Short Communication

Natural Occurrence of Decarbamoylsaxitoxin in Tropical Dinoflagellate and Bivalves†

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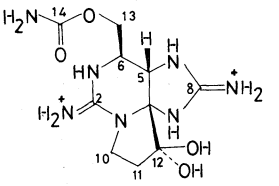
The incidence of paralytic shellfish poisoning (PSP) in tropical and subtropical waters has only become known in recent years. Worth *et al.*[1] were the first to report outbreaks of PSP-like shellfish poisoning in Papua New Guinea and mention its possible relationship with a red tide of a dinoflagellate, *Pyrodinium bahamense*, which was later amended to *P. bahamense* var. *compressa*.[2] The nature of the causative toxins, however, had not been clarified until the authors identified toxins of the saxitoxin family in Palauan specimens of the same species of dinoflagellate as well as in bivalves infested by the organism.[3] This dinoflagellate was characterized by the presence of gonyautoxins V, VI ($GTX_{5,6}$) and a new toxin tentatively coded PBT.[3] The former two toxins were determined as the derivatives of saxitoxin (STX, **1**) and neosaxitoxin (neoSTX) with a sulfonatocarbamoyl function, respectively.[4] In the present paper we report the isolation and chemical structure of PBT.

Bivalves, *Spondylus butleri*, were collected at Arumizu Bay, Palau, where *P. bahamense* var. *compressa* was proliferating. Minced whole body was extracted three times at room temperature using 75% ethanol acidified to pH 3 with HCl. After concentration under reduced pressure, the extracts were washed with chloroform. The aqueous phase was freed from chloroform under vacuum, adjusted to pH 5.5, and treated successively with activated charcoal, Bio-Gel P-2 (Bio-Rad Laboratories) and Bio-Rex 70 (Bio-Rad Laboratories, 100～200 mesh, H^+ form) as described previously.[4] The mixture of neoSTX, PBT and STX obtained from the last column was rechromatographed on a Bio-Rex 70 (−400 mesh) column (0.5 × 100 cm) by a linear gradient elution with acetic acid (0～0.2 N). PBT was eluted from the column between neoSTX and STX, with considerable overlapping with neoSTX. Concomitant neoSTX was eliminated by chromatographing the sample on a column of LiChroprep Si 60 (Merck) with pyridine–ethyl acetate–acetic acid–water (30 : 30 : 6 : 8). The eluates were monitored both by mouse assay and TLC to obtain pure PBT fractions. The residues recovered from the combined fractions were further treated on a Bio-Gel P-2 column. The homogeneity of PBT thus obtained was confirmed by TLC and electrophoresis as described previously.[4] From 1.05 kg of the bivalve meat, 4.9 mg of PBT and 22 mg of STX were obtained.

The CMR and PMR spectral data of PBT are listed in Table I, in comparison with those of STX and decarbamoylsaxitoxin (dcSTX, **2**) which was prepared from STX after the method of Koehn *et al.*[5] The absence of a C-14 signal and the upfield shift of the C-13 signal in



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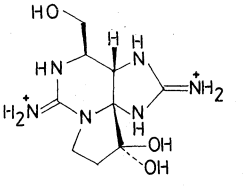

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† Studies on Paralytic Shellfish Poisoning in Tropical Waters. Part VIII.

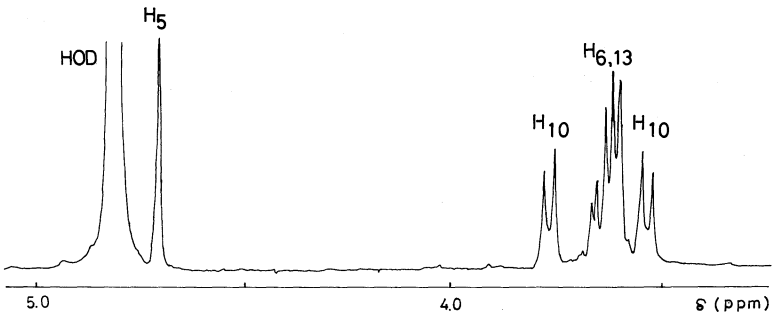

FIG. 1. Proton NMR Spectrum of PBT (400 MHz) after Deuterium Replacement at C-11.

