Media Effects on Antioxidant Activities of Phenols and Catechols

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Received March 18, 1999

Abstract: The H-atom donating activities of 2,6-di-*tert*-butyl-4-methylphenol (BHT), 2,6-di-*tert*-butyl-4-methoxyphenol (DBHA), 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), and 3,5-di-*tert*-butylcatechol (DTBC) toward the nitrogen-centered 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were measured by stopped flow methods in hexane, 1-propanol, *tert*-butyl alcohol, and acetone. Decreases in these activities on transferring from hexane to the hydrogen bond accepting (HBA) solvents, the kinetic solvent effect (KSE), are attributed to hydrogen bonding from the phenolic group. Steric hindrance accounts for a lower decrease observed for the highly hindered BHT and DBHA compared to PMHC. The catechol, DTBC, a very active H-atom donor to DPPH in hexane, showed a dramatic loss of activity in HBA solvents, especially acetone. Higher H-atom donating activities of BHT, DBHA, and PMHC were observed toward the oxygen-centered radical of 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl (DBMP), and the decreases in activity in the HBA solvents paralleled those found with DPPH. Thus the KSE was found to be independent of the nature of the abstracting radical for DPPH and DBMP. The inhibition of the oxygen uptake (IOU) method was used to determine the antioxidant activities (k_{inh}) of α-tocopherol, PMHC, catechol, and DTBC during free radical autoxidation of styrene and mixtures of styrene and *tert*-butyl alcohol. The k_{inh} of α-tocopherol and PMHC dropped to one-tenth of the values with increasing *tert*-butyl alcohol content due to the HBA activity of the alcohol compared to styrene.

The reactions of oxygen-centered radicals with biological molecules such as lipid membranes are implicated in various degenerative human diseases, and consequently the mechanism and antioxidant activities of natural and synthetic antioxidants continue to receive a great deal of interest.[1] The reactions of phenolic antioxidants in relevant biological systems such as heterogeneous aqueous/lipid phases are subject to complex differential solvation and diffusion phenomena. Consequently the antioxidant activities of phenols are reported to be markedly reduced in model heterogeneous phases of micelles or lipid bilayers compared to nonprotic solvents. Such effects were attributed in a qualitative way to hydrogen bonding of the phenolic hydroxyl group, resulting in inhibition of the hydrogen transfer of the phenolic hydrogen atom to peroxyl radicals in the rate-controlling step.[2] Recent reports by Ingold et al. have provided the first clear quantitative evidence of the role that hydrogen bonding plays on the H-atom donor activities of phenolic antioxidants.[3] These reports provide a very useful guide for further studies of the effects of media on the activities of antioxidants since they cover a wide range of solvents, protic and nonprotic, and established two significant principles for the kinetic solvent effects (KSE)[4] on phenolic antioxidant activities: (1) The magnitude of the KSE is determined by the strength of the interaction between the hydrogen bond donor (e.g., the antioxidant, HBD) and the hydrogen bond accepting solvent (HBA), protic or nonprotic, and (2) the KSE on H-atom abstractions from hydroxyl groups are independent of the nature of the abstracting radical.[3] These principles provide a basis for our extended studies of the effect of media on the H-atom donating activity of various classes of antioxidants and on antioxidant activities during radical chain peroxidation.

We now report on the effects of various media on the H-atom donating ability of several classes of phenolic antioxidants including the following: **1**, the two hindered *o*-di-*tert*-butylphenols, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and 2,6-di-*tert*-butyl-4-methoxyphenol (DBHA); **2**, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), an antioxidant of the vitamin E class; and **3**, 3,5-di-*tert*-butylcatechol (DTBC). The phenols BHT and DBHA are well-known synthetic antioxidants, and PMHC, in homogeneous solution, is expected to mimic the behavior of vitamin E, nature's most active lipid-soluble phenolic antioxidant.[5] The catechol derivative DTBC was selected because it contains the structural unit that appears to be the main center responsible for the antioxidant activity of the flavonoids (vide infra). These studies were carried out by

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(1) For recent reports and some reviews on this subject see: (a) Barclay, L. R. C.; Vinqvist, M. R.; Antunes, F.; Pinto, R. E. *J. Am. Chem. Soc*. **1997**, *119*, 5764−5765. (b) Barclay, L. R. C.; Antunes, F.; Egawa, Y.; McAllister, K. L.; Mukai, K.; Nishi, T.; Vinqvist, M. R. *Biochim. Biophys. Acta* **1997**, *1328*, 1−12.

