for 1 h at room temperature, and after the addition of water (0.1 mL), the resultant mixture was stirred for 10 min. Ether (20 mL) was added to the mixture which was washed with 3% $Na_2S_2O_3$ (aqueous) (0.15 mL) and then brine, dried over $MgSO_4$, and concentrated to give 7.5 mg of a colorless oil 1, pure by ^1H NMR and TLC (R_f 0.24, 20% EtOAc–benzene). The yield was 87% based on 7. 1: IR (neat) 3400, 1710, 968 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.1–2.5 (m, 12 H), 1.19 (d, 3 H, J = 6.2 Hz), 2.34 (t, 2 H, J = 7.1 Hz), 3.2–4.4 (m, 2 H), 3.79 (sextet, 1 H, J = 6.2 Hz), 5.1–5.7 (m, 2 H).

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Registry No. 1, 58654-19-6; 2, 64418-66-2; 3, 93757-41-6; 4, 35005-46-0; (*E*)-5, 93757-42-7; (*Z*)-5, 93757-43-8; 5 ethyl ester deriv, 93757-44-9; 6, 93757-45-0; 6 ethyl ester deriv, 93757-46-1; 7, 93757-47-2; 8, 93757-48-3; *tert*-butyl 11-bromo-10-oxo-11-dodecenoate, 93781-83-0.

Communications

Autoxidation of Micelle-Solubilized Linoleic Acid. Relative Inhibitory Efficiencies of Ascorbate and Ascorbyl Palmitate

Summary: The autoxidation of linoleic acid in sodium dodecyl sulfate micelles is inhibited by water-soluble ascorbate and, to a much greater extent, by lipid-soluble ascorbyl palmitate.

Sir: The autoxidation of membrane-bound polyunsaturated fatty acids occurs by a free radical mechanism to yield lipid hydroperoxides as primary products.[1] Despite the known susceptibility of membrane lipids to oxidation, relatively few quantitative studies on the autoxidation of biological molecules in model membranes have been reported. Pioneering studies by Barclay and Ingold have demonstrated that the same rate law describes the autoxidation of egg lecithin in both homogeneous solution and as multilamellar liposomes.[2] More recent studies have described facets of the autoxidation of linoleic acid in sodium dodecyl sulfate (SDS) micelles[3,4] and of dilinoleoylphosphatidylcholine liposomes.[4] Despite the segregation of reagents provided by the micelles and liposomes, these studies have shown that water-soluble antioxidants can inhibit the autoxidation of lipophilic substrates. This result has important implications for biological systems since several naturally occurring hydrophilic antioxidants have been suggested to play a role in protecting cell membranes from lipid peroxidation in vivo.[5] For this reason, we have undertaken a study of the relative efficiencies of water-soluble ascorbate (1) and lipid-soluble ascorbyl palmitate (2) in inhibiting the autoxidation of micelle-solubilized linoleic acid. Since no significant difference in intrinsic reactivity is expected for two such structurally similar compounds, a comparison of the antioxidant efficiencies of 1 and 2 should provide a useful probe of effects due to the biphasic medium.

The system under study is similar to that described by Barclay et al.[3] The autoxidation of linoleic acid (6.90 mM) in SDS/phosphate buffer (50 mM each, pH 7.0) was initiated by using the lipophilic azo compound di-*tert*-butyl hyponitrite[6] (DBHN, 0.306 mM). As shown in Figure 1,

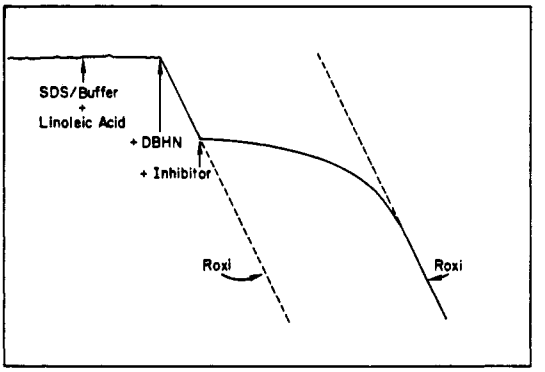

Figure 1. Typical oxygen electrode trace for the autoxidation of linoleic acid (6.90 mM) in SDS micelles at 40.0 °C. The initiator (DBHN, 0.306 mM) and inhibitor (2, 1.48 μM) were added at the points shown on the trace. Note that the rate of oxygen consumption (R_{oxi}) returns to its original value after the added inhibitor is consumed.

