The Journal of Antibiotics (2019) 72:174–177
https://doi.org/10.1038/s41429-018-0131-2

JARA Japan Antibiotics Research Association
The Society for Actinomycetes Japan

BRIEF COMMUNICATION

Bioactive secondary metabolites from an endophytic fungus *Phoma* sp. PF2 derived from *Artemisia princeps* Pamp.

Jung Wha Kim[1] · Hyun Gyu Choi[1] · Ji Hoon Song[2] · Ki Sung Kang[2] · Sang Hee Shim[1]

Received: 17 August 2018 / Revised: 9 November 2018 / Accepted: 13 November 2018 / Published online: 12 December 2018

Abstract

Two new isochromanone derivatives, (3*S*,4*S*)-3,8-dihydroxy-6-methoxy-3,4,5-trimethylisochroman-1-one (**1**) and methyl (*S*)-8-hydroxy-6-methoxy-5-methyl-4a-(3-oxobutan-2-yl)benzoate (**2**), together with six known compounds (**3–8**) were isolated from the cultures of an endophytic fungus *Phoma* sp. PF2 obtained from *Artemisia princeps*. The chemical structures of the isolated compounds were elucidated by interpretation of spectroscopic data (1D, 2D NMR, HRESIMS, and CD) and calculation of ECD. All the isolated compounds (**1–8**) showed moderate inhibitory activities on nitric oxide levels in lipopolysaccharide-induced RAW264.7 machrophage cells.

Endophytes are endosymbiotic microorganisms, which grow in the internal tissues of living plants without causing apparent harm to the host [1]. Though the exact underlying mechanisms of the relationship of endophytes with the host plant are still under study, it is evident that endophytes have mutualistic symbiotic correlations with their host plant [2]. There have been reports that secondary metabolites produced from endophytes may protect the host plants by exhibiting antifungal, antibacterial, antiviral, plant growth, and regulatory activities [3]. In addition, endophytes play various indispensable functions for plant development, stress tolerance, and adaptation in nature [4]. Furthermore, due to their rich biodiversity, endophytic fungi have been considered as potential sources for the new drug candidates, which possess chemically novel structures [5].

During our ongoing effort to investigate the novel bioactive substances from endophytic fungi, *Phoma* sp. PF2 was isolated from the leaves of *Artemisia princeps* Pamp. (Asteraceae). *Phoma* sp., a genus of coelomycetous fungi, has been reported to produce various bioactive secondary metabolites, such as cytotoxic α-pyrones (phomones C-F) [6], thiodiketopiperazine (phomazine B) [7], antiangiogenic polyketide (phomaketide A) [8], anti-inflammatory polyketide (phomaketide C) [8], and antibacterial polyketide (barceloneic acid C) [9]. *A. princeps*, a perennial herb native to Korea, China, and Japan, has been traditionally used to treat inflammation, diarrhea, bacterial infection, and circular disorders [10]. Recent studies have found that the extract of *A. princeps* and its constituents show gastroprotective [11], neuroprotective [12], anti-oxidant [10], anti-inflammatory [13], anti-obesity [14], and anti-diabetic activities [14].

In this study, chemical investigation of the cultures of an endophytic fungus *Phoma* sp. PF2 derived from *A. princeps* resulted in the isolation and identification of eight compounds (**1**−**8**), including two new isochromanone derivatives (**1** and **2**) (Fig. 1). Since *A. princeps* is well known to have anti-inflammatory effect, the anti-inflammatory activities of the isolated compounds (**1**−**8**) were evaluated by measuring the inhibitory activity of nitric oxide (NO) production levels in the lipopolysaccharide (LPS)-induced RAW264.7 macrophage cells.

