1,3,7-TRIMETHYLGUANINE FROM THE LICHEN STEREOCAULON RAMULOSUM

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ABSTRACT

Chromatographic fractionation of an acetone extract of the lichen Stereocaulon ramulosum afforded 1,3,7-trimethylguanine (1), perlatic acid (2), methyl β-orcinolcarboxylate (3), atranorin (4) and galactitol (5). The structures were elucidated using NMR spectroscopy and mass spectrometry. Cinco compuestos fueron aislados del extracto acetónico del Stereocaulon ramulosum, 1,3,7-trimetilguanina (1), acido perlatólico (2), β-orcinolcarboxilato de metilo (3), atranorina (4) y galactitol (5). Las estructuras moleculares fueron elucidadas por métodos espectroscópicos de RMN y espectrometría de masas.

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INTRODUCTION

Lichen are complex organisms consisting of a symbiotic association of a fungus and an alga. The morphology, physiology and biochemistry of a lichen is different to that of the isolated fungus and alga. Lichen have, among other things, developed a number of strategies to minimize UV damage. The synthesis or bioaccumulation of different compounds that directly or indirectly absorb UV energy is one such strategy. Previous phytochemical studies of Stereocaulon ramulosum revealed the presence of perlatic acid, methyl β-orcinolcarboxylate and atranorin, from a sample that was collected from Pongo 3800 m.a.s.l. (La Paz, Bolivia) in November. The presence of perlatic acid was especially interesting, as it shows activity as an antioxidant and a photoprotector of UVB (280-315 nm). In this paper, we describe a similar study of the same species from the same habitat but collected in June. From this we isolated and determined the structure of 1,3,7-trimethylguanine (1), perlatic acid (2), methyl β-orcinolcarboxylate (3), atranorin (4) and galactitol (5), see Figure 1 for structures.

Fig 1. 1,3,7-trimethylguanine 1, perlatic acid 2, methyl β-orcinolcarboxylate 3, atranorin 4, galactitol 5.
RESULTS AND DISCUSSION

Compound 1 was obtained as a white crystals m.p. 238 °C. The elemental composition C₈H₁₁ON₅ was deduced from the EIMS spectrum (m/z 193) and ¹³C NMR spectrum. The IR spectrum exhibited strong absorptions at υₘₐₓ 3340, 1702 and 1658 cm⁻¹ indicative, for N-H, C=O, C=N group and an aromatic ring. The ¹H NMR spectrum revealed the presence of three methyl groups at δ 3.39 s, δ 3.57 s, δ 3.98 s and one aromatic proton at δ 7.50 s. The ¹³C NMR spectrum displayed signal for eight carbon atoms, including four non-protonated unsaturated carbons, one protonated and three methyls. The ¹³C NMR data suggest that compound 1 is a guanine derivate. HMBC and COSY correlations observed were in agreement with the guanine skeleton. Important correlations were those observed in the ¹H-¹H COSY experiment and the HMBC (Table 1). In the HMBC spectrum, δ 3.39 correlate with C-2 (δ 151.9) and C-6 (δ 155.9); δ 3.57 with C-2 (δ 151.9) and C-4 (δ 149.1); δ 3.98 with C-5 (δ 107.6) and C-8 (δ 141.8). The singlet aromatic proton at δ 7.50 correlated with C-6 (δ 155.9), C-5 (δ 107.6), C-4 (δ 149.1) and methyl carbon at δ 34.0 (Figure 2). From the above evidence, compound 1 was characterized as 1,3,7-trimethylguanine (Figure 3). The same compound was previously found in different species of sponges, although this is the first time it is reported from a lichen.

The structures of perlatolic acid, atranorin, methyl β-orcinolcarboxylate and galactitol were elucidated by spectroscopy of ¹H NMR, ¹³C NMR, COSY, HMQC, HMBC and by comparison of their spectroscopy data with those reported in the literature.

![Fig 2. HMBC connectivities in 1](image)

![Fig 3. 1,3,7-trimethylguanine](image)

### Table 1. ¹H (400 MHz) and ¹³C (100 MHz) NMR data and HMBC correlations for compound 1 in CDCl₃

<table>
<thead>
<tr>
<th>Carbon</th>
<th>¹³C (δ)</th>
<th>¹H (δ)</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>N¹-CH₃</td>
<td>28.3</td>
<td>3.39 s</td>
<td>C-2, C-6</td>
</tr>
<tr>
<td>C-2</td>
<td>151.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>N³-CH₃</td>
<td>30.2</td>
<td>3.57 s</td>
<td>C-2, C-4</td>
</tr>
<tr>
<td>C-4</td>
<td>149.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-5</td>
<td>107.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C-6</td>
<td>155.9</td>
<td>-</td>
<td>C-6, C-2</td>
</tr>
<tr>
<td>N⁷-CH₃</td>
<td>34.0</td>
<td>3.98 s</td>
<td>C-5, C-8</td>
</tr>
<tr>
<td>CH-8</td>
<td>141.8</td>
<td>7.50 s</td>
<td>N¹-CH₃, C-5, C-4, C-6</td>
</tr>
</tbody>
</table>

EXPERIMENTAL SECTION

**General**

All melting points were recorded with a Reichert microscope. The UV and IR spectra were recorded with a Varian Cary 2290 and a Perkin-Elmer 298 spectrometer, respectively. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were recorded in CDCl₃ and MeOD using a Bruker DRX400 spectrometer with an inverse multinuclear 5-mm probe head equipped with a shielded gradient coil. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine-shape gradient pulses. EIMS were recorded with a JEOL SX102 spectrometer at 70 eV. Column chromatography was run on Merk silica gel 60 and TLC was carried out on Silica gel GF₂₅₄.
Plant Material
The lichen *Stereocaulon ramulosum* Rausch were collected from Pongo-La Paz Bolivia at 3800 m.a.s.l. in June 2002. Voucher specimens (JV-001) were deposited at the Herbarium National of Bolivia.

