

experiments will be necessary to evaluate in detail the meaning of the apparent values. The conclusions that both enantiomers A and B bind and that one is converted to enzyme-bound TDP appear to be a necessary consequence of any analysis. This confirms the central point of this study.

**Consequences for the Structure of the Active Site.** The inhibition of pyruvate dehydrogenase by methyl acetylphosphonate also involves catalysis by the enzyme of formation of the covalent adduct with TDP, phosphalactyl-TDP. The pyruvate binding site of the enzyme to which TDP binds must recognize the common features of the substrate and inhibitor with regard to monoanionic state,  $\alpha$ -keto group, and the methyl group adjacent to the carbonyl group. This is based on a survey of other potential inhibitors and substrates.<sup>2</sup>

It is reasonable that a monocation serves as the binding site for the substrate anionic center and a hydrogen bond donor associates with the keto group. The donor also can act as a general acid catalyst in the step in which TDP adds to pyruvate (see i and ii in Figure 2).

The addition of TDP to the keto group of the substrate or analogue generates a chiral center. In Figure 2 we have arbitrarily drawn the D-lactyl and D-phosphalactyl adducts for i and ii. When the D,L mixture of A and B isomers of phosphalactyl-TDP binds, presumably the TDP and anionic portions determine the position of binding. However, when isomer B binds (see Figure 2) the general base that removes the hydroxyl proton is in the wrong position to promote the reaction. This picture gives a simple direct relationship between our results

and a reasonable minimal functional set at the active site. The site is certainly more complex and we are conducting further studies to obtain more detail.

**Acknowledgment.** We thank Professor P. A. Frey for the pyruvate dehydrogenase we used in this work.

## References and Notes

- (1) Supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada. Part of this work is from the Doctoral Thesis submitted by David C. Pike.
- (2) Kluger, R.; Pike, D. C. *J. Am. Chem. Soc.* **1977**, *99*, 4504.
- (3) Breslow, R. *J. Chem. Soc.* **1958**, *80*, 3719.
- (4) Holzer, H.; Beaucamp, K. *Angew. Chem.* **1959**, *71*, 776.
- (5) Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991.
- (6) Jencks, W. P. "Catalysis in Chemistry and Enzymology"; McGraw-Hill: New York, 1969; pp 127-131.
- (7) Wolfenden, R. *Acc. Chem. Res.* **1972**, *5*, 10.
- (8) Lienhard, G. E. *Science* **1973**, *180*, 149.
- (9) Westerik, J. O.; Wolfenden, R. *J. Biol. Chem.* **1974**, *249*, 6351.
- (10) Thompson, R. C. *Biochemistry* **1974**, *13*, 5495.
- (11) Wiseman, J. S.; Abeles, R. H. *Biochemistry* **1979**, *18*, 427.
- (12) Chen, R.; Gorenstein, D. G.; Kennedy, W. P.; Lowe, G.; Nurse, D.; Schultz, R. M. *Biochemistry* **1979**, *18*, 921, and references cited therein.
- (13) Pike, D. C. Ph.D. Thesis, University of Toronto, Toronto, 1979.
- (14) Suchy, J.; Mieyal, J. J.; Bantle, G.; Sable, H. Z. *J. Biol. Chem.* **1972**, *247*, 5905.
- (15) Ullrich, J. *Methods Enzymol.* **1970**, *18A*, 109.
- (16) Reed, L. J.; Koike, M.; Levitch, M. E.; Leach, F. R. *J. Biol. Chem.* **1958**, *232*, 143.
- (17) Maldonado, M. E.; Oh, K.-J.; Frey, P. A. *J. Biol. Chem.* **1972**, *247*, 2711.
- (18) Dixon, M.; Webb, E. C. "Enzymes"; Academic Press: New York, 1964.
- (19) Moe, O. A., Jr.; Hammes, G. G. *Biochemistry* **1974**, *13*, 2547.
- (20) Barrera, C. R.; Namibira, G.; Hamilton, L.; Munk, P.; Eley, M. H.; Linn, T. C.; Reed, L. J. *Arch. Biochem. Biophys.* **1972**, *148*, 343.
- (21) Gutowski, J. A.; Lienhard, G. E. *J. Biol. Chem.* **1976**, *251*, 2863.

