Kinetic Solvent Effects on Phenolic Antioxidants Determined by Spectrophotometric Measurements

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The effects of polar (acetonitrile and *tert*-butyl alcohol) and apolar (cyclohexane) solvents on the peroxyl-radical-trapping antioxidant activity of some flavonoids, catechol derivatives, hydroquinone, and monophenols have been studied. The inhibition rate constants k_{inh} of the antioxidants have been determined by following the increase in absorbance at 234 nm of a dilute solution of linoleic acid at 50 °C containing *small amounts* of antioxidant and radical initiator. Despite the low concentration of linoleic acid, the peroxidation process has been confirmed to be a free radical chain reaction described by the classical kinetic laws for this process. However, in the evaluation of k_{inh}, a careful analysis of the peroxidation curve, absorbance versus time, must be done because the final oxidation products of phenols *may* absorb at 234 nm. Phenols with two *ortho*-hydroxyls are the most active antioxidants, with inhibition rate constants in the range of $(3-15) \times 10^5$ $M^{-1}\cdot s^{-1}$ (in cyclohexane). Nevertheless, it has been observed that in *tert*-butyl alcohol (a strong hydrogen bond acceptor) the rate constants dramatically decline to values not detectable by the present kinetic method. In acetonitrile (a weaker hydrogen bond acceptor) instead, the phenols with two *ortho*-hydroxyls scavenge the peroxyl radicals with rate constants close to those in cyclohexane. From the kinetic solvent effect, the equilibrium constant of the first solvation step of hydroquinone with *tert*-butyl alcohol has been determined at 50 °C, $K_1 = 2.5 \pm 0.5$ M^{-1}.

Keywords: *Antioxidant; peroxyl radical; flavonoids; catechols; kinetic solvent effect; inhibition rate constant; linoleic acid peroxidation; hydrogen bonding; conjugated diene hydroperoxides*

INTRODUCTION

The antioxidant activity of phenols in a lipid autoxidation process depends on their ability to transfer a phenolic H atom to peroxyl radicals (Barclay, 1993). The mechanism of H-atom transfer between phenol and peroxyl radical involves the prior equilibrium formation of an H-bonded complex between the species, reaction 1 (Foti et al., 1994). Within this complex a direct H transfer *may* occur, yielding the products provided that a suitable orientation is achieved, reaction 2 (Foti et al., 1994). In solvents with high dielectric constants and when the redox potential difference between ROO$^\bullet$ and ArOH, $\Delta E_{ROO^\bullet-ArOH}$, is >0, a one-electron-transfer reaction from phenol to peroxyl radical is more likely involved (Jovanovic et al., 1992; Simic et al., 1992; Valgimigli et al., 1996). The overall H-atom abstraction from ArOH takes place in two consecutive steps.

$$ROO^\bullet + ArOH \rightleftharpoons (ROO^\bullet\cdots HOAr)_{\text{solvent cage}} \quad (1)$$

$$(ROO^\bullet\cdots HOAr)_{\text{solvent cage}} \rightarrow (ROOH\cdots{}^\bullet OAr)_{\text{solvent cage}} \rightarrow \text{diffusion} \quad (2)$$

$$ROO^\bullet + HOAr \rightarrow (ROO^-\cdots{}^{+\bullet}HOAr)_{\text{solvent cage}} \rightarrow (ROOH\cdots{}^\bullet OAr)_{\text{solvent cage}} \rightarrow \text{diffusion} \quad (3)$$

$$ROO^\bullet + HOAr \rightarrow (ROO^-\cdots{}^{+\bullet}HOAr)_{\text{solvent cage}} \rightarrow ROO^- + H^+ + ArO^\bullet \quad (4)$$

After the electron transfer, a proton is abstracted in a second step from the radical cation ArOH$^{\bullet+}$ by the peroxyl anion ROO$^-$ (reaction 3) or by a molecule of solvent (reaction 4).

Polar solvents make the electron-transfer reaction easy because of the stabilizing effect on the ionic pair; therefore, they could accelerate the overall process of H transfer. However, at the same time, they can hinder the formation of the complex between ArOH and ROO$^\bullet$ because of the preferential formation of a hydrogen-bonded complex between ArOH (as hydrogen bond donor, HBD) and a molecule of solvent (as hydrogen bond acceptor, HBA). In this case the overall H transfer would be slowed. Actually, it will be the fine balance of these "divergent" effects of solvation that determines the final effect of the solvent S on the apparent inhibition rate constant k_{inh}^S(ROO$^\bullet$ + ArOH). The experimental results for ArOH = α-tocopherol (α-TOH) in a number of solvents show that the interference with the ArOH/ROO$^\bullet$ complex is prevailing and the values of k_{inh}^S decrease as the HBA ability of the solvent increases (Valgimigli et al., 1996, 1999). Actually, most of the data on the kinetic solvent effects concern the H-atom abstraction from ArOH by alkoxyl radicals [*tert*-butoxyl radicals (BO$^\bullet$) and cumyloxyl radicals (CumO$^\bullet$)] and 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH$^\bullet$) (Valgimigli et al., 1995, 1996; MacFaul et al., 1996). With these radicals and ArOH = α-TOH, its analogues, and a few other phenols, a notable lowering of the rate constants proportional to the HBA ability of the solvent has been

