JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Identification of Volatile Compounds Responsible for Prune Aroma in Prematurely Aged Red Wines

ALEXANDRE PONS,*,†,§ VALÉRIE LAVIGNE,†,§ FRÉROT ERIC,# PHILIPPE DARRIET,§ AND DENIS DUBOURDIEU§

R&D Division, Seguin Moreau SA, Z. I. Merpins, B.P. 94, 16103 Cognac, France; Corporate R&D Division, Firmenich S.A., P.O. Box 239, CH-1211 Geneva 8, Switzerland; and UMR 1219 Oenologie, Institut des Sciences de la Vigne et du Vin, Université de Bordeaux 2, 351 cours de la Liberation, 33405 Talence cedex, France

The premature aging of red *Vitis vinifera* L. wines is mainly associated with the formation of an intense off-flavor reminiscent of prunes. The compounds responsible for this deterioration in red wine flavor have not previously been identified. Sensory descriptive analysis associated with a gas chromatography−olfactometry (GC-O) technique was first performed to find characteristic odoriferous zones of 15 aged red wines with or without a marked prune aroma. Afterward, high-pressure liquid chromatography, gas chromatography, and multidimensional gas chromatography coupled with mass spectrometry (MDGC-MS) were used to identify the odorants reminiscent of prunes in prematurely aged red wines and in the dried fruit. Three compounds were detected with a strong odor of prunes: γ-nonalactone, β-damascenone, and 3-methyl-2,4-nonanedione. The perception threshold of the latter β-diketone in a model hydroalcoholic solution is 16 ng/L. Identified for the first time in aged red wines, this very powerful volatile compound was also suggested to produce the characteristic prune aroma of prematurely aged red wines. The presence of 3-methyl-2,4-nonanedione was also detected in prunes for the first time.

KEYWORDS: Red wine; prunes; premature aging; 3-methyl-2,4-nonanedione; MDGC-MS; flavor; aroma

INTRODUCTION

The reputation of famous red Bordeaux wines is strongly associated with their aging potential. Indeed, these wines conserve the flavor nuances of young wines while developing specific varietal nuances. However, this ideal aging does not occur in every wine. Premature aging or untypical aging (UTA) is a well-known phenomenon in white wines (*1–4*). Premature-aging aroma phenomena may also reflect the defective aging of red wines. Prematurely aged red wines develop several aromatic nuances reminiscent of prunes and figs. In our experience of red wine tasting, the presence of these overriding odors affects the quality and subtlety of the wine flavor and may shorten its shelf life.

Despite their importance for the wine industry, the chemical compounds responsible for these off-flavors were not previously known. Previous studies, based on gas chromatography−olfactometry (GC-O) analysis and quantitative GC-MS, were aimed at determining the aroma profile of these wines, as well as identifying and quantifying the odorants that affect aged red wines from Spain (*5, 6*). Using the GC-AEDA approach with three judges, followed by statistical treatment of the response (ANOVA) to check for significant differences among the wines and judges, Ferreira et al. (*5*) suggested that the prune nuance of old Spanish wines was possibly related to their 4-propylguaiacol content. However, this compound has an odor reminiscent of cloves, which does not correspond to the prune aroma of prematurely aged red wines. Cutzach (*7*) considered that high concentrations of sotolon in old sweet fortified wines (0.1−1 mg/L) may explain the desired prune nuances. To our knowledge, such high concentrations are not usual in old red wines, in which they remain below sotolon's perception threshold (*8*).

The prior research described above used conventional GC-O, using a single column, by which coelution of odorous compounds is likely to occur, followed by MS detection to identify unknown compounds. GC-O is an efficient tool for studying the impact of odorants, as it is capable of establishing a hierarchy among volatile compounds. GC-O may be used as a screening methodology for evaluating the odoriferous zones with specific interest, but it is not sufficient.

In view of the large spectrum of volatile compound concentrations in wines (0.1 ng/L−100 g/L), it is extremely interesting to use these valuable chromatographic methods coupled with various kind of detectors (olfactometry, mass spectrometry) to identify odor-active compounds present in wine at very low

* Author to whom correspondence should be addressed [e-mail alexandre.pons@oenologie.u-bordeaux2.fr; telephone (33) 5 40 00 64 90; fax (33) 5 40 00 64 68].
† Seguin Moreau SA.
§ Université de Bordeaux 2.
# Firmenich S.A.

10.1021/jf073513z CCC: $40.75 © 2008 American Chemical Society
Published on Web 06/10/2008

concentrations. This type of "global" approach was used by Guth (*9*), combining nonselective liquid−liquid extraction, HPLC purification, and preparative gas chromatography enrichment to identify wine lactone. Another possible solution for identifying trace odorants is nonselective, two-dimensional gas chromatography: GC×GC or multidimensional gas chromatography (MDGC). As recently described by Eyres (*10*), compared to GC×GC, traditional heart cut MDGC-O is more suitable for identifying the compounds responsible for a specific odor by GC-O in a complex matrix.

The main goal of this research was to identify key aroma compounds in prematurely aged red wines with an intense prune aroma. The starting point of this work was the surprising sensory impression of dried fruit (prunes) in the aroma of prematurely aged red wine. This observation led us to study the flavor of prunes in the same way, to assist in identifying this aroma in wines. Indeed, we assumed that the compounds with an odor of prunes found in wines would be more concentrated in prunes.

