# Kinetics and Mechanism of Degradation of Ampicillin in Solution
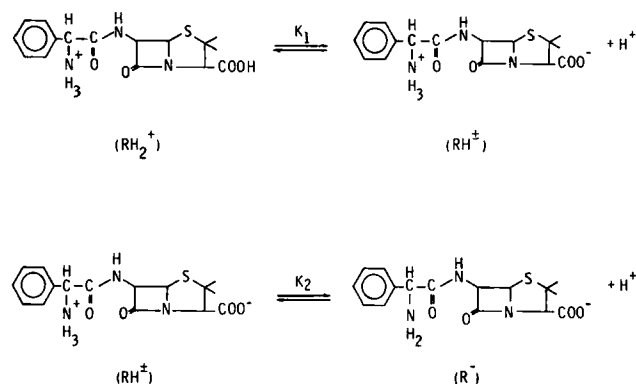
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**Abstract** ☐ The kinetics of degradation of ampicillin ($\alpha$-aminobenzyl penicillin) in solution was investigated at 35° and constant ionic strength of 0.5 over a pH range of 0.8 to 10. The observed rates, obtained by measuring the remaining intact penicillin, were shown to observe first-order kinetics and were shown to be significantly influenced by general acid and general base catalysis. The catalytic rate constants imposed by citrate and phosphate ions were calculated. In 0.08 $N$ hydrochloric acid solution the apparent rate of reaction was shown to be accelerated with increasing neutral salt concentration and retarded due to the presence of alcohol. In a pH 4.94 buffer no primary salt effect was observed. The apparent heats of activation for ampicillin degradation in solution were determined to be 16.4, 18.3, and 9.2 kcal./mole, respectively, in buffers of pH 1.35, 4.93, and 9.78. The pH-rate profile in buffer solutions showed a minimum at a pH of 4.85. However, at zero buffer concentration the maximum stability was shifted to a pH of 5.85. The agreement between the calculated theoretical curve and the experimental points (buffer-free) supports the hypothesis presented concerning the reactions involved in the degradation of ampicillin in solution.

**Keyphrases** ☐ Ampicillin solution—degradation kinetics, mechanism ☐ Kinetics—ampicillin degradation ☐ Degradation rate, ampicillin—alcohol, salt effect ☐ Heat of activation, apparent—ampicillin hydrolysis ☐ pH profile—ampicillin solution degradation

*Scheme I*

Among the modern semisynthetic penicillin group of drugs, ampicillin ($\alpha$-aminobenzyl penicillin) has been shown to be the most acid-stable (1), effective against Gram-negative as well as Gram-positive organisms (2, 3), and well-absorbed from the gastrointestinal tract (3, 4). In contrast to classical monobasic penicillins, ampicillin is an amphoteric compound and it behaves essentially as an aliphatic amino acid. This was shown in a recent series of investigations of ampicillin pH-solubility profile and dissociation constants (5).[1] Being amphoteric, ampicillin in solution exists mainly in three different forms, that is, cation, zwitterion, and anion. This may be represented as shown in Scheme I, where $K_1$ and $K_2$ are the dissociation (ionization) constants, $RH_2^+$, $RH^\pm$, and $R^-$ are the cationic, zwitterionic, and anionic species of ampicillin, respectively.

The kinetics of penicillin G reactions have been reported earlier by Brodersen (7, 8); Schwartz has published the kinetics of phenethicillin (9). The general acid-base catalytic effect and salt effect on the degradation of penicillin G has recently been reported by Finholt et al. (10). According to Brodersen (7, 8) the activation energies for the penicillin G reactions at pH's 1.20, 4.54, and 9.57 are 17.5, 20.9, and 9.8 kcal./mole, respectively. Schwartz et al. (9) reported the activation energy for phenethicillin at pH 6.6 to be 17.6 kcal./mole. Doyle et al. (1) determined the acid stabili-

ties of several penicillins in 0.1 $N$ HCl solutions in the presence of 50% alcohol at 35°.

In general, the drugs of the penicillin group are characterized by a pronounced susceptibility to attack on the $\beta$-lactam ring by acid-base reagents, metal ions, penicillinases, organic catalytic agents, and even water molecules (11, 12). The kinetics of ampicillin have not been reported in the literature. The purpose of this study was to investigate the kinetics of hydrolytic reactions of ampicillin in solution.

## EXPERIMENTAL

**Materials**—Ampicillin anhydrate,[2] was purified with cold water and dried in a desiccator under reduced pressure; m.p. 202–203° (dec.). All other chemicals were reagent grade. Water used for buffers was doubly distilled from 2% acidic potassium permanganate solution in all-Pyrex apparatus and was freshly boiled before use.

**Buffer Solutions**—For the general investigations the buffers used were: at pH < 2, KCl–HCl; at pH 2–8, McIlvaine buffer (citric acid–$Na_2HPO_4$) (13); at pH 8–10, Clark-Lubs buffer (boric acid–NaOH) (14). For the general acid-base catalysis, the buffers used were: citric acid–potassium citrate and $Na_2HPO_4$–$NaH_2PO_4$. A constant ionic strength of 0.5 was maintained for each buffer (except in investigations concerned with the primary-salt effect) by adding an appropriate amount of KCl. The relative amount of the ionic species in the multiacid-base buffers was calculated from the equations given by Laitinen (15). The solutions were freshly prepared and the pH's were measured at 35° by a research pH meter[3] and SC-glass electrodes.

**Analytical Procedure**—An iodometric method was used for determination of the residual intact ampicillin. The procedure reported by Finholt et al. (10) was followed except that thyodene was used as an indicator and 5 ml. of pH 4 citrate buffer was used. Each milliliter of 0.01 $N$ iodine was found to be equivalent to 0.398 mg. of ampicillin anhydrate.

**Kinetic Procedures**—Exactly 257 mg. of ampicillin (99% pure) was quantitatively transferred to each of a series of 100-ml. volumetric flasks and sufficient buffer was added to bring the solution up to volume. The buffer solution had previously been brought to the desired temperature. The flasks were stored in a constant tempera-

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[1] The procedures used to determine the dissociation constants were essentially the same as that reported by Rapson and Bird (6).

Table I—Citric Acid–Disodium Phosphate Buffer Composition[a] and Observed Rate Constants of Degradation of Ampicillin at 35°C. and $\mu = 0.5$

| Obs. pH | pH Required | Total Citrate[b] × 10² | H₃A × 10² | H₂A⁻ × 10² | HA⁻ × 10² | A⁼ × 10² | Total Phosphate[c] × 10² | H₃PO₄ × 10² | H₂PO₄⁻ × 10² | HPO₄⁼ × 10² | $k_{obs}$ × 10³ hr⁻¹ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2.05 | 2.0[d] | 9.90 | 9.19 | 0.72 | — | — | 0.20 | 0.09 | 0.10 | — | 58.85 |
| 2.34 | 2.4 | 9.40 | 7.87 | 1.53 | — | — | 1.20 | 0.32 | 0.88 | — | 56.02 |
| 2.55 | 2.6 | 8.90 | 6.79 | 2.10 | 0.01 | — | 2.18 | 0.41 | 1.77 | — | 52.97 |
| 2.96 | 3.0 | 7.94 | 4.42 | 3.45 | 0.06 | — | 4.11 | 0.34 | 3.76 | — | 42.77 |
| 3.56 | 3.6 | 6.78 | 1.65 | 5.09 | 0.12 | — | 6.44 | 0.14 | 6.29 | — | 24.73 |
| 3.91 | 4.0 | 6.14 | 0.70 | 4.62 | 0.82 | — | 7.71 | 0.06 | 7.64 | 0.01 | 15.34 |
| 4.48 | 4.6 | 5.32 | 0.35 | 2.92 | 2.05 | — | 9.35 | 0.02 | 9.25 | 0.07 | 7.65 |
| 4.67 | 4.8 | 5.07 | 0.05 | 2.39 | 2.61 | 0.06 | 9.86 | 0.01 | 9.72 | 0.12 | 6.43 |
| 4.91 | 5.0 | 4.85 | 0.02 | 1.69 | 3.01 | 0.11 | 10.30 | — | 10.10 | 0.10 | 5.37 |
| 5.31 | 5.4 | 4.42 | 0.01 | 0.75 | 3.34 | 0.32 | 11.15 | — | 10.62 | 0.53 | 5.53 |
| 5.71 | 5.8 | 3.95 | 0.01 | 0.26 | 2.97 | 0.71 | 12.09 | — | 10.74 | 1.35 | 6.80 |
| 5.86 | 6.0 | 3.68 | — | 0.19 | 2.52 | 0.96 | 12.63 | — | 10.53 | 2.10 | 11.23 |
| 6.25 | 6.4 | 3.07 | — | 0.07 | 2.68 | 0.32 | 13.85 | — | 9.25 | 4.62 | 13.61 |
| 6.44 | 6.6 | 2.72 | — | 0.05 | 1.07 | 1.63 | 14.55 | — | 8.11 | 6.44 | 17.79 |
| 6.85 | 7.0 | 1.76 | — | 0.01 | 0.37 | 1.39 | 16.47 | — | 5.50 | 10.97 | 26.81 |
| 7.19 | 7.2 | 1.30 | — | — | 0.18 | 1.11 | 17.39 | — | 4.18 | 13.20 | 31.32 |
| 7.55 | 7.6 | 0.63 | — | — | 0.04 | 0.59 | 18.73 | — | 2.10 | 16.63 | 32.79 |
| 7.94 | 8.0 | 0.27 | — | — | 0.01 | 0.26 | 19.45 | — | 0.93 | 18.52 | 53.78 |

