

required ligand-metal complex concentration, it must have been taken up by the DNA from the surrounding solution. The negative peak that followed the DNA peak represented the ligand-metal complex removed from the solution. The two peak areas were equal.

Figure 7B shows the behavior when an additional 20 equivalents of streptonigrin-Zn<sup>2+</sup> were introduced with the DNA sample. The negative peak was nearly absent, although the positive peak height due to the complex was the same as before. The apparent stoichiometry determined from several such elution curves, in which the negative peak was nearly absent, was 1 mole of streptonigrin/20–25 moles of DNA-phosphorus in the presence of 7 M equivalents of Zn<sup>2+</sup>.

The column behavior in the absence of Zn<sup>2+</sup> is shown in Fig. 7C. The initial negative peak suggested that the DNA displaced I from its position on the column and emerged first. The displaced streptonigrin appeared as the subsequent positive peak. This finding further indicates that complexation did not occur in the absence of Zn<sup>2+</sup>.

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## ACKNOWLEDGMENTS

The author thanks Mr. John L. Davenport for financial support, Dr. John Douros of the National Cancer Institute for the streptonigrin, and Dr. Stephen G. Schulman, College of Pharmacy, University of Florida, for many valuable discussions. The author also thanks Lederle Laboratories, Division of American Cyanamid Co., for the "Lederle Pharmaceutical Faculty Award" for 1977 based on the subject of this paper.

# 3-Quinuclidinyl Benzilate Hydrolysis in Dilute Aqueous Solution

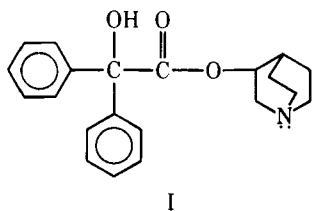
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Received March 9, 1977, from the *Environmental Research Division, Chemical Systems Laboratory, Department of the Army, Aberdeen Proving Ground, MD 21010*. Accepted for publication January 9, 1979.

**Abstract** □ The hydrolysis kinetics of 3-quinuclidinyl benzilate were determined over the pH 0–14 range at different temperatures in solutions buffered to a constant pH. Analysis of data extrapolated to zero buffer concentrations permitted construction of an overall rate expression for pH 0–14 and 0–100°. Reaction mechanisms are discussed.

**Keyphrases** □ Quinuclidinyl benzilate—hydrolysis in dilute aqueous solution, kinetics, pH, temperature □ Aminoalcohol esters—quinuclidinyl benzilate, hydrolysis in dilute aqueous solution, kinetics □ Hydrolysis kinetics—quinuclidinyl benzilate in dilute aqueous solution, pH, temperature

3-Quinuclidinyl benzilate (I), like atropine, is a muscarinic cholinergic antagonist of considerable pharmacological interest (1). Among the properties of greatest pharmaceutical significance is its hydrolytic behavior in dilute aqueous solution. In the present research, sufficient information was gained to permit hydrolysis rate prediction in the absence of high general base or salt concentrations at any desired temperature (0–100°) or pH (0–14).



## EXPERIMENTAL

**Reagents and Materials**—Technical grade I was dissolved in chloroform, decolorized with charcoal, and extracted into aqueous hydrochloric acid. The acidic solution was made slightly basic and back-extracted with chloroform. Solvent evaporation left a solid residue, which was recrystallized twice from ethyl acetate to give a product of at least 98% purity.

*Anal.*—Calc. for: C, 74.75; H, 6.87; N, 4.15; O, 14.23. Found: C, 74.6; H, 7.1; N, 4.3; O, 14.4.

Compound I-HCl was prepared by addition of hydrogen chloride-saturated ether to a chloroform solution of I; the crystalline precipitate was dried carefully. All solutions were made with carbon dioxide-free distilled water. Commercial grade buffers were used without additional purification. Ether was purified of peroxide by filtration through an alumina column.

