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Toxicon 48 (2006) 152–159

TOXICON

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Isolation and identification of pectenotoxins-13 and -14 from *Dinophysis acuta* in New Zealand

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Received 25 November 2005; accepted 10 April 2006
Available online 9 May 2006

Abstract

Two novel pectenotoxins (PTXs), PTX-13 and -14, were isolated from extracts of *Dinophysis acuta* collected from the west coast of South Island, New Zealand. The compounds were identified as oxidized analogues of PTX-2 by NMR spectroscopic and LC-MS studies. PTX-13 (32*R*-hydroxyPTX-2) corresponds to the unidentified analogue PTX-11x reported by [Suzuki et al., 2003. Liquid chromatography-mass spectrometry of spiroketal stereoisomers of pectenotoxins and the analysis of novel pectenotoxin isomers in the toxic dinoflagellate *Dinophysis acuta* from New Zealand. J. Chromatogr. A 992, 141–150]. PTX-13 underwent slow deuteration at the 13β-position during NMR analysis. PTX-14 corresponds to the 32,36-dehydration product of PTX-13, and may be an artifact.

Keywords: *Dinophysis acuta*; Pectenotoxins; New Zealand; NMR; LC-MS

1. Introduction

Pectenotoxins (PTXs; Fig. 1) are group of lactones found in *Dinophysis* species and shellfish from around the world. PTX-2 is the most common PTX in algae, having been found in *Dinophysis acuta*, *Dinophysis acuminata*, *Dinophysis norvegica*, *Dinophysis fortii*, and *Dinophysis rotundata* (Lee et al., 1989; MacKenzie et al., 2005; Miles et al., 2004a; Suzuki et al., 1998, 2003). In addition to PTX-2, several oxidized PTXs, including PTX-11 and -12, have recently been identified from samples of *Dinophysis* (MacKenzie et al., 2005; Miles et al., 2004a; Suzuki et al., 2003, 2006). PTXs have also recently been found in several heterotrophic dinoflagellate species of the genus *Protoperidinium* that had been observed feeding on *Dinophysis* spp. in a

Abbreviations: MeCN, acetonitrile; MeOH, methanol; NOESY, nuclear Overhauser enhancement spectroscopy; PTX, pectenotoxin; SA, seco acid; TOCSY, total correlation spectroscopy

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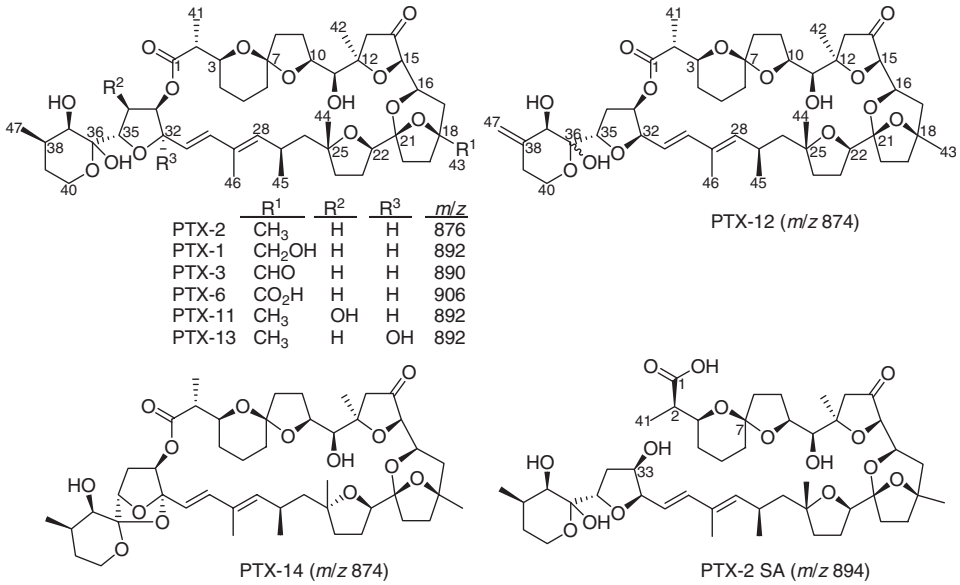
0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.04.005



