Structures of New Pectenotoxin Analogs, Pectenotoxin-2 Seco Acid and 7-*epi*-Pectenotoxin-2 Seco Acid, Isolated from a Dinoflagellate and Greenshell Mussels

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Two new pectenotoxin analogs, pectenotoxin-2 seco acid (PTX2SA) and 7-*epi*-pectenotoxin-2 seco acid (7-*epi*-PTX2SA), were isolated from the dinoflagellate *Dinophysis acuta* and greenshell mussels, *Perna canaliculus*. Their structures were elucidated by NMR and negative ion FAB CID MS/MS experiments.

Pectenotoxin-2 (**1**) is a polyether macrolide toxin involved, together with okadaic acid (OA) and dinophysistoxin-1 (DTX1), in diarrhetic shellfish poisoning.[1] The toxin is produced by the dinoflagellate *Dinophysis fortii* and is metabolized in shellfish to other PTXs shown in Figure 1. The absolute configuration of PTX6 and stereoisomers at C7 were recently determined.[2,3] Although hepatotoxic, PTX2 has attracted renewed attention because of its selective as well as potent cytotoxicity against human lung, colon, and breast cancer cell lines.[4,5] Therefore, we continued the search for new PTX analogs. In 1996 November, greenshell mussels, *Perna canaliculus*, cultivated at Marlborough Sounds, New Zealand, were exposed to a dinoflagellate bloom within which *D. acuta* was one of the dominant species. In Ireland *D. acuta* is the progenitor of diarrhetic shellfish toxins, DTX2 and OA.[6] Analysis of mussels from New Zealand and *D. acuta* from Ireland revealed the presence of new pectenotoxin analogs produced by *D. acuta*. In this paper, we report structural elucidation of the new analogs, pectenotoxin-2 seco acid (**2**) and 7-*epi*-pectenotoxin-2 seco acid (**3**).

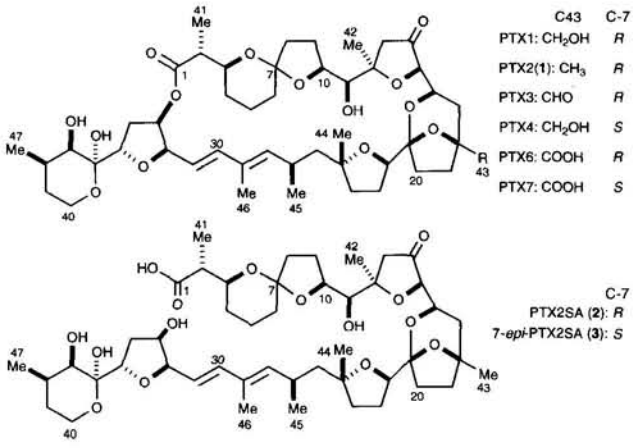

Figure 1. Structures of pectenotoxins (PTXs).

Digestive glands of mussels (1 kg) from New Zealand were extracted with 80% MeOH. The residue from the extract was partitioned between EtOAc and H_2O. The new analogs in the organic layer were chromatographed on an alumina column (ICN Biomedicals) with $CHCl_3$-MeOH (1:1), MeOH, and finally 1% NH_4OH-MeOH (1:1). The residue in the last eluate was chromatographed successively on a silica gel column (Merck) with $CHCl_3$ and $CHCl_3$-MeOH (7:3, 1:1), on a reversed phase ODS-Q3 column (Fuji Gel) with MeOH-H_2O (1:1, 6:4, 8:2) and MeOH, and on a Toyopearl HW-40 column (Tosoh) with MeOH-H_2O (1:1). Final HPLC purification on a Develosil ODS-7 column (Nomura Chemicals) with MeOH:MeCN:H_2O (2:1:2) led to the isolation of **2** and **3** in 180 μg and 340 μg yields, respectively. Both **2** and **3** showed UV spectra comparable with that of **1**.[7] Through the isolation procedure, a small portion of **2** was epimerized to **3** as is the case with PTX6 and PTX7.[3] Elution of **2** and **3** from the columns was monitored by UV absorption at 235 nm.