**Instrumentation**—A spectrophotometer<sup>1</sup> with 1-cm quartz cells or 10-cm silica cells was used for absorptiometric determinations. The pH was determined with a pH meter<sup>2</sup>. Solvent contamination with stopcock grease was avoided through the use of separators with polytef stopcocks for liquid-liquid extractions. The reaction solutions were kept at a constant temperature with water baths<sup>3</sup>, or, in some cases, the reacting mixtures were sealed in glass vials immersed in appropriate refluxing solvents that kept the temperature constant.

**Procedure**—Products were isolated from a pH 11.5 reaction mixture that was kept 4 hr at 60°. Only benzoic acid and 3-quinuclidinol were found; these compounds were identical to authentic samples according to IR and melting-point analyses.

<sup>1</sup> Beckman DK-2.

<sup>2</sup> Beckman model G.

<sup>3</sup> Forma 2095S and Aminco 4-8600.

To determine the I hydrolysis rates, concentration analyses were made at appropriate intervals. The reaction solutions were buffered to a constant pH, adjusted to 0.055 ionic strength with sodium chloride, and thermostated to within  $\pm 2^\circ$ . Kinetic experiments above pH 9 were performed under a nitrogen atmosphere to reduce carbon dioxide absorption.

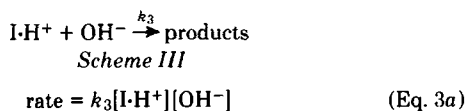
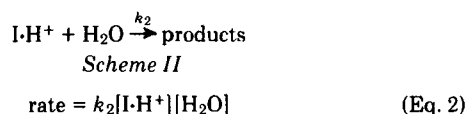
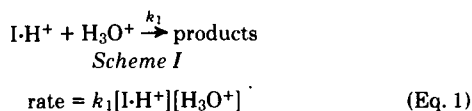
Because of the limited solubility of I at high pH, two analytical procedures were employed. Each reaction mixture for studies below pH 7 was at an initial I-HCl concentration of 0.0023 M. Ten-milliliter aliquots of the reaction mixture were taken at appropriate intervals, mixed with 2 ml of 1.3 M H<sub>2</sub>SO<sub>4</sub>, and extracted with 10 ml of ether to remove benzoic acid. The aqueous extraction layer was collected and analyzed spectrophotometrically for I (as the protonated species, I-H<sup>+</sup>) at 258 nm using a 1-cm light path cell.

For kinetic studies above pH 9, initial concentrations of 0.00026 M I-HCl were used. Forty-milliliter aliquots of the reaction mixtures were extracted with mixtures of 40 ml of ether and 8 ml of 1.3 M H<sub>2</sub>SO<sub>4</sub>. Aqueous layers were analyzed spectrophotometrically as already described with 10-cm light path silica cells.

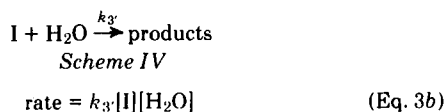
No appreciable error was introduced in the analyses due to I hydrolysis after extraction of the acidified mixtures since the molar absorptivities of I and benzoic acid were not significantly different. The molar absorptivities at 258 nm were 479 and 481, respectively. Also, the hydrolysis reaction in acid at 25° was extremely slow. There was a slight shift in the spectrum when benzoic acid was converted to the benzoate ion, but this shift was not noticeable at 258 nm.

**Data Analysis**—The linearity of semilogarithmic I absorbance plots (and, hence, of concentration) invariably indicated first-order kinetics. The pseudo-first-order rate constants,  $k_{obs}$ , were calculated from these lines by a least-squares treatment. The  $k_{obs}$  values determined at three different buffer concentrations were extrapolated back to zero buffer concentration to eliminate buffer effects and to obtain the first-order rate constants for the water species, *i.e.*, H<sub>3</sub>O<sup>+</sup>, OH<sup>-</sup>, and H<sub>2</sub>O. The intercept of each plot was designated  $k_0$ . The kinetic runs gave pseudo-first-order reactions with respect to the I disappearance rate because of the large excess or constancy of the water species, whether OH<sup>-</sup>, H<sub>3</sub>O<sup>+</sup>, or H<sub>2</sub>O.

