

[Chem. Pharm. Bull.]
31(4)1335—1344(1983)

Stabilization of Ampicillin Analogs in Aqueous Solution. V.¹⁻³⁾ Kinetic Study of the Effects of Aldehydes on the Degradation of Cephalexin in Aqueous Solution

HIROSHI FUJIWARA, SUSUMU KAWASHIMA,* and MASAKO OHASHI

School of Pharmacy, Hokuriku University, Kanagawa-machi, Kanazawa 920-11, Japan

(Received August 30, 1982)

The degradation of cephalexin (CEX) was inhibited by the addition of furfural or benzaldehyde at pH 6.00—8.50. This was due to the formation of adducts of CEX and the aldehydes at this pH region. In the acidic region (pH < 5.00) no such effects of the additives were observed because the adducts were not formed.

At the pH region where the inhibitory effects are observed, CEX exhibits rate enhancement of the β -lactam cleavage due to intramolecular catalysis by the side-chain α -amino group. Since the formation constant of the adducts increased with increase of pH, the α -amino group seemed to be involved in the formation of the adducts, and this is presumably no longer available for intramolecular catalysis. The degradation rates of the adducts were smaller (about 1/10—1/1000) than those of CEX. The adducts were considered to be Schiff bases from the infrared (IR) spectra and the kinetic properties of products prepared by freeze-drying of alkaline solutions containing CEX and an aldehyde (furfural or benzaldehyde).

Keywords—cephalexin; furfural; benzaldehyde; cephalexin-aldehyde adduct; formation constant of adduct; Schiff base; freeze-dried product; intramolecular catalysis

It was found that the degradation of ampicillin was successfully inhibited by the formation of an adduct between ampicillin and an aromatic aldehyde (benzaldehyde or furfural), in neutral and alkaline solutions.²⁾ These adducts were Schiff bases formed by the reaction of the α -amino group of ampicillin with aldehyde moiety of the aromatic aldehydes.³⁾

This report describes a kinetic study of the interaction between aromatic aldehydes and cephalexin (CEX), which is similar in structure to ampicillin.

Experimental

Materials—Cephalexin monohydrate (959 $\mu\text{g}/\text{mg}$) was kindly supplied by Shionogi & Co., Ltd. Furfural was of the highest commercial grade and was used after further purification by distillation under reduced pressure. Benzaldehyde and all other chemicals were of the highest reagent grade available and were used as received.

Buffer Solutions—The buffers used for the kinetic studies (pH 3.00—8.50) and CEX assay (pH 4.0) were described in the previous papers.^{2,3)} The pH values of buffers were read with a research pH meter, Toa model HM-18ET at the experimental temperature. The buffers were adjusted to an ionic strength of 0.5 with potassium chloride except when primary salt effects were to be investigated.

I₂-Colorimetry of CEX—All reagents used for I₂-colorimetry were the same as reported previously.^{2,3)} As mentioned in the following section, CEX in a reaction solution with aldehydes was determined after adjusting the reaction solution to pH 4.0, allowing it to stand for 20 min, and then hydrolyzing it for 90 min with 1 N NaOH at room temperature. In other cases, CEX assay was carried out by I₂-colorimetry as previously described.^{2,3)}

Kinetic Procedures—All kinetic experiments were undertaken at 35°C with a precision of $\pm 0.1^\circ\text{C}$. CEX was dissolved in an appropriate buffer [with or without benzaldehyde (2.5×10^{-3} — 2.5×10^{-2} M) or furfural (2.5×10^{-3} —0.1 M)], which had been preheated at 35°C, to produce 2.5×10^{-4} M final concentration. Samples were withdrawn at suitable intervals, cooled on ice and assayed for intact CEX by I₂-colorimetry as described above. These procedures were carried out two or more times. The pH values of the reaction solutions were measured at 35°C initially and at the end of the experiment. No significant changes in pH were observed.

Preparation of CEX-Aldehyde Adducts—An aqueous solution (adjusted to pH 8.0 with 1 N NaOH) containing 0.1 M CEX and 0.1 M aldehyde (benzaldehyde or furfural) was lyophilized after reacting for 72 h

at 0°C.

Nuclear Magnetic Resonance (NMR) Measurements—The spectra were obtained at 23°C on a JEOL JNM-FX-100 spectrometer. Samples were dissolved in dimethyl sulfoxide- d_6 at a concentration of *ca.* 5 w/v%. Since no interactions were observed between CEX or each aldehyde and tetramethylsilane (TMS) in NMR spectroscopy, TMS was used as an internal standard. The technique of D_2O addition was also used.

Infrared (IR) Measurements—The spectra were measured with a JASCO DS-701G spectrometer by the KBr method.

Bioassay—Antibacterial activity was assayed by the cylinder plate method with *Staphylococcus aureus* 209P as a test organism and with crystalline cephalixin as the standard, using brain heart infusion (Difco) agar.

