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# Radical-Scavenging Activity and Mechanism of Resveratrol-Oriented Analogues: Influence of the Solvent, Radical, and Substitution

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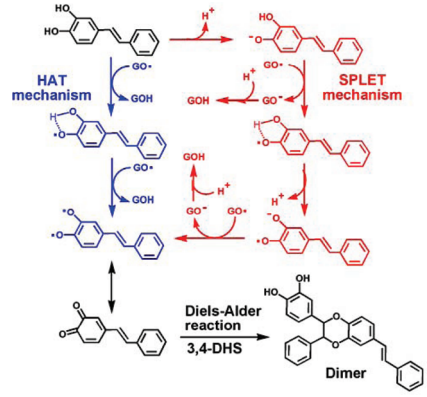


Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, 3,5,4′-THS) is a well-known natural antioxidant and cancer chemopreventive agent that has attracted much interest in the past decade. To find a more active antioxidant and investigate the antioxidative mechanism with resveratrol as the lead compound, we synthesized 3,5-dihydroxy-*trans*-stilbene (3,5-DHS), 4-hydroxy-*trans*-stilbene (4-HS) 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-3-methoxy-*trans*-stilbene (3-MeO-4-HS), 4-hydroxy-4′-methoxy-*trans*-stilbene (4′-MeO-4-HS), 4-hydroxy-4′-methyl-*trans*-stilbene (4′-Me-4-HS), 4-hydroxy-4′-nitro-*trans*-stilbene (4′-$NO_2$-4-HS), and 4-hydroxy-4′-trifluoromethyl-*trans*-stilbene (4′-$CF_3$-4-HS). The radical-scavenging activity and detailed mechanism of resveratrol and its analogues (ArOHs) were investigated by the reaction kinetics with galvinoxyl (GO$^\bullet$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH$^\bullet$) radicals in ethanol and ethyl acetate at 25 °C, using UV−vis spectroscopy. It was found that the reaction rates increase with increasing the electron-rich environment in the molecules, and the compound bearing *o*-dihydroxyl groups (3,4-DHS) is the most reactive one among the examined resveratrol analogues. The effect of added acetic acid on the measured rate constant for GO$^\bullet$-scavenging reaction reveals that in ethanol that supports ionization solvent besides hydrogen atom transfer (HAT), the kinetics of the process is partially governed by sequential proton loss electron transfer (SPLET). In contrast to GO$^\bullet$, DPPH$^\bullet$ has a relatively high reduction potential and therefore enhances the proportion of SPLET in ethanol. The relatively low rate constants for the reactions of ArOHs with GO$^\bullet$ or DPPH$^\bullet$ in ethyl acetate compared with the rate constants in ethanol prove that in ethyl acetate these reactions occur primarily by the HAT mechanism. The contribution of SPLET and HAT mechanism depends on the ability of the solvent to ionize ArOH and the reduction potential of the free radical involved. Furthermore, the fate of the ArOH-derived radicals, i.e., the phenoxyl radicals, was investigated by the oxidative product analysis of ArOHs and GO$^\bullet$ in ethanol. The major products were dihydrofuran dimers in the case of resveratrol, 4,4′-DHS, and 4-HS and a dioxane-like dimer in the case of 3,4-DHS. It is suggested from the oxidative products of these ArOHs that the hydroxyl group at the 4-position is much easier to subject to oxidation than other hydroxyl groups, and the dioxane-like dimer is formed via an *o*-quinone intermediate.

## Introduction

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (Figure 1) is a naturally occurring phytoalexin with a stilbene structure found in at least 70 plant species, a number of which are dietary components including grapes, mulberries, and peanuts.[1] Its relatively high concentration in red wine (0.1−14.3 mg/L)[1] has led to its being proposed as the main protagonist for the so-called "French paradox"; that is, despite fat-rich diets, mortality

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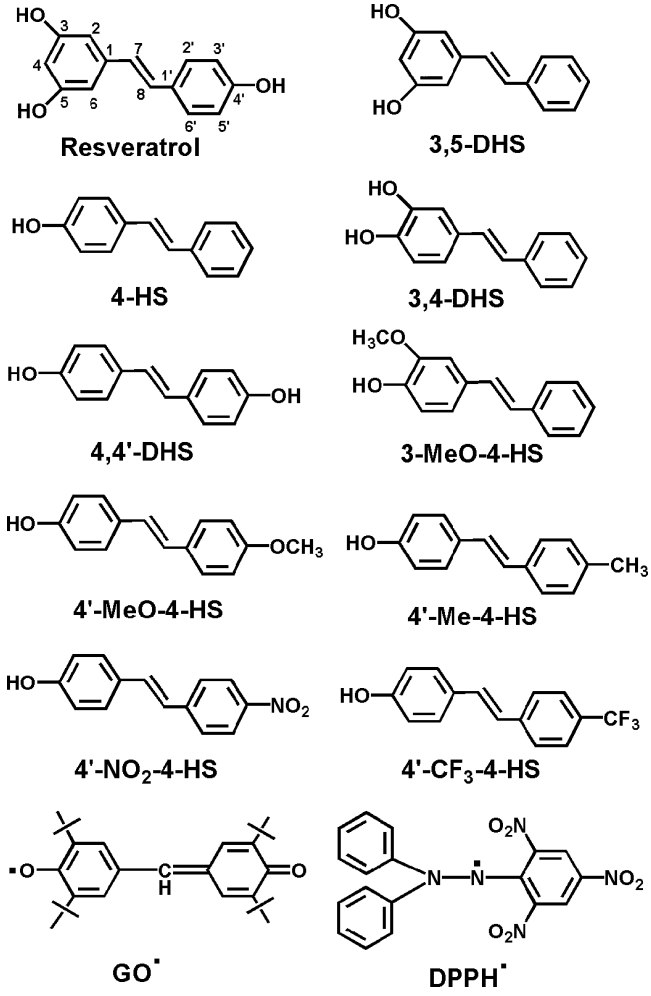


