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A carbon–carbon-coupled dimeric bergenin derivative biotransformed by *Pleurotus ostreatus*

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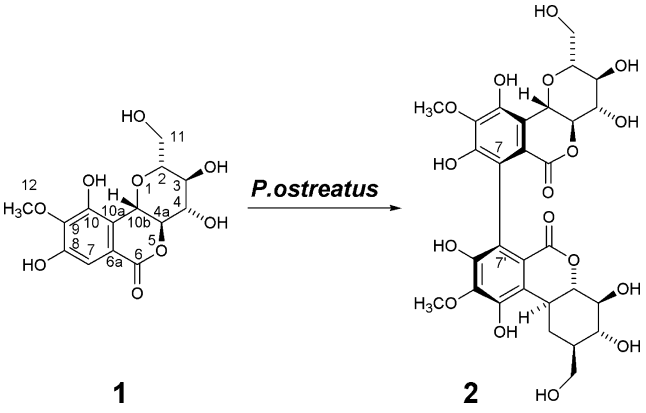
Abstract—A novel C–C-coupled dimer derivative of bergenin was produced by the biotransformation of cultured mycelia of white rot fungus *Pleurotus ostreatus*. Its structure was elucidated by detailed spectroscopic analysis.

Bergenin (**1**), a hydrolyzable tannin derivative, was isolated from several medicinal plants and exhibited various biological activities,[1–6] such as antihepatotoxic and antiulcerogenic effects.[7,8] It contains five hydroxyl groups, which are considered to be potentially active. Using lipase and protease, Mozhaev et al. successfully produced a solution-phase combinatorial library of 167 acylated derivatives of bergenin. In that study, a three-step enzymatic acylation/hydrolysis strategy was applied.[9] By coupling with a variety of fatty acids on hydroxyl groups through chemical catalysis, Takahashi et al. synthesized a series of esterified derivatives of bergenin with greatly enhanced antioxidant activity.[10] Some other chemical modifications of bergenin derivatives were also reported.[11,12]

Compared with chemical catalysis, biocatalysis has shown more potentiality in higher selectivity and higher reactivity over a broad range of operating conditions. Biotransformation, which is at the interface between biology and chemistry, is increasingly important for establishing a diverse library of chemical structure. It is well known that white rot fungi have the ability to degrade lignin. Since lignin, due to its molecular weight, cannot be absorbed by fungal hyphae, the degradation is initiated by the action of extracellular enzymes, such as lignin peroxidase, manganese-dependent peroxidase, manganese-independent peroxidase, and laccase. These enzymes are nonspecific and therefore able to degrade aromatic xenobiotics, such as pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, dyes, polymers, and wood preservatives.[13] *Pleurotus ostreatus*, as an edible fungus belonging to white rot basidiomycete, is widely cultivated. Recently, its ability to degrade nonylphenol in aqueous phase and soil was tested together with other three white rot fungi.[14] Our study on the biotransformation of bergenin (**1**) with white rot fungus *P. ostreatus* led to the isolation of a novel C–C-coupled dimeric bergenin derivative (**2**). This article describes the isolation of **2** and its structural elucidation by detailed spectroscopic analysis.



Fresh fruiting bodies of *P. ostreatus* (Jacq. ex Fr.) Kum were purchased from a local market. The fruiting bodies were initially cultivated on a 2% agar slant[15] and incu-

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bated for 14 days in darkness at 28 °C. The mycelia was reinoculated and maintained in the same medium at 4 °C.

For small-scale transformation experiments, the mycelia were reinoculated in the same 2% agar slants for 7 days. The activated mycelia (1 cm^2) was inoculated into a 100 mL flask containing 30 mL PDA medium (the same as 2% agar slant but without agar) and agitated at 80 rpm with a rotary shaker in darkness at 28 °C. After 14 days of incubation, 30 mg bergenin[16] (dissolved in 1 mL DMSO, filter-sterilized with 0.22 μm filter) was added. The culture liquid was sampled at the intervals of 3 days and analyzed by HPLC[17] (Fig. 1). It was noticed that a new peak for the product appeared and it reached the highest level at the 15th day (Fig. 2).

