Proton Inventory Study of a Water-Catalyzed Hydrolysis

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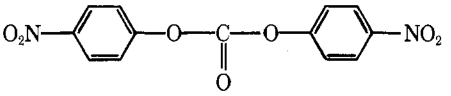
The neutral water reaction of bis(4-nitrophenyl) carbonate in H_2O–D_2O has been subjected to a proton inventory analysis. A plot of observed rate constants vs. the atom fraction of deuterium is linear, indicating that a single proton contributes to the solvent isotope effect of 2.24. This result is not consistent with a "one-water" mechanism nor with a "two-water" cyclic concerted mechanism but does agree with a "two-water" general base mechanism in which the transition state is reached at an early stage. It was also shown that urea, tetraalkylammonium salts, KBr, and other structure-making or structure-breaking additives cause only minor changes in the hydrolysis rates. These data suggest that hydrolytic enzymes do not achieve appreciable rate accelerations by perturbing the water structure at their active sites.

"Water reactions" refer to hydrolyses in which only substrate and water participate.[2,3] Acidic and basic catalysis play no role. Labile carboxylic acid derivatives such as acetic anhydride,[4] acetylimidazole,[5] ethyl trifluoroacetate,[6] and δ-thiovalerolacetone[7] all react with water at 25 °C. The hydrolyses are characterized by sizable negative entropies of activation and by solvent isotope effects greater than unity (typically, $\Delta S^{\ddagger} = -30$ to -45 eu and $k_{H_2O}/k_{D_2O} = 2$–3).[2] One virtue of water reactions is their relatively simple mechanism.[8] Another is their potential usefulness in the study of water properties.[9] In the present paper we describe a "proton inventory" of the hydrolysis of bis(4-nitrophenyl) carbonate (I)



I

at a pH where the neutral water reaction dominates. Proton inventories require the measurement of reaction rates in several different mixtures of protium oxide (water) and deuterium oxide. This information discloses the number of protons contributing to the solvent isotope effect.[10,11] We also determined the rates of the water reaction of I in the presence of large amounts of urea, tetramethylammonium chloride, and other structure-making or structure-breaking additives.

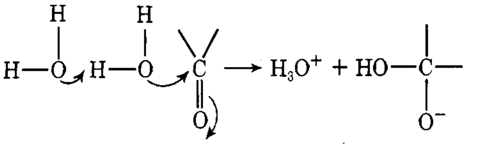
Experimental Section

Materials. Bis(4-nitrophenyl) carbonate (I) was prepared by the method of Fife and McMahon.[12] The carbonate, crystallized twice from benzene, melted at 139–141 °C (lit.[12] mp 138–140 °C). Alkaline hydrolysis of the material produced 2 molar equiv of *p*-nitrophenoxide. Acetonitrile (Eastman Spectrograde) was purified by distillation over P_2O_5 and K_2CO_3. Deuterium oxide (99.7%) and deuterium chloride (99%) were purchased from Diaprep. All inorganic salts were dried in an oven and organic additives (except the tetramethylammonium salts) were crystallized or distilled prior to use.

Kinetics. Hydrolysis rates of I were measured by following the appearance of *p*-nitrophenol at 320 nm using a Cary 14 spectrophotometer fitted with a 1.0 slidewire. A 3.00-ml solution of 0.01 N HCl in an appropriate mixture of D_2O–H_2O was equilibrated in a 1.00-cm cuvette placed within the thermostated cell chamber of the Cary 14. Reactions were initiated by adding 20 μl of a concentrated solution of I in acetonitrile with the aid of a small glass stirring rod. Concentrations of I in the cuvette were approximately 3×10^{-5} M. We followed the reactions to greater than 80% completion, and took infinity values at 10 half-lives. First-order rate constants, calculated in the usual manner, were reproducible to within 3% in most cases. Mr. Josef Nemeth, Urbana, Ill., determined the atom fraction of deuterium in a "pure" DCl/D_2O solution.

