AMINO ACID CONSTITUENTS OF STENDOMYCIN

Sir:

Isolation and properties of the antifungal antibiotic stendomycin were described by THOMPSON and HUGHES[1]. The fatty acid constituents and the occurence of a dehydrobutyrine moiety in the molecule were reported from this Laboratory[2,3]. The present communication deals with the amino acid constituents of stendomycin*.

Quantitative amino acid analysis[4] of acid hydrolysates of the antibiotic revealed the presence of allothreonine (2 moles), serine (1 mole), glycine (1 mole), alanine (1 mole), proline (1 mole), valine (3 moles), alloisoleucine (*ca* 1.3 moles), leucine (*ca* 0.1 mole), ammonia (1 mole cf. ref. 3) and of a basic component (1 mole), emerging shortly after ammonia. The identity of alloisoleucine was confirmed by epimerization with boiling acetic anhydride: this led to the emergence of isoleucine with a concomittant decrease in the amount of alloisoleucine in the mixture. On paper chromatograms a fast moving ninhydrin positive spot was found (cf. ref. 1) originating from a mixture of two dipeptides: valylvaline and (valine, alloisoleucine). Obviously the conditions of hydrolysis applied for these analyses were not vigorous enough to break all peptide bonds. On prolonged hydrolysis with constant boiling hydrochloric acid at 110°C, gradually additional amounts of valine and alloisoleucine were released as shown in Table 1. The analytical data of Table 1 can be best interpreted by assuming that the majority of the stendomycin molecules contain one alloisoleucine and four valine residues, while in a minor part one of the valines is replaced by alloisoleucine or leucine.

The identity of the amino acid constituents of the antibiotic was established by comparisons of the hydrolysis products and of their 2,4-dinitrophenyl derivatives on paper chromatograms and thin layer chromatograms with authentic samples and also through the elution volumes in the quantitative amino acid analysis[4]. For final confirmation, a larger sample of the antibiotic was hydrolysed and the hydrolysis products separated by ion exchange chromotography followed by countercurrent distribution**. The amino acid components thus isolated were compared with authentic samples, this time with the application of i.r. and n.m.r. spectra. For allothreonine, serine, valine and alloisoleucine analytical values (C, H, N, neutr. equiv.),

Table 1. Amino acid content of stendomycin hydrolysates*

Amino acid	Duration of hydrolysis in hours					
	20	64	90	160	400	740
Allothreonine	1.87	1.76	1.70	1.67	1.27	0.86
Serine	0.89	0.77	0.70	0.69	0.27	0.10
Proline	1.00	0.92	0.92	0.95	0.95	0.93
Glycine	0.97	1.00	0.99	0.99	1.02	1.05
Alanine	1.00	1.00	1.01	1.01	1.04	1.02
Valine	2.79	3.28	3.40	3.61	3.84	3.38
Alloisoleucine	1.22	1.40	1.46	1.46	1.46	1.35
Leucine	0.10	0.12	0.11	0.14	0.12	0.11
NH_3	1.3	1.1	1.5	1.9	2.5	2.6
Basic component	1.00	0.94	**	**	**	**

* 1.7 mg samples of stendomycin salicylate (cf. ref. 1) were hydrolyzed with constant boiling hydrochloric acid at 110°C in evacuated sealed ampoules.

** On prolonged hydrolysis the basic component gradually decomposed and methylamine appeared.

* In the first report on stendomycin (ref. 1) two varieties of this antibiotic were described. According to our recent studies the difference between stendomycins A and B is limited to the anion which accompanies the single but strong cationic center of the antibiotic, the substituted guanidino group of the basic component. This does not mean that the name stendomycin refers to a single homogeneous compound. There are at least five groups of stendomycins, each group containing a different fatty acid. Within each group new subdivisions are created, *e. g.* by the presence or absence of leucine. Careful and continued efforts to separate individual members of the stendomycin family failed. The antibiotic produced intractable emulsions in many of the solvent systems in which countercurrent distribution was attempted. Moreover, in most of the solvent systems the distribution coefficients were markedly concentration dependent. For these reasons our investigations were carried out on a reasonably pure (constant amino acid analysis, single spot on thin layer chromatograms) but not homogeneous material.

** *E. g.* serine and allothreonine were separated by countercurrent distribution in the system N-butanol-0.01 N hydrochloric acid, through 6,600 transfers.

in excellent agreement with the calculated ones, were obtained. The specific rotation of the isolated amino acids was also determined. The basic component, first isolated by ion exchange chromatography and countercurrent distribution as the dihydrochloride, could also be obtained as the diflavianate by direct crystallization from the mixture of amino acids in the hydrolysate. This new amino acid, according to the data of elemental analysis and n. m. r. spectra is a N,N'-dimethyl derivative of 2-iminohexahydro-4-pyrimidyl-glycine, the "cyclic arginine" which occurs in capreomycin[5].

During the separation of serine and allothreonine by countercurrent distribution, a compound giving a faint ninhydrin color was observed to accompany allothreonine. Crystallization from alcohol yielded pure allo-D-threonine. Evaporation of the mother liquors and sublimation of the residue *in vacuo* gave N-methyl-L-threonine[6], $[\alpha]_D^{25}$ $-17°$ (c 2, 5 N HCl) identified through its n.m.r. spectra and analytical data inculuding N-methyl determination and neutralization equivalents. Due to its poor color yield with ninhydrin, this amino acid is just noticeable on STEIN MOORE chromatograms[4]; it emerges near to the position of aspartic acid.

While our studies on the basic constituent of stendomycin are still in progress, and will be reported separately, its more conventional amino acids are now sufficiently known to point to the marked similarity between the amino acid compositions of stendomycin, fortuitin[7], and peptidolipin N. A.[8]. A noteworthy feature of stendomycin is the unusually high incidence of amino acids of the D configuration: the alloisoleucine, allothreonine, alanine, and all but one of the valine residues, belong to the D series. The proline, serine, N-methyl threonine and, according to the CLOUGH-LUTZ-JIRGENSON rule the bacic component, are L amino acids.

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