Extraordinary Kinetic Behavior of the α-Tocopheroxyl (Vitamin E) Radical[1,†]

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Rate constants which have been reported for the bimolecular self-reaction of α-tocopheroxyl radicals vary by about 5 orders of magnitude. We have found that the observed bimolecular rate constant can vary by about a factor of 7 during a single, but typical experiment, e.g., in chlorobenzene at 37 °C from ca. 7×10^3 M^{-1} s^{-1} initially to ca. 1×10^3 M^{-1} s^{-1} finally. The overall reaction involves a disproportionation with the transfer of a hydrogen atom from the 5-methyl group of one radical to the phenoxyl oxygen atom of the other radical forming α-tocopherol and an *o*-quinone methide. In the slow regime (which corresponds to the true reaction of two α-tocopheroxyl radicals) this disproportionation has a deuterium kinetic isotope effect of 3.7. The bizarre kinetic behavior observed with α-tocopheroxyl radicals has been traced to a very minor impurity which will be present in any normal sample of α-tocopherol. The impurity in question is a bisphenol in which two α-tocopherol moieties have become linked through their 5-methyl carbon atoms. This bisphenol is a "natural" impurity in α-tocopherol since it will be formed upon exposure of α-tocopherol to air. The coupling of two *o*-quinone methide molecules yields a spiro-dimer which is then reduced to the bisphenol, probably by unoxidized α-tocopherol.

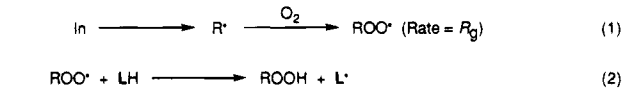
α-Tocopherol, α-TOH, is chemically and biologically the most active lipid-soluble, phenolic antioxidant present in mammalian tissues.[3] This form of vitamin E[4] inhibits the free-radical-chain autoxidation (peroxidation) of polyunsaturated fatty acid esters, LH, present in cell membranes and other *in vivo* lipid "pools". The principal elementary reactions involved in α-TOH-inhibited lipid peroxidation in a homogeneous system are shown in Scheme 1.[5] Each molecule of α-TOH terminates two peroxidation chains. The first is terminated via reaction 5 which yields a molecule of lipid hydroperoxide, LOOH, and an α-tocopheroxyl radical, α-TO•. The second chain is terminated by the fast coupling of an LOO• radical with the α-TO• radical, reaction 6. Accordingly, the α-TOH-inhibited rate of lipid peroxidation is given by:

$$d[LOOH]/dt = R_g k_p[LH]/2k_{inh}[\alpha\text{-TOH}] \qquad (I)$$

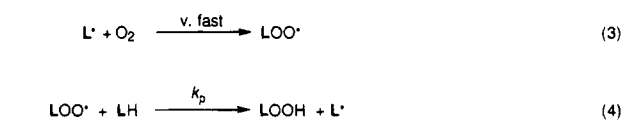
where R_g is the rate of generation of the radicals which initiate the autoxidation chain. In homogeneous, lipid-like solvents,[6] k_{inh} for α-TOH has a higher value (3.2×10^6 M^{-1} s^{-1} at 30 °C)[7,8] than for any other lipid-soluble biological antioxidant, and hence α-TOH is the most powerful natural inhibitor of lipid peroxidation

Scheme 1. α-Tocopherol Inhibited Peroxidation

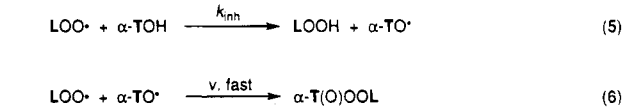
Initiation

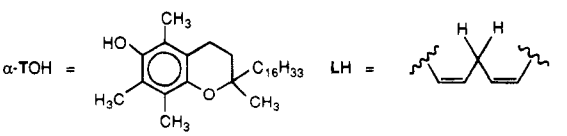


Autoxidation Chain



Inhibition by α-Tocopherol





We have recently demonstrated that the foregoing, and universally accepted, scheme for inhibition by α-TOH breaks down completely in at least one nonhomogeneous system.[9–11] Specifically, in the absence of vitamin C and

† Dedicated to Professor Glen A. Russell on the occasion of his 70th birthday.

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(3) Burton, G. W.; Ingold, K. U. *Acc. Chem. Res.* **1986**, *19*, 194–201.

(4) "Vitamin E" in commerce generally means α-tocopheryl acetate which must be hydrolyzed to give the biologically active phenol. Less active components of natural vitamin E are β-, γ-, and δ-tocopherol which have fewer methyl groups attached to the phenolic ring than does α-tocopherol.

(5) LH is a bis-allylic group in a polyunsaturated fatty acid ester (phospholipid, triglyceride, cholesterol ester, etc.), L• and LOO• are the derived carbon-centered radical and lipid peroxyl radical, respectively, and ROO• is the peroxyl radical which initiates lipid peroxidation. This radical is often generated by the thermal decomposition of an azo-initiator, RN=NR, for *in vitro* experiments.

(6) That is, in non-hydrogen bonding and nonpolar solvents.

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Scheme 2. Tocopherol-Mediated Peroxidation

$$\alpha\text{-TO}^{\bullet} + \text{LH} \xrightarrow{k_{TMP}} \alpha\text{-TOH} + \text{L}^{\bullet} \quad (7)$$

$$\text{L}^{\bullet} + \text{O}_2 \xrightarrow{\text{v. fast}} \text{LOO}^{\bullet} \quad (3)$$

$$\text{LOO}^{\bullet} + \alpha\text{-TOH} \xrightarrow{k_{inh}} \text{LOOH} + \alpha\text{-TO}^{\bullet} \quad (5)$$

ubiquinol-10,[12] α-TOH functions as a *prooxidant* when it is present in an aqueous dispersion of human low density lipoprotein (LDL). The explanation for this "about face" is that LDL particles are physically too small (ca. 21 nm diameter) to "support" more than one radical at a time[13] and an α-TO• radical which is formed in a LDL particle (via reaction 5, Scheme 1) is too water-insoluble to "escape" from that LDL particle into the surrounding aqueous environment. Under these conditions, the α-TO• radical abstracts a hydrogen atom from LH to reform an α-TOH molecule, reaction 7 (Scheme 2). Although reaction 7 is slow ($k_{TMP} \approx 0.1$ M^{-1} s^{-1})[10] it is faster (under most experimental conditions) than the rate at which a second radical will diffuse from the water into the LDL particle and destroy the α-TO• radical (reaction 6). The overall result is a radical-chain process which we have christened Tocopherol-Mediated Peroxidation (TMP) (see Scheme 2). The occurrence of TMP means that α-TOH can function as a prooxidant for LDL. Indeed, we have found[9-11] that the rate of peroxidation of α-TOH-enriched LDL is faster than that of native LDL and, even more remarkably, that the "early" rate of oxidation of LDL which still contains α-TOH (and so is nominally "inhibited") can be faster than the "later" rate of oxidation after all the α-TOH has been consumed!

The discovery of TMP in LDL and the importance of effectively inhibiting LDL peroxidation *in vivo* (since the peroxidation of LDL is implicated as an initiator of atherosclerosis)[14] prompted us to reexamine reactions of the α-TO• radical in homogeneous solutions. Particular interest attaches to the kinetics of peroxyl radical trapping by α-TO• (reaction 6, Scheme 1) and the bimolecular self-reaction of α-TO•, reaction 8, since each of these two reactions destroys two radicals and each therefore provides a two-chain-terminating event.

$$\alpha\text{-TO}^{\bullet} + \alpha\text{-TO}^{\bullet} \xrightarrow{2k_{TO}} \text{Non-radical products} \quad (8)$$

The products formed upon oxidation of α-TOH (and related phenols) with peroxyl radicals indicate that reaction 6 principally involves addition of the lipid peroxyl radical to the position *para* to the oxygen atom which formally bears the unpaired electron in α-TO• to yield an 8a-peroxy-α-tocopherone.[15,16] This coupling of peroxyl radicals with phenoxyl radicals (including α-TO•)[19] is a very fast reaction with measured rate constants for a variety of peroxyl and aryloxyl radicals lying in the range $1-8 \times 10^8$ M^{-1} s^{-1}.[18-21]

By way of contrast, there can be no doubt that reaction 8 is rather slow for a radical–radical reaction but, surprisingly, there is no consensus whatever regarding the magnitude of $2k_{TO}$. Indeed, the experimentally observed values for this rate constant at ambient temperatures,[22-28] $2k_{obs}$, vary by 5 orders of magnitude (see Table 1)! Even if the lowest $2k_{obs}$ value (0.061 M^{-1} s^{-1})[28] is discarded (which may be justified)[29] the other values reported for this rate constant vary by a factor of

Table 1. Literature Values for the Rate Constant for the Bimolecular Self-Reaction of α-Tocopheroxyl Radicals, $2k_{obs}$, at Ambient Temperatures

solvent	$2k_{obs}/(M^{-1} s^{-1})$	reference
benzene	6000	22
benzene	3000	23
ethanol	1400	24
heptanol	560[a]	24
benzene	880	25
chloroform	190	25
chloroform	180	26
cyclohexane	350[b]	27
benzene	0.061	28

[a] A similar value was obtained in methyl heptanoate.[24] [b] If corrected using the value found for ϵ_{424} in the present work, this would rise to ca. 800.

(12) Both of these compounds can help to "export" the radical character from a radical-containing LDL particle into the aqueous phase.[9-11] This action converts α-TOH from a prooxidant in LDL into an antioxidant.[9-11]

(13) Because *most* radical–radical reactions are very fast.

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(15) (a) Winterle, J.; Dulin, D.; Mill, T. *J. Org. Chem.* **1984**, *49*, 491–495. (b) Yamauchi, R.; Matsui, T.; Kato, K.; Ueno, Y. *Agric. Biol. Chem.* **1989**, *53*, 501–511. (c) Matsuo, M.; Matsumoto, S.; Iitaka, Y.; Niki, E. *J. Am. Chem. Soc.* **1989**, *111*, 7179–7185. (d) Yamauchi, R.; Matsui, T.; Satake, Y.; Kato, K.; Ueno, Y. *Lipids* **1989**, *24*, 204–209. (e) Yamauchi, R.; Matsui, T.; Kato, K.; Ueno, Y. *Lipids* **1990**, *25*, 152–158. (f) Liebler, D. C.; Baker, P. F.; Kaysen, K. *J. Am. Chem. Soc.* **1990**, *112*, 6995–7000. (g) Liebler, D. C.; Kaysen, K. L.; Burr, J. A. *Chem. Res. Toxicol.* **1991**, *4*, 89–93. (h) Liebler, D. C.; Burr, J. A. *Biochemistry* **1992**, *31*, 8278–8284. (i) Yamauchi, R.; Miyake, N.; Kato, K.; Ueno, Y. *Lipids* **1993**, *28*, 201–206. (j) Liebler, D. C.; Burr, J. A.; Matsumoto, S.; Matsuo, M. *Chem. Res. Toxicol.* **1993**, *6*, 351–355. (k) Yamauchi, R.; Yagi, Y.; Kato, K. *Biochim. Biophys. Acta* **1994**, *1212*, 43–49.

(16) Analogous 4-peroxycyclohexadienones are formed during the oxidation of numerous other phenols with peroxyl radicals.[17,18]

(17) Ingold, K. U. *Chem. Soc. Spec. Publ.* **1970**, *24*, 285–293.

(18) Mahoney, L. R.; DaRooge, M. A. *J. Am. Chem. Soc.* **1975**, *97*, 4722–4731.

