as the active oxidant in mixed-function oxygenases has recently been suggested.[15]

By analogy with transition metal complexes of ethylene and acetylene,[16,17] where there is considerable decrease in C–C bond order with concomitant increase in metal–carbon bonding, structures **1** and **2** can be regarded as different (and extreme) forms representing a π complex between oxygen and a metal atom. The metal in structure **1** will have a formal oxidation state of two in excess of that in structure **2**. Thus, complexes of transition metals with molecular oxygen are often considered to have the former structure.[18,19] Catalysis by metalloenzymes that mimic singlet oxygen may therefore involve complexes of type **1** or **2** as the "enzyme-bound singlet-oxygen." The oxygenation reactions of diperoxychromium(VI) oxide etherate show that the oxygen moiety in peroxy complexes of metals in their higher oxidation states can have considerable "singlet" character. The chemical activity of metal–oxygen complexes containing oxygen bonded to metal atoms in their lower oxidation states is being examined.

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Henry W.-S. Chan
University Chemical Laboratory
Cambridge, CB2 1EW, England
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Degradation of Saxitoxin to a Pyrimido[2,1-*b*]purine[1]

Sir:

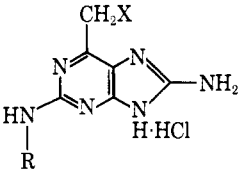
Paralytic shellfish poisoning is a severe form of food intoxication. The responsible toxic principle (LD_{50} 5–10 μg/kg (mouse), intraperitoneally) is saxitoxin, produced by the dinoflagellate *Gonyaulax catenella*[2] and accumulated in some otherwise edible species of shellfish. Previously,[3] we reported the isolation of 3-methyl-6,7-dihydro-5*H*-pyrrolo[1,2-*c*]pyrimidin-1-one from the reaction of saxitoxin with phosphorus and hydriodic acid. Saxitoxin has now been converted under a variety of mildly oxidative conditions to a crystalline product, $C_9H_{10}N_6O_2 \cdot HCl$ (**1**). This compound retains nine of the ten carbon atoms and six of the seven nitrogen atoms of saxitoxin.[3] We now wish to report the structure of this C_9 compound **1** and related degradation products.[4]

Saxitoxin, treated in dilute sodium hydroxide solution with 0.8% hydrogen peroxide at 25° followed by catalytic decomposition of excess peroxide and ion exchange chromatography, gave a 25% yield of crystalline salt **1**: $C_9H_{10}N_6O_2 \cdot HCl$; uv (pH 6) 324 ($\epsilon$ 21,000), 261 sh (4760), 236 (12,8000), 208 (22,650); nmr δ 5.23 (s, 2), 5.08 (t, 2, J = 7 Hz), 3.48 (t, 2, J = 7 Hz). These two triplets suggest the presence of a propionyl residue attached to an iminium nitrogen in **1**, *e.g.*, 1-(2-methoxycarbonylethyl)pyridinium bromide shows two triplets at δ 4.93 and 3.23 for its methylenes.

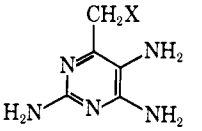
A 3-carbon residue was removed upon heating **1** in alkali, giving **2**, $C_6H_8N_6O \cdot HCl$: uv (pH 6) 308 (ϵ 10,720), 266 (7750), 228 (21,000), 206 (18,250); nmr δ 4.90 (s). The latter absorption is suggestive of the methylene singlet of benzyl alcohols. The C_6 product **2** was resistant to further acidic and alkaline hydrolytic conditions, indicating that the two guanidino groups[5] in saxitoxin probably are intact in the stable aromatic nucleus of **2**. Assuming a benzylic hydroxymethyl moiety in **2**, $C_5H_5N_6$ remains with which to construct a stable system containing two guanidines with no hydrogen attached to carbon. On this basis, the C_6 compound was assigned the probable structure 2,8-diamino-6-hydroxymethylpurine hydrochloride (**2**).

