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Kinetic Study of the Degradation of Cefotiam Dihydrochloride in Aqueous Solution and of the Reaction with Aminoglycosides[1)]

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The kinetics of the degradation of cefotiam in aqueous solution was studied at 25°C and an ionic strength of 0.6 over the pH range of 2 to 12. The degradation was a pseudo first order reaction in this pH range. The rate-pH profile showed a characteristic maximum near the isoelectric point of cefotiam (pH 5.8). The optimal pH of an aqueous solution of cefotiam dihydrochloride for stability was calculated to be 8.0.

The degradation pathways were shown to involve spontaneous hydrolysis of four ion species and hydroxide ion-catalyzed hydrolysis of the monoanion. Aminoglycosides accelerated the degradation of cefotiam by a pseudo second order reaction. From analysis of the rate-pH profile of the second order rate constants of these aminoglycosides, it appeared that the reaction was substantially a third order reaction with regards to cefotiam, aminoglycoside and hydroxide ion. Therefore, the combined use of cefotiam and aminoglycosides should be avoided at alkaline pH and/or at high concentration.

Keywords——cefotiam; β-lactam; antibiotic; SCE-963; kinetics; stability; interaction with aminoglycoside; aminolysis

Knowledge of the stability of a drug is essential for formulation design, for the prediction of shelf-life of the preparation, and for safe and effective use of the medication. In particular, the stability in aqueous solution is of great interest to practitioners.[2)] Incompatibility interactions in mixtures for intravenous administration, such as occurrence of precipitation, turbidity, coloration or discoloration, are easily detectable before the mixtures are administered to a patient, but when a chemical reaction without visible consequences occurs before and/or during the administration, it may result in inappropriate dosing as a result of high degradation and the formation of untoward products. To avoid these undesirable outcomes, fundamental studies on a drug substance as regards stability and physico-chemical interactions with other components and/or the adjuvant of the additive are essential.

β-Lactam antibiotics are typical drugs which may suffer the above problems. In the current study, the kinetics of the degradation of cefotiam (Fig. 1), a new cephem antibiotic developed by Numata *et al.*,[3)] and of the reaction with aminoglycosides were studied.

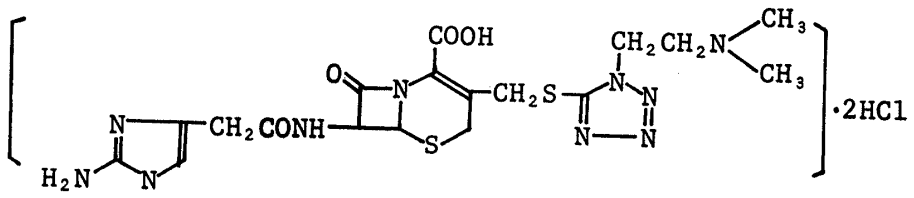

Fig. 1. Chemical Structure of Cefotiam·2HCl

Experimental

Materials——Cefotiam·2HCl (827 μg/mg, Lot No. OBH-227, Takeda Chemical Industries Co., Ltd., Osaka, Japan), amikacin sulfate (780 μg/mg, Lot No. BKE-77, Banyu Pharmaceutical Co., Ltd., Osaka, Japan), dideoxykanamycin B sulfate (692 μg/mg, Lot No. RPK 97, Meiji Seika Kaisha Co. Ltd., Osaka,

Japan), kanamycin sulfate (690 μg/mg, Batch No. RKD 19912, Meiji Seika Kaisha Co., Ltd., Osaka, Japan) were kindly supplied by each manufacturer and used without further purification. Other reagents were all of reagent grade.