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(20) Griva, A. P.; Denisov, E. T. *Int. J. Chem. Kin.* **1973**, *5*, 869–877.

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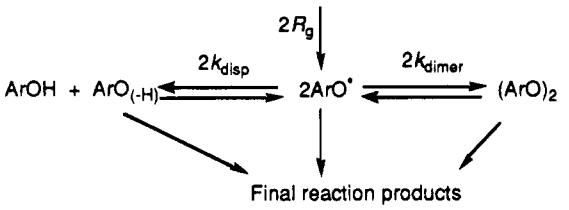
(29) This rate constant was measured by monitoring the decay of α-TO• by ESR after mixing 5 mM DPPH• with 15 mM α-TOH.[28] The initial [α-TO•] appears to have been assumed to be 5 mM. However, there is no evidence to suggest that the mixing and the recording of the EPR spectra were carried out expeditiously. It therefore seems highly probable that when the kinetic measurements were started the actual concentration of α-TO• was considerably less than 5 mM, leading to a low value for $2k_{obs}$.

Scheme 3. Bimolecular Decay of Aryloxyl Radicals



nearly 40. "Solvent effects" cannot be invoked to explain this variation of $2k_{obs}$ in view of the range in values found in benzene and in view of the fact that some workers have reported that this rate constant increases with an increase in solvent polarity[24] while others have reported the reverse[25] (see Table 1).

One of us had previously attributed "low" $2k_{obs}$ values to the known fact[30-32] that the kinetics of the decay of a great many aryloxyl radicals are complicated by the reversible formation of a metastable dimer and/or disproportionation products (see Scheme 3).[23] It was pointed out[23] that if aryloxyl "decay was monitored under anything other than *'initial'* conditions, i.e., in a completely *'fresh'* solution of ArO•, there is a high probability that the reversible and irreversible decay processes will become mixed in varying proportions. The measured value for $2k_{obs}$ will (then) be less than the true value for the *initial* dimerization and/or disproportionation".[33]

Probably the most reliable method for determining the "true" value of $2k_{obs}$, i.e., $2k_{TO}$ (cf. Table 1) and also for investigating any "complications" due to the reversible formation of metastable products is to subject a concentrated solution of α-TOH in an inert solvent to a steady, continuous flux of radicals all of which react only with the α-TOH and generate α-TO• radicals. The α-TO• radicals are generated at a constant rate = R_g, and their concentration is monitored as a function of time. Under these conditions (hereinafter referred to as the Constant Radical Flux method or CRF) most α-TO• radicals will decay by their bimolecular self-reaction, reaction 8. If metastable intermediates play no role in the overall kinetics of decay, the α-TO• radical concentration will grow smoothly to a steady-state (i.e., a plateau in the plot of [α-TO•] vs time) given by:

$$[\alpha\text{-TO}^\bullet]_{ss} = (R_g/2k_{obs})^{1/2} = (R_g/2k_{TO})^{1/2} \qquad \text{(II)}$$

On the other hand, if metastable intermediates do play a role in the overall kinetics the grow-in of the α-TO• to its final plateau concentration, $[\alpha\text{-TO}^\bullet]_{ss}$, will be delayed until equilibrium between the intermediates and the free α-TO• has been established. In this case, the growth in the α-TO• concentration will show two stages: (i) formation of the metastable intermediate(s) with an initial pseudo-steady-state concentration of α-TO• (i.e., a pseudo-plateau) given by:

$$[\alpha\text{-TO}^\bullet]_{pss} = (R_g/2k_{obs})^{1/2} = (R_g/2k_d)^{1/2} \qquad \text{(III)}$$

(30) Weiner, S. A. *J. Am. Chem. Soc.* **1972**, *94*, 581–584.
(31) Mahoney, L. R.; Weiner, S. A. *J. Am. Chem. Soc.* **1972**, *94*, 585–590.
(32) Weiner, S. A.; Mahoney, L. R. *J. Am. Chem. Soc.* **1972**, *94*, 5029–5033.
(33) The measured decay may even follow first-order kinetics under such circumstances.[32] It is also worth noting that α-TO• radicals have been reported to dimerize at low temperatures.[34]

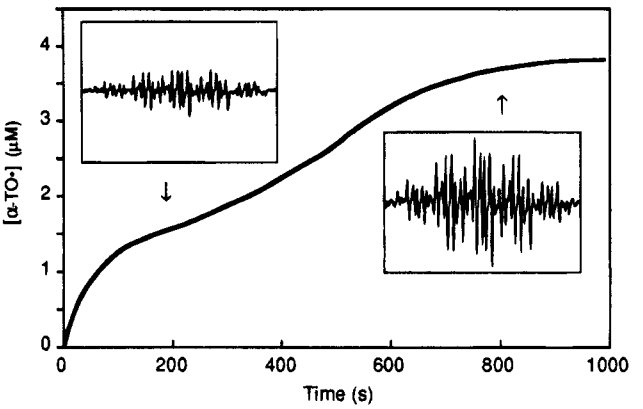


Figure 1. Smooth line: concentration of α-TO• measured by its 424 nm absorption as a function of time. Initial conditions, [α-TOH] = 2 mM in chlorobenzene, [BONNOB] = 1.2 mM, 37 °C. Boxes: EPR spectra recorded under identical conditions at the times indicated by the two arrows. The EPR spectra were recorded with the same instrument settings.

where $2k_d$ refers to the bimolecular α-TO• disproportionation (or dimerization) which yields the metastable intermediate(s) (see Scheme 3); (ii) as the equilibration of α-TO• with the intermediate(s) becomes established the α-TO• concentration will again increase until it reaches its final plateau, $[\alpha\text{-TO}^\bullet]_{ss}$.

Our initial measurements using the CRF method showed a two-stage growth of the α-TO• concentration (see, e.g., Figure 1) which supported the idea that metastable intermediates played a role in the overall decay process. However, a closer examination showed that the time dependence of the α-TO• concentration was not amenable to any simple kinetic treatment based on Scheme 3 since the precise shapes of these curves were found to depend on such factors as: (i) the number of initiating radicals employed (i.e., R_g × initiation time), (ii) the concentration of α-TOH, and (iii) even the "age" of tocopherol stock solutions. Herein, we report our extensive investigations of the α-TO• bimolecular self-reaction both by the CRF method (*vide supra*) and by direct measurements of the kinetics of α-TO• decay. The bizarre kinetic behavior of α-TO• radicals with their "variable" $2k_{obs}$ values (cf. Table 1) was (eventually) found to have a relatively simple explanation.

Results

Constant Radical Flux (CRF) Experiments. The time evolution of the α-TO• concentration following the addition of a concentrated stock solution of di-*tert*-butyl hyponitrite (BONNOB) in chlorobenzene to a "fresh" solution of α-TOH in chlorobenzene at 37 °C is shown in Figure 1. In this experiment the α-TO• concentration was

$$\underset{\text{BONNOB}}{[(CH_3)_3CON]_2} \rightarrow 2(CH_3)_3CO^\bullet + N_2 \qquad (9)$$

$$(CH_3)_3CO^\bullet + \alpha\text{-TOH} \rightarrow (CH_3)_3COH + \alpha\text{-TO}^\bullet \qquad (10)$$

monitored via its near UV absorption, λ_{max} = 424 nm (see insert in Figure 2), versus a reference wavelength, λ_{ref} = 440 nm (which is an isosbestic point for the diphenylpicrylhydrazyl method for α-TO• radical generation, *vide infra*). For α-TO• radicals in chlorobenzene at 37 °C $\epsilon_{424} - \epsilon_{440}$ was found to be 4500 M^{-1} cm^{-1} (see Experimental Section). The rate of radical generation from BONNOB,

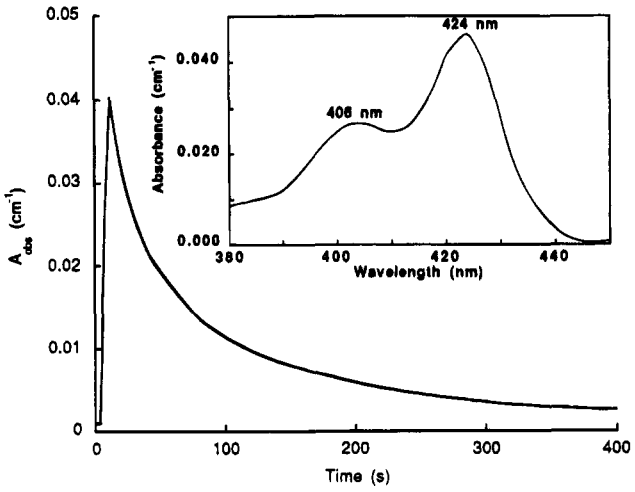


Figure 2. Decay of α-TO• following the addition of DPPH. Initial conditions: [α-TOH] = 2.5 mM in chlorobenzene, [DPPH•] = 10 μM, 37 °C. $A_{obs} = A_{424} - A_{440}$. Inset: spectrum taken 5 s after addition of DPPH.

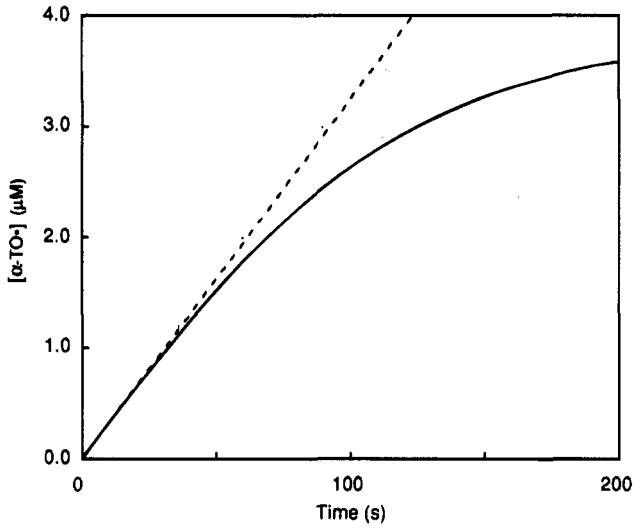


Figure 3. Smooth line: Growth of α-TO• as a function of time following the addition of 1.0 mM BONNOB to 0.5 mM [α-TOH] in chlorobenzene at 37 °C. The α-TO• concentration was calculated from $\epsilon_{424} - \epsilon_{440} = 4500\ M^{-1}\ cm^{-1}$. Dotted line: initial slope = $R_g = 1.2 \times 10^{-8}\ M\ s^{-1}$.

R_g, was most accurately and most directly measured from the *initial* rate of formation of α-TO•, i.e., $R_g = d[\alpha\text{-TO}^\bullet]_{t\to 0}/dt$ (see Figure 3 for the results of a typical experiment designed to measure R_g). This procedure yielded $R_g = 12 \times 10^{-6}$ [BONNOB] M s^{-1} in chlorobenzene at 37 °C.[35] From the known rate of radical generation in the experiment with 1.2×10^{-3} M BONNOB which is shown in Figure 1, viz., $R_g = 12 \times 10^{-6} \times 1.2 \times 10^{-3} = 1.4 \times 10^{-8}$ M s^{-1} and the α-TO• concentration at the first, (pseudo) plateau ($[\alpha\text{-TO}^\bullet]_{pss} \sim 1.4 \times 10^{-6}$ M) we can calculate via eq III that the rate constant for the bimolecular decay of α-TO• radicals to form putative metastable intermediates, $2k_{obs}\ (= 2k_d) \approx (1.4 \times 10^{-8})/(1.4 \times 10^{-6})^2 \approx 7.1 \times 10^3\ M^{-1}\ s^{-1}$. This rate constant is larger than any previously reported value for the bimolecular self-reaction of α-TO• radicals (cf. Table 1). As can also be seen in Figure 1, after ca. 900 s the concentration of α-TO• reaches a true plateau, $[\alpha\text{-TO}^\bullet]_{ss} \sim 3.8 \times 10^{-6}$ M, which yields via eq II, $2k_{obs} = (2k_{TO}) \approx (1.4 \times 10^{-8})/(3.8 \times 10^{-6})^2 \approx 1.0 \times 10^3\ M^{-1}\ s^{-1}$, a value which falls within the range of previously reported values. It should be noted that the first (pseudo) plateau is never a real plateau since the α-TO• concentration increases slowly with time in this region and then, fairly suddenly, it increases more rapidly until it reaches the final (true) plateau (see Figure 1).

