

Solvent effect and proton inventory in the hydrolysis of *p*-methylphenyl trichloroacetate

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ABSTRACT: Hydrolysis of *p*-methylphenyl trichloroacetate in water–acetonitrile mixtures was studied as a function of water concentration in the range 5.5–55.5 M. The proton inventory technique, in H₂O–D₂O mixtures, shows, for a value of D atom fraction in the solvent $n = 0.5$, deviations from the expected value (for a reaction with one proton being transferred) of 7.5 and 12.3%, for experiments in the presence of 16.6 and 33.3 M L₂O (L = H or D), respectively. Theoretical treatment of the data obtained at [L₂O] = 16.6 M using the Gross–Butler equation are consistent with a cyclic transition-state structure with three protons involved. Conversely, similar experiments in the presence of [L₂O] = 33.3 M show that multiple water molecules are involved in the transition state of the reaction. Copyright © 2006 John Wiley & Sons, Ltd.

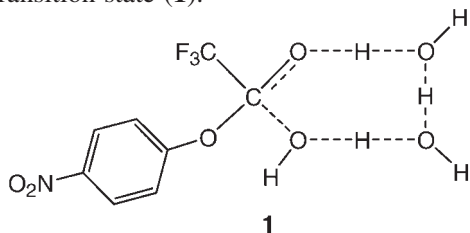
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KEYWORDS: hydrolysis; *p*-methylphenyl trichloroacetate; transition state; proton inventory; kinetics

INTRODUCTION

Proton transfer is the most common enzyme-catalyzed reaction, i.e. enzymes can catalyze efficiently proton transfer reactions involving what would otherwise be high-energy intermediates.¹ In this context, there is considerable interest in the role of hydrogen bonding in view of the fact that low-barrier hydrogen bonds (LBHB) may play an important role in the proton transfer mechanism in enzyme catalysis.² However, Warshel and Papazyan³ postulated that LBHB do not offer a catalytic advantage over ordinary hydrogen bonds. In fact, ordered water molecules seem to play a critical role in several enzyme-catalyzed processes, which provide a scaffold to impart proximity, orientation and nucleophilicity to water molecules.

It is known that in the water-catalyzed hydrolysis of esters the rate constants have a strong dependence on the water concentration. For example, reaction of *p*-nitrophenyl trifluoroacetate is thought to proceed with three protons undergoing bonding changes in an eight-membered transition state (**1**).⁴



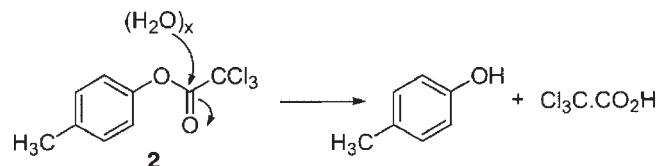
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A great number of both experimental^{4–8} and theoretical^{8–12} studies have been devoted to the hydrolysis reactions of aldehydes, ketones, esters and amides.^{13–17} It has been observed that the nucleophile attack on a carbonyl group is mediated by water molecules that previously hydrated the carbonyl group.¹² The concerted reaction involves proton transfers with carbon–nucleophile bond formation avoiding the energetic cost of desolvation. Engbersen and Engberts¹³ suggested that the strongly negative entropy observed in these reactions indicates that the transition state is highly hydrated compared with the reagents in the ground state.

In order to examine the effects of the composition of the solvent in water–acetonitrile mixtures in the structure of the transition state, we decided to examine the hydrolysis of *p*-methylphenyl trichloroacetate, where the presence of the α -chloro substituents should facilitate the hydration and the reaction should therefore be sensitive to the composition of the solvent mixture.

RESULTS AND DISCUSSION

The hydrolysis reaction of *p*-methylphenyl trichloroacetate (**2**) was carried out in aqueous acetonitrile and resulted in the formation of *p*-cresol and trichloroacetic acid (Scheme 1). First-order rate constants for the hydrolysis of **2**, measured in the range 0.001–0.1 M HCl, showed that the reaction was independent of acid concentration and the average value in the plateau region corresponds to the first-order rate constant of the



Scheme 1

spontaneous water reaction ($k_0 = 2.69 \times 10^{-3} \text{ s}^{-1}$, Fig. 1S in the Supplementary Material, available in Wiley Interscience).

