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Abyssomicins G and H and atrop-Abyssomicin C from the Marine *Verrucosispora* Strain AB-18-032†

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Abstract Abyssomicin C is a complex polyketide-type antibiotic and the first natural inhibitor of the *p*-aminobenzoate biosynthesis produced by the marine *Verrucosispora* strain AB-18-032. We have now isolated three novel naturally produced abyssomicins, among them the even more active atrop-abyssomicin C. The chemical structures were elucidated by mass spectrometry and NMR spectroscopy.

Keywords antibiotics, tetronic acid, structure elucidation, abyssomicins, *Verrucosispora*

Abyssomicin C (**1**) which has been recently described by our groups is a polycyclic polyketide-type antibiotic detected in a screening for inhibition of the *p*-aminobenzoate (*p*ABA) pathway [2, 3]. To our knowledge, **1** is the first natural inhibitor of the *p*ABA biosynthesis pathway derived from a bacterial source. It shows antibiotic activity against Gram-positive bacteria including pathogenic *Staphylococcus aureus* strains. The MIC value of **1** against *S. aureus* N315 (MRSA) and *S. aureus* Mu50 (multiresistant and intermediate resistance against vancomycin) were in the range of 4 μg/ml and 13 μg/ml, respectively [2].

1 has been isolated along with two inactive derivatives, abyssomicins B (**2**) and D (**3**), from the rare actinomycete strain *Verrucosispora* AB-18-032 that was isolated from a sediment sample collected in the Sea of Japan at a depth of 289 m. The structures were elucidated by means of mass spectrometry, NMR spectroscopy and X-ray structure determination. Due to their unique structure, the abyssomicins are attractive leads for chemical synthesis of novel inhibitors. It is not surprising that several synthetic chemistry groups have directed their interest towards the synthesis of **1** [4]. Two successful total syntheses have been published so far, the first by Sorensen and co-workers [5] and the second by Nicolaou and Harrison [6]. Interestingly, the Nicolaou group synthesized a second derivative, atrop-abyssomicin C (**4**), which was obtained simultaneously with **1**.

Herein, we describe the isolation and spectroscopic characterization of three novel abyssomicins from *Verrucosispora* AB-18-032: **4** and two novel derivatives, abyssomicin G and H (**5**, **6**). A detailed analysis of culture filtrates from fermentations of *Verrucosispora* revealed a number of additional signals in the chromatograms of LC-ESI-MS runs, which were related to abyssomicins according to their UV-visible properties. Subsequently, besides the previously known **1**, **2** and **3**, three novel compounds (**4**~**6**) were purified by adsorption chromatog-

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raphy and size-exclusion chromatography, followed by preparative reversed-phase HPLC as described previously [2], but using acid free solvents.

In submerged cultures of *Verrucosispora* AB-18-032 **4** is the major product reaching maximal yields of 60 mg/litre, followed by **2** with 8.0 mg/litre, whereas **1**, **3**, **5** and **6** are minor congeners. Nomenclature of the new compounds is based on the historic course of our abyssomicin research. For structure elucidation, **4**∼**6** were analyzed by mass spectrometry and by 1D and 2D NMR spectroscopy (Table 1). The high-resolution ESI-FTICR mass spectra of **4**∼**6** showed masses of *m*/*z* 347.14892 Da (**4**), 400.13646 Da (**5**), 349.16389 Da (**6**), respectively, corresponding to the molecular formulae $C_{19}H_{22}O_6$ (**4**) [$(M+H)^+_{theor}$=347.14895, Δm=0.08 ppm], $C_{19}H_{23}NO_7$ (**5**) [$(M+Na)^+_{theor}$=400.13667, Δm=0.5 ppm], $C_{19}H_{24}O_6$ (**6**) [$(M+H)^+_{theor}$=349.16457, Δm=1.9 ppm].

NMR experiments were measured on a DRX500 NMR spectrometer (Bruker, Karlsruhe, Germany) equipped with a 5 mm diameter broad band inverse probehead with z gradients. Spectra were recorded in and referenced to [D_4]methanol (3.30 ppm; 49.0 ppm). 2D COSY, NOESY, HMQC and HMBC experiments were measured with standard Bruker parameters (XWinNMR 3.2). LC-MS experiments were performed on a 2000 Q Trap mass spectrometer (Applied Biosystems/MDS Sciex) coupled to an Agilent 1100 HPLC system (Agilent, Waldbronn, Germany). FTICR-ESI mass spectra were recorded on an APEX II FTICR mass spectrometer (4.7 T, Bruker-Daltonics, Bremen, Germany). Spectra were calibrated using PEG 400 as an internal standard.

