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Identification of 45-hydroxy-46,47-dinoryessotoxin, 44-oxo-45,46,47-trinoryessotoxin, and 9-methyl-42,43,44,45,46,47,55-heptanor-38-en-41-oxoyessotoxin, and partial characterization of some minor yessotoxins, from *Protoceratium reticulatum*

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Abstract

Preparative HPLC purification of a side-fraction obtained during purification of 44,55-dihydroxyyessotoxin (**6**) afforded fractions containing previously unidentified yessotoxin analogues. Careful analysis of these fractions by HPLC-UV, LC–MS^3, and NMR spectroscopy, revealed the identities of some of these analogues as 45-hydroxy-46,47-dinoryessotoxin (**1**), 44-oxo-45,46,47-trinoryessotoxin (**2**) and 9-methyl-42,43,44,45,46,47,55-heptanor-38-en-41-oxoyessotoxin (**5**). Numerous other analogues were present but could only be characterized by HPLC-UV and LC–MS^3 due to their low abundance. The HPLC-UV and LC–MS^3 data confirm the presence of large numbers of yessotoxin analogues, some of which may be oxidative degradation products, in extracts of Protoceratium reticulatum. Compound-**1** is the first 46,47-dinoryessotoxin to be identified.

Keywords: Yessotoxin; Shellfish; *Protoceratium reticulatum*

1. Introduction

Yessotoxins (Fig. 1) are sulfated polyethers produced by the dinoflagellates *Protoceratium reticulatum* and *Gonyaulax polyedrum* in many parts of the world (Draisci et al., 1999; Konishi et al., 2004; Paz et al., 2004; Samdal et al., 2004a; Satake et al., 1997a; 2002; Souto et al., 2005; Stobo et al., 2003). These toxins and their metabolites can accumulate in filter-feeding shellfish to the extent that the shellfish give a toxic response in the mouse bioassay for lipophilic marine biotoxins (Yasumoto et al., 1978). The first yessotoxin analogue identified was yessotoxin (**8**) isolated from the Japanese scallop *Patinopecten yessoensis* (Murata et al., 1987). A number of oxidized yessotoxins (e.g. **13** and **14**), apparently arising from metabolism of **8**

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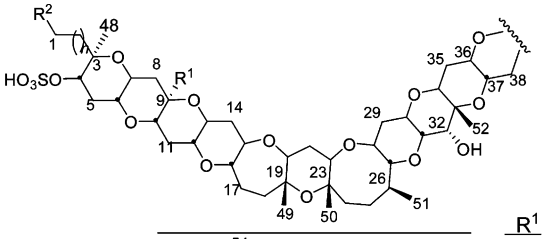


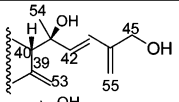
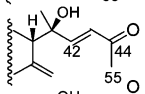
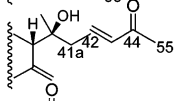
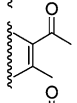
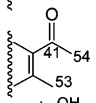
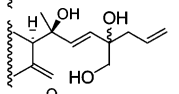
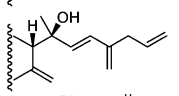
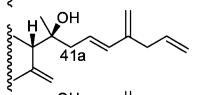
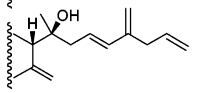
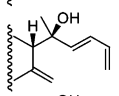
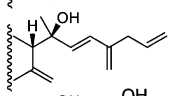
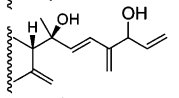
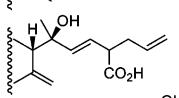
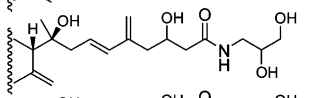
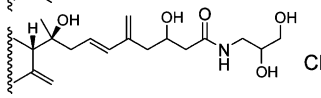
			R^1	R^2	*n*	*m/z*
1	45-OHdinorYTX		H	OSO_3H	1	1131
2	44-oxotrinorYTX		H	OSO_3H	1	1117
3	41a-homo-44-oxotrinorYTX		H	OSO_3H	1	1131
4	YTX enone		H	OSO_3H	1	1147
5	9-MeYTX enone		CH_3	OSO_3H	1	1061
6	44,55-diOHYTX		H	OSO_3H	1	1175
7	ketoYTX		H	OSO_3H	1	1047
8	YTX		H	OSO_3H	1	1141
9	41a-homoYTX		H	OSO_3H	1	1155
10	9-Me-41a-homoYTX		CH_3	OSO_3H	1	1169
11	trinorYTX		H	OSO_3H	1	1101
12	1a-homoYTX		H	OSO_3H	2	1155
13	45-OHYTX		H	OSO_3H	1	1157
14	CarboxyYTX		H	OSO_3H	1	1173
15	41a-homoYTX amide		H	OSO_3H	1	1290
16	9-Me-41a-homoYTX amide		CH_3	OSO_3H	1	1304

Fig. 1. Structures of selected yessotoxin analogues shown in their sulfonic acid forms. Values for *m/z* are for monoanions observed in the LC–MS^3 method used in this study.