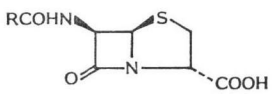
Communications to the Editor

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF A MODIFIED NUCLEAR ANALOGUE OF PIPERACILLIN

Sir:

Several bisnorpenicillin derivatives have been reported in the literature recently but these compounds have generally shown less activity than their conventional analogues. VANDERHAEGHE[1], for example, found that bisnorpenicillin V (**1**) possessed lower antibacterial activity than penicillin V and reported that replacement of the two C-2 methyl groups of penicillin had little or no effect on its sensitivity to β-lactamases. Previous work in these laboratories, leading to a new synthesis of benzyl 6-aminobisnorpenicillanate (**2**)[2], resulted in the preparation of several novel bisnorpenicillins[3] but, once again, these derivatives proved to have generally disappointing activity. Some encouragement was derived, however, from the improved activity of bisnorampicillin (**3**) and bisnorcarbenicillin (**4**) compared to their conventional counterparts against *Klebsiella pneumoniae*.



1 R = $PhOCH_2-$

3 R = $PhCH(NH_2)-$

4 R = $PhCH(COOH)-$

Recent years have seen the disclosure of certain *N*-acylated ampicillin derivatives (*e.g.* piperacillin[4] and VX-VC 43[5]) which, like carbenicillin, display broad-spectrum activity. We report herein the synthesis of a novel α-ureido bisnorpenicillin (**8**) with an expanded spectrum of activity compared to its conventional penicillin analogue.

A key intermediate in the synthesis of bisnorpenicillins is the amine (**2**) but preparation of this nucleus from penicillin V using the disclosed procedure[2] proved inefficient on a scale larger than that reported. A similar but more productive synthesis of **2** was therefore utilised (Scheme 1) in which the 4-mercaptoazetidin-2-one (**5**), derived from benzylpenicillin using established methodology[6], was treated with the bromoacrylate (**6**) in the presence of base to give benzyl bisnorbenzylpenicillin (**7**). This material underwent a Delft cleavage[7] of the side-chain to give the required nucleus (**2**), reproducibly in 40~50% yield. Acylation (acid chloride/pyridine) and deprotection (H_2, Pd/C) then gave "bisnorpiperacillin" (**8**).

The comparative activity of piperacillin and bisnorpiperacillin against a number of Gram-negative and Gram-positive bacteria is shown in Table 1. Generally the activity of the two compounds was similar against non-β-lactamase-producing Gram-negative bacteria such as *Escherichia coli* NCTC 10418, *Haemophilus influenzae* 2074, *Neisseria gonorrhoeae* WHOV

Scheme 1.

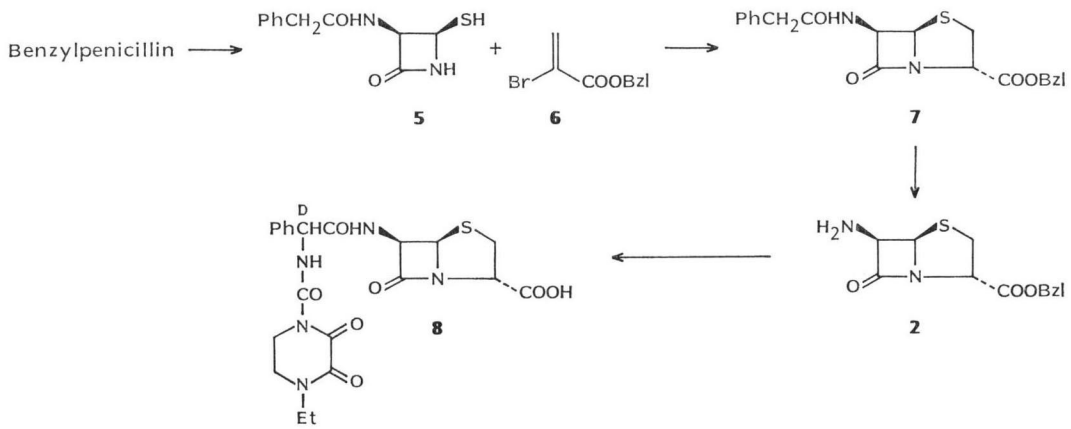


Table 1. Antibacterial spectrum of bisnorpiperacillin and piperacillin.

