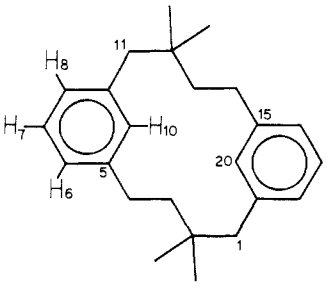

1

The observed induced shifts are explained satisfactorily by the single anticlinal[12] conformation which is very close to the one observed in the crystalline state. However, it is difficult to exclude the possibility that the weighted average of several different conformers is very close to this structure. To address this point, NMR spectra of **1** at lower temperature are examined. As the temperature is lowered, a sharp methyl singlet becomes broad and resolves to two singlets at −100 °C, indicating that some dynamic process is slowed down at this temperature. On the other hand, the signals due to the aromatic protons remain unchanged, and no minor signal could be detected even at the lowest temperature (−100 °C). The behavior of these NMR signals is consistent with the hypothesis that only one anticlinal conformer is present in **1** and that it interconverts to its mirror image by a flipping of the two benzene rings. The negligible population of conformers other than this conformer is further supported by molecular mechanics calculations.[13]

Since there is an increasing interest for determining the precise three-dimensional geometry of macrocycles consisting of polyaromatic rings such as calixarenes, crown ethers, cryptands, and cavitands, this type of calculation might have wide applicability for this purpose. Further studies on the conformational analysis of polyaromatic crown ethers and calixarenes are currently in progress in our laboratory.

Supplementary Material Available: Information listing calculations of the ring current induced shift values of benzene protons, preparation of **1** and the reference compound, and molecular mechanics calculations, figures representing torsion angles, low-temperature ^{1}H NMR spectra, and perspective drawings of the calculated structures of **1**, and tables listing observed and calculated induced shifts for [2.2]cyclophanes and high-energy conformers **1b–1d** and *R* factors, fractional coordinates, isotropic temperature factors, anisotropic thermal parameters, and bond distances and angles for **1** (19 pages). Ordering information is given on any current masthead page.

(11) The crystals of **1** belong to a monoclinic system with cell dimensions a = 10.794 (3) Å, b = 20.127 (4) Å, c = 9.832 (2) Å, β = 110.01 (2)°, and V = 2008.5 (8) Å^3. The space group is $P2_1/c$, Z = 4. The empirical formula is $C_{24}H_{32}$, molecular weight is 320.50, and calculated density is 1.060 g/cm^3. The three-dimensional X-ray data were collected by the use of graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Rigaku AFC-5 automatic four-circle diffractometer up to a maximum 2θ of 55.0°. Of 3249 total unique reflections, 1844 were considered observed at the leve of $|F_o| > 4.0\sigma|F_o|$. Data were corrected for Lorentz and polarization effect by the usual way but not for absorption as the linear absorption coefficient is small enough [λ(Mo Kα) = 2.3 cm^{-1}]. The structure was solved by the direct method (MULTAN78). All non-hydrogen atoms were located on the initial E synthesis. Hydrogen atoms were included in the calculated positions (C–H 1.08 Å). Block-diagonal least-squares refinements with 24 anisotropic non-hydrogen atoms and 32 isotropic hydrogens have converged to a conventional *R* factor of 0.082. All the calculations were done on a HITAC M-200H computer of the Hiroshima University with a structure analysis program system UNICS3 [Sakurai T.; Kobayashi, K. *Rep. Inst. Phys. Chem. Res.* **1979**, *56*, 69]. Further results of the crystallographic experiment are available as Supplementary Material.