(2) (a) Barclay, L. R. C.; Edwards, C. D.; Mukai, K.; Egawa, Y.; Nishi, T. *J. Org. Chem.* **1995**, *60*, 2739−2744. (b) Pryor, W. A.; Strickland, T.; Church, D. F. *J. Am. Chem. Soc.* **1988**, *110*, 2224−2229. (c) Castle, L.; Perkins, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 6382−6384. (d) Barclay, L. R. C.; Baskin, K. A.; Dakin, K. A.; Locke, S. J.; Vinqvist, M. R. *Can. J. Chem.* **1990**, *68*, 2258−2269.

(3) (a) Valgimigli, L.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1996**, *118*, 3545−3549. (b) MacFaul, P. A.; Ingold, K. U.; Lusztyk, J. *J. Org. Chem.* **1996**, *61*, 1316−1321. (c) Valgimigli, L.; Banks, J. T.; Ingold, K. U.; Lustyk, J. *J. Am. Chem.* **1995**, *117*, 9966−9971.

(4) Abbreviations used in the order in which they appear in the text: KSE, kinetic solvent effect; HBD, hydrogen bond donor; HBA, hydrogen bond acceptor; BHT, 2,6-di-*tert*-butyl-4-methylphenol; DBHA, 2,6-di-*tert*-butyl-4-methoxyphenol; PMHC, 2,2,5,7,8-pentamethyl-6-hydroxychroman; DTBC, 3,5-di-*tert*-butylcatechol; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DBMP, 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxyl; IOU, inhibition of oxygen uptake; AIBN, azo-bis-isobutyrylnitrile.

(5) (a) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1995**, *107*, 7053−7065. (b) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194−201.

10.1021/ja990878u CCC: $18.00
Published on Web 06/17/1999

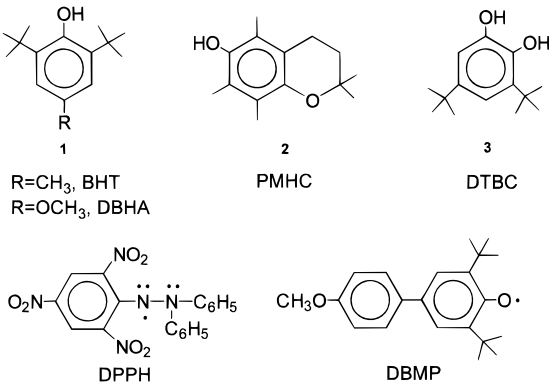


Figure 1. Structures of antioxidants and radicals.

Table 1. Second-Order Rate Constants,[a] M^{-1} s^{-1}, at 30 °C for H-Atom Abstraction from Antioxidants by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Radical

	antioxidants[b]			
solvent	BHT	DBHA	PMHC	DTBC
hexane	1.13	37.6	6.77×10^3	20.3×10^3
1-propanol	0.31	5.23	2.31×10^2	80.4
tert-butyl alcohol	0.41	4.27	2.23×10^2	56.1
acetone	0.16	1.14	1.99×10^2	16.7

[a] Each rate constant is an average of two or three determinations and the experimental error was usually within 5%. [b] The ratio of excess antioxidant to DPPH varied depending on the activity found in trial experiments (see Experimental Section). Linear plots of k_{obs} versus [ArOH] gave satisfactory correlations of 0.90−0.99.

using two hydrogen atom abstracting agents, 1,1-diphenyl-2-picrylhydrazyl (DPPH), a nitrogen-centered radical, and 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxy (DBMP), an oxygen-centered radical. (See Figure 1 for structures of the antioxidants and radicals.) The reactions were followed by monitoring the decay of the main absorption bands in the visible spectra for DPPH or DBMP.

In addition, the *antioxidant activities* of α-tocopherol, PMHC, catechol, and DTBC were determined by employing the inhibition of oxygen uptake method (IOU) during oxidation initiated by peroxyl radicals, generated under controlled conditions from azo-bis-isobutyrylnitrile (AIBN), in styrene and in mixtures of styrene and *tert*-butyl alcohol, a protic solvent.

Results

Kinetic Measurements Employing the Nitrogen-Centered Radical, DPPH. Second-order rate constants, k_2, were obtained by following the decay of DPPH absorption at 528 nm on a diode array spectrometer in the presence of excess H-atom donor under conditions of pseudo-first-order kinetics. The k_2 values were determined from plots of k_{obs} versus [ArOH] according to eq 1.

$$k_{obs} = k_0 + k_2[\mathrm{ArOH}] \quad (1)$$

The k_2 values for H-atom abstraction by DPPH from BHT, DBHA, PMHC, and DTBC in four solvents, hexane, 1-propanol, *tert*-butyl alcohol, and acetone, are given in Table 1.

