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Structure−Activity Relationship and Mechanism of the Tocopherol-Regenerating Activity of Resveratrol and Its Analogues

JIAN-GUO FANG AND BO ZHOU*

State Key Laboratory of Applied Organic Chemistry, Lanzhou University, Lanzhou, Gansu 730000, China

The present study investigated the mechanism of the synergistic antioxidant activity of α-tocopherol with resveratrol (3,5,4′-trihydroxy-*trans*-stilbene, 3,5,4′-THS) and its synthetic analogues, that is, 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-*trans*-stilbene (4-HS), and 3,5-dihydroxy-*trans*-stilbene (3,5-DHS). The reaction kinetics of α-tocopheroxyl radical with resveratrol and its analogues were studied in sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB) micelles using the stopped-flow electron paramagnetic resonance (EPR) technique. It was found that resveratrol and its analogues could regenerate α-tocopherol with rate constants (k_{REG}) in the order of 3,4-DHS > 4,4′-DHS > resveratrol ≥ 4-HS ≥ 3,5-DHS in SDS and CTAB micelles. It was found that the analogues bearing *o*-dihydroxyl groups (3,4-DHS) and *p*-dihydroxyl groups (4,4′-HS) exhibited remarkably higher α-tocopherol-regenerating activity than those bearing no such groups (resveratrol, 4-HS, and 3,5-DHS). In addition, the α-tocopherol-regenerating activity of resveratrol and its analogues was correlated with their electrochemical behaviors, suggesting that electron transfer might play a critical role during the regeneration reaction.

KEYWORDS: Reveratrol; α-tocopherol; antioxidant; antioxidant synergism; lipid peroxidation; structure−activity relationship

INTRODUCTION

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) (**Figure 1**) is a phytoalexin and a naturally occurring phenolic compound belonging to a stilbene family found in a wide variety of dietary sources including grapes, plums, and peanuts (*1, 2*). Its relatively high concentration in red wine (0.1−14.3 mg/L) (*2*) and near absence in white varietals and grape juice have led to its being proposed as the agent responsible for the so-called "French paradox"; that is, mortality from coronary heart disease is lower in some regions of France than in other western countries due to the regular drinking of wine despite a high fat intake (*3*). Growing evidence suggests that resveratrol plays a role in the prevention of human diseases, such as heart disease, neurodegenerative disease, and cancer (*1, 2*). The wide range of biological effects elicited by resveratrol is believed to be due to its powerful antioxidant property (*1, 4*), because free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNA are considered to be associated with a wide variety of chronic health problems, such as atherosclerosis, cancer, and aging (*5, 6*). Therefore, research interest in the antioxidative activity of resveratrol is growing (*7, 8*).

In the antioxidant family vitamin E plays a central role. It is well-known that α-tocopherol (TOH), the principal component and the most active form of vitamin E, is the major endogenous lipid-soluble chain-breaking antioxidant in human plasma and low-density lipoprotein (LDL) (*9*). It can effectively trap the lipid peroxyl radical ($LOO^{\bullet}$) to inhibit the free-radical-initiated lipid peroxidation within lipid domains by its own conversion into an oxidized product, α-tocopheroxyl radical ($TO^{\bullet}$) (**Figure 2**). The original form can be regenerated by another coexisting antioxidant, such as vitamin C, ubiquinol-10, and green tea polyphenols (*10−13*). This regeneration reaction enhances the antioxidant capacity of vitamin E and, therefore, has led to the clinical investigation of vitamin C used in concert with vitamin E supplementation (*14*). We previously found that resveratrol and its analogues exhibited a synergistic

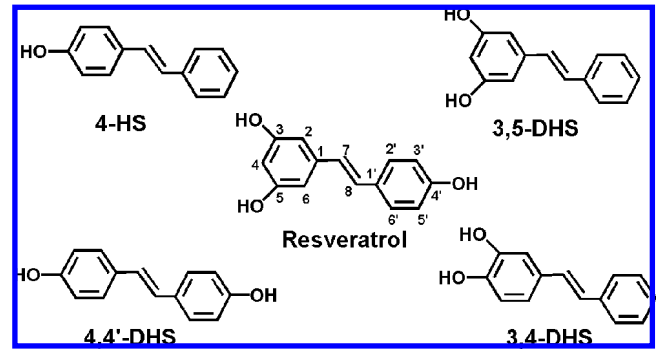

Figure 1. Molecular structures of resveratrol and its analogues.

