The Surprisingly High Reactivity of Phenoxyl Radicals[1]

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Abstract: Rate constants have been measured in nonaqueous media for hydrogen atom abstraction by the phenoxyl radical from some biologically important phenols and related compounds. Although the thermochemistry for these reactions must be very similar to the thermochemistry for H atom abstraction from the same substrate by a peroxyl radical, the phenoxyl rate constants, k_5, are ca. 100–300 times greater than the (already well-known) peroxyl rate constants, k_1. For example, with α-tocopherol in benzene/di-*tert*-butyl peroxide (1:3, v/v) $k_5^{293K} = 1.1 \times 10^9$ M^{-1} s^{-1} vs $k_1^{303K} = 3.2 \times 10^6$ M^{-1} s^{-1} in a similar nonpolar medium, and with ubiquinol-10 in the same solvent mixture $k_5^{293K} = 8.4 \times 10^7$ M^{-1} s^{-1}, while the corresponding value for k_1 is 3.5×10^5 M^{-1} s^{-1}. The greater reactivity of the phenoxyl radical has been traced to the fact that the Arrhenius preexponential factors are much larger than for the corresponding peroxyl radical reactions, i.e., $A_5 \sim 10^2 A_1$. For example, with α-naphthol $\log(A_5/M^{-1}\ s^{-1}) = 8.9$ and $E_5 = 2.2$ kcal/mol vs $\log(A_1/M^{-1}\ s^{-1}) = 6.4$ and $E_1 = 1.7$ kcal/mol. The preexponential factors for H-atom donors more reactive than α-naphthol are even greater; for example, with α-tocopherol in CH_3CN/di-*tert*-butyl peroxide (1:2, v/v) $\log(A_5/M^{-1}\ s^{-1}) = 10.0$ and $E_5 = 2.0$ kcal/mol, and with ubiquinol-0 in benzene/di-*tert*-butyl peroxide (1:3, v/v) $\log(A_5/M^{-1}\ s^{-1}) = 10.5$ and $E_5 = 3.5$ kcal/mol. The role that intermediate hydrogen-bonded complexes between the reacting radical and the phenolic hydrogen donor may play in these reactions is discussed, and it is pointed out that our results are likely to be relevant to *in vivo* radical chemistry.

Phenols are extensively employed in living organisms and in technological applications to protect lipids and other natural or synthetic organic materials from oxidative degradation.[3-5] This antioxidant activity is due to the ability of phenols to trap the chain-carrying peroxyl radicals with the formation of a hydroperoxide and a resonance-stabilized aryloxyl radical, reaction 1. In most systems the aryloxyl radical is too unreactive to continue the chain, and so it "sits around" until it encounters a second peroxyl with which it reacts rapidly, reaction 2. Under some

$$ROO^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet} \quad (1)$$

$$ROO^{\bullet} + ArO^{\bullet} \rightarrow \text{nonradical products} \quad (2)$$

conditions this simple antioxidant behavior is not observed because the aryloxyl radicals continue the oxidative chain either by reacting with hydroperoxide,[6] the reverse of reaction 1, or by reacting with the organic substrate it is "supposed" to protect,[7] reaction 3. In the particularly important case of the peroxidation of low-

$$ArO^{\bullet} + RH \rightarrow ArOH + R^{\bullet} \xrightarrow{O_2} ROO^{\bullet} \quad (3)$$

density lipoprotein[8] even the biologically most important phenolic antioxidant, α-tocopherol (vitamin E),[5] has been shown to have a strong prooxidant effect (via reaction 3) in the absence of vitamin C and ubiquinol-10[10-12] (two other biologically important radical-trapping antioxidants).

There is voluminous literature regarding the absolute rate constants for H atom abstraction from phenols by peroxyl radicals[5,13-17] (reaction 1) and by numerous other radicals.[18] By way of contrast, there is very little information on the absolute rate constants for H atom abstractions by aryloxyl radicals.[17] Moreover, what little information there is on this topic[17] generally involves indirect (and hence not very reliable) kinetic procedures, and furthermore, it usually relates to reactions involving sterically hindered aryloxyls.[22] Such radicals undergo their bimolecular

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(22) Kinetic studies on H atom abstractions by aryloxyl radicals are largely confined to 2,4,6-tri-*tert*-butylphenoxyl[23-25] and related phenoxyls,[26] α-tocopheroxyl,[27] and tocopheroxyls that are sterically more encumbered than α-tocopheroxyl.[28] The same holds true for ESR studies of aryloxyl/phenol equilibria.[29]

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self-reactions (reaction 4) only slowly, if at all. They are therefore

$$ArO^{\bullet} + ArO^{\bullet} \rightarrow \text{nonradical products} \quad (4)$$

relatively persistent, and hence, it is easy to monitor their kinetic behavior.

In view of the clear-cut importance of H atom abstractions by aryloxyl radicals in certain biologically relevant systems[8-12] it appeared worthwhile to initiate a program aimed at obtaining some reliable kinetic data for H atom abstractions by sterically unprotected and, hence, transient (with $2k_4 \sim 10^9$ M^{-1} s^{-1})[30] aryloxyl radicals. We have begun with the simplest aryloxyl, i.e., the phenoxyl radical, and have employed the technique of (time-resolved) laser flash photolysis (LFP) to study H atom abstractions from several phenols by this radical, reaction 5.

$$PhO^{\bullet} + ArOH \rightarrow PhOH + ArO^{\bullet} \quad (5)$$

To our surprise, this phenoxyl radical has been found to be roughly 100–300 times more reactive than peroxyl radicals, i.e., $k_5 \sim [(1\text{–}3) \times 10^2]k_1$ for the same ArOH.

Results

Various potential methods for generating high concentrations of phenoxyl "instantaneously" (i.e., during the 10-ns laser pulse) were explored.[31] The most satisfactory was also the simplest, viz., LFP (355 nm, 40 mJ/pulse, 10 ns/pulse) at 293 K of 1.4 M phenol dissolved in di-*tert*-butyl peroxide/acetonitrile (2:1, v/v) or di-*tert*-butyl peroxide/benzene (3:1, v/v). This produced ca. 10^{-4}–10^{-5} M phenoxyl, which could be monitored via its absorption at 400 nm (reactions 6 and 7).

$$Me_3COOCMe_3 \xrightarrow{h\nu} 2Me_3CO^{\bullet} \quad (6)$$

$$Me_3CO^{\bullet} + PhOH \xrightarrow{\text{fast}[32]} Me_3COH + PhO^{\bullet} \quad (7)$$

Addition of a second phenol, ArOH, allows two additional reactions to occur, reaction 8 and reaction 5. Reaction 8 will be

$$Me_3CO^{\bullet} + ArOH \rightarrow Me_3COH + ArO^{\bullet} \quad (8)$$

"instantaneous" because all of the *tert*-butoxyls generated in the laser pulse will be "instantaneously" consumed in reaction 7 (and 8). Provided an appropriate concentration of ArOH is employed ("appropriate" being determined by the reactivity of ArOH), reaction 5 will compete effectively with the destruction of phenoxyl radicals via their bimolecular self reaction:

$$PhO^{\bullet} + PhO^{\bullet} \rightarrow \text{nonradical products} \quad (9)$$

Under such conditions, the $ArO^{\bullet}$ concentration will show an initial "instantaneous" rise from zero during the laser pulse (reaction 8) and thereafter will show a slow, pseudo-first-order growth (reaction 5). Provided the $ArO^{\bullet}$ radical absorbs in a region which is not "masked" by $PhO^{\bullet}$ absorptions, the two-step growth in the $ArO^{\bullet}$ concentration can be easily monitored. The experimentally derived pseudo-first-order rate constant for the slow, second stage,

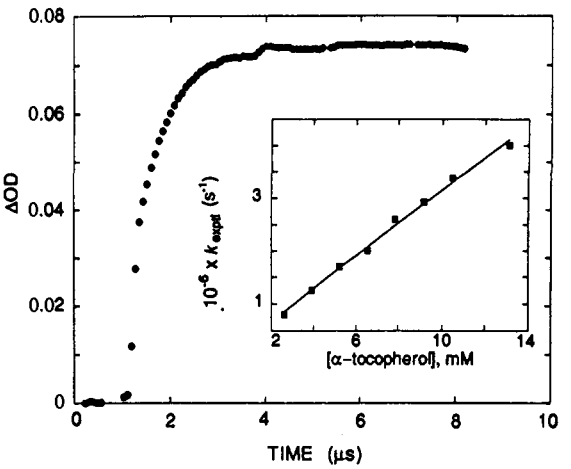

Figure 1. Growth of the α-tocopheroxyl radical's absorption at 430 nm following 355-nm LFP of 1.4 M phenol in di-*tert*-butyl peroxide/acetonitrile (2:1, v/v) containing 8×10^{-3} M α-tocopherol at 293 K. Inset: variation in the experimental first-order grow-in rate constant as a function of the α-tocopherol concentration. The slope of this straight line is equal to k_5.

k_{exptl}, is related to the desired rate constant, k_5, via the equation

$$k_{exptl} = k_0 + k_5[ArOH]$$

provided the reverse reaction rate constant, k_{-5}, is negligible in comparison with k_5 (as would be expected since all the reactions studied are exothermic). Thus, the slope of a "quenching" plot of k_{exptl} vs [ArOH] yields k_5.

