venom phospholipase A_2 binds to the phospholipid substrate in the mixed micelle where the enzymatic reaction occurs.[11] In the present work we show that the mixed micelle system is ideally suited for inhibition studies with phospholipase A_2 since both the inhibitor and substrate can compete for the binding to the active site of the enzyme in the surface of the mixed micelle and is free from the complexities that arise from lipid-lipid interactions. Although the mixed-micelle system is perhaps a poor model of a biological membrane, it provides a reliable method for ranking inhibitors according to their relative free energies of interaction with the enzyme.

Mixed micelles for inhibition studies were prepared by sonicating fixed amounts of Triton X-100 (40 mM) and substrate (dipalmitoyl phosphatidylcholine, 5 mM) with variable amounts of inhibitor in water-containing $CaCl_2$ (10 mM).[12] Enzyme was added, and the reaction velocity was determined in a pH-stat at pH 8.0 and 40 °C.[9] The initial enzymatic velocity as a function of the concentration of inhibitor **1c** is shown in Figure 1. Two-chain analogue **1c** is seen to produce 50% inhibition (IC_{50} value) at a concentration of 5 μM. This compound is the most potent phospholipase A_2 inhibitor reported to date, being significantly more effective than amide analogues[13] and fluorinated ketone analogues.[4] The IC_{50} of methyl phosphonate **2** was found to be 1.25 mM, some 250-fold higher than for **1c**, demonstrating the critical role of the phosphonate anion in the interaction with the enzyme. The IC_{50} values measured for single-chain phosphonates **1a** and **1b** were 0.75 mM and 2.3 mM, respectively, showing that the upper alkyl chain of **1c** plays a significant role in the binding to the enzyme.[14]

Since mixed micelles were used and the fact that the inhibition is seen with levels of **1c** some 1000-fold lower than the amount of substrate make it highly unlikely that the inhibition is due to an inhibitor-induced change in the structure of the substrate aggregate. Rather it appears that these phosphonate-containing phospholipid analogues are interacting tightly with the catalytic site on the enzyme.[15] The phosphonate group may be coordinating to the active-site calcium. Perhaps the most important contribution of this work is the demonstration that the potency of inhibition correlates in a reasonable way with the chemical structure of the inhibitors provided that a mixed micelle assay is used.[7] The possibility of using phosphonates such as **1c** together with sulfur-substituted compounds such as thiophosphonates to probe the role of the calcium ion in the catalysis is particularly intriguing and is under active investigation.

Acknowledgment. The generous support of this research by the National Institutes of Health (HL-36235) and helpful discussion with Professor Ed Dennis and Trevor Payne are gratefully acknowledged. Mass spectra were obtained at the UCSF resource (A. L. Burlingame Director, NIH RR01614).

Supplementary Material Available: Experimental procedures and physical data for the preparation of all new compounds and enzyme inhibition analysis (5 pages). Ordering information is given on any current masthead page.

(11) Roberts, M. F.; Deems, R. A.; Dennis, E. A. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1950.
(12) Substrate hydrolysis by phospholipase A_2 is totally dependent on enzyme-bound calcium (ref 6).
(13) Davidson, F. F.; Hajdu, J.; Dennis, E. A. *Biochem. Biophys. Res. Commun.* **1986**, *137*, 587.
(14) The higher potency of ethanolamine-containing inhibitor **1a** compared to choline-containing inhibitor **1b** is consistent with our earlier findings (see ref 4 for a complete discussion).
(15) It should be pointed out that the use of bulk concentrations (mol/vol) to express inhibitor potencies is useful in comparing a series of inhibitors according to potency as long as the same mixed micelle assay is used in all cases. However, the kinetics of phospholipase A_2 hydrolysis of mixed micelles is sensitive to both the bulk and surface (mol/surface) substrate concentrations: Hendrickson, H. S.; Dennis, E. A. *J. Biol. Chem.* **1984**, *259*, 5734. In the present study, the bulk concentration of substrate used (5 mM) is sufficient to ensure that all of the enzyme is bound to phospholipid in the micelle. Thus, the observed inhibition is due to a competition between substrate and inhibitor for the binding to the enzyme in the surface of the micelle.

