corresponding alkene,[17,18] which suggests a possible intermediate. Since alternative pathways are possible, however, we have sought to probe the mechanism of the coupling reaction in the potentially most symmetrical case. Treatment of $Os_3(CO)_{10}(NCMe)(^{13}CH_2)$, prepared from >90% ^{13}C-enriched diazomethane, with an excess of unenriched diazomethane, results in an equimolar mixture of $HOs_3(CO)_{10}(^{13}CH{=}CH_2)$ and $HOs_3(CO)_{10}(CH{=}^{13}CH_2)$, as judged by ^{13}C NMR spectroscopy.[19] Equilibration of the carbon atoms in the vinyl complex itself is not facile,[20] so formation of the carbon–carbon bond and equilibration of the two carbon atoms must *precede* scission of the carbon–hydrogen bond.[21] The most straightforward inference is that two bridging methylenes are coupled into either a di-σ- or a π-C_2H_4 complex; subsequent hydrogen transfer in the unsaturated intermediate would generate the observed vinyl derivative.[22] The remaining reactions expressed in reaction 3 presumably proceed analogously, with formation of the β-substituted alkenyl product preferred for steric reasons.[23]

We conclude that the interaction of diazomethane (diazoalkanes) with reactive triosmium compounds can lead not only to transfer of a methylene (alkylidene) group but also, as found for metal surfaces,[4] to the facile formation of a dicarbon moiety. Ethylene is not readily observed in the triosmium case due to formation of the stable vinyl complex; however, addition of a strong ligand (such as carbon monoxide[16]) can reverse the conversion and force release of the alkene. Further coupling reactions of $Os_3(CO)_{10}(NCMe)(\mu\text{-}CH_2)$ and related compounds are under active investigation.

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(19) With complete proton decoupling two singlets, equally intense, are seen at δ 69.0 and 101.0. The absence of ^{13}C satellites indicates that essentially no $HOs_3(CO)_{10}(^{13}CH{=}^{13}CH_2)$ is formed; qualitatively, the signal intensity indicates that little label is lost. Off-resonance decoupling shows that the downfield signal corresponds to $CH(C_\alpha)$ and the upfield signal to $CH_2(C_\beta)$.

(20) Potentially, equilibration of the C_α and C_β sites of the vinyl ligand could occur by reductive elimination of the Os–C_α and Os–H bonds to give a π-ethylene complex which could then rotate about the Os–C_2H_4 bond and reform the vinyl complex with C_α and C_β interchanged. In order to test this possibility, we prepared $HOs_3(CO)_{10}(CD{=}CDH)$ by treating $H_2Os_3(CO)_{10}$ with C_2D_2. The ^1H NMR spectrum of the product verified that H was essentially absent at C_α and that the deuterium atoms were cis as expected. The labeled vinyl complex was then treated with ethereal CH_2N_2 under the same conditions as the alkylidene coupling reaction (0 °C in CH_2Cl_2 followed by warming to ambient temperature). The ^1H NMR spectrum of the recovered vinyl complex indicated that exchange between the bridging hydride and deuterium at C_α had not occurred, whereas complete H/D exchange at C_β had taken place. (The latter process occurs in the presence of a variety of bases; cf. $HOs_3(CO)_{10}(CH{=}CH_2)$ + $PPhMe_2$: Churchill, M. R.; DeBoer, B. G.; Shapley, J. R.; Keister, J. B. *J. Am. Chem. Soc.* **1976**, *98*, 2356.) We conclude that C_α and C_β are not equilibrated under these conditions.

(21) The requirement of equivalence eliminates two alternative mechanisms which would preserve a distinction, e.g.,

$$\text{(i)}\ Os_3(\mu\text{-}C^*H_2) \rightarrow HOs_3(\mu\text{-}C^*H) \xrightarrow{+CH_2} HOs_3(\mu\text{-}C^*HCH_2)$$

$$\text{(ii)}\ Os_3(\mu\text{-}C^*H_2) \xrightarrow{+CH_2} Os_3(\mu\text{-}C^*HCH_3) \rightarrow HOs_3(\mu\text{-}C^*HCH_2)$$

(22) The possibility of terminal alkylidenes cannot be eliminated in the triosmium coupling reaction, but considering the pronounced preference for bridging over terminal configurations in the observed structures, the intermediacy of even one terminal form seems unlikely for such a facile reaction. A different possibility is direct formation of a C_2 intermediate by intramolecular attack of diazomethane at the methylene center. However, since $Os_3(CO)_{11}(CH_2)$, under the conditions of its synthesis, does not react with diazomethane to form $HOs_3(CO)_{10}(CH{=}CH_2)$, the coordination site provided by the labile NCMe ligand is necessary for the coupling reaction to proceed. On the other hand, the presently available data do not eliminate the possibility, suggested by a referee, that coupling occurs intramolecularly after formation of a diazomethane complex $Os_3(CO)_{10}(CH_2)(CH_2N_2)$.

