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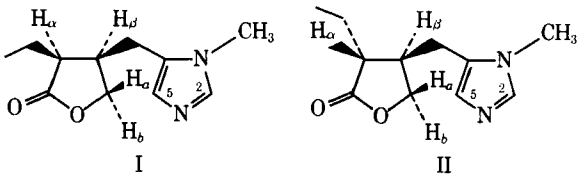
Hydrolysis and Epimerization Kinetics of Pilocarpine in Aqueous Solution

MATHEWS A. NUNES and EINAR BROCHMANN-HANSSENˣ

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

d'Heureuse (13) showed that epimerization of pilocarpine to isopilocarpine under anhydrous alkaline conditions occurs exclusively at the α-carbon of the lactone ring. Chung *et al.* (14) and Link and Bernauer (21) found evidence that pilocarpine undergoes some epimerization in aqueous solution at alkaline pH, based on the differences in specific rotation of the two epimers.

Since the rates of both hydrolysis and epimerization are pH dependent and occur simultaneously, they may be considered as competing reactions. A study of kinetic parameters should, therefore, take into account both mechanisms. The purposes of this investigation were to develop a method for quantitating epimerization and to determine the rates of hydrolysis and epimerization of pilocarpine in aqueous solution.

EXPERIMENTAL

Reagents—The following were used: pilocarpine nitrate[1], mp 174–175° [lit. (22) mp 174–179°], $[\alpha]_D^{23}$ + 80.0° (c 2, water) [lit. (22) $[\alpha]_D$ + 79.5 to +82.0° (c 2, water)]; isopilocarpine nitrate[2], mp 158–158.5° [lit. (23) mp 159°], $[\alpha]_D^{23}$ + 39.4° (c 2, water) [lit. (24) $[\alpha]_D$ + 38.8° (water)]; deuterium oxide[3], 99.85 mole %; sodium deuteroxide[4], 40% in deuterium oxide; deuterium chloride[4], 38% in deuterium oxide; methanol-[OD][4]; and nitrogen gas, 99.996+% pure. All other chemicals were of analytical reagent grade.

Apparatus—Consumption of base was recorded by pH-stat titrimetry[5]. The reactions were carried out in a water-jacketed reaction vessel of 75-ml capacity and stirred with a magnetic stirrer. Solution temperature was maintained using a circulating water bath with proportional temperature control. The NMR spectra were prepared with a 100-MHz spectrometer[6], and the expanded spectra were integrated using a curve resolver[7].

Procedure—*NMR Spectra*—All 100-MHz NMR spectra were recorded in deuterium oxide with tetramethylsilane as the external reference. For the analysis of the proportion of epimers, the NMR spectrum of the terminal methyl group of the α-ethyl substituent was recorded with a 10-fold scale expansion and a 1.0-ppm offset. The methyl triplet of each epimer was integrated with a curve resolver.

Hydroxide-Ion-Catalyzed Reactions—The experimental procedure used for determination of the rate of base consumption was similar to that of Chung *et al.* (14), except that potassium hydroxide was used in place of sodium hydroxide to minimize the sodium effect on the electrode at high pH values. Furthermore, considerably higher concentrations of pilocarpine were used to facilitate sampling for NMR spectroscopy. Pilocarpine nitrate (2.5 g) was dissolved in 50 ml of distilled water in a water-jacketed reaction vessel surrounded by a styrofoam insulator. The vessel was continuously flushed with a stream of nitrogen which had passed through a series of three gas-washing towers containing 10% sulfuric acid, 10% sodium hydroxide, and distilled water, respectively. When the desired temperature was reached, the solution was rapidly brought to the preset pH with 2 *N* potassium hydroxide and the pH-stat recorder was returned to zero to start recording the rate of reaction. The consumption of base was recorded over 8–10 half-lives. The pseudo-first-order rate constants were obtained from Guggenheim plots (25) with the aid of a least-squares program (26) and a calculator[8]. As soon as the addition of base was discontinued, two 1-ml samples of solution were withdrawn and brought to pH < 1 with concentrated hydrochloric acid. The samples were kept at room temperature and in the dark for 1 hr, shell frozen in a dry ice–acetone bath, and lyophilized. The dry residues were dissolved in deuterium oxide (0.35 ml), and the 100-MHz NMR spectra were recorded.

