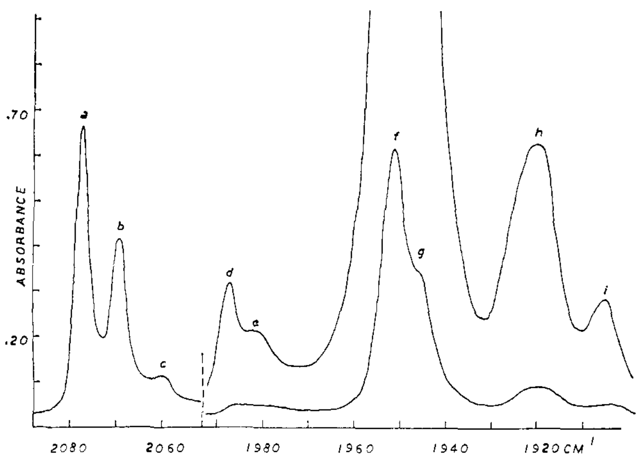

Figure 2. Infrared spectra of ^{13}CO-enriched $Mo(CO)_5As(C_6H_5)_3$ originating from the reaction of $Mo(CO)_5NHC_5H_{10}$ and $As(C_6H_5)_3$ in hexane solution. Peaks a, d, f, and g are for the all-^{12}CO species; peaks b, e, and h are for the $^{13}CO_{eq}$ species; peak i is for the $^{13}CO_{ax}$ species; and peak c is for the di-$^{13}CO_{eq}$ species.[6]

The substitution reaction with triphenylarsine to form $Mo(CO)_5As(C_6H_5)_3$ was performed using the ^{13}CO enriched $Mo(CO)_5NHC_5H_{10}$ sample. Care was taken to avoid complications due to the second-order reaction process. This was checked by simultaneously performing identical decomposition kinetic experiments with $Mo(CO)_5NHC_5H_{10}$ in the absence and presence of triphenylarsine. These reactions were followed employing infrared spectroscopy in the carbonyl stretching region. The experiments were run at 38° in hexane with concentrations of $Mo(CO)_5NHC_5H_{10}$ and $As(C_6H_5)_3$ being 4.72×10^{-3} and 1.50×10^{-2} *M*, respectively. Under these conditions no contribution from k_2 was observed and the sole reaction product was $Mo(CO)_5As(C_6H_5)_3$, obtained in 100% yield. The rate constants k_1 were found to be 1.15×10^{-4} and 1.20×10^{-4} sec^{-1}, respectively, for the two cases. During this reaction there was *no* change in the relative abundance of axial and equatorial carbonyl groups in the $Mo(CO)_5NHC_5H_{10}$ species.

The infrared spectrum in the CO stretching region for the substitution product $Mo(CO)_5As(C_6H_5)_3$, enriched in ^{13}CO, is shown in Figure 2. Now 34.0 ± 0.5% of the molecules have a single equatorial ^{13}CO (peak b) and 7 ± 1% of the molecules are axially substituted with ^{13}CO (peak i). Therefore, *the experiment unequivocally indicates a redistribution of equatorial and axial carbonyl groups during the substitution process.*

This equilibration may occur in the transition state. Although it is difficult to envisage a transition state in which all the CO's are equivalent, a possible model is illustrated by Brown for the $Mn(CO)_5X$ species.[7] Owing to the bulkiness of the piperidine base, this possibility is highly unlikely. The alternative is, assuming a dissociative process, an equilibration of the axial and equatorial CO groups in the five-coordinate intermediate through either a trigonal bipyramid or a highly distorted square pyramid.[8] From the ^{13}CO distribution in $Mo(CO)_5As(C_6H_5)_3$, it appears that there has been almost a complete statistical scrambling of the axial and equatorial positions (prediction would be for 35.3 ± 0.6% ^{13}CO equatorial and 8.8 ± 0.2% ^{13}CO axial in the product based on the percentages in $Mo(CO)_5NHC_5H_{10}$).[10]

The accuracy of this experiment would be greatly improved if it were possible to preferentially label the axial position in the starting material. Studies are in progress in our laboratories for elucidating the photochemical conditions necessary for specifically labeling metal carbonyl species.

Acknowledgments. Financial support from the Petroleum Research Fund, administered by the American Chemical Society, under Grants No. 1705-G3 (D. J. D.) and 1404-G3 (M. Y. D.), is gratefully acknowledged. We also wish to thank the State University of New York at Buffalo Computing Center for generous donation of computer time.