* Corresponding author (fax +86-931-8915557; e-mail bozhou@lzu.edu.cn).

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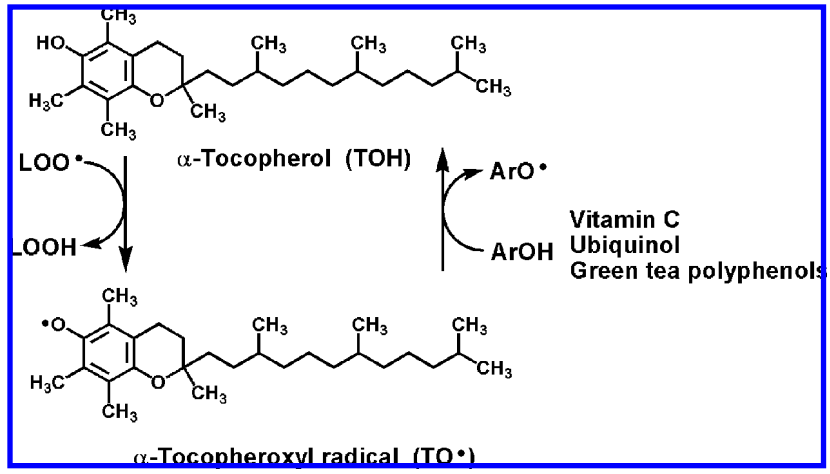


Figure 2. Mechanism for the synergistic antioxidant activity of phenolics (ArOHs) and α-tocopherol (TOH).

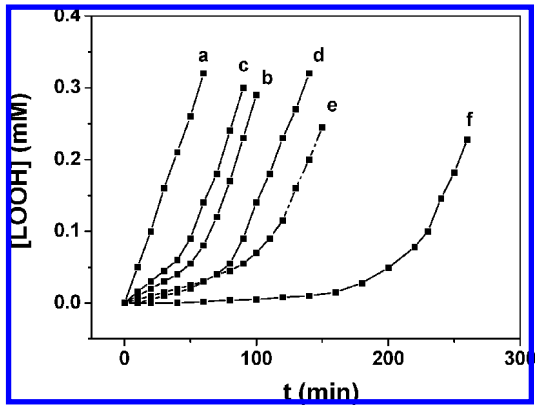


Figure 3. Formation of total hydroperoxides (LOOH) during the peroxidation of linoleic acid (LH) in 0.1 M sodium dodecyl sulfate (SDS) at pH 7.4 and 37 °C, initiated with AAPH and inhibited by resveratrol and its analogues (ROHs) and/or α-tocopherol (TOH): (a) uninhibited peroxidation; (b) inhibited with TOH; (c) inhibited with resveratrol; (d) inhibited with 3,4-DHS; (e) inhibited with resveratrol and TOH; (f) inhibited with 3,4-DHS and TOH. $[LH]_0 = 15.2$ mM, $[AAPH]_0 = 6.3$ mM, [ROHs] = 11.2 μM, [TOH] = 5 μM.

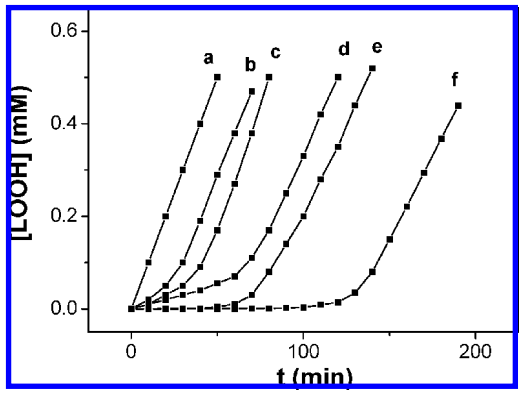


Figure 4. Formation of total hydroperoxides (LOOH) during the peroxidation of linoleic acid (LH) in 15 mM cetyl trimethylammonium bromide (CTAB) at pH 7.4 and 37 °C, initiated with AAPH and inhibited by resveratrol and its analogues (ROHs) and/or α-tocopherol (TOH): (a) uninhibited peroxidation; (b) inhibited with TOH; (c) inhibited with resveratrol; (d) inhibited with 3,4-DHS; (e) inhibited with resveratrol and TOH; (f) inhibited with 3,4-DHS and TOH. $[LH]_0 = 15.2$ mM, $[AAPH]_0 = 6.3$ mM, [ROHs] = 11.2 μM, [TOH] = 5 μM.