[1] Sigma Chemical Co.
[2] Pierce Chemical Co.
[3] Bio-Rad Laboratories.
[4] Stohler Isotope Chemicals, Inc.
[5] Radiometer pH stat–titrimeter assembly, consisting of PHM-28 pH meter, TTT11 titrator, SBR2C titrigraph, SBU1a syringe burette, TTA3 titration assembly, GK2301C combination electrode, and B104 5-ml syringe.
[6] Joelco JNM-4H-100.
[7] Dupont model 310.
[8] Hewlett-Packard model 9100.

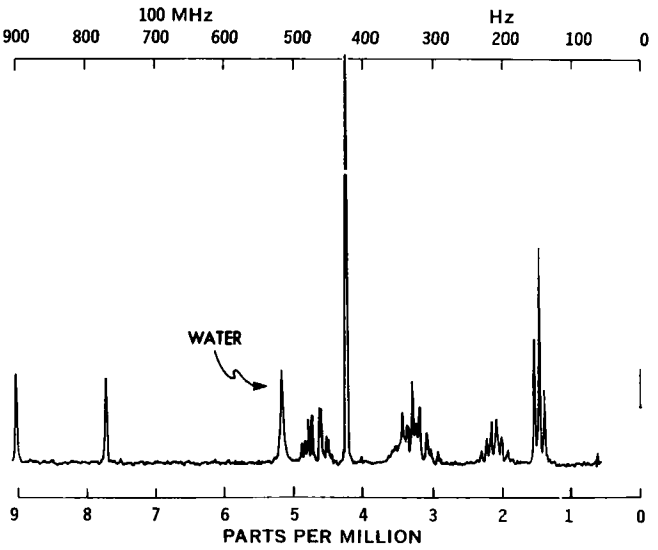


Figure 1—*NMR spectrum of pilocarpine nitrate in deuterium oxide.*

RESULTS AND DISCUSSION

The 60-MHz NMR spectrum of pilocarpine was reported (19) where NMR spectroscopy was used to determine hydrolysis of pilocarpine solutions buffered to pH 6.1. The NMR spectrum of isopilocarpine has not been reported previously, and Link and Bernauer (21) were unable to detect any change in the NMR spectrum of pilocarpine after partial epimerization.

The 100-MHz NMR spectra of pilocarpine nitrate and isopilocarpine nitrate are illustrated in Figs. 1 and 2, respectively. The principal differences in the spectra of the two epimers are caused by the γ-protons of the lactone ring and the terminal methyl protons of the α-ethyl substituent. For pilocarpine, H_a is centered at 4.55 ppm (J_{gem} = 9.0 Hz, J_v = 3.0 Hz) and H_b is at 4.80 ppm (J_{gem} = 9.0 Hz, J_v = 6.0 Hz), while isopilocarpine shows a signal for H_a at 4.48 ppm (J_{gem} = 10.0 Hz, J_v = 6.2 Hz) and H_b at 4.93 ppm (J_{gem} = 10.0 Hz, J_v = 6.3 Hz). The terminal methyl groups of the ethyl substituents appear as triplets with a coupling con-

