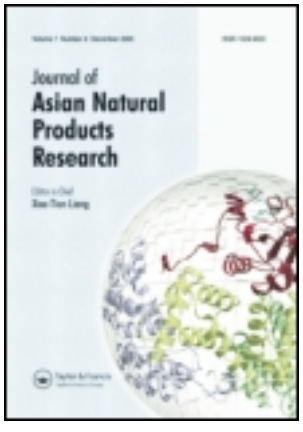

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Two new ylangene-type sesquiterpenoids from cultures of the fungus *Postia* sp.

Qiong-Ying Fan[a], Ze-Jun Dong[b], Zheng-Hui Li[b], Xia Yin[b], Xiao-Yan Yang[b], Tao Feng[b], Kun Wei[b], Ji-Kai Liu[b]* and Bao-Hua Zhao[a]*

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Two new ylangene-type sesquiterpenoids, postinins A (**1**) and B (**2**), were isolated from cultures of the fungus *Postia* sp. Structures **1** and **2** were elucidated on the basis of extensive spectroscopic analysis. The bioactivity evaluation showed that both compounds had significant inhibitory activities against protein tyrosine phosphatase 1B, and SH2-containing cytoplasmic tyrosine phosphatase-1 and -2 with IC_{50} values of 1.6–6.2 μg/ml.

Keywords: *Postia* sp; ylangene-type sesquiterpenoids; bioactivity; protein tyrosine phosphatase inhibitory activity

1. Introduction

We focused on natural products from mushrooms since 1997, and a large number of new and bioactive compounds have been reported from both fruiting bodies and fermentation broth [1–5]. In recent years, more and more sesquiterpenoids have been found from cultures of higher fungi. For instance, trefolane A [6] and conosilane A [7] are two novel sesquiterpenoids from cultures of *Tremella foliacea* and *Conocybe siliginea*, respectively, while irlactins A–D are also four ring-rearranged carbon skeletons from cultures of *Irpex lacteus* [8]. Boledulins A–C were three non-isoprenoid botryane sesquiterpenoids that have been isolated from cultures of the basidiomycete *Boletus edulis*, and boledulin A showed certain cytotoxicities against five human cancer cell lines [9]. In the course of searching for new and biologically active compounds, we have investigated the culture of the fungus *Postia* sp. It resulted in the isolation of two new ylangene-type sesquiterpenoids, postinins A (**1**) and B (**2**) (Figure 1). In this paper, we report the isolation, structural elucidation, and bioactivities of the two compounds.

2. Results and discussion

Compound **1** was obtained as white powder. Its molecular formula was determined to be $C_{15}H_{22}O_2$ by HR-EI-MS at *m/z* 234.1619 $[M]^+$, which indicated five degrees of unsaturation. The IR spectrum showed absorption bands for OH (3431 cm^{-1}) and C=C (1631 cm^{-1}). The ^{13}C NMR and DEPT spectra (Table 1) showed 15 carbon signals attributed to three methyls, two methylenes, eight methines (one oxygenated, one olefinic, and one formyl carbon), and two quaternary carbons (one olefinic). ^1H NMR spectrum showed a signal of CHO group at δ_H 9.47 (1H, s) and an olefinic proton signal at δ_H 6.81 (1H, s). Besides these, two doublets at δ_H 0.84 and 0.82 (each 3H, d, $J = 6.8$ Hz, Me-14 and Me-15) were assigned as two methyls connected to C-13

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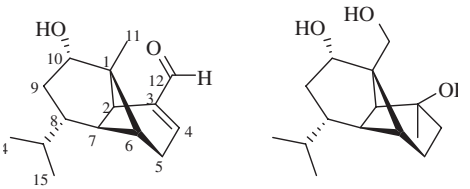

1 2

Figure 1. Structures of compounds **1** and **2**.

(CH, 32.1), as evidenced by $^{1}H-^{1}H$ COSY and HMBC correlations (Figure 2). A methyl at δ_H 0.80 (3H, s) was connected to C-1, as supported by the HMBC correlations of H_3-11 at δ_H 0.80 (3H, s) with C-1 at δ_C 43.9 (s), C-2 at δ_C 35.2 (d), C-10 at δ_C 75.3 (d), and C-6 at δ_C 44.5 (d). These data suggested that compound **1** possessed the ylangene-type backbone with similarities to the known compound isolemnalol [10]. One of the differences between them is that the OH group is placed at C-10 (δ_H 4.08; δ_C 75.3) in **1**, instead of at C-5 (δ_H 4.27; δ_C 70.6) in isolemnalol [10], which was confirmed by the $^{1}H-^{1}H$ COSY correlations of H-10 at δ_H 4.08 with H-9a at δ_H 1.87, and the HMBC correlations from H-10 to C-1, C-8, and C-11. Another difference between them is that the methyl group is replaced by the CHO group at C-12, as supported by the HMBC correlations from H-12 at δ_H 9.47 (1H, s) to C-3 at δ_C 149.0 (s) and C-4 at δ_C 148.9 (d). The relative configurations of chiral carbons were established by a Rotating-frame Overhauser Spectroscopy (ROESY) experiment (Figure 2). As shown in Figure 2, the structure of **1** was restricted in a rigid skeleton by the 6/4/6 ring system. Therefore, the observed ROESY correlations of H-6/H-8 and H-6/H-10 suggested that H-6, H-8, and H-10 were in the same side. Thus, the structure of **1** was established as postinin A, as shown in Figure 1.

