Finally, drug complexation with starch can be a useful method of retarding absorption of a very irritant drug that might complex with starch and thereby reduce its toxicity. Starch has been used in this sense as an antidote for iodine poisoning. In a similar manner, the starch complexing affinity could explain the possible binding to bacterial excretion products in certain dermatitis conditions, thus providing the rationale of the wide use of starch as anti-irritant in dusting powders.

As the precipitation and isolation of "amylose-drug" complex is possible, a highly important question about the stability of a drug enclosed in such a complex form should not remain long unanswered. The nature of these complexes appear to suggest their possible use in a sustained dosage form, since the release of the drug from the complex is possibly caused by the enzymatic hydrolysis of the macromolecule.

SUMMARY AND CONCLUSION

Amylopectin (amylose-free) was found to have very little interaction for benzoic acid, *p*-hydroxybenzoic acid, and sorbic acid, while amylose was found to produce complexes rapidly. Potato starch, arrowroot starch, corn starch, rice starch, and a number of commercial starches rich in amylose were all found to complex with the three drugs tested. A number of selected pharmaceuticals showed interaction with potato starch sols, and in many cases, depending on the solubility of the pharmaceutical, the complex precipitated off the solution after reaching a saturation concentration.

Unruptured potato starch granules were found to adsorb fatty acids and fatty alcohols from methanol or petroleum ether solutions.

A correlation of the appearance and behavior of these complexes with those of "amylose-alcohols" used for the fractionation of starch, and with those of "amylose-iodine" reported in the literature, make one assume that the drug-starch complexing takes the same route and may be the same mechanism as that of "starch-iodine" and "starch-alcohols" complexes.

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Keyphrases

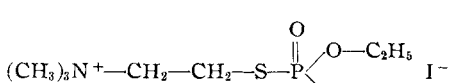
Complexes–starch
Starch starch–fraction sols–complexing behavior
Fatty acids–starch affinity
Molecular weight determination–starches
Spectrophotometry turbidity analysis
Solubility–complex formation analysis

Kinetics and Mechanism of Degradation of Echothiophate Iodide in Aqueous Solution

By ANWAR HUSSAIN, P. SCHURMAN, V. PETER, and G. MILOSOVICH

The degradation of echothiophate iodide was found to occur by two different mechanisms depending on the pH of the solution. In alkaline media (pH range 9.5–12), the major reaction was S-P bond cleavage to yield (2-mercaptoethyl)trimethylammonium iodide. This reaction was found to be first order with respect to the concentration of the compound and first order with respect to hydroxyl ion concentration. In weakly acidic media (pH range 2.4–5), the loss of 1 mole of ethanol through C—O bond cleavage was the predominant reaction. The rate of this reaction was also found to be first order with respect to the concentration of echothiophate iodide. The reaction rate constants and the energies of activation were determined for the two reactions and the degradation products were isolated and characterized.

ALTHOUGH ECHOTHIOPHATE IODIDE (I), a potent long-acting cholinesterase inhibitor, has been recognized in the USP (1) to be unstable in aqueous solution, the chemical degradation of the compound has not been thoroughly investigated. However, it is known that I

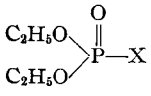


(I)

Received April 28, 1967, from the Pharmaceutical Development Department, Ayerst Laboratories, Inc., Rouses Point, New York, NY 12979
Accepted for publication October 26, 1967.
Presented to the Basic Pharmaceutics Section, APhA Academy of Pharmaceutical Sciences, Las Vegas Meeting, April 1967.
The authors acknowledge the assistance of Dr. A. Smith, Dr. C. Orzech, and Mr. R. Hobson in developing analytical procedures and for their helpful discussions. The authors also thank Dr. G. Schilling for the interpretation of the NMR spectra.

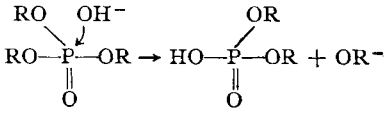
hydrolyzes quantitatively in alkaline media to (2-mercaptoethyl)trimethylammonium iodide and diethylphosphoric acid (1, 2).

Most of the past studies on the degradation of organophosphorus compounds related to I have dealt with the effect of substituents on their alkaline rates of hydrolysis (2, 3). Heath (3) found that the rate of alkaline hydrolysis of the X group from compounds of the type $RR'P(O)X$, where R and R' are alkoxy or alkylamine groups and X is an acidic group, is influenced greatly by the nature of the X group. Thus, the second-order rate constant for the hydrolysis of

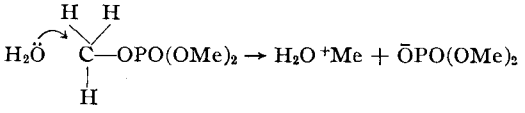


where X is OC_2H_5 is 4.86×10^{-2} M^{-1} min.$^{-1}$ at 25° compared to 81 M^{-1} min.$^{-1}$ where X is $S—C_2H_4S—C_2H_5$.

