Kinetic Study of the Quenching Reaction of Singlet Oxygen by Flavonoids in Ethanol Solution

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The quenching rate of singlet oxygen ($^1O_2$) by seven kinds of flavonoids (flavone, flavonol, chrysin, apigenin, rutin, quercetin, and myricetin) with 2,3-double bonds has been measured spectrophotometrically in ethanol at 35 °C. The overall rate constants k_Q ($=k_q + k_r$, physical quenching + chemical reaction) increased as the number of OH groups substituted to the flavone skeleton (that is, the total electron-donating capacity of flavonoids) increases. The existence of catechol or pyrogallol structure in the B-ring is essential for the $^1O_2$ quenching of flavonoids. Log k_Q was found to correlate with their peak oxidation potentials, E_P; the flavonoids that have smaller E_P values show higher reactivities. Similarly, log k_Q values of flavonoids correlate with the energy level of the highest occupied molecular orbital (E_{HOMO}), calculated by the PM3 MO method, and the longest wavelength $\pi\pi^*$ excitation energy (E_{ex}). The contribution of the chemical reaction (k_r) was found to be negligible in these flavonoids. The k_Q values of rutin, quercetin, and myricetin [$(1.21\sim5.12) \times 10^8$ M^{-1} s^{-1}] were found to be larger than those of lipids [$(0.9\sim6.4) \times 10^4$ M^{-1} s^{-1}], amino acids ($<3.7 \times 10^7$ M^{-1} s^{-1}), and DNA (5.1×10^5 M^{-1} s^{-1}). The result suggests that these flavonoids may contribute to the protection of oxidative damage in foods and plants, by quenching $^1O_2$.

1. Introduction

Flavonoids are natural polyphenolic compounds widely distributed in foods and plants. Flavonoids display pronounced biological activities, protecting against coronary heart disease (CHD),[1,2] cancer,[3−5] inflammation,[5] etc. The biological activities of flavonoids have been related to their properties as antioxidants, and many studies have been performed on the inhibition of lipid peroxidation in biological systems or model media [microsomes, mitochondria, liposomes, low-density lipoprotein (LDL), etc.].[6−10] A notable feature of flavonoids is their high reactivity toward active free radical species. Several kinetic studies have been performed on the reaction of flavonoids with active free radicals (N_3•, HO•, O_2^{-}•, *t*-BuO•, and LOO•) by use of pulse radiolysis techniques[11−14] and high-performance liquid chromatography (HPLC).[15,16]

In a previous work, to clarify the structure−activity relationship in the scavenging reaction of free radical by flavonoids, we have measured the second-order rate constants (k_S and k_R) for the reaction of six kinds of flavonoids (flavone, chrysin, flavonol, apigenin, rutin, and quercetin) with 2,6-di-*tert*-butyl-4-(4-methoxyphenyl)phenoxyl (ArO•, abbreviated to aroxyl) and 5,7-diisopropyltocopheroxyl (Toc•) radicals in ethanol, 2-propanol/water (5:1 v/v), and aqueous Triton X-100 micellar solutions (5.0 wt %) (reactions 1 and 2):[17]

$$\text{ArO}\bullet + \text{flavonoid} \xrightarrow{k_S} \text{ArOH} + \text{flavonoid}\bullet \quad (1)$$

$$\text{Toc}\bullet + \text{flavonoid} \xrightarrow{k_R} \text{TocH} + \text{flavonoid}\bullet \quad (2)$$

The rate constants (k_S and k_R) obtained in micellar solution showed notable pH dependence. The k_S and k_R values of flavonoids increase in the order

$$\text{flavone} < \text{chrysin} < \text{flavonol} < \text{apigenin} < \text{rutin} < \text{quercetin} \quad (3)$$

independent of both pH value and solvent system. Rutin and quercetin, with 3′- and 4′-OH groups at the B-ring, showed high reactivity, indicating that the catechol structure in the B-ring is the obvious radical target site for flavonoids.[11,12] It was found that quercetin and rutin have high activity in vitamin E regeneration.[17]

Illumination with excess photosynthetically active radiation (PAR) is a stress factor for plants, known as photoinhibition. It has been found that photoinhibition of photosynthesis in broad bean leaves is accompanied by singlet oxygen ($^1O_2$) production.[18] Recently, it has been reported that $^1O_2$ is generated in the UVA-irradiated skin of live mice.[19] Flavonoids are widely present in foods and plants in high concentration and may function as quenchers of $^1O_2$ in biological systems. Consequently, measurements of the quenching rate of $^1O_2$ with flavonoids are very important. However, the examples are very limited, as described below.

The quenching rates k_Q ($= k_q + k_r$, physical quenching + chemical reaction) of singlet oxygen with catechins [(+)-catechin (CA), epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), and epigallocatechingallate (EGCG)] in CH_3CN have been reported by Jovanovic et al.,[14] by a laser flash photolysis method:

$$^1O_2 + \text{flavonoid} \xrightarrow{k_Q} \text{physical quenching } (k_q) + \text{chemical reaction } (k_r) \quad (4)$$

where the former results in energy transfer and de-excitation of the singlet state but no chemical change in the energy acceptor. The latter results in modification of the target. The

