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Degradation Kinetics of Sodium Sulbactam in Aqueous Solutions

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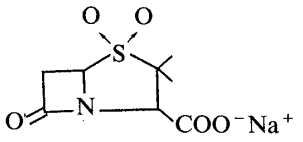
The degradation kinetics of sodium sulbactam in various buffer solutions have been investigated over a pH range of 0.74 to 9.95 at 35 °C and at an ionic strength of 0.5. The amounts of intact sodium sulbactam and a degradation product were determined by an ion-pair reversed-phase high-performance liquid chromatographic method. The degradation kinetics were consistent with a scheme in which sodium sulbactam is degraded to 2-amino-3-methyl-3-sulfinobutanoic acid and formylacetic acid *via* 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoic acid, which is a labile intermediate. The observed degradation rates at various pHs followed pseudo-first-order kinetics. The pH-rate profile showed that the maximum stability of sodium sulbactam occurred in the pH range of 3.0 to 7.0, and that sodium sulbactam is much more stable than potassium clavulanate over the whole range of pH examined.

Keywords——sulbactam; beta-lactamase inhibitor; sulbactam aqueous solution stability; sulbactam degradation kinetics; clavulanate

Introduction

Sodium sulbactam, sodium (2*S*,5*R*)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]-heptane-2-carboxylate 4,4-dioxide (I), is a potent and semisynthetic β-lactamase inhibitor.[1] The combined use of the inhibitor with penicillins or cephalosporins *in vitro* and *in vivo* is, therefore, effective against β-lactamase-producing bacteria.[2–4]



I

In a previous paper,[5] we discussed the alkaline degradation pathways of sodium sulbactam based on the structural investigation of the degradation products. The present paper deals with the kinetic aspects of the degradation reactions which occur in buffer solutions of various pHs.

Experimental

Reagents and Materials——Sodium sulbactam was supplied by Pfizer Taito Co., Ltd. (Tokyo, Japan). 2-Amino-3-methyl-3-sulfinobutanoic acid (II) (λ_{max} 229 nm in methanol), which had been identified as a degradation product of sodium sulbactam,[5] was prepared by the following procedures; an aqueous solution of sodium sulbactam was mixed with a half volume of 1 N NaOH solution, and the mixture was kept standing at room temperature for 10 min, then neutralized with 1 N HCl solution. This procedure quantitatively converted sodium sulbactam into II (confirmed by high-performance liquid chromatography (HPLC)). The resulting solution was used as a standard solution for the

HPLC determination of II in the kinetic study. Tetrabutylammonium bromide (TBAB) and buffer salts were purchased from Nakarai Chemicals Co. (Kyoto, Japan) and were used without further purification. Deionized distilled water and distilled methanol were used to prepare the buffer solutions and the HPLC eluents.

Kinetic Procedures——The buffer solutions used were hydrochloric acid–potassium chloride for pH 0.74, 0.88, 1.09, 1.63, 1.80 and 2.22, lactic acid–sodium lactate for pH 2.60, 2.83, 3.08, 3.48, 3.76, 4.66 and 4.95, monobasic–dibasic phosphate for pH 5.38, 5.97, 6.45, 6.84, 7.29 and 7.81, boric acid–sodium borate for pH 8.31 and 8.81, and sodium bicarbonate–sodium carbonate for pH 9.30 and 9.95. The ionic strengths of all buffer solutions were adjusted to 0.5 by addition of potassium chloride, if necessary. The pH values at the experimental temperature were measured on a pH meter (model F-8, Horiba Co., Kyoto). An accurately weighed amount of sodium sulbactam was dissolved in the various buffer solutions, which had been preheated to the desired temperature, to make a final concentration of 4×10^{-3} M. A 5-ml aliquot of each solution was sealed in a small glass vial and kept in a thermostated water-bath at 35 ± 0.1 °C. A 100-μl aliquot of the solution was accurately withdrawn at appropriate time intervals and diluted with 900 μl of the HPLC eluent. The diluted solution was immediately subjected to chromatography under the conditions described below. The concentrations of the product II and remaining sodium sulbactam were determined by referring to the calibration lines.

