[Chem. Pharm. Bull. 32(9)3651—3661(1984)]

Kinetics and Mechanism of the Degradation and Epimerization of Sodium Cefsulodin in Aqueous Solution[1)]

TOSHIO FUJITA and AKIRA KOSHIRO*

Department of Pharmacy, Yamaguchi University Hospital, 1144 Kogushi, Ube 755, Japan

(Received October 5, 1983)

The kinetics of the degradation and epimerization of cefsulodin in various buffer solutions were studied at 25 °C and 0.6 ionic strength. The overall degradation was a pseudo-first-order reaction in the pH region studied. The rate law of the degradation could be approximated in terms of specific acid-base and water catalyzed hydrolysis, that is k_1 (total degradation rate constant) = $k_{H^+} \times a_{H^+} + k_0 + k_{OH^-} \times a_{OH^-}$. The apparent activation energies of the degradation reaction were 20.7, 22.3, 23.0 and 27.7 kcal mol^{-1} at pH values of 2, 4, 6, and 9, respectively. The epimerization of cefsulodin was proved to be catalyzed by hydroxide ion from the epimerization rate constant–pH profile, solvent effects using ethanol, and the apparent activation energies (which were 27.0 and 26.1 kcal mol^{-1} for the apparent forward and reverse epimerization reactions at pH 9.0, respectively). The mechanism of epimerization of cefsulodin is proposed to involve removal of the α-proton of the benzyl side chain by hydroxide ion to form an anioic intermediate.

Interactions of cefsulodin with amines and aminoglycosides were also examined. The reaction was pseudo-second-order and the second-order rate constants for various amines and aminoglycosides were compared. It was found that intramolecular catalysis is the predominant factor for amines. An equation is proposed for the second-order rate constants of aminoglycosides.

Peaks of unknown products in the alkaline reaction solutions could be separated under the high performance liquid chromatography conditions of the current study.

Keywords——cephem; cephalosporin; antibiotic; β-lactam antibiotic; cefsulodin sodium; kinetics; hydrolysis; epimerization; aminolysis; chemical stability

Cefsulodin (Fig. 1) is a semisynthetic cephem antibiotic which has characteristic potent antibacterial activity against *Pseudomonas*. Its activity is comparable to that of aminoglycosides. β-Lactam antibiotics are used as parenteral solutions in many cases and their stabilities in aqueous solutions have been extensively studied.[2-7)]

Cefsulodin has an α-substituted benzyl side chain and optically active stereoisomers exist, as with ampicillin, carbenicillin, sulbenicillin, cephalothin, and cephaloglycin. Although these optically active β-lactam antibiotics with benzyl side chains are used as mixtures of epimers in various ratios, as described for ampicillin, carbenicillin,[8,9)] sulbenicillin,[10)] and latamoxef (moxalactam),[11-13)] cefsulodin is used as the pure D(−) epimer, which can be obtained effectively by using Amberlite XAD-2.[14)] In addition, cefsulodin is stable to epimerization below neutral pH but sensitive to epimerization in alkaline solution.[15)]

Aminolysis by amine drugs or adjuvants is a well known interaction[16-19)] in parenteral

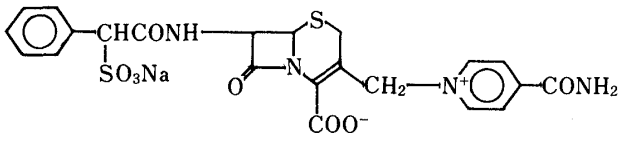

Fig. 1. Structure of Sodium Cefsulodin

admixtures of β-lactam antibiotics, and is of clinical importance. The present study deals with the kinetics and mechanisms of degradation and epimerization of cefsulodin in aqueous solution. The interaction of cefsulodin with amines was also examined.

Experimental

Materials——D(−)-Sodium cefsulodin (*Anal.* Calcd for $C_{22}H_{19}N_4NaO_8S_2 \cdot 1.15H_2O$: C, 45.94; H, 3.73; N, 9.74. Found: C, 45.66; H, 3.78; N, 9.70) and L(+)-sodium cefsulodin (*Anal.* Calcd for $C_{22}H_{19}N_4NaO_8S_2$: C, 47.65; H, 3.45; N, 10.10. Found: C, 47.92; H, 4.01; N, 10.14) were kindly supplied by the manufacturer (Takeda Chemical Industries Co., Ltd., Osaka, Japan) and used without further purification. Ribostamycin sulfate (Meiji Seika Co., Ltd., Tokyo, Japan), kanamycin sulfate (Meiji Seika Co., Ltd., Tokyo, Japan) and amikacin sulfate (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan) were also generously supplied by the manufacturers. Other reagents were all of reagent grade.

Buffer Solution——Buffers were as follows: pH 2.0—4.0 (disodium citrate–hydrochloric acid), pH 5.0 (sodium acetate–acetic acid), pH 6.0 and 7.0 (potassium dihydrogen phosphate–disodium hydrogen phosphate), pH 8.0 and 9.0 (sodium tetraborate–hydrochloric acid), pH 10.0 (sodium tetraborate–sodium hydroxide), pH 11.0 and 12.0 (disodium hydrogen phosphate–sodium hydroxide). The ionic strength of all buffers was adjusted to 0.6 with KCl.

Kinetics of Degradation of Cefsulodin in Buffer Solutions——D(−)- and L(+)-sodium cefsulodin (2—3 mg) were each dissolved in 10 ml of buffer preequilibrated to the desired reaction temperature (25 °C for most of the reactions and 37 and 47 °C for the measurements of activation energies). The residual amount of cefsulodin in each reaction solution was determined periodically by high performance liquid chromatography (HPLC). At pHs above 9.0, aliquots of the reaction solution were neutralized by adding appropriate amounts of 0.1 N hydrochloric acid and stocked in ice until analysis. Data analysis was carried out on a PC-8001 computer (Nippon Electric Co., Ltd., Tokyo, Japan).