The curve shown in Figure 1 could be reproduced within experimental error by the "reverse" addition of a concentrated chlorobenzene solution of α-TOH to the BONNOB in chlorobenzene at 37 °C. Furthermore, the same curve could be obtained in oxygen-saturated as in nitrogen-saturated solutions.

One possible explanation for the "two phase" kinetics illustrated in Figure 1 which did not involve metastable intermediate(s) was that two different radicals were present which had identical UV spectra but very different decay kinetics.[38] This possibility was eliminated by a high resolution electron paramagnetic resonance (EPR) spectroscopic study of an argon-purged α-TOH/BONNOB solution in chlorobenzene at 37 °C. Throughout the *entire* experiment the same EPR spectrum was obtained (see inserts in Figure 1). This spectrum was readily identified as arising from the α-TO• radical.[8,28,34,39] Thus, the "two phase" kinetics would appear to be due entirely to the α-TO• radical.

In case the BONNOB-derived *tert*-butoxyl radicals were in some way responsible for the two phase kinetics, the foregoing UV-monitored CRF experiments were repeated using an azo compound as the radical generator, 2,2′-azobis(2,4-dimethylvaleronitrile), AMVN, see Scheme 4. To produce a significant concentration of α-TO• radicals with AMVN as the radical generator, oxygen is required (in contrast to BONNOB, *vide supra*). This is because the carbon-centered radicals (R• in Scheme 4) abstract the phenolic hydrogen atom from α-TOH at a much lower rate than do the peroxyl radicals, but the R•

(34) Mukai, K.; Tsuzuki, N.; Ishizu, K.; Ouchi, S.; Fukuzawa, K. *Chem. Phys. Lipids* **1981**, *29*, 129–135.

(35) (a) Pryor and co-workers[36] have used the classic autoxidation induction period method of Boozer *et al.*[37] in chlorobenzene at 37 °C to obtain $R_g = 11.3 \times 10^{-6}$ [BONNOB] s^{-1} and $R_g = 3.5 \times 10^{-6}$ [AMVN] s^{-1}. Our own results are in good agreement with these earlier data. (b) R_g values were measured via the initial rate of α-TO• formation at 30, 37, 40, 50, and 60 °C. The combined data for each initiator could be expressed in Arrhenius form as:

$$\log(R_g/([\text{BONNOB}]s^{-1})) = 14.9 - 28.1/\theta$$

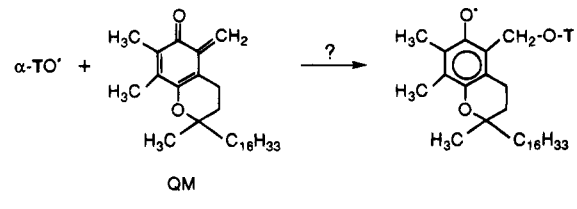
$$\log(R_g/([\text{AMVN}]s^{-1})) = 15.4 - 29.7/\theta$$

where $\theta = 2.3RT$ kcal/mol.

(36) Cosgrove, J. P.; Church, D. F.; Pryor, W. A. *Lipids* **1987**, *22*, 299–304.

(37) Boozer, C. E.; Hammond, G. S.; Hamilton, C. E.; Sen, J. N. *J. Am. Chem. Soc.* **1955**, *77*, 3233–3237.

(38) One of several possibilities we considered was that the first, (pseudo) plateau in [α-TO•] might correspond to the steady-state concentration determined by the α-TO•/α-TO• reaction while the second plateau in 424 nm absorbance might correspond to a slower reaction involving a different radical such as that which might conceivably be formed if α-TO• coupled to the quinone methide, QM, which is an expected product of the α-TO•/α-TO• reaction (*vide infra*), i.e.,

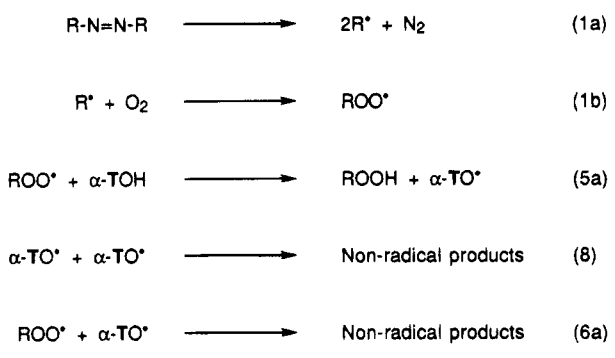


QM

(39) Boguth, W.; Niemann, H. *Biochim. Biophys. Acta* **1971**, *248*, 121–130.

Scheme 4. Radical Generation from AMVN

($R^{\bullet}$ = $(CH_3)_2CHCH_2\dot{C}(CH_3)C{\equiv}N$)



radicals couple with α-TO• just about as readily as do the ROO• (LOO•) radicals. With AMVN under O_2-saturation (760 Torr) the rate of radical generation in chlorobenzene at 37 °C was found from the initial slope of the α-TO• grow-in curves to be given by $R_g = 2.8 \times 10^{-6}$ [AMVN] s^{-1}.[35]

Two-phase curves similar to that shown in Figure 1 were obtained using oxygen-saturated chlorobenzene solutions containing AMVN and α-TOH. Indeed, with the same α-TOH concentrations, identical two-phase curves could be produced using either AMVN (+ O_2) or BONNOB (± O_2) provided these compounds were used at concentrations which gave the same R_g *and* provided that [α-TOH]/[AMVN] ≥ 0.1.[40] At smaller [α-TOH]/[AMVN] ratios the final steady-state concentration of α-TO• decreased (see Figure 4), a phenomenon not observed in the CRF experiments with BONNOB. The reason that $[\alpha\text{-TO}^{\bullet}]_{ss}$ (eq II) declines at constant R_g but at reduced [α-TOH] when using AMVN (Figure 4) is that at low [α-TOH] the destruction of α-TO• by ROO• (reaction 6a) becomes increasingly competitive with the formation of α-TO• by ROO• (reaction 5a). A simple steady-state kinetic analysis of Scheme 4 as [α-TOH] → 0 (so that reaction 8 becomes unimportant relative to reaction 6a) yields:

$$k_{5a}[\text{ROO}^{\bullet}][\alpha\text{-TOH}] = k_{6a}[\text{ROO}^{\bullet}][\alpha\text{-TO}^{\bullet}] \quad \text{(IV)}$$

Thus, the asymptotic slope of plots of $[\alpha\text{-TO}^{\bullet}]_{ss}$ vs [α-TOH] at constant R_g (e.g., [AMVN] = 1.2×10^{-2} M, T = 45 °C, $R_g = 1.1 \times 10^{-7}$ M s^{-1}, see Figure 4) yields $k_{5a}/k_{6a} = 7.4 \times 10^{-3}$. Since k_{5a} must be roughly equal to k_{inh} (reaction 5, Scheme 1), i.e., $k_{5a} \approx 3.2 \times 10^6$ M^{-1} s^{-1},[7,8] we calculate that $k_{6a} \approx 4.3 \times 10^8$ M^{-1} s^{-1}. This value for k_{6a} lies in the middle of the range of rate constants which have been reported for other peroxyl/aryloxyl reactions (viz.,[18-21] $1-8 \times 10^8$ M^{-1} s^{-1} including a value of 3×10^8 M^{-1} s^{-1} for the peroxyl/α-TO• reaction[19]). More importantly, the present value for k_{6a} has been obtained by a more direct method than many of the procedures used heretofore to derive related rate constants which have generally relied on the analyses of complex kinetic schemes and computer fitting.[18-21] Our value for k_{6a} is therefore likely to be fairly reliable.

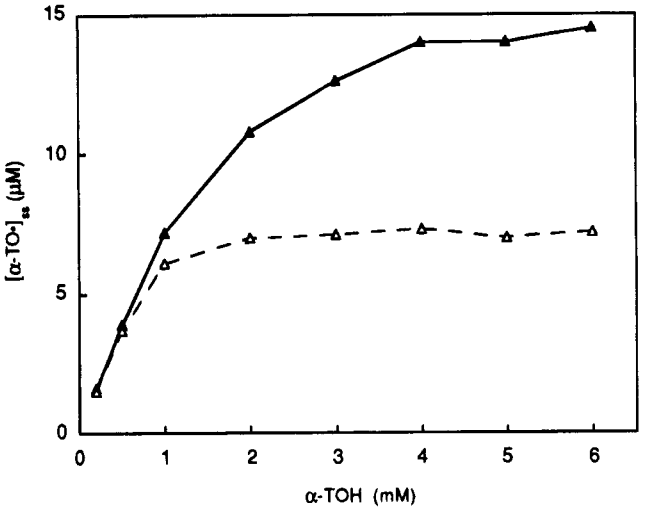


Figure 4. Plots of $[\alpha\text{-TO}^{\bullet}]_{ss}$ vs [α-TOH] for the AMVN/O_2 reaction with α-TOH at various concentrations in chlorobenzene at 45 °C. Key: ▲, [AMVN] = 40 mM; △, [AMVN] = 10 mM.

Since the two-phase grow-in of α-TO• in the CRF experiments did not depend on the initial source of radicals (BONNOB or AMVN/O_2) we explored other features of grow-in kinetics with the following results:

(1) The concentrations of α-TO• at the first (pseudo) plateau, $[\alpha\text{-TO}^{\bullet}]_{pss}$, and at the final (true) plateau, $[\alpha\text{-TO}^{\bullet}]_{ss}$, were independent of the initial concentration of α-TOH but were proportional to the square root of the initiator concentration as would be expected, i.e., both $[\alpha\text{-TO}^{\bullet}]_{pss}$ and $[\alpha\text{-TO}^{\bullet}]_{ss}$ are $\alpha\ R_g^{1/2}$ (see eqs II and III).

(2) The time taken to reach the first (pseudo) plateau, τ_{first}, was inversely proportional to R_g and was roughly independent of the initial concentration of α-TOH, i.e., $\tau_{first} \approx [\alpha\text{-TO}^{\bullet}]_{pss}/R_g$.

(3) The time taken to reach the final (true) plateau, τ_{final}, and the time delay between the first and second plateaus, $\tau_{final} - \tau_{first}$ (see Figure 5A) were inversely proportional to R_g. However, τ_{final} was not independent of [α-TOH] but increased as the initial α-TOH concentration was increased, i.e., $\tau_{final} \alpha [\alpha\text{-TOH}]/R_g$. The time delay between the first and final plateaus (see Figure 5B) could be approximately represented by:

$$\tau_{final} - \tau_{first} \approx 0.01_2[\alpha\text{-TOH}]/R_g \quad \text{(V)}$$

both at 37 °C and at 50 °C.

