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CHEMISTRY

THE HYDROLYSIS AND AMINOLYSIS OF ETHYLTHIOACETATE

BY

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1. *Introduction*

A direct reaction between acylated coenzyme A (Co A) or similar acylated –SH compounds and amines or amino groups has been frequently suggested in order to explain the biosynthesis of the peptide bond. There is a wealth of recent literature on the hydrolysis and aminolysis of acyl Co A compounds and similar thiol esters, but the kinetic data on these fundamental processes are rather contradictory [1–6].

In this preliminary paper some results are presented of our investigation on the reaction kinetics of the hydrolysis of ethyl thioacetate (E.T.A.) and the aminolysis of this thiol ester by glycine, E.T.A. being an uncomplicated model compound for acylated Co A and glycine the most simple amino acid.

All our measurements of reaction velocities have been carried out at a temperature of 37° C, the reacting compounds being dissolved in borate buffers. E.T.A. was freshly distilled from K_2CO_3 before the experiments. During the reaction the amount of E.T.A. in samples of the reaction mixture was determined by reaction with hydroxylamine and ferric chloride according to the method of LIPMANN and TUTTLE [6]. The manipulations were substantially the same as those described for the determination of phenyl benzoyl phosphate in an earlier paper [7].

As the colour, due to the ferric acetyl hydroxamate faded slowly, rigorous standardizing is required in order to obtain reproducible results.

2. *Hydrolysis*

The rate of hydrolysis appeared to be first order with respect to the concentrations of both E.T.A. and OH^- ions up to a pH of about 8.7. This finding is in agreement with the data of HAWKINS and TARBELL [3] but it is contrary to the results of the work of SCHAEFGEN [1] and to that of RYLANDER and TARBELL [2] on the hydrolysis of E.T.A. in aqueous acetone.

Consequently the velocity of hydrolysis of E.T.A. in slightly alkaline aqueous solutions can be given as:

$$v_H = K_H\ [\text{E.T.A.}]\ [OH^-] \tag{1}$$

or

$$v_H = K_{H\ obs.}\ [E.T.A.] \tag{2}$$

with the observed pseudo first order hydrolysis constant

$$K_{H\ obs.} = K_H\ [OH^-] \tag{3}$$

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

TABLE 1

Hydrolysis at 37° C of a solution of 0.0025 m E.T.A. in 0.2 molar borate buffers of various pH. For the calculations of $[OH^-]$ K_{water} at 37° C was taken as 2.4×10^{-14}.

Exp. No.	pH	OH^- (mol. ml^{-1})	$K_{H\ obs.} \times 10^6$ ($sec.^{-1}$)	$K_H \times 10^{-2}$ ($ml.mol.^{-1}sec.^{-1}$)
1	8.33	5.13×10^{-9}	8.1	15.8
4	8.71	1.23×10^{-8}	16.5	13.4
16	8.92	2.00×10^{-8}	22.9	11.5
19	9.67	1.12×10^{-7}	61.4	5.5

From these data it can be seen that the reaction involving OH^- ions seems to cease from being the rate controlling one at $pH > 8.7$.

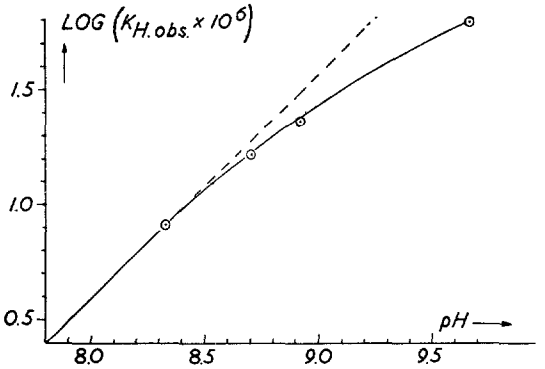

Fig. 1. Observed pseudo first order hydrolysis constants plotted against the pH. The experimental conditions are described in table 1. The dotted line is the theoretical one, derived according to equation (3) with $K_H = 15.8 \times 10^2$ ml. $mol.^{-1}$ $sec.^{-1}$

Obviously, a stepwise mechanism is involved and a reaction scheme of the following general type might be assumed:

$$\text{E.T.A.} + H_2O \leftrightarrows C_1$$
$$C_1 + OH^- \leftrightarrows C_2$$
$$C_2 \rightarrow \text{final products.}$$

Similar schemes have been rejected by SCHAEFGEN [1] but accepted by NODA c.s. [5] the sequence of the first two reactions being also still open to discussion. In our opinion the second or third step could be rate controlling at low pH whereas at higher pH the first step would become rate controlling, causing the reaction to be independent of pH.

