Kinetics—Rate constants were calculated from spectrophotometric measurements of concentration changes. Standard kinetic procedures were followed. The ionic strength of all reaction mixtures was maintained at 10^{-1} *M* with sodium chloride.

CONCLUSIONS

1. Covalent addition of bisulfite ion occurs to Position C_4 or C_6 of 2-aminopyrimidinium ion derivatives so long as these positions are not blocked by alkyl substituents and the pH of the system is such that the reactants exist to an appreciable extent as the monoanion (HSO_3^-) and the cation (pyrimidinium ion).
2. Alkyl substituents at any position in the pyrimidinium ring reduce the extent and rate of addition.
3. The covalent adducts are zwitterions, between pH 3 and 5, and have very low water solubility.
4. The mechanism of addition appears to involve attack of both the bisulfite ion and sulfite ion on the pyrimidinium ion. The reverse reaction involves both a nonbase-catalyzed and a base-catalyzed decomposition of the neutral adduct.

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Kinetics of the Hydrolysis of Pilocarpine in Aqueous Solution

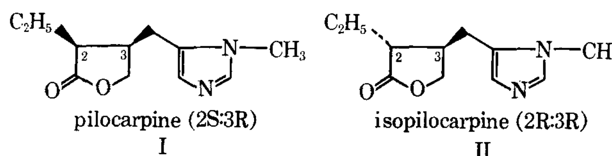
PING-HONG CHUNG, TING-FONG CHIN, and JOHN L. LACH

Abstract ☐ The kinetics of the hydrolysis of pilocarpine in aqueous solution were investigated utilizing pH-stat titrimetry and polarimetry. A cyclic mechanism was proposed for the hydrolysis, which is catalyzed by both hydrogen ion and hydroxide ion. The appropriate rate constants, equilibrium constants, and the energy of activation for the hydroxide-ion catalyzed hydrolysis were calculated. The hydrolysis in high alkaline pH was found to be accompanied by some epimerization. An optimum condition for the preparation of pilocarpine ophthalmic solution was also suggested.

Keyphrases ☐ Pilocarpine in aqueous solution—hydrolysis ☐ Kinetics—pilocarpine hydrolysis ☐ Hydrolysis, pilocarpine—hydrogen-ion catalyzed ☐ TLC—separation ☐ UV spectrophotometry—identity ☐ Titration, pH-stat—pilocarpine degradation determination ☐ Polarimetry—pilocarpine cyclization

Pilocarpine is used topically as a miotic in the treatment of glaucoma. The isomer, isopilocarpine, although qualitatively similar in its pharmacological effects, is almost completely inactive as a miotic (1). However, little clinical data are available concerning isopilocarpine. Pilocarpine solutions ranging from 0.5 to 10% have been used, although there seems to be no advantage in concentrations above 4%. Pilocarpine is less irritating than physostigmine salicylate and can be employed for long periods without producing undesirable side effects (2).

Although pilocarpine was isolated early in 1875, its structure and that of the isomer were elucidated by Jowett (3, 4) in 1900; total synthesis was achieved by several workers in different ways during the 1930's (5–7). In 1966, the absolute configurations of these compounds were established as (2S:3R) (I) and (2R:3R) (II) for pilocarpine and isopilocarpine, respectively (8).



Pilocarpine possesses a γ-lactone with two asymmetric centers. In the dry state and at high temperature, the two isomers, pilocarpine and isopilocarpine, interconvert, isopilocarpine predominating at equilibrium (8). This property has been used for the preparation of isopilocarpine hydrochloride from pilocarpine hydrochloride (9). It has been shown recently that, in the presence of alkali, isomerization may proceed *via* the enol intermediate (10).

In aqueous solution, pilocarpine presents at least two possible pathways of degradation, including hy-

drolysis to pilocarpic acid and epimerization to isopilocarpine. The most important of these is the opening of the lactone ring. As long as the lactone is intact, the alkaloid maintains its stereochemical configuration (11, 12).

Pilocarpine is relatively stable in solutions of acidic pH (12–14). As the pH increases, pilocarpine progressively becomes unstable (12, 13, 15, 16), especially at elevated temperatures. Its stability has also been shown to be affected by certain buffers. Phosphate and carbonate catalyze the degradation of pilocarpine, whereas borate does not (16, 17). Additions of 0.5% of methylcellulose slightly improve the stability of pilocarpine solutions (18). In all cases, the degradation has been accompanied by a marked drop in pH (14, 16–18), phosphate buffers failing to maintain a constant pH (17).

Since stability studies appearing in the literature were of a preliminary nature, providing limited kinetic information, the purpose of this study was to obtain additional data dealing with the rates of hydrolysis and the mechanisms involved.

EXPERIMENTAL

Reagents—The following were used: pilocarpine nitrate USP; hydrochloric acid, reagent grade; sodium hydroxide, reagent grade; potassium chloride, reagent grade; dibasic potassium phosphate trihydrate, reagent grade; monobasic potassium phosphate, reagent grade; citric acid monohydrate USP; silica gel G, TLC grade; Whatman No. 4 chromatography paper (4 cm. × 25 cm.); methanol, reagent grade; chloroform, reagent grade; and *n*-butanol, reagent grade.

