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Hydrolysis and Epimerization Kinetics of Hetacillin in Aqueous Solution

AKIRA TSUJI, YOSHITAKA ITATANI, and TSUKINAKA YAMANA ˣ

Abstract □ Methods were developed for quantitating epimerization to epihetacillin and hydrolysis to ampicillin in the alkaline degradation of hetacillin, and both rates in deuterium oxide at 35° and in water at various temperatures were determined. In each case, plots of log k for the epimerization against pH or pD yielded straight lines with a positive slope, which verified the first-order dependence on the hydroxide ion or deuteroxide ion. The activation energy of the epimerization process was 21.2 kcal/mole. In aqueous solution at high pH, epimerization rather than conversion to ampicillin represents a major pathway of hetacillin degradation, although the β-lactam ring of the hetacillin molecule is highly resistant to attack by the hydroxide ion.

Keyphrases □ Hetacillin—epimerization and hydrolysis in aqueous solution, effect of pH and temperature □ Epimerization—hetacillin in aqueous solution, effect of pH and temperature □ Hydrolysis—hetacillin in aqueous solution, effect of pH and temperature □ Antibacterials—hetacillin, epimerization and hydrolysis in aqueous solution, effect of pH and temperature

methods for quantitating epimerization of hetacillin and to determine the rates of hydrolysis and epimerization of hetacillin in aqueous basic solution by utilizing NMR and optical rotatory dispersion spectroscopy. The preliminary NMR observations were reported previously (7).

EXPERIMENTAL

Materials—Hetacillin potassium[1] and ampicillin sodium[2] were used as supplied.

Epihetacillin was synthesized by a reported procedure (4). Two grams of hetacillin potassium was placed in 20 ml of distilled water, and the

Hetacillin, which was developed to improve the aqueous stability of concentrated ampicillin solutions and to increase oral absorption of ampicillin, is a condensation product of ampicillin and acetone (1) and is often used instead of ampicillin.

In the degradation of hetacillin (I) in aqueous solution, at least two reactions are possible: hydrolytic interconversion to ampicillin (II) (2, 3) and epimerization to 6-epihetacillin (III) (4), which produces inactive 6-epiampicillin (5). The rates of both hydrolysis (2, 3, 6) and epimerization (7) depend on pH and occur simultaneously (Scheme I). Although detailed kinetics of the hydrolysis of hetacillin to ampicillin were reported (2, 3, 6, 8–10), much less is known about the kinetic behavior of the C-6 epimerization reaction.
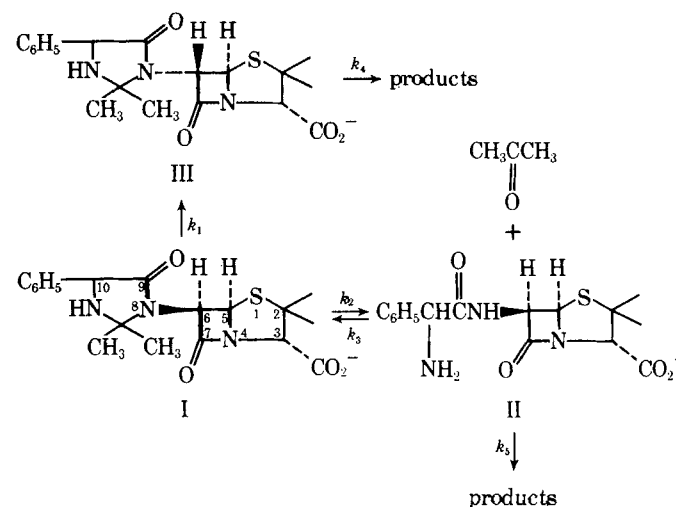
The present investigation was undertaken to develop

Scheme I

[1] Banyu Pharmaceutical Co., Tokyo, Japan.
[2] Takeda Chemical Ind., Osaka, Japan.

Table I—Chemical Shifts[a] (Parts per Million) of Protons of Hetacillin, Epihetacillin, and Ampicillin at 35°

Penicillin	Solvent	Phenyl	C-10	C-6	C-5	C-3	CCH₃			
Hetacillin potassium	Deuterium oxide[b]	7.48 m	4.76	5.08 d (4.0)	5.58 d (4.0)	4.30	1.67	1.55	1.51	1.46
Epihetacillin	Deuterium oxide[b]	7.44 m	4.85	4.74 d (2.0)	5.53 d (2.0)	4.39	1.61	1.56	1.52	1.48
	Dimethyl sulfoxide-d₆[c,d]	7.40 m	4.68	4.58 d (2.0)	5.38 d (2.0)	4.41	1.54	1.45	1.40	1.38
Ampicillin sodium	Deuterium oxide[b,e]	7.40	—[f]	5.46 s	5.46 s	4.19	1.51	1.47	—	—

[a] J values in Hertz in parentheses. [b] Relative to sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionate as internal standard. [c] Relative to tetramethylsilane as internal standard. [d] Assignments from Ref. 4. [e] Assignments from Ref. 13. [f] Not observable under water peak.

solution pH was maintained at 11.50 for 90 min at 25° with the aid of a pH-stat. The reaction solution was acidified to pH 4.5 with 1 N HCl at 0° to precipitate epihetacillin as a white powder. The collected crystals were washed exhaustively with cold water and dried over phosphorus pentoxide, mp 163° [lit. (4) mp 164–165°]. The identity was confirmed by elemental analysis, IR and NMR spectroscopy, and TLC.

