

Two Pyrone Derivatives from Fungus *Phellinus Igniarius*

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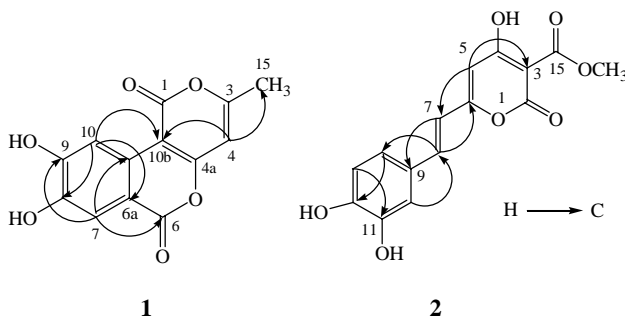
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Abstract: Two new pyrone derivatives phelligridins A **1** and B **2** were isolated from the fruit body of *Phellinus igniarius*. Their structures were elucidated as 8, 9-dihydroxy-3-methyl-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione and 4-hydroxy-6-(3',4'-dihydroxystyryl)-3-methoxycarbonyl-2-pyrone, respectively, by means of spectral methods, including IR, MS, 1D and 2DNMR spectra.

Keywords: *Phellinus igniarius*, pyrones, phelligridins A and B.

Phellinus igniarius, a fungus belonging to Polyporaceae family, preferably hosts on the stems of aspen, robur and birch, and its fruit body is used to treat fever, abdominalgia and bloody gonorrhoea in Chinese traditional medicine¹. Polysaccharides², phenolic pigment³ and peroxidases^{4,5} have been reported from this fungus. We report here the isolation and structural elucidation of two new pyrone derivatives named as phelligridins A **1** and B **2**, from the fruit body of the same fungus.

Figure 1 The key HMBC correlations of **1** and **2**



The fruit bodies of *P. igniarius* was extracted with 95% ethanol, and then the concentrated extract suspended in water was partitioned with EtOAc and *n*-BuOH successively. The EtOAc extract was subjected to chromatography on normal phase silica gel, Sephadex LH-20 and reverse phase HPLC to yield compounds **1** and **2**.

Compound **1** was obtained as pale yellow powder, mp>300°C. Its IR spectrum

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(KBr) showed absorption bands for hydroxyl (3070 cm^{-1}), conjugated carbonyl (1674 cm^{-1}) groups and aromatic rings (1618 , 1591 and 1512 cm^{-1}). The EIMS spectrum exhibited a molecular ion peak at m/z 260 [M^+], and the molecular formula was determined as $C_{13}H_8O_6$ by HREIMS at m/z 260.0332 (calcd. for $C_{13}H_8O_6$ 260.0321). The 1H NMR spectrum of **1** did not show any spin coupling resonance but six singlets attributed to two hydroxyl protons at δ 10.74 (br s, 1H, 9-OH) and 10.07 (br s, 1H, 8-OH), two aromatic protons at δ 8.32 (s, 1H, H-10) and 7.52 (s, 1H, H-7), one vinyl proton at δ 6.60 (s, 1H, H-4), and a methyl group at δ 2.32 (s, 3H, H-15).

Table 1 $^1H^a$ and $^{13}C^b$ NMR data of compounds **1** and **2**

No.	1		2	
	δ_H	δ_C	δ_H	δ_C
1		160.22 s		
2				159.2 s
3		162.40 s		92.5 s
4	6.60 s	99.30 d		176.1 s
4a		160.82 s		
5			6.17 s	102.1 d
6		158.60 s		161.0 s
6a		111.34 s		
7	7.52 s	114.37 d	6.68 d (16)	115.8 d
8		146.85 s	7.23 d (16)	136.6 d
9		153.64 s		126.6 s
10	8.32 s	110.40 d	7.06 s	114.2 d
10a		126.75 s		
10b		98.03 s		
11				147.9 s
12				145.6 s
13			6.77 d (8.5)	116.1 d
14			6.99 d (8.5)	120.9 d
15	2.32 s	19.39 q	3.75 s	168.8 s
				51.7 q

OCH₃

^a NMR data were measured in DMSO at 500 MHz for proton and at 125 MHz for carbon. Proton coupling constants (J) in Hz are given in parentheses. The assignments were based on DEPT, 1H - 1H COSY, HMQC and HMBC experiments.

