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Abyssomicin E, a Highly Functionalized Polycyclic Metabolite from *Streptomyces* Species[#]

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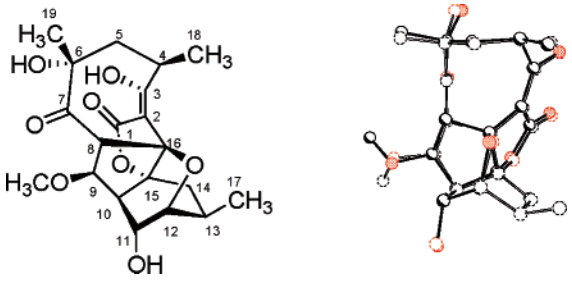
ABSTRACT



Abyssomicin E (1), a new polycyclic metabolite with a C19 skeleton, was isolated from *Streptomyces* sp. (HKI0381). Its chemical structure was determined by comprehensive NMR and MS spectroscopic analyses. For the first time in this recently discovered class of compounds, the absolute stereochemistry was directly established by subsequent single-crystal X-ray diffraction study using anomalous dispersion with copper radiation.

In our efforts to exploit structural diversity of microbial natural products by physicochemical metabolite pattern analysis, a new highly functionalized metabolite with a C_{19} skeleton, named abyssomicin E (**1**), was isolated from *Streptomyces* sp. (HKI0381). Abyssomicin E (**1**) is the first example in this very new class of natural products that was initially fully elucidated on the basis of NMR and MS data. We are presenting those results in full detail to facilitate future studies. However, the structure of **1**, including its absolute stereochemistry, was later also provided by X-ray crystallographic analysis.

Streptomyces sp. (HKI0381) was isolated from a soil sample from Senegal (Ile de Paradis). On the basis of the morphological characteristics and the results of a 16S rRNA gene sequence comparison with GenBank, the isolate was assigned as *Streptomyces* sp.[1] The closest phylogenetic neighbors are *Streptomyces* sp. EF-14 and *Streptomyces eurythermus* which were isolated from potato scab. Both strains share a 16S rRNA gene sequence similarity with strain HKI0381 of 98.9% and 98.8%, respectively.[1]

Strain HKI0381 was cultured for 96 h in a 300 L fermentor in soybean/mannitol medium (20 g L^{-1}, D-mannitol, 20 g L^{-1} degreased soybean meal, pH 7.0 prior to sterilization) at 28 °C, with 50 L/min aeration and stirring at 200 rpm. The culture filtrate was separated by centrifugation from the mycelium and subjected to XAD-16 resin. After washing

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(1) http://www.ncbi.nlm.nih.gov/BLAST/.

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with deionized water, a crude product was obtained through elution with methanol. The eluate was concentrated in vaccuo and lyophilized. The residue was later redissolved in 30 L of water and subjected to chromatographic solid-phase extraction on Amberchrom 161 (30 μm, 6 L) with H_2O, aqueous MeOH (20%, 40%, 60%, 80%), and MeOH (3000 mL of each eluent, 1000 mL min^{-1}, 17 bar) to provide seven compound-containing fractions.[2] Fraction 6 was partitioned between ethyl acetate and water, and the organic fraction gave 2.02 g of a dry residue, which was further chromatographed successively on Sephadex LH-20 (methanol) and on a silica gel column (chloroform and chloroform/methanol) to yield 42.1 mg of crude compound **1**. After repeated recrystallization with methanol−water (99:1), 5 mg of purified compound **1** was obtained as colorless prisms.

Compound **1** displayed its molecular ion in ESIMS at *m*/*z* 395.2 $[M + H]^+$, and HRESIMS yielded $C_{20}H_{26}O_8$ as its molecular formula (found 394.16119, calcd 394.16121).[3]

Partial structure **1a** was readily established by analysis of ^1H, ^{13}C, and 2D NMR data (Figure 1, Table 1). The ^1H,^1H-

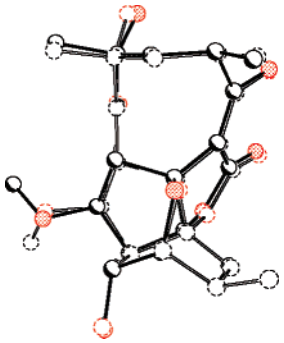

Figure 1. Overlay of the crystal structures of the two conformers A (bold bonds and solid atoms) and B (hollow bonds and dotted atoms) of abyssomicin E (**1**).

