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Phelligridimer A, a Highly Oxygenated and Unsaturated 26-Membered Macrocyclic Metabolite with Antioxidant Activity from the Fungus *Phellinus igniarius*

Ying Wang,[†,‡] Su-Juan Wang,[†] Shun-Yan Mo,[†] Shuai Li,[†] Yong-Chun Yang,[†] and Jian-Gong Shi*[,†]

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College (Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine, Ministry of Education), Beijing 100050, People's Republic of China, and Key Laboratory for Atmospheric Chemistry (LAC), Centre for Atmosphere Watch and Services (CAWAS), Chinese Academy of Meteorological Sciences (CAMS), CMA, Beijing 100081, People's Republic of China

shijg@imm.ac.cn

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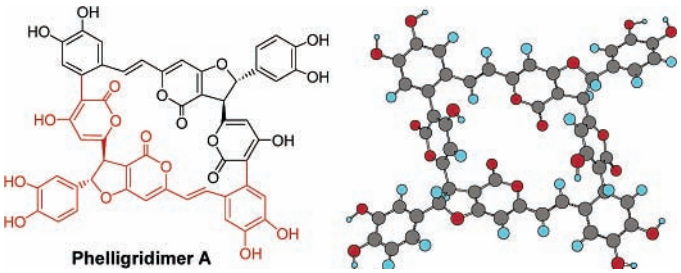
ABSTRACT



A highly oxygenated and unsaturated 26-membered macrocyclic metabolite, phelligridimer A (1), has been isolated from the Chinese medicinal fungus *Phellinus igniarius*. Its structure was elucidated by spectroscopic methods. A possible biogenesis of 1 mediated by the fungal metabolite hispidin was postulated. Phelligridimer A showed antioxidant activity (IC_{50} of 10.2 μM) but was inactive to several human cancer cell lines (IC_{50} > 50 μM) and enzymes PTP1B (IC_{50} > 25 μM) and thrombin (IC_{50} > 10 μM).

Mushrooms have been proved to be one of the most productive sources producing a large and diverse variety of secondary metabolites with interesting chemical structures and significant bioactivities.[1] As part of our recently initiated program to systematically assess the chemical and biological diversity of several traditional Chinese medicines, we carried out on the chemical investigation of the fruit body of the fungus *Phellinus igniarius* (DC. ex Fr.) Quél that has long been used for the treatment of fester, bellyache, and bloody gonorrhea in China.[2] Subsequent investigation on the EtOAc soluble portion of the ethanolic extract of this fungus has resulted in isolation and structural characterization of more

* To whom correspondence should be addressed. Tel: 86-10-83154789. Fax: 86-10-63017757.

[†] Chinese Academy of Medical Sciences and Peking Union Medical College.

[‡] Chinese Academy of Meteorological Sciences.

(1) For some examples, see: (a) Liu, J. K. *Heterocycles* **2002**, *57*, 157. (b) Bell, P. J. L.; Karuso, P. *J. Am. Chem. Soc.* **2003**, *125*, 9304. (c) Brady, S. F.; Bondi, S. M.; Clardy J. *J. Am. Chem. Soc.* **2001**, *123*, 9900. (d) Ohta, T.; Kita, T.; Kobayashi, N.; Obara, Y.; Nakahata, N.; Ohizumi, Y.; Takaya, Y.; Oshima, Y. *Tetrahedron Lett.* **1998**, *39*, 6229.

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

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than twenty metabolites including several antioxidant and/or cytotoxic phelligridins A−G with unusual structural features of pyrano[4,3-*c*][2]benzopyran-1,6-dione or furo-[3,2-*c*]pyran-4-one.[3] Continuing our work on the *n*-BuOH-soluble portion of the ethanolic extract, a highly oxygenated and unsaturated macrocyclic metabolite with an unprecedented 26-membered ring system, designated as phelligridimer A (**1**), has been characterized. We report herein the isolation, structural elucidation, postulated biogenetic formation, and biological activity of **1** (Figure 1).