Compound **1** was obtained as an amorphous white powder and its molecular formula was deduced as $C_{13}H_{16}O_5$ from the negative mode HRESIMS ion peak at *m/z* 251.0914 $[M-H]^-$ (calcd for $C_{13}H_{15}O_5$, 251.0919), indicating six degrees of unsaturation. The ^1H NMR spectrum of **1** showed the signals of three methyls [δ_H 1.22

These authors contributed equally: Jung Wha Kim, Hyun Gyu Choi

Supplementary material The online version of this article (https://doi.org/10.1038/s41429-018-0131-2) contains supplementary material, which is available to authorized users.

✉ Sang Hee Shim
sangheeshim@duksung.ac.kr

[1] College of Pharmacy and Innovative Drug Center, Duksung Women's University, Seoul 01369, Republic of Korea

[2] College of Korean Medicine, Gachon University, Seongnam 13120, Republic of Korea

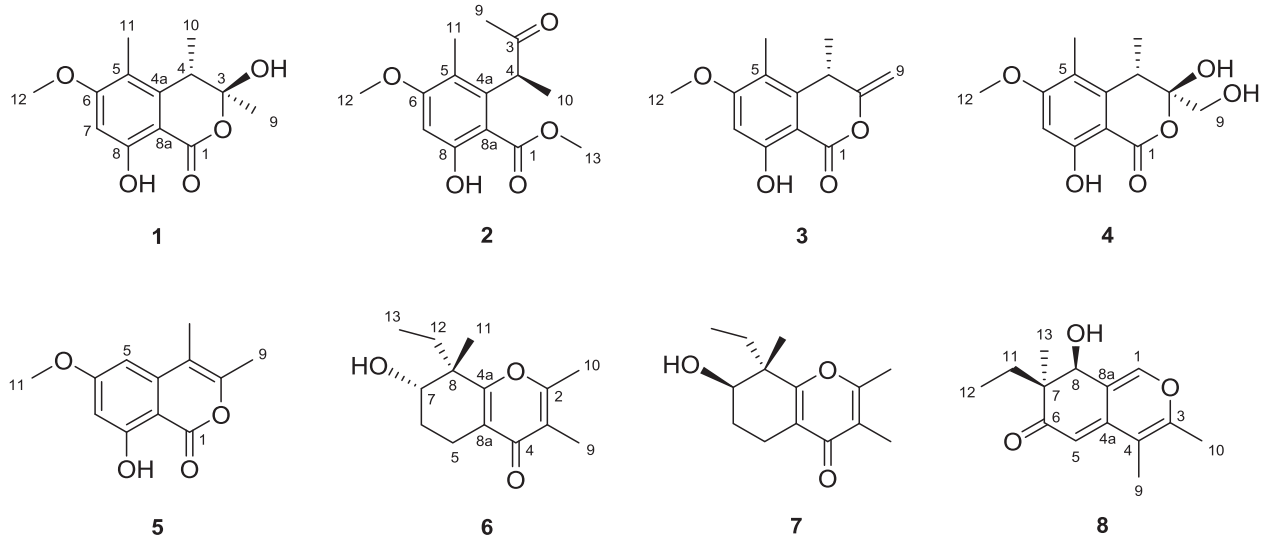

Fig. 1 Chemical structures of **1–8** isolated from *Phoma* sp. PF2 fungi derived from *Artemisia princeps*

Table 1 ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectral data of 1 and 2 in $CDCl_3$ (δ in ppm, J values in parentheses)

Position	1		2	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		169.2		170.8
3		104.2		207.6
4	3.25, q (7.1)	38.7	4.03, q (7.2)	51.1
4a		142.1		142.5
5		115.5		118.6
6		164.6		162.9
7	6.38, s	97.4	6.41, s	98.2
8		163.0		162.9
8a		99.0		104.3
9	1.77, s	25.8	1.93, s	27.8
10	1.22, d (7.1)	17.2	1.33, d (6.8)	15.4
11	2.09, s	10.0	2.07, s	11.7
12	3.86, s	55.7	3.82, s	55.7
13			3.77, s	50.9
8-O<u>H</u>	11.29, s		11.14, s	

