occurs prior to ring formation. Photoisomerization of this kind is not possible for vinyl sulfide 2.

Pyrex-filtered irradiation of $10^{-2}$ $M$ degassed benzene solutions of 2 at room temperature resulted only in polymer formation and not the expected *trans*-dihydrothiophene 9. Cycloaddition of dipolarophiles to chemically generated thiocarbonyl ylides is known[8,9] and in the present case would provide evidence for the intermediacy of 12 in the photolysis of 2. When a $10^{-2}$ $M$ degassed benzene solution of 2 was irradiated in the presence of 2 equiv of $N$-phenylmaleimide, polymer formation was eliminated and *a 90% yield of a single cycloadduct* 13 (mp 264°, $m/e$ 447.1321) was isolated.[10,11]

This trapping experiment is generally significant because, for the first time in stilbene-like photocyclizations,[7] the stereochemistry of the dihydro intermediate



14, R = $C_6H_5$; R' = $CH_3$
15, R = $CH_3$; R' = $C_6H_5$
13
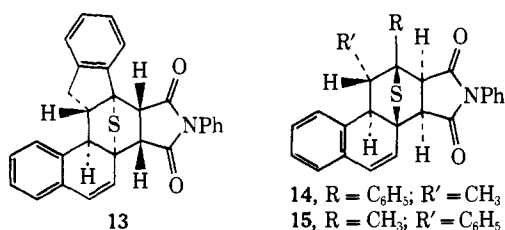
(*e.g.*, 12) can be clearly investigated.[12] From our observations, we conclude that conrotatory cyclization of 2 occurs in the photoexcited state to give the *trans*-thiocarbonyl ylide 12 (R = $o$-$C_6H_4CH_2$ = R', R'' = H), which then undergoes addition to $N$-phenylmaleimide to give adduct 13. The fate of intermediate 12 in the absence of dipolarophile is being studied.

The $N$-phenylmaleimide trapping experiment has provided information regarding the mechanism of dihydrothiophene formation in the photolysis of 1. Pyrex-filtered irradiation of $10^{-2}$ $M$ degassed benzene solutions of 1a or 1b in the presence of 2 equiv of $N$-phenylmaleimide resulted in the usual formation of *cis*-dihydrothiophene 5 (15%) but no 6. Instead, a single cycloadduct 14 was isolated (mp 266–267°, $m/e$ 449.1457) in 60% yield. Irradiation of benzene solutions of *trans*-dihydrothiophene 6 and $N$-phenylmaleimide did not produce any 14. These data indicate that thiocarbonyl ylide 12 (R = $C_6H_5$, R' = $CH_3$, R'' = H), presumably formed by conrotatory photocyclization of 1a, is the precursor to both 6 and 14.

That thiocarbonyl ylide 12 and dihydrothiophene 6 both possess trans stereochemistry is consistent with a suprafacial hydrogen migration occurring in 12 to give 6. Both a [1,4] hydrogen shift and two consecutive

(8) J. D. Bower and R. H. Schlessinger, *J. Amer. Chem. Soc.*, 91, 6891 (1969), and references cited therein.
(9) H. Buter, S. Wassenaar, and R. M. Kellogg, *J. Org. Chem.*, 37, 4045 (1972).
(10) The nmr spectral data for cycloadducts 13–15 are consistent with the assigned stereochemistry. X-Ray analysis of 13 and 14 is in progress and will serve to provide a totally unambiguous stereochemical proof. Stereochemical consequences of photogenerated thiocarbonyl cycloaddition to dipolarophiles will be discussed in a forthcoming paper.
(11) This cycloadduct forming process is truly synthetically useful and must proceed with high efficiency. Irradiation of a benzene solution of 2 (5.0 g), and $N$-phenylmaleimide in a conventional preparative photoreactor under an Argon atmosphere gave 6.9 g (85% yield) of analytically pure 13 in 3.25 hr. We are attempting to utilize this highly efficient method for the construction of steroid and steroid-like ring systems.
(12) Chapman and coworkers attempted without success to trap an intermediate with furan and maleic anhydride in the photocyclization of $N$-phenylenamines: O. L. Chapman, G. L. Eian, A. Bloom, and J. Clardy, *J. Amer. Chem. Soc.*, 93, 2918 (1971).