Compound **2** was obtained as white powder. Its molecular formula $C_{15}H_{26}O_3$ was deduced by the HR-EI-MS at *m/z* 254.1884 $[M]^+$, indicating three degrees of unsaturation. The IR spectrum of **2** revealed the presence of hydroxy (3431 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 1) showed 15 carbon signals attributed to three methyls, four methylenes (one oxygenated), six methines (one oxygenated), and two quaternary carbons (one oxygenated). These data suggested that compound **2** possesses the same carbon skeleton as that of **1** including the hydroxy group substituted at C-10. One difference is that the methyl at C-

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** ($CDCl_3$, δ in ppm and *J* in Hz).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1		43.9, qC		59.8, qC
2	2.90, d (6.6)	35.2, CH	1.91, d (6.3)	51.1, CH
3		149.0, qC		86.0, qC
4	6.81, s	148.9, CH	1.92, m; 1.68, m (overlap)	32.7, CH_2
5	2.67, dt (20.8, 3.0); 2.50, dt (20.7, 2.9)	32.2, CH_2	1.81, m	21.5, CH_2
6	1.61, s	44.5, CH	2.22, m	38.9, CH
7	2.28, m	40.8, CH	2.12, d (2.6)	37.3, CH
8	1.75, m	41.9, CH	1.83, m	42.9, CH
9	1.87, m; 1.72, m	31.8, CH_2	1.68, m (overlap)	32.8, CH_2
10	4.08, d (5.9)	75.3, CH	4.25, dd (7.0, 4.1)	73.4, CH
11	0.80, s	16.2, CH_3	3.95, d (8.8); 3.56, d (8,8)	70.4, CH_2
12	9.47, s	191.4, CH	1.27, s	25.7, CH_3
13	1.49, m	32.1, CH	1.51, m	31.3, CH
14	0.84, d (6.8)	19.4, CH_3	0.83, d (6.8)	19.8, CH_3
15	0.82, d (6.8)	19.9, CH_3	0.92, d (6.8)	20.8, CH_3

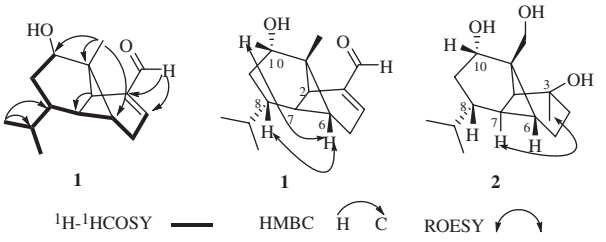

Figure 2. Key 2D NMR correlations of compounds **1** and **2**.

11 in **1** is replaced by a hydroxymethyl in **2** as supported by the HMBC correlation from H-11a at δ_H 3.95 (1H, d, $J = 8.8$ Hz) to C-1 at δ_C 59.8 (s). Another difference is that the formyl group of C-12 in **1** is replaced by a methyl in **2**, as well as two sp^3 carbons in **2** instead of two olefinic carbons in **1**, as supported by the HMBC correlations from Me-12 at δ_H 1.27 (3H, s) to C-3 at δ_C 86.0 (s) and C-4 at δ_C 32.7 (t). Detailed analysis of other 2D NMR data suggested that the other parts of **2** were the same as those of **1**. The ROESY correlation of H_3-12/H-7 determined the relative configuration of C-3, as shown in Figure 2. Thus, the structure of **2** was established as postinin B, as shown in Figure 1.

The bioactivities of compounds **1** and **2** were evaluated in the enzyme inhibition assay against several protein-tyrosine phosphatases (PTPs). As summarized in Table 2, both compounds showed significant inhibitory activities against protein-tyrosine phosphatase 1B (PTP1B), SH2-containing cytoplasmic tyrosine phosphatase-1 (SHP1) and -2 (SHP2) with IC_{50} values of 1.6–6.2 μg/ml. Compound **1** showed more potent inhibitory activities than compound **2**, especially against PTP1B and SHP1. However, both compounds did not show any activity against leukocyte antigen-related protein (LAR) at 50 μg/ml.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). UV spectra were obtained by using a Shimadzu UV-2401A spectrometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer (Bruker, Karlsruher, Germany) with KBr pellets. 1D and 2D NMR experiments were performed on an AVANCE III-600 spectrometer (Bruker) with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Mass spectra (MS) were recorded on Bruker HCT/Esquire (Bruker) or APIQSTAR Pulsar 1 (Advanced Bionics, Los Angeles, CA, USA) spectrometer. Preparative high-pressure liquid chromatography was performed on an Agilent 1100 series with a Zorbax SB-C18 (5 mm, 9.4 mm × 150 mm) column (Agilent Technologies, Santa Clara, USA). Preparative medium-pressure liquid chromatography (MPLC) was performed on a

Table 2. The inhibitory activities of compounds **1** and **2** against PTPs (IC_{50}: μg/ml).

