JOURNAL OF PHARMACEUTICAL SCIENCES

JULY 1982
VOLUME 71 NUMBER 7

RESEARCH ARTICLES

Kinetics and Mechanism of Hydrolysis of Labile Quaternary Ammonium Derivatives of Tertiary Amines

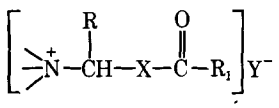
JOSEPH B. BOGARDUS * and TAKERU HIGUCHI ˣ

Received June 29, 1981, from the *Pharmaceutical Chemistry Department, School of Pharmacy, University of Kansas, Lawrence, KS 66044.* Accepted for publication October 22, 1981. *Present Address: College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Abstract □ The kinetics and mechanism of hydrolysis of *N*-(4-hydroxy-3,5-dimethylbenzyl)pyridinium bromide and similar quaternary derivatives of niacinamide, *N*,*N*-dimethylaniline, and trimethylamine were investigated. pH–Rate profiles at 25° for formation of tertiary amine and 4-hydroxymethyl-2,6-dimethylphenol indicated that the zwitterionic quaternary phenoxide was the reactive species in alkaline solution. The apparent rate of hydrolysis was strongly inhibited by addition of small amounts of product tertiary amine, which is consistent with the presence of an intermediate in the reaction pathway. A mechanism was proposed for the hydrolysis and methanolysis of these compounds involving the reversible formation of the quinone methide, 4-methylene-2,6-dimethyl-2,5-cyclohexadien-1-one, followed by a trapping reaction with solvent or nucleophiles. Replacement of the phenolic hydrogen with methyl or acetyl groups greatly stabilized the molecule which is in agreement with the proposed mechanism. For the ester, the rate of amine release was limited by specific base catalyzed hydrolysis of the ester group. Compounds of this type may be useful in prodrug design for tertiary amines. The possibility of quinone methine intermediates in the degradation of structurally similar drugs, such as epinephrine, was discussed.

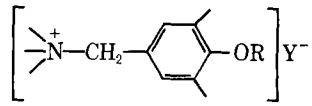
Keyphrases □ Hydrolysis—labile quaternary ammonium derivatives, tertiary amines □ Amines—tertiary, kinetics, mechanism of hydrolysis, labile quaternary ammonium derivatives □ Kinetics—labile quaternary ammonium derivatives, mechanism of hydrolysis, tertiary amines

In recent years a number of approaches have been investigated for preparation of prodrugs (1, 2). Tertiary amines are unusual in that derivatization forms quaternary ammonium compounds. Quaternary salts resulting from simple alkylation, however, are generally chemically stable and therefore are not useful as prodrugs. A class of labile quaternary ammonium salts of the following general type have been investigated previously (3–6):



In this salt R and R_1 are hydrogen, alkyl, or aryl; X is oxygen or sulfur; and Y is a halogen. These compounds hydrolyze to form tertiary amine (>N—), aldehyde (RCHO), carboxylic acid (R_1COXH), and HY. Applications of this approach have included the preparation of soft quaternary germicides, anticholinergic agents, and antitumor agents.

In the present report, the kinetics and mechanism of hydrolysis of a different type of labile quaternary salt were studied. The general structure is:



where R is H, CH_3, or CH_3CO and Y^- is Br^-. Simple tertiary amines were used as model compounds, although the results should be useful in the design of prodrugs. The 4-hydroxybenzyl structure of the quaternizing agent was chosen, since it is known to be a major factor in the instability of drugs such as epinephrine (7, 8). An unstable quinone methide was found to be an intermediate in the hydrolysis of the quaternary salts, and it is likely that the degradation of drugs such as epinephrine proceeds by an analogous mechanism.

EXPERIMENTAL

The following general sequence was used to prepare the quaternary salts *N*-(4-hydroxy-3,5-dimethylbenzyl)pyridinium bromide (I), *N*-(4-hydroxy-3,5-dimethylbenzyl)-3′-carbamoylpyridinium bromide (II), and *N*-(4-hydroxy-3,5-dimethylbenzyl)phenyldimethylammonium bromide (VIII): Formaldehyde and base were used to hydroxymethylate

0022-3549/82/0700-0729$01.00/0

2,6-dimethylphenol. The benzyl bromide was then prepared using hydrobromic acid–acetic acid. This compound was reacted with the corresponding tertiary amine to form the quaternary salt. Satisfactory elemental analyses were obtained on all stable compounds. NMR and IR spectra were run on commercial instruments[1,2]. Melting points were uncorrected.

4-Hydroxymethyl-2,6-dimethylphenol (III)—Equimolar amounts of 2,6-dimethylphenol[3], formaldehyde, and aqueous potassium hydroxide solution were reacted for 24 hr at 5° (9). The product precipitated upon neutralization and was recrystallized from chloroform, mp 101–102° [lit. (9) 105°]. UV (H_2O) 270, 220 nm max; IR (KBr): 3340 cm^{-1}; NMR [$(CD_3)_2SO$]: δ7.98 (s, 1H), 6.80 (s, 2H), 4.90 (s, 1H), and 2.17 (s, 6H) ppm.

4-Bromomethyl-2,6-dimethylphenol—A solution containing 0.5 g of III, 1 ml of 48% hydrobromic acid, and 10 ml of acetic acid was stirred for 0.5 hr at 6°. Cold chloroform (40 ml) and ice water (25 ml) were added, the organic layer was dried with calcium sulfate and rotary evaporated at room temperature. The unstable pink crystals could not be further characterized and were used immediately.

***N*-(4-hydroxy-3,5-dimethylbenzyl)pyridinium bromide (I)**—Immediately upon addition of pyridine to a solution of 4-bromomethyl-2,6-dimethylphenol in chloroform the quaternary compound precipitated, mp 190° dec. UV max (H_2O) 258 nm; IR (KBr): 3360 cm^{-1}; NMR [$(CD_3)_2SO$]: δ 9.25 (d, 2H), 8.35 (m, 4H), 7.24 (s, 2H), 5.77 (s, 2H), and 2.17 (s, 6H) ppm.

