Water Catalyzed Hydrolysis of *p*-Nitrotrifluoroacetanilide and Trifluoroacetanilide. Carbonyl ¹⁸O-Exchange Does Not Accompany the Water Reaction

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Abstract: The water catalyzed hydrolyses of *p*-nitrotrifluoroacetanilide (**3**) and trifluoroacetanilide (**8**) were studied at various pH's and temperatures. The activation parameters for the water reactions of **3** and **8** are: $\Delta H^{\ddagger} = 14.4 \pm 0.6$ and 11.7 ± 0.3 kcal/mol and $\Delta S^{\ddagger} = -36.1 \pm 1.8$ and -52.3 ± 0.7 cal/mol·K, respectively. These are consistent with reactions that involve considerable restriction of degrees of freedom of the solvent/substrate in the transition state. A proton inventory analysis of the rate constants for hydrolysis of **3** in media of different mole fraction D_2O indicates the process involves two or more protons in flight or undergoing loosening of their bonding in the transition state. ¹⁸O-Labeled amides were subjected to the hydrolytic conditions for various times up to 3 half-times of hydrolysis and recovered. Mass analysis showed that the ¹⁸O content in the recovered amide did not change during the course of the reaction. All the data support a process where the rate-limiting step for the water reaction involves a concerted or nearly concerted formation of a diol which undergoes subsequent C−N cleavage in preference to OH expulsion.

Introduction

Due to the importance of the amide bond in biological systems it is not surprising that considerable effort has been expended to understand the mechanisms for its hydrolysis in both acid and base, eq 1.[1,2] Experimental evidence pertaining to possible mechanisms for the water promoted hydrolysis of amides is much less available. This is due to the fact that amide hydrolysis

$$R_2NH + R'CO_2^- \xleftarrow{OH^-} R_2NC(=O)R' \xleftarrow{H_3O^+} R_2NH_2^+ + R'CO_2H \quad (1)$$
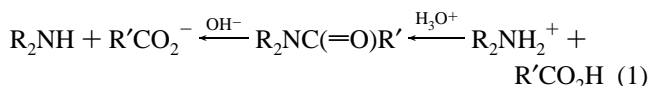
in water in the absence of acid or base is an exceedingly slow process, and there are very few amides where the water reaction or its kinetic equivalent, OH⁻ attack on the protonated amide, has been identified. For the most part, the identified amides capable of hydrolyzing without acid or base catalysis activated in some way, such as **1**,[3] **2**,[3b] **3**,[4] **4**,[5] **5**,[6] and **6**.[7] Without structural activation, water promoted hydrolysis is very slow but observable in some selected cases. For example, Hine and

co-workers[8] have shown that the simplest amide, formamide, exhibits a plateau around neutrality in the pH/rate profile for
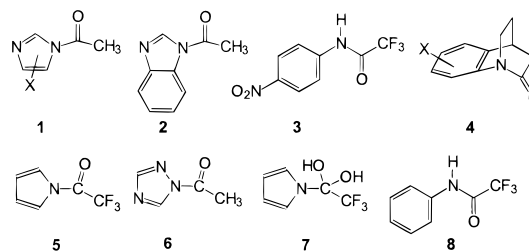
hydrolysis at 80 °C which corresponds to rate constant for water hydrolysis (k_o) of 8.4×10^{-8} s⁻¹, ($t_{1/2} = 95$ days). Quite possibly the water reaction for amides is a general one in the neutral pH region, but it is not usually considered due to the difficulty in detecting it.

There is considerable interest in determining the water hydrolysis rate constants for certain peptides so that these values could be used as comparisons for their enzymatic hydrolyses under comparable conditions.[9−11] New, highly sensitive assay techniques using ¹⁴C radioisotopic detection[9] or post-hydrolytic reaction to form highly fluorescent tracers[10] have been developed to follow reactions to a few percent completion at 25 °C. In a

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recent study, Radzicka and Wolfenden[11] have determined the activation parameters for water hydrolysis of some simple dipeptides by monitoring the hydrolyses between pH 4.2 and 7.8 at several temperatures between 120 and 200 °C. Depending on the structures of the peptides in the latter three studies, the $t_{1/2}$ for the hydrolyses at 25 °C varied from 7 years[9] to 600 years.[11]

Despite the interest in determining the rate of the water reactions, there is no mechanistic information about how this process occurs except in the case of the activated amides **1**−**6**.[3−7] Of particular importance would be the detection of intermediates produced by the addition of water and knowledge of their partitioning between product formation and reversal to form starting material. So far as we know, in only one case, that of *N*-trifluoroacetylpyrrole (**5**), has it been unambiguously determined that a hydrated intermediate can be formed,[12] although from simple mechanistic considerations[13] and by analogy with aldehydes and ketones,[14] a *gem*-diol intermediate (T_o, **7**) is likely to be formed on the reaction pathway for water promoted amide hydrolysis. Should partitioning of the diol between reversal and product formation occur, it could be readily detected through the use of $^{18}O=C$ exchange studies such as those we have used previously to determine the reversal of intermediate formation in acid and base promoted hydrolysis of amides[2,15a−f] and esters.[15gh] Herein we report detailed studies of the water hydrolyses of two trifluormethyl activated acetanilides, **3**[4] and **8,** as well as $^{18}O=C$ studies that show that there is no exchange in the carbonyl oxygen of the amides recovered from the medium during the course of hydrolysis.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were obtained on a Bruker AC-200, infrared spectra on a BOMEM MB-120 spectrometer, and mass spectra on a Fisons Quattro Triple Quadrupole Mass Spectrometer coupled to a Fisons GC 8000 gas chromatograph.

