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Diaporthols A and B: Bioactive diphenyl ether derivatives from an endophytic fungus *Diaporthe* sp.

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ABSTRACT

Diaporthols A (**1**) and B (**2**), two diphenyl ether derivatives, were isolated from cultures of an endophytic fungus *Diaporthe* sp. ECN-137 obtained from the leaves of *Phellodendron amurense*. The structures of **1** and **2** were determined by extensive spectroscopic analyses, and the structure of **2** was confirmed by X-ray crystallographic analysis. Compounds **1** and **2** showed anti-migration activities in TGF-β1-elicited MDA-MB-231 breast cancer cells at a concentration of 20 μM.

Fungal secondary metabolites have contributed greatly to drug discovery as a rich source of medicinally useful compounds.[1] Numerous studies on fungal biosynthetic enzymes producing various fungal metabolites are conducted.[2] Discovery of enzymes that produce unique fungal metabolites is important in engineering new molecules with beneficial effects. Furthermore, identification of gene clusters encoding enzymes for biosynthesis would enable genome mining of new compounds. Therefore, we have been studying new secondary metabolites of endophytic fungi in various plants to find strains possessing novel enzymes and we have reported several polyketides from various strains.[3] Herein, we isolated and elucidated the structure of diaporthols A (**1**) and B (**2**), two diphenyl ether derivatives, from an endophytic fungus *Diaporthe* sp. (Fig. 1). Additionally, we evaluated the anti-migration activities of **1** and **2** in transforming growth factor-β1 (TGF-β1)-elicited MDA-MB-231 breast cancer cells.

Diaporthe sp. ECN-137 was isolated from the healthy leaves of *Phellodendron amurense* (Rutaceae) and identified by sequencing the D1/D2 26S rRNA gene and internal transcript spacers (ITS) of the ribosomal DNA.[4] The whole mycelia of *Diaporthe* sp. ECN-137, which were cultured on 120 plates of 2% malt extract agar (MEA) for 40 days, were extracted three times with MeOH at room temperature and concentrated under reduced pressure to afford a crude extract. The MeOH extract (15.7 g) was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction (2.1 g) was separated on a silica gel column with a gradient elution of $CHCl_3$/ethyl acetate (50:1 to 8:1, v/v) to give fractions 1–9. Fraction 3 (71.2 mg) was recrystallized in EtOAc to afford **1** (6.9 mg) as colorless needles. Fraction 4 (136.8 mg) was subject to further separation on a silica gel column with a gradient elution of *n*-hexane/ethyl acetate (3:1 to 1:1, v/v) to yield **2** (13.6 mg).

Diaporthol A (**1**),[5] obtained as colorless needles, was assigned a molecular formula of $C_{20}H_{18}O_5$ by HR-ESI-MS (*m*/*z* 361.1051 [M+Na]$^+$). The IR spectrum displayed absorption bands for hydroxy (3310 cm^{-1}) and carbonyl (1721 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data (Table 1) of **1** combined with the HMQC, DQF-COSY, and DEPT spectra revealed the presence of signals assigned to three methyl groups [δ_H 1.48 (2 × 3H, s), 2.24 (3H, s)], one oxymethylene group [δ_H 5.10 (2H, br s); δ_C 68.8], one hydroxyl group [δ_H 6.07 (1H, s)], and six olefinic or aromatic protons, including two sets of AX spin systems [δ_H 7.06 (1H, d, *J* = 8.4 Hz, H-4), 6.59 (1H, d, *J* = 8.4 Hz, H-3); 6.30 (1H, d, *J* = 10.0 Hz, H-8), 5.68 (1H, d, *J* = 10.0 Hz, H-9)]. The signals of one carbonyl carbon (δ_C 166.2) and eight quaternary aromatic carbons (δ_C 151.6, 151.3, 147.3, 141.3, 134.8, 126.1, 120.5, 115.2) were observed in the ^{13}C NMR spectrum. The HMBC correlations (Fig. 2) of H-3/C-1, H-4/C-2, C-6, H-8/C-10, C-4, C-6, and H-9/C-5, C-10, C-11(12) indicated a 2,2-dimethyl-2*H*-chromene moiety (unit A). The presence of 2,2-dimethylpyran moiety was inferred by the chemical shifts of an oxygen bearing carbon

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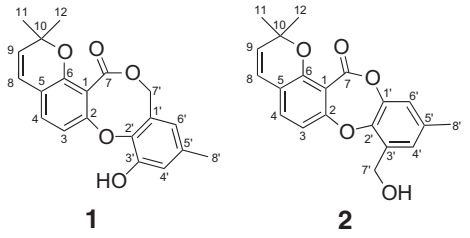

Fig. 1. Structures of diaporthols A (**1**) and B (**2**).

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data for diaporthols A (**1**) and B (**2**) in $CDCl_3$.

No.	**1**		**2**	
	δ_C	δ_H, mult. (*J* in Hz)	δ_C	δ_H, mult. (*J* in Hz)
1	115.2		111.2	
2	151.6[a]		160.2	
3	113.7	6.59, d (8.4)	111.2	6.71, d (7.8)
4	129.6	7.06, d (8.4)	130.6[c]	7.02, d (7.8)
5	120.5		119.6	
6	151.3[a]		155.2	
7	166.2		160.8	
8	121.0[b]	6.30, d (10.0)	120.9[d]	6.23, d (10.0)
9	131.6	5.68, d (10.0)	130.7[c]	5.62, d (10.0)
10	77.9		78.2	
11	27.9[e]	1.48, s[f]	28.0[g]	1.47, s[h]
12	27.9[e]	1.48, s[f]	28.0[g]	1.47, s[h]
1′	126.1		143.7	
2′	141.3		146.0	
3′	147.3		133.2	
4′	117.3	6.84, br s	125.5	6.97, d (2.0)
5′	134.8		136.0	
6′	121.0[b]	6.38, br s	120.9[d]	7.02, d (2.0)
7′	68.8	5.10, br s	60.1	4.86, br s
8′	20.9	2.24, s	20.8	2.30, s
OH		6.07, s		

[a–d]Assignments are interchangeable. [e–h]Signals overlap.

