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Stabilization of Ampicillin Analogs in Aqueous Solution. VI. Kinetic Analysis of the Stabilization Mechanism of Bacampicillin with Benzaldehyde in Aqueous Solution¹⁾

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It was proved that bacampicillin hydrochloride (BAPC) is degraded according to a parallel-consecutive reaction scheme consisting of the direct deactivation reaction of β -lactam cleavage and the process through ampicillin (ABPC) in aqueous solution. In particular, the degradation of the 3-carboxylate of BAPC was faster than that of the β -lactam, and BAPC was transformed quite easily to ABPC in the alkaline region (pH 7.00–9.00). However, the degradation of the β -lactam and that of the ester moiety of BAPC were inhibited due to adduct formation upon addition of benzaldehyde at this pH region. On the other hand, such a stabilization was not observed in the acidic region (pH < 6.00) because no adduct formation developed. The formation constant of the adduct increased with increase of pH.

On the basis of the above results and infrared and mass spectra of the freeze-dried product prepared from an alkaline solution containing BAPC and benzaldehyde, the adduct was concluded to be the Schiff's base formed between the α -amino group of BAPC and benzaldehyde; a similar adduct is formed by ABPC.

Keywords—bacampicillin; ampicillin; benzaldehyde; bacampicillin degradation; bacampicillin stabilization; Schiff's base; parallel-consecutive reaction; I_2 -colorimetry; bacampicillin-ampicillin separation assay

Bacampicillin, which was developed as a prodrug of ampicillin (ABPC) is an ethoxycarbonyloxyethyl ester. This antibiotic is superior to ABPC in bioavailability, and is easily hydrolyzed to ABPC by esterases during gastrointestinal absorption.²⁾ Some data on the stability kinetics of bacampicillin hydrochloride (BAPC) in aqueous solution have been reported.²⁾

In previous papers,³⁾ it was proved that ABPC was stabilized due to the formation of the Schiff's base by the addition of benzaldehyde in alkaline solution. The chemical structure of BAPC is analogous to that of ABPC, and therefore, such stabilization of BAPC was expected to occur with benzaldehyde in the alkaline region.

In this work, thus, we first ran the separation assay of BAPC and ABPC in aqueous solution with and without benzaldehyde. Secondly, by using this analytical procedure, we assessed the mechanism and evaluated the effects of benzaldehyde addition on the degradation of the ABPC prodrug in aqueous solution.

Experimental

Materials—BAPC (659 μ g/mg, Yoshitomi Pharmaceutical Ind., Ltd.) was used as received. ABPC Na (Meiji Seika Kaisha Ltd.) was the normal commercial preparation for injection. Benzaldehyde and all other reagents and solvents were of the highest commercial grade and were used directly.

Reagents—All reagents used for I_2 -colorimetry were the same as used previously.³⁾ A 0.05 M phthalate solution (pH 4.0, for separation assay) was prepared by dissolving 10.22 g of potassium hydrogen phthalate in water to make 1000 ml.

Buffer Solutions—The buffer systems were as follows: at pH 3.00–5.00, $\text{CH}_3\text{COONa}-\text{CH}_3\text{COOH}$; at pH 6.00–7.00, $\text{KH}_2\text{PO}_4-\text{K}_2\text{HPO}_4$ and at pH 8.00–9.00, $\text{H}_3\text{BO}_3-\text{NaOH}$. The ionic strength of these buffers was adjusted to 0.5 by the addition of KCl. The pH of the buffers was measured at the experimental temperature with a Toa pH meter, model HM-18ET.

Analytical Method—a) Simultaneous Determination of BAPC and ABPC in Aqueous Solution: From a solution containing BAPC at less than $1.0 \times 10^{-4} \text{ M}$ with and without benzaldehyde, a sample of 25 ml was pipetted into a 50 ml volumetric flask and adjusted to pH 3.5–4.0 with dil. HCl, followed by the addition of 0.05 M phthalate (pH 4.0) to bring the solution up to volume. After 5 min at room temperature, total penicillin content (BAPC, ABPC and the adducts with benzaldehyde) in the solution (4 ml) was determined by I_2 -colorimetry. Then, 20 ml of the residual buffered solution was adjusted to pH 8.0 with 0.5 ml of 0.1 M borate buffer (pH 9.0) and extracted with 40 ml of toluene.