[1,2] shifts in ground-state 12 are allowed and have been considered.[12,13] Irradiation of monodeuterio-1a (R'' = 90% D, 10% H) gave monodeuterio-6 (R'' = 90% D, 10% H)[14] which indicates that only a 1,4-hydrogen shift is important in the transformation 12 → 6.

Formation of 5 from 1 is unaffected by added dipolarophile suggesting that thiocarbonyl ylide 12 (R = $C_6H_5$, R' = $CH_3$, R'' = H) is not involved in the formation of 5. As an alternative, we have considered vinyl sulfide 4 as a possible intermediate in the formation of 5.[1] Irradiation of 4 did not give any *cis*-dihydrothiophene, 5, but rather resulted in a complex mixture of products, the major component of which was the isomeric *trans*-dihydrothiophene 8 (50% isolated yield). The *cis*-dihydrothiophene 5 was stable to these reaction conditions. Furthermore, irradiation of 4 in the presence of $N$-phenylmaleimide resulted in the formation of adduct 15 (mp 170–172°, $m/e$ 449.1433), but no 14. These results rule out the intermediacy of 4 in the formation of *cis*-dihydrothiophene 5 from 1.

The mechanism of hydrogen migration in various thiocarbonyl ylides is currently being investigated; however, it is clear that only a 1,4-hydrogen migration is occurring in the transformations 1 → 12 → 6 (*vide supra*) and 3 → 12 → 7. The structure of 7 (87% isolated yield, bp 126° at 0.08 Torr) was established by spectral data and the nickel boride desulfurization[15] to 2-($\alpha$-naphthyl)-2-methylbutane. This photoclosure–desulfurization sequence could be synthetically useful for the regiospecific substitution of aromatic rings.

(13) A theoretical treatment of [1,4] *vs.* two consecutive [1,2] shifts has been presented: M. T. Rwetz, *Tetrahedron*, 29, 2189 (1973).
(14) Determined by nmr and mass spectral analysis.
(15) W. E. Truce and F. M. Perry, *J. Org. Chem.*, 30, 1316 (1965); we thank Professor C. A. Brown for suggesting this method of desulfurization.

**Arthur G. Schultz,*  Marvin B. DeTar**
*Department of Chemistry, Cornell University*
*Ithaca, New York  14850*

## Nuclear Magnetic Resonance Relaxation in Lysozyme Crystals

*Sir:*

There has been a great deal of recent interest in the nature of specific interactions between water and protein molecules in a variety of environments including aqueous solutions,[1] frozen aqueous solutions,[2] and whole tissues.[3] Measurements of nmr line width, intensity, self-diffusion coefficients, and transverse and longitudinal relaxation times have been applied to this general study. A major feature of the results obtained

(1) S. H. Koenig, R. D. Brown, and C. F. Brewer, *Proc. Nat. Acad. Sci. U. S.*, 70, 475 (1973).
(2) I. D. Kuntz, *et al.*, *Science*, 163, 1329 (1969).
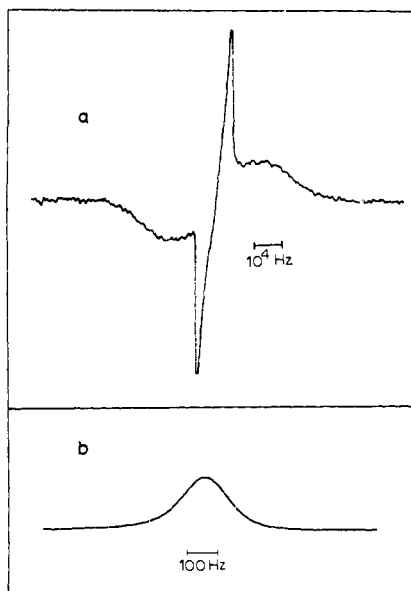(3) J. A. Walter and A. B. Hope, *Progr. Biophys.*, 23, 3 (1971).

