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Extraordinary levels of cadmium and zinc in a marine sponge, *Tedania charcoti* Topsent: inorganic chemical defense agents

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Abstract. The Antarctic marine sponge *Tedania charcoti* has been shown to contain extraordinarily high natural concentrations of cadmium and zinc, which have in turn been correlated to the ability of the crude ethanol extract to modulate protein phosphorylation in chicken forebrain and to inhibit the growth of several test bacteria.
Key words. Cadmium; zinc; Antarctic; marine; sponge; antibiotic; protein phosphorylation; *Tedania charcoti.*

During the course of scientific dredging operations carried out in Prydz Bay, Antarctica, over the summer of 1990/91, many sponges were collected, including two specimens of the marine sponge *Tedania charcoti*, Topsent, 1907 (Poecilosclerida: Tedaniidae) from geographically distinct locations. One specimen of *Tedania charcoti* was collected at Station 55B, Site 15 (trawling from 67°27.27′S, 71°07.38′E to 67°28.56′S, 71°08.31′E) at a depth of 439 m and at a water temperature of −2.1 °C. A second specimen of *Tedania charcoti* was collected at Station 74 (trawling from 67°01.08′S, 78°11.54′E to 67°01.59′S, 78°14.90′E) at a depth of 251–266 m and at a water temperature of −1.6 °C. A sample of *Tedania charcoti* has been lodged with the Queensland Museum, registry number QM G300714.

The aqueous ethanol extract of both these specimens of *Tedania charcoti* proved to have potent antibacterial properties, inhibiting the growth of strains of the bacteria *Staphylococcus aureus*, a *Micrococcus* sp., a *Serratia* sp. and *Escherichia coli*, as well as the ability to modulate protein phosphorylation in chicken forebrain. Detailed chemical investigations revealed the biological activity to be due to extraordinarily high levels of both cadmium and zinc. It would appear that *Tedania charcoti* possesses the ability to sequester and tolerate high concentrations of cadmium and zinc from seawater.

Results and discussion

Over the last two decades the search for unusual biologically active marine natural products has prompted concerted interdisciplinary collaboration[1], bringing together expertise in the areas of taxonomy, pharmacology and chemistry. The use of bioassay directed isolation has been especially useful in directing chemists to valuable metabolites that might otherwise have gone undetected. This approach has successfully targeted numerous unique organic molecules, many of which have been reported to display biological properties of one form or another[1]. Typically, the active agents are secondary metabolites incorporating unusual functional groups indicative of novel biosynthetic origins.

On undertaking the isolation of the active agents in *Tedania charcoti* it rapidly became apparant that we were not dealing with an organic substance. Most telling was the observation that heating a dried portion of the aqueous ethanol extract at flame temperature did not diminish the antibacterial activity. Elution of a water soluble portion of the dried aqueous ethanol extract through Sephadex G10 (with water as the eluent) returned a white, crystalline, antibacterially active fraction. Slow recrystallization of this material from water yielded an abundance of NaCl crystals, plus a selection of fine colourless needle like crystals. Measurement of the space group for these crystals (orthorhombic Pnma, cell dimensions 8.775 3.993 14.591 a/b = 2.198 (0.4550) a/c = 0.6014 b/c = 0.2737) identified them as $KCdCl_3$[2]. This unexpected discovery was confirmed by scanning electron microscopy of the freeze dried crude aqueous ethanol extract which in addition to detecting significant levels of cadmium, also detected high levels of zinc. In order to quantify the amounts of cadmium and zinc present in the original extracts, samples were submitted for atomic absorption spectroscopy, as well as inductively coupled plasma emission spectrometry. The atomic absorption analysis confirmed cadmium concentrations of 320 μg/ml (2.86 mM) and 95μg/ml (0.85 mM), and zinc concentrations of 108 μg/ml (1.66 mM) and 250 μg/ml (3.81 mM), for the aqueous ethanol extracts from the two *Tedania charcoti* specimens respectively. These measurements equate to minimum dry weight heavy metal concentrations of 15,000 and 2000 ppm cadmium, and 5100 and 5000 ppm zinc respectively. Aqueous ethanol solutions of $CdCl_2$ and $ZnCl_2$ at concentrations comparable to those in the natural extracts strongly inhibited the growth of the test bacteria. Similarly, the

combination of Cd and Zn produced effects on the levels of protein phosphorylation equivalent to those found with the sponge extracts. Specifically, and consistent with previous reports[3,4], Zn potently inhibited protein phosphatase activity. Cadmium alone was found to inhibit protein phosphatase activity, however, the effect of Cd alone was much less than that of Zn alone, suggesting that the effects on protein phosphatase activity observed with the sponge extracts were primarily due to the effects of Zn. In addition to effects on protein phosphatase activity, Zn and Cd together elevated basal protein kinase activity, an effect not seen with either Zn or Cd alone. The nature of the synergistic activation of protein kinase is unknown and the subject of further investigation.

The ability of *Tedania charcoti* to both sequester cadmium and zinc from seawater, where they are present in only trace amounts, as well as to tolerate the accummulation of extraordinarily high levels of these toxic heavy metals is noteworthy. At this stage we can only speculate that the high concentration of cadmium and zinc accummulated by *Tedania charcoti* serves as a natural antibiotic and/or an antifouling agent and/or a toxic defense against predation. Logistical difficulties associated with re-collection have prevented a detailed investigation of the mechanism of heavy metal accummulation and storage. In the event that a re-collection can be made it is hoped to further examine these issues. In the meantime it is worth observing that the bioassay directed isolation of 'active marine natural products' need not culminate in the isolation of novel organic secondary metabolites, but may lead to inorganic species. This prospect is all the more noteworthy given the increasing trend towards detecting, isolating and identifying active agents, and the probability that at least some of these active responses may be due to inorganic rather than organic substances.

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