**FIGURE 1.** Molecular structures of resveratrol and its analogues.

from coronary heart disease is lower in France than in other countries due to the moderate consumption of red wine.[2] This compound has attracted much attention during the last years due to its simplicity in structure, its low toxicity in in vivo system, and its activities against a collection of diseases including heart disease, aging, and cancer.[1] The cancer chemoprevention effects, one of the most striking biological activities, elicited by resveratrol can be traced back to its antioxidant activity, because free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNA might play a causative role in cancer.[3] Therefore, the past decade has witnessed tremendous interest in the antioxidant activity of resveratrol,[4] and an effective effort in the synthesis of new resveratrol analogues, aiming to find more effective antioxidants[4e,h] and cancer chemopreventive agents.[5]

In our ongoing research project on bioantioxidants, we previously found that simple structural modification of resveratrol could significantly enhance its antioxidant activity against free radical-induced lipid peroxidation,[6] prooxidant activity on DNA damage in the presence of Cu(II) ions,[7] and cytotoxicity and apoptosis-inducing activity on human promeolocytic leukemia cells.[8] In the antioxidant reaction of resveratrol, the hydrogen abstraction from 4′-OH is more favorable than that from 3-OH or 5-OH as evidenced by stationary $\gamma$-radiolysis and pulse radiolysis experiments,[4i,j] oxidation product analysis,[9] experimental X-ray structure,[10] and theoretical calculations.[11] It was reported previously that the 4′-OH in resveratrol is responsible for its biological activities.[12] Therefore, it is of interest to extend the research and study the structure−activity relationship of resveratrol-oriented analogues by the introduction of electron-donating (ED) and electron-withdrawing groups (EW) in the ortho- or para-position of 4-OH. On the other hand, phenol antioxidants (ArOH) react with free oxygen-centered and nitrogen-center radicals (X$^\bullet$) via three different mechanisms (Scheme 1): (1) a one-step hydrogen atom transfer from phenol to X$^\bullet$ (HAT mechanism), (2) a sequential proton loss electron transfer process from phenoxide anion (ArO$^-$) to X$^\bullet$ (SPLET mechanism), and (3) an electron-transfer process from ArOH to X$^\bullet$ follwed by proton transfer (ET-PT mechanism).[13] To investigate the detailed antioxidant mechanism and the structure basis for the antioxidant activity of resveratrol, we report herein a quantitative kinetic study of the scavenging reaction of



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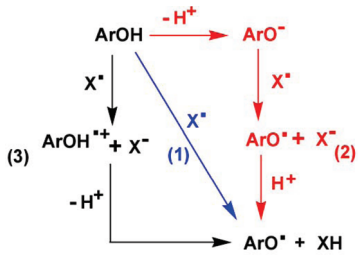
**SCHEME 1. Antioxidative Mechanisms of Phenol Antioxidants (ArOH): (1) HAT Mechanism; (2) SPLET Mechanism; and (3) ET-PT Mechanism**




resveratrol and its analogues (ArOHs) toward galvinoxyl (GO•) or 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical in ethanol and ethyl acetate at 25 °C. The resveratrol-oriented analogues studied were 3,5-dihydroxy-*trans*-stilbene (3,5-DHS), 4-hydroxy-*trans*-stilbene (4-HS), 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-3′-methoxy-*trans*-stilbene (3-MeO-4-HS), 4-hydroxy-4′-methoxy-*trans*-stilbene (4′-MeO-4-HS), 4-hydroxy-4′-methyl-*trans*-stilbene (4′-Me-4-HS), 4-hydroxy-4′-nitro-*trans*-stilbene (4′-$NO_2$-4-HS), and 4-hydroxy-4′-trifluoromethyl-*trans*-stilbene (4′-$CF_3$-4-HS). The oxidation products of ArOHs obtained in the presence of GO• in ethanol were also studied to help elucidate the structure−activity relationship and the fate of the ArOH-derived radicals. The structure−activity relationship and reaction mechanism are discussed based on the kinetic results and oxidation products analysis obtained in this study, providing insight into the mechanistic details and the development of resveratrol-oriented antioxidants.

## Results

**Radical-Scavenging Reactions of Resveratrol and Its Analogues in Ethanol and Ethyl Acetate.** GO• is a relatively stable oxygen radical and has been widely used for evaluating antioxidant activities. Direct measurements of the rate of the reaction between ArOHs and GO• were performed in ethanol at 25 °C by UV−vis spectroscopy. Upon addition of resveratrol to an ethanol solution of GO•, the absorption band at 428 nm due to GO• disappeared immediately as shown in Figure 2. The decay of GO• obeyed pseudo-first-order kinetics when the concentration of resveratrol was maintained at more than 10-fold excess of the concentration of GO•. Plotting this pseudo-first-order rate constant ($k_{obs}$) versus the concentration of resveratrol gave a straight line (the inset of Figure 2), from which the second-order rate constant ($k$) for the GO•-scavenging reaction by resveratrol could be obtained. Other ArOHs gave the same second-order kinetics and their second-order rate constants were listed in Table 1. It can be seen from Table 1 that GO•-scavenging activity of ArOHs follows the sequence of 3,4-DHS > 3-MeO-4-HS > 4,4′-DHS > 4′-MeO-4-HS > 4′-Me-4-HS > resveratrol ≈ 4-HS > 4′-$CF_3$-4-HS > 3,5-DHS. The GO•-scavenging activity of 4-HS is higher than that of 3,5-DHS and is similar to that of resveratrol, indicating that hydrogen transfer from 4′-OH of resveratrol to GO• takes place. By comparing the $k$ values for 4-HS, 3,4-DHS, 4,4′-DHS, 3-MeO-4-HS, 4′-MeO-4-HS, and 4′-Me-4-HS, it is clear that the introduction of ED groups, such as methyl, methoxy, and hydroxyl, in the ortho- or para-position of 4-OH, remarkably increases the GO•-scavenging activity. The compound bearing *o*-dihydroxyl groups (3,4-DHS) is the most reactive one among the ArOHs and its $k$ value is 70 times larger than that of 4-HS or resveratrol. On the other hand, the reaction rate of 4′-$CF_3$-4-HS is 3 times smaller than that of 4-HS, and this may be due to the presence of an EW group, trifluoromethyl.

