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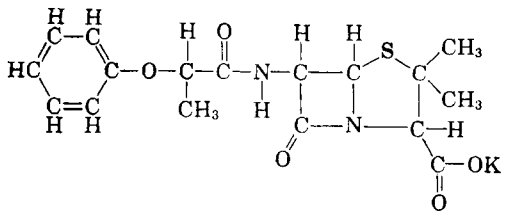
Stability of Potassium Phenethicillin I

Kinetics of Degradation in Aqueous Solution

By M. A. SCHWARTZ, A. P. GRANATEK, and F. H. BUCKWALTER

Kinetic studies of the degradation of potassium phenethicillin in aqueous solution have been carried out and presented with a view toward stability prediction. Phenethicillin, an acid with pKa 2.9, is hydrolyzed by both acid and base with minimum rate of degradation at about pH 6.5 at 35°. In acid solution the ionic form of phenethicillin is hydrolyzed about 13 times as fast as the free acid. The $HPO_4^=$ ion was found to be a general base catalyst. The effect of temperature in the neutral pH region has been determined, and from this data the aqueous solution stability of potassium phenethicillin at 25 and 4° has been predicted.

Potassium phenethicillin[1] is the first commercially produced semisynthetic penicillin.



Its chemical, microbiological and pharmacological properties have been described (1–3). Clinical studies have also been reported (4, 5). The present work was undertaken to enable the prediction of the optimum stability formulating conditions for potassium phenethicillin in aqueous solution.

EXPERIMENTAL

One lot of commercially produced potassium phenethicillin containing approximately 60% of the L-form was used throughout this study. All buffer materials were reagent grade.

The kinetic studies were carried out as follows: 90 ml. of buffer (pH range given in Table I) in a 100-ml. volumetric flask was brought to bath temperature and then 1.0 ml. of a freshly prepared 0.1 *M* aqueous solution of potassium phenethicillin was added with mixing. At appropriate intervals, 2.0-ml. samples of the reaction mixture were taken and added to 8.0 ml. of cold 0.5 *M* pH 6 citrate-phosphate buffer to quench the reaction. These solutions were immediately frozen and kept in this state until just before assay. The phenethicillin potency of these samples was determined by the microbiological turbidimetric assay method with *Staph. aureus* strain FDA209-P as the inoculum.

In order to determine the effect of freezing the quenched samples until assay, a solution of 80 mcg./ml. of potassium phenethicillin in the strong pH 6 buffer was divided into 10-ml. samples and frozen. One sample was assayed each day for 13 days. No significant loss of potency was observed despite a day-to-day variability of 14.5%. To avoid this, all samples from a single kinetic run were assayed on the same day. The maximum time these were kept frozen was 6 days.

The ionic strength of all the buffers was adjusted to 0.5 with potassium chloride. The pH was determined with a Beckman Zeromatic pH meter using glass and calomel electrodes.

The pKa of phenethicillin was determined in 0.5 *M* potassium chloride by taking the pH of a half-neutralized 0.01 *M* solution.

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[1] The trade name of Bristol Laboratories, a division of Bristol-Myers Co., for α-phenoxyethyl penicillin is Syncillin. The generic name is phenethicillin or penicillin 152.