Trialkylphosphoric acid esters, however, have been thoroughly investigated by many workers (4–6). Barnard *et al.* (4) stated that in a trisubstituted phosphate ester, in which both carbon and phosphorus centers are subject to attack, hydroxide and water will tend to be selective for phosphorus and carbon, respectively. From their isotope experiments using water enriched in oxygen-18, they concluded that trimethylphosphate undergoes hydrolysis in alkaline medium with phosphorus-oxygen fission. There action was postulated as a simple nucleophilic displacement at the phosphorus atom:



In neutral and acidic media, however, the hydrolysis proceeded with carbon-oxygen bond fission. Since the reaction rate in water was not significantly changed by the presence of up to 3 M perchloric acid, it was presumed by these authors that this reaction proceeded *via* an S_{N2}-type displacement on carbon with water as the attacking species:



On the basis of the above information and the structure of (I), with both phosphorus and carbon centers open to attack, similar reactions may be anticipated, as shown in the following scheme. It was the primary purpose of this investigation to determine the mechanism of degradation of echothiophate iodide in neutral and weakly acidic media. A further objective was the determination of reaction rate constants and activation energies over a wide pH range.

RESULTS AND DISCUSSION

Order and Nature of the Degradative Steps—The results of this study indicated that in aqueous buffered solutions, the degradation of echothiophate iodide was first order with respect to the compound over a broad pH and temperature range.

In alkaline media, above pH 9, the reaction was followed measuring (2-mercaptoethyl)trimethylammonium iodide (II) formation by iodometric and spectrophotometric determinations, and by measuring consumption of hydroxyl ion on a pH stat. These results are shown in Figs. 1–3. As expected, from the ionic character of the reactant species (Scheme I), a negative primary salt effect was observed in the alkaline pH range. This is evident in Fig. 4, where the logarithm of the observed pseudo first-order rate constant, k_1 [OH^-], determined at pH 11.5, was plotted against the

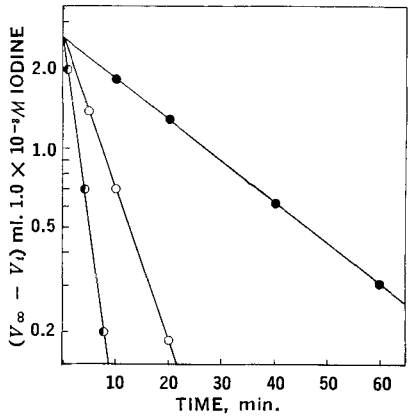


Fig. 1—Hydrolysis of echothiophate iodide as determined iodometrically at pH 10.85 and at ●, 25°; ○, 37°; and ◐, 45°.

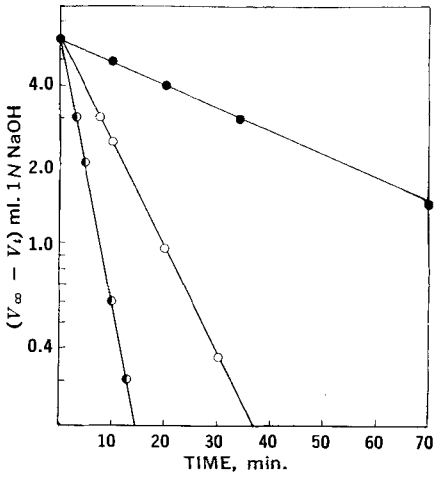
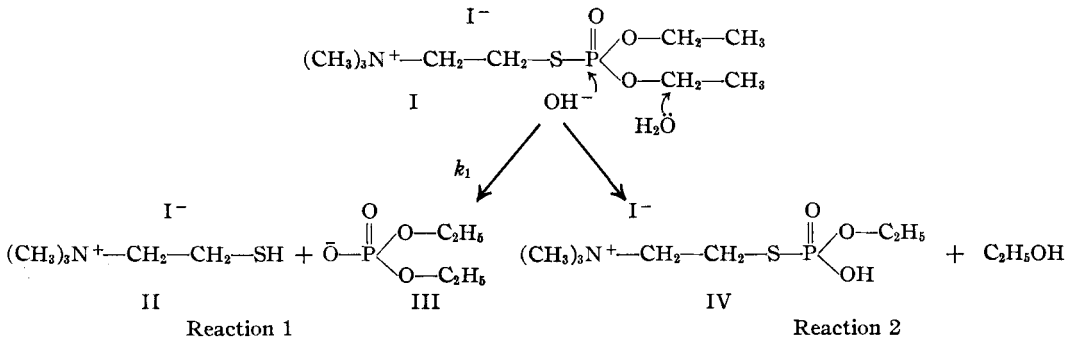


Fig. 2—Hydrolysis of echothiophate iodide as determined from pH stat measurements at pH 10.5 and at ●, 25°; ○, 37°; and ◐, 45°.



Scheme I

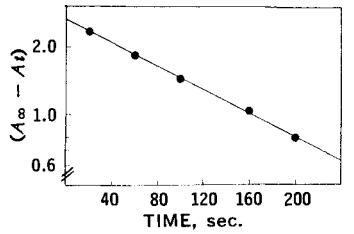


Fig. 3—Spectrophotometric observation at 226 mμ of the hydrolysis of echothiophate iodide at pH 11.8 and 25°.

