RECENT OBSERVATIONS IN WINE OXIDATION[•]

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1. INTRODUCTION

The oxidation of wine has been recognized as a major issue in the chemistry of wine for over 100 years, and was a key issue addressed by Pasteur when he wrote about his studies of wine (Pasteur, 1875). He proposed that wine aging is in fact only wine oxidation. We do know now that there are many other chemical reactions that occur during wine aging, such as ester equilibration, glycoside hydrolysis, and the rearrangement of condensed tannins, but it would still appear that for most wines, their shelf life is limited by their capacity to retain a suitable flavor after absorbing a particular amount of oxygen.

There have been a number of reviews and perspectives on wine oxidation, including those by Danilewicz (2003), Li (Li *et al.*, 2008), Singleton (1987) and Waterhouse and Laurie (2006). This paper will address a few recent developments in key oxidation reactions, in particular reactions of quinones and the Fenton reaction to yield aldehydes and ketones.

2. QUINONES

The initial reaction of oxygen with wine components necessitates the catalytic power of iron or copper in their reduced salt forms. This reaction produces the oxidized form of the metal as well as the reduced form of oxygen, superoxide, or in the acidic environment of wine, the hydroperoxyl radical. This radical has not been observed in wine, but it may exist and be difficult to detect because its spin trapped products are unstable and thus difficult to observe. That radical next reacts very quickly with phenolic substances to reduce the oxygen to the next stage, hydrogen peroxide, and the phenol is converted to the phenolic radical. This reaction is very favored for catechol-containing phenolics because that resulting radical is very stable as a semi-quinone. The semi-quinones disproportionate to yield quinone and the catechol. The oxidized metal will also react with catechols to yield quinones, so both products of the initial oxygen/metal reaction lead to quinone.

Quinones are reactive as electrophiles, and understanding the first stage of wine oxidation involves ascertaining the products of the reaction with quinones.

Quinones will react with nucleophiles and reducing agents, so the first issue is to consider which of these components wine contains. Generally, good nucleophiles include sulfur, nitrogen, oxygen and, under the right conditions, carbon. Nitrogen nucleophiles are amines or related organic substances. While these are very good nucleophiles, under the

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[•] QUAD. VITIC. ENOL. UNIV. TORINO, 31, 2009-2010 acidic conditions in wine, amines are all protonated, eliminating their nucleophilic properties, so in general these can be discounted and such products have generally not been reported.

Sulfur nucleophiles are very important and two classes are described in the literature. First are the reduced form of sulfur, the thiols (also known as mercaptans and sulfanyl compounds). These are important because while they react quickly with quinones in most cases, they are often found in small amounts and so generating just a small amount of quinone by oxidation can deplete these substances. A recent study (Nikolantonaki *et al.*, 2010) compared the reaction of three thiols with oxidized forms of catechin and epicatechin and showed that steric hindrance was a key to reactivity, as would be expected for nucleophiles.

The most reactive are primary (1°) thiols, such as 2-furanmethanethiol, while next in reactivity are the secondary (2°) such as 3-sulfanylhexan-1-ol, also widely known at 3-mercaptohexanol, and the slowest to react are the tertiary (3°) thiols such as 4-methyl-4-sulfanylpentan-2-one (fig. 1).

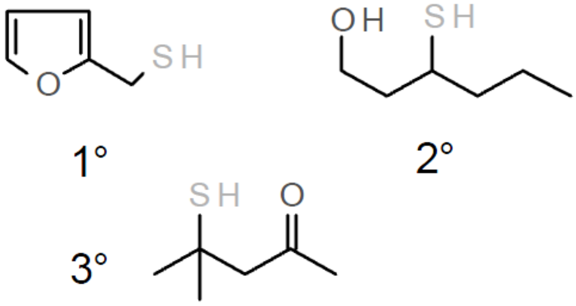

Fig. 1 - Thiols with different steric limitations found in wine

Related nucleophiles include sulfur containing amino acids and hydrogen sulfide (Singleton, 1987). In the latter case, the reaction will eliminate the “reduced” aroma often observed in wine production, where hydrogen sulfide is released by yeast as part of their normal fermentative metabolism. And a number of other sulfur nucleophiles have been observed to react with wine quinones, including the first observation of this reaction (Cheynier *et al.*, 1986) (fig. 2).

