Hydrolysis of Substituted Trifluoroacetanilides. Nucleophilic Catalysis by Imidazole

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Abstract: The hydrolysis of a number of ring-substituted trifluoroacetanilides is markedly accelerated by imidazole buffer. It is found that this is due strictly to nucleophilic attack by imidazole base, with no general acid or general base catalysis observed. Spectrophotometric evidence is presented for the formation of a tetrahedral intermediate, attributed to addition of imidazole to the carbonyl carbon of trifluoroacetanilide. Specific acid protonation of the tetrahedral intermediate does take place during the rate-limiting breakdown to give hydrolysis products and an isotope effect of 4.3. A plot of log k_5 (rate constant for the breakdown step) *vs.* σ for the ring substituents showed two segments. When electron-donating substituents were present ($\sigma < 0$) ρ, the slope of the plot, was +0.8, while when the substituents were electron attracting ($\sigma > 0$) ρ was +2.1. This difference is interpreted in terms of a change in the relative importance in the transition state of proton donation to the anilide –N< as compared to the breaking of the $\gtrdot CN^{+} \lessdot$ bond. Comparison of the results obtained with observations on catalysis of amide hydrolysis by α-chymotrypsin leads to the conclusion that the hydrolysis of trifluoroacetanilides in the presence of imidazole is not a good model for the enzymatic reaction.

The alkaline hydrolysis of substituted trifluoroacetanilides was the subject of a recent paper.[1] The catalysis of anilide hydrolysis by bases other than hydroxyl ion has been investigated by a number of workers. Eriksson and Holst[2] reported that $H_2PO_4^-$ and HCO_3^- ions catalyze the hydrolysis, perhaps acting as a bifunctional "general acid–general base" catalysis. A similar role for these ions in the hydrolysis of 4-hydroxybutyranilide was postulated by Cunningham and Schmir.[3] Eriksson and Bratt[4] investigated the hydrolysis of trifluoroacetanilide in the presence of a number of amines. They proposed that the amines and the conjugate acids act as general base and general acid catalysts for the formation and breakdown, respectively, of the tetrahedral intermediate, while the free bases also act directly on the substrate *via* nucleophilic attack.

Pratt and Lawlor[5] studied the effect of a large number of bases on the hydrolysis of *N*-(4-nitrophenyl)dichloroacetamide. They concluded that with this substrate no nucleophilic attack occurred but that three modes of catalysis could be distinguished: general base catalysis of formation of the tetrahedral intermediate; general acid catalysis of intermediate breakdown, either to product or to starting materials; and general base catalysis of product formation from the tetrahedral intermediate (not all three kinds of catalysis were seen with all the buffers examined). Drake, Schowen, and Jayaraman[6] also used amine buffers in exploring the hydrolysis of *N*-methyltrifluoroacetanilide. They concluded that the buffer played no role in formation of the tetrahedral intermediate (which was formed only by addition of HO^- to the substrate). The conjugate acid did contribute to breakdown of the intermediate to products by general acid catalysis, but the contribution of the free base (*i.e.*, general base catalysis of the breakdown) was small.

My interest in the hydrolysis of various anilides is for the light which they may shed on the hydrolysis of amides by proteases, especially the serine proteases such as chymotrypsin and subtilisin. Since the imidazole portion of a histidine residue plays a crucial role in the action of these enzymes, it seemed worthwhile to investigate in more depth the role imidazole might play in hydrolysis of anilides.

Results

At low concentrations of imidazole buffer (<0.1 *M*) the pseudo-first-order rate of hydrolysis of trifluoroacetanilide increases linearly as buffer concentration increases. The slope of the plot of rate *vs.* total buffer concentration is the apparent second-order rate constant of buffer catalysis, k_2'.[7] The slope was determined at several different buffer compositions, and the values of k_2' obtained were plotted *vs.* fraction of free base in the buffers (Figure 1). The fact that the resultant line intercepts the ordinate at zero when the fraction of free base equals zero shows that there is no general acid catalysis occurring in the present case; that is, imidazolium ion is not contributing to the hydrolysis of trifluoroacetanilide.

