JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

A Method To Quantify Quinone Reaction Rates with Wine Relevant Nucleophiles: A Key to the Understanding of Oxidative Loss of Varietal Thiols

Maria Nikolantonaki and Andrew L. Waterhouse*

Department of Viticulture and Enology, University of California, Davis, California 95616, United States

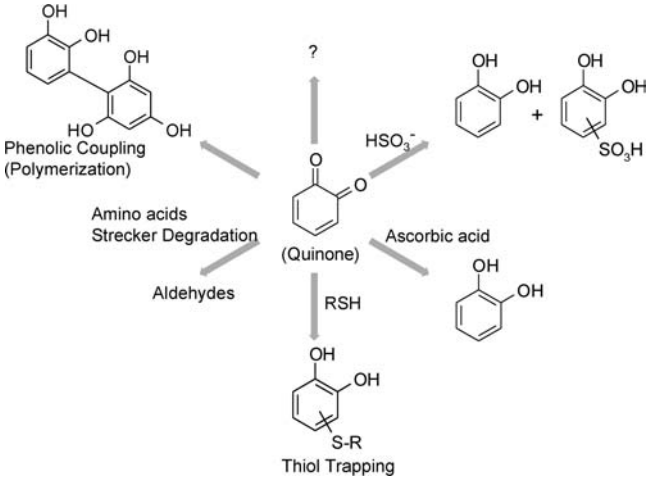
ABSTRACT: Quinones are key reactive electrophilic oxidation intermediates in wine. To address this question, the model 4-methyl-1,2-benzoquinone was prepared to study how it reacts with wine nucleophiles. Those investigated included the varietal volatile thiols 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), and 2-furanmethanethiol (2FMT); hydrogen sulfide (H_2S); glutathione (GSH); sulfur dioxide; ascorbic acid (AA); and the amino acids methionine (Met) and phenylalanine (Phe) in the first kinetic study of these reactions. Products were observed in fair to quantitative yields, but yields were negligible for the amino acids. The reaction rates of 4-methyl-1,2-benzoquinone toward the nucleophiles were quantified by UV−vis spectrometry monitoring the loss of the quinone chromophore. The observed reaction rates spanned three orders of magnitude, from the unreactive amino acids (Met and Phe) (K_{Nu} = 0.0002 s^{-1}) to the most reactive nucleophile, hydrogen sulfide (K_{H_2S} = 0.4188 s^{-1}). Analysis of the kinetic data showed three categories. The first group consisted of the amino acids (Met and Phe) having rates of essentially zero. Next, phloroglucinol has a low rate (K_{Phl} = 0.0064 s^{-1}). The next group of compounds includes the volatile thiols having increasing reactions rates K as steric inhibition declined (K_{4MSP} = 0.0060 s^{-1}, K_{3SH} = 0.0578 s^{-1}, and K_{2FMT} = 0.0837 s^{-1}). These volatile thiols (4MSP, 3SH, 2FMT), important for varietal aromas, showed lower K values than those of the third group, the wine antioxidant compounds (SO_2, GSH, AA) and H_2S (K_{Nu} = 0.3343−0.4188 s^{-1}). The characterization of the reaction products between the nucleophiles and 4-methyl-1,2-benzoquinone was performed by using HPLC with high-resolution MS analysis. This study presents the first evidence that the antioxidant compounds, H_2S, and wine flavanols could react preferentially with oxidation-induced quinones under specific conditions, providing insight into a mechanism for their protective effect.

KEYWORDS: *tannin, mercaptan, antioxidant, kinetics, oxidation, sulfite*

Quinones are reactive chemical species that are formed in abundance during the oxidation of wine. They are known to strongly react with nucleophilic compounds, and to date, there has been little work aimed at understanding their interaction with wine relevant nucleophiles. However, it is hypothesized that quinone−nucleophile reactions may be central to wine aging,[1,2] and more specifically, these reactions may govern changes in wine characteristics due to oxygen introduction and subsequent consumption, as occurs during the production and bottle aging phases. For instance, antioxidants (sulfur dioxide, glutathione, ascorbic acid), desirable aroma volatile thiols (i.e., 3-sulfanylhexanol), undesirable aroma thiols (i.e., hydrogen sulfide), amino acids (i.e., phenylalanine, methionine), and numerous polyphenols (such as epicatechin and other flavanols) all represent nucleophilic species in wine that are likely to react with quinones (Scheme 1).

The main preservative utilized in wine to prevent oxidative spoilage is sulfur dioxide. This acid is in equilibrium with hydrogen sulfite (HSO_3^-, the dominant form at wine pH) and sulfite (SO_3^{2-}). These sulfur species can convert *o*-quinones back to *o*-dihydroxyphenols and react directly with *o*-quinones to form sulfonic acids (Scheme 1).[2] Often sulfur dioxide is used in combination with ascorbic acid and glutathione, in an attempt to lower SO_2 additions and to avoid its negative impact on the organoleptic quality of a wine but also because of its claimed harmful effect on human health, particularly for asthmatics.[3] Ascorbic acid is mostly added just prior to bottling

Scheme 1. Structural Hypothesis of Reaction Products between 4-Methyl-1,2-benzoquinone and Wine Relevant Nucleophiles



Received: May 8, 2012
Revised: July 18, 2012
Accepted: August 6, 2012
Published: August 6, 2012

