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**), perlatolic 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.[1,2] 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.[3,4]

Previous phytochemical studies of *Stereocaulon ramulosum* revealed the presence of perlatolic 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 perlatolic acid was especially interesting, as it shows activity as an antioxidant and a photoprotector of UVB (280-315 nm).[5] 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**), perlatolic acid (**2**), methyl β-orcinolcarboxylate (**3**), atranorin (**4**) and galactitol (**5**), see Figure 1 for structures.

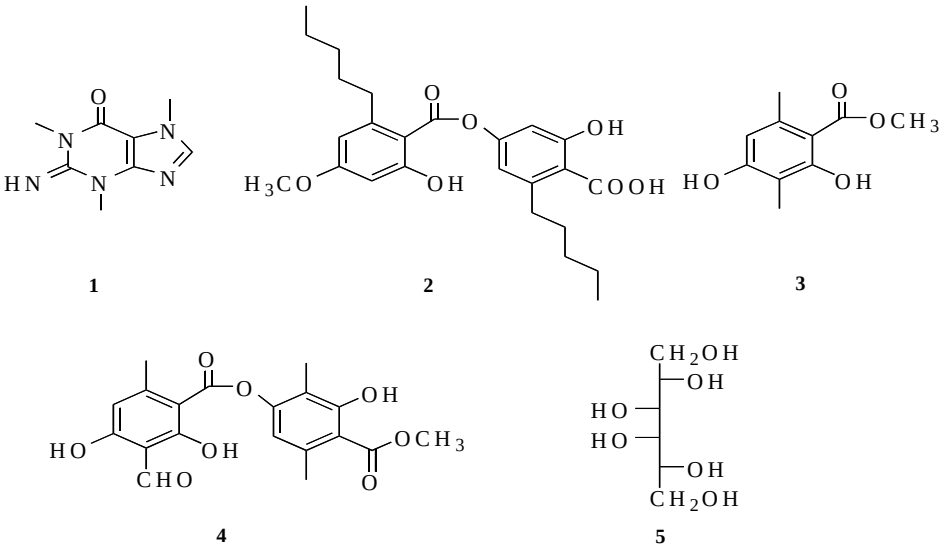

Fig 1. 1,3,7-trimethylguanine **1**, perlatolic acid **2**, methyl β-orcinolcarboxylate **3**, atranorin **4**, galactitol **5**.

RESULTS AND DISCUSSION

Compound **1** was obtained as a white crystals m.p. 238 ^{o}C. The elemental composition $C_8H_{11}ON_5$ was deduced from the EIMS spectrum (*m/z* 193) and ^{13}C NMR spectrum. The IR spectrum exhibited strong absorptions at υ_{max} 3340, 1702 and 1658 cm^{-1} indicative, for N-H, C=O, C=N group and an aromatic ring. The ^{1}H 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 ^{13}C NMR spectrum displayed signal for eight carbon atoms, including four non-protonated unsaturated carbons, one protonated and three methyls. The ^{13}C 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 ^{1}H-^{1}H 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,[6,7,8,9] 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 ^{1}H NMR, ^{13}C NMR, COSY, HMQC, HMBC and by comparison of their spectroscopy data with those reported in the literature.[10,11,12,13]

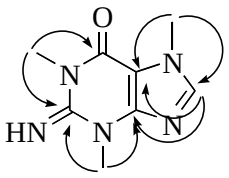

Fig 2. HMBC connectivities in **1**

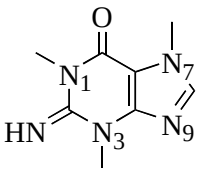

Fig 3. 1,3,7-trimethylguanine

Table 1. *^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data and HMBC correlations for compound **1** in $CDCl_3$*

