Kinetics of Base-Catalyzed Hydrolysis of Trimethadione

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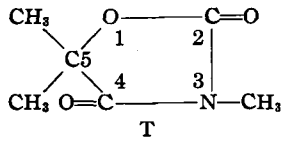
An infrared method is reported for determination of intact trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione) in presence of products of hydrolysis. Kinetics have been determined for hydroxide ion–catalyzed hydrolysis of trimethadione and a mechanism is proposed in which there exists a rapid equilibrium between a cyclic and an acyclic structure. The rate of hydrolysis of trimethadione at pH 10 and 30° is 10^5 to 10^6 times that for acyclic compounds of similar structure.

TRIMETHADIONE (3,5,5-trimethyl-2,4-oxazolidinedione) is an anticonvulsant drug which is often administered in the form of an aqueous solution. Although it is recognized that trimethadione is rapidly decomposed in alkaline solution, the kinetics of this reaction have not been studied.

The trimethadione structure (T) can be considered as representing a lactone, a urethan (1,2,3 positions), and a cyclic amide (3,4,5 positions). Because of the multiplicity of labile



T

bonds in trimethadione, a complex mechanism of degradation is to be expected. Decomposition might occur through hydrolytic cleavage of the lactone, urethan, or amide moiety of the cyclic compound. Rekker and Nauta (1) identified products obtained on alkaline hydrolysis of trimethadione (T) as the salt of a carbamylo-α-hydroxy acid (A) and an N-methyl-α-hydroxy amide (B).

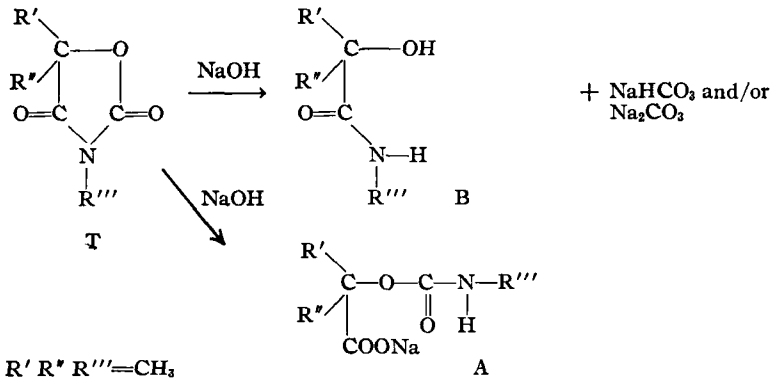
The present study was directed toward the development of an analytical procedure which would permit quantitative determination of residual quantities of intact trimethadione and toward elucidation of the kinetics and mechanism for the base-catalyzed hydrolysis of this drug.

EXPERIMENTAL

Reagents.—Trimethadione[1] U. S. P. was recrystallized twice from ethanol-water, m.p. 45–47°. The two decomposition products of trimethadione, N-methyl-α-hydroxyisobutyramide and N-methylcarbamylo-α-hydroxyisobutyric acid, were synthesized according to the method described by Rekker and Nauta (1, 2). Both compounds were recrystallized from toluene, m.p. 78–79° and m.p. 114–115°, respectively. All other chemicals were of reagent grade.

Apparatus.—A Perkin-Elmer model 137 B Infracord spectrophotometer, 0.099-mm. NaCl cell; Beckman pH meters, model H-2 and model 76; constant temperature bath ±0.01°; 1-ml. Gilmont micropipet-buret[2] with motorized syringe drive;[3] and a JKM "Stat"[4] were utilized in this study.

Kinetic Studies.—Two-gram portions of trimethadione were dissolved in distilled water in 100-ml. volumetric flasks. The solutions were brought to volume at the temperature of the bath. This solution was then transferred to a 250-ml. beaker



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[1] Marketed as Tridione by Abbott Laboratories, North Chicago, Ill.
[2] Manostat Corp., 26 N. Moore Street, New York 13, N. Y.
[3] Emil Greiner Co., New York 13, N. Y.
[4] JKM Instrument Co., Inc., Durham, Pa.

which was immersed to 90% of its length in the thermostatically controlled bath. Dipping beneath the surface of the liquid in the beaker were a glass-calomel electrode pair, a mechanical stirring device, and a 1-ml. micropipet-buret. The micropipet-buret contained a 12 *N* sodium hydroxide solution which was fed into the reaction vessel as required to maintain constant pH. Because of the high concentration of drug employed (2%) and the resulting consumption of large quantities of hydroxide ion, it was not practical to employ buffer systems to maintain constant pH. At no time did the volume of 12 *N* sodium hydroxide added exceed 0.5 ml. (0.5% of the total volume); therefore, no corrections were required to account for volume change during the reaction.

In most studies the pH in the reaction vessel was maintained by constant monitoring of pH and manual addition of sodium hydroxide solution from the micropipet-buret. For studies in the vicinity of pH 8, however, the reaction was followed for as long as 6 days and several modifications were necessitated. In these studies the reaction vessel was fitted with a rubber dam cover with a 22-gauge hypodermic needle providing an opening to avoid pressure changes. A pH stat, consisting of a Beckman model 76 expanded scale pH meter, a JKM "Stat" unit, and a motorized micropipet-buret, was employed to maintain constant pH. Maximum variation during a study was less than 0.02 pH.