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10.1021/jf0006527 CCC: $20.00
Published on Web 11/28/2000

observed. For these reactions Ingold and co-workers (Avila et al., 1995; Banks et al., 1996) have provided the way to quantify the effect of hydrogen bonding on the rate constants with the simple hypothesis of considering the fraction of phenolic antioxidant bonded to the polar solvent unreactive toward the abstracting radicals, reactions 5 and 6.

$$\mathrm{ArOH}_{\mathrm{reactive}} + \mathrm{S} \overset{K}{\rightleftharpoons} (\mathrm{ArOH}\cdots\mathrm{S})_{\mathrm{unreactive}} \qquad (5)$$

where S is the polar solvent and K is the equilibrium constant

$$\mathrm{ArOH}_{\mathrm{reactive}} + \mathrm{RO}^{\bullet} \xrightarrow[\mathrm{S}]{k_6{}^{\mathrm{s}}} \mathrm{ArO}^{\bullet} + \mathrm{ROH} \qquad (6)$$

$$k_6{}^{\mathrm{s}} = k_6/(1 + K[\mathrm{S}]) \qquad (7)$$

where k_6 is the rate constant in apolar medium. It is worthwhile observing that the reduction in the rate of hydrogen atom abstraction is *independent* of the reacting free radical BO$^{\bullet}$, CumO$^{\bullet}$, or DPPH$^{\bullet}$ (Valgimigli et al., 1995) (see also eq 7). It depends only on the strength of the interaction between phenol and solvent or, in other terms, on the value of K. This experimental conclusion has been extended to peroxyl radicals ROO$^{\bullet}$ as well (Valgimigli et al., 1996).

Among the natural phenolic compounds to investigate for their antioxidant properties, flavonoids continue to receive great attention because they are widespread in nature and effective antioxidants (especially those containing a catecholic structure on the B ring) (Jovanovic et al., 1994). Some of these compounds have already been investigated in an aqueous micellar system containing linoleic acid (Foti et al., 1996). However, the solvent effects on this class of compounds (and simple dihydroxybenzenes) for the reaction with peroxyl radicals ROO$^{\bullet}$ are poorly documented, and the few published results are limited to model compounds (Barclay et al., 1999). This kind of investigation would be of outstanding importance because flavonoids (normally ingested with the diet) are mainly concentrated in the gastrointestinal tract in the human body (Jovanovic et al., 1994), where their radical-trapping antioxidant activity can be limited by the presence of water. Consequently, the actual antioxidant activity of representative molecules of this class of compounds together with simple monophenols and dihydroxybenzenes (Figure 1) was evaluated in cyclohexane, acetonitrile, and *tert*-butyl alcohol. Specifically, the solvent effect on their activity in a lipid peroxidation process was taken into account, in view of the role this adverse process has on human health (Halliwell, 1987; Bowry and Ingold, 1999) and the quality of preserved food (St. Angelo, 1992).

The method used in a previous paper (Foti et al., 1996) for determining the values of k_{inh} (relative to α-tocopherol) for flavonoids was based on the spectrophotometric measurement at 234 nm of the rate of formation of conjugated diene hydroperoxides from linoleic acid (Pryor et al., 1993). In the present work, this spectrophotometric method was slightly modified, to adapt it to a homogeneous phase, and, finally, its reliability was also examined.

MATERIALS AND METHODS

General. Linoleic acid (99%) was purchased from Sigma. The radical initiator 2,2′-azobis(isobutyronitrile) (>98%) (AIBN) was from Merck. Quercetin dihydrate (99%), chrysin (98%),

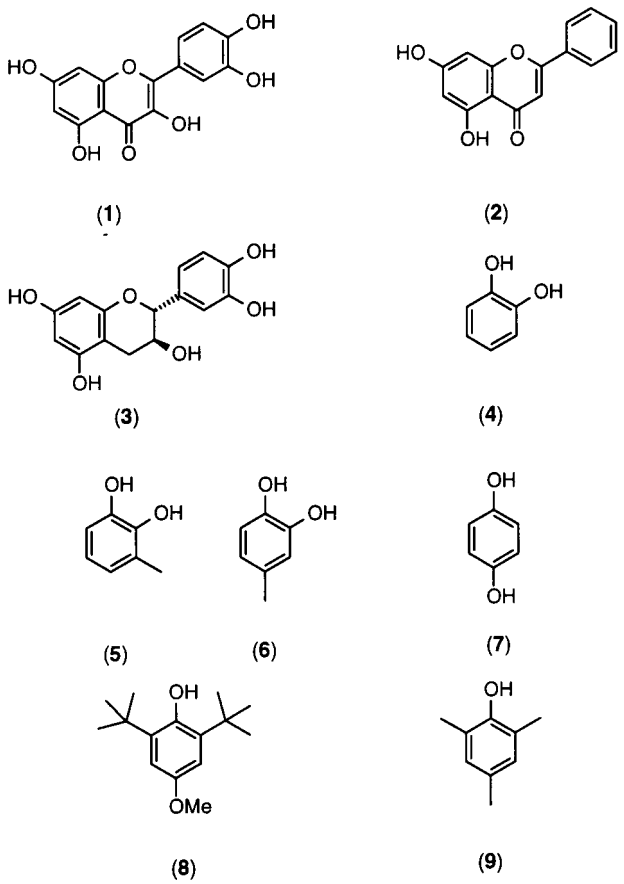


Figure 1. Representative structures of flavonoid and phenolic antioxidants.

