Kinetic Study of the Hydrolysis of 1-(4-Nitrophenyl)-3-methyltriazene in Aqueous Solution and in the Presence of Surfactants

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Abstract □ The hydrolysis of 1-(4-nitrophenyl)-3-methyltriazene in aqueous solution has been studied over a pH range of 3–14. The effect of the anionic and cationic surfactants (sodium lauryl sulfate and hexadecyltrimethylammonium bromide) on the rate of hydrolysis was investigated. The quaternary ammonium bromide causes a rate decrease at all pH values studied, while sodium lauryl sulfate enhances the acid-catalyzed hydrolysis and decreases the observed rate constants in the pH-independent region. The results are discussed in terms of the current theory of micellar effects.

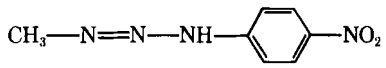
Keyphrases □ 1-(4-Nitrophenyl)-3-methyltriazene—kinetic study of the hydrolysis, effects of sodium lauryl sulfate and hexadecyltrimethylammonium bromide

The hydrolysis of arylmonoalkyltriazenes has been studied extensively (1–3). These substances are important as *in vivo* metabolites of the corresponding aryldialkyltriazenes, a class of compounds possessing antitumor (4–6), carcinogenic (7–9), and mutagenic (10–12) activity.

The pH-rate profiles in aqueous solution of various 1-aryl-3-methyltriazenes (1) and 1-phenyl-3-propyltriazenes (3) show two different regions; the rate of hydrolysis decreases sharply with increasing pH and then becomes almost constant. The plateau region is shifted to lower pH values by electron-withdrawing substituents in the aryl moiety. It has been postulated that the acid-catalyzed reaction involves an alkanediazonium ion as an intermediate or a protonated triazene as a discrete intermediate whose formation is rate determining. In the pH-independent region, a mechanism consisting of a simple unimolecular heterolysis of a nitrogen–nitrogen bond with anilide ion departing from an alkanediazonium ion was formulated (3).

Furthermore, the influence of serum albumins on the rates of some arylmonomethyltriazenes in buffered aqueous solution was also investigated (13). It was shown that the presence of these proteins in solution caused a marked reduction in the decomposition rates of the triazenes. The experimental results were interpreted by assuming that the substrates interact rapidly and reversibly with a binding site on the protein and that they cannot decompose when bound to this site.

The aim of the present work was to complete the pH–rate profile for a representative triazene, 1-(4-nitrophenyl)-3-methyltriazene (I), above pH 10 since systematic data of kinetic runs in this pH region were lacking. Furthermore, we studied the influence of the addition of cationic and anionic surfactants, above their critical micelle concentration (CMC), on the decomposition rate. During the past decade studies of this phenomenon have been stimulated by the hope that a micellar-catalyzed reaction might provide a basic model for interpretation of some aspects of enzymatic catalysis since micelles can be regarded as a model for enzymes and biomembranes (14–16). The effect of the micelles on drug stability has been recently reviewed (17).



EXPERIMENTAL SECTION

Materials—1-(4-Nitrophenyl)-3-methyltriazene (I) was prepared as described previously (18), mp 112–113°C [lit. (18) mp 111–113°C]. Hexadecyltrimethylammonium bromide and sodium lauryl sulfate were purified by methods described previously (19). The buffers used were: formate (pH 3.0–4.2), succinate (pH 4.5–6.0), phosphate (pH 6.5–8.0), borate (pH 9.0), carbonate (pH 10.0–11.0), and phosphate (pH 11.0–12.0).

Kinetic Methods—The triazene was dissolved in acetonitrile to give a 2×10^{-2} M stock solution which was stored in the refrigerator. This solution (10 μL) was added to a volume of the buffer in a quartz cell thermostated at 25 ± 0.1°C to obtain a concentration of the substrate on the order of 10^{-5} M. The ionic strength was kept constant at 0.01. The pH values were determined with a digital pH-meter[1] at the temperature of the kinetic run for reactions in water. The analytical pH was used for reactions in the presence of surface-active agents—as the apparent pH in micelles. The decomposition reactions were followed spectrophotometrically[2] at 350 nm when the disappearance of protonated or neutral triazene was followed, or at 470 nm when anionic triazene was present. These wavelengths were chosen to permit maximum variation and minimum interference with the spectrum of the decomposition product. The spectrum at the end of the reaction was coincident with that of *p*-nitroaniline. Absorbances were recorded as a function of time. Pseudo-first-order rate constants were calculated using a least-squares method. Correlation coefficients were between 0.994 and 0.999.

