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Pinnatoxins and spirolides in Norwegian blue mussels and seawater

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ABSTRACT

Fast-acting cyclic imines belonging to the pinnatoxin and pteriatoxin group of toxins were originally identified in shellfish of the genera *Pinna* and *Pteria* in Japan, after food poisoning events in China linked to consumption of *Pinna* spp. Recently, a range of new and known pinnatoxin analogs has been identified in shellfish, sediment, and seawater samples from Australia and New Zealand. Although the structurally closely-related spirolide toxins are better known, and have a worldwide distribution including Norway and other parts of Europe, the presence of pinnatoxins has not been reported in European waters or shellfish. Here we report results from a survey of Norwegian blue mussels for the presence of pinnatoxins and spirolides, by LC-MS/MS analysis of extracts obtained as part of Norway's routine monitoring programme for regulated algal toxins during late autumn and early winter 2009. Spirolides and pinnatoxin G were widespread (pinnatoxin G (**1**), spirolide C (**2**), iso-spirolide C (**3**), 13-desmethylspirolide C (**4**), 13,19-didesmethylspirolide C (**5**), and 20-methylspirolide G (**6**) were detected in 69%, 13%, 60%, 22%, 33%, and 77%, respectively, of the shellfish samples) and, although levels were generally low, concentrations of up to 115 μg/kg of pinnatoxin G (**1**) and 226 μg/kg of 13-desmethylspirolide C (**4**) were detected. We also analyzed stored extracts from passive sampling disks deployed as part of a separate study in autumn 2007. All the stored extracts contained 20-methylspirolide G (which predominated at most locations), most contained pinnatoxin G (73%) and 13,19-didesmethylspirolide C (67%), but iso-spirolide C (36%) and 13-desmethylspirolide C (52%) were also detected in many of the samples. These results suggest that pinnatoxins may be much more widespread than previously suspected, and indicate that they or related compounds could be responsible for sporadic incidents of rapid-onset symptoms during mouse bioassays of shellfish in Europe and elsewhere. The toxicological significance of these levels of pinnatoxins and spirolides is at present unclear. However, although pinnatoxins appear to be less toxic than spirolides by intraperitoneal injection in the mouse bioassay, recently published preliminary toxicological data indicate that pinnatoxins may be as much as an order of magnitude more toxic than spirolides by oral ingestion via food.

1. Introduction

The fast-acting cyclic imine group of toxins (FAO/IOC/WHO, 2004) has been reported in shellfish from North America, Europe, Japan and New Zealand (Cembella and Krock, 2008). This group of toxins contains a range of structurally-related toxin groups including spirolides and

Abbreviations: MBA, mouse bioassay; NMRI, Naval Medical Research Institute; SPATT, solid-phase adsorption toxin tracking; TEF, toxic equivalency factor.

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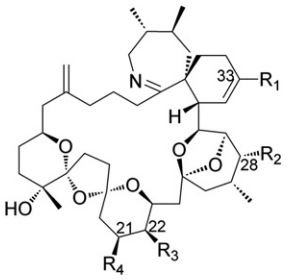
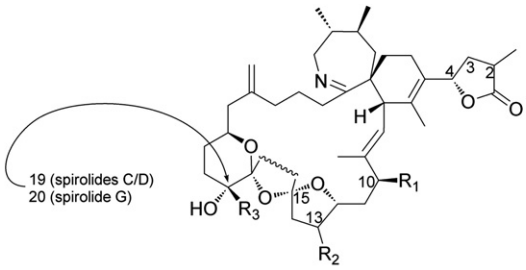
pinnatoxins (Fig. 1), as well as pteriatoxins, gymnodimines, prorocentrolides, and spiro-prorocentrimine (Cembella and Krock, 2008). Very recently, pinnatoxins F and G were isolated from Australian shellfish, identified by spectroscopic methods, and proposed as the progenitors of all the known pinnatoxins and pteriatoxins (Selwood et al., 2010). Pinnatoxins E and F have also been identified in New Zealand shellfish (Selwood et al., 2010), and a novel dinoflagellate that produces pinnatoxin F has now been isolated from Rangaunu Harbour in Northland, New Zealand (Rhodes et al., 2010). Very recently, dinoflagellates have been isolated from South Australia and Japan and shown to produce pinnatoxins F and G, respectively, in culture (Rhodes et al., 2010; Smith et al., in press). Most cyclic imine toxins are highly toxic to mice by i.p injection, causing death in as little as 5 min (Munday, 2008), and recent data show that some of these compounds are also highly toxic by the oral route (Munday, 2008; Rhodes et al., 2010). Indeed, pinnatoxins have been implicated in poisoning of people eating shellfish in China (Uemura et al., 1995), although evidence for the role of toxins in the intoxication event appears to be circumstantial (Cembella and Krock, 2008) and the presence of pinnatoxins or pteriatoxins in these shellfish does not appear to have been demonstrated.

