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Laser Flash Photolysis Studies on the First Superoxide Thermal Source. First Direct Measurements of the Rates of Solvent-Assisted 1,2-Hydrogen Atom Shifts and a Proposed New Mechanism for This Unusual Rearrangement[1]

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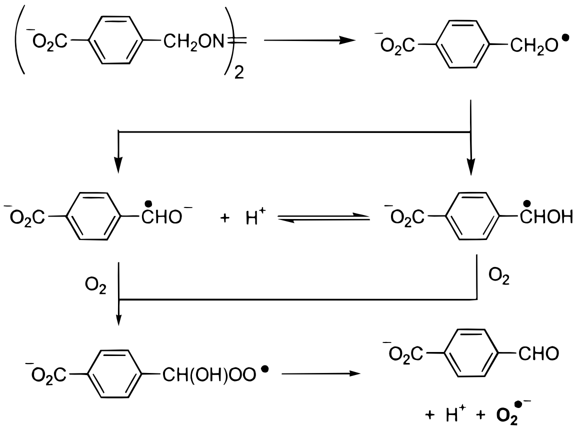
Abstract: The thermal decomposition of bis(4-carboxybenzyl)hyponitrite (SOTS-1) in aerated water under physiological conditions has previously been shown to give the superoxide radical anion in a yield of 40 mol % (Ingold, K. U.; et al. *J. Am. Chem. Soc*. **1997**, *119*, 12364). The absolute kinetics of the elementary reactions involved in the cascade of events leading from the first-formed water-soluble benzyloxyl radical to superoxide have been determined by laser flash photolysis. On the basis of these kinetics it is concluded that SOTS-1 will be suitable for studies of superoxide-induced oxidative stress in most biological systems. A water-assisted 1,2-H shift converting benzyloxyl into the benzyl ketyl radical is an important step in the above reaction cascade. The kinetics of the 1,2-H shift assisted by H_2O, D_2O, and a number of nucleophilic alcohols have been measured for the first time. These data have led to a proposed new mechanism involving the initial formation of a ketyl radical anion and an oxonium cation which generally collapse to give the neutral ketyl radical as the first observable product on the time scale of our experiments (ca. 80 ns).

Although the superoxide radical anion, $O_2^{\bullet-}$, is the major free radical formed in vivo, quantitative studies of its reactions with biologically relevant targets have been seriously handicapped by the lack of any simple procedure which would generate superoxide at a constant, known, and controlled rate for a reasonable length of time under physiological conditions. To overcome this problem, we recently invented the first superoxide thermal source, bis(4-carboxybenzyl)hyponitrite (SOTS-1).[4] In aerated water, pH ∼7, at 37 °C, SOTS-1 was demonstrated to decompose to give the products shown in eq 1 in roughly the amounts indicated.[4] The mechanism by which the SOTS-1

$$\underset{\text{SOTS-1}}{(^-O_2CC_6H_4CH_2ON{=})_2} \xrightarrow[H_2O]{O_2} (1.0\ N_2) + 1.2\ ^-O_2CC_6H_4CHO + 0.8\ ^-O_2CC_6H_4CH_2OH + 0.4\ O_2^{\bullet-}\ (0.4\ H^+) \quad (1)$$

products were assumed to be formed[4] (and for which strong supporting evidence is given herein) is shown in Scheme 1. The in-cage dismutation of the geminal alkoxyl radical pairs (to give 0.8 aldehyde and 0.8 alcohol) has been ignored in Scheme 1 because our present work is focused entirely on the fate of the "free" alkoxyl radicals, i.e., on those radical pairs which separate by diffusion out of the solvent cage in which they were formed.

Scheme 1



If SOTS-1 is to be useful in studies on the effects of superoxide in biologically relevant systems, it is essential that all of the initially formed alkoxyl radicals which have diffused from the solvent cage in which they were formed, $^-O_2CC_6H_4CH_2O^\bullet$, undergo the formal 1,2-hydrogen atom shift to give ketyl radicals, $^-O_2CC_6H_4C^\bullet HOH$ (or radical anions, $^-O_2CC_6H_4CHO^{\bullet-}$) *rapidly and exclusively* (see Scheme 1). This is vital because alkoxyl radicals are very much more reactive than superoxide in oxidative reactions such as H-atom abstractions. Thus, unless the 1,2-H-atom shift is very fast, any observed oxidative damage to a biological target (such as low-density lipoproteins, enzymes, DNA, cells in culture, etc.) might be due to the alkoxyl radical rather than to the superoxide. In contrast, the ketyl radical and its anion will be very unreactive in H-atom abstraction reactions. They are resonance-stabilized benzylic-type radicals which will be good reducing, but poor oxidizing, agents. This pair of radicals is therefore unlikely to cause significant *direct* damage to biological targets, and their lifetimes in most biological

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(4) Ingold, K. U.; Paul, T.; Young, M. J.; Doiron, L. *J. Am. Chem. Soc.* **1997**, *119*, 12364−12365.

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milieus should be quite sufficient for them to react largely or exclusively with dioxygen *provided* their reactions with dioxygen are fast.

In this paper we report the results of a nanosecond laser flash photolysis (LFP) study of SOTS-1, some related compounds, and most of the transient radical intermediates and reactions shown in Scheme 1. Our results prove that the 1,2-H-atom shift (alkoxyl to ketyl radical rearrangement) is very rapid in water. The kinetics of this rearrangement in acetonitrile assisted by water and by other catalysts or potential catalysts were examined, and a new mechanism is proposed for this unusual reaction. We have also demonstrated that the alkoxyl, ketyl, and ketyl anion radicals all react with dioxygen at close to the diffusion-controlled limit. Finally, we report herein for the first time the detailed synthesis of SOTS-1 and some related compounds.

Results

Formation and UV−Visible Spectrum of Benzyloxyl Radicals. Because benzyloxyl radicals undergo a very rapid 1,2-H-atom shift in water (vide infra), we employed *very thoroughly dried* acetonitrile as solvent and this, in turn, necessitated (for reasons of solubility) the use of the diethyl ester of SOTS-1 (Et-SOTS) in place of the free acid. LFP (266 nm) of a solution of Et-SOTS in *dry* acetonitrile at room temperature gave the transient spectra (shown in the Supporting Information) which we assign to the corresponding alkoxyl radical, reaction 2.

$$\underset{\text{Et-SOTS}}{(EtOC(O)C_6H_4CH_2ON{=})_2} \xrightarrow[\text{MeCN}]{\text{266 nm}} EtOC(O)C_6H_4CH_2O^\bullet \quad (2)$$

The spectrum of this alkoxyl radical shows a band maximum at about 325 nm together with a weak, broad absorption in the visible region between 400 and 550 nm and is congruent with expectations. That is, earlier work from this laboratory[5] has shown that a variety of arylcarbinyloxyl radicals have an absorption in the UV and a weaker, broad absorption in the visible. For benzyloxyl the visible band has $\lambda_{max} = 460$ nm and $\Delta OD_{460}/\Delta OD_{320} = 0.62$ in acetonitrile. Electron-donating para-substituents produced a red shift in the visible band and an increase in its intensity. Electron-withdrawing para-substituents were not investigated experimentally, but theoretical calculations[5] indicated that they would induce a blue shift of the visible band and a reduction in its intensity.

The Et-SOTS-derived alkoxyl radical has a lifetime of 2.0 μs in *dry* acetonitrile. We attribute its decay mainly to H-atom abstraction from the solvent, reaction 3.

$$EtOC(O)C_6H_4CH_2O^\bullet + CH_3CN \rightarrow EtOC(O)C_6H_4CH_2OH + {}^\bullet CH_2CN \quad (3)$$

Because of the presence of a minor impurity in the Et-SOTS (vide infra) and a similar impurity in benzyl hyponitrite, we generated the unsubstituted benzyloxyl radicals by 308 nm LFP of benzyl *tert*-butyl peroxide, reaction 4a, and dibenzyl peroxide, reaction 4b.

$$PhCH_2OOCMe_3 \xrightarrow{\text{308 nm}} PhCH_2O^\bullet + {}^\bullet OCMe_3 \quad (4a)$$

$$PhCH_2OOCH_2Ph \xrightarrow{\text{308 nm}} 2\ PhCH_2O^\bullet \quad (4b)$$

(The switch to 308 nm LFP was made to avoid excitation of aldehyde which is produced "instantaneously" from a benzyloxyl radical of the non-N_2-separated, caged geminate radical pair.) The derived benzyloxyl radicals had essentially the same lifetime (1.8 μs, eq 4a, and 1.6 μs, eq 4b) as the ethoxycarbonylbenzyloxyl radical.

The benzyloxyl radical is known to react rapidly with dioxygen.[5] The same holds true for the alkoxyl radical derived from Et-SOTS, reaction 5, for which we found $k_5 = 1.0 \times 10^9$ M^{-1} s^{-1} at room temperature in acetonitrile. Although this

$$EtOC(O)C_6H_4CH_2O^\bullet + O_2 \rightarrow EtOC(O)C_6H_4CHO + HOO^\bullet \quad (5)$$

reaction is very fast (and yields the conjugate acid of superoxide) it is much too slow in air-saturated water ($[O_2] \approx 3 \times 10^{-4}$ M) to compete with the 1,2-H-atom shift (vide infra).

