

## KINETICS OF HYDROLYSIS OF ACETYLTHTIOCHOLINE BY OXIMES

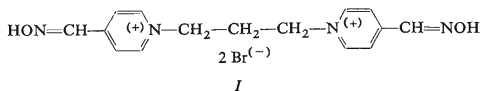
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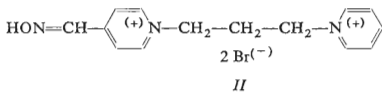
Received February 22nd, 1973

The effect of pH and temperature on the hydrolysis of acetylthiocholine by 1,3-bis(4-pyridiniumalldoxime)propane dibromide and by 1-(4-pyridiniumalldoxime)-3-pyridiniumpropane dibromide was studied. The kinetics of the hydrolysis of acetylthiocholine by both oximes was characterized by kinetic constants. Some thermodynamic parameters of these reactions were also determined.

It has been known from literature that esters and thio esters of alcohols of acidic character are hydrolyzed by oximes. The reaction is catalyzed by a weakly alkaline medium. The ester is decomposed during the reaction to the free alcohol and the acylated oxime<sup>1</sup>. An effect similar to that of oximes also show cysteine and imidazole<sup>2</sup>. In view of the role of certain oximes in the therapy of poisoning by organophosphorus compounds<sup>3-5</sup>, considerable attention has been focused on problems of interaction of oximes with esters. The hydrolysis of acetylthiocholine by 2-PAM, 4-PAM, and DAM\* has been recorded in the literature. The kinetic constants of the reaction of several thioesters with 2-PAM were determined by O'Neill and coworkers<sup>6</sup>. The hydrolysis of butyrylthiocholine<sup>7,8</sup>,  $\alpha$ -naphthyl acetate<sup>7,8</sup>, *o*-nitrophenyl butyrate<sup>7,9</sup>, and indoxyl acetate<sup>7</sup> has also been investigated. Another compound whose effect has been examined and described in literature in addition to that of 2-PAM, is obidoxime<sup>7-10</sup>. In this study the hydrolysis of acetylthiocholine by 1,3-bis(4-pyridiniumalldoxime)propane dibromide (trimedoxime, *I*) and by 1-(4-pyridiniumalldoxime)-3-pyridiniumpropane dibromide (*II*), and the effect of pH and temperature on this hydrolysis were studied.



\* *Abbreviations used:* 2-PAM, 2-pyridiniumalldoxime methiodide; 4-PAM, 4-pyridiniumalldoxime methiodide; DAM, diacetylmonoxime; obidoxime, 1,3-bis(4-pyridiniumalldoxime)-2-oxapropane dichloride; trimedoxime, 1,3-bis(4-pyridiniumalldoxime)propane dibromide



## EXPERIMENTAL

**Material.** 1,3-Bis(4-pyridiniumaldoxime)propane dibromide (I) was prepared by quarterization of pyridine-4-aldoxime by 1,3-dibromopropane<sup>11</sup> and its m.p. was 241°C (value recorded in the literature<sup>11</sup> 238–241°C),  $pK_{a1}$  7.70,  $pK_{a2}$  8.77. 1-(4-Pyridiniumaldoxime)-3-pyridiniumpropane dibromide (II) was prepared by the procedure described earlier<sup>12</sup> and its m.p. was 242°C (value recorded in the literature<sup>13</sup> 212°C),  $pK_a$  8.17. Acetylthiocholine iodide was a product of the Research Institute for Pure Chemicals, Brno. 5,5'-Dithio-bis(2-nitrobenzoic) acid (DTNB, Ellman reagent) was from Serva, Heidelberg. The remaining chemicals used were of G. R. purity grade (Lachema, Brno).

The hydrolysis of acetylthiocholine by both oximes was carried out in 0.05M Tris-HCl buffer (pH 7.2, 7.6 and 8.0) in the presence of DTNB. The reaction of the latter with the arising thiocholine gave a colored complex whose absorbance at 412 nm was measured in Vitatron photocolormeter (Sci. Inst., Holland) in 1 cm glass cells. The cells were temperature-controlled at  $\pm 0.5^\circ\text{C}$  during the measurement by a Höppler ultrathermostat. The reaction mixture in the cell contained 1.2 ml of Tris-HCl buffer, 0.2 ml of the buffered oxime solution, and 0.4 ml of 5 mM DTNB. The reaction was initiated by the addition of 0.2 ml of 0.5 mM acetylthiocholine iodide