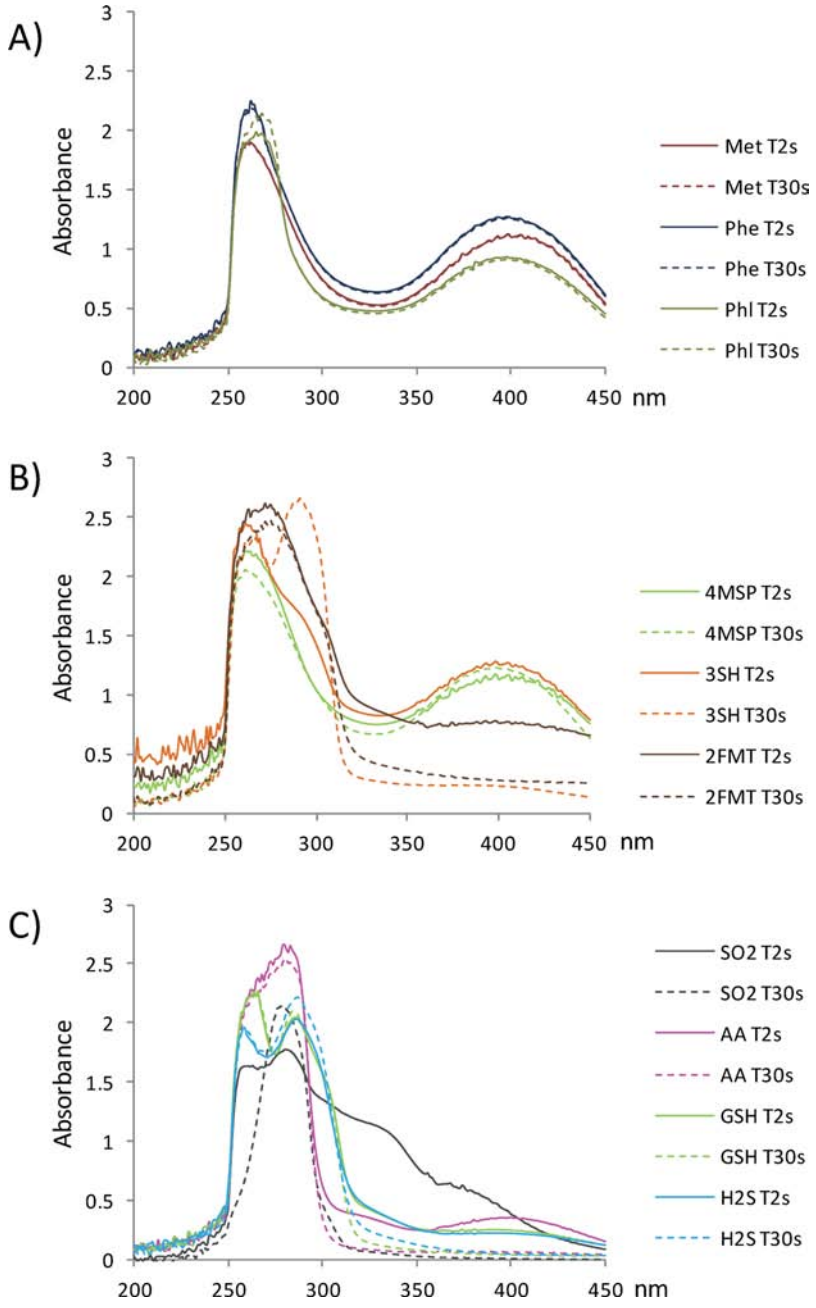


Figure 1. Monitoring of the reaction between 4-methyl-1,2-benzoquinone (Q4MeC) and different wine relevant nucleophiles (A) methionine (Met), phenylalanine (Phe), and phloroglucinol (Phl); (B) 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), and 2-furanmethanethiol (2FMT); and (C) sulfur dioxide (SO_2), ascorbic acid (AA), glutathione (GSH), and hydrogen sulfide (H_2S) in winelike solution at 10 °C, by the changing of UV−vis spectra of Q4MeC (400 nm) and the reaction addition products (250−280 nm) at T2s (2 s) and T30s (30 s).

due to its ability to rapidly scavenge molecular oxygen,[4] although it is now appreciated that SO_2 needs to be present to reduce the resulting hydrogen peroxide and dehydroascorbic acid. The potential of ascorbic acid to recycle *o*-quinones back to *o*-dihydroxyphenols has also been suggested by various authors[5−7] (Scheme 1), but this mechanism remains to be confirmed, considering the latest findings by Makhotina and Kilmartin.[8] In their study, no indication of a rapid interaction between ascorbic acid and *o*-quinones was seen on the cyclic voltammograms of wine polyphenols together with ascorbic acid. The natural sulfur-containing tripeptide glutathione is known to be capable of performing nucleophilic reactions with quinone (Scheme 1)[9] and to exhibit potent protection for important aroma compounds such as esters and monoterpenes,[10] but its scavenging potential compared to that of SO_2 and ascorbic acid remains poorly studied.

In addition to the beneficial effects of the nucleophilic antioxidant agents mentioned above, other wine relevant nucleophiles such volatile thiols can react with quinones, resulting in the loss of varietal character. The loss of varietal wine flavor is known to be correlated not only to varietal aroma loss (i.e., volatile thiols)[11,12] but also to the formation of aldehydes (i.e., methional, phenylacetaldehyde)[13−15] and sotolon [3-hydroxy-4,5-dimethyl-2(5*H*)-furanone].[16−18] At present, research has implicated the oxidation products of phenolic compounds in both mechanistic pathways.[19,20]

dx.doi.org/10.1021/jf302017j | *J. Agric. Food Chem.* 2012, 60, 8484−8491

Indeed, volatile thiols can react with the *o*-quinones derived from phenolic substances, particularly those with a catechol group, by a Michael-type addition scenario[20] (Scheme 1). These adducts are nonvolatile and their formation certainly underpins the loss of wine aroma intensity. The efficacy of the addition reaction between volatile thiols and *o*-quinones strongly depends upon the nucleophilic strength of the thiol, the electrophilic reactivity of the quinone, and the oxidation rate of each catechol.[11,20,21] In addition, the formation of potent aldehydes via the Strecker degradation of the structurally related amino acids is a widely known and a well-investigated reaction.[22] The Strecker degradation involves the interaction of sugar-derived α-dicarbonyl compounds with free amino acids. In a mechanistic point of view, any α-dicarbonyl compound with extended conjugation, including *o*-benzoquinones, is a potential Strecker candidate. Rizzi[19] reported the formation of Strecker aldehydes (i.e., methional and phenylacetaldehyde) in low yields from polyphenol [i.e., (+)-catechin, (−)-epicatechin, caffeic acid, and chlorogenic acid] derived quinones and α-amino acids (i.e., methionine and phenylalanine). However, their study used a neutral aqueous solution without added ethanol at 22 °C, conditions that are different from the acidic wine matrix.

The objective of this study was to characterize the competitive kinetics of wine relevant nucleophile with quinones. In particular, the aim of this work was to measure and rationalize the electrophilic nature of a model oxidized polyphenol, 4-methyl-1,2-benzoquinone (Q4MeC), toward important wine relevant nucleophiles. These reactions were further investigated by analyzing the structures of the reaction products via HPLC separation and structural analysis by high-resolution mass spectrometry.

