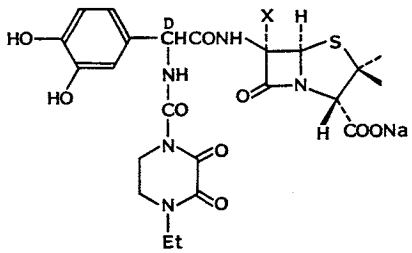
ANTIBACTERIAL ACTIVITY OF CATECHOLIC PIPERACILLIN ANALOGUES

Sir:

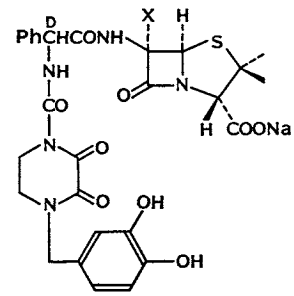
A number of β-lactam antibiotics containing a catechol moiety have now been described which show potent activity against Gram-negative bacteria including *Pseudomonas aeruginosa*[1~3]. In structure-activity relationship studies, catecholic analogues are usually considerably more active than either of the corresponding 3- or 4-hydroxyphenyl compounds[4~6]. Previous publications from these laboratories have described 3,4 - dihydroxyphenylglycylpiperazinyl derivatives[1,5]. We now wish to report the antibacterial properties of piperacillin analogues with a catechol group at various positions in the molecule and the effect on activity of introducing a C(6)α-formamido substituent in the nucleus. Full details of the chemical syntheses of these compounds will be published subsequently.

The activity of the catecholic piperacillin derivatives is shown in Table 1. It can be seen that 3,4-dihydroxyphenylpiperacillin (**1**) was more active than piperacillin itself (**7**) against a number of Gram-negative bacteria but particularly against *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and *P. aeruginosa*. Potent β-lactamase-producing strains of these species, however, were still resistant to **1** and the Gram-positive activity was reduced about 4-fold compared with piperacillin. The introduction of a C(6)α-formamido substituent (**2**) conferred stability to β-lactamase[1] and led to a considerably enhanced level of activity against piperacillin-resistant organisms extending the spectrum to include *Acinetobacter calcoaceticus* and constitutive β-lactamase-producing strains of species such as *E. cloacae*. The improvement in Gram-negative activity, however, was achieved at the expense of activity against Gram-positive cocci.

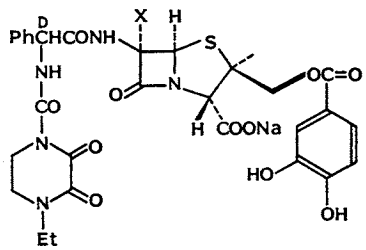
Other piperacillin analogues were synthesised with the catechol moiety incorporated into the



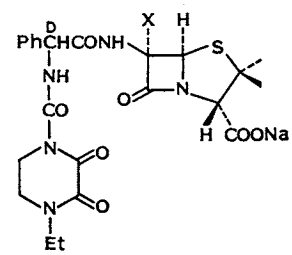
1 X = H
2 X = NHCHO



3 X = H
4 X = NHCHO



5 X = H
6 X = NHCHO



Piperacillin (**7**) X = H

8 X = CH_2OC(=O)– (OAc, OAc)

Table 1. Antibacterial activity[a] (μg/ml) of catecholic penicillins.

Organism	1	2	3	4	5	6	7	8
Escherichia coli DC2	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	≤0.03	4.0
E. coli DCO	≤0.03	≤0.03	≤0.03	≤0.03	0.12	0.12	1.0	>64
E. coli DCO (RTEM)	16	≤0.03	32	≤0.03	4.0	0.25	>64	>64
E. coli JT425 (C+)	4.0	0.12	1.0	0.06	1.0	0.5	16	>64
Klebsiella pneumoniae T767	0.12	0.06	0.5	≤0.03	0.25	0.12	4.0	>64
Enterobacter cloacae N1	0.5	0.25	1.0	1.0	4.0	0.25	4.0	>64
E. cloacae P99 (C+)	>64	2.0	16	2.0	16	2.0	>64	>64
Proteus mirabilis 977	0.5	0.25	2.0	1.0	8.0	32	0.5	>64
Providencia stuartii T90	16	1.0	16	0.5	16	16	16	>64
Pseudomonas aeruginosa K799 WT	0.25	0.5	0.5	0.5	0.12	0.5	4.0	>64
P. aeruginosa Dalgleish (PSE-4)	64	0.12	32	0.12	4.0	0.25	>64	>64
Acinetobacter calcoaceticus WIGI	>64	1.0	>64	0.5	32	1.0	>64	>64
Staphylococcus aureus Oxford	2.0	>64	2.0	>64	4.0	>64	0.5	>64
Streptococcus pyogenes CN10	0.06	1.0	0.25	1.0	0.5	2.0	<0.03	>64
Enterococcus faecalis I	8.0	>64	4.0	>64	16	>64	2.0	>64

[a] Serial dilution in Diagnostic Sensitivity Test Agar (Oxoid) against an inoculum of 10^4 cfu. MIC values taken as the lowest concentration to inhibit growth disregarding a single colony after overnight incubation at 37°C.

C+: Constitutive β-lactamase-producing strain.

N-4 substituent of the diketopiperazine ring, again with and without a C(6)α-formamido substituent in the nucleus. It can be seen that both compounds (**3** and **4**) showed activity very similar to that of the corresponding 3,4-dihydroxyphenylglycyl compounds. Surprisingly even introducing the catechol substituent into the C(2)β-methyl group of piperacillin conferred potent activity. Compound **5** was more active than piperacillin against Gram-negative bacteria other than *Proteus mirabilis* and, judging from MIC values against *E. coli* DCO (RTEM), *E. cloacae* P99 and *P. aeruginosa* Dalgleish (PSE-4) possibly more stable to β-lactamase. The introduction of a C(6)α-formamido substituent further stabilised the molecule to β-lactamase attack and compound **6** was highly active against Gram-negative bacteria, excluding *Proteus/Providencia* sp. but had little Gram-positive activity.

Not all catecholic piperacillin analogues showed potent activity. For example compound **8** which had a catechol incorporated into the C(6)α-substituent was active only against a supersensitive strain of *E. coli*. In this instance the vicinal hydroxy groups were protected as acetates but these were shown to be hydrolysed readily under the test conditions, and the activity in Table 1 reflects that of the catechol.

The reasons for the improved Gram-negative activity of catecholic compounds compared with the corresponding phenyl or monohydroxyphenyl analogues appears to be related to an enhanced penetration through the cell wall[7]. The effect is most marked when the organisms are grown under iron-limited conditions such as on the surface of conventional agar media[8], suggesting that iron-regulated outer membrane proteins are involved in antibiotic uptake as has been reported for catecholic cephalosporins[9].

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