THE SYNTHESIS AND ANTIBACTERIAL ACTIVITIES OF NOVEL 7α SUBSTITUTED CEPHALOSPORINS

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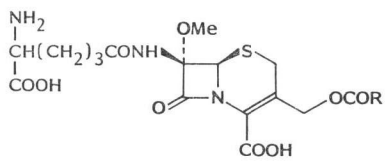
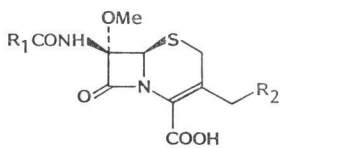
The cephamycins are chemically related to the cephalosporin family of antibiotics[1~3]. A common feature of this group of naturally-occurring compounds exemplified by cephamycins A~C (**1**) is the presence of a 7α-methoxy substituent which renders them highly resistant to hydrolysis by β-lactamases. Recent years have seen the preparation of many semisynthetic cephamycin derivatives, two of which, *viz.*, cefoxitin (**2a**)[4] and cefmetazole (**2b**)[5] are now used clinically. Certain semisynthetic penicillins bearing a 6α-methoxy substituent have been prepared in our laboratories, *e.g.* temocillin (**3**)[6], and shown to possess significant antibacterial activity as well as stability towards β-lactamases. The synthesis of penicillins and cephalosporins bearing substituents other than methoxy at the 6α(7α) position have also been reported but the compounds described were found to show reduced antibacterial activity[7~10] compared to their 6α(7α) unsubstituted analogues. However, we have found that 6α-hydroxymethyl analogues of certain penicillins (including piperacillin[11]), exemplified by (**4a**), possess antibacterial activity[12]. We have also prepared selected 7α-hydroxymethylcephalosporins[13], whose synthesis and *in vitro* antibacterial properties we now report.

Introduction of the hydroxymethyl (CH_2OH) group into the 7α-position of the cephalosporin nucleus involved procedures similar to those employed by the Merck group[10]. The ester (**5a**) was converted into the Schiff's base (**6a**) by treatment with *p*-nitrobenzaldehyde. Treatment of a *N*,*N*-dimethylformamide (DMF) solution of **6a** with anhydrous potassium carbonate formed the carbanion at the 7α-position which was

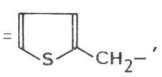


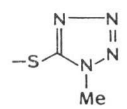
1

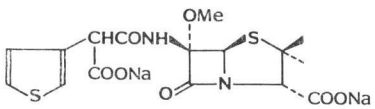
Cephamycin A R = *p*-$HOSO_2OC_6H_4CH{=}C(OMe)-$
Cephamycin B R = *p*-$HOC_6H_4CH{=}C(OMe)-$
Cephamycin C R = NH_2-



2

a R_1 =  , $R_2 = -OCONH_2$

b $R_1 = NCCH_2SCH_2-$, R_2 = 



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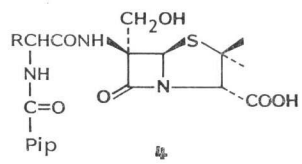
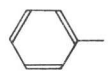
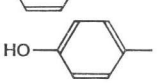
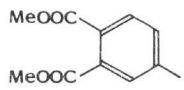
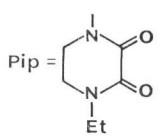


4

a R = 

b R = HO- 

c R = 

Pip = 

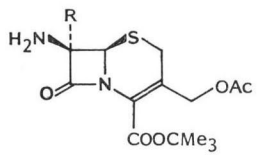
quenched with gaseous formaldehyde giving the alcohol (**6b**). It was found that a temperature of −22°C was critical in this reaction in order to avoid $\Delta^3 \rightarrow \Delta^2$ isomerisation whilst still maintaining an acceptable reaction rate.

Condensation of formaldehyde with the 7β-benzylideneaminocephem nucleus has been assigned by Merck workers as occurring at the 7α-position[10]. This assignment is strengthened in our case by the comparable activities of 6α-CH_2OH piperacillin analogues (**4a**～**c**)[12] with the corresponding 7α-CH_2OH cefoperazone analogues (**10a,c,d**) described below, and by the existence of a positive 25 (±5)% nuclear Overhauser effect between the C-6α hydrogen and C-7 CH_2 group.

Conversion of **6b** into the amine (**5b**) was effected by treatment with preformed 2,4-dinitrophenylhydrazine tosylate salt in ethanol. The 7β-acetamido derivatives (**7a,b,e**) were prepared by coupling the appropriate side-chain acids (**8a, b** and **9a**) to **5b** *via* their acid chlorides (**8c, d** and **9b**), generated using oxalyl chloride in the presence of a catalytic amount of DMF. At this point the protected *p*-hydroxyphenyl analogue (**7b**) was catalytically hydrogenated to the free hydroxyl compound **7c**. Successive treatment with trifluoroacetic acid and sodium ethyl hexanoate (SEH) in methyl isobutyl ketone (MIBK) transformed the esters (**7a**, **7c** and **7e**) into the corresponding C-4 carboxylic acid sodium salts (**10a,c,e**) respectively.

