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# CONFORMATIONAL ANALYSIS OF MEROPENEM AND DESMETHYL MEROPENEM: THE EFFECT OF 1β-METHYL GROUP ON CARBAPENEM ANTIBIOTICS [1]

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**Abstract:** The conformations of meropenem and desmethyl meropenem have been studied using MM calculations and $^1$H NMR experiments. It was found that the preferred conformation of meropenem was relatively linear compared with desmethyl meropenem in aqueous solution. Copyright © 1996 Elsevier Science Ltd

Since the discovery of thienamycin,[2] many research groups have made significant effort to find new carbapenem antibiotics. The susceptibility to renal dehydropeptidase-I (DHP-I) and the chemical instability of the carbapenem antibiotics have been understood as an impediment in clinical use. In the course of our study, meropenem[3] (Fig. 1) was developed as the first carbapenem antibiotic that requires no coadministration drug. Meropenem has potent antibacterial activity against a wide range of gram-positive and gram-negative bacteria,[4] high physicochemical stability,[5] resistance against DHP-I,[6] and reduced side effects.[7] In the case of meropenem, the introduction of a 1β-methyl group on the carbapenem skeleton improved not only its stability against DHP-I as previously reported,[8] but also antibacterial activity against gram-negative bacteria including *pseudomonas aeruginosa*. The introduction of the 1β-methyl group in imipenem did not increase the activity[4] against *pseudomonas aeruginosa*, although a significant improvement in stability[6] against DHP-I by its introduction was observed with imipenem. Furthermore, it was reported[9] that the introduction of the 1β-methyl group on the 2-arylcarbapenem skeleton did not increase the stability against DHP-I and rather decreased slightly it. Such a

variety of the effect of the 1β-methyl group on the biological activities could not be explained by the simple steric hindrance of the 1β-methyl group. These results prompted us to start the study of the comparison of the conformation between meropenem and desmethyl meropenem. The effect of the 1β-methyl group on the biological activities of meropenem is of interest in connection with its conformation. It has already been reported[10] that the crystal conformation of meropenem (trihydrate) was essentially consistent with its solution conformation in water. However little attention has been paid to the effect of the 1β-methyl group on its conformation. In this paper, we will discuss about the conformations of meropenem and desmethyl meropenem studied using MM calculations and $^1$H NMR experiments.

To gain insight into the possible reasons for the superior stability against DHP-I and the anti-pseudomonal activity, investigations were undertaken preliminarily to study the conformation of meropenem and desmethyl meropenem using the MM calculations.[11] The most stable conformers of meropenem and desmethyl meropenem were investigated by the MM3* calculations using vacuous parameters. The relative spatial arrangement between the carbapenem skeleton and pyrrolidine moiety was focused on, because we thought that the pyrrolidine moiety would be affected by the 1β-methyl group of meropenem. The results showed that the torsional angles of φ2 and φ3 of meropenem were essentially different from that of desmethyl meropenem in their most stable conformers (Table 1). The torsional angle of φ2 of meropenem was slightly different between the MM3* calculation results and the X-ray data,[12] however, these two conformers were essentially identical (Table 1). The distribution of possible conformers within 10 KJ/mol of the most stable conformer were investigated using MM3* calculation (Fig. 2). The obvious differences of φ2 and φ3 were observed between meropenem and desmethyl meropenem. Similarly, the obvious differences in distance 1, distance 2, and distance 3 were observed in the distribution of possible conformers between meropenem and desmethyl meropenem. These results indicated that the spatial arrangement of the carbapenem skeleton and the pyrrolidine moiety were different between meropenem and desmethyl meropenem.

The results of these MM calculations encouraged us to further investigate the conformational preferences of meropenem and desmethyl meropenem by $^1$H NMR studies.[13] The investigation of the conformation of meropenem studied by $^1$H and $^{13}$C NMR in aqueous solution was already reported.[10] At this time the conformation of meropenem was verified by supplementary examination. There was no significant difference in

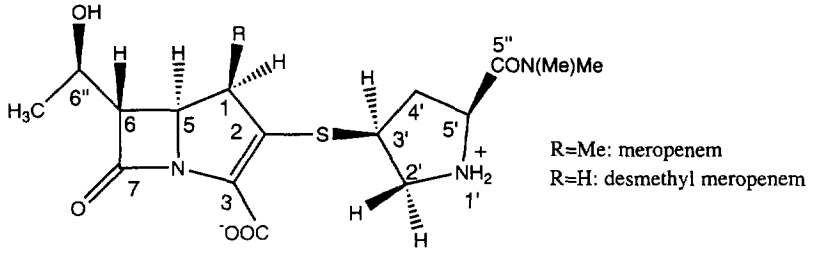



Fig. 1 Molecular formulas of meropenem and desmethyl meropenem with the numbering system used in this paper.

