quately compared with the peptidase action of this enzyme until a complete kinetic study of all of the above-mentioned effects as a function of pH is performed for a peptide substrate. Furthermore, it would be extremely desirable to have more information about the nature of any intermediates formed during the catalytic process. On the basis of the unusual pH–activity behavior displayed by the ester substrate O-(N-benzoylglycyl)-L-3-phenyllactate[106] in comparison with the peptidase substrate N-(N-carbobenzyloxyglycyl)-L-phenylalanine, the proposal has been made that these two substrates are hydrolyzed by different mechanisms.[4,5,8] In particular it was suggested that some nucleophile, B, on the enzyme catalyzes peptide hydrolyses while hydroxide ion replaces B as a nucleophile in ester hydrolyses. The present results invalidate the basis for this proposal (at least as a general mechanistic hypothesis) since the pH dependences of k_{cat}, k_{cat}/K_m, and the activity of carboxypeptidase A toward O-acetyl-L-mandelate throughout the substrate concentration range are all qualitatively similar to the pH–activity curve found for N-(N-carbobenzyloxyglycyl)-L-phenylalanine. The qualitative differences between hydrolyses of O-acetyl-L-mandelate and of O-(N-benzoylglycyl)-L-3-phenyllactate could be due to different rate-determining steps in a common mechanism or to a variety of other causes, including some of the complicating factors mentioned above. It is our opinion that the data presently available are insufficient to make any firm distinctions between the mechanisms of peptide and ester hydrolyses catalyzed by carboxypeptidase A.

(106) Vallee and co-workers found that the pH–activity profile for the carboxypeptidase A catalyzed cleavage of this substrate rose between pH 5.5 and 7.0, exhibited a plateau between pH 7.0 and 9.0, rose again to a maximum at pH 10.5, and then declined;[4,5] these results were confirmed at other substrate concentrations in the region pH 7.65–9.70 by McClure, *et al.*;[8] in contrast, the pH–activity profile for N-(N-carbobenzyloxyglycyl)-L-phenylalanine was bell shaped, being approximately symmetrical about pH 7.5.[4,5,12]

Acknowledgment. The authors wish to thank Mr. H. Kuki for making the orthogonal polynomial curve-fitting program available from the library of the IBM 7094 Computation Center of the University of Chicago, and Mr. E. A. Peterson for his expert programming.

Amide Hydrolysis. Superimposed General Base Catalysis in the Cleavage of Anilides[1]

Richard L. Schowen and George W. Zuorick

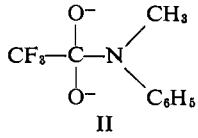
Contribution from the Department of Chemistry, University of Kansas, Lawrence, Kansas. *Received October 16, 1965*

Abstract: The base-catalyzed hydrolysis of 2,2,2-trifluoro-N-methylacetanilide (I) at pH 9.5–10 follows the kinetic law $-d[I]/dt = [I]\{k_0 + [HO^-](k_1 + \sum_i k_i[B_i])\}$ which corresponds to the superimposition of general base catalysis upon specific hydroxide ion catalysis. The results are in accord with a rate-determining elimination of N-methylaniline from an intermediate adduct or with a less likely general base catalyzed attack by hydroxide ion in a concerted displacement reaction.

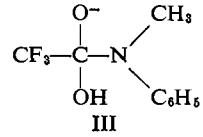
Biechler and Taft[2] and Bruylants and Kèzdy[3] have shown that 2,2,2-trifluoro-N-methylacetanilide (I) and other acyl-activated amides undergo hydrolysis according to the kinetic law of eq 1. A similar, but

$$-d[I]dt = [I](k_1[HO^-] + k_2[HO^-]^2) \quad (1)$$

much more complex, expression has recently been given by Mader[4] for 2,2,2-trifluoroacetanilide. Biechler and Taft[2] attributed the term second order in hydroxide ion to intermediacy of the dinegative ion II, but the data are equally consistent with the general base catalyzed decomposition of III, the k_1 term representing solvent catalysis and the k_2 term hydroxide ion catalysis. As part of a general investigation of catalysis in these and related systems, we have examined this system for general catalysis. Solvent isotope effects will be reported at a later time.



II



III

Results

Table I and Figure 1 show the dependence of the observed first-order rate constant for hydrolysis of I on sodium glycinate concentration in glycine–sodium glycinate buffers. The hydroxide ion dependences of the slopes and intercepts of these lines (Table II) allow determination of rate constants for the individual bases.

