ent-Homoabyssomicins A and B, Two New Spirotetronate Metabolites from *Streptomyces* sp. Ank 210

Muna Ali Abdalla, Prem P. Yadav, Birger Dittrich, Anja Schüffler, Hartmut Laatsch*

SUPPLEMENTARY INFORMATION

General Experimental Procedures. The optical rotations were measured on a Perkin-Elmer polarimeter (model 241). IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer with KBr pellets. NMR spectra were measured on a Varian Inova 600 (599.740 MHz) and a Varian Unity 300 (300.145 MHz) spectrometer. ESI mass spectra were recorded on a Quattro Triple Quadrupol mass spectrometer, with a Finnigan TSQ 7000 with nano-ESI API ion source. HRESI mass spectra were measured on a Micromass LCT mass spectrometer coupled with a HP 1100 HPLC and a diode array detector. Data collection of diffraction intensities was performed on a Bruker SMART 6000 area detector diffractometer with CuK α radiation generated from a 5 kW rotating anode. Flash chromatography was carried out on silica gel (230-400 mesh). Thin-layer chromatography (TLC) was performed on Polygram SIL G/UV₂₅₄ (Macherey-Nagel & Co.). R_f values were measured on Polygram SIL G/UV₂₅₄ (Macherey-Nagel & Co.). Size exclusion chromatography was done on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Ltd., purchased from Sigma-Aldrich Chemie).

Isolation and Taxonomy

The *Streptomyces* strain ANK 210 has been derived from a forest soil sample (Kaiserslautern) and was isolated on YMG agar at room temperature (YMG agar: 2 g/L yeast extract, 5 g/L malt extract, 5 g/L glucose, 15 g/L agar, 30 mg/L cycloheximide). Its almost complete 16S rRNA gene sequence (GenBank Accession Nr. HQ124331) showed similarities (98.8%) to *Streptomyces miharaensis* NBRC 13791 (GenBank Accession Nr. AB184482). A voucher specimen of *Streptomyces*

sp. strain Ank 210 was deposited in the culture collection at the Institute of Organic and Biomolecular Chemistry, Göttingen, Germany.

Fermentation and Isolation

Well-grown agar plates were used to inoculate 100 of 1 L Erlenmeyer flasks each containing 250 ml of M_2^+ medium. The culture was incubated on the shaker (95 rpm) at 28 °C for 7 days. The resulting brown culture broth was mixed with *ca.* 1 kg diatomaceous earth (Celite) and filtered by means of a filter press to afford the aqueous filtrate and a mycelial fraction. The aqueous fraction was extracted with Amberlite XAD-16 resin and eluted with MeOH. The MeOH eluate was evaporated till to the aqueous residue and the latter one extracted with EtOAc.

The mycelium was three times extracted with EtOAc followed by acetone (1×) and the EtOAc and acetone phases were evaporated to dryness. As the three crude extracts showed no differences on TLC, they were combined to obtain 5.8 g of a brown residue. The crude extract was dissolved in methanol, defatted with cyclohexane and subjected to silica gel column chromatography using $CH_2Cl_2/MeOH$ gradient (column 3×60 cm, 0 to 20 % MeOH). Fraction FII was purified on Sephadex LH-20 using MeOH followed by RP-18 using MeOH/ H_2O gradient (10 to 50 % MeOH) to deliver 12 mg *ent*-homoabyssomicins A (1) and 5 mg B (2). In a similar way, fraction FIII was purified on Sephadex LH-20 using MeOH followed by silica gel (cyclohexane/EtOAc, gradient 0 to 100 % EtOAc) afforded indole-3-carboxylic acid, 4.7 mg 2-hydroxy-1-(4-hydroxy-3-methoxy-phenyl)- ne, and 17 m lypropylenglycole. Fraction FIV was purified on RP-18 using MeOH/ H_2O (4:6) to afford 6.3 mg benadrostin.