catechol (>98%), 4-methylcatechol (97%), 2,6-di-*tert*-butyl-4-methoxyphenol (>97%), and 2,4,6-trimethylphenol (97%) were bought from Fluka; 3-methylcatechol (99%) and catechin hydrate (98%) were from Aldrich, and hydroquinone (99%) was from Sigma. Cyclohexane was purchased from Lab-Scan, acetonitrile from Lichrosolv, *tert*-butyl alcohol from Aldrich, and silica gel from Merck. All chemicals and solvents were of analytical grade, and most of them were used without any further purification. AIBN was recrystallized twice from methanol and then stored at −20 °C. 4-Methylcatechol, 3-methylcatechol, and catechol were recrystallized from ethanol/cyclohexane. Linoleic acid was always passed over silica gel in a glass pipette before use. Kinetics were recorded on a Beckman DU-65 spectrophotometer equipped with a thermostated cuvette housing and a PC to process the kinetic data. The temperature of the cuvette housing was set at 50 ± 0.5 °C.

Solutions of Linoleic Acid. When the experiments were run in acetonitrile, the solutions of linoleic acid were left in contact with silica gel (∼1.0 g/25 mL of solution), and occasionally shaken, for 15−20 min. The final concentration of the solutions in the various solvents was (5−23) × 10^{-3} M for the determination of the orders of reaction, whereas in the experiments on the antioxidant activities of compounds it was always (1.1−1.3) × 10^{-2} M. The solutions were prepared immediately prior to use.

Solutions of AIBN. In acetonitrile concentrated stock solutions (0.1 M) were prepared, stored at 5 °C, and used within a week. In cyclohexane and *tert*-butyl alcohol, solutions of ∼2.2 × 10^{-3} M were prepared (to dissolve the material, the solution was sonicated). The final concentration of AIBN in the experiments for the determination of antioxidant activities was (0.7−1.1) × 10^{-3} M. For the orders of reaction, final concentrations in the range of (0.7−70) × 10^{-3} M were used.

Solutions of Antioxidants. Stock solutions [(3−9) × 10^{-4} M] of the antioxidants were prepared in the solvent in use

immediately before the experiments were begun. For the solutions in cyclohexane of some antioxidants (quercetin, catechin, catechol, and hydroquinone) the addition of acetonitrile (200 μL in 5.0 mL of solution) was necessary. The final concentrations of antioxidant in the reaction system were $8 \times 10^{-7}-5 \times 10^{-6}$ M.

Procedure in Cyclohexane and *tert*-Butyl Alcohol. Linoleic acid solution (1 mL) and AIBN solution (1 mL) were mixed in a UV cell, and the resulting solution was stirred in the sample compartment of the spectrophotometer at 50 °C. Neat cyclohexane or *tert*-butyl alcohol were used as blank. The progress of the peroxidation was observed by recording the absorbance at 234 nm until a linear plot was obtained (~8−10 min), and then scalar amounts of antioxidant solution (5−50 μL) were added and the kinetics followed until the antioxidant was consumed (induction period) and the slop of the curve reassumed the initial value.

Procedure in Acetonitrile. In this solvent a slightly different procedure from the above was used. A linoleic acid solution (2 mL) containing scalar amounts of antioxidant solution (5−50 μL) was stirred in a UV cell placed in the cuvette housing at 50 °C. Neat acetonitrile was used as blank. After thermal equilibration (8−10 min), 20 μL of the stock solution of AIBN was added and the inhibited kinetics followed by recording the absorbance at 234 nm until the antioxidant was entirely consumed and the uninhibited peroxidation process defined.

Determination of the Molar Extinction Coefficients of Conjugated Diene Hydroperoxides (CD). The values of ϵ_{234} for linoleic acid's CD in the various solvents were determined according to the procedure adopted by Pryor et al. (1993).

Kinetic Aspects. The radical initiation rate R_i was measured with the induction period method (Burton and Ingold, 1981) by using 2,6-di-*tert*-butyl-4-methoxyphenol (in cyclohexane) and catechol (in acetonitrile) as scavengers. For both it has been assumed that *two* peroxyl radicals are scavenged per molecule of antioxidant. The *oxidizability* of linoleic acid $k_p/(2k_t)^{1/2}$ (Barclay, 1993) was consequently calculated through eq 11. The induction period τ in the curves of peroxidation corresponds to the time interval between the addition of antioxidant and the point of intersection of tangents to the uninhibited and inhibited tracts of the curve. Finally, the factor 0.97 was used to correct the concentrations of all compounds for the thermal expansion of solvents at 50 °C.

RESULTS

Kinetic Method. Linoleic acid (H_2L) represents a good model to study the kinetics of lipid peroxidation because it has two mobile bis-allylic hydrogens (at position 11) having a C−H bond energy of only 75 kcal/mol (Simic et al., 1992). In fact, it is rather simple to start a free radical chain process of peroxidation on this fatty acid with the help of a radical initiator (e.g., azocompounds), reactions 8−10. The rate constant for the propagation step k_p is comparatively large, ~50 $M^{-1}\cdot s^{-1}$ (Bowry and Stocker, 1993). The initial oxidation products of this process are conjugated diene hydroperoxides HLOOH (CD) with a strong absorption band at 234 nm (the molar extinction coefficients are 25500 $M^{-1}\cdot cm^{-1}$ in cyclohexane, 30500 $M^{-1}\cdot cm^{-1}$ in *tert*-butyl alcohol, and 29100 $M^{-1}\cdot cm^{-1}$ in acetonitrile).