TABLE I.—KINETIC STUDIES OF THE DEGRADATION OF POTASSIUM PHENETHICILLIN

Run No.	Temp., °C.	pH	Buffer Ingredients[a] HCl	H_3PO_4	$H_2PO_4^-$	$HPO_4^=$	$H_3Cit.$	$H_2Cit.^-$	$HCit.^=$	$Cit.^{\equiv}$	NaOH	k' ($hr.^{-1}$)[b]	$\sigma k'$
1	35	1.0	0.1	...	...	...	...	...	...	...	...	1.55	0.07
2	35	1.5	0.032	...	...	...	...	...	...	...	...	0.602	0.06
3	35	2.0	...	0.048	0.052	...	...	...	...	...	...	0.256	0.01
4	35	2.3	...	0.007	0.001	...	0.082	0.014	0.0004	...	...	0.219	0.001
5	35	2.6	...	0.004	0.018	...	0.067	0.022	0.0002	...	...	0.158	0.011
6	35	2.9	...	0.0033	0.038	...	0.043	0.036	0.001	...	...	0.109	0.004
7	35	3.1	...	0.0031	0.042	...	0.037	0.039	0.0009	...	...	0.0798	0.0006
8	35	3.3	...	0.002	0.055	...	0.022	0.047	0.002	...	...	0.0644	0.0003
9	35	3.9	...	0.0007	0.076	...	0.006	0.047	0.008	...	...	0.0179	0.0002
10	35	5.0	...	...	0.101	0.002	...	0.014	0.025	0.010	...	0.00480[c]	0.001
11	35	5.6	...	...	0.107	0.0086	...	0.002	0.015	0.024	...	0.00485	0.0005
12	35	6.0	...	...	0.105	0.021	...	...	0.007	0.029	...	0.00528	0.0005
13	35	6.7	...	...	0.045	0.055	...	...	...	...	...	0.00857	0.0002
14	35	6.7	...	...	0.0225	0.0275	...	...	...	...	...	0.00635	0.0002
15	35	7.4	...	...	0.0142	0.0858	...	...	...	...	...	0.0118	0.0001
16	35	7.4	...	...	0.0071	0.0429	...	...	...	...	...	0.00668	0.0002
17	35	8.0	...	...	0.0048	0.0952	...	...	...	...	...	0.0141	0.0002
18	35	11.15[d]	...	...	...	...	...	...	...	...	0.002[e]	787[f]	...
19	35	10.85[d]	...	...	...	...	...	...	...	...	0.001[e]	758[f]	...
20	35	11.40[d]	...	...	...	...	...	...	...	...	0.003[e]	731[f]	...
21	55	6.7	...	...	0.045	0.055	...	...	...	...	...	0.0452	0.001
22	65	6.7	...	...	0.045	0.055	...	...	...	...	...	0.113	0.005

[a] Approximate molar concentrations, calculated from the following pKa: H_3PO_4 = 1.96, $H_2PO_4^-$ = 6.70, $H_3Cit.$ = 3.08, $H_2Cit.^-$ = 4.75, $HCit.^=$ = 5.40. [b] Observed first-order constant. [c] Extrapolated value. [d] Initial pH. [e] Initial Concentration. [f] Second-order rate constant (L. $mole^{-1}$ $hr.^{-1}$).

RESULTS AND DISCUSSION

Order of Reaction.—At constant temperature and pH the hydrolysis of phenethicillin is an overall first-order reaction as shown by the linear log concentration-time plots of Fig. 1. These are typical of the results obtained throughout this study.

Runs 18 through 20 in Table I were made using 0.001 *M* potassium phenethicillin with 0.001, 0.002, and 0.003 *M* hydroxyl ion. The data are plotted as second-order reactions as in Fig. 2. The rate constants were determined from the slopes by the method of least squares.

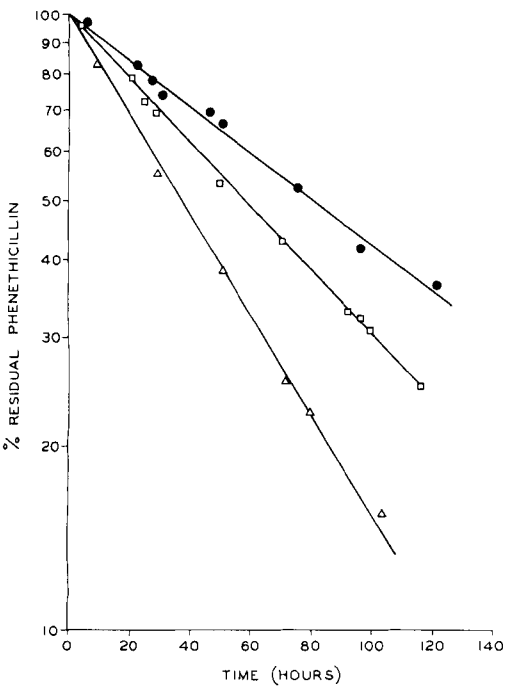

Fig. 1.—First-order plots of degradation of phenethicillin at 35° at several pH's. Δ, pH 3.9; ● pH, 6.7; □, pH 7.4.