Deuterium oxide (99.75%)[3], deuterium chloride (20% in deuterium oxide)[3], and sodium deuteroxide (40% in deuterium oxide)[3] were used for the preparation of the deuterium solvent buffer. All other chemicals were analytical reagent grade.

Apparatus—NMR spectra were recorded on a 100-MHz spectrometer[4] operating in a field sweep mode. Optical rotatory dispersion measurements were made on a recording spectropolarimeter[5], using thermostated 1-cm cells. The apparent pH values of the reaction solution were measured using microelectrodes and a pH meter[6], both before and at the end of the reaction. When no buffer or a low buffer capacity was used, a constant pH was maintained during the reaction by a pH-stat[7]. The pD values in deuterium solvent were obtained by proper correction (11) to the pH meter reading.

Kinetic Procedure—*NMR Study*—Hetacillin potassium (80 mg) was dissolved in 2 ml of deuterium oxide buffer at 35°. The time was recorded when the pH of the reaction buffer was corrected to the desired pD values by addition of 1 N NaOD by a pH-stat. As soon as possible after the pH adjustment, 1 ml of the reaction solution was placed in the NMR sample tube and left in the probe of the instrument[4].

The spectra were recorded at suitable time intervals, with sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)propionate as the internal reference. The temperature was maintained at 35 ± 0.5° by a variable-temperature probe unit[4]. The NMR spectra of the phenyl groups and C-3 protons were recorded with a twofold-scale expansion, and their signals were integrated on a spectrometer. The same procedure was used for epihetacillin and ampicillin sodium.

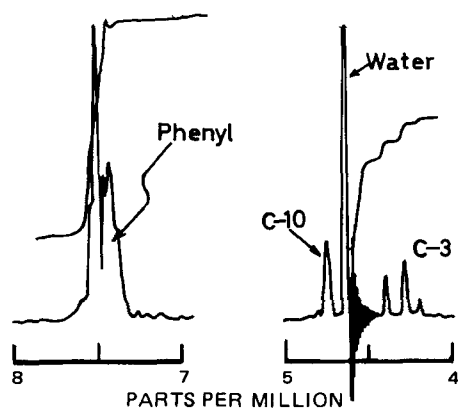*Optical Rotatory Dispersion Study*—The reaction was initiated by

Figure 1—*NMR spectrum (100 MHz) of a synthetic mixture of hetacillin (50%), epihetacillin (40%), and ampicillin (10%) in 0.2 M NaDCO₃–Na₂CO₃ buffer at pD 10.6.*

dissolving exactly weighed hetacillin potassium or epihetacillin into a buffer preheated to a suitable temperature (±0.05°) to give a final concentration of 6×10^{-4} M. The 3 ml was replaced in a cell, which was thermally equilibrated (±0.05°) in the thermostated compartment of the spectrometer, and the optical rotatory dispersion change at 240 nm as the function of time was recorded. The reactions in deuterium solvent were also carried out in a similar manner as in water.

The buffers used in NMR and optical rotatory dispersion studies were NaD₂PO₄–Na₂DPO₄, NaDCO₃–Na₂CO₃, Na₂DPO₄–Na₃PO₄, H₃BO₃–Na₂B₂O₇, NaHCO₃–Na₂CO₃, and Na₂HPO₄–Na₃PO₄. The ionic strength of each solution was adjusted to 0.5 by the addition of potassium chloride. The pH and pD drifts during the reaction were within ±0.02 and ±0.05 unit for optical rotatory dispersion and NMR studies, respectively.

TLC Study—TLC was also employed to identify the degradation products and to verify the NMR and optical rotatory dispersion results. Aliquots, 10 µl, of the degradation solution ($\sim 1 \times 10^{-2}$ M) were applied to silica gel G precoated glass plates[3] (5 × 20 cm) of 250-µm thickness. Chromatograms were run for 15 cm in acetone–acetic acid (95:5). Spots were visualized by spraying 0.5% aqueous permanganate reagent or by exposure of the plates to iodine vapor.

Hetacillin, epihetacillin, ampicillin, and epiampicillin showed R_f values of 0.32, 0.45, 0.13, and 0.25, respectively. TLC of the standard solutions of the penicilloic acids of ampicillin and epihetacillin, synthesized by the method similar to that described by Schwartz and Delduce (12) for benzylpenicilloic acid, revealed virtually single spots with R_f values of 0.05 and 0.17, respectively. All spots, except for epiampicillin, were detected by TLC of the degradation solution of hetacillin at high pH.