antioxidant activity with α-tocopherol in micelles (*15*). The present study was to (i) study the mechanism by which resveratrol and its analogues act as antioxidants synergistically with α-tocopherol and (ii) investigate the reaction kinetics of α-tocopheroxyl radical with resveratrol and its analogues (ROHs), that is, 3,4-dihydroxy-*trans*-stilbene (3,4-DHS), 4,4′-dihydroxy-*trans*-stilbene (4,4′-DHS), 4-hydroxy-*trans*-stilbene (4-HS), and 3,5-dihydroxy-*trans*-stilbene (3,5-DHS) (**Figure 1**) in sodium dodecyl sulfate (SDS) and cetyl trimethylammonium bromide (CTAB) micelles.

MATERIALS AND METHODS

Materials. Resveratrol and its analogues 3,4-DHS, 4,4′-DHS, 4-HS, and 3,5-DHS were synthesized as previously described (*16, 17*). This method produced exclusively the *trans*-isomer. Their structures were fully identified using ^{1}H NMR and EI-MS (see below), and the data were consistent with those reported in the literature (*16, 17*). The purity (>98%) of each compound was checked using high-performance liquid chromatography (HPLC).

Resveratrol: ^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.26 (1H, t, J = 1.8 Hz, H-4), 6.54 (2H, d, J = 1.8 Hz, H-2 and H-6), 6.83 (2H, d, J = 9.0 Hz, H-3′ and H-5′), 6.89, 6.99 (each 1H, d, J = 16.5 Hz, H-7 or H-8), 7.40 (2H, d, J = 9.0 Hz, H-2′ and H-6′); MS, *m/z* 228 [M^+], 211, 199, 181, 157, 115.

3,4-DHS: ^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.81 (1H, d, J = 8.1 Hz, H-5), 6.91 (1H, dd, J = 8.4, 1.8 Hz, H-6), 7.00 (1H, s, J = 7.8 Hz, H-2), 7.07, 7.12 (each 1H, d, J = 16.2 Hz, H-7 or H-8), 7.20 (1H, t, J = 7.5 Hz, H-4′), 7.32 (2H, t, J = 7.8 Hz, H-3′ and H-5′), 7.51 (2H, d, J = 7.8 Hz, H-2′ and H-6′); MS, *m/z* 212 [M^+], 195, 165, 141, 115, 77.

3,5-DHS: ^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.33 (1H, t, J = 2.4 Hz, H-4), 6.61 (2H, d, J = 1.5 Hz, H-2 and H-6), 7.10 (2H, s, H-7 and H-8), 7.24 (1H, t, J = 7.8 Hz, H-4′), 7.35 (2H, t, J = 8.1 Hz, H-3′ and H-5′), 7.55 (2H, d, J = 7.8 Hz, H-2′ and H-6′); MS, *m/z* 212 [M^+], 195, 165, 141, 115, 77.

4-HS: ^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.84 (2H, d, J = 8.7 Hz, H-3 and H-5), 7.08, 7.15 (2H, d, J = 16.5 Hz, H-7 or H-8), 7.23 (1H, dd, J = 7.8, 2 Hz, H-4′), 7.34 (2H, t, J = 8.1 HZ, H-2 and H-6), 7.45 (2H, b, J = 8.7 HZ, H-3′ and H-5′), 7.54 (2H, d, J = 7.5 Hz, H-2′ and H-6′); MS, *m/z* 196 [M^+], 181, 165, 139, 115, 77.

4,4′-DHS, ^{1}H NMR (300 MHz, $(CD_3)_2CO$), δ 6.82 (4H, d, J = 8.7 Hz, H-3, H-5, H-3′, and H-5′), 6.96 (2H, s, H-7 and H-8), 7.39 (4H, d, J = 8.7 Hz, H-2, H-6, H-2′, and H-6′); MS, *m/z* 212 [M^+], 197, 165, 141, 115, 77.

Linoleic acid (chromatographic pure, Sigma-Aldrich, St. Louis, MO), *dl*-α-tocopherol (biochemical reagent, >99.9%, Merck, Darmstadt, Germany) and 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH; Sigma-Aldrich) were kept under nitrogen in a refrigerator. The surfactants SDS and CTAB were recrystallized from ethyl alcohol and acetone/water (9:1, v/v), respectively.

