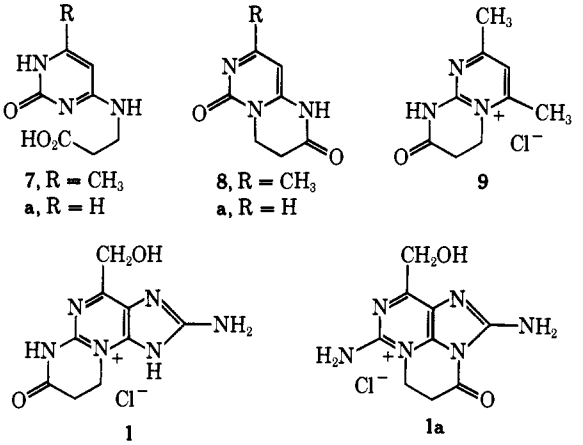
methylpurine (**2**). The synthetic purines were identical in all respects with the respective degradation products derived from saxitoxin.

Thus, the C_9 salt **1** can be envisaged as derived from quaternization and lactamization of the C_6 purine base **2** with a bridging propionyl unit. There exist ten isomeric attachments involving alternately the alkyl and acyl ends of the 3-carbon group at N-1, -2, -3, -7, -8, and -9. The problem became manageable when the lactam in **1** was cleaved in a mild phosphorus and hydriodic acid treatment to yield an oxopurinyl acid **5**: $C_9H_{11}N_5O_3 \cdot HCl$; uv (pH 1–6), 319 ($\epsilon$ 16,4000), 258 (7100), 222 (15,300); nmr δ 4.95 (t, 2, J = 7 Hz), 3.42 (t, 2, J = 7 Hz), 3.0 (s, 3). Since we have observed that a 9-alkyl-2,8-diaminopurine remains unchanged under the conditions leading to formation of **5**, we surmised that the 2-amino group in **1** was hydrolyzed to the oxo function because of a vicinal quaternary nitrogen.

A realistic spectral model for **5** is 8-amino-3,6-dimethyl-2-oxopurine (**5a**). Synthesis of the intermediate 4,5-diaminopyrimidine **6** was achieved following the pattern for the 6-hydrogen analog **6a**.[8] Ring closure with cyanogen bromide gave the 3-methylpurine model **5a**: uv (pH 1) 318 (ϵ 17,520), 256 (7920), 221 (14,420); pH 6, 321 (15,800), 251 (5620), 223 (15,760), practically identical with that of the degradation product **5**. Synthetic confirmation of the oxopurinyl acid as 8-amino-3-(2-carboxyethyl)-6-methyl-2-oxopurine hydrochloride (**5**) was made as follows: the rearrangement[9] of *N*-(1*H*-2-oxo-4-pyrimidinyl)-β-alanine (**7a**) to 1*H*-1,2,3,4-tetrahydro-2,6-dioxopyrimido[1,2-*c*]pyrimidine (**8a**) in acetic anhydride proceeded in excellent yield as did the rearrangement of the corresponding 6-methyl derivative, **7** → **8**. The lactam in **8** was opened in boiling water, and subsequent nitration, reduction, and cyclization with cyanogen bromide led to **5**, identical in all respects with the oxopurinyl acid derived from the C_9 compound **1**.

Synthesis of **5** therefore establishes the site of the propionyl alkyl terminal. Completion of the lactam ring may be *via* acylation of either N-2 or N-9, and the remaining structures for the C_9 hydrochloride are now funneled to **1** and **1a**. Titration of the C_9 salt **1** showed two acidic protons, pK_{a_1} 6.65 and pK_{a_2} 9.05. The first pK_a is close to that of 6.90 determined for the electronically similar pyrimidinium lactam **9**. The uv absorption of the C_9 salt also exhibited a two-step change corresponding to two deprotonations upon change in pH. The C_9 free base with one less acidic proton, $C_9H_{10}N_6O_2$, displayed its longest wavelength maximum at 337 (13,250) in methanol, shifted to 353 (12,830) upon addition of base, indicating the dissociation of the purine C-9 hydrogen.