**Figure 1.** (Curve a) The proton magnetic resonance spectrum of tetragonal lysozyme crystals recorded as the derivative of the absorption mode signal. (Curve b) The proton magnetic resonance spectrum of the narrow component in tetragonal lysozyme crystals recorded as the absorption mode spectrum with no modulation.

in semisolids is the presence of a narrow component in the nmr spectrum identified with water which has several unusual properties such as not freezing at temperatures well below the normal freezing point of pure water. Since the protein crystal occupies a pivotal point in structural biochemistry, the present work was undertaken to study both the protein and the water in the protein crystal and compare the results with similar experiments conducted in solution.

Many workers have reported nmr spectra of water in a variety of environments including protein powders, protein crystals, and whole tissues such as muscle.[4-7] If the spectrometer is adjusted so that the broadest components of the spectrum may be observed, a result such as that shown in Figure 1a is typically observed. The broadest component has a width of approximately 30,000 Hz and the superimposed component is more than a factor of 10 narrower. It is well known that modulation conditions required to easily observe the broad component severely distort the narrow component so that many investigators have chosen to observe only the narrow component as shown in Figure 1b. It is clear that the line width is considerably narrower when this is done, and, because the lines remain quite broad even in a tuned high resolution magnetic field, the line width is often taken as a measure of the transverse relaxation time, $T_2$. This approach must be used cautiously because some assumptions about line shape must be made.

The line shape for the narrow component of the nmr spectrum of lysozyme crystals is neither Lorentzian nor Gaussian suggesting that the transverse relaxation
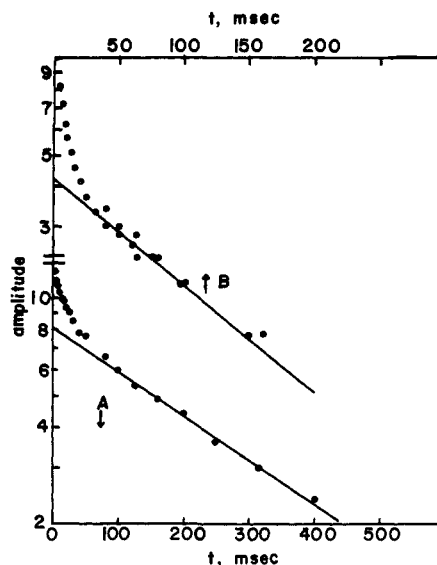
(4) M. E. Fuller and W. S. Brey, Jr., *J. Biol. Chem.*, **243**, 274 (1968).

(5) D. J. Blears and S. S. Danyluk, *Biochim. Biophys. Acta*, **154**, 17 (1968).

(6) G. J. Kruger and G. A. Helcke in "International Conference on Magnetic Relaxation," R. Blinc, Ed., Ljubljana, 1966.

(7) "Physiochemical State of Ions in Water and Tissue," *Ann. N. Y. Acad. Sci.*, **204**, 1973.

**Figure 2.** (Curve A) Amplitude of the proton free induction decay in a 180–90 sequence *vs.* time at 12.2° for monoclinic lysozyme crystals saturated with water. (Curve B) Proton spin echo amplitude *vs.* time at 12.2° for monoclinic lysozyme crystals saturated with water.

time, $T_2$, may not be simply defined. Some additional insight is provided by direct observation of the transverse and longitudinal relaxation times using pulsed nmr methods. Since local magnetic inhomogeneities in a sample such as protein crystals or tissue are large, $T_2^*$ is expected to be significantly different from $T_2$. For this reason a Carr–Purcell sequence was used to measure $T_2$ at 56.4 MHz and a 180–90 pulse sequence was used at both 56.4 and 100 MHz to measure $T_1$. Typical results for monoclinic lysozyme crystals grown in 2% sodium nitrate[8] solution in the absence of buffer are shown in Figure 2.

