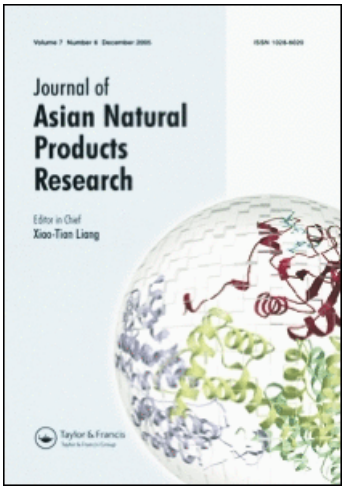

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

9, 10-Dihydrophenanthrene derivatives from *Pholidota yunnanensis* and scavenging activity on DPPH free radical

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To cite this Article Guo, X. -Y. , Wang, J. , Wang, N. -L. , Kitanaka, S. and Yao, X. -S.(2007) '9, 10-Dihydrophenanthrene derivatives from *Pholidota yunnanensis* and scavenging activity on DPPH free radical', Journal of Asian Natural Products Research, 9: 2, 165 — 174
To link to this Article: DOI: 10.1080/10286020500480522
URL: http://dx.doi.org/10.1080/10286020500480522

Journal of Asian Natural Products Research, Vol. 9, No. 2, March 2007, 165–174

9, 10-Dihydrophenanthrene derivatives from *Pholidota yunnanensis* and scavenging activity on DPPH free radical

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(Received 27 June 2005; revised 20 September 2005; in final form 29 September 2005)

Three new 9,10-dihydrophenanthrene derivatives named phoyunnanins A–C, together with six known 9,10-dihydrophenanthrene constituents, 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (**4**), lusianthridin (**5**), eulophiol (**6**), 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) and imbricatin (**8**), were isolated from the 60% ethanolic extract of air-dried whole plant of *Pholidota yunnanensis* Rolfe. The structures of phoyunnanins A–C were established as 6-[2′-(3′,3″-dihydroxy-5′-methoxybibenzy)]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (**1**), 6-[6′-(*trans*-3′,3″-dihydroxy-5′-methoxystilbeny)]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (**2**) and 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,6′-biphenanthrene (**3**), respectively, on the basis of the spectroscopic analysis. All eight compounds (**1–8**) were found to show the DPPH free radical scavenging activity with EC_{50} from 8.8 to 55.9 μM.

Keywords: *Pholidota yunnanensis*; Orchidaceae; Dihydrophenanthrene; Phoyunnanins A–C; Free radical scavenging activity

1. Introduction

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 folk medicine for the treatment of cough, rheumatism, stomachache, bellyache and traumatism [1,2]. Previous chemical study on this plant has resulted in the isolation of cyclopholidone, cyclopholidonol, 25-methylenecycloartanyl *p*-hydroxy-transcinnamate, 25-methylenecyclopholidonyl *p*-hydroxy-transcinnamate, cycloneolitsol, *n*-nonacosane, *n*-dotriacontanoic acid, *n*-octacostyl ferulate and β-sitosterol [3–5]. In the course of our study on the constituents of *P. yunnanesis*, three new 9,10-dihydrophenanthrene derivatives named phoyunnanins A–C (**1–3**), together with five known related compounds, were isolated.

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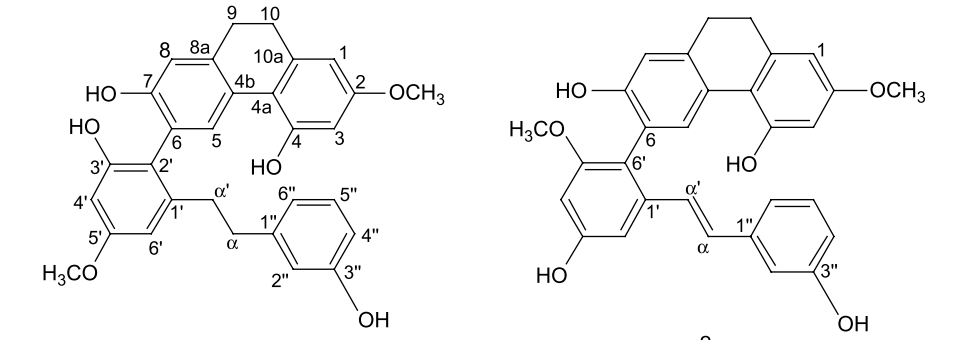
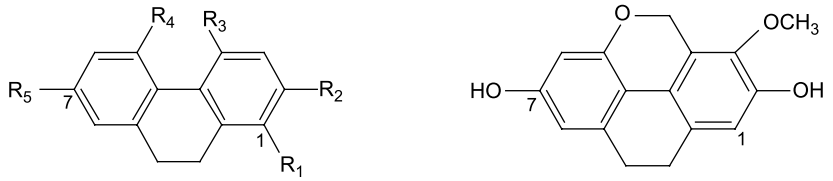
Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2007 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500480522