Determination of Linoleic Acid Hydroperoxides. The linoleic acid micelles were prepared by mixing appropriate amounts of linoleic acid,

Table 1. Inhibition of AAPH-Initiated Peroxidation of Linoleic Acid by Resveratrol and Its Analogues (ROHs) and α-Tocopherol (TOH) in Sodium Dodecyl Sulfate (SDS) and Cetyl Trimethylammonium Bromide (CTAB) Micelles[a,b]

micelle	ROH	$R_p/10^{-8}$ M s^{-1}	$R_{inh}/10^{-8}$ M s^{-1}	$t_{inh}/10^3$ s	$k_{inh}/10^{4c}$ M^{-1} s^{-1}	n'[d]	SE (%)
SDS	none	8.3					
SDS	resveratrol	9.0	2.8	3.1	1.3	0.8	
SDS	4-HS	9.2	4.0	2.1	1.2	0.6	
SDS	3,5-DHS	7.8	3.8	1.5	1.7	0.4	
SDS	4,4′-DHS	8.0	2.6	3.2	1.4	0.9	
SDS	3,4-DHS	8.2	0.8	4.9	2.9	1.4	
SDS	TOH	8.1	1.1	3.0	3.6	2.0	
SDS	resveratrol + TOH	7.0	0.9	6.3	2.0	1.2	∼0
SDS	4-HS + TOH	7.9	0.8	5.3	2.3	1.0	∼0
SDS	3,5-DHS + TOH	8.3	1.0	4.8	2.1	0.9	∼0
SDS	4,4′-DHS + TOH	7.4	0.5	6.6	3.1	1.3	∼0
SDS	3,4-DHS + TOH	6.9	∼0	12.6	*e*	2.4	59.5
CTAB	none	16.8					
CTAB	resveratrol	16.0	2.7	2.7	0.7	2.0	
CTAB	4-HS	19.8	3.2	1.7	1.0	1.2	
CTAB	3,5-DHS	15.6	4.2	2.4	0.5	1.8	
CTAB	4,4′-DHS	14.8	2.8	2.6	0.8	1.9	
CTAB	3,4-DHS	14.0	1.6	4.1	0.9	3.0	
CTAB	TOH	16.7	2.0	1.2	2.0	2.0	
CTAB	resveratrol + TOH	12.3	0.1	4.4	10.9	2.2	12.8
CTAB	4-HS + TOH	17.8	0.3	2.5	6.4	1.3	∼0
CTAB	3,5-DHS + TOH	13.6	0.3	3.0	6.3	1.5	∼0
CTAB	4,4′-DHS + TOH	14.3	0.2	4.5	7.5	2.3	18.4
CTAB	3,4-DHS + TOH	11.7	∼0	7.8	*e*	4.0	47.2

[a] The reaction conditions and the initial concentration of the substrates are the same as described in the legends of **Figures 3** and **4** for reactions conducted in SDS and CTAB micelles, respectively. Data are the average of three determinations, which were reproducible with deviation of <±10%. [b] Because the *n* value of α-tocopherol is generally assumed to be 2 (*29*), the apparent rate of chain initiation, R_i, can be determined from the inhibition period and/or the decay rate of α-tocopherol. R_i is taken as 3.1 and 8.3 nM s^{-1} in SDS and CTAB micelles, respectively. [c] Calculated from the equation $R_{inh} = k_p R_i[LH]/(nk_{inh}[AH])$ by taking $k_p = 37$ M^{-1} s^{-1} (*30*). [d] $n' = R_i t_{inh}/([ROH]_0 + [TOH]_0)$. [e] Could not be calculated because R_{inh} is approximately zero.

