THE CONFIGURATION OF STENDOMYCIDINE

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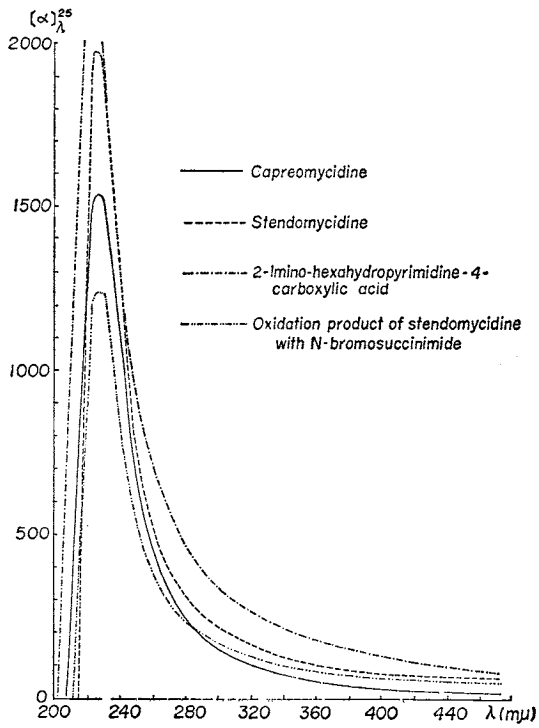
The absolute configuration of stendomycidine (I), a constituent of the peptide antibiotic stendomycin, was determined. Optical rotatory dispersion (ORD) measurements showed the configuration at the α-carbon atom to be that of an L-amino acid. Degradation of stendomycidine with N-bromosuccinimide yielded 2-methylimino-3-methyl-hexahydropyrimidine-4-carboxylic acid (II), a compound with only one center of asymmetry. A comparison of the ORD spectrum of compound II with that of 2-imino-hexahydropyrimidine-4-carboxylic acid (III), prepared from authentic L-α,γ-diaminobutyric acid, showed that the second center of asymmetry also has the L-configuration. Hence, the L-*erythro* configuration was assigned to stendomycidine. Comparison of the nmr spectra of stendomycidine and capreomycidine added some additional support to the above assignment.

In a previous communication[1], structure I was proposed for stendomycidine, a constituent of the peptide antibiotic stendomycin[2]. Because at that time the configuration of I was not firmly established, further studies had to be undertaken; their results are discussed in the present paper.

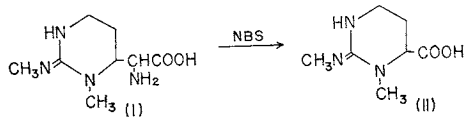
In 1965, JENNINGS, KLYNE, and SCOPES[3] proved that the absolute configuration of α-amino acids could be established from the optical rotatory dispersion measured at low wavelengths. At about 225 mμ (in 0.5 N HCl) L-amino acids exhibit a positive, D-amino acids a negative COTTON effect. The question whether or not this rule can be extended to amino acids with two centers of asymmetry was in part already settled by HAUSMANN, BORDERS, and LANCASTER[4], who demonstrated its validity using

Fig. 1. The ORD spectra of capreomycidine, stendomycidine, 2-imino-hexahydropyrimidine-4-carboxylic acid, and of the product of oxidation of stendomycidine with N-bromosuccinimide; all in 0.5 N HCl. The circular dichroism spectra of these compounds were in harmony with the ORD spectra.

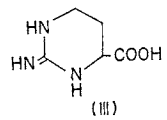


threonines as examples. For the present study, additional comfirmation was obtained through the ORD spectra of the diasymmetric amino acids L-isoleucine, D-alloisoleucine, N-methyl-L-threonine, and N-methyl-L-allothreonine. Under the same conditions stendomycidine (I) showed a strong positive COTTON effect with a peak at 227 mμ (Fig. 1); therefore, the L-configuration had to be assigned to its α-carbon atom.

In order to establish the configuration at the β-carbon, stendomycidine was oxidized with N-bromosuccinimide[5] to yield 2-methylimino-3-methyl-hexahydropyrimidine-4-carboxylic acid (II). The ORD spectrum of this compound



showed a positive COTTON effect with a peak at 219 mμ (Fig. 1). Because compound II is not an amino acid, it seemed necessary to prepare a model compound with known stereochemistry and establish the sense of the COTTON effect as a function of configuration. For this purpose 2-imino-hexahydropyrimidine-4-carboxylic acid (III) was synthesized



from L-glutamic acid via L-α-amino-γ-guanidino-butyric acid in several steps as described by RUDINGER, PODUSKA, and ZAORAL[6]. Compound III (in 0.5 N HCl) gave a positive COTTON effect as shown in Fig. 1. From this it follows that compound II also has the L-configuration. Since the β-carbon atom of stendomycidine is not involved in the reaction with N-bromosuccinimide, the stereochemistry of compound II must correspond to that of the β-carbon atom in compound I. Therefore, stendomycidine is an L-*erythro* amino acid (Fig. 2).