The known compounds were identified as 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (**4**) [6], 4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (lusianthridin) (**5**) [7], 1,5-dihydroxy-2,7-dimethoxy-9,10-dihydrophenanthrene (eulophiol) (**6**) [9], 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) [10] and imbricatin (**8**) [11], respectively, by the spectroscopic analysis and comparison with the data reported in the literature. The stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out to determine the antioxidant activity of these compounds with resveratrol as a positive control. All the 9,10-dihydrophenanthrene derivatives showed the DPPH free radical scavenging activity with EC_{50} from 8.8 to 55.9 μM. In this paper, we describe the isolation and structural elucidation of three new compounds (**1**–**3**) from the air-dried whole plant of *P. yunnanesis* and scavenging activity on DPPH free radical of all the compounds (**1–8**) (figure 1).





5 $R_1 = R_4 = H$, $R_2 = OCH_3$, $R_3 = R_5 = OH$
6 $R_1 = R_4 = OH$, $R_2 = R_5 = OCH_3$, $R_3 = H$
7 $R_1 = R_4 = H$, $R_2 = R_3 = R_5 = OH$

8

Figure 1. Structures of the compounds **1**–**8**.

2. Results and discussion

Phoyunnanin A (**1**) was obtained as amorphous powder and its molecular formula was determined by HREI-MS measurement to be $C_{30}H_{28}O_6$. The IR spectrum showed absorption bands at 3364, 1589 and 1454 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The UV spectrum exhibited the characteristic absorption for dihydrophenanthrene derivatives at 204 (log ε 5.02), 280 (4.57) and 299 nm (4.42) [6–8]. The ^{1}H NMR (table 1) spectrum revealed the signals due to four methylenes at δ 2.76 (4H, m, H_2-9, H_2-10) and 2.65 (4H, m, H_2-α, H_2-α′), two phenyl methoxyls at δ 3.75 (3H, s, 5′-OCH_3) and 3.73 (3H, s, 2-OCH_3), two sets of *meta*-couple aromatic protons at δ 6.41 (1H, d, $J = 2.5$ Hz, H-3), 6.39 (1H, d, $J = 2.5$ Hz, H-1), 6.44 (1H, d, $J = 2.6$ Hz, H-6′), and 6.40 (1H, d, $J = 2.6$ Hz, H-4′), and six additional aromatic protons at δ 8.21 (1H, s, H-5), 6.93 (1H, t, $J = 7.7$ Hz, H-5″), 6.85 (1H, s, H-8), 6.54 (1H, ddd, $J = 7.7$, 1.7, 0.5 Hz, H-4″), 6.49 (1H, t, $J = 1.7$ Hz, H-2″), and 6.43 (1H, m, H-6″). The ^{13}C NMR (table 1) spectrum combined with DEPT135 and HMQC spectra showed the signals due to 24 aromatic carbons, four methylenes and two phenyl methoxyls. The 9,10-dihydrophenanthrene and bibenzyl skeletons were constructed on the basis of the ^{1}H–^{1}H COSY and HMBC experiments. In the ^{1}H–^{1}H COSY spectrum, the correlations between H-5″ (δ 6.93) and H-4″ (δ 6.54), H-5″ and H-6″ (6.43), and H-4″ and H-2″ (δ 6.49) indicated the presence of a *meta*-substituted (C-1″, 3″) aromatic system. In the HMBC spectrum, the correlations between H-5″ and C-1″, C-3″; H-4″ and C-2″, C-6″; H-2″ and C-3″, C-4″, C-6″, C-α; H-6′ and C-5′, C-2′, C-4′, C-α′; H-4′ and C-3′, C-5′, C-2′, C-6′; H-α, α′ and C-1″, C-2″, C-6″, C-6′, C-1′ determined the bibenzyl skeleton. The HMBC correlations between H-3 and C-4, C-2, C-4a, C-1; H-1 and C-2, C-4a, C-3, C-10; H-5 and C-7, C-8a, C-4a; H-8 and C-7, C-6, C-4b, C-9; and H-9, 10 and C-1, C-8, C-4a, C-4b, C-8a, C-10a established the skeleton of 9,10-dihydrophenanthrene. The position of one of the hydroxyls was determined to be at C-4 by the HMBC correlations between the hydroxyl proton at δ 8.59 and C-4, C-4a. Furthermore, the HMBC correlation between H-5 and C-2′ confirmed that the two moieties were linked at C-6 and C-2′. In the NOESY experiment, the correlations between the methoxyl protons at δ 3.73 and H-1, H-3, and between the methoxyl protons at δ 3.75 and H-4′, H-6′ determined that the two methoxyls were located at C-2 and C-5′, respectively. In addition, the numbers and the positions of other hydroxyls could be established by the molecular formula and structural information. Consequently, the structure of phoyunnanin A was elucidated as 6-[2′-(3′,3″-dihydroxy-5′-methoxybibenzy)]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (**1**). The principal HMBC and NOESY correlations are shown in figure 2.

