dine formation (eq 2) can be obtained from eq 12 by dropping all carbon dioxide containing terms. The reciprocal of such a reduced equation is given by eq 18.

$$\frac{1}{k'} = \frac{1}{k_2} + \frac{[H^+]}{k_2 K_{a1}}$$ (18)

A plot, Figure 4, of $1/k'$ vs. $[H^+]$, Table IV, yields a line whose intercept divided by the slope provides another value of K_{a1}, $(2.4(+0.7, -0.9)) \times 10^{-7}$, to be compared with the value of $(3.9 \pm 0.4) \times 10^{-7}$ obtained titrimetrically.

Rauen's results[2b,c] are included in Figure 4 but have not been used in the calculation of the slope or intercept. From the intercept, $k_2 = (6.3 \pm 1) \times 10^{-4} \sec^{-1}$.

Hydrolysis of Substituted Trifluoroacetanilides. Some Implications for the Mechanism of Action of Serine Proteases

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Abstract: The hydrolysis of seven trifluoroacetanilides having various substituents in the aniline ring was investigated at low and high pH. The rate constants for all reactions postulated to occur were obtained separately, and Hammett ρ coefficients for each rate constant were calculated. Positive or zero ρ values were obtained for the reactions: addition of OH^- to trifluoroacetanilide to form a tetrahedral intermediate (k_1); expulsion of OH^- from the intermediate (k_{-1}); and donation of a proton to the intermediate by water (k_2) to form products. The OH^- catalyzed breakdown of the intermediate (k_3) had a negative ρ coefficient. The rate-limiting step for k_3 is considered to involve a proton transfer, while that for k_2 is concluded to be heavy-atom rearrangement. The implications of these findings for the mechanism of action of serine proteases (where hydrolysis of anilide substrates has a negative ρ value) is discussed.

The alkaline hydrolysis of substituted acetanilides has been studied by a number of workers and recently reviewed by O'Conner.[1] Biechler and Taft[2] showed that the pseudo-first-order rate constant for the hydrolysis of trifluoroacetanilide displayed a parabolic dependence on OH^- concentration. They postulated a model in which abstraction of a proton by hydroxide (*i.e.*, ionization of the neutral form of trifluoroacetanilide) produced a nonreactive species. From their data they calculated a pK_a of 11.9 in water. Mader[3] measured the pK_a of trifluoroacetanilide spectrophotometrically and showed it to be 9.5. He proposed a mechanism for the hydrolysis in which a reactive intermediate arises either by addition of hydroxide to the un-ionized form or water to the ionized form of trifluoroacetanilide. Eriksson and coworkers[4,5] studied the effect of various bases as catalysts for the hydrolysis of trifluoroacetanilide. They postulated that the tetrahedral intermediate was formed by addition of hydroxide ion to the un-ionized substrate. Ionization of the trifluoroacetanilide in their model represented a nonhydrolytic pathway. Bender and coworkers[6,7] assumed that the intermediate for the hydrolysis of acetanilide was formed by addition of OH^-. With this substrate no ionization of substrate takes place in the range of OH^- concentrations studied. Schowen and his coworkers[8-11] used a model similar to that of Bender for analyzing their data on the hydrolysis of *N*-methyltrifluoroacetanilide. Again, no ionization is possible with this substrate.

The hydrolysis of acylanilides might be thought a reasonable model reaction to compare with the enzymatic hydrolysis of amides, much as the catalyzed hydrolysis of esters has been used as a model of enzymatic hydrolysis of esters.[12-14] In fact, published data have not supported the analogy for the anilide case. Studies of the hydrolysis of *N*-acetyltyrosylanilides by chymotrypsin[15-18] have yielded negative ρ values with respect to the effect of substituents in the anilide moiety on the rate of acylation of enzyme by the substrate. In contrast, ρ values derived from studies of the hydroxide ion catalyzed hydrolysis of anilides[6-11] have been positive.

