Chain-Breaking Naphtholic Antioxidants: Antioxidant Activities of Polyalkylbenzochromanol, Polyalkylbenzochromenol, and 2,3-Dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan Compared to an α-Tocopherol Model in Sodium Dodecyl Sulfate Micelles

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The antioxidant activities, k_{inh}, and stoichiometric factors, n, for 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]chroman (*2*), 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]-3-chromene (*3*), and 2,3-dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan (*4*) were measured in 0.50 M sodium dodecyl sulfate micelles (SDS) by the oxygen uptake method during thermally-initiated peroxidation of polyunsaturated fatty acids (PUFA): linoleic (LA), linolenic (LE), and arachidonic (AR) acids. The k_{inh} values of *2*–*4* were all higher than that of the α-tocopherol model compound 6-hydroxy-2,2,5,7,8-pentamethylchroman (*1*) under all conditions used. For example, the k_{inh} of *2* and *4* was 6.60 × 10^4 and 12.6 × 10^4 M^{-1} s^{-1}, respectively, compared to 5.37 × 10^4 for *1* during inhibited peroxidation of LA, initiated by the water-soluble initiator, azobis(amidinopropane·hydrochloride) (ABAP). The antioxidant activities, measured by the k_{inh}/k_p ratios, for *2* (1147), *3* (806), and *4* (1712) were significantly higher than that of *1* (623). Similarly, the k_{inh}/k_p ratios of *2* (792), *3* (601), and *4* (1281) during inhibited peroxidation of LE initiated by lipid-soluble di-*tert*-butyl hyponitrite (DBHN) were higher than that found for *1* (538), and the k_{inh}/k_p values of *2* (761), *3* (641), and *4* (1006), for inhibited peroxidation of AR initiated by DBHN, were higher than that of *1* (311). The higher antioxidant activities of *2*–*4* are attributed to enhanced electron delocalization and resulting increased stabilization of the phenolic radicals, compared to that from *1*, formed in the rate-determining step of the hydrogen atom transfer reaction to trap peroxyl radicals. The stoichiometric values for peroxyl radical trapping were found for *2* (1.5–1.7), *3* (1.4–1.5), and *4* (1.3–1.5) compared to $n = 2$ for the model compound *1*.

Introduction

The peroxidation of polyunsaturated fatty acids (PUFA) is of continuing interest because of the variety of pathological events implicated in free radical oxidation of PUFA in natural phospholipid membranes. Considerable activity has centered on the design and testing of synthetic chain-breaking phenolic antioxidants of the α-tocopherol class which mimic the properties of vitamin E but possess greater antioxidant activity.[1] In this regard, we discovered recently that polyalkylchromanols containing a second fused aromatic ring are significantly more active (by four to five times) as antioxidants in solution than α-tocopherol, and a naphthofuran possessed 10 times the activity of α-tocopherol and appears to be the most active antioxidant known of this class.[2] Such active antioxidants are of particular significance because the antioxidant activities of the usual α-tocopherol class are known to be remarkably reduced in aqueous model membrane systems compared to those in homogeneous solution.[3b,4a] Therefore, it is important to determine if the polyalkylbenzochromanols and hydroxynaphthofuran derivative retain their superior antioxidant activities in heterogeneous aqueous/lipid systems.

This paper reports on the antioxidant activities of these compounds in sodium dodecyl sulfate (SDS) micelles. Micelles are frequently employed as simple model environments which mimic natural biphasic systems in peroxidation reactions,[3] and they permit the use of *quantitative kinetic methods* using water-soluble as well as lipid-soluble initiators.[4] The objectives of this research are to determine the antioxidant activities of 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]chroman (*2*), the corresponding chromene (*3*), and 2,3-dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan (*4*), compared to that of the α-tocopherol model compound 6-hydroxy-2,2,5,7,8-pentamethylchroman (*1*) in SDS micelles, by using a water-soluble initiator, azobis(2-amidinopropane·HCl) (ABAP), or a lipid-soluble one, di-*tert*-butyl hyponitrite (DBHN), to control the rate of chain initiation (R_i) during peroxidation of PUFA.

