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NOTE

New cytotoxic spectinabilin derivative from ant-associated *Streptomyces* sp. 1H-GS5

Shuang-he Liu[1], Mei-dong Xu[2], Hui Zhang[2], Huan Qi[2], Ji Zhang[1], Chong-xi Liu[1], Ji-dong Wang[2], Wen-sheng Xiang[1] and Xiang-jing Wang[1]

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Insects are the most abundant and diverse animal class on earth and they are associated with an amazing variety of microorganisms. The ability of insects to live in unique niche habitats is often facilitated by their associated microbe,[1] which perform many important host functions, especially against pathogens, parasitoids or predators by producing metabolites[2,3] and the enormous insect-associated microbial communities are a potential source of novel antibiotic metabolites.[4,5] To find more insect-associated microorganisms-derived bioactive metabolites, the study of the carpenter ants-associated microorganisms was carried out and a strain *Streptomyces* sp. 1H-GS5 exhibiting good activities against some lines of tumor cell was obtained. In the further investigation of chemical components from the broth of *Streptomyces* sp. 1H-GS5, a new spectinabilin derivative (**1**) along with the known compound, spectinabilin (**2**) (Figure 1), were isolated. Here the report deals with the details of the isolation, structure elucidation and the bioactivity of the new compound.

The strain *Streptomyces* sp. 1H-GS5 was isolated from the head of ant (*Camponotus japonicas* Mayr) collected from the Northeast Agricultural University, Harbin of Heilongjiang province, China. The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: KP784764 in the GenBank, National Center for Biotechnology Information) exhibited a high-sequence similarity of 99% with that of *Streptomyces spectabilis* NBRC 13424 (accession no: AB184393). The strain *Streptomyces* sp. 1H-GS5 has been deposited in the Pharmaceutical Research Culture Collection, Zhejiang Hisun Group Co., Ltd, with accession no: HS-HX-087.

Strain was maintained on a YMS medium containing soluble starch (Bei Jing Ao Bo Xing, Beijing, China) 10 g, yeast extract (Bei Jing Ao Bo Xing) 2 g, KNO_3 1 g and agar 20 g in 1.0 l tap water, pH 7.0. The seed medium consisted of glucose (Bei Jing Ao Bo Xing) 20 g, soybean flour (Cormwin, Beijing, China) 15 g and yeast autolysate (Bei Jing Ao Bo Xing) 5.0 g in 1.0 l tap water, pH 7.0. Both the media were sterilized at 121 °C for 20 min. Slant cultures were incubated for 6–7 days at 28 °C.

A total of 10 ml of sterile water was added to the slant of the YMS medium. The spores were scraped and transferred onto a sterile tube containing glass beads; the spore suspension was then filtered through six layers of a sterile filter cheesecloth and adjusted to 10^7–10^8 c.f.u. ml^{-1}. A 2.0 ml of the spore suspension was inoculated into a 250-ml flask containing 25 ml of seed medium and incubated at 28 °C for 24 h, shaken at 250 r.p.m. Then, 8.0 ml of the culture was transferred into 1 liter Erlenmeyer flask containing 100 ml of the producing medium consisting of corn starch (Cormwin) 10%, soybean powder (Bei Jing Ao Bo Xing) 1%, cotton flour (Cormwin) 1%, α-amylase (Bei Jing Ao Bo Xing) 0.02%, NaCl 0.1%, K_2HPO_4 0.2%, $MgSO_4 \cdot 7H_2O$ 0.1%, $CaCO_3$ 0.7%, cyclohexanecarboxylic acid 0.1%, pH 7.0, before sterilization. Fermentation was carried out at 28 ° C for 7 days on a rotary shaker at 250 r.p.m.

The final 30 l of broth from 300 producing fermentations was filtered. The resulting cake was washed with water. Both the supernatant and the wash water were discarded. Methanol (6 l) was used to extract the washed cake and the MeOH extract was evaporated under reduced pressure to 1 liter at 50 °C and the resulting concentrate was extracted three times using an equal volume of EtOAc. The combined organic layer was concentrated to give a yellow oily residue (14 g). The yellow oily residue was subsequently subjected to silica gel column chromatography (Qingdao Haiyang Chemical Group, Qingdao, China; 100–200 mesh) using an *n*-hexane-EtOAc stepwise system (100:0–50:50, v/v) to obtained four fractions (fractions 1–4) based on the TLC profiles. The fraction 2 was subjected to Sephadex LH-20 (GE Healthcare, Glies, UK) gel column eluting with $CHCl_3$/MeOH (1:1, v/v) to give fraction 2–1. The fraction 2–1 was further separated by semi-preparative HPLC (Agilent 1100, Zorbax SB-C18, 5 μm, 250 × 9.4 mm inner diameter; Agilent, Palo Alto, CA, USA) using CH_3OH/H_2O mixture (9:1, v/v) with the flow rates of 1.5 ml min^{-1} at