Some typical experimental results for the reaction of the phenoxyl radical with α-tocopherol in di-*tert*-butyl peroxide/acetonitrile are shown in Figure 1. The mean value found for k_5^{293K} (an average of seven determinations with 1.4 M phenol) in this solvent mixture is 3.1×10^8 M^{-1} s^{-1}, and the reaction is ca. 3.5 times faster in the less polar di-*tert*-butyl peroxide/benzene solvent mixture (cf. Table 1). In both solvents the magnitude of the rate constant was shown to be independent of the phenol concentration over the range 1.4–0.5 M. Table 1 also includes mean k_5 values for a number of other phenols and two ubiquinols[33] (for structures see Scheme 1). Also included in Table 1 are values of k_1 (generally measured at 303 K) which have been derived from the literature.[14,15,34-39] The peroxyl radical which has provided most of the Table 1 kinetic data for reaction 1 was poly(peroxystyryl)peroxyl, and the solvent was styrene.[14,15,34,39]

Attempts were made to measure the deuterium kinetic isotope effect (DKIE) for three phenoxyl/phenol reactions using the technique originally developed to measure DKIEs for peroxyl/phenol reactions.[40,41] However, these experiments were successful

(28) See, for example: Mukai, K.; Kohno, Y.; Ishizu, K. *Biochem. Biophys. Res. Commun.* **1988**, *155*, 1046–1050. Nagaoka, S.; Okauchi, Y.; Urano, S.; Nagashima, U.; Mukai, K. *J. Am. Chem. Soc.* **1990**, *112*, 8921–8924.

(29) See, for example: Jackson, R. A.; Husseini, K. M. *J. Chem. Soc., Chem. Commun.* **1992**, 967–968. Lucarini, M.; Pedulli, G. F.; Cipollone, M. Unpublished results.

(30) See ref 17, pp 142–159.

(31) Using 355-nm LFP irradiation, the following potential phenoxyl sources were investigated: diphenyl oxalate, phenyl *p*-chlorobenzoate, diphenyl carbonate, allyl phenyl ether, and α-(4-cyanophenoxy)acetophenone. Only the first two of these compounds yielded phenoxyl and then either in poor yield or partially "masked" by the absorptions of other transients.

(32) In di-*tert*-butyl peroxide/benzene (2:1, v/v), $k_7 = 3.3 \times 10^8$ M^{-1} s^{-1} at 295 K.[19]

(33) Values of k_5 were actually measured for eight different ubiquinols having different numbers, *n*, of isoprenoid (C_5) units in their "tail". Values of (*n*); individual experimental values of $10^{-7}k_5/M^{-1}s^{-1}$; and, when more than two individual measurements were made, the mean ($\pm 1\sigma$) value of $10^{-7}k_5/M^{-1}s^{-1}$ follow: (0) 8.8, 8.6, 10.0, 9.1; 9.1 (±0.5); (1) 7.3, 8.3; (2) 7.6, 8.3; (3) 8.1, 8.9; (4) 8.1, 8.6; (5) 7.5, 8.8; (6) 8.0, 9.3; (10) 8.9, 7.6, 8.3, 8.8, 8.4 (±0.5).

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(41) In this technique all exchangeable protons are replaced by deuterons by the simple expedient of adding a few drops of D_2O to the solvent (benzene, styrene, etc). Rate constants, k^D, measured under these conditions are then compared with rate constants, k^H, measured in the presence of a few drops of H_2O. (Values of k^H in the presence of H_2O are usually essentially identical to the rate constants measured under normal, "dry", conditions.)

Table 1. Rate Constants for H Atom Abstraction from Some Phenols by Phenoxyl, k_5, and Peroxyl, k_1, Radicals

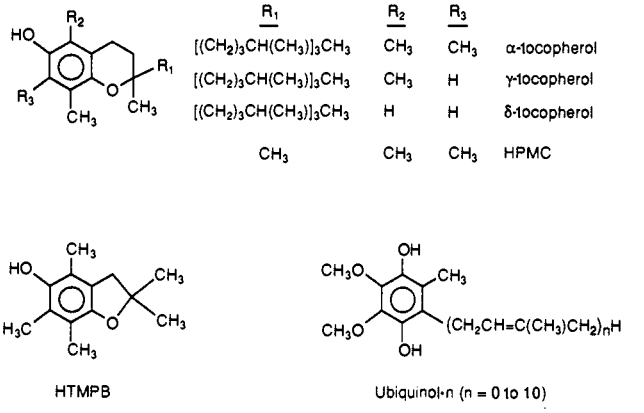
ArOH	conc range[a] (mM)	λ(ArO•)[b] (nm)	10^{-7} k_5^{293K} [c] (M^{-1} s^{-1})		$10^{-5}k_1^{303K}$ [d] (M^{-1} s^{-1})	ref[e]
			CH_3CN[f]	C_6H_6[f]		
α-tocopherol (OH)	1–13	440	31 ± 3	110[g]	32[g]	15
α-tocopherol (OD)	1–8	440		94[h]	5.9[h]	15
γ-tocopherol	2–10	430	8.9 ± 0.5	25	14	15
δ-tocopherol	5–50	430	2 ± 1		4.4	15
HPMC[i]	0.6–3.0	430	37[j]		38	15
HTMPB[k]	0.8–7.0	430	24[j]		47	34
α-naphthol	90–310	510		2.3 ± 0.5	2.4 (1.5)[l]	14 (35)
β-naphthol	100–320	470		0.45 ± 0.06	(0.33)[l,m]	(36)
ubiquinol-0	1–40	430		9.1 ± 0.5[n]	3.1	39
ubiquinol-10	1–7	430		8.4 ± 0.5[n]	3.5[o,p]	39

[a] Range of [ArOH] used in the "quenching" studies. [b] Wavelength at which the growth of [ArO•] was monitored. [c] Errors (corresponding to 1σ) are given when three or more separate quenching plots (cf. Figure 1) were used to derive mean k_5 values. Average values for k_5 are given without errors when only two separate quenching plots were made, the agreement between the k_5 values obtained in the two experiments being always better than ±20% and often better than ±10%. With two substrates only a single quenching plot was made (cf. footnote *j*) because insufficient material was available for a second experiment. [d] Values from the literature for ROO• = poly(peroxystyryl)peroxyl in neat styrene as solvent at 303 K unless otherwise noted. [e] Literature reference to k_1 value. [f] Cosolvent used with the di-*tert*-butyl peroxide: peroxide/acetonitrile (2:1, v/v); peroxide/benzene (3:1, v/v). [g] Without and with (6) drops of H_2O. [h] With (6) drops of D_2O. [i] 6-Hydroxy-2,2,5,7,8-pentamethylchroman, an α-tocopherol analogue in which the 2-phytyl group has been replaced by a methyl group. [j] Single quenching plot. [k] 2,3-Dihydro-5-hydroxy-2,4,6,7-tetramethyl-2-phytylbenzofuran, an α-tocopherol analogue with a CH_2 group removed from the heterocyclic ring. [l] ROO• = $(CH_3)_3COO$•. [m] Same rate constant was obtained with $C_2H_5C(CH_3)_2OO$•. [n] Values of k_5 do not depend on the length of the chain attached to the ubiquinol head group; see ref 33. [o] For the reaction of ubiquinol-6 with $C_6H_5CH(CH_3)OO$• at 50 °C, $k = 3.2 \times 10^5$ M^{-1} s^{-1}, and for the same peroxyl reacting with ubiquinol-9, $k = 3.4 \times 10^5$ M^{-1} s^{-1} at 50 °C and 2.7×10^5 M^{-1} s^{-1} at 40 °C; seee ref 37. [p] Ubiquinol-10 has been reported to be ca. 10% as effective as α-tocopherol for inhibiting the autoxidation of methyl linoleate in hexane at 37 °C; see ref 38.

only in the case of α-tocopherol,[42] for which $k_5^H/k_5^D = 110 \times 10^7/94 \times 10^7 = 1.17$ (see Table 1).

