New Stilbenoids from *Pholidota yunnanensis* and Their Inhibitory Effects on Nitric Oxide Production

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Six new stilbenoids, a (bibenzyldihydrophenanthrene) ether designated phoyunnanin D (1), a bis(dihydrophenanthrene) ether designated phoyunnanin E (2), and four stilbenes designated phoyunbene A—D (3—6), were isolated from the air-dried whole plant of *Pholidota yunnanensis* ROLFE. The new compounds were identified as 7-[2-(3-hydroxyphenethyl)-4-hydroxy-6-methoxyphenoxy]-4-hydroxy-2-methoxy-9,10-dihydrophenanthrene (1), 1-[(9,10-dihydro-4-hydroxy-2-methoxy-7-phenanthrenyl)oxy]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (2), *trans*-3,3′-dihydroxy-2′,4′,5-trimethoxystilbene (3), *trans*-3,4′-dihydroxy-2′,3′,5-trimethoxystilbene (4), *trans*-3,3′-dihydroxy-2′,5-dimethoxystilbene (5), and *trans*-3-hydroxy-2′,3′,5-trimethoxystilbene (6) based on spectroscopic evidence. Furthermore, the inhibitory effects of compounds 1—6 on nitric oxide production in a murine macrophage-like cell line (RAW 264.7) activated by lipopolysaccharide and interferon-γ were examined.

Key words *Pholidota yunnanensis*; Orchidaceae; (bibenzyldihydrophenanthrene) ether; bis(dihydrophenanthrene) ether; stilbene; nitric oxide production inhibitor

Pholidota yunnanensis ROLFE (Orchidaceae) is a perennial herb distributed in Yunnan, Guangxi, Sichuan, and Guizhou provinces in China. The whole plant or pseudobulb is used as a folk medicine for the treatment of cough, rheumatism, stomachache, and trauma.[1,2] In previous investigations on this plant, five triterpenes together with *n*-nonacosane, *n*-dotriacontanoic acid, *n*-octacostyl ferulate, and β-sitosterol were reported.[3—5] The 60% ethanol extract of the air-dried whole plant of *P. yunnanensis* showed inhibitory effects on nitric oxide (NO) production in murine macrophages (RAW 264.7) activated by lipopolysaccharide (LPS) and interferon (IFN)-γ in our preliminary screening (inhibition %=92.1%, 30 mg/ml). Therefore the study of its chemical constituents was undertaken to identify the NO production inhibitors.

The 60% ethanol extract of *P. yunnanensis* was suspended in H_2O and partitioned with $CHCl_3$, EtOAc, and *n*-BuOH successively. The $CHCl_3$ layer (inhibition %=90.3%, 30 mg/ml) and EtOAc layer (inhibition %=97.1%, 30 mg/ml) that showed NO production inhibitory activity were isolated by repeated column chromatography on silica gel, ODS, Sephadex LH-20, and preparative HPLC (ODS), respectively, to afford four new stilbenes (**3**—**6**) and two new stilbenoids (**1**, **2**). In this report, we describe the isolation, structural elucidation, and inhibitory effects on NO production by activated murine macrophages of these new compounds.

Results and Discussion

Phoyunnanin D (**1**), an amorphous powder, was subjected to high-resolution electron impact ionization (HR-EI)-MS and exhibited $[M]^+$ at *m*/*z* 484.1874 to give the molecular formula $C_{30}H_{28}O_6$. The UV spectrum showed the presence of aromatic rings (205, 280 nm). The IR spectrum exhibited the presence of hydroxyl groups (3382 cm^{-1}) and aromatic rings (1593, 1458 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) showed signals assignable to an ABX coupling system [δ 6.64 (1H, dd, J=8.7, 2.8 Hz, H-6), 6.69 (1H, d, J=2.8 Hz, H-8), and 8.27 (1H, d, J=8.7 Hz, H-5)], two pairs of *meta*-coupled aromatic protons [δ 6.38 (1H, d, J=2.5 Hz, H-1), 6.43 (1H, d, J=2.5 Hz, H-3); δ 6.41 (1H, d, J=2.7 Hz, H-3′), 6.50 (1H, d, J=2.7 Hz, H-5′)], four aromatic protons [δ 6.59 (1H, dd, J=7.8, 0.9 Hz, H-6″), 6.61 (1H, ddd, J=7.8, 2.5, 0.9 Hz, H-4″), 6.63 (1H, m, H-2″), 7.02 (1H, t, J=7.8 Hz, H-5″)], four methylene protons [δ 2.69 (4H, m, H_2-9, H_2-10), 2.73 (4H, m, H_2-7″, H_2-8″)], and two aromatic methoxyl protons [δ 3.80 (3H, s, 2-OCH_3), 3.70 (3H, s, 6′-OCH_3)]. The ^{13}C-NMR spectrum (Table 1) combined with the distortionless enchancement by polarization transfer (DEPT) and ^{1}H-detected heteronuclear multiple-quantum coherence (HMQC) spectra exhibited the signals for four methylenes, two methoxyls, and 24 aromatic carbons that consisted of 11 protonated carbons, seven quaternary carbons, and six oxygenated carbons. The presence of a dihydrophenanthrene moiety and a bibenzyl moiety was confirmed by the ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) correlations (Table 1). This was further supported by the nuclear Overhauser effect spectroscopy (NOESY) correlations between the methylene protons at δ 2.69 and H-1, H-8, and between the methylene protons at δ 2.73 and H-3′, H-2″, and H-6″. In the NOESY experiment, the correlations between the methoxyl protons at δ 3.80 and H-1, H-3, and between the methoxyl protons at δ 3.70 and H-5′ further identified the positions of the two methoxyls located at C-2 and C-6′, respectively. Acetylation of **1** with acetic anhydride and pyridine (1 : 1, v/v) afforded a triacetate product (**1a**), indicating the presence of three hydroxyls. As the total number of methoxyl and hydroxyl groups was five, the remaining oxygen should be ether. The positions of these hydroxyls were deduced from NMR data (see Experimental) of **1a** in which the signals of H-3, H-3′, H-5′, H-2″, and H-4″ were shifted downfield of 0.18, 0.34, 0.33, 0.21, and 0.21 ppm, respectively, and the signal of H-5