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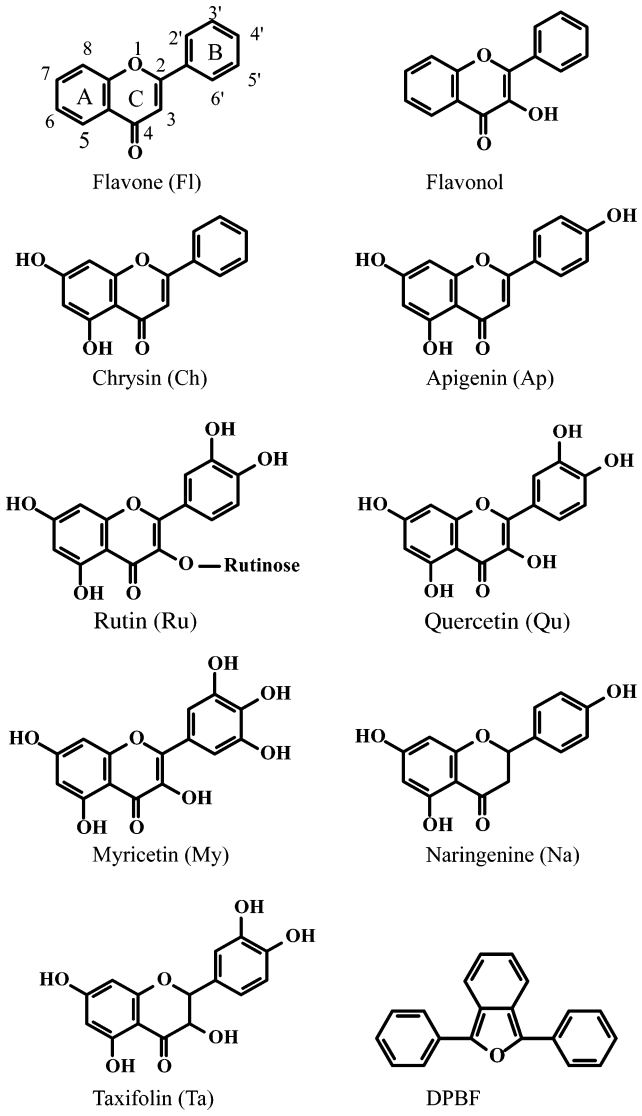


Figure 1. Molecular structures of flavone (Fl), flavonol, chrysin (Ch), apigenin (Ap), rutin (Ru), quercetin (Qu), myricetin (My), naringenin (Na), taxifolin (Ta), and DPBF and numbering system.

notable differences in the rate constants (k_Q) have not been observed for catechins; the values obtained are 1.1×10^8, 9.6×10^7, 1.1×10^8, 2.2×10^8, and 2.2×10^8 M^{-1} s^{-1} for CA, EC, EGC, ECG, and EGCG, respectively. The difference in the rate constants is less than 3-fold. A kinetic study of the quenching reaction of $^1O_2$ by 13 kinds of flavonoids (from the flavonol, flavone, flavanone, and flavane families) in CH_3OH has been performed by Tournaire et al.,[20] using a near-IR $^1O_2$ luminescence method. They reported that the efficiency of the physical quenching (k_q) is mainly controlled by the presence of a catechol moiety on ring B, whereas the structure of ring C (particularly the presence of a hydroxyl group activating the 2,3-double bond) is the main factor determining the efficiency of the chemical reactivity (k_r) of flavonoids with $^1O_2$. The total reactivity scale (k_Q) is dominated by k_q, which is in general higher than k_r. (+)-Catechin showed the highest overall quenching rate ($k_Q = k_q + k_r = 5.8 \times 10^6$ M^{-1} s^{-1} in CH_3OH) among the above flavonoids. However, the k_Q value obtained for (+)-catechin is about 20 times smaller than that (1.1×10^8 M^{-1} s^{-1}) reported by Jovanovic et al.[14]

In the present work, the quenching rates k_Q of $^1O_2$ by seven kinds of flavone derivatives (flavone, flavonol, chrysin, apigenin, rutin, quercetin, and myricetin; see Figure 1), having different numbers ($n = 0 \sim 6$) of OH substituents on a flavone skeleton, have been measured in ethanol at 35 °C, by a competition reaction method[21−23] (see Schemes 1 and 2). The rate constants of two structurally related flavonoids (naringenin and taxifolin) without a 2,3-double bond were measured to investigate the effect of π-conjugation between A- (resorcinol) and B- (phenol, catechol and pyrogallol) rings on the reaction rate. The chemical reaction (k_r) of flavonoids with $^1O_2$ has been studied spectrophotometrically, by reacting flavonoids with $^1O_2$. PM3 molecular orbital (MO) calculations and the measurements of UV−vis absorption spectra were performed for these flavonoids. From the results, the structure−activity relationship in the $^1O_2$ quenching reaction of flavonoids has been discussed.

2. Experimental Section

2.1. Materials. All the flavonoids used in the present work are commercially available: flavone (Kanto Chemicals), flavonol (Wako Chemicals, Japan), chrysin (Aldrich), apigenin (Tokyo Kasei Organic Chemicals, Japan), rutin (Nakarai Chemicals, Japan), quercetin (Aldrich), myricetin (Aldrich), naringenin (Aldrich), and taxifolin (Aldrich). 3-(1,4-Epidioxy-4-methyl-1,4-dihydro-1-naphthyl)propionic acid (endoperoxide, EP) (see Scheme 1) was prepared by the published procedure.[21] 2,5-Diphenyl-3,4-benzofuran [DPBF (Tokyo Kasei Chemicals, Japan) (see Figure 1)] is commercially available.

2.2. Measurements. The measurements of rate constant were performed on a Shimadzu UV-2100S spectrophotometer. All measurements were performed at 35.0 ± 0.5 °C.

