

## Do Peroxyl Radicals Obey the Principle That Kinetic Solvent Effects on H-Atom Abstraction Are Independent of the Nature of the Abstracting Radical?

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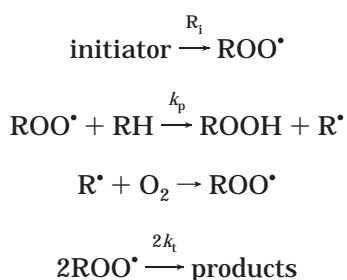
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The oxidation by molecular oxygen of lipids containing polyunsaturated fatty acids or their esters, a reaction called autoxidation or peroxidation, has important effects on the structural and functional properties of biomembranes. Many pathological events, such as heart disease, cancer, atherosclerosis, rheumatoid arthritis, as well as the degenerative processes associated with aging and with the action of foreign toxic substances, are often related to the membrane damage caused by lipid peroxidation, which is believed to represent the major pathway by which free radicals mediate their cytotoxic effects.<sup>1–3</sup>

In the autoxidation of lipids, RH, activated C–H bonds (those in a bisallylic position) are cleaved by peroxyl radicals, ROO•, to give an hydroperoxide molecule, ROOH, and a lipid radical, R•, which by reaction with O<sub>2</sub> regenerates the peroxyl radical (Scheme 1). Phenolic antioxi-

### Scheme 1



dants (AH), such  $\alpha$ -tocopherol (vitamin E), are able to inhibit this reaction by scavenging the chain carrying peroxyl radicals by transfer of a hydrogen atom (eq 1); the resulting radical from the antioxidant, A•, is generally too unreactive to continue the chain.



Although only a negligible dependence on solvent is commonly observed in atom-transfer homolytic reactions,<sup>4</sup> the occurrence of dramatic solvent effects has been recently reported on the rates of hydrogen abstraction

from phenols by cumyloxy, *tert*-butoxy, and DPPH radicals (eq 2). Thus, when X is the cumyloxy radical and AH is phenol,  $k_2$  at 25 °C is 86, 28, 0.58, and 0.3610<sup>7</sup> M<sup>-1</sup> s<sup>-1</sup> in CCl<sub>4</sub>, C<sub>6</sub>H<sub>6</sub>, CH<sub>3</sub>CN, and (CH<sub>3</sub>)<sub>3</sub>COH, respectively.<sup>5</sup> This solvent effect has been found to be essentially identical for the same substrate and independent of the nature of the attacking radical and has been attributed to hydrogen-bond formation between the substrate AH and hydrogen-bond-accepting solvents.<sup>5</sup> A dependence on the nature of the medium has also been found in the reactivity of peroxyl radicals toward phenols. Thus,  $\alpha$ -tocopherol ( $\alpha$ -TOH) reacts very rapidly with peroxyl radical in organic solvents ( $3.2 \times 10^6$  M<sup>-1</sup> s<sup>-1</sup> at 30 °C in styrene),<sup>6</sup> while the rate of this reaction is about 100 and 1000 times lower in SDS micelles ( $3.7 \times 10^4$  M<sup>-1</sup> s<sup>-1</sup> at 40 °C) and in phospholipid bilayers ( $3 \times 10^3$  M<sup>-1</sup> s<sup>-1</sup> at 25 °C).<sup>7,8</sup> Although the reduced reactivity observed in the latter systems might be attributed to solvent effects due to the formation of hydrogen bonding between  $\alpha$ -TOH and water in the proximity of the polar interface,<sup>9</sup> recent results are not consistent with this interpretation,<sup>10</sup> since the rate constant for the reaction of peroxyl radicals with  $\alpha$ -tocopherol in water has been estimated to be as large as  $8 \times 10^5$  M<sup>-1</sup> s<sup>-1</sup>. However, it should be pointed out that this estimate has been obtained from the absolute rate constant for hydrogen atom abstraction by *tert*-butoxy radicals from 6-hydroxy-2,5,7,8-tetramethylchroman-2-acetic acid,<sup>10</sup> i.e., the water-soluble model of  $\alpha$ -tocopherol, by assuming that the kinetic solvent effect on reaction 1 is independent of the nature and reactivity of the abstracting radical X.<sup>5</sup> This implies that any interaction of the abstracting radical with the solvent does not substantially affect its reactivity and that the solvent effect is only due to the complexation of the substrate by the solvent. Since the estimated value of  $k_2$  in water is much larger than that found in SDS micelles or in liposomes, it seems likely that only a small fraction of the observed rate reduction in SDS and phospholipid bilayers can be attributed to hydrogen bonding of  $\alpha$ -tocopherol to water.

