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Structural Comparison of 1β-Methylcarbapenem, Carbapenem and Penem: NMR Studies and Theoretical Calculations

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Abstract—Structural comparisons of meropenem (**1**), desmethyl meropenem (**2**) and the penem analogue (**3**) which contain the same side chains at both C-2 and C-6 were performed using ^{1}H NMR measurements together with 3-21G* level of ab initio MO and molecular mechanics calculations. The ab initio MO calculations reproduced the skeletons of these strained β-lactam rings in good agreement with the crystallographic data. ^{1}H NMR measurements in aqueous solution together with molecular modeling studies indicated that there were conformational differences of the C-2 and C-6 side chains in this series of compounds. These observations suggested that the conformational differences could affect their biological activities.

Introduction

The discovery of thienamycin (**4**) in 1976, which exhibited excellent antimicrobial activity and contained a unique carbapenem skeleton, marked an epoch for β-lactam antibiotics.[1] Subsequently, a number of naturally occurring carbapenems were isolated and extensive synthetic work on carbapenem derivatives was conducted world-wide to afford numerous number of chemically modified carbapenems. Among them, attention focused on the 1β-methylcarbapenems, the first of which was reported by Christensen et al. in 1984,[2] owing to its high stability against dehydropeptidase-I (DHP-I) without loss of antimicrobial activity. Independently, penems, which were designed as hybrids of penicillins and cephalosporins, were synthesized by Woodward et al. in the middle of the 1970s[3] before the discovery of thienamycin. Therefore, the first penems had a 5,6-*cis* configuration with a 6-acylamino group, which was similar to the penicillin and cephalosporin antibiotics. After the discovery of thienamycin, the penem derivatives having a 5,6-*trans* configuration with a 1-hydroxyethyl moiety at C-6 were synthesized and it was found that they showed improved chemical stability together with similar biological activities to those of the carbapenems.[4] However, the carbapenems and penems showed different modes of action in these biological properties, e.g. affinities for the penicillin-binding proteins (PBPs) and susceptibility to renal DHP-I.[5] These disparities were considered to be effected by not only subtle structural biases, simple steric hindrance and physicochemical properties such as chemical stability and p*K*a value but also by conformational differences of the C-2 and C-6 side chains.

Meropenem (**1**) is a 1β-methycarbapenem antibiotic that exhibits potent antibacterial activity against a wide range of gram-positive and gram-negative bacteria, and its stability against renal DHP-I is significantly higher than that of desmethyl meropenem (**2**) (Figure 1).[6]

Recently, we reported a computational study of the conformations of **1** and **2** using ^{1}H NMR measurements and NOE enhancements together with molecular mechanics calculations. This study found that the preferred conformation of **1** in aqueous solution was a relatively linear one, due to the steric interaction between the 1β-methyl and the pyrrolidine substituents, when compared with **2**.[7]

The chemical stability of penems has been reported to be higher than that of imipenem, *N*-formimidoylthienamycin.[8] However, the stability of penem (**3**) against DHP-I was found not to be the highest among **1**, **2** and

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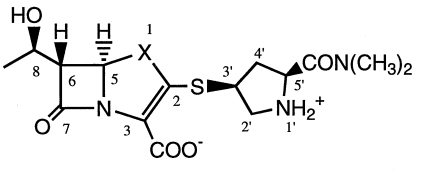
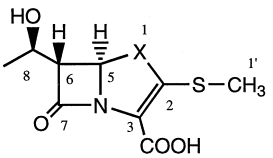


X= CH(β-CH_3) meropenem (1)

X= CH_2 desmethyl meropenem (2)

X= S penem (3)

Figure 1. Chemical structures of meropenem (**1**) and related compounds (**2**) and (**3**).



X= CH(β-CH_3) **1a**

X= CH_2 **2a**

X= S **3a**

Figure 2. Chemical structures of model compounds for ab initio Mo calculations (**1a**, **2a** and **3a**).

3. A reason for this could be its high binding affinity to DHP-I.[9] This suggested that the conformation of **3** in aqueous solution might be different from those of the others and prompted us to extend the conformational studies of these compounds having the same C-2 and C-6 side chains.

This paper describes a direct comparison of 1β-methylcarbapenems, carbapenems and penems using structural analyses by ^{1}H NMR studies and theoretical calculations.

