STUDIES ON THE STRUCTURES OF MEROPENEM(SM-7338) AND IT'S PRIMARY METABOLITE

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The structure and solution conformation of meropenem was examined by using ^{1}H and ^{13}C NMR spectroscopy and nuclear Overhauser enhancement experiments. Similar to the X-ray crystal structure, the close spacing of 1β-methyl substituent to the β-lactam ring and the accessible conformation of C-2 side chain in relation to the carbapenem skeleton was confirmed. The structure of the primary metabolite of meropenem by dehydropeptidase-I was shown to be the β-lactam ring-opened product by comparing the spectroscopic data with those of meropenem, and confirmed by the preparation and structural analysis of its crystalline derivative. This metabolite existed as a mixture of 1-pyrroline and 2-pyrroline isomers, and the coexistence of two isomers at equilibrium in aqueous solution was observed by NMR.

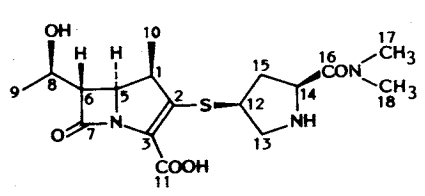
Thienamycin and the related naturally occurring carbapenems are antibiotics possessing potent antibacterial activities against a wide range of Gram-positive and Gram-negative bacteria. While possessing excellent antibacterial activity they are chemically unstable and easily metabolized by renal dehydropeptidase-I (DHP-I)[1,2]. Meropenem(SM-7338), (1*R*,5*S*,6*S*)-2-[(3*S*,5*S*)-5-dimethylaminocarbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acid, (**1**) (Fig. 1) is a novel 1β-methylcarbapenem antibiotic which exhibits an extremely broad spectrum of antibacterial activity and is highly stable against DHP-I[3]. The effect of the 1β-methyl substituent on the conformation of carbapenem ring system is of interest in connection with the chemical and biological stability. The NMR spectroscopy was carried out to study the solution conformation of 1β-methylcarbapenem derivatives. The solution conformation was compared with the X-ray crystal structure.

Furthermore, in the previous investigations[4~6], it has been reported that penem and carbapenem compounds are metabolized by DHP-I, resulting in the formation of β-lactam ring-opened hydrolytic products as the main metabolites. Although meropenem is highly stable against DHP-I digestion, it was also of interest to determine the structure of the metabolite to investigate the degradation pathway of meropenem.

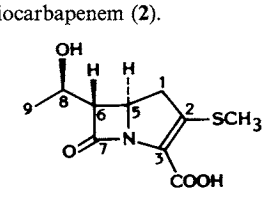
The Structure and Solution Conformation of Meropenem

To maintain high quality as pharmaceutical, the stability of the substance in solid state is an

Fig. 1. Structure of meropenem (**1**) and 2-methylthiocarbapenem (**2**).



1



2

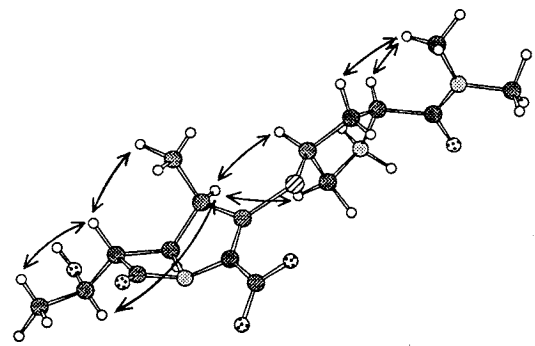
important characteristic. The lyophilized product **1**, which is obtained as an amorphous powder, shows poor stability. However, the crystalline **1** obtained by crystallization from the aqueous solution as trihydrate has excellent stability in solid state. The crystal structure of this meropenem trihydrate was determined by X-ray crystal structure analysis[7]. This revealed the formation of hydrogen bonds between all the functional groups of meropenem and water of crystallization was confirmed, which results in an extensive matrix structure. The IR spectrum exhibits the absorption band at 1750 cm^{-1}, assigned to the carbonyl group of β-lactam ring, shifted to a relatively low stretching frequency compared with other carbapenem compounds. Thus, it is suggested that the good stability of meropenem trihydrate in solid state is attributable to the hydrogen bond formation between the functional groups of **1** and water of crystallization.

The proton assignments for **1** were obtained from the 2D ^1H-^1H COSY data. The coupling constants of the carbapenem ring protons are consistent with the stereochemistry, that is, 1-H–5-H *cis*-(J=9.2 Hz), 5-H–6-H *trans*-(J=3.3 Hz) and 6-H–8-H *trans*-(J=6.3 Hz) stereochemistry.

