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Peroxyl Radical Clocks

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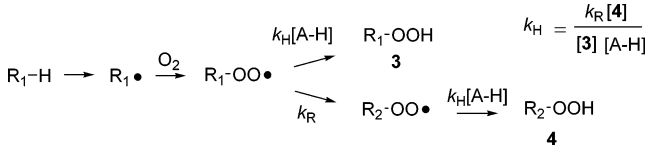


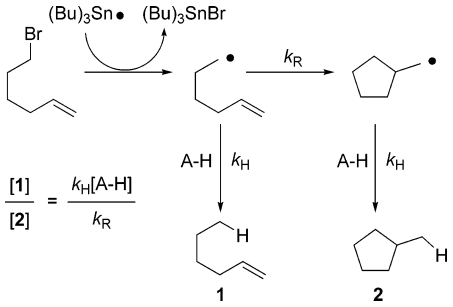
A series of peroxyl radical clocks has been developed and calibrated based on the competition between the unimolecular β-fragmentation (k_β) of a peroxyl radical and its bimolecular reaction with a hydrogen atom donor (k_H). These clocks are based on either methyl linoleate or allylbenzene and were calibrated directly with α-tocopherol or methyl linoleate, which have well-established rate constants for reaction with peroxyl radicals ($k_{H-tocopherol} = 3.5 \times 10^6$ M^{-1} s^{-1}, $k_{H-linoleate} = 62$ M^{-1} s^{-1}). This peroxyl radical clock methodology has been successfully applied to determine inhibition and propagation rate constants ranging from 10^0 to 10^7 M^{-1} s^{-1}.

Introduction

There are many methods for determining absolute rate constants of radical−molecule reactions. The rotating sector, flash photolysis, and pulse radiolysis approaches have provided platforms for kinetic studies of autoxidation, radical polymerization, and other important radical−molecule reactions.[1] Indeed, the determination of absolute rate constants has been the cornerstone for recent advances in free-radical chemistry.

Knowledge of rate constants has been critical to understanding the chemistry of oxygen-centered radicals. These species carry the chain for radical reactions carried out under atmospheric oxygen since alkyl radicals undergo diffusion-controlled reaction with molecular oxygen to yield peroxyl radicals (eq 1). Peroxyl radicals are therefore the predominant chain-carrying species in the reaction of organic compounds with molecular oxygen, and as such, the rate constants for peroxyl radical reactions have been the focus of extensive investigation. Among the most important reactions that peroxyl radicals undergo are atom-transfer reactions with H-atom donors that can either serve to propagate a chain reaction (k_p, eq 2) or break it by forming a radical that does not react with O_2, e.g., phenolic antioxidants, ArO-H (k_{inh}, eq 3).[2,3] The establishment of rate constants for these important reactions stands today as a monument to the exploration of reactivity by physical organic methods.

SCHEME 1. 5-Hexenyl Radical Cyclization Clock



$$R^\bullet + O_2 \rightarrow ROO^\bullet \quad (1)$$

$$ROO^\bullet + R\text{-}H \rightarrow ROOH + R^\bullet \quad (2)$$

$$ROO^\bullet + ArO\text{-}H \rightarrow ROOH + ArO^\bullet \quad (3)$$

Radical clocks are an indirect method for determining radical−molecule reaction kinetics by competition between a unimolecular reaction having a known rate constant and a bimolecular reaction with an unknown rate constant. For example, the 5-hexenyl radical cyclization (Scheme 1) employs a competition between a 5-*exo*-*trig* cyclization (k_R) and abstraction of a hydrogen atom from a substrate A−H (k_H). A simple

(1) (a) Burnett, G. M.; Melville, H. W. In *Techniques of Organic Chemistry*; Friess, S. L., Lewis, E. S., Weissberger, A., Eds.; Interscience: New York, 1963; Vol. VIII, Part II, Chapter 20. (b) Ingold, K. U. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, Chapter 2. (c) Walling, C. *Free Radicals in Solution*; Wiley: New York, 1957. (d) Howard, J. A. *Adv. Free Radical Chem.* **1972**, *4*, 49.

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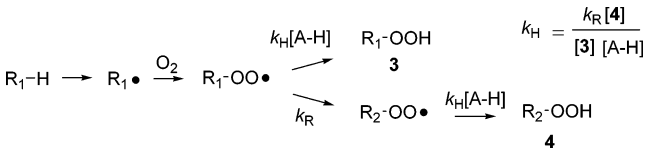
SCHEME 2. Concept of the Peroxyl Radical Clock



kinetic analysis reveals that the product ratio [**1**]/[**2**] is directly proportional to k_H/k_R. Once k_R for the unimolecular reaction has been established, an experiment to determine the product ratio for a given concentration of AH can be performed and the unknown rate constant k_H can be derived.

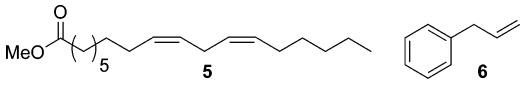
The radical clock method is ideally suited to applications involving carbon-centered radicals due to the vast literature documenting the kinetics of skeletal rearrangements of alkyl radicals. As a result, dozens of clocks have been used for determining reaction rate constants ranging from 10^{-1} to 10^{12} M^{-1} s^{-1}.[4] There is of course a requirement that radical clocks must be "standardized" to an absolute rate constant. In the case of radical cyclization, a number of methods have been used to calibrate various clocks.

