Hydrolysis Kinetics of Benzocaine and Homologs in the Presence of a Nonionic Surfactant

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Abstract □ The kinetics of alkaline hydrolysis of benzocaine (ethyl *p*-aminobenzoate), *n*-butyl *p*-aminobenzoate, and ethyl *p*-*n*-butylaminobenzoate in the presence of a nonionic surfactant (polyoxyethylene 24 monocetyl ether) were investigated. The influence of the nonionic surfactant on the solubility and on the wavelength of maximum absorbance in the UV portion of the spectrum of the three compounds was also studied. At surfactant concentrations above the CMC, the rate of hydrolysis of the esters is decreased, the solubility is enhanced, and there is a bathochromic shift in the wavelength of maximum absorbance. As a result of the investigation, the partition coefficients of the compounds between the micellar phase and the aqueous phase, the location of the compounds within the micellar phase, and the hydrolytic rate constants within the two phases were determined. It was concluded that benzocaine and the homologs are probably associated with the polyoxyethylene portion of the micelle and that the observed change in the rate of hydrolysis can be attributed to both a decrease in the rate of hydrolysis and an increase of solubility within the micellar phase.

Keyphrases □ Benzocaine and homologs—surfactant (polyoxyethylene 24 monocetyl ether) effect on alkaline hydrolysis, solubility, and UV absorbance □ *p*-Aminobenzoate esters—nonionic surfactant effect on alkaline hydrolysis, solubility, and UV absorbance □ Surfactant, nonionic, effect—benzocaine and homolog hydrolysis, solubility, and UV absorbance

Several investigations have concerned the influence of micelles on the kinetics of reaction, and some of the main types of reactions studied are those concerning hydrolysis (1) and oxidation (2). Relationships between the charge on the micelle and the charge on the reactant have been discussed (3, 4) as has secondary valence force catalysis (5). Reviews concerning the effects of surfactants on kinetics have also been presented (6, 7). Some publications relate to the present work, that is, inhibition of hydrolysis in the presence of surfactants (8–10). The significance of partition coefficients or saturation ratios on the rates of hydrolysis also has been investigated (11–13).

Due to the interest in this type of research, it was decided to study the kinetics of hydrolysis of a few compounds differing only in the number of methylene groups. The importance of the partition coefficient of the compounds between the micellar and aqueous phases as it affects the observed kinetics was also investigated. The data obtained regarding the change of wavelength of maximum absorbance (λ_{max}) of benzocaine and its homologs in the surfactant solutions were used to provide an indication of the location of the compounds within the micellar phase. The studies were conducted to determine the influence of the molecular structure of the substrates upon the observed rates of hydrolysis in the presence of surfactants. Benzocaine and its homologs were selected because the kinetics of alkaline hydrolysis have been well established (14) and the rate of hydrolysis of benzocaine is known to be altered in the presence of surfactants (15). The surfactant (polyoxyethylene 24 monocetyl ether) was used because of its resistance to hydrolysis as a result of possessing ether rather than ester linkages. Preliminary experiments indicated that the nonionic surfactant increased the solubility of benzocaine appreciably.

EXPERIMENTAL

Materials—A commercial sample[1] of ethyl *p*-aminobenzoate (benzocaine, I) was purified by recrystallization (15), and *n*-butyl *p*-aminobenzoate (II)[2] was purified in a similar manner. Ethyl *p*-*n*-butylaminobenzoate (III) was synthesized and purified (16). The characteristics of the esters were checked by their melting points and their spectral characteristics in water (wavelengths of maximum absorbance and molar absorptivity) (14, 16, 17). The nonionic surfactant polyoxyethylene 24 monocetyl ether[3], mol. wt. 1300 (18), was purified by partitioning between benzene and a saturated solution of sodium chloride in water in a manner similar to that previously reported (mp 43.5–44.5°) (19).

