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KINETICS OF THE HYDROLYSIS
OF N-TRIFLUOROACETYLAMINO ACIDS

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ABSTRACT

The order of reaction, rate constants, and activation energies for the alkaline hydrolysis of N-trifluoroacetyl glycine, -tryptophan, -phenylalanine, -norleucine, -valine, - ϵ -aminocaproic acid, and -lysine, were determined. The activation energies were found to be all in the same range. The reaction is second order for all compounds except for N-trifluoroacetyllysine. The hydrolysis of N-trifluoroacetyllysine was found to be complicated. A hypothesis for the mechanism of this reaction is suggested.

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It has been shown that N-Trifluoroacetyl amino acids are useful intermediates in the synthesis of peptides.¹ It therefore seemed of interest to study the kinetics of the alkaline hydrolysis of the protecting group and to investigate whether the group R in the molecule $R-CH(NHCOCF_3)-COOH$ has any influence on the rate or the mechanism of the hydrolysis.

EXPERIMENTAL

The N-trifluoroacetyl amino acids used in this study were prepared by E. Schallenberg.¹ An "automatic recording titrator," described by Neilands and Cannon,² was used to perform and follow the course of the hydrolysis.

Three ml of an aqueous solution of N-trifluoroacetyl amino acid of known concentration was placed in a thermally regulated cell (capacity about 6 ml). The cell was equipped with standard Beckman pH meter electrodes, stirrer, and gas inlet and outlet holes. The system was swept with CO_2 -free nitrogen and kept under a very slow stream of nitrogen during the course of the reaction. The solution was brought to the desired pH as quickly as possible by the addition of a known amount of base through the gas-inlet hole by means of a graduated pipette fitted with a syringe needle. After the titration motor had been turned on, the amount of base added from the syringe to the solution, necessary to maintain the given pH, was recorded against time; 0.1 N to 0.5 N KOH was used.

All the N-trifluoroacetyl amino acids used, except that of glycine, are insoluble in water. They were dissolved under cooling by addition of one equivalent of base.

RESULTS

N-Trifluoroacetyl glycine

The hydrolysis of this compound is a second-order reaction. This is shown by the following results.

(1) A plot of the logarithm of the fraction of N-trifluoroacetyl-glycine remaining unhydrolyzed $(1-f)$ vs time at a given pH is a straight line, as would be expected from the equation

$$-\frac{dc_A}{dt} = k [\text{OH}] c_A = k' c_A,$$

where c_A is the concentration of substrate at the time t , k is the rate constant, and k' the apparent rate constant. The diversion from linearity in the latter part of the hydrolysis seen in the examples shown in Figs. 1 and 2 is due to some uncertainties in $t = 0$ and $t = 100\%$ hydrolysis, which is inherent in the method.

(2) Comparisons of the plots of $\log(1-f)$ vs time for different initial concentrations of N-trifluoroacetyl-glycine show that the rate of hydrolysis is proportional to the first order of the concentration, since the slopes of the lines are the same (Fig. 3).

(3) Additions of sodium trifluoroacetate or glycine to the solution of N-trifluoroacetyl-glycine do not change the rate of hydrolysis (Fig. 3).

(4) A plot of $\log k'$ vs pH is a straight line with a slope of 1 at each temperature (Fig. 4), as expected from the equation

$$\log k' = \log k - 14 + \text{pH}.$$

(5) A plot of $\log k'$ vs $1/T$ is a straight line for a given pH (Fig. 5), as expected from the following equations:

$$\text{since } k = A e^{-E/RT},$$

$$\log k' = \log A - 0.4343 E/RT - 14 + \text{pH}$$

The activation energy E obtained from this plot is given in Table III, the relevant data for k' and k in Table I.

Other N-Trifluoroacetyl-amino Acids

The order of reaction, rate constants, and activation energies for N-trifluoroacetyl-valine, -norleucine, -phenylalanine, -tryptophan, and -aminocaproic acid were determined by the same method. The data for k' and k are given in Table II, the activation energies in Table III.

In all these cases the hydrolysis is a second-order reaction. The pH dependence, straight lines with a slope of one, is shown in Fig. 6; the temperature dependence, straight lines for a given pH, is shown in Fig. 5.

The apparent rate constants at a given pH and temperature were not closely reproducible, for as yet unknown reasons. Fatigue of the glass electrode at high pH, small amounts of contaminating CO_2 , and a slow drift

Fig. 1. Hydrolysis of TFA-glycine, 0.01 mM/cc, at 40°C .

Fig. 2. Hydrolysis of TFA- glycine, 0.01 mM/cc, at 30° and 20°C.

