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# Hydrolysis of *p*-Nitrotrifluoroacetanilide Catalyzed by Water and Imidazole[1]

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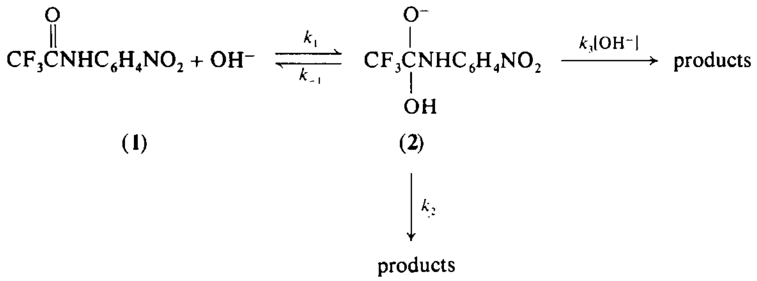
The hydrolyses of *p*-nitrotrifluoroacetanilide catalyzed by water and imidazole were examined at 70°C. The pH–rate constant profile of the hydrolysis in $H_2O$ was examined in the pH range 0.0–11.4. The hydrolysis was independent of pH in the region from pH 1.0 to 4.5, presumably a water-catalyzed reaction. The rate constant and the $D_2O$ solvent isotope effect for this reaction were $1.0 \times 10^{-4}$ $sec^{-1}$ and 3.7, respectively. Both natural imidazole and imidazolium cation catalyzed hydrolysis. The rate constant of the hydrolysis catalyzed by neutral imidazole was determined to be $5.4 \times 10^{-3}$ $M^{-1}$ $sec^{-1}$ and the $D_2O$ solvent isotope effect was 1.8.

## INTRODUCTION

Recently the mechanism of the hydrolyses of anilides has been of much interest, since the hydrolyses of acylanilides are reasonable models of enzymatic hydrolyses of amides (*1–15*). Extensive studies were made of the mechanism of their alkaline hydrolyses, which showed two reactions for the breakdown of the tetrahedral intermediate; i.e., a reaction proceeding through the monoanionic tetrahedral intermediate (**2**) by acid catalysis of a water molecule ($k_2$) (*1–14*) and another reaction proceeding through a dianionic tetrahedral intermediate produced by the reaction of second hydroxide ion with **2** ($k_3[OH^-]$) as shown in Scheme 1.



$$\underset{(1)}{CF_3\overset{\overset{O}{\|}}{C}NHC_6H_4NO_2} + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \underset{(2)}{CF_3\underset{OH}{\overset{O^-}{C}}NHC_6H_4NO_2} \xrightarrow{k_3[OH^-]} \text{products}$$

$$(2) \xrightarrow{k_2} \text{products}$$

SCHEME 1

In a previous paper (*15*), it was found that $\alpha$-cyclodextrin catalyzed the hydrolyses of anilides. There, the secondary hydroxyl group(s) of $\alpha$-cyclodextrin functioned as an acid catalyst in the breakdown of the monoanionic intermediate produced by nucleophilic attack of a hydroxyl ion of the cyclodextrin at the carbonyl carbon atoms of the anilides.

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[1] This paper is dedicated to Dr. W. S. Johnson.

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However, there is only scanty information on the catalyses of the hydrolyses of anilides by water and imidazole (*4*, *5*, *9*), though these are important in enzymatic reactions.

In this paper, the hydrolysis of *p*-nitrotrifluoroacetanilide (**1**) is examined at 70°C in the pH range of 0.0–11.4. The catalyses of hydrolysis of **1** by water and imidazole are shown. Furthermore, $D_2O$ solvent isotope effects on these reactions are reported.

## EXPERIMENTAL

*Materials.* **1** was synthesized from trifluoroacetic anhydride and *p*-nitroaniline; mp, 150°C [lit (*16*), 147°C]. All water used in the kinetic studies was doubly distilled.

