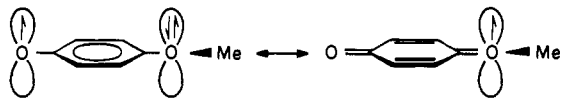
Table I. Relevant X-ray Diffraction Data on Some 4-Alkoxyphenols

structural parameter	angles, deg: dihedral Ar-O-C	angles, deg: interbond Ar-O-C	bond lengths, Å: Ar-OR	bond lengths, Å: ArO-R
2	88.6	113.5	1.408	1.443
3(A)[a]	14.5	117.0	1.389	1.463
3(B)[a]	18.0	116.7	1.392	1.448
8	8.3	117.6	1.377	1.397
9	25.3	117.2	1.397	1.420

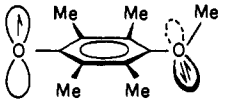
[a] There are two symmetry-unrelated molecules in the unit cell for 3.

chroman ring system, we synthesized **3** for which we find $n \sim 2.0$, $k_{inh}^{H}/k_{inh}^{D} = 5.5$ and $k_{inh} = (21.4 \pm 8.1) \times 10^5$ M^{-1} s^{-1} at 30 °C. Therefore, in vitro at least,[13] the magic of **1**'s antioxidant powers resides in some difference in the properties of the fused chroman ring system of **1** and **3** and those of the simple aromatic ring of **2**.

The initial clue to the origin of this difference came when we found that k_{inh} for **2** was only 1.5 times larger than that for pentamethylphenol. For other pairs of 4-methoxyphenols and 4-methylphenols, both unsubstituted elsewhere and substituted with alkyl groups in the 2 and/or 6 positions, the former compounds are ~5 times as reactive as the latter.[5,6,8a] The methoxy group in **2** is not, therefore, exerting a "normal" accelerating effect in reaction 1. The normal enhancement of k_{inh} by a 4-methoxy group is due to stabilization of the phenoxyl formed in reaction 1 by delocalization of the unpaired electron to the p-type orbital of the methoxyl oxygen, **4** ↔ **5**.[15] Such an interaction would be



4 **5**



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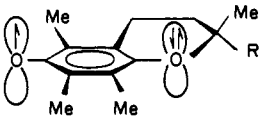

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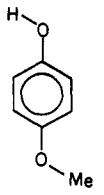
prohibited if the methoxyl in **2** were twisted out of the plane of the aromatic ring, i.e., **6**. However, for **1** and **3**, the fused ring structure should hold the p-type lone pair of the chroman oxygen more or less perpendicular to the aromatic plane, thereby stabilizing the product phenoxyl, **7**. The magnitude of the stabilization of **7** relative to **6** can be estimated to be ~3 kcal/mol.[17]

This stereoelectronic explanation for the high in vitro reactivity of **1** and **3** relative to **2** was tested by X-ray analysis of **2** and **3**, and 4-methoxyphenol (**8**) and 2,6-di-*tert*-butyl-4-methoxyphenol (**9**), as two examples of compounds not having alkyl substituents in positions 3 and 5. Some of the more significant structural parameters are listed in Table I. It can be seen that the Ar–O–C dihedral angle is ~90° for **2** (cf. **6**). However, it is only ~16° for **3** (cf. **7**), which is of similar magnitude to the angles found for **8** and **9**.



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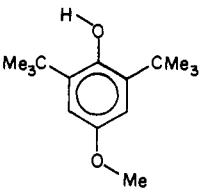

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In summary, the chroman ring system maintains a near-optimal orientation of the ethereal oxygen p-type lone pair with respect to the aromatic ring which, in combination with alkyl substitution at the other four ring positions, explains the superior chain-breaking antioxidant properties of α-tocopherol and **3**. Full details of this and other kinetic work and of the X-ray analyses will be published elsewhere.

Acknowledgment. We thank Dr. J. A. Howard and Dr. J. N. Thompson for helpful discussions.

