

**Kinetic Approach to the Development in  $\beta$ -Lactam Antibiotics. II.<sup>1)</sup>  
Prodrug. (1). Simultaneous Determination of Hetacillin and  
Ampicillin, and Its Application to the Stability of  
Hetacillin in Aqueous Solution<sup>2)</sup>**

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(Received March 1, 1974)

An analytical procedure for the simultaneous determination of hetacillin and ampicillin was developed by use of iodometric titration method. The method is shown to yield accurate results in the analysis of synthetic mixtures of both antibiotics. The hydrolytic behavior of hetacillin at low initial concentration was studied over a wide pH range at 35° and  $\mu=0.5$  by the proposed analytical method, the colorimetric determination of acetone and by a thin-layer chromatography.

The hydrolysis of hetacillin in the range of pH 0—9.5 undergoes to form ampicillin and acetone, while that in high alkaline region above pH 9.5 was found to be relatively complex and to produce simultaneously both ampicillin and epihetacillin. The log  $k_f$ -pH profile for hetacillin disappearance gave a bell-shaped curve with a maximum rate constant ( $k_f \approx 2 \text{ hr}^{-1}$ ) near pH 6. The activation energies for  $k_f$  were evaluated to be 23.8 and 16.7 kcal/mole at pH 6.60 and pH 9.10, respectively.

The comparative stabilities of hetacillin and ampicillin depending upon their initial concentrations were also examined at pH 6.60 and pH 9.10. By means of an analog computer-simulation, a proposed scheme taken into account the concentration dependency of both ampicillin hydrolysis and hetacillin-ampicillin equilibrium was found to be sufficient to interpret the experimental data. Although time for 10% loss in activity (utilization time) for hetacillin is much close to that for ampicillin at 35° because of the rapid conversion to ampicillin, relatively large temperature dependency on  $k_f$  produced a remarkable prolonged effect on the utilization time for concentrated hetacillin solution at 25°.

Hetacillin is a prodrug of ampicillin. This drug is the condensation product resulting from the reaction between ampicillin and acetone.<sup>4)</sup> Superior chemical<sup>5)</sup> and therapeutical<sup>6)</sup> properties of hetacillin compared with ampicillin have been discussed. Several investigators,<sup>7-10)</sup> however, have reported that hetacillin does not offer any therapeutic advantage over ampicillin because of its rapid conversion to ampicillin *in vivo*.

Hetacillin hydrolyzes in aqueous solution into ampicillin and acetone.<sup>7-9)</sup> For studies related to hetacillin hydrolysis, it is desirable to develop a method for the simultaneous deter-

- 1) Part I in this series: T. Yamana, A. Tsuji, and Y. Mizukami, *Chem. Pharm. Bull.* (Tokyo), **22**, 1186 (1974).
- 2) Part of this work was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.
- 3) Location: 13-1 Takara-machi, Kanazawa 920, Japan.
- 4) G.A. Hardcastle, Jr., D.A. Johnson, C.A. Panetta, A.I. Scott, and S.A. Sutherland, *J. Org. Chem.*, **31**, 897 (1966).
- 5) M.A. Schwartz and W.L. Hayton, *J. Pharm. Sci.*, **61**, 906 (1972).
- 6) W.M.M. Kirby and A.C. Kind, *Ann. N. Y. Acad. Sci.*, **145**, 291 (1967).
- 7) J.T. Smith and J.M.T. Hamilton-Miller, *Chemotherapy*, **15**, 366 (1970).
- 8) L. Magni, B. Örtengren, B. Sjöberg, and S. Wahlqvist, *Scand. J. Invest.*, **20**, 195 (1967).
- 9) R. Sutherland and O.P.W. Robinson, *British. Med. J.*, **24**, 804 (1967).
- 10) For recent reviews, see a) G.N. Rolinson and R. Sutherland, in "Advances in Pharmacology and Chemotherapy," Vol. 11, ed. by S. Garattini, A. Goldin, F. Hawking, and I.J. Kopin, New York, 1973, p. 151; b) R.E. Notari, *J. Pharm. Sci.*, **62**, 865 (1973).

mination of hetacillin and ampicillin. Regarding the assay of the intact hetacillin, the following two methods based on the product analysis have been employed in the past. These utilize techniques such as the colorimetric determination of either free acetone by 2,4-dinitrophenylhydrazine<sup>7,8)</sup> or formed ampicillin by hydroxamic acid assay.<sup>7,9)</sup> The latter procedure was based on the fact that  $\beta$ -lactam ring in hetacillin is almost unreactive with hydroxylamine. From these product analyses only, it is impossible to elucidate precisely the hydrolytic behavior of hetacillin.

