THE SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF SOME β-LACTAMASE STABLE 6α-(HYDROXYMETHYL)PENICILLINS

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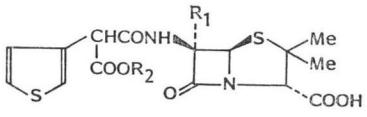
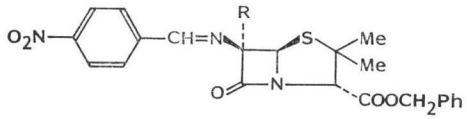
The emergence of bacterial resistance to antibiotics has long been a problem in the clinic. In the case of penicillins and cephalosporins, resistance is often due to the action of β-lactamase, enzymes which inactivate the antibiotic by hydrolysis of the β-lactam ring[1]. Certain penicillins[2] are available which display activity against β-lactamase producing Gram-positive organisms, and some hitherto resistant Gram-negative bacteria can be treated by a number of cephalosporins. However, despite attempts by several groups[3~8], it is only recently with the development of temocillin (BRL 17421)[9] (**1**) that a β-lactamase stable penicillin with good Gram-negative activity has been produced. We now wish to report the preparation and antibacterial activity of some 6α-(hydroxymethyl)penicillins[10], which are further examples of penicillins showing activity against a variety of β-lactamase producing Gram-negative bacteria.

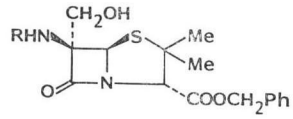
Introduction of the hydroxymethyl group was accomplished by a modification of the route employed by Merck workers[4,11]. Condensation of *p*-nitrobenzaldehyde with benzyl 6β-aminopenicillanate in the presence of 4 Å molecular sieves, afforded the Schiff's base **4**. Treatment of **4** in *N*,*N*-dimethylformamide at 0°C with anhydrous potassium carbonate followed by gaseous formaldehyde gave the 6α-(hydroxymethyl) analogue (**5**), which with toluene-*p*-sulfonic acid monohydrate in ethyl acetate afforded the protected nucleus (**6**) as its toluene-*p*-sulfonic acid salt. The free amine was liberated from this salt by reaction with aqueous sodium hydrogen carbonate, and could be acylated with the appro-

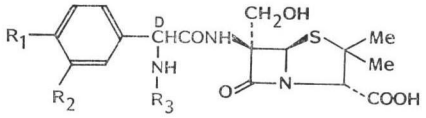


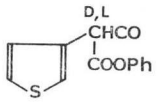
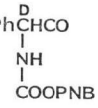
1 $R_1 = OCH_3$, $R_2 = H$
2 $R_1 = CH_2OH$, $R_2 = Ph$
3 $R_1 = CH_2OH$, $R_2 = H$

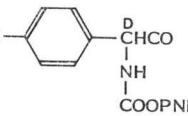


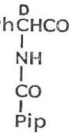
4 R = H
5 $R = CH_2OH$

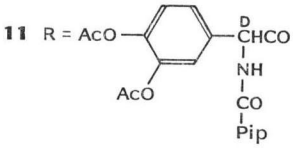




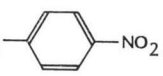
6 R = H **7** R =  **8** R = 

9 R = 

10 R = 

11 R = 

12 $R_1 = R_2 = R_3 = H$
13 $R_1 = OH$, $R_2 = R_3 = H$
14 $R_1 = R_2 = H$, $R_3 = CO\text{–}Pip$
15 $R_1 = OH$, $R_2 = H$, $R_3 = CO\text{–}Pip$
16 $R_1 = R_2 = OAc$, $R_3 = CO\text{–}Pip$

PNB = 

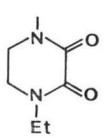
Pip = 

Table 1. The relative activities *in vitro* of compounds **14**~**16** and piperacillin.

