

APPENDIX

Figures 12 and 13 show the computer circuits used in simulating the solutions shown in this work. Of prime concern and utility here is the "nonlinear delay input" by programmed relay shown in Fig. 12. The remaining programs required (immediate start, zero-order, and delayed start, first-order) have been published and discussed previously (3).

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Kinetics of Deterioration of Trimethylene Bis-(4-formylpyridinium Bromide) Dioxime in Dilute Aqueous Solutions

By R. I. ELLIN, D. E. EASTERDAY, P. ZVIRBLIS, and A. A. KONDRITZER

The degradation of trimethylene bis-(4-formylpyridinium bromide) dioxime, TMB-4, occurs by two mechanisms. The first is a hydrolytic reaction of the acid form of the oxime catalyzed by hydrogen ion, and the second, a dehydration reaction. The latter may proceed by either of two pathways—a hydroxyl ion catalyzed dehydration of the acid form of the oxime, or a spontaneous dehydration of the oximate species. Velocity and various thermodynamic constants were determined for each of the mechanisms postulated. General equations were derived that relate the half-life of TMB-4 solutions to pH and temperature.

FOLLOWING THE introduction of pyridinium oximes for the management of intoxication by organophosphorus anticholinesterase compounds, an active search has taken place for more effective compounds. As a result, 1,1'-trimethylene bis-(4-formylpyridinium bromide) dioxime, referred to as TMB-4, was synthesized and shown to be a potent reactivator of phosphorylated cholinesterases (1, 2). Grob has reported (3) that TMB-4 in one-tenth to one-fifteenth the dose of 2-PAM iodide was more effective in humans against the weakening of the response of a muscle to electrical stimulation of its motor nerve. A better therapeutic index for TMB-4 has been demonstrated in laboratory animals (4), and the use of a mixture of 2-PAM and TMB-4 has been reported to be superior to any single oxime tested (5). In view of these reports the establishment of the conditions of maximal stability of TMB-4 in aqueous solution becomes important and worthwhile.

Mechanisms for the degradation of pyridinium aldioximes in aqueous solution have been postulated in previous reports (6, 7). Recent studies by Kosower (8) support the conclusion that the rate-limiting step for the degradation of pyridinium oximes in basic solution is the formation of cyanopyridinium ion. The mechanisms postulated for the breakdown of TMB-4 are presented in Scheme I. The reaction of TMB-4 in basic solution may be explained by an E1cB unimolecular elimination mechanism (9). Hydroxyl ion attack at each methine hydrogen atom results in the removal of a proton and the formation of a carbanion as the rate-controlling step. Subsequent loss of hydroxide ion from the oximino nitrogen leads to the formation of a triple bond; for TMB-4 the corresponding dinitrile would be the final product of this process. Hydroxide ion attack on the cyano group or addition to the pyridine ring forms dicarbamido and dihydroxy dipyridinium ions, respectively. The latter, on further reaction with hydroxide ion, readily lose a proton to form the corresponding dipyrindone. Reaction of TMB-4 with hydrogen ion leads to various states of equilibrium involving the splitting out of hydroxylamine and the formation of

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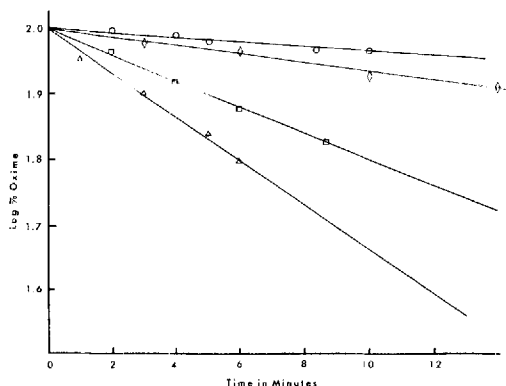


Fig. 2.—Degradation of TMB-4 in perchloric acid solutions at 71°. Key: O, 0.096 *N*; ◇, 0.19 *N*; □, 0.38 *N*; △, 0.70 *N*.

experimental solutions may be determined quantitatively by measuring the absorbance at 345 μ in 0.1 *N* alkaline solution.

Buffered solutions were placed in a thermostatically controlled bath and were allowed to reach the temperature of the bath. A relatively small volume of a TMB-4 solution was added to obtain a final concentration of 1 mg./ml. Samples of 0.5 ml. were removed and diluted to 100 ml. with 0.1 *N* sodium hydroxide and the concentrations of the oxime were determined spectrophotometrically at 345 μ .

RESULTS

Influence of Oxime Concentration.—The rate of disappearance of TMB-4 in both acid and alkaline solutions was found to be directly proportional to the concentration of the oxime, indicating that the degradation is of the first order with respect to the concentration of the oxime. The calculated reaction rate constants obtained at the various temperatures and hydrogen-ion concentrations are given in Table I. Typical plots of the results are shown in Figs. 2 and 3.