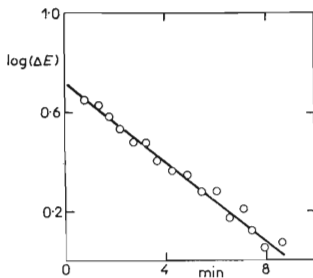


FIG. 1

Example of Calculation of Rate Constants ( $k_{\text{obs}}$ ) of Hydrolysis of Acetylthiocholine by Oxime by Guggenheim Method

Measured at pH 7.6 and 24°C. 0.05 mM acetylthiocholine, 5 mM, 1,3-bis(4-pyridiniumaldoxime)propane dibromide.  $k_{\text{obs}} = 2.303 \text{ tg } \alpha = 0.191 \pm 0.06 \text{ min}^{-1}$ .

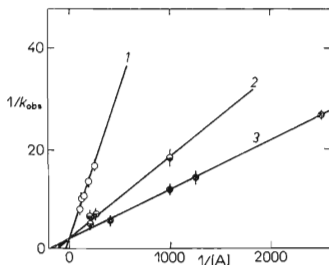


FIG. 2

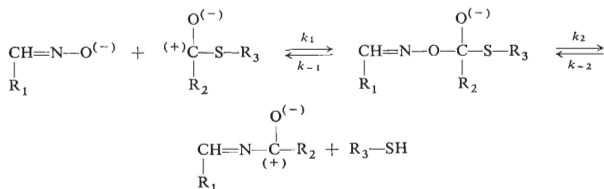
Effect of pH on Dependence of Rate of Hydrolysis of Acetylthiocholine ( $k_{\text{obs}}$ ) on Concentration of 1,3-Bis(4-pyridiniumaldoxime)propane Dibromide ([A]) in Double Reciprocal Plot

1 pH 7.1; 2 pH 7.6 and 3 pH 8.0. The points represent the arithmetic mean and the abscissas the standard deviation.

whose final concentration in the reaction mixture was 0.05 mM. The absorbance change during the hydrolysis was recorded by a recorder. The requirements of the kinetics of the first order (pseudomonomolecular reaction) were fulfilled since the molar excess of the oxime as one of the reacting components was 0.4 to 50 mM and therefore the record of the time profile of the absorbance had the character of an exponential curve. The corresponding rate constants ( $k_{\text{obs}}$ ) were calculated from these curves by the graphical method of Guggenheim<sup>14,15</sup>, namely by plotting the absorbance change ( $\Delta E$ ) in 30-s intervals *versus* reaction time ( $t$ ), as obvious from Fig. 1 given as an example. The interpolation of the straight lines through the experimental points was effected by the method of least squares in Minsk-22 computer. The rate constants ( $k_{\text{obs}}$ ) were calculated from the slopes ( $\text{tg } \alpha$ ) of the corresponding curves by using the formula  $k_{\text{obs}} = -2.303 \text{ tg } \alpha$ . It has been found in control experiments that the rate of the hydrolysis of acetylthiocholine by the two oximes is not affected by the presence of DTNB. The reaction rate was unaltered in its absence. The kinetic and thermodynamic constants were calculated also in the computer and regression analysis was used for the interpolation of the straight lines.

## RESULTS

The reaction mechanism of the hydrolysis of thioesters by oximes can be described by the following scheme<sup>6</sup>



The thioester reacts with the dissociated form of the oxime thus giving rise to an intermediary unstable complex which decomposes to the acylated oxime and the thioalcohol. Since the second part of the reaction is irreversible ( $k_{-2} = 0$ ), the kinetic equation of the reaction can be written as



where A is the oxime, B the thioester, AB the intermediary complex, C the acylated oxime, and D the mercaptane;  $k_1$ ,  $k_{-1}$ , and  $k_2$  are the corresponding rate constants. The dissociation constant is given by the expression  $K_{\text{diss}} = (k_{-1} + k_2)/k_1$ .