Results and Discussion

Examination of I_2 -Colorimetry for Determining the Degradation Rate of Cephalixin with Benzaldehyde in Aqueous Solution

The kinetic runs for intact CEX (initial concentration, 2.5×10^{-4} M) in 0.1 M borate buffer (pH 8.00, $\mu=0.5$) solution with or without benzaldehyde (0.005 or 0.01 M) were carried out at 35°C. Residual intact CEX was assayed I_2 -colorimetrically using a procedure developed for ampicillin.^{2,3)}

The degradation of CEX without benzaldehyde followed pseudo-first-order kinetics. This result is shown by the linear log residual percent *versus* time plot in Fig. 1. The time courses of CEX with benzaldehyde, however, did not display pseudo-first-order kinetics. Thus, residual CEX was less than that without benzaldehyde, especially at the initial stage (Fig. 1).

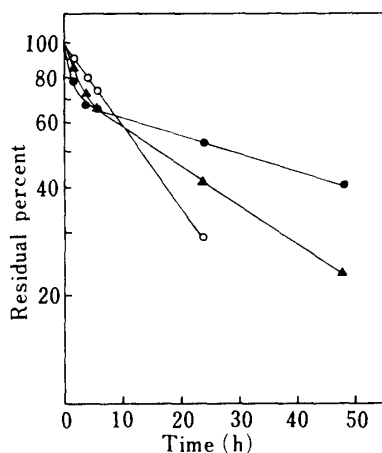


Fig. 1. Time Courses (followed by I_2 -colorimetry) of the Degradation of Cephalexin with and without Benzaldehyde in 0.1 M Borate Buffer of pH 8.00 at 35°C and $\mu=0.5$

○, cephalexin 2.5×10^{-4} M; ●, cephalexin with benzaldehyde 1.0×10^{-2} M; ▲, cephalexin with benzaldehyde 0.5×10^{-2} M.

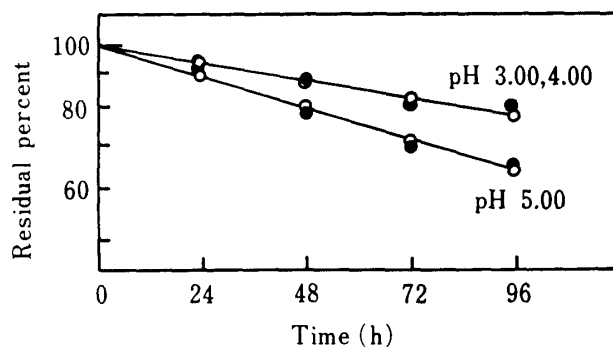


Fig. 2. Pseudo-first-order Plots for the Degradation of Cephalexin with and without Benzaldehyde in 0.1 M Acetate Buffers of pH 3—5 at 35°C and $\mu=0.5$

○, cephalexin 2.5×10^{-4} M; ●, cephalexin with benzaldehyde 2.5×10^{-2} M.

In contrast, the time courses of CEX degradation at the same concentration as above in the buffer solution (0.1 M acetate, $\mu=0.5$) at pH 3—5 and 35°C showed pseudo-first-order kinetics regardless of the addition of benzaldehyde, and the degradation rates were the same with and without benzaldehyde at each pH (Fig. 2).

Table I shows CEX levels determined by I_2 -colorimetry and bioassay of a buffered solution, which initially contained 2.5×10^{-4} M CEX in the presence or absence of 2.5×10^{-2} M benzalde-

TABLE I. Determination of Cephalixin by I_2 -Colorimetry and Bioassay

	I_2 -Colorimetry ^{a)} ($\times 10^4 M$)		Bioassay, ($\times 10^4 M$)	
	pH 4.00	pH 8.00	pH 4.00	pH 8.00
Cephalixin alone	2.4 $\pm$ 0.08	2.5 $\pm$ 0.03	2.8 $\pm$ 0.14	2.5 $\pm$ 0.10
Cephalixin with benzaldehyde	2.4 $\pm$ 0.10	1.0 $\pm$ 0.06	2.4 $\pm$ 0.14	2.3 $\pm$ 0.09

a) The conditions of the method were the same as for ampicillin assay. Samples used for the analysis were 0.1 M borate buffer solutions of pH 8.00 containing $2.5 \times 10^{-4} M$ cephalixin with and without $2.5 \times 10^{-2} M$ benzaldehyde after standing for 72 h at 0°C.

hyde and had been allowed to react for 72 h at 0°C, pH 4.00 or 8.00. The values given by both assays were consistent with each other at pH 4.00, while the value by I_2 -colorimetry was half the value by bioassay at pH 8.00 in the case of the addition of benzaldehyde. Thus, these results indicate that biologically active CEX in the solution with benzaldehyde (pH 8.00) could not be determined successfully under the conditions of the I_2 -colorimetry. For CEX assay, the reaction solution was allowed to stand for 5 min at room temperature and at pH 4.0, treated with 1 N sodium hydroxide for 20 min at room temperature, then subjected to I_2 -colorimetry. It seemed that the adduct formed from CEX and benzaldehyde was not well hydrolyzed to products which consume iodine quantitatively under the conditions of this method.^{2,3)} Thus, the standing time at pH 4.0 and the degradation time with alkali were extended in 0.1 M borate buffer of pH 8.00 in which CEX ($2.5 \times 10^{-4} M$) had been allowed to react with benzaldehyde ($2.5 \times 10^{-2} M$) for 48 h at 0°C.