Phoyunnanin B (**2**) was isolated as amorphous powder and the HREI-MS showed the molecular formula to be $C_{30}H_{26}O_6$. The UV spectrum exhibited the absorption at 215 (log ε 4.87), 279 (4.63) and 298 (4.64) nm similar to those of **1**. The IR spectrum showed the absorption bands at 3279, 1612, 1500 and 1454 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The ^{1}H NMR (table 1) spectrum revealed the signals due to two methylenes at δ 2.76 (4H, br s, H_2-9, H_2-10), two phenyl methoxyls at δ 3.67 (3H, s, 5′-OCH_3) and 3.72 (3H, s, 2-OCH_3), two *trans*-olefinic protons at δ 6.96 (1H, d, $J = 16.4$ Hz, H-α) and 7.00 (1H, d, $J = 16.4$ Hz, H-α′), one set of *meta*-coupled aromatic protons at δ 6.92 (1H, d, $J = 2.2$ Hz, H-2′) and 6.58 (1H, d, $J = 2.2$ Hz, H-4′), and eight additional aromatic protons at δ 8.12 (1H, s, H-5), 7.05 (1H, t, $J = 8.0$ Hz, H-5″), 6.80 (1H, m, H-6″), 6.79 (1H, s, H-8), 6.78 (1H, m, H-2″), 6.64 (1H, ddd, $J = 8.0$, 2.2, 0.9 Hz,

Table 1. ^{1}H NMR and ^{13}C NMR data of compounds **1**–**3**.[a]