These data are consistent with eq 2, the values of the

$$-d[I]/dt = [I]\{k_0 + [HO^-](k_1 + \sum_i k_i[B_i])\} \quad (2)$$

(1) This research was supported by the National Institutes of Health under Research Grant No. GM-12477-01. For further details, see G. W. Zuorick, M.S. Thesis, University of Kansas, Aug 1965. A preliminary report of this work has appeared: R. L. Schowen and G. W. Zworick, *Tetrahedron Letters*, 3839 (1965).

(2) S. S. Biechler and R. W. Taft, Jr., *J. Am. Chem. Soc.*, **79**, 4927 (1957).

(3) A. Bruylants and F. Kèzdy, *Record Chem. Progr.*, **21**, 213 (1960).

(4) P. M. Mader, *J. Am. Chem. Soc.*, **87**, 3191 (1965).

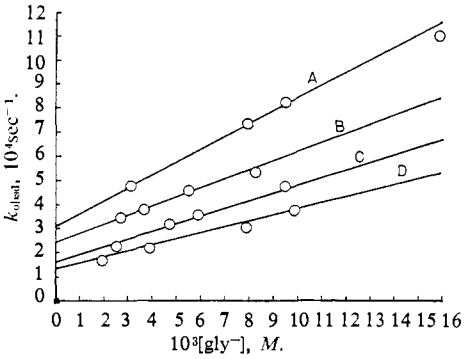


Figure 1. The hydrolysis of 2,2,2-trifluoro-N-methylacetanilide in glycine–sodium glycinate buffers at 25.5°. See Tables I and II.

rate constants, k_1, given in Table III. The summation is over all bases, B_i, in the solution.

Table I.[a,b] The Hydrolysis of 2,2,2-Trifluoro-N-methylacetanilide in Glycine–Sodium Glycinate Buffers at 25.50 ± 0.04°

[Gly], 10^3M	[Gly$^-$], 10^3M	R = [gly]/[gly$^-$]	[HO$^-$], 10^5M	k_{obsd}, 10^4 sec^{-1}
1.52	3.17	0.48	12.60	4.8
3.79	7.93	0.48	12.60	7.4
4.55	9.51	0.48	12.60	8.3
7.59	15.86	0.48	12.60	11.1
2.11	2.77	0.76	8.32	3.5
2.81	3.70	0.76	8.32	3.8
4.22	5.55	0.76	8.32	4.6
6.32	8.32	0.76	8.32	5.4
2.50	2.38	1.05	5.25	2.3
5.00	4.76	1.05	5.25	3.2
6.26	5.94	1.05	5.25	3.6
10.00	9.52	1.05	5.25	4.8
2.87	1.98	1.45	3.98	1.7
5.74	3.96	1.45	3.98	2.2
11.48	7.93	1.45	3.98	3.1
14.35	9.91	1.45	3.98	3.8

[a] The abbreviation gly$^-$ stands for glycinate ion. [b] Ionic strength brought to 0.0100 M with potassium chloride in all cases.

Table II. Slopes and Intercepts of the General Base Catalysis Lines as a Function of Hydroxide Ion Concentration

[HO$^-$], 10^5M	Slope, M^{-1} sec^{-1}	Intercept, sec^{-1}	Line[a]
12.60	5.2	31	A
8.32	3.8	24	B
5.25	3.1	16	C
3.98	2.5	14	D

[a] See Figure 1.

Discussion

These results exclude a mechanism having the unimolecular decomposition of II as the rate-determining step corresponding to the k_2 term of eq 1. We have tentatively excluded the rate-determining formation of II from III, a previously suggested[2] mechanism which would exhibit general base catalysis, on the grounds that an oxygen-to-oxygen proton transfer reaction[5] is unlikely to be slower than the decomposition of II to products.

(5) M. Eigen, W. Kruse, C. Maass, and L. DeMaeyer, *Progr. Reaction Kinetics*, **2**, 285 (1964).

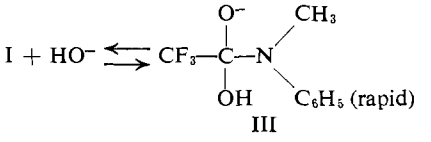
Table III. Rate Constants for Various Catalyzing Bases in the Hydrolysis of 2,2,2-Trifluoro-N-methylacetanilide at 25.5°

Catalyzing base	k_i	K_i,[b] M
...[a]	(5×10^{-5} sec^{-1})	...
H_2O	2.0 M^{-1} sec^{-1}/55.5 M	$10^{-15.7}$
Gly	1.9×10^2 M^{-2} sec.$^{-1}$	$10^{-11.7}$[c]
Gly$^-$	3.3×10^2 M^{-2} sec^{-1}	$10^{-4.2}$[c]
HO$^-$[d]	2×10^3 M^{-2} sec^{-1}	$10^{1.7}$