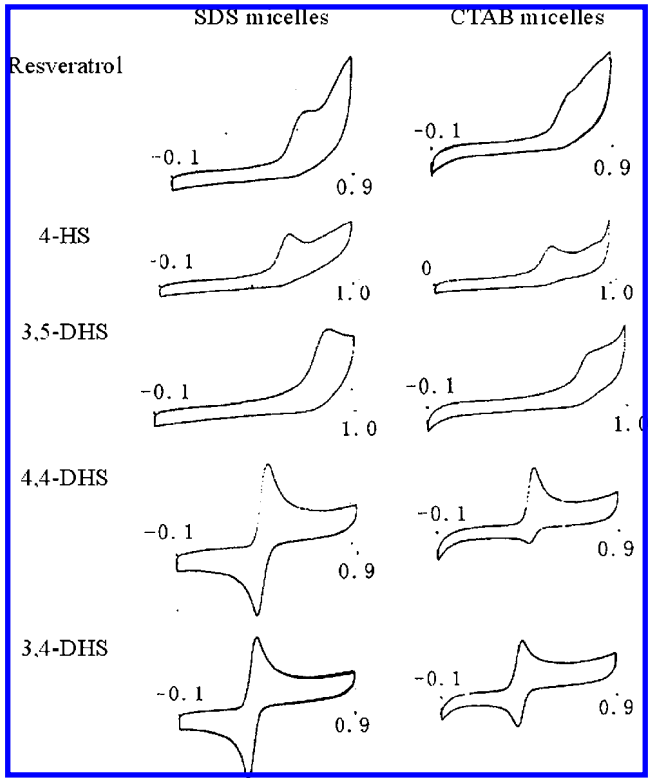


Figure 5. Cyclic voltammograms of resveratrol and its analogues (ROHs) recorded at a glassy carbon electrode in 0.1 M sodium dodecyl sulfate (SDS) and 30 mM cetyl trimethylammonium bromide (CTAB) micelles at pH 7.4.

TOH, resveratrol, and its analogues, when required, in an aqueous solution of 0.1 M SDS or 15 mM CTAB (pH 7.4) followed by vigorous mixing in a vortex mixer for 2 min. AAPH was directly dissolved in phosphate-buffered saline (pH 7.4) and injected into the micelles to initiate the peroxidation.

Table 2. Rate Constants for the Reactions α-Tocopheroxyl Radical (TO•) with Resveratrol and Its Analogues (ROHs) in Micelles[a,b]

ROH	SDS		CTAB	
	k_{REG} 10^2 (M^{-1} s^{-1})	Ep (V vs SCE)	$k_{REG}10^2$ (M^{-1} s^{-1})	Ep (V vs SCE)
resveratrol	0.19	0.62	0.20	0.67
4-HS	0.11	0.64	0.14	0.66
3,5-DHS	0.10	0.85	0.19	0.79
4,4′-DHS	0.61	0.40	0.28	0.43
3,4-DHS	1.35	0.34	2.99	0.36

[a] Determined by EPR in sodium dodecyl sulfate (SDS) and 15 mM cetyl trimethylammonium bromide (CTAB) micelles at pH 7.4 and room temperature under air. [b] The values of k_{REG} and Ep are given with errors usually <10%.

The linoleic acid micelles were placed in an open vessel of 2 mL in volume, thermostated at 37 °C and provided with a magnetic stirrer. The formation of linoleic acid hydroperoxides (LOOH) was determined using HPLC as described previously (*18*). Briefly, the reaction mixture was sampled at various time intervals and subjected to HPLC analysis on a Gilson liquid chromatograph equipped with a Zorbax ODS reversed-phase column (6 × 250 mm, DuPont Instruments), then eluted with a methanol/water mixture (9:1, v/v). The flow rate was set at 1.0 mL/min. Every determination was repeated three times, and the experimental deviations were within ±10%.

Determination of Oxidation Potentials. The oxidation potentials of ROHs were determined on a PAR 173 potentiostat with a glassy carbon electrode in phosphate-buffered micelles (pH 7.4) at room temperature, as described previously (*19*). The potential was recorded relative to a saturated calomel electrode (SCE).

Determination of α-Tocopheroxyl Radical. Electron paramagnetic resonance (EPR) was carried out on a Bruker ER 200D spectrometer operated in the X-band with 100 kHz modulation, a modulation amplitude of 0.25 mT, a time constant of 0.2 s, and a microwave power of 25 mW. A flat quartz flow cell (0.4 × 5.5 × 60 mm) was used for

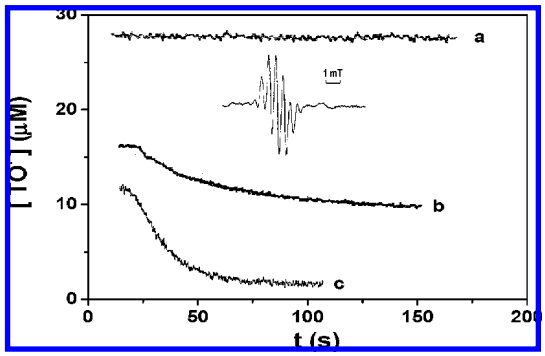


Figure 6. Decay of α-tocopheroxyl radical (TO•) in 15 mM cetyl trimethylammonium bromide (CTAB) micelles at pH 7.4 and room temperature under air: (a) intrinsic decay; (b) in the presence of resveratrol (0.78 mM); (c) in the presence of 3,4-DHS (0.13 mM). (Inset) EPR spectra of the TO• recorded in CTAB micelles.

the stopped-flow determination of the reaction kinetics as described previously (*20*). α-Tocopheroxyl radical was generated by vigorously stirring α-tocopherol (1 mM) and excess lead oxide with a vortex mixer for 3 min in 0.1 M SDS or 15 mM micelles (pH 7.4) at room temperature.