$$R{-}N{=}N{-}R \xrightarrow{k_d e} 2R^{\bullet} + N_2 \text{ (initiation)} \quad (8)$$

$$R^{\bullet} + O_2 \xrightarrow{\text{fast}} ROO^{\bullet}$$

where the rate of radical production $= d[ROO^{\bullet}]/dt = R_i = 2k_d e[R_2N_2]$.

$$ROO^{\bullet}\ (HLOO^{\bullet}) + H_2L \xrightarrow{k_p} ROOH\ (HLOOH) + HL^{\bullet}\ \text{(propagation)} \quad (9)$$

$$HL^{\bullet} + O_2 \xrightarrow{\text{fast}} HLOO^{\bullet}$$

$$2HLOO^{\bullet} \xrightarrow{k_t} \text{nonradical products (termination)} \quad (10)$$

The consumption of oxygen or linoleic acid over time represents the overall rate of oxidation $-V_{O_2}$ and is correlated to the involved rate constants and the concentrations of initiator and linoleic acid via the simple equation (Burton and Ingold, 1981)

$$-V_{O_2} = \frac{k_p}{(2k_t)^{1/2}}[H_2L]R_i^{1/2} \quad (11)$$

There exists another useful and readily obtainable parameter to evaluate the extent of oxidation, namely, the rate of CD formation, V_{CD}. However, CDs are not stable compounds and decompose easily (Pryor et al., 1993). Hence, the rate of oxidation measured via CD formation is lower than the real value $-V_{O_2}$. It has been demonstrated that $-V_{O_2} = 1.19V_{CD}$ (Pryor et al., 1993). Thus, eq 11 becomes

$$V_{CD} = \frac{k_p}{1.19(2k_t)^{1/2}}[H_2L]R_i^{1/2} \quad (12)$$

Obviously, $V_{CD} = (dA_{234}/dt)/\epsilon$, where A_{234} is the absorbance at 234 nm and ϵ the extinction coefficient of HLOOH in the solvent in use. With the CD method it is not possible to explore a large range of linoleic acid concentration because even the purest commercially available acid already contains ~0.1−0.3% of hydroperoxides; hence, at high concentration the initial absorbance of the solution would be too high for an accurate investigation. For this reason the concentration of the solutions of linoleic acid was kept in the range of 5−23 mM. The peroxidation process was initiated with AIBN at 50 °C, generally in comparatively low concentration, 0.7−1.1 mM. To verify whether eq 12 applies to these systems, the orders of reaction with respect to linoleic acid and AIBN were determined in cyclohexane, acetonitrile, and *tert*-butyl alcohol. The results are summarized in Table 1.

Peroxyl radicals do *not* self-quench in the presence of sufficient concentrations of *true* antioxidants, and reaction 10 is substituted by reactions 13 and 14 (Burton and Ingold, 1981).

$$ArOH + HLOO^{\bullet} \xrightarrow{k_{inh}} ArO^{\bullet} + HLOOH \quad (13)$$

$$ArO^{\bullet} + HLOO^{\bullet} \xrightarrow{k'_t} \text{termination} \quad (14)$$

Equation 12 becomes

$$V_{CD,inh} = \frac{k_p}{1.19k_{inh}}\frac{[H_2L]}{2[ArOH]}R_i \quad (15)$$

The steady-state kinetic treatment on the transient species ($ArO^{\bullet}$ and $HLOO^{\bullet}$) shows that the rate of ArOH consumption is equal to $R_i/2$ and the duration time of ArOH (induction period, τ) well-defined, $\tau = 2[ArOH]_{t=0}/R_i$. To determine the inhibition rate constants, the

Table 1. Values of the Orders of Reaction[a] with Respect to [H_2L] and [AIBN] and Values of Oxidizability of Linoleic Acid $k_p/(2k_t)^{1/2}$ and $2k_de$ for AIBN at 50 °C

solvent	order of H_2L	order of AIBN	$k_p/(2k_t)^{1/2}$ ($M^{-1/2}\cdot s^{-1/2}$)	$2k_de^b$ (s^{-1})
C_6H_{12}	0.99 ± 0.05	0.61 ± 0.04	0.027 ± 0.003	(1.36 ± 0.09) × 10^{-6}
CH_3CN	1.02 ± 0.05	0.55 ± 0.04	0.030 ± 0.003	(2.00 ± 0.07) × 10^{-6}
$(CH_3)_3COH$	1.04 ± 0.05	0.55 ± 0.05	nd[c]	nd[c]

[a] Orders of reaction for the uninhibited peroxidation of linoleic acid (eq 12). The results are the average of four groups of experiments, and the straight lines obtained were excellent, $r > 0.998$. [b] Determined with the induction period method by using 2,6-di-*tert*-butyl-4-methoxyphenol as antioxidant (see Materials and Methods). [c] Not determined because in this solvent the technique of the induction period (see Materials and Methods) did not allow the determination of R_i because no phenol showed a definite induction period.

