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Kinetic Analysis of the Effect of Polyhydric Alcohols on Ampicillin Degradation in the Presence and Absence of Aldehydes in Aqueous Solution

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Ampicillin degradation is accelerated by some carbohydrates. The kinetics of the degradation in the presence of polyhydric alcohols (xylitol, glycerin, ethyleneglycol) was studied in alkaline solution, because these alcohol were expected to exhibit a similar accelerating effect.

In these experiments, the degradation of ampicillin obeyed apparent-first-order kinetics and the rate constants increased with increasing concentration of polyhydric alcohols. The rate constant, however, was not affected by monohydric alcohols such as ethanol, propanol, and ethyleneglycol-monomethylether.

The degradation of ampicillin was accelerated only by polyhydric alcohols which have adjacent hydroxy groups. This suggests that nucleophilic attack of a hydroxy anion on the β-lactam ring became easier as a result of simultaneous hydrogen bonding among the two adjacent hydroxy groups of alcohols and the amide-carbonyl and β-lactam carbonyl group of ampicillin.

The rate accelerating effect of polyhydric alcohols on the degradation of ampicillin was depressed by the addition of aldehydes. This inhibition seems to be attributable to the steric hindrance and resonance effect of Schiff's base formation between the α-amino group of ampicillin and the aldehyde.

Keywords——ampicillin degradation; xylitol; polyhydric alcohol; benzaldehyde; furfural; Schiff's base; ampicillin–xylitol interaction; ampicillin–aldehyde interaction

Xylitol has frequently been used in infusion fluids, as have glucose, fructose, *etc.*, and it is given clinically as a mixed injection with ampicillin. Thus, it is important to investigate the effect of xylitol on the degradation of ampicillin in aqueous solution. In this work, the interaction between xylitol and ampicillin was studied. Further, the effect of the addition of other alcohols was also examined in order to analyze the acceleration mechanism. The stabilization of ampicillin in the presence of alcohols by adding aldehydes in aqueous solution was evaluated.

Experimental

Materials——Ampicillin sodium (Meiji Seika Kaisha Ltd.) was used as supplied. Furfural, benzaldehyde, ethanol, butanol, propanol, ethyleneglycol, ethyleneglycol-monomethylether and trimethyleneglycol (Wako Chemical Ind., Ltd.) were of the highest commercial grade available. Glycerin was of pharmacopoeial quality. Xylitol used was kindly supplied by Taiho Pharm. Co., Ltd.

I_2-Colorimetry——Ampicillin was determined by I_2-colorimetry as described previously.[1] Intact ampicillin in aqueous solution can be assayed by that method.

Kinetic Procedures——The buffer solutions for the kinetic run on ampicillin degradation consisted of phosphate (0.02, 0.04, 0.05, 0.10 and 0.15 M). The ionic strength of these buffers was adjusted to 0.5 by the addition of KCl. The reaction temperature was always maintained at 35 ± 0.5 °C. Ampicillin was dissolved in an appropriate buffer solution (with or without aldehyde), which contained various concentrations of alcohols and which had been preheated to the desired temperature. At suitable intervals, samples were withdrawn and assayed immediately. The pH

of the solution was read initially and at the end of the experiment with a pH-meter (Toa pH-meters model HM-20 E). No significant pH change of buffer solution was observed during any experiment. Further, the admixed alcohols did not interfere with the measurement of ampicillin by I_2-colorimetry.

Results and Discussion

Kinetics of Xylitol-Accelerated Degradation of Ampicillin and Effect of Aldehyde Addition

Time courses of the degradation of ampicillin in phosphate buffer (pH 8.00, $\mu=0.5$) were studied at 35 °C. In every case, a plot of the logarithm of residual ampicillin *versus* time was linear (Fig. 1). From the results of Fig. 1, it was found that ampicillin degraded according to apparent-first-order kinetics. In addition, the degradation of ampicillin increased with increasing xylitol concentration, but the acceleration by xylitol was inhibited by the addition of aldehyde. Further, as Fig. 2 shows, a linear relation between the observed first-order-rate constant (k_{obs}) for ampicillin degradation and the concentration of xylitol was obtained. The slope of the straight line was constant regardless of buffer concentration, while it became smaller with increasing aldehyde concentration. The accelerating effect of xylitol on the β-lactam ring cleavage seems to be independent of buffer concentration.[2]