Extraction and Isolation
*Stereocaulon ramulosum* (254.0 g) were extracted successively with petroleum ether, CH$_2$Cl$_2$, Me$_2$CO and EtOH, to yield a crude organic extract (14.6 g) on evaporation in vacuo. This extract was subjected to CC on silica gel eluted with CH$_2$Cl$_2$-MeOH mixtures of increasing polarity to afford guanine 1 (40 mg), perlatic acid (2) (250 mg), methyl β-orcinolcarboxilate (3) (65 mg), atranorin (4) (800 mg), and galactitol (5) (32 mg).

1,3,7-trimethylguanine (1); white crystals (MeOH-(CH$_3$)$_2$CO); m.p. 238 °C; IR (KBr) $\nu_{max}$ 3340, 1702, 1658 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1. EIMS (70 e/v) $m/z$ [M]+ 193 (100), 164 (8), 136 (5), 137(5), 109 (30), 82 (10), 67 (10), 55 (10) (C$_8$H$_{11}$ON$_5$).

Perlatic acid (2); white crystals (MeOH-(CH$_3$)$_2$CO); m.p. 107-108 °C; $^1$H NMR (CDCl$_3$, 400 MHz); $\delta$ 0.85 (CH$_3$-ε), 0.9 (CH$_3$-ε'), 1.2-1.5 (CH$_2$-γγ', δδ'), 1.6-1.8 (CH$_2$-β'), 2.9 (CH$_2$-α), 3.0 (CH$_2$-α'), 3.8 (OCH$_3$), 6.39 (CH$_3$), 6.65 (CH-5'), 6.75 (CH$_3'$); $^{13}$C NMR (CDCl$_3$, 100 MHz); $\delta$ 13.9, 13.9, 22.4, 22.6, 31.3, 31.6, 32.0, 36.4, 37.2, 55.4, 99.0, 103.5, 109.5, 111.9, 116.1, 148.4, 150.1, 155.0, 164.9, 166.5, 169.4, 175.5; EIMS (70 e/v) $m/z$ [M]+ 444 (2), 386 (19), 368 (24), 277 (35), 256 (40), 221 (69), 180 (31), 164 (84), 131 (81), 124 (100), 107 (64) (C$_{25}$O$_7$H$_{30}$).

Methyl β-orcinolcarboxilate (3); white crystals ((CH$_3$)$_2$CO); m.p. 140 °C; $^1$H NMR (CDCl$_3$, 400 MHz); $\delta$ 3.9 (3H, s, -CO$_2$Me), 2.4 (3H, s, Ar-Me, C-8), 2.1 (3H, s, Ar-Me, C-9), 6.2 (1H, s, Ar-H). $^{13}$C NMR (CDCl$_3$, 100 MHz); $\delta$ 172.6, 162.6, 159.5, 139.8, 110.6, 108.9, 104.9, 51.5, 23.8, 7.5. EIMS (70 e/v) $m/z$ [M]+ 196 (2), 194 (100), 165 (5), 137 (7), 109 (58) (C$_{10}$H$_{12}$O$_4$).

Atranorin (4); white crystals; m.p. 196 °C; $^1$H NMR (CDCl$_3$, 400 MHz); 6.44 (1H, s, H-5), 6.56 (1H, s, H-5'), 2.71 (3H, s, H-8), 10.39 (1H, s, H-9), 2.11 (3H, s, H-8'), 2.59 (3H, s, H-9'), 4.05 (3H, s, OMe-1'), 12.53 (1H, s, OH), 12.59 (1H, s, OH). $^{13}$C NMR (CDCl$_3$, 100 MHz); $\delta$ 103.8, 169.3, 108.8, 167.7, 113.1, 152.6, 169.9, 25.7, 194.0, 117.0, 163.1, 110.5, 152.2, 116.2, 140.1, 172.4, 24.2, 9.6, 52.5. EIMS (70 e/v) $m/z$ [M]+ 374 (9), 196 (76), 179 (97), 164 (100), 159 (19), 136 (45), 107 (6) (C$_{19}$O$_8$H$_{18}$).

Galactitol (5); white crystals (MeOH), m.p. 189 °C; $^1$H NMR (DMSO-d$_6$, 400 MHz): $\delta$ 3.3 – 4.5. $^{13}$C NMR (DMSO-d$_6$, 100 MHz); $\delta$ 64.1, 71.6, 69.9, 69.9, 71.6.

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REFERENCES