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# Diaporthin and Orthosporin from the Fruiting Body of *Daldinia concentrica*

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**In our continuing study on the chemical constituents in the fruiting bodies of *Daldinia concentrica*, diaporthin and orthosporin were isolated. Their chemical structures were assigned based on various spectral studies. Diaporthin and orthosporin, phytotoxins previously found in *Aspergillus ochraceus*, were isolated from wood-rotting mushroom *D. concentrica* for the first time.**

**KEYWORDS:** ***Daldinia concentrica*, Diaporthin, Orthosporin, Phytotoxin**

*Daldinia concentrica* (Bolton) Ces. & De Not. belonging to Xylariaceae is an inedible wood-rotting fungus. There is no stipe and the fruit body is attached to the host wood by a broad, flat area underneath the cushion-shaped fruit body. The spore-bearing surface is the outside of the fruit body, and spores leave a slightly darker area of wood around the fungus.

The chemical constituents of *D. concentrica* were first investigated by Allport and Bu'Lock (1958, 1960), who identified characteristic metabolites in its stromata and mycelia, and Anke *et al.* (1995) who reported several compounds with antimicrobial and nematocidal activities. More recent studies on *Daldinia* spp. provided new bioactive metabolites including benzoquinones (Qin and Liu, 2004b), avbinaphthyl (Hashimoto *et al.*, 1994a), cytochalasins (Buchanan *et al.*, 1995, 1996), daldiniapyrone (Quang *et al.*, 2002b), daldinones (Quang *et al.*, 2002b), heptentriols (Wang and Liu, 2004), triterpenoids (Quang *et al.*, 2002a, b; Stadler *et al.*, 2001), azaphilone derivatives (Hashimoto *et al.*, 1994b), benzophenone (Hashimoto *et al.*, 1994a), and steroids (Qin and Liu, 2004a). In our previous study, 1-(3,4,5-trimethoxyphenyl) ethanol and caruilignan C, lignans originated from the host plant lignin that was decomposed by lignase, were isolated as neuroprotective substances (Lee *et al.*, 2002). As part of our ongoing study on chemical constituents in the fruiting bodies of *D. concentria*, two phytotoxins, diaporthin and orthosporin, have been isolated. In this paper, we describe the isolation procedure and structural determination of these compounds.

The fruiting bodies of *D. concentrica* were collected at Keryong Mountain, Chungnam Province, Korea, in September 2005, and identified by the staff of mushroom taxonomy laboratory at the National Institute of Agricultural Science and Technology, RDA. The dried fruiting bodies (460 g) of *D. concentrica* were cut into small pieces and extracted with 70% aqueous MeOH at room temperature for 2 days. The methanolic extract was filtered and concentrated under reduced pressure. The resultant 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 eluting with a gradient with increasing amount of MeOH in $CHCl_3$. Each fraction eluted with $CHCl_3$ only and $CHCl_3$-MeOH (20 : 1, v/v) was concentrated. The $CHCl_3$ fraction was further purified by consecutive Sephadex LH-20 column chromatography eluted with MeOH and preparative silica gel-TLC developed with $CHCl_3$-MeOH (10 : 1, v/v), followed by preparative reversed phase-TLC developed with 50% aqueous MeOH to give compound **1** (3 mg). The other fraction, $CHCl_3$-MeOH (20 : 1, v/v), was subjected to a column of Sephadex LH-20 and eluted with 50% aqueous MeOH to afford compound **2** (2 mg).

The molecular weight of compound **1** was established by the ESI-mass measurement, which provided a quasi-molecular ions at *m/z* 273.3 $[M+Na]^+$ in positive mode and *m/z* 249.3 $[M-1]^-$ in negative mode, suggesting the molecular weight of 250. The $^1H$ NMR spectrum of compound **1** in $CDCl_3$ exhibited signals due to an exchangeable hydroxyl proton at $\delta$ 11.05, two meta-coupled aromatic methine protons at $\delta$ 6.33 and 6.47, an aromatic singlet methine proton at $\delta$ 6.29, an aromatic methoxyl methyl protons at $\delta$ 3.87, and 2-hydroxy propyl moiety at $\delta$ 4.27, 2.66, 2.59 and 1.31. The $^{13}C$ NMR spectrum revealed signals attributable to two $sp^2$ quaternary carbons at $\delta$ 139.0 and 99.9, four oxygenated $sp^2$ quaternary carbons at $\delta$ 166.9, 166.2, 163.6 and 154.6, four methines at $\delta$ 106.1, 101.4, 100.5 and 65.5, an aromatic methoxyl carbon at $\delta$ 55.7, a methylene carbon at $\delta$ 43.0 and one