The lipid-soluble, radical-trapping antioxidant vitamin E is complemented *in vivo* by the lipid-soluble, radical-trapping antioxidant ubiquinol-10[11,12] and by the water-soluble radical-trapping antioxidant vitamin C (ascorbic acid).[11,12] Our measurements of k_5 for eight different ubiquinols[33] demonstrate that their reactivities toward the phenoxyl radical do not depend on the number of isoprenoid units in their hydrophobic "tail" (cf. also Table 1, where it can be seen that k_5 is essentially identical

Scheme 1



for a "tail" of 50 carbon atoms (ubiquinol-10) as for no "tail" (ubiquinol-0)). It is clear, therefore, that the phenoxyl/ubiquinol reactions exclusively involve the abstraction of a hydrogen atom from a hydroxyl group in the hydroquinone "head" of the ubiquinol. The ubiquinols are about 10% as reactive toward phenoxyl as α-tocopherol (cf. Table 1 and ref 33). The same factor of about 10 difference in the reactivities toward peroxyl radicals of α-tocopherol and various ubiquinols has also been reported[37-39] (cf. also Table 1 and footnotes *o* and *p* to this table).[47]

Vitamin E and vitamin C have long been known to interact to provide better protection against lipid peroxidation when used

(42) With the other two phenols which were examined, viz., α- and β-naphthol,[43] the experiments with D_2O yielded puzzling results. In the presence of H_2O the behaviors of the absorptions due to the naphthoxyl radicals were completely normal; that is, the initial instantaneous jump after the laser flash was followed by a slow growth which yielded essentially the same k_5 values as in the absence of H_2O. However, with D_2O there was no slow growth after the initial jump, the absorption due to the naphthoxyl radicals merely undergoing a slow *decrease* in intensity. This unexpected behavior we attribute principally to the fact that the DKIE for hydrogen atom abstraction by *tert*-butoxyl radicals from phenol, k_7^H/k_7^D, is substantially larger than for its abstractions from α- and β-naphthol, k_8^H/k_8^D. For phenol, Das *et al.*[19] have reported that in *tert*-butanol/di-*tert*-butyl peroxide (1:1, v/v) at 295 K $k_7^H = 1.5 \times 10^7$ M^{-1} s^{-1} and $k_7^H/k_7^D = 4.8$. In benzene/di-*tert*-butyl peroxide (1:1, v/v) at 295 K there is an increase in k_7^H to 3.3×10^8 M^{-1} s^{-1}.[19] Under our conditions, viz., benzene/di-*tert*-butyl peroxide (1:3, v/v) at 293 K, we find $k_7^H = 2.0 \times 10^8$ M^{-1} s^{-1} and $k_7^H/k_7^D = 4.5$. That is, as would be expected in the much less polar and non-hydrogen-bonding solvent mixture, there is a substantial increase in k_7^H and a small, but significant, decrease in k_7^H/k_7^D. In benzene/di-*tert*-butyl peroxide (1:2, v/v) Das *et al.*[19] have reported that $k_8^H = 2.0 \times 10^9$ and 1.0×10^9 M^{-1} s^{-1} for α- and β-naphthol, respectively. Under our conditions in benzene/peroxide (1:3, v/v) with α-naphthol we find $k_8^H = 1.1_3 \times 10^9$ M^{-1} s^{-1} and $k_8^H/k_8^D = 1.0_3$, while with β-naphthol we find $k_8^H = 6.9_5 \times 10^8$ M^{-1} s^{-1} and $k_8^H/k_8^D = 1.1_4$. With both naphthols, hydrogen atom abstraction by *tert*-butoxyl radicals is approaching the diffusion-controlled limit (as is also the case with the phenoxyl/α-tocopherol reaction), and as would therefore be expected, there is a very substantial decrease in the DKIEs for these two compounds relative to phenol.[44] As a consequence, in the presence of D_2O the "instantaneous" ratio of radical concentrations immediately following LFP, $[C_6H_5O^\bullet]_{t=0}/[\text{naphthoxyl}]_{t=0}$, will be very considerably smaller than the same ratio in the absence of D_2O.[45] This fact, *combined* with the likelihood that there is a fairly large DKIE for the phenoxyl/naphthol reactions,[46] means that in the presence of D_2O the naphthoxyl radicals produced "instantaneously" simply undergo decay (presumably largely by their bimolecular self-reactions) after the laser pulse. That is, in the presence of D_2O too few phenoxyl radicals are formed *and* the phenoxyl/naphthol (OD) reactions are too slow to show the "normal", second stage, slow growth of the naphthoxyl radical absorptions after the laser pulse.

(43) These were chosen because the DKIEs for peroxyl radical abstraction from α- and β-naphthol have been carefully measured,[35,36] the values for k_1^H/k_1^D at 303 K being 5.4[35] and 6.7,[36] respectively. The lower reactivities toward phenoxyl of the two naphthols in comparison with a-tocopherol (cf. Table 1) also implied that they might show considerably larger k_5^H/k_5^D values than α-tocopherol.

(44) The same is true for α-tocopherol relative to phenol.[20] That is, for the *tert*-butoxyl/α-tocopherol reaction $k_8^H = 3.8 \times 10^9$ M^{-1} s^{-1} at 298 K in benzene/di-*tert*-butyl peroxide (1:1, v/v) and $k_8^H/k_8^D = 1.31$.[20] However, in "wet acetonitrile" the rate of hydrogen abstraction is substantially slower, $k_8^H = 6.6 \times 10^8$ M^{-1} s^{-1}, and the DKIE is enhanced, $k_8^H/k_8^D = 2.69$.[20]

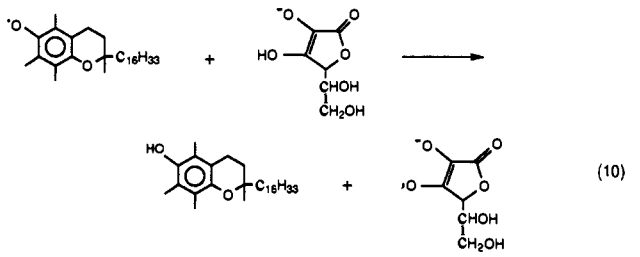
(45) It should be noted that increasing the naphthol concentration will merely reduce the $[C_6H_5O^\bullet]_{t=0}/[\text{naphthoxyl}]_{t=0}$ ratio further and hence will make matters even worse. Before we realized this we did, in fact, increase the maximum concentrations of both naphthols by up to a factor of 10 in D_2O relative to the maximum concentrations employed in the absence of D_2O.

(46) Since neither of these reactions is particularly fast, we estimate that k_5^H/k_5^D will be ca. 2–6, provided reaction 5 involves rate-controlling hydrogen atom abstractions (as seems highly probable in the nonpolar di-*tert*-butyl peroxide/benzene solvent mixture).

(47) A factor of only about 3 difference in the reactivities toward peroxyl radicals of α-tocopherol and ubiquinol-3 has, however, been reported in *tert*-butanol and CCl_4 as solvents.[48] In aqueous dispersions of phospholipids, such as liposomes[48,49] and large unilamellar vesicles,[50] ubiquinols and α-tocopherol would apppear to have a rather similar reactivity toward peroxyl radicals.

