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NEUROTOXINS OF GONYAULAX EXCAVATA AND BAY OF FUNDY SCALLOPS

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SUMMARY: Neurotoxins isolated and characterized from cultured G. excavata cells and from scallops are described and their relative abundance in both sources is compared.

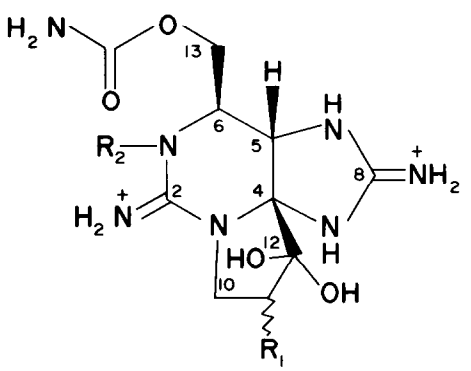
The potent neurotoxins of the saxitoxin family have become important tools in neurophysiological research, because of their highly specific inhibition of the inward sodium current in excitable cells (1,2). These toxins are produced by dinoflagellates of the genus Gonyaulax indigenous to both the Pacific and Atlantic coasts of the United States and Canada and their accumulation in shellfish during "red tide" episodes has often serious economic and public health repercussions in the affected coastal areas. The complexity of toxin mixtures in both dinoflagellates and shellfish is now well recognized (3), but only some of the toxins, including saxitoxin (STX, 1), neosaxi toxin (neoSTX, 2), and 11-hydroxysaxitoxin sulfate (11-OSO_3-STX, 3) have been characterized (4-7).

We report here the isolation of toxins from both laboratory-cultured (8) Gonyaulax excavata (an Atlantic Coast species also referred to as G. tamarensis var. excavata) and from scallops (Placopecten magellanicus) collected in the Bay of Fundy and document distinctive differences in relative toxin content between the two sources. In addition to the known toxins 1, 2, and 3, we have isolated and characterized three related compounds 4, 5, and 6.

Cells and scallop hepatopancreas were extracted with distilled water and after removal of organic soluble components ($CHCl_3$/MeOH), the aqueous toxin extract was purified by BioGel P4 and BioGel P2 chromatography (0.1N HOAc). Subsequent ion exchange chromatography (BioRex 70, H^+ form, eluted with H_2O, 1 mM HCl, 20 mM HCl) separated the toxins into a less-basic and more-basic toxin fraction. From a less-basic fraction derived from both cells and scallops were isolated (by further chromatography over BioRex 70, BioGel P2 and TLC as required) the same four components, namely 11α- and 11β-hydroxysaxitoxin sulfate (3 and 4) and 11α- and 11β-hydroxyneosaxitoxin sulfate (5 and 6), of which one (11α-(OSO_3)STX, 3, obtained from scallops) has been previously identified. The basic fraction of cells contained only the known neoSTX (2), whereas scallops yielded STX (1), neoSTX (2), and an unknown compound.

Characterization of the four less-basic toxins is based on correlation of their spectral data with known compounds and chemical interconversions. Structure analysis of 11α-(OSO_3)STX (3) has been described previously (7). The identification of the corresponding β-epimer 4 is based on a) the NMR data (270 MHz; D_2O solvent) for 3 and 4 (Table I), which show an analogous pattern except for the chemical shifts of the H10 protons and the H10/H11-coupling pattern (see discussion below),

b) hydrolysis of 4 to the hydroxy-ketone 8 which like the hydroxy-ketone 7 derived from 3 is rapidly degraded by periodate under conditions where the sulfate esters 3 and 4 are stable, c) correlation of the NMR spectra (Table I) of the β-hydroxy compound 8 and its known α-epimer 7, and d) epimerization of pure 3 and of pure 4 to equilibrium mixtures containing 3 and 4 in a ratio of ca 4:1 (as determined by TLC and NMR).