Apparatus—The following were used: radiometer pH-stat (a combination of type TTT1c titrator, SBR2c titrigraph, SBU1a syringe burette, and TTA3 titration assembly), with G2222C glass electrode, K4122 calomel electrode, and B101 0.5-ml. syringe; modified pH-stat titration assembly (19), with G202C glass electrode and K401 calomel electrode; Precision Scientific Co. Temp-Trol circulating system; radiometer PHM4c pH meter; Zeiss polarimeter with sodium lamp, 589-mμ interference filter, and 40-cm. tube; and Beckman DK-2 recording spectrophotometer.

Procedure—(*a*) *Preliminary Test*—A 25-ml. portion of an aqueous 2% pilocarpine nitrate solution was placed in each of three 50-ml. volumetric flasks. One of these was brought to strong alkalinity with sodium hydroxide; another was brought to strong acidity with hydrochloric acid. The flasks were then made up to 50 ml. with water and heated in a boiling water bath for 3 hr. After cooling, the solutions were neutralized to approximately the same pH and were chromatographed using TLC [plates: microscope slides coated with a slurry of 30 g. silica gel G in 65 ml. chloroform–methanol (3:1 v/v) and activated at 100° for 1 hr.; solvent system: chloroform–methanol (1:1 v/v); spots located using iodine vapor] and paper chromatography (paper: 4-cm. × 25-cm. Whatman No. 4 filter paper impregnated with 0.2 *M* pH 6.8 phosphate buffer prepared by mixing approximately equal volumes of solutions of 0.2 *M* KH_2PO_4 and 0.2 *M* K_2HPO_4; mobile phase: water-saturated *n*-butanol; spots located using iodine vapor).

(*b*) *Hydroxide-Ion Catalyzed Hydrolysis*—The pH-stat was utilized to follow hydrolytic degradation. Fifty milliliters of water and an appropriate quantity of potassium chloride (for adjusting ionic strength to 0.1) were placed in the reaction vessel, which was then flushed with nitrogen and sealed from contact with the atmosphere in the manner recommended by Chong *et al.* (19). As the temperature reached the desired value, the pH-stat was turned on to adjust the pH to the preset value. A 0.5-ml. aliquot of stock solution (sample) was introduced into the vessel. When the desired pH equilibrium was achieved, the recording pen was returned to zero to start recording the rate of reaction, and the reaction was allowed to proceed either to completion (for pH 11–12) or until sufficient data were obtained (for pH 8–10). The pseudo-first-order rate constants were then obtained either from a Guggenheim plot or the initial rate method.

(*c*) *Hydrogen-Ion Catalyzed Hydrolysis*—Since the hydrogen-ion catalyzed hydrolysis is negligibly small in comparison to the opposing cyclization reaction, comparable information would be obtained more easily and accurately by following the rate of cyclization. A polarimeter was used to follow the rate of cyclization, because there is a large change in optical activity when pilocarpic acid is cyclized to pilocarpine.

RESULTS AND DISCUSSION

In the preliminary study, as would be expected, all of the heat-treated solutions showed no characteristic absorption in the UV region, except that all exhibited a comparable magnitude of end-absorption. However, it should be noted here that pilocarpine does exhibit a maximum at 215 mμ. The thin-layer chromatograms for the heat-treated acidic and neutral (pilocarpine nitrate solution without addition of acid or alkali) solutions showed a single spot, which had the same R_f value as the reference spot, whereas that for the heat-treated alkaline solution exhibited a much smaller R_f value in spite of producing a single spot. This did indicate that pilocarpine is stable in acidic solution but labile in alkaline solution. By repeating the chromatographic separation in a 0.2 *M* pH 6.8 phosphate buffer-treated paper, using water-saturated *n*-butanol as the mobile phase, the alkaline degraded spot and the reference spot showed significantly different and reproducible R_f values, 0.19 and 0.88, respectively. Repeating the test using weaker alkali, the degraded solution displayed two spots with R_f values of 0.19 and 0.88, respectively, indicating only partial degradation as opposed to one spot in strongly alkaline degraded solution. Authentic pilocarpine and isopilocarpine, when chromatographed by the same techniques, showed, however, almost the same R_f value. Therefore, the described chromatographic techniques could not separate the two isomers and epimerization, if present, would not be detected.

From the titration curves, the heat-treated acidic and neutral solutions showed a single pKa value, 6.85, which is similar to that of pilocarpine nitrate; on the other hand, the alkaline degraded solution, when acidified with hydrochloric acid and titrated immediately with alkali, gave two pKa values, 4.05 and 7.50, respectively. However, if the acidified degraded solution was allowed to stand for several hours, the amount of alkali consumed in the titration for the pKa_1 value, 4.05, was decreased, but that for the pKa_2 value, 7.50, was not affected, except that pKa_2 was shifted toward 6.85. This decrease in alkali consumption probably indicates that the free carboxylic acid group in the degraded solution has undergone cyclization.

When freshly prepared pilocarpine base was dissolved in water and immediately titrated with hydrochloric acid, a pKa value of 6.85 for the conjugate acid was produced. However, upon standing, the same solution of pilocarpine base generated an additional inflection in its titration curve, but the equivalent amount of total acid consumed was unchanged, indicating that an acid created from lactone hydrolysis immediately protonated on the original basic group of the molecule to form a zwitterion.

