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Stabilization of Ampicillin Analogs in Aqueous Solution. IV.[1,2] Effect of Addition of Furfural on the Degradation of Ampicillin in the Presence of Various Carbohydrates in Aqueous Solution

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It is well known that the degradation of ampicillin is accelerated by various kinds of carbohydrates in neutral and alkaline aqueous solutions. Thus, the kinetics of ampicillin degradation in the presence of carbohydrate and furfural were investigated to determine how the rate-accelerating effect of carbohydrate is inhibited by furfural.

The rate accelerating effect of carbohydrates increased with increasing molecular weight of the carbohydrates. However, the accelerating effects of sucrose, glucose, maltose and dextran 40 were all inhibited by the addition of furfural at pH 7—9. This inhibition is due to the resistance of the Schiff base, which is formed between ampicillin and furfural, to the action of carbohydrate, presumably because of steric hindrance.

Keywords——ampicillin; furfural; Schiff base; sucrose, glucose, dextran 40, maltose; acceleration by carbohydrate; stabilization of ampicillin; formation constant; kinetics of ampicillin degradation

In a previous paper,[2] we showed that the degradation of ampicillin was inhibited by Schiff base formation between ampicillin and furfural in alkaline aqueous solutions. The formation of the schiff base was deduced from the nuclear magnetic resonance spectra, infrared spectra and chemical properties.[2]

On the other hand, ampicillin is other dispensed or administered in solutions containing various carbohydrates such as glucose, and it is known that these carbohydrates accelerate the degradation of ampicillin at neutral and alkaline pH.[3a,b]

In this paper, therefore, the kinetics of the stabilizing effect of furfural was investigated when furfural was added to solutions containing ampicillin and various carbohydrates.

Experimental

Materials——Ampicillin sodium and furfural used were those described previously.[2] Sucrose and maltose (Wako Chemical Ind. Ltd.) were of the highest commercial grade and glucose was of pharmacopoeial quality (JPX). Dextran 40 used was kindly supplied by Otsuka Pharm. Co., Ltd.

Reagents——All reagents used for I_2-colorimetry were those described in the previous paper.[2] No consumption of iodine was obtained with furfural and/or carbohydrates within the concentrations ranges in these experiments.

Kinetic Procedures——All kinetic studies were carried out in aqueous buffer solutions (phosphate or borate) at 35 ± 0.1°C. Ampicillin was dissolved in an appropriate buffer solution (with or without furfural), which contained various concentrations of carbohydrate and which had been preheated to the desired temperature.

The initial concentrations of ampicillin and furfural were 2.5×10^{-4} and 0.05—0.10 M, respectively. The concentrations of sucrose, glucose, maltose and dextran 40 were 0.025—0.3, 0.2—0.6, 0.1—0.3 and 0.001—0.003 M, respectively. The molarity of dextran 40 was calculated on the basis of a molecular weight of *ca.* 40000, according to JPX.

At suitable intervals, samples were withdrawn, cooled on ice and assayed for intact ampicillin by I_2-colorimetry as described previously.[2] These procedures were carried out two or more times and the values were considered to be accurate to within ±1%. No significant changes in pH of the buffer solutions were observed during any experiment.

Results and Discussion

Kinetics of Sucrose-accelerated Degradation of Ampicillin in Buffer Solutions at pH 8.00

Time courses of the degradation of total ampicillin in 0.03 and 0.10 M phosphate buffer (pH 8.00, μ=0.5), at 35°C, which initially contained ampicillin and various concentrations of sucrose are shown in Fig. 1. In every case, a plot of the logarithm of residual percent *versus* time was linear, and the slope increased with increasing sucrose concentration. Thus, the degradation of ampicillin was accelerated by the addition of sucrose.

In Fig. 2, the values of the observed pseudo-first-order rate constants (k_{obs}) are plotted *versus* sucrose concentration. This shows that the rate of degradation of ampicillin increases linearly with increasing sucrose concentration and the slope is constant regardless of buffer concentration.