2.3. Calculations. The semiempirical MO calculations were performed with MOPAC 2000 ver. 1.0 on Windows XP with parameters in ref 24. The optimized geometries, energy parameters, and MO coefficients were calculated by the semiempirical PM3 method. Molecular geometries were totally optimized.

3. Results

3.1. Overall Rate Constants (k_Q) for the Reaction of $^1O_2$ with Flavonoids. Singlet oxygen was generated by the thermal decomposition of the endoperoxide (EP).[21,22] 2,5-Diphenyl-3,4-benzofuran (DPBF) was used as standard compound. The overall rate constants k_Q ($=k_q + k_r$) for the reaction of $^1O_2$ with flavonoids were determined in ethanol by eq 5 derived from the steady-state treatment of Scheme 2:[23]

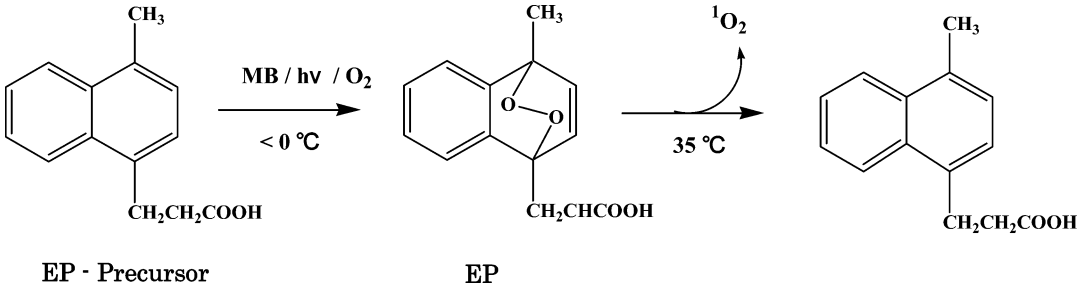
$$S_0/S_S = 1 + [(k_q + k_r)/k_d][\text{flavonoid}] \qquad (5)$$

where S_0 and S_S are slopes of the first-order plots of disappearance of $^1O_2$ acceptor, DPBF, in the absence and presence of flavonoid, respectively. k_d is the rate of deactivation of $^1O_2$ in ethanol.

Figure 2 shows an example of the interaction between DPBF (5.98×10^{-5} M) and EP (2.94×10^{-4} M) without flavonoids in ethanol solution at 35 °C. By the reaction, the appearance of absorption of EP precursor at $\lambda_{max} = 288$ nm due to the thermal decomposition of EP (and, thus, the production of $^1O_2$) and the disappearance of DPBF at $\lambda_{max} = 411$ nm due to the chemical reaction between DPBF and $^1O_2$ produced were observed simultaneously at 288 and 411 nm, respectively (see Table 1). The pseudo-first-order rate constant (S_0) was obtained by following the decrease in absorbance at 411 nm of the DPBF.

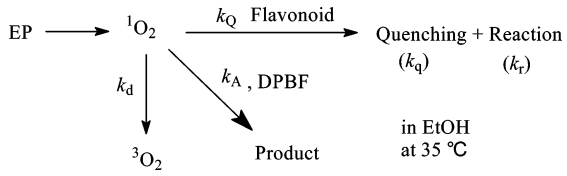
Similarly, solutions containing EP (3.69×10^{-4} M), DPBF (7.02×10^{-5} M), and various amounts of rutin ($0 \sim 1.17 \times 10^{-3}$ M) in ethanol were reacted at 35 °C. The disappearance of DPBF was measured at 411 nm.[22,23] A plot of S_0/S_S vs concentration of rutin is shown in Figure 3. The overall rate

SCHEME 1



EP - Precursor EP

SCHEME 2



constants (k_Q) were calculated by using the value of k_d in ethanol ($k_d = 8.3 \times 10^4$ s^{-1}), reported by Merkel and Kearns.[25] Similarly, flavonoids were reacted with $^1O_2$ in ethanol. S_0/S_S vs [flavonoid] plots for apigenin and myricetin are also shown in Figure 3. The k_Q values obtained were summarized in Table 2, together with those reported for α- and γ-tocopherol,[26−29] ubiquinol-10,[30] and γ-tocopherol hydroquinone (plastoquinol model).[30] The experimental error in k_Q value for each flavonoid was ±8% at maximum.

3.2. Chemical Reaction of $^1O_2$ with Flavonoids. Endoperoxide (EP) was prepared by the reaction of 3-(4-methyl-1-naphthyl)propionic acid (EP precursor) with $^1O_2$ produced by the photosensitization reaction of methylene blue (MB) in ethanol (see Scheme 1).[21] The NMR measurement indicates that the powder sample of EP includes about 19% unreacted EP precursor. The UV absorption spectra of (a) EP (including about 24% EP precursor) and (b) EP precursor in ethanol at 25 °C are shown in Figure 4, where both [EP] and [EP precursor] are 1.50×10^{-4} M. EP precursor shows absorption maxima at λ_{max} (ϵ) = 299 (sh) (4470), 288 (6560), 279 (5340), and 233 (9430), as listed in Table 1. In fact, the UV absorption spectrum of EP in ethanol shows a maximum at $\lambda_{max} = 288$ nm due to about 24% EP precursor in addition to a maximum at $\lambda_{max} = 229$ nm ($\epsilon = 6510$) due to intrinsic EP. Rutin shows two absorption maxima [λ_{max} (ϵ) = 361 nm (17 500) and 259 nm (20 300)] at different wavelength regions (see Table 1).

