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One New Ten-membered Lactone from *Phomopsis* sp. B27, an Endophytic Fungus of *Annona squamosa* L.

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【**ABSTRACT**】 **AIM:** To study the chemical constituents of *Phomopsis* sp. B27, an endophytic fungal strain of *Annona squamosa* L. **METHODS:** The extract was isolated and purified by column chromatography. The structures of chemical components were determined by spectroscopic analyses including 1D-, 2D-NMR and MS data. **RESULTS:** Five compounds were isolated and determined as a ten-membered lactone, namely phomolide C (**1**), 1-methoxy-8-hydroxy-9,10-anthraquinone (**2**), 1,8-dihydroxy-9,10-anthra- quinone (1,8-DHA) (**3**), cytosporone C (**4**) and altiloxin A (**5**). **CONCLUSION**: Compound **1** is a new ten-membered lactone; and compounds **1-5** were isolated from the genus *Phomopsis* for the first time.

【**KEY WORDS**】 *Phomopsis* sp. B27; Chemical constituent; Spectroscopic analyses

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1 Introduction

Endophytic fungi can be found virtually in all terrestrial plants [1] and are viewed as an outstanding source of bioactive natural products because there are so many of them occupying literally millions of unique biological niches (higher plants) growing in so many unusual environments[2]. Recently, increasing attention has been paid to plant commensal microorganisms as a source of new bioactive substances[3-5].

During the course of our searching for new bioactive microbial metabolites, five compounds, namely phomolide C, a ten-membered lactone (**1**), 1-methoxy-8-hydroxy-9,10-anthraquinone (**2**), 1,8-dihydroxy-9,10-anthra-quinone (1,8-DHA) (**3**), cytosporone C (**4**) and Altiloxin A (**5**), were isolated and identified from the endophytic fungus *Phomopsis* sp. B27 isolated from the roots of *Annona squamosa* L. collected in Xiamen, Fujian Province. Herein, we report the isolation, structure elucidation, and antitumor and antibacterial activities of these compounds.

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2 Apparatus and Material

2.1 *Apparatus and Material*

Column chromatography: Qingdao silica gel (200-300 mesh); Merck silica gel 60 RP-18; Sephadex LH-20: Pharmacia products; TLC: Qingdao precoated plates, silica GF_{254} plates; NMR Spectra: Bruker DRX-600 spectrometer with TMS as internal standard; ESI-MS: Thermo-Finnigen LCQ-Advantage spectrometer. Optical rotations were obtained on a Perkin-Elmer 341 polarimeter with MeOH as solvent. The IR spectra were measured in KBr on a Nicolet FT-IR 360.

2.2 *Culture Conditions and Extraction*

The strain B27 was cultured on PDA plates (10 L) at 28℃ for 14 days. The cultured was chopped and extracted with EtOAc-MeOH-AcOH (80 : 15 : 5, *V/V/V*, 10 L) at room temperature overnight for three times. The extracts were collected through filtration and concentrated under vacuum to remove organic solvents to produce an aqueous solution. The aqueous solution was extracted three times with petroleum ether (PE) and acetyl acetate (EtOAc) to afford PE extract (1.2 g) and EtOAc extract (6.0 g), respectively.

2.3 *Isolation*

The PE extract (1.2 g) was subjected to flash column chromatography over reversed-phase C-18 silica gel (30 g) eluted with a stepwise MeOH-water (30%; 50%; 70%; 100%; *V/V*, 400 mL for each gradient). Four fractions PE-A-D were collected. PE-C (150 mg) was subjected to column chromatography over normal phase silica gel (6.0 g) and eluted with

PE-EtOAc (20 : 1, *V*/*V*) to yield **2** (6 mg), and eluted with methanol to afford a wash-off PE-C-Me (88 mg). PE-C-Me was subjected to column chromatography over Sephadex LH-20 (40 g) eluted with methanol, and further isolated by repeated column chromatography over normal phase silica gel (2.5 g) eluted with $CHCl_3$-MeOH (50 : 1, *V*/*V*) and normal phase silica gel (0.5 g) eluted with PE-EtOAc (3 : 1, *V*/*V*) to yield **1** (7 mg).

The EtOAc extract (6.0 g) was subjected to flash column chromatography over reversed-phase C-18 silica gel (180 g) eluted with a stepwise MeOH-water (30%; 50%; 70%; 100%; *V*/*V*, 2 L for each gradient) and collected as 200 mL/fraction. The collections were combined according to the TLC results to 10 fractions EA-A-J.

