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A Unique Highly Oxygenated Pyrano[4,3-*c*][2]benzopyran-1,6-dione Derivative with Antioxidant and Cytotoxic Activities from the Fungus *Phellinus igniarius*

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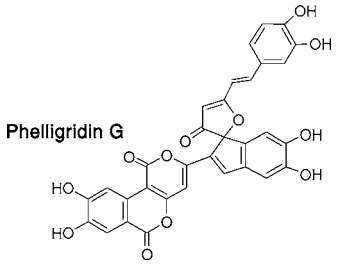
ABSTRACT



A unique pyrano[4,3-*c*][2]benzopyran-1,6-dione derivative with an unprecedented carbon skeleton, phelligridin G (1), has been isolated from the fruiting body of the fungus *Phellinus igniarius*. Its structure was elucidated by detailed spectroscopic analysis. A possible biogenetic origin of 1 mediated by the fungal metabolite precursor 4-hydroxy-6-methyl-2-pyrone was postulated. Phelligridin G (1) showed antioxidant activity inhibiting rat liver microsomal lipid peroxidation and moderate selective cytotoxic activities against human cancer cell lines.

Secondary metabolites produced by the fruiting bodies of mushrooms frequently exhibit a great variety of structures and interesting biological activities.[1] *Phellinus igniarius* (DC. ex Fr.) Quél., a fungus belonging to the Polyporaceae family, preferably hosts on the stems of aspen, robur, and birch. Some *Phellinus* species have been reported as pathogens of aspen (*Populus tremuloides* Michx.)[2] and *Pinus pinaster*,[3] and interesting chemical constituents have been isolated from them.[1f,4] The fruiting body of *P. igniarius* has long been used for the treatment of fester, bellyache, and bloody gonorrhea as a traditional Chinese medicine.[5] In previous papers, we reported several cytotoxic compounds with unusual structural features including pyrano[4,3-*c*][2]benzopyran-1,6-dione analogues phelligridins A and C−E from the ethanolic extract of the fruiting body of *P. igniarius*.[6] In a continuation of

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(1) For some examples, see: (a) Liu J. K. *Heterocycles* **2002**, *57*, 157. (b) Tan, R. X.; Jensen, P. R.; Williams, P. G.; Fenical, W. *J. Nat. Prod.* **2004**, *67*, 1374. (c) Kim, J. P.; Yun, B. S.; Shim, Y. K.; Yoo, I. D. *Tetrahedron Lett.* **1999**, *40*, 6643. (d) Ohta, T.; Kita, T.; Kobayashi, N.; Obara, Y.; Nakahata, N.; Ohizumi, Y.; Takaya, Y.; Oshima, Y. *Tetrahedron Lett.* **1998**, *39*, 6229. (e) Vidari, G.; Che, Z. L.; Garlaschelli, L. *Tetrahedron Lett.* **1998**, *39*, 6073. (f) Lourenco, A.; Lobo, A. M.; Rodriguez, B.; Jimeno, M. L. *Phytochemistry* **1996**, *43*, 617. (g) Gonzalez, A. G.; Bermejo Barrera, J.; Mediavilla Perez, M. J.; Toledo Marante, F. J.; Perales A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 551.

(2) Trifonov, L. S.; Chakravarty, P.; Hiratsuka, Y.; Ayer W. A. *Eur. J. For. Pathol.* **1992**, *22*, 441.

(3) Otjen, L.; Blanchette, R.; Effland, M.; Leatham, G. *Holzforschng* **1987**, *41*, 343.

(4) Ayer, W. A.; Cruz, E. R. *J. Org. Chem.* **1993**, *58*, 7529.

(5) Jiangsu New Medical College. *Dictionary of Traditional Chinese Medicine*; Shanghai Science and Technology Publishing House: Shanghai, 1977; p 1967.