At appropriate time intervals, 10-ml. portions of the aqueous alkaline reaction mixture were withdrawn and pipeted directly into 50-ml. separatory flasks. The reaction was quenched immediately by acidification to approximately pH 3 with concentrated hydrochloric acid. Trimethadione was shown to be stable at pH 3.

Determination of Intact Trimethadione.—At the end of the kinetic run each of the solutions in the separatory flasks was extracted with five 2-ml. portions of chloroform. After the addition of each portion of chloroform the separatory flask was shaken for approximately 1 minute. The chloroform extracts were filtered through a pledget of cotton into a 10-ml. volumetric flask. The cotton was rinsed with chloroform and the washings were collected in the same flask. The combined extracts were then brought to 10 ml. with chloroform and the trimethadione content of the solution was determined by measuring the absorbance at 5.49 μ, using the base-line technique (3).

An experiment was conducted in which 10-ml. portions of the aqueous alkaline solution of trimethadione were withdrawn at intervals and immediately extracted with chloroform without previous adjustment to pH 3. The chloroform extracts were then assayed as described above and the results were compared with data obtained from acid-quenching of the reaction. Both procedures gave the same result for concentration of intact trimethadione.

Determination of Decomposition Products.—The acid decomposition product, N-methyl-carbamylo-α-hydroxyisobutyric acid, was extracted from the reaction mixture with ether and was determined by titration with aqueous sodium hydroxide. A 7-Gm. quantity of anhydrous sodium sulfate was added to a 10-ml. sample of the reaction mixture from which trimethadione had been extracted. The resulting slurry was then extracted with four 10-ml. and two 5-ml. portions of ether. The combined ether extracts were evaporated and the residue was titrated with standard sodium hydroxide, approximately 0.01 *N* or 0.1 *N*.

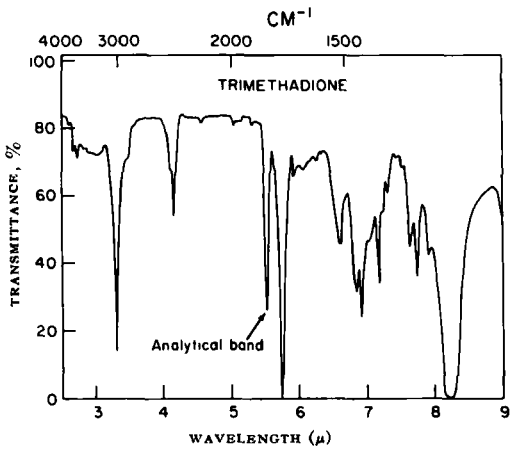


Fig. 1.—Infrared absorption spectrum for trimethadione in chloroform.

Direct determination of the amide decomposition product, N-methyl-α-hydroxyisobutyramide, in the reaction mixture was not practical; however, the reaction scheme indicates that formation of 1 mole of the amide must be accompanied by liberation of 1 mole of carbonate. Determination of carbonate in the reaction mixture, therefore, provided an indirect means of determining the quantity of amide formed.

The 10-ml. samples of reaction mixture to be used for carbonate determination were immediately acidified with 0.5 ml. of a sodium biphosphate solution (70 mg./ml.) to quench the reaction. The samples were then placed in a closed system and acidified with 1 ml. of concentrated sulfuric acid. The carbon dioxide evolved was flushed from the solution by bubbling nitrogen through the system for 1 hour and passing the effluent gases through two traps containing 30 ml. of standard barium hydroxide approximately 0.05 *N* in the first trap and 0.02 *N* in the second trap. The precipitate of barium carbonate was allowed to settle and a clear 10-ml. aliquot of the remaining barium hydroxide solution in each trap was titrated with 0.05 *N* hydrochloric acid to determine the equivalents of barium hydroxide precipitated by the carbon dioxide.

NMR Data.—A nuclear magnetic resonance spectrum[5] was obtained to confirm the structure of one of the products of decomposition, the N-methyl-α-hydroxyisobutyramide. The sample was run as a saturated solution in $CDCl_3$.

RESULTS AND DISCUSSION

Specificity of Assay for Intact Trimethadione.—Previously reported assay methods for trimethadione consist of determination of total nitrogen (4), determination of alkali consumed during hydrolysis in strong base (5), and measurement of carbon

[5] Furnished through the courtesy of Varian Associates, Palo Alto, Calif.

dioxide evolved on acidification after alkaline hydrolysis (6). The assays are unsatisfactory for distinguishing intact drug from decomposition products and for the last two assays the results obtained, even with pure trimethadione, are highly dependent on the conditions under which the assay is conducted.

Trimethadione is transparent in the visible and ultraviolet regions of the spectrum but exhibits a distinctive absorption band in the infrared at 5.49 μ. This absorption is similar to that exhibited by lactones (7); this band disappears upon destruction of the cyclic structure.

None of the possible decomposition products of trimethadione show absorption at 5.49 μ. Therefore, determination of absorbance of a chloroform extract of the aqueous reaction medium provides an assay specific for intact drug. Figure 1 shows the infrared spectrum of trimethadione and Fig. 2 shows the standard curve of absorbance *versus* concentration.