These observed phenomena are consistent with the fact that the lactone ring in the pilocarpine molecule was hydrolyzed in an alkaline condition and subsequently recyclized when acidified. This has also been confirmed by Baeschlin *et al.* (20). Since the hydrolysis of a lactone produces an acid and since reaction at a constant pH is desirable, a pH-stat that not only keeps the pH constant but also records the rate of reaction was used to follow the hydroxide-ion catalyzed hydrolysis of pilocarpine. The theory behind the use of a pH-stat was discussed by Jacobsen *et al.* (21) and will not be discussed here.

For hydroxide-ion catalyzed hydrolysis, a radiometer pH-stat with a closed-type reaction vessel, as suggested by Chong *et al.* (19), and a Precision Scientific Temp-Trol circulating system were used. In the 11–12 pH range, the entire course of the reaction was recorded; pseudo-first-order rate constants were obtained either from Guggenheim plots (22) or by the differential method (22). Both methods were equally satisfactory as applied to the result in this pH range. At pH 8–10, the reaction at each pH level studied was allowed to proceed until sufficient data were obtained. This procedure was used since the recording of the entire course of the reaction would have taken more than 1 day to complete. The initial rate method was used to obtain the pseudo-first-order rate constants. Since all initial rate *versus* initial concentration plots resulted in straight lines at constant pH, the rate law would have the follow-

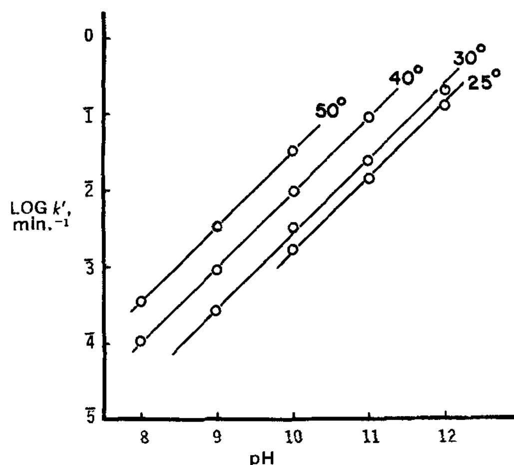


Figure 1—*pH profile of the hydrolysis of pilocarpine in alkaline pH.*

ing form:

$$-\frac{d\,(\text{pilocarpine})}{dt} = k'\,(\text{pilocarpine}) \qquad \text{(Eq. 1)}$$

where k' is the pseudo-first-order rate constant. When log k' was plotted against pH, a straight line was obtained with a slope of 1. Therefore, the rate law for the hydroxide-ion catalyzed hydrolysis of pilocarpine would be:

$$-\frac{d\,(\text{pilocarpine})}{dt} = k_{OH^-}\,(\text{pilocarpine})\,(OH^-) \qquad \text{(Eq. 2)}$$

where k_{OH^-} is the second-order rate constant for the specific hydroxide-ion catalysis.

At pH 7, however, the rate constant was found to be slightly smaller than expected. Intuitively, it could be argued that the *N*-methylimidazole moiety could be acting as a catalyst. Consequently, the possibility of intermolecular catalysis was investigated, using added quantities of *N*-methylimidazole. At pH 10 and up to a concentration of 0.1 *M* of *N*-methylimidazole, no appreciable catalytic effect was observed. (At this pH, *N*-methylimidazole exists entirely in its basic form.)

The effect of ionic strength on alkaline hydrolysis of pilocarpine was also studied at this pH, and no noticeable effect up to the 1.0 *M* potassium chloride level was observed.

This rate constant deviation at pH 7, therefore, probably is due to other facts. For example, the opposing (cyclization) reaction may become significant at this pH, or it may be due to the limitation of pH-stat performance at high buffer capacity and very slow reaction rate. Since recording of sufficient data at this pH would take almost 1 day, the drift in electrode potential could cause a significant error. It should be pointed out that the error recorded by a pH-stat is proportional to the buffer capacity of the system and the magnitude of the potential drift (21).

The temperature effect at different pH's for the hydroxide-ion catalyzed hydrolysis was also studied at 25, 30, 40, and 50°. The pH profiles at these temperatures are shown in Fig. 1 and are consistent with the mentioned rate law. Arrhenius plots for the two pH levels, 9 and 10, are shown in Fig. 2. Both plots show reasonable straight lines of the same slope. The energy of activation was found to be 11.0 kcal./mole after correction for enthalpy of ionization of water. This is in good agreement with the 10.8–12.1 kcal./mole values, as reported by Grace and Symons (23), for the hydroxide-ion catalyzed hydrolysis of substituted and unsubstituted γ-butyrolactones.

In acidic pH, however, pilocarpine is fairly stable. As pointed out by Grace and Symons (23), most γ-lactones are not hydrolyzed appreciably in acid. Therefore, it is difficult to follow the hydrogen-ion catalyzed hydrolysis directly. However, since Long *et al.* (24) reported that the hydrolysis of γ-lactone was catalyzed by hydrogen ion, and since the cleaved acid would recyclize to the lactone in acidic pH, it can be expected that the rate expression would take the form of opposing reactions. Since these reactions would reach equilibrium after a period of time, and since the equilibrium position would lie far toward the lactone in acidic pH, it would be simpler to follow the rate of cyclization than to follow the rate of hydrolysis to obtain this information.

In view of the fact that the opening of the lactone ring in a pilocarpine molecule is accompanied by a large drop in its optical activity (to about 20% of its original value), the rate of cyclization was followed by measuring the optical rotation change with a polarimeter.