The rate of formation of complex AB is given by  $k_1[\text{A}][\text{B}]$  decreased by the rate of the reversible reaction, *i.e.* by  $k_{-1}[\text{AB}]$ , and by the rate of its decomposition. The total rate of formation of complex AB equals zero (1)

$$k_1[\text{A}][\text{B}] - k_{-1}[\text{AB}] - k_2[\text{AB}] = 0. \quad (1)$$

Since the concentration of the free thioester  $[B]$  equals its initial concentration  $[B]_0$ , decreased by its concentration in the complex, i.e.  $[B]_0 - [AB]$ , then

$$k_1([B]_0 - [AB])[A] - (k_{-1} + k_2)[AB] = 0 \quad (2)$$

and thus

$$[AB] = \frac{k_1[B]_0[A]}{k_{-1} + k_2 + k_1[A]} \quad (3)$$

The factor limiting the total reaction rate is the rate of decomposition of complex  $[AB]$ , i.e.  $v = k_2[AB]$ . After the transfer of this expression to equation (3) we obtain for rate  $v$

$$v = \frac{k_1 k_2 [B]_0 [A]}{k_{-1} + k_2 + k_1 [A]}, \quad (4)$$

which by the substitution of the expression for  $K_{\text{diss}}$  can be simplified to  $v = (k_2[B]_0[A])/K_{\text{diss}} + [A]$ . By using the expression  $v = k_{\text{obs}}[B]_0$ , where  $k_{\text{obs}}$  is the observed rate constant, and modification of the equation we arrive at

$$\frac{1}{k_{\text{obs}}} = \frac{1}{k_2} + \frac{K_{\text{diss}}}{k_2[A]}, \quad (5)$$

i.e. the equation of a straight line. If  $1/k_{\text{obs}}$  is plotted *versus* the reciprocal values of the oxime concentration,  $1/[A]$ , we obtain a straight line whose slope is  $K_{\text{diss}}/k_2$ , its intersection with the axis of abscissas  $1/K_{\text{diss}}$ , and its intersection with the axis of ordinates  $1/k_2$ .

Since the hydrolysis of acetylthiocholine is brought about only by the dissociated form of the oxime, the kinetics of its hydrolysis was measured at three different pH-values (7.1, 7.6 and 8.0). The corresponding correction was made for the spontaneous hydrolysis of acetylthiocholine. The dependence of the reciprocal values of the observed rate constant ( $1/k_{\text{obs}}$ ) on the reciprocal values of the oxime concentration ( $1/[A]$ ) for compound *I* is shown in Fig. 2, for compound *II* in Fig. 3. These measurements were made at 24°C. As obvious from both Figures, the pH of the medium affects the dissociation constant ( $K_{\text{diss}}$ ) only whereas the decomposition rate constant ( $k_2$ ) is independent of pH. The calculated kinetic constants are given in Table I.

The effect of temperature on the kinetic constants of hydrolysis of acetylthiocholine by oximes was examined by measurements carried out at four different temperatures (13, 24, 34 and 50°C) and at pH 7.6. The dependence of  $1/k_{\text{obs}}$  on

TABLE I

pH-Dependence of Kinetic Constants of Hydrolysis of Acetylthiocholine by 1,3-Bis(4-pyridiniumaldoxime)propane Dibromide (*I*) and 1-(4-Pyridiniumaldoxime)-3-pyridiniumpropane Dibromide (*II*)

Measured at 24°C. Mean values of the constants  $\pm$  the standard deviation of the mean are given.

pH	$k_2$ $\text{min}^{-1}$	$K_{\text{diss}}$ mM	$k^{\text{II}}$ $\text{M}^{-1} \text{min}^{-1}$
<i>I</i>			
7.1	0.501 $\pm$ 0.031	28.46 $\pm$ 1.67	17.60 $\pm$ 2.38
7.6	0.482 $\pm$ 0.097	8.12 $\pm$ 0.82	59.36 $\pm$ 8.64
8.0	0.509 $\pm$ 0.016	5.28 $\pm$ 0.15	94.51 $\pm$ 5.49
<i>II</i>			
7.1	0.291 $\pm$ 0.009	31.21 $\pm$ 0.32	9.32 $\pm$ 0.82
7.6	0.366 $\pm$ 0.031	12.52 $\pm$ 0.21	29.23 $\pm$ 3.12
8.0	0.258 $\pm$ 0.026	3.58 $\pm$ 0.09	72.07 $\pm$ 8.93

TABLE II

Temperature-Dependence of Kinetic Constants of Hydrolysis of Acetylthiocholine by 1,3-Bis(4-pyridiniumaldoxime)propane Dibromide (*I*) and 1-(4-Pyridiniumaldoxime)-3-pyridiniumpropane Dibromide (*II*)

Measured at pH 7.6. Mean values of the constants  $\pm$  the standard deviation of the mean are given.