Effect of Ethanol on the Degradation of Cefsulodin——Approximately 2—3 mg of D(−)-sodium cefsulodin was dissolved in 0.1 M tetraborate buffer (pH 9.0 $\mu=0.6$ with KCl) containing various amounts (0—20%) of ethanol and reacted at 25 °C.

Interactions with Amines and Aminoglycosides——D(−)-Cefsulodin (3 mg) was dissolved in the buffer with various amounts of ethylenediamine and kanamycin or a ten-fold molar excess of amines and aminoglycosides and reacted at 25 °C.

Determination of Cefsulodin——Cefsulodin in each reaction solution was determined by the HPLC method. A 50 μl aliquot of the reaction solution and 50 μl of the internal standard solution (phthalic acid 3.75×10^{-3} M for mobile phase A or tegafur 6.233×10^{-3} M for mobile phase B) were mixed and 3 μl of the mixture was injected into the chromatograph. HPLC was performed on a model 638-50 liquid chromatograph with a model 635M detector set at 260 nm (Hitachi, Tokyo, Japan). Chromatograms were analyzed by integration by the built-in data processor in the model 638-50 liquid chromatograph. A reversed phase column (μBONDAPAK C_{18}, 30 cm × 3.9 mm internal dimension, Nihon Waters Ltd., Tokyo, Japan) was eluted with mobile phase A (0.0168 M dibasic ammonium phosphate–acetic acid–methanol (100 : 1.68 : 5.98) containing 5×10^{-3} M triethylamine) or B (0.0388 M ammonium acetate, 2.92×10^{-4} M dibasic ammonium phosphate and 9.363×10^{-6} M triethylamine–acetonitrile–methanol–dimethylformamide–acetic acid (1000 : 7.06 : 1.05 : 1.31 : 0.30)) at a flow rate of 1.2 ml/min.

Results and Discussion

Separation of D(−)- and L(+)-Cefsulodin and the Unknown Degradation Products

Figure 2A shows the chromatogram of D-(−)-(**1**) and L(+)-cefsulodin (**2**) and phthalic acid (**6**) as an internal standard with mobile phase A. Good separation was achieved, as in other reported procedures,[15,20] among D(−)- and L(+)-cefsulodin, isonicotinamide (**3**) (degraded C-3 side chain fragment) and probably the isomers of lactone derivatives of the despyridinium products (**4** and **5**).[20a] For the determination of D(−)-cefsulodin in the reaction solution below neutral pH, where both the epimerization and the formation of unknown products were undetectably slow or did not occur, the chromatographic conditions shown in Fig. 2A were sufficient. However, at alkaline pH, peaks of unknown products were detected between the peaks of the D(−)-and L(+)-epimers by using mobile phase A thinned with water in a ratio of 23 : 77. The thinned mobile phase A effectively separated these unknown products but the chromatography was relatively time-consuming, as shown in Fig. 2B. Using the mobile phase B which is more complex and contains a trace amount of dimethylformamide to block more effectively the residual silanol moieties of the reversed

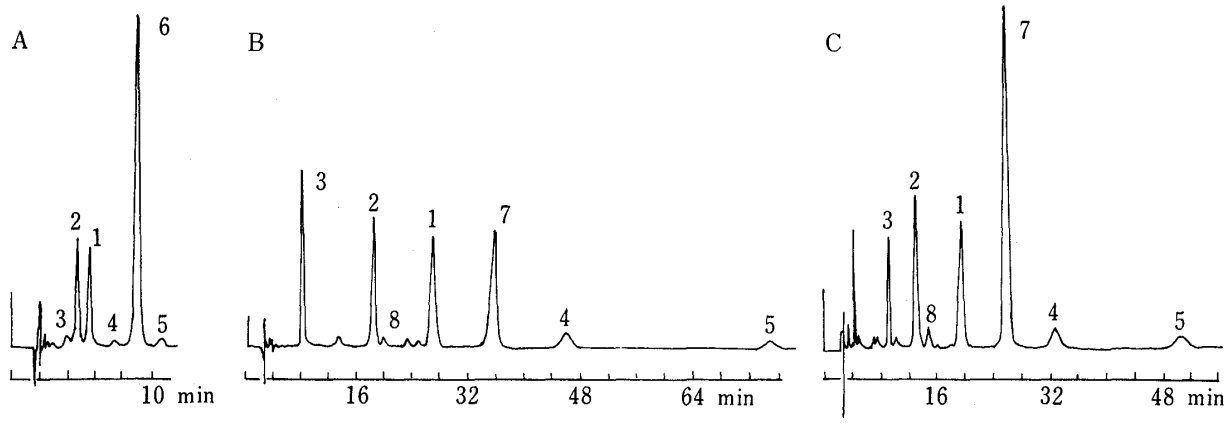

Fig. 2. Chromatograms of Cefsulodin and Its Degradation Products

A, mobile phase A; B, mobile phase A thinned with water; C, mobile phase B. 1, D(−)-cefsulodin; 2, L(+)-cefsulodin; 3, nicotinamide; 4 and 5, probably the isomers of lactone derivatives of the despyridinium products;[20a] 6, phthalic acid (internal standard in A); 7, tegafur (internal standard in B and C); 8, unknown products.

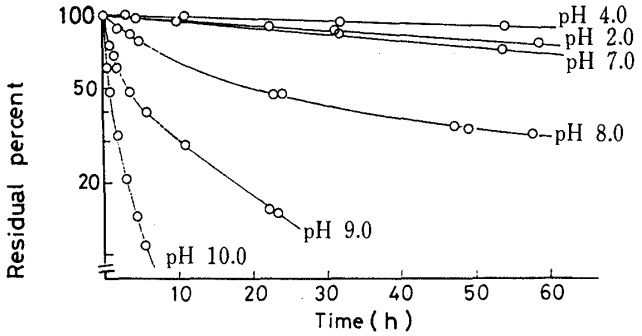

Fig. 3. Time Courses of the Degradation of D(−)-Cefsulodin in Various Buffer Solutions at 25 °C and $\mu=0.6$

phase column,[21] more satisfactory results were obtained (Fig. 2C). This solvent system was used for the determination of cefsulodin in alkaline reaction solutions. Slight modifications of the composition ratio may be necessary depending on the column conditions.