The rate constant for the hydrolysis of **2** depends strongly on the composition of the solvent and, in water–acetonitrile mixtures, a 10-fold increase in water concentration results in an ~ 2700 -fold increase in the rate constant (Table 1).

The strong dependence of the rate constant on the water concentration observed in Table 1 is strongly indicative of a reaction involving several molecules of water (Scheme 1) and consistent with the equation

$$k_{\text{obs}} = k_1 [\text{H}_2\text{O}]^x \quad (1)$$

Hence the logarithmic form of Eqn (1) should allow us to estimate the order of the reaction in relation to water:

$$\log k_{\text{obs}} = \log k_1 + x \log [\text{H}_2\text{O}] \quad (2)$$

According to Eqn (2), a plot of $\log k_{\text{obs}}$ versus $\log [\text{H}_2\text{O}]$ is expected to be linear, with slope x , the apparent order with respect to water. In most cases, the relation observed is not linear because of a variety of effects, of which changes in polarity as a function of the solvent composition is most evident and, responsible for major effects in the observed rate constants. As a consequence, the value of the slope is not expected to be an accurate value for the order of the reaction, but to give an indication of the number of water molecules involved in the rate-limiting transition state. However, experimentally, the plot of the observed rate constant for the hydrolysis of **2** as a function of water concentration, shows considerable

Table 1. Rate constants for the hydrolysis of *p*-methylphenyl trichloroacetate as a function of the concentration of water in H_2O – CH_3CN mixtures at 25°C

$[\text{H}_2\text{O}]$ (M)	k_{obs} (10^{-3} s^{-1})
5.50	0.0670
11.0	0.174
16.6	0.367
22.4	0.630
27.5	1.91
33.3	3.03
38.9	8.91
43.7	22.3
50.1	70.6
55.0	184

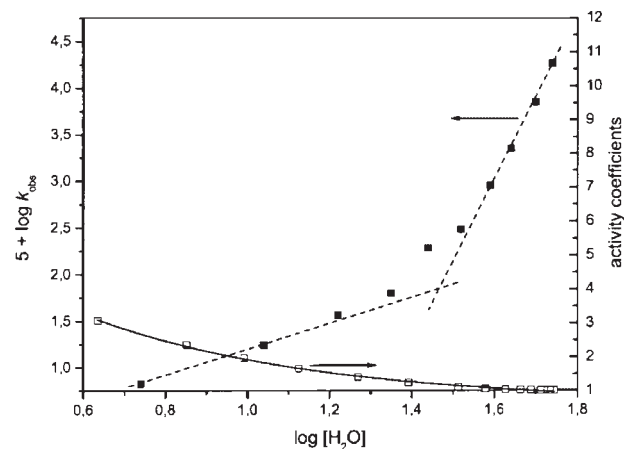


Figure 1. Plots of $\log k_{\text{obs}}$ versus $\log [\text{H}_2\text{O}]$ for the hydration of *p*-methylphenyl trichloroacetate in H_2O – CH_3CN mixtures (filled squares, left axis) and activity coefficients of water in H_2O – CH_3CN mixtures (open squares, right axis)

curvature (Fig. 1). Indeed, it is approximately linear in the water concentration range from 5.5 to $\sim 22 \text{ M}$ ($\log [\text{H}_2\text{O}] = 1.35$), with a slope which indicates a kinetic order of 1.6 ± 0.1 , and this kinetic order is 3.1 ± 0.1 if we use the activity of water in acetonitrile instead of the concentration of water (plot not shown).¹⁸ Above 33.3 M ($\log [\text{H}_2\text{O}] = 1.52$), there is a steep increase in slope and, in the high water content region, the calculated limiting slope indicates that the kinetic order in relation to water is 8.0 ± 0.4 , a result consistent with the increase in hydrogen bonds between water molecules provoked by the decrease in molar fraction of the organic solvent (the kinetic order is 9.9 ± 0.1 using the activity data).¹⁸