Specificity of Assays for Decomposition Products.—The product N-methyl-carbamylo-α-hydroxyisobutyric acid was determined by ether extraction of acidified aqueous solution, followed by evaporation and titration of residue with standard alkali. Presumably a product of further hydrolysis, α-hydroxyisobutyric acid, might also respond to such a procedure. It has been reported (1) that the sodium salt of carbamylo-α-hydroxy acid is not sensitive to alkali even at 100°; however, prolonged exposure to excess alkali did result in further hydrolysis. Below pH 10.8, however, it was found that there was a loss of less than 1% per hour. It would appear that for the duration and conditions of the present studies the carbamylo-α-hydroxyisobutyric acid can properly be considered as a final product.

The possibility of cleavage at the 2,3 position yielding another acid product of equivalent weight identical to N-methyl-carbamylo-α-hydroxyisobutyric acid was eliminated on examination of NMR

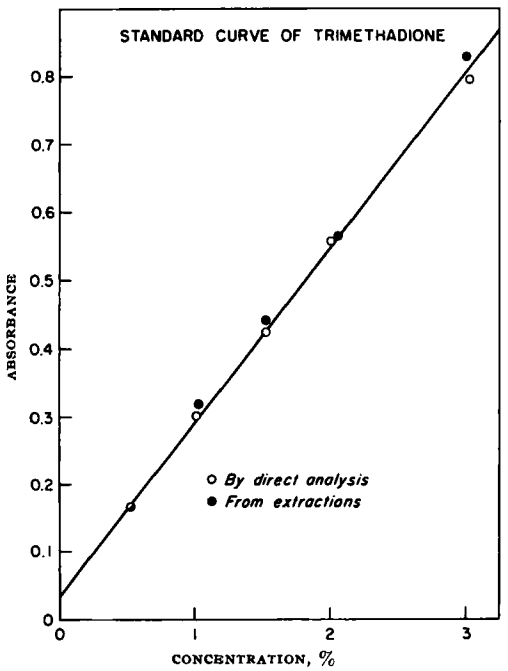


Fig. 2.—Standard curve for infrared determinations of trimethadione in chloroform. The open circles represent trimethadione added to chloroform; the solid circles represent chloroform extractions of aqueous solutions of trimethadione.

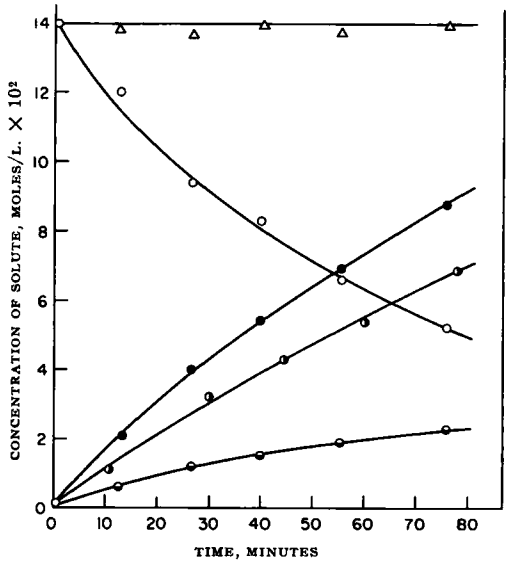


Fig. 3.—Verification of stoichiometry of trimethadione hydrolysis at pH 10 and 30°. Key: ○, trimethadione; ◑, N-methyl-α-hydroxyisobutyramide; ◑, N-methyl-carbamylo-α-hydroxyisobutyric acid; ●, ◑ plus ⊖; △, total.

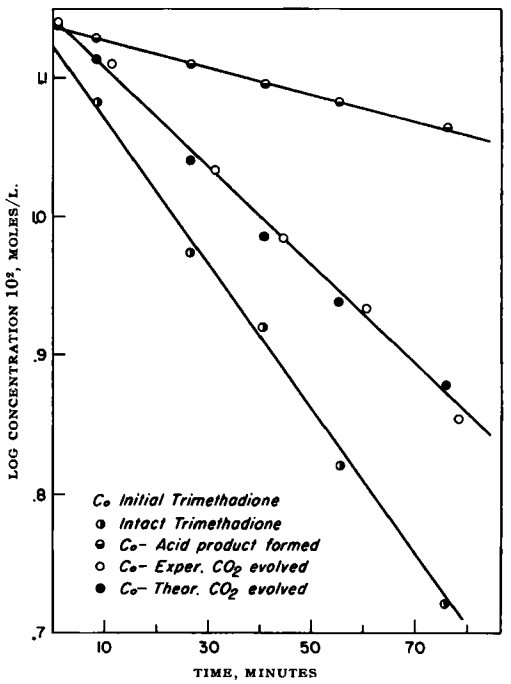
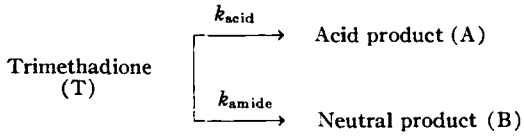


Fig. 4.—First-order disappearance of trimethadione and appearance of products.

spectra obtained for the acid product. The alternate structure for the acidic component would require the resonance of the N-methyl to be single, since there is no adjacent proton; the resonance is, in fact, doubled indicating that the N atom carries a proton as required for the N-methyl-carbamylo-α-hydroxyisobutyric acid structure. Additional resonance assignments and the integrated intensities confirm the above structural assignment.

