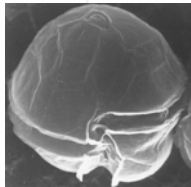




# Mass Spectral Characterization of Unknown Spirolides in AOSH2, a Canadian Strain of *Alexandrium ostenfeldii*



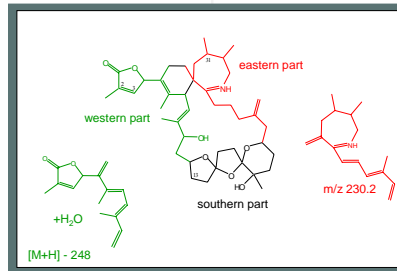
*Alexandrium ostenfeldii*

Spirolide	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>	MW (g/mol)
A	H	CH <sub>3</sub>	CH <sub>3</sub>	H	H	691.5
B	H	CH <sub>3</sub>	CH <sub>3</sub>	H	OH	693.5
C	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	705.5
13-desMe-C	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	691.5
13,19-di-desMe-C	CH <sub>3</sub>	CH <sub>3</sub>	H	H	OH	677.5
D	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	H	H	707.5
13-desMe-D	CH <sub>3</sub>	CH <sub>3</sub>	H	H	H	693.5
G	CH <sub>3</sub>	CH <sub>3</sub>	H	OH	H	691.5
20-Me-G	CH <sub>3</sub>	CH <sub>3</sub>	H	OH	OH	705.5

Structures of to date elucidated spirolides

## AOSH2 produces 11 unknown spirolides

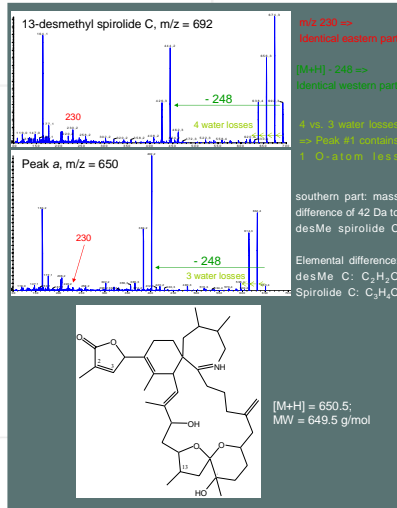
The Canadian strain of *A. ostenfeldii* produces a high variety of spirolides. Only two of them are known compounds, namely spirolide C and 20-methyl spirolide G. All other compounds show mass spectral fragments, which are characteristic for spirolides, such as several subsequent water losses from the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



Spirolide sub-structures (example: 13-desmethyl spirolide C)

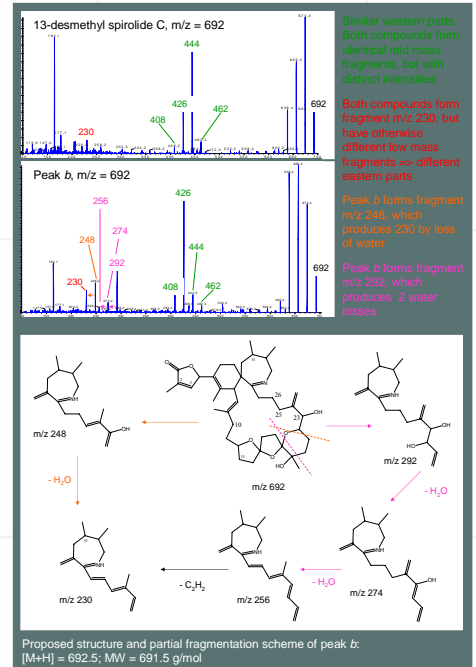
## Proposed structures for unknown spirolides

### Peak a (m/z 650)



The eastern and western parts of peak a are conserved as in C-type spirolides, however the southern part is missing a C<sub>3</sub>H<sub>4</sub>O structural element in relation to spirolide C. Thus the MS/MS spectrum of peak a is consistent with a spirolide structure with only two ether rings instead of the typical three.

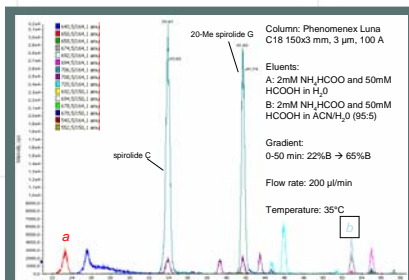
### Peak b (m/z 692)



Peak b is closely related to desMe C, which share the same molecular mass. Variations in the intensities of the mid mass fragments together with water losses from low mass fragments of peak b indicate a shift of the 10-hydroxylation from the western part of desMe C to the eastern part of peak b. The hydroxylation could be located at position 23 (as indicated) or alternatively at positions 25 or 26. The exact structure remains to be elucidated by NMR.

## 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.



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.