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Stopped-Flow Kinetic Study of the Aroxyl Radical-Scavenging Action of Catechins and Vitamin C in Ethanol and Micellar Solutions

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Kinetic study of the aroxyl radical-scavenging action of catechins (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG)) and related compounds (methyl gallate (MG), 4-methylcatechol (MC), and 5-methoxyresorcinol (MR)) has been performed. The second-order rate constant (k_s) for the reaction of these antioxidants with aroxyl radical has been measured in ethanol and aqueous Triton X-100 micellar solution (5.0 wt %). The k_s values decreased in the order of EGCG > EGC > MC > ECG > EC > MG ≫ MR in ethanol, indicating that the reactivity of the OH groups in catechins decreased in the order of pyrogallol B-ring > catechol B-ring > gallate G-ring > resorcinol A-ring. The structure−activity relationship in the free radical-scavenging reaction by catechins has been clarified by the detailed analyses of the pH dependence of k_s values. From the results, the pK_a values have been determined for catechins. The monoanion form at catechol B- and resorcinol A-rings and dianion form at pyrogallol B- and gallate G-rings show the highest activity for free radical scavenging. It was found that the free radical-scavenging activities of catechins are 3.2−13 times larger than that of vitamin C at pH 7.0.

KEYWORDS: Tea catechins; epigallocatechin gallate; free radical-scavenging activity; reaction rate; antioxidant activity; structure−activity relationship; pH dependence; pK_a value

INTRODUCTION

Catechins (tea flavan-3-ols) are well-known as typical water-soluble polyphenolic antioxidants. Catechins are widely found in plants and foods in high concentrations (*1, 2*). Especially, green and black teas contain considerable amounts of catechins (epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG); see **Figure 1**) (*1–4*). Catechins are found in blood and tissues following oral ingestion (*5–7*), prevent human plasma oxidation, and act as inhibitors of low-density lipoprotein (LDL) oxidation (*8–11*). It has been proven that drinking tea, especially green tea, is associated with a lower incidence of human cancer (*12–15*). The cancer preventive effects often have been attributed to antioxidant actions. Catechins are effective antioxidants against linoleic acid (or methyl linoleate) peroxidation in homogeneous and micellar solutions and phospholipids bilayers (*16–19*).

Catechins (EC, ECG, EGC, and EGCG) may function as scavengers of active oxygen radical species in biological systems. Several kinetic studies have been performed for the reaction of catechins with active free radicals (LOO• (eq 1) (*16–21*), N_3• (*22–24*), HO• (*22*), O_2^{-}• (*23*), and *t*-BuO• (*22*). Furthermore, catechins show high activity for the quenching of singlet oxygen (eq 2). The quenching rates (k_Q) of singlet oxygen by EGC and EGCG are higher than those of α-tocopherol and biological hydroquinones (ubiquinol-10, α-tocopherol hydroquinone, etc.) (*25, 26*).

$$\mathrm{LOO}\bullet + \mathrm{Catechin} \xrightarrow{k_{inh}} \mathrm{LOOH} + \mathrm{Catechin}\bullet \qquad (1)$$

$$^1\mathrm{O}_2 + \mathrm{Catechin} \xrightarrow{k_Q} {}^3\mathrm{O}_2 + \mathrm{Catechin} \qquad (2)$$

Now it is generally accepted that the B-ring in the catechins (see **Figure 1**), having catechol or pyrogallol moieties, is responsible for the most of the antioxidant activity (*16–28*). A mechanistic study has been performed for the reaction of the methylperoxide (CH_3OO•) radical with EC, indicating that the hydrogen abstraction process from catechins takes place with a huge tunneling effect (*29*). Catechins have various phenolic OH groups in a molecule, and the dissociation of OH protons proceeds by increasing pH values. Consequently, we can expect notable pH dependence for the free radical-scavenging rates of catechins (*30*). However, the pH dependence on the reaction rates (k_{inh}) of catechins with the LOO• radical has not been reported, as far as we know.

In previous works (*31–33*), we measured the reaction rates (k_s) of α-, β-, γ-, δ-tocopherols (α-, β-, γ-, δ-TocH) with 2,6-

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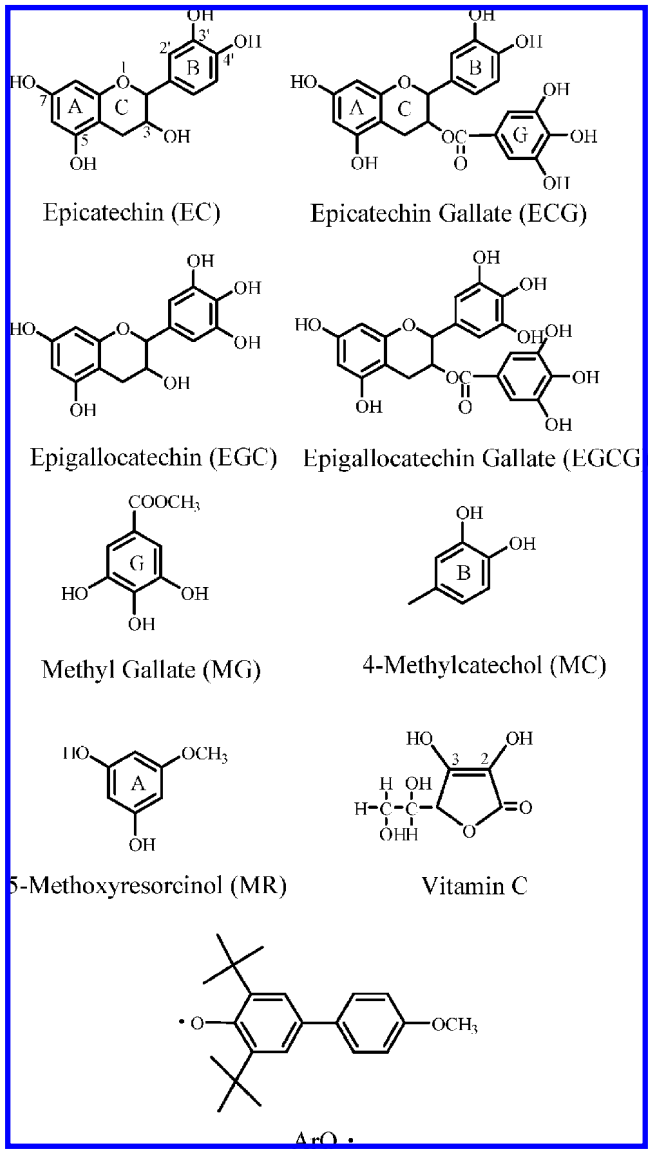

Figure 1. Molecular structures of catechins (EC, ECG, EGC, and EGCG), related compounds (MG, MC, and MR), vitamin C, and aroxyl radical (ArO·).

di-*t*-butyl-4-(4-methoxyphenyl)phenoxyl (aroxyl, ArO•) (see **Figure 1**) (eq 3) in ethanol and Triton X-100 micellar solution (pH 7.0), using stopped-flow spectrophotometry. ArO• can be regarded as a model for active oxygen radicals (LOO• and others) in biological systems.

$$\mathrm{ArO}\bullet + \mathrm{TocH} \xrightarrow{k_s} \mathrm{ArOH} + \mathrm{Toc}\bullet \qquad (3)$$

The second-order rate constants (k_s) obtained are listed in **Table 1**. The relative rates (α:β:γ:δ = 100:44:47:20) agreed well with those obtained for the reaction of TocH with poly(peroxystyryl)peroxyl radicals (100:41:44:14) in chlorobenzene using the O_2 consumption method (eq 1) (*34*). The result suggests that the relative reactivity of TocH in homogeneous solution probably does not depend on the type of oxyradicals (ArO• and LOO•) used (*31, 32*). It was found that the relative ratio of k_s values (100:21:20:2.9) of α-, β-, γ-, δ-TocH in micellar dispersion has good correlation with the relative biopotency ratios for rat fetal resorption, rat hemolysis, and chicken muscle dystrophy (*33*).

Table 1. Second-Order Rate Constants (k_s) for the Reaction of the Aroxyl (ArO·) Radical with Catechins (EC, ECG, EGC, and EGCG) and Related Antioxidants in Ethanol and Triton X-100 Micellar Solution (5.0 wt%) at pH 4.0 and 7.0 at 25.0 °C, and the Ratio (k_{s1}(Micelle)/k_s(Ethanol))

	ethanol	micelle (pH 7.0)	micelle (pH 4.0)	ratio
antioxidant	k_s/M^{-1} s^{-1}	k_s/M^{-1} s^{-1}	k_{s1}[a]/M^{-1} s^{-1}	k_{s1}[a] (micelle)/k_s (ethanol)
EC	8.61×10	8.03×10^2	7.40×10^2	8.6
ECG	1.07×10^2	1.59×10^3	7.80×10^2	7.3
EGC	3.77×10^2	2.13×10^3	1.65×10^3	4.4
EGCG	4.69×10^2	3.20×10^3	2.70×10^3	5.8
MG	1.56×10	5.99×10^2	8.59×10	5.5
MC	2.67×10^2	2.22×10^3	1.87×10^3	7.0
MR	$\sim 10^{-2}$	2.17	1.00	
vitamin C	insoluble	2.51×10^2	2.50×10	
α-tocopherol[b]	5.12×10^3	5.12×10^5	5.12×10^5	100
β-tocopherol[b]	2.24×10^3	1.05×10^5	1.05×10^5	47
γ-tocopherol[b]	2.42×10^3	1.00×10^5	1.00×10^5	41
δ-tocopherol[b]	1.00×10^3	1.49×10^4	1.49×10^4	15
tocol[b]	0.56×10^3	3.53×10^3	3.53×10^3	6.3
ubiquinol-10[b]	4.70×10^3	1.25×10^5	1.21×10^5	26

[a] The k_{s1} values were used instead of the k_s values at pH 4.0. [b] The values reported in ref *33*.

