

(d, 6 H,  $J_{PH} = 11.4$  Hz, phosphate methyl H).

Compound **9** was obtained as follows. A solution of 2.86 g of diphenylpropanetrione in 4 mL of anhydrous  $CH_2Cl_2$  (3 M solution) was added dropwise to a solution of 2.33 g of dimethylphosphorus dimethylphosphoric anhydride in 1 mL of  $CH_2Cl_2$  (12 M solution) at 0-5 °C with stirring under a dry  $N_2$  atmosphere. After the reaction was completed, the solution became yellow and the solvent was removed at 20 °C under reduced pressure. The product could not be distilled but was NMR pure. Due to its hygroscopic nature no satisfactory elemental analysis could be obtained:  $^1H$  NMR ( $CDCl_3$ , 35 °C)  $\delta$  3.70 (d, 6 H,  $J_{PH} = 11.25$  Hz, P(IV) methoxy H), 3.87 (d, 6 H,  $J_{PH} = 15.75$  Hz, P(V) methoxy H), 7.35 (m, 6 H, aromatic H), 7.80 (m, 4 H, aromatic H);  $^1H$  NMR ( $CD_2Cl_2$ , -80 °C)  $\delta$  3.75 (d, 6 H,  $J_{PH} = 11.25$  Hz, P(IV) methoxy H), 3.90 (d, broadened,  $J_{PH} = 15.75$  Hz, P(V) methoxy H), 7.47 (m, 6 H, aromatic H), 7.85 (m, broadened, 4 H, aromatic H);  $^{31}P$  NMR ( $CD_2Cl_2$ , -80 °C)  $\delta$  -57.7 (d,  $J_{PP} = 27$  Hz, P(V)), -7.7 (d,  $J_{PP} = 27$  Hz, P(IV)).

**Triethoxy-3-(oxobutyl)phosphonium Fluoroborate (10).** Diethyl 3-(oxobutyl)phosphonate was obtained as follows. Addition of an equivalent amount of acetic acid to a solution of 2,2,2-triethoxy-5-methyl-2,2,3,3-tetrahydro-1,2-oxaphosphole in hexane (~0.1 M solution) resulted in an exothermic reaction with formation of ethyl acetate and the phosphonate. After evaporation of the hexane the residue could be distilled to give pure phosphonate in a nearly quantitative yield:  $^1H$  NMR ( $CDCl_3$ , 35 °C)  $\delta$  1.27 (t, 6 H,  $J_{HH} = 7$  Hz, ethoxy methyl H), ca. 1.90 (m, 2 H,  $PCH_2$ ), 2.15 (s, 3 H,  $CH_3CO$ ), ca. 2.71 (m, 2 H,  $CH_2CO$ ), 4.07 (dq, 4 H,  $J_{PH} = J_{HH} = 7$  Hz, ethoxy ethyl H).

Reaction of equivalent amounts of diethyl 3-(oxobutyl)phosphonate and triethylxonium fluoroborate<sup>30</sup> in dry  $CH_2Cl_2$  (0.1 M solution) at room temperature for several hours resulted in the formation of the phosphonium salt **10**. The pure compound could be isolated by precipitation in ether, followed by thorough washing with ether. The compound was very hygroscopic, and elemental analysis constantly gave low C and N values:  $^1H$  NMR ( $CH_2Cl_2$ , 35 °C)  $\delta$  1.47 (t, 9 H,  $J_{HH} = 7$  Hz, ethoxy methyl H), 2.21 (s, 3 H,  $CH_3CO$ ), 3.30-2.30 (m, 4 H,  $PCH_2CH_2$ ), 4.55 (dq, 6 H,  $J_{PH} = J_{HH} = 7$  Hz, ethoxy ethyl H).

**Spectroscopic Study of the Reactions of Compounds 1-9 with  $FSO_3H$  in  $CH_2Cl_2$ .** Fluorosulfonic acid was distilled at

atmospheric pressure under a stream of dry nitrogen (bp 167 °C). Due to the very hygroscopic nature of this acid, the storage time is limited, and therefore mainly freshly distilled acid was used. For all the compounds investigated, 1 M solutions were made in  $CH_2Cl_2$  and an aliquot (~0.4 mL) was transferred to an NMR sample tube, after which it was cooled to -85 °C. Next, approximately 0.5 equiv of  $FSO_3H$  was added, and after thorough mixing of the sample the NMR spectra of the sample were investigated in the temperature range of -80 to -10 °C. It should be mentioned that product formation (dealkylation) and keto-enol tautomerization could never be completely eliminated, and therefore, especially at higher temperatures, the measurement times were limited. Therefore, several aliquots of each compound were investigated independently. The activation energy for the equilibrium of **3** and **3'** was determined from line-broadening measurements of the methoxy doublets of both compounds in the temperature range of -50 to -15 °C in which a coalescence of these signals was observed. This experiment was repeated for a large number of independent samples in order to eliminate secondary effects due to decomposition as much as possible. The activation energy for the equilibrium of **2** and **2'** was determined from line-broadening measurements of the  $^{31}P$  NMR signal in the temperature range of -90 to -60 °C.

**Acknowledgment.** We thank Dr. J. W. de Haan and Mr. L. J. M. van de Ven for their assistance in recording and interpreting the  $^{13}C$  and  $^{31}P$  NMR spectra. This investigation has been supported by the Netherlands Foundation for Chemical Research (SON) with financial aid from the Netherlands Organization for the Advancement of Pure Research (ZWO).