[a]The buffers were made from Reference 13, and all citrate and phosphate ions are concentrations in moles /l. [b]The ionization constants of citric acid and citrate ions at 35° are p$K_1$ = 3.11, p$K_2$ = 4.75; p$K_3$ = 6.42, from R. G. Bates and G. D. Pinching, J. Am. Chem. Soc., 71, 2374(1949). [c]The ionization constants of phosphoric acid and phosphate at 35° ($\mu = 0.5$) are p$K_1$ = 1.96; p$K_2$ = 6.70, from M. A. Schwartz, A. P. Granatek, and F. H. Buckwalter, J. Pharm. Sci., 51, 523(1962). [d]The buffer was made by mixing 990 ml. of 0.1 M citric acid with 10 ml. of 0.2 M Na₂HPO₄ to make a liter.

ture bath which was regulated by a thermostat (Haake) with ±0.1° precision. Samples were taken at proper intervals and assayed immediately.

## RESULTS AND DISCUSSION

**Order of Reaction and Observed Rate Constant**—At constant pH, temperature, and total ionic strength ($\mu = 0.5$), the degradation of ampicillin was found to observe pseudo first-order kinetics with respect to the substrate. Figure 1 shows the results of several of the experimental runs at 35° and various pH's. The observed rate constant, $K_{obs}$, at pH 2–8 along with the buffer constituents (citrate-phosphate) are listed in Table I. The measured pH for each of these buffers was found to be a little lower than the original value. This was believed to be due to the secondary salt effect.

**General Acid and General Base Catalysis**—The rates of degrada-

tion of ampicillin in citric acid–potassium citrate buffer solutions at constant pH and 35° were shown to be significantly affected by general acid catalysis. Table II shows that at pH 3.73 the observed first-order rate constants increased linearly with the buffer concentration. When these buffers were maintained at a constant ionic strength ($\mu = 0.5$), by the addition of KCl, the observed rate constants again increased proportionately with the buffer concentration (Table II-B). However, because of the primary salt effect, the absolute rates were slightly higher in each case than when the ionic strength was varied. No significant secondary salt effect on the pH changes of these buffers was observed in the present studies.

The observed rate constant in the citric acid–potassium citrate buffer was actually a summation of several catalytic rate constants catalyzed by the buffer species plus the rate at zero buffer concentration. Accordingly, the observed rate may be expressed by the following equation.

$$k_{obs.} = k_0 + k_{H_3A}[H_3A] + k_{H_2A^-}[H_2A^-] + k_{HA^-}[HA^-] + k_{A^=}[A^=] \quad (Eq. 1)$$

where $k_0$ = the rate constant at zero buffer concentration; other $k$'s are catalytic rate constants imposed by citrate buffer; [H₃A] = concentration of undissociated citric acid; [H₂A⁻] = concentration of dihydrogen citrate ion; [HA⁻] = concentration of monohydrogen citrate ion; [A⁼] = concentration of citrate ion.

The total citrate concentration, $C_A$,

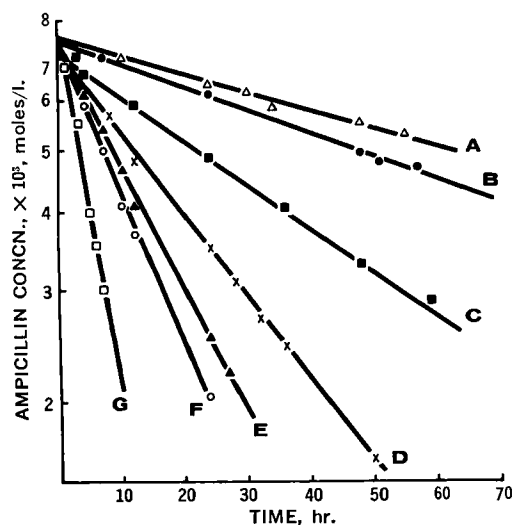$$C_A = [H_3A] + [H_2A^-] + [HA^-] + [A^=] \quad (Eq. 2)$$



Figure 1—Plots of the observed pseudo first-order kinetic degradation of ampicillin in solution at different pH's and 35° ($\mu = 0.5$). Key: A, pH 4.95; B, pH 4.48; C, pH 3.91; D, pH 6.85; E, pH 2.96; F, pH 2.55; G, pH 1.20.

Table II—General Acid Catalysis of Degradation of Ampicillin in Solution at pH 3.73 and 35°C.

| Total citrate × 10, moles/l. | KCl, × 10 moles/l. | $\mu$ × 10 | $k_{obs.}$ × 10³ hr⁻¹ |
|---|---|---|---|
| | A | | |
| 1.50 | — | 1.44 | 42.5 |
| 1.20 | — | 1.16 | 34.3 |
| 0.75 | — | 0.72 | 22.6 |
| 0.32 | — | 0.36 | 13.0 |
| 0.16 | — | 0.18 | 7.4 |
| | B | | |
| 1.50 | 3.55 | 5.0 | 44.4 |
| 1.20 | 3.84 | 5.0 | 35.7 |
| 0.75 | 4.28 | 5.0 | 25.3 |
| 0.32 | 4.64 | 5.0 | 14.8 |
| 0.16 | 4.82 | 5.0 | 9.8 |

**Table III**—List of Citric Acid–Potassium Citrate Buffer Concentrations and Observed Rate Constants on Degradation of Ampicillin at 35°C. and $\mu = 0.5$

| pH | Citrate Buffers[a] | Observed Rate Constant $\times 10^3$ hr.$^{-1}$ at Total Buffer Concn. moles/l. 0.2 | 0.16 | 0.15 | 0.10 | 0.05 | 0.02 | $k_0{}^b \times 10^3$ |
|---|---|---|---|---|---|---|---|---|
| 2.25 | 95 ml. 0.2 $M$ citric acid + 4 ml. 0.2 $M$ potassium citrate | 120.2 | — | 100.4 | 78.3 | 56.0 | 46.5 | 38.5 |
| 2.76 | 85 ml. 0.2 $M$ citric acid + 15 ml. 0.2 $M$ potassium citrate | 97.8 | 83.0 | — | 59.5 | 41.0 | 29.5 | 22.0 |
| 3.12 | 75 ml. 0.2 $M$ citric acid + 25 ml. 0.2 $M$ potassium citrate | 82.9 | — | 66.3 | 50.4 | 33.8 | 23.0 | 16.0 |
| 3.73 | 50 ml. 0.2 $M$ citric acid + 25 ml. 0.2 $M$ potassium citrate | — | — | 44.4 | — | — | — | 5.0 |
| 4.75 | 50 ml. 0.2 $M$ citric acid + 50 ml. 0.2 $M$ potassium citrate | 34.2 | — | 25.5 | 15.5 | 9.44 | 4.40 | 1.20 |
| 6.80 | 1 ml. 0.2 $M$ citric acid + 99 ml. 0.2 $M$ potassium citrate | — | — | 3.45 | 3.13 | 1.84 | 1.03 | 0.85 |

[a] The original buffer concentration was 0.2 mole/l. Proper dilutions were made for other concentrations and each buffer was adjusted to $\mu = 0.5$ by the addition of KCl. [b] The rate at buffer-free condition was obtained by extrapolation.