Degradation of Cefsulodin in Buffer Solutions

Figure 3 depicts the time courses of decrease of D(−)-cefsulodin. The decrease obeyed pseudo first-order kinetics up to pH 7.0. However, double-exponential decreases were observed in the pH region above 7.0.

Figure 4A shows a rapid decrease of D(−)-cefsulodin and the formation of L(+)-cefsulodin at pH 9.0 and 25 °C. The opposite reaction at 37 °C is shown in Fig. 4B. The concentration of the D(−)-epimer was slightly larger than that of the L(+)-epimer at equilibrium. After the concentrations of both epimers had reached equilibrium, the attenuation curves of both epimers were parallel, indicating that they decreased at the same rate. In both cases (Fig. 4A and 4B), the decrease of total cefsulodin (D(−)+L(+)) obeyed pseudo-first-order kinetics. It was concluded from these results that the degradation of cefsulodin can be represented by Chart 1. Solving the differential equations for this model, we can derive Eqs. 1 and 2, which give the amounts of D(−)- and L(+)-cefsulodin, respectively, as a function of time.

$$D_t=\frac{1}{k_2^+ + k_2^-}\left[k_2^-(D_0+L_0)\exp(-k_1 t) + (k_2^+ D_0 - k_2^- L_0)\exp(-(k_1+k_2^++k_2^-)t)\right] \quad (1)$$

$$L_t=\frac{1}{k_2^+ +k_2^-}[k_2^+(L_0+D_0)\exp(-k_1 t) + (k_2^- L_0-k_2^+ D_0)\exp(-(k_1+k_2^+ +k_2^-)t)] \quad (2)$$

where k_1 is the pseudo-first-order degradation rate constant of both cefsulodin epimers, k_2^+ and k_2^- are the apparent rate constants of epimerization of D(−)- to L(+)-epimer and L(+)- to D(−)-epimer, respectively, and D_0 and L_0 are the initial amounts of D(−)- and L(+)-cefsulodin, respectively.

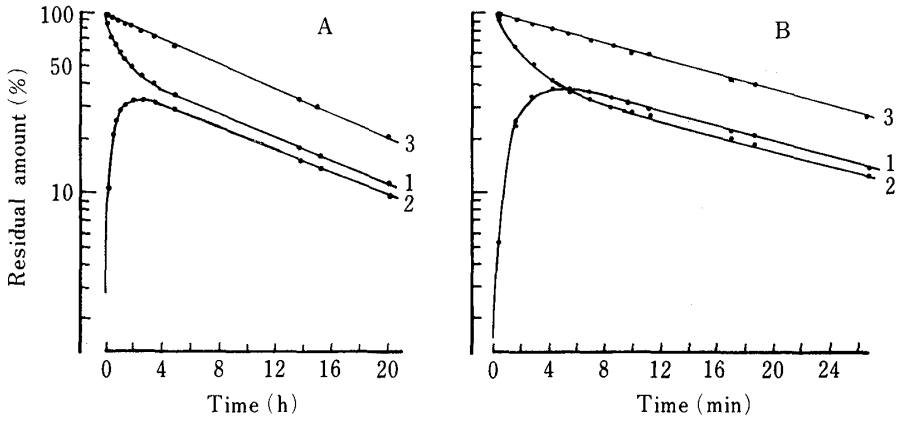

Fig. 4. Time Courses of the Residual Amount (%) of Cefsulodin in Various Reaction Solutions at 25 °C and $\mu=0.6$

A, pH 9.0 and 25 °C; B, pH 9.0 and 37 °C. 1, D(−)-cefsulodin; 2, L(+)-cefsulodin; 3, total cefsulodin.

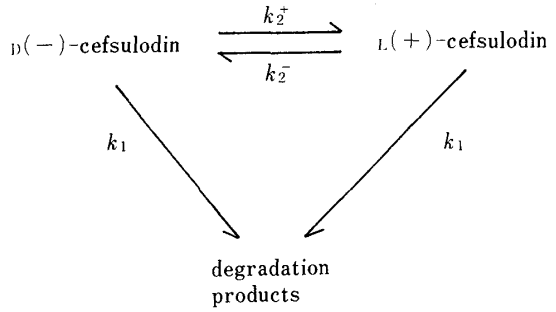

Chart 1. Model of the Degradation and Epimerization of Cefsulodin in Aqueous Solution

Using Eqs. 1 and 2, we can obtain the rate constant k_1 from the attenuation graph at the postequilibrium phase and the value of $(k_1+k_2^+ +k_2^-)$ by the residual method provided that L_0 is zero. At the same time, $k_2^-/(k_2^+ +k_2^-)$ and $k_2^+/(k_2^+ +k_2^-)$ are obtained as the intercepts of the $\exp(-k_1 t)$ term of either D_t (Eq. 1) or L_t (Eq. 2), respectively. All the parameters, k_1, k_2^+, and k_2^-, could be obtained by the above procedures. Or otherwise, including the case where L_0 is zero, these parameters were also obtained by means of a least-squares program "MULTI" for a micro-computer.[22] The results are listed in Table I. The buffer effects of citrates at the concentration used in the current study were not distinct. Further, the buffer effects at pHs above 10 could not be detected, probably because of marked hydroxide ion catalysis. In these cases, the rate constants were obtained as averages. At pHs 6.0—9.0, the buffer effects were clear both with k_1 and with k_2s (Table I). The rate constants were obtained by extrapolation.

