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Structural determination of pteriatoxins A, B and C, extremely potent toxins from the bivalve *Pteria penguin*

Noboru Takada,[a] Naoyashi Umemura,[a] Kiyotake Suenaga[b] and Daisuke Uemura[a,*]

[a]*Department of Chemistry, Graduate School of Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*
[b]*Research Center for Materials Science, Nagoya University, Chikusa, Nagoya 464-8602, Japan*

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Abstract—Pteriatoxins A, B and C, extremely potent toxins, were isolated from the Okinawan bivalve *Pteria penguin*. Their structures were determined based on NMR and MS/MS spectral analyses. Pteriatoxins have polyether macrocycles composed of 6,7-spiro, 5,6-bicyclo and 6,5,6-trispiro ketal rings, the same as in pinnatoxins.

In a previous paper, we reported the isolation and structural determination of pinnatoxins B and C.[1] In our continuing work on shellfish poisons, we observed that a moray eel vomits the viscera of *Pteria penguin*. We confirmed that the aqueous 75% EtOH extract of viscera of *P. penguin* shows acute toxicity, and successfully isolated pteriatoxins A, B and C as extremely toxic and minor components from *P. penguin*. We report here the isolation and structural determination of pteriatoxins A, B and C.

The aqueous 75% EtOH extract of viscera (82 kg) of *P. penguin* was partitioned between EtOAc and H_2O. The aqueous fraction was chromatographed on TSK-G3000S polystyrene gel (50% EtOH), DEAE Sephadex A-25 (0.02 M phosphate buffer), CM Sephadex C-25 (0.2 M phosphate buffer), reversed-phase HPLC (Develosil 300 ODS, MeCN–H_2O–TFA) and reversed-phase HPLC (Develosil 300 C8, MeCN–H_2O–TFA) guided by acute toxicity against mice to give pteriatoxin A (**1**)[2] and both pteriatoxins B (**2**) and C (**3**)[3] in a 1:1 mixture. Since there was too little of these toxins to weigh, the weights of pteriatoxins A (20 μg) and B:C (8 μg) were estimated by the S/N (signal-to-noise) ratio in ^{1}H NMR spectra.[4] These pteriatoxins showed significant acute toxicity against mice, with LD_{99}s of 100 and 8 μg/kg, respectively. Since the toxic symptoms and ^{1}H NMR spectra of pteriatoxins A, B and C resemble those of pinnatoxins,[5] we supposed that pteriatoxins were pinnatoxin analogs.

The molecular formula of **1** was determined to be $C_{45}H_{70}N_2O_{10}S$ by ESIMS (m/z 831.4824, calcd for $C_{45}H_{71}N_2O_{10}S$ $[M+H]^+$, 831.4829). The analyses of ^{1}H

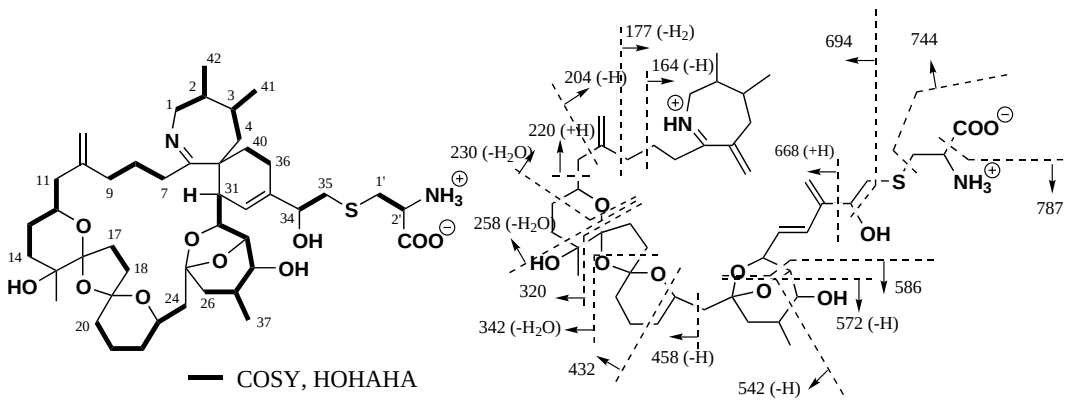

Figure 1. Partial structures and fragmentation pattern of pteriatoxin A (**1**).

* Corresponding author. Tel./fax: +81-52-789-3654; e-mail: uemura@chem3.chem.nagoya-u.ac.jp

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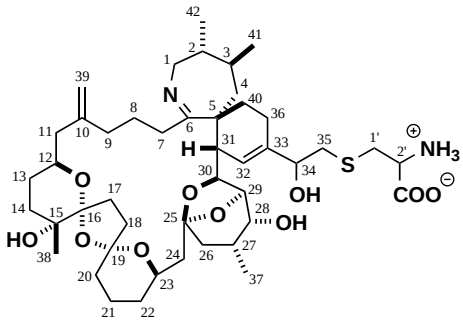
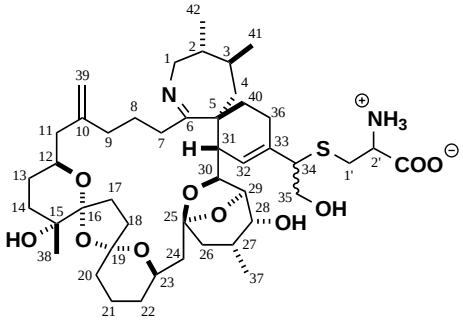
Table 1. ^{1}H NMR data for pteriatoxins A (**1**), B (**2**) and C (**3**)[a]