**Registry No.** 1, 26192-22-3; 1', 75421-19-1; 2, 75444-60-9; 2', 75421-20-4; 3, 1665-79-8; 3', 75444-07-4; 4, 4903-06-4; 4', 75421-21-5; 5, 2908-28-3; 5', 75421-22-6; 6, 4850-55-9; 6', 75421-23-7; 7 (isomer 1), 4130-26-1; 7 (isomer 2), 75444-08-5; 7' (isomer 1), 75421-24-8; 7' (isomer 2), 75444-09-6; 8, 16190-84-4; 8', 75421-25-9; 9, 75421-26-0; 10, 75421-28-2; 11, 75421-29-3; trimethyl phosphite, 121-45-9; methyl vinyl ketone, 78-94-4; 3-(*p*-chlorobenzylidene)-2,4-pentanedione, 19411-75-7; biacetyl, 431-03-8; phenanthrenequinone, 84-11-7; diphenylpropanetrione, 643-75-4; benzil, 134-81-6; *o*-nitrobenzaldehyde, 552-89-6; dimethylphosphorochlorite, 813-77-4; dimethyl phosphate, 813-78-5; dimethylphosphorus dimethylphosphoric anhydride, 1067-83-0; diethyl 3-(oxobutyl)phosphonate, 1067-90-9.

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## Relation of the Transition-State Structure for the Water-Catalyzed Hydrolysis of 1-Acetylimidazolium Ion to Solvent Hydrophobicity: Proton Inventories in Water-Acetonitrile Mixtures<sup>1</sup>

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The transition-state structure for the water-catalyzed hydrolysis of 1-acetylimidazolium ion has been probed in solvent systems which may mimic the hydrophobic nature of an enzyme's active site. The kinetic solvent deuterium isotope effects,  $k_{H_2O}/k_{D_2O}$ , are 2.58, 2.49, and 2.10 in water, in 0.5 vol fraction of acetonitrile in water, and in 0.9 vol fraction of acetonitrile in water, respectively. The proton inventory investigations suggest all three solvent systems entertain a transition-state structure composed of a catalytic proton bridge between the reorganizing substrate and a water molecule acting as a general-base catalyst. A "compression" of the transition-state structure in the solvent system containing the largest amount of acetonitrile is suggested to be responsible for the diminished kinetic solvent deuterium isotope effect. The reaction has been shown to be second order with respect to water.

The proton inventory technique has recently been used to help elucidate transition-state structures for a number

of organic<sup>3-12</sup> and enzyme-catalyzed reactions.<sup>13-20</sup> There is no doubt that the enzymes for which the organic reac-

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Table I. First-Order Rate Constants for the Hydrolysis of 1-Acetylimidazolium Ion in 0.5 Vol Fraction of Acetonitrile<sup>a</sup>

$n^e$	no. of runs	$10^5 k_n, ^b \text{ s}^{-1}$
0.000	5	$1176 \pm 11^c$
0.240	6	$980 \pm 13$
0.480	6	$789 \pm 4$
0.720	5	$623 \pm 14$
0.959 <sup>d</sup>	5	$472 \pm 7$

<sup>a</sup> Reaction solutions had  $10^{-2}$  N HCl-DCl, and the ionic strength was maintained at 0.2 M with NaCl. <sup>b</sup> Rate constants were calculated by using a nonlinear least-squares program. <sup>c</sup> Error limits are standard deviations. <sup>d</sup> Atom fraction deuterium of exchangeable protons was calculated from an analysis of a "100%" D<sub>2</sub>O-acetonitrile sample by Mr. Josef Nemeth.<sup>34</sup> <sup>e</sup> Atom fraction of deuterium.

Table II. First-Order Rate Constants for the Hydrolysis of 1-Acetylimidazolium Ion in 0.9 Vol Fraction of Acetonitrile<sup>a</sup>

$n^e$	no. of runs	$10^6 k_n, ^b \text{ s}^{-1}$
0.000	5	$675 \pm 5^c$
0.243	3	$575 \pm 3$
0.486	4	$489 \pm 4$
0.729	3	$404 \pm 6$
0.972 <sup>d</sup>	6	$323 \pm 9$

<sup>a</sup> Reaction solutions had  $10^{-2}$  N HCl-DCl, and the ionic strength was maintained at 0.025 M with NaCl. <sup>b</sup> Rate constants were calculated by using a nonlinear least-squares program. <sup>c</sup> Error limits are standard deviations. <sup>d</sup> Atom fraction deuterium of exchangeable protons was calculated from an analysis of a "100%" D<sub>2</sub>O-acetonitrile sample by Mr. Josef Nemeth.<sup>34</sup> <sup>e</sup> Atom fraction of deuterium.

tions are studied as model systems may have relatively hydrophobic active sites which are not being adequately mimicked in the aqueous model system studies. The

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Table III. First-Order Rate Constants for the Hydrolysis of 1-Acetylimidazolium as a Function of Acetonitrile Concentration<sup>a</sup>

vol fract CH <sub>3</sub> CN <sup>c</sup>	no. of runs	$10^4 k_{\text{obsd}}, \text{ s}^{-1}$
0.00	4	$527 \pm 8$
0.10	9	$399 \pm 17$
0.20	4	$303 \pm 12$
0.30	4	$222 \pm 2$
0.40	5	$166 \pm 3$
0.50	4	$122 \pm 6$
0.60	4	$87.1 \pm 0.7$
0.70	4	$56.4 \pm 1.3$
0.75	3	$42.5 \pm 0.6$
0.90	5	$6.88 \pm 0.08$
0.00 (D <sub>2</sub> O)	3	$204 \pm 2$

<sup>a</sup> Reaction solutions contained  $10^{-2}$  N HCl, and the ionic strength was maintained at 0.025 M with NaCl. <sup>b</sup> Error limits are standard deviations. <sup>c</sup> Before mixing.

