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Influence of Ionic Strength upon the Proton Inventory for the Water-Catalyzed Hydrolysis of *N*-Acetylbenzotriazole[1]

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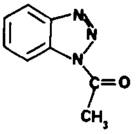
Proton inventory investigations of the hydrolysis of *N*-acetylbenzotriazole at pH 3.0 (or the equivalent point on the pD rate profile) have been conducted at two different temperatures and at ionic strengths ranging from 0 to 3.0 *M*. The solvent deuterium isotope effects and proton inventories are remarkably similar over this wide range of conditions. The proton inventories suggest a cyclic transition state involving four protons contributing to the solvent deuterium isotope effect for the water-catalyzed hydrolysis. The hydrolysis data are described by the equation $k_n = k_0 (1 - n + n\phi_a^*)^4$ with $\phi_a^* \sim 0.74$, where k_0 is the observed first-order rate constant in protium oxide, n is the atom fraction of deuterium in the solvent, k_n is the rate constant in a protium oxide–deuterium oxide mixture, and ϕ_a^* is the isotopic fractionation factor.

INTRODUCTION

Recent investigations of the mechanism of several acyl transfers to water using the proton inventory technique have suggested both cyclic (*1–3*) and acyclic (*4–11*) transition state structures may be involved. The factors which determine the nature of the transition state structure in such cases have not been discovered. As part of a continuing effort to explore this problem the water-catalyzed hydrolysis of *N*-acetylbenzotriazole (**1**) has been explored using the proton inventory technique.



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This investigation was suggested by the recent work of Reboud-Ravaux (*12*) and by the importance of *N*-acetylbenzotriazole as a protein reagent and as a substrate for α-chymotrypsin.

The hydrolysis of *N*-acetylbenzotriazole is pH independent in the region pH 2.5–6.0 and is characterized by a large negative entropy of activation (−43 eu) and a large solvent deuterium isotope effect, k_{H_2O}/k_{D_2O}, of about 3.0. These observa-

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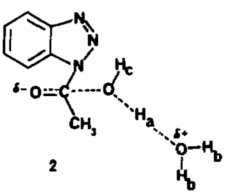
tions led Reboud-Ravaux to consider transition states **2–6** for the water-catalyzed hydrolysis (*12*).



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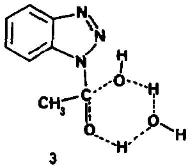

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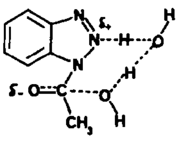

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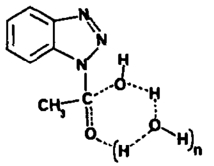

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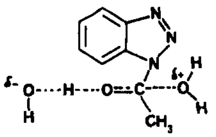

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Each of these transition states, except **5** with $n = 1$, involved two water molecules. She used the fact that water falls on the Brönsted line for general base catalysis ($\beta = 0.38$), among other things, to rule out the cyclic (**3–5**) and acid-catalyzed (**6**) transition states. In light of repeated warnings (*13*, *14*) about the reliability of the Brönsted value as a measure of the extent of proton transfer, the rejection of these transition states seemed to us to require additional justification. The proton inventory studies reported here allow additional consideration of transition state structures **2–6**, as well as others, and illustrate the additional information that can be obtained from such studies.

RESULTS

The hydrolysis of *N*-acetylbenzotriazole was studied at pH 3.0, or the equivalent point on the pD rate profile, in protium oxide, deuterium oxide, and mixtures of the two. The pH(D) was controlled using HCl or DCl, and the studies were conducted at ionic strengths (KCl) of 0.0, 0.5, 1.0, 1.5, 2.0, 2.4, and 3.0.

Table 1 and Fig. 1 show the dependence of the observed first-order rate constants on the isotopic composition of the solvent system of atom fraction deuterium, n, at 40°C ($\mu = 1.0$; KCl). Inspection shows the proton inventory of Fig. 1 exhibits significant downward curvature, thus implicating the involvement of multiple protons in the transition state for the water-catalyzed hydrolysis.