Thus, the acidity and ultraviolet absorptions of the C_9 compound are singularly compatible with **1**, which accommodates two hydrogens dissociable below pH 12, whereas **1a** has only one hydrogen dissociable under these conditions. This structural assignment was further substantiated by another series of degradations of the C_9 compound **1**. When **1** was treated successively with diazomethane, alkali, and phosphorus and hydriodic acid, an *N*-methyl derivative of **3** was isolated;



δ 3.4 (s, 3), 3.0 (s, 3). Of the six possible *N*-methyl derivatives of **3**, 8-amino-6-methyl-2-methylaminopurine (**3a**) should derive from structure **1**. Primary synthesis of **3a** beginning with the known[10] 4-amino-2-chloro-6-methyl-5-nitropyrimidine and using conventional 8-aminopurine synthesis[11] yielded **3a**, identical with the degradation product and confirming the assignment of structure **1**[12] to the C_9 salt derived from saxitoxin.

(8) D. J. Brown, *J. Appl. Chem.*, **9**, 203 (1959).
(9) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1762, 1770 (1964).
(10) A. Albert, D. J. Brown, and H. C. S. Wood, *J. Chem. Soc.*, 3822 (1954).
(11) A. Albert and D. J. Brown, *ibid.*, 2060 (1954).
(12) This appears to be the first occurrence of the pyrimido[2,1-*b*]-purine ring system.
(13) Miller Research Fellow.
(14) National Institutes of Health Predoctoral Fellow.

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The Structure of Nisin

Sir:

We wish to report the structure of the peptide antibiotic nisin as shown in Figure 1.

Cleavage of nisin with cyanogen bromide yielded three fragments, 22–34 (I), the structure of which we reported earlier,[1] and two containing the amino acids of the sequence 1–21 (II) which differed only in the presence or absence of cleavage at methionine-17.

Peptides II were separately cleaved with trypsin (substrate–enzyme, 20:1, 0.2 *N* Tris hydrochloride buffer, pH 7.8, 0.01 *M* $CaCl_2$, 4 days, 25°). Unreacted peptide (less than 10%) and salts were separated from fragments by passing the acidified digest over a Sephadex G-25 column (0.2 *N* acetic acid). The tryptic fragments were separated by countercurrent distribution (*n*-butyl alcohol–water–acetic acid, 3:4:1) and identified by amino acid analysis to be the peptides comprising residues 1–12 (III) and 13–21 (IV), respectively.

Carboxypeptidase A reaction with the nonapeptide IV having the methionyl-glycyl bond intact liberated homoserine and asparagine at nearly equal rates. Esterification of the remaining peptide with methanol (1.0 *N* in hydrochloric acid, 4 hr, 25°) and reduction with sodium borohydride (0.25 *M*) in Tris acetate buffer

(1) E. Gross and J. L. Morell, *J. Amer. Chem. Soc.*, **92**, 2919 (1970).

(0.5 *N*, pH 8.5) at 0° for 18 hr resulted in the disappearance of β-methyllanthionine and the formation of one residue of β-methyllanthioninol.

Edman degradation showed the disappearance of β-methyllanthionine after the first cycle.[1]

Desulfurization with Raney nickel W-6[1] of **peptide IV** with the methionyl–glycyl bond intact and seven cycles of Edman degradation established the sequence shown.

Carboxypeptidase A treatment of the fragment with the bond 17–18 cleaved removed in the order of rate [HS,Leu],[2] Ala, Gly, as well as the originally exocyclic homoserine and asparagine. Edman degradation unequivocally established glycine and β-methyllanthionine to be H_2N termini. Use of the amino acid oxidases on hydrolysates of the desulfurized peptide showed that only α-aminobutyric acid is of the D configuration.

Hydrazinolysis of **peptide III** confirmed lysine to be in the COOH-terminal position. Edman degradation of peptide III removed isoleucine in the first cycle; however, no change in analysis was observed after the second cycle. Desulfurization with Raney nickel W-6 of peptide III before and after addition of methyl mercaptan to the unsaturated amino acids and ten steps of Edman degradation gave the sequence shown.

Mercaptoethylamine was added to the unsaturated amino acids and the COOH-terminal lysine residue removed by treatment with carboxypeptidase B. Subsequent esterification and reduction with sodium borohydride, under the conditions described for peptide IV, resulted in the disappearance of the residue of β-methyllanthionine and the formation of β-methyllanthioninol.

Two steps of Edman degradation successively removed isoleucine and **β-methylthialysine** while the third one caused disappearance of lanthionine after hydrolysis.[1] These results require the assignment of the β-methyllanthionine bridge between residues 8 and 11 and that of lanthionine between residues 3 and 5 or 3 and 7. The latter ambiguity was removed by fixing the position of dehydroalanine as residue 5.

The peptide consisting of residues 1–11 was treated with hydrochloric acid–glacial acetic acid at 110° for 10 min.[3] An aliquot was allowed to react with *o*-phenylenediamine[4] and after Edman degradation showed the loss of 1 residue of isoleucine and 0.5 residue of leucine.

