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BRIEF COMMUNICATION

New antioxidants from the culture broth of *Hericium coralloides*

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Abstract

In our effort to find antioxidants from the higher fungi, we isolated three new compounds (**1**–**3**) with a known compound, spirobenzofuran (**4**), from the culture broth of *Hericium coralloides*. Bioassay-guided fractionation led to the isolation of these compounds, and we determined the chemical structures through spectroscopic methods. These compounds exhibited antioxidant activity in the range of IC_{50} values of 29-66 μM in the 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assay.

Free radicals and reactive oxygen species have been recognized to be triggered in the leading role of various diseases including cardiovascular disease, inflammation, skin-aging, and diabetes [1–4]. Antioxidants can provide a crucial role in the defense against the development of oxidative related disorders.

In our ongoing effort to search for new natural antioxidants from the fungal metabolite, we found that the culture broth of *H. coralloides* showed significant free radical-scavenging activity. *H. coralloides* is an edible mushroom, which belongs to the family Hericiaceae and people use it as a traditional food and medicine in Korea and China. It is mainly distributed in Asia, Europe, and North America. *H. coralloides* has been reported to produce corallocins A–C, inducers of nerve growth factor and brain-derived neurotrophic factor, nematicidal fatty acids, and novel laccase [5–7]. In this study, we describe the isolation and structure determination of three new antioxidative compounds (**1**–**3**, Fig. 1) along with one known compound, spirobenzofuran (**4**), as well as their antioxidant activities.

We cultured a fungal strain of *H. coralloides* at 27 °C for 4 weeks with agitation of 120 rpm in a 1 L flask containing 400 mL of potato dextrose broth medium. We extracted the cultured broth with acetone at room temperature followed by filtration and concentration of the filtrate under reduced pressure to remove acetone, and partitioned the resultant with ethyl acetate. We subjected the EtOAc-soluble layer (199.0 mg) to reversed-phase medium pressure liquid chromatography eluted with a gradient of increasing methanol in water (10–95% aq. MeOH) to provide an active fraction (100.5 mg). The active fraction was further separated through the preparative reversed-phase High-performance liquid chromatography (HPLC) using 28% aq. methanol to afford four compounds **1** (4.8 mg), **2** (2.0 mg), **3** (1.5 mg), and **4** (15.0 mg).

Compound **4** was identified as spirobenzofuran by comparing NMR spectroscopic data with previously published data [8, 9]. Although spirobenzofuran was totally synthesized, its absolute stereochemistry still remains unknown [10]. In this study, we determined the absolute stereochemistry via application of the modified Mosher's methods[11, 12]. Spirobenzofuran (4 mg) was treated with (*R*)-(-)-α-methoxy−α-(trifluoromethyl)phenylacetic acid chloride (8 μL) and 4-(dimethylamino)pyridine (1 crystal) in pyridine (300 μL) at room temperature for 24 h. The mixture was evaporated to dryness under reduced pressure. The product was further separated by reversed-phase HPLC eluted with a gradient of 70–100% aq. MeOH to obtain bis-(*S*)-MTPA ester (3 mg). The bis-(*R*)-MTPA ester (1.6 mg) was analyzed using the same procedures. Analysis of $\Delta\delta_H$ (δ_S–δ_R) values revealed that the absolute configuration of H-12 was *R* (see supplementary information).

Compound **1** was obtained as a colorless oil with the specific rotation value of −145.3° ($[\alpha]_D$; 25 °C, $c = 0.1$, MeOH) and exhibited UV maxima (log ε) at 299 (3.3) and

