Proton Inventory of the Water-Catalyzed Hydrolysis of *p*-Nitrotrifluoroacetanilide

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A proton inventory study was made of the water-catalyzed hydrolysis of *p*-nitrotrifluoroacetanilide at pH 4.0, 70°C. Multiple protons in the transition state were demonstrated; although two or three protons were shown to be involved, they were not distinguishable. Neither imidazolyl cation nor acetic acid catalyzed the water-catalyzed hydrolysis. The water-catalyzed hydrolysis proceeds through acid catalysis by a water molecule of the breakdown of the tetrahedral intermediate between the anilide and another water molecule. Acid catalysis by the first water molecule is probably assisted by proton transfer from the second water molecule.

INTRODUCTION

Recently many studies of the hydrolyses of acetanilides (*1–20*), were carried out mainly because such reactions are good models of hydrolyses of amides catalyzed by hydrolytic enzymes. Most of the studies were concerned with alkaline hydrolysis, wherein a monoanionic tetrahedral intermediate between an anilide and hydroxide ion breaks down to products via two pathways, i.e., a direct breakdown of the monoanionic intermediate by acid catalysis, and another reaction proceeding through a dianionic tetrahedral intermediate produced by reaction of the monoanionic tetrahedral intermediate with a second hydroxide ion (*4–12, 17*).

In the previous paper in this series (*19*), however, the authors found a pH-independent region from pH 1.0 to 4.5 in the hydrolysis of *p*-nitrotrifluoroacetanilide (**1**), definitely attributable to a water-catalyzed hydrolysis. Knowledge of the mechanism of the water-catalyzed hydrolysis, especially of proton transfers, is very important in understanding the mechanism of enzymatic hydrolyses of amides, since water molecules obviously play important roles as reactant and solvent in enzymatic reactions.

In this paper, proton transfers in the water-catalyzed hydrolysis of **1** are examined by the proton inventory technique. As fully described in previous reviews (e.g., *21, 23*), the rate constant in H_2O (mole fraction $1 - n$)–D_2O (mole fraction n) mixtures (k_n) may be represented by Eq (1):

$$k_n = k_0 \prod_i^v \frac{(1 - n + n\varphi_i^T)}{(1 - n + n\varphi_i^R)}, \tag{1}$$

where k_0 is the rate constant in H_2O, and φ_i^R and φ_i^T represent the isotopic fractionation factors of the *i*th site in the reactant and transition state. In most cases, φ_i^R can be taken as unity, and thus

$$k_n = k_0 \prod_i^v (1 - n + n\varphi_i^T). \tag{2}$$

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Consequently, a plot of k_n versus n can give information concerning the number of the protons transferred in the transition state and concerning the tightness of binding of the protons.

EXPERIMENTAL

Materials. 1 was synthesized from trifluoroacetic anhydride and *p*-nitroaniline; mp 150°C [lit. *(24)* 147°C). D_2O was obtained from Merck (minimum isotopic purity, 99.7 atom %). All water used in the kinetic studies was doubly distilled. Imidazole was recrystallized three times from benzene.

Kinetics. The rate constants of the cleavage of 1 were determined at 70°C, pH 4.0, and $I = 0.2\ M$ by the method of Guggenheim *(25)* as described in the previous paper *(19)*. All the rate constants were extrapolated to zero buffer(acetate) concentrations and have the accuracy of $\pm 1\%$. It was assumed that the correction of the pH meter reading in H_2O–D_2O mixtures (the difference between the pH meter reading and the real pH) increases linearly from 0 to 0.4 *(26)* when the mole fraction of D_2O increases from 0 to 1.0. This assumption has little effect on the results of the proton inventory studies, since the pH-rate constant profile definitely shows a plateau in the pH region 1.0–4.5 *(19)*.

