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The Structure of a Crystalline Derivative of Saxitoxin. The Structure of Saxitoxin[1]

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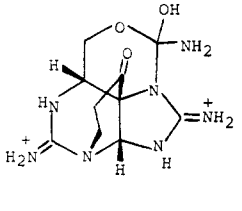
Abstract: The crystalline ethyl hemiketal of saxitoxin, the paralytic shellfish poison, was obtained, and its structure was deduced as **2b** by X-ray crystallography. Carbon-13 magnetic resonance studies demonstrate that saxitoxin in solution exists primarily as a carbamate rather than a cyclol, and that in aqueous solution, saxitoxin exists as the hydrate **2a**.

Saxitoxin, the paralytic shellfish poison, is one of the most toxic known compounds of low molecular weight. The toxin accumulates in normally edible bivalves such as *Saxidomus giganteus* and *Mytilus californianus* after they injest the marine dinoflagellate *Gonyaulax catenella*.[3] Oxidative degradation showed that saxitoxin hydrate dihydrochloride ($C_{10}H_{15}N_7O_3 \cdot 2HCl \cdot H_2O$) is a tetrahydropurine, and structure **1** was proposed on the basis of ^1H NMR and chemical evidence.[4]



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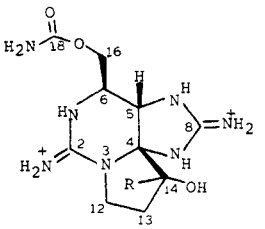


2a; R = OH
2b; R = OEt
2c; R = D

Saxitoxin dihydrochloride is an amorphous, hygroscopic material which has never been crystallized.[5] Despite numerous diverse efforts in the past, no crystalline derivative of saxitoxin was ever obtained.[6] We have now obtained a crystalline ethyl hemiketal dihydrochloride, the structure of which was deduced as **2b** by X-ray crystallography. On the basis of this evidence and ^{13}C NMR studies, we now assign structure **2a** to the hydrate of saxitoxin.

X-Ray Diffraction Analysis

Crystals of saxitoxin ethyl hemiketal dihydrochloride (**2b**) suitable for an X-ray analysis were mounted in glass capillary tubes to prevent the loss of solvate molecules. These crystals were surveyed and a 1 Å data set (maximum $\sin\theta/\lambda = 0.5$) was collected on a Syntex $P\bar{1}$ diffractometer equipped with a graphite monochromator and copper radiation (λ 1.5418 Å). All diffraction data were collected at room temperature. Details of the crystal survey and data collection parameters are summarized in Table I.

Atomic scattering factors for C, N, O, and Cl were taken from The International Tables for X-ray Crystallography.[7] Anomalous dispersion corrections for Cl were included.[8] The scattering factor for H is that given by Stewart, Davidson, and Simpson.[9] No corrections were made for absorption (μ = 32.2 cm^{-1}). Routine crystallographic calculations were facilitated by the CRYM crystallographic computer system.[10]

The weights used throughout the refinement were set equal to $1/\sigma^2(F_o^2)$. $\sigma^2(F_o^2)$ was based on the variance of the intensity calculated by the formula: $\sigma^2(I) = S + \alpha^2(B_1 + B_2) + (dS)^2$ where S is the total counts collected during the scan, B_1 and B_2 are the numbers of counts collected for each background, α is the scan time to total background time ratio, and d is an empirical constant of 0.02.

The phase problem was solved using a computer program developed at Oak Ridge National Laboratory.[11] This program facilitated a rather conventional phasing using direct methods, and the portion of the program utilizing transforms of a known molecular fragment was not used. After the intensities were reduced to normalized structure factor magnitudes, $|E|$, triples were selected which met these conditions: (a) their indices add to zero; (b) each had $|E| \geq 1.5$; and (c) the magnitude of the product[12]

$$A(\mathbf{h},\mathbf{k}) = \frac{^2\sigma_3}{\sigma_2^{3/2}} |E(-\mathbf{h})E(\mathbf{k})E(\mathbf{h}-\mathbf{k})|$$

was greater than 1.3.