The results are shown in Fig. 3. It is apparent from Fig. 3 that quantitative values for CEX with benzaldehyde were obtained when the standing and degradation times were 20 and 90 min, respectively, and that those values were in good agreement with those obtained for the same CEX concentration without benzaldehyde.

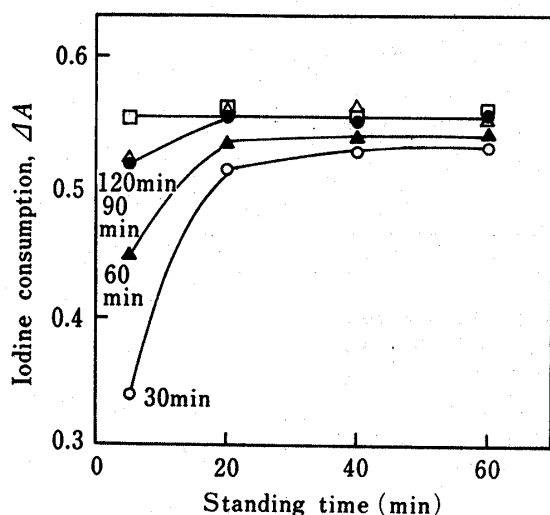


Fig. 3. Relationships between Iodine Consumption and Standing Time at pH 4.0, at Various Reaction Times with Sodium Hydroxide

□, cephalixin $2.5 \times 10^{-4} M$; ●, ○, ▲, △, cephalixin with benzaldehyde $2.5 \times 10^{-2} M$.

Figures on the plot are times (min) of reaction with sodium hydroxide, except for □.

Degradation of Cephalixin with Aldehydes in Aqueous Solution

The time courses of the degradation of intact CEX in buffer of pH 6.00, 7.00 (0.1 M phosphate) and 8.00 (0.1 M borate) with or without benzaldehyde (or furfural) at 35°C are shown in Figs. 4 and 5.

In each case, the plot of log residual percent of CEX *versus* time was linear. Further, the slope decreased with increasing addition of benzaldehyde or furfural, indicating that the degradation of CEX was inhibited by these aldehydes. The plot of the pseudo-first-order rate constants obtained from the slopes against the concentration of each aldehyde was not linear. Thus, these results suggest the formation of adducts of CEX and the aldehydes.^{2,3)}

Assuming the reaction, therefore, to occur according to the scheme in Chart 1, the change of the total concentration, $[C]_T$, of intact CEX present in the solution is given by

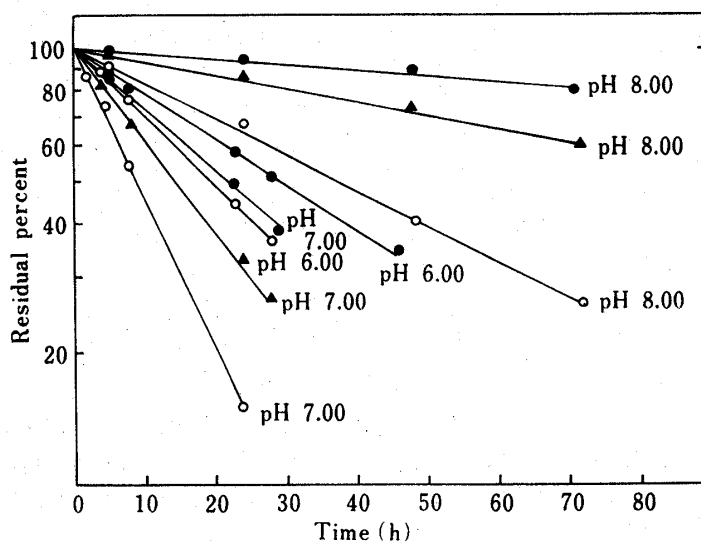


Fig. 4. Pseudo-first-order Plots for the Degradation of Cephalexin with and without Benzaldehyde in 0.1 M Phosphate Buffer (pH 6.00 and 7.00) and 0.1 M Borate Buffer (pH 8.00) at 35°C and $\mu=0.5$

○, cephalexin 2.5×10^{-4} M; ▲, cephalexin with benzaldehyde 0.5×10^{-2} M; ●, cephalexin with benzaldehyde 1.0×10^{-2} M.