The Solvent-Assisted 1,2-Hydrogen Atom Shift. (i) Water. The rearrangement of primary alkoxyl radicals, $RCH_2O^\bullet$, to hydroxyalkyl radicals, $RC^\bullet HOH$, was first detected by EPR spectroscopy[6,7] and, to date, has only been investigated by this technique.[8,9] All of the early work on this rearrangement was carried out in aqueous systems,[6−8] and since 1,2-H-atom shifts were unknown in organic solvents it was simply assumed that water was essential to the reaction. Then, in 1996, Elford and Roberts[9] demonstrated that the rearrangement of $H_2C{=}CHCH_2O^\bullet$ was also induced by alcohols. In cyclopropane at 220 K containing different concentrations of EtOD, it was found that the $[H_2C{=}CHC^\bullet HOD]/[H_2C{=}CHC^\bullet HOH]$ ratio rose from 0.59 at 0.3 M EtOD to ca. 1 at [EtOD] = 0.6−1.8 M and then declined to 0.61 at 2.4 M EtOD and to 0.38 at 3.6 M EtOD.[9] From these results Elford and Roberts[9] concluded that there must be more than one mechanism for this alcohol-assisted 1,2-H-atom shift. Interestingly, under the same conditions a simple primary alkoxyl radical, $CH_3CH_2O^\bullet$, did not undergo a propanol-induced 1,2-H shift, presumably because the rearranged ketyl radical, $CH_3C^\bullet HOH$, does not benefit from allylic stabilization.[9] Earlier, Gilbert et al.[8c] had added methanol as a (competitive) hydrogen atom donor (giving ${}^\bullet CH_2OH$) to an aqueous system in which the $EtCH_2O^\bullet$ radical was being generated and estimated the pseudo-first-order rate constant for the $EtCH_2O^\bullet \rightarrow EtC^\bullet HOH$ reaction in neat water to be 8×10^6 s^{-1}. However, this rate constant is overestimated because it was not recognized at the time the work was done that methanol also catalyzes a C-to-O 1,2-H-atom shift (vide infra),

Since the ketyl radical from SOTS-1 benefits from benzylic stabilization, the water-assisted rearrangement of the alkoxyl radical was expected to be fast, and such proved to be the case.

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(7) In water the analogous rearrangement of aminyl radicals, viz., $RR'CHN^\bullet H \rightarrow RR'C^\bullet NH_2$, occurs and was actually discovered before the alkoxyl radical rearrangement, see: Anderson, N. H.; Norman, R. O. C. *J. Chem. Soc. (B)* **1971**, 993−1003.

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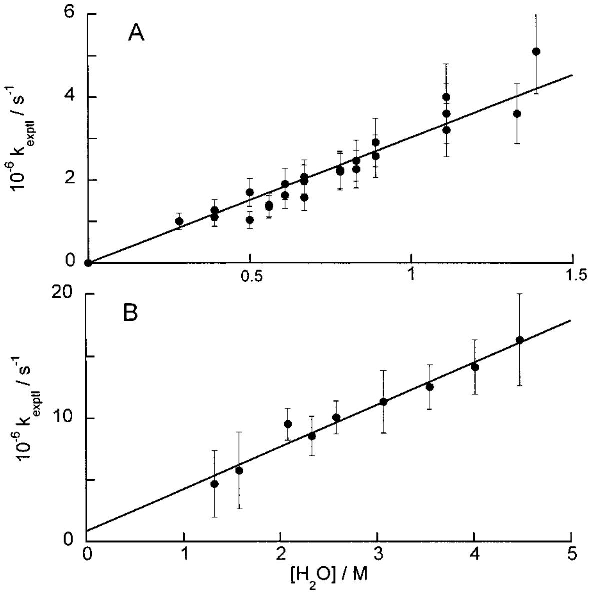

Figure 1. Effect of water on the experimental pseudo-first-order rate constant for the 1,2-H shift reactions, $EtOC(O)C_6H_4CH_2O^\bullet \rightarrow EtOC(O)C_6H_4C^\bullet HOH$ (A) and $C_6H_5CH_2O^\bullet \rightarrow C_6H_5C^\bullet HOH$ (B), in deoxygenated acetonitrile at room temperature, the radicals being generated by 266 nm LFP of Et-SOTS and dibenzylhyponitrite, respectively. In panel B the error bars correspond to 1σ for 4−10 separate measurements at each H_2O concentration. For both panels, measured k_{exptl} values are given in the Supporting Information.[10]

Et-SOTS in dry acetonitrile to which known concentrations of water had been added was subjected to 266 nm LFP, and the change in absorbance was monitored at 320 nm under dioxygen-free conditions. The alkoxyl radical formed in the laser pulse has an absorbance at this wavelength. In the presence of low concentrations of water there was an initial decrease in the OD at 320 nm (the "dip") which was followed by a slow growth and slower decay, while at higher water concentrations there was no dip, only a (faster) growth and then a slow decay (see Supporting Information). The growth curves all followed pseudo-first-order kinetics.[10] A plot of k_{exptl} vs [H_2O] gave a straight line (Figure 1A), the slope of which gives the rate constant for the process investigated. This we identify as formation of the ketyl radical, reaction 6, and from the relation,

$$EtOC(O)C_6H_4CH_2O^\bullet + H_2O \rightarrow EtOC(O)C_6H_4C^\bullet HOH + H_2O \quad (6)$$

$k_{exptl} = k_0 + k_6[H_2O]$, we obtained $k_6 = 3.1 \times 10^6$ M^{-1} s^{-1}. (For the analogous D_2O-catalyzed reaction, $k_6^{D_2O} = 2.1 \times 10^6$ M^{-1} s^{-1}, which yields an H_2O/D_2O solvent kinetic isotope effect = 3.1/2.1 = 1.5.) In pure water, the pseudo-first-order rate constant for the 1,2-H-atom shift is predicted (by a linear extrapolation) to be 1.7×10^8 s^{-1}.[11] It should be noted that the water-assisted rearrangement in acetonitrile did not directly yield the ketyl radical anion as an "instantaneous" and identifiable product; i.e., *reaction 7 was not observed* (however, vide infra).

$$EtOC(O)C_6H_4CH_2O^\bullet + H_2O \nrightarrow EtOC(O)C_6H_4CHO^{\bullet -} + H_3O^+ \quad (7)$$

The "dip" in the 320 nm absorbance vs time profiles at low water concentrations was traced to contamination of the Et-SOTS (and SOTS-1) by the corresponding aldehyde, i.e., $EtOC(O)C_6H_4CHO$. This thermal decomposition product was shown (^1H NMR) to be present at 1−2% even in the most carefully prepared SOTS samples. LFP (266 nm) of the aldehyde in deoxygenated acetonitrile gave an intense transient signal with a maximum at about 320 nm which can readily be assigned to the triplet state of the aldehyde. The sensitivity of the 320 nm absorbance vs time profiles to this SOTS thermal decomposition product is attested to by the fact that at a fixed water concentration the dip became more pronounced when the acetonitrile solution of Et-SOTS was held at room temperature for as little as 10−15 min. A dip was also induced by the deliberate addition of traces of aldehyde to a water-containing system which otherwise showed no dip. The dip was minimized in the kinetic studies by working with the purest Et-SOTS available and the shortest sample preparation time possible.

Unsubstituted benzyloxyl radicals generated by 266 nm LFP of dibenzylhyponitrite in deoxygenated acetonitrile containing low concentrations of water (not unexpectedly) also exhibited a dip in the 320 nm absorbance vs time trace. However, at higher water concentrations pseudo-first-order growth curves were obtained, and a plot of k_{exptl} vs [H_2O] gave a straight line[10] (Figure 1B), from which k_8 was determined to be 3.4×10^6 M^{-1} s^{-1}.

$$PhCH_2O^\bullet + H_2O \rightarrow PhC^\bullet HOH + H_2O \quad (8)$$

We also briefly explored the water-catalyzed 1,2-H-atom shift with 4-methyl- and 4-methoxybenzyloxyl radicals which were generated by 266 nm LFP of the corresponding hyponitrites in deoxygenated acetonitrile. For 4-methylbenzyloxyl the 1,2-H shift had $k = 1.0 \times 10^6$ M^{-1} s^{-1} (Supporting Information), but no rearranged radical was formed (on our time scale) from 4-methoxybenzyloxyl, the relevant kinetic traces showing no growth, only decay, which implies that $k < 5 \times 10^5$ M^{-1} s^{-1}.

To determine the magnitude of the C−H/C−D deuterium kinetic isotope effect (DKIE) for the water-catalyzed 1,2-H(D) shift of benzyloxyl radicals, reaction 9, perdeuteriodibenzylhyponitrite was subjected to 266 nm LFP in deoxygenated acetonitrile in the presence of known concentrations of water.

$$C_6D_5CD_2O^\bullet \xrightarrow{H_2O} C_6D_5C^\bullet DOD \quad (9)$$

The plot of k_{exptl} vs [H_2O] yielded $k_9 = 1.9 \times 10^6$ M^{-1} s^{-1} (Supporting Information). That is, k_8/k_9 (the deuterium kinetic isotope effect, DKIE, for this 1,2-H shift) = 3.4/1.9 = 1.8. Experiments with perdeuteriodibenzyl peroxide were not successful.

(ii) Solvents Other Than Water. Our ability to measure directly the rate constants for the water-assisted 1,2-H-atom

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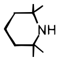
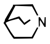
[10] The pseudo-first-order rate constants in LFP experiments are generally considered reliable to ±20%. Unless otherwise noted, 20% error limits are therefore shown on the plots of k_{exptl} vs substrate concentration from which the second-order rate constants are derived. Most plots of k_{exptl} vs [substrate] gave least-squares lines which pass through the origin or have a small positive intercept on the k_{exptl} axis. However, a few such lines had negative intercepts on this axis, which makes no physical sense. In those cases where there was a significant negative intercept, the bimolecular rate constant was obtained from a line which was forced through the origin. To indicate when this was done, a point is shown on the graphs at the origin.