[1]Life Science and Biotechnology Research Center, Department of Bioengineering, School of Life Science, Northeast Agricultural University, Harbin, China and [2]Department of New Drug Screening, Zhejiang Hisun Pharmaceutical Co., Ltd., Taizhou, China
Correspondence: Dr J-d Wang, Department of New Drug Screening, Zhejiang Hisun Pharmaceutical Co., Ltd., Taizhou 318000, China.
E-mail: jdwang@hisunpharm.com
or Professor X-j Wang, Life Science and Biotechnology Research Center, Department of Bioengineering, School of Life Science, Northeast Agricultural University, No. 59, Mucai Street, Xiangfang District, Harbin 150030, China.
E-mail: wangneau@163.com
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room temperature to yield compounds **1** (t_R 14.7 min, 6.2 mg) and **2** (t_R 18.3 min, 25.0 mg). ^{1}H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The electrospray ionization mass spectrometry and high resolution electrospray ionization mass spectrometry spectra were taken on a Q-TOF Micro LC–MS–MS mass spectrometer (Waters Co, Milford, MA, USA).

Compound **1** was isolated as pale yellow oil with $[\alpha]_D^{25}$ − 25.8 (c (g per 100 ml) 0.06, EtOH) and UV (EtOH) λ_{max} nm (log ε): 263

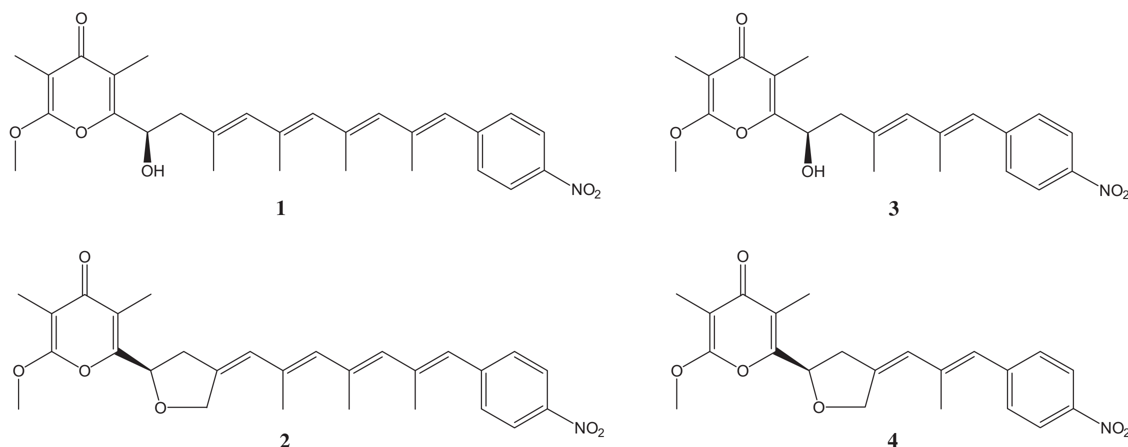

Figure 1 Structures of **1**, **2**, **3** and **4**.