Table I lists the buffers used and the observed results. The k' values were all calculated by the method of least squares. The standard deviations, $\sigma k'$, were obtained essentially by the method described by McLeod, *et al.* (6).

pH Dependence.—Figure 3 shows the dependence of the rate of hydrolysis of phenethicillin on pH. The dashed curve shows the actual values found while the solid curve is corrected for buffer effects. The effect of buffers is discussed in a subsequent section.

The curvature on the acid side indicates that more than one species is involved in the reaction. Since phenethicillin is an acid with pKa 2.9, one may calculate the proportions of unionized and ionized material as a function of pH from the following relationships

$$f_{HP} = \frac{[H^+]}{[H^+] + Ka} \text{ and } f_{P^-} = \frac{Ka}{[H^+] + Ka} \quad \text{(Eq. 1)}$$

where f_{HP} = fraction of phenethicillin as the free acid [HP] and f_{P^-} = fraction of phenethicillin ionized [P^-].

The overall hydrogen ion catalyzed reaction may be written in the form

$$-\frac{dP_T}{dt} = k_1 [H^+] [HP] + k_2 [H^+] [P^-] = k' P_T \quad \text{(Eq. 2)}$$

where k_1 and k_2 are the rate constants for the hydrolysis of acid and ion, respectively, P_T is the total drug concentration, and k' is the observed pseudo first-order rate constant. Dividing through by $P_T[H^+]$

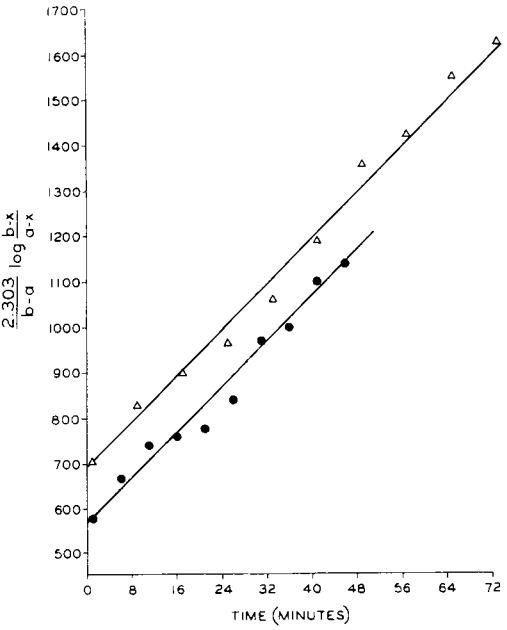

Fig. 2.—Second-order plots of reaction of phenethicillin with hydroxyl ion at 35°. a = Initial molar concentration of phenethicillin, b = initial molar concentration of $[OH^-]$. ●, b = 0.003, a = 0.001; Δ, b = 0.002, a = 0.001.

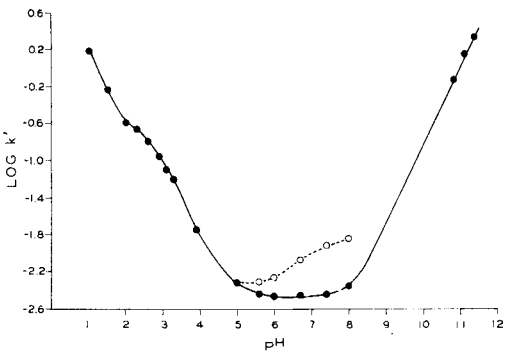

Fig. 3.—pH dependence of rate of hydrolysis of phenethicillin at 35°.

