Stability of a 1β-Methylcarbapenem Antibiotic, Meropenem (SM-7338) in Aqueous Solution

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The stability and the degradation products of 1β-methylcarbapenem, meropenem in aqueous solution were investigated. In pH 4—8 dilute solution, pseudo-first-order degradation was observed, and good stability of meropenem in aqueous solution was demonstrated by the effect of 1β-methyl group against hydrolysis of β-lactam ring. As degradation products, the β-lactam hydrolyzed product and the dimer product resulting from intermolecular aminolysis of β-lactam ring by the amine of the second molecule were described.

Key words meropenem; stability; aqueous solution; kinetics; degradation product

Meropenem (**1**) is a novel 1β-methylcarbapenem antibiotic which exhibits a potent antibacterial activity against a wide range of gram-positive and gram-negative bacteria, and is highly stable against renal dehydropeptidase-I (DHP-I).[1]

While possessing excellent antibacterial activity, thienamycin (**2A**) is chemically unstable in aqueous solution because of the high reactivity of β-lactam ring due to its highly strained structure, which is closely related to the potent antibacterial activity, and intermolecular aminolysis of β-lactam by the primary amine in the side chain.[2] Imipenem (*N*-formimidoylthienamycin, **2B**) was designed to improve the chemical stability by amidination of the primary amine, which repressed the intermolecular aminolysis.[3,4] Meropenem was also designed to prevent the aminolysis by decreasing the basicity and nucleophilicity of the basic part in the C-2 side chain.

It has also been reported that the 1β-methylcarbapenem has a higher stability against DHP-I and a higher chemical stability than the corresponding non-1β-substituted carbapenem.[5] Details of the effect on DHP-I susceptibility have been reported,[1,6] however, the detailed chemical stability of 1β-methylcarbapenem is aqueous solution has not been reported.

We described here chemical stability studies of 1β-methylcarbapenem, meropenem in aqueous solution, demonstrate its improved stability against chemical hydrolysis due to the effect of 1β-methyl group, and its expected resistance to intermolecular aminolysis.

Experimental

Materials Meropenem was synthesized as reported previously.[1] 2-(*N*-Morpholino)ethanesulfonic acid (MES) and 3-(*N*-morpholino) propanesulfonic acid (MOPS) were purchased from Dojin Chemicals Co., Ltd. Monobasic potassium phosphate was purchased from Nacalai Tesque Inc.

Measurement of pK_a The apparent pK_a value of meropenem was determined potentiometrically by measuring the pH of solutions prepared by mixing meropenem solution with 0.1 N hydrochloric acid or 0.1 N potassium hydroxide solution. The initial concentration of meropenem was 200 mg/50 ml. The dissociation constant of carboxylic acid was 2.9 and that of pyrrolidine amine was 7.4.

High-Performance Liquid Chromatography Analysis The column, Sumipax ODS 5 μm (6 mm i.d. × 15 cm, Sumitomo Chemical Analysis Center, Japan), was kept at 40 °C during analysis. The elution pattern was recorded by a UV detector which was set at a wavelength of 220 nm. The mobile phase was 0.1% triethylamine-phosphate buffer (pH 5.0): methanol = 5:1 (v/v). Flow rate of the mobile phase was set at 1.0 ml/min.

Kinetic Runs Aqueous buffer solutions of 0.224 M monobasic potassium phosphate, 0.1 M MES (μ=0.05) and 0.1 M MOPS (μ=0.05) were adjusted to the desired pH with concentrated aqueous sodium hydroxide. These buffers, to be used as solvents for kinetic runs, were maintained at the reaction temperature. The kinetic studies were carried out at 25, 35 and 40 °C. The residual meropenem concentrations were determined by high-performance liquid chromatography.

In the hydrolysis reaction with pseudo-first-order rate, the rate dependence on pH is consistent with the following equation[7]:

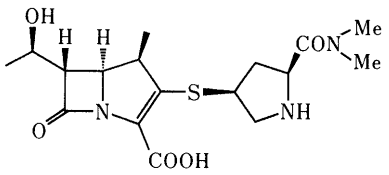
$$k_{pH} = k_H a_H + k_0 + k_{OH} (K_W / a_H)$$

where k_{pH} is the apparent first-order rate constant at a given pH; k_H and k_{OH} are the second-order rate constants for the hydrogen-ion-catalyzed degradation and hydroxide-ion-catalyzed degradation, respectively; k_0 is the first-order rate constant for spontaneous or water-catalyzed degradation; K_w is the dissociation constant of water; and a_H is the hydrogen ion activity. The values of K_w: 10^{-14} at 25 °C, $10^{-13.65}$ at 35 °C and $10^{-13.47}$ at 40 °C were used.[8]