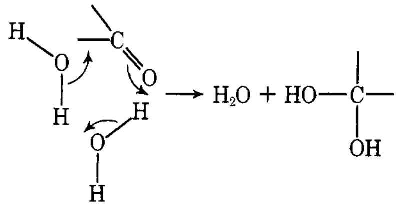
Results and Discussion

Water reactions of labile carboxylic acid derivatives, including I, are believed to involve *two* water molecules as shown in eq 1.[12] One water molecules functions as a general base



(1)

whereas the other functions as a nucleophile. This mechanism accords with a solvent isotope effect greater than unity and with the known susceptibility of labile substrates to general base catalysis. Yet neither line of evidence precludes kinetic equivalents of eq 1 such as the cyclic concerted process in eq 2.[13,14] In fact, even a "one-water" mechanism is consistent with



(2)

a solvent isotope effect near 2 provided that the bond between the water oxygen and the carbonyl carbon is well formed in the transition state. These ambiguities prompted us to carry out a "proton inventory" experiment in order to secure the total number of protons contributing to the solvent isotope effect.

A proton inventory is a rather specialized physical organic method, and a brief discussion of its attributes might be useful.[10,11,15] The observed rate constant k_n in a H_2O–D_2O mixture is related to the rate constant k_0 in pure water by eq 3.

$$k_n/k_0 = \prod_{i}^{\mathrm{TS}} (1 - n + n\phi_i^{*}) \Big/ \prod_{j}^{\mathrm{GS}} (1 - n + n\phi_j) \qquad (3)$$

The parameter n signifies the atom fraction of deuterium. All transition state protons i that contribute to the isotope effect possess a term in the right-hand numerator of eq 3. These terms are multiplied as are the terms in the denominator corresponding to the reactive ground state protons j. Each proton site is associated with an isotopic fraction factor ϕ which expresses the degree of deuteration of the site. For example, since ϕ for $R_2OH^+ = 0.69$, the $[R_2OD^+]/[R_2OH^+]$ ratio equals 0.69 (not 1.0) in a 50% D_2O/50% H_2O mixture. Lists of fractionation factors for various types of exchangeable protons can be found elsewhere.[11] Analysis of the water reaction of bis(4-nitrophenyl) carbonate is particularly simple because the substrate has no reactive protons and the other reactant, water, has a $\phi = 1$ by definition. Consequently, the denominator is unity and we need consider only the protons of the transition state. If only one proton in the transition state contributes to the solvent isotope effect, then a plot of k_n vs. n should be linear. Nonlinearity would arise from two or more transition state terms in eq 3.

Table I. Observed First-Order Rate Constants for the Hydrolysis of Bis(4-nitrophenyl) Carbonate in Mixtures of 0.01 N HCl/H_2O and 0.01 N DCl/D_2O at 50.0 °C[a]

Atom fraction of deuterium (n)	No. of runs	$k_n \times 10^4$, min^{-1}
0.000	10	1795 ± 47[c]
0.099	3	1696 ± 32
0.198	4	1604 ± 20
0.297	3	1501 ± 14
0.395	3	1382 ± 20
0.494	4	1278 ± 32
0/593	4	1172 ± 30
0.692	3	1066 ± 20
0.791	3	987 ± 21
0.889	2	874 ± 10
0.986[b]	20	802 ± 20

[a] Ionic strength = 0.05 with KCl. [b] Atom fraction of deuterium in "100%" 0.01 N DCl/D_2O was determined by J. Nemeth, Urbana, Ill. Other n values are based on this number. [c] Error limits are standard deviations.

Most of our kinetic studies of I were carried out in 0.01 M aqueous HCl at 50.0 °C. We are certain that the hydrolysis is a true neutral water reaction under these conditions because the observed rates in 0.10 N HCl, 0.01 N HCl, 0.001 N HCl, and at pH 4.65 and 5.29 differ only slightly (0.177, 0.180, 0.178, 0.185, and 0.186 min^{-1} at 50.0 °C, respectively). The latter two rate constants were secured using an acetate buffer and extrapolating to zero buffer concentration (acetate catalyzes the hydrolysis by a general base mechanism[12]). Fife and McMahon[12] obtained a $k_{obsd} = 0.168$ at 0.01 N HCl and 50.0 °C, a satisfactory agreement. The solvent isotope effect, k_{H_2O}/k_{D_2O}, was found to equal 2.24 which is substantially smaller than the value of 2.88 published previously.[12] The source of the discrepancy remains unclear.