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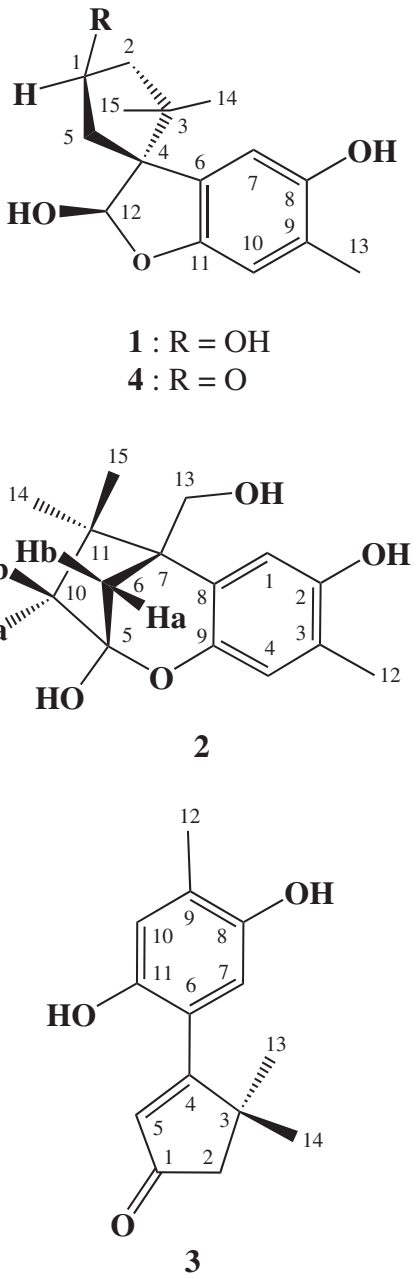

Fig. 1 Structures of compounds **1–4**

202 (4.1) nm. Its molecular formula was determined as $C_{15}H_{20}O_4$ through a high-resolution electron ionization (EI) mass measurement (*m/z* 264.1362 (M^+), Δ 0.0 mmu). The ^{1}H NMR spectrum of **1** showed signals due to two aromatic methine at δ 6.75 and 6.49, one acetal at δ 5.56, one oxygenated methine at δ 4.43, two methylenes at δ 2.77/1.93 and 1.97/1.76, and three methyls at δ 2.14, 0.88, and 0.84. In the ^{13}C NMR spectrum, the following were evident as we can see in Table 1: total 15 carbons including two oxygenated sp^2 quaternary carbons at δ 152.4 and 150.2, two sp^2 quaternary carbons at δ 129.6 and 125.3, two

Table 1 ^{1}H and ^{13}C NMR spectral data for 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
1	70.6	4.43 (m) [b]	114.4	6.68 (s)	211.5	
2	50.0	1.97 (dd, 13.7, 8.2)	149.3		53.6	2.43 (s)
		1.76 (dd, 13.7, 5.1)				
3	45.0		125.1		46.1	
4	63.8		118.7	6.47 (s)	184.7	
5	40.4	2.77 (dd, 14.4, 5.1)	106.2		132.2	6.22 (s)
		1.93 (dd, 14.4, 8.2)				
6a	129.6		41.3	1.99, (dd, 11.6, 1.3)	120.5	
6b				2.27, (d, 11.6)		
7	113.8	6.75 (s)	53.7		115.5	6.72 (s)
8	150.2		127.3		148.9	
9	125.3		148.1		128.7	
10a	112.1	6.49 (s)	55.9	1.91 (dd, 14.4, 1.3)	119.5	6.64 (s)
10b				1.83 (d, 14.4)		
11	152.4		46.4		149.1	
12	104.6	5.56 (s)	16.2	2.11 (s)	16.4	2.15 (s)
13	16.7	2.14 (s)	63.2	4.01 (d, 11.0)	28.4	1.36 (s)
				3.80 (d, 11.0)		
14	26.0	0.84 (s)	27.0	0.74 (s)	28.4	1.36 (s)
15	24.5	0.88 (s)	27.5	1.10 (s)		