To obtain the product, biotransformation was scaled up by adding 1 g bergenin (dissolved in 15 mL DMSO) into a 1000 mL flask, in which mycelia of *P. ostreatus* had been incubated for 14 days in 500 mL PDA medium. Fifteen days after the addition of **1**, the culture medium was harvested, and through a repeated separating procedure,[18] the purified product (compound **2**) was obtained in 1% yield.

Compound **2** was a brown amorphous solid. $[\alpha]_D^{27} = 26.19$ (0.003, H_2O). The NMR data were found to be in highly agreement with those of **1** (Tables 1 and 2). The ^1H spectral data of **2** were very similar to those of **1** except for the absence of the proton signal at 6.98 (s, H-7). In ^{13}C NMR spectra, the signal of C-7 in **1** (δ 109.5) was significantly shifted to lower field of δ 121.4 in **2**. The DEPT spectra indicated that C-7 was converted to a quaternary carbon in **2** from a tertiary carbon in **1**, suggesting that C-7 position of **2** was substituted. The negative FABMS of **2** exhibited a molecular ion peak at *m/z* 654 $[M]^-$, and the HRESIMS (*m/z* 653.1353 $[M-H]^-$ for $C_{28}H_{30}O_{18}$-H, calcd 653.1353) assigned its molecular formula as $C_{28}H_{30}O_{18}$. These observations indicated that **2** was a dimer of **1** coupled between C-7 of two bergenin units. The absolute configuration of **2** was determined as *R* according to a strong negative Cotton effect at 239 nm and a strong positive Cotton effect at 272 nm in the circular dichroism (CD) spectra (*c* 0.76 mM, H_2O, Δ_{239} −2.94, Δ_{272} +2.30), similar to the CD spectroscopic data of ellagitannins.[19]

Compounds **1** and **2** were tested for their in vitro antioxidant activity as described,[20] and it can be found easily that SC_{50} of **2** (2.13 mmol/mL) doubled that of **1** (1.07 mmol/mL). This can be made plain with **2** being the dimer of **1**.

In the parallel contrast tests, the culture without substrate and the medium containing substrate but without fungus were incubated in the same condition as described for the biotransformation of **1**. The HPLC analysis showed that both incubations had not resulted in the generation of **2**. It was concluded that compound **2**

Table 1. ^1H NMR data for compounds **1** and **2** (δ in ppm, *J* in Hz)

Position	1	Position	2
2	3.56 (t, 7.6)	2, 2′	3.58 (t, 7.7)
3	3.21 (m)	3, 3′	3.17 (m)
4	3.65 (m)	4, 4′	3.72 (t, 9.7)
4a	3.98 (t, 9.9)	4a, 4a′	3.87 (br s)
7	6.98 (s)		
10b	4.95 (d, 10.4)	10b, 10b′	4.92 (d, 10.2)
11	3.44 (m)	11, 11′	3.42 (m)
12	3.77 (s)	12, 12′	3.79 (s)
OH-3	5.35 (br s)	OH-3, OH-3′	5.44 (br s)
OH-4	5.56 (br s)	OH-4, OH-4′	5.55 (br s)
OH-11	3.84 (br s)	OH11, OH-11′	3.83 (br s)
OH-arom	8.41 (br s)	OH-arom	8.59 (br s)
	9.66 (br s)		

Recorded at 500 MHz in DMSO-d_6 with TMS as internal standard.

Table 2. ^{13}C NMR data (δ in ppm) for compounds **1** and **2**

Position	1	Position	2
2	81.7	2, 2′	81.5
3	70.7	3, 3′	70.6
4	73.7	4, 4′	73.7
4a	79.8	4a, 4a′	79.1
6	163.3	6, 6′	161.9
6a	118.0	6a, 6a′	116.3
7	109.5	7, 7′	121.4
8	150.9	8, 8′	149.5
9	140.6	9, 9′	139.6
10	148.0	10, 10′	146.3
10a	116.0	10a, 10a′	115.3
10b	72.2	10b, 10b′	72.6
11	61.1	11, 11′	61.2
12	59.8	12, 12′	60.0

Recorded at 125 MHz in DMSO-d_6 with TMS as internal standard.

