Gluconic Acid, Its Lactones, and SO$_2$ Binding Phenomena in Musts from Botrytized Grapes

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Intramolecular gluconic acid esterification reactions led to the formation of two lactones, γ- and δ-gluconolactone (glucono-1,4-lactone and glucono-1,5-lactone). The presence of the first has not yet been reported in must or wine. These lactones are in equilibrium with gluconic acid, γ- and δ-gluconolactone representing, respectively, 5.8 and 4.1% of the acid level. Correlations between must SO$_2$ binding power, gluconic acid, and consequently its lactones are shown. The SO$_2$ affinity of a mixture containing this acid and γ- and δ-gluconolactone was determined, and gluconic acid appeared to be indirectly responsible for ~8% of the bindable SO$_2$ in musts from botrytized grapes.

KEYWORDS: SO$_2$ binding; gluconic acid; γ- and δ- gluconolactone; intramolecular esterification

INTRODUCTION

In sweet white table wines made from grapes infected by desirable *Botrytis cinerea*, called noble rot, sulfur dioxide is an essential preservative. It is used to stop alcoholic fermentation and to avoid any fermentative phenomena during conservation of these wines. Sometimes the maximum authorized level of this additive does not allow effective antiyeast protection due to high carbonyl compound levels. In fact, these compounds react with SO$_2$ to give carbonyl bisulfite and thus inactivate it with respect to fermentative yeast.

In wine, SO$_2$-binding compounds arise either from alcoholic fermentation or from grapes themselves. The substances produced during alcoholic fermentation have long been studied (*1*), but this is not the case for those stemming from grapes. At this level, the importance of 5-oxofructose, which is sometimes responsible for more than half of the whole bound SO$_2$, was recently established (*2*). Even though we managed to highlight the correlation between gluconic acid levels and SO$_2$-binding powers, the exact role of this compound and its derivatives was still unclear.

That gluconic acid occurs in musts and wines from botrytized grapes is well established (*3*), and it is one of their major constituents. Studies on derivatives such as oxogluconic acids and δ-gluconolactone have been made. 2- and 5-oxogluconic acids have been characterized in botrytized musts and wines (*4*), but their role in SO$_2$-binding phenomena is very negligible (*5*). The simultaneous presence of δ-gluconolactone with the acid produced by glucose oxidation was demonstrated in refs *6*

and *7*. The possible role of this lactone in SO$_2$-binding phenomena was suggested in ref *8*; after determining the dissociation constant of its carbonyl bisulfite and showing that it is able to bind SO$_2$, these authors estimated the content of δ-gluconolactone as being 2−10% of the corresponding acid one.

The present paper investigates gluconic acid and its lactones in musts from botrytized grapes. Ratios between gluconic acid and the corresponding lactones are determined, their relationship with SO$_2$-binding power is discussed, and their role in SO$_2$-binding phenomena is demonstrated.

MATERIALS AND METHODS

Gluconic Acid Analysis. This was done with ionic HPLC. Levels were calculated by comparison of peak areas with those obtained after injections of aqueous solutions containing the reference product provided by Sigma-Aldrich (Saint Quentin Fallavier, France), like all of the chemicals used in this work. The Dionex 4500 ion chromatograph was equipped with three columns: an ion pack anion trap column (Dionex ATC-1 10-32), an ion pack precolumn (Dionex AG11, 50 mm × 4 mm), and an ion pack column (Dionex AS11, 250 mm × 4 mm). The system functioned in gradient mode with a mobile phase of water/methanol (20%, v/v) and NaOH: 0/0.5, 2/0.5, 20/35 (time in minutes/NaOH concentration in millimolar). Samples were diluted 50 times, filtered on a 0.22 μm membrane, and introduced into the eluant flow by a 10 μL injection loop. Eluant hydroxide ions were neutralized before the conductometric detection cell with the ASRS Ultra suppressor (Dionex) working in chemical mode.

