Identification of Zinc and Cadmium Complexes of the Mycotoxin Sporidesmin A by Electrospray Mass Spectrometry

William Henderson,*[a] Christopher O. Miles,[b] and Brian K. Nicholson[a]

[a] *Department of Chemistry, University of Waikato, Private Bag 3105, Hamilton, New Zealand; E-mail w.henderson@waikato.ac.nz*

[b] *New Zealand Pastoral Agriculture Research Institute Ltd., Ruakura Agricultural Research Centre, Private Bag 3123, Hamilton, New Zealand*

The fungal toxin sporidesmin A (spdA), responsible for the occurrence of facial eczema in livestock, forms a dianionic thiolato complex of the type $[(spdA)_2M]^{2-}$ with zinc and cadmium, identified by electrospray mass spectrometry; such complexes may be important in the protection against facial eczema offered by zinc salts.

Sporidesmin A (spdA) **1** is a toxin produced by the saprophytic fungus *Pithomyces chartarum*, and is primarily responsible for causing facial eczema in livestock. Facial eczema has been reported to occur in New Zealand, Australia, France, South Africa and South America;[1] in New Zealand alone, annual losses to facial eczema have been estimated[1] at NZ$ 63 m. Dosing livestock with large quantities of zinc salts has been known for many years to offer the most effective preventative treatment against the effects of facial eczema.[1] Zinc is well known to form a diverse range of thiolate complexes of chemical and biochemical importance,[2] and it has been suggested[3] that the observed protection might be due to the formation of a complex between the administered zinc and the reduced form of sporidesmin A **2a**.

Here we report the application of electrospray mass spectrometry (ESMS) to the identification of complexes formed between reduced sporidesmin A **2a** and zinc and cadmium ions, both of which are known to form highly stable thiolate complexes. We also report some preliminary studies on a non-toxic thioether analogue, sporidesmin D **2b**. ESMS is a relatively new but extremely versatile technique which, to date, has been applied to only a relatively small number of inorganic systems. Specific examples of the use of ESMS in bioinorganic systems to date include studies on iron bleomycin[4] and the interaction of zinc ions with angiotensin peptides.[5]

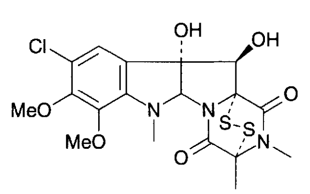
Sporidesmin A itself gives a strong molecular ion peak in the positive ion ESMS spectrum due to $[spdA]H^+$ at *m/z* 474, together with a peak due to the dimer $[spdA]_2H^+$ at *m/z* 947. Electrospray mass spectral data† are summarised in Table 1. In a typical metal complexation experiment, to an aqueous acetonitrile (1 : 1) solution of purified sporidesmin A[6] and $M(NO_3)_2$ (M = Zn or Cd) (6 : 1 molar ratio) was added a large excess of freshly prepared aqueous $NaBH_4$.‡ The predominant species in the negative ion ESMS spectrum at *m/z* 505 is assigned to the dianionic species $[(spdA)_2Zn]^{2-}$ **3**, whilst a smaller peak due to the sodium adduct $Na[(spdA)_2Zn]^-$ is found at *m/z* 1035. Because zinc has a very strong tendency[2] to form tetrahedral tetrathiolatozincate complexes $[(RS)_4Zn]^{2-}$, it is highly probable that the species $[(spdA)_2Zn]^{2-}$ (and also the sodium adduct) is a thiolato complex of this type. Consistent with this, the $[(spdA)_2Zn]^{2-}$ species was found to be stable to high cone voltages (up to 80 V was investigated) without noticeable fragmentation.

When an excess of $NaBH_4$ reducing agent was not employed, additional peaks in the spectrum at *m/z* 743 and 979 were observed and assigned to the species $[(spdA)_3Zn]^{2-}$ and $[(spdA)_4Zn]^{2-}$, respectively; a typical spectrum is illustrated in Fig. 1. These may be hydrogen-bonded adducts of $[(spdA)_2Zn]^{2-}$ with one and two neutral sporidesmin A molecules, thereby retaining the four-coordination at zinc typically found in thiolate complexes. Analogous spectra were

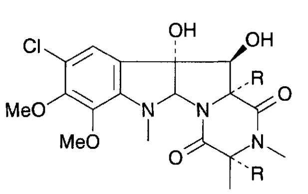
Table 1 Electrospray mass spectral data

System	Ion mode	Ions[a]
1	+	$[spdA]H^+$ (474), $[spdA]_2H^+$ (947)
2a + Zn^{2+}	−	$[(spdA)_2Zn]^{2-}$ (505), $[(spdA)_3Zn]^{2-}$ (743), $[(spdA)_4Zn]^{2-}$ (979), $Na[(spdA)_2Zn]^-$ (1035)
2a + Cd^{2+}	−	$[(spdA)_2Cd]^{2-}$ (530), $[(spdA)_3Cd]^{2-}$ (767), $[(spdA)_4Cd]^{2-}$ (1003), $Na[(spdA)_2Cd]^-$ (1083)
2b	+	$[spdD]H^+$ (504), $[spdD]_2H^+$ (1008) $[spdD]_2NH_4^+$ (1025), $[spdD]_3H^+$ (1528)
2b + Ag^+	+	$[spdD]Ag^+$ (612), $[spdD]Ag(MeCN)^+$ (653), $[spdD]_2Ag^+$ (1115)