Temperature °C	$k_2$ $\text{min}^{-1}$	$K_{\text{diss}}$ mM	$k^{\text{II}}$ $\text{M}^{-1} \text{min}^{-1}$
<i>I</i>			
13	0.184 $\pm$ 0.003	6.45 $\pm$ 0.40	28.50 $\pm$ 0.20
24	0.482 $\pm$ 0.097	8.12 $\pm$ 0.82	59.36 $\pm$ 8.64
34	0.776 $\pm$ 0.046	8.40 $\pm$ 0.36	92.20 $\pm$ 9.19
50	1.651 $\pm$ 0.050	7.58 $\pm$ 0.18	217.21 $\pm$ 2.20
<i>II</i>			
13	0.251 $\pm$ 0.012	13.89 $\pm$ 0.25	18.01 $\pm$ 1.68
24	0.366 $\pm$ 0.031	12.52 $\pm$ 0.21	29.23 $\pm$ 3.12
34	0.826 $\pm$ 0.052	13.38 $\pm$ 0.27	61.98 $\pm$ 4.48
50	2.120 $\pm$ 0.121	12.42 $\pm$ 0.18	171.00 $\pm$ 2.06

TABLE III

Thermodynamic Parameters Characterizing Hydrolysis of Acetylthiocholine by 1,3-Bis(4-pyridiniumaldoxime)propane Dibromide (I) and 1-(4-Pyridiniumaldoxime)-3-pyridiniumpropane Dibromide (II)

Measured at pH 7.6.

Temperature °C	$K_{\text{diss}}^{\pm}$ mM	$\Delta G^{\circ}$ kcal mol <sup>-1</sup>	$\Delta S^{\circ}$ e.u.
I			
13	3.08	-3.28	+11.4
24	3.89	-3.26	+11.0
34	4.00	-3.36	+11.0
50	3.62	-3.60	+11.2
II			
13	2.95	-3.31	+11.6
24	3.65	-3.50	+11.8
34	2.82	-3.55	+11.5
50	2.64	-3.80	+11.8

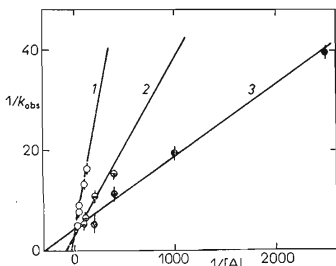


FIG. 3

Effect of pH on Dependence of Rate of Hydrolysis of Acetylthiocholine ( $k_{\text{obs}}$ ) on Concentration of 1-(4-Pyridiniumaldoxime)-3-pyridiniumpropane Dibromide [A] in Double Reciprocal Plot

1 pH 7.1; 2 pH 7.6 and 3 pH 8.0. The points represent the arithmetic mean and the abscissas the standard deviation.

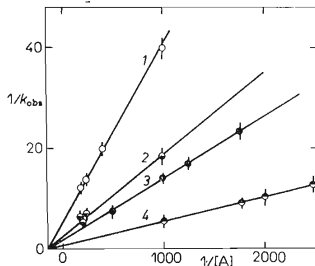


FIG. 4

Effect of Temperature on Dependence of Rate of Hydrolysis of Acetylthiocholine ( $k_{\text{obs}}$ ) on Concentration of 1,3-Bis(4-pyridiniumaldoxime)propane Dibromide [A] in Double Reciprocal Plot

1 13°; 2 24°; 3 34° and 4 50°C. The points represent the arithmetic mean and the abscissas the standard deviation.

$1/[A]$  for compound *I* is shown in Fig. 4 and for compound *II* in Fig. 5. The slope of the straight lines decreases with increasing temperature; the lines, however intersect at one point on the axis of abscissas.  $K_{\text{diss}}$  is thus temperature-independent whereas  $k_2$  increases with increasing temperature. The calculated constants are given in Table II.

The activation energy ( $E_{\text{act}}$ ) of the decomposition of complex AB was calculated from the dependence of the decomposition rate constant  $k_2$  on temperature in the Arrhenius plot (Fig. 6).  $E_{\text{act}}$  for compound *I* is  $10.74 \pm 0.89 \text{ kcal mol}^{-1}$  and for compound *II*  $11.68 \pm 0.32 \text{ kcal mol}^{-1}$ . The standard free energy ( $\Delta G^\circ$ ) was calculated from the dissociation constant of the ionized form of the oxime ( $K_{\text{diss}}^\pm$ ) according to formula  $\Delta G^\circ = 2.303 RT \log K_{\text{diss}}^\pm$ . The standard change of entropy was calculated from formula  $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$ , where  $\Delta H^\circ = 0$ , since the dissociation constant  $K_{\text{diss}}^\pm$  is temperature independent. The values of  $\Delta G^\circ$  and  $\Delta S^\circ$  for both compounds are given in Table III.