For each of these triples the quantity[13]

$$D(\mathbf{h},\mathbf{k}) = \langle (|E(\mathbf{h}+\mathbf{l})|^2 - 1) || E(\mathbf{l})| \geq 1.5; |E(\mathbf{k}+\mathbf{l})| \geq 1.5 \rangle_{\mathbf{l}}$$

was computed. Only those triples with $D > 0$ were retained for manipulation. Phases were obtained by a multisolution approach which utilized $D(\mathbf{h},\mathbf{k})$ as the weight assigned to each triple in convergence mapping.[14] Phase extension and refinement were accomplished by a weighted tangent formula[15]

$$\tan\phi(h) = \frac{S}{C} = \frac{\langle w(\mathbf{h},\mathbf{k}) \sin[\phi(\mathbf{k}) + \phi(\mathbf{h}-\mathbf{k})] \rangle_{\mathbf{k}}}{\langle w(\mathbf{h},\mathbf{k}) \cos[\phi(\mathbf{k}) + \phi(\mathbf{h}-\mathbf{k})] \rangle_{\mathbf{k}}}$$

where $w(\mathbf{h},\mathbf{k}) = D(\mathbf{h},\mathbf{k})t(\mathbf{k})t(\mathbf{h}-\mathbf{k})$ and $t(\mathbf{h}) = ((S^2 + C^2)^{1/2}| \sum_{\mathbf{k}} w(\mathbf{h},\mathbf{k})$ is the consistency of the phase for reflection **h** as determined on the previous cycle.

Four phase sets were produced; an E map based on the set with the highest overall self-consistency ($\sum_{\mathbf{h}}|E(\mathbf{h})|t(\mathbf{h})/$

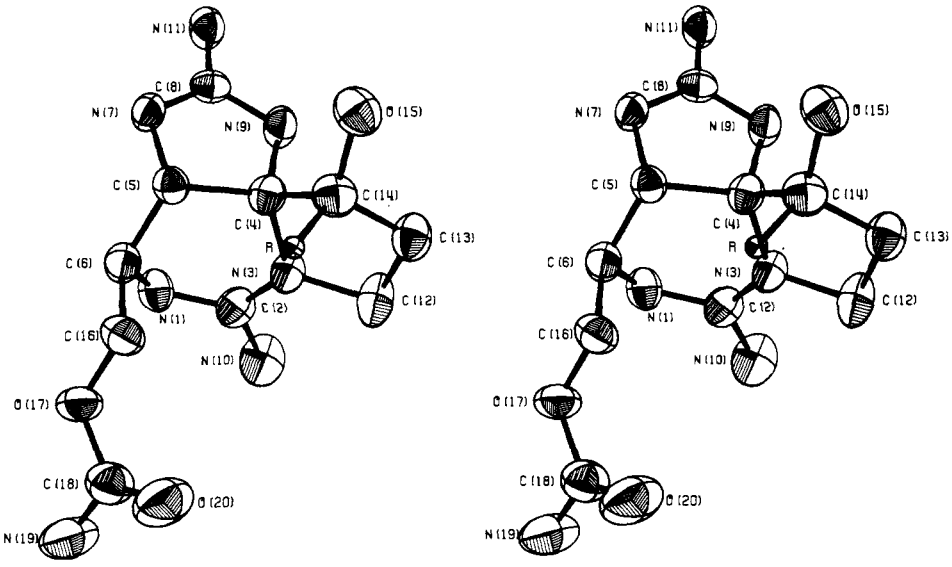


Figure 1. Stereoview of saxitoxin ethyl hemiketal dihydrochloride, viewed down the c axis, where R = $O\text{-}CH_2CH_3$.

