Antioxidant Mechanism of Flavonoids. Solvent Effect on Rate Constant for Chain-Breaking Reaction of Quercetin and Epicatechin in Autoxidation of Methyl Linoleate

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The rate of oxygen depletion, as measured by electron spin resonance spectroscopy (oximetry using a spin probe), in a homogeneous solution of peroxidating methyl linoleate (initiated by an azo initiator) in the presence or absence of antioxidants was converted to second-order rate constants for the inhibiting reaction of quercetin and epicatechin. In the non-hydrogen-bonding solvent chlorobenzene at 50 °C, k_{inh} had values of 4.3×10^5 M^{-1} s^{-1} for quercetin and 4.2×10^5 M^{-1} s^{-1} for epicatechin, respectively. In the hydrogen-accepting "water-like" solvent *tert*-butyl alcohol, the values were 2.1×10^4 and 1.7×10^4 M^{-1} s^{-1}, respectively. The solvent effect (factor of 20) is more significant than for α-tocopherol (factor of 4), and the two flavonoids have efficiencies comparable to that of α-tocopherol in scavenging peroxyl radicals in the nonpolar solvent but not in the hydrogen-bonding solvent.

Keywords: *Quercetin; epicatechin; antioxidant mechanism; hydrogen atom transfer; solvent effect*

INTRODUCTION

Flavonoids are polyphenolic compounds that are found in many foods of plant origin and which are known to possess antioxidant ability both in living organisms (*1*) and in food and beverages (*2*, *3*). Large values are in general found for rate constants for reaction between activated oxygen species such as hydroxyl or alkoxyl radicals (*4*, *5*). However, a high reactivity toward especially the hydroxyl radical does not imply that flavonoids are good chain-breaking antioxidants because the majority of organic molecules would react quickly with this radical.

To evaluate the antioxidant ability of polyphenols, their reactivity as H-atom donors toward the peroxyl radicals, which are the chain-carrying species in the autoxidation of lipids, must be measured. There are results reported from only a few studies of such reactions, most of them referring to experiments performed under poorly reproducible conditions. However, in two recent papers, Bors and co-workers investigated the reactivity of flavonoids toward peroxyl radicals from diphenylmethane (*6*) and methyl linoleate (*7*) in homogeneous and micellar solutions. In both cases flavonoids did not behave as *classical* phenolic antioxidants as both the stoichiometric factor and the inhibition rate constants showed a strong dependence not only on the nature of the oxidation substrate but also on the antioxidant concentration (in methyl linoleate the flavonoid efficiency decreased with increasing concentration). However, at the time these studies were carried out the importance of solvent effects in autoxidation reactions were not yet fully recognized. Ingold and co-workers and other groups have subsequently emphasized the fact that hydrogen bonding may induce dramatic changes in the H-atom donor activities of phenolic antioxidants (*8*). We have accordingly undertaken an investigation of the antioxidant ability of two flavonoids, that is, quercetin (QC), a flavon-3-ol, and epicatechin (EC), a flavan-3-ol, which have been reported to be among the most effective flavonoids and each has been reported to be the most active of its own class (*9*). The selection has thus been restricted to flavonoids with a catechol group on the B ring (see Figure 1) because this structural element has been found to be essential for high antioxidant activity (*10*−*13*).

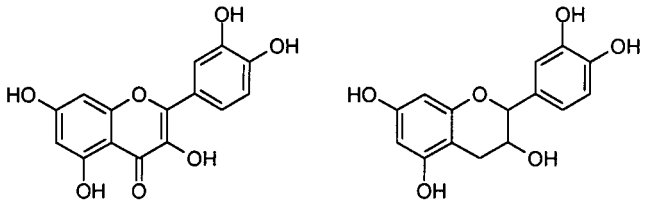

Figure 1. Structures of a flavon-3-ol and a flavan-3-ol with a cathecol group on the B ring.

To measure the inhibition rate constants of QC and EC and the effect of solvent on the nature of inhibition reaction and its rate, the autoxidation of methyl linoleate (ML) was studied at 50 °C in the hydrogen-bond-accepting (HBA) solvent *tert*-butyl alcohol and in the non-hydrogen-bonding solvent chlorobenzene, by using as thermal initiator α,α′-azoisobutyronitrile (AIBN). The autoxidation of cumene in chlorobenzene was also studied using the same initiator of autoxidation. The rate of the oxygen consumption, measured by ESR spectroscopy (*14*), was compared for peroxidating sys-

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tems in the absence and in the presence of either flavonoid or α-tocopherol (vitamin E).