The total amount of ABPC (ABPC and its adduct with benzaldehyde) in the resulting aqueous layer (4 ml) was assayed by the same method. The amount of BAPC in the solution was calculated from the difference between the total penicillin and ABPC content.

b) I_2 -Colorimetry: The assay procedure was the same as described previously.³⁾

$\text{p}K_a$ Measurement—The apparent dissociation constant, K_a , for BAPC was determined at 35°C by a potentiometric titration.⁴⁾ A hundred milliliters of $1.0 \times 10^{-3} \text{ M}$ solution of BAPC, adjusted with KCl to a constant ionic strength of 0.5, was titrated with 0.1 N NaOH ($\mu=0.5$ with KCl) with bubbling of N_2 through solution. The $\text{p}K_a$ value obtained was 6.80 ± 0.04 .

Kinetic Procedures—Exactly weighed BAPC was transferred to a volumetric flask which contained buffer with or without benzaldehyde and sufficient buffer was added to bring the solution up to volume. The buffer solution had previously been heated to the desired temperature. The flask was stored in a constant temperature bath which was regulated to 35°C by a thermostat with $\pm 0.1^\circ\text{C}$ precision. The initial concentrations of BAPC and benzaldehyde were 1.0×10^{-4} and 1.0×10^{-3} – $5.0 \times 10^{-3} \text{ M}$, respectively.

Samples were taken at suitable intervals, cooled and assayed immediately for intact BAPC and/or ABPC by the simultaneous determination method described above.

Preparation of Freeze-Dried Product—Aqueous solution containing BAPC ($5 \times 10^{-4} \text{ M}$) and benzaldehyde ($5 \times 10^{-2} \text{ M}$) was adjusted to pH 8.0 by the addition of NaOH and stored in a cooled bath (0°C) followed by lyophilization after 72 h.

Determination of Infrared (IR) and Mass Spectra (MS)—IR spectra were recorded with a JASCO DS-701G grating infrared spectrometer using the KBr method. MS were measured with a JEOL JMS-D-100 mass spectrometer.

Data Analysis—The amounts of BAPC and/or ABPC were generated by an NEC PC-8800 microcomputer using the MULTI program⁵⁾ according to the appropriate reaction scheme.

Results and Discussion

Estimation of BAPC

The relationship between the concentration of BAPC (or ABPC) below $5.0 \times 10^{-4} \text{ M}$ and the extent of iodine consumption (converted into absorbance, ΔA) obtained by I_2 -colorimetry was linear (Fig. 1). In addition, the slopes of the calibration curves were the same. Thus, it was found that the extent of iodine consumption by BAPC in this assay method was the same as that by ABPC.

In order to study the time course of BAPC transformation in aqueous solution, the ester and free ABPC must be determined independently. It is well known that BAPC in aqueous solution can be eliminated by extraction with toluene.²⁾ The extractive effect, however, may be pH-dependent in view of the ionization of the α -amino group ($\text{p}K_a$ 6.80) of BAPC. Thus, the effects of solution pH and the amount of toluene on the extraction efficiency were examined.

The quantity of ABPC ester in the aqueous layer which contained $2.5 \times 10^{-2} \text{ M}$ of the ester was determined, after extraction of the solution at various pH values with various amounts of toluene (Table I). BAPC was efficiently separated at pH 8.0 with two volumes of toluene (Table I). Moreover, none of the ABPC in the aqueous solution was extracted into the toluene layer under these conditions. Mixtures of BAPC and ABPC were prepared with various concentrations of each so as to give a total concentration of $2.0 \times 10^{-4} \text{ M}$ in aqueous solution, in order to investigate how the coexistence of ABPC affects the extraction. When