Mechanism of the 1,5-Sigmatropic Hydrogen Shift in 1,3-Pentadiene

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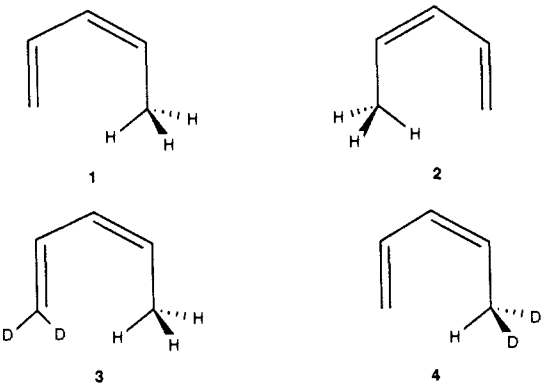
Some years ago Roth and Konig[1] reported a surprisingly large value (5.1 at 473 K) for the primary deuterium kinetic isotope effect (KIE) in the 1,5-sigmatropic rearrangement of 1,3-pentadiene (**1** ⇄ **2**). Extrapolation to room temperature, using the



observed activation parameters, led moreover to a value (12.2) which seemed unreasonably large. MINDO/3[2] calculations predicted a value (2.5) similar to those reported for analogous processes. The reaction therefore seems unlikely to involve a normal thermal rearrangement, and normal ground-state tunnelling seems ruled out by the necessarily large change in geometry. Dewar et al.[2] suggested that the discrepancy might be due to tunnelling from vibrationally excited states (vibrationally assisted tunnelling; VAT), a suggestion supported by an approximate calculation of the VAT rate. The activation energy calculated by MINDO/3 was moreover higher than that observed by 10 kcal/mol, and the KIE corrected for VAT agreed with experiment.

While subsequent ab initio calculations by Dormans and Buck[3] have supported these conclusions, they have recently been challenged by Jensen and Houk[4] on the basis of further ab initio calculations allowing for correlation by Møller-Plesset (MP) perturbation theory.[5] The fact that the calculated activation energy agreed with experiment was taken as evidence that VAT plays a negligible role. The KIE[6] (2.52) calculated by using the 3-21G basis set, but without allowance for correlation, agreed with the MINDO/3 value and was likewise far less than that observed. Jensen and Houk dismissed this discrepancy on the grounds that better agreement with experiment might be expected if correlation were included. They implied that such a calculation would have been impracticable.

The difference between the MINDO/3 and MP activation energies is, however, too small for reliable conclusions to be drawn. It is, for example, the same as the error (10 kcal/mol) in the activation energy calculated by Breulet and Schaefer[7] for a simpler reaction (ring opening of cyclobutene) by using a better ab initio procedure than that of Jensen and Houk. On the other hand,

(1) Roth, W. R.; Konig, J. *Liebigs Ann. Chem.* **1966**, *699*, 24. Roth, W. R. *Chimia* **1966**, *20*, 229.
(2) Dewar, M. J. S.; Merz, K. M., Jr.; Stewart, J. J. P. *J. Chem. Soc., Chem. Commun.* **1985**, 166.
(3) Dormans, G. J. M.; Buck, H. M. *J. Am. Chem. Soc.* **1986**, *108*, 3253. Note that an incorrect geometry was used for the transition state.
(4) Jensen, F.; Houk, K. N. *J. Am. Chem. Soc.* **1987**, *109*, 3139.
(5) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.
(6) Hess, B. A., Jr.; Schaad, L. J.; Pancir, J. *J. Am. Chem. Soc.* **1985**, *107*, 149.
(7) Breulet, J.; Schaefer, H. F., III. *J. Am. Chem. Soc.* **1984**, *106*, 1221.