Similar results were obtained at pH's 7.00 and 9.00 as shown in Fig. 3. On the other hand,

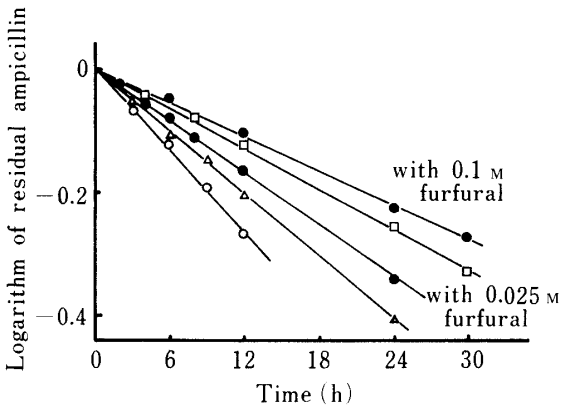


Fig. 1. Apparent-First-Order Plots for the Degradation of Ampicillin in the Presence of Various Concentrations of Xylitol with and without Furfural in 0.04 M Phosphate Buffer at pH 8.00, 35 °C ($\mu=0.5$)

○●, with 0.06 M xylitol; △, with 0.04 M xylitol; □, with 0.02 M xylitol.
Closed circles indicate furfural addition.

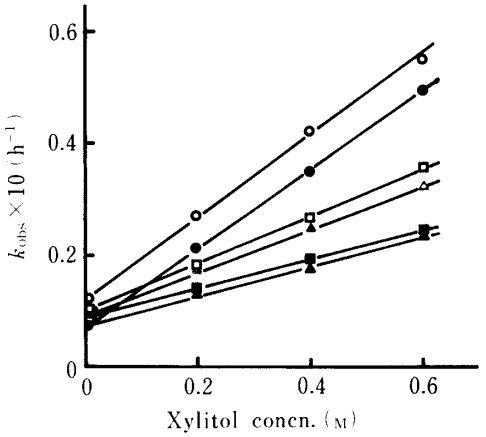


Fig. 2. Effect of Xylitol on the Apparent-First-Order Rate Constant of Ampicillin Degradation with or without Aldehydes in Phosphate Buffer of pH 8.00 ($\mu=0.5$) at 35 °C

○, 0.02 M phosphate buffer; ●, 0.04 M phosphate buffer; ■, 0.04 M phosphate buffer with 0.05 M benzaldehyde; □, 0.04 M phosphate buffer with 0.025 M benzaldehyde; ▲, 0.04 M phosphate buffer with 0.10 M furfural; △, 0.04 M phosphate buffer with 0.05 M furfural.

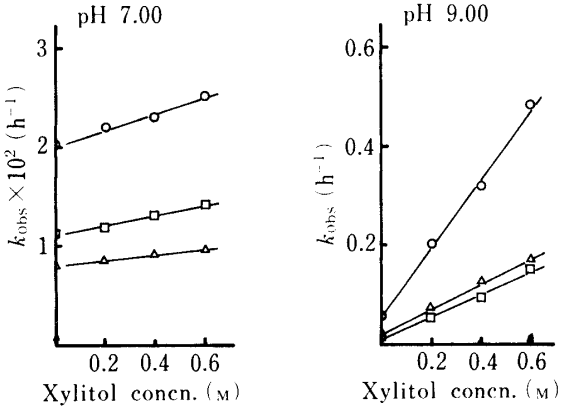


Fig. 3. Effect of Xylitol on the Apparent First-Order Rate Constant of Ampicillin Degradation with or without Aldehydes in Phosphate Buffer of pH 7.00 and Carbonate Buffer of pH 9.00 at 35 °C ($\mu=0.5$)

○, ampicillin; □, ampicillin with 0.025 M benzaldehyde; △, ampicillin with 0.1 M furfural.

no accelerating effect of xylitol on ampicillin degradation was found at pH's 3.00 and 5.00. As can be seen in Figs. 2 and 3, a plot of k_{obs} of ampicillin degradation *versus* xylitol concentration was linear. Thus, Eq. 1 is valid.

$$k_{obs}=k_a+k_{a\cdot al}[Al] \tag{1}$$

where k_a = apparent-first-order rate constant for the hydrolysis of ampicillin, $k_{a\cdot al}$ = catalytic second-order-rate constant of alcohol, and [Al] = alcohol concentration.