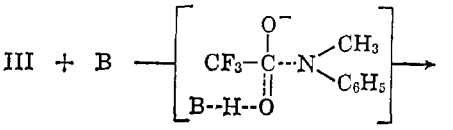
[a] We are uncertain of the origin of this small term. [b] These are equilibrium constants for the reaction $B_i + H_2O = B_iH^+ + HO^-$, corrected to units of M. [c] B. B. Owen, *J. Am. Chem. Soc.*, **56**, 24 (1934). [d] Determined by H. Jayaraman. Solvent isotope effects on this reaction will be discussed in a forthcoming paper by Schowen, Jayaraman and Kershner; this paper will present evidence excluding the third, concerted displacement mechanism mentioned in the text.

Equation 2 is consistent with three mechanisms in which general base catalysis is superimposed on an initial specific catalysis by hydroxide ion.[6] Mechanism A represents "classical general base catalysis" of the decomposition of III, while mechanism B ("inverse classical general base catalysis") corresponds to acid catalysis of the decomposition of the Biechler–Taft intermediate II by the conjugate acid of the general base. Mechanisms such as A and B are kinetically

Mechanism A



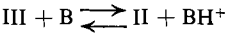
III

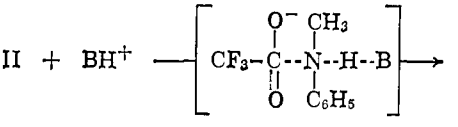


$BH^+ + CF_3CO_2^- + C_6H_5NCH_3^-$

$BH^+ + C_6H_5NCH_3^- \longrightarrow B + C_6H_5NHCH_3$ (rapid)

Mechanism B



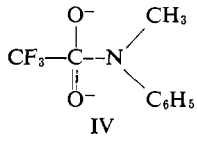


$B + CF_3CO_2^- + C_6H_5NHCH_3$

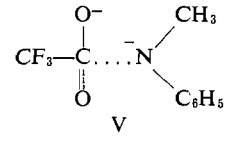
indistinguishable but may potentially be separated by the use of linear-free-energy data and the "reacting-bond" and "solvation" rules of Swain and his coworkers.[7,8] We are now engaged in this task. A third, less likely mechanism would consist of general base catalyzed attack by hydroxide ion in a concerted displacement of N-methylanilide ion (see footnote *d*, Table III). If the role of the BH moiety[8a] is merely to stabilize the remainder of the activated complex by "solvation," then the structure of this part of the activated complex will govern the choice of the system

(6) This is similar to the "catalysis of catalysis" found by J. F. Kirsch and W. P. Jencks, *J. Am. Chem. Soc.*, **86**, 833 (1964), for ester hydrolysis.
(7) C. G. Swain and E. R. Thornton, *ibid.*, **84**, 817 (1962).
(8) (a) C. G. Swain, D. A. Kuhn, and R. L. Schowen, *ibid.*, **87**, 1553 (1965); (b) C. G. Swain and J. Worosz, *Tetrahedron Letters*, 3199 (1965).

between mechanisms A and B. If the substrate moiety resembles the reactants of the rate-determining step (IV), BH will solvate the more basic, alkoxide-like oxygen rather than the amine-like nitrogen and mechanism A will prevail. If structure V, resembling



IV



V

products, is more nearly correct, then BH will solvate the amide-like nitrogen in preference to the carboxylate-like oxygen (mechanism B). The distinction of A and B therefore promises information on this partial structure of the activated complex.

Brønsted Catalysis Law. Figure 2 shows that the Brønsted catalysis law[9] (eq 3) holds approximately

$$\log k_i = \beta \log K_i + c \qquad (3)$$

for the bases water, glycinate ion, and hydroxide ion with $\beta \sim 0.3$. The point for glycine is surprisingly far above the line. Possible reasons for this apparently enhanced catalytic efficiency for glycine are (a) it functions as an acid catalyst as well as a base catalyst, and (b) it acts as a bifunctional catalyst. We hope that the matter will be elucidated by work now in progress in this laboratory.

Conclusions. Our results show either mechanism A or B to be the most likely route for basic hydrolysis of 2,2,2-trifluoro-N-methylacetanilide and we suspect that all amides for which kinetic terms second order in hydroxide ion have been detected are also subject to superimposed general base catalysis.