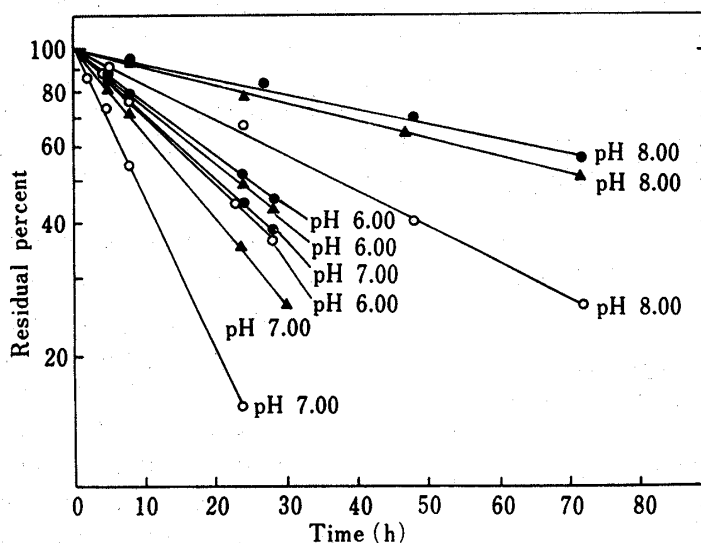


Fig. 5. Pseudo-first-order Plots for the Degradation of Cephalexin with and without Furfural in 0.1 M Phosphate Buffer (pH 6.00 and 7.00) and 0.1 M Borate Buffer (pH 8.00) at 35°C and $\mu=0.5$

○, cephalexin 2.5×10^{-4} M; ▲, cephalexin with furfural 0.5×10^{-2} M; ●, cephalexin with furfural 1.0×10^{-2} M.

Eq. (1):

$$-\frac{d}{dt} ([C] + [CA]) = -\frac{d}{dt} [C]_T \quad (1)$$

$$= k_x [C] + k_c [CA]$$

where $[C]$ is the concentration of free CEX, $[CA]$ is the concentration of the adduct, and k_x and k_c are the pseudo-first-order rate constants of β -lactam cleavage of CEX and the adduct,

respectively.

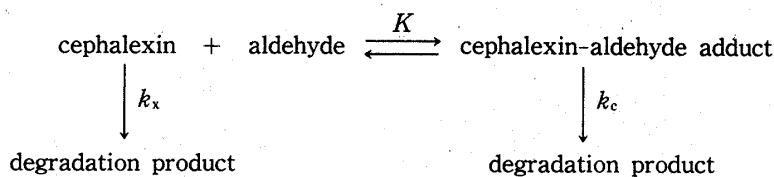


Chart 1

If 1:1 adduct formation is assumed the formation constant, K , can be expressed as follows:

$$K = \frac{[CA]}{[C][A]} \quad (2)$$

where $[A]$ = concentration of aldehyde.

From Eqs. (1) and (2), Eq. (3) is obtained:

$$\begin{aligned}
 -\frac{d}{dt} [C]_T &= \left\{ \frac{k_x}{1 + K[A]} + \frac{k_c \cdot K[A]}{1 + K[A]} \right\} [C]_T \\
 &= k_{\text{obs}} [C]_T
 \end{aligned} \quad (3)$$

where k_{obs} is the observed pseudo-first-order rate constant of intact CEX. Eq. (4) is then obtained from Eq. (3):

$$k_{\text{obs}} = \frac{k_x + k_c \cdot K[A]}{1 + K[A]} \quad (4)$$

Each aldehyde was used in more than 10-fold excess over CEX and was little decomposed within this experimental pH range at 35°C.^{2,3)} Further, since the overall reaction with aldehyde was observed to follow pseudo-first-order kinetics (Figs. 4 and 5), the reversible reaction rate for adduct formation may be very fast as compared to the degradation rate of CEX or the adduct.

Eq. (5) is derived from Eq. (4) by putting q equal to $(1 - k_c/k_x)$:

$$\frac{k_x}{k_x - k_{\text{obs}}} = \frac{1}{qK} \cdot \frac{1}{[A]} + \frac{1}{q} \quad (5)$$

Linear double reciprocal plots based on Eq. (5) were obtained by using k_{obs} values at pH 6.00 and 7.00 as shown in Fig. 6. In each case, $1/q$ and $1/qK$ were calculated by the least-squares method from the slope and intercept, and K and k_c were obtained. For degradation of CEX at pH 6.00 and 7.00 in the presence or absence of aldehyde, the rate constant, k_{obs} , was determined experimentally as a function of buffer concentration in the range of 0.05–0.15 M. Fig. 7 shows the k_{obs} versus buffer concentrations plots.

Because a linear relationship was found between k_{obs} and buffer concentrations in each plot, the degradations of CEX⁴⁾ and also the adduct were recognized to undergo catalysis by phosphate buffer species.