(a) Materials. *p*-Nitrotrifluoroacetanilide (**3**) and trifluoroacetanilide (**8**) were prepared by the dropwise addition (at 0 °C) of trifluoroacetic anhydride to an equimolar amount of the appropriate aniline dissolved in pyridine. In the case of *p*-nitrotrifluoroacetanilide (**3**) crystallization from acetone−hexane (40:60%, by volume) gave yellowish crystals, yield 34%, mp 149−150 °C (lit. mp 147 °C).[4,17] ^{1}H NMR (CDCl$_3$): δ = 7.84 (m, 2H,), 8.23 (m, 2H,), 9.53 (bs, 1H, NH); ^{13}C NMR (CD$_3$CN): δ = 156.3 (q, $J_{F,C=O}$ = 38 Hz), 145.7, 142.9, 125.7, 121.7, 116.6 (q, $J_{F,C}$ = 285 Hz); IR (Nujol) 1744.6 cm^{-1}.

Trifluoroacetanilide (**8**) was crystallized also from acetone−hexane (60:40%, by volume) to give pinkish crystals, yield 66%, mp 89−90 °C (lit. mp 86.5 °C)[16]. ^{1}H NMR (CDCl$_3$): δ 7.23−7.72 (m, 5H), 8.23 (s, NH); ^{13}C NMR (CDCl$_3$): δ = 155.2 (q, $J_{F,C=O}$ = 37 Hz), 135.0, 129.2, 126.4, 120.0, 115.7(q, $J_{F,C}$ = 287 Hz).

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[18]O-Labeled trifluoroacetic acid was made by exchange of trifluoroacetic acid (0.9 g, 0.008 mol) with ^{18}O-water (0.4 g, 0.022 mol). The mixture was heated at reflux overnight with a little dry HCl as a catalyst.

[18]O-Labeled *p*-nitrotrifluoroacetanilide was made exactly as above from *p*-nitroaniline and ^{18}O-labeled trifluoroacetyltriflate (TFAT). The synthesis of TFAT was done according to a procedure of Taylor et al.[17] A larger amount of P$_2$O$_5$ (13.0 g, 0.046 mol) was added to account for extra water introduced with ^{18}O-labeled trifluoroacetic acid. The crude amide was purified by column chromatography using silica gel and an eluant of 20% (by volume) ethyl acetate in chloroform. Final crystallization from acetone−hexane yielded 0.43 g of pure material with about 12% ^{18}O-enrichment in the carbonyl group (by mass spectrometry). ^{18}O-labeled trifluoroacetanilide was made twice from ^{18}O-Labeled TFAT and aniline in the manner described above, yield 45%, with about 16% and 44% [18]O incorporation.

DCl (Aldrich, 37 wt % solution in D$_2$O, 99.5% D), D$_2$O (99.9%), and H$_2$[18]O (^{18}O, 95−98%, Cambridge Isotope Laboratories) were used without further purification.

All solutions used for kinetics studies were made using degassed (CO$_2$ free, O$_2$ free), deionized water (Osmonics-Aries water purifying system).

Dimethoxyethane (HPLC grade, Fisher) was dried over Na (48 h, reflux) under Ar atmosphere. Fresh, dry DME was used to make stock solutions of the amides.

(b) Kinetics. (i) H$_2$O. Kinetic data were obtained by observing the rate of change (increase for **3**, decrease for **8**) in absorbance of $0.5-1.0 \times 10^{-4}$ M aqueous solution of amides at the wavelength of maximum change (400 nm, **3**; 250 nm, **8**) with a Cary 210 UV−vis spectrophotometer interfaced as previously described.[15] At temperatures below 80 °C the reactions were initiated by injecting 30 μL of a stock solution of amide in DME (1×10^{-2} M) to a 1.0 cm quartz cuvette containing 3.0 mL of acid solution of appropriate concentration ($\mu =$ 0.2 (KCl), **3**; $\mu = 1.0$ (KCl), **8**) which had been previously equilibrated in the instrument cell holder for 15 min. Each determination was repeated in triplicate, and the rate constants are derived from the fits of the abs. vs time profiles to a standard exponential model: the values reported in the tables are the averages with errors being the standard deviation of the mean.

Data for slower reactions of **8** and for **3** at temperatures above 80 °C were obtained by measuring absorbance of individual 5 mL aliquots of sample in the appropriate medium which were thermostated in sealed ampoules (flushed with Argon, then flame sealed) in a boiler designed for conducting isothermal experiments at elevated temperatures. Ampoules were removed at various times and cooled, and the absorbance of the contents measured at 25 °C. Rate constants were obtained by fitting the absorbance vs time profile to a standard exponential model using nonlinear least squares methods. The reactions were followed to at least two half-times and in all cases exhibited clean first-order kinetics.

(ii) D$_2$O. DCl solutions were made by adding aliquots of concentrated DCl (37 wt %) to D$_2$O ($\mu = 0.2$ (KCl)). The exact acid concentrations were determined by titration against standardized base using phenolphthalein as an indicator. Hydrolysis kinetics in D$_2$O and D$_2$O−H$_2$O mixtures were performed as above. Solutions of different mole fraction D$_2$O were made up by mixing the appropriate amounts of H$_2$O and D$_2$O and adding to 100 mL of this 1.0 mL of 1.00 N HCl to bring the [L$_3$O$^+$] to 10^{-2} M.

(c) ^{18}O-Exchange. A typical exchange experiment was conducted as follows. To 192 mL of the acid solution of desired concentration ($\mu = 0.2$ (KCl) for **3** and $\mu = 1.0$ (KCl) for **8**) in a 200 mL volumetric flask was added 8 mL of 0.0125 M solution of the appropriate amide in DME. The aqueous solutions were made CO$_2$ and O$_2$ free by bubbling argon through them for 15 min. The contents of the volumetric flask were transferred in 10 mL portions to several ampoules (16−20) which were then flame-sealed and placed in the boiler. The ampoules were kept at a given temperature for 10 min for thermal equilibration after which four ampoules were removed at times corresponding to 1/2, 1, 2, and 3 half-times of hydrolysis. At each time of withdrawal, the contents of the four ampoules were combined and extracted with 3×10 mL of freshly distilled CH$_2$Cl$_2$. The combined extracts were washed with saturated NaCl and water. The

