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Solvent Effects on the Antioxidant Activity of Vitamin E[1]

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Vitamin E (α-tocopherol, TOH) is the major radical-trapping, lipid-soluble antioxidant in mammals.[4,5] It protects biological membranes from damage by trapping the lipid peroxyl radicals, $ROO^\bullet$, which would otherwise destroy the membrane lipids, RH, in a two-step chain reaction.

$$\mathrm{ROO^\bullet + RH \rightarrow ROOH + R^\bullet} \qquad (1)$$

$$\mathrm{R^\bullet + O_2 \rightarrow ROO^\bullet} \qquad (2)$$

This inhibition of lipid peroxidation is effected via a very facile transfer of the phenolic hydrogen atom of TOH to peroxyl radicals.[4,6]

$$\mathrm{ROO^\bullet + TOH \rightarrow ROOH + TO^\bullet} \qquad (3)$$

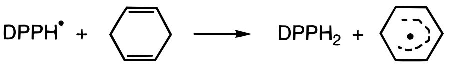
To understand and, hopefully, to model the ability of a biological membrane to withstand oxidative stress requires knowledge of the absolute rate constant for reaction 3, $k^{\mathrm{S}}_{\mathrm{ROO/TOH}}$, in biologically relevant systems. However, this rate constant is very likely to be highly dependent on the solvent, S, because we have found that rate constants for abstraction of hydroxylic hydrogen atoms show dramatic solvent effects.[7−11] These kinetic solvent effects (KSEs) can be accounted for *quantitatively* on the basis of hydrogen bonding between the hydrogen bond donor (HBD) substrate (or antioxidant) and hydrogen bond acceptor (HBA) solvent.[7,8,11] Because the extent of the HBD/HBA interaction depends only on the HBD and HBA, it was predicted that the magnitude of a KSE would be independent of the reactivity of the attacking radical.[7] This prediction was confirmed for hydrogen atom abstraction from TOH (and phenol) by highly reactive *tert*-alkoxyl radicals (reaction 4) and by the very

$$\mathrm{RO^\bullet + TOH \rightarrow ROH + TO^\bullet} \qquad (4)$$

unreactive 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^\bullet$, reaction 5) in about a dozen solvents chosen so that the

$$\mathrm{DPPH^\bullet + TOH \rightarrow DPPH_2 + TO^\bullet} \qquad (5)$$

rate constants for each radical plus substrate reaction changed by about 2 orders of magnitude.[8,12] Only *tert*-butyl alcohol gave anomalous results in that the rate constants for hydrogen atom abstraction by $DPPH^\bullet$ (but not by $RO^\bullet$) were ca. 5 times greater than expected. Subsequently, rate constants for hydrogen atom abstraction from 1,4-cyclohexadiene (CHD) by $DPPH^\bullet$, $k^{\mathrm{S}}_{\mathrm{DPPH/CHD}}$, provided further evidence that the reactivity of $DPPH^\bullet$ is enhanced by a factor of about 3 in *tert*-butyl alcohol relative to its reactivity in many other solvents.[13]



(6)

In the present work, we set out to determine whether solvent effects on the $ROO^\bullet$/TOH reaction mirrored those of reaction 4 or reaction 5.

Results

Peroxyl radicals were generated "instantaneously" by UV (300−400 nm) flash photolysis of dicumyl ketone in oxygen-saturated (ca. 5 mM [O_2]) solutions at 298 K in the presence of various concentrations of TOH (see Scheme 1). Dicumyl ketone was chosen as the peroxyl radical precursor for two reasons. First, decarbonylation of the 2,2-dimethyl-2-phenylacetyl radical is sufficiently rapid ($k_8 = 1.5 \times 10^8$ s^{-1})[14] that oxygen trapping to give the acylperoxyl radical, $PhCMe_2C(O)OO^\bullet$, can be ignored.[15] Second, the tertiary cumylperoxyl radicals undergo only a slow bimolecular self-reaction ($2k_{11} = 6 \times$

Scheme 1

$$\mathrm{(PhCMe_2)_2CO \xrightarrow{h\nu} Ph\dot{C}Me_2 + PhCMe_2\dot{C}O} \qquad (7)$$

$$\mathrm{PhCMe_2\dot{C}O \longrightarrow Ph\dot{C}Me_2 + CO} \qquad (8)$$

$$\mathrm{Ph\dot{C}Me_2 + O_2 \longrightarrow PhCMe_2OO^\bullet} \qquad (9)$$

$$\mathrm{PhCMe_2OO^\bullet + TOH \longrightarrow PhCMe_2OOH + TO^\bullet} \qquad (10)$$

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[12] In any particular solvent, $k^{\mathrm{RO^\bullet}}/k^{\mathrm{DPPH^\bullet}}$ was ca. 10^6 for TOH and ca. 10^{10} for phenol.

