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Styrylpyrones from the medicinal fungus *Phellinus baumii* and their antioxidant properties

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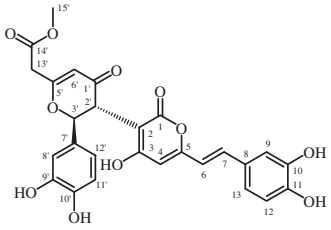
ABSTRACT

During the search for natural antioxidants from basidiomycetes, a new styrylpyrone, baumin (**1**), was isolated from the cultivated medicinal fungus *Phellinus baumii*, together with known compounds, davallialactone (**2**), hispidin (**3**), hypholomine B (**4**), interfungin A (**5**), inoscavin A (**6**), and phelligridin D (**7**), which were previously isolated from the medicinal fungi *Phellinus ignarius*, *Phellinus linteus*, and *Inonotus xeranticus*. Their structures were elucidated by extensive spectroscopic methods. These compounds exhibited antioxidant activity through Fenton reaction inhibition via iron chelation and free radical scavenging.

Several fungi belonging to genera *Phellinus* and *Inonotus* have been used as traditional medicines for treatment of gastrointestinal cancer, liver or heart diseases, and stomach ailments without adverse effects.[1–3] Polysaccharides, particularly β-glucan, have been considered responsible for their biological activity, and a number of polysaccharides and protein-bound polysaccharides are used clinically for the treatment of cancer.[2] Although their immuno-augmentative activity is overwhelmingly regarded as the primary medicinal effect of these fungi, other curative properties can also be found in these medicinal fungi. Recently, it has been reported that these fungi produce a large variety of yellow polyphenol pigments with a styrylpyrone skeleton, which has been reported to exhibit antioxidative, anti-platelet aggregation, and anti-inflammatory activities.[4–9] In a previous study, we isolated



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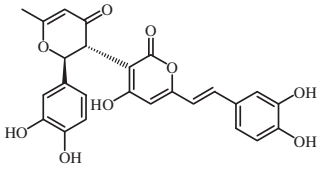

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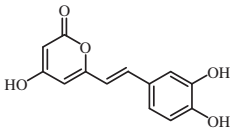

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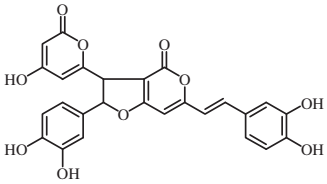

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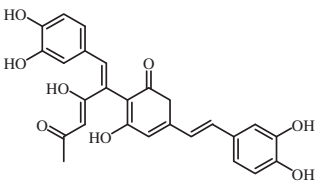

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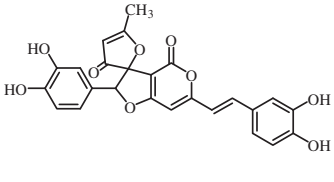

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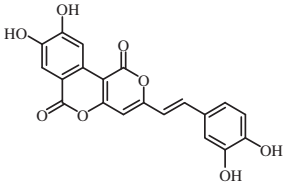

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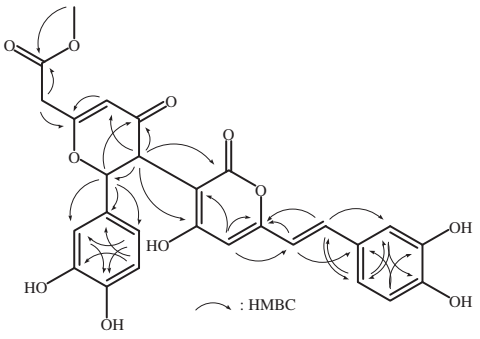

Figure 1. HMBC correlations of **1**.

diverse styrylpyrone-class compounds from the fruiting body of the medicinal fungi *Inonotus xeranticus* and *Inonotus obliquus*.[4–6] Our continuous investigation of fungal antioxidative constituents has resulted in isolation of a new styrylpyrone, baumin (**1**), from the cultivated medicinal fungus *Phellinus baumii*, together with six known compounds, davallialactone (**2**), hispidin (**3**), hypholomine B (**4**), interfungin A (**5**), inoscavin A (**6**), and phelligridin D (**7**), which were previously isolated from the medicinal fungi *Phellinus ignarius*, *Phellinus linteus*, and *I. xeranticus* as free radical scavengers.[4–7] Cultivated fungus *P. baumii* is known to alleviate severe side-effects of antitumour drugs[10] and to exhibit various biological activities, such as anticancer, anti-inflammatory, antioxidative, and anti-allergic effects.[11–13] Nevertheless, active substances are still unknown. In this Letter, we describe the isolation and structure determination of the isolated compounds **1**–**7** from the medicinal fungus *P. baumii*, and the investigation for their antioxidant activity as well.