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**Table 1.** $^{1}H$ and $^{13}C$ NMR spectral data of compounds **1** and **2**[a]

| No. | Compound **1** | | Compound **2** |
|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | $\delta_H$ |
| 1 | | 166.2 | |
| 3 | | 154.6 | |
| 4 | 6.29 (1H, s)[b] | 106.1 | 6.25 (1H, s) |
| 4a | | 139.0 | |
| 5 | 6.33 (1H, d, *J*=1.8) | 101.4 | 6.14 (1H, d, *J*=1.7) |
| 6 | | 166.9 | |
| 6-$OCH_3$ | 3.87 (3H, s) | 55.7 | |
| 7 | 6.47 (1H, d, *J*=1.8) | 100.5 | 6.18 (1H, d, *J*=1.7) |
| 8 | | 163.6 | |
| 8-OH | 11.05 (1H, s) | | |
| 8a | | 99.9 | |
| 9 | 2.59 (1H, dd, *J*=14.5, 8.1) | 43.0 | 2.60 (1H, d, *J*=6.5) |
| | 2.66 (1H, dd, *J*=14.5, 4.3) | | |
| 10 | 4.27 (1H, m) | 65.5 | 4.15 (1H, m) |
| 11 | 1.31 (1H, d, *J*=6.2) | 23.3 | 1.20 (3H, d, *J*=6.2) |

Compound **1** was recorded at 600 MHz for proton and 150 MHz for carbon in $CDCl_3$ and compound **2** was recorded at 300 MHz for proton in $CD_3OD$.

[a]Proton resonance integral, multiplicity and coupling constant (*J*=Hz) in parenthesis.

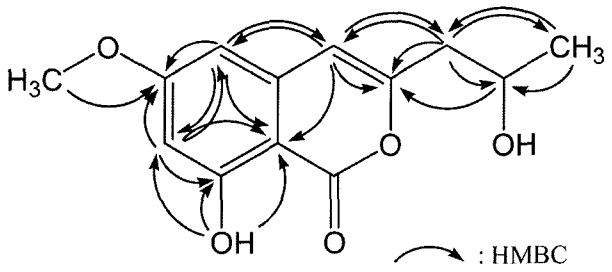
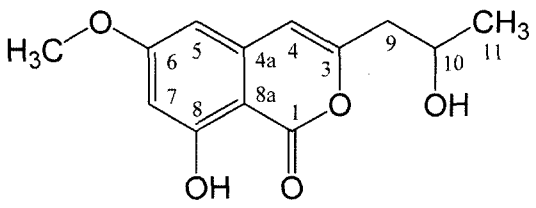
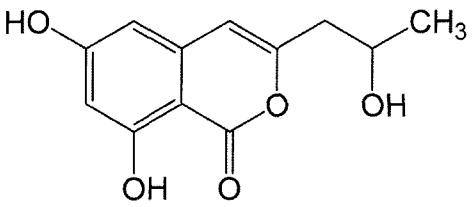



**Fig. 1.** HMBC correlations of compound **1**.

methyl carbon at $\delta$ 23.3 (Table 1). The proton-bearing carbons were established by the aid of HMQC spectrum. The structure of compound **1** was determined on the basis of HMBC correlations, as summarized in Fig. 1. The HMBC correlations of H-9 to C-3 and C-4 revealed that a 2-hydroxypropyl moiety was attached to C-3 of 8-hydroxy-6-methoxyisocoumarin moiety, and other crucial cross-peaks were observed from H-4 to C-3, C-5, C-8a and C-9, H-5 to C-4, C-6, C-7 and C-8a, H-7 to C-5, C-6, C-8 and C-8a, 6-$OCH_3$ to C-6 and 8-OH to C-7, C-8 and C-8a. Therefore, the structure of compound **1** was determined to be an isocoumarin with 2-hydroxypropyl side chain (Fig. 2). In extensive literature search based on structure information, compound **1** was identified as diaporthin, which was first reported as a phytotoxin from *Cryphonectria parasitica*. The ESI-mass measurements of compound **2** gave a quasi-molecular ions at *m/z* 259.3 $[M+Na]^+$ in positive mode and *m/z* 235 $[M-1]^-$ in negative mode, implying that the molecular weight of compound **2** was 14 less than compound **1**. The $^{1}H$ NMR spectrum of




**diaporthin**




**orthosporin**

**Fig. 2.** Structures of diaporthin and orthosporin.

compound **2** in $CD_3OD$ was almost identical to compound **1** except for disappearance of an aromatic methoxyl signal, and this spectral data was consistent with the molecular weight of 236 that proposed by the ESI-mass spectrometry. Thus, the structure of compound **2** was determined as de-*O*-methyldiaporthin (Fig. 2), which was known as orthosporin. Orthosporin was isolated from the plant pathogenic fungus *Drechslera siccans* that causes irregular brown spots on leaves of oats and both perennial and Italian ryegrass (Hallock *et al.*, 1988) and also from the culture broth of *Rhynchosporium orthosporum*, which causes leaf scald on ochard grass (Ichihara *et al.*, 1989). Diaporthin and orthosporin were co-isolated from the culture broth of *Aspergillus ochraceus* that was known as a producer of mycotoxins. The biosynthesis of diaporthin arises intracellularly by methylation of orthosporin, and the influence of ethionine, methylation inhibitor, on the concentration of orthosporin in the fermentation broth might only occur if extracellular orthosporin arises through demethylation of diaporthin (Harris and Mantle, 2001).

In this paper, we found for the first time that wood-rotting mushroom *D. concentrica* produced the phytotoxins, diaporthin and orthosporin.

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