RESULTS AND DISCUSSION

Kinetic Study by NMR Spectroscopy—Table I summarizes the 100-MHz spectral data of hetacillin, epihetacillin, and ampicillin in deuterium oxide and dimethyl sulfoxide-d₆. Assignments were made on the basis of previous results (4, 13). Although the principal differences in the spectra of the two epimers were due to the characteristic protons at C-5 and C-6 ($J = 4.0$ Hz in hetacillin and $J = 2.0$ Hz in epihetacillin), these signal areas were unsuitable for monitoring the kinetics of hetacillin degradation because of considerable interference of other proton signals.

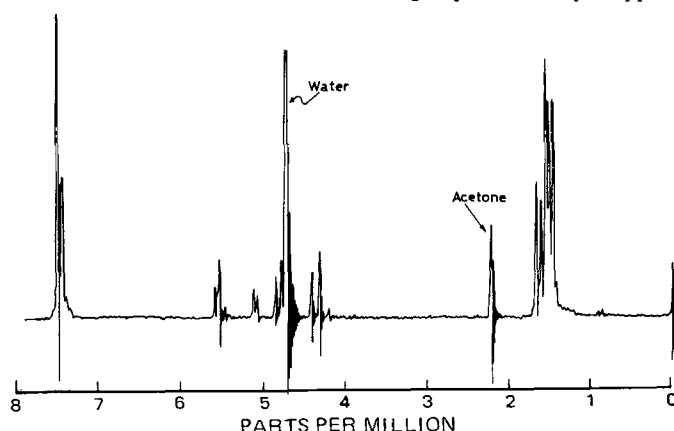The chemical shifts for the C-3 methine groups differed by 0.1 ppm

Figure 2—*NMR spectrum (100 MHz) of a reaction mixture of hetacillin after 40 min in 0.2 M NaDCO₃–Na₂CO₃ buffer at pD 10.6 and 35°.*

Table II—Recovery of Hetacillin, Epihetacillin, and Ampicillin from Synthetic Mixtures by NMR Spectroscopic Method

Sample	Quantities Added, %			Quantities Found, %[a]		
	Hetacillin	Epihetacillin	Ampicillin	Hetacillin	Epihetacillin	Ampicillin
1	95	0	5	93	0	6
2	75	0	25	75	0	25
3	50	50	0	50	52	0
4	45	55	0	44	56	0
5	50	40	10	48	42	10
6	25	60	15	26	62	15

[a] Calculated using Eq. 1.

among these β-lactam compounds and their β-lactam cleavage products and provided the best means for quantifying epimerization and also hydrolysis of hetacillin. A typical NMR spectrum of a synthetic mixture of hetacillin (50%), epihetacillin (40%), and ampicillin (10%) in 0.2 M carbonate buffer at pD 10.6 is depicted in Fig. 1. Signals due to the phenyl groups in these compounds all fell in the area of 7.40–7.50 ppm. If the total integration values for the range represent 100% of the phenyl groups present, then the amount of the three compounds may be calculated from each integrated value of the respective C-3 proton as follows:

$$\text{percent compound} = (5I/I_T) \times 100 \qquad (Eq. 1)$$

where I_T = total integral value of the phenyl groups in the area of 7.40–7.50 ppm, and I = integral value of the C-3 methine proton for hetacillin, epihetacillin, or ampicillin.

The recovery of known synthetic mixtures of the three compounds at lower pD than 10.6 was satisfactory, with standard deviation of less than ±2% (Table II). Although such tests at a more alkaline pD gave relatively poor recovery for hetacillin and epihetacillin because of rapid epimerization and for ampicillin because of facile β-lactam cleavage, each concentration in the kinetic solutions at the desired time after hetacillin dissolution in such a high alkaline buffer at the kinetic temperature might be determined reasonably by this analytical method.

NMR spectra of the degradation solution of hetacillin in alkaline deuterium oxide solution showed that each proton for hetacillin at 4.30 (C-3), 5.08 (C-6), and 4.76 (C-10) ppm decreased in intensity, accompanied by an increase and then a decrease in intensity of proton resonance at 4.39 ppm due to the C-3 proton and at 4.85 ppm due to the C-10 proton of epihetacillin and at 4.19 ppm due to the C-3 proton of ampicillin. Two signals also appeared at 3.30 and 3.52 ppm, assigned to the C-3 protons of the β-lactam ring opening products, probably the penicilloic acids, of ampicillin and epiampicillin. However, there were no detectable signals corresponding to intact epiampicillin. During the reaction processes studied, the total integrated values for the phenyl proton area were constant and gave an I_T value corresponding to five protons. The typical NMR spectrum for the degradation after 40 min in 0.2 M carbonate

buffer at pD 10.6 and 35° is illustrated in Fig. 2.