(23) Substitution at C_α leads to significant destabilization. See: Clauss, A. D.; Tachikawa, M.; Shapley, J. R.; Pierpont, C. G. *Inorg. Chem.* **1981**, *20*, 1528.

Strutures of Two Novel Toxins from *Protogonyaulax*

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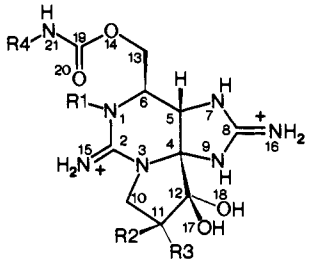
Saxitoxin (**1**) and several other potent neurotoxins (**2**, **4**, **6**–**8**) have been isolated from shellfish and dinoflagellates of the genus *Protogonyaulax*.[2-5] We have recently described a novel class of



	R1	R2	R3	R4
1	H	H	H	H
2	H	H	OSO_3^-	H
3	H	H	OSO_3^-	SO_3^-
4	H	OSO_3^-	H	H
5	H	OSO_3^-	H	SO_3^-
6	OH	H	H	H
7	OH	H	OSO_3^-	H
8	OH	OSO_3^-	H	H

related substances,[6] significant in that they have relatively low in vivo toxicity until they are hydrolyzed to the previously known toxins, and have now established the structures of two of these as carbamoyl-*N*-sulfo-11α-hydroxysaxitoxin sulfate (**3**) and the 11β-epimer **5**.[7] The structure determinations of these new substances are of particular interest since (a) compounds **3** and **5** are the most widespread and abundant toxins produced by dinoflagellates found along the Alaskan coast from Southeast Alaska to the Aleutians, (b) this is the first report of the *N*-sulfocarbamoyl group in a natural product, (c) the attenuation of toxicity associated with sulfonation of the carbamoyl group introduces a new aspect of structure–activity relationships in this group of neurotoxins, and (d) establishment of structure **5** by X-ray crystallography coupled with the chemical interconversions described herein confirms the structural assignments for **2** and **4** previously made principally from spectroscopic data.[3,5]

Compounds **3** and **5** were obtained as white solids by chromatography[8] of extracts from several batch cultures of *Proto-*

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(2) (a) Schantz, E. J.; Ghazarossian, V. E.; Schnoes, H. K.; Strong, F. M.; Springer, J. P.; Pezzanite, J. O.; Clardy, J. *J. Am. Chem. Soc.* **1975**, *97*, 1238. (b) Bordner, J.; Thiessen, W. E.; Bates, H. A. Rapoport, H. *Ibid.* **1975**, *97*, 6008.

(3) Boyer, G. L.; Schantz, E. J.; Schnoes, H. K. *J. Chem. Soc., Chem. Commun.* **1978**, 889.

(4) Shimizu, Y.; Hsu, C.; Fallon, W. E.; Oshima, Y.; Miura, I.; Nakanishi, K. *J. Am. Chem. Soc.* **1978**, *100*, 6791.

(5) (a) Fix Wichmann, C.; Boyer, G. L.; Divan, C. L.; Schantz, E. J.; Schnoes, H. K. *Tetrahedron Lett.* **1981**, *22*, 1941. (b) Shimizu, Y.; Hsu, C. P. *J. Chem. Soc. Chem. Commun.* **1981**, 314.

(6) Hall, S.; Reichardt, P. B.; Nevē, R. A. *Biochem. Biophys. Res. Commun.* **1980**, *97*, 649.

(7) In ref 6, these were designated C1 and C2, respectively.

(8) (a) BioGel P2/0.1 M acetic acid; toxins elute as described in ref 6. (b) IRP64 (H^+ form/water; toxins **3** and **5** elute unretained; the other toxins are bound and subsequently eluted with 0.1 M acetic acid. (c) BioGel P2/water; **3** and **5** elute at ca. 135 and 145% bed volume, respectively; the other toxins are bound and are eluted with 0.1 M acetic acid. (d) The data of ref 6 show **3** and **5** to be the most abundant toxins in the mixture produced by this clone, accounting for over a third of the total toxins on a molar basis.