[11] Obviously, time-resolved studies on the 1,2-H shift of the $^-O_2CC_6H_4CH_2O^\bullet$ radical in neat water would require picosecond techniques. If such studies are undertaken, it should be remembered that the visible absorption band of arylcarbinyloxyl radicals are substantially red-shifted in water relative to those in nonaqueous solvents. See: Baciocchi, E.; Bietti, M.; Lanzalunga, O.; Steenken, S. *J. Am. Chem. Soc.* **1998**, *120*, 11516−11517.

Table 1. Bimolecular Rate Constants for the Additive Assisted 1,2-H-Atom Shift: $4\text{-EtOC(O)C}_6\text{H}_4\text{CH}_2\text{O}^\bullet \rightarrow 4\text{-EtOC(O)C}_6\text{H}_4\text{C}^\bullet\text{HOH}$ in Acetonitrile at Room Temperature

Additive	$10^{-6}\,k$ (M^{-1} s^{-1})	α_2^H	Additive	$10^{-6}\,k$ (M^{-1} s^{-1})	α_2^H
H_2O	3.1	0.353	CF_3CH_2OH	< 0.5	0.567
D_2O	2.1		$CH_3CH_2OCH_2CH_3$	< 0.5	
CH_3OH	4.5	0.367	CH_3CO_2H	< 0.5	0.550
CH_3CH_2OH	10	0.328	$H_2NCH_2CH_2NH_2$	< 0.5	0.00
$(CH_3)_2CHOH$	26[a]	0.324		< 0.5	0.00
$(CH_3)_2CHOD$	17			< 0.5	
$(CH_3)_3COH$	6.9	0.319	CH_3CH_2SH	< 0.5	0.00
$HOCH_2CH_2OH$	7.6	0.328			

[a] If the data obtained in benzene are also considered (see Figure 4), $k = 27 \times 10^6$ M^{-1} s^{-1}.

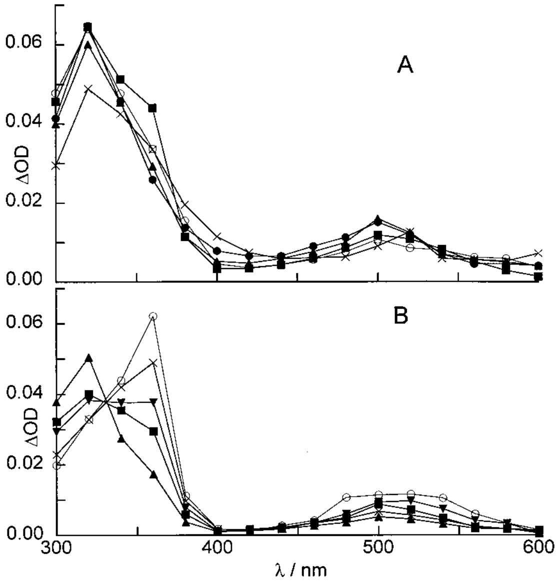


Figure 2. (A) Transient spectra recorded 100 ns after 266 nm LFP of SOTS-1 in deoxygenated phosphate buffer at various pH's. Key: pH 6.6 (●), 7.2 (▲), 7.6 (■), 8.1 (×), and 8.6 (○). (B) Transient spectra (normalized at 420 nm) recorded 6 μs after 266 nm LFP of SOTS-1 in deoxygenated aqueous buffer at various pH's. Key: pH 7.2 (▲), 7.6 (■), 7.8 (▼), 8.1 (×), and 8.6 (○).

shifts encouraged us to explore briefly the ability of other additives to catalyze this reaction. The results of these experiments with alkoxyl radicals derived from Et-SOTS in deoxygenated, dry acetonitrile at room temperature are summarized in Table 1. Their consideration is reserved for the Discussion.

The Ketyl Radical/Ketyl Radical Anion Equilibrium. Rearrangement (1,2-H shift) of the initially generated alkoxyl radical in water is instantaneous on our time scale.[11] LFP (266 nm) of SOTS-1 in deoxygenated aqueous buffer at pH's ranging from 6.6 to 8.6 gives initially, e.g., 100 ns after the laser pulse (Figure 2A), essentially the same absorption spectra. This is a "classic" ketyl radical spectrum, and its identity is confirmed by its slow evolution (complete after 6 μs) to an equilibrium mixture (pH dependent, vide infra) of the ketyl radical and radical anion (see Figure 2B). The ketyl radical anion's spectrum is also "classic" with a band maximum at about 360 nm and a weaker, but still relatively strong, absorption in the visible (480−530 nm). The rate of decay of the ketyl radical's absorption, monitored at 320 nm, was equal to the rate of growth of the anion radical's absorption, monitored at 360 nm. Spectral evolution occurs with an isosbestic point (ca. 340 nm), further confirming that the ketyl radical evolves directly into the anion radical, reaction 10. That equilibrium 10 is established 6 μs after

$$^{-}O_2CC_6H_4C^{\bullet}HOH \rightleftharpoons {}^{-}O_2CC_6H_4CHO^{\bullet -}\ (+\ H^+) \quad (10)$$

the laser pulse is proven by the fact that, thereafter, the only change in the spectrum at all pH's is a uniform decrease in intensity; i.e., the rate of decay is the same at all wavelengths. By fitting an $[H^+]$ titration curve to the values of ΔOD at 360 nm from Figure 2B, the pK_a of the ketyl radical was found to be about 7.8.

The foregoing results unequivocally prove that in water at about neutral pH the initially produced alkoxyl radical is very rapidly converted into the neutral ketyl radical (reaction 11)

$$^{-}O_2CC_6H_4CH_2O^{\bullet} + H_2O \rightarrow {}^{-}O_2CC_6H_4C^{\bullet}HOH + H_2O \quad (11)$$

and that the latter deprotonates relatively slowly to give the anion radical (reaction 10). This reaction is catalyzed by the phosphate buffer (10−100 mM, pH 7.5) with a rate constant of about 10^8 M^{-1} s^{-1}.[12]

Reactions of the Ketyl Radical and Its Anion with Dioxygen. The rate constants for reactions 12 and 13 were measured at pH 6.6 and 11.8, respectively,

$$^{-}O_2CC_6H_4C^{\bullet}HOH + O_2 \xrightarrow{k_{12}} {}^{-}O_2CC_6H_4CHO + HOO^{\bullet}\ (H^+ + O_2^{\bullet -}) \quad (12)$$

$$^{-}O_2CC_6H_4CHO^{\bullet -} + O_2 \xrightarrow{k_{13}} {}^{-}O_2CC_6H_4CHO + O_2^{\bullet -} \quad (13)$$

at room temperature in water which was saturated with oxygen and with oxygen/nitrogen mixtures of known composition. At these two pH's the ketyl radical and ketyl radical anion are formed "instantaneously" upon LFP of SOTS-1 in water. The rate constants obtained were $k_{12} = 3.9 \times 10^9$ M^{-1} s^{-1} and $k_{13} = 2.6 \times 10^9$ M^{-1} s^{-1}.

We have previously demonstrated using both Fe(III) cytochrome *c* and tetranitromethane (TNM) that thermal decomposition of SOTS-1 in aerated water at 37 °C yields ca. 40 mol % superoxide[4] (reaction 1). We have now briefly investigated superoxide formation following 266 nm LFP of SOTS-1 using TNM (2×10^{-3} M) by monitoring growth of the nitroform anion at ca. 355 nm.[13] Reaction 14 is nearly diffusion-controlled, k_{14}

$$O_2^{\bullet -} + C(NO_2)_4 \rightarrow O_2 + NO_2^{\bullet} + {}^{-}C(NO_2)_3 \quad (14)$$

$= 1.9 \times 10^9$ M^{-1} s^{-1},[13a] and under our conditions any superoxide generated will be trapped with a half-life of 1.8×10^{-7} s. In anaerobic phosphate buffer, pH 6.6, the absorption due to the ketyl radical (monitored at 320 nm) decayed and was replaced by the absorption due to the nitroform anion, see Figure 3A. There was an isosbestic point (ca. 345 nm), and the rate constants for decay and growth were identical, viz., 1.2×10^5 s^{-1}. Clearly, in the absence of dioxygen, the ketyl radical

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(12) We thank Professors G. Merényi and M. Newcomb for many helpful comments about the ketyl radical/radical anion equilibrium.

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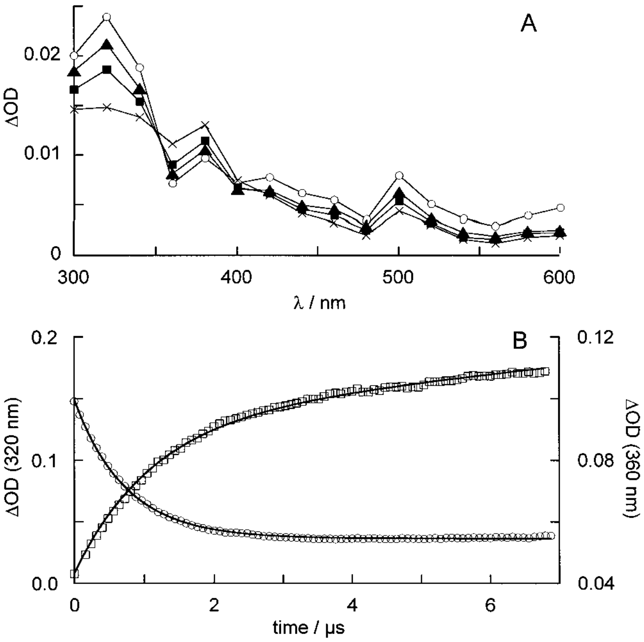