Adherence to stoichiometry during the course of the reaction, as illustrated in Fig. 3, supports the acceptability of the determination of liberated carbon dioxide as an indirect means of determining the quantity of N-methyl-α-hydroxyisobutyramide produced.

Order of Reaction with Respect to Trimethadione. —Figure 4 illustrates that overall disappearance of trimethadione is first order with respect to intact drug. The nature of Fig. 3 suggests that at constant hydroxide ion concentration the decomposition occurs by formation of an acid product and an amide *via* two parallel first-order reactions. The proposed reaction is



The symbols T, A, B represent trimethadione, N-methyl-carbamylo-α-hydroxyisobutyric acid, and N-methyl-α-hydroxyisobutyramide, respectively, and k_{acid} and k_{amide} represent the corresponding rate constants. The rate equation at constant hydroxide ion concentration is

$$\frac{-dT}{dt} = \frac{dA}{dt} + \frac{dB}{dt} \qquad \text{(Eq. 1)}$$

or $$-\frac{dT}{dt} = kT = k_{acid}T + k_{amide}T \qquad \text{(Eq. 2)}$$

where $$k = k_{acid} + k_{amide} \qquad \text{(Eq. 3)}$$

and $$ln\ (T_0/T) = kt \qquad \text{(Eq. 4)}$$

or $$T = T_0e^{-kt} \qquad \text{(Eq. 5)}$$

where T_0 is the initial concentration of trimethadione and T is the concentration at any time t. The rate of formation of the acidic product can be expressed as

$$\frac{dA}{dt} = k_{acid}T \qquad \text{(Eq. 6)}$$

$$= k_{acid}T_0e^{-kt} \qquad \text{(Eq. 7)}$$

and by integration

$$A = -\frac{k_{acid}T_0e^{-kt}}{k} + \text{constant} \qquad \text{(Eq. 8)}$$

$$A = A_0 + \frac{k_{acid}}{k}T_0(1 - e^{-kt}) \qquad \text{(Eq. 9)}$$

where A_0 is the initial concentration of the acid product and A is the concentration at any time t. If $A_0 = 0$ (Eq. 10), then

$$A = \frac{k_{acid}}{k}T_0(1 - e^{-kt}) \qquad \text{(Eq. 11)}$$

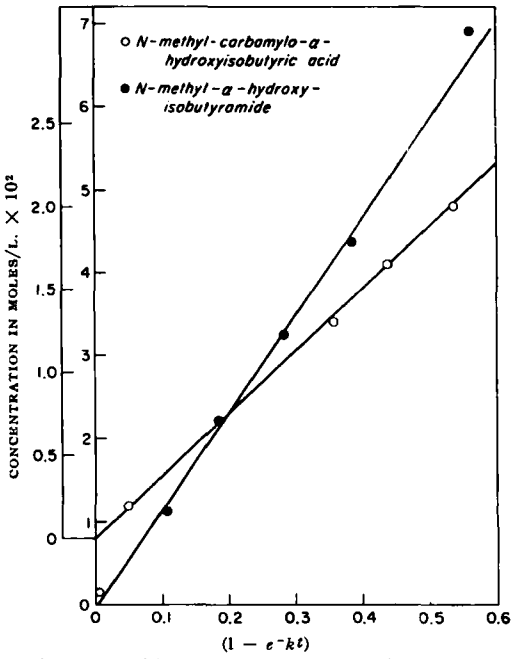


Fig. 5.—Evidence for parallel pseudo-first order reactions for hydrolysis of trimethadione to N-methyl-carbamylo-α-hydroxyisobutyric acid and N-methyl-α-hydroxyisobutyramide at pH 10.0 and 30°.

A similar expression derived for the neutral product is

$$B = \frac{k_{amide}}{k}T_0(1 - e^{-kt}) \qquad \text{(Eq. 12)}$$

Equations 11 and 12 indicate that for the base catalyzed hydrolysis of trimethadione, a plot of the concentration of either A or B against $(1 - e^{-kt})$ should yield a straight line. At zero time the curve should pass through the origin and at infinite time the function should have a value of unity.

The value for k, the overall first-order rate constant, was obtained by plotting logarithm of the concentration of residual trimethadione *versus* time at various hydroxide ion concentrations. It was then possible to check the relationship expressed in Eq. 11 using the concentrations of acidic product formed at various hydroxide ion concentrations. A plot of the acidic product formed in the degradation of trimethadione at pH 10.0 and 30° *versus* $(1 - e^{-kt})$ yielded a straight line through the origin as predicted by Eq. 11 (See Fig. 5). Also shown in Fig. 5 is a linear plot of the neutral or amide product formed at pH 10.0 and 30° *versus* $(1 - e^{-kt})$. The value of k_{acid}, the rate constant for the formation of acidic material, was calculated from the slope by Eq. 11.

$$\text{Slope} = \frac{k_{acid}}{k}T_0 \qquad \text{(Eq. 13)}$$

The value of k_{amide}, the rate constant for the formation of neutral material at pH 10.0 and 30°, was also obtained in the same manner. Values for k_{amide} at other pH values were obtained by evaluat-

ing k_{acid} in the above manner at each pH value and then subtracting k_{acid} from k according to Eq. 3. The data are found in Table I.