The observed first-order rate constant in protium oxide of $2.49 \times 10^{-4}\ \mathrm{sec}^{-1}$ is in good agreement with the literature value of $2.2 \times 10^{-4}\ \mathrm{sec}^{-1}$ at 40°C (*12*). The solvent

TABLE 1

FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF *N*-ACETYLBENZOTRIAZOLE IN MIXTURES OF 10^{-3} *M* HCl-H_2O AND DCl-D_2O AT 40 ± 0.05°C[a]

Atom fraction of deuterium (n)	No. of runs	$10^7 k_n$ sec^{-1}	$10^7 k_n$, sec^{-1} (calcd) Brönsted method[b]	γ method[c]
0.000	5	2495 ± 7[d]	2495	2495
0.247	5	1913 ± 10	1991	1917
0.495	5	1468 ± 20	1537	1445
0.742	5	1059 ± 6	1132	1067
0.989[e]	5	775 ± 5	774	768

[a] Ionic strength was maintained at 1.0 *M* with KCl.

[b] Calculated based on the observed Brönsted β value of 0.38 with $\phi_a^* = 0.404$ and $\phi_b^* = 0.868$ in Eq. [11].

[c] Calculated based on the γ value of 0.26 with $\phi^* = 0.742$ in Eq. [13].

[d] Error limits are standard deviations.

[e] Atom fraction of deuterium in "100%" 10^{-3} *M* DCl-D_2O as determined by Mr. Josef Nemeth.

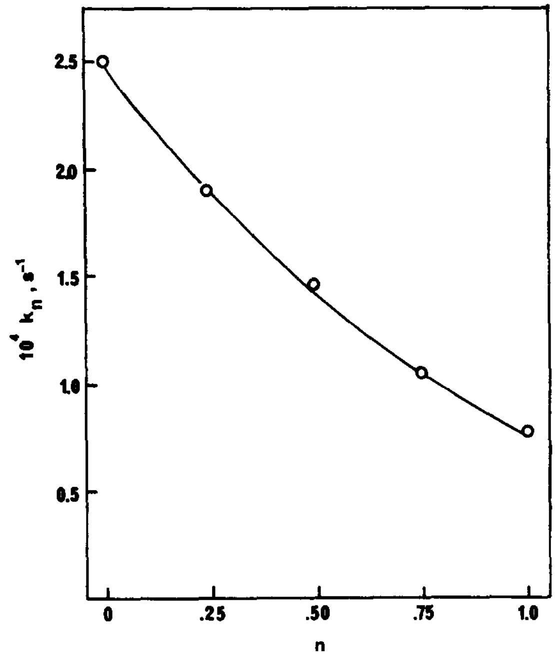


FIG. 1. Dependence of the observed first-order rate constants for the water-catalyzed hydrolysis of *N*-acetylbenzotriazole on the atom fraction of deuterium in the solvent at 40°C. The data are taken from Table 1. The solid line was calculated based upon the transition state model described by Eq. [13] with $\phi_a^* = 0.742$.

deuterium isotope effect of 3.22 (40°C) compares favorably also with the value at 25°C of 3.09 (*12*).

Table 2 lists the rate constants for the water-catalyzed hydrolysis of 1 at seven different ionic strength values at 50°C. Figure 2 shows the proton inventory curve of the reaction at μ = 1.0 at 50°C. Here again visual inspection reveals significant downward curvature. The solvent deuterium isotope effect is remarkably constant (Table 2) over this range of ionic strengths (i.e., k_{H_2O}/k_{D_2O} ranges from 3.21 to 3.37 with a mean value of 3.31 ± 0.06). Proton inventory curves for the data at the other ionic strengths are not included, but they all exhibit the same downward curvature. Tables 1 and 2 also include the calculated values of the rate constants based on models for the transition state discussed below, and the solid lines of Figs. 1 and 2 are based on this transition state model (8).

Figure 3 is a plot of the log k_{obs} for hydrolysis against the ionic strength (μ) in solutions of three different atom fraction deuterium (n) at 50°C.

