LETTERS TO THE EDITORS

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Cephalosporin C, a New Antibiotic containing Sulphur and D-α-Aminoadipic Acid

A SPECIES of *Cephalosporium* produces a hydrophilic penicillin, called cephalosporin *N*, which yields D-α-aminoadipic acid on hydrolysis[1]. Crude preparations of cephalosporin *N* have been found to show strong absorption in ultra-violet light at 260 mμ, although highly purified cephalosporin *N* shows very little absorption at this wave-length. A new antibiotic with an absorption maximum at 260 mμ has now been isolated from the crude preparations in the form of a crystalline sodium salt. It resembles cephalosporin *N* in certain physical, chemical and biological properties, but differs from it sharply in others.

Cephalosporin *C* sodium salt ($[\alpha]_D^{20}$ + 103° ; $\lambda_{max.}$ 260 mμ, $\varepsilon_{max.}$ 9,500) separates from aqueous ethanol or aqueous propanol in monoclinic crystals. Its equivalent weight, determined by titration, is 480 ± 15. X-ray crystallographic measurements were kindly made by Dr. Dorothy Hodgkin and Mrs. M. Mackay, who reported as follows : unit cell dimensions : a = 13·12 A. ; b = 4·97 A. ; c = 17·82 A. ; β = 106° 30′. Space group, $P2_1$; number of molecules in the unit cell, 2 ; density, 1·38. The molecular weight, calculated from these figures, is 470 ± 15.

On drying *in vacuo* at room temperature, cephalosporin *C* sodium salt loses two moles of water which are rapidly regained on exposure to laboratory air. The results of elementary analysis, together with the value for the molecular weight, indicate that a possible molecular formula for the crystalline hydrate is $C_{16}H_{20}O_8N_3SNa,2H_2O$; but this formula is only a provisional one and may need revision.

The infra-red spectrum of cephalosporin *C* sodium salt (in paraffin paste) shows bands at the following wave-lengths : 2·94μ, 3·06μ, 5·61μ, 5·77μ, 6·05μ, 6·29μ, 6·57μ, 7·17μ and 7·36μ. It is of interest that a band in the region of 5·61μ is shown by the common penicillins and by cephalosporin *N*, and has been attributed in the former to the C=O of the fused β-lactam-thiazolidine ring system[2]. The band at 5·77μ could be due to an ester or lactone grouping.

Cephalosporin *C* gives a ninhydrin reaction. Electrometric titration indicates that it is a monoaminodicarboxylic acid, having two acidic groups with pK values of 3·1 and <2·6 respectively and a basic group with a pK value of 9·8. When subjected to ionophoresis on paper in a collidine–acetate buffer at pH 7, it migrates towards the anode at almost the same rate as cephalosporin *N*.

Unlike cephalosporin *N* and benzylpenicillin, cephalosporin *C* is stable in aqueous solution at pH 2·5 at room temperature. It is rapidly inactivated, however, with loss of its characteristic absorption spectrum, at pH 12. After it has been kept in aqueous solution at pH 12 for two hours, back titration shows that two acidic groups per mole have been formed. Under similar conditions only one acidic group is liberated from cephalosporin *N* and benzylpenicillin.

Cephalosporin *C* is not inactivated by penicillinase from *B. subtilis*, strain 569[3]. It loses activity, though much less rapidly than cephalosporin *N* or benzylpenicillin, in the presence of a crude preparation of penicillinase from *B. cereus* (*NRRL* 569). The inactivation is accompanied by the liberation of an acidic group and loss of absorption at 260 mμ. It appears to be caused by an enzyme other than penicillinase which is present in the preparation. The ratio (cephalosporinase activity/penicillinase activity) was much lower with highly purified penicillinase[4] from *B. cereus* than with the crude material.

On hydrolysis with acid, cephalosporin *C*, like cephalosporin *N*, yields one mole of carbon dioxide and D-α-aminoadipic acid. The product obtained by allowing cephalosporin *C* to react with 1 : 2 : 4-fluorodinitrobenzene yields, on hydrolysis, a substance which behaves like dinitrophenyl-α-aminoadipic acid on paper chromatograms. It therefore appears that cephalosporin *C* contains a residue of D-α-aminoadipic acid and that the α-amino group is free. The pK value of this group suggests that the α-carboxyl group is also free.

Hydrolysis of cephalosporin *C* with acid yields little, if any, penicillamine. However, the carbon skeleton of penicillamine appears to be present in the molecule. Hydrolysis of the product obtained from cephalosporin *C* by hydrogenolysis with Raney nickel yields, in addition to α-aminoadipic acid, an amino-acid which behaves like valine on paper chromatograms.

Cephalosporin *C* is less active than cephalosporin *N* against most of the bacteria tested, although the two compounds show similar activities against a strain of *Bact. coli*. Against *Staph. aureus* and *Salm. typhi*, cephalosporin *C* shows an activity[5] of 8–10 units per mgm.

In 1951 a strain of *Cephalosporium* isolated in the United States was reported to produce an antibiotic called synnematin[6]. Abraham, Newton, Crawford, Burton and Hale[7] directed attention to the possibility that synnematin was identical with cephalosporin *N*. Synnematin was later separated into two components called synnematin *A* and synnematin *B*, the former being apparently less soluble in methanol than the latter[8]. The behaviour of crude synnematin *B* on paper chromatograms buffered with sodium citrate, pH 5·5, and run with methanol[6] appears to be very similar to that of cephalosporin *N* (R_F = 0·44). However, the reported behaviour of crude synnematin *A* under these conditions (R_F = 0) is different from that of cephalosporin *C* (R_F = 0·34).

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