

THE CONFIGURATION OF THE
ALLOTHREONINE RESIDUE
IN THE ANTIBIOTIC
LL-A-0341

Sir:

In previous communications¹⁾ we have commented that L-isoleucine and D-alloisoleucine are found as constituents of peptide antibiotics and, with one notable exception²⁾, no D-isoleucine or L-allothreonine has been noted. These observations led us to the "rule of alpha epimerization" and to conclude that L-amino acids are the origin of all D-amino acids in peptide antibiotics¹⁾. It can be expected that this rule might be less valid for threonine and allothreonine residues. The hydroxyl group in these amino acids could allow epimerization on the β -carbon atom through oxidation followed by stereospecific reduction. D-Threonine has been reported rarely in microbial products³⁾, and L-configuration has been tentatively assigned⁴⁾ to the allothreonine in telomycin⁵⁾. The configuration of an allothreonine residue in the telomycin-related antibiotic LL-A-0341 has not been determined⁶⁾. We have been able to secure this missing, and for us not insignificant, piece of information*.

A sample (48 mg) of LL-A-0341-B was hydrolyzed with 6 N HCl (2 ml) in an evacuated, sealed ampoule at 115°C for 24 hours. The hydrolysate was evaporated and the residue chromatographed on a column (1.5×22 cm) of Dowex 50×12 cation-exchange resin⁷⁾. Dilute HCl, first 0.18 N (255 ml), and then 0.36 N (400 ml), was used for elution; 3-ml fractions were collected and examined on thin-layers of silica gel in the system butanol - butanone - diethylamine - water (10:10:2:5)⁸⁾. Allothreonine, together with serine and threonine, was detected in fractions No. 83~85 and in less contaminated form in fractions 85~90. The contents of these latter fractions were chromatographed on tlc in the system used

for detection. The allothreonine thus isolated was repurified by adsorption on a short column of Dowex 50×12 resin and eluted with 0.36 N HCl. Quantitative amino acid analysis⁸⁾ verified the absence of other amino acids. The optical rotatory dispersion curve of the purified material in 0.5 N HCl (Carey 60 Spectropolarimeter, 0.5 cm cell) exhibits a negative COTTON effect, with a minimum at 227 nm. This clearly demonstrates⁹⁾ that the isolated allothreonine has the D-configuration**. Earlier studies¹⁰⁾ proved that, in threonine and allothreonine, this COTTON effect due to the carbonyl chromophore is independent of the configuration at the β -carbon atom.

The D-configuration of the allothreonine in LL-A-0341-B suggests that it originates from L-threonine and supports the "rule of alpha epimerization"¹⁾. It seems rather likely that the observation made with LL-A-0341-B can be extended to the very similar telomycin as well.

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AGNES BODANSZKY
MIKLOS BODANSZKY

Department of Chemistry,
Case Western Reserve University,
Cleveland, Ohio, U.S.A.

KATO L. PERLMAN
D. PERLMAN

School of Pharmacy,
University of Wisconsin,
Madison, Wisconsin, U.S.A.

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** By the same method, the 3-hydroxyproline and β -hydroxyisoleucine residues of LL-A-0341-B were shown to have the L-configuration.

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