The Journal of Antibiotics (2016) 69, 400–402
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www.nature.com/ja

NOTE

New *p*-terphenyls from the fruiting bodies of *Pseudomerulius curtisii* and their antioxidant activity

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The Journal of Antibiotics (2016) **69,** 400–402; doi:10.1038/ja.2015.117; published online 25 November 2015

Reactive oxygen species is one of the main factors that cause the oxidation of polyunsaturated fatty acid in the surface of biomembranes, and it has been associated with the beginning of many diseases and the degenerative processes of aging.[1–4] Accumulating excess reactive oxygen species damages lipids, proteins, carbohydrates and DNA, leading to oxidative stress, loss of cell function and apoptosis or necrosis.[5,6] These biochemical processes are common in various diseases, such as cancers, inflammation, atherosclerosis and cardiovascular disease.[7] An intake of antioxidants has been posited to reduce the risk of developing some of these pathologies related with oxidative stress.[8] During the search for natural antioxidants, we found that the fruiting bodies of *Pseudomerulius curtisii* (previous name, *Paxillus curtisii*) in the family *Tapinellaceae* exhibited potent free radical scavenging activity.[9] *P. curtisii*, a brown-rot fungus, is distinguished by its characteristic cinnamon odor and is difficult to find due to its rarity in nature. It is known to produce diverse *p*-terphenyls, curtisians A-V, that were reported as both antioxidants and neuroprotectants.[10–15] In our continuous study, three new *p*-terphenyls (**1–3**, Figure 1) were isolated from the fruiting bodies of *P. curtisii* and characterized.

The fruiting bodies of *P. curtisii* were ground, and extracted twice with MeOH at room temperature. After removal of the MeOH under reduced pressure, the resulting extract was partitioned between ethyl acetate and water. The ethyl acetate extract was chromatographed on a column of silica gel eluting with a gradient of increasing methanol (2–50%) in chloroform to afford antioxidant fractions. The fractions were collected, concentrated under reduced pressure and subjected to a column of Sephadex LH-20 eluting with chloroform–methanol (1:1, v/v) to give an active fraction. The active fraction was re-chromatographed on a Sephadex LH-20 column eluting with methanol, followed by C_{18} Sep-pak cartridge with a gradient of increasing methanol (25–100%) in water. Finally, an antioxidant fraction showing free radical scavenging activity was separated by a reversed-phase preparative HPLC eluted with a gradient of increasing methanol (40–60%) in water to afford three active compounds **1** (29.8 mg), **2** (18.6 mg) and **3** (2.4 mg).

Compound **1** was obtained as a dark brown powder with the specific rotation value of +39.2° ($[\alpha]_D$; *c* 2.63, MeOH) and exhibited UV maxima (log ε) at 260 (4.3) and 205 (4.8) nm. Its molecular formula of $C_{34}H_{30}O_8$ was established by high resolution FAB-mass spectrometry (*m/z* 589.1838 $[M+Na]^+$, Δ+0.2 mmu). The ^1H NMR spectrum of **1** in CD_3OD exhibited signals due to five methines in the aromatic region of δ 7.02–7.20 assignable to a phenyl group, eight aromatic methines at δ 7.08 and 6.82 attributable to two 1,4-disubstituted benzenes, four methylenes at δ 2.67, 2.47, 2.33/2.21 and 2.24/2.14, two methines at δ 5.07 and 2.84, and one methyl at δ 1.60 (Table 1). In the ^{13}C NMR spectrum, two carbonyl carbons at δ 175.8 and 172.5, six oxygenated sp^2 quaternary carbons at δ 158.1, 158.1, 142.5, 142.4, 134.8 and 134.7, six sp^2 quaternary carbons at δ 141.7, 139.5, 124.9, 124.9, 123.9 and 123.8, 14 sp^2 methine carbons

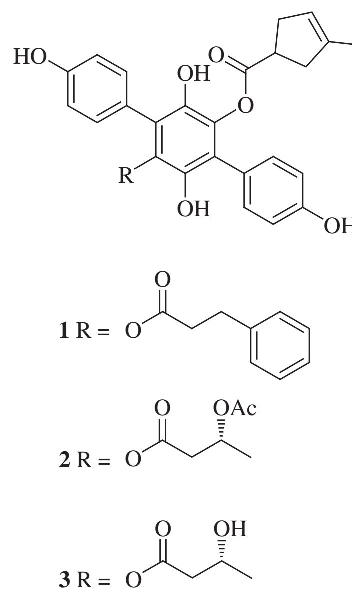

Figure 1 Structures of compounds **1**–**3**.