Figure 5 shows the rate–pH profiles of the pseudo-first-order rate constant, k_1, of the degradation reaction and the pseudo-first-order rate constants, k_2^+ and k_2^-, of the apparent reversible reaction of epimerization. The k_2s could not be measured below pH 7.0. The overall degradation of cefsulodin can be regarded as a simple acid-base catalyzed reaction and Eq. 3 seems to be valid.

TABLE I. Rate Constants of Degradation and Epimerization of Cefsulodin in Various Buffer Solutions at 25 °C and Ionic Strength of 0.6

pH	Buffer system		Rate constants in various buffer systems (h^{-1})			Extrapolated or average rate constants (h^{-1})
2.0	Citrate-HCl	k_1	4.26×10^{-3} (0.063 M)	4.30×10^{-3} (0.032 M)	4.29×10^{-3} (0.016 M)	4.29×10^{-3} [b]
3.0	Citrate-HCl	k_1	2.66×10^{-3} (0.079 M)	2.51×10^{-3} (0.040 M)		2.59×10^{-3} [b]
4.0	Citrate-HCl	k_1	2.57×10^{-3} (0.124 M)	2.64×10^{-3} (0.062 M)		2.61×10^{-3} [b]
5.0	Acetate	k_1	2.65×10^{-3} (0.2 M)	2.74×10^{-3} (0.1 M)		2.69×10^{-3} [b]
		k_1	3.05×10^{-3} (0.2 M)	3.01×10^{-3} (0.1 M)	2.64×10^{-3} (0.05 M)	2.64×10^{-3} [a] (0.815)
6.0	Phosphate	k_1	3.68×10^{-3} (0.2 M)	3.44×10^{-3} (0.15 M)	3.34×10^{-3} (0.05 M)	2.99×10^{-3} [a] (0.975)
		k_1	3.59×10^{-3} (0.2 M)	2.97×10^{-3} (0.1 M)	2.64×10^{-3} (0.05 M)	2.36×10^{-3} [a] (1.000)
7.0	Phosphate	k_1	4.97×10^{-3} (0.2 M)	4.01×10^{-3} (0.1 M)	3.53×10^{-3} (0.05 M)	3.05×10^{-3} [a] (1.000)
8.0	Tetraborate-HCl	k_1	1.54×10^{-3} (0.086 M)	1.22×10^{-2} (0.043 M)	9.90×10^{-3} (0.022 M)	8.31×10^{-3} [a] (0.995)
		k_2^+	7.88×10^{-2}	4.49×10^{-2}	3.55×10^{-2}	1.85×10^{-2} [a] (0.992)
		k_2^-	9.69×10^{-2}	5.07×10^{-2}	4.31×10^{-2}	2.00×10^{-2} [a] (0.979)
9.0	Tetraborate-HCl	k_1	8.94×10^{-2} (0.131 M)	6.91×10^{-2} (0.066 M)	5.43×10^{-2} (0.033 M)	4.41×10^{-2} [a] (0.995)
		k_2^+	4.97×10^{-1}	3.81×10^{-1}	2.69×10^{-1}	2.11×10^{-1} [a] (0.983)
		k_2^-	6.04×10^{-1}	4.52×10^{-1}	3.32×10^{-1}	2.56×10^{-1} [a] (0.993)
10.0	Tetraborate-NaOH	k_1	0.34 (0.149 M)	0.33 (0.075 M)	0.40 (0.075 M)	0.36 [b]
		k_2^+	1.30	1.86	1.91	1.69 [b]
		k_2^-	1.60	2.34	1.93	1.96 [b]
11.0	Phosphate-NaOH	k_1	3.38 (0.182 M)	3.72 (0.091 M)		3.55 [b]
		k_2^+	13.7	14.6		14.2 [b]
		k_2^-	16.5	17.7		17.1 [b]
12.0	Phosphate-NaOH	k_1	37.8 (0.135 M)	29.3 (0.067 M)		33.6 [b]

a) Rate constant obtained by extrapolation to zero buffer concentration.
b) Average. Correlation coefficient of the regression line is given in parenthesis.

$$k_1 = k_{H^+} \times a_{H^+} + k_0 + k_{OH^-} \times a_{OH^-} \quad (3)$$

Analysis of the k_1s at pH 2.0—12.0 by a weighted least-squares method[22] according to Eq. 3 afforded values of $1.60\times10^{-1}\ h^{-1}\ M^{-1}$ for k_{H^+}, $2.62\times10^{-3}\ h^{-1}$ for k_0, and $3.69\times10^{3}\ h^{-1}\ M^{-1}$ for k_{OH^-}. The solid line in Fig. 5 is the predicted rate–pH profile generated by using the above parameters and Eq. 3. A fairly good fit between the experimental values and predicted line was obtained, suggesting that the overall degradation can be approximated by a simple specific acid-base catalyzed degradation.

The values of k_2^+ and k_2^-, the rate constants of the apparent reversible reaction or epimerization reaction, increased markedly with increase of pH, as shown in Fig. 5. The effect of ethanol on the degradation and epimerization rate at pH 9.0, 25 °C is shown in Fig. 6. The values of k_1 and k_2s decreased with increase of ethanol content, namely with decrease of the dielectric constant.[23] At pH 9.0, cefsulodin molecules are all in anionic form. Therefore, the

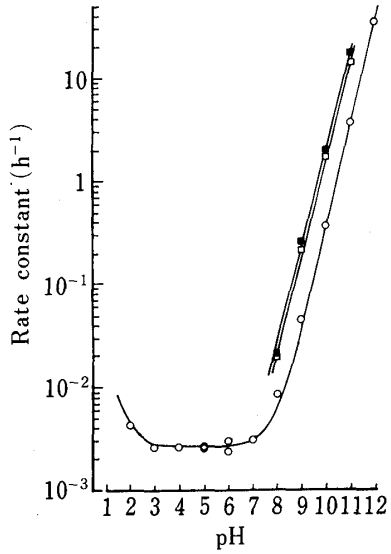

Fig. 5. Rate-pH Profile of the Degradation and the Epimerization Rate Constants at 25 °C and $\mu=0.6$

○, k_1; □, k_2^+; ■, k_2^-.