(3 H, d, $J = 7.1$ Hz), 1.77 (3 H, s), and 2.09 (3 H, s)], a methoxy group [δ_H 3.86 (3 H, s)], a methine [δ_H 3.25 (1 H, q, $J = 7.1$ Hz)], and an aromatic proton [δ_H 6.38 (1 H, s)], indicating the presence of a pentasubstituted aromatic ring (Table 1). The ^{13}C NMR spectrum exhibited 13 carbon resonances, which comprised four methyls (δ_C 10.0, 17.2, 25.8, and 55.7), two methines (δ_C 38.7 and 97.4), and seven quaternary carbons (δ_C 99.0, 104.2, 115.5, 142.1, 163.0, 164.6, and 169.2). The HMBC spectrum correlation signals from H-4 (δ_H 3.25) to C-3 (δ_C 104.2), C-4a (δ_C 142.1), and C-8a (δ_C 99.0), and from H-7 (δ_H 6.38) to C-5 (δ_C 115.5),

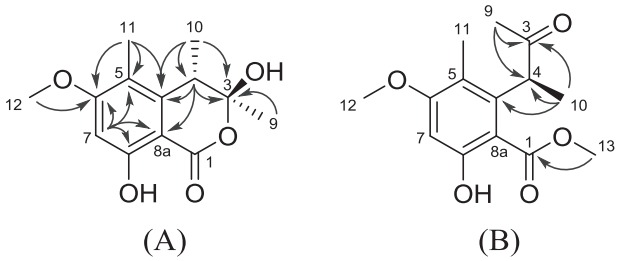

Fig. 2 Key HMBC correlations of **1** (**a**) and **2** (**b**)

C-6 (δ_C 164.6), C-8 (δ_C 163.0), and C-8a revealed the presence of an 1-isochromanone skeleton in the structure (Fig. 2a). In addition, the correlations from H_3-9 (δ_H 1.77) to C-3, from H_3-10 (δ_H 1.22) to C-3, C-4 (δ_C 38.7), and C-4a, from H_3-11 (δ_H 2.09) to C-4a, C-5, and C-6, and from H_3-12 (δ_H 3.86) to C-6 in the HMBC spectrum established the position of three methyls and a methoxy group. Since **1** had stereogenic centers at C-3 and C-4, the stereochemistry of this compound should be established. The strong ROESY correlations between H_3-9 (δ_H 1.77) and H_3-10 (δ_H 1.22) deduced that the two methyls of C-9 and C-10 were in the same face of the molecule. Therefore, the methyl group at C-10 and the hydroxyl group at C-9 were found to be in "anti" orientation. The absolute configuration at C-3 and C-4 of **1** was determined by the measurement of CD spectrum, as well as ROESY data. It displayed positive Cotton effects at 238 and 270 nm, establishing the absolute configuration of C-4 to be in *S* (Fig. 3a) [15]. Accordingly, the absolute stereochemistry of C-3 was also concluded to be in *S* based on the relative stereochemistry obtained by ROESY data [15]. Based on these results, compound **1** was