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Received 28 July 2015; revised 30 September 2015; accepted 19 October 2015; published online 25 November 2015

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

	1		*2*		*3*
No.	δ_C	δ_H	δ_C	δ_H	δ_H
1,1"	124.9		124.8, 124.9		
2,6,2",6"	132.6, 132.7	7.08 (d, *J*=8.4)[b], 7.08 (d, *J*=8.4)	132.7	7.18 (d, *J*=8.4), 7.14 (d, *J*=8.4)	7.18 (d, *J*=8.4), 7.14 (d, *J*=8.4)
3,5,3",5"	115.9, 116.0	6.82 (d, *J*=8.4), 6.82 (d, *J*=8.4)	116.0, 116.1	6.83 (d, *J*=8.4), 6.82 (d, *J*=8.4)	6.80 (d, *J*=8.4), 6.79 (d, *J*=8.4)
4,4"	158.1		158.2, 158.3		
1',4'	123.8, 123.9		123.9, 124.0		
2',5'	134.7, 134.8		134.5, 134.8		
3',6'	142.4, 142.5		142.4, 142.7		
1a	175.8		175.8		
2a	42.9	2.84 (m)	43.0	3.06 (m)	3.10 (m)
3a	40.9	2.24 (m), 2.14 (m)	40.9	2.39 (m), 2.20 (m)	2.39 (m), 2.20 (m)
4a	139.5		139.5		
5a	123.2	5.07 (br. s)	123.2	5.12 (br. s)	5.10 (br. s)
6a	37.0	2.33 (m), 2.21 (m)	37.0	2.45 (m), 2.33 (m)	2.46 (m), 2.31 (m)
7a	16.2	1.60 (s)	16.1	1.63 (s)	1.63 (s)
1b	172.5		169.8		
2b	36.1	2.47 (t, *J*=7.2)	40.5	2.59 (dd, *J*=16.4, 6.4) 2.42 (dd, *J*=16.4, 5.6)	2.42 (m), 2.33 (m)
3b	32.3	2.67 (t, *J*=7.2)	68.1	5.00 (m)	3.95 (m)
3b–Ac			21.1, 172.0	1.92 (s)	
4b	141.7		19.6	1.06 (d, *J*=6.4)	0.98 (d, *J*=6.4)
5b	129.4	7.02 (m)			
6b	129.5	7.20 (t, *J*=6.8)			
7b	127.2	7.07 (m)			
8b	129.5	7.20 (t, *J*=6.8)			
9b	129.4	7.02 (m)			

[a]All spectra were recorded at 400 MHz for proton and at 100 MHz for carbon.
[b]Proton resonance multiplicity and coupling constant (*J*=Hz) in parenthesis.

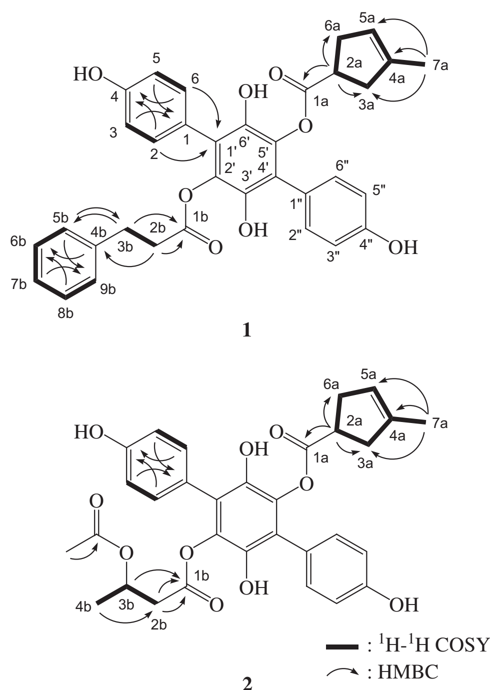

Figure 2 ^{1}H-^{1}H COSY and HMBC correlations of compounds **1** and **2**.