MATERIALS AND METHODS

Materials. All chemicals were commercially available, chosen of the highest purity and used as received. Methyl linoleate, isopropylbenzene (cumene), chlorobenzene, 3,3′,4′,5,7-pentahydroxyflavone dehydrate (quercetin), 3′,4′,5,7-tetrahydroxyflavan-3-ol (epicatechin), and 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) were purchased from Aldrich-Sigma (St. Louis, MO), α,α′-azoisobutyronitrile was from Fluka (Buchs, Switzerland), and *all-rac*-α-tocopherol and 2-methyl-2-propanol were from Merck (Darmstadt, Germany).

Apparatus. The ESR spectra were recorded on a Bruker ECS 106 spectrometer (Bruker, Rheinstetten, Germany) by using the following settings: microwave frequency, 9.42 GHz; power, 0.80−2 mW; modulation amplitude, 0.5 G; center field, 3360 G; sweep time, 81 s; time constant, 81 ms.

Samples. The samples were air-saturated at room temperature and introduced (∼100 μL) into a capillary tube with an internal diameter of ∼2 mm. A second capillary tube having a thinner diameter and sealed at one end was introduced into the sample tube so as to leave very little dead volume space. The tube was then put into the ESR cavity kept at 50 °C, and the first spectrum was recorded after ∼1 min to allow for the temperature equilibration time.

Line Width. The ESR spectra of a solution of 3.5×10^{-5} M TEMPO in *tert*-butyl alcohol or in chlorobenzene at 50 °C recorded in air atmosphere and in nitrogen atmosphere were used as standards for measuring the line width of TEMPO under air and in the absence of oxygen.

RESULTS AND DISCUSSION

Antioxidant Activity of Flavonoids in *tert*-Butyl Alcohol. The autoxidation of 0.242 M methyl linoleate in *tert*-butyl alcohol was initiated by 0.0344 M AIBN at 50 °C both in the presence and in the absence of α-tocopherol (αTOH), quercetin (QC), or epicatechin (EC) and monitored by ESR following the line width variations of 3.5×10^{-5} M TEMPO (*14*). The problem of a possible interference of the nitroxide radical with the autoxidation reaction has been previously discussed (*14*, *15*) and ruled out for concentrations of TEMPO up to 5×10^{-5} M. Nonetheless, periodic controls were performed to ensure that the ESR signal from TEMPO (integration of absorption) was constant over the time taken by the measurement of the oxygen consumption.

ESR spectra of the peroxidating solutions were recorded as a function of time at intervals of a few seconds over a period of ∼1 h, that is, the time requested for oxygen depletion. For each ESR spectrum the reciprocal of the square root of the signal height, $I^{-1/2}$, of the central line of TEMPO was measured and plotted against time. The measure of $I^{-1/2}$ is equivalent to the measure of the line width, although it has the advantage of being more accurate, especially in the presence of large oxygen concentrations (*14*).

The line widths were then converted to concentration of oxygen, based on the fact that the oxygen concentration corresponding to the value of $I^{-1/2}$ at time $t = 0$ is given by the oxygen solubility in *tert*-butyl alcohol, 1.73×10^{-3} M (*15*), whereas the value of $I^{-1/2}$ when no more oxygen is present was the one measured on a deoxygenated solution of TEMPO in *tert*-butyl alcohol.

The time traces of the oxygen consumption observed during the uninhibited peroxidation of ML or in the presence of QC or αTOH are shown in Figure 2. Values for $-d[O_2]/dt$, obtained from a linear regression of the experimental data, are reported in Table 1.

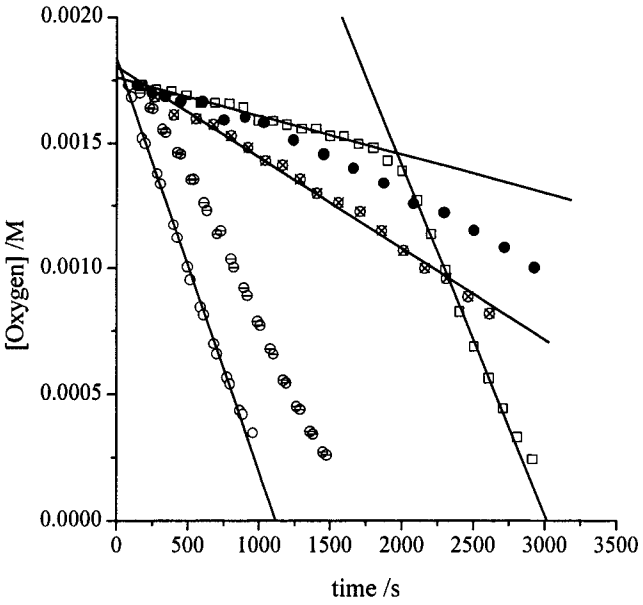

Figure 2. Oxygen depletion during autoxidation of ML (0.242 M) in *t*-ButOH initiated by AIBN (0.0301 M) at 50 °C in the absence (○) or presence of 4.00×10^{-5} M (⊖), 1.98×10^{-4} M (⊗), or 4.20×10^{-4} M (●) quercetin or in the presence of 8.39×10^{-5} M (□) α-tocopherol in a closed system with no headspace.