Thus, Eqs. (6) and (7) hold:

$$k_x = k_x^0 + k_{\text{xp}}[P] \quad (6)$$

$$k_c = k_c^0 + k_{\text{cp}}[P] \quad (7)$$

where $[P]$ is total phosphate buffer concentration, k_{xp} and k_{cp} are the catalytic rate constants of

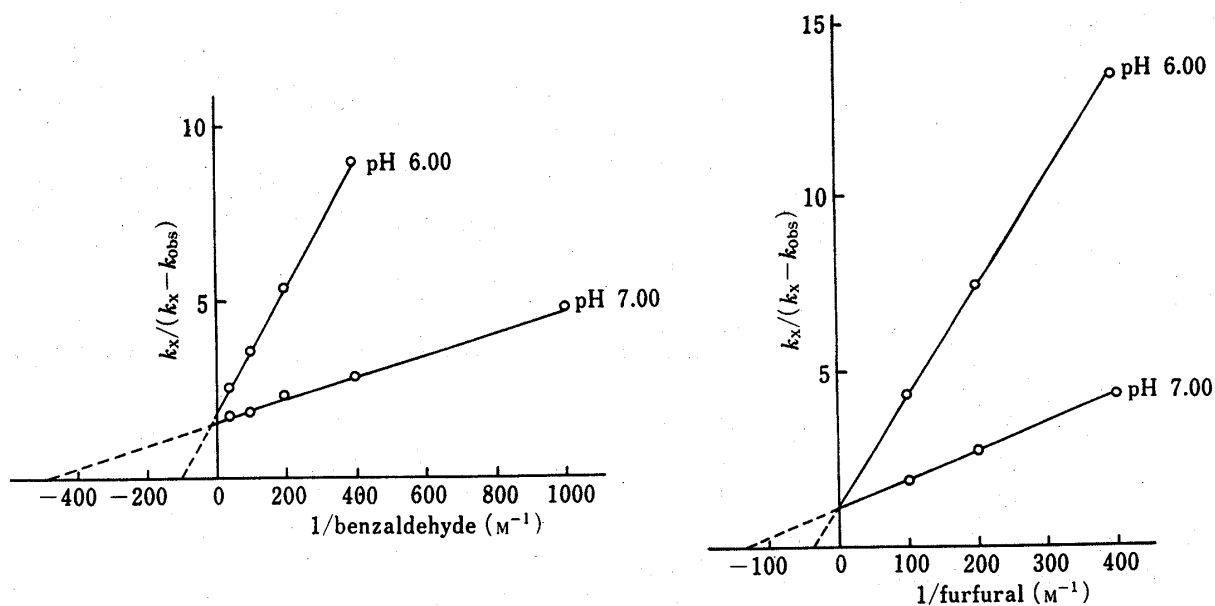


Fig. 6. Double Reciprocal Plots for the Adduct Formation between Cephalixin and Aldehyde in 0.1 M Phosphate Buffer, pH 6.00 and 7.00

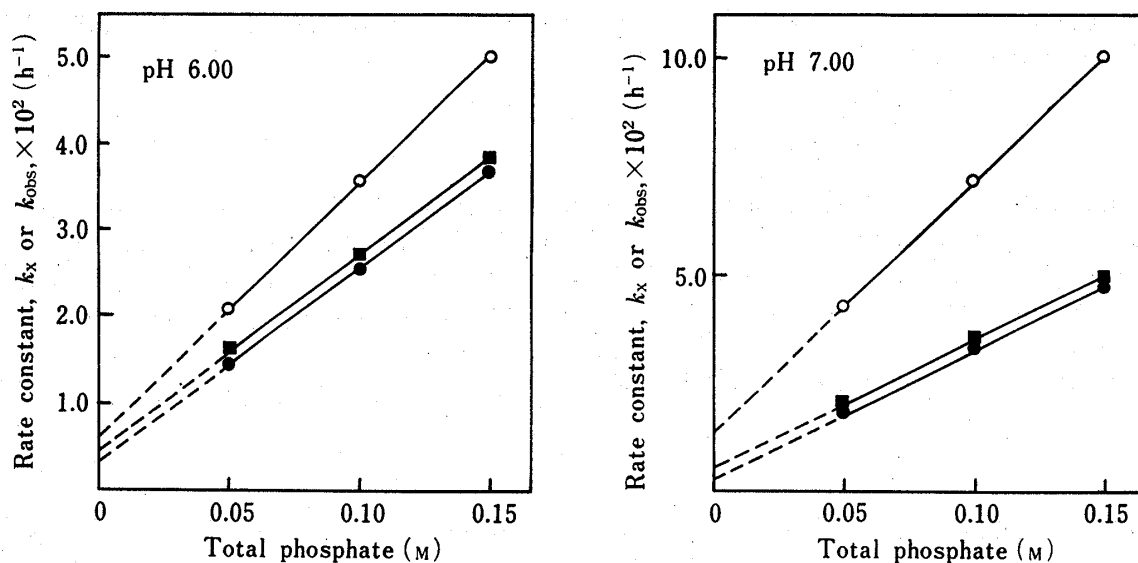


Fig. 7. Plots of First-order Rate Constant, k_{obs} (●, ■) or k_x (○) versus Buffer Concentration at pH 6.00 and 7.00 at 35°C and $\mu=0.5$

●, with benzaldehyde; ■, with furfural.
Initial concentrations of CEX and benzaldehyde or furfural were 2.5×10^{-4} and 1.0×10^{-2} M, respectively.