In this study, red wine and fruit extracts were prepared by liquid−liquid extraction and analyzed using different chromatographic techniques. Once GC-O had located odorous zones reminiscent of prematurely aged wine and prunes, a new volatile compound was isolated from both wine and prunes and its sensory properties were analyzed.

MATERIALS AND METHODS

Chemicals and Reference Compounds. Dichloromethane (Chromasolv grade), sodium acetate (99%), and hydroxylamine hydrochloride (99%) were from Sigma-Aldrich (St Quentin Fallavier, France). Anhydrous sodium sulfate was supplied by Prolabo. Trizma base (99%) and L-(+) tartaric acid (99.5%) were from Fluka, and ethanol (Lichrosolv grade) was from Merck. Pentane (Normapur quality) from Prolabo was distilled to improve its quality. Reference compound 3-methyl-2,4-nonanedione was a gift from Firmenich S.A. (Geneva, Switzerland).

Wine Samples. Red wines used in this study were from several origins (Bordeaux, Burgundy, and Valdepeñas) and vintages (from 1949 to 2004), produced according to standard winemaking procedures (grapes were destemmed and crushed before being put into tanks, alcoholic fermentation was brought to dryness and followed by malolactic fermentation), with or without wood contact. They were all analyzed in 2006. Four of the 15 wines were not prematurely aged.

Wine Extraction. *Organic Solvent Selection.* Several organic solvents (pentane, diethyl ether, and dichloromethane), alone or mixed, were used to obtain organic extracts of prematurely aged red wine, following procedure 1 described below. A drop of each extract was put on a smelling strip, and the aroma was compared with that of the original wine. Moreover, GC-O analysis was performed to determine the best solvent extractability of the first odoriferous zone (OZ 1).

Procedure 1 (Standard Extraction Procedure). Red wines (100 mL) were extracted three times with pentane/dichloromethane (1/1, v/v; 3 × 10 mL). The organic layer was dried over Na_2SO_4 and then concentrated to 0.5 mL under nitrogen flow (approximately 100 mL/min).

Procedure 2 (Specific to the Identification of Prune Aroma). Red wine (1.5 L) with a marked prune aroma was extracted with pentane/dichloromethane (1/1, v/v; 3 × 160 mL). The resulting organic phases obtained in 10 runs were mixed, dried over Na_2SO_4, and concentrated to 300 mL by distilling the solvent with a Rotavapor system. The organic phase was treated three times with an aqueous Trizma buffer (0.1 M; pH 10, 50 mL). Then, the organic extract containing neutral and basic compounds was dried over Na_2SO_4 and concentrated to 2 mL under nitrogen flow.

Extracting Volatiles from Prunes and Figs. Commercially dried Agen plums (*Prunus domestica* L.), without sodium sorbate treatment, and figs (800 g) were sliced separately and then extracted for 4 h with 200 mL of dichloromethane/ethanol (90:10, v/v) with mechanical stirring. The organic phase was then dried over Na_2SO_4 and concentrated to 500 μL under nitrogen flow.

High-Pressure Liquid Chromatography (HPLC) Purification. *Chromatographic Conditions.* The procedure was based on the method first described by Ferreira (*11*) and later adapted by Pineau (*12*). The HPLC fractionation of wine was accomplished with a Waters (Milford, MA) HPLC, consisting of one pump, an automated gradient controller, and a manual injector. The column used was a Novapak C18 (3.9 × 318 mm, 4 μm). The chromatographic conditions included a flow rate of 0.5 mL/min and an injection volume of 270 μL. The linear program gradient involved phase A, water, and phase B, ethanol, 0% B reaching 100% B in 50 min. An automated fraction collector (Bio-Rad) was connected to the end of the column to collect 1 mL of the eluted solvent every 2 min. The HPLC eluate was recovered in 25 separate fractions, evaluated for their smell as described below. The fractions with remarkable odors were re-extracted and analyzed.

Flavor Fraction Re-extraction. The alcohol content of the fractions eluted by HPLC was adjusted to 12% (v/v). Then, the solution was extracted three times with 0.5 mL of a pentane/dichloromethane mixture (1:1, v/v). The solvent extract was concentrated under nitrogen to 200 μL and used for GC-O analysis, as well as for further purification by MDGC coupled with olfactometry and mass spectrometry.

Capillary Gas Chromatography−Olfactometry (GC-O). The analysis was carried out alternately by two operators on a Hewlett-Packard HP5890 series II (Agilent Technologies, Palo Alto, CA) coupled with olfactory detection using an ODO-1 installation [Scientific Glass Engineering (SGE), Ringwood, Australia]. A 2 μL sample of the extract (procedure 1) was introduced onto a polar BP20 capillary column (SGE, 50 m, 0.25 mm i.d., 0.25 μm film thickness) or an SPB1-type fused silica nonpolar capillary column (Supelco, 30 m, 0.25 mm i.d., 1 μm). The carrier gas was He (Linde Gas, Bordeaux, France), 5.3 grade, with a flow rate of 1 mL/min for all of the analyses. The injector, in splitless mode (purge time = 1 min; purge flow = 50 mL/min) was set at 230 °C. Oven temperature was initially set at 45 °C for 1 min, then raised to 240 at 3 °C/min, and held at that temperature for 20 min. Linear retention indices (LRI) were obtained by simultaneous injection of samples and a series of alkanes (C_7−C_{23}) (*13*).