Given the relative ease with which conventional radical clock experiments are conducted, it may prove useful to have radical clock methods for the determination of rate constants for reactions of peroxyl radicals. In fact, we have previously reported studies that provide the basis for the development of peroxyl radical clocks[5] based on the autoxidation of lipids such as methyl linoleate, and herein, we provide a detailed discussion of the methodology and describe extended studies of these clocks with a variety of H-atom donors.

As is true with any clock, it is desirable to cover a broad time domain to maximize possible applications. We describe the development of a series of peroxyl radical clocks based on the competition (Scheme 2) between a unimolecular peroxyl radical rearrangement (k_R) and a bimolecular H-atom transfer (k_H), where k_H collectively refers to compounds that may propagate (k_p) or inhibit (k_{inh}) radical chain reactions. By modifying the structure of the clock (R_1-H), we have been able to vary the rate constant for the rearrangement reaction (k_R), providing a range of clocks amenable to studying peroxyl radical reactions having rate constants between 10^0 and 10^7 M^{-1} s^{-1}.

Results and Discussion

Design of Peroxyl Radical Clocks. Methyl linoleate (**5**) and allylbenzene (**6**) were chosen as substrates for the development of a series of peroxyl radical clocks. The autoxidation mechanism of methyl linoleate has been studied in great detail, and it is known to involve β-fragmentations of several intermediate peroxyl radicals.[6] In addition, theoretical calculations have been carried out on peroxyl radicals derived from linoleate as well as those formed from allylbenzenes. The calculations suggest that the rate constants associated with allylbenzene-derived peroxyl radical fragmentations have values that are complimentary to the fragmentations associated with methyl linoleate.[7] Therefore, we envisioned that these two substrates would provide a range of values for peroxyl radical fragmentations useful for the development of a series of clocks.



The autoxidation of **5** is known to proceed by a series of free-radical reactions, with the product distribution highly dependent on the hydrogen atom donor (H-donor) present (Scheme 3). When autoxidations of **5** were carried out in the presence of α-tocopherol (>0.01 M) or other good H-donors, only the kinetically controlled oxidation products (**11**−**13**) were observed. Oxygen partitions itself across the three positions of the pentadienyl radical (**7**) to form the nonconjugated (**9**) and conjugated (**8** and **10**) peroxyl radicals with partitioning coefficients of α and 1-α, respectively. Since β-fragmentation of the conjugated peroxyl radicals (**8** and **10**) does not compete with trapping by α-tocopherol (α-TOH), only the *cis,trans*-conjugated hydroperoxides (**11** and **12**) are observed.[8] In contrast to the conjugated peroxyl radicals, the nonconjugated peroxyl radical (**9**) undergoes a rapid β-fragmentation ($k_{\beta I}$), reforming the original pentadienyl radical (**7**).[9] Competing with this β-fragmentation is H-atom transfer (k_H) to the peroxyl radical to form the nonconjugated hydroperoxide **13**. This competition between β-fragmentation and H-atom transfer establishes the basis for what we call the fast peroxyl radical clock (ML_{fast}) derived from **5**.

When autoxidations of **5** are carried out in the presence of H-donors that do not compete with $k_{\beta I}$, only the thermodynamic conjugated hydroperoxides (**11**, **12**, **18**, and **19**) are observed (Scheme 3). Although it is assumed that upon initial H-atom abstraction the nonconjugated peroxyl radical (**9**) is formed, $k_{\beta I}$ is the predominant pathway (relative to k_H) under these reaction conditions and there is a negligible amount of **9** present in product mixtures. Only the conjugated peroxyl radicals (**8** and **10**) and their subsequent reactions are relevant in the thermodynamically controlled autoxidation of **5**. Upon formation of **8** and **10**, one possible pathway is bond rotation followed by β-fragmentation to form the new pentadienyl radical (**14** and **15**) with the *trans,cis*-conformation. Oxygen partitions itself at the transoid or cisoid ends of **14** and **15** to form either the *trans,cis*- (**8** and **10**) or *trans,trans*-conjugated peroxyl radicals (**16**

(2) Ingold, K. U. *Acc. Chem. Res*. **1969**, *2*, 1.

(3) (a) Cipollone, M.; Di Palma, C.; Pedulli, G. F. *Appl. Magn. Reson*. **1992**, *3*, 99. (b) Pedulli, G. F.; Lucarini, M.; Pedrielli, P.; Sagrini, M.; Cipollone, M. *Res. Chem. Intermed*. **1996**, *22*, 1. (c) Lucarini, M.; Pedulli, G. F., Valgimigli, L. *J. Org. Chem*. **1998**, *63*, 4497.

(4) (a) Griller, D.; Ingold, K. U. *Acc. Chem. Res*. **1980**, *13*, 317. (b) Newcomb, M. *Tetrahedron* **1993**, *49*, 1151. (c) Newcomb, M.; Toy, P. H. *Acc. Chem. Res.* **2000**, *33*, 449.

