Stability of Methicillin

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This paper presents the results of investigations on some of the factors influencing the stability of methicillin in solution. Rates of decomposition of methicillin in aqueous solution were determined which can be useful in a qualitative prediction of incompatibility with special reference to the effect of pH.

METHICILLIN (I), 6-(2,6-dimethoxybenzamido) penicillanic acid, was the first synthetic penicillin used in medicine that was highly active against staphylococci resistant to other penicillins. Preliminary investigation showed that this compound was unstable in acid solution and was absorbed only to a small extent after oral administration. Therefore, it is administered by the parenteral route, usually intramuscularly or intravenously. As such, it is used in aqueous solution and often mixed with other injectables, especially for intravenous administration.

This investigation was conducted to realize the maximum useful life of products in which methicillin is incorporated. This report deals with the results of investigations on some of the factors influencing stability of methicillin in solution.

EXPERIMENTAL

The kinetic studies were carried out in the same way as similar studies on phenethicillin (1), except that (*a*) the buffers used to quench the reactions were 0.2 *M* ethylenediaminetetraacetate, pH 8.0 (for experiments in acid solution) and pH 6.7 (for all other experiments), and (*b*) a microbiological cup plate diffusion assay was employed with *Sarcina lutea*, FDA No. 1001, as the test organism.

A single lot of production grade sodium methicillin was used throughout the study, and the ionic strength of all solutions was adjusted to 0.5 with potassium chloride. The initial concentration of methicillin was 10^{-3} *M* in all the experiments.

The buffers used (1) were HCl, pH 1–1.6; phosphate, pH 2–3; citrate-phosphate or acetate, pH 3–5.5; phosphate, pH 5.9–8.1; and carbonate, pH 9–10.

The ionization constant, pK′, for methicillin was determined at ionic strength 0.5 by measuring the pH of a series of solutions of sodium salt which had been partially neutralized with a standard HCl solution. The following equation used for the calculation includes a correction for the dissociation of acid which is important below pH 4.

$$pK' = pH - \log\frac{[A^-]_T - [HCl] + [H^+]}{[HCl] - [H^+]}$$

The result at 25° was pKa = 2.74 ± 0.03.

The pH of the buffers was measured at 25° since the ionization constants of acidic buffers generally do not vary greatly with temperature. In the calculation of hydroxyl ion concentrations, pKa at higher temperatures was used.

RESULTS AND DISCUSSION

All rates were pseudo first order with respect to methicillin concentration, and the rate constants ($k_{obs.}$) were determined from the slopes of the log concentration time plots. Figure 1 shows the pH dependence of the degradation rates of methicillin at 75°. The shape of this curve is typical of that of penicillins in general (1, 2) and shows specific catalysis of degradation by $[H^+]$ and $[OH^-]$ ions. The curvature on the acid side is due to the ionization of the penicillin (1). The entire curve of Fig. 1 is described by

$$k_{obs.} = k_1[H^+]f_{HP} + k_2[H^+]f_p^- + k_{OH^-}[OH^-] + k_o$$

where k_o is the rate constant for reaction with k_{OH^-}, the rate constant for alkaline hydrolysis, f_{HP}, the fraction of the methicillin existing in the nonionized form, and f_p^-, the fraction ionized. The latter two terms were calculated from the pH of the solution and the ionization constant of methicillin by

$$f_{HP} = \frac{[H^+]}{[H^+] + K_a}$$

and

$$f_p^- = \frac{K_a}{[H^+] + K_a}$$

The rate constants in acid solution, k_1 and k_2, were determined at 25, 30, and 35° by the same method used with phenethicillin (1). The values at 75° were determined by extrapolation of the usual Arrhenius type plots. The rate constants are shown in Table I.

Phosphate buffers were used between pH 5.9 and 8.1. The effect of phosphate on degradation rate is

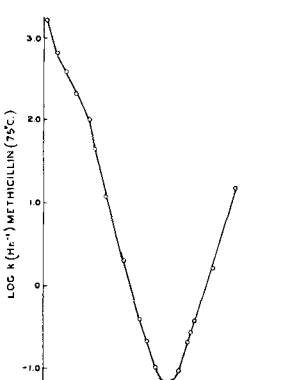

Fig. 1.—pH dependence of degradation rate of methicillin at 75°.

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TABLE I.—RATE CONSTANTS (HR.$^{-1}$)

	35°	75°
k_1	600	...
k_2	3200	...
k_o	...	0.032
k_{OH^-}	...	8000
k_4	...	2.0

shown in Fig. 2. At each pH the slope of the line represents the rate constant for catalysis by phosphate, and the intercept represents the degradation rate in the absence of phosphate. The latter values were used in the pH rate profile of Fig. 1.

