for 50% of the wine tasters in a dry white wine for Champagne elaboration. Moreover, it was not possible to assay 1-nonen-3-one in the rotten grape bunches and grape juices for which there was detection in wine (data not shown).

Table 1. 1-Nonen-3-one Content Determined by MDGC-MS in Various Wines

wine sample	variety	vintage	1-nonen-3-one (ng/L)
red Burgundy	Pinot noir	2003	<LOQ[a]
red Burgundy	Pinot noir	2004	40 ± 4
red Burgundy	Pinot noir	2006	<LOQ
red Burgundy	Pinot noir	2006	127
dry white wine for Champagne	Chardonnay	2005	<LOQ
dry white wine for Champagne	Chardonnay	2005	15
dessert Bordeaux white	Sémillon	2004	75
dessert Bordeaux white	Sémillon	2007	80

[a] LOQ: limit of quantification (15 ng/L).

Evidence for the Assay of 1-Octen-3-One in White Wines at Concentrations Far over the Olfactory Perception of This Compound. Methods for the quantification of carbonyl compounds in wines have been extensively developed by gas chromatography−mass spectrometry using the derivatization with perfluorated compounds, i.e., PFBOA and PFBHA.[32,36−38] Concerning the quantification of 1-octen-3-one in wines, Culleré et al.[32] proposed a derivatization method. This method was adapted using a deuterated internal standard 1-octen-3-one-8,8,8-d3, and the quantification was validated in a white wine for a range of concentrations from 20 to 250 ng/L by GC-MS in SIM mode (see Materials and Methods). 3-Decanone could be used as secondary internal standard. This method was applied for the quantification of 1-octen-3-one in numerous wine samples, particularly white wines, marked by a strong mushroom off-flavor. 1-Octen-3-one could be assayed in white wines from various varieties (Pinot meunier, Sauvignon blanc, and Pinot noir) at concentrations ranging from LOQ (15 ng/L) up to 385 ± 18 ng/L in a Sauvignon wine marked by a strong mushroom flavor, i.e., concentrations far over the olfactory perception threshold of this compound (3 ng/L in water; 30 ng/L in model medium close to wine, 40 ng/L in a neutral white wine, and 70 ng/L in a neutral red wine), and sometimes over the

Table 2. 1-Octen-3-one Content Determined in Various Wine Samples

wine sample	variety	vintage	1-octen-3-one (ng/L)
dry white wine for Champagne	Pinot noir	1995	180
dry white Bordeaux	Sauvignon blanc	2004	385 ± 18
dry white wine for Champagne	Pinot noir	2005	150
dry white wine for Champagne	Pinot noir	2005	230 ± 9
dry white wine for Champagne	Pinot meunier	2005	115
dry white wine for Champagne	Pinot meunier	2006	90
dry white wine for Champagne	Pinot meunier	2006	120
dry white wine for Champagne	Pinot meunier	2006	202 ± 13
dry white wine for Champagne	Pinot meunier	2007	<LOQ[a]
dry white wine for Champagne	Pinot meunier	2007	<LOQ

[a] LOQ: limit of quantification (15 ng/L).

Table 3. 1-Octen-3-one, 1-Nonen-3-one, and 1-Octen-3-ol Concentrations in White Wine Samples Marked or Not by a Mushroom Off-Flavor[a]

wine sample	variety	vintage	1-octen-3-one (ng/L)	1-nonen-3-one (ng/L)	1-octen-3-ol (μg/L)	mushroom intensity
dry white wine for Champagne	Pinot meunier	2006	106 ± 10	31 ± 3	2	strong
dry white wine for Champagne	Pinot meunier	2006	120 ± 10	20 ± 2	5	strong
dry white Alsace	Pinot gris	2007	20 ± 3	23 ± 3	17	weak
dry white Alsace	Pinot gris	2007	115 ± 10	20 ± 2	5	strong

[a] Limit of quantification (LOQ): 15 ng/L for 1-nonen-3-one and for 1-octen-3-one.