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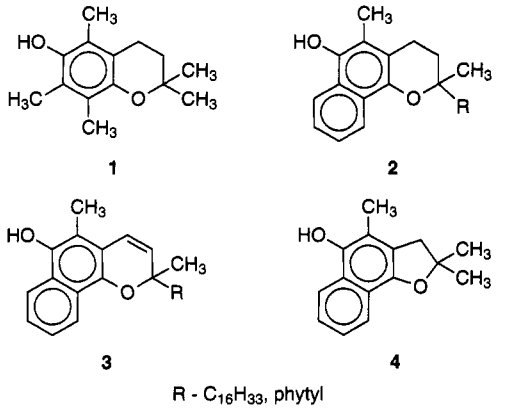

The classical equation for oxygen uptake, eq 1, is known to be applicable to PUFA solubilized in SDS micelles.[4]

$$\frac{-dO_2}{dt} = \frac{k_p}{2k_t^{1/2}}[RH]R_i^{1/2} \quad (1)$$

Here k_p is the rate constant for propagation (e.g., for hydrogen abstraction from the PUFA by peroxyl radicals) and $2k_t$ the rate constant for termination by recombination of peroxyl radicals. The oxidizability of a substrate, RH, is given by eq 2.

$$\frac{k_p}{2k_t^{1/2}} = \frac{-dO_2/dt}{[RH]R_i^{1/2}} \quad (2)$$

For quantitative kinetic studies, the rate of chain initiation, R_i, must be known and controlled, and this is done in the present study by using azo initiators with known rate constants of decomposition, k_i, so that the R_i is given by eq 3 where e is the efficiency of initiation.

$$R_i = 2ek_i[RN{=}NR] \quad (3)$$

The R_i is measured by adding an efficient phenolic inhibitor (ArOH), known to trap two peroxyl radicals (e.g., the stoichiometric factor, n, is 2), and by measuring the inhibition τ period during which oxidation is suppressed. Under these controlled conditions, eq 4 applies.

$$R_i = n[ArOH]/\tau \quad (4)$$

During inhibition by an efficient phenolic antioxidant, such as the hydroxychromans of the α-tocopherol class, the peroxyl radicals are terminated by mechanisms represented by eqs 5 and 6.

$$ROO\dot{} + ArOH \xrightarrow{k_{inh}} ROOH + Ar\dot{O} \quad (5)$$

$$ROO\dot{} + Ar\dot{O} \xrightarrow{fast} ROOArO \quad (6)$$

During inhibition, a steady state approximation is applied, eq 7, and oxygen uptake is represented by eq 8

$$R_i = k_{inh}n[ArOH][ROO\dot{}] \quad (7)$$

$$-\frac{dO_2}{dt} = \frac{k_p}{k_{inh}}\frac{[RH]R_i}{n[ArOH]} \quad (8)$$

The stoichiometric factor, n, for other antioxidants can be determined by measurement of the rate of chain initiation, R_i, using antioxidants with a known n value and application of eq 4.