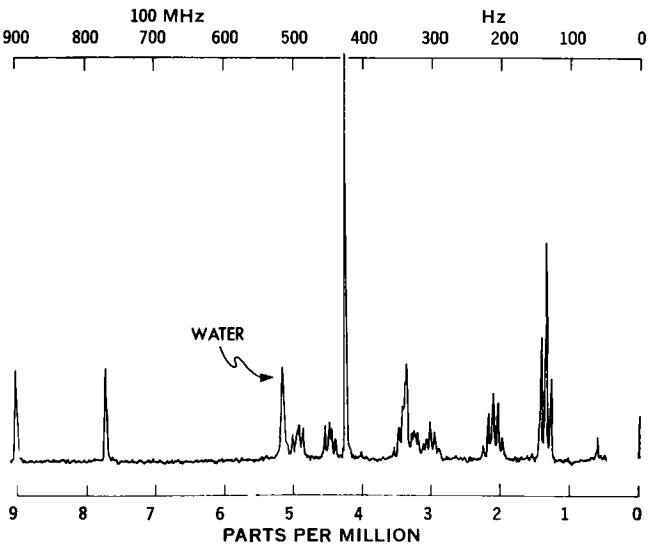


Figure 2—*NMR spectrum of isopilocarpine nitrate in deuterium oxide.*

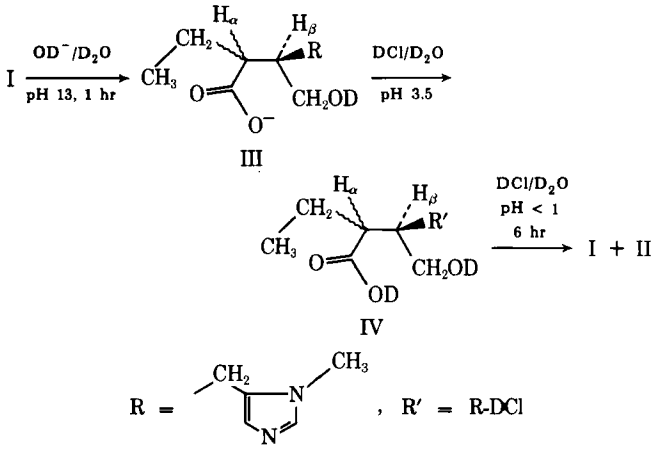
Table I—NMR Spectral Data of Pilocarpine Nitrate (I), Isopilocarpine Nitrate (II), Pilocarpate (III), Pilocarpic Acid (IV), Isopilocarpate (V), and Isopilocarpic Acid (VI) in Deuterium Oxide

	CH_3 / CH_2—	CH_3 / CH_2—	H_α	H_β	—CH_2 Bridge	N—CH_3	H_a	H_b	C-5 Proton of Imidazole	C-2 Proton of Imidazole
I	1.46 (t)	2.07 (m)	←———	3.27 (m)	———→	4.25 (s)	4.55 (dd)	4.80 (dd)	7.72 (s)	9.06 (s)
II	1.36 (t)	2.08 (m)	←———	3.21 (m)	———→	4.25 (s)	4.48 (dd)	4.93 (dd)	7.72 (s)	9.06 (s)
III	1.31 (t)	1.86 (m)	2.26 (m)	2.65 (m)	3.04 (m)	3.99 (s)	←	3.96 (m)	→	7.18 (s)	7.92 (s)
IV	1.31 (t)	2.05 (m)	←	2.71 (m)	→	3.21 (m)	4.25 (s)	←	4.04 (m)	→	7.72 (s)	9.06 (s)
V	1.31 (t)	1.98 (m)	2.36 (m)	2.72 (m)	3.12 (m)	4.02 (s)	←	3.96 (s)	→	7.18 (s)	7.92 (s)
VI	1.31 (t)	2.06 (m)	←	2.76 (m)	→	3.25 (d)	4.27 (s)	←	4.02 (d)	→	7.72 (s)	9.06 (s)

stant of 7.25 Hz; however, the chemical shifts differ by 0.1 ppm for the two epimers. The spectral data are summarized in Table I.

Pilocarpic acid and isopilocarpic acid, as well as their respective anions, are all encountered during hydrolysis and recyclization. For spectral identification of these intermediates, pilocarpine and isopilocarpine were hydrolyzed with base to the sodium salts of the γ-hydroxy acids. The solutions were then neutralized with deuterochloride to pH 3.5 to give the deuterochloride salts of the free acids[9]. At this pH, the rate of lactone formation is slow (14). Finally, acid was added to pH < 1 for cyclization of the lactones. These reactions are illustrated in Schemes I and II. Deuterated reagents and solvents were used to avoid the large water signal in the NMR spectra. This resulted in partial exchange of the α-proton of pilocarpine for deuterium as epimerization proceeded and, consequently, a reduction in the NMR signal of the α-proton relative to those of nonexchangeable protons.

When pilocarpine and isopilocarpine were hydrolyzed in alkaline solution to the anions of the γ-hydroxy acids (III and V), the NMR triplets of the terminal methyl group of the ethyl substituents shifted upfield to 1.31 ppm for both epimers. The γ-protons (H_a and H_b) also shifted upfield and overlapped the *N*-methyl group. The spectra of the hydroxy acids (IV and VI) showed that the methyl triplets of the ethyl substituents were in the same position as in the anions, whereas the γ-protons had shifted slightly downfield toward their position in the closed lactones, resonating slightly above 4 ppm. After complete hydrolysis and recyclization of pilocarpine according to Scheme I, the NMR spectrum of the product was different from that of the original pilocarpine salt. Each γ-proton still appeared as a doublet of doublets for pilocarpine; however, there were also peaks corresponding to the γ-protons of isopilocarpine. The multiplet due to H_α, H_β, and the methylene bridge between the two rings was also different from either pure pilocarpine or isopilocarpine. The signal for the methyl group of the ethyl substituent clearly showed six peaks, corresponding to the methyl triplets of pilocarpine and isopilocarpine,