The first purpose in this paper is to establish a precise and convenient method for the simultaneous determination of hetacillin and ampicillin. Differentiation of the two compounds may be accomplished by taking advantage of the presumed stability of hetacillin toward nucleophiles such as hydroxylamine. The iodometric titration method can serve this purpose.

The second purpose is to investigate the hydrolytic behavior of hetacillin in the whole pH range and relative stabilities of hetacillin and ampicillin in aqueous solutions. Recently, Schwartz and Hyton<sup>5)</sup> have discussed the utilization time<sup>11)</sup> of both antibiotics depending on their initial concentrations by the computer-simulated analysis of kinetic data available in literature.<sup>7,8)</sup> Their predictions, however, lack experimental confirmation.

### Experimental

**Materials**—Crystalline potassium hetacillin and sodium ampicillin used were kindly supplied by Banyu Pharm. Co., Ltd. and Takeda Chemical Ind. Ltd., respectively. Epihetacillin was prepared according to the procedure reported by Johnson, *et al.*<sup>12)</sup> All other chemicals were of reagent grade.

**Analytical Method**—Hetacillin and ampicillin can be determined simultaneously in a mixture of these two antibiotics by the iodometric titration method: From reaction mixture (total penicillin  $5 \times 10^{-3}\text{M}$ ), two samples of 2 ml are pipetted into separate conical flasks. To these samples, 5 ml of 1N NaOH are added. After standing the respective flasks for 5 min and 60 min at room temperature, 5 ml of 0.2M phthalate buffer solution of pH 4.5, 5 ml of 1N HCl and 10 ml of 0.01N iodine are added in each flask. These flasks are kept for 20 min in darkness at room temperature. Excess of iodine is titrated with 0.01N sodium thiosulfate. The amounts of  $a_5$  ml and  $a_{60}$  ml of 0.01N sodium thiosulfate are consumed respectively, where subscripts, 5 and 60, refer to the reaction time treated with 1N NaOH. A 3rd 2 ml sample of the reaction mixture was treated with 5 ml of phthalate buffer solution of pH 4.5 and 10 ml of 0.01N iodine for 20 min in darkness at room temperature. The mixture is titrated with 0.01N sodium thiosulfate. Thus, the amount of  $b$  ml of 0.01N sodium thiosulfate is consumed.

The amounts of  $(b-a_5)$  ml and  $(b-a_{60})$  ml can be used to calculate the concentrations of hetacillin and ampicillin as will be described in the latter section.

**Kinetic Procedure**—Exactly weighed hetacillin or ampicillin was dissolved in the buffer solution which had previously been brought to the desired temperature (usually to  $35^\circ \pm 0.1^\circ$ ). Unless otherwise stated, the initial concentration of penicillins was  $5 \times 10^{-3}\text{M}$ . At intervals, samples were withdrawn, cooled, diluted with  $\text{H}_2\text{O}$ , if necessary, and assayed for intact hetacillin and/or ampicillin.

The buffer solutions were the same mixtures as used in an earlier paper.<sup>1)</sup> Ionic strength of the buffers was brought to 0.5 by the addition of potassium chloride. The pH of buffers was measured at the experimental temperature with a Radiometer model PHM 26 pH meter, and those of HCl and NaOH solutions at  $35^\circ$  and  $\mu=0.5$  were determined by calculations.<sup>1)</sup> In the experiments when a higher initial concentration of the antibiotics was used, the pH of the solution was maintained by a Radiometer TTT-2 pH-stat.

**Data Analysis by Analog Computation**—The amounts of hetacillin and/or ampicillin were generated by the analog computer programmed with the appropriate reaction scheme. The analog computer used was a Hitachi ALS-250.

**Product Analysis**—a) Thin-Layer Chromatography (TLC): The hydrolysis of hetacillin was monitored periodically by TLC as follows. Aliquots of hydrolysis solution ( $1 \times 10^{-2}\text{M}$ ), 10  $\mu\text{l}$ , were applied onto silica gel G plates ( $5 \times 20$  cm) of 250  $\mu$  thickness. Chromatograms were run 15 cm with a solvent system consisting of acetone-acetic acid (95:5). Spots appeared by use of 0.5% aqueous permanganate reagent and by exposure of the plates to iodine vapor.

b) Determination of Acetone: At various time intervals, aliquots (3 ml) of the hydrolysis solution of hetacillin ( $5 \times 10^{-3}\text{M}$ ) were withdrawn and analyzed for free acetone by the method of Greenberg and Lester<sup>13)</sup> as slightly modified in this laboratory.