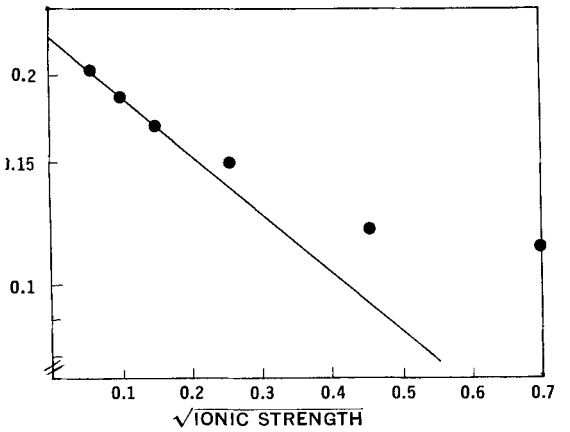


Fig. 4—Log $k_1[OH^-]$, the observed first-order rate constant of thiol formation, as a function of square root of ionic strength at 22° at pH 11.5. pH maintained with dilute NaOH, ionic strength changed by varying concentration of NaCl.

square root of ionic strength according to the Brönsted-Bjerum equation. Since this relationship was derived on the assumption of low ionic strength, the experimental values deviate rapidly from linearity. The initial slope, however, has a value of 0.9, close to the theoretically predicted value of 0.98 for sodium chloride. Since the rate of degradation at these hydroxyl ion concentrations was not influenced by changes in concentration or species of buffer, as shown in Figs. 5 and 8, the hydrolysis appears to be a specific hydroxide ion catalyzed reaction.

In acidic media, pH range 2.4–4.6, there was no detectable free thiol in degraded echothiophate iodide solutions, although solutions of thiol alone are stable under these conditions (less than 1% lost in 24 hr. at 70°). It was found, however, that in this pH range 1 mole of ethanol was formed for every

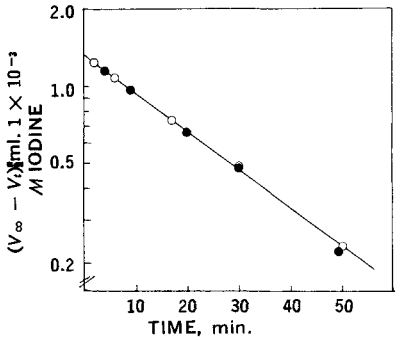


Fig. 5—Effect of change of molarity of phosphate buffer on the rate of hydrolysis of echothiophate iodide at pH 10.85 and ionic strength of 1.0 at 25°. Key: O, 0.1 M; ●, 0.2 M.

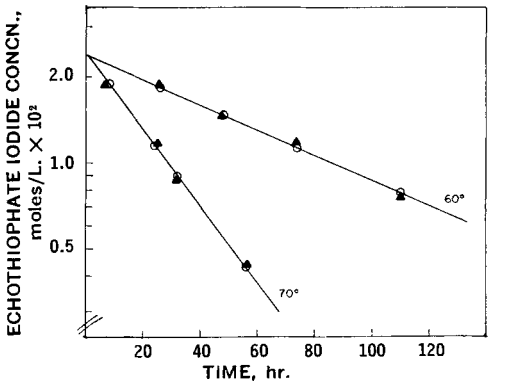


Fig. 6—Disappearance of echothiophate iodide at pH's 2.4 and 4.6 and at 60° and 70°. Key: O, found by direct analysis of echothiophate; ▲, calculated from the concentration of ethanol and based on the observed fact that one molecule of ethanol was formed for every molecule of echothiophate iodide degraded.

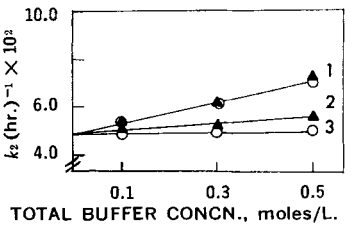


Fig. 7—Plot showing the effect of buffer concentration upon the rate of ethanol formation from echothiophate iodide at 74° and total ionic strength of 0.5. Key: ▲, 1, pH 5.2 acetate and pH 6.3 phosphate; O, 2, pH 4.6 acetate and 3, pH 2.4 phosphate.

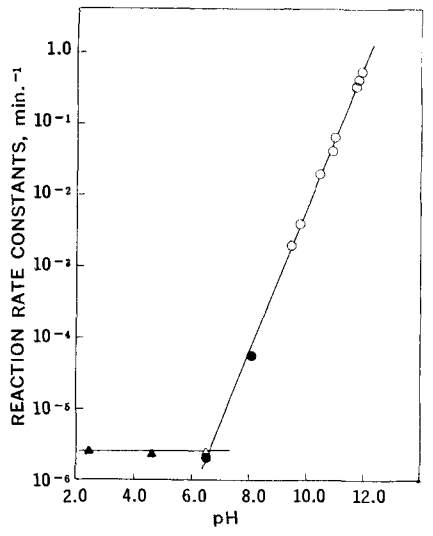


Fig. 8—pH profile of $k_1[OH^-]$ *and* k_2 *at 25°. Key:* O, $k_1[OH^-]$, ▲, k_2 *determined directly;* ●, $k_1[OH^-]$, △, k_2 *determined using the observed overall rate constant* $k_1[OH^-] + k_2$ *and Eq. 5.*

mole of I lost. Furthermore, the rate of loss of I from solution and the rate of appearance of ethanol were essentially the same. As shown in Fig. 6, the residual concentrations of I obtained by direct analysis are the same as those calculated from the concentrations of ethanol liberated. It is evident that for all practical purposes the reaction involving ethanol formation was responsible for the entire observed loss of I.