Analytical Procedures——A liquid chromatograph (TRI ROTAR-V, Japan Spectroscopic Co., Ltd., Tokyo, Japan) equipped with a variable-wavelength detector (UVIDEC-100-V, Japan Sepctroscopic Co.) was used with a stationary phase of Chemcosorb 5ODS-H (Chemco Sientific Co., Ltd., Osaka, Japan) packed in 15 cm × 4.6 mm i.d. stainless steel tubing. A precolumn packed with Chemcosorb 5ODS-H (3 cm × 4.6 mm i.d.) was used to guard the main column. The eluent used was an aqueous solution containing 5 mM TBAB, 1 mM Na_2HPO_4, and 1 mM NaH_2PO_4 mixed with methanol at a volume ratio of 2 : 1. The flow rate was maintained at 0.7 ml/min (80 kg/cm^2). The detection wavelength was 235 nm. All operations were performed at ambient temperature. For sulbactam assay, the six standard solutions (10—100 μg/ml) were prepared by dissolving sodium sulbactam in H_2O–HPLC eluent = 1 : 9 (v/v). The calibration graph, which was obtained daily, was constructed by plotting peak height *vs.* concentration. For the assay of II, the standard solutions of sodium sulbactam (200—2000 μg/ml) were quantitatively converted to II according to the procedures described above. The calibration graph, which was also obtained daily, was constructed by plotting peak height *vs.* concentration (equivalent to sodium sulbactam). The calibration graphs from sodium sulbactam and II were both linear and passed through the origin.

Data Analysis——The numerical calculations were carried out on a microcomputer (MZ-2000, Sharp Co., Osaka, Japan) with programming in BASIC.

Results

HPLC Results and Degradation Pathways

Figure 1 shows the time-dependent changes in the chromatograms of sodium sulbactam

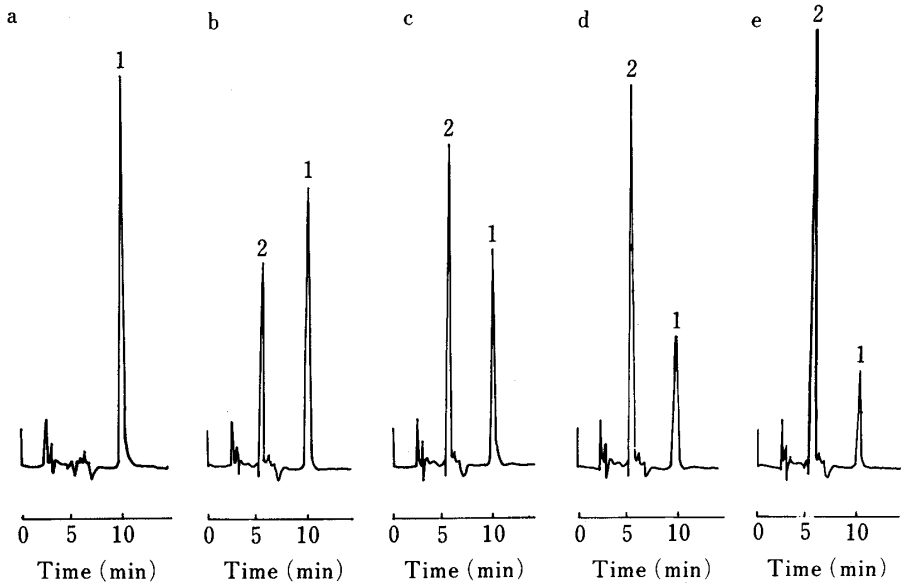


Fig. 1. Chromatograms of Sodium Sulbactam Degraded in 0.3 M Borate Buffer at pH 8.81, 35 °C and $\mu=0.5$

Reaction times: a, 0 h; b, 6 h; c, 12 h; d, 16 h; e, 24 h. Key: 1, sodium sulbactam; 2, 2-amino-3-methyl-3-sulfinobutanoic acid. HPLC conditions, see the text.