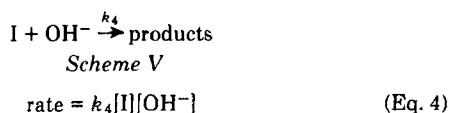
Initial data inspection led to the assumption, later confirmed by theoretical curves, that the important reactions were analogous to those observed by Hussain and Schurman (2) with 2-dimethylaminoethanethiol propionate, *i.e.*:



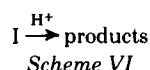
or:



and:



Although Eqs. 3a and 3b are kinetically equivalent, Eq. 3b was ruled out effectively by several authors for related reactions (2-5). The rigid structure of I cannot be contorted to give the intermediate required by Bruce and Mautner (6), who would favor Eq. 3b. Equation 3a represents the important reaction in the present instance. Also, since the hydrogen-ion concentration is quite low in the region where the free base form (I) is significant, the following reaction was not considered:



The dissociation constant for I-H<sup>+</sup>,  $K_a = [\text{I}][\text{H}_3\text{O}^+]/[\text{I-H}^+]$ , and the protolysis constant for water,  $K_w = [\text{H}^+][\text{OH}^-]$ , were utilized in an overall rate expression:

$$k_0 = \frac{k_3K_w + k_4K_aK_w/[\text{H}_3\text{O}^+] + k_1[\text{H}_3\text{O}^+]^2 + k_2[\text{H}_2\text{O}][\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a} \quad (\text{Eq. 5})$$

Values of  $K_w$  at 0.055 ionic strength were calculated according to the equations of Harned and Owen (7), assuming the electrolyte to be sodium chloride, where  $K_{w0.055} = K_{w0.0}(\alpha_{\text{H}_2\text{O}}/\gamma_{\text{H}^+}\gamma_{\text{OH}^-})$ . Values of  $k_4$  were obtained from experiments above pH 11, where  $k_4 = k_0/[\text{OH}^-]$ . The individual values of  $k_4$  were fitted to an Arrhenius equation by a least-squares plot.

The value of  $K_a$  could not be determined directly by potentiometric titration owing to solubility problems and to hydrolysis during titration. Therefore, at 25°, a least-squares data plot for pH 6.8, 8.1, and 9.05 was used to calculate  $k_3$  and  $K_a$ . The resulting equation derived from Eq. 5, with omission of terms in  $k_1$  and  $k_2$ , was:

$$k_0[\text{H}_3\text{O}^+] = k_3K_w + \{(k_4K_w/[\text{H}_3\text{O}^+]) - k_0\}K_a \quad (\text{Eq. 6})$$

The ordinate  $k_0[\text{H}_3\text{O}^+]$  was plotted against the abscissa  $(k_4K_w/[\text{H}_3\text{O}^+]) - k_0$  to give  $K_a$  as the slope and  $k_3K_w$  as the intercept. In this calculation, the average  $k_4$  value at 25°, 155 M<sup>-1</sup> hr<sup>-1</sup>, was used.

Approximate  $k_3$  values were calculated from 45 and 87° data at pH 6.86, which were used along with the 25° value for an initial Arrhenius plot of  $k_3$ . The Arrhenius plot values were used to calculate  $K_a$  at four temperatures; in turn, these values were used to correct the preliminary values of  $k_3$ , *i.e.*:

$$k_3 = \left\{ k_0/[\text{OH}^-] - k_4 \left( \frac{K_a}{[\text{H}_3\text{O}^+] + K_a} \right) \right\} \left( \frac{[\text{H}_3\text{O}^+] + K_a}{[\text{H}_3\text{O}^+]} \right) \quad (\text{Eq. 7})$$

The corrections were small. The final Arrhenius plot of  $k_3$  (25, 45, and 87°) provided the necessary input for refined  $K_a$  calculations.

The reaction of I-H<sup>+</sup> with water at 25° in the pH range of minimum reaction, ~pH 3.6, was too slow for a direct rate determination. Therefore, the value for  $k_2$  was estimated by means of a Bronsted plot (8) of  $\log k_B/q$  versus  $\log (p/q)K_a$ , where  $k_B$  is the catalytic constant of the base and  $K_a$  is the dissociation constant of the aminomethane, HPO<sub>4</sub><sup>2-</sup>, and acetate. This plot was extrapolated to the  $\log (p/q)K_a$  for water (9), *i.e.*, 1.744, to obtain  $k_2$ , which is also  $k_B$  for water.

