

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 Nemeth³⁴ 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 ± 0.05 °C. Reactions were initiated by injecting 10 μ L of a solution which was 3×10^{-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.

Acknowledgment. We thank Professor John Albery for providing the computer program GAMISO and Mr. Kenneth Davis for his assistance in modifying it to run on our Micromation computer.

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Registry No. 1a, 31346-45-9.

Substrate Hydrophobicity and Its Influence on the Transition-State Structure for the Water-Catalyzed Hydrolysis of Acyl Esters¹

William P. Huskey,² Christina T. Warren,² and John L. Hogg*

Department of Chemistry, Texas A&M University, College Station, Texas 77843

Received June 25, 1980

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, γ , 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.³ 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.⁴ 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 ± 0.05 °C^a

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

^a Ionic strength was maintained at 0.5 M with KCl.

^b Error limits are standard deviations. ^c Atom fraction of deuterium in "100%" 10^{-2} N DCl- D_2O as determined by Mr. Josef Nemeth.²⁵ ^d 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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(3) Fife, T. H.; McMahon, D. M. *J. Am. Chem. Soc.* 1969, 91, 7481-7485.

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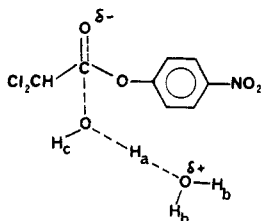


Figure 1. Catalytic "proton-bridge" transition-state structure suggested by Fife and McMahon³ for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate.

Table II. 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 ± 0.05 °C^a

n^d	no. of runs	$10^2 k_n, s^{-1} b$
0.000	12	6.11 ± 0.16
0.248	4	4.66 ± 0.07
0.497	6	3.64 ± 0.19
0.745	4	2.73 ± 0.04
0.993 ^c	5	2.02 ± 0.03

^a Ionic strength was uncontrolled (i.e., no additional salt was added). ^b Error limits are standard deviations. ^c Atom fraction of deuterium in "100%" 10^{-2} N DCl- D_2O as determined by Mr. Josef Nemeth.²⁵ ^d Atom fraction of deuterium.

Table III. First-Order Rate Constants for the Hydrolysis of *p*-Nitrophenyl Dichloroacetate in 10^{-2} N HCl (DCl)^a Mixtures of Atom Fraction of Deuterium n at 0.1 Mol Fraction of *t*-BuOL in L_2O ($L = H$ or D) at 25.00 ± 0.05 °C

n	no. of runs	$10^5 k_n, s^{-1} b$
0.000	3	541 ± 7
0.247	2	415 ± 14
0.494	3	310 ± 8
0.742	3	222 ± 3
0.989 ^c	4	159 ± 3

^a Protiated solutions were actually 1.5×10^{-2} N HCl due to a mistake in calculation. Therefore, mixtures have variable HCl-DCl concentrations (see Discussion). ^b Error limits are standard deviations. ^c Atom fraction of deuterium in "100%" deuterated solvent system as determined by Mr. Josef Nemeth.²⁵

tions which the ionic strength was controlled at 0.5 M with potassium chloride (Table I) or left uncontrolled (Table II). The observed solvent deuterium isotope effects, k_{H_2O}/k_{D_2O} , are 2.93 ($\mu = 0.5$ M) and 3.02 ($\mu = 0.01$ M). These values compare very favorably with the literature values of 3.10 (10^{-1} N HCl)³ and 3.15 (10^{-2} N HCl, $\mu = 0.01$ M).⁴ The actual rate constants, however, show some dependence upon ionic strength. In general, the rate constants tend to decrease with increasing ionic strength. There is excellent agreement between our values and those of Engbersen and Engberts under identical conditions in both pure protium oxide and pure deuterium oxide. Figure 2 collects the proton inventory data under the two sets of conditions.