Pteriatoxin A (**1**)



Pteriatoxins B (**2**) and C (**3**)

Pteriatoxin A (**1**)				Pteriatoxin B (**2**)				Pteriatoxin C (**3**)			
Atom	^{1}H (ppm)	Atom	^{1}H (ppm)	Atom	^{1}H (ppm)	Atom	^{1}H (ppm)	Atom	^{1}H (ppm)	Atom	^{1}H (ppm)
1a	3.60	**22a**	1.24	**1a**	3.60	**22a**	1.26	**1a**	3.60	**22a**	1.26
1b	4.30	**22b**	1.68	**1b**	4.30	**22b**	1.65	**1b**	4.30	**22b**	1.65
2	1.70	**23**	4.05	**2**	1.72	**23**	4.04	**2**	1.72	**23**	4.04
3	1.41	**24a**	1.92	**3**	1.43	**24a**	1.91	**3**	1.41	**24a**	1.91
4a	1.83	**24b**	2.00	**4a**	1.78	**24b**	2.02	**4a**	1.82	**24b**	2.02
4b	2.02	**25**		**4b**	2.07	**25**		**4b**	2.03	**25**	
5		**26a**	1.62	**5**		**26a**	1.63	**5**		**26a**	1.63
6		**26b**	1.72	**6**		**26b**	1.72	**6**		**26b**	1.72
7	3.62 (2H)	**27**	2.21	**7**	3.65 (2H)	**27**	2.23	**7**	3.65 (2H)	**27**	2.23
8a	1.97	**28**	3.78	**8a**	1.86	**28**	3.78	**8a**	1.86	**28**	3.85
8b	2.09	**29**	4.56	**8b**	2.08	**29**	4.61	**8b**	2.08	**29**	4.54
9a	1.80	**30**	3.86	**9a**	1.79	**30**	3.92	**9a**	1.79	**30**	3.86
9b	2.20	**31**	3.55	**9b**	2.22	**31**	3.61	**9b**	2.22	**31**	3.61
10		**32**	5.36	**10**		**32**	5.32	**10**		**32**	5.30
11a	2.19	**33**		**11a**	2.20	**33**		**11a**	2.20	**33**	
11b	2.38	**34**	4.22	**11b**	2.41	**34**	3.61	**11b**	2.41	**34**	3.63
12	4.10	**35**	2.80	**12**	4.09	**35**	3.72	**12**	4.09	**35**	3.75
13a	1.31	**36**	2.34 (2H)	**13a**	1.33	**36a**	2.25	**13a**	1.33	**36a**	2.35
13b	1.69			**13b**	1.69	**36b**	2.55	**13b**	1.69	**36b**	2.48
14a	1.54	**37**	1.02 (3H)	**14a**	1.54	**37**	1.04 (3H)	**14a**	1.54	**37**	1.02 (3H)
14b	1.93	**38**	1.23 (3H)	**14b**	1.96	**38**	1.24 (3H)	**14b**	1.96	**38**	1.24 (3H)
15		**39**[b]		**15**		**39a**	4.86	**15**		**39a**	4.86
16				**16**		**39b**	4.96	**16**		**39b**	4.96
17a	1.63	**40a**	1.88	**17a**	1.66	**40a**	1.95	**17a**	1.66	**40a**	1.91
17b	2.22	**40b**	2.03	**17b**	2.22	**40b**	2.07	**17b**	2.22	**40b**	2.02
18a	1.86	**41**	1.08 (3H)	**18a**	1.86	**41**	1.10 (3H)	**18a**	1.86	**41**	1.08 (3H)
18b	2.07	**42**	1.23 (3H)	**18b**	2.08	**42**	1.23 (3H)	**18b**	2.08	**42**	1.23 (3H)
19		**1′a**	3.04	**19**		**1′a**	2.83	**19**		**1′a**	2.83
20a	1.52	**1′b**	3.13	**20a**	1.53	**1′b**	3.08	**20a**	1.53	**1′b**	3.08
20b	1.90	**2′**	3.74	**20b**	1.90	**2′**	3.63	**20b**	1.90	**2′**	3.63
21a	1.66			**21a**	1.67			**21a**	1.67		
21b	1.86			**21b**	1.85			**21b**	1.85		

[a] Recorded at 800 MHz in CD_3OD.
[b] Not observed.

