The Journal of Antibiotics (2019) 72:625–628
https://doi.org/10.1038/s41429-019-0184-x

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BRIEF COMMUNICATION

Three new meroterpenoids from culture broth of *Perenniporia medulla-panis* and their antioxidant activities

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Received: 17 January 2019 / Revised: 7 March 2019 / Accepted: 22 March 2019 / Published online: 25 April 2019

Abstract

Three new meroterpenoids (**1**–**3**) together with one known compound (**4**) were isolated from the culture broth of *Perenniporia medulla-panis*, a wood-rotting fungus in the family Polyporaceae. Their structures were elucidated by NMR and HRESIMS analyses. These compounds exhibited antioxidant activity with IC_{50} values ranging from 12.8 to 190.3 μM in the 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assay.

Perenniporia medulla-panis is a wood-rotting fungus belonging to the family Polyporaceae which comprises ~100 species. It is distributed in all forests around the world [1, 2]. Previous studies have reported that the genus *Perenniporia* produces various secondary metabolites, including naphthalenones, sesquiterpenoids, diterpenoids, and triterpenoids which possess antimicrobial and antifungal activities as well as cytotoxic activity [3–7]. In our search for novel bioactive secondary metabolites from fungal strains, we found that the EtOAc-soluble layer of the culture broth of *P. medulla-panis* showed 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities, leading to the isolation of three new xylopyranosyl meroterpenoids (**1**–**3**), along with one known compound (**4**). In this paper, we report the isolation, structure determination, and antioxidant activities of these compounds (**1**–**4**, Fig. 1).

The fungal strain *P. medulla-panis* (KACC43440) used in this study was received from the National Institute of Agricultural Sciences, Wanju, Republic of Korea. To prepare the culture medium of *P. medulla-panis*, the fungus was grown in potato dextrose agar at 27 °C for 1 week. For preculture, agar plugs of *P. medulla-panis* were then inoculated into a 4 l flask containing 1.8 l of potato dextrose broth. *P. medulla-panis* was cultured in seven flasks under stationary conditions (27 °C and 4 weeks). The culture broth of *P. medulla-panis* (12.6 l) was extracted with acetone at room temperature. The acetone extract was filtered and evaporated under reduced pressure to yield a crude extract. The crude extract was partitioned with EtOAc. The EtOAc-soluble portion (4.5 g) was subjected to silica gel column chromatography eluted with $CHCl_3$–MeOH to yield two active fractions. One (521.0 mg) was separated using reversed-phase medium pressure liquid chromatography (MPLC), followed by reversed-phase HPLC eluted with 42% aqueous MeOH containing 0.04% trifluoroacetic acid to yield three compounds **1** (3.2 mg), **2** (2.1 mg), and **4** (3.1 mg). The other (810.0 mg) was fractionated by MPLC, followed by Sephadex LH-20 column chromatography eluted with MeOH to obtain compound **3** (2.9 mg).

Compound **1** was obtained as a yellow oil with a specific rotation value of −149.0° (25 °C, $c = 0.1$, MeOH). Its molecular formula of $C_{21}H_{26}O_8$ was elucidated from the HRESIMS ion peak at *m/z* 405.1542 $[M\text{-}H]^-$ (calcd for 405.1549, $C_{21}H_{25}O_8$). It displayed UV maxima (log ε) at 203 (4.37) and 289 (4.06)nm. The ^1H NMR spectrum (Table 1) of **1** showed the presence of three aromatic methines at δ 7.05, 6.71, and 6.50, two olefinic methines at δ 7.49 and 5.09, an oxygenated methane at δ 6.37, two methylenes at δ 2.31 and 2.25, and two methyls at δ 1.64 and 1.56. The ^{13}C NMR spectrum (Table 1) of **1** revealed the presence of 16 carbon signals including a carboxyl carbon at δ 176.8, two oxygenated sp^2 quaternary carbons at

Supplementary information The online version of this article (https://doi.org/10.1038/s41429-019-0184-x) contains supplementary material, which is available to authorized users.

