Comparison of the Efficiencies of Several Natural and Synthetic Antioxidants in Aqueous Sodium Dodecyl Sulfate Micelle Solutions

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Abstract: This paper reports the effectiveness of 18 natural and synthetic antioxidants in a system in which linoleic acid undergoes autoxidation in sodium dodecyl sulfate (SDS) micelles. Rate constants are reported for the reaction of 14 of these antioxidants with the linoleic acid derived peroxyl radical. Because a micellar system allows rapid equilibration of antioxidants from the water phase into the oil phase, and because the standard kinetic equations for autoxidation apply, absolute rate constants can be obtained more easily than in a bilayer vesicle or membrane system in which equilibrium concentrations are attained more slowly. In this micellar system, α-tocopherol (α-T) is the most effective of the four tocopherols. However, diphenylphenylenediamine (DPPD) and ethoxyquin are even better antioxidants than is α-T, as is a benzofuran analogue of vitamin E synthesized by Burton and Ingold.[18] In this micellar system, α-T reacts with peroxyl radicals with a rate constant k_{inh} = 4×10^4 M^{-1} s^{-1}. In homogeneous solutions of chlorobenzene as solvent and with styrene as the autoxidizable substrate, this value for α-T is 320×10^4 M^{-1} s^{-1}. The other three tocopherols also have lower values of k_{inh} in the aqueous system than in chlorobenzene–styrene. In contrast 2,6-di-*tert*-butyl-*p*-cresol (BHT) has virtually the same value of k_{inh} in chlorobenzene–styrene and in linoleic acid–SDS, indicating that hydrogen bonding of water to the phenolic group or to the peroxyl radical does not retard the rate of hydrogen atom transfer in the case of BHT. However, BHT is the only phenol studied that has two bulky *tert*-butyl groups ortho to the phenolic function, and this is known to hinder solvation of the phenol. Thus hydrogen bonding to the phenolic function may contribute to the rate retardation observed in SDS micelles relative to chlorobenzene for the other phenolic antioxidants studied. A detailed examination of all of the data suggests that the lower reactivity of the tocopherols toward the peroxyl radical in this micellar system also is due in part to hydrogen bonding to the para ether oxygen atom, which reduces its ability to conjugate with the phenoxy radical.

The autoxidation of polyunsaturated fatty acids (PUFA) has been implicated in a number of diseases, including atherosclerosis[1-3] and cancer,[4-12] and in the aging process itself.[5-14] The major line of defense against PUFA autoxidation in cellular membranes is thought to be chain-breaking antioxidants, the most important of which are the tocopherols.[15,16] It therefore is important to understand the behavior of antioxidants and to have measures of the rates at which they react with peroxyl radicals to stop PUFA autoxidations.

Quantitative in vitro studies of the autoxidation of organic compounds and the inhibition of autoxidation by various synthetic and natural antioxidants, generally in homogeneous solution in organic solvents, have been performed by a number of groups, most notably Ingold and co-workers,[15-18] as well as others.[19,20] This field has been critically reviewed by a number of authors, and the phenomena are well understood.[7,21-23]

In contrast, autoxidation and antioxidant studies in aqueous solutions using either purified lipids in liposomes or biological membrane preparations have proven more difficult to quantify, and generally only semiquantitative or relative rate data are reported.[24-36] Systems containing biological membranes (such

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as red blood cells) and even synthetic liposomes are quite complex, but micellar systems are relatively simple and uniform and well-understood although heterogeneous. Therefore, micellar solutions offer a takeoff point[7] for studies of more complex membrane systems; that is, they represent a system in which antioxidants equilibrate rapidly from the aqueous phase into the model "membrane", but where some separation of aqueous and oil-like phases occurs and some of the complexities that arise in such systems might begin to be understood. For this reason, we here present a quantitative study of the effectiveness of 18 antioxidants that are often used in biological systems. Our model consists of linoleic acid solubilized in an aqueous solution of sodium dodecyl sulfate (SDS) at pH 7.4 in phosphate buffer, all treated to remove adventitious metals.