Position	**1**		**2**		Position	**3**	
	δ_H	δ_C	δ_H	δ_C		δ_H	δ_C
1	6.39 (1H, d, $J = 2.5$)	106.1	6.38 (1H, br s)	106.1	1		118.4
2		159.4		159.3	2		157.6
3	6.41 (1H, d, $J = 2.5$)	101.6	6.38 (1H, br s)	101.6	3	6.58 (1H, s)	99.4
4		156.0		155.9	4		155.1
4a		115.8		116.0	4a		115.8
4b		126.2		125.6	4b		126.3
5	8.21 (1H, s)	132.9	8.12 (1H, s)	133.1	5	8.24 (1H, d, $J = 8.5$)	130.2
6		120.9		121.6	6	6.70 (1H, dd, $J = 8.5, 2.7$)	113.4
7		154.0		153.9	7		156.1
8	6.85 (1H, s)	115.6	6.79 (1H, s)	115.3	8	6.67 (1H, d, $J = 2.7$)	114.7
8a		139.4		138.8	8a		140.2
9	2.76 (2H, m)	30.5	2.76 (2H, br s)	30.5	9	2.56 (2H, m)	30.6
10	2.76 (2H, m)	31.6	2.76 (2H, br s)	31.6	10	2.52 (2H, m)	28.4
10a		141.5		141.4	10a		141.1
1′		144.3		139.3	1′	6.40 (1H, br s)	106.1
2′		118.4	6.92 (1H, d, $J = 2.2$)	104.3	2′		159.3
3′		157.0		158.6	3′	6.40 (1H, br s)	101.6
4′	6.40 (1H, d, $J = 2.6$)	100.0	6.58 (1H, d, $J = 2.2$)	99.8	4′		155.9
5′		160.8		160.1	4a′		116.0
6′	6.44 (1H, d, $J = 2.6$)	107.3		119.7	4b′		125.7
1″		145.0		140.2	5′	8.10 (1H, s)	132.6
2″	6.49 (1H, t, $J = 1.7$)	116.2	6.78 (1H, m)	113.8	6′		122.6
3″		158.1		158.4	7′		153.7
4″	6.54 (1H, ddd, $J = 7.7, 1.7, 0.5$)	113.4	6.64 (1H, ddd, $J = 8.0, 2.2, 0.9$)	115.4	8′	6.78 (1H, s)	115.2
5″	6.93 (1H, t, $J = 7.7$)	129.8	7.05 (1H, t, $J = 8.0$)	130.4	8a′		138.6
6″	6.43 (1H, m)	120.4	6.80 (1H, m)	119.0	9′	2.75 (2H, m)	30.5
α	2.65 (2H, m)	37.9	6.96 (1H, d, $J = 16.4$)	129.7	10′	2.75 (2H, m)	31.6
α′	2.65 (2H, m)	37.5	7.00 (1H, d, $J = 16.4$)	128.5	10a′		141.4
2-OCH_3	3.73 (3H, s)	55.3	3.72 (3H, s)	55.3	2-OCH_3	3.66 (3H, s)	55.7
5′-OCH_3	3.75 (3H, s)	55.3	3.67 (3H, s)	55.9	2′-OCH_3	3.73 (3H, s)	55.2
5-OH	8.59 (1H, br s)				7’-OH	7.15 (1H, s)	

[a]Measured in acetone-d_6 at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, with assignments confirmed by DEPT135, ^{1}H–^{1}H COSY, HMQC, HMBC and NOESY experiments.