Kinetic Determinations—Known concentrations of I or its homologs in surfactant solutions or water were prepared by using freshly boiled distilled water. The hydrolysis was carried out in the presence of a relatively high concentration of sodium hydroxide (approximately 0.045 *N*) so that there was no significant decrease in the hydroxide-ion concentration during the reaction. The kinetic studies, in duplicate, were conducted in a spectrophotometer[4] equipped with a dual thermospacer set through which water, from a thermostatted bath, was continuously circulated to maintain the cells at a constant temperature of 30°. Hydrolysis of the esters was followed by observing the change in the absorbance at the λ_{max} of the ester in the surfactant solution. From the values of the absorbances at zero and infinite time, the first-order rate constants at 0.045 *N* NaOH were obtained by plotting the logarithm of the percentage ester remaining against time and correcting for the actual sodium hydroxide concentration.

Solubility Studies—The solubilities of the esters were determined in duplicate by shaking an excess quantity of the ester in solutions containing different surfactant concentrations. The mixtures were shaken at 30° for 5 days in a water bath shaker. An aliquot of the clear supernatant liquid was diluted with water and analyzed spectrophotometrically, employing a suitably diluted surfactant solution as a blank.

UV Absorbance Studies—The wavelength of maximum absorbance of the esters in hexane solutions, aqueous surfactant solutions, and aqueous polyethylene glycol 1000 solutions was determined by dissolving suitable amounts of the esters in the solvent and determining the absorbances at different wavelengths.

RESULTS AND DISCUSSION

The solubilities of the esters are given in Table I. There is a slight increase in the solubility of each ester in the presence of the surfactant (concentration 1×10^{-5}%) as compared to the solubility of the ester in water. This slight increase in solubility may be

[1] British Drug Houses.
[2] Matheson Coleman and Bell.
[3] Texofor A24, Glover's Chemicals Ltd.
[4] Beckman DU-2.

Table I—Effect of Surfactant Concentration on the Properties of the Esters at 30°

Ester	Surfactant Concentration, %	Solubility of Ester, %	$\frac{S_e - S_{ew}}{C_s}$ [a]	Partition Coefficient	$10^3 k$ [b], min^{-1}
I	0	0.123	—	—	11.3
	1.0×10^{-5}	0.127	—	—	11.1
	0.1	0.143	1.53	165	9.07
	0.5	0.196	1.15	121	6.85
	1.0	0.267	1.13	118	4.87
	5.0	0.825	1.10	115	1.56
II	0	2.20×10^{-2}	—	—	7.32
	1.0×10^{-5}	2.35×10^{-2}	—	—	7.77
	0.1	4.44×10^{-2}	1.49	1030	3.24
	0.5	0.126	1.39	955	1.19
	1.0	0.224	1.35	917	0.674
	5.0	1.08	1.42	965	0.138
III	0	6.48×10^{-4}	—	—	6.87
	1.0×10^{-5}	6.60×10^{-4}	—	—	7.03
	0.1	3.85×10^{-3}	0.188	4996	1.11
	0.5	1.66×10^{-2}	0.187	4971	0.288
	1.0	3.28×10^{-2}	0.188	4950	0.180
	5.0	0.16	0.186	4912	0.0407

[a] Moles of ester solubilized per mole of surfactant. [b] Pseudo-first-order rate constant in 0.045 *N* NaOH.

due to the interaction of the esters with the polyoxyethylene portion of the surfactant. Guttman and Higuchi (20) reported on the effects of macromolecules, such as polyethylene glycols, which give rise to an alteration in the solubility of a number of organic compounds. Above the critical micelle concentration (CMC), the solubilities of the ester increased significantly, as is to be expected when micelles are formed that offer a suitable medium for the dissolution of the solutes.