Figure 3. (A) Transient spectra following 266 nm LFP of SOTS-1 in deoxygenated phosphate buffer, pH 6.6, in the presence of 2×10^{-3} M tetranitromethane. Key: spectra were recorded 0.24 (○), 4.8 (▲), 10 (■), and 22 μs (×) after the laser pulse. (B) Traces of ΔOD at 320 and 360 nm after 266 nm LFP of SOTS-1 in aerated phosphate buffer, pH 6.6, containing 2×10^{-3} M tetranitromethane. The decay at 320 nm (○) followed pseudo-first-order kinetics ($k_{\text{exptl}} = 1.3 \times 10^6$ s^{-1}). The growth at 360 nm (□) was analyzed as a first-order growth ($k_{\text{exptl}} = 9 \times 10^5$ s^{-1}) combined with a zero-order growth ($k = 2.5 \times 10^3$ ΔOD s^{-1}).

can reduce TNM, reaction 15 (for which we found $k_{15} = 6.4 \times 10^7$ M^{-1} s^{-1}).

$$^{-}O_2CC_6H_4C^{\bullet}HOH + C(NO_2)_4 \rightarrow {}^{-}O_2CC_6H_4CHO + NO_2^{\bullet} + {}^{-}C(NO_2)_3 + H^+ \quad (15)$$

Identical experiments with SOTS-1 carried out under aerobic conditions also showed decay of the ketyl radical's 320 nm absorption and growth of the nitroform anion's 360 nm absorption (Figure 3B). However, our results differ from the results of rather similar experiments in which LFP was used to generate various alkyl radicals (but *not* ketyl radicals) in oxygenated water containing TNM.[14] In this other work,[14] the rate constant for superoxide release from the alkylperoxyl radicals could be determined from the grow-in of the nitroform anion absorbance. The decay of the ketyl radical from SOTS-1 was followed at 320 nm, and the rate constant for its reaction with dioxygen was found to be $k = 13 \times 10^5$ s^{-1} under the reaction conditions employed. However, the grow-in of the 360 nm absorbance of the nitroform anion continued after all of the ketyl radical had decayed, suggesting that some other reaction is occurring, see Figure 3B. This "side reaction" was accounted for by analyzing the grow-in with a kinetic model for a first-order growth combined with a linear (zero-order) growth (possibly due to hydrolysis of TNM). The calculated rate constant for the first-order growth ($k = 9 \times 10^5$ s^{-1}) must be considered equal, within the experimental error, to the rate constant for ketyl radical decay. This fact, together with the absence of a lag phase (i.e., the grow-in is not S-shaped), necessarily implies that, under our conditions, the formation of the ketylperoxyl radical (reaction 16) is slow relative to its decomposition to aldehyde and superoxide (reaction 17).

$$O_2CC_6H_4C^{\bullet}HOH + O_2 \xrightarrow{k_{12}} {}^{-}O_2CC_6H_4CH(OO^{\bullet})OH \quad (16)$$

$$^{-}O_2CC_6H_4CH(OO^{\bullet})OH \rightarrow {}^{-}O_2CC_6H_4CHOH + O_2^{\bullet -} + H^+ \quad (17)$$

Therefore, the rate of superoxide formation by reaction 17 cannot be measured in our system because it is faster than ketyl radical decay. This puts a lower limit on the rate constant for reaction 17 (which must be $\geq 13 \times 10^5$ s^{-1}) which is 160 times faster than the rate at which superoxide is released by the structurally comparable non-ketyl radical, α-methoxy-α-methylbenzylperoxyl radical.[14] It should be noted that although the rate of reaction 16 could have been increased by saturating with oxygen rather than with air, this would have led to significant superoxide formation via the O_2/alkoxyl radical reaction 5. It should also be pointed out that the superoxide/TNM reaction 14 is not rate limiting; i.e., k_{14} [TNM] $= (1.9 \times 10^9$ M^{-1} s^{-1}) $(2 \times 10^{-3}$ M) $= 38 \times 10^5$ s^{-1} $> 13 \times 10^5$ s^{-1}.

Syntheses of SOTS-1 and Et-SOTS. The best synthetic routes to these two compounds are given in the Experimental Section. Additional synthetic details are available as Supporting Information.

Discussion

On the Suitability of SOTS-1 as a Thermal Source of Superoxide in Biological Systems. Destruction of the cage-escaped, freely diffusing 4-carboxybenzyloxyl radicals in water by the 1,2-H-atom shift is so rapid ($k_6 \approx 1.7 \times 10^8$ s^{-1}) that even a diffusion-controlled reaction of these radicals with a biomolecule will be of negligible importance unless the concentration of the biomolecule exceeds ca. 100 mM (which is improbable). That is, all free 4-carboxybenzyloxyl radicals generated by thermolysis of SOTS-1 will rearrange to ketyl radicals in any biological system or reasonable mimic thereof. The ketyl radical and its more slowly formed anion react with oxygen at, or close to, the diffusion-controlled limit ($k_{12} = 3.9 \times 10^9$ M^{-1} s^{-1}, $k_{13} = 2.6 \times 10^9$ M^{-1} s^{-1}). Most biomimetic systems, including cells in culture, are studied under air, which means that the concentration of dioxygen will be $\sim 3 \times 10^{-4}$ M in the aqueous phase (pH 7, 37 °C). In such systems, the ketyl radical and ketyl radical anion will be destroyed by their reactions with dioxygen in roughly 1 μs, which is probably too short a time for any other reaction to occur. However, in mammals the dioxygen concentrations in many tissues are 1 or 2 orders of magnitude below air saturation. If SOTS-1 is used in whole animal studies of oxidative stress injury and response, the possible incursion of reductive stress (by the ketyl radical and its anion) should not be ignored.

It seems likely that dioxygen adds to the carboxybenzyl ketyl radical to yield a very short-lived peroxyl radical which decays to aldehyde and superoxide (reaction 17) since an addition/elimination mechanism appears to be a common feature in the reactions of α-hydroxyalkyl (and dialkoxyalkyl) radicals with dioxygen.[15] Peroxyl radicals are very much less reactive than alkoxyl radicals. The very short half-life of any peroxyl radicals

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(14) Emanuel, C. J.; Newcomb, M.; Ferreri, C.; Chatgilialoglu, C. *J. Am. Chem. Soc.* **1999**, 121, 2927−2928. Emanuel, C. J.; Newcomb, M. Private communication.

(15) See, e.g.: Bothe, E.; Schuchmann, M. N.; Schulte-Frohlinde, D.; von Sonntag, C. *Z. Naturfosch.* **1983**, *38b*, 212−219. von Sonntag, C.; Schuchmann, H.-P. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1229−1253. Biette, M.; Baciocchi, E.; Steenken, S. *J. Phys. Chem. A* **1998**, *102*, 7337−7342.

derived from SOTS-1 ($\leq 5 \times 10^{-7}$ s) means that they will not cause any direct oxidative damage in any biological or biomimetic system.

Some Insights into the Mechanism of the 1,2-H-Atom Shift. The only reasonable previous estimate of the rate constant for a water-assisted carbon-to-oxygen 1,2-H-atom shift is that reported by Gilbert et al.[8c,16] These workers found that when methanol was added to an aqueous system in which the $EtCH_2O^{\bullet}$ radical was being continuously generated, both the $^{\bullet}CH_2OH$ and the $EtC^{\bullet}HOH$ radicals could be observed by ESR. The $[^{\bullet}CH_2OH]/[EtC^{\bullet}HOH]$ ratio increased linearly as the $[CH_3OH]/[H_2O]$ ratio was increased from 0 to 1.3 (corresponding to $[CH_3OH] = 18.4$ M and $[H_2O] = 14.1$ M). It was assumed that the 1,2-H shift was not catalyzed by methanol, and on this basis Gilbert et al.[8c] calculated that $k_{18}/k_{19} = 1.86$.

$$EtCH_2O^{\bullet} + CH_3OH \rightarrow EtCH_2OH + {}^{\bullet}CH_2OH \quad (18)$$

$$EtCH_2O^{\bullet} + H_2O \rightarrow EtC^{\bullet}HOH + H_2O \quad (19)$$

By taking $k_{18} = 2.6 \times 10^{5}$ M^{-1} s^{-1}, they obtained $k_{19} = 1.4 \times 10^{5}$ M^{-1} s^{-1}, corresponding to a pseudo-first-order rate constant for the 1,2-H shift in pure water of 8×10^{6} s^{-1}. However, our results indicate that water and methanol have comparable activity as catalysts for the 1,2-H shift of the $EtOC(O)C_6H_4CH_2O^{\bullet}$ radical (Table 1). Assuming the same holds true for the propyloxyl radical, it can readily be calculated that Gilbert et al.'s k_{19} value should be reduced by a factor of about 0.6. That is, for the propyloxyl radicals the water-assisted 1,2-H shift should have $k_{19} \approx 8.4 \times 10^{4}$ M^{-1} s^{-1} and a pseudo-first-order rate constant in neat water of about 5×10^{6} s^{-1}. The corresponding rate constants for benzyloxyl (k_8) and 4-ethoxycarbonylbenzyloxyl (k_6) are both about 40 times greater. Thus, our work confirms earlier conclusions[9] that alkoxyl radical 1,2-H shifts are accelerated by resonance stabilization of the carbon-centered radical product.

The kinetic data in Table 1 demonstrate that *only* hydroxylic compounds, *but not all* hydroxylic compounds, produce an enhancement in the rate of the 1,2-H-atom shift to the point where it can be observed and measured by our technique (i.e., $k_{exptl} \geq 5 \times 10^{5}$ s^{-1}). These data place the following constraints on mechanistic speculation:

1. *Only hydroxylic compounds catalyze the rearrangement.* Since water (O−H bond dissociation energy (BDE) = 119 kcal/mol) is almost as active a catalyst as, for example, methanol and *tert*-butyl alcohol (O−H BDEs = 104 kcal/mol), the transition state is very unlikely to involve rupture of the O−H bond of the catalyst. Nevertheless, the small solvent deuterium kinetic isotope effect (DKIE) for $EtOC(O)C_6H_4CH_2O^{\bullet}$ rearrangement in H_2O vs D_2O (1.5) and in i-PrOH vs i-PrOD (1.5) implies that there must be some stretching of the catalyst's O−H bond in the transition state.