The observed reaction order is indicative that at low water content the reaction behaves as if it were a third-order reaction in relation to the water concentration and that, at the limit of high water concentration, multiple water molecules (probably a considerable part of those forming the cybotactic region) are participating in the reaction. Clearly, the observed effects may be interpreted as reflecting changes in the structure of the solvent mixture, which in turn will reflect variation of the transition-state structure with solvent composition in the hydrolysis of **2**. A similar behavior has been reported for the hydration reaction of 2,2-dichloro-1-(3-nitrophenyl)ethanone in H_2O –THF mixtures.⁷

In Fig. 1, we also plot the activity coefficients of water in acetonitrile versus $\log [\text{H}_2\text{O}]$ as determined by French measurement of vapor pressures of acetonitrile–water mixtures over the whole composition range using the static method.¹⁸ It is clear that there is only a very small effect on the activity coefficient of water above 33.3 M and, in this region, as the concentration of acetonitrile in the mixture becomes higher, the activity coefficient increases by only about 10% compared with pure water. It is important to note that very small changes in activity coefficients are observed in the same region where the

increase in concentration of water has a strong effect on the observed rate constant. The observed experimental results could be explained according to a 'microdomains' model, where the limit of water concentration at which acetonitrile can be accommodated within the cavities of ordinary water is ~ 37 M ($\log[\text{H}_2\text{O}] \approx 1.56$).^{19,20} Below this limit there are two microdomains, the first highly structured consisting predominantly of coordinated water molecules and the second relatively disordered containing mostly acetonitrile and hydrogen-bonded water molecules.^{20,21} Indeed, for $\log[\text{H}_2\text{O}] < 1.35$ the solvent mixture is dominated by the formation of acetonitrile–water clusters and, as a consequence, in this acetonitrile-rich region, the rate constant decreases linearly with decreasing water concentration. In the water-rich region, the decrease in water concentration is accompanied by a sharp decrease in k_{obs} and, most probably, the observed effect is related to the effect of enhanced cooperative water–water hydrogen bonding.^{22,23} In the intermediate region ($\log[\text{H}_2\text{O}]$ from 1.52 to 1.35), the statistical weight of each type of microdomain is changing and the observed dependence of the rate constant for the hydrolysis reactions follows the change in composition of the solvent.²⁰

Since the slopes of plots of $\log k_{\text{obs}}$ versus $\log[\text{H}_2\text{O}]$ may be affected by the non-ideal behavior of the solvent mixture,^{19,24} we applied the proton inventory technique in order to gain further insight into the structure of the transition state for the hydrolysis of **2** in H_2O – CH_3CN mixtures. With this technique it is possible to obtain information about the number of hydrogen atoms that are effectively transferred in the rate-determining step of the reaction.

The proton inventory technique is based on the fact that the solvent isotope effect, k_0/k_n , for a variety of proton-transfer reactions in H_2O – D_2O mixtures is a function of n , the atom fraction²⁵ of deuterium in the mixture. This happens because not all the exchangeable protons causing the isotope effect have the same composition as the bulk solvent. Equation (3), known as the Gross–Butler equation, shows the theoretical treatment of this phenomenon for rate processes:

$$k_n = k_0 \left[\prod_i^v (1 - n + n\phi_i^{\text{T}}) / \prod_j^v (1 - n + n\phi_j^{\text{R}}) \right] \quad (3)$$

where k_n and k_0 are the rate constants for a solvent whose D atom fraction is equal to n and for a pure protiated solvent, respectively, ϕ are the fractionation factors for every proton which exchanges with the solvent during the process under study and the superscripts R and T refer to reagents and transition state, respectively; n is the atom fraction of deuterium in the solvent and varies from 0 (pure protiated solvent) to 1 (pure deuterated solvent).