Table 1 ^{1}H and ^{13}C NMR data of compounds 1 and 2

Position	δ_H (J in Hz)		δ_C	
	1[a]	*2*[b]	*1*[a]	*2*[b]
1			162.2 (s)	162.3 (s)
2			99.9 (s)	98.4 (s)
3			180.8 (s)	179.6 (s)
4			119.1 (s)	118.8 (s)
5			156.2 (s)	156.4 (s)
6	4.98 (1H, dd, 8.0, 6.2)	5.20 (1H, dd, 5.9, 5.1)	67.0 (d)	72.8 (d)
7	2.62 (1H, dd, 13.2, 8.0)	3.02 (1H, dd, 15.2, 5.9)	45.9 (t)	38.0 (t)
	2.52 (1H, dd, 13.2, 6.2)	2.90 (1H, brdd, 15.2, 5.1)		
8			131.1 (s)	139.3 (s)
9	5.81 (1H, s)	6.10 (1H, s)	134.5 (d)	126.5 (d)
10			133.7 (s)	134.6 (s)
11	5.81 (1H, s)	5.89 (1H, s)	134.1 (d)	135.3 (d)
12			135.8 (s)	136.2 (s)
13	5.96 (1H, s)	6.02 (1H, s)	133.9 (d)	134.9 (d)
14			139.6 (s)	139.9 (s)
15	6.46 (1H, s)	6.56 (1H, s)	127.9 (d)	128.6 (d)
16			144.8 (s)	144.9 (s)
17	7.44 (1H, d, 8.8)	7.59 (1H, d, 8.2)	129.5 (d)	128.6 (d)
18	8.20 (1H, d, 8.8)	8.20 (1H, d, 8.2)	123.5 (d)	123.9 (d)
19			145.8 (s)	145.8 (s)
1a	4.04 (3H, s)	3.91 (3H, s)	55.5 (q)	56.0 (q)
2a	1.87 (3H, s)	1.69 (3H, s)	6.9 (q)	7.3 (q)
4a	2.00 (3H, s)	1.88 (3H, s)	9.4 (q)	9.5 (q)
8a	1.88 (3H, s)	4.78 (1H, d, 14.4)	18.3 (q)	69.9 (t)
		4.66 (1H, d, 14.3)		
10a	1.93 (3H, s)	1.95 (3H, s)	19.2 (q)	17.9 (q)
12a	2.03 (3H, s)	2.01 (3H, s)	19.5 (q)	20.0 (q)
14a	2.09 (3H, brs)	2.08 (3H, brs)	19.5 (q)	19.8 (q)

[a]Chemical shifts are reported in parts per million (δ), using $CDCl_3$ (δ_H 7.26 p.p.m.; δ_C 77.0 p.p.m.) as an internal standard, with coupling constants (J) in Hz.
[b]Chemical shifts are reported in parts per million (δ), using DMSO-d_6 (δ_H 2.50 p.p.m.; δ_C 39.5 p.p.m.) as an internal standard, with coupling constants (J) in Hz.

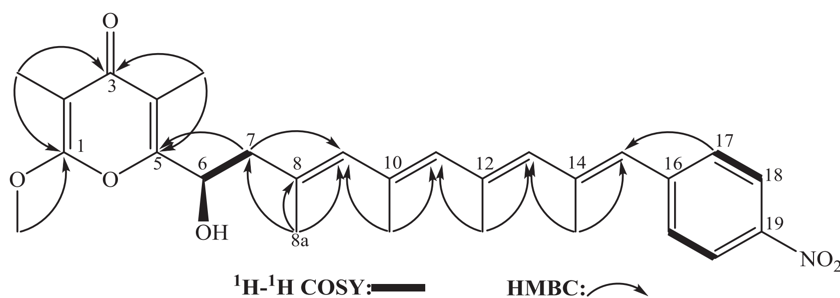

Figure 2 Key ^{1}H–^{1}H COSY and HMBC correlations of **1**.

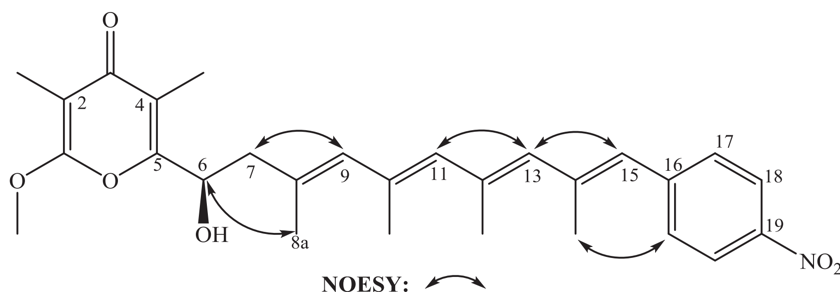

Figure 3 Key NOESY correlations of **1**.

(4.15), 361 (3.98). From high resolution electrospray ionization mass spectrometry measurements in combination with ^{1}H and ^{13}C NMR data (Table 1), the molecular formula of **1** was determined to be $C_{28}H_{33}NO_6$ (found: 480.2357 $[M+H]^+$, calcd: 480.2381). The IR spectrum revealed that **1** possessed a conjugated carbonyl group (1668 cm^{-1}) and a nitro group (1517 and 1341 cm^{-1}). The structure of **1** was mainly determined by NMR spectral analysis as follows.