To determine the dependence of optical activity on pH, 0.0625 *M* solutions of pilocarpine, which give a reading of about 5° in a 40-cm. polarimeter tube, and similar solutions that had been hydrolyzed completely at pH 12 were adjusted to constant ionic strength (0.2) and to different pH values. Their optical rotations were measured immediately after the adjustment of pH. The optical rotation–pH profiles obtained are shown in Fig. 3. In this figure, it can be clearly seen that the optical rotation–pH profile for the hydrolyzed product, pilocarpic acid, exhibits two inflections which correspond exactly to its two pKa values. This is obvious if the titration curve is superimposed on the optical rotation–pH profile (Fig. 4). In fact, this provides another way for the determination of pKa values of a compound possessing optical activity. This approach is superior to the potentiometric method, since it is not affected by the acidity or alkalinity of the solvent. The presence of optical activity in the completely hydrolyzed product also revealed that the assumption made by Anderson (17), which states that "all products of the degradation other than isopilocarpine have no optical activity," was not valid.

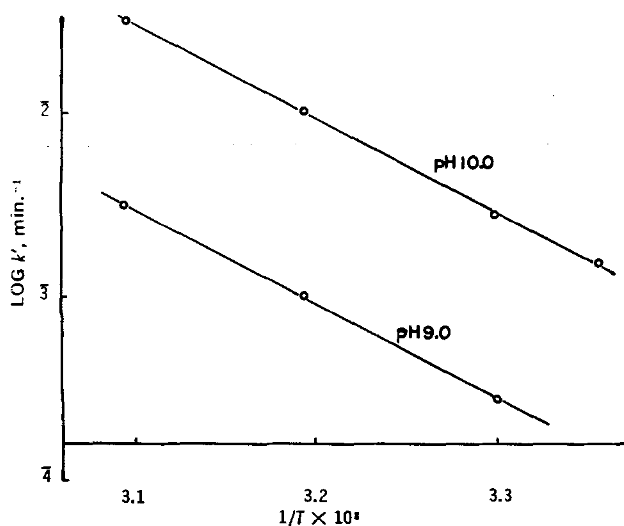


Figure 2—*Arrhenius plot of hydroxide-ion catalyzed hydrolysis of pilocarpine.*

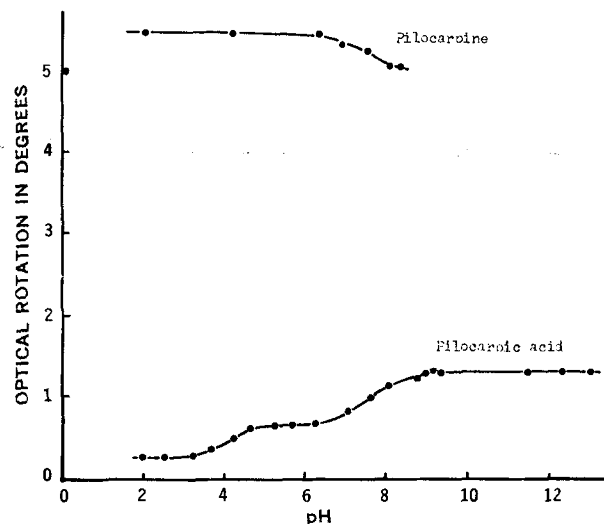
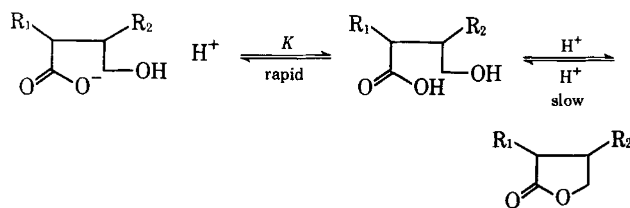


Figure 3—*Optical rotation–pH profile of pilocarpine and pilocarpic acid.*

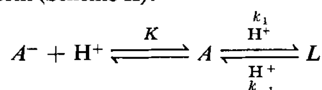
To follow the rate of cyclization, a pilocarpine solution was hydrolyzed at pH 12 for 1 hr. to achieve complete hydrolysis. The hydrolyzed solution was then brought to acidic pH with hydrochloric acid and diluted to a final concentration corresponding to that of a 0.0625 *M* hydrolyzed pilocarpine solution. The pH of the solution was measured, and the cyclization was immediately followed by measuring the optical rotation at different times. These results are shown in Fig. 5. As cyclization proceeds, the optical rotation increases rapidly at first, then gradually slows down, and finally converges to a fixed value which is smaller than the original optical rotation of the corresponding concentration of pilocarpine (Fig. 5). This could indicate that the opposing reactions, namely cyclization and hydrolysis, have attained an equilibrium. Since the rate of the optical rotation change is higher at lower pH, both reactions would be catalyzed by hydrogen ion. If this statement is valid, one should also be able to attain this equilibrium by starting with pilocarpine instead of pilocarpic acid. However, it has been found that the equilibrium optical rotation obtained from pilocarpine is higher than that obtained from pilocarpic acid (Fig. 5). When analyzed for lactone, both equilibrium solutions showed almost equal amounts of lactone remaining, clearly indicating that some epimerization occurred during or after hydrolysis. From the work of Døpke and d'Heureuse (10) and from the fact that the equilibrium optical rotation of the cyclized solution was the same no matter how long the starting compound, pilocarpic acid, remained in an alkaline condition, the former is more likely the case. A study of the extent of epimerization is being conducted in this laboratory and will be reported later.

