

## SUMMARY

**Title of Project:** Bioactive Natural Products from Sri Lankan Lichens: Culturing of the Mycobiont in the Laboratory

**Institution Where the Research is Carried Out:** Department of Chemistry,  
University of Peradeniya

**Chief Scientific Investigator:** Veranja Karunaratne

**Period of Contract:** June 15 1996 to December 2000

### **Scientific Background and Scope/Objectives of Project:**

There is general scarcity of research on Sri Lanka lichen flora. The World Conservation Monitoring Center has designated Sri Lanka as a "hotspot" in terms of biodiversity. Of this rich biodiversity, unmatched in the region, the non-flowering plants (cryptogams) are least studied.

Therapeutic value of plants is well known. However, tropical lichens, which show great promise as a source of therapeutics, are one of the least studied cryptogams. In Sri Lanka, both taxonomy and chemistry of these lichens had remained completely unexplored until 1996, when I initiated a research program to explore the lichen flora of Sri Lanka for biologically active compounds.

Therefore the research work was undertaken to explore the potential of Sri Lankan lichen flora, hitherto unexplored national resource, as a source of new bioactive compounds.

### **Experimental method:**

The research carried out under the above grant focussed on the isolation of bioactive compounds from four lichen species collected in Sri Lanka: *Usnea* sp., *Heterodermia diademata*, *Roccella montagnei* and *Leproloma sipmanianum*.

Bioassays used in this study are the antifungal assay against *Cladosporium cladosporioides*, and the mosquitolarvicidal assay against the 2<sup>nd</sup> instar larvae of *Aedes*

*aegypti*. Approximately 50 lichen species collected mostly from the montane region were subjected to the above bioassays.

### Results:

From the CH<sub>2</sub>Cl<sub>2</sub> extract of an *Usnea* sp. collected from Ambewela, three compounds, namely, Ambewelamide A, Ambewelamide B and Usnic acid were separated. Ambewelamide A and B are new members of a family of highly modified dithiadiketopiperazines and constitute the first examples of this family of compounds isolated from a lichen. Ambewelamide A was found to possess potent *in vitro* cytotoxicity (murine leukemia P388: IC<sub>50</sub> 8.6 ng/ML) and significant *in vitro* antineoplastic activity (P388: %T/C 140 @ 160 µg/Kg). A Canadian patent application was filed based on the promising bioactivity of this compound. The MeOH extract of the same lichen furnished two new depsidone lactones.

Seven compounds were isolated from *Roccella montagnei* lichen collected from Bamunukotuwa in the Kurunegala district. The MeOH extract yielded methylorsellinate, erythrin, erythritol, rocellic acid, β-sitosterol, and β-carotene and atranorin. The CH<sub>2</sub>Cl<sub>2</sub> extract yielded aspisilin.

*Heterodermia diademata* collected from Labukelle, Nuwara Eliya District, furnished 5-chloro-3-formyl-4-hydroxy-2-methoxybenzoic acid, methyl β-orcinol carboxylate and zeorin. The CH<sub>2</sub>Cl<sub>2</sub> extract gave atranorin and chloroatranorin.

*Leproloma sipmanianum* specimens were collected in the upland area of Nuwara Eliya on proterozoic rocks of gneiss and quartz around Beragala. This is the first report of the lichen from the Asian region. The butterfly *Talicadia nyseus nyseus* (Guer) (Red Pierrot) was found to be closely associated with *Leproloma sipmanianum*. The

CH<sub>2</sub>Cl<sub>2</sub> extract of *Leproloma sipmannianum* contained five compounds namely, (+) usnic acid, atranorin,  $\beta$ -sitosterol, zeorin, and a long chain fatty acid ester. Upon comparison, the extracts of the adult *Talicadia nyseus nyseus* contained all of the above compounds. The presence of the above five compounds in the butterfly was confirmed by a combination of TLC and GCMS. The presence of lichen substances in the adult *Talicadia nyseus nyseus* indicates that their larvae feed on *Leproloma sipmannianum*. To the best of our knowledge, there are no previous reports of the butterfly family (Lycaenidae) feeding on lichens.

