A NEW APPROACH FOR PLTX-LIKE COMPOUNDS DISCOVERY IN *OSTREOPSIS* CF. *OVATA* SAMPLES

M. Varra[1], L. Tartaglione[1], V. Miele[1], E. Passfield[3], A. Selwood[3], S. Murray[3], A. Penna[4], A. Turner[5], T. Harwood[3], C. Dell'Aversano[1,2]

[1] Department of Pharmacy, School of Medicine and Surgery, University of Naples Federico II, via D. Montesano 49, 80131 Naples, Italy;
email: varra@unina.it
[2] NBFC, National Biodiversity Future Center, Palermo, Italy;
[3]Cawthron Institute, 98 Halifax Street East, Nelson 7010, New Zealand
[4] Department of Biomolecular Science, University of Urbino, Viale Trieste 296, 61121, Pesaro, Italy
[5] Weymouth Laboratory, Centre for Environment, Fisheries and Aquaculture Science, Weymouth, DT4 8UB, UK

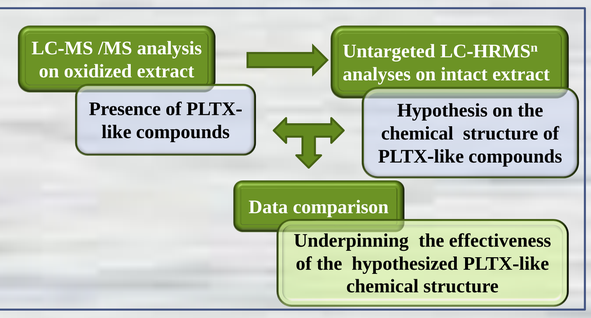
INTRODUCTION

A wide array of different palytoxin (PLTX)-like compounds, many of them present only in traces, has been identified analyzing different strain *of Ostreopsis* sp. As a result, the characterization of the whole toxin profile of some *Ostreopsis* cf. *ovata* strains can result challenging[1].

AIMS & WORFLOW

***Ostreopsis* cf. *ovata* cells**

Two *Ostreopsis* cf. *ovata* samples, one collected along the New Zealand coastlines and the second from the Italian coastlines, have been analyzed for the presence of PLTX-like compounds by means of two different methods: the micro-scale oxidation[2] method followed by targeted liquid chromatography coupled to mass spectrometry (LC-MS/MS) analyses and LC-high resolution MS (LC-HRMS) untargeted analyses of both the untreated and oxidized extracts[3]. The combination of the two aforementioned methods played a key role in establishing a new strategy for PLTX-like compound discovery.



WORKFLOW & RESULTS

LC-HRMSⁿ (n = 1, 2) ON CBA 1410 OXIDIZED EXTRACT (MEDITERRANEAN *O.* cf. *OVATA* STRAIN)

A Mediterranean strain of *Ostreopsis* cf. *ovata* (CBA1410), which contained OVTX-a as the major component of the toxin profile (Table 1) was used as reference sample. An aliquot of CBA1410 extract was oxidized on SPE cartridges using HIO_4 following a previously published procedure[2]. The resulting oxidized extracts were analysed by LC-HRMSⁿ (n=1,2) (Figures 2,3). Then, a New Zealand *O.* cf. *ovata* strain, CAWD 221, was subjected to the same oxidation process and both the intact and the oxidized extracts were analysed by LC-HRMSⁿ (n=1,2) (Figures 4-7).

