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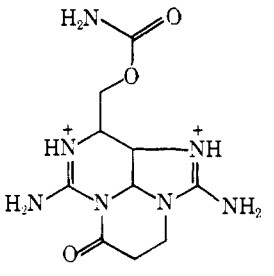
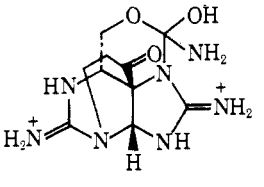
The Structure of Saxitoxin[1]

Sir:

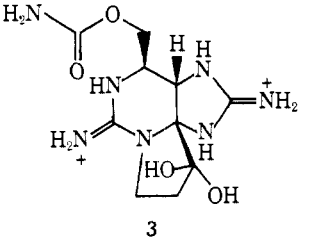
Saxitoxin is the neurotoxin isolated from toxic Alaska butter clams (*Saxidomus giganteus*), toxic mussels (*Mytilus californianus*), and axenic cultures of *Gonyaulax catanella*.[2] Recently it was also found to be present in aged extracts of scallops collected during a *G. tamarensis* bloom.[2] It is among the most toxic substances known with an LD_{50} of 5–10 μg/kg (mouse, ip). The chemistry of saxitoxin has been complicated by its noncrystalline, highly polar, nonvolatile nature and even the molecular formula has been a subject of debate.[3b] As a result of extensive chemical and spectroscopic work, structures **1**[3a] and **2**[3b] have previously been reported in the literature. We have succeeded in crystallizing the *p*-bromobenzenesulfonate saxitoxin and now wish to report the structure deduced from a single-crystal X-ray diffraction study as **3**.



1



2



3

Purified saxitoxin hydrochloride ($C_{10}H_{17}N_7O_4 \cdot 2HCl$) was dissolved in water and 2 equiv of sodium *p*-bromobenzenesulfonate was added. The crystals resulting from this were washed and recrystallized from water. A satisfactory

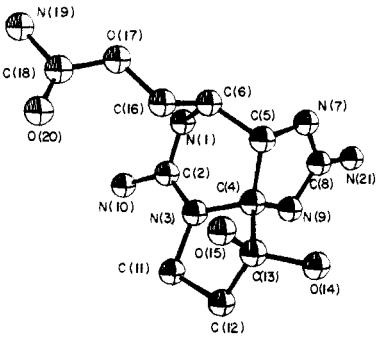


Figure 1. A computer generated perspective drawing of saxitoxin crystallized as the *p*-bromobenzenesulfonate. For clarity the hydrogen atoms of the saxitoxin portion and all atoms of the sulfonate are omitted. The absolute configuration is as shown.

biological assay (4088 mu/mg) was obtained.[4] These crystals were subjected to a single-crystal X-ray diffraction study. A crystal of 0.1 × 0.2 × 0.6 mm was mounted in a Lindemann capillary with mother liquor. The crystals formed in the common, chiral, unambiguously determined, space group $P2_12_12_1$ with a = 31.095 (4), b = 12.180 (1), c = 7.8057 (9) Å. A calculated density of 1.74 g/cm^3 (for Z = 4 and mol wt = 773) was interpreted to mean that one unit of $C_{10}H_{19}N_7O_4 \cdot 2C_6H_4BrSO_3$ formed the asymmetric unit. Reflection data for all unique diffraction maxima with $2\theta \leq 114°$ were collected on a fully automated four-circle diffractometer using an ω-scan technique because of the pronounced spread of the reflections. A total of 2340 reflections were measured and after correction for Lorentz, polarization, and background effects 1895 (81%) were judged observed ($F_o \geq 3\sigma(F_o)$).

The bromine and sulfur atoms were easily located and three-dimensional electron density syntheses revealed all 43 non-hydrogen atoms.[7] Least-squares refinements with anisotropic temperature factors lowered the conventional discrepancy index to 6.3%. Placement of all hydrogens followed by further least-squares refinement lowered the discrepancy index to 5.6%. Inclusion of anomalous scattering factor contributions for Br and S, followed by still further least-squares refinement lowered the discrepancy index to the current value of 5.2% for the structure and 5.7% for its mirror image, a statistically significant difference.[8] A careful remeasurement of the 20 most enantiomorph sensitive reflections also indicated that the structure and not its mirror image was the correct absolute configuration. Figure 1 shows a computer generated drawing of the final X-ray model. Tables I, II, III, and IV contain the final fractional coordinates, bond distances, bond angles, and structure factors, respectively, and can be found in the supplemental material.

In general the bond distances and angles agree well with accepted values. Saxitoxin contains three rings and is conveniently described as a 3,4,6-trialkyl tetrahydropurine. The 3 and 4 positions are bridged by a three-carbon fragment to form the third ring which contains a hydrated ketone as a novel structural element. The 2 and 8 positions of the purine ring contain NH_2 groups which form the two guanidine moieties of saxitoxin. Position 6 is substituted by a $-CH_2OCONH_2$ fragment which adopts a pseudo-axial configuration. The crystal structure reveals 13 possible H bonds but none of these is intramolecular. There is a close approach between O(14) and N(9) of 2.82 Å but the hydrogen of O(14) is turned away from N(9).