RESULTS AND DISCUSSION

Figure 1 shows a plot of the rate constant of the water-catalyzed hydrolysis of 1 (k_n) versus the mole fraction of D_2O in the H_2O–D_2O mixtures (n). From the results in
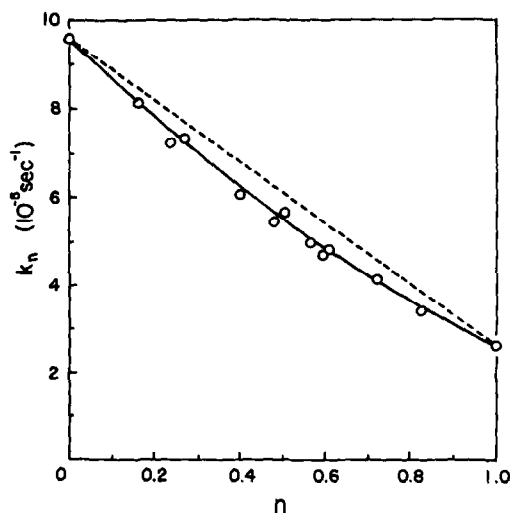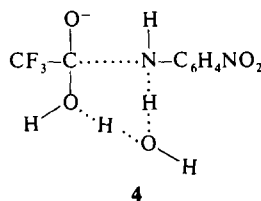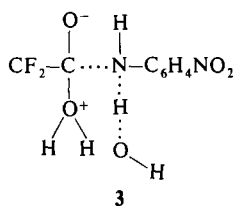

FIG. 1. Dependence of the rate constant of the water-catalyzed hydrolysis of 1 (k_n) on the mole fraction of D_2O of the H_2O–D_2O mixtures at 70°C. The solid line is the theoretical line calculated on the assumption of a two proton transfer mechanism $(\varphi_1^T = \varphi_2^T = 0.52)$, whereas the dotted line is for a one proton transfer mechanism.

Fig. 1, any mechanism which involves one proton in the transition state is absolutely ruled out, since it should show a linear relation between k_n and n. Transition state (2) is also unlikely. In this case, both φ_1^T and φ_2^T, which refer to the two protons attached to the oxygen atom of the attacking water molecule, should be equal to 0.52. This value is too low for the two protons in 2, since it should be between 1.0 and 0.69. The former value is for the protons of reactant water, and the latter is for the proton attached to the completely positively charged oxygen atom (23). Thus, it is concluded that the

$$
\begin{array}{c}
O^- H \\
| \quad | \\
CF_3-C-N-C_6H_4NO_2 \\
| \\
O^+ \\
/ \quad \backslash \\
H \quad H
\end{array}
$$

2
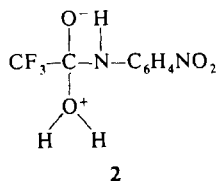
formation of a tetrahedral intermediate between 1 and a water molecule is not sufficient for the cleavage of 1, but other water molecule(s) are necessary.

Two structures 3 and 4, of the transition state of the water-catalyzed hydrolysis of 1 are consistent with the proton inventory evidence.

$$
\begin{array}{c}
O^- \quad H \\
| \qquad | \\
CF_2-C\cdots N-C_6H_4NO_2 \\
| \qquad \vdots \\
O^+ \quad H \\
/ \backslash \quad \vdots \\
H \quad H \quad O \\
\qquad \backslash H
\end{array}
\qquad
\begin{array}{c}
O^- \qquad H \\
| \qquad\qquad | \\
CF_3-C\cdots\cdots N-C_6H_4NO_2 \\
| \qquad\qquad \vdots \\
O \qquad\quad H \\
/ \backslash \qquad \vdots \\
H \quad H \quad O \\
\qquad\qquad \backslash H
\end{array}
$$

3   4

In the formation of transition state 3, a water molecule attacks the carbonyl carbon atom of 1 as a nucleophile, while another water molecule functions as a general acid catalyst, donating a proton to the nitrogen atom [and not as a general base catalyst as proposed by Hogg *et al.* in the hydrolysis of acetylimidazolium ion (27)]. In 4, two water molecules also function as nucleophile and acid catalyst. In 4, however, partial proton transfer from the attacking water molecule to the acid-catalyzing water molecule enhances reaction.

The solid line in Fig. 1 is the theoretical line calculated for reaction through transition state 4 under the assumption that φ_1^T for the proton between two water oxygen atoms is equal to that for the proton between the oxygen atom of water and the nitrogen atom of 1 (φ_2^T), i.e., both are 0.52. φ^Ts for other protons can be reasonably taken as 1.0 (21). There are many other combinations of φ_1^T and φ_2^T which show curves that almost superimpose the solid line in Fig. 1. Thus, the values of φ^Ts cannot be determined. However, it can be said that transition state 4 is consistent with the proton inventory results.