Table 1. Rates of Autoxidation of 0.242 M Methyl Linoleate in the Absence ($R_{ox} = -d[O_2]/dt$) and in the Presence (R_{inh}) of Quercetin, Epicatechin, and α-Tocopherol in *tert*-Butyl Alcohol at 50 °C Monitored by ESR by Using the Line Width Variations of 3.5×10^{-5} M TEMPO

antioxidant	[AH]/ 10^{-5} M	R_i/ 10^{-8} M s^{-1}	R_{ox}/ 10^{-7} M s^{-1}	R_{inh}/ 10^{-7} M s^{-1}	R_{inh}/R_{ox}
quercetin	0	8.4 ± 0.8[a]	16.8		
	1.2			16.7	0.994
	2.0			10.9	0.649
	4.0			9.42	0.561
	9.8			4.24	0.252
	19.8			3.26	0.194
	42			1.95	0.116
	105			1.82	0.108
epicatechin	0	7.3 ± 0.6[b]	13.3		
	3.9			7.55	0.568
	6.2			6.72	0.505
	13.0			4.22	0.317
	14.8			3.46	0.260
	20.8			3.24	0.244
	39.0			2.21	0.166
	62.4			1.78	0.134
α-tocopherol	5.80	7.3 ± 0.6[b]	13.3	1.48	

[a,b] Rate of initiation for 0.0344 and 0.0301 M AIBN, respectively.

Figure 2 shows that the rate of oxygen consumption during lipid peroxidation was reduced in the presence of QC compared to the rate observed in its absence. Therefore, QC is able to slow the oxidation of ML, although much less than αTOH, which is one of the most efficient natural antioxidants. Actually, in the latter case a definite induction period, τ, during which the rate of oxidation is strongly reduced, was observed. The length of τ, which depends on the antioxidant concentration [AH], provides the stoichiometric factor, n, that is, the number of radical chains interrupted by a single antioxidant molecule. The value of n is given by eq 1, where R_i is the rate of initiation (*16*).

$$n = R_i\tau/[AH] \quad (1)$$

In the case of αTOH, where each molecule inactivates two peroxyl radicals (eqs 2 and 3), $n = 2$.

$$\alpha TOH + ROO^\bullet \rightarrow \alpha TO^\bullet + ROOH \quad (2)$$

$$\alpha TO^\bullet + ROO^\bullet \rightarrow \text{adducts} \quad (3)$$

The rate constant for the chain-breaking reaction, that is, the rate constant for the reaction of an antioxidant with the peroxyl radicals of the oxidation substrate, can be determined from the slope of the inhibited oxygen consumption, $(-d[O_2]/dt)_{inh}$ (eq 4), when a clear induction period is observed (*16*). The comparison of this rate with the rate measured in the presence of αTOH provides the value for k_{inh} for the other antioxidant when k_{inh} for αTOH is known under the same experimental conditions.

$$(-d[O_2]/dt)_{inh} = (k_p[RH]R_i/nk_{inh}[AH]) + R_i \quad (4)$$

With QC, however, which induced only a retarding of the autoxidation, no definite induction period could be detected. Thus, eq 4, derived under the assumption that the peroxyl radicals are all quenched by the antioxidant, does not hold anymore. The value of k_{inh} can instead be obtained in this case by using eq 5, derived by taking into account also the self-termination of the peroxyl radicals (*17*).

$$(-d[O_2]/dt)_{inh} = (k_p[RH]/2k_t) \times \{-k_{inh}[AH] + \sqrt{k_{inh}^2[AH]^2 + 2k_tR_i}\} + R_i \quad (5)$$

However, because this equation requires knowledge of k_p and $2k_t$, the rate constants for the propagation and termination steps of the autoxidation reaction, respectively, the inhibition rate constant for QC was estimated by using the method proposed by Darley-Usmar et al. (*17*), which is especially suitable for moderate inhibitors of oxidation. This method depends on measuring the rate of oxygen uptake in the presence of different concentrations of a given antioxidant at constant initiator and ML concentrations. The rate of oxygen consumption is analyzed according to eq 6, in which the ratio of the rates of inhibited and uninhibited oxygen consumptions is expressed as a function of the antioxidant concentration, and regression analysis provides a composite rate constant k_{AH} carrying information on the rate constant k_{inh}, characterizing the particular antioxidant under study.

$$(-d[O_2]/dt)_{inh}/(-d[O_2]/dt)_{ox} = 1 - \beta\{(k_{AH}[AH] + 1) - (k_{AH}^2[AH]^2 + 1)^{1/2}\} \quad (6)$$

where

$$\beta = k_p[RH]/\sqrt{2k_tR_i} + k_p[RH] \quad (7)$$

and

$$k_{AH} = k_{inh}/\sqrt{2k_tR_i} \quad (8)$$

The value of k_{AH} for the antioxidant studied was obtained by a nonlinear least-squares fitting of the ratios of the rates of oxygen consumption measured at variable concentrations of the inhibitor. Experiments were performed in *tert*-butyl alcohol at 50 °C by using QC concentrations in the range of 1×10^{-5}–1×10^{-3} M, and the resulting plot is shown in Figure 3. The parameters obtained by fitting these data to eq 6 are reported in Table 2.