Experimental Section

Materials. Linoleic acid, 2,6-di-*tert*-butyl-*p*-cresol (BHT), the commercial mixture of 2,5-di-*tert*-butyl- and 3,5-di-*tert*-butyl-4-hydroxyanisole that is sold as BHA, 2-methyl-1,4-naphthoquinone (menadione), 4-(dimethylamino)-1,2-dihydro-1,5-dimethyl-2-phenyl-3*H*-pyrazol-3-one (4-(dimethylamino)antipyrine or aminopyrine), and β-carotene were obtained from Sigma Chemical Co. All four of the tocopherols were gifts from Eisai Chemical Co. The samples of 2,2,5,7,8-pentamethyl-6-hydroxychroman, 2,2,5,7,8-pentamethyl-6-hydroxy-3-chromene, and 2,2,4,6,7-pentamethyl-6-hydroxy-2,3-dihydrobenzofuran were gifts from Dr. K. U. Ingold. The 2,5,7,8-tetramethyl-6-hydroxylchroman-2-carboxylic acid (Trolox C) was a gift from Hoffmann-La Roche, Inc. *N,N'*-Diphenyl-*p*-phenylenediamine (DPPD) was from Eastman Kodak. 1,3-Dimethyl-2-thiourea was from Aldrich Chemical Co. 4,4'-(Isopropylidenedithio)bis(2,6-di-*tert*-butylphenol) (Probucol) was a gift from Dow Chemical Co. 1,2-Dihydro-6-ethoxy-2,2,4-trimethylquinoline (ethoxyquin) was a gift from Monsanto Chemical Co. 2-(4-Isobutylphenyl)propionic acid (ibuprofen) was a gift from Upjohn. 2,2'-Azobis(2-amidinopropane) dihydrochloride was obtained from Wako Chemical Co. Sodium dodecyl sulfate (SDS) was obtained from Bio-Rad. The 0.5 M SDS solution was prepared in 0.05 M NaH_2PO_4 and Na_2PO_4 (pH 7.4), which was passed through a Chelex 100 (200–400 mesh) column to remove trace metals. (The concentrations are given for the bulk solution, not the micelle.) Antioxidant solutions of 0.05–0.1 M were made by dissolving the antioxidants in a suitable solvent (methanol, acetone, or water). These solutions were then diluted into 0.5 M SDS solutions to give solutions that were 0.1–5.0 mM, depending upon the antioxidant used. The antioxidant solutions were prepared before each experiment and were stored under nitrogen. ABAP solutions were prepared with distilled water and were stored at −30 °C.

Kinetic Analysis. The kinetic equations for homogeneous autoxidation of linoleic acid is a free-radical process, and inhibition of this process by a chain-breaking antioxidant can be described by reactions 1–7, where

$$\text{initiator} \rightarrow 2R^{\bullet} \quad (1)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (2)$$

$$ROO^{\bullet} + LH \rightarrow ROOH + L^{\bullet} \quad (3)$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet} \quad (4)$$

$$LOO^{\bullet} + LH \rightarrow LOOH + L^{\bullet} \quad (5)$$

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet} \quad (6)$$

$$A^{\bullet} + LOO^{\bullet} \rightarrow \text{nonradical products} \quad (7)$$

LH is linoleic acid and AH is a chain-breaking antioxidant. From the steady-state kinetic analysis of this system, the rate of oxygen consumption during the inhibition period can be expressed as shown in eq 8.[7] In eq 8, k_p is the rate constant for eq 5, [LH] is the concentration

$$-d[O_2]/dt = (k_p[LH]R_i]/(nk_{inh}[AH]) \quad (8)$$

of linoleic acid (which is assumed to be unchanged in the experiment), k_{inh} is the rate constant for eq 6, [AH] is the concentration of antioxidant, n is the number of LOO• radicals trapped by each inhibitor molecule and R_i is the rate of initiation.

We have used as the initiator the water-soluble azo compound, 2,2-azobis(2-amidinopropane) dihydrochloride (ABAP), to achieve a constant rate of initiation. A water-soluble initiator was chosen to model the conditions that we believe most accurately reflect the usual situation in a biological cell subjected to oxidative stress, where superoxide generation in the aqueous milieu is the primordial radical-generating event and other water- and oil-soluble organic radicals are produced from the subsequent reactions of superoxide. ABAP decomposes to give two alkyl radicals, R•, which either recombine in the solvent cage or react with oxygen to give peroxyl radicals, as shown in eq 9–12. The production

$$RN{=}NR \rightarrow [2R^{\bullet}] + N_2 \quad (9)$$

$$[2R^{\bullet}] \rightarrow R{-}R \quad (10)$$

$$[2R^{\bullet}] \rightarrow 2R^{\bullet} \quad (11)$$

$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet} \quad (12)$$

of radicals that can initiate autoxidation is given by eq 13, where k_d is the rate constant for eq 9, the decomposition of ABAP, and e, the efficiency, is the fraction of alkyl radicals that react with oxygen to form peroxyl radicals and initiate the autoxidation of LH, eq 11 and 12.

$$R_i = 2ek_d[ABAP] \quad (13)$$

Autoxidation Procedures. Autoxidations were carried out under 760 Torr of O_2 in an automatic recording gas pressure transducer apparatus similar to those described by Howard and Ingold.[37] The apparatus consists of a calibrated Validyne DP 15-30 pressure transducer and a CD-12 transducer connected to a strip-chart recorder. The transducer and reaction vessel were both thermostated at 37.0 °C (±0.01 °C).