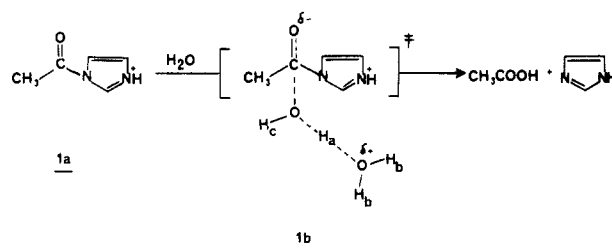
Table IV. Volume Fractions of Acetonitrile before Mixing and Corresponding Volumes after Mixing along with Water Concentrations for Mixtures<sup>a</sup>

vol fract of CH <sub>3</sub> CN before mixing	vol after mixing, mL	[H <sub>2</sub> O], M
0.00	20.00	55.34
0.10	$19.78 \pm 0.18$	50.36
0.20	$19.67 \pm 0.18$	45.01
0.30	$19.56 \pm 0.18$	39.61
0.40	$19.60 \pm 0.18$	33.88
0.50	$19.59 \pm 0.18$	28.25
0.60	$19.61 \pm 0.18$	22.58
0.70	$19.69 \pm 0.18$	16.86
0.75	$19.73 \pm 0.18$	14.02
0.90	$19.84 \pm 0.18$	5.58

<sup>a</sup> Water solutions contained enough NaCl and HCl to give an ionic strength of 0.025 M and a concentration of  $10^{-2}$  N HCl for 20 mL of the final water-acetonitrile mixture.

present study is directed at probing the influence of solvent system hydrophobicity upon solvent deuterium isotope effects and proton inventories.

Because of the importance of acyl-transfer and hydrolysis reactions in biochemical systems, we have chosen 1-acetylimidazolium ion (**1a**) hydrolysis as a suitable model



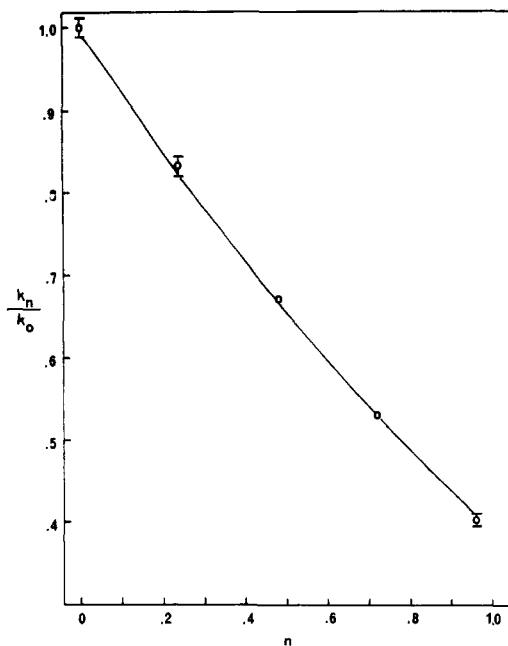
system for this study. Previous investigations had shown that the pH-independent, water-catalyzed hydrolysis of 1-acetylimidazolium ion probably involved a transition-state structure such as that shown in **1b**.<sup>6,7,21-24</sup> We chose to see what, if any, influence the addition of significant amounts of organic cosolvent (acetonitrile) would have on the nature of the transition state for this well-characterized

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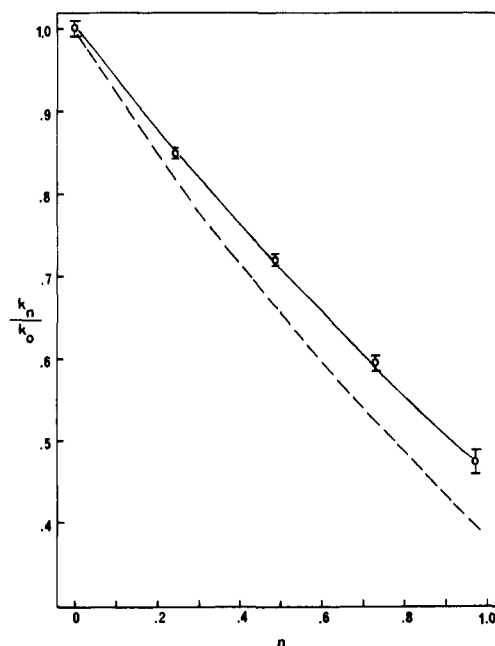
**Figure 1.** Proton inventory for the water-catalyzed hydrolysis of 1-acetylimidazolium ion in water-acetonitrile (0.5 vol fraction) solution. The solid line was calculated on the basis of the transition-state model described by eq 1. Where error bars are omitted the circles encompass them.

reaction. Since transition states such as 1b have been implicated in a large number of systems, the results of the present investigation were expected to be particularly useful. Details of the proton inventory technique are available in several excellent reviews and will not be presented here.<sup>25-27</sup>

### Results

Tables I and II contain the first-order rate constants for the hydrolysis of 1-acetylimidazolium ion in 0.5 and 0.9 volume fraction of acetonitrile in protium oxide-deuterium oxide mixtures of atom fraction of deuterium  $n$ . All solutions contained  $2 \times 10^{-2}$  N HCl (DCI) to maintain the solutions in the pH-independent region of the pH-rate profile. The ionic strength was maintained at 0.2 M with sodium chloride in the 0.5 vol fraction of acetonitrile solution and at 0.025 M in the 0.9 vol fraction of acetonitrile solution. This was necessary due to a salting-out influence at high acetonitrile concentrations. The ionic strength was shown to have no influence on the solvent deuterium isotope effect as can be seen from the data in Table III. The solvent isotope effect in water at pH 2 ( $\mu = 0.025$  M) is  $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 2.58$ . This value is in excellent agreement with the literature value of 2.58 ( $\mu = 0.2$  M).<sup>6</sup> Table III collects the data for the hydrolysis reaction in various volume fractions of acetonitrile with  $10^{-2}$  N HCl ( $\mu = 0.025$  M). Since reaction mixtures were prepared in terms of volume fractions before mixing, the volume change on mixing for each solution prepared was measured, and these results are shown in Table IV.