Organism	MIC (μg/ml)[c]			
	14	**15**	**16**	Piperacillin
Escherichia coli ESS	0.1	0.1	<0.06	<0.06
E. coli JT 4[a]	100	100	1.0	>128
E. coli JT425[b]	50	50	8.0	16
E. coli NCTC 10418	25	5.0	0.12	0.5
Pseudomonas aeruginosa NCTC 10662	>100	>100	4.0	4.0
P. aeruginosa Dalgleish[a]	>100	>100	8.0	>128
Serratia marcescens US 32	50	5.0	2.0	1.0
Klebsiella aerogenes A	5.0	2.5	0.12	2.0
Enterobacter cloacae N1	25	5.0	4.0	1.0
Proteus mirabilis C977	10	2.5	4.0	0.5
P. mirabilis 889[b]	5.0	2.5	4.0	>128
P. rettgeri	50	50	8.0	0.5
Staphylococcus aureus Oxford	>100	>100	>128	0.5
S. aureus Russell[a]	>100	>100	>128	>128
Streptococcus pyogenes CN10	25	25	16	0.12

[a] β-Lactamase producing strain (plasmid-mediated).
[b] β-Lactamase producing strain (non-plasmid-mediated).
[c] Determined by serial dilution in nutrient agar containing 5% defibrinated horse blood, inoculum 0.001 ml of an undiluted overnight broth culture (approximately 10^6 cfu).

Table 2. Stability of **14** and **15** to cell-free β-lactamase preparations from Gram-negative and Gram-positive bacteria.

Enzyme preparation	Class of β-lactamase[12]	Stability[a]		
		14	**15**	Piperacillin
Escherichia coli R$^+$TEM	Plasmid mediated penicillinase III a	NDH[b]	NDH	5.3×10^3
E. coli JT414	Chromosomally mediated cephalosporinase Ib	2	NDH	20
Enterobacter cloacae 10005	Chromosomally mediated cephalosporinase Ia	3	6	NDH
Proteus mirabilis 889	Chromosomally mediated penicillinase II	NDH	NDH	5.3×10^3
Klebsiella pneumoniae A	Chromosomally mediated penicillinase IV	NDH	NDH	NDH
Staphylococcus aureus MB9	Gram-positive penicillinase	NDH	NDH	9.6×10^2

[a] Figures are rates of hydrolysis (μM/hour) for 1 cell enzyme unit, (the concentration of β-lactamase produced after 7 hours growth in nutrient broth No. 2 at 37°C).
Reaction mixtures containing 0.1 mM substrate were made up in 0.05 M phosphate buffer (pH 7).
Samples removed in duplicate at 0, 10, 20 and 40 minutes, and the residual substrate determined by a tape bioassay method[13] using *E. coli* ESS.
[b] No detectable hydrolysis at 5 cell enzyme units.

priate side-chain acids to afford a series of 6β-acylamino-6α-(hydroxymethyl)penicillanates (**7**~**11**). Hydrogenolysis of **7** over 10% palladium on charcoal catalyst afforded the half-ester (**2**), which on hydrolysis with aqueous sodium tetraborate yielded 6α-(hydroxymethyl)ticarcillin (**3**). Deprotection (10% Pd/C; H_2) of **8** and **9** gave the 6α-(hydroxymethyl)ampicillin (**12**) and amoxicillin (**13**) derivatives respectively, which could be further acylated to afford the piperacillin derivatives **14** and **15**. Preparation of **14** could also be effected by hydrogenolysis of the benzyl ester (**10**), and in a similar manner, deprotection of **11** gave the 3,4-diacetoxyphenyl-

penicillin (**16**).

The stereochemistry of the hydroxymethylation reaction has been assigned by the Merck group[4] as occurring from the α-face of the Schiff's base (**4**). We have confirmed this by demonstrating a positive nuclear Overhauser effect ($16\pm9\%$) between the 5α-proton and the 6-methylene group in the 6α-(hydroxymethyl)piperacillin ester (**10**).

Several of the compounds prepared including an α-carboxy analogue (**3**) and α-amino derivatives **12** and **13** were essentially inactive. However compounds **14**~**16**, which are structurally related to piperacillin, inhibited many clinically derived Gram-negative bacteria (Table 1). In particular the 3,4-diacetoxyphenyl derivative (**16**) showed good activity against β-lactamase producing strains of *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. None of these compounds displayed significant activity against Gram-positive organisms.

The stabilities of compounds **14** and **15** to isolated bacterial β-lactamases, compared with piperacillin, are shown in Table 2. Neither of the 6α-(hydroxymethyl)penicillins were significantly hydrolysed, whereas piperacillin was inactivated by enzymes isolated from *E. coli*, *P. mirabilis* and *Staphylococcus aureus*. Thus, incorporation of the 6α-(hydroxymethyl) group into the penicillin nucleus, like the 6α-methoxy substituent in temocillin, confers improved stability to bacterial β-lactamases.

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