11) "Utilization time" indicates the period for 10% loss of total penicillin activity.

12) D.A. Johnson, D. Mania, C.A. Panetta, and H.H. Silvestri, *Tetrahedron Letters*, 1968, 1903.

13) L.A. Greenberg and D. Lester, *J. Biol. Chem.*, 154, 177 (1944).

## Result and Discussion

### Simultaneous Determination of Hetacillin and Ampicillin in Mixture

1) **Calibration Curve**—Solution containing known amount of hetacillin and ampicillin was assayed according to the analytical method. Figure 1 shows the typical calibration curves. In any case, the value of the slope,  $S$ , of the calibration curve for ampicillin was found to be almost independent of the reaction time with sodium hydroxide, whereas that for hetacillin showed an apparent dependence on the reaction time up to *ca.* 60 min at room temperature. These situations are expressed in Fig. 2 with plots of  $S$  values *vs.* the reaction time. In the present analytical procedure, 5 min and 60 min as the reaction time were employed because of convenience in operation.

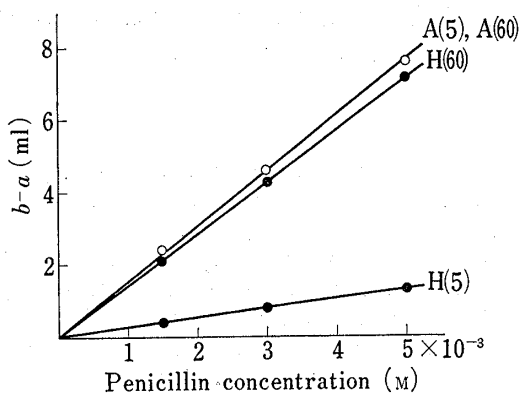


Fig. 1. Typical Calibration Curves for Hetacillin (H) and Ampicillin (A)

The parenthetical values refer to the reaction time (min) with sodium hydroxide at room temperature. These antibiotics were dissolved in 0.2M phosphate buffer of pH 6.60 and the solution was analyzed.

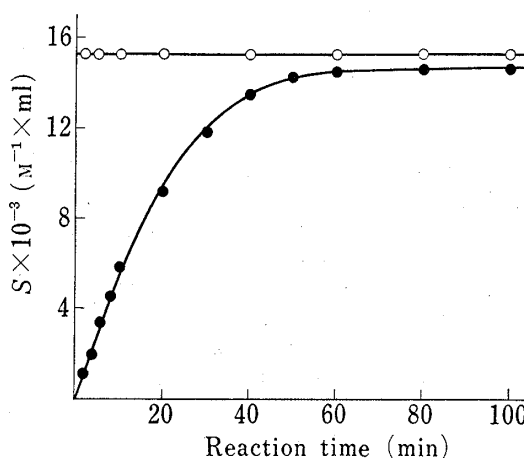


Fig. 2. Relationship between the Slope ( $S$ ) of the Calibration Curve for Hetacillin (●) or Ampicillin (○) and Reaction Time with Sodium Hydroxide

2) **Recovery Experiment**—Mixtures of hetacillin ( $C_H$ , M) and ampicillin ( $C_A$ , M) were prepared so that varying concentrations of each existed with total concentration being  $5 \times 10^{-3}$  M in a buffer solution. When aliquots of the resultant solutions were analyzed in the proposed manner, the following simultaneous equations are valid:

$$b - a_5 = S_5^H \cdot C_H + S_5^A \cdot C_A \quad (1)$$

and 
$$b - a_{60} = S_{60}^H \cdot C_H + S_{60}^A \cdot C_A \quad (2)$$

where symbols for  $S$ ,  $H$  and  $A$ , refer to hetacillin and ampicillin and the subscripts, 5 and 60, are the reaction time (min) treated with sodium hydroxide. Because of  $S_5^A = S_{60}^A = S^A$ , each concentration can be calculated from

$$C_H = \frac{a_{60} - a_5}{S_{60}^H - S_5^H} \quad (3)$$

and 
$$C_A = \frac{S_{60}^H(b - a_5) - S_5^H(b - a_{60})}{S^A(S_{60}^H - S_5^H)} \quad (4)$$

The results obtained under three different conditions are recorded in Table I and show that the method is accurate with standard deviation 1.7%. The proposed method can be very useful for stability study of hetacillin in aqueous solution.