Fig. 3. Hydrolysis of TFA-glycine at 40°C, different initial concentrations.

Fig. 4. Hydrolysis of TFA-glycine: pH dependence.

Fig. 5. Hydrolysis of TFA-amino acids: temperature dependence.

Table I

Hydrolysis of N-Trifluoroacetyl-glycine				
t	C _o	pH	k' x 10 ⁻⁴	k
40°	0.01	10.23	1.62	0.952
	"	10.51	2.86	0.881
	"	10.58	5.22	1.37
	"	11.01	12.0	1.17
	"	11.50	39.7	1.25
	"	11.53	46.1	1.36
30°	"	10.65	1.6	0.36
	"	10.99	3.73	0.39
	"	11.51	10.2	0.31
	"	12.0	40.6	0.40
20°	"	11.0	1.14	0.114
	"	11.51	4.1	0.23
	"	11.87	11.0	0.15
40°	0.01	11.02	12.5	1.24
	0.02		12.1	1.21
40°	0.01	11.07	12.6	1.22
40°	0.01	11.09	16.6	1.59
	0.01 + 0.01 glycine	11.09	16.2	1.55
42	0.01	11.09	22.9	2.19
	0.01 + 0.01 Na TFA	11.09	26.8	2.56

t = °C

C_o = Initial concentration of N-trifluoroacetyl-glycine in mM/cc, total of 3 cck' = apparent rate constant = slope of curve of log (1-f) vs time x $\frac{2.303}{60}$ k = absolute rate constant = k' / [OH]⁻¹ mol⁻¹ sec⁻¹.

Table II

Hydrolysis of N-Trifluoroacetyl amino Acids					
TFA-	t	C _o	pH	k' x 10 ⁻⁴	k
Valine	40°	0.01	11.39	1.59	0.0647
		"	11.45	2.41	0.0858
		0.007	11.45	12.1	0.429
		0.01	11.47	15.8 ^a	0.536
		"	11.47	52.7	1.78
		"	11.62	3.74 ^a	0.0900
		0.02	11.70	2.89	0.0579
		0.01	11.70	54.1	3.60
		"	11.81	31.9	0.494
		"	11.56 ^b	9.59 ^b	0.265
	30°	0.01	11.33	1.00	0.0466
		"	11.71	2.41	0.0469
		"	11.56 ^c	1.70 ^c	0.0469
Norleucine	50°	0.003	11.11	12.6	0.978
		0.007	11.66	34.0 ^a	0.742
		"	11.77	20.2	0.342
		"	11.77	115.0	1.96
		"	11.77	48.6 ^b	0.828
		0.01	11.91	66.1	0.832
		"	11.54 ^b	28.4 ^b	0.820
	40°	0.01	11.54	8.7	0.251
		"	11.92	16.2	0.194
		"	11.54 ^d	6.79 ^d	0.196
		"	11.54 ^b	7.69 ^d	0.221
Phenyl- alanine	50°	0.01	11.47	4.51	0.153
		"	11.48	3.20	0.106
		"	11.50	4.21	0.133
		"	11.47 ^b	4.09 ^b	0.137
	40°	0.02	11.47	1.26	0.0427
		0.003	11.47	1.38	0.0469
		"	11.47	1.47	0.0498

Table II (continued)

		0.01	11.48	1.72	0.0570
		"	11.47 ^b	1.46	0.049
Tryptophan	50°	0.01	11.08	2.71	0.226
		"	11.19	1.44	0.0929
		"	11.19	2.82	0.182
		"	11.15 ^b	2.23 ^b	0.158
		"	11.69	7.52	0.154
	40°	"	11.42 ^e	4.06	0.154
		"	11.42	1.06	0.04
		"	11.42	0.75 ^f	0.028
		"	11.42	0.89 ^b	0.034
		"	11.69	1.42	0.039
Lysine	50°	0.01	11.47	69.1	
	40°	0.01	11.19	15.1	
		"	11.47	19.3	
		"	11.80	26.4	
	30°	"	11.24	5.19	
		"	11.47	6.45	
		"	11.80	8.42	
ε-Aminocaproic acid	40°	0.01	10.77	6.01	1.06
		"	11.26	14.2	0.78
		"	11.35	17.4	0.78
	50°	0.01	10.65	31.6	7.0
		"	10.76	24.1	4.2
		"	10.97	35.2	3.8
		"	11.07	41.2	3.5
		0.0053	11.09	32.6	2.65