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Table 1. The NMR Data of Compounds **1** and **2** (in Acetone-d_6)[a,b]

1				**2**			
Position	δ_H	δ_C	HMBC	Position	δ_H	δ_C	HMBC
1	6.38 (1H, d, J=2.5)	106.0	C-2 (^2J); C-3, 4a, 10 (^3J)	1		133.9	
2		159.6		2		152.0	
3	6.43 (1H, d, J=2.5)	101.6	C-2, 4 (^2J); C-1, 4a (^3J)	3	6.68 (1H, s)	100.8	C-2, 4 (^2J); C-1, 4a (^3J)
4		156.1		4		152.6	
4a		115.5		4a		115.8	
4b		127.6		4b		125.6	
5	8.27 (1H, d, J=8.7)	129.7	C-7, 8a, 4a (^3J)	5	8.29 (1H, d, J=8.2)	130.2	C-7, 8a, 4a (^3J)
6	6.64 (1H, dd, J=8.7, 2.8)	112.7	C-7 (^2J); C-8, 4b (^3J)	6	6.72 (1H, dd, J=8.2, 2.7)	113.6	C-7 (^2J); C-4b, 8 (^3J)
7		157.8		7		156.4	
8	6.69 (1H, d, J=2.8)	114.3	C-7 (^2J); C-6, 4b, 9 (^3J)	8	6.70 (1H, d, J=2.7)	115.0	C-7 (^2J); C-6, 4b, 9 (^3J)
8a		139.7		8a		139.8	
9	2.69 (2H, m)	30.8	C-8a, 10 (^2J); C-8, 4b, 10a (^3J)	9	2.62 (2H, m)	30.0	C-8a, 10 (^2J); C-8, 4b, 10a (^3J)
10	2.69 (2H, m)	31.4	C-10a, 9 (^2J); C-1, 4a, 8a (^3J)	10	2.62 (2H, m)	23.8	C-10a, 9 (^2J); C-1, 4a, 8a (^3J)
10a		141.6		10a		134.0	
1′		135.1		1′	6.38 (1H, d, J=2.5)	106.0	C-2′ (^2J); C-3′, 4a′, 10 (^3J)
2′		137.4		2′		159.6	
3′	6.41 (1H, d, J=2.7)	108.7	C-4′ (^2J); C-1′, 5′, 8″ (^3J)	3′	6.43 (1H, d, J=2.5)	101.6	C-2′, 4′ (^2J); C-1′, 4a′ (^3J)
4′		155.9		4′		156.1	
5′	6.50 (1H, d, J=2.7)	99.8	C-4′, 6′ (^2J); C-1′, 3′ (^3J)	4a′		115.5	
6′		154.3		4b′		127.6	
1″		144.4		5′	8.26 (1H, d, J=8.7)	129.8	C-7′, 8a′, 4a′ (^3J)
2″	6.63 (1H, m)	116.1	C-4″, 6″, 7″ (^3J)	6′	6.64 (1H, dd, J=8.7, 2.8)	112.6	C-7′ (^2J); C-8′, 4b′ (^3J)
3″		158.2		7′		157.7	
4″	6.61 (1H, ddd, J=7.8, 2.5, 0.9)	113.6	C-2″, 6″ (^3J)	8′	6.69 (1H, d, J=2.8)	114.2	C-7′ (^2J); C-6′, 4b′, 9′ (^3J)
5″	7.02 (1H, t, J=7.8)	130.0	C-1″, 3″ (^3J)	8a′		139.7	
6″	6.59 (1H, dd, J=7.8, 0.9)	120.4	C-2″, 4″, 7″ (^3J)	9′	2.68 (2H, m)	30.7	C-8a′, 10′ (^2J); C-8′, 4b′ 10a′ (^3J)
7″	2.73 (2H, m)	37.1	C-1″, 8″ (^2J); C-2″, 6″, 2′ (^3J)	10′	2.68 (2H, m)	31.4	C-10a′, 9 (^2J); C-1′, 4a′, 8a′ (^3J)
8″	2.73 (2H, m)	33.2	C-2′, 7″ (^2J); C-1′, 3′, 1″ (^3J)	10a′		141.6	
2-OCH_3	3.80 (3H, s)	55.3	C-2 (^3J)	2-OCH_3	3.72 (3H, s)	56.0	C-2 (^3J)
6′-OCH_3	3.70 (3H, s)	56.0	C-6′ (^3J)	2′-OCH_3	3.74 (3H, s)	55.3	C-2′ (^3J)

a) Assignments were confirmed by DEPT, HMQC, HMBC and NOESY spectra. *b*) *J* values (in parentheses) are reported in Hz.

was shifted upfield of 0.42 ppm in comparison with that of **1**. Furthermore, the signals of C-4, C-4′, and C-3″ were shifted upfield of 6.7, 6.5, and 6.2 ppm, respectively, and those of C-3, 4a, 3′, 5′, 2″, and 4″ were shifted downfield of 7.0, 5.3, 7.0, 6.5, 6.4, and 6.6 ppm, respectively. All the evidence confirmed that the locations of the three hydroxyls were at C-4, C-4′, and C-3″, and the ether linkage should be between C-7 and C-1′. Thus the structure of phoyunnanin D (**1**) was elucidated to be 7-[2-(3-hydroxyphenethyl)-4-hydroxy-6-methoxyphenoxy]-4-hydroxy-2-methoxy-9,10-dihydrophenanthrene, a new (bibenzyldihydrophenanthrene) ether.