(48) Landi, L.; Cabrini, L.; Fiorentini, D.; Stefanelli, C.; Pedulli, G. F. *Chem. Phys. Lipids* **1992**, *61*, 121–130.

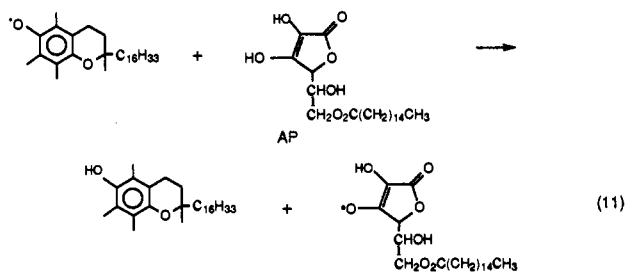
together than when either is used alone. This mutual reinforcing action, or synergism, is due to the "regeneration" of vitamin E from its radical by the ascorbate anion, reaction 10, as first suggested by Golumbic.[51] The facility with which this reaction



(10)

occurs is attested to by the fact that it can take place across the phase boundary in water–lipid systems; that is, the ascorbate anion (in the aqueous phase) has been demonstrated to reduce the α-tocopheroxyl radical in a lipid phase such as phospholipid vesicles[52,53] and LDL particles.[12]

There have been several measurements of the rate constant for reaction 10 at ambient temperatures in water-containing systems,[54–57] all of which indicate that it is a fairly fast reaction. For example, $k_{10} = 1.55 \times 10^6$ M^{-1} s^{-1} in a homogeneous solution of propanol (50%), water (40%), acetone (10%), and CCl_4 (0.04 M),[54] $k_{10} = 2 \times 10^5$ M^{-1} s^{-1} in an aqueous dispersion of phosphatidylcholine liposomes,[55] and in positively charged hexadecyltrimethylammonium chloride[56] and bromide[57] micelles (which would attract the ascorbate anion) values of $k_{10} = 7.2 \times 10^7$ [56] and 9×10^5 M^{-1} s^{-1} [57] have been reported. By way of contrast, in benzene as solvent the reduction of the α-tocopheroxyl radical by a nonionized, lipid-soluble form of vitamin C, ascorbic acid 6-palmitate, AP (reaction 11), is fairly slow with $k_{11} \approx 2.8 \times 10^3$ M^{-1} s^{-1} at 37 °C.[58,59] A very similar rate constant for reaction 11, viz., 3×10^3 M^{-1} s^{-1}, has also been obtained in hexadecyltrimethylammonium bromide micelles at room temperature.[57]



(11)

Our method of generating phenoxyl radicals (via reactions 6 and 7) required the use of a relatively nonpolar organic solvent and hence the use of ascorbyl palmitate rather than the ascorbate anion as the substrate, reaction 12. A suitable solvent was found

$$\mathrm{PhO^{\bullet}} + \underset{\text{(AP)}}{C_{22}H_{38}O_7} \rightarrow \mathrm{PhOH} + C_{22}H_{37}O_7^{\bullet} \qquad (12)$$

to be di-*tert*-butyl peroxide/tetrahydrofuran (2:1, v/v) containing 1.4 M phenol. In this case, it was the loss of the phenoxyl radical after the laser pulse which was monitored (via its absorption at 400 nm). Excellent pseudo-first-order decay traces were obtained (see Figure 2), and from the plot of k_{exptl} vs [ascorbyl palmitate] (see inset in Figure 2) we obtained $k_{12}^{293K} = 5.9 \times 10^6$ M^{-1} s^{-1}.[64]

The addition of a few drops of H_2O to the above described system had a negligible kinetic effect[64] ($k_{12}^{293K}(H_2O) = 5.6 \times 10^6$ M^{-1} s^{-1}), but the addition of D_2O "switched off" the phenoxyl/AP reaction completely. That is, the addition of AP produced no increase in the rate at which the phenoxyl radicals decayed in the absence of AP ($k_0 \sim 5.8 \times 10^5$ s^{-1}; see inset in Figure 2) even up to the maximum AP concentration achievable, viz., 0.2 M, above which concentration emulsions are produced. Since the phenoxyl decay rate accelerating effect of 0.02 M AP ($k_{exptl} \sim 7 \times 10^5$ s^{-1}; see inset in Figure 2) in the "dry" system (and in the H_2O experiment) is quite readily distinguishable from k_0, we must conclude that deuterated AP (or AP$^-$)[64] is at least 10 times less reactive toward phenoxyl radicals than the undeuterated material. That is, k_{12}^H/k_{12}^D (or $k_{12^-}^H/k_{12^-}^D$)[64] > 10. Such a very large DKIE (which is only consistent with a rate-controlling hydrogen atom transfer) is unusual but not without precedent. That is, DKIEs of 10 or greater have been found for the reactions of peroxyl radicals with sterically hindered phenols.[65]

Discussion[66]

The most striking fact to emerge from the kinetic data given in Table 1 is that the phenoxyl radical is roughly 100–300 times as reactive as a peroxyl radical in abstracting phenolic hydrogen atoms. That is, $k_5^{293K} \sim (1\text{–}3) \times 10^2 k_1^{303K}$ for all phenols and over a range in k_5 or k_1 values of about 100. The large magnitude of these k_5:k_1 ratios was unexpected because, in the only previous investigation of which we are aware,[25] the k_5:k_1 ratio was only about 10. In this earlier study,[25] the phenol was 2,4,6-tri-*tert*-butylphenol, and at 60 °C k_5 was estimated to be ca. 3.5×10^5 M^{-1} s^{-1} and k_1 to be ca. 2.8×10^4 M^{-1} s^{-1}. It is more usual to see differences in reactivities becoming smaller as the reactions become faster not the reverse, hence, a part of our surprise.

All the reactions covered by the kinetic data given in Table 1 are undoubtedly exothermic. However, for any particular phenol the exothermicities of reactions 5 and 1 must be nearly equal because the O—H bond dissociation energies (BDEs) for phenol and for hydroperoxides are certainly very similar, and perhaps

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(59) Rate constants for the reactions of sterically more crowded tocopheroxyl radicals with (un-ionized) ascorbic acid and ascorbic acid 6-fatty acid esters generally appear to be somewhat smaller.[60–63] For example,[61] at 25 °C in benzene/ethanol/H_2O (2:1:0.1, v/v) a tocopheroxyl radical having two *ortho*-isopropyl substituents reacted with ascorbic acid and ascorbic acid 6-stearate with k = 230 and 350 M^{-1} s^{-1}, respectively.
(60) Mukai, K.; Fukuda, K.; Ishizu, K.; Kitamura, Y. *Biochem. Biophys. Res. Commun.* **1987**, *146*, 134–139.
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(63) Mukai, K.; Nishimura, M.; Kikuchi, S. *J. Biol. Chem.* **1991**, *266*, 274–278.
(64) We cannot completely rule out the possibility that the polarity of this solvent system is sufficient for the AP to be ionized and that reaction 12 should be reformulated to involve the AP anion, reaction 12′. However, for reasons

$$\mathrm{PhO^{\bullet}} + C_{22}H_{37}O_7^- \rightarrow \mathrm{PhOH} + C_{22}H_{36}O_7^- \qquad (12')$$

discussed later this would appear to be rather unlikely.
(65) For example, for 2,6-di-*tert*-4-methylphenol $k_1^H/k_1^D = 24$ at 176 K,[35] 11.0 at 239 K,[35] and 10.6 at 338 K.[40] Similarly large isotope effects have been found with various other 4-substituted 2,6-di-*tert*-butylphenols.[35]
(66) We will refrain from any discussion of the *origins* of the differences in reactivity of the various phenols because this topic has been very extensively covered in publications relating to the kinetics of H atom abstraction by peroxyl radicals.[3–7,14–16,23–25,33,34]

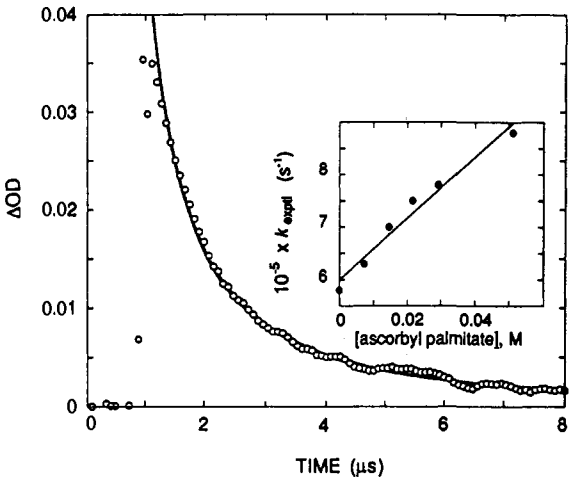

Figure 2. Growth and decay of the phenoxyl radical's absorption at 400 nm following 355-nm LFP of 1.4 M phenol in di-*tert*-butyl peroxide/tetrahydrofuran (2:1, v/v) containing 0.052 M ascorbic acid 6-palmitate at 293 K. The solid line is the best first-order fit to the decay curve. Inset: variation in the experimentral first-order decay rate constant as a function of the concentration of ascorbic acid 6-palmitate. The slope of this straight line is equal to k_{12}.

even identical,[67] hence, the remainder of our surprise that $k_5/k_1 \sim (1\text{–}3) \times 10^2$.