In 3, three protons can have φ^Ts other than 1.0; e.g., the two protons in the attacking water molecule (φ_1^T) and the proton between the oxygen atom of the acid-catalyzing water molecule and the nitrogen atom of 1 (φ_2^T). For example, the calculated line almost superimposes the solid line in Fig. 1 (and fits the experimental points satisfactorily) when $\varphi_1^T = 0.83$, and $\varphi_2^T = 0.4$. The combination of $\varphi_1^T = 0.74$ and $\varphi_2^T = 0.5$

can also give a theoretical line which fits the experimental points well. The values of φ_1^T (0.83 or 0.74) and φ_2^T (0.4 or 0.5) are reasonable for the value of the protons on the attacking water molecule and for the value of the bridging proton (23). Therefore, transition states **3** and **4** are not distinguishable from the results of proton inventory only. However, the following discussion strongly favors transition state **4**.

Neither the imidazolyl cation nor acetic acid showed measurable catalysis of the water-catalyzed hydrolysis of **1** at pH 2.0, when the concentration of either of them was changed from 0.0 to 0.5 M. At pH 2.0, almost all of the imidazole and acetic acid are in the acidic form. Furthermore, the catalyses of the breakdown of the monoanionic tetrahedral intermediate between **1** and hydroxide ion by imidazolyl cation and acetic acid are also negligible (19), since the rate of formation of this tetrahedral intermediate is much smaller than the rate of the water-catalyzed hydrolysis of **1**. If the water-catalyzed hydrolysis of **1** proceeds through transition state **3**, the imidazolyl cation and acetic acid should have led to better catalyses than water by functioning as general acids instead of water. The imidazolyl cation and acetic acid are in general better acid catalysts than water if one considers their pK_as: imidazolyl cation (6.95), acetic acid (4.75), and water (15.74) (28). Thus, the much more effective catalysis by water than by imidazolyl cation or by acetic acid (as acid catalysts) strongly supports transition state **4**. In transition state **4**, the acid catalysis by water molecule (donating a proton to the nitrogen atom of **1**) may be assisted by concurrent proton transfer from the other water molecule (attacking the carbonyl carbon atom of **1**). Thus, the acid catalysis by the water molecule may be much more effective than expected from its pK_a. The imidazolyl cation is not suitable for abstraction of a proton from the attacking water because of electrostatic repulsion. The small catalytic activity of acetic acid may be attributable to hydrogen bonding between the hydrogen atom of the attacking water and the carbonyl oxygen atom of acetic acid rather than hydrogen bonding between the hydrogen atom of the attacking water and the ether oxygen atom of acetic acid.

In the methanolyses of N-methyltrifluoroacetanilides, Schowen and co-workers proposed transition states containing three protons. One proton was located between the nitrogen atom of the anilides and the oxygen atom of the methanol molecule acting as an acid catalyst while the other two protons originated from two other methanol molecules attached to the lone-pair electrons of the methanol molecule acting as an acid catalyst. Surprisingly, the latter two protons showed a much higher value of φ^T (1.2) than of φ^R (0.74) (12). However, no such drastic reversal in the isotopic fractionation factors was observed in the water-catalyzed hydrolysis of **1**. In view of these facts, transition state **4** is more likely, since the increase in the electrophilicity of the oxygen atom of the acid-catalyzing water molecule is compensated by proton transfer from the attacking water molecule. Thus, the strengthening of hydrogen bonds between the oxygen atom and the surrounding water molecules is not necessary.

Transition state **4** is more favorable than transition state **3** from an energetic viewpoint. The reaction through transition state **3** shows charge separation, generating a hydroxide ion adjacent to a hydronium ion, whereas the reaction through transition state **4** requires no charge separation.[1]

Thus, the present proton inventory study definitely shows that the water-catalyzed

[1] This argument was suggested by a referee and is acknowledged with thanks.

hydrolysis of **1** proceeds through acid catalysis by a water molecule in the breakdown of the tetrahedral intermediate between **1** and another water molecule. The reaction probably proceeds with the aid of acid catalysis by a second water molecule (transition state **4**).

ACKNOWLEDGMENTS

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