Next, the hydrolysis of trifluoroacetanilide was investigated over a much wider range of imidazole concentrations. The results obtained in a typical experiment are shown in Figure 2. There are two features of this result which are important to note. First, the increase in hydrolysis rate over the rate in the absence of buffer appears to have a hyperbolic dependence on imidazole concentration. This is confirmed by the linearity of the double-reciprocal plot of $1/(k_{obsd} - k_0)$ *vs.* 1/[imidazole] (Figure 2 inset).

Secondly, the rate of anilide hydrolysis as buffer concentration increases becomes much larger than $k_1[OH^-]$, the rate of tetrahedral intermediate formation by attack of hydroxide on the carbonyl carbon. This means that the imidazole catalysis is not a matter of general base assisted breakdown of the oxyanion tetrahedral intermediate to products. Thus, Drake, *et al.*,[6] postulated

(1) C. E. Stauffer, *J. Amer. Chem. Soc.*, **94**, 7887 (1972).
(2) S. O. Eriksson and C. Holst, *Acta Chem. Scand.*, 20, 1892 (1966).
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(6) D. Drake, R. L. Schowen, and H. Jayaraman, *J. Amer. Chem. Soc.*, **95**, 454 (1973).
(7) W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill, New York, N. Y., 1969.

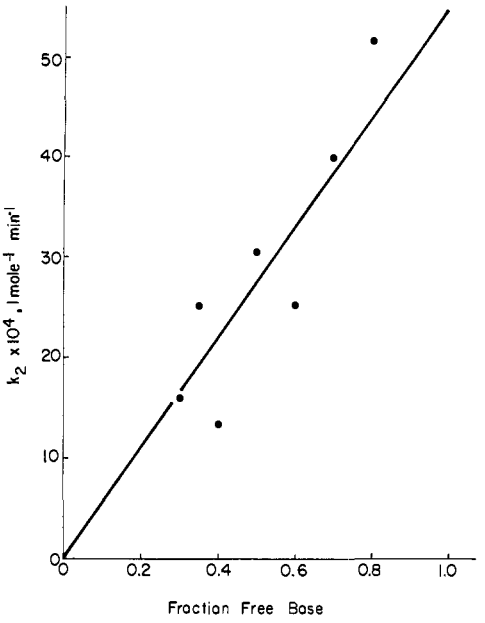


Figure 1. Relationship between the apparent second-order rate constant for buffer catalysis, k_2', and buffer composition. Hydrolysis of 10^{-4} M trifluoroacetanilide in imidazole buffers at 30°.

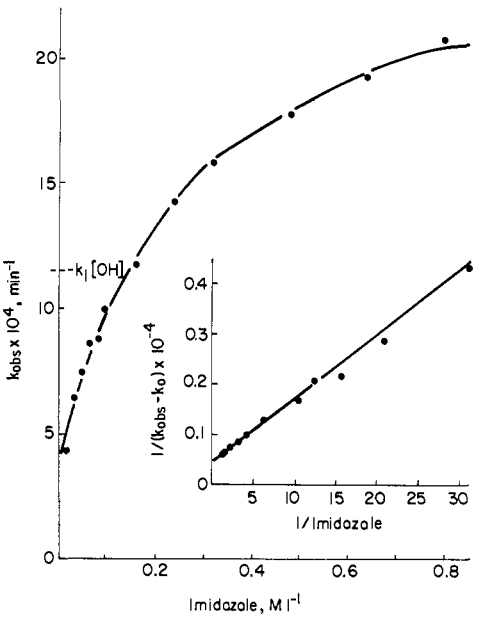


Figure 2. Hydrolysis of 10^{-4} M trifluoroacetanilide in imidazole buffer at 30°: [Im/ImH$^+$] = 4; pH 7.65; k_1[OH$^-$] calculated using previously measured value of k_1.[1]

a reaction sequence for hydrolysis of *N*-methyltrifluoroacetanilide according to Scheme I.

