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Phenoxybenzamine stability in aqueous ethanolic solutions. I. Application of potentiometric pH stat analysis to determine kinetics

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Summary

The kinetics of decomposition of phenoxybenzamine (I) were determined as a function of apparent pH in 1 : 1 absolute ethanol : water. Decomposition proceeds through reversible cyclization to an unprotonated ethylenimonium ion [N-benzyl-N-(1-phenoxy-2-propyl)ethylenimonium ion, II], which then reacts with both water and ethanol to form solvolysis products. Both I and solvolysis products are weak bases that exist partially protonated. Decomposition of I involves the formation of titratable hydrogen ion from protonated I as II is formed. Solvolysis of II yields titratable hydrogen ion as the products are formed. At apparent pH values of 4.50 and below, all titratable hydrogen ion may be assigned to the cyclization process. At apparent pH values of 6.50 and above all titratable hydrogen ion may be assigned to the solvolysis process. This unambiguous assignment of titratable hydrogen ion to the individual processes allows individual rate constants to be determined from pH stat data only. Complementary analytical methods such as chloride ion analyses are unnecessary to elucidate the kinetics of I decomposition. The rate of cyclization was pH-dependent due to reaction of unprotonated I only. The pK_a of the conjugate acid of I was 5.01 and the specific rate constant for cyclization was 0.293 min^{-1}. The reversibility of the cyclization reaction was found to be minimal. The rate constant for the reaction between II and chloride ions to form I was 4×10^{-6} $M \cdot min^{-1}$. The solvolysis was found to be pH-independent with a rate constant of 0.0289 min^{-1}.

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Introduction

Phenoxybenzamine (I) is a 2-chloroethylamine and exhibits the characteristic instability of this group of compounds in aqueous or semiaqueous solutions. The drug is commercially available as the hydrochloride salt in 10 mg capsules for oral use. In addition, it is available for investigational purposes in ampules containing 100 mg of drug in 2 ml of 1 : 1 acidified ethanol : propylene glycol mixture. This solution, in which the drug exhibits adequate stability, is diluted with Sodium Chloride Injection or Dextrose Injection prior to intravenous or intra-arterial infusion.

The decomposition of 2-chloroethylamines in aqueous or semiaqueous solutions involves an initial cyclization during which chloride ion is produced, followed by hydrolysis of the cyclic intermediate during which hydrogen ion is produced. Early studies of the kinetics of 2-chloroethylamine decomposition were based on titration of these ions alone (Bartlett et al., 1947a and b) or combined with a thiosulfate titration, which quantitates the 2-chloroethylamine plus the cyclic intermediate (Golumbic et al., 1946; Hanby et al., 1947b; Harvey and Nickerson, 1953). Analysis of the rates of appearance of chloride ion and hydrogen ion permitted the rates of disappearance of the 2-chloroethylamine and appearance of the ethanolamine product, respectively, to be determined. The concentration of the cyclic intermediate could be estimated in the absence of thiosulfate data or directly calculated if thiosulfate data were available.

Later workers have applied more sophisticated analytical techniques to the study of the kinetics of 2-chloroethylamine decomposition. These techniques include spectrophotometry (Geissman et al., 1952; Friedman and Boger, 1961), fluorometry (Mellett and Woods, 1960), polarography (Mantsavinos and Christian, 1958; Zallen et al., 1961) and NMR spectroscopy (Pettit et al., 1965). Analytical limitations existing among these methods include lack of specificity and sensitivity and unsuitability to accurately quantitate the rapid cyclization reaction characteristic of this class of compounds at certain pH values.

The stability of the 2-chloroethylamines is pH-dependent. For example, I has been reported to be stable in acidified non-aqueous solutions, but unstable in neutral or alkaline solutions (Martindale: The Extra Pharmacopoeia, 1977). No data are available quantitating the rates of hydrolysis of I as a function of pH. The present work was undertaken to investigate this pH dependency. Kinetic determinations in this study utilized potentiometric pH stat analysis. During the course of the study, unique conditions were observed which permitted the sole use of this analytical method to define I decomposition kinetics. This analytical approach was confirmed by comparing the pH stat data with data obtained by chloride ion analysis and HPLC. The hydrochloride salt of I is sparingly water-soluble (Harvey and Nickerson, 1955; British Pharmacopoeia, 1973), thus the studies were performed in 1 : 1 absolute ethanol : water. The following paper will describe the kinetics in solvents of differing proportions of ethanol and water.

Materials and Methods

Materials

Phenoxybenzamine hydrochloride (I-HCl; Dibenzyline, lot 2-6DI, Smith Kline and French Laboratories, Philadelphia, PA) and 2-(N-benzyl-2-hydroxyethylamine)-1-phenoxypropane (III; SK + F no. 1010, RFD-2624-297-2, Smith Kline and French Laboratories, Philadelphia, PA) were used as received. 2-(N-benzyl-2-ethoxyethylamino)-1-phenoxypropane (IV) was synthesized by reaction of I-HCl in absolute ethanol in the presence of sodium according to the method used by Gump and Nikawitz (1950) for the synthesis of ethyl 2-dibenzylaminoethyl ether. The structure of the resulting compound, an oil, was confirmed by IR and NMR spectroscopy. Absolute ethyl alcohol was USP reagent quality and sodium nitrate was purified grade. All other chemicals were either reagent grade or primary standard grade.

The distilled water and ethyl alcohol were boiled for at least 15 min to remove dissolved carbon dioxide and then stored in tightly sealed bottles. The solvent used in all kinetic and pK_a determinations was prepared by mixing equal volumes of water and ethyl alcohol and adjusting to 0.05 M sodium nitrate to maintain constant ionic strength.

Instrumentation

Rate of hydrogen ion formation. A pH stat (Radiometer A/S, Copenhagen, Denmark) which consisted of a TTT2 titrator, SBR2c titrigraph and ABU11 Autoburette (0.25 ml burette) was used. The electrode pair was a Radiometer G202C glass electrode and a K401 saturated calomel electrode. A jacketed reaction vessel modeled after that described by Chong et al. (1967) was used to give a virtually airtight system. The delivery tube was positioned between the reference and glass electrodes in order that stirring would propel titrant directly toward the glass electrode for fastest response. An air space of approximately 5 mm existed above the solution after positioning the stopper. Equilibration of the air space with the atmosphere was prevented by placing a length of flexible plastic tubing over the inlet tube and immersing the other end in propylene glycol. The small bore D4332 delivery tube (Radiometer A/S, Copenhagen, Denmark) was required by the small volume of titrant and slow titration rate involved at certain pH values. Chemically resistant type S52312 grease prevented titrant leakage from the stopcock.