Heart-Cut Multidimensional Gas Chromatography−Olfactometry−Mass Spectrometry (MDGC-O-MS). *General Setup of the System.* The MDGC separations were performed on three capillary columns with different stationary phases or film thicknesses on two GC ovens: Oven I was a Hewlett-Packard 5890 series II, whereas oven II was an Agilent 6890 coupled with a 5973 quadrupole mass spectrometer. The two chromatographs were connected with a temperature-controlled transfer line set at 230 °C (West 4400 from ILS, Lyon, France). The outlet of the precolumn was connected to a sniff port (ODO I; SGE France) to determine odor retention time in this configuration and to the second column, via a Gerstel MCS 2 multicolumn switching system. A cryotrap was also placed at the start of the second column for cryofocusing. The end of the second column was split (1:1) via a crosspiece (Gerstel) between MS detection (Agilent) and the sniffing port (ODP II, Gerstel). For oven I, only 10% of the total flow was transferred to the deactivated fused silica column connected to ODO I, whereas 50% of the flow was transferred to ODP II in oven II. The MDGC system was operated under constant pressure to maintain the balance between the two columns throughout the oven temperature program.

MDGC Conditions for Wine Extract Analysis. The injector temperature was set at 230 °C. The sample was transferred from the precolumn to the main column via the MCS (Gerstel) system at a defined cut time. Preseparation was performed using a nonpolar SPB1 fused silica capillary column (30 m, 0.25 mm i.d., 0.25 μm). The GC oven was programmed from 45 °C (1 min) to 250 at 3 °C/min and held for 40 min to ensure that all compounds were eluted. The cut time (28.8−29.8 min) was selected with the ODO I system. During this period the cryogenic trap was maintained at −50 °C. The second column was a 50 m × 0.25 i.d. × 0.25 μm BP20 (SGE France). Column head pressures were 333 kPa (oven I) and 222 kPa (oven II) at 45 °C. Prior to final identification, agreement of mass spectrometric and olfactometric data were confirmed by fitting oven II to another column (SPB1, 60 m × 0.25 mm i.d., 1 μm). The temperature of oven II was initially set at 45 °C for 29.8 min, then raised to 240 at 3 °C/min, and held at that temperature for 30 min. Helium 5.3 (Linde Gas) was used as the

Table 1. Identifying the Odor-Active Regions in the Red Wine Extracts with Prematurely Aged Aromas

odoriferous zone	odor descriptors[a]	LRI[b]	LRI[c]	compound identified[d]	identification methods[e]
OZ1	prune[e]	1742	1213 (1369)	3-methyl-2,4-nonanedione	HPLC-MDGC-MS, RI, RC
OZ2	dried fruit, applesauce[e]	1841	1369	β-damascenone	GC-MS, RI, RC
OZ3	overripe peach[e]	2041	1325	γ-nonalactone	GC-MS, RI, RC

[a] Odor descriptors generated by the two assessors during GC-O. [b] Retention index (LRI) of odor peak on a BP20 (50 m × 0.25 mm, 0.25 μm) column by GC-O. [c] Retention index of odor peak on an SPB1 (30 m × 0.25 mm, 1 μm) column by GC-O. LRI of minor peak resulting from the keto−enol equilibrium of the compound is given in parentheses. [d] Compounds identified as odor-active regions responsible for the odor perceived. [e] Compounds identified on the basis of hyphenated HPLC-MDGC-MS or GC-MS techniques by comparing their mass spectra and retention indices (RI) with reference databases and their odors with reference compounds (RC).

carrier gas, with a flow rate of 2 mL/min. The temperatures of the ion source and transfer line were 250 and 230 °C, respectively. The electron energy for the EI mass spectra was 70 eV, and CI was initiated using methane 4.5 grade (Linde Gas) as the reactant gas. The MS was operated in the scan mode only (*m/z* from 45 to 250). Identification was performed by comparing linear retention indices and mass spectrometric data for sample constituents with those of authentic reference compounds.

Determining the Flavor of 3-Methyl-2,4-nonanedione Keto−Enol Forms. During the HRGC of the pure compound on a nonpolar column, the keto and enol forms were separated and their odors assessed by a selected jury. The concentration of pure compound in dichloromethane was 0.5 μg/mL. Chromatographic conditions were as previously described in the GC-O section. Separation was performed on a nonpolar SPB1 (30 m, 0.25 mm, 1 μm) column.

Sensory Analysis. *HPLC Fractions.* A 100 μL sample of each fraction collected was put on a "smelling strip" to evaluate the olfactometric properties of the hydroalcoholic fraction. After this, and only with those that were found to smell of prune, extractions were carried out as previously described.