It must be emphasized that the assumption that the kinetic solvent effect for peroxyl radicals is negligible as for other radicals has not yet been unambiguously proved.<sup>11</sup> Indeed, suggestions contradicting this assumption have been proposed by various authors, who pointed out that peroxyl radicals are likely to be hydrogen-bonded

(5) (a) Valgimigli, L.; Banks, J. T.; Ingold, K. U.; Luszyk, J. *J. Am. Chem. Soc.* **1995**, *117*, 9966. (b) Avila, D. V.; Ingold, K. U.; Luszyk, J.; Green, W. H.; Procopio, D. P. *J. Am. Chem. Soc.* **1995**, *117*, 2929.

(6) Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc.* **1985**, *107*, 7053.

(7) (a) Barclay, L. R. C.; Baskin, K. A.; Locke, S. J.; Schaeffer, T. D. *Can. J. Chem.* **1987**, *65*, 2529. (b) Pryor, W. A.; Strickland, T.; Church, D. F. *J. Am. Chem. Soc.* **1988**, *110*, 2224. (c) Castle, L.; Perkins, M. J. *J. Am. Chem. Soc.* **1986**, *108*, 6381.

(8) (a) Barclay, L. R. C.; Baskin, K. A.; Locke, S. J.; Vinquist, M. R. *Can. J. Chem.* **1989**, *67*, 1366. (b) Barclay, L. R. C.; Baskin, K. A.; Dakin, K. A.; Locke, S. J.; Vinquist, M. R. *Can. J. Chem.* **1990**, *68*, 2258.

(9) Iwatsuki, M.; Tsachiya, J.; Komuro, E.; Yamamoto, Y.; Niki, E. *Biochim. Biophys. Acta* **1994**, *1200*, 19.

(10) Valgimigli, L.; Ingold, K. U.; Luszyk, J. *J. Am. Chem. Soc.* **1996**, *118*, 3545.

(11) The ratio between the rate constants  $k_2$  (RO• +  $\alpha$ -tocopherol) measured in benzene and in *tert*-butyl alcohol is 17, while the ratio between the rate constant  $k_1$  (ROO• +  $\alpha$ -tocopherol) in styrene and in *tert*-butyl alcohol is 14.

(1) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Clarendon Press: Oxford, 1989.

(2) Comporti, M. In *Free Radicals: From Basic Science to Medicine*; Poli, G., Albano, E., Dianzani, M. U., Eds.; Birkhauser Verlag: Basel, 1993.

(3) Scott, G. *Chem. Brit.* **1995**, 879–882.

(4) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 2nd ed.; VCH: Weinheim, 1988.

**Table 1. Oxidizability Values and Absolute Rate Constants for Propagation,  $k_p$ , and Termination,  $2k_t$ , Measured for the Autoxidation of Cumene (1.1 M) in Various Solvents at 50 °C (The Initiation Rate ( $R_i$ ) Was Measured for Each Experiment and the Range Employed Is Reported)**