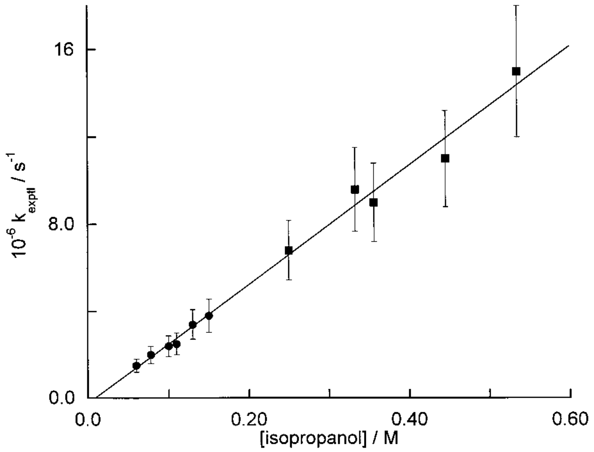

Figure 4. Effect of 2-propanol on the pseudo-first-order rate constant for the 1,2-H shift in $EtOC(O)C_6H_4CH_2O^{\bullet}$ generated by LFP of Et-SOTS.[10] Key: ●, in deoxygenated acetonitrile, 266 nm LFP; ■, in deoxygenated benzene, 308 nm LFP.

2. *The nucleophilicity of the hydroxylic catalyst is very important.* This is borne out both by the failure of the very poorly nucleophilic alcohol, CF_3CH_2OH (solvent nucleophilicity value, $N = -2.78$),[18] to catalyze the reaction and by the fact that the rate constants for the 1,2-H shift generally increase with an increase in solvent nucleophilicity;[18] viz., for H_2O, MeOH, EtOH, and i-PrOH, 10^{-6} k/M^{-1} s^{-1} = 3.1, 4.5, 10, and 26, respectively, with $N = -0.26$, 0.01, 0.09, and 0.09, respectively (there being apparently no N value for *tert*-butyl alcohol). The inability of acetic acid to catalyze the reaction is also likely to be due to the poor nucleophilicity of this compound ($N = -2.05$).[18]

3. *Nucleophilicity is not enough* since primary, secondary, and tertiary amines, ether, and ethanethiol do not catalyze the rearrangement. This is most simply explained by a mechanism in which the catalyst is required to use its hydroxylic hydrogen atom to form a hydrogen bond to the alkoxyl radical in the transition state, it being well known that primary and secondary amines and thiols are much weaker hydrogen bond donors than alcohols and water. To put these observations into more quantitative terms we turn to the α_2^H values of Abraham et al.[19] which define the relative hydrogen-bond-donating (HBD) abilities of different compounds on a scale ranging from 0 (little or no HBD character) to ca. 1.0 (very strong HBD). The α_2^H values for most of the additives have been included in Table 1. It is clear that water and the five alkanols are moderate HBDs, that trifluoroethanol and acetic acid are strong HBDs, and that the amines and thiol are, at best, very weak HBDs. We conclude that while hydrogen bonding from the additive to the alkoxyl radical is necessary for the 1,2-H shift to occur, it is ineffective *unless* the HBD is also a good nucleophile (see 2 above).

4. *Only one hydroxyl group participates in the rearrangement* under the conditions of our experimental rate measurements. This is indicated by the fact that all plots of k_{exptl} vs [catalyst] gave excellent straight lines (see Figures 1 and 4 and Supporting Information), combined with the fact[20] that alcohols and water exist as isolated monomeric molecules in acetonitrile at the very

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[16] For the $MeCH_2O^{\bullet} \rightarrow MeC^{\bullet}HOH$ reaction in water, Berdnikov et al.[6] estimated a completely impossible pseudo-first-order rate constant of the order of 10^{19} s^{-1} which was based on their failure to detect the $Me_2C^{\bullet}OH$ radical on the addition of 2-propanol (0.5 M) and an assumed rate constant for the $MeCH_2O^{\bullet} + Me_2CHOH$ reaction equal to that for the $HO^{\bullet} + Me_2CHOH$ reaction, viz., 2.0×10^{9} s^{-1}. Since the rate constant for the $Me_3CO^{\bullet} + Me_2CHOH$ reaction actually is 1.8×10^{6} M^{-1} s^{-1},[17] it is obvious that Berdnikov's pseudo-first-order rate constant must be revised down to 10^{16} s^{-1}, a value which is, however, still impossibly high kinetically and is more than 9 orders of magnitude greater than the corresponding value for the $EtCH_2O^{\bullet} \rightarrow EtC^{\bullet}HOH$ reaction, see text.

[17] Paul, H.; Small, R. D., Jr.; Scaiano, J. C. *J. Am. Chem. Soc*. **1978**, *100*, 4520−4527.

[18] Schadt, F. L.; Bentley, T. W.; Schleyer, P. v. R. *J. Am Chem. Soc.* **1976**, *98*, 7667−7674.

[19] Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Duce, P. P.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 699−711.

[20] Reimers, J. R.; Hall, L. E. *J. Am. Chem. Soc.* **1999**, *121*, 3730−3744.

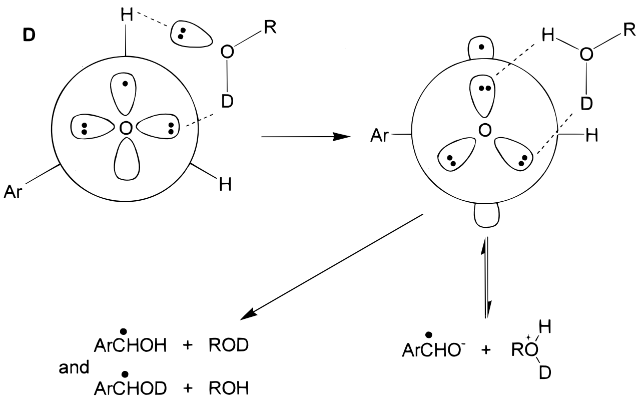
high (>0.9) mole fractions of acetonitrile employed in our kinetic work.[21]

5. *The substrate exhibits a substantial DKIE*, viz., 1.8 for $C_6H_5CH_2O^\bullet$ vs $C_6D_5CD_2O^\bullet$ with catalysis by water. There is, therefore, substantial breaking of the substrate's C−H bond in the transition state.

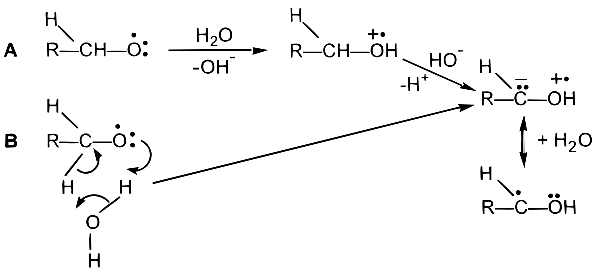
6. *The acidity of the C−H hydrogen atom is important*, as was first pointed out by Elford and Roberts.[9] Thus, the rate of the water-assisted 1,2-H shift becomes slower when electron-donating groups are present on the ring (substituent, $10^{-5}\ k/\mathrm{M}^{-1}\ \mathrm{s}^{-1}$: H, 34; CH_3, 10; CH_3O, <5). These polar effects are not large, and the electron-withdrawing EtOC(O) group may even slightly decrease the rate ($10^{-5}\ k/\mathrm{M}^{-1}\ \mathrm{s}^{-1} = 31$) relative to that of the unsubstituted radical. Indeed, Merényi[25] has estimated that the pK_a's of $PhCH_2O^\bullet$ and $CH_3CH_2O^\bullet$ are, very roughly, −3 and 1, respectively, yet the rate constants for their water-assisted 1,2-H shifts differ by less than a factor of 100, vide supra.

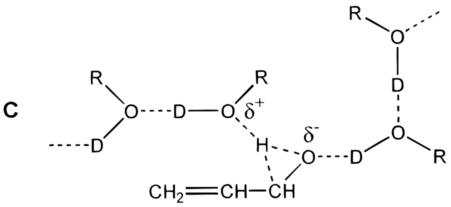
7. *The first observable product is the neutral ketyl radical, but* see below.

Scheme 2



There may possibly also be an eighth constraint. This comes from Elford and Roberts's[9] interesting EPR study of the EtOD-assisted rearrangement of the $H_2C{=}CHCH_2O^\bullet$ radical (vide supra). The hydrogen atom which "arrives" at the alkoxyl radical's oxygen atom comes, in part, from the hydrogens of the α-CH_2 group in the radical and, in part, from the alcohol's deuterium atom. Furthermore, the OD/OH ratio in the product ketyl radical depends in a nonlinear manner on the EtOD concentration (vide supra). This eighth constraint rules out the two mechanisms for the 1,2-H shift, **A** and **B**, which had been proposed earlier,[8c] because both would imply exclusive incorporation of deuterium into the ketyl radical in Elford and Roberts's experiments. In place of these mechanisms, Elford and Roberts suggested a variety of competing processes, viz., **B** (implicitly at low [EtOD] leading to D incorporation), **C** (at high [EtOD] leading to H incorporation), and an S_N2'-like reaction involving nucleophilic assistance by interaction of the alcohol oxygen atom with the C=C bond in the allyloxyl radical, a process which can be ignored in the present instance as it could not occur with benzyloxyl radicals.