Temperature Dependency.—The velocity constants of the degradation of TMB-4 at 50, 59.7, and 71° in perchloric acid solutions ranging from 0.1 to 1.0 *N* (reaction 1 of Scheme I) were used to calculate the respective values for the catalytic constant, k_{H^+} , at normal hydrogen-ion concentration for each of the temperatures used. In Fig. 4 the rate constants are plotted against the reciprocal of the absolute temperature. The straight line relationships obtained indicate that the mechanism of the hydrolysis reaction is not altered by changes in temperature.

The temperature dependency of reaction 2 in Scheme I is graphically illustrated in Fig. 5 and indicates that the postulated dehydration mechanism is not affected by changes in temperature.

The effect of pH on the rate of degradation of TMB-4 at various temperatures is shown in Fig. 6. Between approximately pH 4.5 and 8, the rate is linear with hydroxyl-ion concentration; above approximately pH 8, the reaction becomes nonlinear. This phenomenon may be interpreted as representing

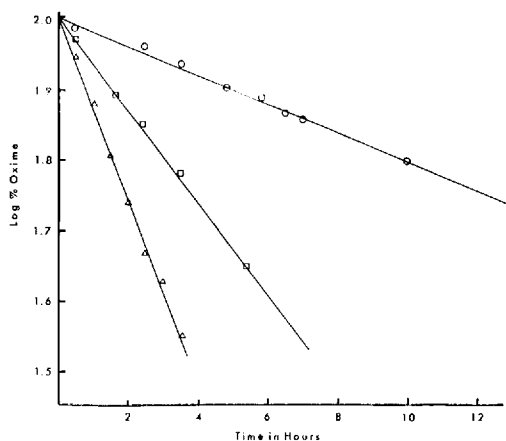


Fig. 3.—Degradation of TMB-4 in 0.1 *N* sodium hydroxide. Key: O, 71°; □, 81°; △, 90°.

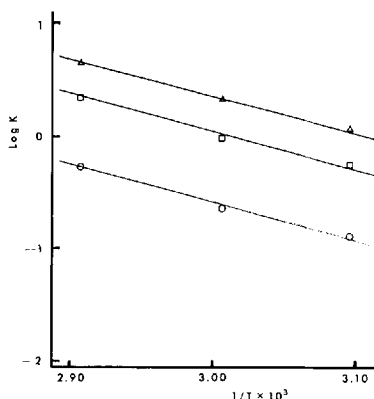


Fig. 4.—Arrhenius plot of hydrogen-ion catalysis of decomposition of TMB-4. Key: △, pH 0.11; □, pH 0.42; O, pH 1.0.

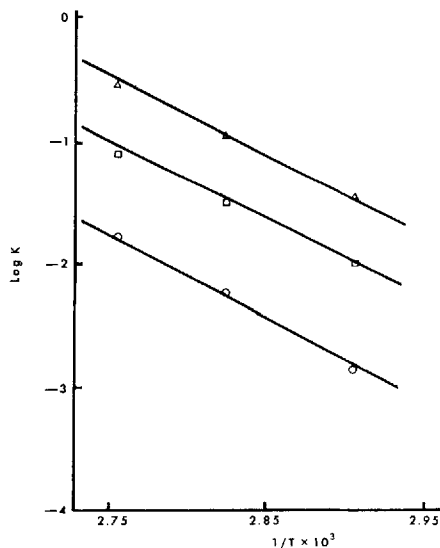


Fig. 5.—Arrhenius plot of hydroxyl-ion catalysis of decomposition of TMB-4. Key: O, pH 6; □, pH 7; △, pH 10.

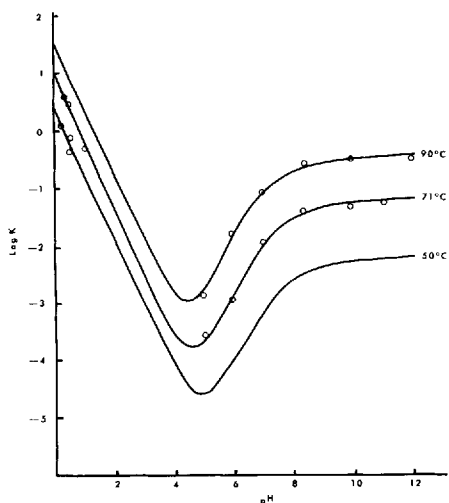


Fig. 6.—The pH profile for the breakdown of TMB-4 at 50, 71, and 90°. The curves represent values calculated from the equation derived from experimental data. The illustrated circles represent experimental values.