at δ 132.7 (×2), 132.6 (×2), 129.5 (×2), 129.4 (×2), 127.2, 123.2, 116.0 (×2) and 115.9 (×2), a methine carbon at δ 42.9, four methylene carbons at δ 40.9, 37.0, 36.1 and 32.32 were evident (Table 1). These spectral data suggested that **1** was a *p*-terphenyl derivative common in the fruiting bodies of *P. curtisii*.

Table 2 Antioxidant activities of compounds 1–3.

	IC_{50} (μM)[a]	
Compounds	*ABTS*[b]	*DPPH*[c]
Compound **1**	47.8±3.4	287.1±4.0
Compound **2**	83.0±0.4	239.0±3.5
Compound **3**	239.9±4.3	>400.0
BHA[d]	17.4±2.3	114.9±3.8
Trolox	24.0±1.2	65.8±3.3

[a]IC_{50} values represent the concentration that caused a 50% loss of antioxidant activity.
[b]2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).
[c]α,α-Diphenyl-β-picrylhydrazyl.
[d]Butylated hydroxyanisole.

All proton-bearing carbons were assigned by interpretation of the HMQC spectrum. Interpretation of ^{1}H-^{1}H COSY and HMBC spectral data of **1**, along with ^{1}H and ^{13}C NMR data, suggested the presence of a phenylpropionyl group (δ_H 7.20, 7.07, 7.02, 2.67, 2.47; δ_C 172.5, 141.7, 129.5, 129.4, 127.2, 36.1, 32.3) and 3-methylcyclopent-3-enecarboxyl (δ_H 5.07, 2.84, 2.33, 2.24, 2.21, 2.14, 1.60; δ_C 175.8, 139.5, 123.2, 43.0, 40.9, 37.0, 16.1) in **1** (Figure 2). The phenylpropionyl moiety was established by the ^{1}H–^{1}H COSY spectrum and HMBC correlations, which showed the long-range correlations from the methylene protons at δ 2.67 to the carbon at δ 129.4 and from the methylene protons at δ 2.47 and 2.67 to the carbonyl carbon at δ 172.5. The 3-methylcyclopent-3-enecarboxyl moiety was also

established by the ^{1}H-^{1}H COSY correlations between H-2a to H-6a and HMBC correlations from the methyl protons at δ 1.60 to the carbons at δ 139.5, 123.2, and 40.9 and from the methine proton at δ 2.84 to the carbonyl carbon at δ 175.8. Aromatic methine protons at δ 7.08 showed the long-range correlation to the carbons at δ 158.1 (C-4, C-4″) and 123.8/123.9 (C-1′, C-4′), suggesting the presence of two *p*-hydroxybenzenes. These partial structures and comparison of the ^{1}H and ^{13}C NMR spectra of **1** with those of known *p*-terphenyls indicated that the structure of **1** was a new *p*-terphenyl derivative substituted with phenylpropionyl and 3-methylcyclopent-3-enecarboxyl groups on the central aromatic ring.

Compound **2** was obtained as a dark brown powder with the specific rotation value of +15.3° ($[\alpha]_D$; *c* 1.54, MeOH) and exhibited UV maxima (log ε) at 260 (4.3) and 204 (4.7) nm. Its molecular formula $C_{31}H_{30}O_{10}$ was established by high resolution FAB-mass spectrometry (*m/z* 563.1918 $[M+H]^+$, Δ 0.0 mmu). The ^{1}H and ^{13}C NMR spectra suggested that **2** was also a *p*-terphenyl derivative. Interpretation of ^{1}H–^{1}H COSY and HMBC spectra, along with ^{1}H and ^{13}C NMR data, suggested the presence of a 3-acetoxybutyryl group (δ_H 5.00, 2.59, 2.42, 1.92, 1.06; δ_C 172.0, 169.8, 68.1, 40.5, 21.1, 19.6) and 3-methylcyclopent-3-enecarboxyl group (δ_H 5.12, 3.06, 2.45, 2.39, 2.33, 2.20, 1.63; δ_C 175.8, 139.5, 123.2, 43.0, 40.9, 37.0, 16.1) in **2** (Figure 2).

