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Characterization of 27-hydroxy-13-desmethyl spirolide C and 27-oxo-13,19-didesmethyl spirolide C. Further insights into the complex Adriatic *Alexandrium ostenfeldii* toxin profile

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ABSTRACT

Alexandrium ostenfeldii is a widespread toxic dinoflagellate that has recently bloomed across the Adriatic Sea, seriously threatening both shellfish consumers and aquacultures. In 2007 we reported on preliminary studies carried out on field samples and cultures of *A. ostenfeldii*. At the time, along with three major spirolides – among which 27-hydroxy-13,19-didesmethyl spirolide C (**3**) proved to be a novel compound – a number of new minor spirolides were detected. Unfortunately, for all of them only Mass Spectrometry-based structural hypotheses could be ventured due to their very small amount. In the present paper we report on isolation and High Resolution Mass Spectrometry- and NMR-based structural elucidation of two of those minor spirolides detected in our previous study.

1. Introduction

The widespread toxic dinoflagellate *Alexandrium ostenfeldii* is the producer of different classes of marine biotoxins depending on its geographical origin. Canadian *A. ostenfeldii* was reported to only produce spirolides (SPXs) (Cembella et al., 1999); while paralytic shellfish poisoning (PSP) toxins have been detected in *A. ostenfeldii* strains isolated from New Zealander seawater (Mackenzie et al., 1996). Even more complex was the toxin content of Scandinavian *A. ostenfeldii* extracts, in which both spirolides and PSP-toxins were individuated (Cembella et al., 2000).

Around the onset of the new millennium, following a massive bloom of *A. ostenfeldii* across the Adriatic Sea, we investigated the toxin profile of such a microorganism. Preliminary studies based on combination of liquid chromatography with tandem mass spectrometry (LC-MS/MS) identified the Adriatic *A. ostenfeldii* as the producing organism of only spirolide toxins (Ciminiello et al., 2006). Among the spirolides known at the time, 13-desmethyl spirolide C (**1**, Fig. 1) was unambiguously detected as the major component of the *A. ostenfeldii* toxic extract. Successive LC-MS/MS- and NMR-based studies (Ciminiello et al., 2007) – carried out on much larger extracts obtained from Adriatic *A. ostenfeldii* cultures – led us to isolate and structurally characterize two further major spirolides, identified as 13,19-didesmethyl spirolide C (**2**, Fig. 1) and 27-hydroxy-13,19-didesmethyl spirolide C (**3**, Fig. 1), respectively. While the former (**2**) had been previously isolated and structurally characterized in 2006 (MacKinnon et al., 2006), the latter (**3**) proved an unreported compound. In the wake of this study, we have recently elucidated the whole relative stereochemistry of **2** and shed some light on its conformational behavior in

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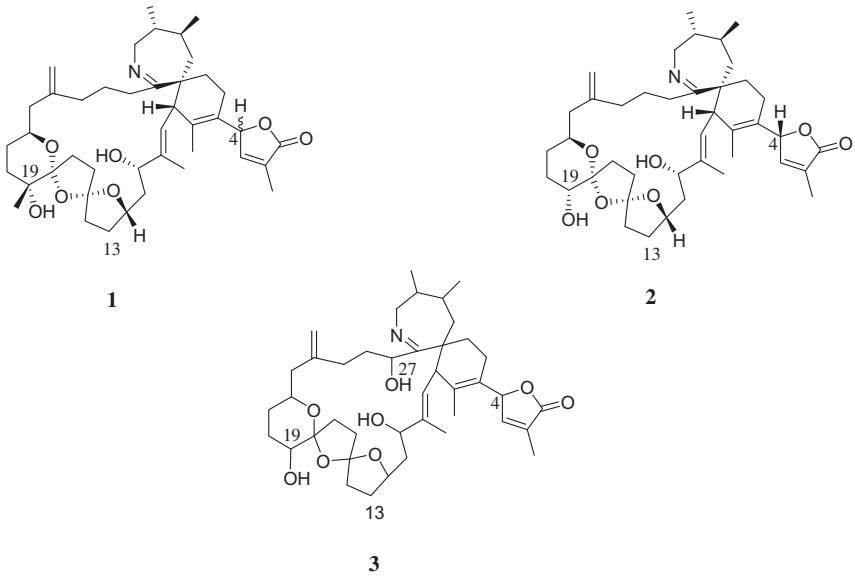

