BIOCHEMICAL AND MICROBIOLOGICAL STUDIES ON 6-SUBSTITUTED PENICILLINS

Sir:

Penicillins have been shown to inhibit the biosynthesis of the bacterial cell wall.[1] These antibiotics also inhibit the enzyme, transpeptidase. This enzyme catalyzes transpeptidation of two linear peptidoglycan strands resulting in the formation of an interpeptide bridge with concomitant elimination of D-alanine.[2] In addition, HARTMANN *et al.*[3] recently have suggested that a glycosidase, obtained from the supernatant of *Escherichia coli* W7 cell wall extract, is also sensitive to penicillin inhibition. However, in this case, the amount of penicillin (2,000 units/ml) required to exhibit a 20% inhibition of glycosidase was far greater than that required to inhibit transpeptidase, and the significance of glycosidase as a secondary target site of penicillin remains uncertain.

STROMINGER *te al.*[2] have postulated that the 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. Because penicillin, an LD-dipeptide, resembles D-alanyl-D-alanine in conformation, he further proposed that addition of a methyl group in the C_6 α position of penicillin might enhance the binding of the antibiotic to the enzyme. A series of 6-α substituted penicillins has, therefore, been synthesized in our laboratories, and examined for activities in both the transpeptidase system and by antibacterial assay.

In Table 1, the concentrations required for 50% inhibition of the transpeptidase and the corresponding MIC values are given for 6-substituted penicillin G (benzyl penicillin) and 6-substituted penicillin V (phenoxymethyl penicillin) derivatives. The concentrations required to inhibit the enzyme were found to be much lower than the MIC values. This discrepancy probably reflects the degree of permeability of the drug through the cell wall of this organism.* We have found that any substitution in the C_6 α position of penicillin in our series reduces both enzyme inhibition and antibacterial activity. The lack of enhanced activity for the 6-methyl penicillins could perhaps be explained by either of the following hypotheses; (1) the 6-methyl group is not a determinant in substrate-enzyme binding, or (2) substitution on the β-lactam increases its chemical stability, thus lowering its ability to acylate the enzyme molecule.

To test the second hypothesis, the rates of hydrolyses of the penicillin V and penicillin G derivatives were determined in aqueous media at constant pH (Table 2). In fact, 6-substitution does reduce the reactivity

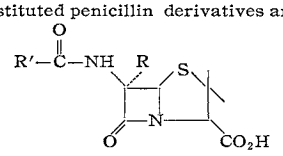
Table 1. Inhibition of growth and transpeptidase by 6-substituted penicillins

	6-Substitution[c]	MIC (mcg/ml)[a]	Concentrations required for 50% inhibition (mcg/ml)[b]
A. Penicillin V derivatives	-H	100	0.1
	$-CH_3$	>200	100.0
	$-OCH_3$	100	1.0
	$-SCH_3$	>200	100.0
B. Penicillin G derivatives	-H	13.5	0.1
	$-CH_3$	>200	100.0
	$-OCH_3$	200	1.0
	$-OC_2H_5$	>200	10.0

a) The MIC values were determined by the two-fold tube dilution method with *E. coli* Y10 (*E. coli* Y10 was kindly provided by Dr. J.L. STROMINGER of Harvard University, Cambridge, Mass.).

b) Transpetidase was prepared from *E. coli* Y10 and assayed according to the method of STROMINGER *et al.*[4]

c) The 6-substituted penicillin derivatives are available as follows:



Where R=$-CH_3$, by the method of workers at Squibb—E.H.W. BOHME, H.E. APPLEGATE, B. TOEPLITZ, J.E. DOLFINI & J.Z. GOUGOUTAS, J. Amer. Chem. Soc. 93: 4234, 1971

Where R=$-OCH_3$ or $-OC_2H_5$, by the method of workers at Merck—L.D. CAMA, W.J. LEANZA, T.R. BEATTIE & B.G. CHRISTENSEN, J. Amer. Chem. Soc. 94: 1408, 1972

Where R=$-SCH_3$ by a method developed in these laboratories which will be published shortly.

* STROMINGER *et al.*[2] has observed a similar difference in MIC and transpeptidase inhibition levels with benzyl penicillin.

Table 2. The relative rates of base hydrolysis of 6-substituted penicillins at pH 10.0*

Pen G		Pen V	
6H	1.0	6H	1.0
6-OCH_3	0.2	6-CH_3	0.1
6-OC_2H_5	0.1	6-OCH_3	0.3
		6-SCH_3	0.1

* Rate constant determined by titration at constant pH of the penicilloic acid product.

of the β-lactam in all cases tested; however, this effect appears to be primarily steric rather than polar* and the implication in an enzymatic reaction is not clear.

From our data we cannot definitively determine whether 6-substitution of penicillin does or does not enhance reversible enzyme binding. However, we have established that 6-methoxy penicillin derivatives are better enzyme inhibitors than the corresponding 6-methyl derivatives. The reasons for this difference between 6-methyl and 6-methoxyl penicillins as well as the question of enzyme binding are presently under investigation.

Peter P. K. Ho
Richard D. Towner

Joseph M. Indelicato
Wayne A. Spitzer
Gary A. Koppel

The Lilly Research Laboratories,
The Lilly and Company,
Indianapolis, Indiana 46206,
U.S.A.

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3) Hartmann, R.; J. V. Holtje & U. Schwartz: Targets of penicillin action in *E. coli*. Nature 235 : 426~429, 1972
4) Izaki, K.; M. Matsuhashi & J.L. Strominger: Biosynthesis of the peptidoglycan of bacterial cell walls. XIII. J. Biol. Chem. 243: 3180~3192, 1968

* From an examination of the polar substituent constants σ_I, σ^*, or σ^m one would predict that the rate of hydrolysis of the 6-methoxy penicillin would be faster and the 6-methyl penicillin slower than the parent penicillin. Since, in fact, both these substituents lower the rate of hydrolysis, the primary effect of 6-substituents must be steric in nature.