Table I. Data for the Inhibited Autoxidation of Linoleic Acid in SDS Micelles at 40.0 °C

ascorbate (1)		ascorbyl palmitate (2)	
1 (μM)	$-d(O_2)/dt$ $\times 10^9$ (M s^{-1})	2 (μM)	$-d(O_2)/dt$ $\times 10^9$ (M s^{-1})
0	82.4	0	77.4
46.0	12.8	1.02	8.57
69.0	9.96	1.63	6.48
115	7.80	2.45	5.50
195	6.11	3.67	4.30
333	5.18	5.71	3.78
564	4.80	7.75	3.39

the rate of oxidation is quite slow in the absence of added initiator but proceeds at a constant and rapid rate immediately upon addition of the DBHN.[7] Similarly, the addition of either lipophilic or hydrophilic antioxidants has an immediate inhibitory effect on the reaction. This reflects the fact that diffusion into the micelle is known to be a fast process,[8] so that initiators and inhibitors can be added to the bulk aqueous phase and will equilibrate into the micelle too fast for any delay to be observed in the kinetic traces.

The rate of oxidation of micelle-solubilized linoleic acid was studied as a function of added inhibitor concentration

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(2) Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6478.
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(4) Barclay, L. R. C.; Locke, S. J.; MacNeil, J. M.; VanKessel, J.; Burton, G. W.; Ingold, K. U. *J. Am. Chem. Soc.* **1984**, *106*, 2479.
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(6) Kiefer, H.; Traylor, T. G. *Tetrahedron Lett.* **1966**, 6163.
(7) Oxygen concentrations in solution were measured either with a YSI Model 53 oxygen electrode or with a Validyne pressure transducer Model DP15.
(8) Bolt, J. D.; Turro, N. J. *J. Phys. Chem.* **1981**, *85*, 4029.

0022-3263/85/1950-0281$01.50/0

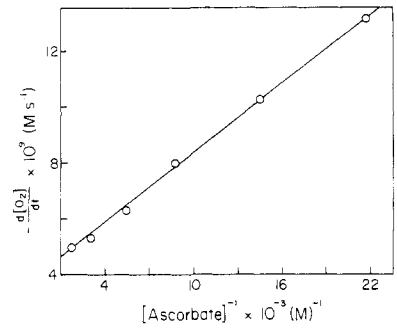

Figure 2. Plot of the rate of oxygen consumption vs. the reciprocol of the inhibitor concentration. This plot was constructed from the data for the ascorbate-inhibited reaction taken from Table I.

Table II. k_{inh} Values for Ascorbate and Ascorbyl Palmitate

ascorbate		ascorbyl palmitate	
run	$k_{inh} \times 10^{-3}$ (M^{-1} s^{-1})	run	$k_{inh} \times 10^{-5}$ (M^{-1} s^{-1})
1	3.24	1	2.08
2	2.97	2	1.99
3	3.43	3	2.23
	av 3.21 ± 0.23		av 2.10 ± 0.12

under 1 atm air. Data from representative kinetic runs are shown in Table I. These results show that both compounds **1** and **2** are capable of inhibiting the autoxidation; however, ascorbate is a much less effective antioxidant than is **2**.

Studies in homogeneous solution have shown that the mechanism of an inhibited autoxidation can be represented by eq 1–5, in which RH represents the substrate being

$$\text{initiator} \xrightarrow{R_i} \text{R}\cdot \quad (1)$$

$$\text{R}\cdot + \text{O}_2 \xrightarrow{k_o} \text{ROO}\cdot \quad (2)$$

$$\text{ROO}\cdot + \text{RH} \xrightarrow{k_p} \text{ROOH} + \text{R}\cdot \quad (3)$$

$$\text{ROO}\cdot + \text{InH} \xrightarrow{k_{inh}} \text{ROOH} + \text{In}\cdot \quad (4)$$

$$(n-1)\text{ROO}\cdot + \text{InH} \xrightarrow{k_t} \text{inert products} \quad (5)$$

oxidized and InH is the inhibitor.[9] The rate law describing this sequence is given by eq 6. Consistent with eq 6, we

$$-\text{d}(\text{O}_2)/\text{d}t = R_i + k_p(\text{RH})R_i/nk_{inh}(\text{InH}) \quad (6)$$

find that excellent linear correlations ($r^2 > 0.990$) are obtained when the rate of oxygen consumption is plotted against the reciprocol of the inhibitor concentrations. (However, see the Note Added in Proof). Figure 2 shows such a plot for data obtained from the ascorbate-inhibited reaction.