Results and discussion

Structural trends in 1β-methylcarbapenem, carbapenem and penem using model structures with theoretical calculations

At the beginning of the structural comparisons of these β-lactams, we intended to reproduce the structures of these highly strained and conjugated ring systems by theoretical calculations. However, typical molecular mechanics calculations (MMs) were not well applicable to these systems, since they lacked appropriate parameters for β-lactams. And semi-empirical MO methods could not also reproduce the subtle structural differences among these ring systems as reported before.[10a] Thus, the 3-21G* level of ab initio MO calculations were performed using the Gaussian 94 program[11] for the corresponding model compounds **1a**, **2a** and **3a**,[12] respectively, because these calculations were generally used to evaluate the structures of the sulfur-containing compounds and they could be achieved within the practical computational time (Figures 2 and 3).

The initial structures were constructed by modifying an X-ray crystallographic structure of meropenem (**1**) and resulting initial structures were then fully optimized using the 3-21G* basis sets. The calculated structures (3-21G*) are shown in Figure 5 (**1aA**, **2aA**, **3aA**) and the tortional angle at the C-2 position is defined as ϕ (X(1)-C(2)-S-C(1′)) and the tortional angle at C-6 is defined as ψ (C(5)-C(6)-C(8)-C(9)), for convenience of the discussion. It should be noted that these structural optimizations with 3-21G* theory reproduced well the highly strained structures in comparison with the structures derived from X-ray crystallography.[1d,13,14] The corresponding parameters from the X-ray analysis of meropenem (**1**),[13] thienamycin analogue (**5**)[1d] and ritipenem analogue (**6**)[14] are listed in Table 1 along with the ab initio (3-21G*) parameters for **1aA**, **2aA**, **3aA** (Figure 5).

In addition, 'pyramidality' which is defined as 360-(ΣN) where (ΣN) is the sum of the three bond angles around the β-lactam *N* atom, is often referred to as an important parameter related to the antibacterial activity of these β-lactams. The contribution of lactam 'pyramidality' is usually interpreted as increasing the reactivity towards nucleophilic attack by perturbation of lactam

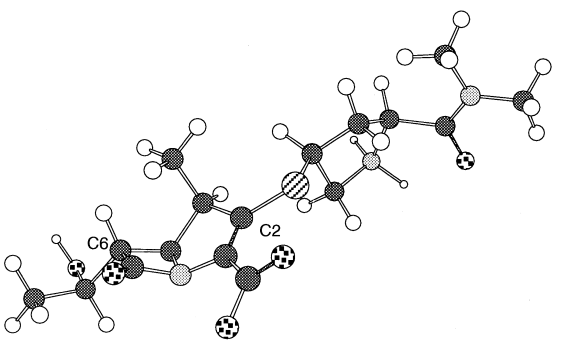


Figure 3. X-Ray crystal structure of meropenem (**1**).

amine resonance.[10] The calculated and the observed values of this parameter for compounds **1**, **5** and **6** were in good agreement (30.0 for **1aA**, 30.9 for **1**; 31.5 for **2aA**, 34.0 for **5**; 27.4 for **3aA**, 25.3 for **6**). Thus, both calculated and observed values of 'pyramidality' indicate that the chemical stability of penems is the highest. It is noted that in these calculations, we also observed another possible conformer which contained an intramolecular hydrogen bond (**1aD**) (Figure 5). However, in the optimized structure of **1aD**, 'pyramidality' of the *N* atom at the bridge head of the β-lactam rings was not coincident with that of the observed value in **1** (15.5 for **1aD**, 30.9 for **1**) and so was not taken into consideration.

Next, the conformational trends of the C-2 and C-6 side chains were investigated. The tortional angle ϕ was systematically rotated by increments of 30° and each initial conformation was fully optimized using the 3-21G* basis set. In the C-2 side chains, generally two energetically local minima were found. In the case of **1a**, the most stable conformer (**1aA**) had $\phi = -23.0°$ and a second stable conformer (**1aB**) (0.7 kcal/mol less stable)