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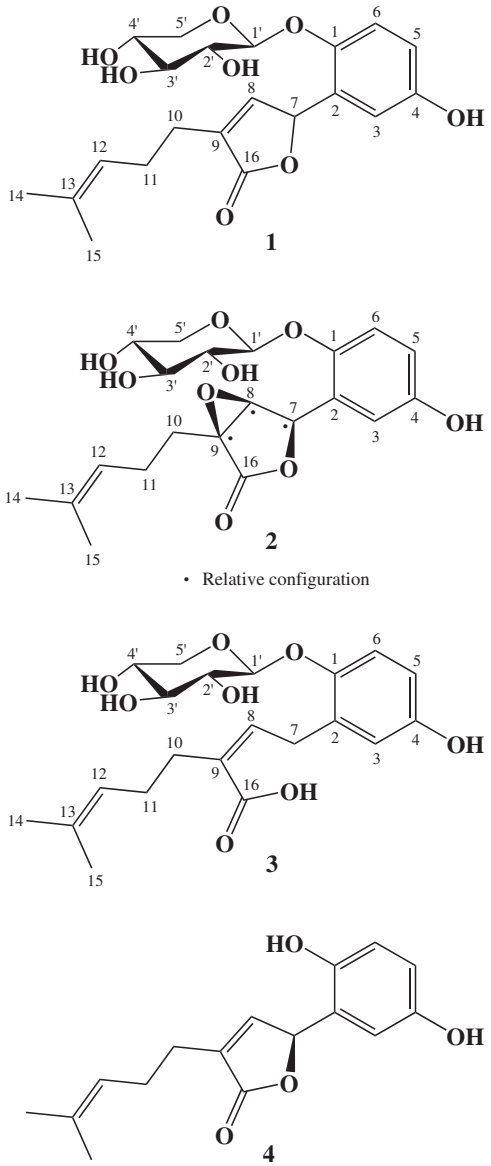

Fig. 1 Structures of compounds **1**–**4**

δ 154.6 and 149.2, three sp^2 quaternary carbons at δ 134.1, 133.0, and 128.1, five sp^2 methine carbons at δ 151.7, 124.1, 119.3, 117.1, and 113.0, an oxygenated methine carbon at δ 79.7, two methylene carbons at δ 27.1 and 26.3, and two methyl carbons at δ 25.9 and 17.9. The ^{1}H and ^{13}C NMR data of **1** were similar to those of fornicin A. The ^{1}H NMR spectrum of **1** also indicated the presence of a sugar unit due to an anomeric proton at δ 4.75, three oxygenated methine protons at δ 3.56, 3.43, and 3.40, and an oxygenated methylene protons at δ 3.91/3.29. The sugar unit of **1** was assigned using ^{1}H NMR coupling patterns and NOESY correlations. The coupling constant (7.3 Hz) of the anomeric proton indicated the presence of a β-form. The coupling constants of H-2′/H-3′, H-3′/H-4′, and H-4′/H-5′ at 9.2 Hz, 8.5 Hz, and 11.5 Hz, respectively, suggested axial–axial relationships. Furthermore, the NOESY correlations of H-1′/H-3′, H-1′/H-5b′, and H-4′/H-5a′ established the presence of β-xylose. The absolute configuration of β-xylose was determined to be a d-configuration using acid hydrolysis, sugar derivatization, and HPLC analysis. Compound **1** (1.0 mg) were hydrolyzed using 2 N CF_3COOH (2 ml) at 90 °C for 3 h. After cooling at room temperature, the residue was extracted with EtOAc to remove aglycone. The aqueous layer, including the sugar unit, was evaporated to dryness under reduced pressure. The sugar unit of **1** was compared with a xylose standard (R_f, 0.43) using silica TLC eluting with $CHCl_3$:MeOH (2:1, v/v). The residue was dissolved in pyridine (100 µl) containing L-cysteine methyl ester hydrochloride (2.0 mg) at 60 °C for 1 h. Then, *o*-tolylisothiocyanate (10 µl) was added and heated at 60 °C for another 1 h. The reaction mixture was immediately analyzed by HPLC. HPLC was performed on a reversed-phase C_{18} column (5 µm, 4.6 mm × 150 mm) with isocratic conditions of 25% ACN containing 0.1% formic acid for 30 min at a flow rate of 1.0 ml min^{-1}, with UV detection at 250 nm. A peak at 13.40 min coincided with a derivative of D-xylose (t_R of L-xylose: 12.45 min) [8–10]. The linkage between the fornicin A unit and the β-D-xylose unit was determined by the HMBC correlation from H-1′ to C-1 (Fig. 2). The stereochemistry of C-7 still remains to be investigated due to its limited amount. Accordingly, the structure of **1** was determined to be a new β-D-xylopyranosyl meroterpenoid and was named perennipin A.