(4) The addition of a fairly large quantity of α-TOH to a reaction which had already achieved the final (true) plateau, $[\alpha\text{-TO}^{\bullet}]_{ss}$, caused the α-TO• concentration to fall to its first (pseudo) plateau level, $[\alpha\text{-TO}^{\bullet}]_{pss}$. This decrease in the α-TO• concentration followed second-order kinetics:

$$d[\alpha\text{-TO}^{\bullet}]/dt = 2k_{obs}[\alpha\text{-TO}^{\bullet}]^2 \quad \text{(VI)}$$

with $2k_{obs} \sim 8 \times 10^3$ M^{-1} s^{-1} at 37 °C. That is, decay from $[\alpha\text{-TO}^{\bullet}]_{ss}$ to $[\alpha\text{-TO}^{\bullet}]_{pss}$ is "fast" with a rate constant approximately equal to that which determines the α-TO• concentration at the first (pseudo) plateau, i.e., from eq III:

(40) A relatively high [α-TOH]/[AMVN] ratio ensures that α-TO•/peroxyl radical reactions are unimportant. It is also worth noting that if reverse addition is employed, i.e., if α-TOH is added to a prewarmed, oxygenated solution containing AMVN there is a very rapid initial formation of α-TO• which is produced by the AMVN-derived peroxyl radicals, ROO•, present at their steady-state concentration, $[\text{ROO}^{\bullet}]_{ss}$. This steady-state concentration is determined by R_g and the rate constant for the bimolecular self-reaction of ROO•, $2k_t$. For $R_g = 1.2 \times 10^{-7}$ M s^{-1} the initial yield of α-TO• (which must equal $[\text{ROO}^{\bullet}]_{ss}$) was $\sim 9 \times 10^{-7}$ M. Since $R_g = 2k_t[\text{ROO}^{\bullet}]^2_{ss}$, we can calculate that $2k_t \approx 1.5 \times 10^5$ M^{-1} s^{-1}.

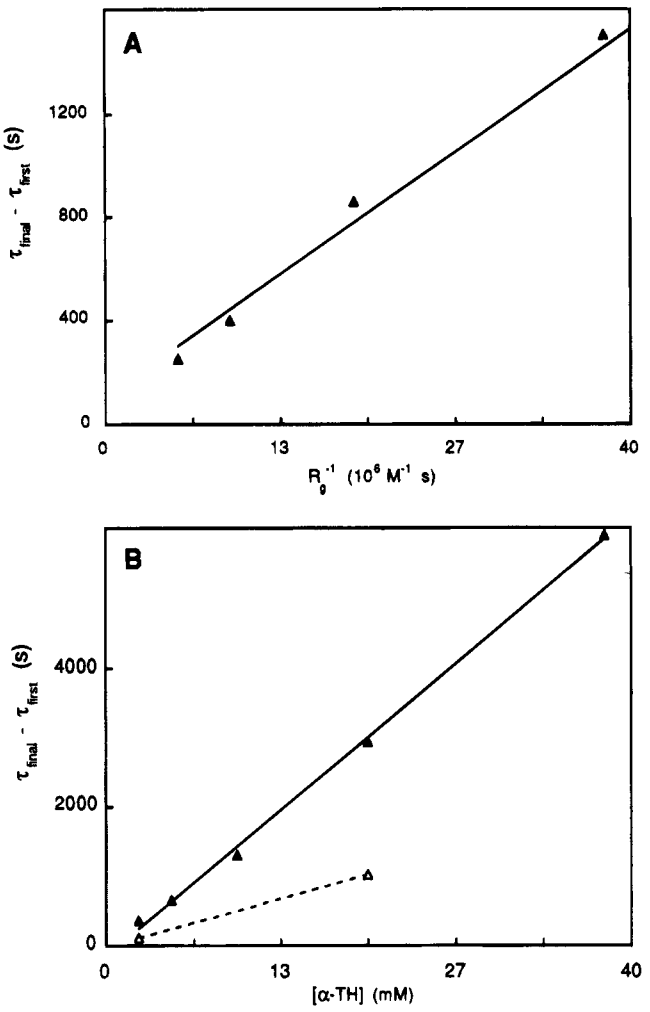


Figure 5. A: Plot of $\tau_{final} - \tau_{first}$ vs R_g^{-1} for [α-TOH] = 5 mM in chlorobenzene at 37 °C with BONNOB as the radical source. B: Plot of $\tau_{final} - \tau_{first}$ vs [α-TOH] using BONNOB as the radical source in chlorobenzene at 37 °C: $R_g = 5.8 \times 10^{-8}$ M s^{-1}; ▲, α-TOH; △, d_3-α-TOH.

$$2k_{obs} = R_g/[\alpha\text{-TO}^{\bullet}]^2_{pss} \qquad \text{(VII)}$$

(5) Attempts to demonstrate the formation of metastable intermediate(s) during the decay of α-TO• radicals by the "temperature jump" technique[41] were uniformly unsuccessful. For example, α-TO• radicals were generated at 0 °C (and at 20 °C) by the relatively fast reaction of the diphenylpicrylhydrazyl radical, DPPH• (2 mM) with α-TOH (10 mM), reaction 11.[28,39,42]

$$\alpha\text{-TOH} + \text{DPPH}^{\bullet} \rightarrow \alpha\text{-TO}^{\bullet} + \text{DPPH}_2 \qquad (11)$$

The solutions were allowed to stand for ca. 30 min and were then rapidly heated to 60 °C in the cavity of an EPR spectrometer. No increase (however brief in duration) of the α-TO• concentration could be detected, a disappointingly "negative" result which implies that the two stage kinetics shown in Figure 1 cannot be due to the formation of metastable intermediates during the bimolecular self-reaction of α-TO• radicals.

Direct Kinetic Studies of the Decay of Preformed α-TO• Radicals. In the hope of unraveling the mysterious kinetic behavior of α-TO• radicals which had been revealed by the CRF experiments we undertook some direct studies of α-TO• decay. As others have found previously,[24-26,28,39,43] one of the most convenient procedure for rapidly generating known concentrations of α-TO• is to mix a concentrated stock solution of α-TOH into a dilute, thermostated solution of DPPH•. As mentioned above, reaction 11 is fairly fast and so one observes a quick and complete loss of (purple) DPPH• and the simultaneous grow-in of (orange) α-TO•, followed by the much slower bimolecular decay of the α-TO• radicals (monitored at 424 nm relative to the isobestic point reference wavelength of 440 nm, see Figure 2).

Under all experimental conditions the α-TO• radicals appeared to decay with rather good second-order kinetics. However, and as we might expect from the CRF experiments, the derived bimolecular rate constants for α-TO• decay varied when the experimental conditions were changed. For experiments with "fresh" α-TOH solutions in chlorobenzene in which [DPPH•] ≪ [α-TOH], the observed second-order rate constants, $2k_{obs}$, at 10, 20, 30, 40, 50, and 60 °C were 3000, 3500, 4600, 5100, 6600, and 7100 M^{-1} s^{-1}, respectively, data which yield:[44]

$$\log(2k_{obs}/(M^{-1}\ s^{-1})) = 6.1 - 3.4/\theta$$
$$(\theta = 2.3RT \text{ kcal/mol}) \qquad \text{(VIII)}$$

From this Arrhenius equation we obtain $2k_{obs} = 5.0 \times 10^3$ M^{-1} s^{-1} for αTO• decay in chlorobenzene at 37 °C, a value which is in fair agreement with the 7.1×10^3 M^{-1} s^{-1} obtained from the CRF experiments via $[\alpha\text{-TO}^{\bullet}]_{pss}$, R_g and eq III. At 23 °C this Arrhenius equation yields $2k_{obs} = 3900$ M^{-1} s^{-1}, a value which is in quite satisfactory agreement with the 3000 M^{-1} s^{-1} found in benzene at this temperature during earlier work at these laboratories[23] (see Table 1). There is, in fact, a small solvent effect on the rate of bimolecular decay of α-TO• radicals measured under these "initial" conditions where [DPPH•] ≪ [α-TOH] with $2k_{obs}$ decreasing slightly as the solvent polarity was increased.[45]

For experiments in which the DPPH• concentration was not very much smaller that the α-TOH concentration the decay of the α-TO• radicals also followed rather good second-order kinetics, but there was a dramatic decline in the rate at which they decayed. For example, a chlorobenzene solution of α-TOH and DPPH• was prepared at 37 °C which initially contained 2.0×10^{-3} M [α-TOH] and 2.5×10^{-4} M [DPPH•]. In this solution there was a very rapid consumption of 12.5% of the initial α-TOH with the formation of 2.5×10^{-4} M [α-TO•], via reaction 11. These radicals proceeded to decay with second-order kinetics. The decay of the final 14×10^{-6} M of the α-TO• radicals produced in this experiment is shown in Figure 6 and, from the decay rate, $2k_{obs}^{37°C}$ is calculated to be 1.9×10^3 M^{-1} s^{-1} (see line b in inset in

(41) Brokenshire, J. L.; Roberts, J. R.; Ingold, K. U. *J. Am. Chem. Soc.* **1972**, *94*, 7040-7049.

(42) The rate constant for reaction 11 has been reported to be 1600 M^{-1} s^{-1} at 25 °C in benzene.[43] In the present work at 37 °C we found $k_{11} = 2200$ M^{-1} s^{-1} in chlorobenzene, 1200 M^{-1} s^{-1} in CH_2Cl_2, 800 M^{-1} s^{-1} in ethanol, 220 M^{-1} s^{-1} in acetone, and 200 M^{-1} s^{-1} in methyl ethyl ketone.

(43) Boguth, W.; Repges, R.; Pracht, I. *Int. Z. Vitam. Forsch.* **1969**, *39*, 438-446.

(44) For comparison, $\log(2k_{obs}/(M^{-1}\ s^{-1})) = 8.2-6.6/\theta$ in ethanol[24] and $6.1-4.3/\theta$ in heptanol.[24]

(45) At 25 °C, $2k_{obs} \times 10^{-3}/(M^{-1}\ s^{-1}) = 15$, 6.0, 5.6, 5.1, 4.5, 4.1, and 2.6 in isooctane, acetone, acetonitrile, benzene, CH_2Cl_2, chlorobenzene, and methanol, respectively. In view of our final conclusions regarding the nature of this "fast" decay of α-TO• radicals, the origin of this solvent effect was not explored.

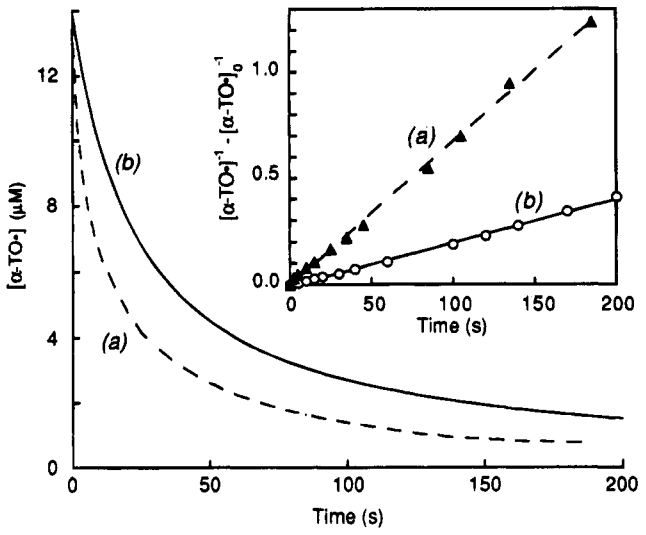


Figure 6. Decay of 1.4×10^{-5} M α-TO• in chlorobenzene at 37 °C. The radical source was DPPH•. Initial conditions: (a) [α-TOH] = 2.0 mM, [DPPH•] = 0.020 mM. (b) [α-TOH] = 2.0 mM, [DPPH•] = 0.25 mM. Inset: second-order decay plots.