Rate of chloride ion formation. A Corning Digital 110 expanded scale pH meter (Corning Glass Works, Medfield, MA) equipped with a Corning 476126 solid state specific chloride ion electrode and Thomas 4857-H10 double-junction saturated calomel reference electrode (Arthur H. Thomas, Philadelphia, PA) were used to measure chloride ion concentration.

The pH was maintained with the pH stat equipped with the standard Radiometer TTA3 titration assembly which had a 2.5 ml burette. The electrode pair was a Radiometer G202C glass electrode and a K701 double-junction saturated calomel reference electrode.

HPLC studies. A Perkin-Elmer Model 1220 liquid chromatograph with built-in UV detector (set at 254 nm) and Model 165 recorder (Perkin-Elmer, Norwalk, CT)

was used. A 0.5 m long by 2.6 mm i.d. stainless steel column was packed with Permaphase ODS (octadecyltrimethoxysilane) stationary phase and support (E.I. duPont de Nemours, Wilmington, DE). The mobile phase was 7:3 and 6:4 95% ethanol:water in two pH 4.00 studies and 6:4 absolute ethanol:water in one pH 6.50 study. Pump flow rates were 0.75 ml/min (pH 4.00 studies) and 0.50 ml/min (pH 6.50 study).

pK_a determinations. Titrant was delivered from the Radiometer Autoburette (0.25 ml burette) into the airtight system described above. The pH measurements were made with either the Radiometer TTT2 titrator or the Corning Digital 110 pH meter.

Analog computer analysis. A Pace TR-48 analog computer and Model 1130 Variplotter recorder (Electronic Associates, West Long Branch, NJ) aided the kinetic analysis, particularly in the evaluation of the second-order rate constant for reaction between the cyclic intermediate and chloride ion to reform I. Analog computer programing and modeling principles are well described in the literature (Korn and Korn, 1956; Jenness, 1965; Stice and Swanson, 1965; EAI Applications Reference Library, 1969).

Kinetic procedures

Rate of hydrogen ion formation. A 40.00 ml volume of solvent was placed in the reaction vessel and maintained at 30 ± 0.02°C, the temperature used for all kinetic and pK_a studies. The solution was adjusted to the desired pH either by addition of titrant or external addition of 0.1 M nitric acid. The instrument was then maintained in an operational state for at least 5 min to permit attainment of pH steady-state.

The volume of titrant required to initially adjust the solution to the desired pH upon addition of I-HCl was determined through a preliminary study at each pH. This procedure facilitated determination of the initial course of rapid reactions and the total volume of hydroxide ion consumed during the reaction. Titration was continued until hydrogen ion production ceased or through at least 8 estimated half-lives. Concentrations of I-HCl and sodium hydroxide titrant were approximately $4.7\text{–}4.8 \times 10^{-4}$ M and 8.9×10^{-2} M, respectively. The change in ionic strength of the solution due to pH adjustment and titrant addition was negligible. Under all conditions of study, negligible volumes of titrant were consumed in the absence of drug.

Rate of chloride ion formation. The pH stat maintained the pH of drug solutions at pH 4.50 and above. Double-junction reference electrodes were used in these studies and in the subsequent chloride ion assay to prevent chloride ion contamination from the reference electrodes. Maximum sample dilution by titrant during the pH 4.50 study was less than 2.7%; in all other studies sample dilution was less than 1.5%. The buffer capacity of highly acidic solutions maintained the pH constant for solutions of pH 3.01 and below.

Aliquots of 5.00 ml were withdrawn at known time intervals and quenched with 10.00 ml of 0.15 M nitric acid. Chloride ion concentration was determined by the standard addition method of potentiometric analysis (Mowbray, 1969). The potential was determined before and after exactly 0.5 ml of approximately 9.0×10^{-3} M

sodium chloride solution was added to the quenched aliquot. Correction was made for the chloride ion introduced as the HCl salt of I. Remaining I determined from these data were plotted semilogarithmically versus time to calculate $k_{1,app}$, the apparent first-order rate constant for loss of total intact drug.

HPLC studies. A 40.00 ml volume of solvent was maintained at pH 4.00 (two studies) and pH 6.50 (one study) with the pH stat. Initial I-HCl concentrations were approximately 9.5×10^{-4} M and 2.2×10^{-3} M, respectively. Approximately 0.5 ml samples were withdrawn from the reaction vessel at known time intervals and either a 5.00 or a 10.00 μl aliquot was immediately injected. Kinetic analyses of I (pH 4.00) or III and IV (pH 6.50), identified by comparison with retention times of the known compounds, were based on peak heights.

