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NOTE

Photipyrones A and B, new pyrone derivatives from the plant endophytic fungus *Pestalotiopsis photiniae*

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In order to obtain more secondary metabolites in one single strain possessing genetic potential to produce different compounds, one easiest way is to vary the culture conditions. This approach was termed 'one strain many compounds' (OSMAC).[1] As either endophytic or pathogenic fungus on living plant, the genera *Pestalotiopsis* spp. are prolific fungi, and our prior chemical studies of the species of this genus have led to the isolation of a variety of new/novel different bioactive compounds,[2–15] which revealed that the genera *Pestalotiopsis* spp. possessed the biogenetic potential to produce different secondary metabolites with a broad range of bioactive effects. Photinides A–F with unique benzofuranone-derived γ-lactone skeleton have been isolated from the extract of *Pestalotiopsis photiniae* (L461) grown in SA media culture.[5] In contrast, the crude isolate of the same fungus cultivated in rice-media culture in static condition produced two new δ-lactone derivatives named photipyrones A (**2**) and B (**3**), along with four known analogues namely LL-P880α (**1**),[16] LL-P880β (**4**),[17] 1′-hydroxy-4-methoxy-6-pentyl-2H-pyran-2-one (**5**),[18] and 1′,2′-dihydroxy-4-methoxy-6-pentyl-2H-pyran-2-one (**6**)[16] (Figure 1) completely different from photinides A–F from SA media culture. In this note, we will present the isolation, structure elucidation and bioactivities of these compounds.

The culture of *P. photiniae* was isolated from the plant *Roystonea regia* (H.B.K.) Cook collected from Jianfeng Mountain, Hainan Province, People's Republic of China, in April 2005. The isolate was identified by one of the authors (LG) based on morphology and sequence analysis of the ITS region of the ribosomal DNA and was assigned the accession number L461 in LG's culture collection at the Institute of Microbiology, Chinese Academy of Sciences, Beijing. The fungal strain was cultured on slants of potato dextrose agar (PDA) at 25 °C for 10 days. The agar plugs were used to inoculate 250 ml Erlenmeyer flasks, each containing 50 ml of media (0.4% glucose, 1% malt extract and 0.4% yeast extract), and the final pH of the media was adjusted to 6.5 before sterilization. Flask cultures were incubated at 25 °C on a rotary shaker at 170 rpm for 5 days. Fermentation was carried out in four 500 ml Fernbach flasks, each containing 75 g of rice. Spore inoculum was prepared by suspension in sterile, distilled H_2O to give a final spore/cell suspension of 1×10^6 ml^{-1}. Distilled H_2O (100 ml) was added to each flask and the contents were soaked overnight before autoclaving at 15 lb in^{-2} for 30 min. After cooling to room temperature, each flask was inoculated with 5.0 ml of the spore inoculum and incubated at 25 °C for 40 days.

The fermented material was extracted with methyl ethyl ketone (MEK; 3×500 ml), and the organic solvent was evaporated to dryness under vacuum to afford 4.9 g of crude extract, which was fractionated by silica-gel normal chromatography using CH_2Cl_2–MeOH gradient elution. The fraction eluted with 98:2 CH_2Cl_2–CH_3OH was separated by Sephadex LH-20 column chromatography (Pharmacia, Uppsala, Sweden) eluted with MeOH to afford a subfraction of 80 mg. Purification of the fraction by semipreparative reversed-phase HPLC (Agilent Zorbax SB-C_{18} column, Agilent Technologies Inc, Wilmington, DE, USA); 5 μm; 9.4 × 250 mm; 2 ml min^{-1}) afforded LL-P880α (**1**; 2.0 mg, t_R 35.8 min; 2.5 mg, 15% MeCN in H_2O over 5 min, 15–100% over 100 min); photipyrone A (**2**; 1.0 mg, t_R 24.5 min; same gradient as in purification of **1**); photipyrone B (**3**; 1.1 mg, t_R 16.7 min; same gradient as in purification of **1**); LL-P880β (**4**; 3.0 mg, t_R 21.0 min; same gradient as in purification of **1**); 1′-hydroxy-4-methoxy-6-pentyl-2H-pyran-2-one (**5**; 3.5 mg, t_R 39.0 min; same gradient as in purification of **1**); 1′, 2′-dihydroxy-4-methoxy-6-pentyl-2H-pyran-2-one (**6**; 1.2 mg, t_R 20.3 min; same gradient as in purification of **1**). Optical rotations were measured on a PerkinElmer 241 polarimeter (PerkinElmer, Lodz, Poland) and UV data were recorded on Shimadzu Biospec-1601 spectrophotometer (Shimadzu, Kyoto, Japan). IR data were recorded using a Nicolet Magna-IR 750 spectrophotometer (Nicolet Company, Madison, WI, USA). ^{1}H and ^{13}C NMR data were acquired with Varian Mercury-400, -500 and -600 spectrometers using solvent