The presence of fast-acting neurotoxins in Norwegian shellfish was described in 2001 (Ramstad et al.), and a range of spirolides have since been found (Rundberget et al., 2009, 2007; Aasen et al., 2005b; Aasen et al., 2006) in shellfish and seawater from Norway. Production of spirolides is generally associated with the dinoflagellate *Alexandrium ostenfeldii* (Cembella and Krock, 2008), although *Alexandrium peruvianum* has also recently been reported to produce spirolides (Touzet et al., 2008). Occasionally, unexplained rapid-onset symptoms consistent with cyclic imine toxins have been observed in mouse bioassays conducted on Norwegian shellfish samples that did not contain measurable amounts of spirolides by LC-MS. Despite this, no other fast-acting cyclic imine toxins have been reported in Norwegian shellfish samples.

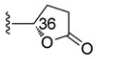
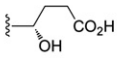
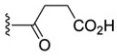
The availability in our laboratory of standards and LC-MS methods for spirolides and, more recently, pinnatoxins allowed us to undertake a survey of the occurrence of



Pinnatoxins



Spirolides

	m/z [M+H]$^+$	R_1	R_2	R_3	R_4
Pinnatoxin G (**1**)	694		OH	H	H
Pinnatoxin A	712		OH	H	H
Pinnatoxins B, C	741		OH	H	H
Pinnatoxin F	766		H	OH	CH_3
Pinnatoxin E	784		H	OH	CH_3
Pinnatoxin D	782		H	OH	CH_3

	m/z [M+H]$^+$		R_1	R_2	R_3	
Spirolide C (**2**)	706		OH	CH_3	CH_3	$\Delta^{2,3}$
iso-Spirolide C (**3**)	706	Structure undefined				
13-Desmethylspirolide C (**4**)	692		OH	H	CH_3	$\Delta^{2,3}$
13,19-Didesmethylspirolide C (**5**)	678		OH	H	H	$\Delta^{2,3}$
Spirolide D	708		OH	CH_3	CH_3	–
13-Desmethylspirolide D	694		OH	H	CH_3	–
Spirolide G	692		H	H	H	$\Delta^{2,3}$
20-Methylspirolide G (**6**)	706		H	H	CH_3	$\Delta^{2,3}$

Fig. 1. Structures of selected pinnatoxins (pinnatoxins B and C are pairs of diastereoisomers at C-36) and spirolides. Note that the modified atom-numbering proposed by Selwood et al. (2010) has been used here for pinnatoxin analogs, that this numbering system differs from those used for spirolides, and that the standard atom-numbering for spirolides A–D differs from that of spirolide G after C-15. The lactone junctions (36*S* in pinnatoxins E and F, and 4*S* in the spirolides) are depicted with the same stereochemistry as found for gymnodimine (Stewart et al., 1997) and are consistent with recent structural data on 13-desmethylspirolide C (Bourne et al., 2010).