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[15] The elimination of acylperoxyl radicals is important, because they are very much more reactive than alkylperoxyl radicals, see: Zaikov, G. E.; Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1969**, *47*, 3017−3029.

10.1021/jo982360z CCC: $18.00

Table 1. Absolute Rate Constants ($M^{-1}\ s^{-1}$ at 298 ± 2 K) for Hydrogen Atom Abstraction from α-Tocopherol by Cumylperoxyl Radicals ($k^{S}_{ROO/TOH}$), *tert*-Butoxyl Radicals ($k^{S}_{RO/TOH}$), and 1,1-Diphenyl-2-picrylhydrazyl Radicals ($k^{S}_{DPPH/TOH}$)

	solvent	$10^{-5}\ k^{S}_{ROO/TOH}$	$10^{-8}\ k^{S}_{RO/TOH}$ [a]	$10^{-2}\ k^{S}_{DPPH/TOH}$ [a]
1	hexane	200[b]	99[c]	74[c]
2	carbon tetrachloride	140	42	36
3	benzene	34[d]	31	18
4	toluene	30[d]		
5	chlorobenzene	27	36	27
6	anisole	15	20	14
7	acetic acid	8.8	7.7	6.2
8	*tert*-butyl alcohol	5.6[f]	1.8	5.7
9	acetonitrile	3.8	9.4	4.9
10	ethyl acetate	2.3	2.9	1.6

[a] Data from ref 8. [b] In cyclohexane at 293 K, a value of 68 × $10^{5}\ M^{-1}\ s^{-1}$ was obtained by pulse radiolysis.[18] [c] Value in pentane. [d] In styrene at 303 K, a value of 32 × $10^{5}\ M^{-1}\ s^{-1}$ was obtained from the rate of the TOH-inhibited oxidation of styrene.[6] [e] Measured at 303 K. [f] Measurements of the rate of the TOH inhibited oxidation of methyl linoleate at 310 K in *tert*-butyl alcohol gave 5.1 × $10^{5}\ M^{-1}\ s^{-1}$ [19] and of the TOH-inhibited oxidation of linoleic acid at 303 K in *tert*-butyl alcohol gave 2.3 × $10^{5}\ M^{-1}\ s^{-1}$.[20]

$10^{3}\ M^{-1}\ s^{-1}$)[16] so they can be exclusively trapped by the added TOH.[17]

$$2PhCMe_2OO^{\bullet} \rightarrow \text{nonradical products} \qquad (11)$$

Hydrogen atom abstraction from TOH by the cumylperoxyl radicals was monitored by following the pseudo-first-order growth of the 420 nm absorption of the tocopheroxyl radicals, k_{obs}. At least three separate measurements of k_{obs} were made at each concentration of TOH employed, and at least five different concentrations of TOH were employed in each solvent. Absolute second-order rate constants, $k^{S}_{ROO/TOH}$, in each solvent, S, were obtained by least-squares fitting of plots of k_{obs} vs [TOH] according to

$$k_{obs} = k_o + k^{S}_{ROO/TOH}\ [TOH]$$

Excellent straight lines were obtained in all solvents ($R \geq 0.98$). Values of $k^{S}_{ROO/TOH}$ are given in Table 1 together with our earlier kinetic data for reaction 4 ($k^{S}_{RO/TOH}$) and reaction 5 ($k^{S}_{DPPH/TOH}$).

Discussion

Where comparison is possible, the present values of $k^{S}_{ROO/TOH}$ are in satisfactory agreement with earlier direct[18] and indirect[6,19,20] rate constant determinations in the same (or similar) solvents (see footnotes b, d, and f in Table 1). The agreement between the values of $k^{S}_{ROO/TOH}$ determined in this work in benzene (34 × 10^{5} $M^{-1}\ s^{-1}$) and in toluene (30 × $10^{5}\ M^{-1}\ s^{-1}$) and that determined by measurement of the TOH-inhibited autoxidation of styrene (32 × $10^{5}\ M^{-1}\ s^{-1}$)[6] is quite remarkable and speaks well for both techniques.

