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

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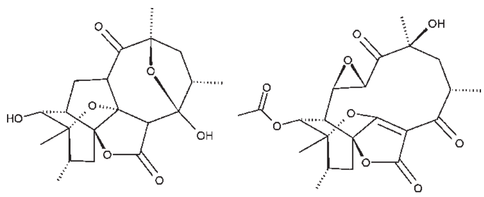
ABSTRACT



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***ent*-Homoabyssomicins A (1) and B (2) are new complex polycyclic spirotetronate metabolites isolated from *Streptomyces* sp. isolate Ank 210. The structures of 1 and 2 were elucidated by detailed spectroscopic analyses of 1D and 2D NMR data. The absolute configuration of 1 was established by subsequent single-crystal X-ray diffraction studies.**

In our endeavor to explore secondary metabolites from microbial sources, we isolated two complex polycyclic compounds from the terrestrial *Streptomyces* sp. Ank 210, namely *ent*-homoabyssomicins A (**1**) and B (**2**), which are two new spirotetronate natural products. In this letter we report details of the isolation and characterization of compounds **1** and **2** on the basis of NMR and MS data. The absolute stereochemistry of **1** was assigned by single-crystal X-ray diffraction (Figure 1).

The producing *Streptomyces* sp. strain Ank 210 was obtained from a forest soil sample (Kaiserslautern) and 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

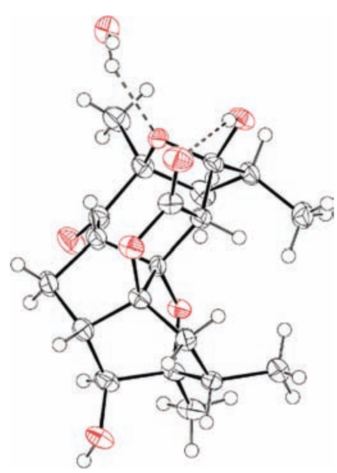

Figure 1. ORTEP plot of *ent*-homoabyssomicin A (**1**).

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Table 1. ^{13}C and ^{1}H NMR Data (125 MHz, 600 MHz) of *ent*-Homoabyssomicins A (**1**) and B (**2**) in CD_3OD

	ent-Homoabyssomicin A (**1**)			*ent*-Homoabyssomicin B (**2**)		
No.	δ_C	δ_H (mult.; *J* in Hz)	HMBC	δ_C	δ_H (mult.; *J* in Hz)	HMBC
1	177.5			170.5		
2	51.5	3.51 (s)	C-1, 3, 8, 16	103.0		
3	110.1			200.5		
4	45.0	2.38 (m)	C-3, 6	42.5	2.74 (m)	C-3, 6
5a	40.4	2.15 (dd, 13.4, 11.4)	C-3, 6, 7	41.9	2.28 (dd, 16.2, 2.4)	C-3, 6, 7
5b		2.03 (dd, 13.4, 10.6)			1.73 (dd, 16.2, 5.4)	
6	87.0			81.2		
7	212.8			207.9		
8	53.2	4.41 (dd, 11.1, 6.0)	C-7, 10	49.8	3.92 (d, 1.8)	C-8, 10
9a	27.8	1.85 (ddd, 13.5, 11.2, 6.8)	C-11, 16	56.8	3.31 (dd, 3.4, 2.0)	C-11
9b		2.01 (m)				
10	52.1	2.41 (d, 6.7)	C-15	46.1	3.17 (t, 3.0)	C-15
11	85.8	3.46 (s)	C-15	69.3	4.79 (d, 2.7)	C-15, 21
12	77.7			87.7		
13	29.0	2.37 (m)	C-12, 15	31.0	2.55 (m)	C-12, 15
14a	32.0	2.54 (dd, 14.1, 12.2)	C-15	36.3	2.79 (dd, 12.8, 11.0)	C-15, 16
14b		1.37 (dd, 14.1, 3.3)			1.45 (dd, 12.8, 3.3)	
15	89.3			79.1		
16	86.4			179.9		
17	14.5	1.21 (d, 7.1)	C-3	17.8	1.17 (d, 6.7)	C-3
18	24.8	1.39 (s)	C-5, 7	26.4	1.45 (s)	C-5, 7
19	21.0	1.05 (s)	C-11, 13	19.1	1.47 (s)	C-11, 12, 13
20	16.7	1.07 (d, 7.2)	C-14	16.4	1.07 (d, 7.2)	C-14
21				170.0		
22				20.8	2.10 (s)	C-21

the Institute of Organic and Biomolecular Chemistry (Göttingen, Germany).