Hydroxide Ion Dependency.—The influence of hydroxide ion on the rate constant for trimethadione decomposition is illustrated in Fig. 6. Also shown in Fig. 6 is the influence of hydroxide ion on the rate constants for appearance of the decomposition products, N-methyl-carbamylo-α-hydroxyisobutyric acid and N-methyl-α-hydroxyisobutyramide. All plots show a hydroxide ion dependency of an order that is non-integral but greater than one.

Temperature Dependency.—Rate constants for the overall disappearance of trimethadione were obtained over the temperature range of 30 to 60° at a pH of 9.25. The data are presented as an Arrhenius plot in Fig. 7, and the apparent heat of activation for the process was determined as approximately 26.0 kilocalories per mole.

Mechanism.—Over the pH range studied, the pseudo-first order rate coefficient for the disappearance of trimethadione and the rate coefficients for the parallel pseudo-first order appearance of decomposition products, N-methyl-α-hydroxyisobutyramide and N-methyl-carbamylo-α-hydroxyisobutyric acid, all show hydroxide ion dependencies which are non-integral but of an order greater than one. The amide is the principal product below about pH 8.75, with the acid product becoming increasingly important above pH 9.50.

In postulating a mechanism to satisfy these requirements, the data illustrated in Fig. 8, showing hydroxide ion uptake during the reaction, become extremely useful. Figure 8 indicates an initial uptake of hydroxide ion greater than the equivalent quantity of trimethadione disappearing, followed by a region in which the hydroxide uptake is proportional to the trimethadione disappearing but is less than equivalent to the trimethadione disappearing. This observation suggests a relatively rapid reaction of hydroxide ion with trimethadione to produce a species which is in equilibrium with the trimethadione. On the basis of these observations the mechanism proposed is

TABLE I.—EXPERIMENTAL RATE CONSTANTS FOR THE BASE-CATALYZED HYDROLYSIS OF TRIMETHADIONE AT 30° C.

pH	k, Hr.$^{-1}$	k_{acid}, Hr.$^{-1}$	k_{amide}, Hr.$^{-1}$
10.75	7.314	2.710	4.604
10.50	2.696	0.873	1.823
10.25	1.325	0.359	0.966
10.00	0.676	0.145	0.531
9.75	0.292	0.047	0.245
9.50	0.119	0.019	0.100
9.25	0.0684	...	...
9.00	0.0284	...	...
8.75	0.0141	...	...
8.00	0.00251	...	...

Development of a rate expression can be considerably simplified if it can be assumed that the cyclic structure I is in rapid equilibrium with the acyclic structure III. An equilibrium quotient may then be written as

$$K = \frac{\text{III}}{(\text{I})\,(\text{OH}^-)} \qquad \text{(Eq. 14)}$$

The fraction of total trimethadione present in the cyclic form is

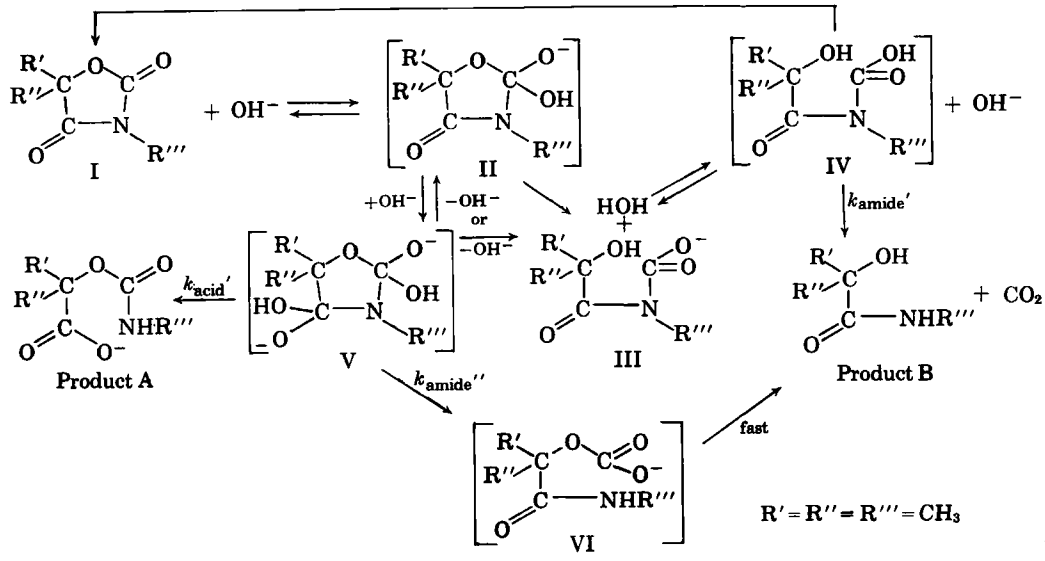
$$\frac{1}{1 + K\,(\text{OH}^-)} \qquad \text{(Eq. 15)}$$

and the fraction of total trimethadione in the acyclic form III is

$$\frac{K\,(\text{OH}^-)}{1 + K\,(\text{OH}^-)} \qquad \text{(Eq. 16)}$$

The existence of this equilibrium could not be verified directly, since at a pH where a significant fraction of trimethadione would theoretically exist in the acyclic structure, hydrolysis is extremely rapid. Structure IV apparently is extremely unstable, for in no case was it possible to isolate this compound after acidification of the reaction mixture.