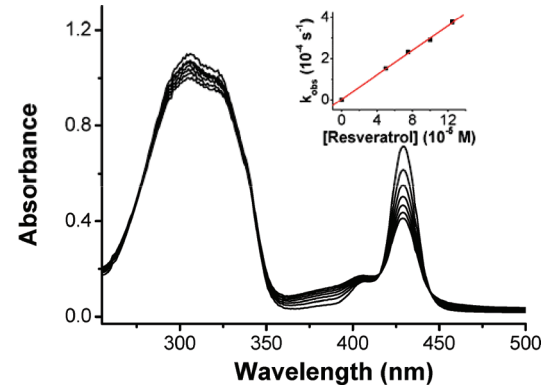



**FIGURE 2.** Spectral changes observed upon addition of resveratrol (50 μM) to an ethanol solution of GO• (5 μM) at 298 K (interval: 3 min). Inset: Plot of the pseudo-first-order rate constant ($k_{obs}$) vs. the concentration of resveratrol.

**TABLE 1. Rate Constants for Radical-Scavenging Reactions of ArOHs at 25 °C**[a]

| | $k$ ($M^{-1}$ $s^{-1}$) (GO•) | | $k$ ($M^{-1}$ $s^{-1}$) (DPPH•) | |
|---|---|---|---|---|
| ArOHs | ethanol | ethyl acetate | ethanol | ethyl acetate |
| Resveratrol | 29.8 | 15.7 | 95.1 | 1.6 |
| 3,5-DHS | 2.1 | 1.1 | 1.2 | 0.05 |
| 4-HS | 29.4 | 9.7 | 106.9 | 0.6 |
| 3,4-DHS | 2147.6 | 1468.0 | −[b] | 83.1 |
| 4,4′-DHS | 414.0 | 108.6 | 2982.2 | 6.4 |
| 3-MeO-4-HS | 1095.6 | 101.0 | 2298.1 | 9.1 |
| 4′-MeO-4-HS | 202.9 | 33.0 | 358.0 | 1.9 |
| 4′-Me-4-HS | 92.3 | 15.7 | 151.0 | 1.1 |
| 4′-$NO_2$-4-HS | −[c] | −[c] | 6.1 | 0.3 |
| 4′-$CF_3$-4-HS | 9.9 | 3.2 | 23.5 | 0.7 |

[a] Data are the averages of three reproducible determinations with a deviation of less than ±10%. [b] Reaction rate is too fast to be determined. [c] Reaction rate could not be measured because of spectral overlapping.

It has been reported that ethyl acetate and ethanol have identical hydrogen bond-accepting activity ($\beta_2^H$ = 0.45).[13c] However, ethyl acetate has much lower dielectric constants ($\varepsilon$ = 6.02)[13c] than ethanol ($\varepsilon$ = 24.30)[13c] and hence has a lower ability to support ionization of the substrate. To investigate the GO•-scavenging reaction mechanism by ArOHs, the reaction kinetics was also performed in ethyl acetate at the same temperature. The results are summarized in Table 1. The structure−activity relationship obtained in ethyl acetate is similar to that obtained in ethanol. However, the rate constants for the reactions of GO• with ArOHs in ethyl acetate are 2−10 times smaller than that in ethanol.

The DPPH• has been widely used to measure the radical-trapping abilities of natural antioxidants and served the mechanistic studies in the past.[13] In contrast to GO• ($E^0_{red}$ vs. SCE = 0.05 V),[14] DPPH• has a relatively high reduction potential ($E^0_{red}$ vs. SCE = 0.18 V),[14] thereby facilitating the electron transfer process. The reaction rates of DPPH• with ArOHs were determined by monitoring the decay of DPPH• at 517 nm in ethanol and ethyl acetate, and the results are summarized in

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**TABLE 2. Effect of Acetic Acid on Rate Constants of ArOHs in Ethanol at 25 °C[a]**