From the dissociation constants

$$K_1 = \frac{[H^+][H_2A^-]}{[H_3A]} \qquad \text{(Eq. 3)}$$

$$K_2 = \frac{[H^+][HA^=]}{[H_2A^-]} \qquad \text{(Eq. 4)}$$

$$K_3 = \frac{[H^+][A^=]}{[HA^-]} \qquad \text{(Eq. 5)}$$

and from Eqs. 1 and 2 the following overall rate expression, $k_{obs.}$, was obtained.

$$k_{obs.} =$$
$$k_0 + C_A \frac{k_{H_3A}[H^{+3}] + k_{H_2A^-}[H^+]^2 K_1 + k_{HA^=}[H^+]K_1K_2 + k_{A^=}K_1K_2K_3}{[H^+]^3 + [H^+]^2 K_1 + [H^+]K_1K_2 + K_1K_2K_3} \qquad \text{(Eq. 6)}$$

Based on the pKa values: $pK_1 = 3.11$, $pK_2 = 4.75$, and $pK_3 = 6.42$ (16) and the pH, the relative amount of each of the buffer species could be calculated.

At pH 2, there are about 93% undissociated citric acid molecules and 7% dihydrogen citrate ions. The total citrate concentration and
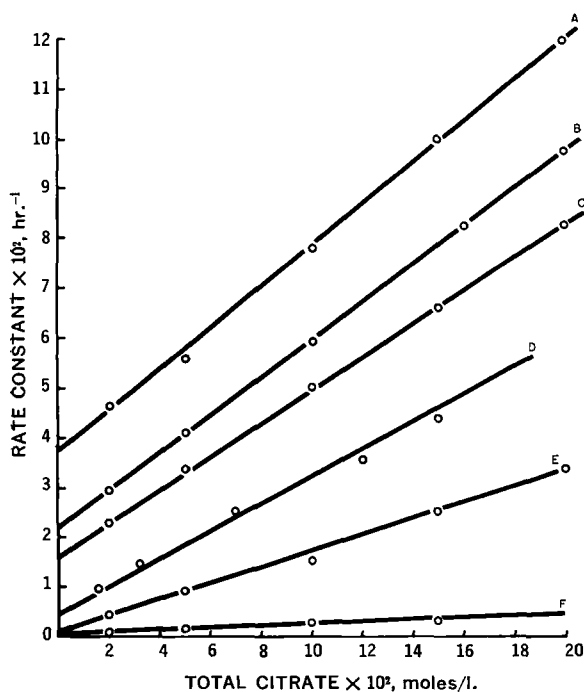
**Figure 2**—*Plots showing the citrate-buffer catalytic effect on the observed rate constants of ampicillin degradation at constant pH and 35° ($\mu = 0.5$). All the studies were in citric acid–potassium citrate buffers. Key: A, pH 2.25; B, pH 2.76; C, pH 3.12; D, pH 3.73; E, pH, 4.75; F, pH 6.80.*

overall rate, therefore, may be simplified to:

$$C_A = [H_3A] + [H_2A^-] \qquad \text{(Eq. 7)}$$

$$k_{obs.} = k_0 + C_A \frac{k_{H_3A}[H^+] + k_{H_2A^-}K_1}{[H^+] + K_1} \qquad \text{(Eq. 8)}$$

Similarly, at pH's 3–4, the undissociated citric acid, di- and mono-hydrogen citrate ions are equally important. The total citrate concentration and the overall rate may be simplified to

$$C_A = [H_3A] + [H_2A^-] + [HA^-] \qquad \text{(Eq. 9)}$$

$$k_{obs.} = k_0 + C_A \frac{k_{H_3A}[H^+]^2 + k_{H_2A^-}[H^+]K_1 + k_{HA^-}K_1K_2}{[H^+]^2 + [H^+]K_1 + K_1K_2} \qquad \text{(Eq. 10)}$$

At pH > 6, the citrate buffer consists essentially of monohydrogen citrate ion and unprotonated citrate ions. Accordingly,

$$C_A = [HA^-] + [A^=] \qquad \text{(Eq. 11)}$$

$$k_{obs.} = k_0 + C_A \frac{k_{HA^-}[H^+] + k_{A^=}K_3}{[H^+] + K_3} \qquad \text{(Eq. 12)}$$

Based on the rate constants in various buffer concentrations listed in Table III, plots were constructed by plotting the observed rate constants *versus* the total citrate concentration at several pH's as shown in Fig. 2. From the slopes, the citrate buffer catalytic rate constants on degradation of ampicillin at 35° were calculated and are shown to be in the following order: $k_{H_3A} = 0.42$ l. mole$^{-1}$hr.$^{-1}$ > $k_{H_2A^-} = 0.24$ l. mole$^{-1}$hr.$^{-1}$ > $k_{HA^-} = 0.07$ l. mole$^{-1}$hr.$^{-1}$ > $k_{A^=} = 0.002$ l. mole$^{-1}$hr.$^{-1}$. Slopes were calculated using these rates which are in reasonable agreement with the slopes observed in Fig. 2, as shown in Table IV.

The observed rate constants were also shown to be increased with buffer concentration in the phosphate buffers. At pH's 6–7, only di- and monohydrogen phosphate ions are important. Accordingly, the total phosphate concentration and the overall rate may be written as:

$$k_{obs.} = k_0 + k_{H_2PO_4^-}[H_2PO_4^-] + k_{HPO_4^-}[HPO_4^=] \qquad \text{(Eq. 13)}$$

$$C_A = [H_2PO_4^-] + [HPO_4^=] \qquad \text{(Eq. 14)}$$

The dissociation constant of dihydrogen phosphate ion ($pK_2 = 7.21$) (17)

$$K_2 = \frac{[H^+][HPO_4^=]}{[H_2PO_4^-]} \qquad \text{(Eq. 15)}$$

**Table IV**—Slopes of Citric Acid–Potassium Citrate Buffer Catalysis in Ampicillin Degradation

| pH | Slopes $\times 10$ Calcd.[a] | Observed[b] |
|---|---|---|
| 2.25 | 4.02 | 4.07 |
| 2.76 | 3.65 | 3.80 |
| 3.12 | 3.29 | 3.35 |
| 3.73 | 2.60 | 2.60 |
| 4.75 | 1.58 | 1.60 |
| 6.80 | 0.22 | 0.22 |

[a] The slopes were calculated from Eqs. 8, 10, and 12. [b] The observed slopes from the lines in Fig. 2.