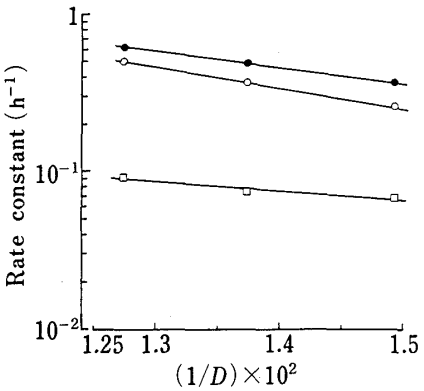

Fig. 6. Solvent Effects on the Degradation and Epimerization of Cefsulodin in Aqueous Solution at 25 °C and $\mu=0.6$

□, k_1; ○, k_2^+; ●, k_2^-.

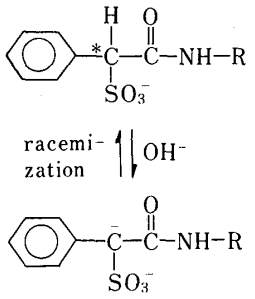

Chart 2. Mechanism of the Epimerization of Cefsulodin

results shown in Fig. 5 indicate that the counter reactant of the epimerization is an anion,[24] that is hydroxide ion, in both hydrolysis (k_1 term) and the epimerization reaction (k_2^+ and k_2^- terms). The fact that the counter reactant of epimerization is hydroxide ion leads to the hypothesis for the mechanism of epimerization shown in Chart 2. The elimination of a proton by hydroxide ion may occur firstly to give the optically inactive anion intermediate. Then, the latter is reprotonated to form D(−) and/or L(+) optical isomers. In view of the hydroxide ion dependency of the epimerization, reprotonation of the anion intermediate seems to be quite rapid compared to deprotonation, and the latter may be the rate-limiting step. Thus, the epimerization is described by the apparent forward and reverse reactions shown in Chart 1. On the basis of the above epimerization mechanism, the ratio of D(−)- and L(−)-epimers at equilibrium should be 1 : 1. However, the amount of D(−)-epimer at equilibrium was slightly larger than that of L(+)-epimer, indicating that the conformation of D(−)-epimer is more stable than that of L(+)-epimer.

Unlike the other β-lactam antibiotics with an α-substituted benzyl side chain, cefsulodin is fully purified to the D(−)-form for clinical use. D(−)-Epimers are generally far more potent than L(+)-epimers. For example, L(+)-sulbenicillin has only 2.7% potency as compared to D(−)-sulbenicillin.[10] Furthermore, pure D(−)-cefsulodin scarcely epimerized below neutral pH, although carbenicillin was shown to epimerize rapidly under mild conditions and pure D(−)-carbenicillin is not superior to the DL mixture in clinical effectiveness.[25] Thus, cefsulodin injection has the great advantage of chemical purity over the other β-lactam antibiotics having an α-sustituted benzyl side chain. However, the decrease of potency due to

conversion to the L(+)-epimer is very sensitive to alkali. Thus, consideration should be paid to the decrease of potency not only due to hydrolysis but also due to epimerization to the inactive epimer.

Effect of Temperature

Figure 7 shows the Arrhenius plots of the degradation constants, k_1s, at pHs 2, 4, 6 and 9 and epimerization rate constants, k_2s, at pH 9.0. From the slope, the apparent activation energies for k_1 at pHs 2, 4, 6 and 9 were calculated to be 20.7, 22.3, 23.0 and 27.7 kcal mol^{-1}, respectively, and values of 27.0 and 26.1 kcal mol^{-1} were obtained, respectively, for k_2^+ and k_2^- at pH 9.0. The hydroxide ion catalysis in epimerization is also apparent from the similar values of apparent activation energies to that of degradation at pH 9.0, which seems to include the heat of ionization of water.

At pH 2.0, the fraction ratio of ionized and unionized species is 1:4 (pK_a 2.6[15]). Thus, degradation may occur by the simultaneous proton-catalyzed hydrolysis of both ion species. The linearity of the Arrhenius plots of the rate constants at pH 2.0 suggests that the activation energies of acid hydrolysis of both species are almost equal. The activation energies at pH 4.0 and 6.0 should reflect those of water-catalyzed hydrolysis of the ionized species whose mole fraction is almost 1.0 at this pH region. Similarly, the apparent activation energy at pH 9.0 seems to be that of hydroxide-catalyzed hydrolysis of the ionized species, including the heat of ionization of water (13.1 kcal mol^{-1}). Thus, the unbuffered degradation rate constant at temperature T can be written as:

$$k_{pH}^{T} = a_{H^+} k_{H^+} \exp(-20.7\times10^3\ (298.15-T)/(\boldsymbol{R}T\times298.15)) + k_0 \exp(-22.7\times10^3\ (298.15-T)/(\boldsymbol{R}T\times298.15)) + k_{OH^-}(10^{-14}/a_{H^+}) \exp(-27.7\times10^3\ (298.15-T)/(\boldsymbol{R}T\times298.15)) \quad (4)$$

where $\boldsymbol{R}$ is the gas constant and 22.7×10^3 is the average of the apparent activation energies at pH 4.0 and 6.0. For example, the predicted k_{pH} in unbuffered solution at pH 6.0 and 35 °C is calculated to be 9.27×10^{-3} h^{-1}, 3.5 times larger than that at 25 °C (2.66×10^{-3} h^{-1}). This agrees well with the general rule that an increase of temperature by 10 °C increases the reaction rate by 2—3 times.