The ca. 100 fold difference in rates between reactions 5 and 1 could, in principle, be due to a difference of ca. 2.7 kcal/mol in their activation energies (i.e., $E_5 \approx E_1 - 2.7$ kcal/mol) or to a ca. 100 fold difference in their preexponential factors (i.e., $A_5 \approx 10^2 A_1$ M^{-1} s^{-1}) or some combination of these two extremes. The immediately available evidence suggested that it was differences in the preexponential factors of reactions 5 and 1 which were mainly responsible for the high k_5:k_1 ratios. Thus, the Arrhenius parameters for reaction 1 have been very carefully measured by a direct ESR method[80] over a significant range in $1/T$ for α- and β-naphthol and the corresponding deuterated (OD) compounds.[35,36] For α-naphthol (OH) and (OD) (T = 168–199 K)[35]

$$\log(k_1^{H}/M^{-1}\,s^{-1}) = (6.4 \pm 0.8) - (1.7 \pm 0.6)/\theta$$

$$\log(k_1^{D}/M^{-1}\,s^{-1}) = (6.4 \pm 0.7) - (2.7 \pm 0.6)/\theta$$

For β-naphthol (OH) and (OD) (T = 180–293 K)[36]

$$\log(k_1^{H}/M^{-1}\,s^{-1}) = (6.4 \pm 0.3) - (2.6 \pm 0.3)/\theta$$

$$\log(k_1^{D}/M^{-1}\,s^{-1}) = (6.5 \pm 0.4) - (3.9 \pm 0.4)/\theta$$

where $\theta = 2.3RT$ kcal/mol and the errors correspond to 1σ.[81]

Inspection of Table 1 reveals that the magnitudes of k_5^{293K} for α- and β-naphthol are $10^{7.4}$ and $10^{6.7}$ M^{-1} s^{-1}, respectively. These values are well above the $10^{6.4}$ M^{-1} s^{-1} found by Howard and co-workers[35,36] for the preexponential factors for peroxyl radical attack on these two phenols. Thus, it is clear that $A_5 \gg A_1$ for the two naphthols.

Although it seemed likely that $A_5 \gg A_1$ for the other phenols (since the measured A-factors for reaction 1 with a variety of phenols[82] are also "unusually low" when compared with the "normal" value for a simple atom-transfer reaction of $10^{8.5\pm0.5}$ M^{-1} s^{-1} [83]), we decided to measure the Arrhenius parameters for reaction 5 for some selected phenolic substrates. Rate constants for reaction 5 were measured over a temperature range with α-tocopherol, α-naphthol, β-naphthol, and ubiquinol-0. The Arrhenius plots are shown in Figure 3, and the derived Arrhenius equations are given in Table 2.[84] It is obvious that the ca. 100 fold enhancement of k_5 over k_1 for each phenolic substrate is due mainly, or entirely, to a ca. 100 fold enhancement in the magnitude of the preexponential factor for H atom abstraction by the phenoxyl radical relative to the peroxyl radical.

The generally accepted explanation for "unusually low" A-factors (and, in part,[85] "low" activation energies) in H atom transfers between two oxygen atoms is that such reactions occur via prior equilibrium formation of a hydrogen-bonded complex of the oxygen-centered radical with the OH-containing substrate followed by a rate-controlling atom transfer within the complex,[25,34,35,87,88] reaction 13.

$$XO^{\bullet} + HOAr \underset{-a}{\overset{a}{\rightleftarrows}} XO^{\bullet}\cdots HOAr \underset{-b}{\overset{b}{\rightleftarrows}} XOH\cdots{}^{\bullet}OAr \underset{-c}{\overset{c}{\rightleftarrows}} XOH + {}^{\bullet}OAr \quad (13)$$

For the phenols listed in Table 1 the overall processes leading to products via reactions 1 and 5 are likely to be nearly irreversible since these processes are quite exothermic. If reaction $-a$ is negligible compared to reaction b, i.e., $k_{-a} < k_b$, the experimental rate constant will be

(67) Surprisingly, measured and estimated values of the O—H bond dissociation energy (BDE) for phenol cover a much wider range than do the values for the O—H BDE in hydroperoxides: BDE (PhO—H) in kcal/mol, 84,[68,69] 85.4,[70] 86.5,[71] 88.2,[72] 88.3,[25] 88.9–90,[73] and 91.6;[74] BDE (ROO—H) in kcal/mol, 87 (R = H),[75] 87.7 (R = H),[76] 88.0 (R = tetralyl),[24,25] 88.1 (R = cumyl),[77] 88.5 (R = H or CH_3),[78] 89.6 (R = H or $(CH_3)_3C$).[79]

(68) Mulder, P.; Saastad, O. W.; Griller, D. *J. Am. Chem. Soc.* **1988**, *110*, 4090–4092.

(69) Recent work using the same experimental technique as in ref 68 (photoacoustic calorimetry) indicates that this value is probably ca. 2 kcal/mol too low: Wayner, D. D. M.; Mulder, P. Unpublished results.

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(80) This involved measurement of the pseudo-first-order rate constants for decay of (relatively persistent) $(CH_3)_3COO^{\bullet}$ and $C_2H_5C(CH_3)_2OO^{\bullet}$ in the presence of known concentrations of the phenols.

(81) For α-naphthol these equations yield $k_1^{H}/k_1^{D} = 1.5 \times 10^5/2.8 \times 10^4 = 5.4$ at 303 K, and for β-naphthol $k_1^{H}/k_1^{D} = 3.3 \times 10^4/4.9 \times 10^3 = 6.7$ at the same temperature. For β-naphthol the errors have been calculated by us from the original experimental data.[35]

(82) The most reliable Arrhenius parameters for reaction 1 come from the direct ESR method for measuring k_1.[35,36] For phenol,[36] $\log(A_1/M^{-1}\,s^{-1}) = 7.2$ and E_1 = 5.2 kcal/mol; for various sterically hindered 2,6-di-*tert*-butyl 4-substituted phenols,[35] $\log(A_1/M^{-1}\,s^{-1})$ are in the range 3.7–4.7 and E_1 values are in the range 0.4–1.0 kcal/mol. More conventional, though generally less accurate, kinetic procedures have also yielded unusually low preexponential factors (and activation energies) for reaction 1.[24,40]

(83) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley: New York, 1976.

(84) (a) For α-tocopherol in the benzene solvent system k_5 is about 3 times as large as in the CH_3CN solvent system (see Table 1). The value of $\log(A_5/M^{-1}\,s^{-1})$ for α-tocopherol in the former solvent will therefore probably be ca. 10.5 (since the activation energy is not likely to be smaller than the 2.0 kcal/mol found in the latter solvent). (b) Note that for ubiquinol-0 $\log(A_5/M^{-1}\,s^{-1}$/OH group) = 10.2.