In order to elucidate the conformation in solution, a nuclear Overhauser enhancement (NOE) study was carried out with particular interest on the 1β-methyl and C-2 side chain conformation. The following enhancements were observed upon irradiation and measurement of the NOE difference spectra; 1-H–8-H, 1-H–12-H, 1-H–13-H, 6-H–9-H, 6-H–10-H, 17- or 18-H–14-H and 17- or 18-H–15-H (shown in Fig. 2). The observation of an NOE between 6-H–10-H is due to the steric interaction caused by the rigidity of the carbapenem skeleton. The presence of steric interaction between 6-hydroxyethyl and C-1 was also supported by an NOE (1-H–8-H). Further, with regard to the C-2 side chain conformation, in addition to the accessibility of dimethylamide group and pyrrolidine ring (17- or 18-H and 15-H), it is confirmed that the relative position of C-2 side chain against carbapenem skeleton is fixed at an angle that the steric interaction is expected (1-H–12-H and 1-H–13-H). After calculation of the distance between the protons, based on the X-ray analysis data, all NOEs were found to correspond to the interaction between protons 2.29~2.46 Å apart except 1-H–8-H (4.59 Å). The accessibility of 6-hydroxyethyl and C-2 side chain moiety to C-1 position in the solution is of interest in connection with the reactivity and stability of **1**.

In order to consider the effect of 1β-methyl substitution to the solution conformation of carbapenem skeleton, the NMR data was compared with 2-methylthiocarbapenem (**2**)[6]. There were significant differences in the chemical shift of 6-H (**1**: 3.39 ppm, **2**: 3.46 ppm), and $J_{5,6}$ (**1**: 3.3 Hz, **2**: 2.4 Hz). The large $J_{5,6}$ for **1** may be attributable to the change of dihedral angle (5-H–C-5–C-6–6-H) caused by 1β-methyl substituent. Changes were also observed in ^{13}C NMR analysis specifically a downfield shift at C-1 and C-5 and an upfield shift at C-6. The downfield shifts are due to α and β-effect of 1β-methyl substitution, and the upfield shift is due to the steric interaction with 1β-methyl substituent, respectively. The close chemical shift value of carbonyl carbon of the β-lactam ring (**1**: 168.4 ppm, **2**: 169.3 ppm) indicate that these two compounds have almost the same degree of amide resonance, suggesting that the same chemical reactivity in the

Fig. 2. Selected NOE enhancement for meropenem.



solution is expected.

Thus, with regard to the solution conformation of **1**, the close spacing of 1β-methyl group to one side of β-lactam and 6-hydroxyethyl group to the other side is suggested, indicating that conformation similar to the crystal can be considered in aqueous solution. This solution conformation around β-lactam ring may correlate to the stability and the reactivity of **1**. The high stability against DHP-I can be attributed to this conformational properties, that is, the 1β-methyl substitution of β-lactam ring hindering the interaction of **1** and the enzyme. Furthermore, the 1β-methyl substituent, by steric repulsion, is likely to be contributing to a less folded, flat carbapenem ring system compared to carbapenems without 1β substitution. This is possibly another factor contributing to the stability of **1** against enzymatic and chemical hydrolysis, and also to the excellent antibacterial activity of **1**.

The Structure of Primary Metabolite

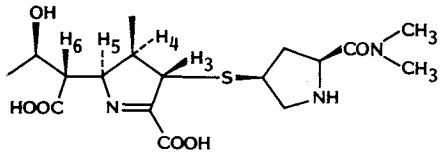
The enzymatic degradation product of **1** was obtained by treating **1** with DHP-I. The HPLC analysis of the reaction mixture revealed a single peak, whose retention time was identical with that of the metabolite detected in the urine sample of a mouse treated with **1**. After purification by column chromatography of metabolite (**3**), **3** was subjected to UV and IR analysis. As a result, absorption at *ca.* 300 nm in the UV spectrum, and IR absorption at 1750 cm^{-1} (carbonyl of β-lactam group), both of which are characteristic for carbapenems were not detectable. The molecular ion peak of the metabolite was observed at *m/z* 402 in the mass spectrum. These results suggest that **3** is a β-lactam ring-opened product, similar to metabolites of other carbapenem compounds. It is also noteworthy that the ^1H NMR spectrum of **3** revealed several peaks corresponding to the 1β-methyl substituent, suggesting that the metabolite existed as a mixture in aqueous solution.