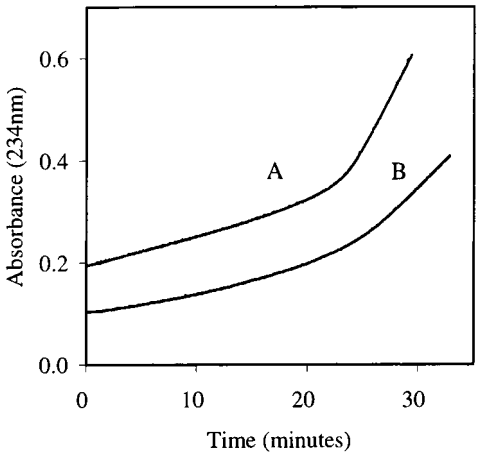


Figure 2. Peroxidation curves of linoleic acid at 50 °C: (A) in the presence of 3.98 × 10^{-6} M quercetin ([H_2L] = 1.08 × 10^{-2} M, [AIBN] = 2.90 × 10^{-3} M; solvent, acetonitrile); (B) in the presence of 1.55 × 10^{-6} M hydroquinone ([H_2L] = 1.11 × 10^{-2} M, [AIBN] = 1.50 × 10^{-3} M; solvent, cyclohexane).

integrated form of eq 15 is usually utilized

$$\frac{k_{inh}}{k_p} = -\frac{\epsilon_{234}}{1.19}[H_2L]\frac{\ln(1 - t/\tau)}{\Delta A_{234}} \qquad (16)$$

where ϵ_{234} is the molar extinction coefficient of CD at 234 nm, [H_2L] is the linoleic acid concentration, ΔA_{234} is the increase of absorbance at time t, and τ is the induction period (obtainable from the curve A_{234} versus time; see also Kinetic Aspects under Materials and Methods). Figure 2 shows some of these curves, whereas in Table 2 the values of k_{inh}/k_p obtained in cyclohexane and acetonitrile (in *tert*-butyl alcohol there is no induction period) for compounds **1**−**9** are presented. These values can be turned into absolute rate constants k_{inh} because the value of k_p for linoleic acid is known ($k_p \sim$ 50 $M^{-1}\cdot s^{-1}$).

If the antioxidants are not able to capture all peroxyls (retarders rather than antioxidants), in other words, if a fraction of reaction 10 persists in the system, eq 15 is not valid. In this case a steady-state kinetic analysis of the reactions involved has led to the following (Foti, unpublished results):

$$\frac{V_{CD}}{V_{CD,inh}} - \frac{V_{CD,inh}}{V_{CD}} = \frac{nk_{inh}[ArOH]}{(2k_tR_i)^{1/2}} \qquad (17)$$

[V_{CD} and $V_{CD,\,inh}$ are the rates of reaction in the absence and presence, respectively, of antioxidant, and n is the stoichiometric factor of phenol (i.e., the number of peroxyls that react with one molecule of antioxidant, generally $n \approx 2$)] with the simplification of neglecting the possible bimolecular self-quenching of aryloxyl radicals $ArO^\bullet$. Here the rate of ArOH consumption is no longer constant as above (now its value is $R_i/2 - k_t[HLOO^\bullet]^2$), and as a macroscopic consequence there is the disappearance of a definite induction period, τ (see Figure 3).

Table 2. Antioxidant Efficiencies k_{inh}/k_p[a] and Absolute Rate Constants[b] k_{inh} for the Reaction ArOH + $HLOO^\bullet$ in Cyclohexane and Acetonitrile,[c] at 50 °C

	$10^{-3}k_{inh}/k_p$		$10^{-4}k_{inh}$ ($M^{-1}\cdot s^{-1}$)	
compound	C_6H_{12}	CH_3CN	C_6H_{12}	CH_3CN
quercetin (**1**)	6.0	2.2	30	11
chrysin (**2**)	0.5	na[d]	2.5	
catechin (**3**)	nd[e]	nd		
catechol (**4**)	10	6.0	50	30
3-methylcatechol (**5**)	17	4.0	85	20
4-methylcatechol (**6**)	30	8.0	150	40
hydroquinone (**7**)	5.4	na	27	
2,6-di-*tert*-butyl-4-methoxyphenol (**8**)	2.3	na	11	
2,4,6-trimethylphenol (**9**)	1.4	na	7	

[a] Experimental error ±20%. Average of the values of three to four experiments. [b] Calculated with k_p = 50 $M^{-1}\cdot s^{-1}$ for linoleic acid. [c] In *tert*-butyl alcohol no compound behaves as antioxidant but rather as retardant. [d] Low antioxidant activity. The present kinetic method does not allow estimation of the values of k_{inh}/k_p roughly inferior to (1−2) × 10^2. [e] Activity not detectable by the present technique, but this compound does react with peroxyl radicals (see Discussion).