Table II. Effect of Additives on the Water-Catalyzed Hydrolysis of Bis(4-nitrophenyl) Carbonate in 0.01 N HCl at 50.0 °C

Registry no.	Additive	Concn, M	$k_{obsd} \times 10^4$, min^{-1}
	None		1794 ± 47[a]
7758-02-3	KBr	0.99	1393 ± 21
	KBr	2.00	922 ± 54
7447-40-7	KCl	0.99	1642 ± 31
75-05-8	CH_3CN	1.00	1511 ± 23
67-68-5	DMSO	1.12	1891 ± 58
123-91-1	Dioxane	1.03	1312 ± 51
75-65-0	t-BuOH	1.04	1487 ± 60
57-13-6	Urea	1.00	1950 ± 43
	Urea	3.00	2008 ± 13
	Urea	4.00	2176 ± 91
	Urea[b]	4.00	1195
632-22-4	Tetramethylurea	1.03	1934 ± 13
75-57-0	Tetramethylammonium chloride	1.00	1730 ± 30
	Tetramethylammonium chloride	2.00	1550 ± 60
1643-19-2	Tetrabutylammonium bromide	1.00	1110 ± 34

[a] Error limits are standard deviations from usually three runs. [b] Hydrolysis carried out in D_2O; single run only.

Table I lists first-order rate constants, k_n, for the water reaction of I at different atom fractions of deuterium. Since a plot of k_n vs. n (Figure 1) is linear,[16,17] we conclude that only a *single* proton contributes to the solvent isotope effect. Let us now consider the significance of this result with respect to possible transition states of the water reaction. In the general base mechanism (eq 1) the solvent isotope effect must derive solely from the proton which is transferred from the "nucleophilic" water to the "general base" water. The fact that our inventory detected no secondary isotope effect from the three remaining water protons signifies that extensive proton transfer to the "general base" water is not achieved in the transition state. Otherwise both protons of the "general base" water would be transformed into hydronium-like protons. Their fractionation factor would then change from 1.0 in the ground state to a value resembling 0.69 in the transition state, and a three-proton inventory would have been observed. Note that the fourth proton in eq 1 (namely the nontransferred proton on the "nucleophilic" water) is converted from an -OH proton of water into an -OH proton of a tetrahedral intermediate; one would not expect the site to add an important term to eq 3. In summary, the linear k_n vs. n plot is indeed consistent with a general base mechanism provided that the transition state is reached at an early stage of the reaction.[18] Moreover, we can eliminate a "one water" hydrolysis with considerable bond formation between the oxygen and the carbonyl carbon because this would lead to a two-proton inventory. Lack of a two-proton inventory also renders unlikely a cyclic concerted mechanism (eq 2) in which two protons are transferred.

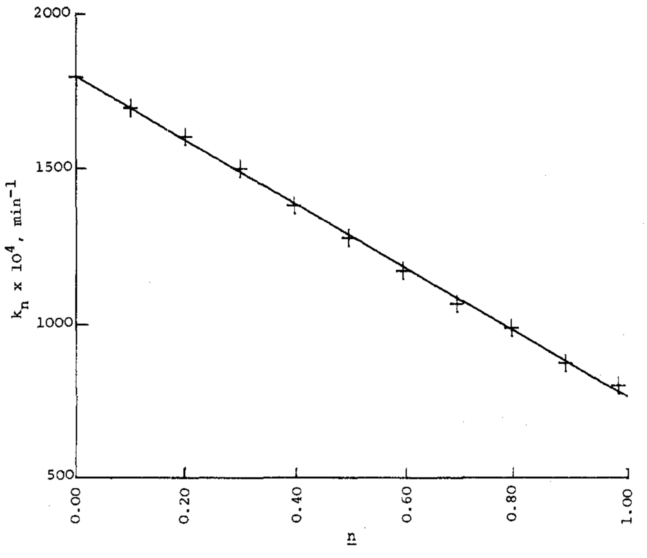


Figure 1. Dependence of k_n on the atom fraction of deuterium n in mixtures of protium and deuterium oxides. The data are taken from Table I.