The rate dependence in phosphate buffer was consistent with the following equation[9]:

$$k' = k_{pH} + k_{HPO_4^{2-}} [HPO_4^{2-}]$$

where k' is the apparent rate constant; $k_{HPO_4^{2-}}$ is the second-order rate constant for the phosphate ion catalyzed degradation; and $[HPO_4^{2-}]$



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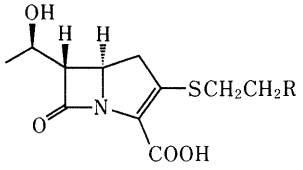

2A : R = NH_2

2B : R = NHCH=NH

Fig. 1. Structure of Meropenem (**1**), Thienamycin (**2A**) and Imipenem (**2B**)

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is the phosphate ion concentration.

Identification of Meropenem Dimer An aqueous solution of equimolar amounts of meropenem and sodium hydrogencarbonate was lyophilized to give sodium salt of meropenem. The solution of this salt (400 mg) in water (1 ml) was kept at 30 °C and monitored by HPLC. The HPLC peak area ratio after 6 h was: meropenem 47%, degradation product A 11% and degradation product B 38%. The solution of the mixture was neutralized with diluted hydrochloric acid and poured into acetone to afford 320 mg of amorphous powder. This crude product was purified by CHP-20P absorption column chromatography and the fractions containing product B were corrected and lyophilized to afford the dimer product (25 mg) as white powder. UV $\lambda_{max}^{H_2O}$ 298 nm. MS *m/z*: 767 (M + H). IR (KBr): 3400, 1750, 1655, 1600 cm^{-1}. ^{1}H-NMR (D_2O) δ: 0.95 (d, J=7.3 Hz, 28-CH_3 of **3B**), 1.07 (d, J=7.3 Hz, 28-CH_3 of **3A**), 1.22 (d, J=7.3 Hz, 10-CH_3), 1.26 (d, J=6.3 Hz, 27-CH_3), 1.32 (d, J=6.3 Hz, 9-CH_3), 1.7—2.0 (m, 15-H and 32-H), 2.7—3.0 (m, 19-H, 24-H and 32-H), 2.97 (s, N-CH_3), 3.00 (s, N-CH_3), 3.06 (s, N-CH_3), 3.20 (s, N-CH_3), 3.0—3.2 (m, 15-H), 3.2—3.8 (m, 1-H, 6-H, 13-H and 30-H), 4.00 (m, 20-H), 4.0—4.4 (m, 5-H, 8-H, 12-H, 23-H, 26-H and 29-H), 4.68 (m, 23-H), 5.06 (m, 15-H).

Results and Discussion

Stability in Aqueous Solution The degradation pattern of **1** in aqueous solution (5 mg/ml, 13 mM) was investigated by HPLC; degradation products were product A as major product and product B as minor product with other products only in trace amounts. It was observed that product A is preferentially formed under both acidic and alkaline conditions, while product B tended to be formed in a neutral pH region. In addition, an increasing tendency of product B was observed when higher concentration of **1** was used. Thus, product B is presumed to be the product of an intermolecular reaction, bimolecular aminolysis, of **1**.

The degradation kinetics of **1** in dilute aqueous solution (1 mg/ml, 2.6 mM) with non nucleophilic MES and MOPS buffer at pH 4—8 were investigated at 25, 35 and 40 °C. In dilute solution of **1**, plots of log residual *versus* time were linear (Fig. 2), indicating that the degradation of **1** follows pseudo-first-order decomposition. The pH *versus* degradation rate constant plot, shown in Table 1, indicates that **1** is relatively more unstable under acidic and alkaline conditions than under neutral condition, which is consistent with other β-lactam compounds.[4,7]

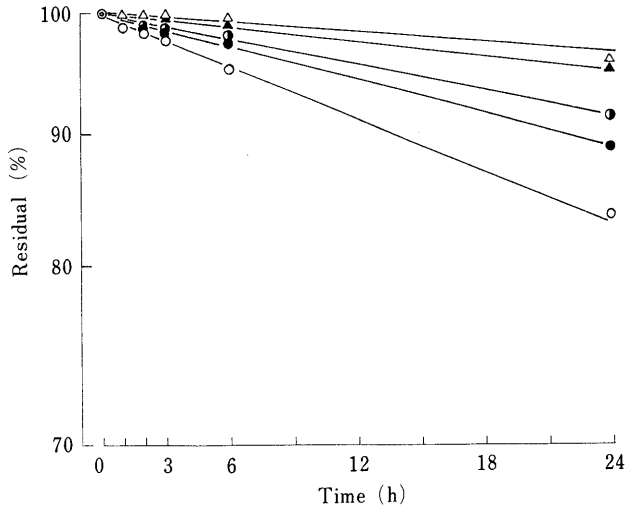


Fig. 2. Apparent First-Order Plots Followed by HPLC for the Degradation of Meropenem at Various pH, in MES and MOPS Buffers, 25 °C and μ=0.05

Initial potency of meropenem: 1 mg/ml. pH; —△—, 6.0; —▲—, 7.0; —◑— 8.0; —○—, 4.1; —●—, 5.0.

