their Larmor frequencies, (3) application of the spin-locking field (which will rapidly destroy the X components of magnetization) along y for the relaxation period t_{max}, and (4) removal of the spin-locking field and acquisition of the fid, during t_2. Double Fourier transformation yields the 2D spectrum (Figure 3). The spectrum itself is displayed along the diagonal, while cross-peaks represent transient Overhauser effects in the rotating frame.

Interproton effects identified from the 2-D experiment on the tetrasaccharide include (A1,A2), (A2,A3), (A3,A4), (A2,A4-(diaxial)), (A1,B2), (A1,B3), (B2-B3), (B3,B4), (C2,C3), (C4,C5), (C6,C6'), and (D4,D5). An effect tentatively identified as (C2,B5) suggests a particular conformation about the B–C glycosidic linkage.

Relaxation processes in the rotating frame are sensitive to low-frequency motions (of the order of $\omega_1 = \gamma H_1$). Such motions might occur in the form of slow conformational changes, and the influence of these on the effects is of interest. However, most processes will be fast compared to ω_1, and the experiment may be thought of as a way to restore the extreme narrowing condition for higher molecular weight compounds. We think a suitable acronym for this type of experiment is CAMELSPIN (cross-relaxation appropriate for minimolecules emulated by locked spins). Ice-skating enthusiasts will have no difficulty with the origin of the term.

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Free Radicals in Lipid Bilayers: New Probes of Lipid Radical Dynamics

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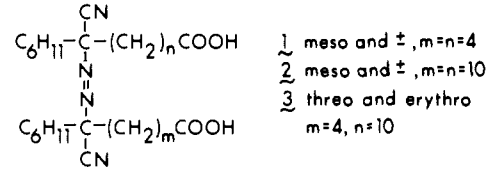
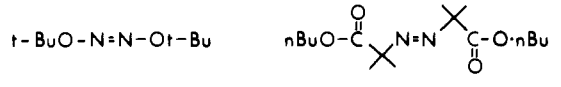
Free radical reactions in membranes have been implicated in several instances of destructive membrane oxidation[1] but the details of the initiation and propagation reactions of radicals in membranes are not well understood. In order to investigate the effect of phospholidpid bilayers on the formation and fate of lipidic radicals, we prepared[2] the amphipathic 1,2-diazenes **1**, **2**, and **3**.





4 **5**

Each of these diazenes has two diastereomers which were isolated and studied separately.[3] Other 1,2-diazenes have been studied as free radical initiators in model membranes. For example, di-*tert*-butyl hyponitrite (**4**) and 2,2'-azobis(2-(*n*-butylcarboxy)-propane) (**5**) have been examined as free radical initiators in phospholipid bilayers.[4,5] However, these studies suffer from a lack of information about the microenvironment surrounding the initiators in the bilayers. This uncertainty raises questions about the magnitude of bilayer cage effects in these systems.[4] Initiators **1–3** are designed to be oriented in the bilayer and thus resolve locational ambiguities that plague initiators **4** and **5**.[6]

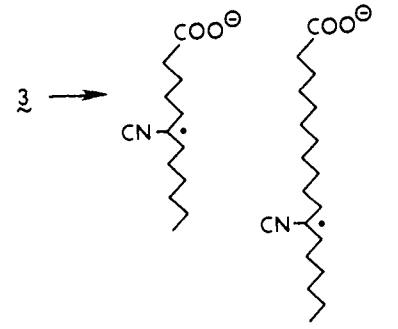


Figure 1.