A slight influence of buffer concentration on the rate of ethanol formation determined at different hydroxyl ion concentration is seen in Fig. 7. At pH's below 5, the buffer effect was minimal. Since the rate constants were determined at low buffer concentration below pH 5, the contribution of buffer to the overall degradation was insignificant.

Thus, it appeared on the basis of these results that echothiophate iodide degrades in aqueous solution by at least two different mechanisms, depending upon the pH.

Variation of Reaction Rate Constants with pH—The pseudo first-order rate constants for formation of thiol and ethanol are shown as a function of pH in Fig. 8. These rate constants were determined directly from first-order concentration–time plots at pH's greater than 9 for thiol formation and pH's less than 5 for ethanol formation. It is seen from Fig. 8 that the plot has a zero slope at low pH and slope of unity at high pH.

In the intermediate pH range of 5–9, where both reactions contribute to the overall degradation, the individual rate constants were calculated from the overall rate of degradation of (I) and the final concentration of ethanol.

A rate equation for loss of I, based on Scheme I and Fig. 8, can be written:

$$-\frac{d[I]}{dt} = k_1[I]\,[OH^-] + k_2[I] = k_{obs.}[I] \quad \text{(Eq. 1)}$$

and

$$k_{obs.} = k_1[OH^-] + k_2$$

where [I] is the concentration of echothiophate iodide, k_1 is the rate constant for formation of thiol

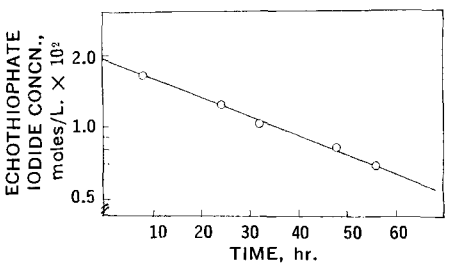


Fig. 9—Plot showing the disappearance of echothiophate iodide as a function of time at pH 6.3 and 60°.

TABLE I—FINAL CONCENTRATION OF ETHANOL AS A FUNCTION OF pH AT 60°, $[I]_o = 2.4 \times 10^{-2}$ *M*

pH	Ethanol Concentration × 10²
2.4	2.4
4.6	2.4
6.3	1.2
8.1	0.24
9.5	0.0

(II), k_2 is the rate constant for formation of ethanol and $k_{obs.}$ is the observed rate constant for loss of echothiophate iodide.

Since pH was maintained constant during an experiment, Eq. 1 can be integrated to give,

$$\log [I]_t = \log [I]_o - \frac{(k_1[OH^-] + k_2)t}{2.303} \quad \text{(Eq. 2)}$$

It can be shown (7) that for the case $[E_o] = 0$ at $t = 0$, the formation of ethanol with time may be described by,

$$[E]_t = \frac{k_2[I]_o}{(k_1[OH^-] + k_2)}\,(1 - e^{-(k_1[OH^-] + k_2)t}) \quad \text{(Eq. 3)}$$

where $[E]_t$ is the concentration of ethanol at time t. At $t = \infty$ Eq. 3 becomes,

$$[E]_\infty = \frac{k_2[I]_o}{(k_1[OH^-] + k_2)} \quad \text{(Eq. 4)}$$

$(k_1\,[OH^-] + k_2)$ can be obtained from the slope of log $[I]_t$ *versus* t plots, Fig. 9, according to Eq. 2. Since $[I]_o$ is known, and $[E]_\infty$ can be measured after $[I]_t = 0$, $k_1\,[OH^-]$ and k_2[1] can be calculated for the intermediate pH range.

According to Eq. 4, the final concentration of ethanol will be a function of pH. At high pH, k_1 $[OH^-]$ is large compared to k_2 and $[E]_\infty$ is very small, while at low pH, k_1 $[OH^-]$ is small compared to k_2 and $[E]_\infty$ approaches $[I]_o$. This is shown in Table I.

In the above scheme and equations, it was assumed that ethanol production was due solely to direct hydrolysis of echothiophate iodide as caused by Reaction 2, and the formation of ethanol from diethylphosphoric acid (III) was negligible. This assumption seems entirely valid in view of the much slower rate of hydrolysis of dialkylphosphates under these conditions (8, 9).

Both the USP assay method and the method used in this study for echothiophate iodide are based upon alkaline hydrolysis followed by iodometric determination of the liberated thiol. Although one might

[1] k_2 at pH 6.3 was determined by extrapolation of the data shown in Fig. 8 to zero buffer concentration.

expect that the degradation product (IV) produced by Reaction 2 would behave similarly, it appears that IV is not readily hydrolyzed to thiol under these basic conditions, probably because the negative charge acquired in basic solution serves to hinder attack by the negatively charged hydroxyl ion. It is known, for example, that subsequent stages of alkaline hydrolysis of triethylphosphoric acid are extremely slow after the first ethanol group is cleaved (10).