The ^{13}C NMR and DEPT spectra of **1** displayed 13 carbon signals, which consist of one methyl, three sp^2 methines and nine sp^2 quaternary carbons (six oxygenated) (see **Table 1**). The protonated carbon signals were unambiguously assigned by HMQC experiment and the oxygenated carbon signals were assigned on the basis of their chemical shift values ($\delta_c > 145$ ppm). In the HMBC spectrum of **1**, two and three bond correlations from both hydroxyl protons to C-10, C-8 and C-9; H-4 to C-4a, C-10b, C-3, C-15; H-10 to C-8, C-9, C-6a, C-10b and H-7 to C-6, C-8, C-9 and C-10a (see **Figure 1**) unequivocally established the locations of two hydroxyl group and the connections among 12 carbons except for C-1, since C-1 did not correlate to any proton. However, the connection between C-1 and C-10b, two quaternary carbons, was readily established on the basis of their chemical shift values. All above evidences indicated that **1** possesses a highly oxygenated and highly conjugated structure. To satisfy ten degrees of unsaturation of the molecule and six oxygenated quaternary sp^2 carbons, the structure

of **1** must own two lactone rings. Based on the partial structure established by HMBC experiment, two reasonable lactone rings could only be formed between C-6 and C-4a and between C-1 and C-3. Accordingly, the structure of **1** was determined to be 8,9-dihydroxy-3-methyl-1*H*, 6*H*-pyrano[4, 3-*c*][2]benzopyran-1, 6-dione.

Compound **2** was obtained as orange powder, mp 218-220°C. Its IR spectrum (KBr) showed absorption bands for hydroxyl (3394 cm⁻¹), conjugated carbonyl (1647 cm⁻¹) and ketone (1707 cm⁻¹) groups, as well as aromatic rings (1608, 1520 and 1506 cm⁻¹). The FABMS spectrum showed a quasi-molecular ion peak at *m/z* 305.0 [M+H]⁺, and the molecular formula was established as C₁₅H₁₂O₇ by HRFABMS at *m/z* 305.0606 [M+H]⁺ (calcd. for C₁₅H₁₃O₇ 305.0661). The ¹H NMR spectrum of **2** showed signals attributed to a *trans* substituted double bond unit at δ 7.23 (d, 1H, *J*=16 Hz, H-8) and 6.68 (d, 1H, *J*=16Hz, H-7), a trisubstituted double bond unit at 6.17 (s, 1H, H-5), and a 1, 3, 4-trisubstituted phenyl unit at δ 6.77 (d, 1H, *J*=8.5Hz, H-13), 6.99 (br d, 1H, *J*=8.5Hz, H-14) and 7.06 (br s, 1H, H-10), in addition to a methoxyl group at 3.75 (s, 3H) and two phenolic hydroxyl groups at δ 9.56 (br s, 1H) and 9.13 (br s, 1H). The presence of these units and groups were confirmed by ¹³C NMR and DEPT spectra which displayed 15 carbon signals including six sp² methines, eight sp² quaternary carbons (six oxygenated) and a methoxyl carbon (see **Table 1**). The protonated carbon signals were assigned by the HMQC experiment. The connections among these units along with C-3 and C-4 were elucidated by long range correlations from H-7 to C-5, C-6, C-9, H-8 to C-6, C-10, C-14, and H-5 to C-3, C-4 C-6 and C-7 in the HMBC spectrum of **2** (see **Figure 1**). However, C-2 did not correlate to any proton, and C-15 correlated only to the methoxyl protons in the HMBC spectrum. Both of them were assigned to directly connect to the quaternary carbon C-3 because of a high field chemical shift of C-3 (δ 92.5). Finally, the lactone ring between C-2 and C-6 was established according to ten degrees of unsaturation of the molecule. Therefore, the structure of **2** was determined as 4-hydroxy-6-(3', 4'-dihydroxystyryl)-3-methoxycarbonyl-2-pyrone. A similar compound hispidin [6-(3', 4'-dihydroxystyryl)-4-hydroxy-2-pyrone] was reported as the metabolite of fungus *Inonotus hispidus*.⁶

Acknowledgments

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