THE OCCURRENCE OF DEHYDROBUTYRINE IN STENDOMYCIN

Sir:

Isolation and properties of the antifungal antibiotic stendomycin were described by THOMPSON and HUGHES[1]. In a recent communication[2] in this Journal the fatty acid constituents of the antibiotic were reported. We present here the results of experiments which show that a dehydrobutyryl residue occurs in the molecule of stendomycin.

Acid hydrolysis (6 N HCl, 110°C, 16 hours) of stendomycin liberates 1.4 moles of ammonia. Since no aspartyl, glutamyl or carbamoyl residues are present, a labile amino acid had to be aussumed as the source of ammonia. Moreover the antibiotic exhibits an ultraviolet absorption spectrum which could not be correlated with the already recognized amino acid constituents. This spectrum shows no distinct bands but a rather general absorption is found, weak at 260 mμ and gradually increasing with decreasing wavelength. E.g. at 240 mμ the specific absorption coefficient (in alcohol) is about 44. Considering the molecular weight of about 1,600 for stendomycin* the molar absorption coefficient is about 7,000. On hydrolysis the u. v. absorption disappears. These data would be in harmony with the presence of a dehydro-amino acid residue in the sequence, therefore a search for the keto acid which on hydrolysis should be produced from such a residue, was undertaken.

Stendomycin (3.0 g) was dissolved in 6 N hydrochloric acid (100 ml) and the solution was heated under reflux in an atmosphere of nitrogen. After 22 hours the mixture was cooled, diluted with water (200 ml) and filtered to remove the fatty acids[2]. The filtrate was treated with a 0.43 % solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid (300 ml). Two hours later the yellow precipitate was collected, washed with 2 N hydrochloric acid, with water and dried. Two hundred forty five mg of a 2,4-dinitrophenylhydrazone (m.p. 197~198°C (dec.)) were obtained. On acidification of the solution of this compound in sodium bicarbonate a purified product, m. p. 199~200°C (dec.), was recovered. The n.m.r. spectrum of the 2,4-dinitrophenylhydrazone in deuterated acetic acid (CD_3COOD) exhibited in addition to the peaks of the aromatic protons of the 2,4-dinitrophenyl group, a triplet (3 protons) at δ 0.83 and a quartet (2 protons) δ 2.3. This spectrum corresponds to the 2,4-dinitrophenylhydrazone of α-keto butyric acid. A comparison (mixed m. p., thin layer chromatograpy, i. r. and n.m.r. spectra) with an authentic sample (m. p. 200~201°C, lit.[3] 198°C) confirmed this identification and led to the conclusion that stendomycin contains a dehydrobutyrine residue.

Hydrogenation of stendomycin in ethanol in the presence of a palladium on charcoal catalyst yielded a product in which most of the u. v. absorption of the parent antibiotic was absent. Analysis[4] of an acid hydrolysate of this material (dihydrostendomycin) gave, besides the amino acids present in stendomycin, also one mole of butyrine (α-amino butyric acid); most of the original ammonia was found to be absent. The presence of butyrine in the hydrolysate was confirmed also by thin layer chromatography.

To our best knowledge dehydrobutyrine so far has not yet been found in peptide antibiotics. However, dehydroalanine was reported as a constituent of nisin[5], dehydrotryptophan was found in telomycin[6] and the albonoursins are diketopiperazines[7] from dehydroamino acids.

This work was supported by a National Institute for Health Grant No. AI 07515-01.

* From the values of amino acid analyses the molecular weight of about 1,600 can be calculated for stendomycin. However, the name stendomycin denotes here a family of antibiotics. Individual members of this family differ from each other in respect of their fatty acid constituents (cf. ref. 2). Furthermore, some members contain leucine and there is evidence that to a minor extent dehydrobutyrine is replaced by dehydroalanine in some of these closely related structures.

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(Received August 21, 1967)

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