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BRIEF COMMUNICATION

Spiciferone analogs from an endophytic fungus *Phoma betae* collected from desert plants in West China

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Abstract

Endophytic fungi from desert, arid, and grassland areas are an ecologically important but unique group with poor chemical investigation. During our ongoing study to mine bioactive secondary metabolites from unique fungal environments, a new shunt product spiciferone F (**1**) including two new analogs spiciferones G (**2**) and H (**3**) together with four known ones spiciferone A (**4**), spiciferol A (**5**), **6**, and **7** were isolated from endophytic fungus *Phoma betae* inhabiting in plant *Kalidium foliatum* (Pall.) Moq from Ningxia Province of West China. The planar, relative, and absolute configurations of these new compounds were elucidated by nuclear magnetic resonance, high-resolution electrospray ionization mass spectrometry, and electronic circular dichroism experiments. According to the shunt products, intermediates and analogs isolated from this endophytic fungus, the possible biosynthetic pathway of spiciferones was reconstructed. Compounds **1**–**7** were evaluated cytotoxic activities against three cancer cell lines HCT 116, HeLa, and MCF7, and only did **1** display strong biological effect against MCF7 with a half-maximal inhibitory concentration value at 7.73 ± 0.11 μM compared with the *cis*-platinum (14.32 ± 1.01 μM).

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During the long period of evolution, the diversities of fungi (except insects) are far more than those of other organisms. Flooded in every corner over the world, to compete with other competitors for limited space and nutrition or to prevent being killed by other predators, fungi evolve different survival strategies, one of which is chemical defense to produce diverse and bioactive secondary metabolites (SMs) to adapt to abiotic/biotic factors of the environment. Now SMs from fungal origin have been the vital sources of agricultural chemicals and pharmaceutical molecules. Endophytic fungi from desert, arid, and grassland areas are an ecologically important but unique group with poorl chemical investigation. During our ongoing study to mine bioactive SMs from unique fungal environments [1–4], chemical investigation of endophytes inhabiting desert, arid, and grassland plants of West China was initiated in our lab [5, 6]. Chemical investigation of an endophytic fungus *Phoma betae* (number: AL-5-1) led to obtaining a series of spiciferone analogs including three new analogs spiciferone F–H (**1–3**) together with four known ones spiciferone A (**4**) [7], spiciferol A (**5**) [8], **6**[9, 10], and **7** [8] (Fig. 1). In this report, the isolation, structural elucidation, and biological activities of compounds **1–7** were present.

The molecular formula of **1** was determined to be $C_{14}H_{16}O_2$ on the basis of high-resolution electrospray

