

**Phellinins B and C, new free radical scavengers from the cultured broth of
Phellinus sp. PL009**

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***Phellinus* sp. PL009로부터 분리한 신규 항산화 화합물 phellinin B 및 C**

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Objectives

Mushrooms are ubiquitous in nature, and some of them are nutritionally functional foods and important sources of physiologically beneficial medicines. The styrylpyrone pigments are produced in the mushrooms belonging to the genera *Gymnopilus*, *Hypholoma*, and *Pholiota* in the Agaricales, and among the Aphyllophorales they are strictly restricted to the Hymenochaetaceae including *Phellinus* and *Inonotus*. Recently, the number of findings of novel styrylpyrones with various biological activity from the fruiting body of *Phellinus* and *Inonotus* sp. keeps rising sharply, and styrylpyrones have been focused on their structural diversity and biological activity. Previously, we reported four major antioxidants, hispidin, hypholomine B, 3,14'-bihispidinyl and 1,1-distyrylpyrylethan from the cultured broth of *Phellinus linteus* and *Inonotus xeranticus*. These compounds were proposed to play an important role in biological activity of these fungi. The objective of this study is to search new styrylpyrone compounds with antioxidant activity from the cultured broths of different *Phellinus* isolates.

Materials and Methods

Antioxidant fractions of the cultured broths obtained from *Phellinus* isolates were analysed using reversed-phase HPLC that was monitored by photodiode array detector. A selected strain *Phellinus* sp. PL009 showed different HPLC profile compared to other strains. *Phellinus* sp. PL009 was mass-cultured and cultured broth were centrifuged to separate the broth filtrate and the mycelium cake. The mycelial cake was extracted with 0.5 liter of 80% acetone. The acetone extract was partitioned with ethyl acetate and subjected to a column of Sephadex LH-20, followed by ODS column chromatography and preparative ODS-TLC to provide phellinin B (**1**) and C (**2**). Phellinins B and C were identified based on extensive spectroscopic methods and evaluated scavenging activity against DPPH, ABTS, and superoxide radicals.

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Results

Two new antioxidants, phellinins B (**1**) and C (**2**), were isolated from strain *Phellinus* sp. PL009. Phellinin B was a mixture of isomers of an open chain (**1a**), *trans*-hemiketal (**1b**), and *cis*-hemiketal (**1c**) by same ratio, and phellinin C (**2**) was obtained as isomeric pairs of *trans*-ketal (**2a**) and *cis*-ketal (**2b**). The structures and relative configurations of these compounds were elucidated by NMR experiments. These compounds significantly scavenged free radicals such as DPPH, ABTS, and superoxide radicals.

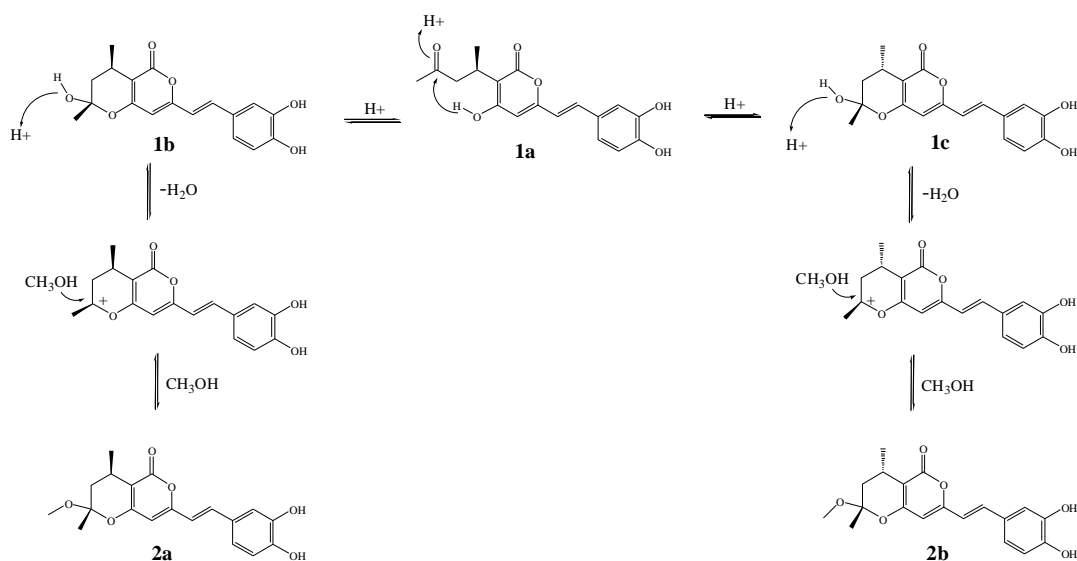


Figure 1. Structures of phellinins B (**1**) and C (**2**).

Table 1. Free radical scavenging activity of phellinins B and C

Compounds	TEAC ^{a,b}		Superoxide ^e
	DPPH ^c	ABTS ^d	IC ₅₀ (μM) ^b
Phellinin B	0.49±0.60	0.46±0.08	16.82±2.47
Phellinin C	5.38±0.87	2.06±0.25	61.84±4.73
Caffeic acid	0.11±0.23	0.18±0.35	16.54±1.10
BHA	0.34±0.11	0.12±0.26	> 500

^aExpressed as IC₅₀ of μM compound / IC₅₀ of μM trolox.

^bResults presented as the mean (n=3) ±SD.

^cα,α-diphenyl-β-picrylhydrazyl. ^d2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid).

^eXanthin/xanthin oxidase