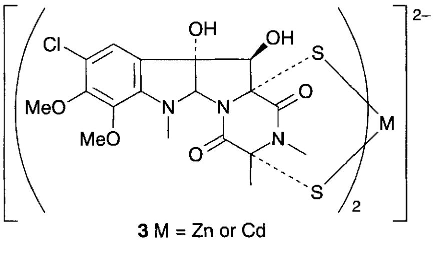
[a] All peaks are identified by the peak in the isotope distribution pattern having the greatest intensity.



1



2a R = S⁻
b R = SMe



3 M = Zn or Cd

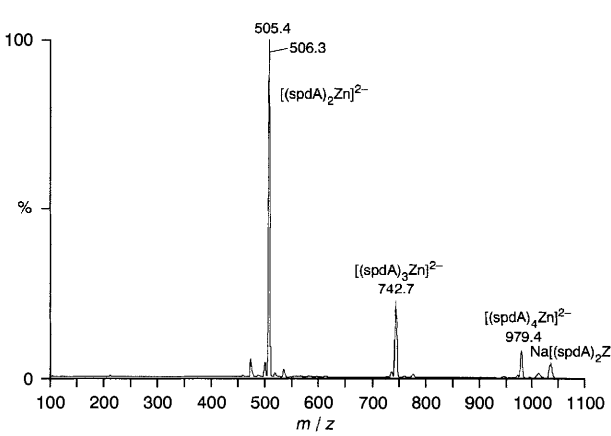


Fig. 1 Electrospray mass spectrum ($MeCN–H_2O$, cone voltage 30 V) of a mixture of sporidesmin A, $Zn(NO_3)_2$ and $NaBH_4$ reducing agent

also obtained with $Cd(NO_3)_2$, with the expected mass shifts for the corresponding sporidesmin complexes. For all assigned peaks there was excellent agreement between the observed and calculated isotope distribution patterns.

We also investigated metal ion adducts of the thioether analogue sporidesmin D (spdD), which was synthesised *via* reaction of **1** with $NaBH_4$, pyridine and MeI in ethanol.[7] A single crystal X-ray structure analysis of **2b** indicated that the sulfur lone pairs are ideally disposed for metal coordination. ESMS (positive ion mode) of a mixture of spdD and $AgNO_3$ in MeCN–H_2O (1 : 1) (Table 1) indicated that Ag^I readily forms adducts of the type $[(spdD)Ag]^+$, $[(spdD)Ag(MeCN)]^+$ and $[(spdD)_2Ag]^+$. Although the exact nature of these adducts remains speculative, it is not unreasonable to suggest that $[(spdD)_2Ag]^+$ contains a distorted tetrahedral AgS_4 coordination geometry, in keeping with the structures of other Ag^I–thioether complexes.[8]

This preliminary study demonstrates that electrospray mass spectrometry is a versatile technique for the identification of metal ion adducts of mycotoxic sporidesmins. Tetrathiolatozincate complexes of the type $[(spdA)_2Zn]^{2-}$ **3** may be important species formed in the well-known protection afforded by zinc salts against the toxic effects of sporidesmin A. ESMS has the potential to allow the investigation of competitive complexation of sporidesmin A with other metal ions known to form stable thiolate complexes, or with zinc complexes containing other ancillary (*e.g.* thiolate) ligands. Such studies may lead to a better understanding of the coordination chemistry of toxic epidithiodioxopiperazine mycotoxins—such as sporidesmin and gliotoxin—with biologically important metals, and to the development of improved measures for the control of facial eczema. The recent implication of gliotoxin in candidiasis in humans[9] provides further impetus for such studies.

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Footnotes

† Electrospray mass spectra were recorded on a VG Platform II instrument. The sample was injected into the spectrometer *via* a Rheodyne injector fitted with a 10 μl sample loop. A Thermo Separation Products SpectraSystem P1000 LC pump delivered the solution to the mass spectrometer source at a flow rate of 0.01 ml min^{-1}, and nitrogen was employed both as a drying and nebulizing gas. Cone voltages were varied between 20 and 80 V, while voltages of 30 V were typically found to be satisfactory.

‡ These conditions were found to give the simplest spectra; use of lower spdA : Zn ratios and lesser amounts of $NaBH_4$ reductant leads to more complex spectra.

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