RESULTS AND DISCUSSION

Hydrolysis in Aqueous Solution—Figure 1 shows the dependence on pH of the first-order rate constant for the decomposition of I which is consistent with that obtained for 1-(4-nitrophenyl)-3-propyltriazene by Sinnot *et al.* (3). Two routes for the triazene decomposition were confirmed: a pH-independent pathway and an acid-catalyzed reaction with a negative slope (~1, Table I). The acid-catalyzed reaction pathway was interpreted as an $A\text{-}S_E2(N)$ reaction involving a transition state in which the proton is almost completely transferred and little N—N bond-breaking has occurred, whereas the pH-independent reaction was considered as an S_N1 reaction on nitrogen without participation of water (3).

Above pH 12.5, a third region was observed with a negative slope (~1); in this case the formation of the anionic triazene was shown by a change in the visible spectrum (Fig. 2). We suggest that the decrease in log k_{obs} in this pH region is essentially due to the diminution of the reactive neutral triazene involved in the acid–base equilibrium.

The suggested overall mechanism of the reaction is described in Scheme I, where T is the unprotonated, TH^+ the protonated, and T^- the deprotonated, triazene.

$$T^- + H^+ \underset{K_2}{\rightleftharpoons} T + H^+ \underset{K_1}{\rightleftharpoons} TH^+ \xrightarrow{k_1} \text{products}$$

$$\downarrow k_2$$

$$\text{products}$$

Scheme I

[1] pHM84 Radiometer or 632 Metrohm pH meters.
[2] Model 552 and Lambda 5 spectrophotometers; Perkin-Elmer.

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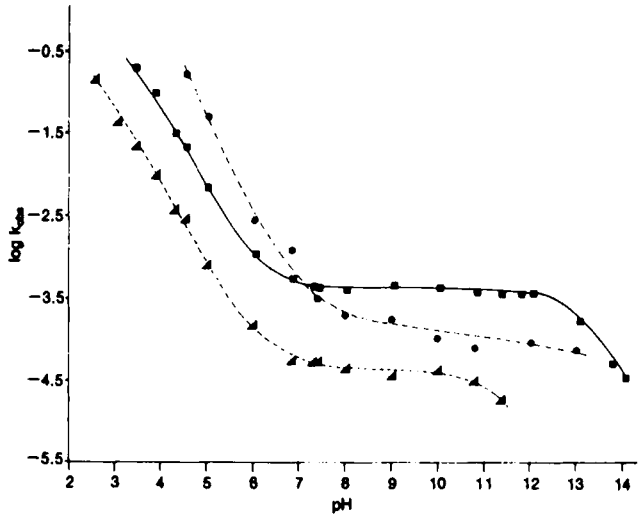

Figure 1—*Log k_{obs}-pH profile of I. Key: (■) in buffered aqueous solution at 25°C and μ = 0.01, calculated according to Eq. 1; (▲) in presence of 2 × 10^{-2} M hexadecyltrimethylammonium bromide; (●) in presence of 2 × 10^{-2} M sodium lauryl sulfate.*

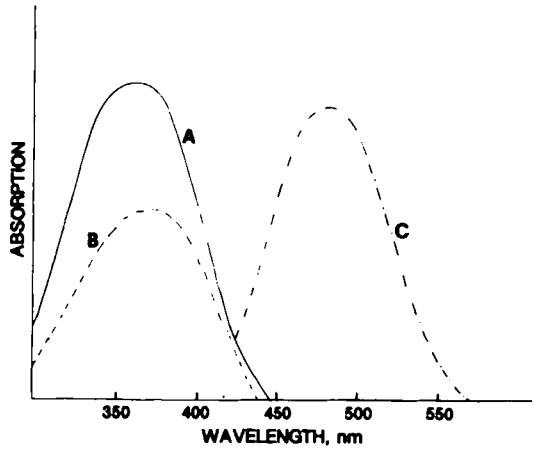

Figure 2—*Changes in visible absorption spectra in buffered aqueous solution. Key: (A) neutral triazene, pH 8.45 (I); (B) p-nitroaniline, pH 8.45; (C) the anionic form of triazene, 1M NaOH (I).*

Equation 1 represents the rate law for the mechanistic scheme:

$$k_{obs} = \frac{k_1[H^+]^2 + k_2K_1[H^+]}{[H^+]^2 + K_1[H^+] + K_1K_2} \qquad \text{(Eq. 1)}$$

The curve fitting of this equation is reported in Fig. 1 according to the following parameters: $k_1 = 0.5\ s^{-1}$; $k_2 = 3.5 \times 10^{-4}\ s^{-1}$; $K_1 = 7.9 \times 10^{-4}$ M; $K_2 = 1.0 \times 10^{-13}$ M. The estimated value of $pK_2 = 13.0$ is reasonable as compared, for example, with the $pK_a = 12.31$ measured for the deprotonation of *p*-nitroformanilide (20).