| | $k$ ($M^{-1}$ $s^{-1}$) (GO•) | | | | | | $k$ ($M^{-1}$ $s^{-1}$) (DPPH•) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | [$CH_3COOH$] ($\mu$M) | | | | | | [$CH_3COOH$] ($\mu$M) | | | | | |
| ArOHs | 0 | 10 | 50 | 1000 | 5000 | 10000 | 0 | 10 | 50 | 1000 | 5000 | 10000 |
| Resveratrol | 29.8 | 22.6 | 14.5 | 11.6 | 11.3 | 10.9 | 95.1 | 54.1 | 26.0 | 3.9 | 2.5 | 2.0 |
| 3,5-DHS | 2.1 | 1.4 | 1.0 | 0.8 | 0.8 | 0.8 | 1.2 | 0.9 | 0.7 | 0.5 | 0.5 | 0.5 |
| 4-HS | 29.4 | 20.2 | 9.0 | 4.7 | 4.6 | 4.6 | 106.9 | 61.4 | 33.5 | 3.9 | 2.1 | 1.9 |
| 3,4-DHS | 2147.6 | 1516.6 | 686.6 | 508.0 | 462.6 | 432.6 | —[b] | —[c] | —[c] | —[c] | —[c] | —[c] |
| 4,4′-DHS | 414.0 | 231.0 | 93.4 | 68.0 | 57.2 | 64.4 | 2982.2 | 1331.5 | 562.7 | 41.9 | 27.2 | 27.0 |
| 3-MeO-4-HS | 1095.6 | 515.2 | 294.6 | 135.2 | 117.6 | 111.8 | 2298.1 | 919.2 | 448.3 | 81.9 | 27.7 | 28.0 |
| 4′-MeO-4-HS | 202.9 | 109.8 | 36.8 | 21.4 | 19.2 | 18.3 | 358.0 | 260.5 | 130.0 | 9.4 | 6.2 | 5.9 |
| 4′-Me-4-HS | 92.3 | 37.8 | 15.5 | 11.7 | 10.8 | 9.1 | 151.0 | 91.9 | 57.8 | 6.5 | 2.9 | 2.9 |
| 4′-$NO_2$-4-HS | —[d] | —[d] | —[d] | —[d] | —[d] | —[d] | 6.1 | 4.5 | 2.6 | 0.6 | 0.5 | 0.4 |
| 4′-$CF_3$-4-HS | 9.9 | 5.9 | 3.0 | 1.6 | 1.5 | 1.3 | 23.5 | 21.3 | 12.3 | 3.9 | 0.5 | 0.43 |

[a] The $k$ values were given with errors usually less than 10%. [b] Reaction rate is too fast to be determined. [c] No determination. [d] Reaction rate could not be measured because of spectral overlapping.

Table 1. A similar structure−activity relationship was also obtained in the case of DPPH•, that is, the $k$ value increases with the introduction of ED groups (methyl, methoxy, and hydroxyl) and decreases with the introduction of EW groups (nitro and trifluoromethyl), but the reaction rate for the two radicals (DPPH• and GO•) was appreciably different. In ethanol that supports ionization solvent the reaction rate is quicker in the case of DPPH• than in the case of GO•. The differences and details will be discussed in the following sections.

**Effect of Acetic Acid on the Rates of Radical-Scavenging Reactions.** In solvents that support ionization, such as alcohols and water, ArOH may be in equilibrium with the corresponding phenolate anion ($ArO^-$) [eq 1], which is a much stronger electron donor as compared to the parent ArOH. If $ArO^-$ acts as the electron donor and the SPLET mechanism is operative in solvents that support ionization, the addition of acid will suppress the ionization of ArOH and the measured rate constant might therefore be expected to decrease. When the electron donor is the parent molecule (ArOH) and the ET-PT mechanism occurs, the addition of acid will increase or remain the measured rate constant.[13d] Recently, Litwinienko and Ingold have clearly demonstrated the occurrence of SPLET in the reaction of DPPH• with some phenolic compounds in methanol and ethanol by studying the effect of added acetic acid on the measured rate constant.[13a,c,d]

$$ArOH \rightleftharpoons ArO^- + H^+ \quad (1)$$

To rationalize the mechanism and actual electron donor, the effect of acetic acid on the radical-scavenging rates of ArOHs in ethanol was examined. The results are summarized in Table 2. Addition of acetic acid to the ethanol-containing resveratrol from 10 $\mu$M to 10 mM reduced the rate of the GO•-scavenging reaction by a factor of 3 and to the limiting value (Table 2), suggesting that the actual electron donor is $ArO^-$ and SPLET does occur in nonacidified ethanol and acid reduces the rate by eliminating SPLET to leave only HAT (vide infra). The limiting value and 5- to 10-fold decline in the rate of the GO•-scavenging reaction were also found in the case of 3,4-DHS and other analogues (Table 2). Similar results were obtained in the case of DPPH•, but the effect of acetic acid on the reaction rate became much more pronounced. For example, the rate was decreased 50- and 100-fold for resveratrol and 4,4′-DHS (Table 2), respectively. The mechanistic details in the radical-scavenging reaction will be discussed in the following sections.

**TABLE 3. Oxidative Dimerization of ArOHs with Galvinoxyl Radical in Ethanol at Room Temperature**

| ArOHs | time (h) | conversion (%) | isolated yield (%) |
|---|---|---|---|
| resveratrol | 8 | 78 | 41 |
| 4-HS | 8 | 85 | 42 |
| 4,4′-DHS | 4 | 80 | 56 |
| 3,4-DHS | 3 | 100 | 87 |

**Oxidative Products of Resveratrol and Its Analogues in the Presence of GO• in Ethanol.** Whether the electron-transfer or the hydrogen atom-transfer process from ArOH to free radical will result in the formation of the same phenoxyl radical, ArO•. To investigate the fate of ArO•, we also isolated and identified the reaction products of resveratrol, 4-HS, 4,4′-DHS, and 3,4-DHS with GO• in ethanol at room temperature (Table 3). The major products (Figure 3) were dihydrofuran dimers in the case of resveratrol, 4-HS, and 4,4′-DHS and a dioxane-like dimer in the case of 3,4-DHS by characterizing with HRMS (ESI) and 1D and 2D NMR (see the Supporting Information). It is suggested from the oxidative products that the hydroxyl group at the 4-position is much easier to subject to oxidation than other hydroxyl groups, and the dioxane-like dimer is formed via an *o*-quinone intermediate (vide infra). The oxidative product analysis also suggests the importance of *o*-dihydroxyl groups and 4-hydroxyl groups. It is worth noting that the oxidative coupling products of ArOHs in the presence of GO• were obtained in good yields and conversions (Table 3). This provides an efficient approach for the synthesis of resveratrol-based dimers.

