tive spin density on the ligand as observed.[13] Donation of ligand electrons into empty 7s, 7p, or 6d orbitals will also produce the correct sign but is less likely because of their relatively high energies. Exchange polarization of filled metal orbitals will also give the correct sign but the magnitudes calculated and observed for other lanthanide and actinide compounds[14] appear to be much smaller than observed in the present COT complexes. This interpretation is consistent with the Mössbauer isomer shift in $(COT)_2Np$[5] and does not disagree with the simple MO formulation presented earlier.[4,15] We hope that a more detailed model will result from further experimental and theoretical studies in progress.

Acknowledgment. We thank R. G. Hayes for helpful discussions and advice.

(13) J. Owen and J. H. M. Thornley, *Rep. Progr. Phys.*, **29**, 675 (1966).

(14) R. E. Watson and A. J. Freeman, *Phys. Rev. Lett.*, **6**, 277 (1961); J. M. Baker and W. B. J. Blake, *Phys. Lett. A*, **31**, 61 (1970); W. Kolbe and N. Edelstein, *Phys. Rev. B*, **4**, 2869 (1971); M. T. Eastman, H. G. Hecht, and W. B. Lewis, *J. Chem. Phys.*, **54**, 4141 (1971).

(15) The one-electron MO description in ref 4 should be regarded as one of the several configurations because of the mixing of f orbitals resulting from spin–orbit coupling.

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The Structure of Saxitoxin[1]

Sir:

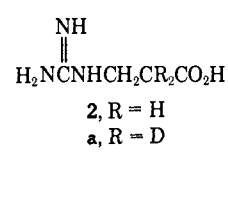
Saxitoxin, the powerful neurotoxin produced by the dinoflagellate *G. catenella*, has been degraded to a pyrimido[2,1-*b*]purine containing nine of the ten carbons and six of the seven nitrogens of the original toxin. Establishment of structure **1** for this key degradation product,[2] together with additional data now to be presented, allows us to formulate a complete structure for saxitoxin.

Determination of the molecular formula of saxitoxin has been difficult because of its noncrystalline, highly polar, nonvolatile nature.[3] Characterization of the pure toxin as the dihydrochloride, $C_{10}H_{17}N_7O_4 \cdot 2HCl$, has been reported;[4] however, we found that combustion analyses of saxitoxin, dried to constant weight at 110° (10^{-5} mm) (with no loss of biological activity) were consistent with $C_{10}H_{15}N_7O_3 \cdot 2HCl$. That 1 mol of water of solvation reconciles the difference in the two formulas was indicated by exchange with $H_2{}^{18}O$. Upon dissolution of saxitoxin in $H_2{}^{18}O$ followed by drying at room temperature, ^{18}O analysis[5] showed that one (98%) of the four oxygens was ^{18}O. Subjecting this sample to the more vigorous drying conditions left the molecule containing three oxygens and no ^{18}O. Also, since Dumas and Kjeldahl nitrogen determinations were identical, the toxin contains no N–N or N–O bonds.

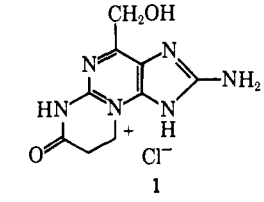
Oxidation of saxitoxin with 15% hydrogen peroxide at pH 4.6 yielded guanidine (0.8 mol), the pyrimidopurine **1** (0.15 mol), and 3-guanidinopropionic acid (**2**) (0.3 mol). Formation of 140 mol % of guanidine residues under these oxidizing conditions established the presence of two guanidine residues in the toxin and confirmed the absence of any N–N bonding.

Isotopic labeling was used to investigate the origin of the propionyl residue of **2**. Two methylene hydrogens (δ 2.37, m)[6] of saxitoxin underwent slow, reversible isotopic exchange in water at room temperature. When deuterium-exchanged toxin (no signal at 2.37) was oxidized using protium reagents as above, 3-guanidinopropionic-*2*-d_2 acid (**2a**) (δ 3.66, s) was obtained. Oxidation of nonexchanged saxitoxin with D_2O_2[7] generated **2** containing no deuterium. Thus, the propionyl moiety of **2** maintains its integrity throughout the oxidation of saxitoxin and must be present as such and bonded to the same guanidino group in the toxin.

