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NOTE

Angiolactone, a new Butyrolactone isolated from the terrestrial myxobacterium, *Angiococcus* sp.

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DESCRIPTION

Myxobacteria are recognized as one of the outstanding prokaryotic sources of bioactive compounds in nature. More than 100 core structures with diverse biological activities had been elucidated, most of which were novel.[1] In the continuous screening program for anti-infectives focusing on novel, rare, and unexplored myxobacterial taxa, great potential was discovered in *Angiococcus disciformis* SBAn001. The strain was routinely cultivated in TS-6 medium (Tryptone (Difco, BD Biosciences, San Jose, CA, USA) 0.6%, $MgSO_4 \cdot 7H_2O$ 0.2%, soluble starch (Roth, Karlsruhe, Germany) 0.4%, HEPES 1.19%, pH adjusted to 7.2 with KOH before autoclaving).

Shake flask fermentation was performed in 10 liters volume TS-6 medium for 10 days at 30 °C, 160 r.p.m., after which on the 3rd day amberlite resin XAD-16 was added (2% v/v). The resin was filtered from the fermented broth and washed twice with distilled water, followed by extraction with methanol (500 ml × 2). The MeOH extract was then dried *in vacuo* yielding a crude extract of 126 mg fraction. This was then partitioned between 30 ml of hexane, CH_2Cl_2 and MeOH to afford 33.6, 20.6 and 42.3 mg fractions, respectively. The MeOH fraction was subsequently purified by semi-preparative reverse-phase HPLC to yield the UV absorbing (λ_{max} 260 nm) compound **1** (Figure 1; $t_R = 24.2$ min; 0.3 mg). A detailed account of the spectroscopic analysis leading to the assignment of angiolactone (**1**) is presented below.

HRESI (+)MS analysis of **1** (Table 1) revealed a pseudomolecular ion ($[M+Na]^+$) indicative of a molecular formula ($C_{23}H_{26}O_4$) requiring 11 double bond equivalents. Examination of the NMR (methanol-d_4) data (Table 2, Supplementary Figures S1–S4) revealed 15 sp^2 carbon resonances, 12 of which were attributed as aromatic (δ_C 115.3–157.4), an olefinic double bond (δ_C 105.8, 153.2) and an ester carbonyl (δ_C 175.1) accounting for 7 double bond equivalent and requiring that **1** be tricyclic. Analysis of the 1D and 2D NMR data revealed equivalent aromatic protons H-2″ plus H-6″ (δ_H 7.00, d, 7.6) and H-3″ plus H-5″ (δ_H 6.63, d, 8.3), which showed HMBC correlations to their same HSQC-correlated ^{13}C resonances, the quaternary carbons C-1″ (δ_C 127.5), C-4″ (δ_C 157.4) and to the methylene C-6 (δ_C 41.2), indicating the presence of a 4-hydroxybenzyl moiety (Figure 1). COSY correlations from the aromatic methines H-2′ to H-6′ extended by HMBCs to the olefinic methine H-5 (δ_H 5.25) led to the construction of a mono-substituted aromatic ring attached to an exocyclic double bond (Figure 1). Moreover, HMBCs from H-2 (δ_H 2.83) to the ester carbonyl C-1 (δ_C 175.1) and to an oxy quaternary carbon C-3 (δ_C 80.8), and from H_2-6 (δ_H 2.96, 2.65) to C-2 (δ_C 52.2), C-3, and the oxygenated sp^2 carbon C-4 (δ_C 153.2) and together with long-range correlations from H-5 to C-3 and C-4 corraborated the presence of a substituted furanone ring. The final substituent on the furanone ring by examination of the COSY spectra revealed an isolated spin system (H_3-11- H-2) consistent with an isopentyl group (Figure 1). On the basis of these data, the planar

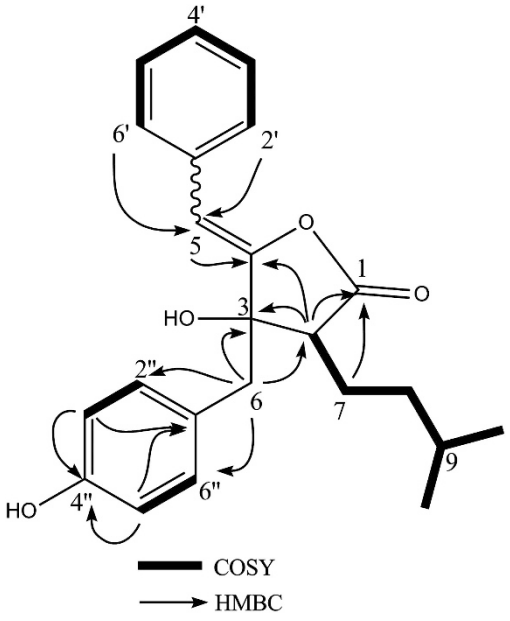

Figure 1 Key 2D NMR correlations (500 MHz, methanol-d_4) for angiolactone (**1**).