Figures 1 and 2 are proton inventories for the water-catalyzed hydrolysis of 1-acetylimidazolium ion prepared



**Figure 2.** Proton inventory for the water-catalyzed hydrolysis of 1-acetylimidazolium ion in water-acetonitrile (0.9 vol fraction) solution. The solid line was calculated from eq 6. The dashed line was calculated on the basis of eq 1 for the system with no acetonitrile. Where error bars are omitted the circles encompass them.

**Table V.** Comparison of Observed Rate Constants  $(k_n/k_0)_{\text{obsd}}$  for the Hydrolysis of 1-Acetylimidazolium Ion in 0.5 Vol Fraction (before Mixing) of Acetonitrile with Rate Constants  $(k_n/k_0)_{\text{calcd}}$  Calculated from Eq 1 and 2

$n$	no. of runs	$(k_n/k_0)_{\text{obsd}}^a$	$(k_n/k_0)_{\text{calcd}}$	
			eq 1	eq 2
0.000	5	$1.000 \pm 0.012^b$	1.000	1.000
0.240	6	$0.833 \pm 0.013$	0.825	0.827
0.480	6	$0.671 \pm 0.005$	0.668	0.671
0.720	5	$0.530 \pm 0.005$	0.529	0.530
0.959	5	$0.401 \pm 0.008$	0.407	0.403

<sup>a</sup> Calculated from mean values of rate constants in Table I. <sup>b</sup> Error limits were propagated from standard deviations.

**Table VI.** Comparison of Observed Rate Constants  $(k_n/k_0)_{\text{obsd}}$  for the Hydrolysis of Acetylimidazolium Ion in 0.9 Vol Fraction (before Mixing) of Acetonitrile with Rate Constants  $(k_n/k_0)_{\text{calcd}}$  Calculated from Eq 3, 6, and 7

$n$	no. of runs	$(k_n/k_0)_{\text{obsd}}^a$	$(k_n/k_0)_{\text{calcd}}$		
			eq 3	eq 6	eq 7
0.000	5	$1.000 \pm 0.010^b$	1.000	1.000	1.000
0.243	3	$0.848 \pm 0.008$	0.850	0.849	0.853
0.486	4	$0.721 \pm 0.008$	0.714	0.712	0.717
0.729	3	$0.596 \pm 0.010$	0.588	0.589	0.593
0.972	6	$0.476 \pm 0.014$	0.475	0.478	0.480

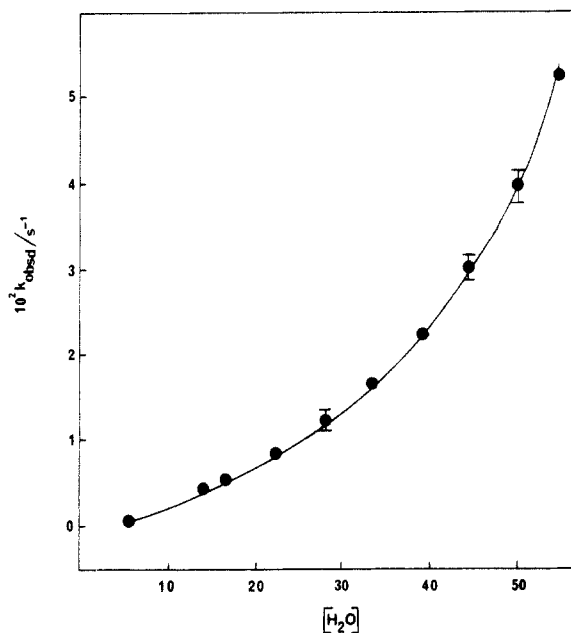
<sup>a</sup> Calculated from mean values of rate constants in Table II. <sup>b</sup> Error limits were propagated from standard deviations.

from the data of Tables V and VI. The kinetic solvent deuterium isotope effects measured were  $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}} = 2.49$  and 2.10 for 0.5 and 0.9 vol fraction of acetonitrile, respectively. Both proton inventories exhibit significant downward curvature. Figure 3 shows the dependence of hydrolysis rate constants on the concentration of water (i.e., data of Table IV) while Figure 4 is a plot of  $\ln k_{\text{obsd}}$  vs.  $\ln [\text{H}_2\text{O}]$ . The slope of the line in Figure 4 is 1.8 with a correlation coefficient of 0.995.

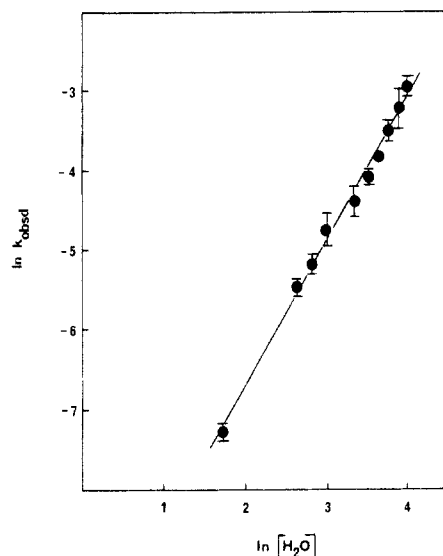
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**Figure 3.** Dependence of the observed rate constant for the water-catalyzed hydrolysis of 1-acetylimidazolium ion upon the concentration of water. Where error bars are omitted the circles encompass them.



**Figure 4.** Dependence of  $\ln k_{\text{obsd}}$  on  $\ln [\text{H}_2\text{O}]$ . The slope of the least squares fit line shown is 1.8 and the correlation coefficient  $r$  is 0.995.