*Kinetics.* The cleavage of **1** at 70°C was followed at 400 nm on a Cary Model 14 PM spectrophotometer equipped with a thermostatted cell compartment. The reaction was initiated by the addition of 15 μl of stock solution of **1** in acetonitrile to 3 ml of thermoequilibrated buffer, followed by thorough mixing of the solution. The initial concentration of **1** was $10^{-4}$ *M*. All the reactions followed first-order kinetics. The observed rate constant to zero buffer concentration ($I = 0.2$ *M*). For the deuterium (pH > 8), or by the method of Guggenheim (*17*) at lower pH (pH < 8). HCl, formate, acetate, phosphate, borate, carbonate, and NaOH buffers were used. All the rate constants of the hydrolysis in aqueous solutions ($k$) were obtained by extrapolating the observed rate constant to zero buffer concentration ($I = 0.2$ *M*), For the deuterium oxide experiments. pD was determined using the equation: pD = pH meter reading + 0.4 (*18*).

The p$K_a$ of **1** (see Eq. [1]) was determined spectrophotometrically at 350 nm. Since there was appreciable hydrolysis during the measurements, the absorbancies were extrapolated to the time of mixing.

$$CF_3\overset{\overset{O}{\|}}{C}\overset{H}{N}-C_6H_4NO_2 \overset{K_a}{\rightleftharpoons} CF_3\overset{\overset{O}{\|}}{C}N^{-}-C_6H_4NO_2 \qquad [1]$$

(**1a**) (**1b**)

## RESULTS

Figure 1 shows the pH–$k_{cor}$ profile of the hydrolysis of **1** at 70°C. Here, $k_{cor}$ is the rate constant corrected for the ionization shown in Eq. [1]. It has been widely accepted that **1a** is the only reactive species in hydrolysis, whereas **1b** is unreactive (*4*, *6*, *8–10*). Therefore, $k_{cor}$ was obtained by dividing the rate constant, $k$, by the proportion of unionized species (**1a**) at that pH. We measured spectrophotometrically the p$K_a$ of **1** (7.6 ± 0.1) at 70°C, and all the rate constants were corrected for this ionization.

In Fig. 1, a straight line of slope 1 is observed above pH 9.6. However, the slope is larger than 1.0 below pH 9.6. This result, which is consistent with the finding by Pollack and Dumsha (*10*), can be explained by Eq. [2] obtained for Scheme 1 using the steady-state assumption.

$$k_{cor} = \frac{k_1(k_2 + k_3[OH^-])[OH^-]}{k_{-1} + k_2 + k_3[OH^-]} \qquad [2]$$

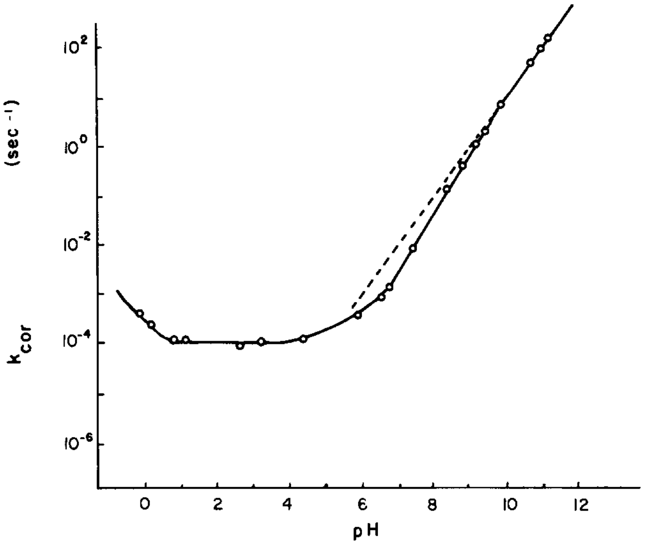

FIG. 1. The pH–$k_{cor}$ profile of the hydrolysis of 1 at 70°C, $I = 0.2\ M$.