Inhibition of Hydrolysis by Hexadecyltrimethylammonium Bromide—Cationic micelles of hexadecyltrimethylammonium bromide inhibit the hydrolysis rate at any pH value considered (Table II). As expected, cationic micelles of hexadecyltrimethylammonium bromide inhibit the acid hydrolysis of I. The variation of rate constant with surfactant concentration is generally based on the assumption that substrate, in this case triazene (T), is distributed between the aqueous and micellar pseudophases, with the first-order rate constants designated as $k_{w'}$ and $k_{m'}$. The micellar surfactant concentration, $[D_n]$, is assumed to be the total surfactant concentration [D] less that of monomeric surfactant which is assumed to be given by critical micelle concentration (CMC), so that $[D_n] = [D] - CMC$; K_s is the equilibrium constant for substrate binding.

$$T + D_n \underset{}{\overset{K_s}{\rightleftharpoons}} TD_n$$

$$\downarrow k_{w'} \qquad \downarrow k_{m'}$$

products products

$$K_s = \frac{[TD_n]}{[T][D_n]}$$

Scheme II

Following Scheme II the relation between k_{obs} and $[D_n]$ is shown by:

$$k_{obs} = \frac{k_{w'} + k_{m'}K_s[D_n]}{1 + K_s[D_n]} \qquad \text{(Eq. 2)}$$

and its rearrangement gives:

$$\frac{1}{k_{w'} - k_{obs}} = \frac{1}{k_{w'} - k_{m'}} + \frac{1}{(k_{w'} - k_{m'})K_s([D] - CMC)} \qquad \text{(Eq. 3)}$$

By plotting $1/(k_{w'} - k_{obs})$ against $1/([D] - CMC)$ it is possible to calculate

Table I—Kinetic Data of the Pseudo-First-Order Decomposition of I [a]

Buffer Conc.	Buffer	Final pH	k_{obs}, $s^{-1} \times 10^4$ H_2O	Hexadecyltrimethylammonium Bromide 2×10^{-2} M	Sodium Lauryl Sulfate 2×10^{-2} M
0.0032	HCl	2.50	—	1490	—
0.016	Sodium chloroacetate	3.02	—	447	—
0.030	Sodium formate	3.42	1720	227	—
0.017	Sodium formate	3.85	837	99	—
0.013	Sodium formate	4.26	271	38.6	—
0.012	Sodium succinate	4.50	181	29.8	1760
0.076	Sodium succinate	5.00	58.8	8.29	537
0.040	Sodium succinate	6.00	9.33	1.56	29.8
0.0059	Sodium phosphate	6.82	4.71	0.58	13.0
0.0044	Sodium phosphate	7.30	3.75	0.56	4.67
0.0042	Sodium phosphate	7.40	3.63	0.57	3.40
0.0036	Sodium phosphate	8.00	3.44	0.47	2.14
0.027	Sodium borate	9.00	3.91	0.38	1.90
0.057	Sodium carbonate	10.00	3.60	0.45	1.14
0.025	Sodium carbonate	10.80	3.30	0.34	0.88
0.0022	Sodium hydroxide	11.35	3.07	0.20	—
0.0056	Sodium hydroxide	11.75	3.07	—	—
0.010	Sodium hydroxide	12.02	3.11	0.032	1.04
0.10	Sodium hydroxide	13.00	1.41	—	0.84
0.50	Sodium hydroxide	13.70	0.44	—	—
1.0	Sodium hydroxide	14.00	0.29	—	—

[a] In buffered aqueous solution and in the presence of hexadecyltrimethylammonium bromide and sodium lauryl sulfate at 25°C.

Table II—Rate of Hydrolysis of 1-(4-Nitrophenyl)-3-methyltriazene in the Presence of Hexadecyltrimethylammonium Bromide

Hexadecyltrimethylammonium Bromide, M × 10^3	k_{obs}, s^{-1} × 10^4 [a]			
	pH 4.50	pH 7.30	pH 8.45	pH 12.02
–	181	3.75	3.61	3.11
1	—	3.44	—	0.20
2	164	2.27	3.31	0.091
3	135	—	2.77	0.057
4	107	1.67	2.48	0.050
6	79.5	—	2.04	0.048
8	63.1	—	1.35	0.037
10	52.7	0.82	0.88	0.038
20	29.8	0.56	0.61	0.032

[a] Values determined at 25°C and μ = 0.01.