Phoyunnanin E (**2**), an amorphous powder, showed UV absorption at 212, 278, and 297 nm, similar to dihydrophenanthrenes.[6] The IR spectrum showed the presence of hydroxyl groups (3394 cm^{-1}) and aromatic rings (1612, 1589, 1485 cm^{-1}). The molecular formula was determined to be $C_{30}H_{26}O_6$ based on the HR-EI-MS. The ^{1}H-NMR spectrum (Table 1) showed the signals due to two sets of ABX coupling systems [δ 6.64 (1H, dd, J=8.7, 2.8 Hz, H-6′), 6.69 (1H, d, J=2.8 Hz, H-5′), 8.26 (1H, d, J=8.7 Hz, H-8′); δ 6.70 (1H, d, J=2.7 Hz, H-8), 6.72 (1H, dd, J=8.2, 2.7 Hz, H-6), 8.29 (1H, d, J=8.2 Hz, H-5)], a pair of *meta*-coupled aromatic protons [δ 6.38 (1H, d, J=2.5 Hz, H-1′), 6.43 (1H, d, J=2.5 Hz, H-3′)], an aromatic proton [δ 6.68 (1H, s, H-3)], four methylene protons [δ 2.68 (4H, m, H_2-9′, H_2-10′), 2.62 (4H, m, H_2-9, H_2-10)], and two aromatic methoxyl protons [δ 3.72 (3H, s, 2-OCH_3), 3.74 (3H, s, 2′-OCH_3)]. The ^{13}C-NMR spectrum (Table 1) combined with DEPT and HMQC spectra showed signals for four methylenes, two methoxyls, and 24 aromatic carbons that consisted of nine protonated carbons, nine quaternary carbons, and six oxygenated carbons. The HMBC correlations (Table 1) established the skeleton of two dihydrophenanthrenes, which was further confirmed by the NOESY correlations between the methylene protons at δ 2.68 and H-1′, H-8′, and between the methylene protons at δ 2.62 and H-8. In the NOESY spectrum, the correlations between the methoxyl protons at δ 3.74 and H-1′, H-3′, and between the methoxyl protons at δ 3.72 and H-3 further confirmed that the two methoxyls were located at C-2′ and C-2, respectively. Acetylation of **2** with acetic anhydride and pyridine (1 : 1, v/v) also afforded a triacetate product (**2a**), suggesting the presence of three hydroxyls. As the total number of methoxyl and hydroxyl groups was five, the remaining oxygen should be ether. The positions of the three hydroxyls were determined by comparing the ^{13}C-NMR data of **2a** (see Experimental) with those of **2**. In the ^{13}C-NMR spectrum of **2a**, the signals of C-4, C-7, and C-4′ showed upfield shifts of 6.2, 5.7, and 6.7 ppm, respectively, and the signals of C-3, C-4a, C-6, C-8, C-3′, and C-4a′ showed downfield shifts of 7.1, 5.2, 7.1, 7.0, 7.0, and 5.2 ppm, respectively. As the above evidence indicated, the locations of the three hydroxyls were at C-4, C-7, and C-4′, the two dihydrophenanthrene moieties were connected at C-1 and C-7′ through an ether bond. Thus the structure of phoyunnanin E (**2**) was elucidated to be 1-[(9,10-dihydro-4-hydroxy-2-methoxy-7-phenanthrenyl)oxy]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene.

Phoyunbene A (**3**), white needles, mp 170—171 °C, showed a molecular ion at *m/z* 302.1157 $[M]^+$ in the HR-EI-MS to give the molecular formula $C_{17}H_{18}O_5$. The UV spectrum showed absorption at 202, 223, and 317 nm, similar to stilbene derivatives.[7] The IR spectrum exhibited the presence of hydroxyl groups (3360 cm^{-1}), aromatic rings (1609, 1589, 1500 cm^{-1}), and *trans*-olefinic (999 cm^{-1}). The ^{1}H-NMR spectrum showed signals due to two *trans*-olefinic protons at δ 7.23 (1H, d, J=16.4 Hz, H-α) and 6.93 (1H, d, J=16.4 Hz, H-α'), a pair of *ortho*-coupled aromatic protons at δ 7.07 (1H, d, J=8.7 Hz, H-6′) and 6.72 (1H, d, J=8.7 Hz, H-5′) indicating the presence of a 1,2,3,4-tetrasubstituted benzene ring, three aromatic protons at δ 6.58 (1H, t, J=1.8 Hz, H-2), 6.54 (1H, t, J=1.8 Hz, H-6), and 6.26 (1H, t, J=1.8 Hz, H-4) suggesting the presence of a 1,3,5-trisubstituted benzene ring, and three aromatic methoxyl protons at δ 3.84, 3.81, and 3.76. The ^{13}C-NMR spectrum (see Experimental) combined with the HMQC spectrum showed signals due to two olefinic carbons at δ 124.3 and 129.0, 12 aromatic carbons, and three methoxyls. All the evidence indicated that **3** is a characteristic *trans*-stilbene. This was further supported by the HMBC correlations between H-α and C-1, C-2′, and C-6′, and between H-α' and C-2, C-6, and C-1′. The HMBC correlations between the methoxyl protons at δ 3.76 and C-5, between the methoxyl protons at δ 3.81 and C-2′, and between the methoxyl protons at δ 3.84 and C-4′ indicated that the three methoxyls were located at C-5, C-2′, and C-4′, respectively. The NOESY experiment further confirmed that the locations of the methoxyls were at C-5, C-2′, and C-4′ by the correlations between 5-OC$\underline{H}_3$ and H-4, H-6, between 2′-OC$\underline{H}_3$ and H-α, and between 4′-OC$\underline{H}_3$ and H-5′. According to the molecular formula and the structural information, two hydroxyls were present and located at C-3 and C-3′. Thus the structure of phoyunbene A (**3**) was elucidated to be *trans*-3,3′-dihydroxy-2′,4′,5-trimethoxystilbene.