Procedure for the Kinetic Study of the Degradation of Cefotiam in Buffer Solution——Cefotiam·2HCl was dissolved in buffers previously equilibrated to 25°C to make 1.67×10^{-3} M solution. For the estimation of the activation energy, the degradation was also examined at 37 and 47°C. The reaction temperatures were regulated by using a Thermo Unit C-600 (Taiyo Scientific Industrial Co. Ltd., Tokyo, Japan) with 0.01°C precision. The buffer solutions were as follows: pH 2.00—4.03 (0.2—0.05 M disodium citrate–0.2—0.05 N HCl); pH 5.00, 5.02 (0.2—0.05 M sodium acetate–acetic acid); pH 6.00—7.00 (0.2—0.05 M potassium dihydrogen phosphate–disodium hydrogen phosphate); pH 7.86—10.0 (0.2—0.05 M sodium tetraborate–0.2—0.05 N sodium hydroxide); pH 10.69—12.00 (0.2—0.05 M disodium hydrogen phosphate–0.2—0.05 N sodium hydroxide). Ionic strengths of all the buffers were adjusted to 0.6 with KCl.

Data analysis was caaried out using an ACOS-800 computer (Nippon Electric Co., Ltd., Tokyo, Japan) and an MZ-80C computer (Sharp Corporation, Osaka, Japan).

Kinetics of the Reaction of Cefotiam with Aminoglycosides——Buffer solutions of cefotiam·2HCl (1.67×10^{-3} M) containing various concentrations of amikacin sulfate (1.70, 9.76 and 16.7×10^{-3} M) were reacted at 25°C. The residual cefotiam was determined at appropriate time intervals. Other aminoglycosides were reacted at ten-fold higher concentrations than cefotiam (1.67×10^{-2} M) at 25°C. The slight pH changes of the buffers on dissolving these reactants were corrected by adding 1—5 N hydrochloric acid or sodium hydroxide solution. The pH was measured with a model F-5 pH meter (Hitachi-Horiba, Kyoto, Japan).

Determination of Cefotiam——Cefotiam was determined by a high performance liquid chromatography (HPLC) method, by a slight modification of the published procedure.[4] A model 638-50 chromatograph (Hitachi, Tokyo, Japan) was used for HPLC. Column, μBONDAPAK C_{18} (3.9 mm × 30 cm, Nihon Waters Ltd., Japan); mobile phase, 0.03 M diammonium hydrogen phosphate–acetic acid–methanol (100: 3: 8.6—10.72), (the content of methanol was varied appropriately to give the optimal separation); flow rate, 1 ml/min; detection, 250 nm using a model 635M UV detector (Hitachi, Tokyo); internal standard, tegafur (6.233×10^{-3} M aqueous solution).

Each reaction solution (50 μl) and the internal standard solution (50 μl) was admixed and 3 μl of the mixture was injected into the chromatograph.

pK_a Determination of Aminoglycosides——Each aminoglycoside sulfate was dissolved in 20 ml of purified water to make a final concentration of *ca.* 1.08—1.30 M. Seven ml of 0.2 N sodium hydroxide was added and the solution was titrated with 0.2 N hydrochloric acid using a recording auto titrator, type RAT-11 (Hiranuma Sangyo Co., Ltd., Mito, Japan). The pK_a was read at the half-neutralization point of the titration curve.

Results and Discussion

Determination of Cefotiam in the Reaction Solution

Figure 2a shows a chromatogram of cefotiam and tegafur. A calibration graph with a good linear regression lines (peak height ratio=0.871×[mg/ml]−0.014 (r=0.9992)) was obtained up to the concentration of 2 mg/ml, as shown in Fig. 2b.

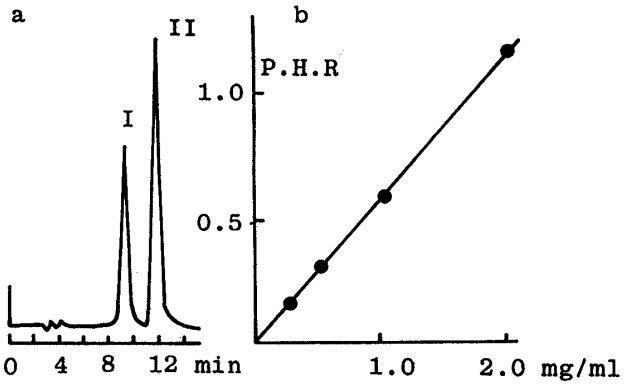

Fig. 2. Chromatogram of Cefotiam (I) and Tegafur (II) (a) and the Calibration Graph of Cefotiam (b)

P.H.R.: Peak height ratio.