3. *Influence of oxygen on the breakdown of E.T.A.*

We found an overwhelming effect of oxygen on the breakdown of E.T.A. in aqueous solutions. During all measurements of reactions with low velocity, the reaction rate began to increase considerably after four or more hours of incubation. This is not a case of simple autocatalysis, as addition of acetate or ethyl mercaptane had no effect at all on the reaction velocity. When, however, a stream of oxygen was bubbled slowly through the solution during a hydrolysis experiment the effect was striking as is illustrated in fig. 2 where the course of the concentration of E.T.A. is plotted against the time of incubation during similar hydrolysis experiments in the presence and absence of oxygen.

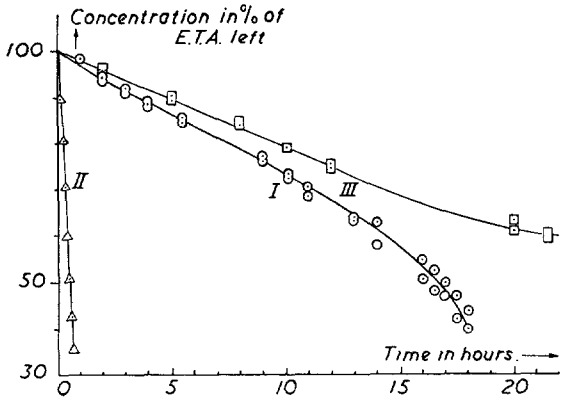

Fig. 2. Course of the concentration of E.T.A. with the time of incubation during the hydrolysis of 0.0025 m E.T.A. solutions in borate buffer at pH 8.3. I. Under normal conditions without complete exclusion of oxygen. II. When oxygen is bubbled slowly through the solution. III. In deaerated buffer under nitrogen atmosphere.

Further investigation on this phenomenon is still in progress.

4. *Isolation of acetyl glycine*

A check through literature failed to reveal satisfactory data about the isolation of compounds obtained by aminolysis of E.T.A. Furthermore, the statement by SCHWYZER [4] that E.T.A. would not yield any aminolysis products at all made it quite desirable to try to isolate acetyl glycine in a reasonable yield after aminolysis of E.T.A. by glycine. To this end 3 ml of freshly distilled E.T.A. was added to 70 ml of a 1 molar glycine solution in deaerated borate buffer at pH 8.4. The reaction mixture was stirred vigorously and kept at a temperature of 37° C under nitrogen atmosphere for 12 hours. During this time the pH was measured every thirty minutes and kept between 8.3 and 8.6 by addition of a solution of 10 N NaOH. Then the reaction mixture was brought to pH 5.2 by addition of a few drops of 5 N sulphuric acid and evaporated to dryness in vacuum at a temperature of about 35° C. The crystalline residue was redissolved

in 20 ml of distilled water, brought to pH 3 by addition of 5 N sulphuric acid solution and put in the refrigerator overnight. The next morning 1.2 gr. of precipitate (M.P. 204° C) was filtered off, this quantity of crude acetyl glycine being sufficient for our purpose. After four recrystallizations from hot water the white crystalline compound had a constant melting point of 208° C (corr.) (Lit.: 207–208° C for acetyl glycine [8]).

Micro-analysis [1]):

	Calc.	Found
C	41.03	41.54
H	6.00	6.02
N	11.97	11.76

Our kinetic data on the simultaneous hydrolysis and aminolysis of E.T.A. in alkaline aqueous solutions are rather complicated. As the measurements are still continued these data will be published later. The authors wish to thank Dr. C. A. SALEMINK for providing the E.T.A. and Mr. E. M. DUYVIS and Mr. P. H. WIERSEMA for carrying out the reaction velocity measurements.

5. *Summary*

The rate of the alkaline hydrolysis of ethylthioacetate (E.T.A.) has been determined and proved to be of first order in E.T.A. and of an order varying between one and zero in the OH^- ions. Oxygen has been shown to lead to rapid decomposition of E.T.A. Acetyl glycine has been isolated in good yield and high purity from the reaction between E.T.A. and glycine at pH 8.4.

[1]) The micro-analysis was carried out at the Organic-Chemical Institute T.N.O., Utrecht.

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