(85) The intrinsic activation energy (i.e., E for a thermoneutral reaction) for the transfer of a hydrogen atom between two heavy atoms, X and Y, depends on the triplet repulsion energy curve for the $X^{\bullet}/Y^{\bullet}$ pair. This is due to the necessary occurrence of parallel electron spins on X and Y during the course of H transfer, i.e., $[X\uparrow\cdots H\downarrow\cdots Y\uparrow]^{\ddagger}$. Since the triplet repulsion between two oxygen atoms is less than between oxygen and carbon or between two carbon atoms, the two oxygen atoms can approach more closely to one another and the activation energy for H transfer is lowered.[25,86]

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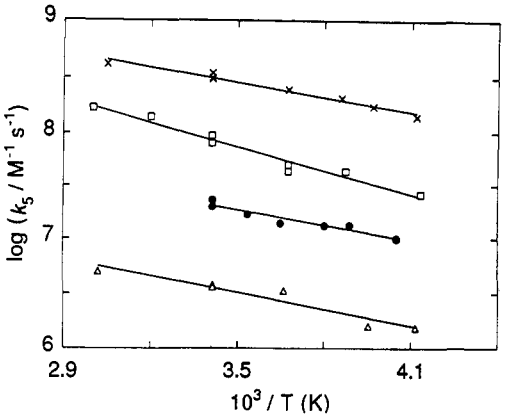


Figure 3. Arrhenius plots for some phenoxyl radical/phenol reactions: α-tocopherol, ×, in di-*tert*-butyl peroxide/acetonitrile (2:1, v/v) plus 1.4 M phenol; and ubiquinol-0, □; α-naphthol, ●; and β-naphthol, △, in di-*tert*-butyl peroxide/benzene (3:1, v/v) plus 1.4 M phenol.

Table 2. Arrhenius Parameters for H Atom Abstraction from Some Phenols by Phenoxyl Radicals

ArOH	solvent[a]	T/K (range)	$\log(A_5/M^{-1}\,s^{-1})$[b]	E_5/kcal mol^{-1} [b]
α-tocopherol	P/CH_3CN (2:1, v/v)	243–328	10.0 ± 0.2	2.0 ± 0.2
α-naphthol	P/C_6H_6 (3:1, v/v)	247–293	8.9 ± 0.3	2.2 ± 0.3
β-naphthol	P/C_6H_6 (3:1, v/v)	243–331	8.3 ± 0.3	2.3 ± 0.4
ubiquinol-0	P/C_6H_6 (3:1, v/v)	242–333	10.5 ± 0.2	3.5 ± 0.3

[a] P = di-*tert*-butyl peroxide. Note that the solvent also contains 1.4 M phenol. [b] Errors correspond to one standard deviation.

$$k_{exptl} = k_a$$

In this case, the Arrhenius preexponential factor will be "high" since, in the absence of steric constraints, it will be equal to the A-factor for a diffusion-controlled reaction, i.e., $A \sim 10^{11.5}$ M^{-1} s^{-1} in solvents of "normal" viscosity.[89] This would be the situation for a simple, straightforward H atom abstraction that was thermodynamically favored and for which the activation energy for step b, E_b, was very small. In such a case, the experimental activation energy would be roughly equal to the activation energy for diffusion (typically ca. 3 kcal/mol in normal solvents).[89] Such a "direct" H atom abstraction would be indistinguishable (except in the microscopic timing of events) from a process involving a strong attraction (i.e., a strong hydrogen bond) between the reactants *provided* this attraction resulted in an orientation of the reactants suitable for the formation of the products (*vide infra*) since, in this case, once the two reactants had become associated, they inevitably and without a measurable barrier would yield the final products even if E_b were not "very small".

On the other hand, if reaction -a competes effectively with reaction b, i.e., $k_{-a} > k_b$, we have

$$k_{exptl} = K_a k_b$$

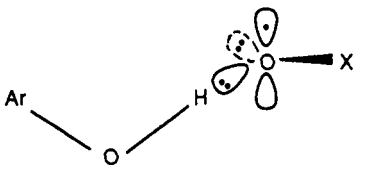
and

$$\log(A_{exptl}/M^{-1}\,s^{-1}) = \log(A_b/s^{-1}) + (\Delta S_a/2.3R)$$

Thus, A_{exptl} will be reduced below $A_b(\sim 10^{13.5}\ s^{-1})$ by the loss of entropy caused by formation of the H-bonded complex. This can cause A_{exptl} to become considerably smaller than the "normal" value for a hydrogen atom abstraction; that is, it can lead to $A_{exptl} \ll 10^{8.5}$ M^{-1} s^{-1}. In this case, moreover, there will also be a reduction in the measured activation energy compared with E_b by an amount equivalent to the (negative) heat of formation of the H-bonded complex, i.e.,

$$E_{exptl} = E_b + \Delta H_a$$

This situation (i.e., $k_{-a} > k_b$) would apply when the initial hydrogen-bonded complex was suitably oriented for product formation but there was only a very weak association between the reactants, always provided that E_b was not "very small". This situation would also apply even if E_b was "very small" when there was a strong association of the reactants but the hydrogen-bonded complex held the reactants in orientations not suitable for product formation. This last situation could apply to reaction 13 because the formation of a hydrogen bond from ArOH to the oxygen-centered radical, XO•, seems more likely to involve an oxygen 2p-type lone pair of electrons than the unpaired electron, i.e.,



A hydrogen bond of this type would hold the reactants in a nonproductive encounter, i.e., in a mutual orientation which did not lie on the pathway to the products. The stronger the hydrogen bond to an oxygen lone pair of XO• the more likely it would be for an encounter between the reactants to yield a nonproductive complex. Reaction might only be able to occur after dissociation and diffusion apart (step -a) and reassociation (step a), a process which might occur thousands of times before the "redocking" of the XO•/ArOH pair led to an orientation suitable for product formation.

For reaction 1 the unusually low values which have nearly always been found for A_{exptl} therefore imply that $k_{-a} > k_b$, which means either that there is only a very weak association between ROO• and ArOH or that there is a strong association which is improperly aligned for reaction. In contrast, for reaction 5 our results require that $k_{-a} < k_b$, which means either that there is a "direct" hydrogen atom abstraction from ArOH by PhO• or that a hydrogen-bonded intermediate complex is formed in which the reactants are correctly positioned to yield the products. Phenol (pK_a = 9.95)[90] is more acidic than organic hydroperoxides (pK_a = 11.5–12.8)[90] which means that PhO^- is a weaker base than ROO^-. On this basis, it seems reasonable to argue that PhO• will be a weaker base than ROO• and, therefore, that a PhO•/ArOH hydrogen-bonded complex is likely to be weaker than a ROO•/ArOH complex.[91] Thus, it appears likely that in reaction 5 there is a "direct" hydrogen atom abstraction from ArOH by PhO• (with any intermediate hydrogen-bonded complex playing only a minor role), whereas, in reaction 1 the ArOH and ROO• form a relatively strong hydrogen-bonded complex which hinders the overall reaction because it is incorrectly oriented to yield the products.

The preexponential factors found for reaction 5 range from values "normal" for metatheses reactions[83] (α- and β-naphthol, $\log(A_5/M^{-1}\,s^{-1}$ = 8.9 and 8.3, respectively) to the "high" values which imply that the reaction is partially diffusion-controlled (α-tocopherol, $\log(A_5/M^{-1}\,s^{-1})$ = 10.0 in CH_3CN (but probably ca. 10.5 in C_6H_6),[84a] and ubiquinol-0, $\log(A_5/M^{-1}\,s^{-1}$/OH group)[84b] = 10.2).[92] The activation energies for all the phenoxyl/phenol reactions are small (2.0–3.5 kcal/mol), which puts them in the range expected for reactions which are diffusion-controlled

(89) Beckwith, A. L. J.; Bowry, V. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4983–4992.

(90) Jencks, W. P.; Regenstein, J. In *Handbook of Biochemistry and Molecular Biology*, 3rd ed., *Physical and Chemical Data*; Fasman, G. D., Ed.; CRC Press: Cleveland, OH, 1976; Vol. 1, Section D, pp 314, 316.