| solvent                    | $R_i/s^{-1}$                 | $k_p/(2k_t)^{1/2}/M^{-1/2} s^{-1/2}$ | $2k_t/M^{-1} s^{-1}$ | $k_p^a/M^{-1} s^{-1}$ |
|----------------------------|------------------------------|--------------------------------------|----------------------|-----------------------|
| isooctane                  | $(0.81-1.21) \times 10^{-8}$ | $1.99 \times 10^{-3}$                | $2.5 \times 10^5$    | $0.99 \pm 0.17$       |
| benzene                    | $(0.95-1.75) \times 10^{-7}$ | $1.54 \times 10^{-3}$                | $2.4 \times 10^5$    | $0.75 \pm 0.09$       |
| acetonitrile               | $(0.85-1.32) \times 10^{-7}$ | $1.97 \times 10^{-3}$                | $1.4 \times 10^5$    | $0.74 \pm 0.08$       |
| <i>tert</i> -butyl alcohol | $(0.83-1.42) \times 10^{-7}$ | $1.03 \times 10^{-3}$                | $6.0 \times 10^5$    | $0.80 \pm 0.07$       |
| pyridine                   | $(1.23-2.72) \times 10^{-7}$ | $1.23 \times 10^{-3}$                | $4.5 \times 10^5$    | $0.82 \pm 0.08$       |

<sup>a</sup> Errors correspond to twice the standard deviation (2 $\sigma$ ). True uncertainties are believed to be at least twice as big.

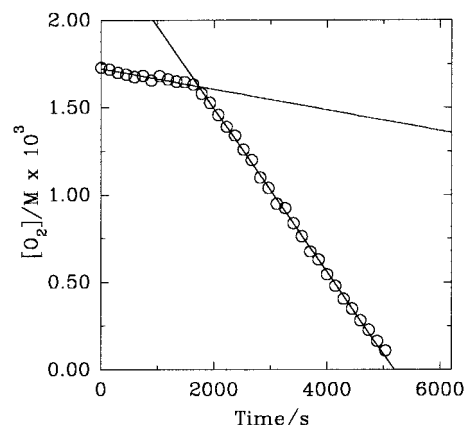
in hydroxylic solvents and this could, in principle, reduce their reactivity.<sup>8,9,12</sup> Experimental data making the picture more confusing are those recently reported on hydrogen abstraction reaction from hydrocarbons by DPPH, a radical related to peroxy radicals in its electronic structure.<sup>13</sup> The rate of this reaction, although largely solvent independent, is higher in *tert*-butyl alcohol than in the other investigated solvents, including isooctane and benzene.

To check if peroxy radicals obey the principle that kinetic solvent effects on hydrogen abstraction are independent of the nature of the abstracting radical, we have measured the rate constants for the H-abstraction from cumene by peroxy radicals,  $k_p$ , in five solvents characterized by different hydrogen-bond-donating/accepting properties (isooctane,  $\alpha = 0$ ,  $\beta = 0$ ; benzene,  $\alpha = 0$ ,  $\beta = 0.10$ ; acetonitrile,  $\alpha = 0.19$ ,  $\beta = 0.31$ ; *tert*-butyl alcohol,  $\alpha = 0.68$ ,  $\beta = 0.5$ ; pyridine,  $\alpha = 0$ ,  $\beta = 0.64$ ).<sup>14</sup> This reaction was chosen because it has been recently demonstrated that the H-abstraction from hydrocarbons by cumyloxy radicals does not show any measurable kinetic solvent effect.<sup>15</sup>

$$-\frac{d[O_2]}{dt} = \frac{k_p[RH]\sqrt{R_i}}{\sqrt{2k_t}} + R_i \quad (3)$$

The values of  $k_p$  have been determined by studying the azoisobutyronitrile (AIBN)-initiated oxidation of cumene, a well-known free-radical chain autoxidation reaction. The oxidation rate is given by eq 3, where RH is the cumene concentration,  $2k_t$  is the chain termination rate constant, and  $R_i$  is the rate of free-radical chain initiation that was measured in each solvent using a phenolic antioxidant (see Table 1). The rate of oxygen consumption has been measured in a closed tube using a method based on the variation of the EPR spectral line width of a stable nitroxide radical dissolved in solution, as described elsewhere.<sup>16</sup> Figure 1 shows, as an example, the decrease with time of the oxygen concentration observed during the autoxidation of cumene at 50 °C in benzene containing AIBN and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), while the oxidizability,  $k_p/(2k_t)^{1/2}$ , of cumene obtained in the five investigated solvents is reported in Table 1.