Ground fruiting bodies of *P. baumii* were extracted twice with MeOH. The methanolic extract was partitioned between *n*-hexane and H_2O and then between ethyl acetate and H_2O. Repeated chromatographic separations of the ethyl acetate-soluble fraction led to the purification of seven antioxidants, **1**–**7**.[14]

Baumin (**1**) was obtained as a yellow powder and its molecular weight was established as 522 by ESIMS, providing molecular ion peaks at *m*/*z* 545.5 $[M+Na]^+$ in positive mode and at *m*/*z* 521.4 $[M-H]^-$ in negative mode. The molecular formula of **1** was determined to be $C_{27}H_{22}O_{11}$ by high resolution ESI-mass. The UV maxima at 261 and 376 nm were similar to those of known compound **2**, suggesting that **1** was a styrylpyrone derivative. The ^1H NMR spectrum in CD_3OD showed six aromatic methine signals assignable to two 1,2,4-trisubstituted benzenes at δ 6.97 (1H, d, J = 2.0 Hz), 6.90 (1H, dd, J = 8.0, 2.0 Hz), and 6.74 (1H, d, J = 8.0 Hz), and 6.84 (1H, d, J = 1.6 Hz), 6.67 (2H, m, overlapped), two olefinic methine peaks attributable to a *trans*-1,2-disubstituted double bond at δ 7.21 (1H, d, J = 16.0 Hz) and 6.46 (1H, d, J = 16.0 Hz), four methines at δ 5.95 (1H, s), 5.79 (1H, d, J = 13.4 Hz), 5.59 (1H, s), and 4.31 (1H, d, J = 13.4 Hz), a methylene singlet at δ 3.46 (2H, s), and a methyl singlet at δ 3.73 (3H, s). The ^{13}C NMR spectrum revealed the presence of 27 carbons comprised of one methyl, 10 sp^2 methines, and 13 quaternary carbons, including an α,β-unsaturated ketone carbonyl, two ester carbonyl, and seven oxygenated sp^2 carbons. Proton-bearing carbons were assigned with the aid of an HMQC spectrum. These spectroscopic data were very similar to those of davallialactone, except for the presence of additional methoxyl methyl (δ_H 3.72, δ_C 52.9), a methylene (δ_H 3.46, δ_C 41.0), and an ester carbonyl at δ 170.3, instead of a methyl group in davallialactone. The structure of **1** was determined by interpretation of the HMBC spectrum in comparison with NMR spectra of davallialactone (Fig. 1). HMBC correlations of H-4 to C-2, C-5, and C-6, H-6 to C-5 and C-8, H-7 to C-9 and C-13, H-9 to C-11 and C-13, H-12 to C-8 and C-10, and H-13 to C-7, C-9, and C-11 were observed, and their chemical shift values were in good agreement with the corresponding protons and carbons of the styrylpyrone moiety in davallialactone.[4] In addition, HMBC correlations from H-2′ to C-1′ and C-6′, H-3′ to C-1′ and H-6′ to a sp^2 quaternary carbon (C-5′) revealed the presence of the 2,3-dihydropyran-4-one moiety and long-range correlations from H-3′ to C-7′, C-8′, and C-12′ implied that the 3,4-dihydroxyphenyl group should be connected to C-3′. The remaining partial structure of methyl acetate was connected to C-5′ on the basis of a long-range correlation of H-13′ to C-5′. Other long-range correlations from HMBC data were in accordance with those of davallialactone. Therefore, the structure of compound **1** was established as a new davallialactone derivative named baumin.[15]

Compounds **2**–**7** were identified as davallialactone,[16] hispidin,[7] hypholomine B,[7] interfungin A,[5] inoscavin A,[17] and phelligridin D,[18] respectively, on the basis of their NMR spectral data, which were in good agreement with those published previously. In addition, these compounds exhibited the same retention times as corresponding authentic compounds in analytical HPLC. Compounds **2**, **5**, **6**, and **7** were previously isolated from the fruiting body of *I. xeranticus*[4–6] and compounds **3** and **4** were isolated as free radical scavengers from the mycelial culture of *I. xeranticus* and *P. linteus*.[7] Compound **1** also showed free radical scavenging activity with IC_{50} values of 11.7 and 7.5 μM against ABTS and DPPH radicals, respectively, and was less active than davallialactone, with IC_{50} values of 2.8 and 1.2 μM, against ABTS and DPPH radicals, respectively.