either spontaneous degradation of the oximate species or dehydration of the oxime catalyzed by hydroxyl ion. Either possibility would support the conclusion that above approximately pH 4.5 the mechanism of degradation can be due predominantly to reaction 2 (Scheme 1). On the assumption that the rate of the water reaction, *i.e.*, k_{H_2O} is negligible, and by use of the initial rate constants obtained for the reaction in acid solution and the rate constants for the reaction in alkaline solution, one obtains a general expression for the observed velocity constant of the degradation of TMB-4 (Eqs. 1 and 2).

$$\text{rate} = k_{H^+}(H^+) \frac{(H^+)}{K_a + (H^+)} + k_{OH^-} \frac{K_w}{K_a + (H^+)} \quad (\text{Eq. 1})$$

or

$$\text{rate} = k_{H^+}(H^+) \frac{(H^+)}{K_a + (H^+)} + k_{Ox} \frac{K_a}{K_a + (H^+)} \quad (\text{Eq. 2})$$

where k_{H^+} = catalytic constant for the hydrogen-ion catalysis, k_{OH^-} = catalytic constant for the hydroxylion catalysis, k_{Ox} = rate constant for degradation of oximate, and K_a = acid dissociation constant,

TABLE II.—THERMODYNAMIC CONSTANTS FOR DEGRADATION REACTIONS AT 25° C.

Reaction	E_a Kcal./mole	ΔH^* Kcal./mole	ΔF^* Kcal./mole	ΔS^* cal./mole deg.	A , hr. ⁻¹
TMB(H) ⁺ + H ₂ O ⁺	14.6	14.0	23.2	-30.8	1.01×10^{10}
TMB(H) ⁺ + OH ⁻	22.8	22.2	20.2	6.7	1.70×10^{18}
TMB(O) ⁻ spontaneous	24.2	23.6	27.3	-12.5	1.13×10^{14}

TABLE III.—PREDICTED STABILITY OF TMB-4 AT pH OF MINIMUM DECOMPOSITION

t , °C.	pH _{Min.}	$t_{1/2}$, yr.
0	6.13	2400
10	5.90	570
20	5.69	140
25	5.60	80
30	5.49	40
40	5.31	12
50	5.13	4
60	4.96	1.3
70	4.81	0.5
80	4.67	0.2
90	4.52	0.08
100	4.39	12 days

oxime \rightleftharpoons oximate, calculated from the equation, $-\log K_a = 3.41 + 1422/T$, which was derived from titration with alkali between 25 and 90°.

If the reaction is determined in solutions wherein the hydrogen ion is the only effective catalyst, Eqs. 1 and 2 reduce to $\text{rate} = k_{H^+}(H^+)$; at relatively high pH values, Eq. 1 reduces to $\text{rate} = K_w k_{OH^-}/K_a$ and Eq. 2 to $\text{rate} = K_{Ox}$.

The activation energies, E_a , were determined from the slopes of the Arrhenius-type plots in Figs. 4 and 5. The relationship between the rate constant and the equilibrium constant, ΔK^* , was used to determine the free energy of activation, ΔF^* , the frequency factor, A , and the entropy of activation, ΔS^* . The values for the latter two characteristics were calculated from Eqs. 3 and 4.

TABLE IV.—PREDICTED STABILITY OF TMB-4 IN AQUEOUS SOLUTIONS, $t_{1/2}$ (HALF-LIFE)

Temp., °C.	pH						
	3	4	5	6	7	8	13
10	1.4 yr.	14.4 yr.	140 yr.	560 yr.	93 yr.	13 yr.	3.3 yr.
20	0.6 yr.	5.9 yr.	57 yr.	115 yr.	15 yr.	2.2 yr.	0.8 yr.
30	95 days	2.6 yr.	24 yr.	23 yr.	2.7 yr.	146 days	73 days
40	44 days	1.2 yr.	9.6 yr.	4.7 yr.	197 days	37 days	19 days
50	21 days	212 days	3.7 yr.	1.0 yr.	44 days	10 days	6 days
60	11 days	106 days	1.3 yr.	91 days	11 days	3 days	2 days
70	5.5 days	56 days	170 days	25 days	3 days	21 hr.	16 hr.
80	2.9 days	29 days	54 days	7 days	18 hr.	7 hr.	5 hr.
90	1.7 days	16 days	17.5 days	2 days	7 hr.	2.6 hr.	2.2 hr.

$$k = \frac{RT}{Nh} e^{\Delta S^*/R} \cdot e^{-\Delta H^*/RT} \quad (\text{Eq. 3})$$

and

$$A = \frac{RT}{Nh} e^{\Delta S^*/R} \quad (\text{Eq. 4})$$

The calculated values for the thermodynamic constants are listed in Table II. By substituting the appropriate constants into Eqs. 1 and 2, the observed reaction rate can be expressed, in terms of hr.⁻¹, as either Eqs. 5 or 6.