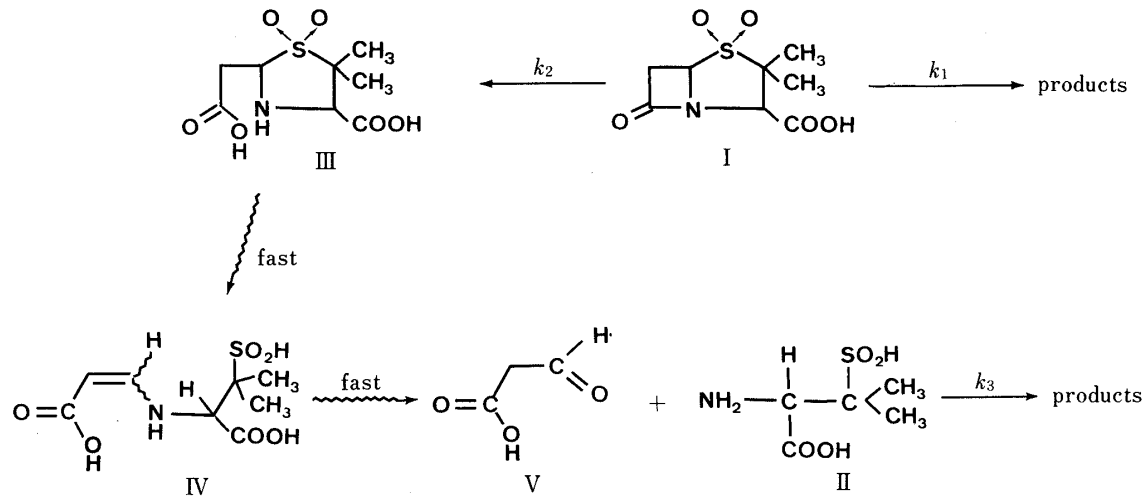


Chart 1. Degradation Pathways of Sulbactam

degraded at pH 8.81; peak 1 is due to intact sulbactam, and peak 2 has the same retention time as II. When the reaction was continued until 24 h (Fig. 1e), about 75% of the initial amount of sodium sulbactam was degraded and no degradation product other than II was found on the chromatogram. The minor peaks observed regardless of the reaction time are presumably base line fluctuations. The degradation in other pH regions (acidic and neutral) gave almost the same results as in Fig. 1. This indicates that the degradation of sodium sulbactam in buffer solutions of pH 0.74 to 9.95 proceeds according to the pathways (Chart 1) which we proposed in the previous paper[5] for the alkaline degradation of sodium sulbactam.

Woodard *et al.*[6] reported that thiazolidine sulfones that bear no substituent group on nitogen, such as III in Chart 1, suffer rapid fission of the C–S bond. It is also reported[7] that 5-carboxy-6-methyl-6-sulfino-4-aza-2-heptenoic acid (IV) thus formed is unstable in acidic, neutral and weakly alkaline media, but is rather stable at pH > 13. Thus, the intermediate products III and IV in Chart 1 obtained in the present experiments are too unstable to be detected chromatographically, and formylacetic acid (V), which is a co-product with II, gives an HPLC peak only when the eluate is alkalized.[5] The presence of V in the sodium sulbactam solutions degraded in the pH range of 0.74—9.95 was supported by the postcolumn alkalization of the eluate. In the acidic region, V could be decarboxylated to yield acetaldehyde.[8] The product II thus formed is quite stable, but the chromatographic response (peak 2 in Fig. 1) decreases gradually on prolonged exposure of product II to the buffer solutions.

