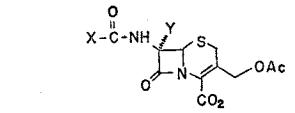
BIOCHEMICAL AND MICROBIOLOGICAL STUDIES ON 7-METHOXYCEPHALOSPORINS

Sir:

Penicillins and cephalosporins are known to interfere with the biosynthesis of the bacterial cell wall[1]. Further, it has been shown that these antibiotics inhibit the cell wall enzyme transpeptidase which catalyzes the linkage of two linear peptidoglycan strands[2]. STROMINGER *et al.*[2] have postulated that transpeptidase performs its function by reacting with the D-alanyl-D-alanine end of a terminal pentapeptide found in the peptidoglycan strands, thus forming an acyl-enzyme intermediate with simultaneous elimination of D-alanine. In addition, based on the structural resemblance to D-alanyl-D-alanine, these workers proposed that a 6-α-methyl penicillin or 7-α-methyl cephalosporin might be expected to have increased antimicrobial properties.

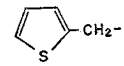
The recent discovery of naturally occurring 7-α-methoxy cephalosporins which have increased gram-negative activity[3,4] seemed to substantiate the STROMINGER proposal. However, in a recent study of penicillins, we have demonstrated that addition of a 6-α-methyl group or a 6-α-methoxy group results in a reduction of both transpeptidase inhibition and overall activity[5]. Moreover, STROMINGER *et al.*[6] have recently shown that a 7-α-methyl desacetoxy cephalosporin derivative is also less active than the parent compound in enzyme inhibition and in antimicrobial properties. In a continuation of this investigation we now present the results of enzyme inhibition, MIC, and aqueous hydrolysis studies on 7-α-methoxy cephalosporin.

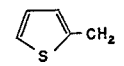
Table 1 compares the concentrations required for 50% inhibition of the transpeptidase and the corresponding MIC values for two representative cephalosporins (**I, III**) and their corresponding 7-α-methoxy derivatives (**II, IV**). We found that, contrary to the results obtained in the penicillin series, the introduction of a 7-α-methoxy group to cephalosporins results in antibiotics that are better transpeptidase enzyme inhibitors than their unsubstituted counterparts.



I. X = $CO_2H-\underset{NH_2}{CH}-(CH_2)_3-$; Y = -H (Cephalosporin C)

II. X = $CO_2H-\underset{NH_2}{CH}-(CH_2)_3-$; Y = $-OCH_3$

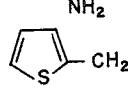
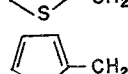
III. X =  ; Y = -H (Cephalothin)

IV. X =  ; Y = $-OCH_3$

To test the effect of 7-α-methoxy substitution on the chemical stability of the cephalosporin β-lactam, the relative rates of hydrolysis of compounds **I**~**IV** were determined in aqueous solution at pH 10 (Table 2). We established that 7-α-methoxy substitution in cephalosporins has no pronounced effect on the reactivity of the β-lactam. This result is in contrast to the 3~5 fold decrease in reactivity found with 6-methoxy substitution in penicillins V and G[5].

The differences in reactivity resulting from methoxy substitution in penicillins and cephalosporins may be explained by steric considera-

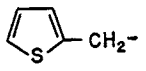
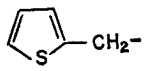
Table 1. Inhibition of growth and transpeptidase by 7-substituted cephalosporins

Cephalosporins	X	7-Substituent Y	MIC* (mcg/ml)	Conc. required for 50% Inhibition**
I	$CO_2HCH(CH_2)_3-$ NH_2	$-H$	25.0	10.0
II	$CO_2HCH(CH_2)_3-$ NH_2	$-OCH_3$	3.5	0.1
III		$-H$	3.5	10.0
IV		$-OCH_3$	25.0	0.1

* The MIC values were determined by the two-fold tube dilution method with *E. coli* Y 10. (*E. coli* Y 10 was kindly provided by Dr. J. L. STROMINGER of Harvard University, Cambridge, Massachusetts.)

** Transpeptidase was prepared from *E. coli* Y 10 and assayed according to the method of STROMINGER *et al.*[10]

Table 2. The relative rates af base hydrolysis of 7-substituted cephalosporins at pH 10*

Cephalosporins	X	7-Substituent Y	Relative rates
I	$CO_2HCH(CH_2)_3-$ with NH_2	H	1.1
II	$CO_2HCH(CH_2)_3-$ with NH_2	$-OCH_3$	1.4
III		H	1.0
IV		$-OCH_3$	1.3

* Rate constants determined by following the loss of the 260 nm chromophore at constant pH[11].

tions.[8] In penicillins the β-face of the β-lactam is subject to severe steric hindrance, and attack of a nucleophile is restricted to the unhindered α-face. The addition of a 6-α-methoxy substituent would therefore have the effect of sterically impeding α attack by the nucleophile thus giving the molecule greater stability. Attack at the α-face of cephalosporins would also be hampered by addition of a 7-α-methoxy group, but the β-face of cephalosporins is much less hindered than in penicillins. As a result, the net effect of steric hindrance at the α-face should be diminished and countered by the expected polar effect on the methoxy group.

While the chemical stability is unchanged, and despite the increase in transpeptidase inhibition, the MIC values are not always improved by the addition of a 7-α-methoxy in cephalosporins (see Table 1). The relative differences in MIC and enzyme inhibition within each pair of cephalosporins probably reflect the degree of permeability of the antibiotic through the cell wall of this organism. Cell wall permeability may be related to the lipophilic character of the antibiotic,[9] and this property could be affected in varying degrees by the introduction of the 7-α-methoxy group.

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