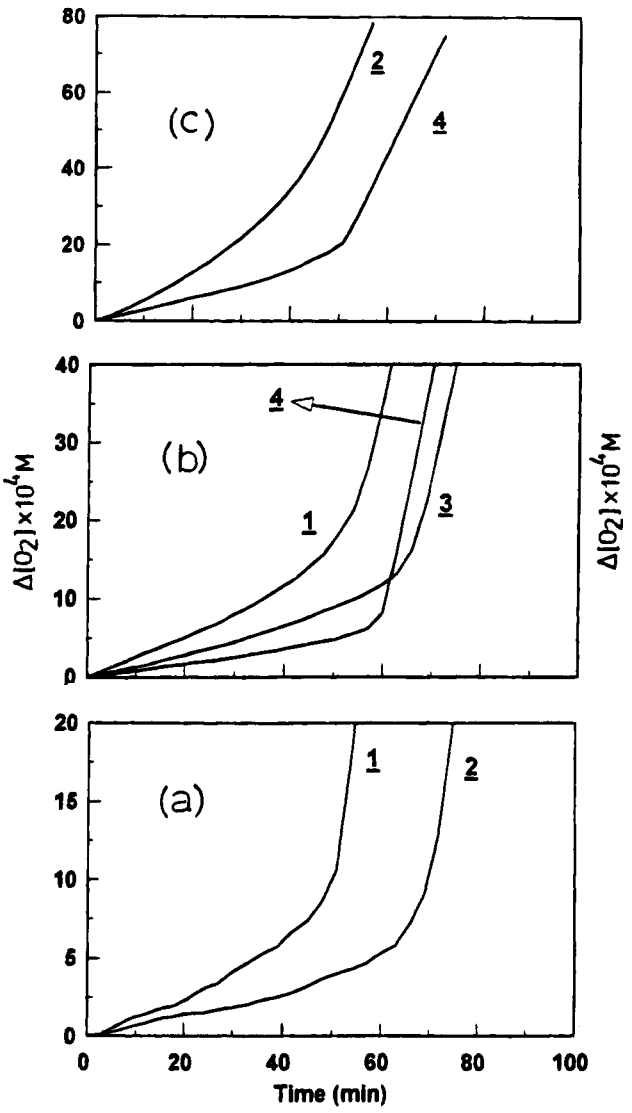

Figure 1. Traces of oxygen uptake showing effects of antioxidants on peroxidation of PUFA in 2.00 mL of 0.50 M SDS micelles at 37 °C: (a) linoleic acid, 129 μmol, initiated by ABAP, 35 μmol, inhibited by **1**, 1.85×10^{-8} mol, inhibited by **2**, 3.37×10^{-8} mol; (b) linolenic acid, 131 μmol, initiated by ABAP, 35 μmol; inhibited by **1**, 1.85×10^{-8} mol, inhibited by **3**, 3.02×10^{-8} mol, inhibited by **4**, 3.26×10^{-8} mol; (c) arachidonic acid, curve **2**, 303 μmol, curve **4**, 212 μmol, initiated by DBHN, curve **2**, 11.5 μmol, curve **4**, 9.58 μmol, inhibited by **2**, 4.49×10^{-8} mol, inhibited by **4**, 5.32×10^{-8} mol.

Results

1. Profile of Kinetic Peroxidation–Inhibition Methods. In order for quantitative kinetic studies to be applied for determination of the activities of antioxidants certain basic conditions should be met, namely (1) there must be a definite inhibition period during which peroxidation is suppressed so that this period (τ) can be measured, (2) there needs to be a chain reaction remaining during this inhibition period so that the steady state approximation and eq 8 are applicable during this period, and (3) the rate of chain initiation (R_i) must be known and controlled. Some typical profiles of inhibited oxygen uptake are illustrated in Figure 1 a–c to show how the

kinetic oxygen uptake method employing the pressure transducer system is used to meet these conditions. Figure 1a shows experimental oxygen uptake traces by compounds *1* and *2* for inhibition of peroxidation of linoleic acid initiated by water-soluble ABAP. Figure 1b gives traces for inhibition, by compounds *1*, *3*, and *4*, of peroxidation of linolenic acid initiated by ABAP. Similar results were obtained (not shown) when the peroxidation of linolenic acid was initiated by lipid-soluble DBHN. Figure 1c gives the traces for inhibition, by compounds *2* and *4*, of peroxidation of arachidonic acid initiated by DBHN. The relative ability of these compounds as antioxidants in micelles can be judged in a very qualitative manner from these experimental oxygen uptake studies to be 4 > 3 or 2 > 1. However, for a quantitative evaluation it is more significant to derive the rate constants for antioxidant activity.

2. Determinations of Antioxidant Activities (k_{inh} or k_{inh}/k_p) and Stoichiometric Factors (n). The antioxidant activities represented by the absolute rate constants for inhibition, k_{inh} (eq 5), are determined by measuring the oxygen uptake during the course of the inhibition period. The results are applied to the integrated form of the inhibition, eq 9, as used before,[2] and

$$\Delta[O_2]_t = {}^{-}k_p/k_{inh}[RH] \ln (1 - t/\tau) \qquad (9)$$

the k_{inh} is obtained from a plot of the linear equation, $\Delta[O_2]_t$ versus -ln $(1 - t/\tau)$, where the slope is equal to k_p $[RH]/k_{inh}$.