Compound **2** was obtained as a yellow oil with a specific rotation value of −80.0° (25 °C, $c = 0.1$, MeOH). Its molecular formula was established as $C_{21}H_{26}O_9$ based on an HRESIMS ion peak at *m/z* 421.1492 [M-H]$^-$ (calcd for 421.1499, $C_{21}H_{25}O_9$). It exhibited UV maxima (log ε) at 202 (4.36) and 288 (3.47)nm. The ^{1}H and ^{13}C NMR spectra (Table 1) of **2** were closely related to those of **1**, except for the presence of an epoxy unit. The main difference between **1** and **2** was that an olefinic methine proton at δ 7.49, an sp^2 quaternary carbon at δ 133.0, and an sp^2 methine carbon at δ 151.7 in **1** were replaced by an oxygenated methine proton at δ 4.37, an oxygenated quaternary carbon at δ 60.3, and an oxygenated methine carbon at δ 64.8 in **2**. Furthermore, the HMBC correlations from H-11 to C-9, from H-10 to C-9, from H-8 to C-7, and from H-7 to C-8 suggested the presence of an epoxy unit (Fig. 2). The relative configuration of **2** was determined by selective 1D NOE and NOESY experiments. Irradiation at a resonance frequency of H-8 produced a strong NOE peak with H-7. Furthermore, NOESY correlations of H-7/H-8 and H-8/H-10 indicated the same face of these protons. The β-xylose unit in **2** was elucidated by axial–axial coupling constants of H-2′, H-3′, H-4′, and H-5′ and an anomeric proton of 7.5 Hz coupling constant. The absolute configuration of the β-xylose unit was determined to be a d-configuration by the same methods as in

Table 1 ^{1}H and ^{13}C NMR spectral data of compounds **1–3** in CD_3OD[a]

No.	1		2		3	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	149.2		149.0		150.1	
2	128.1		127.6		133.1	
3	113.0	6.50 (d, $J = 2.9$)[b]	113.9	6.60 (d, $J = 2.9$)	117.7	6.62 (d, $J = 2.9$)
4	154.6		154.6		154.0	
5	117.1	6.71 (dd, $J = 8.7$, 2.9)	116.0	6.76 (dd, $J = 8.8$, 2.9)	114.6	6.57 (dd, $J = 8.8$, 2.9)
6	119.3	7.05 (d, $J = 8.7$)	119.2	7.08 (d, $J = 8.8$)	118.9	6.94 (d, $J = 2.9$)
7	79.7	6.37 (d, $J = 1.2$)	78.0	5.76 (s)	31.3	3.77 (m)
8	151.7	7.49 (d, $J = 1.2$)	64.8	4.37 (s)	140.5	5.97 (t, $J = 7.5$)
9	133.0		60.3		133.7	
10a	26.3	2.31 (m)	26.5	2.14 (m)	36.1	2.26 (dd, $J = 7.5$, 7.2)
10b				1.80 (m)		
11a	27.1	2.25 (m)	24.0	2.17 (m)	28.8	2.11 (m)
11b				2.07 (m)		
12	124.1	5.09 (m)	123.7	4.98 (m)	124.8	5.08 (m)
13	134.1		134.3		133.2	
14	25.9,	1.64 (s)	25.7	1.53 (s)	25.9	1.63 (s)
15	17.9	1.56 (s)	17.8	1.52 (s)	17.9	1.56 (s)
16	176.8		174.7		172.2	
1′	105.1	4.75 (d, $J = 7.3$)	105.0	4.80 (d, $J = 7.5$)	105.0	4.67 (d, $J = 7.2$)
2′	75.0	3.43 (dd, $J = 9.2$, 7.3)	74.8	3.46 (dd, $J = 8.9$, 7.5)	75.0	3.43 (dd, $J = 8.2$, 7.2)
3′	78.1	3.40 (dd, $J = 9.2$, 8.5)	78.1	3.40 (t, $J = 8.9$)	78.0	3.40 (t, $J = 8.2$)
4′	71.1	3.56 (m)	71.1	3.57 (m)	71.2	3.56 (m)
5a′	67.1	3.91 (dd, $J = 11.5$, 5.3)	67.2	3.91 (dd, $J = 11.5$, 5.5)	67.1	3.88 (dd, $J = 11.4$, 5.3)
5b′		3.29 (m, overlap)		3.28 (m, overlap)		3.26 (dd, $J = 11.4$, 10.3)