In the following development, T represents total trimethadione in solution (essentially I + III), K is the equilibrium quotient as defined above, and



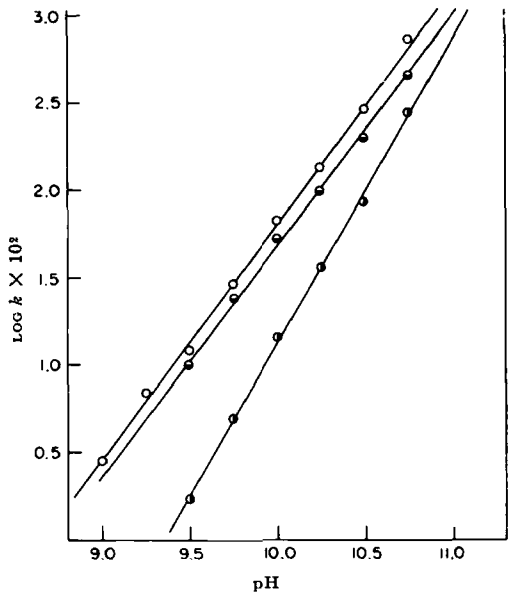


Fig. 6.—Influence of hydroxide ion on the pseudo-first order rate constant for hydrolysis of trimethadione at 30°. Also shown is the effect of hydroxide ion on the rate constants for appearance of products. Key: O, trimethadione; ◒, N-methyl-α-hydroxyisobutyramide; ◑, N-methyl-carbamylo-α-hydroxyisobutyric acid.

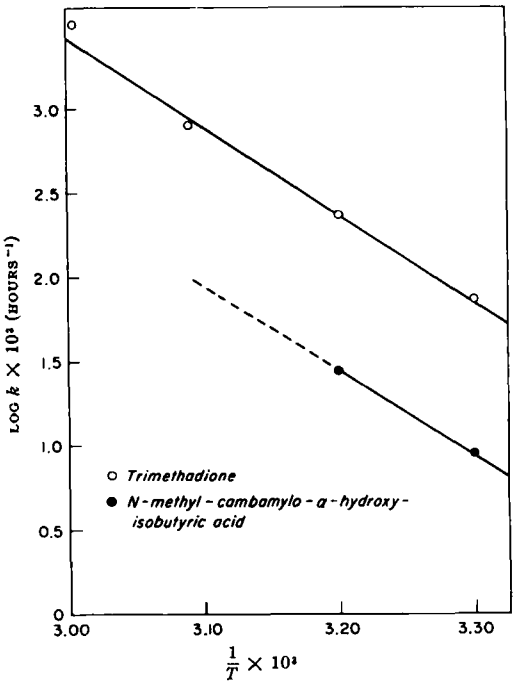


Fig. 7.—Arrhenius-type plots showing the temperature dependency for hydrolysis of trimethadione and appearance of N-methyl-carbamylo-α-hydroxyisobutyric acid at pH 9.25.

$k_{amide'}$, $k_{amide''}$, and $k_{acid'}$ are rate coefficients for formation of decomposition products. The overall rate of disappearance of trimethadione can be represented as

$$\text{Rate} = (\text{III})\,k_{amide'} + (\text{I})\,(OH^-)^2\,k_{amide''} + (\text{I})\,(OH^-)^2\,k_{acid'} \quad \text{(Eq. 17)}$$

When Eqs. 15 and 16 are substituted into Eq. 17, then

$$\text{Rate} = \frac{(T)\,K\,(OH^-)\,k_{amide'}}{1 + K\,(OH^-)} + \frac{(T)\,(OH^-)^2\,k_{amide''}}{1 + K\,(OH^-)} + \frac{(T)\,(OH^-)^2\,k_{acid'}}{1 + K\,(OH^-)} \quad \text{(Eq. 18)}$$

or

$$\frac{\text{Rate}}{T} = \frac{K\,(OH^-)\,k_{amide'}}{1 + K\,(OH^-)} + \frac{(OH^-)^2\,k_{amide''}}{1 + K(OH^-)} + \frac{(OH^-)^2\,k_{acid'}}{1 + K\,(OH^-)} \quad \text{(Eq. 19)}$$