Furthermore, a plot of k_{obs} *versus* xylitol concentration was linear even if aldehydes were added, and this is similar to the findings with carbohydrates previously described.[2] That is to say, the accelerating effect of carbohydrates on ampicillin degradation is inhibited by Schiff's base formation[1-4] between ampicillin and aldehydes.

Equation 2, then, it considered to be valid.[2]

$$k_{obs}=\frac{k_a+k_sK[S]}{K[S]+1}+\frac{k_{a\cdot al}+k_{s\cdot al}K[S]}{K[S]+1}[Al] \tag{2}$$

where k_s = first-order-rate constant for the degradation of the Schiff's base, $k_{s\cdot al}$ = catalytic second-order-rate constant of alcohol for the degradation of Schiff's base, K = formation constant of Schiff's base and [S] = aldehyde concentration.

Bundgaard and Klixbüll[5] have recently reported that 4-imidazolidinone formation between the α-aminoamide side-chain function of ampicillin and various carbonyl compounds is valid in the stabilization of ampicillin in basic aqueous solution. Hetacillins in which the 4-imidazoridinone structure is present, is stable in acidic aqueous solution and can be recrystallized at pH 2—3.[6] However, adducts which were obtained in basic solutions of ampicillin and aldehydes were not formed in acidic solution and dissociated to the original ampicillin and aldehydes immediately at pH < 4. Thus, the adducts in this study were estimated to be Schiff's bases on the basis of the above physicochemical properties and ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectra.[3]

According to Eq. 2, it is necessary for the aldehyde concentration to be constant during reaction in order to obtain a linear relationship between k_{obs} and [Al]. In this run, since aldehyde used are more than 100 times greater than that of ampicillin, aldehyde concentrations may be regarded as constant even when though the two substances react. From Eq. 2, the slope of k_{obs} *versus* alcohol concentration, that is, the second-order-rate constant, is given by Eq. 3.

$$\text{slope}=\frac{k_{a\cdot al}+k_{s\cdot al}K[S]}{K[S]+1} \tag{3}$$

Further, assuming that the degradation of the Schiff's base formed between ampicillin and aldehydes is not accelerated by xylitol, $k_{s\cdot al}$ is considered to be zero. Then, Eq. 4 is obtained.

$$\text{slope}=\frac{k_{a\cdot al}}{K[S]+1} \tag{4}$$

The calculated values obtained from Eq. 4 by using $k_{a\cdot al}$, K and [S], and the experimental values are summerized in Table I. In each case, the values are in good agreement. Consequently, it was proved that the degradation of the Schiff's base formed between ampicillin and aldehyde is not accelerated by xylitol.

Interaction between Alcohols and Ampicillin

It was considered that the degradation of ampicillin may be accelerated by other alcohols in the same way as by xylitol. First, the interaction between glycerin and ampicillin was

TABLE I. Observed and Calculated Second-Order-Rate Constants for the Reactions of Xylitol and Aldehyde with Ampicillin in Buffer Solutions of Various pH Values at 35 °C

	Second-order rate constant ($M^{-1} h^{-1}$)					
	pH 7.00		pH 8.00		pH 9.00	
	Observed	Calculated	Observed	Calculated	Observed	Calculated
	0.0075		0.07		0.71	
Furfural added						
0.05 M			0.038	0.039		
0.1 M	0.003	0.004	0.025	0.026	0.27	0.26
Benzaldehyde added						
0.01 M			0.042	0.039		
0.025 M	0.005	0.004	0.025	0.024	0.21	0.22