In the present work, we measured the rate constants (k_s) for the reaction of catechins (EC, ECG, EGC, and EGCG) and related compounds (methyl gallate (MG), 4-methylcatechol (MC) and 5-methoxyresorcinol (MR)) (see **Figure 1**) with ArO• radicals in ethanol and aqueous Triton X-100 micellar solution (5.0 wt %) at pH 4−13 (eq 3). MG, MC, and MR are considered to be a model of the gallate G-ring, catechol B-ring, and resorcinol A-ring in catechins, respectively. The k_s values obtained in micellar solution showed notable pH dependence. Vitamin C (Vit C) is also one of the representative water-soluble antioxidants. The measurement of the k_s value of Vit C at pH 3.5−11 was also performed for comparison. Recently, detailed kinetic studies have been performed for the reaction of catechins with the 5,7-di-isopropyl-tocopheroxyl (5,7-Di*i*Pr-Toc•) radical in homogeneous and micelle solutions at pH 4−12 (*35*). The present study was performed to obtain more insight into the structure−activity relationship of radical scavenging by catechins and to clarify the role of catechins as antioxidants in biological systems.

MATERIALS AND METHODS

Chemicals. EC, EGC, ECG, and EGCG were obtained from Funakoshi (Japan). MC (Wako Chemicals, Japan), MG (Tokyo Kasei Organic Chemicals, Japan), and MR (Aldrich) are commercially available. The aroxyl radical (ArO•) was prepared according to the method of Rieker et al. (*36*). All buffer solutions were prepared using distilled water treated with a Millipor Q system. The pH of the solution was adjusted using an appropriate buffer (0.2 M): CH_3COOH-CH_3COONa at pH 3.5−5.0, KH_2PO_4−Na_2HPO_4 at pH 6.0−9.5, $NaHCO_3$−Na_2CO_3 at pH 10−11.5, and Na_2CO_3−NaOH at pH 12−13.

Measurements. Since catechins and related compounds are unstable at basic pH conditions, all of the reactions were carried out under nitrogen atmosphere. A continuous flow of nitrogen gas was bubbled through aqueous Triton X-100 micellar solutions (10.0 wt %) (0.2 M buffer) containing ArO•. Similarly, aqueous solutions (not buffer solution) of catechins and related compounds were prepared under nitrogen atmosphere and reacted immediately with the above micellar solution containing ArO•. The ArO• radical is fairly stable in ethanol and Triton X-100 micellar solution at 13 ≥ pH ≥ 4.

The kinetic data were obtained with a Unisoku Model RS-450 stopped-flow spectrophotometer by mixing equal volumes of solutions of catechins (or vitamin C) and ArO•. The shortest time for mixing two solutions and recording the first data point (that is, dead time) was

Table 2. pH Dependence of the Second-Order Rate Constants (k_s) for the Reaction of Catechins (EC, ECG, EGC, and EGCG) with the Aroxyl (ArO·) Radical in 5.0 wt% Triton X-100 Micellar Solution

	k_s/M^{-1} s^{-1}			
pH	EC	ECG	EGC	EGCG
4	6.39×10^2	7.81×10^2	1.62×10^3	2.75×10^3
5	7.43×10^2	7.79×10^2	1.65×10^3	2.72×10^3
6	7.51×10^2	9.82×10^2	1.76×10^3	2.98×10^3
7	8.03×10^2	1.59×10^3	2.13×10^3	3.20×10^3
8	8.69×10^2	2.17×10^3	2.91×10^3	5.74×10^3
8.5	1.28×10^3	3.40×10^3	8.05×10^3	1.68×10^4
9	3.42×10^3	7.67×10^3	1.26×10^4	3.26×10^4
9.25			9.12×10^3	2.45×10^4
9.5	4.32×10^3	8.02×10^3	8.57×10^3	2.23×10^4
10	3.80×10^3	9.08×10^3	9.35×10^3	3.19×10^4
10.5	2.05×10^3	6.80×10^3	1.45×10^4	4.85×10^4
11	1.48×10^3	6.00×10^3	1.66×10^4	5.62×10^4
11.5	1.30×10^3	5.03×10^3	1.38×10^4	4.32×10^4
12	1.26×10^3	4.83×10^3	1.11×10^4	2.45×10^4
12.5	9.00×10^2	6.82×10^3	8.64×10^3	2.85×10^4
13	4.85×10^2	8.61×10^3	1.63×10^3	3.02×10^4

10−20 ms. The reaction was monitored with either single wavelength detection or with a photodiode array detector attached to the stopped-flow spectrophotometer. All measurements were performed at 25.0 ± 0.5 °C. Experimental errors in the rate constants (k_s) were estimated to be about 5 and 8% in ethanol and micellar solutions, respectively.

RESULTS

Rate Constants (k_s) of the Aroxyl-Scavenging Reaction with Catechins and Related Compounds in Ethanol. Measurements of the rate constant (k_s) for the reaction of ArO· with catechins (CAs) (EC, ECG, EGC, and EGCG) and related compounds (MR, MC, and MG) were performed in ethanol solution (eq 3). The decay rate of ArO· was measured by following the decrease in absorbance at 376 and/or 580 nm of the ArO· (**Figure 2**A) (*31, 33*). The pseudofirst-order rate constants (k_{obsd}) at 376 nm were linearly dependent on the concentration of catechins ([CA]), and thus the rate equation is expressed as

$$-\mathrm{d[ArO\cdot]}/\mathrm{d}t = k_{obsd}[\mathrm{ArO\cdot}] = k_s[\mathrm{CA}][\mathrm{ArO\cdot}] \quad (4)$$

where k_s is the second-order rate constant for oxidation of catechins by ArO· radical. The rate constants (k_s) were obtained by plotting k_{obsd} against [CA], as shown in **Figure 2**B. Similar measurements were performed for related compounds. The k_s values obtained are summarized in **Table 1**, together with those reported for α-, β-, γ-, δ-tocopherol, tocol, and ubiquinol-10. As listed in **Table 1**, the rate of the scavenging reaction decreases in the following order in ethanol solution.

$$\mathrm{EGCG} > \mathrm{EGC} > \mathrm{MC} > \mathrm{ECG} > \mathrm{EC} > \mathrm{MG} \gg \mathrm{MR} \quad (5)$$

The k_s value of EGCG is 5.4 times larger than that of EC. The k_s values of EC, ECG, EGC, and EGCG are approximately 1 to 2 orders of magnitude smaller than those of α-tocopherol (5.12×10^3 M^{-1} s^{-1}) and ubiquinol-10 (4.70×10^3 M^{-1} s^{-1}) in ethanol.

pH Dependence of the Rate Constants (k_s) of the Aroxyl-Scavenging Reaction with Catechins, Related Compounds, and Vitamin C in Micellar Solution. Measurements of the rate constant (k_s) for the reaction of ArO· with catechins and related compounds were performed at various pH values in Triton X-100 micellar solution under nitrogen atmosphere, as described in the Materials and Methods section. The rate constants (k_s) were obtained by plotting k_{obsd} against [CA] at each pH (data are not shown). The k_s values obtained are summarized in **Tables 2** and **3**.