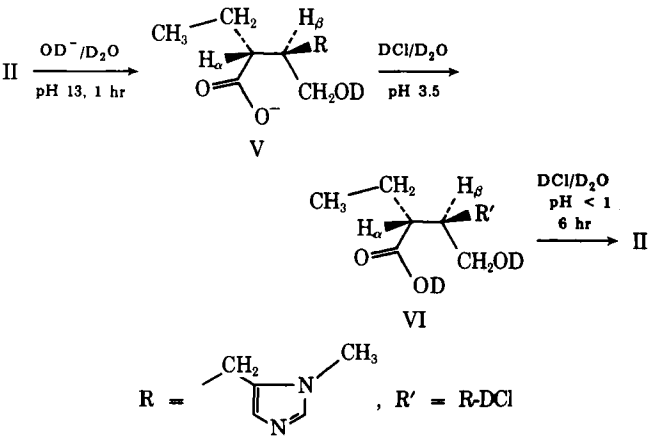


Scheme I—Effects of base on pilocarpine (I); recyclization of the lactone in acid medium

and provided the best means of quantitating epimerization. For this purpose, the triplets were recorded with a 10-fold scale expansion and an offset of 1.0 ppm (Fig. 3). Integration of the two triplets corresponding to pilocarpine and isopilocarpine showed that the mixture produced according to Scheme I at room temperature consisted of about 80% pilocarpine and about 20% isopilocarpine.

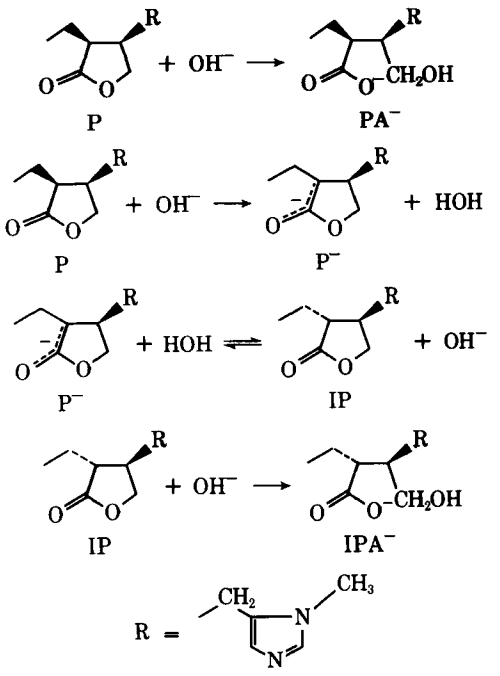
Several workers have shown that once the lactone is hydrolyzed and the hydroxy acid is in the anion form, no further epimerization takes place (12, 13). In agreement with this, NMR spectroscopy revealed that the ratio of pilocarpine to isopilocarpine was constant at constant temperature when the solutions were kept at pH 13 for various periods ranging from a few minutes to 2 days. To determine if the rate of cyclization of the lactone affected this ratio, a completely hydrolyzed solution was separated into two parts. One part was brought quickly to pH < 1 for rapid cyclization while the other was maintained at pH 3.5 for 6 hr before being brought to a pH < 1. When the methyl regions were integrated, the ratio of pilocarpine to isopilocarpine was the same in both solutions. Therefore, the spectra of III and IV in Scheme I and Table I do not represent pure pilocarpate and pilocarpic acid but mixtures of the epimers in the same ratio as that determined after recyclization of the lactone ring. When isopilocarpine was completely hydrolyzed and recyclized according to Scheme II, the spectrum of the product was identical with that of the isopilocarpine salt before hydrolysis. This indicated that isopilocarpine did not epimerize under these conditions to an extent that could be detected by NMR spectroscopy. Therefore, the equilibrium constant for epimerization is overwhelmingly in favor of the *trans*-epimer.

