

RESEARCH NOTE

## Chemistry of *Heterodermia microphylla*, a lichen new to Sri Lanka

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Lichens are biologically distinct symbiotic entities composed of an algal or cyanobacterial photobiont and a fungal mycobiont<sup>1</sup>. Lichens synthesize a great variety of metabolites with different structures and potential biological activities<sup>2,3</sup>. In our continuing research on the chemistry of tropical lichens, we have investigated a foliose type lichen belonging to the family Physciaceae, *Heterodermia microphylla* (Kurok.) Skorepa (Figure 1), a lichen which has not been reported from Sri Lanka prior to this study. The thallus is appressed and marginally ascendant; its upper surface, and sometimes both surfaces, may contain a cortex<sup>4</sup>. The lower surface is corticated with rhizinae which are found only along the margin<sup>5</sup>. The photobiont of *Heterodermia* is a green alga, which is a chlorococcoid. The thallus of *H. microphylla* has lobes slightly disjunct or adjunct, more or less plane, not ascending, with short lateral lobes<sup>6</sup>. Underside of the thallus is non-corticate and pale. *H. microphylla* is present in Asia and New Zealand. This lichen can be found at altitudes of 1500-2300 m growing on tree trunks

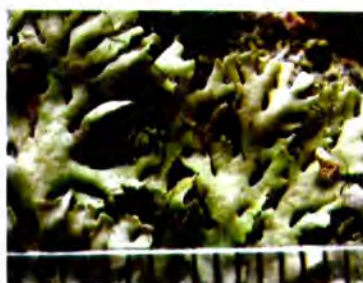


Figure 1: A part of the thallus (upper surface) of *H. microphylla*

in sheltered but open woodland and occasionally on rock. The genus *Heterodermia* is useful in determining the ecological continuity of forest ecosystems<sup>7</sup>.

*H. microphylla* growing on a rock beside a small stream was collected from Labukelle, Central Province. Dried, cleaned specimens of *H. microphylla* (26.05 g) were sequentially extracted with 2.5 L of hexane and  $\text{CH}_2\text{Cl}_2$  (2.5 L).

The hexane extract (242 mg) when subjected to flash chromatography (eluent: 50% hexane/ $\text{CH}_2\text{Cl}_2$ ) yielded the following: Methyl-5-chloro-3-formyl-2,4-dihydroxy-6-methylbenzoate (**1**): 6mg<sup>8</sup>; Methyl- $\beta$ -orcinolcarboxylate (**2**): 8 mg; m.p. 144-143 °C, lit.<sup>9</sup> 140-141 °C; Zeorin (**3**): 42 mg; m.p. 236-242 °C lit.<sup>10</sup> 240-243 °C.

The  $\text{CH}_2\text{Cl}_2$  extract (460 mg) when subjected to Medium Pressure Liquid Chromatography (MPLC) (hexane to  $\text{CH}_2\text{Cl}_2$ ) followed by flash chromatography (eluent: 1% MeOH/ $\text{CH}_2\text{Cl}_2$ ) yielded: Atranorin (**4**): 43 mg; m.p. 194-196 °C, (lit m.p.<sup>11</sup> 196 °C); and Chloroatranorin (**5**): 6 mg; m.p. 207-210 °C (lit. m.p.<sup>12</sup> 208-208.5 °C).

Upon close examination, the Nuclear Magnetic Resonance (NMR) data (<sup>1</sup>H and <sup>13</sup>C) of compound (**1**) were identical to methyl chlorohematommate, isolated from the lichen *Lethariella canariensis*, its only other reported source in nature<sup>8</sup>. The hexane extract also yielded methyl- $\beta$ -orcinolcarboxylate (**2**) and the triterpenoid zeorin (**3**). In addition to chloroatranorin (**5**), the  $\text{CH}_2\text{Cl}_2$  extract yielded atranorin (**4**). The latter

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exhibited moderate larvicidal activity against the 2<sup>nd</sup> instar larvae of *Aedes aegypti* (90 and 100 % mortality at 100 ppm after 24 and 48 h, respectively).<sup>13</sup>

The Thin Layer Chromatography (TLC) bioassay<sup>14</sup> indicated the antifungal activity of compound (4) against *Colletotrichum gloeosporioides*. A quantitative spore germination assay<sup>15</sup> corroborated the above result (Table 1) indicating that atranorin (4) was comparable in activity to the standard benlate (methyl-1-(butylcarbonyl)-2-benzamidazolecarbamate) against *C. musae*.

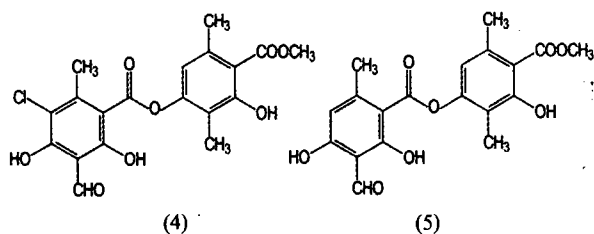
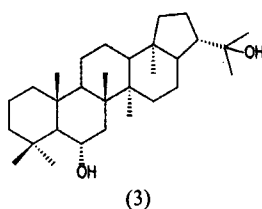
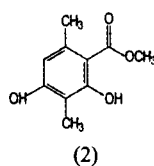
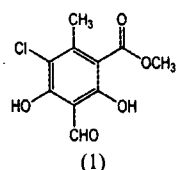
From a chemotaxonomic point of view, this is the first report of the occurrence of compound 1 in the genus *Heterodermia*, and the family Physciaceae.

**Table 1:** Spore germination results for atranorin

Fungus	Compound	% Germination
<i>C. gloeosporioides</i>	4 <sup>a</sup>	58.2 ± 3.3
	control <sup>b</sup>	74.2 ± 1.4
	benlate <sup>a</sup>	33.3 ± 1.1
<i>C. musae</i>	4	26.2 ± 2.3
	control	66.8 ± 1.1
	benlate	32.4 ± 3.3

<sup>a</sup> Concentration of both compound 4 and benlate was 10 ppm

<sup>b</sup> Negative control with no compound



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