

Occurrence of Hydroxypropanedial in Certain Musts and Wines

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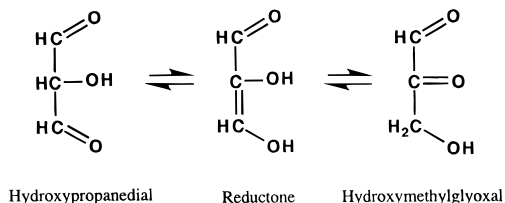
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The identification of a reductone found in sweet botrytized white table wines is described. A derivation method with *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA), which permitted the identification of carbonyl and α -dicarbonyl compounds, was used. On the basis of analysis by GC and GC/MS and of $^1\text{H-NMR}$ data, the compound has been identified as hydroxypropanedial. This triose reductone is possibly characterized by a large sulfur dioxide combination power and redox properties. The compound was isolated from musts and wines produced from botrytized grapes. It is normally present in very low concentrations in dry white or red wines, but this carbonyl compound occurs in huge levels if *Botrytis cinerea* and other damaging molds with associated moldy and acetic characters are present in spoiled berries.

Keywords: *Hydroxypropanedial; reductones; α -dicarbonyls; wine*

INTRODUCTION

Hydroxypropanedial, also known as triose reductone, presents a tautomeric equilibrium with 3-hydroxy-2-oxopropanal and with 3-hydroxy-2-hydroxyprop-2-enal. Hydroxypropanedial is the simplest reductone. Reductones have reductive properties because enediol may be transformed into dicarbonyl. They may also constitute oxidoreduction systems (Rabotnova et al., 1963). Oxidoreduction reactions in wine have been implicated in different transformations during the maturation and aging process (Paul, 1962, 1963). Reductones preserve organoleptic qualities, fix aromas (Yamanaka et al., 1994; Yamanaka and Tsunoda, 1995), and inhibit bacterial development (Shimohara et al., 1981).



The presence of hydroxypropanedial in wines was proposed by Barraud (1954), who determined a compound, namely "R", with a very similar structure but which was not a reductone. Later, Paul (1963) gave details of the occurrence of natural reductones in musts and wines, but these were not clearly determined. Reductones have been identified in different foods (Bagdanka, 1959; Shpan, 1959) and in beverages (Masiar, 1958; Owades and Zientora, 1961; Postolatii, 1992).

Reductones may react with amino acids and form browning compounds (Yamagushi, 1969; Shimohara et al., 1974). Products of the browning reaction are also observed with most dicarbonyl compounds such as glyoxal, methylglyoxal, and diacetyl (Velisek and Davidek, 1978). These compounds have also been detected in wine produced by the metabolism of yeast or lactic acid bacteria (de Revel and Bertrand, 1994).

The determination of natural reductones with oxidoreduction reactions (iodometry) is difficult because

sulfur dioxide interacts. Moreover, other methods [determination by tartrazine, formation of indolphenolic derivatives (Nomura and Fukuda, 1959), or reaction with 2,4-dinitrophenylhydrazine] have been used, but these methods in our conditions of study do not give us satisfaction. In this paper, the identification of hydroxypropanedial, a triose reductone, is reported in musts and wines; high levels of hydroxypropanedial are shown in sweet table wines, particularly in "Sauternes", and initial results are presented.

MATERIALS AND METHODS

Aldehydes and dicarbonyl compounds were purchased from Aldrich Chemicals and used without further purification.

Carbonyl Analysis. The method of detection is derived from that used for carbonyl compounds with low molecular weight in alcoholic drinks such as wines, beers, or brandies (Guillou, 1992; de Revel and Bertrand, 1993). We used *O*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBOA) added to the carbonyl function to form an oxime. PFBOA-derived products are very volatile and are easy to detect by gas chromatography/mass spectrometry (GC/MS) or a gas chromatography/electron capture detector (GC/ECD). This method is now used routinely in our laboratory for different types of brandies (Hervé, 1996), Port wines (Ferreira, 1993), and dry white and red wines (Guillou, 1992).