γ- and δ-Gluconolactone Identification. This was performed after derivatization thanks to hexamethyldisilazane (*9, 10*). Sample (0.1 mL) was dried with a rotavapor, and 0.2 mL of anhydrous pyridine, 0.7 mL of hexamethyldisilazane, and 0.1 mL of trifluoroacetic acid were added to the dry residue. The reaction was performed in a stoppered flask for 3 h at 60 °C. After cooling, 1 μL of extract was injected into the GC with an MS detector. Chromatographic conditions were the following:

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Hewlett-Packard HP 6890 gas chromatograph coupled with a mass spectrometer (HP 5972, electronic impact 70 eV, eMV = 2 kV, detection mode was total ion chromatogram; column CP Sil 5CB (50 m × 0.25 mm, 0.2 μm); helium 5.6 Aga pressure, 70 kPa; injector temperature, 250 °C; detector temperature, 280 °C; oven temperature, 50 °C for 1 min programmed at a rate of 2 °C/min to 100 °C and then programmed at a rate of 5 °C/min to 250 °C, the final step lasting 20 min; split mode; split flow, 60 mL/min.

Derivative spectra were compared to the NBS 75K database and to reference product derivatives prepared from sodium gluconate and δ-gluconolactone.

γ- and δ-Gluconolactone and Gluconic Acid Relative Parts. These were estimated in synthetic solutions. Buffer solutions were prepared by dissolving 4 g/L tartaric acid and sodium gluconate to a 5.4 g/L gluconic acid level, and the mixture was partially neutralized to the required pH (4 M NaOH). First, an aqueous solution with pH 3.80 was kept at 25 °C and was daily analyzed to determine the delay necessary to establish an equilibrium between acid and lactone. Then, this test was renewed in pH 3.60, 3.80, and 4.00 buffer solutions (aqueous and aqueousalcoholic 14%, v/v). The samples were analyzed after equilibrium had been established between the different forms.

Quantification was performed after derivatization and GC injection as described before. Instead of an MS, the detector was a flame ionization one, and the response factors of the three derivative isomers were considered to be the same, as in fact is the case.

Determination of SO₂-Binding Power. The TL50 value was the total SO₂ level necessary to obtain 50 mg/L free SO₂. This was established on the basis of the work of Kielhöfer and Würdig (*11*), who were the first to draw SO₂-binding curves representing free SO₂ versus bound SO₂. Free and total SO₂ were determined by iodometry (*12*). Samples at pH 5 (12 M NaOH), to accelerate SO₂-binding phenomena, received four increasing and known SO₂ additions (thanks to a potassium bisulfite solution) in order to obtain a range of free SO₂ contents including the value of 50 mg/L. One hour after addition, free SO₂ was assayed. Then the straight line representing total SO₂ versus free SO₂ was plotted to express the TL50.

Equilibrium Constant for the Dissociation of Carbonyl Bisulfite Determination. The dissociation constant of the bisulfitic combination characterizes the affinity of a compound for SO₂. K_d was calculated according to the mass action law. The analytical methods used made it possible to establish only the total concentration of carbonyl compounds, so it was necessary to consider that $(C_{total}) = (C_{combined}) + (C_{free})$. In solution, where only one SO₂-binding compound is present, $(C_{combined}) = (SO_{2combined})$. Considering that $(SO_{2free}) = (HSO_3^-)$ because at our study pH the hydrogenosulfite level is nearly free SO₂, it appears that

$$K_d = \frac{(SO_{2free}) \times [(C_{total}) - (SO_{2combined})]}{(SO_{2combined})}$$

which means that

$$\frac{(SO_{2free})}{(SO_{2combined})} = \frac{1}{(C_{total})} \times (SO_{2free}) + \frac{K_d}{(C_{total})}$$

Thus, the curve $(SO_{2free})/(SO_{2combined})$ versus (SO_{2free}) expressed in millimolar is a straight line with which we could determine K_d as shown in **Figure 1**.