In addition to free radical scavenging activity, the protective effect of the compounds isolated against the Fenton reaction was evaluated by the ferrous and hydrogen peroxide-induced DNA single strand breakage method.[19] Due to its importance in biochemistry, medicine, food, and environmental chemistry, Fenton chemistry is an extensively researched subject.[20] The presence of iron in a reaction system containing hydrogen peroxide is truly catalytic and leads to production of a strong oxidant, hydroxyl radical. Thus, inhibition of the Fenton reaction has been used in assessment of antioxidative activity, including hydroxyl radical scavenging and iron chelation activity. All of the isolated compounds inhibited supercoiled DNA single strand breakage induced by the Fenton reaction, in a final concentration of 30 μM, as shown in Figure 2. All compounds, except for **3**, exhibited a potent DNA protective effect comparable to that of iron chelator, desferrioxiamine (DFO). Antioxidants BHA and trolox showed no inhibitory activity against DNA breakage, suggesting that inhibition of the Fenton reaction by compounds **1**–**7** was due to iron chelation rather than radical scavenging. To prove whether or not the isolated compounds act as iron chelators, iron chelation activity was estimated by the modified CAS (chrome azurol S) assay method.[21] The CAS

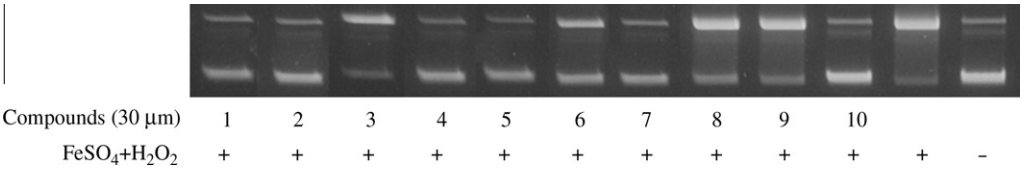

Figure 2. Agarose gel electrophoresis showing the effect of compounds **1**–**7**, **8** (BHA), **9** (Trolox), and **10** (DFO) on single strand breaks induced by hydroxyl radicals in pBR 322 DNA.

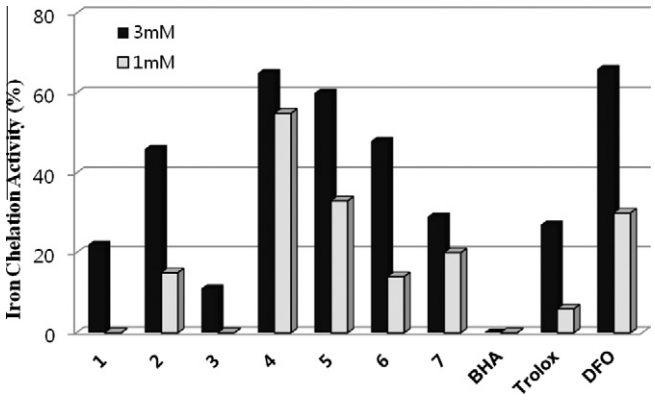

Figure 3. Iron chelation activity of compounds **1**–**7**, measured by CAS assay.

assay method has been used widely for detection of siderophore from microorganisms. This assay is based on competition for iron between the ferric complex of an indicator dye, chrome azurol S, and an iron chelator or siderophore. The color of the CAS reagent changes from dark blue to pink when iron is removed from CAS by an iron chelator with a higher affinity for iron. As a result of the CAS assay, compounds **2**, **4**–**6**, and **7** exhibited potent iron chelation activity, which was comparable to that of the iron chelator, DFO, as shown in Figure 3. Thus, these compounds are thought to possess both free radical scavenging activity and iron chelation activity. Consequently, these styrylpyrone compounds provide a new class of antioxidant with both free radical scavenging activity and an iron chelation effect, and might be considered strong candidates for use as drugs or nutraceuticals against oxidative damage.