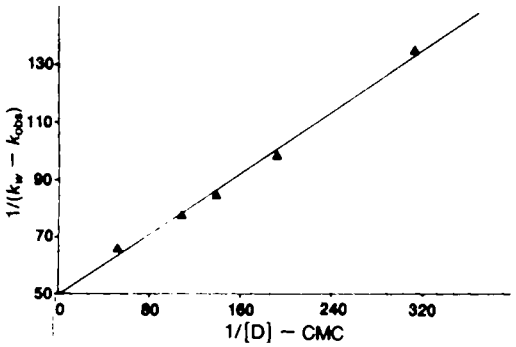

Figure 3—*The effect of hexadecyltrimethylammonium bromide on* k_{obs} *at pH 4.50 according to Eq. 2.*

$k_{w'}$, $k_{m'}$, and K_s. However, this plot is sensitive to CMC values and, as previously shown, this problem can be solved by assuming CMC as an adjustable parameter (21); a representative plot is shown in Fig. 3.

At pH 4.50 the best fit is obtained by taking CMC = 8 × 10^{-4} M, which is identical to the value determined by the surface tension method (22); then K_s = 180 M^{-1}. Similar treatment with data obtained at pH 7.30, 8.45, and 12.02 gives the results listed in Table III. Under these conditions $k_{m'}$ is very small relative to $k_{w'}$. From the estimated binding constants and pH-rate profile in micelles, it can be observed that the substrate in its neutral form is moderately bound to hexadecyltrimethylammonium bromide micelles while the anionic form, which is present at pH 12.02, is strongly bound (K_s = 35,000 M^{-1}). Also, the equilibrium between neutral triazene and its anionic form is strongly shifted by hexadecyltrimethylammonium bromide toward the anionic form, *i.e.*, the apparent pK_a value of triazene is changed by stabilization of the anionic form by cationic micelles. These effects of micelles on equilibria have been previously reported by Bunton *et al.* (23).

Hydrolysis in Presence of Sodium Lauryl Sulfate—The anionic sodium lauryl sulfate micelles enhance the rate of acid-catalyzed hydrolysis by a factor of ~10 at pH 4.5–6.0, as shown in Table I. Otherwise, sodium lauryl sulfate inhibits the uncatalyzed reaction by a smaller factor (~2).

For a given hydrogen ion concentration, the rate constants go through a maximum with increasing concentration of the anionic surfactant (Table IV). At pH 4.50 the largest enhancement occurs at 2 × 10^{-2} M sodium lauryl sulfate and the rate constant is approximately 10-fold higher than that determined in water.

The kinetic micellar effect could be interpreted by separation of the contributions of the reaction in the aqueous and micellar pseudophases, as reported (21). The quantitative treatment requires knowledge of the binding constant of triazene with micellar sodium lauryl sulfate. Unfortunately, we cannot measure or estimate K_s. However, the results in the acid-catalyzed region confirm the importance of the stabilization of the protonated form of the triazene in anionic micelles causing a change in its pK_a value.

The "Uncatalyzed" Reaction in Presence of Sodium Lauryl Sulfate and Hexadecyltrimethylammonium Bromide—The inhibition caused by both anionic and cationic micelles is not surprising since the transition state for the S_N1 decomposition of the triazene should have a strong zwitterionic character and, therefore, should be destabilized in the less polar micellar medium, whereas the initial state should be stabilized. Inhibitory effects by hexadecyltrimethylammonium bromide micelles have been previously found in the S_N1 reaction of 1-bromo-2-phenylpropane (24) and of 3-bromo-3-phenyl propionate ion (25) and by both hexadecyltrimethylammonium bromide and sodium lauryl sulfate on the S_N1 reaction of α-phenylallyl butanoate (26). These effects are comparable to the well-known effects of solvent on S_N1 reactions (27, 28).