Values of  $k_B$  for each base were determined as follows. First, values of  $k_B$  for three of the bases that had been used to establish the  $k_0$  values (H<sub>2</sub>BO<sub>3</sub><sup>-</sup>, tromethamine, and HPO<sub>4</sub><sup>2-</sup>) were calculated from the slopes of:

$$k_{obs} = k_{Bi}[\text{buffer base}] + k_0 \quad (\text{Eq. 8})$$

where the buffer base concentration was taken as one-half of the total buffer concentration since the acid to base ratio was always 1:1. Then,  $k_B$  was calculated from  $k_{Bi}$  by correcting for the dissociation of I-H<sup>+</sup> to I and assuming that the reactivity of I was 1/29 (*i.e.*,  $k_3 = 29k_4$ ) as great as that of I-H<sup>+</sup>. (See Appendix for details.) The value of  $k_B$  for hydroxide ion was taken as equal to  $k_3$ , and the dissociation constant for water, the conjugate acid of hydroxide ion, was taken as  $1.8 \times 10^{-16}$ .

To obtain  $k_B$  for acetate ion, which was run at pH = pKa of acetic acid, the calculation required that the water reaction be included:

$$k_B = \frac{k_{obs} - k_3[\text{OH}^-] - k_2[\text{H}_2\text{O}]}{[\text{acetate ion}]} \quad (\text{Eq. 9})$$

Thus, on the first round,  $k_2$  was considered to be zero in the calculation of acetate  $k_B$ , which was used in determining an approximate value of  $k_2$ . This value, in turn, was used to refine the acetate  $k_B$  and so on in a rapidly convergent iterative process.

For determining  $k_2$  at 100°, an aqueous I-H<sup>+</sup> solution was adjusted with hydrochloric acid to pH 3.6 and ionic strength 0.055. From the relative densities of water at 25 and 100° and the molar water concentration at 25°, the molar water concentration at 100° is 53.4. The value of  $k_{obs}$  was corrected by subtraction of  $k_3[\text{OH}^-]$  to obtain  $k_0$ . The rate was then:

$$k_2 = k_0/[\text{H}_2\text{O}] = k_0/53.4 \quad (\text{Eq. 10})$$

At the pH in question, correcting  $k_2$  for  $k_1[\text{H}_3\text{O}^+]$  was unnecessary.

From the values of  $k_2$  at 25 and 100°, an Arrhenius equation was derived.

The calculation of  $k_1$  involved the assumption that ionic strength has little effect. [This assumption was necessary because the reaction rate at 0.055 ionic strength, *i.e.*, 0.055 N HCl, and 25 or 45° is so low. From data for 1 and 0.5 N HCl at 25°, one may estimate that the rate increase due to the ionic strength of 1 N HCl over the rate at ionic strength 0.055