Table 1. The most stable conformers of meropenem and desmethyl meropenem.

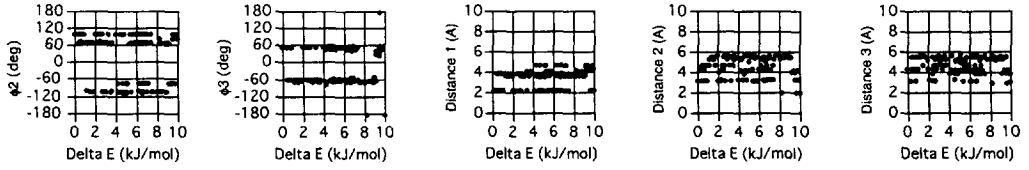
| | ϕ1 | ϕ2 | ϕ3 | ϕ4 | angle | dist1 | dist2 | dist3 |
|---|---|---|---|---|---|---|---|---|
| MM3* | | | | | | | | |
| meropenem | 61.792 | 103.694 | 59.513 | -144.306 | 98.487 | 2.408 | 3.388 | 4.554 |
| desmethyl meropenem | 61.411 | -108.726 | -62.766 | -128.167 | 97.885 | 3.404 | 5.204 | 5.686 |
| X-ray[a)] | | | | | | | | |
| meropenem | 67.4 | 157.9 | 49.4 | -111.2 | 105.0 | 2.404 | 2.515 | 3.699 |

a) data from ref. 12.

ϕ1: O(6")-C(6")-C(6)-H(6) ϕ2: C(3)-C(2)-S-C(3') angle: C(2)-S-C(3') dist2: H(1)-Hα(2')

ϕ3: C(2)-S-C(3')-H(3') ϕ4: H(5')-C(5')-C(5")-O(5") dist1: H(1)-H(3') dist3: H(1)-Hβ(2')

Meropenem




Desmethyl meropenem

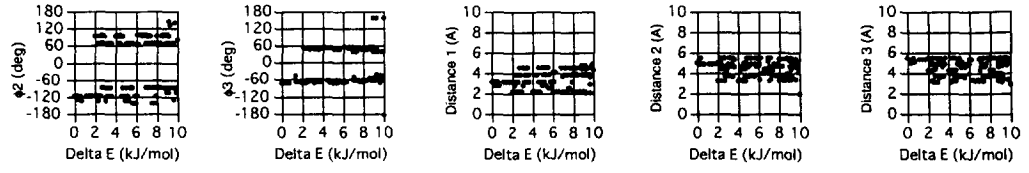



Fig. 2 Possible conformers of meropenem and desmethyl meropenem calculated by MM3*.

the J-coupling between meropenem and desmethyl meropenem (Table 2), however, the chemical shifts of 5'-H, 4'α-H, and 2'β-H of meropenem were different from desmethyl meropenem (> 0.05 ppm). These results suggested that the conformations of the carbapenem skeleton and pyrrolidine ring were not changed in these two compounds and the relative spatial arrangement of these two parts were different. The NOE data of meropenem was compared with desmethyl meropenem to consider the spatial arrangement in these two compounds (Fig. 3). The following enhancements were observed upon irradiation and measurement of the NOE difference spectra in meropenem; 1α-H-3'-H (5.1 %), 1α-H-2'α-H (3.6 %). However, there was no NOE (0.5 % >) between the carbapenem skeleton and pyrrolidine ring in desmethyl meropenem. These NOE data of meropenem and desmethyl meropenem were consistent with the MM3* calculation (except the distance 1 in desmethyl meropenem) and X-ray data. Fig. 4 shows the representative low energy conformations of meropenem and desmethyl meropenem in aqueous solution confirmed by the $^1$H NMR and MM calculation studies. The conformation of meropenem was extended and that of desmethyl meropenem was a bent conformation.

We found that the preferred conformation of meropenem in aqueous solution was relatively linear compared with desmethyl meropenem due to the steric interaction between the 1β-methyl and the pyrrolidine substituents. In the case of imipenem, it was suggested that the 1β-methyl group had little effect on its molecular conformations, because it has a flexible chain moiety. These results suggests that this conformational difference between meropenem and desmethyl meropenem is one of the reasons for the improvement in the biological activities by the introduction of 1β-methyl group on the carbapenem skeleton.

**Acknowledgment**

The authors are grateful to Ms. Maki Tamura for her technical assistance.