The degradation kinetics of sodium sulbactam and II at pH 1.09 (HCl–KCl) and 8.81 (0.3 M boric acid–sodium borate) at 35 °C and $\mu = 0.5$ were simultaneously followed by HPLC. The degradation rate constants were evaluated according to Chart 1 by using the following equations;

$$[\mathrm{I}]/C_0 = e^{-(k_1+k_2)t} \tag{1}$$

$$[\mathrm{II}]/C_0 = \frac{k_2}{(k_1+k_2)-k_3}\left[e^{-k_3 t} - e^{-(k_1+k_2)t}\right] \tag{2}$$

where [I] and [II] are the concentrations of sodium sulbactam and II at time t, respectively; C_0 is the initial concentration of sodium sulbactam; and k_i is the first-order rate constant (k_1, degradation of sodium sulbactam other than hydrolysis of the β-lactam moiety; k_2, hydrolysis of the β-lactam moiety of sodium sulbactam; k_3, degradation of II). The experimental data were analyzed on the basis of Eqs. 1 and 2 by means of the non-linear least-squares method

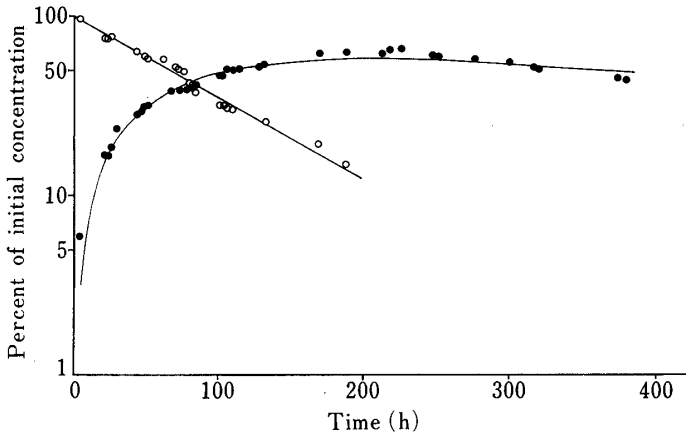


Fig. 2. Degradation Kinetics of Sodium Sulbactam (○) and II (●) during the Degradation of Sodium Sulbactam in HCl–KCl Buffer at pH 1.09, 35 °C and $\mu=0.5$

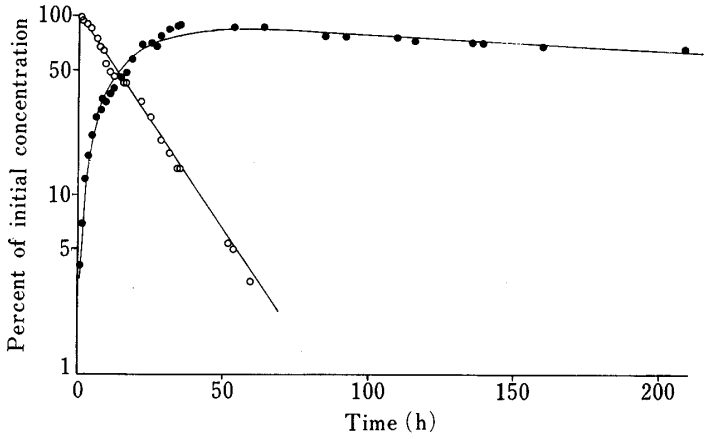


Fig. 3. Degradation Kinetics of Sodium Sulbactam (○) and II (●) during the Degradation of Sodium Sulbactam in 0.3 M Borate Buffer at pH 8.81, 35 °C and $\mu=0.5$

(MULTI).[9] The pertinent values of rate constants for the degradation at pH 1.09 are estimated to be $k_1=0.164\times10^{-2}$ (h^{-1}), $k_2=0.862\times10^{-2}$ (h^{-1}), and $k_3=0.176\times10^{-2}$ (h^{-1}). Those at pH 8.81 are $k_1=0.231\times10^{-2}$ (h^{-1}), $k_2=0.0526$ (h^{-1}), and $k_3=0.240\times10^{-2}$ (h^{-1}). The theoretical curves obtained by using these k values are shown in Figs. 2 and 3, where the circles indicate the experimental values.