TABLE I. Influence of pH and Quantity of Toluene on Extraction of BAPC with Toluene from Aqueous Solution

pH	BAPC added $\times 10^2$ M	BAPC found in aqueous layer $\times 10^2$ M	Extractive efficiency %	Quantity of toluene
3.99	2.50	1.30	48.0	} 5 fold
5.00	2.50	0.19	92.4	
6.27	2.50	0.16	93.6	
7.15	2.50	0.019	99.2	
7.98	2.50	0	100	
7.98	2.50	0	100	4 fold
7.98	2.50	0	100	3 fold
7.98	2.50	0.003	99.9	2 fold
7.98	2.50	0.013	99.5	1 fold

TABLE II. Recoveries of BAPC and ABPC from Synthetic Mixtures

Quantities added		Quantities found			
BAPC $\times 10^4$ M	ABPC $\times 10^4$ M	BAPC $\times 10^4$ M	% recovery ^{a)}	ABPC $\times 10^4$ M	% recovery ^{b)}
1.25	0.75	1.28	102	0.73	97.3
1.00	1.00	0.98	98.0	1.03	103
0.75	1.25	0.74	98.7	1.24	99.2
0.50	1.50	0.49	98.0	1.52	101
0.25	1.75	0.25	100	1.74	99.4
0	2.00	0	100	2.01	100

a) Average, 99.5; S.D., 1.39. b) Average, 100; S.D., 1.91.

TABLE III. Determination of BAPC or ABPC and Its Adducts with Benzaldehyde

a)	BAPC found		ABPC found	
	$\times 10^4$ M	% recovery	$\times 10^4$ M	% recovery
Sample I (1)	2.50	100	2.51	100
Sample II (2)	2.49	99.6	2.49	99.6
(3)	0.01	0.4	2.52	101
(4)	0	0	2.50	100
(5)	2.53	101	0	0

a) Number in Chart 1.

aliquots of the resultant solution were analyzed by the proposed method, the results shown in Table II were obtained.

Thus, it was confirmed that BAPC and ABPC in aqueous solution could be determined separately by this analytical method because the found values were in fair agreement with the added values in every mixture. In addition, this analytical method was unaffected by the addition of the degradation products.

Determination of BAPC and ABPC in Aqueous Solution Containing Benzaldehyde

In alkaline solution, ABPC forms the Schiff's base with benzaldehyde.³⁾ Since BAPC is also assumed to exhibit a similar interaction, separation assay of the penicillins in the presence

sample I: 2.5×10^{-4} M BAPC or ABPC buffered solution (0.1 M borate, pH 8.00) standing for 72 h at 0°C , I_2 -colorimetry (1)

sample II: 2.5×10^{-4} M BAPC or ABPC buffered solution (0.1 M borate, pH 8.00) with 2.5×10^{-3} M benzaldehyde standing for 72 h at 0°C

Chart 1

Fig. 1. Calibration Curve for Bacampicillin (○) and Ampicillin (●) Obtained by I_2 -Colorimetry

Fig. 2. Typical Calibration Curve of Bacampicillin and Ampicillin in the Presence and Absence of Benzaldehyde by Separation Assay

○, bacampicillin; ●, ampicillin; △, bacampicillin with benzaldehyde.

Fig. 3. Time Courses for Bacampicillin and Ampicillin during Degradation with or without Benzaldehyde at pH 8.00 (0.1 M Borate), 35°C and $\mu=0.5$

○, ●, bacampicillin + ampicillin; △, ▲, ampicillin; □, ■, bacampicillin. The closed symbols show the courses in the presence of benzaldehyde. Initial concentrations of bacampicillin and benzaldehyde were 1.0×10^{-4} and 3.5×10^{-3} M, respectively. The lines are the least-squares best fits to the experimental points consistent with Chart 3 ($k_e=0.534 \text{ h}^{-1}$, $k_b=0.252 \text{ h}^{-1}$, $k_a=0.013 \text{ h}^{-1}$, $k_{se}=0.249 \text{ h}^{-1}$, $k_{sb}=0.074 \text{ h}^{-1}$, and $k_{sa}=0.0097 \text{ h}^{-1}$).

of benzaldehyde was studied. After standing for 72 h at 0 °C (conditions under which ABPC formed the Schiff's base), the buffered solution (0.1 M borate, pH 8.00) containing BAPC or ABPC with or without benzaldehyde was processed as shown in Chart 1 followed by the determination of the penicillins in each sample solution (Table III).