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Orientation Dependence of ^{2}H NMR Spin-Lattice Relaxation Rates for Cholesterol in Macroscopically Oriented Multibilayers†

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Establishing the time scales and amplitudes of molecular motions in the anisotropic environment of lipid bilayers is a task well suited to NMR, since it provides a window precisely in the range where the frequencies of most molecular motions in lipid bilayers are concentrated (10^0–10^{10} Hz).[1] In this regard, ^{2}H NMR continues to play a leading role in studies of dynamic disorder and molecular mobility in these systems.[2a–e] A potentially valuable source of information on molecular dynamics in lipid bilayers can be provided by measurements of the dependence of the ^{2}H spin–lattice relaxation times on the angle between the bilayer normal (director) and the magnetic field (T_1 anisotropy). Such measurements not only put constraints on models of membrane dynamics but also facilitate the identification of the most significant relaxation pathways for ^{2}H nuclei in such systems.[3a–d] Measurements of the T_1 anisotropy for labeled cholesterol in oriented DPPC multibilayers, presented in this note, serve a 2-fold purpose: they allow us to test the validity of a simple model for cholesterol motion and to demonstrate the utility of oriented systems for studies of molecular dynamics.

In the absence of orientational averaging,[4] anisotropic T_1 behavior can be observed in the partially relaxed line shapes of unoriented dispersions.[3b,d] A distinct advantage of oriented systems is that they circumvent the orientational averaging effects of rapid lateral diffusion over the curved liposomal surfaces, a physical process which would thwart any attempt to observe anisotropic spin–lattice relaxation.[4,5] In addition, the enhanced sensitivity and resolution afforded by oriented samples obviously permit a more rapid and straightforward measurement of T_1 anisotropies, particularly when there are overlapping powder patterns in the unoriented spectrum, as is the case for the labeled cholesterol of this study. The fused rings of cholesterol present a rigid moiety whose molecular motion will relax ^{2}H nuclei at different sites at rates modulated *only* by the respective angles (Θ_i) between the C–^{2}H bonds and the motional averaging axis. We exploit this fact to test a simple model for cholesterol dynamics in lipid bilayers, since a correct description of this system should simultaneously predict the angular-dependent relaxation profiles for *all* labeled sites on the steroid ring system.

†N.R.C.C. No. 29629.

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(5) Note that orientational averaging by rapid lateral diffusion is a *sufficient*, but not a *necessary* condition, to explain the angular independence of T_1 in the L_α phase of disaturated lecithins.[3c]

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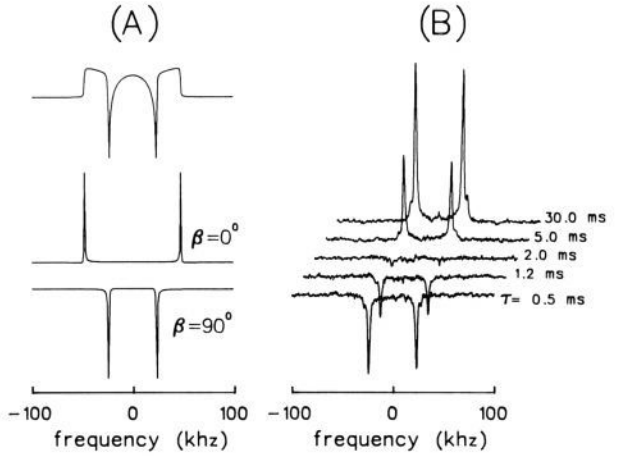

Figure 1. (A) Simulations of partially recovered (T_1) ^{2}H NMR spectra in the region of the null ($\tau \approx (\ln 2)T_1$). Powder line shape (top) and oriented line shapes (bottom) corresponding to two particular orientations (β) of the motional averaging axis with respect to the magnetic field are shown. (The T_1 anisotropy used for the powder simulation and the relative intensities of the oriented spectra are arbitrary and have been chosen for the purpose of illustration only). (B) Partially recovered spectra (T_1) of α-[C_7-$^2H_1$] cholesterol/DPPC (50 mol %) at 30 °C, oriented at β = 90°, as a function of the delay τ in the inversion recovery sequence: 180_x–τ–90_x–t–90_y–t–ACQ. ^{2}H NMR spectra were obtained on a home-built spectrometer (30.7 MHz) using a 10-mm solenoidal sample coil. π pulse 7.0 μs, recycle time 50 ms ($\gg 5T_1$), 30 K accumulations.