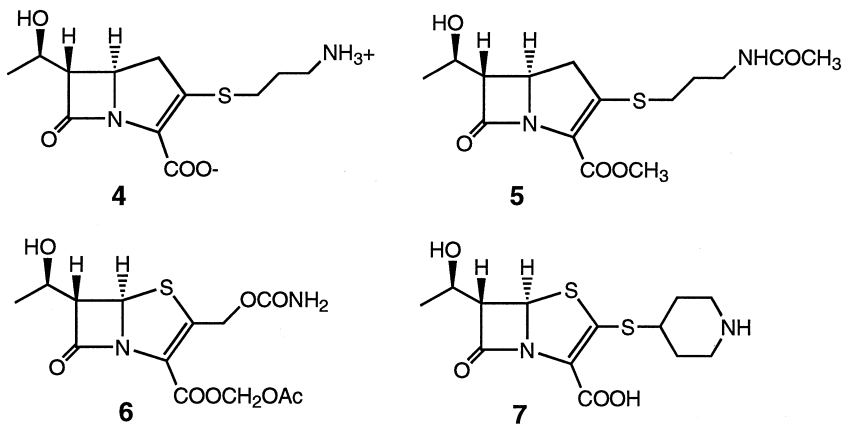


Figure 4. Chemical structures of thienamycin (**4**), related compounds (**5**), (**6**) and (**7**).

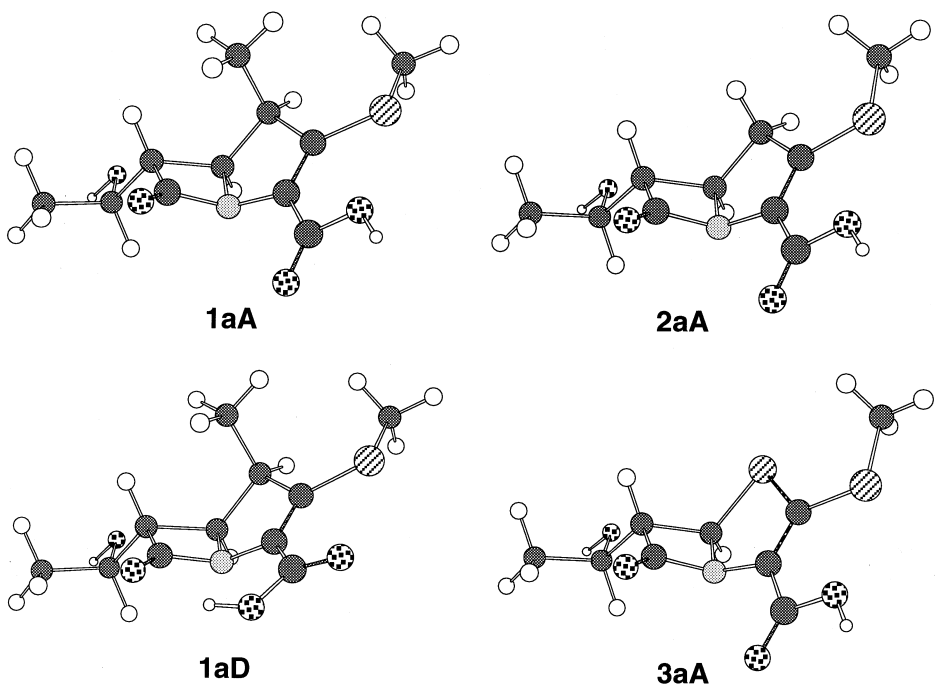


Figure 5. Structures for **1aA**, **2aA**, **3aA** and **1aD** calculated by 3-21G* level of ab initio method.

Table 1. Structural parameters from the X-ray diffraction analysis of **1**, **5**, **6** and from ab initio (3-21G*) calculations for **1aA**, **2aA**, **3aA**

	1 (X-ray)	1a (3-21G*)	5 (X-ray)	2a (3-21G*)	6 (X-ray)	3a (3-21G*)
Bond distances						
X(1)-C(2)	1.54	1.55	1.54	1.55	1.74	1.79
C(2)-C(3)	1.35	1.33	1.38	1.33	1.38	1.34
C(3)-N(4)	1.44	1.42	1.44	1.44	1.41	1.42
N(4)-C(5)	1.50	1.50	1.52	1.50	1.47	1.48
C(5)-C(6)	1.55	1.55	1.55	1.56	1.51	1.55
C(6)-C(7)	1.53	1.54	1.52	1.54	1.54	1.54
N(1)-C(7)	1.39	1.42	1.42	1.40	1.37	1.42
X(1)-C(5)	1.54	1.55	1.58	1.55	1.84	1.84
Bond angles						
X(1)-C(2)-C(3)	111.1	111.0	111.2	110.9	114.6	112.6
X(1)-C(5)-N(3)	103.8	102.7	103.2	103.1	104.6	103.4
X(1)-C(5)-C(6)	123.7	121.5	120.8	120.1	119.0	118.0
C(2)-C(3)-N(4)	109.4	111.0	109.4	110.4	111.3	113.4
C(3)-N(4)-C(5)	109.2	109.0	111.0	110.2	114.9	113.9
C(3)-N(4)-C(7)	127.5	127.9	123.2	124.7	128.2	125.7
N(4)-C(5)-C(6)	88.0	87.7	87.8	87.8	90.7	88.3
N(4)-C(7)-C(6)	92.7	91.1	92.8	92.3	93.3	90.9
C(5)-X(1)-C(2)	101.6	101.6	103.3	103.6	91.0	91.4
C(5)-C(6)-C(7)	85.1	86.1	87.2	86.0	83.9	85.7
C(5)-C(6)-C(8)	114.6	112.1	118.2	115.1	117.4	112.0
Pyramidality	30.9	30.0	34.0	31.5	25.3	27.4