The results shown in Table III indicate clearly that BAPC and ABPC in the presence of benzaldehyde can also be determined simultaneously in alkaline solution. In addition, it was found that BAPC was assayed as total intact BAPC even if an adduct is formed between BAPC and benzaldehyde. As shown in Fig. 2, calibration curves obtained by the analytical procedure described in Experimental for ABPC, BAPC and BAPC with benzaldehyde (ten fold excess over BAPC) all fell on the same line.

Degradation of BAPC with and without Benzaldehyde in Aqueous Solution

Figure 3 shows the time courses of the change of total penicillin (the sum of BAPC and ABPC) and ABPC in 0.1 M borate buffer solution which initially contained 1.0×10^{-4} M BAPC at pH 8.00 ($\mu=0.5$) and 35 °C. The amount of total penicillin coincided with that of ABPC at about 7 h, and the subsequent degradation obeyed good pseudo-first-order kinetics. This first-order kinetic disappearance is the same as that of ABPC. Moreover, the degradation of BAPC obtained from both time courses also showed pseudo-first-order kinetics.

Thus, assuming that the degradation of the ABPC prodrug proceeds by the parallel consecutive reaction mechanism shown in Chart 2,⁶⁾ the changes of BAPC and ABPC are given by Eqs. 1 and 2, respectively:

$$[B] = [B]_0 e^{-(k_e + k_b)t} \quad (1)$$

$$[A] = \frac{k_e}{(k_e + k_b) - k_a} [B]_0 (e^{-k_a t} - e^{-(k_e + k_b)t}) \quad (2)$$

where $[B]$ and $[A]$ = concentration of BAPC and ABPC, respectively; $[B]_0$ = initial concentration of BAPC; k_e = first-order rate constant of ester hydrolysis of BAPC; k_a and k_b = first-order rate constants of β -lactam cleavage of ABPC and BAPC, respectively.

Chart 2

From the results in Fig. 3, k_e , k_b and k_a , which were calculated by the nonlinear least-squares method from Eqs. 1 and 2 using the MULTI computer program,⁵⁾ were 0.534, 0.252 and 0.013 h^{-1} , respectively. This degradation constant, k_a , was in good agreement with the value (0.012 h^{-1}) calculated from the slope of the pseudo-first-order plot of ABPC degradation shown in Fig. 3 and also the degradation rate constant ($k_a = 0.013 \text{ h}^{-1}$) which was independently obtained from the study of ABPC under the same conditions. Therefore, it was ascertained that BAPC is degraded according to the proposed process through ABPC. In this study, the degradation route of the ester moiety other than the carbonate ester group of BAPC has not been elucidated. However, the rate of liberation of ABPC from BAPC exhibited first-order kinetics with no indication of a lag time (Fig. 3). This suggests that even if an intermediate is formed in the ester moiety hydrolysis, it is highly unstable and spontaneously degrades into ABPC as shown in the case of pivampicillin^{6,7)} or is soluble in aqueous solution.

TABLE IV. Pseudo-First-Order Rate Constants for the Degradation of BAPC with and without Benzaldehyde at Various pH Values, 35°C and $\mu=0.5$

BAPC $\times 10^{-4}$ M	Benzaldehyde added $\times 10^{-3}$ M	Rate constant (h^{-1})	
		pH 3.00	pH 8.00
1.0	0	0.0097	0.786
	1.0		0.695
	2.5		0.610
	3.5	0.0097	0.572

Fig. 4. Effect of Benzaldehyde Concentration on the Pseudo-First-Order Rate Constant (k_{obs}) for Bacampicillin Degradation at Various pH Values of Buffer at 35°C and $\mu=0.5$

Δ , pH 7.00; $\circ$, pH 8.00; ∇ , pH 8.50; $\square$, pH 9.00. The solid lines were calculated from Eq. 8 and the parameters obtained from the rate constant, benzaldehyde concentration and Eq. 9.