Phoyunbene B (**4**) was obtained as an oil. The molecular formula was determined to be $C_{17}H_{18}O_5$ identical to that of **3**. The UV spectrum showed absorption at 219 and 318 nm. The ^{1}H- and ^{13}C-NMR data (see Experimental) of **4** were similar to those of **3** and indicated the presence of a *trans*-stilbene skeleton, which was further established by the HMBC correlations (see Experimental). The HMBC correlations between the methoxyl protons at δ 3.76 and C-5, between the methoxyl protons at δ 3.84 and C-3′, and between the methoxyl protons at δ 3.85 and C-2′ indicated the positions of the three methoxyls at C-5, C-3′, and C-2′, respectively. In the NOESY spectrum, the correlations between 5-OC$\underline{H}_3$ and H-4, H-6, and between 2′-OC$\underline{H}_3$ and H-α further confirmed the locations of the two methoxyls. Thus phoyunbene B (**4**) was determined to be *trans*-3,4′-dihydroxy-2′,3′,5-trimethoxy stilbene, an isomer of compound **3**.

Phoyunbene C (**5**) was obtained as an oil, and the UV spectrum showed the presence of aromatic rings (219, 303 nm). The molecular formula was determined to be $C_{16}H_{16}O_4$ based on HR-EI-MS. The ^{1}H- and ^{13}C-NMR data (see Experimental) of **5** suggested the presence of a *trans*-stilbene skeleton, which was further established by the HMBC correlations (see Experimental). In the HMBC spectrum, the correlations between the methoxyl protons at δ 3.78 and C-2′, and between the methoxyl protons at δ 3.76 and C-5, indicated that positions of the two methoxyls were located at C-2′ and C-5. The NOESY correlations between 5-OCH_3 and H-4, H-6, and between 2′-OCH_3 and H-α, further confirmed the locations of the two methoxyls. Thus the structure of phoyunbene C (**5**) was determined to be *trans*-3,3′-dihydroxy-2′,5-dimethoxystilbene.

Phoyunbene D (**6**), a yellow prism, mp 128—129 °C, exhibited UV absorption at 223 and 301 nm. The IR spectrum showed the presence of hydroxyl groups (3356 cm^{-1}), aromatic rings (1597, 1474 cm^{-1}), and *trans*-olefinic (999 cm^{-1}). The molecular formula was determined to be $C_{17}H_{18}O_4$ by HR-EI-MS. The ^{1}H- and ^{13}C-NMR data (see Experimental) indicated that **6** is a characteristic *trans*-stilbene. This was supported by the HMBC correlations (see Experimental), and the locations of the three methoxyls were determined to be at C-5, C-2′, and C-3′ based on the HMBC correlations between the methoxyl protons at δ 3.77 and C-5, between the methoxyl protons at δ 3.81 and C-2′, and between the methoxyl protons at δ 3.85 and C-3′. In the NOESY spectrum, the correlations between 5-OCH_3 and H-4, H-6, between 2′-OCH_3 and H-α, and between 3′-OCH_3 further confirmed the positions of the three methoxyls. Thus the structure of phoyunbene D (**6**) was determined to be *trans*-3-hydroxy-2′,3′,5-trimethoxystilbene.

Macrophages play major roles in inflammation and host defense mechanisms against bacterial and viral infections.[8] The NO radical produced by the oxidation of L-arginine by NO synthase (NOS) is an effective molecule for the antiinflammatory and antimicrobial effects of macrophages. How-

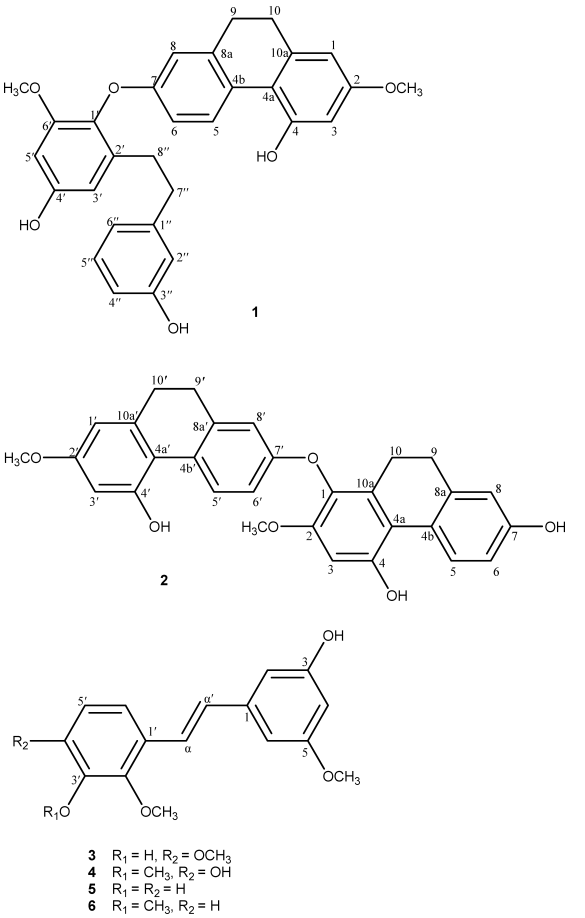

Fig. 1. Structures of Compounds **1**—**6** Isolated from *Pholidota yunnanensis*