In order to confirm that the product of DHP-I digestion was not altered during the process of purification, the enzymatic hydrolysis of **1** was conducted in D_2O under the existence of DHP-I at 37°C and continuously monitored by ^1H NMR spectroscopy. The ^1H NMR spectrum of this mixture was consistent with that of isolated **3** and indicated that **1** was completely degraded. Almost no change in ^1H NMR spectrum was observed under the same condition without DHP-I.

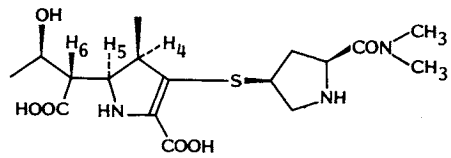
To further investigate the structure of **3**, **1** was subjected to chemical hydrolysis, which was expected to yield the same β-lactam ring-opened product. The chemical hydrolysis of **1** was monitored by using HPLC analysis. Under acidic (pH < 2) and alkaline (pH > 13) conditions, **1** was immediately hydrolyzed, and one major degradation product was formed. The hydrolyzed products were isolated and purified by absorption column chromatography. HPLC analysis, UV, IR and NMR spectra demonstrated that these hydrolyzed products were identical to metabolite **3** obtained by DHP-I treatment of **1**. As a result, the chemical hydrolysis proved to be a convenient alternative method to prepare the metabolite.

The ^1H NMR spectra, with the upfield chemical shift for 6-H compared with **1** (2.58 ppm for **3**, 3.46 ppm for **1**) and large $J_{5,6}$ coupling constant of 8.3 Hz (**1**: $J_{5,6}=3.3$ Hz) also indicate that **3** is a β-lactam ring-opened product. The ^1H NMR data also indicated that some proton peaks exist as mixture of signals as well. For instance, β-methyl (C-4) proton at 0.84 and 0.99 ppm, and 5-H proton at 3.87 and 4.25 ppm had approximately the same intensity ratio. These results suggest that hydrolyzed product **3** exists as a mixture of double bond isomers in aqueous solution, 1-pyrroline (**3A**) and 2-pyrroline (**3B**) (Fig. 3). Among these isomers, 1-pyrroline isomer (**3A**) is possibly the main component of the mixture, judged from the relative intensity of the NMR signals. Storing the solution of **3** at room temperature

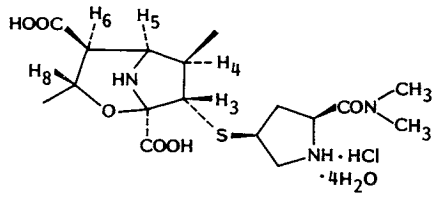
Fig. 3. Structure of metabolites of meropenem.



3A



3B



3C

resulted in an equilibration mixture of **3A** and **3B** at the ratio of 4:1.

In the previous investigations regarding the stereochemistry of β-lactam ring-opened 1-pyrroline type product, the conformation at C-3 position, a new asymmetric center, was either elucidated as a mixture or remained unclarified[4,6], which suggests the difficulty in elucidating the conformation. However, *trans* conformation at C-3 and C-4 of **3A** was clearly suggested by NMR data of 3-H, a doublet at 3.80 ppm ($J_{3,4}=2.3$ Hz). This result was probably due to the steric interaction between the β-methyl and *S*-pyrrolidine substituent.

In order to confirm the structure and the stereochemistry of the metabolite, a crystalline sample was prepared. The crystalline hydrolysis product (**3C**) was obtained as a tetrahydrate of mono HCl salt by crystallization from aqueous hydrochloric acid solution of **1**. As expected, **3C** possesses the same retention time with **3A**/**3B** in HPLC analysis. However, the ^{1}H NMR and IR spectrum of **3C** was unidentical to that of **3A**/**3B**. From mass spectrum data (*m*/*z* 402) and upfield shifts of 3-H and 5-H proton of **3C** in NMR compared with **3A**/**3B**, (ppm, 3-H; 2.81 for **3C**, 3.80 for **3A**, 5-H; 3.67 for **3C**, 4.25 for **3A**, 3.87 for **3B**), the structure of **3C** was speculated to be a bicyclo compound with a novel structure (Fig. 3). A large coupling constant, $J_{3,4}=7.9$ Hz, indicates the *trans* stereochemistry of the bicyclo form. This bicyclic structure was also confirmed by X-ray crystal structure analysis of **3C**.