As an example, the oxidation of 3,4-DHS in the presence of GO• and the structural characterization of its dimer will be discussed. The HRMS (ESI) analysis of the major product showed an $[M+H]^+$ of 423.1597 (calculation: 423.1591),

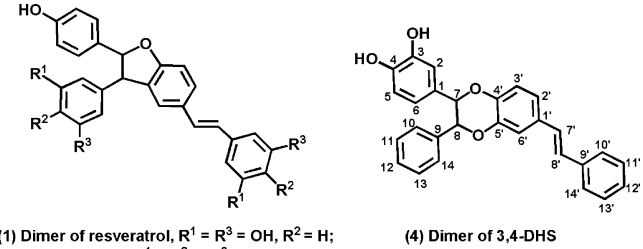
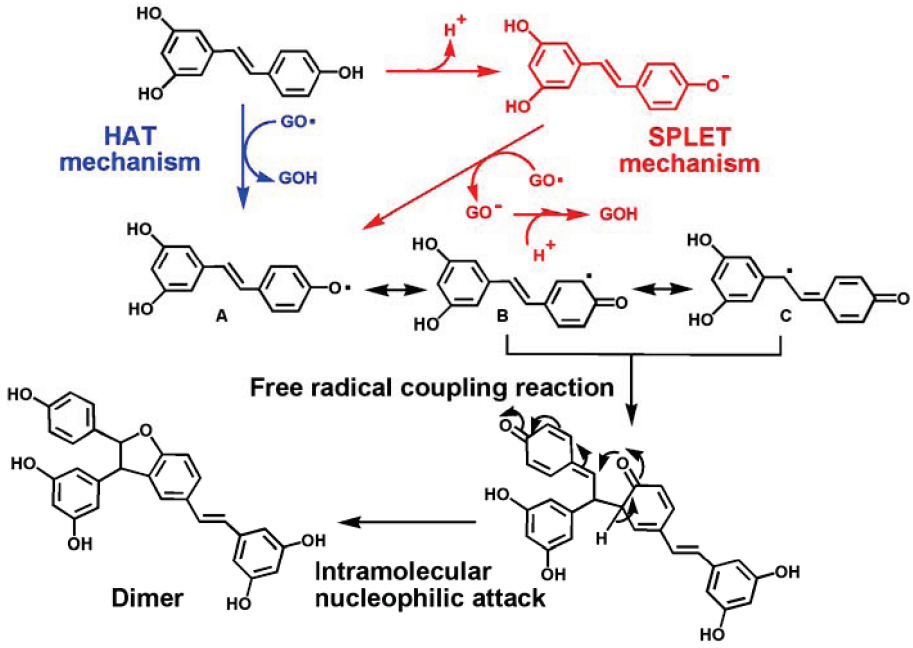



**FIGURE 3.** Oxidation products of resveratrol and its analogues obtained in the presence of GO• in ethanol.

**SCHEME 2. GO•-Scavenging Reaction Mechanism by Resveratrol in Ethanol**




demonstrating the structure of a dehydrodimer, and its $^1$H NMR spectrum gave additional information that allowed its identification with the structure. Despite the fact that the product was a dimer, only two phenolic OH (a singlet at $\delta$ 8.01), two aliphatic protons (doublets at $\delta$ 4.92 and 5.05, $J = 8.0$ Hz, H-7 and H-8), two *trans*-olefinic protons (two doublets at $\delta$ 7.13 and 7.19, with a large coupling constant of 16.0 Hz, H-7′ and H-8′), and thirteen aromatic protons were present. The correct structural assignments could be made on the basis of homonuclear bidimensional correlation ($^1$H,$^1$H-COSY), heteronuclear $^1$H,$^{13}$C-HMQC, and long-range $^1$H,$^{13}$C-HMBC correlation experiments. The long-range correlation between the doublet at $\delta$ 6.51 (H-6) and the aliphatic carbon at $\delta$ 81.1 (related to the doublet at $\delta$ 4.92, C-7), as well as between the doublet at $\delta$ 6.77 (H-2) and the carbon at $\delta$ 81.1 (related to the doublet at $\delta$ 4.92, C-7), allowed us to establish that the 3,4-dihydroxybenzoyl moiety was bonded to C-7. The long-range correlation between the multiplet at $\delta$ 7.26 (H-10 and H-14) and the aliphatic carbon at $\delta$ 81.3 (related to the doublet at $\delta$ 5.05, C-8) allowed us to establish that the phenyl moiety was bonded to C-8. All these data were consistent with the proposed "closed" and dioxane-like dimer formed via an *o*-quinone intermediate (vide infra). Moreover, more than 8 values for the $^3J$ coupling constant between H-7 and H-8 in the dimer suggested a predominant pseudo-*trans*-axial arrangement for these two aliphatic protons. Furthermore, enantiomeric composition of the dimer was evaluated by HPLC analysis on a chiral column and it was found that the dimer was a racemic mixture (see the Supporting Information).