Figures 3, 4, and 5 give typical plots of the mole percents for hetacillin, epihetacillin, and ampicillin, calculated from the integral value of the C-3 proton for each compound using Eq. 1, as a function of time for the degradation of hetacillin at pD 9.8, 10.6, and 11.4, respectively. The completed curves were generated from an analog computer[8] according to Scheme I. The kinetic parameters used are listed in Table III; the rate constants, k_4 and k_5, were determined directly from the degradation of epihetacillin and ampicillin under the same conditions, and the k_3 value was assumed to be zero. In all cases, reasonably good agreement was found between the experimental values for each species and the computer-drawn curves (Figs. 3–5). These results indicate that the present NMR method is sufficiently accurate for simultaneous determination of hetacillin, epihetacillin, and ampicillin from a kinetic solution.

The reaction of epihetacillin at pD 9.8, 10.6, and 11.4 gave no detectable amount of hetacillin. This result is consistent with the previous report (5) of the irreversible conversion of hetacillin into epihetacillin. The degradation products from hetacillin in deuterium oxide at high pD were also confirmed by TLC studies to be in accordance with the NMR observations. These results strongly support the assumption that the degradation of hetacillin, in the pD ranges examined, follows the reaction route illustrated in Scheme I. The reversible reaction of ampicillin and acetone to form hetacillin gave only a negligible contribution under the present experimental conditions.

Kinetic Study by Optical Rotatory Dispersion Spectroscopy—By utilizing the large difference in optical rotatory dispersion maxima near 240 nm between hetacillin ($[\Phi]_{240\,nm} = 3.40 \times 10^3$) and epihetacillin ($[\Phi]_{238\,nm} = 2.12 \times 10^3$), Mitscher et al. (14) proposed a useful method for monitoring the kinetics of epimerization of hetacillin in aqueous solution. Loss of the 238-nm optical rotatory dispersion peak with time in glycine buffer at pH 11 and 23.8° followed a biexponential process in accordance with the following general equation (14):

$$\text{rotation} = Ae^{-\alpha t} + Be^{-\beta t} \qquad (Eq. 2)$$

Figure 3—Time course for hetacillin (O), epihetacillin (●), and ampicillin (△) determined by NMR spectroscopy during the degradation of hetacillin in 0.1 M NaDCO₃-Na₂CO₃ buffer at pD 9.8 and 35°. Curves were generated by the analog computer simulation according to Scheme I and the kinetic parameters listed in Table III.
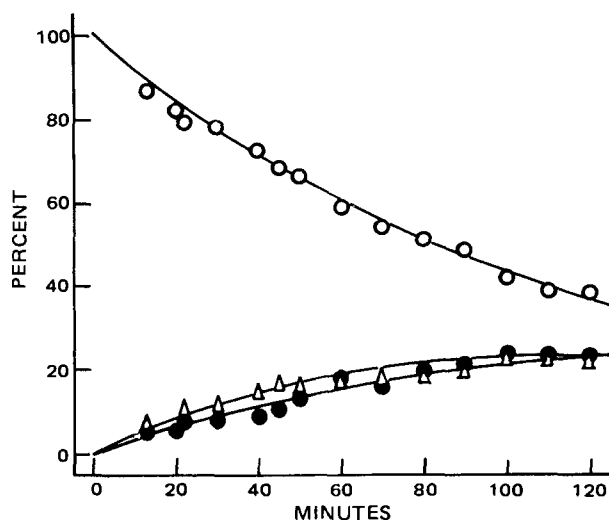

Figure 4—Time course for hetacillin (O), epihetacillin (●), and ampicillin (△) determined by NMR spectroscopy during the degradation of hetacillin in 0.2 M NaDCO₃-Na₂CO₃ buffer at pD 10.6 and 35°. Curves were generated by the analog computer simulation according to Scheme I and the kinetic parameters listed in Table III.
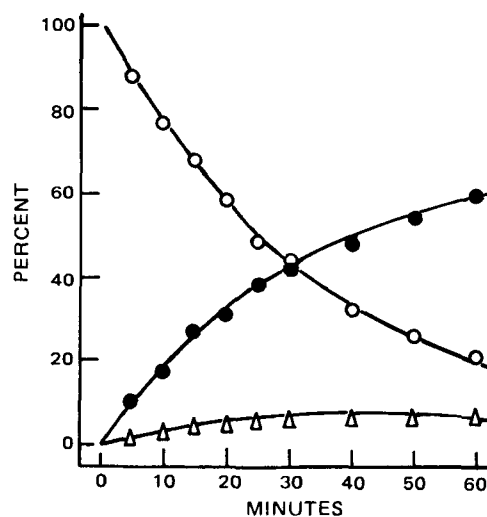
Table III—Various Rate Constants[a] for the Degradation of Hetacillin, Epihetacillin, and Ampicillin Determined by NMR and Optical Rotatory Dispersion (ORD) Spectroscopic Methods in Water and Deuterium Oxide under Various Conditions at μ = 0.5.