However, it is the plateau in the pH 1.0–4.5 region that is of special importance. This plateau shows that $k_{cor}$ is not expressed sufficiently by Eq. [2] but should also involve a second term ($k_4$), which is *independent* of pH. As described later, the $k_4$ term corresponds to the water-catalyzed hydrolysis of **1**. Furthermore, an increase in rate constant was observed in the highly acidic region, which is obviously attributable to proton-catalyzed hydrolysis. Consequently, $k_{cor}$ should be expressed by Eq. [3] instead of Eq. [2].[2]

$$k_{cor} = \frac{k_1(k_2 + k_3[OH^-])[OH^-]}{k_{-1} + k_2 + k_3[OH^-]} + k_4 + k_5[H^+] \qquad [3]$$

Evaluated values of the parameters, obtained by fitting of the calculated values to experimental values in Fig. 1, are shown in Table 1.

TABLE 1

RATE CONSTANTS FOR THE HYDROLYSIS OF 1

| Rate constants | Values at 70°C |
|---|---|
| $k_1$ | $(4.2 \pm 0.2) \times 10^{3\,a}$ |
| $k_2/k_{-1}$ | $(1.5 \pm 0.4) \times 10^{-1}$ |
| $k_3/k_{-1}$ | $(3.0 \pm 0.2) \times 10^{4\,a}$ |
| $k_4$ | $(1.0 \pm 0.05) \times 10^{-4\,b}$ |
| $k_5$ | $(3.0 \pm 0.3) \times 10^{-4\,a}$ |

[a] Values given in molar$^{-1}$ seconds$^{-1}$.
[b] Values given in seconds$^{-1}$.

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[2] In Eq. (3), the first-order dependence of the proton-catalyzed hydrolysis was assumed, which is most likely in the limited number of experiments performed.

$D_2O$ solvent isotope effects on $k_4$ were measured in the plateau region [pH (pD) 1.2, 2.8, and 4.5] as shown in Table 2. The ratio of $k_4$ in $H_2O$ to that in $D_2O$ is 3.7, irrespective of pH.

TABLE 2

$D_2O$ EFFECTS ON THE RATE CONSTANT OF THE WATER-CATALYZED HYDROLYSIS OF 1 $(k_4)^a$

| pH (pD) | $k_4(H_2O)/k_4(D_2O)$ |
|---|---|
| 1.2 | 3.8 ± 0.2 |
| 2.8 | 3.7 ± 0.2 |
| 4.5 | 3.7 ± 0.2 |

[a] 70°C, $I = 0.2$ $M$.

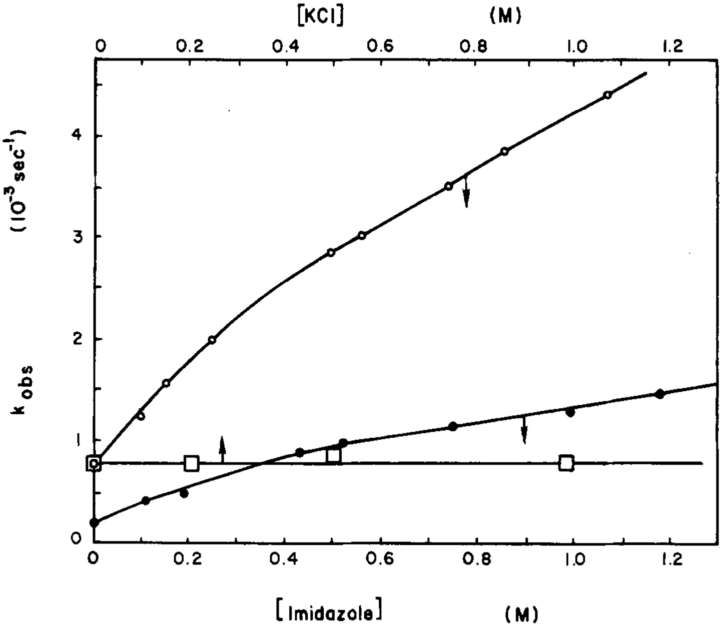


FIG. 2. The dependence of the rate of the hydrolysis of 1 on the concentration of imidazole at 70°C; ○, pH 6.2 in $H_2O$; ●, pD 6.2 in $D_2O$; □, pH 6.2 in $H_2O$.