Observed Rate Constants

The degradation of sodium sulbactam was studied at various pHs and buffer concentrations at a constant temperature (35 °C) and ionic strength (0.5). Figures 4, 5, and 6 show semilogarithmic plots of residual percent of sodium sulbactam *versus* time in the acidic, neutral, and alkaline regions, respectively. The observed first-order rate constants, k_{obs}, were estimated by means of the least-squares method from the slopes of the plots.

General Acid–Base Catalysis

The catalytic effects of the buffer salts used in the kinetic studies were investigated with various buffer concentrations at constant pH, temperature, and ionic strength. The results are given in Table I.

The catalytic effects of phosphate ions over a pH range of 5.38—7.81 are shown in Fig. 7,

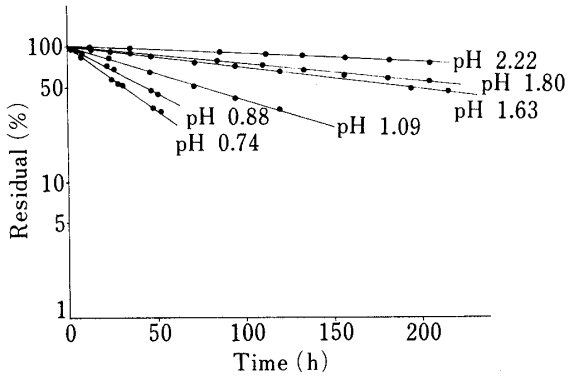


Fig. 4. Observed First-Order Plots for the Degradation of Sodium Sulbactam at Various pH Values in the Acidic Region at 35 °C and $\mu=0.5$

The buffer salts used were HCl–KCl.

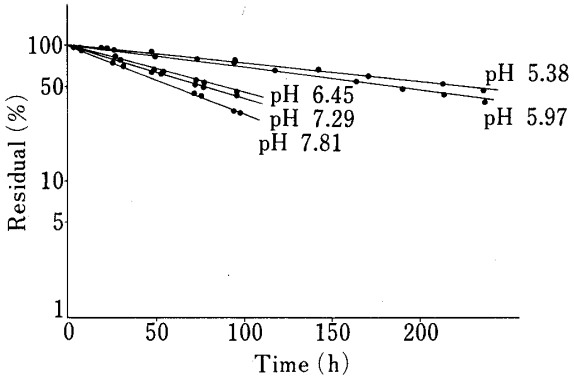


Fig. 5. Observed First-Order Plots for the Degradation of Sodium Sulbactam at Various pH Values in the Neutral Region at 35 °C and $\mu=0.5$

Total buffer concentration: 0.3 M for pH 5.38, 5.97; 0.25 M for pH 6.45; 0.18 M for pH 7.29; 0.15 M for pH 7.81. The buffer salts used were dihydrogen and monohydrogen phosphates.

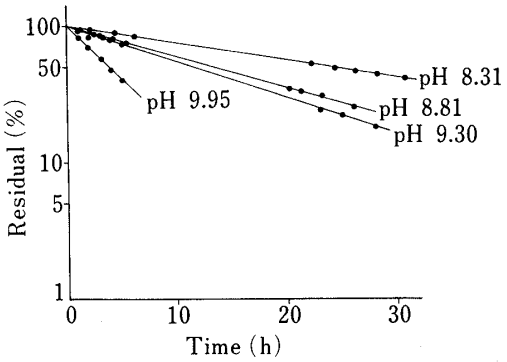


Fig. 6. Observed First-Order Plots for the Degradation of Sodium Sulbactam at Various pH Values in the Alkaline Region at 35 °C and $\mu=0.5$

Total buffer concentration: 0.3 M for pH 8.31, 8.81, 9.30; 0.25 M for pH 9.95. For the buffer salts used, see Experimental.