Table V—List of Phosphate Buffer Concentrations and Observed Rate Constants on Degradation of Ampicillin at 35°C. and $\mu$ = 0.5

| pH | Phosphate Buffer Concn.[a] | Observed Rate Constants × 10³ at Total Buffer Concn. moles/l. | | | | | $k_0$ × 10³[b] |
| | | 0.20 | 0.16 | 0.12 | 0.08 | 0.04 | |
|---|---|---|---|---|---|---|---|
| 6.60 | 62 ml. $NaH_2PO_4$ + 38 ml. $Na_2HPO_4$ | 23.9 | 19.2 | 14.6 | 10.0 | 4.95 | 0.75 |
| 7.11 | 29 ml. $NaH_2PO_4$ + 71 ml. $Na_2HPO_4$ | 29.0 | 23.6 | 18.2 | 12.0 | 6.46 | 0.90 |

[a] The original buffer was 0.2 mole /l. for both $NaH_2PO_4$ and $Na_2HPO_4$. Proper dilutions were made for other concentrations and were adjusted to onic strength $\mu$ = 0.5 by adding KCl. [b] The rate at buffer-free condition was obtained by extrapolation.

and the overall rate may be shown as:

$$k_{obs.} = k_0 + C_A \frac{k_{H_2PO_4^-}[H^+] + k_{HPO_4^-}K_2}{[H^+] + K_2} \qquad (Eq. 16)$$

Table V shows the phosphate buffer rate constants at varied buffer concentrations at pH's 6.60 and 7.11. From Eq. 16 the catalytic rate constants on the degradation of ampicillin at 35° were obtained and shown to be $k_{H_2PO_4^-}$ = 0.077 l.mole⁻¹hr.⁻¹ < $k_{HPO_4^-}$ = 0.166 l.mole⁻¹ hr.⁻¹. Similarly, the rate constants at varied buffer concentrations were also obtained for citrate phosphate buffer systems as shown in Figs. 3 and 4 by plotting the observed rate constants *versus* total citrate (acid buffers) or total phosphate (neutral buffers) concentration. In the present investigations, no observable buffer catalysis induced by borate ion was shown, probably the uncharged boric acid as well as the dihydrogen borate ion were imposing a negligible effect in comparison to the hydroxy-ion catalysis.

**Effect of Salt**—At constant temperature (35°) and pH, a linear positive salt effect was observed for ampicillin degradation in 0.08 N hydrochloric acid solution. A positive primary-salt effect is normally expected for ion-ion interactions between similarly positive-charged ions. The observed rate constant increased linearly with the ionic strength of the solvent as shown in Fig. 5. However, the data plotted according to the method of Bronsted and Bjerrum (18) resulted in a slope of nonintegral value. This may be due to either the high salt concentration in the system, or the complexity of ampicillin degradation in acid.

No salt effect was shown on ampicillin degradation in a pH 4.94 buffer as shown in Fig. 5.
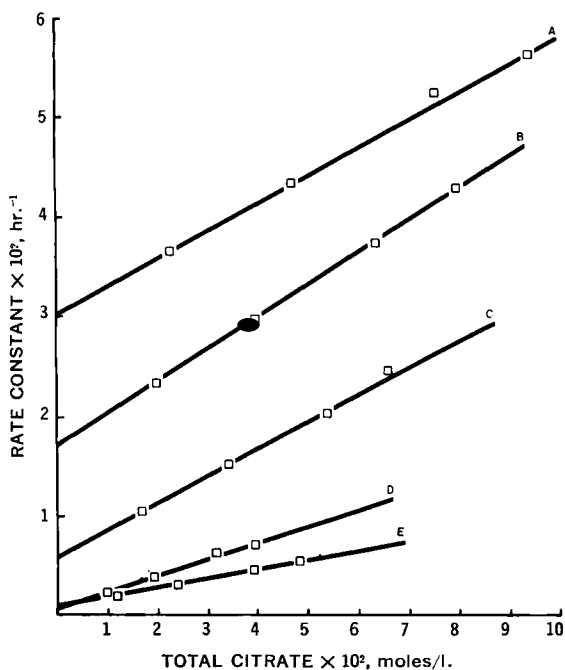
**Effect of Alcohol**—Table VI shows 0.08 N hydrochloric acid solution and hydroalcoholic mixtures in the observed rate constants of ampicillin degradation decreases with an increase in the volume of alcohol. As the volume of ethanol increases, the dielectric constant of the solvent medium decreases. Accordingly, the dependence of the observed rates on the dielectric constant can be expressed by Eq. 17, as described by Scatchard (19).

$$\log k_{obs.} = \log k_0 - \frac{NZ_AZ_B e^2}{2.303\ RT\ d_{AB}} \cdot \frac{1}{\epsilon} \qquad (Eq. 17)$$

where $k_0$ is the rate constant in a medium of infinite dielectric constant, and $\epsilon$ is the dielectric constant of the medium. A plot constructed by plotting log $k_{obs.}$ against the reciprocal of the dielectric constant of the solvent medium is shown in Fig. 6. The linear negative slope obtained indicates a decreasing rate as the dielectric constant of the solvent decreases.

The pH of the solvent mixtures would be expected to increase due to the presence of alcohol. However, the actual measured pH change was from 1.20 to 1.25 as the alcohol was increased from 0 to 50%. Accordingly, the decreased rate was essentially a result of the change in solvent medium.

In the acidic solution, the ampicillin cation is assumed to interact with the hydrated proton (ion-ion interaction) to form as an intermediate a double positively charged activation complex. This inter-
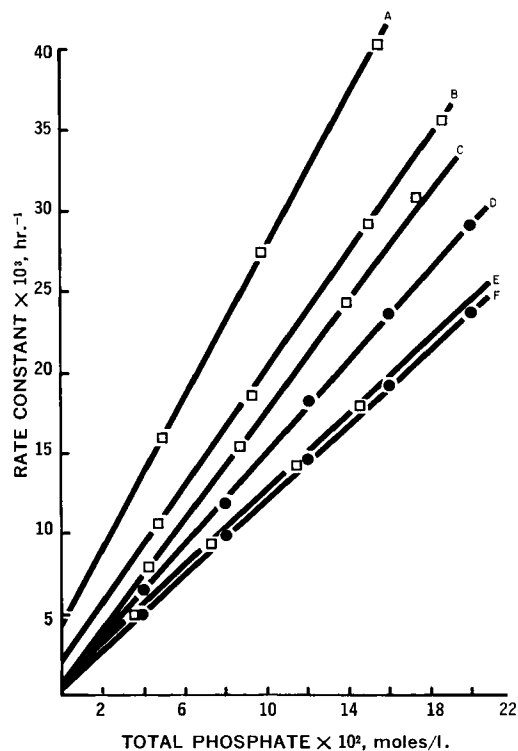


Figure 3—*Plots showing the buffer catalytic effect on the observed rate constants of ampicillin degradation at constant pH and 35° ($\mu$ = 0.5). The plots were made by plotting the rate constant against the total citrate concentration since these studies were in citrate-phosphate buffers. Key: A, pH 2.44; B, pH 2.96; C, pH 3.56; D, pH 5.71; E, pH 4.91.*



**TOTAL PHOSPHATE × 10², moles/l.**

Figure 4—*Plots showing the buffer catalytic effect on the observed rate constants of ampicillin degradation at constant pH and 35° ($\mu$ = 0.5). The solid circles are the studies in $NaH_2PO_4$-$Na_2HPO_4$ buffers and the squares are the studies in the citric acid-phosphate buffers. The plots are constructed by plotting the rate constants against the total phosphate concentration of the buffer. Key: A, pH 7.97; B, pH 7.55; C, pH 7.20; D, pH 7.11; E, pH 6.47; F, pH 6.60.*
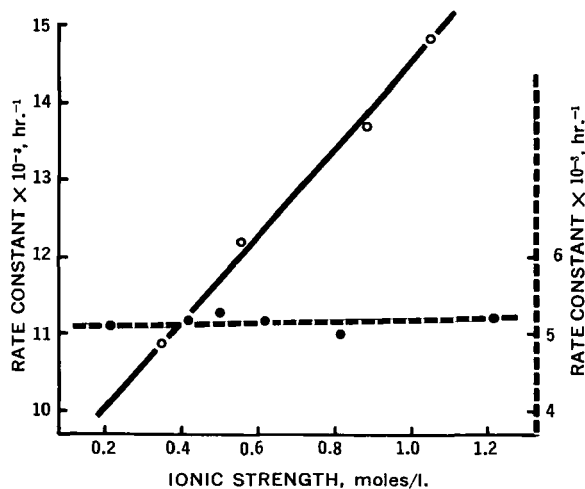
**Figure 5**—*Salt effect on ampicillin degradation in solution. Key: O, at pH 1.20 (0.08 N HCl); ●, at pH 4.94.*

mediate should be more stable in a solvent of higher dielectric constant and accordingly, the reaction rate increases with the increasing dielectric constant (20).