TABLE I. COMPARISON OF CMR (100 MHz) AND PMR (400 MHz) OF PBT, SAXITOXIN AND DECARBAMOYLSAXITOXIN

CMR chemical shifts are expressed in ppm downfield from TMS (solvent, 50% CD_3CN in H_2O; internal standard, dioxane = 67.4 ppm). PMR chemical shifts are expressed in ppm downfield from TMS (solvent, 99.9% D_2O; internal standard, *tert*-BuOH = 1.23 ppm). Coupling constants in parentheses are given in Hz.

No. C, H	PBT		STX		dcSTX	
	CMR	PMR	CMR	PMR	CMR	PMR
14	—		158.4 (s)		—	
8	158.0 (s)		158.2 (s)		158.0	
2	156.1 (s)		156.1 (s)		156.1	
4	82.5 (s)		82.6 (s)		82.4	
5	56.5 (d)	4.71 (1)	56.8 (s)	4.73 (1)	56.5	4.70 (1)
6	55.7 (d)	3.63 (m)	53.2 (d)	3.81 (5, 9, 12)	55.7	3.63 (m)
10	42.9 (t)	3.77 (10)	42.9 (t)	3.79 (10)	42.9	3.76 (10)
		3.54 (10)		3.57 (10)		3.54 (10)
11	32.9 (t)	2.39 (m)	33.0 (t)	2.39 (m)	32.9	2.39 (m)
12	98.7 (s)		98.6 (s)		98.7	
13	61.4 (t)	3.63 (m)	63.2 (t)	4.01 (5, 12)	61.3	3.63 (m)
		3.63 (m)		4.29 (9, 12)		3.63 (m)

the CMR spectrum suggest that PBT lacks the carbamoyl function. Conforming to the shift of the C-13 signal, those for the 13-protons of PBT shifted to a higher field. Moreover, the double quartet pattern of the 13-protons observed for STX was transformed into a single complex multiplet, indicating the removal of any rotational restriction imposed by the carbamoyl function (Fig. 1). Complete agreement in both CMR and PMR spectra can be seen between PBT and dcSTX (Table I). A comparison of STX, PBT and dcSTX by TLC with various combinations of adsorbents and solvents are presented in Table II. PBT is indistinguishable from dcSTX in all the systems employed. In electrophoresis, the relative mo-

TABLE II. TLC *Rf* VALUES FOR PBT, SAXITOXIN AND DECARBAMOYLSAXITOXIN

Solvent[a]	I	II	III		IV	
Adsorbent[b]	A	A	A	B	A	B
PBT	0.24	0.40	0.32	0.77	0.49	0.46
STX	0.24	0.40	0.31	0.85	0.55	0.59
dcSTX	0.24	0.40	0.32	0.77	0.49	0.46

[a] I, *tert*-BuOH–H_2O–AcOH = 2 : 1 : 1; II, EtOH–H_2O–AcOH = 12 : 5 : 3; III, $CHCl_3$–EtOH–AcOH–H_2O–NH_4OH = 10 : 30 : 2 : 10 : 1; IV, Py–AcOEt–AcOH–H_2O = 15 : 7 : 3 : 6.

[b] A, silicagel 60 precoated (Merck); B, silicagel 60 silanized (Merck).

bility of PBT and dcSTX to STX (1.00) was found identical (1.05). Carbamoylation of PBT with chlorosulfonylisocyanate under the conditions described by Tanino *et al.*[6] afforded a 55% yield of a compound which was indistinguishable from STX by both TLC and electrophoresis. The specific activity of PBT based on the mouse assay[7] was estimated to be 4200 ± 500 mouse units per mg, which was compatible with the reported value for dcSTX (3700 ± 400).[5] All these results unanimously support that PBT is dcSTX (**2**).

The present work is the first to confirm the occurrence of dcSTX in nature. Despite the extensive studies conducted on toxins involved in PSP in northern waters, its natural occurrence has not yet been reported. In tropical waters, on the other hand, its occurrence seems rather common. Toxic crabs[8] from coral reefs also contain a component identical to dcSTX (=PBT) in chromatographic and electrophoretic properties, although their toxins derive from a red calcareous alga, *Jania* sp.[9] The wide distribution of dcSTX among tropical specimens may be taken as a distinction of PSP in warm waters.

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