Our determinations were carried out in aqueous buffer solution (4 g/L tartaric acid, pH 3.8 using 4 M NaOH). A solution initially containing 5.4 g/L gluconic acid was divided into five fractions receiving SO₂ additions (66, 132, 198, 264, and 330 mg/L). The bottles, which were filled and hermetically stopped, were kept at 25 °C. After 7 days, once equilibrium had been established, free and total SO₂ were assayed.

SO₂ Combination Estimation. As indicated above, according to the mass action law

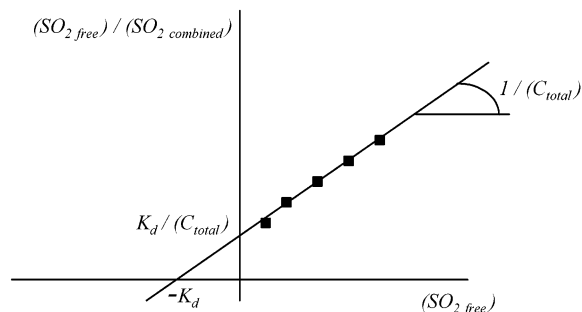$$K_d = \frac{(C_{free}) \times (HSO_3^-)}{(C_{combined})}$$

Figure 1. $(SO_{2free})/(SO_{2combined})$ ratio versus (SO_{2free}) in a solution containing only one SO₂ binding compound.
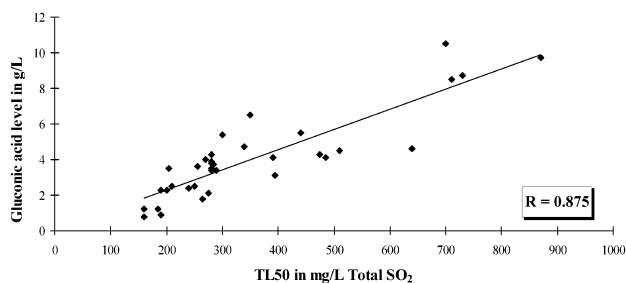

Figure 2. Gluconic acid levels versus TL50.

Because $(C_{total}) = (C_{combined}) + (C_{free})$, by knowing the hydrogenosulfite level (which was nearly the same as free SO₂), the estimation of the quantity of compound combined with SO₂ was possible:

$$\frac{(C_{combined})}{(C_{total})} = 1 - \frac{K_d}{(HSO_3^-) + K_d}$$

Because SO₂-binding power was TL50, these conditions (free SO₂ = 50 mg/L) were used to estimate the fraction of the compound bound to SO₂ and to calculate its contribution to SO₂ binding.

Appropriation of Grapes and Preparation of Musts. During the 2000 harvest, grapes infected by desirable *B. cinerea* (noble rot) were harvested in the Sauternes area (close to Bordeaux). Each batch was composed of 1−2 kg of berries, which were pressed with a small vertical wine-press. The musts obtained were centrifuged at 9000*g* for 15 min and kept at −18 °C until analysis. Thirty-five different musts were prepared with this technique and are shown in **Table 1**.

RESULTS AND DISCUSSION

Gluconic Acid Levels. In musts obtained with TL50 values ranging between 160 and 870 mg/L total SO₂, gluconic acid levels were almost always >1 g/L and could reach >10 g/L. As shown in **Figure 2**, these levels correlated well with the SO₂-binding power of musts. This confirms observations made in the 1997 and 1998 vintages (*2*). The production of gluconic acid by *B. cinerea* has already been described (*13*), but these significant increases cannot be due to only this fungus; berry glucose oxidation by acetic acid bacteria, in particular *Gluconobacter* as demonstrated earlier (*14*), should also be taken into account.