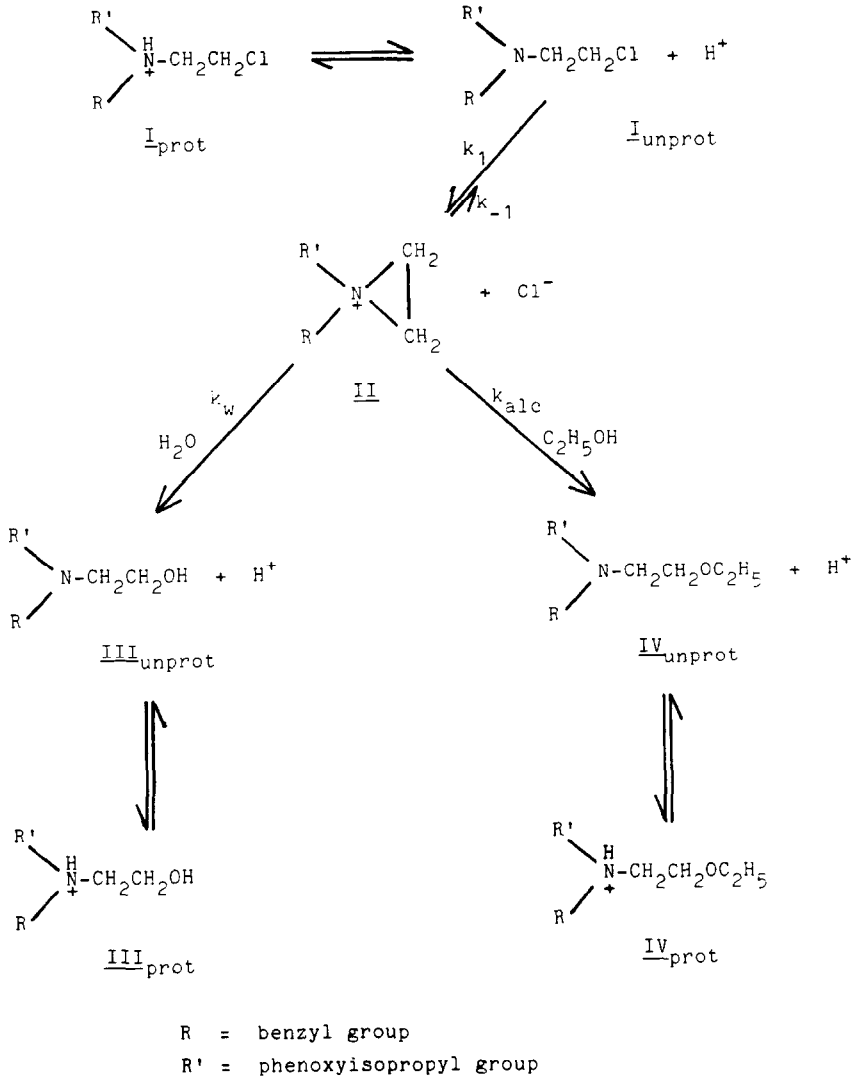
pK_a determinations

The rapid decomposition of I in solution at a pH equal to its pK_a (the apparent rate constant equals one-half the specific rate constant for cyclization) required rapid pK_a determination. The apparent pK_a was determined by half-neutralization of I-HCl with sodium hydroxide, followed by pH measurement 30 s later. At this time less than 10% decomposition has occurred. The longer time required for pK_a determination by automatic potentiometric titration would have produced greater error. The apparent pK_a values of the conjugate acids of III and IV were determined by half-neutralization of the free bases with hydrochloric acid. Stability was not a problem for these compounds and pH measurements were made between 30 and 60 s. The concentrations of each compound ranged from approximately 4.6×10^{-4} M to 6.0×10^{-4} M. No corrections were necessary for significant hydrogen ion or hydroxyl ion concentrations (Albert and Serjeant, 1971).

The pH meters and pH stat were standardized against aqueous buffers. Hence, experimentally measured pH values and mixed solvent apparent acid dissociation constant values (in the negative logarithmic form, $p_sK'_a$) of the conjugate acids of I, III and IV are reported. These are not thermodynamic values referred to the standard state in 1 : 1 ethanol : water. Conversion to thermodynamic values would require correction of the dissociation constants for ionic strength of the medium and of both pH values and dissociation constants for a combination of liquid junction potential and medium effect for hydrogen ion (Ong et al., 1964; Bates, 1973).

Results and Discussion

The high degree of reactivity of the alkyl and arylalkyl 2-chloroethylamines (Golumbic et al., 1946; Hanby et al., 1947b; Chapman and James, 1954; Chapman and Triggle, 1963; Kerwin et al., 1947), including I (Harvey and Nickerson, 1953), is generally postulated to involve the formation of an ethylenimonium ion. The decomposition of I in 1 : 1 ethanol : water is presented in Scheme 1. The important characteristics of this decomposition are: (1) equilibrium between protonated and unprotonated I with only the unprotonated drug being reactive (Cohen et al., 1948); (2) formation of equimolar chloride ion as cyclization occurs; (3) solvolysis of the



Scheme 1.

ethylenimonium ion II to the unprotonated products III and IV with liberation of equimolar hydrogen ion; and (4) equilibrium of the unprotonated and protonated solvolysis products.

Order of the cyclization reaction

The cyclization rate of I-HCl in 1 : 1 ethanol : water was determined from the rate of appearance of chloride ion in solution. Semilogarithmic plots of remaining I

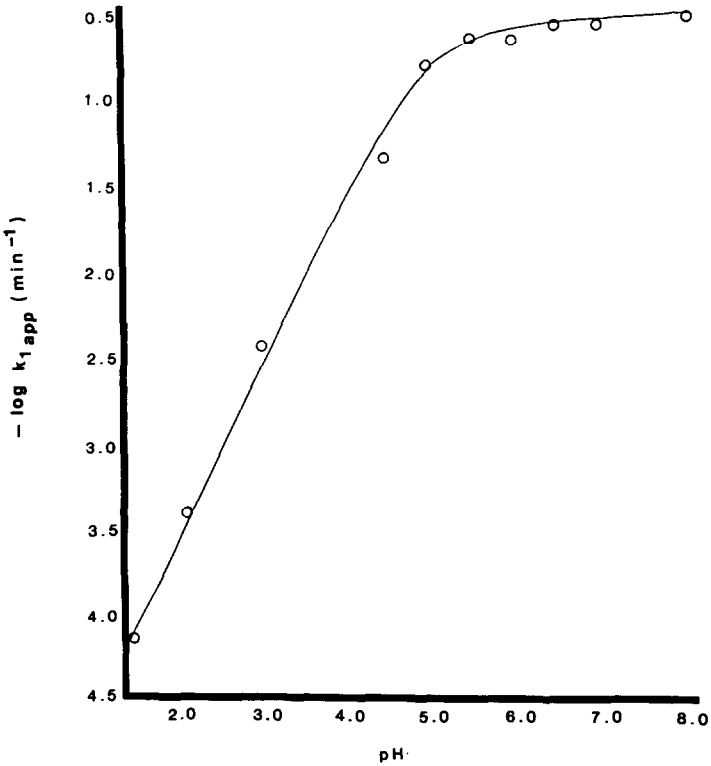


Fig. 1. The pH–rate profile for decomposition of I in 1:1 absolute ethanol:water, based on chloride ion analysis. The line is calculated from Eqn. 3 and the mean k_1 determined from all chloride ion analyses.

versus time were linear over the time period studied. Rates were first-order with respect to I over the pH range of 1.47–8.00.

pH–rate profile of the cyclization reaction

The pH–rate profile based upon the rate constants determined by chloride ion analysis is presented in Fig. 1. The rate law for cyclization of I according to Scheme 1 is given by Eqn. 1,

$$-\frac{d(I_T)}{dt} = k_{1,app}(I_T) \tag{1}$$

where $k_{1,app}$ is the apparent first-order rate constant for loss of total intact drug and (I_T) is the total concentration of phenoxybenzamine, both protonated and unprotonated, present at any time. This assumes that k_{-1}, the specific second-order rate constant for conversion of II and chloride ion to I, is negligible, as will be established in a subsequent section.

Only unprotonated I is reactive. Therefore, $k_{1,app}$ is equal to the unprotonated

TABLE 1

APPARENT pK_a VALUES OF THE CONJUGATE ACIDS OF PHENOXYBENZAMINE (I) AND SOLVOLYSIS PRODUCTS (III AND IV) IN 1:1 ABSOLUTE ETHANOL:WATER

Compound	$p_sK'_a$
I	5.01 ± 0.02 [a]
III	6.67 [b]
IV	6.70 [b]

[a] Mean $\pm$ S.D. (n = 4).