The results with DPPH show that for BHT, DBHA, and PMHC, the order of H-atom donating activity parallels the known antioxidant activities determined earlier in styrene/chlorobenzene mixtures,[5] but the absolute values differ for the different reactions (free radical chain autoxidation with peroxyl radicals[5] compared to H-atom abstraction by DPPH). For DBHA and BHT, the protic solvents 1-propanol and *tert*-butyl alcohol caused a *decrease* in the H-atom transfer, and acetone exerted an additional retarding effect. In contrast, the H-atom donating ability of PMHC is about the same in acetone and the two alcohols, as shown in the similar rate constants in these media, indicative of the same degree of solvent effect. The catechol, DTBC, is a much more active H-atom donor than the other phenols in hexane but exhibited a larger solvent effect, so that DBTC appears to be in a class by itself in this regard.

Table 2. Second-Order Rate Constants,[a] M^{-1} s^{-1}, at 30 °C for H-Atom Abstraction from Antioxidants by the 2,6-Di-*tert*-butyl-4-(4′-methoxyphenyl)phenoxy (DBMP) Radical

	antioxidant[b]		
solvent	BHT	DBHA	PMHC
hexane	160	44.3×10^2	(93.9×10^3)[6]
1-propanol	41.1	5.77×10^2	4.40×10^3
tert-butyl alcohol	63.4	6.42×10^2	5.64×10^3
acetone	19.3	2.77×10^2	3.28×10^3

[a] See Table 1, footnote *a*. [b] See Table 1, footnote *b*. Linear plots of k_{obs} versus [ArOH] gave satisfactory correlations of 0.90−0.99.

Kinetic Measurements Employing the Oxygen-Centered Radical, DBMP. The second-order rate constants, k_2, for H-atom abstraction were obtained by following the decay of DBMP absorption at 370 nm in hexane and at 374 nm in the alcohols and acetone on the diode array spectrometer in the presence of excess H-atom donor under conditions of pseudo-first-order kinetics. The k_2 values were obtained from plots of eq 1 as before. The k_2 values for H-atom abstraction by DBMP from BHT, DBHA, and PMHC are given in Table 2. The higher rate constants for H-atom abstraction by DBMP reflect the effect of the more reactive oxygen-centered radical, compared to the values from the nitrogen-centered radical of DPPH. The drop in k_2 observed by BHT and DBHA on transferring from hexane to the alcohols or acetone for H-atom abstraction by DBMP parallels the results shown in Table 1 for abstraction by DPPH.[6]

Antioxidant Activities of α-Tocopherol, PMHC, and Catechols in *tert*-Butyl Alcohol/Styrene. To determine the effect of a protic solvent on the peroxyl radical trapping activity of these phenols, we adapted the method developed by Ingold et al., namely, the measurement of inhibited oxygen uptake (IOU) by the antioxidants during controlled initiation of styrene peroxidation by the azo initiator AIBN.[5] In our studies, solvent mixtures of styrene, the substrate, and the alcohol were used. Sufficient styrene was required to ensure a kinetic chain length of at least 10 during the inhibition periods in the presence of antioxidants. We conducted a series of measurements with various amounts of *tert*-butyl alcohol to determine the effect of this protic solvent on the absolute rate constant for the rate-determining step of inhibition, k_{inh}, eq 2.

$$\mathrm{ROO}^{\bullet} + \mathrm{ArOH} \xrightarrow{k_{inh}} \mathrm{ROOH} + \mathrm{ArO}^{\bullet} \quad (2)$$

The phenoxyl radical, $\mathrm{ArO}^{\bullet}$, will undergo rapid termination with a second peroxyl radical, eq 3, so that the stoichiometric factor, n, for trapping peroxyl radicals by phenolic antioxidants is 2. The expression for suppressed oxygen uptake during the inhibition period is given by eq 4. In calculations, we used the reported value[5] of 41 M^{-1} s^{-1} for the propagation rate constant, k_p, for peroxidation of styrene at 30 °C.

