CHEMISTRY

THE HYDROLYSIS AND AMINOLYSIS OF ETHYL THIO-ACETATE. I. KINETIC ANALYSIS OF THE ALKALINE HYDROLYSIS

BY

J. Th. G. OVERBEEK and V. V. KONINGSBERGER

(Van 't Hoff Laboratory, University of Utrecht)

(Communicated at the meeting of April 24, 1954)

1. *Introduction*

In a preceding paper [1] concerning the rôle of nucleic acids in the biosynthesis of the peptide bond an acylating interaction of enzyme systems involving coenzyme A (CoA) or similar—SH compounds has been assumed. Recently analogous actions of acyl CoA [2–6] and acyl glutathione [6] derivatives were made probable, but the data on the kinetics of some simpler reactions of the same type, such as the hydrolysis and the amino-lysis of thiolesters are still contradictory [7–12].

Preliminary results of our investigations on the reaction kinetics of the hydrolysis and the aminolysis of ethyl thioacetate (E.T.A.) have been communicated previously [12]; we now present our completed data on the alkaline hydrolysis.

2. *Experimental part*

As it was described in an earlier paper [12] all our measurements of reaction velocities have been carried out at a temperature of 37° C., the reacting compounds being dissolved in 0.2 m boric acid—0.05 m borate buffers. Furthermore, the experiments were performed in nitrogen atmosphere in order to exclude oxygen [12]. Before the experiments E.T.A. was freshly distilled from K_2CO_3 also under exclusion of oxygen. The amount of E.T.A. in samples of the reaction mixture was determined according to the method of LIPMANN and TUTTLE [13], which had been rigorously standardized in order to obtain reproducible results.

The sample was mixed with a 2 m hydroxylamine solution at pH 6 and was kept 30 minutes at 37° C, the formation of acetyl hydrozamic acid being completed after 5–15 minutes. The colour, due to the ferric acetyl hydroxamate faded slowly, which made it necessary to measure extinctions at well determined times (30 minutes) after the addition of a 1.66 % $FeCl_3$ solution in 1 N HCl. These measurements were carried out at an average wavelength of 530 mμ by means of an "Engel" colorimeter.

From the extinctions measured the percentages of E.T.A. left after increasing times of incubation were calculated. First order rate constants

were determined by plotting the logarithm of the concentration of E.T.A. left against the time of incubation; second order rate constants were calculated from curves in which the reciprocal value of the concentration of E.T.A. left was plotted against the time of incubation.

The pH of the reaction mixture was determined by means of a "Philips pH-meter G.M. 4491" preferably with the liquid and the glass- and calomel electrodes at a temperature of 37° C. The apparatus was adjusted with the aid of a temperature-independent "Electrofact" standard buffer solution (pH 7) before every experiment. Some pH data obtained at a lower temperature (29° C) were corrected to 37° C by means of an empirical correction.

From the obtained pH data the OH⁻ion activities were calculated with K_{water} at 37° C $= 2,4 \times 10^{-14}$. These OH⁻ion activities were approximately converted into OH⁻ion concentrations with the use of the ionic activity functions $\dfrac{\gamma_H \gamma_{OH}}{a_{H_2O}}$ [14] of water in NaCl-solutions according to:

$$(1) \qquad [OH] = \frac{2,4 \times 10^{-14}}{\text{antilog}\,(-pH)} \frac{1}{\sqrt{\gamma_H \gamma_{OH}/a_{H_2O}}} .$$

Concentrations are indicated by symbols between square brackets.

3. *Hydrolysis*

The results of our measurements dealing with the alkaline hydrolysis of E.T.A. are summarized in table 1.

TABLE 1

Hydrolysis at 37° C of 0.0025 m and 0.00125 (exp. 44, 45) m E.T.A. solutions at various pH. In exp. 1, 4, 19 and 46 borate buffers were used. In exp. 43, 44 and 45 the high pH was obtained by using solutions of NaOH. In these experiments [OH] was calculated from the composition of the mixtures. The pH was calculated from: $pH = pK_{water} + \log f \pm [OH]$. In exp. 43 and 44 the concentrations of E.T.A. and OH⁻ions were equal.