In a typical experiment, 10 mL of 0.5 M SDS was placed in the reaction vessel, which was then purged with O_2 for 15–20 min while the solution was rapidly stirred. Sufficient time was allowed for the solution and vessel to come to thermal equilibrium, and then a known amount of ABAP was injected into the SDS solution. Then, linoleic acid was injected, and sufficient time was allowed for the oxygen uptake to become constant. After linear oxygen consumption was established, a known amount of antioxidant, dissolved in 0.5 M SDS, was injected.

Measurement of R_i. The induction-period method was used to determine R_i due to the thermal decomposition of ABAP, by using eq 14,

$$R_i = [AH]_0/T \quad (14)$$

where $[AH]_0$ is the concentration of antioxidant at time zero and T is the lag time. α-Tocopherol was used to determine R_i since its stoichiometric factor, n, is known to be close to 2.0.[16] The induction period, T, was taken as the time from injection until linear oxygen consumption resumed. The value of T was determined graphically[16,38,39] on the plot of oxygen consumption versus time as the point of intersection of a line of the rate of oxygen uptake after the inhibitor was consumed and a line tangent to the curve with a slope equal to half of the slope of the line after the inhibitor was consumed. (See Figure 1 for an example.) The efficiency, e, for ABAP in this system was found to be 0.49, calculated with the k_d value of 3.72×10^{-7} s^{-1} that we determined in 0.5 M SDS and phosphate buffer by using the method of nitrogen evolution and our pressure transducer.

Measurement of n. Stoichiometric factors for the autoxidants were determined by using eq 14. Since α-T was used to determine R_i, the n values for the other antioxidants are relative to $n = 2$ for α-T.

Measurement of k_{inh}. The k_{inh} values were determined by a method used previously.[16,38] If eq 14 is written for a general time point, t, eq 15

$$n([AH]_0 - [AH])/R_i = t \quad (15)$$

is obtained, where [AH] is the concentration of inhibitor at time t. If the value of AH from eq 15 is substituted into eq 8 and the resulting equation is integrated, eq 16 is obtained. The amount of oxygen ab-

$$-d[O_2] = [-k_p/k_{inh}][LH] \ln (1 - t/T) \quad (16)$$

sorbed, $-d[O_2]$, must be corrected for the amount of nitrogen released in eq 9 and oxygen absorbed in eq 2 by the decomposition of ABAP. The

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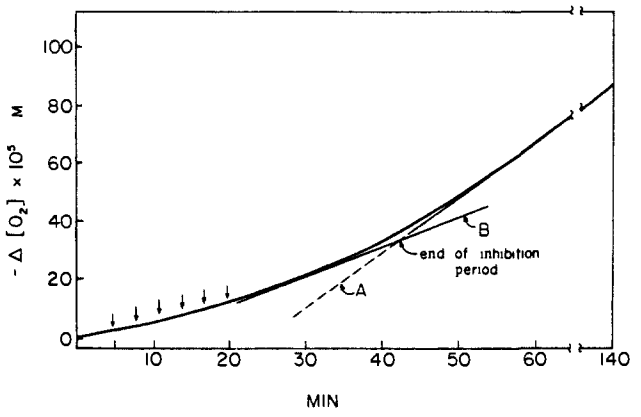


Figure 1. The inhibited oxygen curve for 2.85 μM δ-tocopherol in 0.5 M SDS, 0.5 M phosphate buffer (pH 7.4), and 46.7 mM linoleic acid, at 37 °C initiated by 4.83 mM ABAP. The inhibition period, T, is determined by the intersection of lines A and B. The arrows indicate points at which data was calculated.

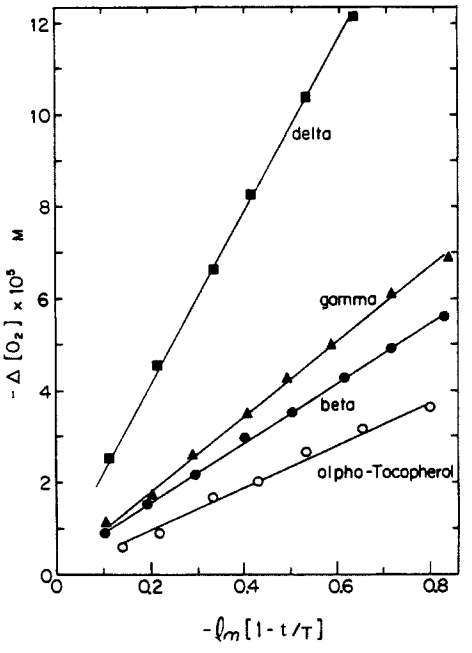


Figure 2. Plot of $-[O_2]$ against $-\ln(1 - t/T)$ for α-, β-, γ-, and δ-tocopherol (1.45, 1.22, 1.93, and 2.85 μM, respectively). The slope of these lines is equal to $k_p[LH]/k_{inh}$.