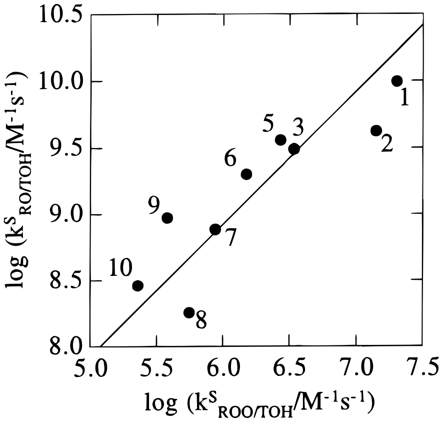

Figure 1. Comparison of kinetic solvent effects for H-atom abstraction from TOH by peroxyl and *tert*-alkoxyl radicals. The straight line in the figure has a slope of 1.0 and $R = 0.84$.

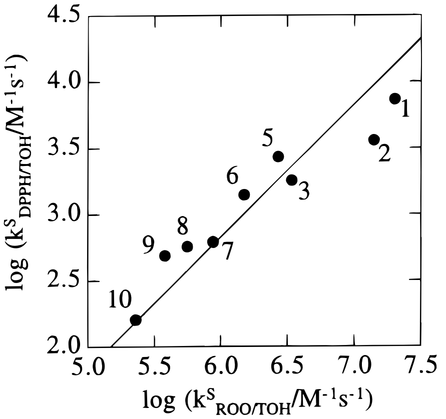

Figure 2. Comparison of kinetic solvent effects for H-atom abstraction from TOH by peroxyl and DPPH$^{\bullet}$ radicals. The straight line in the figure has a slope of 1.0 and $R = 0.90$.

As expected, values of $k^{S}_{ROO/TOH}$ are strongly solvent dependent and the magnitude of the KSEs on reactions 3−5 are essentially identical. That is, Figure 1 shows a plot of log $k^{S}_{RO/TOH}$ vs log $k^{S}_{ROO/TOH}$, and it can be seen that all of the points lie close to the straight line shown that has been drawn with the theoretical slope of 1.0. Figure 2 shows a similar plot of log $k^{S}_{DPPH/TOH}$ vs log $k^{S}_{ROO/TOH}$. These two figures add further support to our view that KSEs for abstraction of hydroxylic hydrogen atoms are, to a first approximation, independent of the attacking radical, depending instead on the HBD ability of the substrate and the HBA ability of the solvent.[7,8,11,21]

A closer examination of the two figures reveals that the kinetic data for the ROO$^{\bullet}$/TOH reaction correlates better with that for the DPPH$^{\bullet}$/TOH reaction ($R = 0.90$, Figure 2) than with that for the RO$^{\bullet}$/TOH reaction ($R = 0.84$, Figure 1). The better correlation seen in Figure 2 arises largely because *both* $k^{S}_{ROO/TOH}$ and $k^{S}_{DPPH/TOH}$ are larger in *tert*-butyl alcohol (**8**) than in acetonitrile (**9**) and ethyl acetate (**10**). In contrast, $k^{S}_{RO/TOH}$ is lowest in *tert*-butyl alcohol and decreases along the solvent series,

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[17] Primary and secondary alkylperoxyl radicals undergo much faster bimolecular self-reactions (Ingold, K. U. *Acc. Chem. Res.* **1969**, *2*, 1−9), and exclusive trapping by the added TOH could not be guaranteed.

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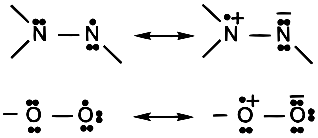
acetonitrile > ethyl acetate > *tert*-butyl alcohol, an order which is consistent with the relative HBA abilities of these three solvents.[22] Our present results indicate, therefore, that ROO$^•$ radicals have an enhanced reactivity in *tert*-butyl alcohol comparable in magnitude to the previously discovered enhanced reactivity of DPPH$^•$ radicals in this solvent[8,13] (but not in methanol or ethanol).[13] These two radicals are isoelectronic and both are stabilized by conjugative electron delocalization:



therefore, a similar *tert*-butyl alcohol-induced increase in their reactivities, whatever its cause, is not entirely unreasonable. However, the situation must be somewhat more complex, because one of us has previously demonstrated that there is no KSE in a reaction in which a C−H bond is broken by an attacking peroxyl radical (reaction 1, with RH = cumene and ROO$^•$ = cumylperoxyl).[21] Specifically, $k^{S}_{ROO/RH}$ is the same within experimental error in *tert*-butyl alcohol, isooctane, benzene, acetonitrile and pyridine.[21] This result was not expected because the rate constant for C−H bond breaking by DPPH$^•$, $k^{S}_{DPPH/CHD}$ (reaction 6) was 3 times greater in *tert*-butyl alcohol than in other solvents.[13]