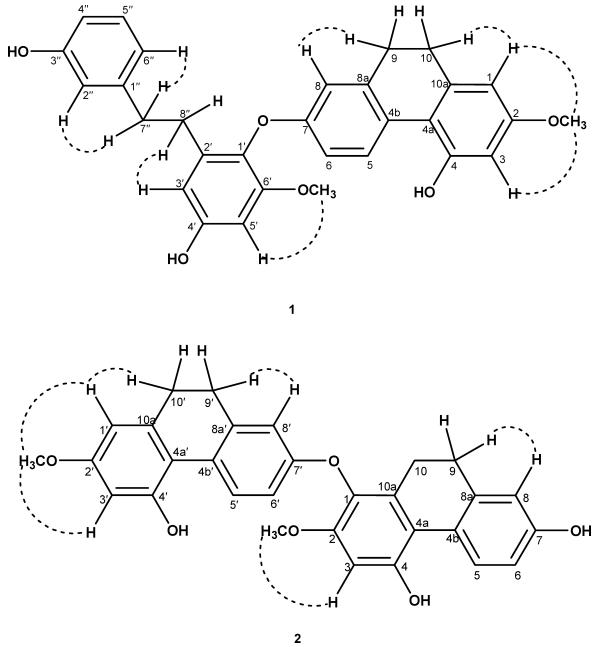

Fig. 2. The Principle NOESY Correlations of Compounds **1** and **2**

ever, excessive production of NO may lead to severe injury to host cells and tissues during acute and chronic inflammation.[9] Compounds **1**—**6** were examined for their inhibitory effects on NO production in a murine macrophage-like cell line (RAW 264.7) that was activated by LPS and IFN-γ. In the assay, resveratrol (IC_{50}=29.8 μM), which has been reported to have inhibitory effects on NO production in LPS-activated RAW 264.7 macrophages by down-regulation of the inducible NOS and mRNA, was used as a positive control.[10,11] Four stilbenes, phoyunbene A (**3**), phoyunbene B (**4**), phoyunbene C (**5**), and phoyunbene D (**6**), showed inhibitory effects on NO production without cytotoxicity with IC_{50} values of 32.9, 7.5, 49.0, and 87.3 μM, respectively. The two ethers (**1**, **2**) showed cyctotoxic effects at the test concentrations. Phoyunbene B (**4**) showed much stronger inhibitory effects than resveratrol.

Experimental

General Procedures Melting points were determined on a Yanaco micromelting point apparatus and uncorrected. UV spectra were measured in MeOH using a Shimadzu UV2401PC spectrophotometer. IR spectra were run on KBr disks with a Shimadzu FTIR8900 spectrophotometer. HR-EI-MS and ESI-MS were recorded on a Finnigan MAT95 mass spectrometer and a Bruker Esquire 2000 mass spectrometer, respectively. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance spectrometer at 400 and 100 MHz with TMS as an internal standard. Silica gel (200—300 mesh, Qingdao Haiyang Chemical Co., Ltd, China), Sephadex LH-20 (Amersham Biosciences AB, Sweden), and ODS-A 120-S150 (YMC Co., Ltd., Japan) were used for column chromatography. TLC was prepared with silica gel G (Qingdao Haiyang Chemical) and the spots were detected by spraying with 10% H_2SO_4, followed by heating to 105 °C. Preparative HPLC was performed on a Shimadzu LC-8A apparatus equipped with a Shimadzu SPD-10A UV-VIS detector using an ODS column (PHEP-ODS, Shim-pack, 20×250 mm, 5 μM).

Plant Material The air-dried whole plant of *P. yunnanensis* was purchased in Liuzhou (China) in April 2003 and was identified by Prof. Luoshan Xu (China Pharmacutical University, Naijing, China). A voucher specimen (YGXYPY-2003) of this herbal medicine is deposited in Shenzhen Research Center of Traditional Chinese Medicines and Natural Products, Shenzhen, China.

Extraction and Isolation The air-dried whole plant of *P. yunnanensis* (2.3 kg) was extracted twice with 60% ethanol (23 l) under reflux for 2 h. The combined 60% ethanol extract was concentrated under reduced pressure to afford a dark-brown residue (270 g, 11.7%). The 60% extract (250 g) was suspended in water (3.0 l) and partitioned successively with $CHCl_3$ (3.0 l×3), EtOAc (3.0 l×3), and *n*-BuOH (3.0 l×3). $CHCl_3$ layer (45.0 g) was subjected to silica gel column chromatography (700 g, ϕ 7.0×50.0 cm) eluted with a cyclohexane/EtOAc (100 : 0—0 : 100, v/v) gradient to afford 13 fractions. Fraction 9 (0.8 g) was separated with medium-pressure liquid chromatography (MPLC) on ODS (ϕ 1.5×26.0 cm) eluted with MeOH/H_2O (45 : 55, 70 : 30, v/v) and preparative HPLC [MeOH/H_2O (58 : 42, v/v), flow rate 10 ml/min, UV-VIS detector 275 nm] to give **6** (10.5 mg). Fraction 11 (10.4 g) was subjected to MPLC on silica gel (ϕ 2.0×29.0 cm) eluted with $CHCl_3$/MeOH (70 : 1, 60 : 1, v/v) to give 6 fractions. Fraction 6-2 was purified by MPLC on ODS (ϕ 1.5×26.0 cm) eluted with MeOH/H_2O (60 : 40, 80 : 20, v/v) and preparative HPLC [MeOH/H_2O (53 : 47, v/v), flow rate 10 ml/min, UV-VIS detector 254 nm] to give **4** (78.4 mg) and **5** (163.2 mg). Fraction 12 (5.7 g) was isolated by MPLC on ODS (ϕ 2.0×24.0 cm) eluted with MeOH/H_2O (35 : 65, 50 : 50, v/v) and Sephadex LH-20 column chromatography (ϕ 1.6×29.4 cm) eluted with MeOH/H_2O (60 : 40, v/v) to give **3** (15.0 mg). The EtOAc layer (55.0 g) was subjected to silica gel column chromatography (500 g, ϕ 5.2×49.0 cm) eluted with a $CHCl_3$/MeOH (100 : 0—0 : 100, v/v) gradient to afford 13 fractions. Fraction 5 (6.0 g) was subjected to MPLC on ODS (ϕ 2.5×22.9 cm) eluted with a MeOH/H_2O (30 : 70—100 : 0, v/v) gradient to obtain 9 fractions. Fraction 5—9 was purified by Sephadex LH-20 (ϕ 1.6×29.4 cm) eluted with MeOH/H_2O (60 : 40, 70 : 30, v/v) and preparative HPLC [MeOH/H_2O (60 : 40, v/v), flow rate 10 ml/min, UV-VIS detector 278 nm] to give **1** (15.2 mg). Fraction 6 (5.2 g) was subjected to MPLC on ODS (ϕ 2.5×25.0 cm) eluted with a MeOH/H_2O (30 : 70—100 : 0, v/v) gradient to give 9 fractions. Fraction 6—8 was purified by Sephadex LH-20 column chromatography (ϕ 1.6×29.4 cm) eluted with MeOH/H_2O (70 : 30, 90 : 10, v/v) and preparative HPLC [MeOH/H_2O (60 : 40, v/v), flow rate 10 ml/min, UV-VIS detector 278 nm] to give **2** (25.4 mg).