(5) (a) Tallman, K. A.; Pratt, D. A.; Porter, N. A. *J. Am. Chem. Soc*. **2001**, *123*, 11827. (b) Porter, N. A.; Wujek, D. G. *J. Am. Chem. Soc*. **1984**, *106*, 2626.

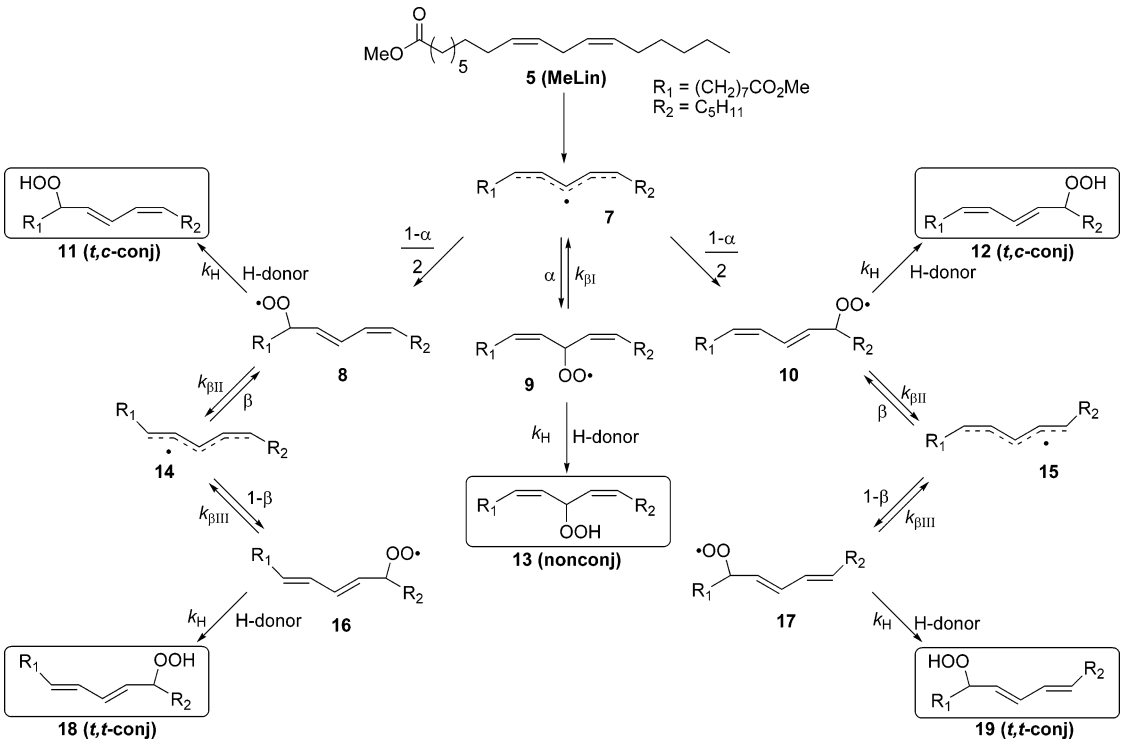
(6) (a) For the identification of the kinetic products, see: Tallman, K. A.; Roschek, B., Jr.; Porter, N. A. *J. Am. Chem. Soc*. **2004**, *126*, 9240 and ref 5a. For the thermodynamic products, see: (b) Chan, H. W.-S.; Levett, G. *Lipids* **1977**, *12*, 99. (c) Porter, N. A. *Acc. Chem. Res*. **1986**, *19*, 262. (d) Porter, N. A.; Caldwell, S. E.; Mills, K. A. *Lipids* **1995**, *30*, 277.

(7) Pratt, D. A. Ph.D. Dissertation, Vanderbilt University, Nashville, TN, December 2003.

(8) A value of 430 s^{-1} has been reported (see ref 5b), but we have reevaluated the rate constants for fragmentation of the conjugated peroxyl radicals.

(9) The mechanism for this β-fragmentation is under investigation. There may be a contribution from a bis-allylic rearrangement through a radical−dioxygen complex. Regardless of the mechanism, the kinetics remain unchanged and does not influence the concept of the clock. For more information regarding this radical−dioxygen complex, see: (a) Olivella, S.; Solé, A. *J. Am. Chem. Soc*. **2003**, *125*, 10641. (b) Mills, K. A.; Caldwell, S. E.; Dubay, G. R.; Porter, N. A. *J. Am. Chem. Soc*. **1992**, *114*, 9689. (c) Porter, N. A.; Mills, K. A.; Carter, R. L. *J. Am. Chem. Soc*. **1994**, *116*, 6690. (d) Porter, N. A.; Mills, K. A.; Caldwell, S. E.; Dubay, G. R. *J. Am. Chem. Soc.* **1994**, *116*, 6697. (e) Lowe, J. R.; Porter, N. A. *J. Am. Chem. Soc*. **1997**, *119*, 11534.

SCHEME 3. General Mechanism of Methyl Linoleate Oxidation



and **17**) with partitioning coefficients of β and 1-β, respectively. The conjugated peroxyl radicals can undergo β-fragmentation to reform the original pentadienyl radical, denoted by $k_{\beta II}$ and $k_{\beta III}$. Competing with this fragmentation is hydrogen atom transfer to the peroxyl radical to form either the *trans,cis*- (**11** and **12**) or the *trans,trans*-conjugated hydroperoxides (**18** and **19**). This competition between hydrogen atom transfer and β-fragmentation establishes our slow peroxyl radical clock (ML_{slow}).