**Table I—Kinetic Data for Hydrolysis of 3-Quinuclidinyl Benzilate (I) in 0.055 Ionic Strength Aqueous Solutions**

Buffer <sup>a</sup>	Total Molar Concentration	$K_{obs}$ , hr <sup>-1</sup>	pH
	15°		
Boric acid	0.02	0.01545	9.15
	0.04	0.01664	9.15
	0.06	0.01781	9.15
		0.01427 <sup>b</sup>	9.15
NaOH	0.01243	0.8572	—
	0.00268	0.2012	—
	25°		
HCl	0.03	0.0001077	1.40
	0.5 <sup>c</sup>	0.0003783	0.30
	1.0 <sup>c</sup>	0.0007820	0.00
CH <sub>3</sub> CO <sub>2</sub> H NaH <sub>2</sub> PO <sub>4</sub> plus Na <sub>2</sub> HPO <sub>4</sub>	0.03	0.0006065	4.62
	0.010	0.0006177	6.75
	0.018	0.0007639	6.84
	0.026	0.0009833	6.88
Tromethamine		0.000377 <sup>b</sup>	6.80
	0.006	0.009007	8.01
	0.02	0.010427	8.08
	0.04	0.011004	8.10
Boric acid		0.008899 <sup>b</sup>	8.10
	0.006	0.03956	0.97
	0.02	0.04171	9.03
	0.04	0.05105	9.05
NaOH	0.06	0.05213	9.08
		0.3796 <sup>b</sup>	9.05
	0.00237	0.3956	—
	0.00593	0.8546	—
	0.01185	1.819	—
	35°		
Boric acid	0.02	0.1351	8.90
	0.04	0.1324	8.85
		0.1386	8.90
NaOH		0.1319 <sup>b</sup>	8.90
	0.00248	0.7213	—
	0.01243	3.203	—
	45°		
HCl NaH <sub>2</sub> PO <sub>4</sub> plus Na <sub>2</sub> HPO <sub>4</sub> <sup>d</sup>	1.03 <sup>c</sup>	0.004146	-0.01
	0.010	0.00528	6.86
	0.015	0.00623	6.86
	0.02	0.00673	6.86
Boric acid		0.0039 <sup>b</sup>	6.86
	0.02	0.3124	8.83
	0.04	0.3038	8.82
	0.06	0.3669	8.83
NaOH		0.2732 <sup>b</sup>	8.83
	0.00248	1.224	—
	65.5°		
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> · 10H <sub>2</sub> O <sup>d</sup>	0.01	1.416	8.94
	0.015	1.495	8.94
	0.02	1.643	8.94
		1.178 <sup>b</sup>	8.94
	87°		
NaH <sub>2</sub> PO <sub>4</sub> plus Na <sub>2</sub> HPO <sub>4</sub> <sup>d</sup>	0.01	0.5026	6.86
	0.015	0.6109	6.86
	0.02	0.6535	6.86
		0.3627 <sup>b</sup>	6.86
	100°		
HCl		0.001566	3.60

<sup>a</sup> Buffers other than NaOH and HCl consisted of equal concentrations of the buffer base and its conjugate acid. <sup>b</sup> Calculated  $k_0$  for buffer concentration. <sup>c</sup> Ionic strength same as molar concentration. <sup>d</sup> The pH for these experiments was estimated from the pKa of the buffer at the given temperature. [H. W. Robinson, *J. Biol. Chem.*, 82, 775 (1969). R. G. Bates, "Electronic pH Determinations," Wiley, New York, N.Y., 1954, p. 86. R. P. Bell, "Acid-Base Catalysis," Oxford University Press, London, England, 1941, pp. 11-35.]

is about 12%. According to Aksnes and Prue (10), it might have been higher.] The calculation was:

$$k_1 = k_{obs} - k_2[H_2O] \quad (\text{Eq. 11})$$

The values of  $k_1$  at 25 and 45° were incorporated in an Arrhenius equation.

## RESULTS AND DISCUSSION

### Kinetic Rate Data and Equilibria—Kinetic rate data and associated

**Table II—General Base Catalysis Data for Hydrolysis of 3-Quinuclidinyl Benzilate (Protonated Species)**

Base	$p/q$	$K_a$	$k_B^a$
Water	1/1	55.5	$2.94 \times 10^{-7}$
Acetate	1/2	$2.399 \times 10^{-5}$	0.00272
Phosphate	2/3	$1.585 \times 10^{-5}$	0.0460
Tromethamine	1/1	$7.943 \times 10^{-9}$	0.1257
Borate	3/1	$8.913 \times 10^{-10}$	1.0036
Hydroxide	1/1	$1.80 \times 10^{-16}$	4514

<sup>a</sup>  $k_B$  calculated from:

$$k_B = \frac{k_{obs} - k_0}{[\text{buffer base}]} \left[ \frac{[H_3O^+] + K_a}{[H_3O^+] + K_a/29} \right]$$

pH values are summarized in Table I. Data used for the Bronsted plot to estimate  $k_2$  at 25° are shown in Table II. A plot of  $\log k_{B/q}$  versus  $\log (p/q)K_a$  using the values in Table II was linear ( $r = 0.9996$ ) with a slope of 0.58, supporting the contention that I hydrolysis is subject to general base catalysis.