### Discussion

The kinetic solvent deuterium isotope effect of 2.49 for the hydrolysis of 1-acetylimidazolium ion in 0.5 vol fraction of acetonitrile is in good agreement with the value of 2.58 reported in an earlier study for a system containing no acetonitrile.<sup>6,28</sup> The solid line of Figure 1 was calculated from eq 1, and it adequately describes the proton inventory

$$k_n/k_0 = (1 - n + 0.55n)(1 - n + 0.84n)^2 \quad (1)$$

curve with 0.5 vol fraction of acetonitrile. This is essentially identical with the expression used to describe the proton inventory reported earlier for this system in the absence of acetonitrile. The difference in the two ex-

pressions is clearly not significant in that a fractionation factor of 0.84 vs. 0.83 for  $\text{H}_b$  (see 1b) cannot be distinguished experimentally.

The data of Figure 1 can also be described in terms of eq 2. This equation implicates two transition-state pro-

$$k_n/k_0 = (1 - n + 0.54n)(1 - n + 0.71n) \quad (2)$$

tons, but it is difficult to imagine a structure which is chemically reasonable on consideration of the fractionation factors. The goodness of fit of the data to eq 2 and 3 can be seen from the calculated values in Table V.

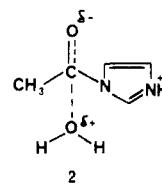
Since the proton inventory in 0.5 vol fraction of acetonitrile can be described by using the same equation which describes the proton inventory in water, the simplest interpretation is that the rate-determining transition-state structure is the same as that proposed earlier (i.e., 1b).

Increasing the acetonitrile content to 0.9 volume fraction produces a significant change in the kinetic solvent deuterium isotope effect and on the proton inventory as shown in Figure 2. The dashed line of Figure 2 was calculated from eq 1 to illustrate the difference in the proton inventories. Possible explanations of the decreased solvent isotope effect at higher acetonitrile concentrations include a change in the order dependence of the observed rate constants on water concentration, the appearance of a new partially rate-determining step along the reaction path, Hammond's postulate type shifts in the transition state, or a change in the geometry of the transition state perpendicular to the reaction coordinate.

A change in the order dependence on water concentration was considered because the proton inventory with 0.9 vol fraction of acetonitrile can be described by eq 3. The

$$k_n/k_0 = (1 - n + 0.68n)^2 \quad (3)$$

fractionation factor of 0.68 is very close to that for a hydronium ion site (i.e., 0.69).<sup>29</sup> A transition-state structure such as 2 would be consistent with eq 3. This transition



state contains only one water molecule, as opposed to two in 1b, and one would expect the order with respect to water to change when transition state 2 became important.

Figure 3 shows the dependence of the observed rate constant on water concentration. The reaction mixtures for the water order-dependence studies were prepared by volume before mixing; therefore, changes in volume on mixing for each solution were measured and corrected for (Table IV). The percentage volume changes ranged from 0.4% to 2.1% while the rate constants for this experiment had errors ranging from 0.9% to 4.9%. The dependence of  $\ln k_{\text{obsd}}$  on  $\ln [\text{H}_2\text{O}]$  shown in Figure 4 clearly shows that the line (slope = 1.8) passes through the point with a volume fraction of acetonitrile of 0.9 and is consistent with the stoichiometry of the transition state being constant throughout the range of water concentrations used. Thus, 2 seems to be eliminated as a viable alternative.

Figure 4 also suggests that new partially rate-determining steps are not appearing with higher acetonitrile concentrations. If a partially rate-determining step appeared, it might have transition state and reactant state

(28) Each run in the earlier study actually contained 1.6% acetonitrile since 50  $\mu\text{L}$  of a stock substrate solution in acetonitrile was injected to initiate each kinetic run.

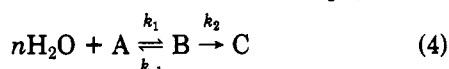
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Table VII. Values from the  $\gamma$ -Method Analysis of the Proton Inventories

vol fract of CH <sub>3</sub> CN <sup>d</sup>	$\gamma$	$10^5 k_1, s^{-1}$ <sup>a</sup>	$10^5 k_0, s^{-1}$ <sup>a</sup>	$k_0/k_1$ <sup>a</sup>
0.0	$0.394^b \pm 0.112^c$	$1899 \pm 27$	$4946 \pm 118$	$2.60 \pm 0.07$
0.5	$0.575 \pm 0.073$	$448 \pm 5$	$1177 \pm 21$	$2.63 \pm 0.06$
0.9	$0.645 \pm 0.115$	$31.5 \pm 0.3$	$67.5 \pm 1.3$	$2.14 \pm 0.05$

<sup>a</sup> Rate constants were calculated from the best fit to a second-order polynomial. <sup>b</sup> For the system with no acetonitrile  $\gamma$  was calculated by using rate constants from ref 6. Rate constants at  $n = 0.098$  and  $n = 0.294$  were omitted from the analysis. <sup>c</sup> Error limits are standard deviations or are propagated from standard deviations. <sup>d</sup> Before mixing.

isotopic fractionation factors which would be right for diluting the observed isotope effect, but the water order dependence plot of Figure 4 would not be expected to be linear. For a general reaction scheme as in eq 4, the ob-



served rate constant is given in eq 5. The log of eq 5 should

$$k_{\text{obsd}} = \frac{k_1 k_2 [\text{H}_2\text{O}]^n}{k_2 + k_{-1}} \quad (5)$$

be nonlinear in  $\log [\text{H}_2\text{O}]$  if  $k_1$ ,  $k_2$ , or  $k_{-1}$  change with water concentration. Similarly, Figure 4 should be nonlinear if a new reaction pathway involving additional water molecules is appearing. The linearity of the plot in Figure 4 suggests that neither of these two hypotheses based on the appearance of new rate-determining or partially rate-determining transition states can account for the observed differences in the proton inventories in water and 0.9 vol fraction of acetonitrile.