***N* - (4-hydroxy-3,5-dimethylbenzyl)-3′-carbamoylpyridinium bromide (II)**—The product precipitated from a solution of 1.6 g of niacinamide and 4-bromomethyl-2,6-dimethylphenol (prepared from 2 g of III) in 3 ml of dry dimethylformamide, mp 202°. NMR [$(CD_3)_2SO$]: δ 9.66 (s, 1H), 9.16 (m, 2H), 8.60 (s, 2H), 8.27 (t, 1H), 7.30 (s, 2H), 5.84 (s, 2H), 3.3 (s, 1H), and 2.22 (s, 6H) ppm.

***N* - (4-hydroxy-3,5-dimethylbenzyl)phenyldimethylammonium bromide (VIII)**—The procedure for I was followed with the substitution of *N*,*N*-dimethylaniline, mp 131–132°. IR (KBr): 3350 cm^{-1}; NMR [$(CD_3)_2SO$]: δ 7.67 (m, 5H), 6.50 (s, 2H), 4.84 (s, 2H), 3.50 (s, 7H), and 2.03 (s, 6H) ppm.

***N*-(4-acetoxy-3,5-dimethylbenzyl)pyridinium bromide (IV)**—A 0.5-g portion of I was acetylated with 1 ml of acetic anhydride in 3 ml of warm pyridine. The product crystallized upon cooling, mp 258°. UV max (H_2O) 258 nm; NMR [$(CD_3)_2SO$]: δ 9.25 (d, 2H), 8.33 (m, 3H), 7.33 (s, 2H), 5.80 (s, 2H), 2.33 (s, 3H), and 2.10 (s, 6H) ppm.

4-Dimethylaminomethyl-2,6-dimethylphenol—Mannich condensation of equimolar amounts of 2,6-dimethylphenol, formaldehyde, and dimethylamine in aqueous solution yielded the product (10). Recrystallization from benzene or ethanol—water gave long needles, mp 112° [lit. (10) 120–122°]. NMR (acetone-d_6): δ 6.83 (s, 2H), 3.22 (s, 2H), 2.19 (s, 6H), and 2.12 (s, 6H) ppm.

4-Hydroxy-3,5-dimethylbenzyltrimethylammonium Iodide (IX)—A literature procedure (10) for the reaction of 4-dimethylaminomethyl-2,6-dimethylphenol with methyl iodide in ether gave significant quantities of two byproducts (11). The water-soluble fraction containing IX and tetramethylammonium iodide was used. NMR (D_2O): δ 7.17 (s, 2H), 4.33 (s, 2H), [3.22 (s, $(CH_3)_4N^+$)], 3.07 (s, 9H), and 2.28 (s, 6H) ppm.

4-Hydroxymethyl-2,6-dimethylanisole—Two milliliters of dimethylsulfate was slowly added to 30 ml of a cold alkaline aqueous solution containing 1.0 g of III. The reaction mixture (two layers) was kept alkaline with 10% aqueous potassium hydroxide. After stirring 2 hr, the mixture was warmed gently and allowed to stand for 1 hr. The product was obtained as a yellow oil by ether extraction and solvent evaporation. NMR (CCl_4): δ 6.80 (s, 2H), 4.30 (s, 2H), 3.90 (s, 1H), 3.58 (s, 3H), and 2.17 (s, 6H) ppm.

4-Bromomethyl-2,6-dimethylanisole—A 0.5-g sample of 4-hydroxymethyl-2,6-dimethylanisole was converted to the bromide in 25 ml of 48% hydrobromic acid containing 1 ml of sulfuric acid. The product was extracted into 60 ml of chloroform, washed with 5% sodium bicarbonate solution, and dried with calcium sulfate. Evaporation of solvent gave a yellow oil. NMR (CCl_4): δ 6.91 (s, 2H), 4.28 (s, 2H), 3.65 (s, 3H), and 2.24 (s, 6H) ppm.

***N*-(4-methoxy-3,5-dimethylbenzyl)pyridinium bromide (VII)**—One milliliter of 4-bromomethyl-2,6-dimethylanisole was reacted with an equal volume of dry pyridine at 60°. Upon cooling, an oil separated which subsequently crystallized. Recrystallization from acetone afforded the pure product, mp 173–174°. UV max (H_2O) 257 nm; NMR (D_2O): δ 8.67 (m, 5H), 7.27 (s, 2H), 5.80 (s, 2H), 3.80 (s, 3H), and 2.34 (s, 6H) ppm.

Rate Studies—Reaction rates were measured in the thermostated cell compartment of a spectrophotometer[4]. Inorganic salts and nucleophiles were reagent grade and water was redistilled from an all-glass apparatus. Pseudo first-order rate constants were calculated[5] from the slopes of $\ln|A_\infty - A_t|$ *versus* time plots using the least-squares method. Correlation coefficients were consistently >0.998 and infinity values were taken after 10 half-lives. The pH of all solutions was measured[6] before and after the reactions and the change was <0.04. The reaction was initiated by injection of a concentrated stock solution into the buffer in the spectrophotometer cell. Wavelengths for the kinetic measurements were: I, 260 nm; II, 265 nm; IV, 250 and 285 nm; VIII, 250 nm; IX, 260 nm.

Reactions with half-lives <10 sec were followed using a stopped-flow spectrophotometer[7]. Transmittance–time data were converted to absorbance and then analyzed as before.

A high-performance liquid chromatograph (HPLC) with 254 nm detector was used[8]. The mobile phase consisting of pH 9.1, 5 m*M* borax buffer was pumped through the strong anion exchange column at 1.0 ml/min resulting in retention times of 3.5 (III) and 8.3 min (VI). Reaction solutions containing III and VI were extracted with 15 ml each of chloroform–ether–chloroform; the combined extracts were evaporated to dryness and reconstituted with 5 ml of methanol. The methanol solution was injected on the HPLC. The relative peak areas for III and VI were measured planimetrically. The aqueous phase from the extraction contained negligible amounts of the two compounds.