The rate constants of meropenem with hydrogen ion, water and hydroxide ion and the activation energies were calculated by nonlinear regression based on the degradation data at 25, 35 and 40 °C (see Table 2). The reactivity of **1** with hydrogen ion, water and hydroxide ion is significantly lower than that of non 1β-methylcarbapenem, imipenem **2B** (k_H($M^{-1}h^{-1}$): 6500, k_{OH} ($M^{-1}h^{-1}$): 7700, k_0 (h^{-1}): 0.01 at 35 °C),[4] and is almost the same as a penicillin such as carbenicillin (k_H ($M^{-1}h^{-1}$): 52.2,

Table 1. Apparent First-Order Rates of Meropenem

pH	k_{pH},[a] $h^{-1} \times 10^3$			k_{obs},[b] $h^{-1} \times 10^3$
	25 °C	35 °C	40 °C	25 °C
4.0	3.079	8.821	16.582	6.869
4.5	2.236	4.470	—	—
5.0	1.465	2.617	5.817	3.233
5.5	1.308	2.096	—	—
6.0	0.844	1.878	2.761	3.253
6.5	0.950	1.999	—	—
7.0	1.014	2.597	3.478	4.400
8.0	1.725	3.834	5.987	5.056

a) Measured in nonnucleophilic buffers, MES (pH 4.0—6.0) and MOPS (pH 6.5—8.0). *b*) Apparent rate in phosphate buffer, $[H_2PO_4^-]+[H_2PO_4^{2-}]$= 0.224 M.

Table 2. Calculated Reactivities and Activation Energies of Meropenem in Nonnucleophilic Buffers

Parameter	k_H, $M^{-1}h^{-1}$	k_0, h^{-1}	k_{OH}, $M^{-1}h^{-1}$
Rate-constant at 25 °C	26.9	0.001	681
Rate-constant at 35 °C	68.8	0.002	808
Rate-constant at 40 °C	132.7	0.003	1120
E_a (kcal/mol)	19.4	14.8	5.7

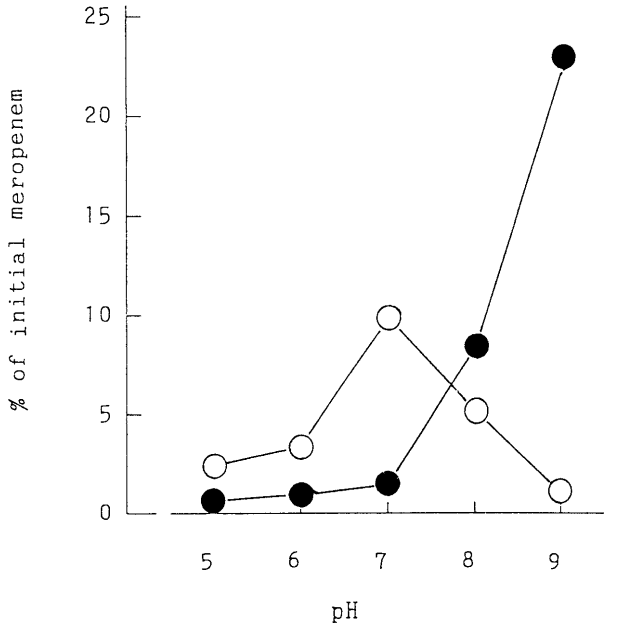
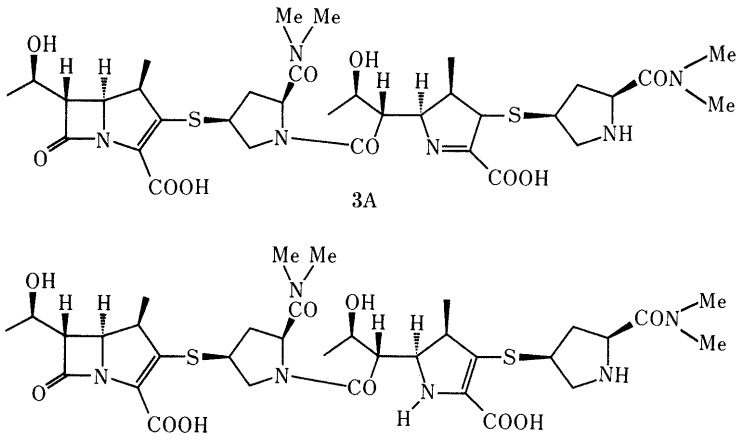


Fig. 3. The pH Profiles of Degradation Product of Meropenem in 0.2 M MOPS Buffer at 23 °C

Percent of initial meropenem, 24 h after dissolution. Initial potency of meropenem: 30 mg/ml. ●, product A; ○, product B.