EXPERIMENTAL SECTION

Reagent and Chemicals. Amberlyst A-26(OH) ion-exchange resin, periodic acid, L-methionine, L-phenylalanine, glutathione, 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 2-furanmethanethiol, 3-sulfanylhexan-1-ol, and sodium hydrosulfide dihydrate were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). 4-Methylcatechol, phloroglucinol dihydrate, sodium bisulfite, and formic acid were purchased from Acros Organics (Morris Plains, NJ). Acetonitrile, methanol, L-tartaric acid, and ascorbic acid were purchased from Fisher Bioreagents, Fisher Scientific (Fair Lawn, NJ). Tetrahydrofuran (THF) and anhydrous ethyl ether were from EMD Chemicals Inc. (Gibbstown, NJ). 4-Methyl-4-sulfanylpentan-2-one (1% in polyethylene glycol) was supplied by Interchim (St. Pedro, CA). Water was purified using a Milli-Q system (Millipore, Billerica, MA). All chemicals were of analytical grade or of the highest available purity.

Preparation of 4-Methyl-1,2-benzoquinone by Periodate Resin. Periodate resin was prepared according to the procedure first described by Harrison and Hodge[23] and later adapted by Jongberg et al.[24] The *o*-quinone 4-methyl-1,2-benzoquinone (Q4MeC) was prepared by dissolving 7.8 mg of 4-methylcatechol in 2.50 mL of acetonitrile. The solution was degassed using a magnetic stirrer for 10 min under argon. An aliquot of 100 mg of activated periodate resin was added to the solution and left to react while being stirred for 5 min still under argon. The quinone solution was used within 30 min. The amount of phenol converted to quinone was estimated to be ∼95% by HPLC−UV−vis, which gives a quinone concentration of 23 mM.

Kinetic Studies. The reaction of the studied wine relevant nucleophiles (SO_2, ascorbic acid, glutathione, phloroglucinol, 3SH, 4MSP, 2FMT, H_2S, methionine, and phenylalanine) with Q4MeC was monitored by the disappearance of Q4MeC in winelike solution (12% vol, 5 g/L tartaric acid, pH = 3.5) at an absorbance of 400 nm at 10 °C in an Agilent 8453 UV−vis spectrophotometer (Agilent Technologies, Palo Alto, CA). Absorbance−time data were recorded in real time and analyzed over at least four half-lives. The shortest time for mixing two solutions and recording the first data point (dead time) was 0.5 s. Solutions of 1 mM Q4MeC and 4 mM of each nucleophile were mixed, with nucleophile always preceding quinone, in the thermostated cell of the spectrophotometer, and the reaction products were identified by peaks observed in the absorption spectra (250−300 nm) (Figure 1). All solutions were essentially ethanolic (12% v/v), although the addition of Q4MeC solutions imparted some acetonitrile (<1% vol), while 3SH, 2FMT, 4MSP, and phloroglucinol reactions contained some additional ethanol (<2% vol) carried over from the stock solution prepared in these solvents. 3SH, 2FMT, and 4MSP stock solutions were prepared according to Ellman's method using DTNB.[25] All measurements were performed 10 times.

HPLC−HRMS Analysis of Reaction Products between 4-Methyl-1,2-benzoquinone and Wine Relevant Nucleophiles. For HPLC−MS analysis, samples (5 μL) were brought up in water:acetonitrile (50:50) and injected onto a Phenomenex Polymerx RP-1 column (2.1 mm × 100 mm). A standard reverse-phase linear gradient with water and acetonitrile was run over 30 min at a flow rate of 250 μL/min, and the eluent was monitored for negative anions by a Thermo Fisher Scientific LTQ Orbitrap operated in the centroided mode. Source parameters were 5.5 kV spray voltage, a capillary temperature of 275 °C, and a nitrogen sheath gas setting of 20 mL/min. Spectral data were acquired at a resolution setting of 60 000 fwhm with the lockmass feature, which typically results in a mass accuracy <2 ppm.

RESULTS AND DISCUSSION

Rate Constants of the Reaction of 4-Methyl-1,2-benzoquinone with Wine Relevant Nucleophiles. Measurement of the rate constant (K) of the reaction of 4-methyl-1,2-benzoquinone (Q4MeC) with nucleophiles such as amino acids [methionine (Met), phenylalanine (Phe), volatile odoriferous thiols (3SH, 4MSP, 2FMT, H_2S), basic antioxidant compounds (SO_2, ascorbic acid (AA), glutathione (GSH)], and a model phenol [phloroglucinol (Phl)] were performed in model wine solution (12% vol, 5 g/L tartaric acid, pH 3.5). The decay rate of Q4MeC was measured by following the decrease in UV−vis absorbance at 400 nm of Q4MeC at 10 °C (Figure 1). In the absence of nucleophiles, the decay of Q4MeC consistently exhibited zero-order kinetics. This behavior provided convincing evidence that the reactions of Q4MeC without nucleophiles were not significant under these experimental conditions. When Q4MeC was mixed with a model solution containing a 4-fold excess of each nucleophile (relative to the quinone concentration), excellent pseudo-first-order absorbance traces were observed when the reactions were monitored at 400 nm. Thus, the observed reaction rate constants were calculated by the first-order-rate equation 1, while the plotted $\ln(Q/Q_0)$ of Q4MeC concentration at different time intervals was fit via linear regression (Table 1).

$$-\frac{\mathrm{d}[\mathrm{Q4MeC}]}{\mathrm{d}t} = K[\mathrm{Q4MeC}] \quad (1)$$

The rate constants for the reaction of the wine relevant nucleophiles with the model quinone are summarized in Figure 2. Comparing the first-order rate constants measured by UV−vis absorbance at 400 nm, we can compile a nucleophilicity scale toward Q4MeC in the winelike solution:

$$\mathrm{Met} \approx \mathrm{Phe} < \mathrm{Phl} < 4\mathrm{MSP} < <3\mathrm{SH} < 2\mathrm{FMT} < <SO_2 \approx \mathrm{AA} \approx \mathrm{GSH} \approx H_2S$$

Such a scale can be used to rationalize the selectivity of Q4MeC in addition-type reactions. Consequently, the nucleophiles can be grouped in three categories. The first group consisted of