Figure 6). The magnitude of this decay rate constant is commensurate with the "slow" $2k_{obs}$ values calculated from $[\alpha\text{-TO}^\bullet]_{ss}$, in the CRF experiments. For comparison, in an otherwise identical experiment except that only 1.0% of the initial α-TOH was converted to α-TO• radicals (i.e., 2.0×10^{-5} M [DPPH•] with 2.0×10^{-3} M [α-TOH]), the final 14×10^{-6} M of the α-TO• radicals decayed with much faster second-order kinetics (see Figure 6 and inset line a) to yield $2k_{obs}^{37°C} = 5.9 \times 10^3$ M^{-1} s^{-1}. This $2k_{obs}$ has a magnitude commensurate with the "fast" $2k_{obs}$ values calculated from $[\alpha\text{-TO}^\bullet]_{pss}$, in the CRF experiments. Even more interesting results were obtained upon the addition of a second and equal aliquot of DPPH• to the solution resulting from the first of these two experiments after the first "crop" of α-TO• radicals had decayed completely. Thus, 25% of the initial α-TOH had now been converted to α-TO• radicals. This second crop of α-TO• radicals also decayed with clean second-order kinetics but more slowly than the first crop with the decay of the final 14×10^{-6} M [α-TO•] yielding $2k_{obs}^{37°C} = 1.0 \times 10^3$ M^{-1} s^{-1}. In contrast, a 25-fold increase in the initial α-TOH concentration (to 50 mM) followed by the addition of a 2.5×10^{-4} M aliquot of DPPH• gave a fast decay ($2k_{obs}^{37°C} = 5.9 \times 10^3$ M^{-1} s^{-1}) and the decay rate was not reduced by the addition of a second 2.5×10^{-4} M DPPH• aliquot.

Thus, it appeared as though the consumption of α-TOH by its oxidation to α-TO• radicals caused the rate of the bimolecular self-reaction of these radicals to decline. That is, from the experiments described above it is seen that 0.5% and 1% consumption of the α-TOH yielded $2k_{obs}^{37°C} = 5.9 \times 10^3$ M^{-1} s^{-1}, a 12.5% consumption yielded 1.9×10^3 M^{-1} s^{-1} and a 25% consumption yielded 1.0×10^3 M^{-1} s^{-1}. This peculiar phenomenon is clearly connected to the two-phase grow-in of the α-TO• radicals in the CRF experiments. Some additional experiments were therefore carried out:

(1) Two hours after making up a chlorobenzene solution containing 1.0×10^{-4} M [DPPH•] and 1.0×10^{-3} M [α-TOH] at 20 °C the solution was heated to 40 °C and BONNOB (2×10^{-3} M) was added. This produced a relatively rapid single-phase grow-in of α-TO• to a plateau concentration which was equal to that produced more slowly and via a two phase grow-in to the final (true) plateau in an experiment which was identical except that no DPPH• was used.

(2) To a 5×10^{-3} M α-TOH solution was added ca. 1×10^{-6} M copper stearate (an oxidation catalyst) and, after 2 days storage at room temperature exposed to air, the solution was heated to 40 °C and BONNOB was added to a concentration of 5×10^{-3} M. This also produced a relatively rapid single-phase grow-in of α-TO• to the same plateau concentration ($[\alpha\text{-TO}^\bullet]_{ss}$) as was finally reached without the copper stearate pretreatment. However, this plateau was reached in about one-third of the time with the copper stearate.

(3) An α-TOH (3×10^{-3} M) plus BONNOB (2×10^{-3} M) solution in chlorobenzene was heated to 50 °C for 400 s, and then the solution was cooled to 20 °C and DPPH• (5×10^{-6} M) was added. The "new" α-TO• radicals generated via the DPPH• reaction decayed at the "slow" rate with $2k_{obs}^{20°C} = 8 \times 10^2$ M^{-1} s^{-1} (vs 3.7×10^3 M^{-1} s^{-1} for a "fresh" α-TOH solution).

Deuterium Kinetic Isotope Effects (DKIE). Addition of DPPH• to a solution of α-tocopherol deuterated at the hydroxylic position[46] (i.e., α-TOD) reduced the rate of formation of the α-TO• radicals by about a factor of 6 relative to their rate of formation from α-TOH, i.e., $k_{11}^H/k_{11}^D \sim 6$. To our surprise, such deuteration reduced the rate constant for the "early/fast" decay of α-TO• by about 30%. However, there was no DKIE on the rate constant for the "late/slow" decay of the α-TO• radicals.

The oxidation of α-TOH with a wide variety of oxidizing agents yields products which are most readily rationalized as being formed via a quinone 5-methide, QM, such as methide dimers in nonpolar solvents and methide solvolysis products in hydroxylic solvents.[15j,48] The α-TO• radical must have been formed in many of these product studies and so it appears reasonable to suggest that QM is formed in the bimolecular self-reaction of α-TO• radicals. The apparently exclusive formation of 5-methide-derived products can be rationalized in terms of the higher spin density at the 5-methyl position of α-TO• relative to the 7-methyl position as revealed by EPR spectroscopy.[8,34,39] That is, we can rewrite eq 8 as equation 8′. If this reaction is a straightforward but very slow, kinetically controlled, radical–radical disproportionation[49] and is also the rate-controlling step in the α-TO• bimolecular self-reaction, then replacement of the 5-methyl group by a 5-trideuteromethyl group would be expected to reduce the measured rate constant.[50]

Both [5-CD_3]-α-tocopherol (d_3-α-TOH) and [5,7-$(CD_3)_2$]-α-tocopherol (d_6-α-TOH) were available from earlier

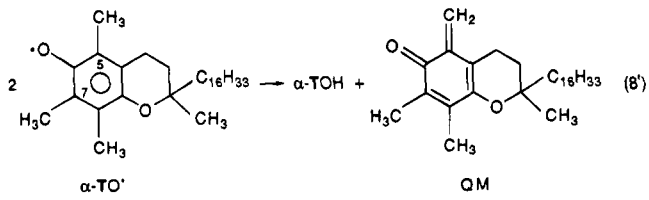
(46) Achieved by the addition of a few drops of D_2O to the solvent.[8,47]

(47) Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1962**, *40*, 1851–1864.

(48) (a) Nelan, D. R.; Robeson, C. D. *J. Am. Chem. Soc.* **1962**, *84*, 2963–2965. (b) Skinner, W. A.; Alaupovic, P. *J. Org. Chem.* **1963**, *28*, 2854–2858. (c) Skinner, W. A.; Parkhurst, R. M. *J. Org. Chem.* **1966**, *31*, 1248–1251. (d) Sumarno, M.; Atkinson, E.; Suarna, C.; Saunders, J. K.; Cole, E. R.; Southwell-Keeley, P. T. *Biochim. Biophys. Acta* **1987**, *920*, 247–250. (e) Suarna, C.; Craig, D. C.; Cross, K. J.; Southwell-Keeley, P. T. *J. Org. Chem.* **1988**, *53*, 1281–1284. (f) Suarna, C.; Southwell-Keeley, P. T. *Lipids* **1989**, *24*, 56–60. (g) Suarna, C.; Baca, M.; Southwell-Keeley, P. T. *Lipids* **1992**, *27*, 447–453. (h) Kohar, I.; Suarna, C.; Southwell-Keeley, P. T. *Lipids* **1993**, *28*, 1015–1020. (i) Suarna, C.; Southwell-Keeley, P. T. *Lipids* **1991**, *26*, 187–190.

(49) As is the case for many other alkylated phenoxyl radicals, see e.g., Roginskii, V. A. *Izv. Akad. Nauk SSSR, Ser. Khim.* **1985**, 1987–1996.

(50) As is the case for some other slow, kinetically-controlled, radical-radical disproportionations, see e.g., Bowman, D. F.; Gillan, T.; Ingold, K. U. *J. Am. Chem. Soc.* **1971**, *93*, 6555–6561.



biokinetic studies on vitamin E.[51] Oxidation of these two deuterated tocopherols with DPPH· at 40 °C under conditions where [DPPH·] ≪ [d_n-α-TOH] showed that the "initial/fast" decay rate was reduced by about a factor of 4.1 for both the d_3- and d_6-α-TO· radicals relative to the rate for unlabelled (d_0) α-TO· radicals, viz., $2k_{obs}^{40°C}$ (d_3) ≈ $2k_{obs}^{40°C}$ (d_6) ≈ 2.0 × 10^3 M^{-1} s^{-1} vs $2k_{obs}^{40°C}$ (d_0) ≈ 8.2 × 10^3 M^{-1} s^{-1}. Similarly, the "final/slow" decay rates obtained with relatively higher concentrations of DPPH· were reduced by a factor of ca. 3.7 for both the d_3- and d_6-α-TO· radicals relative to d_0-α-TO·, viz., $2k_{obs}^{40°C}$ (d_3) ≈ $2k_{obs}^{40°C}$ (d_6) ≈ 3.0 × 10^2 M^{-1} s^{-1} vs $2k_{obs}^{40°C}$ (d_0) ≈ 1.1 × 10^3 M^{-1} s^{-1}. The fact that d_3-α-TO· and d_6-α-TO· radicals undergo their bimolecular self-reactions with the same rate constants (within experimental error) and with rate constants significantly lower than those for d_0-α-TO· radicals rules out the possibility that one regime involves a disproportionation to give the 5-methide and the other a disproportionation to give the 7-methide.[52] This conclusion was confirmed by CRF experiments using both BONNOB and AMVN/O_2 to generate the α-TO· radicals. In these experiments, the α-TO· concentrations at the first (pseudo) plateau ([α-TO·]$_{pss}$) and at the final (true) plateau ([α-TO·]$_{ss}$) were both reduced by about a factor of 2 (corresponding to a reduction of about four in $2k_{obs}$) for both the d_3-, and d_6-α-TO· radicals relative to the d_0-α-TO· radical.

Unraveling the Mystery. At this point in our experiments we despaired of finding an explanation for our observation that what appeared to be the same overall reaction (8') had two distinct kinetic regimes with similar DKIEs since it is an axiom of chemical kinetics that an elementary reaction can have only one rate constant (under the same experimental conditions). On reexamining our basic assumptions we realized that we had, perhaps unjustly, ruled out an "impurity effect". We had done this because the d_0-α-TOH and the two d_n-α-TOH's were ≥96% pure by HPLC (monitored at 295 nm) with no single impurity constituting more than 1.1% of the total material. Furthermore, the [α-TO·]$_{ss}$/[α-TO·]$_{pss}$ ratio was quite reproducible (≈3.5) and was the same for radicals produced from d_0-, d_3-, and d_6-α-TOH. Moreover, when mixtures of phenols are subjected to attack by free radicals it is generally found that the phenol which, when used by itself is the most active radical trap, is the first phenol to be consumed.[53] Only after the concentration of this phenol falls below that of the less active phenols are the latter also consumed.[53] Since α-TOH is one of the most active phenolic radical traps known,[7,8] we initially felt that traces of *phenolic* impurities in our α-TOH should have no effect on the rates of decay of the α-TO· radicals. This would not necessarily be true for hydroquinones, QH_2, because the cross-reaction, 12, might be sufficiently rapid that even a small amount of

$$\alpha\text{-TO}^{\bullet} + \text{QH}^{\bullet} \rightarrow \alpha\text{-TOH} + \text{Q} \qquad (12)$$

QH_2 could accelerate the decay of the α-TO· radicals.[54] However, oxygen reacts rapidly with semiquinone radicals, QH·, and hence oxygen would be expected to influence the rate of decay of α-TO· radicals if their decay was accelerated by a hydroquinone. Experimentally oxygen had no effect on the α-TO· decay rates (*vide supra*). Furthermore, among the several minor impurities present in the α-TOH used in these experiments (96.2% pure by HPLC) there was no trace of the only likely hydroquinone contaminant, α-tocopherolquinol (α-TQH_2); see Experimental Section.