Interaction with Amines and Aminoglycosides

Figure 8 shows the effects of ethylenediamine and kanamycin on the degradation of cefsulodin. At the concentration of amines used in the current study, the degradation obeyed

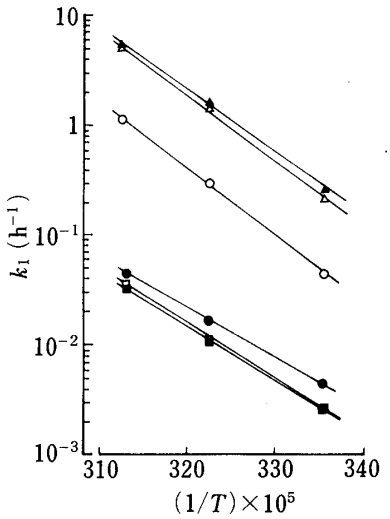

Fig. 7. Arrhenius Plots of the Rate Constants of the Degradation and Epimerization at μ= 0.6

k_1: ●, pH 2.0; ■, pH 4.0; □, pH 6.0; ○, pH 9.0. pH 9.0: △, k_2^+; ▲, k_2^-.

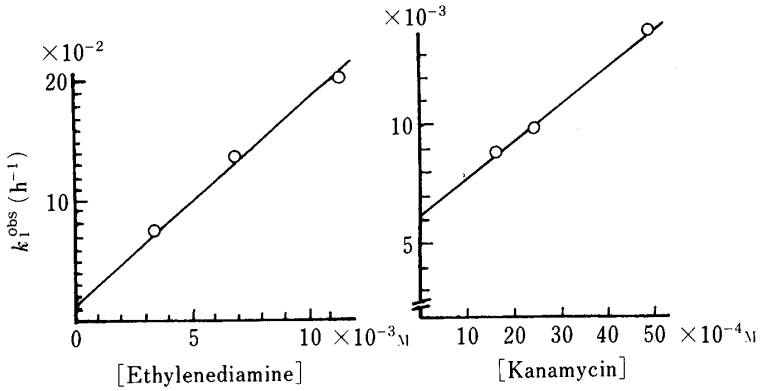

Fig. 8. Effects of Amines on the Degradation of Cefsulodin at 25 °C and $\mu=0.6$
Ethylenediamine, pH 8.0; kanamycin, pH 7.0.

TABLE II. Pseudo Second-Order Rate Constants of the Reaction of Amines with Cefsulodin at 25 °C and $\mu=0.6$

Amines	pK_a (°C)	k_{AM} ($h^{-1}M^{-1}$)			
		pH 7.0	pH 8.0	pH 9.0	pH 10.0
Ethylenediamine	9.70[a] (25)		17.0		
Tromethamine	7.73[a] (25)		4.47		
Methylamine	10.7[a] (25)		0.64		
Isobutylamine	10.7[a] (25)		0.44		
n-Propylamine	10.7[a] (25)		0.37		
ε-Aminocaproic acid	10.7[a] (25)		0.37		
Amikacin	8.39[b] (25)	1.44	22.2	98.5	300
Kanamycin	7.86[b] (25)	1.21	23.9	98.0	358
Ribostamycin	7.97[b] (25)	1.40	22.5	76.0	227

a) Determined by the half-neutralization method.
b) Reference 27.

pseudo-first-order kinetics. In both cases, the values of pseudo-first-order rate constant increased linearly with increase of the concentration of amine or aminoglycoside, indicating a pseudo-second-order reaction of cefsulodin with these amines.

The pK_as and the pseudo-second-order rate constants for amines and aminoglycosides are listed in Table II. No Brønsted relationship was observed between the pK_as and the second-order rate constants. The rate constants for tromethamine and kanamycin were larger than those of other amines with high pK_as. Further, ethylenediamine was remarkably reactive compared to other alkylamines although the pK_a values were almost equal. This substantiates the suggestion that the rate-limiting factor is intramolecular catalysis by a functional group adjacent to the amine moiety.[26]

The pseudo-second-order rate constants of aminoglycosides were also larger than those of alkylamines. This may arise from the larger number of amine moieties per molecule and catalysis by adjacent hydroxy moieties of aminoglycosides.

The reaction of cefsulodin with aminoglycosides was studied at alkaline pH. The results are listed in Table II. Figure 9 shows the pH-rate profile of the apparent second-order reaction rate constant, k_{AM}, for each aminoglycoside. The equation for the second-order rate constant may include the direct molecular interaction of aminoglycoside and cefsulodin and the hydroxide ion-catalyzed interaction. From an analysis of the data by a weighted least-squares

method,[22] it was found that Eq. 5 gave reasonable parameters.

$$k_{AM}=(k_{AH(OH)}a_{OH^-})\frac{a_{H^+}}{K_a+a_{H^+}}+(k_A+k_{A(OH)}a_{OH^-})\frac{K_a}{K_a+a_{H^+}} \quad (5)$$

The rate constants in Eq. 5 are listed in Table III and the predicted k_{AM}–pH profile of each aminoglycoside is shown by solid lines in Fig. 9.

Equations 6 and 7 also gave reasonable parameters as listed in Table IV with virtually the same sum of squares as obtained with Eq. 5.

$$k_{AM}=(k_{AH(OH)}a_{OH^-})\frac{a_{H^+}}{K_a+a_{H^+}}+(k_{A(OH)}a_{OH^-})\frac{K_a}{K_a+a_{H^+}} \quad (6)$$

$$k_{AM}=(k_A+k_{A(OH)}a_{OH^-})\frac{K_a}{K_a+a_{H^+}} \quad (7)$$

Equations 6 and 7 generated identical k_{AM}–pH profiles, and these profiles were also identical with those generated from Eq. 5 (shown in Fig. 9). As previously mentioned, $k_{AH(OH)}a_{OH^-}a_{H^+}$ in Eq. 6 and k_AK_a in Eq. 7 are equivalent mathematically[27] and $k_{AH(OH)}a_{OH^-}a_{H^+}+k_AK_a$ in Eq. 5 also has the same meaning. From these results and the rate law proposed by Tsuji *et al.*,[28] it is considered that the dominant reactions involved are hydroxide ion-catalyzed aminolysis by unionized molecules and nucleophilic aminolysis by unionized aminoglycoside molecules.