In the case of the reaction of penicillins with sucrose in aqueous solutions, it was reported that the relation between the observed pseudo-first-order rate constants and sucrose concentration is nonlinear by Hem *et al.*,[3b] while it was found to be strictly linear by Bundgaard *et al.*[4] The results shown in Fig. 2 support the findings of Bundgaard *et al.*, and the accele rating effects of ampicillin degradation due to the ampicillin-sucrose complexation reported by Hem *et al.* were not observed.

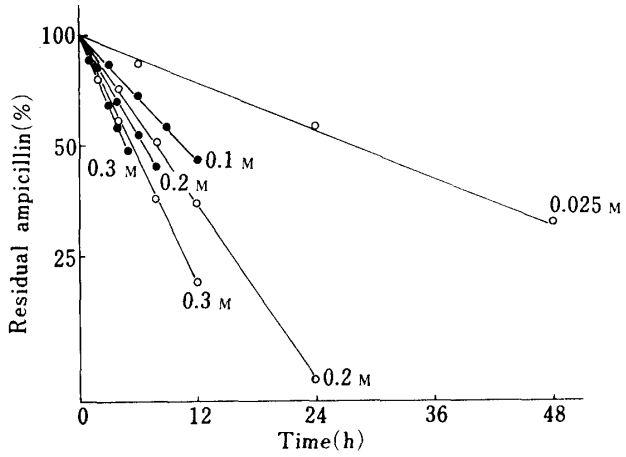

Fig. 1. Pseudo-first-order Plots for the Degradation of Ampicillin in the Presence of Various Concentrations of Sucrose at pH 8.00, 35°C and μ=0.5

○, in 0.03 M phosphate buffer; ●, in 0.10 M phosphate buffer.

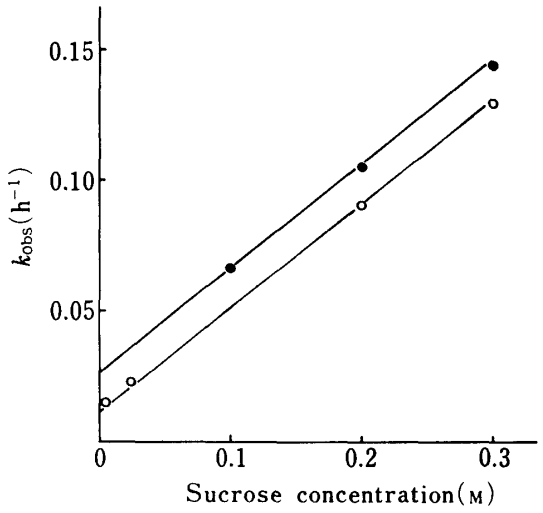

Fig. 2. Effect of Sucrose on the Pseudo-first-order Rate Constant for the Degradation of Ampicillin in Aqueous Solution at pH 8.00, 35°C and μ=0.5

○, in 0.03 M phosphate buffer; ●, in 0.10 M phosphate buffer.
The intercepts at zero sucorse concentration coincide with the found values.[3]

Kinetics and Mechanism of the Degradation of Ampicillin with Sucrose in Aqueous Solutions in the Presence of Furfural

The rates of degradation of intact ampicillin in aqueous sucrose solutions (0.03 M, μ=0.5, phosphate buffer) in the presence of furfural were measured at 35°C and pH 8.00 (Fig. 3). The pseudo-first-order plots of intact ampicillin were linear. Further, the slope decreased with increasing furfural concentration when the same concentration of sucrose was added. This suggests that the sucrose-accelerated degradation of ampicillin was inhibited by the addition of furfural.