coupling systems of H_2-5/H-4/H_3-18, H_2-14/H-13/H_3-17, and H-8/H-9/H-10/H-11/H-12 were evident from COSY data. Although a correlation between H-13 and H-12 was not observed, a direct connection between C-13 and C-12 was deduced from HMBC correlations of H-14α and H_3-17 with C-12. In addition, H-8, H-10, and H-14 demonstrated ^1H−^{13}C long-range correlations with both C-16 and C-15, indicating that these two oxygenated quaternary carbons are immediate neighbors. Thus, a bicyclic moiety with a five- and a six-membered ring was deduced. Moreover, HMBC interaction between H-12 and C-16 was deduced, which suggested the occurrence of an oxygen bridge between C-16

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(3) Abyssomicin E: colorless, mp 76−79° C; $[\alpha]_D^{23.5}$ + 98.9 (*c* 0.46, MeOH); UV (MeOH) λ max (log ϵ) 229 nm (4.19) nm; IR (KBr) ν_{max} 3391, 2972, 2933, 1713, 1700, 1631, 1460, 1370, 1301, 1206, 1182, 1168, 1138, 1117, 1090, 1043, 1026, 1009, 969, 942, 923, 815 cm^{-1}; ESI-MS *m*/*z* 395.2 $([M + H]^+$, 46%), 412.3 $([M + NH_4]^+$, 100%), 417.2 $([M + Na]^+$, 35%), 806.0 $([2M + NH_4]^+$, 30%), 810.6 $([2M + Na]^+$, 55%); HRESI-MS *m*/*z* 394.16119 (calcd for $C_{20}H_{26}O_8$ 394.16121).

Table 1. NMR Spectral Data and HMBC Correlations for **1**[a]

no.	^1H (δ_H, mult., *J*, Hz)	^{13}C (δ_C)	HMBC (C → H)
1		174.1 (s)	
2		97.8 (s)	4
3		182.0 (s)	5α, 4, 18
4	2.80 (m)	36.0 (d)	5α, 5*β*, 18
5α	2.00 (dd, 4.7, 14.5)	37.9 (t)	4, 18, 19
5*β*	2.86 (d, 14.5)		
6		78.5 (s)	5, 19
7		209.6 (s)	5α, 5*β*, 8, 9, 19
8	3.26 (d, 8.0)	65.1 (d)	9, 10
9	4.45 (dd, 4.0, 8.0)	77.0 (d)	11, OMe
10	2.63 (d, 4.0)	50.9 (d)	12, 14α, 14*β*
11	4.64 (d, 4.0)	67.6 (d)	9, 10, 12
12	3.72 (d, 4.0)	76.2 (d)	14α, 17
13	2.50 (m)	24.2 (d)	11, 14*β*, 17
14α	2.32 (dd, 11.4, 13.8)	32.5 (t)	12, 17
14*β*	1.07 (dd, 5.4, 13.8)		
15		84.6 (s)	8, 10, 11, 14α, 14*β*
16		85.3 (s)	8, 10, 12, 14α
17	1.00 (3H, d, 6.8)	18.6 (q)	12, 13, 14α, 14*β*
18	1.31 (3H, d, 6.9)	19.3 (q)	5*β*, 4
19	1.26 (3H, s)	27.9 (q)	5α
OMe	3.29 (3H, s)	58.0 (q)	9
3-OH	11.27 (br s)		

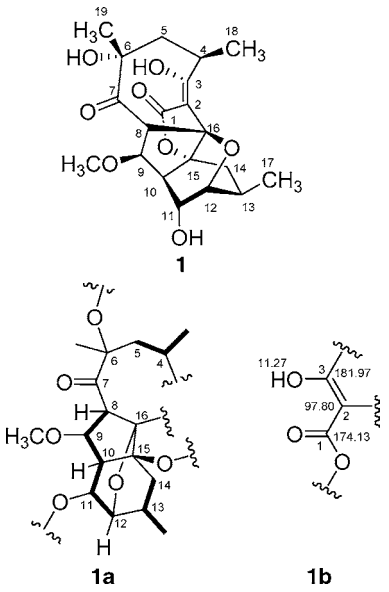
[a] Data were recorded in $CDCl_3$ on Bruker AM-300 MHz (^1H, ^{13}C) and Bruker DRX-500 MHz spectrometers (COSY, HMBC, ROESY); chemical shifts are given in parts per million by referencing the signals of $CDCl_3$.

and C-12. Furthermore, the methoxyl group was determined to be at C-9 from the mutual HMBC correlations between the protons and carbons of the OMe group and C-9/H-9.