The chemical shifts of the protons on the γ and δ carbon atoms are virtually the same in compounds I and IV (Fig. 3). The chemical shifts of the α and β protons

Fig. 2. The absolute configuration of stendomycidine in FISCHER projection.

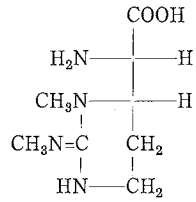


Fig. 3. The NMR spectra of (a) stendomycidine monohydrochloride and (b) capreomycidine monohydrochloride in D_2O.

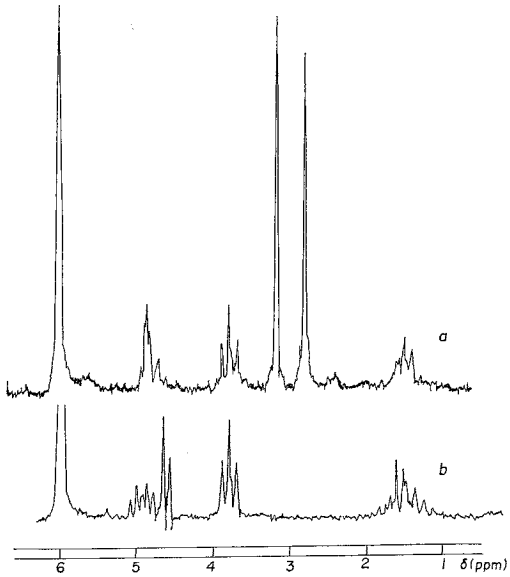
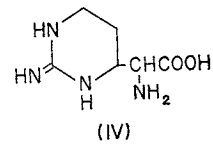


Table 1. Chemical shifts and coupling constants of stendomycidine (Ste) and capreomycidine (Cap) in D_2O.

		Ste·2HCl	Cap·2HCl	Ste·HCl	Cap·HCl
60 Mc	J_α	5 cps	···	···	5 cps
	$\delta_{\gamma\delta}$	1.30 ppm	1.30 ppm	1.35 ppm	1.35 ppm
100 Mc	J_α	5.5 cps	···	···	5 cps
	$\delta_{\gamma\delta}$	1.33 ppm	1.32 ppm	1.35 ppm	1.35 ppm


(IV)

(in the dihydrochlorides) show less similarity, but this is not unexpected[1] since in compound I a methyl substituent is present on a neighboring nitrogen atom. Previous work on diastereomeric N-methyl threonines[7] and α,β-diaminobutyric acids[4] showed that there is a marked difference in the nmr spectra, *e.g.*, the chemical shifts, of the diastereoisomers. Moreover, the coupling constants of the protons on the α-carbon atom are also affected. HAUSMANN and his associates[4] have attributed the effect on the coupling constant to a difference in the dihedral angles between the α and β protons. This angle is a consequence of the preferred conformation, which is due to repulsion of identically charged groups.

The coupling constant of the α proton in compound I is the same as that of the corresponding proton in compound III and this adds further support to the above proposed identities in their relative stereochemistry. The nmr data are summarized in Table 1. These details add further support to our contention[1] that compound I and IV have the same relative stereochemistry*.

Experimental

Instruments. Optical rotatory dispersion and circular dichroism spectra were run on a Cary Model 60 spectropolarimeter in 1 cm quartz cells at a concentration of 0.1 % in 0.5 N HCl. NMR spectra were obtained on a Varian Model A 60-A spectrometer in 2 N DCl unless otherwise stated. External TMS was used as a standard.

Stendomycidine monohydrochloride. A sample of stendomycidine dihydrochloride[1] (69.6 mg) was dissolved in 95 % ethanol (1.5 ml). Pyridine was added to the solution until it was neutral to pH indicator paper. Absolute ethanol (1.5 ml) was then added and the solution placed in the cold at 4°C overnight. The white crystals which formed were filtered and washed with two 5 ml portions of absolute ethanol. After drying 57.6 mg of stendomycidine monohydrochloride was obtained. The material was recrystallized by dissolving in a minimum amount of water and adding a five-fold excess of absolute ethanol.

Anal.: Calc'd for $C_8H_{16}N_4O_2 \cdot HCl$: C 40.6, H 7.2, N 23.7.
Calc'd for $C_8H_{16}N_4O_2 \cdot HCl \cdot H_2O$: C 37.7, H 7.5, N 22.0.
Found: C 37.3, H 7.3, N 21.9.

