magnitude too small to account for the volume difference between the transition states and products of the maleic anhydride reactions.

Table II. Partial Molal Volumes of *endo*- and *exo*-5-Norbornene-2,3-dicarboxylic Anhydride at Infinite Dilution

Adduct	Solvent: Nitromethane	Solvent: Dichloromethane
endo, cc/g mol	126.9 ± 0.4	123.7 ± 0.4
exo, cc/g mol	127.4 ± 0.4	124.1 ± 0.4

According to Hoffmann and Woodward,[9] the general rule for preferential formation of *endo* Diels–Alder adducts can be explained by secondary π-electron interactions at nonbonding atoms. Such a concept may also explain the abnormally small volumes of the transition states for the maleic anhydride reactions. For the acetylenic dienophile, with which secondary interactions cannot occur, the transition state is somewhat *larger* than the product, as expected.

In contrast with previous high-pressure studies, the results support a concerted four-center mechanism for the reactions examined.

Acknowledgment. The authors gratefully acknowledge the financial support of the U. S. Army Research Office—Durham. They also thank Professor Peter Beak for helpful discussion.

(9) R. Hoffmann and R. B. Woodward, *J. Amer. Chem. Soc.*, 87, 4388 (1965).

R. A. Grieger, C. A. Eckert
Department of Chemistry and Chemical Engineering
University of Illinois, Urbana, Illinois
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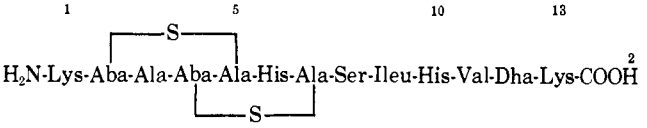
Nisin. The Assignment of Sulfide Bridges of β-Methyllanthionine to a Novel Bicyclic Structure of Identical Ring Size

Sir:

The presence of the rarely encountered amino acids lanthionine, β-methyllanthionine, dehydroalanine, and β-methyldehydroalanine in the antibiotic nisin[1] required a number of approaches new to the structural elucidation of peptides. Sulfide bridges of β-methyllanthionine had hitherto not been determined in peptides and, previously, it had not been necessary to distinguish between genuine alanine residues and those resulting from the desulfurization of β-methyllanthionine.

The antibiotically active tridecapeptide of the following structure was isolated from nisin after cleavage with



cyanogen bromide.

The peptide was degraded to the greatest possible extent prior to chemical modification of the sulfide bridges. Desulfurization was a prerequisite for the determination of the linear sequence. Conversion of the peptide to the tetrasulfonate and selective cleavage of the aminoacyl bond of serine were critical factors in the assignment of the sulfide bridges.

Chymotryptic digestion of the tridecapeptide liberated the tripeptide Val-Dha-Lys. Aminopeptidase cleaved the valyldehydroalanine bond and caused the release of pyruvyllysine.[3]

Carboxypeptidase A removed histidine and isoleucine from the decapeptide at nearly equal rates. The indication that histidine occupied the terminal position was confirmed by the tritium labeling technique.[4]

Edman degradation of the octapeptide removed lysine in the first cycle. Differential amino acid analysis of the peptide from the second cycle showed that none of the monoamino acids had been removed. It demonstrated, however, that one residue of β-methyllanthionine had been destroyed, presumably *via* β elimination since cysteine and cystine were present in the hydrolysate. The amino acid analysis after the third cycle did not indicate any additional change. The phenylthiocarbamyl derivative of the β-methyllanthionine residue will only cyclize, and subsequently decompose, under the more stringent conditions of hydrolysis.

Desulfurization of the octapeptide with Raney nickel W-6[5] was followed by five cycles of stepwise degradation providing the H_2N-terminal pentapeptide sequence. The positions of the remaining amino acids were established by a time study of their release by carboxypeptidase A.

The **original alanine residue** was assigned to position 3 following Edman degradation of the tetrasulfonic acid derivative of the undecapeptide amide. This amide is formed as the result of the loss of the terminal dipeptide due to the oxidation and decomposition of dehydroalanine during the preparation of the tetrasulfonate.