The results of calculating the ratio of moles of ester solubilized per mole of surfactant, $(S_e - S_{ew})/C_s$, where S_e = molar solubility of the ester, S_{ew} = molar solubility of the ester in water, and C_s = molar concentration of the surfactant, are provided in Table I. It can be seen that above the CMC the solubilization of the esters per molecule of surfactant is essentially constant, indicating that the solubilizate does not alter the composition of the micelles in such a way so as to affect the solubilizing power of the micelles. The number of molecules of II solubilized per molecule of surfactant is appreciably greater than for I as a result of the increase of the hydrocarbon portion of the molecule which makes the butyl ester more suitable for dissolution within the micellar environment. This result is similar to that reported by Patel (21), where the percentage binding to the surfactant increases with increasing hydrocarbon chain length of the solubilizate if the solubilized molecules are small. The value of $(S_e - S_{ew})/C_s$ for III is considerably smaller than are the values for the other two esters, even though the number of methylene groups has increased. It is logical to expect that I and II would be associated with the micelle in such a way that the hydrocarbon portion of the molecules would be directed toward the lipophilic interior of the micelle and that the polar portion of the molecules would be directed toward the hydrophilic polyoxyethylene part of the micelle (22). As a result of this arrangement between the solubilizate and the surfactant molecules in the micelle, the configuration of the two esters would be linear, permitting closer packing within the micelle and resulting in a relatively high value for $(S_e - S_{ew})/C_s$. In contrast to this situation, III has a nonpolar hydrocarbon group on opposite ends of the molecule and this would tend to discourage a linear configuration of the ester and the close packing arrangement if the polar portion of the ester is associated with the polar part of the micelle and the nonpolar portions of the ester are associated with the hydrocarbon interior of the micelle. The value of $(S_e - S_{ew})/C_s$ in Table I would tend to support this concept. In addition, there is a rank-order correlation between the values of $(S_e - S_{ew})/C_s$ and the difference between the λ_{max} for water and for the solution containing 5% surfactant in Table III, which indicates that both I and II have the greatest degree of interaction with the surfactant micelles.

Table II—Reaction Rate Constants of Unsolubilized (k_a) and Solubilized (k_m) Esters at 30° in 0.045 *N* NaOH

Ester	$10^3 k_a$, min^{-1}	$10^4 k_m$, min^{-1}	$10^3 k_m/k_a$
I	10.4	1.88	17.9
II	6.4	0.332	5.19
III	6.64	0.335	5.05

The partition coefficients for the distribution of the esters between the micellar and aqueous phases can be calculated from the following relationships:

$$K = \frac{C_m}{C_a} = \frac{A_m/V_m}{C_a} \qquad \text{(Eq. 1)}$$

where C_m = the concentration of the ester in the micellar phase, C_a = the concentration of the ester in the aqueous phase, A_m = the amount of ester in the micellar phase ≈ (total amount of ester present in the solution − the amount of ester in the aqueous phase), and V_m = the volume of the micellar phase ≈ the volume or weight of the surfactant corrected for the CMC, taken to be 0.001 g/100 ml (18).

The results of these calculations are given in Table I; the value of the partition coefficient increases appreciably with an increase in the length of the hydrocarbon chain of the solubilizate. The smaller the water solubility of the compound, the greater is the tendency for the ester to be partitioned in favor of the micellar phase. Consequently, even though the uptake of III per molecule of surfactant is less by a factor of almost 10, this compound is mostly in the surfactant phase due to its extremely low solubility in water.

The pseudo-first-order rate constants were averaged (Table I), and none of the individual rate constants differs from the average by more than 1%. The value of the second-order rate constant (liter $minute^{-1}$ $mole^{-1}$) for I in solution containing no surfactant (0.253) is similar to earlier studies (0.252) (23) and the value of the second-order rate constant for III (0.152) in water at 30° is comparable to the calculated value (0.183) obtained from the data of Karlen and Agren (14).

The reduction in the rate of hydrolysis of II and III in water as compared to I can be attributed to the replacement of either the ethyl group or hydrogen atom by the butyl group, which possesses greater electron-releasing properties (14, 24), thus hindering the nucleophilic attack of the hydroxide ion at the acyl carbon atom.

It can be seen from Table I that the rate of hydrolysis of each ester is reduced appreciably in high concentrations of the surfactant and that this reduction in the rate takes place when the concentration of the surfactant is above the CMC. This effect on the rate of hydrolysis can be explained from the knowledge that a considerable amount of the ester is contained within the micelle and by postulating that the rate of hydrolysis in the micellar phase is smaller than the rate in the aqueous phase.