RESULTS

pH–Rate Profiles for Hydrolysis of I, II, and IV—In neutral to alkaline aqueous solutions, the quaternary ammonium salts of pyridine (I) and niacinamide (II) rapidly cleave to form the corresponding tertiary amine and III, as shown in Scheme I. The products were identified by UV, NMR, and TLC analyses. Repetitive UV scanning did not indicate the presence of an intermediate during the reaction. The rate was pH-de-

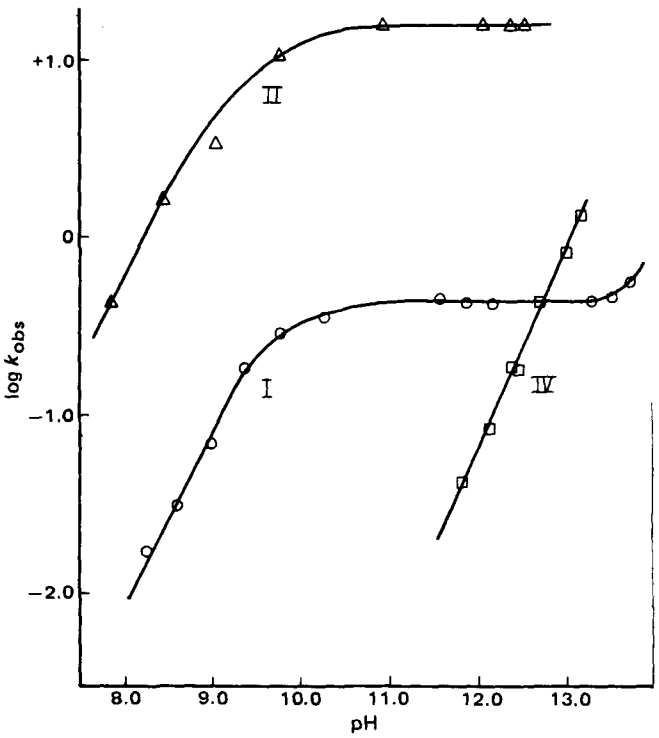
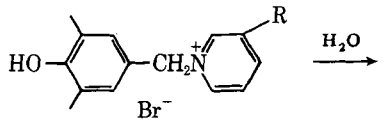
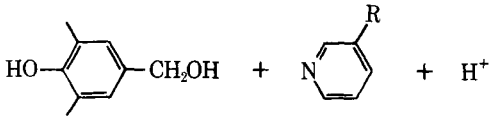


Figure 1—*Log* k_{obs}*–pH profiles for I, II, and IV at 25°,* $\mu = 1.0$ *(KCl). Parameters for the solid lines are: I,* (○) $k_{max} = 0.450\ min^{-1}$, $K_a' = 2.82 \times 10^{-10}$ *and* $k_{OH} = 0.250\ M^{-1}\ min^{-1}$; *II,* (△) $k_{max} = 15.6\ min^{-1}$ *and* $K_a' = 3.55 \times 10^{-10}$; *IV* (□) $k_{es} = 6.60\ M^{-1}\ min^{-1}$.

[1] Varian T-60.
[2] Beckman IR-33.
[3] Aldrich Chemical Co.
[4] Cary models 14, 15, or 16.
[5] Hewlett-Packard model 9810.
[6] Radiometer model 26.
[7] Durrum-Gibson.
[8] Dupont model 820.



I: R = H
II: R = $CONH_2$



III

Scheme I

pendent and the spectrophotometrically determined log k_{obs}–pH profiles at 25° are shown in Fig. 1.

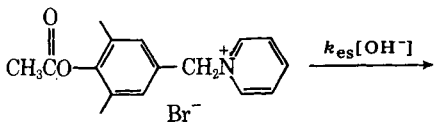
The reaction rates were increased by buffers and the buffer data are shown in Tables I and II. All data in Fig. 1 were extrapolated to zero buffer concentration. The lines for I and II in Fig. 1 were calculated according to:

$$k_{obs} = k_{max}\left(\frac{K_a'}{K_a' + a_H}\right) + k_{OH}K_w/a_H \qquad \text{(Eq. 1)}$$

where k_{obs} is the pseudo first-order rate constant at zero buffer concentration, k_{max} is the rate constant in the plateau region, K_a' is the dissociation constant of the reactant, k_{OH} is the apparent second-order rate constant for the effect of hydroxide on the conjugate base of I or II, K_w is the ion product of water, and a_H is the hydrogen ion activity. The rate of hydrolysis of II at high pH could not be accurately determined using the stopped-flow technique, and thus, k_{OH} could not be determined.

For the hydrolysis of I under conditions of pH < pK_a' and [I] $\simeq 10^{-4}$ *M*, the apparent rate constant became smaller during the reaction. This rate-retarding effect was due to the pyridine produced upon hydrolysis of I, according to the Law of Mass Action. The rate constants reported for this pH region were for the initial portion of the reaction where first-order conditions were well approximated or were obtained with more dilute solutions of I in longer path length UV cells. A study of this effect is reported in the next section.

Also shown in Fig. 1 is the effect of pH on the hydrolysis of IV, the acetate ester of I. As shown in Scheme II, this compound decomposed by consecutive kinetics:



IV

$$CH_3COO^- + I \longrightarrow III + Pyridine$$

Scheme II

After hydrolysis of equal concentrations of I and IV at pH 12.5, the UV spectra of the products were identical. In the pH region studied, the rate of decomposition of I was three- to ten-fold faster than the hydrolysis of the ester. This allowed the rate constant for ester hydrolysis to be determined as the terminal slope of ln $|A_t - A_\infty|$ *versus* time plots.

The second-order rate constant for ester hydrolysis, k_{es}, was calculated from the slope of a plot of k_{obs} *versus* K_w/a_H according to:

$$k_{obs} = k_{es}K_w/a_H \qquad \text{(Eq. 2)}$$

Table I—Reaction Conditions and Buffer Data for the Hydrolysis of I[a]

pH	Buffer Species	Conc. Range, *M*	Number of Concs.	Number of k_{obs}
8.25	Borate	0.14–0.24	2	4
8.58	Borate	0.14–0.24	2	3
9.00	Borate	0.15–0.20	2	4
9.34	Carbonate	0.1–0.6	5	11
9.77	Carbonate	0.1–0.4	4	8
10.26	Carbonate	0.1–0.4	3	7
11.54–13.72	Hydroxide	—	—	—

[a] At 25°, μ = 1.0.

