E2-13: Cytotoxic alkaloids from the flatworm *Prostheceraeus villatus* and its tunicate prey *Clavellina lepadiformis*

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Flatworms (Phylum:Platyhelminthes) are brightly coloured animals with unprotected soft bodies. *Prostheceraeus villatus* (Class: Turbellaria, Order: Polycladida) is a relatively large flatworm. Flatworms in the Order Polycladida are carnivores that feed on a variety of small animals. It is well documented that shell-less molluscs which are also brightly coloured and soft bodied, utilise defensive allomones, often sequestered from dietary organisms, to thwart predators, By analogy it is reasonable to hypothesise flatworms might also use defensive chemicals as one method to deter predation. The flatworm *P.villatus*, and its tunicate prey *Clavellina lepadiformis* were collected in sufficient numbers making the chemical characterisation of this pair possible.

The flatworms and their tunicate prey were collected by hand using SCUBA at depths of -3 to - 20m. Freshly collected specimens were preserved in MeOH. The $CHCl_3$ soluble fraction of the crude extracts of *P.villatus* was fractionated by size-exclusion chromatography on Sephadex LH 20 and by reversed phase HPLC. A similar chromatographic procedure was used to fractionate the crude extract of the tunicate *C.lepadiformis*. The pure compounds which resulted from chromatography were characterised mainly by MS and NMR (1D and 2D) spectroscopy.

Chromatographic separation of the crude extract of the flatworm gave 3 pure compounds. Spectral characterisation of these metabolites established the major compound to be the known alkaloid lepadin A (*Figure 1*). Lepadin A was identified by comparison of its NMR and MS data with literature values. Closely related structures of the remaining 2 metabolites, lepadin B and C were established by comparison of their MS and NMR data (1D and 2D) with those of lepadin A. Similar spectral characterisation of the tunicate metabolites showed that the tunicates contain the same 3 alkaloids in about the same proportions.

Lepadin A and B exhibited significant *in vitro* cytotoxicity against a number of human cancer cell lines.

The current study is the first to show that flatworms sequester secondary metabolites from a prey organism.

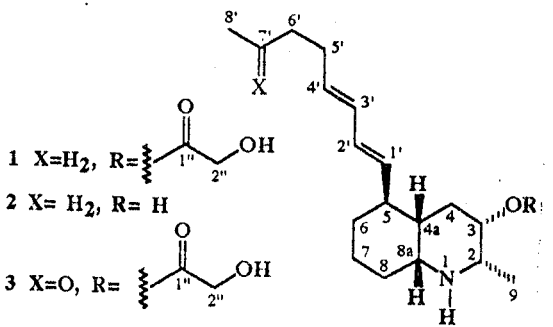

Figure 1: Structures of lepadin A (1), lepadin B (2) and lepadin C (3)