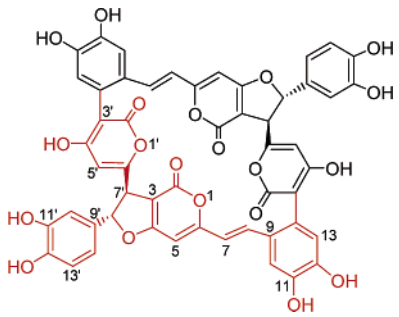


Figure 1. Structure of phelligridimer A (**1**).

Fractionation of the *n*-BuOH-soluble portion (60 g)[4] via middle-pressure liquid chromatography over reversed-phase silica gel (C-18) eluting with a gradient of increasing MeOH (0−100%) in acidic water containing 0.1% HOAc gave a partially purified mixture that was further purified by chromatography over Sephadex LH-20 by using $CHCl_3$−MeOH (1:1) as mobile phase to afford phelligridimer A (**1**) (152 mg), a yellow amorphous powder, mp >205 °C dec, insoluble in EtOAc, $CHCl_3$, and $(Me)_2CO$, slightly soluble in MeOH and H_2O, and soluble in DMSO and pyridine, $[\alpha]^{20}_D$ −2.0 (*c* 0.1, DMSO). The IR spectrum of **1** showed the presence of hydroxyl (3386 cm^{-1}), conjugated carbonyl (1684 cm^{-1}) groups, aromatic rings (1550 and 1429 cm^{-1}), and C−O bonds (1286, 1171, and 1109 cm^{-1}). The presence of aromatic ring and conjugated carbonyl functional groups was also indicated by the UV absorption bands at λ_{max}^{MeOH} (logϵ) 204 (5.03), 281 (4.41), and 385 (4.59) nm. To unambiguously determine the molecular weight and formula of **1**, different mass spectroscopic techniques were used. The negative mode electron spray ionization (ESI) and positive mode fast atom bombardment (FAB) mass spectra of **1** exhibited a quasi-molecular ion peak at *m*/*z* 975 $[M - H]^-$ and 977 $[M + H]^+$, respectively, and the positive-mode high-resolution FT-MALDI-MS at *m*/*z* 977.15597 $[M + H]^+$ established the molecular formula $C_{52}H_{32}O_{20}$ (calcd 977.15674 for $C_{52}H_{33}O_{20}$) requiring 37 sites of unsaturation. However, the ^1H and ^{13}C NMR spectra of **1** exhibited resonances of 16 protons and 26 carbons (Table 1), respectively, which were only half the numbers of proton and carbon atoms expected from the molecular formula. These spectroscopic data suggested that **1** possessed a symmetric structure. And, therefore, each signal in the NMR spectra of **1** represented a pair of overlapping resonances with a completely equivalent chemical and magnetic environment.

Table 1. NMR Data for the Half Structure of Phelligridimer A (**1**)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
2		163.0 s	2′		157.7 s
3		99.3 s	3′		101.7 s
4		170.4 s	4′		165.0 s
5	6.51 s	95.0 d	5′	6.31 s	101.7 d
6		162.8 s	6′		159.6 s
7	6.60 d (15.5)	116.8 d	7′	4.34 d (6.5)	51.4 d
8	6.95 d (15.5)	133.9 d	8′	5.90 d (6.5)	89.6 d
9		126.1 s	9′		129.6 s
10	7.12 s	112.2 d	10′	6.82 brs	113.5 d
11		145.3 s	11′		145.6 s
12		147.2 s	12′		146.4 s
13	6.55 s	118.5 d	13′	6.76 brs	115.5 d
14		125.2 s	14′	6.76 brs	118.0 d

[a] NMR data were recorded in DMSO-d_6 at 500 MHz for proton and at 125 MHz for carbon.