It is also our suspicion that this mechanism is of *general application* in amide hydrolysis. As was noted above, the second-order base terms are observed in acyl-activated amides, *i.e.*, those having an electron-withdrawing substituent on the carbonyl function. "Ordinary" amides thus have a relatively electron-releasing substituent at this position. Application of the reacting-bond rule of Swain and Thornton[7,10] leads to the prediction that "ordinary" amides will have both oxygen and nitrogen more basic in the transition state than acyl-activated amides. Then the solvation rule[8a] predicts that the proton involved in general catalysis will be bound more tightly to the substrate moiety (to oxygen for mechanism A or nitrogen for mechanism B) in the "ordinary" cases and β for the general catalysis will become small. The ratio k_2/k_1 thus will decrease and the second-order (k_2) term will become difficult to detect experimentally. This prediction appears to be confirmed by the data of Biechler and Taft[2] for trifluoro-N-methylacetanilide ($k_2/k_1 = 190$), difluoro-N-methylacetanilide ($k_2/k_1 = 34$), and chloro-N-methylacetanilide ($k_2/k_1 = 2$). Thus β decreases steadily with electron donation to the carbonyl function; the k_2 term will first disappear experimentally and finally, when $\beta = 0$, the mechanism merges into specific hydroxide ion catalysis.[11] It is tempting to speculate that proteolytic enzymes may function in part by electron supply or withdrawal at substrate carbonyl groups in order to adjust β to a value optimal for the particular acid–base combination present in the active site.

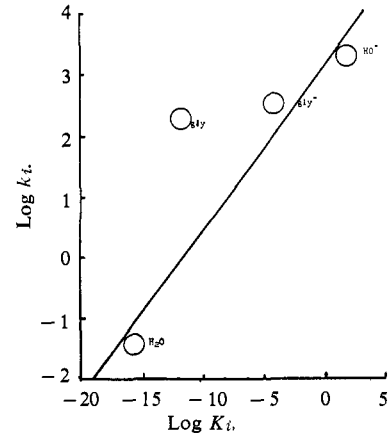


Figure 2. Brønsted plot for superimposed general base catalysis of the hydrolysis of 2,2,2-trifluoro-N-methylacetanilide.

A superimposed general base catalyzed cleavage of the carbon–carbon bond appears to us to be the most reasonable mechanism also for the hydrolysis of chloral hydrate[12] and perhaps of β-diketones[13] rather than the simple proton transfer proposed previously.[12,13]

Experimental Section[14]

Materials. Carbonate-free sodium hydroxide (Fisher certified) solutions were prepared and standardized as described by Day and Underwood.[15] Glycine (Matheson Coleman and Bell) and potassium chloride were dried at 110° before using. N-Methylaniline (Eastman) was distilled (bp 196° at 1 atm) using Pyrex glassware. Trifluoroacetic anhydride (Eastman) was used as supplied.

2,2,2-Trifluoro-N-methylacetanilide was prepared according to the method of Bourne and co-workers[16] in which trifluoroacetic anhydride and N-methylaniline were allowed to react in diethyl ether. After 2 hr, ice–water was added to precipitate the amide, which was recrystallized five times from petroleum ether (bp 30–60°), mp 25–26° (lit.[16] mp 26–27°).

Kinetics. A Cary Model 14 recording spectrophotometer with constant temperature cell holder (with Beckman or Pyrocell glass-stoppered 1-cm silica cells) was used. The temperature was controlled at 25.50 ± 0.04°. The rate of reaction was determined by measuring the change in absorbance at 285 mμ.

(9) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1961, p 218 ff.

(10) Or of the equivalent arguments given by C. G. Swain and R. L. Schowen, *J. Org. Chem.*, **30**, 615 (1965).

(11) Our value of k_2/k_1 (*cf.* Table III) is 1000 rather than 190; the discrepancy results from the incursion of more complex kinetics than was recognized by Biechler and Taft[2] at pH values above 10: H. Jayaraman, unpublished data. The phenomenon in question may well affect the other values of k_2/k_1.

(12) C. Gustafsson and M. Johanson, *Acta Chem. Scand.*, **2**, 42 (1948).

(13) R. G. Pearson and E. A. Mayerle, *J. Am. Chem. Soc.*, **73**, 926 (1951).

(14) Melting points and boiling points are uncorrected.

(15) R. A. Day, Jr., and A. L. Underwood, "Quantitative Analysis, Laboratory Manual," Prentice-Hall, Inc., Englewood Cliffs, N. J., 1958.

(16) E. J. Bourne, S. H. Henry, C. E. M. Tatlow, and J. C. Tatlow, *J. Chem. Soc.*, 4014 (1952).