Assuming that cyclization of pure pilocarpic acid is followed, the simplified mechanism of cyclization may be stated as in Scheme I:



Scheme I

or in symbolic form (Scheme II):



Scheme II

where A^-, A, and L represent pilocarpate, pilocarpic acid, and pilocarpine, respectively; K is the association constant of pilocarpic acid (*i.e.*, $K = 1/K_a$); and k_1 and k_{-1} represent the rate constants of the

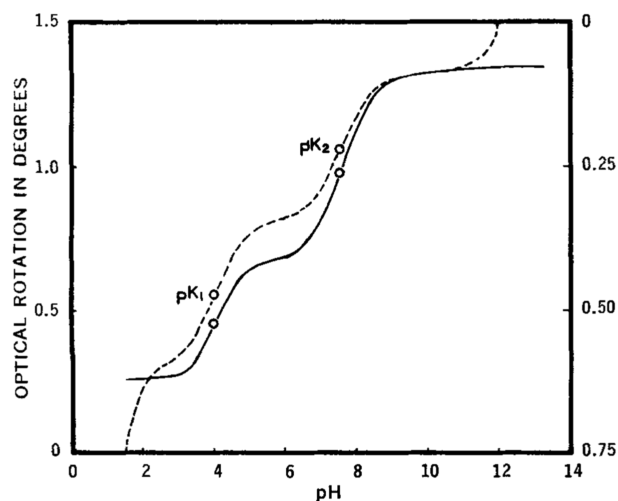


Figure 4—*Optical rotation–pH profile and titration curve of pilocarpic acid. Key: - - - -, titration curve; and —, optical rotation–pH profile.*

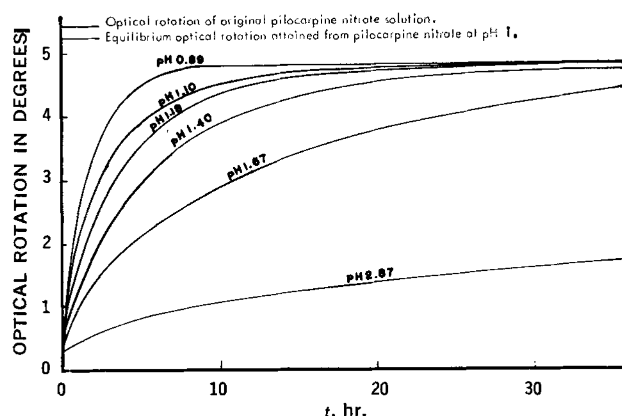


Figure 5—*Optical rotation* versus *time plot for cyclization of pilocarpic acid.*

hydrogen-ion catalyzed cyclization and hydrolysis, respectively (*i.e.*, $k_1 = k_{-H^+}$, and $k_{-1} = k_{H^+}$).

If one starts with an initial concentration, C_o, then the material balance equation is

$$C_o = (A^-) + (A) + (L) = (A_T) + (L) \qquad \text{(Eq. 3)}$$

where $(A_T) = (A^-) + (A)$. (A) can be expressed in terms of C_o and (L):

$$(A) = \frac{K(H^+)}{K(H^+) + 1}[C_o - (L)] = F[C_o - (L)] \qquad \text{(Eq. 4)}$$

where $F = K(H^+)/[K(H^+) + 1]$.

The rate of formation of pilocarpine is

$$\frac{d(L)}{dt} = k_1(H^+)(A) - k_{-1}(H^+)(L) \qquad \text{(Eq. 5)}$$

Combination of Eqs. 4 and 5 gives

$$\frac{d(L)}{dt} = k_1F(H^+)C_o - (k_1F + k_{-1})(H^+)(L) \qquad \text{(Eq. 6)}$$

which can then be integrated if the pH is kept constant. Upon integration of this equation, using the fact that $(L) = 0$ when $t = 0$, at constant hydrogen-ion concentration, gives

$$\ln \frac{k_1FC_o}{k_1FC_o - (k_1F + k_{-1})(L)_t} = (k_1F + k_{-1})(H^+)t \qquad \text{(Eq. 7)}$$

At equilibrium, $d(L)/dt = 0$, and Eq. 6 gives

$$(L)_{eq} = \frac{k_1F}{k_1F + k_{-1}} C_o \qquad \text{(Eq. 8)}$$

Combination of Eqs. 7 and 8 gives rise to

$$\ln \frac{(L)_{eq}}{(L)_{eq} - (L)_t} = (k_1F + k_{-1})(H^+)t \qquad \text{(Eq. 9)}$$

From the titration curve of pilocarpic acid, K was found to be 1.12×10^4 l./mole.

At pH ≤ 2, $F = K(H^+)/[K(H^+) + 1] = 1$, and Eq. 9 can be reduced to

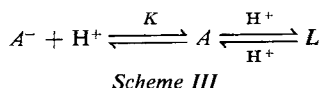
$$\ln \frac{(L)_{eq}}{(L)_{eq} - (L)_t} = (k_1 + k_{-1})(H^+)t \qquad \text{(Eq. 10)}$$

Equation 10 states that a plot of $\log [(L)_{eq}/\{(L)_{eq} - (L)_t\}]$ *versus* t will be a straight line, and the slope is given by

$$\text{slope} = (k_1 + k_{-1})(H^+)/2.303 \qquad \text{(Eq. 11)}$$

It should be noted that this slope is hydrogen-ion concentration dependent.