## Communications to the Editor

### Nonsymmetry of the Hydrogen Bond in 1-Phenylamino-7-phenylimino-1,3,5-cycloheptatriene<sup>1</sup>

Sir:

There is currently considerable interest in intramolecularly hydrogen-bonded systems, such as the enol of malondialdehyde and the title compound, in which it is necessary to distinguish between a truly symmetric hydrogen bond with a single well potential function and a pair of rapidly equilibrating tautomers in which the proton experiences a symmetric double well potential. Several criteria, including X-ray photoelectron spectra,<sup>2</sup> hydrogen-deuterium-tritium chemical-shift isotope effects,<sup>3</sup> and deuteron quadrupole coupling constants,<sup>4</sup> have been successfully applied to this problem. Such systems have also been the subject of several sophisticated quantum mechanical calculations.<sup>5</sup> X-ray and neutron diffraction methods on the other hand have been less successful in that it is difficult to distinguish between a symmetric molecular structure and a symmetric Fourier map which arises from static or dynamic disorder associated with the existence of tautomers.<sup>6</sup> We have recently described<sup>7</sup> a method, based on studies of spin-lattice relaxation times, which can locate the position of a proton in an unsymmetrical hydrogen bond, and we now show that it can be extended to resolve the position(s) of a proton in potentially symmetric situations.

The spin-lattice relaxation method utilizes the strong dependence on internuclear separation ( $1/r^6$ ) of the contribution of the bridging proton to the observed  $T_1$ 's of neighboring, fully substituted <sup>13</sup>C atoms. The precise contribution is found from a comparison of the  $T_1$ 's for the protio and deuterio species. The relaxation times for various proton bearing carbons provide the necessary knowledge of the rotational diffusion pa-

rameters which together with the C-H bond lengths allow calculation of the unknown internuclear distances.

We now report a study of the title compound<sup>8</sup> (Figure 1). An X-ray diffraction structure<sup>9</sup> of the analogous *N,N'*-dimethyl compound was found to be consistent with the existence of a symmetric hydrogen bond or with a disordered structure due to a statistical distribution of tautomers. The diphenyl derivative is symmetric on the <sup>13</sup>C chemical shift time scale (26 °C) so that only two <sup>13</sup>C resonances, viz., those of C(1), C(7), and ipso pair, provide relaxation data. Furthermore, because of the proximity of the other protons and the nitrogen nuclei, the contribution of the bridging proton to the relaxation times of these positions is relatively small. For this reason we have studied the compound fully labeled with <sup>15</sup>N.<sup>10</sup> The pertinent data are presented in Table I.

**Table I.** <sup>13</sup>C and <sup>15</sup>N Spin Lattice Relaxation Times (seconds) for 1-Phenylamino-7-phenylimino-1,3,5-cycloheptatriene (0.30 M) in Deuteriobromobromine at 26 °C

	$\delta$ , ppm <sup>a</sup>	$T_1^{\text{obsd}}$ (H)	$T_1^{\text{obsd}}$ (D)	$T_1^{\text{DD}}$ (H) <sup>b</sup>
2,6	113.9	0.387 ± 0.005	0.387 ± 0.004	
3,5	132.3	0.463 ± 0.006	0.441 ± 0.006	
4	121.0	0.504 ± 0.007	0.540 ± 0.008	
para	122.8	0.391 ± 0.003	0.396 ± 0.004	
1,7	150.3	8.8 ± 0.2	11.1 ± 0.2	40 ± 4
ipso	143.7	9.7 ± 0.1	12.1 ± 0.2	46 ± 4
<sup>15</sup> N	164.8 <sup>c</sup>	4.17 ± 0.04	24.8 ± 0.3	4.71 ± 0.05

<sup>a</sup> The assignments are based on single-frequency off-resonance decoupling experiments and long-range (three-bond) <sup>13</sup>CH splittings. <sup>b</sup> Contribution of the labile proton to the relaxation time.<sup>7</sup> <sup>c</sup> Downfield from external 1.0 M aqueous <sup>15</sup>NH<sub>4</sub>Cl.