The diacetoxyphenylcephalosporin ester (**7d**) was prepared by a *N,N*-dicyclohexylcarbodiimide mediated coupling of the appropriate side-chain acid (**11**) to the nucleus (**5b**). The ester (**7d**) was subsequently converted to the sodium salt (**10d**) in the usual way.

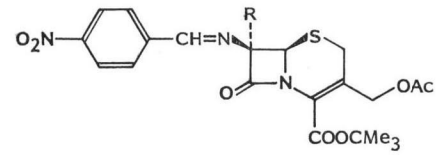
Certain 7α-hydroxymethyl substituted acylaminocephalosporins have been shown to possess significant antibacterial activity. It can be seen from Table 1 that compounds (**10a**, **c** and **d**)



5

a R = H

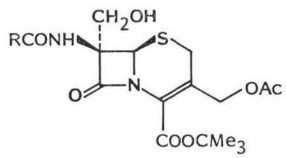
b R = CH_2OH



6

a R = H

b R = CH_2OH



7

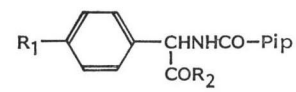
a R = PhCHNHCO–Pip

b R = Z–O– –CHNHCO–Pip

c R = HO– –CHNHCO–Pip

d R = MeOOC, MeOOC, –CHNHCO–Pip

e R = –CHCOO– –Me, S



8

a R_1 = H, R_2 = OH

b R_1 = O–Z, R_2 = OH

c R_1 = H, R_2 = Cl

d R_1 = O–Z, R_2 = Cl

Z = $PhCH_2OC(=O)$–

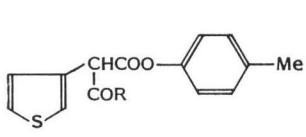
Table 1. The relative activities *in vitro* of **10a**, **10c**, **10d** and cefoperazone.

Organism	MIC (μg/ml)*			
	10a	**10c**	**10d**	Cefoperazone
Escherichia coli ESS	<0.02	0.05	<0.06	<0.01
E. coli JT4[a]	5.0	2.5	<0.06	>100
E. coli JT425[b]	2.5	1.0	<0.06	1.0
E. coli NCTC 10418	2.5	1.0	<0.06	0.05
Pseudomonas aeruginosa NCTC 10662	>100	50	2.0	5.0
P. aeruginosa Dalgleish[a]	>100	25	2.0	25
Serratia marcescens US 32	5.0	5.0	2.0	5.0
Klebsiella pneumoniae A	0.5	0.25	0.12	0.05
Enterobacter cloacae N1	10	10	8.0	1.0
Proteus mirabilis C977	2.5	2.5	16	0.5
P. mirabilis 889[b]	1.0	0.5	2.0	10
P. rettgeri	10	10	8.0	2.5
Staphylococcus aureus Oxford	>100	>100	>128	1.0
S. aureus Russell[a]	>100	>100	>128	5.0
Streptococcus pyogenes CN10	100	>100	32	0.1

[a] β-Lactamase-producing strain (plasmid-mediated).

[b] β-Lactamase-producing strain (non-plasmid-mediated).

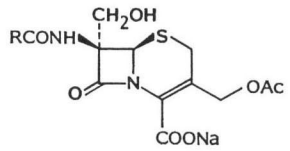
* Determined by serial dilution in nutrient agar containing 5% defibrinated horse blood, inoculum 0.001 ml of an undiluted overnight broth culture (approximately 10^8 cfu).



9

a R = OH

b R = Cl



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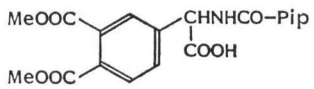

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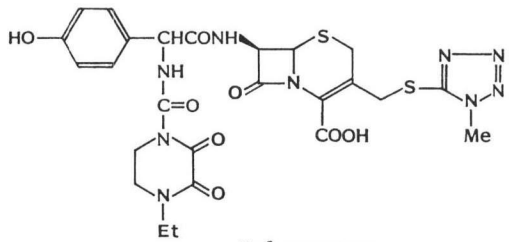

Cefoperazone

which can be considered as structurally related to cefoperazone[14], inhibited many Gram-negative bacteria with MIC values of less than 10 μg/ml. The less active of these three compounds, *viz.* **10a** and **10c**, still demonstrated improved activity compared with cefoperazone against certain β-lactamase producing organisms such as *Escherichia coli* JT4 and *Proteus mirabilis* 889, although they were usually less potent against other Gram-negative bacteria, including *E. coli* NCTC 10418 and *P. mirabilis* C977. Presumably the 7α-hydroxymethyl group, like the 7α-methoxy substituent in the cephamycin series, confers improved stability to β-lactamases.

However, the diacetoxyphenyl example (**10d**) showed much improved activity at least the equal of cefoperazone against the cefoperazone sensitive *E. coli* NCTC 10418 and *Pseudomonas aeruginosa* NCTC 10662, while again displaying stability to β-lactamases as expressed by its MIC values

against *E. coli* JT4, *P. aeruginosa* Dalgleish and *P. mirabilis* C977.

All three compounds (**10a**, **c** and **d**) had little or no Gram-positive activity. The α-carboxycephalosporin (**10e**) showed no significant antibacterial activity.

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