The constants in Eq. 19 are evaluated in the following manner. At any constant hydroxide concentration, the observed pseudo-first order rate coefficients listed in Table I may be expressed as

$$k_{amide} = \frac{K\,(OH^-)\,k_{amide'}}{1 + K\,(OH^-)} + \frac{(OH^-)^2\,k_{amide''}}{1 + K\,(OH^-)} \quad \text{(Eq. 20)}$$

$$k_{acid} = \frac{(OH^-)^2\,k_{acid'}}{1 + K\,(OH^-)} \quad \text{(Eq. 21)}$$

$$k = k_{acid} + k_{amide} \quad \text{(Eq. 22)}$$

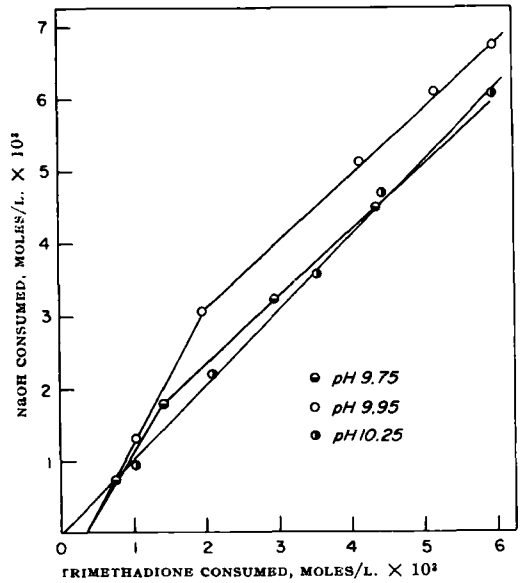


Fig. 8.—Plots showing uptake of hydroxide ion *versus* quantity of trimethadione degrading during hydrolysis reaction.

Using Eq. 21 and substituting observed values for k_{acid} at several hydroxide ion concentrations, it is possible to solve for the two unknowns, $k_{acid'}$ and K. These constants were determined from data obtained in the pH range 9.50 to 10.75. Using Eq. 20 and substituting the known value for K, and selecting values for k_{amide} at several hydroxide ion concentrations from Table I, simultaneous equa-

tions may be employed to determine $k_{amide'}$ and $k_{amide''}$. The coefficients thus calculated are

$$k_{acid'} = 0.965 \times 10^{7}\ \text{hr.}^{-1}$$
$$k_{amide'} = 0.074 \times 10^{1}\ \text{hr.}^{-1}$$
$$k_{amide''} = 1.491 \times 10^{7}\ \text{hr.}^{-1}$$
$$K = 2.564 \times 10^{3}$$

Using the above coefficients in the overall rate equation (Eq. 19) the theoretical rate of disappearance of trimethadione was calculated for the pH range of 7.5 to 11.0. The calculated rate appears as a solid line in Fig. 9. The rate expression predicts that the overall rate for disappearance of trimethadione should be first order with respect to hydroxide ion below pH 8.75 and above pH 11.0, and that the order is non-integral between pH 8.75 and 11.0. The experimentally determined rates are in good agreement with the theoretical and the rate does become first order with respect to hydroxide ion below pH 8.75. It was not feasible to study the reaction above pH 11.0 to examine for first-order hydroxide ion dependency because of the extremely rapid reaction under these conditions.

Comparison of Hydrolysis Rates for Cyclic and Acyclic Compounds.—Trimethadione is much more sensitive to alkaline hydrolysis than are acyclic compounds of similar structure. At pH 10 and 30° trimethadione is hydrolyzed at a rate approximately 10^{6} times that of ethyl-N-methylcarbamate (8), and approximately 10^{5} times the rate at which ethyl carbamate (urethan) (8) is hydrolyzed under similar conditions.

Rekker and Nauta (1) demonstrated that, in the presence of alkali, carbamylo-α-hydroxy esters are not hydrolyzed directly but are first converted to a trimethadione-type cyclic intermediate which then hydrolyzes to produce as products an N-substituted-α-hydroxy amide and the salt of a carbamylo-α-hydroxy acid. (See reaction scheme below.)

The extreme sensitivity of the trimethadione structure to alkaline hydrolysis (when compared with acyclic structures) suggests that the trimethadione structure might be of interest as an enzyme model or as an intermediate in the decomposition or metabolism of related drugs.

Pharmaceutical Significance.—From the theoretical rate equation proposed, half-life values for hydrolysis of trimethadione were calculated as a function of pH and are listed in Table II. It should be noted that these values are for solutions in which constant hydroxide ion concentration is maintained throughout the course of the reaction. Since trimethadione is usually employed in high concentration (5%) and since hydroxide ion is consumed in the hydrolysis reaction, the reaction presumably is self-limiting in unbuffered or weakly buffered solutions.