(Figure 6) has a $\phi = +117.0°$. The former value is similar to that found in the X-ray crystallographic structure of **1** ($\phi = -31.5°$). This result indicated that there is one more possible conformation of the C-2 side chain of **1a**. It is also interesting that among **1aA**, **2aA** and **3aA**, the ϕ value of **1aA** ($\phi = -23.0°$) is much larger than that for **2aA** and **3aA**, which are comparable ($\phi = -5.8°$ and $-2.6°$ respectively). This observation suggests that the steric repulsion between the 1β-methyl moiety and S-methyl side chain on C-2 brings about the observed conformational change of the side chain in **1a**. In addition, generally three energetically local minima were found in the C-6 side chains by our initial conformational searches using the MM3* force field.[15] Then two of them were selected for further studies because there was more than 2.0 kcal/mol energy difference between them and the third one. Each structure was then fully optimized using the 3-21G* basis set and it was found that two of them were important to discuss. In **1a** a stable conformer with ($\psi = +171.0°$) (**1aA**) corresponded to that of the reported crystallographic structure.[13] However, a conformer with ($\psi = -87.0°$) was 1.0 kcal/mol more stable (**1aC**) (Figure 6) and the corresponding conformation of **3a** (**3aC**) (Figure 6) was also found to be the most stable. This observation again suggested that there were also other conformational possibilities of the C-6 side chain. These calculations did not consider the solvation effect but the energy differences were not large enough to obtain a preferred conformation. And this result would be supported by an observed ψ value of $-66.3°$ in the X-ray crystallographic data of penem **6** (ritipenem).[14] One possible reason for this conformational bias would be a difference in the steric repulsion between H_5 and the C-6 functionality, which is dependent on structural distortion of these ring systems.

Conformational studies in aqueous solution of 1, 2 and 3 by NMR

Next, we performed conformational analyses of **1**, **2** and **3** in aqueous solution, which was assumed to reflect their conformations in physiological conditions. The theoretical calculations indicated the possibility of different conformations of the C-2 and C-6 side chains in this series of compounds. The ^{1}H NMR measurements and NOE experiments focused on the relative orientation of the side chains and the β-lactam ring. The chemical shifts and *J*-coupling values of **1**–**3** are listed in Table 2. No significant difference in the *J*-coupling values between meropenem and desmethyl meropenem was found, however, the chemical shifts of $H_{5'}$, $H_{4'\alpha}$ and $H_{2'\beta}$ of meropenem were different from those of desmethyl meropenem (> 0.05 ppm). This suggests that the conformations of the carbapenem skeleton and the pyrrolidine moiety were not changed though the relative

spatial arrangement between them were different. The NOE data of meropenem was compared with desmethyl meropenem, to consider the spatial arrangement in these two compounds (Figure 7). The following enhancement were observed upon irradiation and measurement of the NOE difference spectra in meropenem **1**; $H_{1\alpha}$-$H_{3'}$ (5.1%), $H_{1\alpha}$-$H_{2'\alpha}$ (3.6%). There was, however, no NOE (0.5% >) between the carbapenem skeleton and pyrrolidine ring in desmethyl meropenem **2**, thus indicating a conformational bias in **1**.