[b] The average of two determinations, differing by not more than 0.01 pH units in each case.

fraction of I_T, $f_{I,unprot}$, times k_1, the specific first-order rate constant for conversion of unprotonated I to II (Eqn. 2).

$$k_{1,app} = f_{I,unprot} k_1 \tag{2}$$

Eqn. 3 expresses $k_{1,app}$ in terms of ${}_sK'_{aI}$, the mixed solvent apparent acid dissociation constant of the conjugate acid of I.

$$k_{1,app} = \frac{{}_sK'_{aI}}{{}_sK'_{aI} + (H^+)} \cdot k_1 \tag{3}$$

This equation has previously been applied to cyclization reactions of tertiary 2-chloroethylamines (Cohen et al., 1948).

The $p_sK'_a$ value of I was found to be 5.01 (Table 1), which is similar to the reported value of 5.1 in 1:1 methanol:water at 25°C (Beddoe and Smith, 1971). Using Eqn. 3, a value of k_1 was calculated from each of the ten $k_{1,app}$ versus pH data points of Fig. 1 and the observed $p_sK'_{aI}$ of 5.01. k_1 equals 0.293 ± 0.045 min^{-1} (mean $\pm$ S.D.).

Rate of II solvolysis determined from pH stat titration

In general, the specific rate constant for cyclization of alkyl and arylalkyl 2-chloroethylamines is larger than the rate constant for the subsequent ethylenimonium ion hydrolysis. This has been established in studies of many different 2-chloroethylamines. Such studies (Bartlett et al., 1947a and b; Chapman et al., 1952; Chapman and James, 1954; Cohen et al., 1948), including those of I (Harvey and Nickerson, 1953), have been conducted in neutral or basic aqueous or semiaqueous solutions. Under these conditions the 2-chloroethylamines are unprotonated. The production of hydrogen ion must therefore be due only to solvolysis of the ethylenimonium ion. None of the hydrogen ion arises from deprotonation of the 2-chloroethylamines. Consequently, the rate of production of hydrogen ion under these conditions is a measure of the rate of the solvolysis step.

Potentiometric pH stat analyses of the appearance of hydrogen ion were conducted at experimentally measured pH values where I is largely unprotonated.

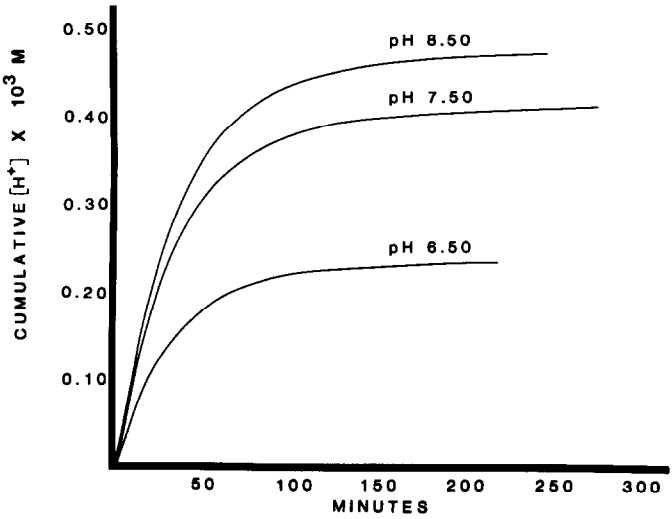


Fig. 2. pH stat curves of cumulative hydrogen ion concentration at pH 6.50, pH 7.50 and pH 8.50 for representative studies in 1:1 absolute ethanol:water.

Studies were performed at pH values of 6.50, 7.50 and 8.50. Fig. 2 shows the cumulative hydrogen ion concentration data for the decomposition of I at these pH values. Each plot possesses a sigmoidal shape characteristic of the concentration of C in a simple series first-order A to B to C reaction (Moore and Pearson, 1981) represented by Scheme 2.

$$A \xrightarrow{k_1} B \xrightarrow{k_2} C$$

Scheme 2

Potentiometric pH stat data at pH 4.50 indicates that the value of k_{-1} (in Scheme 1) is very small, as will be shown. As an approximation, therefore, the rate of formation of C at any time t is given by Eqn. 4.

$$\frac{dC}{dt} = \frac{k_1 k_2 A_0}{(k_2 - k_1)} \cdot (e^{-k_1 t} - e^{-k_2 t}) \tag{4}$$

In this equation, A_0 represents $(I_T)_0$, the total concentration of both protonated and unprotonated phenoxybenzamine at time zero, and k_2 is the first-order rate constant for II solvolysis, the sum of the individual rate constants k_w and k_{alc} for reaction of II with water and ethanol, respectively.

When k_1 is much greater than k_2 and at times sufficiently late in the study, only the second exponential term is of quantitative significance. Estimates of k_2 may be obtained from Eqn. 4 by substitution of hydrogen ion concentration for C and adjustment for $f_{Products,unprot}$, the fraction of both III and IV that is unprotonated at

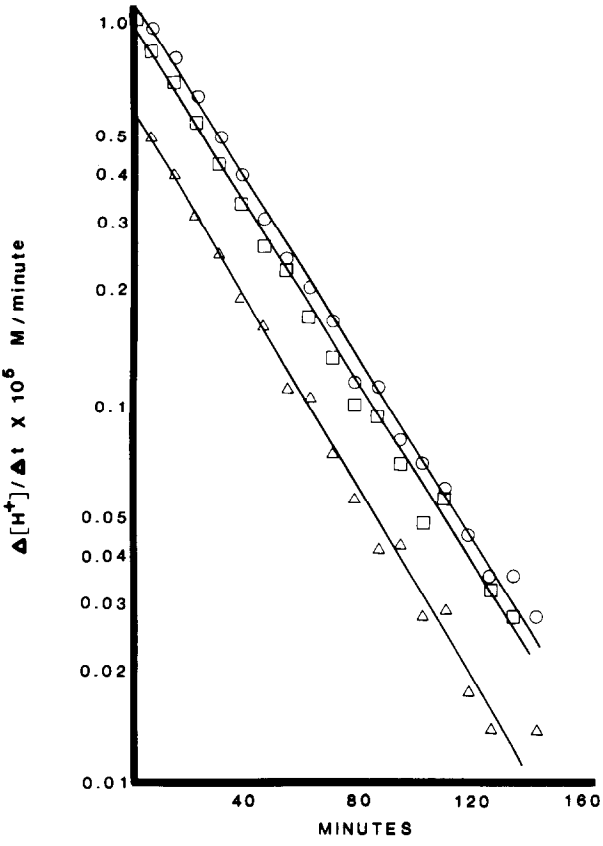


Fig. 3. Apparent first-order plots of the finite increments of hydrogen ion concentration and time versus time at pH 6.50 (Δ), pH 7.50 (□) and pH 8.50 (○) for representative studies in 1:1 absolute ethanol:water.