From Ramboda in Nuwara Eliya district, a new lichen species growing on proterozoic rocks of gneiss and quartz was discovered. This species has been named as *Lepraria atrotomentosa*.

In May 1999, we (Peradeniya Botanic Gardens and Departments of Botany and Chemistry, University of Peradeniya) organised the first national workshop on Sri Lankan lichens. In this effort we were able to make very valuable contacts with Dr. G. N. Hariharan of the Swaminathan Research Institute, Chennai, India and Ms. Pat Wolseley of the British Natural History Museum, UK. About 15 scientists participated in this event.

**Papers published:**

- (1) Williams, D., Bombuwala, K., Lobkovsky, E., De Silva, E.D., Karunaratne, V., Allen, T.M., Clardy, J & Andersen, R.J. 1998: Ambewelamide A and B, Antineoplastic Epidithiapiperazinediones Isolated from the Lichen *Usnea* sp. *Tetrahedron Letters*, **39**: 9579-9582.
- (2) V. Karunaratne, K. Bombuwela and E.D. De Silva, Novel Antineoplastic Compounds from Sri Lankan *Usnea* sp. (1998), *Proceedings of the Award Winning Papers, University of Peradeniya Research Sessions*, **3A**, 83.
- (3) K. Bombuwela, V. Subramaniam, K.S. De Silva, V. Karunaratne, N.K.B. Adikaram and H.M.T.B. Herath, Antitumor and Antifungal Activity of Usnic acid and Atranorin. (1999), *Cey. J. Science (Physical Science)*, **6(1)**, 8-12.

- (4) V. Karunaratne, Lichen Substances: Biochemistry, Ecological Role and Economic Importance. (1999), *Cey. J. Science (Physical Sciences)*, **6(1)**, 13-28.
- (5) K. Bombuwela and V. Karunaratne, Identification of lichen Substances from *Roccella Montagnei*. (2001), *Proceedings of the Award Winning Papers, University of Peradeniya Research Sessions*, **4A**, 21.
- (6) C. Nayanakantha, K. Bombuwela, V. Karunaratne, N.K.B. Adikaram and D.S.A. Wijesundara, Studies on Sri Lankan Lichen Flora. (2001), *Proceedings of the Award Winning Papers, University of Peradeniya Research Sessions*, **4A**, 77.
- (7) A. Orange, P. Wolseley, V. Karunaratne and K. Bombuwela, Two Leprarioid Lichens New to Sri Lanka. (2001), *Bibliotheca Lichenologica*, **78**, 327-333.
- (8) V. Karunaratne, K. Bombuwela and N.K.B. Adikaram, Constituents of the lichen *Heterodermia diademata*, Fitoterapia (submitted).
- (9) V. Karunaratne, K. Bombuwela and N.K.B. Adikaram, Antifungal Constituents of the lichen *Roccella montagnei*, Fitoterapia (submitted).
- (10) V. Karunaratne, K. Bombuwela, S. Kathirgamanathar, D. N. Karunaratne, K.B. Ranawana, D.S.A. Wijesundara, A. Weerasooriya, and E.D. De Silva, An association between the butterfly *Talicauda nyseus* and the lichen *Leprolomasipmanium* as evidenced from chemical studies, *Current Science* (submitted).