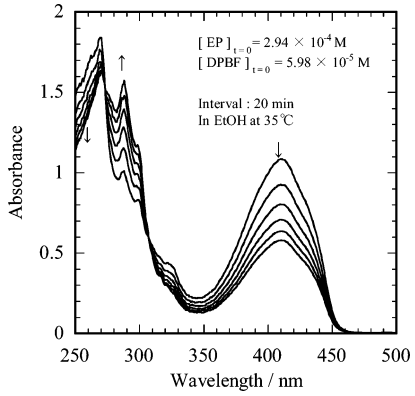


Figure 2. Change in electronic absorption spectrum of DPBF and endoperoxide (EP) during reaction of DPBF with EP in ethanol solution at 35 °C. $[DPBF]_{t=0} = 5.98 \times 10^{-5}$ M and $[EP]_{t=0} = 2.94 \times 10^{-4}$ M. The spectra were recorded at 20 min intervals. The arrow indicates decreasing absorbance of DPBF and increasing absorbance of EP precursor with time, respectively.

TABLE 1: UV−Vis Absorption Maxima (λ_{max}) and Molar Extinction Coefficients (ϵ) of the Flavonoids and Related Compounds in Ethanol, and Energy Level of HOMO (E_{HOMO})

flavonoids	λ_{max}, nm	ϵ, L mol^{-1} cm^{-1}	E_{HOMO}, eV
flavone	294	23 300	−9.29
	251	19 400	
flavonol	343	19 000	−8.97
	305	13 600	
	240	21 300	
chrysin	313	17 600	−9.24
	269	43 600	
	246 (sh)	21 200	
apigenin	336	19 800	−9.16
	289 (sh)	12 300	
	269	17 300	
rutin	361	17 500	−9.06
	295 (sh)	7610	
	259	20 300	
quercetin	371	20 300	−9.05
	301 (sh)	6440	
	256	19 000	
myricetin	377	59 200	−9.06
	305	18 200	
	255	50 000	
naringenin	290	52 000	−9.27
taxifolin	291	51 600	−9.18
EP	229	6510	
EP precursor	299 (sh)	4470	
	288	6560	
	279	5340	
	233	9430	
DPBF	411	19 700	
	311	7260	
	262	25 900	

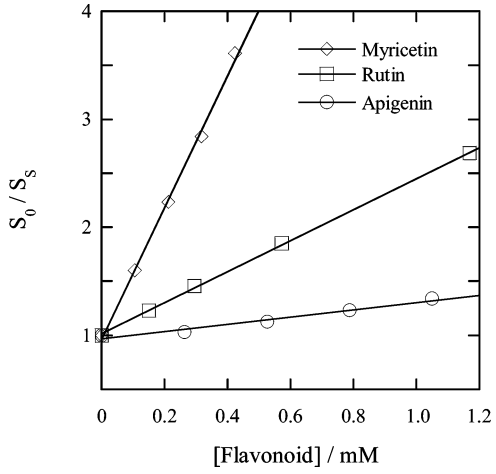


Figure 3. Plot of S_0/S_S vs concentrations of apigenin, rutin, and myricetin.

Figure 5 shows an example of the interaction between rutin (4.50×10^{-5} M) and EP (1.25×10^{-4} M) in ethanol solution at 35 °C. The spectra were recorded at 30 min intervals. At 35

TABLE 2: Second-Order Rate Constants (k_Q) and Relative Rate Constants [$100k_Q$(AO)/k_Q(α-Toc)] for the Reaction of $^1O_2$ with Flavonoids and Related Compounds in Ethanol, and Peak Oxidation Potentials (E_P)[a]

antioxidants	*n*	k_Q, M^{-1} s^{-1}	$100k_Q$(AO)/ k_Q(α-Toc)	E_P, V vs Ag/AgCl[b]
flavone	0	$<3 \times 10^5$	<0.145	
flavonol	1	5.33×10^6	2.59	
chrysin	2	2.01×10^7	9.76	0.794
apigenin	3	2.84×10^7	13.8	0.658
rutin	4	1.21×10^8	58.7	0.360
quercetin	5	4.57×10^8	222	0.178
myricetin	6	5.12×10^8	249	
naringenin	3	3.68×10^6	1.79	0.688
taxifolin	5	9.37×10^6	4.54	0.248
α-tocopherol		2.06×10^8 [c]	100	
γ-tocopherol		1.38×10^8 [c]	67.0	
γ-tocopherol hydroquinone (γ-TQH_2) (plastoquinol model)		1.17×10^8 [c]	56.8	
ubiquinol-10 ($UQ_{10}H_2$)		1.58×10^8 [c]	76.7	
β-carotene		1.58×10^{10}	7670	

[a] *n* denotes number of OH groups substituted to flavone skeleton [b] Values reported by Hotta et al. (*31*). [c] Values reported in a previous paper (*30*).

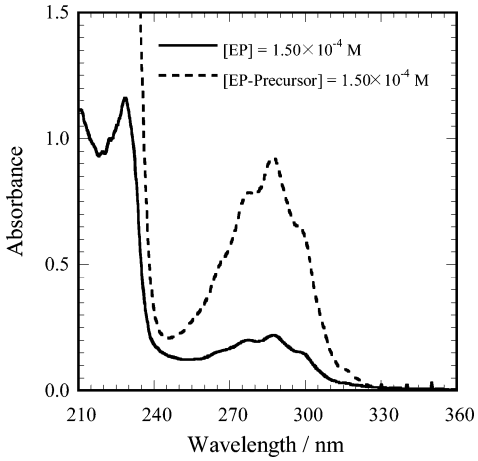


Figure 4. UV absorption spectra of endoperoxide (EP) and EP precursor in ethanol at 35 °C. [EP] = [EP precursor] = 1.50×10^{-4} M.