Supplementary Material Available: Crystal data, data collection information, atomic positional and thermal parameters for **2**, **3**, **8**, **9** (11 pages). Ordering information is given on any current masthead page.

(13) In vivo, however, **3** does not show vitamin E activity[14] and so the phytyl is vital.

(14) Skinner, W. A.; Parkhurst, R. M.; Scholler, J.; Alaupovic, P.; Crider, Q. E.; Schwarz, K. *J. Med. Chem.* **1967**, *10*, 657. Skinner, W. A.; Parkhurst, R. M. *Lipids* **1970**, *5*, 184.

(15) For 4-methoxyphenol this stabilization amounts to 4.3 kcal/mol relative to phenol or 2.5 kcal/mol relative to 4-methylphenol.[16]

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

(17) Based on the fact that the k_{inh} values for **1** and **3** are about 10 times the value for **2** and the relationship between k_{inh} and phenolic O–H bond strengths given in ref 16.

(18) N.R.C.C. Research Associate 1978–1980.

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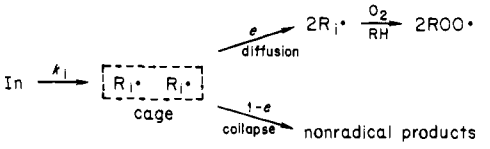
Autoxidation of a Model Membrane. A Comparison of the Autoxidation of Egg Lecithin Phosphatidylcholine in Water and in Chlorobenzene[1]

Sir:

A large body of quantitative kinetic information regarding the autoxidation of many organic substrates in homogeneous solution is now available, and the overall process is very well understood.[2] In contrast, the autoxidation of biological membranes, though known to occur readily and to be associated with many important pathological events,[3] is totaly lacking in quantitative kinetic data. In this communication we report some results from a kinetic study of the thermally initiated autoxidation of egg lecithin phosphatidylcholine at 30 °C in homogeneous solution in chlorobenzene and as bilayer dispersions (vesicles or model membranes) in 0.1 M aqueous NaCl. Our results provide answers to three simple, but extremely important, questions concerning the autoxidation of lecithin bilayers, answers which we hope will prove relevant to the autoxidation of biomembranes.

(1) Is There a Large Cage-Effect in a Lecithin Bilayer? In kinetically controlled autoxidations an initiator, In, decomposes to produce two radicals.[2] These may react together within the



(1) Issued as N.R.C.C. No. 18859.

(2) See, e.g.: (a) Mayo, F. R. *Acc. Chem. Res.* **1968**, *1*, 193. (b) Ingold, K. U. *Ibid.* **1969**, *2*, 1. (c) Reich, L., Stivala, S. S. "Autoxidation of Hydrocarbons and Polyolefins"; Marcel Dekker: New York, 1969. (d) Howard, J. A. *Adv. Free-Radical Chem.* **1972**, *4*, 49. (e) Howard, J. A. In "Free Radicals"; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 2, Chapter 12, pp 3–62. (f) Hendry, D. G.; Mill, T.; Piszkiewicz, L.; Howard, J. A.; Eigenmann, H. K. *J. Phys. Chem. Ref. Data* **1974**, *3*, 937.

(3) See: (a) Wolman, M. *Israel J. Medical Sci. Suppl.* **1975**, *11*, 1–245. (b) Mead, J. F. *Free Radicals Biol.* **1976**, *1*, 51. (c) *Tocopherol, Oxygen Biomembr. Proc. Int. Symp.* **1978**, 1–374.

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solvent cage or diffuse from the cage as "free" radicals. Autoxidation chains are started only by that fraction of radicals, e, that escape from the cage. The value of e will decrease as the viscosity of the medium increases.[4] For egg lecithin bilayers the microviscosity at 30 °C can be calculated from published data[5] to lie between 58 and 96 cP. These viscosities are much greater than that of chlorobenzene at this temperature, viz., 0.73 cP.

