Isomerization Kinetics of Panipenem in Aqueous Solution

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The isomerization kinetics of panipenem (INN: (+)-(5*R*,6*S*)-3-[(*S*)-1-(acetimidoylpyrrolidin-3-yl)thio]-6-[(*R*)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, CAS No. 87726-17-8) in aqueous solution were investigated. An equilibrium between the *Z*-form and *E*-form was observed, and it was found that the isomerization rates were affected by the pH of the solution. Under acidic conditions, the isomerization rates were small. However, the isomerization rates were increased with the pH value. This phenomenon resulted from the extent of proton dissociation from the acetimidoyl group.

Key words panipenem; *Z*-isomer; *E*-isomer; equilibrium; kinetics; mechanism

Panipenem (PAPM, Fig. 1) is a synthesized carbapenem antibiotic discovered and developed at Sankyo Co., Ltd. This compound has a broad antibacterial spectrum and potent bactericidal activity against both Gram-positive and Gram-negative bacteria, and this compound is not readily hydrolyzed by microbial β-lactamase.[1—4]

The pyrrolidinylthio group of the C2 side chain of the carbapenem nuclear structure, enhances the activity against Gram-negative bacteria, while the acetimidoyl group, at the 1 position of the pyrrolidinylthio group, enhances the activity against *Pseudomonas aeruginosa*.

In the previous report, the stability of panipenem in aqueous solution was investigated in terms of pH, ion strength, temperature and buffer catalysis, and compared with other β-lactam antibiotics.[5—11]

As for its stereochemistry, panipenem exists as either the *Z*-form or *E*-form depending on the position of the acetimidoyl group.[12] A certain isomer type can be obtained depending on the solvent used in the recrystallization process; the *E*/*Z* mixture, *E*-form, and *Z*-form can be obtained, by using acetone, methanol, and ethanol as the recrystallization solvent, respectively. In this report, the isomerization kinetics of panipenem in aqueous solution was investigated. The data in this report were obtained as the basic information for the formulation development.

Experimental

Materials Panipenem (*E*-form and *Z*-form) was obtained from Sankyo Co., Ltd. and was used without further purification. 2-(*N*-morpholino) ethanesulfonic acid (MES, pK_a=6.15) and 3-(*N*-morpholino) propanesulfonic acid (MOPS, pK_a=7.20) were purchased from Dojin Chemicals Co., Ltd. All other chemicals were of reagent grade.

Kinetic Runs The buffer used as the kinetic medium was 0.05 M MES (pH 5—6) or 0.05 M MOPS (pH 7—9), which were adjusted to the desired pH values with concentrated aqueous sodium hydroxide. The buffered solutions in the test tube were maintained at the reaction temperature (±0.1 °C) with a thermostatic water bath. The pH value of the buffered solutions was measured before and after the kinetic runs. The concentration of panipenem

in the reaction solutions was 1 mg/ml in every experiment (1 mg/ml≈3 mM). Samples were taken out from the test tube at specified times, and were diluted with ice-cooled buffer (pH 5.0). The concentration of each form of panipenem (*Z*-form or *E*-form) was determined by high-performance liquid chromatography (HPLC).

High-Performance Liquid Chromatography Analysis A μ-Bondasphere C18 (3.9 mm I.D.×150 mm, Nihon Waters K.K.) column was ice-cooled and kept at 0 °C during analysis. The mobile phase was 0.05 M acetate buffer (pH 5.0):CH$_3$CN=94:6 (v/v). The flow rate of the mobile phase was 1.0 ml/min. The elution profiles were monitored and recorded by a UV detector at 280 nm.