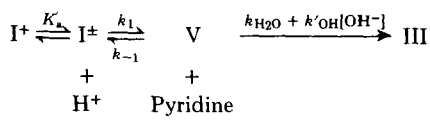
Table II—Reaction Conditions and Buffer Data for the Hydrolysis of II[a]

pH	Buffer Species	Conc. Range, *M*	Number of Concs.	Number of k_{obs}
7.85	Imidazole	0.05–1.0	4	8
8.45	Tromethamine	0.04–0.8	5	11
9.02	Borate	0.12–0.24	2	5
9.76	Carbonate	0.025–0.25	4	9
10.91	Butylamine	0.05–0.42	4	8
11.43	Phosphate	0.025–0.1	3	6
12.05–12.5	Hydroxide	—	—	—

[a] At 25°, μ = 1.0.

This reaction was first order in hydroxide ion, as indicated by the slope of 1.1 in Fig. 1. At pH 12.75 the rates of decomposition of I and IV were equal, *i.e.*, $k_{max} = k_{es}K_w/a_H$.

Effect of Added Pyridine on the Hydrolysis of I—In the pH-independent region for hydrolysis, addition of small amounts of pyridine at constant pH greatly decreased the observed rate constants. Figure 2 shows the kinetic effect of added pyridine at three pH values. The rate-retarding effect of pyridine decreased as pH increased, in the region where the hydrolysis reaction (Fig. 1) was strictly pH-independent. This mass law effect of pyridine was analogous to the common ion effect observed in many S_N1 reactions (12). A possible explanation for these data was a mechanism involving reversible dissociation of I to pyridine plus an intermediate (V). As shown in Scheme III, the intermediate can either react with pyridine to form starting material or with solvent irreversibly to form III:



Scheme III

By applying the steady-state assumption to the intermediate, the rate law for loss of I may be expressed as shown in Eq. 3 where $[I]_T = [I^+] + [I^\pm]$ and [P] is the pyridine concentration:

$$\frac{-d[I]_T}{dt} = \frac{k_1(k_{H_2O} + k'_{OH}K_w/a_H)[I]_T}{k_{-1}[P] + k_{H_2O} + k'_{OH}K_w/a_H}\left(\frac{K_a'}{K_a' + a_H}\right) \qquad \text{(Eq. 3)}$$

The observed rate constant is defined as:

$$k_{obs} = \frac{k_1K_a'}{(K_a' + a_H)\left(1 + \left[\frac{k_{-1}[P]}{k_{H_2O} + k'_{OH}K_w/a_H}\right]\right)} \qquad \text{(Eq. 4)}$$

In the absence of added pyridine, $k_{-1}[P] \ll (k_{H_2O} + k'_{OH}K_w/a_H)$ and the rate constant is given by Eq. 1 with $k_{max} = k_1$ in the pH region where the k_{OH} term is negligible. Inversion and rearrangement of Eq. 4 gives:

$$\frac{1}{k_{obs}} = \left(\frac{k_{-1}[P]}{k_1(k_{H_2O} + k'_{OH}K_w/a_H)} + \frac{1}{k_1}\right)\left(1 + \frac{a_H}{K_a}\right) \qquad \text{(Eq. 5)}$$

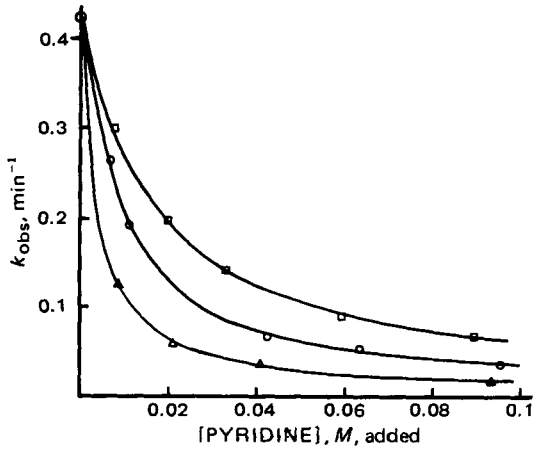


Figure 2—*Effect of added pyridine on the rate constant for hydrolysis of I at three pH values. Key:* (△) *pH 12.15,* (○) *pH 12.45,* (□) *pH 12.74.*

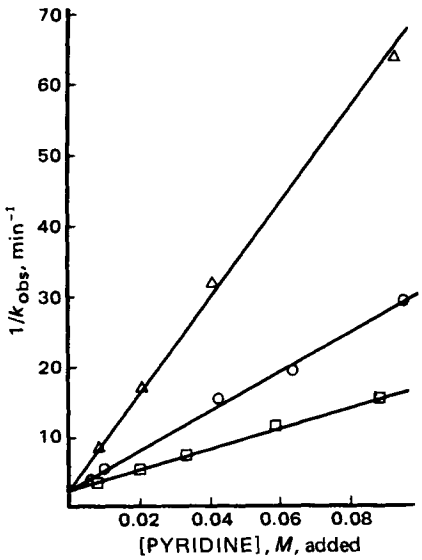


Figure 3—*Inverse rate constant for hydrolysis of I as a function of added pyridine concentration according to Eq. 5. Key:* (△) *pH 12.15,* (○) *pH 12.45,* (□) *pH 12.74.*

Equation 5 predicts a linear pH-dependent relationship between $1/k_{obs}$ and pyridine concentration, with slope = $k_{-1}/[k_1(k_{H_2O} + k'_{OH}K_w/a_H)]$ and intercept $1/k_1$. Figure 3 is an inverse plot according to Eq. 5 for the data in Fig. 2. Division of the intercept in Eq. 5 by the slope allows elimination of k_1 giving a hydroxide-dependent ratio, R:

$$R = \frac{k_{H_2O} + k'_{OH}K_w/a_H}{k_{-1}} \qquad \text{(Eq. 6)}$$

Figure 4 is a plot of R *versus* K_w/a_H with slope equal to $k'_{OH}/k_{-1} = 0.29$ and zero intercept. Thus, the reaction of the intermediate with water (k_{H_2O}) was negligible compared to k_{-1}. The slope of Fig. 3 indicates that the intermediate is 3.4-fold more reactive with pyridine than with hydroxide ion.

Effect of Added Substances on the Hydrolysis of I—The hydrolysis of I exhibited significant salt and solvent effects (Table III). The rate of hydrolysis at pH 12.8 decreased by one-half as ionic strength increased from 0.08 to 1.0. Identical rates of hydrolysis were obtained when potassium chloride–potassium iodide mixtures were used as electrolyte even though iodide is a stronger nucleophile in most displacement reactions. Addition of the polar nonelectrolytes, acetonitrile and dimethylformamide, at ~4 and 7% (v/v), respectively, strongly increased the observed rate at constant ionic strength.