(6) The k_2 value for H-atom abstraction from PMHC by DPPH in hexane is in reasonable agreement with that reported (7.40×10^3 M^{-1} s^{-1}).[2b] Our value for the reaction of PMHC with DBMP in hexane is considered less accurate since the rate of decay of the DBMP radical appeared to reach the limit of the diode array used.

$$ROO^{\bullet} + ArO^{\bullet} \xrightarrow{fast} \text{nonradical products} \quad (3)$$

$$-d[O_2]/dt = k_p/k_{inh} \times [RH]R_i/n[ArOH] \quad (4)$$

The rate of radical chain initiation, R_i, is known for initiation by the azo initiator AIBN, and can be measured by the induction period method by using eq 5, where τ is the length of the induction period during suppressed oxygen uptake.

$$R_i = n[ArOH]/\tau \quad (5)$$

The antioxidant activity, represented by the absolute rate constant for inhibition, k_{inh}, is determined by measuring the oxygen uptake during the course of the inhibition period. For calculations, the integrated form of the inhibition period, eq 6, was used as before.[7]

$$\Delta[O_2]_t = -k_p/k_{inh}[RH] \ln(1 - t/\tau) \quad (6)$$

The rate constants were obtained from the slopes of the plots of $\Delta[O_2]_t$ versus $-\ln(1 - t/\tau)$, which equal $k_p[RH]/k_{inh}$. The k_{inh} were first determined in neat styrene for the antioxidants, α-tocopherol, PMHC, catechol, and DTBC. Then, for each antioxidant the values were determined for known mixtures of styrene and *tert*-butyl alcohol, carefully controlling the amount of alcohol in the mixture so that the kinetic chain length was at least 10 during the inhibition period, a necessary condition for this method.[7] The profiles of the results, indicating the effect of the varying concentrations of *tert*-butyl alcohol on the k_{inh} values[8] of α-tocopherol and PMHC, are plotted in Figure 2. The results for catechol and TBHC are plotted in Figure 3. The plots show that *tert*-butyl alcohol causes an initial sharp drop in the k_{inh} values, followed by a tailing off as the solvent mixture is made richer in the protic solvent.

Discussion

As noted above (Tables 1 and 2) there are significant drops in the second-order rate constants for H-atom abstraction on transferring from hexane to the two alcohols or acetone. These solvents all have about the same hydrogen bond accepting ability (HBA).[9] One would expect that they would have about the same degree of hydrogen bond donor (HBD) interaction with the phenolic hydroxyl group, and consequently a comparable drop in H-atom donating activity of HBD to HBA would be expected. What is *actually* observed is that the effects depend markedly on the structure of the antioxidants. This is clear from the ratios of second-order rate constants for transferring from hexane to the HBA solvents, summarized in Table 3.

The hindered phenols BHT and DBHA exhibit similar $k_{hexane}/k_{solvent}$ values for 1-propanol and *tert*-butyl alcohol, but the effect of acetone is significantly higher. On the other hand, the three solvents have about the same effect, although larger, on the vitamin E model PMHC. We attribute these differences to the steric hindrance provided by the *ortho* tertiary butyl groups in BHT and DBHA, which hinder the HBD of these phenols to HBA interaction of the alcohols. Space-filling models indicate that the alcohols must come within contact distance to the *ortho*

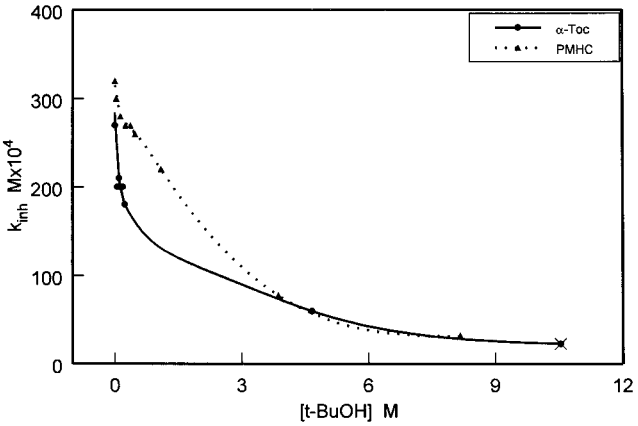


Figure 2. Profiles of antioxidant activities of α-tocopherol (α-toc), ●, and PMHC, ▲, during inhibited oxidation of styrene, initiated by AIBN (typically 0.0235 M), showing the effects of added *tert*-butyl alcohol. Each point represents the average of three experiments, with error limits within 12.5% for each point. The concentration of styrene varied from 8.7 (neat) to 3.8 M for experiments with α-toc ([α-toc] was 2 to 3 μM) and 8.7 to 1.1 M for experiments with PMHC ([PMHC] was 1.5 to 3 μM). The rate of chain initiation, $R_i = 2[ArOH]/\tau$, was within the range $(2.5-3.9) \times 10^{-9}$ M s^{-1} for these experiments. The point × is from the α-toc inhibited peroxidation of linoleate in *tert*-butyl alcohol (Barclay, L. R. C.; Baskin, K. A.; Locke, S. J.; Schaefer, T. D. *Can. J. Chem.* **1987**, *65*, 2527−2540).