DISCUSSION

Theory

Studies of reaction rates in mixtures of protium oxide and deuterium oxide (i.e., proton inventories) allow one to relate the rate constant, k_n, in solvent composed

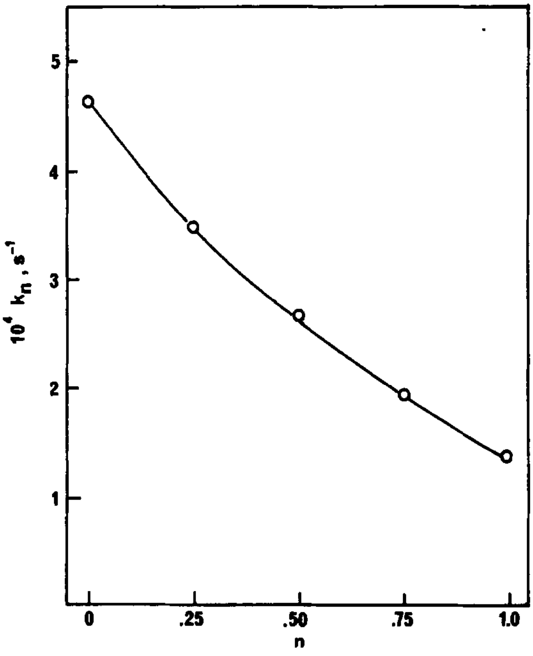


FIG. 2. Dependence of the first-order rate constants for the water-catalyzed hydrolysis of *N*-acetylbenzotriazole on the atom fraction of deuterium in the solvent at 50°C and ionic strength of 1.0. The data are taken from Table 2. The solid line was calculated based upon the transition state model described by Eq. [13] with $\phi_a^* = 0.737$.

TABLE 2

FIRST-ORDER RATE CONSTANTS FOR THE HYDROLYSIS OF *N*-ACETYLBENZOTRIAZOLE IN MIXTURES OF 10^{-3} *M* HCl-H_2O AND DCl-D_2O AT 50 ± 0.05°C AS A FUNCTION OF IONIC STRENGTH

Ionic strength(*M*)[a]	Atom fraction of deuterium(*n*)	No. of runs	$10^7 k_n$, sec^{-1}	$10^7 k_n$, sec^{-1} (calcd) Brönsted method[b]	 γ Method[c]
0.00	0.000	4	5476 ± 30[d]	5476	5476[c]
	0.247	5	4129 ± 19	4355	4207
$\frac{k_{H_2O}}{k_{D_2O}} = 3.32$	0.495	4	3142 ± 20	3347	3172
	0.742	5	2306 ± 9	2447	2341
	0.989[e]	5	1651 ± 10	1650	1685
0.50	0.000	5	5026 ± 40	5026	5026[d]
	0.247	5	3857 ± 50	4012	3861
$\frac{k_{H_2O}}{k_{D_2O}} = 3.21$	0.495	5	2868 ± 30	3100	2912
	0.742	5	2149 ± 17	2285	2149
	0.989	5	1565 ± 8	1563	1547
1.00	0.000	5	4628 ± 40	4628	4628[e]
	0.247	5	3485 ± 25	3675	3536
$\frac{k_{H_2O}}{k_{D_2O}} = 3.36$	0.495	8	2671 ± 60	2819	2651
	0.742	5	1926 ± 80	2054	1943
	0.989	5	1378 ± 15	1377	1387
1.50	0.000	3	4108 ± 22	4108	4108[f]
$\frac{k_{H_2O}}{k_{D_2O}} = 3.32$	0.495	3	2332 ± 30	2509	2378
	0.989	3	1237 ± 8	1238	1264
2.00	0.000	5	3507 ± 10	3507	3507[g]
	0.247	5	2670 ± 10	2784	2694
$\frac{k_{H_2O}}{k_{D_2O}} = 3.37$	0.495	5	1998 ± 10	2132	2032
	0.742	5	1480 ± 6	1553	1499
	0.989	5	1040 ± 4	1041	1079
2.40	0.000	3	3152 ± 10	3152	3152[f]
$\frac{k_{H_2O}}{k_{D_2O}} = 3.31$	0.495	3	1821 ± 31	1926	1825
	0.989	3	952 ± 20	952	970
3.00	0.000	3	2574 ± 22	2574	2574[f]
$\frac{k_{H_2O}}{k_{D_2O}} = 3.25$	0.495	3	1491 ± 18	1581	1490
	0.989	3	791 ± 12	791	792

[a] Maintained with KCl.