Table III lists the rate constants for the neutral hydrolysis of *p*-nitrophenyl dichloroacetate in *t*-BuOL/ L_2O mixtures (where $L = H$ or D) with the mole fraction of alcohol equal to 0.1. These rate constants compare very favorably with those reported in the literature for pure protium oxide and deuterium oxide at 0.1 mol fraction of *t*-BuOL.⁴ The solvent deuterium isotope effect of $k_{H_2O}/k_{D_2O} = 3.40$ is slightly smaller than the literature value of 3.70. Figure 3 shows a plot of k_n vs. the atom fraction of

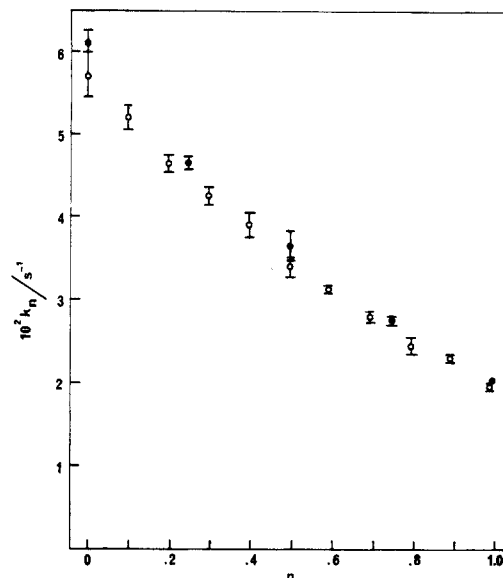


Figure 2. Proton inventory for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate: open circles are for the reaction in 10^{-2} M HCl (DCl) with the ionic strength controlled at 0.5 M (KCl); filled circles are for the reaction in 10^{-2} M HCl (DCl) with the ionic strength unaltered (i.e., $\mu = 10^{-2}$ M). The data are from Tables I and II.

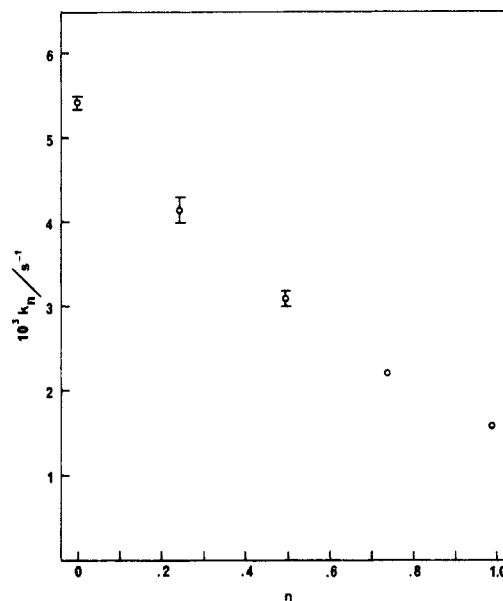


Figure 3. Proton inventory for the hydrolysis of *p*-nitrophenyl dichloroacetate in 10^{-2} M HCl (DCl) with 0.1 mol fraction of *t*-BuOH (*t*-BuOD). Where error bars are omitted the circles encompass them. The data are from Table III.

deuterium for the data of Table III.

Using the densities determined (see Experimental Section) for the *t*-BuOL/ L_2O solutions and the known mole fraction of *t*-BuOL, one can calculate the molar concentration of H_2O or D_2O in the "pure" *t*-BuOL/ L_2O solutions. These data and the corresponding rate constants in the absence of *t*-BuOL and at 0.1 mol fraction of *t*-BuOL/ L_2O can be used to make a crude "water-order" plot. The data used to make this plot (Figure 4) are collected in Table IV. Identical plots are obtained by using the data from the present study or the literature data.⁴ Although the lines in Figure 4 are drawn between only two data points, the agreement between the data sets and between protiated and deuterated solvents is excellent. The slopes which, in many cases, are interpreted as the