Having eliminated all of the more obvious and many of the less obvious ways by which the α-TO· bimolecular self-reaction might exhibit two-phase kinetics (cf. Figure 1) we recalled some of S. Holmes's sound advice.[57] This forced us to postulate that among the minor impurities in our α-TOH there must be a new type of compound, XH_2, which was not a hydroquinone but which could act as an accelerator for the decay of α-TO· radicals.

The quantity of XH_2 which would have to be present in our α-TOH could be estimated from the time delay between the first and final α-TO· concentration plateaus, eq V (*vide supra*), viz.,

$$\tau_{final} - \tau_{first} \approx 0.01_2[\alpha\text{-TOH}]/R_g \qquad (V)$$

If we now assume that XH_2 (like QH_2) can trap two α-TO· radicals, viz.,

$$\alpha\text{-TO}^{\bullet} + XH_2 \rightleftharpoons \alpha\text{-TOH} + XH^{\bullet} \qquad (13)$$

$$\alpha\text{-TO}^{\bullet} + XH^{\bullet} \rightarrow \alpha\text{-TOH} + X \qquad (14)$$

then eq V implies that the initial [XH_2]/[α-TOH] mole ratio in unoxidized α-TOH is ca. 0.006.[58] The kinetics of α-TO· decay in the presence of XH_2 would be given by:

(51) See e.g., Ingold, K. U.; Burton, G. W.; Foster, D. O.; Hughes, L.; Lindsay, D. A.; Webb, A. *Lipids* **1987**, *22*, 163–172. Cheng, S. C.; Burton, G. W.; Ingold, K. U.; Foster, D. O. *Lipids* **1987**, 22, 469–473. Traber, M. G.; Ingold, K. U.; Burton, G. W.; Kayden, H. J. *Lipids* **1988**, *23*, 791–797. Burton, G. W.; Ingold, K. U.; Foster, D. O.; Cheng, S. C.; Webb, A.; Hughes, L.; Lusztyk, E. *Lipids* **1988**, *23*, 834–840.

(52) After the present work was completed, an interesting publication by Liebler *et al.*[15j] appeared in which the peroxyl radical (AMVN/O_2 at 60 °C) induced oxidation products from 2,2,5,7,8-pentamethylchroman-6-oxyl (PMC) and its 5-trideuteromethyl isotopomer were compared. Among other products, the undeuterated material gave the 5,5'-spirodimer (SD, see below) which arises via dimerization of the quinone 5-methide, QM (R = CH_3), in about a 5-fold higher yield than was obtained from the [5-CD_3] PMC. With the latter compound only, a novel 5,7'-spirodimer was also formed indicating an isotopically-induced shift to the formation of some quinone 7-methide. The authors[15j] pointed out that the radical which reacts with the chroman-oxyl radical, PMCO·, to form QM (R = CH_3) could have been a peroxyl or an alkoxyl or another PMCO· radical. Our results would appear to rule out this last possibility.

(53) See e.g., Niki, E.; Tsuchiya, J.; Yoshikawa, Y.; Yamamoto, Y.; Kamiya, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 497–501.

(54) The hydroquinone, ubiquinol-10, is less reactive than α-TOH toward peroxyl radicals, but when the two compounds are used together the ubiquinol-10 is consumed first, both in homogeneous solutions[55] and in lipid dispersions.[10,11,55,56]

(55) Yamamoto, Y.; Komuro, E.; Niki, E. *J. Nutr. Sci. Vitaminol.* **1990**, *36*, 505–511.

(56) Sato, K.; Niki, E.; Shimasaki, H. *Arch. Biochem. Biophys.* **1990**, *279*, 402–405. Frei, B.; Kim, M. C.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 4879–4883. Cross, C. E.; Forte, T.; Stocker, R.; Louie, S.; Yamamoto, Y.; Ames, B. N.; Frei, B. *J. Lab. Clin. Med.* **1990**, *115*, 396–404. Stocker, R.; Bowry, V. W.; Frei, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1646–1650.

(57) "When you have eliminated the impossible, whatever remains, however improbable, must be the truth". Holmes, S. as quoted by Doyle, A. C. in *The Sign of Four*.

(58) A detailed kinetic analysis indicates that the initial [XH_2]/[α-TOH] mole ratio would be slightly lower, viz., 0.0045.

$$2k_{obs} = 2k_{TO} + 2k_{14}K_{13}[XH_2]/[\alpha\text{-TOH}] \quad (IX)$$

which, for the initial/fast decay, can be written as:

$$2k_{obs} = 2k_{TO} + 2k_{14}K_{13}(0.006) \quad (X)$$

The decay rate will decrease as XH_2 is consumed and hence [α-TO•] in the CRF experiments will increase. When all the XH_2 has been consumed, the final/slow decay would be given by:

$$2k_{obs} = 2k_{TO} \quad (XI)$$

Both the CRF method and the direct measurement of decay rates (via DPPH• addition) yield $2k_{obs} \approx 8 \pm 2 \times 10^3$ M^{-1} s^{-1} and $\approx 1.1 \pm 0.2 \times 10^3$ M^{-1} s^{-1} for the initial/fast and final/slow reactions, respectively, at 37 °C. Inserting these quantities into eq X yields $2k_{14}K_{13} \approx 1.2 \times 10^6$ M^{-1} s^{-1}.

The situation with the deuterated α-tocopherols was particularly interesting. For α-TOD there can be no DKIE on the final/slow reaction but there could be small DKIEs on K_{13} and k_{14} which would affect the initial/fast reaction. Experimentally, there was no DKIE with α-TOD for the final/slow reaction but the initial/fast reaction rate constant was reduced by ca. 30%. With d_3-α-TOH and d_6-α-TOH, the delay in the grow-in of the α-TO• radicals to the final (true) plateau concentration was ca. one-third as long as was found with α-TOH, see eq V, i.e.,

$$\tau_{final} - \tau_{first} \approx 0.004[d_3\text{- or } d_6\text{-}\alpha\text{-TOH}]/R_g \quad (XII)$$

This implies that there is only one-third as much of the putative XH_2 impurity in our two deuterated tocopherols (both of which were made and purified at the NRCC[59] whereas the α-TOH was commercial material). Comparison of the impurities in the α-TOH and in the d_3- and d_6-α-TOH by HPLC revealed that one impurity was present in the α-TOH at the required level of ca. 0.6 mol % and that this same impurity (presumably deuterated) was present in the d_3- and d_6-α-TOH at the required level of ca. 0.2 mol %. This impurity was identified (by comparison with authentic material synthesized for the purpose)[48a] as the bibenzyl reduction product of the spiro-dimer, SD, which is formed by the coupling of two quinone 5-methide molecules (which are themselves produced by the disproportionation of α-TO• radicals), see Scheme 5. The SD was also present as an impurity at 1.1 mol % in α-TOH and at 0.5 mol % in the d_3- and d_6-α-TOH.

Proof that XH_2 was the accelerating impurity which we had been forced to hypothesize was present and responsible for increasing the rate of the decay of the α-TO• radicals was simple:

(1) XH_2 was consumed during the reaction with the last traces gone at the same time that the α-TO• concentration reached its final plateau, $[\alpha\text{-TO}^\bullet]_{ss}$.

(2) An analogue of XH_2 having R = CH_3 was synthesized and was shown to produce a dramatic decrease in the α-TO• concentration when it was added to a CRF experiment which had reached the $[\alpha\text{-TO}^\bullet]_{ss}$ final plateau. For example, the [α-TO•] concentration in a BONNOB-initiated CRF experiment in chlorobenzene at 40 °C was reduced from $[\alpha\text{-TO}^\bullet]_{ss}$ by the expected factor, viz., 3.8, upon the addition of 0.6 mol % XH_2 (R = CH_3) and by a factor of 10 upon the addition of 4 mol % XH_2 (R = CH_3) (i.e., at $[\alpha\text{-TOH}]/[XH_2(R = CH_3)] = 25$). In both cases the XH_2 (R = CH_3) was quantitatively converted into the spiro-dimer, SD (R = CH_3), and at a rate consistent with:

$$-d[XH_2\ (R = CH_3)]/dt = d[SD\ (R = CH_3)]/dt = 0.5R_g \quad (XIII)$$

As the XH_2 (R = CH_3) was consumed the α-TO• concentration increased from its initial (pseudo) steady-state value, $[\alpha\text{-TO}^\bullet]_{pss}$, to the final plateau level, $[\alpha\text{-TO}^\bullet]_{ss}$.

(3) The α-TOH homologue, 2,2,5,7,8-pentamethylchroman-6-ol (PMC), in which the 16 carbon atom phytyl "tail" of α-TOH has been replaced by a methyl group, is crystalline (unlike α-TOH) and hence can readily be purified. CRF experiments with PMC gave a smooth, single-phase grow-in of the pentamethylchromanoxyl radical[60] (PMCO•) and when this radical was generated by the DPPH• method it was found that $2k_{obs}$ was uninfluenced by the initial [DPPH•]/[PMC] ratio. The CRF-derived value for $2k_{obs}^{37°C}$ (eq II) was 890 M^{-1} s^{-1} [61] and the DPPH•-derived value for $2k_{obs}^{25°C}$ was 710 M^{-1} s^{-1} (based on $\epsilon_{424} - \epsilon_{440} = 4500$ M^{-1} s^{-1}), values which are identical within experimental error to the true values for $2k_{TO}$ at these two temperatures. Our measured rate constants for the bimolecular self-reaction of PMCO• radicals are in rather satisfactory agreement with kinetic data for this radical which were obtained by Roginskii and Krasheninnikova[62] using the BONNOB-induced CRF method. From their Arrhenius equation, viz.,[62]

$$\log(2k^{PMCO\bullet}/(M^{-1}\ s^{-1})) = 8.12 - 7.09/\theta \quad (XIV)$$

we obtain $2k^{PMCO\bullet} = 1320$ M^{-1} s^{-1} at 37 °C and 830 M^{-1} s^{-1} at 25 °C. Thus, PMCO• is relatively[60] "well-behaved" in comparison with α-TO•. *However*, in both the CRF and the DPPH• experiments the behavior of PMCO• deteriorates upon the addition (and formation)[60] of XH_2 (R = CH_3). Indeed, for the same XH_2/phenol ratios, the kinetic behavior of α-TO• containing its "natural" impurity XH_2 or a mixture of XH_2 and added XH_2 (R = CH_3) is no different from the behavior of PMCO• + XH_2 (R = CH_3).