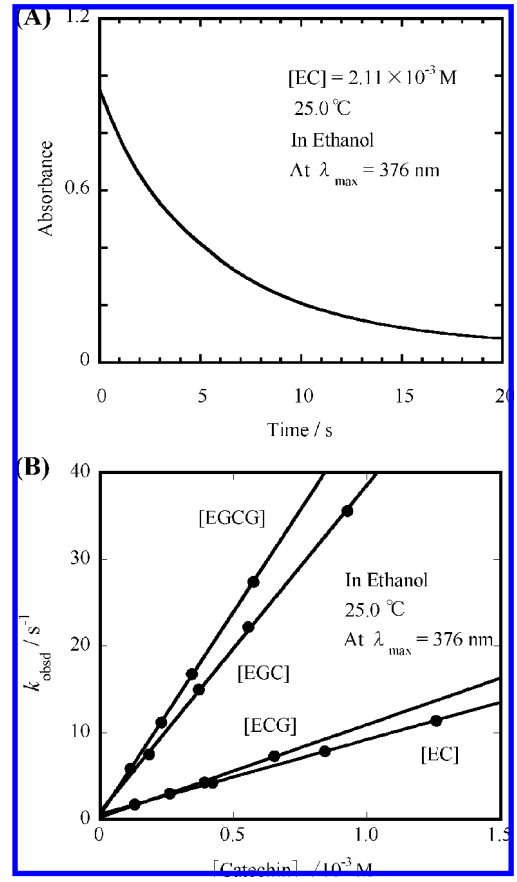

Figure 2. (**A**) Change in absorbance of aroxyl radical (ArO·) at 376 nm during the reaction of ArO· with epicatechin (EC) in ethanol at 25.0 °C. $[\mathrm{ArO\cdot}]_{t=0}$ = ∼0.056 mM and $[\mathrm{Epicatechin}]_{t=0}$ = 2.11 mM. (**B**) Dependence of pseudo first-order rate constant (k_{obsd}) on concentrations of catechins (EC, ECG, EGC, and EGCG) in ethanol at 25.0 °C.

In order to ascertain that the time of the decomposition reaction of catechins at high pH conditions is slower than the decay time of ArO· due to the reaction of ArO· with catechins, the reaction between an aqueous solution of catechins and a buffer solution without ArO· radical was performed at high pH regions (pH 11−13) under nitrogen atmosphere, as a blank test. For instance, the changes of the absorption spectra (λ_{max} = 323 nm at pH 11.0, 321 nm at pH 12.0, and 321 nm at pH 13.0) of EGCG were small at t < 5 s and were almost negligible at these pH conditions. However, a new broad absorption peak appeared at λ_{max} ∼580 nm at t > 10 s and increased gradually (data are not shown). As shown in **Figure 2**A, by reacting the ArO· radical with EC in ethanol solution, the absorption of ArO· at 376 nm decreases rapidly until t = 20 s. The second-order rate constants (k_s) of catechins (EC, ECG, EGC, and EGCG) are 10^3−10^4 M^{-1} s^{-1} at pH > 8, as listed in **Table 2**, and are 2 to 3 orders of magnitude larger than that (k_s = 8.61 × 10 M^{-1} s^{-1}) of EC in ethanol solution. Therefore, the reaction time is less than 5 s, and the decomposition of catechins is negligible even if the reactions were performed in the high pH region. The reaction between the 5,7-di-isopropyl-tocopheroxyl radical and catechins was also performed under similar conditions in a previous work (*35*).

As shown in **Figure 3A**−**D**, the k_s values of catechins remain constant in the low pH region and show similar values at pH 4−6. The ratios of k_s values between catechins at pH 4.0 are

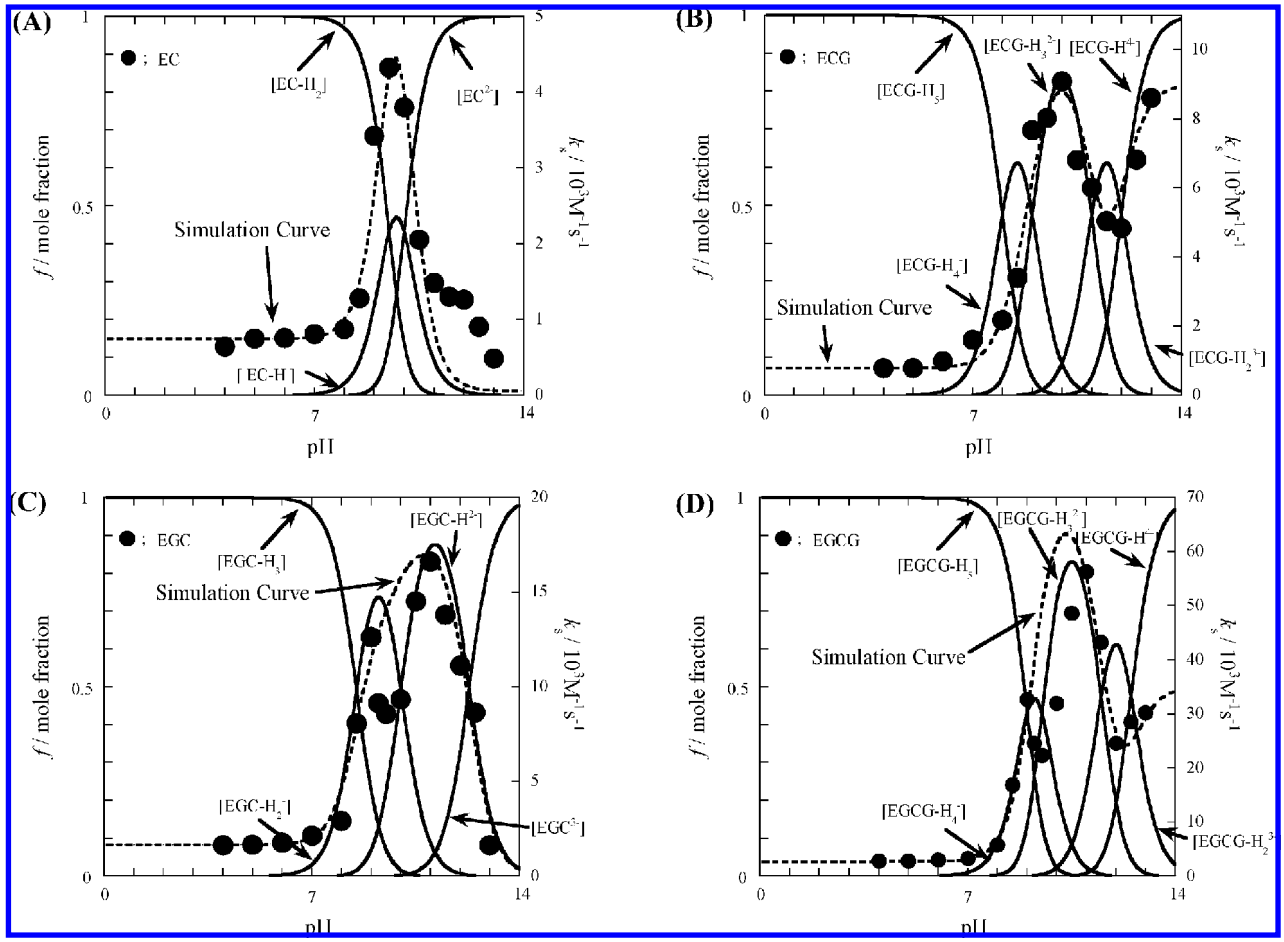

Figure 3. (**A**) Plots of second-order rate constant (k_s) for EC (●) versus pH and of mole fraction (f) of three EC species (EC-H_2, EC-H^-, and EC^{2-}) vs pH (—). The dotted line is a simulation curve. (**B**) Plots of second-order rate constant (k_s) for ECG (●) vs pH and of mole fraction (f) of five ECG species (ECG-H_5, ECG-H_4^-, ECG-H_3^{2-}, ECG-H_2^{3-}, and ECG-H^{4-}) vs pH (—). The dotted line is a simulation curve. (**C** and **D**) Similar plots for EGC and EGCG, respectively. All of the reactions were performed in 5.0 wt % Triton X-100 micellar solution at 25.0 °C.

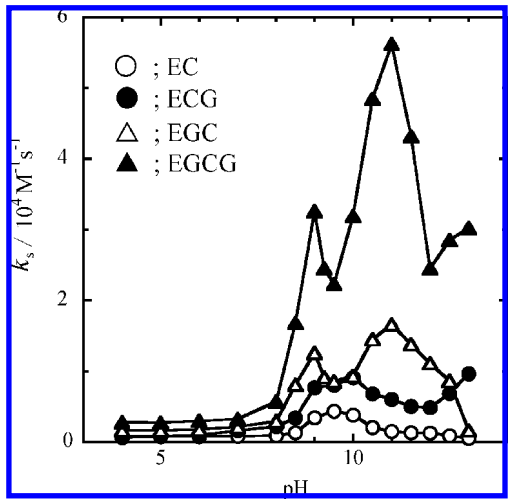

Figure 4. Plots of second-order rate constant (k_s) for the reaction of EC (○), ECG (●), EGC (△), and EGCG (▲) with the aroxyl radical (ArO·) vs pH. All of the reactions were performed in 5.0 wt % Triton X-100 micellar solution at 25.0 °C.

less than 4.3 in micellar solution. The rate constants of catechins increase rapidly at about pH 7. At pH 7−12, the rate constants increase in the order of EC < ECG < EGC < EGCG independent of pH values, except for the case of ECG and EGC at pH 9.5 (see **Figure 4** and **Table 2**). The rate constants of EC and ECG show a maximum at pH 9.5 and 10.0, respectively. The rate constants of EGC and EGCG show two peaks at pH 9.0 and 11.0. The rate constants of MG and MC also show notable pH dependence and increase by increasing pH value, as shown in **Figure 5A** and **B**. The k_s values of MR at pH 6−12 are much smaller than those reported for EC, ECG, EGC, EGCG, MG, and MC.