Theoretical Analysis

$$Z\text{-isomer} \underset{k_r}{\overset{k_f}{\rightleftharpoons}} E\text{-isomer} \tag{1}$$

Scheme 1 represents the reversible reaction between the *Z*-form isomer and *E*-form isomer.[13] The constant values, k_f and k_r, are the rate constants of the forward reaction and reverse reaction, respectively. According to Scheme 1, the reaction can be represented by Eq. 2.

$$\frac{d}{dt}[Z] = -k_f[Z] + k_r[E] \tag{2}$$

where [*Z*] and [*E*] are the concentration of the *Z*-form and *E*-form, respectively. The degradation of panipenem was negligible in all the experiments in this report. Thus, the total concentration was maintained during the kinetic runs.

$$[Z]+[E]=\text{const.}=[Z]_0+[E]_0 \tag{3}$$

where $[Z]_0$ and $[E]_0$ are the initial concentrations of the *Z*-form and *E*-form, respectively. From Eqs. 2 and 3, Eq. 4 can be obtained.

$$\frac{d}{dt}[Z] = -(k_f+k_r)\cdot[Z] + k_r\cdot([Z]_0+[E]_0) \tag{4}$$

Equation 5 can be obtained by integrating Eq. 4, with "$[Z]=[Z]_0$ at $t=0$" as the initial condition.

$$(k_f+k_r)\cdot t = \ln \frac{k_f[Z]_0 - k_r[E]_0}{(k_f+k_r)\cdot[Z] - k_r\cdot([Z]_0+[E]_0)} \tag{5}$$

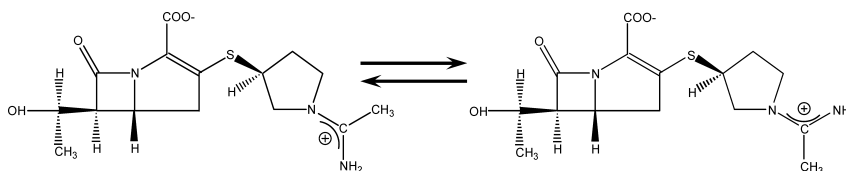Equation 5 can be transformed to Eq. 6, and [*Z*] can be expressed as a function of time.

Fig. 1. Chemical Structure of Panipenem (Left, *Z*-Form; Right, *E*-Form)

$$[Z] = \frac{(k_f[Z]_0 - k_r[E]_0) \cdot \exp\{-(k_f + k_r) \cdot t\} + k_r \cdot ([Z]_0 + [E]_0)}{k_f + k_r} \quad (6)$$

In Eq. 6, k_f and k_r are difficult to obtain from experimental data. However, in the equilibrium state, both $[Z]$ and $[E]$ are maintained constant, and the values $[Z]_{eq}$ and $[E]_{eq}$, which are the concentrations of the Z-form and E-form in the equilibrium state, can be directly determined from experimental data. In the equilibrium state, the left hand side of Eq. 2 is equal to zero, giving Eq. 7.

$$k_f[Z]_{eq} = k_r[E]_{eq} \quad (7)$$

As mentioned in Eq. 3, the total concentration "$[Z] + [E]$" is maintained during the kinetic runs, which leads to Eq. 8.

$$[Z] + [E] = \text{const.} = [Z]_0 + [E]_0 = [Z]_{eq} + [E]_{eq} \quad (8)$$

Equation 9 can be obtained from Eqs. 5, 7, and 8.

$$\ln\frac{1}{[Z] - [Z]_{eq}} = (k_f + k_r) \cdot t - \ln\frac{[Z]_0[E]_{eq} - [E]_0[Z]_{eq}}{[Z]_0 + [E]_0} \quad (9)$$

By plotting "time" against "$\ln\{1/([Z] - [Z]_{eq})\}$", a straight line with slope "$k_f + k_r$" will be obtained.