The ^{1}H NMR spectrum of **3C** in alkaline condition revealed the existence of **3A** as a minor component, which suggests that the ring-opened type and bicyclo type compounds coexist in aqueous solution. The ^{1}H NMR spectra of **3C** recorded immediately after dissolution in NaOH/D_2O (pD=12) demonstrated that the ratio of **3A** to **3C** is *ca.* 1:4. Compound **3C** is converted gradually into a mixture of **3A** and **3B** by storing the solution at room temperature. On the other hand, the spectra of **3C** in the neutral condition, pD=6~7.5, is identical with that of the mixture of **3A** and **3B**. These results suggests that the new bicyclo form **3C**, is converted completely into ring-opened products **3A** and **3B**, in neutral aqueous solution.

Experimental

IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer. NMR spectra were taken with

Jeol JNM-GX270 (270 MHz) FT spectrometer, in D_2O using 2,2-dimethyl-2-silapentane-5-sulfonate or residual HOD (δ: 4.80 ppm) for ^1H NMR and dioxane for ^{13}C NMR as an internal reference. Mass spectra were obtained on Hitachi DF/GC/MS M-80 and M-003, and Jeol JMS-HX110 and JMA-DA5000 spectrometers. UV spectra were recorded on a Hitachi 330 UV-VIS spectrophotometer. HPLC condition for the analysis of the hydrolysis of meropenem is as follows: Novapak C-18 column (4 μm, 8 mm × 10 cm, Waters); Mobile phase: 0.005 M phosphate buffer (pH 7.0)-methanol (100:20); Detector wavelength: UV 220 nm.

Meropenem Trihydrate

Meropenem amorphous powder (3.0 g) was dissolved in water (30 ml) at 30°C and cooled to 5°C, and then acetone (150 ml) was added. After stirring for 1 hour, the resulting crystalline material was collected by filtration, washed with acetone and dried under reduced pressure to afford meropenem trihydrate (2.28 g) as white crystals: FAB-MS m/z 384 (M+H), 298, 175, 143; IR (KBr) cm^{-1} 3400, 1750, 1650, 1580, 1390; ^1H NMR (D_2O, 2,2-dimethyl-2-silapentane-5-sulfonate as standard) δ: 1.20 (d, J=7.3 Hz, 10-CH_3), 1.28 (d, J=5.9 Hz, 9-CH_3), 1.97 (m, 15-Hα), 2.29 (s, N–CH_3), 3.06 (s, N–CH_3), 3.12 (m, 15-Hβ), 3.37 (dq, J=9.2 and 7.3 Hz, 1-H), 3.45 (dd, J=4.6 and 11.9 Hz, 13-Hα), 3.46 (dd, J=3.3 and 5.9 Hz, 6-H), 3.76 (dd, J=6.6 and 11.9 Hz, 13-Hβ), 4.05 (m, 12-H), 4.24 (dq, J=5.9 and 5.9 Hz, 8-H), 4.24 (dd, J=3.3 and 9.2 Hz, 5-H), 4.77 (m, 14-H). ^{13}C-NMR (D_2O) δ: 16.6 (q, C-10), 20.9 (q, C-9), 34.4 (t, C-15), 36.6 (q, N–C), 37.4 (q, N–C), 41.3 (d, C-12), 43.3 (d, C-1), 53.0 (t, C-13), 56.7 (d, C-5), 59.0 (d, C-14), 59.6 (d, C-6), 65.9 (d, C-8), 134.6 (s, C-3), 138.2 (d, C-2), 168.4 (s, C-7), 168.7 (s, C-16), 177.7 (s, C-11). Some connectivities established by NOE difference experiments in D_2O are as follows:

Proton irradiated	Proton affected
1-H	8-H, 12-H, 13-H
6-H	9-H, 10-H
N–CH_3	14-H, 15-H

Anal Calcd for $C_{17}H_{25}N_3O_5S \cdot 3H_2O$: C 46.67, H 7.14, N 9.60, S 7.33.
Found: C 46.32, H 7.41, N 9.41, S 7.24.

Enzymatic Hydrolysis of **1**

Renal DHP-I, which was partially purified from swine kidney by CAMBELL's method was used[8]. A solution of **1** (30 mg) in 0.1 M Tris buffer (5 ml) and a DHP-I solution was kept at 37°C, the resulting solution was periodically monitored by HPLC. After 5 hours, the absence of **1** and formation of a new single peak was observed and solution was concentrated by lyophilization. The crude product was purified by using CHP-20P absorption gel column and the selected fractions were lyophilized to afford the metabolite. The UV spectra showed the disappearance of the absorption at 300 nm (carbapenem chromophore) and the absorption at 277 nm was observed with a reduced intensity. The NMR spectra revealed two peaks corresponding to the 1β-methyl substituent of **1** in a ratio *ca.* 1:4, indicating that the metabolite is a mixture with a similar structure. SIMS-MS m/z 402 (M+H), 385, IR (KBr) cm^{-1} 3450, 1710, 1650, 1600.