Buffer	Solvent	pH or pD	Temperature	k_1[b]	k_2	k_4	k_5	Analytical Method
0.1 M Phosphate	Deuterium oxide	6.0	35°	—	1.60	—	—	NMR
0.1 M Phosphate	Deuterium oxide	6.0	35°	—	—	0.53	—	NMR
0.1 M Borate	Water	9.7	35°	0.88	—	0.08	—	ORD
0.1 M Carbonate	Deuterium oxide	9.8	35°	0.20	0.30	0.10	0.42	NMR
0.1 M Carbonate	Water	9.9	35°	1.10	—	0.09	—	ORD
0.1 M Carbonate	Water	10.3	35°	1.80	—	0.12	—	ORD
0.2 M Carbonate	Deuterium oxide	10.6	35°	1.30	0.30	0.11	1.20	NMR
0.2 M Carbonate	Deuterium oxide	10.6	35°	1.55	—	0.10	—	ORD
0.1 M Carbonate	Water	10.7	35°	3.30	—	0.14	—	ORD
0.1 M Phosphate	Water	11.1	35°	7.18	—	0.20	—	ORD
0.1 M Phosphate	Deuterium oxide	11.4	35°	8.00	0.30	0.12	7.00	NMR
0.1 M Phosphate	Deuterium oxide	11.4	35°	8.38	—	0.10	—	ORD
0.025 M Phosphate	Water	11.4	35°	22.3	—	0.25	—	ORD
0.05 M Phosphate	Water	11.4	35°	22.0	—	0.27	—	ORD
0.1 M Phosphate	Water	11.4	35°	22.3	—	0.38	—	ORD
Sodium hydroxide	Water	11.4	35°	22.1	—	0.23	—	ORD
Sodium hydroxide	Water	11.6	35°	—	—	0.27	—	ORD
Sodium hydroxide	Water	12.0	35°	—	—	0.65	—	ORD
Sodium hydroxide	Water	12.3	35°	—	—	1.54	—	ORD
Sodium deuteroxide	Deuterium oxide	12.4	35°	—	—	0.69	—	NMR
Sodium deuteroxide	Deuterium oxide	12.4	35°	—	—	0.65	—	ORD
Sodium hydroxide	Water	12.5	35°	—	—	1.65	—	ORD
0.1 M Phosphate	Water	11.0	30°	2.80	—	0.08	—	ORD
0.1 M Phosphate	Water	11.4	30°	7.90	—	0.15	—	ORD
0.1 M Phosphate	Water	11.5	30°	11.0	—	0.16	—	ORD
0.1 M Phosphate	Water	11.2	25°	2.00	—	0.05	—	ORD
0.1 M Phosphate	Water	11.4	25°	4.20	—	0.08	—	ORD
0.1 M Phosphate	Water	11.6	25°	5.90	—	0.09	—	ORD

[a] Rate constants are defined in Scheme I. [b] The value was obtained from the assumption that $(k_1 + k_2)$ values determined by the optical rotatory dispersion method would be almost equal to k_1 values (see text).

where A, B, α, and β are constants. Although Mitscher et al. (14) suggested that the results of rotation change reflect epimerization followed by hydrolysis, theoretical implications of Eq. 2 or detailed kinetics of epimerization of hetacillin by means of the optical rotatory dispersion method have not been reported.

The rotation at any time during the reaction of hetacillin, R_t, may be the sum of the rotation of the individual species of hetacillin (I), ampicillin (II), epihetacillin (III), epiampicillin (IV), and penicilloic acids of the last three compounds, V–VII, as follows:

$$R_t = \Phi_I[I] + \Phi_{II}[II] + \Phi_{III}[III] + \Phi_{IV}[IV] + \Phi_V[V] + \Phi_{VI}[VI] + \Phi_{VII}[VII] \quad (Eq. 3)$$

where Φ_i refers to the molar rotation coefficient of the ith species. In aqueous solution at pH >10, the amount of intact β-lactam compounds

II and IV may be negligible because of their slow formation rate and the fast degradation rate of the β-lactam rings. These speculations were confirmed using TLC for the degradation of hetacillin at pH >10 and 35°. Equation 3 can be reduced to:

$$R_t = \Phi_I[I] + \Phi_{III}[III] + \Phi_V[V] + \Phi_{VI}[VI] + \Phi_{VII}[VII] \quad (Eq. 4)$$

and the following approximate relationship can be derived:

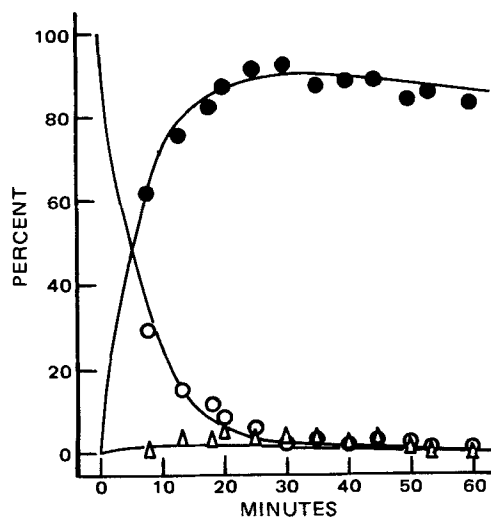$$[V] + [VI] + [VII] = C_0 - ([I] + [III]) \quad (Eq. 5)$$