For musts made from grapes without *Botrytis cinerea* attack, white wines and red wines, 2 mL samples were mixed with 5 mg of PFBOA powder (Aldrich 19, 448-4) and 50 μL of lindane (Aldrich H-4500) at a concentration of 600 mg/L as internal standard. For musts made from berries infected with *B. cinerea* (noble rot) and for sweet wines ("moelleux" and "liqueureux"), the preparation was different: 0.1 mL of must or wine was mixed with 1.9 mL of a 15% hydroalcoholic solution containing PFBOA (5 mg) and 50 μL of lindane (500 mg/L).

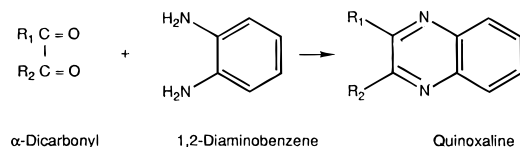
The reaction was done in a stoppered flask for 1 h at room temperature. The oximes were extracted with 2 mL of ether/hexane (1:1 v/v, SDS, *pestipurs*) for 2 min. One microliter of extract was injected into the GC (with ECD or MS detectors).

Chromatographic conditions were as follows: (for GC/ECD) column, apolar (CPSIL 5CB, Chrompack, 25 m \times 0.32 mm \times 0.12 μm); injector and detector temperatures, 250 $^{\circ}\text{C}$; oven temperature, 60 $^{\circ}\text{C}$ programmed at a rate of 2 $^{\circ}\text{C}/\text{min}$ to 210 $^{\circ}\text{C}$, the final step lasting 10 min; splitless time, 20 s; (for GC/MS) Hewlett-Packard HP 5890 gas chromatograph coupled with a mass spectrometer (HP 5970, electronic impact = 70 eV, eMV = 2.7 kV and mode in total ion chromatogram or TIC);

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column, Carbowax 20 M (AML) (50 m × 0.25 mm × 0.12 μm); injector and detector temperatures, 250 °C; oven temperature, 60 °C programmed at a rate of 3 °C/min to 200 °C, the final step lasting 30 min; splitless time, 20 s. This method have been validated: sensitivity, linearity, and repeatability have been studied and previously published (Guillou, 1996).

α-Dicarbonyl Analysis. The method for determining the α-dicarbonyls was adapted from that described previously by Moree-Testa and Saint-Jalm (1981) and Bednarski et al. (1989). α-Dicarbonyl compounds were detected after reaction with 1,2-diaminobenzene ($R_1 = H$; $R_2 = CH_2OH$) and were identified by GC/MS as quinoxaline derivatives.



Preparation of Sample. In 50 mL of wines at pH 8 (NaOH), 5 mL of an aqueous solution of 1,2-diaminobenzene (Sigma, *o*-phenylenediamine P-9029) at 5.4 g/L was added, and the whole was further kept for 2 h at 25 °C. The mixture was acidified with 4 N sulfuric acid to pH 2.5 and extracted three times with 5 mL of dichloromethane. The organic phase was isolated and evaporated to form quinoxaline yellow-brown crystals, which were then diluted in 1 mL of methanol and injected in GC/MS. Apparatus and conditions were the same as described above for PFBOA derivative analysis. Two microliter volumes were injected in this case.

Synthesis of Hydroxypropanedial. Hydroxypropanedial or triose reductone was synthesized according to the method of William and Waring (1926). Thirty grams of copper acetate was added in dihydroxyacetone solution (4.6 g in 100 mL of water). The reaction mixture was kept 48 h at 60 °C. It formed a red precipitate of copper oxide. The only products were hydroxypropanedial and acetic acid. Reaction yield was superior to 98%, and chromatographic purity was better than 80%.

Wine Samples. Wines were obtained from different regions in France where desirable *B. cinerea* infection occurs on grapes. Commercial red and white wines originated from the Bordeaux region.