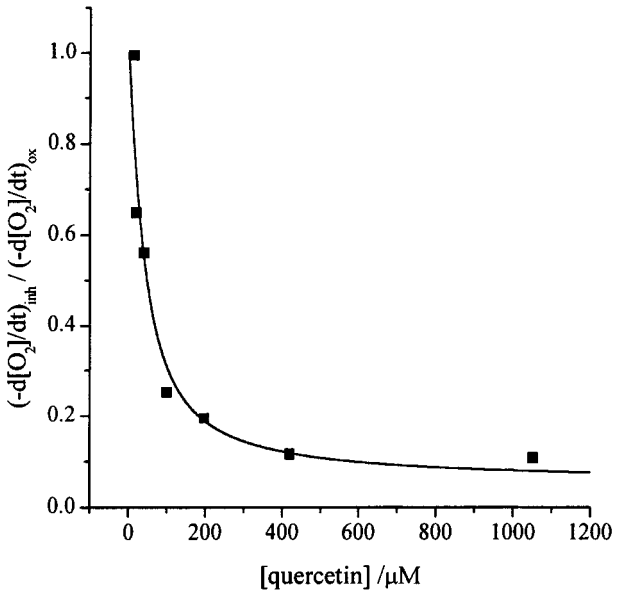

Figure 3. Ratio of the rates of oxygen uptake in the presence and absence of quercetin in *tert*-butyl alcohol at 50 °C at constant AIBN (0.0301 M) and ML (0.242 M).

Table 2. Inhibition Rate Constants of Quercetin and Epicatechin in *tert*-Butyl Alcohol at 50 °C

antioxidant	k_{AH}	β	$k_{inh}/10^4$ M^{-1} s^{-1}
quercetin	0.0172 ± 0.0043	0.946 ± 0.064	2.1 ± 0.5
epicatechin	0.0158 ± 0.0012	0.903 ± 0.018	1.7 ± 0.2
α-tocopherol			62.8[a]

[a] Reference *21*.

To calculate k_{inh} from k_{AH}, we estimated the rate of initiation, R_i, as $(8.4 \pm 0.8) \times 10^{-8}$ M s^{-1} from the length of the induction period requested to consume a given amount of αTOH, and we extrapolated the rate of termination, $2k_t$, of peroxyl radicals from ML at 50 °C as 1.7×10^7 M^{-1} s^{-1} from the value of E_a obtained by using for log A the reasonable value of 11.5 (*18*) and the reported value for $2k_t$ (8.8×10^6 M^{-1} s^{-1}) obtained at 30 °C in chlorobenzene (*19*). Because the termination rate constants of peroxyl radicals experience only a moderate, although not negligible, solvent effect (*20*), we have chosen the value in chlorobenzene in the absence of the actual value in *tert*-butyl alcohol.

The inhibition rate constant for QC at 50 °C in *tert*-butyl alcohol determined by this method is $k_{inh} = 2.1 \times 10^4$ M^{-1} s^{-1}.

The AIBN-induced oxidation of ML in *tert*-butyl alcohol at 50 °C was similarly studied in the presence of EC in the concentration range of 4×10^{-5}–6×10^{-4} M as inhibitor; the experimental oxygen uptake plots are shown in Figure 4 and the corresponding data are reported in Table 1.

Also in this case the oxidation of ML was retarded in the presence of the flavonoid, whereas no definite induction period was observed. Therefore, the determination of k_{inh} was done by using the Darley-Usmar method (see Figure 5). No EC concentrations $>6 \times 10^{-4}$ M could be used because of its low solubility in *tert*-butyl alcohol. The inhibition rate constant for EC was

