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Two Neuroprotective Compounds from Mushroom *Daldinia concentrica*

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Abstract In the course of our screening for neuroprotective agents, a new compound (**1**) was isolated together with a known compound, caruilignan C (**2**), from the fruiting body of *Daldinia concentrica.* Their structures were determined on the basis of various spectral studies. Both compounds exhibited neuroprotective effect against iron-induced neurodegeneration in a primary culture of mouse cortical neurons.

Key words: Neuroprotective compound, mushroom, *Daldinia concentrica*

Iron, which produces hydroxyl radicals via the Fenton reaction, has been suggested to result in lipid peroxidation, protein peroxidation, and DNA cross-linkage, thereby resulting in cell death [7, 9]. In the search for protective agents against iron-induced neurodegeneration from basidiomycetes and ascomycetes, we have isolated compounds **1** and **2** (Fig. 1) from the methanolic extract of the fruiting body of *Daldinia concentrica*, which was previously reported to produce daldinol, daldinals [3], daldinins [4], and concentricol [8]. In this paper, we describe the isolation, physicochemical properties, structure elucidation, and neuroprotective activity of **1** and **2**.

D. concentrica was collected at Keryong mountain, Chungnam Province, Korea, in September 1999, and identified by the staff at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The dried fruiting body (460 g) of *D. concentrica* was cut into small pieces and extracted with 70% aqueous MeOH at room temperature for 2 days. The MeOH extract was filtered and concentrated under reduced pressure. The liquid residue was partitioned between ethyl acetate and water. The ethyl acetate soluble portion was concentrated *in vacuo*, and the residue was chromatographed on a column of silica gel and eluted with $CHCl_3$-MeOH mixtures. The fraction eluted with $CHCl_3$-MeOH (50:1) was then concentrated, subjected to a column of Sephadex LH-20, and eluted with MeOH, followed by ODS gel column chromatography with 60% aqueous MeOH to give **1** (3 mg) and **2** (5 mg). The physicochemical properties of **1** and **2** are described in Table 1.

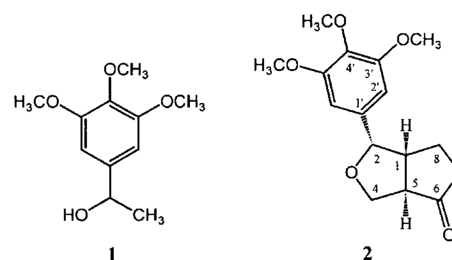

Fig. 1. Structures of compounds **1** and **2**.

The molecular formula for **1** was determined to be $C_{11}H_{16}O_4$ by high resolution EI mass measurement (*m/z* 212.1040 M^+ -0.9 mmu). Its IR absorption at 3,437 cm^{-1} implied the presence of a hydroxyl group. The ^{1}H-NMR spectrum of **1** in $CDCl_3$ exhibited signals for three aromatic methoxyl groups at δ 3.87 (6H) and 3.84 (3H), two

Table 1. Physicochemical properties of compounds **1** and **2**.

	1	**2**
Appearance	Yellow brown solid	Yellow brown solid
Molecular formula	$C_{11}H_{16}O_4$	$C_{18}H_{18}O_5N_2$
HREI-MS (*m/z*)		
found	212.1040	294.1099
calcd	212.1049	294.1103
UV λ_{max}^{MeOH} nm (log e)	210 (4.53)	210 (4.3)
	245 (sh 3.82)	245 (sh 3.68)
	268 (3.37)	270 (3.03)
IR ν_{max} (KBr) cm^{-1}	3437, 2930, 1593	2942, 1770, 1592
Solubility		
soluble in	MeOH, DMSO	MeOH, DMSO
slightly soluble in	$CHCl_3$	$CHCl_3$
insoluble in	H_2O, *n*-hexane	H_2O, *n*-hexane

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Table 2. ^{1}H and ^{13}C-NMR spectral data of compounds **1** and **2**.

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1	4.83 (q, *J*=6.36)[a]	70.5	3.14 (m)	48.4
2	1.45 (d, *J*=6.36)	25.2	4.61 (d, *J*=6.6)	86.1
4			4.21 (dd, *J*=3.6, 9.0) 4.39 (t, *J*=9.0)	70.2
5			3.45 (dt, *J*=3.6, 9.0, 9.0)	46.0
6				177.9
8			4.36 (dd, *J*=2.1, 9.6) 4.52 (dd, *J*=6.6, 9.6)	69.8
1'		141.7		134.5
2'	6.61 (s)	102.2	6.56 (s)	102.8
3'		153.2		153.6
4'		137.0		138.0
5'		153.2		153.6
6	6.61 (s)	102.2	6.56 (s)	102.8
3'-OCH$_3$	3.87 (s)	56.1	3.87 (s)	56.1
4'-OCH$_3$	3.84 (s)	60.7	3.84 (s)	60.7
5'-OCH$_3$	3.87 (s)	56.1	3.87 (s)	56.1