Table I. Efficiencies for Radical Production from Diazenes 1-3[a] in Phospholipid Bilayers at 60.00 °C

diazene[a]	k ± SE in C_6H_5Cl[a-c]	e ± SE in C_6H_5Cl	k ± SE in DPPC[c,d]	e ± SE in DPPC[d]
meso-1	21.1 ± 0.4	0.512 ± 0.014	35.5 ± 0.5	0.054 ± 0.001
(±)-1	14.5 ± 0.1	0.596 ± 0.024	5.7 ± 0.2	0.041 ± 0.006
2a	23.7 ± 0.3	0.483 ± 0.021	13.2 ± 0.2	0.077 ± 0.008
2b	12.8 ± 0.4	0.507 ± 0.028	5.2 ± 0.2	0.069 ± 0.011
3a	*e*	*e*	18.7 ± 1.3	0.101 ± 0.017
3b	*e*	*e*	5.3 ± 0.3	0.109 ± 0.011

[a] Stereoisomers of diazenes **2** and **3** have not been identified and are indicated here as "a" and "b".[3] [b] SE = standard error of linear regression. [c] First-order rate constant × 10^6, s^{-1}; weighted mean of at least three independent measurements. [d] DPPC = dipalmitoylphosphatidylcholine in pH 7 buffer with 1 mM EDTA. [e] Not determined.

Free radicals generated by decomposition of diazenes can react with each other or escape the initial encounter and react with atmospheric oxygen at a diffusion-controlled rate.[7] The resulting peroxyl radicals are scavenged by hydrogen atom donors such as α-tocopherol (vitamin E) and the ratio of radicals escaped (and α-tocopherol scavenging) to radicals produced from the diazene is termed the escape efficiency.[8,9]

The efficiencies of our initiators were experimentally determined via reverse-phase HPLC by monitoring the α-tocopherol loss relative to the loss of the diazene in question at 60.00 °C under atomspheric oxygen. Since each α-tocopherol is known to scavenge two peroxyl radicals,[8] the efficiencies were calculated as the coefficient of the linear regression of [α-tocopherol] vs. [diazene]. Efficiencies were independent of diazene concentration in the range studied (3–10% in DPPC), and efficiency plots were linear to at least three diazene half-lives (<0.6% diazene in DPPC). The results thus suggest that the diazene does not, in itself, significantly modify the bilayer medium. Our results are presented in Table I.

In chlorobenzene at 60 °C, 2,2'-azobis(isobutyronitrile) (AIBN) gives escape efficiencies of 0.59.[10] Under the same conditions,

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(2) Petter, R. C.; Mitchell, J. C.; Brittain, W. J.; McIntosh, T. J.; Porter, N. A. *J. Am. Chem. Soc.* **1983**, *105*, 5700.
(3) The diazenes **2** and **3** were prepared in a way analogous to the method used to prepare **1**.[2] Diastereomers were separated by chromatography on a C-18 column with CH_3OH/H_2O/HOAc. A solvent mixture of (v/v) 750/250/2 was used for **1**, 810/190/2 for **3**, and 950/50/2 for **2**. The first eluting isomer is designated "a", the second "b". The configuration of the stereoisomers of **2** and **3** is not known.
(4) Barclay, L. R. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 6478.
(5) Winterle, J. S.; Mill, T. *J. Am. Chem. Soc.* **1980**, *102*, 6336.
(6) A low-angle X-ray analysis of **1** incorporated into DPPC multilamellar vesicles has been published.[2] This study confirms the fact that **1** forms a homogeneous phase with DPPC. Preliminary results indicate that similar conclusions can be drawn about **2** in DPPC.
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initiators **1** and **2** show comparable efficiencies as do **4** and **5**. In multilamellar vesicles (liposomes) of dipalmitoylphosphatidylcholine (DPPC) at 60.00 °C with a pH 7 buffer, 1.0 mM EDTA, under atmospheric oxygen, all of our initiators show a *pronounced* decrease in efficiency from the value obtained in chlorobenzene.

This result is in contrast to the efficiencies obtained[5] with **5** in liposomes at 50 °C ($e = 0.25$). We suggest that the high escape efficiency exhibited by **5** in bilayers may be attributed to its localization in a region of low microviscosity, e.g., near the terminal methyl groups of the acyl chains.[11] The same reasoning would account for the slightly higher efficiency of **2** in bilayers compared to **1** since the diazene linkage for **2** would be in a region of lower microviscosity than that of **1**.