In Fig. 4, the k_{obs} values at pH 8.00 are plotted *versus* sucrose concentration. The

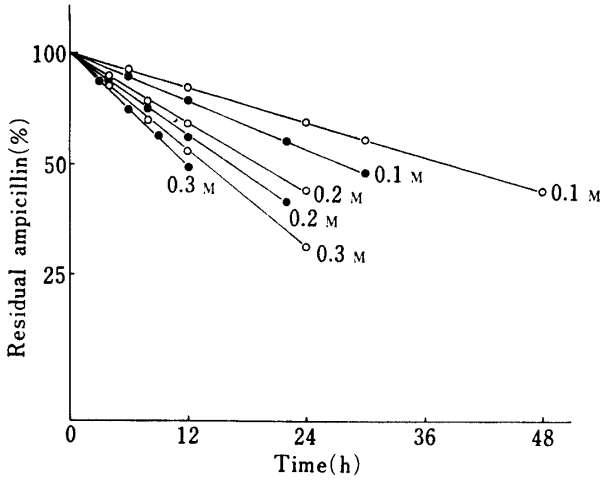

Fig. 3. Pseudo-first-order Plots for the Degradation of Ampicillin in the Presence of Various Concentrations of Furfural and Sucrose in 0.03 M Phosphate Buffer of pH 8.00 ($\mu=0.5$) at 35°C

○, with 0.1 M furfural; ●, with 0.05 M furfural.
The concentrations in the figure are those of sucrose.

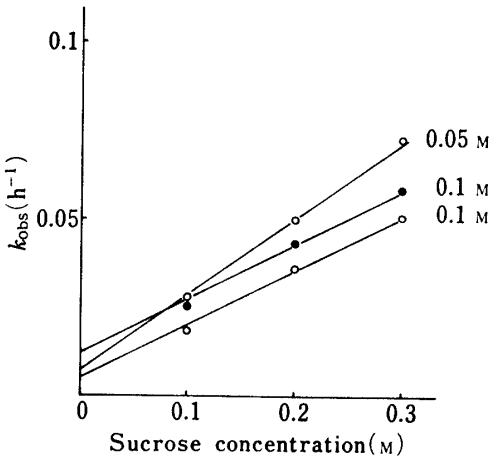

Fig. 4. Effect of Furfural on the Pseudo-first-order Rate Constant for the Degradation of Ampicillin in the Presence of Sucrose in Aqueous Solution at pH 8.00 ($\mu=0.5$), 35°C

○, in 0.03 M phosphate buffer; ●, in 0.10 M phosphate buffer.
The concentrations in the figure are those of furfural, and the intercepts at zero sucrose concentration coincide with the found values.[3]

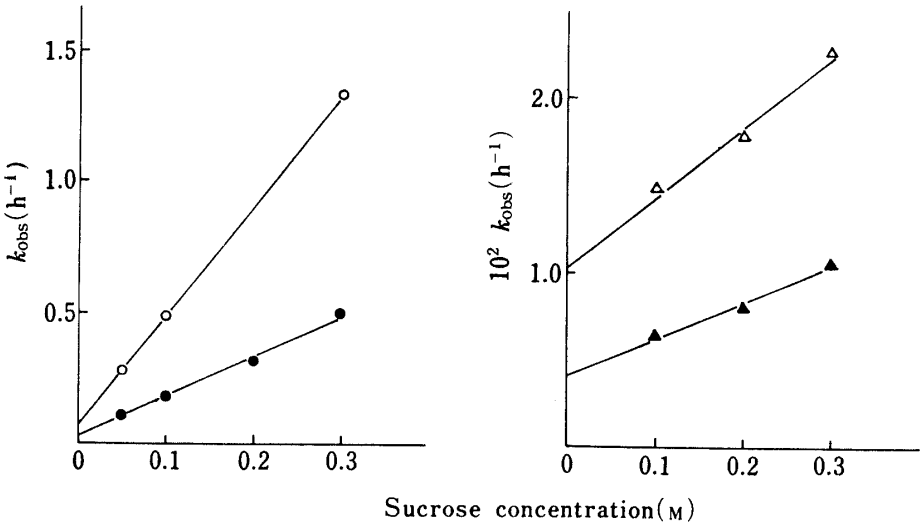

Fig. 5. Effect of Sucrose on the Pseudo-first-order Rate Constant for the Degradation of Ampicillin with or without Furfural in Aqueous Solution at Various pH Values ($\mu=0.5$), 35°C