Degradation of Cefotiam in the Buffer Solution

As shown in Fig. 3, the degradeation of cefotiam obeyed pseudo-first-order kinetics in all buffer solutions. General acid-base catalyzed hydrolysis, the buffer effect, was observed in phosphate and borate buffers (Fig. 4) and k_{obs}^{pH}, the rate constant purely dependent on pH, was obtained by extrapolation to zero buffer concentration. The k_{obs}^{pH} at each pH is listed in Table I and shown in Fig. 5.

The rate–pH profile showed an unusual pattern having the character-

istic maximum near the isoelectric point of cefotiam, pH 5.8 (Fig. 5), as was presented by Ichimura *et al.*[5] Rate-pH profiles of similar unusual pattern were reported to occur in the hydrolysis of oxazolidines, the condensates of ephedrine with benzaldehydes.[6] Cefotiam has three hydrogen-accepting moieties and the values of pK_a are 2.6 for pK_{a_1}, 4.6 for pK_{a_2}, and 7.0 for pK_{a_3}.[4] Thus, the specific acid-base catalyzed hydrolysis may involve the four ion species of AH_3^{2+}, $AH_2^{2+/-}$, $AH^{+/-}$, and A^-, and k_{pH} can be represented by Equation 1.

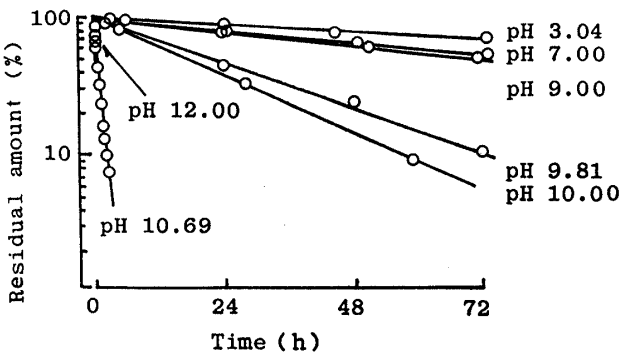

Fig. 3. Time Courses of the Degradation of Cefotiam in the Buffer Solution at 25°C and Ionic Strength 0.6

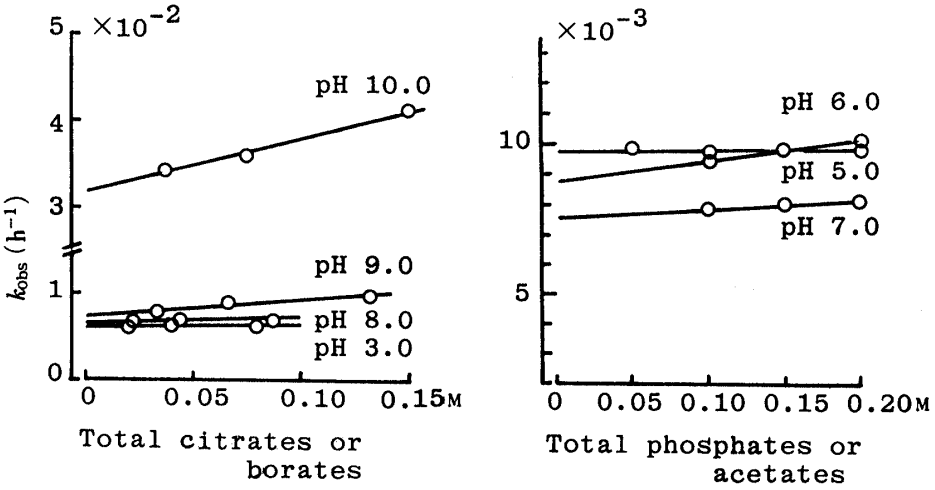

Fig. 4. The Effect of the Buffer Components on the Degradation of Cefotiam
Components of buffers: refer to the text or Table I.