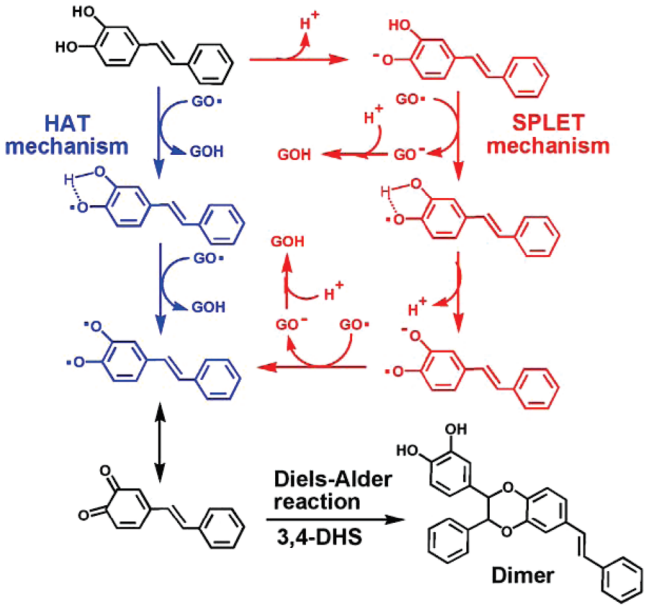
## Discussion

Resveratrol's significant antioxidant and cancer chemoprevention activities, along with its low molecular weight and lack of toxicity, make this molecule an ideal lead compound for development of new antioxidants and cancer chemoprevention agents. Also, the detailed antioxidant mechanism of resveratrol and its analogues is important for understanding their biological activities. The present work studied the radical-scavenging activity of nine resveratrol-oriented analogues with the different structural features that enables us to deduce a clear picture on the structural determinants for the activity and a structure−activity relationship. Furthermore, the study on the reaction kinetics gives us important information for understanding the reactive mechanism of these antioxidants.

**Structure−Activity Relationship.** The oxidative product of resveratrol demonstrates that the hydroxyl group at the 4′-position is much easier to subject to oxidation than other hydroxyl groups. This is in accordance with that obtained from the UV−visible spectra change mentioned above and the previous observation by stationary $\gamma$-radiolysis and pulse radiolysis experiments,[4i,j] experimental X-ray structure,[10] and theoretical calculations.[11] Theoretical calculations[10] showed that the most stable phenolate in resveratrol is obtained after extraction of the proton in the 4′-position, which is ∼7 kcal/mol lower than those having extracted the protons in the 3- and 5-position. Therefore, the 4′-OH in resveratrol provides the most acidic hydrogen and its removal from the 4′-OH is highly preferred. Pulse radiolysis experiments also indicated that spectral and kinetic properties of the phenoxyl radicals show great similarity between resveratrol and 4-HS; thus, 4′-OH of resveratrol scavenges free radicals more effectively than its 3-OH and 5-OH.[4i,j] The oxidation reaction of 4′-OH resulted in the formation of phenoxyl radicals or semiquinone ("A", "B", and "C"), whose existence was also elucidated by the electron paramagnetic resonance[4h] and pulse radiolysis experiments.[4i,j] Successively, the coupling of one radical "B" and one radical "C", followed by tautomeric rearrangement and intramolecular nucleophilic attack to the intermediate quinone, gave the dihydrofuran dimer as shown in Scheme 2.

It can be seen from Table 1 that the radical-scavenging activity of ArOHs increases with the introduction of ED groups (methyl, methoxy, and hydroxyl) in the ortho- or para-position of 4-OH. On the contrary, the activity decreases in the presence of EW groups (trifluoromethyl and nitro). It has been proven that the bond dissociation energy (BDE) of O−H regulates the

**SCHEME 3. GO•-Scavenging Reaction Mechanism by 3,4-DHS in Ethanol**




antioxidative potency in phenolic compounds.[15] Therefore, enhancement in the radical-scavenging activity of ArOHs can be explained by the fact that ED groups reduce the BDE, and EW groups have the reverse effect. The compound bearing *o*-dihydroxyl groups (3,4-DHS) is the most reactive one among the examined ArOHs. It can also be understood because the oxidative intermediate, *o*-hydroxyphenoxyl radicals, is more stable due to the intramolecular hydrogen bonding interaction, as evidenced recently from experiments by spectrophotometric measurement[16] and theoretical calculations.[15a] The theoretical calculation showed that the hydrogen bond in the *o*-OH phenoxyl radical is approximately 4 kcal/mol stronger than that in the parent catechol, and that the BDE of catechol is 9.1 kcal/mol lower than that of phenol and 8.8 kcal/mol lower than that of resorcinol.[15a] In addition, it should be easier to further oxidize the *o*-OH phenoxyl radical and/or *o*-semiquinone anion to form the final *o*-quinone (Scheme 3) as evidenced from the formation of the dioxane-like dimer of 3,4-DHS (Figure 3) in the presence of GO• in ethanol at room temperature. Obviously, this dimer is the [4+2] Diels−Alder adduct of the *o*-quinone intermediate and another molecule of 3,4-DHS.

**Mechanism.** Table 2 shows that the GO•-scavenging reaction rates of ArOHs in ethanol were decreased approximately 3- to 10-fold by the addition of acetic acid. This result is fully consistent with the partial dissociation of these ArOHs and the occurrences of SPLET in nonacidified ethanol. It is also noticeable that the limiting rate constants of ArOHs in ethanol at high concentration of acetic acid are similar to rate constants for the same GO•-scavenging reaction in ethyl acetate having similar hydrogen bond-accepting activity and low ability to ionize ArOHs (Tables 1 and 2). This indicates that in acidified ethanol, these limiting rate constants correspond to the HAT reaction. Therefore, the experimental rate constant is the sum of the rate constant for the HAT process and the rate constant for reaction of radical with the phenoxide anion (SPLET) [eq 2]. Schemes 2 and 3 show cooperation between hydrogen-abstraction and electron-transfer processes in the GO•-scavenging reaction by resveratrol and 3,4-DHS in ethanol. The antioxidant mechanism of the resveratrol, 3,5-DHS, and 4-HS was well investigated previously by pulse radiolysis experiments and their anionic phenoxyl radicals (semiquinone anion) were also identified from the transient absorption spectra in alkaline solution.[4j] It should be pointed out that the relative contribution of SPLET and HAT processes in the radical-scavenging reaction depends on the experimental conditions such as the ability of the solvent to ionize ArOHs and the character of the attacking radical. The relatively low rate constants for the reactions of ArOHs with GO• in ethyl acetate compared with the rate constants in ethanol (Table 1) clearly indicates that in ethyl acetate having low ability to ionize ArOHs the reactions occur primarily by the HAT mechanism. In contrast to GO•, DPPH• has a relatively high reduction potential and therefore more easily undergoes the electron-transfer reaction. The extremely high rate constants for the reactions of ArOHs with DPPH• in ethanol compared with the rate constants in ethyl acetate (Table 1), taken together with the dramatic decreases in the rate constants in ethanol which are produced by added acetic acid (Table 2), prove that in ethanol these reactions proceed primarily via the SPLET mechanism.