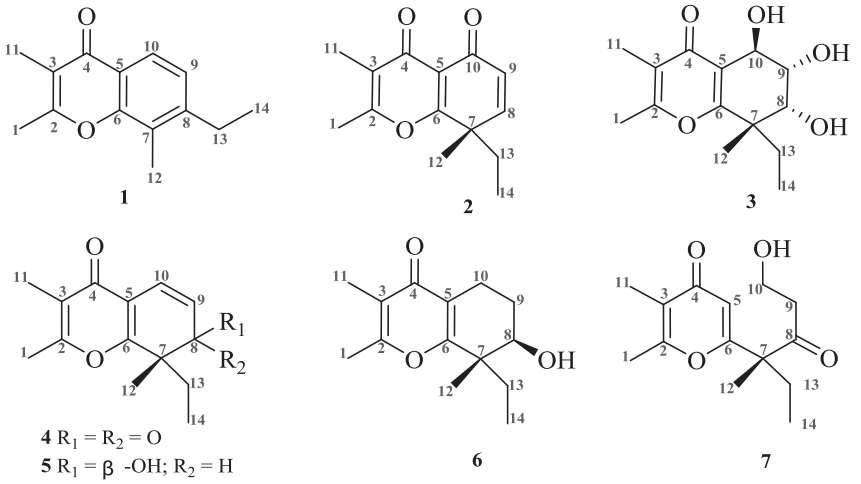

Fig. 1 The structures of the compounds **1**–**7**

ionization mass spectrometry (HRESIMS) (*m/z* 217.1225 $[M + H]^+$; calcd. *m/z* 217.1229) with seven degrees of unsaturation. The ultraviolet (UV) (231 nm or so) of **1** suggested the existence of α, β-unsaturated carbonyl group, and infrared (IR) spectra (2930–2950, 1719, 1643, and 1575) of **1** implied the existence of aliphatic chain, carbonyl groups, and aromatic ring. The ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra together with heteronuclear multiple bond correlation (HMBC) spectra of **1** (Table 1) revealed the presence of a carbonyl carbon, eight olefinic carbons (two of which are protonated), one methylene unit, four methyls in structure **1**, which accounted for all the NMR resonances, and implied two rings found in structure **1**. Analysis of coupling constant ($J = 7.5$ Hz) in ^{1}H NMR spectrum revealed a ethyl present in **1**, which was supported by ^{1}H H-COSY correlations (Fig. 2). The remaining connectivities was determined on the basis of HMBC correlations (Fig. 2). The correlations from 13-CH_2- to C-7, C-8, and C-9, from 14-Me to C-8 and C-13, from 12-Me to C-6, C-7, and C-8 put a methyl and an ethyl on C-7 and C-8, respectively. The key cross-points from H-10 to C-5, C-6, C-8, and C-9, from H-9 to C-5, C-7, and C-13 in the HMBC spectra established a *tetra*-substituted phenyl ring with two coupled aromatic protons at C-9 and C-10, respectively. The HMBC correlation between H-9 and H-10 to C-4 confirmed that the carbonyl group (C-4) was connected with C-5. The key correlations from 1-Me to C-2 and C-3, from 11-Me to C-2, C-3, and C-4 established a fragment corresponding to C-1–C-2–C-3(C-11)–C-4. Considering the chemical shift values of C-2 and C-6 together with only an oxygen atom left in the molecular formula, an ether bond must be formed between C-2 and C-6 (Fig. 2). Thus, the structure of **1** was determined.

The molecular formula of **2** was determined to be $C_{14}H_{16}O_3$ on the basis of HRESIMS (*m/z* 255.0995 $[M + Na]^+$; calcd. *m/z* 255.0997). The UV (225 nm or so) of **2** suggested the existence of α, β-unsaturated carbonyl group, and IR spectra (2931–2970, 1678, 1638, and 1616) of **2** implied the existence of aliphatic chain, α, β-unsaturated carbonyl group and aromatic ring. The NMR data of **2** especially the two-dimensional (2D) NMR spectra revealed the same fragments including the 2,3-dimethyl-4*H*-pyran-4-one and the quaternary carbon (C-7) bearing a methyl and a ethyl (C-13 and C-14) as those found in compounds **4**–**7** [7–12]. Analysis of the ^{13}C NMR spectra of **2** and **4** revealed that the chemical shift value of C-6 was down-fielded to δ_c 177.4 in **2** compared with δ_c 170.8 ppm in **4**. In addition, the chemical shift value of C-8 (200.9 ppm) in **4** was up-fielded to be δ_c 183.5 ppm in **2**. This implied that the carbonyl group at C-8 in **4** might be transferred to be at C-10 in **2**, which formed two pairs of α, β-unsaturated carbonyl group units (Fig. 1) leading to the chemical shift value of C-6 to be more down-field. Additionally, due to the fact that the carbonyl group (C-10) was connected with two double bonds (C-5/C-6 and C-8/C-9), this made the chemical shift value of C-10 in **2** to be more up-field (δ_c 183.5 ppm). These hypotheses were further confirmed by the ^{1}H H-COSY and HMBC correlations (Fig. 2). Thus, the planar structure of **2** was determined. Considering the same biosynthesis and same rotation between **2** and **4**, the absolute configuration of C-7 was suggested to be *R* [9].