recognition threshold estimated at 120 ng/L in neutral white wine (Table 2 and Figure 2). In these wines, 1-octen-3-ol concentrations were much lower than the olfactory perception threshold of this compound [2 μg/L in water; 20 μg/L in model medium close to wine and 40 μg/l in a neutral white wine].[4] In Champagne base wines (wines before Champagne process), during the same vintage (2006) depending on the plot origin (Aÿ, Hautvillers, Vandières, Oeuilly, or of unknown origin) the concentrations assayed in dry white wines could vary a lot (Figure 2). In 2006, wines elaborated from a vine plot in Aÿ presented median concentrations at 160 ng/L, while median concentrations were close to 100 ng/L in plots from Hautvillers, Vandières, or an unknown location and were only 20 ng/L in plots from Oeuilly. Also depending on the vintage, the proportion of wines with 1-octen-3-one could change, some wine samples from 2005 and 2006 vintages presenting elevated concentrations while the base Champagne wine samples from 2007 presenting concentrations lower than the LOQ (Table 2). Moreover, an exceptional elevated concentration for 1-octen-3-one (180 ng/L) was detected in a Pinot noir wine from 1995 vintage.

The correlation was determined between the intensity of the mushroom flavor in 44 wines estimated on a scale from 0 to 5 by an expert tasting committee (5 persons) (0, no odor; 1, discrete odor; 2, just perceived odor; 3, recognized odor; 4, clear off-flavor; 5, strong off-flavor) and the concentrations of 1-octen-3-one following the described method (Figure 3). The coefficient of correlation (R^2 0.756) shows that there was a certain correlation between the perceived intensity of mushroom flavor and the content in 1-octen-3-one. These results were in accordance with the concentrations necessary to recognize the mushroom flavor in a supplemented white wine and with the values given by Culleré et al.[32]

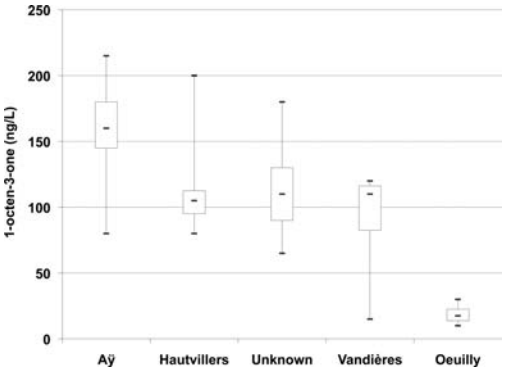

Figure 2. Box-plots illustrating the content of 1-octen-3-one in some base Champagne wines from different vine parcels (vintage 2006).

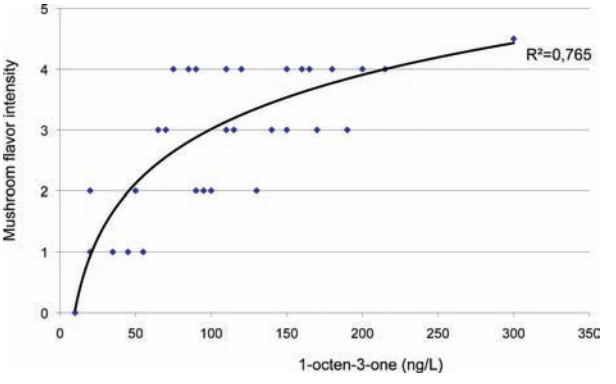

Figure 3. Correlation between the mushroom flavor intensity and 1-octen-3-one concentrations in Champagne base wines.

In order to progress in the study of 1-nonen-3-one, 1-octen-3-one, and 1-octen-3-ol and their sensory contribution to wine's mushroom off-flavor, these three compounds were assayed during 2008 in some dry white wine samples marked or not by the odor (Table 3). It appeared that 1-octen-3-ol concentrations were always at concentrations much lower than the olfactory perception threshold of this compound, whereas 1-nonen-3-one and mainly 1-octen-3-one were detected at concentrations over their olfactory perception threshold and recognition threshold in wines presenting the mushroom flavor.

Study of 1-Octen-3-One Reactivity with Nitrogen Compounds in Wine and in Wine-Like Solution. Carbonyl compounds can react with various nucleophile compounds. Recently, this phenomenon was evidenced for 1-octen-3-one with glycine.[39] We studied in a naturally contaminated white wine with 1-octen-3-one the intensity and concentration of this compound by GC-O and GC-MS, in the presence of an amino acid, glycine, and a tripeptide, glutathione. Both were added at the same molar concentration (32.5 μM). In the following weeks and after 1 and 2 months, the intensity of 1-octen-3-one perceived by GC-O by 2 experts assessors significantly decreased (Table 4). This diminution was confirmed by GC-MS analysis of the assayed 1-octen-3-one in the wines at the beginning and after two months. The diminution of 1-octen-3-one concentrations was associated with an important decrease of mushroom off-flavor in wines.