For the remaining quaternary carbons, the partial fragment **1b** was proposed (Scheme 1). The combination of fragments **1a** and **1b** at C-4−C-3 and C-2−C-16 resulted in an eight/five/six-membered ring skeleton which could satisfy all correlations detected in the HMBC spectrum. In the proposed skeleton, the enormous shift dispersion of the olefinic C-2 (δ_C 97.8 ppm) and C-3 (δ_C 182.0 ppm) was consistent with the conjugating effects of the C-1 carbonyl. The molecular formula of **1** implied the existence of an additional oxygen bridge between any two carbons among C-1, C-6, C-11, and C-15 because all of them had to be oxygenated based on their chemical shifts The lack of acidic properties, e.g., formation of a clear spot on silica gel TLC (R_f = 0.35, $CHCl_3/Me_2CO$ = 9:1), suggested that **1** contained a lactone between the carboxylate at C-1 and the oxygen at C-15. Accordingly, the oxygen substituents at C-6 and C-11 could only be hydroxyl groups.

As for the relative stereochemistry, NOE correlations (NOESY) between H-5α and Me-19, H-5α and H_3-18, H-8 and H_3-19, and H-8 and H-11 revealed H-8, H-11, C-19, and C-18 to be α-oriented. The NOEs between H-9 and H-10, H-10 and H-12, H-10 and H-14*β*, H-12 and H-14*β*, H-13 and H-12, and H-14α and H_3-17 indicated a *β*-orientation for H-9, H-10, H-12, and H-13. The *β*-orientation of H-12 led to an α-orientation of the oxirane substitution at C-16. Only the stereochemistry of the lactone attachment was still to be determined.

Scheme 1. Structure of Compound **1** and Partial Chemical Structures of **1a**,**b**[a]



[a] **1a**: Bold bonds indicate connectivities as deduced by COSY data.

In the course of our studies, a single crystal of **1** was obtained after several recrystallizations. X-ray diffraction confirmed the NMR spectroscopic structure elucidation and established the absolute stereochemistry of all chiral centers as 4*R*,6*R*,8*S*,9*R*,10*S*,11*R*,12*R*,13*R*,15*R*,16*S* as well as the *Z*-type double bond between C-2 and C-3 by anomalous dispersion with copper radiation (Figures 1 and 2). The

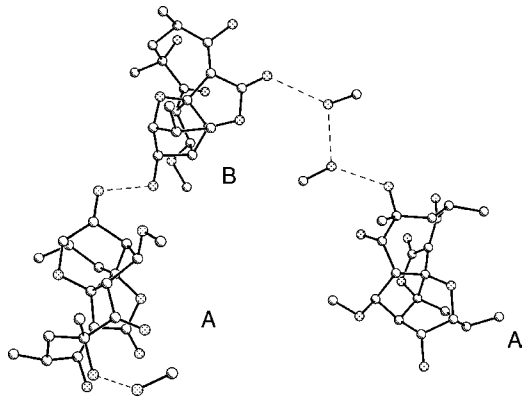
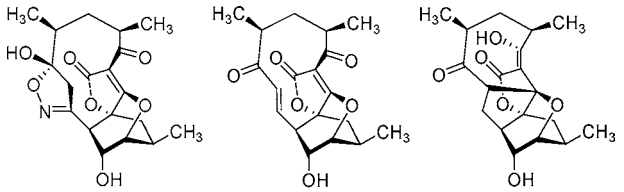


Figure 2. X-ray crystallographic structure of **1** consisting of conformers A and B in a 2:1 ratio (dashed lines represent hydrogen bonds).

crystal of **1** was shown to consist of two conformers in a 2:1 ratio. The most obvious conformational deviation was the spatial position of the 9-OMe arising from the rotation of the σ-bond between C-9 and OMe. The macrocyclic ring exhibited observable structural deviations of C-6 and its substituents. The complete structure of compound **1** was finally determined as shown, and the compound was named abyssomicin E.[7]

Three related compounds, abyssomicins B−D (Scheme 2), were recently discovered from a marine actinomycete,