2-Imino-hexahydropyrimidine-4-carboxylic acid[6]. α-Amino-γ-guanidinobutyric acid dihydrochloride[6] (314 mg) was dissolved in a small amount of water and applied to a column of Dowex 1-X 8 (OH^- cycle). The column was eluted with water. Fractions were collected until the eluant was no longer basic (approx. 50 ml). The solution was evaporated to 10 ml and allowed to stand for about a week at room temperapture. The crystals of III which formed were filtered and washed with a small amount of water. They were dried at 60°C at 0.05 mm; mp>300°C. $[\alpha]_D^{24}$ +23.6° (*c* 2, 5 N HCl). For nmr spectrum, cf. Fig. 4.

Anal.: Calc'd for $C_5H_9N_3$: C 41.9, H 6.3, N 29.4.
Found: C 41.8, H 6.3, N 29.3.

Oxidation of stendomycidine with N-bromosuccinimide. A solution of stendomycidine dihydrochloride (336 mg) in water (915 ml) was adjusted to pH 5.25 with 1 N NaOH.

* The absolute configuration of capreomycidine (IV) was established by B. W. BYCROFT, D. CAMERON, L. CROFT and A. JOHNSON (Chem. Comm., 1968 : 1301~1302).

Fig. 4. The NMR spectrum of 2-imino-hexahydropyrimidine-4-carboxylic acid.

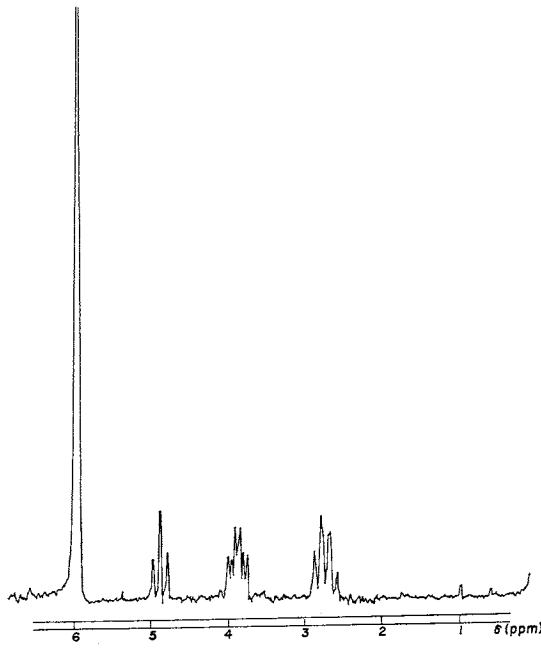
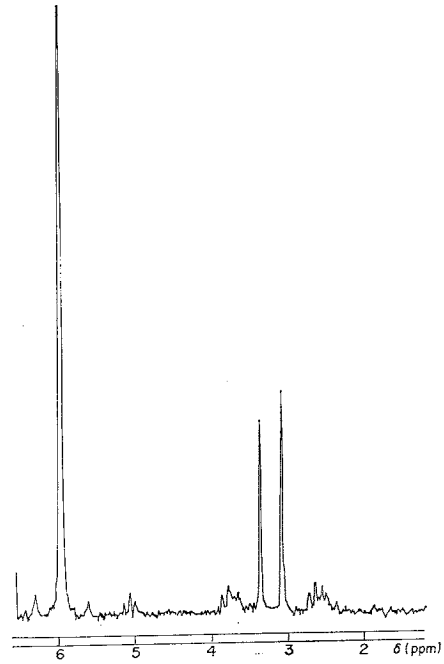


Fig. 5. The NMR spectrum of the N-bromosuccinimide oxidation product of stendomycidine.



N-Bromosuccinimide (865 mg) was added in small portions with vigorous stirring. The solution was stirred at room temperature for three more hours. The solution was extracted with chloroform to remove N-bromosuccinimide and succinimide. The solvent was evaporated and the residue redissolved in a methanol-chloroform mixture (1 : 4). The solution was applied to a column of silica gel (2.5 cm × 65 cm) equilibrated with the same solvent system which was also used initially for elution. The concentration of methanol was gradually increased. A total of 200 fractions of 6 ml was collected. The fractions were scanned by thin-layer chromatography on silica-gel with methanol-chloroform (1 : 1) as the mobile phase; spots were detected with *t*-butyl hypochlorite-starch-iodide[9]. Three bands were detected and the corresponding fractions, 62~95, 125~155, and 156~180 were pooled. The nmr spectra showed that the desired material (II) was present in fractions 156~180 (eluted with methanol). The material recovered from these fractions was not analytically pure and was characterized only through its nmr spectrum (Fig. 5), which is in harmony with the spectrum expected for 2-methylimino-3-methyl hexahydropyrimidine-4-carboxylic acid (hydrochloride). The ORD spectrum of the degradation product is shown in Fig. 1.

Acknowledgements

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