Temperature Dependency—Since the hydrolysis reaction may proceed by either of the two pathways, Reactions 1 and 2, it was necessary to determine the temperature dependency for each. The effect of temperature on Reaction 1 was determined iodometrically at pH 10.85 and on a pH stat at pH 10.5, since at these pH's, Reaction 2 was negligible. Arrhenius-type plots of log k_1 OH^- *versus* $1/T$ were observed to give straight lines, as shown in Fig. 10. The apparent activation energy was calculated from the slopes and found to be 22 Kcal./mole with a standard error of 1 Kcal./mole. Since this apparent energy of activation includes the heat of ionization of water (approximately 12 Kcal./mole), the energy of activation for the hydroxyl reaction would be 10 Kcal./mole.

The effect of temperature on Reaction 2 was determined at pH 2.4 and pH 4.6, since at these pH's Reaction 1 was negligible. The results obtained are shown in Fig. 11 where the log k_2, the observed first-order rate constant, was plotted against $1/T$. The heat of activation calculated from the slope of these lines is 23 Kcal./mole. The activation energy of the hydrolysis of trimethylphosphate in neutral and acidic solution was found to be 22.7 Kcal./mole (4).

The observed overall first-order rate constants for the degradation of echothiophate iodide, calculated at 25°, are shown as a function of pH in Table II.

In Fig. 12, the observed overall first-order rate constant $k_{obs.}$ was plotted against the pH of the solution. The line was calculated from Eq. 1 using $k_1 = 59.7\ M^{-1}$ min.$^{-1}$ and $k_2 = 2.3 \times 10^{-6}$ min.$^{-1}$ determined at pH 12 and pH 2.4, respectively, and the points were experimentally determined.

Degradation Products in Acidic Media—The degradation products in solutions at pH 4.6 were identified as ethanol and the monoethylester (IV).

The ethanol was determined quantitatively by gas chromatography. The monoethylester (IV) was isolated as the sodium salt (Na IV), and found to possess no cholinesterase inhibitor properties (11). Thin-layer chromatographic analysis of (Na IV)

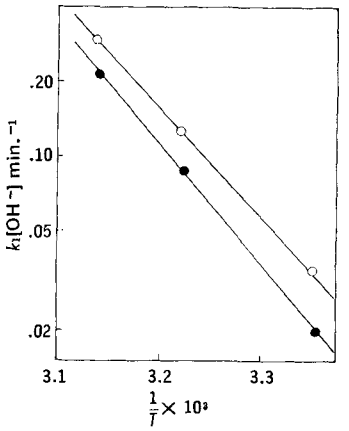


Fig. 10—Arrhenius plots showing the temperature dependency of Reaction 1. Key: ○, pH stat at pH iodometrically at pH 10.85; ●, pH stat at pH 10.5.

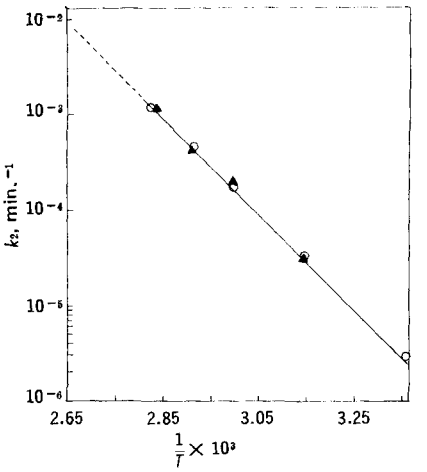


Fig. 11—Temperature dependency of Reaction 2. The log of the observed rate plotted against the reciprocal of the absolute temperature. Key: ○, pH 2.4; ▲, pH 4.6.

TABLE II—OBSERVED OVERALL FIRST-ORDER RATE CONSTANT AS A FUNCTION OF pH AT 25°

pH	$k_{obs.}$ min^{-1}
2.4	2.4×10^{-6}, 2.2×10^{-6}[a]
4.6	2.2×10^{-6}[a]
6.4	4.4×10^{-6}[a]
8.1	5.9×10^{-6}[a]
9.5	2.1×10^{-3}
9.8	4.0×10^{-3}
10.5	2.2×10^{-2}
10.9	4.1×10^{-2}
11.0	6.1×10^{-2}
11.7	3.5×10^{-1}
11.9	5.5×10^{-1}

[a] Calculated for 25° using the apparent activation energies.

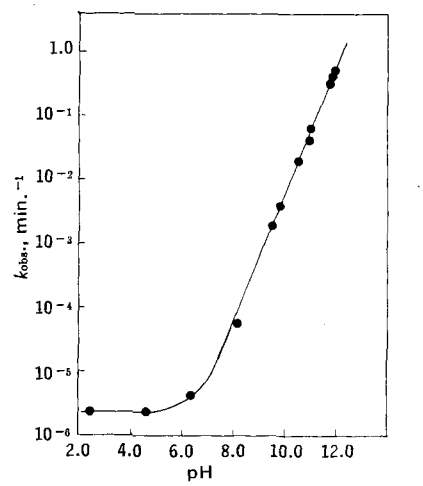


Fig. 12—Curve relating the observed overall first-order rate constant with pH at 25°. The solid line was calculated from Eq. 1, circles represent experimentally determined values.