21 22 23

Table I. Crystal and Data Collection Parameters

Compd	$C_{12}H_{21}N_7O_4 \cdot 2HCl \cdot H_2O$ (418.28)
Crystal size	0.2 × 0.2 × 0.3 mm
Cell dimensions	a = 9.213 (2); b = 11.837 (3); c = 18.573 (7) A; V = 2024 (1) A^3
Space group	$p2_12_12_1$
Density	1.37 g/cm³ (calcd 1.372, for Z = 4)
Scan	Mode, $\theta/2\theta$; rate, 2° min in 2θ
Background count time	Peak scan time/2 (at both ends of scan)
No. of reflections	1223; 1133 nonzero[a]

[a] Reflections with intensities less than 3σ were set equal to zero with zero weight.

$\sum_{\mathbf{h}}|E(\mathbf{h})|$) contained 24 of the 26 nonhydrogen atom positions among the 29 largest peaks. The remaining two atoms (the carbons of the ethoxy group) were located by Fourier techniques.

This trial structure refined routinely to an R index of 0.044: $R = \sum||F_o| - |F_c||/\sum|F_o|$. The goodness of fit was 2.57: GOF = $[\sum w(F_o^2 - F_c^2)^2/(m - s)]^{1/2}$ (where m is the number of observations and s is the number of parameters refined). Hydrogen positions were calculated wherever possible. The remaining hydrogens were located by difference Fourier techniques. While the hydrogen parameters were added to the structure factor calculations in the latter stages of refinement, these parameters were not allowed to refine. The final cycles of full-matrix least-squares refinement contained all the nonhydrogen coordinates in one matrix, and the anisotropic temperature factors and scale factor in a second matrix. The shifts calculated in the final cycle of refinement were all less than one-seventh of the corresponding standard deviations. A final difference Fourier revealed no missing or misplaced electron density.

The refined structure of saxitoxin ethyl hemiketal (**2b**) was plotted using the ORTEP computer program[16] (Figure 1). Bond distances and angles, along with their standard deviations, are presented in Table II. Atomic coordinates, temperature factors, and structure factor tables appear in Tables III-V.[17] The absolute configuration of the molecule was determined by the method of Ibers and Hamilton.[18] The configuration pictured refined to an R index of 0.044, while the enantiomer refined to an R index of 0.055. The differences in discrepancy indices (Hamilton's R'') established the correct enantiomer at the 0.5% level of significance (i.e., with 99.5% confidence).[19]

Results

The structure of saxitoxin ethyl hemiketal dihydrochloride, deduced by X-ray crystallography, is shown in Figure 1 and as structure **2b.** Spectral data (^1H NMR and ^{13}C NMR) suggest that saxitoxin has not undergone any skeletal rearrangement in forming hemiketal **2b,** and this is confirmed by its facile reconversion to saxitoxin, identical in all respects to an authentic sample, upon treatment with water at 20°.

The ^{13}C NMR spectrum of aqueous saxitoxin (Table VI) reveals a lack of the expected ketone signal for C-14. Instead, a signal typical of a ketone hydrate (δ 98.9 ppm) is observed. This assignment is confirmed by reduction to **2c** which eliminates the 98.9 ppm signal and is independently known to involve reduction of the ketone position.[4]

A cyclol structure incorporating N-7 or N-1 via the carbamate was originally introduced in structure **1** to explain its pK_a data. Saxitoxin has two pK_a values, 8.24 and 11.60, in water. The first increases to 9.05 in 50% ethanol, which was interpreted as indicating proton dissociation from the cyclol oxygen.[4]

The cyclol hypothesis is vitiated by our present ^{13}C NMR studies. The signal assigned to C-18 (159.0 ppm) is typical for a carbamate (i.e., ethyl carbamate, 157.8 ppm).[20] Predicting the chemical shift for the tautomeric cyclol is more enigmatic due to a lack of data for similar compounds. The closest analogy in the literature is a cyclol constituted by two amides, one hydroxyl, and one alkyl group attached to the central carbon which absorbs at 94.4 ppm.[21] Attempting to estimate the chemical shift using standard chemical shift parameters is treacherous, as it is well known that these parameters are not additive for a carbon highly substituted with electronegative groups.[22] As a model, we prepared tetramethylurea diethyl acetal and observed a chemical shift of 113.2 ppm for the central carbon. This allows us to predict[23] that C-18 would have a chemical shift of 104 ppm if it were present as a cyclol. Unmistakably,