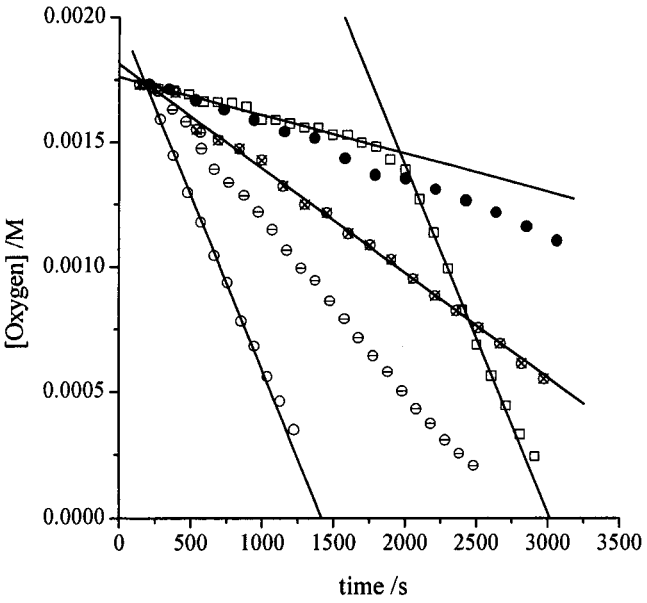

Figure 4. Oxygen depletion during autoxidation of ML (0.242 M) in *t*-ButOH initiated by AIBN (0.0344 M) at 50 °C in the absence (○) or presence of 8.19×10^{-5} M (⊖), 1.30×10^{-4} M (⊗), or 3.90×10^{-4} M (●) epicatechin or in the presence of 8.39×10^{-5} M (□) α-tocopherol in a closed system with no headspace.

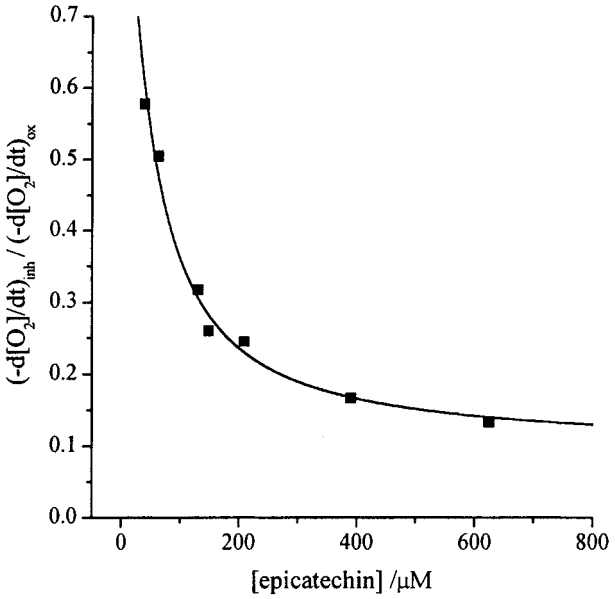

Figure 5. Ratio of the rates of oxygen uptake in the presence and absence of epicatechin in *tert*-butyl alcohol at 50 °C at constant AIBN (0.0344 M) and ML (0.242 M).

calculated as 1.7×10^4 M^{-1} s^{-1} by using $2k_t = 1.7 \times 10^7$ M^{-1} s^{-1} as before and $R_i = (7.3 \pm 0.6) \times 10^{-8}$ M s^{-1}.

The efficiency of QC and EC as antioxidants can be estimated by comparing their inhibition rate constants with that for αTOH under the same experimental conditions. Recently the inhibition rate constant was measured in *tert*-butyl alcohol at 37 °C to have the value of 5.1×10^5 M^{-1} s^{-1} (*21*). By assuming a log *A* of 8 for this radical molecule bimolecular reaction, on the basis of the fact that the measured log *A* factors for reaction 2 with a variety of phenols have been found to be lower than the value of 8.5 ± 0.5 for a simple atom-transfer reaction (*22*, *23*), k_{inh} for αTOH can be calculated as 6.28×10^5 M^{-1} s^{-1} at 50 °C. Therefore, QC and EC are 30 and 36 times less effective, respectively, than αTOH in inactivating peroxyl radicals in *tert*-butyl alcohol. On the other hand, the values of k_{inh} for the two flavonoids in *tert*-butyl alcohol are similar to those measured, under the same experimental conditions (*24*), for the synthetic antioxidants BHT (2,6-di-*tert*-butyl-4-methylphenol, $k_{inh} = 1.8 \times 10^4$ M^{-1} s^{-1}) and BHA (2,6-di-*tert*-butyl-4-methoxyphenol, $k_{inh} = 6.5 \times 10^4$ M^{-1} s^{-1}).