Fig. 5. Double-Reciprocal Plot for the Adduct Formation between Bacampicillin and Benzaldehyde in Borate Buffer of pH 8.00 According to Eq. 9

The time courses of the total penicillin and ABPC analogues in the presence of benzaldehyde (3.5×10^{-4} M) are shown in Fig. 3. It is clear that the amounts of all the penicillins and ABPCs became the same at about 9 h and subsequently followed pseudo-first-order kinetics in the same manner as in the absence of benzaldehyde. The slope of this straight line, further, was smaller than that in the absence of benzaldehyde. This is evidently because ABPC was stabilized owing to Schiff's base formation with benzaldehyde.³⁾ The BAPC degradation obtained from both time courses also showed pseudo-first-order kinetics, and was definitely inhibited compared to that of BAPC alone.

Effect of Benzaldehyde Concentration on BAPC Degradation in Aqueous Solution

Each BAPC degradation showed pseudo-first-order kinetics and tended to be inhibited by the addition of various concentrations of benzaldehyde. The pseudo-first-order rate constants for BAPC degradation under various conditions are summarized in Table IV.

All the degradations of BAPC obeyed good pseudo-first-order kinetics and were increasingly inhibited with increasing addition of benzaldehyde in the alkaline region also. Pseudo-first-order rate constants calculated from these slopes were plotted against the concentration of benzaldehyde; as can be seen in Fig. 4, there was no linear relationship.^{3,8)}

From the above results, the reaction process of BAPC in aqueous solution with

benzaldehyde is considered to be similar to that of ABPC³⁾ as shown in Chart 3. Thus, the degradation of BAPC can be expressed by Eq. 3, since the total concentration of intact BAPC or ABPC present in the solution is determined by the I₂-colorimetry:

$$-\frac{d}{dt}([B] + [BB]) = -\frac{d}{dt}[B]_T = (k_e + k_b)[B] + (k_{se} + k_{sb})[BB] \quad (3)$$

where k_{se} = first-order rate constant of ester hydrolysis of the BAPC adduct; k_{sb} = first-order rate constant of β -lactam cleavage of the BAPC adduct; $[B]$ = concentration of free BAPC; $[BB]$ = concentration of BAPC adduct.

Chart 3

Substituting $k = k_e + k_b$, $k' = k_{se} + k_{sb}$, Eq. 3 gives Eq. 4:

$$-\frac{d}{dt}[B]_T = k[B] + k'[BB] \quad (4)$$

If 1:1 adduct formation between BAPC and benzaldehyde is assumed, the formation constant (K_B) can be expressed as follows:

$$K_B = \frac{[BB]}{[B][BZ]} \quad (5)$$

where $[BZ]$ is benzaldehyde concentration. The equilibrium rates of adduct formation of BAPC with benzaldehyde in the solution seem to be very fast compared to the degradation rates of BAPC and the adduct, because the overall reaction was observed to follow first-order kinetics.

Equation 6 can be derived from Eqs. 4 and 5.

$$-\frac{d}{dt}[B]_T = \left\{ \frac{k + k'K_B[BZ]}{1 + K_B[BZ]} \right\} [B]_T = k_{obs}[B]_T \quad (6)$$

As Eq. 6 should provide a pseudo-first-order reaction,³⁾ Eqs. 7 and 8 can be obtained.

$$[B]_T = [B]_0 e^{-k_{obs}t} \quad (7)$$

$$k_{obs} = \frac{k + k'K_B[BZ]}{1 + K_B[BZ]} \quad (8)$$

Equation 8 is converted to Eq. 9 by substituting q for $(1 - k'/k)$.

$$\frac{k}{k - k_{\text{obs}}} = \frac{1}{qK_B[BZ]} + \frac{1}{q} \quad (9)$$

A linear double reciprocal plot based on Eq. 9 was obtained from the results at pH 8.00 (Fig. 5). K_B (246.1 M^{-1}) and k' (0.323 h^{-1}) were calculated by the least-squares method from this slope and intercept.