In penem (**3**), there was also no NOE (0.5% >) enhancement between the penem skeleton and pyrrolidine ring and so the conformation of the C-2 side chain was not obvious. However, the NOE pattern around the 6-(1-hydroxyethyl) moiety of penem **3** is different from

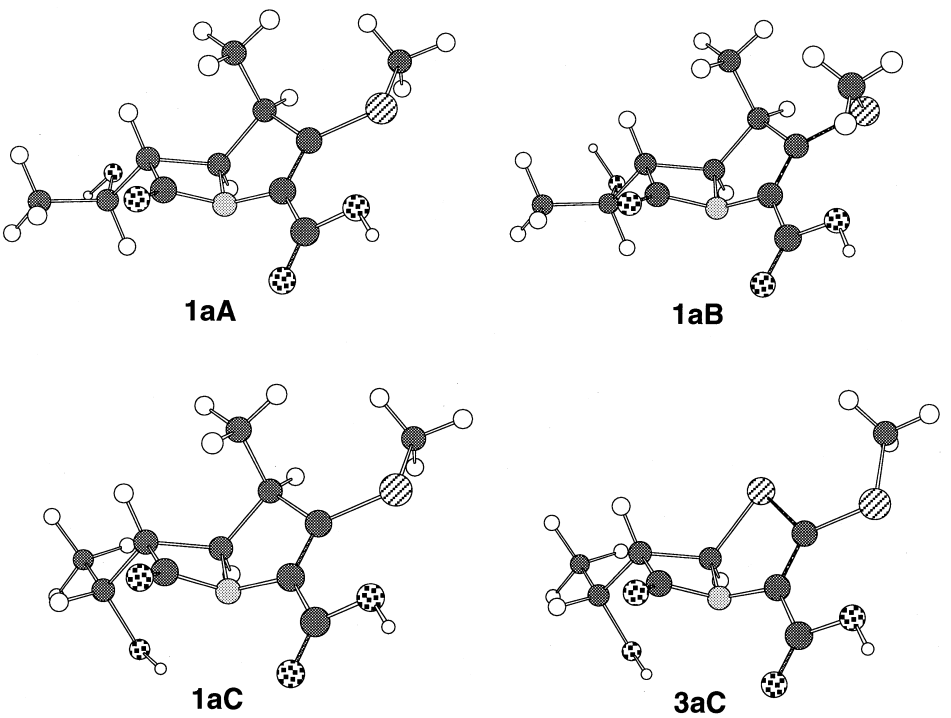


Figure 6. Structures for **1aA**, **1aB**, **1aC** and **3aC** calculated by 3-21G* level of ab initio method.

Table 2. ^{1}H NMR chemical shifts of meropenem (**1**), desmethyl meropenem (**2**) and penem (**3**) in aqueous solution. (500 MHz, pH 7.4, 30 °C in 50 mM phosphate buffer)

	Meropenem	Desmethyl meropenem	Penem
1-Me	1.137, 3H, d, *J*-7.3 Hz		
6-Me	1.216, 3H, d, *J* = 6.4 Hz	1.209, 3H, d, *J* = 6.2 Hz	1.151, 3H, d, *J* = 6.0 Hz
4′β	1.837, 1H, ddd, *J* = 7.5, 8.5, 14.5 Hz	1.803, 1H, ddd, *J* = 7.5, 8.5, 14.5 Hz	1.862, 1H, ddd, *J* = 9.0 Hz
N-Me(Me)	2.920, 3H, s and 2.993, 3H, s	2.909, 3H, s and 2.992, 3H, s	2.838, 3H, s and 2.909, 3H, s
4′α	2.967, 1H, ddd, *J* = 7.0, 7.5 Hz	2.882, 1H, ddd, *J* = 6.5, 8.5, 14.5 Hz	2.924, 1H, ddd, *J* = 8.0, 9.0, 15.0 Hz
1α	3.319, 1H, dq, *J* = 7.0, 7.5 Hz	3.172, 1H, dd, *J* = 8.5, 17.5 Hz	
1β		3.118, 1H, dd, *J* = 8.5, 17.5 Hz	
2′β	3.338, 1H, dd, *J* = 5.0, 12.0 Hz	3.275, 1H, dd, *J* = 6.0, 12.5 Hz	3.433, 1H, dd, *J* = 5.5, 12.5 Hz
6	3.389, 1H, dd, 2.5, 6.0 Hz	3.348, 1H, dd, *J* = 2.7, 6.1 Hz	3.794, 1H, dd, *J* = 1.5, 6.0 Hz
2′α	3.623, 1H, dd, *J* = 6.0, 12.0 Hz	3.586, 1H, dd, *J* = 6.5, 12.0 Hz	3.732, 1H, dd, *J* = 7.0, 12/5 Hz
3′	3.949, 1H, ddt, *J* = 5.0, 6.0, 7.5 Hz	3.904, 1H, ddt, *J* = 6.0, 6.5, 7.5 Hz	3.979, 1H, dddd, *J* = 5.5, 7.0, 7.5, 8.0 Hz
Root of OH and 5	4.150–4.197, 2H, m	4.125–4.190, 2H, m	4.100, 1H, dq, *J* = 1.5, 6.0 Hz and 5.569, 1H, d, *J* = 1.5 Hz
5′	4.650, 1H, t, *J* = 8.5 Hz	4.541, 1H, t, J = 8.5 Hz	4.650, 1H, t, *J* = 9.0 Hz