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[7] T. L. Brown, *Inorg. Chem.*, 7, 2673 (1968).

[8] A similar observation was made when tungsten hexacarbonyl was irradiated in a frozen hydrocarbon medium. Infrared spectra indicate that the pentacarbonyl species produced in the frozen-glass state most likely has a square-pyramidal configuration, whereas when the solid is melted a trigonal-bipyramidal configuration is produced.[9]

(9) I. W. Stolz, G. R. Dobson, and R. K. Sheline, *J. Amer. Chem. Soc.*, **84**, 3589 (1962); **85**, 1013 (1963).

(10) If the reaction proceeds through a trigonal-bipyramidal intermediate, the per cent distribution between equatorial and axial carbonyl groups is expected to be 83% $^{13}CO_{eq}$ and 17% $^{13}CO_{ax}$, starting with the mono-equatorially labeled species. This is very close to the expected statistically scrambling distribution of CO groups in a pentacarbonyl species (80% $^{13}CO_{eq}$ and 20% $^{13}CO_{ax}$).

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A New Highly Anisotropic Dinitroxide Ketone Spin Label. A Sensitive Probe for Membrane Structure

Sir:

Recently we introduced a short versatile procedure for the conversion of a ketone group into a stable mononitroxide free radical.[1] Such nitroxides have enjoyed wide use as spin labels, the esr spectra of which yield valuable information, *inter alia*, about the orientation, motion, and polarity within the molecular structure of membranes and membrane models.[2] We now describe a procedure, patterned after our previous one,[1] for the conversion of a ketone group into a stable dinitroxide spin label. We also describe experiments which show such dinitroxides to have potential as new sensitive probes for membrane structure studies.

Amino acid **2**, prepared from amino ketone **1**[3] by the method of Rassat,[4] was reduced with $LiAlH_4$ in refluxing ether to afford dinitroxide precursor **3**, mp 119–120°.[5] A solution of **3** (0.5 g), 5α-cholestan-3-one

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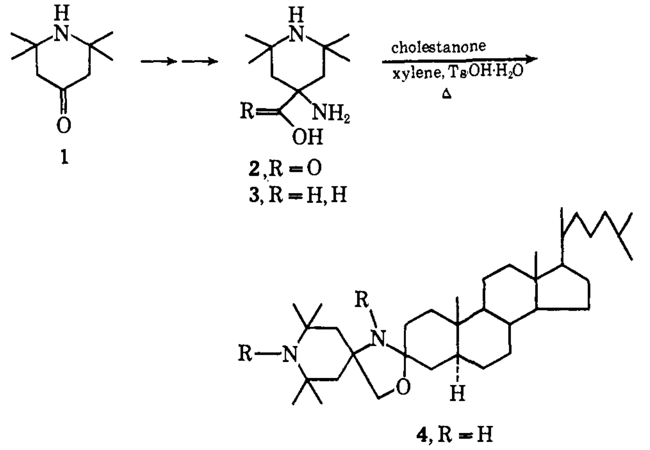
(1) J. F. W. Keana, S. B. Keana, and D. Beetham, *J. Amer. Chem. Soc.*, **89**, 3055 (1967).

(2) See, *inter alia*, (a) O. H. Griffith and A. S. Waggoner, *Accounts Chem. Res.*, **2**, 17 (1969); (b) P. Jost, A. S. Waggoner, and O. H. Griffith, "Membrane Structure and Function," L. Rothfield, Ed., Academic Press, New York, N. Y., 1971, Chapter 2; (c) P. Jost and O. H. Griffith in "Physical Methods in Pharmacology," C. Chignell, Ed., Vol. 2, Appleton-Century-Crafts, New York, N. Y., 1971, Chapter 2; (d) L. J. Libertini, A. S. Waggoner, P. C. Jost, and O. H. Griffith, *Proc. Nat. Acad. Sci. U. S.*, **64**, 13 (1969); (e) O. H. Griffith, L. J. Libertini, and G. B. Birrell, *J. Phys. Chem.*, in press; (f) I. C. P. Smith in "Biological Applications of Electron Spin Resonance Spectroscopy," J. R. Bolton, D. Borg, and H. Schwartz, Ed., Interscience–Wiley, New York, N. Y., 1971; (g) H. M. McConnell and B. G. McFarland, *Quart. Rev. Biophys.*, **3**, 91 (1970).