°C, EP decomposes and generates singlet oxygen and EP precursor. The arrow indicates an increase in absorbance of EP precursor at 288 nm with time. However, the absorption of rutin at $\lambda_{max} = 361$ nm shows no change in the intensity. The chemical reaction between $^1O_2$ and rutin is very slow, and the measurable change in the absorption spectrum of rutin was not observed. Similar measurements were performed for the reaction between EP and flavonoids with and without a 2,3-double bond. The chemical reaction expected was not observed for all the flavonoids studied. Consequently, the k_Q values obtained for flavonoids are thought to be due to physical quenching (k_q); that is, $k_Q \approx k_q$.

It has been reported that tocopherols can act as efficient scavengers of $^1O_2$.[26−29] It was shown that α-tocopherol scavenges $^1O_2$ by a combination of physical quenching (k_q) and chemical reaction (k_r). For instance, the k_Q value ($k_Q = k_q + k_r$) of α-tocopherol is 2.5×10^8 M^{-1} s^{-1} in pyridine. Because $k_q \gg k_r$, the quenching process is almost entirely "physical"; that is, α-tocopherol deactivates about 120 $^1O_2$ molecules before being destroyed by chemical reaction (eq 4).[26,27] As described in the Introduction, Tournaire et al.[20] reported that the flavonoids with a hydroxyl group activating the 2,3-double bond at the

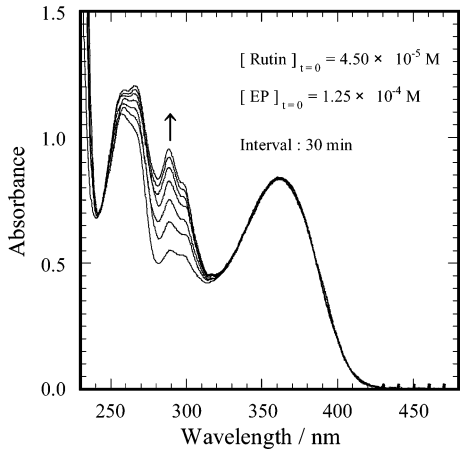


Figure 5. Increase of the absorption of EP precursor at 288 nm due to the generation of singlet oxygen from endoperoxide (EP) in ethanol at 35 °C. [EP] = 1.25×10^{-4} M and [rutin] = 4.50×10^{-5} M. The spectra were recorded at 30 min intervals.

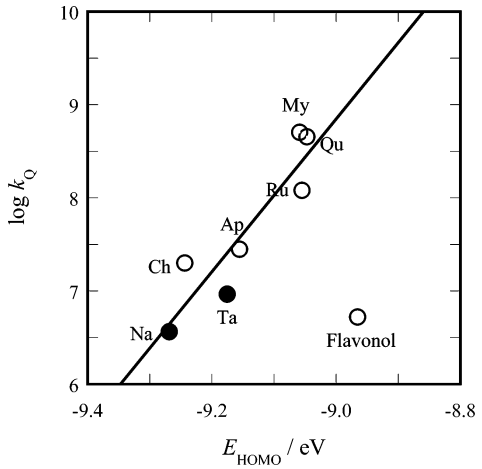


Figure 6. Plot of log k_Q vs E_{HOMO} for flavonol, chrysin, apigenin, rutin, quercetin, myricetin, naringenin, and taxifolin.

C-ring show chemical reactivity, although k_q is in general higher than k_r. On the other hand, the chemical reaction was not observed for the flavonoids without the 2,3-double bond. However, the result obtained here shows that the chemical reaction is almost negligible in all the flavonoids studied. The reason for such a difference in the chemical reactivity (k_r) is not clear at present.

3.3. Molecular Orbital Calculations of Flavonoids. Molecular orbital calculations were performed for the flavonoids by the semiempirical PM3 method.[24] The energy levels of the highest occupied molecular orbital (E_{HOMO}) obtained are listed in Table 1. The values of log k_Q for flavonoids have been plotted against E_{HOMO}. As shown in Figure 6, log k_Q of flavonoids except for flavonol correlates well with E_{HOMO} with a slope of 8.2 ± 1.6 eV^{-1} (correlation coefficient = 0.91). The flavonoids that have higher E_{HOMO} values show higher reactivities with singlet oxygen.

3.4. UV−Vis Absorption Spectra of Flavonoids. Measurements of UV−vis absorption spectra have been performed for the flavonoids in ethanol solution. Absorption spectra of flavonoids with 2,3-double bonds are shown in Figure 7. The wavelengths of absorption maxima (λ_{max}) and molar absorption coefficients (ϵ) obtained are listed in Table 1, indicating that the values of ϵ at λ_{max} are similar to each other in these flavonoids. The λ_{max}^L values of the longest wavelength in flavonoids, except for flavonol, increase with increasing number

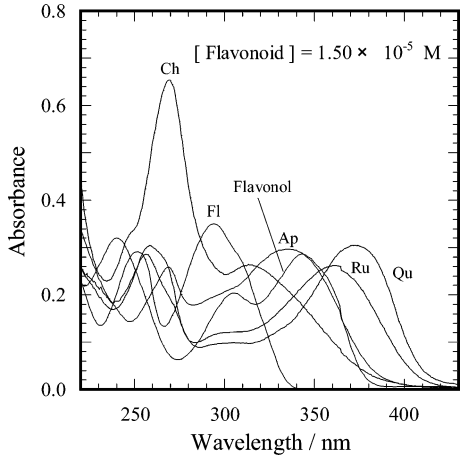


Figure 7. Absorption spectra of flavone, flavonol, chrysin, apigenin, rutin, and quercetin. [Flavonoid] = 1.50×10^{-5} M.