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Table I. ^{1}H NMR Data[a]

proton	2	3	4	5
H-5	4.41 d (1.0)	4.42	4.44 d (1.0)	4.45 d (1.0)
H-6	3.46 ddd (1.0, 5.6, 9.0)	3.50 dd (5.3, 9.5)	3.41 ddd (1.0, 6.0, 9.0)	3.51 ddd (1.0, 5.4, 9.3)
H-13	3.64 dd (5.4, 11.9)	3.76 dd (5.5, 11.8)	3.67 dd (5.5, 12.0)	3.81 dd (5.0, 11.9)
H-13	3.85 dd (9.3, 11.2)	3.96 dd (9.8, 11.8)	3.87 dd (9.0, 11.8)	3.99 dd (9.5, 11.8)
H-10	3.76 d (12.1)	3.77 d (12.2)	3.75 dd (8.8, 11.0)	3.79 dd (7.5, 10.8)
H-10	3.60 dd (5.0, 12.7)	3.63 dd (4.8, 12.2)	3.18 dd (7.0, 11.0)	3.23 dd (7.0, 11.0)
H-11	4.44 d (4.8)	4.43 d (5.0)	4.56 dd (7.5, 8.5)	4.58 dd (7.6, 7.0)

[a] Chemical shifts in ppm with reference to $CHCl_3$ at δ 7.27 as internal standard. Data in parentheses are coupling constants in Hz.

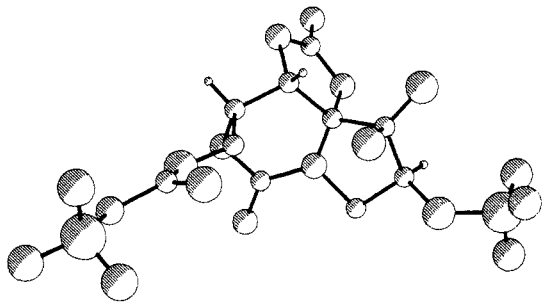

Figure 1. PLUTO plot of the structure of **5** as determined by X-ray crystallography. The positions of protons on carbons 5, 6, and 11 have been calculated and inserted for clarity.

gonyaulax clone PI07. Recrystallization of **5** from hot water gave large, gem-like crystals which dried (10 μm) to a monohydrate[9] with loss of crystallinity. Compound **3** is much more soluble in water but was obtained as small prisms from methanol–water mixtures.[10]

Both **3** and **5** exhibit a zero net charge at acidic pH, as judged by the lack of retention on carboxylate resins, and electrophoretic comparison[11] with **1**, **2**, and **4**. The ^{1}H NMR (270 MHz, D_2O) spectrum of toxin **5** (Table I) established the structural similarity between **5** and the known 11-β-hydroxysaxitoxin sulfate (GTX3, **4**)[5], and the nearly identical ^{13}C NMR spectra (50 MHz, D_2O) of both **4** and **5**, showing resonances of 10 carbons, supported assignment of the same carbon skeleton to both compounds. The structural relationship was confirmed by conversion (0.1 N HCl, 100 °C for 5 min) of **5** to a compound identical with natural **4** (by ^{1}H and ^{13}C NMR, TLC, and electrophoresis). The ^{1}H NMR spectrum of **4** in Me_2SO (270 MHz) allows identification of all OH and NH resonances and shows a prominent two-proton signal at δ 6.58 for the carbamate NH_2; in the spectrum of **5**, this signal is replaced by a sharp one-proton signal at δ 9.37. Hydrolysis of **5** to **4** eliminates the δ 9.37 resonance and restores the two-proton signal at δ 6.58. Given the combustion analysis data,[9] these results indicate an *N*-sulfocarbamoyl substituent and lead to structure **5** for this toxin.

The structure of **5** was independently established by single-crystal X-ray analysis[12] (Figure 1), which in turn confirmed the stereochemistry previously assigned to the C-11 epimers **2** and **4** on the basis of NMR data.[3,5]

Given the structure for **5**, the assignment of structure **3** to the other toxin follows directly from (a) the obvious similarity (Table I) between the ^{1}H NMR spectra of **3** and the known toxin 11α-hydroxysaxitoxin sulfate (**2**),[3,5] (b) the likewise very similar10-carbon pattern of the respective ^{13}C NMR spectra (50 MHz, D_2O), and (c) facile conversion of **3** to toxin **2**, as established by TLC and ^{1}H NMR data, upon mild acid hydrolysis, (d) the shift of the carbamate-NH_2 signal (2 H) from δ 6.61 in the ^{1}H Me_2SO–NMR spectrum of **2** to δ 9.28 (1 H, OCON*H*-SO_3) in the spectrum of **3**, and (f) the epimerization of pure β-epimer **5** to α-epimer **3** (confirmed by ^{1}H NMR and TLC) and the analogous conversion of pure **3** to **5** (an equilibrium mixture contains **5** and **3** in a ratio of ca. 1:3.5).