Table II. Distances and Angles

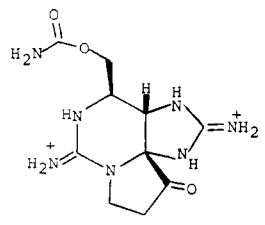
Atom	Atom	Distance, Å	Atom	Atom	Atom	Angle, deg	Atom	Atom	Atom	Angle, deg
N(1)	C(2)	1.340 (9)	C(6)	N(1)	C(2)	118.1 (5)	N(9)	C(8)	N(7)	111.2 (6)
N(1)	C(6)	1.456 (9)	N(3)	C(2)	N(1)	117.1 (6)	N(11)	C(8)	N(7)	125.7 (6)
C(2)	N(3)	1.335 (8)	N(10)	C(2)	N(1)	122.3 (6)	N(11)	C(8)	N(9)	123.1 (6)
C(2)	N(10)	1.319 (9)	N(10)	C(2)	N(3)	120.3 (6)	C(8)	N(9)	C(4)	111.2 (5)
N(3)	C(4)	1.462 (8)	C(4)	N(3)	C(2)	121.7 (5)	C(13)	C(12)	N(3)	102.5 (5)
N(3)	C(12)	1.486 (9)	C(12)	N(3)	C(2)	124.0 (6)	C(14)	C(13)	C(12)	107.0 (6)
C(4)	C(5)	1.549 (9)	C(12)	N(3)	C(4)	113.9 (5)	C(13)	C(14)	C(4)	102.9 (5)
C(4)	N(9)	1.449 (8)	C(5)	C(4)	N(3)	113.9 (5)	O(15)	C(14)	C(4)	112.9 (5)
C(4)	C(14)	1.570 (9)	N(9)	C(4)	N(3)	110.8 (5)	O(21)	C(14)	C(4)	102.4 (5)
C(5)	C(6)	1.525 (9)	C(14)	C(4)	N(3)	100.2 (5)	O(15)	C(14)	C(13)	109.2 (6)
C(5)	N(7)	1.447 (8)	N(9)	C(4)	C(5)	102.5 (5)	O(21)	C(14)	C(13)	114.3 (6)
C(6)	C(16)	1.508 (9)	C(14)	C(4)	C(5)	117.8 (5)	O(21)	C(14)	O(15)	114.4 (5)
N(7)	C(8)	1.298 (9)	C(14)	C(4)	N(9)	112.0 (5)	O(17)	C(16)	C(6)	107.3 (5)
C(8)	N(9)	1.333 (9)	C(6)	C(5)	C(4)	110.9 (5)	C(18)	O(17)	C(16)	116.9 (5)
C(8)	N(11)	1.337 (9)	N(7)	C(5)	C(4)	102.3 (5)	N(19)	C(18)	O(17)	111.6 (6)
C(12)	C(13)	1.525 (10)	N(7)	C(5)	C(6)	113.0 (5)	O(20)	C(18)	O(17)	122.7 (7)
C(13)	C(14)	1.510 (10)	C(5)	C(6)	N(1)	110.2 (5)	O(20)	C(18)	N(19)	125.4 (7)
C(14)	O(15)	1.383 (8)	C(16)	C(6)	N(1)	110.9 (5)	C(22)	O(21)	C(14)	116.9 (5)
C(14)	O(21)	1.397 (8)	C(16)	C(6)	C(5)	113.1 (5)	C(23)	C(22)	O(21)	107.6 (7)
C(16)	O(17)	1.418 (8)	C(8)	N(7)	C(5)	112.5 (5)				
O(17)	C(18)	1.339 (9)								
C(18)	N(19)	1.315 (10)								
C(18)	O(20)	1.207 (10)								
O(21)	C(22)	1.443 (8)								
C(22)	C(23)	1.480 (14)								

Table III. Heavy-Atom Parameters and Their Standard Deviations[a]