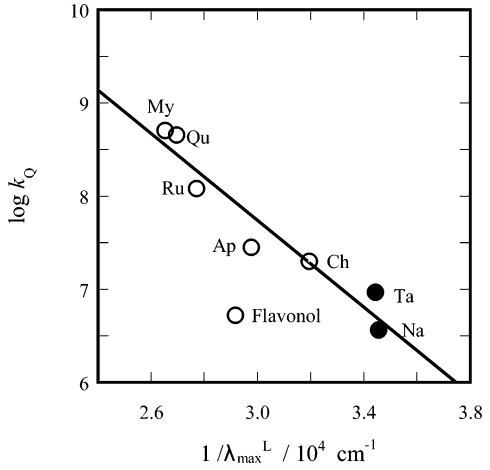


Figure 8. Plot of log k_Q vs $1/\lambda_{max}^L$ for flavonol, chrysin, apigenin, rutin, quercetin, myricetin, naringenin, and taxifolin.

of OH groups substituted to the flavone skeleton. The plot of log k_Q vs inverse absorption maximum ($1/\lambda_{max}^L$) is shown in Figure 8. The good correlation ($R = 0.96$) with a slope of $-(2.33 \pm 0.30) \times 10^{-4}$ cm^{-1} was observed for flavonoids except for flavonol, indicating that flavonoids with larger λ_{max}^L values show faster $^1O_2$ quenching rates.

4. Discussion

4.1. Structure−Activity Relationship of the $^1O_2$ Quenching Reaction by Flavonoids in Ethanol Solution. As shown in Figure 1, the flavonoids except for naringenin and taxifolin are OH derivatives of flavone, in which the aromatic A- and B-rings are π-conjugated to each other by a 2,3-double bond. As listed in Table 2, the k_Q values obtained are less than $<3 \times 10^5$ M^{-1} s^{-1} for flavone, 5.33×10^6 M^{-1} s^{-1} for flavonol, and 2.01×10^7 M^{-1} s^{-1} for chrysin. The reactivity of flavone without an OH group is very weak and almost negligible. Flavonol with a 3-OH group at the C-ring shows low reactivity. Chrysin has higher reactivity than flavonol, clearly indicating that the resorcinol A-ring contributes to the $^1O_2$ quenching action by flavonoids. The reaction rate ($k_Q = 2.84 \times 10^7$ M^{-1} s^{-1}) of apigenin, with a 4′-OH group at the B-ring, is larger than that of chrysin, suggesting that the 4′-OH group at the B-ring contributes to the $^1O_2$ quenching. Further, the k_Q values obtained for rutin ($k_Q = 1.21 \times 10^8$ M^{-1} s^{-1}) and quercetin ($k_Q = 4.57 \times 10^8$ M^{-1} s^{-1}), which have 3′- and 4′-OH groups at the B-ring, are 6.0 and 23 times larger than that for chrysin. The result indicates that the catechol structure in the B-ring of rutin and quercetin mainly contributes to the quenching of $^1O_2$. The k_Q value (5.12×10^8 M^{-1} s^{-1}) obtained for myricetin, which has 3′-, 4′-, and 5′-OH groups at the B-ring, is larger than those of rutin and quercetin.

As described above, the quenching rates of $^1O_2$ oxygen for flavonoids increase in the order

$$\text{flavone } (n = 0) < \text{flavonol } (n = 1) < \text{chrysin } (n = 2) < \text{apigenin } (n = 3) < \text{rutin } (n = 4) < \text{quercetin } (n = 5) < \text{myricetin } (n = 6) \quad (6)$$

in ethanol solution, where n is the number of OH substituents in these flavonoids. The result clearly indicates that the quenching rate increases with increasing number of OH substituents, that is, the electron-donating capacity of these flavonoids. Especially, the existence of catechol or pyrogallol structure in the B-ring is essential for the $^1O_2$ quenching of flavonoids, although the resorcinol structure in the A-ring also contributes partly (4∼17%) to the $^1O_2$ quenching.

Apigenin and naringenin have 5- and 7-OH groups at the A-ring and a 4′-OH group at the B-ring. Further, only the apigenin has a 2,3-double bond, which is responsible for π-conjugation between A- and B-rings. In fact, the quenching rate (k_Q) of apigenin (2.84×10^7 M^{-1} s^{-1}) is 7.7 times larger than that of naringenin (3.68×10^6 M^{-1} s^{-1}) without a 2,3-double bond in ethanol. Similarly, the k_Q of quercetin (4.57×10^8 M^{-1} s^{-1}) and rutin (1.21×10^8 M^{-1} s^{-1}) are 49 and 13 times larger than that of taxifolin (9.37×10^6 M^{-1} s^{-1}) in ethanol, respectively. The result indicates that the existence of the 2,3-double bond in quercetin, rutin, and apigenin is important for $^1O_2$ quenching.