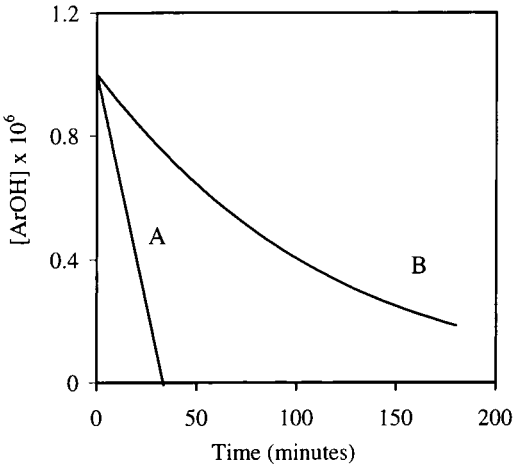


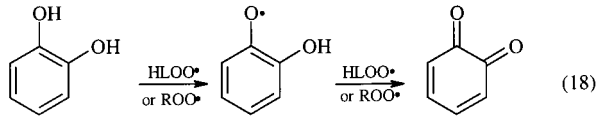
Figure 3. Theoretical trends [ArOH] versus time during an inhibited peroxidation process of linoleic acid: (A) for an effective antioxidant $[ArOH]_t = [ArOH]_0 - (R_i/2)t$ (the calculation was done for $[ArOH]_0 = 1 \times 10^{-6}$ M; $R_i = 1 \times 10^{-9}$ M/s); (B) for a weak antioxidant $[ArOH]_{t+\Delta t} = [ArOH]_t - (R_i/2 - k_t[HLOO^\bullet]_t^2)\Delta t$, $[HLOO^\bullet]_t = -k_{inh}/(2k_t)[ArOH]_t + [(k_{inh}/(2k_t)[ArOH]_t)^2 + R_i/(2k_t)]^{1/2}$ (the calculation was done with a numerical method for $[ArOH]_0 = 1 \times 10^{-6}$ M; $R_i = 1 \times 10^{-9}$ M/s, $k_{inh} = 9 \times 10^3$ $M^{-1}\cdot s^{-1}$, $2k_t = 3 \times 10^6$ $M^{-1}\cdot s^{-1}$).

DISCUSSION

The data presented in Table 1 show that in the absence of antioxidant the order of reaction for AIBN

is slightly larger than the expected value of 0.5, whereas for linoleic acid it is undoubtedly 1 in any solvent as eq 12 implies. Others have obtained similar results for AIBN (Barclay et al., 1987), and the general explanation is that at lower concentration some first-order chain termination of peroxyl radicals occurs simultaneously with their canonical bimolecular self-quenching (reaction 10). Nevertheless, this "side reaction" does not impair the conclusion that reactions 8−10 (and hence eq 12) describe the kinetics of the process. In fact, despite the low concentration of linoleic acid, the chain reaction length ν (namely, the number of oxygen molecules absorbed per initiating radical, $-V_{O_2}/R_i$) was always sufficiently large (owing to the low value of R_i) to allow a chain oxidation process (ν, in the range of 7−18 according to [H_2L] and [AIBN]).

In the presence of an antioxidant, the inhibited process of peroxidation is governed by eq 15, and through its integrated version (eq 16) the ratio k_{inh}/k_p is readily obtained. Although the procedure is simple, caution must be used in the evaluation of this ratio because the observed increase in the absorbance ΔA_{234} *may* be partly due to the formation of oxidation products from the phenol. For instance, catechol and hydroquinone react with peroxyls, yielding as end products *o*-quinone and *p*-quinone, respectively, which absorb at 234 nm, reactions 18 and 19. The formation of these



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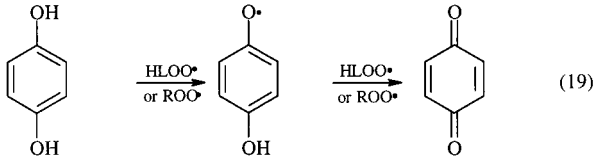


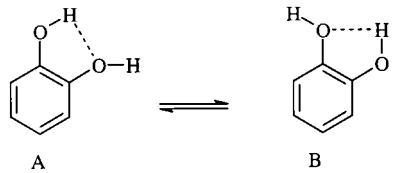
(19)

products occurs at a constant rate equal to $R_i/2$ (the rate of antioxidant consumption); therefore, the contribution to ΔA_{234} at time t is $\Delta\epsilon_{234}(R_i/2)t$, where $\Delta\epsilon_{234}$ is the difference in the extinction coefficients at 234 nm between product and reactant. Thus, to minimize this interference, it is necessary to use low concentrations of radical initiator, so that the term $R_i/2$ is small, and the calculation of k_{inh}/k_p should be done at the beginning ($t < \tau/2$) of the oxidation curve when the contribution of $\Delta\epsilon_{234}(R_i/2)t$ is negligible. The direct reaction between antioxidant and radical initiator at the concentration in use will provide the term $\Delta\epsilon_{234}R_i/2$. As it is possible to observe, this term is independent of the antioxidant concentration.

From Table 2 it is evident that the presence of two *ortho*-hydroxyls in the phenols has a marked effect on their reactivity. In fact, a 2−5-fold increase in the rate constants of catechol derivatives with respect to hydroquinone has been detected and well beyond this range with respect to 2,6-di-*tert*-butyl-4-methoxyphenol because in this case the rate constants are 5−13 times larger (for a combination in the latter of statistical effect due to the presence of only one OH and steric hindrance caused by *tert*-butyl groups). The explanation for this strong antioxidant activity must be found in the facile formation of the transient aryloxyl radical ArO$^\bullet$ for catechol structures, because the presence of a hydrogen bond between the oxygen with the unpaired electron and the adjacent hydroxyl lowers the activation energy of reaction 18 (Xi and Barclay, 1998; Barclay et al., 1999) (vide infra). No intramolecular hydrogen bond is possible instead with the radical involved in the reaction 19. From the values of k_{inh}, a difference of 0.4 kcal/mol in Gibbs energy of activation $\Delta\Delta G^{\#}_{323}$ can be estimated between reactions 19 and 18. Greater values are calculated for 3-methylcatechol (0.7 kcal/mol) and 4-methylcatechol (1.1 kcal/mol) for the presence on the aromatic ring of electron-donating alkyl groups providing additional stabilization for the transient aryloxyl radical. It would appear that the behavior emphasized above is not observed with quercetin because the ratio k_{inh}/k_p is as great as that for hydroquinone (in C_6H_{12}: 6.0×10^3 versus 5.4×10^3, respectively) despite the presence of two *ortho*-hydroxyls on its B ring. This would suggest that some (unknown) side reaction occurs with quercetin when it is added to linoleic acid/AIBN solutions. No formation of absorbing oxidation products at 234 nm was observed when quercetin reacted with peroxyls from AIBN, in the absence of linoleic acid.