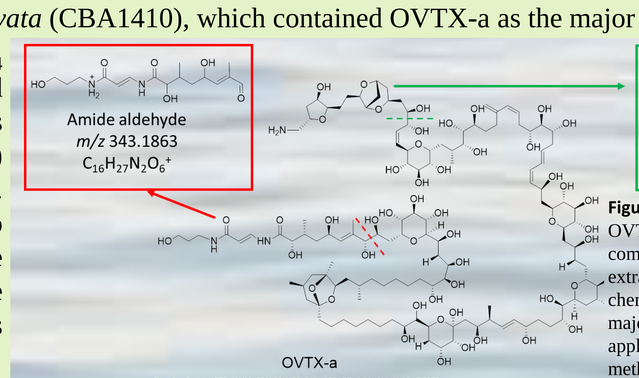


Figure 1. The chemical structure of OVTX-a, the major toxic component of the CBA1410 extract and, in green squares, the chemical structure of the two major products obtained from the application of the oxidation method on the extract CBA1410.

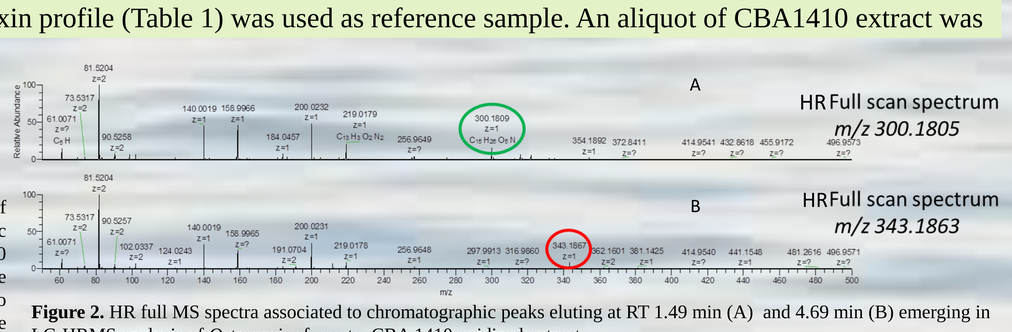
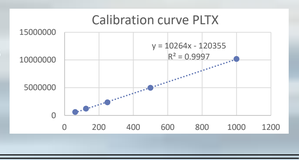


Figure 2. HR full MS spectra associated to chromatographic peaks eluting at RT 1.49 min (A) and 4.69 min (B) emerging in LC-HRMS analysis of *Ostreopsis* cf. *ovata* CBA 1410 oxidized extract.

CBA 1410
OVTX-a quantification in starting samples

Calibration curve PLTX W:M 1:1

ng/mL	Area
1000	10181003
500	4973647
250	2336683
125	1202518
62.5	591611



	OVTX-a peak area	µg/mL
CBA 1410	40120730	3.92

Table 1. Quantitative analysis: calibration curve of Palytoxin standard analyzed by LC-HRMS and OVTX-a content in *Ostreopsis* cf. *ovata* CBA 1410 extract.

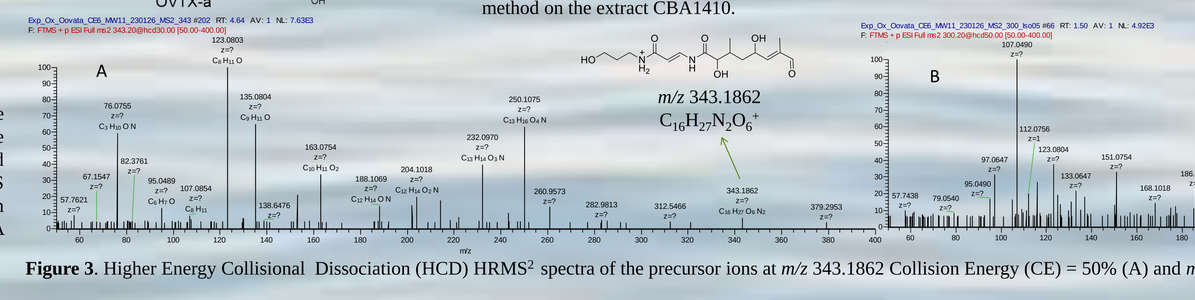


Figure 3. Higher Energy Collisional Dissociation (HCD) HRMS^2 spectra of the precursor ions at *m/z* 343.1862 Collision Energy (CE) = 50% (A) and *m/z* 300.1805 CE = 30% (B), in *O.* cf. *ovata* CBA 1410 oxidized extract