We propose a fourth mechanism, **D**, which is shown in Scheme 2. This mechanism is congruent with the seven constraints on the mechanism listed above, *provided* the initial ketyl radical anion/oxonium ion pair, $ArCHO^{\bullet-}/ROHD^+$ collapses to the neutral product pairs, $ArC^\bullet HOH/ROD$ and $ArC^\bullet HOD/ROH$, more rapidly than the two ions diffuse apart. Specifically, this mechanism involves a hydroxylic catalyst to form a hydrogen bond to the oxygen of the alkoxyl radical (constraint 1), a nucleophilic (H-bonded) alcohol to remove a proton from the benzylic position (constraints 2, 3, and 6), one molecule of a hydroxylic catalyst (constraint 4), some C−H

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[21] If it were to be argued that there is a prior equilibrium of the alkoxyl radical with the added water or alcohol, XOH, which lies strongly on the side of the XOH-complexed radical,[22] the fact that the kinetics of the XOH-assisted 1,2-H shifts are first order in XOH would not reflect the actual stoichiometry of the rearrangement process, i.e.,

$$\mathrm{XOH} + \mathrm{ArCH_2O^\bullet} \rightleftarrows [(\mathrm{XOH})(\mathrm{ArCH_2O^\bullet})] \xrightarrow[\text{slow}]{\mathrm{XOH}} \mathrm{ArC^\bullet HOH} + 2\ \mathrm{XOH} \quad (20)$$

The relative abilities of organic molecules to act as hydrogen bond acceptors (HBAs) are best described by Abraham et al.'s[23] β_2^{H} values, which range from 0.00 for non-HBA solvents, such as alkanes, to 1.00 for HMPA, the strongest HBA. For a relatively low concentration of an XOH having $\alpha_2^{\mathrm{H}} \approx 0.35$ (see Table 1) in acetonitrile, it can be calculated[19,23] that almost all (ca. 95%) of the XOH will be H-bonded to an acetonitrile molecule and only a small amount (ca. 5%) will not be H-bonded. Since neat acetonitrile is ca. 19 M, the addition of 0.1 and 1.0 M concentrations of this XOH will mean that only ca. 0.5% (0.1 × 100/19) and ca. 5%, respectively, of the acetonitrile molecules will actually be involved in a hydrogen bond with XOH. The β_2^{H} value for the benzyloxyl radical is unknown but would be expected to be not too dissimilar from that of benzyl alcohol, viz.,[23,24] 0.42. This is essentially the same as the β_2^{H} value for acetonitrile (0.44), and therefore the percentage of benzyloxyl radicals expected to be involved in H-bonds with XOH will be approximately the same as for acetonitrile, viz., 0.5% and 5% with 0.1 and 1.0 M XOH, respectively. Therefore, if there was a prior equilibrium to form a hydrogen-bonded intermediate, [(XOH)-($ArCH_2O^\bullet$)], which subsequently reacted with a second XOH to give the rearranged radical (reaction 20), then a 10-fold change in the XOH concentration (0.1−1.0 M) would also change the concentration of the intermediate by 10, and the reaction kinetics would be second order in XOH. The involvement of two XOH molecules in the 1,2-H shift occurring as shown in reaction 20 would be kinetically invisible *only* if β_2^{H} for the benzyloxyl radical was greater than 1.0, which can be ruled out. That is, it can be calculated[19,23] that the percentage of benzyloxyl radicals H-bonded by 0.1 and 1.0 M H_2O (ratio of these percentages in parentheses) would be 3.4/26 (7.6), 13/61 (4.7), and 47/90 (1.9) for $\beta_2^{\mathrm{H}} = 0.75$, 1.0, and 1.3, respectively, and thus this 10-fold change in H_2O concentration would increase the rate by factors of 76, 47, and 19, respectively, and the first two factors would certainly be seen to be different from 10.

[22] We thank Prof. M. Newcomb for bringing this point to our attention.

[23] Abraham, M. H.; Grellier, P. L.; Prior, D. V.; Morris, J. J.; Taylor, P. J. *J. Chem. Soc., Perkin Trans. 2* **1990**, 521−529

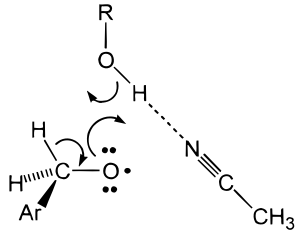
[24] We measured the β_2^{H} value for dibenzyl hyponitrite and obtained a value of 0.35.

[25] (a) Merényi, G. Private communication. (b) The pK_a of $PhCH_2O^\bullet$ was estimated to be very roughly 11 units lower than the pK_a of 7.8 found in this work for $^-O_2CC_6H_4C^\bullet HOH$ by using a thermodynamic cycle based on the bond dissociation enthalpies of the C−H (~88 kcal mol^{-1}) and O−H (~104 kcal mol^{-1}) bonds in $PhCH_2OH$, i.e., the pK_a of $PhCH_2O^\bullet$ is lower by ca. (104−88)/1.4 = 11 units than the pK_a of the ketyl radical. Hence, the pK_a of $PhCH_2O^\bullet$ is very roughly −3. A similar calculation for ethanol suggests a pK_a for its alkoxyl radical of ca. 1.

bond-breaking in the transition state, and hence a small C−H/C−D substrate DKIE (constraint 5) together with some O−H bond stretching and a small O−H/O−D solvent DKIE (constraint 1), and rapid collapse of the ion pair (constraint 7).

Mechanism **D** also provides a simple explanation of the intriguing equality of H-atom and D-atom transfer in the Elford and Roberts [9] experiments at [EtOD] = 0.6−1.8 M. There are several possible explanations for their observation that H-atom transfer was favored over D-atom transfer at both high and low [EtOD]. The most likely is that their experimental conditions (cyclopropane solvent at 220 K) would encourage the ethanol molecules to self-associate into dimers and oligomers, and these might induce reaction mechanism(s) that simply do not occur with monomeric ethanol in acetonitrile at 295 K. It has also been suggested[26] that in acetonitrile a molecule of acetonitrile might serve in lieu of a second molecule of alcohol to assist the 1,2-H shift, mechanism **E**. An attempt to explore mechanism **E** using the non-HBA solvent, isooctane, with added alcohols was unsuccessful because the time window between the end of the laser pulse and the decay of benzyloxyl by reaction with the isooctane became too narrow for meaningful kinetic studies. However, meaningful (though difficult) kinetic studies could be done in benzene with added 2-propanol. The fascinating results are shown in Figure 4, where a single straight line has been drawn through the points obtained by plotting $k_{\mathrm{exptl}}/\mathrm{s}^{-1}$ vs [2-propanol] in acetonitrile *and* in benzene. Although a molecule of benzene could, in principle, act in place of the molecule of acetonitrile shown in mechanism **E**, this appears somewhat improbable because benzene ($\beta_2^{\mathrm{H}} = 0.14$)[23] is a very much weaker HBA than acetonitrile ($\beta_2^{\mathrm{H}} = 0.44$).[23] Certainly, we would not expect one single line to correlate the kinetics in benzene and acetonitrile.

E



Finally, the excess of H-transfer over D-transfer observed by Elford and Roberts in some of their experiments might possibly be due to a loss of deuterium from the alcohol by exchange with hydroxylic compounds produced during the reaction. However, if, as seems likely,[26] the D/H transfer ratios are correct they may be specific to allyloxyl in cyclopropane with added EtOD. This would mean that there is not one unique mechanism for the 1,2-H shift in alkoxyl radicals. This would not be too unusual as certain other radical rearrangements have been demonstrated to occur by more than one mechanism.[27] Thus, for example, the concerted mechanism **B** (which would have led to exclusive deuterium incorporation in Elford and Roberts' experiments) could not be ignored, particularly in view of the small effect of pK_a (benzyloxyl vs aliphatic alkoxyl, vide supra) on the rates of the water-assisted 1,2-H shift in these radicals.[25a]

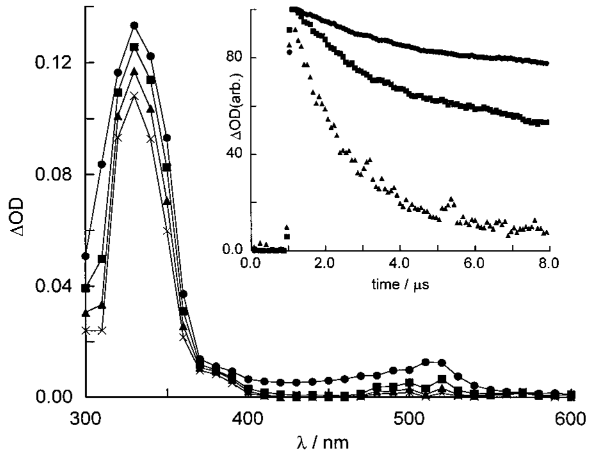


Figure 5. Transient spectra following 266 nm LFP of Et-SOTS in deoxygenated ethanol at room temperature. Key: spectra were recorded 0.08 (●), 0.96 (■), 2.4 (▲), and 4.4 μs (×) after the laser pulse. Inset: Normalized decays, monitored at 320 (top), 360 (middle), and 520 nm (bottom).