In this paper I report some studies on the hydroxide-catalyzed hydrolysis of a number of meta- and para-substituted trifluoroacetanilides. This work was under-

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(2) S. S. Biechler and R. W. Taft, Jr., *J. Amer. Chem. Soc.*, **79**, 4927 (1957).
(3) P. M. Mader, *ibid.*, **87**, 3191 (1965).
(4) S. O. Eriksson and C. Holst, *Acta Chem. Scand.*, **20**, 1892 (1966).
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(6) M. L. Bender and R. J. Thomas, *J. Amer. Chem. Soc.*, **83**, 4183 (1961).
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(8) R. L. Schowen and G. W. Zuorick, *ibid.*, **88**, 1223 (1966).
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(11) L. D. Kershner and R. L. Schowen, *ibid.*, **93**, 2014 (1971).
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(14) M. L. Bender and F. J. Kézdy, *Annu. Rev. Biochem.*, **34**, 49 (1965).
(15) W. F. Sager and P. C. Parks, *J. Amer. Chem. Soc.*, **85**, 2678 (1963).
(16) L. Parker and J. H. Wang, *J. Biol. Chem.*, **243**, 3729 (1968).
(17) T. Inagami, A. Patchornik, and S. S. York, *J. Biochem. (Tokyo)*, **65**, 809 (1969).
(18) M. Caplow, *J. Amer. Chem. Soc.*, **91**, 3639 (1969).

Table I. Rate Constants for Hydrolysis of Meta- and Para-Substituted Trifluoroacetanilides

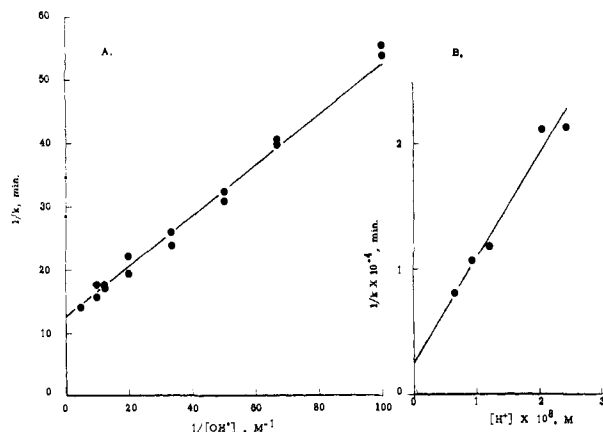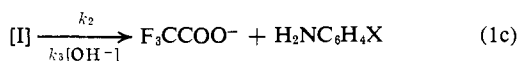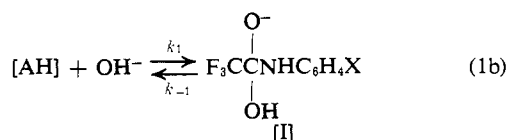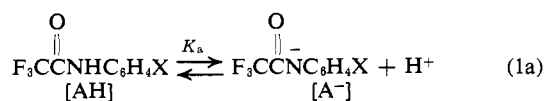| Ring substituent	$k_1 \times 10^{-3}$, M^{-1} min^{-1}	$k_{-1} \times 10^3$, min^{-1}	$k_2 \times 10^4$, min^{-1}	k_3, M^{-1} min^{-1}
m-Cl	2.16 ± 0.12	7.3 ± 2.1	3.5 ± 1.1	3.4 ± 0.34
p-Cl	2.09 ± 0.09	8.0 ± 12.0	5.9 ± 0.4	2.18 ± 0.28
m-OCH₃	1.63 ± 0.04	3.8 ± 0.9	2.8 ± 1.9	2.24 ± 0.14
p-F	1.70 ± 0.04	4.7 ± 1.6	4.5 ± 0.1	2.66 ± 0.21
H	1.74 ± 0.04	13.1 ± 3.6	4.0 ± 2.4	2.70 ± 0.18
m-CH₃	1.70 ± 0.03	6.3 ± 5.8	2.3 ± 0.4	2.62 ± 0.15
p-CH₃	1.38 ± 0.03	18.8 ± 13.3	4.9 ± 1.8	3.10 ± 0.17
p-OCH₃	1.07 ± 0.03	3.9 ± 2.5	3.0 ± 0.4	2.96 ± 0.21

Figure 1. Plots of k_{obsd} vs. appropriate functions for hydrolysis of N-trifluoroacetanilide under various conditions: (A) high pH, $K_a \gg$ [H$^+$], $k_2 \ll k_3$[OH]$^-$; plot of $1/k_{obsd}$ vs. $1/$[OH$^-$]; (B) low pH, $K_a \ll$ [H$^+$], $k_2 \gg k_3$[OH$^-$]; plot of $1/k_{obsd}$ vs. [H$^+$].

taken with the aim of clarifying the connection between anilide hydrolysis as a model reaction and the mechanism of action of serine proteases.