Linoleic acid is a useful substrate because the k_p has been determined in SDS micelles under these conditions[3b] and the k_{inh} are thus obtained directly. Preliminary experiments indicated that the more active antioxidants, such as the furan *4*, suppressed oxygen uptake almost completely so that oxygen uptake during inhibition was difficult to measure with sufficient precision and the resulting kinetic chain length was very low, less than 5 throughout. Therefore, it was desirable to carry out measurements with more reactive PUFA, such as linolenic acid and arachidonic acid, with additional reactive doubly allylic positions which should result in increased kinetic chain lengths during the inhibition stages.

Typical plots of oxygen uptake, $\Delta[O_2]_t$, versus -ln $(1 - t/\tau)$ (eq 9) during inhibition periods by the antioxidants *1*–*4* as shown in Figure 2a–c indicate that the data follow the linear equation as required. Also, the increase in oxygen uptake during the inhibition periods follows the increase in reactive methylene units—arachidonic acid > linolenic acid > linoleic acid as expected.

Experimental results for determinations of antioxidant activities are given in Tables 1–4. Complete sets of data are provided for the systems: inhibition, by PMHC (*1*), of peroxidation of linoleic acid initiated by water-soluble ABAP (Table 1a) and when initiated by lipid-soluble DBHN (Table 1b) to illustrate the reproducibility in k_{inh} values for different amounts of *1*, substrate, and initiator. The remaining tables provide only ranges used of these variables and standard deviations of results. For the substrates linolenic acid and arachidonic acid, antioxidant activities are reported as ratios of k_{inh}/k_p since the k_p values are not known under these conditions.

The stoichiometric factor, *n*, for antioxidants *2*–*4* was determined relative to the value of $n = 2$, known for the α-tocopherol model compound (PMHC, *1*) by determination of the rate of chain initiation, R_i, using PMHC in each experiment, and *n* was calculated using eq 4. The