RESULTS AND DISCUSSION

Synergistic Inhibition on Linoleic Acid Peroxidation by Resveratrol, Its Analogues, and α-Tocopherol. Peroxidation of linoleic acid or its ester as a model of unsaturated lipids gives different hydroperoxides depending on the reaction conditions (*21*). Hydroperoxide substitution at C-9 or C-13 positions produces either *trans*,*trans* or *cis*,*trans* conjugated dienes, which are the major products in the absence of antioxidants or in the presence of a small amount of antioxidant, for example, millimolar concentrations of α-tocopherol (*21*). They showed characteristic ultraviolet absorption at 235 nm (*22*) that was used to monitor the formation of the total hydroperoxides formed during the peroxidation after separation of the reaction mixture by HPLC.

A set of representative kinetic curves of the total hydroperoxides formation during the peroxidation of linoleic acid in SDS micelles is shown in **Figure 3**. It can be seen (line a in **Figure 3**) that, upon AAPH initiation, concentration of the hydroperoxide increased quickly and linearly with time in the absence of antioxidants, demonstrating the fast linoleic acid peroxidation. The slope of this line corresponds to the rate of propagation, R_p. The peroxide formation was remarkably inhibited by the addition of α-tocopherol (line b) during the so-called "inhibition period". After the inhibition period, the rate of hydroperoxide formation increased to close to the original rate of propagation, corresponding to the exhaustion of the antioxidant. The turning point from the inhibition period to the restoration of propagating rate refers to the inhibition time, t_{inh}. During the inhibition period, the concentration of the hydroperoxides also increased approximately linearly with time, and the slope of this line was designated R_{inh}, which reflects the antioxidative potential of the antioxidant. Addition of resveratrol or 3,4-DHS showed also a clear inhibition period, demonstrating that these compounds acted well as chain-breaking antioxidants in SDS micelles (lines c and d). Other ROHs (4,4′-DHS, 4-HS, and 3,5-DHS) also showed similar inhibition periods (data not shown). Addition of 3,4-DHS together with TOH significantly prolonged the inhibition period of the latter (line f). The t_{inh} of TOH and 3,4-DHS when they were used together in SDS micelles was approximately 60% [(synergistic efficiency SE %) (*23*), eq 1] longer than the sum of the t_{inh} values when the two antioxidants were used individually, demonstrating clearly the antioxidant synergism of 3,4-DHS with TOH. The peroxidation was completely inhibited by 3,4-DHS and TOH in combination in SDS micelles. Similar results were also obtained in CTAB micelles (**Figure 4**). On the basis of the inhibition period and synergistic efficiency, the antioxidant activity follows the sequence 3,4-DHS > 4,4′-DHS > resveratrol ≈ 4-HS > 3,5-DHS.

$$\mathrm{SE}\,\% = \{t_{inh}(\mathrm{TOH}+\mathrm{ArOH}) - [t_{inh}(\mathrm{TOH}) + t_{inh}(\mathrm{ArOH})]\}/[t_{inh}(\mathrm{TOH}) + t_{inh}(\mathrm{ArOH})] \times 100 \quad (1)$$