	R^1	R^2	R^3	m/z
PTX-2	CH_3	H	H	876
PTX-1	CH_2OH	H	H	892
PTX-3	CHO	H	H	890
PTX-6	CO_2H	H	H	906
PTX-11	CH_3	OH	H	892
PTX-13	CH_3	H	OH	892

Fig. 1. Structures of PTX-1, PTX-2, PTX-2 SA, PTX-3, PTX-6, PTX-11, PTX-12, PTX-13, and PTX-14. Values of *m*/*z* are for $[M+NH_4]^+$ ions.

net haul sample (Miles et al., 2004a). Filter-feeding shellfish usually metabolize PTXs absorbed during ingestion of *Dinophysis* cells. Blue (*Mytilus edulis* (Miles et al., 2004b) and *Mytilus galloprovincialis* (Miles et al., 2004b; Suzuki et al., 2001a)) and green-lipped (*Perna canaliculus* (Miles et al., 2004b; Suzuki et al., 2001a)) mussels and the New Zealand scallop (*Pecten novaezelandiae* (Suzuki et al., 2001b)) rapidly hydrolyze PTX-2 to form PTX-2 seco acid (SA), which is usually the most abundant PTX found in these shellfish species. However, PTX-11 (Suzuki et al., 2006) and PTX-12 (Miles et al., 2004a) appear to be much more resistant to enzymatic hydrolysis than PTX-2, and these compounds appear to accumulate to a greater extent in mussels than does PTX-2 (Miles et al., 2004a; Suzuki et al., 2003). Oxidized derivatives of PTX-2, such as PTX-1, -3, and -6 accumulate in Japanese scallops (*Patinoecten yessoensis*) instead of PTX-2 SA (Suzuki et al., 1998), suggesting that these shellfish lack the enzymes necessary to hydrolyze PTXs to their SAs. PTX-1 (Yasumoto et al., 1984, 1985), PTX-2 (Miles et al., 2004b; Yasumoto et al., 1984, 1985; Yoon and Kim, 1997a, b) and PTX-11 (Suzuki et al., 2006) are toxic to mice by intraperitoneal injection in the mouse bioassay for lipophilic marine biotoxins (Yasumoto et al., 1978), but PTX-2 SAs are not (Miles et al., 2006, 2004b). Thus, hydrolysis of PTXs to PTX SAs constitutes a detoxification mechanism. Some earlier studies indicated that PTXs were both diarrhetic (Ishige et al., 1988) and orally toxic (Ogino et al., 1997) to mice, and could therefore constitute a serious threat to the health of shellfish consumers. However, recent oral dosing studies failed to elicit signs of diarrhetic activity or toxicity, even at doses as high as 5 mg/kg (Miles et al., 2004b; Suzuki et al., 2006), suggesting that these compounds may be less of a threat than originally supposed and confirming that they cannot be classed together with okadaic acid and dinophysistoxin analogues as "diarrhetic shellfish poisons". In view of these findings, the current regulation of PTXs in units of "okadaic acid equivalents" by the European Union (European Union, 2002) requires revision.

During the recent isolation of PTX-11 we observed several early eluting UV-absorbing peaks during HPLC analyses that appeared to correspond to novel PTX analogues (Miles et al., 2004b). Here we report isolation of two of these compounds from an extract of New Zealand *D. acuta*, and their identification as PTX-13 (32α-hydroxyPTX-2) and a cyclized 32α,36-oxido analogue, PTX-14.

2. Experimental procedures

2.1. PTX-13

In a previous study (Miles et al., 2004b), an early eluting side-fraction (eluting at 5.2 min) was