Table 1. Regression Equations and Mean Reaction Rate Constants of 4-Methyl-1,2-benzoquinone Disappearance in the Presence of Different Wine Relevant Nucleophiles [methionine (Met), phenylalanine (Phe), phloroglucinol (Phl), 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), 2-furanmethanethiol (2FMT), sulfur dioxide (SO_2), ascorbic acid (AA), glutathione (GSH), hydrogen sulfide (H_2S)]

nucleophiles	regression equations $\ln(Q/Q_0)$[a]	mean[b] reaction rate constants (s^{-1})	relative mean[b] reaction rate constants (s^{-1})
Met	$y = -0.0055 - 0.0002x$	−0.0002	0.003
Phe	$y = -0.0082 - 0.0005x$	−0.0005	0.009
Phl	$y = -0.1623 - 0.0064x$	−0.0064	0.110
4MSP	$y = -0.0258 - 0.0060x$	−0.0060	0.100
3SH	$y = -0.2295 - 0.0578x$	−0.0578	1.000
2FMT	$y = -0.3409 - 0.0837x$	−0.0837	1.440
SO_2	$y = -0.3549 - 0.3343x$	−0.3343	5.800
AA	$y = -0.5987 - 0.3471x$	−0.3471	6.000
GSH	$y = -0.6169 - 0.3808x$	−0.3808	6.600
H_2S	$y = -0.7535 - 0.4188x$	−0.4188	7.200

[a]Q, the 4-methyl-1,2-benzoquinone concentration; Q_0, the 4-methyl-1,2-benzoquinone concentration at time zero. [b]Data are means of 10 replicates determination; SD is noted in Figure 2

amino acids (methionine and phenylalanine) having rates of essentially zero. The formation of Strecker aldehydes (methional, phenylacetaldehyde) after the reaction of polyphenol-derived quinones and α-amino acids (Met, Phe) was reported in the literature under basic conditions (0.1 M phosphate buffer at pH 7.17).[19] However, under our experimental acidic conditions (12% vol, tartaric acid 5 g/L, pH 3.50) the lack of reactivity between quinones and amino acids suggests that the hypothesis by Rizzi does not occur under winelike conditions.[19] Perhaps, the difference in pH between the two reaction media has an important role in controlling the rate of the α-amino addition.[26,27] The loss of reactivity for amino acids with the quinone, comparing neutral and acidic conditions, can be rationalized by the different degree of amino acid protonation.[28] Next, phloroglucinol, has a very low rate (K = 0.0064). The coupling or "polymerization" reactions of tannins when subjected to oxidation have often been described qualitatively in the literature.[29,30] This data is the first quantitative assessment of one route to this important reaction, albeit in a model system. The next group of compounds includes the volatile thiols having increasing reactions rates K as steric inhibition declines. Q4MeC reactivity (Table 1) spans one order of magnitude on passing from 4MSP (K_{4MSP} = 0.0060) to the most reactive volatile thiol, 2FMT (K_{2FMT} = 0.0837). The tertiary thiol, 4MSP, was much less reactive with quinones than the secondary (3SH) and primary thiols (2FMT), and their rates are quite close to the rate of phloroglucinol. The differences in relative rates are in complete accord with the data published by Nikolantonaki et al.,[11] which compared the reactivity of the same sulfur volatile compounds with (+)-catechin and (−)-epicatechin under wines oxidation conditions. The odoriferous volatile thiols (4MSP, 3SH, 2FMT) showed lower K values (K = 0.0060, 0.0578, and 0.0837, respectively) than those of the third group of the wines antioxidant compounds (SO_2, GSH, AA; K = 0.3343, 0.3471, and 0.3808, respectively) and H_2S (K_{H_2S} = 0.4188). The result indicates that, compared to the varietal thiols, the antioxidant compounds (SO_2, GSH, AA) and H_2S would react preferentially with oxidation-induced quinones, resulting in the preservation of varietal aromas if they were present in a wine undergoing oxidation.

Identification of Reaction Products between 4-Methyl-1,2-benzoquinone and Wine Relevant Nucleophiles Using HPLC−HRMS. In order to better understand and confirm the proposed reaction mechanisms and products formed, samples from the reaction solutions were taken at the end, and all products were characterized by HPLC coupled to

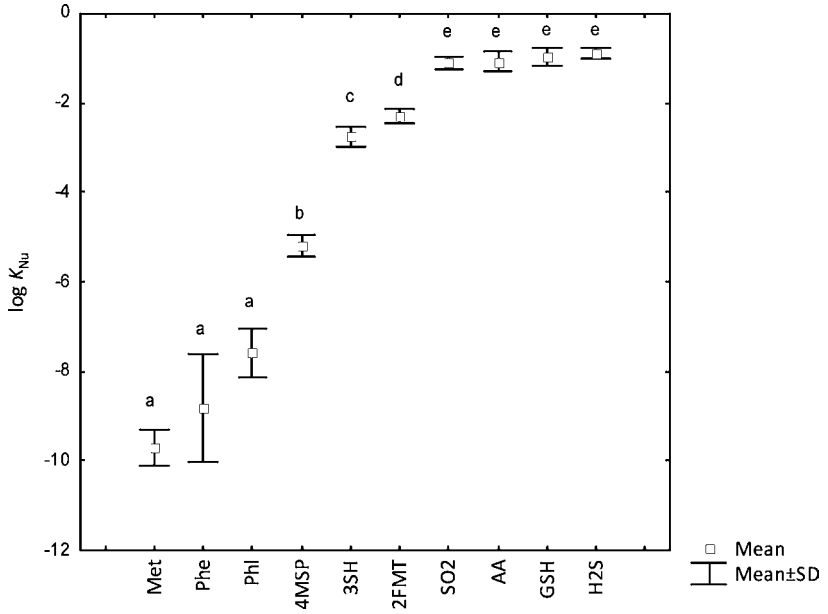
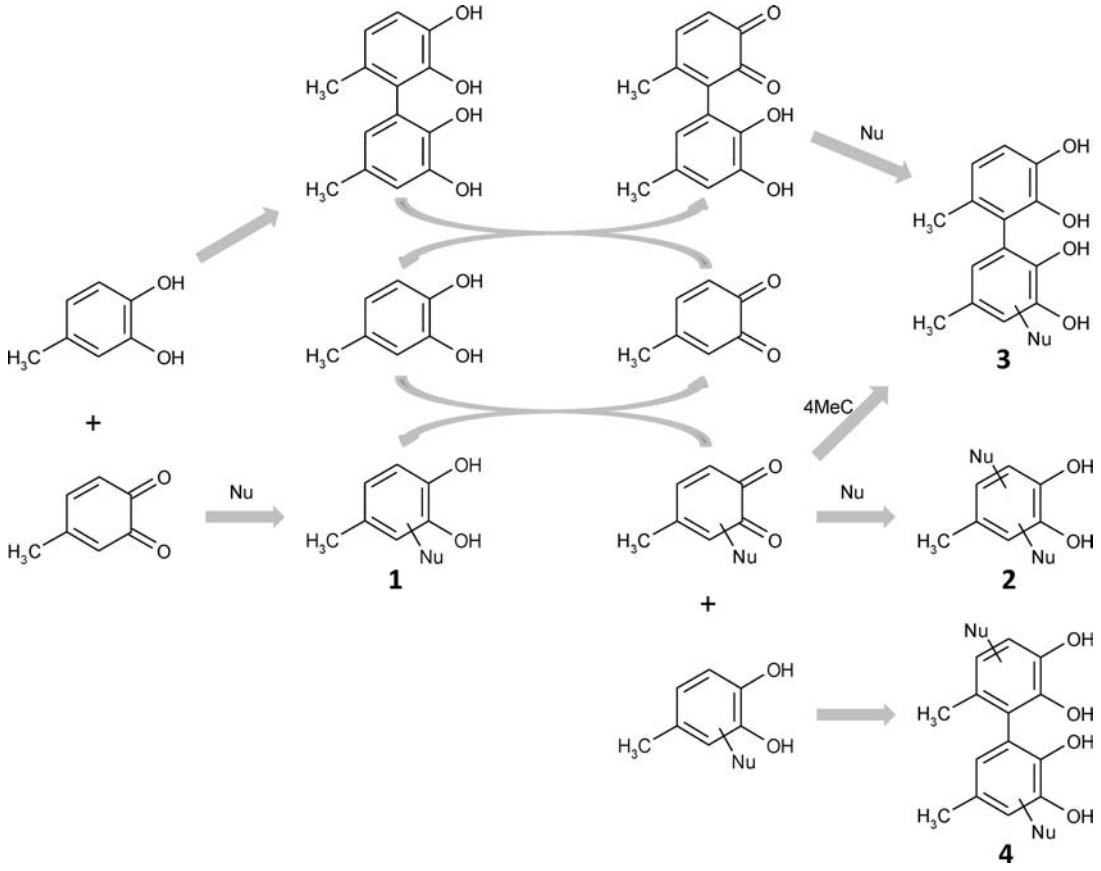