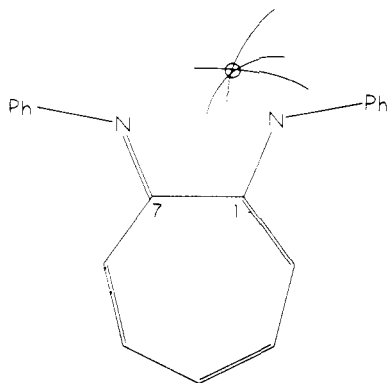


Figure 1. Position of the labile proton in 1-phenylamino-7-phenylimino-1,3,5-cycloheptatriene.

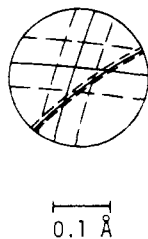


Figure 2. Inset from Figure 1 indicating propagated errors (---) in the computed loci (—).

The value of  $T_1^{DD}$  (H) for the  $^{15}\text{N}$  nuclei correspond to internuclear N-H distance which precludes a symmetric structure for any reasonable C(1)-N-H bond angles. It is therefore necessary to determine loci of proton positions, relative to the probe nuclei, on the basis of rapidly equilibrating tautomers. It is not possible to do this explicitly, but the loci have been found by successive approximation and are depicted in Figures 1 and 2. It is the nature of the problem that a true skeletal geometry for the molecule is unavailable through diffraction methods. Nevertheless, a reasonable geometry can be predicted. That in Figure 1 is based on the X-ray structure of the dimethyl derivative except that averaged C(1)-N and C(7)-N bond lengths found in that structure have been increased and decreased, respectively, by  $0.04 \text{ \AA}$ .<sup>11</sup> The N-C(ipso) bond lengths have been assumed to be  $1.42 \text{ \AA}$ , the value found in *N,N'*-diphenyl-6-aminofulvene-2-aldimine.<sup>13</sup>

Figure 2 indicates the precision of the method, the outer pairs of loci (dashed lines) being based on the fully propagated errors in the calculations.<sup>14</sup> It is gratifying to note that there is a region of mutual intersection which defines the position of the proton. We believe that the greatest uncertainty is, in fact, that of the skeletal geometry. The N-H bond length, which is essentially independent of the skeletal geometry and is found with high precision ( $1.072 \pm 0.004 \text{ \AA}$ ) is a vibrationally averaged value. The C(1)-NH bond angle is  $116 \pm 3^\circ$  in which, as can be seen from Figure 2, the uncertainty is associated primarily with the precision indices of the two loci calculated from the  $^{13}\text{C}$  relaxation times. The position of the proton clearly establishes the presence of a symmetric double-well potential function for the hydrogen bond in this molecule. This agrees with observation<sup>16</sup> of a reasonably large (203-kHz) deuteron quadrupole coupling constant for the N-deuterated species. The proton is, in fact, located in the same position as a peak found near each nitrogen atom in a difference electron density synthesis described in the X-ray diffraction study.<sup>9</sup>

The procedure embodied in this example appears to be applicable to a number of related systems. In addition, it underscores the utility of  $^{15}\text{N}$  as a sensitive probe for determining nitrogen-hydrogen internuclear distances.

**Acknowledgment.** The authors are indebted to the National Science Foundation for the support of this research under Grant No CHE76-2-879 and to Dr. R. E. Benson for his interest in this work.