In order to progress on the characterization of a reaction product between 1-octen-3-one, glycine, or glutathione, analysis were conducted in wine-like solution in the presence of proportionally important concentrations of 1-octen-3-one with glycine or glutathione. The analysis of the model solutions after two weeks permitted us to obtain adducts by HPLC-mass spectrometry and NMR (Figure 4).

Concerning the mixture of glycine and 1-octen-3-one, NMR data were in agreement with results obtained by Cheng et al.[39] Briefly, the ^{1}H NMR spectrum of the mixture showed the presence of two products: glycine and *N*-1-(3-oxo-octyl)glycine (GLY-OCT). The mixture showed that the signals at δ 6−6.5 of the ABC system of the three vinylic protons of 1-octen-3-one disappeared and those in the region δ 3−3.8 appeared, which would correspond to NMR signals of the complex system AA′BB′ from ethylene protons partially overlapped by the glycine side bands. In addition, a CH_2 singlet signal appeared at δ 3.62, which is downfield compared with the ^{1}H NMR spectrum of glycine. The triplet at δ 2.72 ppm of CH_2 at C-4 of 1-octen-3-one disappeared, and a triplet appeared at 2.52 ppm. The multiplet at δ 1.58 ppm of CH_2 at C-5 of 1-octen-3-one was shifted to δ 1.55 ppm. The other signals at δ 1.28 and 0.86 ppm corresponding, respectively, at two CH_2 at C-6 and C-7 and CH_2 at C-8 of 1-octen-3-one, were unchanged. On the basis of this spectroscopic information, and by comparison with results of Cheng et al.,[39] the formation of *N*-1-(3-oxo-octyl)glycine could be postulated.

Concerning the mixture of glutathione and 1-octen-3-one, the resulting product was investigated by HPLC-MS, then with one- and two-dimensional NMR spectrometry. In HPLC-MS, besides the glutathione ion, with a mass of 308.1 ± 0.05 (M+H), the ion for a new compound was detected with a mass of 434.19 ± 0.05 (M+H) by positive electrospray (Figure 5). The NMR spectra of the mixture confirmed the presence of two products: glutathione and a glutathione adduct attributed to *S*-1-(3-oxo-octyl)glutathione (GS-OCT). Assignments of all ^{1}H and ^{13}C signals of

Table 4. Incidence of Glycine or Glutathione Supplementation in a Base Champagne Wine on the Evolution of 1-Octen-3-one Concentration and on the Intensity of the Mushroom Flavor Estimated by Gas Chromatography−Olfactometry

		after 65 days		
	day 0	test[a]	Gly	Glu
1-octen-3-one (ng/L)	100 ± 7	92 ± 10	26 ± 5	30 ± 5
1-octen-3-one intensity[b]	3−3	3−3	1−1	1−1

[a] Test, without supplementation; Gly, supplementation with glycine at 2.44 mg/L (32.54 μM) final concentration; Glu, supplementation with glutathione at 10 mg/L (32.54 μM) final concentration. [b] Intensity of mushroom odor perceived by GC-O by 2 assessors in wine extracts (0, no odor; 1, weak; 2, discrete; 3, strong).

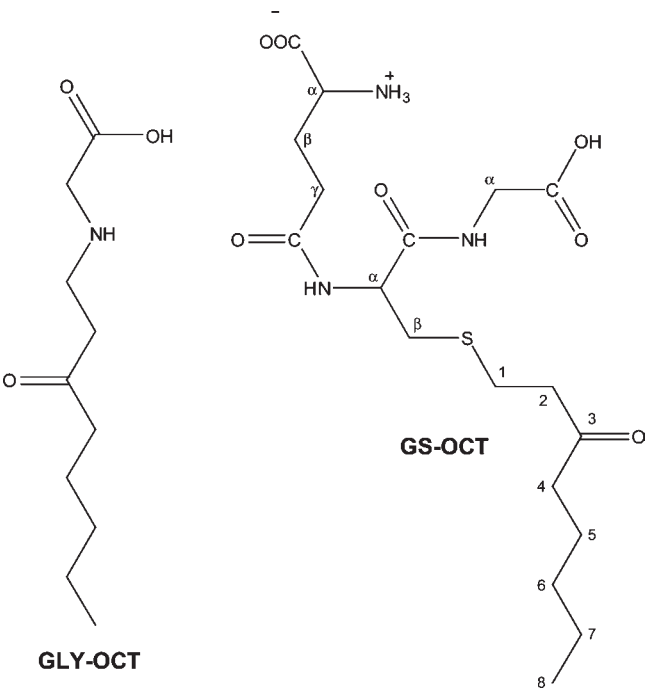

Figure 4. Structures of the 1-octen-3-one conjugates of glycine (GLY-OCT) and glutathione (GS-OCT).