For the half structural unit of **1**, the ^1H NMR spectrum displayed a pair of doublets assignable to a 1,2-disubstituted *trans*-double bond at δ 6.60 and 6.95 (d each, J = 15.5 Hz, H-7 and H-8), another pair of doublets ascribed to two mutually coupled vicinal protons at δ 4.34 and 5.90 (d each, J = 6.5 Hz, H-7′ and H-8′), and seven singlets due to olefinic and/or aromatic protons at δ 6.31, 6.51, 6.55, 6.76, 6.76, 6.82, and 7.12 (s each, H-5′, H-5, H-13, H-13′, H-14′, H-10′, and H-10), in addition to five exchangeable phenolic hydroxyl protons at δ 9.09, 9.13, 9.18, 9.50, 11.55 (brs each, *OH*-11′, *OH*-11, *OH*-12′, *OH*-12, *OH*-4′). The ^{13}C NMR and DEPT spectra showed 26 carbon signals consisting of 11 methines and 15 quaternary carbons, of which 10 quaternary carbons were distinguished to be oxygenated sp^2 hybrid carbons on the basis of their chemical shift values (δ >145 ppm). Above spectroscopic data confirmed that **1** was a highly oxygenated and highly unsaturated symmetric dimer.

The structure of **1** was subsequently established by a comprehensive analysis of 2D NMR spectroscopy. The heteronuclear single quantum coherence (HSQC) spectrum of **1** led to the unambiguous assignment of resonances of protons and protonated carbons in the NMR spectra of **1** (Table 1). The heteronuclear multiple bond correlation (HMBC) spectrum played the most important role in the establishment of structural moieties and their connection relationships in **1**. A series of HMBC correlations from H-5 to C-3 (δ 99.3), C-4 (δ 170.4), C-6 (δ 162.8), and C-7 (δ 116.8), from H-7 to C-5 (δ 95.0), C-6, and C-9 (δ 126.1),

(3) (a) Wang, Y.; Mo, S. Y.; Wang, S. J.; Li, S.; Yang, Y. C.; Shi, J. G. *Org. Lett.* **2005**, *7*, 1675. (b) Mo, S. Y.; Wang, S. J.; Yang, Y. C.; Chen X. G.; Shi, J. G. *J. Nat. Prod.* **2004**, *67*, 823. (c) Mo, S. Y.; Yang, Y. C.; He W. Y.; Shi, J. G. *Chin. Chem. Lett.* **2003**, *14*, 704. (d) Mo, S. Y.; He W. Y.; Yang, Y. C.; Shi, J. G. *Chin. Chem. Lett.* **2003**, *14*, 810. (e) Mo, S. Y.; Yang, Y. C.; Shi, J. G. *Huaxue Xuebao* **2003**, *61*, 1161. (f) Mo, S. Y.; Yang, Y. C.; Shi, J. G. *Zhongcaoyao* **2004**, *35*, 1095.

(4) Details of the fungal material and the procedure to yield the *n*-BuOH-soluble portion were described previously (see ref 3b).

from H-8 to C-6, C-14 (δ 125.2), and C-10 (δ 112.2), from H-10 to C-8 (δ 133.9), C-11 (δ 145.3), C-12 (δ 147.2), and C-14, and from H-13 to C-9, C-12, and C-11 (Figure 2), in