The ^{1}H NMR spectrum of **1** in $CDCl_3$ clearly showed a 1,4-disubstituted phenyl group at δ 7.44 (2H, d, J=8.8 Hz), 8.21 (2H, d, J=8.8 Hz), 4 olefinic resonances at δ 6.46 (1H, s), 5.96 (1H, s), 5.81 (2H, s), one oxygenated methine proton at δ 4.98 (1H, dd, J=8.0, 6.2 Hz), a methoxy group at δ 4.04 (3H, s), a methylene at δ 2.62 (1H, dd, J=13.2, 8.0 Hz), 2.52 (1H, dd, J=13.2, 6.2 Hz) and six singlet olefinic methyls at δ 2.09, 2.03, 2.00, 1.93, 1.88 and 1.87. The ^{13}C NMR and HSQC spectra revealed a carbonyl carbon at δ_C 180.8, an oxygen-bearing methine at δ_C 67.6, a methoxy group at δ_C 55.5, a methylene carbon at δ_C 45.9, six methyl resonances at δ_C 19.5, 19.5, 19.2, 18.3, 9.4 and 6.9 in additon to 8 sp^2 methines and 10 sp^2 quaternary carbons. The observed HMBC correlations from H_3-2a to C-1, C-2 and C-3, from H_3-4a to C-3, C-4 and C-5, and from H_3-1a to C-1 revealed the presence of a 2-methoxy-3,5-dimethyl-γ-pyrone moiety. By detailed comparison of the ^{1}H and ^{13}C NMR data (Table 1) of **1** with those of spectinabilin (**2**) suggested that **1** was similar to spectinabilin. The differences between **1** and spectinabilin were that the furan ring in **2** was absent in **1** and a hydroxy and a methyl groups were substituted at C-6 and C-7 respectively, in **1**. The ^{1}H–^{1}H COSY correlation (Figure 2) of δ_H 4.98 and δ_H 2.62, 2.52, and the observed HMBC correlations (Figure 2) from C-8a methyl group (δ_H 1.88) to δ_C 45.9 (C-7), 131.1 (C-8), 134.5 (C-9) (Figure 1) supported the assignment. Consequently, the planar structure of **1** was established. The geometry of the four trisubstituted double bonds from C-8 to C-15 were elucidated as all *E* on the basis of the NOESY correlations (Figure 3) of H-6/H-8a, H-7/H-9, H-11/H-13, H-13/H-15, H-14a/H-17 and by the analogy to spectinabilin (**2**).[6]

Furthermore, the structure of **1** was very similar to that of an aureothin derivative (**3**, Figure 1) reported in the literature[7] except of two additional couples of olefinic and methyl structural fragments in **1**. By comparison of the optical values of compounds **1** $\left([\alpha]_D^{25} - 25.8\right)$ and **3** $\left([\alpha]_D^{22} - 23\right)$, the stereochemistry of C-6 in **1** was also assigned as 6*R*.[8]

Compound **2** was also obtained as pale yellow oil. Its structure was elucidated as spectinabilin by analysis of its spectroscopic data and comparison with literature values.[6]

The cytotoxicity of compounds **1** and **2** were assayed for growth-inhibition activity *in vitro* against three human tumor cell lines, A549, HCT-116 and HepG2 using CCK8 method as described in our previous papers.[9,10] As a result, **1** exhibited good cytotoxic activities with IC_{50} values of 3.0, 5.8 and 3.7 ug ml^{-1}, respectively, while the values of **2** were 34.3, 47.0 and 37.2 ug ml^{-1}.

It was reported that aureothin (**4**, Figure 1), an analog of **2** with a shorter carbon chain, has been shown that the tetrahydrofuran moiety is biosynthesized in two steps, hydroxylation followed by cyclization.[6,11,12] The aureothin derivative (**3**) is a biosynthetic intermediate of aureothin. Corresponding to compounds **3** and **4**, compound **1** may be a biosynthetic intermediate en route to spectinabilin (**2**).

Structure-activity relationships for related aureothin and spectinabilin/neoaureothin derivatives have been investigated.[13,14] The results showed that the size of the polypropionate backbone has a clear impact on the biological activities of these pyrone natural products and the longest congener exhibits enhanced cytostatic effects, whereas antifungal activity increases dramatically with shorter chain lengths in comparison with polypropionate homologs.[14] In our research, compound **1** exhibited much better cytostatic effects than spectinabilin (**2**), thus, compound **1** is not only a biosynthetic intermediate of **2**, but also a potential lead for further exploration.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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