Both neutral imidazole and imidazolium cation showed considerable catalyses of the hydrolysis of 1. Figure 2 depicts the dependence of the $k_{obs}$ of the hydrolysis of 1 on the concentration of imidazole at pH (pD) 6.2 in $H_2O$ and $D_2O$. $k_{obs}$ increases linearly with the concentration of the imidazole at higher concentrations of imidazole, but at lower concentrations of imidazole the increase is gradual. The former straight line corresponds to catalysis by neutral imidazole, whereas the latter gradual increase is due to the breakdown of 2 catalyzed by imidazolium cation as well as by hydroxide ion and water (5). In this reaction, KCl did not show any measurable effect.

Table 3 shows the rate constant of the hydrolysis of **1** catalyzed by neutral imidazole ($k_{\mathrm{Im}}$) determined by the method of Eriksson and Bratt (*5*). Here, the p$K_a$ of imidazole at 70°C was taken as 6.32 in $H_2O$ and 6.68 in $D_2O$ (*19*),[3] whereas the p$K_a$ of **1** in $D_2O$ was taken as 8.2 (*20*).[4] The $k_{\mathrm{Im}}$ in $H_2O$ is 1.8-fold larger than that in $D_2O$.

TABLE 3

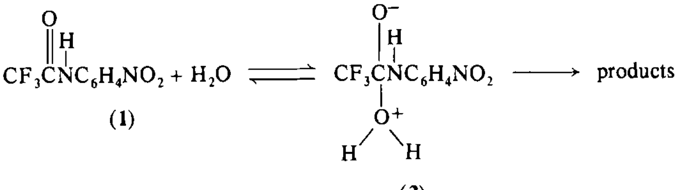
The Rate Constant of the Hydrolysis of 1 Catalyzed by Neutral Imidazole[a]

| Solvent | $k_{\mathrm{Im}}$ ($10^{-3}\ M^{-1}\ \mathrm{sec}^{-1}$) | $k_{\mathrm{Im}}(H_2O)/k_{\mathrm{Im}}(D_2O)$ |
|---|---|---|
| $H_2O$ | $5.4 \pm 0.4$ | $1.8 \pm 0.4$ |
| $D_2O$ | $3.0 \pm 0.4$ | |

[a] 70°C.

## DISCUSSION

The $k_4$ term associated with the plateau in Fig. 1, involves the water-catalyzed reaction as shown in Eq. [4].



$$\mathrm{CF_3\overset{\overset{O}{\|}}{C}\overset{\overset{H}{|}}{N}C_6H_4NO_2 + H_2O \rightleftharpoons CF_3\underset{\underset{\underset{H\quad H}{O^+}}{|}}{\overset{\overset{O^-}{|}}{C}}\overset{\overset{H}{|}}{N}C_6H_4NO_2 \longrightarrow products} \qquad [4]$$

(1)

(3)

The magnitude of the $D_2O$ solvent isotope effect of $k_4$ (3.7) is close to that in the hydrolysis of trifluoro-*N*-methylacetanilide catalyzed by a hydroxide ion (and a water molecule) (3.3), where hydroxide ion and the water molecule function as nucleophile and acid catalysts, respectively (*13*). This result indicates that the breakdown of the dipolar intermediate (**3**) to products in Eq. [4] proceeds through intramolecular proton transfer from the oxygen atom of the water to the nitrogen atom of **1**. If another water molecule were to transfer a proton to the nitrogen atom of *3* as an acid catalyst, a larger value of the $D_2O$ effect would be expected.

The scheme depicted by Eq. [5], in which a proton functions as an acid catalyst in the breakdown of **2**, cannot be correct for the plateau rate, although it gives a pH-independent term ($[OH^-]\ [H^+]$). If the reaction in Eq. [5] were to take place together with the two processes of the breakdown of **2** (the $k_2$ and $k_3\ [OH^-]$) in Scheme 1, $k_{\mathrm{cor}}$ would be expressed by Eq. [6].