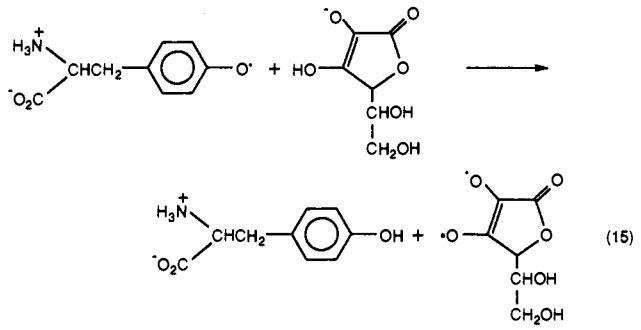
(91) We thank an anonymous referee for this suggestion.

(92) Much lower preexponential factors have been reported for reactions of the sterically hindered 2,4,6-tri-*tert*-butylphenoxyl radical with various substituted phenols. Substituents, $\log(A/M^{-1}\,s^{-1})$, E (kcal/mol):[23] 4-*tert*-butyl, 5.5, 4.8; 3,5-dimethyl, 6.4, 6.8; 4-bromo, 6.5, 7.5; 3-carboethoxy, 5.9, 8.2; 2,4,6-trichloro, 4.6, 5.5. The preexponential factor is of a similar magnitude for the identity reaction with 2,4,6-tri-*tert*-butylphenol.[87]

or which proceed at rates sufficiently fast to be influenced by diffusion. Because the reaction rate is largely controlled by diffusion, there cannot be any substantial DKIE, cf. α-tocopherol, for which $k_5^H/k_5^D = 1.17$. Conversion of the phenoxyl radical/phenol pair into the phenol/aroxyl radical pair (step *b* of reaction 13) most probably occurs via hydrogen atom transfer. However, an electron transfer followed by proton transfer cannot be ruled out by our experiments:

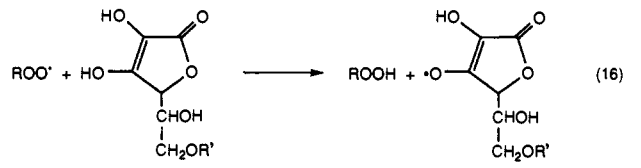
$$PhO^{\bullet}\cdots HOAr \rightarrow PhO^{-}\cdots [HOAr]^{\bullet+} \rightarrow PhOH\cdots {}^{\bullet}OAr \quad (14)$$

Ascorbyl palmitate is a slightly better trap for phenoxyl radicals (k_{12} (or $k_{12'}$)[64] = 5.9 × 10⁶ M⁻¹ s⁻¹) than β-naphthol (k_5 = 4.5 × 10⁶ M⁻¹ s⁻¹). The available kinetic data on the reactions of α-tocopheroxyl with ascorbyl palmitate ($k_{11} \approx 3 \times 10^3$ M⁻¹ s⁻¹)[57-59] and with the ascorbate anion (k_{10} = 7 × 10⁷ to 2 × 10⁵ M⁻¹ s⁻¹)[54-57] imply that the ascorbate anion would be a very effective trap for phenoxyl radicals. This implication is fully supported by a pulse radiolysis study of the tyrosyl radical/ascorbate reaction (15) in aqueous buffer (pH 7), for which a rate constant k_{15} =

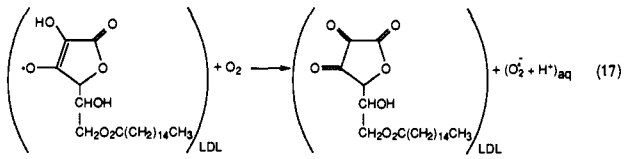


4.4 × 10⁸ M⁻¹ s⁻¹ was obtained at room temperature.[93,94] We would expect the phenoxyl radical, PhO•, and the tyrosyl radical to be quite similar in reactivity, with the former probably being slightly more reactive.[95] These kinetic data lead us to conclude that the phenoxyl radical/ascorbate acid 6-palmitate reaction which we have studied probably involves hydrogen atom abstraction from neutral AP, reaction 12, rather than hydrogen atom abstraction from the AP anion, reaction 12',[64] even in the presence of a few drops of H_2O (or D_2O).

Barclay *et al.*[97] have measured the rate constant for reaction of poly(peroxystyryl)peroxyl radicals with AP at 30 °C, reaction 16 (R′ = $C_{16}H_{31}O$), and obtained k_{16} = 1.1 × 10⁶ M⁻¹ s⁻¹ (in styrene).[98] Thus, in nonpolar solvents AP is about as reactive toward peroxyl radicals as γ-tocopherol (see Table 1). Surprisingly, k_{16} is only about a factor of 5 lower than the rate constant found in the present work for reaction of phenoxyl radicals with (probably nonionized)[64] AP, viz., k_{12} = 5.9 × 10⁶ M⁻¹ s⁻¹ (in di-*tert*-butyl peroxide/tetrahydrofuran (2:1, v/v) with 1.4 M phenol). Either our measured value of k_{12} has been reduced by this rather good hydrogen-bond-acccepting solvent mixture from a higher value it would have in a nonpolar solvent or the phenoxyl/peroxyl reactivity ratio of ca. 100 found with phenols does not extend to nonphenolic hydrogen atom donors.



Although lipid-soluble esters of ascorbic acid are somewhat less effective peroxyl-radical-trapping agents than α-tocopherol in homogeneous nonpolar media, in lipid micelles dispersed in water such esters have been demonstrated to be as effective, or even slightly more effective, than α-tocopherol.[98b,102] This we attribute to the same phenomenon which makes ubiquinol-10 an outstandingly effective inhibitor of the peroxidation of low-density lipoprotein (LDL)[11,12] whereas α-tocopherol in LDL functions as a prooxidant in the absence of ubiquinol-10 and ascorbate.[10-12] The prooxidant action of vitamin E has been attributed to the inability of the highly lipophilic α-tocopheroxyl radical to "escape" from the LDL particle in which it is generated.[10-12] The α-tocopheroxyl radical abstracts a hydrogen atom from a polyunsaturated fatty acid moiety, thereby generating a lipid radical and setting in-train the tocopherol-mediated peroxidation process.[10-12] In contrast, when a hydrogen atom is abstracted from the highly lipophilic ubiquinol-10, the resultant semiquinone radical can "export" its radical character from the LDL particle into the aqueous phase by reacting with oxygen to generate superoxide.[11,12] Thus, there is no possibility of an oxidative chain reaction in an LDL particle which contains a molecule of ubiquinol-10.[11,12] We would expect that the same situation might well hold for ascorbyl palmitate and related compounds; that is, the semidione radical formed in an LDL particle via reaction 16 (R′ = $OC(CH_2)_{14}CH_3$) could also "export" superoxide into the aqueous phase, reaction 17.



In conclusion, phenoxyl radicals are unexpectedly reactive toward phenols. With the more reactive phenols, including, in particular, the biologically important α-tocopherol (vitamin E), the overall reaction (hydrogen atom transfer) occurs at a rate

(93) Hunter, E. P. L.; Desrosiers, M. F.; Simic, M. G. *Free Radical Biol. Med.* **1989**, *6*, 581–585.

(94) With Trolox, a water-soluble analogue of α-tocopherol, the reaction with the tyrosyl radical had a rate constant of 3.1 × 10⁸ M⁻¹ s⁻¹ under these conditions.[93] There is a clear implication here that the ascorbate anion is at least as good as, and probably better than, α-tocopherol at trapping phenoxyl radicals.

(95) This is a deduction based on the well-established fact that *p*-cresol is a somewhat better donor of its phenolic hydrogen to numerous radicals than is phenol[17-21,96] and on measured and estimated O—H BDEs for phenol and for 4-alkyl-substituted phenols, which generally indicate that the O—H BDEs in the latter are ca. 1–2 kcal/mol weaker than in phenol.[68,70,72-74]

(96) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1963**, *41*, 1744–1751.

(97) Barclay, L. R. C.; Dakin, K. A.; Zahalka, H. A. *Can. J. Chem.* **1992**, *70*, 2148–2153.