observed oxygen consumption, $-d[O_2]_{obsd}$, was corrected with the rates of reactions 2 and 9 to obtain the following expression (eq 17) for $-d[O_2]$.

$$-d[O_2] = -d[O_2]_{obsd} - k_d[ABAP]t + 2ek_d[ABAP]t \quad (17)$$

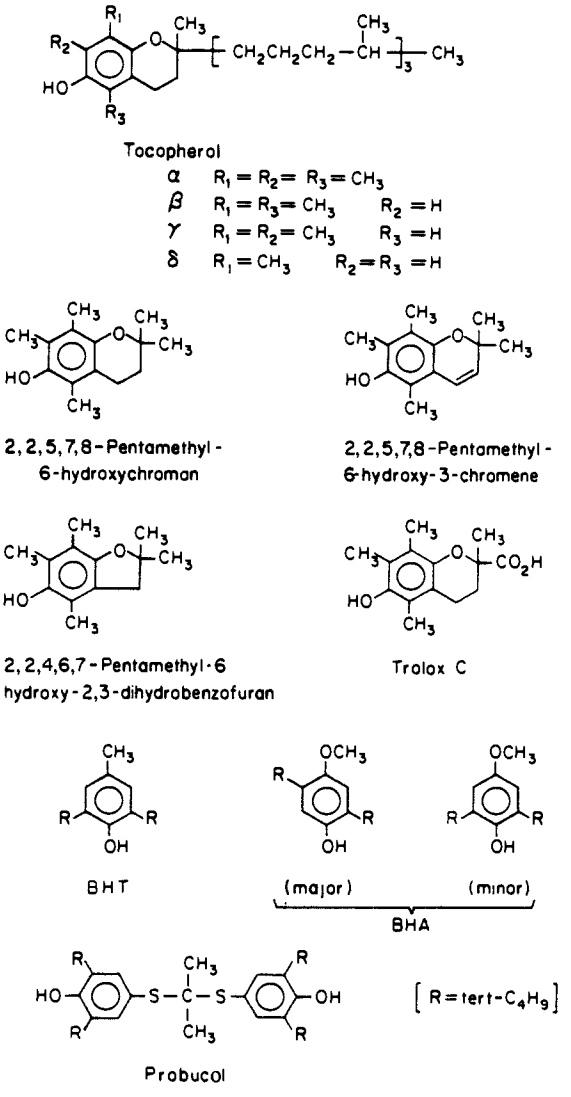
The rates of nitrogen evolution and oxygen absorption by ABAP in 0.5 M SDS solutions were measured with our pressure transducer apparatus. In eq 17, t equals a time on the inhibited oxygen curve after t_0, the time when the inhibitor is injected and assuming that $[ABAP]_0 = [ABAP]$.

Results

Figure 1 is a typical curve of the points during the inhibited oxygen absorption period for δ-tocopherol. The method used to determine the inhibition period T and the points from which data was calculated are illustrated. Similar curves for the other antioxidants were used to determine k_{inh} values.

Plots of $-d[O_2]$ versus $-\ln(1 - t/T)$ as required for eq 1 are shown in Figure 2 for all four tocopherols. The points in Figure 1 are those plotted in Figure 2 for the case of δ-tocopherol. Excellent linear correlations of the slopes (r^2 = ca. 0.990) were obtained for this plot for all the antioxidants studied. Plots of eq 16 like those shown in Figure 2 were used to calculate k_{inh} values. The linoleic acid concentration of 46.7 mM was such that no more than 10% of the oxidizable PUFA was consumed during any given experiment.

Chart I. Structures of Phenolic Compounds Used



In our experience, plots such as Figure 2 are a superior method for obtaining values of k_{inh} than is the point-by-point collection of the rates of oxygen use versus $1/[AH]$ values, although this latter method has been used by other workers.[21] The method using eq 16 allows all of the data to be collected with a single injection of antioxidant and avoids the undesirable necessity for waiting until the new rate of oxygen usage becomes steady after each new injection. For inefficient inhibitors, the autoxidizable substrate can become depleted if it is necessary to perform many injections and wait long periods for equilibration.

Table I collects the values of k_{inh} for each of the antioxidants studied, and Charts I and II give the structures of the compounds studied. Three independent measurements were made for each antioxidant over the concentration range shown in Table I, and the average of the values is reported. Equation 16 actually gives values of the ratio of k_{inh}/k_p, and these have been converted to absolute values of k_{inh} by use of the value of k_p = 37 M^{-1} s^{-1} for linoleic acid in 0.5 M SDS micellar solutions recently determined by Barclay et al.[40] Because of the difficulties inherent in measuring the absolute values of radical rate constants, the absolute values of k_{inh} are probably accurate only to about ±50%, but the relative values (that is the values of k_{inh}/k_p) are thought to be accurate to ±15%. The stoichiometric factor, n, for each antioxidant in Table I also is an average of three determinations and is relative to α-T as a standard (with n taken as 2) and is considered to be accurate within ±10%.