[a]NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C

[b]Proton multiplicity and coupling constants in parenthesis

1. Thus, the structure of **2** was determined to be a new *β*-D-xylopyranosyl meroterpenoid and was named perennipin B.

Compound **3** was isolated as a yellow oil with a specific rotation value of −70.0° (25 °C, $c = 0.1$, MeOH). Its molecular formula was determined to be $C_{21}H_{28}O_8$ based on a HRESIMS ion peak at *m/z* 407.1701 $[M\text{-}H]^-$ (calcd for 407.1706, $C_{21}H_{27}O_8$). It exhibited UV maxima (log ε) at 204 (4.51) and 287 (3.47)nm. The ^{1}H and ^{13}C NMR spectra (Table 1) of **3** were similar to those of **1**, except for the five-membered ring unit. The ^{1}H NMR spectrum of **3** exhibited an olefinic proton at *δ* 5.97 and a methylene proton at *δ* 3.77. In addition, the carboxylic acid unit of C-16 was determined by the HMBC correlations from H-10 to C-9 and C-16 and from H-8 to C-16. Therefore, the planar structure of **3** was determined as shown in Fig. 2. The *β*-xylose unit was established by the coupling constant (7.2 Hz) of the anomeric proton, the axial–axial coupling constants (7–12 Hz) of H-2′/H-3′, H-3′/H-4′, and H-4′/H-5′, and the NOESY correlations of H-1′/H-3′, H-1′/H-5b′, and H-4′/ H-5a′. The absolute configuration of the *β*-xylose unit was determined to be a d-configuration by the same methods as in **1**. Hence, compound **3** was identified as a new *β*-D-xylopyranosyl meroterpenoid and was named perennipin C.

Compound **4** was identified as (+)-fornicin A by comparison of the ^{1}H NMR spectroscopic data and positive specific rotation (+6.6°, 24 °C, $c = 0.1$, MeOH) with previously reported in the literature [11, 12].

The antioxidant activities of compounds **1–4** were evaluated using 2,2-azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assays as previously described [13–15]. The ABTS and DPPH radical-scavenging assays were performed in triplicate. As positive controls, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and butylated hydroxyl-anisole (BHA) were used. Compounds **1–3** displayed no DPPH radical-scavenging activity at a concentration of 400 μM. However, compound **4** showed significant DPPH radical-scavenging activity with an IC_{50} value of 106.0 μM. The positive controls, BHA and Trolox, showed IC_{50} values of 139.3 and 80.9 μM, respectively (Table 2). Interestingly, compounds **1–4** showed antioxidant activity against ABTS radical-scavenging activity with IC_{50} values ranging from 12.8 to 190.3 μM. Compound **4** showed much higher ABTS radical-scavenging activity than **1** and **2**, indicating that the attachment between the *β*-D-xylose unit and the meroterpenoid unit decreased the ABTS radical-scavenging activity. However, the double bond at C-8 and C-9 in **1** exhibited significant ABTS radical-scavenging activity compared to the epoxy unit at C-8 and C-9 in **2**. Furthermore, the presence of carboxylic acid

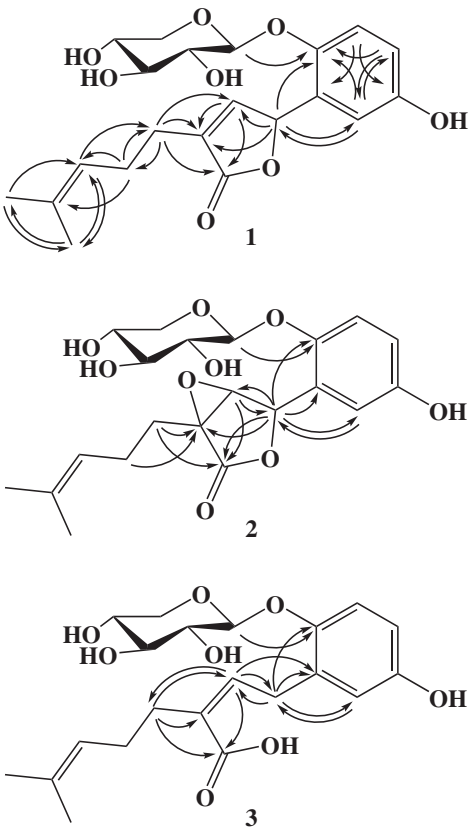

Fig. 2 HMBC correlations of compounds **1**–**3**

Table 2 ABTS and DPPH radical-scavenging activities of compounds **1**–**4**

Compounds	IC_{50} (μM)[a]	
	ABTS	DPPH
1	64.8 ± 8.6	Inactive[b]
2	190.3 ± 13.0	Inactive
3	12.8 ± 0.5	Inactive
4	39.0 ± 0.9	106.0 ± 2.8
BHA	22.3 ± 2.7	139.3 ± 2.3
Trolox	20.1 ± 2.6	80.9 ± 1.9

[a]Results were performed in triplicate ($n = 3$) ± SD

[b]No activity at the concentration of 400 μM

at C-16 in **3** significantly increased the ABTS radical-scavenging activity with an IC_{50} value of 12.8 μM, compared to that of **1** (64.8 μM). Compounds **1**–**4** exhibited no antibacterial activity at a concentration of 100 μg/disk against *Bacillus subtilis*, *Escherichia coli*, *Propionibacterium acnes*, and *Staphylococcus aureus*.

Acknowledgements This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future Planning (NRF-2016R1A2B2014430). The authors thank Ms Ji-Young Oh, Center for University Research Facility (CURF) at Chonbuk National University, for NMR measurement.

Compliance with ethical standards

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

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