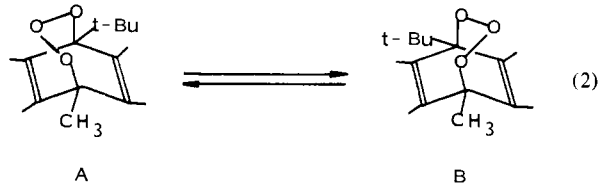
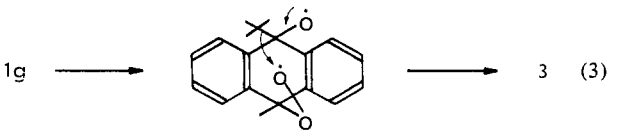


A B (2)

lescence or broadening of the corresponding ^1H NMR (80 MHz) signals was not observed even at 78.0 °C, indicating that energy barriers to rotation of the *tert*-butyl group and to the process A ⇄ B are pretty high.[7]

The stereochemistry of **1g** (Figure 1) was determined by X-ray crystallography.[11] The molecule situates on a crystallographic mirror plane. The O(2) atom is located in either of two positions that deviate by 0.68 Å from the mirror plane. The lengths of two O–O bonds are 1.361 and 1.428 Å, respectively, and two C–O bonds are 1.490 and 1.497 Å, respectively. The O–O–O angle of 113.3° is smaller than the value for ozone (116°).[1b] The calculated value for the unsubstituted 1,2,3-trioxolane is 104.4 ± 1.6°.[12] The fact that the C(9)–C(8)–C(7) angle is larger than the C(2)–C(3)–C(4) angle is ascribable to peri interaction between the *tert*-butyl group and two hydrogen atoms at C(7) and C(7′).

Thus the salient features of the stereochemistry of **1g** are (1) that the two O–O bond distances (1.361 and 1.428 Å) are very different from each other and are unusually short as compared to O–O bonds of typical organic peroxides (1.48 Å)[13,14] and (2) that both of the C–O bonds (1.490 and 1.497 Å) are definitely longer than C–O bond for other alkyl peroxides (1.22–1.48 Å).[13b-e,14] It is interesting to note that the thermal decomposition of **1g** was formally initiated by selective cleavage of the shorter (stronger) peroxide bond, i.e., cleavage of O(2)–O(3) rather than O(1)–O(2) (eq 3). Further study to elucidate this point is in progress.



(3)

(7) Minimum barriers to the *tert*-butyl rotation and to the oxygen flipping A ⇄ B were estimated as 20 and 19 kcal/mol, respectively, by using eq 4 (T

$$\Delta G_c^{\ddagger} = -RT \ln (\pi h \Delta\nu/(2^{1/2}kT)) \quad (4)$$

= 351 K; $\Delta\nu$ = 2.4 Hz for the *tert*-butyl rotation and 14 Hz[8] for the oxygen flipping). These data suggest that optical resolution of **1g** at room temperature may be possible. Rotational barriers for the *tert*-butyl group are typically less than 13 kcal/mol.[9] Boat–chair interconversion barrier of a cyclic trisulfide has recently been estimated to be $\Delta G_c^{\ddagger}$ = 17.9 kcal/mol.[10]

(8) A similar $\Delta\nu$ value was obtained for **1b**.[2d]

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(14) One of the referees pointed out that these structural features are peculiar and appear inexplicable. At this stage we can only say that the trioxide **1g** is a peculiar species due to some interactions and constraints, which are not entirely obvious.

Acknowledgment. This work was supported by the Ministry of Education of Japan under a special research project. We thank Dr. Isao Saito for his helpful suggestions.

Supplementary Material Available: Listings of atomic coordinates and thermal parameters in crystalline **1g** (3 pages). Ordering information is given on any current masthead page.