a: Average slope of the initial and later parts of the curve

b: Average value of all values given above

c: Estimated from the curve between the two experimental points above

d: Estimated from the data at pH 11.92

e: Estimated from the data at pH 11.15 and 11.69

f: Estimated from the data at pH 11.69

Table III

Activation Energies of the Hydrolysis of N-Trifluoroacetylmino Acids

N-Trifluoroac- etylmino Acid	E (kcal/deg/mole)	Most Probable Error*(50% Sig- nificant)kcal	2 x Standard Deviation of the Mean* (95% Significant) kcal
Glycine	21.0	0.54	11.6
Tryptophan	30.4	4.77	14.0
Norleucine	23.1	5.5	16.3
Phenylalanine	20.8	3.0	8.9
Valine	31.2	10.2	30.2
Lysine	21.1	0.54	1.6
ϵ -Aminocaproic acid	31.8	1.68	5.0

* These values were calculated according to Reference 4.

of the zero-point setting of the pH meter may be contributing factors. The consequence of this spread in k' values is a large error in the activation energies, as shown in the table.

N-Trifluoroacetyllysine

Hydrolysis of this compound is first-order in respect to N-trifluoroacetyllysine, as shown by a straight-line plot of $\log (1-f)$ vs time (Fig. 7). It is of an apparent order less than one in respect to hydroxylion. This is shown by the fact that the straight-line plot of $\log k'$ vs pH has a slope of less than one for each temperature (Fig. 8). The over-all reaction has an activation energy very similar to the activation energies of the other N-trifluoroacetylmino acids investigated. (Table II).

One possible mechanism of the hydrolysis that would account for these results can be expressed as follows:

Fig. 6. Hydrolysis of TFA-amino acids: pH dependence.

Fig. 7. Hydrolysis of TFA-lysine, 0.01 mM/cc, at 30° and 40°C.

Fig. 8. Hydrolysis of TFA-lysine, pH dependence.

It can be shown that where $k_a \gg k_b$, the slope of a plot of $\log k'$ vs pH would be less than one. The assumption of the presence of about 10% of BAH^+ in the pH range used in this study is reasonable in view of the measured pK value of about 9.8.

Hydrolysis of N-Trifluoroacetyl amino Acids in Ethanol

A series of hydrolyses was performed in alcoholic solutions of some of the N-trifluoroacetyl amino acids. The solvent was 40% ethanol, the base was 0.1N potassium hydroxide in 40% ethanol. Some representative examples of the apparent rate constants are shown in Table IV. The hydrolyses proceeded much more slowly than in aqueous solution. The higher the pH the greater the difference in the apparent rate constants.

The mechanism of the hydrolysis seems to be complicated in the presence of ethanol. The plots of $\log (1-5)$ vs time do not show a good linearity beyond about 30% hydrolysis; neither do the plots of $\log k'$ vs pH. It therefore seems that there are other reactions besides hydrolysis going on. Esterifications and alcoholyses are two possibilities.

Table IV

Hydrolysis of N-Trifluoroacetyl amino Acids in Aqueous and Alcoholic Solution*					
TFA-	t	pH	$k' \times 10^{-4}$ in aqueous solution	$k' \times 10^{-4}$ in alcoholic solution	$\frac{k' \times 10^{-4} \text{ aq.}}{k' \times 10^{-4} \text{ alc.}}$
Glycine	40	10.50	2.86	0.90	3.1
		11.02	12.0	2.05	6.0
		11.50	39.7	6.45	6.1
	30	11.0	3.73	0.51	7.4
		11.62	14.9	1.5	10.0
	20	11.07	1.14	0.38	3.0
Phenyl- alanine	40	11.60	0.72	5.2	7.2
		11.50	1.50	0.78	1.9
		11.90	4.80	1.29	3.7

*The alcoholic solution was 40% ethanol

DISCUSSION

In order to answer the question of the influence of the R-group of $R-CH(NHCOCF_3)-COOH$ on the rate of the hydrolysis of the $-NHCOCF_3$ group, the data for the activation energies have to be compared. The "most probable error" of E is not reliable for this purpose because it gives only the range into which 50% of the values will fall. The term "2 x standard deviation of the mean" gives the range into which 95% of the values will fall. Both sets of data are given in Table III. A comparison of these figures shows that the activation energies for the hydrolysis of the seven N-trifluoroacetyl-amino acids investigated are in the same range, and no influence of the R-group can be demonstrated.

It is of interest that the activation energy for the hydrolysis of the amide group of L-asparagine ($E = 20.55$ kcal/mole) and for L-asparagyl-glycine ($E = 19.22$ kcal/mole)³ is in the same range as the activation energy for the hydrolysis of the trifluoroacetyl group described here.

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