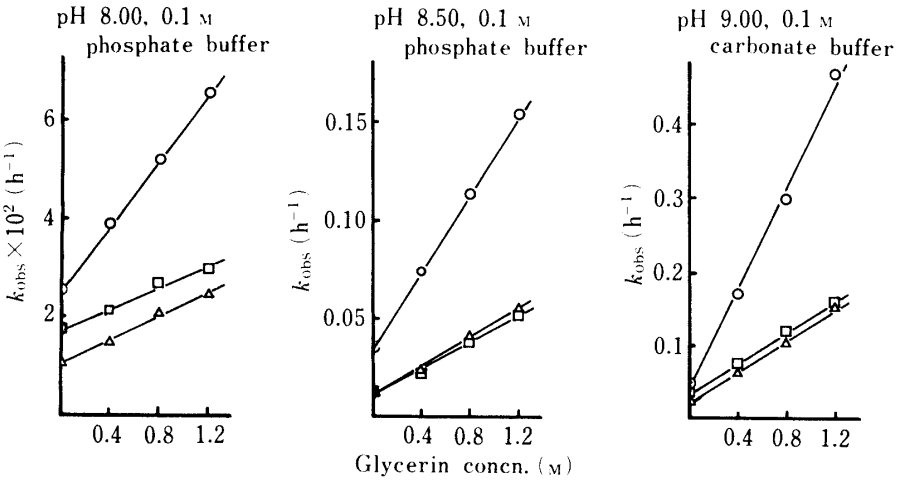


Fig. 4. Effect of Glycerin on the Apparent-First-Order Rate Constant for the Degradation of Ampicillin with or without Aldehydes in Buffer Solution

○, ampicillin; □, ampicillin with 0.025 M benzaldehyde; △, ampicillin with 0.1 M furfural.

investigated. Ampicillin was degraded according to apparent-first-order kinetics at pH's 8.00, 8.50 and 9.00, even if glycerin was added and benzaldehyde or furfural was added simultaneously with glycerin. Then, the apparent-first-order rate constant (k_{obs}) of ampicillin degradation was plotted against the concentration of glycerin (Fig. 4). At every pH, the plot of k_{obs} *versus* glycerin concentration was linear. From these results, it was concluded that the degradation of ampicillin was accelerated by the addition of glycerin and the accelerating effect was inhibited by the addition of aldehydes in the same manner as in the case of xylitol.

Similar experiments on the mutual interaction of ampicillin and alcohols were next carried out by the use of ethanol, butanol, propanol, ethyleneglycol-monomethylether and trimethyleneglycol as monohydric alcohols. The degradation of ampicillin showed apparent first-order kinetics with each alcohol. No accelerating effect on the degradation of ampicillin, however, was caused by the monohydric alcohols (Table II).

Ethyleneglycol promoted the degradation of ampicillin, as did glycerin. There was a linear relation of k_{obs} against ethyleneglycol concentration. Further the second-order-rate constant obtained from the slope became smaller with the addition of aldehydes together with the glycerin.

Second-order-rate constants calculated by means of Eq. 4 are also given in Table II.

TABLE II. Observed and Calculated Second-Order-Rate Constants for the Reactions of Alcohols and Aldehydes with Ampicillin in Buffer Solutions of Various pH Values at 35 °C ($\mu=0.5$)