The relative changes in free energy of the reactants and the tetrahedral intermediate in a system with a high concentration of acetonitrile must be considered to discuss Hammond's postulate shifts in the transition state. Acetonitrile solvates ions more poorly than does water, based on consideration of the dielectric constants<sup>30</sup> of 36.0 and 78.4 for acetonitrile and water, respectively, at 25 °C, but acetonitrile preferentially solvates cations over anions.<sup>31</sup> Since there is a negative charge in the tetrahedral intermediate, it is possible that it is more poorly solvated than is the reactant, and, therefore, the intermediate is raised in energy with respect to the relative energy differences between the reactant and the intermediate in water. Raising the relative energy of the tetrahedral intermediate due to solvation effects shifts the transition state parallel to the reaction coordinate to give a "later" transition state. A late version of 1b in which H<sub>a</sub> is more completely transferred would give, presumably, three protons contributing to the solvent isotope effect with fractionation factors of about 0.69. However, such transition-state models do not simulate the experimental proton inventory unless the fractionation factors are about 0.69 and 0.83 for H<sub>a</sub> and H<sub>b</sub>, respectively. This is an unlikely situation.

A solvation effect exerting its major influence perpendicular to the reaction coordinate (i.e., an anti-Hammond effect) may be used to explain the observed effects in 0.9 volume fraction of acetonitrile.<sup>32</sup> Equations 6 and 7 both

$$k_n/k_0 = (1 - n + 0.63n)(1 - n + 0.86n)^2 \quad (6)$$

$$k_n/k_0 = (1 - n + 0.59n)(1 - n + 0.89n)^2 \quad (7)$$

generate curves which simulate the experimental proton inventory very well as can be seen from the calculated values in Table VI. The difference in eq 6 and 7 and the model used to explain the inventory in water and 0.5 vol fraction of acetonitrile is the increase in fractionation factors for H<sub>a</sub> and H<sub>b</sub> from 0.55 and 0.83, respectively. These larger fractionation factors give a smaller isotope effect for both the primary contribution of H<sub>a</sub> and the secondary contribution of H<sub>b</sub> and suggest tighter binding of both "types" of protons in the transition state. This is consistent with a "compression" of the transition state structure 1b in 0.9 vol fraction of acetonitrile in much the same way that acetonitrile promotes ion pairing. This compression increases the force constants of the bonds of the reacting water molecules to give rise to the observed effect and is still consistent with the order dependence on water. The linearity of Figure 4 suggests that the decrease in rate as one proceeds to higher acetonitrile concentrations is merely a reflection of the decreasing water concentration. If one divides the observed rate constants for the reactions with varying amounts of acetonitrile by the concentration of water raised to the second power one obtains a constant of  $(17 \pm 3) \times 10^{-6}$  which would be the intrinsic rate constant,  $k'$ , defined in eq 8.

$$k_{\text{obsd}} = k' [\text{H}_2\text{O}]^2 \quad (8)$$

In order to get a better handle on the transition-state fractionation factors and their associated errors, we have also used the  $\gamma$  method of Albery to analyze the data.<sup>27</sup> Albery has devised a scheme for analyzing proton inventory data using a curvature parameter,  $\gamma$ , defined in eq 9,

$$\gamma = 8 \ln [y_{0.5}/y_{1.0}^{1/2}] [\ln y_{1.0}]^{-2} \quad (9)$$

$$y_n = \frac{k_1^{\text{RS}}}{k_0} \prod_i (1 - n + n\phi_i) = \frac{\text{TS}}{j} (1 - n + n\phi_j) \quad (10)$$

where  $y_n$  is the transition-state term of the Gross-Butler equation expressed in eq 10. For a general equation (eq 11), Albery describes the fractionation factors  $\phi_a$  and  $\phi_b$  in eq 12 and 13.  $\Lambda_A$  is found by solving the quadratic eq 14, and  $\Lambda_B$  is  $(1 - \Lambda_A)$ .

$$y_n = (1 - n + n\phi_a)^a (1 - n + n\phi_b)^b \quad (11)$$

$$\phi_a = \exp \left[ \frac{1}{a} (\ln y_1) \Lambda_A \right] \quad (12)$$

$$\phi_b = \exp \left[ \frac{1}{b} (\ln y_1) \Lambda_B \right] \quad (13)$$

$$a\gamma = \Lambda_A^2 + \frac{a}{b} (1 - \Lambda_A)^2 \quad (14)$$

$\gamma$  was calculated for each proton inventory by using a program, provided courtesy of Professor Albery, which fits the data to a second-order polynomial, extracts  $k_n$  at  $n = 0.5$  and  $n = 1.00$ , and uses  $y_{0.5}$  and  $y_{1.0}$  in eq 9. Table VII shows the  $\gamma$  values and Table VIII the fractionation factors for each proton inventory calculated from eq 12 and 13.

(30) King, E. J. In "Physical Chemistry of Organic Solvent Systems"; Covington, A. K., Dickenson, T., Eds.; Plenum: New York, 1973; Chapter 3.

(31) See page 371 of ref 30.

(32) For a discussion of parallel vs. perpendicular effects, see: Thornton, E. K.; Thornton, E. R. In "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 1, pp 65-66.