Antioxidant Activity of Flavonoids in Chlorobenzene. It has recently been reported that the rate constants for the hydrogen transfer reaction from the hydroxyl group of a phenol to a free radical show dramatic solvent effects. Ingold and co-workers thus found that in HBA solvents these rate constants are strongly decreased compared to those in non-hydrogen-bonding solvents (*8*). Such a kinetic solvent effect has been measured for phenol and αTOH and found to be of similar magnitude both in the reaction with stable radicals such as DPPH and with more reactive species such as peroxyl or alkoxyl radicals. The rate constant for the hydrogen atom transfer from αTOH to cumylperoxyl radicals at 25 °C thus decreases by ~2 orders of magnitude on passing from hexane, 2.0×10^7 M^{-1} s^{-1}, to ethyl acetate, 2.3×10^5 M^{-1} s^{-1} (*25*). This solvent effect has been explained in terms of hydrogen bonding between αTOH, which acts as hydrogen donor, and the solvent, which acts as hydrogen acceptor. In terms of the transition-state theory, the more solvating medium induces an additional stabilization of the reactants compared to the transition state, in effect increasing the activation energy of the reaction. The magnitude of this solvent effect is determined by the strength of the interaction between ArOH and the solvent and is, at least for simple phenols, independent of the nature of the radical abstracting the hydrogen atom.

Also, polyphenols are expected to be subjected to strong kinetic solvent effects in the reaction with peroxyl radicals. A decrease by 50-fold of k_{inh} for the 3,5-di-*tert*-butylcatechol has also been observed on passing from an organic solution of styrene in chlorobenzene to sodium dodecyl sulfate micelles containing ML (*26*). Therefore, to explore the solvent effect on flavonoids in a medium having a lower hydrogen-bond-accepting ability (*27*) than *tert*-butyl alcohol ($\beta_2^H = 0.49$), we have studied the autoxidation of ML in chlorobenzene ($\beta_2^H = 0.09$) in the absence and presence of QC and EC.

The autoxidations of 0.242 M ML in chlorobenzene at 50 °C were initiated by 0.035 M AIBN by using as inhibitor either QC, EC, or αTOH. Because flavonoids are scarcely soluble in chlorobenzene, they were first dissolved in *tert*-butyl alcohol, and then a small amount of this concentrated solution was added to chlorobenzene (the total amount of alcohol did not exceed 10% v/v). Oxygen consumption time traces obtained in the presence and in the absence of QC are reported in Figure 6. To convert the ESR data into oxygen concentrations, the value of the oxygen solubility in chlorobenzene in equilibrium with air, 1.61×10^{-3} M (*28*), was used.

Figure 6 shows that QC in chlorobenzene behaves as a chain-breaking inhibitor rather than as a retarder as was found in *tert*-butyl alcohol, as is evidenced by a clear induction period. The length of this induction period was found to depend on the flavonoid concentration, which enabled us to obtain the stoichiometric factor *n* (eq 1). The averaged values of *n* were 1.8 for QC and 1.7 for EC (see Table 3), thus indicating that the antioxidant mechanism of flavonoids in chlorobenzene is similar to that of αTOH (eqs 2 and 3).

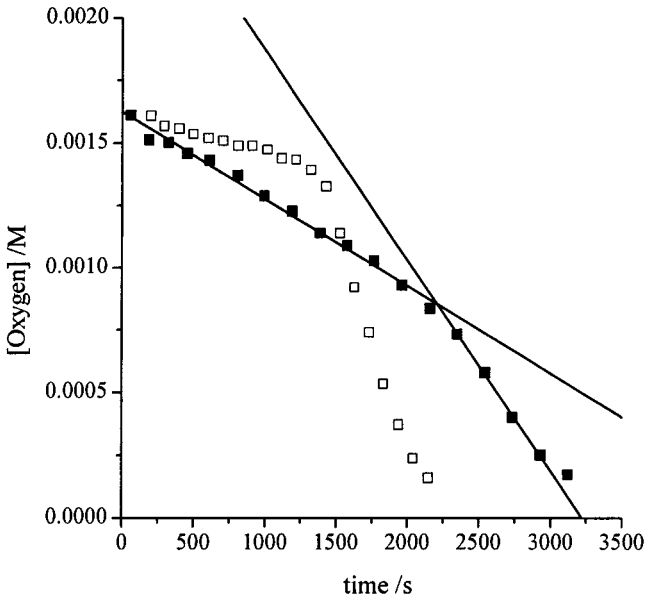

Figure 6. Oxygen depletion during autoxidation of ML (0.242 M) in chlorobenzene initiated by AIBN (0.035 M) at 50 °C in the presence of 1.4×10^{-4} M (■) quercetin or 7.98×10^{-5} M (□) α-tocopherol in a closed system with no headspace.

Table 3. Inhibition Rate Constants and Stoichiometric Factors for Quercetin and Epicatechin Measured in Chlorobenzene at 50 °C during the Peroxidation of Methyl Linoleate

antioxidant	$k_{inh}/10^5$ M^{-1} s^{-1}	n
quercetin	4.30 ± 0.7	1.8
epicatechin	4.18 ± 0.4	1.7
α-tocopherol	35.5[a]	2

[a] Reference *25*.