[a]Proton resonance multiplicity and coupling constant (*J*=Hz) in parenthesis. All spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C.

aromatic hydrogens at δ 6.61 (2H), and an ethanol moiety at δ 4.83 and 1.45. The ^{13}C-NMR spectrum revealed signals attributable to four sp^2 quaternary carbons at δ 153.2 (2C), 141.7, and 137.0, three methines at δ 102.2 (2C) and 70.5, three aromatic methoxyl carbons at δ 60.7 and 56.1 (2C), and methyl carbon at δ 25.2 (Table 2). The chemical equivalence of two aromatic methines at δ 6.61 and two aryl methoxyl methyls at δ 3.87 suggested a symmetrical trimethoxyl aromatic ring. Its ^{13}C chemical shifts and intensity at δ 102.2, 137.0, 141.7, and 153.2 were well matched with the corresponding carbons of 3,4,5-trimethoxyl galloyl group. Therefore, the structure of **1** was determined to be 1-(3,4,5-trimethoxyphenyl)ethanol, a novel natural compound, and confirmed based on the HMBC correlations between the methine proton at δ 4.83 and carbons at δ 102.2 and 141.7.

Compound **2** was identified as a lignan caruilignan C on the basis of the above physicochemical properties and NMR spectral data (Table 2). Caruilignan C was recently isolated from *Artemisia caruifolia* as a cytotoxicant to tumor cell lines [6]. The current study is the first to report its neuroprotective activity against iron-induced neurodegeneration as well as its isolation from *D. concentrica*.

Compounds **1** and **2** appeared to originate from the lignin constituent of the host plant, which was decomposed by lignase in *D. concentrica*. The degradative reaction involved in the phenolic syringaresinol model was previously described and would seem to be common with other white-rot fungi [5]. Thus, **1** and **2** were apparently biogenerated from the host lignin by the same reaction as in the syringaresinol model.

The biological activity of **1** and **2** was estimated based on their neuroprotective effect against iron-induced neurotoxicity in mouse cortical cell cultures. The mouse cortical cell cultures were established from gestation day-15 ICR mouse embryos, as previously described by Choi [1]. The cortical cells were plated on 48-well plates (1×10^6 cells/dish) precoated with 100 μg/ml poly-D-lysine and 4 μg/ml laminin. The cortical cells were grown in an Eagle's minimal essential medium (Earles salt, supplied glutamine-free), supplemented with 5% fetal bovine serum, 5% horse serum, 2 mM glutamine, and 20 mM glucose, at 37°C in a humidified atmosphere of 5% CO_2. After 7 days of culture, the nonneuronal cell division was stopped by the addition of cytosine arabinoside (10 μM). Two days later, the cultures were shifted into a growth medium identical to the plating medium, but lacking the fetal serum. The cultures were allowed to mature for 12 days before being used for the experiments. The cortical cell cultures were washed with the MEM medium without serum and incubated with the samples for 1 h. The cultures were then exposed to 50 μM iron (II) sulfate for 24 h along with the samples. After 24 h incubation, the cultures were assessed for the extent of neuronal damage by measuring the levels of lactate dehydrogenase (LDH) efflux in the bathing medium [2]. As shown in Fig. 2, compounds **1** and **2** revealed neuroprotective activity in a dose-dependent manner with IC_{50} values of 25 and 12 μM, respectively, against oxidative injury induced by iron in the primary culture of mouse cortical cells. Both compounds did not exhibit protection against DNA single strand breaks *in vitro* [10] (data not shown), suggesting that **1** and **2** did not act as an iron chelator. The detailed mechanism of action of compounds **1** and **2** for the neuroprotection is under further investigation.

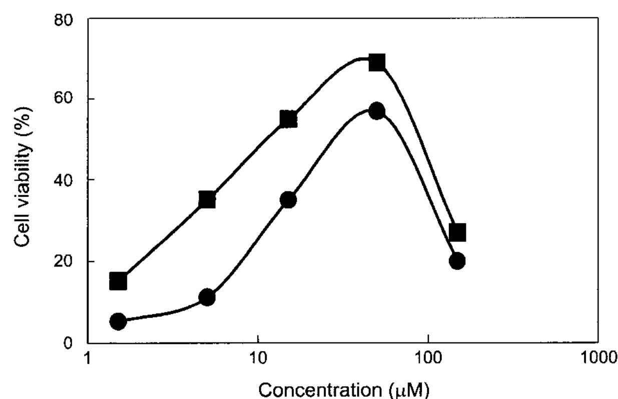

Fig. 2. Protective activity of compounds **1** (●) and **2** (■) against 50 μM iron (II) sulfate-induced neurotoxicity in primary cultured mouse cortical neurons.

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