Figure 2. The first-order rate constants (K) of the reaction of each nucleophile [methionine (Met), phenylalanine (Phe), phloroglucinol (Phl), 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), 2-furanmethanethiol (2FMT), sulfur dioxide (SO_2), ascorbic acid (AA), glutathione (GSH), hydrogen sulfide (H_2S)] with 4-methyl-1,2-benzoquinone, in model wine at 10 °C.

Scheme 2. Structural Hypothesis of Reaction Products between 4-Methyl-1,2-benzoquinone Nucleophiles, Including Structures Having One or Two Skeletons of 4-Methylcatechol and One or More Nucleophilic Moieties (types 1−4)



electrospray ionization high-resolution mass spectrometry. Structural hypothesis on the reaction products were first formulated according to the structure of each nucleophile and the available knowledge concerning *o*-quinone reactivity with both sulfur compounds[5,9,20,31−34] and amino acids.[19] The molecular ions $[M - H]^-$ corresponding to the expected products were screened on the full ion chromatograms over the mass range 100−1100. Only products that incorporated the model molecule (4MeC) in their structure were considered. Therefore, the screening included structures having one or two skeletons of the model 4MeC and one or more nucleophilic moieties (Scheme 2; structure types **1**−**4**). The main reaction products of all samples are listed in Table 2. As the structures are derived from MS data, the isomeric information is very limited, and in most cases, the available data cannot distinguish between multiple isomeric possibilities.

Reaction Products between 4-Methyl-1,2-benzoquinone and 3-Sulfanylhexanol. The HPLC/ESI-MS analysis of the reaction medium where the Q4MeC was incubated with 3SH clearly showed three products at m/z = 255.1048 ($[M - H]^-$), indicating that these compounds could result from the nucleophilic addition of 3SH onto the Q4MeC in a similar manner as that recently reported by Nikolantonaki et al.,[20] where this volatile thiol was studied reacting with the *o*-quinone derived from (+)-catechin, (−)-epicatechin, and caftaric acid under wine oxidation conditions. The presence of three isomeric products with the same molecular ion mass (i.e., m/z = 255.1048) in the reaction mixture could result from 3SH nucleophilic attack on the three different electrophilic carbon centers of the aromatic ring of the *o*-quinone derived from 4MeC (Scheme 2; structure type **1**). Moreover, high-resolution ESI-MS analyses showed that these three products have the same molecular formula, i.e., $C_{13}H_{20}O_3S$. Our mass data cannot distinguish which structure is which, but the ratio of these three products based on MS ionization peak areas is 1:8:1. The HPLC/ESI-MS analysis also revealed another four products at m/z = 387.1643 with the same molecular formula, i.e., $C_{19}H_{32}O_4S_2$, which indicates a double addition of 3SH onto the oxidized 4MeC (Scheme 2; structure type **2**). Thus, the formation of this product results from a second nucleophilic addition of 3SH onto the *o*-quinones derived from the oxidation of the initially formed adducts. The differences appear to be due to positional and diasteriomeric isomers, as racemic 3SH is used. In addition to these compounds, intermediate 3SH adducts of dimeric 4MeC derivatives were observed in low yield (Scheme 2; structure type **3**). This compound gave a signal at m/z 377.1406, which corresponds to the molecular formula $C_{20}H_{26}O_5S$ and thus to a structure in which one 3SH unit is linked to two 4MeC moieties. This product could be formed by two different routes (see Scheme 2). One is via the formation of the catechol dimer via reaction between quinone and catechol. This product can then react with Q4MeC via a redox couple, yielding the quinone of the dimer which can then react with nucleophile. Another possibility is that an initial nucleophile product reacts with Q4MeC to form a bond between the catechol rings. Further work is needed to elucidate which pathway is operative, but such products are observed in several cases.