Characterization of Gluconic Acid Lactones. A buffer solution (pH 3.8) initially containing gluconic acid was derivatized. Analysis by gas chromatography showed two additional peaks numbered 1 and 2 (**Figure 3**), which were not present after sodium D-gluconate crystals derivatization. Once silylated, sodium D-gluconate crystals gave only peak 3 and δ-gluconolactone crystals gave only peak 1. As expected, MS spectra of peaks 1 and 3 corresponded according to the NBS75K library and in comparison with reference compounds, respectively, with those of the silylated derivatives of δ-gluconolactone and
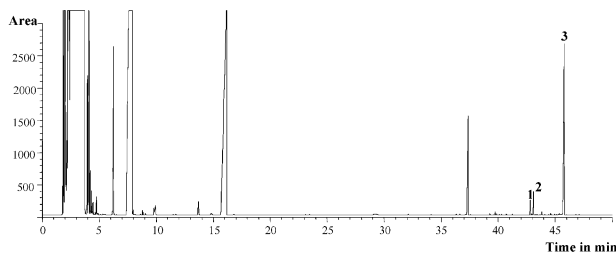
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Figure 3. Chromatogram of model medium after silylation: 1, δ-glucono-lactone derivative; 2, unknown peak; 3, gluconic acid derivative.

Table 1. SO$_2$-Binding Power (TL50), Sugars Contents, and pH in Samples

picking date	TL50 (mg/L of total SO$_2$)	sugars (g/L)	pH
Sept 21, 2000	250	349	3.93
	240	368	3.98
	350	323	3.87
	280	320	3.68
Oct 5, 2000	340	405	3.73
	390	409	3.39
	270	473	3.34
Oct 9, 2000	255	386	3.93
	280	407	4.03
	280	360	4.11
	300	355	3.93
	185	276	3.80
	160	262	3.80
	160	289	3.75
	190	291	3.80
Oct 12, 2000	205	252	3.80
	190	262	3.80
	210	281	3.75
	200	278	3.80
Oct 20, 2000	870	233	3.61
	730	215	3.76
	710	196	3.86
	700	204	3.84
	280	225	3.52
	280	239	3.76
	285	233	3.66
	290	239	3.54
Oct 30, 2000	510	257	3.52
	485	278	3.62
	640	254	3.59
	475	247	3.58
	395	228	3.78
	265	207	3.88
	275	260	3.81
	440	265	3.72

gluconic acid. As shown in **Figure 4**, peak 2 mass spectrum matched the silylated derivative of the γ-gluconolactone in the NBS75K library (identical fragmentation and comparable abundances). To our knowledge, the presence of this compound in wine has not yet been reported. Gluconic acid is a hydroxy-acid; the hydroxyl groups in positions 4 and 5 react with the carbon atom of the acid group according to an intramolecular esterification reaction, as specified for various hydroxyacids (*15*), and give, respectively, glucono-1,4-lactone (γ-glucono-lactone) and glucono-1,5-lactone (δ-gluconolactone) as shown in **Figure 5**. Due to the high sugar contents of the musts, which interfered with analysis, it was not possible to highlight these compounds in musts from botrytized grapes. Nevertheless, we managed to characterize these lactones in dry white wine from botrytized grapes (**Figure 6**).
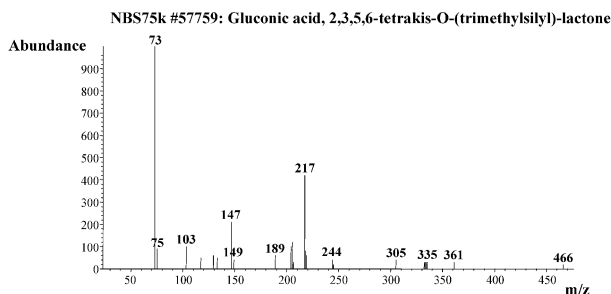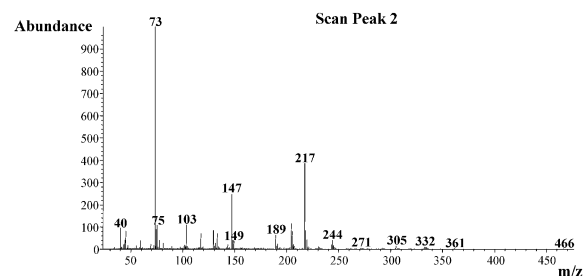