An examination of the oxidizability values shows that these are slightly solvent dependent, the smaller (1.03



**Figure 1.** Oxygen consumption observed during the autoxidation of 1.1 M cumene in *tert*-butyl alcohol in the presence of  $4.6 \times 10^{-2}$  M AIBN and  $8.0 \times 10^{-5}$  M  $\alpha$ -tocopherol, at 50 °C. The two different slopes correspond to the inhibited and uninhibited autoxidation, the latter being observed when  $\alpha$ -tocopherol is completely consumed. The time,  $\tau$ , at which this change is observed corresponds to the induction period that provides the value of  $R_i$  by means of the following equation,  $R_i = 2[\alpha\text{-tocopherol}]/\tau$ .

$\times 10^{-3} M^{-1/2} s^{-1/2}$ ) and the larger ( $1.99 \times 10^{-3} M^{-1/2} s^{-1/2}$ ) being found in *tert*-butyl alcohol and isooctane, respectively. Since the observed variations could be due to a difference in the values of either  $k_p$  or  $k_t$  or of both,<sup>17</sup> we determined the value of  $2k_t$ , under the same conditions employed in the autoxidation experiments, by following the decay of the EPR signal due to the peroxy radical that consists of a single broad line (peak to peak width ca. 12 G) centered at  $g = 2.0149$ . The cumyloxy radicals were generated by photolyzing oxygen-saturated solutions of *tert*-butyl peroxide in the presence of a large amount of cumene.

The decay traces followed good second-order kinetics in all cases (see Figure 2), but the measured rate constants showed some variation with cumene concentration.<sup>18</sup> This effect was previously observed and was attributed to the occurrence of the reactions shown in Scheme 2, the relative importance of which depends on the cumene concentration.<sup>19</sup> Although the true value of  $2k_t$  could, in principle, be determined by analyzing the decay traces using the cumbersome equation that has been worked out from the complete kinetic treatment,<sup>19</sup> we preferred to carry out the decay experiments under

(12) Roberts, B. P. *J. Chem. Soc., Perkin Trans. 2* **1996**, 2719.

(13) Valgimigli, L.; Ingold, K. U.; Luszyk, J. *J. Org. Chem.* **1996**, *61*, 7947.

(14) Kamlet, M. J.; Abboud, J. L.; Abraham, M. H.; Taft, R. W. *J. Org. Chem.* **1983**, *48*, 2877 and references therein.

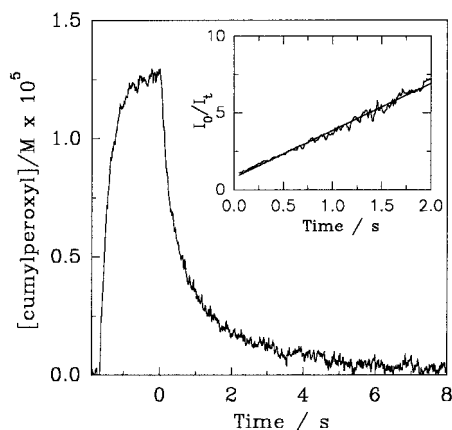
(15) Avila, D. V.; Brown, C. E.; Ingold, K. U.; Luszyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 6576.

(16) (a) Cipollone, M.; Di Palma, C.; Pedulli, G. F. *Appl. Magn. Reson.* **1992**, *3*, 99. (b) Pedulli, G. F.; Lucarini, M.; Pedrielli, P.; Sagrini, M.; Cipollone, M. *Res. Chem. Intermed.* **1996**, *22*, 1.

(17) Experimental data for the autoxidation of styrene in different solvents have been previously reported (Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1964**, *42*, 1044) and the observed differences attributed to changes in the rate constant for chain propagation.

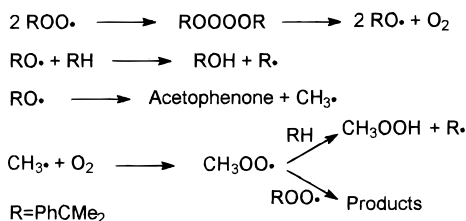
(18) The EPR measured values of  $2k_t$  at 50 °C in benzene are 9.0, 5.4, 3.0, 2.4, and  $1.9 \times 10^5 M^{-1} s^{-1}$ , at cumene concentrations of 0.125, 0.25, 0.87, 1.1, and 1.81 M, respectively.