$$\frac{k'}{[H+]} = k_1 f_{HP} + k_2 f_{P^-} \qquad \text{(Eq. 3)}$$

Since $f_{HP} + f_{P^-} = 1$, we may rearrange Eq. (3) to

$$\frac{k'}{[H^+]} = (k_1 - k_2) f_{HP} + k_2 \qquad \text{(Eq. 4)}$$

A plot of $k'/[H^+]$ as a function of f_{HP} should give a straight line with slope $k_1 - k_2$ and intercept k_2. Such a plot is shown in Fig. 4. The constants derived from the best fit line as determined by the method of least squares are[2]: k_1 = 12.5 ± 5.7 L. mole^{-1} hr.$^{-1}$ and k_2 = 162 ± 6.5 L. mole^{-1} hr.$^{-1}$ at 35°. The greater affinity of the negatively charged ion for proton probably accounts for its faster reaction rate.

[2] The errors noted here and subsequently in this paper represent the standard deviation.

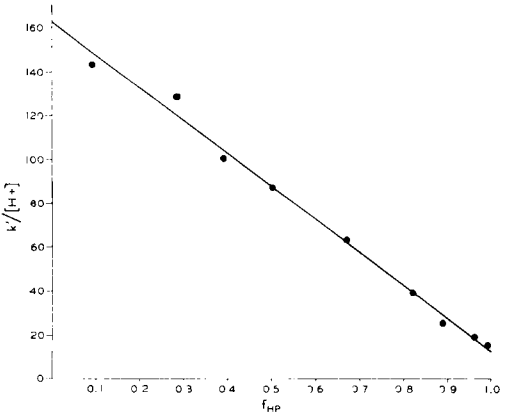

Fig. 4.—Plot of Eq. 4 from which was determined k_1 and k_2 at 35°.

The second-order constant for alkaline hydrolysis was determined directly to be 758 ± 28 L. mole^{-1} hr.$^{-1}$.

From these values the pH of minimum degradation rate may be determined as follows

$$k' = k_2 [H^+] + k_{OH^-} [OH^-] = k_2 [H^+] + \frac{K_w k_{OH^-}}{[H^+]} \qquad \text{(Eq. 5)}$$

since all the phenethicillin is in the ionic form in this pH region. Differentiating k' with respect to $[H^+]$, we obtain

$$\frac{dk'}{d[H^+]} = k_2 - \frac{K_w k_{OH^-}}{[H^+]^2} = 0 \qquad \text{(Eq. 6a)}$$

$$[H^+]_{min.} = \sqrt{\frac{k_{OH^-} K_w}{k_2}} \qquad \text{(Eq. 6b)}$$

At 35° this results in $pH_{min.}$ = 6.5. This, incidentally, is the same value observed in the case of benzyl penicillin (7).

Effect of Buffers.—In the pH range 5.5 to 7.0 where specific acid and base effects were calculated to be negligible, appreciable rates of degradation were observed. This led to the conclusion that water and/or one or more of the buffer ingredients were catalytic species. Examination of the results indicated that the $HPO_4^=$ ion was a general base catalyst. This effect may be formulated as follows

$$k' = k_2[H^+] + k_{OH^-}[OH^-] + k_0 + k_{HPO_4^=} [HPO_4^=] \qquad \text{(Eq. 7a)}$$

which when transposed becomes

$$k' - k_2[H^+] - k_{OH^-}[OH^-] = k'' = k_0 + k_{HPO_4^=} [HPO_4^=] \qquad \text{(Eq. 7b)}$$

A plot of k'' against $HPO_4^=$ should therefore be linear with slope $k_{HPO_4^=}$ and intercept k_0, where k_0 represents the rate of reaction with H_2O. Figure 5 is such a plot, and by the method of least squares the 35° values of k_0 = 0.00325 ± 0.0001 and $k_{HPO_4^=}$ = 0.094 ± 0.003 were obtained. Unprotonated citrate ion appears to have no catalytic effect on the reaction rate.