Table IV contains apparent second-order rate constants and experimental conditions for the effect of several nucleophilic substances on the hydrolysis of I. A Brønsted plot of this data (log k_2^{app} *versus* pK'_a) showed a slope of ~0.1 indicating that the reaction was essentially insensitive to changes in basicity. The effect of imidazole was pH-dependent. As pH increased the apparent catalytic constant for imidazole decreased. In Fig. 5a, the apparent second-order rate constants for imidazole have been plotted against K_w/a_H. If the rate effect of imidazole anion was negligible compared to the neutral molecule, a description of the kinetic data gives:

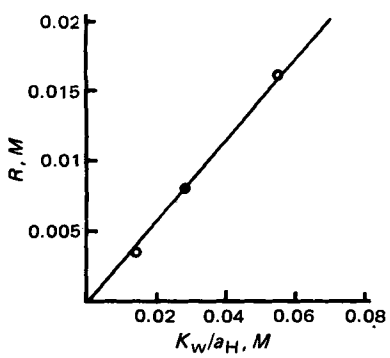


Figure 4—*Dependence of the ratio,* R, *on hydroxide ion concentration according to Eq. 6.*

Table III—Effect of Variations in Salt and Solvent Concentrations on the Hydrolysis of I

Conditions[a]	Ionic Strength	k_{obs}, min^{-1}	$\frac{k_{obs}}{k_{max}}$
0.92 *M* KCl	1.0	0.415	1
0.08 *M* KOH	0.08	0.800	1.9
0.4 *M* KI	1.0	0.414	1.0
0.8 *M* KI	1.0	0.410	0.99
1.15 *M* CH_3CN	1.0	0.611	1.5
1.02 *M* *N*,*N*-dimethylformamide	1.0	1.243	3.0

[a] All solutions contained 0.08 *M* KOH.

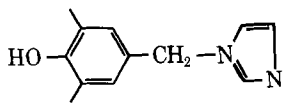
$$k_2^{app} = k_0\left[\frac{a_H}{K'_a + a_H}\right] \qquad \text{(Eq. 7)}$$

where k_0 is the second-order rate constant for the effect of neutral imidazole on the hydrolysis of I, and K'_a is the apparent dissociation constant for imidazole as an acid. Inversion and rearrangement gives:

$$\frac{1}{k_2^{app}} = \frac{1}{k_0} + \frac{K_w}{k_0K'_ba_H} \qquad \text{(Eq. 8)}$$

Equation 8 predicts a linear relationship between reciprocal rate constant and K_w/a_H as shown in Fig. 5b. The second-order rate constant for imidazole in Table IV was calculated from the intercept of Fig. 5b.

Upon hydrolysis of a concentrated solution of I in the presence of imidazole, *N*-(4-hydroxy-3,5-dimethylbenzyl) imidazole (VI) crystallized and was isolated as a product.

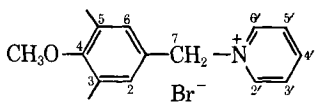


VI

The effect of added imidazole on the product composition for the hydrolysis of I at pH 12.70 was determined by HPLC. Figure 6 shows the effect of imidazole on the molar ratio of imidazole- and hydroxide-substituted products, $[VI]/[III]_\infty$, at the end of the reaction. The kinetic effect of added imidazole at pH 12.70, $k_2^{app} = 0.22\ M^{-1}\ min^{-1}$, can be interpolated from Fig. 5a. Therefore, the ratio of rate constants for the effects of imidazole and hydroxide at this pH was $k_2^{app}/k_{max} = 0.5\ M^{-1}$. Comparison of this value with the slope of Fig. 6, 14 M^{-1}, indicates that 28-fold more imidazole was appearing in the product than could be accounted for by the apparent second-order rate dependency.

The potent nucleophile cyanide ion had no effect on the rate of hydrolysis of I at pH 12.47 (Table IV). The low standard deviation for duplicate runs at each concentration of cyanide ion ($0.446 \pm 0.010\ min^{-1}$) indicated the absence of any effect other than that attributable to salt composition.

Stability of the *O*-methyl Quaternary Salts—No change in absorption spectrum was detectable when *N*-(4-methoxy-3,5-dimethylbenzyl)pyridinium bromide(VII) was dissolved in water, 1.0 *M* KOH, or 1.0 *M* NaCN and scanned with time over a 1-hr period. Replacement of the phenolic hydrogen by a methyl ether completely eliminated the instability of I in solution.



VII

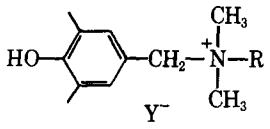
When the NMR spectrum of VII in alkaline deuterium oxide (pD 13.1) was scanned over a 6-day period at room temperature, the benzylic protons (position 7) and the 2′ and 6′ protons of the pyridine ring were completely exchanged. Under these conditions, no decomposition of VII could be detected. Similar exchange has been reported for 1-methyl and 1-oxypyridinium ions (15). The analogous quaternary derivative of niacinamide was also stable in base but formed an apparent cyanide adduct with characteristic absorption maximum at 340 nm (16).

Hydrolysis of Aliphatic Quaternary Compounds—Analogous quaternary derivatives of *N*,*N*-dimethylaniline (VIII) and trimethylamine (IX) hydrolyzed similarly to I and II except that rate inhibition by the product amine was a more serious problem:

Table IV—Apparent Second-Order Rate Constants for the Effect of Nucleophiles on the Hydrolysis of I[a]

Species	pK'_a	k_2^{app}, M^{-1} min^{-1}	pH	Conc. Range, M
Iodide	—	0	12.75	0.4–0.8
Azide	4.0[b]	0.11[c]	12.75	(0.8)
Acetate	4.61[b]	0.168	12.61	0.08–0.94
Acetate	—	0.152	12.14	0.1–0.6
Methoxylamine	4.73[d]	0.23[c]	12.19	(0.4, 1.0)
Imidazole	7.21[b]	0.45[e]	—	0.05–1.0
Morpholine	8.87[b]	0.36	12.81	0.08–0.88
Cyanide	9.3[b]	0	12.47	0.1–0.8
Glycinate	9.76[b]	0.206	12.45	0.08–0.8
Glycinate	—	0.210	12.90	0.08–0.8
n-Butylamine	10.9[f]	0.68	12.88	0.07–0.7
Hydroxide	15.74	0.25	—	—

[a] At 25°, μ = 1.0. [b] Reference 13. [c] $(k_{obs} - k_0)/[N]$. [d] Reference 14. [e] pH independent. [f] pK'_a = pH − log[$\alpha_n/(1-\alpha_n)$], where α_n is the fraction of nonionized amine.