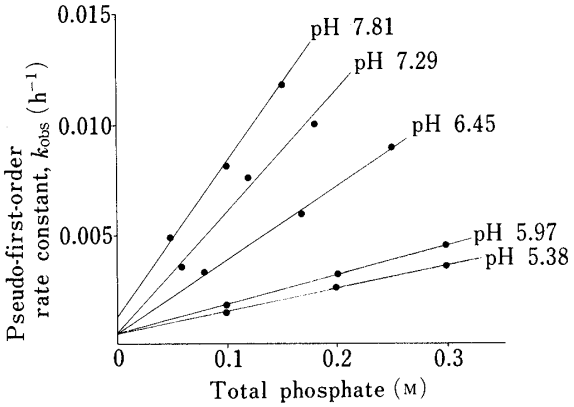


Fig. 7. Dependence of the Pseudo-First-Order Rate Constant, k_{obs}, on Total Phosphate Buffer Concentration at Various pHs at 35 °C and $\mu=0.5$

which shows that the observed first-order rate constant increased linearly with the buffer concentrations at a constant pH. Taking account of the pK_a value (6.59)[10] of the dihydrogen phosphate ion at 35 °C ($\mu=0.5$), the observed first-order rate constants, k_{obs}, in the phosphate buffer systems used can be written as follows;

$$k_{obs}=k_{pH}+k_{H_2PO_4^-}[H_2PO_4^-]+k_{HPO_4^{2-}}[HPO_4^{2-}] \quad (3)$$

where k_{pH} represents the rate constant at zero buffer concentration; $k_{H_2PO_4^-}$ and $k_{HPO_4^{2-}}$ are the second-order rate constants due to dihydrogen and monohydrogen phosphate ions, respectively; $[H_2PO_4^-]$ and $[HPO_4^{2-}]$ are the concentrations of dihydrogen and monohydrogen phosphate ions, respectively. By using total phosphate concentration $[Ph]_T$, Eq. 3 can be rearranged to

$$k_{obs}=k_{pH}+(k_{H_2PO_4^-}f_{H_2PO_4^-}+k_{HPO_4^{2-}}f_{HPO_4^{2-}})[Ph]_T \quad (4)$$

where $f_{H_2PO_4^-}$ and $f_{HPO_4^{2-}}$ represent the mole fractions of dihydrogen and monohydrogen phosphate ions, respectively, in total phosphate concentration; thus $pK_a=pf_{HPO_4^{2-}}-pf_{H_2PO_4^-}+pH$ and $f_{H_2PO_4^-}+f_{HPO_4^{2-}}=1$. The phosphate catalytic rate constants ($k_{H_2PO_4^-}$ and $k_{HPO_4^{2-}}$) obtained from Eq. 4 and the slopes in Fig. 7 are listed in Table II together with those for other buffer systems obtained in a similar manner.

TABLE I. Effects of Buffer Concentration and pH on Rate Constants (k_1+k_2 in Chart 1) for Degradation of Sulbactam at 35 °C and at an Ionic Strength of 0.5

pH (Buffer)	$10^3\times k_{obs}$ (h^{-1})			$10^3\times k_{pH}$ (h^{-1})
	0.10 M	0.20 M	0.30 M	
0.74 (HCl–KCl)	—	—	—	21.5
0.88 (HCl–KCl)	—	—	—	16.8
1.09 (HCl–KCl)	—	—	—	10.0
1.63 (HCl–KCl)	—	—	—	3.84
1.80 (HCl–KCl)	—	—	—	2.39
2.22 (HCl–KCl)	—	—	—	1.48
2.60 (Lactate)	1.59	1.89	2.03	1.40
2.83 (Lactate)	1.35	1.66	1.98	1.03
3.08 (Lactate)	0.796	1.01	1.34	0.504
3.48 (Lactate)	0.770	1.04	1.46	0.401
3.76 (Lactate)	0.884	1.22	1.59	0.521
4.66 (Lactate)	1.21	1.93	2.77	0.413
4.95 (Lactate)	1.08	1.78	2.46	0.378
5.38 (Phosphate)	1.44	2.55	3.53	0.417
5.97 (Phosphate)	1.73	3.22	4.45	0.410
6.45 (Phosphate)	3.27[a]	5.87[b]	9.02[c]	0.428
6.84 (Phosphate)	3.49[d]	8.12[e]	10.4[f]	0.429
7.29 (Phosphate)	3.40[g]	7.73[h]	9.93[i]	0.487
7.81 (Phosphate)	4.76[j]	8.04[k]	11.7[l]	1.21
8.31 (Borate)	11.4	20.8	29.0	2.80
8.81 (Borate)	21.1	41.8	52.7	6.93
9.30 (Carbonate)	30.8	52.4	62.0	17.2
9.95 (Carbonate)	148[a]	179[b]	191[c]	130