The remainder of the peptide was reduced with Raney nickel W-6 and passed over a Dowex 50 column in the hydrogen form. The effluent (water) contained the peptide Pyr-Leu-Ala-Aba-Pro-Gly-Ala (V)[5] while the eluent (1.2 *M* *N*-ethylmorpholine) yielded the peptide Ileu-Aba-Ala-Ileu-NH_2 (VI).

These two peptides were useful in assigning the configurations of the α-carbon atoms of lanthionine as each contains one of the alanine residues generated by desulfurization.

The COOH-terminal alanine residue of peptide V was removed by treatment with carboxypeptidase A. Exposure of the hydrolysates of the remaining peptide and peptide VI to the amino acid oxidases showed the amino acids to be of the L configuration with the exception of alanine and aminobutyric acid in positions 3[6] and 8, respectively.

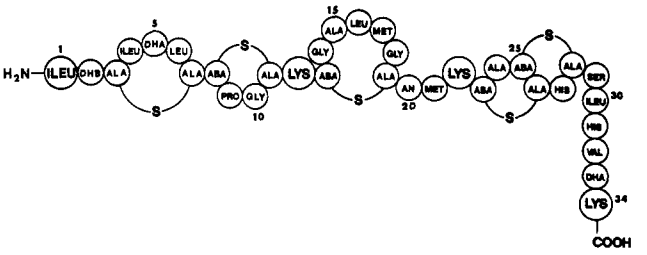

Figure 1. The structure of nisin: ABA = aminobutyric acid; DHA = dehydroalanine; DHB = dehydrobutyrine (β-methyldehydroalanine).

It is interesting to observe that the α-carbon atom of the first amino acid in lanthionine and β-methyllanthionine is always of the D configuration.

The correlation of the configurations of the β-carbon atoms of the four residues of β-methyllanthionine with threonine will be reported later.

With the elucidation of the structure of nisin, *sulfide bridges*, contributed by lanthionine and β-methyllanthionine, have been assigned for the first time and a pentacyclic heterodetic type of peptide—heretofore unknown—has been revealed. The unique bicyclic structure of the COOH-terminal fragment[1] consisting of rings with 13 members each is singular in the molecule. While there is present one additional 13-membered ring, none of its amino acids participates in a second cyclic structure. The two remaining rings are larger in size. They are comprised of 5- and 7-amino acids, respectively, and thus give rise to 16- and 22-membered rings.

Certain biological properties were predicted for nisin on the basis of structural considerations invoking the addition of essential sulfhydryl groups to the α,β-unsaturated amino acids and ion-binding capacity possible in view of the presence of the polycyclic structure. Indeed, the intact parent molecule as well as the H_2N-terminal fragment[1] display, for instance, these activities: antimalarial action,[7] release of lysosomal enzymes, and lysis of erythrocytes.[8]

Studies are in progress to further explore the biological activity of peptides with α,β-unsaturated amino acids and of those in which α,β-unsaturated amino acids and lanthionine and/or β-methyllanthionine are linked biosynthetically *via* amides and keto acids.[9]

(2) HS = homoserine.

(3) Under these conditions dehydrobutyrine is stable as shown by the recovery of aminobutyric acid after reduction with Raney nickel W-6 and the presence of β-methyl-*S*-benzylcysteine upon addition of benzyl mercaptan.

(4) H. B. T. Dixon and V. Moret, *Biochem. J.*, 94, 463 (1965).

(5) Pyr = pyruvyl.

(6) Lanthionine is subject to racemization *via* a mechanism as yet not understood. Amino acid analysis of the esterified and reduced ($NaBH_4$) peptide consisting of residues 1–11 showed 60% *meso*-lanthionine and 40% DL-lanthionine. L-Lanthionine prepared from L-chloroalanine and L-cysteine [(*cf.* G. B. Brown and V. du Vigneaud, *J. Biol. Chem.*, 140, 167 (1941)] when exposed to the conditions of standard acid hydrolysis was transformed to *meso*-lanthionine and DL-lanthionine at a ratio of 0.43:0.57.

(7) E. Gross and J. Morell, *J. Amer. Chem. Soc.*, 89, 2791 (1967).

(8) B. Mohit, J. van Brunt, and E. Gross, in preparation.

(9) E. Gross and J. L. Morell in "Peptides: Chemistry and Biochemistry," B. Weinstein and S. Lande, Ed., Marcel Dekker, New York, N. Y., 1970, p 389.

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