However, unless and until multiple mechanisms for 1,2-H shifts in alkoxyl radicals are demonstrated, we prefer to follow William of Occam's advice and opt for one mechanism, i.e., **D**, as shown in Scheme 2. The main *experimental* evidence against this mechanism is the fact that the first detectable product in water is the neutral ketyl radical and not the ketyl radical anion. In the hope of observing the initial formation of at least some of the radical anion, we subjected Et-SOTS to 266 nm LFP in neat ethanol. Immediately after the laser pulse (0.08 μs) the spectrum is due mainly to the neutral ketyl radical, but there is also clear evidence at 520 nm for the radical anion (see Figure 5). This 520 nm signal decays much more rapidly than the signal due (mainly) to the neutral at 320 nm, presumably because the anion is rapidly protonated[28] (see inset in Figure 5). Decay at 360 nm, the band maximum for the anion radical, occurred with mixed (non-first-order) kinetics. Thus, at least some ketyl radical anion is formed as the initial product of the ethanol-assisted 1,2-H shift of a benzyloxyl radical in ethanol. The same results were obtained in neat 2-propanol and in 2-propanol/isooctane (1:1, v/v). These observations lend further experimental support to mechanism **D** (Scheme 2, which includes reversible dissociation of the initially formed, intimate ion pair).

Conclusion

Our kinetic work indicates that SOTS-1 is suitable for studies of superoxide-induced oxidative stress in most, if not all, biological systems.

A new mechanism for the water- or alcohol-assisted 1,2-H shift in alkoxyl radicals is proposed on the basis of extensive kinetic data and is supported by some spectroscopic evidence.

Experimental Section

Analytical Equipment. ^{1}H NMR spectra (200 MHz) and ^{13}C NMR spectra (50 MHz) were recorded on a Bruker AM-200, unless otherwise noted. Mass spectra were obtained with an HP 5970 mass-selective detector connected to an HP 5890 gas chromatograph, EI at 70 eV. Infrared spectra were measured with a Perkin-Elmer 1600 FT-IR

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(26) Roberts, B. P. Private communication.

(27) See, e.g.: Beckwith, A. L. J.; Crich, D.; Duggan, P. J.; Yao, Q. *Chem. Rev.* **1997**, *97*, 3273−3312. Choi, S.-Y.; Crich, D.; Horner, J. H.; Huang, X.; Martinez, F. N.; Newcomb, M.; Wink, D. J.; Yao, Q. *J. Am. Chem. Soc.* **1998**, *120*, 211−212. Crich, D.; Huang, X.; Beckwith, A. L. J. *J. Org. Chem.* **1999**, *64*, 1762−1764. Choi, S. Y.; Crich, D.; Horner, J. H.; Huang, X.; Newcomb, M.; Whitted, P. O. *Tetrahedron* **1999**, *55*, 3317−3326.

(28) In contrast, the small, ca. 500 nm, absorption shown in Figure 2A (in water) is not due to the ketyl radical anion. This follows from the (fairly noisy) kinetic traces at pH 6.6, 7.6, and 8.1, which showed no growth or decay following the laser pulse for up to 8 μs. However, at pH 8.6 this absorption showed a clear increase in intensity as the anion radical grew in.

spectrophotometer. Elemental analyses were carried out using a Perkin-Elmer 240C elemental analyzer.

Materials. Reagents were of analytical grade, and solvents were dried over 4 Å molecular sieves. Silver hyponitrite was synthesized by reaction of silver nitrate with sodium hyponitrite[29] and was stored in the dark at −20 °C.

General Procedure for the Synthesis of Hyponitrites. Silver hyponitrite was added in small portions to a stirred solution of the benzylic bromide in a dry solvent in the dark at 0 °C. After all the silver hyponitrite had been added the reaction mixture was allowed to warm to room temperature, and when the bromide had completely reacted (as determined by frequent GC analysis) the silver bromide was removed by filtration. If the reaction was not complete within 8 h, the reaction mixture was stored in the refrigerator at 4 °C overnight and stirred again at room temperature the following day until all the bromide was consumed. The crude product was obtained from the filtrate by evaporation of the solvent under reduced pressure without heating. After purification (vide infra) the organic hyponitrites were stored at −78 °C since they decompose slowly at −20 °C.

Synthesis of SOTS-1. (i) α-Bromotoluic Acid Chloride. A mixture of α-bromotoluic acid (Aldrich, 10.0 g, 0.046 mol) and thionyl chloride (Aldrich, 25 mL, 0.31 mol) was heated to reflux for 3 h and then allowed to cool to room temperature, which caused the acid chloride to crystallize. The excess thionyl chloride was removed by distillation under vacuum. The acid chloride was used without further purification: white solid, yield 10.1 g (93%); mp 53−55 °C; ^{1}H NMR ($CDCl_3$) δ 8.12−8.06 (m, 2H, ArH), 7.56−7.50 (m, 2H, ArH), 4.51 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$) δ 167.7 ($C{=}O$), 145.2 ($C_{Ar}COCl$), 132.8 ($C_{Ar}CH_2Br$), 131.8, 129.5 ($C_{Ar}H$), 31.3 (CH_2Br); IR (KBr) ν_{max} 3100, 3035, 2978, 1792, 1211, 851 cm^{-1}. Anal. Calcd for C_8H_6BrClO: C, 41.15; H, 2.59; Br, 34.22; Cl, 15.18. Found; C, 41.01; H, 2.57; Br, 33.93; Cl, 15.05.

(ii) Benzyl α-Bromotoluate. A mixture of α-bromotoluic acid chloride (3.0 g, 0.012 mol), benzyl alcohol (10 mL, 0.097 mol), and sodium bicarbonate (3.0 g, 0.036 mol) was stirred at room temperature for 5 h and then filtered. The filtrate was purified on a silica gel column using dichloromethane as eluent ($R_f = 0.56$). After evaporation of the solvent, a colorless oil was obtained, which gave white crystals on storage at room temperature: yield 3.4 g (86%); mp 63−64 °C; ^{1}H NMR ($CDCl_3$) δ 8.07−8.02 (m, 2H, ArH), 7.47−7.34 (m, 7H, ArH), 5.36 (s, 2H, CH_2O), 4.48 (s, 2H, CH_2Br); ^{13}C NMR ($CDCl_3$) δ 165.8 (C=O), 142.5 ($C_{Ar}CO_2R$), 135.9 ($C_{Ar}CH_2OR$), 131.0 ($C_{Ar}CH_2Br$), 130.2, 129.0, 128.6, 128.3, 128.2 ($C_{Ar}H$), 66.8 (OCH_2), 32.2 (CH_2Br); IR (KBr) ν_{max} 3087, 3037, 2957, 1716, 1278, 861 cm^{-1}; EI MS m/e (relative intensity) 306 [$M^+ + 2$] (19), 304 [M^+] (19), 225 (27), 199 (54), 197 (54), 91 (100). Anal. Calcd for $C_{15}H_{13}BrO_2$: C, 59.04; H, 4.29; Br, 26.18. Found; C, 59.22; H, 4.20; Br, 26.28.

(iii) Bis(4-benzylcarboxybenzyl)hyponitrite. The crude product obtained by reaction of benzyl α-bromotoluate (2.15 g, 7.0 mmol) with silver hyponitrite (2.0 g, 7.3 mmol) in dry dichloromethane (20 mL) was purified by recrystallization from THF/methanol at −78 °C to give white crystals: yield 0.42 g (23%); mp 83−85 °C (decomposed with vigorous gas evolution); ^{1}H NMR ($CDCl_3$) δ 8.09−8.04 (m, 4H, ArH), 7.43−7.35 (m, 14H, ArH), 5.36 (s, 4H, $PhCH_2O$), 5.30 (s, 4H, CH_2OR); ^{13}C NMR ($CDCl_3$) δ 166.0 ($C{=}O$), 140.7 ($C_{Ar}CO_2R$), 135.9 ($C_{Ar}CH_2ON{=}R$), 130.2, 129.9, 129.5, 128.5, 128.2, 128.1 ($C_{Ar}H$), 74.8 ($CH_2ON{=}R$), 66.7 (CH_2OR); IR (KBr) ν_{max} 3057, 3041, 2982, 2931, 1699, 1295, 848 cm^{-1}.

(iv) Bis(4-carboxybenzyl)hyponitrite (SOTS-1). A potassium hydroxide solution (3.0 g of KOH in 20 mL of methanol and 10 mL of water) was added dropwise to a solution of bis(4-benzylcarboxybenzyl)hyponitrite (1.60 g, 3.1 mmol) in 15 mL of anhydrous THF at 0 °C. The resulting yellow solution was stirred for 3 h at 0 °C and was then allowed to warm to room temperature. If precipitation occurred during this time, THF or water was added to redissolve the precipitate. After 1 h at room temperature, the solution was diluted with 40 mL of cold water and extracted with diethyl ether (30 mL × 3). The aqueous phase was then acidified at 0 °C with concentrated HCl to pH 2−3. The white precipitate was filtered off, washed several times with cold methanol, and dried over P_2O_5 at −20 °C: yield 0.95 g (92%); mp sintering up to 215 °C with no obvious gas evolution; ^{1}H NMR (600 MHz, Na phosphate buffer in D_2O (pD 7.6), 278 K) δ 7.99−7.86 (m, 4H, ArH), 7.47−7.43 (m, 4H, ArH), 5.35 (s, 4H, CH_2OR); ^{13}C NMR (150 MHz, Na phosphate buffer in D_2O (pD 7.6), 278 K) δ 176.3 ($C{=}O$), 139.5 ($C_{Ar}CO_2R$), 137.1 ($C_{Ar}CH_2OR$), 130.0, 129.3 ($C_{Ar}H$), 75.7 (CH_2OR); IR (KBr) ν_{max} 2950, 1689, 1291 cm^{-1}.