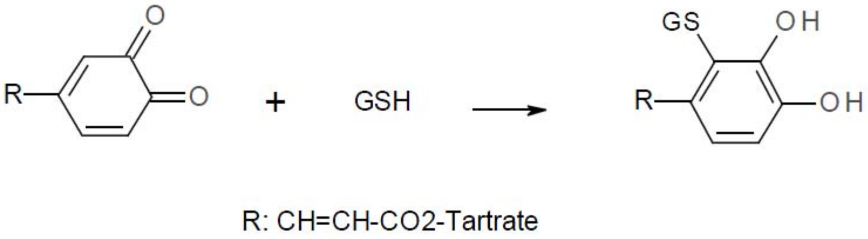

Fig. 2 - Formation of Grape Reaction Product

The other sulfur nucleophile of importance in wine is sulfite. This is commonly used to prevent oxidation of wine, although from a chemical perspective it does not prevent oxidation, but mitigates the effects of oxidation. In fact, its use will accelerate the consumption of oxygen, however, it then removes or reacts with the initial products of oxidation, in particular the quinones. Danilewicz has shown that two products are observed when a model quinone, that of 4-methyl catechol, reacts with sulfite (Danilewicz *et al.*, 2008). One is an addition product, and in the alternate reaction some of the quinone is reduced back to the catechol (fig. 3).

