SHORT REPORT

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Two New Germicidins from the Endophytic *Streptomyces* sp. A00122 of *Camptotheca acuminata*

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Abstract: Two new germicidins, namely, germicidin D (**1**) and germicidin E (**2**), together with two known ones germicidin (**3**) and acetamide (**4**) were isolated from the endophytic *Streptomyces* sp. A00122 of *Camptotheca acuminata*. The structures of the isolated compounds were elucidated by spectroscopic analyses, including 1D- and 2D-NMR experiments HR-Q-TOF mass spectrometry. Antimicrobial assays with compounds **1** and **2** were carried out; they had shown no effect on the growth of tested bacteria or yeasts.

Keywords: Germicidin; germicidin D; germicidin E; spectroscopic analysis; antimicrobial activity.

1. Microbe Source

Camptotheca acuminata is a plant native to mainland China, and produces a promising antineoplastic agent camptothecin (CPT) [1]. As the endophytes may play important roles in the synthesis or transformation of these bioactive compounds [2], we embarked on the isolation of endophytic microorganisms from *Camptotheca acuminata* and the search for novel bioactive compounds from them [3,4].

The strain A00122 was isolated from roots of *Camptotheca acuminata* collected from Youxi Beizhai Nature Conservation Area. The 16S rDNA sequencing (GenBank accession number: EU009996) was performed to characterize it as a *Streptomyces* species.

2. Previous Studies

Endophytic microorganisms from pharmaceutical plants have been widely recognized as an important source of novel bioactive natural products, such as antibiotics, antitumor and anti-infection agents, and plant growth regulators8 [5,8].

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3. Present Study

The spores of A00122 grown on YMG plates were used to inoculate YMG agar medium (yeast extract 5 g/L, malt extract 10 g/L, glucose 5 g/L, agar 15 g/L, pH 7.2, 20 mL/plate), and the plates were incubated at 28 °C. The fermentations were performed twice. During the first time, 8 L were cultivated for 11 days, and the second time, 5 L for 7 days. The cultures were diced and extracted with EtOAc/MeOH/AcOH (80: 15: 5), and then partitioned between EtOAc and double distilled H_2O, respectively, to afford *A1* (1.2 g, EtOAc extract of 8 L fermentation) and *A2* (0.65 g, EtOAc extract of 5 L fermentation).

A1 (1.2 g) was subjected to MPLC (*RP-18* (80 g); gradient aq. acetone (30, 50 and 70%, resp. 1.5 L/each)) to afford *A1A-A1H*. *A1B* (170 mg) was subjected to column chromatography (CC) (*Sephadex LH-20* (140 g); MeOH) to produce two fractions *A1B1* and *A1B2*. *A1B2* (30 mg) was subjected to MPLC (*RP-18* (45 g); acetone/ddH_2O, 1 : 9) to afford fractions *A1B2a* and *A1B2b*. *A1B2a* (15 mg) was subjected to MPLC (*RP-18* (45 g); acetone/ddH_2O, 1 : 4), and further purified by CC (SiO_2; petroleum ether/ethyl acetate, 4 : 1; 7 : 2) to obtain **4** (12 mg). *A1B2b* (7 mg) was purified by CC (SiO_2; petroleum ether/ethyl acetate, 9 : 1; 8 : 1) to obtain **3** (3 mg).

A2 (0.65 g) was subjected to MPLC (*RP-18* (80 g); gradient aq. acetone (30, 50 and 70%, resp. 1 L/each)) to afford *A2A-A2D*. *A2B* (80 mg) was purified by CC (*Sephadex LH-20* (45 g); MeOH), and subjected to MPLC (*RP-18* (30 g); acetone/ddH_2O, 1 : 3) to afford fractions *A2B1* and *A2B2*. *A2B1* (10 mg) was crystallized in acetone/ddH_2O solution at r.t. to obtain **2** (4 mg). *A2B2* (12 mg) was subjected to MPLC (*RP-18* (30 g); acetone/ddH_2O, 1 : 3) to obtain **1** (9 mg).

The chemical structures of the isolated compounds **1 - 4** were elucidated by analysis of 1D- and 2D-NMR data and HR-Q-TOF mass spectrometry. Germicidin D (**1**) and germicidin E (**2**) are new germicidin derivatives, while germicidin (**3**)[9] and N-acetamide (**4**) are known ones.

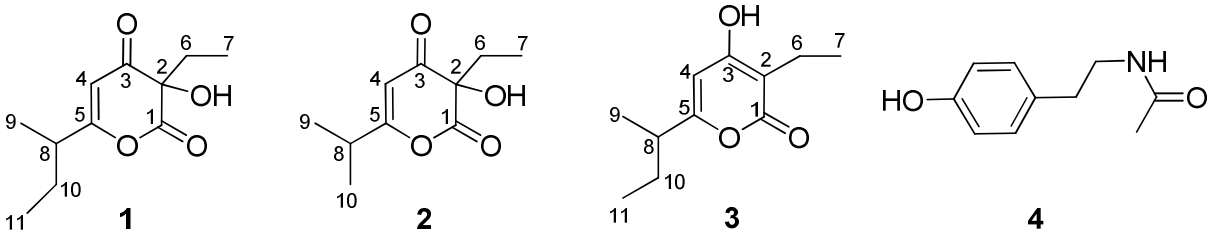

Figure 1. The chemical structures of compounds **1 - 4**.