At the outset, it should be noted that the detection of anisotropic relaxation behavior in oriented and unoriented samples is quite different; Figure 1A compares simulated partially relaxed spectra in both systems at a fixed time delay τ following an inverting pulse. Since anisotropic relaxation effects in powder spectra manifest themselves in the region of the null[6a,b] ($\tau = (\ln 2)T_1$) where the S/N ratio is the poorest, detection of these effects in unoriented systems places stringent demands on the spectrometer. Moreover, if 0.1-ms increments in the delay period τ should be required to adequately define the effects of anisotropic T_1 behavior in the partially relaxed line shapes, such behavior is easily missed if the set of τ delays does not include sufficient (or any) points in the region of the null.[6a] On the other hand, even though separate inversion recovery experiments are required for each orientation in oriented systems, the improvement in S/N ratio, resolution and experimental flexibility more than compensates for the additional experiments, particularly since it allows the unequivocal and unambiguous detection of T_1 anisotropies.

A typical inversion recovery experiment for one orientation is shown in Figure 1B; the same experiment repeated at different angles was used to determine the T_1 profiles shown in Figure 2. These profiles vary significantly with label position; the relative independence of T_1 with orientation for the C-6 position contrasts with the corresponding profile for the axial deuterons. Note that in the absence of orientational averaging, such anisotropic relaxation would invalidate the use of dePaking,[7] a procedure used previously[8] for the analysis of partially relaxed powder spectra of multiply-labeled cholesterol. Assuming that cholesterol undergoes 3-fold reorientation about its motional averaging axis, relaxation profiles $T_1(\beta,\Theta_i)$ were calculated by using the general formalism of Torchia and Szabo,[9] where β is the angle between the magnetic field and the motional averaging axis, and Θ_i specifies the angle between each C–^{2}H bond vector and the averaging axis. A similar modeling approach has been used recently to analyze