Scheme I

$$F_3CCON(CH_3)C_6H_5 \underset{k_{-1}}{\overset{k_1[OH^-]}{\rightleftharpoons}} F_3C{-}\overset{O^-}{\overset{|}{C}}(OH){-}N(CH_3)C_6H_5 \xrightarrow[k_4[BH^+],\ k_5[B]]{k_2[H_2O],\ k_3[OH^-]} \text{products}$$

The pseudo-first-order rate equation for this reaction is

$$k_{obsd} = \frac{k_1[OH^-](k_2 + k_3[OH^-] + k_4[BH^+] + k_5[B])}{k_{-1} + k_2 + k_3[OH^-] + k_4[BH^+] + k_5[B]} \quad (1)$$

At high buffer strengths, where $k_4[BH^+] + k_5[B]$ becomes much greater than the other rate constants, the value of k_{obsd} should approach a limiting value of $k_1 \cdot [OH^-]$. Drake, *et al.*,[6] reported that this was found in their experiments. My limiting value, by contrast, is well above the value of $k_1[OH^-]$.

A somewhat similar scheme is that of Pratt and Lawlor[5] with the additional postulated kinetic steps of general base catalyzed formation of the tetrahedral intermediate ($k_6[B]$) and general acid catalyzed reversal of the same step ($k_{-6}[BH^+]$). In this case the expression for the pseudo-first-order rate constant for anilide hydrolysis becomes

$$k_{obsd} = \frac{(k_1[OH^-] + k_6[B])(k_2 + k_3[OH^-] + k_4[BH^+] + k_5[B])}{k_{-1} + k_2 + k_3[OH^-] + k_4[BH^+] + k_5[B] + k_{-6}[BH^+]} \quad (2a)$$

As the buffer concentration becomes large and all terms not containing [B] or [BH$^+$] become insignificant, this reduces to

$$k_{obsd} = \frac{k_6[B](k_4[BH^+] + k_5[B])}{k_4[BH^+] + k_5[B] + k_{-6}[BH^+]} \quad (2b)$$

i.e., k_{obsd} should become first order in buffer and increase indefinitely as buffer concentration increases. This is not the case in the present work, nor did any of the earlier workers report such a phenomenon.[4-6]

The simplest scheme which is consistent with the present data is that of nucleophilic attack by imidazole to form a tetrahedral intermediate which breaks down (unassisted by any general acid catalysis) to give the products (Scheme II).

Scheme II

$$F_3CCONHC_6H_5 \underset{k_{-4}}{\overset{k_4[Im]}{\rightleftharpoons}} F_3C{-}\overset{O^-}{\overset{|}{C}}(Im^+){-}\overset{H}{N}{-}C_6H_5 \xrightarrow{k_5} F_3CC(=O)Im^+ + HN^-C_6H_5 \xrightarrow{H_2O,\ \text{fast}} F_3CCO_2H + Im + H_2NC_6H_5$$

There are several questions to be considered. (1) Does the attacking imidazole molecule necessarily give up a proton, either through general base catalysis during formation of the intermediate or by ionization of the intermediate itself? (2) Does proton transfer play any role at all in the rate-limiting step of the reaction? (3) Is there any evidence for the formation of the intermediate, and its accumulation at large buffer concentrations, when intermediate breakdown is the rate-limiting reaction?

The first question can be answered simply no. A run was made using *N*-methylimidazole as the buffer. The results were very nearly the same as when imidazole was the buffer species (Figure 3). Thus, deprotonation of the N-3 position in imidazole (a process impossible with *N*-methylimidazole) plays no part in the reaction considered here.