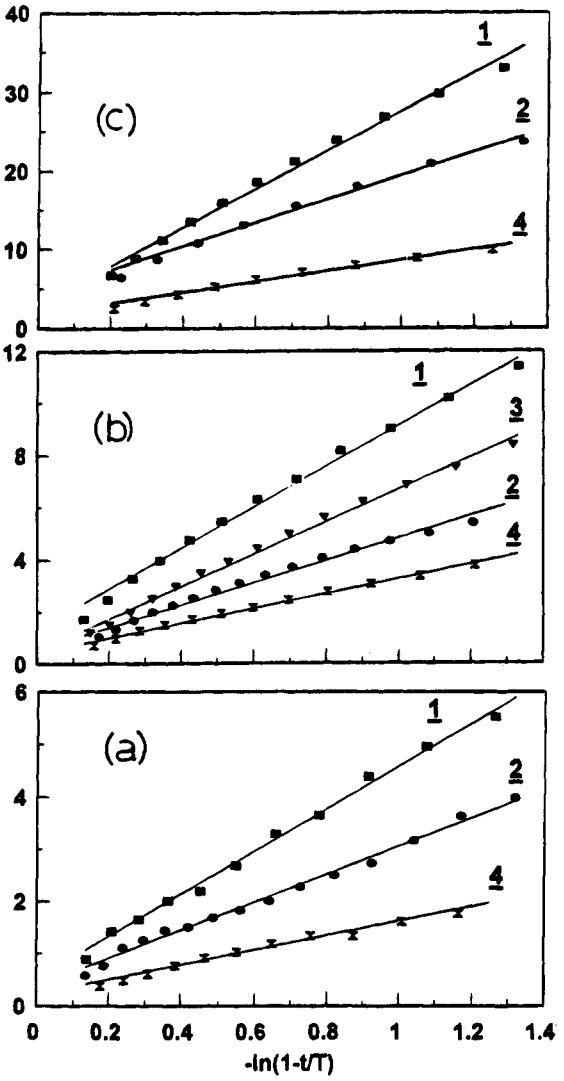

Figure 2. Plots of oxygen uptake during inhibition periods versus -ln $(1 - t/\tau)$ during peroxidation of PUFA in 0.50 M SDS at 37 °C. The k_{inh} values are obtained from the slopes, which equal k_p $[RH]/k_{inh}$. (a) linoleic acid, ABAP, **1** and **2** amounts as in Figure 1a, **4**, 2.55×10^{-8} mol; (b) linolenic acid, ABAP, **1**, **3**, and **4** amounts as in Figure 1b, **2**, 3.37×10^{-8} mol; (c) arachidonic acid, DBHN, **2** and **4** amounts as in Figure 1c, **1**, 4.94×10^{-8} mol. Key: **1**, 6-hydroxy-2,2,5,7,8-pentamethylchroman; **2**, 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]chroman; **3**, 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]-3-chromene; **4**, 2,3-dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan.

n values, reported in Tables 2–4, were found to vary somewhat with the conditions used but were in the ranges *2* ($n = 1.5$–1.7), *3* ($n = 1.4$–1.5), and *4* ($n = 1.3$–1.5).

Discussion

The accurate determination of antioxidant activities during lipid peroxidation requires a reliable method to measure the rate of peroxidation both during and after inhibition. Various reviews are available on the methods used.[5] The direct oxygen uptake method using a sensitive, calibrated pressure transducer systems has unique

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Table 1. Antioxidant Activity of Pentamethylhydroxychroman (PMHC, 1) in SDS[a] Micelles at 37 °C

	1 (mol × 10^8)	substrate[b] (μmol)	Initiator[b] (μ mol)	τ (s × 10^{-3})	R_i[c] (M s^{-1} × 10^8)	ν[d] range	k_{inh}[e] (M^{-1} s^{-1} × 10^{-4})
(a)	2.59	LA (129)	ABAP (34.9)	4.05	5.05	4–8	4.77
	1.73		34.3	2.88	4.70	6–10	5.42
	1.85		34.9	3.06	4.81	5–11	4.64
	2.47		34.9	3.75	5.21	3–6	6.64
							5.37 ± 0.79
							$k_{inh}/k_p = 14.5 \times 10^2$
(b)	4.56	129	DBHN[f] (9.00)	2.78	12.3	4–6	3.21
	8.91[g]	322	19.7	2.48	14.0	3–6	4.08
	8.91[g]	322	19.1	2.62	13.0	3–6	4.60
							3.96 ± 0.57
							$k_{inh}/k_p = 10.7 \times 10^2$
					R_i range		k_{inh}/k_p[i]
(c)	1.85–2.47	LE[h] (131)	ABAP (34–35)		4.11–5.30	6–24	623 ± 75(6)
(d)	3.56–6.17	LE (131, 263)	DBHN (3.78–12.7)		5.65–16.6	5–20	538 ± 72(8)
(e)	3.70–6.17	AR[j] (212, 303)	DBHN (9.52–11.6)		7.33–12.8	9–50	323 ± 16(3)

[a] 2.00 mL, 0.50 M sodium dodecyl sulfate in phosphate buffer, pH 7.0 used in each run unless noted otherwise. [b] LA, linoleic acid; ABAP, azobis(amidinopropane hydrochloride). [c] The rate of chain initiation, measured from the PMHC inhibition period using the relationship $R_i = 2[ArOH]/\tau$, where τ = inhibition period. [d] The kinetic chain length during the inhibition period. [e] The micellar reaction volume, as previously estimated,[4b] was used in calculations. The k_{inh} values calculated from plots of oxygen uptake during inhibition periods vs -ln $(1 - t/\tau)$, where the slope = $k_p[RH]/k_{inh}$ and k_p = 37 M^{-1} s^{-1} for linoleic acid in SDS micelles.[3b] [f] Di-*tert*-butyl hyponitrite. [g] 4.00 mL, 0.50 M SDS used in these experiments. [h] LE, linolenic acid. [i] The number in parentheses gives the number of separate experiments. [j] AR, arachidonic acid.