[b] Calculated based on the observed Brönsted β value of 0.38 with $\phi_b^* = 0.868$ and $\phi_a^* = 0.391, 0.404, 0.386, 0.392, 0.386, 0.393$, and 0.40 for $\mu = 0, 0.5, 1.0, 1.5, 2.0, 2.4$, and 3.0, respectively, in Eq. [11].

[c] Calculated based on the γ value of 0.25 which is the maximum positive γ value for these data. This gives $\phi_a^* = 0.742$ in Eq. [13].

[d] Calculated based on a γ value of 0.25 which is slightly larger than the calculated γ (Table 3) but is consistent with the data at other ionic strengths. This gives $\phi_a^* = 0.742$ in Eq. [13].

[e] Calculated based on a γ value of 0.25 (Table 3) with $\phi_a^* = 0.737$ in Eq. [13].

[f] No γ value was calculated but a $\phi_a^* = 0.742$ was used in Eq. [13] to calculate these numbers.

[g] Calculated based on a γ value of 0.24 (Table 3) with $\phi_a^* = 0.742$ in Eq. [13].

of atom fraction deuterium n to the rate constant in protium oxide, k_0, by

$$k_n = k_0 \prod_i^{TS} (1 - n + n\phi_i^*) / \prod_j^{RS} (1 - n + n\phi_j). \qquad [1]$$

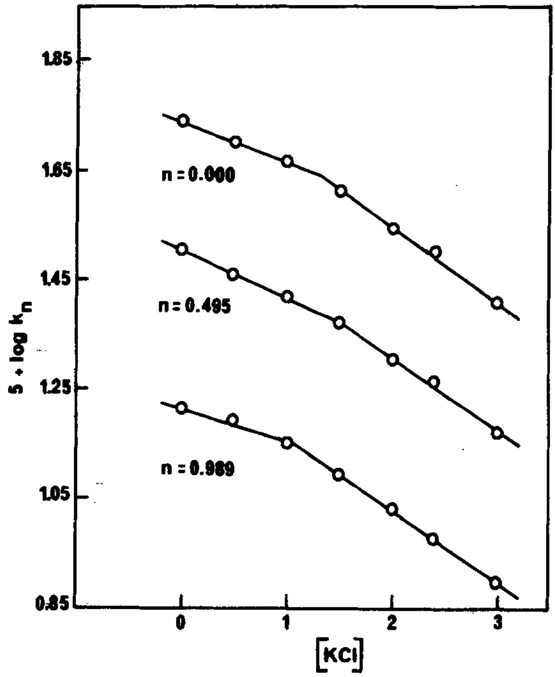


FIG. 3. Dependence of the observed first-order rate constants for the water-catalyzed hydrolysis of *N*-acetylbenzotriazole upon the ionic strength at 50°C in solutions of atom fraction of deuterium *n*. The data are from Table 2.

In this equation, ϕ_i^* and ϕ_j refer to transition state (TS) and reactant state (RS) isotopic fractionation factors, respectively. These factors represent the deuterium to protium preference for the exchangeable hydrogenic site in question relative to an average solvent site. If the only exchangeable reactant state sites have fractionation factors of unity and/or are solvent sites then Eq. [1] reduces to

$$k_n = k_0 \prod_i^{\mathrm{TS}} (1 - n + n\phi_i^*). \qquad [2]$$

Such is the case here.

Thus, an analysis of the variation of k_n with n allows, in theory, the determination of the values of ϕ_i^*. A plot of k_n versus n (i.e., a proton inventory) yields information about the identity and number of transition state protons giving rise to isotope effects. Additional details of the proton inventory technique are available in several excellent reviews (*15, 16*).

Our analysis will center on the use of the γ method developed by Albery with comments on the use of the Brönsted β value as an aid in interpreting proton inventories (*17–19*). The γ method provides a quantitative indication of the minimum number of protons contributing solvent isotope effects in the transition state. This allows the formulation of transition state models and an estimation of the fractionation factors of the protons involved. The curvature parameter γ is defined by

$$\gamma = 8 \ln [y_{0.5}/\sqrt{y_{1.0}}][\ln y_{1.0}]^{-2}, \qquad [3]$$

with the value of y_n being defined by

$$y_n = \frac{k_1}{k_0} \prod_{j}^{\mathrm{RS}} (1 - n + n\phi_j) = \prod_{i}^{\mathrm{TS}} (1 - n + n\phi_i^*). \qquad [4]$$