On the other hand, the formation and degradation of ABPC are described by Eq. 10.

$$\frac{d}{dt}[A]_T = k_e[B] - k_a[A] + k_{se}[BB] - k_{sa}[AB] \quad (10)$$

Because 1 : 1 adduct formation occurs between ABPC and benzaldehyde,³⁾ Eq. 11 is valid and Eq. 12 is then obtained by using Eqs. 5, 10 and 11:

$$K_A = \frac{[AB]}{[A][BZ]} \quad (11)$$

$$\begin{aligned} \frac{d}{dt}[A]_T &= \{k_e[B] + k_{se}[BB]\} - \{k_a[A] + k_{sa}[AB]\} \\ &= \left\{ \frac{k_e + k_{se}K_B[BZ]}{1 + K_B[BZ]} \right\} [B]_T - \left\{ \frac{k_a + k_{sa}K_A[BZ]}{1 + K_A[BZ]} \right\} [A]_T \\ &= k_{OB}[B]_T - k_{OA}[A]_T \end{aligned} \quad (12)$$

$$k_{OB} = \frac{k_e + k_{se}K_B[BZ]}{1 + K_B[BZ]} \quad (13)$$

$$k_{OA} = \frac{k_a + k_{sa}K_A[BZ]}{1 + K_A[BZ]} \quad (14)$$

where $[A]$ is the concentration of free ABPC, $[AB]$ is the concentration of ABPC adduct; k_{sa} is the first-order rate constant of β -lactam cleavage of the ABPC adduct. Since benzaldehyde is present in a large excess compared to ABPC and/or BAPC, and can be regarded as almost constant during the reaction k_{OB} and k_{OA} shown in Eqs. 13 and 14 are constant. Then, Eq. 15 is obtained from Eqs. 7 and 12.

$$[A]_T = \frac{k_{OB}}{k_{\text{obs}} - k_{OA}} [B]_0 (e^{-k_{OA}t} - e^{-k_{\text{obs}}t}) \quad (15)$$

From the results in Fig. 3, k_{OB} (0.402 h^{-1}) and k_{OA} (0.012 h^{-1}) were calculated by using Eqs. 7 and 15. By applying these rate constants (k_{OB} , k_{OA}), k_a and k_e (calculated with Eqs. 1 and 2), K_B and k' (calculated with Eq. 9), and K_A ³⁾ (calculated from the results of ABPC adduct formation) to Eqs. 13 and 14, k_{se} , k_{sa} and k_{sb} were obtained as 0.249 h^{-1} , 0.0097 h^{-1} and 0.074 h^{-1} , respectively. The above rate constants, k_a and k_{sa} , were in good agreement with the result obtained from the previous study of the ABPC adduct.³⁾

Catalytic Effect of Buffer on the Adduct Degradation in Aqueous Solution

The double reciprocal plot at pH 7.00 ($\mu = 0.5$, 35°C) based on Eq. 9 showed a linear relation. The formation constant and degradation rate constant of the adduct were also calculated from these results. Figure 6 shows plots of the first-order rate constants calculated as mentioned above *versus* buffer concentration. The linearity of these plots suggests that the β -lactam and ester moiety of BAPC and its adduct were hydrolyzed by general acid-base catalysis.⁹⁾

The relation between each rate constant and buffer concentration, consequently, can be expressed in the form of Eq. 16 as a general formula.

Fig. 6. Plots of First-Order Rate Constant, k_i ($\circ$), k_{se} ($\bullet$), k_a ($\square$), k_{sa} ($\blacksquare$), k_b ($\triangle$), k_{sb} ($\blacktriangle$) versus Buffer Concentration at pH 7.00, 35°C and $\mu=0.5$

TABLE V. Rate Constants of Bacampicillin, Ampicillin and Their Adducts in Phosphate Buffer at pH 7.00, 35°C and $\mu=0.5$