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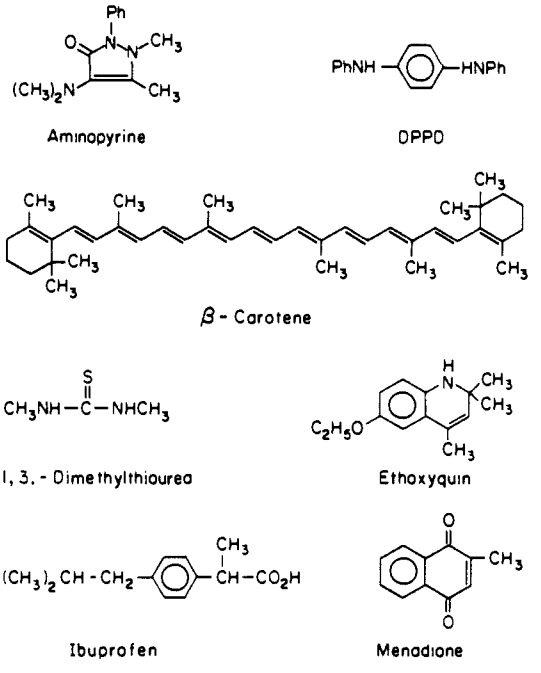
Table I. Values of k_{inh} for Antioxidants in SDS Micelles at 37 °C

antioxidant	this work: $k_{inh} \times 10^{-4}$ [a,b]	antioxidant concn × 10^6 M	stoichiometric factor,[c] n
α-tocopherol	3.7	0.97–1.95	(2.00)
β-tocopherol	2.2	1.22–3.66	2.10
γ-tocopherol	2.2	0.96–2.88	2.01
δ-tocopherol	0.89	1.89–3.82	2.17
pentamethyl-6-hydroxychromene	9	0.66–1.67	1.90
pentamethyl-6-hydroxychroman	15	0.78–1.58	2.08
pentamethyl-6-hydroxydihydrobenzofuran	18	0.85–1.67	1.81
BHT	1.1	1.95–5.81	2.21
BHA	1.0	7.17–9.56	1.86
DPPD	15	0.20–0.30	1.84
Trolox C	11	0.73–1.43	2.05
ethoxyquin	20	0.26–0.53	2.19
Probucol	0.52	8.94–17.93	7.66
1,3-dimethyl-2-thiourea	0.40	13.55–27.18	5.37
menadione	*d*	21.81	
aminopyrine	*d*	3.01–26.46	
ibuprofen	*d*	9.71–42.45	
β-carotene	*d*	12.40–51.81	

[a] Using 0.5 M SDS, 0.05 M buffer (pH = 7.4), 46.7 mM linoleic acid, and 4.85–5.24 mM ABAP. Calculated from the raw data with a value of k_p = 37 M^{-1} s^{-1} (see text for discussion). [b] Average of three measurements ± 15%. [c] Average of three measurements ± 10%, with α-tocopherol as a standard. [d] Too small to measure in our SDS system by this technique.

Chart II. Structures of Nonphenolic Compounds



Discussion

We here present data on the reactivity of a series of antioxidants with the peroxyl radical from linoleic acid in SDS micelles at 37 °C. Before discussing the data, it is worth stating several caveats and generalizations: (1) A micelle, while modeling some features of the oil–water separation that occurs in a bilayer vesicle, is considerably more permeable. Thus, micelles allow components such as antioxidants and initiators to equilibrate rapidly into the oil phase so that quantitative kinetic data can be obtained more easily;[41] this is not true in bilayers. However, our data show that rates from a micellar system do not parallel data obtained in homogeneous solution perfectly, demonstrating a sensitivity of inhibitor rate constants to the nature of the system used for their measurement. Therefore, caution must be exercised in extrapolating data from micelles to more complex (and more realistic) bilayer systems. (2) In the micellar system, variables can be expected to influence the data obtained and perhaps even the *relative* order of effectiveness of antioxidants. The types of variables that can be expected to have an effect include the nature of the initiator (including its charge type and its water or oil solubility), the nature and charge type of the buffer and the surfactant, the size and complexity of model (i.e., micelle or vesicle), and the nature of the charge type of metals and/or chelated metals that may be present.