Phoyunnanin A (**1**): Amorphous powder; UV (MeOH) λ_{max} nm (log ε): 205 (4.92), 280 (4.47); IR (KBr) ν_{max} cm^{-1}: 3382 (OH), 1593, 1458 (phenyl nucleus); HR-EI-MS: *m/z* 484.1874 $[M]^+$ (Calcd for $C_{30}H_{28}O_6$: 484.1886); ESI-MS: *m/z* 507 $[M+Na]^+$, 483 $[M-H]^-$; ^{1}H- and ^{13}C-NMR data, see Table 1. **1** was acetylated with acetic anhydride and pyridine (1 : 1, v/v) in the usual manner to give the triacetate **1a**: a solid; UV (MeOH) λ_{max} nm (log ε)=204 (4.97), 282 (4.36); ESI-MS: *m/z* 633 $[M+Na]^+$, 609 $[M-H]^-$; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 7.85 (1H, d, *J*=8.7 Hz, H-5), 7.23 (1H, t, *J*=7.8 Hz, H-5″), 6.97 (1H, dt, *J*=7.8, 1.1 Hz, H-6″), 6.89 (1H, ddd, *J*=7.8, 2.3, 1.1 Hz, H-4″), 6.84 (1H, m, H-2″), 6.83 (1H, d, *J*=2.6 Hz, H-5′), 6.79 (1H, d, *J*=2.7 Hz, H-1), 6.78 (1H, d, *J*=2.8 Hz, H-8), 6.75 (1H, d, *J*=2.6 Hz, H-3′), 6.70 (1H, dd, *J*=8.7, 2.8 Hz, H-6), 6.61 (1H, d, *J*=2.7 Hz, H-3), 3.81 (3H, s, 2-OCH$_3$), 3.75 (3H, s, 6′-OCH$_3$), 2.79 (4H, br s, H_2-7″, H_2-8″), 2.75 (4H, m, H_2-9, H_2-10), 2.27 (3H, s, OAc), 2.25 (3H, s, OAc), 2.19 (3H, s, OAc); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 169.6 (2×O<u>C</u>OCH$_3$), 169.4 (–O<u>C</u>OCH$_3$), 159.4 (C-2), 158.0 (C-7), 154.1 (C-6′), 152.0 (C-3″), 149.4 (C-4, C-4′), 144.1 (C-1″), 141.9 (C-10a), 140.9 (C-8a), 139.4 (C-1′), 137.1 (C-2′), 130.0 (C-5″), 128.3 (C-5), 126.5 (C-4b), 126.4 (C-6″), 122.5 (C-2″), 120.8 (C-4a), 120.2 (C-4″), 115.7 (C-3′), 115.1 (C-8), 113.4 (C-6), 112.3 (C-1), 108.6 (C-3), 106.3 (C-5′), 56.4 (6′-OCH$_3$), 55.8 (2-OCH$_3$), 36.7 (C-7″), 32.8 (C-8″), 31.0 (C-10), 30.4 (C-9), 21.3 (–OCO<u>C</u>H$_3$), 21.0 (–OCO<u>C</u>H$_3$), 20.9 (–OCO<u>C</u>H$_3$).

Phoyunnanin B (**2**): Amorphous powder; UV (MeOH) λ_{max} nm (log ε): 212 (4.96), 278 (4.74), 297 (4.59); IR (KBr) ν_{max} cm^{-1}: 3394 (OH), 1612, 1589, 1485 (phenyl nucleus); HR-EI-MS: *m/z* 482.1719 (Calcd for $C_{30}H_{26}O_6$: 482.1729); ESI-MS: *m/z* 505 $[M+Na]^+$, 483 $[M+H]^+$, 481 $[M-H]^-$; ^{1}H- and ^{13}C-NMR data, see Table 1. **2** was acetylated with acetic anhydride and pyridine (1 : 1, v/v) in the usual manner to give the triacetate **2a**: a solid; UV (MeOH) λ_{max} nm (log ε)=205 (4.94), 280 (4.65); ESI-MS: *m/z* 631 $[M+Na]^+$, 607 $[M-H]^-$; ^{1}H-NMR (400 MHz, acetone-d_6) δ: 8.01 (1H, d, *J*=8.6 Hz, H-5), 7.85 (1H, d, *J*=8.7 Hz, H-5′), 7.07 (1H, dd, *J*=8.6, 2.5 Hz, H-6), 7.04 (1H, d, *J*=2.5 Hz, H-8), 6.93 (1H, s, H-3), 6.79 (1H, d, *J*=2.8 Hz, H-8′), 6.78 (1H, d, *J*=2.8 Hz, H-1′), 6.71 (1H, dd, *J*=8.7, 2.8 Hz, H-6′), 6.61 (1H, d, *J*=2.8 Hz, H-3′), 3.81 (3H, s, 2′-OCH$_3$), 3.79 (3H, s, 2-OCH$_3$), 2.75 (4H, m, H_2-9′, H_2-10′), 2.70 (4H, m, H_2-9, H_2-10), 2.32 (3H, s, OAc), 2.26 (3H, s, OAc), 2.25 (3H, s, OAc); ^{13}C-NMR (100 MHz, acetone-d_6) δ: 169.6 (–O<u>C</u>OCH$_3$), 169.4 (2×–O<u>C</u>OCH$_3$), 159.4 (C-2′), 157.8 (C-7′), 152.9 (C-2), 150.7 (C-7), 149.4 (C-4′), 146.4 (C-4), 141.9 (C-10a′), 140.9 (C-8a′), 140.4 (C-8a), 138.3 (C-1), 135.0 (C-10a), 130.0 (C-4b), 128.4 (C-5′), 128.2 (C-5), 126.6 (C-4b′), 122.0 (C-8), 121.0 (C-4a), 120.7 (C-6, C-