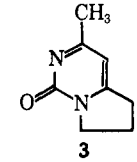
Further structurally definitive conclusions follow from the exchangeability of the α-CH_2 protons in the propionyl residue. Model compounds of the type $H_2NC(=NH)NHCH_2CH_2COR$ (R = aryl, alkyl) also showed exchange of the α-CH_2 protons in D_2O. However, the corresponding acids, esters, amides, and guanidides [R = OH, alkoxyl, NH_2, $NHC(=NH)NH_2$] failed to undergo H–D exchange even at elevated temperatures. The presence of a 2-guanidinoethyl alkyl ketone in saxitoxin thus is assured. The keto group accounts for the ir absorption in saxitoxin at 5.7 μ which disappeared upon reduction (H_2, PtO_2, or $NaBH_4$) to dihydrosaxitoxin. In dihydrosaxitoxin the methylene hydrogens were no longer exchangeable, and the Baeyer–Villiger type peroxide degradation (see below) did not occur.



2, R = H
a, R = D



1



3

(1) Supported in part by the U.S. Army Research Office, Durham, N. C.

(2) J. L. Wong, M. S. Brown, K. Matsumoto, R. Oesterlin, and H. Rapoport, *J. Amer. Chem. Soc.*, **93**, 4633 (1971).

(3) These properties precluded the use of mass spectrometry and X-ray crystallography. Also, suitable derivatives (salts or acyl, alkyl, or silyl derivatives) with properties amenable to these physical methods could not be prepared despite herculean efforts.

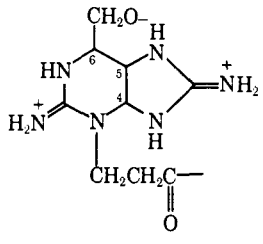
(4) E. J. Schantz, J. M. Lynch, G. Vayvada, K. Matsumoto, and H. Rapoport, *Biochemistry*, **5**, 1191 (1966).

(5) C. D. Snyder and H. Rapoport, *ibid.*, **7**, 2318 (1968).

(6) Nmr spectra were taken in D_2O using sodium trimethylpropanesulfonate as internal standard (δ 0) at 100 and 220 HMz.

(7) Procedure of P. Askenasy and R. Rose, *Z. Anorg. Allg. Chem.*, **189**, 27 (1930), using deuterium oxide.

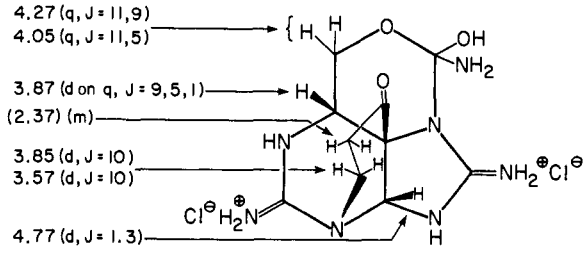
Mild alkaline oxidation of saxitoxin with 0.8% hydrogen peroxide at 25° led to the isolation in good yield and identification of the pyrimidopurine **1**[2] which contains all but one each of the original complement of carbon and nitrogen atoms. As important as the product itself is the manner in which **1** was formed. Saxitoxin was readily oxidized over the pH range 3–12 to yield **1**, consuming 1 mol of oxygen/mol of toxin when oxygen was used as the oxidant. The conclusion therefore can be drawn that saxitoxin contains a tetrahydropurine nucleus which gives rise to **1**, and this is consistent with the conversion of saxitoxin to the pyrrolopyrimidine **3** upon treatment with phosphorus and hydriodic acid.[8] Both **1** and **3** contain a 3,6-dialkylpyrimidine although they were generated in totally different environments. These data are now summarized in structure **4** which is



4

lacking only (a) the carbonyl terminus of the propionyl group[9] and (b) the single carbon and nitrogen atoms lost in the formation of **1**.

Since the propionyl residue is part of a ketone, the carbonyl must be bonded to C-4, -5, or -6. Analysis of the nmr spectra permits a unique assignment to C-5, based on the AMX type of splitting patterns for the C-6 H and the C-6 CH_2 as shown in structure **5**.