Acknowledgments

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14. Ground fruiting bodies of *Phellinus baumii* (1 kg) were extracted twice with 5 L of MeOH at room temperature for 2 days. Following removal of MeOH under reduced pressure, the resulting solution was partitioned between *n*-hexane and H_2O and then ethyl acetate and H_2O. The ethyl acetate-soluble fraction was subjected to a column of Sephadex LH-20 eluted with MeOH to give three fractions (Fr-1, Fr-2, and Fr-3), which were divided by monitoring with HPLC (LaChrom Elite HPLC, Hitachi Co., Tokyo, Japan). HPLC conditions were as follows: column, TSK gel ODS-100 V (4.6 × 150 mm, Tosoh Co., Tokyo, Japan); solvent, a gradient with an increasing amount of MeOH (10 → 90%) in water/0.04% trifluoroacetic acid (TFA); flow rate, 1.0 mL/min; detector, photodiode array detector. Compounds **1**–**5** were purified from Fr-1 as follows; Fr-1 was separated by solid phase extraction by eluting with 50–60% aqueous MeOH to provide two fractions. One fraction was concentrated under reduced pressure to provide compound **4** (60 mg). The other fraction was separated on a column of Sephadex LH-20 with 70% aqueous MeOH to afford three fractions, Fr-1-1, Fr-1-2, and Fr-1-3. The Fr-1-1 fraction was purified by preparative reversed-phase HPLC on a column (Cosmosil RP-18, 20 × 150 mm) and eluted with 40% aqueous MeOH containing 0.04% TFA to give compounds **1** (8 mg) and **2** (20 mg). Fr-1-2 and Fr-1-3 fractions were purified, respectively, by reversed-phase TLC with 60% aqueous MeOH to provide **3** (13 mg) and **5** (7 mg), respectively. Fraction Fr-2 was separated by solid phase extraction, eluting with 50–60% aqueous MeOH, followed by Sephadex LH-20 column chromatography, and eluted with 70% aqueous MeOH to give compound **6** (4 mg). Compound **7** (8 mg) was purified from Fr-3 using Sep-pak ODS cartridge, and eluted with 50–60% aqueous MeOH.
15. Yellow powder; $[\alpha]_D$ +61 (*c* 0.1, MeOH); UV λ_{max} (MeOH) (log ε) 261 (4.17), 376 (3.98) nm; IR ν_{max} 3242, 2957, 1662, 1607, 1556, 1420, 1381, 1283, 1196 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) 7.21 (1H, d, *J* = 16.0 Hz, H-7), 6.97 (1H, d, *J* = 2.0 Hz, H-9), 6.90 (1H, dd, *J* = 8.0, 2.0 Hz, H-13), 6.84 (1H, d, *J* = 1.6 Hz, H-8′), 6.74 (1H, d, *J* = 8.0 Hz, H-12), 6.67 (2H, overlapped, H-11′, H-12′), 6.46 (1H, d, *J* = 16.0, H-6), 5.95 (1H, s, H-4), 5.79 (1H, d, *J* = 13.4 Hz, H-3′), 5.59 (1H, s, H-6′), 4.31 (1H, d, *J* = 13.4 Hz, H-2′), 3.73 (3H, s, H-15′), 3.46 (2H, s, H-13′); ^{13}C NMR (CD_3OD, 100 MHz) 195.5 (C-1′), 171.9 (C-5′), 170.3 (C-14′), 169.7 (C-3), 166.5 (C-1), 160.5 (C-5), 148.6 (C-11), 147.1 (C-10′), 146.7 (C-10), 146.0 (C-9′), 137.1 (C-7), 129.9 (C-7′), 128.8 (C-8), 121.9 (C-13), 120.5 (C-12′), 116.6 (C-6), 116.5 (C-12), 115.8 (C-11′), 115.6 (C-8′), 114.8 (C-9), 106.7 (C-6′), 100.6 (C-4), 98.4 (C-2), 84.9 (C-3′), 52.9 (C-15′), 48.4 (C-2′), 41.0 (C-13′); ESI-mass (positive mode) *m*/*z* 545.5 $[M+Na]^+$, (negative mode) *m*/*z* 521.4 $[M-H]^-$; High resolution ESI-mass *m*/*z* 523.1238 $[M+H]^+$ (calcd for $C_{27}H_{23}O_{11}$, 523.1241).
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