The rates constants for the hydrolysis reaction of **2** were determined in different molar fractions of

Table 2. Observed rate constants for the hydration of *p*-methylphenyl trichloroacetate in $\text{DCI}/\text{D}_2\text{O}$ – $\text{H}_2\text{O}/\text{CH}_3\text{CN}$ at different deuterium molar fractions n

n	$k_{\text{n,obs}} (10^{-4} \text{ s}^{-1})$	
	$[\text{L}_2\text{O}] = 16.6 \text{ M}$	$[\text{L}_2\text{O}] = 33.3 \text{ M}$
0	3.010	28.04
0.1	2.700	25.72
0.2	2.520	22.74
0.3	2.340	20.56
0.4	2.080	18.28
0.5	1.880	16.25
0.6	1.700	14.97
0.7	1.540	12.61
0.8	1.320	11.13
0.9	1.160	10.42
1.0	1.056	9.00

L_2O – CH_3CN mixtures (Table 2). The total water concentration ($[\text{L}_2\text{O}]$, where $\text{L} = \text{H}$ or D) was maintained constant at 16.6 and 33.3 M to obtain information about the number of protons involved in the transition states of both regions of the water order plot (Fig. 1).

The results are consistent with a normal isotope effect with values for $K_{\text{H}_2\text{O}}/K_{\text{D}_2\text{O}}$ of 2.85 and 3.11, at $[\text{L}_2\text{O}]$ 16.6 and 33.3 M, respectively, and they are similar to those for the reaction of other activated aryl esters.^{14,26} In both cases a downward curvature is obtained when the observed rate constants are plotted against the molar fraction of deuterium (Fig. 2). The degree of curvature depends on the increase in the molar concentration of L_2O in the mixture, showing, for a value of $n = 0.5$, deviations from the expected value (for a reaction with one proton being transferred) of 7.5 and 12.3% for $[\text{L}_2\text{O}] = 16.6$ and 33.3 M, respectively. Thus, an increase in the molar concentration of L_2O in the solvent mixture

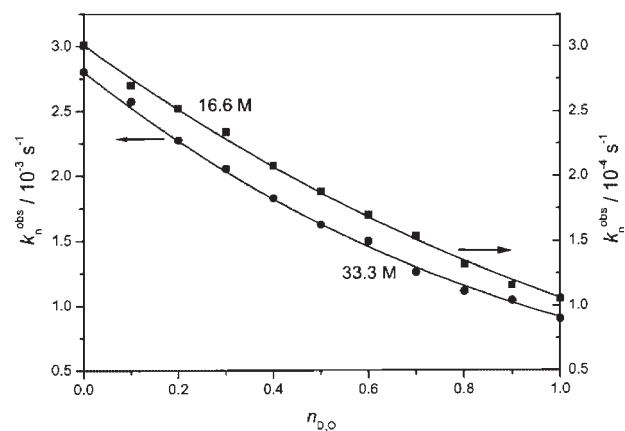


Figure 2. Rate constants for the hydrolysis of *p*-methylphenyl trichloroacetate in L_2O – CH_3CN mixtures as a function of the deuterium molar fraction, n , for $[\text{L}_2\text{O}] = 16.6$ and 33.3 M. Theoretical Gross–Butler curves correspond to three protons (■) and multiprotons (●) intervening in the transition state

causes an increase in the number of protons that take part in the transition state. The experimental data shown in Fig. 2 are consistent with three or more protons being involved in the process. The arguments follow.

Theoretical treatment of the data (Fig. 2) was performed using the Gross–Butler equation for a reaction where there is no contribution of the reagent to the observed isotope effect:²⁵

$$k_n^{\text{obs}} = k_0^{\text{obs}} (1 - n + n\phi^T)^m \quad (4)$$

where m corresponds to the number of protons that contributed to the observed isotope effect.

Thus, the solid lines in Fig. 2 represent the best fits obtained for the experimental data assuming three protons and multiprotons participating in the transition state for $[\text{L}_2\text{O}] = 16.6$ and 33.3 M, respectively.