The answer to the second question is yes; a proton

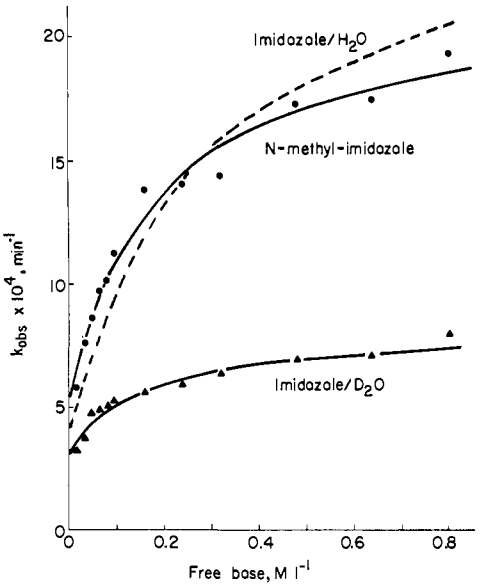


Figure 3. Hydrolysis of 10^{-4} M trifluoroacetanilide at 30° in N-methylimidazole buffer and in imidazole buffer in D_2O. The dotted line represents catalysis by imidazole in H_2O. $[B]/[BH^+]$ = 4 in each case.

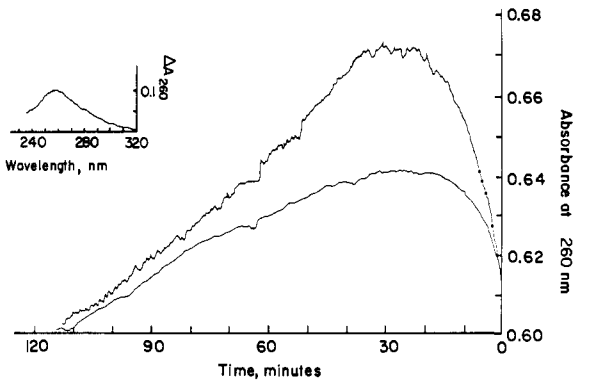


Figure 4. Initial increase in absorbance at 260 nm when 30 μl of 0.01 M trifluoroacetanilide in p-dioxane was added to 3.0 ml of imidazole buffer at 30°. The Gilford spectrophotometer was zeroed with just the buffer in the light path, and the anilide stock solution was added at t = 0: upper curve, [Im] = $[ImH^+]$ = 1.0 M; lower curve, [Im] = $[ImH^+]$ = 0.5 M; inset, difference spectrum, scanned in a Cary 14 10 min after trifluoroacetanilide was added to the buffer; sample cell, 5-mm path length, 0.5 M [Im], 1.0 M $[ImH^+]$, 3.2×10^{-4} M anilide, and a 5-mm path length cell containing water; reference cell, 5-mm path length, same buffer, a separate 5-mm path length cell, 3.2×10^{-4} M anilide in water.

is transferred during the rate-limiting step. A run made with imidazole buffer in D_2O gave a lower limiting rate, as shown in Figure 3. The value of $k_{lim,H_2O}/k_{lim,D_2O}$ was 4.3, implicating proton transfer at the rate-limiting step. There was no evidence for any general acid catalysis by imidazolium ion (Figure 1). Another possibility is that specific acid catalysis by H_3O^+ occurs during the rate-limiting step. However, in experiments with trifluoroacetanilide at three different pH's (6.87, 7.23, and 7.65) the limiting rates at high buffer concentrations were the same within experimental error indicating that the limiting step rate constant was zero order in H^+. These two facts taken together indicate that a proton from a water molecule is donated to the intermediate during the breakdown step, accounting for the observed isotope effect.