Fig. 1. Stereostructure of 13-desmethyl spirolide C (**1**) devoid of configuration at C4; full stereostructure of 13,19-didesmethyl spirolide C (**2**); planar structure of 27-hydroxy-13,19-didesmethyl spirolide C (**3**).

solution (Ciminiello et al., 2009). This represents a step towards a better understanding of the molecular bases of spirolide biological activity, which is still far from being totally assessed. To this day pharmacological studies have just classified spirolides as fast-acting toxins, since, once intraperitoneally injected into a 20-g mouse, they induce rapid onset of symptoms akin to those reported for the acute toxicity of PSP-toxins, followed by death within minutes (Hu et al., 1995, 1996; Richard et al., 2001).

In addition to the above mentioned spirolides (**1**–**3**), our LC-MS/MS investigation of the Adriatic *A. ostenfeldii* toxin profile disclosed the presence of a number of co-occurring minor spirolides, for which we could only venture MS-based structural hypotheses, as they had been isolated in too small amounts for any NMR investigation (Ciminiello et al., 2007).

In the present paper, we report on isolation and structural characterization of two of these minor spirolides. High resolution MS (HRMS), HRMS/MS and NMR experiments were used to identify them as the new 27-hydroxy-13-desmethyl spirolide C (**4**, Fig. 2) and 27-oxo-13,19-didesmethyl spirolide C (**5**, Fig. 3), respectively.

2. Material and methods

2.1. Chemicals

All organic solvents were of distilled-in-glass grade (Carlo Erba, Milan, Italy). Water was distilled and passed through a MilliQ H_2O purification system (Millipore Ltd., Bedford, MA, USA). Formic acid (95–97%, Laboratory grade) and ammonium formate (AR grade) were purchased from Sigma Aldrich (Steinheim, Germany). Spirolide 13-desMeC standard solution was kindly provided by Dr Michael A. Quilliam (Institute for Marine Biosciences, National Research Council of Canada, Halifax, NS, Canada).

2.2. Cultures of Alexandrium ostenfeldii

Alexandrium ostenfeldii (Paulsen) Balech and Tangen (1985) was collected in the North-Western Adriatic Sea along the Emilia-Romagna coast (Italy) in November 2003. The dinoflagellate was isolated by the capillary pipette method (Hoshaw and Rosowski, 1973) and after an initial growth in microplates, unialgal cultures were grown in sterile Erlenmeyer flasks sealed with cotton plugs at 20 °C under a 16:8 h LD cycle (ca. 90 μmol m^{-2} s^{-1} from cool white lamps); nutrients were added at the f/2 concentration (Guillard, 1975), and H_2O salinity was adjusted to 30 Practical Salinity Units (psu, which corresponds to grams of salts per liter of solution). Cells from a total culture volume of 60 L were collected at stationary phase on the 30th day of

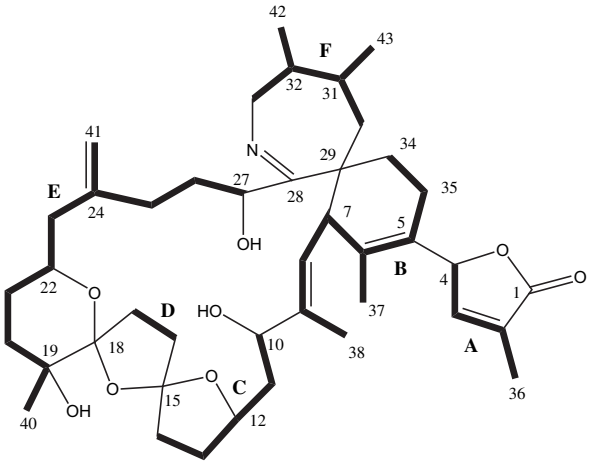

Fig. 2. Planar structure of 27-hydroxy-13-desmethyl spirolide C (**4**). Bold lines represent the six spin systems (A–F) emerging from COSY and z-TOCSY experiments.