a particular pH, thus the fraction of products that yield titratable hydrogen ion. The rate of hydrogen ion production may be expressed in logarithmic form as the ratio of the finite increments of hydrogen ion concentration and time (Eqn. 5).

$$\log \frac{\Delta(H^+)}{\Delta t} \simeq -\log \frac{k_1 k_2 f_{\text{Products,unprot}} (I_T)_0}{k_2 - k_1} - \frac{k_2 t}{2.303} \qquad (5)$$

Semilogarithmic plots of the potentiometric pH stat data over the pH 6.50 to pH 8.50 range are given in Fig. 3. The observed solvolysis rate constants obtained from the terminal slopes of these plots (Table 2) are pH-independent; k_2 equals 0.0289 ± 0.0008 min^{-1} (mean ± S.D. for the eight runs).

pH dependency of titratable hydrogen ion

The cumulative hydrogen ion concentration data over the pH 6.50 to 8.50 range (Fig. 2) demonstrated unexpected pH dependency. A possible explanation for this

TABLE 2

FIRST-ORDER RATE CONSTANTS FOR SOLVOLYSIS OF ETHYLENIMONIUM ION II BETWEEN pH 6.50 AND pH 9.50 [a]

pH	k_2 (min^{-1})
6.50	0.0294 (0.0292, 0.0296)
7.50	0.0287 (0.0289, 0.0285)
8.50	0.0282 (0.0272, 0.0291)
9.50	0.0292 (0.0296, 0.0288)

[a] Mean; individual values are reported in parentheses.

observation is the pH-dependent formation of additional product. Harvey and Nickerson (1953) have suggested that a small amount (calculated at about 5.5% by the present investigators) of a piperazinium dimer is produced as a result of decomposition in a 7 : 3 ethanol : water 0.020 M solution of I. Formation of this product results in chloride ion but no hydrogen ion production. In the present study the drug concentration (5×10^{-4} M) and solvent (1 : 1 ethanol : water) conditions would tend to decrease the proportion of piperazinium salt formed from 2-chloroethylamines (Bartlett et al., 1947a and b; Hanby and Rydon, 1947a). Therefore, dimer formation is an unreasonable explanation for the observed results.

To better define the nature of the pH dependency of titratable hydrogen ion, the decomposition of I was followed by potentiometric pH stat titration at pH 3.50, pH 4.50 and pH 5.50. The results are presented in Fig. 4. In contrast to the results at and above pH 6.50, cumulative hydrogen ion concentration plateau levels increased with decreasing pH. Fig. 5 presents the fraction of hydrogen ion titrated, i.e. the number of moles of sodium hydroxide added at the plateau divided by the initial number of moles of I_T.

The explanation for the pH dependency of titratable hydrogen ion was suggested by the apparent pK_a values of the conjugate acids of 2-chloroethylamines and their ethanolamine analogs. Cohen et al. (1952) found that the apparent pK_a values of N-methyl-bis(2-chloroethyl)amine and its first hydrolysis product, N-methyl-N-(2-chloroethyl)ethanolamine in water at 37°C were 5.97 and 7.02, respectively. The differences in apparent pK_a values of the N-ethyl analogs of the same two compounds in 1 : 3 acetone : water and in 2 : 1 acetone : water at 25°C were 1.19 and 1.61 units, respectively, the ethanolamine having the higher value in each case (Bartlett et al., 1947b). The greater acidity of the original compounds is due to the more electron-withdrawing nature of a chloro group relative to a hydroxy or alkoxy group, which increases the acidity of acids and bases (March, 1968).

A large difference in apparent pK_a values between I and its solvolysis products, such as observed with the bis(2-chloroethyl)amines, could produce the pH dependency of Fig. 5. The apparent pK_a values of the two solvolysis products, III and IV, were determined (Table 1) and found to be 1.66 and 1.69 units higher, respectively, than that of I. The direction and magnitude of the apparent pK_a differences between

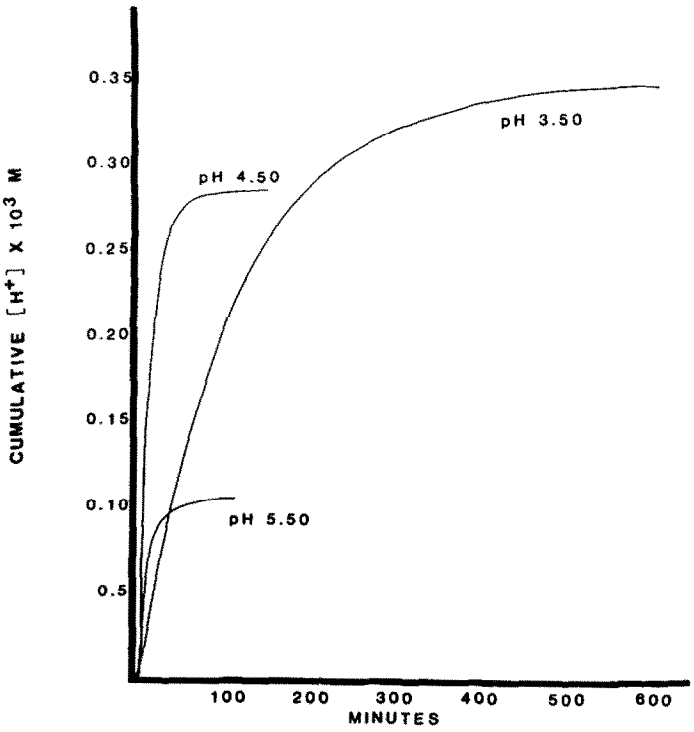


Fig. 4. pH stat curves of cumulative hydrogen ion concentration at pH 3.50, pH 4.50 and pH 5.50 for representative studies in 1 : 1 absolute ethanol : water.

reactant and products are consistent with those observed for the bis(2-chloroethyl)amines.