Table III—Binding Constants and Micellar Rate Constants Calculated in Presence of Hexadecyltrimethylammonium Bromide

pH	K_s, M^{-1}	CMC, M × 10^3	$k_{w'} - k_{m'}$, s^{-1}	$k_{w'} = k_{obs(w)}$, s^{-1}
4.50	180	0.8	2.1 × 10^{-2}	1.81 × 10^{-2}
7.30	440	0.4	3.5 × 10^{-4}	3.75 × 10^{-4}
8.45	70	0.8	6.0 × 10^{-4}	3.61 × 10^{-4}
12.02	35000	0.2	3.1 × 10^{-4}	3.11 × 10^{-4}

Table IV—Rate of Hydrolysis of 1-(4-Nitrophenyl)-3-methyltriazene in the Presence of Sodium Lauryl Sulfate

Sodium Lauryl Sulfate, M × 10^3	k_{obs}, s^{-1} × 10^2 [a]
—	1.81
4	2.16
6	5.2
8	10.3
10	13.7
20	17.6
30	17.2
40	17.5
50	15.2
60	13.6
80	15.0

[a] Values at 25°C, μ = 0.01, and pH 4.50.

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Kinetics and Mechanism of Enzymatic Hydrolysis of Pivampicillin Monolayers

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Abstract □ A study of the enzymatic hydrolysis of pivampicillin (an insoluble penicillin) extended as a monolayer on the aqueous interface at a constant surface pressure has been performed. Penicillinase promotes intensive hydrolysis of the pivampicillin monolayers, inducing their solubility. However, no action was observed with dog liver esterase. The hydrolytic process, which was dependent on the film surface pressure and on the quantity of the injected enzyme, is of the Michaelis-Menten type in two dimensions.

Keyphrases □ Pivampicillin—enzymatic kinetics, surface monolayers, penicillinase, esterases □ Kinetics—enzymatic, pivampicillin, surface monolayers, Michaelis-Menten

The catalytic action of an enzyme can generally be measured by the quantity of transformed substance or by the product generated per unit of time. The processes of enzymatic kinetics in bulk materials are usually Michaelis-Menten in character (1). Under these conditions, both soluble substrates and enzyme-substrate complexes are in a homogeneous system. However, insoluble substrates are normally in the form of micellar aggregates, which act as the real substrate. In such cases, study of the kinetics is complicated by the difficulty of knowing the concentration of substrate in contact with the enzyme.

This difficulty can be overcome by extending the insoluble substrate as a monolayer film over an aqueous mass which contains the soluble enzyme. Under these conditions, all the molecules of substrate are equally exposed to the action of the enzyme. Thus, it is possible to quantitate the amount of substrate in contact with the enzyme (by controlling its surface concentration in the film) and the amount of enzyme reacting with the surface (by controlling its concentration in the subphase). This system makes it possible to obtain information about action of the enzyme sites in the substrate molecules.

The first studies of enzymatic kinetics using the surface monolayer technique were performed by Hughes (2) in 1935. Measuring the decrease in surface potential during the hydrolysis process, Hughes observed that the hydrolysis rate of lecithin in monomolecular films catalyzed by phospholipase A_2 decreased when the surface concentration of the lecithin molecules in the interface increased. This method is, however, subject to significant errors in interpretation.

Since that time, several authors have used the monolayer technique to measure the activity of lipolytic processes catalyzed by lipases and phospholipases. Short-chain phospholipids and triglycerides were used as substrates so that the reaction products would be soluble and, therefore, diffuse into the aqueous subphase, leaving the monolayer. Garner and Smith (3) performed these determinations in a constant area, measuring the decrease of the monolayer surface pressure as the substrate was dissolved by the lipolytic effect of the enzyme. An inherent difficulty of this method is that the surface concentration of the film molecules vary continually during the process; therefore, the decrease of the surface pressure is linear with respect to time only in the first moment. Lagocki *et al.* (4), Dervichian (5), and Verger and de Haas (6) used methods with constant pressure, measuring the kinetic rate by the decrease of the area covered by the film (directly connected with the number of substrate molecules which escape from the surface).

The method of "zero-order trough" or "two-compartment trough" proposed by Verger and de Haas (6) is particularly interesting. In this method the enzymatic reaction takes place in a different compartment than the one which acts as a substrate reservoir. In this way, the number of the substrate molecules in the reaction compartment can be kept constant during the entire process. The process develops in a pseudo-zero-order manner, *i.e.*, the decrease of the monolayer area is proportional to time, yielding a straight line, the course and slope of which give the rate of the kinetic process.

Kinetic studies have been performed on monolayers of insoluble penicillins (7). The penicillin molecules are transformed by hydrolysis into soluble products that leave the monolayer and dissolve in the aqueous subphase. The number of hydrolyzed molecules can be determined by the monolayer surface decrease, maintaining a constant surface pressure at all times (Fig. 1).

For this type of study, pivampicillin is a particularly interesting test drug. It is a penicillin which is insoluble in neutral solutions and soluble in acidic media. Pivampicillin is activated by esterases, which transform it into ampicillin (8), and it is

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