(4) Addition of 0.6 mol % XH_2 (R = CH_3) to a solution of α-TOH and subjecting this solution to CRF conditions doubled the time delay between the first and final α-TO• concentration plateaus, i.e., eq V now became:

$$\tau_\delta = \tau_{final} - \tau_{first} \approx 0.024[\alpha\text{-TOH}]/R_g \quad (XV)$$

Thus, to within experimental uncertainties, the 0.6 mol % XH_2 (R = $C_{16}H_{33}$) present in our usual "fresh" α-TOH solutions is the sole source of the "extra" time, τ_δ, required to achieve $[\alpha\text{-TO}^\bullet]_{ss}$ in a CRF experiment. By inference, the "natural" XH_2 impurity in α-TOH is the main source of the variation in α-TO• decay rates ($2k_{obs}$) we found in our experiments and presumably variable amounts of this impurity in different samples of α-TOH account for

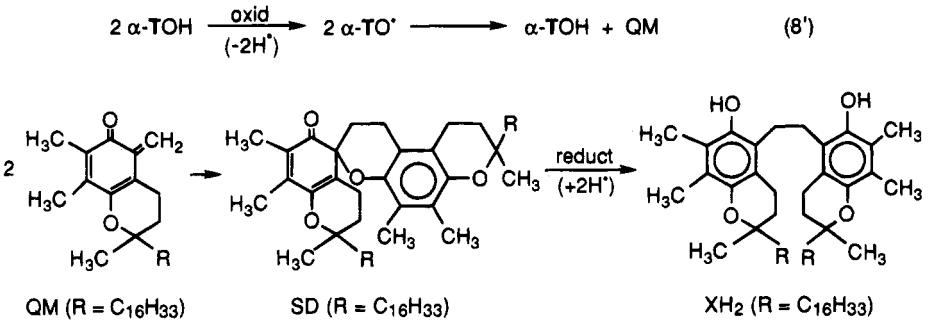
(59) Ingold, K. U.; Hughes, L; Slaby, M.; Burton, G. W. *J. Labelled Compds. Radiopharm.* **1987**, *24*, 817–831.

(60) The PMCO• concentration actually passed through a maximum at about $t = 2[PMCO^\bullet]/R_g$ (typically ~ 50 s) before falling slowly to its final plateau, $[PMCO^\bullet]_{final} \sim 0.7[PMCO^\bullet]_{max}$. The cause of this fall-off was not investigated but it probably results from the formation of small quantities of the termination accelerator, XH_2 (R = CH_3), as a reaction product since this compound has been positively identified in several studies of the products formed upon the oxidation of PMC with radicals.[15c,48b,g]

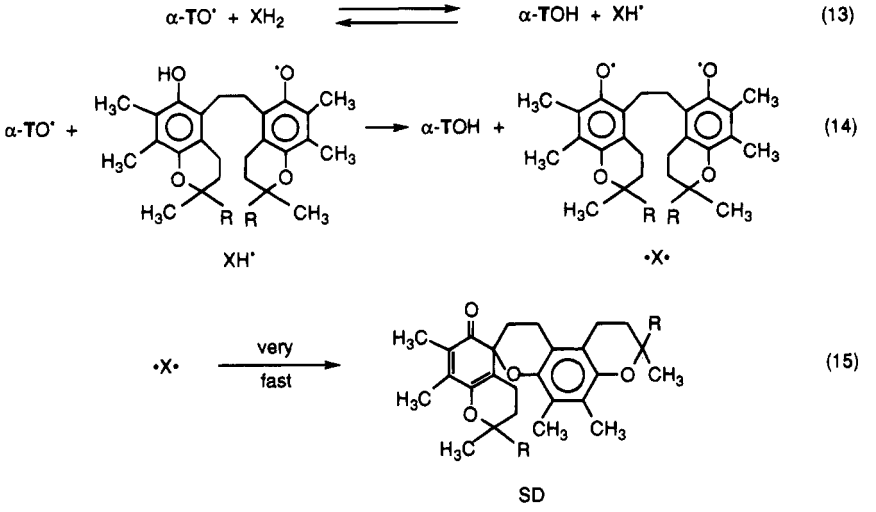
(61) Based on $[PMCO^\bullet]_{max}$.[60]

(62) Roginskii, V. A.; Krasheninnikova, G. A. *Dokl. Akad. Nauk. SSSR* **1987**, *293*, 157–162.

Scheme 5. Origin of the Mysterious Accelerator, XH_2



Scheme 6. Proposed Mechanism of Acceleration of α-TO• Decay by XH_2



much, if not all,[29] of the variation in $2k_{obs}$ found by different workers and tabulated in Table 1.

Discussion

Reported rate constants for the bimolecular self-reaction of α-TO• radicals cover a large range (see Table 1) which cannot be attributed to the choice of solvent, small changes in temperature, or (in general)[28,29] experimental error. Much of the variation in $2k_{obs}$ found by different workers can most probably be attributed to varying levels of an accelerating impurity in the α-TOH employed. Indeed, *in a single experiment* at 37 °C (see Figure 1) the values of $2k_{obs}$ can decrease from ca. 7×10^3 M^{-1} s^{-1} to ca. 1×10^3 M^{-1} s^{-1}. The substantial DKIE (3.7) for the slow bimolecular decay of α-TO• when the 5-methyl group is replaced by a trideuteromethyl group provides the first *kinetic* proof that the rate-controlling step is a disproportionation with transfer of a hydrogen from the 5-methyl group of one α-TO• radical to the other α-TO• radical so as to yield α-TOH and the quinone methide, QM (reaction 8′). The fast decay is simply due to the more rapid destruction of a pair of α-TO• radicals by the bis-phenol, XH_2, than by their self-disproportionation. The observed DKIE for the fast decay was very misleading. It is, in fact, an experimental artifact which arose because the [α-TOH]/[XH_2] ratio in our α-TOH was ca. 3 times as great as in our d_3- and d_6-α-TOH.

Acceleration of the rates of the bimolecular self-reactions of free radicals is rather uncommon[63] and generally ordinary phenols do not accelerate the decay of aryloxyl radicals. We propose that the unique feature of XH_2 is the ability of this bis-phenol to react in a stepwise manner with two α-TO• radicals to form two α-TOH molecules and a biradical, •X•. This biradical rapidly collapses to the spiro-dimer, SD, which drives the equilibrium reaction to the right (see Scheme 6). According to this scheme, oxygen should have no effect on the rate of α-TO• decay. This was also found experimentally.

The O–H bond strengths in α-TOH, XH_2, and XH• should be practically identical which simplifies the kinetic treatment. That is, since there are two OH bonds in XH_2, statistical considerations dictate that the equilibrium constant,[64] $K_{13} \approx 2$. The equilibrium concentration of XH• is therefore given by:

$$[XH^\bullet] = 2[\alpha\text{-}TO^\bullet][XH_2]/[\alpha\text{-}TOH] \qquad (XVI)$$

Furthermore, since [α-TOH] ≫ [XH_2] it is clear that [α-TO•] ≫ [XH•]. In a CRF experiment at the (pseudo) steady-state we have:

$$R_g = 2k_{obs}[\alpha\text{-}TO^\bullet]^2_{pss} = 2k_{TO}[\alpha\text{-}TO^\bullet]_{pss} + 2k_{14}[\alpha\text{-}TO^\bullet]_{pss}[XH^\bullet] \qquad (XVII)$$

That is,

$$2k_{obs} = R_g/[\alpha\text{-}TO^\bullet]^2_{pss} = 2k_{TO} + 2k_{14}[XH_2]/[\alpha\text{-}TOH] \qquad (XVIII)$$

(see also eq IX). On taking $2k_{14} = 5 \times 10^5$ M^{-1} s^{-1}, the

(63) Other examples are the $O_2^{\bullet-}$ self-reaction catalyzed by superoxide dismutase and the α-TO• self-reaction catalyzed by ubiquinol.[10,11]

(64) Since [α-TOH] ≫ [XH_2] this will be a true equilibrium.

calculated and experimental grow-in curves for [α-TO•] under CRF conditions were found to correspond.[65]

In a matched set of experiments, the slow (i.e., true) bimolecular self-reaction of α-TO• radicals in chlorobenzene yielded the following rate constants: $(2k_{obs})_{final} = 2k_{TO} = 2k_8' = 600$, 860, 1180, and 2200 M^{-1} s^{-1} at 15, 25, 37, and 55 °C, respectively. These kinetic data yield the Arrhenius equation:[71]

$$\log(2k_{TO}/(M^{-1}\ s^{-1})) = 7.4-6.1/\theta \qquad \text{(XIX)}$$

Summary

The rate-controlling step in the bimolecular self-reaction of α-TO• involves H-atom transfer from the 5-methyl group of one radical to the phenoxyl oxygen atom of the other radical. This reaction yields, therefore, free α-TOH and an *o*-quinone methide, QM. Replacement of the 5-methyl group, or the 5- and 7-methyl groups, by trideuteromethyl groups retards the reaction by an equal amount, the deuterium kinetic isotope effect being 3.7 in both cases.

The α-TO• bimolecular self-reaction exhibits some highly unusual kinetics. The observed rate constant for decay, $2k_{obs}$, can vary by about a factor of 7 in a single, but typical experiment, e.g., at 37 °C from ca. 7000 M^{-1} s^{-1} initially to ca. 1000 M^{-1} s^{-1} finally (see Figure 1). This bizarre behavior has been traced to acceleration of the α-TO• bimolecular self-reaction by a very minor (ca. 0.5 mol %) impurity which is present in commercial α-TOH. This impurity, XH_2, is derived from a spiro-dimer, SD, of QM by reduction. Hence, XH_2 is a "natural" product of α-TOH oxidation since the α-TOH can provide the reducing equivalents required to convert SD to XH_2.[72] The really novel feature about XH_2 as an accelerator is that it is a bis-phenol in which the two phenolic rings are not in conjugation. The driving force for acceleration is believed to arise from the initial conversion of XH_2 to a biradical, •X•, by two successive thermoneutral reactions with two α-TO• radicals, followed by a rapid collapse of •X• to give SD.

Implications for Lipid Peroxidation. The slowness with which α-TO• radicals undergo their bimolecular self-reaction and the fact that they do not react with oxygen[23] cause α-TOH to be a prooxidant not only for aqueous dispersions of lipids such as low density lipoprotein[9-11] but also for bulk lipids when the α-TOH is used at high concentrations.[73] The acceleration of the α-TO• bimolecular reaction which is afforded by relatively small amounts of XH_2 suggests that this compound (and structurally related bis-phenols) should greatly decrease the prooxidant activity of α-TOH in lipids. That is, XH_2 will act as a *synergistic* antioxidant with α-TOH in much the same way as does ascorbic acid (vitamin C).[74] Indeed, XH_2 reacts with α-TO• ($k_{13} \approx 2k_{14} = 5 \times 10^5$ M^{-1} s^{-1}) more rapidly in nonpolar media than would (nonionized) ascorbic acid[75] or even a ubiquinol.[76] The effectiveness of ascorbic acid as a co-antioxidant for α-TOH in lipid storage tests[74a,b] may therefore be due, in part, to reduction of SD to XH_2 by the ascorbic acid since this reducing agent is employed in the synthesis of XH_2 from α-TOH.[48a] There is an intriguing, if somewhat remote, possibility that XH_2 also plays a role *in vivo*.