The question about possible intermediate formation may be readily answered in the affirmative. When trifluoroacetanilide was added to imidazole buffer and the absorbance at 260 nm followed as a function of time, there was an initial *increase* with a half-life of about 5 min, followed by the expected *decrease* due to hydrolysis of the anilide (Figure 4). The size of the increase was proportional to the concentration of imidazole present. Also, a difference scan of the intermediate *vs.* anilide plus buffer (Figure 4, inset) showed that the wavelength of maximum increase was at 260 nm. Since no similar absorbance increase was seen when trifluoroacetanilide was placed in high concentrations of ^-OH, the absorbing species cannot be the tetrahedral adduct formed by addition of hydroxide ion to the anilide molecule. The only other reasonable possibility is the imidazoyl intermediate as depicted in Scheme II.

At high levels of imidazole, the breakdown of intermediate is rate limiting, not its formation, implying that there is a build-up of intermediate under these conditions. While the intermediate would be expected to be somewhat unstable, it could break down either *via* k_{-4} or k_5. If $k_{-4} \gg k_5$, then there would be essentially an equilibrium situation with regard to anilide, imidazole, and intermediate. The half-life for formation of intermediate of ~5 min is much shorter than the half-life for its breakdown to products (100–300 min, from values of k_5, Table I), indicating that the existence of an equilibrium situation (after the initial accumulation of intermediate) is a reasonably good approximation.

Table I. Rate Constants for Nucleophilic Hydrolysis of Substituted Trifluoroacetanilides[a]

Ring substituent	$k_5 \times 10^3$, min^{-1}	k_{-4}/k_4, M
m-Cl	14.0 ± 1.9	1.61 ± 0.27
p-Cl	6.82 ± 0.44	0.70 ± 0.08
m-OCH_3	2.90 ± 0.18	0.37 ± 0.04
p-F	3.31 ± 0.48	0.83 ± 0.19
H	2.22 ± 0.68	0.28 ± 0.02
m-CH_3	1.94 ± 0.17	0.41 ± 0.07
p-CH_3	1.83 ± 0.30	0.57 ± 0.17
p-OCH_3	1.26 ± 0.17	0.59 ± 0.14
H, N-methylimidazole	1.62 ± 0.08	0.18 ± 0.02
H, imidazole in D_2O	0.52 ± 0.04	0.15 ± 0.03

[a] All runs in imidazole buffer in H_2O at 30°, unless noted otherwise.

With these three answers in hand, I propose that the likeliest mechanism for imidazole catalysis of trifluoroacetanilide hydrolysis is that shown in Scheme III.

Scheme III

$$F_3CCONHC_6H_5 + Im \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} F_3CC(O^-)(Im^+)-NC_6H_5 \xrightarrow[H_2O]{k_5} H_2NC_6H_5 + F_3CCOIm^+$$

$$H_2NC_6H_5 + F_3CCO_2H + Im \xleftarrow[fast]{} OH^-$$

The pseudo-first-order rate constant for this process,

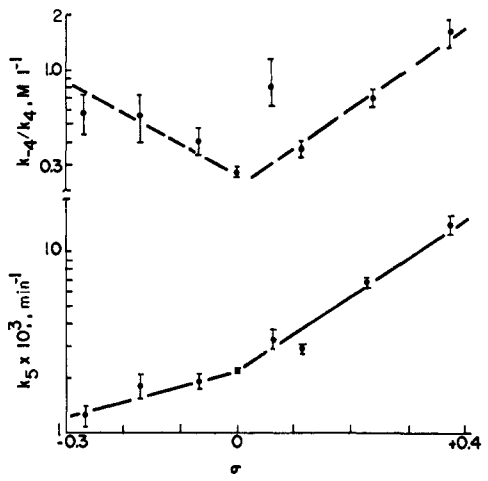


Figure 5. Free energy relationship of log rate constant *vs.* σ for the ring substituent. Vertical bars represent ± 1 standard deviation. The lines (the slopes of which represent Hammett ρ values) were drawn by inspection.

k_n (assuming equilibrium as discussed above), is given by

$$k_n = \frac{-dA}{dt}\frac{1}{A} = \frac{k_5[\mathrm{Im}]}{(k_{-4}/k_4) + [\mathrm{Im}]} \qquad (3)$$

Experimentally, $k_n = k_{obsd} - k_0$, where k_0 is the hydrolysis rate obtained by extrapolation to zero buffer concentration.

