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# Synthesis and Biological Activities of an α-Methyl and a β-Methyl Carbapenem and the Corresponding Unsubstituted Compound†

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**Abstract**—The carbapenem potassium salts **4**, **7** and **8** were prepared. Their rates of β-lactam hydrolysis and their biological activities, particularly their β-lactamase inhibiting properties, were examined and explained on the basis of their different substitution and pyramidality. 

Carbapenems are broad spectrum antibiotics with high activity against Gram-positive and Gram-negative bacteria.[1] Because of their strained bicyclic ring system they are very reactive. Investigations have shown that a β-methyl group in position 1 of the bicyclic molecule has a stabilizing influence without significantly decreasing the biological activity.[2] In contrast, an α-methyl group in position 1 decreases the antibiotic potential.[3] Consequently, to achieve higher antibiotic activity, most endeavours were focused on the β-methyl series. Concerning the activity as β-lactamase inhibitors, investigations have also been limited to the β-methyl and 1-unsubstituted derivatives.[4] Surprisingly, as far as we know, an α-methyl carbapenem has never been examined in this context.

1-β-Methyl carbapenam **2** is an intermediate of many carbapenem syntheses. It is known that **2** can undergo a partial epimerisation to the corresponding α-methyl isomer during a silica gel column chromatography.[5] This procedure was used to prepare the 1-α-methyl carbapenam **5**.

Starting with **1**, the carbapenam **2** was prepared by the known cyclisation reaction with $Rh_2(OAc)_4$ as catalyst.[2,3,6] Subsequently, **2** was chromatographed several times to give pure α-methyl compound **5**. The preparation of **5** is even easier by stirring **2** in EtOAc with $SiO_2$ at rt for about 2 h, affording better yields. Likewise, the pure isomeric **5** could be obtained after filtration and evaporation under reduced pressure.

The β- as well as the α-methyl compounds could be methylated to the carbapenems **3** and **6** with diazomethane. Then they were both hydrogenolyzed in EtOAc with 10% Pd on C as catalyst to remove the *p*-nitrobenzyl protective group. This reaction has to be carried out with care in order to avoid hydrogenation of the C=C double bond. Therefore, the hydrogen absorption was observed permanently. The reaction was stopped slightly before consumption of the expected amount of hydrogen (4 equiv). The reaction mixture was then filtered at 0 °C and the filtrate extracted with 1 equiv of $KHCO_3$ in water. Compounds **4** and **7** were obtained in aqueous solutions in 100% and 58% yields, respectively, as determined by UV-spectroscopy.[7] According to HPLC,[8] the purity of **4** and **7** was higher than 97%. The carbapenem **8**, which is unsubstituted in position 1, was prepared in an analogous way (Scheme 1).

Table 1 shows the half-lives of hydrolysis of the carbapenem potassium salts **4**, **7** and **8** under physiological conditions (pH = 7.4, 37 °C), as determined by UV-spectroscopy.

The pyramidality at the β-lactam nitrogen atom has often been used to explain the reactivity of bicyclic β-lactams.[9–11] When compared to the 1-unsubstituted carbapenem **8**, the pyramidality at the β-lactam nitrogen decreases with a β-methyl substitution. In other words, the β-methyl group flattens the bicyclic structure, whereas the α-methyl substitution does not affect the folding noticeably. Therefore the half-lives of hydrolysis of **7** and **8**, being in the same order of magnitude, as well as that of **4**, being significantly higher, can be explained on this basis (Table 1; see also Scheme 2).

Another important influence is exercised by steric hindrance. It is known, that a serine residue of penicillin binding proteins (PBPs) and β-lactamases open the β-lactam ring of bicyclic β-lactam antibiotics from the α-side.

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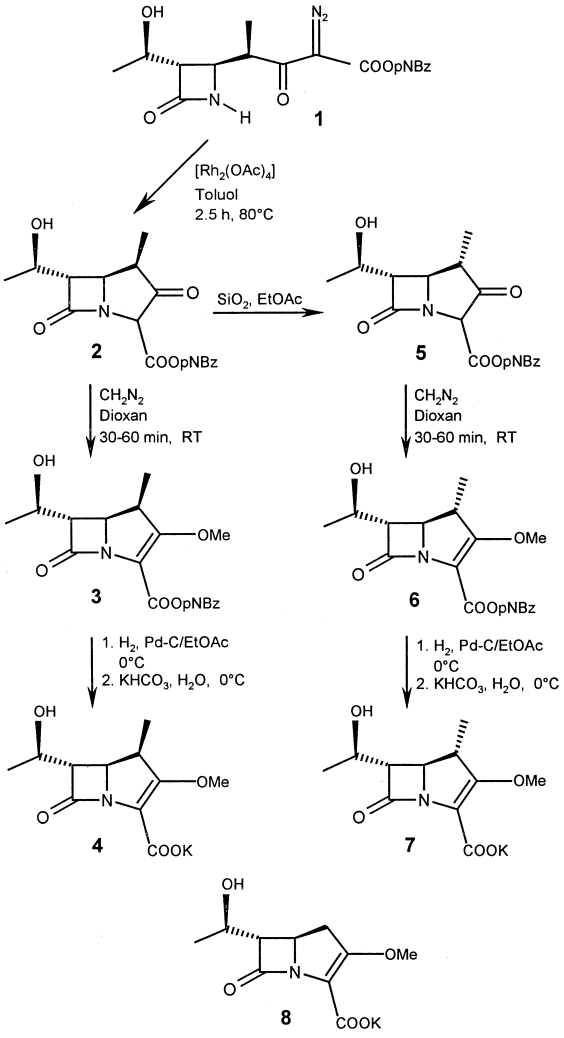