Evidently no change in reaction mechanism occurs due to the presence of alcohol in the reaction medium.

**Apparent Heat of Activation**—The temperature dependence of the hydrolytic reactions of ampicillin in solution was determined by measuring the pseudo first-order rate constants at various pH's and a constant ionic strength of 0.5. These determinations were made in buffers of pH 1.35 (HCl-KCl), pH 4.93 (citric acid-$Na_2HPO_4$), and pH 9.78 (boric acid-NaOH). In each of these buffer systems, ampicillin exists in only one molecular form. Table VII shows the observed rate constants at temperatures ranging from 30 to 50.5° and the corresponding Arrhenius-type plots are shown in Fig. 7. The calculated heats of activation are 16.4 ± 0.2 kcal./mole at pH 1.35; 18.3 ± 0.1 kcal./mole at pH 4.93; and 9.2 ± 0.5 kcal./mole at pH 9.78. These energies are similar to those observed for penicillin G in solutions of similar pH. This suggests that the side chain does not affect the mechanism of β-lactam ring cleavage either in acid, neutral, or basic solutions. However, the nature of the side chain does significantly influence the rate of reaction.

It should be noted that the calculated overall heat of activation at pH 9.78 was actually 22.3 ± 0.5 kcal./mole. However, the heat of ionization of water was included in this value. By employing the value of 13.05 kcal./mole (21) as the heat of ionization of water, the net heat of activation of ampicillin degradation in basic solution, 9.2 kcal./mole, was obtained.

**pH-Rate Profile**—By plotting the logarithm of the observed rate constants (Table I) at pH's 2-8 for the hydrolysis of ampicillin against the pH's of the buffer at 35° (the dotted line of Fig. 8), a
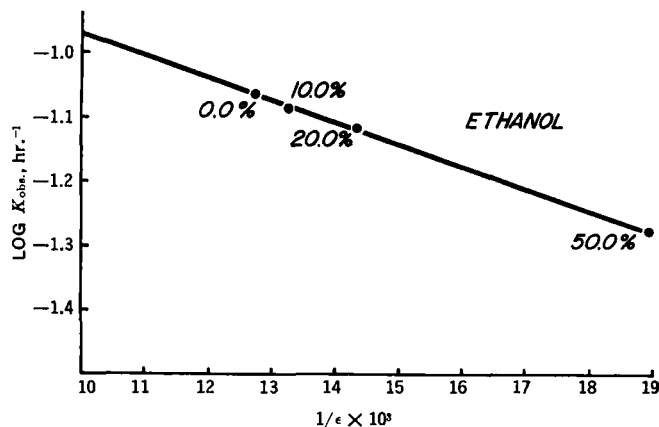


**Figure 6**—*Effect of dielectric constant of the solvent on the observed rate constant for ampicillin degradation reaction in 0.08 N hydrochloric acid solution.*
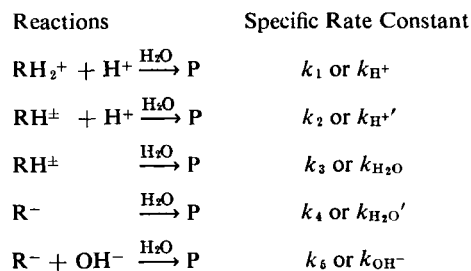
**Table VI**—Effect of Alcohol on Degradation of Ampicillin in 0.08 N HCl at 35°C. and μ = 0.08

| Vol. Ethanol, %[a] | ε[b] | $K_{obs.} \times 10^3$ hr.$^{-1}$ | $t_{0.5, hr.}$ |
|---|---|---|---|
| 0.0 | 78.5 | 86.7 | 7.99 |
| 10 | 75.4 | 82.2 | 8.43 |
| 20 | 69.8 | 76.7 | 9.03 |
| 50 | 52.8 | 53.4 | 12.97 |

[a] The solutions were made by proper dilution from concentrated HCl solution by adding ethanol. [b] Data from J. Wyman, Jr., *Chem. Rev.*, **19**, 213(1936).

minimum rate at a pH of 4.85 was observed. The observed rate in this pH-apparent rate profile was actually a summation of a series of catalytic reaction rates induced by the buffer species, hydrogen and hydroxyl ions and water molecules. The minimum rate lies on the isoelectric point of ampicillin and this seemed to suggest that the isoelectric molecular form of this amphoteric penicillin is more resistent to attack than its positively and negatively charged species. The solid line of Fig. 8 is an overall pH-rate profile in which the line represents the calculated theoretical curve while the points are the experimental results. These latter values are the rates at zero buffer concentration (pH's 2-8) which were obtained from the intercepts of the lines of Figs. 2-4, and the observed hydrogen and hydroxyl ion catalytic rates at pH's below 2 and above 7.

The pH-rate profile suggested that there are at least five reactions which contribute to the overall velocity of ampicillin degradation in solution. These are represented by Scheme II, where $RH_2^+$, $RH^\pm$,

| Reactions | Specific Rate Constant |
|---|---|
| $RH_2^+ + H^+ \xrightarrow{H_2O} P$ | $k_1$ or $k_{H^+}$ |
| $RH^\pm + H^+ \xrightarrow{H_2O} P$ | $k_2$ or $k_{H^+}'$ |
| $RH^\pm \xrightarrow{H_2O} P$ | $k_3$ or $k_{H_2O}$ |
| $R^- \xrightarrow{H_2O} P$ | $k_4$ or $k_{H_2O}'$ |
| $R^- + OH^- \xrightarrow{H_2O} P$ | $k_5$ or $k_{OH^-}$ |

*Scheme II—Ampicillin Hydrolytic Reactions*

and $R^-$ are cationic, zwitterionic, and anionic species of ampicillin. The overall velocity is, obviously, equal to the sum of the rate of these reactions as shown in the following equation:

$$v = -\frac{d[P]_T}{dt} = k_1[RH_2^+][H^+] + k_2[RH^\pm][H^+]$$
$$+ k_3[RH^\pm] + k_4[R^-] + k_5[R^-][OH^-] \quad (Eq. 18)$$

where $[P]_T$ is the total ampicillin concentration. By introducing the dissociation constants, $K_1$ and $K_2$, of ampicillin at 35° ($pK_1 = 2.60$, $pK_2 = 7.05$), and the dissociation constant of water, $K_w$,

$$K_1 = \frac{[RH^\pm][H^+]}{[RH_2^+]} \quad (Eq. 19)$$

$$K_2 = \frac{[R^-][H^+]}{[RH^\pm]} \quad (Eq. 20)$$

**Table VII**—Apparent Heat of Activation of Ampicillin Degradation, in Solution at Constant pH and Ionic Strength μ = 0.5

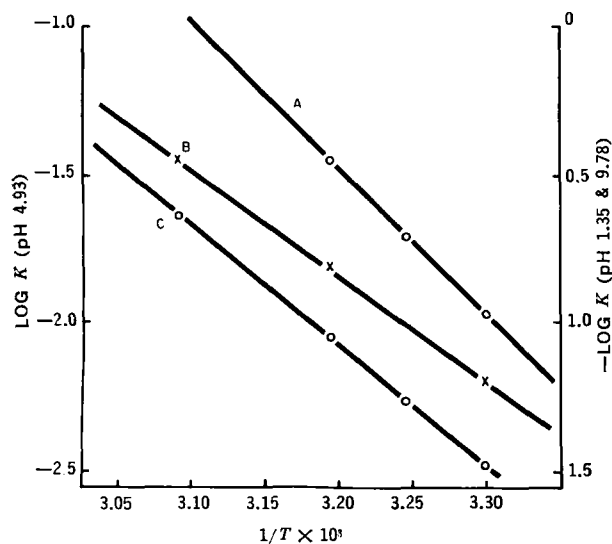| Buffer pH | Rate Constant × 10³ hr.$^{-1}$ | | | | ΔH kcal./mole |
|---|---|---|---|---|---|
| | 30° | 35° | 40° | 50.5° | |
| 1.35 | 63.95 | 107.9 | 155.4 | 357.4 | 16.4 ± 0.2 |
| 4.93 | 3.34 | 5.37 | 8.91 | 23.03 | 18.3 ± 0.1 |
| 9.78 | 109.2 | 214.8 | 355.6 | — | 9.2 ± 0.5 |