CEX and the adduct, and k_x^0 and k_o^0 are the buffer-free rate constants of CEX and the adduct, respectively. Therefore, substituting Eqs. (6) and (7) in Eq. (4) yields the following equation for the pseudo-first-order rate constant, k_{obs} , in phosphate buffer of pH 6.00 or 7.00:

$$k_{\text{obs}} = \frac{1}{1 + K[A]} (k_x^0 + k_o^0 \cdot K[A]) + \frac{1}{1 + K[A]} (k_{\text{xp}} + k_{\text{cp}} \cdot K[A]) [P] \quad (8)$$

By using Eq. (6) to analyze the results for CEX in the absence of aldehyde and Eq. (8) for CEX in the presence of aldehyde (these results are shown in Figs. 8 and 9), the buffer-free rate constant and the second-order catalytic rate constant of phosphate for CEX (k_x^0 , k_{xp}) and the adduct (k_a^0 , k_{ap}) can be calculated. These rate constants are summarized in Table II.

TABLE II. Kinetic Parameters of Cephalixin and Its Furfural and Benzaldehyde Adducts at Various pH Values at 35 °C and $\mu=0.5$

pH	Formation constant		Rate constant of cephalixin		Rate constant of benzaldehyde adduct		Rate constant of furfural adduct	
	K_B	K_F	$10^2 k_x^0$	k_{xp}	$10^2 k_c^0$	k_{cp}	$10^2 k_a^0$	k_{ap}
	(M ⁻¹)		(h ⁻¹)	(M ⁻¹ h ⁻¹)	(h ⁻¹)	(M ⁻¹ h ⁻¹)	(h ⁻¹)	(M ⁻¹ h ⁻¹)
6	98.9	34.8	0.62	0.293	0.00089	0.138	0.00062	0.0281
7	480	135.6	1.43	0.578	0.016	0.245	0.0072	0.0541
8	747	212.3	1.86		0.19		0.061	
8.6	782	223.5	1.92		0.78		0.23	

On the other hand, similar investigations of the reactions in various concentrations of borate buffer at pH 8.00 and 8.50 yielded the finding that k_{obs} was independent of borate buffer concentration at either pH. Thus, the formation and degradation constants of the adduct at these pHs were obtained from double reciprocal plots of the results obtained in 0.1 M borate buffer at each pH, as shown in Table II. From Table II, it can be seen that the slower degradation rate of the adduct (k_a^0) at pH 6.00–8.50 and the smaller catalytic effect of phosphate on the adduct (k_{ap}) at pH 6.00 and 7.00 contribute to the stabilization of CEX by the addition of aldehydes, as is observed in the case of ampicillin.^{2,3)}

Effects of pH on the Kinetic Parameters of CEX Adducts with Aldehydes in Aqueous Solution

Cephalixin-aldehyde adducts were not formed by the addition of aldehydes in the acidic region, pH 5.00 (Fig. 2). The degradation of CEX, however, was depressed by these additives at above pH 6.00. The values of $\log k_x^0$ and $\log k_a^0$ were plotted *versus* pH to yield the pH-rate profiles, respectively (Fig. 8).

The pH-rate profile for CEX was curved at pH 6.00–8.50, as shown in Fig. 8. Yamana *et al.*⁴⁾ stated that this was due to direct intramolecular nucleophilic attack of the side-chain α -amino group on the β -lactam. Thus, according to their study, the pseudo-first-order rate constant, k_x^0 , of CEX can be expressed as follows within this pH region:

$$k_x^0 = k_0 + k_b \frac{K_a}{K_a + aH} \quad (9)$$

where K_a represents the dissociation constant of the conjugated acid of the α -amino group ($pK_a=6.88^{4)}$, and k_b and k_0 represent the rate constants for intramolecular nucleophilic reaction of the α -amino group and for spontaneous or water-catalyzed degradation, respectively. Values of $1.50 \times 10^{-2} \text{ h}^{-1}$ for k_0 and $4.88 \times 10^{-3} \text{ h}^{-1}$ for k_b were obtained from the values