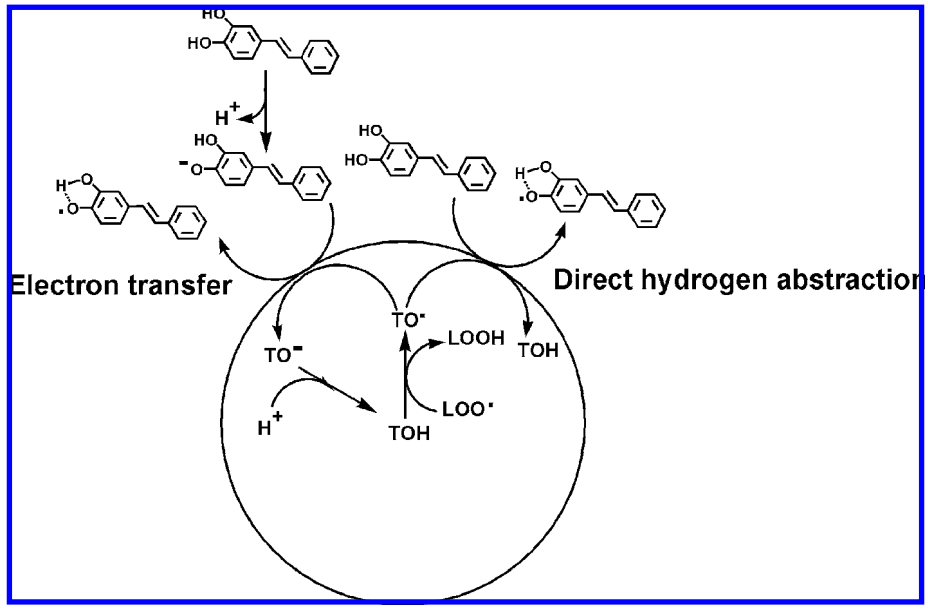
The kinetic parameters deduced from **Figures 3** and **4** are listed in **Table 1**. The kinetics of linoleic acid peroxidation initiated by azo compounds and its inhibition by chain-breaking antioxidant (AH) have been discussed in detail in our previous paper (*24*). In **Table 1**, k_{inh} is the rate constant for the chain inhibition by the antioxidant (eq 2), and n is the stoichiometric factor that designates the number of peroxyl radicals trapped by each antioxidant molecule.

$$\mathrm{LOO^{\bullet}} + \mathrm{ROH} \xrightarrow{k_{inh}} \mathrm{LOOH} + \mathrm{RO^{\bullet}} \quad (2)$$

Determination of Oxidation Potentials. To rationalize the structure−activity relationship and mechanism of the antioxidant synergism of ROHs and TOH, the oxidation potentials were determined using a cyclic voltammetric method (**Figure 5** and **Table 2**). It can be seen (**Figure 5** and **Table 2**) that the analogues bearing *o*-dihydroxyl or *p*-dihydroxyl groups (3,4-DHS and 4,4′-DHS) showed lower oxidation potentials and reversible cyclic voltammograms, and those bearing no such groups (resveratrol, 4-HS and 3,5-DHS) showed higher oxidation potentials and irreversible cyclic voltammograms. The results demonstrate that 3,4-DHS and 4,4′-DHS exhibited higher activity, whereas resveratrol, 4-HS and 3,5-DHS were less active.

Direct Determination of the Rate of α-Tocopherol Regeneration Reaction. TOH, the most biologically and chemically active form of vitamin E, is considered to be the major antioxidant in human plasma and low-density lipoprotein (LDL) (*9*). The antioxidative activity of TOH can be restored when the α-tocopheroxyl radical (TO•) is reduced by the coexistent antioxidant (**Figure 2**), such as L-ascorbic acid (vitamin C) (*10*) and green tea polyphenols (*11, 12*). To rationalize if resveratrol and its analogues (ROHs) have similar mechanisms, the decay kinetics of α-tocopheroxyl radical (TO•) in the presence of the former were determined by stopped-flow EPR technique as described previously (*20*). α-Tocopheroxyl radical (TO•) was produced by oxidizing TOH with PbO_2 in micelles. α-Tocopheroxyl radical (TO•) is usually more persistent in micelles than in homogeneous solutions. The rate constants of the bimolecular self-reaction of TO• were reported to be 3×10^3 M^{-1} s^{-1} in benzene/di-*tert*-butyl peroxide (*25*) and 75 and 15 M^{-1} s^{-1} in SDS and CTAB micelles (*12*), respectively. Therefore, the reaction kinetics of TO• could be easily determined in micelles at ambient temperature (*20*). The inset of **Figure 6** shows the EPR spectrum of TO• recorded in CTAB micelles. Addition of resveratrol remarkably increased the decay of TO•, which was found to be pseudo-first-order in the presence of a large excess of resveratrol (line b in **Figure 6**). 3,4-DHS reacted with TO• more rapidly than resveratrol (line c in **Figure 6**). Other ROHs showed similar enhanced decay (data not shown). Plotting this first-order rate constant k_{obs} versus the concentration of ROHs gave a straight line from which the second-order rate constant, k_{REG}, between TO• and ROHs, could