The HRESIMS (*m/z* 291.1202 $[M + Na]^+$; calcd. *m/z* 291.1208) of compound **3** gave its molecular formula as $C_{14}H_{20}O_5$ with five degrees of unsaturation. The UV (255 nm or so) of **3** suggested the existence of α, β-unsaturated carbonyl group, and IR spectra (3413, 2850–2934, 1657, and 1589) of **3** implied the existence of free hydroxyls, aliphatic chain, α, β-unsaturated carbonyl group, and aromatic ring. The NMR spectra of **3** revealed the same fragments of (2, 3-dimethyl-4*H*-pyran-4-one and quaternary

Table 1 ^{1}H NMR and ^{13}C NMR data of compounds **1–3** and **6** recorded at 500 and 125 MHz, respectively

Pos.	**1**[a]		**2**[a]		**3**[a]		**6**[a]	
	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)
1	10.8	2.39, s	17.5	2.33, s	17.7	2.30, s	17.7	2.26, s
2	161.4		158.9		162.3		160.8	
3	116.2		123.3		120.0		118.8	
4	178.4		175.1		180.7		179.1	
5	120.5		117.6		116.8		118.2	
6	154.4		177.4		166.6		165.8	
7	123.9		43.5		43.0		42.4	
8	147.8		148.2	6.50, d, (10.0)	73.5	3.92, d, (2.0)	72.9	3.82, t, (5.0)
9	124.8	7.18, d, (7.5)	129.9	6.34, d, (10.0)	72.3	4.02, dd, (2.0, 8.0)	25.3	1.90, m 1.88, m
10	122.8	7.98, d, (7.5)	183.5		69.4	4.93, d, (7.0)	17.7	2.59, dt, (7.0, 17.5) 2.51, dt, (7.0, 17.5)
11	10.0	2.06, s	9.8	1.96, s	9.4	1.92, s	9.7	1.92, s
12	18.5	2.44, s	25.5	1.46, s	23.1	1.28, s	22.5	1.25, s
13	26.9	2.75, q, (7.5)	32.8	2.08, m1.72, m	26.4	1.88, m1.81, m	26.6	1.78, q, (6.0)
14	14.5	1.24, t, (7.5)	9.2	0.69, t, (7.5)	7.5	1.02, t, (7.5)	8.8	0.98, t, (7.5)

NMR nuclear magnetic resonance

[a]Recorded in $CDCl_3$.

carbon (C-7) connected with the 12-methyl and the ethyl (C-13 and C-14)) as those found in its analogs. Considering the molecular formula, degrees of unsaturation, and chemical shift values, it indicated that compound **3** must possess three free hydroxyl groups at C-8, C-9, and C-10, respectively. This was supported by the observation of three oxymethine units with chemical shift values at δ_c 73.5, 72.3, and 69.4 ppm in the ^{13}C NMR spectrum, and three mutual-coupling protons at δ_H 4.93 (d, 8.0 Hz), 4.02 (dd, 8.0, 2.0 Hz), and 3.92 (d, 2.0 Hz) in the ^{1}H NMR spectrum, respectively, which was further enhanced by ^{1}H H-COSY correlations (Fig. 2). Finally, the planar structure of **3** was determined by HMBC correlations (Fig. 2). The relative configuration was established by analysis of the coupling constants and NOESY correlations. The small coupling constant between H-8 and H-9 ($J = 2.0$ Hz) implied their *cis*-configuration, whereas the big coupling constant between H-9 and H-10 ($J = 8.0$ Hz) suggested the *trans*-configuration of these two protons. The NOESY correlations from H-8 to 12-Me put these protons on the same side of the cyclohexene ring. Thus, the relative configuration of **3** was determined. On account of same biosynthesis of **2–7**, the stereochemistries of C-8, C-9, and C-10 were postulated to be *R*, *R*, and *R*, respectively.

To further determine the absolute configurations of **2** and **3**, electronic circular dichroism (ECD) approach combined with quantum-chemical calculations adopting time-dependent density functional theory was used to determine the absolute configuration of **2** and **3**. The calculated ECD spectrum of the (7*S*)-isomer of (**2**) was red-shifted 1 nm according to the UV correction and matched well with the experimental ECD spectrum of **2**. Thus, the stereochemistry at C-7 of **2** was determined to be *S*. In the same approach, the calculated ECD spectrum of the (7*S*, 8*R*, 9*R*, and 10*R*)-isomer (**3**) was red-shifted **12** nm according to the UV correction and matched well with the experimental ECD spectrum of **3**. Thus, the stereochemistry of **3** was determined, which was consistent with their biogenetic origin. In addition, the absolute configuration of **4** was first established by ECD methods same as the results suggested by X-ray analysis and modified Mosher's experiments [10] (Figure S1).