(3) Available from Aldrich Chemical Co.

(4) A. Rassat and P. Rey, *Bull. Soc. Chim. Fr.*, 815 (1967).

(0.8 g), and toluenesulfonic acid monohydrate (10 mg) in 50 ml of xylene was refluxed for 60 hr with continuous water removal by means of a Dean–Stark trap, leading to oxazolidine **4**, one epimer of which had mp 197–199° (98% yield).[5] Oxidation of **4** with 4 equiv of purified *m*-chloroperoxybenzoic acid in refluxing ether smoothly gave, after silica gel chromatography, dinitroxide **5**[5] as yellow plates, mp 209–210° (59% yield).



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2, R = O
3, R = H,H

4, R = H
5, R = O

In order to assess the potential of dinitroxides such as **5** as structure probes in the field of membrane biophysics, the spin label was incorporated into a dipalmitoyl lecithin multilayer utilizing the general procedure of Griffith.[2d,6] Such phospholipid multilayers have been shown by both X-ray[7] and mononitroxide spin-labeling[2,8] studies to orient spontaneously with the long molecular axis perpendicular to the glass slide. Thus an aliquot of a solution containing 1 ml of chloroform, 20 mg of β,γ-dipalmitoyl-DL-α-lecithin,[9] 8 mg of cholesterol, and 35 μl of a 10^{-3} *M* chloroform solution of **5**[10] was spread on a 60 × 5 × 0.1 mm glass slide and warmed at 43° until a clear thin film remained. The edges of the multilayer film were trimmed and then esr spectra were measured with the glass slide parallel to the pole faces of the magnet ($\theta = 0°$), perpendicular ($\theta = 90°$), and at several intermediate orientations. These spectra together with chloroform-solution and rigid-glass spectra of dinitroxide **5** are presented in Figure 1.

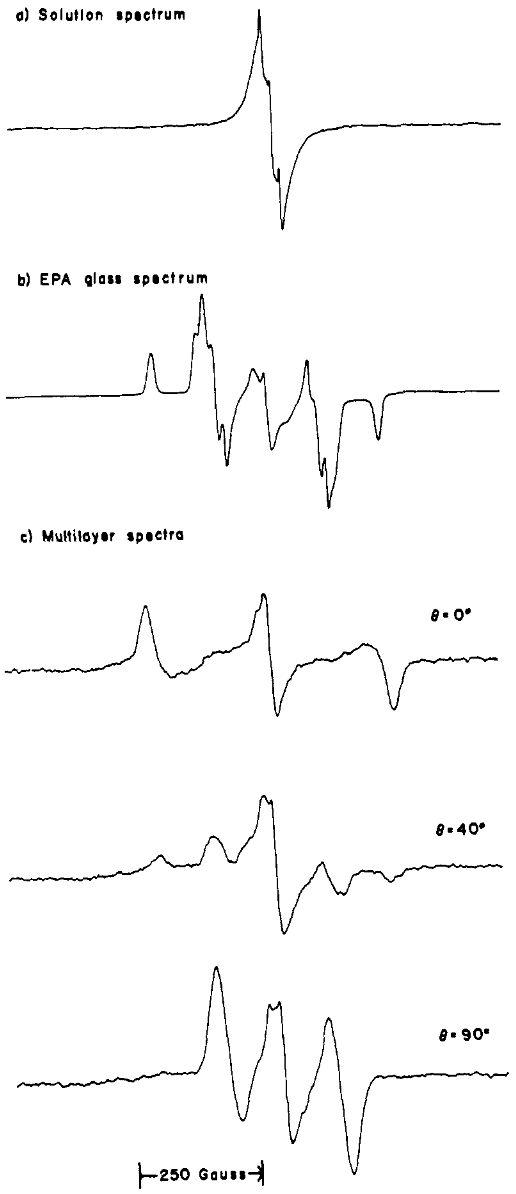

Figure 1. X-Band esr spectra of **5** at low concentration in the following media: (a) $\sim 10^{-4}$ *M* chloroform solution; (b) ethyl ether–isopentane–ethanol (EPA) rigid glass spectrum at −190° (a half-field transition (not shown) was observed at about one-half the mean resonant field of the $\Delta m = \pm 1$ transitions); (c) dipalmitoyl lecithin multilayer spectra with $\theta = 0$, 40, and 90° (see text). Absorption observed in the center portion of spectra b and c is due to a small contaminant of mononitroxide in the sample.