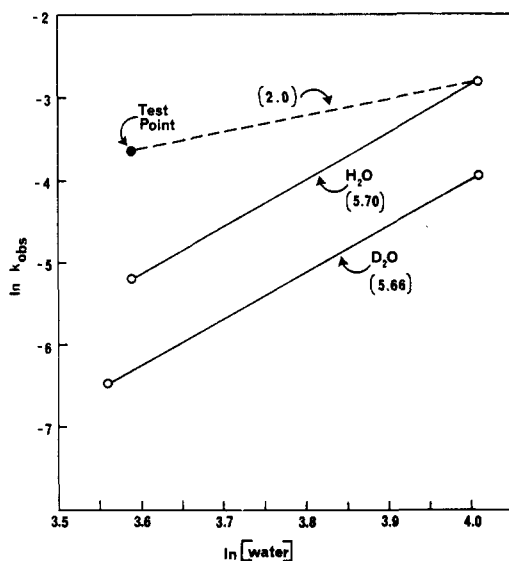


Figure 4. "Water-order" plot of $\ln k_{\text{obs}}$ vs. $\ln [\text{water}]$. The values in parentheses are the slopes of the lines and represent the order of the hydrolysis of *p*-nitrophenyl dichloroacetate with respect to water. The test point is included to show the value that would be required to give a slope of 2.0 which would be consistent with the transition state of Figure 1. The data are from Table IV.

Table IV. Values of $-\ln k_{\text{obsd}}$ for the Hydrolysis of *p*-Nitrophenyl Dichloroacetate as a Function of $[\text{L}_2\text{O}]$ at Various Mole Fractions of *t*-BuOL

$[\text{L}_2\text{O}]$, M	\ln of $[\text{L}_2\text{O}]$	mole fraction of <i>t</i> -BuOL	$-\ln k_{\text{obsd}}^c$	ref
55.3	4.01	0	2.80 (H ₂ O)	this study
55.3	4.01	0	3.90 (D ₂ O)	this study
55.3	4.01	0	2.81 (H ₂ O)	4
55.3	4.01	0	3.95 (D ₂ O)	4
36.4 ^a	3.59	0.1	5.18 (H ₂ O)	4
35.2 ^b	3.56	0.1	6.50 (D ₂ O)	4
36.4 ^a	3.59	0.1	5.22 (H ₂ O)	this study
35.2 ^b	3.56	0.1	6.44 (D ₂ O)	this study

^a Based on a measured density of 0.955 for the protiated solvent system with a mole fraction of *t*-BuOH of 0.1. ^b Based on a measured density of 0.999 for the deuterated solvent system with a mole fraction of *t*-BuOD of 0.1. ^c L₂O type in parentheses.

order of the hydrolysis with respect to water are approximately 5.7 in both cases. This point is considered in more detail in the Discussion. A reasonable error analysis leads to the conclusion that these plots may indicate the involvement of from four to seven water molecules in the transition state for hydrolysis. Figure 4 shows a hypothetical point on the plot that would be required for the slope to be 2 for the protiated solvent system. This is clearly well outside our experimental error but bears on possible catalytic proton-bridge transition-state structures. Because of the general disagreement over the meaning of such plots (vide infra) and the limited data available in the present study, we base our interpretation only partly on the "water-order" plot.

Discussion

The proton inventories presented here for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate under a variety of conditions all show a significant amount of downward curvature. This curvature is indicative of multiple proton contributions in the rate-limiting transition state to the solvent deuterium isotope effect.⁵ Once

Table V. Values of γ Calculated for the Proton Inventories for Hydrolysis of *p*-Nitrophenyl Dichloroacetate

conditions	γ value ^a	γ^{-1}	$\gamma^{-1}_{\text{min}}^b$
10 ⁻² N HCl (DCl), $\mu = 0.50$ M	0.19 ± 0.17	5.26	2.77
10 ⁻² N HCl (DCl), $\mu = 0.01$ M	0.16 ± 0.16	6.25	3.15
10 ⁻² N HCl (DCl), 0.1 mol fraction of <i>t</i> -BuOL in L ₂ O	0.28 ± 0.10	3.57	2.63

^a Calculated by using a computer program, GAMISO, kindly provided by J. Albery. ^b Calculated by assuming maximum positive error.

the proton inventories are in hand it becomes necessary to check various transition-state structures for consistency with the inventories. A variety of techniques is employed in this procedure.⁵