The hydrolysis of a number of ring-substituted trifluoroacetanilides (1) was examined in imidazole buffers, and the results were analyzed according to eq 3. Values of k_5 and k_{-4}/k_4 are given in Table I and plotted in Figure 5 as log rate constant *vs.* σ values for the respective ring substituents. The lines in Figure 5 were drawn by inspection, so that the slopes (Hammett ρ values) given in Table II do not have any associated confidence limits. The signs and the approximate values of ρ, however, are unmistakable.

Table II. Hammett ρ Values for Nucleophilic Attack by Imidazole

Rate constant	ρ	Rate constant	ρ
k_5, $\sigma < 0$	+0.8	k_{-4}/k_4, $\sigma < 0$	−1.6
k_5, $\sigma > 0$	+2.1	k_{-4}/k_4, $\sigma > 0$	+2.1

Discussion

It was rather surprising to find that imidazole catalyzed trifluoroacetanilide hydrolysis only by nucleophilic attack. Pratt and Lawlor[5] found that imidazole acted as a general acid catalyst in the breakdown of the tetrahedral intermediate of *N*-(4-nitrophenyl)dichloroacetamide, but such a role for imidazole in the present instance is ruled out by the data presented in Figure 1. Pollack and Dumsha[8] conclude that Tris buffer contributed *via* general acid catalysis to the breakdown of the tetrahedral intermediate formed by addition of OH^- to *p*-nitrotrifluoroacetanilide. Eriksson and Bratt[4] concluded that imidazole contributed to trifluoroacetanilide hydrolysis by both general acid catalysis of tetrahedral intermediate breakdown and by direct nucleophilic attack on the substrate. The conditions they used covered a much wider range of pH than in the present study, but values of k_{obsd} at pH 7.65 found by me (Figure 2) compare quite well with Eriksson and Bratt's results obtained at pH 7.05 and 8.25 (Figures 2 and 3 of ref 4).

General base catalysis (in the present instance) of either formation of the tetrahedral intermediate[5] or breakdown of intermediate to product[6] is ruled out on kinetic grounds. The rate of hydrolysis does not increase indefinitely with increasing buffer concentration as required by the former case (eq 2b), nor does the limiting rate at high buffer concentration approach the value of $k_1[OH^-]$, as predicted in the latter mechanism (eq 1).

Ruling out the general acid–base mechanisms leaves only nucleophilic and specific acid catalysis to account for the increased rates of hydrolysis which were seen. The observed isotope effect of 4.3 is fully consistent with a rapid protonation of an acid of $pK_a \sim 11$[9] during the step associated with the rate constant k_5. This acid is identified as the tetrahedral adduct shown in Scheme III.

The presence of a tetrahedral intermediate in the hydrolysis of amides has been pretty much taken as given in recent work.[1-8] Thus, Menger and Donohue[10] not only assumed the presence of the tetrahedral intermediate during the hydrolysis of *N*-acylpyrroles but claimed to have determined the value of an ionization constant for the intermediate based upon kinetic results. Similarly, Cunningham and Schmir[3] postulated a tetrahedral intermediate (a hydrated iminolactone) in their study of hydrolysis of 4-hydroxybutyranilide. Robinson[11] obtained spectrophotometric evidence for the formation of a tetrahedral intermediate during the hydrolysis of 1,3-diphenyl-2-imidazolinium chloride. As in this study, he found an initial increase in absorbance after mixing substrate and catalyst (hydroxide ions) followed by a slower decrease due to hydrolysis. The time scale in his studies was milliseconds as compared to minutes in this work. However, this difference in time scale merely reflects reaction rates not the inherent probability of observing the tetrahedral intermediate. As an example of a slow rate of reaction, Rogers and Bruice[12] recently reported the isolation of a tetrahedral intermediate fully capable of further acyl migration (for instance, to a water molecule giving hydrolysis). By analogy to these previous results, I conclude that the increase in absorbance shown in Figure 4 does indeed represent a preequilibrium formation and accumulation of the tetrahedral intermediate resulting from addition of an imidazole molecule to the carbonyl carbon of the anilide. The kinetics of the preequilibrium stage of this reaction have not yet been fully explored.

