ON THE N-METHYL-L-THREONINE RESIDUE IN STENDOMYCIN

Sir:

In a previous communication[1] to this Journal, the isolation of N-methyl-L-threonine from acid hydrolysates of stendomycin[2~4] was briefly mentioned. We wish to report here some experiments that led to the final assignment of the configuration for this amino acid with two centers of asymmetry.

The specific rotation of the N-methyl-amino acid from stendomycin was in good agreement with the values reported[5] for N-methyl-L-threonine. This agreement, however, could not be considered as conclusive evidence for their identity, especially because the rotation of the optically active forms of N-methylallothreonine was not known from literature, where only properties of N-methyl-DL-allothreonine have been published[6]. It seemed possible that the specific rotations of the optically active N-methylallothreonines are not sufficiently different from the rotations in the normal series to permit the assignment of configuration solely on this basis. N-Methyl-L-threonine is more levorotatory than L-threonine which represents an exception to the rule[7] that in the L-series the N-methyl derivatives have more positive rotation than the amino acids from which they are derived. The contribution from the second center of asymmetry (the β carbon atom) could be the reason for this discrepancy[7], which cautions against the complete reliance in the assignment of configuration on a comparison with only one of the diastereoisomers.

In order to ascertain the correctness of the above assignment a sample of authentic N-methyl-L-threonine was prepared from L-threonine through the N-benzyl-, and benzylmethyl derivatives[8]. A sample of N-methyl-L-allothreonine was obtained through the application of the same procedure to L-allothreonine which in turn was secured by the epimerization method of ELLIOTT[9]. The optical rotations of the products, summarized in Table 1, show that the methylamino acid in stendomycin is indeed N-methyl-L-threonine.

The nmr spectra of N-methyl-L-threonine and N-methyl-L-allothreonine (Fig. 1) are different especially in respect to the chemical shifts of the doublets corresponding to the C-methyl groups. It is interesting to note that a mixture of equal amounts of N-methylthreonine and N-methylallothreonine exhibits an nmr spectrum which is different from those of its components. The formation of a molecular compound is probably the best explanation for this observation. The nmr spectrum of the natural compound is identical with that of authentic N-methyl-L-threonine.

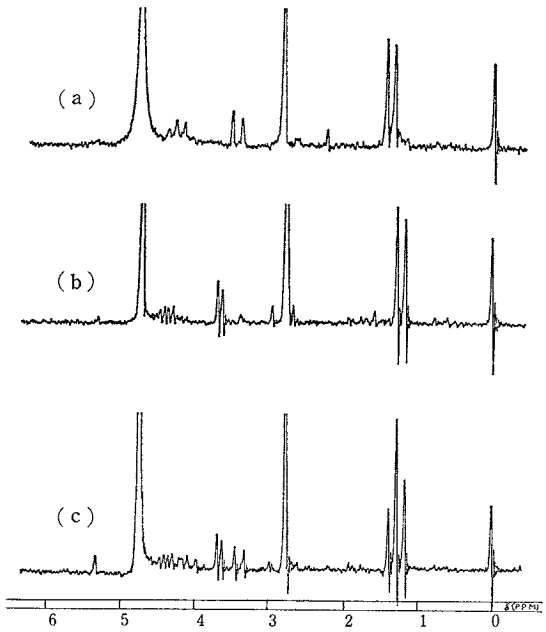
In the quantitative amino acid analysis of the hydrolysate of stendomycin according to the procedure of SPACKMAN, STEIN and MOORE[10], N-methylthreonine appears close to the position of aspartic acid but with an extremely low ninhydrin color yield. In these chromatograms N-methylallothreonine and N-methylthreonine cannot be distinguished from each other. Since in the recordings of the amino acid analyses often a small doublet was observed, some doubt existed about the nature of the second material revealed at the aspartic acid position. It could be a trace of aspartic acid formed as a secondary degradation product, *e.g.*, by the oxidation of proline, but the possibility of the presence of N-methylallothreonine (in addition to N-methyl-L-threonine) could not be excluded. Since stendomycin contains two D-allothreonine residues, this problem had to be investigated.

Table 1. Optical rotations of diastereomeric threonines and N-methylthreonines

Amino acid	$[\alpha]_D^{24}$ (c 2, H_2O)	$[\alpha]_D^{24}$ (c 2, 5 N HCl)
L-Threonine	−28.4°	−15.0°
L-Allothreonine	+10.0°	+31.8°
NMe-L-Threonine (synth.)	−33°	−16°
NMe-L-Threonine (natural)	−30°	−17°
NMe-L-Allothreonine	+ 5°	+16°

The values of specific rotations of threonine and allothreonine are quoted from literature (ref. 7, pages 2238~2239). For N-methyl-L-threonine, IZUMIYA (ref. 6) reported $[\alpha]_D = -35°$ in H_2O and EBATA *et al.*, (ref. 5) $[\alpha]_D = -15.9°$ in 5 N HCl.

Fig. 1. NMR spectra of (a) N-methyl-L-threonine, (b) N-methyl-L-allothreonine, (c) mixture of N-methyl-L-threonine and N-methyl-allothreonine. All spectra at 60 MHz with D_2O as solvent (1% DSS used as standard)



Stendomycin (10 g) was hydrolysed with constant boiling hydrochloric acid at reflux temperature in an atmosphere of nitrogen for 60 hours. After removal of the hydrochloric acid by evaporation and of the fatty acids[3] by extraction with hexane the resulting mixture of amino acid hydrochlorides was chromatographed on a column of cation exchange resin (Dowex 50W-X12 4.5 cm × 40.6 cm). Dilute hydrochloric acid in gradually increasing concentration was used for elution. The first major peak appeared when the concentration of the hydrochloric acid reached 0.36 N. The weight of the hydrochlorides (3.5 g) isolated from this first peak indicated that in addition to the allothreonine (2 moles), serine and N-methyl-L-threonine residues (calcd. for the hydrochlorides 3.5 g) no other major component is present in the mixture. The latter was treated with N_2O_3 in order to deaminate* serine and allothreonine. The N-methylamino acids which remained unchanged in this reaction, were separated from the hydroxy acids by counter-current distribution (40 transfers) in the system methylisobutylketone-0.01 N hydrochloric acid. The nmr spectrum of the N-methylamino acid containing fraction was found to be identical with the one shown under (a) in Fig. 1. Moreover, the spectrum clearly revealed the absence of N-methylallothreonine.

These experiments allow the conclusion that stendomycin contains an N-methyl-L-threonine residue and no N-methylallothreonine**.

This study was supported by the National Institutes of Health Grant No. AI 07515.

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(Received October 21, 1968)

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* For deamination, N_2O_3 was generated from sodium nitrite and hydrochloric acid. The gas was transported with a stream of nitrogen into the reaction vessel containing the amino acid hydrochlorides in 3N hydrochloric acid.

** The fact that the N-methylthreonine residue has the L configuration is in harmony with the general observation (ref. 11) that, in microbial peptides, the diasymmetric amino acids belonging to the normal series have the L configuration while those belonging to the allo series are D-amino acids.

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