The Hamiltonian for a dinitroxide spin label is $\mathcal{H} = \mathcal{H}_{\text{Zeeman}} + \mathcal{H}_{\text{hyperfine}} + \mathcal{H}_{\text{exchange}} + \mathcal{H}_{\text{dipolar}}$. The dramatic anisotropic effect observed in Figure 1 is due to $\mathcal{H}_{\text{dipolar}}$, where $\mathcal{H}_{\text{dipolar}} = D(S_z^2 - {}^1/_3S^2) + E(S_x^2 - S_y^2)$. The observed dipolar splitting, *d* (distance in gauss between the two outermost lines), is related to the constants *D* and *E* (in units of $g\beta$) and the angles θ and ϕ by the following expression which follows from first-order perturbation theory:[11a] $d = |(3\cos^2\theta - 1)D + 3(\sin^2\theta\cos 2\phi)E|$, where θ and ϕ are the usual polar coordinates describing the position of one of the N–O groups (point dipole approximation) *r* ångströms away from the other N–O group taken to be at the origin. Thus[11b] maximum dipolar splitting is observed when $\theta = 0°$ ($d = 2D$), *i.e.*, the magnetic field is parallel to the line *r*, connecting the two N–O groups.

$2D$ as estimated from the rigid glass spectrum of **5** is 455 G; for $\theta = 0°$ in the multilayer, $d = \sim 450$ G;[12]

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(5) All new compounds showed acceptable elemental analyses. Spectra were consistent with the assigned structure.

(6) T. Marriott, P. Jost, and O. H. Griffith, manuscript in preparation.

(7) Y. K. Levine and M. H. F. Wilkins, *Nature* (*London*), in press.

(8) J. C. Hsai, H. Schneider, and I. C. P. Smith, *Biochem. Biophys. Acta*, **202**, 399 (1970).

(9) Available from Sigma Chemical Co.

(10) Dilute stock solutions of **5** suffer slow reduction to a mononitroxide over a period of days. Dinitroxide may be regenerated with ease by shaking with Ag$_2$O followed by centrifugation. Dinitroxide **5** regenerated in this way gave results identical with those derived from freshly prepared **5**.

(11) (a) D. B. Chesnut and W. D. Phillips, *J. Chem. Phys.*, **35**, 1002 (1961); (b) S. P. McGlynn, T. Azumi, and M. Kinoshita, "Molecular Spectroscopy of the Triplet State," Prentice-Hall, Englewood Cliffs, N. J., 1969, p 333; M. Bersohn and J. C. Baird, "An Introduction to Electron Paramagnetic Resonance," W. A. Benjamin, New York, N. Y., 1966, Chapter 8.

(12) Since we do not have perfect orientation in the multilayers, the two outermost lines are broadened somewhat and it is difficult to decide exactly which points should be used for the dipolar splitting measurement.

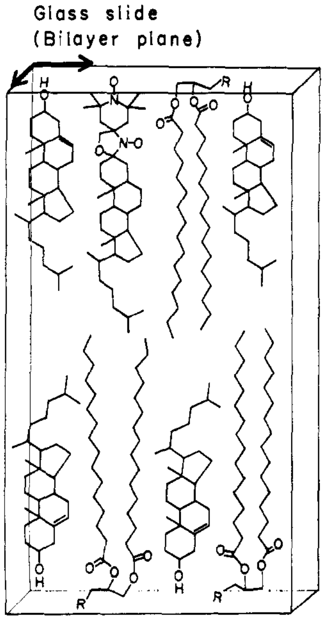
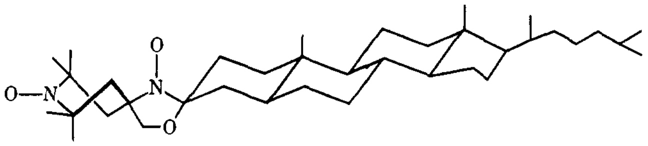


Figure 2. R = $^-O_3POCH_2CH_2N^+(CH_3)_3$. Schematic representation of a section of dipalmitoyl lecithin bilayer containing cholesterol showing the relative orientation of the dinitroxide spin label **5**.

for $\theta \approx 90°$, $d = \sim 250$ G.[12] While an exact calculation of E is not possible from our data, it is clear that $|E| \ll |D|$.