Consequently, it is assumed that the overall rate of hydrolysis

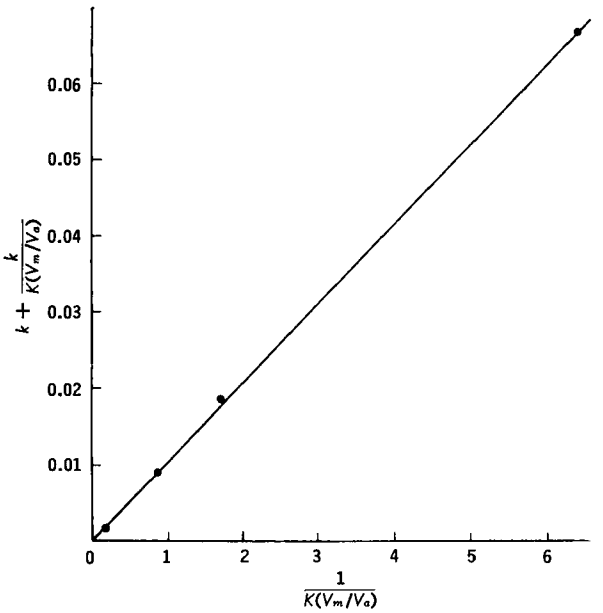

Figure 1—*Plot of Eq. 5 for I.*

can be expressed according to the following equation:

$$-(V_a + V_m)\frac{d\bar{C}}{dt} = k_a V_a C_a + k_m V_m C_m \qquad \text{(Eq. 2)}$$

where V_m = the volume of the micelles ≈ volume or the weight of surfactant added (corrected for the concentration of free molecules), V_a = the volume of aqueous alkaline solution, C_m = the concentration of ester in the micelle, C_a = the concentration of ester in the aqueous solution, k_m = the pseudo-first-order rate constant of solubilized ester, k_a = the pseudo-first-order rate constant of unsolubilized ester, and $\bar{C}$ = the average concentration of ester which is equal to $(V_a C_a + V_m C_m)/V_a + V_m$.

The partition coefficient of the ester is given by:

$$K = \frac{C_m}{C_a} \qquad \text{(Eq. 3)}$$

The combination of Eq. 2 with Eq. 3 leads to Eq. 4, where k is the measured pseudo-first-order rate constant:

$$k = -\frac{d \ln \bar{C}}{dt} = \frac{k_a - k_m}{1 + K\frac{V_m}{V_a}} + k_m \qquad \text{(Eq. 4)}$$

This mathematical treatment is similar to that reported by Tong *et al.* (11) for the decomposition of indoaniline dyes in the presence of surfactants. They assumed that the dyes were solubilized in the surfactant micelles, the dyes were distributed between the aqueous and micellar phases with a constant partition coefficient, and the rate constant for the decomposition of the solubilized dye was different in general from that of the free dye.

In the present work, it was not assumed that the partition coefficient remained constant with increasing concentrations of surfactants, since it was of particular interest to correlate the partition coefficient, the measured pseudo-first-order rate constants, and other physical properties of the system with the molecular structure of the esters. Consequently, the partition coefficients were determined by solubility experiments.

To obtain the values of k_a and k_m, Eq. 4 was rearranged as shown in Eq. 5:

$$k + \frac{k}{K\frac{V_m}{V_a}} = \frac{k_a}{K\frac{V_m}{V_a}} + k_m \qquad \text{(Eq. 5)}$$

Equation 5 was then plotted (Fig. 1) using the experimental values of k, K, V_m, and V_a; the results for k_a and k_m as determined by linear regression analysis are given in Table II.