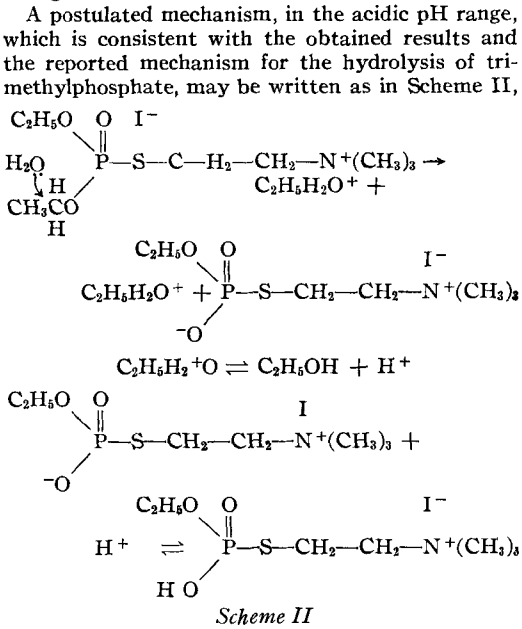
showed one spot with R_f value 0.7. The NMR spectra in D_2O was consistent with the structure of the monoethylester (12). Molecular weight determinations using perchloric acid as a titrant, according to the method described by Pifer *et al.* (13), gave a value of 377.3, corresponding to that of Na IV.

Elemental analysis of the sodium content corresponded to the theoretical value calculated for (Na IV) and the X-ray diffraction pattern showed no sodium iodide, indicating existence of Na IV, as such, in the solid state. A solution of Na IV was degraded at 121° in 3 *N* HCl, and the presence of free thiol (II) was detected by thin-layer chro matography.

Mechanism of Degradation in Weakly Acidic Media—The observed rate of formation of ethanol from echothiophate iodide was not influenced by changes in concentration of hydroxyl ion. The slight buffer catalysis observed was considered insignificant in the overall reaction.

The rate of hydrolysis of triethylphosphate (5) and trimethylphosphate (4) were also investigated in neutral and acidic media. The reported values for the observed first-order rate constants for formation of ethanol from triethylphosphate at 101° at pH 1.0 and 4.9 were 3.85×10^{-3} min.$^{-1}$ and 4.2×10^{-3} min.$^{-1}$, respectively. The observed first-order rate constants for formation of methanol from trimethylphosphate at 101° in water and in 3 *M* perchloric acid were reported to be 2.2×10^{-3} min.$^{-1}$ and 2.0×10^{-3} min.$^{-1}$, respectively. The observed first-order rate constant for the formation of ethanol from echothiophate iodide at pH 2.6 and 101° calculated by extrapolation from data shown in Fig. 11 was found to be 6.1×10^{-3} min.$^{-1}$.

A postulated mechanism, in the acidic pH range, which is consistent with the obtained results and the reported mechanism for the hydrolysis of trimethylphosphate, may be written as in Scheme II,



Scheme II

where the rate determining step would be the initial attack by H_2O on echothiophate iodide.

EXPERIMENTAL

Reagents and Apparatus—Echothiophate iodide (98% potency) and (2-mercaptoethyl)trimethylammonium iodide were obtained from the Research Department, Ayerst Laboratories, Division of Ayerst, McKenna and Harrison Ltd., Montreal.

Buffer, standard sodium hydroxide, iodine, and perchloric acid solutions were prepared using reagent grade materials.

Water used in the studies was prepared by distilling tap distilled water from an acidic permanganate solution.

The instruments used in this study were a Cary model 14 spectrophotometer, a pH stat, catalog No. S-30240, an Aerograph 204B gas chromatograph, and a Beckman pH meter.

Kinetic Procedures—The hydroxyl and hydrogen ion concentrations of the system were maintained by using buffer solutions or excess base. Buffer systems used in the study were: phosphate, pH's 2.4, 6.3, 10.8–11; acetate, pH 4.6; glycine, pH 8.1; borate, 9.5–10; and dilute NaOH (carbonate free) for pH's above 11.

The ionic strength of the solutions was adjusted to the desired value by the addition of sodium chloride. Most of the determinations were run at a total ionic strength of 0.5 unless otherwise specified.

Rate of Formation of (2-Mercaptoethyl)trimethylammonium Iodide—The rate of degradation of echothiophate iodide in alkaline pH range was determined by measuring the rate of formation of (2-mercaptoethyl)trimethylammonium iodide by three independent methods: iodometrically, spectrophotometrically, and on a pH stat.

When the reaction was followed iodometrically, solutions of 0.200 Gm. of the compound in 50 ml. of water and 50 ml. of 0.20 *M* buffer of the desired pH were equilibrated in separate flasks in a constant-temperature bath and then mixed. Periodically, 5-ml. samples (2 mg./ml.) were quickly discharged into a 50-ml. conical flask containing 5 ml. of 0.1 *M* phosphoric acid in order to quench the reaction. The thiol was then titrated with 1×10^{-3} *M* iodine solution under a blanket of nitrogen using starch as an indicator to obtain V_t (ml. of iodine solution at time t). A final titration was performed to obtain V_∞ (ml. of iodine solution at infinite time which corresponds to essentially complete hydrolysis).