TABLE I. Recovery of Hetacillin and Ampicillin from Synthetic Mixtures of Various pH Values

pH	Quantities added		Quantities found			
	Hetacillin 10 <sup>3</sup> M	Ampicillin 10 <sup>3</sup> M	Hetacillin		Ampicillin	
			10 <sup>3</sup> M	% recovery	10 <sup>3</sup> M	% recovery
1.38 <sup>a)</sup>	3.50	1.50	3.52	100	1.52	101
	3.00	2.00	3.00	100	1.95	97.5
	2.50	2.50	2.44	97.6	2.48	99.2
	2.00	3.00	2.01	101	3.05	102
	1.50	3.50	1.48	98.3	3.51	100
	1.00	4.00	1.01	101	4.06	102
6.60 <sup>b)</sup>	4.00	1.00	4.05	102	1.02	102
	3.50	1.50	3.51	100	1.51	101
	2.50	2.50	2.53	101	2.51	100
9.10 <sup>c)</sup>	4.00	1.00	3.88	97.0	1.02	102
	3.00	2.00	2.99	99.7	1.98	99.0
	1.00	4.00	1.03	103	3.86	96.5
			average	100.1		100.2
			SD	1.68		1.77

a) determined in 0.05N HCl

b) determined in 0.2M phosphate buffer

c) determined in 0.2M carbonate buffer

### Hydrolytic Pathway of Hetacillin

The hydrolytic behavior of hetacillin at low concentration ( $5 \times 10^{-3} \text{M}$ ) was investigated over a wide pH range at  $35^\circ$  and  $\mu=0.5$ . The formation of ampicillin and acetone in the hetacillin hydrolysis was confirmed by TLC technique and by the colorimetric determination, respectively. In Fig. 3 the time-courses of the relevant species are typically shown for pH 1.38. The concentration of the original hetacillin decreases according to first-order kinetics and liberated ampicillin rises to a maximum value of 51% after about 8 hr and then decrease in an approximately linear fashion. Since this reaction pathway is of first-order consecutive type ( $\text{H} \xrightarrow{k_f} \text{A} \rightarrow$ ), the concentration of ampicillin at time  $t$  can be calculated from the expression:

$$C_A = \frac{k_f}{k_a - k_f} (e^{-k_f t} - e^{-k_a t}) C_0 \quad (5)$$

where  $C_0$  is the initial concentration of hetacillin. The calculated amounts<sup>14)</sup> of ampicillin as a function of time are in relatively good agreement with the experimental values in the wide pH range 0–9.5 (e.g., see Fig. 3).

Recently, Durbin and Rydon<sup>15)</sup> have obtained the evidence that hetacillin and ampicillin are in equilibrium in aqueous solution. In the pH region investigated, however, equimolecular quantities ( $5 \times 10^{-3} \text{M}$ ) of ampicillin and acetone did not yield the measurable amount of hetacillin. Such a reverse reaction (with rate constant,  $k_r$ ) can be negligible under these experimental conditions.

An interesting behavior was observed in the alkaline hydrolysis of hetacillin. In the pH range around 9.5–12, the first-order plots showed apparent curvature,<sup>16)</sup> but at higher

14) The rate constants,  $k_f$  and  $k_a$ , used for the calculation were determined under the same conditions when each of the penicillins was a starting material.

15) A.K. Durbin and H.N. Rydon, *Chem. Commun.*, 1970, 1249.

16) The  $k_f$ 's were estimated from the slopes of the graph in the initial stage of these reactions.



as epihetacillin, and that the reaction above pH 12 (see Fig. 4) is consistent with the hydrolysis (with rate constant,  $k_h$ ) of epihetacillin isomerized rapidly from hetacillin. We suspect that the difference in the reactivity between hetacillin and its epimer reflects the curvature observed in the first-order plots for the data in the pH region from 9.5 to 12. These results indicate that the transformation (with rate constant,  $k_e$ ) into epihetacillin is the predominant process of hetacillin alkaline degradation rather than acetone liberation to produce ampicillin.

Consequently, the overall reaction pathway of hetacillin degradation may be represented by the scheme in Chart 1.

The effect of temperature on the rate of the hydrolysis of hetacillin was determined at two different pH values and a constant ionic strength of 0.5. Their Arrhenius plots are shown in Fig. 5. The apparent energies of activation are 23.8 and 16.7 kcal/mole in buffers of pH's 6.60 and 9.10, respectively.