Reaction Products between 4-Methyl-1,2-benzoquinone and 2-Furanmethanthiol. The HPLC/ESI-MS analysis of the

Table 2. MS Analytical Results of Major Peaks from the Reaction of 4-Methyl-1,2-benzoquinone (Q4MeC) and Different Nucleophiles [methionine (Met), phenylalanine (Phe), phloroglucinol (Phl), 4-methyl-4-sulfanylpentan-2-one (4MSP), 3-sulfanylhexan-1-ol (3SH), 2-furanmethanethiol (2FMT), sulfur dioxide (SO_2), glutathione (GSH), hydrogen sulfide (H_2S)]

hypothetical structures	HRMS calcd	HRMS found	retention time (min)	peak ratio[a]
3SH−Q4MeC Adducts				
$[(Q + SH - 2H) - H]^-$, $C_{13}H_{19}O_3S$	255.1049	255.1048	17.07:17.87:18.31	1:8:1
$[(Q + 2SH - 4H) - H]^-$, $C_{19}H_{31}O_4S_2$	387.1658	387.1643	19.43:19.70:20.92:21.89	1:1:1:9
$[(2Q + SH - 4H) - H]^-$, $C_{20}H_{25}O_5S$	377.1417	377.1406	16.95:17.93	1:1
2FMT−Q4MeC Adducts				
$[(Q + SH - 2H) - H]^-$, $C_{12}H_{11}O_3S$	235.0423	235.0427	18.02:18.59:18.88	2:6:1
$[(Q + 2SH - 4H) - H]^-$, $C_{17}H_{15}O_4S_2$	347.0406	347.0399	21.87:22.81	1:1.2
$[(2Q + SH - 4H) - H]^-$, $C_{19}H_{17}O_5S$	357.0791	357.0771	17.14:17.87	1:8
4MSP−Q4MeC Adducts				
$[(Q + SH - 2H) - H]^-$, $C_{13}H_{17}O_3S$	253.0892	253.0892	17.02:18.59	3:1
$[(Q + 2SH - 4H) - H]^-$, $C_{19}H_{27}O_4S_2$	383.1345	383.1333	21.21:23.05	2.3:1
$[(2Q + SH - 4H) - H]^-$, $C_{20}H_{23}O_5S$	375.1260	375.1243	16.23:17.05	1:1
H_2S−Q4MeC Adducts				
$[(Q + SH - 2H) - H]^-$, $C_7H_7O_2S$	155.0161	155.0178	13.32	
$[(2Q + SH - 4H) - H]^-$, $C_{14}H_{13}O_4S$	277.0529	277.0524	15.14:16.99	2:1
$[(2Q + 2SH - 6H) - H]^-$, $C_{14}H_{13}O_4S_2$	309.0249	309.0240	13.41	
SO_2−Q4MeC Adducts				
$[(Q + S - 2H) - H]^-$, $C_7H_7O_5S$	203.0008	203.0019	15.81:16.29:20.04	5:1:2
GSH−Q4MeC Adducts				
$[(Q + GSH - 2H) - H]^-$, $C_{17}H_{22}N_3O_8S$	428.1122	428.1102	10.92:11.37:14.46	16:2:1
$[(Q + 2GSH - 4H) - H]^-$, $C_{27}H_{37}N_6O_{14}S$	733.1770	733.1770	13.35	
Phl−Q4MeC Adducts				
$[(Q + P - 2H) - H]^-$, $C_{13}H_{11}O_5$	247.0601	247.0603	10.94:13.03:13.37	2:10:1
$[(2Q + 2P - 6H) - H]^-$, $C_{26}H_{21}O_{10}$	493.1129	493.1102	9.73:13.02	2:1
$[(2Q + P - 4H) - H]^-$, $C_{20}H_{17}O_7$	369.0968	369.0960	13.49	
Met−Q4MeC Adducts				
$[(Q + Met - 2H) - H]^-$, $C_{12}H_{16}NO_4S$	270.0794	–	not detected	
Phe−Q4MeC Adducts				
$[(Q + Phe - 2H) - H]^-$, $C_{16}H_{16}NO_4$	286.1073	–	not detected	

[a]Adducts formation ratio based on compounds MS ionization peak areas.

reaction medium where the Q4MeC was incubated with 2FMT showed three major products at m/z = 235.0427 ($[M - H]^-$), indicating that these compounds with molecular formula $C_{12}H_{12}O_3S$ could result from the nucleophilic addition of 2FMT onto the *o*-quinone species derived from the oxidation of 4MeC in a manner similar to that of 3SH (Scheme 2; structure type **1**). Also, the nucleophile 2FMT attacks at two different electrophilic carbon centers of the *o*-quinone and produced two diadducts (Scheme 2; structure type **2**); these were confirmed by the presence of signals at m/z 347.0399, corresponding to the molecular formula $C_{17}H_{16}O_4S_2$. In addition, two 4MeC oxidation products formed with catechol dimer structure as above (Scheme 2; structure type **3**) were detected at m/z 357.0771. These products, like their homologous compounds produced in the 3SH−4MeC reaction mixture described above, were formed in a very low yield.

Reaction Products between 4-Methyl-1,2-benzoquinone and 4-Methyl-4-sulfanylpentan-2-one. The main products, which were formed in this model reaction, were addition products including one or two 4MSP and one 4MeC skeleton similar to these detected in the reaction medium where Q4MeC was incubated with 3SH or 2FMT. Six products in total, two of each showing molecular ions $[M - H]^-$ at m/z = 253.0892, 383.1333, and 375.1243 were detected. They correspond to single or double 4MSP adducts to one or two 4MeC molecules. Moreover, high-resolution ESI-MS analyses confirmed that these two product pairs had the same molecular formula, i.e., $C_{13}H_{18}O_3S$ (Scheme 2; structure type **1**), $C_{19}H_{28}O_4S_2$ (Scheme 2; structure type **2**), and $C_{20}H_{24}O_5S$ respectively (Scheme 2; structure type **3**).

Reaction Products between 4-Methyl-1,2-benzoquinone and Hydrogen Sulfide. Products having incorporated one H_2S and one Q4MeC were screened via the TIC data. One peak was clearly detected at 155.0178, and the presence of a main MS/MS fragment at 123.0439 confirmed that the quinone had incorporated one H_2S (Scheme 2; structure type **1**). The TIC also showed evidence of two polymerized products in this reaction medium. It revealed the presence of one chromatographic peak at m/z 309.0240 and two at m/z 277.0524, in accordance with addition products involving two 4MeC and one and/or two H_2S units, respectively. High-resolution ESI-MS analyses confirmed that the two products at m/z 277.0524 had the same molecular formula, $C_{14}H_{14}O_4S$, and likely resulted from the coupling of an initial monoadduct 4MeC−H_2S with another quinone residue (Scheme 2; structure type **3** or, alternatively, a S-bridged dimer, structure not shown). In the same manner, the product at m/z 309.0240 indicates the dimerization of the monoadduct 4MeC−H_2S (Scheme 2; structure type **4**). These dimeric adducts appear to result from the coupled oxidation−reduction of the initial monomeric adducts with Q4MeC, as the adducts have lower oxidation potential due to the S substitution. The resulting *o*-quinone

reacts further, in one case with a catechol (Scheme 2; structure type **3**) and in the other case with a monoadduct 4MeC−SH by intramolecular coupling (Scheme 2; structure type **4**).

