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DINOFLAGELLATE NEUROTOXINS RELATED TO SAXITOXIN: STRUCTURES OF TOXINS C3 AND C4, AND CONFIRMATION OF THE STRUCTURE OF NEOSAXITOXIN[1]

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Abstract: By x-ray crystallography of the 11β epimer, toxins C3 and C4 are shown to be 21-sulfo-N-1-hydroxysaxitoxin-11α- and 11β-hydroxysulfate, confirming the position and identity of the 3 substituents which, with the parent compound, form the array of 12 saxitoxins found in Protogonyaulax.

In the analysis of toxins from cultured Protogonyaulax of the northeast Pacific (2) the expected compounds 1, 3, 5, 7, 9, and 11 were generally found to accompany somewhat larger amounts of the 21-sulfo derivatives 2, 4, 6, and 8. Although composition varied greatly among isolates, the above substances were all eventually found in relatively high concentrations. Compounds 10 (C3) and 12 (C4), the corresponding derivatives of 9 (gonyautoxin 1, GTX 1) and 11 (gonyautoxin 4, GTX 4) were at first notable for their absence but were finally detected in the mother liquors from crystallizations of 4 and 6 (3), and as trace constituents in analyses of Protogonyaulax clone PI07 (4).

The behavior of 10 and 12 (5,7) is largely analogous to that of 4 and 6 (2,3). Compounds 10 and 12 epimerize (TLC, NMR), the conversion of 12 to 10 predominating. Hydrolysis (0.1M HCl, 100°C, 5 minutes; 6) converts 10 to 9 and 12 to 11 (TLC). Preliminary assays indicate that the mouse intraperitoneal potencies of crude 10 and 12 increase by factors of 40x and 7x with these conversions.

	R1	R2	R3	R4	
1	H	H	H	H	STX
2	H	H	H	SO_3^-	B1
3	H	H	OSO_3^-	H	GTX 2
4	H	H	OSO_3^-	SO_3^-	C1
5	H	OSO_3^-	H	H	GTX 3
6	H	OSO_3^-	H	SO_3^-	C2
7	OH	H	H	H	NEO
8	OH	H	H	SO_3^-	B2
9	OH	H	OSO_3^-	H	GTX 1
10	OH	H	OSO_3^-	SO_3^-	C3
11	OH	OSO_3^-	H	H	GTX 4
12	OH	OSO_3^-	H	SO_3^-	C4

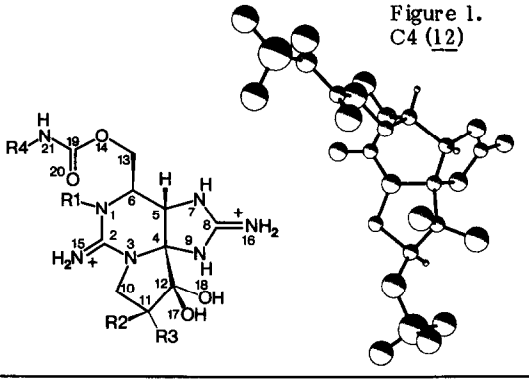

Figure 1. C4 (12)

Table 1. ^{1}H-NMR data[a].

	H-5	H-6[b]	H-10[b]	H-10	H-11	H-13	H-13
10	4.50,s	3.76,dd (6,6)	3.77,d (12)	3.63,dd (4.6,11.9)	4.46,d (4.5)	4.12,dd (6.7,11.6)	3.87,dd (6.4,11.3)
12	4.53,s	3.79,dd (7,7)	3.78,dd (7,10)	3.25,dd (7.0,10.7)	4.58,dd (7,8)	4.14,dd (7.0,11.3)	3.92,dd (6.7,11.6)

a) Chemical shifts in ppm from internal chloroform = 7.27. Data in parentheses are coupling constants in Hz.
b) Assignments approximate due to overlap.

