

Communications to the editor

ON THE STRUCTURE OF A NEW
ANTIBIOTIC TL-119

(STUDIES ON ANTIBIOTICS FROM
THE GENUS *BACILLUS*. VI)

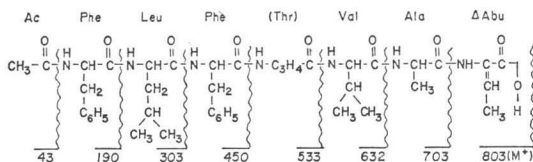
Sir:

TL-119 is a peptide antibiotic produced by a strain of *Bacillus subtilis*. An empirical formula of $C_{42}H_{87}N_7O_9$ and the presence of threonine (1), alanine (1), valine (1), leucine (1) and phenylalanine (2) have already been reported.¹⁾ In this communication, we report on the structure elucidation carried out chiefly by mass spectrometry.

To verify the suspected presence of additional residues indicated by mass spectral data, TL-119 was hydrogenated in the presence of platinum oxide in acetic acid and the hydrogenated product was hydrolyzed with hydrochloric acid by the manner described in the preceding paper.¹⁾ When the hydrolyzate was analyzed by an automatic amino acid analyzer, an additional amino acid, α -aminobutyric acid, was found in equimolar ratio to the other amino acid residues. Moreover, when the ethereal extract of acid hydrolyzate of the intact antibiotic was methylated and analyzed by gas chromatography-mass spectrometry, methyl α -ketobutyrate was found. This confirmed the presence of one residue of α -aminodehydrobutyric acid in the antibiotic that could not be recovered by the usual acid hydrolysis. Strong end absorption in the uv spectrum¹⁾ of this antibiotic can be explained by this dehydroamino acid residue.²⁾ The occurrence of this amino acid in a peptide antibiotic was first reported in stendomycin.²⁾

From the mass spectrum* of TL-119, the following sequence was deduced (Fig. 1). In

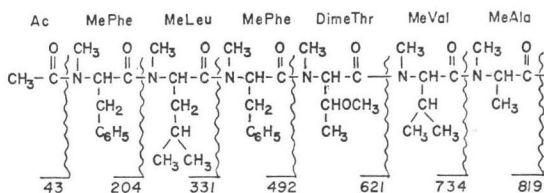
Fig. 1. Fragmentation pattern of TL-119



* Mass spectrum was measured by Hitachi RMU-6 mass spectrometer through direct inlet system at 250°C. Ionization voltage was 70 eV.

this interpretation, we assumed that dehydration at the threonine residue took place during mass spectral fragmentation to give an ion m/e 533 (relative abundance: 27%). The presence of the threonine residue as well as other residues in this order was also supported by the mass spectrum of the permethylated product of the antibiotic (Fig. 2). However, no information about the C-terminal group was obtained with the permethylated product.

Fig. 2. Fragmentation pattern of permethylated TL-119.



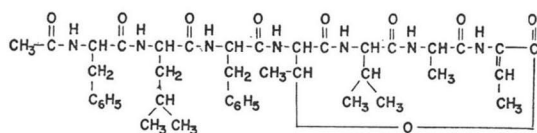
The N-acetylated terminal of TL-119 is compatible with the fact that this antibiotic is a neutral substance and negative to ninhydrin reaction.¹⁾

A lactone linkage between the C-terminal amino acid, α -aminodehydrobutyric acid residue, and the hydroxy group of the threonine residue can be suggested to be present in the intact antibiotic.

In peptide lactone antibiotics,³⁾ the carbonyl stretching bands of lactone linkages are located in the neighborhood of 1740 cm^{-1} generally, but it is observed at 1723 cm^{-1} in TL-119.¹⁾ The slight shift to lower wavenumbers is explainable by the conjugated carbonyl system of the α -aminodehydrobutyric acid residue.

When TL-119 was treated with 0.1N NaOH in 50% aqueous methanol, it was converted into an acid, TL-119 acid, of which infrared spectrum shows an absorption at 1695 cm^{-1} . The mass spectrum shows an ion at m/e 803 ($M-H_2O$). The acid was esterified with diazomethane to give TL-119 acid methyl ester. The infrared spectrum of this compound shows a peak at 1728 cm^{-1} , and the mass spectrum indicates molecular ion peak at m/e

Fig. 3. Postulated structure of TL-119.



835. Chromic acid oxidation with the acid completely destroyed the threonine residue, which was confirmed by automatic amino acid analysis with the hydrolyzate of the oxidized product. On the other hand, chromic acid oxidation did not have any effect on the threonine residue of the intact antibiotic.

The possibility that the hydroxy group of the threonine and the C-terminal carboxyl group might be linked with other unknown residues is ruled out by the empirical formula $C_{42}H_{57}N_7O_9$, deduced from elemental analytical data,¹⁾ and the mass spectral molecular ion mass number (m/e 803).

Based on our studies, we conclude that the structure of TL-119, without the stereochemistry of the amino acid residues, is as shown in Fig. 3.

YUZO NAKAGAWA
TSUYAKO NAKAZAWA
JUN'ICHI SHOJI

Shionogi Research Laboratory,
Shionogi & Co., Ltd.
Fukushima-ku, Osaka 553
Japan

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