4a′), 115.0 (C-8′), 113.3 (C-6′), 112.3 (C-1′), 108.6 (C-3′), 107.9 (C-3), 56.5 (2-OCH_3), 55.8 (2′-OCH), 30.9 (C-10′), 30.4 (C-9′), 29.4 (C-9), 23.5 (C-10), 21.3 (2×–OCO$\underline{C}H_3$), 21.0 (–OCO$\underline{C}H_3$).

Phoyunbene A (**3**): White needles; mp 170—171 °C; UV (MeOH) λ_{max} nm (log ε): 202 (6.78), 223 (6.83), 317 (4.75); IR (KBr) ν_{max} cm^{-1}: 3360 (OH), 1609, 1589, 1500 (phenyl nucleus), 999 (*trans*-olefinic); HR-EI-MS: *m/z* 302.1157 $[M]^+$ (Calcd for $C_{17}H_{18}O_5$: 302.1154); ESI-MS: *m/z* 303 $[M+H]^+$, 301 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.23 (1H, d, *J*=16.4 Hz, H-α), 7.07 (1H, d, *J*=8.7 Hz, H-6′), 6.93 (1H, d, *J*=16.4 Hz, H-α'), 6.72 (1H, d, *J*=8.7 Hz, H-5′), 6.58 (1H, t, *J*=1.8 Hz, H-2), 6.54 (1H, t, *J*=1.8 Hz, H-6), 6.26 (1H, t, *J*=1.8 Hz, H-4), 3.84 (3H, s, 4′-OCH_3), 3.81 (3H, s, 2′-OCH_3), 3.76 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.5 (C-5), 159.7 (C-3), 149.8 (C-4′), 147.2 (C-2′), 141.5 (C-1), 140.6 (C-3′), 129.0 (C-α'), 125.3 (C-1′), 124.3 (C-α), 117.3 (C-6′), 108.8 (C-5′), 106.6 (C-2), 104.8 (C-6), 101.5 (C-4), 61.3 (2′-OCH_3), 56.7 (4′-OCH_3), 55.7 (5-OCH_3); HMBC: H-2 (^2J C-3; ^3J C-4, C-6, C-α'), H-4 (^2J C-3, C-5; ^3J C-2, C-6), H-6 (^2J C-5; ^3J C-2, C-4, C-α'), H-5′ (^2J C-4′; ^3J C-1′, C-3′), H-6′ (^3J C-2′, C-4′, C-α), H-α (^3J C-1, C-2′, C-6′), H-α' (^3J C-2, C-6, C-1′), 5-O$\underline{C}H_3$ (^3J C-5), 2′-O$\underline{C}H_3$ (^3J C-2′), 4′-O$\underline{C}H_3$ (^3J C-4′).

Phoyunbene B (**4**): An oil; UV (MeOH) λ_{max} nm (log ε): 219 (4.91), 318 (4.93); HR-EI-MS: *m/z* 302.1150 (Calcd for $C_{17}H_{18}O_5$: 302.1154); ESI-MS: *m/z* 325 $[M+Na]^+$, 303 $[M+H]^+$, 301 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.22 (1H, d, *J*=16.7 Hz, H-α), 7.21 (1H, d, *J*=8.7 Hz, H-6′), 6.88 (1H, d, *J*=16.7 Hz, H-α'), 6.62 (1H, d, *J*=8.7 Hz, H-5′), 6.57 (1H, t, *J*=1.8 Hz, H-2), 6.53 (1H, t, *J*=1.8 Hz, H-6), 6.26 (1H, t, *J*=1.8 Hz, H-4), 3.85 (3H, s, 2′-OCH_3), 3.84 (3H, s, 3′-OCH_3), 3.76 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.5 (C-5), 159.7 (C-3), 152.9 (C-2′), 152.0 (C-4′), 142.2 (C-3′), 141.5 (C-1), 128.3 (C-α'), 124.1 (C-1′), 124.0 (C-α), 122.2 (C-6′), 113.2 (C-5′), 106.5 (C-2), 104.7 (C-6), 101.4 (C-4), 61.6 (2′-OCH_3), 61.1 (3′-OCH_3), 55.6 (5-OCH_3); HMBC: H-2 (^2J C-3; ^3J C-4, C-6, C-α'), H-4 (^2J C-3, C-5; ^3J C-2, C-6), H-6 (^2J C-5; ^3J C-2, C-4, C-α'), H-5′ (^2J C-4′; ^3J C-1′, C-3′), H-6′ (^3J C-2′, C-4′, C-α), H-α (^3J C-1, C-2′, C-6′), H-α' (^3J C-2, C-6, C-1′), 5-O$\underline{C}H_3$ (^3J C-5), 2′-O$\underline{C}H_3$ (^3J C-2′), 3′-O$\underline{C}H_3$ (^3J C-3′).