Alcohol	pH	Aldehyde concn. (M)	Second-order rate constant ($M^{-1}h^{-1}$) Obsd.	Calcd.[a]	Alcohol	pH	Aldehyde concn. (M)	Second-order rate constant ($M^{-1}h^{-1}$) Obsd.	Calcd.[a]
Glycerin	5.0, 7.0	0	0		Propylene glycol	8.0	0	0.014	
	8.0	0	0.025				0.025 (B)	0.005	0.005
		0.025 (B)	0.011	0.016			0.1 (F)	0.005	0.005
		0.1 (F)	0.012	0.013		9.0	0	0.16	
	8.5	0	0.103				0.025 (B)	0.05	0.06
		0.025 (B)	0.032	0.033			0.1 (F)	0.05	0.05
		0.1 (F)	0.037	0.038	Ethyleneglycol monomethylether	8.0	0	0	
	9.0	0	0.34		Trimethylene glycol	8.0	0	0	
		0.025 (B)	0.111	0.107					
		0.1 (F)	0.114	0.123					
Ethyleneglycol	8.0	0	0.008		Ethanol	8.0	0	0	
		0.025 (B)	0.003	0.002	*n*-Propanol	8.0	0	0	
		0.1 (F)	0.004	0.002	*n*-Butanol	8.0	0	0	
	9.0	0	0.075						
		0.025 (B)	0.025	0.024					
		0.1 (F)	0.028	0.029					

a) calcd. $= \frac{k_{a\cdot al}}{K[S]+1}$. (B), benzaldehyde; (F), furfural.

From the foregoing results, it was found that the alcohols which had an accelerating effect on the degradation of ampicillin were polyhydric alcohols which had adjacent hydroxy groups, such as xylitol, glycerin and ethyleneglycol.

Plots of the logarithm of second-order-rate constant *versus* pH became linear with a slope of unity in each case. The results in Tables I and II show specific acid-base catalysis, and therefore Eqs. 5 and 6 were applied.

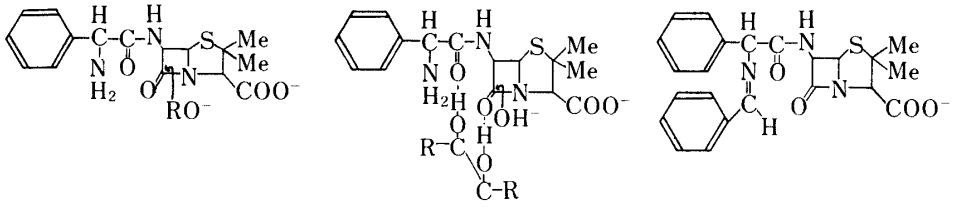
$$k_{al} = a\,OH\,k_{OH\cdot al} \tag{5}$$

$$\log k_{al} = pH - pk_w + \log k_{OH\cdot al} \tag{6}$$

The third-order-rate constants for hydroxy anion catalysis obtained from Eq. 6 were 35000, 15000, 4000 and 7500 $M^{-2}h^{-1}$ for xylitol, glycerin, ethyleneglycol and propyleneglycol, respectively. These values seem to increase with increasing number of hydroxy groups in the alcohol.

Bundgaard and Larsen[7] obtained similar results in an examination of the acceleration effect of carbohydrate and alcohols on the degradation of ampicillin. They suggested that an alkoxide anion derived from the hydroxy group of the carbohydrate and alcohol attacks as a nucleophile at the carbonyl group of β-lactam (Chart 1, I). However, in our experiments, such alcohols as ethyleneglycol and propyleneglycol accelerated the degradation of ampicillin, while ethanol which easily forms an alkoxide anion, did not show such an effect. Further, only polyhydric alcohols which had adjacent hydroxy groups accelerated the degradation of ampicillin. These results are not explicable by Bundgaard's hypothesis.

It is known that hydrogen bonds are generated between the amide group of ampicillin and solvent alcohols as ethanol and isopropanol.[8,9] Consequently, monohydric alcohols form



I II III

Chart 1

hydrogen bond to the amide group of ampicillin. Further, in alcohols which contain adjacent hydroxy groups, it may be considered that one of hydroxy groups forms a hydrogen bond to the amide group and the other forms a similar bond to the β-lactam carbonyl (Chart 1, II). The degradation of ampicillin, then, seems to be accelerated by the easier nucleophilic attack of a hydroxy anion on the β-lactam ring based on the above hydrogen bonding effects.

The accelerating effect on ampicillin degradation in aqueous solution was depressed by the addition of furfural and benzaldehyde. This effect may be attributed to Schiff's base formation between ampicillin and furfural or benzaldehyde; steric hindrance and resonance effects then inhibited the access of the alcohols to the β-lactam ring (Chart 1, III).

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