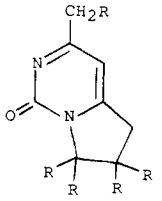
	x	y	z	u_{11}	u_{22}	u_{33}	u_{12}	u_{13}	u_{23}
N(1)	7509 (6)	3931 (4)	3387 (2)	43 (3)	46 (4)	37 (3)	−7 (3)	−5 (3)	−5 (3)
C(2)	8876 (7)	4268 (5)	3508 (3)	39 (4)	52 (5)	32 (4)	2 (4)	−3 (4)	2 (4)
N(3)	9109 (5)	4918 (4)	4084 (2)	30 (3)	51 (4)	30 (3)	−1 (3)	1 (3)	−9 (3)
C(4)	7949 (6)	5593 (5)	4406 (3)	32 (4)	42 (4)	33 (4)	−2 (3)	5 (3)	1 (3)
C(5)	6431 (6)	5034 (5)	4363 (3)	34 (4)	32 (4)	33 (4)	−2 (3)	−1 (3)	2 (3)
C(6)	6519 (7)	3883 (5)	3997 (3)	34 (4)	40 (4)	47 (4)	−3 (4)	−3 (4)	4 (3)
N(7)	5609 (5)	5853 (4)	3953 (2)	28 (3)	37 (3)	44 (3)	−1 (3)	−6 (3)	5 (3)
C(8)	6400 (7)	6696 (5)	3739 (3)	43 (4)	32 (4)	31 (4)	5 (4)	3 (3)	−4 (3)
N(9)	7738 (5)	6635 (4)	4010 (2)	29 (3)	44 (3)	46 (3)	−7 (3)	−2 (3)	2 (3)
N(10)	9979 (6)	3901 (5)	3119 (3)	58 (4)	89 (5)	38 (3)	1 (4)	6 (3)	−25 (3)
N(11)	5953 (6)	7545 (4)	3320 (2)	36 (3)	46 (3)	51 (3)	−8 (3)	2 (3)	4 (3)
C(12)	10565 (7)	5133 (6)	4399 (3)	35 (4)	81 (6)	45 (4)	−10 (4)	−2 (4)	−9 (5)
C(13)	10213 (7)	5930 (6)	5020 (3)	41 (4)	57 (5)	37 (4)	−4 (4)	−4 (3)	−6 (4)
C(14)	8611 (7)	5808 (6)	5173 (3)	45 (5)	47 (5)	36 (4)	1 (4)	5 (4)	−7 (4)
O(15)	8104 (5)	6790 (3)	5488 (2)	57 (3)	49 (3)	43 (2)	−4 (3)	4 (2)	−5 (2)
C(16)	6938 (7)	2945 (5)	4506 (3)	48 (5)	33 (4)	47 (4)	−1 (4)	5 (4)	−3 (4)
O(17)	7045 (5)	1940 (3)	4094 (2)	54 (3)	36 (3)	60 (3)	12 (3)	−16 (2)	−1 (2)
C(18)	8092 (8)	1210 (6)	4278 (4)	59 (5)	42 (5)	53 (5)	5 (4)	−3 (4)	−6 (4)
N(19)	8149 (7)	350 (5)	3832 (3)	74 (5)	66 (4)	74 (4)	29 (4)	−18 (4)	−8 (4)
O(20)	8782 (6)	1299 (4)	4828 (3)	80 (4)	79 (4)	90 (4)	28 (3)	−38 (4)	−22 (3)
O(21)	8240 (4)	4821 (3)	5542 (2)	53 (3)	50 (3)	30 (2)	1 (3)	−6 (2)	6 (2)
C(22)	8746 (8)	4720 (7)	6275 (3)	74 (6)	76 (6)	39 (4)	10 (5)	−9 (4)	3 (4)
C(23)	7920 (13)	3794 (10)	6619 (5)	155 (11)	154 (11)	72 (7)	−23 (10)	−32 (8)	45 (7)
O(24)	3038 (5)	7339 (3)	2764 (2)	55 (3)	57 (3)	41 (2)	9 (2)	−7 (2)	4 (2)
Cl(25)	3257 (1)	4725 (1)	2798 (1)	43 (1)	54 (1)	50 (1)	1 (1)	−2 (1)	−6 (1)
Cl(26)	309 (1)	8201 (1)	3585 (1)	38 (1)	55 (1)	53 (1)	−2 (1)	0 (1)	10 (1)