Determination of the Olfactory Thresholds. Perception thresholds were determined using the method described by Boidron (*14*). These were obtained by directional triangular tests of five increasing concentrations in dilute model wine solution [L-(+)-tartaric acid 5 g/L, 12% vol, pH 3.5). The solutions were presented in glasses corresponding to Association Française des Normes (AFNOR) standards. The sensory panel consisted of 40 people between 20 and 40 years old, who received weekly training sessions. The odor perception threshold corresponded to the minimum concentration below which 50% of tasters statistically failed to detect the difference from the control.

Hydroxylamine Test. In an aqueous medium, the reaction between carbonyl compounds and hydroxylamine is quasiquantitative (*15*). Red wine (100 mL) with an intense prune odor was supplemented with hydroxylamine hydrochloride (0.1 g) and sodium acetate (0.2 g). An organic extract of this mixture was prepared, as previously described (procedure 1).

RESULTS AND DISCUSSION

The most important descriptors related to the oxidative character of aged red wines were identified by a trained panel. The terms selected were "prunes", "figs", and "overripe fruit". Among the various solvents tested (pentane, pentane/dichloromethane, dichloromethane, and diethyl ether), the pentane/dichloromethane (1:1, v/v) extract (see procedure 1) best represented the initial odor of aged red wines. GC-O analysis of the extract revealed the odor zones corresponding to the wine flavors.

After concentration, the extracts were injected by GC-O. In addition, prune and fig extracts were prepared and analyzed by

Table 2. Distribution of the Three Main Odoriferous Zones Found in Prematurely Aged Red Wines (Several Vintages and Origins), Prunes, and Figs by GC-O

material[a]	appellation	vintage	odoriferous zones[d]
W1	Pessac Leognan	2004[b]	1 + 2
W2	Graves	2003[b]	1 + 2
W3	Pessac Leognan	2003[b]	1 + 2
W4	Saint-Emilion	2001[b]	1 + 2
W5	Valdepeñas	1976[b]	1 + 2 + 3
W6	Bordeaux	1998[b]	1 + 2 + 3
W7	Bordeaux	1999[b]	1 + 2 + 3
W8	Bordeaux	2001[b]	1 + 2
W8	Bordeaux	2003[b]	1 + 2
W9	Bordeaux	1990[b]	1 + 2 + 3
W10	Burgundy	1949[b]	1 + 2+ 3
W11	Medoc	2004[c]	2
W12	Bordeaux	2004[c]	2
W13	Medoc	2000[c]	2
W14	Saint Emilion	2004[c]	2
W15	Saint Emilion	2004[c]	2
P1			1 + 2 + 3
F1			2

[a] Wines (W), prunes (P), and figs (F) studied by GC-O. [b] Wines with a strong prune flavor. [c] Wines without any marked prune flavor. [d] Odoriferous zones perceived by the two assessors using GC-O (BP20 column).

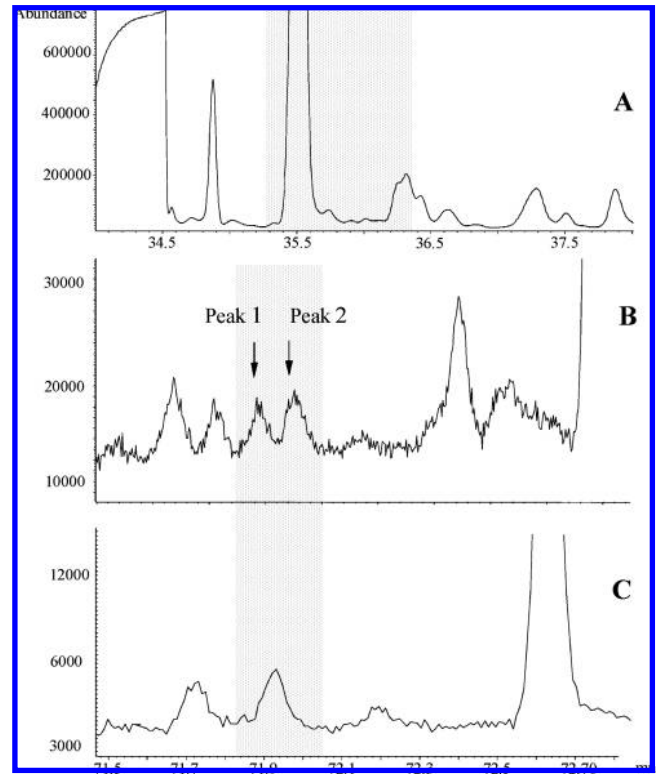

Figure 1. Example of an MDGC separation of an organic red wine extract. (**A**) GC-MS chromatogram (SPB1 column; 30 m × 0.25 mm, 0.25 μm) of the wine's crude organic extract washed with Trizma buffer. The shaded region represents the area where the intense prune odor was perceived during GC-O. It was heart-cut to the second column (BP20 column; 50 m × 0.25 mm, 0.25 μm) by MDGC. (**B**) Expanded MS chromatogram corresponding to the fraction (isolated in **A**), showing the unresolved peaks responsible for the prune odor. (**C**) HPLC-MDGC separation with the localization of the peak associated with the prune odor.