The five-membered ring containing the guanidine group is planar with a maximum deviation from the least-squares plane of 0.001 Å. In addition, four (N(3), C(4), C(11), and C(12)) of the five atoms in the other five-membered ring

form a plane with a maximum deviation of 0.01 Å, while C(13) is 0.62 Å from this plane. The six-membered ring also has a four atom plane (N(3), C(4), C(5), and C(6)) with a maximum deviation of 0.001 Å. N(1) and C(2) are 0.87 Å and 0.57 Å, respectively, from this plane; both are on the same side. The guanidine moiety (N(1), C(2), N(3), and N(10)) is planar with a maximum deviation of 0.003 Å. All of the C···N bonds of both guanidines are the same within experimental error (1.32 (1) Å) except N(9)-C(8) which is 1.36 (1) Å.

Structure **3** is compatible with all of the published chemical and spectral data of saxitoxin if an equilibrium exists between **3** and its keto form. Thus under vigorous drying conditions (110° at 10^{-5} mm to constant weight)[3b] saxitoxin is dehydrated which reconciles the proposed molecular formulas. Presumably the ketone hydrates readily in saxitoxin because of the strongly electron withdrawing guanidinium groups on the α carbon. It is entirely possible that the two forms of saxitoxin seen on countercurrent distribution are the ketone and ketone hydrate which revert to a single form in acid.[6] We would also attribute the very weak ketone absorption observed in the ir spectrum to a small amount of keto form.[9] The keto form is also responsible for the reduction (H_2-PtO_2 or $NaBH_4$) reaction which eliminates the ketone ir absorption.[9]

The two hydroxyl groups of the hydrated form are diastereotopic. Vigorous drying followed by rehydration with $H_2{}^{18}O$ incorporates one ^{18}O which is subsequently lost upon vigorous drying.[3b] From inspection of molecular models it is clear that the bottom surface of the molecule is more accessible to attack. Thus O(14)H is probably the fragment lost as water to form the ketone and added to generate the ketone hydrate.

The NMR[3b] spectral assignments are now δ 4.27 (1 H, q, J = 11, 9 Hz) and 4.05 (1 H, q, J = 11, 5 Hz) to the two H's on C(16), δ 3.87 (1 H, d of q, J = 9, 5, 1 Hz) to the lone H on C(6), and δ 4.77 (1 H, d, J = 1 Hz) to the bridgehead H on C(5). The dihedral angle of 72° between these last two hydrogens in the crystalline state explains their relatively small coupling. The protons on C(11) are responsible for the resonances at δ 3.85 and 3.57. The δ 2.37 multiplet is attributed to the protons on C(12) when saxitoxin is in the keto form.

Details of the results of X-ray crystallography and spectral analysis will be presented in a subsequent paper.

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Supplementary Material Available. The fractional coordinates (Table I), important bond distances (Table II), important bond angles (Table III), and structure factors (Table IV), 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 Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.50 for photocopy or $2.50 for microfiche, referring to code number JACS-75-1238.

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(4) The biological activity is in fact greater than anticipated. Purified saxitoxin has a specific toxicity of 5500 ± 500 mu/mg.[5] Thus the bis-*p*-bromobenzenesulfonate derivative should have had 2400–2900 mu/mg rather than the observed 4088 mu/mg. Possible explanations for this enhanced activity are (1) preferential crystallization of one of the two forms of saxitoxin[6] or (2) synergistic effects with the reagent.
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A Biogenetically Patterned Synthesis of the Morphine Alkaloids

Sir:

Since the original conception[1] and refinement[2] of the idea that the morphine alkaloids arise in the plant via oxidative coupling of a phenolic benzyltetrahydroisoquinoline derivative, a great deal of effort has been devoted to elucidating the actual biosynthetic pathway and to applying the biogenetic postulate to a laboratory synthesis of these alkaloids. While the biosynthetic pathway has been thoroughly delineated[3] (Scheme I, **1** → **6**), the latter goal has proved elusive. The many attempts to effect para-ortho oxidative coupling of (±)-reticuline (**1**) using $K_3Fe(CN)_6$,[3b,4a-d] MnO_2-silica gel,[4e] $AgCO_3$-Celite,[4d] and $VOCl_3$[4d,f] have, with one exception, afforded only the para-para coupling product (±)-isosalutaridine (**7**, yields of 0.3–4%) and/or the ortho-para coupling product (±)-isoboldine (**8**, yields of 0.4–53%); the exception was Barton and coworkers'[3b] detection via isotope dilution techniques of a 0.03% yield of (±)-salutaridine (**2**) from ferricyanide oxidation of **1**. Similarly negative results have also been obtained with *N*-acylnorreticuline derivatives.[4b,e,5] However, we wish now to report realization of the long-sought laboratory analogy for the in vivo para-ortho coupling of **1** to **2**, by thallium tristrifluoroacetate (TTFA) coupling[6] of *N*-acylnorreticuline derivatives **9** and **10**.

Treatment of (±)-*N*-norreticuline[7] with trifluoroacetic anhydride and K_2CO_3 in CH_2Cl_2, followed by stirring in aqueous CH_3OH, afforded the *N*-trifluoroacetyl derivative **9**, mp 127–130° (petroleum ether-$CHCl_3$), mp 148–152° (CH_3OH-$CHCl_3$). Oxidation of **9** with 1.0 mol equiv of