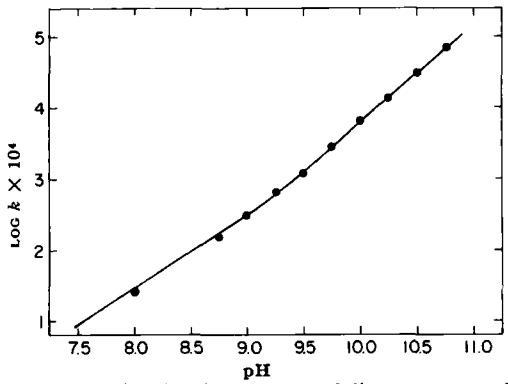


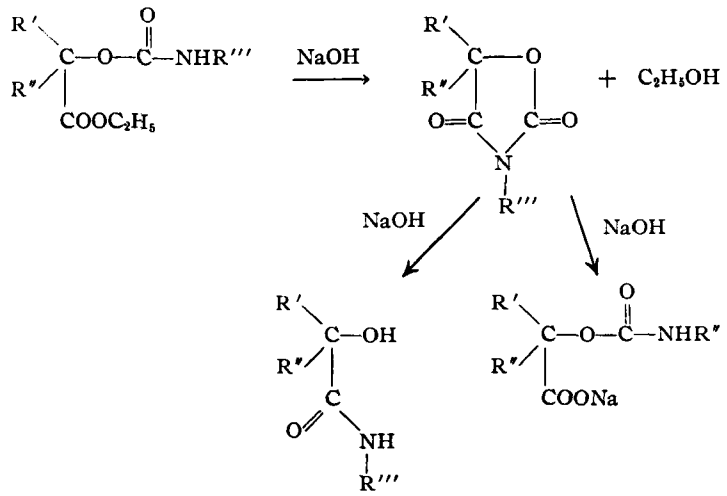
Fig. 9.—Plot showing the rate of disappearance of trimethadione as a function of hydroxide ion concentration over the pH range 8.0 to 11.0. The theoretical curve as calculated from the rate equation is shown as a solid line. The circles are experimental points determined at 30°.

TABLE II.—HALF-LIFE PERIODS, $t^1/_2$, FOR THE DEGRADATION OF TRIMETHADIONE AT CONSTANT pH AND 30° C. CALCULATED FROM THE THEORETICAL RATE EQUATION

pH	Half-life, hr.
7.0	2472
8.0	247
9.0	25.3
10.0	1.11
11.0	0.047

SUMMARY

1. An infrared method is reported for determination of trimethadione in the presence of products of its hydrolytic decomposition.



2. The kinetics of the base catalyzed hydrolysis of trimethadione (3,5,5-trimethyl-2,4-oxazolidinedione) have been determined for the pH range 8.0 to 10.75 at 30°. Pseudo–first order rate coefficients are reported for the disappearance of trimethadione and for the parallel pseudo–first order appearance of the decomposition products N-methyl-carbamylo-α-hydroxyisobutyric acid and N-methyl-α-hydroxyisobutyramide. The amide is the principal product below pH 8.75, with the acid becoming an increasingly important product above pH 9.50.

3. The overall rate of disappearance of trimethadione in aqueous solution, at constant hydroxide ion concentration, was found to be in agreement with a rate equation of the form

$$\frac{\text{Rate}}{T} = \frac{K(OH^-)\,k_{amide'}}{1 + K(OH^-)} + \frac{(OH^-)^2\,k_{amide''}}{1 + K(OH^-)} + \frac{(OH^-)^2\,k_{acid'}}{1 + K(OH^-)}$$

4. A mechanism was proposed in which there exists a rapidly reversible equilibrium between the cyclic trimethadione and an acyclic structure.

5. Trimethadione is much more sensitive to alkaline hydrolysis than acyclic compounds of similar structure. Trimethadione hydrolyzes approximately 1,000,000 times as fast as ethyl-N-methylcarbamate and approximately 100,000 times as fast as ethyl carbamate (urethan) at pH 10 and 30°.

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Determination and Identification of *p*-Hydroxyamphetamine as the O,N-Diacetyl Derivative

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Therapeutic solutions of hydroxyamphetamine hydrobromide, such as the ophthalmic solution U.S.P. and nasal solution, N.N.R. (N.N.D.), may be assayed gravimetrically by treating with acetic anhydride in the presence of bicarbonate and quantitatively isolating the O,N-diacetylhydroxyamphetamine thus formed. The properties of the readily crystallizable derivative serve to identify the parent substance.

TESTS AND STANDARDS for New and Nonofficial Remedies" (1) includes monographs for a 1% nasal solution and a 1% ophthalmic solution of *p*-hydroxyamphetamine hydrobromide. The assay specified for the former solution involves liberation of hydroxyamphetamine base with potassium carbonate, extraction of the base into ether, addition of excess standard acid to the extract, and back-titration after evaporation of the ether. The assay specified for the latter solution is based on measurement of its absorbance at 225 mμ.

Vincent, Krupski, and Fischer (2) have reported an alkalimetric method in which the solution containing a salt of hydroxyamphetamine is passed through a column of Amberlite IR-45. The base so formed is titrated after being eluted with ethanol. Varga and Vastagh (3) have developed a bromometric assay applicable to therapeutic solutions of hydroxyamphetamine.

The alkalimetric methods and the procedure of Varga and Vastagh are relatively nonspecific. In regulatory work it would be necessary to supplement them with experimental data providing some assurance that consumption of reagent is due only to the substance to be quantitated. The N.N.R. (1) alkalimetric method is, in addition, somewhat tedious. Even with the salting-out effect produced by the specified high concentration of potassium carbonate (15 Gm. for a 20-ml. sample), seven extractions are required, and there are other difficulties related to the low specific gravity and high vapor pressure of ether. The N.N.R. spectrophotometric method, although convenient, is applicable only in the laboratory of the manufacturer since it requires employing as a blank the menstruum used in preparing the solution.

In the course of investigating alternative

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