From 1D and 2D NMR spectra, **4** was identified as atrop-abyssomicin C [6]. **5** has the same molecular formula as **2**. 2D NMR data from COSY, TOCSY, HMQC, and HMBC experiments were found to be similar to **2**, except for chemical shift differences in the western molecule part adjacent to the oxabicyclooctane system (C(7)∼C(9)). The connectivity of C(7) through C(10) was established based on HMBC correlations. H-C(8) couples to C(7), C(9), and C(10), and H-C(10) couples to C(7), C(8), C(9) in addition

Table 1 ^{1}H- and ^{13}C-NMR shifts of atrop-abyssomicin C, abyssomicins B, G, and H[a]

No.	atrop-abyssomicin C (**4**)		abyssomicin B (**2**)		abyssomicin G (**5**)		abyssomicin H (**6**)	
	δ (^{1}H) [ppm]	δ (^{13}C) [ppm]	δ (^{1}H) [ppm]	δ (^{13}C) [ppm]	δ (^{1}H) [ppm]	δ (^{13}C) [ppm]	δ (^{1}H) [ppm]	δ (^{13}C) [ppm]
1	—	171.5	—	172.0	—	172.0	—	172.1
2	—	106.9	—	104.5	—	102.2	—	101.7
3	—	201.2	—	200.2	—	200.0	—	200.2
4	2.65	44.7	3.36	43.0	3.58	43.1	3.33	44.1
5	1.85	39.2	1.32	40.6	1.95	40.9	1.99	40.6
	1.44		1.18		1.40		1.43	
6	2.50	50.0	1.91	37.4	2.94	46.0	2.73	47.6
7	—	204.2	—	116.1	—	211.3	—	214.4
8	6.52	129.8	2.86	42.4	3.77	46.8	2.65	36.5
			2.26		3.48			
9	6.65	140.3	—	159.1	—	151.7	2.17	21.0
							1.91	
10	3.18	51.1	3.16	50.6	3.73	47.4	2.24	45.6
11	4.40	67.6	4.21	67.7	4.52	70.4	4.44	71.0
12	4.62	85.1	4.81	85.6	4.64	85.6	4.55	86.7
13	2.79	26.7	2.83	26.2	2.79	26.2	2.67	26.0
14	2.69	34.6	2.67	34.4	2.74	38.3	2.60	39.0
	1.45		1.41		1.21		1.13	
15	—	81.8	—	79.1	—	78.1	—	79.9
16	—	180.1	—	185.3	—	187.9	—	187.0
17	1.16	18.8	1.14	18.6	1.13	19.0	1.11	19.1
18	1.11	16.9	1.05	17.0	1.04	16.9	1.08	17.0
19	1.14	18.0	1.08	14.4	1.07	21.4	1.04	21.2

[a] CD_3OD.

to correlations to C(11), C(14), C(15), and C(16). The most striking difference in chemical shifts of the ^{13}C-NMR spectrum is 116 ppm *versus* 211 ppm for C(7) of **2** and **5**, respectively. This leads to the conclusion that, as in **1** and **3**, C(7) is the carbonyl of a ketone. Chemical shifts of C(8) with 3.77 ppm and 3.48 ppm in ^{1}H and 46.8 ppm in ^{13}C, C(9) with ^{13}C shift of 151.7 ppm, and C(10) with ^{1}H shift of 3.73 ppm and ^{13}C shift of 47.4 ppm combined with the molecular formula derived from HR-ESI-FTICR-MS are in good agreement with the proposed structure, bearing an oxime at C(9) (Fig. 1). **5** is stable under room temperature and does not spontaneously rearrange to **2**.