$$k_{pH} = \sum_{n=0}^{3}[k_{H^+(AH_n)} \times a_{H^+} + k_{0(AH_n)} + k_{OH^-(AH_n)} \times a_{OH^-}] \times f_{(AH_n)} \quad (1)$$

where AH_n stands for n hydrogenated cefotiam molecules and k_{H^+}, k_0 and k_{OH^-} are the rate constants of acid-catalyzed, spontaneous and hydroxide ion-catalyzed hydrolysis, respectively. $f_{(AH_n)}$ is the mole fraction of an ion species corresponding to the specified n value and its values are given by Equations 2—5.

$$f_{AH_3^{2+}} = (a_{H^+})^3/K \quad (2)$$

$$f_{AH_2^{2+/-}} = (a_{H^+})^2 K_{a_1}/K \quad (3)$$

$$f_{AH^{+/-}} = (a_{H^+}) K_{a_1} K_{a_2}/K \quad (4)$$

$$f_{A^-} = K_{a_1} K_{a_2} K_{a_3}/K \quad (5)$$

where $K = (a_{H^+})^3 + (a_{H^+})^2 K_{a_1} + (a_{H^+}) K_{a_1} K_{a_2} + K_{a_1} K_{a_2} K_{a_3}$.

TABLE I. Pseudo First Order Rate Constants of the Degradation of Cefotiam at 25 °C and μ=0.6

pH (Buffer)	$k_{obs}^{pH} \times 10^3$ (h^{-1})
2.00 (Citrate)	6.397
2.03 (Citrate)	5.569
3.00 (Citrate)	5.958
3.04 (Citrate)	5.414
4.03 (Citrate)	6.225
5.00 (Acetate)	9.747
5.02 (Acetate)	9.227
6.00 (Phosphate)	8.802
7.00 (Phosphate)	7.575
7.86 (Borate)	5.615
8.00 (Borate)	6.454
8.00 (Borate)	6.625
8.88 (Borate)	8.236
9.00 (Borate)	7.415
9.00 (Borate)	7.248
9.81 (Borate)	33.710
10.00 (Borate)	31.525
10.69 (Borate)	104.6
11.00 (Phosphate)	399.9
12.00 (Phosphate)	3951.8

On the hypothesis that the dominant reaction pathways are the spontaneous hydrolysis of each ion species and hydroxide ion-catalyzed hydrolysis of A^- anion, and that other hydrolysis pathways are negligible. Equation 1 is reduced to Equation 6.

$$k_{obs} = k_{0(AH_3)} \times f_{AH_3^{2+}} + k_{0(AH_2)} \times f_{AH_2^{2+/-}} + k_{0(AH)} \times f_{AH^+} + [k_{0(A)} + k_{OH^-} \times a_{OH^-}] \times f_{A^-} \quad (6)$$

The apparent first order rate constants at pH 2.0 to 12.0 were analyzed according to Equation 6 by the linear least-squares method using the values of $f_{(AH_n)}$ and $f_{(A^-)} \times a_{OH^-}$ calculated from Equations 2 to 5. The solution using the data in Table I with some omissions (k_{obs}^{pH}: 104.6, 31.525, 7.248 and 5.615×10^{-3} h^{-1}, respectively) gave the following micro rate constants:

$k_{0(AH_3)} = 6.059 \times 10^{-3} h^{-1}$; $k_{0(AH_2)} = 5.547 \times 10^{-3} h^{-1}$;
$k_{0(AH)} = 1.016 \times 10^{-2} h^{-1}$; $k_{0(A)} = 5.068 \times 10^{-3} h^{-1}$; $k_{OH^-} = 3.947 \times 10^2 M^{-1} h^{-1}$.

Using these results and Equation 6, the predicted rate-pH profile was generated (solid line in Fig. 5). Fairly good agreement with the experimental values was observed. This indicates that the hypothesis regarding the reaction pathways is reasonable. The value of $k_{0(AH)}$, the rate constant of the amphoteric ion species $AH^{+/-}$, was characteristically higher than those of other ion species. In this type of amphoteric ion, there may be intramolecular neutralization between the anion and cation moieties and the net charge of the whole molecule may be zero. This suggests that the water-catalyzed hydrolysis is most likely to occur with the unionized molecule. The rate-pH profile also indicates that the optimal pH for cefotiam solution is 8.0 from the viewpoint of stability.

