CONFORMATION AND ABSOLUTE CONFIGURATION OF β-METHYLLANTHIONINE

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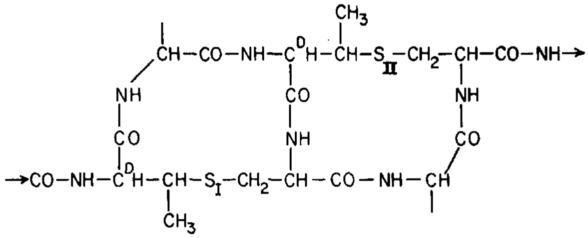
Summary. If the bicyclic peptide ring proposed by Gross et al. (1,2) does in fact exist in nisin and related antibiotics, then the unusual β-methyllanthionine component must be significantly distorted from its conformation in the free state, as determined by x-ray structure analysis. The torsion angles about the S-C_β bonds are 50-100° from the torsion angles in models of the sulfur-bridged peptide ring proposed for nisin. The chirality of the methylated β-carbon atom is (S). The conformation of the amino acid differs from that of meso-lanthionine only by a 180° rotation of a carboxyl group about the C^D_α-$C_\beta(CH_3)$ bond.

Nisin and subtilin, unsaturated peptides from Streptococcus lactis and Bacillus subtilis, each contain four residues of β-methyllanthionine and one residue of lanthionine (3,4). At least in the case of nisin, these residues function as sulfide bridges to form five loops in the peptide chain (1,2), three of which are the smallest heterodetic rings yet found in peptides. Two of these 13-atom rings share a peptide linkage in a bicyclic structure as shown.



The bridge-head α-carbon atom in each α-aminobutyric acid residue (aba) is reported to have the D configuration (2,5). We report an x-ray determination of the configuration of both α- and β-carbon atoms in the hydrolysis fragment β-methyllanthionine, aba-S-ala. A comparison is made of the fragment's conformation with that of meso-lanthionine (6), ala-S-ala, which is present in another ring in nisin, followed by a comparison with model nisin structures.

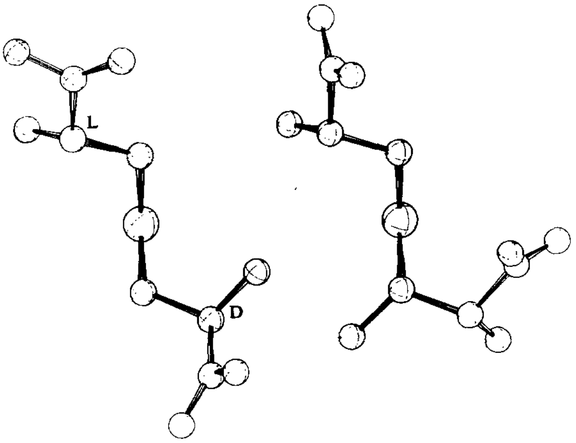
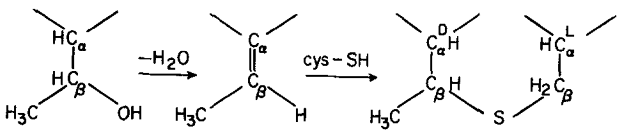


Fig. 1. Comparison of conformations of meso-lanthionine (left) and β-methyllanthionine (right). Hydrogen atoms are not shown. Letters indicate configuration of α-carbon atoms.

Results and Discussion. We have confirmed the assignment (2,5) of the D configuration to the α-carbon atom in the methylated half of the molecule, and find further that the chirality (8) about the methylated β-carbon atom is (S). Therefore one of the precursors of β-methyllanthionine, L(α), R(β)-threonine (9), must have undergone a change of configuration at both the α and β positions. Available evidence on the biosynthesis of β-methyllanthionine (9) suggests that this could occur during the addition of a sulfhydryl group from L-cysteine to the unsaturated intermediate β-methyldehydroalanine farther down the chain towards the amino terminus.



The two molecules in the asymmetric unit have torsion angles which are equivalent within 12°. This conformational similarity of the two molecules arises only in part from the similarity of their crystal environments. For if torsional angles are compared with those of the related amino acid meso-lanthionine (6) (Fig. 1 and Table 1), the added importance of intramolecular forces in determining the innermost torsion angles is suggested by the

Table 1. Selected torsional angles in *meso*-lanthionine, β-methyllanthionine, and peptide model (Fig. 2). Zero of angle is defined with front and rear bond superposed in projection down middle bond. Angle is positive if right-handed rotation of either front or rear bond is required for their superposition.