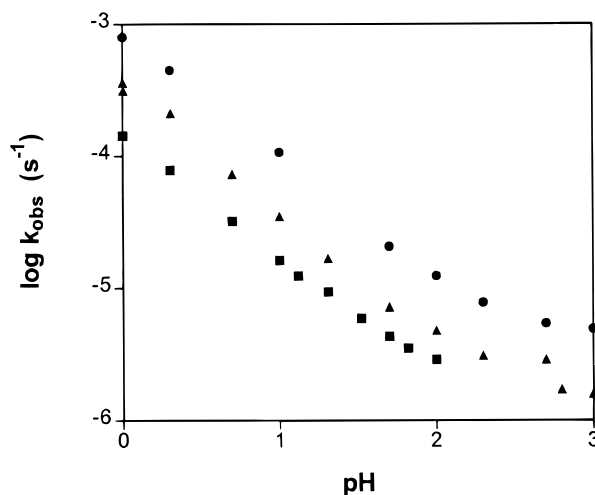**Figure 1.** Plot of the log k_{obs} vs pH for the hydrolysis of trifluoroacetanilide (**8**) at three different temperatures. Data of Table 1: ■, $T = 72$ °C; ▲, $T = 82.4$ °C; ●, $T = 100$ °C.

Table 1. Pseudo-First-Order Rate Constants of the Hydrolysis of Trifluoroacetanilide (**8**) at Different pH Values and Temperatures ($\mu = 1.0$ (KCl))

pH	$k_{obs} \times 10^6$ (s^{-1})		
	$t = 72.0$ °C	$t = 82.4$ °C	$t = 100.0$ °C
0.00	143 ± 3	363 ± 12	799 ± 93
0.004		315 ± 35	
0.301	78.3 ± 0.5		449 ± 20
0.305		212 ± 30	
0.70	32.3 ± 0.1	73.1 ± 2.1	
1.00	16.3 ± 0.2	35.1 ± 2.5	107 ± 3
1.12	12.4 ± 0.4		
1.31	9.40 ± 0.22	16.9 ± 3.0	
1.52	5.92 ± 0.02		
1.70	4.33 ± 0.08	7.2 ± 2.6	20.7 ± 1.2
1.82	3.53 ± 0.09		
2.00	2.89 ± 0.34	4.85 ± 0.25	12.4 ± 0.5
2.31		3.12 ± 0.28	7.79 ± 0.21
2.70		2.9 ± 1.6	5.43 ± 1.04
2.80		1.73 ± 0.15	
		1.6 ± 1.7	4.93 ± 0.22
3.10		1.81 ± 0.07	

organic layer was dried (MgSO$_4$) and stripped of solvent (rotary evaporator) to yield the residual amide which was subjected to analysis with a Fisons quattro triple quadrupole mass spectrometer coupled to a Fisons GC 8000 gas chromatograph (DB-5HS column, 30 m, i.d. 0.25 mm, 40 °C, 4 psi with helium carrier gas). For mass analysis, 15−20 scans of the ^{16}O and ^{18}O peaks were done for each sample, and the ^{18}O-content was evaluated as % ^{18}O = $I_{M+2}/(I_{M+} + I_{M+2})$ where I = peak intensity and M and M + 2 refer to the molecular ions. The % ^{18}O vs time data are given in Table 7.

Results

(a) Hydrolysis. Given in Table 1 are the pseudo-first-order rate constants, k_{obs}, for the hydrolysis of trifluoroacetanilide (**8**) at various acid concentrations ($\mu = 1.0$ (KCl): in Figure 1 are graphical representations of the k_{obs} vs pH data at three different temperatures (72.0, 82.4, and 100.0 °C). These data can be satisfactorily fit by linear regressions of the type: $k_{obs} = k_{H_3O^+}$ [H$_3$O$^+$] + k_{H_2O}. Linear least squares fitting of the k_{obs} versus [H$_3$O$^+$] data in the region of maximum curvature of the plots (pH 1−3) yields the rate constants, $k_{H_3O^+}$ and k_{H_2O}, given in Table 2.

The pH-rate constant profile of the hydrolysis of *p*-nitrotrifluoroacetanilide (**3**) was examined in the pH range 0.0−11.4 by Komiyama and Bender.[4a,b] The reported pH-independent

Table 2. Rate Constants for Acid ($k_{H_3O^+}$) and Water (k_{H_2O}) Hydrolysis of Trifluoroacetanilide (**8**) at Different Temperatures ($\mu = 1.0$ (KCl))

temp (°C)	$k_{H_3O^+} \times 10^4$ ^{a}(M^{-1} s^{-1})	$k_{H_2O} \times 10^6$ ^{a}(s^{-1})
72.0	1.69 ± 0.04	1.01 ± 0.14
82.4	2.77 ± 0.12	1.67 ± 0.13
100.0	8.36 ± 0.13	3.92 ± 0.09

^{a} Errors computed from standard deviations on the plots of k_{obs} vs [H$_3$O$^+$], data of Table 1.

Table 3. Pseudo-First-Order Rate Constants of the Hydrolysis of *p*-Nitrotrifluoroacetanilide (**3**) at Different pH Values, $T = 70.2 \pm 0.2$ °C ($\mu = 0.2$ (KCl))

pH	$k_{obs} \times 10^5$ (s^{-1})	pH	$k_{obs} \times 10^5$ (s^{-1})	pH	$k_{obs} \times 10^5$ (s^{-1})
−0.28	64.1 ± 0.1^a	0.70	11.6 ± 0.1^b	1.73	6.05 ± 0.05
0.0	34.0 ± 0.1^b	0.70	12.1 ± 0.1^d	1.97	5.6 ± 0.2
0.0	36.2 ± 0.1^c	1.00	8.3 ± 0.1^d	2.25	5.6 ± 0.2
0.30	21.9 ± 0.1^c	1.52	6.5 ± 0.2	3.0^e	5.2 ± 0.3
0.70	12.1 ± 0.1^c				

^{a} Determined at 300 nm, by observing disappearance of **3**; $\mu = 1.91$. ^{b} Determined at 400 nm by observing appearance of *p*-nitroaniline; $\mu = 1.0$. ^{c} Determined at 300 nm; $\mu = 1.0$ (KCl). ^{d} Determined at 400 nm; $\mu = 0.2$ (KCl). ^{e} Value quoted in the table extrapolated to [buffer] = 0 from formate buffer: three different concentrations of formate buffer were used: 0.05 M, $k_{obs} = 5.80 \pm 0.01 \times 10^{-5}$ s^{-1}; 0.10 M, $k_{obs} = 6.92 \pm 0.01 \times 10^{-5}$ s^{-1}; 0.20 M, $k_{obs} = 8.05 \pm 0.01 \times 10^{-5}$ s^{-1}.