From the data presented in Table 2 another peculiar property of the *ortho*-dihydroxybenzenes is readily recognized. As may be observed, in neat acetonitrile their antioxidant activity is only slightly reduced. For example, for catechol k_{inh} is 5×10^5 $M^{-1}\cdot s^{-1}$ in cyclohexane and 3×10^5 $M^{-1}\cdot s^{-1}$ in acetonitrile. On the contrary, hydroquinone and monophenols showed a dramatic decrease in the inhibition rate constants in the same polar solvent because no definite induction period was observed (in other words, they behaved like retarders). In *tert*-butyl alcohol, even phenols with catechol structures showed in any case the same collapse in the antioxidant activity. The β scale of HBA basicities shows that *tert*-butyl alcohol is a better HBA solvent than acetonitrile (Kamlet et al., 1983). In fact, the values of K determined by eq 7 at 25 °C when ArOH = phenol (see reaction 5) are 3.5 M^{-1} for acetonitrile and 14 M^{-1} for *tert*-butyl alcohol (Banks et al., 1996), whereas the concentrations of neat solvents are, respectively, 17.8 and 10.5 M. Therefore, the term K_{t-BuOH}[*t*-BuOH] > K_{MeCN}[MeCN] (see eq 7) and the kinetic solvent effect in *tert*-butyl alcohol, namely, the reduction of k_{inh}, is expected to exceed that in acetonitrile, in agreement with our current results.

The different behaviors of *ortho*-dihydroxybenzenes with respect to hydroquinone can be understood by considering the kind and strength of the hydrogen bond involved with the solvent *and* the different stability of the transient semiquinone radicals produced in reactions 18 and 19. In solution, catechol (and the other correlated compounds) carries out a relatively rapid swing between structures A and B. Both the structures



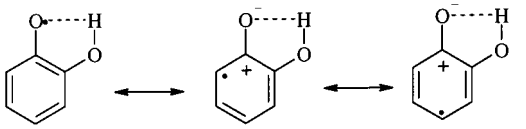
(20)

A B

have an easily extractable H atom, whereas the other is intramolecularly bonded. Recently, it has been observed that 2-methoxyphenol, ubiquinols, and other (poly)methoxyphenols are reactive toward free radicals despite the phenolic OH being engaged in a strong intramolecular hydrogen bond (de Heer et al., 2000).

This suggests that a distinction between a linear hydrogen bond (with an external molecule of solvent) and a nonlinear hydrogen bond (intramolecular hydrogen bond) must be made because only the former prevents the H-atom abstraction by a free radical (de Heer et al., 2000). Evidently, catechol can form only one linear hydrogen bond with the free OH in an HBA solvent. Thus, it could still react with peroxyls by the intramolecularly bonded OH.

The semiquinone radical from catechol can be partially depicted as an internal radical ion pair by virtue of the resonance canonical structures represented in 21. Thus, this radical may have a polar character

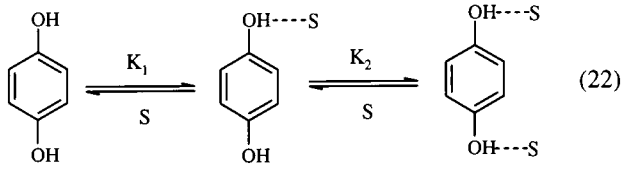


(21)

(Jackson and Hosseini, 1992), and the intramolecular H bonding with the *ortho*-hydroxyl is expected to be stronger than that in the noncharged catechol (A and B). In consequence, the transition state leading to this radical would also be more strongly H bonded than catechol and the rate of reaction 18 increased. Protic solvents are expected to interfere with this stabilization as a consequence of *intermolecular* H bonding (Barclay et al., 1999).

With this in mind, the experimental results in acetonitrile would then indicate this solvent may interact only with the free −OH of A/B and may interfere rather weakly with the internal stabilization of the semiquinone radical. Furthermore, by virtue of the polar character of the semiquinone (see 21), acetonitrile might stabilize the transition state leading to this radical. The large solvent effect observed in *tert*-butyl alcohol allows one to suppose instead that an additional and relatively strong interaction between the intramolecularly bonded hydrogen and a molecule of solvent can exist [a kinetic solvent effect has also been observed with (poly)-methoxyphenols (de Heer et al., 2000)] or that a severe destabilization of the semiquinone radical can slacken the inhibition step.