We carried out a set of peripheral experiments to determine if additives that perturb water structure also perturb the water reaction rates of I. The data are presented in Table II. Tetraalkylammonium ions, strong structure-making species,[19] are seen to decrease the hydrolysis rates. Urea, an effective structure-breaker,[20] increases the rates. Dioxane and KBr do not fit this pattern; they are weakly structure-breaking[21] and yet decrease the rates. The most striking feature of Table II is the small variation in rate constants. Several explanations for this are possible. (1) The water reaction of I does not require properly oriented water molecules. Hence the hydrolysis is insensitive to the diffusional average water structure. (2) The water reaction does in fact demand two properly oriented water molecules, but these molecules are members of solvation shells encasing the substrate. If the structure of the solvation shell is only slightly affected by the additives, their presence will not change k_{obsd}. (3) The additives modify $\Delta H^{\ddagger}$ and $T\Delta S^{\ddagger}$ in a compensatory manner so that the rate effects are small. Engbersen and Engberts[9] have shown clearly that this third factor is important. However, from the point of view of catalysis, which is concerned primarily with rates and not the component activation parameters, the lesson from Table II and the work of others[22,23] is clear. Nonreactive additives have a disappointing rate effect on the water reactions of carboxylic acid derivatives.[24] Our data suggest that hydrolytic enzymes do not achieve appreciable rate accelerations by perturbing the microscopic environment at the active sites.[25]

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Registry No.—I, 5070-13-3; water, 7732-18-5.

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(16) Analysis of the data in Table I by means of a polynomial regression program indicates that quadratic and higher terms in eq 3 are insignificant. We thank Professor R. L. Schowen and Mr. Daniel P. Quinn for carrying out these calculations.
(17) This result contrasts with that of B. D. Batts and V. Gold, *J. Chem. Soc. A*, 984 (1969), who found a slight curvature in the k_n vs. n plot for the water reaction of acetic anhydride at 25.0 °C. Since our runs were carried out at 50.0 °C, direct comparison of the two systems is difficult.
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Metalation of Cumene with *n*-Pentylsodium in the Presence of *N,N,N′,N′*-Tetramethylethylenediamine. Preparation of α-Cumylsodium

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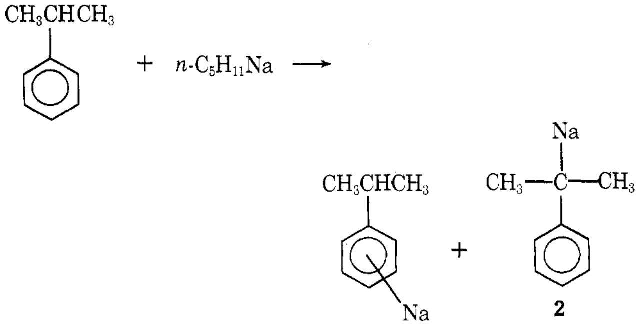
The metalation of cumene with *n*-pentylsodium was reinvestigated to assess the effect of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) on this reaction. It was found that in the presence of TMEDA, cumene was metalated by *n*-pentylsodium giving a 65% yield of α-cumylsodium with an isomeric purity of 95% after a 24-h reaction period. In the initial stages of the reaction, a kinetically controlled metalation occurred giving principally *o*-, *m*-, and *p*-isopropylphenylsodium. At longer reaction times, these compounds isomerized to α-cumylsodium in a thermodynamically controlled process.

In 1963 Professor Benkeser and co-workers[2] reported that cumene was metalated by *n*-pentylsodium in a kinetically controlled process to give a mixture of *m*- and *p*-isopropylphenylsodium (**1b** and **1c**) and trace amounts of α-cumylsodium (**2**). In the presence of excess cumene, the ring sodium compounds **1b** and **1c** slowly converted to the α compound **2** in a thermodynamically controlled process. The α-sodium compound was shown to be unstable and decomposed to α-methylstyrene and sodium hydride.[3]



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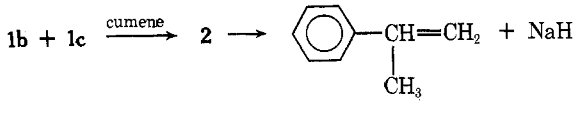
a, ortho
b, meta
c, para



Recently, Trimitsis and co-workers[4] reported that the presence of *N,N,N′N′*-tetramethylethylenediamine (TMEDA) greatly altered the reactivity of *n*-pentylsodium in metalation reactions. Thus, 1,3-dimethylnaphthalene and