Results

The simplest model for trifluoroacetanilide hydrolysis is essentially that proposed by Eriksson and Holst[4] and shown in eq 1.

$$\underset{\text{[AH]}}{F_3CCNHC_6H_4X} \overset{K_a}{\rightleftarrows} \underset{\text{[A}^-\text{]}}{F_3CCNC_6H_4X} + H^+ \tag{1a}$$

$$\text{[AH]} + \text{OH}^- \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \underset{\text{[I]}}{F_3CCNHC_6H_4X} \tag{1b}$$

$$\text{[I]} \xrightarrow[k_3\text{[OH}^-\text{]}]{k_2} F_3CCOO^- + H_2NC_6H_4X \tag{1c}$$

The kinetic model of eq 1 may be easily solved using the "Theory of Graphs" procedure[19-21] to obtain the pseudo-first-order rate constant k_{obsd} ($=$ d ln A_t/dt) as given in eq 2.

$$k_{obsd} = \frac{k_1 K_w(k_2 + k_3\text{[OH}^-\text{]})}{k_1 K_w + (\text{[H}^+\text{]} + K_a)(k_{-1} + k_2 + k_3\text{[OH}^-\text{]})} \tag{2}$$

Eriksson used a similar expression[4,5] but without the factor $k_1 K_w$ in the denominator. He used the steady-

(19) E. L. King and Carl Altman, J. Phys. Chem., **60**, 1375 (1956).

(20) M. V. Vol'kenshtein, "Enzyme Physics," Plenum Press, New York, N. Y., 1969.

(21) H. J. Fromm, Biochem. Biophys. Res. Commun., **40**, 692 (1970).

state assumption, taking [I] to be negligible and defining A_t = [AH] + [A$^-$]. In fact, [I] is not negligible. The fraction of total anilide present as the intermediate may be estimated from eq 3.

$$\text{[I]}/A_t = k_{obsd}/(k_2 + k_3\text{[OH]}) \tag{3}$$

Using the values of k_2 and k_3 obtained experimentally and given in Table I, I found that I/A_t ranges from 0.05 to 0.10, definitely not a negligible fraction.[22]

The individual rate constants in eq 1 can be evaluated by making measurements under two separate conditions.

I. high pH, $K_a \gg$ [H$^+$], $k_2 \ll k_3$[OH] (4)

$$k_{obsd} = \frac{k_1 K_w k_3\text{[OH}^-\text{]}}{k_1 K_w + K_a(k_{-1} + k_3\text{[OH}^-\text{]})} \tag{4a}$$

$$\frac{1}{k_{obsd}} = \frac{k_1 K_w + k_{-1} K_a}{k_1 K_w k_3} \frac{1}{\text{[OH}^-\text{]}} + \frac{K_a}{k_1 K_w} \tag{4b}$$

From a plot of $1/k_{obsd}$ vs. $1/$[OH$^-$], one obtains the indicated intercept and slope (Figure 1a). From the intercept k_1 is calculated using experimental values of K_a and taking K_w to be 1.48 $\times$ 10^{-14} at 30°. The slope is used, along with known values of k_1, k_{-1} (see below), K_a, and K_w to calculate k_3

II. low pH, $K_a \ll$ [H$^+$], $k_2 \gg k_3$[OH$^-$] (5)

$$k_{obsd} = \frac{k_1 K_w k_2}{k_1 K_w + \text{[H}^+\text{]}(k_{-1} + k_2)} \tag{5a}$$

$$\frac{1}{k_{obsd}} = \frac{1}{k_2} + \frac{(k_{-1} + k_2)}{k_1 k_2 K_w}\text{[H}^+\text{]} \tag{5b}$$

The intercept of the plot of $1/k_{obsd}$ vs. [H$^+$] gives k_2, and k_{-1} is readily calculated from the slope (Figure 1b).

The rate constants for all the compounds are summarized in Table I. The precision of these data is indicated by the standard error associated with each rate constant, which has been evaluated by standard statistical methods as outlined in the Experimental Section.

In Figure 2 are plots of the log rate constant vs. σ. The slopes of these lines were calculated by least mean

(22) In the theory of graphs method for solving complex kinetic equations, the instantaneous concentration of each species present is expressed as a fraction of the total amount of that material present.[19,20] Thus, in the present case, the determinant for, e.g., I is expressed as some function [I] = f[A_t] where A_t is the total amount of anilide present in all forms. The kinetic equation then becomes $-dA_t/dt = A_t \cdot f(k_i, [OH])$, i.e., a pseudo-first-order equation with respect to anilide. The assumptions in this method are essentially steady state, in that $dI/dt = 0$, but the only concentration which must remain constant is that of the catalyst, [OH$^-$] in the present case.[19] The concentrations of various intermediate species are not neglected in this procedure.