Table 2. Antioxidant Activity and Stoichiometric Factor (n) of 6-Hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]chroman (2) in SDS Micelles at 37 °C

	2 (mol × 10^8)	substrate (μmol)	initiator (μmol)	R_i (range) (M s^{-1} × 10^8)	ν range	n[b]	$k_{inh}/k_{p\times10^{-2}}$
(a)	3.43–4.58	LA (257)	ABAP (34.7–34.9)	5.93–6.07	5–18	1.63 ± 0.05	17.8 ± 1.4(4)
						(k_{inh} = 6.60 ± 0.50 × 10^4 M^{-1} s^{-1})	
(b)	2.24–4.49	LE (131)	ABAP (34.4–35.0)	4.46	3–12	1.52 ± 0.08	11.5 ± 1.5(4)
(c)	6.73–8.98	LE (263)	DBHN (10.2–12.2)	11.0–15.3	5–16	1.54 ± 0.04	7.92 ± 0.73(5)
(d)	4.49–5.61	AR (303)	DBHN (8.98–11.5)	9.16–12.6	10–29	1.65 ± 0.08	7.61 ± 0.70(3)

[a] For other experimental conditions and notes on calculations, see Table 1, footnotes a–i. [b] The stoichiometric factor, based on $n = 2$ for pentamethylhydroxychroman (**1**) and the R_i measured with **1** using the relationship $R_i = n[PMHC]/\tau$.

Table 3. Antioxidant Activity and Stoichiometric Factor (n) of 6-Hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]-3-chromene (3) in SDS Micelles at 37 °C[a]

	3 (mol × 10^8)	substrate (μmol)	initiator (μmol)	R_i (range) (M s^{-1} × 10^8)	ν range	n	k_{inh}/k_p
(a)	1.26–3.77	LE (131)	ABAP (34.4–34.8)	4.26	6–16	1.50 ± 0.08	806 ± 98(4)
(b)	5.47–10.9	LE (263)	DBHN (10.5–11.4)	11.5–12.9	7–21	1.47 ± 0.04	601 ± 173(3)
(c)	4.80–6.12	AR (213, 303)	DBHN (8.63–9.22)	8.70–9.63	7–25	1.42 ± 0.02	641 ± 73(3)

[a] For other experimental conditions and notes on calculations, see Table 1, footnotes a–j, and Table 2, footnote b.

advantages for these measurements. This method provides a continuous record of the inhibited and uninhibited peroxidation, the results are reproducible within the necessary error limits to measure inhibition rate constants, and the kinetic chain length is readily determined from this method. This is an important requirement because, as emphasized by others,[1a,b] for the steady state approximation to be applicable (e.g., eqs 7 and 8), it is essential that the inhibited autoxidation is still a *measurable chain reaction* whatever the method of peroxidation employed. Measurements of k_{inh} or k_{inh}/k_p of compounds *1*–*4* by the kinetic oxygen uptake method gives reproducible results within 15% error, except in the experiments employing benzochromenol (*3*) with DBHN-sensitized peroxidation of linolenic acid, where errors approaching 30% resulted. We attribute this to a noted thermal instability of the inhibitor (*3*). Solutions of *3* darkened very rapidly, and the initial UV spectrum[7] also changed.

The antioxidant activities and stoichiometric factors of *1*–*4* in SDS micelles are summarized in Table 5 for comparison with results recently found in homogeneous solution in chlorobenzene.[2] Remarkable reductions in antioxidant activities are observed in SDS micelles for all four compounds compared to values in solution. Similar reductions in antioxidant activities of hindered phenols of the chromanol class were discovered before in micelles[3b,4a] and phospholipid bilayers.[8] These effects in aqueous micelles and bilayers were attributed to hydrogen bonding by water of the lone pair of electrons on the *para* ether oxygen of chromanol inhibitors[3b,4a] (thereby lowering the so-called stereoelectronic effect needed for optimum inhibition[6]) and by hydrogen bonding by water of the phenolic hydroxyl group which inhibits the hydrogen atom transfer of the rate-determining step, (5).[3b,8] Our earlier results indicated that the latter

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Table 4. Antioxidant Activity and Stoichiometric Factor (n) of 2,3-Dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan (4) in SDS Micelles at 37 °C[a]

