tom-made test apparatus similar to that described in the literature [7, 8]. The apparatus consists of a pulley, a motor and a balance. The gel with the thickness of 0.3–0.4 mm was placed between two glass-plates. A piece of string was wound by means of a motor working at a constant speed of 140 cm/min. The shearing stickness values were represented by the reading on the spring balance attached to the set up when two glass-plates were separated with the applied force of motor.

2.4. Determination of release rate of CC from the bioadhesive gels and the ointment

Drug release studies were carried out using the apparatus for disintegration of vaginal tablets described in BP with some modifications [9, 10]. 2 g sample was applied on top of the perforated plate assembly. The cage was immersed into buffered medium held at 37 ± 0.5 °C and rotated at 50 rpm. The amounts of released CC were determined spectrophotometrically at 232 nm after 30 min, 1, 2, 3, 4, 5, 20, 22, and 24 h.

2.5. Determination of viscosity of the formulations containing CC

Viscosity of bioadhesive gels and the ointment containing CC were measured using a Brookfield viscometer (Mode RVT, Stoughton, MA, 02072, USA). The viscosity measurements were carried out just after preparation and one month later. Each measurement was repeated three times.

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Neuropogonines A, B and C, new depsidon-type metabolites from *Neuropogon* sp., an Antarctic lichen

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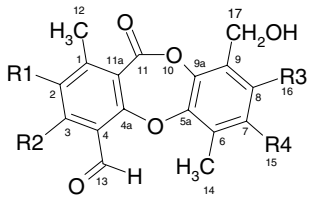
Lichens from special regions of the globe have been suggested as a source of chemically diverse bioactive metabolites [1]. Antarctica is a particularly interesting biosphere due to the special climatic conditions and the distance to other continents. Amongst the aromatic structures from lichens the depsidon-type metabolites form a major group [2–5]. They were identified as constituents of various genera and species of lichens. However, the determined substitution pattern of the hitherto known tricyclic depsidone do not reflect the chemical diversity of these compounds. Here we report structures and biological activities of new depsidones, neuropogonines A, B and C (**1**, **2**, **3**), which were isolated from the Antarctic lichen *Neuropogon* sp.

A 150 g sample of dried *Neuropogon* sp. collected on the Antarctic Livingstone Island in the course of the Bulgarian Antarctic Expedition in 1995, was extracted twice by 500 ml MeOH/$CHCl_3$ (3 : 1) for 48 h. The combined extracts were evaporated and the residue (1.6 g) was subjected to CC on silica gel 60 (Merck, column 5 cm × 40 cm, elution by a) $CHCl_3$, b) $CHCl_3$/MeOH (9 : 1). Thereby five components were eluted which stained blueish on TLC with 1% vanillin/conc. H_2SO_4. Final purification was achieved by preparative HPLC (Lichrospher 100, RP-18, 7 µm, 10 × 250 mm; gradient 95% water/0.1% TFA to 95% acetonitrile, 28 min, 4 ml/min, detection at 210 nm) affording **1**, **2** and **3** in addition to protocetraric (**4**) and usnic acids (**5**) [6].



	R1	R2	R3	R4
1	OH	H	H	CH_2OH
2	H	OH	OCH_3	COOH
3	H	OH	H	COOH
4	H	OH	OH	COOH

4 and **5** were readily identified as known metabolites [6] on the basis of their mass spectrometric data and NMR measurements. Compounds **1**, **2** and **3** were shown as new structures (EI-MS: **1**: MW 344, m/z 344.08981 (M^+, calcd. 344.08979 for $C_{18}H_{16}O_7$); **2**: MW 388, m/z 388.08099 (M^+, calcd. 388.08251 for $C_{19}H_{16}O_9$); **3**: MW 358, m/z 271.06408 ([$M-CO_2$, $-CO$, $-CH_3$], calcd. 271.06708 for $C_{15}H_{11}O_5$).

The presence of aromatic carboxylic groups in **1**, **2** and **3** was confirmed by λ_{max} 1725 cm^{-1} in the IR spectra and λ_{max} 314–320 nm in the UV-VIS spectra.

Conclusive evidence for the structures of **1**, **2** and **3** was furnished by the ^{1}H, ^{13}C, DEPT, COSY, HSQC and HMBC NMR spectra (Table 1). Each of the aromatic protons appeared as a singlet. In the ^{1}H NMR spectrum of **1** the position of the phenolic hydroxyl group at C-2 was attested by a signal at 8.30 ppm. However, the ^{1}H NMR spectra of **2** and **3** showed hydroxyl proton signals at 11.92 ppm and 11.95 ppm, respectively, due to the neighbourhood of the aldehyde group. The number of aldehyde, carboxylic, hydrogen- or oxygen-bonded and quaternary carbons was readily suggested by the ^{13}C and DEPT spectra. C,H-long-range coupled spectra (HMBC) were of pivotal importance for the assignment of structures. Thus in **2**, $^3J_{H,C}$ couplings between H-12, C-2 and C-11a confirmed the substitution at C-2 by hydrogen.

Thus the physico-chemical data suggest that neuropogonines **1**, **2** and **3** are new members of the depsidon family of lichen metabolites which are produced in addition to protocetraric and usnic acids (**4**, **5**) by the Antarctic lichen *Neuropogon*.