Effect of Temperature

Figure 6 shows Arrhenius plots of the apparent first order rate constants at 3.0, 6.0, 7.0

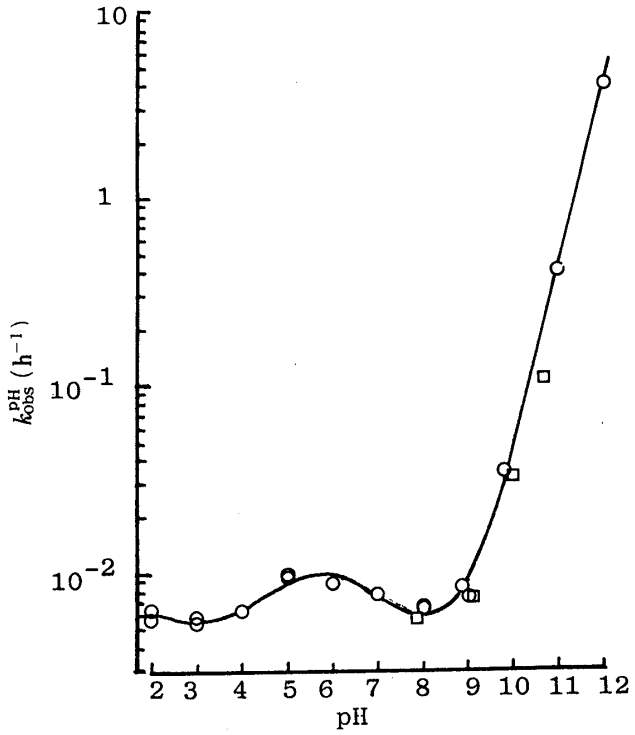

Fig. 5. Rate-pH Profile of the Degradation of Cefotiam in the Buffer Solutions at 25°C and Ionic Strength 0.6

○ and □, observed rate constants (the data indicated by □were omitted from the analysis) solid line, predicted rate-pH profile calculated by Equation 6.

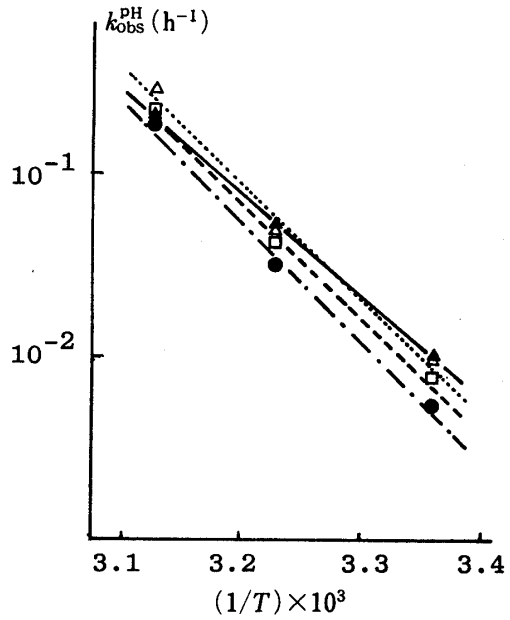

Fig. 6. Effect of Temperature on the Degradation of Cefotiam at Various pHs

●, pH 3.0; △, pH 6.0; □, pH 7.0; ▲, pH 9.0

and 9.0. The apparent activation energies were calculated to be 30.58, 29.07, 28.31 and 26.19 kcal mol^{-1}, respectively.

Applying these values to Arrhenius' equation, the values of k_{pH} at other temperatures can be calculated, making it possible to predict the residual amount of cefotiam at any given pH and temperature.

pK_a of Aminoglycoside

The titration of an alkalinized solution of aminoglycoside sulfate (pH: above 12.0) with 0.2 N hydrochloric acid gave the titration curves shown in Fig. 7. The values of pK_a were estimated from the half-neutralization points. The contents of sulfate could also be determined (Table II).