### pH-Rate Profile

Figure 6 shows the pH dependency of the apparent first-order rate constants,  $k_{app}$ ,<sup>20)</sup> of hetacillin hydrolysis at 35° and  $\mu=0.5$ , and also illustrates the pH-rate profile of ampicillin hydrolysis.<sup>21)</sup>

The pH-rate profile for hetacillin represents a relatively complex and bell-shaped curve with a maximum around pH 6. In the neutral pH region between 4 and 8, since hetacillin is rapidly hydrolyzed to ampicillin with the half-life of 17–20 min at 35°, the rate of the hydrolysis is close to that of ampicillin. Below pH 3 and above pH 9, however, the stability of hetacillin is superior to that of ampicillin. For example, in aqueous solution at pH 1.0 and 35°, the half-life for the  $\beta$ -lactam hydrolysis of total penicillin is calculated to be 12.5 hr, whereas that for ampicillin is 3.5 hr.

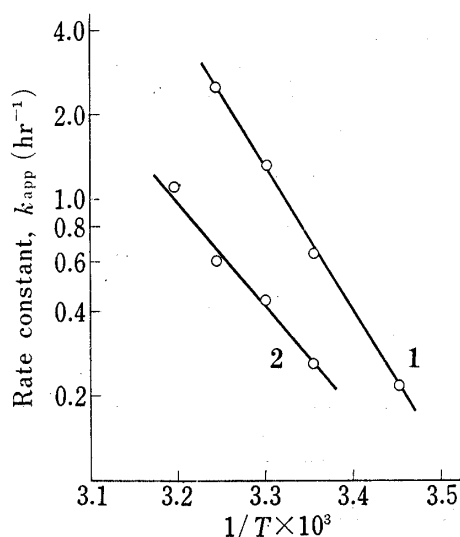


Fig. 5. Arrhenius Plots of Apparent First-Order Rate Constants,  $k_{app}$ , for the Hydrolysis of Hetacillin at Different pH Values and  $\mu=0.5$

1: in 0.2M phosphate buffer of pH 6.60  
2: in 0.2M carbonate buffer of pH 9.10

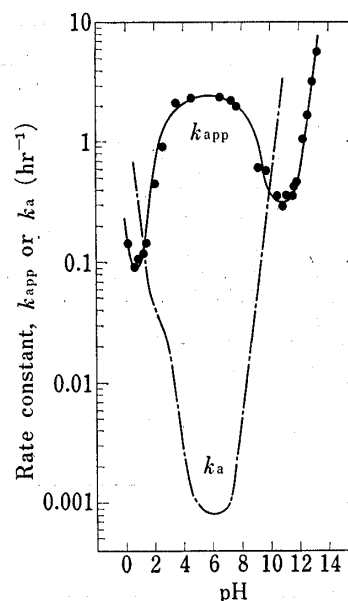


Fig. 6.  $\log k$ -pH Profile for the Hydrolysis of Hetacillin and Ampicillin at 35° and  $\mu=0.5$

The circles represent the experimentally determined values ( $k_{app}$ ) for Hetacillin. The dashed line is the  $\log k_a$ -pH profile for ampicillin from ref. 21.

20) The rate constants,  $k_{app}$ , in Fig. 6 have not been corrected for buffer catalysis, because separate studies in acetate, phosphate and carbonate buffers indicated no or negligible buffer concentration effect on the reaction rates.

21) J.P. Hou and J.W. Poole, *J. Pharm. Sci.*, **58**, 447 (1969).

Durbin and Rydon<sup>15)</sup> showed the experimental evidence, using NMR technique, for the existence of Schiff base intermediate in the interconversion between hetacillin and ampicillin. This reaction scheme may lead to a bell-shaped pH-rate profile which has been recognized<sup>22)</sup> in the number of Schiff base formation and hydrolysis and other related reactions. As has been discussed about these reactions, the bell-shaped pH-rate curve of the hetacillin hydrolysis can probably be interpreted in terms of change in rate-determining step at an appropriate pH.

The positive unity slope of the  $\log k_{app}$ -pH profile at  $\text{pH} > 12$  indicates that the reaction proceeds exclusively *via* hydroxide-ion catalysis. This reaction is attributed to the direct  $\beta$ -lactam cleavage of epihetacillin with the rate constant of  $k_h$ . It is noteworthy that the apparent second-order rate constant for  $k_h$  ( $20 \text{ M}^{-1} \text{ hr}^{-1}$ ) is *ca.* 20–100 times lower than those for ampicillin<sup>21)</sup> ( $2 \times 10^3 \text{ M}^{-1} \text{ hr}^{-1}$ ) and other semisynthetic penicillins<sup>1)</sup> ( $4 \times 10^2$ – $2 \times 10^3 \text{ M}^{-1} \text{ hr}^{-1}$ ).