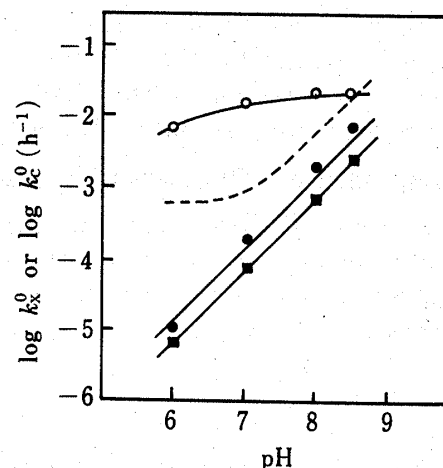


Fig. 8. $\log k$ -pH Profile for the Degradation of Cephalixin (O) and the Adduct (●, ■) at 35°C and $\mu=0.5$

The solid lines were calculated from Eq. (11) using $k_{cOH}=675 \text{ M}^{-1} \text{ h}^{-1}$ (benzaldehyde adduct) and $302 \text{ M}^{-1} \text{ h}^{-1}$ (furfural adduct), and the dashed line is the $\log k$ -pH profile for ampicillin, taken from ref. 9. The points are experimental values. ●, benzaldehyde adduct; ■, furfural adduct.

of k_x^0 at pH 6.00–8.50 by using the least-squares method.

In contrast, the plots of $\log k_c^0$ versus pH became linear with a positive slope of unity on addition of benzaldehyde or furfural (Fig. 8). Consequently, k_c^0 can be given by the following equation:

$$k_c^0 = k_{cOH} \cdot aOH \quad (10)$$

where k_{cOH} = specific rate constant for the adduct degradation, and aOH = the activity of the hydroxy ion.

Eq. (11) is obtained from Eq. (10):

$$\log k_c^0 = \log k_{cOH} - pK_w + pH \quad (11)$$

Since pK_w is 13.68⁵⁾ at 35°C, k_{cOH} values for benzaldehyde and furfural adducts were obtained as 675 $\text{M}^{-1} \text{h}^{-1}$ and 302 $\text{M}^{-1} \text{h}^{-1}$, respectively, by substituting the results of Fig. 8 into Eq. (11). These two pH-rate profiles are similar to that of ampicillin, which does not exhibit direct intramolecular attack of the side-chain α -amino group. This indicates the disappearance of such rate enhancement of the β -lactam opening of CEX and suggests that the formation of these adducts is due to the reaction between aldehydes and the α -amino group of CEX. Further, the specific catalytic rate constant of hydroxy ion, k_{cOH} , for the furfural adduct was smaller than that for the benzaldehyde adduct. It may be considered that the nucleophilic attack of hydroxy ion on the β -lactam carbonyl group becomes less favorable due to enhancement of the amino resonance and/or the larger steric hindrance effects in the furfural adduct.^{3,6)}

pH Dependence of Formation Constant of the Adducts

As shown in Table II, the apparent formation constant, K , increased with increase of pH. Because the adduct formation was not observed in the acidic region, the adduct was assumed to be formed between each aldehyde and the anionic species, $[C^-]$, of CEX. Thus, the absolute formation constant, K_s , can be expressed as follows:

$$K_s = \frac{[CA]}{[C^-][A]} \quad (12)$$

Eq. (13) can be derived from Eqs. (2) and (12):

$$K = K_s \frac{K_a}{K_a + aH} \quad (13)$$

Values of K_s of 800 M^{-1} (benzaldehyde adduct), and 230 M^{-1} (furfural adduct) were obtained from K listed in Table II by means of the least-squares method.

Fig. 9 shows plots of K versus pH; the solid lines for the two kinds of aldehydes represent the theoretical K values calculated from K_s values. The theoretical and found values were in good agreement with each other for both adducts.

Chemical Structure of the Adducts

Since no differences in biological activities were recognized between CEX and freeze-dried product which was prepared from a solution containing CEX and an aldehyde (benzaldehyde or furfural) and these aldehydes were not contained in free form in the products, the freeze-dried products seemed to be mixtures of CEX and the adducts.

Attempts to separate and purify these two components of the mixture yielded unsatisfactory results. Thus, ^1H and ^{13}C NMR spectra were measured for each freeze-dried product dissolved in deuterated dimethyl sulfoxide, and after D_2O addition. The observed signals in the adducts could not be assigned because of overlapping with those of CEX, but the aldehyde proton signal at δ 9.66 (furfural) or at δ 10.06 (benzaldehyde) was not detected in the corresponding adducts in the region of δ 9–11.

Three protons at δ 7.04–8.28 (furan ring) were observed in the furfural adduct. These

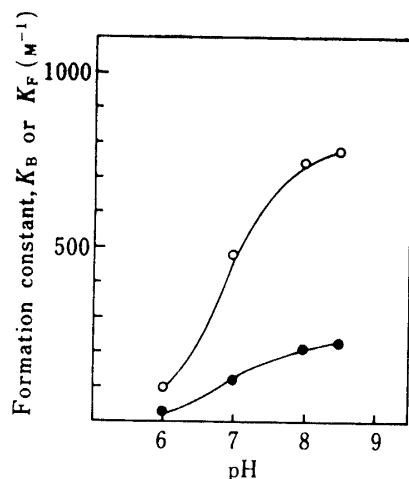


Fig. 9. Plots of the Formation Constants, K_B (○, Benzaldehyde Adduct), K_F (●, Furfural Adduct), of Cephalixin-Aldehyde Adduct *versus* pH in Aqueous Solution at 35°C and $\mu = 0.5$

The solid lines were calculated by means of Eq. (13) from K_s , K_a and aH values by the least-squares method, while the points represent experimental values.