If k_p is designated the rate constant for phosphate catalysis and k' the rate constant for all other factors, then between pH 5.9 and 8.1

$$k_{obs.} = k' + k_p[P_T]$$

where $[P_T]$ represents total phosphate concentration. The slope of each of the lines in Fig. 2 is then k_p at that particular pH. Since the species present in phosphate buffers in this pH range are $H_2PO_4^-$ and HPO_4^{-2}, we may write

$$k_p[P_T] = k_3[H_2PO_4^-] + k_4[HPO_4^{-2}]$$

or dividing through by $[P_T]$:

$$k_p = k_3 f_{H_2PO_4^-} + k_4 f_{HPO_4^{-2}}$$

where the f's refer to the fraction of the total phosphate as that species. Since $f_{H_2PO_4^-} + f_{HPO_4^{-2}} = 1$, a plot of k_p against $f_{HPO_4^{-2}}$ should be linear with intercept k_3 when $f_{HPO_4^{-2}}$ equals zero, and k_4 at $f_{HPO_4^{-2}}$ equals unity. This plot in Fig. 3 shows that k_3 is essentially zero and $k_4 = 2.0$ at 75° within experimental error. The result is similar to that with phenethicillin (1), where HPO_4^{-2} catalyzed the reaction and $H_2PO_4^-$ did not. Similar treatment showed that carbonate, citrate, and acetate buffers had no effect on the degradation rates of methicillin.

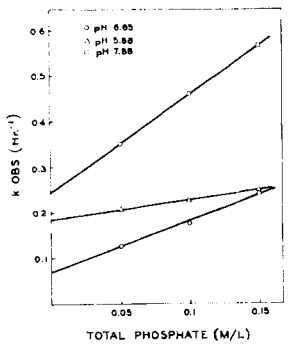

Fig. 2.—Dependence of rate of degradation of methicillin at 75° on phosphate concentration at various pH values.

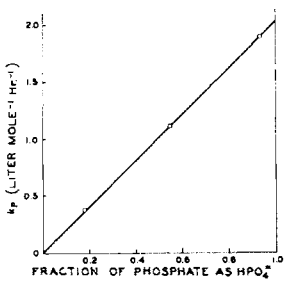

Fig. 3.—Plot of rate constant for phosphate catalysis against HPO_4^{-2} which determined k_3 and k_4.

While the degradation of methicillin as a function of pH follows generally the same pattern as that of phenethicillin (1), the great acid instability of the former causes two differences which merit consideration. First, the pH of maximum stability of methicillin is higher than that of phenethicillin. The following equation gives the hydrogen ion concentration at which the rate will be a minimum (1)

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

This depends then on the ratio k_{OH^-}/k_2. The smaller this ratio is, the higher will be the pH where degradation is minimal. Therefore, the great acid instability of methicillin accounts for the higher pH of maximum stability.[1]

Second, and of greater importance in formulation, the pH range of maximum stability of methicillin is relatively narrow compared to that of phenethicillin (1). With the latter, the rate of reaction with water is greater than the sum of the acid and alkaline degradation rates between pH 5.5 and 7.3. Thus, in this region the over-all reaction is independent of pH almost completely and is due to reaction with water almost entirely. On the other hand, the opposite is true with methicillin. In the pH region around the minimum in the pH rate profile, the over-all reaction rate is due almost entirely to the sum of the rates of acid and alkaline hydrolysis; reaction with water plays only a minor role. The reaction rate is highly dependent upon pH, except close to the minimum. Thus, more care is required with the manufacture and formulation of products of methicillin than with the more acid-stable penicillins.

A previous paper from these laboratories (4) discussed the stability of sodium methicillin as dry powder in vials, as solution in vials, and mixed with a number of common intravenous solutions. Many of the latter mixtures had pH values in the range 4–5. These had a utility time[2] shorter than the normal solution of sodium methicillin (pH about 6.5) or mixtures with other intravenous solutions where the resulting pH was 5–7. This behavior is directly in accord with expectation after considering Fig. 1.

SUMMARY

The rates of decomposition of methicillin in aqueous solution have been determined over the pH range 1–11. The behavior of this drug is similar to that of other penicillins, except that its high acid instability narrows the range of pH of maximum stability relative to that observed with more acid-stable penicillins. At least qualitative prediction of incompatibility may be made with knowledge of the factors influencing degradation, especially the effect of pH.

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[1] It might be argued here that a decrease in k_{OH^-} would cause a similar shift in pH of maximum stability. However, k_{OH} for all the penicillins studied to date are all the same order of magnitude (3).

[2] Utility time may be defined as time for 20% loss in potency to occur.