$$v = k_{HAT}[ArOH][GO^{\bullet}] + k_{SPLET}[ArO^{-}][GO^{\bullet}] \qquad (2)$$

## Conclusion

Resveratrol and its analogues (ArOHs), that is, 3,5-DHS, 4-HS, 3,4-DHS, 4,4′-DHS, 3-MeO-4-HS, 4′-MeO-4-HS, 4′-Me-4-HS, 4′-$NO_2$-4-HS, and 4′-$CF_3$-4-HS, are effective scavengers against GO• and DPPH• in ethanol and ethyl acetate. The observation that the activity of ArOHs increases with increasing the electron-rich environment in the molecules, and the compound bearing *o*-dihydroxyl groups (3,4-DHS) is the most reactive one among the examined ArOHs, gives us useful information for antioxidant drug design. The SPLET and HAT mechanisms are responsible for the radical-scavenging reaction and the relative contribution depends on the ability of the solvent to ionize ArOH and the reduction potential of the free radical involved.

## Experimental Section

**Materials.** Resveratrol and its analogues, that is, 3,5-DHS, 4-HS, 3,4-DHS, 4,4′-DHS, 3-MeO-4-HS, 4′-MeO-4-HS, 4′-Me-4-HS, and 4′-$NO_2$-4-HS, were synthesized by the Wittig−Horner reaction.[17] 4′-$CF_3$-4-HS was synthesized by the Perkin reaction.[18] Their structures were fully identified by using $^1$H and $^{13}$C NMR and EI-MS (see the Supporting Information) and the data were consistent with those reported in the literature.[18] The purity (>98%) of each compound was all checked by using high-performance liquid chromatography (HPLC).

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The galvinoxyl radical (GO•) (from Acros) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) (from Aldrich-Sigma) radical were purchased with the highest purity available and used as received.

**Spectral and Kinetic Measurement.** Typically, an aliquot of ArOHs at more than 10-fold excess of the concentration of GO• was added to a quartz (10 mm i.d.) that contained GO• ($5 \times 10^{-6}$ M) in ethanol solution. This led to a hydrogen-transfer reaction from ArOH to GO•. UV−visible spectra changes associated with this reaction were measured at room temperature with a Hitachi 557 spectrophotometer. The rates of hydrogen transfer were determined by monitoring the absorbance change at 428 nm due to GO•. In the case of DPPH•, the rates followed second-order kinetics and were measured by monitoring the decay of DPPH• at 517 nm ([ArOH]/[DPPH•] = 1/1). The rates in the presence of acid were determined in the same manner.

**Oxidation Products Analysis of Resveratrol and Its Analogues in the Presence of Galvinoxyl Radical in Ethanol.** Resveratrol (60 mg, 0.26 mmol) and 220 mg of GO• (0.52 mmol) were mixed in 30 mL of ethanol and the solution was stirred for 8 h at room temperature. The products were purified by silica gel chromatography and eluted with chloroform−methanol (5:1/v:v) to afford dimer (24 mg) and recover unreacted resveratrol (29 mg) with the conversion and yield of 78% and 41%, respectively.

**Dimer of resveratrol:** $^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 4.62 (d, $J$ = 8.0 Hz, 1H), 5.48 (d, $J$ = 8.0 Hz, 1H), 6.19 (d, $J$ = 2.0 Hz, 2H), 6.25 (d, $J$ = 2.0 Hz, 2H), 6.53 (d, $J$ = 2.0 Hz, 2H), 6.84 (d, $J$ = 8.0 Hz, 2H), 6.88 (d, $J$ = 8.0 Hz, 1H), 6.92, 7.08 (AB system, d, $J$ = 16.0 Hz, 2H), 7.24 (d, $J$ = 8.0 Hz, 2H), 7.25 (d, $J$ = 8.0 Hz, 1H), 7.45 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $(CD_3)_2CO$) $\delta$ 57.7, 94.2, 102.4, 102.7, 105.7 (2C), 107.4 (2C), 110.1, 116.1 (2C), 123.9, 127.2, 128.5 (3C), 129.1, 131.7, 132.1, 132.5, 140.7, 145.1, 158.4, 159.5 (2C), 159.7 (2C), 160.5; HRMS (ESI) for $C_{28}H_{22}O_6$ *m*/*z* calcd for $[M + H]^+$ 455.1489, found 455.1481, error = 1.8 ppm.

4-HS ( 98 mg, 0.5 mmol) and 424 mg of GO• (1.0 mmol) were mixed in 40 mL of ethanol and the solution was stirred for 8 h at room temperature. The products were purified by silica gel chromatography and eluted with petroleum ether−acetone (4:1/v:v) to afford the dimer (41 mg) and recover unreacted 4-HS (52 mg) with the conversion and yield of 85% and 42%, respectively.