squares and the values of Hammett ρ and the associated standard errors are given in Table II. The ρ values for

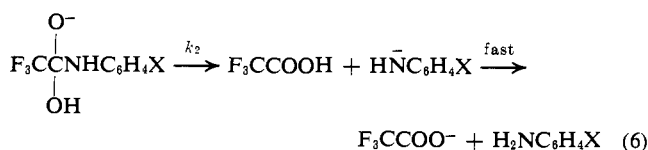**Table II.** Hammett ρ Values Associated with the Rate Constants

Constant	ρ
k_1	$+0.42 \pm 0.08$
k_{-1}	-0.08 ± 0.48
k_2	$+0.19 \pm 0.27$
k_3	-0.30 ± 0.06

most of the rate constants are rather small, but they are in line with small effects of ring substituents on the ionization constant of the trifluoroacetanilides. The plot of log K_a vs. σ has a slope of $+0.79 \pm 0.14$, whereas reported ρ values for ionization of similar compounds are $+3.47$ for N-methylanilines[11] and $+3.5$ for two acetanilides (from data given in ref 7). It appears that the high electronegativity of the trifluoromethyl group decreases about fourfold the sensitivity of the amide system to electron-donating or -withdrawing substituents in the ring.

Discussion

The ρ value of $+0.4$ for k_1 is consistent in sign and in magnitude (if the fourfold correction for insensitivity is made) with the reported values of $+1.0$ for addition of hydroxide to anilides[6] and $+1.18$ found for the reaction of hydroxide with N-methyltrifluoroacetanilides.[11] Such a positive ρ value would be expected for addition of hydroxide ion to the amide carbonyl carbon.

The situation with respect to the rate constants k_{-1} and k_2 is confused. Statistically, the ρ values shown in Table II for both of these rate constants are not significantly different from zero. Bender and Thomas,[6] studying the hydrolysis of acetanilides, found a ρ value of -1 associated with the ratio of rate constants k_2/k_{-1}. Kerschner and Schowen[11] found two values of ρ for the rate constant which they designated as k_1: $+0.07$ for N-methyltrifluoroacetanilides with $pK_b < 9$, and $+1.1$ for substrates with $pK_b > 9$. Their "rate constant" k_1 is equivalent to $k_1 k_2/k_{-1}$ as used in this paper. They found that k_1 (k_a in their terminology) had a ρ value of $+1.18$; thus the ρ values for k_2/k_{-1} in their work would be -1.11 ($pK_b < 9$) and -0.08 ($pK_b > 9$). Eriksson and Holst[4] found the ratio of rate constants k_2/k_{-1} to be 0.025 for both trifluoro- and trichloroacetanilide, implying no substituent effect on that ratio with respect to changes in the acyl group. From the data in Table II (and from the principle that ρ values are additive in a logarithmic sense) I calculate a ρ of $+0.27$ $\pm$ 0.75 [$+0.19 - (-0.08)$] associated with k_2/k_{-1} for the trifluoroacetanilides. Bender and Thomas[6] themselves pointed out that on the basis of a reaction mechanism such as shown in eq 6 one would expect a positive ρ value for k_2, since electron-withdrawing constituents would be expected to stabilize the anionic leaving group. Although the value of $+0.19$ found in this study is in agreement with this expectation, the
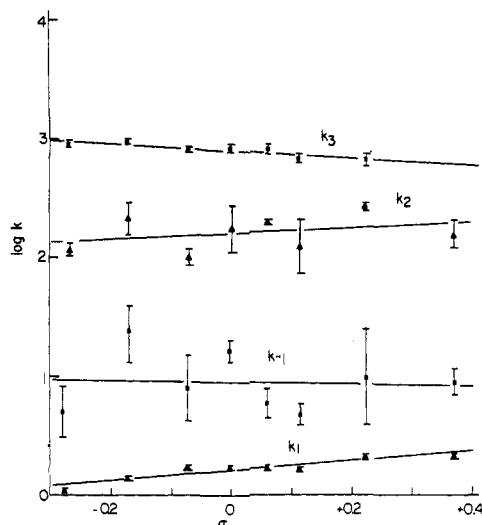
$$\underset{\substack{|\\ \text{OH}}}{\overset{\substack{\text{O}^-\\|}}{F_3C\overset{|}{C}NHC_6H_4X}} \xrightarrow{k_2} F_3CCOOH + H\overline{N}C_6H_4X \xrightarrow{\text{fast}}$$