those of meropenem **1** and desmethyl meropenem **2** (Figure 7). In compound **3**, no significant difference of J-coupling value between H_6 and H_8 (6.0 Hz) was observed compared with those of **1** and **2**. But NOE enhancements between H_5-H_8 and H_5-H_9 were observed, whereas no NOE enhancements between these protons were observed in carbapenems **1** and **2**. These observations suggest that the orientation of the hydroxyethyl moiety in penem **3** is different from those in meropenem **1** and in desmethyl meropenem **2**. The relationship between H_6 and H_8 was synclinal in meropenem **1** and desmethyl meropenem **2**, it would be anticlinal in penem **3** (Figure 8). Moreover, in the other penem compound (**7**) (Figure 4) NOE enhancements between H_5-H_8 and H_5-H_9 were similar. This result suggested that the conformation of the hydroxyethyl moiety at C-6 obtained in **3** would be observed commonly in penems.

The NMR studies of **1** and **2** showed that there is a conformational difference of the C-2 side chain in the series of compounds. Furthermore, it also indicated that a conformational difference of the hydroxyethyl moiety only existed in penem **3**.

Conformational analysis of 1, 2 and 3 using MM3 force field

With the structural information from the ^{1}H NMR studies in hand, we performed some molecular modeling

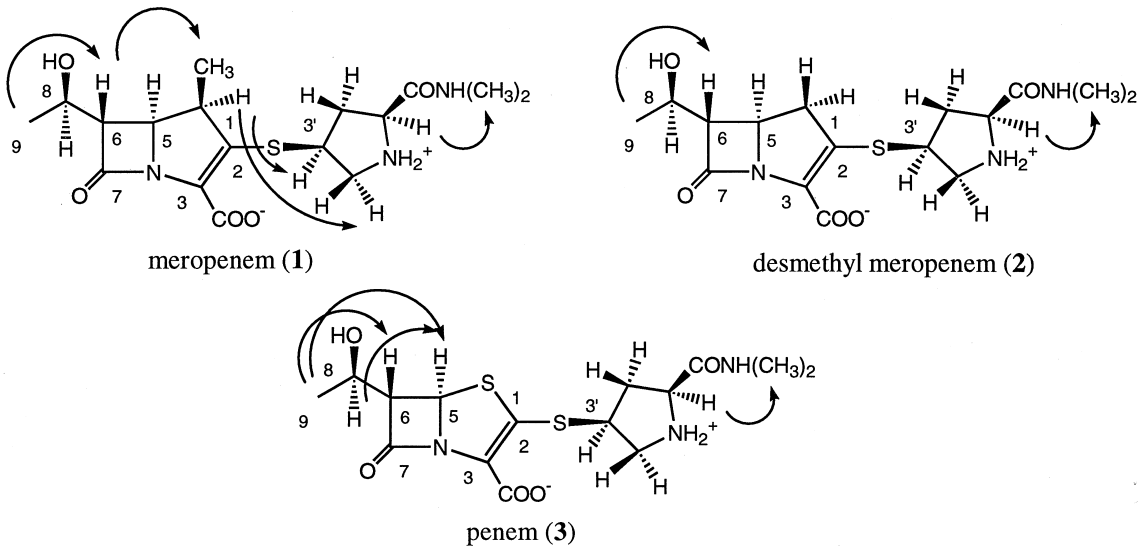


Figure 7. Intramolecular NOE enhancements of meropenem, desmethyl meropenem and penem. Only significant enhancements are indicated.