In the present study we will make some use of the so-called " γ method" of Albery.^{5c} This procedure allows the calculation of a value, γ , which is related to the overall solvent isotope effect k_1/k_0 and the solvent isotope effect when the atom fraction of deuterium, n , is 0.5 (i.e., $k_{0.5}/k_0$) by eq 1. The midpoint of the proton inventory is the point

$$\gamma = 8 \ln [(k_{0.5}/k_0)/(k_1/k_0)^{1/2}] / \ln (k_1/k_0)^2 \quad (1)$$

at which the curvature (i.e., departure from linearity) is maximal. Albery has shown that $\gamma = 1$ when the proton inventory is linear and γ approaches zero as the number of sites contributing to the isotope effect becomes large. Thus, the values of γ can be calculated for each proton inventory on the basis of the experimental rate constants. Albery has provided us with a computer program, GAMISO, which utilizes all values of k_n and n to determine the γ values. With this program the values of γ tabulated in Table V were obtained. Albery has shown that for a given experimental value of γ the number of transition-state sites contributing to the solvent deuterium isotope effect must be greater than γ^{-1} . Although the errors in the γ values in Table V are rather large, one can use the maximal error limit in the positive direction to estimate the minimum values of γ^{-1} for the three proton inventories. Thus, the γ values indicate contributions of anywhere from three to greater than six protons in the transition states. This is certainly in accord with the water-order plots (Figure 4) which suggest four to six water molecules in the transition states.

Attempts to correlate the proton inventories with the transition state (or a modification) of Figure 1 give the following result. If one assumes that the two protons (H_b in Figure 1) attached to the water molecule acting as the base will have isotopic fractionation factors between unity (no transfer of H_a in the transition state) and 0.69 (the fractionation factor for a hydronium ion), one can estimate their fractionation factors by using eq 2. In eq 2 the value

$$\phi_b^* = 0.69^\beta \quad (2)$$

of β has been assumed to be the Brønsted value for general-base catalysis or some other measure of the extent of proton transfer.^{5b} Once ϕ_b^* is estimated, it can be sub-

(5) For detailed discussions of the theory of the proton inventory technique, see: (a) Schowen, K. B. In "Transition States of Biochemical Processes"; Gandour, R. D., Schowen, R. L., Eds.; Plenum: New York, 1978; Chapter 6; (b) Schowen, R. L. In "Isotope Effects of Enzyme-Catalyzed Processes"; Cleland, W. W., O'Leary, M. H., Northrop, D. B., Eds.; University Park Press: Baltimore, MD, 1977; (c) Albery, J. In "Proton Transfer Reactions"; Caldin, E., Gold, G., Eds.; Wiley: New York, 1975; Chapter 9.

stituted into eq 3, the general equation for a transition

$$k_n = k_0(1 - n + n\phi_a^*)(1 - n + n\phi_b^*)^2 \quad (3)$$

state such as in Figure 1, along with $k_{1,0}$ and k_0 to calculate a ϕ_a^* value. In the extreme case of complete proton transfer in the transition state, the equation would take on the form of eq 4 where $\phi_a^* = 0.69$. It can be shown that

$$k_n = k_0(1 - n + n\phi_a^*)^3 \quad (4)$$

a series of transition states of the type shown in Figure 1 with $\beta \approx 0.5-1.0$ is reasonably consistent with the proton inventory curves and the associated error in each point. However, these transition states involve only two water molecules and, thus, are at odds with the number of water molecules indicated by the γ values and water-order plots.