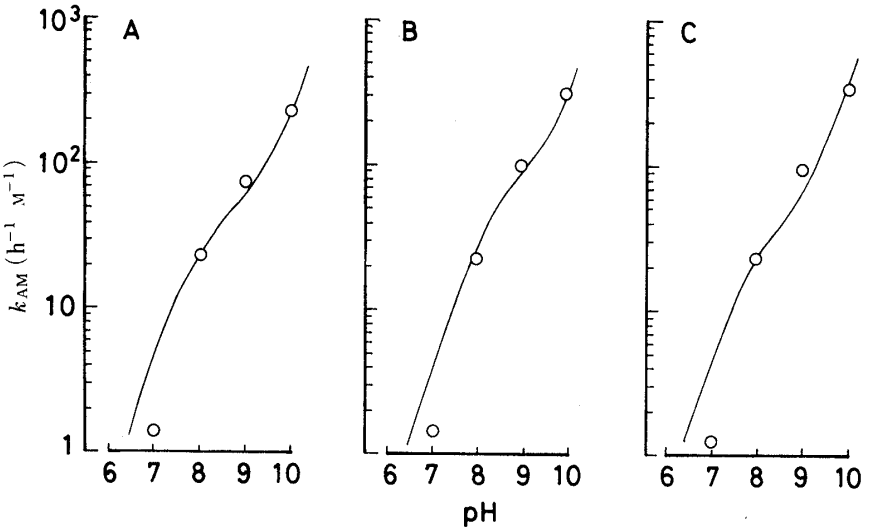

Fig. 9. Rate-pH Profile of the Pseudo-Second-Order Rate Constants of the Interaction of Cefsulodin with Aminoglycosides at 25 °C and $\mu=0.6$

A, ribostamycin; B, amikacin; C, kanamycin.

TABLE III. Micro Rate Constants for the Pseudo Second-Order Rate Constants of Reaction of Aminoglycoside with Cefsulodin in Eq. 5 at 25 °C and $\mu=0.6$

Aminoglycoside	$k_{AH(OH)}$ ($h^{-1}M^{-2}$)	k_A ($h^{-1}M^{-1}$)	$k_{A(OH)}$ ($h^{-1}M^{-2}$)
Kanamycin	4.99×10^5	3.54×10	3.36×10^6
Amikacin	8.58×10^6	6.36×10	2.26×10^6
Ribostamycin	6.02×10^6	3.73×10	1.92×10^6

TABLE IV. Micro Rate Constants for the Pseudo Second-Order Rate Constants of Reaction of Aminoglycoside with Cefsulodin in Eqs. 6 and 7 at 25 °C and $\mu=0.6$

Aminoglycoside	$k_{AH(OH)}$ ($h^{-1}M^{-2}$)	k_A ($h^{-1}M^{-1}$)	$k_{A(OH)}$ ($h^{-1}M^{-2}$)
Kanamycin	4.93×10^{7}	3.57×10	3.36×10^{6}
Amikacin	3.45×10^{7}	8.47×10	2.26×10^{6}
Ribostamycin	4.61×10^{7}	4.30×10	1.92×10^{6}

Pharmaceutical Considerations

Cefsulodin is an excellent β-lactam antibiotic with an α-substituted benzyl side chain; the active D(−)-epimer can be obtained in pure form and is optically stable at ordinary pH. However, epimerization to the less active L(+)-form occurs rapidly at alkaline pH. Equation 1 can be transformed to Eq. 8.

$$D_t=\left[\frac{1}{k_2^+ + k_2^-}(k_2^- + k_2^+ \exp(-k_2 t))\right]\times[D_0 \exp(-k_1 t)] \tag{8}$$

where k_2 is the summation of k_2^+ and k_2^- and represents the equilibrium rate constant. The first bracket in Eq. 8 shows the ratio of D(−)-cefsulodin to total cefsulodin. The activity of L(+)-cefsulodin against *P. aeruginosa* is negligible compared to that of D(−)-cefsulodin.[14] Thus, the conversion to the L(+)-epimer involves loss of the strong intrinsic activity of the D(−)-epimer against *P. aeruginosa*. As shown in Table V, the $t_{0.8}$s for k_2 at pH 8.0 and 9.0 are 5.8 and 0.48 h, respectively, by which times the ratio of D(−)-cefsulodin is about 90% because the equilibrium constant is almost 1. These rates are much faster than the rate of degradation by hydrolysis. From Eq. 8, the total remaining D(−)-cefsulodin at these times at pH 8.0 and 9.0 are 87 and 88%, respectively. Thus attention should be paid to the problem of inactivation by epimerization at alkaline pH in addition to hydrolysis and aminolysis. Injections with high

TABLE V. Predicted $t_{0.9}$ and $t_{0.5}$ of the Total Degradation and the Equilibrium of Epimerization[a] of Cefsulodin at 25 °C and $\mu=0.6$ in Unbuffered Solution

pH		Predicted rate constant (h^{-1})	$t_{0.9}$ (h)	$t_{0.5}$ (h)
2.0	k_1	4.25×10^{-3}	24.8	163
3.0	k_1	2.86×10^{-3}	36.8	242
4.0	k_1	2.72×10^{-3}	38.7	255
5.0	k_1	2.70×10^{-3}	39.0	257
6.0	k_1	2.74×10^{-3}	38.5	253
7.0	k_1	3.05×10^{-3}	34.5	227
8.0	k_1	6.25×10^{-3}	16.9	111
	k_2	3.86×10^{-2}	5.8[a]	18.0
9.0	k_1	3.82×10^{-2}	2.8	18.1
	k_2	4.67×10^{-1}	0.48[a]	1.48
10.0	k_1	3.58×10^{-1}	0.29	1.94
	k_2	3.65	0.06[a]	0.19
11.0	k_1	3.55	0.03	0.20
	k_2	3.13×10^{1}	7.1×10^{-3} [a]	2.2×10^{-2}
12.0	k_1	3.55×10^{1}	3.0×10^{-3}	2.0×10^{-2}

a) 80% equilibrium time ($t_{0.8}$). k_2: refer to Eq. 8.

pH (aminophylline, 5-fluorouracil, and tegafur injections, for example) should not be admixed with cefsulodin, as with other β-lactam antibiotics injections.