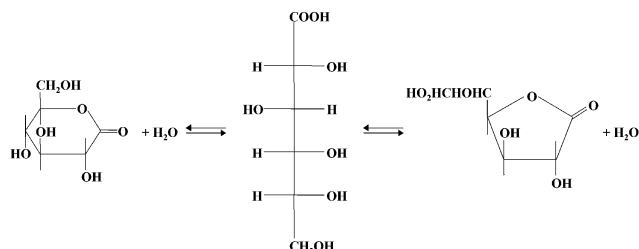**Figure 4.** Mass spectrum of peak 2 and γ-gluconolactone according to the NBS75K library.

Figure 5. Equilibrium between gluconic acid and γ- and δ-gluconolactone.
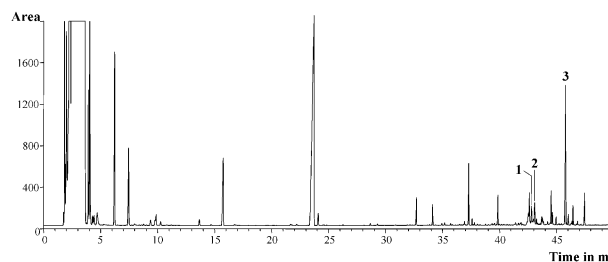

Figure 6. Chromatogram of dry white wine from botrytized grapes after silylation: 1, δ-gluconolactone derivative; 2, γ-gluconolactone derivative; 3, gluconic acid derivative.

Relative Parts of Gluconic Acid and Its Lactones. Twenty-four hours after dissolution of sodium D-gluconate, γ- and δ-gluconolactone proportions remained constant as indicated in **Figure 7**.

Analysis of aqueous synthetic solutions with pH ranging between 3.60 and 4.00 showed that the relative levels of γ- and δ-gluconolactone remained constant as shown in **Figure 8**. In this pH range, γ-gluconolactone was more abundant than δ-gluconolactone, the average value of the ratio between the contents of these two isomers being 1.41. Thus, at the pH of musts from botrytized grapes, γ- and δ-gluconolactone levels were, respectively, about 5.8 and 4.1% of the gluconic acid level. Experiments performed in aqueous alcoholic solutions showed very similar results: at the pH of wines from botrytized grapes, γ- and δ-gluconolactone levels were, respectively, about 6.1 and 4.1% of the gluconic acid level. Because gluconic acid is not metabolized during alcoholic fermentation (*16*), the SO$_2$ bindable to these compounds in musts and corresponding wines is very probably the same.
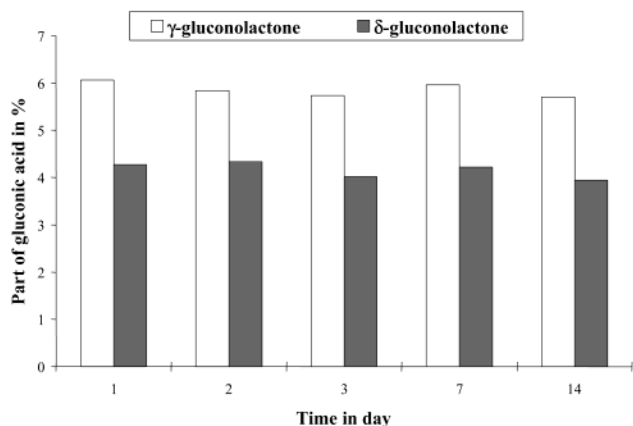
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Figure 7. Relative part of γ- and δ-gluconolactone after sodium gluconate dissolution in synthetic buffer medium at pH 3.80.
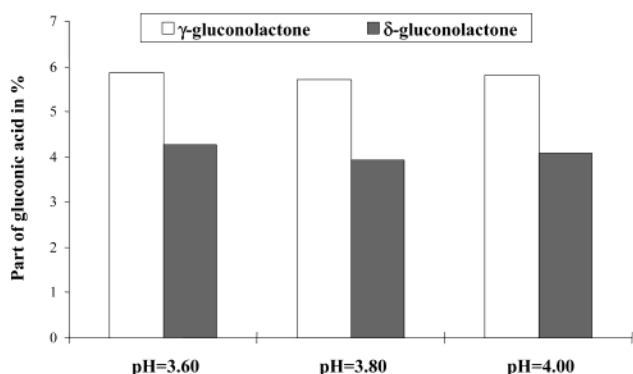