RESULTS AND DISCUSSION

Carbonyl Analysis. By the PFBOA derivation method, we detected the presence of one substance (peaks 5 and 10, Figure 1) in great concentrations, particularly in the case of botrytized sweet white table wines (Figure 1). In other wines, this substance was found in smaller levels. Since the substance was a PFBOA derivate, the identification of the molecule was complicated.

PFBOA Derivation, GC Analysis. The dicarbonyl compounds derived formed several peaks, since oxime has two geometrical isomers; according to the quantity of reagent (PFBOA) present, the reaction formed a monooxime or a dioxime. This also occurred for glyoxal, methylglyoxal, and the unidentified compound particularly present in botrytized sweet wines (Figures 1 and 2).

Comparison with other dicarbonyls allowed an evaluation of molecular weight for the unknown derived compound. The molecular weights were proportional to the retention time observed in the chromatogram (Figure 3). The equation made it possible to estimate the molecular weight of the derived compound: monooxime (peak 1) and dioxime (peak 2). The unidentified molecule after deduction of the PFBOA had a molecular weight between 88 and 90.

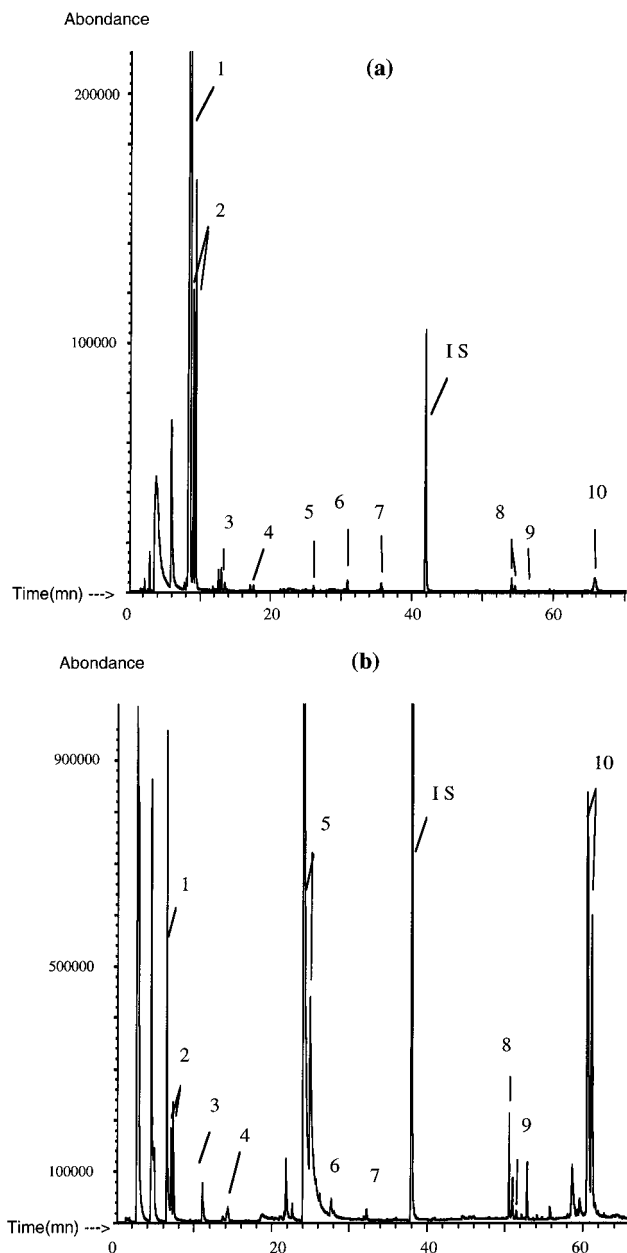


Figure 1. Gas chromatogram of the extract of PFBOA-derivatized carbonyl compounds of a sweet white wine detected in ECD: (1) excess of PFBOA; (2) acetaldehyde; (3) glyoxal monooxime; (4) methylglyoxal monooxime; (5) unknown compound monooxime; (6) glyceraldehyde; (7) dihydroxyacetone; (IS) lindane; (8) glyoxal dioxime; (9) methylglyoxal dioxime; (10) unknown compound dioxime; (a) wine from grapes slightly attacked by rot; (b) wine from grapes strongly attacked by desirable and undesirable rot.