	Catalytic rate constant ($M^{-1} h^{-1}$) $k_{ip}^{a)}$			Buffer-free rate constant (h^{-1}) $k_i^{0 a)}$		
	BAPC	ABPC	Adduct	BAPC	ABPC	Adduct
Ester cleavage	0.346		0.090	0.050		0.019
β -Lactam cleavage	2.02	0.236 ^{b)}	0.387 0.080 ^{b)}	0.008	0.0009 ^{b)}	0.007 0.0005 ^{b)}

a) $i=e, a, b, se, sa$ and sb . b) The values of this run were in good agreement with data obtained in a previous study of ampicillin.³⁾

$$k_i = k_{ip}[P] + k_i^0 \quad (16)$$

($i=e, a, b, se, sa$ and sb)

Here, k_{ep} , k_{ap} and k_{bp} are the catalytic rate constants for the ester, β -lactam of ABPC and β -lactam of BAPC, respectively, and, k_{sep} , k_{sap} and k_{sbp} are the catalytic rate constants for the adduct ester, β -lactam of the ABPC adduct and β -lactam of the BAPC adduct, respectively. Then, k_e^0 , k_a^0 , k_b^0 , k_{se}^0 , k_{sa}^0 and k_{sb}^0 are the buffer-free rate constants of ester, β -lactam of ABPC, β -lactam of BAPC, the adduct ester, β -lactam of the ABPC adduct and β -lactam of the BAPC adduct, respectively. The term $[P]$ is total phosphate buffer concentration. Various parameters which were calculated from the results shown in Fig. 6 are summarized in Table V. Further, the formation constant of BAPC adduct (K_B) was almost constant regardless of the buffer concentration and on average was $156.4 M^{-1}$.

These results suggest that the smaller catalytic effect of phosphate on the adduct and the slower degradation rate of the adduct compared to that of BAPC alone contribute to the stabilization of BAPC by the addition of benzaldehyde.

Effect of pH on the Kinetic Parameters of the BAPC-Adduct with Benzaldehyde in Aqueous Solution

No effects of benzaldehyde addition ($3.5 \times 10^{-3} M$) on BAPC degradation were recognized in 0.1 M acetate buffer of pH 3.00, 4.00 and 5.00 or 0.15 M phosphate of pH 6.00 ($\mu=0.5$), as in the case of ABPC.³⁾ Therefore, the adduct could not be formed between BAPC and

Fig. 7. $\log k_i$ -pH Profiles for the Degradation of Bacampicillin in the Acidic Region (35 °C, $\mu=0.5$)

○, k_e ; △, k_b ; □, k_a . Each rate constant was obtained from a run in 0.1 M acetate buffer.

Fig. 8. $\log k_i^0$ -pH Profiles for the Degradation of Bacampicillin, Ampicillin and Their Adducts at 35 °C and $\mu=0.5$

○, k_e^0 ; △, k_b^0 ; □, k_a^0 ; ●, k_{se}^0 ; ▲, k_{sb}^0 ; ■, k_{sa}^0 . The straight lines (pH 7.00) were calculated from Eq. 18 using k_{iOH} listed in Table VI, while the points are experimental values.

TABLE VI. Specific Rate Constants for the Degradation of Bacampicillin, Ampicillin and Their Adducts at 35 °C and $\mu=0.5$

	Ester cleavage $\times 10^3 \text{ M}^{-1} \text{ h}^{-1}$	β -Lactam cleavage	
		BAPC, $\times 10^3 \text{ M}^{-1} \text{ h}^{-1}$	ABPC, $\times 10^3 \text{ M}^{-1} \text{ h}^{-1}$ ^{a)}
BAPC or ABPC	100.9	41.9	3.10
Adduct	71.6	36.9	2.10

^{a)} The values of this run were in good agreement with data obtained in a previous study of ampicillin.³⁾

benzaldehyde in the acidic region.

However, since the apparent degradation rate of BAPC was smaller than that of ABPC³⁾ under the same conditions and the time courses of total penicillin followed pseudo-first-order kinetics, it is clear that $k_b, k_e < k_a$. The changes of these rate constants with pH are shown in Fig. 7. As can be seen in Fig. 7, BAPC is relatively stable at pH below 5. On the other hand, the pseudo-first-order rate constant of BAPC became smaller with increase of benzaldehyde concentration above pH 7.00 (Fig. 4). In addition, plots of the logarithm of each rate constant against pH gave a slope of unity (at pH > 8.00 for k_a, k_b) as shown in Fig. 8.