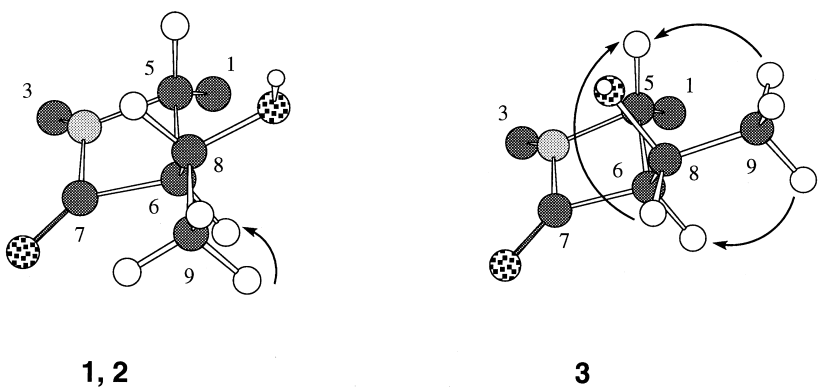


Figure 8. Conformational differences and NOE enhancements of 1-hydroxyethyl moiety in carbapenems (**1**, **2**) and penem (**3**) (some parts of structures were omitted for clarity).

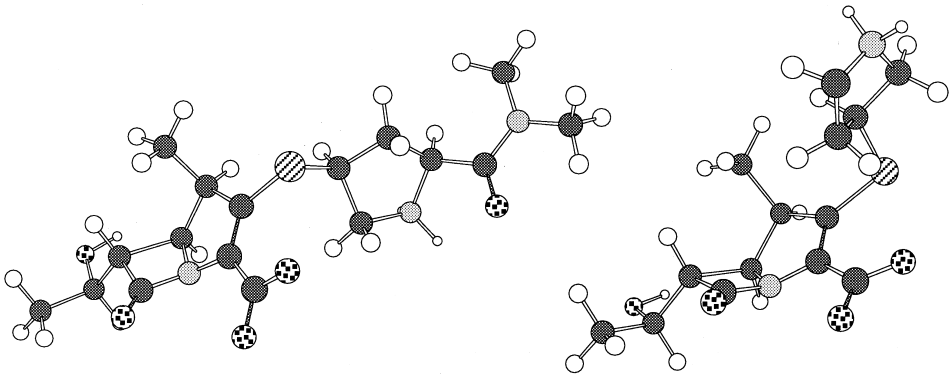


Figure 9. Extended conformation A (left) and bent conformation B (right) of meropenem (**1**) (side chain on pyrrolidine ring was omitted for clarity).

studies. In these molecular studies, we wanted to make more realistic models which would reproduce the results obtained from ^{1}H NMR spectral analyses. To do this, we had used the whole structures of **1**, **2** and **3** instead of using model structures in order to investigate conformational differences of the C-2 side chains and also perform conformational searches and structural minimizations under consideration of the aqueous media. We performed 2000 steps of Monte Carlo conformational searches for the structures of **1**, **2** and **3** using Macromodel version 5.5 program.[15] The MM3* force field together with (GB/SA) water continuum treatment[16] implemented in the program was used to optimize the generated structures using less computational time. In these conformational analyses, two general families of conformations were observed in the conformational analysis of **1**, **2** and **3** in which the basic nitrogen on the C-2 side chain was placed relatively below (resembles an extended conformation) or upper (resembles a bent conformation) to the skeleton (Figure 9, A and B), although the structures of β-lactam rings were not accurately reproduced.

In meropenem **1**, the most stable family of conformations were in an extended form (A) which was similar to that found in the crystal structure (Figure 3). The bent form (B) was 1.2 kJ/mol less stable. However, in desmethyl meropenem **2** and penem **3**, the folded conformations (B) were 2.0 kJ/mol and 0.9 kJ/mol more stable than that of A. These differences in conformations were mainly derived from the difference in tortional angles at the C(1)-C(2)-S-C(3′) positions. In meropenem **1**, this tortional angle in the favored conformation (A) is $-75°$, whereas, in the disfavored conformation it is 89°. However, in desmethyl meropenem **2** and penem **3**, those in the favored conformations were $+73°$ and $+74°$ and those in the disfavored conformations were $-76°$ and $-77°$, respectively.