The tridecapeptide was oxidized with performic acid to form the bissulfone. Treatment of the bissulfone with 1 *N* sodium bisulfite solution for 7 hr at 105° resulted in β elimination and the addition of bisulfite to the newly formed α,β-unsaturated amino acids. Performic acid treatment converted the sulfinic acids to sulfonic acids.

The heptapeptide comprising residues 2–8 was esterified by treatment with 2.5 *N* methanolic hydrogen chloride for a period of 28 hr at room temperature. Complete release of serine methyl ester was demonstrated by amino acid analysis. The selective removal of the serine residue presumably proceeds *via* N→O acyl shift and transesterification. The resulting methyl ester was reduced to the alcohol by treatment with sodium borohydride in 0.5 *N* trisacetate buffer (pH 8.5) at 0° for 23 hr.

The peptide alcohol was allowed to react with phenylisothiocyanate and the addition product was hydrolyzed. The hydrolysate contained one residue each of alanine, histidine, and β-methyllanthioninol. In agreement with the earlier observation made during the attempted stepwise degradation of residue 2, the β-methyllanthionine residue was destroyed during hydrolysis. The absence of β-methyllanthionine from the

(1) E. Gross and J. L. Morell, *FEBS Lett.*, 2, 61 (1968).
(2) Aba = aminobutyric acid; Dha = dehydroalanine.
(3) Free dehydroalanine is not stable and decomposes with the formation of ammonia and pyruvic acid; *cf.* E. Gross and J. L. Morell (*J. Amer. Chem. Soc.*, 89, 2791 (1967)), for the formation of pyruvyllysine from nisin.
(4) H. Matsuo, Y. Fujimoto, and T. Tatsuno, *Biochem. Biophys. Res. Commun.*, 22, 69 (1966).
(5) H. R. Billick and H. Adkins, *Org. Syn.*, 3, 176 (1955).

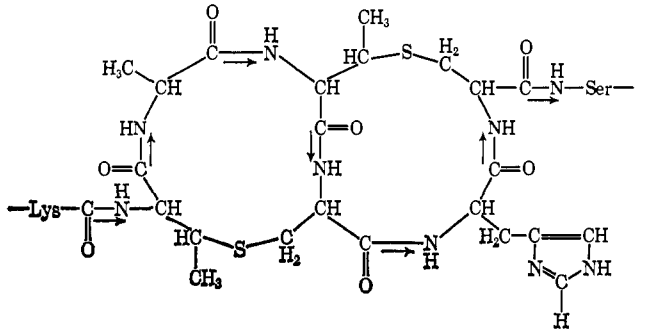
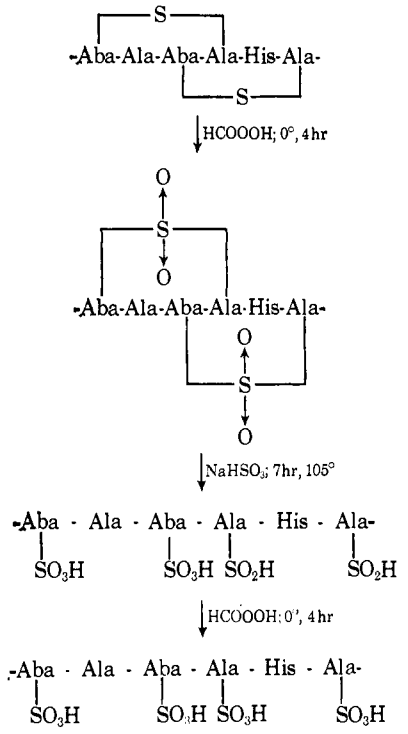


Figure 1. Bicyclic structure of identical ring size formed by sulfide bridges of β-methyllanthionine (arrows parallel to peptide bonds indicate the direction of the peptide chain).

hydrolysate rules out a sulfide bridge between residues 4 and 5. The **thioether linkages** may therefore be assigned unequivocally to residues 2 and 5 and 4 and 7, respectively.