The autoxidation of egg lecithin[6] is a self-initiated, autocatalytic process. For kinetic studies the self-initiation process was "swamped out" by the addition of a sufficient quantity of a suitable thermal initiator, and all measurements were made at very small extents of oxidation. Many commonly employed thermal radical sources were found to initiate the autoxidation of egg lecithin in chlorobenzene. However, in the aqueous dispersion only, di-*tert*-butyl hyponitrite (DBHN) was an effective initiator that dissolved completely in the bilayer and did not partition (≤0.1%) into the aqueous layer (the solubility of DBHN in H_2O was found to be 1.3×10^{-5} M at 22 °C).[7] The rate constant, k_i, for decomposition of DBHN is 3.2×10^{-6} s^{-1} at 30 °C in homogeneous solution[8] and has been shown to be virtually independent of solvent polarity (95% ethanol to isooctane) and viscosity (Nujol).[8] We assume that the same rate constant can be used for the lecithin bilayer. The rate of chain initiation, $\rho = 2ek_i$[DBHN], was measured by the inhibitor method[9] using nature's antioxidant, α-tocopherol, which we have shown is the most efficient phenolic chain-breaking antioxidant known and which, in homogeneous solutions, traps exactly two peroxyl radicals.[10] The molecular structure of α-tocopherol should make it particularly suitable for inhibiting the autoxidation of lipid bilayers. In calculating the efficiency of initiation by DBHN in the bilayer, it has been *assumed* that the α-tocopherol resides wholly in the bilayer because of its low solubility in H_2O (≤1×10^{-7} M at 22 °C) and that it traps two peroxyls. The latter assumption cannot be verified, but a change to one or three peroxyls would not change our overall conclusions.

Autoxidations were carried out under 760 torr of O_2 in an automatic recording gas absorption apparatus. In chlorobenzene at egg lecithin concentrations[11] in the range $(2.5–12.5) \times 10^{-3}$ M, and with [DBHN] = $(0.75–11.7) \times 10^{-3}$ M and [α-tocopherol] = $(4.0–50.0) \times 10^{-6}$ M, the measured values of ρ were in the range $(0.31–4.81) \times 10^{-8}$ M s^{-1} and the mean value for e was 0.66 ± 0.06. The aqueous dispersions were prepared by dissolving the lecithin, DBHN, and α-tocopherol in methylene chloride/benzene, removing the solvent, and vortex mixing the residue in 0.1 M NaCl under N_2 for 5 min. This yielded a mixture of uni- and multilamellar vesicles, the majority of which had diameters in the range 2–6 μm. At egg lecithin levels of $(5–50) \times 10^{-6}$ mol[11] in 2 mL of H_2O and with [DBHN] = (0.2–0.8) mol L^{-1} (lecithin) and [α-tocopherol] = $(0.4–2.0) \times 10^{-3}$ mol L^{-1} (lecithin), the measured ρ values were in the range $(0.44–2.80) \times 10^{-7}$ mol L^{-1} (lecithin) s^{-1} and the mean value for e was 0.043 ± 0.008. Provided our assumptions are valid, it would appear that the initiation efficiency is reduced in the bilayer, presumably because of the high microviscosity of the latter. This is, no doubt, one of the factors that retards the autoxidative degradation of biomembranes.

(2) Is the Kinetic Rate Law for Autoxidation the Same for Biomembranes As for Homogeneous Systems? At oxygen partial pressures above ~100 torr the autoxidation of most organic substrates, RH, in homogeneous solution can be represented by the following reactions:

$$\text{In} \xrightarrow{k_i} 2R_1\cdot \xrightarrow[RH]{O_2} 2ROO\cdot \quad (1)$$

$$ROO\cdot + RH \xrightarrow{k_p} ROOH + R\cdot \quad (2)$$

$$R\cdot + O_2 \xrightarrow{\text{fast}} ROO\cdot \quad (3)$$

$$2ROO\cdot \xrightarrow{2k_t} \text{nonradical products} \quad (4)$$

The rate of oxygen absorption[12] is given by

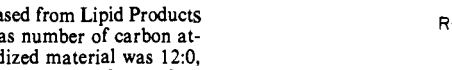
$$\frac{-d[O_2]}{dt} = \frac{k_p[RH](2ek_i[In])^{1/2}}{(2k_t)^{1/2}} = \frac{k_p[RH]\rho^{1/2}}{(2k_t)^{1/2}} \quad (5)$$