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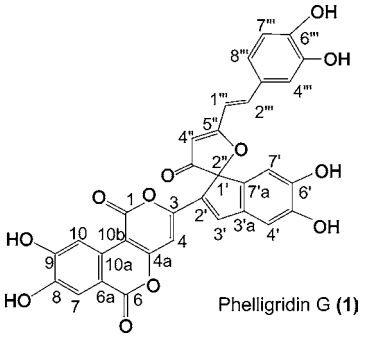


Figure 1. Structure of phelligridin G (**1**).

our work, another cytotoxic and antioxidant pyrano[4,3-*c*]-[2]benzopyran-1,6-dione derivative with an unprecedented carbon skeleton (**1**, Figure 1) has been isolated from an antioxidant fraction of the same extract. Compound **1** is another unique example that may be biogenetically synthesized from the fungal metabolite 4-hydroxy-6-methyl-2-pyrone by coupling with activated 3,4-dihydroxybenzoyl-SCoA and co-occurring 3,4-dihydroxybenzaldehyde.[7] Details of the isolation, structural elucidation, postulated biogenetic formation, and biological activities of **1** are presented below.

The fungal material of *P. igniarius*[8] was collected in the Dandong district of Liaoning province, China. The air-dried and powdered fruiting bodies (5 kg) were exhaustively extracted with 95% EtOH at room temperature, and the gum (193 g) obtained by concentrating the EtOH extract in vacuo was suspended in water and then partitioned with EtOAc and *n*-BuOH successively. The EtOAc-soluble portion (95 g) was fractionated via silica gel column chromatography eluting with a gradient increasing acetone (0−50%) in $CHCl_3$ followed by increasing MeOH (20−100%) in $CHCl_3$ to give 24 fractions (a_1 to a_{24}). The fraction a_{20} (4.5 g) showed antioxidant activity[9] and was further separated via middle-pressure liquid chromatography over reversed silica gel (C-18) using step-gradient elution increasing MeOH (0−100%) in acidic H_2O (contain 0.1% acetic acid), and then purified by chromatography over Sephadex LH-20 eluting with $CHCl_3$−MeOH (3:1) to afford phelligridin G (**1**) (17 mg).

Phelligridin G (**1**) was obtained as an orange amorphous powder (MeOH), mp >300 °C. The absorption bands in its IR spectrum suggested the presence of hydroxyl (3394 and 3240 cm^{-1}), conjugated carbonyl (1685 cm^{-1}), aromatic rings (1589, 1545 and 1518 cm^{-1}), and C−O bonds (1298, 1149 and 1109 cm^{-1}). The negative ESIMS of **1** gave a quasi-molecular ion peak at *m*/*z* 593.4 $[M - H]^-$, while the positive

Table 1. NMR Data for Phelligridin G (**1**) Recorded in DMSO-d_6[a]

no.	δ C	δ H	no.	δ C	δ H
1	159.6		4′	112.4	7.00, s
3	153.9		5′	147.9	
4	96.5	6.02, s	6′	147.7	
4a	160.7		7′	110.1	6.61, s
6	159.2		7′a	135.3	
6a	112.4		3″	197.9	
7	115.2	7.47, s	4″	103.9	6.25, s
8	147.9		5″	185.6	
9	154.3		1‴	112.7	7.10, d, 16.0
10	111.3	8.28, s	2‴	142.1	7.54, d, 16.0
10a	127.4		3‴	126.7	
10b	100.1		4‴	115.7	7.16, d, 1.5
1′	94.9		5‴	146.5	
2′	132.7		6‴	149.8	
3′	142.1	7.86, s	7‴	116.6	6.79, d, 8.5
3′a	132.9		8‴	122.9	7.10, dd, 8.5, 1.5

[a] NMR data were measured at 500 MHz for proton and at 125 MHz for carbon.