[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

sp^2 methine carbons at δ 113.8 and 112.1, one acetal carbon at δ 104.6, one oxygenated methine carbon at δ 70.6, two quaternary carbons at δ 63.8 and 45.0, two methylene carbons at δ 50.0 and 40.4, and three methyl carbons at δ 26.0, 24.5, and 16.7. In the evidence of ^{1}H and ^{13}C NMR spectra, compound **1** was close to that of spirobenzofuran (**4**), except for the replacement of the keto group at C-1 of spirobenzofuran with the hydroxyl group. The ^{1}H-^{1}H COSY spectrum displaying the presence of one structural fragment (H-5/H-1/H-2) supports this. The critical HMBC correlations from H-2 and H-5 to the methine carbon at δ 70.6 (C-1) were evident (Fig. 2). Thus, we determined the structure of **1** as a new spirobenzofuran derivative, and this compound was named hydrospirobenzofuran. The relative configuration of **1** was determined by nuclear Overhauser effect (NOE) correlations between H-1 and H-15, H-12 and H-15. Although the absolute configuration of compound **1** remains to be determined due to the limited amount of the compound available, it appears to be a reduced form of

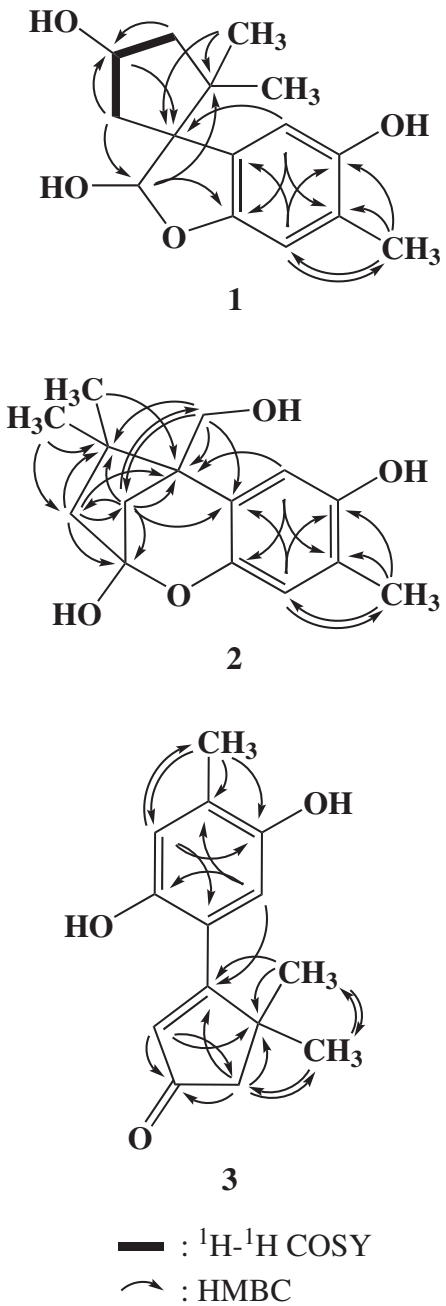

Fig. 2 Two-dimensional NMR correlations of compounds **1–3**

spirobenzofuran at C-1. Thus, the proposed stereochemistry is illustrated in Fig. 1.