$$k = 1.01 \times 10^{10} e^{-14,600/RT} (H^+) + 1.70 \times 10^{18} e^{-22,800/RT} \left[\frac{K_a}{K_a + (H^+)} \right] \quad (\text{Eq. 5})$$

or

$$k = 1.01 \times 10^{10} e^{-14,600/RT} (H^+) + 1.13 \times 10^{14} e^{-24,200/RT} \left[\frac{K_a}{K_a + (H^+)} \right] \quad (\text{Eq. 6})$$

Agreement of the values calculated by means of the equations with those obtained experimentally is shown in Fig. 6, where the lines are calculated and the open circles represent experimental data.

pH of Minimum Hydrolysis.—The equation for the hydrogen-ion concentration at which the rate of degradation is minimal can be derived by differentiating Eqs. 1 and 2 with respect to (H⁺) and equating to zero, as described previously for 2-PAM iodide (7). It may be expressed as:

$$\text{pH}_{\text{Min.}} = \frac{1}{2} \left(\text{p}K_a - 4.05 + \frac{2102}{T} \right)$$

Calculated values for pH_{Min.} at various temperatures are given in Table III and vary inversely with temperature, ranging from 6.13 to 0° to 4.39 at 100°.

The over-all reaction rate of the decomposition of TMB-4 can be expressed as the half-life, *t*_{1/2}, of the reaction; half-lives can be calculated for any pH and temperature. Predicted stability data for pH from 3 to 13 and for temperatures from 40 to 90° are shown in Table IV.

Isolation and Identification of Degradation Product.—Three grams of TMB-4 and 300 ml. of 0.1 *N* sodium hydroxide were heated for 24 hr. in a water bath at 90°. About 3% of the original oxime remained when determined by ultraviolet analysis. To a 25-ml. aliquot, enough picric acid solution in ethanol was added to produce a definite turbidity. The mixture was heated for 15 min. on a steam bath and then was cooled. The resulting yellow precipitate was collected by filtration, dried, and recrystallized from alcohol-water and then from water. The product melted at 234–238°. The elemental analysis for the compound was: C, 43.6%; H, 3.1%; O, 37.2%. The analytical results agree with the calculated elemental content of the dipicrate of 1,1'-trimethylene bis-(4-pyridone): C, 43.6%; H, 2.9%; O, 37.2%.

DISCUSSION

The decomposition of TMB-4 proceeds both by a proton catalyzed hydrolysis mechanism and a

dehydration reaction in which the cyanopyridinium intermediate is formed. The experimental data indicate that the dehydration may be explained equally well either by a hydroxyl-ion catalyzed reaction of the oxime, represented by *k*_{OH⁻}, or by spontaneous dehydration of the oximate ion, represented by *k*_{ox}.

A comparison of the thermodynamic constants for the dehydration reaction of TMB-4 with those found for the monoquaternary pyridinium aldoxime, 2-PAM (7), may lead to the conclusion that the predominant mechanisms involved differ for the two compounds. In the dehydration reaction for 2-PAM, activation energies assigned for *k*_{ox} and *k*_{OH⁻} are 28 and 17 Kcal., respectively. The activation energy for *k*_{ox} is determined directly from the observed reaction rates in 0.1 *N* alkali; whereas, that for *k*_{OH⁻} is determined from extrapolated values of observed rates at lower pH. The activation energy for the dehydration reaction of TMB-4 is about 24 Kcal. for both *k*_{OH⁻} and *k*_{ox}. The entropy of activation, Δ*S*^{*}, for the dehydration reaction of 2-PAM is more positive for *k*_{ox} than for *k*_{OH⁻}. In the case of TMB-4 the opposite is true; Δ*S*^{*} for *k*_{OH⁻} has the more positive value. If it is assumed that the least sterically hindered mechanism would occur more readily, a base-catalyzed mechanism could be assigned to the degradation of TMB-4 and a spontaneous degradation mechanism to 2-PAM.

The data in this study, with that given in the investigation of 2-PAM (7), provide a means for optimizing conditions for long-term storage of a mixture of the two compounds in solution. The pH-rate profiles of the two aldoximes are similar but not congruent. TMB-4 is the more stable of the two with respect to the dehydration reaction; whereas, 2-PAM is the more stable with respect to the proton catalyzed hydrolysis mechanism. The pH of maximum stability for the two compounds differs by about 1.2 pH units. A pH of 4.5 to 5.0 would be practical for the prolonged storage of mixtures of the two oximes in solution, provided storage temperatures were below 30°. At temperatures appreciably higher than 30°, the stabilities of both oximes in solution decrease to such an extent that long-term storage becomes impractical. If storage temperatures above 30° are anticipated, then the mixture should be packaged as a dry powder; provisions and directions for preparing solutions extemporaneously should be supplied.

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