For egg lecithin in chlorobenzene the initiated oxidation follows this rate law, i.e., the rate of oxidation is proportional to [egg lecithin] × [In]$^{1/2}$ and is almost independent of the O_2 pressure (760–159 torr). In an aqueous dispersion it must be remembered that the egg lecithin is *not* diluted by the water. That is, *the egg lecithin concentration in a bilayer*[11] *is the same as that in bulk material.* Nevertheless, the rate of the DBHN-initiated oxidation of aqueous dispersions of egg lecithin is proportional to [In]$^{1/2}$ and is almost independent of the O_2 pressure (760–159 torr). We therefore conclude that the classical rate law (5) is obeyed since we cannot envisage any other situation that could lead to these kinetic observations.

(3) Is the Oxidizability of Egg Lecithin the Same in Homogeneous Solution As in an Aqueous Dispersion? The oxidizability of an organic substrate is defined as being the value of $k_p/(2k_t)^{1/2}$ at the temperature in question. For egg lecithin in chlorobenzene $k_p/(2k_t)^{1/2}$ has a value of 0.61 ± 0.09 M$^{-1/2}$ s$^{-1/2}$ at 30 °C. For the egg lecithin dispersion in 0.1 M aqueous NaCl, the value of $k_p/(2k_t)^{1/2}$ which is obtained on the assumption that the egg lecithin concentration in the bilayer is 1.0 M[11] is (1.65 ± 0.25) × 10^{-2} M$^{-1/2}$ s$^{-1/2}$ at 30 °C. That is, the oxidizability of egg lecithin in vesicles is only 2.7% of that for the homogeneous material. We are unaware of any published work that might have led us to anticipate this result!

The reduced oxidizability of egg lecithin in the aqueous dispersion is unlikely to be due to a simple increase in $2k_t$. That is, the values of $2k_t$ for secondary alkylperoxyls in homogeneous solution are generally ~10^7 M^{-1} s^{-1} at 30 °C.[2b,d-f] A reaction having such a rate constant is proceeding at close to the diffusion-controlled limit in a medium having the high viscosity of an egg lecithin vesicle.[5] There must, therefore, be a dramatic reduction of k_p in the aqueous dispersion. *We suggest that the reason for this reduction in k_p lies in the polarity of the peroxyl radical.*

As far as we are aware, the fact that peroxyls must have a significant dipole moment has not been explicitly recognized, though it has long been accepted that the stabilizing canonical structure (**2**) makes an ~40% contribution to the peroxyl structure.[13] We estimate that the dipole moment of the peroxyl



1 (60%) 2 (40%)

moiety is ~2.6 D. Because of its high polarity, the peroxyl portion of the radical will rapidly diffuse *out* of the nonpolar, autoxidizable, hydrocarbon environment in which it was formed and *into* the polar, nonautoxidizable, surface region of the bilayer.[14] Chain

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(6) Samples prepared at the N.R.C.C. and purchased from Lipid Products (U.K.) behaved identically. The fatty acid (given as number of carbon atoms:number of double bonds) content of the unoxidized material was 12:0, 0.23%; 14:0, 0.12%; 16:0, 35.4%; 16:1, 1.9%; 18:0, 11.8%; 18:1, 29.0%; 18:2, 19.0%; 20:4, 2.6%.

(7) Ineffective initiators included azobis(isobutyronitrile), azocumene, and 2,2,3,3-tetraphenylbutane, all of which could be seen by phase-contrast microscopy to have crystallized out of the aqueous egg lecithin dispersion.