Compound **1** was isolated as a white powder. The molecular formula of **1** was determined to be $C_{11}H_{16}O_4$ by its HR-Q-TOF-MS (*m/z* 235.1064 $[M + Na]^+$) and NMR data (Table 1). $[\alpha]^{20}_D$: + 3.40^o (*c* 0.50, $CDCl_3$). The IR spectrum showed the absorptions for carbonyl groups (1745 cm^{-1}). The ^{13}C-NMR and DEPT spectra of **1** exhibited 11 carbon signals corresponding to three CH_3, two CH_2, two CH, as well as four quaternary carbon atoms. Inspection of HSQC, HMBC, and ^{1}H,^{1}H-COSY experiments readily revealed a germicidin-type structure of **1** [9]. The HMBC correlations from Me(7) to C(2) and C(6), from Me(9) to C(5), C(8) and C(10), from Me(11) to C(8) and C(10), from H_2-C(6) to C(1) and C(3), from H-C(4) to C(2) and C(5), along with the ^{1}H,^{1}H-COSYs H-C(6)↔Me(7), Me(9)↔H-C(8)↔H_2-C(10) ↔Me(11), led to the establishment of the structure of **1**. (Figures 1 and 2). Thus, compound **1** was determined to be 6-sec-butyl-3-ethyl-3-hydroxy-2H-pyran-2,4(3H)-dione, named as germicidin D (Figure 1).

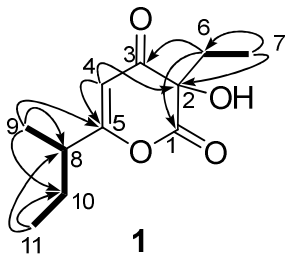

Figure 2. ^{1}H-^{1}H COSY correlations (—) and selected HMBC (H→C) correlations of **1**.

Compound **2** was isolated as colorless needle crystals. The molecular formula of **2** was determined to be $C_{10}H_{14}O_4$ by its HR-Q-TOF-MS (*m/z* 221.1073 $[M + Na]^+$) and NMR data (Table 1). $[\alpha]^{20}_D$: - 2.73^o (*c* 0.11, $CDCl_3$). The IR spectrum showed the absorption bands for carbonyl groups (1744cm^{-1}). The structure of **2** was established by comparison of its NMR data with those of **1**. The spectroscopic data of both compounds were similar, except for a 2-propyl group instead of a 2-butyl chain was bonded to C(5) in compound **1,** which was confirmed by the HMBC correlations from Me(9) and Me(10) to C(5) and C(8), and the ^{1}H,^{1}H-COSYs Me(9)↔H-C(8)↔Me(10). Thus, compound **2** was determined to be 3-ethyl-3-hydroxy-6-isopropyl-2H-pyran-2,4(3H)-dione, named as germicidin E.

Table 1. ^{1}H- and ^{13}C- NMR Data of **1** and **2** (150 and 600 MHz, resp., in $CDCl_3$), δ in ppm, *J* in Hz.

Position	**1**		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	-	167.8 (*s*)	-	167.6 (*s*)
2	-	91.5 (*s*)	-	91.6 (*s*)
3	-	191.7 (*s*)	-	191.6 (*s*)
4	5.72 (*s*)	104.7 (*d*)	5.72 (*s*)	103.3 (*d*)
5	-	175.5 (*s*)	-	176.4 (*s*)
6	2.01 (*q*, *J* = 7.5)	30.7 (*t*)	2.02 (*q*, *J* = 7.5)	30.7 (*t*)
7	1.02 (*t*, *J* = 7.5)	7.2 (*q*)	1.00 (*t*, *J* = 7.5)	7.2 (*q*)
8	2.45 (*m*)	40.1 (*d*)	2.19 (*septet*, *J* = 6.8)	33.0 (*d*)
9	1.24 (*dd*, *J* = 6.9, 2.2)	17.1 (*q*)	1.26 (*d*, *J* = 6.8)	19.2(*q*)
10	1.58 (*m*) 1.74 (*m*)	26.5 (*t*)	1.25 (*d*, *J* = 6.8)	19.3 (*q*)
11	0.94 (*t*, *J* = 7.5)	11.4 (*q*)	-	-

The antimicrobial activities of **1** and **2** were tested against bacteria (*Escherichia coli* (CMCC (B) 44103), *Bacillus subtilis* (CMCC (B) 63501), *Bacillus pumilus* (CMCC (B) 63202) and *Staphylococcus aureus* (CMCC (B) 26003)), and yeast (*Candida albicaus* (AS 2.538)) using Oxford plate assay system. The results showed that compounds **1** and **2** had no effects on the growth of tested bacteria or yeast at a concentration of 1mg/ml with a loading volume of 20 μl.

Germicidin **3** was first isolated from the submerged culture of *Streptomyces viridochromogenes* NRRL B-1551, has an inhibitory effect on the germination of its own arthrospores at a concentration as low as 200 pM [9]. The germination inhibition activities of **1** and **2** need to be further investigated.

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Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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