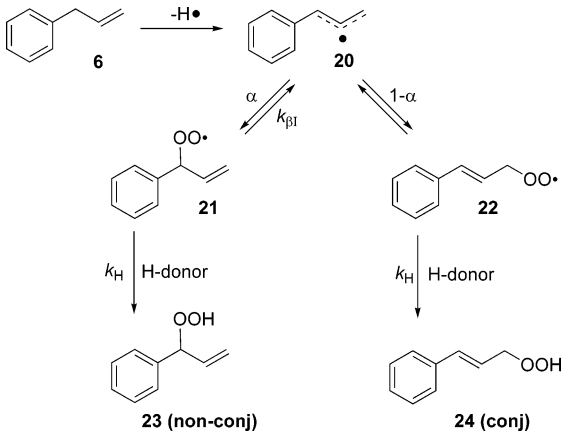
Steady-state analysis of the mechanism presented in Scheme 3 leads to equations which describe the product distribution as a function of oxygen partitioning, β-fragmentation, and [H-donor] for autoxidations carried out under kinetically or thermodynamically controlled conditions. Kinetically controlled reactions (ML_{fast}) are represented by eq 4, which describes the product ratio as a function of α, $k_{\beta I}$, k_H, and [H-donor]. Equation 5 represents the thermodynamically controlled autoxidation (ML_{slow}), which describes the product distribution as a function of β, $k_{\beta II}$, $k_{\beta III}$, k_H, and [H-donor].

$$ML_{fast}: \quad \frac{[\mathbf{11}+\mathbf{12}]}{[\mathbf{13}]} = \frac{k_{\beta I}}{k_H[\text{H-donor}]}\left(\frac{1-\alpha}{\alpha}\right) + \frac{1-\alpha}{\alpha} \quad (4)$$

$$ML_{slow}: \quad \frac{[\mathbf{11}+\mathbf{12}]}{[\mathbf{18}+\mathbf{19}]} = \frac{k_H[\text{H-donor}]}{k_{\beta II}(1-\beta)} + \frac{k_{\beta III}}{k_{\beta II}}\left(\frac{\beta}{1-\beta}\right) \quad (5)$$

The autoxidation of methyl linoleate provides rate constants for the β-fragmentation clock reaction in the 10^6 and 10^2 s^{-1} regimes, vide infra. Allylbenzene (**6**) was also chosen as a substrate since we expected that the nonconjugated peroxyl radical derived from it (**21**, below) would have a rate constant for β-fragmentation intermediate to those of the nonconjugated (**9**) and conjugated (**8** and **10**) peroxyl radicals used in the ML_{fast} and ML_{slow} clock reactions, respectively, suggesting it would be useful for clocking reactions in the intermediate time regime

SCHEME 4. General Mechanism of Allylbenzene Oxidation



(ca. 10^4 s^{-1}). This expectation was based on the 3 kcal/mol lower radical stabilization energy (RSE) of a benzyl group as compared to an allyl group and, hence, α-vinylbenzyl radical as compared to the pentadienyl (or α-vinylallyl) radical. Indeed, we carried out density functional theory calculations that suggested that the C−OO• bond in **21** is 10.5 kcal/mol, compared to 7.4 kcal/mol in **9** and 14.2 in **8** and **10**.[10] According to a published plot of experimental rate constants for β-fragmentation vs calculated C−OO• bond dissociation enthalpies,[10] we estimated that the rate constant for β-fragmentation of **21** would therefore be 9×10^4 s^{-1}.

The mechanism for the autoxidation of allylbenzene (**6**), shown in Scheme 4, is very similar to that of methyl linoleate (**5**, Scheme 3). H-atom abstraction from the benzylic position yields an α-vinylbenzyl radical (**20**), which is trapped by O_2 generating the nonconjugated and conjugated peroxyl radicals

(10) These calculations were done at the (RO)B3P86/6-311G(d,p)//(U)B3P86/6-311G(d, p) level of theory as in ref 11.

21 and **22**, respectively.[11] The fraction of α-vinylbenzyl radicals trapped at the benzylic position and leading to the nonconjugated peroxyl radical is defined as α, analogous to the partitioning of O_2 at the central position of the nonconjugated dienes. Similarly, this nonconjugated peroxyl radical (**21**) undergoes β-fragmentation ($k_{\beta I}$) in competition with H-atom transfer (k_H), setting up the intermediate peroxyl radical clock (AB). Kinetic analysis of the mechanism in Scheme 4 (eq 6) leads to the same relationship between product ratio, α, $k_{\beta I}$, k_H, and [H-donor] as defined in eq 4 for the kinetically controlled autoxidation of methyl linoleate.

$$\text{AB:} \qquad \frac{[\mathbf{24}]}{[\mathbf{23}]} = \frac{k_{\beta I}}{k_H[\text{H-donor}]}\left(\frac{1-\alpha}{\alpha}\right) + \frac{1-\alpha}{\alpha} \qquad (6)$$