The ESI-MS spectrum of PTX2SA gave an ion peak corresponding to $(M-H)^-$ at *m/z* 875, implying that **2** was 18 mass units larger than PTX2 ($C_{47}H_{70}O_{14}$). The HR-FAB MS data gave the molecular formula of **2** as $C_{47}H_{72}O_{15}$ (MNa^+ 899.4797 Δ +2.8 mmu). From the MS data it was conceived that 1,33-lactone of **1** was hydrolyzed in **2**. In support of this, **2** was easily labeled with a fluorogenic reagent 9-

Table 1. ^1H NMR chemical shifts (δ) of PTX2 (**1**), PTX2SA (**2**), and 7-*epi*-PTX2SA (**3**)

Pos	1	2	3	Pos	1	2	3
2	2.31	2.38	2.32	24	1.43	1.58	1.59
3	3.46	3.63	3.91		1.62	1.73	1.74
4	1.18	1.25	1.29	26	1.56	1.54	1.54
	1.51	1.62	1.57		1.71	1.65	1.64
5	1.59	1.58	1.67	27	2.59	2.71	2.72
	1.84	1.83	1.82	28	5.27	5.33	5.35
6	1.69	1.65	1.59	30	6.48	6.29	6.32
	1.72	1.69	1.66	31	5.42	5.69	5.70
8	1.54	1.49	1.71	32	4.77	4.41	4.42
	2.50	2.48	1.85	33	5.48	4.21	4.21
9	1.65	1.90	1.91	34	2.11	2.05	2.08
	2.07	2.05	2.02		2.22	2.13	2.14
10	4.29	4.24	3.99	35	4.50	4.48	4.49
11	4.01	3.79	3.70	37	3.29	3.26	3.27
13	1.98	2.16	2.28	38	2.13	2.09	2.11
	2.85	2.82	2.77	39	1.27	1.20	1.23
15	3.81	3.87	3.93		1.67	1.62	1.62
16	4.27	4.21	4.25	40	3.69	3.59	3.62
17	1.32	1.25	1.28		3.99	3.89	3.92
	2.09	2.07	2.11	41	1.09	1.17	1.12
19	1.68	1.67	1.69	42	1.20	1.21	1.33
	1.91	1.86	1.90	43	1.34	1.34	1.32
20	2.00	2.01	2.02	44	1.22	1.23	1.21
	2.19	2.09	2.11	45	0.97	0.95	0.98
22	3.83	3.83	3.86	46	1.70	1.79	1.79
23	1.68	1.79	1.82	47	0.95	0.92	0.94
	2.04	1.95	1.97				

CHD_2OD taken as 3.31 ppm.

anthryldiazomethane which reacts with carboxylic acids.[8] Further structural elucidation of **2** was carried out mainly by comparing the NMR data between **1** and **2**.[9] On the ^{1}H-^{1}H COSY and TOCSY spectra, eight partial structures (H2-H6, H8-H11, H13, H15-H17, H19-H20, H22-H24, H26-H35, and H37-H40) were elucidated. Proton connectivities in these partial structures were exactly the same as those in **1** including the positions of interruption by quaternary carbons. Assembling the partial structures by measuring long range ^{1}H-^{13}C correlations around quaternary carbons by the HMBC spectrum was prevented by the scarcity of **2**. In the ROESY spectrum NOE correlations from H3 to H8, from Me42 to H10, H11, H13, and H15, from Me43 to H17 and H19, from Me44 to H20, and from H37 to H34 were observed as are in **1**. Thus, stereostructures and connectivities of **2** should be identical with those of **1**. Both UV maximum and NOEs indicated that the geometries of the conjugated double bonds were both *E* as in **1**. Proton chemical shift value of H33 was markedly shifted upfield from 5.48 ppm in **1** to 4.21 ppm in **2**, supporting the postulated hydrolysis of the 1,33-ester bond in **1** (Table 1). Slight differences of ^{1}H chemical shifts and coupling constants observed between **1** and **2** on H10, H11, H31, and H32 presumably arose from conformational changes as the result of the ring opening of the macrolactone.