$$F_3COO^- + H_2NC_6H_4X \quad (6)$$

Figure 2. Hammett relationship plots of log k vs. σ for each of the rate constants in the hydrolysis of N-trifluoroacetanilide. The vertical bars represent one standard deviation. Relationships between actual values of log rate constants and the ordinate are: log k_1 = log k; log k_{-1} = log k − 3; log k_2 = log k − 5.5; log k_3 = log k − 3.
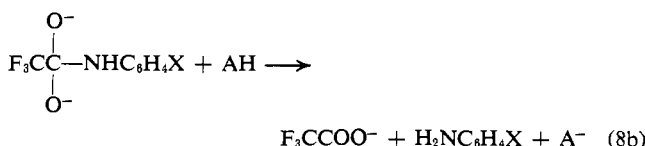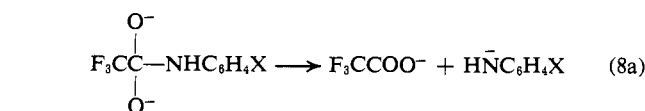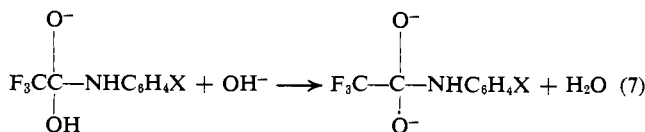
data are not sufficiently precise to unequivocally confirm or refute the prediction.

The catalysis of breakdown on the tetrahedral intermediate by water (k_2) probably goes through a transition state such as Ia or Ib, with the products being

Ia   Ib
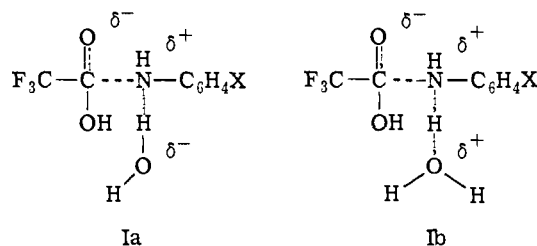
F_3CCOOH, $H_2NC_6H_4X$, and OH^- or H_2O, respectively. These are similar to structure XI in the paper by Kershner and Schowen.[11] The small positive value of ρ found for k_2 implies that in the transition state the breaking of the C–N bond is more complete than is the formation of the N–H bond.
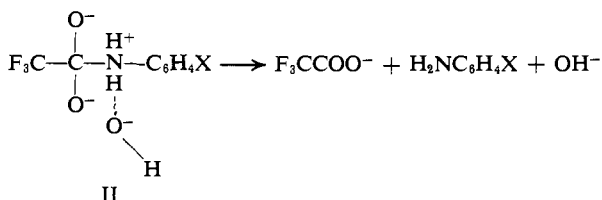
The catalysis of breakdown of the intermediate by hydroxide ion has been suggested to occur via one of two mechanisms[7,11] according to eq 7 and 8.

$$\underset{\substack{|\\ \text{OH}}}{\overset{\substack{\text{O}^-\\|}}{F_3C\overset{|}{C}{-}NHC_6H_4X}} + OH^- \longrightarrow \underset{\substack{|\\ \text{O}^-}}{\overset{\substack{\text{O}^-\\|}}{F_3C{-}\overset{|}{C}{-}NHC_6H_4X}} + H_2O \quad (7)$$

$$\underset{\substack{|\\ \text{O}^-}}{\overset{\substack{\text{O}^-\\|}}{F_3C\overset{|}{C}{-}NHC_6H_4X}} \longrightarrow F_3CCOO^- + H\overline{N}C_6H_4X \quad (8a)$$

$$\underset{\substack{|\\ \text{O}^-}}{\overset{\substack{\text{O}^-\\|}}{F_3C\overset{|}{C}{-}NHC_6H_4X}} + AH \longrightarrow$$

$$F_3CCOO^- + H_2NC_6H_4X + A^- \quad (8b)$$

The tetrahedral intermediate loses a proton to yield the oxydianion (eq 7). If the electron-withdrawing

strength of the substituents is great enough to appreciably stabilize the anilide ion, the oxydianion will break down according to eq 8a.[7,11] On the other hand, for electron-donating substituents or those substituents which are weaker electron withdrawers, contribution of a proton to the anilide nitrogen by the acidic species AH is necessary. This species may be H_3O^+ or H_2O. The possible transition states for reactions 8a and 8b have been discussed by Kerschner and Schowen.[11]