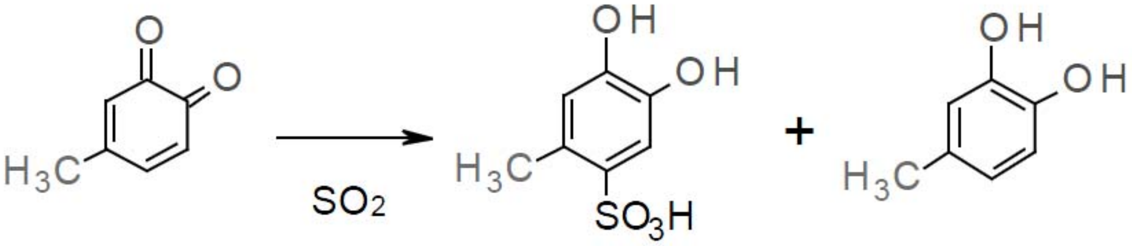

Fig. 3 - Reaction of sulfite with quinine

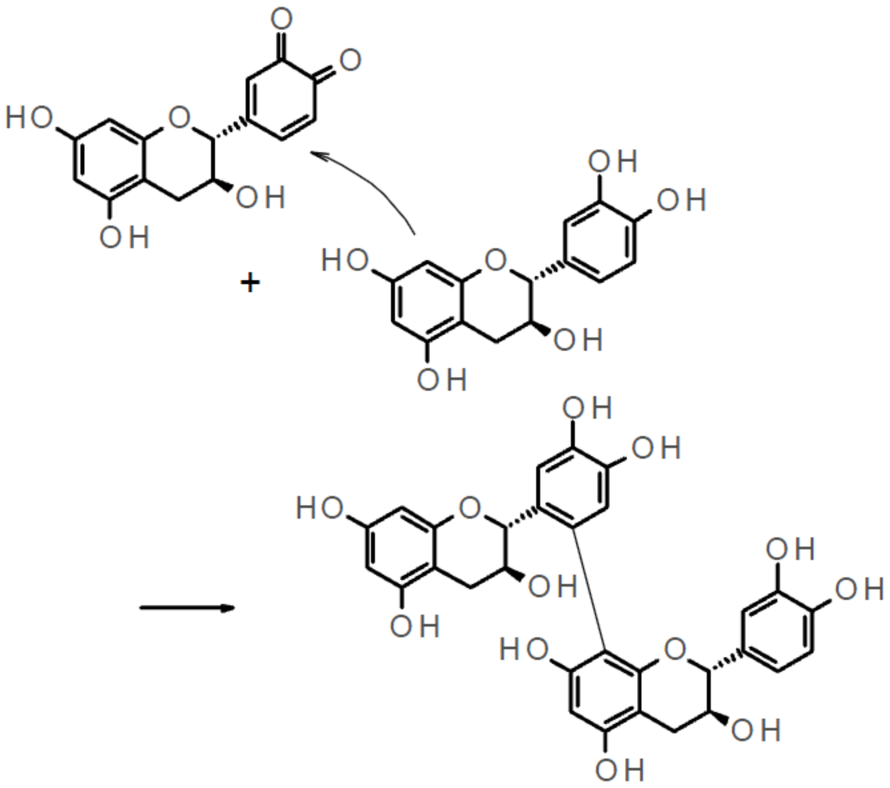

Fig. 4 - Catechin coupling reaction

The next important quinone reaction is that with carbon nucleophiles, and here specifically the phloroglucinol group, found on the A-ring of flavonoids. This nucleophilic property is utilized in one of the assays used to assess the constituents in condensed tannin (Kennedy, Jones, 2001). When this reaction occurs, it could bridge condensed tannin chains, between a B-ring on one chain, oxidized to the quinone, and the A-ring of a subunit on another chain and this reaction has been observed in model systems (fig. 4) (Jimenez-Atienzar *et al.*, 2004; Poupard *et al.*, 2008). Early work on catechin oxidation reported dimerization, but the products were not identified (Oszmianski, Lee, 1990). This type of reaction has also been observed between the oxidized form of a flavan-3-ol and the A-ring of an anthocyanin (Duenas *et al.*, 2006).

There are potentially other nucleophiles of interest that could react with wine quinones. One would include amino acids that would react in a Strecker-type reaction to yield aldehydes (Rizzi, 2006) (fig. 5), one exception of nitrogen acting as a nucleophile. These Strecker aldehydes have been reported as important aroma substances in wine, but their origin is not clearly related to quinone reactions (Escudero *et al.*, 2000).

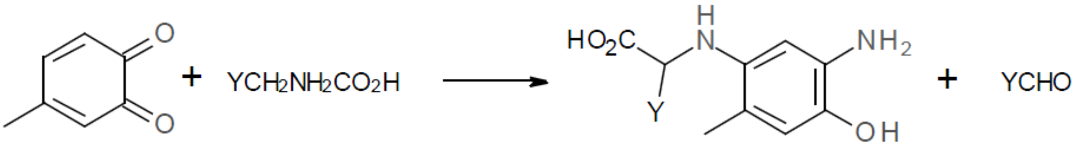

Fig. 5 - Strecker aldehydes from quinones

3. FENTON REACTION

In the next stage of oxidation, hydrogen peroxide, produced with the quinone, can now react with reduced iron, Fe^{+2}, to produce the hydroxyl radical, ·OH. This radical will oxidize all organic substances and appears to do so in proportion to the molar fraction of substrates in a mixture. As wine has a very high concentration of ethanol, ~2M, it is the target of most of the oxidation. A recent study has found that when wine is oxidized, the predominant radical observed by spin-trapping and EPR observation is the ethoxyl radical that would be formed as the first product of ethanol oxidation (fig. 6).

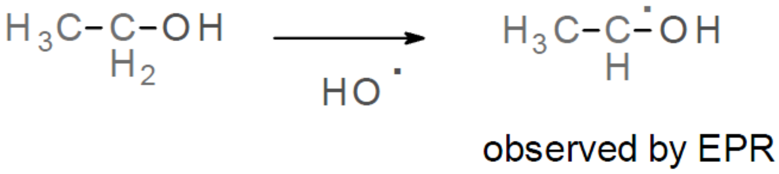

Fig. 6 - Fenton formation of ethoxyl radical

The ethoxyl radical can react with oxygen and subsequently yield acetaldehyde, or, in the absence of oxygen, react with Fe^{+3}, from the Fenton reaction, and reduce the iron to the +2 state, oxidizing the radical to acetaldehyde. There are a large number of similar reactions with other organic substances that can product products with related reaction pathways.

The next most likely oxidation reaction is that of glycerol, as it exists at about 7 g L^{-1} in wine. Glycerol has two sites for oxidation, and the reaction products of both are observed, glyceraldehyde and 1,3 dihydroxyacetone, but a number of minor additional products can be detected. The oxidation can be detected in model systems as well as in wine under oxidizing conditions (Laurie, Waterhouse, 2006b).