The rate constant of most interest to us is k_{inh}, which characterizes the ability of an antioxidant to trap the chain-carrying peroxyl radicals. Since the quantities [RH], R_i, and n are known[10] and the value of k_p can be estimated,[12] k_{inh} can be derived from correlations such as that shown in Figure 2. The values of k_{inh} obtained from three independent determinations for each inhibitor are recorded in Table II. A word of caution is necessary in interpreting these values. For the ascorbyl palmitate inhibited reaction, both the antioxidant and the substrate are located in the micellar pseudophase; in this circumstance, the value of k_{inh} corresponds to the rate constant for reaction 4. In contrast, the reaction of aqueous-based ascorbate with micellar peroxyl radicals probably occurs at the water–detergent interfacial region.[4] Due to the uncertainty in the reaction volume, and hence in the effective reagent concentrations, the k_{inh} value for ascorbate is not a true indication of the rate constant for reaction 4. However, a comparison of observed k_{inh} values indicates that **2** is ca. 65 times more reactive than ascorbate in this micellar system. Such a large difference in reactivity cannot be accounted for by the modest structural differences between **1** and **2** and must arise from the localization of lipophilic reagents provided by the micelle. It is interesting that the observed relative reactivities are rather close to the volume ratio of aqueous to organic phases, which is approximately 80:1 in our system.[13] This implies that the ascorbate ion has access to a significant portion of the micelle.[14]

These results demonstrate that autoxidant data obtained in homogeneous organic solvents may not parallel data from biphasic aqueous media. In homogeneous solution, ascorbate and ascorbyl palmitate should have roughly comparable effectiveness as antioxidants; however, in our system, where the segregation of the organic components influences reaction rates, this is not the case. One might ask: What are the relative k_{inh} values for these two antioxidants in a bilayer vesicle, where diffusion rates are much slower than in micelles? Because diffusion is very much slower in a vesicle, this question is not easily answered;[15] the utility of our micellar system is that it is a useful "halfway house", combining the biphasic environment of a biological system with the rapid diffusive mixing that is characteristic of homogeneous solutions.[16] Our data clearly point out, however, that antioxidant rate constants cannot be extrapolated from homogeneous solutions to biological systems without caution.

Note Added in Proof: Preliminary results from our laboratories indicate that our k_{inh} values may show a dependence on oxygen pressure, with different values being obtained under 1 atm air or oxygen. This is unexpected and is in contrast to the usual situation in homogeneous solution. One possible rationalization of this is that termination reactions involving L· radicals may be competitive with those involving LOO· radicals in this micellar system. If this explanation were the correct one, then the rate constants we obtain could not be interpreted as those for eq 4; rather, the apparent rate constants we observe would be a composite of rate constants for several steps. Our present results indicate that the ratio of k_{inh} values for ascorbate and ascorbyl palmitate are much less sensitive to oxygen pressure than are the individual values of k_{inh}, leaving our conclusions unchanged.

(9) (a) Mahoney, L. R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 547. (b) Howard, J. A. In "Advances in Free Radical Chemistry"; Williams, G. H., Ed.; Academic Press: New York, 1972; Vol. 4, p 49.

(10) Unexpectedly, we have determined that the stoichiometric factor n (the number of oxidative chains stopped per inhibitor molecule) is only 1.5 for ascorbyl palmitate, compared to 2 found for several other antioxidants.[11] This nonintegral value may indicate that a more complex termination than that indicated by reaction 5 is operative. The value n = 1.5 has also been used in calculating k_{inh} for ascorbate.

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(12) A value of k_p of 120 M^{-1} s^{-1} was estimated based on studies of the autoxidation of methyl linoleate in homogeneous solution: Howard, J. A.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 785.

(13) The volume of the organic phase was calculated by using a molar density for SDS of 0.25 L mol^{-1}: Corkill, J. M.; Goodman, J. F.; Walker, T. *Trans. Faraday Soc.* **1967**, *63*, 768.

(14) A similar explanation has been proposed to account for the reactivities of micellar olefins toward permanganate ion: Menger, F. M.; Doll, D. W. *J. Am. Chem. Soc.* **1984**, *106*, 1109.

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Acknowledgment. This work was supported in part by a contract from the National Foundation for Cancer Research and NIH Grant HL-16029.

Registry No. 1, 50-81-7; 2, 137-66-6; SDS, 151-21-3; DBHN, 14976-54-6; linoleic acid, 60-33-3.

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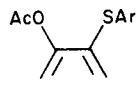
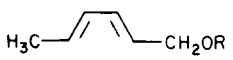
Regioselective Diels–Alder Reactions. A Synthesis of the Left-Hand Portion of CC-1065

Summary: Diene **2b** reacted regioselectively with dienophiles. Adduct **6** was converted into the left-hand portion of CC-1065 in 10% overall yield.