The quantity "$(L)_{eq}/[(L)_{eq} - (L)_t]$" can be determined from optical rotation measurements, as derived from Scheme III:



Scheme III

From this mechanism, the material balance can be written as:

$$C_o = (A^-) + (A) + (L) = (A_T)_t + (L)_t = (A_T)_{eq} + (L)_{eq} \quad \text{(Eq. 12)}$$

for any time t and at equilibrium. For convenience, the following terms are defined:

$[\alpha]_L$ = optical rotation of pilocarpine with concentration C_o
$[\alpha]_{AT}$ = optical rotation of 100% hydrolyzed pilocarpine solution of the same concentration
R_t = optical rotation of sample solution at time t
R_{eq} = optical rotation of sample solution at equilibrium

Since optical rotation is an additive property of a system, the optical rotation of the sample solution can be considered as having contribution from the optically active species present. Thus,

$$R_t = [\alpha]_{AT} \cdot \frac{(A_T)_t}{C_o} + [\alpha]_L \cdot \frac{(L)_t}{C_o} \quad \text{(Eq. 13)}$$

$$R_{eq} = [\alpha]_{AT} \cdot \frac{(A_T)_{eq}}{C_o} + [\alpha]_L \cdot \frac{(L)_{eq}}{C_o} \quad \text{(Eq. 14)}$$

Subtraction of Eq. 13 from Eq. 14 leads to

$$R_{eq} - R_t = \frac{1}{C_o} [[\alpha]_{AT}\{(A_T)_{eq} - (A_T)_t\} + [\alpha]_L\{(L)_{eq} - (L)_t\}] \quad \text{(Eq. 15)}$$

Rearrangement of Eq. 12 produces

$$(A_T)_t - (A_T)_{eq} = (L)_{eq} - (L)_t \quad \text{(Eq. 16)}$$

Combination of Eqs. 15 and 16 renders

$$(L)_{eq} - (L)_t = \frac{C_o}{[\alpha]_L - [\alpha]_{AT}} \cdot (R_{eq} - R_t) \quad \text{(Eq. 17)}$$

$(L) = 0$ when $5 = 0$, and $R_t = R_o$. As a result, Eq. 17 is reduced to

$$(L)_{eq} = \frac{C_o}{[\alpha]_L - [\alpha]_{AT}} \cdot (R_{eq} - R_o) \quad \text{(Eq. 18)}$$

Dividing Eq. 18 by Eq. 17 gives

$$\frac{(L)_{eq}}{(L)_{eq} - (L)_t} = \frac{R_{eq} - R_o}{R_{eq} - R_t} \quad \text{(Eq. 19)}$$

Equation 19 suggests that the quantity "$(L)_{eq}/[(L)_{eq} - (L)_t]$" can be obtained from measurement of the optical rotation of the sample solution at the beginning, at any time t, and at equilibrium, without knowing the concentration.

In the 100% hydrolyzed solution of pilocarpine, isopilocarpic acid is present in much smaller amounts than pilocarpic acid. Since there is a smaller contribution to optical rotation change when isopilocarpic acid is cyclized (about one-half of the value when pilocarpic acid is cyclized), and since isopilocarpic acid cyclizes at a much faster rate ($t_{1/2}$ = 182 sec. at pH 1) (25), to the first approximation, the derived equations can be used to obtain information about cyclization of pilocarpic acid in the hydrolyzed pilocarpine solution.

Thus, the plots of $\log [(L)_{eq}/\{(L)_{eq} - (L)_t\}]$ *versus* t for different pH values are illustrated in Fig. 6. Reasonable straight lines are indicated.

Taking the logarithm of Eq. 11 yields

$$\log (\text{slope}) = \log [(k_1 + k_{-1})/2.303] - \text{pH} \quad \text{(Eq. 20)}$$

which implies that a plot of log (slope) against pH will have a straight line with a slope equal to -1, with intercept being $\log [(k_1 + k_{-1})/2.303]$. This plot is shown in Fig. 7, and the $(k_1 + k_{-1})$ so obtained is 4.10 l./mole/hr.

At pH greater than 2, Eq. 20 will no longer hold, since $F = K(H^+)/[K(H^+) + 1] \neq 1$, and the slope is now given by

$$\text{slope} = \left[k_1 \frac{K(H^+)}{K(H^+) + 1} + k_{-1}\right] (H^+)/2.303 \quad \text{(Eq. 21)}$$

Upon rearrangement of Eq. 21, Eq. 22 is obtained:

$$\frac{2.303\,(\text{slope})}{(H^+)} = k_1 \frac{K(H^+)}{K(H^+) + 1} + k_{-1} \quad \text{(Eq. 22)}$$