Scheme 2. Structures of Abyssomicins B−D (**2**−**4**)



2 **3** **4**

Verrucosispora strain AB 18−302, and attracted much attention because of their unprecedented complex structures.[5] The absolute stereochemistry of **1** is in accordance with the configurations of abyssomicins B−D that were determined indirectly by the Mosher and Helmchen methods and were later confirmed by total synthesis.[4−6]

Abyssomicin E is most closely related to abyssomicin D, which is the 6,9-deoxy analogue of **1**. The close constitutional relationship of abyssomicins D and E is also reflected in their similar crystal structures. The oxygenation at C-6 in **1** is a structural variation that is otherwise not found in this

(4) Crystal Data: $C_{20}H_{26}O_8 \cdot CH_4O$, $M_r = 426.45$ g mol^{-1}, colorless prism, size 0.50 × 0.30 × 0.20 mm^3, monoclinic, space group $P2_1$, $a = 9.118(3)$, $b = 19.012(3)$, $c = 11.942(5)$ Å, $\beta = 93.65(2)°$, $V = 2066.0(12)$ Å^3, $T = -80$ °C, $Z = 4$, $\rho_{calcd} = 1.371$ g cm^{-3}, μ(Cu K$_\alpha$) = 8.99 cm^{-1}, $F(000) = 912$, 8773 reflections in $h(-11/11)$, $k(-23/23)$, $l(-14/14)$, measured in the range $3.71° \le \Theta \le 74.20°$, completeness $\Theta_{max} = 99.6\%$, 8003 independent reflections, $R_{int} = 0.071$, 7326 reflections with $F_o > 4\sigma(F_o)$, 553 parameters, 1 restraint, $R1_{obs} = 0.057$, $wR2_{obs} = 0.158$, $R1_{all} = 0.073$, $wR2_{all} = 0.210$, GOF = 1.087, Flack parameter 0.1(2), largest difference peak and hole = 0.407/−0.409 e Å^{-3}. The intensity data for the compounds were collected on a Nonius CAD4 diffractometer, using graphite-monochromated Cu K$_\alpha$ radiation. Data were corrected for Lorentz and polarization effects but not for absorption effects.[8] The structures were solved by direct methods (SHELXS[9]) and refined by full-matrix least squares techniques against F_o^2 (SHELXL-97[10]). The hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically.[10] The absolute configuration could be determined. XP (SIEMENS Analytical X-ray Instruments, Inc.) was used for structure representations.

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(7) The systematic name of abyssomicin E is (2*R*,3*R*,4*R*,4a*S*,5*R*,-5a*S*,7*R*,9*R*,12a*R*,12b*S*)-4,7,10-trihydroxy-5-methoxy-2,7,9-trimethyl-12-oxo-1,2,3,4,4a,5,5a,6,7,8,9,11,12a,12b-tetradecahydrobenzo[*g*]cycloocta[*cd*]-pentalene-6,11-dione. Note: this name applies a numbering system that is different from the one shown.

compound class. Little is known about the biosynthesis of the abyssomicins which most likely should be similar to that of the kijanimicin-type antibiotics.[11] It can be reasoned that oxygenation of the polyketide backbone at C-6 requires additional enzymatic oxygenation of the polyketide skeleton.

In our general bioactivity profiling program, e.g., antimicrobial, cytotoxic, and antiviral testing, as well as various enzyme assays, **1** was not active.[12−14] This result is in agreement with the absence of antibiotic or other biological activity of the closely related abyssomicin D.[5] In contrast, abyssomicin C shows a promising antibiotic activity against a variety of Gram-positive bacteria including pathogenic *Staphylococcus aureus* strains and drug-resistant strains.

In summary, we have discovered a new member of a very small class of highly functionalized complex microbial secondary metabolites. It is the first account of NMR and MS related structure determinations of abyssomicins, and it is the first report on the direct determination of absolute configuration.

Acknowledgment. Fruitful discussions with Dr. Jian He (Leibniz-Institute for Natural Product Research and Infection Biology) and Dr. Xueshi Huang (Leibniz-Institute for Natural Product Research and Infection Biology) are gratefully acknowledged. Spectroscopic data were recorded by HKI's NMR and MS units. X.-M. Niu acknowledges funding by a DAAD-Leibniz postdoctoral fellowship.

Supporting Information Available: 1D and 2D NMR spectra as well as CIF data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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