Table 4. Pseudo-First-Order Rate Constants for the Water Catalyzed Hydrolysis of *p*-Nitrotrifluoroacetanilide (**3**) at Different pH Values and Temperatures ($\mu = 0.2$ (KCl))

pH	temp (°C)	$k_{obs} \times 10^5$ (s^{-1})	pH	temp (°C)	$k_{obs} \times 10^5$ (s^{-1})
1.64	46.3	1.16 ± 0.02	1.97	50.4	1.52 ± 0.02
1.64	50.1	1.77 ± 0.01	1.97	61.2	3.23 ± 0.06
1.64	64.7	3.93 ± 0.21	1.97	70.2	5.6 ± 0.2
1.80	46.3	1.14 ± 0.04	1.97	75.3	7.70 ± 0.09
1.80	50.1	1.78 ± 0.01	2.25	51.2	1.38 ± 0.01
1.80	64.7	4.00 ± 0.10	2.25	61.1	3.12 ± 0.03
1.80	82.4	15.9 ± 2.7	2.25	70.4	5.6 ± 0.2

Table 5. Activation Parameters for the Hydrolysis of *p*-Nitrotrifluoroacetanilide (**3**) and Trifluoroacetanilide (**8**)

amide	$\Delta H^{\ddagger}$ (kcal/mol)	$\Delta S^{\ddagger}$ (cal/K mol)	$\Delta G^{\ddagger}_{100\,C}$ (kcal/mol)
3	14.4 ± 0.6	−36.1 ± 1.8	27.9 ± 1.1
8	11.7 ± 0.3	−52.3 ± 0.7	31.2 ± 0.6
8^a	14.0 ± 1.3	−35.4 ± 3.7	27.2 ± 2.4

^{a} H$_3$O$^+$ catalyzed reaction.

region from 1.0−4.5 in that pH profile resulting from the water catalyzed reaction was of special importance to us. Therefore the rate constants at different temperatures, solvent deuterium kinetic isotope effect (SKIE), and ^{18}O-exchange studies were performed in this region to learn more about the mechanism. We controlled the pH with known [HCl] to avoid using buffers such as formate which were used in the previous study.[4a,b] The rate constants for hydrolysis of **3** in H$_2$O (pH = −0.28 to 3.0) at 70.2 °C are presented in Table 3,[18] while the rate constants in H$_2$O at four different pH's (1.64, 1.80, 1.97 and 2.25) in the temperature range 46.3−82.4 °C are given in Table 4. Given in Table 5 are the activation parameters $\Delta H^{\ddagger}$, $\Delta S^{\ddagger}$, and $\Delta G^{\ddagger}$ (calculated using the standard Eyring equation) for the acid and water catalyzed hydrolysis of trifluoroacetanilide and for the water catalyzed reaction of *p*-nitrotrifluoroacetanilide (using the data of Tables 2 and 4, respectively).

(b) SKIE and Proton Inventory Studies. The solvent kinetic isotope effects (SKIE) for the hydrolysis of **3** were measured in the pH independent region (pH = 2.0) at 70.2 °C. The rate constants in DCl/D$_2$O are given in Table 6. The $(k_{obs})_{H_2O}/(k_{obs})_{D_2O}$ ratio is 3.3 ± 0.2 at [LCl] = 10^{-2} M.

Table 6. Pseudo-First-Order Rate Constants for the Water Catalyzed Hydrolysis of *p*-Nitrotrifluoroacetanilide (**3**) at Different Mole Fractions of D_2O, $[L_3O^+] = 10^{-2}$ M, $T = 70.2 \pm 0.3$ °C, $\mu = 0.2$ (KCl)

mole fraction (*n*)	$k_{obs} \times 10^5$ (s^{-1})	mole fraction (*n*)	$k_{obs} \times 10^5$ (s^{-1})
0.0	5.59 ± 0.18	0.8	2.3 ± 0.1
0.2	4.3 ± 0.1	1.0	1.71 ± 0.05
0.4	3.53 ± 0.08	1.0^a	1.59 ± 0.02
0.6	2.7 ± 0.2		

^{a} At pL = 2.20.

Table 7. ^{18}O Content in Amide Recovered at Various Times from the Hydrolysis Medium during the Hydrolysis of *p*-Nitrotrifluoroacetanilide (**3**), $\mu = 0.2$ (KCl), and Trifluoroacetanilide (**8**), $\mu = 1.0$ (KCl).

amide	temp (°C)	pH	temp^a	% ^{18}O
3	100.0	1.80	0	11.3 ± 0.2
			$1/2t_{1/2}$	11.1 ± 0.3
			$t_{1/2}$	11.5 ± 0.3
			$1^1/_2t_{1/2}$	11.1 ± 0.3
			$2t_{1/2}$	11.2 ± 0.4
			$3t_{1/2}$	11.3 ± 0.3
8^b	70.0	0.0	0	16.1 ± 0.2
			$t_{1/2}$	16.1 ± 0.2
		1.0	0	15.8 ± 0.1
			$t_{1/2}$	15.5 ± 0.1
		2.0	0	15.9 ± 0.1
			$t_{1/2}$	16.0 ± 0.2
	100.0	1.0	0	43.3 ± 0.3
			$t_{1/2}$	43.8 ± 0.3
			$2t_{1/2}$	43.8 ± 0.4
		3.0	0	44.0 ± 0.3
			$t_{1/2}$	43.7 ± 0.3
			$2t_{1/2}$	43.9 ± 0.2

^{a} $t_{1/2}$ hydrolysis computed from the data in Tables 1 and 3.