Compound **2** was obtained as a colorless oil with the specific rotation value of +53.4° ($[\alpha]_D$; 25 °C, $c = 0.1$, MeOH) and exhibited UV maxima (log ε) at 297 (4.2) and 208 (4.7) nm. Its molecular formula was determined as $C_{15}H_{20}O_4$ via high-resolution EI mass measurement (*m/z* 264.1361 (M^+), Δ−0.1 mmu). The ^{1}H NMR spectrum of **2** revealed signals for aromatic methines at δ 6.68 and 6.47, one oxygenated methylene at δ 4.01/3.80, two methylenes at δ 2.27/1.99 and 1.91/1.83, and three methyls at δ 2.11, 1.10, and 0.74. In the ^{13}C NMR spectrum, the following were evident as we can see in Table 1: two oxygenated sp^2 quaternary carbons at δ 149.3 and 148.1, two sp^2 quaternary carbons at δ 127.3 and 125.1, two sp^2 methine carbons at δ 118.7 and 114.4, one ketal carbon at δ 106.2, one oxygenated methylene carbon at δ 63.2, two methylene carbons at δ 55.9 and 41.3, two quaternary carbons at δ 53.7 and 46.4, and three methyl carbons at δ 27.5, 27.0, and 16.2. We elucidated the presence of five-membered ring by HMBC correlations from H-10 to C-5, C-6, C-7, and C-11, from H-14 and H-15 to C-7, C-10, and C-11, from H-6 to C-10, and C-11, and from H-13 to C-7 and C-11. The dihydrobenzopyran moiety was suggested using the long-range correlations from H-1 to C-3 C-7, and C-9, from H-4 to C-2 and C-8, from H-6 to C-5, C-7, and C-8, and from H-13 to C-6, C-7, and C-8. Finally, through elimination, we connected C-5 to C-9 by ethereal linkage. Other HMBC correlations from H-13 to C-6 and C-8 and from H-12 to C-2, C-3, and C-4 supported the structure of **2**, as shown in Fig. 2. Therefore, we determined the structure of compound **2** to be a new sesquiterpene with dihydrobenzopyran moiety and named sesquibenzopyran. Proton signals were completely assigned by the NOESY spectrum, which showed NOE correlations between H-6b and H-15, H-15 and H-10b, H-13 and H-15, and H-6b and H-10b.

Compound **3** was obtained as a brown oil with UV maxima (log ε) at 360 (3.2), 291 (3.4), 221 (3.9), and 202 (4.1) nm. Its molecular formula was determined as $C_{14}H_{16}O_3$ via a high-resolution EI mass measurement (*m/z* 232.1096 (M^+), Δ−0.3 mmu). The ^{1}H NMR spectrum of **3** exhibited signals due to two aromatic methines at δ 6.72 and 6.64, one olefinic methine at δ 6.22, one methylene at δ 2.43, and three methyls at δ 2.15 and 1.36, and 1.36. In the ^{13}C NMR spectrum, the following results were obtained (Table 1): 14 carbon peaks including one ketone carbon at δ 211.5, two oxygenated sp^2 quaternary carbons at δ 149.1 and 148.9, three sp^2 quaternary carbons at δ 184.7, 128.7, and 120.5, three sp^2 methine carbons at δ 132.2, 119.5, and 115.5, one methylene carbon at δ 53.6, one quaternary carbon at δ 46.1, and three methyl carbons at δ 28.4 and 16.4 (× 2). We determined the chemical structure using the HMBC spectrum, which showed the long-range correlations from H-7 to C-9 and C-10, from H-10 to C-6 and C-8, and from H-12 to C-8, C-9, and C-10 indicated the presence of 2,5-dihydroxy-4-methylphenyl. Long-range correlations from H-13 and 14 to C-2, C-3, and C-4, from H-2 to C-1, C-3, and C-4, and from H-5 to C-1, C-2, and C-3 established the presence of a cyclopentenone moiety. Finally, we connected C-4 to C-6 using the long-range correlation of H-7 with C-4, suggesting that the compound **3** was a new cuparene derivative. This compound was named coralcuparene.

Table 2 Free radical-scavenging activities of compounds **1**–**4**

Compounds	IC_{50} (μM)[a]	
	ABTS[b]	DPPH[c]
1	66.0 ± 2.4	121.0 ± 7.9
2	29.0 ± 1.8	87.3 ± 3.8
3	62.5 ± 1.1	118.9 ± 1.3
4	29.6 ± 1.5	90.8 ± 9.6
BHA	18.5 ± 1.6	78.3 ± 2.0
Trolox	25.2 ± 0.7	55.7 ± 6.8

[a] Results presented as the mean ($n = 3$) ± SD

BHA (butylated hydroxyanisole) and Trolox were used as positive controls

[b] 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)