Reaction Products between 4-Methyl-1,2-benzoquinone and Sulfur Dioxide. The HPLC/ESI-MS analysis of the reaction medium where the Q4MeC was incubated with SO_2 showed that the majority (89%) of the Q4MeC was reduced back to the 4MeC and only 11% of the substrate gave three addition products (sulfonates) with SO_2 in a ratio of 5:1:2, based on their MS ionization peak areas. More precisely, three addition products at m/z = 203.0001 were detected (Scheme 2; structure type **1**). The high-resolution ESI-MS analyses showed that these three products have the same molecular formula, $C_7H_8O_5S$.

Reaction Products between 4-Methyl-1,2-benzoquinone and Glutathione. The main compounds formed in this model reaction were addition products including one or two GSH and one 4MeC skeleton similar to those detected in the reaction medium where Q4MeC was incubated with the volatile thiols 3SH, 2FMT, and 4MSP. Three monoadducts in a ratio 1:19:2, based on their MS ionization peak areas, all showing molecular ion $[M - H]^-$ at m/z = 428.1102 were detected. They correspond to single GSH adducts with one 4MeC molecule (Scheme 2; structure type **1**), and the use of high-resolution ESI-MS analyses confirmed they had the same molecular formula, $C_{17}H_{23}N_3O_8S$. Products resulting from double GSH addition to the oxidized catechol moiety (Scheme 2; structure type **2**) were also screened on the full MS data. One peak was clearly detected on the extracted LC−MS chromatogram at m/z 733.1770 by having the molecular formula $C_{27}H_{38}N_6O_{14}S_2$ and was thus assigned to the second nucleophilic addition of GSH onto the quinone deriving from the oxidation and addition to the initially formed monoadducts. No molecules were found with two 4MeC moieties.

Reaction Products between 4-Methyl-1,2-benzoquinone and Ascorbic Acid. The HPLC−MS analysis of the reaction medium where the Q4MeC was incubated with AA showed the complete reduction of Q4MeC back to 4MeC. The potential of ascorbic acid to recycle *o*-quinones back to *o*-dihydroxyphenols has also been suggested by various authors,[5−7] and this result is the first explicit evidence that the suggested mechanism can take place under wine acidic conditions. This also helps explain the oxygen consumption stimulation effect of AA, observed by winemakers who add it to eliminate oxygen from crushed must. Thus, the AA does not directly consume oxygen but accelerates the conversion of oxygen and catechol to hydrogen peroxide and quinone, which AA then rapidly reduces,[35] driving the equilibrium in favor of the products.

On the other hand, the application of cyclic voltametry by Makhotkina and Kilmartin[8] to the study of ascorbic acid showed no enhancement effects on the cyclic voltammograms of wine polyphenols and wines, suggesting that AA was a weak or slow reducing agent. However, since the AA itself was oxidized at the electrode (while the SO_2 was not) much of the AA at the electrode was depleted and little was present to reduce the quinone before the electrode potential was reversed, so little enhancement might be expected.

Reaction Products between 4-Methyl-1,2-benzoquinone and α-Amino Acids. The proposed reaction by Rizzi[19] of products forming during the first steps of Strecker degradation of methionine or phenylalanine using *o*-quinone as reactant was screened by the full HPLC−MS ion chromatogram. The extracted m/z 270.0794 and 286.1073, corresponding, respectively, to methionine and phenylalanine adduct to a Q4MeC moiety, were not observed. As a result, under our low pH model wine conditions, we could not observe Rizzi's Strecker-type reaction. This would point to a Fenton-type oxidation of the related alcohols as the source of the aldehydes thought to affect wine aroma so strongly.[13−15]

These results provide mechanistic insights into the understanding of why some wines retain their varietal aromas for decades in the face of bottle aging and oxidation, whereas others lose their varietal aromas after relatively little oxygen exposure, or why the oxidation of a wine may sometimes rid it of undesirable thiols and other times it may not. The kinetic study described above has provided a useful quantitative characterization of the electrophilic character of a quinone (Q4MeC) under acidic winelike conditions. The results clearly demonstrate that sulfites, ascorbate, and/or glutathione can provide a protective effect by acting as sacrificial nucleophiles, suppressing varietal thiol consumption during wine aging. Further studies may now be built upon these results to assess these nucleophiles reacting with quinones of wine phenolics, as well as reactions in oxidized wines, studies which we hope will lead to predictive estimates of the result of wine oxidation and aging based on initial composition.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +1-530-752-4777. Fax: +1-530-752-0382. E-mail: alwaterhouse@ucdavis.edu.

Funding

We sincerely thank the American Vineyard Foundation (AVF) for its financial support.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We express our thanks to Nick Emil Gislason for contributing to some preliminary data for this study.

REFERENCES

(1) Waterhouse, A. L.; Laurie, V. F. Oxidation of wine phenolics: A critical evaluation and hypotheses. *Am. J. Enol. Vitic.* **2006**, *57*, 306−313.

(2) Danilewicz, J. C. Interaction of sulfur dioxide, polyphenols, and oxygen in a wine-model system: Central role of iron and copper. *Am. J. Enol. Vitic.* **2007**, *58*, 53−60.

(3) Walker, R. Sulphiting agents in foods: Some risk/benefit considerations. *Food Addit. Contam.* **1985**, *2*, 5−24.

(4) Bradshaw, M. P.; Barril, C.; Clark, A. C.; Prenzler, P. D.; Scollary, G. R. Ascorbic acid: A review of its chemistry and reactivity in relation to a wine environment. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 479−498.

(5) Cilliers, J. J. L.; Singleton, V. L. Caffeic acid autoxidation and the effects of thiols. *J. Agric. Food Chem.* **1990**, *38*, 1789−1796.

(6) Danilewicz, J. C. Review of reaction mechanisms of oxygen and proposed Intermediate reduction products in wine: Central role of iron and copper. *Am. J. Enol. Vitic.* **2003**, *54*, 73−85.

(7) S. Isaacs, N.; van Eldik, R. A mechanistic study of the reduction of quinones by ascorbic acid. *J. Chem. Soc., Perkin Trans. 2* **1997**.