The values of k_a should be similar to the pseudo-first-order rate constant for the hydrolysis of the ester in solutions containing no surfactant, since k_a represents the rate of hydrolysis outside of the micellar phase. It can be seen from Tables I and II, however, that the values of k_a for the three esters are somewhat smaller than the values of k at 0% surfactant concentration. While it is presumed that the surfactant is completely dissociated below the CMC, some type of complex formation may possibly exist between the ester molecules and the individual surfactant molecules. This type of interaction could account for the smaller values of k_a as compared to the rate constant in solutions containing 0% surfactant. This reduction in the rate of hydrolysis at low concentrations of surfactant is similar to the work of Lach and Pauli (25) who reported on the stabilizing and solubilizing effect of compounds, such as polyethylene glycol 4000 which does not form micelles, on benzocaine in aqueous solution. Consequently, from previous work (20, 25) and from the present work regarding both solubility and kinetics, it seems reasonable to postulate that there is some interaction between the ester and the surfactant below the CMC.

The value of k_m is the pseudo-first-order rate constant for the hydrolysis of the ester within the micellar phase. The values of k_m are considerably smaller than the values of k_a, indicating that conditions conducive to rapid hydrolysis of the ester are no longer present within the micelle. A number of factors have been suggested to explain the reduction in the rate of reactions within the micellar phase. For example, it has been shown that the rate of hydrolysis of I is reduced when the polarity of the medium is decreased by altering the ratio of organic solvent to water in the solvent system (26). Thus, it would seem logical to expect that the decrease in the rate of hydrolysis of the three esters is at least partly due to the less polar nature of the micellar phase. Another factor that may be responsible for the alteration of rates is due to enrichment of one or both of the reactants in the micelles. A catalytic effect of micelles has been observed when both reactants are enriched within the micelle. This catalytic effect has been noticed

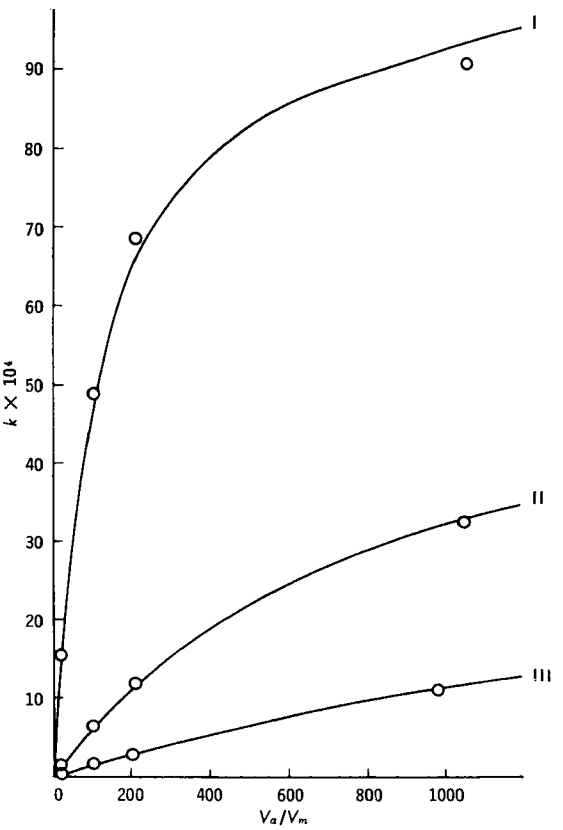

Figure 2—*Plot of observed rate constants* (○) *and calculated rate constants* (——) *from Eq. 4 as a function of* V_a/V_m *for Compounds I–III.*

Table III—Wavelength of Maximum Absorbance in Different Solvents

Solvent	I, λ_{max}, nm	II, λ_{max}, nm	III, λ_{max}, nm
Water	286	285	305
1×10^{-5}% surfactant in water	286	286	305
0.1% surfactant in water	286.2	290	307.5
0.5% surfactant in water	287	293	308
1% surfactant in water	289	294	308
5% surfactant in water	293	294	308
Hexane	270	271	291
50% polyethylene glycol 1000 in water	294.5	296	312

when, for example, both the reactants and the micelles are ionic in nature (27). Conversely, when one reactant is enriched and the other reactant is excluded, a decrease in the rate is observed. This situation exists in the present study since it has already been shown that the esters are concentrated within the micelles and it would be expected that the hydroxide ions would be excluded, at least to some extent, from the nonpolar regions of the micelle, thus leading to a decrease in the rate of reaction. A third factor that would tend to alter the rate of hydrolysis within the micellar phase is due to the interaction between the esters and the surfactant molecules (28). The complex so formed could sterically hinder the approach of the attacking agent and/or the complex could alter the electronic structure of the esters to render them less susceptible to attack by the nucleophilic reagent.