In this work, no evidence has been found for hydrolysis of the trifluoroacetanilides studied via the pathway of eq 8a. Instead, the transition state for the rate-determining step appears to be one in which the $\geqslant C-N \leqslant$ bond is still relatively intact, but a proton has been almost fully donated to the nitrogen, as depicted in structure II. In reaching such a transition state, elec-

$$F_3C-\overset{\overset{\displaystyle O^-}{|}}{\underset{\underset{\displaystyle O^-}{|}}{C}}-\overset{\overset{\displaystyle H^+}{|}}{\underset{\underset{\displaystyle H}{|}}{N}}-C_6H_4X \longrightarrow F_3CCOO^- + H_2NC_6H_4X + OH^-$$
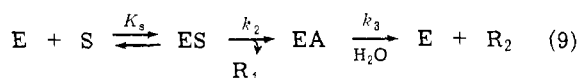
II

tron-withdrawing groups on the ring would decrease the ease with which a proton is transferred to the amide nitrogen, thus decreasing the rate constant k_3 and resulting in a negative value of ρ. This was observed in the present work. Previous workers have not reported this, because they have not been able to determine uniquely the rate constant k_3 in the systems used. Kerschner and Schowen[11] designate the second-order rate constant as k_2, equivalent to k_3k_1/k_{-1}, as specified in eq 1 above. Subtracting out their value for ρ of k_1, they found ρ for k_3/k_{-1} to be -0.9 ($pK_b < 9$) or $+1.2$ ($pK_b > 9$). The ρ value for k_3/k_{-1} in this study was -0.22 (± 0.54), agreeing in sign with Kerschner and Schowen's value for substrates with $pK_a < 9$. On the basis of a ρ value of -0.3 for k_3, then, a transition state structure such as II seems reasonable, with the rate-limiting factor being the transfer of a proton to the amide nitrogen.

To briefly recapitulate, the addition of a hydroxide ion to the carbonyl carbon (k_1) is enhanced by electron-withdrawing substituents, yielding a positive ($+0.4$) ρ value. The breakdown of this intermediate with direct donation of a proton by water (k_2) is marginally enhanced by electron-withdrawing groups, and this rate constant is associated with a small positive ρ coefficient. The rate-limiting factor in the water-catalyzed breakdown is probably the separation of the C–N atoms in the tetrahedral intermediate. On the other hand, the hydroxide ion catalyzed breakdown of the tetrahedral intermediate is the only step found to be enhanced by electron donation and to have a negative ρ value. In this case, transfer of a proton from an acid (H_2O) to the amide N is probably rate limiting. The role of proton transfer and heavy atom rearrangement as rate limiting steps in amide hydrolysis has been discussed by Kerschner and Schowen.[11]

Implications for Mechanism of Action of Serine Proteases. The initial reason for carrying out this work was to evaluate the base-catalyzed hydrolysis of trifluoroacetanilides as a possible model for the hydrolysis of N-acyl-L-tyrosylanilides by chymotrypsin.[15-18] This

approach to understanding more details of the action of the enzyme has apparently worked when ester substrates were employed.[12-14,23] One clear difference presents itself, however; with ester substrates, ρ values are positive in both the enzymatic and the model cases, while the anilide substrates reported ρ values are negative in the enzymatic case[15-17] but positive in the model systems.[6,7,11] As discussed above, however, the rate constants used to represent breakdown of the tetrahedral intermediate have been composites of two or three rate constants for the system, and the connection between them and the steps in the enzymatic system which they are presumed to model has not been at all clear. The uncomplicated rate constants obtained in the present study do allow some comparisons to be drawn, however.

In the simplest case, serine proteases require a kinetic model such as that of eq 9 to explain observed phenomena[14] where R_1 is the alcohol or amine moiety

$$E + S \underset{}{\overset{K_s}{\rightleftharpoons}} ES \underset{R_1}{\overset{k_2}{\longrightarrow}} EA \overset{k_3}{\underset{H_2O}{\longrightarrow}} E + R_2 \qquad (9)$$

of the substrate, R_2 is the acid portion, and EA is an acylated enzyme intermediate. Caplow[18] suggested that, at least for amide substrates (with which $k_2 \ll k_3$), a tetrahedral intermediate with a finite lifetime may also exist between the enzyme–substrate ES complex and the acyl enzyme. Fersht and Requena[24] found that with a series of N-formylphenylalanine amides the kinetic pK for k_{cat} was altered, depending on the substrate used, and furthermore this pK was not always reflected in the pK for the corresponding K_M. They explained their results on the basis of the presence of a tetrahedral intermediate as indicated in eq 10 with the additional proviso that at high pH k_2 is the rate-limiting step in formation of EA from ES, while at low pH k_1 is the rate-limiting step.