For a transtition state involving "*a*" protons, each contributing equally to the isotope effect, Eq. [4] simplifies to

$$y_n = (1 - n + n\phi^*)^a. \qquad [5]$$

A transition state having two different types of protons would be consistent with

$$y_n = (1 - n + n\phi_a^*)^a(1 - n + n\phi_b^*)^b. \qquad [6]$$

The fractionation factors are related by

$$\phi_a^* = \exp\left[\frac{1}{a}(\ln y_1)\Lambda_A\right], \qquad [7]$$

$$\phi_b^* = \exp\left[\frac{1}{b}(\ln y_1)\Lambda_B\right], \qquad [8]$$

$$a\gamma = \Lambda_A^2 + \left[\frac{a}{b}(1 - \Lambda_A)^2\right]. \qquad [9]$$

Thus, Λ_A is found by solving the quadratic Eq. [9], and Λ_B is $(1 - \Lambda_A)$. This allows the calculation of values for ϕ_a^* and ϕ_b^*.

Thus, Albery's method of analysis consists of (i) calculation of the value of γ from the k_n and n data, (ii) assessment of the minimum number ($\geq\gamma^{-1}$) of protons involved in the transition state, and (iii) construction of possible transition state models consistent with γ^{-1} and the values of the fractionation factors calculated.

Gamma was calculated for the proton inventories using a computer program, provided through the courtesy of Professor Albery, which fits the data to a second-order polynomial, extracts $k_{0.5}$ and $k_{1.0}$, and uses them in Eq. [3].

Model Analysis

Reboud-Ravaux suggested transition state **2** as being most consistent with the data available for the water-catalyzed hydrolysis of *N*-acetylbenzotriazole (*12*). This structure involves "general base" catalysis by one water molecule to assist the attack of another water molecule on the carbonyl carbon. Such transition state structures have been suggested before for such hydrolysis reactions based upon proton inventory investigations. It has been suggested that the fractionation factor for H_c in **2** would remain unity and that fractionation factors for H_b could be estimated from a knowledge of the Brönsted β value using Eq. [10] since ϕ_b^* should lie between 1.0 (the value for H_2O) and 0.69 (the value for H_3O^+).

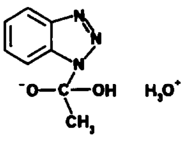
$$\phi_b^* = (\phi_b^{\mathrm{RS}})^{1-}{}_{\beta}(\phi_b^{\mathrm{PS}})^{\beta}. \qquad [10]$$

Substitution of the value of ϕ_b^* calculated from Eq. [10] into Eq. [11] allows one to obtain a value of ϕ_a^* consistent with this Brönsted value (*20*).

$$k_n = k_0(1 - n + n\phi_a^*)(1 - n + n\phi_b^*)^2. \qquad [11]$$

This treatment of the data obtained at 40°C yields $\phi_b^* = 0.868$ and $\phi_a^* = 0.404$. It can easily be seen from the values calculated in Table 1 using this method that not enough curvature is generated. A similar analysis of the data at 50°C (Table 2) yields the same result at all ionic strengths.

In general, more curvature will be generated in a proton inventory through the involvement of additional protons making identical contributions to the isotope effect. A transition state similar to **2** in which the proton had been fully transferred could be envisioned as in **7**.



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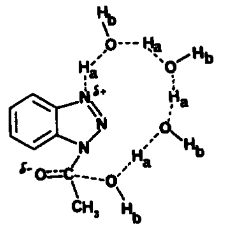

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Such a transition state would have a hydronium ion ($\phi_b^* = 0.69$) and a gem-diol-like proton (H_a). Even if H_a had a fractionation factor of unity then the equation describing this inventory would be of the form of

$$k_n = k_0(1 - n + 0.69n)^3. \qquad [12]$$

Such an equation generates a maximum solvent isotope effect of 3.04, a value well below the experimental value in all cases. When one considers what is known about gem-diol-type protons, one is led to include a term for H_a involving a fractionation factor of about 1.25 (*21a*). Thus, the inclusion of such a term would further diminish the curvature. This analysis implies the involvement of additional protons and this is confirmed by the γ analysis.