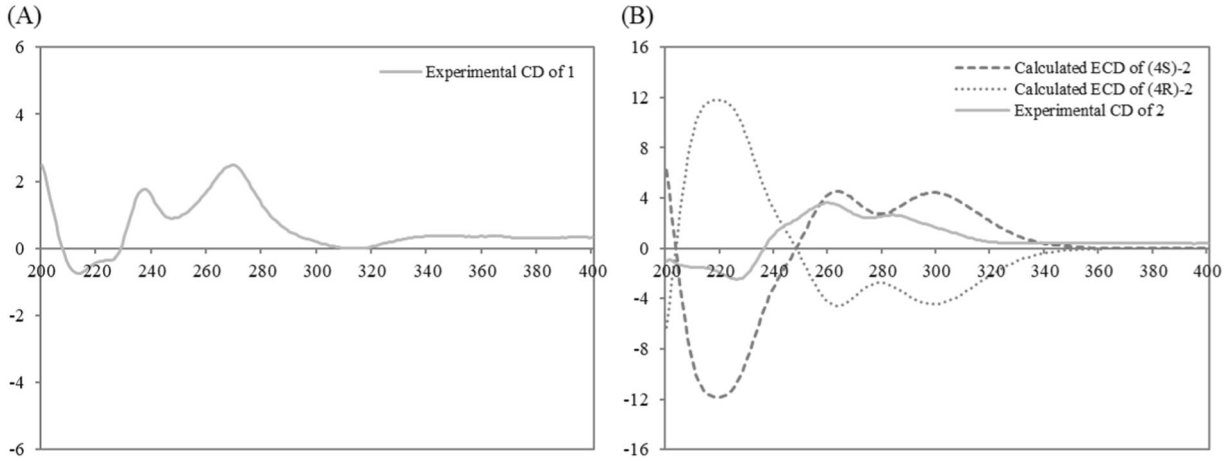

Fig. 3 **a** Experimental CD spectrum of compound **1**. **b** Comparison of the experimental CD spectrum (solid) and the calculated ECD spectra (dashed) of compound **2**

assigned as (3*S*,4*S*)-3,8-dihydroxy-6-methoxy-3,4,5-trimethylisochroman-1-one.

Compound **2** was isolated as an amorphous white powder and the molecular formula, $C_{14}H_{18}O_5$, was established by a HRESIMS peak in the negative ion mode at *m/z* 265.1074 $[M-H]^-$ (calcd for $C_{14}H_{17}O_5$, 265.1076). The ^{1}H NMR spectrum of **2** was similar to those of **1,** except for the presence of two methoxy groups [δ_H 3.77 (H_3-13) and 3.82 (H_3-12)] and the downfield shifted appearance of a quartet methine signal [δ_H 4.03 (1 H, q, $J = 7.2$ Hz, H-4)] (Table 1). The ^{13}C NMR spectrum of **2** showed 14 carbon signals including overlapped quaternary carbons at δ_C 162.9 (C-6 and C-8). Compared to that of compound **1**, it showed an additional methoxy (δ_C 50.9, C-13) group and a ketone (δ_C 207.6, C-3) group, respectively, instead of a hemiacetal carbon at δ_C 104.2 (C-3) shown in **1**. Considering 6 degrees of unsaturation of this molecule, two carbonyl carbons were deduced to be present in **2**, suggesting the opening of the lactone ring moiety of an 1-isochromanone skeleton. The HMBC correlation from H_3-13 (δ_H 3.99, s) to C-1 (δ_C 170.8) indicated that the additional methoxy group was attached to C-1, which supported the above assumption. Moreover, the HMBC correlation signals from H_3-9 (δ_H 1.93, s) to C-3 (δ_C 207.6) and C-4 (δ_C 51.1) and from H_3-10 [δ_H 1.33, d ($J = 6.8$ Hz)] to C-3, C-4, and C-4a (δ_C 142.5) revealed the presence of an acetyl group linked to the methyl-bearing methine carbon C-4. (Fig. 2b). The absolute configuration of C-4 was determined by comparing the experimental CD spectrum with the calculated ECD spectrum. The experimental CD spectrum of **2** displayed a negative Cotton effect at 226 nm and positive Cotton effects at 260 and 283 nm, which was more consistent with those in the calculated ECD spectrum of 4*S*-form than that of 4*R*-form of **2** (Fig. 3b). Therefore, the stereochemistry of the chiral carbon C-4 in **2** was established to be *S*, which agreed with the absolute stereochemistry of C-4 in compound **1**. From these data, the structure of compound **2** was concluded as methyl (*S*)-8-hydroxy-6-methoxy-5-methyl-4a-(3-oxobutan-2-yl)benzoate.