**Scheme 1.**

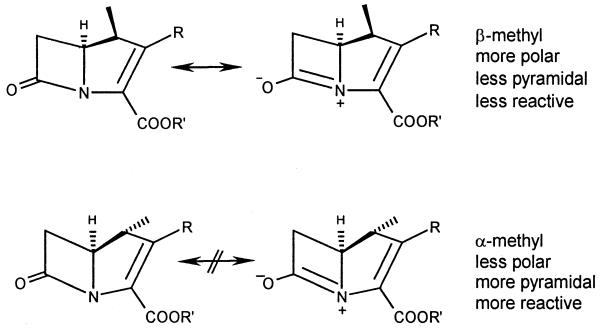
**Table 1.** Half-lives of hydrolysis (pH = 7.4, 37 °C) in phosphate buffer

| Compound | $t_{1/2}$ |
|---|---|
| **7** (α-Methyl) | 3.25 h |
| **8** | 5.75 h |
| **4** (β-Methyl) | 72 h |

However, according to stereoelectronic theory, in a non-enzymatic process a nucleophile attacks the β-lactam carbonyl predominantly from the β-side, due to the presence of the nitrogen lone electron pair. Since the β-methyl group is sitting above and close to the carbonyl group, it partially prevents the nucleophilic water approaching from the β-side. Consequently, the higher half-life of hydrolysis of **4** can be explained also on this basis.

The antibacterial activities of the carbapenems **4**, **7** and **8** were compared after determination of the minimal inhibitory concentrations (MIC, μg/mL), as depicted in Table 2.

The α-methyl compound **7** was more active against Gram-positive, whereas the β-methyl derivative **4** showed




**Scheme 2.**

**Table 2.** MIC-values in μg/mL after an incubation period of 18 h at 37 °C, DIFCO Nutrient Agar

| | **7** (α-Methyl) | **4** (β-Methyl) | **8** | CeCl |
|---|---|---|---|---|
| *S. aur* 1104 | <0.5 | 25 | 1 | <0.5 |
| *S. aur. res.* | 1 | 25 | 2 | 5 |
| *Staph.* 25768 | 5 | >50 | 5 | 10 |
| *Staph. Innsbruck* | >50 | >50 | 25 | >50 |
| *E. coli* 1103 | 10 | 1 | 2 | 2 |
| *E. coli* TEM | 5 | 0.5 | 1 | 25 |
| *E. cloacae* | 25 | 5 | 5 | >50 |
| *E. coccus* | >50 | >50 | 50 | 50 |
| *Ps. aer.* | >50 | >50 | >50 | >50 |
| *Ps. aer. res.* | >50 | >50 | >50 | >50 |

better MIC-values against Gram-negative bacteria. Polar β-lactam antibiotics, e.g., the 3rd generation cephalosporins, are by far more active against Gram-negative bacteria, whereas the more lipophilic compounds, such as the penicillins, predominantly inhibit Gram-positive bacteria. Therefore, the different activities of **4** and **7** can be explained on the basis of their polarity. Compounds **4** and **7** are indeed different in this respect. This can be detected by means of the $R_f$-values[12] of their *p*-nitrobenzyl esters **3** and **6**, the β-methyl compound **3** being by far more polar. This is also illustrated by the higher participation of the zwitterionic formula (Scheme 2) arising from the enhanced amide resonance of the less pyramidal β-methyl compound **4**.

The β-lactamase inhibiting activities of **4**, **7** and **8** were investigated with the nitrocefin test[13] using the isolated (cell free) resistance enzymes[14] of *Enterobacter cloacae* and *Escherichia coli* 205 TEM. The $IC_{50}$-values are summarized in Table 3.

The tested substances are almost equal inhibitors of the Class A enzyme of *E. coli* TEM. However, with the Class C β-lactamase of *E. cloacae* the inhibiting properties vary considerably, the α-methyl carbapenem **7** being 25 times more active than the β-methyl isomer **4**. As can be seen from its low $IC_{50}$-value, **7** is a potent β-lactamase inhibitor. Similar or even better values are known from some oxapenems[15] or a penem-sulfone.[16] Further investigations will reveal whether this surprising quality of compound **7** is also a property of other 1-α-methyl carbapenems.

**Table 3.** β-Lactamase inhibition activities ($IC_{50}$, mol per liter) after 15 min of preincubation with the enzyme at 37 °C

| | *E. cloacae* | *E. coli* TEM |
|---|---|---|
| **4** (β-Methyl) | $2.1\times10^{-6}$ | $1.3\times10^{-7}$ |
| **8** | $5.1\times10^{-7}$ | $3.4\times10^{-7}$ |
| **7** (α-Methyl) | $8.2\times10^{-8}$ | $2.3\times10^{-7}$ |

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