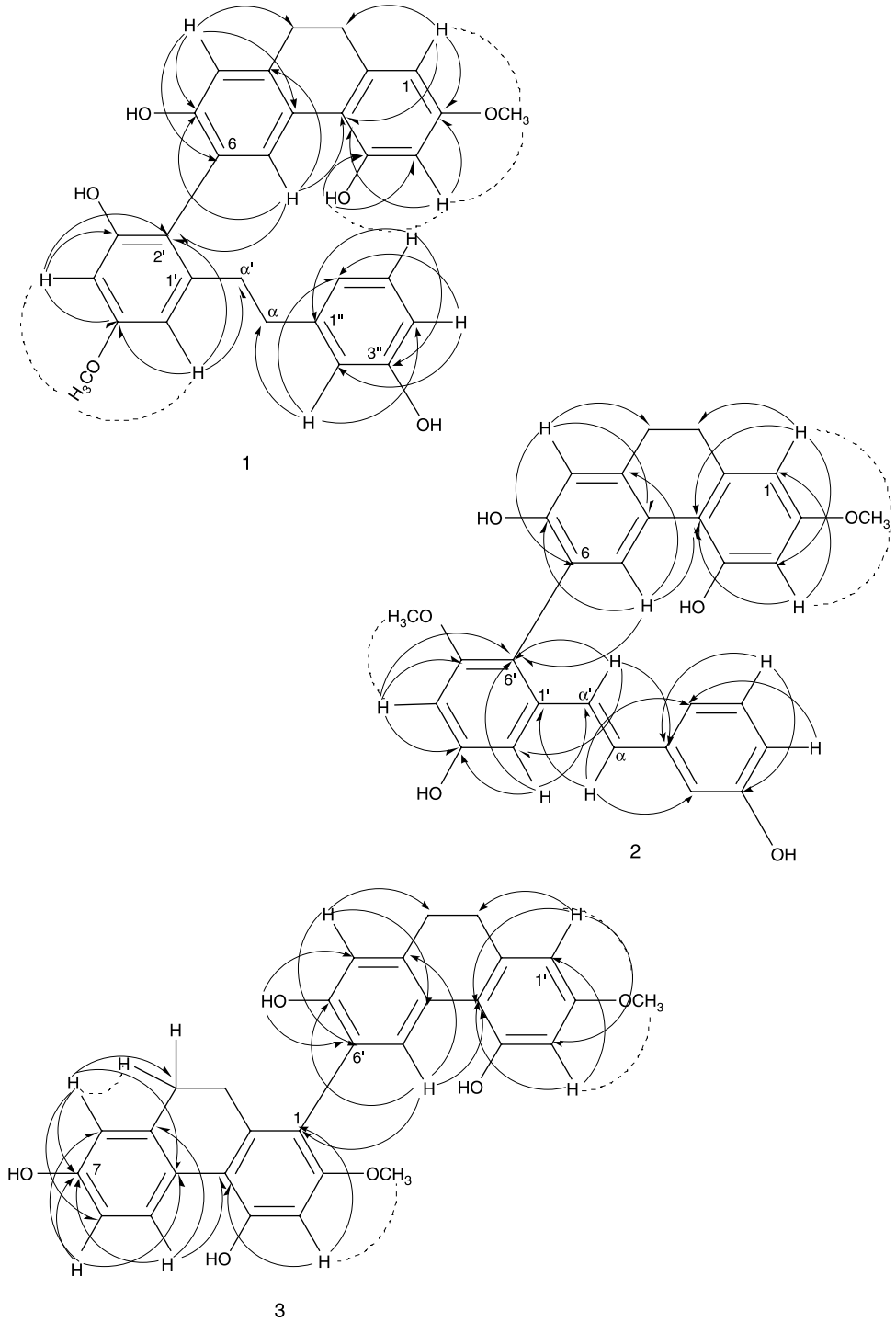


Figure 2. Key HMBC and NOESY correlations of compounds **1**–**3**.

H-4″), and 6.38 (2H, br s, H-1, H-3). The $^{1}H-^{1}H$ COSY experiment showed correlations between H-5″ (δ 7.05) and H-4″ (δ 6.64), and H-5″ and H-6″ (δ 6.80) indicating the presence of a *meta*-substituted (C-1″, 3″) aromatic system. The ^{13}C NMR (table 1) spectrum combined with DEPT135 and HMQC spectra showed the signals due to two olefinic carbons at δ 129.7 and 128.5, 24 aromatic carbons, two methylenes and two phenyl methoxyls. All the above evidence indicated the presence of a dihydrophenanthrene skeleton and a *trans*-stilbene skeleton in the structure of **2** which were further constructed by the HMBC correlations (figure 2). The moiety of dihydrophenanthrene in **2** was quite similar to that in **1**. Furthermore, the HMBC correlation between H-5 and C-6′ confirmed that the two moieties were linked at C-6 and C-6′. The HMBC correlation between the methoxyl protons at δ 3.67 and C-5′, and the NOESY correlation between the methoxyl protons at δ 3.67 and H-4′ further confirmed that the methoxyl was located at C-5'. The position of the methoxyl at δ 3.72 was the same as that of **1.** The numbers and the positions of the hydroxyls could be established on the basis of the molecular formula and the structural information. Thus, the structure of phoyunnanin B was characterised as 6-[6′-(*trans*-3′,3″-dihydroxy-5′-methoxystilbeny)]-4,7-dihydroxy-2-methoxy-9,10-dihydrophenanthrene (**2**). The principal HMBC and NOESY correlations are shown in figure 2.