A proton inventory study was performed with **3** in the pH independent region (pH = 1.97). In Table 6 are presented the rate constants of water catalyzed reaction at various mole fractions (*n*) of D_2O in H_2O at 70.2 °C: a graphical illustration of these (not shown) is bowed down.

(c) ^{18}O-Exchange. Whether ^{18}O-exchange accompanies the hydrolyses of either amide was determined by mass analysis of amide recovered from the hydrolytic media at various times up to $3t_{1/2}$ hydrolysis. The hydrolysis solutions contained 4% DME for reasons of solubility. The experimental data for ^{18}O-exchange for **3** and **8** (the latter in both 1 M acid and at pH 1, 2, and 3) are given in Table 7. In all cases, the results show that the percentage of ^{18}O remains, within experimental error, unchanged up to 3 half-times of hydrolysis.

Discussion

There are three essential pieces of information that need to be considered in order to establish the mechanistic possibilities for the water reaction for anilides **3** and **8**. These are (1) the activation parameters for the reaction for both amides; (2) the solvent SKIE and proton inventory conducted for **3** (this work and ref 4b); and (3) the lack of any observable ^{18}O-exchange in either amide recovered from the hydrolysis medium. We will deal with these data in turn.

(1) In the case of the hydrolysis of trifluoroacetanilide (**8**), there is no plateau in the pH vs k_{obs} profiles shown in Figure 1, but fitting of the pH vs k_{obs} data in Table 1 to the linear regression, $k_{obs} = k_{H_3O^+}[H_3O^+] + k_{H_2O}$, allows one to determine the acid and water hydrolytic constants at the three temperatures (Table 2). By comparison, at 72 °C the water reaction for amide **8** is some 60-fold slower than that for **3** at 70 °C: 1.0×10^{-6} s^{-1} vs 5.6×10^{-5} s^{-1}. From the activation parameters given

in Table 5 it can be seen that the enthalpies lie in the 11.5−15 kcal/mol range, while the entropies of activation for both amides are large and negative: at 100.0 °C, the entropies contribute 48% (**3**) and 62% (**8**) to the free energy of activation, $\Delta G^\ddagger$.

Water reactions have been also observed for the hydrolyses of activated esters such as alkyl and aryl haloacetates and substituted phenyl acetates,[19a] as well as for methyl,[19b] ethyl,[19b,c] isopropyl,[19b] and *tert*-butyl trifluoroacetates.[19b] It is notable that for these the activation enthalpies are fairly low (7−14 kcal/mol) while the entropies are large and negative (−34 to −50 cal/mol·K), the exception being *tert*-butyl trifluoroacetate which hydrolyses by an S_N1 mechanism.[19b] The former activation parameters would be the expected ones for a hydrolysis process involving a transition state that restricts the degrees of freedom of more than one water molecule.[19a,b] The activation parameters can be rationalized by processes involving ordered transition states involving several waters such as in **9**−**11**. Whether these processes are noncyclic (as in **9**), concerted (**10**), or cyclic leading to diol tetrahedral intermediates (**11** to **12**) is not known. Analogous structures and transition states could rationalize the activation parameters for the two amides in this study.
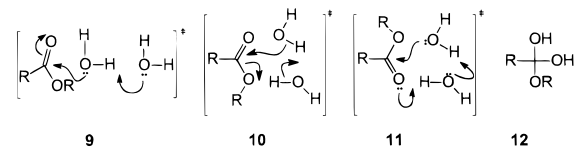

(2) In earlier studies Komiyama and Bender[4a,b] had determined that the pH independent region for hydrolysis of **3** extends from 1.0 to 4.5 with a reported SKIE of 3.7 in this domain at $T = 70$ °C. Given that our initial hydrolysis experiments with **3** indicated that the reaction rate constant for the water reaction was roughly half that reported,[4a] ($5.6 \pm 0.2 \times 10^{-5}$ s^{-1} vs $1.0 \pm 0.05 \times 10^{-4}$ s^{-1}), we decided to repeat the hydrolysis experiments with **3** at pH 1−3 using HCl so that we could avoid the use of formate buffers.[20] It was necessary to repeat all the hydrolysis measurements since the 2-fold disparity between our data and the previous ones[4a,b] could have significant bearing on the interpretation of the SKIE. The SKIE that we determined for the hydrolysis of **3** at [LCl] = 10^{-2} M, $T = 70.2$ °C is 3.3 $\pm$ 0.2. These data are consistent with the SKIE of 3.7 at pH = 4.0, $T =70$ °C that was originally reported,[4a,b] the only difference being the absolute values of the rate constants.

(3) The fact that there is no observable ^{18}O-exchange for the water reaction of either amide comes as a surprise and rules out some of the available mechanisms while placing constraints on others. Most easily ruled out is a two- or more-step process such as in eq 2 where a symmetric diol intermediate (hydrate) is reversibly produced from the addition of water with rate

(18) The data in Table 3 indicate that below pH 1 there is an acid promoted reaction. At higher acid concentrations, the abs. vs time profile at 400 nm shows some upward drift indicative of some decomposition of the *p*-nitroaniline product. At 300 nm, the disappearance of **3** exhibits clean first-order kinetics. The dependence of the rate on [HCl] seems slightly less than first-order, and the cause of this is under investigation. We thank a referee for alerting us to inconsistencies in our original data reported for the acid domain.