Only linear hydrogen bonds are possible with hydroquinone, and even in acetonitrile they are strong enough to prevent the H abstraction by the peroxyls. The following equilibrium can be written:



(22)

$K_2 = f \times K_1$ (f is a factor < 1)

Despite the complexity of the mechanism of H-atom transfer (see the Introduction), a treatment for the kinetic solvent effects similar to that developed by Ingold and co-workers (Avila et al., 1995) can be attempted for hydroquinone. If both species $Ph(OH)_2$ and Ph(OH)-(OH- - -S) are considered to be reactive toward the peroxyl radicals, the sum of their concentrations must be used in eq 15, $k_{inh,Ph(OH)_2} \sim 2k_{inh,Ph(OH)(OH- - -S)}$. Thus, the following equation for the kinetic solvent effect can be derived:

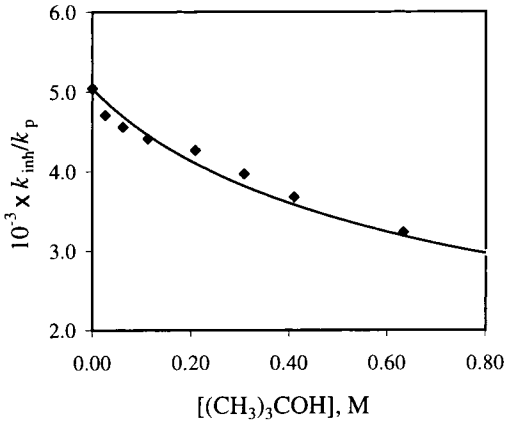


Figure 4. (◆) Experimental values of k_{inh} for hydroquinone (5.4×10^{-6} M) at 50 °C for increasing amounts of *tert*-butyl alcohol in cyclohexane as solvent ($[H_2L] = 1.03 \times 10^{-2}$ M, $[AIBN] = 2.50 \times 10^{-3}$ M). The solid line represents the kinetic solvent effect calculated by eq 23 for $f = 0.1$ and $K_1 = 2.5$ M^{-1} (see eq 23 and Discussion).

$$k^s_{inh,Ph(OH)_2} = k_{inh,Ph(OH)_2} \frac{(1 + 0.5K_1[S])}{(1 + K_1[S] + fK_1^2[S]^2)} \quad (23)$$

$k_{inh,Ph(OH)_2}$ is the rate constant in apolar solvent.

Figure 4 represents the progressive reduction of k_{inh} for hydroquinone caused by the presence of increasing amounts of *tert*-butyl alcohol (0.026−0.634 M) in solution. Unfortunately, these kinetic data cannot be used for an accurate calculation of K_1 by eq 23, unless the value of f (namely, K_2) is known. Anyhow, for values of f from 0.5 to 0.05, the best fit between experimental values of k_{inh} and theoretical trend was achieved for K_1 in the range of 2−3 M^{-1} (at 50 °C). Values of $K_1 > 3$ M^{-1} are scarcely plausible (with our current kinetic data and eq 23) because they would imply a drastic reduction of the equilibrium constant for the second solvation step ($K_2 \leq 0.01K_1$). On the other hand, for values of $K_1 < 2$ M^{-1} the fit of the experimental data with eq 23 is achieved only for $K_2 \sim K_1$ ($f \geq 0.9$). The observed significant drop from 14 M^{-1} for phenol/*tert*-butyl alcohol (at 25 °C; Banks et al., 1996) to ∼2.5 M^{-1} for hydroquinone/*tert*-butyl alcohol (at 50 °C) is mainly attributable to the higher temperature of the latter system more than electronic effects of the hydroxyl in the *para*-position. The solid line in Figure 4 represents eq 23 for $f = 0.1$ *and* (best fit) $K_1 = 2.5$ M^{-1}.

Among compounds **1**−**9**, catechin must be distinguished for its singular and completely unforeseeable behavior, because when it was added to the solution of linoleic acid/AIBN, no antioxidant effect was observed. Actually, the antioxidant activity of this flavonoid is presumably masked by the formation of products that strongly absorb at 234 nm. As a matter of fact, the direct reaction between catechin and peroxyls from AIBN has shown a nonlinear strong growth in the absorbance at 234 nm.

In conclusion, the peroxyl-radical-trapping antioxidant activity of catecholic compounds, hydroquinone, and monophenols is markedly dependent on the HBA activity of the solvents, being most effective in apolar organic solvent, whereas in *tert*-butyl alcohol they do not behave like antioxidants. This behavior has been observed with quercetin and chrysin, as well, and probably can be extended to other flavonoids. In acetonitrile all compounds with catechol structures (including quer-

cetin) have preserved their strong antioxidant activity. This finding has a notable importance because the HBA ability of water is close to that of acetonitrile (Valgimigli et al., 1996), thereby roughly k_{inh}^{water}(quercetin + HLOO•) $\approx$ $k_{inh}^{acetonitrile}$(quercetin + HLOO•) $\geq 1 \times 10^5$ $M^{-1}\cdot s^{-1}$ on the grounds of the principle that the kinetic solvent effects depend only on the HBA abilities of the solvents.

Finally, the CD method for the determination of the antioxidant activities in homogeneous phase is reliable with most of the phenolic compounds (**1**−**9**). For quercetin a relatively low antioxidant activity has been pointed out by this method and for catechin, *seemingly* no activity at all.

ACKNOWLEDGMENT

We are grateful to Prof. M. Piattelli (Università di Catania, Italy) for his kind review of the manuscript. We thank Prof. L. R. Barclay (Mount Allison University, Sackville, NB, Canada) and Dr. Alessandro Bagno (Centro CNR, Meccanismi Reazioni Organiche, Padova, Italy) for helpful discussions.

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Received for review May 26, 2000. Revised manuscript received October 12, 2000. Accepted October 13, 2000.

JF0006527