Comment [MA1]: homoabyssomicia

Comment [MA2]: homoabyssomicinb

ent-Homoabyssomicin A (1): White crystals, UV absorbing at 254 nm, $R_f = 0.45$ ($\text{CH}_2\text{Cl}_2/5\%$ MeOH); a crystal suitable for single-crystal X-ray diffraction studies was obtained by slow evaporation of a solution in $\text{CHCl}_3/5\%$ MeOH at 4 °C; $[\alpha]_D^{20} = -22.1$ ($c = 0.117$, CH_3OH); UV/VIS: λ_{max} ($\log \epsilon$) ($\text{CH}_3 \text{CN}$): 276 sh (2.80), 223 (2.93); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3428, 2928, 1762, 1726, 1636, 1460, 1383, 1250, 1111, 1075, 1030; CD ($c = 1.89 \times 10^{-4}$ M, MeOH, 22 °C): λ_{max} 202 nm ($\Delta\epsilon$ 3.35); ^1H NMR (600 MHz, MeOH) and ^{13}C NMR (125 MHz, MeOH); (+)-ESIMS: $m/z = 379$ ($[\text{M} + \text{H}]^+$, 90), 779 ($[2\text{M} + \text{Na}]^+$, 50); (+)-HRESIMS: $m/z = 379.17512$ $[\text{M} + \text{H}]^+$, (calcd. for $[\text{C}_{20}\text{H}_{27}\text{O}_7]^+$, 379.17513).

ent-Homoabyssomicin B (2): White solid, UV absorbing at 254 nm, $R_f = 0.48$ ($\text{CH}_2\text{Cl}_2/5\%$ MeOH); $[\alpha]_D^{20} = +41.7$ ($c = 0.034$, CH_3OH); UV/VIS: λ_{max} ($\log \epsilon$) ($\text{CH}_3 \text{CN}$): 287 (3.10), 241 (3.56), 219 sh (3.51); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3428, 1742, 1656, 1454, 1396, 1020, 666; CD ($c = 1.085 \times 10^{-4}$ M, MeOH, 22 °C): $\lambda_{\text{max}} = 207$ nm ($\Delta\epsilon$ 14.57), 236 nm ($\Delta\epsilon$ 7.48); ^1H NMR (600 MHz, MeOH) and ^{13}C NMR (125 MHz,

MeOH) data see table 1; (+)-ESIMS: $m/z = 457$ ($[M + Na]^+$, 100), 891 ($[2M + Na]^+$, 50); (+)-HRESIMS: $m/z = 457.14686$ $[M + Na]^+$, (calcd. for $[C_{22}H_{26}O_9Na]^+$, 457.14690).

Figure S1: ^1H NMR spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin A (**1**)

Figure S2: ^{13}C NMR spectrum (125 MHz, MeOD) of *ent*-homoabyssomicin A (**1**)

Figure S3: ^1H , ^1H COSY spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin A (**1**)

Figure S4: HMBC spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin A (**1**)

FILENAME: lastsch/na210b2f_6ghsgc2

Figure S5: HSQC spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin A (**1**)

Figure S6: ^1H NMR spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin B (**2**)

Figure S7: ^{13}C NMR spectrum (125 MHz, MeOD) of *ent*-homoabyssomicin B (**2**)

Figure S8: ¹H, ¹H COSY spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin B (**2**)

Figure S9: HMBC spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin B (**2**)

Figure S10: HSQC spectrum (600 MHz, MeOD) of *ent*-homoabyssomicin B (**2**)

Crystal Data for *ent*-Homoabyssomicin A (**1**)

Formula	C ₂₀ H ₂₆ O ₇ , H ₂ O
Formula Weight	396.42
Crystal System	orthorhombic
Space group	P2 ₁ 2 ₁ 2 ₁ (No. 19)
a, b, c [Å]	7.579(1), 15.663(2), 16.461(3)
V [Å ³]	1954.1(5)
Z	4
D(calc) [g/cm ³]	1.347
Mu(CuK α) [/mm]	0.870
F(000)	848
Crystal Size [mm]	0.02 × 0.09 × 0.15

Data Collection

Temperature (K)	100
Radiation [Å]	CuK α , 1.54180
Θ Min-Max [Deg]	3.9, 72.3
Dataset ($h_{\min}$: $h_{\max}$; $k_{\min}$: $k_{\max}$; $l_{\min}$: $l_{\max}$)	-9: 9; -19:18; -15:19
Tot., Uniq. Data, R(int)	19265, 3581, 0.073
Observed data [$I > 0.0 \sigma(I)$]	2653

Refinement

N _{ref} , N _{par}	2653, 366
R, wR2, S	0.0314, 0.0292, 1.30
Weighting scheme	w = 1/[$\sigma^2(F_o)$]
Max. and Av. Shift/Error	0.00, 0.00
Flack x	0.06(19)
Min. and Max. Resd. Dens. [e/Å ³]	-0.19, 0.17