Measurement of the reaction rate for Vit C was also performed, by varying pH values. The k_s value of Vit C increased with increasing pH, showed similar values at pH 5−9, and increased until pH 11 (see **Table 3** and **Figure 5C**). The pH dependence of k_s values observed will be explained in the following section. As listed in **Table 1**, the rate constants (k_s) decrease in the order of

$$\text{EGCG} > \text{MC} > \text{EGC} > \text{ECG} > \text{EC} > \text{MG} > \text{Vit C} \gg \text{MR} \quad (6)$$

in micellar solution at pH 7.0. The result indicates that the k_s values of catechins at pH 7.0 are 3.2−13 times larger than that of Vit C.

Analyses of the pH Dependence on the Reaction Rates (k_s) of Catechins, Related Compounds, and Vitamin C. *a. 4-Methylcatechol (MC), 5-Methoxyresorcinol (MR), and Methyl Gallate (MG).* MC is dibasic and can exist in three different molecular forms, that is, the undissociated form (MC-H_2),

monoanion (MC-H^-), and dianion (MC^{2-}), depending on the pH value (see **Figure 6A**). The equilibrium reactions of MC have the following form:

$$\text{MC-H}_2 \overset{K_{a1}}{\rightleftharpoons} \text{MC-H}^- \overset{K_{a2}}{\rightleftharpoons} \text{MC}^{2-} \qquad (7)$$

If we assume that k_{s1}, k_{s2}, and k_{s3} are the reaction rates for the MC-H_2, MC-H^-, and MC^{2-} forms of MC, respectively, the total rate k_s will be expressed as follows (*35*):

$$k_s = k_{s1} f(\text{MC-H}_2) + k_{s2} f(\text{MC-H}^-) + k_{s3} f(\text{MC}^{2-}) \qquad (8)$$

where f(MC-H_2), f(MC-H^-), and f(MC2-) are mole fractions for three different molecular forms of MC in micellar solution.

By comparing the observed pH dependence of k_s with the pH dependence of mole fraction (f), the values of k_{si} ($i = 1-3$) were determined: for instance, at pH 4 only the undissociated form of MC exists in solution, that is, f(MC-H_2) = 1, and we can immediately determine the k_{s1} value. At 9 < pH < 11, both the undissociated and monoanion forms exist in solution. Consequently, we can determine the k_{s2} value, using eq 8. The k_{s3} value is considered to be small compared to k_{s2} and negligible because the dianion form (MC^{2-}) of MC does not have any OH proton to reduce ArO• (*30, 33, 35*). By using these k_{s1}, k_{s2}, and k_{s3} values and eq 8, we simulated the experimental data (*35, 37*). As shown in **Figure 5**B, good accordance between the observed rate constants (k_s) and simulation curve was obtained, suggesting that each reaction rate (k_{si}) and pK_{ai} value (see **Table**s **4** and **5**) estimated is reasonable. The k_{s2} value is 29 times as large as the k_{s1} value. The result indicates that the reaction rate k_{si} increases by increasing the anionic character of MC, that is, the electron-donating capacity of MC (*30, 33, 35*). Similarly, the analysis of the pH dependence of k_s value was performed for MR. The k_{s2} value for the monoanion (MR-H^-) is 56 times as large as the k_{s1} value for the undissociated form (MR-H_2).

MG is tribasic and can exist in four different molecular forms in micellar solution, depending on the pH value (see **Figure 6C**). By comparing the observed pH dependence of k_s with the pH dependence of the mole fraction, the values of k_{si} and pK_{ai} were determined, where the k_{s4} value was assumed to be 0 M^{-1} s^{-1}. The reaction rates (k_{si}) increased remarkably with increasing the anionic character of MG. The results obtained show that both the monoanion (MG-H_2^-) and dianion (MG-H^{2-}) forms of the G-ring of MG mainly contribute to the scavenging of the free radical at pH 8−12. However, in a lower pH region (pH < 8), both the undissociated form (MG-H_3) and the monoanion (MG-H_2^-) contribute to the scavenging of free radicals.

b. Epicatechin (EC) and Epicatechin Gallate (ECG). EC is tetrabasic and can exist in five different molecular forms in micellar solution, depending on the pH value. As listed in **Tables 2** and **3**, the reaction rates (k_s) of MR (a model of A-ring in EC) are very small, and are about 2−3 orders of magnitude smaller than those of EC at all pH regions. The result suggests that the 5- and 7-OH groups at the A-ring of EC do not contribute to the scavenging of ArO•. The pH dependence of k_s in EC will be explained by considering only the dissociation of protons of 3′- and 4′-OH groups at the B-ring, as shown in **Figure 7A**. By comparing the observed pH dependence of the k_s value with the simulation curve, the values of k_{si} and pK_{ai} (see **Table**s **4** and **5**) were determined. As shown in **Figure 3A**, only the undissociated form (EC-H_2) of the B-ring contributes to the scavenging of the free radical at pH < 8.

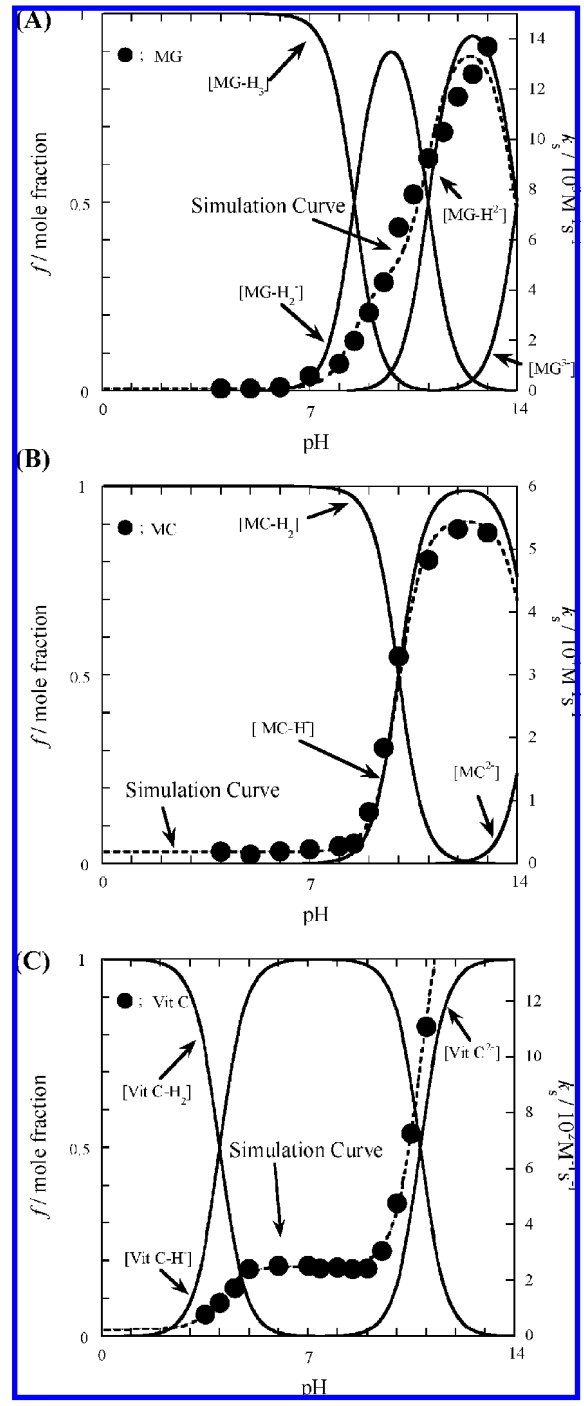

Figure 5. (**A**) Plots of second-order rate constant (k_s) for MG (●) vs pH and of the mole fraction (f) of four MG species (MG-H_3, MG-H_2^-, MG-H^{2-}, and MG^{3-}) vs pH (—). The dotted line is a simulation curve. (**B**) Plots of second-order rate constant (k_s) for MC (●) vs pH and of the mole fraction (f) of three MC species (MC-H_2, MC-H^-, and MC^{2-}) versus pH (—). The dotted line is a simulation curve. (**C**) Plots of second-order rate constant (k_s) for Vit C (●) vs pH and of the mole fraction (f) of three Vit C species (Vit C−H_2, Vit C−H^-, and Vit C^{2-}) vs pH (—). The dotted line is a simulation curve. All of the reactions were performed in 5.0 wt % Triton X-100 micellar solution at 25.0 °C.

However, in the higher pH region, both the EC-H_2 and EC-H^- forms contribute to scavenging.