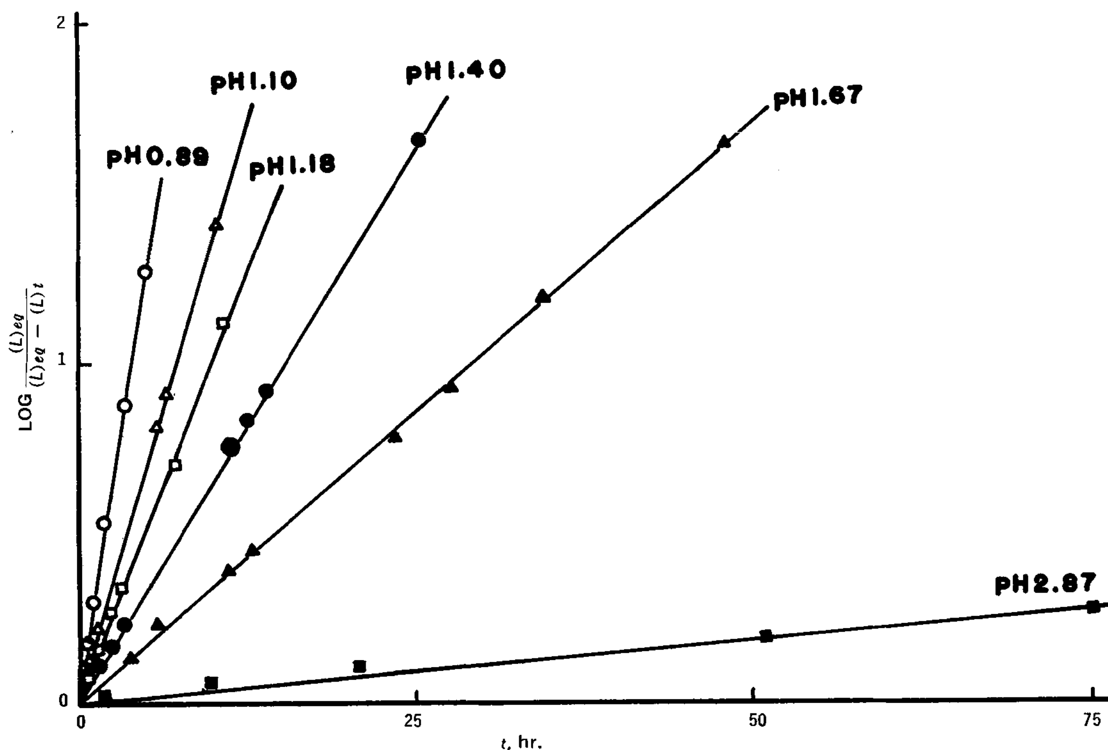


Figure 6—*Cyclization of pilocarpic acid at different pH's.*

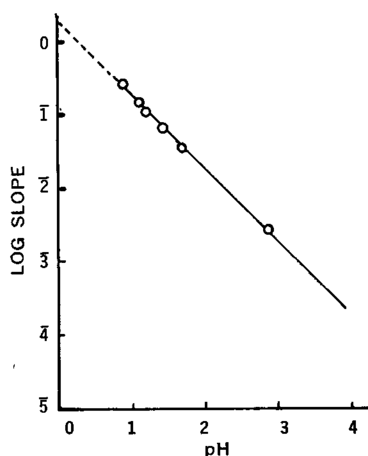


Figure 7—*pH profile of the hydrolysis of pilocarpine in acid pH (log slope versus pH plot).*

which denotes that a plot of 2.303 (slope)/(H^+) against $k(H^+)/[K(H^+) + 1]$ will be a straight line with slope equal to k_1 and intercept equal to k_{-1}. However, at a pH higher than 3, the change in pH value becomes significant as the cyclization proceeds. It is, therefore, necessary to use a pH-stat or buffer to maintain the pH constant. The rate of cyclization at a pH higher than 3 was found to be so slow that utilization of a pH-state was impractical. The use of a buffer such as citrate not only failed to maintain the pH constant but also catalyzed the reactions. For this reason, the application of Eq. 22 was unsuccessful.

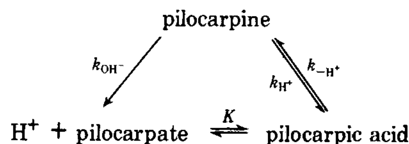
Nevertheless, k_1 and k_{-1} can be calculated from the results obtained in Fig. 7 and the data obtained from the analysis of the cyclized solution for pilocarpine. This is because at low pH (for example, pH = 1), the hydrolyzed product would exist essentially in its acidic form and the ratio k_1/k_{-1} would be equal to $(\text{pilocarpine})_{eq}/[C_o - (\text{pilocarpine})_{eq}]$. Thus, the results from Fig. 7 and from the analysis of cyclized solution give

$$k_1 + k_{-1} = 4.10 \text{ l./mole/hr.} \quad \text{(Eq. 23)}$$

$$k_1/k_{-1} = 29.3 \quad \text{(Eq. 24)}$$

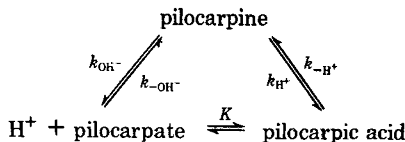
Solution of the simultaneous Eqs. 23 and 24 results in $k_1 = 3.97$ l./mole/hr. and $k_{-1} = 1.35 \times 10^{-1}$ l./mole/hr.

Up to this point, the mechanism for the hydrolysis of pilocarpine can be written as the cyclic equilibrium process shown in Scheme IV:



Scheme IV

where k_{H^+} is equal to k_{-1}, and k_{-H^+} is equal to k_1. By considering "the principle of microscopic reversibility," a reversible reaction for the hydroxide-ion catalyzed pathway can be inferred. Consequently, the complete cyclic mechanism may be stated as in Scheme V:



Scheme V

Here, k_{-OH^-} is defined as the first-order rate constant for the cyclization of pilocarpate to pilocarpine and may be calculated from k_{OH^-}, k_{H^+}, k_{-H^+}, and K. The k_{-OH^-} so calculated is 2.49×10^{-6}/hr.