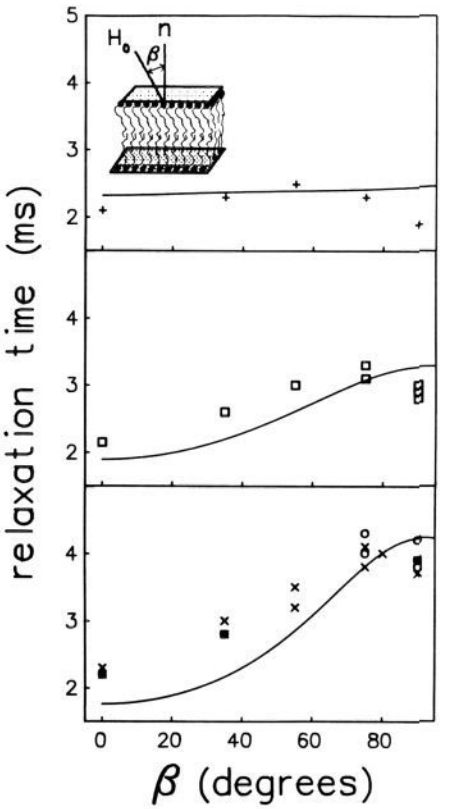

Figure 2. Experimental (symbols) and calculated (solid lines) ^{2}H spin–lattice relaxation times as a function of the angle β between the magnetic field direction and the director (see insert) for several labeled positions of cholesterol in DPPC (50 mol %) oriented multibilayers: positions (Θ_i, symbol) are indicated as C_7 (75°, ×), $C_{2,4}$ axial (75°, ○) (bottom), $C_{2,4}$ equatorial (68°, □) (middle) and C_6 (56°, +) (top). All curves have been calculated assuming that the C–^{2}H bond jumps between three equivalent sites at the same exchange rate of 3×10^8 rad s^{-1}. Therefore these profiles are distinguished only by the angle Θ_i between the C–^{2}H bond and the motional axis as taken from Taylor et al.[10] Calculations were performed with a modification of a line shape simulation program[6b] based on the formalism of Torchia and Szabo.[9] Note that for each value of β the relaxation rate has been powder averaged over all azimuthal angles. From the fitting of the experimental inversion recovery data, the standard error for any T_1 value is estimated to be ≤5%. The estimated accuracy of the β values is ±3°.

T_1 anisotropies in powder spectra of cholesterol,[3d] with the important distinction that by assuming $S_{mol} = 1$, the angle Θ_i could be determined directly from the quadrupolar splitting. In our analysis, the angles Θ_i were those determined by an order parameter analysis[10] where $S_{mol} < 1$. Thus, we have explicitly neglected any contribution to relaxation by the motions of the motional averaging axis. At this stage, it would be premature to rule out other models; we note only that this simple model does reproduce many significant features of the observed relaxation behavior. Refinements in this model as well as testing of alternative models[8,11] of cholesterol dynamics using the orientation dependence of ^{2}H relaxation rates will be the subjects of a more detailed study to be reported elsewhere. As the use of oriented multibilayers becomes more commonplace, particularly for the study of peptide/lipid systems,[12a–c] we anticipate that the approach discussed here for the lipid/cholesterol system will be used to investigate molecular dynamics in related systems. In this regard,

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oriented bilayers allow the anisotropy in both relaxation rates, T_{1Z}^{-1} and T_{1Q}^{-1}, to be measured simultaneously, a well-established approach in liquid crystals[13a-c] but relatively unexploited in lipid bilayers. These T_{1Q} experiments as well as extensions of the approach described herein to other oriented systems, particularly those containing proteins, are the focus of our current investigations.

Acknowledgment. We gratefully acknowledge Dr. E. J. Parish for the generous gift of deuteriated cholesterols. We also thank Dr. R. G. Griffin for a copy of a computer program for calculating T_1 anisotropies.

Registry No. DPPC, 2644-64-6; cholesterol, 57-88-5.

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Unsaturated Transition-Metal Complexes in Solution: Naked $Cr(CO)_5$ in Cyclohexane Solution Observed by Picosecond IR Transient Absorption

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We have made the first identification of a "naked" square pyramid $Cr(CO)_5$ ground singlet state molecule in cyclohexane solution, which has a vibrational frequency equivalent to the gas phase, and measured its reaction rate with cyclohexane. We tentatively have inferred the reaction rates for another precursor species, probably the trigonal bipyramid triplet state of $Cr(CO)_5$, which reacts to add cyclohexane faster than the square pyramid structure. The method used picosecond UV photodissociation of $Cr(CO)_6$ at 266 nm to generate $Cr(CO)_5$ and probed the transient species via picosecond IR absorption.

Coordinatively unsaturated organometallic complexes are widely accepted[1-3] as reactive intermediates involved in homogeneous catalysis. Photodissociation of transition-metal complexes has been used to generate intermediates in matrices[4,5] and gases.[6-8] True naked intermediates have been characterized by IR absorption in gases, but prior matrix and solution work, including nanosecond resolved spectroscopy in hydrocarbon solution,[9] have always found saturated coordination. Recently, solution phase transient absorption in the visible was reported[10-13] which had very fast transient risetimes of less than 1 ps,[12] which was attributed to fast solvent attachment. Such a conclusion is not compatible with the transient infrared results presented here; although the transient shifts in electronic spectra may be a unique probe of continuous bond strength at the metal center, these experiments must separate the effects of multiple species identified in our work. We have used computer simulation to show that spectral overlap in the electronic spectra of *both* intermediates and products of photolysis can create transient absorption signals which are dominated by the fast creation of the initial species and do not necessarily reflect solvent reaction rates. In our experiments we obtained transient infrared absorption spectra, which are sensitive to CO coordination, with a laser developed in our laboratory[14] having a 20 ps pulse width (FWHM) and 5 cm^{-1} of wavelength resolution.