ECG has seven OH groups in a molecule and can exist in eight different molecular forms in micellar solution, depending on the pH value. We assumed that the two OH groups in the

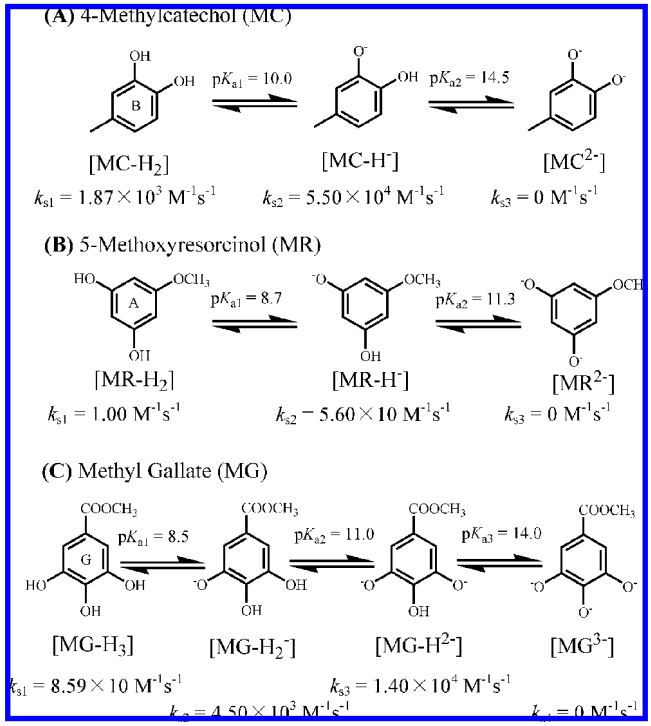

Figure 6. (**A**) Three different molecular forms of MC (MC-H_2, MC-H^-, and MC^{2-}), (**B**) three different molecular forms of MR (MR-H_2, MR-H^-, and MR^{2-}), and (**C**) four different molecular forms of MG (MG-H_3, MG-H_2^-, MG-H^{2-}, and MG^{3-}) in aqueous solution and their reaction rates, k_{si}.

Table 3. pH Dependence of the Second-Order Rate Constants (k_s) for the Reaction of MG, MC, MR, and Vitamin C with the ArO· Radical in 5.0 wt% Triton X-100 Micellar Solution

	$k_s/M^{-1}\ s^{-1}$			
pH	MG	MC	MR	VitaminC
3.5				7.88×10
4	8.55×10	1.87×10^3		1.18×10^2
4.5				1.72×10^2
5	8.69×10	1.41×10^3		2.41×10^2
6	1.53×10^2	1.95×10^3	1.00	2.51×10^2
7	5.99×10^2	2.22×10^3	2.17	2.51×10^2
7.5				2.44×10^2
8	1.08×10^3	2.78×10^3	8.00	2.46×10^2
8.5	1.98×10^3	3.20×10^3		2.40×10^2
9	3.11×10^3	8.19×10^3	3.93×10	2.43×10^2
9.5	4.32×10^3	1.84×10^4		3.06×10^2
10	6.50×10^3	3.29×10^4	5.05×10	4.77×10^2
10.5	7.82×10^3			7.25×10^2
11	9.25×10^3	4.83×10^4	3.61×10	1.11×10^3
11.5	1.03×10^4			
12	1.17×10^4	5.32×10^4	2.58×10	
12.5	1.26×10^4			
13	1.37×10^4	5.26×10^4		

A-ring do not contribute to aroxyl radical scavenging and that the OH groups of the B- and G-rings in ECG have pK_{ai} values similar to those in EC and MG, respectively, as shown in **Figure 7B**. As listed in **Tables 2** and **3**, the k_s value of MG is smaller than that of EC at pH 4−7. However, the rate increases rapidly by increasing pH and shows a value larger than that of EC at pH > 7. The result suggests that the OH groups at the G-ring of MG contribute to the scavenging of ArO· radical. The values of k_{si} ($i = 1-5$) determined are listed in **Table 4**. As shown in **Figure 3B**, good accordance between the observed k_s values and simulation curve was obtained.

Table 4. Reaction Rates (k_{si}, $i = 1 \sim 5$) for Undissociated, Monoanion, Dianion, Trianion, and Tetraanion Forms of Catechins (EC, ECG, EGC, and EGCG) and Related Compounds (MG, MC, and MR), and Vitamin C in 5.0 wt% Triton X-100 Micellar Solution

	$k_{si}/M^{-1}\ s^{-1}$				
antioxidant	k_{s1}	k_{s2}	k_{s3}	k_{s4}	k_{s5}
EC	7.40×10^2	9.00×10^3	5.10×10		
ECG	7.80×10^2	3.00×10^3	1.00×10^4	2.50×10^3	9.00×10^3
EGC	1.65×10^3	1.40×10^4	$(1.80 \times 10^4)^a$	0	
EGCG	2.70×10^3	4.50×10^4	$(7.00 \times 10^4)^a$	$(1.00 \times 10^4)^a$	$(3.50 \times 10^4)^a$
MG	8.59×10	4.50×10^3	1.40×10^4	0	
MC	1.87×10^3	5.50×10^4	0		
MR	1.00	5.60×10	0		
vitamin C	2.50×10	2.50×10^2	1.70×10^3		

[a] The values were tentatively determined.

c. Epigallocatechin (EGC) and Epigallocatechin Gallate (EGCG). As the pK_{ai} values of EGC have not been reported, we tried to determine the pK_{ai} values, by comparing the pH dependence of the k_s value with the simulation curve. If EGC takes only the undissociated form (EGC-H_3, see **Figure 7C**) at pH 4.0, the k_s value at pH 4.0 corresponds to the k_{s1} value for EGC-H_3. By comparing the increase of k_s at pH 7−8.5 with the simulation curve, we can determine the pK_{a1} and k_{s2} values. The k_{s4} value due to the trianion form (EGC^{3-}) is considered to be small compared to k_{s3} and negligible ($k_{s4} = 0$). By comparing the decrease of the k_s value at pH 11−13 with the simulation curve, the pK_{a3} and k_{s3} values were determined. By varying the pK_{a2}, we tried to simulate the change of the k_s value at pH 8.5−11. The pH dependence of the k_s value shows two peaks at pH 9.0 and 11.0. However, two peaks were not found in the simulation curve, by varying the pK_{a2} value, as shown in **Figure 3C**. The reason is not clear at present. We could only determine the k_{s1}, k_{s2}, k_{s4}, pK_{a1}, and pK_{a3} values for EGC.

A similar analysis was performed for EGCG, by taking the pK_{ai} ($i = 1-3$) values obtained for MG into account. The k_{s1}, k_{s2}, and pK_{a1} values obtained will be correct, but the others are considered to be tentative. All of the k_{si} and pK_{ai} values obtained are listed in **Tables 4** and **5**.

d. Vitamin C. pH dependence of the k_s value of Vit C has also been analyzed in a similar manner. The reaction rates k_{s1}, k_{s2}, and k_{s3} for three different molecular forms (Vit C-H_2, Vit C-H^-, and Vit C^{2-}) of Vit C were determined, by varying the k_{si} ($i = 1-3$) values and pK_{a1} and pK_{a2} values. As listed in **Table 5**, the values of pK_{a1} (4.0) and pK_{a2} (10.8) obtained for Vit C are similar to those reported (4.17 and 11.57) (*37, 38*). The k_{s2} value is 10 times as large as the k_{s1} one. Furthermore, the k_{s3} value is 6.8 times as large as the k_{s2} value, although the dianion form (Vit C^{2-}) has no OH proton at the C_2 and C_3 positions to reduce the ArO· radical. We can expect electron transfer between the ArO· radical and Vit C^{2-}, as recently reported for the reaction of ArO· with caffeic acid, chlorogenic acid, and Trolox (*39, 40*).

DISCUSSION

Structure−Activity Relationship in the Aroxyl-Scavenging Reaction by Catechins in Ethanol and Micellar Solutions. *a. In Ethanol Solution.* The rate of the scavenging reaction of ArO· with catechins and related compounds decreases in the order indicated in eq 5 in ethanol. The reactivity of MR ($k_s \sim 10^{-2}\ M^{-1}\ s^{-1}$) in ethanol is very small and almost negligible. However, MC shows higher reactivities: $k_s = 2.67 \times 10^2\ M^{-1}\ s^{-1}$. The result indicates that the catechol structure in the B-ring is the obvious radical target site for EC. The rate constants (k_s)