To summarize, the peroxyl radical/α-tocopherol reaction exhibits the expected large kinetic solvent effects. However, our work reveals that there are still some relatively small (≤a factor of ca. 5) KSEs on hydrogen atom abstraction reactions which are not understood at present. To be specific, *tert*-butyl alcohol enhances the rate constants for (i) C−H bond breaking by DPPH$^•$,[13] (ii) O−H bond breaking by DPPH$^•$,[8] and (iii) O−H bond breaking by ROO$^•$ (this work) but has no special effect on (iv) C−H bond breaking by ROO$^•$,[21] (v) C−H bond breaking by RO$^•$,[25] (vi) O−H bond breaking by RO$^•$,[8,10] or (vii) O−H bond breaking by R$^•$.[26] The reason for the deviant behavior of *tert*-butyl alcohol solvent on the kinetics of a few hydrogen atom abstraction reactions remains to be determined.

Experimental Section

Materials. Solvents were of the purest grade commercially available and were used as received. 2*R*,4′*R*,8′*R*-α-tocopherol (natural vitamin E) was purified chromatographically on silica gel as described previously.[8] Dicumyl ketone was synthesized from dibenzyl ketone (Aldrich, purified by crystallization from hexane to >99% by GC−MS) "piecemeal" in three steps with purification after each step, more or less following a literature procedure.[27] Briefly, dibenzyl ketone was slowly added to 2.4 equiv NaH in dry THF under argon at 0 °C, the mixture was allowed to warm to room temperature with stirring (30 min), 2.4 equiv of methyl iodide was added, and the mixture was refluxed for 2 h. After the addition of cold (0 °C) water, the crude dimethylated derivative was extracted with ethyl ether, the ether was removed under vacuum, and the derivative was purified by crystallization from hexane. This process was repeated twice more using half the quantities of NaH and MeI, with purification by recrystallization of the intermediate trimethylated derivative. The dicumyl ketone was obtained in pure form (>99%, GC−MS) in an overall yield of 40%: mp 111−113 °C, lit. 111−112 °C,[27] 110−115 °C (dec);[28] ^{1}H NMR (200 MHz, CDCl$_3$) δ: 1.35 (s, 12 H), 7.2 (m, 2 H), 7.3 (m, 8 H), lit.[27] (CDCl$_3$) δ: 1.26 (s, 12 H), 7.1 (bs, 10 H).

Kinetic Measurements. Conventional flash photolysis work was performed in a commercial PRA FP-1000 flash system. The system was operated at 6.0 kV, and the exciting light was filtered through Pyrex. Transient absorptions were monitored at 420 nm. The signals were captured by a Tektronix 24.32 digital scope interfaced to a PowerMac 7100 computer that provided suitable processing facilities. Experiments were performed in oxygen-saturated (760 Torr) solvents, using a 10 cm optical path quartz cuvette. The solvents contained ca. 50 mM dicumyl ketone[29] and 0.5−2.5 mM α-tocopherol. At least five different concentrations of TOH were employed for each solvent, and every measurement was repeated at least three times. The pseudo-first-order growth of the TO$^•$ absorbance was monitored at 420 nm. Absolute second-order rate constants, $k^{S}_{ROO/TOH}$, were obtained by least-squares fitting of the observed pseudo-first-order rate constant, k_{obs}, vs [TOH].

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(22) For acetonitrile, ethyl acetate, and *tert*-butyl alcohol, β_2^{H} = 0.44, 0.45, and 0.49, respectively,[23] and β = 0.31, 0.45, and ≥0.6, respectively.[24]

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(28) Gould, I. R.; Zimmt, M. B.; Turro, N. J.; Baretz, B. H.; Lehr, G. F. *J. Am. Chem. Soc.* **1985**, *107*, 4607−4612.

(29) The dicumyl ketone was added to the solvent at such a concentration that the OD at 320 nm in a 7 mm cuvette was ca. 1.0.