Table 3 lists the γ values obtained for the proton inventories of *N*-acetylbenzotriazole under a variety of conditions. The mean value of γ is 0.22 ± 0.05. Since the inverse of γ is indicative of the minimum number of protons contributing isotope effects, the maximum positive error limit suggests the involvement of at least four protons (i.e., $0.27^{-1} = 3.7$). A likely transition state is shown as **8**. More striking examples of such cyclic transition states are found for the water-catalyzed hydration of 1,3-dichloroacetone (*21b*), and the mutarotation of tetramethylglucose (*21c*). The low values of γ, 0.26 ± 0.05 and 0.07 ± 0.11, obtained in these cases suggest that at least five or six sites must be involved, somewhat as in the cyclic transition states. In **8** it seems likely that the four protons "in flight" (H_a) contribute the entire solvent isotope effect. One could argue that H_{a1} might have a slightly different fractionation factor than the three protons undergoing transfer between oxygen atoms. Alternately, all four H_a protons could exhibit identical fractionation factors. These two cases have been considered using the γ method. Only the case in which all four H_a fractionation factors are assumed to be equal adequately duplicates the proton inventories, as can be seen from the calculated values in Tables 1 and 2. Thus, the cyclic transition state of **8**, represented by Eq. [13], is consistent with all the proton inventories reported.

$$k_n = k_0(1 - n + n\phi_a^*)^4. \qquad [13]$$

Such a cyclic transition state has been proposed for other water-catalyzed hydrolyses (*1–3*) and is consistent with the large negative entropy of activation, since the water molecules involved in the transition state suffer loss of translational and rotational freedom (*12*).

The fact that the water point falls on the Brönsted line for general base catalysis is not inconsistent with the proposed cyclic transition state. This seems only to indicate that water is acting in some capacity as a base. It is true that the Brönsted coefficient is frequently used as measure of the degree of proton transfer. However, one should not necessarily be able to use a value derived from a wide range of bases in this way, since it is reasonable to expect that the position of the proton in the transition state will change with the strength of the base (*22*).

The cyclic transition state proposed could occur either in the nucleophilic attack of water to form the tetrahedral intermediate (**8**) or in the breakdown of a tetrahedral intermediate to form products. Jencks and Fox concluded that it is

TABLE 3

VALUES OF THE CURVATURE PARAMETER, γ, FOR THE PROTON INVENTORIES OF THE WATER-CATALYZED HYDROLYSIS OF *N*-ACETYLBENZOTRIAZOLE AT DIFFERENT IONIC STRENGTHS

Temperature (°C)	Ionic strength (*M*)[a]	γ Value[b]
40	1.0	0.26 ± 0.05
50	0.0	0.19 ± 0.05
50	0.5	0.14 ± 0.05
50	1.0	0.25 ± 0.09
50	2.0	0.24 ± 0.03

[a] Maintained with KCl in 10^{-3} *M* $HCl-H_2O$ and $DCl-D_2O$ mixtures.

[b] Calculated using a computer program, GAMISO, provided by Professor J. Albery.

only N-4 in 1-acetyl-1,2,4-triazole which becomes protonated (*23a*). By analogy we would expect N-3 of *N*-acetylbenzotriazole to act as a base in the proposed cyclic transition state. The protonation of N-3 of 1-acetyl-1,2,4-triazole is more favorable than that of N-2 for the following reasons, based on CNDO-2 calculatons (*23b*):

(i) 1,2,3-benzotriazole-1H and 1,2,3-benzotriazole-3H are identical.

(ii) 1,2,3-triazole-1H is more stable than 1,2,3-triazole-2H.

(iii) Conversion of 1,2,3-triazole-1H to 1,2,3-triazole-1H,3H triazolium ion is the favored conversion.

(iv) Stable protonated triazoles are the ones in which the protons are maximally separated.

Furthermore, space-filling models constructed for a cyclic transition state such as **8** give the best arrangement for the involvement of N-3.