It is clear from these data that both transverse and longitudinal relaxation is at least biphasic at time scales greater than 1 msec. The values for the fast components of the relaxation behavior are similar but not identical with those reported for tetragonal lysozyme crystals which contain significantly larger amounts of water.[9] The biphasic character of the nuclear relaxation requires that there are two populations of protons observed in the narrow component of these samples. The simplest explanation of this result is to suggest that there is one population of water molecules, A, comprising 67% of the total water characterized by $T_{2A}$ = 6.8 msec and $T_{1A}$ = 320 msec, and a second fraction comprising 33% of the total water characterized by $T_{2B}$ = 132 msec and $T_{1B}$ = 22 msec. Since an average relaxation is not observed, it is necessary in this model that the chemical exchange of water between these two populations be slow compared with the relaxation times involved. In this context it is interesting to note that the values of $f_A$, the fraction of A-type protons, yield water populations which are very similar to the numbers associated with "non free" water in such measurements as protein crystal density[10] and dielectric relaxation.[11,12]

(8) M. Sundaralingam, private communication.

(9) L. K. Steinrauf, *Acta Crystallogr.*, **12**, 77 (1959).

(10) M. F. Perutz, *Trans. Faraday Soc.*, **42**, 187 (1946).

(11) S. C. Harvey and P. Hoekstra, *J. Phys. Chem.*, **76**, 2987 (1972).

(12) B. Pennock and H. P. Schwan, *J. Phys. Chem.*, **73**, 3600 (1969).

An alternative explanation for biphasic behavior in protein solution studies has been advanced which involves a transfer of magnetization between two proton populations such as the protein protons and the water protons.[13] The values of $T_1$ and $T_2$ measured for the two regions of the relaxation data are also consistent with this approach. The values of $T_1$ and $T_2$ obtained in these crystalline systems indicate that a major fraction of the water molecules in the crystal are essentially liquid as suggested by several other types of measurement.

These results show quite clearly that line width measurements in such heterogeneous systems as tissue or suspensions must be interpreted with care since the line width and its saturation behavior may not necessarily be interpreted in terms of a single relaxation time or be related in a simple way to molecular motion.

(13) R. Kimmich and F. Noack, Z. Naturforsch. A, 25, 1680 (1970).

J. E. Jentoft, R. G. Bryant*

Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455
Received September 18, 1973

## Congestion. A Conformation-Dependent Measure of Steric Environment. Derivation and Application in Stereoselective Addition to Unsaturated Carbon[1]

*Sir:*

Although steric effects play an important role in determining the course of many chemical reactions,[2] in general it has proved difficult to evaluate them in a quantitative manner. Taft has achieved some success in quantitatively accounting for steric effects in terms of substituent constants, $E_s$, in cases where conformational changes in the parent structure are not important.[3] However no generally applicable method appeared to exist which would allow automatic prediction of reaction stereoselectivity for computer analysis of synthesis.[4,5]

Stereochemistry may be conveniently divided into effects which are conformation independent (sym-

(1) This research was supported by the National Institutes of Health, Grant RR-00578, and in part by Hoffmann-La Roche and Merck Foundation.

(2) M. S. Newman, Ed., "Steric Effects in Organic Chemistry," Wiley, New York, N. Y., 1956.

(3) R. W. Taft, Jr., in ref 2, p 587.

(4) E. J. Corey and W. T. Wipke, Science, 166, 178 (1969); E. J. Corey, W. T. Wipke, R. D. Cramer III, and W. J. Howe, J. Amer. Chem. Soc., 94, 421 (1972), and following papers.