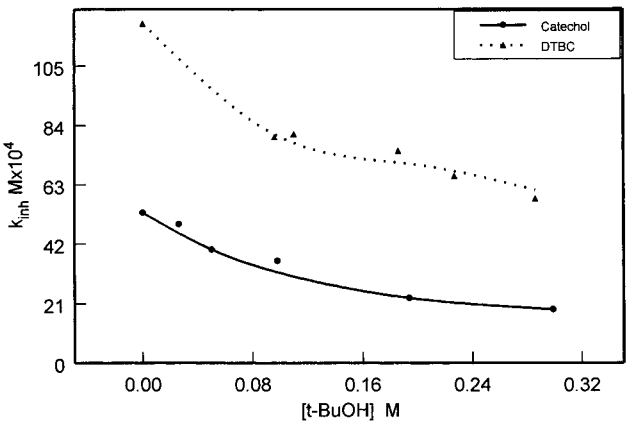


Figure 3. Profiles of antioxidant activities of catechol, ●, and DTBC, ▲, during inhibited oxidation of styrene, initiated by AIBN (typically 0.0235 M), showing the effects of added *tert*-butyl alcohol. Each point represents the average of three experiments, with error limits within 15% for each point. The concentration of styrene varied from 8.7 (neat) to 7.0 M for experiments with catechol ([catechol] was 1.1 to 3.5 μM) and 8.7 to 6.5 M for experiments with DTBC ([DTBC] was 0.80 to 2.2 μM).

Table 3. Ratios of H-Atom Donor Activities, $k_{hexane}/k_{solvent}$

	$k_{hexane}/k_{solvent}$ for		
antioxidant	1-propanol	*tert*-butyl alcohol	acetone
DPPH as abstracting radical			
BHT	3.6	2.8	7.1
DBHA	7.2	8.8	33
PMHC	29	30	34
DTBC	253	362	12.2×10^2
DBMP as abstracting radical			
BHT	3.9	2.5	8.3
DBHA	7.7	6.9	16

(7) Barclay, L. R. C.; Vinqvist, M. R.; Mukai, K.; Itoh, S.; Morimoto, H. *J. Org. Chem.* **1993**, *58*, 7416−7420.

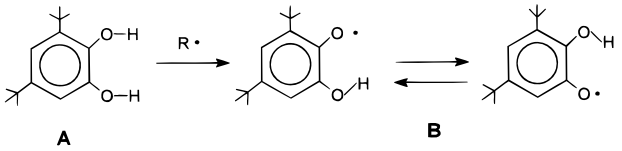
(8) The absolute inhibition rate constants (M^{-1} s^{-1})determined in neat styrene are as follows: α-tocopherol, 2.74×10^6, PMHC, 3.20×10^6, compared to literature values of 3.20×10^6 and 3.80×10^6, respectively.[5a] For DTBC and catechol the values are 1.20×10^6 and 0.53×10^6, compared to literature values of 1.49×10^6 and 0.55×10^6, respectively: Xi, F.; Barclay, L. R. C. *Can. J. Chem.* **1998**, *76*, 171−182.

(9) The hydrogen bond accepting ability (HBA) of these solvents is expressed as *b* values: *tert*-butyl alcohol, 0.49; 1-propanol, 0.45 (Laurence, C.; Berthelot, M.; Sraidi, K. *J. Phys. Chem.* **1989**, *93*, 3799−3802). The corresponding β_2^H values are the following: *tert*-butyl alcohol, 0.49; primary alcohols, 0.45; and acetone 0.50 (Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Morris, J. J. *J. Chem. Soc., Perkin 2* **1990**, 521−529).

tertiary butyls to approach the phenolic hydroxyl. On the other hand, the HBA carbonyl oxygen of acetone does not show this kind of hindrance. In the case of PMHC, the *ortho* methyls do not present the same degree of steric hindrance to solvent interaction with the phenolic hydroxyl. Steric hindrance has been invoked before[7,10] to account for differences in antioxidant activities of hindered phenols, and these results provide new, quantitative evidence for such effects.