Calibration of Clocks. The values for k_β and the oxygen partition coefficients (α and β) must be derived from controlled autoxidations of **5** or **6** in the presence of a H-atom donor with a well-established k_H. Thus, the ML_{fast} and AB clocks were calibrated by measuring the ratio of oxidation products as a function of α-TOH concentration ($k_H = 3.5 \times 10^6$ M^{-1} s^{-1} at 37 °C).[12] The rate constant for α-TOH is too high to reliably determine product ratios for the ML_{slow} clock. Therefore, this clock was calibrated using methyl linoleate itself (**5**, $k_H = 62$ M^{-1} s^{-1} at 37 °C) as the H-atom donor.[13]

Autoxidations of **5** (0.1−2.7 M depending on H-donor present) or **6** (3.0−4.3 M), initiated by 2,2′-azobis-(4-methoxy-2,4-dimethylvaleronitrile) (MeOAMVN), were carried out in benzene or chlorobenzene and incubated at 37 °C in the presence of varying concentrations of α-TOH. In the case of the ML_{slow} clock, α-TOH was omitted and the concentration of methyl linoleate was varied. Conditions were chosen such that a negligible amount of H-atom donor was consumed since the kinetic analysis assumes a constant concentration of H-atom donor. Higher concentrations of substrate and longer reaction times were required for **6**, as compared to autoxidations of **5**, to obtain sufficient yields of oxidation products for analysis. This is consistent with the higher C−H BDEs predicted for these compounds.[7]

The products of methyl linoleate oxidation were measured by HPLC analysis of the hydroperoxides for the ML_{fast} clock, whereas the oxidation products for ML_{slow} were reduced to the corresponding alcohols with triphenylphosphine. These analysis conditions provided optimal separation of the oxidation products. In addition to methyl linoleate, autoxidations were also carried out with *cis,cis*-6,9-pentadecadiene (**25**), which has been shown to give nearly identical results.[6a] Whereas the oxidation products from **5** were analyzed by HPLC, the products derived from **25** were reduced to the corresponding alcohols, identified by comparison to authentic samples, and analyzed by GC. The hydroperoxides formed upon autoxidation of **6** were reduced to the corresponding alcohols with triphenylphosphine and subsequently analyzed by HPLC or GC. The nonconjugated and conjugated alcohols observed under these conditions were identified by comparison to authentic samples. Utilizing these various substrates, we take advantage of complimentary methods of analysis.

In addition to measuring the product ratio derived from each oxidation, the concentration of H-donor was also monitored by HPLC. The kinetic expressions require that the reactions be carried out under pseudo-first-order conditions and hence a constant concentration of H-donor during the course of the reaction. Reaction conditions were chosen so that less than 5% of H-donor was consumed.

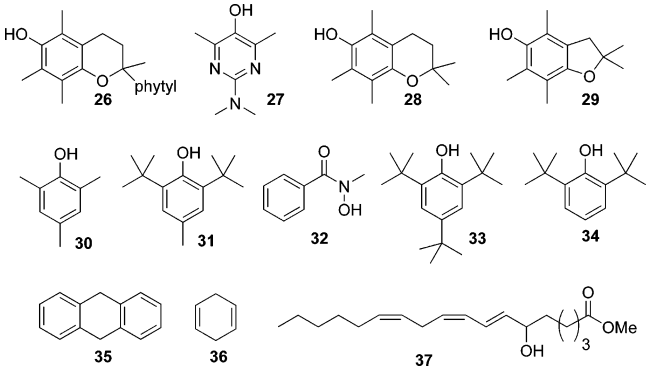
The product ratios for each clock were plotted as a function of H-donor concentration (i.e., ML_{fast}: *trans,cis*-conjugated/nonconjugated, AB: conjugated/nonconjugated, and ML_{slow}: *trans,cis*-conjugated/*trans,trans*-conjugated). When the data was fit by eqs 4−6, the values for the partitioning coefficients (α) and β-fragmentations ($k_{\beta I}$, $k_{\beta II}$, and $k_{\beta III}$) were obtained (Table 1). The values for ML_{fast} are on the order of 10^6 s^{-1}, whereas the $k_{\beta I}$ for AB is slightly less. For the ML_{slow} clock, the fragmentation rate constants are 690 and 50 s^{-1} for $k_{\beta II}$ and $k_{\beta III}$, respectively. From the data it is clear that these substrates provide a wide range of values for k_β and, hence, can be used to clock a wide variety of H-donors.

TABLE 1. Experimental Rate Constants for β-Fragmentation (k_β) and Partitioning Coefficients (α or β) for Peroxyl Radical Clocks

clock	k_β (s^{-1})	α or β
ML_{fast}[a] (**5**)	2.57 (±0.27) × 10^6	0.45 (±0.05)
(**25**)	2.94 (±0.30) × 10^6	0.46 (±0.04)
AB[a]	2.62 (±0.27) × 10^5	0.74 (±0.12)
ML_{slow}[b]	690 (±70), 50 (±5)	0.686[c]

[a] Calibrated with α-TOH. [b] Calibrated with methyl linoleate. [c] Previously determined, ref 6a. Errors reported are 95% confidence limits of the rate constant as determined by *t* tests. Error has been propagated to include experimental error, as well as the error associated with literature values for the propagation rate constant of α-TOH and methyl linoleate (±10%).