[a] The values have been multiplied by 10^4. The temperature factor is in the form: $T = \exp[-(u_{11}h^2 + u_{22}k^2 + u_{33}l^2 + u_{12}hk + u_{13}hl + u_{23}kl)]$.

saxitoxin exists primarily as the carbamate under these conditions. Thus the hydrate of saxitoxin has structure **2a**, and saxitoxin has structure **3**.



3



4a; R = H
4b; R = D

Discussion

The observation that saxitoxin normally exists as a hydrate accounts for the vigorous conditions required to remove this molecule of water from saxitoxin, and the incorporation of one ^{18}O upon dissolution in $H_2{}^{18}O$.[4] The existence in aqueous solution of a small fraction of saxitoxin as ketone is supported by the low intensity ketone absorption at 1775 cm^{-1} and deuterium exchange at the C-13 methylene.[4] It is well-known that carbonyls in proximity to electron-withdrawing groups tend to form hydrates (i.e., chloral and ninhydrin) and this has recently been observed for a number of cyclic guanidino ketones.[26] The hydrate structure at C-14 will undoubtedly prove significant in under-

Table IV. Hydrogen Atom Parameters[a]

	Atom bearing H	X	Y	Z
H(27)	5	6095	4979	4853
H(28)	6	5466	3716	3746
H(29)	12	10895	4360	4608
H(30)	12	11175	5468	4062
H(31)	13	10853	5752	5489
H(32)	13	10461	6811	4889
H(33)	16	7949	3128	4774
H(34)	16	6240	2803	4903
H(35)	22	9983	4632	6305
H(36)	22	8626	5414	6567
H(37)	23	8329	3118	6315
H(38)	23	8203	3714	7240
H(39)	23	6841	3928	6544
H(40)	19	7944	629	3400
H(41)	19	8963	−93	3802
H(42)	15	7424	6600	5694
H(43)	1	7451	3494	2938
H(44)	10	11062	4262	3123
H(45)	10	9747	3752	2722
H(46)	9	8522	7169	3934
H(47)	7	4889	5660	3726
H(48)	11	6702	7890	3211
H(49)	11	4958	7373	3120
H(50)	24	2210	7600	3080
H(51)	24	3010	6540	2650

[a] The values for the coordinates have been miltiplied by 10^4. The value for the isotropic temperature was fixed at 3.0 Å^2.

standing saxitoxin's biological activity as a sodium ion channel blocker.

Since our data do not support the existence of a cyclol in saxitoxin, it cannot be responsible for the 8.24 pK value, and another explanation must be sought. The hydrate is not responsible since dihydrosaxitoxin (**2c**, R = H) has the same pK values as saxitoxin.[27] Considering the spatial arrangement of the two guanidines, one guanidine could have a pK at least three units below the other. This assumes the positive charges of the guanidinium groups at average positions on C-2 and C-8, separated by 3.48 Å. The two amino groups of 1,2-diaminoethane, which are separated by 3.86 Å,[28] have pK values of 7.00 and 10.09.[29] Hence it is reasonable that a guanidine may be responsible for the 8.24 pK.

The deficiency of such a rationalization for the pK is its failure to explain the change in pK from 8.24 to 9.05 as the solvent is changed from water to 50% aqueous ethanol, respectively.[4] Such solvent dependency is well-documented in support of proton dissociation from oxygen rather than from protonated nitrogen.[30] If indeed this dissociation in saxitoxin does involve a protonated nitrogen, then a unique behavior is being manifest. Perhaps the decrease in solvent polarity results in less interaction between the two guanidinium ions, or increased intramolecular hydrogen bonding between the carbamate and guanidinium ion, stabilizing the latter. Alternatively, cyclol formation may be catalyzed by the addition of alkali. Attempts to test this possibility by observing the ^{13}C NMR spectrum were thwarted by the instability of saxitoxin at higher pH's in the times required. At present the pK data still await a full explanation.