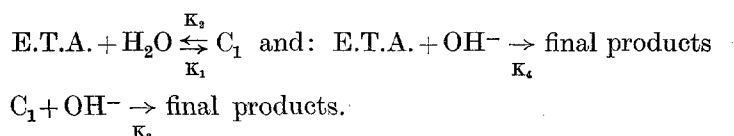| Exp. No.	pH	$[OH] \times 10^9$ (mol. ml.⁻¹)	$K_{H \cdot obs} \times 10^6$ (sec.⁻¹)	$\{K_{H \cdot obs}/[OH]\} \times 10^{-2}$ (ml. mol.⁻¹ sec.⁻¹)
1	8,34	6,6	8,3	12,5
4	8,68	14,9	16,5	11,1
46	9,01	32,0	30,6	9,6
19	9,56	114	61,6	5,4
44	10,71	1250	—	1,9
43	11,01	2500	—	1,7
45	11,92	24.000	3610	1,5

At all pH's investigated the rate of alkaline hydrolysis of E.T.A. was first order with respect to the concentration of E.T.A. Up to a pH of about 8,7 the rate is also proportional to the concentration of OH⁻ions. Above this pH the second order rate constant begins to decrease rapidly. However, at very high pH, when the rate constant has diminished to about

10 % of its original value, the rate of hydrolysis again is almost exactly first order with respect to the concentrations of both E.T.A. and OH⁻ions.

This phenomenon could only be explained by the assumption that the alkaline hydrolysis of E.T.A. occurs by two different pathways. One of these, prevailing at a relatively low pH-range, involves a stepwise mechanism as was assumed before [12]; the other one, involving a rate of hydrolysis which is first order with respect to the OH⁻ion concentration over the whole pH-range, determines the velocity of hydrolysis for the greater part at high pH.

The first step of the stepwise mechanism is either a monomolecular activation of E.T.A., or, more probably a reaction between E.T.A. and water. A reaction scheme of the following type is assumed:

$$\text{E.T.A.} + \text{H}_2\text{O} \underset{K_1}{\overset{K_2}{\rightleftharpoons}} C_1 \quad \text{and:} \quad \text{E.T.A.} + \text{OH}^- \overset{K_4}{\rightarrow} \text{final products}$$

$$C_1 + \text{OH}^- \underset{K_3}{\rightarrow} \text{final products.}$$

A reaction kinetic analysis of this scheme gives the following equation for the disappearance of E.T.A. with the time of incubation [1]).

$$
(2) \quad
\begin{cases}
V_H = \text{velocity of hydrolysis} = \\
\quad = -\dfrac{d[\text{E.T.A.}]}{dt} = K_1' \dfrac{K_3}{K_2}\,[\text{E.T.A.}]\,[\text{OH}]\dfrac{1}{1+(K_3/K_2)[\text{OH}]} + \\
\qquad\qquad\qquad\qquad\qquad + K_4\,[\text{E.T.A.}]\,[\text{OH}]
\end{cases}
$$

with $K_1' = K_1[\text{H}_2\text{O}]$.

When the observed hydrolysis constant is defined as:

$$K_{H \cdot \text{obs}} = \frac{V_H}{[\text{E.T.A.}]}$$

it follows from (2) that $K_{H \cdot \text{obs}}$ should be a pseudo first order rate constant if the reaction velocity measurements are carried out in buffered solutions or in the presence of an excess of OH⁻ions for:

$$(3) \qquad K_{H \cdot \text{obs}} = [\text{OH}]\left\{ K_1' \frac{K_3}{K_2} \cdot \frac{1}{1+(K_3/K_2)[\text{OH}]} + K_4 \right\} = \text{constant.}$$

For very low concentrations of OH⁻ions equation (3) may be written as:

$$\lim_{[\text{OH}]\to 0} K_{H \cdot \text{obs}} = [\text{OH}]\left\{ K_1' \frac{K_3}{K_2} + K_4 \right\}$$

or:

$$(4) \qquad\qquad \lim \frac{K_{H \cdot \text{obs}}}{[\text{OH}]} = K_1' \frac{K_3}{K_2} + K_4 .$$

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[1]) For the derivation of this equation: See appendix.

For very high OH⁻ion concentrations, however, eq. (3) can be given as:

$$\lim_{[OH]\to\infty} K_{H\cdot obs} = K_1' + K_4\,[OH]$$

or:

(5)
$$\lim \frac{K_{H\cdot obs}}{[OH]} = K_1'\,\frac{1}{[OH]} + K_4$$

K_1' and K_4 are determined by plotting $\dfrac{K_{H\cdot obs}}{[OH]}$ against $\dfrac{1}{[OH]}$ (eq. 5) and extrapolating to $\dfrac{1}{[OH]} = 0$.

By plotting $\dfrac{K_{H\cdot obs}}{[OH]}$ against $[OH]$ (eq. 4) and extrapolating to $[OH]=0$

$K_1'\,\dfrac{K_3}{K_2} + K_4$ can be determined; as K_1' and K_4 are already known $\dfrac{K_3}{K_2}$ can be calculated.

