LETTERS TO THE EDITORS

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Isolation of Penicillaminic Acid and D-α-Aminoadipic Acid from Cephalosporin *N*

CEPHALOSPORIN *N*, an antibiotic produced by a species of *Cephalosporium*, was recently reported to be a new type of penicillin. The physical properties of this substance indicated that it contained a basic group as well as acidic groups[1]. The purest preparation of cephalosporin *N* so far obtained, which appears from analysis of a counter-current distribution curve to be at least 50 per cent homogeneous, has now been found to yield D-α-aminoadipic acid on hydrolysis. While L-α-aminoadipic acid is known to occur in Nature[2,3], the D-isomer does not appear to have been isolated previously from a natural source.

The preparation of cephalosporin *N* was hydrolysed in *N* hydrochloric acid at 100° for six hours, and the hydrolysate was separated by electrophoresis into neutral and acidic fractions.

The neutral fraction was oxidized with bromine water and the acidic material formed was again separated by electrophoresis. On treatment with charcoal and crystallization from aqueous acetone this material yielded colourless prisms of penicillaminic acid (ββ-dimethylcysteic acid) (found: C, 30·6; H, 6·0; N, 6·9 per cent; $C_5H_{11}O_5NS$ requires: C, 30·7; H, 5·6; N, 7·1 per cent).

The acidic fraction contained an amino-acid which behaved on two-dimensional paper chromatograms like α-aminoadipic acid. This amino-acid was obtained in a homogeneous state by chromatography on a column of 'Amberlite *XE*-59', eluting with 0·2 *M* ammonium acetate buffer, *p*H 5[4]. It crystallized from water, melting point 205–207°, $[\alpha]_D^{20} = -26°$ ($c = 1$ in 6 *N* HCl) (found: C, 44·2; H, 7·0; N, 9·0; $C_6H_{11}O_4N$ requires: C, 44·7; H, 6·9; N, 8·7).

This substance showed no depression in melting point on mixing with a sample of authentic D-α-aminoadipic acid. Electrometric titration showed the presence of two acidic groups of *pK* 2·2 and 4·4 respectively and a basic group of *pK* 9·8. An X-ray powder photograph was kindly taken by Dr. D. Hodgkin, who reported that it was identical with a photograph of authentic D-α-aminoadipic acid. Oxidation with chloramine-*T*[5] and addition of 2 : 4-dinitrophenyl hydrazine to the resulting solution yielded an acidic 2 : 4-dinitrophenylhydrazone, melting point 130–131°, which appeared to be derived from γ-aldehydo-*n*-butyric acid (found: C, 44·8; H, 4·2; N, 18·4 per cent; $C_{11}H_{12}O_6N_4$ requires C, 44·5; H, 4·7; N, 18·9 per cent). The same compound was formed on oxidation of DL-α-aminoadipic acid.

The preparation of cephalosporin *N* contains a basic group of *pK* 9·8 and forms a dinitrophenyl derivative with fluorodinitrobenzene. This derivative yields a compound on hydrolysis which behaves like DNP-α-aminoadipic acid on paper chromatograms. It is therefore concluded that the α-amino group of α-aminoadipic acid is free in the active material. Since the *pK* values of the basic group in this material and in the amino-acid are very similar, it appears likely that α-aminoadipic acid is linked to the rest of the molecule through its δ-carboxyl group.

The evidence so far available is not incompatible with the assumption that cephalosporin *N* is an α-aminoadipyl derivative of the essential core of the penicillin molecule. A compound of this structure would be expected to have the physical properties of the antibiotic. The existence of glutamyl and aspartyl derivatives is also possible. Traces of glutamic acid and aspartic acid were liberated from the purest preparation of cephalosporin *N* on hydrolysis with *N* hydrochloric acid at 100°, and larger amounts from cruder preparations.

The presence of a free amino group obviously makes it possible to prepare derivatives of cephalosporin *N* by reactions which cannot be applied to benzylpenicillin. It is of interest that the DNP-derivative, which, unlike the parent substance, can be extracted into ether or ethyl acetate, has a higher activity than the latter against *Staph. aureus* and a much lower activity against *Salm. typhi*.

We are indebted to Dr. J. P. Greenstein for a sample of synthetic D-α-aminoadipic acid, to Dr. Borsook for a sample of L-α-aminoadipic acid, and to Drs. Fones, Gaudry and Work for samples of DL-α-aminoadipic acid.

G. G. F. NEWTON
E. P. ABRAHAM
Sir William Dunn School of Pathology,
University of Oxford. Aug. 1.

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A Colorimetric Method, based on Metallic Complex Formation, for the Detection of Aureomycin in Presence of Amino-Acids and Proteins

EXPERIMENTS involving the feeding of aureomycin to animals are now so common that it would be an advantage to have a rapid and reliable chemical method for its detection in body fluids and similar media. This is the more desirable since microbiological methods of assay are not easy to carry out because of the instability of the antibiotic in ordinary bacteriological media, even at 25°[1,2]. There is one remarkable chemical property of aureomycin, apparently not yet recorded in the literature, which might be made use of to this end, namely, its ability in alkaline solution to form stable, yellow complexes, soluble in *n*-butanol, with certain divalent cations only, notably Mg, Ca, Co, Ni, Cu and Sr. Zn, Cd, Mn, Ba, Hg and Pb do not form stable coloured complexes. Aureomycin alone gives, of course, a yellow colour with excess of ammonia or caustic soda, but this fades quite quickly and irreversibly at 30°, a purple fluorescence developing in its place. Shaking with *n*-butanol then extracts no colour.

Many divalent cations also form stable complexes with amino-acids[3], and hence their colours (if any) with aureomycin may not be stable in bacteriological media. It happens, however, that the affinity of aureomycin for Ca^{++} and Co^{++} is so great that this limitation is scarcely felt with these cations. Thus, to demonstrate the calcium or cobalt colours in