(98) (a) There are also extensive measurements on the kinetics of peroxyl radical/ascorbate anion reactions in aqueous (mixed-solvent) systems at room temperature,[99] e.g., for $CH_3OO^{\bullet}$ + ascorbate (pH 7) k = 1.8 × 10⁶ M⁻¹ s⁻¹,[99a,100] and a measurement of the rate constant for $CH_3OO^{\bullet}$ + ascorbic acid (pH 3.1) for which k_{16}(R′ = H) = 4 × 10⁵ M⁻¹ s⁻¹;[99a] that is, the acid is only about 25% as active in trapping peroxyl radicals as is the anion. This last rate constant is in fair agreement with a value reported from a study of the inhibited autoxidation of methyl linoleate in *tert*-butanol/methanol (3:1, v/v) at 37 °C by (presumably nonionized) ascorbic acid, viz., k_{16}(R′ = H) = 7.5 × 10⁴ M⁻¹ s⁻¹.[101] In the *tert*-butanol/methanol solvent and in neat *tert*-butanol k_1 for α-tocopherol was 5.1 × 10⁵ M⁻¹ s⁻¹.[101] (b) Interestingly, for the azo-initiated autoxidation of linoleic acid/SDS micelles dispersed in water at 37 °C the antioxidant activity of ascorbyl palmitate has been shown to be 1.09 times greater than the antioxidant activity of α-tocopherol.[102]

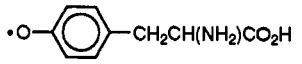
(99) See for example: (a) Neta, P.; Huie, R. E.; Mosseri, S.; Shastri, L. V.; Mitall, J. P.; Maruthamuthu, P.; Steenken. S. *J. Phys. Chem.* **1989**, *93*, 4099–4104. (b) Neta, P.; Huie, R. E.; Maruthamuthu, P.; Steenken, S. *J. Phys. Chem.* **1989**, *93*, 7654–7659. (c) Alfassi, Z. B., Huie, R. E.; Kumar, M.; Neta, P. *J. Phys. Chem.* **1992**, *96*, 767–770.

(100) Much of the kinetic work on the peroxyl radical/ascorbate reaction[99] has been carried out using highly reactive halogenated peroxyl radicals such as $Cl_3COO^{\bullet}$.

(101) Niki, E.; Saito, T.; Kawakami, A.; Kamiya, Y. *J. Biol. Chem.* **1984**, *259*, 4177–4182.

(102) Pryor, W. A.; Cornicelli, J. A.; Devall, L. J.; Tait, B.; Trivedi, B. K.; Witiak, D. T.; Wu, M. *J. Org. Chem.* **1993**, *58*, 3521–3532.

which is close to the diffusion-controlled limit. The reaction is only ca. 1 order of magnitude slower for the other biologically important reducing agents, γ-tocopherol and ubiquinol. The potential significance of such fast reactions in biological systems should not be overlooked because, in such systems, the tyrosyl radical,



would be expected (as indicated above) to behave kinetically in much the same way as the phenoxyl radical behaved in the present study. In particular, we note that the tyrosyl radical has been demonstrated to catalyze the oxidative cross-linking of proteins[103] and that radical-induced protein oxidation is a chain reaction.[104]

Experimental Section

Materials. Spectrograde acetonitrile and benzene (BDH) were used as received. Di-*tert*-butyl peroxide (Aldrich) was passed twice through neutral alumina before use. Phenol (Aldrich) was purified by several recrystallizations from cyclohexane, dried under vacuum, and stored in a desiccator until needed. 2*R*,4′*R*,8′*R*-α-, γ-, and δ-tocopherols, 6-hydroxy-2,2,5,7,8-pentamethylchroman (HPMC), and *all-rac*-2,3-dihydro-5-hydroxy-2,4,6,7-tetramethyl-2-phytylbenzofuran (HTMPB) were available from earlier work in this laboratory. α- and β-Naphthol (Aldrich) were sublimed and then recrystallized from cyclohexane/ethanol. L-Ascorbic acid 6-palmitate (95%, Aldrich) was recrystallized from ethanol/H_2O and dried under vacuum. The ubiquinols were obtained by reduction of the corresponding ubiquinones with ascorbic acid using one procedure for ubiquinone-0 and a different procedure for ubiquinone-1 to -6 and -10, as described below for ubiquinol-0 and -10.

To a three-necked flask containing 50 mL of H_2O and a stirring bar were added ubiquinone-0 (200 mg, 1.10 mmol) and ascorbic acid (380 mg, 2.16 mmol). The solution was sparged with nitrogen continuously throughout the reaction. The flask was immersed in a water bath at 40–50 °C, and through an addition funnel connected to the flask, 2 mL of a 0.05 M aqueous solution of NaOH was added one drop at a time with vigorous stirring and an interval of 5 min between each drop. During this addition the solution slowly changed from the orange color of the ubiquinone to the colorless ubiquinol-0. When the reduction was complete, the solution was maintained at 40–50 °C for a further 10 min, after which it was concentrated on a rotary evaporator. Extraction with CH_2Cl_2 (2 × 30 mL), drying (anhydrous Na_2SO_4), filtration, and removal of the solvent under vacuum gave ubiquinone-0 (160 mg, 98–99% pure by GC) as a very pale yellow oil, which readily solidified at 0 °C.

To a three-necked flask containing 100 mL of absolute EtOH and a stirring bar were added ubiquinone-10 (200 mg, 0.23 mmol) and ascorbic acid (80 mg, 0.45 mmol). The flask was then sonicated until most of the solid material had dissolved. After flushing with nitrogen for a few minutes the flask was placed in a water bath at 50 °C, the nitrogen bubbling was maintained, and 2 mL of a 0.05 M aqueous solution of NaOH was added drop by drop with vigorous stirring. Subsequently, either 3 mL of H_2O or 3 mL of H_2O containing 40 mg of ascorbic acid was added rapidly to the ethanol solution, and the temperature of the reaction mixture was maintained for 1–2 h until the solution became a very pale orange color. After isolation as described above for ubiquinol-0, the ubiquinol-10 was obtained as a pale yellow solid (150 mg, 95–98% pure by TLC).

Laser Flash Photolysis (LFP). Excitation was provided by a Lumonics HY 750 Nd:YAG laser (third harmonic, 355 nm; 10-ns pulses, 40 mJ/pulse) using an experimental system which has been described elsewhere.[105] None of the phenols used in the present work absorbed at 355 nm at the concentrations at which they were employed. The laser's energy is absorbed solely by the di-*tert*-butyl peroxide which had to be employed as the major component of the solvent (peroxide/acetonitrile, 2:1 v/v; peroxide/benzene, 3:1 v/v) in order to obtain reasonably strong signals. All solutions were deoxygenated by bubbling with pure nitrogen for 5 min prior to laser excitation. Four to six transient growth traces of the aryloxyl radial, ArO•, derived from the phenolic substrate, ArOH, were averaged (with correction for fluorescence) for each ArOH concentration employed.

The following describes a typical experiment. Solutions of phenol (2.1 M) in di-*tert*-butyl peroxide and α-tocopherol (0.04 M) in acetonitrile were prepared. To a standard, 7 × 7 mm^2 Suprasil quartz tube were added 2.00 mL of the phenol solution and a total of 1.00 mL of acetonitrile made up first from 0.05 mL of the α-tocopherol solution plus 0.95 mL of pure acetonitrile. To a second quartz tube was added a fresh 2.00 mL of the phenol solution together with 0.10 mL of the α-tocopherol solution plus 0.90 mL of pure acetonitrile. This was continued with new quartz tubes until 1.00 mL of the acetonitrile solution of α-tocopherol was added to a fresh 2.00 mL of the phenol solution. In all these tubes the final concentration of phenol was 1.4 M, while the concentration of α-tocopherol ranged from 6.67×10^{-4} to 1.33×10^{-2} M. After degassing each tube by sparging with nitrogen the laser experiment was carried out and the growth of the absorption due to the α-tocopheroxyl radical was analyzed kinetically, as described in the Results section.

Samples for measurements of the deuterium kinetic isotope effects were prepared as described above but using benzene as the cosolvent with di-*tert*-butyl peroxide. To these samples were added 6 drops of H_2O or 6 drops of D_2O (MSD Isotopes, 99.8% d). The cell was shaken gently and then allowed to stand for 5 min prior to LFP.

Temperature variation in LFP experiments was provided by passage of a stream of precooled or preheated nitrogen over the outside of the reaction vessel which itself was placed in a nonsilvered supracil Dewar vessel. Temperatures were measured with a thermocouple after thermal equilibrium had been established.

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