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Table I. Kinetic Isotope Effects (k_H/k_D) for the 1,5-Hydrogen Shift of 1,3-Pentadiene Using MP2/3-21G Frequencies at 473 K

reactant	calcd	exptl[1]
1,3-pentadiene	2.53	
1,1-dideuterio-1,3-pentadiene	2.54	5.10
5,5-dideuterio-1,3-pentadiene	2.58	5.10

calculations of isotope effects are not expected to be in error by more than the errors in the calculated frequencies of molecular vibrations, which are usually <10%. It therefore seems extremely unlikely that a calculated isotope effect could be in error by a factor of two, due simply to neglect of electron correlation.

Since this problem involved issues of general significance and since it seemed well within the scope of available supercomputers, we decided to calculate the deuterium kinetic isotope effect for the rearrangement of **1** at the MP2/3-21G level. The calculations reported below took 30 h of cpu time on a CRAY X-MP/24 computer.

The s-trans conformation of **1** and the transition state geometries were optimized at the MP2/3-21G level by using the GAUSSIAN 82 program.[8] The transition state was optimized under C_s symmetry, since this corresponds to the lowest saddle point at the MP2 level.[4] MP2/3-21G force constant matrices were calculated for the optimized geometries by using GAUSSIAN 82. The force constant matrices were mass weighted and converted to frequencies with a program[9] that inputs the GAUSSIAN 82 archive file. The KIEs were calculated by using the rigid-rotor-harmonic-oscillator approximation.[10]

The experimental value[1] for the primary KIE was determined from the 1,1-dideuterio (**3**) and 5,5-dideuterio (**4**) derivatives of **1**, ignoring possible secondary deuterium KIEs. We calculated KIEs for **1**, **3**, and **4** both to compare with experiment and to estimate the secondary isotope effects. Table I contains the results. While none of the calculated KIEs are close to the experimental value, all are close to those from the RHF/3-21G (2.52) and MINDO/3 (2.50) calculations. The KIEs calculated for **3** and **4** differed very little from that for **1**, indicating that the secondary KIEs are small.

These results support the arguments presented by Dewar et al. for the intervention of VAT in this reaction. If VAT is indeed involved, the true activation energy for the reaction must be significantly larger than the experimental one and so larger than the value calculated by Jensen and Houk, the agreement between the latter and experiment being coincidental. For reasons indicated above, this could well be the case.

It should be remembered that *no* current procedure, ab initio or semiempirical, can lead by itself to reliable predictions of reaction mechanisms. The best approach is to combine experiment with calculation, including data for as many different properties and for as many different molecules as possible. Isotope effects and entropies of activation are particularly useful in this connection because they are more directly related to the mechanism of a reaction than enthalpies of activation and because it is also easier to calculate them with the requisite accuracy.

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(8) Binkley, J. S.; Frisch, M. J.; Defrees, D. J.; Raghavachari, K.; Whiteside, R. A.; Schlegal, H. B.; Fluder, E. M.; Pople, J. A. Carnegie Mellon University: Pittsburgh, PA.

(9) To be submitted to Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN.

(10) van Hook, W. A. In *Isotope Effects in Chemical Reactions*; Collins, C. J., Bowman, N. S., Eds.; Van Nostrand: New York, 1970.

Trajectories of Proton-Transfer Reactions. Experimental Determination of the Magnitude of Primary Deuterium Isotope Effects for Proton Transfers Occurring at Acute Angles

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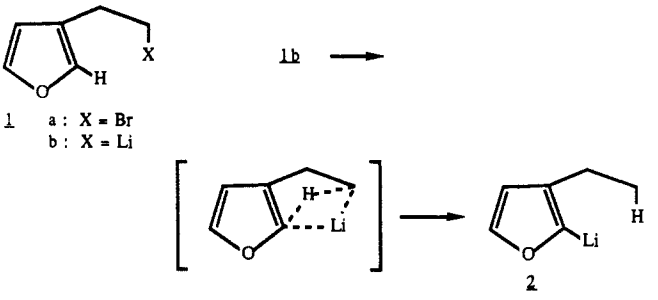
Proton-transfer reactions represent one of the most fundamental classes of chemical reactions. Although most proton-transfer reactions preferentially occur at or near angles of 180°,[1,2] some can deviate significantly from a linear trajectory.[3,4a,b] However, little is known about (a) the energy costs associated with deviations from linearity and (b) correlations between the angle of a proton transfer and the magnitude of its kinetic isotope effect.[5] This communication attempts to address both of these issues.