In this way, a novel bicyclic structure of identical ring size has been assigned to nisin. Figure 1 traces the direction of the peptide chain of the bicyclic portion of the molecule as it is seen in the Stuart–Briegleb model. The two sulfur atoms constrain "head" and "tail" of the peptide chain to a center where four atoms are shared.

Thirteen-membered ring structures with one sulfur atom are new to peptide chemistry. Other heterodetic cyclic peptides with sulfur in the ring are of larger size.

Nisin has been found to be membrane active in experiments with lysosomes from which it releases enzymes. It shall be interesting to see whether this activity is associated with the unique ring size or whether it is due to the presence of other structurally unique features.

With the exception of residues 2 and 4, all α-carbon atoms possess the L configuration as determined with the amino acid oxidases and by the susceptibility of the peptides to reaction with amino peptidase and carboxypeptidase A. The configurations of the β-carbon atoms of the methyllanthionine residues are now being determined.

Erhard Gross, John L. Morell

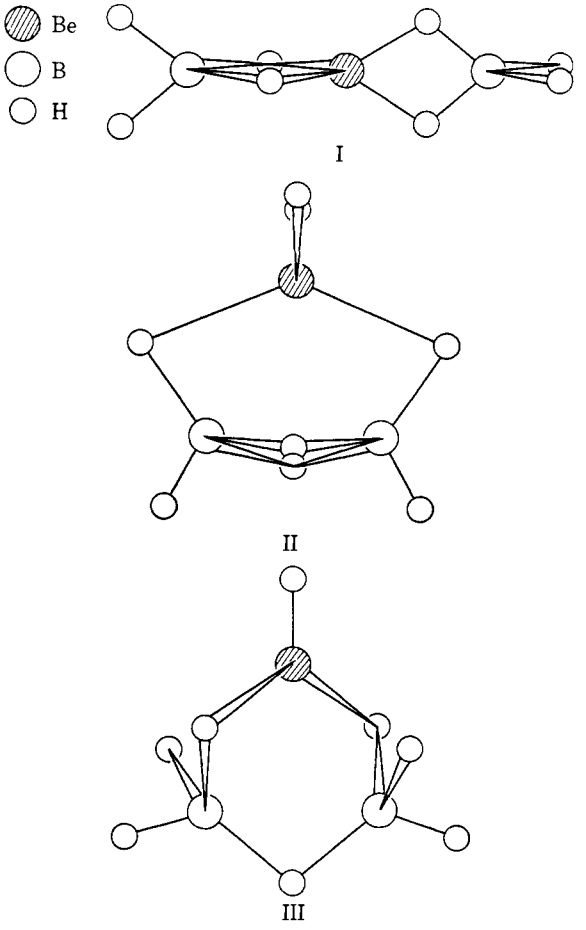
Section on Molecular Structure, Laboratory of Biomedical Sciences
National Institute of Child Health and Human Development
Bethesda, Maryland 20014

Received February 9, 1970

Electric Deflection and Dipole Moment of Beryllium Borohydride

Sir:

From electron diffraction experiments, several structures for BeB_2H_8 have been proposed, the most recent being the rather surprising models II and III.[1,2] In



fact, these latter models must now be questioned since very recent efforts to reproduce the diffraction data have not been successful.[3] Although the nonzero dipole moment reported by one of us[4] would seem to eliminate I and favor II or III, unfortunately the dielec-

(1) A. Almenningen, G. Gundersen, and A. Haaland, *Acta Chem. Scand.*, 22 859 (1968).
(2) G. Morgan and T. Cook, *J. Amer. Chem. Soc.*, **91**, 774 (1969).
(3) A. Almenningen, A. Haaland, and G. L. Morgan, University of Oslo, private communication; also, G. Gundersen and K. W. Hedberg, Oregon State University, unpublished results.
(4) J. W. Nibler and J. McNabb, *Chem. Commun.*, 134 (1969).