## References and Notes

- (1) Part 16 of the series "Studies in Nuclear Magnetic Resonance Spectroscopy". For part 15, see *J. Natural Prod.*, in press.
- (2) R. S. Brown, *J. Am. Chem. Soc.*, **99**, 5497 (1977); R. S. Brown, A. Tse, T. Nakashima, and R. C. Haddon, *ibid.*, **101**, 3157 (1979).
- (3) L. J. Altman, D. Laungani, G. Gunnarsson, H. Wennerström, and S. Forsén, *J. Am. Chem. Soc.*, **100**, 8264 (1978).
- (4) W. Egan, G. Gunnarsson, T. W. Bull, and S. Forsén, *J. Am. Chem. Soc.*, **99**, 4568 (1977).
- (5) G. Karlström, H. Wennerström, B. Jönsson, S. Forsén, J. Almlöf, and B. Roos, *J. Am. Chem. Soc.*, **97**, 4188 (1975); G. Karlström, B. Jönsson, B. Roos, and H. Wennerström, *ibid.*, **98**, 6851 (1976); E. M. Fluder and J. R. de la Vega, *ibid.*, **100**, 5265 (1978).
- (6) I. Olovsson and P.-G. Jönsson in "The Hydrogen Bond", P. Schuster, G. Zundel, and C. Sandorfy, Eds., North-Holland Publishing Co., Amsterdam, 1976, Chapter 8.
- (7) L. M. Jackman and J. C. Trewella, *J. Am. Chem. Soc.*, **98**, 5712 (1976).
- (8) W. R. Brasen, H. E. Holmquist, and R. E. Benson, *J. Am. Chem. Soc.*, **83**, 3125 (1961).
- (9) P. Goldstein and K. N. Trueblood, *Acta Crystallogr.*, **23**, 148 (1967).
- (10) The labeled compound was prepared by the literature method<sup>8</sup> using 95%  $^{15}\text{N}$ -enriched aniline.
- (11) The difference of  $0.08 \text{ \AA}$  corresponds to the bond alternation of the carbon-oxygen bonds in tropolones.<sup>12</sup>
- (12) H. Shimanouchi and Y. Sasada, *Acta Crystallogr., Sect. B*, **29**, 81 (1973); T. A. Hamor and J. E. Derry, *ibid.*, **29**, 2649 (1973).
- (13) H. L. Ammon and V. Mueller-Westerhoff, *Tetrahedron*, **30**, 1437 (1974).
- (14) The rotational diffusion parameters were calculated from the  $T_1$  values for the 2(6), 3(5), 4, and para (para') carbon atoms by a nonlinear least-squares fitting of the equation  $1/T_1 = \gamma_C^2 \gamma_H^2 \hbar^2 r_{CH}^{-6} (A \cos^2 \Psi + B \sin^2 \Psi)$  using a vibrationally averaged value of  $r_{CH}$  ( $1.107 \text{ \AA}$ ). This equation assumes the molecule can be treated as a planar uncoupled rotor.<sup>15</sup> The  $T_1$  values were corrected for the small contributions of the "ortho" protons. The values of  $A$  and  $B$  so determined were then used to evaluate the unknown internuclear distances.
- (15) (a) W. T. Huntress, *J. Chem. Phys.*, **48**, 3524 (1968); *Adv. Magn. Reson.*, **4**, Chapter 1 (1970).
- (16) L. M. Jackman and J. C. Trewella, unpublished results.

L. M. Jackman,\* J. C. Trewella  
Department of Chemistry  
The Pennsylvania State University  
University Park, Pennsylvania 16802  
Received May 22, 1979

## New Conjunctive Reagents.

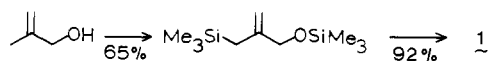
### 2-Acetoxyethyl-3-allyltrimethylsilane for Methylene-cyclopentane Annulations Catalyzed by Palladium(0)

Sir:

The increasing number of cyclopentanoid natural products and heightened interest in potentially anti-aromatic systems such as the pentalenes suggest the need for novel annulating approaches to cyclopentane systems.<sup>1,2</sup> We report herein that 2-acetoxyethyl-3-allyltrimethylsilane (**1**) serves as a novel annulating agent with olefins bearing electron-withdrawing groups in the presence of a palladium(0) catalyst<sup>3</sup> according to eq 1.



The requisite conjunctive reagent **1** was prepared by metalating  $\alpha$ -methylallyl alcohol<sup>4</sup> (2 equiv of *n*-C<sub>4</sub>H<sub>9</sub>Li in ether,



2 equiv of TMEDA,  $0^\circ\text{C}$ , then add THF,  $0^\circ\text{C} \rightarrow$  room temperature), followed by quenching with trimethylsilyl chloride.