According to the Gross–Butler theory, the equations

$$(k_n/k_0)^{1/3} = 1 + (\phi^T - 1)n \quad (5)$$

$$\ln(k_n/k_0) = -m(1 - \phi^T)n \quad (6)$$

are adequate for reactions where the numbers of protons participating in the rate-limiting step of the reaction are 3 and infinity, respectively.²⁵ Hence the linear plot observed for the experimental data at $[\text{L}_2\text{O}] = 16.6$ and 33.3 M indicates that the number of protons participating in the reaction increases from 3, for $[\text{L}_2\text{O}] = 16.6$ M, to a multiproton reaction at water concentration 33.3 M (correlation coefficients >0.99 , Fig. 3).

The slopes of the lines in Fig. 3, based on a three-proton and multi-proton model, with each proton having an identical fractionation factor, are 0.707 ± 0.003 and 0.869 ± 0.001 , respectively. The first value is very similar

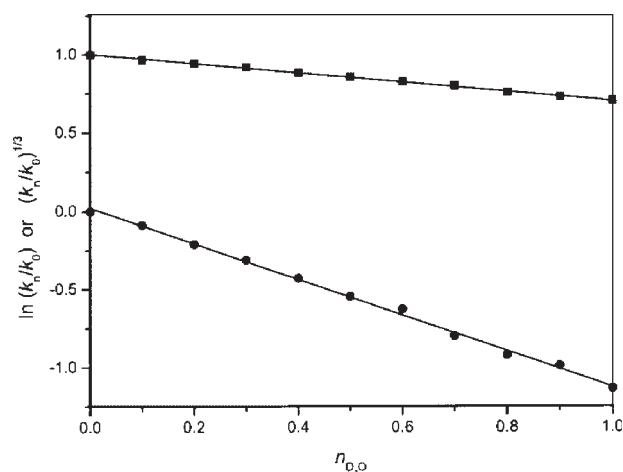
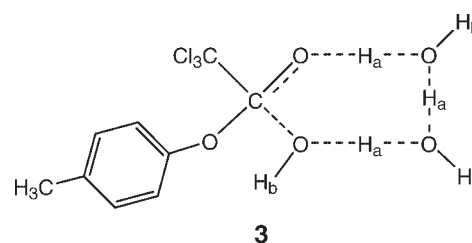


Figure 3. Plots of $\ln(k_n/k_0)$ or $(k_n/k_0)^{1/3}$ for the hydrolysis of *p*-methylphenyl trichloroacetate in L_2O – CH_3CN mixtures as a function of the deuterium molar fraction, n , for $[\text{L}_2\text{O}] = 16.6$ (■) and 33.3 M (●)

to that observed by Venkatasubban *et al.* for *p*-nitrophenyl trifluoroacetate, a fractionation factor of 0.697 ± 0.005 for a three-proton model.⁴

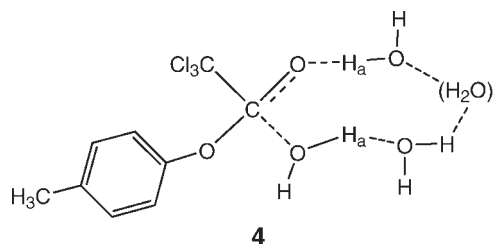


Structure **3** is consistent with the observed effect and we can see that the three hydrogens H_a are most likely responsible for the global isotope effect, since the hydrogens H_b contribute little or nothing, since they are expected to have a fractionation factor of 1.