Table II. Comparison of k_{inh} Values in Aqueous Micellular and Organic Solutions

antioxidant	linoleic acid in 0.5 M SDS,[a] × 10^{-4} M^{-1} s^{-1}	linoleic acid in 0.015 M SDS,[b] × 10^{-4} M^{-1} s^{-1}	styrene in chlorobenzene,[c] × 10^{-4} M^{-1} s^{-1}
α-tocopherol	3.7	6.0	320.0
β-tocopherol	2.2		130.0
γ-tocopherol	2.2		140.0
δ-tocopherol	0.89		44.0
pentamethyl-6-hydrochromene	9		250.0
pentamethyl-6-hydroxychroman	15		380.0
pentamethyl-6-hydroxydihydrobenzofuran	18		570.0
BHT	1.1	3.4	2.4
BHA	1.0	2.5	11
DPPD	15		117.0
Trolox C	11	1.0	110.0
ethoxyquin	20		
Probucol	0.52		
1,3-dimethyl-2-thiourea	0.40		

[a] Calculated with the value of k_p = 37 M^{-1} s^{-1} in SDS micelles at 37 °C measured by Barclay et al.[19a,19b] [b] In SDS, 0.015 M; sodium phosphate buffer, 50 mM (pH 7.0); linoleic acid, 3 mM, 40.0 °C; di-*tert*-butyl hyponitrite, 0.3 mM; the data were calculated with k_p = 100 M^{-1} s^{-1}. Measured with use of an O_2 electrode.[21] [c] Burton and Ingold et al.[16,18] in chlorobenzene–styrene solution, initiated by azobis(isobutyronitrile), 30.0 °C, k_p = 41 M^{-1} s^{-1}. Measured with use of a transducer.

Our values of k_{inh} for a number of natural and synthetic antioxidants in micellar systems are given in Table I and are compared in Table II with the limited set of values determined recently by Castle and Perkins[21] in a similar micellar system but with use of a different methodology. Our values also are compared with those determined in homogeneous solution in chlorobenzene with styrene as the oxidizable substrate by Burton and Ingold and their collaborators.[16,18] Burton and Ingold have argued that their chlorobenzene–styrene system produces a scale of k_{inh} values that is generally applicable.[42] (Note, however, that styrene has no abstractable hydrogens and forms a polymeric peroxyl radical rather than a monomeric unsaturated fatty acid peroxyl radical.[41]) Thus, the discussion below assumes that the value of k_{inh} is affected to only a small extent by replacing the linoleic acid peroxyl radical with the polystyryl peroxyl radical; i.e., that all ROO• radicals have about the same reactivity toward antioxidants. Differences observed between the set of data obtained in chlorobenzene–styrene and linoleic acid–SDS micelles are assumed to arise almost entirely from the effects of changing solvent and moving from homogeneous solution to micelles.

Our values agree quite well for three of the compounds measured by us and by Castle and Perkins; the small variations undoubtedly are due to the different methodologies used. (We used a different calculation method and a transducer, as discussed above.) The explanation for the 10-fold smaller value they get for Trolox C, however, is not clear. They used an oil-soluble

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(42) Burton, G. W.; Cheeseman, K. H.; Doba, T.; Ingold, K. U. In Biology of Vitamin E; CIBA Symposium No. 101; Pittman: London, 1983; pp 4–14, and see especially the discussion section, pp 15–16.

initiator whereas ours is water-soluble. Since Trolox C is water soluble at pH 7.4, it is possible that some of the radicals from our initiator are directly intercepted by Trolox C in our system; this would lower the effectiveness of the initiator and would be analogous to scavenging superoxide in the cytosol before it reaches the bilayer in a biological system. However, *all* of the initiator radicals are not scavenged in our system before they reach the micelle, since even for Trolox C we observe substantial kinetic chain lengths, indicating that normal autoxidation of linoleate occurs in the micelle. (See the discussion below.)

One of the most striking results from our study becomes apparent when the k_{inh} values for α-T and BHT are compared in the chlorobenzene–styrene and SDS–linoleate systems. The value for BHT is quite similar in both the organic and the aqueous system; however, k_{inh} for α-T is 100-fold smaller in the aqueous system relative to chlorobenzene. In fact, the values of k_{inh} for all of the antioxidants are much more similar in the SDS–linoleate system than was found in chlorobenzene–styrene; i.e., the antioxidant scale is greatly compressed in the micellar system. The fact that BHT has the same value of k_{inh} in both chlorobenzene and water implies that hydrogen bonding to the phenolic hydrogen of BHT does *not* reduce its rate of reaction with a peroxyl radical in a micellar system. BHT is the only phenol that was examined both in chlorobenzene and aqueous micelles that has two ortho *tert*-butyl groups, and these bulky groups are known to cause a noticeable steric hindrance to the approach of solvent molecules.[43] However, this hindrance is absent in phenols possessing a single ortho *tert*-butyl group (as in BHA) or two ortho methyl groups (as is the case for α-T).[43] Thus, rate retardation due to hydrogen bonding by water molecules to the phenolic function in BHT may not occur, but solvation of the phenolic function in BHA, α-T, and the other phenols studied might be expected. This might make the other phenols, including α-T, less reactive toward peroxyl radicals in the SDS system relative to BHT, as is observed.