Bilayer-Induced Diastereomeric Kinetic Differentiation

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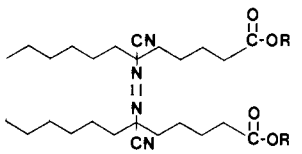
While the physical aspects of lipid bilayers and monolayers have been the focus of considerable investigation,[2] use of bilayers as oriented hosts for chemical reactants has received far less attention.[3] In this communication, we report the synthesis of a new amphipathic diazene, **1**, and the thermal decomposition of **1** in



1, meso, (±); R = H

2, meso, (±); R = CH_3

phosphatidylcholine multilamellar vesicles (MLV's).[2,4] Our results support the notion that reactivity and the stereochemical course of reaction may be affected by utilizing lipid bilayers as the solvent medium.

The free radical initiator, **1**, was prepared by standard procedures[5] from methyl 6-oxododecanoate. The meso and (±) diastereomers of **1**[6] were readily separated by chromatography. The isomer that eluted last on reverse-phase high-pressure liquid chromatography (HPLC, C18: methanol/water/acetic acid;

(1) (a) Department of Chemistry. (b) Department of Anatomy.

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(5) The synthesis is a modification of procedures used for AIBN preparation. Methyl 6-oxododecanoate was reacted with hydrazine hydrate to give the azine, which was then reacted with HCN(1) to afford the cyanohydrazine. Bromine oxidation gave a mixture of **2** meso and **2** (±). Overall yield was 30% for the three-step sequence.

(6) 1 meso: mp 108.5–110 °C; anal. ($C_{26}H_{44}N_4O_4$) C, H. 1 (±): mp 114.5–116 °C. anal. ($C_{26}H_{44}N_4O_4$) C, H. Both diastereomers exhibited similar ^1H and ^{13}C NMR. ^{13}C (15 MHz, CD_2Cl_2) δ 14.1 (CH_3), 22.9, 24.2, 24.6, 29.3, 31.8, 33.9, 37.2, 37.5, 77.5 (quaternary C), 117.9 (C≡N), and 179.5 (COOH); ^1H (250 MHz, CD_2Cl_2) δ 0.88 (t, 6 H, CH_3's), 1.3 (m, 2 H), 1.7 (m, 4 H), 2.0–2.3 (m, 8 H), 2.4 (m, 4 H); UV (CH_3OH) λ_{max} = 345 (ϵ = 21).

(7) We carried out low-angle X-ray studies to convince ourselves that **1** does incorporate into the lipid bilayer. A discussion of the low-angle X-ray work is presented in supplementary material to the manuscript.

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Table I. Rate Constants for the Decomposition of **1** and **2** at 60.00 ± 0.02 °C

diazene	solvent[a]	10^6k, s^{-1} [b]	$k_{meso}/k_{(\pm)}$
meso-**2**	C_6H_5Cl	15.5 ± 0.6	1.1
(±)-**2**	C_6H_5Cl	14.0 ± 1.4	
meso-**1**	C_6H_5Cl	21.1 ± 0.4	1.5
(±)-**1**	C_6H_5Cl	14.5 ± 0.1	
meso-**1**	DPPC	35.5 ± 0.5	6.2
(±)-**1**	DPPC	5.7 ± 0.2	

[a] Diazenes were typically 0.002 M in chlorobenzene and 5-10 mol % in DPPC. [b] The rates presented are weighted means from N_2 evolution and HPLC analysis and are given ± the standard error of linear regression analysis.

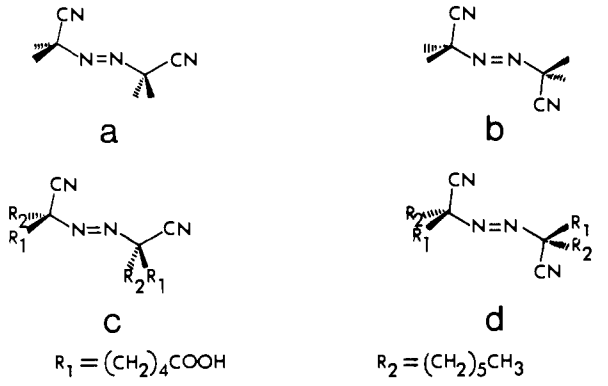

Figure 1. Conformation of diazenes: (a) AIBN in non-*S* conformation; (b) AIBN in *S* conformation; (c) *meso*-**1** in non-*S* conformation; (d) (±)-**1** in *S* conformation.

