OXIME ETHERS AS STRUCTURE ANALOGS OF CLAVULANIC ACID

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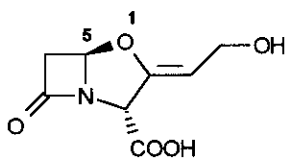
Abstract - The reaction of racemic *p*-nitrobenzyl 1-aza-3,7-dioxobicyclo-[3.2.0]heptane-2-carboxylate with methoxyamine leads to an oxime ether. The corresponding potassium salt is stable in neutral aqueous solution. However, it neither shows any significant activity as an antibacterial nor as a β-lactamase inhibitor.

According to Knowles, clavulanic acid (**1**)[1] relates to the group of irreversible β-lactamase inhibitors,[2] implying a labile bond between atoms 1 and 5. Obviously the carbapenems (**2**) (and carbapenams) do not fit into this class of compounds because of their stable C-C bond and, consequently, they were classified as reversible inhibitors.



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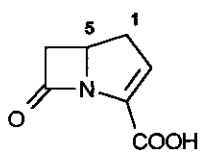

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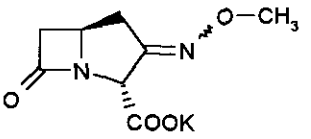
An important feature in the structure of clavulanic acid is the exocyclic double bond of the hydroxy-ethylidene side chain, which is essential for its activity as a β-lactamase inhibitor. If the double bond is hydrogenated leading to a single bond, the weak antibacterial activity indeed remains but the activity as a β-lactamase inhibitor is lost.[3] It was interesting for us to examine the oxime ether (**3**) to see whether the incorporation of an exocyclic double bond in carbapenams would eventually lead to active inhibitors as is true with the oxapenams.



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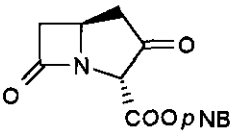

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Compound (**3**), as a mixture of E/Z - isomers, was tested with several bacterial strains (*Staphylococcus aureus, Penicillin resistant S. aureus., S. aureus* 25768, *S. aureus Innsbruck, Escherichia coli* 1103, *E. coli TEM, Enterobacter cloacae, Pseudomonas aeruginosa* 30055, *P. aeruginosa. res.)*. However, in no case compound (**3**) showed any antibacterial activity.

The inhibition effect on isolated β-lactamases was examined by means of the nitrocefin test.[5] Table 1 shows the IC_{50} value of the oxime ether in comparison to clavulanic acid and sulbactam.

Penicillinases from	Oxime ether (3)	Clavulanic acid	Sulbactam
Enterobacter cloacae	$> 1.9 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-6}$
Escherichia coli 205 TEM R^+	$6.4 \cdot 10^{-4}$	$4 \cdot 10^{-8}$	$8 \cdot 10^{-7}$

Table 1:

Concentrations in mmol/mL, necessary to inhibit the isolated β-lactamases by 50%, i.e. IC_{50}, after a 15 min preincubation period at 37 ^{0}C.

The half-life of **3** in phosphate buffer, pH 7.4 and 37 ^{0}C, is 11 days.

Obviously the oxime ether (**3**) is much less active than clavulanic acid and sulbactam. Interestingly, an exocyclic double bond, leading to potent β-lactamase inhibitors with the oxapenam moiety as shown for clavulanic acid, appears by far less appropriate with the carbapenam system.

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EXPERIMENTAL

The ^{1}H NMR spectra were recorded on a Varian VXR 400 S spectrometer using $Me_3SiCD_2CD_2CO_2Na$ as an internal standard. The IR spectra were recorded with a Perkin Elmer 1420 Ratio Recording Spectrophotometer.

Potassium 3-Methoxyimino-7-oxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (3)

4[4] (118 mg, 0.39 mmol) and $CH_3ONH_2 \cdot HCl$ (39 mg, 0.47 mmol) were stirred in dry pyridine (5 mL) at rt. After 2 h the solution turned yellow and excess pyridine was evaporated at 45°C in vacuo. After 45 min a yellow residue remained which was dissolved in CH_2Cl_2 (15 mL). The organic solution was washed with saturated $NaHCO_3$ solution (5 mL) and twice with portions of brine (5 mL). The aqueous layer was reextracted twice with 5 mL portions of CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and the solvent evaporated in vacuo. The crude product consisted of a 1:1 mixture of E/Z isomers. It was chromatographed on silica gel 60 (0.063-0.040 mm) with toluene/EtOAc 9:1. The E/Z isomers (R_f 0.64 and R_f 0.59 with EtOAc, silica gel plates) of the oxime ether were separated in 20 and 22% yields. The individual isomeric intermediates (16.7 mg, 0.05mmol) were hydrogenolyzed in EtOAc (3 mL) using 10% Pd/C (20 mg) at 0°C. After 60 min at 0°C, 4.1 mL of H_2 have been absorbed (theoretical amount 4.5 mL). After extraction with water (1.5 mL), containing $KHCO_3$, (5.0 mg, 0.05 mmol), and lyophilisation of the aqueous solution in high vacuum at -30°C, the potassium salts of the oxime ethers were accessible as colourless non-crystalline solids (yields approx. 7.5 mg, 64%, purity by NMR approx. 77%) ^{1}H NMR (D_2O) **Isomer A**: δ 2.66 (dd, 1H, ^2J 19.2, $^3J_{4\text{-}5}$ 6.4, 4-H_{exo}); 2.94 (dd, 1H, ^2J 16.5, $^3J_{5\text{-}6}$ 2.0, 6-H_{endo}); 3.10 (ddd, 1H, ^2J 19.2, $^3J_{4\text{-}5}$ 6.3, $^4J_{2\text{-}4}$ 1.6, 4-H_{endo}); 3.49 (dd, 1H, ^2J 16.5 , $^3J_{5\text{-}6}$ 4.7, 6-H_{exo}); 3.90 (s, 3H, -OC$\underline{H}_3$); 4.13 (m, 1H, 5-H); 4.87 (broad signal, 1H, 2-H). **Isomer B**: δ 2.68 (dd, 1H, ^2J 17.8, $^3J_{4\text{-}5}$ 7.4, 4-H_{exo}); 2.96 (dd, 1H, ^2J 16.4, $^3J_{5\text{-}6}$ 1.9, 6-H_{endo}); 3.07 (ddd, 1H, ^2J 17.8 , $^3J_{4\text{-}5}$ 7.3 , $^4J_{1\text{-}4}$ 0.7, 4-H_{endo}); 3.48 (dd, 1H, ^2J 16.4 , $^3J_{5\text{-}6}$ 4.6, 6-H_{exo}); 3.83 (s, 3H, -OC$\underline{H}_3$); 4.17 (m, 1H, 5-H); 4.94 (broad signal, 1H, 2-H).

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