LC-HRMSⁿ (n = 1, 2) OF CAWD 221 OXIDIZED EXTRACT (NEW ZEALAND *O.* cf. *OVATA* STRAIN)

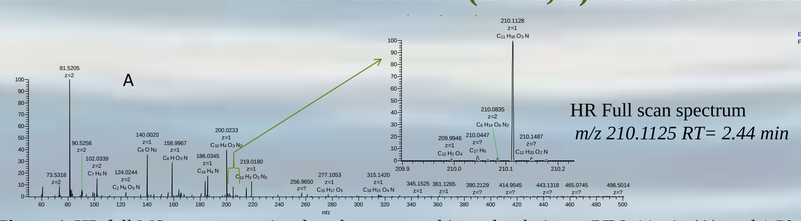


Figure 4. HR full MS spectra associated to chromatographic peaks eluting at RT 2.44 min (A) and 1.50 min (B) emerging in LC-HRMS analysis of *Ostreopsis* cf. *ovata* CAWD221 oxidized extract.

LC-HRMSⁿ (n = 1, 2) OF UNTREATED CAWD 221 EXTRACT

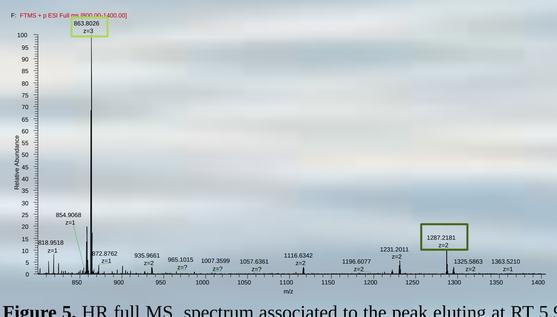


Figure 5. HR full MS spectrum associated to the peak eluting at RT 5.98 min in LC-HRMS of the untreated CAWD 221 extract. Green squares highlight the multiply charged ions associated to compound 1 with the dominant $[M+H+Ca]^{3+}$ ion at *m/z* 863.4693.

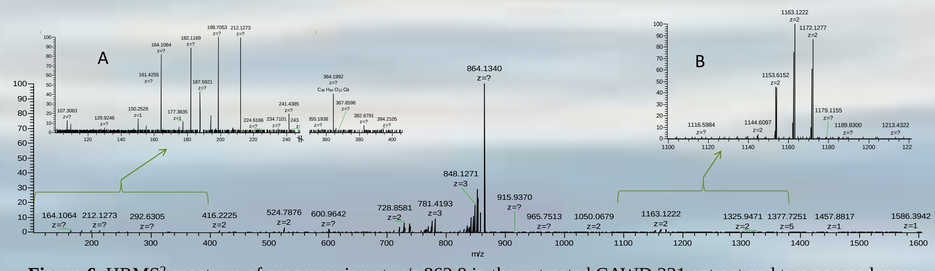
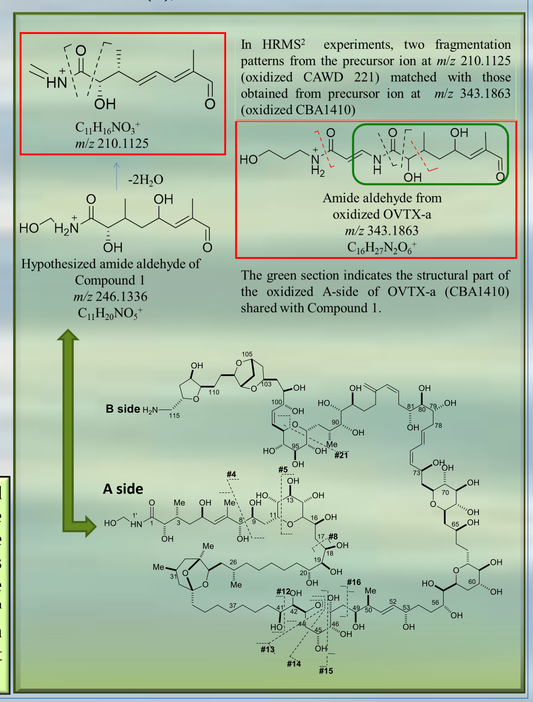