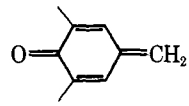


VIII: R = phenyl, Y = Br
IX: R = CH_3, Y = I

Initial rate data for VIII at neutral pH and the highest practicable dilution allowed estimation of k_{max} as shown in Table V. Stopped-flow measurements were not possible due to the instability of VIII in all solvents in which it was soluble. Hydrolysis of the trimethylammonium salt (IX) was studied using 10-cm UV cells in which first-order kinetics were obtained. Constant values of k_{max} were measured at pH 12.5 and 12.9.

Table V summarizes the hydrolysis data for I, II, VIII, and IX as a function of the pK'_a of the tertiary amine leaving group. When these data are plotted as log k_{max} *versus* pK'_a, heterocyclic and aliphatic ammonium compounds appear to fall on separate lines with slope ≃0.8, according to the limited data available. The aliphatic quaternary ammonium compounds are ~250 times more reactive than the pyridinium compounds. More data would be necessary to determine the existence of any possible structure–reactivity relationships.

Reactions in Methanol—The quaternary salts react in methanol to form amine and 4-methoxymethyl-2,6-dimethylphenol, which was identified by TLC comparison with a synthesized sample. In this solvent VIII exhibited unexpectedly high absorptivity, a different λ_{max} (285 nm), and a monoexponential decline in absorbance with $t_{1/2}$ = 17 sec. An identical rate constant and λ_{max} were reported previously (18) for the reaction of the quinone methide, 4-methylene-2,6-dimethyl-2,5-cyclohexadien-1-one (V), with methanol:



V

Apparently VIII completely dissociated to amine and V by the time the spectrophotometric data were obtained. The reaction of I and II was biexponential and showed a broad rise and fall in absorbance with time. An added complication was that the rates of both steps were sensitive to small amounts of water in the methanol. Due to this problem and the kinetic complexity of A ⇌ B → C systems, reactions in methanol were not studied further.

When the quaternary compounds were dissolved in polar aprotic solvents, *e.g.*, acetonitrile, dimethylformamide, or dimethyl sulfoxide, the dissociation equilibrium was again rapidly established. Since the reaction of V with these solvents was not possible, condensation reactions occurred to form dimeric products (19).

DISCUSSION

Mechanism of Hydrolysis—The intermediate in the aqueous reaction of these quaternary compounds was probably the quinone methide (V). A generalized form of Scheme III involving unimolecular dissociation of phenoxide zwitterion to V and tertiary amine, followed by trapping of the intermediate with solvent or nucleophiles, is consistent with the data. A kinetically indistinguishable pathway is possible, involving reaction of hydroxide with the protonated form of the quaternary compounds. If this mechanism were operative, compounds with the phenolic

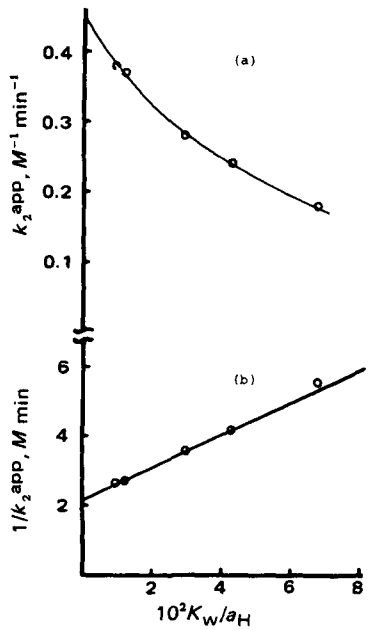


Figure 5—*Apparent rate constant for the effect of imidazole as a function of hydroxide ion concentration (a); data plotted according to Eq. 8 (b).*

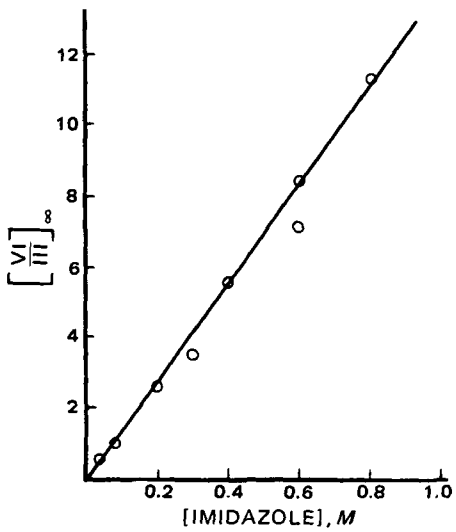


Figure 6—*Ratio of imidazole to hydroxide substitution in the product as a function of imidazole concentration for hydrolysis of I at pH 12.70, 25°, μ = 1.0 (KCl).*

Table V—Effect of Leaving Group Basicity on the Rates of Hydrolysis for I, II, VIII, and IX

Compound	pK'_a[a]	k_{max}, min^{-1}
I	5.52[b]	0.45
II	3.55[b]	15.6
VIII	5.15[c]	$10^3 \pm 10^1$
IX	9.75[c]	0.039

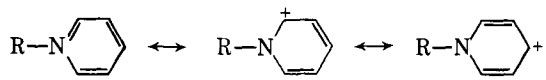
[a] pK'_a of the conjugate acid of the amine leaving group. [b] Reference 13. [c] Reference 17.

hydrogen replaced by a methyl group should exhibit comparable rates of hydrolysis to the phenolic derivatives. The complete stability of the methoxy compounds under conditions where their phenolic derivatives were quite unstable rules out a mechanism involving nucleophilic attack of hydroxide ion.

Although the proposed quinone methide intermediate (V) was too unstable in water to be observed spectrophotometrically, its presence was essential in explaining the kinetic data. Moreover, spectrophotometric detection of this compound upon methanolysis of the quaternary derivatives strongly suggested that the same intermediate occurred in water.