The rate constants for the inhibition of ML autoxidation in chlorobenzene by QC and EC were determined (see Table 3) by comparing the slopes of the oxygen uptake traces obtained in the presence of the flavonoid and in the presence of αTOH (eq 4), under conditions where the chain lengths were in the range four to six for the various flavonoid concentrations. It should be noted that QC and EC are characterized by similar inhibition rate constants in both media, despite their structural difference. Thus, the higher resonance stabilization expected for the phenoxyl radical of QC seems to play only a minor role in its antioxidant ability.

These rate constants are about 8 times slower than that of α-tocopherol under the same conditions. The value of 3.55×10^6 M^{-1} s^{-1} of αTOH at 50 °C has been calculated from the value of 2.7×10^6 M^{-1} s^{-1} at 25 °C of the rate constant for the hydrogen transfer reaction from αTOH to cumylperoxyl radicals (*25*) by assuming a log *A* of 8 (*23*). An examination of Tables 2 and 3 shows that the antioxidant efficiency in *tert*-butyl alcohol is reduced both for αTOH and for the flavonoids, but the effect is much larger for the flavonoids.

The antioxidant activity of the flavonoids may also be compared with the antioxidative activity of the hindered phenols BHT and BHA, which are currently used as food additives and for which k_{inh} has been measured in chlorobenzene at 50 °C to have values of 2.4×10^4 and 1.1×10^5 M^{-1} s^{-1}, respectively (*24*). Thus, the two investigated flavonoids are about 18 and 4 times more reactive than BHT and BHA, respectively.

It is also interesting to compare the kinetic data obtained in the present study for QC and EC with those recently measured for catechols in the inhibition of the autoxidation of neat styrene at 30 °C (*26*). The reported k_{inh} values are 5.5×10^5 M^{-1} s^{-1} for the unsubstituted catechol and 1.49×10^6 M^{-1} s^{-1} for 3,5-di-*tert*-butylcatechol (DTBC). These rate constants are very similar to those measured in the present work for QC and EC, an observation that strongly supports the suggestion that the catechol moiety is important for the antioxidant activity of flavonoids.

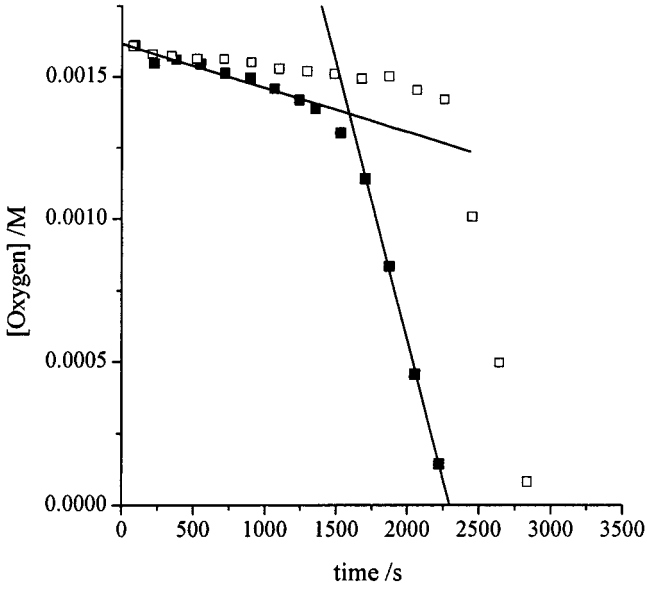

Figure 7. Oxygen depletion during autoxidation of cumene (4.31 M) in chlorobenzene initiated by AIBN (0.023 M) at 50 °C in the presence of 6.05×10^{-5} M (■) quercetin or 8.17×10^{-5} M (□) α-tocopherol in a closed system with no headspace.

The scavenging ability of QC and EC was also tested toward cumylperoxyl radicals. Cumene was chosen as oxidation substrate because cumene as a hydrocarbon does not contain carbonyl groups such as ML, which may behave as hydrogen bond acceptors for the hydroxyl protons of the flavonoids. Although the oxygen consumption traces showed a clear induction period in the presence of either QC or EC (see Figure 7), the chain lengths during these induction periods, even at high cumene concentrations (4.3 M), were too short (about two) to allow the determination of the inhibition rate constant. From these experiments we were able to obtain only the stoichiometric factor, which resulted again to be ∼2 for both flavonoids.

Careful examination of the traces of oxygen consumption recorded during the autoxidation of ML in the presence of both QC and EC shows that, when the induction period is over, the oxygen uptake is weakly retarded with respect to control samples (Figure 6). This retarding effect, observed after the catechol group has been completely oxidized to quinone, is presumably due to the A ring of the flavonoid, which should be still available for reacting with peroxyl radicals (*7*). Another possibility is that the retarding effect is due to the quinone itself. Actually, it has been reported that coenzyme Q, a quinone ubiquitous in biological systems, behaves as a weak antioxidant (*29*).

