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Pinnatoxins B and C, the most toxic components in the pinnatoxin series from the Okinawan bivalve *Pinna muricata*

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Abstract—Pinnatoxins B and C, the most toxic components in the pinnatoxin series, were successfully purified from the Okinawan bivalve *Pinna muricata*. Their gross structures were determined based on NMR and MS/MS spectral analyses. Their stereostructures were mainly determined by transformation reactions. Pinnatoxins B and C, C-34 epimers of each other, have an amphoteric macrocycle composed of 6,7-spiro, 5,6-bicyclo and 6,5,6-trispiro ketal rings, the same as in pinnatoxins A and D. © 2001 Elsevier Science Ltd. All rights reserved.

In our continuing work on the identification of seafood poison(s) resulting from ingestion of the bivalve *Pinna* sp.,[1] we previously reported the isolation and structural determination of the major toxic component, pinnatoxin A, and an alkaloidal marine toxin, pinnamine. However, there was such a small proportion of the most potent toxic component(s) in *P. muricata* that purification became very difficult. Finally, we have now successfully obtained pinnatoxins B and C in a 1:1 mixture. We report here the isolation and structural determination of these two compounds.

The aqueous 80% EtOH extract of viscera (21 kg) of *P. muricata* was partitioned between EtOAc and H_2O. The aqueous fraction was chromatographed on TSK G-3000S polystyrene gel (50% EtOH), Sephadex LH-20 (MeOH), DEAE Sephadex A-25 (0.02 M phosphate buffer), reversed-phase MPLC (ODS, MeCN–H_2O–TFA) and reversed-phase HPLC (ODS, MeOH–H_2O–TFA) guided by acute toxicity against mice. Final purification was achieved by reversed-phase HPLC (ODS, MeOH–H_2O–TFA) to give pinnatoxins B (**1**) and C (**2**) in a 1:1 mixture (0.3 mg, LD_{99} 22 μg/kg).[2]

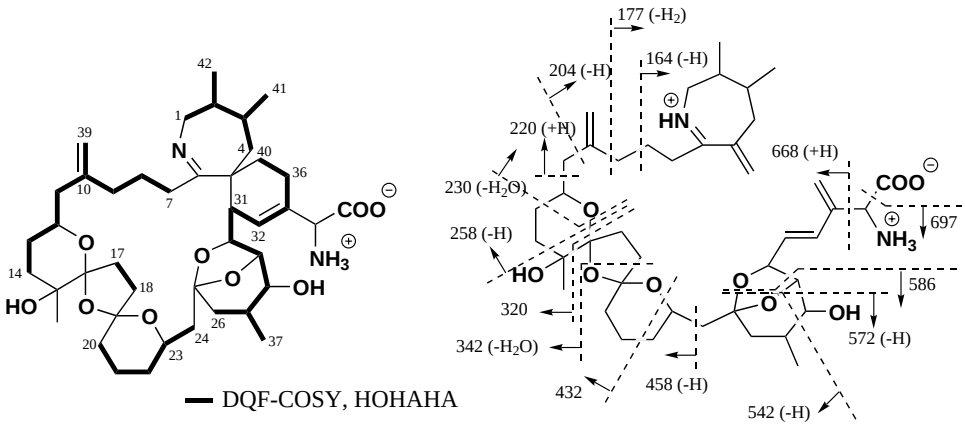

Figure 1. Partial structures and fragmentation patterns of Pinnatoxins B (**1**) and C (**2**).

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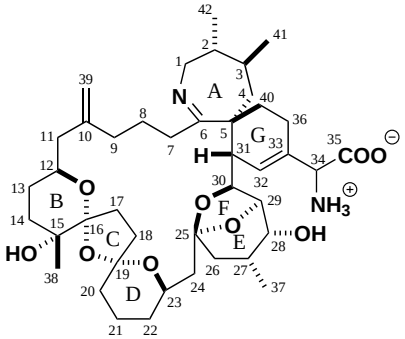
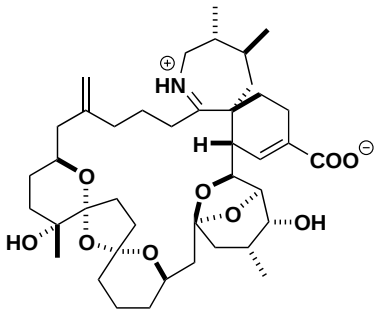
Table 1. ^{1}H NMR data for pinnatoxins B (**1**) and C (**2**)[a]