In the point dipole approximation $2D$ is related to the distance r between two N–O groups by the expression $2D = 55.6 \times 10^3 \times r^{-3}$.[2c,13] Measurements on molecular models of various low-energy conformations of the piperidine ring of **5** provide values for r of 3.8–5.2 Å. For twist-boat **6**, r = 5.0 Å, corresponding to $2D$ = 445 G,[14] in reasonable agreement with $2D$ = 455 G estimated from the outermost lines of the rigid glass spectrum (see above). The model of **6** also shows that line r forms an angle γ of about 20° with the long molecular axis about which **6** probably can rotate in the multilayer, undoubtedly a factor contributing to line broadening in the multilayer spectra.[12]



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A section of the labeled dipalmitoyl lecithin–cholesterol–dinitroxide multilayer is diagrammed in Figure 2. While we undoubtedly do not have perfect orientation of label **5** in the multilayer, the difference, nevertheless, between the maximum and minimum dipolar splittings observed here is about 200 G. When this anisotropy is compared with that typically observed[2] for mononitroxide spin labels, namely about 16–20 G, it is evident that the esr spectrum of the dinitroxide label is much more sensitive to orientation within a multilayer than are conventional mononitroxides. This large anisotropy should also facilitate studies involving molecular motion within membrane and membrane model systems.

Acknowledgment. We thank the National Science Foundation (GP-27367), the National Institutes of Health (1 RO3 MH 17209), and the donors of the Petroleum Research Fund, administered by the American Chemical Society (GF 674), for generous financial support. We also thank Professor O. H. Griffith and Dr. P. Jost for stimulating discussions and the use of some of their equipment.

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[13] N. Hirota and S. I. Weissman, *J. Amer. Chem. Soc.*, **86**, 2538 (1964).

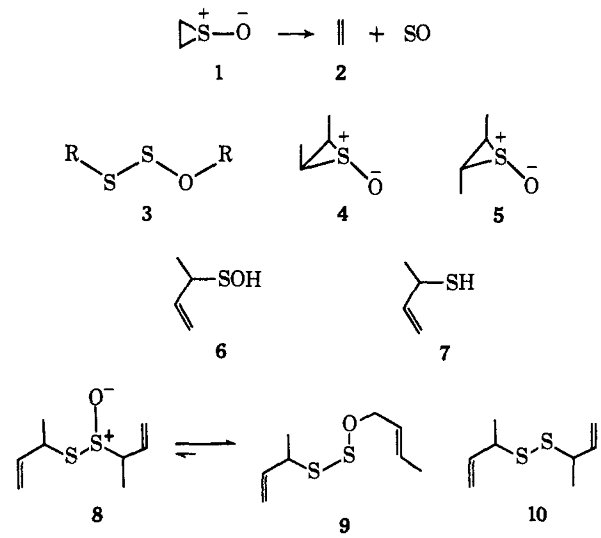
[14] It is evident that the calculated value of $2D$ should be extremely sensitive to the value chosen for r. Therefore, the presence of other conformations of **5** would lead to additional line broadening (see above and ref 12).

Rearrangement of Strained Dipolar Species. I. Episulfoxides. Demonstration of the Existence of Thiosulfoxylates

Sir:

It has been generally assumed that thermal decomposition of episulfoxides (**1**) proceeds to the olefin (**2**) by elimination of sulfur monoxide (Scheme I).[1] Such a process may be concerted, with retention of configuration of the liberated olefin, when it proceeds in the manner of a nonlinear cheletropic reaction.[2] We have observed, however, that in the presence of a suitably disposed hydrogen atom, there occurs a more facile pathway, namely rearrangement to allylic sulfenic acids. These substances may be intercepted or they may dimerize to a new structural type, the thiosulfoxylate (**3**).[3]

Scheme I



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[1] (a) G. E. Hartzell and J. N. Paige, *J. Amer. Chem. Soc.*, **88**, 2616 (1966); (b) G. E. Hartzell and J. N. Paige, *J. Org. Chem.*, **32**, 459 (1967).

[2] R. B. Woodward and R. Hoffmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 781 (1969).

[3] Esters of sulfoxylic acid, $S(OH)_2$, have been described: A. Meuwsen and H. Gerbhard, *Chem. Ber.*, **69**, 937 (1936); Q. E. Thompson, *J. Org. Chem.*, **30**, 2703 (1965). We can find no mention of thiosulfoxylates.