The reaction we have utilized to probe these questions is the conversion of 2-(3-furyl)ethyllithium, **1b**, to 3-ethyl-2-lithiofuran, **2**. This reaction appeared to be an ideal case to study for the



1 a: X = Br
b: X = Li

2

following reasons. First, semiempirical calculations on anions **1b** and **2** (with and without lithium) indicated that the proton-transfer process should possess a large thermodynamic driving force (ΔH_{reac} ranged from −15 to −20 kcal/mol).[6] Second, the cal index intramolecular proton transfers must occur at angles which are close to 90°.[7] Third, since this reaction is carried out in an aprotic solvent, problems associated with solvent insertion in the transition state become unimportant.

In our first experiment, lithium alkyl **1b** was generated in situ by a lithium–halogen exchange reaction (2 equiv of *t*-BuLi/THF, −78 °C to 0 °C, 2 h) on bromide **1a**. After addition of quinone

(1) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265.

(2) For example, hydrogen bonds (which are considered to be on a reaction coordinate for proton transfer) are often linear. See: (a) Strohbusch, F.; Marshall, D. B.; Eyring, E. M. *J. Phys. Chem.* **1978**, *82*, 2447. (b) Scott, R.; Vinogradov, S. *J. Phys. Chem.* **1969**, *73*, 1890. (c) Jones, R. E.; Templeton, D. H. *Acta Crystallogr.* **1958**, *11*, 484.

(3) Maercker et al. have presented strong evidence that the 1,4 proton shift in 4,4-diphenylbutyllithium occurs in an intramolecular fashion. See: Maercker, A.; Passlack, M. *Chem. Ber.* **1983**, *116*, 710.

(4) Both Menger[4a] and Tidwell[4b] have suggested that certain norbornyl derivatives undergo intramolecular proton abstraction reactions at angles which may be as small as 100°.[5] However, since these proton-transfer reactions were performed in protic solvents, solvent insertion in the transition state cannot be ruled out. Under these conditions it is difficult to determine angular relationships with certainty. See: (a) Menger, F. M.; Chow, J. F.; Kaiserman, H.; Vasquez, P. C. *J. Am. Chem. Soc.* **1983**, *105*, 4996. (b) Abad, G. A.; Jindal, S. P.; Tidwell, T. T. *J. Am. Chem. Soc.* **1973**, *95*, 6326.

(5) (a) For some theoretical treatments of the effects of nonlinear proton-transfer transition states on isotope effects, see: More O'Ferrall, R. A. *J. Chem. Soc. B* **1970**, 785. Anhede, B.; Bergman, N.-A. *J. Am. Chem. Soc.* **1984**, *106*, 7634. Zhou, P.; Vitale, A. A.; San Fillippo, J.; Saunders, W. H. *J. Am. Chem. Soc.* **1985**, *107*, 8049. (b) For an interesting overview on the effects of directionality on chemical reactivity, see: Menger, F. M. *Tetrahedron* **1983**, *39*, 1013.

(6) The calculations use the MNDO method and involve either simple anions or monomeric lithium alkyls which have been fully optimized. However, the energies reported here are only meant to suggest the high exothermicity of this process and not to represent an accurate quantitative measure of ΔH_{react}.

(7) According to these calculations, the pertinent CHC angles are as follows: **1b** (without lithium) = 82°, **2** (without lithium) = 79°, **1b** (with lithium) = 104°, and **2** (with lithium) = 93°.

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