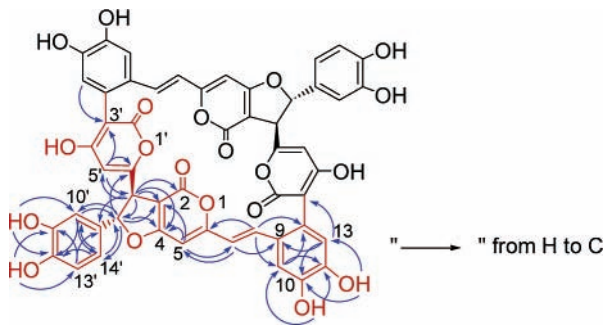


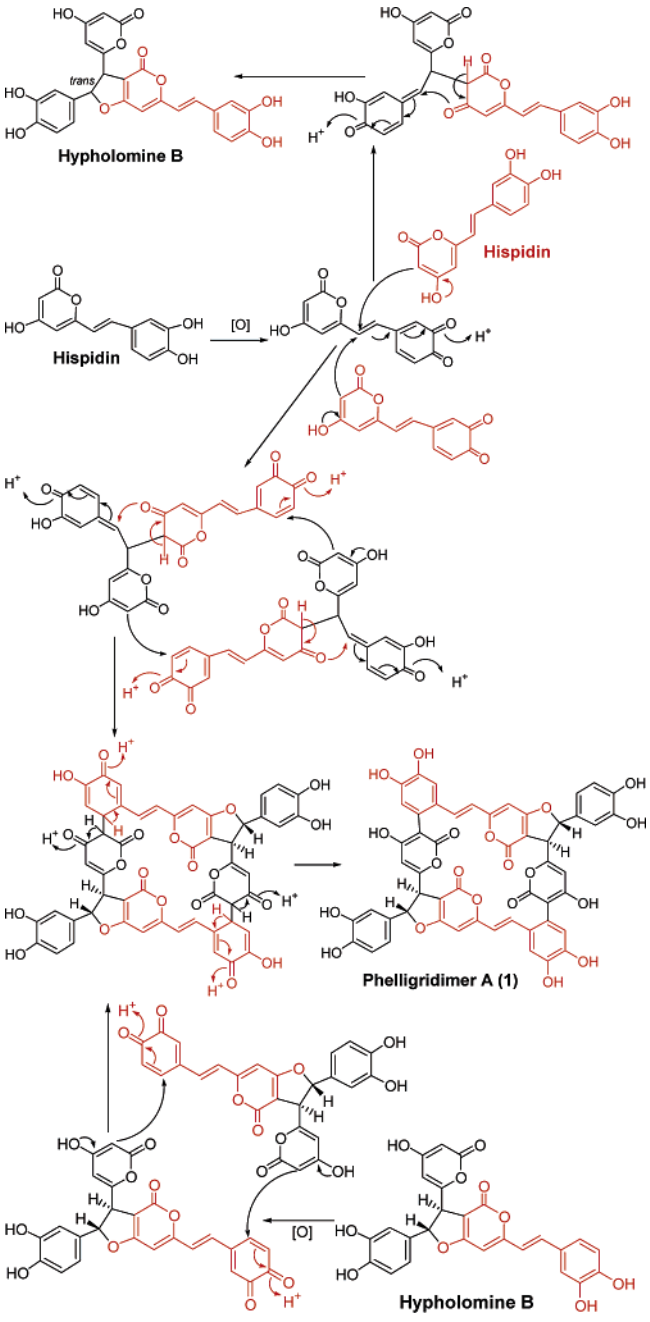
Figure 2. Key HMBC correlations of phelligridimer A (**1**).

combination with a quaternary carbon resonance at δ 163.0 (C-2), as well as a careful comparison of chemical shift values of above protons and carbons with those of hispidin[5] and the hispidin units of phelligridins A−F,[3b] unequivocally revealed the presence of a 3,14-disubstituted hispidin moiety in the half structure of **1**. Likewise, the occurrence of a 3′,7′,8′-trisubstituted 7′,8′-dihydrohispidin moiety in the half structure of **1** was unambiguously demonstrated by HMBC correlations from H-5′ to C-3′ (δ 101.7), C-4′ (δ 165.0), C-6′ (δ 159.6), and C-7′ (δ 51.4), from H-7′ to C-5′ (δ 101.7), C-8′ (δ 89.6), and C-9′ (δ 129.6), from H-8′ to C-6′, C-7′, C-9′, C-10′ (δ 113.5), and C-14′ (δ 118.0), from H-10′ to C-8′, C-11′ (δ 145.6), C-12′ (δ 146.4), and C-14′, from H-13′ to C-9′ and C-11′, and from H-14′ to C-8′, C-10′, and C-12′ (Figure 2), together with an uncorrelated quaternary carbon resonance at δ 157.7 (C-2′) comparable to C-2 of hispidin.[5] Additionally, the connection pattern between the two moieties of the half structure was evidenced by HMBC correlations from H-7′ to C-2, C-3 and C-4 and from H-8′ to C-3 and C-4 (Figure 2), as well as the chemical shift values of C-4 and C-8′, indicating a direct linkage between C-3 and C-7′ and an oxygen-bridged linkage between C-4 and C-8′ to form a dihydrofuro[3,2-*c*]pyran-4-one moiety in the half structure of **1**. The vicinal coupling constant between H-7′ and H-8′ (J = 6.5 Hz) suggested a trans orientation[6] of the two protons of the dihydrofuro[3,2-*c*]pyran-4-one moiety, which was further confirmed from an NOE enhancement of H-5′ by irradiation of H-8′ that in turn enhanced by irradiation of H-5′, as well as from enhancements of H-10′ and H-14′ by irradiation of H-7′ in the NOE difference spectrum of **1**. Accordingly, the half structure of **1** was established to be similar to hypholomine B isolated from the fungus *Hypholoma fasciculave* and characterized on the basis of spectroscopic analysis of its permethyl derivative.[7]