Phoyunnanin C (**3**) was isolated as amorphous powder and its molecular formula was determined by HREI-MS measurement to be $C_{30}H_{26}O_6$ which was the same as that of **2**. The UV spectrum showed the absorption at 216 (log ε 4.95), 279 (4.75) and 298 nm (4.63) similar to those of dihydrophenanthrene derivatives [6–8]. The IR spectrum showed the absorption bands at 3310, 1589 and 1439 cm^{-1} ascribable to hydroxyl and aromatic functional groups. The ^{1}H NMR (table 1) spectrum exhibited the signals assignable to four methylenes at δ 2.52 (2H, m, H_2-10), 2.56 (2H, m, H_2-9) and 2.75 (4H, m, H_2-9′, H_2-10′), two phenyl methoxyls at δ 3.66 (3H, s, 2-OCH_3) and 3.73 (3H, s, 2′-OCH_3), a phenolic hydroxyl at δ 7.15 (1H, s), one set of ABX system aromatic protons at δ 8.24 (1H, d, $J = 8.5$ Hz, H-5), 6.70 (1H, dd, $J = 8.5$, 2.7 Hz, H-6), and 6.67 (1H, d, $J = 2.7$ Hz, H-8), and five additional aromatic protons at δ 8.10 (1H, s, H-5′), 6.78 (1H, s, H-8′), 6.58 (1H, s, H-3), and 6.40 (2H, br s, H-1′, 3′). The ^{13}C NMR (table 1) spectrum showed the signals due to twenty-four aromatic carbons, four methylenes and two phenyl methoxyls. All the above evidence indicated the presence of a dimeric structure formed by the unsymmetrical coupling of two dihydrophenanthrenes which were further confirmed by the HMBC correlations (figure 2). The two moieties was linked at the C-1 and C-6′ that was determined by the HMBC correlation between H-5′ and C-1. As shown in figure 2, the NOESY correlations between the methoxyl protons at δ 3.66 and H-3, and the methoxyl protons at δ 3.73 and H-1′, H-3′ further determined the two methoxyls were located at C-2 and C-2′. The comparison of the NMR data of **3** with those of **1** indicated that they had the same 9,10-dihydrophenanthrene moiety just like lusianthridin (**5**) (see Experimental section). Thus, the structure of phoyunnanin C was established as 4,4′,7,7′-tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,6′-biphenanthrene (**3**), i.e. a dimer of lusianthridin.

4,4′,7,7′-Tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (**4**) [6], lusianthridin (**5**) [7], eulophiol (**6**) [9] and 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**7**) [10] were isolated from this genus for the first time. Imbricatin (**8**) [11] was isolated from this species for the first time.

The stable free radical DPPH which showed a characteristic absorption band at 517 nm in ethanol solution has been widely used to test the free radical scavenging ability of various samples [12]. When the test samples donate an electron or hydrogen radical to DPPH, DPPH

becomes a more stable reduced form and the absorption vanishes [13]. In the assay of the antioxidant effects against DPPH free radical, these 9,10-dihydrophenanthrene derivatives (**1–8**) showed the EC_{50} at 55.9, 47.3, 26.7, 15.6, 22.3, 27.7, 10.0 and 8.8 μM, respectively. Resveratrol was used as a positive control with EC_{50} at 21.2 μM. The bioassay results implied that the structure of 9,10-dihydrophenanthrene contributed much to the ability of DPPH free radical scavenging.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco micromelting point apparatus and are uncorrected. UV spectra were measured in MeOH using a Shimadzu UV2401PC spectrophotometer. IR spectra were run in KBr disks with a Shimadzu FTIR8900 spectrophotometer. HREI-MS and ESI-MS spectra were recorded on a Finnigan MAT95 mass spectrometer and a Bruker Esquire 2000 mass spectrometer, respectively. ^{1}H NMR 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 Co.) and the spots were detected by spraying with 10% H_2SO_4 followed by heating to 105 °C. The preparative HPLC was performed on a Shimadzu LC-8A apparatus equipped with a Shimadzu SPD-10A UV-VIS detector and an ODS column (PHEP-ODS, Shim-pack, 20 × 250 mm, 5 μm).

3.2 Plant material

The air-dried whole plant of *Pholidota yunnanensis* was purchased from Liuzhou (China) in April 2003 and was identified by Professor Luoshan Xu (China Pharmaceutical University, Naijing, China). A voucher specimen (YGXYPY-2003) of this herbal medicine is deposited in the Shenzhen Research Centre of Traditional Chinese Medicines and Natural Products, Shenzhen, China.

3.3 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 with $CHCl_3$ (3.0 L × 3), EtOAc (3.0 L × 3) and *n*-BuOH (3.0 L × 3), successively. $CHCl_3$ extract (45.0 g) was subjected to silica gel column chromatography (700 g, Φ 7.0 × 50.0 cm) eluted by cyclohexane/EtOAc (100:0–0:100, v/v) gradiently to afford 13 fractions. Fraction 9 (0.8 g) was separated by medium pressure liquid chromatography (MPLC) on ODS (Φ 1.5 × 26.0 cm) eluted by MeOH/H_2O (45:55, 60:40, v/v) and preparative HPLC [MeOH/H_2O (50:50, v/v), flow rate 10 ml/min,