Organism	MIC (μg/ml)[a]	
	Bisnorpiperacillin	Piperacillin
Bacteroides fragilis WS1	100	25
Enterobacter cloacae N1	0.2	1.0
Escherichia coli NCTC 10418	1.0	1.0
〃 〃 Ba 78 (R-TEM)[b]	2.5	100
Haemophilus influenzae 2074	≦0.02	≦0.02
〃 〃 AS 74100[b]	0.2	50
Klebsiella pneumoniae A	0.2	5.0
〃 〃 BED 10 (R-TEM)	10	>100
Neisseria gonorrhoeae WHO V	≦0.001	≦0.001
〃 〃 US 4077[b]	0.02	>100
Proteus mirabilis C977	0.2	0.2
〃 〃 889[b]	>100	>100
Pseudomonas aeruginosa NCTC 10662	10	5.0
〃 〃 S1	10	5.0
Serratia marcescens US32	0.5	1.0
〃 〃 OG 9 (R-TEM)	5.0	100
Staphylococcus aureus T131	1.0	0.5
〃 〃 Russell[b]	100	>100
Streptococcus faecalis I	25	5.0
〃 *pyogenes* CN10	0.2	0.05

[a] Determined by serial dilution in an appropriate agar medium. Plates inoculated with 1 μl of an undiluted overnight broth culture ($\sim 10^6$ cfu) and incubated at 37°C either aerobically or anaerobically for 18 hours.
[b] Ampicillin-resistant strain.

Table 2. The relative stabilities of bisnorpiperacillin and piperacillin to the β-lactamases of *E. coli* (R-TEM) and *K. pneumoniae*.

β-Lactamase isolated from	Incubation time	Residual activity (%)*	
		Bisnorpiperacillin	Piperacillin
E. coli (TEM-1) (plasmid-mediated)	10 minutes	100	35
	20 minutes	100	20
	60 minutes	100	<5
K. pneumoniae (chromosomal enzyme)	10 minutes	100	70
	20 minutes	95	55
	60 minutes	85	5

* Piperacillin and its bisnor analogue (20 μg/ml) were mixed with either 0.001 cell enzyme units† of *E. coli* R-TEM β-lactamase or 0.1 cell enzyme units of *Klebsiella* β-lactamase and residual antibiotic concentrations determined using a paper-tape bioassay as described previously[8].
† 1 cell enzyme unit=enzyme obtained from $\sim 10^9$ sonicated bacteria.

and *Proteus mirabilis* C977. However, it can be seen that against some β-lactamase-producing bacilli, for example, *K. pneumoniae* A and *Enterobacter cloacae* N1, and particularly those strains producing R-TEM β-lactamase such as *E. coli* Ba 78, *H. influenzae* AS 74100, *K. pneumoniae* BED 10, *N. gonorrhoeae* US 4077 and *Serratia marcescens* OG9, bisnorpiperacillin was considerably more active than piperacillin. Strains of *Bacteroides fragilis, Pseudomonas aeruginosa* and most Gram-positive cocci on the other hand proved to be from two to five-fold more sensitive to piperacillin than the bisnor analogue.

The improved activity of bisnorpiperacillin compared to piperacillin against the Gram-negative bacteria was shown to be related to an

increased stability to the β-lactamases produced by these organisms. Table 2 shows, for example, that there is little or no hydrolysis of bisnorpiperacillin in the presence of β-lactamase isolated from *E. coli* (TEM-1) or *K. pneumoniae* whereas under the same conditions piperacillin was almost completely destroyed.

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