Since the value of $k_{\text{H}_2\text{O}}/k_{\text{D}_2\text{O}}$ is 2.85, at $[\text{L}_2\text{O}] = 16.6$, it is very difficult in this particular case to distinguish a two-proton from a three-proton mechanism. This experimental difficulty is related to the relatively small global isotope effect and to the associated precision necessary to distinguish between these mechanistic possibilities. Despite this fact, we believe that the choice of an eight-membered transition state, rather than a six-membered transition state involving two water molecules, is appropriate given that the former has bond angles that accommodate approximately linear hydrogen bonds.^{4,27}

The water-assistance mechanism in ester hydrolysis has a substantial advantage in comparison with a single water attack. Molecular orbital calculations carried out by Hori *et al.*¹⁰ for the oxygen exchange accompanying the alkaline hydrolysis of methyl acetate showed that the activation barrier for the water-assisted mechanism is lower [$3.8 \text{ kcal mol}^{-1}$ ($1 \text{ kcal} = 4.184 \text{ kJ}$)] than that of the hydroxide attack without water assistance ($21.8 \text{ kcal mol}^{-1}$). In addition, results reported by Guthrie¹² have shown that a water-assisted mechanism involving proton transfer from water to carbonyl oxygen in a cyclic transition state avoids the cost of desolvation of the negative charge generated by the nucleophilic attack and, therefore, favors a concerted mechanism.

The data for hydrolysis at $[\text{L}_2\text{O}] = 33.3$ M are indicative of a highly hydrated transition state. Structure **4** is consistent with a large number of water molecules participating in the transition state.



Cyclic transition-state structures are frequently postulated in the literature,^{4–6,9,10} including for proton transfer

in water^{12,28} and acid-¹² and base-catalyzed^{10–12} hydrolysis of esters. In pure water or in water-rich solvent mixtures, water-to-water hydrogen bonds predominate and a multiple number of protons participate in the transition state for the hydrolysis of *p*-methylphenyl trichloroacetate. As this structure is disrupted by the addition of the organic cosolvent, the number of protons participating in the transition state gradually decreases, reaching the minimum value of three, which corresponds to the structure **3**. Clearly, in aqueous solutions and in mixtures of water and organic solvents the role of hydrogen bonding is fundamental. It is difficult to distinguish between the contribution of low-barrier hydrogen bonds and ordinary hydrogen bonds in terms of catalytic advantage.² However, it seems that in this particular reaction, the highly hydrogen-bonded water structure typical of the water-rich region provides a reaction cage which favors proximity, orientation and, as a consequence, increases the rate of the reaction.

EXPERIMENTAL

Preparation of *p*-methylphenyl trichloroacetate. A solution of trichloroacetyl chloride (0.1 mol), *p*-methylphenol (0.1 mol) and pyridine (0.1 mol) in diethyl ether (250 ml) was refluxed for 2.5 h. The solvent was evaporated and the resultant solid was passed through a column of silica gel HF 60, eluted with hexane–acetone (9:1, v/v). The solvent was removed and a clear yellow solid was obtained in 83% yield, m.p. 51 °C.

Kinetics. The aqueous solutions were prepared from deionized, doubly distilled water. Deuterium oxide and deuterium chloride with a minimum isotopic purity of 99.9 atom-% of deuterium were purchased from Aldrich and manipulated under a nitrogen atmosphere.

The rate constants of *p*-methylphenyl trichloroacetate hydrolysis were followed spectrophotometrically with a Hewlett-Packard Model 8452 spectrometer equipped with a thermostated water-jacketed cell holder at 25.0 ± 0.05 °C. In a typical run, the reaction was initiated by injection of 20 μ l of a 10^{-2} M stock solution of the substrate in acetonitrile (Merck, HPLC grade) to 3 ml of aqueous solvent mixture equilibrated at 25.0 ± 0.05 °C. The absorbance (*A*) decay was recorded at 244 nm and absorbance versus time data were stored directly on a microcomputer. First-order rate constants, k_{obs} , were estimated from linear plots of $\ln(A_{\infty} - A_t)$ against time for at least a 90% reaction using an iterative least-squares

program; correlation coefficients, ρ , were >0.999 for all kinetic runs and, between replicates, the standard deviation for the first-order rate constants was always $<2\%$. The pH was maintained at 2.0 with HCl and the solutions were prepared immediately before use, except in the study of the solvent isotope effect, where the solutions were left standing for 15 h at room temperature in order to allow the H/D balances to be reached.

Acknowledgements

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