The value of the ratio of the rates of hydrolysis of the ester within the micelle phase to that within the aqueous phase should be approximately the same if the micellar environment causes a proportional decrease in the rate of hydrolysis in the two phases. It can be seen from Table II that the values of k_m/k_a are considerably smaller for II and III. This suggests that the micellar environment has a greater inhibiting effect on the rate of hydrolysis when the nonpolar portion of the substrate molecule has been increased. As a result, the values of both k_m and K depend upon the chain length of the solubilizate.

The effect of the two constants, k_m and K, on the observed rate constant is particularly important for esters with a large partition coefficient and in solutions when the ratio V_m/V_a is high since, under these conditions, nearly all of the ester is solubilized within the micelle and the observed rate constant approaches the value of k_m. This phenomenon can be seen by comparing the observed rate constants for Table I with the value of k_m in Table II.

Figure 2 illustrates the comparison of the observed and calculated rate constants in relation to the variable V_a/V_m. The calculated rate constants were plotted by using the average value for K and the values of k_m and k_a (from Table II) for each ester and by employing Eq. 4. This graph indicates that there is good agreement between the experimental results and the postulation that two main factors, namely the partition coefficient and the pseudo-first-order rate constant in the micellar phase, account for the major portion of the decrease in the observed rate when the surfactant concentration is increased for this series of compounds. It will be noted that when K is large (Compound III) in comparison with V_a/V_m, Eq. 4 becomes linear (Fig. 2).

The λ_{max} of each ester increases as the polarity of the solvent increases from hexane to water (Table III); as a result, the excited state of the esters is considered to be more polar than the ground state (29). Since the quinoid form of substituted benzene derivatives (30) contributes substantially to UV absorption, it is expected that the excited state would receive a significant contribution from the more highly polar quinoid structure (31). Such structures with separated charges are stabilized by solvent polarity in accordance with simple electrostatic theory. Hence, the polar solvent, water, facilitates excitation and a red-shift is observed as compared to the nonpolar solvent, hexane. The λ_{max} for each of the three esters shows a further red-shift in the solvent composed of polyethylene glycol 1000 (IV) and water. The composition of this solvent was selected so as to simulate the concentration of water in the polyoxyethylene portion of the micelle where there are about two or three water molecules per oxygen atom (32). This enhanced red-shift suggests that there is a further degree of interaction of the solvent with the ester molecules (33), thus encouraging the stabilization of the more polar form of the esters.

Similarly, in the presence of the surfactant, a further red-shift, compared to water, is observed, indicating a degree of interaction between the esters and the surfactant. The shift for III is not as great as it is with the two other esters, which implies that there is less interaction with the surfactant. Riegelman (15) suggested that if the surfactant concentration is increased, the characteristics of the exterior of the micelle approaches those of a mixture of IV and water. Consequently, the λ_{max} of the solubilizate at high surfactant concentrations should approach that of IV and water if the esters are located in the exterior portion of the micelle. Both I and II have λ_{max} values in the 5% surfactant solution that are close to the λ_{max} in the 50% mixture of IV and water, indicating that these two compounds are located mainly within the polyoxyethylene portion of the micelle. The relatively smaller shift for III indicates that it is located in a different portion of the micelle and that this compound is not bound to the surfactant to the same extent as the other two esters. Other evidence for the smaller degree of interaction of III compared with the other two esters was given in the discussion of $(S_e - S_{ew})/C_s$.

The UV studies thus provide some evidence for the location of the esters within the micellar phase and further evidence for interaction between the esters and the surfactant. As a result of this interaction, the ester will become more polar or quinoid in character, which will increase the electron density at the carbonyl portion of the esters. Since the alkaline hydrolysis of esters occurs as a result of a nucleophilic attack of the hydroxide ion on the carbonyl carbon atom, the rate of hydrolysis would be expected to decrease in the micelle where a more polar form of the ester exists and where there is a greater electron density at the site of attack.