Effect of Temperature.—For purposes of stability prediction the rate of reaction was run at pH 6.7 as a function of temperature. The Arrhenius plot

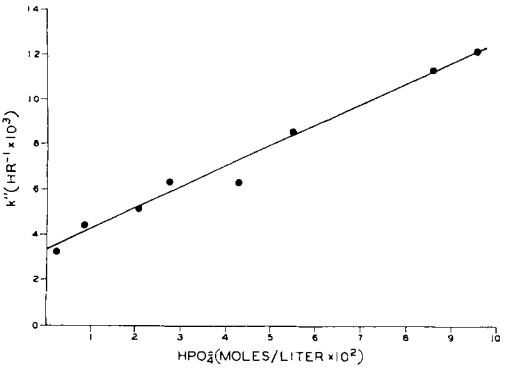

Fig. 5.—Plot showing catalytic effect of $HPO_4^=$ ion on rate of hydrolysis of phenethicillin at 35°

from these data is shown in Figure 6. The energy of activation was calculated to be 17.6 ± 0.3 Kcal. per mole.

Prediction of Stability.—For a solution of phenethicillin not containing phosphate the rate of degradation at room and refrigerator temperatures may be calculated from k_0 determined at 35° and the heat of activation at pH 6.7. This assumes that the latter is the same as the heat of activation for the reaction with water.[3] In this way the following values were calculated k_0 = 0.00134 hr.$^{-1}$ at 25° (room temperature) and k_0 = 0.000129 hr.$^{-1}$ at 4° (refrigerator temperature).

Further application of this data to product formulation will be considered in a subsequent paper.

SUMMARY AND CONCLUSIONS

Degradation rates for potassium phenethicillin have been determined over the pH range 1–11 and as a function of temperature in the neutral region. On the acid side, both the free acid and ionized species undergo degradation at different rates with the constant for the ion being about 13 times that of the acid. The half-life of potassium phenethicillin at 35° and pH 1.5 is about 1 hour. A comparable value for penicillin G is about 4 minutes (7). This fifteenfold increase in potassium phenethicillin stability over penicillin G at pH 1.5 and 35° may account for the greater serum levels achieved with oral administration of potassium phenethicillin.

The pH of the minimum rate of degradation of potassium phenethicillin is 6.5, although the rate of degradation does not increase appreciably until the pH is brought below 5.5 or above 7. Secondary phosphate ion catalyzes the hydrolysis while unprotonated citrate ion does not have a catalytic effect. Optimum aqueous solution stability conditions for potassium phenethicillin would therefore be provided by maintaining the pH of the preparation between 6 and 6.5 using a buffer such as citrate which has no effect on its hydrolysis in this pH range. Applications of these data to product formulation are considered in a subsequent paper (8).

[3] This was found to be true as will be shown in a subsequent paper.

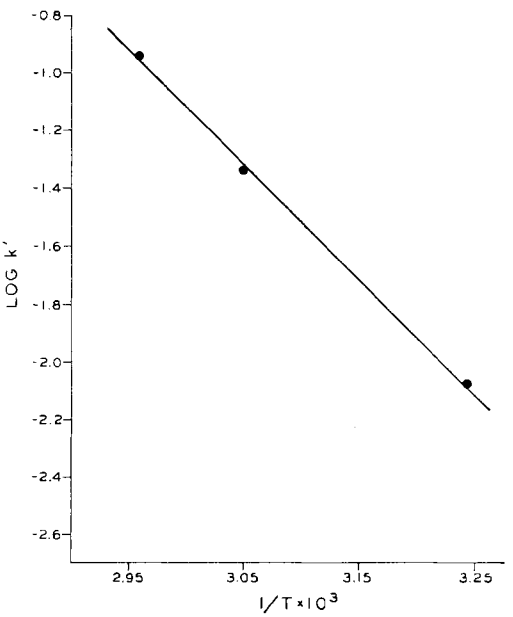

Fig. 6.—Arrhenius plot for temperature dependence of rate of hydrolysis of phenethicillin at pH 6.7.

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