Table 2. ^{13}C-NMR data for 10

carbon	shift, ppm
2[b]	159.4
4	81.8
5	57.7
6	62.1
8[b]	158.8
10	52.0
11	78.3
12	98.0
13	64.6
19	154.2

a) Chemical shifts in ppm from internal dioxane = 67.6.
b) Assignments may be reversed.

Compound 12 crystallizes (8) in space group $P2_12_12_1$ with cell dimensions a = 12.037(8) Å, b = 16.255(10) Å, c = 11.652(9) Å, and Z = 4. D_c for $C_{10}H_{17}O_{12}N_7S_2 \cdot 4H_2O$, mw 563.40, is 1.642 $g \cdot cm^{-3}$. Final refinements of the diffraction data attained an R factor of 0.067 for the 3σ data set. A plot of the structure is shown in Figure 1 with the calculated positions of hydrogens on carbons 5, 6, and 11 shown for clarity. Note the oxygen bonded to N-1.

^{1}H-NMR data (270 MHz, D_2O) for 10 and 12 (Table 1) are consistent with those for the other saxitoxins (2, 3, 9-11). It is noteworthy that the chemical shifts observed for H-5, H-6, and both H-13 in 10 and 12 are significantly downfield from the corresponding shifts for 4 and 6 (3), consistent with the presence of N-1-OH in 10 and 12. ^{13}C-NMR data (67.9 MHz, H_2O/D_2O, broad-band decoupled) for 10 (Table 2) correlate well with those for 1-8 (2,9). Of particular interest are the resonances for C-6 (cf. 7, 61.6; 8, 62.0), C-10 (cf. 3, 51.1; 4, 51.5), C-11 (cf. 3, 77.7; 4, 78.1), and C-19 (cf. 2, 154.8; 4, 154.6; 6, 154.7; 8, 154.3). The resonance at 154-155 ppm appears diagnostic for the sulfamate carbonyl carbon in the saxitoxins (2).

The structural relationships among compounds 1-8, 9, and 11 have been established (2, 3, 6, 10-12). The data presented here linking 10 and 12 with 9 and 11, coupled with the x-ray structure for 12, secure the structures for the entire array and in particular serve to confirm the position and identity of the N-1-hydroxyl substituent of neosaxitoxin, 7 (9,13).

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4. A preliminary report has recently appeared suggesting the same structures for two compounds isolated from a Japanese strain of Protogonyaulax: T. Noguchi, Y. Onoue, J. Maruyama, K. Hashimoto, S. Nishio, and T. Ikeda. Bull. Japan. Soc. Sci. Fish. 49: 1931 (1983).
5. Under the previously described analytical conditions (6), toxins 10 and 12 elute from BioGel P2 overlapping but slightly later than 4 and 6 respectively. Each migrates to slightly higher Rf on TLC than 4 and 6, respectively. When sprayed with 1% hydrogen peroxide and heated 15 minutes at 120°C (L. J. Buckley, M. Ikawa, and J. J. Sasner, Jr. J. Agric. Food Chem. 24: 107 (1976)) compounds 10 and 12 tend, like 7, 8, 9, and 11, to form spots with yellowish fluorescence rather than the blue fluorescence obtained with compounds 1-6.
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7. Preparative chromatography of the mixed group C toxins (4, 6, 10, 12) under neutral or weakly acidic conditions on a number of media failed to give useful separation. Facile resolution of 10 and 12 from 4 and 6 was finally achieved (2) by chromatography on BioGel P-2 in 0.1M pyridine, under which conditions 10 and 12 elute before and well separated from 4 and 6. Compounds 10 and 12 can be resolved from each other by chromatography on the same gel with either water or 0.1M acetic acid and then crystallized by methods similar to those used for 4 and 6 (2,3).
8. A crystal of 12 was mounted in a capillary with a trace of mother liquor and data collected at room temperature using graphite monochromated MoK_α radiation. Of the 1733 reflections collected, 1535 were classified as observed. The structure was solved by direct methods using the program MULTAN 80 and atoms verified in difference Fourier maps using the program set CRYM.
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