EA-G (385 mg) was chromatographed over normal phase silica gel (6.0 g) and eluted with $CHCl_3$-MeOH (15 : 1, *V*/*V*) to yield fraction EA-G-4. EA-G-4 (101 mg) was purified by repeated column chromatography over normal phase silica gel (5.0 g) eluted with PE-EtOAc (5 : 1, *V*/*V*), and normal phase silica gel (0.8 g) eluted with chloroform-methanol (100 : 1, *V*/*V*) and normal-phase silica gel (0.3 g) eluted with chloroform-methanol (200 : 1, *V*/*V*) to yield **5** (2 mg).

EA-H (1.8 g) was subjected to column chromatography over Sephadex LH-20 (140 g) eluted with methanol to afford 10 fractions according to the TLC results. Fraction 1 (10 mg) was chromatographed over normal phase silica gel (0.5 g) and eluted with PE-EtOAc (6 : 1, *V*/*V*) to yield **4** (4 mg).

EA-I (2.08 g) was subjected to column chromatography over Sephadex LH-20 (140 g) eluted with methanol to afford 12 fractions according to the TLC results. Fraction 2 (270 mg) was chromatographed over normal phase silica gel (6.0 g) and eluted with PE-EtOAc (5 : 1, *V*/*V*) to yield **3** (35 mg).

3 Results

3.1 *Structure elucidation and identification*

Compound 1 $[\alpha]_D^{20}$ −27.5 (*c* 10.0, MeOH), was obtained as white powder. Its molecular formula was determined to be $C_{18}H_{30}O_6$ by positive HR-Q-TOF-ESI-MS (*m/z* 365.269 4 $[M + Na]^+$, 381.245 5 $[M + K]^+$).

The ^{13}C-NMR (DEPT) spectra of **1** showed 18 carbon signals for one methyl, nine methylenes, six methines and two quaternary carbons including one carbonyl at δ 175.6 (C-1) (Table 1). The ^{1}H NMR spectrum showed a methyl triplet at δ 0.82 (*t*, *J* = 6.6 Hz), four oxy protons at δ 3.56, 4.31, 4.73 and 3.87, and two olefinic protons at δ 5.50 and 5.75. Addi-

Table 1 The NMR data of Compound 1 in MeOD (δ, *J* in Hz)

No.	^{1}H	^{13}C	HMBC	^{1}H-^{1}H COSY
1		175.6s		
2	2.36 (1H, dd/q, 10.8) 2.03 (1H, q,11.4)	32.4t	C-3, C-4, C-1	H-3
3	2.30(1H, q, 11.4) 1.56(1H, t, 12.0)	26.6t	C-2, C-5, C-4, C-1	H-2
4	3.56(1H, d, 10.8)	75.5d		H-3, H-5
5	4.31(1H, s)	72.3d	C-3, C-4, C-7, C-6	H-4, H-6
6	5.75(1H, d,15.6)	132.3d	C-5, C-8, C-7	H-5, H-7
7	5.50(1H, d,15.0)	130.6d	C-5, C-8, C-6	H-6
8		96.1s		
9	4.73(1H, s)	70.8d	C-12, C-1	H-11
11	2.10(1H, q,13.8) 1.69(1H, d,13.8)	22.9t	C-12	H-11
12	1.45(1H, m) 1.34(1H, m)	24.9t	C-11, C-15, C-14, C-13 C-16	H-13
13	3.87(1H, s)	69.3d	C-12, C-14	H-14, H-12
14	1.44(1H, m) 1.33(1H, m)	35.8t	C-17, C-12/C-15, C-16, C-13	H-13
15	1.32(1H, m) 1.22(1H, m)	25.0t	C-17 C-18	
16	1.24(2H, m)	29.1t	C-18	
17	1.25(2H, m)	22.3t		
18	1.21(2H, m)	31.6t	C-17, C-16	H-19
19	0.82(3H, t, 6.6)	13.0q	C-17, C-18	H-18