Medium Effects on the Antioxidant Ability of Quercetin and Epicatechin. The rate constants obtained in the present study for the reaction of QC and EC with the peroxyl radicals from ML undergo a substantial decrease when the medium is changed from chlorobenzene (4.3×10^5 and 4.2×10^5 M^{-1} s^{-1}) to *tert*-

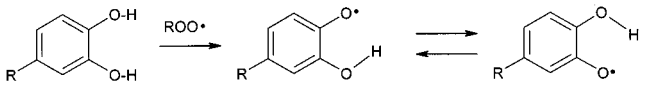

Figure 8. Stabilization of the aroxyl radical in the B ring of flavonoids by intramolecular H-bonding.

butyl alcohol (2.1×10^4 and 1.7×10^4 M^{-1} s^{-1}, respectively). Thus, the reduction for the flavonoids appears to be larger (a factor of 20) than that found for αTOH, which shows only a 4-fold decrease for the reaction with cumylperoxyl radicals (*25*).

Kinetic solvent effects for catechols have been reported to be stronger than for simple phenols in their reaction with free radicals. Actually, the rate of H-atom transfer from 3,5-di-*tert*-butylcatechol to 1,1-diphenyl-2-picrylhydrazyl (DPPH) has been found to decrease by a factor of 360 by changing the solvent from hexane to *tert*-butyl alcohol, whereas 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) undergoes a decrease of only 30 times in the two solvents (*30*). A similar decrease by a factor of 36 in the value of k_{inh} for the reaction of αTOH with cumylperoxyl radicals has been measured by Valgimigli et al. (*25*) in the change from hexane to *tert*-butyl alcohol.

The stronger solvation of the semiquinone radicals of QC and catechin in hydrogen-bonding solvents compared to the flavonoids is further seen by the lowering of the reduction potential by ~700 mV when water is compared with dimethylformamide (*31*).

It is worth pointing out that, because under our experimental conditions chlorobenzene contains 10% of *tert*-butyl alcohol, k_{inh} is expected to be higher in neat chlorobenzene. Thus, the kinetic solvent effect for flavonoids is presumably even larger than that reported here.

Barclay et al. (*30*) have recently suggested that the main factor controlling the activity of catechols as antioxidant (and therefore also of most flavonoids) is the stabilization by intramolecular H-bonding of the aroxyl radical formed after the inactivation of the (first) peroxyl radical (Figure 8).

Solvents with high HBA ability will interfere with this stabilization through an intermolecular H-bonding. Thus, the combined effect of a greater stabilization of the catechol (or flavonoid) due to better solvation and of a destabilization of the aroxyl radical primarily formed is expected to increase the bond dissociation energy of the polyphenol relative to hydrocarbon solvents. This effect, together with steric hindrance for the approach of the peroxyl radical to the solvent complexed phenol, will reduce the rate constant of the H-atom abstraction.

CONCLUSION

The antioxidant ability of two flavonoids has been evaluated by determining the absolute kinetic constants of the H-transfer reaction to peroxyl radicals in a hydrogen-bond-accepting solvent and in a non-hydrogen-bonding solvent in order to compare antioxidant mechanisms in aqueous solutions and in apolar lipids. The rate constants are strongly affected by the nature of the solvent in which the autoxidation takes place. Clearly, one parameter such as a single rate constant is not sufficient to describe the antioxidant activity of a given compound but has to be combined with knowledge of the antioxidant environment. We have found a substantial decrease (a factor of 20) in the inhibition rate constants of flavonoids toward the peroxyl radicals from methyl linoleate by changing the solvent from the non-hydrogen-bonding solvent chlorobenzene to the hydrogen-bond-accepting solvent *tert*-butyl alcohol. This reduction in rate constant is much larger than the 4-fold reduction found for α-tocopherol. Apparently the hydrogen-bond-accepting solvents affect for the flavonoid not only the energy of the phenolic antioxidant as for α-tocopherol but also the energy of the intermediate aroxyl semiquinone radical.

Such considerations will be even more important in the heterogeneous phases of phospholipid bilayers, where the localization of antioxidants should be known in order to understand the effectiveness of their antioxidant activities. α-Tocopherol seems to be located in the lipophilic part of membranes in which its chromane ring is stabilized by the hydrogen bonding with ester carbonyl groups of phospholipids, in effect resulting in a decrease of several orders of magnitude in the value of k_{inh} (*32*). Flavonoids are more hydrophilic than α-tocopherol, and they are located in the polar surface region of the phospholipid bilayers (*33*). Flavonoids are thus likely to have a role in protecting the membranes against radicals formed in the aqueous phase or to interact with α-tocopherol on the polar surface. Our further studies will focus on the interaction between flavonoids and α-tocopherol to provide a basis for a discussion of antioxidant processes in interphases of heterogeneous systems.

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