The six known compounds were assigned as (4*S*)-clearanol C (**3**) [15], (3*R*,4*S*)-3,8-dihydroxy-3-hydroxymethyl-6-methoxy-4,5-dimethylisochroman-1-one (**4**) [15], polygonolide (**5**) [16], (7*S*,8*S*)-8-ethyl-7-hydroxy-2,3,8-trimethyl-5,6,7,8-tetrahydro-4*H*-chromen-4-one (**6**) [17], (7*R*,8*S*)-8-ethyl-7-hydroxy-2,3,8-trimethyl-5,6,7,8-tetrahydro-4*H*-chromen-4-one (**7**) [17], and leptosphaerone (=(7*S*,8*S*)-7-ethyl-7,8-dihydro-8-hydroxy-3,4,7-trimethyl-6*H*-2-benzopyran-6-one) (**8**) [18] by comparing their spectroscopic data with previously reported literature values (Fig. 1). To the best of our knowledge, the known compounds **3–6** and **8** were isolated from *Phoma* sp. for the first time.

Several previous researches have reported the anti-inflammatory activities of isochromanone-type compounds. In particular, 8-dihydroxy-3-methyl-isochromanone was found to inhibit LPS-induced vascular inflammation in human umbilical vein endothelial cells through inhibition of mitogen-activated protein kinase and nuclear transcription factor-kappa B (NF-κB) signaling pathway activation [19]. In addition, 1-phenyl-6,7-dihydroxy-isochroman was reported to attenuate LPS-stimulated inflammatory response through down-regulation of inducible isoforms of nitric oxide synthase and cyclooxygenase (COX)-2, as well as of NF-κB in primary cultured rat microglial cells [20]. Therefore, to investigate the anti-inflammatory activities of the isochromanones, the compounds (**1–8**) isolated from the endophytic fungus in this study were subjected to the inhibition assay on NO production in LPS-induced RAW264.7 macrophage cells (S17).

Most isolates except for **6** did not show the cell cytotoxicity at 50 μM on RAW264.7 cells in preliminary MTT assay. All the isolated compounds exhibited moderate inhibitory activities on NO production in LPS-stimulated RAW264.7 cells.

In summary, eight secondary metabolites (**1**–**8**), including two new isochromanone derivatives, (3 *S*,4 *S*)-3,8-dihydroxy-6-methoxy-3,4,5-trimethylisochroman-1-one (**1**) and methyl 6-hydroxy-4-methoxy-3-methyl-2-(3-oxobutan-2-yl)benzoate (**2**), were isolated from the ethyl acetate extract of the cultures of an endophytic fungus *Phoma* sp. (PF2) derived from *A. princeps*. Among the isolates, **1** and **4** showed inhibitory activities on NO production against LPS-activated RAW264.7 mouse macrophage cells without cell cytotoxicities. These results provide further information about the chemical characteristics of the endophytic fungus *Phoma* sp., as well as its potential use as a natural source for anti-inflammatory agents.

Acknowledgements The present study was supported by the National Research Foundation of Korea 210 (NRF-2018R1A2B6001733).