tionally, tionally, the ^{1}H NMR spectrum showed overlapped proton signals at δ 1.21-1.45 for seven methylenes and a broad triplet at δ 0.91 for a terminal methyl, suggesting the presence of an nonane moiety (from C-11 to C-19) which was supported by DEPT spectra and HMBC correlations. The ^{1}H-^{1}H-COSY spectra of **1** demonstrated the connectivities from C-2 to C-7. The HMBC correlations from H-9 to C-1 (δ 175.6) and the relatively lower-field resonance of H-9 (at δ 4.73) suggested that **1** was a 10-membered lactone. Three degrees of unsaturation attributed to carbonyl, carbon-carbon double band and the ring of lactone. The $J_{H\text{-}6/H\text{-}7}$ value 15.6 Hz revealed a *trans*- configuration of the olefinic protons. The downshift of C-4 (δ 75.5), C-5 (δ 72.3), C-8 (δ 96.1) and C-13 (δ 69.3) revealed the presence of four more oxy carbons besides C-9. However, the acetylation of **1** revealed the presence of only three free hydroxyl groups, suggesting an epoxy ring. The epoxide moiety at C-8 and C-13 was evident from the upfield shifts of C-8 and C-13, and further supported by the yield of 4,5,8-triacetyl product in acetylation. Therefore, the remaining one degree of unsaturation was assigned to the six- membered epoxy ring. Therefore, compound **1** was elucidated as (*E*)-2-hexyl-9,10,12*a*-trihydroxy-2,3,4,4*a*,7,8,9,10-octahydropyrano[3,2-*b*]oxecin-6(12*aH*)-one. However, the relative configuration of **1** remained to be unresolved due to insufficient NOE correlations. The structures of **2**–**5** were elucidated by comparison of the spectral data (^{1}H NMR and ^{13}C NMR) with those reported to be 1-methoxy-8-hydroxy-9,10-anthraquinone(**2**)[6-7], 1,8- dihydroxy-9,10-anthraquinone(1,8-DHA) (**3**)[8], cytosporone C (**4**)[9] and Altiloxin A (**5**)[10], respectively (Fig. 1).

3.2 *Bioactivities of compounds* **1-5**

The cytotoxic activity of compounds **1-5** were measured by MTT method [11] in HepG2 and HeLa cell lines. The antimicrobial activities of **1-5** were measured by disk diffusion method[12], using *Bacillus subtilis* CMCC63501 *Escherichia coli* CMCC44103, *Staphylococcus aureus* ATCC9763, *Candida albicans* AS2.538 as indicator organisms. The results showed that **1-5** displayed no antitumor activities at 10 μg·mL^{-1} and displayed no antimicrobial activity at 50 μg/disk.

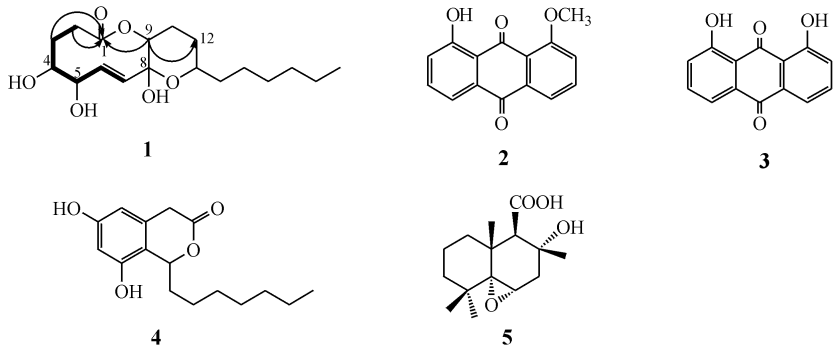

Fig. 1 The selected ^{1}H-^{1}H COSY( ) and HMBC correlation ( ) for 1 and the structure of compounds 1 – 5

4 Discussion

The genus *Phomopsis* is a rich source of biologically active metabolites including antimicrotubule phomopsidin[13], antimalarial and antitubercular phomoxanthones[14], antifungal phomoxanthone A[15] and phomodiol[16] and phomolide A,B[17] and the plant growth regulator cytochalasin H[18].

From the extracts of the culture of the strain *Phomopsis* sp. B27, one new (**1**) and four known (**2-5**) compounds were isolated. These five compounds belong to four types of structures including ten-membered lactone (**1**), anthraquinones (**2-3**), octaketide (**4**) and sesquiterpene (**5**). They were isolated from the genus *Phomopsis* for the first time.

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番荔枝内生菌拟茎点霉 B27 中分离到的一个新十元大环内酯

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【摘　要】 **目的：**研究番荔枝内生真菌拟茎点霉 B27 的化学成分。**方法：**通过色谱层析柱对提取物进行分离纯化, 并通过波谱解析(一维、二维的核磁共振谱和质谱)确定化合物的结构。**结果：**分离纯化得到 5 个化合物, 鉴定为十元大环内酯 phomolide C (1), 1-甲基-8-羟基-9,10-蒽醌 (2), 1,8-双羟基-9,10-蒽醌 (3), cytosporone C (4) 和 Altiloxin A (5)。**结论：**化合物 **1** 是新化合物; 化合物 **1 - 5** 首次从拟茎点霉属菌株中分离得到。

【关键词】 拟茎点霉 B27; 化学成分; 波谱解析

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