Epimerization requires the abstraction of the α-proton to produce a carbanion which may be stabilized by delocalization to the enolate (13). Subsequent reprotonation gives the thermodynamically more stable *trans*-epimer. The α-protons of γ-lactones are more acidic than those of open esters (27), and Döpke and d'Heureuse (13) showed the enolate to be an intermediate during the epimerization of pilocarpine in sodium ethoxide. In such anhydrous medium, the reaction proceeds smoothly without significant opening of the lactone ring. Isopilocarpine will produce the same carbanion under these conditions, as demonstrated by deuterium exchange. Isopilocarpine nitrate was refluxed with sodium



Scheme II—Effect of base on isopilocarpine (II); recyclization of the lactone in acid medium

[9] The pH measurements were performed with a pH meter standardized with aqueous buffers and expressed in pH units, ignoring the difference between the ionization products of water and deuterium oxide.



Scheme III—Hydroxide-ion-catalyzed hydrolysis and epimerization of pilocarpine (P). PA⁻ = pilocarpate, P⁻ = delocalized carbanion = IP⁻, IP = isopilocarpine, and IPA⁻ = isopilocarpate.

methoxide in methanol-[OD] for 18 hr and acidified with deuterium chloride. The solution was evaporated to dryness and the residue was dissolved in deuterium oxide. The NMR spectrum revealed that the multiplet corresponding to H_α, H_β, and the protons of the methylene bridge integrated for three protons instead of four, indicating complete exchange of the α-proton. The spectrum showed no evidence of pilocarpine salt.

The overall effect of aqueous base on pilocarpine may be considered to be the result of three principal reactions: hydrolysis to pilocarpate, epimerization to isopilocarpine, and hydrolysis to isopilocarpate. These reactions are illustrated in Scheme III, which represents the hydroxide-ion-catalyzed hydrolysis of pilocarpine to pilocarpate (P → PA⁻), the base-catalyzed epimerization of pilocarpine (P → P⁻ and P⁻ → IP), and the hydrolysis of isopilocarpine (IP → IPA⁻). The rate of disappearance of pilocarpine in alkaline solution follows pseudo-first-order kinetics (14). The rate of epimerization and subsequent hydrolysis of isopilocarpine (P → P⁻ → IP → IPA⁻) must also be governed by rate-limiting pseudo-first-order reactions. Thus, the rate of disappearance of pilocarpine is controlled by two competing pseudo-first-order reactions, and Scheme III may be abbreviated:

$$P + OH^- \xrightarrow{k_H} PA^-$$

$$P + OH^- \xrightarrow{k_E} IPA^-$$

Scheme IV

The rate law may be written in the following form:

$$\frac{-d(P)}{dt} = (k_H + k_E)(OH^-)(P) \quad \text{(Eq. 1)}$$

$$= k_{exp}(OH^-)(P) \quad \text{(Eq. 2)}$$

$$= k_{exp}'(P) \quad \text{(Eq. 3)}$$

where k_H is the second-order rate constant for the specific hydroxide-ion-catalyzed hydrolysis of pilocarpine, k_E is the second-order rate constant for the specific hydroxide-ion-catalyzed epimerization and hydrolysis of the isopilocarpine thus produced, k_{exp} is the experimentally determined second-order rate constant, and k_{exp}' is the experimentally determined pseudo-first-order rate constant. A plot of log k_{exp}' against pH yielded straight lines with slopes of one, verifying a first-order dependence on hydroxide ion. The pH profiles at various temperatures are illustrated in Fig. 4.

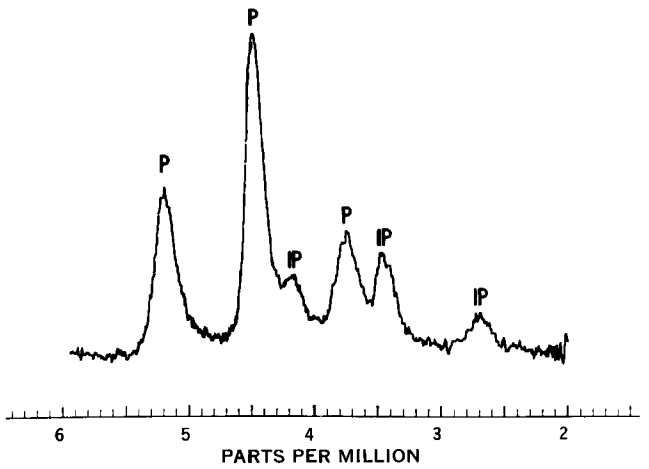


Figure 3—*Expanded NMR spectrum of the terminal methyl group of the α-ethyl substituent of pilocarpine deuterochloride after partial epimerization. P and IP designate pilocarpine and isopilocarpine, respectively.*