(19) (a) Kirby, A. J. In *Comprehensive Chemical Kinetics*, Bamford, C. H., Tipper, C. F. H., Eds.; Elsevier: Amsterdam, 1972; Vol. 10, pp 57−207. (b) Barnes, D. J.; Cole, M.; Lobo, S.; Winter, J. G.; Scott, J. M. W. *Can. J. Chem.* **1972**, *50*, 2175. (c) Hegazi, M.; Mata-Segreda, J. F.; Schowen, R. L. *J. Org. Chem.* **1980**, *45*, 307 and references therein.

(20) The possible source for the discrepancy between our results and those of Komiyama and Bender[4a,b] could arise from the use of formate buffers. Experimentally this requires that the k_{obs} values be several different [buffer], the reported value being found by extrapolation to [formate] = 0. In our study we have avoided the use of buffers by controlling the pH with HCl.

$$(2)$$

Scheme 1
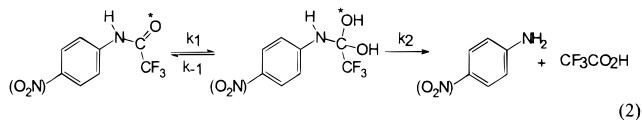
limiting breakdown to product. This leaves us with a few alternative possibilities that need to be considered in the light of the proton inventory data accumulated here and in the previous study.[4a,b]

(4) As has been well established, the proton inventory technique[21] provides information about the number of protons undergoing change in their bonding in the transition state relative to the ground state. Equation 3 expresses the relationship between the rate constant observed in mixtures of L_2O (L = H, D) with known isotopic composition and the fractionation factors (ϕ) for the exchangeable protons undergoing change in their bonding; n is the atom fraction of D in the medium, and i and j represent the sites undergoing exchange in the transition and reactant states, respectively.

$$k_n = k_o \prod_i^{TS}(1 - n + n\Phi_i)/\prod_J^{RS}(1 - n + n\Phi_j) \qquad (3)$$

The ϕ's for the various hydrogens relate to the tightness of their bonding and are less than unity if the overall bonding is weak as is the case for H's in flight in the transition state or for H's involved in hydrogen bonds where the overall bonding is loose.[22] The former case relates to primary kinetic isotope effects, while the latter relates to secondary kie's which can contribute significantly to the overall effect. The data for the hydrolysis of **3** determined at [LCl] = 10^{-2} M (Table 6), when plotted (not shown), indicate a significant downward bowing which is consistent with a transition state having two or more protons in flight or undergoing significant bond loosening.[20]

We can analyze the expected SKIE on k_{obs} for various possibilities for the hydrolysis of **3** in a fashion analogous to what we have presented before for the acid and base promoted hydrolyses of amides.[15] The ϕ values for the exchangeable H's in the ground state (H_2O) are unity which considerably simplifies the overall SKIE based on eq 3 into $k_D/k_H = \Pi\phi^{TS}$. We assume that the transition state for water addition is rate limiting with the position of the TS being roughly 0.5–0.7 along the reaction coordinate. The primary DKIE for a proton in flight between O and O or O and N is based on a TS fractionation factor of 0.4–0.5, or slightly higher (0.6–0.7) if the proton transfers are nonlinear as required for a cyclic transition state. For OH^-, the ϕ is 1.22 with each of its three solvating water H-bonds having $\phi = 0.7$;[23] a developing hydroxide or one involved in proton abstraction then has two waters of solvation rather than the three in $HO^-(H_2O)_3$. Finally, ϕ for each of the hydrogens in H_3O^+ is 0.69, while those for the OH's in a diol[24] and any anionic tetrahedral intermediate are 1.0. In what follows we will use bold **H**'s to signify the hydrogens in the TS with ϕ values different from unity.

Mechanistic Possibilities. (a) Diol with Equivalent Oxygens. Should the diol actually be the intermediate, its formation must be rate limiting, otherwise it is expected that ^{18}O-exchange would be observed. From the proton inventory and activation parameter data, the process requires two or more protons in flight and restriction of at least two waters in the transition state. Viable possibilities are given in **13** and **14** which are cyclic and open versions of a two-or-more-water process. An analogous cyclic TS involving three H_2O molecules is suggested for the hydration of 1,3-dichloroacetone: this process shows a $k_H/k_D = 2.7$.[25] Transition states for hydration involving two or three waters could be feasible geometrically. An analogy might be the proton exchange between the oxygens of benzoic acid in methanol, for which it has been shown that there are two solvent molecules involved in an eight-membered ring.[26] Fitting all the proton inventory data of Table 6 to the simplified form of eq 3, $k_D/k_H = \Pi(1 - n - n\phi^{TS})$, where the fractionation factors
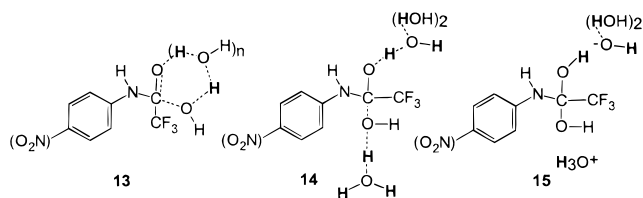
for the protons in flight in TS **13** are equal gives best fit values of $\phi^{TS} = 0.50$ if there are two protons in flight from two waters, and $\phi^{TS} = 0.67$ if there are three waters involved, the fit for the latter case being slightly better than for two waters. Fitting of the limited data to a model for TS **14** leading to the diol/$H_3O^+/HO^-(H_2O)_2$ encounter complex **15** is not possible given the fact that there are at least two unequal protons in flight, three more undergoing changes in bonding in the developing hydronium and hydroxide ions, and at least two waters of solvation for the developing OH^-.[27] Large SKIEs for k_H/k_D between 6.9 and 8.5 can be computed for this process on the basis of the assumptions made above which probably rules out this mechanism. Furthermore, from an intuitive perspective, TS **14** seems an unlikely way of creating the diol because of the simultaneous generation of OH^- and H_3O^+ in **15**, although interestingly, in the microscopic reverse direction, the requirement for collecting both OH^- and H_3O^+ for the reversal of **15** provides a convenient explanation for the lack of ^{18}O-exchange.