Figure 8. Relative part of γ- and δ-gluconolactone in aqueous synthetic buffer medium at different pH values.

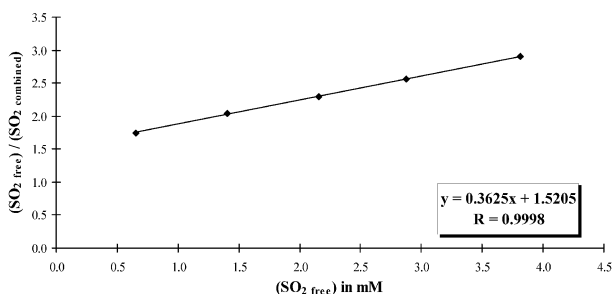**Figure 9.** $(SO_{2free})/(SO_{2combined})$ ratio versus (SO_{2free}) in a pH 3.80 aqueous solution containing 4.924 g/L gluconic acid, 0.279 g/L γ-gluconolactone, and 0.197 g/L δ-gluconolactone.

Sulfur Dioxide Affinity. Due to its chemical structure, gluconic acid is not able to form carbonyl bisulfite. The affinity for SO₂ of a solution containing 4.924 g/L gluconic acid, 0.279 g/L γ-gluconolactone, and 0.197 g/L δ-gluconolactone was determined and is shown in **Figure 9**. This mixture affinity was the same as that of a monocarbonyl compound characterized by a concentration of 2.76 mM, which is nearly the sum of γ- and δ-gluconolactone concentrations (2.68 mM) and a constant dissociation of the carbonyl bisulfite, K_d = 4.19 mM, which is very close to that determined for δ-gluconolactone bisulfite in refs *8* and *17*, that is, respectively, 4.0 and 3.9 mM.

Thus, a total gluconolactone concentration of 100 mg/L explained 5.6 mg/L bound SO₂ for a free SO₂ level of 50 mg/L. Insofar as the sum of the of γ- and δ-gluconolactone corresponds to ~10% of gluconic acid, these lactones in the musts analyzed were able to bind up to 58 mg/L SO₂ for 50 mg/L free SO₂. In these conditions, gluconic acid is therefore indirectly responsible for 7.7 ± 2.4% of the whole bindable SO₂ in musts from botrytized grapes.

With the help of GC-MS of silylated derivatives, we have managed to highlight for the first time in wine γ-gluconolactone formed by a gluconic acid intramolecular esterification reaction between the acid and hydroxyl groups on C-4. With δ-gluconolactone, this compound is in balance with gluconic acid. We confirm that this acid correlates well with SO₂-binding powers. It is well-known to be a *Gluconobacter* metabolite (*18*). This genus has even been defined from acetic acid bacteria strains with high glucose oxidative activity (*19*).

The affinity for SO₂ of γ- and δ-gluconolactone has clearly been determined. The carbonyl bisulfite dissociation constant is such that the combination is not irreversible and may dissociate to give bisulfite again when free SO₂ levels diminish. Nevertheless, even if they are not as important as 5-oxofructose, these lactones are among the most important compounds of musts from botrytized grape SO₂-binding balance sheet.

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