Negative ion collision induced dissociation (CID) MS/MS experiments were carried out on $(M - H)^-$ ion (*m/z* 875) of **2** to further confirm the proposed structure (Figure 2). Prominent product ions at *m/z* 789, 743, and 717 arising from cleavages around C36 were observed. Product ions at *m/z* 549, 493, 465, 367 and 340 explained connectivities from C16 to C24, and ions at *m/z* 283, 167, 155 and 127 were in accordance with the structure around C7 and C12. Other prominent product ions were generated by bond cleavages at the sites characteristic of ether rings, as established in previous experiments on amphidinol, yessotoxin, and maitotoxin.[10] All of these data allowed us to assign the structure of PTX2SA as shown in **2**.

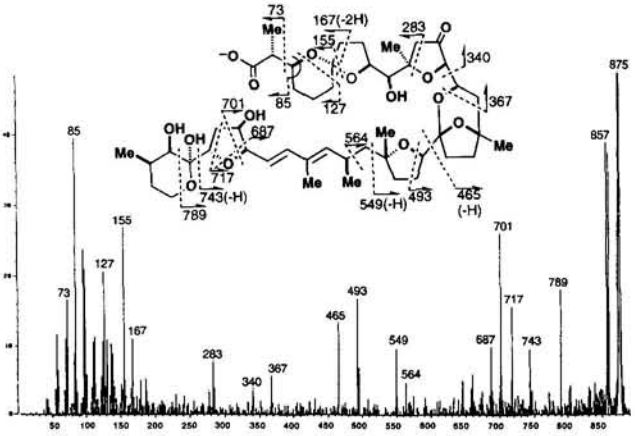

Figure 2. Negative ion CID MS/MS spectrum of PTX2SA (**2**).

In the ESI-MS spectrum of 7-*epi*-PTX2SA was observed an ion peak corresponding to $(M-H)^-$ at *m/z* 875. The CID FAB MS/MS experiments carried out on the $(M-H)^-$ ion as the precursor produced the same fragment ions as those of **2**. These results suggested that **2** and **3** were stereoisomers as are PTX1 and PTX4, and PTX6 and PTX7.[3] Connectivities and signal shapes of **3** deduced from NMR spectra were identical with those of **2**. Also observed, respectively, were NOE correlations from Me42 to H10, H11, H13, and H15, from Me43 to H17 and H19, from Me44 to H20 and from H37 to H34. However, an NOE correlation between H3 and H8 was not observed. Instead of this key NOE, an NOE from Me41 to H10 was observed. This result indicated that the oxygen substituent at C7 in **3** is axially disposed as in PTX4 and PTX7.[3] In support of this, the resonance for H3 in **3** was shifted downfield by 0.28 ppm compared with that of **2** (Table 1). Therefore, the configuration at C7 was deduced to be *S*. Proton chemical shift value of H33 was also shifted upfield from 5.48 ppm in **1** to 4.21 ppm in **3**. Moreover, on the HMBC spectrum of **3**, a correlation from Me41 to C1 (179.3 ppm) was observed. This carbon chemical shift value well matched that of a carboxylic acid but not that of an ester carbon. These results confirmed that the 1,33-ester bond in **1** was cleaved to produce a carboxylic acid moiety at C1 and a hydroxy group at C33. Consequently, the structure of 7-*epi*-PTX2 seco acid was unambiguously deduced to be **3**.

Neither PTX2SA nor 7-*epi*-PTX2SA showed cytotoxicity against KB cells at a dose 1.8 μg/ml, while PTX2 did at 0.05 μg/ml, indicating the importance of the cyclic structure of **1** to exert the potency. In contrast to *D. fortii*, *D. acuta* did not contain PTX2. The absence of **2** and **3** in Japanese scallops heavily contaminated by PTX2 confirmed that they were not the artifacts of PTX2 produced during purification.

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7 PTX2SA (**2**): UV (MeOH) 234.5 nm (ε 17000). 7-*epi*-PTX2SA (**3**): UV (MeOH) 237 nm (ε 26000). Extremely small amounts of **2** and **3** hampered measuring their specific rotations.
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