Scheme 1. Mechanism for the Synergistic Antioxidant Activity of 3,4-DHS and TOH in Micelles[a]



[a] The circle denotes the surface of the micelles. LOO•, propagating peroxyl radical; LOOH, linoleic acid hydroperoxides; TO•, α-tocopheroxyl radical; TOH, α-tocopherol.

be obtained (**Table 2**). This provides unambiguous evidence that these comounds could reduce α-tocopheroxyl radical to regenerate α-tocopherol (eq 3). The rate constant for TOH regeneration by ROHs, k_{REG}, decreased in the order 3,4-DHS > 4,4′-DHS > resveratrol ≥ 4-HS ≥ 3,5-DHS in SDS and CTAB micelles. On the other hand, the Ep values increased in the order 3,4-DHS < 4,4′-DHS < resveratrol ≈ 4-HS < 3,5-DHS, as listed in **Table 2**. The structure−activity relationship obtained from this experiment is similar to that obtained from the inhibition of linoleic acid peroxidation by HPLC method noted above.

$$TO^{\bullet} + ROH \xrightarrow{k_{REG}} TOH + RO^{\bullet} \quad (3)$$

Because the rate of this TOH regeneration reaction ($k_{REG} \approx 10^2$ M^{-1} s^{-1}) is approximately 2 orders of magnitude slower than that of the antioxidation reaction (eq 2), $k_{inh} \approx 10^4$ M^{-1} s^{-1}], the antioxidant synergism can only be observed when the rate of the TOH regeneration reaction is high enough to compete with the antioxidation reaction.

Structure−Activity Relationship and Mechanism for the TOH Regeneration Reaction. It is worth noting that the tocopherol-regenerating activity of ROHs is correlated with the electrochemical behavior of the molecule (**Table 2**), implying that the electron transfer between α-tocopheroxyl radical (TO•) and ROHs might also contribute to the reaction as shown in eq 4.

$$TO^{\bullet} + RO^{-} \xrightarrow{k_{REG}} TO^{-} + RO^{\bullet} \quad (4)$$

It is well-known that the phenoxide (ArO^-) more easily undergoes electron transfer oxidation, producing relatively stable phenoxyl radical ($ArO^{\bullet}$) in alkaline solutions. Resveratrol, with a pK_{a1} of 6.4 (*26*), partially dissociates under the present experimental conditions (pH 7.4); this makes the electron transfer reaction feasible. Therefore, the electron transfer regeneration of α-tocopherol by ArO^- (eq 4) may take place simultaneously with the direct hydrogen abstraction (eq 3). Obviously, the relative contribution of these processes depends on the nature of the reaction medium and the pH, as well as the oxidation potential and the acid dissociation constant (pK_a) of ROHs. Mukai et al. have proven that the rate constant for TOH regeneration reaction by green tea polyphenols (*11*) and flavonols, such as quercetin and rutin (*27*), in micellar solution was pH-dependent and greater with increasing pH value.

The present study demonstrates that analogues bearing *o*-dihydroxyl groups (3,4-DHS) or bearing *p*-dihydroxyl groups (4,4′-HS) exhibited remarkably higher activity than those bearing no such groups (resveratrol, 4-HS, and 3,5-DHS) in the tocopherol regenaration reaction. This can be explained because the *o*-hydroxyl phenoxyl radical, the oxidation intermediate for 3,4-DHS, is more stable due to the intramolecular hydrogen-bonding interaction, as evidenced recently from theoretical calculations (*28*) (**Scheme 1**). The theoretical calculation showed that the hydrogen bond in the *o*-OH phenoxyl radical was approximately 4 kcal/mol stronger than that in the parent catechol and that the bond dissociation energy (BDE) of catechol was 9.1 kcal/mol lower than that of phenol and 8.8 kcal/mol lower than that of resorcinol (*28*). In the case of 4,4′-DHS, the intermediate formation of *p*-OH phenoxyl radical and/or *p*-semiquinone radical anion can be stabilized by the 4′-OH group by resonance through the trans double bond.

In summary, this work provides unambiguous evidence that resveratrol and its analogues can effectively reduce α-tocopheroxyl radical to regenerate α-tocopherol in the order 3,4-DHS > 4,4′-DHS > resveratrol ≥ 4-HS ≥ 3,5-DHS in SDS and CTAB micelles. It was observed that resveratrol analogues bearing *o*-dihydroxyl or *p*-dihydroxyl groups exhibited remarkably high synergistic antioxidant activities.

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