	4 (mol × 10^8)	substrate (μmol)	initiator (μmol)	R_i (M s^{-1} × 10^8)	v range	n	$k_{inh}/k_p \times 10^{-2}$
(a)	2.55	LA (129)	ABAP (34.8)	5.26	3–5		35.9
	4.77	LA (129)	ABAP (34.8)	5.26	3–4		32.2 (k_{inh} = 12.6 × 10^4 M^{-1} s^{-1})
(b)	3.26–4.35	LE (131)	ABAP (34.5–34.6)	5.26	2–6	1.51 ± 0.02	17.1 ± 1.2(3)
(c)	8.67–10.84	LE (263)	DBHN (11.0–11.8)	13.9–15.6	4–6	1.29 ± 0.05	12.8 ± 0.8(3)
(d)	5.32–7.97	AR (212, 303)	DBHN (9.58–10.7)	9.88–11.4	6–13	1.36 ± 0.06	10.1 ± 0.8(5)

[a] For experimental conditions and calculation methods, see Table 1, footnotes *a–j*.

Table 5. Summary of Antioxidant Activities and Stoichiometric Factors, n

	SDS micelles					solution[b]		
	k_{inh} ($M^{-1}s^{-1}$ × 10^{-4})	$k_{inh}/k_p \times 10^{-2}$				n_{range}	k_{inh} (M^{-1} s^{-1} × 10^{-6})	n
antioxidant	LA + ABAP[a]	LA + ABAP[a]	LE + ABAP	LE + DBHN	AR + DBHN			
PMHC (1)	5.37	14.5	6.23	5.38	3.23	(2.0)	3.8	2.0
benzochromanol (2)	6.60	17.8	11.5	7.92	7.61	1.5–1.7	11.4	1.5
benzochromenol (3)			8.06	6.01	6.41	1.4–1.5	10.2	1.4
naphtholfuran (4)	12.6	34.1	17.1	12.8	10.1	1.3–1.5	28.7	1.6

[a] The k_{inh} value using the combination LA + DBHN is 3.96 × 10^4 M^{-1} s^{-1} and k_{inh}/k_p = 10.7 × 10^2. [b] Values in styrene, taken from ref 2.

solvation effect is more important.[8] Recent independent evidence employing as antioxidant a polyalkyl-5-hydroxyindan system[9] (e.g., lacking the *para* ether oxygen) provides convincing evidence that the hydrogen bonding between a protic solvent and phenolic group is the more important effect on lowering antioxidant activities of phenols.

The α-naphthol derivatives *2–4* exhibit significantly higher antioxidant activities than the α-tocopherol model compound PMHC (*1*), despite the overall lowering of antioxidant activities. It is evident that the second aromatic ring in *2–4* causes enhanced electron delocalization and stabilization of the incipient phenolic radical so that enhanced antioxidant activity is observed. The effect is most pronounced with compound *4*. This latter compound was discovered to be the most active known phenolic antioxidant in solution, 10 times more active than α-tocopherol.[2] In the aqueous SDS system, compound *4* is two to three times more active than the model compound *1*, and it is clearly the most active antioxidant of this group. The ether oxygen in *1*, incorporated into a five-membered ring, imparts additional stabilization of the intermediate radical by stereoelectronic stabilization provided by the coplanar lone pair on this oxygen.[6]

The water-soluble initiator, ABAP, is a very convenient one to initiate peroxidation of PUFA in micelles, because one can initiate the reaction by adding small volumes of this initiator to the system after thermal equilibrium is obtained. Pryor *et al.* found that the combination of ABAP and linoleic acid was a convenient method to "screen" the antioxidant potencies of a wide variety of antioxidants.[3a] Our results show that experiments using the water-soluble ABAP result in somewhat higher antioxidant activities than those employing lipid-soluble DBHN. We suspect that the reason for this is that a portion of the initial peroxyl radicals from ABAP is trapped in the aqueous phase before reaction with the lipid, although we found that both ABAP and linoleic acid (and presumably linolenic and arachidonic acids) partition at least 90% into the micellar phase of SDS.[4a] We emphasize again the need for an appreciable chain length when assessing the activities of antioxidants in heterogeneous aqueous/lipid phases of micelles or membranes; especially when testing the activities of water-soluble antioxidants.[10] The k_{inh}/k_p values obtained for PMHC, using DBHN as initiator, exhibit a decrease in the order linoleic acid (10.7 × 10^2) > linolenic acid > arachidonic acid with experimental ratios 1:2:3.3 respectively, close to that one would expect if the k_p follows the ratio of number of activated methylene groups; namely, 1:2:3 in these PUFA.