6 has the same molecular formula as **3**. However, 1D and 2D spectra are more similar to **1**, except for major chemical shift deviations for C(8) and C(9). The connectivity of C(7) through C(10) was established based on COSY correlations of H-C(9) to H-C(8) and H-C(10) and HMBC correlations. HMBC correlations are found between H-C(8) and C(6), C(7), C(9), and C(10), between H-C(9) and C(7), C(8), C(10), and C(11), and between H-C(10) and C(8), C(9), C(11), C(14), C(15), and C(16). The assignment of chemical shifts shows that in **1**, C(8) and C(9) are connected *via* a double bond, whereas in **6**, C(8) and C(9) are reduced to methylene units (Fig. 1). **5** and **6** are novel members of the abyssomicin family and were named abyssomicin G and H (Fig. 1). Antibacterial tests performed as described in [2] for **5** (MIC >1 mM) and **6** (MIC >1 mM) showed no antibacterial activities.

Remarkably, **4**, previously described only by synthetic chemists, is also naturally produced by *Verrucosispora* AB-18-032, as assumed previously by Nicolaou and Harrison [7]. In fact in our fermentations **4** appeared as the main product. Reconsideration of experimental data from previous cultivations of *Verrucosispora* AB-18-032 which led to the discovery of **1** indeed confirmed the presence of **4**. However, **4** depleted during previous purifications or was converted to **1** by use of acidic HPLC solvents. The extremely high similarity of both compounds with regard to retention time and UV spectra obscured an earlier observation of this loss. The antibacterial assay performed by our groups confirmed a stronger inhibitory effect for **4** compared to **1** as found by Nicolaou and Harrison [6] describing a 1.5 fold lowered MIC value for **4** against MRSA. Abyssomicins G (**5**), which is a putative precursor of **2** displays no antibiotic activity and thus confirms the crucial role of an intact Michael-acceptor system located at C(7)~C(9). The biosynthesis of **5** might occur *via* addition of ammonia and subsequent *N*-oxidation by a monooxygenase. *N*-oxidized natural products have been found to be often biosynthesized *via* a hydroxylamine intermediate catalyzed by flavin monooxygenases [8]. The

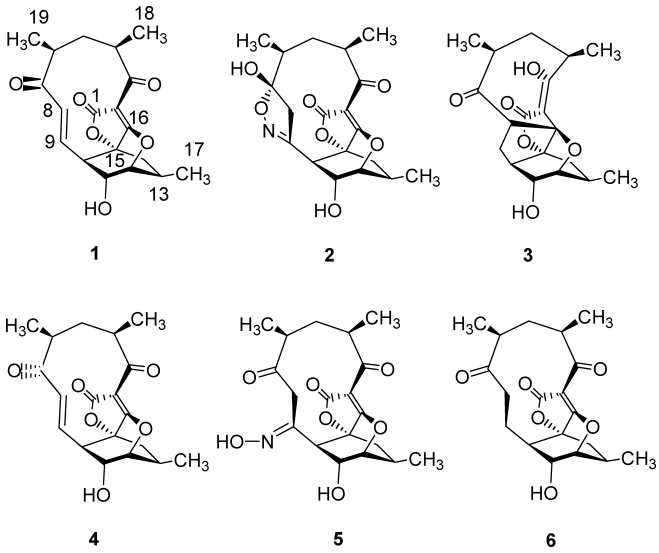

Fig. 1 Structural formulae of abyssomicins C (**1**) B (**2**), D (**3**), G (**5**), H (**6**), and atrop-abyssomicin C (**4**).

postulated oxime formation has been described by the Townsend group for nocardicin A and was found to be mediated by a cytochrome P450 enzyme [9]. More strikingly, in **6** with MIC values of >1 mM the Michael system C(7)~C(9) is destroyed by reduction of the alkene at C(8)~C(9) to the corresponding alkane. This underscores the likely action of **1** and **4** as antibacterials with Michael-acceptor properties. Furthermore, this assumption is strongly supported by MIC-assays of Nicolaou and Harrison [7] performed on **1** analogs showing that reduction of the ketone at C(7) to the corresponding hydroxy group leads to complete loss of antibacterial activity. **3** and **6** are products of a formal hydrogen addition. As the experiments from Nicolaou and Harrison show, the incubation of an NADH analogue with **4** lead to the formation of **3** [7]. Incubations of **1** and **4** in our lab with $NaBH_4$ in THF lead to the formation of both **3** and **6**. For the synthesis of **6** a Michael addition of a hydrogen equivalent or the action of an enoylreductase of a polyketide synthase seems possible. Current experiments by our groups directed to the elucidation of the biosynthetic assembly of abyssomicins will further address these questions.

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