UV-VIS detector 220 nm] to give **7** (12.0 mg). Fraction 10 (1.7 g) was subjected to Sephadex LH-20 column chromatography (Φ 2.0 × 42.0 cm) eluted by MeOH/H_2O (60:40, v/v) to give three fractions. Fraction 10-3 was purified by MPLC on ODS (Φ 1.0 × 25.0 cm) eluted by MeOH/H_2O (60:40, v/v) and preparative HPLC [MeOH/H_2O (53:47, v/v), flow rate 10 ml/min, UV-VIS detector 220 nm] to yield **6** (99.6 mg) and **9** (5.1 mg). EtOAc extract (55.0 g) was subjected to silica gel column chromatography (500 g, Φ 5.2 × 49.0 cm) eluted by $CHCl_3$/MeOH (100:0–0:100, v/v) gradiently to afford 13 fractions. Fraction 4 (5.6 g) was subjected to MPLC on ODS (Φ 2.5 × 22.9 cm) eluted by MeOH/H_2O (30:70–75:25, v/v) gradiently to give four fractions. Fraction 4-4 was purified by preparative HPLC [MeOH/H_2O (50:50, v/v), flow rate 10 ml/min, UV-VIS detector 220 nm] to give **5** (8.5 mg). Fraction 5 (0.6 g) was separated by Sephadex LH-20 column chromatography (Φ 2.0 × 42.0 cm) eluted by $CHCl_3$/MeOH (1:1, v/v) to give three fractions. Fraction 5-3 was subjected to ODS column chromatography (Φ 2.5 × 33.0 cm) eluted by MeO/H_2O gradiently to give nine fractions. Fraction 5-3-7 was purified with preparative HPLC [MeOH/H_2O (54:46, v/v), flow rate 10 ml/min, UV-VIS detector 220 nm] to yield **1** (18.0 mg). Fraction 6 (0.7 g) was purified by MPLC on ODS column chromatography (Φ 2.5 × 25.0 cm) eluted by MeOH/H_2O (30:70–80:20, v/v) gradiently to give eight fractions. Fractions 6-5 and 6-8 were purified with preparative HPLC [MeOH/H_2O (51:49, v/v), flow rate 10 ml/min, UV-VIS detector 220 nm], respectively, to yield **2** (12.5 mg) and **3** (22.9 mg). Fraction 7 (0.6 g) was separated by Sephadex LH-20 column chromatography (Φ 2.0 × 42.0 cm) eluted by MeOH/H_2O (40:60–60:40) to furnish seven fractions. Fractions 7-5 and 7-7 were purified with ODS column chromatography (Φ 2.0 × 21.5 cm) eluted by MeOH/H_2O (30:70–60:40, v/v) and preparative HPLC (flow rate 10 ml/min, UV-VIS detector 220 nm) using MeOH/H_2O (35:65, v/v) and MeOH/H_2O (52:48, v/v) as the mobile phase, respectively, to give **8** (5.9 mg) and **4** (14.2 mg).

3.3.1 Phoyunnanin A (1). Amorphous powder; UV (MeOH) λ_{max} (log ε): 204 (5.02), 280 (4.57), 299 (4.42) nm; IR (KBr) ν_{max}: 3364, 1589, 1454 cm^{-1}; HREI-MS: *m/z* $[M]^+$484.1884 (calcd for $C_{30}H_{28}O_6$, 484.1886); ESI-MS: *m/z* 507 $[M + Na]^+$, 485 $[M + H]^+$, 483 $[M - H]^-$; ^1H NMR and ^{13}C NMR data: see table 1.

3.3.2 Phoyunnanin B (2). Amorphous powder; UV (MeOH) λ_{max} (log ε): 215 (4.87), 279 (4.63), 298 (4.64) nm; IR (KBr) ν_{max}: 3279, 1612, 1500, 1454 cm^{-1}; HREI-MS: *m/z* $[M]^+$482.1728 (calcd for $C_{30}H_{26}O_6$, 482.1729); ESI-MS: *m/z* 505 $[M + Na]^+$, 483 $[M + H]^+$, 481 $[M-H]^-$; ^1H NMR and ^{13}C NMR data: see table 1.