The stoichiometric factors of *2–4*, relative to $n = 2$ for PMHC,[3a,6] are similar to those found in homogeneous solution (Table 5). Various factors may contribute to make the n factors of *2–4* nonintegral values, less than 2 compared to PMHC. These include possible peroxidation of the naphthalene ring of *2–4*,[11] and ArO "wasting" reactions that result from self-reaction and reaction with the substrate causing some prooxidant activity of the antioxidant.[12,13] The low stoichiometric factors reported earlier for *2* and *3*, 0.3 and 0.2, and lower antioxidant activities than α-tocopherol in SDS[3a] are somewhat surprising. We do not have an explanation for such remarkable differences between these data and our current results. They are presumably related to the different procedures used to measure peroxidation; development of conjugated diene hydroperoxide shown by UV[3a] versus the oxygen uptake methods used here.

Experimental Section

Materials. The inhibitors used were prepared by known procedures and their identities checked by ^{1}H NMR and UV spectra. Pentamethylhydroxychroman (*1*),[14] 6-hydroxy-2,5-dimethy-2-phytylbenzo[7,8]chroman (*2*),[7] 6-hydroxy-2,5-dimethyl-2-phytylbenzo[7,8]-3-chromene (*3*),[7] and 2,3-dihydro-5-hydroxy-2,2,4-trimethylnaphtho[1,2-*b*]furan were prepared

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by the literature methods. The PUFA, linoleic, linolenic, and arachidonic acids were obtained from NU CHEK PREP (purity > 99%) and stored in sealed vials at −30 °C until used. Azobis-(2-amidinopropane·hydrochloride) (ABAP) was obtained from Polysciences, Inc. Di-*tert*-butyl hyponitrite (DBHN) was prepared as described.[15] Sodium dodecyl sulfate (SDS) was electrophoresis purity from Bio-Rad. The phosphate buffer (pH 7.0) was prepared from 0.05 M each of NaH_2PO_4 and Na_2HPO_4 in deionized distilled water containing 1×10^{-4} M EDTA. The buffer was passed through a column of Bio-Rad Chelex 100 (50−100 mesh) to remove traces of heavy metal ions.

Preparations of Solutions in SDS Micelles. Known concentrations of the initiator, DBHN, in 0.50 M SDS−phosphate buffer were prepared by vortex stirring, under argon, known amounts of DBHN and the actual concentration obtained measured from the UV spectrum of a sample diluted in methanol and the concentration calculated from the corrected molar absorptivity at 227 nm.[4b] The solutions of inhibitors *1*−*4* of known concentrations in 0.50 M SDS buffer were prepared by vortex stirring, under argon, of films of the inhibitors from methanol, and the concentrations determined by measuring the UV spectra of diluted samples compared to their known molar absorptivities for *2*−*4*[2,7] and compared to that determined for *1* in methanol at 292 nm, $\epsilon = 3200$.

The Autoxidation/Inhibition Procedure. Autoxidations were carried out at 37 °C/760 Torr under oxygen in a sensitive dual-channel stainless steel apparatus that is described elsewhere.[2,16] In the present study, a calibrated cell was first charged with 2.00 mL of 0.50 M SDS buffer containing a known concentration of of DBHN. The reference cell contained the buffer. When the system reached temperature equilibrium at 37 °C, a known volume of PUFA was injected into the sample cell. For reactions initiated with ABAP, the sample containing the PUFA in 0.50 M SDS buffer was first equilibrated at 37 °Cand then the reaction initiated by injecting a known amount of initiator dissolved in buffer. As soon as the oxygen uptake reached a constant rate, known amounts of the inhibitors were injected and the oxygen uptake recorded until the rate returned to the uninhibited rate. The rate of chain reaction, R_i, was measured for each reaction run by injecting known amounts of PMHC/SDS.

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