3A

3B

Fig. 4. Structure of Dimer Product of Meropenem (**3A** and **3B**) Formed by Intermolecular Aminolysis

k_{OH} ($M^{-1}h^{-1}$): 1210, k_0 (h^{-1}): 0.002 at 35 °C).[7]

The degradation kinetics in phosphate buffer which has a nucleophilicity was also investigated. The degradation rate constants at various pH are also shown in Table 1. A comparison of the value of the catalytic constant of the phosphate ion with those of other β-lactam compounds shows that **1** is more stable than **2B** and several penicillins. The phosphate ion catalyzed constant from **1** is about 40 times lower than that of **2B**, although the hydroxide ion catalyzed constant for **1** is almost 10 times lower than that of **2B**. ($k_{HPO_4^{2-}}$ ($M^{-1}h^{-1}$), **1**: 0.0158 (at 25 °C), **2B**[4]: 0.58 (at 23 °C), carbenicillin[10]: 0.064 (at 35 °C)).

To determine the behavior of the intermolecular reaction of **1**, its chemical degradation of **1** in aqueous solution at high concentration was also investigated. The pH profile of degradation product of **1** in MOPS buffer at initial concentration of 30 mg/ml, show in Fig. 3, indicated that product B (intermolecular reaction product) was preferentially formed in the neutral pH region. This could be explained by the aminolysis taking place in the neutral pH, because the amino group exists as a free form at neutral and alkaline pH. At higher alkaline pH, although the amino group exists as a free form, the β-lactam hydrolysis reaction by hydroxide ion is predominant and product B is observed as a minor product.

However, **1** was highly stable even at high concentration ($T_{0.9}$ (h), time required to reach 90% of the initial potency is *ca.* 15 h at pH 7 and *ca.* 11 h at pH 8; conditions are described in Fig. 3). The weak basicity of secondary amino group of pyrrolidine, and the lower reactivity to the bimolecular reaction is considered to be one of the factors accounting for the excellent stability of **1** in aqueous solution.

Degradation Product in Aqueous Solution The results of HPLC analysis confirmed degradation product A to be the β-lactam hydrolyzed product described in a previous paper.[10]

Degradation product B exhibited a peak at 298 nm, characteristic UV absorption of carbapenem compounds, and also showed an absorption at 1750 cm^{-1} which indicates the existence of a carbonyl group of a β-lactam ring by IR spectrum, suggesting that degradation product B has a carbapenem skeleton. Furthermore, the molecular ion peak of this degradation product was observed at $m/z = 767$ in the mass spectrum. From these spectroscopic results, degradation product B is thought to be a dimer formed by intermolecular aminolysis, a reaction between the amino group of pyrrolidine ring of the first molecule of **1** with the β-lactam ring of the second molecule. The fact that product B has antibacterial activity and also tends to be formed in high concentration supports this conclusion.

The ^{1}H-NMR spectrum of the dimer, with the upfield chemical shift for one of 6-H compared with **1** (2.7—3.0 and 3.2—3.8 ppm for dimer, 3.46 ppm for **1**) and with the peaks of 4 singlet *N*-methyl proton also indicate the existence of **1** and a β-lactam ring opened structure. The proton peak of the dimer corresponding to the 1β-methyl protons of **1** were observed as three doublets, and among them two upfield doublets had a pattern closely resembling that of the β-lactam ring-opened product, and the sum of integration of these two peaks corresponds to three protons. Therefore, the dimer was also belived to exist as a mixture of double bond isomers, 1-pyrroline (**3A**) and 2-pyrroline (**3B**) (see Fig. 4). 20-H proton assigned as a characteristic peak of the 1-pyrroline ring was observed at 4.00 ppm. The ^{1}H-NMR spectrum of dimer suggested that the 1-pyrroline isomer existed as a main component in aqueous solution and that the ratio of **3A** and **3B** varied with pH of this solution. As to the relationship between **3A**/**3B** ratio and pH, the ratio was about 8/1 at pH 6.5, 5/1 at pH 9 and about 2/1 at pH 4.5 respectively.

The mixture of meropenem and dried sodium carbonate was therefore selected as the injection form for the purpose of pH control and dissolution, for a pharmaceutical application of **1**.[11]

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