Compliance with ethical standards

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

References

1. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: a review. Prikl Biokhim Mikrobiol. 2008;44:153–8.
2. Aly AH, Debbab A, Proksch P. Fungal endophytes: unique plant inhabitants with great promises. Appl Microbiol Biotechnol. 2011;90:1829–45.
3. Lee C, Kim S, Li W, Bang S, Lee H, Lee HJ, et al. Bioactive secondary metabolites produced by an endophytic fungus *Gaeumannomyces* sp. JS0464 from a maritime halophyte *Phragmites communis*. J Antibiot. 2017;70:737–42.
4. Wani ZA, Ashraf N, Mohiuddin T, Riyaz-Ul-Hassan S. Plant-endophyte symbiosis, an ecological perspective. Appl Microbiol Biotechnol. 2015;99:2955–65.
5. Smith SA, Tank DC, Boulanger LA, Bascom-Slack CA, Eisenman K, Kingery D, et al. Bioactive endophytes warrant intensified exploration and conservation. PLoS ONE. 2008;3:e3052.
6. Sang XN, Chen SF, Tang MX, Wang HF, An X, Lu XJ, et al. α-Pyrone derivatives with cytotoxic activities, from the endophytic fungus *Phoma* sp. YN02-P-3. Bioorg Med Chem Lett. 2017;27:3723–5.
7. Kong F, Wang Y, Liu P, Dong T, Zhu W. Thiodiketopiperazines from the marine-derived fungus *Phoma* sp. OUCMDZ-1847. J Nat Prod. 2014;77:132–7.
8. Lee MS, Wang SW, Wang GJ, Pang KL, Lee CK, Kuo YH, et al. Angiogenesis inhibitors and anti-inflammatory agents from Phoma sp. NTOU4195. J Nat Prod. 2016;79:2983–90.
9. Xia X, Kim S, Bang S, Lee HJ, Liu C, Park CI, et al. Barceloneic acid C, a new polyketide from an endophytic fungus *Phoma* sp. JS752 and its antibacterial activities. J Antibiot (Tokyo). 2015;68:139–41.
10. Hirano A, Goto M, Mitsui T, Hashimoto-Hachiya A, Tsuji G, Furue M. Antioxidant *Artemisia princeps* extract enhances the expression of filaggrin and loricrin via the AHR/OVOL1 pathway. Int J Mol Sci. 2017;18:E1948.
11. Kim JI, Park SW, Lim JJ, Sohn SI, Shin JS, Park SC, et al. Gastroprotective effects of the isopropanol extract of *Artemisia princeps* and its gastroretentive floating tablets on gastric mucosal injury. Acta Pharm. 2017;67:479–94.
12. Lee SG, Lee H, Nam TG, Eom SH, Heo HJ, Lee CY, et al. Neuroprotective effect of caffeoylquinic acids from *Artemisia princeps* Pampanini against oxidative stress-induced toxicity in PC-12 cells. J Food Sci. 2011;76:C250–C256.
13. Chen CC, Lin MW, Liang CJ, Wang SH. The anti-inflammatory effects and mechanisms of eupafolin in lipopolysaccharide-induced inflammatory responses in RAW264.7 macrophages. PLoS ONE. 2016;11:e0158662.
14. Yamamoto N, Kanemoto Y, Ueda M, Kawasaki K, Fukuda I, Ashida H. Anti-obesity and anti-diabetic effects of ethanol extract of *Artemisia princeps* in C57BL/6 mice fed a high-fat diet. Food Funct. 2011;2:45–52.
15. Tayone WC, Kanamaru S, Honma M, Tanaka K, Nehira T, Hashimoto M. Absolute stereochemistry of novel isochromanone derivatives from *Leptosphaeria* sp. KTC 727. Biosci Biotechnol Biochem. 2011;75:2390–3.
16. Furuta T, Fukuyama Y, Asakawa Y. Polygonolide, an isocoumarin from Polygonum hydropiper possessing anti-inflammatory activity. Phytochemistry. 1986;25:517–20.
17. Nakajima H, Fujimoto H, Kimura Y, Hamasaki T. Importance of the ketone function for the phytotoxicity of spiciferone A, a phytotoxin produced by the fungus *Cochliobolus spicifer*. Biosci Biotechnol Biochem. 1993;57:1938–9.
18. Liu JY, Liu CH, Zou WX, Tian X, Tan RX. Leptosphaerone, a metabolite with a novel skeleton from *Leptosphaeria* sp. IV403, an endophytic fungus in *Artemisia annua*. Helv Chim Acta. 2002;85:2664–7.
19. Fu R, Chen Z, Wang Q, Guo Q, Xu J, Wu X. XJP-1, a novel ACEI, with anti-inflammatory properties in HUVECs. Atherosclerosis. 2011;219:40–48.
20. Trefiletti G, Togna AR, Latina V, Marra C, Guiso M, Togna GI. 1-Phenyl-6,7-dihydroxy-isochroman suppresses lipopolysaccharide-induced pro-inflammatory mediator production in human monocytes. Br J Nutr. 2011;106:33–36.