The experimentally determined second-order rate constant (k_{exp}) was 6.96×10^2 liters/mole/hr at 25°, or 8% lower than the value reported by Chung *et al.* (14). To determine if this difference was due to a concentration effect, control experiments were performed in which the concentration of pilocarpine nitrate ranged from 0.02 to 6%. The observed rate constants were the same over the entire concentration range.

Since the hydroxide-ion-catalyzed reactions of pilocarpine are competing pseudo-first-order reactions, the fraction of pilocarpine that disappears by each pathway during the reaction is constant as is the ratio of products from each pathway. Consequently, the ratio of pilocarpate to isopilocarpate at the end of the reaction is equal to the ratio of the rate constant for hydrolysis of pilocarpine (k_H) to the rate constant for epimerization to isopilocarpine and its hydrolysis (k_E), *i.e.*:

$$\frac{(PA^-)}{(IPA^-)} = \frac{k_H}{k_E} \quad \text{(Eq. 4)}$$

Epimerization does not occur after the lactone ring has been opened, whether in the anion or in the free acid form. Therefore, the NMR spectra recorded after complete hydrolysis and recyclization, when integrated, gave the ratio of epimers in the solution at any stage during the hydroxide-ion-catalyzed reactions. The spectra showed that the percentage of isopilocarpine formed during these reactions was independent of pH but dependent on temperature (Table II). At least three separate experiments were performed at each pH value and temperature, and two samples of

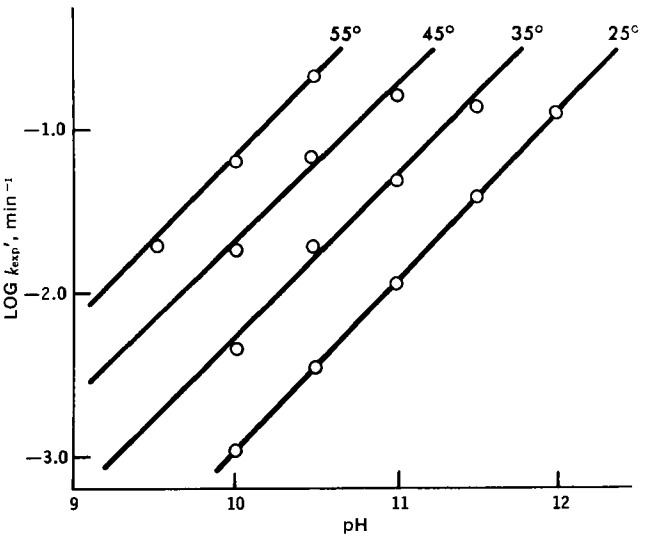


Figure 4—*The pH profile of the disappearance of pilocarpine in alkaline pH.*

Table II—Epimerization of Pilocarpine to Isopilocarpine after Complete Hydrolysis and Recyclization (Percent)

pH	Temperature 25°	35°	45°	55°
9.5	—	—	29.6	32.3
10.0	20.5	26.3	30.4	32.0
10.5	20.8	27.0	29.5	32.3
11.0	20.0	25.9	30.3	—
11.5	21.0	25.7	—	—
12.0	20.8	—	—	—

each hydrolyzed solution were analyzed for the extent of epimerization. Thus, the percentages of isopilocarpine recorded in Table II are the average values from six or more spectra. The coefficient of variation was 5.0%.