It is instructive to reinterpret the ^{1}H NMR spectrum of saxitoxin, illuminating the errors which led to incorrect structure **1**. All the previous assignments[4] are correct, except for the point of attachment of the proton corresponding to the furthest downfield signal [δ 4.77 (d, J = 1.3 Hz)] previously assigned to a C-4 proton and now assigned to the C-5 proton. This surprisingly large chemical shift must be due to spatial deshielding by the two hydrate oxygens.[31] Previously, this chemical shift was ascribed to the influence

Table VI. ^{13}C NMR Data[a] for Aqueous Saxitoxin (**2a**) and Dihydrosaxitoxin-*14-d* (**2c**)

C atom	2a (mult)	2c
2[b]	157.9 (s)	156.1
4	82.6 (s)	82.5
5[c]	57.3 (d)	57.1
6[c]	53.2 (d)	52.1
8[b]	156.1 (s)	155.0
12	43.0 (t)	42.9
13	33.1 (t)	28.0
14	98.9 (s)	73.7
16	63.3 (t)	62.3
18[b]	159.0 (s)	158.1

[a] Ppm relative to TMS. Assignments based on multiplicities in proton off-resonance decoupled spectra, predicted chemical shifts, and comparison of **2a** and **2c**. [b] These three assignments may be interchanged. [c] These two assignments may be interchanged.

of the gem-diguanidinium ions and led to the conclusion that the proton was on C-4, coupled to the C-6 proton via a four-bond coupling constant. This led to structure **1** where C-14 is connected to C-5 instead of C-4.[4] The remarkably small vicinal coupling constant between the protons attached to C-5 and C-6 now is explained by the 89° dihedral angle observed between them in **2b.**

Attachment of the three-carbon bridge from N-3 to C-4 would appear to have been required in any case by our original isolation of 8-methyl-2-oxo-2,4,5,6-tetrahydropyrrolo[1,2-*c*]pyrimidine (**4a**) from the action of phosphorus–hydrogen iodide–acetic acid on saxitoxin.[32] However, we have since established that this degradation product does not reflect the intact skeleton of saxitoxin since reaction in a fully deuterated medium led to incorporation of five deuterium atoms as shown in **4b.**[33] This implies extensive rearrangement, which we are now studying, and does not restrict the attachment of the three-carbon bridge to C-4.

In early studies on saxitoxin, it was noted that an unsymmetrical peak was seen on countercurrent distribution, which led to the hypothesis of interconvertible saxitoxins A and B. The equilibrium must involve the ketone hydrate, since its reduction leads to a symmetrical peak.[5] Nonhydrated saxitoxin (**3**) is not present to an appreciable extent in aqueous solution, and is thus unlikely to be responsible. Presumably, reversible hemiketal formation with the ethanol and/or butanol of the organic phase accounts for the observed behavior.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 220 MHz and are reported as δ values downfield from internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (δ 0). Ir spectra were observed with a Perkin-Elmer 421 spectrometer. Optical rotation was measured using a Bendix 143A automatic polarimeter attached to a chart recorder. Elemental analyses were provided by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley.

Natural abundance Fourier transform ^{13}C NMR spectra of aqueous **2a** and **2c** were recorded at 6° and 15.09 MHz and are reported as δ values downfield from external TMS (δ 0). ^{13}C NMR spectra of dimethylformamide dimethyl acetal and tetramethylurea diethyl acetal were recorded at 37° and 20.00 MHz and are reported as δ downfield from internal TMS. We are grateful to Mr. T. McGrath and Dr. J. Crowley for assistance in obtaining the spectra.

Thin-Layer Chromatography. Plates coated with Macherey and Nagel MN 300 cellulose were developed with *tert*-butyl alcohol–acetic acid–water (2:1:1) and visualized with Weber reagent[5] after neutralization in an ammonia atmosphere. The R_f values were 0.40 for saxitoxin dihydrochloride and 0.75 for saxitoxin ethyl hemiketal dihydrochloride.