850/150/2) was assigned the (±) structure by resolution with (-)-quinine.

Decomposition of *meso*- and (±)-**1** in chlorobenzene (0.002 M) shows some rate differentiation, the meso compound decomposing 1.5 times faster than the (±) diastereomer (Table I). The greatest diastereomeric kinetic differentiation, however, is seen when **1** is decomposed in aqueous emulsions (pH 7) of DPPC (as multilamellar vesicles), a rate ratio of over 6 being observed. In a typical experiment, DPPC (15 mM) and freshly purified **1** (0.75 mM) in pH 7 phosphate buffer were vortexed to give a milky emulsion of MLVs. Nitrogen evolution was then measured by a pressure transducer,[8] or if HPLC was used to monitor the reaction, 0.90 mM lauric acid was added to the MLVs as internal standard and the decomposition was monitored by sampling from a vessel open to the atmosphere.

One rationale for the kinetic differentiation observed[9] is the possibility that the organizational properties of the lipid bilayer influence the conformation of ground-state diazene or transition state of decomposition. Diazenes similar to **1** such as AIBN have been studied by single-crystal X-ray analysis.[10] The nitriles, diazene, and connecting quarternary carbons all lie in a plane in what is termed the *S* conformation (Figure 1). As shown in the figure, the (±)-diazene can adopt the *S* conformation and place both hydrophilic carboxylate chains on one side of the diazene plane and both hydrophobic hexyl groups on the other side of that plane. The (±) diastereomer may thus adopt the *S* conformation and satisfy the amphipathic requirements of the lipid bilayer. This is not possible for the meso compound. It seems clear then, that the oriented medium could have a dramatic *and different* effect on ground- or transition-state geometries of the meso and (±) diastereomers and thus influence the kinetics of decomposition.

The bilayer-induced diastereomeric differentiation reported here indicates that lipid bilayer hosts may have a significant effect on the course of reactions involving diastereomeric reactants and transition states. Bilayer-induced selectivity in the formation of diastereomeric products would also appear to be possible. These aspects of bilayer-induced stereodifferentiation are currently under investigation in our laboratories.

Supplementary Material Available: A discussion of low-angle X-ray analysis of **1** incorporated into DPPC along with a figure showing a bilayer electron density map is presented (2 pages). Ordering information is given on any current masthead page.

(8) We thank Drs. G. Burton and K. Ingold for their design of a pressure measuring transducer.

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Proton NMR Study of the Mechanism of the Heme-Apoprotein Reaction for Myoglobin

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Myoglobin and hemoglobin are globular proteins which noncovalently enfold protoheme IX. Considerable effort has been extended toward defining their single-crystal structures[1-4] whose unique conformations are the basis for the interpretation of functional studies. The rates of the reversible reaction between protoheme IX (E in Figure 1) and apomyoglobin and apohemoglobin have been studied by optical spectroscopy[5-7] which led to the currently accepted mechanism whereby both apoproteins combine with protoheme IX to yield a single, native conformation and this reaction is complete within a time scale of a millisecond.[8,9] The complete understanding of this reaction is not only highly relevant to the biosynthesis of b-type hemoproteins[10] but also has important ramifications for the interpretation[11-14] of physicochemical measurements made on reconstituted proteins even when using native protoheme IX.

We report herein a real time ^1H NMR analysis of the reconstitution of myoglobin whose results are in essential variance with both the presently accepted mechanism of the reconstitution as well as the nature of the conformation of the native protein. Sperm whale myoglobin was reconstituted[15,16] in situ within a NMR tube by mixing equivalent amounts of protohemin-IX or reduced[17] protoheme IX-CO with apomyoglobin, and the time course of the reaction was followed by ^1H NMR. A in Figure 1 shows that the spectrum immediately after reconstitution consists of two sets of heme resonances: the set X_i arising from "native" metMbH_2O previously assigned by isotope labeling[18] and a set Y_i, whose

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