1 $R_1 = R_2 = H$

2 $R_1 = H, R_2 = OH$

3 $R_1 = \alpha\text{-}OSO_3^-, R_2 = H$

4 $R_1 = \beta\text{-}OSO_3^-, R_2 = H$

5 $R_1 = \alpha\text{-}OSO_3^-, R_2 = OH$

6 $R_1 = \beta\text{-}OSO_3^-, R_2 = OH$

7 $R_1 = \alpha\text{-}OH, R_2 = H$

8 $R_1 = \beta\text{-}OH, R_2 = H$

9 $R_1 = \alpha\text{-}OH, R_2 = OH$

Table I. ^1H NMR Data for toxins 1 - 9

proton	1	2	3	4	5	6	7	8	9
H-11	2.00 m	1.98 m	4.44 d (4.8)	4.56 dd (7.5,8.5)	4.45 d (4.9)	4.57 dd (7.5,7.4)	3.90 d (5.1)	4.07 dd (7,8.1)	3.91 d (5)
H-10	3.20 ddd (8,10,11)	3.18 ddd (7,10.2,9.7)	3.60 dd (5.0,12.7)	3.18 dd (7,11)	3.57 dd (4.9,11.6)	3.21 dd (7.4,10)	3.35 d (11.4)	2.89 dd (7.3,10.6)	3.33 d (11.8)
H-10	3.45 ddd (2.5,10,11)	3.38 ddd (2.7,10.2, 10)	3.76 d (12.1)	3.75 dd (8.8,11)	3.75 d (11.3)	3.78 dd (7.5,10.4)	3.48 dd (5.1,11.8)	3.64 dd (8.4,10.3)	3.47 dd (5,11.5)
H-6	3.47 m (1.3,6,9)	3.70 m (1.1,6.0,6.2)	3.46 dd (5.6,9)	3.41 dd (6,9)	3.70 dd (5.4,6)	3.72 dd (6.5,6)	3.46 m	3.44 ddd (1.1,5.5, 9.6)	3.71 m (6,6.4)
H-13	3.65 (6,12)	3.82 dd (6.2,11.8)	3.64 dd (5.4,11.9)	3.67 dd (5.5,12)	3.83 dd (5.9,12.3)	3.89 dd (5.9,12.2)	3.63 dd (5.1,11.4)	3.65dd (5.5,12)	3.81 dd (6.3,11.6)
H-13	3.88 dd (9,12)	4.00 dd (5.9,11.8)	3.85 dd (9.3,11.2)	3.87 ddd (9,11.8)	4.01 dd (5.8,11.8)	4.04 dd (6,12.6)	3.85 dd (9.2,11.4)	3.88 dd (9.2,11.4)	4.01 dd (6,12.4)
H-5	4.33 d (1.3)	4.42 d (1.1)	4.41 d (1)	4.41 d (1)	4.50	4.54	4.39 d (1.1)	4.43 d (1.1)	4.47

Chemical shifts in ppm downfield from TMS ($CHCl_3$ standard = 7.27 ppm). Coupling constants in parentheses are given in Hz. Proton assignments for compounds 1, 2, 3, 5, 6 and 8 were confirmed by decoupling experiments.

The structure of 11α-(OSO_3)neoSTX (5) is based on the following observations: a) the chemical shifts and coupling patterns of H5, H6, and H13 in 5 (Table I) correlate exactly with the proton pattern in the known neoSTX (2) and indicate an N-1-OH substituent (6,10), b) comparison of the H10/H11 proton patterns of 5 with 3 indicating a C-11-OSO_3^- substituent, c) by hydrolysis of 5

yielding inorganic sulfate (determined by precipitation with $BaCl_2$) and the hydroxy-ketone 9 which, unlike 5, is sensitive to periodate, and d) the correlation of the NMR spectrum of 9 with that of neoSTX (2), and the known hydroxy-ketone 7 (Table I).