PFBOA Derivation, GC/MS Analysis. Normally the mass spectrum of the derived compound showed no clear molecular ion, except when the substance was in a great concentration. The molecular weight was determined by GC/MS chemical ionization with ammonia as reagent gas (chromatographic conditions are the same as for the preceding analyses by GC/MS). These different analyses confirmed the previously found molecular weight of the different oximes. The molecular weight of the unidentified substance was 88.

The compound is a dicarbonyl compound with an OH group. On the basis of these analyses the molecule was identified as hydroxypropanedial.

¹H-NMR analysis of the synthesized product proved the reductone structure. The same analysis of sweet

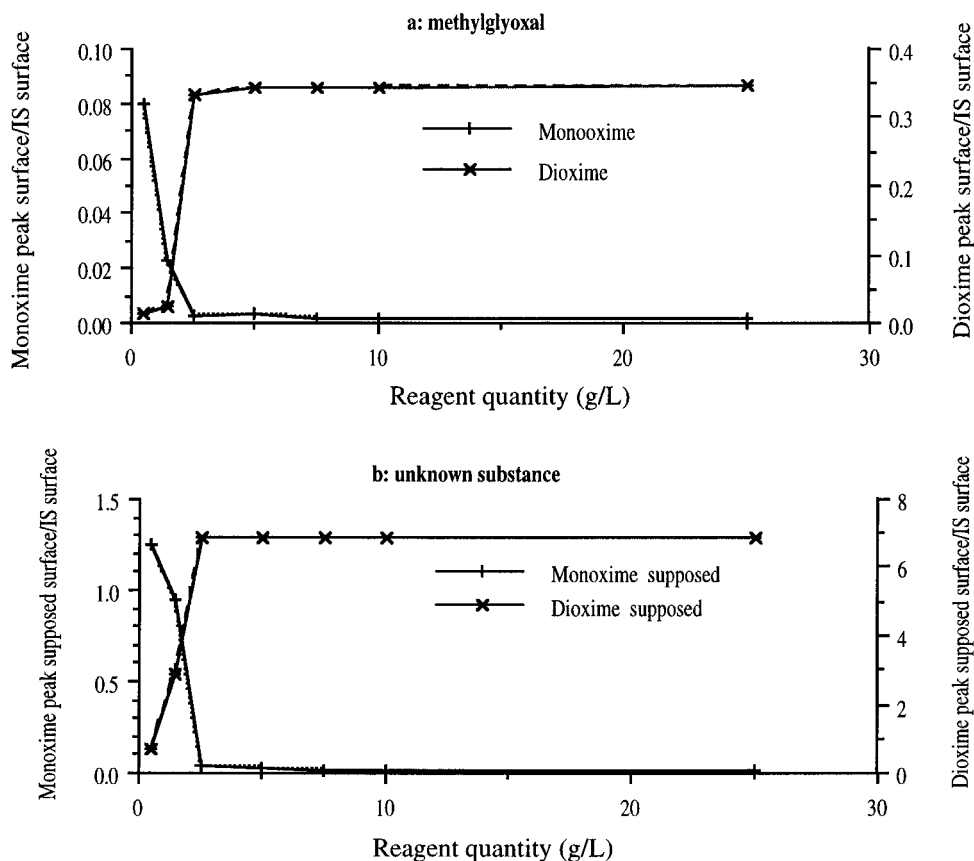


Figure 2. Incidence of PFBOA quantity with the monooxime/dioxime formation rate.