ESIMS exhibited $[M + H]^+$ at *m*/*z* 595.1 and $[M + Na]^+$ at *m*/*z* 617.0. The molecular formula $C_{32}H_{18}O_{12}$ with 24 degrees of unsaturation was established by the HR-MALDI-FTMS which gave the $[M + H]^+$ ion peak at *m*/*z* 595.08711 (calcd 595.08766 for $C_{32}H_{19}O_{12}$). The ^1H NMR spectrum of **1** showed signals attributed to a 1,3,4-trisubstituted phenyl moiety at δ 6.79 (d, 1H, $J = 8.5$ Hz), 7.10 (dd, 1H, $J = 8.5$, 1.5 Hz), and 7.16 (d, 1H, $J = 1.5$ Hz), a *trans*-disubstituted double bond at δ 7.10 and 7.54 (1H each, d, $J = 16.0$ Hz), seven uncoupled aromatic and/or olefinic protons at δ 6.02, 6.25, 6.61, 7.00, 7.47, 7.86, and 8.28 (1H each, s), and six exchangeable phenolic hydroxyl protons at δ 9.23 (s, 5‴-O*H*), 9.43 (s, 5′-O*H*), 9.59 (s, 6′-O*H*), 9.76 (s, 6‴-O*H*), 10.12 (s, 8-O*H*) and 10.73 (s, 9-O*H*). In the ^{13}C NMR spectrum of **1**, all of the 32 carbon signals appeared in the relative lower magnetic field region (δ > 94 ppm), and the DEPT experiments differentiate them to be 12 methines and 20 quaternary carbons (Table 1). The protonated carbons and their bonded protons were unambiguously assigned by the HMQC experiment, and 12 quaternary carbons were assigned to be oxygenated sp^2 hybrid carbons on the basis of their chemical shift values (δ > 145 ppm). These spectroscopic data implied a highly oxygenated and highly unsaturated structure for **1**, which was finally established by the HMBC experiment (see Figure 2).

In the HMBC spectrum, long-range correlations from H-4 (δ 6.02) to C-3, C-4a, and C-10b (δ 153.9, 160.7, and 100.1), H-7 (δ 7.47) to C-6, C-8, C-9, and C-10a (δ 159.2, 147.9, 154.3, and 127.4), H-10 (δ 8.28) to C-8, C-9, C-6a (δ 112.4), and C-10b, −O*H* (δ 10.73) to C-8, C-9 and C-10, and −O*H* (δ 10.12) to C-9, in combination with the chemical shift of C-1 (δ 159.6), demonstrated that **1** contained the basic structural unit 8,9-dihydroxy-1*H*,6*H*-pyrano[4,3-*c*][2]-benzopyran-1,6-dione identical to that of phelligridins A and

(6) (a) Mo, S. Y.; Yang, Y. C.; He W. Y.; Shi, J. G. *Chin. Chem. Lett.* **2003**, *14*, 704. (b) Mo, S. Y.; Wang, S. J.; Yang, Y. C.; Chen X. G.; Shi, J. G. *J. Nat. Prod.* **2004**, *67*, 823.

(7) A biosynthetic pathway for phelligridins A−F and related co-occurring compounds we proposed previously (see ref 6b).

(8) A voucher specimen (No. 200136) has been deposited at the Herbarium of the Department of Medicinal Plants, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, P. R. China.

(9) The inhibitory rate against rat liver microsomal lipid peroxidation was 77.9% at a concentration of 100 μg/mL.