○, in 0.10 M borate buffer of pH 9.00 without furfural;
●, in 0.10 M borate buffer of pH 9.00 with 0.1 M furfural;
The intercepts at zero sucrose concentration coincide with the found values.[3]
△, in 0.05 M phosphate buffer of pH 7.00 without fufural;
▲, in 0.05 M phosphate buffer of pH 7.00 with 0.1 M furfural.

ampicillin degradation followed pseudo-first-order kinetics at pH 7.00 and 9.00 with or without furfural and sucrose, and the relationship between the resulting rate constant (k_{obs}) and sucrose concentration is shown in Fig. 5.

As can be seen in Figs. 4 and 5, the degradation rate of intact ampicillin in the presence of furfural also increased linearly with increasing sucrose concentration at pH 7.00 and 9.00. Furthermore, it was found that the slope decreased with increasing furfural concentration at

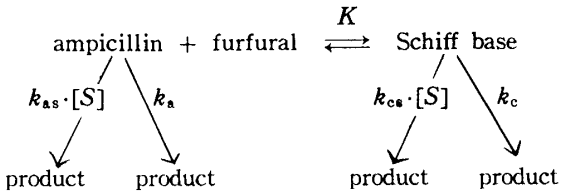

Chart 1

every pH tested.

From the above results, the reaction of these compounds in aqueous solution is assumed to be shown in Chart 1. Then, Eqs. (1) and (2) can be derived from Chart 1:

$$-\frac{d}{dt}[A] = \{k_a + k_{as}[S]\}[A] \tag{1}$$

$$-\frac{d}{dt}[AF] = \{k_c + k_{cs}[S]\}[AF] \tag{2}$$

where k_a=the pseudo-first-order rate constant for hydrolysis of ampicillin in the absence of sucrose, k_c=the pseudo-first-order rate constant for hydrolysis of the Schiff base in the absence of sucrose, k_{as}=apparent second-order rate constant for the reaction of ampicillin with sucrose, k_{cs}=apparent second-order rate constant for the reaction of the Schiff base with sucrose, $[A]$=free ampicillin concentration, $[AF]$=Schiff base concentration and $[S]$=sucrose concentration.

The total concentration, $[A]_T$, of intact ampicillin present in the solution and the formation constant, K, of the Schiff base are described by the following expression

$$[A]_T = [A] + [AF] \tag{3}$$

$$K = \frac{[AF]}{[A][F]} \tag{4}$$

where $[F]$ is the concentration of furfural. Consequently, the overall reaction rate is given by Eq. (5), from Eqs. (1), (2), (3) and (4).

$$-\frac{d}{dt}[A]_T = -\{\frac{d}{dt}[A] + \frac{d}{dt}[AF]\} = \{k_a + k_{as}[S]\}\frac{[A]_T}{K[F]+1}$$
$$+\{k_c + k_{cs}[S]\}\frac{K[F][A]_T}{K[F]+1} = \{\frac{k_a + k_{as}[S] + (k_c + k_{cs}[S])K[F]}{K[F]+1}\}[A]_T \tag{5}$$

For Eq. (5) to result in good first-order kinetics, it is necessary that $[S]$ and $[F]$ are consta nt during experiments and that the reversible formation and dissociation of the Schiff base is very fast compared with the degradations of ampicillin and the Schiff base. The concentration of furfural used for this run was more than 200 times that of ampicillin, and very little loss of furfural was observed at 35°C in the pH range used.[2] The reversible reaction rates of the Schiff base in the solution of ampicillin with furfural should be very fast compared with the degrada-tion rates of free ampicillin and the Schiff base.[2] Thus, Eq. (5) should yield first-order kinetics and k_{obs} will be given by Eq. (6).