(19) Howard, J. A. In *Free Radicals*; Kochi, J. K., Ed.; Wiley-Interscience: New York, 1975; Vol. 2, Chapter 12.



**Figure 2.** Trace of the growth and the decay of cumylperoxy radicals photolytically generated from a 1.1 M solution of cumene in oxygen-saturated benzene in the presence of 1.0 M *tert*-butyl peroxide at 50 °C. Insert: second-order plot of the decay curve observed when shutting off the light.

### Scheme 2



the same experimental conditions employed in the autoxidation of cumene, i.e., at 50 °C and at 1.1 M cumene,<sup>20</sup> using the termination rate constants measured under these conditions to calculate the  $k_p$  values reported in Table 1.

The constancy of  $k_p$  in the five solvents examined demonstrates that there is no significant kinetic solvent effect on hydrogen atom abstraction from cumene by cumyl peroxy radicals and that the small dependence on solvent of the oxidizability of cumene is due entirely to variations of the termination rate constant. Therefore, in the reaction of peroxy radicals with phenols, the solvent effects reported in homogeneous systems can be attributed to the formation of hydrogen bonding between

(20) Measurements made over a wide range of steady-state concentrations of peroxy radicals did not show any appreciable difference in the apparent rate constant  $2k_t$  in the same solvent.

solvent and the hydroxylic proton of the substrate. Thus, it can be concluded that peroxy radicals do obey the principle that kinetic solvent effects on H-atom abstraction are independent of the nature of the abstracting radical.<sup>5a</sup> This enforces the estimated value of  $8 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  for the rate constant for the hydrogen abstraction from  $\alpha$ -tocopherol by peroxy radical in water.

On this basis, it is inferred that the dramatic reduction of reactivity of peroxy radicals toward  $\alpha$ -tocopherol observed in SDS micelles and in liposomes with respect to hydrocarbons can be only partially due to hydrogen bonding of the phenol by water molecules. This low reactivity should instead be attributed largely to other factors, specifically, as Castle and Perkins pointed out,<sup>7c,10</sup> and which we consider probable, to the physical inaccessibility of much of the  $\alpha$ -tocopherol to the attacking peroxy radicals. This inaccessibility has been attributed to the fact that only a small fraction of micelles and liposomes contain at least a molecule of antioxidant and that the hydrophobic phytyl chain inhibits the transfer of  $\alpha$ -tocopherol between micelles or between liposomes, this leaving the majority of them unprotected.

### Experimental Section

Typically, six to eight experiments were performed in each solvent on samples consisting of oxygen-purged solutions of cumene (1.1 M) containing di-*tert*-butyl peroxide (1.0 M) that were photolyzed with the unfiltered light from a 500 W high-pressure mercury lamp directly inside the cavity of a Bruker ESP 300 EPR spectrometer equipped with a ER033M field frequency lock. The temperature was controlled by standard variable-temperature accessories. The decay traces of the EPR signals were collected on a dedicated computer using time constants ranging from 4 to 12 ms. Radical concentrations were measured with respect to a solution of DPPH of known concentration using the signal from a ruby crystal as internal standard. The measurement of oxidizability ( $k_p/(2k_t)^{1/2}$ ) was accomplished by following the autoxidation of 1.1 M cumene at 50 °C according to a previously described EPR method.<sup>16</sup> Typically, six to eight experiments for each solvent were performed on sealed air-saturated solutions containing AIBN ( $(0.1-1) \times 10^{-2} \text{ M}$ ), TEMPO or TEMPO-*d\_6* ( $0.5-1.0 \times 10^{-5} \text{ M}$ ) as spin probe.  $\alpha$ -Tocopherol ( $(5-9) \times 10^{-5} \text{ M}$ ) has been added in each experiment in order to determine  $R_i$  by the inhibitor method (see Figure 1).

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