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signals ($CDCl_3$; δ_H 7.26/δ_C 77.6) as references. The HMQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. ESIMS data were recorded on a Bruker Esquire 3000^{plus} spectrometer. HRESIMS and EIMS data were obtained using a Bruker APEX III 7.0 T spectrometer and APEXII FT-ICR (Bruker, Billerica, MA, USA), respectively.

The IR spectrum of **1** displayed hydroxyl group absorption at 3431 cm^{-1}(br.s) and carbonyl group signals at 1703 cm^{-1}(br.s). The molecular formula of photipyrone B (**2**) was determined to be $C_{11}H_{18}O_5$ by analysis of its HRESIMS (*m/z* 253.1040 $[M+Na]^+$, Δ +0.6), which was consistent with the NMR spectra data (Table 1 and Supplementary Figures S1 and S2). Detailed analysis of the ^1H and ^{13}C NMR data of **2** revealed the presence of structural features similar to those found in the known compound LL-P880α (**1**),[12] except that the methylene unit (C-3) was disappeared, and an additional signal for one oxymethine proton with chemical shift value at δ_H 4.12 p.p.m. was observed, which implied that a methylene unit is replaced by an oximethine group. And this suggestion was also supported by the presence of one more oxygenate carbon in the ^{13}C NMR spectra of **2** (δ_C 67.1). Finally, the structure was confirmed by ^1H–^1H COSY and HMBC correlations (Figure 1). ^1H–^1H COSY correlation revealed one isolated fragment corresponding to C-3–C-2–C-7–C-8–C-9–C-10–C-11. The HMBC correlations from H-2 to C-6, from H-3 to C-4 and C-5 together with from H-5 to C-3, C-4 and C-6 established one α, β-unsaturated γ-lactone moiety. The distinct cross peak from 12-OMe to C-4 in HMBC spectra placed the oxymethyl group attached at C-4. The correlations from CH_3-11 to C-9 and C-10, and that from H_2-10 to C-8 and from H-7 to C-9 confirmed the connectivity of C-7–C-8–C-9–C-10–C-11 determined by ^1H–^1H COSY correlation (Figure 1). Thus the planar structure for **2** was determined. The relative configuration was determined by analysis of ^1H NMR *J*-values. The small coupling constant ($J \approx 0$ Hz) observed between H-2 and H-3 indicated that both protons were *cis* to each other with respect to the corresponding six-membered ring.[19] The absolute configurations of C-2 and C-7 in **2** were postulated to be the same as those found in **1** on accounting for the similar biogenetic source as those of analogues.

Photipyrone C (**3**) was assigned the molecular formula of $C_{11}H_{18}O_5$ (three degrees of unsaturation) on the basis of HRESI analysis (*m/z* 253.1043 $[M+Na]^+$, Δ +0.3) and NMR data (Table 1 and Supplementary Figures S3 and S4). Comparison of the NMR spectra with those of **1** displayed the structural similarity, except that