On the other hand, $t_{0.9}$ in the pH range of 2.0—7.0 at 25 °C is 24.8—39 h (Table V), indicating sufficient stability for clinical use. In this pH region, both aminolysis and epimerization are negligible or do not occur. In fact, it was confirmed that the decrease of D(−)-cefsulodin in mixtures of below neutral pH was relatively small during incubation at 25 °C (the amount of cefsulodin remaining in each case was more than 90% at 6 h).[29] Thus, if the pH of a mixture is adjusted to below neutral pH by adding an appropriate injection such as sodium ascorbate injection, epimerization by an additive with high pH and aminolysis by amine drug injection should be avoided.

Acknowledgements The authors are grateful to Prof. Itsuo Nishioka and Assistant Prof. Yukihiro Shoyama of Kyushu University for facilities for elemental analysis and to Mrs. Yukiko Harima and Mis Eiko Saeki for their assistance in some of the experiments.

References and Notes

1) A part of this work was presented at the 102nd Annual Meeting of the Pharmaceutical Society of Japan, Osaka, April 1982.
2) P. Finholt, G. Jürgensen, and H. Kristiansen, *J. Pharm. Sci.*, **54**, 387 (1965).
3) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, **58**, 447 (1969).
4) T. Yamana and A. Tsuji, *J. Pharm. Sci.*, **65**, 1563 (1976).
5) E. S. Rattie, J. T. Zimmermann, and L. J. Ravin, *J. Pharm. Sci.*, **68**, 1369 (1979).
6) A. Tsuji, E. Nakashima, Y. Deguchi, K. Nishide, T. Shimizu, S. Horiuchi, K. Ichikawa, and T. Yamana, *J. Pharm. Sci.*, **70**, 1120 (1981).
7) H. Bundgaard and C. Larsen, *Arch. Pharm. Chem. Sci. Ed.*, **6**, 184 (1978).
8) K. Yamaoka, S. Narita, T. Nakagawa, and T. Uno, *J. Chromatogr.*, **168**, 187 (1979).
9) P. A. Twomey, *J. Pharm. Sci.*, **70**, 824 (1981).
10) H. Nomura, K. Kawamura, M. Shinohara, Y. Masuda, Y. Okada, and S. Fujii, *J. Takeda Res. Lab.*, **3**, 442 (1972).
11) R. Weise, N. Wright, and P. J. Wills, *Rev. Infec. Dis.*, **4**, S564 (1982).
12) J. A. Ziemniak, D. A. Chiarmonte, D. J. Miner, and J. J. Schentag, *J. Pharm. Sci.*, **71**, 399 (1982).
13) M. Nakamura, K. Sugeno, R. Konaka, H. Yamada, and T. Yoshida, *J. Pharm. Sci.*, **71**, 1188 (1982).
14) H. Nomura, T. Fugono, T. Hitaka, I. Minami, T. Azuma, S. Morimoto, and T. Masuda, *J. Med. Chem.*, **17**, 1312 (1974).
15) I. Aoki, E. Konishi, and M. Kuwayama, *Iyakuhin Kenkyu*, **9**, 1022 (1978).
16) M. A. Schwartz, *J. Pharm. Sci.*, **57**, 1209 (1968).
17) M. A. Schwartz and A. J. Delduce, *J. Pharm. Sci.*, **58**, 1137 (1969).
18) A. Tsuji, T. Yamana, E. Miyamoto, and E. Kiya, *J. Pharm. Pharmacol.*, **27**, 580 (1975).
19) T. Yamana, Y. Mizukami, F. Ichimura, and K. Yokogawa, *Yakuzaigaku*, **38**, 74 (1978).
20) *a*) L. Elrod, Jr., L. B. White, D. C. Wimer, and R. D. Cox, *J. Chromatogr.*, **237**, 515 (1982); *b*) G. R. Granneman and L. T. Sennello, *J. Pharm. Sci.*, **71**, 1112 (1982).
21) *a*) K.-G. Wahlund and A. Sokolowski, *J. Chromatogr.*, **151**, 299 (1978); *b*) T. L. Hafkenscheid and E. Tomlinson, *Int. J. Pharmaceut.*, **17**, 1 (1983).
22) K. Yamaoka, T. Tanigawa, T. Nakagawa, and T. Uno, *J. Pharm. Dyn.*, **4**, 879 (1981).
23) G. Akerlöf, *J. Am. Chem. Soc.*, **54**, 4125 (1932).
24) T. Yamana (ed.), "Kinetics of Drugs," Nankodo, Tokyo, 1982, p. 75.
25) K. Butler, A. R. English, V. A. Ray, and A. E. Timreck, *J. Infec. Dis.*, **122**, Supplement, S1 (1970).
26) T. Yamana, A. Tsuji, E. Miyamoto, and E. Kiya, *J. Pharm. Pharmacol.*, **27**, 771 (1975).
27) T. Fujita, Y. Harima, and A. Koshiro, *Chem. Pharm. Bull.*, **31**, 2103 (1983).
28) A. Tsuji, E. Miyamoto, and T. Yamana, *J. Pharm. Sci.*, **68**, 616 (1979).
29) A. Koshiro, T. Fujita, Y. Harima, and E. Saeki, *Yakuzaigaku*, **43**, 76 (1983).