There have been two general approaches to the treatment of hydrolysis rates in mixed-solvent systems. The first, and admittedly more rigorous, approach is to treat the rates in terms of effects on reactant and transition-state activity coefficients without attempting to elucidate an order with respect to water.⁶ This approach is often considered best due to the frequent problems encountered in the second approach. The assumption is often made that if the dependence on the water concentration can be shown to be the same over a large range of water concentrations in more than one solvent system, then the water order determined may be taken as approximately correct. The critical condition to be met is that the activity coefficients of water, the admixed solvent, the reactant, and the transition state change in such a way as to largely cancel one another. This approach is similar to the one taken by Bell and Critchlow in their studies of 1,3-dichloroacetone hydration in water-dioxane and water-acetonitrile mixtures.⁷ The water order of 3 for hydration was found to be consistent with the γ value of 0.26 ± 0.05 calculated by Albery using their data.^{5c} Similar studies of anhydride hydrolyses have been used to probe the water order.⁸⁻¹²

However, the meaning of plots of $\ln k_{\text{obsd}}$ vs. $\ln [\text{water}]$ in mixed-solvent systems is not totally clear. These plots while often linear have highly variable slopes (often varying dramatically as a function of substrate hydrophobicity).^{6,13} In many cases the slopes change dramatically over rather small changes in water concentration, while other parameters are not varying significantly.⁶ Thus, it has been argued that it is hard to imagine that these changes in slope really reflect changes in transition-state structure. Langford and Tong have suggested that solvolyses are always (pseudo) first order.¹⁴ The influence of added organic cosolvent may alter this interpretation, but the problem of exactly how it does this is certainly a complex one. In a related system, Engberts and Holterman argue that changes in hydrophobic interaction between the initial state and the organic cosolvent play a major role in determining the thermodynamic activation parameters.¹⁵

(6) Engberts, J. B. F. N. In "Water: A Comprehensive Treatise"; Franks, F., Ed.; Plenum Press: New York, 1979; Vol. 6, Chapter 4.
(7) Bell, R. P.; Critchlow, J. E. *Proc. R. Soc. London, Ser. A* 1971, 325, 35.

(8) Cavasino, F. P.; D'Allessandro, S. *Atti. Accad. Sci., Lett. Arti Palermo, Parte 1* 1964-1965, 25, 119; *Chem. Abstr.* 1966, 65, 19949g.

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(10) Nestler, H. J.; Seydel, J. K. *Chem. Ber.* 1967, 100, 1983.

(11) Hawkins, M. D. *J. Chem. Soc., Perkin Trans. 2* 1975, 282.

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(13) Personal communication from J. B. F. N. Engberts.

(14) Langford, C. H.; Tong, J. P. K. *Acc. Chem. Res.* 1977, 10, 258.

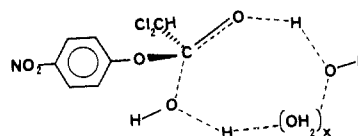


Figure 5. Possible cyclic transition-state structure for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate. The number of water molecules varies with the value of x . Attack on the carbonyl carbon is assisted by the carbonyl oxygen in this transition state.

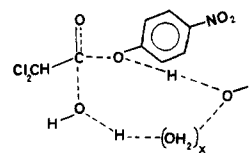


Figure 6. Possible cyclic transition-state structure for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate. The number of water molecules varies with the value of x . Attack on the carbonyl carbon is assisted by the ester oxygen of the leaving group in this transition state.

We, therefore, do not wish to place too much emphasis on a "water-order" plot (especially one consisting of only two points) but will consider a few possible interpretations of the results. The water-order plots require the incorporation of from two to four additional water molecules in the transition state. There are numerous ways that several solvating water molecules could be incorporated into the transition state. If the fractionation factors of the protons of these solvating water molecules did not change on going from the reactant state to the transition state, then no isotope effect would be generated by them. Thus, we could imagine a transition state such as that shown in Figure 1 but with several additional solvating water molecules intimately involved. However, the γ -value analysis requires that the sites actually be contributing to the solvent isotope effect. Hydrolytic reactions which have recently been interpreted in terms of transition-state structures similar to that in Figure 1 in the literature¹⁶ have exhibited γ values around 0.4, a much larger value than in the present study. The recently reported studies interpreted in terms of one-proton mechanisms exhibit γ values very near unity.¹⁶ Thus, we must consider the implications of the γ values obtained in the present study. The γ values and water-order plot for the three proton inventories hint at the involvement of several water molecules in the transition states. Several cyclic transition-state structures can be envisioned for this process. Two possibilities are shown in Figures 5 and 6. A transition-state model similar to that in Figure 5 is discussed by Albery^{5c} for the hydration of 1,3-dichloroacetone ($\gamma = 0.26 \pm 0.05$) studied by Bell and Critchlow.⁷ Figure 6 is

(15) Holterman, H. A. J.; Engberts, J. B. F. N. *J. Am. Chem. Soc.* 1980, 102, 4256.