Reduction of **2** with phosphorus and hydriodic acid yielded the deoxy derivative, $C_6H_8N_6 \cdot HCl$, δ 2.51, formulated as 2,8-diamino-6-methylpurine hydrochloride (**3**). Synthesis of the deoxypurine **3** was accomplished in one step by condensing the known 6-methyl-2,4,5-triaminopyrimidine (**4a**) with cyanogen bromide.

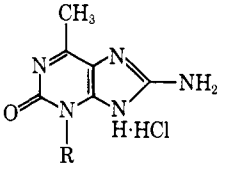
For the synthesis of **2**, the key intermediate was 6-hydroxymethyl-2,4,5-triaminopyrimidine (**4**) which was prepared *via* amination of 2,4-dichloro-6-methoxycarbonyl-5-nitropyrimidine,[6] followed by catalytic hydrogenation of the 5-nitro group and sodium borohydride reduction of the 6-ester function.[7] Condensation of **4** with cyanogen bromide gave 2,8-diamino-6-hydroxy-



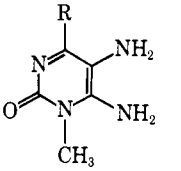
2, X = OH; R = H
3, X = R = H
a, X = H; R = CH_3



4, X = OH
a, X = H



5, R = CH_2CH_2COOH
a, R = CH_3



6, R = CH_3
a, R = H

(1) Supported in part by the U. S. Army Research Office, Durham, N. C.
(2) E. J. Schantz, J. M. Lynch, G. Vayvada, K. Matsumoto, and H. Rapoport, *Biochemistry*, **5**, 1191 (1966).
(3) W. Schuett and H. Rapoport, *J. Amer. Chem. Soc.*, **84**, 2266 (1962).
(4) Satisfactory elemental analyses and chromatographic and spectral characterization were obtained for all compounds mentioned in this communication; 60-MHz nmr spectra were taken in deuterium oxide with tetramethylsilane (δ = 0) as external standard; uv spectra [λ_{max} nm (ϵ)] were taken in water unless otherwise specified.
(5) A greater than 100 mol % yield of guanidine residues (as β-guanidinopropionic acid, guanidine, and **1**) has been obtained from the oxidation of saxitoxin (R. Oesterlin, Ph.D. Thesis, University of California, Berkeley). Details will be reported in a forthcoming publication.
(6) J. Clark and G. Ramage, *J. Chem. Soc.*, 2821 (1958).
(7) M. S. Brown and H. Rapoport, *J. Org. Chem.*, **28**, 3261 (1963).

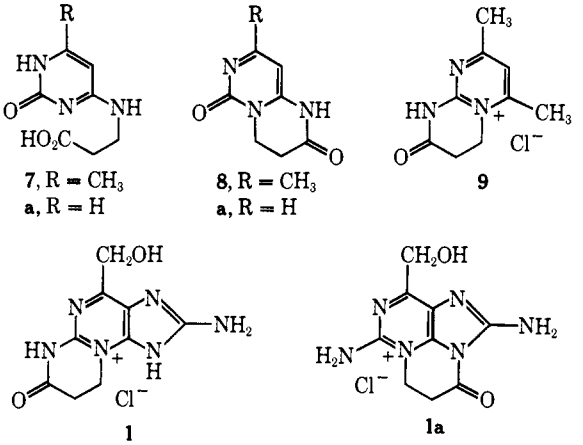
methylpurine (**2**). The synthetic purines were identical in all respects with the respective degradation products derived from saxitoxin.

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.

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(13) Miller Research Fellow.
(14) National Institutes of Health Predoctoral Fellow.

J. L. Wong, M. S. Brown,[13] K. Matsumoto[14]
R. Oesterlin,[14] H. Rapoport*
Department of Chemistry, University of California
Berkeley, California 94720
Received June 14, 1971

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

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