DTBC is a useful model for studying the more complex flavonoids. Flavonoids are widely distributed in fruits and vegetables and are widely used as nutritional supplements. There are several reviews on the antioxidant properties of these polyphenols,[11] and there is theoretical[12] and experimental[13] evidence linking their antioxidant properties to the catechol moiety usually found in their structures. The catechol structure is also present in natural catechol amines, such as L-dopa and dopamine, which are reported to have both toxic and antioxidant effects,[14] and in catechol steroids, where effective antioxidant activity was found in lipoproteins[15] and rat liver microsomes.[16] Many of these studies were carried out in the presence of heavy metal ions, thus it is difficult to evaluate the activities of polyphenols as *radical chain breaking antioxidants* because they may also act as *preventative antioxidants* by complexing metal ions.[17] We use DTBC as a simple model system to study the H-atom donating activity of the catechol system, including effects of solvents, and by implication resolve some of the uncertainties concerning the activities of the more complex natural polyphenols as H-atom donors and antioxidants. It is clear from our current results that DTBC is the most active H-atom donor to DPPH in a hydrocarbon solvent (see Table 1). However, it is also clear that its activity *decreases* substantially, more than any of the other phenols studied, in the alcohols and especially in acetone, where the decrease is more than 3 orders of magnitude from the value in hexane. Evidence was provided earlier to show that the main effect controlling the activity of DTBC as an *antioxidant* is the stabilization by intramolecular H-bonding of the aroxyl radical semiquinone (Scheme 1, **B**) formed in the rate-determining inhibition step.[8] It is expected that protic solvents would interfere with this stabilization through intermolecular H bonding so that the H-atom donating activity is greatly reduced as found.[18] However, it is not completely clear how acetone causes an additional drop in activity of DTBC as a H-atom donor. It appears that acetone is able to act more strongly as a HBA on the diol, perhaps by acting as an effective HBA on both phenolic hydroxyl groups, thus blocking the initial state of the catechol (Scheme 1, **A**) from H-atom abstraction more effectively than the alcohols do. These results with the model system, DTBC, indicate that experiments to measure the H-atom donor or antioxidant activities of polyphenols such as the flavonoids must be carried out with great care, and must take into account the possible effects of traces of metal ions and of the solvent system used. In addition, other isolated phenolic groups in the system may impart some *prooxidant* activity (e.g., start new oxidation chains), because the phenoxyl radical is now known to possess high reactivity[19] even compared to peroxyl radicals (the main chain-carrying radicals in lipid peroxidation).

Scheme 1. Stabilization of the DTBC Aroxyl Semiquinone Radical by Hydrogen Bonding



As indicated in the Results, solvent effects on the second-order rate constants for H-atom abstraction from the phenols by the oxygen-centered radical, DBMP, parallel the results found for DPPH. We have just sufficient data to confirm an important principle, namely, that the kinetic solvent effects (KSE) on H-atom abstractions from hydroxyl groups are independent of the nature of the abstracting radical.[3] Thus the ratios of the KSE observed, $k_{hexane}/k_{solvent}$, for H-atom abstraction from DBHA and BHT by the nitrogen-centered radical DPPH are approximately equal to these ratios for H-atom abstraction by the oxygen-centered radical DBMP for the solvents used, with the exception of DBHA in acetone (see Table 3).

DPPH is frequently used to measure the H-atom donor properties of antioxidants, especially the flavonoids.[20] The oxygen-centered radical, DBMP, provides the same advantage. Although it has been used less frequently, its H-atom abstracting activity appears to follow the antioxidant activity of the tocopherols, at least in a relative manner.[21] While studies with stable radicals such as DPPH and DBMP provide quantitative evidence on H-atom donating activities of antioxidants, actual *antioxidant activities* must measure the ability of the H-atom donors to trap peroxyl radicals. Here the H-atom donating antioxidants must be more active than the substrate to *break* the propagating chain reaction, eq 7. In other words k_{inh} (eq 2)

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must be orders of magnitude greater than k_p.

$$R_s{-}H\ (\text{substrate}) + ROO^{\bullet} \xrightarrow{k_p} R_s^{\bullet} + ROOH \qquad (7)$$