(16) Although the γ values were not reported in the original papers, it can be shown that they are around 0.4 for the neutral, water-catalyzed hydrolysis of 1-acetylimidazolium ion,¹⁷ 1-acetyl-3-methylimidazolium ion,¹⁸ and ethyl trifluoroacetate.¹⁹ The γ values for several recently reported linear proton inventories are all near unity. For examples see the studies on the imidazole-catalyzed hydrolysis of 1-acetylimidazole²⁰ and ethyl trifluoroacetate²⁰ and the intramolecularly catalyzed hydrolysis of phenyl hydrogen malonate anion.²¹

(17) Hogg, J. L.; Phillips, M. K.; Jergens, D. E. *J. Org. Chem.* 1977, 42, 2459-2461.

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(19) Venkatasubban, K. S.; Davis, K. R.; Hogg, J. L. *J. Am. Chem. Soc.* 1978, 100, 6125-6128.

(20) Patterson, J. F.; Huskey, W. P.; Venkatasubban, K. S.; Hogg, J. L. *J. Org. Chem.* 1978, 43, 4935-4938.

(21) Venkatasubban, K. S.; Hogg, J. L. *Tetrahedron Lett.* 1979, 4355-4358.

essentially the same and is closely related to the transition-state structure recently proposed by Komiyama and Bender for the water-catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide.²² We have also recently proposed such cyclic transition-state structures for the water-catalyzed hydrolysis of 1-acetyl-1,2,4-triazole.²³ By assuming various values for x in Figures 5 and 6, one can easily write expressions of the form shown in eq 5 which are consistent

$$k_n = k_0(1 - n + n\phi_a^*)^{x+2} \quad (5)$$

with the proton inventories. These expressions assume that only the protons undergoing transfer give rise to isotope effect contributions. The involvement of such a large number of water molecules is consistent with the water-order plot and the small values of γ . The hydrophobic nature of the substrate molecule may play a role in determining the number of water molecules involved in the transition state. This factor has previously been emphasized by Fife and McMahon in their paper on the hydrolysis of *p*-nitrophenyl esters³ and by Engbersen and Engberts.⁴ The large negative ΔS^\ddagger for hydrolysis of *p*-nitrophenyl dichloroacetate (-30 to -40 eu) suggests a highly hydrated transition state relative to the ground state.⁴ It has been suggested that the interaction with water molecules in the transition state will be more extensive when this species is accommodated in voids in the water structure surrounded by water molecules of enhanced structure.⁶ This may explain the increased curvature seen with hydrophobic substrates and the increase in isotope effect on addition of 0.1 mol fraction of *t*-BuOL.

Conclusion

The proton inventories for the water-catalyzed hydrolysis of *p*-nitrophenyl dichloroacetate suggest cyclic transition states involving several water molecules. The "hydrophobicity" of the substrate may be a major factor in determining the "structure" of the water surrounding the transition state. Clearly the interpretation of these results is complex, and more work on proton inventories for hydrophobic substrates in aqueous and mixed organic-aqueous solvents is underway. The implications of such results for an understanding of the catalytic processes occurring in the hydrophobic pockets of enzymes are obvious. It seems reasonable that the water in the active sites may be unusual in its reactivity and may give rise to effects that are only beginning to be understood.