Bors et al.[11,12] measured the second-order rate constants for the reaction of flavonoids with HO•, N_3•, and *t*-BuO• radicals, at pH 11.5, by a pulse radiolysis technique and reported that three structural groups in flavonoids are important determinants for radical scavenging: (i) the 3′- and 4′-OH groups (catechol structure) in B-ring, which are the obvious radical target site for all flavonoids; (ii) the 2,3-double bond in conjugation with a 4-oxo function, which is responsible for electron delocalization from the B-ring; and (iii) the existence of both 3- and 5-OH groups for maximal radical-scavenging activity. The results obtained in the present work indicate that all the above determinants i−iii proposed for the radical-scavenging by Bors et al.[11,12] are also important for singlet oxygen quenching in flavonoids.

4.2. Correlation between Log k_Q and Peak Oxidation Potentials. In a previous work, the rate of quenching of $^1O_2$ by 17 kinds of tocopherol derivatives, including α-, β-, γ-, and δ-tocopherols, and five structurally related phenols has been measured spectrophotometrically in ethanol at 35 °C.[29] The result indicates that the overall rate constants, k_Q, increase as the total electron-donating capacity of the alkyl substituents on the aromatic ring increases. Log k_Q was found to correlate with their peak oxidation potentials, E_P. Similar correlation was observed for the biological hydroquinones and related compounds.[30]

Measurements of E_P of flavonoids (chrysin, apigenin, rutin, quercetin, naringenin, and taxifolin) were performed in 1:1 (v/v) water−ethanol containing 50 mM KCl and 50 mM phosphate buffer (pH 7.0) by Hotta et al.,[31] using cyclic voltammetry. The E_P values reported are listed in Table 2. The values of log k_Q for flavonoids have been plotted against E_P. As shown in Figure 9, log k_Q of chrysin, apigenin, rutin, and quercetin (compounds with 2,3-double bonds) correlates with E_P with a slope of −2.2

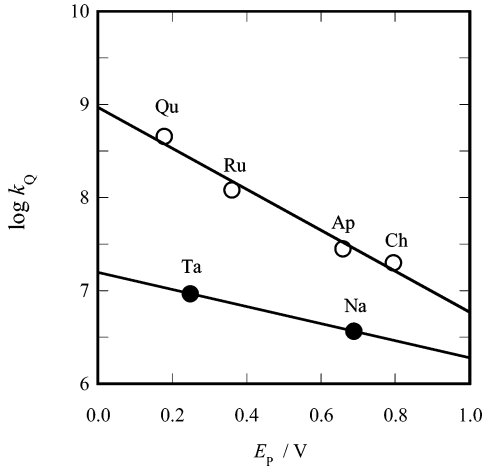


Figure 9. Plot of log k_Q vs E_P for chrysin, apigenin, rutin, quercetin, naringenin, and taxifolin.

$\pm$ 0.2 V^{-1} (correlation coefficient = -0.99). The flavonoids that have smaller E_P values show higher reactivities. The result suggests that the transition state in the above $^1O_2$ quenching reaction by flavonoids has the property of a charge-transfer intermediate.[32] On the other hand, the values of naringenin and taxifolin, without 2,3-double bonds, deviate from the above correlation line. The E_P values reported for naringenin and taxifolin are considered to be smaller than those expected from E_{HOMO} values obtained by MO calculation, as described below.

As reported in a previous work, the rate constants of scavenging of ArO• (k_S) by tocopherol derivatives increase as the total electron-donating capacity of the alkyl groups at the aromatic ring increases.[33] A plot of log k_S and log k_Q versus peak oxidation potential (E_P) was found to be linear and the slope was negative. Linear correlation between the rates of quenching of singlet oxygen (k_Q) and scavenging of peroxyl and aroxyl radicals (k_S) in solution was found.[29] As described in a previous section, the k_S and k_R values of flavonoids increase in the order shown in eq 3, independent of both pH value and solvent system.[17] On the other hand, the E_P values of the above flavonoids reported by Hotta et al. decrease in the order chrysin > apigenin > flavonol > rutin > quercetin. Hendrickson et al.[34] found that the effect of flavonoids on microsomal phenol hydroxylase activity correlates well with the oxidation potential (E_P) for flavonoids aglycons; the flavonoids that have smaller E_P values show higher inhibitions of phenol hydroxylase activity. Further, the correlation between the E_P values of flavonoids and their log IC_{50} values for doxorubicin-induced lipid peroxidation has been reported by Acker et al.[6] These results suggest that the flavonoids with smaller E_P values show higher free radical scavenging and singlet oxygen quenching activities, and thus higher biological activity.

4.3. Correlation of Log k_Q with Energy Level of HOMO and Inverse Absorption Maximum in UV−Vis Absorption Spectra. As shown in Figure 6, the flavonoids that have higher E_{HOMO} values show higher reactivities. The result is reasonable, because the flavonoids that have higher E_{HOMO} values will show smaller ionization potential (I_P), that is, smaller oxidation potential (E_P).

UV−Vis absorption spectra of flavonoids with 2,3-double bonds are shown in Figure 7. The λ_{max}^L values for the longest wavelength $\pi\pi^*$ excitation in flavonoids increase with increasing number of OH groups substituted to the flavone skeleton. As shown in Figure 8, a good correlation between log k_Q and $1/\lambda_{max}^L$, that is, the longest-wavelength $\pi\pi^*$ excitation energy (E_{ex}), was observed for flavonoids. A similar correlation was observed for the carotenoid derivatives having comparatively higher excitation energies [$(2.20\sim2.30) \times 10^4$ cm^{-1}], that is, slower quenching rates [$k_Q = (1.0 \times 10^8)\sim(3.0 \times 10^9)$ M^{-1} s^{-1}].[35] The result suggests that flavonoids with smaller E_{ex} values show higher $^1O_2$ quenching activities and, thus, higher biological activity, as described above.