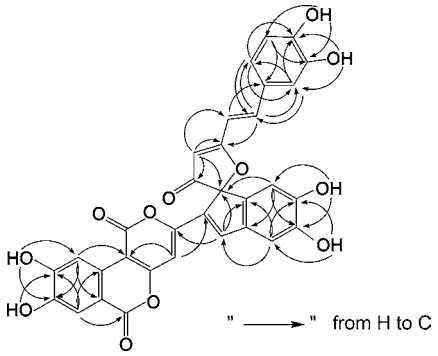
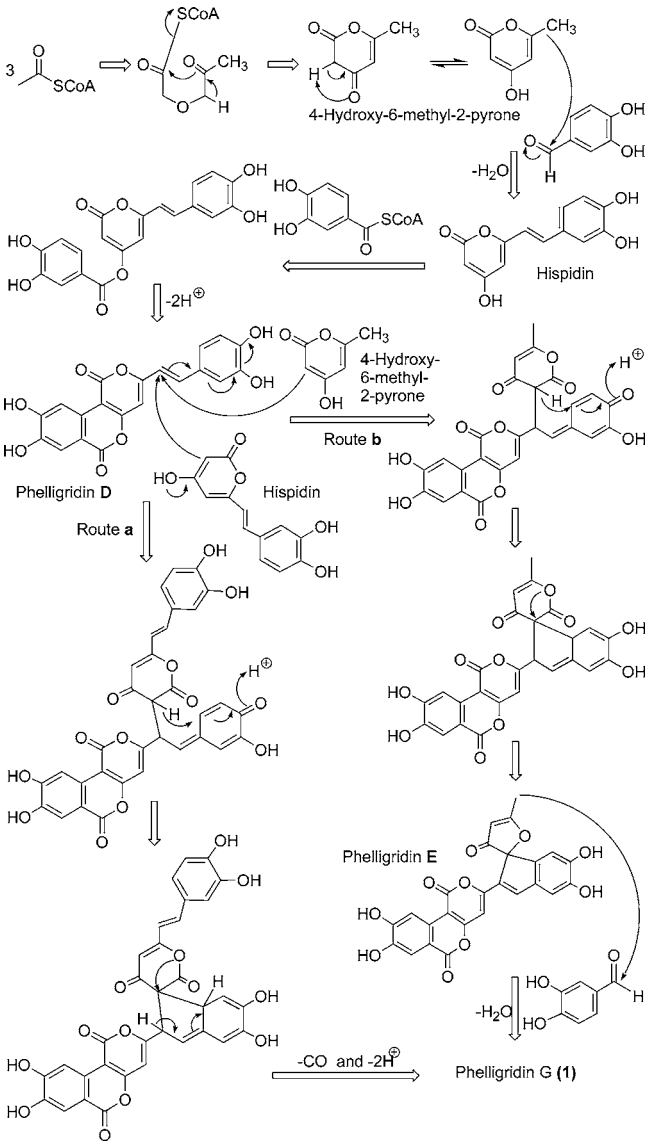


Figure 2. Key HMBC correlations of phelligridin G (**1**).