Table VIII.  $\gamma$ -Method Fractionation Factors for  
 $k_n/k_0 = (1 - n + n\phi_a)(1 - n + n\phi_b)^2$

vol fract of CH <sub>3</sub> CN <sup>c</sup>	$\phi_a$	range of $\phi_a^a$	$\phi_b$	range of $\phi_b$
0.0	0.60	0.53–0.73 <sup>b</sup>	0.80	0.73–0.86
0.5	0.49	0.47–0.52	0.88	0.85–0.90
0.9	0.55	0.52–0.59	0.92	0.89–0.95

<sup>a</sup> Range of  $\phi_a$  and range of  $\phi_b$  were calculated by solving eq 12 and 13 with values for  $\gamma$  at the limits of the standard deviations (see Table VII). <sup>b</sup> Since the lower limit of  $\gamma$  in this case is less than  $1/3$ , a three-proton model cannot be applied, and so the high  $\phi_a$  was calculated by using  $\gamma = 1/3$ . <sup>c</sup> Before mixing

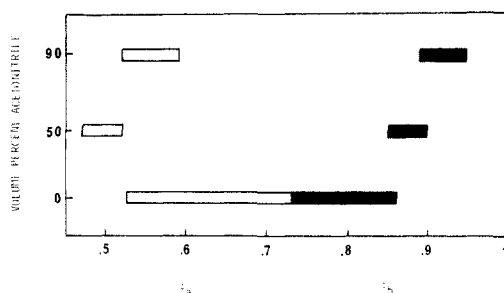


Figure 5. Representation of the ranges of  $\phi_a$  and  $\phi_b$  calculated from the  $\gamma$  method by using  $k_n/k_0 = (1 - n + n\phi_a)(1 - n + n\phi_b)^2$  for the water-catalyzed hydrolysis of 1-acetylimidazolium ion in various solvent systems.

The ranges of the fractionation factors, which give a measure of their precision, were calculated by using the limits of each  $\gamma$  value in Table VII. Figure 5 is a graphical representation of possible fractionation factors for a transition state 1b.

Figure 6 shows expected changes in fractionation factors for various types of shifts in the transition state 1b. A–C are Hammond's postulate type shifts (parallel to the reaction coordinate), so each pair of fractionation factors converges for a hypothetical acetonitrile content in the solvent to a particular fractionation factor. For a late transition state, as discussed earlier, protons H<sub>a</sub> and H<sub>b</sub> approach the bonding situation of hydronium ion protons. Figure 6A shows  $\phi_a$  and  $\phi_b$  converging at 0.69, the accepted value for a hydronium ion fractionation factor in water. Figure 6B describes the expected fractionation factor changes for a late shift accompanied by a hypothetical change in the hydronium ion fractionation factor.  $\phi_a$  must undergo a more dramatic change than  $\phi_b$  to "catch up" to the changing hydronium ion fractionation factor. Figure 6C shows a parallel shift toward reactants where  $\phi_a$  and  $\phi_b$  both approach unity. The two nonconverging lines in Figure 6D represent a shift perpendicular to the reaction coordinate.  $\phi_a$  and  $\phi_b$  do not approach the same value as in the parallel shifts. Figure 5, the representation of  $\gamma$ -analysis fractionation factors, generally looks most like Figure 6D.  $\phi_b$ , the fractionation factor for the two protons contributing the secondary effect, increases with increasing acetonitrile content, but  $\phi_a$ , for the primary effect, does not show any definite trend as the solvent is changed. We believe this is consistent with the proposed perpendicular shift in the transition-state structure. The constancy of  $\phi_a$  could be rationalized by considering that the proton H<sub>a</sub> is not as exposed to the solvent as are the H<sub>b</sub> protons so the solvent influence on this proton is small. Throughout this discussion we have assumed that the proton H<sub>c</sub> in 1b has a fractionation factor of unity. Kurz and Lee have recently considered the possibility that fractionation factors might be different in water and in water-organic

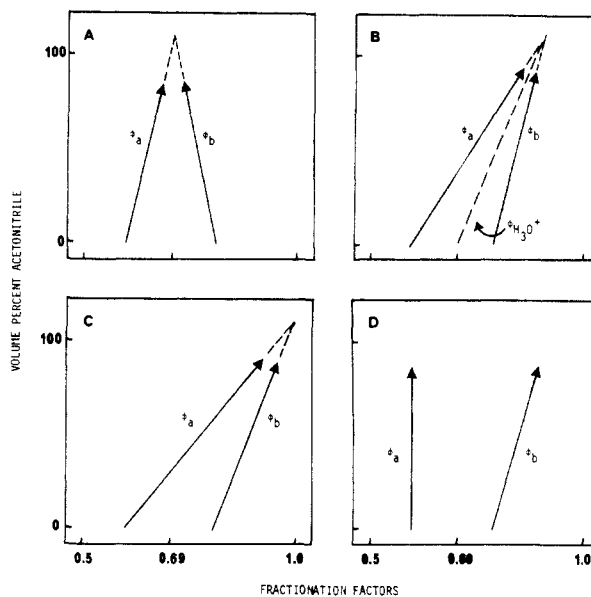


Figure 6. Expected changes in fractionation factors for transition state 1b with increasing acetonitrile content in the solvent: A shows changes expected for a shift parallel to the reaction coordinate toward a late transition state; B shows the same type shift as A, with the fractionation factor for a hydronium ion site also changing; C shows a parallel shift toward reactants; D shows a shift perpendicular to the reaction coordinate.

solvent systems, but consider this unlikely. We, therefore, have briefly considered such a possibility.<sup>35</sup>

### Conclusion

While several of the perturbations in the transition state, reaction coordinate, or reaction scheme discussed in this study may be occurring to some extent as acetonitrile is added to the solvent for water-catalyzed hydrolysis of 1-acetylimidazolium ion, we believe the data best support the idea that a shift perpendicular to the reaction coordinate accounts for most of the changes in the proton inventories. This conclusion lends some validity to model system studies in as much as we have more closely approximated the hydrophobicity of an active site, and the catalytic portion of the transition state remained constant with respect to its stoichiometry, mechanism, and position along the reaction coordinate. The results of this study also show the utility of comparatively using several proton inventories to probe transition-state structures. By calculation of transition-state fractionation factors with Albery's  $\gamma$  method, the relative changes in  $\gamma$  and fractionation factors for several systems can be used to discuss changes in transition-state structures.