The oxidation of organic acids by this reaction has been well characterized, even by Fenton himself. The oxidation of tartaric acid is supposed to yield glyoxylic acid, but the direct observation of glyoxylic acid from tartrate has not been described of late (Fenton, 1894). However, a secondary product from the glyoxylic acid, its reaction with catechin, has been described in convincing detail (Fulcrand *et al*., 1997).

The oxidation of malic and lactic acids is reported to yield pyruvic acid (Merz, Waters, 1949) (fig. 7). This complicates the origin of pyruvate in wine, because yeast also produce it during fermentation.

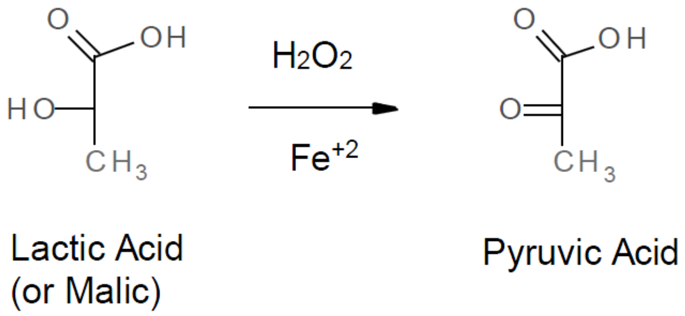

Fig. 7 - Fenton oxidation of lactic acid

Sugars are certainly also targets of the Fenton reaction. However, the daunting number of possible products, along with their intramolecular reactivity has discouraged much study of the reactions involved. However, the expected aldehydes and ketones would certainly contribute to the oxidative reactions in wine.

The Fenton reaction cannot be overcome with antioxidants to trap or intercept the hydroxyl radical. It reacts with nearly all organic substances at diffusion controlled rates, so protective materials would have to be present at tremendously high concentrations to avoid other products. However, it is quite feasible to prevent the Fenton reaction from occurring if sulfites are present. They react very quickly with hydrogen peroxide, removing it from the wine before the damaging Fenton reaction can occur (fig. 8).

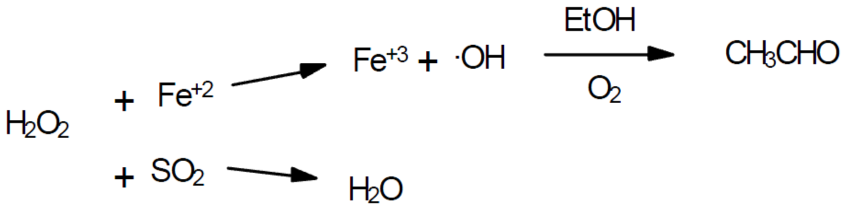

Fig. 8 - Alternate reaction pathways for hydrogen peroxide

4. REACTIONS OF FENTON PRODUCTS

As with the quinones, the aldehydes and ketones formed by the Fenton reaction are reactive electrophiles. They tend to react with different nucleophiles, but the phloroglucinol functional group is a major reaction partner as observed in many studies. These reaction products formed by reactions with anthocyanins or flavan-3-ols, are important to building color in red wine, and do affect yellowing in white wine.

Acetaldehyde is the major product of oxidation and many a student knows that adding acetaldehyde to red wine in an experiment results in darker color. The first report of these reactions with acetaldehyde was described by Saucier *et al.* in 1997, while the first report of pyruvic acid's products came out in 1998 by Fulcrand *et al.* A novel type of condensation product was the pinotin type derived from cinnamates, via a pathway that does not involve oxidation (Schwarz *et al.*, 2003). We observed that glyceraldehyde reacts with anthocyanins and flavan-3-ols, and thus may contribute to the color reactions (Laurie, Waterhouse, 2006a). There has been much activity in the area, and a suitable review of the topic by some of the most important contributors has been published (Fulcrand *et al.*, 2006).

Abstract

The chemistry of wine oxidation is captured in the reactions between the oxidation products, mostly reactive electrophiles, with other wine constituents. An understanding of both components and their reactions can lead to ideas and techniques to control and mitigate or enhance these reactions to allow for the desired development of the wine. Current investigations are yielding much useful information about oxidation reactions in wine.

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