$$k_{obs} = \frac{k_a + k_c\cdot K[F]}{K[F]+1} + \frac{k_{as} + k_{cs}\cdot K[F]}{K[F]+1}[S] \tag{6}$$

The slope obtained from the plots of k_{obs} *versus* sucrose concentration in the presence of furfural (Figs. 4 and 5) is expressed as follows.

$$\text{slope} = \frac{k_{as} + k_{cs}\cdot K[F]}{K[F]+1} \tag{7}$$

From Fig. 4, the values of the slope in the presence of 0.05 and 0.10 M furfural were 0.220

and 0.153 M^{-1} h^{-1}, as determined by the least-squares method. Next, putting k_{cs} equal to zero, Eq. (7) is converted to Eq. (8).

$$\text{slope}' = \frac{k_{as}}{K[F] + 1} \tag{8}$$

The value of k_{as} of Eq. (8) was calculated to be 0.389 M^{-1} h^{-1} from the slope shown in Fig. 2. As described in the previous paper,[2] the formation constant, K, is 16.1 M^{-1} at pH 8.00 regardless of buffer concentration. Then, the slope' values (calculated values) obtained by using Eq. (8) and K value in the presence of 0.05 and 0.10 M furfural were 0.216 and 0.149 M^{-1} h^{-1}, which were in good agreement with the found values. Thus, k_{cs} dose seem to be zero or negligible in comparison to k_{as}. This suggests that the Schiff base formed between ampicillin and furfural does not undergo accelerated degradation in the presence of sucrose. The kinetic parameters obtained at pH 7—9 are summarized in Table I. The results in Table I show that the found values of the slope, obtained by plotting k_{obs} *versus* sucrose concentration, and the calculated values agreed very closely with each other, and thus indicate that the Schiff base was not degraded by sucrose at any pH tested. Further, the second-order catalytic rate constant (k_{as}) increased with increasing pH, and the plots of logarithm of k_{as} *versus* pH became linear with a slope of unity (Fig. 6). This result is in fair agreement with that of Bundgaard *et al.*[4]

Table I. Slope of the Plot of k_{obs} *versus* at Various pH Values with 0.1 M Furfural at 35°C (μ=0.5)

pH	K (M^{-1})[a]	k_{as} (M^{-1} h^{-1})	$\frac{k_{as}+k_{cs}\cdot K[F]}{K[F]+1}$ (M^{-1} h^{-1})	
			Observed	Calculated
7.00	8.4	0.0395	0.0211	0.0215
8.00	16.1	0.389	0.153	0.149
9.00	17.8	4.20	1.54	1.51

a) This value was obtained from ref. 2.

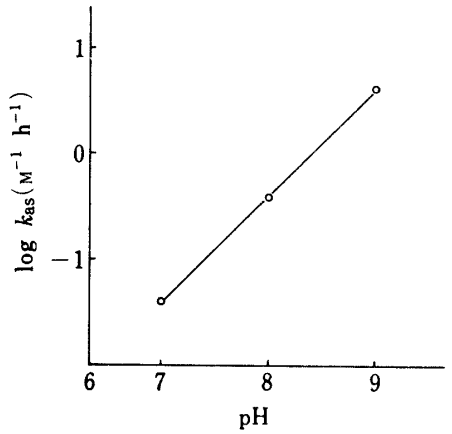

Fig. 6. The logarithm of Second-order Rate Constant for the Sucrose-accelerated Degradation of Ampicillin plotted as a Function of pH

Reactions of Other Carbohydrates

Similar investigations were carried out for glucose, maltose and dextran 40. The time courses of the degradation of intact ampicillin in 0.1 M phosphate buffer (pH 8.00, μ=0.5) at 35°C in the presence of 0.2—0.6 M glucose, 0.1—0.3 M maltose, or 0.001—0.003 M dextran 40 were investigated without or with 0.10 M furfural. In each case, the time courses showed pseudo-first-order kinetics as in the case of sucrose. Plots of the observed first-order rate constants, which were obtained from the above investigations *versus* concentration of each carbohydrate are shown in Fig. 7.