C−E.[6] Meanwhile, long-range HMBC correlations from H-4 to C-2′ (δ 132.7), H-3′ (δ 7.86) to C-1′, C-2′, C-3′a and C-7′a (δ 94.9, 132.7, 132.9 and 135.3), H-4′ (δ 7.00) to C-3′ and C-6′ (δ 142.1 and 147.7) and C-7′a, H-7′ (δ 6.61) to C-1′, C-5′ (δ 147.9) and C-3′a, −O*H* (δ 9.43) to C-4′ (δ 112.4) and C-6′, and −O*H* (δ 9.59) to C-5′ and C-7′ (δ 110.1) unequivocally established the 1′,1′-disubstituted-5′,6′-dihydroxyindene moiety. That this moiety bonded through C-2′ to C-3 of the basic structural unit was indicated by HMBC correlations from H-4 to C-2′ and H-3′ to C-3. Furthermore, the HMBC correlations from H-4″ (δ 6.25, s) to C-1′ of the indene moiety and two lower field carbons at δ 197.9 (C-3″) and δ 185.6 (C-5″) suggested that C-1′ connected through C-3″ and C-4″ (δ 103.9) and then to C-5″. In addition, HMBC correlations from H-1‴ (δ 7.10, d, J = 16 Hz) to C-3‴ (δ 126.7), H-2‴(δ 7.54, d, J = 16 Hz) to C-4‴ and C-8‴ (δ 115.7 and 122.9), H-4‴ (δ 7.16, d, J = 1.5 Hz) to C-2‴, C-5‴ and C-6‴ (δ 142.1, 146.5 and 149.8) and C-8‴, H-7‴ (δ 6.79, d, J = 8.5 Hz) to C-3‴, C-5‴ and C-6‴, H-8‴ (δ 7.10, dd, J = 8.5 and 1.5 Hz) to C-2‴, C-4‴ and C-6‴, as well as −O*H* (δ 9.23) to C-4‴, C-5‴and C-6‴, −O*H* (δ 9.76) to C-5‴, C-6‴ and C-7‴ unambiguously revealed the presence of a *trans*-5‴,6‴-dihydroxystyryl unit in **1**. Moreover, HMBC correlations from both H-1‴ and H-2‴ to C-5″ and H-4″ to C-1‴ indicated that the *trans*-5‴,6‴-dihydroxystyryl unit was linked to C-5″. In consideration of the molecular composition $C_{32}H_{18}O_{12}$ and 24 degrees of unsaturation of **1**, C-1′ of the indene moiety had to be bonded through an oxygen atom to C-5″ to form a spiroindene structure moiety that was supported by the chemical shift values of the C-1′ and C-5″. In comparison, the NMR data of **1** (Table 1) were in good agreement with those of phelligridin E[6b] except that the data assigned to the *trans*-5‴,6‴-dihydroxystyryl unit of **1** replaced those of the methyl group of phelligridin E and the chemical shift value of C-5″ of **1** was upfield shifted by $\Delta\delta$ 6.7 ppm due to the conjugative effect of the *trans*-5‴,6‴-dihydroxystyryl unit. This further confirmed all above elucidations. Consequently, the structure of **1** was unambiguously established as 8,9-dihydroxy-3-{5′,6′-dihydroxy-5″-(*trans*-5‴,6‴-dihydroxystyryl)-3″-oxo-spiro[furan-2″(3″*H*),1′-inden]-2′-yl}-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione and named as phelligridin G. Like the co-occurring phelligridin E and inoscavin A,[6b] phelligridin G (**1**) was also optically inactive and, hence, a racemate, indicating that the biogenetic formation of the chiral center (C-1′) of the spiroindene moiety is nonstereoselective.

Scheme 1. Proposed Biogenesis of Phelligridin G (**1**) from 4-Hydroxy-6-methyl-2-pyrone and 3,4-Dihydroxybenzaldehyde



Phelligridins mainly consist of fungal metabolites possessing the unique basic structural unit 8,9-dihydroxy-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione.[6] For phelligridins and related co-occurring compounds including inoscavin A, we have postulated a biogenetic pathway mediated by the fungal metabolite precursor 4-hydroxy-6-methyl-2-pyrone coupling with activated 3,4-dihydroxybenzoyl-SCoA or 3,4-dihydroxybenzaldehyde and/or 4-hydroxybenzaldehyde.[10] Based on our speculation, phelligridin G (**1**) may be further

(10) (a) See ref 6b. (b) During our investigation, Gill proposed a similar biosynthetic mechanism for inoscavin A; see: Gill, M. *Nat. Prod. Rep.* **2003**, *20*, 615.

biosynthesized from the coupling between phelligridin D and hispidin[11] (route a) and/or from the coupling between phelligridin E and 3,4-dihydroxybenzaldehyde (route b) (see Scheme 1).

Phelligridin G (**1**) showed antioxidant activity inhibiting rat liver microsomal lipid peroxidation with an IC_{50} of 3.86 μM and moderate selective cytotoxic activities against human ovary cancer cell line (A 2780) and human colon cancer cell line (HCT-8) with IC_{50} values of 20.4 and 30.2 μM, respectively.

(11) The widespread fungal metabolite hispidin was previously reported from the fungus *Phellinus igniarius*; see: Kirk, T. K.; Lorenz L. F.; Larsen, M. J. *Phytochemistry* **1975**, *14*, 281.

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Supporting Information Available: MS, HRMS, IR, UV, and 1D and 2D NMR spectra of phelligridin G (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

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