Reaction with Aminoglycosides

The degradation of cefotiam in the presence of various amounts of amikacin sulfate at pH 8.90, 25°C obeyed pseudo first order kinetics, and the reaction rate increased with increase

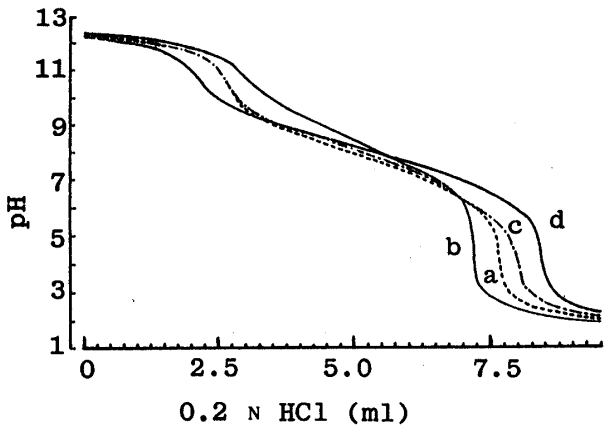

Fig. 7. Titration Curves of Aminoglycosides

a, 2.5×10^{-4} mol kanamycin; b, 2.2×10^{-4} mol amikacin; c, 2.7×10^{-4} mol ribostamycin; d, 2.5×10^{-4} mol dideoxykanamycin B. Initial volume of aminoglycoside solution: 27 ml.

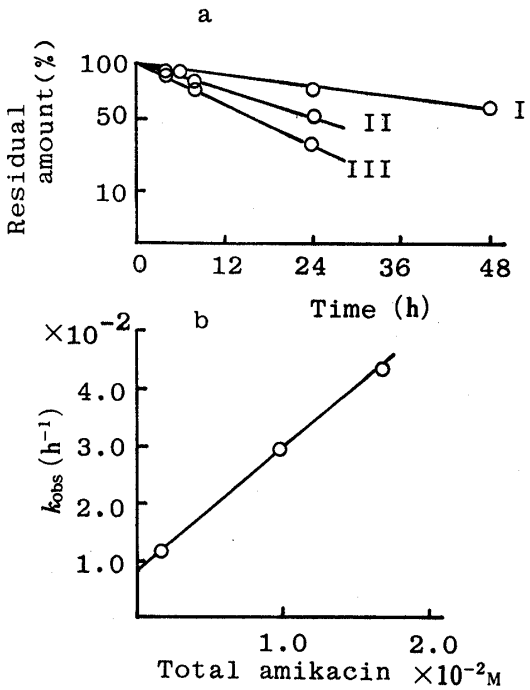

Fig. 8. Time Courses of the Degradation of Cefotiam in the Presence of Various Amounts of Amikacin Sulfate (a) and Plot of Pseudo First Order Rate Constants *versus* Amikacin Concentration (b)

Concentration of amikacin sulfate: I, 1.70×10^{-3} M; II, 9.76×10^{-3} M; III, 1.67×10^{-3} M.

TABLE II. Aminoglycosides Titration Data and Micro Rate Constants

Aminoglycosides	pK_a (25 °C)	Content of H_2SO_4 (M)	$k_{AH}^{OH^-}$ $M^{-2}h^{-1}$	k_A^0 $M^{-1}h^{-1}$	$k_A^{OH^-}$ $M^{-2}h^{-1}$
Kanamycin	7.86 (0.082)[a]	1.76 (0.006)	2.91×10^6	2.11	8.95×10^4
Amikacin	8.39 (0.046)	1.94 (0.041)	8.90×10^5	2.19	8.20×10^4
Ribostamycin	7.97 (0.087)	1.67 (0.091)	2.08×10^6	1.94	7.63×10^4
Dideoxy-kanamycin B	8.08 (0.029)	1.78 (0.080)	2.33×10^6	2.80	1.26×10^5

a) Mean of three determinations (SD in parenthesis).

of amikacin concentration as shown in Fig. 8a. The plot of apparent first order rate constant *versus* amikacin concentration was linear (Fig. 8b), and k_{obs}, the apparent first order rate constant, is described by Equation 8.

$$k_{obs} = k_{pH} + k_{AM}[AM] \tag{8}$$

where k_{AM} is the apparent second order rate constant and [AM] is the molar concentration of amikacin.