(8) Kiefer, H.; Traylor, T. G. *Tetrahedron Lett.* **1966**, 6163.

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(10) Burton, G. W.; Le Page, Y.; Gabe, E. J.; Ingold, K. U. *J. Am. Chem. Soc.*, preceding paper in this issue.

(11) We have assumed that the average molecular weight of egg lecithin is 800 and that its density is 0.8. This makes the egg lecithin concentration in the bilayer 1.0 M, irrespective of the amount of added water.

(12) After correction for N_2 evolution in initiation, O_2 absorption in the initiation process, and O_2 evolution in chain termination.

(13) (a) Adamic, K.; Ingold, K. U.; Morton, J. R. *J. Am. Chem. Soc.* **1970**, *92*, 922. (b) Howard, J. A. *Can. J. Chem.* **1972**, *50*, 1981. (c) Melamud, E.; Silver, B. L. *J. Phys. Chem.* **1973**, *77*, 1896.

propagation will therefore be retarded while the resulting increased local concentration of peroxyls near the surface will increase chain termination. That is, the nonhomogeneous distribution of peroxyls in the bilayer will lead to an apparent decrease in k_p and increase in $2k_t$.

In summary, our results suggest that the physical structure of lecithin bilayers makes them more resistant to autoxidation than would be expected on the basis of their chemical composition. Thus, although these bilayers appear to follow the normal kinetic law for autoxidation, the initiation process appears to be rather inefficient (probably because of its high microviscosity), and oxidizability appears to be reduced (possibly because of the "expulsion" of the peroxyls from the autoxidizable region of the bilayer). We hope that our results will stimulate additional kinetic work on bilayer and biomembrane autoxidation and that our suggestions will prove valuable in understanding these complex systems.

Acknowledgment. We thank Dr. G. W. Burton for his continued help and advice. We are also grateful to Dr. J. A. Howard, Dr. B. F. Johnson, Mrs. Anne Joyce, Mr. D. Lindsay, and Dr. I. C. P. Smith for their assistance and/or helpful suggestions.

(14) A referee suggested that the DBHN may be sequestered only in the polar region of the bilayer, near the head groups, and that this would slow down propagation because only unreactive $-CH_2-$ would be available for reaction. This is equivalent to our own suggestion except insofar as the process which causes the first peroxyl to be near the surface of the bilayer. However, the initiating *tert*-butoxyl does not abstract alkane hydrogen atoms all that rapidly, and so it should have time to diffuse to a position where it can attack the much more reactive allylic and doubly allylic hydrogens (unless it, too, is sequestered in the polar region).

(15) On sabbatical leave from Mount Allison University, Sackville, New Brunswick.

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Mechanistic Aspects of the Photochemistry of Metal-Metal Bonds. Evidence for the Intervention of Two Different Primary Photoproducts in the Photochemistry of $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$

Sir:

The results of recent photochemical and related[1-4] studies on transition-metal compounds containing metal–metal single bonds have been consistently interpreted by a primary photochemical act in which homolytic cleavage of the metal–metal bond occurs (eq 1).[1-4] In particular, for the iron dimer, $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$,

$$M\text{-}M \xrightarrow{h\nu} 2M\cdot \qquad (1)$$

both halogen atom abstraction from halocarbon solvents and substitution of phosphines and phosphites for CO have been attributed to $(\eta^5\text{-}C_5H_5)Fe(CO)_2$.[2] However, flash-photolysis studies on the dimers $Mn_2(CO)_{10}$[5] and $(\eta^5\text{-}C_5H_5)_2Mo_2(CO)_6$[6] have provided evidence for both homolytic cleavage and additional transients, and the roles that the different intermediates play in the net photochemistry are unclear. For example, for the iron dimer, Tyler, Schmidt, and Gray[7] obtained low temperature spectroscopic