**Figure 7**—*Arrhenius-type plots showing temperature dependence of reaction rates of hydrolysis of ampicillin at various pH's. Key:* A, *pH 9.78;* B, *pH 1.35;* C, *pH 4.93.*

the following overall rate expression was obtained.

$$k = \frac{k_1[H^+]^3 + k_2[H^+]^2 K_1 + k_3[H^+]K_1 + k_4 K_1 K_2 + k_5 K_1 K_2 K_w/[H^+]}{[H^+]^2 + [H^+]K_1 + K_1 K_2}$$

(Eq. 21)

At pH < 1.5, ampicillin exists as the cationic species. The velocity of the hydrolytic reaction is attributed exclusively to the hydrogen ion and ampicillin cation. This is supported by the slope of negative unity at the region below pH of 1.5 in the pH-rate profile. Thus in the right-hand side of Eq. 18, only the first term is important. Accordingly,

$$v = -\frac{d[RH_2^+]}{dt} = k[RH_2^+]$$

(Eq. 22)

and

$$k = k_1[H^+]$$

(Eq. 23)

The logarithmic form of Eq. 23,

$$\log k = \log k_1 + \log [H^+]$$

(Eq. 24)

According to Harned and Hammer (21),

$$\alpha_{H^+} \cdot \alpha_{OH^-} = 2.09 \times 10^{-14}$$

(Eq. 25)

$$[H^+] \cdot [OH^-] = 3.61 \times 10^{-14}$$

(Eq. 26)

since

$$[H^+] \cdot \gamma = \alpha_{H^+}$$

(Eq. 27)

$$[OH^-] \cdot \gamma = \alpha_{OH^-}$$

(Eq. 28)

a mean ionic activity coefficient, $\gamma = 0.762$, at 35° and $\mu = 0.5$ was derived. Thus from Eq. 27,

$$\log [H^+] = 0.12 - pH$$

(Eq. 29)

and from Eq. 24,

$$\log k = \log k_1 + 0.12 - pH$$

(Eq. 30)

The specific rate constant catalyzed by hydrogen ion was calculated from Eq. 30 (Table VIII), an average value of $k_1 = 1.38$ l. mole$^{-1}$ hr.$^{-1}$ was obtained.

At pH $\simeq$ p$K_1$ of ampicillin, the shoulder-type break of the pH-rate profile seemed to suggest that the cation and zwitterion are interacting with hydrogen ion in different magnitudes. At this particular region the possibility of interaction between ampicillin cation with water molecules may not be as significant as the interaction with protons. At the region of pH 3–5, if the assumption that the hydrogen ion and water molecule are the catalyzing species is correct, the first three terms in the right-hand side of Eq. 18 are

**Table VIII**—Catalytic Effect of Hydrogen Ions on Degradation of Ampicillin at 35°C. and $\mu = 0.5$

| pH | $(H^+) \times 10$ moles/l. | $k \times 10^3$ hr.$^{-1}$ | $k_{H^+}$ Lm.$^{-1}$ hr.$^{-1a}$ |
|---|---|---|---|
| 0.81 | 2.04 | 269.5 | 1.32 |
| 1.10 | 1.04 | 136.3 | 1.33 |
| 1.10 | 1.04 | 132.0 | 1.27 |
| 1.20 | 0.82 | 107.9 | 1.30 |
| 1.51 | 0.42 | 62.3 | 1.52 |
| 1.51 | 0.42 | 62.4 | 1.53 |

$^a$ Average $k_{H^+} = 1.38$ l. mole$^{-1}$ hr.$^{-1}$

important to the buffer free velocity. Accordingly,

$$v = -\frac{d[P]_T}{dt} = k_1[RH_2^+][H^+] + k_2[RH^\pm][H^+] + k_3[RH^\pm]$$

(Eq. 31)

and

$$k = \frac{k_1[RH_2^+][H^+] + k_2[RH^\pm][H^+] + k_3[RH^\pm]}{[RH_2^+] + [RH^\pm]} =$$

$$k_1[H^+] \cdot f_{RH_2^+} + k_2[H^+] \cdot f_{RH^\pm} + k_3 f_{RH^\pm}$$

(Eq. 32)

where $f_{RH_2^+}$ and $f_{RH^\pm}$ are fractions of the cation and zwitterion of ampicillin. Dividing both sides of Eq. 32 by [H$^+$], Eq. 33 is obtained and from this equation, $k_2$ could be calculated

$$\frac{k}{[H^+]} = k_1 f_{RH_2^+} + k_2 f_{RH^\pm} + \frac{k_3 f_{RH^\pm}}{[H^+]}$$

(Eq. 33)

From the values of $k_1 = 1.38$ and $k_3 = 7.5 \times 10^{-4}$,[4] and the hydrogen ion concentration calculated from Eq. 29, the value for $k_2$ was shown to increase (from 13 to 20 with an average value of 16.8) with the increasing of $f_{RH^\pm}$. This indicated that ampicillin zwitterions may
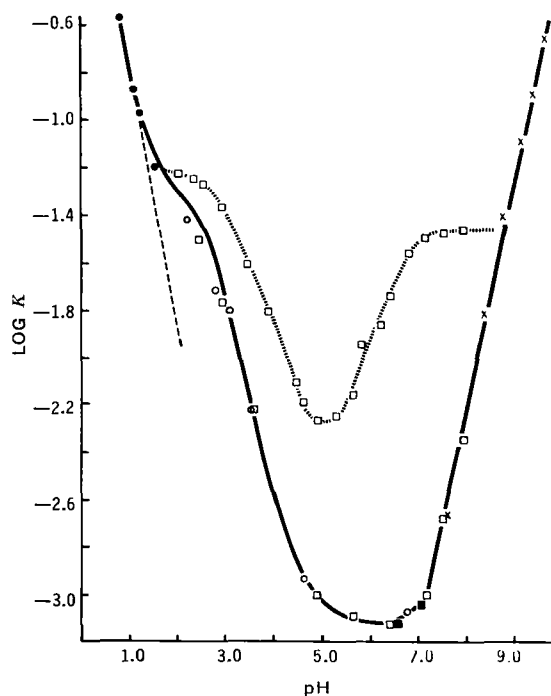


**Figure 8**—*pH-Rate profiles of ampicillin degradation in solution at constant pH and 35°* ($\mu = 0.5$). *The dotted curve is a pH-apparent rate profile and all the studies were in citric acid-phosphate buffers (Table I). The solid curve represents the theoretical line calculated from the rate constants while the points are experimental results. Key: Apparent rate constants in buffers:* ●, *HCl-KCl;* ☐, *Citric acid-phosphate;* ×, *$H_2BO_3$-NaOH. Rate constants at zero buffer concentration:* ○, *citric acid-potassium citrate;* ■, *$NaH_2PO_4$-$Na_2HPO_4$;* ☐, *citric acid-phosphate buffer.*

[4] Lowest rate observed at zero buffer concentration at pH 6.5.