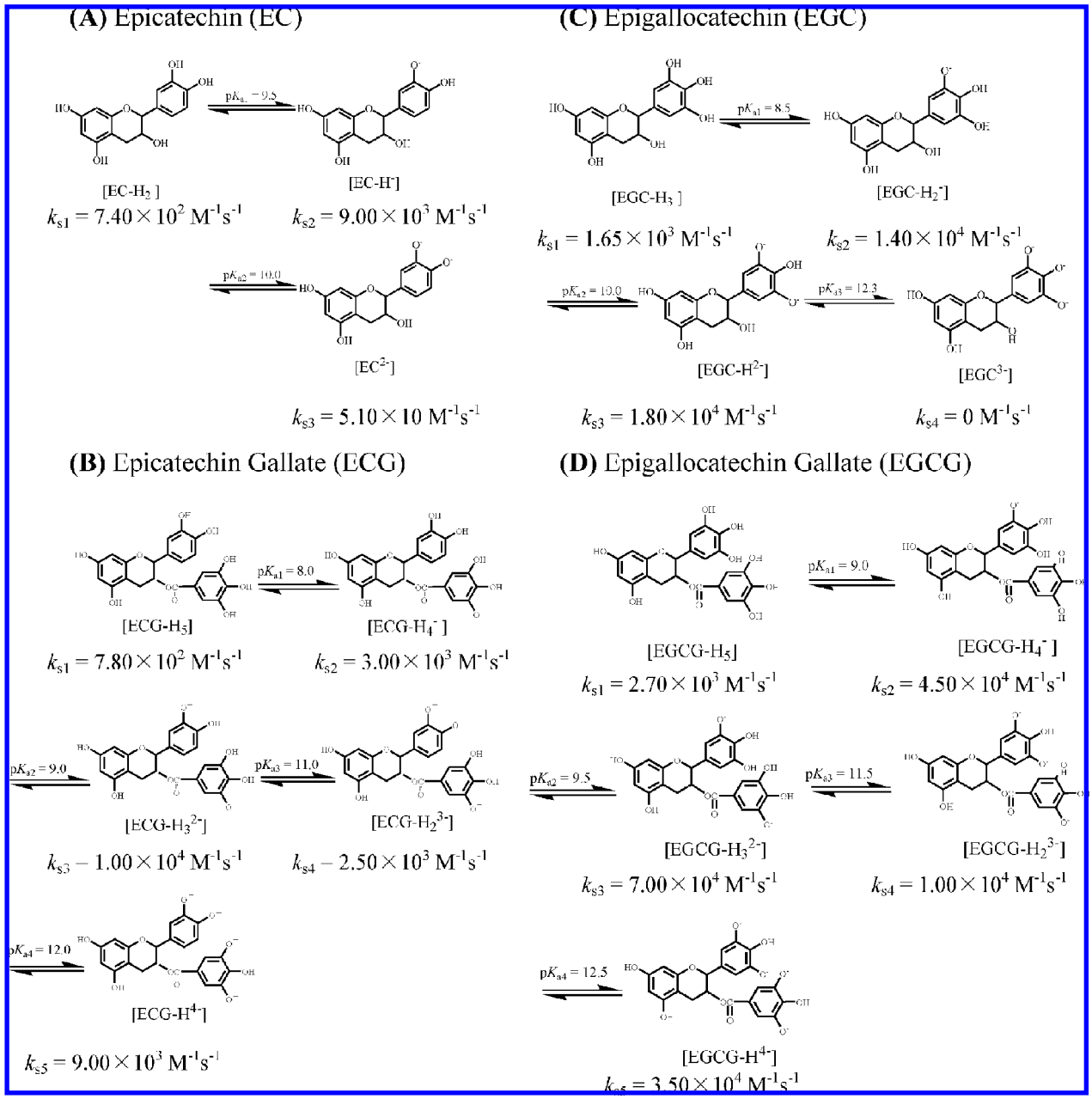

Figure 7. (**A**) Three different molecular forms of EC (EC-H_2, EC-H^-, and EC^{2-}), (**B**) five different molecular forms of ECG (ECG-H_5, ECG-H_4^-, ECG-H_3^{2-}, ECG-H_2^{3-}, and ECG-H^{4-}), (**C**) four different molecular forms of EGC (EGC-H_3, EGC-H_2^-, EGC-H^{2-}, and EGC^{3-}), and (**D**) five different molecular forms of EGCG (EGCG-H_5, EGCG-H_4^-, EGCG-H_3^{2-}, EGCG-H_2^{3-}, and EGCG-H^{4-}) in aqueous solution and their reaction rates, k_{si}.

of EGC and EGCG are 4.2 and 3.1 times larger than those of EC and ECG in ethanol, respectively, showing that the free radical-scavenging activity of the pyrogallol B-ring is higher than that of the catechol B-ring. The k_s value of MG is $1.56 \times 10\ M^{-1}\ s^{-1}$ in ethanol. The value is 3 orders of magnitude larger than that of MR, and corresponds to about 18 and 5.5% of EC and MC, respectively. In conclusion, the reactivity of the OH groups in each ring of catechins decreases in the following order in homogeneous ethanol solution.

Pyrogallol B-ring > catechol B-ring >
gallate G-ring ≫ resorcinol A-ring (9)

In ECG (or EGCG), the EC (or EGC) and MG moieties are not π-conjugated to each other. Therefore, we can expect that the ArO•-scavenging rate of ECG (or EGCG) may be explained by the simple sum of those of EC (or EGC) and MG, as a first approximation. In fact, the k_s value of ECG ($1.07 \times 10^2\ M^{-1}\ s^{-1}$) shows good accordance with the sum ($1.02 \times 10^2\ M^{-1}\ s^{-1}$) of EC ($8.61 \times 10\ M^{-1}\ s^{-1}$) and MG ($1.56 \times 10\ M^{-1}\ s^{-1}$) in ethanol. However, the k_s value of EGCG ($4.69 \times 10^2\ M^{-1}\ s^{-1}$) cannot be explained by the simple sum ($3.93 \times 10^2\ M^{-1}\ s^{-1}$) of EGC ($3.77 \times 10^2\ M^{-1}\ s^{-1}$) and MG ($1.56 \times 10\ M^{-1}\ s^{-1}$). The value of the former is 1.2 times as large as the latter.

For instance, if the B- and G-ring moieties in ECG (or EGCG) approach each other by van der Waals interaction, an electronic π-π interaction between B- and G-rings develops. As a result, the energy level of the charge-transfer intermediate between ECG (or EGCG) and ArO• at the transition state is lowered, and the rate constant (k_s) may increase (*31, 32, 41*). Ab initio MO calculations were performed for ECG by Okajima (*42*). The PM3 optimized structure of ECG showed that the B- and G-rings do not overlap to each other by π-π interaction. Density

Table 5. pK_{ai} Values Obtained for the Reaction of Catechins (EC, ECG, EGC, and EGCG), Related Compounds (MG, MC, and MR), and Vitamin C with the ArO· Radical in Micellar Solution[a]

antioxidant	pK_{a1}[b]	pK_{a2}[b]	pK_{a3}[b]	pK_{a4}[b]
EC	9.5 (B)	10.0 (B)		
ECG	8.0 (G)	9.0 (B)	11.0 (B)	12.0 (G)
EGC	8.5 (B)	(10.0)[c] (B)	12.3 (B)	
EGCG	9.0 (B)	(9.5)[c] (G)	(11.5)[c] (B)	(12.5)[c] (G)
MG	8.5 (G)	11.0 (G)	14.0 (G)	
MC	10.0 (B)	14.5 (B)		
MR	8.7 (A)	11.3 (A)		
vitamin C	4.0	10.8		

[a] A, B, and G in parentheses mean A-, B-, and G-rings in catechins, respectively. [b] Experimental errors in pK_{ai} values are estimated to be about ± 0.3. [c] The values were tentatively determined.

functional theory (DFT) calculations were performed by Leopoldini et al. (*43*) to evaluate the antioxidant activity of 10 types of phenolic antioxidants including EC. The bond dissociation enthalpy (BDE) for the O−H bonds and the ionization potentials (IP) were calculated in the gas phase and in two solvents with different polarity (water and benzene). Conformation of EC changed remarkably depending on the environment in which the EC molecule was placed. Conformation of the ECG and EGCG will also change depending on the environment. However, the structures of ECG and EGCG in ethanol solution are not clear at present. The crystal structures of ECG and EGCG are not reported, either.

b. In Micellar Solution. The k_s values obtained for catechins and related compounds decrease in the order in eq 6 at pH 4−7 in micellar solution. At pH 4−6, these antioxidants will take only the undissociated form similar to that in ethanol solution (see **Figures 3**, **4**, and **5**). In fact, the order of the reaction rates of these antioxidants in micellar solution is the same as that in ethanol, except for the case of MC (see eq 5). The result indicates that, at pH 4−6, the reactivity of the OH groups in the A-, B-, and G-rings of catechins in aqueous micellar solution also decreases in the order in eq 9. Furthermore, the ratios in the rate constants between 4 kinds of catechins are less than ca. 5.4 and 4.3 in ethanol and micellar solutions, respectively.

In a higher pH region, the ArO· radical reacts with phenolate anions rather than with undissociated phenols. Each molecular form shows different ArO· radical-scavenging activity (k_{si}), as listed in **Table 4**. The k_s values obtained in micellar solution decrease in the order of

$$\text{EGCG} > \text{EGC} > \text{ECG} > \text{MG} > \text{EC} \gg \text{MR} \qquad (10)$$

at pH 8−10, as listed in **Tables 2** and **3** and shown in **Figure 4**. The k_s values of MG are larger than those of EC. The order of MC changes depends on pH.