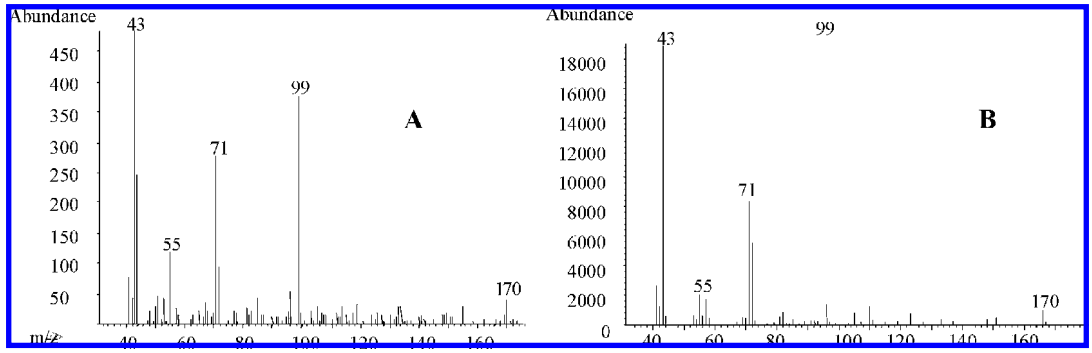

Figure 2. Mass spectra (EI) of unknown compound (**A**) and pure MND (**B**).

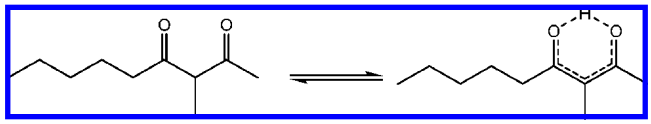

Figure 3. 3-Methyl-2,4-nonanedione keto−enol tautomerism.

GC-O for comparison with the red wine. Wine is a very complex beverage, with over 60 aroma-active compounds detected by GC-O, whereas there were only 40 in prune extract and 21 in fig extract. The main odoriferous zones reminiscent of prune in wine extract are listed in **Table 1**.

The three odoriferous zones perceived by GC-O as strongly reminiscent of prematurely aged red wine aroma were OZ1, OZ2, and OZ3. OZ1 had a strong prune odor. Surprisingly, this odor was detected at two retention times with different intensities on a nonpolar capillary column (SPB1). Odoriferous zones OZ2 and OZ3 were more similar to dried fruit. Only OZ1 and OZ3 seemed to be specific to prematurely aged red wines (**Table 2**). Indeed, OZ2 was detected in all of the wines analyzed, whether or not they had a prematurely aged character. Moreover, OZ3 was found in only the oldest wines with very intense prune aromas, indicating that it enhanced the overall prune aroma of these wines. The three odoriferous zones were also detected in prune extract (**Table 2**), but only OZ2 was detected in fig extract. Consequently, these results show a good agreement between the flavor of prematurely aged red wines and fruit with the same odor. We can, therefore, postulate that the same compounds associated with the prune aroma of prematurely aged red wines are responsible for the flavor of prunes.

Using GC-MS with chemical standards, it was possible to identify the molecules corresponding to the following retention indices: OZ2, β-damascenone ($RI_{polar} = 1841$, $RI_{nonpolar} = 1369$), and OZ3, γ-nonalactone ($RI_{polar} = 2041$, $RI_{nonpolar} = 1325$).

β-Damascenone is a widespread powerful flavoring compound in nature (*16–21*). It has been previously identified in young wines of different origins (*22–24*), as well as aged red wines (*5, 6*), and prunes (*25*). According to Ferreira (*26*), high levels of norisoprenoids such as β-damascenone and β-ionone (but below their perception thresholds in wine) may develop "dry-plum notes" in red wines. However, a recent study (*12*) revealed that β-damascenone apparently had no direct impact on red wine aroma, but was potentially an aroma enhancer.

γ-Nonalactone has been identified in many different wines (*22, 27, 28*). Concentrations are higher in old red wines (*8, 29*) and red wines aged in oak barrels (*30*). These results are in agreement with our findings. Indeed, under our conditions, γ-nonalactone was detected by GC-O in only old red wines marked by an intense prune flavor. This lactone is also found in many stone fruit, for example, apricots (*31, 32*) and peaches (*33*). γ-Nonalactone had not previously been reported as a volatile constituent of prunes.

Table 3. Odor Description of 3-Methyl-2,4-nonanedione in Wine Model Solution Depending on Its Concentration

concentration (μg/L)	descriptors
0.1	minty
1	anise, kernel, prune
10	anise

Identification of 3-Methyl-2,4-nonanedione in Prematurely Aged Red Wine. When hydroxylamine hydrochloride was added to the wine before extraction, as described under Materials and Methods, OZ1 and OZ2 were no longer detectable by GC-O. As aldehydes and ketones react readily with hydroxylamine to form oximes, the compounds associated with these two odoriferous zones may represent carbonyl functions, as already demonstrated in the case of β-damascenone (OZ2). Consequently, knowledge of a specific function of an unknown compound may be of assistance in identifying a fragmentation pattern from an unknown mass spectrum. To collect more information for an unequivocal elucidation of its structure, the volatiles from a total of 15 L of prematurely aged red wine were isolated by liquid−liquid extraction (procedure 2). Acidic compounds were removed by washing the organic extract with Trizma buffer (pH 10).