**Table IX**—Catalytic Effect of Hydroxyl Ions on Degradation of Ampicillin at 35°C. and $\mu = 0.5$

| pH | [OH$^-$], moles/l. | $k \times 10^3$hr.$^{-1}$ | $k_{OH^-} \times 10^{-3}$ l. mole$^{-1}$ hr.$^{-1a}$ |
|---|---|---|---|
| 7.60 | $1.09 \times 10^{-6}$ | 2.00 | 1.82 |
| 7.94 | $2.40 \times 10^{-6}$ | 4.50 | 1.87 |
| 8.40 | $6.92 \times 10^{-6}$ | 15.0 | 2.18 |
| 8.81 | $1.78 \times 10^{-5}$ | 38.3 | 2.16 |
| 9.15 | $3.89 \times 10^{-5}$ | 77.9 | 2.00 |
| 9.40 | $6.92 \times 10^{-5}$ | 124.3 | 1.79 |
| 9.78 | $1.66 \times 10^{-4}$ | 294.8 | 1.77 |

[a] Average $k_{OH} = 1945$ l. mole$^{-1}$hr.$^{-1}$.

possibly precede some parallel reactions, for example, to form concurrently penicillinic acid and penillic acid, or to form penicillinic acid and penicilloic acid, and this latter reaction has been reported for penicillin G by Schwartz (23). The average value of $k_2$ (16.8 l. mole$^{-1}$hr.$^{-1}$) was used to calculate the theoretical line. The theoretical curve fits the points in the pH 3 to 5 regions. However, such was not the case in the pH $\simeq$ p$K_1$ area.

At pH's 5.5 to 6.5, the experimental data show a slope of nearly zero (Fig. 8). This suggests that the reactions in this pH range were, essentially, spontaneous and only water is the catalyzing species interacting with ampicillin zwitterion and anions. The buffer free rate constants in this pH region were found to be from 7.5 to $8.0 \times 10^{-4}$ l. mole$^{-1}$hr.$^{-1}$. Accordingly, it is assumed that $k_3 \simeq k_4 = 7.5 \times 10^{-4}$ l. mole$^{-1}$hr.$^{-1}$. It is noted that at pH $\simeq$ p$K_2$ region of the pH-rate profile (Fig. 8) the break is not as evident as the one observed at pH $\simeq$ p$K_1$ region. This may be due to some extent to the nature of the reaction in this pH region, for example, the zwitterions and anions interact only with water and in a similar magnitude.

At pH $> 7.5$ the data indicated that only one type of reaction was responsible for the rate, which is the hydroxyl ion interacting with the anionic form of ampicillin. The positive unity slope of the pH-rate profile at pH $> 7.5$ supports this assumption. Accordingly, only the last term of the right-hand side of Eq. 18 is of importance. The rate equation:

$$v = -\frac{d[P]_T}{dt} = k[R^-] \qquad \text{(Eq. 34)}$$

and

$$k = k_5 [OH^-] \qquad \text{(Eq. 35)}$$

The logarithmic form of Eq. 35,

$$\log k = \log k_5 + \log [OH^-] \qquad \text{(Eq. 36)}$$

From Eqs. 25 and 28, the following equations were obtained to calculate the hydroxyl-ion concentration and the specific rate constant.

$$\log [OH^-] = pH - 13.56 \qquad \text{(Eq. 37)}$$

$$\log k = \log k_5 + pH - 13.56 \qquad \text{(Eq. 38)}$$

The hydroxyl-ion catalytic rate constant values determined from the above equations are listed in the last column of Table IX. An average value of $k_5 = 1,945$ l. mole$^{-1}$hr.$^{-1}$ was obtained. In basic solution the hydrolytic rate of ampicillin is almost 1,400 times faster than that in acid solution. Evidently, the $\beta$-lactam ring of ampicillin, like penicillin G, is also extremely susceptible toward nucleophilic attack initiated by hydroxyl ion. This seems in good agreement with the observed low heat of activation (9.2 kcal./mole) in basic solutions.

The pH of minimum rate, pH$_{min.}$, of ampicillin degradation in buffer free condition was found to be about 5.8[5] at 35° from the following equation.

$$[H^+] = [k_{OH} \times K_w/k_2]^{0.5} \qquad \text{(Eq. 39)}$$

This value suggests that the zwitterionic form of ampicillin is the most stable species. In contrast, the monobasic penicillins (such as penicillin G and phenethicillin) exhibited a value of pH$_{min.}$ of 6.5 as reported by Brodersen (8) and Schwartz et al. (9), respectively. The difference in pH$_{min.}$ between the amphoteric penicillin and mono-
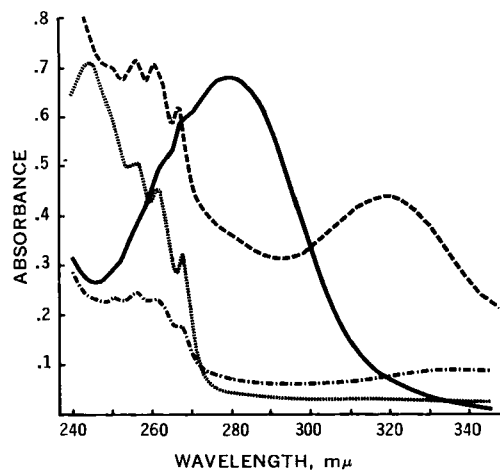
**Figure 9**—*UV absorption spectra of ampicillin reaction mixtures. Key:* ——, in 0.5–1.0 N HCl solution after heating for about 10 min. ($\lambda_{max.}$ 280 $m\mu$); ---, in 0.1–0.5 N HCl solution after heating for about 30 min. ($\lambda_{max.}$ 320 $m\mu$); . . ., in freshly prepared pH 5 buffer solution; ----, in freshly prepared pH 10 buffer solution.

basic penicillins is believed due to the chemical nature of the compound.

**Mechanism of Ampicillin Degradation**—Based on classical knowledge (11) of hydrolytic reactions of penicillin G as well as those of recent reports (6, 22, 23), the data obtained in this study indicate that the amino side-chain group of ampicillin plays a significant role in the rate but not on the mechanism of degradation. Regardless of the complexity of the pathways by which the degradation proceeds, the initial $\beta$-lactam ring structure cleavage appears to be responsible for the overall degradation observed in solution. The postulated degradative reactions are shown in Scheme III.

In acidic solution, ampicillin (I) gave a UV-absorption maximum at $\lambda$ 320–322 $m\mu$ in pH 2–5 solution. This band was shown to increase in intensity with time. By addition of strong acid (0.1 N HCl) a new band at $\lambda$ 228 $m\mu$ was obtained. The initial degradation species observed was probably (via transient oxazolone [II]) $\alpha$-aminobenzyl penicillinic acid (III), while $\alpha$-aminobenzyl penillic acid (IV) was obtained in the stronger acid solution. Pure benzylpenicillinic acid in 95% alcohol absorbs at $\lambda$ 322 $m\mu$ as has been reported earlier (22). However, ampicillin in 0.5–1.0 N HCl, shows an absorption band at $\lambda$ 280 $m\mu$ and no change of this band was observed for several days. This stable degradation product obtained under drastic acidic conditions was possibly $\alpha$-aminobenzylpenamaldic acid (V). The penamaldate band at $\lambda$ 280 $m\mu$ has been reported for benzylpenicillin in base (6, 11). The data presented suggest this open structure of the fused $\beta$-lactam-thiozolidine ring of $\alpha$-aminobenzylpenamaldic acid is also formed in strong acid.

Both $\alpha$-aminobenzylpenicilloic acid (VI) and $\alpha$-aminobenzyl-penilloic acid (VII) were shown to be formed in basic solution. This was confirmed from the comparison of the TLC spots (ninhydrin-positive reaction) of known samples. In a strong basic solution a decarboxylated product, $\alpha$-aminobenzylpenilloic acid, was formed which is similar to the one obtained from an aged ampicillin sample in mild basic solution.

All the aged ampicillin reaction mixtures showed absorption at about $\lambda$ 340 $m\mu$ to $\lambda$ 355 $m\mu$ with loss of the typical benzyl characteristics bands at $\lambda$ 268 $m\mu$, $\lambda$ 262 $m\mu$, and $\lambda$ 257 $m\mu$ of ampicillin.

The absorption spectra of ampicillin at several pH's are shown in Fig. 9. The data presented are not sufficient to positively identify the multiple degradation products formed in the various solutions.