The breakdown of tetrahedral intermediate to give products apparently goes through somewhat different transition states when the ring substituents are electron

(8) R. M. Pollack and T. C. Dumsha, *J. Amer. Chem. Soc.*, **95**, 4463 (1973).

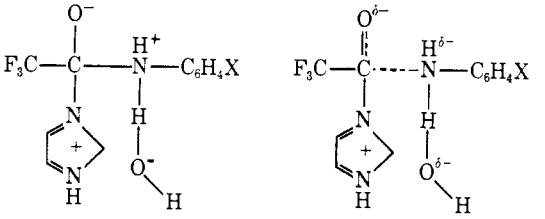
(9) (a) C. A. Bunton and V. J. Shiner, Jr., *J. Amer. Chem. Soc.*, **83**, 42 (1961); (b) R. P. Bell and A. T. Kuhn, *Trans. Faraday Soc.*, **59**, 1789 (1963); (c) ref 7, pp 250–253.

(10) F. M. Menger and J. A. Donohue, *J. Amer. Chem. Soc.*, **95**, 432 (1973).

(11) D. R. Robinson, *J. Amer. Chem. Soc.*, **92**, 3138 (1970).

(12) G. A. Rogers and T. C. Bruice, *J. Amer. Chem. Soc.*, **95**, 4452 (1973).

donating ($\sigma < 0$) then when the ring substituents are electron attracting ($\sigma > 0$). A very similar phenomenon was observed in the case of hydroxide-catalyzed hydrolysis of *N*-methyltrifluoroacetanilides.[13] Kershner and Schowen interpreted this to mean that the rate-limiting transition state for the weakly basic substrates tended toward involving proton transfer, while the transition state for the more basic substrates depended more on heavy-atom reorganization. A similar differentiation may obtain in the present instance, with the compounds having $\sigma < 0$ going through a transition state similar to **1** on the way to products, while those with $\sigma > 0$ pass through a transition state more like **2**.



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This interpretation would be consistent with the stabilization of the anilide ion by electron-withdrawing substituents and enhancement of proton acceptance on the nitrogen atom by electron-donating ring substituents.

The change in sign of the "half-maximum velocity" constant k_{-4}/k_4 perhaps reflects the same tendencies. However, since I did not obtain individual rate constants k_4 and k_{-4}, no detailed interpretation will be attempted.

The real point of this study was to find out if the model system used could provide information pertinent to the mechanism of action of serine proteases such as chymotrypsin. In general, these enzymes have been supposed to act *via* general base–general acid catalysis by the imidazole ring of a histidine, with a serine hydroxyl as the alkoxide base.[14] If this is indeed an accurate conception, then the nucleophilic attack observed in this work does not contribute to any further understanding of the enzymatic mechanism.

Investigations of hydrolysis of a number of anilide substrates by chymotrypsin have indicated that the correlation line between k_2 (the rate of cleavage of the -C(=O)-N(H)- bond) and the σ values of various ring substituents was negative,[15] with a value for ρ of about -2. However, Philipp, *et al.*,[16] gathered together data on hydrolysis by α-chymotrypsin of *N*-acetyl-L-tyrosinamides having a wide range of leaving group pK's which indicated that ρ was essentially zero. In either case, the corresponding reaction in this work with rate constant k_5 and ρ values of $+0.8$ to $+2.1$ is obviously not a model for the events which occur during hydrolysis of amides by α-chymotrypsin.[17]