(5) (a) The widespread fungal metabolite hispidin was previously isolated from the fungus *Phellinus igniarius*, see: Kirk, T. K.; Lorenz L. F.; Larsen, M. J. *Phytochemistry* **1975**, *14*, 281. (b) Kim, J.-P.; Yun, B.-S.; Shim, Y. K.; Yoo, I.-D. *Tetrahedron Lett.* **1999**, *40*, 6643. (c) Ali, N. A. A.; Jansen, R.; Pilgrim, H.; Liberra, K.; Lindequist, U. *Phytochemistry* **1996**, *41*, 927.

A careful comparison of the ^{1}H NMR data of the half structure of **1** and permethyl hypholomine B[7] indicated that H-3′ and H-14 of the half structure were absent, suggesting that the dimerization of the half structure occurred at C-3′ and C-14. This was supported by the quaternary nature of C-3′ and C-14 indicated in the DEPT and HSQC spectra of **1**. Finally, in the HMBC spectrum of **1** a long-range correlation from H-13 to C-3′ (Figure 2) was reasonably explained to be the three bond correlation from H-13 of one-half structure to C-3′ of another, and unequivocally demonstrated that C-14 of the half structure connected to C-3′ of another to form the symmetrically dimeric structure with an unprecedented 26 membered macrocyclic ring system.

Scheme 1. Proposed Biogenesis of Hypholomine B and Phelligridimer A (**1**) from Hispidin



Therefore, the structure of **1** was elucidated as a hypholomine B dimer, and designated as phelligridimer A.

The 3D structure of **1** generated by molecular modeling using the MM2 program is given in Figure 3, illustrating a

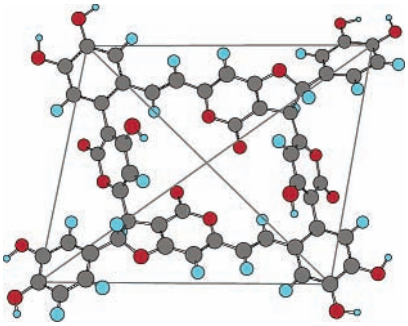


Figure 3. 3D structure of phelligridimer A (**1**).

cagelike structure within a parallelepiped frame, and the two freely rotating aromatic rings extended to a pair of opposite angles of the frame.

For related co-occurring compounds including hispidin, inoscavin A and phelligridins A−G, 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.[3a,b] Based on our speculation, hypholomine B may be further biosynthesized from the oxidation coupling of two hispidin mediates, and phelligridimer A (**1**) may be sequentially or simultaneously formed from oxidation coupling of four hispidin mediates, and/or from oxidation coupling of two molecules of hypholomine B (Scheme 1).

Phelligridimer A (**1**) is the major component of the *n*-BuOH-soluble portion, and showed antioxidant activity inhibiting rat liver microsomal lipid peroxidation with an IC_{50} of 10.2 μM, but was inactive to several human cancer cell lines (IC_{50} > 50 μM) including human ovary cancer cell line (A 2780), colon cancer cell line (HCT-8), hepatoma cell line (Bel-7402), mammary cancer cell line (MCF-7), and lung cancer cell line (A549) and inactive as well to protein tyrosine phosphatase 1B (PTP1B) (IC_{50} > 25 μM) and thrombin (IC_{50} > 10 μM)).

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

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(7) Fiasson, J.-L.; Gluchoff-Fiasson, K.; Steglich, W. *Chem. Ber.* **1977**, *110*, 1047−1057.