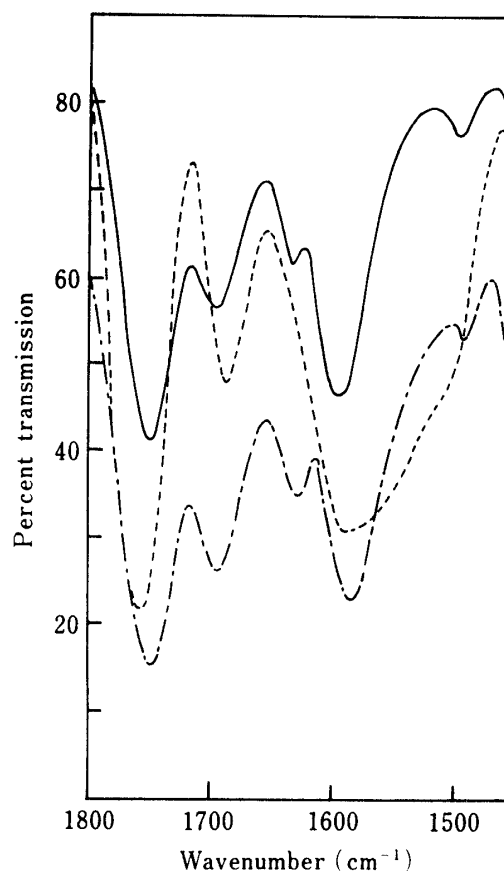


Fig. 10. IR Spectra of Freeze-dried Products

---, cephalixin; —, furfural, adduct, — · —, benzaldehyde adduct.

investigations indicate that the aldehydes are not present in the free form and that the aldehyde moieties are lost in the adducts.

Then, the IR spectra were measured for the same products. Specific absorption bands such as lactam at 1760 cm^{-1} , amide at 1685 cm^{-1} and carboxylate at 1590 cm^{-1} , and a characteristic stretching vibration due to azomethine at 1640 cm^{-1} were observed for each product (Fig. 10). This azomethine band was not observed in freeze-dried CEX. Furthermore, a band due to the furan ring at 880 cm^{-1} was detected in the furfural adduct, while no band due to aromatic aldehyde (1700 cm^{-1}) was seen in either adduct.

As described above, these adducts are not formed in acidic aqueous solution and the adducts formed in alkaline aqueous solution dissociate into the original compounds within a

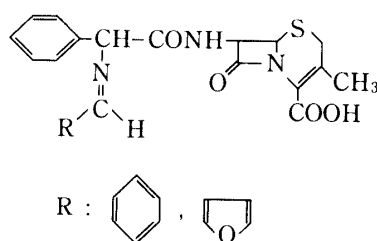


Chart 2

short time on adjusting the pH to the acidic region. Thus, the adducts are not considered imidazo lidinyl⁷⁾ or oxazolidinylidene⁸⁾ structures, but Schiff bases⁹⁾ (Chart 2).

Acknowledgement The authors thank Shionogi & Co., Ltd. for the gift of cephalixin, Prof. K. Yabu for bioassay, Mr. M. Teranishi for NMR spectral measurements and Miss I. Mitsui for technical assistance.

References and Notes

- 1) Presented in part at the 102nd annual meeting of the Pharmaceutical Society of Japan, Osaka, Japan, April, 1982.
- 2) H. Fujiwara, S. Kawashima, and M. Ohhashi, *Chem. Pharm. Bull.*, **30**, 1430 (1982); H. Fujiwara, S. Kawashima, and M. Ohhashi, *ibid.*, **30**, 2181 (1982).
- 3) H. Fujiwara, S. Kawashima, Y. Yamada, and K. Yabu, *Chem. Pharm. Bull.*, **30**, 3310 (1982).
- 4) T. Yamana and A. Tsuji, *J. Pharm. Sci.*, **65**, 1563 (1976).
- 5) T. Yamana (ed.), "Iyakuhin Sokudoron," 1st ed., Nankodo Publishing Co., Tokyo, 1909, p. 23.
- 6) R.M. Sweet and J.F. Dahl, *J. Am. Chem. Soc.*, **92**, 5489 (1970).
- 7) A. Tsuji and T. Yamana, *Chem. Pharm. Bull.*, **22**, 2434 (1974).
- 8) A.C. Davis and A.L. Levy, *J. Chem. Soc.*, **1951**, 3479.
- 9) J.P. Hou and J.W. Pool, *J. Pharm. Sci.*, **58**, 447 (1969).