Figure 5—Time course for hetacillin (O), epihetacillin (●), and ampicillin (△) determined by NMR spectroscopy during the degradation of hetacillin in 0.1 M Na_2DPO_4–Na_3PO_4 buffer at pD 11.4 and 35°. Curves were generated by the analog computer simulation according to Scheme I and the kinetic parameters listed in Table III.
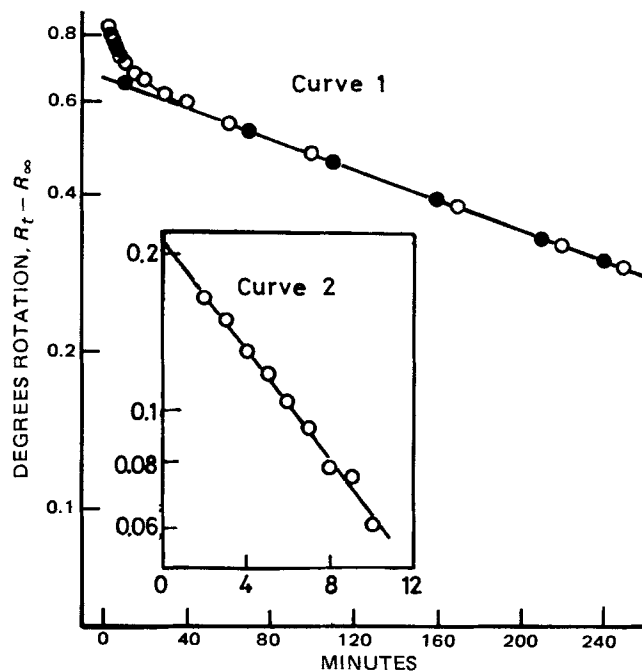

Figure 6—First-order plot for the decrease in optical rotatory dispersion at 240 nm for the degradation of hetacillin (O, 6 × 10⁻⁴ M) and epihetacillin (●, 6 × 10⁻⁴ M) in 0.1 M Na_2HPO_4–Na_3PO_4 buffer at pH 11.1 and 35°. Curve 2 is a first-order plot for epimerization and hydrolysis.
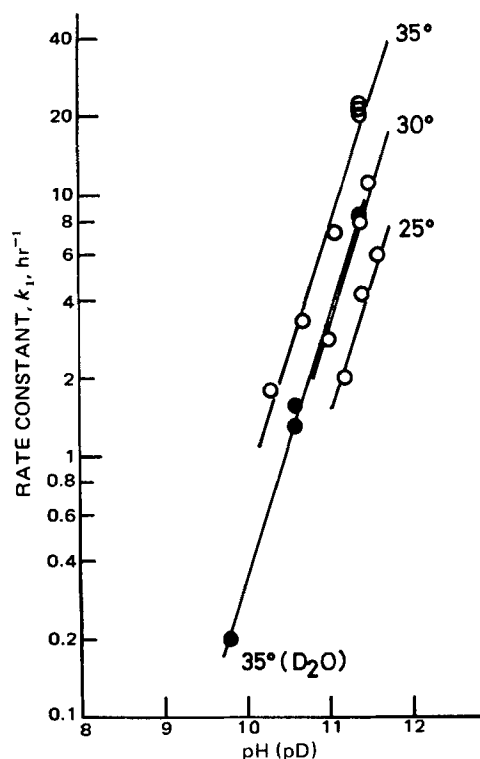
Figure 7—*Log* k-*pH (pD) profiles for the epimerization of hetacillin in water* (O) *at various temperatures and in deuterium oxide* (●) *at 35°.*

where C_0 represents the initial concentration of hetacillin. Results from the degradation of epihetacillin and ampicillin at pH >10 gave, within the experimental error, an approximate and simple relationship at 240 nm:

$$\Phi_V C_0 = \Phi_{VI} C_0 = \Phi_{VII} C_0 = R_\infty \qquad \text{(Eq. 6)}$$

Since the reversed reaction from ampicillin and acetone to hetacillin may be negligible in a sufficiently dilute solution of hetacillin, the time dependence of [I] and [III] can be given by the following equations from Scheme I:

$$[\text{I}] = C_0 e^{-(k_1+k_2)t} \qquad \text{(Eq. 7)}$$

$$[\text{III}] = \frac{k_1}{(k_1 + k_2) - k_4} C_0 [e^{-k_4 t} - e^{-(k_1+k_2)t}] \qquad \text{(Eq. 8)}$$

These specific situations in a highly basic solution can lead to Eq. 9 for the R_t change at 240 nm:

$$R_t - R_\infty = Ae^{-(k_1+k_2)t} + Be^{-k_4 t} \qquad \text{(Eq. 9)}$$

where:

$$A = (\Phi_I C_0 - R_\infty) - (\Phi_{III} C_0 - R_\infty)\frac{k_1}{(k_1 + k_2) - k_4} \qquad \text{(Eq. 10)}$$

$$B = (\Phi_{III} C_0 - R_\infty)\frac{k_1}{(k_1 + k_2) - k_4} \qquad \text{(Eq. 11)}$$

Equation 9 is essentially in agreement with that found by Mitscher *et al.* (14).