In reality, the lack of ^{18}O-exchange simply means that protonation of the leaving group and C–N cleavage is faster than oxygen equilibration and expulsion from the tetrahedral intermediate. Due to the strong electron withdrawing properties
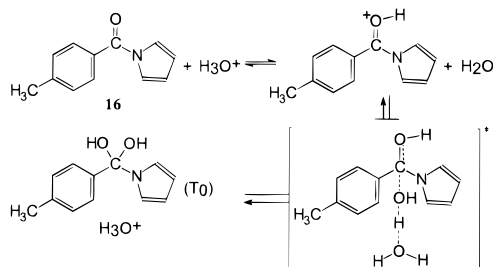
(21) (a) Schowen, R. L. In *Isotope Effects on Enzyme-Catalyzed Reactions;* Cleland, W. W., O'Leary, M. H., Northrop, D. B., Eds.; University Park Press: Baltimore, MD, 1977. (b) Alvarez, F. J.; Schowen, R. L. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Vol. 7, pp 1–60. (c) Kresge, A. J.; More-O'Ferrall, R. A.; Powell, M. F. In *Isotopes in Organic Chemistry*; Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Vol. 7, pp 177–274. (d) Alvarez, F. J.; Schowen, R. L. In *Isotopes in Organic Chemistry* Buncel, E., Lee, C. C., Eds.; Elsevier: Amsterdam, 1987; Vol. 7, pp 1–60.
(22) Kresge, A. J. *J. Am. Chem. Soc.* **1973**, *95*, 3065.
(23) Gold, V.; Grist, S. *J. Chem. Soc., Perkin Trans. 2* **1972**, 89.
(24) Bone, R.; Wolfenden, R. *J. Am. Chem. Soc.* **1985**, *107*, 4772.

(25) Bell. R. P.; Millington, J. P.; Pink, J. M. *Proc. R. Soc. London Ser. A* **1968**, *303*, 1.
(26) (a) Grunwald, E. *Prog. Phys. Org. Chem.* **1966**, *3*, 317. (b) Grunwald, E.; Jumper, C. F.; Meiboom, S. *J. Am. Chem. Soc.* **1963**, *85*, 522.
(27) Note that for any of the models for the various TS, the limited data available means that the derived fractionation factors from fitting are seriously correlated.

of the CF$_3$ group, the starting materials are destabilized[28] which both encourages nucleophilic addition and discourages regeneration of the amide. Thus, the rate constant for expulsion of OH from the diol may be abnormally slow. For related cases where we know diols are formed readily, such as in the hydration of **5** to form **7**[6] or in the acid-promoted hydrolysis of *N*-toluoylpyrrole (**16**, Scheme 1),[15b] reversal with concomitant ^{18}O-exchange[15b] occurs. Acid promoted amide hydrolysis in general gives a T_0/H$_3$O$^+$ encounter complex which, if the amine is basic, undergoes fast proton transfer to N prior to diffusional separation.[15,29] This accounts for the fact that acid-catalyzed hydrolysis of amides, including **8** (Table 7), is not usually accompanied by significant ^{18}O-exchange unless protonation of the amine is inhibited sterically or by electronic factors that reduce the ability to install a proton at the N. Such is the case with **16** which exchanges about 50-fold faster than it hydrolyzes.[15] In the present case, the amine leaving groups[30] are more basic than is pyrrole.[31] Apparently, however it is created, once the diol of **3** or **8** enters the hydrolysis manifold, C−N cleavage with general or specific acid catalysis by H$_3$O$^+$ (0.1−10^{-4} M) occurs more easily than OH expulsion.

**(b) Tetrahedral Intermediate with Inequivalent Oxygens.** A possibility exists that attack of one water is assisted by a second as in **17** to give the encounter complex **18**. The mechanism is similar to that proposed and termed "immature hydronium ion" by Hegazi, Mata-Segreda, and Schowen[19c] for the water hydrolysis of ethyl trifluoroacetate for which a SKIE of 3.6 is observed. Transition states **17** and **14** are similar except for the degree of transfer of the proton from the three waters solvating the developing oxyanion. For TS **17,** the data can be

17   18
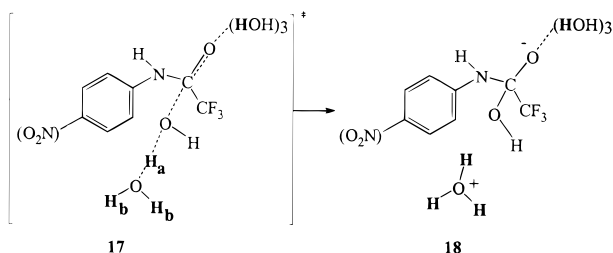
fit by a model having a single proton in flight (fixed value of $\phi^{TS} = 0.4-0.5$) and five others changing their degree of bonding (computed values of $\phi^{TS} = 0.95-0.91$).[24] If the three waters solvating the oxyanion are omitted, as they were in the "immature hydronium ion" mechanism,[19c] and a ϕ^{TS} of 0.5 is set for **H$_a$**, a computed ϕ^{TS} of 0.8 for **H$_b$** is obtained; these values compare favorably with those computed for the analogous H's in the hydrolysis of ethyl trifluoroacetate ($\phi^{Ha} = 0.47$, $\phi^{Hb} = 0.78$).[19c] Note also that for any fits for the latter cases, the ϕ values of **H$_a$** and **H$_b$** are heavily correlated. Even the case of a very late transition state resembling **18**, with its fully formed hydronium ion ($\phi^{Ha} = \phi^{Hb} = 0.69$) and an unspecified number of weakly associated waters all having $\phi = 1.0$, provides a satisfactory fit to the proton inventory data.

A convenient, but not necessarily correct, rationalization for the lack of ^{18}O-exchange is a virtue of this mechanism since the carbonyl oxygen need never become fully equivalent to the one in the attacking water. To do so would require that the

(28) Gambaryan, N. P.; Rokhlin, E. M.; Zeifman, Yu. V.; Ching-Yun, C.; Knunyants, I. L. *Angew. Chem., Int. Ed. Engl.* **1966**, *5*, 947.