SCHEME 5. Hydrogen Atom Donors of Interest



In addition to methyl linoleate, autoxidations were also carried out with *cis,cis*-6,9-pentadecadiene (**25**), which has been shown to give nearly identical results.[6a] Whereas the oxidation products

Application of Peroxyl Radical Clocks. To demonstrate the utility of the peroxyl radical clocks, we investigated the rate of hydrogen atom abstraction from a series of compounds (Scheme 5). Many of these compounds have been studied elsewhere so their rate constants are known. This provides an opportunity to validate the radical clock method. The H-atom donors we have chosen to study vary in structure and span a wide range of rate

(11) Although most of the spin resides in the aromatic ring upon generation of the α-vinylbenzyl radical, we have drawn the radical delocalized across the allyl moiety since trapping is only observed at the allyl group. We have attempted to trap benzyl radicals at the ortho and para positions with no success. See: Pratt, D. A.; Mills, J. H.; Porter, N. A. *J. Am. Chem. Soc*. **2003**, *125*, 5801.

(12) The rate constant for α-TOH at 37 °C was obtained from a linear interpolation of the two flanking rate constants at (a) 30 °C (3.2×10^6 M^{-1} s^{-1}) (Burton, G. W.; Doba, T.; Gabe, E. J.; Hughes, L.; Lee, F. L.; Prasad, L.; Ingold, K. U. *J. Am. Chem. Soc*. **1985**, *107*, 7053) and (b) 50 °C ($4.1 (\pm 0.4) \times 10^6$ M^{-1} s^{-1}): Pratt, D. A.; DiLabio, G. A.; Brigati, G.; Pedulli, G. F.; Valgimigli, L. *J. Am. Chem. Soc*. **2001**, *123*, 4625.

(13) The rate constant for methyl linoleate has been reported as 62 M^{-1} s^{-1} at 30 °C (Howard, J. A.; Ingold, K. U. *Can. J. Chem*. **1967**, 793). A comparison of $k_p/(2k_t)^{1/2}$ values at 30 °C and 37 °C (Cosgrove, J. P.; Church, D. F.; Pryor, W. A. *Lipids* **1987**, *22*, 299) reveals comparable values. Until the absolute rate constant for methyl linoleate is measured at 37 °C, we assume the value to be approximately 62 M^{-1} s^{-1}.

TABLE 2. Rate Constants for Hydrogen Atom Donors Using Peroxyl Radical Clock Method[a]

antioxidant	clock	exptl[b] k_H (M^{-1} s^{-1})	lit.[c] k_H (M^{-1} s^{-1})
27	ML_{fast} (**5**)	9.35 (±1.50) × 10^6	8.6 × 10^6 (50 °C)[d]
	ML_{fast} (**25**)	1.53 (±0.29) × 10^7	
28	ML_{fast} (**5**)	2.68 (±0.48) × 10^6	3.8 × 10^6,[e]
	ML_{fast} (**25**)	3.90 (±0.64) × 10^6	
29	ML_{fast} (**5**)	4.56 (±0.76) × 10^6	5.7 × 10^6,[e]
	ML_{fast} (**25**)	6.15 (±0.92) × 10^6	
30	AB	2.32 (±0.51) × 10^5	8.5 × 10^4,[e]
32	AB	3.10 (±0.60) × 10^5	na
31	AB	2.37 (±2.03) × 10^4	1.4 × 10^4,[e]
31	ML_{slow}	1.88 (±0.77) × 10^4	1.4 × 10^4,[e]
33	ML_{slow}	1.71 (±0.64) × 10^4	1.6 × 10^4 (65 °C)[f]
34	ML_{slow}	3.22 (±0.32) × 10^3	3.1 × 10^3,[e]
35	ML_{slow}	384 (±46)	397[g]
36	ML_{slow}	265 (±33)	362[g]
37	ML_{slow}	367 (±42)	na
6	ML_{slow}	5.5 (±0.6)	10[h]

[a] Errors reported are 95% confidence limits of the rate constant as determined by *t* tests. Error has been propagated to include experimental error, as well as the error in k_β for each clock. Rate constants for ML_{fast} were derived from eq 4, AB from eq 6, and ML_{slow} from eq 5. [b] Values from clocks at 37 °C. [c] Literature values at 30 °C unless otherwise noted. [d] Taken from ref 12b. [e] Taken from ref 12a. [f] Taken from ref 14. [g] Taken from ref 15. [h] Taken from ref 16.

constants and this provides the opportunity to explore the general application of the radical clock method. Some of the compounds we chose to study fall into the antioxidant class given their ability to inhibit autoxidation processes (k_{inh}), whereas others are known to propagate radical chemistry (k_p). Regardless of their classification (k_{inh} or k_p), the fundamental reaction is H-atom transfer and the kinetics remain unchanged.