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Table 1 Physico-chemical properties of 1

	1
Appearance HR-ESI-MS (*m*/z)	Yellow oil
Found	389.1746 $[M+Na]^+$
Calcd	389.1729 ($C_{23}H_{26}O_4Na$)
$[\alpha]_D$ (MeOH) c (0.03)	(+) 39°
UV λ_{max} nm (log ε) (MeOH)	260 (4.55)

Table 2 NMR (500 MHz, methanol-d_4) data for angiolactone (1)

Pos	δ_H, *mult (J in Hz)*	δ_C[a]	*COSY*	*HMBC*
1		175.1		
2	2.83, dd (7.9, 6.7)	52.2	7	1, 3, 6, 7, 8
3		80.8		
4		153.2		
5	5.25, s	105.8		3, 4, 2′, 6′
6a	2.96, d (13.6)	41.2		2, 3, 4, 1″, 2″, 6″
6b	2.65, d (13.6)			2, 3, 4, 1″, 2″, 6″
7	1.82, m	22.8	2, 8a/b	1, 2, 3, 8
8a	1.61, m	37.8	7, 8b	
8b	1.48, m		7, 8a, 9	
9	1.65, m	29.1	8b, 10, 11	
10	0.98[b], d (6.4)	22.8	9	8, 9, 11
11	0.97[b], d (6.4)	22.8	9	8, 9, 10
1′		135.2		
2′	7.40, d (7.6)	129.4	3′	5, 4′, 6′
3′	7.27, dd (7.6, 7.0)	129.1	2′, 4′	1′, 5′
4′	7.17, dd (7.2, 7.0)	127.6	3′, 5′	2′, 6′
5′	7.27, dd (7.6, 7.2)	129.1	4′, 6′	1′, 3′
6′	7.40, d (7.6)	129.4	5′	5, 2′ 4′
1″		127.5		
2″	7.00, d (8.3)	132.8	3″	6, 4″, 6″
3″	6.63, d (8.3)	115.3	2″	1″, 4″, 5″
4″		157.4		
5″	6.63, d (8.3)	115.3	6″	1″, 3″, 4″
6″	7.00, d (8.3)	132.8	5″	6, 2″, 4″

[a]^{13}C NMR resonances obtained from 2D HSQC and HMBC experiments.
[b]Overlapping signals.

structure of angiolactone (**1**) was determined to be 5-benzylidene-4-hydroxy-4-(4-hydroxybenzyl)-3-isopenty-γ-butyrolactone. Despite our efforts in experimenting with different media components to get a better production for **1**, we were unsuccessful in ramping up production of **1** and as a result were not able to acquire enough material to address the stereochemistry and bioactivity of **1**.

Butyrolactones are ubiquitously present in microbes and plants and they have been frequently associated with their role as quorum-sensing signaling molecules for activating antibiotic production in actinomycetes.[2] The diversity of butyrolactones in myxobacteria is however much lower with the known example being

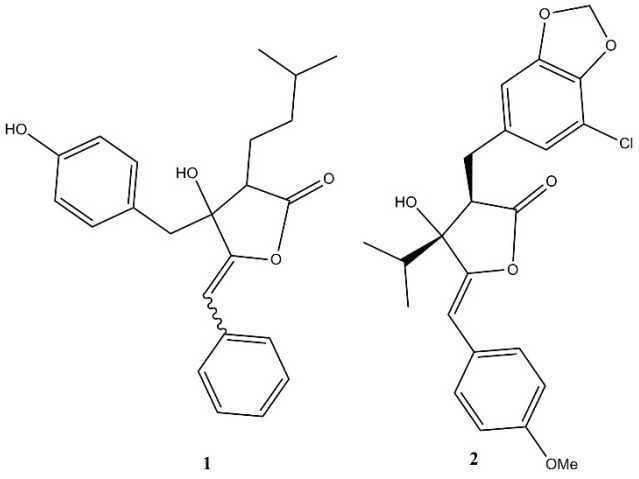

Figure 2 Structures of **1** and **2**.

the structurally unique leupyrrins.[3] The closest known metabolite to **1** was cyanobacterin **2**, a chlorine-containing γ-lactone isolated from the freshwater cyanobacterium *Scytonema hofmanni* (Figure 2).[4] In summary, we have successfully isolated and identified a new butyrolactone (**1**), a compound class, which is rare from myxobacterium.

EXPERIMENTAL PROCEDURE

NMR spectra were obtained on a Bruker Ascend 500 MHz spectrometer equipped with a cryoprobe system (Bruker Biospin GmbH, Waldbronn, Germany), in the solvents indicated and referenced to residual ^{1}H signals in deuterated solvents. ESI-MS were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes under the following conditions: Zorbax C_8 column, 150 × 4.6 mm, eluting with 0.4 ml min^{-1} 95% H_2O/MeCN to 5% H_2O/MeCN (with isocratic 0.01% trifluoroacetic acid) over 22 min, then held for 5 min. HRMS was carried out using an UltiMate 3000 rapid separation liquid chromatography system (Dionex RSLC, Crawford Scientific, Lanarkshire, Scotland, UK) coupled to an UHR-TOF mass spectrometer (Bruker Daltonik MaXis) operating in the positive ESI mode.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)