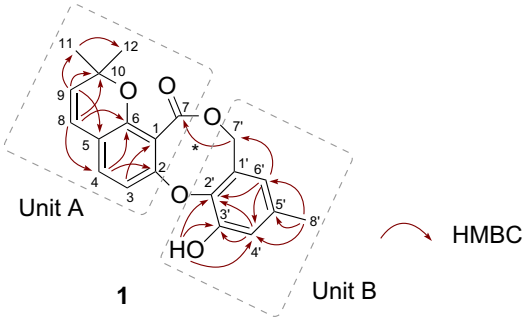

Fig. 2. Selected HMBC correlations of diaporthol A (**1**). *Correlation observed in acetone d_6 but not in $CDCl_3$.

signal at δ_C 77.9 (C-10) and two methine carbons at δ_C 121.0 (C-8) and 131.6 (C-9), attributable to vinyl carbon atoms. Furthermore, the linkage between C-1 and an ester carbonyl group (C-7) was also deduced from the chemical shift of C-1 (δ_C 115.2). The DQF-COSY crosspeak between two *meta*-coupling aromatic protons [δ_H 6.38 (1H, br s, H-6′), 6.84 (1H, br s, H-4′)] and the HMBC correlations 3′-OH/C-2′, C-3′, C-4′, H-4′/C-2′, H_3-8′/C-4′, C-5′, C-6′, and H-6′/C-2′, C-4′, C-7′ indicated the presence of a 1,2,3,5-tetrasubstituted benzene ring (C-1′ to C-6′) with hydroxyl, methyl, and oxymethylene groups at C-3′, C-5′, and C-1′, respectively (unit B). When the HMBC spectrum was measured in acetone d_6, a crucial correlation between H_2-7′/C-7 was observed, which was not observed in $CDCl_3$. Therefore, the ester linkage between C-7 and C-7′ was corroborated. The ether bond between C-2 and C-2′ was established, accounting for the remaining degree of unsaturation and the molecular formula predicted by HR-ESI-MS. Hence, the structure of **1** was determined as shown in Fig. 2.

Diaporthol B (**2**)[6] was obtained as colorless prisms. The HR-ESI-MS data suggested the same molecular formula, $C_{20}H_{18}O_5$ (*m*/*z* 361.1036 $[M+Na]^+$), as **1**. ^{1}H and ^{13}C NMR spectral data for **2** are listed in Table 1. The HMQC, DQF-COSY, and HMBC results (Fig. 3) revealed that **2** contained two phenolic units A and B, which were similar to those in **1**. Although no HMBC correlation was detected to clarify the linkage between units A and B, the NOE between H-3 and H_2-7′ observed in the NOESY spectrum implied the structure shown in Fig. 3. Because the assignment of the linkage with only one NOE was not reliable, the structure was confirmed by single-crystal X-ray diffraction. Single crystals of **2** were obtained as colorless prisms by slow evaporation of the ethyl acetate solution.[7] The structure of **2** was determined as shown in Fig. 4.

Diphenyl ethers are a class of fungal polyketides. Many research groups have been studying its biosynthesis, and some biosynthetic pathways have been proposed. Most fungal depsidones found in lichens are probably formed by the oxidative coupling of depsides, esters of two polyketidic benzoic acid derivatives.[8] Armaleo et al. reported that a polyketide synthase, namely CgrPKS16, is most likely responsible for the oxidative coupling of depsides to depsidones.[9] In contrast, some fungal depsidones and 5*H*,7*H*-dibenzo[b,g][1,5]dioxocin-5-ones (*e.g.* penicillide and purpactin A) are assumed to be produced by the rearrangement of grisadienedione intermediates derived of anthraquinones or anthrones.[10] Recently, the gene cluster for synthesizing diphenyl ethers from anthraquinones *via* benzophenones was identified.[11] Considering the structural similarity with purpactin A, **1** and **2** are presumed to be biosynthesized *via* grisadienediones as previously reported by

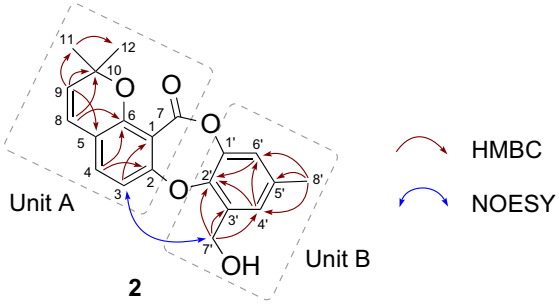

Fig. 3. Selected HMBC and NOESY correlations of diaporthol B (**2**).

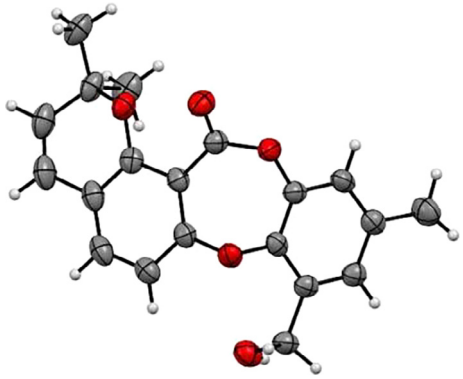

Fig. 4. ORTEP representation of diaporthol B (**2**).