In spite of the factors discussed, which tend to inhibit the rate of hydrolysis within the micelle, it seems both from a mathematical analysis and from experimental evidence that the overall kinetic rate constant approaches a limiting value, k_m, as the concentration of the surfactant is increased, which indicates that those regions of the micellar phase in which the esters are located are not impervious to penetration by the hydroxide ion.

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Hydrolysis and Epimerization Kinetics of Pilocarpine in Aqueous Solution

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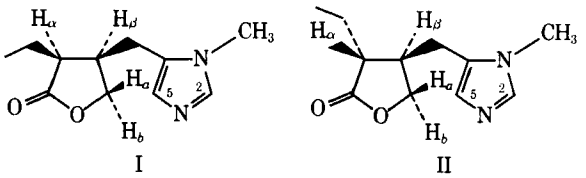
Abstract □ The kinetics of the hydroxide-ion-catalyzed hydrolysis and epimerization of pilocarpine in aqueous solution were investigated utilizing pH-stat titrimetry and NMR spectroscopy. The mechanism of epimerization involves formation of a carbanion stabilized by resonance with the enolate hybrid. Both hydrolysis and epimerization follow pseudo-first-order kinetics, and the appropriate rate constants and energies of activation were calculated. Epimerization was found to occur to a greater extent than previously assumed and must be considered as a major pathway of degradation and inactivation of pilocarpine. The rate of hydroxide-ion-catalyzed epimerization increases more rapidly with temperature than does the rate of hydrolysis, a fact that should be considered if ophthalmic solutions of pilocarpine are sterilized by heat. It is suggested that isopilocarpine may not be a genuine jaborandi alkaloid but an artifact produced by epimerization of pilocarpine during drying, storage, and extraction of the plant material.

Keyphrases □ Pilocarpine—kinetics of hydroxide-ion-catalyzed hydrolysis and epimerization, pH-stat titrimetry and NMR spectroscopy □ Epimerization kinetics and hydrolysis, pilocarpine—determination, pH-stat titrimetry and NMR spectroscopy □ NMR spectroscopy—determination, pilocarpine epimerization kinetics

Pilocarpine, an alkaloid obtained from various species of *Pilocarpus* (Rutaceae), was isolated independently by Gerrard (1) and Hardy (2) in 1875. Later, several closely related alkaloids were isolated, such as isopilocarpine, pilocarpidine, and pilosine. The elucidation of the chemical structures of the jaborandi alkaloids is due primarily to Jowett (3), Pinner and Kohlhammer (4), and Pinner and Schwarz (5). There are two asymmetric centers in the lactone part of the molecule, and Jowett (6) assumed the relationship between pilocarpine and isopilocarpine to be stereochemical (*cis–trans*-isomers). This assumption was supported by the work of Langenbeck (7) and proven by synthesis (8–10). However, it was not until 1966 that Hill and Barcza (11) established the absolute configuration of the two asymmetric centers as 2*S*:3*R* for pilocarpine (I) and 2*R*:3*R* for isopilocarpine (II).



I II

Pilocarpine, which is the *cis*-isomer, can be converted to the more stable isopilocarpine by heating or by treatment with sodium ethoxide followed by acidification (11–13). Both alkaloids are dextrorotatory. Two pathways of degradation of pilocarpine are hydrolysis to pilocarpic acid and epimerization to isopilocarpine, with both mechanisms resulting in loss of pharmacological activity.

Hydrolysis of pilocarpine in aqueous solution is an equilibrium process catalyzed by hydrogen ions and hydroxide ions (14). Several investigators studied the hydrolytic decomposition of pilocarpine at various pH values and temperatures (15–20), and a detailed kinetic study was reported (14). Much less is known about the epimerization reaction. The difficulty of detecting partial epimerization has been due to the fact that there has been no simple way of separating the two diastereoisomers or of quantitating either epimer in the presence of the other. Döpke and