Experimental Section

Materials. *p*-Nitrophenyl dichloroacetate was prepared from *p*-nitrophenol and dichloroacetic anhydride (Aldrich) via a slight modification of the literature procedures.^{3,24} The product had a melting point of 30.5–31.5 °C (lit.²⁴ mp 30.5 °C). The NMR spectrum (CCl₄, Me₄Si) showed the following signals: δ 6.18 (1 H, s), 7.9 (4 H, q). D₂O from Aldrich (99.8 atom % deuterium) was used as obtained. Potassium chloride (Fisher Certified) was oven dried before use. Water was doubly glass distilled before

use. Acetonitrile (Fisher reagent grade) was stirred over calcium hydride overnight, distilled from calcium hydride through a 30-cm fractionating column packed with glass helices, and stored under a nitrogen atmosphere. Both *tert*-butyl alcohol (Fisher) and *tert*-butyl alcohol-*d* (Aldrich) were distilled from calcium hydride and stored under a nitrogen atmosphere.

Solutions. A stock solution of the *p*-nitrophenyl dichloroacetate which was 6×10^{-3} M in acetonitrile was prepared. Stock 10^{-2} N HCl and DCl solutions in H₂O and D₂O, respectively, were prepared by using concentrated hydrochloric acid. The ionic strength was maintained at 0.5 M with potassium chloride or left uncontrolled as desired. The amount of protium introduced into the 10^{-2} N DCl solution in D₂O in this manner was determined on samples of the pure DCl–D₂O solutions by Mr. Josef Nemeth.²⁵ This factor has been considered in the data analysis. Reactions in H₂O–D₂O mixtures were done by using the appropriate volumes of the HCl–H₂O or DCl–D₂O stock solutions.

For the alcohol–water solutions the following procedures were used. Stock solutions of *t*-BuOH/H₂O and *t*-BuOD/D₂O were prepared by weight to give a mole fraction of 0.1 for the alcohol. Densities of mixtures of these solutions with a mole fraction of alcohol of 0.1 were determined by preparing the solutions by weight and weighing 1-mL samples of the solutions. By use of these densities (0.955 ± 0.017 and 0.999 ± 0.007 g/mL for the "H" and "D" solutions, respectively, at room temperature), the approximate volumes of final solutions were calculated along with the proper amount of HCl or DCl to add to H₂O or D₂O so that the final solution would be 10^{-2} N HCl or DCl. The necessary weight of the acidified water solutions was added to a flask followed by the appropriate weight of the *t*-BuOH/H₂O or *t*-BuOD/D₂O to give final solutions which were 10^{-2} N HCl (or DCl) with a mole fraction of alcohol equal to 0.1. Due to an error in calculation the HCl solution was 1.5×10^{-2} N. From these stock solutions of *t*-BuOL/L₂O were prepared mixtures having various atom fractions of deuterium by volume. The differences in HCl (DCl) content due to the error in calculation should have no influence on the results due to the pH-independent nature of the reaction in the region under investigation.

Kinetics. The hydrolysis of *p*-nitrophenyl dichloroacetate was monitored by following the increase in absorbance at 317 nm (330 nm in alcohol–water solutions) with a Cary 118C UV–vis spectrophotometer equipped with a constant-temperature cell compartment and cell holder to control the temperature at 25.00 ± 0.05 °C. Reactions were initiated by injection of 10–25 μ L of the stock *p*-nitrophenyl dichloroacetate solution into 3.00 mL of the appropriate reaction solution. Mixing was facilitated in most instances by inserting a "plunger" directly into the cell to give manual agitation of the solution for 3–4 s. This method appeared to give adequate mixing of all solutions.

Reactions were followed to greater than 80% completion, and infinity values were taken at 10 half-lives. The pH(D) of each reaction solution was measured at the completion of each run by using a Corning 130 pH meter equipped with a combination electrode. First-order rate constants were determined by using a nonlinear least-squares computer program which calculates first-order rate constants from given time and absorbance values. These constants were confirmed by plots of $\log(A_\infty - A_t)$ vs. time.

Acknowledgment. We are especially grateful to Professor John Albery for providing us with a copy of his computer program, GAMISO.

Registry No. 4-Nitrophenyl dichloroacetate, 780-32-5; *p*-nitrophenol, 100-02-7; dichloroacetic anhydride, 4124-30-5.

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