The equilibrium constant of the isomerization reaction (K) can be obtained from $[Z]_{eq}$ and $[E]_{eq}$, according to Eq. 10.

$$K = \frac{[E]_{eq}}{[Z]_{eq}} = \frac{k_f}{k_r} \quad (10)$$

Results

Typical chromatograms are shown in Fig. 2, where the E-form is eluted at 3.3 min and the Z-form at 4.3 min.
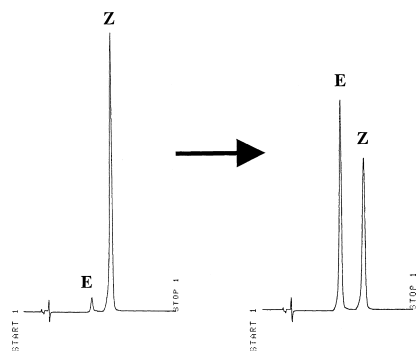
From the HPLC data, the time course of isomerization was obtained and is shown in Fig. 3 (pH 7.0, [PAPM] = 1 mg/ml, 10 °C). From Fig. 3, $[Z]_{eq}$ and $[E]_{eq}$ were 45.1% and 54.6%, respectively. Thus, the equilibrium constant (K) in this case was 1.21 (= 54.6%/45.1%, Eq. 10). In Fig. 3, a non-linear regression analysis was performed based on Eq. 6, and the values $k_f = 0.196\,h^{-1}$ and $k_r = 0.164\,h^{-1}$ were obtained. The experimental values fitted the regression curves well (Fig. 3).

Then, the effect of pH on the isomerization rates was investigated (pH 5—9), and Fig. 4 was obtained based on Eq. 9. The slope value at each pH in Fig. 4 was equal to "$k_f + k_r$", and these slope values were obtained without the non-linear regression analysis. These values were plotted as a function of pH in Fig. 5. As shown in Fig. 5, "$k_f + k_r$" values were increased with the pH values. The influence of pH on "$k_f + k_r$" (regression lines in Fig. 5) will be discussed later in this report.

From Fig. 3, $[Z]_{eq}$ and $[E]_{eq}$ were directly obtained. The equilibrium constants (K) at different pH values were calculated from Eq. 10, and are shown in Table 1. The equilibrium constant was equal to 1.2 at all pH values in these experiments. Therefore, the pH value of the solution was found to have no effect on the equilibrium constant.

As for the effect of the temperature, the isomerization rate and the equilibrium constant (K) were measured at 0, 10, 25, and 40 °C, and the results are shown in Table 2. The equilib-

Fig. 2. Typical Chromatograms of Panipenem (Left, before Kinetic Run; Right, after Kinetic Run)

Fig. 4. The Effect of pH on the Isomerization Rate

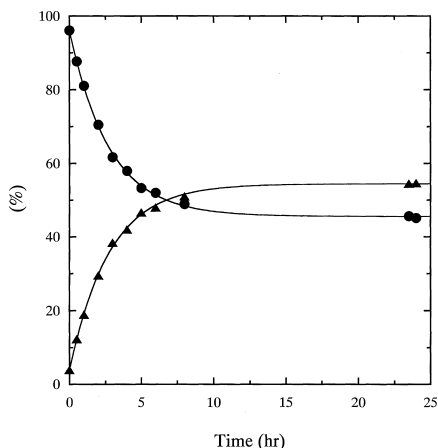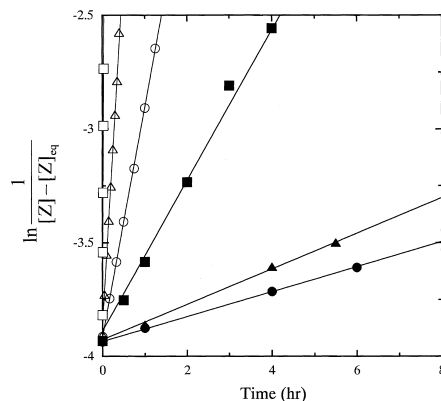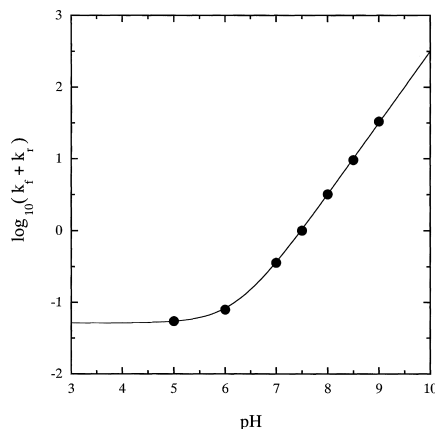[PAPM] = 1 mg/ml, 10 °C. ●: pH 5.0, ▲: pH 6.0, ■: pH 7.0, ○: pH 7.5, △: pH 8.0, □: pH 9.0.