Figure 6. HRMS^2 spectrum of precursor ion at *m/z* 863.8 in the untreated CAWD 221 extract and two zoomed mass ranges *m/z* 100-400 (A), and *m/z* 1100-1400 (B).



In HRMS^2 experiments, two fragmentation patterns from the precursor ion at *m/z* 210.1125 (oxidized CAWD 221) matched with those obtained from precursor ion at *m/z* 343.1863 (oxidized CBA1410)

The green section indicates the structural part of the oxidized A-side of OVTX-a (CBA1410) shared with Compound 1.

Figure 7. The hypothesized structure of Compound 1. The arrow indicates the structure of the modified A-side as deduced from comparative analyses of LC-HRMSⁿ (n=1,2) data acquired on oxidized and untreated extract CAWD221.

DISCUSSION & CONCLUSIONS

LC-HRMS untargeted analyses conducted on the untreated and oxidized Mediterranean strain of *Ostreopsis* cf. *ovata* extract CBA1410 confirmed that OVTX-a was the most abundant PLTX-like compound in the extract and that the oxidative cleavage of OVTX-a gave rise mainly to two aldehyde products, derived from the amino and the amide portions of the molecule[2] (Fig. 1-3 and Table 1). To investigate the content in PLTX-like compounds of other *ostreopsis* sp. extracts, targeted LC-MS/MS analyses were conducted on oxidized extract CAWD 221, a New Zealand *Ostreopsis* cf. *ovata* sample, evidencing the presence of the amino aldehyde ion at *m*/z 300.2, whereas the peak at *m*/z 343.2 could not be detected (data not shown). These data suggested the presence of a PLTX-like compound featuring structural modification at the A-side of the molecule. LC-HRMS untargeted analyses of the oxidized extract confirmed the absence of the ion at *m*/z 343.1862 and showed, besides the ion at m/z 300.1805, the presence of two ions at m/z 246.1336 ($C_{11}H_{20}NO_5^+$) and 210.1125 ($C_{11}H_{16}NO_3^+$) which elemental formulae differed for two water molecules (Fig. 4). LC-HRMS untargeted analyses of the intact extract CAWD 221 evidenced the presence of one interesting ion *m*/z (z=3) 863.4693, RT 5.68 min (Fig. 5). The cross-checked interpretation of the elemental formulae of all the doubly- and triply-charged ions contained in the HRMS spectrum, confirmed the elemental composition of the new compound (Compound 1) $C_{124}H_{216}O_{51}N_2$. The processed HRMS^2 experiments conducted on the precursor ion at m/z 863.4693 (Fig. 6) revealed that this compound was a new PLTX congener presenting a C_5H_7ON moiety and two unsaturation less than OVTX-a ($C_{129}H_{223}O_{52}N_3$). Furthermore, results from LC-HRMS^2 experiments conducted on the oxidized extract selecting as precursor the ion at *m*/z 210.1125 suggested that this compound derived from the modified aldehyde generated from oxidative cleavage of Compound 1 at the A side (Fig. 7). Thus, the whole results accounted for the presence in CAWD 221 extract of a new PLTX-like compound modified at the A-side portion of the molecule, herein reported, at the best of our knowledge, for the first time.

References: Pavaux, A.-S. et al. Front. Mar. Sci., 2020, article 498; [2]Seelwood, A. et al. Toxicon 2012, 60, 810-20; [3]P. Ciminiello et al. J. Am. Chem. Soc. 2012, 134, 1869-1875.