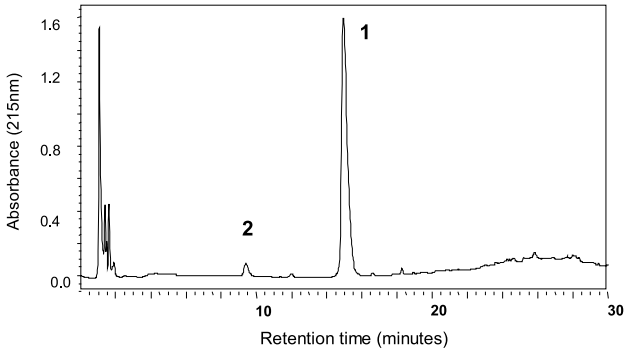

Figure 1. HPLC analysis of *P. ostreatus*–bergenin biotransformed culture.

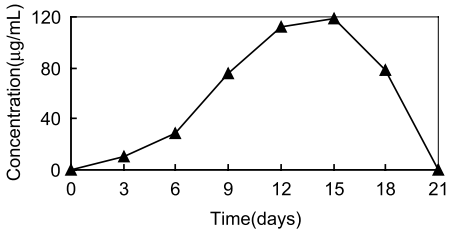

Figure 2. The yield curve of compound **2** in *P. ostreatus*–bergenin culture, analyzed by HPLC.

is a new metabolite derived from the fungal biotransformation of **1**.

When starting this work, we had anticipated that some changes would have occurred on the hydroxyl groups or methoxy group in bergenin. However, the result indicated that all these functional groups were not modified, but dimerization took place between the aromatic carbons from two bergenin units to form a biphenyl structure. This kind of dimerization reaction usually happens under more hasher operating conditions by traditional chemical catalysis. In most of the biotransformation experiments, the formation of C–O bonds is much more common than direct C–C linkage when one phenyl ring coupled with other groups. It was reported that some C–C-linked biarylic compounds were formed by another white rot fungus *Pycnoporus cinnabarinus*.[21] To the best of our knowledge, compound **2** was the first C–C-coupled hydrolyzable tannin derivative biotransformed by white rot fungus. In this study, dehydro-oxidation reaction catalyzed by oxidase may be used to explain the possible mechanism. Further studies on the mechanism of selectivity and associated enzymes in this biocatalysis are under investigation. And to acquire a higher yield of product, the improvement of culture conditions is in progress.

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15. 2% agar slants: consisted of (in g/L) potato 200.0 (peeled, cut up, boiled and filtered), dextrose 20.0, KH_2PO_4 0.3, $MgSO_4$ 0.15, $CaCl_2$ 0.3, peptone 3, agar 20, pH 6.0.
16. Compound **1** (bergenin) was used as the precursor of the biotransformation. It was isolated from dried root of *Bergenia purpurascens* collected in Yunnan, China, by the method reported in Ref. 2.
17. HPLC analysis was conducted on a Waters 2596 system equipped with a 2996 PDA detector. Mobile phase: ($MeOH/H_2O$, 0 min: 5%, 13 min: 10%, 20 min: 30%, 26 min: 90%), column: symmetry C-18 column (5 μm, 3.9 × 150 mm, Waters), flow rate: 1 mL/min, UV detection at 200–400 nm, column temperature: 35 °C.
18. *Isolation and purification of* ***2***. The culture medium was filtered and applied to a Diaion HP-20 column chromatography, eluted successively with H_2O and MeOH. The MeOH fraction was concentrated to dryness under reduced pressure and subjected to HPLC purification to give **2** (10 mg). HPLC purification was performed on a Waters 600 semi-prep HPLC system with a Delta-Pak C-18 column (15 μm, 25 × 100 mm Waters), mobile phase: 15% MeOH in H_2O (v/v); flow rate: 18 mL/min, UV detection at 215 nm.
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