EA
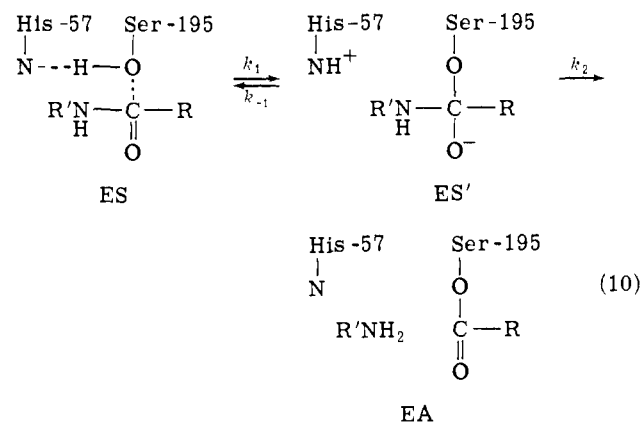
The published studies on the relationship between ring substituents and k_{cat} were carried out at high pH (pH 6.92[15] and 8.0[16,17]) so that the rate k_2 in eq 10 should be rate limiting. This would be analogous to the catalysis of the breakdown of the trifluoroacetanilide tetrahedral intermediate via water catalysis (k_2 in eq 1c), as opposed to the water-catalyzed breakdown of the oxydianion (k_3 in eq 1c). The essentially zero ρ value found for k_2 in the model reaction is not in accord with the

(23) L. Polgár and M. L. Bender, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1335 (1969).

(24) A. R. Fersht and Y. Requena, *J. Amer. Chem. Soc.*, **93**, 7079 (1971).

large negative ρ value found for k_2 of the enzymatic reaction.

This disagreement might be due to one of at least three different factors. (1) The model reaction deals with a tetrahedral intermediate arising from attack of a *hydroxide* ion on the amide carbonyl carbon, whereas in the enzymatic case the intermediate arises from addition of an *alkoxide* ion. This does not seem to make a difference, however, for alcoholysis of acetamides by phenol apparently has a positive value of ρ.[25] Also, ρ values for methanolysis of N-methyltrifluoroacetanilides are in line with corresponding ρ values for hydrolysis of the same substrates.[26] (2) The acid catalysis in the model reaction is due to water hydronium ion, while in the enzymatic case an imidazolium group is postulated to fulfill this role. While no published data speak directly to this point, preliminary work in this laboratory indicates that imidazole-catalyzed hydrolysis of the trifluoroacetanilides studied gives rate constants having large positive ρ values. (3) The enzymatic reaction does not correspond to the model reaction represented by k_2 but rather to the reaction represented by k_3. This possibility may have some merit, since in the hydroxide-catalyzed hydrolysis of substituted acetanilides the rate k_3/k_{-1} for the p-NO_2 derivative[7] fell far above the correlation line found with other derivatives,[6] and in the enzymatic hydrolysis of N-acetyl-L-tyrosylanilides the p-NO_2 derivative also fell above the correlation line.[17] If this explanation for the anomaly is correct, then either the enzymatic tetrahedral intermediate ES' (eq 10) gives rise to an oxydianion (a rather unlikely possibility); the electronic distribution around the carbonyl group of ES' approximates a dianion without generating a second formal negative charge; or the postulated mechanism for the model reaction k_3 is wrong, and it does not, in fact, form an oxydianion at all. The basis for choosing between the possibilities is not clear-cut at this time.

Experimental Section

The trifluoroacetanilides were synthesized as follows. The desired substituted aniline (0.05 mol) was dissolved in 25 ml of pyridine and cooled in an ice bath. Trifluoroacetic anhydride (0.05 mol) was added dropwise over a 20–30-min period with continuous stirring and cooling. The reaction mixture was then allowed to sit at room temperature for 1 hr, and the solvent was removed under vacuum on a rotary evaporator. The residue was dissolved in $CHCl_3$, washed twice with 0.1 N HCl, twice with saturated $NaHCO_3$, and twice with water, and dried over anhydrous Na_2SO_4, and then the solvent was removed (rotary evaporator). The residue was crystallized from hexane–acetone, with a second crop of crystals obtained from the mother liquor. The combined crystals were taken up in $CHCl_3$ and treated with activated charcoal, and the solvent was removed. The residue was recrystallized from hexane–acetone solvent. The melting points of the crystals were determined on a Fisher-Johns hot-stage apparatus. The melting points in Table III are uncorrected.