Figure 9 shows the differences between k_{pH} and k_{obs} in the presence of a 10-fold higher concentration of aminoglycosides than that of cefotiam. The differences were remarkable in the high pH region. Figure 10 shows the rate-pH profiles of second order rate constants with the aminoglycosides. k_{AM} increased remarkably with increase of pH, suggesting that k_{AM} is also a function of a_{H^+} and/or a_{OH^-}, like k_{pH}. Thus it may be reasonable to describe k_{AM} by the use of Equation 9, containing the whole reaction model.

$$k_{AM} = (k_{AH}^{H^+} \times a_{H^+} + k_{AH}^{0} + k_{AH}^{OH^-} \times a_{OH^-}) \frac{a_{H^+}}{K_a + a_{H^+}} + (k_{A}^{H^+} \times a_{H^+} + k_{A}^{0} + k_{A}^{OH^-} \times a_{OH^-}) \frac{K_a}{K_a + a_{H^+}} \tag{9}$$

Because the interaction of aminoglycosides with a β-lactam antibiotic is negligible at neutral pH, the a_{H^+} terms can probably be neglected. The data were analyzed on the basis of the residual four reaction terms by the least-squares method, in the same way as described for the analysis of k_{pH}-rate profile of the degradation of cefotiam. Reasonable positive micro rate constants were obtained only for the two reaction models described by Equations 10 and 11.

$$k_{AM} = (k_{A}^{0} + k_{A}^{OH^-} \times a_{OH^-}) \times f_A \tag{10}$$

$$k_{AM} = k_{AH}^{OH^-} \times a_{OH^-} \times f_{AH} + k_{A}^{OH^-} \times a_{OH^-} \times f_{A^-} \tag{11}$$

$k_{A}^{0} \times f_A$ in Equation 10 and $k_{AH}^{OH^-} \times a_{OH} \times f_{AH}$ in Equation 11 cannot be distinguished mathematically and identical predicted rate-pH profiles were obtained for both models (solid lines in Fig. 10). As shown in Fig. 9, the contribution of aminoglycoside to the degradation of cefotiam becomes negligible in the lower pH region. Little difference between k_{pH} and k_{obs} was observed at low pH. This is explained by the lowered concentration of hydroxide ion in the reaction model described by Equation 11 and lowered unionized fraction in the reaction model described by Equation 10. Thus, we cannot definitely determine whether or not the ionized form of aminoglycoside interacts with β-lactam from the results of the current study.

In the high pH region, a controversial decrease of k_{AM} with increase of pH was reported.[7] This suggests that the nucleophilic attack by unionized aminoglycoside is disturbed by high concentrations of hydroxide ion. Thus, the nucleophilic reaction by aminoglycoside may also be negligible compared to hydroxide ion-catalyzed

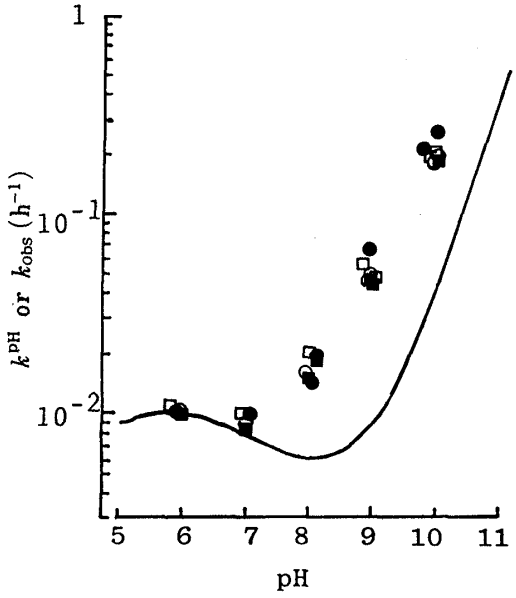

Fig. 9. Comparison of the k_{pH} with the Pseudo First Order Rate Constant in the Presence of Aminoglycoside

□, kanamycin; ■, amikacin; ●, ribostamycin; ○, dideoxykanamycin B. The concentrations of all the aminoglycosides were ten-fold higher than that of cefotiam. Solid line: predicted k_{pH}.

