Isolation of novel spirolides from the marine dinoflagellate *Alexandrium ostenfeldii*

I. Marschallek, B. Krock, A. Cembella

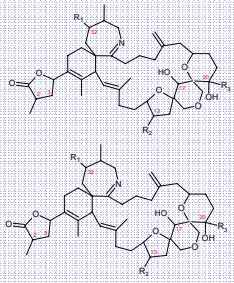
Alfred Wegener Institute for Polar and Marine Research,

Am Handelshafen 12, Bremerhaven, Germany

Abstract

Spirolides are macrocyclic compounds characterised by a tricyclic ether system and a seven-membered cyclic imine moiety. The marine dinoflagellate *Alexandrium ostenfeldii* is the only known proximal source of these biologically active compounds that evoke apparent neurotoxicological symptoms in mice. In recent investigations of a strain (AOSH2) of *A. ostenfeldii* originating from Ship Harbour in Atlantic Canada, we found several previously undescribed spirolides. Precursor scans of characteristic fragment-ions by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) revealed molecular ion masses that did not correspond to known structures and exhibited fragment ion spectra that differed from spirolides of equal molecular weight. An LC-MS/MS method was optimised for the baseline separation of the complex spirolide mixture. Since the unambiguous structural elucidation of these compounds requires nuclear magnetic resonance (NMR) spectroscopy, dinoflagellate batch cultures were harvested to generate sufficient spirolides (microgram range) and high purity components for spectroscopic analysis. Low pressure column chromatography and solid phase extraction (SPE) techniques were employed to remove major matrix compounds from the raw cell extracts. These combined methods provide a feasible scheme for the production of high purity spirolides for structural elucidation.

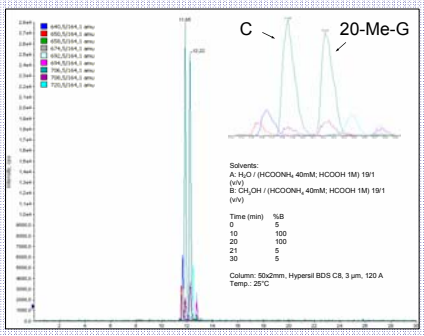
Spirolide structures



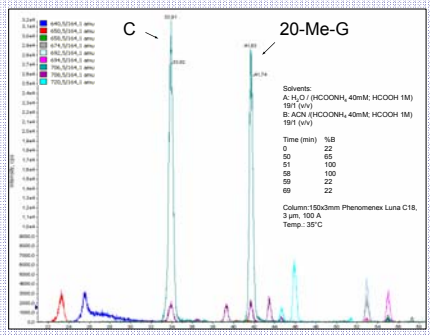
Spirolide	R_1	R_2	R_3	$\Delta^{2,3}$	EMW
A	H	CH_3	CH_3	√	691,5
B	H	CH_3	CH_3	-	693,5
C	CH_3	CH_3	CH_3	√	705,5
13-desMe-C	CH_3	H	CH_3	√	691,5
13,19-didesMe-C	CH_3	H	H	-	677,5
D	CH_3	CH_3	CH_3	-	707,5
13-desMe-D	CH_3	H	CH_3	-	693,5
G	CH_3	H	H	√	691,5
20-Me-G	CH_3	H	CH_3	√	705,5

Spirolide structures; bioactive forms produced by *A. ostenfeldii* cells originating from different populations; compounds indicated with by $\Delta^{2,3}$ have a double bond between carbons 2 and 3 (Aasen *et al.*, 2005)

LC-MS/MS method development

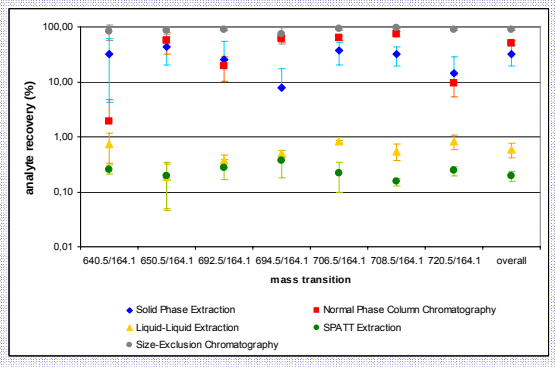


LC-MS/MS profile of spirolide composition in *A. ostenfeldii* (AOSH2 strain) from Ship Harbour, Nova Scotia, generated using a rapid screening method; undetermined peaks show novel spirolides of the given mass transition



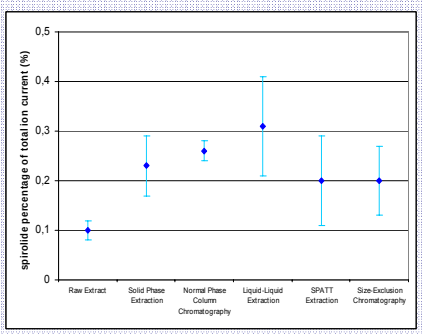
LC-MS/MS method developed for fractionation of purified cell extracts; peak separation of the 708,5/164,1 and 720,5/164,1 mass transition revealed the presence of additional novel spirolide compounds (showing different retention)

Analyte recovery



The extraction techniques revealed crucial differences in spirolide recovery. The column chromatographic steps showed high analyte recovery as well as good reproducibility at least for the major amount of compounds. SPE yielded a moderate analyte recovery, though the high standard deviation shows that the experiments were only poorly reproducible. For SPATT extraction, which is based on Solid Phase Adsorption (first described by MacKenzie *et al*., 2004) and Liquid-Liquid Extraction the recovery rates were insufficient, barely > 1%.

Purification efficiency



Purification was achieved by all of the employed clean-up steps. Although Liquid Liquid Extraction revealed the best clean-up efficiency, the results vary greatly and hence show low reproducibility. The most reliable results were obtained by Normal Phase Column Chromatography.

Summary

The investigations carried out so far have shown that purification of spirolides from the raw extract poses a great challenge. Different purification techniques have been employed in order to create a robust purification scheme, consisting of several clean-up steps. Development of the current LC-MS/MS method provides separation of the target analytes and foms the basis for fractionating. Preliminary results have already shown the applicability of the fractionating method; several of the novel spirolide compounds could already be separated. Forthcoming efforts will require the production of larger amounts of spirolides by mass culturing of *A. ostenfeldii* (AOSH2), hence offering the possibility for successful NMR-spectroscopic and mass spectrometric analyses of the novel compounds. Additionally, further improvement of analyte purification is necessary for confirmation of the recovery and efficiency data.

References:
Aasen, J., MacKinnon, S.L., Walter, J.A., Quilliam, M.A., (2005). Detection and Identification of spirolides in Norwegian shellfish and plankton. Chem. Res. Toxicol. *18*, 509-515
MacKenzie, L., Beuzenberg, V., Holland, P., McNabb, P., Selwood, A., (2004). Solid phase adsorption toxin tracking: a new monitoring tool that simulates the biotoxin contamination of filter feeding bivalves. Toxicon *44*, 901-918