E2-08: Cytotoxic peptides from the marine ponge *Cymbastela sp.*

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Marine sponges, the most primitive multicellular animals, are a rich source of cytotoxic compounds with novel structural features. Notable among these are peptides that contain previously unknown amino acids. Over 100 tropical sponges were screened for cytotoxicity and it was found that the crude extracts of the sponge *Cymbastela* exhibit significant *in vitro* cytotoxicity (L 1210 ED_{50} > 1 μg/ml). Studies were undertaken to isolate and characterise the active principle(s) present in the crude extract and to evaluate their biological properties.

Specimens of *Cymbastela sp.* were collected by hand, using SCUBA on reefs off Madang, Papua New Guinea. Sponge (260 g dry wt) was extracted exhaustively with a mixture of CH_2Cl_2/MeOH (1:1). Evaporation of the organic extract gave an aqueous suspension. MeOH was added to give a 9:1 MeOH/H_2O solution (1l), which was extracted with hexane (4 x 250 ml). Water was added to the MeOH solution to give a 1:1 MeOH/H_2O solution, which was extracted with $CHC1_3$ (4 x 250 ml). The combined $CHCL_3$ layers were concentrated *in vacuo* to yield an orange oil (3.5g). Bioassay guided fractionation, repeatedly by size exclusion chromatography on Sephadex LH-

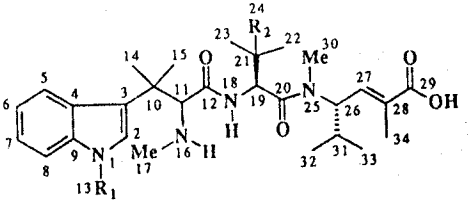
20 eluting with MeOH gave a fraction containing the cytotoxic peptides. Reversed-phase isocratic HPLC (0.05%TFA:MeOH, 1:1) afforded 3 peptides, with potent cytotoxicity. The structure elucidation of the peptides was done with Mass, IR, UV and high resolution NMR spectroscopy.

Interpretation of the spectral data showed that the structure of the major peptide ($C_{30}H_{47}O_4N_4$; 40 mg) to be identical to the known tripeptide hemiasterlin (*Figure 1*), previously isolated from the unrelated sponge *Hemiasterella minor*. Hemiasterlin A ($C_{29}H_{45}O_4N_4$;32 mg) and hemiasterlin B ($C_{28}H_{43}0_4N_4$; 1 mg) had closely related structures as shown in *Figure 1*.

The hemiasterlins showed potent *in vitro* activities against

(i) murine leukaemia P388, (ii) human breast cancer MCF7 (iii) human glioblastoma/astrocytoma U373 and (iv) human ovarian carcinoma HEY. They also showed potent *in vivo* activity in mice injected with P388 leukaemia cells.

Work on an identified sponge of the genus *Cymbastela* shows that it contains metabolites with potent cytotoxicities against a number of human cancer cell lines. These peptides are promising candidates to be developed as clinically useful pharmaceuticals.



1 $R_1 = R_2 = Me$
2 $R_1 = H$, $R_2 = Me$
3 $R_1 = R_2 = H$

Figure 1 - Structures of Hemiasterlins