The mass law effect of added pyridine in the hydrolysis of I supports the proposed unimolecular mechanism. In the absence of added pyridine, the rate of reaction of V with solvent was faster than its reaction with pyridine. The rate-determining step under these conditions was the loss of reactant (k_1) and pH-independent kinetics were observed. Addition of small amounts of pyridine greatly increased the rate of reversion of V to starting material and caused the observed rate constant to exhibit an inverse dependence on pyridine concentration. The approximation that the rate of reaction of quinone methide with hydroxide was much faster than its reaction with pyridine was no longer valid. In this manner addition of pyridine transformed an apparently pH-independent reaction into one which was first-order in hydroxide.

The maximum rate of hydrolysis of the quaternary compounds of this study was quite sensitive to the structure and basicity of the tertiary amine leaving group (Table V). The rate increased with decreasing basicity of the leaving group for structurally similar compounds. Structural influence is demonstrated by the quaternary compounds of dimethylaniline and pyridine reacting at quite different rates, even though the amine leaving groups had similar pK'_a values. The large rate difference between the two structural classes is due to resonance stabilization of the pyridinium derivatives, as opposed to the instability of the aliphatic and aryl-aliphatic amines (20):



Charge delocalization of this type is not possible with salts of aliphatic or aryl-aliphatic amines.

Effect of Additives on the Rate of Hydrolysis—The effect of medium on reaction rates in solution is described by the Brønsted equation:

$$k = k_0 \frac{\gamma_A \gamma_B}{\gamma_\pm} \qquad \text{(Eq. 9)}$$

where k is the observed reaction rate constant, k_0 is the limiting value of the rate constant at zero concentrations of all solutes, and the γ's are the activity coefficients of the reactants and transition state (12). For the unimolecular reactions of the present report, this equation can be expressed as:

$$k = k_0 \frac{\gamma_z}{\gamma_\pm} \qquad \text{(Eq. 10)}$$

with γ_z defined as the activity coefficient of the zwitterionic reactant.

The hydrolysis of I involved a negative salt effect, *i.e.*, the compound was more stable as ionic strength increased. This phenomenon was due to stabilization of the zwitterionic reactant by added salt (a decrease in γ_z) more than the nonpolar transition state. Usually, a unimolecular reaction of an electrically neutral species is insensitive to variations in ionic strength (12). The highly polarized zwitterion of I, however, would be expected to be affected greatly by changes in ionic strength of the medium.

The addition of nonionic solutes at constant ionic strength caused a rate effect opposite to that of added salt. Acetonitrile or dimethylformamide stabilized the nonpolar transition state causing an increase in rate. This effect was exhibited more dramatically by the several thousand-fold increase in rate of decomposition in methanol compared to water. In addition, the reactant in methanol was presumably the nonionized phenol which was essentially unreactive in water.

The presence of several inorganic and organic nucleophilic species increased the rate of hydrolysis of I (Table IV). A Brønsted slope of ~0.1 was found for this data. Charged nucleophiles, *e.g.*, acetate, azide, imidazole anion, glycinate, and hydroxide, exhibited negative deviations compared to neutral substances of similar pK'_a values. The less polar amines, morpholine, and butylamine showed greater than first-order rate dependencies (11) while cyanide of similar basicity had no effect. These data suggest that the nucleophilic substances affect the activity coefficients rather than participating in a general acid–base or nucleophilic reaction with I.

The apparent Brønsted correlation of rate with basicity was probably a kinetic artifact arising from the effects of added solutes on the activity coefficients. The pH dependency of the effect of imidazole on the hydrolysis of I was in accord with this hypothesis. Imidazole anion is generally a much stronger nucleophile than the neutral form, although it was less effective in causing hydrolysis of I. This behavior is consistent with the expected effect of a charged species on the activity coefficients. The lack of kinetic dependency on cyanide ion is also in agreement with this hypothesis. Added sodium cyanide at constant ionic strength should have very little effect on the activity coefficient, although this compound is a strong nucleophile. The k_{OH} term observed in the hydrolysis of I was thought to be due to medium effects of hydroxide ion rather than a nucleophilic or base-catalyzed reaction.

Hydrolysis of the Ester (IV)—When the phenolic group of I was acetylated, the slow step in the overall rate of hydrolysis <pH 12.5 was the hydrolysis of the ester. The rapid hydrolysis of the quaternary compounds can, therefore, be moderated by the choice of a suitable ester function. The second-order rate constant for hydrolysis of IV is ~10-fold smaller than that for hydrolysis of phenyl acetate (13). This value is reasonable considering the rate-retarding steric and electronic effects of the two *ortho* methyl groups (21) and the accelerating effect of the ammonium group.

The results of this preliminary report indicate that quaternization and hydrolysis *via* quinone methides is a possible method for forming useful prodrug derivatives of tertiary amines. The simple amines studied represent a wide range of basicity and structure. Although actual drug molecules were not employed, the results are expected to be easily extrapolated to more complex molecules. Esterification yields a quaternary salt for which the release of the parent drug is controlled by ester hydrolysis. This is desirable since structure–reactivity relationships of esters are well known and the compounds should be substrates for enzymatic cleavage *in vivo*.

Possible Quinone Methide Intermediates in Drug Degradation—The abnormally high reactivity of drugs containing the *o*- or *p*-hydroxybenzyl alcohol structure has been recognized and studied previously (8). In a survey of reactions of several pharmaceuticals with bisulfite, an *o*- or *p*-hydroxyl or amino group was essential for reaction. *meta*-Substituted isomers as well as *o*- or *p*-methoxy derivatives were unreactive with sulfite under the experimental conditions. Substituted benzyl sulfonic acids were determined to be the products in several of the reactions. An explanation for these data would be reversible dehydration to form the quinone methide followed by a rapid reaction with bisulfite to form the sulfonate product.

The reaction of epinephrine with bisulfite also suggests the intermediacy of a quinone methide (7). Below pH 5 the rate of degradation was zero-order in bisulfite concentration indicating a unimolecular mechanism. Additional evidence for a quinone methide intermediate is the specific acid catalyzed racemization of epinephrine observed in the absence of bisulfite (22). The planar intermediate formed by dehydration could react with solvent to form optically inactive epinephrine.