The pH dependences of (i) the k_s value of ECG and (ii) the sum of those of EC and MG are shown in **Figure 8A**. The rate constants of the former can be well explained by the sum of the latter at pH 4−9.5, as observed in ethanol solution. At pH 10−13, the rate constants of the former are smaller than the sum of the latter. However, at all pH regions, the rate constants of EGCG are larger than the sum of those of EGC and MG, as observed in ethanol solution, suggesting the overlap of B- and G-rings in EGCG (see **Figure 8B**). Molecular structures of ECG and EGCG, that is, the overlap of B- and G-rings will vary depending on pH because of the dissociation of various phenolic hydroxyl protons in catechins. The dissociation induces the change in intramolecular electronic π-π interaction between B- and G-rings and thus the change in the reaction rates. The

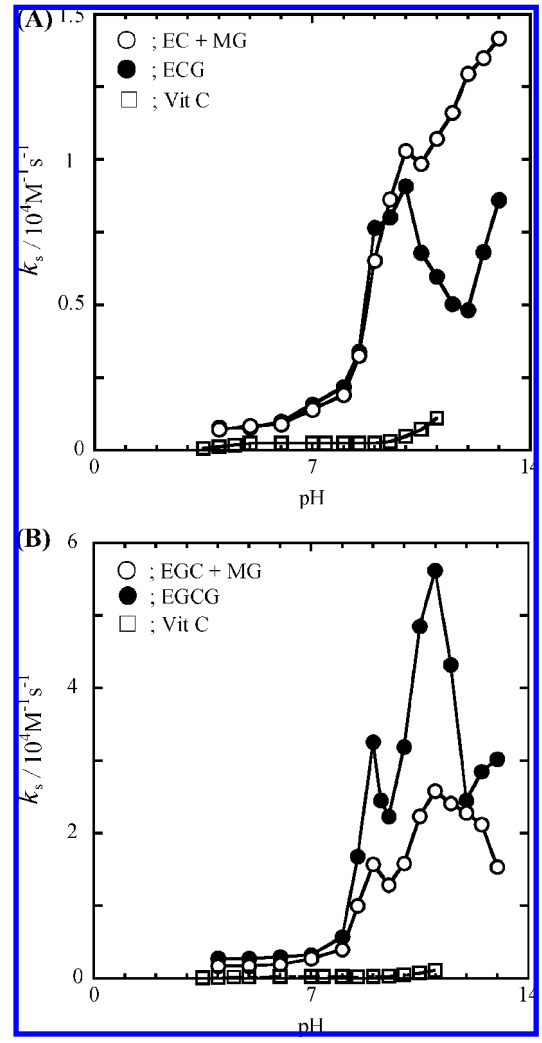

Figure 8. (**A**) pH dependence of the rate constant (k_s) for ECG (●), the sum of those for EC and MG (○), and that for Vit C (□). (**B**) pH dependence of the rate constant (k_s) for EGCG (●), the sum of those for EGC and MG (○), and that for Vit C (□).

reaction rates of catechins in micellar solution depend on many factors compared to those in homogeneous solutions. The pH dependence of the rate constants (k_s) reflects a complex mechanism.

The protective effects of the catechin family of flavonoids against the oxidation of lipids and low-density lipoproteins (LDL) have been studied by several investigators (*8–11*). The results show that the presence of the ortho-trihydroxy group in the B- and G-rings is most important for the antioxidant activities of catechins (*1, 8–11*). A similar tendency in the scavenging effects of catechins on the DPPH radical was previously observed; the scavenging ability decreased in the order of EGCG ∼ ECG > EGC > EC ∼ CA (*44*). Scavenging of nitric oxide and superoxide by green tea has been studied (*3*). EGCG and ECG having a gallate ring showed higher scavenging activity of NO radical than EGC, EC, and CA. The oxidation products of EGCG and EGC were isolated and identified by Valcic et al. (*45, 46*). In all identified products, changes occurred solely in the B-ring of EGCG or EGC, showing that the principal site of antioxidant reactions in EGCG and EGC is the trihydroxyphenyl B-ring, regardless of the presence of a 3-galloyl moiety. As described above, the rate constants (k_s) observed for catechins decrease in the order of

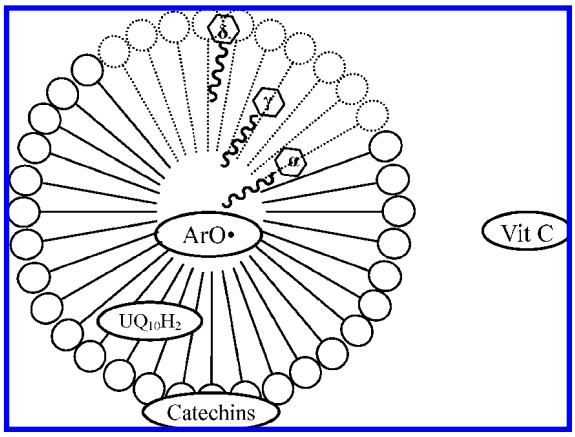

Figure 9. Polarities of the reaction field where α-, β-, γ-, δ-tocopherols, ubiquinol-10, catechins, and vitamin C react with the ArO· radical in the micelle are different from one another.

eq 5 in ethanol and eq 6 at pH 7.0 in micelle solution. The results indicate that the existence of the pyrogallol B-ring is most important for free radical scavenging in catechins.

Effect of the Reaction Field on the Aroxyl Radical-Scavenging Rates (k_s) by Catechins, Vitamin E, and Ubiquinol-10 in Micellar Solution. As described in the Introduction, the relative rates ($100 \times k_s$(TocH)/k_s(α-TocH)) of the k_s values for the reaction with ArO· radical (α:β:γ:δ = 100:44:47:20) in ethanol (*33*) are similar to the relative rates ($100 \times k_{inh}$(TocH)/k_{inh}(α-TocH)) of the k_{inh} values for the reaction with the LOO· radical (100:41:44:14) in chlorobenzene (*34*). However, the relative rates of the k_s values (α:β:γ:δ:tocol = 100:44:47:20:11) in ethanol are very different from that (100:21:20:2.9:0.69) in micellar solution (**Table 1**) (*33*).

The solvent effect on the reaction rates (k_s) of α-tocopherol with ArO· radical has been studied in a previous work (*47*). The k_s values are 5.12×10^3 (ethanol), 1.44×10^4 (diethyl ether), 9.52×10^4 (benzene), and 1.94×10^5 M^{-1} s^{-1} (*n*-hexane) for α-tocopherol. The k_s value of α-tocopherol in *n*-hexane is 38 times as large as that in ethanol. The result indicates that the aroxyl radical-scavenging rates of the tocopherols change notably, depending on the polarity of solvents, that is, the reaction field that the tocopherols react with free radical (ArO·).

As listed in **Table 1**, the ratios of reaction rates, k_{s1}(micelle)/k_s(ethanol), of α-, β-, γ-, δ-tocopherols, tocol, and ubiquinol-10 in ethanol and micelle solutions are 100, 47, 41, 15, 6.3, and 26, respectively. These antioxidants are lipophillic and thus will be localized inside the micelle, as shown in **Figure 9**. Consequently, the local concentration of antioxidants in 5.0 wt% Triton X-100 micelle will become about 20 times as large as that in homogeneous ethanol solution, if we assume that the density of the part of the micelle is 1 g/mL, and the volume that Triton X-100 molecules (5.0 wt%) occupy in micellar solution is 5.0% of the total volume. The ArO· radical molecule is also lipid soluble and will react with these antioxidants inside the micelle. Therefore, if the polarity of the reaction field is similar to that of ethanol, the k_s values of the antioxidants observed in the micelle will become ca. 20 times as large as those in ethanol. In fact, the ratios of the reaction rates (k_{s1}(micelle)/k_s(ethanol) = 15 and 26) obtained for δ-tocopherol and ubiquinol-10, respectively, are similar to that (20) expected. The ratios for α-, β-, γ-tocopherols (100, 47, and 41, respectively) are larger than that (20) expected, suggesting that these antioxidants react with ArO· at a less polar reaction field than that of ethanol. However, the ratio (6.3) for the tocol molecule is smaller than 20, suggesting that the tocol molecule reacts at a more polar reaction field. The reaction rates of α-tocopherol are 9.1 and 145 times larger than those of tocol in ethanol and micellar solutions, respectively. As listed in **Table 1**, the ratios of the reaction rates (k_{s1}(micelle)/k_s(ethanol)) obtained for catechins and related compounds are similar to each other and are 4.4−8.6. The ratios are smaller than those for α-, β-, γ-, δ-tocopherols, and ubiquinol-10 (100, 47, 41, 15, and 26, respectively), suggesting that catechins react with ArO· at a more polar reaction field than that of ethanol, that is, at the position closer to the micelle surface, as shown in **Figure 9**. The order of the ratios of the reaction rates (k_{s1}(micelle)/k_s(ethanol)) will correspond to the order of the polarity of the reaction field for the antioxidants.