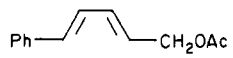
Sir: In the Diels–Alder reaction, the effect of a functional group attached directly to the diene unit has been extensively studied. The regiochemical control conferred by certain substituents coupled with the stereochemical control inherent in a concerted cycloaddition have made the Diels–Alder reaction a powerful synthetic tool.[1] The observation that the regiochemical outcome with dienes such as **1** can be reversed by Lewis acid catalysis has increased the versatility of this reaction.[2] However, there are still many instances (e.g., with unsymmetrical 1,4-dialkyl dienes) wherein regiocontrol cannot be achieved. In the process of devising solutions to this longstanding problem, we studied the effects exerted by substituents *not directly attached* to the diene unit, an idea which has only once been tested.[3] We report herein that dienes **2b** and **3** exhibit synthetically useful regioselectivity with a variety of dienes.



1

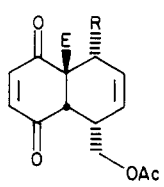


2a, R = CH_3
2b, R = $COCH_3$
2c, R = $COC(CH_3)_3$
2d, R = $COCF_3$
2e, R = $COCH_2Cl$

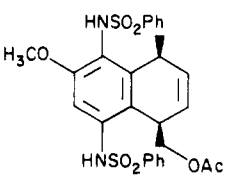


3

While diene **2a** reacted with carbomethoxybenzoquinone[4] in benzene at 25 °C to produce a 60:40 ratio of isomers, **2b** afforded a 20:1 ratio of isomers. The major isomer was assigned structure **4** on the basis of NMR decoupling experiments at 300 MHz. In particular, the methine proton adjacent to the methyl group was coupled only to the vinyl proton and the methyl group, while the proton adjacent to the acetoxymethyl group was coupled to four different protons. Additionally, diene **3** reacted with carbomethoxybenzoquinone to produce a 1.5:1 ratio wherein **5** predominated. In experiments designed to better understand the rationale for the selectivity observed with **2b**, pivalate ester **2c** was treated with carbomethoxybenzoquinone to afford approximately equal amounts of two isomers. Additionally, dienes **2d** and **2e** afforded mostly products derived from the decomposition of the diene when treated with either ethyl propiolate or carbomethoxybenzoquinone. Decomposition also resulted when sorbyl chloride was used.

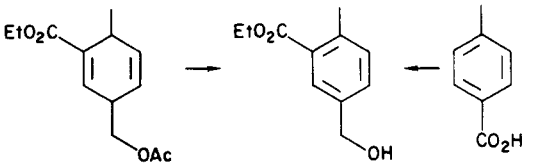


4, R = CH_3
E = CO_2Me
5, R = Ph



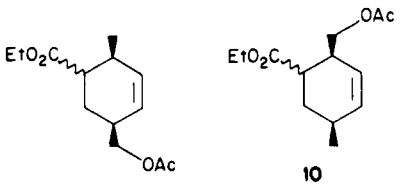
6

Diene **2b** reacted with methoxybenzoquinone bis(benzenesulfonimide)[5] at 25 °C to give, after aromatization with potassium acetate–acetic acid, adduct **6** in 55% yield[6] with a selectivity of greater than 25:1. The structure of **6** was determined by X-ray crystallography. Interestingly, **2b** reacted with ethyl propiolate to produce only one isomer. Its structure was shown to be **7** on the basis of conversion



7 **8**

to **8** using DDQ[7] followed by acetate hydrolysis. Ester **8** was independently synthesized from *p*-toluic acid in three steps.[8] The reaction of **2b** with ethyl acrylate (135 °C) afforded exo/endo mixtures of both **9** and **10**. Ultimately,



9 **10**

the regioselectivity was determined by conversion to **8**.[9] Diesters **9** were favored over **10** by a ratio of 9:1. The origin of the selectivity may arise from secondary orbital overlap involving the acetate carbonyl carbon and the orbital at C-3 of diene **2b**.[10] This analysis would explain the lack of regioselectivity with diene **2c**. Alternatively, if the Diels–Alder transition state involves concerted but not completely synchronous bond formation,[11] the acetoxymethyl group would be expected to destabilize the transition state leading to **10**, while the methyl group would be expected to stabilize the transition state leading to **9**.[12]

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(7) Carretto, J.; Sib, S.; Simalty, M. *Bull. Soc. Chim. Fr.* **1972**, 2312.
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(9) The steps involved acetate hydrolysis (HCl, EtOH) and a three-step aromatization scheme (2.2 equiv of LDA, Br_2; DBN, 50 °C; DDQ, benzene).
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(12) This would be true whether ionic or diradical intermediates were postulated.

0022-3263/85/1950-0283$01.50/0