The characterization of 11β-(OSO_3)neoSTX (6) is based on NMR correlation with the corresponding α compound 5 and 11β-(OSO_3)STX (4). The pronounced shift of the H5, H6, and H13 protons of 6 correlate with those of 11α-(OSO_3)neoSTX (5) and there is a similar match between the H10 and H11 signals of 4 and 6 (Table I). Furthermore, epimerization of 6 yields an equilibrium mixture of 5 and 6 (ca 5:1, by TLC and NMR).

The less-basic fraction, therefore, consists of two epimeric pairs of compounds, 11α- and 11β-(OSO_3)STX, (3) and (4), and 11α- and 11β-(OSO_3)neoSTX, (5) and (6). Although the stereochemistry of the 11-OSO_3 substituent cannot at this stage be rigorously determined, we propose the α configuration for 3 and 5 since that stereochemistry would most readily account for the distinctive H10-H11 coupling pattern. This pattern correlates well with the NMR spectrum of STX (1) where the 11β-H subtends an approximately 90° dihedral angle with the downfield H10 (11). Thus, an 11α-OSO_3 substituent, resulting in a small or zero coupling between 11β-H and 10α-H, would explain the doublet and quartet patterns observed for the two H10-protons in toxins 3 and 5. In the corresponding β-epimers, the 11α-H would subtend angles of approximately 30° and 150° with the two H10 protons and thus give rise to the pattern observed for toxins 4 and 6 where both C-10 protons appear as a doublet of doublets. This assignment is in accord with a previous proposal (12) which suggested, on the basis of steric considerations, the α-configuration for the predominant component 3 in an equilibrium mixture of 3 and 4.

Table II: Distribution of Toxins from G. Excavata and Scallops

Compound	% of total toxin[a]	
	G. excavata	Scallops
α 11-(OSO_3)STX (3)	~9	58
β 11-(OSO_3)STX (4)	41	11
α 11-(OSO_3)neoSTX (5)	~9	3
β 11-(OSO_3)neoSTX (6)	30	<1
neoSTX (2)	11	1
STX (1)	0	20
Unknown toxin	0	7

[a]Percentages determined using a combination of TLC and ion exchange chromatography. Scallops were collected September, 1979.

The distribution of toxic compounds in G. excavata and scallops shows some striking differences. Most notable is the change in the relative amounts of each of the sulfates - the epimers of 11-(OSO_3)STX and 11-(OSO_3)neoSTX (see Table II). In G. excavata, the β-epimers 4 and 6 are the predominant species (ca 70%). The α-epimers 3 and 5 account for only ca 18% of the total toxicity. This

contrasts sharply with Bay of Fundy scallops where the α epimers predominate (ca 60%), whereas the β-epimers (4 and 6) represent only 12% of the total toxicity. The second difference between G. excavata and scallop toxins relates to the composition of the basic toxins. In G. excavata, neoSTX (2) is the only detectable basic toxin. In scallops, neoSTX (2) comprises <5% of the total basic toxins, whereas STX (1) is the major component, accompanied by an as yet uncharacterized compound, which based on NMR is an STX analog. These comparisons suggest that upon accumulation and storage of dinoflagellate toxins in scallops, epimerization of the initially dominant β-epimers to the more stable α-epimers occurs. Furthermore, the dramatically reduced abundance of neoSTX (2) in scallops relative to STX (1) which is absent in dinoflagellate cells suggests the possibility of conversion of neoSTX to the deoxy-compound 1 (3). The compositional trend observed for the sulfate esters, where again the ratio of deoxy-compounds 3 and 4 to N1-OH analogs 5 and 6 is strikingly enhanced in scallops relative to their ratio in cells, tends to support this speculation. We have observed also that increased storage time of frozen scallops causes a decrease in N1-OH-toxin content. For example, scallops analyzed shortly after harvest gave a neoSTX/STX ratio varying between 1.5-1:1 for different collections, whereas an anlysis two years after collection indicated the presence of the deoxy-compounds 1, 3 and 4 only.

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