Scheme 1 assumes that III and IV are the only end products, i.e. there are no competing, non-hydrogen ion producing reactions. The effect of I solvolysis is to convert intact drug with a $p_sK'_a$ of 5.01 to products with values of 6.67 and 6.70. These increases result in products that are more highly protonated than are reactants at any particular pH value, in accord with Eqns. 6 and 7.

$$f_{I,prot} = \frac{(H^+)}{(H^+) + {}_sK'_{aI}} \tag{6}$$

$$f_{Products,prot} = \frac{(H^+)}{(H^+) + {}_sK'_{aIII}} \tag{7}$$

where $f_{I,prot}$ and $f_{Products,prot}$ are the fractions of I and products protonated, respectively. The closeness of the apparent pK_a values of the two solvolysis products permits $f_{Products,prot}$ to be calculated from sK'_{aIII} only. The increase in the fraction of products protonated compared to the fraction of reactant protonated is pH-dependent. Hydrogen ion required to satisfy the increased protonation requirements of the solvolysis products is unavailable for titration and results in a net decrease in

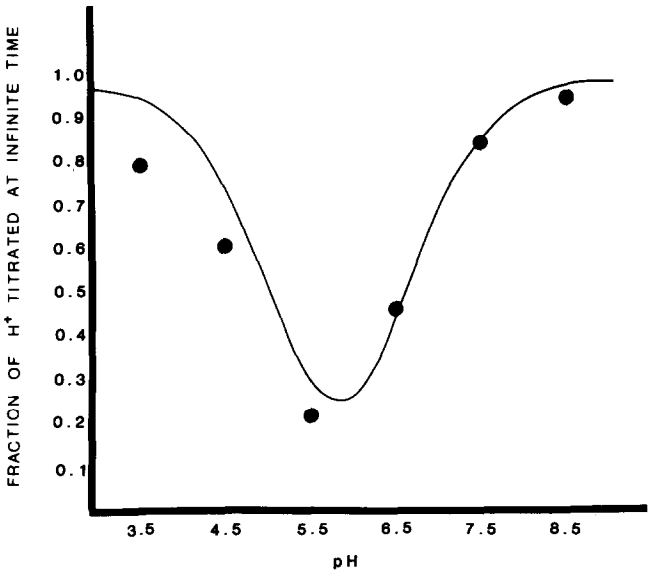


Fig. 5. Fraction of hydrogen ion titrated versus pH after completion of I decomposition in 1:1 absolute ethanol:water. The points represent the observed number of moles of sodium hydroxide added at reaction completion divided by the initial number of moles of I_T. The line is calculated from Eqn. 8.

titratable hydrogen ion at the completion of the reaction, t_{inf}. The decrease in titratable hydrogen ion, $f_{Products,prot}$ minus $f_{I,prot}$, is equal to the increase in the fraction of products protonated compared to the fraction of reactant protonated. The net fraction of hydrogen ion available for titration upon completion of solvolysis, $f_{Hydrogen\ ion\ titrated,t_{inf}}$, is equal to unity (the fraction of hydrogen ion produced by solvolysis) minus the decrease in titratable hydrogen ion, as shown in Eqn. 8.

$$f_{Hydrogen\ ion\ titrated,t_{inf}} = 1 - (f_{Products,prot} - f_{I,prot}) \tag{8}$$

The calculated fraction of hydrogen ion titrated at completion of the reaction is shown in Fig. 5. The bell-shaped nature of this plot is attributable to the relationship between protonated reactant and products. At pH values sufficiently less than or greater than the apparent pK_a values of reactant and products, these compounds are essentially completely protonated or completely unprotonated, respectively. Under these conditions, there is no net change in the degree of protonation in the transition from reactant to products. Hence, the parenthetical term in Eqn. 8 approaches zero and the plot approaches unity. Upon substitution of Eqns. 6 and 7 into Eqn. 8 and differentiating with respect to hydrogen ion concentration, $(H^+)_{min}$, the minimum titratable hydrogen ion concentration, may be calculated (Eqn. 9).

$$(H^+)_{min} = \frac{{}_sK'_{aI} - \sqrt{\frac{{}_sK'_{aI}}{{}_sK'_{aIII}}} \cdot {}_sK'_{aIII}}{\sqrt{\frac{{}_sK'_{aI}}{{}_sK'_{aIII}}} - 1} \tag{9}$$

From this equation, the minimum in Fig. 5 is found to occur at pH 5.84.

The quantitatively good agreement between observed and predicted amounts of hydrogen ion titrated at pH 6.50 and above (Fig. 5) suggests that under the conditions of this study the only decomposition products are III and IV. At these pH values, essentially all titratable hydrogen ion is due to solvolysis of II with very small amounts due to deprotonation of protonated I. Formation of a piperazinium dimer would not result in titratable hydrogen ion at these pH values. Formation of a quaternary ammonium product (Golumbic et al., 1946; Cohen et al., 1952) by reaction of II with III or IV would produce less than the anticipated amount of hydrogen ion since only half of the molecules forming the quaternary ammonium product would have solvolyzed. Formation of such products therefore appears not to occur. Significant error between observed and predicted values at pH 5.50 and below was observed. Predicted amounts of titratable hydrogen ion are sensitive to error in $p_sK'_{aI}$ over this range. Attempts to improve agreement at each of the three pH values studied by substituting lower $p_sK'_{aI}$ values into Eqn. 8 were not successful. A satisfactory explanation for the error is unknown.

Rate of cyclization determined from pH stat titration

At pH 6.50 and above the rate of hydrogen ion accumulation yielded k_2. At pH 4.50 and below the solvolysis products are essentially completely protonated. Therefore, hydrogen ion that is produced as a result of II solvolysis is unavailable for titration. Any hydrogen ion produced during drug decomposition at pH 4.50 and below can thus be unambiguously assigned to deprotonation of protonated I. At pH 4.50 and below a large fraction of I is protonated. Protons produced as I is converted to II, which only exists unprotonated, are available for titration and may be represented by B in a simple A to B series reaction. As expected for the appearance of B, the cumulative hydrogen ion concentration plots at pH 4.50 and below lack the sigmoidal nature of the plots at pH 6.50 and above. The rate of the cyclization reaction based upon these plots is given by Eqn. 10.

$$\frac{d(H^+)}{dt(f_{I,prot})} = -\frac{d(I_T)}{dt} = k_{1,app}(I_T) = k_{1,app}(I_T)_0 e^{-k_{1,app}t} \tag{10}$$

Division of the rate of appearance of hydrogen ion concentration by $f_{I,prot}$ is necessary since only protonated I can produce titratable hydrogen ion. Transformation of Eqn. 10 into the logarithmic form given in Eqn. 11 permits determination of $k_{1,app}$ from the semilogarithmic plots given in Fig. 6.

$$\log \frac{\Delta(H^+)}{\Delta t} \simeq \log k_{1,app} f_{I,prot}(I_T)_0 - \frac{k_{1,app}t}{2.303} \tag{11}$$

Excellent agreement was observed between values of $k_{1,app}$ measured by hydrogen ion and chloride ion analyses (Table 3).