Of the initiators **1**–**3**, **3** has the highest escape efficiency. We speculate that the hydrophobic effect[12] provides a driving force for realignment of the lipid radicals once the diazene linkage is broken. This realignment of radicals would shear apart the radical centers and thus retard coupling of radicals during the initial encounter[11] (see Figure 1).

Registry No. *meso*-**1**, 86550-44-9; (±)-**1**, 86550-45-0; *meso*-**2**, 88377-13-3; (±)-**2**, 88377-14-4; *threo*-**3**, 88377-15-5; *erythro*-**3**, 88377-16-6; DPPC, 2644-64-6; chlorobenzene, 108-90-7.

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Table I. Products of the Reaction of Copper Nitrate and Iodosylbenzene with Olefins in Acetonitrile[a]

substrate	products	yield,[b,c] %	substrate consumed,[c] %
		76	68
	PhCHO	10[d]	
		28	27
		44	
	PhCHO	28[d]	
		28	92
		28	26
		1.8[f]	
		2.6[f]	

[a] See text for experimental procedure. The ratio reactant: copper nitrate:iodosylbenzene = 1:4:8. [b] Percent yields are based on substrate consumed. [c] Rates and yields are somewhat variable and appear to depend on variations in individual preparations of iodosylbenzene. Values reported are typical values obtained after 2-h reactions. [d] Yields of benzaldehyde are calculated based on two benzaldehyde molecules per stilbene. [e] Other products were present in low yield and have not been characterized. [f] Yields of cyclohexenone and cyclohexenol were variable, although always low, and may have been due to competing side reactions.[1]

Copper Ion Mediated Epoxidation of Olefins by Iodosylbenzene

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Metalloenzymes that catalyze the incorporation of oxygen atoms derived from dioxygen into organic substrates usually contain either iron or copper. Our understanding of the mechanisms involved in the reactions of the iron-containing heme oxygenases has been substantially advanced by the characterization of high-valent iron porphyrin oxo complexes capable of epoxidation or hydroxylation of organic substrates. Such iron porphyrin oxo complexes have typically been prepared from single oxygen atom sources such as iodosylbenzene,[1,2] peroxy acids,[3] or amine oxides.[4] Similar reactivity has been observed with manganese and chromium porphyrins as well.[5-7] In the case of the copper enzymes, e.g., tyrosinase[8] or dopamine-β-hydroxylase,[9] our understanding is much more limited. It has been suggested that either a (μ-peroxo)dicopper(II) complex or a high-valent copper oxo complex derived from O–O bond cleavage may be the active species.[9,10] The best model system for this reaction is the binuclear cuprous complex of Karlin and co-workers,[11] which reacts with dioxygen to give ligand hydroxylation. The nature of the copper complex that is directly responsible for the hydroxylation is unknown in this case also.

We have initiated a study of copper-mediated oxygenations using a variety of oxygen atom sources, e.g., dioxygen, superoxide, hydrogen peroxide, iodosylbenzene, etc., in an attempt to observe and characterize some of the intermediates in these reactions and relate them back to the biological systems. We report here an initial success in this endeavor, the observation that olefins can be epoxidized by iodosylbenzene in the presence of copper ions in organic solvents. The occurrence of this reaction leads to the surprising conclusion that porphyrin ligands are not required for metal ion activation of iodosylbenzene, as has recently also been demonstrated by Hecht and co-workers[12] who reported that copper bleomycin could mediate the reaction of iodosylbenzene with olefins in aqueous solution.

In a typical experiment, 174 mg (0.791 mmol) of iodosylbenzene[13] was added all at once to a solution of *trans*-stilbene (17.8 mg, 0.0987 mmol) and cupric nitrate,[14a] $Cu(NO_3)_2 \cdot 2.5H_2O$ (91.5

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(13) Prepared by the method of Saltzman and Sharefkin (Saltzman, H.; Sharefkin, J. G. "Organic Syntheses"; Wiley: New York, 1973; Collect. Vol. V, pp 658-659) and stored at −5 °C to retard the spontaneous disproportionation to iodoxybenzene and iodobenzene.

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