3.3.3 Phoyunnanin C (3). Amorphous powder; UV (MeOH) λ_{max} (log ϵ): 216 (4.95), 279 (4.75), 298 (4.63) nm; IR (KBr) ν_{max}: 3310, 1589, 1439 cm^{-1}; HREI-MS: *m/z* $[M]^+$482.1738 (calcd for $C_{30}H_{26}O_6$, 482.1730); ESI-MS: *m/z* 505 $[M + Na]^+$, 483 $[M + H]^+$, 481 $[M-H]^-$; ^{1} H NMR and ^{13}C NMR data, see table 1.

3.3.4 4,4′,7,7′-Tetrahydroxy-2,2′-dimethoxy-9,9′,10,10′-tetrahydro-1,1′-biphenanthrene (4). Amorphous powder; UV (MeOH) λ_{max} (log ϵ): 213 (4.95), 279 (4.71), 298 (4.55) nm;

ESI-MS *m/z* 505 $[M + Na]^+$, 483 $[M + H]^+$, 481 $[M - H]^-$; ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.27 (2H, d, $J = 8.6$ Hz, H-5, 5′), 6.70 (2H, dd, $J = 8.6$, 2.7 Hz, H-6, 6′), 6.66 (2H, d, $J = 2.7$ Hz, H-8, 8′), 6.58 (2H, s, H-3, 3′), 3.61 (6H, s, 2, 2′-OCH_3), 2.53 (4H, m, H_2-9, H_2-9′), 2.33 (4H, m, H_2-10, H_2-10′); ^{13}C NMR (100 MHz, acetone-d_6) δ: 157.4 (C-2, 2′) 156.0 (C-7, 7′), 154.9 (C-4, 4′), 140.6 (C-10a, 10a′), 140.2 (C-8a, 8a′), 130.2 (C-5, 5′), 126.5 (C-4b, 4b′), 117.4 (C-1, 1′), 115.5 (C-4a, 4a′), 114.7 (C-8, 8′), 113.4 (C-6, 6′), 99.2 (C-3, 3′), 55.6 (2, 2′-OCH_3), 30.6 (C-9, 9′), 27.9 (C-10, 10′).

3.3.5 4,7-Dihydroxy-2-methoxy-9,10-dihydrophenanthrene (5). A solid; UV (MeOH) λ_{max} (log ϵ): 213 (4.90), 277 (4.70), 294 (4.54) nm; IR (KBr) ν_{max}: 3283, 1612, 1458, 1435 cm^{-1}; ESI-MS *m/z* 241 $[M-H]^-$; ^{1}H NMR (400 MHz, acetone-d_6) δ: 8.22 (1H, d, $J = 7.5$ Hz, H-5), 6.69 (1H, m, H-8), 6.68 (1H, dd, $J = 7.5$, 2.7 Hz, H-6), 6.44 (1H, d, $J = 2.6$ Hz, H-3), 6.37 (1H, d, $J = 2.6$ Hz, H-1), 3.74 (3H, s, 2-OCH_3), 2.67 (4H, m, H_2-9, H_2-10); ^{13}C NMR (100 MHz, acetone-d_6) δ: 159.3 (C-2), 156.1 (C-7), 155.9 (C-4), 141.4 (C-10a), 139.8 (C-8a), 129.9 (C-5), 125.9 (C-4b), 115.9 (C-4a), 115.0 (C-8), 113.5 (C-6), 106.0 (C-1), 101.6 (C-3), 55.3 (2-OCH_3), 31.5 (C-10), 30.8 (C-9).

3.4 DPPH free radical scavenging activity

Reduction of DPPH free radical was determined according to the method of Okada [12] with a slight modification. The assay mixture contained an ethanol solution of DPPH (1.0 mM, 0.3 ml), 99% ethanol (2.4 ml) and different concentrations of each test sample solution (3–100 μM, 0.3 ml). The solution was rapidly mixed and the scavenging ability was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm, 10 min later. Resveratrol was used as a positive control and the reaction solution without DPPH was used as a blank control. The scavenging activity was calculated as the percentage of the radical reduction.

Acknowledgements

The authors thank Professor Luoshan Xu, China Pharmaceutical University, for the identification of the plant of *Pholidota yunnanesis* Rolfe; Professor Yang Ye, Shanghai Institute of Materia Medica of Chinese Academy of Science, for HREI-MS experiments. Thanks are also extended to Xue Zhang and Sanlin Jin for NMR experiments, and Hao Gao and Xinluan Wang for ESI-MS experiments in the Shenzhen Research Centre of Traditional Chinese Medicines and Natural Products.

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