Tetrahedron Letters 55 (2014) 5508–5510

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Pinnatoxin H: a new pinnatoxin analogue from a South China Sea *Vulcanodinium rugosum* isolate

Andrew I. Selwood [a,*], Alistair L. Wilkins [b], Rex Munday [c], Haifeng Gu [d], Kirsty F. Smith [a], Lesley L. Rhodes [a], Frode Rise [e]

[a] Cawthron Institute, Private Bag 2, Nelson, New Zealand
[b] Chemistry Department, University of Waikato, Private Bag 2105, Hamilton, New Zealand
[c] AgResearch Ltd, Ruakura Research Centre, Private Bag 3123, Hamilton, New Zealand
[d] Third Institute of Oceanography, SOA, Xiamen 361005, China
[e] University of Oslo, PO Box 1033, Blindern, NO-0315 Oslo, Norway

ARTICLE INFO

Article history:
Received 13 June 2014
Revised 28 July 2014
Accepted 13 August 2014
Available online 19 August 2014

Keywords:
Pinnatoxin
Portimine
Vulcanodinium
Cyclic imine
Algal toxin

ABSTRACT

Pinnatoxin H was isolated from a culture of the dinoflagellate *Vulcanodinium rugosum* isolated from the South China Sea. The structure of pinnatoxin H was elucidated by LC–MS/MS and NMR spectroscopy. It was found to have the same macrocyclic structure and substituents as pinnatoxins D, E and F, but the side chain on the cyclohexenyl ring was an ethenyl group, as found in pinnatoxin G and portimine. The observation that this strain of *V. rugosum* produced only pinnatoxin H and portimine is consistent with previous findings that the profile of pinnatoxins can vary significantly among strains. The acute toxicity of pinnatoxin H to mice was 67 μg/kg (intraperitoneal) and 163 μg/kg (gavage).

The toxin profile of *Vulcanodinium rugosum* varies among strains.[1] This dinoflagellate species produces pinnatoxins E (**2**), F (**3**) and G (**4**) and another cyclic imine, portimine (**5**).[2] Initial LC–MS screening of a *V. rugosum* strain isolated from the South China Sea indicated that it produced **5**, but no known pinnatoxins. However, further LC–MS scanning experiments revealed the presence of a new pinnatoxin analogue.[3] In this Letter we report the isolation and structural elucidation of pinnatoxin H (**1**), and its acute toxicity to mice.

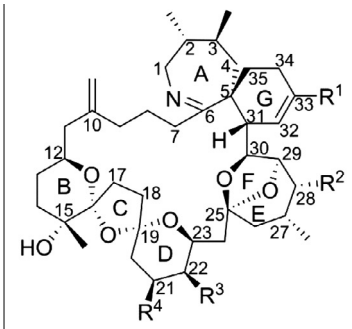
A clonal isolate of *Vulcanodinium rugosum* (Cawthron Institute Culture Collection of Micro-Algae, isolate CAWD198), collected from the South China Sea[3] was cultured at 25 °C, 100 μmol m^{-2} s^{-1} photon irradiance (12:12 h L:D), in K medium.[4] Batch cultures were grown in aerated, sterile plastic bags with inserts containing 0.6 M K_2CO_3/2.4 M $KHCO_3$ in deionised water, as described previously.[5] Cells were harvested from 16 L of culture and the wet cells extracted twice with methanol. The combined extract was diluted 1:1 with 30 mM HCl, washed with *n*-hexane and adjusted to pH 12. Following partitioning into *n*-hexane and removal of the solvent, the residue (60 mg) was dissolved in dichloromethane and subjected to open column chromatography on neutral alumina. The residue of a dichloromethane/propan-2-ol (97.5:2.5 v/v) fraction (5.5 mg) was subjected to two stages of solid phase extraction (silica and Strata-X) to yield **1** as a colourless, amorphous solid (1 mg) (Fig. 1).

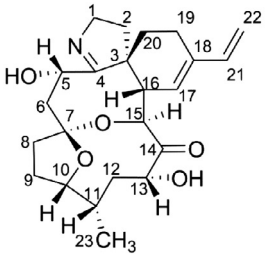
The molecular formula of **1** was established as $C_{43}H_{65}NO_7$ from the ESI-TOFMS [$(M+H)^+$ observed, 708.4834; required for $C_{43}H_{66}NO_7$ 708.4839; 12 rings plus double bonds]. Key collision-induced dissociation fragment ions were consistent with those of **2** and **3** but differed from **4**. This spectroscopic data suggested that **1** consisted of a pinnatoxin macrocycle containing 21-Me and 22-OH substituents with an ethenyl side chain at C-33. This ethenyl substituent is present in **4** and **5**, both of which have been isolated from another strain of *Vulcanodinium rugosum*.[2] The strong UV absorbance for **1** with λ_{max} 230 nm (ε 29,000), is consistent with the presence of 1,3-diene as found in pinnatoxin G (**4**).