Compound **6** was an artifact ever semi-synthesized from spiciferone A by reduction, whereas the ^{13}C NMR data of this compound was not provided [9, 10]. The structure **6** was first isolated naturally and then elucidated based on HRESIMS and NMR experiments, and its NMR data was given in Table 1.

According to the isotope-labeled experimental results [10] together with intermediates or shunt products isolated from this endophytic fungus *P. betae*, the biosynthesis of spiciferones was reconstructed in this note, which, we think, expands what Nakajima et al. [10] ever suggested. The PKSs first biosynthesizes a 10-membered monocylic intermediate (**8**), and then a key retro-Aldol reaction transforms

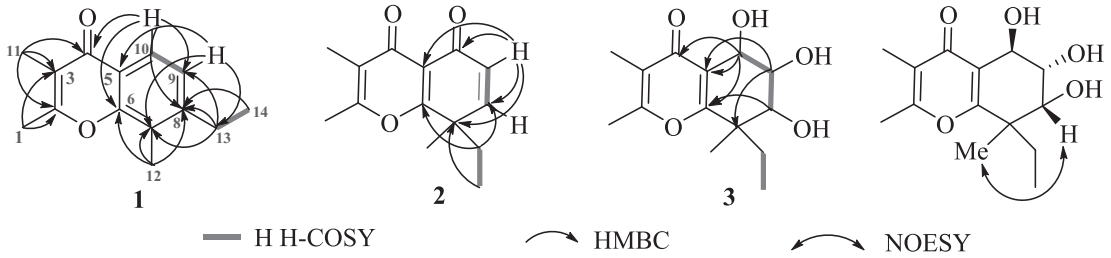

Fig. 2 Key 2D correlations of compounds **1–3**

8 to the key intermediate (**9**). The monocyclic spiciferone congener featuring a butoxyl side chain (**7**) is formed from **9** under reduction and dehydration reactions. Compound **9** shapes another vital intermediate **10**, from which compounds **1–6** are biosynthesized by different dehydration, oxidization, reduction, and rearrangement (Figure S2).

Spiciferones are a small member of phytotoxins which cause blotchy spots on cotyledons of wheat, and the structure–activity relationships revealed that the carbonyl group at C-8 played a vital role in the pathogenic activities [9]. Compounds **1–7** were evaluated cytotoxic activities against three cancer cell lines HCT 116, HeLa, and MCF7, and only did **1** display strong biological effect against MCF7 with half-maximal inhibitory concentration (IC_{50}) value at 7.73 ± 0.11 μM compared with the *cis*-platinum (14.32 ± 1.01 μM).

Diverse fungi including plant pathogen *Cochliobolus spicifer* [11], fungicolous fungus *Pestalotiopsis disseminate* [12], entomopathogen *Lecanicillium* sp. [13], and sponge-associated fungus *Drechslera hawaiiensis* [8] can produce spiciferone analogs. In this report, this member of polyketides was also isolated from the endophytic fungus *P. betae*, implying that horizontal gene transfer of spiciferone gene cluster might exist in these different fungi. Our results suggested that spiciferones possibly possess different activities in these fungi, such as insecticidal effects (entomopathogen *Lecanicillium* sp.), inhibitory activities against competitive microbes (fungicolous fungus *Pestalotiopsis disseminata*), and phytotoxins (plant pathogen *Cochliobolus spicifer*), which need to be further confirmed. To sum up, seven spiciferones including three new ones spiciferones F–H (**1–3**) were isolated from a desert plant endophytic fungus *P. betae*. Our results further supported that endophytic fungi inhabiting in unique biotopes such as desert, arid and grassland areas are an important treasure and could produce a diverse range of new/novel SMs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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