Pinnatoxin B (**1**): 34*S* isomer
Pinnatoxin C (**2**): 34*R* isomer



Pinnatoxin A (**3**)

Pinnatoxin B (**1**)						Pinnatoxin C (**2**)					
Atom	^{1}H[a]	^{13}C[b]	Atom	^{1}H[a]	^{13}C[b]	Atom	^{1}H[a]	^{13}C[b]	Atom	^{1}H[a]	^{13}C[b]
1	3.67, 4.27	50.9	22	1.28, 1.67	31.2	1	3.67, 4.27	50.9	22	1.28, 1.67	31.2
2	1.71	38.5	23	4.05	69.8	2	1.69	38.3	23	4.05	69.8
3	1.42	34.1	24	1.90, 2.01	44.0	3	1.38	34.0	24	1.90, 2.01	44.0
4	1.78, 1.98	34.5	25			4	1.78, 1.98	34.5	25		
5			26	1.62, 1.73	40.5	5			26	1.62, 1.73	40.5
6			27	2.18	30.0	6			27	2.18	30.0
7	3.57 (2H)	34.9	28	3.74	66.0	7	3.57 (2H)	34.9	28	3.86	65.9
8	1.98, 2.08	20.8	29	4.61	80.6	8	1.98, 2.08	20.8	29	4.54	80.5
9	1.88, 1.94	32.7	30	3.91	78.5	9	1.88, 1.94	32.7	30	3.87	78.5
10			31	3.52	43.0	10			31	3.59	43.0
11	2.20, 2.39	45.4	32	5.46	134.4	11	2.20, 2.39	45.4	32	5.44	135.0
12	4.10	68.6	33			12	4.10	68.6	33		
13	1.34, 1.70	28.5	34	4.07	59.5	13	1.34, 1.70	28.5	34	4.11	60.4
14	1.53, 1.91	34.3	35			14	1.53, 1.91	34.4	35		
15			36	2.36, 2.43	22.1	15			36	1.08, 2.30	21.5
16			37	1.05 (3H)	15.6	16			37	1.04 (3H)	15.6
17	1.77, 2.20	30.3	38	1.24 (3H)	21.6	17	1.77, 2.20	30.3	38	1.24 (3H)	21.6
18	1.85, 2.06	37.9	39	4.89, 4.94	121.3	18	1.85, 2.06	37.9	39	4.89, 4.94	121.3
19			40	1.80, 2.03	32.2	19			40	1.80, 2.03	32.2
20	1.53, 1.91	34.4	41	1.10 (3H)	19.7	20	1.53, 1.91	34.4	41	1.09 (3H)	19.7
21	1.66, 1.85	20.3	42	1.23 (3H)	18.6	21	1.66, 1.85	20.3	42	1.23 (3H)	18.6

[a] Recorded at 800 MHz in CD_3OD.
[b] Determined by HMQC experiments.

The molecular formula of both **1** and **2** was determined to be $C_{42}H_{64}N_2O_9$ by ESIMS (*m*/*z* 741.4707, calcd for $C_{42}H_{65}N_2O_9$ $[M+H]^+$, 741.4690), which reflects a 29 MS unit (CH_3N) increase compared with that of pinnatoxin A (**3**). A positive ninhydrin test on a TLC plate for **1** and **2** suggested the presence of an amino group. The ^{1}H NMR spectrum showed duplicate signals (1:1) for H-2, H-3, H-28 to H-37 and H-41, suggesting the presence of epimeric isomers (Table 1). A detailed analysis of DQF-COSY and HOHAHA spectra allowed the five partial structures shown in Fig. 1. The chemical shifts and the coupling constants of protons in