Well-grown colonies of Ank 210 on agar were used to inoculate 100 1-L Erlenmeyer flasks, each containing 250 mL of M_2^+ medium. The culture was incubated on a linear shaker at 28 °C for 7 days. The resulting brown culture broth was mixed with ca. 1 kg of diatomaceous earth (Celite) and pressed through a filter press to afford the filtrate and a mycelial fraction. The filtrate was extracted on Amberlite XAD-16 resin, eluting with MeOH. The mycelium was extracted with EtOAc followed by acetone, and the EtOAc and acetone phases were evaporated to dryness. The MeOH fraction was concentrated to the aqueous residue and extracted with EtOAc. All extracts were combined. The yellowish brown residue (5.8 g) was dissolved in methanol, defatted with cyclohexane, and subjected to silica gel column chromatography. Fraction FII was purified on Sephadex LH-20 (MeOH), followed by RP-18 silica gel ($MeOH/H_2O$ gradient 10 to 50% MeOH), to deliver *ent*-homoabyssomicins A (**1**) and B (**2**) as white solids, which turned yellow on TLC with anisaldehyde/sulphuric acid spray reagent.

The ESI mass spectra of compounds **1** and **2** displayed *pseudo*molecular ion peaks at *m*/*z* 379 $[M + H]^+$ and 457 $[M + Na]^+$, respectively, indicating molecular weights of 378 and 434 Dalton. HRESIMS established the molecular formulas as $C_{20}H_{26}O_7$ and $C_{22}H_{26}O_9$. The NMR data of **1** and **2** are listed in Table 1.

The structure of **1** was elucidated by means of 2D NMR measurements. Analysis of $^{1}H,^{1}H$ COSY, along with TOCSY spectra, revealed the presence of three spin systems *via* correlations between protons H-4, H-5 and CH_3-17, from H-8 to H-11 and between CH_3-20, H-13, and

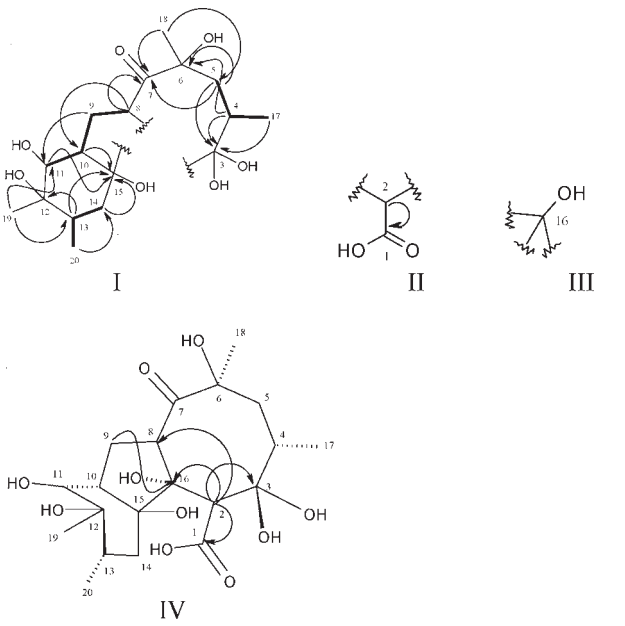

Figure 2. fragments I, II, and IV of *ent*-homoabyssomicin A (**1**) and $^{1}H,^{1}H$ COSY (bold bonds) and selected HMBC correlations (arrows).

H-14 (bold lines in Figure 2). HMBC correlations of CH_3-17 with C-3, from CH_3-18 to C-5 and C-7, and from H-5 to C-3, C-6, and C-7 supported the carbon network between C-3 and C-7. Additional HMBC and COSY correlations confirmed the partial structure I as drawn in Figure 2. A further two carbon chain (fragment II) and one quaternary oxygenated carbon substituent (fragment III) were left, making a total of five bonds that remained to be determined. As fragments II and III must be connected *via* C-3, C-8, and C-15, three diffferent permutations were possible. As a result of the HMBC correlation of H-2 with C-3, C-8, and C-16, the connection of C-2 with C-3 was the most plausible alternative. In this case, C-16 is connected to C-8 and C-15, resulting in substructure IV (Figure 2).

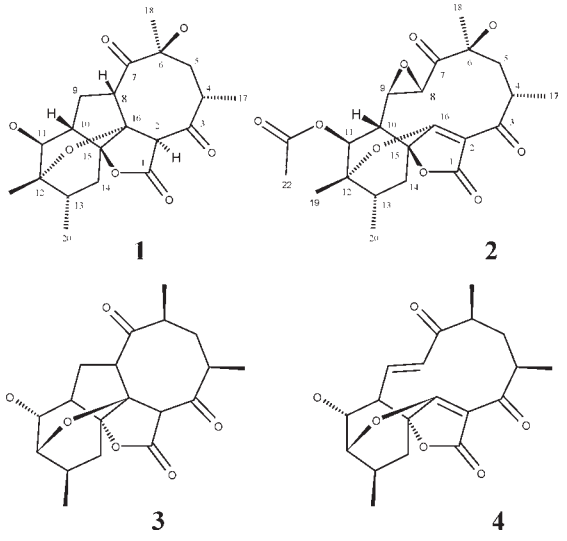

Figure 3. Structures of *ent*-homoabyssomicins A (**1**), B (**2**) and abyssomicins D (**3**), C (**4**).