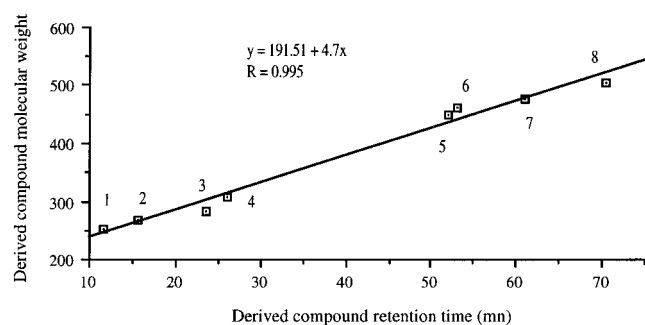


Figure 3. Molecular weight evaluation of compounds (with standard solution of products): (1) glyoxal monooxime; (2) methylglyoxal monooxime; (3) unknown substance, supposed monooxime; (4) hexane-2,3-dione monooxime; (5) glyoxal dioxime; (6) methylglyoxal dioxime; (7) unknown substance thought to be dioxime; (8) hexane-2,3-dione dioxime.

wine purified extract confirmed the hydroxypropanedial presence, because the integration of the proton signals attributed to hydroxypropanedial is in agreement with the structure under consideration.

Hydroxypropanedial was synthesized as described above. On the basis of GC/MS and NMR results, peaks 1 and 2 were confirmed as PFBOA-derived hydroxypropanedial. The product previously synthesized was derived and injected in the same conditions as musts and wines. Retention times of the two peaks were the same in the two different polarity chromatographic systems.

α -Dicarbonyl Analysis. α -Dicarbonyl compounds were detected after reaction with 1,2-diaminobenzene and were identified by GC/MS as quinoxaline derivatives. Glyoxal, methylglyoxal, diacetyl, and pentane-2,3-dione, compounds previously studied in wine (de Revel and Bertrand, 1994), were determined by this

Table 1. Hydroxypropanedial Levels in Different Musts and Wines

	hydroxypropanedial (mg/L)
from healthy grape musts	≤ 2.5
from botrytized grape musts	40–2500
red wines	≤ 1
dry white wines	≤ 2
sweet table wines (moelleux)	≤ 8
sweet botrytized white table wines (liquoreux) (1 determination/wine)	
1	25
2	30
3	33
4	45
5	50
6	62
7	160
8	296
9	331
10	412
11	417
12	493
13	612
14 ^a	1230

^a Wines from sour grapes.

α -dicarbonyl-specific method in Sauternes type wines. Moreover, reaction with 1,2-diaminobenzene confirmed the presence in these types of wines of 3-hydroxy-2-oxopropanal (hydroxymethylglyoxal), a dicarbonyl form of triose reductone (Figure 4).

Hydroxypropanedial Levels in Musts and Wines. Several musts and wines from different origins were analyzed to determine their hydroxypropanedial levels (Table 1). Results show that musts from sound grapes, dry white wines, and red wines had low levels of this compound (generally < 2.5 mg/L). However, both musts and dry wines from very bad quality grapes had higher