Buffer concentration (M); *a*) 0.08, *b*) 0.17; *c*) 0.25; *d*) 0.07; *e*) 0.14; *f*) 0.21; *g*) 0.06; *h*) 0.12; *i*) 0.18; *j*) 0.05; *k*) 0.10; *l*) 0.15. The ionic strengths of all buffer solutions were adjusted to 0.5 by addition of KCl, if necessary.

TABLE II. Catalytic Rate Constants of Phosphate, Lactate, Borate, and Carbonate Buffer Ions ($\mu=0.5$)

Buffer	$k_a\times10^2$ ($M^{-1}h^{-1}$)	$k_b\times10^2$ ($M^{-1}h^{-1}$)
Phosphate[a]	0.362	5.95
Lactate[b]	0.178	0.399
Borate[c]	3.70	29.4
Carbonate[d]	8.56	19.2

a) k_a, $H_2PO_4^-$; k_b, HPO_4^{2-}. *b*) k_a, $CH(CH_3)(OH)COOH$; k_b, $CH(CH_3)(OH)COO^-$. *c*) k_a, H_3BO_3; k_b, $H_2BO_3^-$. *d*) k_a, HCO_3^-; k_b, CO_3^{2-}.

pH-Rate Profile

The pH-rate profile for the degradation of sodium sulbactam was obtained by plotting the values of $\log k_{pH}$ against pH. The k_{pH} values used for the plots were obtained from the intercepts of the plots of k_{obs} *versus* total buffer concentration at various times (Fig. 7). The $\log k_{pH}$–pH profile was fitted to the equation

$$k_{pH}=k_{H_2O}+k_H a_H+k_{OH}(K_W/a_H) \quad (5)$$

where k_H and k_{OH} represent the second-order rate constants for the hydrogen and hydroxide ion-catalyzed degradations, respectively; k_{H_2O} is the rate constant for the spontaneous or water-catalyzed degradation; a_H is the activity of hydrogen ions measured with a glass electrode; and K_W is the autoprotolysis constant for water, 2.09×10^{-14} at 35 °C ($\mu=0.5$). From the experimental values k_{H_2O}, k_H, and k_{OH} were calculated by means of a weighted least-squares method using a microcomputer, the results being 0.402×10^{-3} (h^{-1}), 0.127 ($M^{-1}\,h^{-1}$), and 4.28×10^{2} ($M^{-1}\,h^{-1}$), resepectively. The solid curve illustrated in Fig. 8 was obtained by substituting these k values into Eq. 5, and the closed circles show the experimental data. The pH for the maximal stability of sodium sulbactam, which is given by