**Dimer of 4-HS:** $^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 4.63 (d, $J$ = 8.5 Hz, 1H), 5.49 (d, $J$ = 8.5 Hz, 1H), 6.83 (d, $J$ = 8.0 Hz, 2H), 6.88 (d, $J$ = 8.4 Hz, 1H), 6.98, 7.13 (AB system, d, $J$ = 16.4 Hz, 2H), 7.14 (t, d, $J$ = 8.0, 2.0 Hz, 1H), 7.16 (d, $J$ = 2.0 Hz, 1H), 7.19 (d, $J$ = 8.0 Hz, 2H), 7.19 (d, $J$ = 8.3 Hz, 2H), 7.21 (t, $J$ = 8.0 Hz, 1H), 7.21 (t, d, $J$ = 8.0, 1.5 Hz, 1H), 7.29 (t, d, $J$ = 8.0, 2.0 Hz, 2H), 7.34 (t, d, $J$ = 8.0, 1.5 Hz, 1H), 7.46 (d, $J$ = 8.4 Hz, 1H), 7.49 (t, d, $J$ = 8.0, 2.0 Hz, 2H); $^{13}$C NMR (100 MHz, $(CD_3)_2CO$) $\delta$ 57.1, 94.2, 110.2, 116.1 (2C), 123.6, 126.9 (2C), 127.6, 128.0, 128.4 (2C), 128.5, 129.0 (2C), 129.2, 129.3 (2C), 129.6 (2C), 131.6, 131.7, 132.4, 138.5, 142.5, 160.4; HRMS (ESI) for $C_{28}H_{22}O_2$ *m*/*z* calcd for $[M + H]^+$ 391.1693, found 391.1689, error = 1.0 ppm.

4,4′-DHS (55 mg, 0.26 mmol) and 110 mg of GO• (0.26 mmol) were mixed in 30 mL of ethanol and the solution was stirred for 4 h at room temperature. The products were purified by silica gel chromatography and eluted with chloroform−methanol (15:1/v:v) to afford dimer (32 mg) and recover unreacted 4.4′-DHS (16 mg) with the conversion and yield of 80% and 56%, respectively.

**Dimer of 4,4′-DHS:** $^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 4.53 (d, $J$ = 8.8 Hz, 1H), 5.43 (d, $J$ = 8.8 Hz, 1H), 6.80 (d, $J$ = 8.4 Hz, 2H), 6.83 (d, $J$ = 8.8 Hz, 2H), 6.85 (d, $J$ = 8.4 Hz, 2H), 6.87 (d, $J$ = 8.4 Hz, 1H), 6.96, 6.97 (AB system, d, $J$ = 18.4 Hz, 2H), 7.05 (d, $J$ = 8.8 Hz, 2H); 7.16 (br s, 1H), 7.24 (d, $J$ = 8.4 Hz, 2H), 7.37 (d, $J$ = 8.4 Hz, 1H), 7.39 (d, $J$ = 8.4 Hz, 2H); $^{13}$C NMR (100 MHz, $(CD_3)_2CO$) $\delta$ 57.5, 94.5, 110.2, 116.2 (2C), 116.4 (2C), 116.6 (2C), 123.4, 126.6 (2C), 126.9, 128.1 (2C), 128.4 (2C), 128.7, 130.3 (2C), 130.5, 132.4, 132.9, 133.4, 157.6, 157.9, 158.5, 160.3; HRMS (ESI) for $C_{28}H_{22}O_4$ *m*/*z* calcd for $[M + H]^+$ 423.1591, found 423.1595, error = 0.9 ppm.

3,4-DHS (54 mg, 0.25 mmol) and 107 mg of GO• (0.25 mmol) were mixed in 30 mL of ethanol and the solution was stirred for 3 h at room temperature. The products were purified by silica gel chromatography and eluted with chloroform−methanol (10:1/v:v) to afford dimer (47 mg) with the yield of 87%. The mixture first eluted down was repurified by silica gel chromatography and eluted with petroleum ether and gave 99 mg of galvinoxy-H.

**Dimer of 3,4-DHS:** $^1$H NMR (400 MHz, $(CD_3)_2CO$) $\delta$ 4.92 (d, $J$ = 8.0 Hz, 1H), 5.05 (d, $J$ = 8.0 Hz, 1H), 6.51 (d, $J$ = 8.0 Hz, 1H), 6.68 (d, $J$ = 8.0 Hz, 1H), 6.77 (d, $J$ = 2.0 Hz, 1H), 6.97 (d, $J$ = 8.0 Hz, 1H), 7.13, 7.19 (AB system, d, $J$ = 16.0 Hz, 2H), 7.17 (d, $J$ = 8.0 Hz, 1H), 7.23 (m, 2H), 7.24 (d, $J$ = 7.8 Hz, 1H), 7.25 (m, 1H), 7.26 (m, 2H), 7.27 (d, $J$ = 2.4 Hz, 1H), 7.35 (d, $J$ = 7.8 Hz, 2H), 7.57 (d, $J$ = 7.8 Hz, 2H); $^{13}$C NMR (100 MHz, $(CD_3)_2CO$) $\delta$ 81.1, 81.3, 115.4, 115.5, 115.6, 117.9, 120.6, 120.9, 127.1 (2C), 127.9, 128.1 (2C), 128.8 (2C), 128.9 (2C), 129.2, 129.4, 129.5 (2C), 132.1, 137.7, 138.6, 144.8, 145.2, 145.6, 146.2; HRMS (ESI) for $C_{28}H_{22}O_4$ *m*/*z* calcd for $[M + H]^+$ 423.1591, found 423.1597, error = 1.4 ppm.

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**Supporting Information Available:** 1D and 2D NMR and MS spectral data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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