At this point, the application of MDGC to the organic wine extract is a logical step forward in the analysis of such complex samples. Thus, a heart cut was made in a given region of the chromatogram, and the desired components were transferred to a second, more selective, column. In this way, selected odor regions where coelution occurred in one dimension were heart-cut and resolved on the second column. The choice of column is crucial. Orthogonal column polarities are often recommended, as successfully used by Darriet (*34*) and, more recently, by Laguerche (*35*) and Campo (*36*), to identify 1-octen-3-one, 2-methylisoborneol, and three new esters in red wines.

The first-dimension chromatogram obtained from a wine extract (procedure 2) is complex, as seen in **Figure 1A**, which also shows the transferred section, corresponding to the prune odor retention window. The odor perception time of this odoriferous zone (OZ1; LRI = 1213) was very long (55 s), which may be due to a number of factors, including the concentration of the extract, as well as the perception of several coeluting odorants.

The second dimension chromatogram (**Figure 1B**) had two peaks (peaks 1 and 2) at the retention time of prune odor. However, the mass spectrum for these peaks indicated coelution of other compounds, and it was impossible to obtain a clear mass spectrum. Therefore, in our case, the MDGC-MS method was not capable of identifying a trace compound from a quasi-crude red wine extract. A further purification step was necessary to avoid coelution. HPLC with a C18 reversed-phase column

was used to divide the organic wine extract into 25 fractions (see Materials and Methods). Only fraction 18 smelled of prunes and was, thus, reminiscent of prematurely aged red wine flavor. This fraction was then re-extracted (procedure 2) and analyzed by MDGC with simultaneous sniffing detection.

Following the same procedure, MDGC-MS analysis of the second odoriferous OZ1 on a SPB1 nonpolar column (**Table 1**) revealed the presence of a compound with the same flavor quality and a mass spectrum similar to that of the compound studied. These results suggested the existence of two isomers or, the rarer phenomenon of the separation of two tautomers.

On the basis of MS (EI) data (**Figure 2**) and also MS (CI, CH_3) spectra, which indicated a molecular mass $M = 170$ ($[M + H]^+ = 171$), associated with LRI data in the literature (*37*), the peak corresponding to the odoriferous zone (**Figure 1C**) was identified as 3-methyl-2,4-nonanedione. MDGC-O analysis was repeated for the same extract on a nonpolar SPB1 column (oven II), confirming this result. Using 800 g of organic prune extract, the odoriferous zone in the fruit, assessed by the same protocol, was determined to be the same compound as in red wine.

3-Methyl-2,4-nonanedione (MND) was first mentioned by Guth and Grosch as an off-flavor in reversed soybean oil (*38*). It was then associated with the hay-like off-flavor that develops in dried parsley (*39*) and spinach (*40*). This β-diketone was also reported to contribute significantly to the flavor of green tea (*41*). The keto−enol equilibrium (**Figure 3**), in which the tautomers are only partially interconverted, according to the temperature and polarity of the solvent, was first described by Guth (*38*). On the basis of NMR studies, this author identified the keto and enol tautomers by GC on a nonpolar capillary column. According to recent work by Naef (*37*), studying this compound in green tea, only the keto form was observable on a polar column.

To the best of our knowledge, this is the first time that this very powerful compound has been identified in wine. Guth found that certain furan fatty acids (10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid) were precursors of this compound in soybean oil (*42*). The origin of 3-methyl-2,4-nonanedione in red wine is still unknown.

Odor Characteristics of 3-Methyl-2,4-nonanedione (MND). The odor threshold of MND was first reported by Guth (*38*) in air (0.007−0.0014 ng/L). On the basis of work by Masanetz (*39*), its threshold in water is 30 ng/kg. In our research, the perception threshold of MND in wine model solution, determined by a well-trained panel of 40 persons, was found to be 16 ng/L.

3-Methyl-2,4-nonanedione has a strong anise, hay-like odor. These descriptors may depend on its concentration. Indeed, at low concentrations, MND smells different from the pure compound and is rather reminiscent of prematurely aged red wine (**Table 3**). In small amounts (100 ng/L), it has a minty flavor, at 1 μg/L it is reminiscent of anise, fruit kernels, and prunes, whereas at 10 μg/L its flavor is mainly described as anise.

GC-O studies were performed on a nonpolar column to separate and smell the keto and enol forms of MND. The eluate was sniffed by three assessors. The odors of the two forms were found to be very similar, reminiscent of anise and prune.

Prune Aroma of Prematurely Aged Red Wines. These findings lead us to think that the characteristic prune flavor of prematurely aged red wines might be the result of a combination of several odorants including MND, β-damascenone, and γ-nonalactone. However, from our point of view, the study of the distribution of these compounds in several red wines, marked or not with this flavor, leads us to suggest that MND, one of the most odorant non-thiol compounds in red wine, and γ-nonalactone were more specifically associated with the prune aroma of old red wines. Further research is planned to investigate levels of β-damascenone, γ-nonalactone, and 3-methyl-2,4-nonanedione in aged red wines.

LITERATURE CITED

(1) Lavigne-Cruège, V.; Dubourdieu, D. Role of glutathione on development of aroma defects in dry white wines. In *13th International Enology Symposium*; Trogus, H., Gafner J., Sutterlin, Eds.; International Association of Enology: Montpellier, France, 2002; pp 331−347.