**References and Notes**

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Table 2. $^1$H NMR chemical shifts of meropenem and desmethyl meropenem.
(500MHz, pH7.4, 30°C in 50 mM phosphate buffer)

| | meropenem | desmethyl meropenem | Δ(δmeropenem - δdesmethyl meropenem) |
|---|---|---|---|
| 1-Me | 1.137,3H,d,J=7.3Hz | | |
| 6-OH(Me) | 1.216,3H,d,J=6.4Hz | 1.209,3H,d,J=6.2Hz | 0.007 |
| 4'β | 1.837,1H,ddd,J=7.5,8.5,14.5Hz | 1.803,1H,ddd,J=7.5,8.5,14.5Hz | 0.034 |
| N-Me(Me) | 2.920,3H,s and 2.993,3H,s | 2.909,3H,s and 2.992,3H,s | 0.011 and 0.001 |
| 4'α | 2.967,1H,ddd,J=7.5,8.5,14.5Hz | 2.882,1H,ddd,J=6.5,8.5,14.5Hz | 0.085 |
| 1α | 3.319,1H,dq,J=7.0,7.5Hz | 3.172,1H,dd,J=8.5,17.5Hz | 0.147 |
| 1β | | 3.118,1H,dd,J=8.5,17.5Hz | |
| 2'β | 3.338,1H,dd,J=5.0,12.0Hz | 3.275,1H,dd,J=6.0,12.5Hz | 0.063 |
| 6 | 3.389,1H,dd,J=2.5,6.0Hz | 3.348,1H,dd,J=2.7,6.1Hz | 0.041 |
| 2'α | 3.623,1H,dd,J=6.0,12.0Hz | 3.586,1H,dd,J=6.5,12.0Hz | 0.037 |
| 3' | 3.949,1H,ddt,J=5.0,6.0,7.5Hz | 3.904,1H,ddt,J=6.0,6.5,7.5Hz | 0.045 |
| root of OH and 5 | 4.150-4.197,2H,m | 4.125-4.190,2H,m | |
| 5' | 4.650,1H,t,J=8.5Hz | 4.541,1H,t,J=8.5Hz | 0.109 |

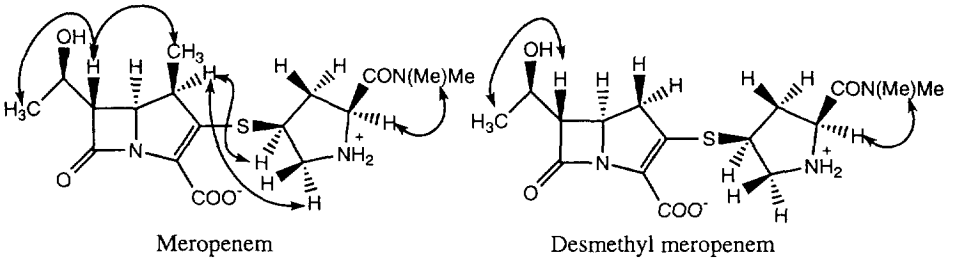


Fig. 3 Intramolecular NOEs of meropenem and desmethyl meropenem. Significant NOE signals are indicated.

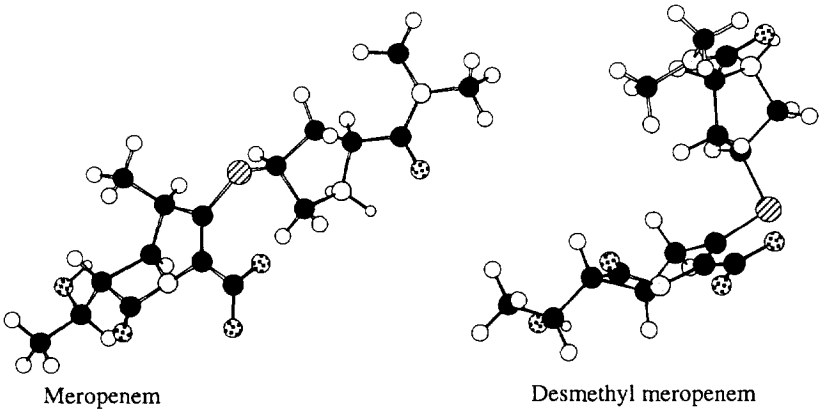


Fig. 4 Representative low energy conformations of meropenem and desmethyl meropenem in aqueous solution.

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13) Meropenem and desmethyl meropenem were dissolved in 0.6 ml of phosphate buffer solution (50 mM sodium phosphate, pH 7.4). Both the 1D and 2D NMR spectra were recorded on JEOL A 500 spectrometer. The chemical shifts (in ppm) were referenced to 2-trimethylsilyl-2,2,3,3,-tetradeuteriopropionic acid, sodium salt as the internal standard. NOE difference spectra were recorded as 32 K data points. Phase sensitive 2D NMR spectra were recorded as 256 $t_1$ blocks of 1024 complex points each in the $t_2$ dimension and averaged 16 scans per block during the recycle delay of 1.0 sec. for DQF-COSY and 3.0 sec. for the NOESY. The 1D and 2D data sets were processed using the program EDL (JEOL Inc., Tokyo, Japan) on a VAX 3200 workstation. The 256 complex points in the $t_1$ dimension were zero-filled to 512 points prior to the FT.

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