A partial structure, 3-acetoxybutyryl moiety, was secured by ^{1}H–^{1}H COSY correlations between H-2b to H-4b and HMBC correlations from the methylene protons (H-2b) at δ 2.59/2.43 and the methine proton (H-3b) at δ 5.00 to the carbonyl carbon at δ 169.8 and from the methyl protons at δ 1.92 and the methine proton at δ 5.00 to the carbonyl carbon at δ 172.0. The 3-methylcyclopent-3-enecarboxyl moiety was established by the ^{1}H-^{1}H COSY correlations between H-2a to H-6a and HMBC correlations from the methyl protons at δ 1.63 to the carbons at δ 139.5, 123.2 and 40.0 and from the methine proton at δ 3.06 to the carbonyl carbon at δ 175.8. Other ^{1}H and ^{13}C NMR peaks were in good agreement with those of compound **1**, establishing that **2** was a new *p*-terphenyl derivative that the phenylpropionyl group in **1** was replaced with the 3-acetoxybutyryl group.

Compound **3** was obtained as a dark brown powder with the specific rotation value of –75.6° ($[\alpha]_D$; *c* 0.21, MeOH) and exhibited UV maxima (log ε) at 264 (4.2) and 203 (4.6) nm. Its molecular formula of $C_{29}H_{28}O_9$ was established by high resolution FAB-mass spectrometry (*m/z* 521.1812 $[M+H]^+$, Δ 0.0 mmu). The ^{1}H NMR spectrum showed the signals due to *p*-terphenyl moiety at δ 7.18, 7.14, 6.80 and 6.79 and a 3-methylcyclopent-3-enecarboxyl group at δ 5.10, 3.10, 2.46, 2.39, 2.31, 2.20 and 1.63 (Table 1). In addition to these partial structures, a methine at δ 3.95, a methylene at δ 2.42/2.33, and a methyl at δ 0.98 attributable to a 3-hydroxybutryl group were evident (Table 1). Comparison of the ^{1}H NMR spectrum with that of compound **2**, in combination with the molecular formula, indicated that the 3-acetoxybutyryl group in **2** was replaced with a 3-hydroxybutryl group in **3**. Thus, the structure of **3** was determined as a new *p*-terphenyl substituted with a 3-methylcyclopent-3-enecarboxyl and a 3-hydroxybutyryl groups on the central aromatic ring.

The absolute configurations of the 3-acetoxybutyryl and 3-hydroxybutyryl substituents in known curtisians were reported to be *S* through saponification, methylation and acetylation procedures.[13,14] From the co-occurrence of known curtisians I-S, the absolute configurations of the 3-acetoxybutyryl in **2** and 3-hydroxybutryl in **3** were deduced to be *S*. However, the 3-methylcyclopent-3-enecarboxyl group in **1–3** remains to be determined.

The antioxidant activity of compounds 1–3 was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and ABTS (the 2,2`-azinobis[3-ethylbenzothiazoline-6-sulfonate]) radical scavenging assay methods.[14] The DPPH and ABTS radical scavenging activities were defined as the amount of antioxidant necessary to decrease the initial radical concentration by 50% (IC_{50}). Although compounds **1–3** were less active than Trolox and butylated hydroxyanisole, which were used as positive controls, they showed significant antioxidant activities in a dose-dependent manner. Compound **1** exhibited antioxidant activity with IC_{50} values of 47.8 μM in the ABTS radical scavenging assay. However, compounds **2** and **3** with IC_{50} values of 83.0 and 239.9 μM, respectively, were less active than compound **1**. Compounds **1–3** showed moderate DPPH radical scavenging activities (Table 2).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a Grant from the Korea Forest Service and by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2013062358), Republic of Korea. This research was supported by 'Research Base Construction Fund Support Program' funded by the Chonbuk National University in 2015.

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