REFERENCES

(1) T. Higuchi and V. Stella, Eds., "Pro-drugs as Novel Drug Delivery Systems," American Chemical Society, Washington, D.C., 1975.

(2) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).

(3) N. Bodor, in "Design of Biopharmaceutical Properties through Prodrugs and Analogs," E. B. Roche, Ed., American Pharmaceutical Association, Washington, D.C., 1977, Chap. 7.

(4) N. Bodor, J. J. Kaminski, and S. Selk, *J. Med. Chem.*, **23**, 469 (1980).

(5) N. Bodor and J. J. Kaminski, *ibid.*, **23**, 566 (1980).
(6) N. Bodor, R. Woods, C. Raper, P. Kearney, and J. J. Kaminski, *ibid.*, **23**, 474 (1980).
(7) T. Higuchi and L. C. Schroeter, *J. Am. Chem. Soc.*, **82**, 1904 (1960).
(8) T. Higuchi and L. C. Schroeter, *J. Am. Pharm. Assoc., Sci. Ed.*, **48**, 535 (1959).
(9) K. Hultzsch, *Ber. Dtsch. Chem. Ges.*, **74**, 1533 (1941).
(10) P. D. Gardner, H. Sarrafizadeh Rafsanjani, and L. Rand, *J. Am. Chem. Soc.*, **81**, 3364 (1959).
(11) J. B. Bogardus, Ph.D. Thesis, University of Kansas, 1973.
(12) L. P. Hammett, "Physical Organic Chemistry," 2nd ed., McGraw-Hill, New York, N.Y., 1970.
(13) W. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, **90**, 2622 (1968).
(14) T. St. Pierre and W. P. Jencks, *J. Am. Chem. Soc.*, **90**, 3817 (1968).
(15) J. A. Zoltewicz, G. M. Kauffman, and C. L. Smith, *ibid.*, **90**, 5939 (1968).
(16) R. N. Lindquist and E. H. Cordes, *ibid.*, **90**, 1269 (1968).
(17) D. D. Perrin, "Dissociation Constants of Organic Bases in Aqueous Solutions," Butterworths, London, England, 1965.
(18) L. J. Filar and S. Winstein, *Tetrahedron Lett.*, **25**, 9 (1960).
(19) K. Fries and E. Brandes, *Justus Liebigs Ann. Chem.*, **542**, 48 (1939).
(20) M. H. Palmer, "The Structure and Reactions of Heterocyclic Compounds," Edward Arnold Publications, London, 1967, p. 23.
(21) N. A. Fischer, G. J. Leary, R. D. Topsom, and J. Vaughan, *J. Chem. Soc. (B)*, **1966**, 782.
(22) L. C. Schroeter and T. Higuchi, *J. Am. Pharm. Assoc., Sci. Ed.*, **47**, 426 (1958).

ACKNOWLEDGMENTS

Adapted in part from a dissertation submitted by J. B. Bogardus to the University of Kansas in partial fulfillment of the Doctor of Philosophy degree requirements.

This work was supported by a National Science Foundation Graduate Traineeship (J.B.B.).

Concentration-Dependent Disappearance of Fluorouracil from Peritoneal Fluid in the Rat: Experimental Observations and Distributed Modeling

JERRY M. COLLINS ˣ, ROBERT L. DEDRICK, MICHAEL F. FLESSNER, and ANTHONY M. GUARINO

Received February 12, 1981, from the *Biomedical Engineering and Instrumentation Branch, Division of Research Services and the Developmental Therapeutics Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205.* Accepted for publication October 6, 1981.

Abstract □ The rate of disappearance of fluorouracil from peritoneal fluid has been experimentally measured and mathematically modeled. The experimental data were obtained following the instillation of 50 ml of dialysis fluid which contained an initial fluorouracil concentration ranging from 24 μM to 12 mM. The rate of disappearance was strongly dependent upon concentration. A distributed model has been formulated which incorporates concepts of diffusion with saturable metabolism and nonsaturable capillary uptake in the tissue surrounding the peritoneal fluid. This model successfully describes the experimental observations and also suggests that the effective penetration depth into tissue is highly dependent upon concentration.

Keyphrases □ Fluorouracil—concentration-dependent disappearance from peritoneal fluids, rats □ Pharmacokinetics—concentration-dependent disappearance of fluorouracil from peritoneal fluid, rats □ Peritoneal fluid—concentration-dependent disappearance of fluorouracil, rats

Although intraperitoneal injections are extensively used for the administration of drugs to rodents, there are few studies which examine the kinetic features of this route (1, 2). When drugs are administered in a small volume, it is usually assumed that absorption occurs through pathways which lead to the portal vein. For larger volumes, as used in peritoneal dialysis, nonportal pathways such as the ventral abdominal wall, diaphragm, and retroperitoneal tissues may have a significant role. Lymphatic uptake, although largely unexplored, is assumed to be quantitatively unimportant for substances with small molecular weights.

The rate of disappearance of drugs from peritoneal fluid is primarily a consequence of the concentration gradient established between peritoneal fluid and surrounding tissue. As drug molecules diffuse into this tissue, they can be carried away by capillary blood, metabolized by enzymes in the tissue, or be bound to tissue constituents. Removal by capillary blood is kinetically a first-order process, while metabolism and tissue uptake or binding are potentially saturable since these processes depend upon a finite number of sites.

The intraperitoneal administration of the pyrimidine analog, fluorouracil, is currently undergoing clinical evaluation for the treatment of several cancers which are initially confined to the abdomen (2, 3). Since the clinical range of intraperitoneal fluorouracil concentration is restricted by therapeutic considerations, a previously developed rat model was used for these peritoneal disappearance studies. The metabolism of fluorouracil is saturable (4), and at least some tissues surrounding the peritoneal cavity are sites for this metabolism (5). No tissue binding has been reported.

Data have been collected on the rate of peritoneal disappearance over a wide range of initial concentrations in order to observe metabolism in both linear and nonlinear regions. A distributed model has been formulated which incorporates concepts of diffusion with chemical reaction and capillary uptake in the surrounding tissue. The nonlinear partial differential equation was solved numerically with the appropriate initial and boundary conditions. The solution suggests that the effective penetration depth into the tissue and the rate of removal from the peritoneal cavity are concentration dependent.

0022-3549/82/0700-0735$01.00/0