Experimental Section

Materials. (2*R*,4′*R*,8′*R*)-α-Tocopherol (natural vitamin E) was a gift from the Henkel Corp. Its composition was examined by HPLC on a 150 × 4.6 mm, 3 μm particle size C-18 column eluted at 1.0 mL/min with MeOH/i-PrOH (20:1, v/v). Using 295 nm detection the following compounds (in order of elution) were identified with their percentage in the mixture being based on the percentage areas of the 295 nm absorptions: α-tocopheryl quinone (0.5%), α-TOH (96.2%), XH_2 (1.0% or 0.5 mol %), SD (0.8%), trimer[78] (0.3%), and unknowns (0.6%). The α-tocopheryl quinone, 1,2-bis(2,2,7,7-tetramethyl-6-hydroxy-chroman-5-yl)ethane (XH_2), spirodimer SD, and trimer were identified by coelution with authentic materials synthesized by nitric acid oxidation of α-TOH for the quinone[79] and by alkaline-ferricyanide oxidation of α-TOH (SD and trimer)[48a,b] and ascorbic acid reduction of SD (XH_2).[48a] 2,2,5,7,8-Pentamethylchroman-6-ol, PMC, was synthesized by a literature method[80] and was purified by recrystallization from H_2O/MeOH to a constant mp. (40 °C, lit.[80] 40–41 °C). Oxidation of PMC with alkaline-ferricyanide[48a,b] gave SD (R = CH_3) and reduction of SD (R = CH_3) with ascorbic acid[48a] gave XH_2 (R = CH_3).[48i] Analyses of the two deuterated tocopherols by the same HPLC method gave for d_3-α-TOH:α-tocopheryl quinone (0.7%), α-TOH (97.0%), XH_2 (0.3_0% or 0.1_5 mol %), SD (0.7%), trimer (0.3%), and for d_6-α-TOH: α-tocopheryl quinone (0.9%), α-TOH (96.1%), XH_2 (0.2_8% or 0.1_4 mol %), SD (0.9%), and trimer (0.8%).

(65) The substantial magnitude of k_{14} can be attributed to the combination of relatively low steric hindrance to this reaction and the well-known fact that intrinsic activation energies (i.e., *E*'s for thermoneutral reactions) for the transfer of a hydrogen atom between two oxygen atoms are much lower than for H-atom transfer between oxygen and carbon or between two carbon atoms.[18,66-70] In addition, we have recently shown that H-atom abstraction from a variety of phenols by the unsubstituted phenoxyl radical can be quite extraordinarily fast, e.g., from α-TOH, $k^{20\ °C} = 1.1 \times 10^9$ M^{-1} s^{-1} in benzene.[70]

(66) Zavitsas, A. A. *J. Am. Chem. Soc.* **1972**, *94*, 2779–2789.

(67) Kreilick, R. W.; Weissman, S. I. *J. Am. Chem. Soc.* **1966**, *88*, 2645–2652. Arick, M. R.; Weissman, S. I. *J. Am. Chem. Soc.* **1968**, *90*, 1654.

(68) Griller, D.; Ingold, K. U. *J. Am. Chem. Soc.* **1974**, *96*, 630–632 and references cited.

(69) The retarding effect of bulky ortho-substituents can be illustrated by comparison of the rate constants for the tri-*tert*-butylphenoxyl/tri-*tert*-butylphenol identity reaction, viz.,[67] ~220–330 M^{-1} s^{-1} (at room temperature) with those for the 4-methoxyphenoxyl/tri-*tert*-butylphenol reaction, viz.,[18] ~ 5×10^5 M^{-1} s^{-1} in the forward direction and 6×10^3 M^{-1} s^{-1} in the reverse direction (at 60 °C).

(70) Foti, M.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1994**, *116*, 9440–9447.

(71) For the α-TOH used in the present work, the initial fast decay of α-TO• radicals can be described by:

$$\log(2k_{initial}/(M^{-1}s^{-1})) = 6.1 - 3.4/\theta$$

(72) Heating α-TOH and SD has been demonstrated to yield XH_2 (together with other products).[48b] Our own preliminary experiments indicate that, although this is a fairly slow reaction at ambient temperatures, it can be catalyzed by acids.

(73) Loury, M.; Bloch, C.; Francois, R. *Rev. Francaise Corps Gras* **1966**, *13*, 747–754. Cillard, J.; Cillard, P.; Cormier, M.; Girre, L. *J. Am. Oil Chem. Soc.* **1980**, *57*, 252–255. Peers, K. E.; Coxon, D. T. *Chem. Phys. Lipids* **1983**, *32*, 49–56. Koskas, J. P.; Cillard, J.; Cillard, P. *J. Am. Oil Chem. Soc.* **1984**, *61*, 1466–1469. Terao, J.; Matsushita, S. *Lipids* **1986**, *21*, 255–260.

(74) (a) Golumbic, C.; Mattill, H. A. *J. Am. Chem. Soc.* **1941**, *63*, 1279–1280. (b) Golumbic, C. In *Biological Antioxidants*; Mackenzie, C. G., Ed.; Josiah Macy, Jr. Foundation: New York, 1946; pp 42–48. (c) Packer, J. E.; Slater, T. F.; Willson, R. L. *Nature* **1979**, *278*, 737–738. (d) Doba, T.; Burton, G. W.; Ingold, K. U. *Biochim. Biophys. Acta* **1985**, *835*, 298–303. (e) Burton, G. W.; Wronska, U.; Stone, L.; Foster, D. O.; Ingold, K. U. *Lipids* **1990**, *25*, 199–210 and references cited.

(75) The reduction of α-TO• to α-TOH by ascorbic acid-6-palmitate has a rate constant of 2.8×10^3 M^{-1} s^{-1} at 37 °C in benzene.[70]

(76) Reduction of α-TO• by a ubiquinol in nonpolar media has a rate constant of ca. 4×10^5 M^{-1} s^{-1} at ambient temperatures.[10,77]

(77) Mukai, K.; Kikuchi, S.; Urano, S. *Biochim. Biophys. Acta* **1990**, *1035*, 77–82.

(78) This compound is a trimer of QM.[48b]

(79) Cohen, N.; Lampresti, R. J.; Neukom, C. *J. Org. Chem.* **1981**, *46*, 2445–2450.

(80) Smith, L. I.; Ungnade, H. E.; Hoehn, H. H.; Wawzonek, S. *J. Org. Chem.* **1939**, *4*, 311–317.

Di-*tert*-butyl hyponitrite was synthesized from *tert*-butyl bromide, sodium hyponitrite, and anhydrous $ZnCl_2$ by Mendenhall's method[81] and was purified by recrystallization from H_2O/MeOH. 2,2′-Azobis(2,4-dimethylvaleronitrile), AMVN (Polyscience, CA), was also purified by recrystallization from H_2O/MeOH. Diphenylpicrylhydrazyl, DPPH• (BDH, Poole, UK), was 98% pure by HPLC (260 nm, MeOH/H_2O 5:1) and had the correct elemental analysis. Solvents were the highest grades commercially available and were used without further purification.

Instruments. Kinetic measurements were made with a Hewlett Packard 8462A-diode array spectrophotometer fitted with a Peltier heating/cooling unit. EPR spectra were recorded on a Varian E104 EPR spectrometer and on a Brucker ER 200D-SRC spectrometer fitted with a customized spectrum analysis software package.[82]

Kinetic Procedures. CRF experiments were normally performed by adding a concentrated stock solution of BONNOB or AMVN to a magnetically stirred, thermostated solution of α-TOH (or d_n-α-TOH or PMC). The absorption difference, $A_{obs} = A_{424} - A_{440}$ (for chlorobenzene, where the 424 nm absorption is due to α-TO• and 440 nm corresponds to an isosbestic point for α-TO•/DPPH•) was monitored at 0.2 s time intervals. The absolute concentration of α-TO• was calculated from the measured difference in extinction coefficients, $\epsilon_{424} - \epsilon_{440}$ (*vide infra*). Background drift and noise limited reproducibility in [α-TO•] to $\pm 0.2\ \mu M$ (1σ). To correct for any baseline drift in long-term CRF experiments (due to the formation of colored products) the final $[\alpha\text{-TO}^\bullet]_{ss}$ was also calculated using the entire spectrum.

In the direct kinetic studies of α-TO• decay the radical's concentration was monitored in the same way.

Measurement of $\epsilon_{424} - \epsilon_{440}$ for α-TO•. EPR spectroscopy served not only to show that only α-TO• could be observed throughout a CRF experiment, but also to measure (via double integration against a Tempo standard) the absolute α-TO• concentration at steady-state, $[\alpha\text{-TO}^\bullet]_{ss}$, in these experiments. Comparison with the UV absorption data obtained in an identical experiment then yielded $\epsilon_{424} - \epsilon_{440}$. For example, with 5 mM AMVN and 2 mM α-TOH in chlorobenzene at 40 °C we obtained $\epsilon_{424} - \epsilon_{440} = (5 \pm 2) \times 10^3\ M^{-1}\ cm^{-1}$.

Since this estimate of $\epsilon_{424} - \epsilon_{440}$ suffers from the intrinsic uncertainties involved in the double integration of a complex EPR spectrum it was refined using the DPPH• methodology. Addition of 12 μL of a concentrated (100 mM) stock solution of α-TOH in chlorobenzene into 600 μL thermostated, dilute (2.0, 5.0, 10, and 15 μM) chlorobenzene solutions of DPPH• gave $(A_{obs})_{max} = (A_{424} - A_{440})_{max} = 0.0088$, 0.020, 0.036, and 0.051 cm^{-1}, respectively. Because some of the α-TO• decays during the finite time it took for the α-TOH/DPPH• reaction to run to completion (i.e., during the α-TO• grow-in period) the measured values of ϵ_{obs} (i.e., $(A_{obs})_{max}/[DPPH^\bullet]_{t=0}$) were extrapolated to zero $[DPPH^\bullet]_{t=0}$ concentration. This procedure yielded $\epsilon_{obs} = \epsilon_{424} - \epsilon_{440} = 4500 \pm 400\ M^{-1}\ cm^{-1}$ at 37 °C in chlorobenzene.[83]

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(83) The availability of both steady-state and transient decay data offers a novel third method for evaluating ε. That is, by comparing the CRF expression, $2k_{obs} = R_g/[\alpha\text{-TO}^\bullet]^2$, with the second order decay equation, $-d[\alpha\text{-TO}^\bullet]/dt = 2k_{obs}[\alpha\text{-TO}^\bullet]^2$, we find $R_g = -d[\alpha\text{-TO}^\bullet]/dt = -(dA_{obs}/dt)/\epsilon_{obs}$, or $\epsilon_{obs} = \epsilon_{424} - \epsilon_{440} = -(dA_{obs}/dt)/R_g$, where $-(dA_{obs}/dt)$ is the rate of decay in A_{obs} at the point where $(A_{obs})_{transient\text{-}decay} = (A_{obs})_{steady\text{-}state}$. Thus, e.g., a mixture of α-TOH (5 mM) and BONNOB (3.8 mM) in chlorobenzene at 37 °C ($R_g = 4.3 \times 10^{-8}\ M\ s^{-1}$ afforded $(A_{obs})_{steady\text{-}state} = 0.014\ cm^{-1}$ at the first (pseudo) plateau ($[\alpha\text{-TO}^\bullet]_{pss}$) while in a matched DPPH• experiment ($[DPPH^\bullet]_{t=0} = 1.0 \times 10^{-5}$ M), the slope of the decay trace, $-(dA_{obs}/dt)$, was $(1.7_0 \bullet 0.1_5) \times 10^{-4}\ cm^{-1}\ s^{-1}$ at $A_{obs} = 0.014\ cm^{-1}$. Therefore, $\epsilon_{obs} \approx (1.7 \times 10^{-4}\ cm^{-1}\ s^{-1})/(4.3 \times 10^{-8}\ M\ s^{-1}) = 4000\ M^{-1}\ cm^{-1}$ which is in reasonable agreement with our more directly measured value of $4500\ M^{-1}\ cm^{-1}$. However, it should be noted that this method for determining ε for a free radical not only depends on the radical being relatively persistent but also on a knowledge of R_g *not* measured via the initial rate of formation of the radical (cf. Figure 3).