Determinations of the specific toxicity[13] of **3** and **5** are complicated by their facile epimerization and hydrolysis of the carbamoyl-*N*-sulfo group. The toxicity of **5** is 500 mouse units/mg and increases 6- to 8-fold to 3700 mouse units/mg after hydrolysis to **4**. The toxicity of **3** is ca. 36 mouse units/mg, when determined on freshly purified material, but increases with storage and handling because of hydrolysis of **2**; the latter has a specific toxicity of 2600 mouse units/mg. It remains possible that **3** itself is not toxic.

Compounds **3** and **5** occur in the extracts of cultured *Protogonyaulax* from several locations along the west coast of North America, from San Francisco to the Aleutian Islands. In contrast to the predominance of **3** in equilibrated mixtures, **5** is consistently the major epimer in fresh extracts, which suggests the possibility that **5** is the natural product, while **3** arises only through epimerization. Although the methodology of this study does not exclude the possibility that these toxins result from bacterial action in the cultures, the distribution of toxins among the various isolates[14] is difficult to interpret unless the toxins are being produced either by the dinoflagellates or by an isolable assemblage of dinoflagellate and bacterium characteristic of each locale.

It should be noted that these toxins may not have been detected in previous studies due to their greatly reduced toxicity and the ease with which they are hydrolyzed under conditions that have previously been considered suitable for manipulating the dinoflagellate neurotoxins.[15,16]

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(9) *Anal.* Calcd for $C_{10}H_{17}N_7S_2O_{11}\cdot H_2O$: C, 24.34; H, 3.88; N, 19.87; S, 13.00; 0, 38.91. Found: C, 24.39; H, 3.88; N, 19.78; S, 12.89; O, 38.59. (Galbraith Laboratories, Knoxville, TN.)

(10) Further chromatography of the mother liquors from these crystallizations revealed two minor constituents, designated C3 and C4, which are the corresponding derivatives of 11α- and 11β-hydroxyneosaxitoxin sulfate[5] GTX 1 and GTX 4. This completes the array we had previously outlined[6] and explains our observation that trace amounts of GTX 1 and 4 resulted from the hydrolysis of crude **3** and **5**, which presumably contained C3 and C4. Complete descriptions of C3 and C4 will appear in a forthcoming full paper.

(11) Paper electrophoresis (Whatman No. 1 paper) at 1200 V for 45 min in pH 4.5 sodium acetate buffer gives the following relative mobilities: saxitoxin (**1**), 1.0; toxin **2**, 0.62; **3**, 0.0; **4**, 0.60; **5**, 0.0. We thank Frank Koehn for these determinations.

(12) Compound **5** crystallizes in space group $P2_12_12_1$ with cell dimensions a = 12.064 (5) Å, b = 16.222 (4), c = 11.723 (9) Å, Z = 4. D_0 = 1.592 g cm^{-3} compared with D_c = 1.400 for $C_{10}H_{17}N_7O_{11}S_2$, suggests that four molecules of water are included in the unit cell. The structure was solved by direct methods using 1764 reflections collected by the θ–2θ scan technique at room temperature using graphite monochromated Mo Kα radiation. Heavy atoms were located and the data refined using programs MULTAN 74, CRYSP, and CRYM to a final R = 0.199. The water molecules were found to be disordered in the lattice. This disorder probably accounts for the difficulty in refining the structure further.

(13) Anon. "Official Methods for Analysis", 12th ed.; revised; A.O.A.C.: Washington, DC, 1975; pp 319–321.

(14) S. Hall, unpublished results.

(15) Drs. Shimizu and Kobayashi (University of Rhode Island) have recently communicated to us their isolation of a toxin (designated GTX8) for which they also propose structure **5**.

(16) **Note Added in Proof**: Since the submission of this manuscript, an account of Shimizu and Kobayashi's work has appeared (Kobayashi, M.; Shimizu, Y. *J. Chem. Soc., Chem. Commun.* **1981**, 827) which (Y. Shimizu, personal communication) contains typographical errors in the structures of **3** and **5** (their **8** and **7**, respectively).