

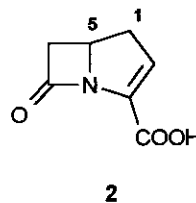
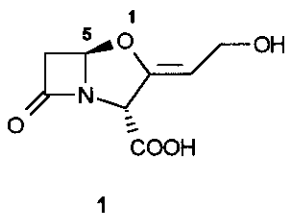
OXIME ETHERS AS STRUCTURE ANALOGS OF CLAVULANIC ACID

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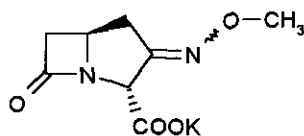
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Abstract - The reaction of racemic *p*-nitrobenzyl 1-aza-3,7-dioxobicyclo-[3.2.0]heptane-2-carboxylate with methoxyamine leads to an oxime ether. The corresponding potassium salt is stable in neutral aqueous solution. However, it neither shows any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

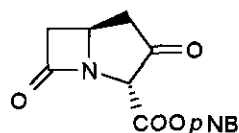
According to Knowles, clavulanic acid (1)¹ relates to the group of irreversible β -lactamase inhibitors,² implying a labile bond between atoms 1 and 5. Obviously the carbapenems (2) (and carbapenams) do not fit into this class of compounds because of their stable C-C bond and, consequently, they were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



An important feature in the structure of clavulanic acid is the exocyclic double bond of the hydroxyethylidene 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.



3



4

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 IC₅₀ value of the oxime ether in comparison to clavulanic acid and sulbactam.

Penicillinases from	Oxime ether (3)	Clavulanic acid	Sulbactam
<i>Enterobacter cloacae</i>	$> 1.9 \cdot 10^{-3}$	$1 \cdot 10^{-4}$	$1 \cdot 10^{-6}$
<i>Escherichia coli</i> 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. IC₅₀, after a 15 min preincubation period at 37 °C.

The half-life of 3 in phosphate buffer, pH 7.4 and 37 °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 ¹H NMR spectra were recorded on a Varian VXR 400 S spectrometer using Me₃SiCD₂CD₂CO₂Na 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 $\text{CH}_3\text{ONH}_2 \cdot \text{HCl}$ (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, 0.05 mmol) 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%) ^1H NMR (D_2O) **Isomer A**: δ 2.66 (dd, 1H, 2J 19.2, $^3J_{4-5}$ 6.4, 4- H_{exo}); 2.94 (dd, 1H, 2J 16.5, $^3J_{5-6}$ 2.0, 6- H_{endo}); 3.10 (ddd, 1H, 2J 19.2, $^3J_{4-5}$ 6.3, $^4J_{2-4}$ 1.6, 4- H_{endo}); 3.49 (dd, 1H, 2J 16.5, $^3J_{5-6}$ 4.7, 6- H_{exo}); 3.90 (s, 3H, $-\text{OCH}_3$); 4.13 (m, 1H, 5-H); 4.87 (broad signal, 1H, 2-H). **Isomer B**: δ 2.68 (dd, 1H, 2J 17.8, $^3J_{4-5}$ 7.4, 4- H_{exo}); 2.96 (dd, 1H, 2J 16.4, $^3J_{5-6}$ 1.9, 6- H_{endo}); 3.07 (ddd, 1H, 2J 17.8, $^3J_{4-5}$ 7.3, $^4J_{1-4}$ 0.7, 4- H_{endo}); 3.48 (dd, 1H, 2J 16.4, $^3J_{5-6}$ 4.6, 6- H_{exo}); 3.83 (s, 3H, $-\text{OCH}_3$); 4.17 (m, 1H, 5-H); 4.94 (broad signal, 1H, 2-H).

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