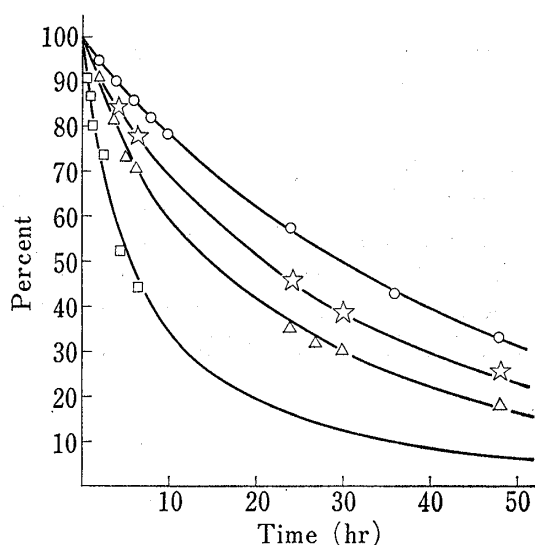


Fig. 7. Hydrolysis of Ampicillin of Various Initial Concentration in 0.2M Phosphate Buffer of pH 6.60 at 35°

The lines are the output of the analog computer, while the points are the experimental values. The kinetic parameters in Table II were used.

initial conc.:

—○—: 0.005M    —△—: 0.1M  
—☆—: 0.05M    —□—: 0.5M

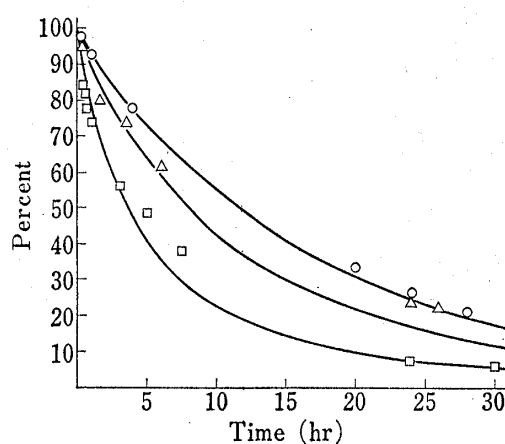


Fig. 8. Hydrolysis of Ampicillin of Various Initial Concentration in 0.2M Carbonate Buffer of pH 9.10 at 35°

The lines are the output of the analog computer, while the points are the experimental values. The kinetic parameters in Table II were used.

initial conc.:

—○—: 0.05 M    —△—: 0.1 M    —□—: 0.5 M

TABLE II. The Kinetic Parameters for the Hydrolysis of Hetacillin and Ampicillin at pH 6.60<sup>a)</sup> and pH 9.10<sup>b)</sup>

Rate constant	pH 6.60 35°	pH 9.10	
		25°	35°
$k_r^c)$ hr <sup>-1</sup>	2.52	0.260	0.602
$k_r^d)$ M <sup>-1</sup> hr <sup>-1</sup>	0.150	0.022	0.040
$k_a^e)$ hr <sup>-1</sup>	0.023	0.018	0.060
$k_d^d)$ M <sup>-1</sup> hr <sup>-1</sup>	0.032	0.012	0.040

a) 0.2M phosphate buffer

b) 0.2M carbonate buffer

c) determined by the proposed analytical method in the hydrolysis of  $5 \times 10^{-3} \text{ M}$  hetacillin

d) determined from the best fit of the experimental data by means of an analog computer

e) determined iodometrically in the hydrolysis of  $5 \times 10^{-3} \text{ M}$  ampicillin

22) W.P. Jencks, in "Progress in Physical Organic Chemistry," Vol. 2, ed. by S.G. Cohen, A. Streitweiser, Jr., and R.W. Taft, Interscience, New York, 1964, p. 63.

This remarkable stability of epihetacillin  $\beta$ -lactam is probably due to the steric hindrance by *gem*-dimethyl of imidazolidine ring toward nucleophilic reagents. The kinetic study on hetacillin epimerization is being investigated and the results will be reported separately.

### Comparative Stabilities of Hetacillin and Ampicillin

The stabilities of the two antibiotics were investigated under identical buffer conditions (0.2M phosphate buffer of pH 6.60 and 0.2M carbonate buffer of pH 9.10).