Structural determination of **1** was performed by NMR spectroscopy. A detailed analysis of NMR spectral data (600 MHz), including ^1H, ^{13}C, DEPT135, COSY, TOCSY, ROESY, HSQC, H2BC, HMBC, 1D-SELTOCSY and 1D-SELROESY spectra determined in CD_3OD and ^1H, COSY, TOCSY and ROESY spectra determined in CD_3OH with pre-saturation of the HOD peaks verified the presence in **1** of a conjugated ethenyl group (C-32, C-33, C-36 and C-37 signals at 128.2 ppm, 139.9 ppm, 140.0 and 112.5 ppm, respectively), together with 27-Me, 22-OH and 21-Me groups (signals at

* Corresponding author. Tel.: +64 3 548 2319; fax: +64 3 546 9464.
E-mail address: andy.selwood@cawthron.org.nz (A.I. Selwood).

http://dx.doi.org/10.1016/j.tetlet.2014.08.056
0040-4039/





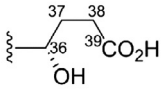
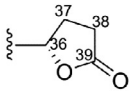
	R^1	R^2	R^3	R^4		m/z [M+H]$^+$
1		H	OH	CH_3	Pinnatoxin H	708
2		H	OH	CH_3	Pinnatoxin E	784
3		H	OH	CH_3	Pinnatoxin F	766
4		OH	H	H	Pinnatoxin G	694

Figure 1. Chemical structures of pinnatoxins and portimine.

22.7 ppm, 69.4 ppm and 18.4 ppm, respectively) (see Table 1 and Supplementary material). Consideration of ROESY and 1D-SELROESY data determined in CD_3OD and in CD_3OH with HOD peak pre-saturation showed that the relative configurations at C-16, C-21, C-22, C-27 and C-31, and at all the other asymmetric carbons of **1** were as previously defined for **2**–**4**, as defined by molecular modelling results and ROESY and NOESY data for pinnatoxins E–G.[6] Structurally significant ROESY correlations observed for **1** included those between H-31 (3.528 ppm) and H-32 (5.23 ppm), H-7a (3.28 ppm), H-27 (2.35 ppm), H-28a (2.00 ppm) and H-35a (1.88 ppm) and between H-22 (3.44 ppm), H-23 (4.14 ppm), 22-OH (4.10 ppm), H-21 (2.10 ppm), H-20a (1.64 ppm) and the 21-methyl group protons (0.96 ppm) (see Supplementary material). Given that the absolute configuration of **1** at C-31 is likely to be same as in all known pinnatoxins, it can therefore be inferred that the absolute configuration at C-16, C-21, C-22, C-27 and all other asymmetric carbons of **1**, and the preferred solution confirmation of the macrocyclic ring are the same as in all other known pinnatoxins.

No signal attributable to C-6 was observed under the conditions used to acquire the ^{13}C spectrum of **1**; namely, a 70° pulse with a pulse repetition rate of 2.86 s. This is presumably due to a combination of this quaternary carbon signal being devoid of an NOE effect due to its lack of directly attached protons, together with partial saturation of its signal. This is due to it possessing a long T_1 and broadening and $^2J_{C-C-D}$ coupling (D has $I = 1$) of any detectable signal because of a deuterium exchange at C-7 (see HMBC-Accordian NMR spectra in Supplementary information). A weak correlation was seen from one of the H-35 protons (that centred at 1.89 ppm) to a carbon signal at 179.2 ppm. This was previously reported for pinnatoxin G[6] (see Table 1), and can be attributed to C-6. Only one C-7 methylene proton signal (that centred at 3.28 ppm) was observed for H-7 in CD_3OD, whereas two similar but distinguishable H-7 proton signals centred at 3.28 and 3.31 ppm, respectively, were observed in the HSQC spectrum of **1** determined in CH_3OH. The exchange of a single H-7 proton for a deuterium has been reported for related compounds when spectra were recorded in CD_3OD.[7] Comparison of ^{13}C chemical shifts determined to two decimal places (see Supplementary material), as opposed to commonly reported one decimal place data (see Table 1) showed that while most chemical shifts typically varied by less than ±0.03 ppm between CD_3OD and CD_3OH solvents, the C-7, C-8, C-14, C-15, 15-Me, C-21 and C-22 chemical shifts in CD_3OH differed from those determined in CD_3OD by −0.32, −0.09, −0.04, −0.09, −0.05, −0.04 and −0.09 ppm, respectively. These variations can be attributed to deuterium exchange at C-7 via an enamine exchange pathway and –OD (rather than –OH) groups at C-15 and C-22, respectively, in the presence of CD_3OD.