[c] α,α-diphenyl-β-picrylhydrazyl (DPPH)

We evaluated the antioxidant activity of compounds **1**–**4** using the ABTS and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay methods. We did the free radical-scavenging assay via the methods described in the previous literature [13] and used antioxidants, butylated hydroxyanisole and Trolox as a positive control. All compounds exhibited antioxidant activity in a dose-dependent manner. Compounds **1**–**4** displayed potent antioxidant activity in the range of IC_{50} values of 29–66 μM in the ABTS radical-scavenging assay. These compounds also showed significant antioxidant activity in the range of IC_{50} values of 87.3–121.0 μM in the DPPH radical-scavenging assay (Table 2). Compounds **2** and **4** were comparable to Trolox in the ABTS radical-scavenging assay. Antimicrobial activity was determined by the conventional paper disk (Advantec, 8 mm in diameter) method. All compounds showed no antimicrobial activity up to 100 μg/disk against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Botrytis cinerea*, and *Rhizoctonia solani*.

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Compliance with ethical standards

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

References

1. Adegbola P, Aderibigbe I, Hammed W, Omotayo T. Antioxidant and anti-inflammatory medicinal plants have potential role in the treatment of cardiovascular disease: a review. Am J Cardiovasc Dis. 2017;7:19–32.
2. Arulselvan P, et al. Antioxidants in the treatment of diabetes. Curr Diabetes Rev. 2011;7:106–25.
3. Furue M, et al. Antioxidants for healthy skin: the emerging role of aryl hydrocarbon receptors and nuclear factor-erythroid 2-related factor-2. Nutrients. 2017;9:e223.
4. Aouacheri O, Saka S, Krim M, Messaadia A, Maidi I. The investigation of the oxidative stress-related parameters in type 2 diabetes mellitus. Can J Diabetes. 2015;39:44–9.
5. Wittstein K, Rascher M, Rupcic Z, Löwen E, Winter B, Köster RW, Stadler M. Corallocins A-C, nerve growth and brain-derived neurotrophic factor inducing metabolites from the mushroom Hericium coralloides. J Nat Prod. 2016;79:2264–9.
6. Stadler M, Mayer A, Anke H, Sterner O. Fatty acids and other compounds with nematicidal activity from cultures of Basidiomycetes. Planta Med. 1994;60:128–32.
7. Zou YJ, Wang HX, Ng TB, Huang CY, Zhang JX. Purification and characterization of a novel laccase from the edible mushroom *Hericium coralloides*. J Microbiol. 2012;50:72–8.
8. Kleinwächter P, Schlegel B, Dörfelt H, Gräfe U. Spirobenzofuran, a new bioactive metabolite from *Acremonium* sp. HKI 0230. J Antibiot. 2001;54:526–7.
9. Ki DW, Kim DW, Hwang BS, Lee SW, Seok SJ, Lee IK, Yun BS. New antioxidant sesquiterpenes from a culture broth of *Coprinus echinosporus*. J Antibiot. 2015;68:351–3.
10. Srikrishna A, Vasantha Lakshmi B. The first total synthesis of a bioactive metabolite, a spirobenzofuran isolated from the fungi *Acremonium* sp. HKI 0230. Tetrahedron Lett. 2005;46:7029–31.
11. Jiao P, Swenson DC, Gloer JB, Wicklow DT. Chloriolide, a 12-membered macrolide from *Chloridium virescens var. chlamydosporum* (NRRL 37636). J Nat Prod. 2006;69:636–9.
12. Kim KH, Choi SU, Lee KR. Gymnopilin K: a new cytotoxic gymnopilin from *Gymnopilus spectabilis*. J Antibiot. 2012;65:135–7.
13. Lee IK, Jung JY, Kim YS, Rhee MH, Yun BS. *p*-Terphenyls from the fruiting bodies of *Paxillus curtisii* and their antioxidant properties. Bioorg Med Chem. 2009;17:4674–80.