A solution of **1** (2.1 mg) in 0.1 M Tris-D_2O buffer solution (0.8 ml, pH 7.0) was stored at 37°C, a solution of DHP-I in D_2O was added and periodically examined by ^1H NMR. After 2 hours, NMR spectrum revealed that **1** was completely degraded to a product which had almost the same with the isolated product. Under the same condition without DHP-I, the spectrum was almost unchanged, indicating that **1** was stable to the neutral condition.

Chemical Hydrolysis of **1**

A solution of **1** (103 mg) in 1 N HCl (4 ml) was kept at 5°C and periodically monitored by HPLC. After 2.5 hours, **1** was degraded completely and a single peak, which had the same retention time with the metabolite, was detected. The solution was neutralized with 1 N NaOH and lyophilized. The resulting product was desalted by using CHP-20P absorption gel column and lyophilized to afford the hydrolyzed product (45 mg) as white amorphous powder. IR, mass and NMR spectra gave the same results with those of the enzymatically degraded product. The ^1H NMR data revealed that the product exists as a

mixture of double bond isomers (**3A** and **3B**) in an aqueous solution. ^{1}H NMR (NaOH/D_2O) δ: 0.84 (d, J=7.3 Hz, 10-CH_3 of **3B**), 0.99 (d, J=7.3 Hz, 10-CH_3 of **3A**), 1.22 (d, J=6.3 Hz, 9-CH_3 of **3A** and **3B**), 1.50 (m, 15-H of **3A**), 1.70 (m, 15-H of **3B**), 2.51 (m, 4-H of **3A**), 2.55~2.65 (m, 6-H of **3B**), 2.58 (dd, J=7.6 and 8.3 Hz, 6-H of **3A**), 2.65~2.75 (m, 15-H of **3A** and **3B**), 2.76 (m, 4-H of **3B**), 2.96 (s, N–CH_3 of **3A** and **3B**), 3.05 (s, N–CH_3 of **3A** and **3B**), 3.05~3.20 (m, 13-H of **3A** and **3B**), 3.45~3.60 (m, 13-H of **3A** and **3B**), 3.80 (d, J=2.3 Hz, 3-H of **3A**), 3.87 (dd, J=5.6 and 8.9 Hz, 5-H of **3B**), 3.95~4.10 (m, 8-H of **3B**, 12-H of **3A** and **3B**), 4.00 (m, 8-H of **3A**), 4.25 (m, 5-H of **3A**), 4.90 (m, 14-H of **3A** and **3B**).

Isolation of Crystalline Product **3C**

A solution of **1** (1.3 g) in 10% HCl (77 g) was kept at 0~5°C. After 1.5 hours, **1** was absent and solution was partially neutralized with 30% NaOH (*ca.* 2.7 g) to pH 2.6 and stirring for 4 hours at 0~5°C. The resulting crystals were collected by filtration, washed with aqueous acetone and dried under reduced pressure to afford **3C** (1.2 g) as off-white crystals: SIMS-MS m/z 402 (M+H), 358; IR (KBr) cm^{-1} 3450, 2970, 2940, 1720, 1650, 1590; ^{1}H NMR (NaOH/D_2O) δ: 1.18 (d, J=7.6 Hz, 10-CH_3), 1.25 (d, J=5.9 Hz, 9-CH_3), 1.40 (m, 15-H), 1.97 (m, 4-H), 2.24 (dd, J=3.0 and 10.9 Hz, 6-H), 2.60 (m, 15-H), 2.81 (d, J=7.9 Hz, 3-H), 3.00 (s, N–CH_3), 3.08 (s, N–CH_3), 3.15 (m, 13-H), 3.65 (m, 13-H), 3.67 (dd, J=3.0 and 5.6 Hz, 5-H), 4.10 (m, 8-H), 4.25 (m, 12-H), 4.90 (m, 14-H).

Anal Calcd for $C_{17}H_{27}N_3O_6S \cdot 4H_2O$, HCl: C 39.07, H 7.21, N 8.04, S 6.13.
Found: C 39.34, H 7.15, N 8.10, S 6.30.

Acknowledgments

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