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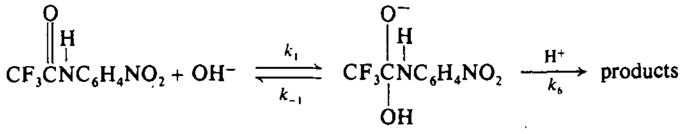
[3] The p$k_a$ in $H_2O$ was calculated by use of $\Delta H =$ 8.4 kcal/mol and the value in $D_2O$ was estimated assuming that the difference of p$K_a$ in $H_2O$ and in $D_2O$ at 70°C is equal to that at 34°C; all values are from (*19*).

[4] This estimation is based on the dependence of the difference of the p$K_a$ in $H_2O$ and in $D_2O$ on the p$K_a$ reported in (*20*). The error of 0.1 pH unit in this value did not cause an error larger than 1% in $k_{\mathrm{Im}}$.



$$CF_3\overset{\overset{O}{\|}}{C}\overset{H}{N}C_6H_4NO_2 + OH^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CF_3\underset{OH}{\overset{O^-}{C}}\overset{H}{N}C_6H_4NO_2 \xrightarrow[k_6]{H^+} \text{products} \quad [5]$$

$$k_{\mathrm{cor}} = \frac{k_1(k_2 + k_3[OH^-] + k_6[H^+])[OH^-]}{k_{-1} + k_2 + k_3[OH^-] + k_6[H^+]} \quad [6]$$

If $k_{-1} \gg k_6\,[H^+] \gg k_2, k_3\,[OH^-]$, $k_{\mathrm{cor}}$ is reduced to a pH-independent term, $k_1k_6\,K_w/k_{-1}$. The ion product of water, $K_w$, is $10^{-12.82}$ at 70°C (*21*). However, $k_6$ would be required to be $1.6 \times 10^5$ times $k_{-1}$, in order that $k_1k_6k_w/k_{-1}$ be equal to the plateau rate ($1.0 \times 10^{-4}$ $\mathrm{sec}^{-1}$). Thus, $k_6[H^+]$ must be larger than $k_{-1}$ in the pH region 1.0 to 4.5. Consequently, the mechanism of Eq. [5] can be ruled out.

The mechanism, in which the tetrahedral intermediate is formed through general acid catalysis by a proton, can be also ruled out, since its breakdown through the $k_2$ step is much faster than that through the $k_3$ step below pH 5. Thus, this mechanism should have given a straight line of slope −1 in the pH–$k_{\mathrm{cor}}$ profile instead of the observed plateau.

The value of the $D_2O$ solvent isotope effect in the hydrolysis of **1** catalyzed by neutral imidazole (1.8) is considerably smaller than that in the hydrolysis of **1** catalyzed by water (3.7). Besides, this value is also smaller than the value for the hydrolysis of trifluoro-*N*-methylacetanilide catalyzed by hydroxide ion and water (3.3) (*13*). These facts indicate that the imidazole-catalyzed hydrolysis of **1** probably proceeds through nucleophilic catalysis rather than general base catalysis. Imidazole catalyzes the hydrolysis of *p*-nitrophenyl acetate also nucleophilically, showing a $D_2O$ effect of 1.0 (*22*). The breakdown of the tetrahedral intermediate between **1** and imidazole can proceed through intermolecular acid catalysis by surrounding water or through intramolecular acid catalysis by the imidazolium cation. This may be responsible for the fact that the $D_2O$ effect is 1.8 rather than 1.0. If a tetrahedral intermediate is formed by general base catalysis by imidazole, followed by its breakdown through acid catalysis by water, a larger value than that in the hydrolysis of trifluoro-*N*-methylacetanilide catalyzed by hydroxide ion and water is likely. General base catalysis by imidazole, followed by intramolecular acid catalysis by imidazolium cation, would be also unfavorable because of the small $D_2O$ effect. The value of the $D_2O$ effect in the imidazole-catalyzed hydrolysis of **1** (1.8) is much smaller than that in the acylation step of the α-chymotrypsin-catalyzed hydrolysis of *N*-acetyl-L-tyrosine *m*-chloroanilide (3.4) (*23*). This fact also points to the nucleophilic catalysis by imidazole in the hydrolysis of **1**. However, before definite conclusions are drawn, the effect of substituents on the $D_2O$ effect must be examined, and are currently being investigated.

## ACKNOWLEDGMENTS

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