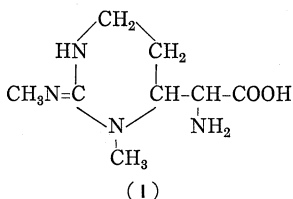


THE STRUCTURE OF STENDOMYCIDINE

Sir:

In an earlier communication¹⁾ a basic amino acid constituent of the antifungal peptide antibiotic stendomycin²⁾ was already briefly mentioned as an N,N'-dimethyl derivative of 2-imino-hexahydro-4-pyrimidyl-glycine and hence related to capreomycin, a basic amino acid from capreomycin³⁾. In this communication the name "stendomycin" and structure I are proposed for the basic amino acid from stendomycin.



A sample of the antibiotic was hydrolyzed with constant boiling hydrochloric acid under reflux in an atmosphere of nitrogen for 60 hours. After removal of the fatty acids⁴⁾ by extraction with hexane and evaporation to dryness, the residual mixture of amino acid hydrochlorides was chromatographed on an ion-exchange column (Dowex 50W-X12 4.5 cm × 40.6 cm, in H⁺ cycle). For elution hydrochloric acid, in gradually increasing concentration, was used. Stendomycin emerged near the end of the elution procedure, when the concentration of the hydrochloric acid was raised to 6 N. The material thus separated was still somewhat contaminated with alioisoleucine. Final purification was achieved by countercurrent distribution (30 transfers) in a system of *n*-butanol-0.001 N HCl (1:1). The dihydrochloride was secured by evaporation of the solvents from the contents of tubes No. 0~5 as a crystalline, but somewhat hygroscopic material. On paper chromatograms and thin-layer chromatograms developed with butanol-acetic acid-water (3:1:1) it gave a single ninhydrin-positive spot (R_f=0.2).

Calc'd for C₈H₁₆N₄O₂·2HCl:

C 35.2, H 6.6, N 20.5, Cl 26.0

Found: C 34.9, H 6.9, N 20.3, Cl 26.1

Addition of a solution of flavianic acid in water to an aqueous solution of the dihydrochloride converted it to the sparingly soluble diflavianate, which crystallized in well formed plates.

Calc'd for C₂₈H₂₈N₈O₁₈S₂:

C 40.6, H 3.4, N 13.5, S 7.7

Found: C 40.3, H 3.6, N 13.7, S 7.7

Subsequently it was found that stendomycin diflavianate can be isolated in pure form directly from the mixture of amino acid hydrochlorides (after the removal of the fatty acids and hydrochloric acid) by the addition of excess flavianic acid to their aqueous solution.

While stendomycin gives a strong reaction with ninhydrin, treatment with nitrous acid yielded a ninhydrin-negative product. Strong alkalis liberated methylamine; the same amine appeared also on prolonged treatment of stendomycin with ammonia*. From these observations, from the values of elemental analysis including N-methyl determinations and mainly from the NMR spectrum (Fig. 1) structure I could be proposed for stendomycin.

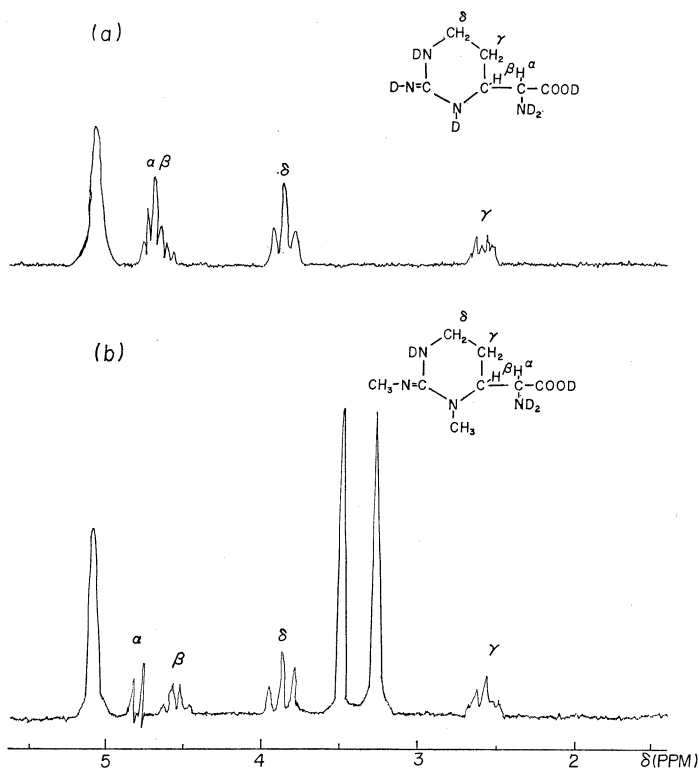
The position of the N-methyl groups could be assigned as shown on Fig. 1 through exclusion of the α-amino group (ninhydrin reaction, positive before and negative after deamination) and by the chemical shifts (3.89 ppm with external TMS as standard) of the protons on the δ-carbon. This value was found to be identical with the one determined on a sample of capreomycin³⁾ received from the Lilly Research Laboratories. Observations made on model compounds show that the chemical shift of protons on a carbon next to a nitrogen atom is changed to lower δ values when a hydrogen on the nitrogen atom is substituted by a methyl group. Therefore, in stendomycin no methyl group is present on the nitrogen adjacent to the δ-carbon.

Stendomycin, while probably derived biogenetically from arginine, has two centers of asymmetry. According to the CLOUGH-LUTZ-JIRGENSONS rule⁵⁾ the α-carbon atom has the L-configuration; this was supported by the ORD spectrum⁶⁾ of the copper complex of stendomycin.

Determination of the configuration of the β-carbon atom is more complex. The

* The exchange of CH₃-N= with NH= on the action of ammonia was observed by Dr. ICHIRO MURAMATSU, presently in this laboratory.

Fig. 1. NMR spectra (100 Mc)
 (a) Capreomycidine dihydrochloride and (b) stendomycidine
 dihydrochloride in D₂O (external TMS as standard).



chemical shift at this center is influenced not only by the relative configuration of the α - and β -carbon atoms, but also by the methyl group on the nitrogen adjoining the β -carbon. On the other hand, the protons on the γ - and δ -carbon atoms appear at 2.56 ppm and 3.89 ppm, respectively, both in stendomycidine and in capreomycidine (*cf.* Fig. 1). The chemical shifts of the protons on the δ -carbon should be independent of the configuration at the β -carbon and also from the presence or absence of a methyl group on the nitrogen adjacent to the β -carbon. The chemical shift on the γ -carbon, however, is a function** of the stereochemistry at the β -center. Hence, the relative stereochemistry at the β - and α -carbons in stendomycidine should be the same as in capreomycidine***.

** The influence of the relative stereochemistry at the α - and β -carbons of diasymmetric amino acids on the chemical shifts of protons on the γ -carbon is shown in threonine and allothreonine (1.33 ppm and 1.28 ppm, respectively, in D₂O with 1% DSS as standard).

*** According to T. TAKITA & K. MAEDA (J. Antibiotics, Ser. A 21 : 512~516, 1968), dihydroviomycidine is the diastereomer of capreomycidine. Their NMR spectra are different. The absolute chirality of capreomycidine has been established by B. W. BYCROFT and his associates at the University of Nottingham, England (personal communication from Dr. BYCROFT).

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