Torsion angle	Lanthionine	β-Methyllanthionine		Nisin Peptide	
		Molecule 1	Molecule 2	Bridge I	Bridge II
$C_\beta\text{-}SC_\beta\text{-}C^L_\alpha$	-85	-88	-86	110	150
$C_\beta\text{-}SC_\beta\text{-}C^D_\alpha$	-99	-98	-100	-170	-160
$S\text{-}C_\beta C^L_\alpha\text{-}C$	-178	-171	-176	170	180
$S\text{-}C_\beta C^D_\alpha\text{-}C$	-178	59	61	90	90
$CH_3\text{-}C_\beta C^D_\alpha\text{-}C$	--	-170	-170	180	180
$N\text{-}C^L_\alpha C\text{-}O$	33	-20	-8	140*	†
$N\text{-}C^D_\alpha C\text{-}O$	13	16	8	20	-140‡

* -40° if peptide nitrogen atom substituted for oxygen.
† variable
‡ 40° if peptide nitrogen atom substituted for oxygen.

equivalence in the two amino acids of the respective angles involving sulfur. At the ends of all three molecules, the torsions about the C_α-C bond depend more on local crystal forces.

A conformational study was made of the bicyclic nisin peptide proposed by Gross *et al.* (1,2). Of the 32 peptide models defined by the location of the five carbonyl oxygen atoms and two sulfur atoms on one side or the other of the bicyclic ring, all contained at least one S-C_β torsion angle which differs appreciably ($\sim$100°) from torsion angles observed in β-methyllanthionine. One of these models is shown in Fig. 2, and seven of its torsion angles can be contrasted with the angles seen in the amino acid (Table 1). It is not known how much of this change is due to electrostatic differences between zwitterion and peptide.

The activity of nisin has been correlated not with ion binding capacity, but rather with the presence in the antibiotic of three α,β

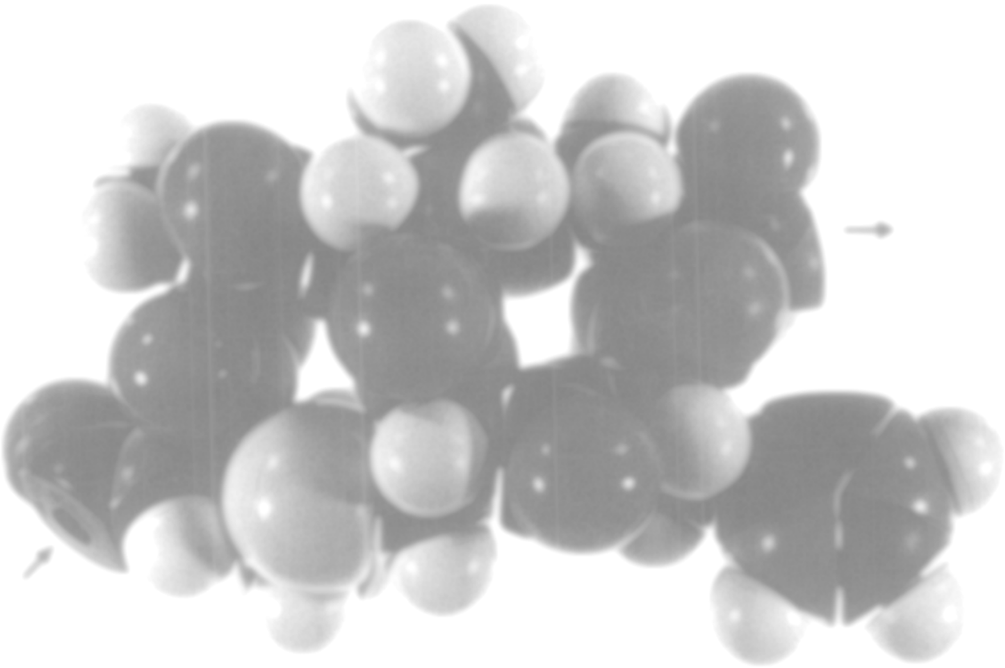


Fig. 2. CPK model of a conformer of the bicyclic peptide fragment (residues 23 to 28) proposed to exist in nisin. Approximate torsional angles measured from this model are given in Table 1. Chemical formula is shown in text.

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unsaturated side chains (10), which can intercept sulfhydryl compounds essential to the organism. In agreement with this, we find that in none of the 32 peptide models is it possible to visualize a bicyclic ionophore in which more than two carbonyl groups could form an acceptable coordination array around a cation.

Methods. 5 mg of β-methyllanthionine from subtilin was supplied by Dr. Gordon Alderton of the U. S. Department of Agriculture (5). Diffraction photographs showed a monoclinic unit cell with a = 11.12, b = 16.37, c = 6.78 Å and β = 91.1°. The space group is $P2_1$ with four molecules of β-methyllanthionine and eight molecules of water in the cell. X-ray data were collected with a diffractometer using graphite monochromatized copper Kα radiation. 1567 intensities had background-corrected counts greater than three times the standard error in the background count. The structure was solved by direct methods using a multiple solution tangent formula procedure (7). After thirty cycles of refinement including the 225 reflections with $|E| \geq 1.5$, the phase set with an R(Karle) index (7) equal 0.23 was used to calculate an E map which revealed the two molecules. Absolute configuration was determined from the anomalous scattering of sulfur. The structure is being refined by least-squares methods, and the current residual is 0.112.

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