NMR, COSY and HOHAHA spectra allowed 10 partial structures, C-1 to C-2 including C-42, C-3 to C-4 including C-41, C-7 to C-9, C-11 to C-14, C-17 to C-18, C-20 to C-24, C-26 to C-31 containing C-37, C-34 to C-35, C-36 to C-40 and C-1′ to C-2′ (Table 1, Fig. 1). As mentioned previously, positive ion ESI MS/MS[6] of pinnatoxins showed a series of prominent fragment ions generated by G ring-opening reactions, followed by bond cleavage.[1] Positive ion ESI MS/MS of **1** showed the same series of prominent fragment ions as the carbocyclic moiety in pinnatoxin A (Fig. 1). Therefore, pteriatoxin A (**1**) had the same polyether macrocycle as in pinnatoxin A. The observation of fragment ion peaks (m/z 787, 744) suggested the presence of an α-amino acid moiety in the side chain. Furthermore, the chemical shifts of H-35 (δ_H 2.80) and H-1′ (δ_H 3.04, 3.13) suggested the presence of a sulfide bond between C-35 and C-1′. The chemical shift of H-34 (δ_H 4.22) suggested the presence of an allylic hydroxy group at C-34. Therefore,

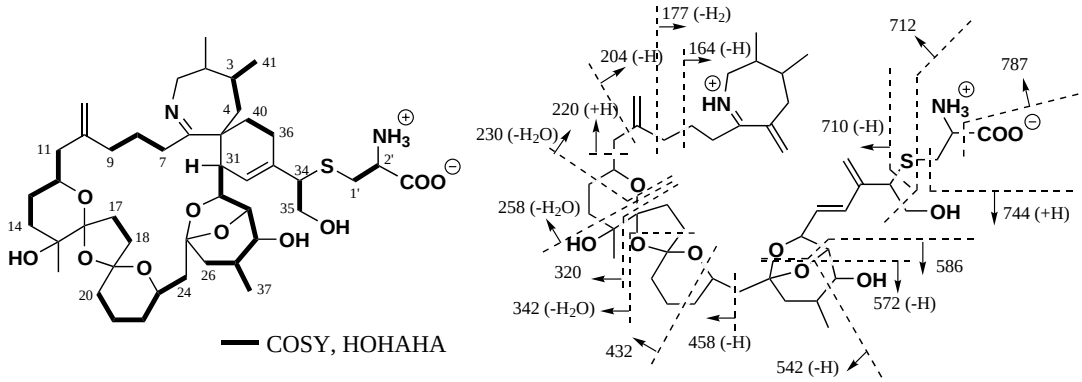


Figure 2. Partial structures and fragmentation pattern of pteriatoxins B (**2**) and C (**3**).

the gross structure of pteriatoxin A was determined to be as shown in **1**.

The molecular formula of both **2** and **3** was determined to be $C_{45}H_{70}N_2O_{10}S$ by ESIMS (*m*/*z* 831.4813, calcd for $C_{45}H_{71}N_2O_{10}S$ $[M+H]^+$, 831.4829). Analysis of the ^1H NMR spectrum showed duplicate signals (1:1) for a set of protons (H-3, H-4, H-28 to H-37, H-40 and H-41), suggesting the presence of epimeric isomers (Table 1). Analyses of ^1H NMR, COSY and HOHAHA spectra allowed nine partial structures, as shown in Fig. 2. Positive ion ESI MS/MS of **2** and **3** showed the same series of prominent fragment ions as the macrocyclic moiety in pinnatoxin A (Fig. 2). Therefore, pteriatoxins B (**2**) and C (**3**) were assumed to have the same polyether macrocycles as in pinnatoxin A. The observation of fragmentation ion peaks (*m*/*z* 787, 744, 710) suggested the presence of a cysteine moiety in the side chain. Furthermore, the observation of another fragment ion peak (*m*/*z* 712), which is not observed in **1**, suggested the presence of a hydroxymethyl group. Therefore, the gross structure of pteriatoxins B and C was determined to be as shown in **2** and **3**. The position of duplicate signals in the ^1H NMR spectrum suggested that pteriatoxins B (**2**) and C (**3**) are C-34 epimers of each other.

As described in our previous paper, the absolute stereochemistries of a series of pinnatoxins have already been clarified. Considering the chemical shifts and the coupling patterns in the ^1H NMR spectra, we can propose that the stereochemistry in the carbocycles of pteriatoxins and pinnatoxins may be superimposed on each other.

Pteriatoxins A, B and C were isolated from the Okinawan bivalve *P. penguin*. Based on an analysis of positive ion ESI MS/MS spectra, they were determined to be pinnatoxin analogs containing a cysteine moiety. Based on the presence of pinnatoxin analogs in both *Pinna* sp. and *Pteri*a sp., the pinnatoxin series may be synthesized by common symbionts.

Acknowledgements

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References

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2. Conditions for the isolation of pteriatoxin A: column, Develosil 300 ODS (4.6×250 mm); solvent, MeCN:H_2O:TFA (20:80:0.1); flow rate, 1.0 mL/min; detection at 215 nm.
3. Conditions for the isolation of pteriatoxins B and C: column, Develosil 300 ODS (4.6×250 mm); solvent, MeCN:H_2O:TFA (17:83:0.1); flow rate, 1.0 mL/min; detection at 215 nm.
4. The weights of pteriatoxins were estimated by comparison of the S/N ratio of 67 μM okadaic acid with those of pteriatoxins in CD_3OD (0.18 mL).
5. (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.
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