5

Also, the long-range 1.3-Hz diequatorial coupling between the C-4 and C-6 H's defines the cis ring fusion and the configuration at C-6 as shown.

Assignment of bonding to the remaining carbon and nitrogen must accommodate the facts that (a) the nitrogen must be at the ammonia level since there are no N–N or N–O bonds in the toxin, and (b) the carbon is at the oxidation level of carbon dioxide since all the C–H's in the nmr are accounted for and no further bonding to any of the carbons of **4** is possible. Therefore, this carbon and nitrogen must be bonded to each other, forming a carbamyl group.

(8) W. Schuett and H. Rapoport, *J. Amer. Chem. Soc.*, 84, 2266 (1962).

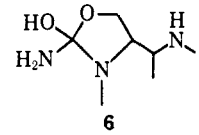
(9) The carbonyl terminus of the propionyl residue at exocyclic N-2 in the pyrimidopurine **1** is of no structural significance since we have found that the purine propionic acid exits in solution at pH 9 and above and lactamizes to N-2 upon neutralization and evaporation to dryness. Thus, structural conclusions can be drawn only from the intermediate propionic acid.

Definitive placement of this carbamyl group follows from a detailed pK study. Saxitoxin has two pK values, 8.24 and 11.60,[4] in water. When the solvent is changed to 20% ethanol, the first pK increases to 8.50, and in 50% ethanol, it becomes 9.05. This behavior of significant decreasing dissociation with decreasing polarity of the solvent is characteristic of proton dissociation from oxygen rather than from positively charged nitrogen.[10] Thus, saxitoxin must contain a hydroxyl group of pK 8.24, the remaining pK being accounted for by the guanidinium ions.

These observations are accommodated by bonding the carbamyl group to the oxymethyl at C-6 to form a carbamate which exists as a cyclol[11] *via* interaction with the NH at position 7. The resulting urea hemiacetal group provides the acidic hydroxyl. Thus saxitoxin has the structure **5**.

(10) E. Grunwald and B. J. Berkowitz, *J. Amer. Chem. Soc.*, 73, 4939 (1951); B. Gutbezahl and E. Grunwald, *ibid.*, 75, 559 (1953).

(11) Equilibrium between the cyclol **5** and the cyclol involving N-1 *via* the carbamate accounts for the interconvertible saxitoxin's A and B seen on countercurrent distribution which revert to a single form in acid.[12] A firm choice between cyclol **5** and cyclol **6** is not possible at this time, although **6** appears sterically less favorable.



6

(12) J. D. Mold, J. P. Bowden, D. W. Stanger, J. E. Maurer, J. M. Lynch, R. S. Wyler, E. J. Schantz, and B. Riegel, *J. Amer. Chem. Soc.*, 79, 5235 (1957).

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Transition Metal Catalyzed Valence Isomerizations. The Role of the Ligand[1]

Sir:

The transition metal catalyzed valence isomerizations of highly strained cyclobutane compounds have been shown to vary with the metal or metal complex utilized.[2] In such systems, Rh(I) complexes bring about cleavages of a cyclobutane to diolefin, whereas the noncomplexed Ag(I) and the complex $PdCl_2(C_6H_5CN)_2$ bring about cyclobutane–dicyclopropane isomerizations. Our initial study showed that the 1,1′-bishomocubane system **1** readily rearranged to the dicyclopropane isomer **2** in the presence of Ag(I).[3] Continued study of this strained system using transition metal complexes has shown that rearrangement to the dienes **3** and **4** can be achieved. For these rearrangements, *i.e.*, to **2** or to **3** and **4**, it has been found that both the combination of the polarizability and the σ donor–π acceptor ability of the ligands of such complexes as well as the σ electron acceptor ability of the

(1) This work was supported by the National Science Foundation, Grant No. GP-8700.

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(3) W. G. Dauben, M. G. Buzzolini, C. H. Schallhorn, and D. L. Whalen, *Tetrahedron Lett.*, 787 (1970); W. G. Dauben, C. H. Schallhorn, and D. L. Whalen, *J. Amer. Chem. Soc.*, 93, 1446 (1971).