The Burton–Ingold group[18] has shown that α-T owes its exceptional reactivity in aprotic solvents to the ability of the chroman-ring ether-oxygen atom to effectively stabilize the phenoxyl radical. The effect of hydrogen bonding by water molecules to the lone pairs on this ether oxygen would be to localize these electrons and allow them to stabilize the phenoxyl radical to a smaller extent. Because BHT has no such ether oxygen, the presence of water should not have an effect on its value of k_{inh}, as is found. For phenols such as the tocopherols that do contain a para ether oxygen, hydrogen bonding to the ether oxygen in the SDS system should reduce the value of k_{inh} relative to the value obtained in chlorobenzene. Furthermore, the rate retardation might be expected to parallel the degree to which the ether oxygen stabilizes the phenoxyl radical. This stabilization is substantially less for BHA than for α-T,[18a] in agreement with prediction, the value of k_{inh} is reduced by just 11-fold in going from chlorobenzene to water for BHA but is reduced 86-fold for α-T. The relative values of k_{inh} for BHT:BHA:α-T are (1):5:133 in chlorobenzene and (1):1:3 in micelles. Thus, as the ether oxygen becomes more important in stabilizing the phenoxyl radical,[18] the ratio of inhibitory rate constants increases very dramatically in chlorobenzene. However, the effect is much less in a micelle, consistent with the hypothesis that hydrogen bonding by water molecules to the ether oxygen decreases the ability of its lone pairs to delocalize and stabilize the phenoxyl odd electron, producing a "leveling effect".

The "super vitamin E" benzofuran derivative synthesized by Burton and Ingold has a value of k_{inh} that is more than 200-fold greater than that of BHT in chlorobenzene, whereas in an aqueous system it is only 16-fold greater. The superior antioxidant properties of the benzofuran derivative result from the ability of its ether oxygen to stabilize its peroxyl radical even more than in α-T, due to improved resonance overlap between the phenoxyl radical and the ether oxygen in the five-membered ring.[18] This resonance contribution of the ether oxygen is reduced in the aqueous environment, consistent with the hypothesis that localization of the ether oxygen atom's unpaired electrons by hydrogen bonding reduces conjugative stabilization of the phenoxyl radical in this case. Note, however, that the benzofuran remains a better antioxidant than α-T; the ratio of values of k_{inh} for the benzofuran to α-T is 2 in chlorobenzene and 5 in the micellar system.

The absolute values of k_{inh} are much larger both for all the tocopherols and for the "super vitamin E" benzofuran derivative in chlorobenzene relative to SDS micelles. It seems logical that the effectiveness of these antioxidants is greater in a lipophilic environment, where they were designed to operate by Nature. It is likely that the ability of water to penetrate into the neighborhood of these inhibitors and reduce their effectiveness by hydrogen bonding to them in micelles probably is greatly diminished or absent in bilayer visicles and biological membranes. Whether a diminished absolute value of k_{inh} for these antioxidants also will be found in natural bilayers must await the determination of these values, a task that will be difficult but possible.

Probucol, a drug with antihyperlipoproteinemic properties, is a diphenol linked by a sulfide bridge. We find it to have a substantial value of k_{inh} and also an unusually high value of n, suggesting that it may scavenge radicals by reaction with both phenolic groups (and perhaps by reaction at sulfur as well). 1,3-Dimethyl-2-thiourea also is found to have a substantial value of k_{inh}; it is known to be an antioxidant, but it is remarkably effective in our system.

A striking feature of our data is that Trolox C, DPPD, and ethoxyquin are all more effective antioxidants in our system than is α-T, whereas this is not true in the chlorobenzene system. For Trolox C, a water-soluble analogue of α-T, this effect may arise because of partial trapping of primordial radicals from the water-soluble initiator, as discussed above, or because hydrogen bonding does not reduce its inhibitory power as much as is true for α-T. Ethoxyquin and DPPD, both secondary amines, are partially protonated and may associate with the Stern layer of the SDS micelle. Our finding of high antioxidant properties for DPPD is consistent with the report of Hamilton and Tappel[44] who found DPPD to be more effective than α-T in an emulsion system in which qualitative rates of autoxidation of lipids were measured. Howard and Yamada[45] have shown that the transition state for the reaction of amines with peroxyl radicals is stabilized by appreciable dipolar contributions; this rate-enhancing effect may be more important in the more polar micellar environment than in chlorobenzene–styrene. Aminopyrine, a tertiary amine that has no N–H hydrogen atom (the atom that is transferred to the peroxyl radical) is inefficient in our system, as expected.