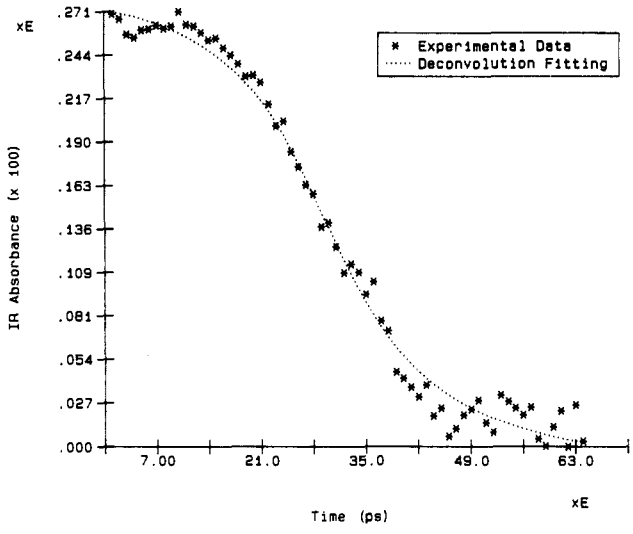

Figure 1. Infrared transient absorption at 1980 cm^{-1} after 266-nm excitation of $Cr(CO)_6$ at a concentration of 3×10^{-3} M in cyclohexane solution. The transient is assigned to "naked" square pyramid $Cr(CO)_5$, and the fit by a kinetic model is shown as a dotted line. The absorbance maximum is consistent with the kinetic model and final product concentration. The 266-nm excitation used 5.4×10^{-2} J/cm^2, and the infrared probe used 1.0×10^{-4} J/cm^2.

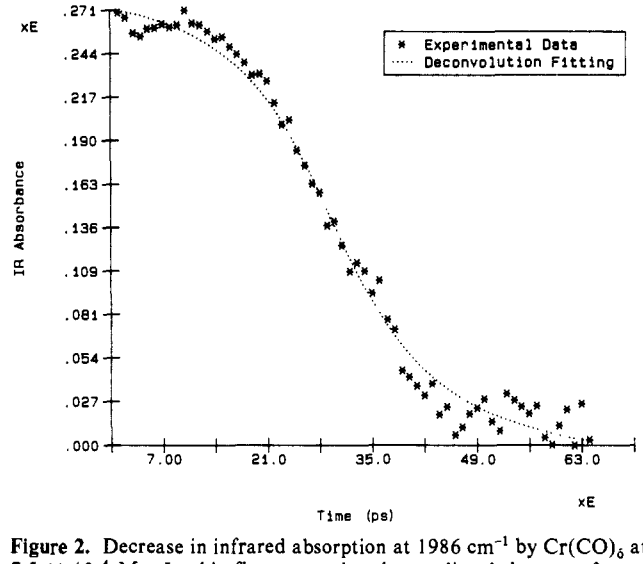

Figure 2. Decrease in infrared absorption at 1986 cm^{-1} by $Cr(CO)_6$ at 7.5×10^{-4} M. In this figure we plot the predicted decrease for an instantaneous decay rate and a Lorenztian excitation pulse of 20 ps FWHM.

Figure 1 shows evidence for a "naked" $Cr(CO)_5$ molecule in cyclohexane at 22 °C. The transient absorption at 1980 cm^{-1} corresponds to the E symmetry carbonyl vibrational mode of "naked" $Cr(CO)_5$ which we have inferred by comparison with gas-phase assignment[7] of this frequency to a square pyramid structure of C_{4v} symmetry. Other vibrational modes (A symmetry)

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