(8) Makhotkina, O.; Kilmartin, P. A. Uncovering the influence of antioxidants on polyphenol oxidation in wines using an electrochemical method: Cyclic voltammetry. *J. Electroanal. Chem.* **2009**, *633*, 165−174.

(9) Cheynier, V.; Trousdale, E.; Singleton, V.; Salgues, M.; Wylde, R. Characterization of 2-*S*-glutathionyl caftaric acid and its hydrolysis in relation to grape wines. *J. Agric. Food Chem.* **1986**, *34*, 217−221.

(10) Roussis, I. G.; Lambropoulos, I.; Tzimas, P. Protection of volatiles in a wine with low sulfur dioxide by caffeic acid or glutathione. *Am. J. Enol. Vitic.* **2007**, *58*, 274−278.
(11) Nikolantonaki, M.; Chichuc, I.; Teissedre, P. L.; Darriet, P. Reactivity of volatile thiols with polyphenols in a wine-model medium: Impact of oxygen, iron, and sulfur dioxide. *Anal. Chim. Acta* **2010**, *660*, 102−109.
(12) Blanchard, L.; Darriet, P.; Dubourdieu, D. Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fractions, and sulfur dioxide. *Am. J. Enol. Vitic.* **2004**, *55*, 115−120.
(13) Wildenradt, H. L.; Singleton, V. L. The production of aldehydes as a result of oxidation of polyphenolic compounds and its relation to wine aging. *Am. J. Enol. Vitic.* **1974**, *25*, 119−126.
(14) Escudero, A.; Cacho, J.; Ferreira, V. Isolation and identification of odorants generated in wine during its oxidation: A gas chromatography−olfactometric study. *Eur. Food Res. Tech.* **2000**, *211*, 105−110.
(15) Loscos, N.; Hernandez-Orte, P.; Cacho, J.; Ferreira, V. Evolution of the aroma composition of wines supplemented with grape flavour precursors from different varietals during accelerated wine ageing. *Food Chem.* **2010**, *120*, 205−216.
(16) Escudero, A.; Asensio, E.; Cacho, J.; Ferreira, V. Sensory and chemical changes of young white wines stored under oxygen. An assessment of the role played by aldehydes and some other important odorants. *Food Chem.* **2002**, *77*, 325−331.
(17) Lavigne, V.; Pons, A.; Darriet, P.; Dubourdieu, D. Changes in the sotolon content of dry white wines during barrel and bottle aging. *J. Agric. Food Chem.* **2008**, *56*, 2688−2693.
(18) 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.
(19) Rizzi, G. P. Formation of Strecker aldehydes from polyphenol derived quinones and α-amino acids in a nonenzymic model system. *J. Agric. Food Chem.* **2006**, *54*, 1893−1897.
(20) Nikolantonaki, M.; Jourdes, M.; Shinoda, K.; Teissedre, P.-L.; Quideau, S.; Darriet, P. Identification of adducts between an odoriferous volatile thiol and oxidized grape phenolic compounds: Kinetic study of adduct formation under chemical and enzymatic oxidation conditions. *J. Agric. Food Chem.* **2012**, *60*, 2647−2656.
(21) Charles-Bernard, M.; Kraehenbuehl, K.; Rytz, A.; Roberts, D. D. Interactions between volatile and nonvolatile coffee components. 1. Screening of nonvolatile components. *J. Agric. Food Chem.* **2005**, *53*, 4417−4425.
(22) Yaylayan, V. A. Recent advances in the chemistry of Strecker degradation and amadori rearrangement: Implications to aroma and color formation. *Food Sci. Tech. Res.* **2003**, *9*, 1−6.
(23) Harrison, C. R.; Hodge, P., Polymer-supported periodate and iodate as oxidizing agents. *J. Chem. Soc., Perkin Trans. 1* **1982**.
(24) Jongberg, S.; Gislason, N. E.; Lund, M. N.; Skibsted, L. H.; Waterhouse, A. L. Thiol−quinone adduct formation in myofibrillar proteins detected by LC−MS. *J. Agric. Food Chem.* **2011**, *59*, 6900−6905.
(25) Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70−77.
(26) Modica, E.; Zanaletti, R.; Freccero, M.; Mella, M. Alkylation of amino acids and glutathione in water by *o*-quinone methide. Reactivity and selectivity. *J. Org. Chem.* **2001**, *66*, 41−52.
(27) Youngblood, M. P. Kinetics and mechanism of the addition of sulfite to *p*-benzoquinone. *J. Org. Chem.* **1986**, *51*, 1981−1985.
(28) Ellenbogen, E. Dissociation constants of peptides. I. A survey of the effect of optical configuration. *J. Am. Chem. Soc.* **1952**, *74*, 5198−5201.
(29) Drinkine, J.; Lopes, P.; Kennedy, J. A.; Teissedre, P.-L.; Saucier, C. Ethylidene-bridged flavan-3-ols in red wine and correlation with wine age. *J. Agric. Food Chem.* **2007**, *55*, 6292−6299.
(30) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740−1746.
(31) Salgues, M.; Cheynier, V.; Gunata, Z.; Wylde, R. Oxidation of grape juice 2-*S*-glutathionyl caffeoyl tartaric acid by *Botrytis cinerea* laccase and characterization of a new substance: 2,5-Di-*S*-glutathionyl caffeoyl tartaric acid. *J. Food Sci.* **1986**, *51*, 1191−1194.
(32) Quideau, S.; Feldman, K. S.; Appel, H. M. Chemistry of gallotannin-derived *o*-quinones: Reactivity toward nucleophiles. *J. Org. Chem.* **1995**, *60*, 4982−4983.
(33) Moridani, M. Y.; Scobie, H.; Salehi, P.; O'Brien, P. J. Catechin metabolism: Glutathione conjugate formation catalyzed by tyrosinase, peroxidase, and cytochrome P450. *Chem. Res. Toxicol.* **2001**, *14*, 841−848.
(34) Tanaka, T.; Mine, C.; Inoue, K.; Matsuda, M.; Kouno, I. Synthesis of theaflavin from epicatechin and epigallocatechin by plant homogenates and role of epicatechin quinone in the synthesis and degradation of theaflavin. *J. Agric. Food Chem.* **2002**, *50*, 2142−2148.
(35) Deutsch, J. C. Ascorbic acid oxidation by hydrogen peroxide. *Anal. Biochem.* **1998**, *255*, 1−7.