The LD_{50} value of **1** in mice by intraperitoneal injection was 67 μg/kg (95% CI 63–79 μg/kg) and by gavage was 163 μg/kg (95% CI 139–175 μg/kg). The symptoms of intoxication by **1** were closely similar to those recorded with other pinnatoxin derivatives.[6,8] At lethal doses, mice became immobile soon after dosing. Their respiration rates subsequently declined, until respiration ceased completely. Cyanosis and exophthalmia were noted shortly before death. Death occurred within 30 min of administration of **1**, whether by injection or by oral administration. Animals surviving beyond this time made a full recovery, and their appearance and behaviour throughout the remainder of the observation period was entirely normal. No abnormalities were recorded at necropsy, and organ weights were within the normal range. The oral LD_{50} value of **1** was only 2.4 times that of the LD_{50} by intraperitoneal injection. In this respect **1** resembles pinnatoxins **3** and **4**, for which ratios between oral and intraperitoneal injection were 2.0 and 3.1, respectively, rather than **2**, which was 48 times less toxic by gavage than by intraperitoneal injection.[8]

In conclusion, pinnatoxin H **1** isolated from *V. rugosum* has close structural similarities to other pinnatoxins with the same polycyclic ether and spiroimine core, but a variation in the substituents. It has similar high acute toxicity to mice as other pinnatoxins, particularly

Table 1
NMR assignments for pinnatoxin H (**1**) in CD_3OD and CD_3OH, together with published assignments for pinnatoxins G (**4**) and E (**2**)

Atom	Pinnatoxin G		Pinnatoxin E		Pinnatoxin H		Pinnatoxin H	
	CD_3OD		CD_3OD		CD_3OD		CD_3OH	
	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	53.6	3.53, 3.88	52.6	3.56, 4.10	53.3	3.514, 3.90	53.3	3.52, 3.90
2	42.2	1.42	40.7	1.57	41.9	1.42	41.9	1.42
2-Me	20.9	1.22	20.3	1.18	20.8	1.17	20.8	1.17
3	35.1	1.37	35.5	1.30	35.4	1.28	35.4	1.28
3-Me	21.5	0.96	21.7	1.03	21.9	0.95	21.9	0.95
4	37.8	1.79, 1.46	36.5	1.93, 1.66	37.7	1.80, 1.47	37.7	1.80, 1.47
5	51.5		51.9		51.7		51.6	
6	179.5		nd		179.2*		nd	
7	34.5	3.13	35.1	3.53	34.5	3.28	34.8	3.28, 3.31
8	22.7	2.16, 1.45	21.8	2.15, 1.74	22.4	2.17, 1.47	22.5	2.18, 1.47
9	34.5	2.39, 1.66	33.8	1.82, 2.17	34.1	2.34, 1.68	34.1	2.34, 1.67
10	147.9		146.5		147.7		147.7	
10-CH_2	110.2	4.75, 4.77	111.3	4.81, 4.86	110.3	4.75, 4.77	110.2	4.75, 4.77
11	47.3	2.33, 2.11	46.9	2.25, 2.37	47.3	2.10, 2.33	47.3	2.10, 2.33
12	69.7	4.14	69.5	4.06	69.2	4.06	69.6	4.06
13	29.8	1.64, 1.24	29.8	1.27, 1.64	29.8	1.22, 1.60	29.8	1.22, 1.61
14	35.6	1.95, 1.48	35.5	1,50, 1.90	35.5	1.46, 1.91	35.5	1.47, 1.91
15	71.3		71.1		71.2		71.3	
15-Me	22.8	1.21	22.9	1.22	22.9	1.20	22.9	1.20
15-OH								4.47
16	113.6		113.7		113.7		113.7	
17	31.5	2.20, 1.78	31.4	2.20, 1.78	31.4	2.19, 1.77	31.4	2.19, 1.77
18	39.1	2.07, 1.83	38.7	2.15, 1.85	38.7	2.14, 1.84	38.7	2.15, 1.85
19	109.9		110.1		110.1		110.1	
20	35.9	1.87, 1.53	37.7	1.66 (2H)	37.8	1.64 (2H)	37.8	1.64 (2H)
21	21.6	1.89, 1.62	32.1	2.09	32.0	2.10	32.0	2.10
21-Me			18.3	0.97	18.4	0.96	18.4	0.96
22	32.2	1.75, 1.21	69.4	3.44	69.4	3.44	69.4	3.44
22-OH								4.10
23	71.0	4.15	73.1	4.09	73.1	4.14	73.1	4.14
24	44.8	2.01, 1.90	40.1	2.21, 1.92	39.9	2.20, 1.91	39.9	2.21, 1.91
25	109.3		109.6		109.2		109.2	
26	42.5	1.65, 1.59	45.4	1.87, 1.41	45.7	1.85, 1.40	45.7	1.84, 1.39
27	30.8	2.33	26.0	2.29	25.9	2.35	25.9	2.35
27-Me	16.9	1.01	22.7	0.99	22.7	0.97	22.7	0.97
28	67.5	3.73	33.8	2.04, 1.55	33.9	2.00, 1.53	33.9	2.00, 1.53
29	81.9	4.47	77.4	4.62	77.4	4.60	77.4	4.60
30	80.4	3.89	81.1	3.86	81.3	3.86	81.3	3.86
31	45.0	3.43	44.7	3.52	45.5	3.528	45.5	3.52
32	127.9	5.25	121.0	5.21	128.2	5.23	128.2	5.24
33	140.2		144.3		139.9		139.8	
34	22.1	2.29 (2H)	21.8	2.26 (2H)	22.1	2.29 (2H)	22.06	2.29 (2H)
35	33.6	1.89, 1.76	34.1	1.82, 1.92	33.7	1.78, 1.88	33.7	1.78, 1.89
36	140.0	6.37	75.7	4.02	140.0	6.37	140.0	6.37
37	112.5	5.17, 5.01	32.1	1.84	112.5	4.99, 5.16	112.5	5.00, 5.15
38			33.7	2.25				
39			180.3					