Fig. 3. The Isomerization of Panipenem in Aqueous Solution

pH 7.0, [PAPM] = 1 mg/ml, 10 °C. ●: Z-form, ▲: E-form.

Fig. 5. The Effect of pH on the Isomerization Rate Constant

[PAPM] = 1 mg/ml, 10 °C.

Table 1.   The Effect of pH on the Equilibrium Constant

pH	K (equilibrium constant)
5.0	1.20
6.0	1.21
7.0	1.21
7.5	1.22
8.0	1.20
8.5	1.21
9.0	1.19

[PAPM]=1 mg/ml, 10 °C.

Table 2.   The Effect of Temperature on the Isomerization

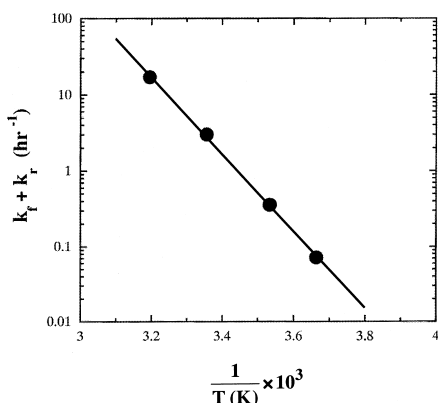| Temperature (°C)	K (equilibrium constant)	$k_f + k_r$ (h^{-1})
0	1.23	0.0719
10	1.21	0.359
25	1.18	3.02
40	1.19	17.0

[PAPM]=1 mg/ml, pH 7.0.

Fig. 6.   Arrhenius Plot for the Isomerization Rate Constants
   [PAPM]=1 mg/ml, pH 7.0.

rium constant was equal to 1.2 at all temperature. Therefore, the temperature of the solution was found to have no effect on the equilibrium constant.

Based on the Table 2 and Eq. 11, the Arrhenius plot was performed, and are shown in Fig. 6.

$$\log_{10} k = \log_{10} A - \frac{E_a}{\ln 10 \cdot RT} \tag{11}$$

In Eq. 11, A is the frequency factor, E_a is the activation energy, R is the gas constant ($R=1.987\,\mathrm{cal\,mol^{-1}\,K^{-1}}$), and T is the absolute temperature.[14] In Fig. 6, the correlation coefficient (r^2) of the regression line was 0.999, and the obtained Arrhenius parameters were, $E_a=23.2\,(\mathrm{kcal\,mol^{-1}})$, and $A=3.10\times10^{17}\,(\mathrm{h^{-1}})$.

The effects of buffer concentration and ionic strength of the solution on the isomerization rate were investigated, and these parameters were found to have no effect on the isomerization rate (data not shown).

Discussion

As shown in Figs. 7 and 8, the Z-form is thought to exist
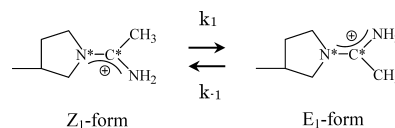
Fig. 7.   Hypothetical Equilibrium between Z_1 and E_1 (Protonated Type)
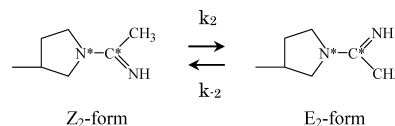
Fig. 8.   Hypothetical Equilibrium between Z_2 and E_2 (Deprotonated Type)

as either Z_1 (protonated type) or Z_2 (deprotonated type), and similarly for the E-form, as either E_1 or E_2. Considering the hypothetical equilibrium in Figs. 7 and 8, Eqs. 12 and 13 are obtained for the forward reaction and the reverse reaction, respectively.

$$v_f = k_f[Z] = k_1[Z_1] + k_2[Z_2] \tag{12}$$

$$v_r = k_f[E] = k_{-1}[E_1] + k_{-2}[E_2] \tag{13}$$

where v_f and v_r are the reaction velocities of the forward reaction and reverse reaction, respectively.

