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DINOFLAGELLATE NEUROTOXINS RELATED TO SAXITOXIN: STRUCTURE AND LATENT ACTIVITY OF TOXINS B1 AND B2[1]

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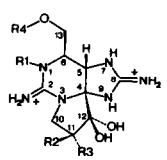
Abstract: Toxins B1 and B2 from cultured dinoflagellates of the genus *Protogonyaulax* are shown to be the carbamoyl-N-sulfo derivatives of saxitoxin and neosaxitoxin, the structures confirmed by synthesis from the corresponding decarbamoyl toxins.

In the analysis of toxins from cultured *Protogonyaulax* of the Northeast Pacific[2], we expected to find saxitoxin (1)[3,4], neosaxitoxin (7)[5], and their four epimeric 11-hydroxysulfate esters (3, 5, 9, and 10)[6-8]. Although each was eventually found in analyses of various clones, they were generally present at lower concentrations than a family of novel substances which had rather low toxicity until hydrolyzed, under mild conditions, to yield the six toxins previously known[9,10]. We found[10] two of the new toxins, C1 and C2, to be 4 and 6, the carbamoyl-N-sulfo derivatives of 11α- and 11β-hydroxysaxitoxin sulfate, 3 and 5. Our results[2] suggest that the new compounds are broadly distributed and have possibly been overlooked in previous studies due to their low toxicity, facile hydrolysis, and altered chromatographic properties[11]. We now report the structures of toxins B1 and B2 as 2 and 8, the carbamoyl-N-sulfo derivatives of 1 and 7.

Hydrolysis (0.1 M HCl, 100°C, 5 min) of B1 and B2 to 1 and 7, respectively (^{1}H-NMR, TLC[9], electrophoresis[12]) and the close similarity of the corresponding ^{1}H-NMR (270 MHz, D_2O; Table 1) and ^{13}C-NMR (50 MHz, D_2O) spectra established the basic ring structures of the toxins. Chromatographic behavior[13] and electrophoretic comparison[12] with the dications 1 and 7 showed that B1 and B2 carry a net charge of +1 in acidic solution, implying the presence of a substituent charged -1. B1 and B2 tested negative for free sulfate, but released inorganic sulfate upon



	R1	R2	R3	R4
1	H	H	H	$CONH_2$
2	H	H	H	$CONHSO_3^-$
3	H	H	OSO_3^-	$CONH_2$
4	H	H	OSO_3^-	$CONHSO_3^-$
5	H	OSO_3^-	H	$CONH_2$
6	H	OSO_3^-	H	$CONHSO_3^-$
7	OH	H	H	$CONH_2$
8	OH	H	H	$CONHSO_3^-$
9	OH	H	OSO_3^-	$CONH_2$
10	OH	OSO_3^-	H	$CONH_2$
11	H	H	H	H
12	OH	H	H	H

Table 1: ^{1}H-NMR data for toxins B1 (2) and B2 (8)[a]

	H-5	H-6	H-10	H-10	H-11	H-13	H-13
1	4.33,d (1.3)	3.47,ddd (1.3,6,9)	3.20,ddd (8,10,11)	3.45,ddd (2.5,10,11)	2.00,m	3.65,dd (6,12)	3.88,dd (9,12)
2	4.34,d (1.3)	3.48,ddd (1.3,6,9.5)	3.20,m (b)	3.40,m (b)	2.00,m	3.72,dd (6,13)	4.00,dd (9.5,12)
7	4.42,d (1.1)	3.70,ddd (1.1,6,6.2)	3.18,ddd (7,10.2,9.7)	3.38,ddd (2.7,10.2,10)	1.98,m	3.82,dd (6.2,11.8)	4.00,dd (5.9,11.8)
8	4.43,d (1.1)	3.76,dt (1.1,6.8)	3.24,m (b)	3.39,m (b)	1.95,m	3.88,dd (6.8,12)	4.14,dd (6.8,12)

a) Chemical shifts in ppm with reference to $CHCl_3$ at δ=7.27 as internal standard. Data in parenthesis are coupling constants in Hz.
b) Reliable determination of the H-10 coupling constants for 2 and 8 was precluded by partial deuterium exchange of the H-11, catalyzed by acetate counterion. Fully exchanged samples showed an H-10 geminal coupling constant of 11 Hz for 2 and 8.

hydrolysis to 1 and 7. Combustion analysis of B2 acetate and titration[14] of sulfate liberated by hydrolysis both revealed 1 equivalent of $-SO_3^-$. By analogy to 6, for which x-ray crystallography had confirmed the substitution, structures 2 and 8 appeared likely for B1 and B2.

To confirm this assignment for B1, decarbamoylsaxitoxin (11), prepared[12] from 1 by hydrolysis, was treated with chlorosulfonyl isocyanate in formic acid at 0°C[15]. Rapid quenching with cold, aqueous ammonium acetate followed by chromatography[13] gave, as the major product, the expected N-sulfo saxitoxin 2, identical with natural B1 (^{1}H-NMR, TLC, electrophoresis), and 1 as the minor product[16]. Similar treatment of decarbamoyl neosaxitoxin (12, from 7 by hydrolysis) gave a major product identical (TLC, electrophoresis) to natural B2, and a small amount of 7.

Compounds 2 and 8 were found to have toxicities of 150 and 180 mouse units per micromole which, following hydrolysis[9], increased to 2400 and 2900 mouse units per micromole, the approximate potencies of authentic 1 and 7[2].

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