GS-OCT were made by the analysis of ^{1}H−^{1}H COSY, ^{1}H−^{1}H ROESY, ^{1}H−^{13}C HSQC, and ^{1}H−^{13}C HMBC 2D NMR data (NMR data see Table 5). α,β-Unsaturated ketones are known to be very reactive with nucleophilic compounds such as glutathione. As previously described in acidic media,[40] general rules concerning kinetics analysis of the reaction between glutathione and unsaturated carbonyls followed on pseudo-first-order conditions. On the basis of this study, these authors observed that, when the double bond in the β-position is not substituted, the rate of reaction with glutathione was the highest. The observation is in accordance with our results. Moreover, in our conditions the reaction between the sulfide group of glutathione seemed to be nonreversible.

In summary, the development of different analytical techniques (MDGC-MS and derivatization then GC-MS) offered the possibility to identify and assay in red and white wines obtained from at least rotten grapes two carbonyl compounds, 1-octen-3-

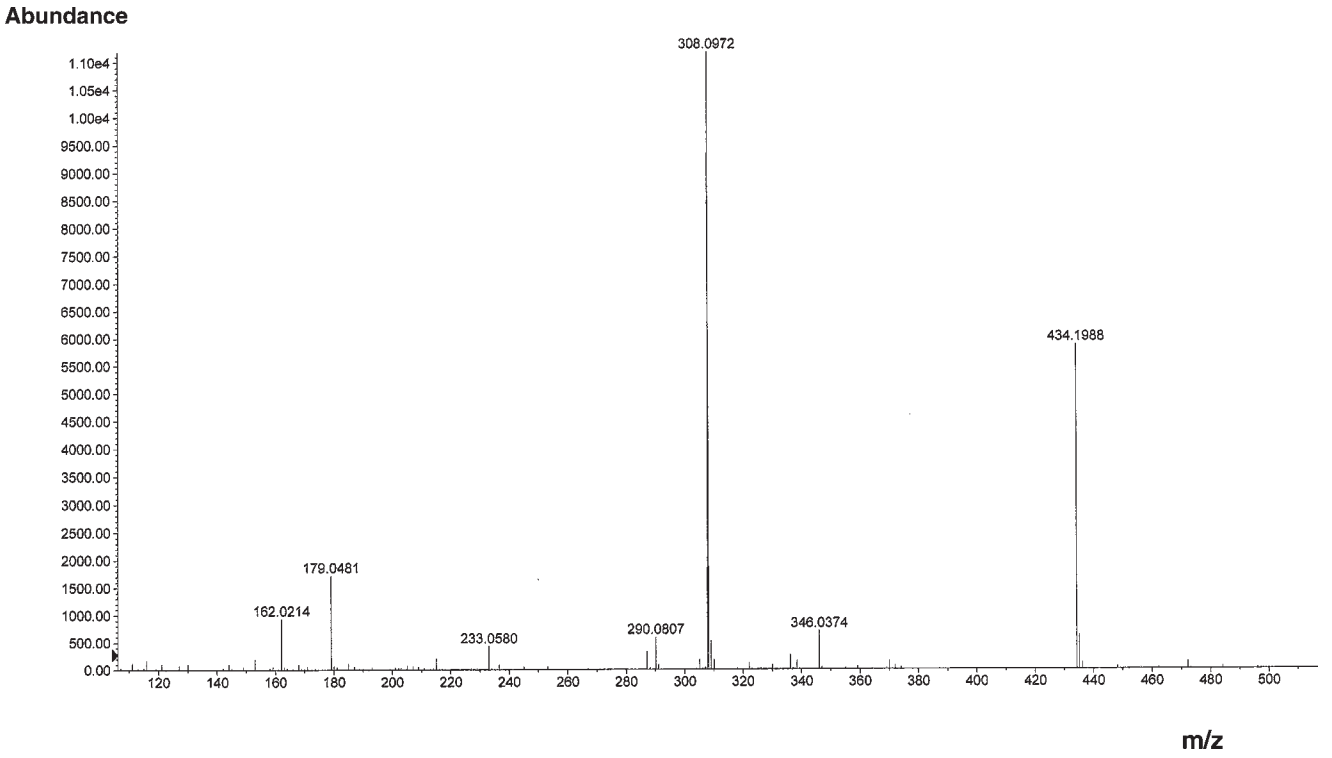

Figure 5. Mass spectra of glutathione and of the adduct between 1-octen-3-one and glutathione in the model wine solution.