Spectrophotometric determinations were made at 226 mμ. Although the ultraviolet absorption spectra of echothiophate iodine and its hydrolytic product, the thiol (pKa 7.6 determined spectrophotometrically), are practically identical in acid media (molar absorptivity values for echothiophate iodide and the thiol at pH 4.2 are 1.3×10^4 and 1.4×10^4 M^{-1} cm.$^{-1}$, respectively), the salt form of the thiol has sufficiently higher molar absorptivity that it can be differentiated in alkaline media (2×10^4 M^{-1} cm.$^{-1}$ at pH 9.1). A fresh 2.6×10^3 *M* solution of echothiophate iodide was prepared in water and a 0.1-ml. portion was transferred to a 1.0-cm. light path silica cell. Three milliliters of 0.1 *M* phosphate buffer of the desired pH was injected into the cell, and the change in absorbance at 226 mμ was followed in the Cary model 14 recording spectrophotometer. After the run was completed, the pH of the reaction mixture was determined to be unchanged.

The rate of the alkaline hydrolysis was also determined from data obtained on a pH stat. In 50 ml. of water 0.200 Gm. of the compound was dis-

solved and the consumption of hydroxyl ion, under nitrogen, was followed at constant temperatures and fixed pH. Not more than 1 ml. of 1.0 N sodium hydroxide was added totally to maintain the pH throughout the reaction.

The effect of buffer concentration upon the alkaline rate of hydrolysis was studied iodometrically at pH 10.85 using 0.1 and 0.2 M phosphate buffers and total ionic strength of 1.0.

The effect of ionic strength upon the rates of hydrolysis was studied in 3.5×10^{-3} N sodium hydroxide containing sodium chloride to give the desired ionic strength. The reaction was followed spectrophotometrically by adding the sodium hydroxide solutions to the echothiophate iodide as described above.

The effect of temperature on the rate of the alkaline hydrolysis was determined iodometrically at pH 10.85, 0.1 M phosphate buffer, and on a pH stat at pH 10.5 as described above.

Rate of Loss of Echothiophate Iodide and Rate of Formation of Ethanol—The degradation of echothiophate iodide below pH 9 was determined following the rate of loss of the compound and the rate of formation of ethanol.

Echothiophate iodide solutions, of concentration 2.4×10^{-2} M were prepared using phosphate, acetate, and glycine buffers of the desired pH and ionic strength of 0.5. The solutions were filled into hard glass ampuls and saturated with nitrogen before sealing. The ampuls were then placed in a constant-temperature bath which was maintained at the desired temperature ±0.05°. After allowing 15 min. for temperature equilibration, the 0-hr. samples were removed and further samples were withdrawn at time intervals suitable to the nature of the system. The reaction was quenched by immersion of the samples in ice water. The samples were then frozen till the end of each experiment.

The residual concentration of echothiophate iodide in the ampuls was determined using a modified USP method, in which potassium iodate as a titrant was replaced by standard iodine solution. A sample containing approximately 10 mg./ml. of echothiophate iodide was transferred into a conical flask. Two milliliters of 1.0 N sodium hydroxide was added, the flask was flushed with nitrogen, and stoppered. The solution was allowed to stand for 10 min. at room temperature. Two milliliters of 1 M phosphoric acid was then added, and the liberated thiol was titrated with 2×10^{-3} moles/L. standard iodine solution using starch as indicator. A residual blank titration for the thiol in solution was performed using the same procedure except the alkaline hydrolysis was omitted. The difference between the volume of iodine solution consumed in the two determinations is equivalent to the amount of thiol literated from the intact molecule. The concentration of echothiophate iodide can then be calculated as follows:

$$\text{moles/L. echothiophate iodide} = \frac{\text{ml. } I_2 \times 2 \times 10^{-3}}{\text{ml. sample size}}$$

The concentrations of ethanol in the ampuls were determined by gas chromatography using a 5-ft., 1/8-in. o.d. stainless steel column packed with 8% w/w didecylphthalate on 80/100 mesh Gas-chrom Z.

The following instrument parameters were used. Nitrogen carrier, 40 ml./min., column temperature 80°, sample size, 3 μl.

The amount of ethanol in the sample was determined by comparison with a standard aqueous ethanol solution.

The effect of buffer concentration on the rate of hydrolysis in the acidic pH range was determined at pH's 2.4, 6.3 phosphate and pH's 4.6 and 5.2 acetate, and ionic strength of 0.5.

The effect of temperature on the acid rate of hydrolysis was determined at elevated temperatures at pH's 2.4 and 4.6. Samples were also left at room temperature, pH 2.4, and the reaction was followed over a 1-year period.

Stability of (2-Mercaptoethyl)trimethylammonium Iodide—The stability of (2-mercaptoethyl)-trimethylammonium iodide was determined at 70° at pH's 2.4, 4.6, 6.3, and 8.1. Fresh 2.4×10^{-2} M solutions were stored in ampuls under nitrogen. The concentration of thiol was determined periodically by titrating with 2×10^{-3} M iodine to the blue starch end point.

Isolation and Characterization of Trimethyl (2-hydroxyethyl) Ammonium Iodide, S-ester with Phosphorothioic Acid, O-ether Ester (Na IV)—Three grams of echothiophate iodide was dissolved in 30 ml. of 1 M acetate buffer pH 4.6. The solution was transferred into a 50-ml. ampul and saturated with nitrogen before sealing. The ampul was kept for 4 days at 80° to ensure complete degradation. The solution was then transferred into a round-bottom flask and evaporated to dryness under vacuum at 80°. The solid residue was extracted with hot isopropanol (3 × 30 ml.), and the extracts were left at refrigerator temperature overnight. The separated crystalline compound had an m.p. 119°.