* Detected in an HMBC-ACCORDIAN spectrum acquired with ^{1}H decoupling.

pinnatoxin F and G, and due to its relative oral potency it can be considered a risk to human health if found in seafood.

Acknowledgments

Janet Adamson and Craig Waugh, Cawthron Institute, are thanked for technical assistance with cultures. Pat Gread, University of Waikato, is thanked for obtaining HR-MS spectra. This study was supported by funding from the New Zealand Ministry for Business, Innovation and Employment (previously New Zealand Foundation for Research, Science and Technology), contract CAWX1317.

Supplementary data

Supplementary data (NMR spectra; LC–MS/MS chromatogram; CID MS/MS spectrum) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.08.056.

References and notes

1. Rhodes, L.; Smith, K.; Selwood, A.; McNabb, P.; Munday, R.; Suda, S.; Molenaar, S.; Hallegraeff, G. *Phycologia* **2011**, *50*, 624–628.
2. Selwood, A. I.; Wilkins, A. L.; Munday, R.; Shi, F.; Rhodes, L. L.; Holland, P. T. *Tetrahedron Lett.* **2013**, *54*, 4705–4707.
3. Zeng, N.; Gu, H.; Smith, K. F.; Rhodes, L.; Selwood, A. I.; Yang, W. *New Zeal. J. Mar. Freshwater Res.* **2012**, *46*, 511–521.
4. Keller, M. D.; Selvin, R. C.; Claus, W.; Guillard, R. R. L. *J. Phycol.* **1987**, *23*, 633–638.
5. Pors, Y.; Wustenberg, A.; Ehwald, R. *J. Phycol.* **2010**, *46*, 825–830.
6. Selwood, A. I.; Miles, C. O.; Wilkins, A. L.; Van Ginkel, R.; Munday, R.; Rise, F.; McNabb, P. S. *J. Agric. Food Chem.* **2010**, *58*, 6532–6542.
7. (a) Hu, T.; Curtis, J. M.; Oshima, Y.; Quilliam, M. A.; Walter, J. A.; Watson-Wright, W. M.; Wright, J. L. *J. Chem. Soc., Chem. Commun.* **1995**, 2159–2161; (b) Falk, M.; Burton, I. W.; Hu, T. M.; Walter, J. A.; Wright, J. L. C. *Tetrahedron* **2001**, *57*, 8659–8665.
8. Munday, R.; Selwood, A. I.; Rhodes, L. *Toxicon* **2012**, *60*, 995–999.