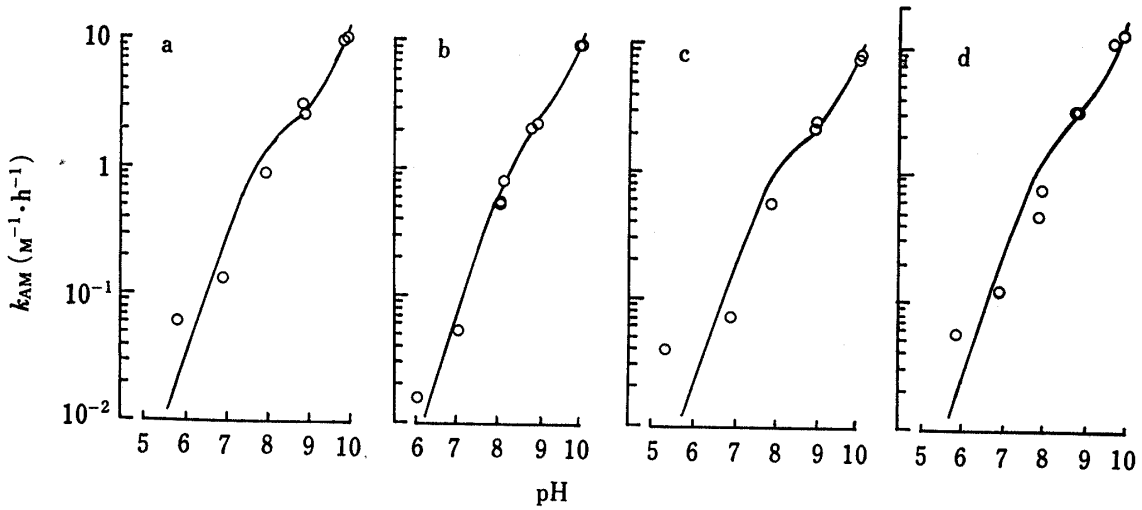

Fig. 10. The Pseudo Second Order Rate Constants of the Reaction of Cefotiam with Aminoglycosides

a, kanamycin; b, amikacin; c, ribostamycin; d, dideoxykanamycin B. Solid line: predicted rate-pH profile generated using the data in Table II and Equations 10 and 11.

hydrolysis at higher pH, as well as at lower pH (pH$<$7). The precise mechanism of the reaction of aminoglycosides with β-lactam antibiotic in the alkaline region is not clear at present. Further study is necessary.

Cephem antibiotics are generally more stable to specific acid-catalyzed hydrolysis compared to penicillins, but more sensitive to spontaneous hydrolysis at neutral pH.[8] The rate-pH profile of cephem in the acidic to neutral regions is a plateau in many cases. In contrast, cefotiam showed accelerated spontaneous hydrolysis near the isoelectric point, but the times required for the decrease to 90% of the initial amount of cefotiam at the pH region of 2 to 9 at 25°C are 11.0 to 18.3 h as calculated from the k_{pH} obtained from Equation 6, indicating sufficient stability of cefotiam in aqueous solution for clinical use.

Cefotiam showed remarkable interaction with aminoglycosides in the alkaline region, as is the case with other β-lactam antibiotics,[7] strongly suggesting that inactivation of both cefotiam and aminoglycoside will occur in an intravenous admixture. However, combined use would be possible provided that the concentration and pH of the admixture are low, because the interaction is a second order reaction and is slow in the neutral pH region.

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References and Notes

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