Equation 14 can be obtained from Eqs. 12 and 13.

$$k_f + k_r = \frac{k_1[Z_1] + k_2[Z_2]}{[Z]} + \frac{k_{-1}[E_1] + k_{-2}[E_2]}{[E]} \tag{14}$$

The dissociation constants for the acetimidoyl group in Figs. 7 and 8, are expressed as Eqs. 15 and 16. The dissociation constants, K_{aZ} and K_{aE}, are considered to have almost the same value, and can thus be represented as K_a (Eq. 17).

$$K_{aZ} = \frac{[Z_2][H^+]}{[Z_1]} \tag{15}$$

$$K_{aE} = \frac{[E_2][H^+]}{[E_1]} \tag{16}$$

$$K_a \equiv K_{aZ} = K_{aE} \tag{17}$$

Therefore, from Eqs. 14—17, Eq. 18 is obtained.

$$k_f + k_r = (k_1 + k_{-1}) + (k_2 + k_{-2})\frac{K_a}{[H^+]} \tag{18}$$

In Eq. 18, when the term "$k_1 + k_{-1}$" is small enough to be neglected, Eq. 18 can be transformed to Eq. 19.

$$\log_{10}(k_f + k_r) = \mathrm{pH} + \log_{10}(k_2 + k_{-2}) - pK_a \tag{19}$$

Based on Eq. 19, the plots of "$\log_{10}(k_f + k_r)$" against "pH" provide a linear relationship with slope=1.

As shown in Fig. 5, a linear correlation of the data set was observed for the alkaline solution. Therefore, a linear regression analysis was performed for 4 data points (pH 7.5—9) as shown in Fig. 5 to provide "slope=1.0071, r^2=0.9994". As "$k_1 + k_{-1}$" in Eq. 18 was small enough to be neglected, Eq. 19 can be applied for the alkaline solution.

Regarding the pK_a value of the acetimidoyl group, it was impossible to obtain this value by photometric methods because of the degradation of panipenem itself during the mea-

surement. Therefore, the pK_a value of the acetimidoyl group was calculated as 10.9, based on the iso-electric point of panipenem which was determined to be 7.15 by titration methods, and the pK_a value of the carboxyl group which was determined to be 3.4 by photometric methods.

Then, from the linear regression analysis of the 4 data points (pH 7.5—9) based on Eq. 18, "k_2+k_{-2}" was calculated to be $2510\,h^{-1}$. The "k_1+k_{-1}" value was calculated to be $0.0514\,h^{-1}$, by substituting "k_f+k_r (at pH 5.0)$=0.0546\,h^{-1}$", "p$K_a=10.9$", and "$k_2+k_{-2}=2510\,h^{-1}$", in Eq. 18. The curve based on Eq. 18, using these 3 parameters (p$K_a=10.9$, $k_1+k_{-1}=0.0514\,h^{-1}$, $k_2+k_{-2}=2510\,h^{-1}$), is shown in Fig. 5, which well fits the experimental values.

In Figs. 7 and 8, considering the molecular bond between the nitrogen and carbon atoms (N*–C*), a double bond restricts the rotation of the Z_1- or E_1-form (protonated form); on the other hand, a single bond rotates freely in the Z_2- or E_2-form (deprotonated form). Therefore, the greater the pH value, the greater the number of molecular species in the deprotonated form, and the faster is their isomerization. The rate of isomerization was related to the degree of rotation of the molecular bond.

Conclusions

In this study, the isomerization kinetics of panipenem in aqueous solution was examined, and it was found that the isomerization rate between the Z-form and E-form was affected by the pH value of the solution. Under acidic conditions, the isomerization rate was small. However, the isomerization rate was increased with the pH value. The phenomenon resulted from the extent of proton dissociation from the acetimidoyl group.

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