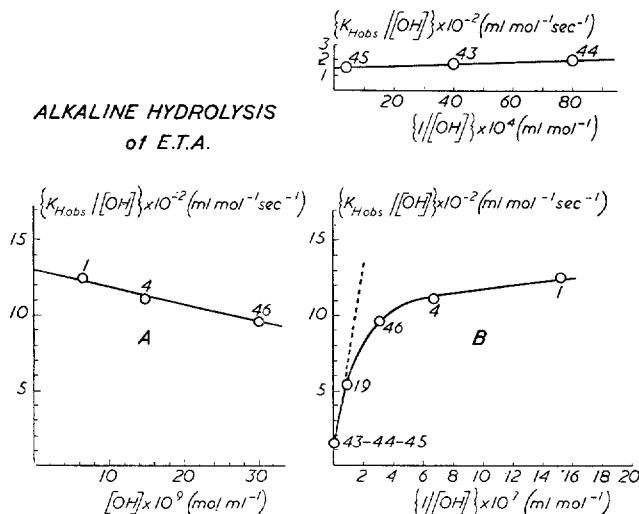Fig. 1A and B and table 2 are illustrations of the procedure outlined above.

Fig. 1.   Alkaline hydrolysis at 37° C of E.T.A.:

A: $\dfrac{K_{H\cdot obs}}{[OH]}$ plotted against $[OH]$ at low $[OH]$

B: $\dfrac{K_{H\cdot obs}}{[OH]}$ plotted against $\dfrac{1}{[OH]}$ with two different scales of $\dfrac{1}{[OH]}$.

TABLE 2

Alkaline hydrolysis of E.T.A.: rate constants determined according to the equations (4) and (5)

$K_1' = 6 \times 10^{-5}$ sec.$^{-1}$.
$K_4 = 1,5 \times 10^2$ ml. mol.$^{-1}$ sec.$^{-1}$.
$K_4 + K_1'\,(K_3/K_2) = 13 \times 10^2$ ml. mol.$^{-1}$ sec.$^{-1}$.
$K_3/K_2 = 1,9 \times 10^7$ ml. mol.$^{-1}$.

These figures will be discussed in the following paper of this series.

The authors wish to thank the students E. M. Duyvis, P. W. Wiersema, W. P. J. D. van der Drift, P. W. Hendrikse and A. H. Wagenaar for their help in carrying out the measurements.

4. Appendix

Derivation of equation (2) for the velocity of hydrolysis of E.T.A.:

According to the proposed reaction scheme the reaction velocity can be written as:

$$(2a) \quad -\frac{d[E.T.A.]}{dt} = K_1[E.T.A.][H_2O] + K_4[E.T.A.][OH] - K_2[C_1].$$

Assuming the existence of a stationary state with respect to C_1 we may write:

$$\frac{d[C_1]}{dt} = 0 = K_1[E.T.A.][H_2O] - K_2[C_1] - K_3[C_1][OH]$$

or:

$$(2b) \qquad [C_1] = \frac{K_1[E.T.A.][H_2O]}{K_2 + K_3[OH]}.$$

After substitution of $[C_1]$ eq. 2a is written as:

$$-\frac{d[E.T.A.]}{dt} = K_1[E.T.A.][H_2O] + K_4[E.T.A.][OH] - K_2\frac{K_1[E.T.A.][H_2O]}{K_2 + K_3[OH]}$$

$$= K_1[E.T.A.][H_2O]\frac{K_3[OH]}{K_2 + K_3[OH]} + K_4[E.T.A.][OH].$$

By substituting $K_1' = K_1[H_2O]$ and rearranging we obtain:

$$(2) \quad \left\{ V_H = -\frac{d[E.T.A.]}{dt} = K_1'\frac{K_3}{K_2}[E.T.A.][OH]\frac{1}{1 + (K_3/K_2)[OH]} + \right.$$
$$+ K_4[E.T.A.][OH].$$

5. Summary

1. Reaction velocities of the alkaline hydrolysis of ethyl thioacetate (E.T.A.) have been measured at 37° C.

2. The rate of the alkaline hydrolysis of E.T.A. proved to be first order in E.T.A. and to increase less than proportionally with the concentration of OH⁻ions.

3. The data obtained are explained by assuming two different pathways for the hydrolysis; one involves an attack of the E.T.A. molecules by OH⁻ions, the other one involves a similar attack but on hydrated E.T.A. molecules.

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