$$pH_{min}=\frac{1}{2}(pk_{OH}-pk_H+pK_W) \quad (6)$$

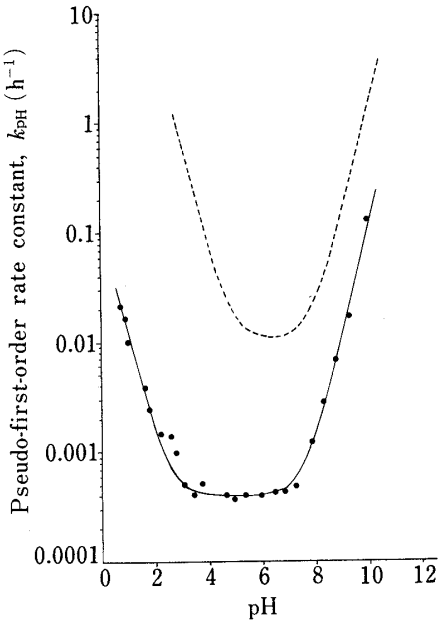


Fig. 8. The pH-Rate Profile of the Degradation of Sodium Sulbactam at 35 °C and $\mu=0.5$

The closed circles indicate experimental values, the solid line shows the theoretical curve according to Eq. 5, and the dotted line represents the curve for potassium clavulanate at 35 °C and $\mu=0.5$.[13]

TABLE III. Rate Constants and Arrhenius Activation Parameters for Degradation of Sodium Sulbactam at $\mu=0.5$

pH	T (°C)	$k_{pH}\times10^2$ (h^{-1})	E_a (kcal/mol)	$\log A$ (h^{-1})
1.80	35	0.239	22.7	13.5
	50	1.34		
	65	6.39		
5.38	35	0.0417	23.3	13.2
	50	0.300		
	65	1.22		
8.81	35	0.693	29.7	19.0
	50	9.62		
	65	51.6		

is estimated to be 5.08. Sodium sulbactam was stable in the pH range of 3.0—7.0.

Temperature Effect

The effect of temperature on the degradation of sodium sulbactam was studied at three different pH values in the acidic, neutral, and alkaline regions at $\mu=0.5$. The rate constants at 35, 50, and 65 °C, the apparent Arrhenius activation energies (E_a), and the values of frequency factor are given in Table III. The Arrhenius parameters for the degradation at pH 1.80 and 5.38 were very similar.

Discussion

In the previous paper,[5] we reported that sodium sulbactam was degraded in aqueous alkaline solutions to yield II and V *via* IV. The present results indicate that Chart 1 proposed for the alkaline degradation of sodium sulbactam is valid over the entire pH range. The ratios $k_2/k_1=5.25$ for pH 1.09 and $k_2/k_1=22.8$ for pH 8.81 show that the β-lactam ring opening is the main route for the degradation of sodium sulbactam. The ratios $k_2/k_3=4.90$ for pH 1.09 and $k_2/k_3=21.9$ for pH 8.81 show that II is much more stable than sodium sulbactam.

It is interesting to compare the degradation pathways of sodium sulbactam with those of penicillins. It is well known that penicillins are hydrolyzed in neutral and alkaline regions to penicilloic acids, and are converted to penicillenic acids in acidic media.[11] However, since the hydrolysis product (III) of the β-lactam ring of sodium sulbactam is unstable compared with penicilloic acids, III undergoes subsequent fission of the C–S bond to yield IV, which is further degraded to II and V.

The pH-rate profile, without a shoulder-type break in the pK_a zone (p$K_a=2.6$ for sodium sulbactam),[12] reveals that the proton-catalyzed degradation rates for the ionized and unionized species of sodium sulbactam are almost the same. In the previous paper,[13] we reported the degradation kinetics of potassium clavulanate, which is also a β-lactamase inhibitor. The pH-rate profile of sodium sulbactam is different from that of potassium clavulanate (dotted line in Fig. 8). The values of k_{H_2O}, k_H, and k_{OH} for potassium clavulanate were 1.07×10^{-2} (h^{-1}), 7.64×10^{2} ($M^{-1}h^{-1}$), and 6.14×10^{3} ($M^{-1}h^{-1}$), respectively. This means that the degradation of sodium sulbactam is about 25 times slower in the neutral region, about 10 times slower in the alkaline region, and 6000 times slower in the acidic region than that of potassium clavulanate. Thus, it follows that sodium sulbactam is much more stable than potassium clavulanate, especially in the acidic region.

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