The peroxyl radical clock experiments were carried out in the same manner as described above for the calibration of each clock, substituting α-TOH with the compound of interest. The clock was chosen on the basis of the expected rate constants for each H-donor, using a clock with a k_β that best matched the expected k_H. If the k_H could not be estimated, then multiple clocks are used to find the best match. The faster antioxidants were clocked with MeLin (**5**) or the diene (**25**) utilizing $k_{\beta I}$ (ML_{fast}) and the product ratios measured by HPLC or GC, respectively. Allylbenzene (**6**) was used for the intermediate antioxidants and ML_{slow} was used for the compounds expected to have the lowest k_H values. The product ratio was determined as a function of the H-donor concentration and plotted in the same way as described above. Using eqs 4−6 for the appropriate clock and substituting the derived values for the partitioning coefficients and k_β from Table 1, the values for k_H were determined (Table 2).

For those compounds whose inhibition rate constants are available in the literature, those obtained by the clock method are in good agreement with previously reported values taking into account the temperature differences. While literature values have been measured in experiments that widely vary in solvent, temperature, and method, the values reported here were derived from experiments carried out in benzene or chlorobenzene at 37 °C by a single method. As a result, comparison of the trends in k_H is straightforward and reliable.

The clock approach involves a competition leading to two different products and the experimental imperative is a determination of the relative amounts of these two products formed at a known concentration of H-atom donor. As a general guide, a unimolecular clock reaction should be selected to time H-atom transfer reactions having bimolecular rate constants that are 0.05−20 times the rate of the clock reaction. In addition, recall that the clock substrates are H-atom donors themselves. Therefore, the concentration of H-atom donor of interest must be chosen so that the reaction rate of the substrate with propagating peroxyl radicals does not compete with the H-atom donor of interest.

Confidence in the analytical protocol can be provided by application of multiple clocks and/or multiple analytical methods. Although most of the antioxidants clocked with AB and diene **25** were analyzed by GC, the analysis could also be carried out with HPLC. In contrast, most analyses of methyl linoleate oxidation mixtures were by HPLC. Multiple analytical methods offer the advantage that the clocking experiments can be carried out by the protocol of choice for a particular antioxidant.

A final *caveat* concerns the fact that the clocks are themselves oxidizable compounds, a requirement of the approach. This can give rise to problems in the analysis if sufficient care is not taken to ensure the appropriate purity of the clock. Commercial methyl linoleate may have substantial amounts (1−2%) of oxidation products as received and blank analyses should always be performed to ensure that clock substrates are uncontaminated by the very products formed in the clock experiment.

Conclusion

Three peroxyl radical clocks were developed based on the competition between the unimolecular β-fragmentation of a peroxyl radical with bimolecular hydrogen atom transfer. These clocks were calibrated with two common hydrogen atom donors, α-TOH and methyl linoleate, which have well-established rate constants for reaction with peroxyl radicals. The clocks are amenable to studying a variety of hydrogen atom transfer reactions with rate constants that range from 10^0 to 10^7 M^{-1} s^{-1}.

Experimental Section

Materials. The initiator, 2,2′-azobis(4-methoxy-2, 4-dimethylvaleronitrile) (MeOAMVN), was dried under high vacuum for 2 h. Benzene and chlorobenzene were passed through a column of neutral alumina and stored over 4 Å molecular sieves. **Clocks:** Methyl linoleate (**5**) was chromatographed on silica (10% EtOAc/hexanes) prior to use. The synthesis of 6,9-pentadecadiene (**25**) was previously reported.[6a] Allylbenzene (**6**) was chromatographed on silica (hexanes) immediately before use to remove any oxidation products. **Antioxidants:** α-TOH (**26**) was purified by flash chromatography (10% EtOAc/hexanes, silica), protected from light. It is crucial that the α-TOH be purified prior to use and dried overnight under high vacuum. NMBHA (**32**)[17] and the benzofuran (**29**)[11a] were synthesized by literature procedures. The synthesis of the pyrimidinol antioxidant (**27**) and **37** is reported in the Supporting Information. All other antioxidants used in this study were commercial and purified if necessary. **Oxidation products:** The oxidation products of MeLin (**5**) and diene (**25**) have been

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characterized in a previous publication.[6a] The oxidation products of allylbenzene (**6**) were purchased.

General Procedure for MeLin and Diene Clock Calibrations (ML_{fast}). Stock solutions of MeLin or diene (1.0 M), MeOAMVN (0.1 M), and α-TOH (1.0 M) were prepared in benzene. For samples containing high concentrations, neat α-TOH (2.2 M) was used. Samples were prepared in 1.0 mL autosampler vials with a total reaction volume of 100 μL. It is important to add the solutions in the following order to avoid premature oxidation: α-TOH (0.05−1.8 M), linoleate or diene (0.10 M), MeOAMVN (0.01 M) and diluted to 100 μL with benzene. The sealed samples were then incubated at 37 °C for 4 h.

After 4 h, the oxidation was stopped by the addition of BHT (50 μL of 1.0 M solution in hexanes), followed by the addition of the internal standard (5 mM benzyl alcohol for linoleate and 5 mM tetradecane for diene). The linoleate samples were diluted to 1.0 mL with hexanes and analyzed by HPLC (0.5% *i*-PrOH/hexanes, 1 mL/min, detection at 207 nm) as their hydroperoxides. The response factors for the nonconjugated and conjugated hydroperoxides are 0.77 and 1.09, respectively, relative to benzyl alcohol. The diene samples were reduced with PPh_3 (50 μL of 1.0 M solution/hexanes) and analyzed by GC (100−180 °C at 5 °C/min, 180−280 °C at 20 °C/min, 10 min). The response factors for the nonconjugated and conjugated alcohols are 1.05 and 1.08, respectively, relative to tetradecane. The ratio of products (conjugated:nonconjugated) was plotted versus the α-TOH concentration. The values for α and k_β were derived from the *y*-intercept and slope, respectively, using eq 4.