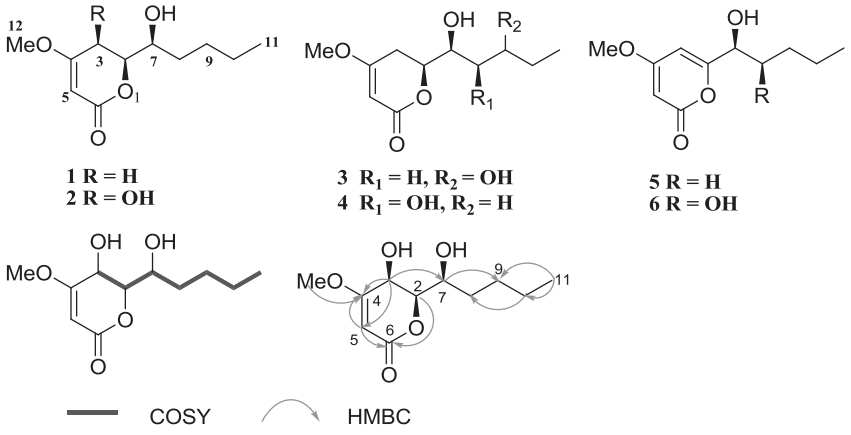

Figure 1 Structures for compounds 1–6, 1H–1H COSY, and Key HMBC for compound 2. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

Table 1 NMR spectroscopic data for Photipyrone B (2) and Photipyrone C (3) in $CDCl_3$

	*Photipyrone B (**2**)*			*Photipyrone C (**3**)*	
Position	δ_H[a] *(J in Hz)*	δ_C[b], *mult.*	*HMBC (H→C#)*	δ_H[a] (J *in Hz)*	δ_C[b], *mult.*
2	4.20 (br. s)	80.1, CH	C-3, C-7	4.38, dt (4.4, 13)	78.1, CH
3	4.12 (br. s)	67.1, CH	C-2, C-4, C-5	2.73, ddd (1.3, 13, 17) 2.30, dd (3.7, 17)	37.5, CH_2
4		172.6, qC			173.1, qC
5	5.18 (s)	91.4, CH	C-3, C-4, C-6	5.14, d (1.3)	90.1, CH
6		166.6, qC			166.6, qC
7	4.10 (m)	71.3, CH	C-2, C-3	3.99, m	73.4, CH
8	1.65 (m) 1.50 (m)	32.6, CH_2	C-2, C-7, C-9, C-10	1.70, m	30.8, CH_2
9	1.65 (m)	27.5, CH_2	C-7, C-8, C-10, 11-Me	3.85, m	72.8, CH
10	1.35 (m)	22.7, CH_2	C-8, C-9, 11-Me	1.54, m	28.8, CH_2
11	0.92 t (7.0)	14.2, CH_2	C-9, C-10	0.96, t (7.6)	9.6, CH_3
12-OMe	3.80 (s)	56.7, CH_3	C-4	3.76, s	56.2, CH_3

[a]Recorded at 400 MHz.
[b]Recorded at 150 MHz.

the methylene unit of C-9 was oxygenated to be one oxymethine group. And this observation was confirmed by the ^{1}H–^{1}H COSY correlations of H-9 with H_2-8 and H_2-10, which determined the planar structure of **3**. Analysis of coupling stands and comparison of the chemical shift values of C-2 and C-7 implied that both carbons in **3** might possess the same absolute configurations at C-2 and C-7 as those found in **1** and **4**.

Secondary metabolites **1–6** were evaluated bioactivities against the human tumor cell line MDA-MB-231. And only compound **3** showed modest inhibitory effects on the growth of MDA-MB-231 with inhibitory rate at 25.0% and 23.0%, respectively, when tested at $10\,\mu g\,ml^{-1}$.

Photipyrones A (**2**) and B (**3**) are new α-Pyrone analogues, members of a general class that is commonly found among different fungi and plants.[20–22] These two new compounds are the first secondary metabolites reported from *Pestalotiopsis photiniae* (L461), which builds up the hypothesis that endophytic fungi, as one kind of unique environmental microbes and relatively rare untouched reservoir, could be the potential sources for new/novel secondary metabolites as the potent lead compounds for agricultural and medicinal agents.

The structural family of compounds **1–6** is completely different from that of photinides A–F isolated from the same fungus cultivated in SA media,[5] which implied that *Pestalotiopsis* spp. might possess multiple different biosynthetic genes with huge biogenetic potential to produce diverse natural products, and variation of the easily accessible cultivation parameters, such as media composition, could trigger the expression of latent biosynthetic genes, which might influence directly or indirectly the transcription, translation, and enzyme activity/specificity in the whole organism,[1] and finally, lead to the produce of different secondary metabolites. Thus, the result from the present experiment promotes us to utilize *Pestalotiopsis* spp. fully to obtain more diverse and interesting bioactive secondary metabolites by using various fermentation media.

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Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)