Table 6. pK_{ai} Values Reported for the Reaction of Catechins (EC, ECG, EGC, and EGCG), Related Compounds (MG, MC, and MR), and Vitamin C with the 5,7-di*i*Pr-Toc· Radical in Micellar Solution[a]

antioxidant	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}
EC	8.64[b] (B)	11.26[b] (B)	9.41[b] (A)	13.4[b] (A)
ECG	8.03 (G)	8.64 (B)	11.26 (B)	11.6 (G)
EGC	no report			
EGCG	7.75[b]	8.0[b]		
MG	8.03[b] (G)	11.6[b] (G)		
MC	9.56[c] (B)	14.00[c] (B)		
MR	9.30[b] (A)	11.3[b] (A)		
vitaminC	4.17[d]	11.57[d]		

[a] A, B, and G in parentheses mean A-, B-, and G-Rings in catechins, respectively. [b] The value reported by Jovanovich et al. (*23*). [c] The value reported by Slabbert (*50*). [d] The value reported by Windholz et al. (*38*).

The rate constants (k_r) for the reaction of catechins with 5,7-di*i*Pr-Toc· radical (eq 11) were reported in a previous work (see **Table 1** in ref *35*). The k_r values for Toc· in ethanol are larger than the corresponding values for ArO·, indicating that the reactivities of Toc· with catechins are intrinsically higher than those (k_s) of ArO·. However, k_r values for Toc· in micelle (at pH 4.0) are smaller than the corresponding ones (k_s) for ArO· in the micelle. The polarity of the reaction field where the phenol group of the α-tocopherol exists in liposome has been reported to be similar to that of ethanol (*48, 49*). The reaction field of the phenoxyl ring of the 5,7-di*i*Pr-Toc· radical molecule with the long phytyl chain will also be the surface of the micelle and will be more polar than that of the ArO· radical molecule. In fact, the ratios of the reaction rates (k_r(micelle)/k_r(ethanol)) obtained for the reaction of the Toc· radical with catechins at pH 4.0 are 3.23, 0.921, 0.557, and 0.438 for EC, ECG, EGC, and EGCG, respectively, and are much smaller than those (4.4−8.6) for ArO·. Catechins are water soluble, and thus, the reaction of catechins with Toc· will occur at the surface of the micelle. In such a case, the k_r values for catechins in micelle will be smaller than those in ethanol.

$$\text{Toc}\cdot + \text{Catechin} \xrightarrow{k_r} \text{TocH} + \text{Catechin}\cdot \qquad (11)$$

Determination of the pK_{ai} Values of Catechins and Related Compounds in Micellar Solution. In a previous work (*35*), the simulations of the pH dependence of the k_r values for the reaction of 5,7-di*i*Pr-Toc· radical with EC, MG, MC, and MR (eq 11) were performed, using the pK_{ai} values reported by Jovanovich et al. (*23*) and Slabbert (*50*) (see **Table 6**). A good accordance between the observed and simulation curves was obtained, indicating that the pK_{ai} values used are valid. The simulation for ECG was performed using the pK_{ai} values

reported for EC and MG. However, if we use the pK_{ai} values listed in **Table 6**, the accordance between the observed and simulation curves was not obtained for the reaction with the ArO• radical. As listed in **Table 5**, the use of about 0.5−1.0 larger pK_{a1} values was necessary for the simulation. The pK_{a2} (and pK_{a3} and pK_{a4}) values are also different from those reported, although the values are not necessarily larger than those reported. As described in a previous section, the ArO• radical molecules will react with catechins at a less polar reaction field than Toc•. The difference in the pK_{ai} values observed for catechins will be due to the difference in the reaction fields between ArO• and Toc• radicals.

In the present work, pH dependence of the k_s value was studied at a more broad pH region (4 < pH < 13) than that of the k_r (4 < pH < 12). As the k_s values of catechins show rapid increase or decrease at the pH 12−13 region (see **Figure 3**), we have succeeded in analyzing the pH dependence of k_s in more detail. Furthermore, the pK_{ai} and k_{si} values of EGC and EGCG have tentatively been estimated for the first time in the present work (see **Table**s **4** and **5**). The determination of the pK_{ai} values for catechins is important because the molecular forms of catechins in biological systems depend on the pK_{ai} values and relate to the antioxidant activity of catechins, as described above.

As listed in **Table 1**, the k_s values of catechins at pH 7.0 are larger than the corresponding values at pH 4.0 (or the k_{s1} values). However, the ratios of the k_s values (k_s (pH = 7.0))/k_{s1} (pH = 4.0)) for catechins (EC, ECG, EGC, and EGCG) are 1.1, 2.0, 1.3, and 1.2, respectively, indicating that the catechins take almost an undissociated form at pH 7.0. However, the ratios of the k_r values (k_r (pH 7.0))/k_r (pH 4.0)) for catechins (EC, ECG, EGC, and EGCG) are 2.6, 3.5, 4.0, and 5.9, respectively (*35*). The differences in the k_r values at pH 4.0 and 7.0 are notable, indicating that the catechins take not only the undissociated form but also the monoanion form with higher reactivity at pH 7.0. The result also indicates that the pK_{a1} values of catechins for the reaction with ArO• in micelle solution are larger than those used in a previous work (see **Table 6**).

Comparison between the Rates of Aroxyl Radical-Scavenging Reaction with Catechins and Natural Antioxidants in Solution. α-Tocopherol (and ubiquinol-10) and Vit C are well known as representative lipid- and water-soluble antioxidants, respectively. As listed in **Table 1**, the rate of the scavenging reaction of ArO• with catechins and these antioxidants decreases in the order of

$$\alpha\text{-tocopherol} > \text{ubiquinol-10} \gg \text{EGCG} > \text{EGC} > \text{ECG} > \text{EC} > \text{Vit C} \quad (12)$$

at pH 7 in micellar solution. The order of the k_s values of these antioxidants in micellar solution is similar to that in ethanol.

The rate constants (k_s) of Vit C showed notable pH dependence. As listed in **Tables 2** and **3** and shown in **Figure 8**, the k_s values of catechins (EC, ECG, EGC, and EGCG) are faster than the corresponding values of Vit C at pH 4−11 in micellar solution. For instance, at pH 7.0, the k_s values of EC, ECG, EGC, and EGCG are 3.2, 6.3, 8.5, and 13 times larger than that of Vit C, respectively. Furthermore, the k_s value of EGCG (3.20×10^3 M^{-1} s^{-1}) is 8.7−19 times larger than those of rutin (2.28×10^2 M^{-1} s^{-1}) and caffeic acids (chlorogenic acid (1.67×10^2), ferulic acid (2.65×10^2), and caffeic acid (3.68×10^2)) at pH 7.0 (*30, 39*). Catechins have high free radical-scavenging activity in micellar solutions. However, the k_s value of EGCG is 160 and 39 times smaller than those of α-tocopherol (5.12×10^5 M^{-1} s^{-1}) and ubiquinol-10 (1.25×10^5 M^{-1} s^{-1}) at pH 7.0, respectively. The reaction field of α-tocopherol (and ubiquinol-10) with ArO• will be inside the micelle (see **Figure 9**). However, catechins, rutin, and caffeic acids will react at the surface of the micelle, showing lower reaction rates.

In addition to the direct scavenging of the LOO• radical, catechins may contribute to the regeneration reaction from α-tocopheroxyl radical to α-tocopherol to protect lipid peroxidation in biological systems (*27*). As reported in a previous work (*35*), the rate constants (k_r) for the reaction of catechins (EC, ECG, EGC, EGCG, and MG) with 5,7-di*i*Pr-Toc• at pH 7.0 in Triton X-100 (5.0 wt%) micellar solution are 1.27×10^3, 1.68×10^3, 2.42×10^3, 3.64×10^3, and 1.36×10^3 M^{-1} s^{-1}, respectively. These values are similar to that of Vit C ($k_r = 2.49 \times 10^3$ M^{-1} s^{-1}) at pH 7.0. The reaction of α-tocopheroxyl with Vit C is well known as the usual tocopherol-regeneration reaction in biomembrane systems.

In fact, the EC and ECG in liposome are reported to act synergistically with α-tocopherol to inhibit lipid peroxidation (*19*). Furthermore, Liu et al. reported that catechins exhibit a synergistic antioxidant effect with α-tocopherol in homogeneous solutions (*16*), in micelles (*17, 51*), and in human LDL (*52, 53*). Recently, the rate constants (k_r) for the reaction of α-tocopheroxyl radical with catechins at pH 7.4 in the sodium dodecyl sulfate (SDS) micelle have been measured by using stopped-flow ESR method (*51*); the rate constants (k_r) reported are 0.45, 1.31, 1.11, 1.91, and 0.43×10^2 M^{-1} s^{-1} for EC, ECG, EGC, EGCG, and GA (gallic acid), respectively. The result of the present work also suggests that catechins contribute to the regeneration reaction of the α-tocopheroxyl radical rather than the direct scavenging of peroxide radical in biological systems, if α-tocopherol and catechins coexist in tissues.

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