Phoyunbene C (**5**): An oil; UV (MeOH) λ_{max} nm (log ε): 219 (5.74), 303 (5.78); HR-EI-MS: *m/z* 272.1044 (Calcd for $C_{16}H_{16}O_4$: 272.1049); ESI-MS: *m/z* 295 $[M+Na]^+$, 273 $[M+H]^+$, 271 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.31 (1H, d, *J*=16.4 Hz, H-α), 7.10 (1H, dd, *J*=7.9, 1.5 Hz, H-6′), 7.02 (1H, d, *J*=16.4 Hz, H-α'), 6.92 (1H, t, *J*=7.9 Hz, H-5′), 6.76 (1H, dd, *J*=7.9, 1.5 Hz, H-4′), 6.60 (1H, t, *J*=1.8 Hz, H-2), 6.57 (1H, t, *J*=1.8 Hz, H-6), 6.29 (1H, t, *J*=1.8 Hz, H-4), 3.78 (3H, s, 2′-OCH_3), 3.76 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.6 (C-5), 159.8 (C-3), 151.5 (C-3′), 147.0 (C-2′), 141.1 (C-1), 132.4 (C-1′), 130.9 (C-α'), 125.5 (C-5′), 124.1 (C-α), 118.0 (C-6′), 116.7 (C-4′), 106.8 (C-2), 105.0 (C-6), 101.9 (C-4), 61.4 (2′-OCH_3), 55.7 (5-OCH_3); HMBC: H-2 (^2J C-3; ^3J C-4, C-6, C-α'), H-4 (^2J C-3, C-5; ^3J C-2, C-6), H-6 (^2J C-5; ^3J C-2, C-4, C-α'), H-4′ (^2J C-3′, C-5′; ^3J C-2′, C-6′), H-5′ (^2J C-6′; ^3J C-1′, C-3′), H-6′ (^3J C-2′, C-4′, C-α), H-α (^3J C-1, C-2′, C-6′), H-α' (^3J C-2, C-6, C-1′), 5-O$\underline{C}H_3$ (^3J C-5), 2′-O$\underline{C}H_3$ (^3J C-2′).

Phoyunbene D (**6**): Yellow prism, mp 128—129 °C; UV (MeOH) λ_{max} nm (log ε): 223 (4.85), 301 (4.85); IR (KBr) ν_{max} cm^{-1}: 3356 (OH), 1597, 1474 (phenyl nucleus), 999 (*trans*-olefinic); HR-EI-MS: *m/z* 286.1198 (Calcd for $C_{17}H_{18}O_4$: 286.1205); ESI-MS: *m/z* 309 $[M+Na]^+$, 287 $[M+H]^+$, 285 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.35 (1H, d, *J*=16.5 Hz, H-α), 7.23 (1H, dd, *J*=8.2, 1.5 Hz, H-6′), 7.05 (1H, t, *J*=8.2 Hz, H-5′), 7.04 (1H, d, *J*=16.5 Hz, H-α'), 6.91 (1H, dd, *J*=8.2, 1.5 Hz, H-4′), 6.60 (1H, t, *J*=1.8 Hz, H-2), 6.57 (1H, t, *J*=1.8 Hz, H-6), 6.29 (1H, t, *J*=1.8 Hz, H-4), 3.85 (3H, s, 3′-OCH_3), 3.81 (3H, s, 2′-OCH_3), 3.77 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, CD_3OD) δ: 162.6 (C-5), 159.8 (C-3), 154.5 (C-3′), 148.1 (C-2′), 141.1 (C-1), 132.5 (C-1′), 131.2 (C-α'), 125.4 (C-5′), 123.8 (C-α), 119.0 (C-6′), 112.8 (C-4′), 106.8 (C-2), 105.0 (C-6), 101.9 (C-4), 61.4 (2′-OCH_3), 56.3 (3′-OCH_3), 55.7 (5-OCH_3); HMBC: H-2 (^2J C-3; ^3J C-4, C-6, C-α'), H-4 (^2J C-3; ^3J C-2, C-6), H-6 (^2J C-5; ^3J C-2, C-4, C-α'), H-4′ (^3J C-2′, C-6′), H-5′ (^2J C-6′; ^3J C-1′, C-3′), H-6′ (^3J C-2′, C-4′, C-α), H-α (^3J C-2′, C-6′, C-1), H-α' (^3J C-2, C-6), 5-O$\underline{C}H_3$ (^3J C-5), 2′-O$\underline{C}H_3$ (^3J C-2′), 3′-O$\underline{C}H_3$ (^3J C-3′).

Inhibition of NO Production by the Activated Macrophage-Like Cell Line RAW 264.7[12] The cells were seeded at 1.2×10^6 cells/ml onto 96-well flat-bottomed plates (Sumitomo Bakelite, #8096R, Tokyo) and then incubated at 37 °C for 2 h. Next, the test sample was added to the culture simultaneously with both *Escherichia coil* LPS (100 ng/ml) and recombinant mouse IFN-γ (0.33 ng/ml). The cells were incubated at 37 °C for approximately 16 h and subsequently chilled on ice. The culture supernatant (100 μl) was placed in duplicate in the well of 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was placed in other wells on the same plate. To quantify nitrite, 50 μl of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% *N*-1-naphthyletylenediamide dihydrochloride) were added to each well. After 10 min, the reaction products were colorimetrically quantitated at 550 nm with subtraction of the background absorbance at 630 nm using a Model 3550 Microplate Reader (BIO-RAD). Cytotoxicity was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method.[13]

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