Figure 6 shows a typical plot of log $(R_t - R_\infty)$ *versus* time for the degradation of hetacillin and epihetacillin in 0.1 M phosphate buffer ($\mu = 0.5$) at pH 11.1 and 35°. Since $k_1 + k_2$ is faster than k_4, $e^{-(k_1+k_2)t}$ ap-

Table IV—Epimerization Rate Constants of Hetacillin at Various Temperatures and $\mu = 0.5$

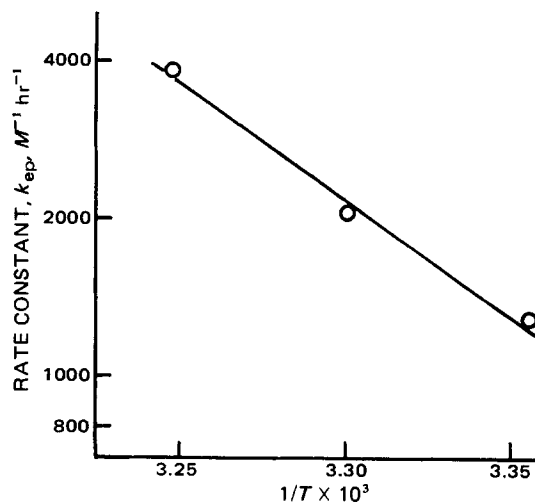| Solvent	Temperature	k_{ep}, M^{-1} hr^{-1}
Water	25°	1.29×10^3
Water	30°	2.05×10^3
Water	35°	3.84×10^3
Deuterium oxide	35°	1.04×10^4

Figure 8—*Arrhenius plot of the second-order epimerization rate constant,* k$_{ep}$, *of hetacillin.*

proaches zero at some time point. There will remain a term in $e^{-k_4 t}$, and the plot of log $(R_t - R_\infty)$ *versus* time will be linear with a slope of $-k_4/2.303$. This linear portion of the curve may be extrapolated to zero time, and the value of $(R_t - R_\infty)$ along this portion of the line represents the contribution of the term in $e^{-k_4 t}$ to the overall value. Thus, subtraction of this term from the observed $(R_t - R_\infty)$ leaves only the term $e^{-(k_1+k_2)t}$. Then a plot of the logarithm of these residuals against time should be linear with a slope of $-(k_1 + k_2)/2.303$ (curve 2 in Fig. 6). The linear portions corresponding to $Be^{-k_4 t}$ were essentially identical at pH > 10 to the portion for epihetacillin itself, obtained from independent measurements at the same concentration under the same conditions as shown in Fig. 6, indicating the validity of Eq. 9 and suggesting the relation of $k_1 \gg k_2, k_4$.

Calculation of each value of k_1 and k_2 is theoretically possible from the intercept value of A or B. However, when taking into account the experimental error and large contribution of k_1 in the $(k_1 + k_2)$ value, it seems reasonable to assume for the data at pH > 10 that the $(k_1 + k_2)$ values thus determined are approximately equal to the epimerization rate constant, k_1. These rate constants are summarized in Table III. The k_1 and k_4 values determined at pD 10.6 and 11.4 by optical rotatory dispersion spectroscopy are in satisfactory agreement with those determined by NMR spectroscopy.

The results in phosphate buffer and sodium hydroxide solution at pH 11.4 (Table III) revealed that the apparent epimerization rate constants, k_1, were almost independent of the buffer concentration used. Since the C-6 epimerization of penicillin is known to proceed by general base catalysis (15, 16), the present results indicate that the general base-catalyzed epimerization rate of hetacillin may be negligible compared with the hydroxide-ion-catalyzed process in the experimental pH range.

pH–Rate Profile and Temperature Dependence for Epimerization of Hetacillin—Plots of log k_1 against pH (pH > 10) or pD for various temperatures in water and in deuterium oxide are illustrated in Fig. 7 and yielded straight lines with a slope of unity in each case, verifying a first-order dependence on the hydroxide ion. The rate law may be written as:

$$k_1 = k_{ep} a_{OH^-} \qquad \text{(Eq. 12)}$$

$$k_1 = k_{ep}(a_{H^+}/K_w) \qquad \text{(Eq. 13)}$$

where k_{ep} represents the second-order rate constant for hydroxide-ion-catalyzed epimerization; and a_{OH^-} and a_{H^+} refer to the activity of the hydroxide ion and hydrogen ion, respectively, which were calculated from pH measurements and the autoprotolytic constant in water (17) and deuterium oxide (18). The rate constants, k_{ep}, consistent with the experimental results in water and deuterium oxide are shown in Table IV.