**Stability of Ampicillin**—The extreme susceptibility of the penicillin group of drugs toward acid, base, and penicillinase is due to the fact that the four membered $\beta$-lactam ring is strained by about 10–20 kcal./mole as compared with the normal peptide $\beta$-lactam ring (11). However, the relative acid stability of these drugs is due to the chemical structure of the side chain. Ampicillin is about 200 times more acid-stable than pencillin G (1). Conceivably the side-chain amino group in ampicillin plays a significant role. This amino group must also be stereospecific, since L(+) isomer of ampicillin (24) showed much less activity than its D(−) isomer

1. In Acid and Neutral Solutions:

2. In Basic Solution:
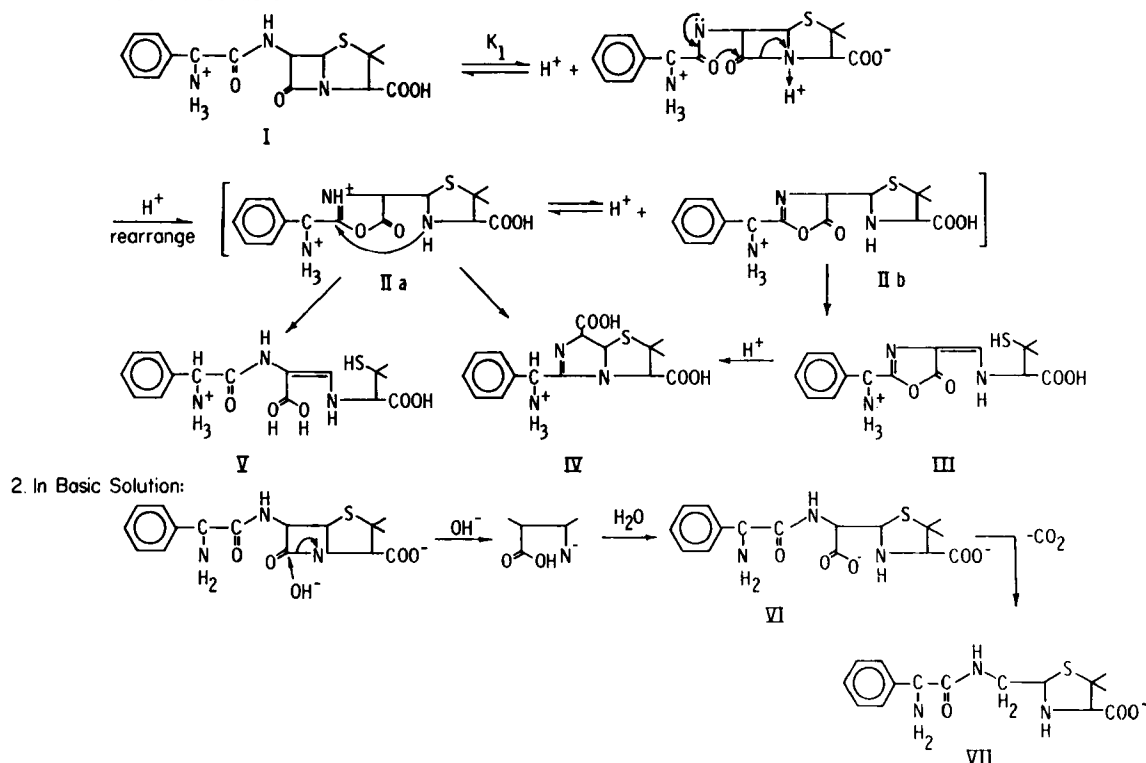
Scheme III—Ampicillin β-Lactam Ring Cleavage

which is used medicinally. On the other hand, the amino group in the para-position of the benzyl group in p-aminobenzylpenicillin was shown to be only seven times more acid-stable over penicillin G (25). Doyle et al. (1) have previously reported that a correlation exists between the penicillin half-lives and their side-chain pKa values. H-bonding, however, may also be a contributing factor for the ampicillin acid stability in addition to the side-chain inductive effect. The H-bond formation presumably can be either intramolecular or intermolecular. The intramolecular H-bonding is probably formed by one of the ampicillin side-chain N—H bond with its neighboring carbonyl oxygen atom to form a N—H—O bond. The intermolecular H-bonding may exist as a result of interactions between a hydrated $NH_3$ group and a hydrated side-chain peptide carbonyl oxygen atom. Any of these interactions would decrease the possibility of electronic rearrangement leading to β-lactam cleavage.

It has been shown in the literature (26) that the $RNH_3^+$ group readily forms hydrogen bonds with its surrounding water molecules. Based on IR absorption spectra, Austin et al. (27) and Grant and Alburn (28) have reported that the charge amino group may be associated with water molecules to form the hydrates of ampicillin. Further investigations are needed to confirm an H-bond formation hypothesis. It is important to note that one should not be misled by assuming that a hydrated form of ampicillin is more stable in the solid state. Grant and Alburn (28) have reported that in the solid state ampicillin monohydrate is less stable than its anhydrous form, especially at elevated temperatures. Conceivably, this is due to the hydrolysis initiated by the water molecule.

## REFERENCES

(1) F. P. Doyle, J. H. C. Nayler, H. Smith, and E. R. Stove, Nature, 191, 1091(1961).
(2) G. N. Rolinson and S. Stevens, Brit. Med. J., 1961, 191.
(3) D. M. Brown and P. Acred, ibid., 1961, 197.
(4) P. Acred, D. M. Brown, D. H. Turner, and M. J. Wilson, Brit. J. Pharmacol., 18, 356(1962).
(5) J. P. Hou and J. W. Poole, unpublished data.
(6) H. D. C. Rapson and A. E. Bird, J. Pharm. Pharmacol., 15, 222T(1963).
(7) R. Brodersen, Acta Chem. Scand., 1, 403(1947).
(8) R. Brodersen, Acta Pharmacol., 3, 345(1947).
(9) M. A. Schwartz, A. P. Granatek, and F. H. Buckwalter, J. Pharm. Sci., 51, 523(1962).
(10) P. Finholt, G. Jurgensen, and H. Kristiansen, J. Pharm. Sci., 54, 387(1965).
(11) "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Eds., Princeton University Press, Princeton, N. J., 1949, pp. 415–454.
(12) M. A. Schwartz, J. Pharm. Sci., 54, 1308(1965).
(13) T. C. McIlvaine, J. Biochem., 49, 183(1921).
(14) W. M. Clark and H. A. Lubs, J. Biol. Chem., 25, 479(1916).
(15) H. A. Laitinen, "Chemical Analysis," McGraw-Hill Co., New York, N. Y., 1960, p. 35.
(16) R. G. Bates and G. D. Pinching, J. Am. Chem. Soc., 71, 1274(1949).
(17) A. Albert and E. P. Serjeant, "Ionization of Constants of Acids and Bases," Wiley, New York, N. Y., 1962, p. 151.
(18) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," 2nd ed., Wiley, New York, N. Y., 1961, p. 150.
(19) G. Scatchard, Chem. Rev., 10, 229(1932).
(20) K. B. Wiberg, "Physical Organic Chemistry," Wiley, New York, N. Y., 1964, p. 390.
(21) H. S. Harned and W. J. Hammer, J. Am. Chem. Soc., 55, 2194(1933).
(22) B. B. Levine, Arch. Biochem. Biophys., 93, 50(1961).
(23) M. A. Schwartz, J. Pharm. Sci., 54, 472(1965).
(24) F. P. Doyle, J. H. C. Nayler, and H. Smith, U. S. pat. 2,985, 648(May, 1961).
(25) A. L. Tosoni, D. G. Glass, and L. Goldsmith, Biochem. J., 69, 476(1958).
(26) R. P. Bell and J. W. Bayles, J. Chem. Soc., 1952, 1518.
(27) K. W. B. Austin, A. C. Marshall, and H. Smith, Nature, 208, 999(1965).
(28) N. H. Grant and H. E. Alburn, ibid., 207, 645(1965).