Thus, each buffer-free degradation rate constant (k_i^0) can be expressed as follows:

$$k_i^0 = k_{iOH} \cdot aOH \quad (17)$$

(i = e, a, b, se, sa and sb)

where k_{iOH} and aOH are specific rate constants for degradation of ABPC, BAPC, and their adducts, and the activity of the hydroxy ion, respectively.

Equation 17 can be converted to Eq. 18.

$$\log k_i^0 = \log k_{iOH} - pK_w + pH \quad (18)$$

As pK_w is $13.68^{3)}$ at 35°C , each specific rate constant can be calculated by substituting the results of Fig. 8 into Eq. 18 (Table VI). From these results it is clear that BAPC is easily hydrolyzed into ABPC in alkaline solution. Furthermore, the β -lactam cleavage of BAPC is about 14 times faster than that of ABPC. The degradation of BAPC, however, is inhibited by the addition of benzaldehyde. This may be largely attributed to difficulty of hydroxy ion attack resulting from steric effects in the BAPC adduct, while the β -lactam cleavage of the BAPC adduct is also inhibited by an enhancement of the usual amino resonance.³⁾

Relationship between Formation Constant and pH

As shown in Fig. 13, the apparent formation constant, K_B , increased with increase of pH. Thus, only non ionic species of BAPC, $[B]_f$, were assumed to be involved in the adduct formation with benzaldehyde. Thus the true formation constant, K_s , can be expressed as follows:

$$K_s = \frac{[BB]}{[B]_f[BZ]} \quad (19)$$

The dissociation constant of BAPC, K_a (1.58×10^{-7} M), is as follows:

$$K_a = \frac{[B]_f \cdot aH}{[BH^+]} \quad (20)$$

where $[BH^+]$ is the cationic species of BAPC. Equation 21 can be obtained from Eqs. 5, 19 and 20.

$$K = K_s \frac{K_a}{K_a + aH} \quad (21)$$

A value of K_s of 277.8 M^{-1} was obtained from the K data (Fig. 9) by means of the least-squares method. Further, the good agreement between the theoretical and experimental

Fig. 9. Plot of the Formation Constant, K_B , of Bacampicillin-Benzaldehyde Adduct as a Function of pH in Aqueous Solution at 35°C and $\mu=0.5$

The solid line was calculated by means of Eq. 21 from K_s , k_a and aH values by the least-squares method, while the points represent experimental values. The dashed line is the plot for ampicillin-benzaldehyde adduct taken from ref. 3.

Fig. 10. IR Spectra of Freeze-Dried Products
----, bacampicillin; —, bacampicillin-benzaldehyde adduct.

values as shown in Fig. 9 supports the validity of the scheme proposed in Chart 3.

Chemical Structure of the Adduct

The IR spectrum of the freeze-dried product which was prepared from a solution containing BAPC and benzaldehyde was measured in order to elucidate the structure of the adduct (Fig. 10). Specific absorption bands such as lactam¹⁰⁾ at 1760 cm^{-1} , amide¹⁰⁾ at 1685 cm^{-1} and azomethine¹¹⁾ at 1640 cm^{-1} were observed. The absorption bands at 1760 and 1685 cm^{-1} were observed while the 1640 cm^{-1} band was not detected in the freeze-dried product of BAPC alone under the same conditions. Furthermore, as no band due to aromatic aldehyde (1700 cm^{-1}) was seen in the adduct, benzaldehyde was not contained in the free form in this adduct.

Chart 4

The MS of the same products were also measured. The molecular ion peaks of BAPC (MW 465.5) and the adduct were detected at m/e 465 and 553, respectively. The latter M^+ peak (m/e 553) is consistent with the molecular weight of the adduct formed between the α -amino group of BAPC and benzaldehyde (MW 553, **a** in Chart 4). The peak at m/e 195 which was recognized in the adduct may be due to structure **b** in Chart 4, because it was not detected in the product of BAPC alone.

Consequently, the BAPC-benzaldehyde adduct is concluded to be the Schiff's base.

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References and Notes

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