Since protic solvents show a remarkable effect on the H-atom donating ability of common antioxidants, it is important to quantitatively determine the effect of a protic solvent on the peroxyl radical trapping efficiency of antioxidants. The effect of *tert*-butyl alcohol on the k_{inh} of PMHC, α-tocopherol, and catechols was determined under controlled conditions during peroxidation of styrene. The protic solvent decreases the k_{inh} of both α-tocopherol and PMHC in a similar manner (Figure 2). At a molar ratio of alcohol to styrene of 1.0 to 1.2, the initial differences in k_{inh} values between PMHC and α-tocopherol are "leveled"[22] and reduced to about one-quarter of their values in styrene. This indicates a strong HBA effect of *tert*-butyl alcohol at the phenolic hydroxyl as it displaces the hydrocarbon as solvent. The k_{inh} of PMHC and α-tocopherol continues to drop as the molar ratio of alcohol to styrene is increased to 7.4 (Figure 2), where one can assume that the solvent effect approximates that of neat *tert*-butyl alcohol. At this point the k_{inh} has decreased by a factor of 10 times less than that in neat styrene. This factor is more than the estimated decrease of 3.9 times for H-atom abstraction from α-tocopherol in water compared to benzene,[3a] and probably reflects a larger difference in the HBA values in our experiments (between styrene and *tert*-butyl alcohol) compared to the HBA difference between benzene and water.[23] These results now indicate clearly that the drop in k_{inh} for α-tocopherol observed earlier in heterogeneous phases of phospholipid bilayers, which is several orders of magnitude compared to homogeneous solution,[2d] must be due to a combination of various effects. The α-tocopherol may in part be physically inaccessible[3a] to the lipid peroxyl radicals due to nonuniform distribution and restricted diffusion in the bilayer.[2c] The hydrogen bond acceptor (HBA) effect alone, although it is significant, accounts for only a portion of the dimunition in antioxidant activity.

The profiles of antioxidant activities of the catechols also exhibit a drop of k_{inh} with increased *tert*-butyl alcohol concentration (Figure 3). We could follow the effect on k_{inh} to mole ratios of *tert*-butyl alcohol to styrene of only 0.03 to 0.04. At higher ratios, there were not satisfactory inhibition periods to measure τ values, since in alcohol the catechols appeared to behave like "retarders" rather than true antioxidants.[17] The "antioxidant activities" of polyphenols such as the flavonoids are often reported in alcohols in terms of the concentration of flavonoid required to decrease the concentration of a monitoring radical (i.e., DPPH) by 50%, the EC_{50} value,[20a] which is different from the determination of the absolute rate constant for H-atom transfer, k_{inh}, which is a quantitative measure of the *antioxidant activity*. Even in chlorobenzene, the solvent commonly used for quantitative measurements of k_{inh},[5,7] the flavonoids do not behave as *classical* phenolic antioxidants.[24] Our results on the catechols provide a basis for additional studies in more biologically relevant systems such as phospholipid bilayers.

(22) The leveling effect of antioxidant activity of α-tocopherol toward that of BHT noted before in phospholipid membranes compared to homogeneous solutions[2d] is now recognized to be partly or even mainly due to restricted diffusion of lipophilic molecules, like α-tocopherol between or within the bilayer or micellar structures.[1,2c,3c]

(23) The β_2^H values of water and benzene are 0.38 and 0.14, respectively,[9] giving a difference of 0.24; using the log $K_B^H = -0.27$ for styrene[9] gives a β_2^H of 0.18 and a difference between *tert*-butyl alcohol and styrene of $0.49 - 0.18 = 0.31$.

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Experimental Section

Materials. The highest grade of solvents was used for all analyses and they were distilled just before use. The stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), was purchased from Sigma and used as received. The 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)phenol, provided by Dr. Kazuo Mukai,[21] was converted to the radical (DBMP) by oxidation with an excess of lead dioxide[25] under argon in the solvent of interest immediately before use. The antioxidants 2,6-di-*tert*-butyl-4-methylphenol (BHT) and 2,6-di-*tert*-butyl-4-methoxyphenol (DBHA) were purchased from Aldrich and stored at −23 °C. The catechol antioxidant, 3,5-di-*tert*-butylcatechol (DTBC), was also purchased from Aldrich and stored at room temperature. The vitamin E model, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), was synthesized as described[26] and stored at −23 °C. It was recrystallized twice from hexane prior to use. Solutions of antioxidants were prepared in known concentrations in the solvent of choice just prior to use. Azo-bis-isobutyrylnitrile (AIBN) from Eastman was recrystallized from methanol and stored at −23 °C. Solutions of known concentration were prepared in chlorobenzene or *tert*-butyl alcohol to initiate reaction in the autoxidation experiments. Styrene from Aldrich was passed through a column of neutral alumina under argon to remove commercial inhibitor and traces of hydroperoxides just before use.