(29) Satterthwait, A. C.; Jencks, W. P. *J. Am. Chem. Soc.* **1974**, *96*, 7031.

(30) pK_a of anilinium and *p*-nitroanilinium are 4.63 and 1.0 (25 °C): *CRC Handbook of Chemistry and Physics*, 50th ed.; Weast, R. C., Ed.; The Chemical Rubber Co.: Cleveland, OH, 1969−1970; p D115.

(31) pK_a of the conjugate acid of pyrrole is −3.8, but this is for protonation on the ring; if the protonation were on N, the pK_a would be lower: Chiang, Y.; Whipple, E. B. *J. Am. Chem. Soc.* **1963**, *85*, 2763.

anionic oxygen be protonated either by external H$_3$O$^+$ or by the nascent H$_3$O$^+$ in the encounter complex **18**. The former pathway is thermodynamically favorable and should occur at the diffusion limit, which, at pH 1, sets the rate constant at roughly 10^9−10^{10} s^{-1}.[32] Proton transfer from the nascent H$_3$O$^+$ would require rotation within the complex and disruption/reformation of hydrogen bonds which could occur in competition with diffusional separation of the encounter complex (i.e., 10^{10}−10^{11} s^{-1} or possibly slower since this process involves overcoming electrostatic attraction).[32] Because of the rapidity of these processes, the only way that the two oxygens could be prevented from becoming protonically equilibrated under the reaction conditions would be if the lifetime of the intermediate with respect to C−N cleavage was less than 10^{-11} s. This would require that the H$_3$O$^+$ in the encounter complex become associated with the developing, but weakly basic, nitrogen lone pair to assist in the aniline's departure. In an effort to explain their proton inventory data, Komiyama and Bender[4b] have implied simultaneous proton transfer from the attacking water to the departing leaving group in the hydrolysis of **3** via transition state **19**. In the limit where this occurs so quickly as to prevent equilibration of the oxygens, the energy barrier for the breakdown process must be sufficiently low that water attack and leaving group departure are essentially concerted. Concerted processes have been suggested for the water promoted hydrolyses of acyl imidazoles (**1,2**)[3b,c] and acyl triazole (**6**)[7] via a cyclic transition state as in **20**, but these amides have remote basic nitrogens with fully formed lone pairs to which H-bonds can be directed. The scenario of a concerted reaction with no intermediate formed during the hydrolysis of the anilides used in this study would require that there be a preexisting hydrogen bond network between the attacking water(s) and the anilino lone pair, which, given its extensive delocalization into the CF$_3$C=O and aromatic ring, is unfavorable. We conclude that a truly concerted displacement of the anilide by water is unlikely and that the reaction, even if proceeding via the immature hydronium ion mechanism[19c] must lead to the diol which irreversibly breaks down to product.

19   20   21
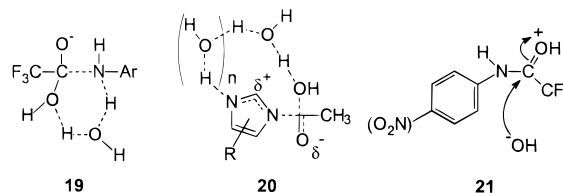
(c) OH$^-$ Attack on a Protonated Amide. As a final topic we consider the possibility that the water reaction really results from its kinetic equivalent, HO$^-$ attack on the protonated amide as in **21**. One can rule this out, at least in the case of **3**, by considering that at [H$_3$O$^+$] = 10^{-2} M, the onset of the water reaction, the [HO$^-$], is ~1.59 × 10^{-11} M at 70 °C[33a] and the amount of protonated amide is calculated to be <10^{-5} M if a pK_a of less than −3 is appropriate for the latter.[33b] Since k_{obs} = 5.6 × 10^{-5} s^{-1} = k_{OH-}[HO$^-$][A-H$^+$], a value for k_{OH-} of > 3.5 × 10^{11} M^{-1} s^{-1} would be necessary to explain the observed rate, this value being about 35- to 100-fold greater than the diffusion limit.[32] An analogous calculation for **8** cannot rule out this possibility for the water reaction, but the strong structural

(32) Eigen, M. *Angew. Chem., Int. Ed. Engl.* **1964**, *3*, 1.

(33) (a) At 70 °C the K_w of water is computed to be 1.59 × 10^{-11}. Harned, H. S.; Owen, B. B. *The Physical Chemistry of Electrolytic Solutions*, 3rd ed.; ACS Monograph Series, No. 137, Reinhold: New York, 1958; p 645. (b) pK_a for amides is usually between 0 and −2; see: Guthrie, J. P *J. Am. Chem. Soc.* **1974**, *96*, 3608 and references therein.

analogies between **3** and **8** suggest the two should hydrolyze by the same mechanisms.

Conclusion

The current study provides the most complete set of data available that have bearing on the ill-defined process of water promoted hydrolysis of an amide. Admittedly the amides in question are trifluoroacetanilides which are both highly disposed toward the formation of hydrated forms and resistant to expulsion of the aniline and OH groups. The available evidence for these systems indicates that the most probable mechanism is a two-step one where the rate limiting step involves two or more waters attacking the amide via an ordered, possibly cyclic, transition state. It is most likely that the first intermediate having any lifetime is the diol (hydrate), and this could be formed in one concerted step or in two steps from a anionic tetrahedral intermediate/H_3O^+ encounter complex which quickly collapses to the diol prior to diffusional separation. The most interesting aspect of this work is the lack of observed ^{18}O-exchange in the $C{=}O$ group of the amide recovered from solution at incomplete hydrolysis. This indicates that even though the amine leaving groups are weakly basic, their departure from the diol intermediate, which undoubtedly requires protonation prior to, or concerted with $C-N$ cleavage, occurs in preference to regeneration of the amide starting material. It remains to be seen how well the information obtained for these amides relates to the water reactions of amides in general.