1, **2** and **3** showed moderate activity against *Mycobacterium vaccae* 10670 (MIC 50 μg/ml) 7.

Experimental

1. Instruments

HREI-MS was recorded on an AMD 402 high-resolution sector-field mass spectrometer (AMD Intectra, Harpstedt, Germany). NMR spectra were recorded on a Bruker DRX 500 instrument, IR spectra as film on a Fourier-transform IR spectrometer equipped with ATR (Mattson, Madison, USA), UV spectra on a Specord 2000 scanning spectrophotometer (Analytik Jena, Jena, Germany).

2. Measurement of antibacterial activity

Antibacterial activity was measured using the agarplate diffusion assay [7].

3. Neuropogonin A (1)

Yield: 15 mg; wax; TLC (Merck silica gel aluminium sheets; $CHCl_3$/MeOH, 8 : 2), R_f 0.15; HPLC (nucleosil 100 RP_{18}; 5 μm; 4.6 × 125 mm, gradient 95%, H_2O/0.5% TFA to 95 acetonitrile, 20 min, 2 ml/min, 210 nm), R_t 9.9 min; UV-VIS (λ_{max} acetonitrile): 265 nm, 315 nm; IR (film; λ_{max}; cm^{-1}): 1725; ^{1}H NMR (500 MHz, in $CDCl_3$, δ in ppm): 2.32 (H-14, s, 3 H), 2.41 (H-12, s, 3 H), 4.68 (H-15, s, br, 2 H), 4.68 (H-17, s, br, 2 H), 5.1.. (17-OH, br, 1 H), 6.57 (H-3, s, 1 H), 6.57 (H-8, s, 1 H), 8.30 (2-OH, br, 1 H), 10.56 (H-13, s, 1 H).
^{13}C NMR (125 MHz, in $CDCl_3$, δ in ppm): 14.2 (C-14), 20.5 (C-12), 52.1 (C-15), 56.7 (C-17), 112.3 (C-4), 115.3 (C-11a), 127.0 (C-6), 132.2 (C-8), 134.7 (C-3), 142.2 (C-5a), 143.4 (C-9), 146.3 (C-1), 153.2 (C-7), 156.0 (C-11), 160.8 (C-2),157.0 (C-9a), 164.4 (C-4a), 192.7 (C-13).

4. Neuropogonin B (2)

Yield: 12 mg; wax; TLC (Merck silica gel aluminium sheets; $CHCl_3$/MeOH, 8 : 2), R_f 0.3; HPLC (nucleosil RP_{18}, 5 μm, 4.6 × 125 mm, gradient 95% H_2O/0.1% TFA to 95% acetonitrile, 20 min, 2 ml/min, 210 nm): R_t 11.78 min, UV-VIS (λ_{max} in acetonitrile): 265 nm, 318 nm; IR (film, λ_{max}, cm^{-1}): 1725; ^{1}H NMR (500 MHz, in $CDCl_3$, δ in ppm): 2.38 (H-14, s, 3 H), 2.48 (H-12, s, 3 H), 3.44 (H-16, s, 3 H), 4.47 (H-17, s, br, 2 H), 5.31 (17-OH, br, 1 H), 6.90 (H-2, s, 1 H), 10.6 (H-13, s, 1 H), 11.92 (3-OH, br, 1 H). ^{13}C NMR (125 MHz, in $CDCl_3$, δ in ppm): 21.3 (C-12), 14.4 (C-14), 57.3 (C-16), 62.3 (C-17), 112.0 (C-4), 115.0 (C-11a), 116.9 (C-2), 123.0 (C-7), 126.5 (C-6), 144.8 (C-5a), 145.0 (C-9), 149.0 (C-1), 151.7 (C-9a), 155.0 (C-11), 159.3 (C-8), 163.8 (C-3), 166.0 (C-4a), 169.0 (C-15), 191.7 (C-13).

5. Neuropogonin C (3)

Yield: 8 mg; wax; TLC (Merck, silica gel aluminium sheets, $CHCl_3$/MeOH, 8 : 2, 0.4, HPLC (nucleosil 100 RP_{18}, 5 μm, 4.6 × 125 mm, gradient 95% H_2O/0.1% TFA to 95% acetonitrile, 20 min, 2 ml/min, 210 nm): R_t 11.52 min, UV-VIS (λ_{max} in acetonitrile): 270 nm, 320 nm, IR (film, λ_{max}, cm^{-1}): 1725; ^{1}H NMR (500 MHz, in $CDCl_3$, δ in ppm): 2.44 (H-14, s, 3 H), 2.48 (H-12, s, 3 H), 4.52 (H-17, s, br, 2 H), 5.28 (17-OH, s, br, 1 H), 6.63 (H-8, s, 1 H), 6.83 (H-2, s, 1 H), 10.57 (H-13, s, 1 H), 11.95 (3-OH, br, 1 H). ^{13}C NMR (125 MHz, in $CDCl_3$, δ in ppm): 14.7 (C-14), 22.1 (C-12), 57.7 (C-17), 112.7 (C-4), 115.0 (C-11a), 117.9 (C-2), 124.0 (C-7), 126.0 (C-6), 133.2 (C-8), 142.2 (C-5a), 145.3 (C-9a), 152.1 (C-1), 160.9 (C-11), 164.1 (C-9), 164.5 (C-3), 165.4 (C-4a), 192.5 (C-13).

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