Such a model could be very useful in illuminating the reasons for catalytic efficiency of serine proteases. A successful model would have to unambiguously show general base catalysis by imidazole of formation of the tetrahedral intermediate with alkoxide or hydroxide ion and general acid catalysis by imidazolium ion of the expulsion of the amine moiety of the substrate. Thus far, no study has met these criteria. Cunningham and Schmir,[3] in looking at catalyzed lactonization of 4-hydroxybutyranilide with concomitant expulsion of aniline, found no increase in rate in the presence of imidazole. Drake, *et al.*,[6] found general acid catalysis of breakdown but no general base catalysis of intermediate formation, with *N*-methyltrifluoroacetanilide as the substrate. In addition, they did not use imidazole as one of their catalyst buffers. Other workers[4,5] have studied the enhancement by imidazole of amide hydrolysis, but their work was not extensive enough to clearly establish the nature of the catalysis. Thus, the need for a model reaction for the hydrolysis of amides by serine proteases remains unfilled.

Experimental Section

The trifluoroacetanilides were those used previously.[1] Imidazole (Aldrich Chemical Corp.) was dissolved in benzene, decolorized with activated charcoal, and recrystallized (mp 89–90°). Stock solutions were 2 *M* imidazole or imidazolium chloride in H_2O (or D_2O and DCl, Merck Chemical Co.). Aliquots of stock buffer solutions to give the desired final concentrations of the base and acid were made to 3.0 ml total and brought to 30°, and 30 μl of 10^{-2} *M* anilide in *p*-dioxane was added to start the reaction. The decrease in absorbance at 260 nm was followed in a Gilford 2400 spectrophotometer (0–0.1 absorbance scale) after the initial pre-equilibrium phase (~20 min) was completed, with the cuvette chamber thermostated at 30°. The zero-order rate of absorbance decrease was divided by the total expected decrease (obtained by comparing A_{260} for 10^{-4} *M* solutions of completely hydrolyzed anilide and unhydrolyzed anilide) to obtain the pseudo-first-order rate constant k_{obsd}. Reaction rates were followed for 20–40 min, and the extent of hydrolysis was usually 2–5% of total anilide and never more than 10%.

Values of k_0 and slopes (k_2') were obtained by extrapolation of values of k_{obsd} over the buffer range 0.02–0.12 *M*, using linear least mean squares to fit the line. The data for k_n *vs.* concentration of imidazole were analyzed using the program HYPER[18] to get the values of $k_5 \pm$ se and $k_{-4}/k_4 \pm$ se as given in Table I.

(13) L. D. Kershner and R. L. Schowen, *J. Amer. Chem. Soc.*, **93**, 2014 (1971).

(14) (a) L. Polgar and M. L. Bender, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1335 (1969); (b) M. L. Bender and F. J. Kezdy, *Annu. Rev. Biochem.*, **34**, 49 (1965).

(15) (a) W. F. Sager and P. C. Parks, *J. Amer. Chem. Soc.*, **85**, 2678 (1963); (b) L. Parker and J. H. Wang, *J. Biol. Chem.*, **243**, 3729 (1968); (c) T. Inagami, A. Patchornik, and S. S. York, *J. Biochem. (Tokyo)*, **65**, 809 (1969); M. Caplow, *J. Amer. Chem. Soc.*, **91**, 3639 (1969).

(16) M. Philipp, R. M. Pollack, and M. L. Bender, *Proc. Nat. Acad. Sci. U. S.*, **70**, 517 (1973).

(17) C. D. Hubbard and J. F. Kirsch, *Biochemistry*, 11, 2483 (1972), have proposed that transient formation of an acylimidazole intermediate takes place during the acylation of α-chymotrypsin by some nonspecific substrates, substituted phenyl benzoates. While this hypothesis is neither confirmed nor refuted, the present work does preclude extension of their proposal to anilide substrates.

(18) W. W. Cleland, *Advan. Enzymol. Relat. Subj. Biochem.*, **29**, 1 (1967).