Kinetic Measurements. 1. Diode Array. Experiments were conducted with an HP-8452A diode array spectrophotometer at 30 °C under an argon atmosphere. The equipment was flushed with the solvent of choice prior to the experiment, and a blank baseline obtained. Solutions were prepared just before use under argon and the concentration of the radical was checked by UV. The decay of the radical with time in the absence of antioxidant was monitored in the solvent prior to each experiment, to ensure the k_{obs} value was not being affected by radical decay. Samples were rapidly mixed in the reaction cuvette with use of an Applied Photophysics Model RX1000 stop flow assembly. The loading syringes were filled with the radical (syringe 1) and the antioxidant (syringe 2), and the cuvette was flushed with an excess volume of these for each measurement to ensure no contamination from the previous injection. Syringe 2 was rinsed with solvent each time the antioxidant concentration was changed. A minimum of five different concentrations of antioxidant were used to obtain the second-order rate constant in each solvent system, with a minimum of three measurements at each antioxidant concentration. At least one duplicate experiment was conducted. The R^2 for the determinations of k_{obs} and k_2 was 0.90−0.99.

(a) Measurements with DPPH Radical. The molar absorptivity was calculated to be $\epsilon = 1.34 \times 10^3$ ($\lambda_{max} = 515$ nm in methanol), assuming 90% purity of the DPPH sample, compared to the literature value of $\epsilon = 1.43 \times 10^3$ ($\lambda_{max} = 525$ nm in chloroform).[27] The literature value was used in all calculations. The actual concentration ratio of [antioxidant]/[DPPH] used in the experiments depended upon the antioxidant used and the solvent: with DBHA, average [DPPH] $= 6.66 \times 10^{-5}$ M, and [DBHA]/[DPPH] varied from 12 to 1684; with BHT, average [DPPH] $= 6.47 \times 10^{-5}$ M, and [BHT]/[DPPH] varied from 47 to 1394; with PMHC, average [DPPH] $= 9.46 \times 10^{-5}$ M, and [PMHC]/[DPPH] varied from 2 to 60; and with DTBC, average [DPPH] $= 8.81 \times 10^{-5}$ M, and [DTBC]/[DPPH] varied from 1 to 101.

(b) Measurements with DBMP Radical. An excess of lead dioxide was placed in a test tube with 2 mL of the solvent of interest under argon. A known amount of 2,6-di-*tert*-butyl-4-(4′-methoxyphenyl)-phenol was added and the sample was vortex mixed for 1 min, then centrifuged on a benchtop centrifuge for 2 min. The solvent was removed and filtered into a 50 mL volumetric with use of a Millex-SR 0.5 mm syringe tip filter. This was repeated with a second aliquot of solvent to obtain all the radical, the volumetric was prepared to the mark under argon, and the concentration of the DBMP radical was determined by using UV. The molar absorptivity was determined to be $\epsilon = 2.41 \times 10^4$ ($\lambda_{max} = 370$ nm in hexane). The actual concentration

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ratio of [antioxidant]/[DBMP] used in the experiments again depended upon the antioxidant used and the solvent: with DBHA, average [DBMP] = 6.11×10^{-5} M, and [DBHA]/[DBMP] varied from 1 to 712; with BHT, average [DBMP] = 8.79×10^{-5} M, and [BHT]/[DBMP] varied from 4 to 308; and with PMHC, average [DBMP] = 7.32×10^{-5} M, and [PMHC]/[DBMP] varied from 1 to 15.

2. Autoxidation/Inhibition Procedures. Autoxidations were carried out at 30 °C/760 Torr under oxygen in a calibrated sensitive dual-channel pressure transducer apparatus that is described elsewhere.[28] The procedure for initiation of the oxidation of styrene and measuring oxygen uptake during inhibition periods reported before[7] was generally followed. The apparatus was first "conditioned" with styrene or combinations of styrene and *tert*-butyl alcohol in both the sample side and reference side. The reference side was injected with antioxidant to prevent self-initiated oxidation. For reactions in neat styrene, the reaction was initiated by injection of a known amount of AIBN in chlorobenzene. In cases where combinations of styrene and *tert*-butyl alcohol were used, the AIBN was injected in a known volume of the alcohol. When a constant uptake of oxygen was reached, a known amount of antioxidant was injected and the suppressed oxygen uptake measured until the rate of oxidation returned to the uninhibited rate. The length of the inhibition period was measured to check the rate of chain initiation (R_i, see text). This procedure was generally repeated three times for determinations of inhibition rate constants for each solvent mixture used.

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Acknowledgment. This manuscript is dedicated to Dr. Keith Ingold on the occasion of his 70th birthday. Support of this research by a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) is gratefully acknowledged.

JA990878U