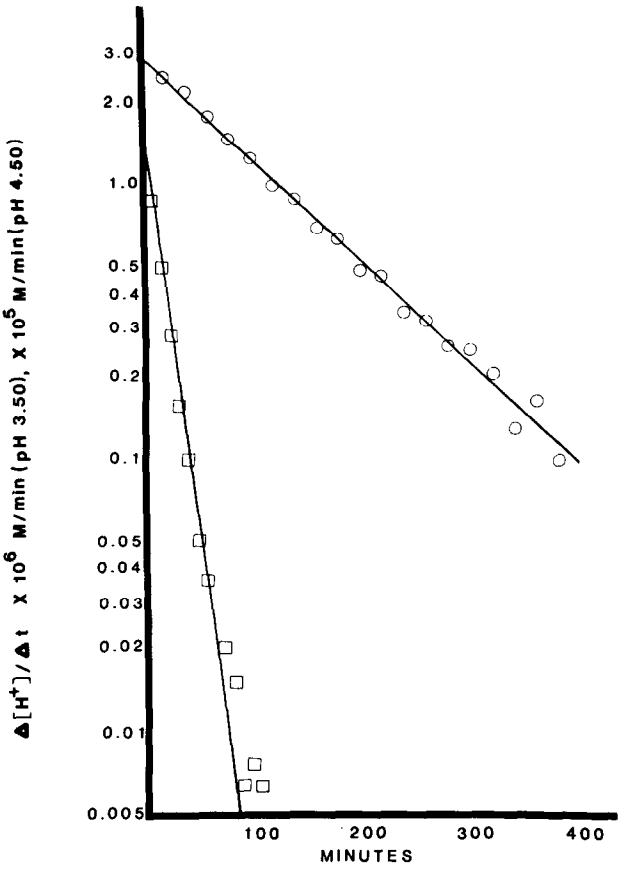


Fig. 6. Apparent first-order plots of the finite increments of hydrogen ion concentration and time versus time at pH 3.50 (○) and pH 4.50 (□) for representative studies in 1:1 absolute ethanol:water.

TABLE 3

APPARENT FIRST-ORDER RATE CONSTANTS AND HALF-LIVES FOR CYCLIZATION OF PHENOXYBENZAMINE (I) AT pH 3.50 AND pH 4.50 IN 1:1 ABSOLUTE ETHANOL:WATER, DETERMINED BY TWO METHODS

pH	Hydrogen ion analysis [a]		Chloride ion analysis	
	$k_{1,app}$ (min^{-1})	$t_{1/2}$ (min)	$k_{1,app}$ (min^{-1})	$t_{1/2}$ (min)
3.50	$8.77 \times 10^{-3} \pm 0.00 \times 10^{-3}$	79.0	8.76×10^{-3} [b]	79.1
4.50	$6.93 \times 10^{-2} \pm 0.39 \times 10^{-2}$	10.0	6.70×10^{-2} [c]	10.1

[a] Results expressed as mean ± S.D. (n = 2).

[b] Calculated from the k_1 of 0.293 min^{-1} determined by chloride ion analysis and Eqn. 3; the rate of chloride ion formation at pH 3.50 was not studied.

[c] Based upon only one study.

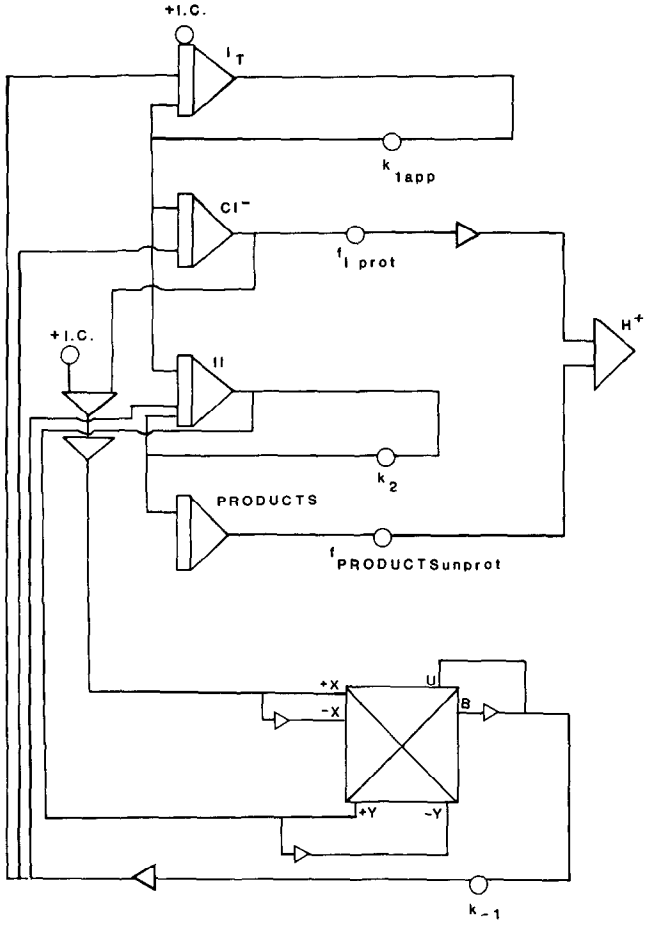


Fig. 7. Analog computer program representing the decomposition of I in 1 : 1 absolute ethanol : water. All computer symbols are standard. All concentration terms are molar. The two initial condition (I.C.) potentiometers were adjusted to produce initial I and chloride ion concentrations in agreement with the observed infinite time product concentrations.

Rate of reverse reaction determined from pH stat titration

The graphically determined values of k_1 and k_2 were based upon negligible contributions of k_{-1} to the cyclization and solvolysis reactions. To determine the accuracy of the calculated values of k_1 and k_2, as well as to evaluate k_{-1}, an analog computer model (Fig. 7) based upon Eqns. 12–16 was utilized.

$$\frac{d(I_T)}{dt} = -f_{I,unprot}k_1(I_T) + k_{-1}(II)\left[(Cl^-) + (I_T)_0\right] \tag{12}$$

$$\frac{d(II)}{dt} = f_{I,unprot}k_1(I_T) - k_{-1}(II)\left[(Cl^-) + (I_T)_0\right] - k_2(II) \tag{13}$$

$$\frac{d(Cl^-)}{dt} = f_{I,unprot}k_1(I_T) - k_{-1}(II)\left[(Cl^-) + (I_T)_0\right] \tag{14}$$

$$\frac{d(Products)}{dt} = k_2(II) \tag{15}$$

$$\frac{d(H^+)}{dt} = (Cl^-)f_{I,prot} + (Products_T)f_{Products,unprot} \tag{16}$$

where $(Products_T)$ is the total concentration of both III and IV, both protonated and unprotonated, present at any time; and (Cl^-) is the concentration of chloride ion present at any time that was originally covalently bound; it does not include chloride ion obtained from the hydrochloride salt of I.