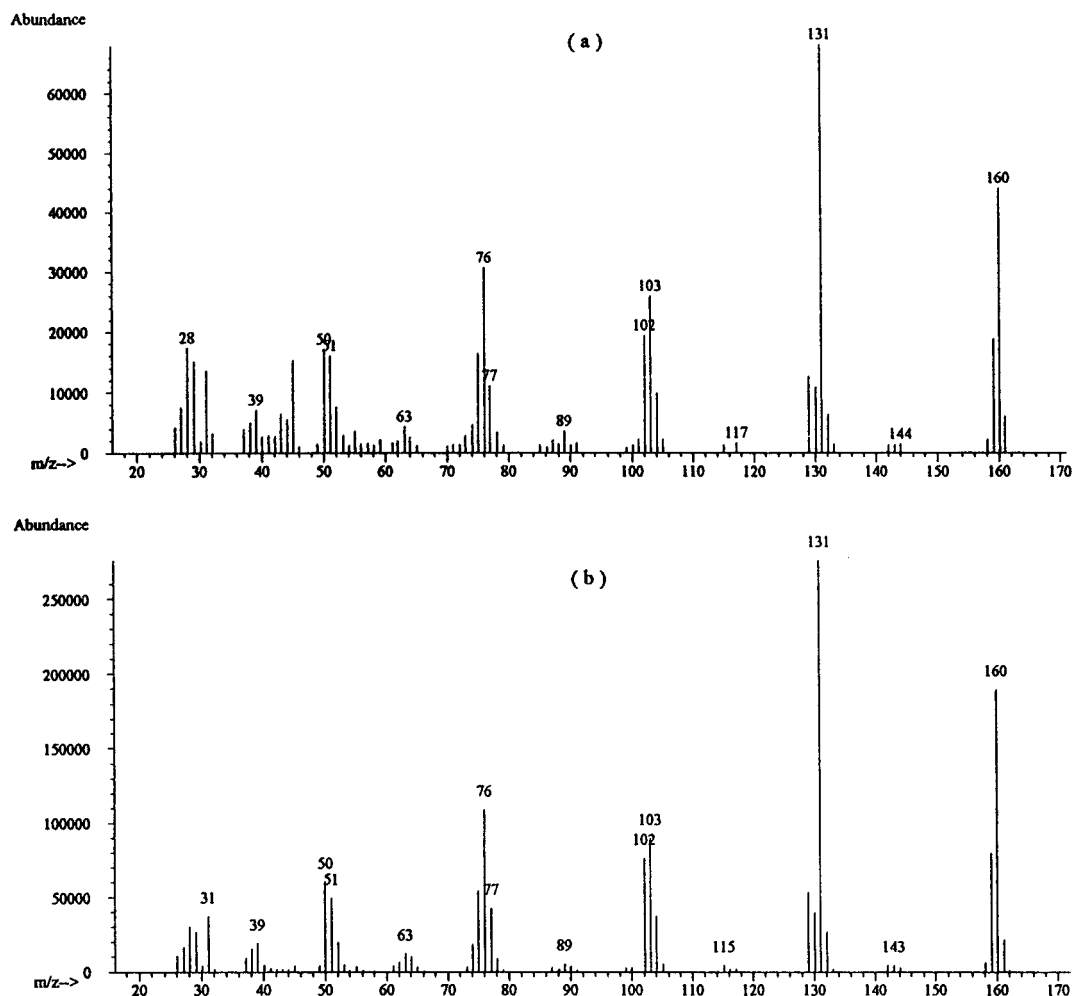


Figure 4. Mass spectrum of quinoxaline of 3-hydroxy-2-oxopropanal: (a) in wine; (b) standard.

concentrations of hydroxypropanedial, up to 20 mg/L for dry white wines with acetic problems.

On the other hand, sweet white table wines showed high levels of this reductone, but there is a great diversity according to the origin and the quality of the grapes. The first results indicate that the hydroxypropanedial concentration is dependent on the development and abundance of "noble rot" (*pourriture noble*). However, higher levels of hydroxypropanedial (to 3500 mg/L in wines) were also found due to the development of *Botrytis* mold (*pourriture grise*) on grapes. Other parasites or grape molds could also produce hydroxypropanedial.

Conclusion. This work confirms the presence of hydroxypropanedial or triose reductone in musts and wines, although further studies are necessary to clarify the presence of a reductone-bound form. Gas chromatography analysis with splitless injection mode may allow the determination of total reductone (free and bound) in musts and wines. GC/MS or GC/ECD after PFBOA derivation gives satisfactory sensitivity and is relatively easy and rapid to perform.

Hydroxypropanedial was identified in musts and white or red wines but generally in very low levels. If the grapes are attacked by mold, high concentrations of this compound may be encountered. Sweet white table wines made from grapes infected by desirable *Botrytis* (noble rot) are rich in triose reductone. If *B. cinerea* attack is very severe, according to the climate and the vintage, levels may be increased even 4–5-fold. If the grapes become spoiled (moldy and acetic), the

hydroxypropanedial concentration may reach 1 g/L, so this compound is a good marker for grape sanitary quality. Knowledge of hydroxypropanedial and other dicarbonyl compounds in botrytized wines is very important because of the role they play in sulfur dioxide combinations.

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