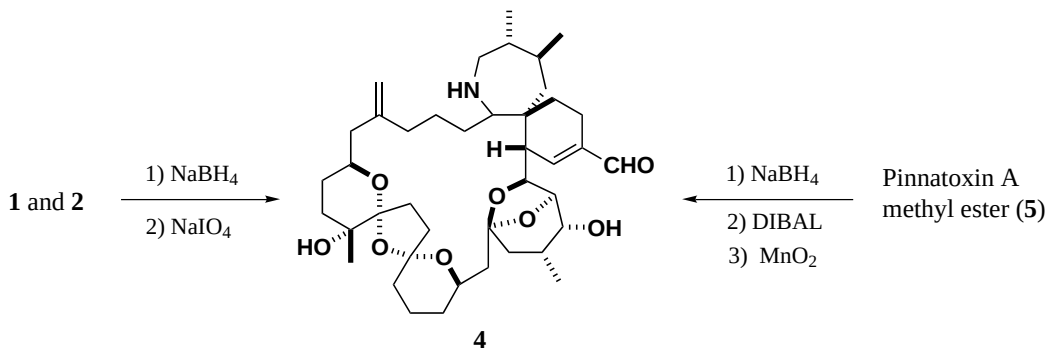


Figure 2. Interrelationship of macrocycles in pinnatoxins.

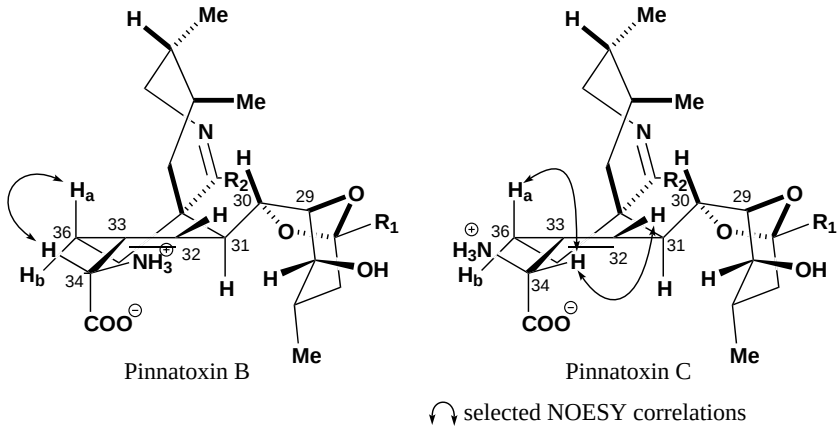


Figure 3. NOESY correlations of pinnatoxins B (**1**) and C (**2**).

these five parts of **1** and **2** strongly resembled those of **3**, expect for H-32 (δ_H 5.46, 5.44 ppm). This result suggested that **1** and **2** consist of the same polyether macrocycles as **3**, which is composed of 6,7-spiro, 5,6-bicyclo and 6,5,6-trispiro ketal rings, and that the side chains in **1** and **2** were different from that in **3**. The presence of an α-amino acid function in **1** and **2** was revealed by characteristic NMR signals, δ_H 4.07 and 4.11 ppm and δ_C 59.5 and 60.4 ppm determined by HMQC experiments, that corresponded to those of typical α-amino acids. The amino acid moiety [-CH(NH_3^+)COO^-] must be connected to C-33 based on the molecular formula. Furthermore, positive ion ESI MS/MS of pinnatoxins showed a series of prominent fragment ions generated by a G ring-opening reaction, the retro Diels–Alder reaction of **3**, followed by bond cleavage of carbocycles (Fig. 1).[3] As expected, the fragment ions of the polyether macrocycle moiety in **1** and **2** were identical to those of **3**. Therefore, the gross structures of pinnatoxins B and C were determined to be **1** and **2**. The stereochemistries of the macrocycles in **1** and **2** were determined as follows (Fig. 2). Reduction of the imino group in the methyl ester of pinnatoxin A with $NaBH_4$[4] followed by transformation of a carboxylic acid to an aldehyde provided aldehyde **4**.[5] This aldehyde **4** was also obtained by reduction of the imino group in **1** and **2** with $NaBH_4$ followed by oxidative cleavage with $NaIO_4$. The coincidence between aldehyde **4** derived from **1** and **2** and that from **3** was confirmed by detailed analysis by NMR, MS and TLC. As a result, the relative stereochemistries of the macrocycles in **1** and **2** were determined to be the same as those in **3**. Recently, Kishi's group achieved the total synthesis of pinnatoxin A (**3**) and *ent*-**3**. They reported that only natural pinnatoxin A was toxic.[6] Therefore, based on this observation, the absolute stereochemistries of the macrocycles in pinnatoxins B and C were suggested to be as shown in **1** and **2**.