Since the epimerization of hetacillin was suggested (5, 15) to occur through a rate-limiting formation of an enolate intermediate resulting from the proton subtraction of the acidic C-6 proton by a hydroxide ion, the ratio $k_{ep}^{D_2O}/k_{ep}^{H_2O} = 2.7$ may be attributed to the difference in base strength between deuteroxide and hydroxide ions. Subsequent reprotonation gives the thermodynamically more stable epihetacillin; then epimerization proceeds irreversibly, as noted previously (5).

An Arrhenius plot for k_{ep} is illustrated in Fig. 8. By applying the method of least squares to the plots, the energy of activation for epimerization to epihetacillin was 21.2 kcal/mole.

In plots of log k_1 *versus* pH at 35° in water, slight positive deviation from the first-order dependence on the hydroxide ion was observed at alkaline pH < 10 (not presented in Fig. 7). This result may have been due to the considerable contribution of the hydrolysis rate, k_2, to the k_1 value estimated from the optical rotatory dispersion method. This result would occur because of the pH-dependent degradation behavior of hetacillin in which the epimerization process is a major pathway in a highly basic solution, whereas hydrolysis to ampicillin becomes increasingly significant in medium basic and neutral solutions (2, 3, 6).

No significant β-lactam cleavage reaction probably would take place in hetacillin itself, since the disappearance of hetacillin was virtually accompanied, even in highly basic solutions, with two competing reactions, the formation of epihetacillin (k_1 reaction) and the conversion to ampicillin (k_2 reaction), as verified by quantitative NMR studies (Figs. 3–5). The extreme stability of the β-lactam in the hetacillin molecule was also revealed in epihetacillin degradation. The k_4 reaction concerned with the disappearance of epihetacillin is controlled by hydrolysis of the β-lactam ring and conversion to epiampicillin. The pH dependency of k_4 at 35° (Table III) indicates that the hydroxide-ion-catalyzed hydrolysis of the β-lactam moiety of epihetacillin is predominant above pH 11.5, and conversion to epiampicillin seems to be significant below this pH.

The second-order rate constant for the β-lactam hydrolysis of epihetacillin was calculated to be 65.8 M^{-1} hr^{-1} at 35°, one-twentieth of the value for the hydrolysis of ampicillin β-lactam (19). The remarkable stability of both β-lactam rings of hetacillin and its epimer may be attributed to the steric hindrance of *gem*-dimethyl groups of the imidazolidine rings toward the attack by a hydroxide ion.

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Reactions of Benzenesulfonohydrazides and Benzenesulfonamides with Hydrogen Chloride or Hydrogen Bromide in Acetic Acid

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Abstract □ Benzenesulfonohydrazides capable of yielding a sulfinic acid intermediate by virtue of a basic nitrogen atom in the second position of the hydrazide moiety produced thiosulfonates when treated with 1 *N* hydrogen chloride in acetic acid and produced disulfides when treated with 1 *N* hydrogen bromide in the same solvent. In two cases, a crystalline mixture of *p*-nitrophenyl *p*-nitrobenzenethiosulfonate and bis(*p*-nitrophenyl) disulfide was isolated from the hydrogen chloride reactions. No reaction product was obtained from either the hydrogen chloride or hydrogen bromide reaction with benzenesulfonohydrazides that were unable to form a sulfinic acid intermediate. Reduction of benzenesulfonamides to disulfides appeared to be possible only with hydrogen bromide in acetic acid. No thiosulfonate was isolated from the treatments of benzenesulfonamides with 1 *N* hydrogen chloride in acetic acid. *p*-

Nitrophenyl *p*-nitrobenzenethiosulfonate and *p*-bromophenyl *p*-bromobenzenethiosulfonate exhibited some antimicrobial activities against Gram-positive bacteria. The latter compound also showed analgesic properties in the phenylquinone test.

Keyphrases □ Benzenesulfonohydrazides and benzenesulfonamides —reaction with hydrogen chloride or bromide in acetic acid, pharmacological activity of thiosulfonates formed □ Thiosulfonates—formed by reaction of benzenesulfonohydrazides and benzenesulfonamides with hydrogen chloride in acetic acid, pharmacological activity screened □ Antimicrobial activity—screened in thiosulfonates formed by reaction of benzenesulfonohydrazides and benzenesulfonamides with hydrogen chloride in acetic acid

While preparing some potential antimicrobial unsymmetrical piperazine compounds, it was necessary to synthesize 1-(*p*-acetamidobenzenesulfonamido)piperazine from 1-(*p*-acetamidobenzenesulfonamido)-4-ethoxycarbonylpiperazine (Ia, Table I). Removal of ethoxycarbonyl groups of piperazine derivatives is usually accomplished by either acidic or alkaline hydrolysis (1, 2).

A nonhydrolytic method utilizing dry hydrogen bromide