From the empirical formula, compound **1** has eight double bond equivalents. Thus, the presence of two carbonyl groups and the absence of double bonds requires the presence of six rings. In substructure IV, three rings are therefore still to be formed. One of these can be accounted for by a lactone, as indicated by the carbonyl at δ 177.5. The remaining two bonds must contain oxygen as a bridge atom, giving rise to a spiroketal skeleton as assigned in structure **1**, and completing the tally of required rings. This structure was completely confirmed by X-ray diffraction (Figure 1).

Compound **1** is closely related to the abyssomicin group.[1] It is, however, a homologue with one more carbon atom. Correspondingly, the spectroscopic data for the spiroketal part of the molecule showed close similarities with those of abyssomicin D (**3**)[2] and other spirotetronate metabolites (Figure 3), although the 6-hydroxy group and the skeletal methyl group at C-12 present in **1** are absent in **3**.

While the configuration of **3** and other abyssomicins is (*R*) at C-4, -11, -12, -13, and -15, the absolute configuration of **1** was assigned as (2*S*,4*S*,6*S*,8*S*,10*S*,11*S*,12*S*,13*S*,15*S*,16*R*) by XRD[3] using a locally modified version[4] of the program XDLSM.[5] The configuration of **1** is therefore enantiomeric to that in the abyssomicins, so that the name *ent*-homoabyssomicin A is suggested for **1**.

Analysis of the ^{1}H,^{1}H COSY and TOCSY spectra of the second compound **2** again revealed the presence of three spin systems (Figure 4) centered around protons H-5, H-4, CH_3-17, from H-8 to H-11 and CH_3-20, H-13, H-14, similar to those of compound **1**. Key HMBC correlations, as shown in Figure 4, confirmed the fragment from C-3 to C-16. Further correlations from H-14 to C-16 suggested the presence of a double bond of an α,β-unsaturated ketone between C-16 and C-2 or, alternatively, that C-16 is a carbonyl group and C-2 an acetal C atom.

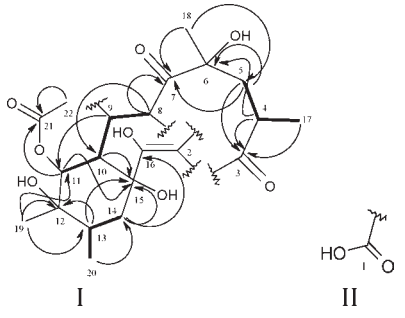

Figure 4. Fragments I and II of *ent*-homoabyssomicin B (**2**) with ^{1}H,^{1}H COSY (bold bonds) and HMBC correlations (arrows).

HMBC correlations, along with COSY correlations, confirmed the partial structure I (Figure 4) for compound **2**. ^{13}C NMR and HSQC data allowed the complete carbon assignment and localized the additional two carbon atoms in an acetyl residue. Analysis of the ^{13}C, HSQC and HMBC data confirmed again a spirotetronate moiety attached at position C-2. The resulting structure was similar to the basic skeleton of abyssomicin C (**3**); however the Michael acceptor in the latter had been epoxidized in *ent*-homoabyssomicin B (**2**).

The relative stereochemistry of *ent*-homoabyssomicin B (**2**), as determined from key NOESY correlations between 14α/20, 14β/10, 14β/13, and 19/22, is in agreement with the

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(3) CCDC 794471.

(4) Dittrich, B.; Hübschle, C. B.; Luger, P.; Spackman, M. A. *Acta Cryst* **2006**, *A62*, 217–223.

(5) Koritsanszky, T.; Richter, T.; Macchi, P.; Volkov, A.; Gatti, C.; Howard, S.; Mallinson, P. R.; Farrugia, L.; Su, Z. W.; Hansen, N. K. *XD – Computer Program Package for Multipole Refinement and Topological Analysis of Electron Densities from Diffraction Data*; Freie Universität: Berlin, Germany, 2003.

configuration of **1**. As both compounds were found in the same strain, identical absolute configurations can be assumed, so that **2** is also an *ent*-homoabyssomicin. The opposite signs of the optical rotations may be due to different conformations and the different chromophors in **1** and **2**.

We presume that *ent*-homoabyssomicin A and B have a biosynthetic origin from the polyketide biosynthesis pathway as proposed for other spirotetronate metabolites.[6]

Compounds **1** and **2** were found to be inactive in the agar diffusion test at a concentration of 40 μg/paper disk against *Escherichia coli*, *Bacillus subtilis*, *Streptomyces viridochromogenes*, *Staphylococcus aureus*, *Candida albicans, Mucor miehei*, and brine shrimps (*Artemia salina*). Abyssomicin C (**4**) is a potent inhibitor of the *p*ABA/tetrahydrofolate biosynthesis.[7] Further SAR studies based on synthetic abyssomicin C analogues by Nicolaou and Harrison suggested that a properly oriented active Michael acceptor is required for antibacterial activity.[8] Missing activities of *ent*-homoabyssomicins A (**1**) and B (**2**) further validated the requirement of a Michael acceptor for activity in these compounds.

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Supporting Information Available. Experimental procedure; ^{1}H, ^{13}C, and 2D NMR spectra (COSY, HSQC and HMBC); HRESI MS, CD, IR, UV, and crystallographic data of *ent*-homoabyssomicin A (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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