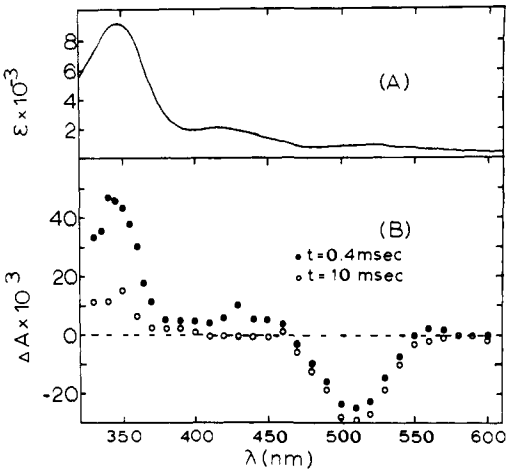

Figure 1. (A) Absorption spectrum of $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$ in cyclohexane. (B) Difference spectrum observed following flash photolysis of $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$ in cyclohexane. ΔA is the absorbance change observed from before the flash to t = 0.4 ms after initiation of the flash (○) and from before the flash to 10 ms after the flash (●). $\Delta A > 0$ corresponds to a decrease in absorbance of the solution after the flash.

(IR) evidence for a photochemical intermediate thought to be $(\eta^5\text{-}C_5H_5)(CO)_2Fe(\mu\text{-}CO)Fe(\eta^5\text{-}C_5H_5)(CO)(L)$ (L = P(*O-i*-Pr)$_3$) but whose structure and composition are uncertain. They suggested that the observed photochemistry of the starting dimer may occur solely via the dinuclear intermediate rather than through $(\eta^5\text{-}C_5H_5)Fe(CO)_2$.

In order to resolve the apparent mechanistic ambiguities in the iron dimer system, we have investigated its photochemistry by flash photolysis under both net photochemical and nonphotochemical conditions in inert solvents (cyclohexane, benzene). Under nonphotochemical conditions (freeze–pump–thaw–degassed, flame sealed), the samples were completely photochromic and stable for a period of at least several weeks. The electronic spectrum of $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$ has a characteristic, intense absorption band at 345 nm which has been assigned to the transition ($\sigma^* \leftarrow \sigma$(Fe–Fe))[8a] but which is probably more appropriately assigned to a $\pi^* \leftarrow \pi$ transition of the $Fe_2(CO)_2$ bridge.[8b] Either UV ($\lambda > 250$ nm) or visible ($\lambda > 400$ nm) flash photolysis of the dimer leads to the formation of two distinct intermediates. Both of the intermediates are present immediately following the flash (50 μs) and can be studied separately because their subsequent decay processes occur on substantially different time scales.

The more short-lived of the intermediates returns to $(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4$ by equal concentration, second-order kinetics within 2 ms after the flash. The difference spectrum for the transient process observed (Figure 1B; t = 0.4 ms) shows a bleaching of the absorption band at 345 nm, and the reaction occurring is almost surely recombination of the monomeric fragments formed by photolysis during the flash (eq 2; $k_2(20 \pm 2\ °C) = 3.2 \times 10^9\ M^{-1}\ s^{-1}$ (cyclohexane), 1.0×10^9 (benzene)).

$$(\eta^5\text{-}C_5H_5)_2Fe_2(CO)_4 \underset{k_2}{\overset{h\nu}{\rightleftharpoons}} 2(\eta^5\text{-}C_5H_5)Fe(CO)_2 \qquad (2)$$

The long-lived (seconds) intermediate, I, also returns to the original dimer, and the difference spectrum for this process (Figure 1B; t = 10 ms) shows that I has a broad absorption centered at 510 nm which decays by first-order kinetics. The decay rate increases in a roughly linear manner with increasing monitoring light intensity. The results obtained suggest that I undergoes both secondary photolysis and a thermal reaction and that both re-

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(5) Hughey, J. L., IV; Anderson, C. P.; Meyer, T. J. *J. Organomet. Chem.* **1977**, *125*, C49.

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