Table III. Physical Constants of Substituted Trifluoroacetanilides

Ring substituent	Mp, °C	Lit. mp, °C	pK_a	σ^c
m-Cl	66–68		8.83	+0.373
p-Cl	123–124	123–124.5[a]	9.23	+0.227
m-OCH_3	71–72		9.46	+0.115
p-F	110–111		9.38	+0.062
H	87–88	89–90,[a] 87–89[b]	9.50	0
m-CH_3	62–64[d]	67–68[b]	9.52	−0.069
p-CH_3	109.5–111	111–112,[a] 112–113[b]	9.61	−0.170
p-OCH_3	113–115	112.5–115[a]	9.65	−0.268

[a] E. J. Bourne, S. H. Henry, C. E. Tatlow, and J. C. Tatlow, *J. Chem. Soc.*, 4014 (1952). [b] K. G. Rutherford, S. Y.-S. Ing, and R. J. Thibert, *Can. J. Chem.*, **43**, 541 (1965). [c] L. N. Ferguson, "The Modern Structural Theory of Organic Chemistry," Prentice-Hall, Englewood Cliffs, N. J., 1963, p 415. [d] This value did not change even after two additional recrystallizations.

The rates of anilide hydrolysis at $[OH^-] > 0.01$ M were followed in a Gilford spectrophotometer, with the cuvette chamber thermostated at 30°. The decrease in absorbance at 260 nm was followed, and the pseudo-first-order rate constant k_{obsd} was evaluated by the method of Swinbourne.[27] In all cases, the initial anilide concentration was 1×10^{-4} M. The reaction solvent was aqueous NaOH (at the concentration noted later) containing 1% p-dioxane (the solvent for the stock anilide solutions). Ionic strength of the solutions was not adjusted, since it has little or no effect on the rate of reaction.[2,3,26] The relationship between k_{obsd} and OH^- concentration was found to be hyperbolic over the range of OH^- concentrations covered, so the data were processed with the HYPER computer program of Cleland[28] to obtain A and B in the equation $k_{obsd} = A[OH^-]/(B + [OH^-])$. The standard errors in k_1 and k_3 were derived from the computer program.

At low pH ($[H^+] \geq 20K_a$) the rate of appearance of aniline was determined by diazotization,[29] starting with a solution of 1×10^{-3} M anilide in buffer of given pH at various dilutions and extrapolated to zero buffer concentration to obtain the rate for the reaction uncomplicated by buffer catalysis. Each extrapolation was based on 6–8 experimental points. The buffer used was N-2-hydroxyethyl-piperazine-N-2-ethanesulfonic acid and NaOH. The buffer effect on rate was linear up to 0.02 M total buffer ion, although in this work concentrations of 0.0025–0.010 M were used. The plot of $1/k_{obsd}$ vs. $[H^+]$ was fit using linear least mean squares statistics. The standard errors for the estimates of k_2 and k_{-1} were also obtained from this analysis of regression.

The ionization constants of the anilides were determined as follows. A difference spectrum was run (on a Cary 14) with 1×10^{-3} M anilide in 0.05 M phosphate buffer pH 6.0 in the reference cell to determine the wavelength of maximum absorption difference between ionized and un-ionized anilide. To 3.0 ml of 0.1 M glycine–NaOH buffer (pH measured on a Radiometer TTT1 pH meter with a scale expander, Model PHA 630) 30 μl of 0.1 M anilide in p-dioxane was added and the absorbance at the desired wavelength was read vs. a water blank. This was repeated with a total of at least 12 buffer solutions at intervals of approximately 0.1 pH unit. The data were fitted with an iterative computer program to give the absorbance of the ionized and un-ionized anilide species and the pK of the anilide. The values obtained are given in Table III.

(25) A. R. Fersht, *J. Amer. Chem. Soc.*, **93**, 3504 (1971).
(26) R. L. Schowen, C. R. Hopper, and C. M. Bazikian, *ibid.*, **94**, 3095 (1972).
(27) E. S. Swinbourne, *J. Chem. Soc.*, 2371 (1960).
(28) W. W. Cleland, *Advan. Enzymol. Relat. Subj. Biochem.*, **29**, 1 (1967).
(29) V. Kratohvil, *Z. Anal. Chem.*, **183**, 267 (1961).