E2-17: Antifungal activity profiles of a lichen substance and its biosynthetic brecursor of *Ramalina farinaceae*

Karunananda Bombuwala[1], Veranja Karunaratne[1], N K B Adikaram[2], B S S Mendis[1]
([1] *Dept of Chemistry*, [2] *Dept of Botany, University of Peradeniya*)

Lichens are ubiquitous and produce a variety of biologically active substances suggesting that they might function in the chemical defense against pathogens. *Ramalina farinaceae* is a lichen growing on the main tree trunk of coconut trees found mainly in the Kurunegala district. The lichen is ash-brown in colour, has an erect thallus (foliose type) and is easily removable. Previously we reported the isolation of erythrin from the MeOH extract of the same lichen. In the TLC bioassay technique against fungus *Cladosporium cladosporioides* erythrin showed low activity while methyl-2-4-dihydroxy-6-methyl-benzoate, also isolated from the same extract, was strongly antifungal active. We were intrigued by this result since both compounds are almost identical in functionality and in general, phenolic compounds are known to exhibit antifungal species. The present work describes the comparison of antifungal activity profiles of erythrin and its biosynthetic precursor, methyl-2,4-dihydroxy-6-methyl-benzoate against a number of fungal species.

Medium pressure liquid chromatography of the MeOH extract of *Ramalina farinaceae* with a solvent gradient of CH_2Cl_2 - 5% MeOH/Ch_2Cl_2 yielded erythrin and methyl-2,4-dihydroxy-6-methyl-benzoate. Both compounds were characterized by extensive NMR and mass spectral analysis.

The 2 compounds were subjected to antifungal assay against 4 fungi, *C.cladosporioides Curvularia trifolii* sp., *Colletotrichum musae* and *Colletotrichum gloeosporioides* using the TLC bioassay technique. While erythrin showed considerably less activity against all 4 fungal species, methyl-2,4-dihydroxy-6-methyl-benzoate exhibits a much higher level of activity and also exceeds the standard compound benlate. Based on the diameter of the inhibition zones, methyl-2.4-dihydroxy-6-methyl-benzoate was 5 times as active as benlate against *C. cladosporioides*. Against the other 3 fungi, it was at least twice as active as erythrin.

Erythrin is found in the lichen in large amounts (8 g, 4.9%) compared to its biosynthetic precursor methyl-2,4-dihydroxy-6-methyl-benzoate (0.50 g, 0.3 %). It is common that lichen substances are accumulated in amounts exceeding 20% of the dry weight. These substances are considered to be imparting protection to the lichen against herbivores. Interestingly, in *Ramalina farinaceae* the storage of erythrin in large amounts (with its relatively low antifungal activity) seems to indicate that perhaps the lichen may be releasing the strongly antifungal active methyl-2,4-dihydroxy-6-methyl-benzoate by hydrolysis when needed.

Financial assistance from NARESA (RG/96/C/04) is acknowledged.