Potentiometers were adjusted to simulate pH 6.50 conditions, utilizing the graphically determined values of $k_{1,app}$ and k_2. The potentiometer representing k_{-1} was then adjusted to obtain the best fit to the experimental data. The procedure was repeated with the pH 8.50 data. Although the analog computer-generated curves were relatively insensitive to variations in k_{-1} due to the minor contribution of the reverse reaction to overall II disappearance, the data were best fit with k_{-1} values between 0 and 8×10^{-6} $M \cdot min^{-1}$. The cumulative hydrogen ion concentration curves at pH 6.50 and above were also quite insensitive to the value of $k_{1,app}$ since the solvolysis step is slow relative to the cyclization step. These curves were highly sensitive to the value of k_2, however. The graphically determined value of k_2 produced a very good computer fit to the data, whereas values plus and minus 10% and 20% different produced very poor fits to the data. The analog computer therefore confirmed the correctness of the graphical evaluation of k_2.

At pH 4.50, $k_{1,app}$ is approximately one-fourth as large as at pH 6.50. Further, at pH 4.50 the rate of hydrogen ion production represents the cyclization reaction. As a result, the pH 4.50 data is more sensitive to both $k_{1,app}$ and k_{-1} than is the pH 6.50 data. The potentiometers were adjusted to simulate the pH 4.50 conditions, based upon the graphically determined estimates of $k_{1,app}$ and the average value of k_2 observed at pH 6.50 and above. The best fit to the data was obtained with k_{-1} values of 0 and 4×10^{-6} $M \cdot min^{-1}$. Only after cyclization was 75% complete did altering the value of k_{-1} from 0 to 4×10^{-6} $M \cdot min^{-1}$ influence the cumulative hydrogen ion concentration curve. Therefore, the values of $k_{1,app}$ obtained from the Fig. 6 plots yield correct values of $k_{1,app}$.

To improve the estimates of k_{-1}, drug decomposition was followed at pH 4.50 in the presence of potassium chloride approximately equimolar to $(I_T)_0$. The curve was thus considerably more sensitive to variations in the magnitude of k_{-1}. The k_{-1} potentiometer was varied between 0 and 12×10^{-6} $M \cdot min^{-1}$, producing significant differences in the shape of the curve. Duplicate experiments yielded the best fit to the data at k_{-1} equal to 4×10^{-6} $M \cdot min^{-1}$.

Conditions for kinetic analysis determined from pH stat data only

As shown, decomposition kinetics of I can be deduced from pH stat data alone. Additional analytical methods are redundant. Based upon titratable hydrogen ion,

three conditions are essential to the complete kinetic analysis of a reaction of the type represented by Scheme 1. First, the reaction intermediate B must be unprotonated at all times. Second, both reactant A and product(s) C must liberate titratable hydrogen ion. These conditions are essential to unambiguously assign titratable hydrogen ion to either the A to B or the B to C process. Any cumulative hydrogen ion concentration curve that includes significant amounts of hydrogen ion from both processes will yield an incorrect rate constant. Third, specific rate constant k_1 must be much greater than rate constant k_2, in order that the A to B process not be a rate-determining step when k_2 is evaluated.

At pH 4.50 and below, the pH is over two units away from the apparent pK_a of the products. Hence, the solvolysis step will contribute negligibly (less than 1% titratable hydrogen ion) to the cumulative curve resulting from the cyclization step. At higher pH values (such as pH 5.00), the solvolysis step will produce increasing proportions of titratable hydrogen ion, potentially increasing the error in the estimate of $k_{1,app}$. The actual contribution of titratable hydrogen ion due to solvolysis to that resulting from cyclization is a function of the three rate constants. When k_2 is small relative to $k_{1,app}$, titratable hydrogen ion resulting from solvolysis is negligible during the initial few drug half-lives that serve as the basis for evaluating $k_{1,app}$ (Eqn. 11).

At pH values above 7.01, the cyclization step will contribute less than 1% titratable hydrogen ion to the cumulative hydrogen ion concentration curve resulting from solvolysis. At lower pH values, the cyclization step will produce increasing proportions of titratable hydrogen ion, potentially increasing the error in the estimate of k_2. At pH 6.50, the cyclization step produces about 3% titratable hydrogen ion. In the present study a valid estimate of k_2 was obtained at this pH since $k_{1,app}$ is almost ten times that of k_2.

No attempt was made to kinetically analyze data within the pH 5.00 to 6.50 range approximately bounded by the pK_a values of drug and products. Within this range, both cyclization and solvolysis processes contribute titratable hydrogen ion. Analytical error is increased due to the decrease in the fraction of titratable hydrogen ion. Also, $k_{1,app}$ is smaller than at higher pH values, invalidating the approximation of Eqn. 5.

Decomposition kinetics determined from HPLC analyses

HPLC analyses yielded peaks representative of I, III and IV, enabling validation of the potentiometric methods of analysis. The cyclization rate of I was determined at pH 4.00 over a 1-h period. Good linearity of semilogarithmic plots of I peak height versus time were observed for over 30 min, after which slight upward deviation occurred as a result of the reverse reaction. Results of the two studies were very similar, with an average $k_{1,app}$ of 2.65×10^{-2} min^{-1}. This value is in excellent agreement with $k_{1,app}$ of 2.61×10^{-2} min^{-1} at pH 4.00 calculated from the potentiometrically determined k_1. Analog computer analyses of the pH 4.00 HPLC data yielded a k_{-1} of 3×10^{-6} $M \cdot min^{-1}$. The appearance of III and IV were determined at pH 6.50 over a 2-h period. Analog computer analysis of III, IV and III + IV peak heights versus time produced satisfactory fitting of the data with k_2 values of 0.037,

0.029 and 0.033 min^{-1}, respectively. The average k_2 of 0.033 min^{-1} is in reasonable agreement with the k_2 of 0.0288 min^{-1} determined by potentiometric titration.

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