Trolox C might be rationalized as more effective in our system because it partially scavenges primordial radicals from ABAP in the aqueous solution before they reach the micelle. If Trolox C (or any of the inhibitors studied) were to capture all of the initiator-derived radicals before they reach the micelle, the kinetic chain length (KCL) would be unity; that is, just one dioxygen molecule would be used by each initiator radical that is produced from ABAP. The KCl can be calculated from the ratio of the rate of oxygen utilization to the rate of production of radicals from the initiator, $(d[O_2]/dt)/R_i$. (The corrected value for the rate of oxygen usage, given by eq 17, actually should be used, but the difference is negligible for the data presented here.) For the plot shown in Figure 1, the KCl is about 300 for δ-T; the value for α-T, a better antioxidant, is about 95. In fact, if the KCL were less than about 20–40, the method used here cannot be applied because the slope of the line in Figure 1 would be too small to allow the calculations to be performed. Trolox C may be more effective in our system than in the chlorobenzene–styrene system because it is ionized in our pH 7.4 buffer. The CO_2^- group will enhance the resonance interaction of the ether oxygen with the phenolic odd electron, whereas in the chlorobenzene–styrene system Trolox contains the un-ionized carboxylic acid function, which may cause the ether oxygen to be ineffective due to its

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electron-withdrawing inductive effect.[46]

Four compounds were investigated and found to have values of k_{inh} that were too small to be measurable in our system; these compounds are listed in Table I but not Table II. Menadione, a quinone, does not behave as an antioxidant in our system; quinones typically are good scavengers of carbon-centered radicals but not of peroxyl radicals.[47] Aminopyrine is a tertiary amine; although sometimes referred to as an antioxidant, it would not be expected to scavenge peroxyl radicals, and it has no activity in our system. Ibuprofen is a benzylacetic acid derivative and also would not be expected to be an antioxidant. We tested it since nonsteroidal antiinflammatory compounds that are inhibitors of prostaglandin synthase often are found to be antioxidants; however, ibuprofen, which is a very effective antiinflammatory drug, has no detectable antioxidant properties in our system. This implies that using antioxidant effectiveness as a screening technique for antiinflammatory drugs has limitations. β-Carotene also has an inhibitor constant that is too small to measure in our system. This result is in agreement with the results of Burton and Ingold,[48] who have shown that β-carotene is quite effective at low oxygen tensions but less so at higher oxygen tensions in their chlorobenzene-methyl linoleate system. Our linoleic acid-aqueous system, of course, is fully oxygenated, and we find that β-carotene has a very low effectiveness as an antioxidant.

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Host-Guest Complexation. 46. Cavitands as Open Molecular Vessels Form Solvates[1,2]

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Abstract: The syntheses, physical properties, and crystal structures of a series of cavitands of general structure I are described. The four methyl groups act as "feet" to support "bowls" whose depths and shapes vary with the character of the R substituents (H, CH_3, Br, and I) and whose curvatures vary with the number of methylenes in the O to O bridges (n = 1-3). These compounds all possess enforced concave surfaces of molecular dimensions (cavitands) and form solvates with simple guest molecules, most of which are complementary to their cavities, such as CH_2Cl_2, $CHCl_3$, SO_2, CH_3CN, $C_6H_5CH_3$, $(CH_2)_6$, and C_6H_6. Nine crystal structures of inclusion complexes have been determined that illustrate a variety of differently shaped bowls and different host-guest contacts. Three examples of compound I are reported in which only three of the four sets of adjacent oxygens are bridged. These compounds provide starting materials for a variety of more elaborate cavitands into which binding and catalyzing functional groups might be incorporated.

Structural recognition in complexation depends directly on stereoelectronic complementarity between surfaces common to hosts and guests in their complexes.[3] At one extreme, complexes contain partners, which are completely organized for complexation *prior* to their becoming paired (preorganized).[4] At the other extreme, complexes are derived from conformationally mobile hosts and guests. In the usual intermediate or latter cases, those molecular parts of each partner that bind one another are very likely to be rigid and relatively free of conformational degrees of freedom once complexed. *Thus most host-guest complexes are more rigid than their hosts and guests taken separately.*

This generalization particularly applies to complexation in solution in which host, guest, and complex are all solvated. Complexes present less surface for solvent contacts than hosts and guests taken separately. Therefore, complexes are less solvated than their uncomplexed partners taken in sum. Even with highly preorganized hosts and guests, rotational degrees of freedom exist for their solvating molecules. Thus desolvation accompanying complexation represents an exchange of increased rigidity of the binding partners for more degrees of freedom for those solvent molecules that are liberated.

In an attempt to eliminate some of the cancelling effects that compose structural recognition in complexation, we have increasingly studied hosts that are highly preorganized for complexation. In this investigation we sought a series of compounds that fulfill the following criteria. (1) The compounds should be cavitands containing *enforced concave surfaces of molecular dimensions*.[5,6] (2) The dimensions should be subject to design.

(1) We warmly thank the National Science Foundation for Grant CHE 81-09532, which helped support this work.
(2) This paper is dedicated to Professor Edward C. Taylor on the occasion of his 65th birthday.
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