Structural differences between **1** and **2** were distinguished by NOESY data (Fig. 3). In addition to the observation of a NOESY correlation between H-34/H-36a in both **1** and **2**, only H-34 assigned for **2** correlated to H-32 in the NOESY spectrum. To minimize the allylic strain of carboxyl group, the bulkiest substituent at C-34, the carboxyl group may be perpendicular to the olefin in G ring.[7] Therefore, we can propose that the stereochemistries at C-34 in pinnatoxins B and C are 34*S* and 34*R*, respectively.

In conclusion, pinnatoxins B and C are the most potent toxins in the pinnatoxin series from the Okinawan bivalve *P. muricata*. The gross structures were determined by analyses of 2D NMR and positive ion ESI MS/MS spectra. These stereostructures were determined by correlation between pinnatoxin A and pinnatoxins B and C. However, we were unable to separate pinnatoxins B and C from each other. Therefore, it should be resolved that the actual toxin is both of them combined or an alternative isomer.

Acknowledgements

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References

1. (a) Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S.; Chen, H. *J. Am. Chem. Soc.* **1995**, *117*, 1155; (b) Chou, T.; Kamo, O.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4023; (c) Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4027; (d) Chou, T.; Kuramoto, M.; Otani, Y.; Shikano,

M.; Yazawa, K.; Uemura, D. *Tetrahedron Lett*. **1996**, *37*, 3871; (e) Takada, N.; Iwatsuki, M.; Suenaga, K.; Uemura, D. *Tetrahedron Lett*. **2000**, *41*, 6425.

2. Conditions for the isolation of pinnatoxins B and C: column, Develosil ODS (4.6×250 mm); solvent, MeCN:H_2O:TFA (20:80:0.1); flow rate, 1.0 mL/min; detection at 216 nm. Although further separation was attempted, successful results were not obtained.
3. (a) Satake, M.; Murata, M.; Yasumoto, T.; Fujita, T.; Naoki, H. *J. Am. Chem. Soc*. **1991**, *113*, 9859; (b) Naoki, H.; Murata, M.; Yasumoto, T. *Rapid Commun. Mass Spectrom*. **1993**, *7*, 179.
4. Reduction of the imino group proceeded stereoselectively to afford a single isomer. We deduced that the hydride attack from outside of the macrocycles in pinnatoxins gave only the 6*S* isomer.
5. Aldehyde **4**: ^{1}H NMR (800 MHz, CD_3OD, –20°C) δ 9.44 (s, 1H), 6.44 (s, 1H), 4.88 (br s, 1H), 4.86 (br s, 1H), 4.67 (m, 1H), 4.40 (dd, J=4.8, 11.6 Hz, 1H), 4.23 (m, 1H), 3.91 (br t, J=11 Hz, 1H), 3.76 (m, 1H), 3.61 (m, 1H), 3.56 (m, 1H), 3.23 (m, 2H), 2.53–2.49 (m, 2H), 2.44–2.39 (m, 2H), 2.35–1.30 (m, 31H), 1.29 (s, 3H), 1.17 (d, J=7.1 Hz, 3H), 1.05 (d, J=6.7 Hz, 3H), 1.03 (d, J=7.1 Hz, 3H); ESIMS m/z 698.4611, calcd for $C_{41}H_{64}NO_8$ $[M+H]^+$ 698.4632.
6. McCauley, J. A.; Nagasawa, K.; Lander, P. A.; Mischke, S. G.; Semones, M. A.; Kishi, Y. *J. Am. Chem. Soc*. **1998**, *120*, 7647.
7. Hoffmann, R. W. *Chem. Rev*. **1989**, *89*, 1841.