The hydrolytic information was obtained by following the rate of cyclization to approach equilibrium. (The hydrolysis of isopilocarpine would take the same mechanism but with different values of rate constants.) Since there wasn't noticeable epimerization indicated in acidic pH (14), the hydrolytic information obtained in this study and the postulated degradative pathway do not take into account the epimerization aspect.

The information obtained in this study is different from the recent publications by Baeschlin and Etter (26, 27), in which certain kinetic parameters were not fully considered.

CONCLUSION

1. Hydrolysis of pilocarpine in aqueous solution is a cyclic equilibrium process which is catalyzed by hydrogen ion and hydroxide ion.
2. The equilibrium position depends on pH; it shifts to pilocarpate at high pH and shifts to pilocarpine at low pH. The equilibrium constant and the rate constants at 25°, as well as the energy of activation for the hydroxide-ion catalyzed hydrolysis, were determined and are as follows:

$$K = 1.12 \times 10^4 \text{ l./mole}$$
$$k_{H^+} = 1.35 \times 10^{-1} \text{ l./mole/hr.}$$
$$k_{-H^+} = 3.97 \text{ l./mole/hr.}$$
$$k_{OH^-} = 7.56 \times 10^2 \text{ l./mole/hr.}$$
$$k_{-OH^-} = 2.49 \times 10^{-6}\text{/hr.}$$

$$Ea_{\text{(for OH}^- \text{ catal. hydrolysis)}} = 11.0 \text{ kcal./mole}$$

3. It is suggested that pilocarpine solutions be prepared at pH 4–5 for acceptable stability and physiological availability.
4. In alkaline pH, pilocarpine also undergoes some epimerization but at a rate much slower than hydroxide-ion catalyzed hydrolysis.

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Solubility Profiles for Several Barbiturates in Hydroalcoholic Mixtures

T. L. BREON and A. N. PARUTA

Abstract ☐ The solubilities of eight physiologically active barbiturates were determined in binary mixtures of alcohol and water. The solubility curves for these substances varied, showing either solubility maxima or asymptotic solubility isotherms. The dielectric requirement of the barbiturates investigated illustrated an approximate inverse relationship with the number of carbon atoms in the molecule. A similar correlation was found with the solubilities in pure water, with the ratios of the solubilities in ethanol, and at the dielectric requirement to the solubility in water. The therapeutic indexes of duration of action and the period of time involved between administration of the drug and the time when the activity is first manifested increased as the relative polarity of these barbiturates declined. An approximate correlation between activity and solubility ratios is considered.

Keyphrases ☐ Barbiturates, solubility—ethanol–water systems ☐ Dielectric requirement—barbiturates ☐ Polarity, barbiturates—activity correlation ☐ Solubility ratios—barbiturates, ethanol–water systems

The pharmacological action exerted by a drug molecule in contact with a biological system is the net result of the interactions and extent of interactions with the complex biological environment. The degree as well as the rate of interaction is governed by many parameters, many of which depend on the physical and chemical properties associated with the drug molecule.

To be physiologically active, a drug must be absorbed and distributed throughout the biological fluids. More specifically, it is noted that these actions occur on a molecular level; under these conditions, it would be assumed that solution properties and characteristics are operative. Many biologically active substances are weak electrolytes, and properties such as the pH of the medium, pKa of the drug, concentration gradients, surface tension, and the aqueous and lipid solubilities of the various species contribute to the overall extent of activity.

The biologically active species, to initiate a response, would be presumed to have interacted with cellular constituents; this process is involved with diffusion and permeability as well as those factors previously discussed. Thus, this study is an initial investigation into the possible approximate correlation between solubility characteristics of several barbiturates and therapeutic activity.

The wide variety of available barbiturates certainly attests to the importance of these materials, with a wide spectrum of uses such as sedatives and anticonvulsants. They are derivatives of barbituric acid with a variety of substitutions in the 5-position, and about 20 of these are presently available as therapeutic agents.

Although the general sedative action of all these barbiturates is about the same, they do vary in the duration of depressant action. Since these barbiturates are chemically different, it would be judicious to study them in attempting to relate known duration of action and chemical structure.

Thus, if in a series of barbiturates, a property such as solubility was determined as a function of polarity, there may be an indication of the relative lipoidal nature of these materials. It would be well to consider the solubility of these types of materials in a manner previously described (1). The cosolvent action on these barbiturates by mixtures of decreasing polarity should be instructive.

It might be expected that the position of the dielectric requirement (DR) and the magnitude of solubility at that point would be indicative of the effect of substituents and the relative polarity of the drug molecule. In view of this possibility, some eight barbiturates with a spectrum of values for the onset and duration of action were studied relative to their solubility behavior in hydroalcoholic solutions.

Several long-, intermediate-, and short-acting barbiturates (2) were chosen to test this hypothesis, including barbital as the comparing standard.

The very important work of Hansch and Anderson (3) should be mentioned here since they showed a definitive correlation of the activity of barbiturates with the log of the partition coefficients for various derivatives. This would suggest the importance of the hydrophobic character of substituted barbiturates in a wide variety of biochemical systems.

The model used by Hansch and Anderson (3) is a partitioning between phases to calculate the coefficients or a measure of lipophilicity of these drugs, *i.e.*, the