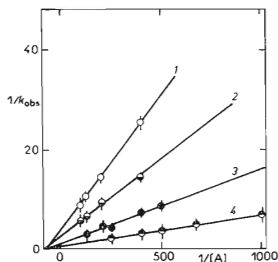


FIG. 5

Effect of Temperature on Dependence of Rate of Hydrolysis of Acetylthiocholine ( $k_{\text{obs}}$ ) on Concentration of 1-(4-Pyridiniumalldoxime)-3-pyridiniumpropane Dibromide  $[A]$  in Double Reciprocal Plot

1 13°; 2 24°; 3 34° and 4 50°C. The points represent the arithmetic mean and the abscissas the standard deviation.

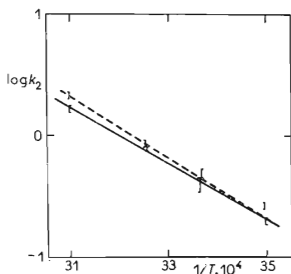


FIG. 6

Dependence of Decomposition Rate Constant ( $k_2$ ) of Complex Acetylthiocholine-Oxime on Temperature ( $T$ ) in Arrhenius Plot

Activation energy of hydrolysis of acetylthiocholine by 1,3-bis(4-pyridiniumalldoxime)propane dibromide:  $10.74 \pm 0.89 \text{ kcal mol}^{-1}$  (full line), activation energy of hydrolysis of acetylthiocholine by 1-(4-pyridiniumalldoxime)-3-pyridiniumpropane dibromide:  $11.68 \pm 0.32 \text{ kcal mol}^{-1}$  (broken line).  $E_{\text{act}} = 2.303 R \text{ tg } \alpha$ . The abscissas represent the mean value  $\pm$  standard deviation.

## DISCUSSION

The two oximes studied are known reactivators of phosphorylated cholinesterases<sup>4,16</sup>. One of them, compound *I*, is used under the designation of TMB-4 or trimedoxime in clinical practice as antidote in case of organophosphorus intoxication<sup>4,17</sup>. The oximes show, however, in addition to their reactivating effect on phosphorylated cholinesterases still other effects which are not always desirable<sup>18</sup>. Besides the parasympatholytic effect<sup>19</sup> the most marked role plays their ability to hydrolyze certain esters. It is assumed that the oximes can inhibit the transacetylation reactions catalyzed by transacetylases as a result of their reaction with acyl-coenzymes A and thus to interfere with the synthesis of certain compounds, *e.g.* of acetylcholine<sup>6,18</sup>.

The oximes studied by us differ by the number of oxime groups in their structure. However, one aldoxime group only participates in the reaction of compound *I* with acetylthiocholine, as follows from the comparison of the bimolecular rate constants of the two compounds, calculated in terms of concentration of the ionized form of the oxime ( $k^{II\pm}$ ). For compound *I*  $k^{II\pm}$  equals  $136\text{M}^{-1}\text{min}^{-1}$  and for compound *II*  $132\text{M}^{-1}\text{min}^{-1}$ . To calculate these constants we used both the average values of the decomposition rate constant  $k_2$  and the average values of dissociation constant  $K_{\text{diss}}$ , calculated in terms of the concentration of the ionized form by the Henderson-Hasselbach equation. The thus calculated dissociation constants ( $K_{\text{diss}}^{\pm}$ ) for compound *I* are 6.18 mM at pH 7.1, 3.89 mM at pH 7.6, and 4.53 mM at pH 8.0 (average value 3.65 mM) and for compound *II* 2.45 mM at pH 7.1, 2.65 mM at pH 7.6, and 1.44 mM at pH 8.0 (average value 2.18 mM).

The ionized forms of compound *I* and compound *II* hydrolyze acetylthiocholine at the same rate and their temperature dependence is also the same as indicated by their identical activation energy ( $10.74 \pm 0.89\text{ kcal mol}^{-1}$  for compound *I* and  $11.68 \pm 0.32\text{ kcal mol}^{-1}$  for compound *II*). The standard change in entropy ( $\Delta S^\circ$ ) of this reaction is also the same for both compounds and its average value is  $+11.4\text{ e.u.}$  The standard binding enthalpy  $\Delta H^\circ$  equals zero since the size of  $K_{\text{diss}}^{\pm}$  is temperature-independent.

*The authors are indebted to Mrs M. Zechovská for technical assistance and to Mrs V. Pacovská for the calculations on the computer.*

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Translated by V. Kostka.