(5) Princeton Simulation and Evaluation of Chemical Synthesis (SECS) program: W. T. Wipke, T. M. Dyott, and J. G. Verbalis, Abstracts 161st National Meeting of the American Chemical Society, Los Angeles, Calif., March 1971, CHLT-22; W. T. Wipke, P. Gund, J. G. Verbalis, and T. M. Dyott, Abstracts, 162nd National Meeting of the American Chemical Society, Washington D. C., Sept 1971, ORGN-17; W. T. Wipke, Proceedings on the Conference on Computers in Chemical Education and Research, No. Ill. Univ., July 1971, p 10–60; W. T. Wipke, P. Gund, T. M. Dyott, and J. Verbalis, Abstracts, 7th Middle Atlantic Regional Meeting of the American Chemical Society, Philadelphia, Pa., Feb 1972, p 42; W. T. Wipke, T. M. Dyott, P. Gund, and C. Still, Abstracts, 164th National Meeting of the American Chemical Society, New York, Sept 1972, CHED-39; W. T. Wipke in "Computer Representation and Manipulation of Chemical Information," Wiley, New York, N. Y., 1974.
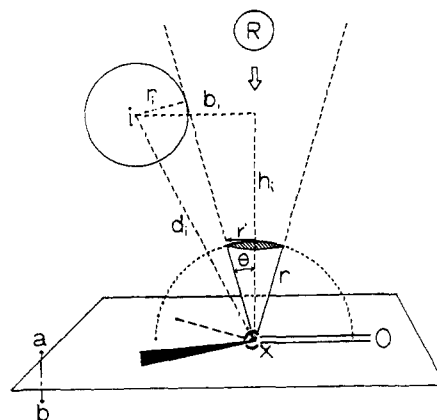


**Figure 1.** Cone of preferred approach of R to x allowed by atom i. The *accessibility* of x on side *a* with respect to i is defined by this solid angle and numerically equals the area on a unit sphere cut by this cone (shaded area).

bolically perceivable relationships, *e.g.*, cis–trans), and conformation dependent (spatial relationships: axial-equatorial, proximity, steric environment). Symbolic stereochemical relationships may be treated algebraically without recourse to atomic coordinates,[6] but treatment of spatial relationships for the general case requires evaluation of a three-dimensional model.[7] We report here a function which affords a semiquantitative estimate of congestion about a reaction center of a molecular structure and which appears to correlate with preferred direction of addition to congested ketones.

We wish to define *steric congestion* at a reaction center as *a property of the substrate molecule in its ground state*—independent of reaction partners or transition-state structure.[9a] To develop a function reflecting this definition, consider the specific case of nucleophilic attack on a ketone[9b] (Figure 1). Let us assume that a nucleophile R of neglibibly small radius preferentially approaches the carbon, x, along a line perpendicular to the plane of the carbonyl group. For each hindering atom, i, we may define a cone of preferred approach, centered on the perpendicular and tangent to the sphere of van der Waals radius $r_i$ surrounding atom i. Intersection of this cone with a sphere of unit radius centered on x defines a spherical cross section of preferred approach. We equate this solid angle[10] with $A_{xa}$ (i), the *accessibility* of x on side a with respect to i (Figure 1). Angle $\theta$ is easily derived from $r_i$, $d_i$ (the distance from x to i), and $h_i$ (the height of i above the plane).

$$A_{xa}(i) = 2\pi r^2(1 - \cos \theta) \tag{1}$$

We further define $C_{xa}$ (i), the *congestion* at x on side a caused by i, as the reciprocal of the accessibility; then

(6) W. T. Wipke and T. M. Dyott, J. Amer. Chem. Soc., in press.

(7) Models built by SYMIN,[5] an interactive Westheimer type molecular mechanics[8] program: W. T. Wipke, P. Gund, T. M. Dyott, and J. G. Verbalis, to be submitted for publication.

(8) Review: J. E. Williams, P. J. Stang, and P. v. R. Schleyer, Annu. Rev. Phys. Chem., 19, 531 (1968).

(9) (a) Thus steric congestion is only one part of the total effect called steric hindrance. (b) J. D. Morrison and H. S. Mosher, "Asymmetric Organic Reactions," Prentice-Hall, Englewood Cliffs, N. J., 1971, Chapter 3.

(10) The demensionless unit of solid angle is the steradian, that solid angle which encloses a surface on the sphere equivalent to the square of the radius.