Marine Biotoxins: 
Determination of Spirolide Profiles 
in Phytoplankton by LC/MS/MS

1. Introduction
2. Search for unknowns
3. Mass spectral characterization
4. Phenotypic profiling of *Alexandrium ostenfeldii* strains
5. Pitfalls
Ecological Chemistry: Allelochemical Effects of Protists

Allelochemistry:

Interaction of biologically active components eliciting specific responses in target organisms.

These highly specific allelochemical compounds are typically secondary metabolites and should be distinguished from low molecular weight inorganic and organic nutrients and complex but poorly defined dissolved organic matter (DOM) that may be utilized as growth substrates by protists.

There are both stimulatory and inhibitory functions to be exploited via production of allelochemicals by protists. Among the putative functions of allelochemicals, their use as agents of chemical defence is most often invoked.
Ecological Chemistry: Allelochemical Effects of Protists

1. Introduction

Lytic effect of *Alexandrium*, here the example of *Oxyrrhis marina* (heterotrophic dinoflagellate).

Black arrows: *Alexandrium*; Red arrows: remains of *Oxyrrhis*

Photos: U. Tillmann

*Oxyrrhis marina*

*Alexandrium ostenfeldii*
### Ecological Chemistry: Allelochemical Effects of Protists

<table>
<thead>
<tr>
<th>Organism</th>
<th>Effect</th>
<th>Chemical Interaction</th>
<th>Ecological Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alexandrium ostenfeldii</em></td>
<td>Accumulation in marine food webs, poisoning of vertebrates and humans</td>
<td>Spirolides</td>
<td>?</td>
</tr>
<tr>
<td></td>
<td>Lysis of other protists</td>
<td>?</td>
<td>Defense against predators and/or competitors</td>
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</table>

**Organism Effect chemical interaction ecological function**

[Image of Spirolides molecule]
During routine monitoring of shellfish aquaculture sites in Nova Scotia, extracts of the digestive glands of blue mussels (*Mytilus edulis*) from Ship Harbour and sea scallops (*Placopecten magellanicus*) (Graves Shoal) elicited a *unique toxic response* in the DSP mouse bioassay……..

Coincident consumer complaints of *mild illness* after shellfish consumption.
“Fast Acting Toxicity”: Lipophilic extracts (DSP toxins)

Symptomology:

not PSP/DSP(!)

strong convulsions

tail whirling

body arching

rapid death (min)

High I.P. Toxicity
“Fast Acting Toxicity”: Occurrence

1. Introduction

- Transect Station
- TACCS Station
- Mussel farm
- Ship Harbour
- Nova Scotia, Canada
“Fast Acting Toxicity”: Occurrence

Limfjorden, Denmark

Later also found in:
Norway,
Scotland
1. Introduction

Scanning electron micrographs of vegetative cells

*Alexandrium tamarensen*  
*Alexandrium ostenfeldii*

LC/MS Tag Oldenburg 20.3.06  
Bernd Krock 2006
Confirmation of spirolides in cultured isolates from Nova Scotia

**Alexandrium tamarense**

Produces PSP toxins, but no spirolides

**Culprit species!!**

**Alexandrium ostenfeldii**

Produces spirolides, but no PSP toxins
## Cause of “Fast Acting Toxicity”

### Novel compounds identified as “spirolides”
- macrocyclic imines
- structural similarity to pinnatoxin & gymnodimine
- pharmacologically active/inactive forms

### Table of Structures

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<td>F</td>
<td>H</td>
<td>CH₃</td>
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Identical mass transitions – different retention times

13-desMe C Strd
MRM: m/z 692.5 > 164.1

AOSH 2
MRM: m/z 692.5 > 164.1

LC/MS Tag Oldenburg 20.3.06
Bernd Krock 2006
2. Search for unknowns

MS/MS spectra of m/z 692.5

13-desMe C Strd: EPI m/z 692.5

AOSH 2: EPI m/z 692.5

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2. Search for unknowns

Characteristic spirolide fragment

M+H = 692.5
m/z = 444.3
m/z = 164.1
Cyclic imino moiety accounts for toxicity and forms a characteristic spirolide fragment

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2. Search for unknowns

Cyclic imino moiety accounts for toxicity and forms a characteristic spirolide fragment
Using the characteristic fragment for the detection of unknown spirolides

AOSH 2: Precursor m/z 164.1
2. Search for unknowns

Spirolide masses

AOSH 2:  Precursor m/z 164.1

11.7 min  m/z 640.6  m/z 650.7

12.0 min  m/z 706.6

12.6 min  m/z 706.6  m/z 720.7

13.0 min  m/z 692.6  m/z 694.6
3. Mass spectral characterization

AOSH 2: m/z = 640.5
3. Mass spectral characterization

AOSH 2: \( m/z = 650.5 \)
3. Mass spectral characterization

AOSH 2: m/z = 692.5
3. Mass spectral characterization

AOSH 2: m/z = 694.5
3. Mass spectral characterization

AOSH 2: m/z = 706.5
3. Mass spectral characterization

AOSH 2: m/z = 720.5
4. Phenotypic profiling of *Alexandrium ostrenfeldii* strains

CCMP 1773, Denmark

678.5 > 164.1

692.5 > 164.1

13,19-didesMe C

13-desMe C
4. Penotypic profiling of *Alexandrium ostrenfeldii* strains

AOSH 1, Canada

692.5 > 150.1
692.5 > 164.1
694.5 > 164.1
706.5 > 164.1

13-desMe C

Spirolide C

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Bernd Krock 2006
4. Penotypic profiling of *Alexandrium ostrenfeldii* strains

**AOSH 2, Canada**

- Spirolide C
- 20-Me Spirolide G

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<tbody>
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<td>720.5</td>
<td>164.1</td>
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LC/MS Tag Oldenburg 20.3.06
5. Pitfalls

Identical retention time and mass transition,

**AOSH 1:** MRM 706.5 > 164.1

**AOSH 2:** MRM 706.5 > 164.1
but different mass spectra

AOSH 1: EPI m/z 706.5; 12.37 min

AOSH 2: EPI m/z 706.5; 12.37 min
5. Pitfalls

Compound or isotopic peak?

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5. Pitfalls

Compound or isotopic peak?

**AOSH 1:** MRM 694.5 > 164.1

**AOSH 2:** MRM 694.5 > 164.1
5. Pitfalls

Product ion spectra reveal isotopic pattern

**AOSH 1:** EPI m/z 694.5; 12.0 min

Isotopic fragments of m/z $^{13}$C$_2$-692.5

**AOSH 2:** EPI m/z 694.5; 12.9 min

Monosotopic fragments of m/z $^{12}$C-694.5
Conclusions:

Triple quadrupole tandem mass spectrometry is a powerful tool for the determination and quantitation of spirolides.

Unknown toxic spirolides can be detected in the precursor ion mode of the characteristic cyclo imino fragments at m/z 150 and 164, respectively.

Structural information can be obtained by product ion spectra of parent ions.

Co-eluting compounds with identical mass transitions can be differentiated by their product ion spectra.

Product ion spectra can be used to differentiate between isotope satellites and monoisotopic peaks.
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