The results of the X-ray structure analyses show that the torsion angles (θ) between B- and C-rings in flavone derivatives without an OH substituent at the 3-position are 0.7∼24.1° (avg 12°), indicating that the molecules take planar structures.[36] Similarly, the torsion angles (θ) in flavonol and quercetin with an OH substituent at the 3-position are 5.5° and 7°, respectively, indicating planar structure.[37,38] Both quercetin and rutin, which is quercetin rutinoside at the 3-position, have OH substituents at 3′-, 4′-, 5-, and 7-positions, and we can expect similar rate constants (k_Q) for these flavonoids. However, the k_Q value of rutin is 3.8 times smaller than that of quercetin in ethanol, as listed in Table 2. The π-conjugation between B- and C-rings in rutin will be weaker than that in quercetin, because the B-ring of rutin is considered to twist much more than that of quercetin by the steric repulsion between the 6′- (or 2′-) ring proton at the B-ring and the rutinose group. In such a case, the energy level of HOMO (E_{HOMO}) of rutin lowers, the oxidation potential (E_P) of rutin increases, the λ_{max}^L value decreases, and thus the k_Q value will decrease.[29,30,35] In fact, the E_P value of rutin (0.360 V vs Ag/AgCl) is larger than that of quercetin (0.178 V),[31] and the λ_{max}^L value of rutin (361 nm) is smaller than that of quercetin (371 nm).

4.4. Comparison between the Quenching Rates (k_Q) of Flavonoids and Biological Compounds. Singlet oxygen reacts with a wide variety of biological targets including lipids, sterols, proteins (amino acids), DNA, etc. Rate constants k_Q ($=k_q + k_r$) for a large number of these reactions have been reported previously (see Table 1 in ref 39). Most reactions of $^1O_2$ with biological targets occur via chemical rather than physical routes. For instance, peroxidation of unsaturated lipids is induced by singlet oxygen. The quenching rates (k_Q) of $^1O_2$ by saturated and unsaturated fatty acids and lipids are 9.0×10^3 M^{-1} s^{-1} for stearic acid, 1.7×10^4 M^{-1} s^{-1} for oleic acid, 4.2×10^4 M^{-1} s^{-1} for linoleic acid, and 6.0×10^4 M^{-1} s^{-1} for egg yolk phosphatidylcholine.[40,41] The quenching rate increases as the number of double bonds in the fatty acid molecule increases. The k_Q values [$(3.68 \times 10^6)\sim(5.12 \times 10^8)$ M^{-1} s^{-1}] observed for eight kinds of flavonoids are 2−4 orders of magnitude larger than those for fatty acids and phospholipid. The result suggests that these flavonoids may contribute to the quenching of $^1O_2$ and prevent lipid peroxidation in cell membranes. Similarly, the k_Q values observed for flavonoids are 1−3 orders of magnitude larger than that (5.1×10^5 M^{-1} s^{-1}) for DNA.

Davies et al.[39,42] reported that proteins will be major targets for $^1O_2$ within cells, as the rate constants for reaction of $^1O_2$ with amino acid side chains in proteins are higher than those with most other cellular targets, and proteins are present at high concentrations when compared to other species within cells. Of the common amino acids present in proteins, only Try, His, Tyr, Met, and Cys react at significant rates at physiological pH values. Rate constants reported for these amino acids are $(0.8\sim3.7) \times 10^7$ M^{-1} s^{-1}. The values are similar to those of chrysin (2.01×10^7 M^{-1} s^{-1}) and apigenin (2.84×10^7 M^{-1} s^{-1}) and 1−2 orders of magnitude smaller than those of rutin (1.21×10^8 M^{-1} s^{-1}), quercetin (4.57×10^8 M^{-1} s^{-1}), and myricetin (5.12×10^8 M^{-1} s^{-1}).

α-, β-, γ-, and δ-Tocopherol and biological hydroquinones, such as ubiquinol-10 ($UQ_{10}H_2$), vitamin K_1 hydroquinone, and

plastoquinol (PQH_2), are well-known as the most popular lipid-soluble antioxidants. γ-Tocopherol hydroquinone (γ-TQH_2) is considered to be a plastoquinol model, because both PQH_2 and γ-TQH_2 have two methyl substituents at 2- and 3-positions and a long alkyl chain at the 6-position, and thus the rate constants k_Q of PQH_2 and γ-TQH_2 are thought to be similar to each other. The quenching rates of $^1O_2$ by these antioxidants have been reported in previous works (see Table 2).[29,30] As described in a previous section, the rate of the quenching reaction of $^1O_2$ with flavonoids increases in the order shown in eq 6 in ethanol solution. The rate constants (k_Q) obtained for rutin (1.21×10^8 M^{-1} s^{-1}), quercetin (4.57×10^8), and myricetin (5.12×10^8) are similar to (or larger than) those of α-tocopherol (2.06×10^8 M^{-1} s^{-1}), γ-tocopherol (1.38×10^8), $UQ_{10}H_2$ (1.58×10^8), and γ-TQH_2 (plastoquinol model) (1.17×10^8). Flavonoids are found in high concentration in foods and plants. The present kinetic study suggests that the above flavonoids function as singlet oxygen quenchers in biological systems (such as cell membranes, photosynthetic systems, etc.) and protect the systems from oxidative damage. However, the quenching rates of these flavonoids are 1−2 orders of magnitude smaller than that (1.58×10^{10} M^{-1} s^{-1}) of β-carotene, which is well-known as a representative $^1O_2$ quencher.

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