The temperature-related equations for the hydrolysis rate constants in units of  $M^{-1} \text{hr}^{-1}$  and the acid dissociation constant of I are:

$$\log_{10} k_1 = (-3388.2/T) + 8.2477 \quad (\text{Eq. 12})$$

$$\log_{10} k_2 = (-2717.5/T) + 2.5827 \quad (\text{Eq. 13})$$

$$\log_{10} k_3 = (-2379.6/T) + 11.5558 \quad (\text{Eq. 14})$$

$$\log_{10} k_4 = (-2531.6/T) + 10.6599 \quad (\text{Eq. 15})$$

and:

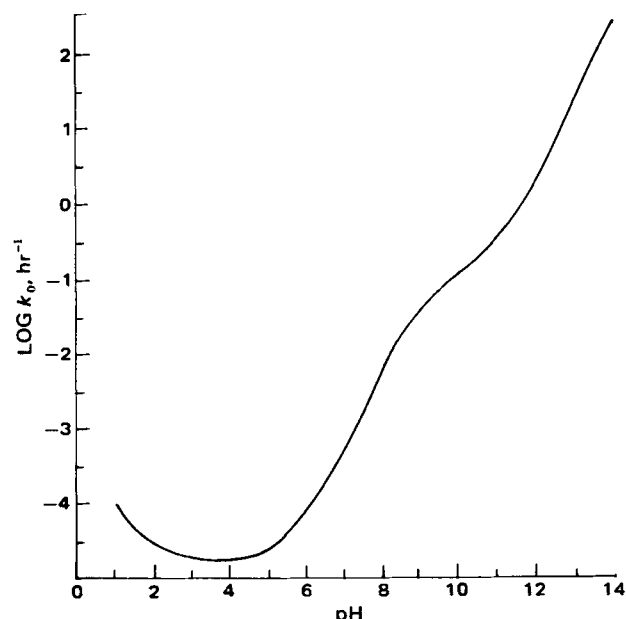
$$\log_{10} K_a = (-2455.8/T) - 1.0647 \quad (\text{Eq. 16})$$

where  $T$  is in °K.

In Table III, the values of  $k_0$  for nine experiments are compared with those obtained by use of Eq. 5 with the constants calculated from Eqs. 12-16. The predicted values were generally within 35% of the experimental values.

The pH of maximum stability in water at 25° containing little or no basic anions is 3.64 (11). From Eq. 5, the observed rate at this pH is  $1.66 \times 10^{-5} \text{hr}^{-1}$ . The water reaction accounts for ~98% of the reaction at this pH. At pH 3, the rate is only  $1.71 \times 10^{-5} \text{hr}^{-1}$ , with the water reaction accounting for 95% of the overall reaction. At pH 4.5, the water reaction accounts for 93% of the total reaction.

Thus, the left side of the pH profile of I hydrolysis (Fig. 1) shows a decreasing rate with increasing pH; the change of the rate initially is 10-fold per pH unit but diminishes as the pH nears 3. The rate over pH



**Figure 1—Hydrolysis rates of 3-quinuclidinyl benzilate from pH 1-14 at  $t = 25^\circ$  and  $\mu = 0.055$ .**

**Table III—Calculated versus Experimental  $k_0$  Values for 3-Quinuclidinyl Benzilate (I) Hydrolysis in Buffer Solutions**

Temperature	pH	$k_0^a, \text{hr}^{-1}$	
		Calculated	Found
15°	9.15	0.0142	0.0143
25°	6.80	0.000356	0.000377
25°	8.10	0.00677	0.00890
25°	9.05	0.0428	0.0380
35°	8.90	0.102	0.132
45°	6.86	0.00522	0.00391
45°	8.83	0.253	0.273
65.5°	8.94	1.428	1.178
87°	6.86	0.522	0.363

<sup>a</sup> Values of  $k_0$  calculated from:

$$k_0 = \frac{k_3 K_w + K_4 K_a K_w / [\text{H}_3\text{O}^+] + k_1 [\text{H}_3\text{O}^+]^2 + k_2 [\text{H}_2\text{O}] [\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a}$$

3–4.5 is, to all intents, constant and is due to the reaction of I-H<sup>+</sup> with water molecules. Above pH 4.5, the rate increases in the manner shown in Fig. 1.