one and 1-nonen-3-one, which were previously evidenced by GC-olfactometry. These compounds, although present in trace concentrations, in the range of ng/L, are present in wines at concentrations above their detection and recognition threshold. Also, the correlation between the perception of a mushroom off-flavor by experienced tasters and the concentration of 1-octen-3-one in wines showed evidence of the contribution of the above-mentioned compounds to the mushroom off-flavor. 1-Octen-3-one, originating from lipid peroxidation, has frequently been cited as being implicated in mushroom off-flavor.[10,13,41] But its contribution to organoleptic defects in wines had not been demonstrated so far. Indeed, its assay in wines had not highlighted concentration levels reaching the perception and recovery threshold.[32] Also, although present in grapes affected by various fungal attacks due to *Uncinula necator*[31] or to *Botrytis cinerea*,[4] this compound is known to be reduced to 3-octanone during alcoholic fermentation in the presence of enone reductase activity.[4,31,42] The presence of 1-octen-3-one measured at concentrations levels of hundreds of nanograms per liter in wine is unusual in view of the previous work.[32,43] The cause of this concentration is probably due to the harvest of grapes affected by bunch rot complexes in which a variety of saprophytic fungal species (*Penicillium* sp., *Clonostachys* sp., *Trichothecium roseum*, *Verticillium* sp., and *Trichoderma* sp.) isolated in the clusters are implicated, through their metabolism, with the pathogen *B. cinerea*.[35,44] We demonstrated that the addition of glutathione or glycine in wine led to a decrease in the levels of 1-octen-3-one. This decrease is associated with a much significant diminution of the mushroom odor. Parallel analysis of the reaction mixture between glycine and 1-octen-3-one or glutathione and 1-octen-3-one in wine-like medium demonstrated the formation of adducts. We postulate that these adducts can be formed in wines naturally,

Table 5. ^{1}H and ^{13}C NMR[b] Spectral Data of GS-OCT at 300 K[a]

position	δ_C	δ_H	ROESY	HMBC
			OCT	
1	26.4	2.79	cys-Hβ	H-2, cys-Hβ
2	42.7	2.87	H-4	H-1, H-4
3	210.1			H-1, H-2, H-4, H-5
4	43.6	2.53	H-2, H-6	H-2, H-5
5	23.9	1.55		H-4, H-6
6	31.5	1.27	H-4	H-4, H-5, H-7, H-8
7	22.6	1.29		H-5, H-6, H-8
8	14.2	0.86		H-7
			γ-glu	
α	54.6	3.79	H-β, H-γ	γ-glu-Hβ, γ-glu-Hγ
β	27.0	2.16	H-α, H-γ	γ-glu-Hα, γ-glu-Hγ
γ	32.1	2.55	H-α, H-β	γ-glu-Hα, γ-glu-Hβ
γ-CO	175.3			γ-glu-Hβ, γ-glu-Hγ, cys-Hα
COO	174.1			γ-glu-Hα, γ-glu-Hβ
			cys	
α	53.3	4.74	H-β	cys-Hβ
β	34.1	2.90	H-α, H-1	cys-Hα, H-1
COO	172.8	3.05		cys-Hα, cys-Hβ, gly-Hα
			gly	
α	42.6	3.92		
COO	174.5			gly-Hα

[a] Main ROESY and HMBC correlations are indicated. [b] ^{1}H and ^{13}C spectra were measured in deuterated water at 600 and 151 MHz, respectively.

depending on their aging modalities, or after supplementation with some nitrogen compounds subject to legal authority. Concerning 1-nonen-3-one, this compound has probably a fungal origin given the lack of detection by GC-O and GC-MS in wines made from grapes from the same grape varieties which were not affected by rot, but the genesis of this compound remains unknown.

■ ASSOCIATED CONTENT

Supporting Information. Chromatogram in selected ion monitoring mode of a white wine extract after derivatization with PFBHA. This material is available free of charge via the Internet at http://pubs.acs.org.

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