From Eq. 4 it follows that the ratio (X) of pilocarpine to isopilocarpine determined from the NMR spectra is equal to the ratio of the rate constants, *i.e.*:

$$\frac{k_H}{k_E} = X \qquad \text{(Eq. 5)}$$

Since $k_{exp} = k_H + k_E$ (Eqs. 1 and 2), substitution gives:

$$k_{exp} = k_H + \frac{k_H}{X} \qquad \text{(Eq. 6)}$$

which may be solved for k_H:

$$k_H = k_{exp}\left(\frac{X}{X+1}\right) \qquad \text{(Eq. 7)}$$

Similarly, k_E may be expressed as:

$$k_E = \frac{k_{exp}}{X+1} \qquad \text{(Eq. 8)}$$

From Eqs. 7 and 8, k_H was calculated to be 5.53×10^2 liters/mole/hr at 25°, while k_E was 1.43×10^2 liters/mole/hr at 25°. The pseudo-first-order rate constant for hydrolysis of isopilocarpine was significantly greater than that of pilocarpine. Therefore, the rate-limiting step in the conversion of pilocarpine to isopilocarpate (Scheme IV) must be epimerization to isopilocarpine. Consequently, k_E is the second-order rate constant for hydroxide-ion-catalyzed epimerization.

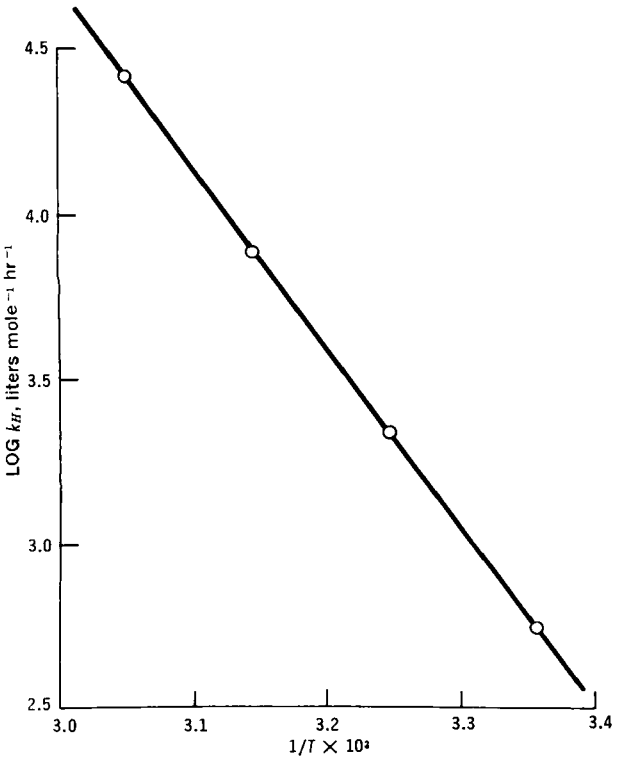


Figure 5—*Arrhenius plot of hydroxide-ion-catalyzed hydrolysis of pilocarpine.*

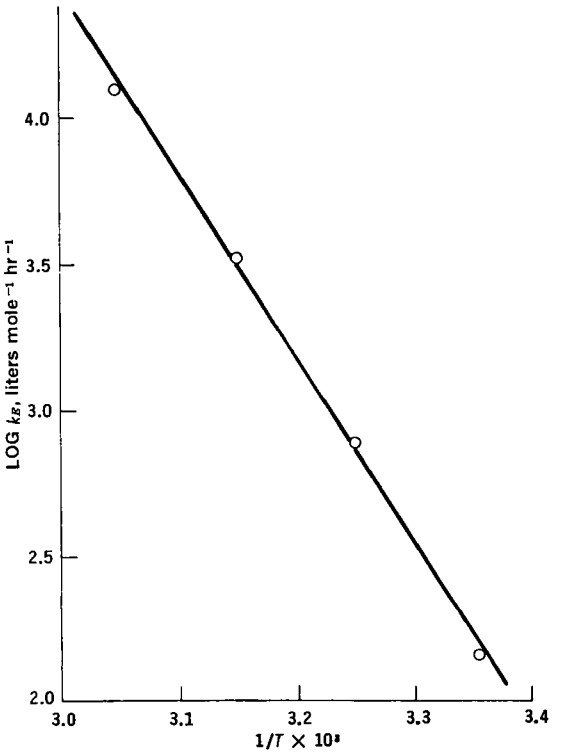


Figure 6—*Arrhenius plot of hydroxide-ion-catalyzed epimerization of pilocarpine.*