(2) Ferreira, V.; Ortega, L.; Escudero, A.; Cacho, J. A comparative study of the ability of different solvents and adsorbents to extract aroma compounds from alcoholic beverages. *J. Chromatogr. Sci.* **2000**, *38*, 469–476.

(3) Versini, G.; Rapp, A.; Ullemeyer, H. 2-Aminoacetophenone: causal component of 'untypical aging flavour', 'naphthalene note', 'hybrid note' of wine. *Vitis* **1993**, *32*, 61–62.

(4) Silva Ferreira, A. C.; Hogg, T.; Guedes de Pinho, P. Identification of key odorants related to the typical aroma of oxidation-spoiled white wines. *J. Agric. Food Chem.* **2003**, *51*, 1377–1381.

(5) Ferreira, V.; Aznar, R.; Lopez, R.; Cacho, J. Quantitative gas chromatography−olfactometry carried out at different dilutions of an extract. Key differences in the odor profiles of four high-quality spanish aged red wines. *J. Agric. Food Chem.* **2001**, *49*, 4818–4824.

(6) Aznar, M.; Lopez, R.; Cacho, J.; Ferreira, V. Identification and quantification of impact odorants of aged red wines from Rioja. GC−olfactometry, quantitative GC-MS and color evaluation. *J. Agric. Food Chem.* **2001**, *48*, 2924–2929.

(7) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Rôle du sotolon dans l'arôme des vins doux naturels, influence des conditions d'élevage et de vieillissement. *J. Int. Sci. Vigne Vin* **1998**, *32* (4), 223–233.

(8) Pons, A. *Recherches sur l'Arôme de Vieillissement Prématuré des Vins*; Université Victor Segalen Bordeaux II: Bordeaux, France, 2006.

(9) Guth, H. Quantitation and sensory studies of character impact odorants of different white wine varieties. *J. Agric. Food Chem.* **1997**, *45*, 3027–3032.

(10) Eyres, G.; Marriot, P. J.; Dufour, J. P. The combination of gas chromatography−olfactometry and multidimensional gas chromatography for the characterization of essential oil. *J. Chromatogr., A* **2007**, *1150*, 770–777.

(11) Ferreira, V.; Hernandez-Orte, P.; Escudero, A.; Lopez, R.; Cacho, J. Semipreparative reversed-phase liquid chromatographic fractionation of aroma extracts from wine and other alcoholic beverages. *J. Chromatogr., A* **1999**, *864*, 77–88.

(12) Pineau, B.; Barbe, J.-C.; VanLeeuwen, C.; Dubourdieu, D. Which impact for β-damascenone on red wines aroma? *J. Agric. Food Chem.* **2007**, *55*, 4103–4108.

(13) Van Del Dool, H.; Kratz, P. A generalization of the retention index system including linear temperature programmed gas−liquid partition chromatography. *J. Chromatogr.* **1963**, *11*, 463–471.

(14) Boidron, J. N.; Chatonnet, P.; Pons, M. Influence du bois sur certaines substances odorantes des vins. *Connaiss. Vigne Vin* **1988**, *22*, 275–294.

(15) Peres, M.; Poirier, P. *Méthodes et Réactions de l'Analyse Organique. II. Méthode de Caractérisation*; Masson et Cie: Paris, France, 1953.

(16) Demole, E.; Enggist, P.; Säuberli, U.; Stoll, M.; Kovats, E. Structure et synthèse de la damascénone (triméthyl-2,6,6-*trans*-crotonoyl-1-cyclohexadiène-1,3), constituant odorant de l'essence de rose bulgare (*Rosa damascena* Mill.). *Helv. Chim. Acta* **1970**, *53*, 541.

(17) Schreier, P.; Drawert, F.; Junker, A. Gaschromatographisch-massens-pektrometrische untersuchung fluchtiger inhaltsstoffe des weines. *Z. Lebensm. Unters. Forsch.* **1974**, *154*, 757–759.

(18) Kotseridis, Y.; Baumes, R. L.; Skouroumounis, G. K. Quantitative determination of free and hydrolytically liberated β-damascenone in red grapes and wines using a stable isotope dilution assay. *J. Chromatogr., A* **1999**, *849* (1), 245–254.

(19) Sen, A.; Laskawy, G.; Schieberle, P.; Grosch, W. Quantitative determination of β-damascenone in foods using a stable isotope dilution assay. *J. Agric. Food Chem.* **1991**, *39*, 757–759.

(20) Etiévant, P. Stoned fruit: apricot, plum, peach, cherry. In *Food Flavours, The Flavour of Fruits*; Morton, I. D., Macleod, A. J., Eds.; Elsevier: Amsterdam, The Netherlands, 1990.

(21) Acree, T.; Braell, P.; Butts, M. The presence of damascenone in cultivars *Vitis Vinifera* (Linnaeus), *rotundifolia* (Michaux) and *labruscana* (Bailly). *J. Agric. Food Chem.* **1981**, *29*, 688–690.

(22) Fang, Y.; Qian, M. Aroma compounds in Oregon Pinot Noir wine determined by aroma extract analysis (AEDA). *Flavour Fragrance J.* **2005**, *20*, 22–29.

