Communications to the editor

NATURALLY-OCCURRING β-LACTAMASE INHIBITORS WITH ANTIBACTERIAL ACTIVITY

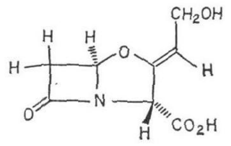
Sir:

A number of important pathogens which are resistant to penicillins and cephalosporins owe their resistance to the production of a β-lactamase. If this β-lactamase could be inhibited adequately *in vivo* it would be expected that infections caused by such organisms could be successfully treated with the penicillins and cephalosporins now already available.

We now wish to report the isolation of certain naturally-occurring substances which are potent β-lactamase inhibitors and which also possess antibacterial activity. These substances, designated MM 14151, MM 4550 and MM 13902 (Belgian Patents 827,926, 827,331 and 827,332) are produced by certain cultures of *Streptomyces* and were detected in fermentation broths using the following test. Agar plates were prepared containing benzylpenicillin at a concentration of 10 μg/ml and seeded with a strain of *Klebsiella aerogenes* NCTC 418 (ATCC 15380) which owes its penicillin resistance to the production of β-lactamase. Broth samples for test were placed in holes cut in the agar and the plates incubated overnight. Samples containing a diffusible β-lactamase inhibitor gave rise to zones of inhibition resulting from the protection of the penicillin present in the agar. In the absence of any β-lactamase inhibitor, bacterial growth occurred as a result of the inactivation of the penicillin by the β-lactamase produced by the organism.

MM 14151 is produced by *Streptomyces clavuligerus* ATCC 27064 and has been given the trivial name clavulanic acid. This compound has been isolated as the sodium salt and shown by HOWARTH, BROWN and KING[2] to be a novel fused β-lactam (Fig. 1) structurally distinct from the penicillins, cephalosporins and cephamycins. Clavulanic acid shows a broad antibacterial spectrum but the level of activity is relatively low. It is, however, a potent progressive inhibitor of the β-lactamase produced by many strains of *Staphylococcus aureus, Escherichia coli, Klebsiella, Proteus, Shigella, Pseudomonas* and *Haemophilus influenzae*. As a result, in the presence of low concentrations (<10 μg/ml) of clavulanic acid many of these β-lactamase-producing organisms are rendered almost as sensitive to penicillins and cephalosporins now commercially available as are non-β-lactamase-producing strains.

Fig. 1



MM 4550 and MM 13902 are co-produced by strains of *Streptomyces olivaceus* (*e.g.* ATCC 21379) and can be distinguished chromatographically and spectroscopically from clavulanic acid and the naturally-occurring penicillins, cephalosporins and cephamycins.

MM 4550, which is a sulphur-containing carboxylic acid, shows a broad-spectrum of antibacterial activity against Gram-positive and Gram-negative bacteria, most of the sensitive organisms being inhibited at concentrations of 5~50 μg/ml. MM 4550 is also an extremely potent inhibitor of a number of β-lactamases including those produced by strains of *Staph. aureus, E. coli, Klebsiella, Citrobacter, Proteus* and *Pseudomonas* and, in the presence of MM 4550 at a concentration of 10 μg/ml or less many of these β-lactamase-producing organisms are inhibited by ampicillin, for example, at a concentration of less than 5 μg/ml.

MM 13902 is also a sulphur-containing carboxylic acid related to MM 4550. Like MM 4550 it is a potent inhibitor of β-lactamase but shows considerably greater antibacterial activity. MM 13902 inhibits a wide range of Gram-positive and Gram-negative bacteria, including penicillin- and cephalosporin-resistant strains, and most of these organisms are inhibited by concentrations less than 5 μg/ml.

The existence of a β-lactamase inhibitor produced by *Streptomyces olivaceus* was first disclosed in Belgian Patent 772,636 from our laboratories.

Subsequently, HATA, *et al.*[1] and also MIYAMURA and OCHIAI[3] have reported β-lactamase inhibitors from *Streptomyces* species but these substances appear to be structurally quite different from clavulanic acid, MM 4550 and MM 13902.

More recently, UMEZAWA, *et al.*[4] have reported the production of two β-lactamase inhibitors by *Streptomyces fulvoviridis* MC 696-SY2 but it is not known whether these are related to the substances reported here.

Clavulanic acid is the first naturally-occurring β-lactamase inhibitor to be fully characterized and the properties of this substance and also those of MM 4550 and MM 13902 are being evaluated.

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