### Experimental Section

**Materials.** 1-Acetylimidazole was prepared by the method of Boyer<sup>33</sup> and had a melting point of 100.5–101.5 °C (lit.<sup>33</sup> mp 101.5–102.5 °C). Acetonitrile was distilled from phosphorus pentoxide and stored under nitrogen. Deuterium oxide (99.8 atom % deuterium, Aldrich) was distilled from barium nitrate. Water was glass distilled. Sodium chloride (Fisher Certified) was dried in an oven at 120 °C overnight.

**Reaction Solutions.** For the proton inventory in 0.5 vol fraction (before mixing) of acetonitrile an H<sub>2</sub>O or D<sub>2</sub>O solution containing sodium chloride and HCl or DCl was mixed with acetonitrile. The final solution had an ionic strength of 0.2 M and contained 10<sup>-2</sup> N HCl or DCl. For 0.9 vol fraction (before mixing) of acetonitrile solutions, the same procedure as above was followed, and the resulting solutions had an ionic strength of 0.025

M and contained  $10^{-2}$  N HCl or DCl. From these stock solutions of  $H_2O$ -acetonitrile and  $D_2O$ -acetonitrile, mixtures with varying atom fractions of deuterium were prepared by volume. The atom fraction of deuterium of exchangeable protons was determined from the results of an analysis by Mr. Josef Nemeth<sup>34</sup> on a sample of "100%"  $D_2O$ -acetonitrile solution. For the order dependence of the observed rate constant on water concentration experiment, solutions were prepared by using the same general procedure, mixing acetonitrile with water solutions such that the final solution had an ionic strength of 0.025 M and contained  $10^{-2}$  N HCl. Changes in volumes on mixing were determined for each point on the water-order plot. The water solution and acetonitrile were added as "volume fraction before mixing" to a buret. The mixture was thoroughly stirred with a glass rod, and a reading was taken from the buret with the rod still in place. The apparatus was

calibrated by using known volumes of water.

**Kinetics.** The hydrolysis of 1-acetylimidazolium ion was monitored by following the decrease in absorbance at 245 nm with a Cary 118C UV-vis spectrophotometer equipped with a constant-temperature cell compartment and cell holder to control the temperature at  $25.00 \pm 0.05$  °C. Reactions were initiated by injecting 10  $\mu$ L of a solution which was  $3 \times 10^{-2}$  M acetylimidazole in acetonitrile into 3.00 mL of the reaction solution. Reactions were followed for at least 3 half-lives, with infinity absorbances being taken at greater than 10 half-lives. First-order rate constants were determined by using a nonlinear least-squares computer program.

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## Substrate Hydrophobicity and Its Influence on the Transition-State Structure for the Water-Catalyzed Hydrolysis of Acyl Esters<sup>1</sup>

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Proton inventories for the "water-catalyzed" hydrolysis of *p*-nitrophenyl dichloroacetate have been completed in  $10^{-2}$  N HCl (DCl) solutions at two different ionic strengths and in a *tert*-butyl alcohol/water mixture of 0.1 mol fraction of *tert*-butyl alcohol. The proton inventories exhibit significant downward curvature in all cases and are consistent with greater than three protons contributing to the solvent deuterium isotope effect. The reaction appears to be fourth order or greater with respect to water on the basis of the study in *tert*-butyl alcohol/water mixtures and the values of the curvature parameter,  $\gamma$ , generated from the proton inventory data. Several possible transition-state structures are considered.

The hydrolysis of *p*-nitrophenyl dichloroacetate has been demonstrated to be pH independent in the region pH 1-3 by Fife and McMahon.<sup>3</sup> Thus, the reaction under observation in this region is the "water-catalyzed" hydrolysis of the ester. The observed solvent isotope effect of  $k_{H_2O}/k_{D_2O} = 3.10$  was indicative of a catalytic proton bridge transition state involving water-catalyzed nucleophilic attack by water as shown in Figure 1. This transition state was suggested by Fife and McMahon. However, a kinetically equivalent transition state could not be ruled out on the basis of their results. Subsequent studies on this system in water/*t*-BuOH mixtures by Engbersen and Engberts lent support to this idea.<sup>4</sup> This study showed a dramatic dependence of the solvent deuterium isotope effect upon the mole fraction of *tert*-butyl alcohol in the solvent system, however. This information along with their studies of the influence of "structure-breaking" additives suggested that proton inventory investigations of this system under a variety of conditions would be useful in

Table I. First-Order Rate Constants for the Hydrolysis of *p*-Nitrophenyl Dichloroacetate in Mixtures of  $10^{-2}$  N HCl- $H_2O$  and  $10^{-2}$  N DCl- $D_2O$  at  $25.00 \pm 0.05$  °C<sup>a</sup>

$n^d$	no. of runs	$10^2 k_n, s^{-1} b$
0.000	4	$5.71 \pm 0.27$
0.099	3	$5.20 \pm 0.15$
0.197	3	$4.67 \pm 0.07$
0.296	3	$4.25 \pm 0.12$
0.394	5	$3.91 \pm 0.15$
0.493	5	$3.39 \pm 0.12$
0.591	3	$3.13 \pm 0.02$
0.690	3	$2.80 \pm 0.04$
0.788	5	$2.46 \pm 0.09$
0.887	3	$2.29 \pm 0.05$
0.985 <sup>c</sup>	3	$1.95 \pm 0.03$

<sup>a</sup> Ionic strength was maintained at 0.5 M with KCl.

<sup>b</sup> Error limits are standard deviations. <sup>c</sup> Atom fraction of deuterium in "100%"  $10^{-2}$  N DCl- $D_2O$  as determined by Mr. Josef Nemeth.<sup>25</sup> <sup>d</sup> Atom fraction of deuterium.

delineating the transition-state structure. We report the results of our investigations here.

### Results

Tables I and II list the rate constants obtained for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate in  $10^{-2}$  N HCl- $H_2O$  and DCl- $D_2O$  mixtures under condi-

(1) This research was supported by the Robert A. Welch Foundation and the National Institutes of Health (Grant No. 1 R01 GM2543301).

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