(23) Ferreira, V.; Cacho, R. L. Quantitative determination of the odorants of young red wines from different grape varieties. *J. Sci. Food Agric.* **2000**, *80* (11), 1659–1667.

(24) Kotseridis, Y. *Etude de l'Arome des Vins de Merlot et de Cabernet Sauvignon de la Region Bordelaise*; Victor Segalen, Bordeaux II: Bordeaux, France, 1999.

(25) Moutounet, M. Formation des substances volatiles au cours de l'élaboration du pruneau. In *Flavours of Fruits and Fruit Juices*; Juris Druck and Verlag, International Federation of Fruit Juice Producers: Zurich, Switzerland, 1978; pp 363−372.

(26) Escudero, A.; Campo, E.; Farina, L.; Cacho, J.; Ferreira, V. Analytical characterization of the aroma of five premium red wines. Insights into the role of odor families and the concept of fruitiness of wines. *J. Agric. Food Chem.* **2007**, *55*, 4501–4510.

(27) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Etude sur l'arôme des vins doux naturels non muscatés. *J. Int. Sci. Vigne Vin* **1998**, *32* (2), 99–110.

(28) Sarrazin, E.; Dubourdieu, D.; Darriet, P. Characterization of key-aroma compounds of botrytized wines, influence of grape botrytization. *Food Chem.* **2007**, *103* (2), 536–545.

(29) Ferreira, V.; Jarauta, I.; Ortega, L.; Cacho, J. Simple strategy for the optimization of solid-phase extraction procedures through the use of solid-liquid distribution coefficients: application to the determination of aliphatic lactones in wine. *J. Chromatogr., A* **2004**, *1025*, 147–156.

(30) Cerdan, T. G.; Goni, D. T.; Azpilicueta, C. A. Accumulation of volatile compounds during ageing of two red wines with different composition. *J. Food Eng.* **2004**, *65* (3), 349–356.

(31) Nitz, S.; Kollmannsberger, H. Changes in flavour composition during thermal concentration of apricot purée. *Z. Lebensm. Unters. Forsch.* **1993**, *197*, 541–545.

(32) Greger, V.; Schieberle, P. Characterization of the key aroma compounds in apricots (*Prunus armeniaca*) by application of the molecular sensory science concept. *J. Agric. Food Chem.* **2007**, *55*, 5221–5228.

(33) Egel, K. H.; Flath, R. A.; Buttery, R. G.; Mon, T. R.; Ramming, D. W.; Teranishi, R. Investigation of volatile constituents in nectarines. 1. Analytical and sensory characterization of aroma components in some nectarine cultivars. *J. Agric. Food Chem.* **1988**, *36*, 549–553.

(34) Darriet, P.; Pons, M.; Henry, R.; Dumont, O.; Findeling, V.; Cartolaro, P.; Calonnec, A.; Dubourdieu, D. Impact odorants contributing to the fungus type aroma from grape berries contaminated by powdery mildew (*Uncinula necator*); Incidence of enzymatic activities of the yeast *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* **2002**, *50*, 3277–3282.

(35) LaGuerche, S.; Dauphin, B.; Pons, M.; Blancard, D.; Darriet, P. Characterization of some mushroom and earthy off-odors microbially induced by the development of rot on grapes. *J. Agric. Food Chem.* **2006**, *54*, 9193–9200.

(36) Campo, E.; Ferreira, V.; Lopez, R.; Escudero, A.; Cacho, J. Identification of three novel compounds in wine by means of a laboratory-constructed multidimensional gas chromatographic system. *J. Chromatogr., A* **2006**, *1122*, 202–208.

(37) Naef, R.; Jaquier, A.; Velluz, A.; Maurer, B. New constituents related to 3-methyl-2,4-nonanedione identified in green tea. *J. Agric. Food Chem.* **2006**, *54*, 9201–9205.

(38) Guth, H.; Grosch, W. 3-Methyl-2,4-nonanedione an intense odour compound formed during flavour reversion of soya-bean oil. *Fat Sci. Technol.* **1989**, *91*, 225–230.

(39) Masanetz, C.; Grosch, W. Hay-like off-flavour of dry parsley. *Z. Lebensm. Unters. Frosch.* **1998**, *206* (2), 114–120.

(40) Masanetz, C.; Guth, H.; Grosch, W. Fishy and hay-like off-flavours of dry spinach. *Z. Lebensm. Unters. Frosch.* **1998**, *206* (2), 108–113.

(41) Guth, H.; Grosch, W. Identification of potent odorants in static headspace samples of green and black tea powders on the basis of aroma extract dilution analysis (AEDA). *Flavour Fragrance J.* **1993**, *8*, 173–178.

(42) Guth, H.; Grosch, W. Detection of furanoid fatty acids in soya-bean oil—cause for the light-induced off-flavour. *Fat Sci. Technol.* **1991**, *93* (7), 249–255.

Received for review December 1, 2007. Revised manuscript received March 31, 2008. Accepted April 16, 2008. We thank the Seguin Moreau cooperage, the Conseil Interprofessionnel des Vins de Bordeaux (CIVB), and the Conseil Régional d'Aquitaine for funding this project.

JF073513Z