Thin-layer Chromatographic Analysis—Alcoholic solutions of (Na IV) and echothiophate iodide were prepared by dissolving 10 mg. in 1 ml. of methanol. Ten microliters of each solution was spotted directly on a chromatographic plate prepared with Silica Gel G. The plate was developed using a solvent mixture of two parts methanol, two parts water, and one part ammonium hydroxide. When placed in an iodine chamber, two spots were visible, one with R_f 0.7 corresponding to that of (Na IV), and the other of R_f 0.48 corresponding to echothiophate iodide. Twenty milligrams of (Na IV) was hydrolyzed with 3.0 N HCl at 121° and the hydrolytic product was identified using the above chromatographic procedure, as (2-mercaptoethyl)trimethylammonium iodide.

Sodium Analysis—The sodium content of the compound was determined by flame photometric analysis. Sodium calculated for $C_7H_{14}INO_3PSNa$, 6.1, found 6.2.

Resistance to Mild Alkaline Hydrolysis—To 20 mg. of (Na IV) in 3 ml. of water, 2 ml. of 1.0 N sodium hydroxide was added. No thiol was detected after 10 min. at room temperature as determined iodometrically with 2×10^{-3} M iodine solution.

SUMMARY

1. The overall degradation rate of echothiophate iodide in aqueous solution has been shown to be first order with respect to the compound over a wide pH and temperature range.

2. In alkaline media above pH 9, the main degradative pathway was the hydrolytic cleavage at

the sulfur–phosphorus bonds for form (2-mercaptoethyl) ammonium iodide. The rate of this reaction was found to be first order with respect to hydroxyl ion. The second-order rate constant at 25° was calculated to be 59.7 M^{-1} min.$^{-1}$. The apparent activation energy was determined to be 22 Kcal./mole with a standard error of 1 Kcal./mole. Since this energy of activation includes the heat of ionization of water, the energy of activation for the hydroxyl reaction would be 10 Kcal./mole.

3. Below pH 5 the main degradative route was the hydrolytic cleavage at the carbon oxygen bonds to form ethanol and trimethyl (2-hydroxylethyl) ammonium iodide, *S*-ester with phosphorothionic and *O*-ethyl ester. The rate of this reaction was not influenced by changes in concentration of hydroxyl ion but was increased slightly by increased buffer concentration. The attacking species were postulated to be a water molecule and the activation energy was determined to be 23 Kcal./mole.

4. The overall first-order rate constant for echothiophate iodide in aqueous solution was presented at 25° as a function of pH.

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Keyphrases

Echothiophate iodide degradation
pH effect—echothiophate iodide degradation
Hydrolysis, echothiophate iodide—temperature effect
Degradation products—acidic, alkaline media
GLC—analysis
TLC—analysis
UV spectrophotometry—analysis
Flame photometry—analysis
Iodometric analysis

Inhibition of Acetylcholinesterase by Chelates III

By ERNEST MARIO* and SANFORD BOLTON†

Inhibition of acetylcholinesterase by 1–1 cupric chelates of 1,3-diaminopropanol-2 and 1,3-diaminopropane is analyzed in the pH range of 8.0–9.0. The terdentate chelate of 1,3-diaminopropanol-2 exhibits weak inhibitory activity at pH values below 8.5 and increased activity at pH values above 8.5. The bidentate chelate of 1,3-diaminopropane exerts significant inhibition at pH 8.0. The bidentate system exhibits essentially noncompetitive inhibition while the terdentate system appears to be essentially competitive. Increased inhibition at elevated pH in the terdentate system is further evidence that the chelate is interacting with an ionizing group(s) on the enzyme surface, as previously reported.

As PART of a continuing study to investigate metal chelate inhibition of the acetylcholinesterase–acetylcholine enzyme reaction system, copper chelates of 1,3-diaminopropanol-2 (AOH) and 1,3-diaminopropane (AH) were prepared. These two structurally similar ligands were chosen because (*a*) the cupric chelates of AOH and AH had been clearly elucidated in an earlier investigation (1) and (*b*) it was of interest to examine the comparative effects of the two ligands, one of which is capable of forming both a 1–1 and 2–1 and the other only a 1–1 chelate.

In the first papers of this series, Bolton (2, 3) described some of the problems which are incurred in a study of this type. Since the chelate solutions contain an equilibrium mixture of several species, it is necessary to monitor closely the absolute concentrations of free ligand, free metal, 1–1 chelate, and 2–1 chelate under specific experimental conditions to determine which specie(s) is responsible for enzyme inhibition. The information obtained from the first two papers in this series may be briefly summarized as follows: (*a*) metal chelates inhibit the acetylcholinesterase–acetylcholine interaction; (*b*) in general, free ligand does not inhibit the interaction; (*c*) cupric

Received July 27, 1967, from the College of Pharmacy, University of Rhode Island, Kingston, RI 02881
Accepted for publication October 19, 1967.
Abstracted in part from a thesis submitted to the Graduate School, University of Rhode Island, in partial fulfillment of Doctor of Philosophy degree requirements.
This investigation was supported by research grant NB 04580-02 from the Institute of Neurological Disease and Blindness, U. S. Public Health Service, Bethesda, Md.
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