General Procedure for Allylbenzene Clock Calibrations (AB). The oxidations were carried out in the same manner as described for the ML_{fast} clock with allylbenzene (2.26 M), MeOAMVN (0.02 M), and α-TOH (0.20−1.07 M) in benzene. Allylbenzene was also calibrated in chlorobenzene to determine if there is a solvent effect on the reaction. The samples were incubated at 37 °C for 4 h. The oxidation was stopped by the addition of excess BHT, followed by the addition of the internal standard (10 mM benzyl alcohol). The hydroperoxides were reduced to their corresponding alcohols with PPh_3, and the samples were diluted with hexanes (1.8 mL). HPLC analysis was carried out using the same conditions described for the methyl linoleate clock. The response factors for the nonconjugated and conjugated alcohols are 1.17 and 0.40, respectively, relative to benzyl alcohol. The values for α and k_β^I were derived from the *y*-intercept and slope, respectively, using eq 6.

General Procedure for Slow MeLin Calibration (ML_{slow}). The oxidations were carried out in a similar manner as the ML_{fast} calibrations using methyl linoleate (0.3−2.7 M) and MeOAMVN (0.01 M) in chlorobenzene for 1 h at 37 °C. In this case, methyl linoleate was its own H-atom donor. The oxidations were stopped by the addition of excess BHT, reduced with PPh_3, and followed by the addition of the internal standard (5 mM cinnamyl or cumyl alcohol). HPLC analysis was carried out using the same conditions described for the ML_{fast} clock. The response factors for the *trans,-cis-* and *trans,trans*-conjugated alcohols are 0.37 relative to cinnamyl alcohol and 0.27 and 0.26, respectively, relative to cumyl alcohol. The values for $k_{\beta II}$ and $k_{\beta III}$ were determined from the slope and the *y*-intercept, respectively, using eq 5.

General Procedure for H-Atom Donor Consumption Experiments. Oxidations were carried out as described above for each compound and the concentration of H-atom donor monitored over time. The oxidation was stopped by the addition of excess phenol (not the phenol being monitored), followed by the addition of the internal standard. The hydroperoxides were reduced to their corresponding alcohols with PPh_3, and the samples were diluted with acetonitrile or methanol (1.8 mL). HPLC analyses were carried out with a C-18 column using methanol and water as the mobile phase. H-atom donors **26**−**29** were analyzed using isocratic conditions (**26**, 100:0; **27**, 50:50, **28**, 70:30; and **29**, 70:30 MeOH,H_2O). H-atom donors **5**, **6**, and **30**−**37** were analyzed using a gradient (MeOH/H_2O) 90:10 for 5 min, ramp to 75:25 in 10 min, ramp to 20:80 in 15 min, ramp to 90:10 in 10 min. Consumption was monitored at a wavelength >260 nm to ensure no peak contamination from the oxidation products. When consumption of NMBHA was monitored, acetonitrile was used instead of methanol due to solubility.

General Procedure for Clocking Experiments. The samples were prepared as described above for each clock in benzene and varying the hydrogen atom donor concentration (see the Supporting Information for specific information). The samples were incubated at 37 °C for 4 h (ML_{fast} and diene), 1−24 h (AB), or 1 h (ML_{slow}). Following the oxidation, BHT (50 mM) and internal standard (5 mM benzyl alcohol for ML_{fast}, 5 mM tetradecane for diene, 5 mM benzyl alcohol for AB, and 5 mM cinnamyl alcohol for ML_{slow}) were added to each sample. MeLin samples (ML_{fast}) were diluted to 1 mL with hexanes and analyzed by HPLC (0.5% *i*-PrOH/hexanes, 1 mL/min, detection at 207 nm). Oxidation mixtures from ML_{slow} clocking experiments were reduced with PPh_3 and analyzed by the same HPLC method. Oxidation mixtures of the diene and AB were reduced with PPh_3 (50 mM) and analyzed by GC: diene (100−180 °C at 5 °C/min, 180−280 °C at 20 °C/min, 10 min), AB (75 °C, 5 min−150 °C at 5 °C/min, 150−280 °C at 25 °C/min, 2.8 min). The rate constants k_H were derived from eqs 4−6 for the respective clock.

Error Analysis. All errors reported are 95% confidence limits of the rate constant as determined by *t* tests, i.e., best fit ± (standard error)(*t**). The standard error was determined from SigmaPlot or Origin and multiplied by *t**. The value for *t** was calculated in Excel using the equation TINV (0.05, df), where df is the degrees of freedom. The degrees of freedom were calculated by subtracting the number of parameters (2) from the total number of data points. The error in the literature values for the k_H of α-TOH and methyl linoleate (±10%) was also propagated into the error.

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Supporting Information Available: Experimental procedures for the synthesis of H-atom donors; plots of calibrations, H-atom donor consumption and clocking experiments, and NMR spectra of all synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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