Syntheses of Et-SOTS. (v) Ethyl α-Bromotoluate. A mixture of α-bromotoluic acid chloride (3.0 g, 0.012 mol), ethanol (30 mL, 0.52 mol), and sodium bicarbonate (3.4 g, 0.04 mol) was stirred at room temperature for 4 h and then filtered, and the filtrate was evaporated under vacuum. The crude product was recrystallized from diethyl ether/pentane (1:2) as white crystals: yield 2.4 g (88%); mp 35−36 °C; ^{1}H NMR ($CDCl_3$) δ 8.04−7.99 (m, 2H, ArH), 7.47−7.43 (m, 2H, ArH), 4.49 (s, 2H, CH_2Br) 4.38 (q, $J = 7.0$ Hz, 2H, OCH_2), 1.39 (t, $J = 7.0$ Hz, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 165.9 ($C{=}O$), 142.4 ($C_{Ar}CO_2R$), 130.3 ($C_{Ar}CH_2Br$), 129.9, 128.9 ($C_{Ar}H$), 61.0 (OCH_2), 32.2 (CH_2Br), 14.2 (CH_3); IR (KBr) ν_{max} 3102, 3038, 2982, 2963, 1720, 1275, 861 cm^{-1}; EI MS m/e (relative intensity) 244 [$M^+ + 2$] (4), 242 [M^+] (4), 199 (10), 197 (10), 163 (100), 135 (23). Anal. Calcd for $C_{10}H_{11}BrO_2$: C, 49.41; H, 4.56; Br, 32.87. Found; C, 48.24; H, 4.53; Br, 33.12.

(vi) Bis(4-ethylcarboxybenzyl)hyponitrite. The crude product obtained by reaction of ethyl α-bromotoluate (1.5 g, 6.2 mmol) with silver hyponitrite (1.3 g, 4.7 mmol) in dry dichloromethane (15 mL) was redissolved in dichloromethane (5 mL)/hexane (15 mL) and then stored at −20 °C to obtain white crystals: yield 0.51 g (42%); mp 68 °C (decomposed with vigorous gas evolution); ^{1}H NMR ($CDCl_3$) δ 8.06−8.01 (m, 4H, ArH), 7.44−7.39 (m, 4H, ArH), 5.31 (s, 4H, CH_2OR), 4.38 (q, $J = 7.2$ Hz, 4H, OCH_2CH_3), 1.40 (t, $J = 7.2$ Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$) δ 166.2 ($C{=}O$), 140.6 ($C_{Ar}CO_2R$), 129.9, 128.8 ($C_{Ar}H$), 126.4 ($C_{Ar}CH_2OR$), 74.8 (CH_2OR), 61.1 (OCH_2CH_3), 14.3 (CH_3); IR (KBr) υ_{max} 3038, 2982, 2963, 1722, 1273, 761 cm^{-1}. Anal. Calcd for $C_{20}H_{22}N_2O_6$: C, 62.16; H, 5.75; N, 7.25. Found; C, 62.31; H, 5.67; N, 7.18.

Synthesis of Benzyl *tert*-Butyl Peroxide. Following a procedure described by Kharasch and Fono,[30] toluene (5 mL), *tert*-butylhydroperoxide (5 mL), and $CuCl_2$ (0.1 g) were stirred at room temperature for 36 h. The $CuCl_2$ was removed by filtration, and the filtrate was washed with dilute $NaHCO_3$ (3 × 15 mL) and then with water. After drying ($MgSO_4$) and removal of the unreacted organic reagents on a rotary evaporator, the desired peroxide was separated in low yield (ca. 3%) on preparative silica gel TLC plates using CH_2Cl_2: ^{1}H NMR ($CDCl_3$) δ 7.4 (m, 5H, ArH), 5.0 (s, 2H, CH_2) 1.2 (s, 9H, $C(CH_3)_3$).

Synthesis of Dibenzyl Peroxide. Following a published procedure,[31] to KO_2 (0.355 g, 5.0 mmol) in dry benzene (15 mL) in a dry flask were added benzyl bromide (0.855 g, 0.6 mL, 5.0 mmol) and 18-crown-6 ether (1.82 g, 5 mmol), and the mixture was stirred vigorously for 5 h at room temperature. Following that, the mixture was poured onto a saturated solution of NaCl and extracted with CH_2Cl_2. The product was isolated in small portions using preparative TLC plates and eluting with hexane/CH_2Cl_2, 1:1 v/v: ^{1}H NMR ($CDCl_3$) δ 7.3 (s, 1OH, ArH), 4.6 (s, 4H, CH_2).

Synthesis of Perdeuteriodibenzylhyponitrite. Benzyl-d_7 chloride (d_7, >98%; Cambridge Isotope, MA; 1.00 g, 7.5 mmol) was reacted with LiBr (3.0 g, 35 mmol) in 10 mL of acetone/methanol (95:5) containing a drop of water at room temperature. The reaction was followed by GC/MS analysis and was stopped after reaching equilibrium. The two benzyl halides were extracted into ether and dried over $MgSO_4$. Evaporation yielded a crude product which was treated with LiBr a second time. The same workup yielded crude benzyl-d_7 bromide (90% bromide by GC/MS), which was reacted without further purification in dry CH_2Cl_2 (15 mL) with silver hyponitrite (2.0 g, 7.3 mmol). The crude perdeuteriodibenzylhyponitite was recrystallized from CH_2Cl_2/pentane (1:3) as white crystals: yield 101 mg (10%); mp 39−41 °C (decomposed with gas evolution); ^{2}H NMR ($CHCl_3$) δ 7.38 (s, br, 10 D, ArD), 5.21 (s, 4D, CD_2).

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Synthesis of Perdeuteriodibenzyl Peroxide. The same procedure was employed as for the unlabeled material but started with 0.67 g of benzyl-d_7 chloride. The product had the same R_f (TLC) as the unlabeled compound and the expected mass spectral fragmentation pattern.

Synthesis of 4-Ethoxycarbonylbenzaldehyde. A mixture of 4-carboxybenzaldehyde (Aldrich, 1.8 g, 0.012 mol) and thionyl chloride (10 mL, 0.12 mol) was refluxed for 3 h and cooled to room temperature, and then the excess thionyl chloride was removed under vacuum. Addition of dry ethanol (30 mL) and anhydrous $NaHCO_3$ (0.5 g) was followed by filtration and removal of the excess ethanol under vacuum. The residue was dissolved in ether and purified by column chromatography on silica gel. After removal of most of the ether and freezing, white crystals of 4-ethoxycarbonylbenzaldehyde were obtained: yield 0.64 g (30%); ^{1}H NMR ($CDCl_3$) δ 10.1 (s, 1H, CHO), 8.2 (d, $J = 6$ Hz, 2H, ArH), 7.9 (d, $J = 6$ Hz, 2H, ArH), 4.25 (q, $J = 7$ Hz, 2H, CH_2), 1.42 (t, $J = 7$ Hz, 3H, CH_3).

Laser Flash Photolysis. A Lumonics HY-750 YAG laser (266 nm, ~25 mJ/pulse, 10 ns pulse duration) and a Lumonics EX-530 Excimer laser (XeCl, 308 nm, 40 mJ/pulse, 6 ns pulse duration) were used for the kinetic experiments. These were carried out in a 7 mm × 7 mm quartz cell at an OD of 0.3−0.7 at the laser wavelength. Details of the experimental setup have been published.[32]

Dry acetonitrile was freshly prepared each day by distillation over calcium hydride and was stored under nitrogen in a rubber septum-sealed vial at room temperature. This acetonitrile was transferred in 1 mL aliquots by hypodermic syringe into small septum-sealed vials containing the alkoxyl radical precursors (e.g., Et-SOTS, 5−7 mg, dibenzylhyponitrite 10−15 mg) under nitrogen. These vials were stored at −20 °C. The laser tubes were oven-dried, cooled under nitrogen, and sealed with rubber septa, following which 2 mL of the dry acetonitrile was injected with a syringe. This acetonitrile was then bubbled with nitrogen (5−10 min) or with oxygen or oxygen/nitrogen mixtures of known composition (30 min) employing a home-built gas mixing apparatus. (The oxygen concentrations were calculated from the known solubility of oxygen in acetonitrile.) Afterward, 20−30 μL of the hyponitrite or peroxide solution was injected. For the 1,2-H shift kinetic experiments, known quantities of the catalyst (H_2O, alcohol) or potential catalyst (e.g., amine) were added to the acetonitrile in the laser tube prior to the bubbling with nitrogen and the addition of the standard solution of the alkoxyl radical precursor. The experimental first-order rate constants at the various catalyst concentrations from which the second-order rate constants were calculated are given as Supporting Information.

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Supporting Information Available: Syntheses of Me-SOTS and *t*-Bu-SOTS and an alternative synthesis of SOTS-1 from *t*-Bu-SOTS; spectra of $EtOC(O)C_6H_4CH_2O^\bullet$ (Figure S1); traces showing effects of H_2O (0−2.5 M) on OD (320 nm) vs time following LFP of Et-SOTS in MeCN (Figure S2); effect of H_2O on the rate of the 1,2-H shift for $4\text{-}CH_3C_6H_4CH_2O^\bullet$ (Figure S3); effect of H_2O on the rate of the 1,2-D shift for $C_6D_5CD_2O^\bullet$ (Figure S4); kinetic plots showing effects of various alcohols on the rate of the 1,2-H shift for $EtOC(O)C_6H_4CH_2O^\bullet$ in acetonitrile and the effect of D_2O on the 1,2-H shift for $EtOC(O)C_6H_4CH_2O^\bullet$ and $C_6H_5CH_2O^\bullet$ (Figure S5); a tabulation of all kinetic data for the effect of H_2O, D_2O, and alcohols on the rate of the 1,2-H shift for $EtOC(O)C_6H_4CH_2O^\bullet$ (Table S1); and a tabulation of all kinetic data for the effect of H_2O and D_2O on the rate of the 1,2-H(D) shift for $C_6H_5CH_2O^\bullet$ and $C_6D_5CD_2O^\bullet$ (Table S2) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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