No.	PTP1B	SHP1	SHP2	LAR
1	1.6	1.6	3.9	NA
2	6.2	5.4	5.3	NA
Ursolic acid[a]	0.8	1.2	1.2	>50

Note: NA, no activity.
[a] Positive control.

Büchi apparatus equipped with Büchi fraction collector C-660, Büchi pump module C-605, and manager C-615 (Büchi, Flawil, Switzerland). Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd, China), RP-18 gel (40–75 μm, Fuji Silysia Chemical Ltd, Kasugai, Aichi, Japan), and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden). Fractions were monitored by TLC (GF_{254}, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China), and spots were visualized by heating silica gel plates immersed in vanillin–H_2SO_4 in EtOH.

3.2 Fungal material and cultivation conditions

Postia sp. was collected from the Bawang Mountains in Hainan Province, China, in September 2006, and authenticated by Prof. Yu-Cheng Dai, Beijing Forestry University, China. Culture medium: glucose 5%, pork peptone 0.15%, yeast powder 0.5%, KH_2PO_4 0.05% and $MgSO_4$ 0.05%. Fermentation was carried out on a shaker at 24°C and 150 rpm for 25 days.

3.3 Extraction and isolation

The culture broth (20 l) was extracted three times with ethyl acetate (EtOAc). The EtOAc layer was evaporated *in vacuo* to give a residue (5.0 g). The residue was subjected to silica gel CC with a gradient elution system of $CHCl_3$–MeOH (from 1:0 to 0:1) to obtain eight fractions (1–8). Fraction 3 (1.2 g) was subjected to preparative MPLC with an RP-C18 column (MeOH–H_2O, from 3:7 to 10:0) to obtain 10 subfractions (3-1–3-10). Fraction 3-4 (180 mg) was separated by silica gel eluted with $CHCl_3$–Me_2CO (from 20:1 to 5:1) to yield fractions 3-4-1–3-4-7. Fraction 3-4-1 (21 mg) was purified by silica gel CC (petroleum ether–EtOAc, 8:1) and Sephadex LH-20 (Me_2CO) to yield **1** (1.3 mg). Fraction 3-4-7 (58 mg) was purified by Sephadex LH-20 (Me_2CO) to give **2** (1.1 mg).

3.3.1 Postinin A (*1*)

White powder; $[\alpha]_D^{24}$ + 13.2 (c = 0.13, MeOH); IR (KBr) ν_{max} 3430, 2955, 2923, 2870, 1630, 1048 cm^{-1}; for ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectral data, see Table 1; HR-EI-MS (positive): *m/z* 234.1619 $[M]^+$ (calcd for $C_{15}H_{22}O_2$, 234.1620).

3.3.2 Postinin B (*2*)

White powder; $[\alpha]_D^{24}$ + 2.3 (c = 0.07, MeOH); IR (KBr) ν_{max} 3431, 2956, 2920, 2853, 1630, 1127 cm^{-1}; for ^1H NMR ($CDCl_3$, 600 MHz) and ^{13}C NMR ($CDCl_3$, 150 MHz) spectral data, see Table 1; HR-EI-MS (positive): *m/z* 254.1884 $[M]^+$ (calcd for $C_{15}H_{26}O_3$, 254.1882).

3.4 Bioactivity assay

Human PTP1B, SHP2, SHP1, and LAR with an N-terminal 6 × His-tag were recombinantly expressed in *Escherichia coli* and purified by Ni-NTA affinity chromatography [11–13]. The enzymatic assay was carried out at room temperature in 96-well plates. After the assay buffer which contained 100 mM of Hepes (pH 6.0), 5 mM of DTT, 0.015% of Brij-35, and PTPase (20 ng PTP1B, 10 ng SHP2, 10 ng SHP2, or 40 ng LAR per well) was incubated with test compound for 15 min, the reaction was initiated by addition of the substrate *p*-nitrophenol phosphate (*p*NPP, Sigma, P4744, St. Louis, MO, USA) at a final concentration of 2 mM. The activity of PTPase-catalyzed hydrolysis of *p*NPP was determined by measuring the amount of *p*-nitrophenol and the absorbance at 405 nm was recorded as the amount of *p*-nitrophenol. The IC_{50} value was determined by the nonlinear curve fitting of the percentage inhibition versus inhibitor con-

centration plot. Ursolic acid (Sigma, U6753) was used for the positive control [14]. All the assays were carried out in triplicate and the average results were presented.

Acknowledgments

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