Carbon	^{13}C (δ)	^{1}H (δ)	HMBC
N^1-CH_3	28.3	3.39 s	C-2, C-6
C-2	151.9	-	-
N^3-CH_3	30.2	3.57 s	C-2, C-4
C-4	149.1	-	-
C-5	107.6	-	-
C-6	155.9	-	C-6, C-2
N^7-CH_3	34.0	3.98 s	C-5, C-8
CH-8	141.8	7.50 s	N^7-CH_3, C-5, C-4, C-6

EXPERIMENTAL SECTION

General

All melting points were recorded with a Reichter microscope. The UV and IR spectra were recorded with a Varian Cary 2290 and a Perkin-Elmer 298 spectrometer, respectively. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ and MeOD usin 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 chromathography was\run on Merk silica gel 60 and TLC was carried out on Silica gel GF_{254}.

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_2Cl_2, Me_2CO 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_2Cl_2-MeOH mixtures of increasing polarity to afford guanine **1** (40 mg), perlatolic 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-$(CH3)_2CO$); m.p. 238 ^oC; IR (KBr) υ_{max} 3340, 1702, 1658 cm^{-1}; ^1H 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) ($C_8H_{11}ON_5$).

Perlatolic acid (**2**); white crystals (MeOH-$(CH_3)_2CO$); m.p. 107-108 ^oC; ^1H NMR ($CDCl_3$, 400 MHz); δ 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,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 (CH3'); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 13.9, 13.9, 22.4, 22.6, 31.3, 31.6, 32.0, 36.4, 37.2, 55.4, 99.0, 103.5, 108.6, 109.5, 111.9, 116.1, 148.4, 150.1, 155.0, 164.9, 165.2, 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) ($C_{25}O_7H_{30}$).

Methyl β-orcinolcarboxilate (**3**); white crystals ($(CH_3)_2CO$); m.p. 140 ^oC; ^1H NMR ($CDCl_3$, 400 MHz); δ 3.9 (3H, *s*, -CO_2Me), 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); δ 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 ^oC; ^1H 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); δ 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), 150 (19), 136 (45), 107 (6) ($C_{19}O_8H_{18}$).

Galactitol (**5**); white crystals (MeOH), m.p. 189 ^oC; ^1H NMR (DMSO-d_6,400 MHz): δ (3.3 – 4.5). ^{13}C NMR (DMSO-d_6, 100 MHz); δ 64.1, 71.6, 69.9, 69.9, 71.6, 64.1

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REFERENCES

1. Purvis, W. *Lichens*, Smithsonian Institution, Washington,D.C in association with The Natural History Museum, Press: London **2000**.
2. Hale, M., *"The Biology of lichens"* Third Edition, Ed. Spottiswoode Ballantyne Ltd., Press: London **1983.**
3. Kranner, I., Cram, J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., Pfeifhofer, H., *PNAS* **2005**, 102, 3141-3146.
4. Behera, B., Verma, N., Sonone, A., Makhija U., *Phytother. Res.* **2005**, 19, 58–64.
5. Mollinedo, P., Vila, J., Sterner,O., *Revista Boliviana de Quimica* **2003**, 1, 21-27.
6. Perry, N., Blunt, J., Munro, M., *Journal of Natural Products* **1987**, 50, 307-308.
7. Landini, D., Maia, A., Rampoldi, A., *J. Org. Chem.* **1986**, 51, 5476-5478.
8. Copp, B., Wassvik, C., Lambert, G., Page, M., *Journal of Natural Products* **2000**, 63, 1168-1169.
9. Van Wagoner, R., Jompa, J., Tahir, A., Ireland, C., *Journal of Natural Products* **1999**, 62, 1168-1169.
10. Hylands P., Ingolfsdottir K., *Phytochemistry* **1985**, 24, 127-129.
11. Sundholm, G., Huneck, S., *Letter to Chemica Scripta* **1980**, 16, 197-200.
12. Sundholm, G., Huneck, S., *Letter to Chemica Scripta* **1981**, 18, 233-236.
13. Konig, G., Wright, A., *Phytochem. Anal*. **1999**, 10, 279-284.