Thus, it was found that the relation between k_{obs} and concentration of carbohydrate was strictly linear, and that the degradation of ampicillin was accelerated by the addition of each carbohydrate,[4] while the accelerating effect was inhibited by furfural as found in the case of sucrose.

The value of $k_{a\cdot carb}$ (from Fig. 7) and the slope according to Eq. (9) can be obtained as

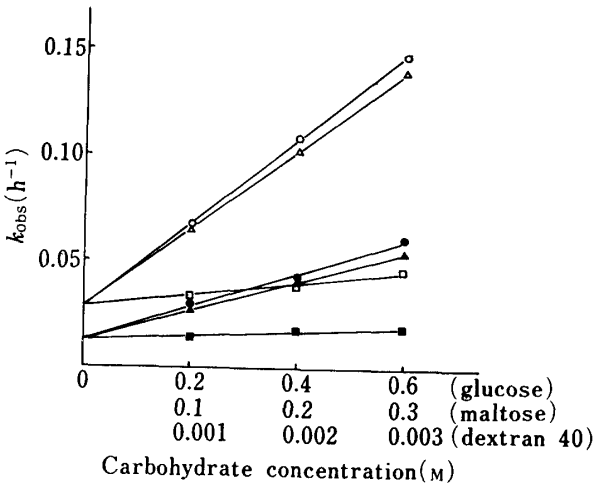

Fig. 7. Effect of Various Carbohydrates on the Pseudo-first-order Rate Constant for the Degradation of Ampicillin with or without 0.1 M Furfural in 0.10 M Phosphate Buffer of pH 8.00 (μ=0.5) at 35°C

○, glucose without furfural; ●, glucose with furfural; △, maltose without furfural; ▲, maltose with furfural; □, dextran 40 without furfural; ■, dextran 40 with furfural.

The intercepts at zero carbohydrates concentrations coincide with the found values.[2]

in the case of sucrose, where $k_{a\cdot carb}$ and $k_{c\cdot carb}$ are the second-order rate constants for the reaction of ampicillin and the Schiff base with carbohydrates (Table II).

$$\text{slope} = \frac{k_{a\cdot carb} + k_{c\cdot carb}K[F]}{K[F] + 1} \qquad (9)$$

The "calculated values" of the slope in Table II are the values obtained by putting $k_{c\cdot carb}$ equal to zero as in the case of sucrose. From Table II, it is apparent that the Schiff base was not degraded by any carbohydrate, because the found value of the slope on addition of furfural was in good agreement with the calculated one. Furthermore, it appeared that the magnitude of the second-order catalytic rate constant of ampicillin ($k_{a\cdot carb}$) was dependent on that of the molecular weight of carbohydrate (Tables I and II). The accelerating effect of carbohydrate on the degradation of ampicillin in aqueous solution, however, was depressed by the addition of furfural.

This effect may be attributed the formation from ampicillin and furfural in alkaline solution of a Schiff base whose steric hindrance inhibits a nucleophilic attack of carbohydrate on the β-lactam carbonyl group.[4]

TABLE II. Slope of the Plot of k_{obs} *versus* Carbohydrate at pH 8.00 with 0.1 M Furfural at 35°C (μ=0.5)

Carbohydrate	$k_{a\cdot carb}$ (M^{-1} h^{-2})	$\frac{k_{a\cdot carb}+k_{c\cdot carb}\cdot K[F]}{K[F]+1}$ (M^{-1} h^{-1})	
		Observed	Calculated
Glucose	0.199	0.080	0.077
Maltose	0.375	0.145	0.144
Dextran 40	6.50	2.50	2.49

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References and Notes

1) This study was presented at the 102nd annual meeting of the Pharmaceutical Society of Japan, Osaka, Japan, April, 1982.
2) Part III: H. Fujiwara, S. Kawashima, Y. Yamada, *Chem. Pharm. Bull.*, **30**, 3310 (1982).
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