Mass Spectral Characterization of Unknown Spirolides in AOSH2, a Canadian Strain of *Alexandrium ostenfeldii*

**AOSH2 produces 11 unknown spirolides**

The Canadian strain of *A. ostenfeldii* produces a high variety of spirolides. Only two of them are known compounds, namely spirolide C and 20-methyl spirolide G. All other compounds show mass spectral fragments, which are characteristic for spirolides, such as several subsequent water losses from the pseudo-molecular ion, characteristic mid mass fragments and the typical vecinal methyl cyclo imino fragment.

**Spirolide profile of the Canadian *A. ostenfeldii* strain AOSH2**

**Spirolide fragmentation**

1) Spirolides with 7 oxygen atoms show 4 subsequent water losses, spirolides with six oxygen atoms show 3 water losses

2) Positive charge is located on the imino nitrogen => low mass fragments are related to the eastern molecule part

3) Mid mass fragments are formed by the neutral loss of the western molecule part and structurally resemble eastern and southern parts.

**Proposed structures for unknown spirolides**

Peak b (m/z 692)

- Both eastern part and structurally resemble eastern and western molecule part
- Both compounds form high intensity fragments but with different low mass fragments.
- Both compounds form peaks 230, which differ in content of formaldehyde.

**Peak a (m/z 650)**

- Both compounds form high intensity fragments but with different low mass fragments.
- Both compounds form peaks 230, which differ in content of formaldehyde.

**Structure of to date elucidated spirolides**

**Summary**

The Canadian strain of *A. ostenfeldii* AOSH2 produces a high variety of spirolides, all of them are of the C,D or G-type with a vecinal dimethylation at the cyclic imino function. These spirolides, unlike A and B-type, are hardly metabolized, but easily accumulated by shellfish. Two of the spirolides could be identified by their mass spectra as spirolide C and 20-Me G, all others are unknown so far. However, structural elements can be deduced by mass spectrum comparison and interpretation. Unambiguous structural elucidation has to be performed by NMR techniques.