In Fig. 7 and 8 are shown the time-courses for ampicillin disappearance at the various initial concentrations from 0.005M to 0.5M. Ampicillin showed the instability in the concentrated aqueous solutions. These rapid hydrolyses of ampicillin depending upon the concentration are known to be attributed to the formation of polymers from this penicillin by the nucleophilic attack of the free  $\alpha$ -amino group to the  $\beta$ -lactam of a second molecule.<sup>5,23,24</sup> A rate law for the overall loss of ampicillin can be formulated as the equation:

$$-\frac{dC_A}{dt} = k_a C_A + k_d C_A^2 + k_t C_A C_D \quad (6)$$

where  $k_d$  and  $k_t$  represent the second- and third-order rate constants for the formation of ampicillin dimer and the trimer, respectively, and  $C_D$  refers to the concentration of the dimer. Below 0.5M, the first two terms in Eq. 6 may contribute significantly to the loss of ampicillin since  $C_D$  is expected to be very small within the experimental periods. The curves generated by the analog computer programmed in accordance with Eq. 6 corresponded the experimental data as seen in Fig. 7 and 8. The kinetic parameters used for the calculation were given in Table II.

Figures 9 and 10 show the time-courses of the relevant species in the hetacillin hydrolysis at various initial concentrations. The first-order plots of hetacillin loss showed curvature with increase of the hetacillin initial concentration from 0.05M to 0.5M. This behavior is

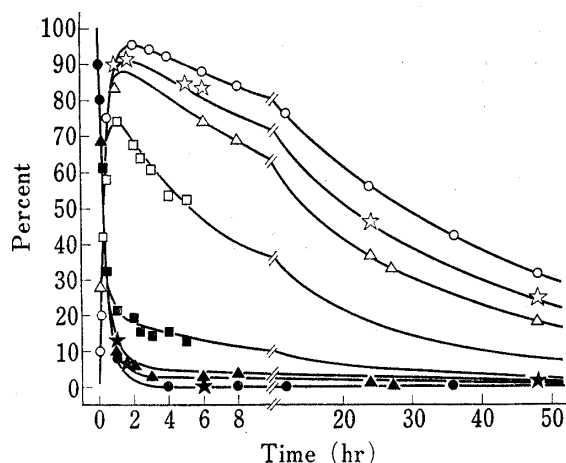


Fig. 9. Hydrolysis of Hetacillin of the Various Initial Concentrations in 0.2M Phosphate Buffer of pH 6.60 at 35°

The lines are the output of the analog computer, while the points are the experimental values. The kinetic parameters in Table II were used.

hetacillin	ampicillin
●—	○—: 0.005M
★—	☆—: 0.05M
▲—	△—: 0.1M
■—	□—: 0.5M

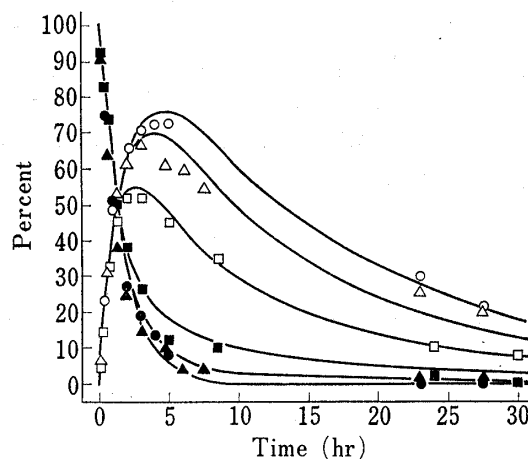


Fig. 10. Hydrolysis of Hetacillin of the Various Initial Concentrations in 0.2M Carbonate Buffer of pH 9.10 at 35°

The lines are the output of the analog computer, while the points are the experimental values. The kinetic parameters in Table II were used.

hetacillin	ampicillin
●—	○—: 0.005M
▲—	△—: 0.1M
■—	□—: 0.5M

- 23) a) H. Smith and A.C. Marshall, *Nature*, **232**, 45 (1971); b) H. Smith, J.M. Dewdney, and A.W. Wheeler, *Immunology*, **21**, 527 (1971).  
 24) E.J. Kuchinkas and G.N. Levy, *J. Pharm. Sci.*, **61**, 727 (1972).



