In an attempt to prepare $(C_4H_9)_2Sn$ the amount of di-isobutyl aluminium hydride added was increased to 8·0 g, but a black unresolved mixture was obtained.

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BIOCHEMISTRY

Production of a Cephalosporinase by *Pseudomonas pyocyanea*

CRUDE preparations of penicillinase from *Bacillus cereus* N.R.R.L. 569, unlike the purified penicillinase from this organism or crude penicillinase from *Staphylococcus aureus* R_1, have been shown to catalyse the hydrolysis of the β-lactam ring of cephalosporin *C* and other derivatives of 7-aminocephalosporanic acid at a significant rate. The enzyme responsible for this hydrolysis was termed a cephalosporinase[1,2]. The maximum rate of hydrolysis (V_{max}) of cephalosporin *C* obtained with a crude enzyme from *B. cereus* was only 5 per cent of that of benzylpenicillin, but this value increased to 33 per cent after selective inactivation of penicillinase in aqueous solution at 60° C. Subsequent studies were made with a crude enzyme from *Bacillus subtilis* N.C.T.C. 6346, induced with cephalosporin *C* and freed from the cells by the action of lysozyme[3]. With this enzyme preparation V_{max} for cephalosporin *C* was about 10 per cent that for benzylpenicillin, but attempts to separate a cephalosporinase from a penicillinase, or selectively inactivate a penicillinase, were unsuccessful.

We have found that a strain of *Pseudomonas pyocyanea* (N.C.T.C. 8230) produces an enzyme which shows a much greater value of V_{max} with cephalosporin *C* or 7-phenacetamidocephalosporanic acid as substrate than with penicillin *N* (which has the same D-α-aminoadipoyl side-chain as cephalosporin *C*) or benzylpenicillin. When *Ps. pyocyanea* was grown in nutrient 'Oxoid' broth the penicillinase or cephalosporinase activity of the culture fluid or whole culture was too small to be detected by the manometric method. Furthermore, no activity was detected after a culture had been subjected to ultrasonic disintegration (60 W at 0° for 2–15 min) or freeze-pressing in an 'X-press'[4]. No detectable amount of enzyme was induced by concentrations of benzylpenicillin sufficient to cause maximum induction of penicillinase in *B. cereus* (1 μg/ml.) or by concentrations of 2 : 6-dimethoxyphenylpenicillin (1 μg/ml.) or cephalosporin *C* (10 μg/ml.), which showed a strong inducing activity in *Staphylococcus aureus* R_1[2]. However, after very high concentrations of benzylpenicillin (10 mg/ml.) or cephalosporin *C* (10 mg/ml.) had been added to a growing culture of the organism, considerable enzyme activity appeared in the culture fluid. A significant but smaller amount of enzyme appeared after the addition of 7-phenacetamidocephalosporanic acid (5 mg/ml.). In some, but not in all, of these experiments the cells underwent partial lysis.

With the crude enzyme, V_{max} at *p*H 7·0 was more than ten times as great for cephalosporin *C* and 7-phenacetamidocephalosporanic acid as for penicillin *N* and benzylpenicillin. But with all these substrates the Michaelis constants were very much higher than those for penicillin *N* or benzylpenicillin with penicillinase from *Staph. aureus* or *B. cereus*. Characteristic changes in ultra-violet absorption spectra, which accompanied the hydrolysis of cephalosporin *C* and deacetylcephalosporin *C* lactone, indicated that the enzyme involved was a β-lactamase.

2 : 6-Dimethoxyphenylpenicillin (methicillin) proved to be relatively highly resistant to the enzyme and a powerful inhibitor of the action of the enzyme on cephalosporin *C*, 7-phenacetamidocephalosporanic acid and benzylpenicillin. Similarly, 3-*o*-chlorophenyl-5-methyl-4-isoxazolylpenicillin (orbenin) was highly resistant to hydrolysis. This compound, and to a lesser extent 6-aminopenicillanic acid and 7-aminocephalosporanic acid, also inhibited the hydrolysis of cephalosporin *C*. α-Aminobenzylpenicillin (ampicillin) was hydrolysed at a rate which was about 15 per cent of that with benzylpenicillin in the concentration used (1 mg/ml.).

The behaviour of 2 : 6-dimethoxyphenylpenicillin to the enzyme from *Ps. pyocyanea* is in striking contrast with its failure to inhibit the hydrolysis of penicillin *N* or benzylpenicillin by staphylococcal penicillinase[2,5], but is analogous with its inhibitory action on a penicillinase from *Klebsiella aerogenes*[6]. The effect of the 2 : 6-dimethoxybenzoyl side-chain on the enzyme from *Ps. pyocyanea* is paralleled in the behaviour of 7-(2 : 6-dimethoxybenzamido) cephalosporanic acid, which is also resistant to hydrolysis by this enzyme. Whether the crude enzyme consists of more than one β-lactamase remains undetermined.

An enzyme produced by a strain of *Enterobacter cloacae* has recently been reported to be highly active against cephalosporin *C* but to have little, if any, activity against benzylpenicillin[7]. It appears that enzymes able to degrade cephalosporin *C* have not been found in *Klebsiella*, but are distributed within a number of other genera of the Enterobacteriaceae and that the species most resistant to the antibiotic are among those which produce these enzymes[7]. However, we have no evidence which would suggest that the production of penicillinases or cephalosporinases is primarily responsible for the high resistance of *Pseudomonas pyocyanea* to any of the penicillins or cephalosporins tested. Methicillin and orbenin, which were relatively insensitive to the enzyme from *Ps. pyocyanea*, and ampicillin, which was hydrolysed at a relatively low rate, showed no significant activity against this organism.

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Phosphoprotein as an Intermediate in Cerebral Microsomal Adenosine-triphosphatase

MICROSOMAL and cell membrane fractions from cerebral cortex contain a powerful ATP-splitting system, activated by Mg^{2+}, Na^+ and K^+ and inhibited by the cardiac glycoside ouabain[1-6]. The nature of the ATP-splitting reaction is not clear; but recently it has been reported that a phosphorylated intermediate is involved in the splitting of ATP by guinea pig kidney cortex[13], human erythrocyte membranes[14] and the electric organ of the electric eel[15]. This note provides evidence that phosphoprotein from cerebral cortex becomes labelled during the splitting of $AT^{32}P$ by the ATPase, and that the time-course of uptake of labelled phosphate from ATP into the phosphoprotein fraction and into inorganic orthophosphate is indicative of the phosphoprotein being a precursor of inorganic phosphate.

In principle, the experiment consisted of the brief incubation of $AT^{32}P$ with a brain enzyme preparation in