Arrhenius plots for hydrolysis of pilocarpine and for epimerization to isopilocarpine are illustrated in Figs. 5 and 6, respectively. By applying the method of least squares to each plot, the energy of activation for the hydrolysis of pilocarpine was found to be 25.02 kcal/mole (r = 0.9995), and for epimerization it was 28.48 kcal/mole (r = 0.9998). The magnitude of the epimerization rate constant, together with its comparative increase with temperature, leads to the conclusion that epimerization represents a major pathway of degradation and inactivation of pilocarpine in aqueous solution. Since the rate of hydroxide-ion-catalyzed epimerization increases more rapidly with temperature than does the rate of hydrolysis, ophthalmic solutions sterilized by heat may suffer significant loss of activity by this mechanism. It may also be surmised that it is not possible to isolate or extract pilocarpine base from its salts or from jaborandi leaves without some concurrent epimerization. It is, therefore, conceivable that isopilocarpine may not be a product of alkaloid biosynthesis in the living plants but may be an artifact produced during drying, storage, extraction of the plant material, and/or isolation of the alkaloids.

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Corneal Absorption of Ophthalmic Drugs

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Abstract □ Corneal absorption of 0.0075% homatropine hydrobromide solution and 0.0025% tropicamide solution in rabbit eyes was reinforced by prebuffering the eyes with specific alkaline buffers or by adding viscolizers and a surfactant or by a combination of these measures. For 0.0075% homatropine hydrobromide solution, prebuffering with an isotonic sodium borate solution (2.6%) and two "biological" buffer solutions, 0.2 *M* cyclohexylaminopropanesulfonic acid and 0.2 *M* tris(hydroxymethyl)methylaminopropanesulfonic acid, was found to increase significantly the amount of corneal absorption of the mydriatic. Cyclohexylaminopropanesulfonic acid provided the greatest amount of increase. Two viscolizers, 0.7% hydroxypropyl methylcellulose (45 cps) and 0.375% guar gum (41 cps), increased the effect of tropicamide more than that of homatropine hydrobromide. Hydroxypropyl methylcellulose, providing slightly greater viscosity than guar gum, resulted in a slightly increased mydriatic effect with each drug. Dilute polysorbate 80 in the two mydriatic solutions gave a greater enhancement to the effect of tropicamide than to that of homatropine hydrobromide for the first 30 min, but the enhanced mydriatic effect of homatropine hydrobromide was longer lasting. The increased absorption of the mydriatics obtained by employing both prebuffer and surfactant was greater than when using either alone.

Keyphrases □ Ophthalmic (corneal) absorption of homatropine hydrobromide and tropicamide—effect of prebuffering and/or adding viscolizers and surfactant, rabbits □ Absorption, ophthalmic (corneal)—effect of prebuffering and/or adding viscolizers and surfactant, homatropine hydrobromide and tropicamide, rabbits □ Homatropine hydrobromide corneal absorption—effect of prebuffering and/or adding viscolizers and surfactant, rabbits □ Tropicamide corneal absorption—effect of prebuffering and/or adding viscolizers and surfactant, rabbits

Pretreatment of the eye with a drop of sterile, isotonic, 2.6% sodium borate solution (pH 9.2) was shown to reduce markedly the amount of an alkaloidal drug required to produce a mydriatic or miotic response in the eye (1). This technique did not affect the stability of ophthalmic solutions, since it was the eye and not the solution that was temporarily buffered.

Good *et al.* (2) introduced 12 "biological" buffers which they claimed to be suitable for biological research. In the current study the buffering effect of available biological buffers, cyclohexylaminopropanesulfonic acid[1] and tris(hydroxymethyl)methylaminopropanesulfonic acid[2], was compared with that of 2.6% sodium borate solution whose buffer action was previously known to be effective. Homatropine hydrobromide and tropicamide were the mydriatics employed for the comparisons; rabbits were used as the test subjects because the biological buffers have not been cleared by the Food and Drug Administration for use on humans.

Another objective of this study was to compare the effect of a guar gum viscolizer[3] with the routinely used viscolizer, hydroxypropyl methylcellulose, as an adjuvant for increasing the physiological effect of mydriatic eyedrops in rabbits.

Since it was reported (3–5) that agents which reduce surface tension will generally increase the permeability of certain biological membranes, another objective was to compare the mydriatic effect of homatropine hydrobromide and tropicamide solutions both with and without polysorbate 80.

[1] CAPS, Calbiochem, Los Angeles, CA 90054
[2] TAPS, Calbiochem, Los Angeles, CA 90054
[3] Jaguar J2S-1, Stein, Hall & Co., New York, N.Y.