Saxitoxin Ethyl Hemiketal Dihydrochloride (2b). An ethanolic solution of saxitoxin dihydrochloride (25 mg/ml) was stored at −13°, and very slowly a crystalline ethyl hemiketal was formed in 40% yield. Crystals suitable for X-ray analysis were dried at 20° over $Mg(ClO_4)_2$ and mounted in glass capillary tubes. More vigorous drying (60°, 0.02 mm, 20 hr) caused destruction of crystallinity, and further drying (110°, 10^{-6} mm, 3 days) results in substantial loss of ethanol and water. Recrystallization from ethanol was accomplished, though with some difficulty: ir (KBr disk) 3200, 1725, 1688, 1655, 1595, 1445, 1335, 1315, 1250, 1220, 1180, 1100, 960, and 900 cm^{-1} (note lack of ketone peak at 1775 cm^{-1} obtained in saxitoxin); ^1H NMR (immediately upon dissolution in D_2O) δ 1.21 (t, J = 6.5 Hz), 2.11 (m), 2.71 (m), 3.51 (m), 3.70 (m), 3.82 (dd, J = 6 and 9 Hz), 3.98 (dd, J = 7 and 11 Hz), 4.27 (dd, J = 9.5 and 10 Hz), 4.8 (obscured by HDO).

Anal. Calcd for $C_{12}H_{21}N_7O_4 \cdot 2HCl \cdot H_2O$: C, 34.5; H, 6.0; N, 23.4. Found: C, 34.6; H, 6.1; N, 23.4.

Saxitoxin reacts with absolute ethanol (20°, 2 days) to form a product identical with **2b** by TLC.

Conversion of Saxitoxin Ethyl Hemiketal Dihydrochloride (2b) to Saxitoxin Hydrate (2a). The ^1H NMR of a solution of saxitoxin ethyl hemiketal dihydrochloride (**2a**) in D_2O (40 mg/ml) was observed immediately upon dissolution and periodically thereafter. A half-life of 40 min (20°) was measured, based on the disappearance of multiplets at 2.11 and 2.71 and appearance of a new multiplet at 2.37. Consistent results were observed by TLC which showed essentially complete reaction after 90 min.

The saxitoxin concentration of the equilibrated solution, measured by toxicity,[5] and oxidative degradation with alkaline hydrogen peroxide,[34] indicated quantitative reaction. A freshly prepared solution of hemiketal **2b** undergoes oxidative degradation to 8-amino-6-(hydroxymethyl)-2-iminopurine-3(2*H*)-propionic acid in 20% yield in less than 1 min, whereas saxitoxin gives a 63% yield in 30 min under the same conditions.[34]

The optical rotation of freshly prepared hemiketal **2b** is $[\alpha]^{H_2O}_D$ +137° (extrapolated to zero time). This decreases to 128°, the value for saxitoxin,[3] with a half-life of 6 min at 20°. The significance of this half-life is not clear.

Dihydrosaxitoxin-*14-d* dihydrochloride was prepared by catalytic deuteration of saxitoxin.[5]

Tetramethylurea diethyl acetal was prepared by treating tetramethylurea with triethyloxonium flouroborate followed by sodium ethoxide:[35] ^1H NMR (neat) δ 1.10 (t, 6), 2.45 (s, 12), 3.36 (q, 4); ^{13}C NMR (neat) δ 15.3 (CH_2CH_3), 38.2 (NCH_3), 57.5 (OCH_2), 113.2 (central C). The product was contaminated with 10% (^1H NMR) tetramethylurea.

Dimethylformamide dimethyl acetal was prepared by treating dimethylformamide with dimethyl sulfate followed by sodium methoxide:[36] ^1H NMR (neat) δ 2.23 (s, 6), 3.24 (s, 3), 4.29 (s, 1); ^{13}C NMR (neat) δ 37.5 (NCH_3), 52.7 (OCH_3), 113.6 (central C).

Supplementary Material Available. Table V will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C., 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JACS-75-6008.

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