Salt Effect

The rate of the reaction is depressed as the ionic strength is increased. This result is similar to the effect observed for the hydrolysis of several other acyl compounds such as 1-acetylimidazolium ion (*24*), acetic and succinic anhydrides (*25, 26*), and acetylpyridinium ion (*27*). Formally the salt effects may be described by the Brönsted–Bjerum equation [14], which expresses the rate constant in terms of the activity coefficients and salting-out constants of the reactants and transition states.

$$k = \frac{f_r}{f^*}. \qquad [14]$$

The related Setschenow equation [15] can be used to estimate a Setschenow constant, K_s.

$$\log k_{\mathrm{obs}} = K_s[\mathrm{salt}]. \qquad [15]$$

Plots of log k_{obs} versus salt concentration (KCl) are linear up to $\mu = 1.5$ with a slope of 0.074. At higher salt concentrations the plot is linear with a slope of 0.113 (Fig. 3) up to $\mu = 3.0$ in water and deuterium oxide. Similar plots are observed in the mixed solvent systems. Because of this nonlinearity it was not possible to determine the Setschenow constants (K_s) for the reaction.

It is usual to ascribe the observed logarithmic decrease of reaction rate with the increase of ionic strength to a decrease in $a_{\mathrm{H_2O}}$ and a requirement for several water molecules in the transition state for hydrolysis or to an effect on the activity coefficient ratio, fr/f^* (*27–31*). However, the fairly constant values of γ obtained at different ionic strengths rule out the above effects on the transition state structure either directly or through changes in the water–solute interactions. This may be due to the highly diffuse charges in the cyclic transition state.

Although most alkali metal and halide ions, which are commonly used to maintain the ionic strength of the medium, have some "structure-breaking" influence, we cannot attribute the observed salt effects to salt-induced changes in water (*32*). However, it could be argued that increasing concentrations of potassium chloride retard the reaction by requiring larger amounts of solvating water which renders it (the solvating water) unreactive.

CONCLUSION

The observed proton inventories at 40 and 50°C for the water-catalyzed hydrolysis of *N*-acetylbenzotriazole can be adequately, but not uniquely, described by the cyclic transition state model **8** involving four protons contributing to the observed solvent deuterium isotope effect. The choice of this model is based primarily on the γ analysis of the proton inventory data. Although the ionic strength affects the rate constants, the proton inventories are, within experimental error, identical over a range of ionic strengths from 0 to 3 *M*. The proposed transition state model is chemically reasonable in view of the known general base catalysis of hydrolysis, the large negative entropy of activation, and the large solvent deuterium isotope effects.

EXPERIMENTAL

Materials. *N*-Acetylbenzotriazole (**1**) was prepared by the method of Staab (*33*) and had mp 49°C (lit. (*33*) 51°C). It was also characterized by ir and ^{1}H nmr. Acetonitrile (Fisher reagent grade) was stirred over calcium hydride overnight, distilled, and stored under nitrogen. Deuterium oxide (Bio-Rad; 99.8 atom% deuterium) was purified by distillation from an all-glass apparatus. Water was twice glass distilled before use. Potassium chloride was oven dried and deuterium chloride (Aldrich; 20% solution in D_2O) was used as obtained.

Kinetics. The hydrolysis of *N*-acetylbenzotriazole was monitored by following the decrease in absorbance at 300 nm using a Cary 118C uv-vis spectrophotometer equipped with a constant temperature cell compartment and holder.

Concentrated hydrochloric acid was used to prepare 10^{-3} *M* HCl in H_2O. Deuterium chloride was used to prepare 10^{-3} *M* DCl in D_2O. The ionic strength was maintained at the desired concentration with potassium chloride. An analysis of the D_2O-DCl solution by Mr. Josef Nemeth showed that the deuterium content was 98.9%. Reactions in H_2O-D_2O mixtures were done using appropriate volumes of HCl in H_2O and DCl in D_2O stock solutions.

Reactions were initiated by injecting 50 μl of a stock solution which was 2.05×10^{-4} *M* in *N*-acetylbenzotriazole in acetronitrile into 3.00 ml of the appropriate HCl, DCl, or HCl-DCl solution. Reactions were followed to greater than 80% completion and infinity absorbances were taken at 10 half-lives. The pH(D) of the reaction solutions was monitored at the start and end of each run. No change in the pH(D) was observed. First-order rate constants were obtained using a nonlinear least-square computer program.

ACKNOWLEDGMENT

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