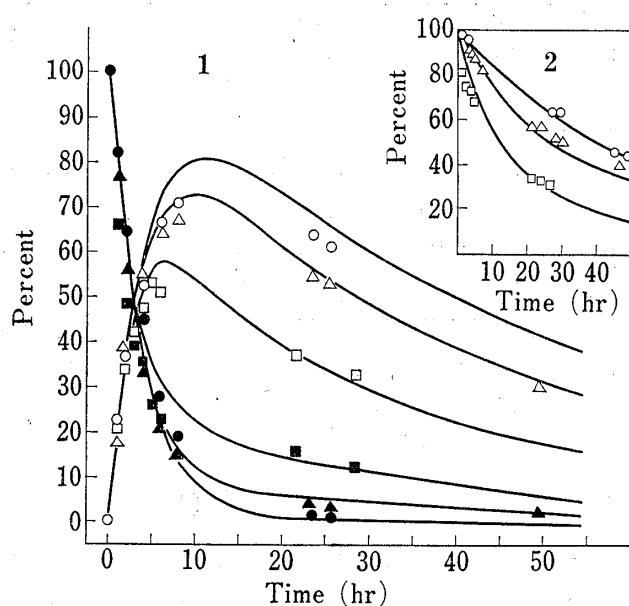


Fig. 11. Hydrolysis of Hetacillin (1) and Ampicillin (2) of the Various Initial Concentrations in 0.2M Carbonate Buffer of pH 9.10 at 25°

The lines are the output of the analog computer, while the points are the experimental values. The kinetic parameters in Table II were used.

hetacillin    ampicillin  
 ●,            ○: 0.005M  
 ▲,            △: 0.1M  
 ■,            □: 0.5M

10% loss in 1 hr under the same conditions. No experimental evidence, however, has been shown yet. The calculated utilization times at 35° and/or 25° for hetacillin and ampicillin solutions of pH 6.60 and pH 9.10 are summarised in Table III. Because of rapid hydrolysis of hetacillin to ampicillin, the utilization time of hetacillin at 35° is much close to that of ampicillin in solutions of both pH's. At 25°, however, large differences (*e.g.*, *ca.* 4 hr at pH 9.10) in the utilization times between both antibiotics existed.

TABLE III. Effect of Initial Concentration on the Stability of Hetacillin and Ampicillin in 0.2M Phosphate Buffer of pH 6.60 and in 0.2M Carbonate Buffer of pH 9.10

Antibiotics	Initial conc.		Utilization time, <sup>b)</sup> hr		
	M	mg/ml <sup>(a)</sup>	pH 6.60 35°	pH 9.10	
				35°	25°
Ampicillin	0.5	185	0.5	0.5	1.5
Hetacillin	0.5	214	1.5	1.9	5.6
Ampicillin	0.1	37.0	1.8	1.3	3.8
Hetacillin	0.1	42.8	3.0	3.0	7.8
Ampicillin	0.005	1.85	4.6	1.8	6.0
Hetacillin	0.005	2.14	5.5	3.2	9.5

a) corresponding to potassium hetacillin and sodium ampicillin

b) Calculated value by an analog computer. The kinetic parameters in Table II were used.

### Consideration for Use of Ampicillin Prodrug

It has recently been recognized that the instability of penicillins may be concerned with not only the formulation of their pharmaceutical dosage forms<sup>25)</sup> but the chemical reactions

25) M.A. Schwartz and F.H. Buckwalter, *J. Pharm. Sci.*, 51, 1119 (1962).

possibly involved in penicillin allergy.<sup>26)</sup> The allergic reactions to penicillins have been attributed to reactions of penicillins with tissue proteins, polymer formation, and contamination impurities.<sup>27)</sup>

A concentrated aqueous solution higher than 0.02M of sodium ampicillin may give the production of its dimer within a day. Several workers<sup>23,24,27)</sup> have reported that further polymerization occurs for several days storage of the solution at room temperature, and claimed that the ampicillin polymer has antigenic determinant. Therefore, major pharmaceutical advantages to use of the prodrug of ampicillin may be its chemical stability in concentrated solution and the prevention of the rapid production of ampicillin polymer. Hetacillin may be impossible to form such an antigenic polymer probably due to its secondary amino group less available for acylation and the great stability of its  $\beta$ -lactam toward nucleophiles. Except for the case of the storage for short periods at low temperature, however, hetacillin was not enough stable to show the above advantage. To avoid the loss of antibiotic activity and any allergic side-effect, it is desirable to use freshly prepared solutions of ampicillin and also hetacillin for therapy.

**Acknowledgement** The authors thank to Banyu Pharm. Co., Ltd. for the gift of potassium hetacillin and Takeda Chemical Ind. for sodium ampicillin. The authors are also indebted to Miss T. Usui and Miss H. Honda for their technical assistance.

26) M.A. Schwartz, *J. Pharm. Sci.*, **58**, 643 (1969).

27) For review, see S. Shaltiel, R. Mizrahi, and M. Sela, *Proc. Roy. Soc. (London), Ser. B*, **179**, 411 (1971).