#### **Communications Presented:**

- (1) B.D.K. Bombuwela, V. Karunaratne, N.K.B. Adikaram, Aweerasooriya and E.D. De Silva, Antifungal Activity of Sri Lankan Lichens. (1997), Faculty of Science, *University of Peradeniya Annual Research Sessions*, 18.
- (2) K. Bombuwela, V. Karunaratne, E. D. De Silva, N.K.B. Adikaram and A. Weerasooriya, Antifungal and Mosquitolarvicidal Activity of Sri Lankan Lichens. (1997), *Proc. Sri Lanka Assoc. Advmt. Sci.* **53**, 269.
- (3) K. Bombuwela, V. Karunaratne, E.D. De Silva, N.K.B. Adikaram, A New Aromatic Polyether Compound from the Coconut Lichen *Ramalina farinacea*. (1997), *Proc. Sri Lanka Assoc. Advmt. Sci.* **53**, 350.
- (4) V. Karunaratne, K. Bombuwela and E.D. De Silva, Ambewelamides A and B, Novel Antineoplastic Compounds from Sri Lankan *Usnea* sp. (1998), *University of Peradeniya Annual Research Sessions*, **85**.
- (5) K. Bombuwela, V. Karunaratne, K.B. Ranawana, A. Weerasooriya, D.S.A. Wijesundara and E.D. de Silva, Sequestration of Lichen Compounds by *Talicia*

- Nyseus Nyseus*. (1998), *University of Peradeniya. Annual Research Sessions*, 26.
- (6) K. Bombuwela, V. Karunaratne and E. D. de Silva A Novel Cytotoxic Compound From a Sri Lankan *Usnea* sp. (1998), *Proc. Sri Lanka Assoc. Advmt. Sci.* 54, 289.
  - (7) K. Bombuwela, V. Karunaratne, K. B. Ranawana, A. Weerasooriya, D.S.A. Wijesundara and E.D. de Silva, Evidence for a Lichen-Butterfly Association. (1998), *Proc. Sri Lanka Assoc. Advmt. Sci.* 54, 206
  - (8) K. Bombuwela, V. Karunaratne, N.K.B. Adikaram and B.S.S. Mendis, Antifungal Activity Profiles of a Lichen Substance and its Biosynthetic Precursor of *Ramalina farinacea*. (1998), *Proc. Sri Lanka Assoc. Advmt. Sci.* 54, 288.
  - (9) K. Bombuwela, V. Karunaratne, K.S. de Silva and H.M.T.B. Herath, Insecticidal Activity of Usnic Acid. (1998), *Proc. Sri Lanka Assoc. Advmt. Sci.* 54, 294.
  - (10) V. Karunaratne, K. Bombuwela, V. Subramaniam and N.K.B. Adikaram, Antifungal and Mosquitolarvicidal Activity of Usnic Acid and Atranorin. (1999), *Proc. Sri Lanka Assoc. Advmt. Sci.* 55, 231.
  - (11) K. Bombuwela and V. Karunaratne, Identification of Lichen Substances in the Lichen *Heterodermia* sp. (1999), *Proc. Sri Lanka Assoc. Advmt. Sci.* 55, 232.
  - (12) K. Bombuwela and V. Karunaratne, Identification of Lichen Substances from the Lichen *Rocella* sp. (1999), *Proc. University of Peradeniya Annual Research Sessions*, 24.
  - (13) C. Nayanakantha, K. Bombuwela, V. Karunaratne, N.K.B. Adikaram and D.S.A. Wijesundara, Studies on Sri Lankan Lichen Flora. (1999), *Proc. University of Peradeniya Annual Research Sessions*, 116.
  - (14) Characterisation of the Lichen Compounds Present in the Butterfly, *Talicia nyseus nyseus*. (2000), V. Karunaratne, K. Bombuwela, K.B. Ranawana, E.D. De Silva and D.S.A. Wijesundara, *Proc. Sri Lanka Assoc. Advmt. Sci.*, 56, 246.
  - (15) Chemistry and Biology of Sri Lankan Lichens. (2000), V. Karunaratne, *Gordon Research Conference*, Plymouth, New Hampshire, USA. (No abstracts are printed at this exclusive conference).
  - (16) Lichen Substances from *Heterodermia leucomelos*. (2000), S. Kathirgamanathar, V. Karunaratne and V. Kumar, *Proc. Faculty of Science and Post Graduate Institute of Science Research Sessions*, 19.
  - (17) Lichens New to Sri Lanka: *Lepraria atrotomentosa* and *Leproloma sipmanianum*. (2000), V. Karunaratne, K. Bombuwela, A. Orange and P. Wolseley, *Proc. Faculty of Science and Post Graduate Institute of Science Research Sessions*, 3.