The hydrolysis of I in the absence of buffers lowers the aqueous solution pH since hydrolysis produces benzoic acid. Thus, an unbuffered I solution that is initially slightly basic (the pK<sub>a</sub> of I-H<sup>+</sup> is 9.3) will hydrolyze at a rate calculable from Eq. 5 but will produce acid, lower the pH, and thus produce an environment where the hydrolysis rate is less than it was initially.

**Comparison with Hydrolysis of Related Compounds**—The hydrolysis kinetics of amino alcohol (and amino mercaptan) esters in which the nitrogen is separated from the alcohol oxygen by two or three carbons was studied as early as 1950 (12). In general, reactivity with hydroxide ion is in the order: protonated tertiary amine > quaternary ammonium > tertiary amine. Values of  $k_4$  (the hydroxide-ion rate constant with the unprotonated species) tend to remain fairly close to one another under a given set of conditions (4, 13–15), as well as to values for esters containing no nitrogen (4, 14, 15). Nitrogen proximity to the carbonyl carbon is unimportant (4, 13, 15).

Nitrogen quaternization by alkylation inductively increases these rate constants up to 32-fold (2, 4, 6, 12, 13, 15). Protonation, however, increases the reactivity up to 40,000-fold (2, 4, 6, 13, 14, 16). The higher increase, therefore, cannot be ascribed only to an inductive effect of the positive charge. The large increases are found in cases where the proton can participate in intramolecular hydrogen bonds with the acyl carbonyl oxygens (4, 17). When rigidity prevents intramolecular hydrogen bonding, the protonated species are not much more reactive than the quaternized species. Thus, in the case of 3-quinuclidinyl acetate, the factor is less than 2 (13, 18). The  $k_3/k_4$  ratio of 29 in this work appears entirely reasonable.

**Significance of Present Study**—Amino alcohol ester hydrolytic stability has been of concern in the pharmaceutical field, and several pH profile determinations are on record (2–6, 16, 19, 20). These usually have ignored both the water reaction ( $k_2$ ), which is generally slow enough to present experimental difficulties, and the acid-catalyzed hydrolysis ( $k_1$ ). The present study provides the means for calculating the hydrolysis rate over wide temperature and pH ranges with all four rate-determining reactions considered.

## APPENDIX

For the calculations of  $K_B$  from  $K_{Bi}$ :

$$k_{Bi} = \frac{[\text{I}\cdot\text{H}^+]k_B}{[\text{I}] + [\text{I}\cdot\text{H}^+]} + \frac{[\text{I}]k_B}{29([\text{I}] + [\text{I}\cdot\text{H}^+])} \quad (\text{Eq. A1})$$

Since:

$$\frac{[\text{I}\cdot\text{H}^+]}{[\text{I}] + [\text{I}\cdot\text{H}^+]} = \frac{[\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a} \quad (\text{Eq. A2})$$

and:

$$\frac{[\text{I}]}{[\text{I}] + [\text{I}\cdot\text{H}^+]} = \frac{K_a}{[\text{H}_3\text{O}^+] + K_a} \quad (\text{Eq. A3})$$

it follows that:

$$k_B = k_{Bi} \div \left( \frac{[\text{H}_3\text{O}^+]}{[\text{H}_3\text{O}^+] + K_a} + \frac{K_a/29}{[\text{H}_3\text{O}^+] + K_a} \right) \quad (\text{Eq. A4})$$

or:

$$k_B = k_{Bi} \left( \frac{[\text{H}_3\text{O}^+] + K_a}{[\text{H}_3\text{O}^+] + K_a/29} \right) \quad (\text{Eq. A5})$$

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