This could be explained by the steric repulsion between the 1β-methyl group and large pyrrolidine substituent, compared with the methyl group in model compounds **1a**–**3a**, which were attached through the sulfur atom to the carbapenem or penem skeleton. The crystal structure of **1** shows that the tortional angle for C(CH3)-C(1)-C(2)-S is $-58.8°$. Therefore, the tortional angle for C(1)-C(2)-S-C(3′) cannot be around $+58.8°$ in order to avoid steric interactions. Similar conformational locks are often discussed as (±) double gauche pentane interactions in hydrocarbons (Figure 10).[17]

However, from the molecular mechanics calculations, the conformations of the C-6 side chains were found to be the same in **1**, **2** and **3** and did not reproduce any conformational bias as that observed in the solution structure of penem **3** and X-ray crystallographic structure of ritipenem (**6**).[14] This inconsistency might be caused by inaccuracies of this method in reproducing the structures of these hindered ring system.

Conclusions

Several examples of calculating the structures of β-lactam antibiotics using molecular mechanics or semi-empirical MO methods were achieved. The calculated

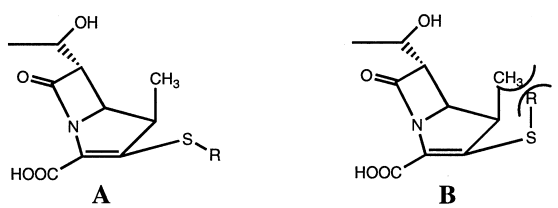


Figure 10. Preferred conformation ((++)-gauche) (A) and forbidden conformation ((+−)-gauche) (B) of meropenem (**1**).

structures did not exactly reproduce the observed structures by X-ray crystallography. This inaccuracy in calculation was partially derived from a lack of proper parameter sets for these strained and conjugated ring system.

However, we found that the 3-21G* level of ab initio MO method could reproduce the structure of β-lactams with comparable accuracy to the X-ray crystallographic structures including the value of 'pyramidality' in these β-lactam ring system in the conformational study. In addition, calculations including side chains at both the C-2 and C-6 positions suggested possible conformational differences in these β-lactam antibiotics. These results showed that 3-21G* level of ab initio MO method is appropriate for calculations of the β-lactam ring systems.

The conformational differences suggested by the theoretical calculations were verified by ^{1}H NMR measurements and NOE enhancements of 1β-methylcarbapenem (**1**), carbapenem (**2**) and penem (**3**) in aqueous solution. These experiments indicated that there are conformational differences in the side chains of carbapenems, 1β-methylcarbapenems and penems in a physiological environment. Those are, the conformation of the C-2 side chain is changed in 1β-methylcarbapenem compared with that of carbapenem and the conformation of the C-6 side chain in the penem is different from that in the carbapenem. This is interesting in connection with the biological properties because modifications of the structure and/or stereochemistry of these C-2 or C-6 side chains would be strongly related to the antibacterial activities or stabilities against DHP-I.[6,18,19] With regard to the biological activities, such as affinities for PBPs or stabilities against hydrolysis by DHP-I among carbapenems, 1β-methylcarbapenems and penems, much attention had been paid to the relative chemical stabilities of the skeletons or steric hindrance caused by incorporation of a methyl group into the carbapenems. However, differences in affinities to these enzymes caused by conformational differences in the side chains could be another reason for varying affinities of these β-lactams to the enzymes.

Experimental

Ab initio MO calculations of 1a, 2a and 3a

Ab initio calculations were performed with the GAUSSIAN 94 program[11] implemented on the Silicon Graphics Indigo 2 workstation. Initial structures were manually constructed by modifying the X-ray crystallographic structure of **1**. In the calculations varying tortional angles at the C-6 functionality, initial conformations were generated using the Monte Carlo conformational searches with structural optimizations using MM3* force field implemented in the Macromodel program.[15] Then, ab initio calculations were performed using 3-21G(*) level of basis set with full geometry optimization. In the calculations varying tortional angles of the C-2 functionality, the tortional angle was changed in increment of 30° and fully optimized using 3-21G* level of ab initio calculations.

NMR studies

Samples 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 s for DQF-COSY and 3.0 s 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.

Molecular modeling of 1, 2 and 3

Conformational searches of **1**, **2** and **3** were carried out on a Silicon Graphics Indigo 2 workstation using MM3* force field implemented in version 5.5 of Macromodel program[15] with (GB/SA) water continuum treatment.[16] Initial structures were derived from X-ray crystal structure of meropenem (Figure 3) and 2000 steps of Monte Carlo conformational searches were conducted. All flexible bonds except the carbapenem ring system were allowed to rotate during the Monte Carlo steps and minimizations were performed for every generated structure up to 2000 iterations. Conformations within 10 kJ/mol of the lowest energy and which had no intramolecular hydrogen bonding were taken into consideration in this modeling study.

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