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Chemical constituents of *Pholidota leveilleana*

Zhi-Peng Yuan, Yu-Jiao Zhong, Xue-Hui Su, Cong-Ying Li, Hong-Jian Du, Ya-Ya Wen, Yan-Fang Li*, Bing Liang

Department of Pharmaceutics & Bioengineering, School of Chemical Engineering, Sichuan University, Chengdu 610065, China

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1. Subject and source

The genus *Pholidota* (Orchidaceae) distributed from tropical Asia to tropical Australia and consists of *ca.* 30 species. Among them, *ca.* 14 species occur in China. *Pholidota leveilleana* Schltr., called 'Dan Ye Shi Xian Tao' in Chinese, is a perennial herb distributed in North and West Guangxi, South Guizhou and SouthEast Yunnan provinces in China (Chen, 1999). The whole plants of *Pholidota leveilleana* were purchased from a local herbal medicine store in Chengdu, Sichuan province, China, in May 2010. The plant was identified by Dr. Yan-Fang Li. A voucher specimen (No. 20100607) of this plant was deposited at the Department of Pharmaceutics & Bioengineering, Sichuan University, Chengdu, China.

2. Previous work

Secondary metabolites of plants from the genus *Pholidota*, including 9,10-dihydrophenanthropyrans, 9,10-dihydrophenanthrenes, bibenzyls, stilbenes, triterpenes, steroids, lignans, benzoxepins, and phenolic compounds, have been reviewed by Bandi and Lee (2011). Among them, 9,10-dihydrophenanthrenes are reported as the major components of this genus. The whole plant of *P. chinensis* has been used as a remedy for acute or chronic bronchitis, toothache, and duodenal ulcer (State Administration of Traditional Chinese Medicine, 2004), and showed anti-fatigue and anti-hypoxia activities (Liu et al., 2004, 2006). Also, the whole plant or pseudobulb of *P. yunnanensis* is used traditionally for various ailments, such as cough, rheumatism, stomachache, and trauma (Bandi and Lee, 2011). Both species are the most popular plants in empirical

* Corresponding author. Tel./fax: +86 28 85405221.
E-mail address: lyf471@yahoo.com.cn (Y.-F. Li).

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study. Some dihydroxyphenanthrens isolated from the latter showed potent cytotoxicity against four human cancer cell lines (HepG2, MCF-7, NCI-H460, and SF-268)(Wang et al., 2006a). Eight 9, 10-dihydrophenanthrene derivatives isolated from *P. yunnanensis* (Guo et al., 2007) and some stilbenes and bibenzyls obtained from *P. chinensis* (Wang et al., 2006b) also exert DPPH free radical-scavenging activity. But there are no reports on chemical constituents of *P. leveilleana*.

3. Present study

Air-dried whole plants of *P. leveilleana* (5.0 kg) were powdered and extracted at room temperature with 95% ethanol (25 L × 3, each time for 72 h). After concentration in *vacuo*, the concentrate was suspended in H_2O (2 L) and partitioned successively with petroleum ether (PE), and ethyl acetate (EtOAc) (each 3 L × 3). The EtOAc solution was concentrated to dryness under reduced pressure. The resulting extract (173 g) was subjected to silica gel chromatography with a step gradient of PE and EtOAc (v/v, 2:1, 1.5:1, 1:1, 1:1.5, 1:2, 1:5, each 3 L) to yield 14 portions (A ~ N).

Portion A (2.68 g) was subjected on MCI CC using acetone and water as the elution solvents (v/v, 7:3, 8:2, 9:1, 1:0, each 200 mL) to afford 3 fractions (A1-A3). Fraction A1 (358 mg) was rechromatographed over MCI resin (acetone/H_2O, v/v, 5:5–7:3, each 100 mL) to give 3 subfractions (A1a-A1c). Subfraction A1b (95 mg) and A1c (87 mg) were combined and separated by preparative TLC (PTLC, CH_2Cl_2/acetone, v/v, 13:1) to afford **2** (110 mg). Compounds **5** (12.4 mg) and **6** (10.4 mg) were obtained from subfraction A1a (116 mg) in the same way. Fraction A2 (628 mg) was further separated by using Toyopearl HW-40F CC (CH_2Cl_2/MeOH, v/v, 1:1) and PTLC (PE/EtOAc, v/v, 2:1) to give subfraction A2a1 (174 mg). Compound **1** (77 mg) was isolated from subfraction A2a1 by using CC over Toyopearl HW-40F (CH_2Cl_2–MeOH, v/v, 1:1) and MCI resin (acetone/H_2O, v/v, 7:3–1:0, each 100 mL).

Portion B (15 g) was subjected to MCI CC (acetone/H_2O, v/v, 5:5–1:0, each 800 mL) to give seven fractions (B1-B7). Fraction B1 (56 mg) was purified by PTLC (CH_2Cl_2/acetone, v/v, 7:1) to afford **8**(8.3 mg); fraction B2 (262 mg) was separated by using Sephadex LH-20 CC (CH_2Cl_2/MeOH, v/v, 1:1) and PTLC (CH_2Cl_2/acetone, v/v, 20:1) to yield **7** (43 mg). Fraction B4 (2.2 g) was subjected to Sephadex LH-20 CC (CH_2Cl_2/MeOH, v/v, 1:1) to give four subfractions (B4a–B4d). Subfraction B4b (600 mg) and B4c (550 mg) were combined and subjected to MCI (acetone/H_2O, v/v, 5:5–8:2, each 150 mL) and Toyopearl HW-40F CC (MeOH) to provide fractions (B4c1-B4c3). Repeated PTLC (CH_2Cl_2/acetone, v/v, 15:1) led to the isolation of **3** (16.5 mg), **4** (240 mg) and **10** (9.4 mg) from fraction B4c2 (282 mg). Compounds **11** (15.3 mg) and **12** (103 mg) were obtained from fraction B4c3 (206 mg) in the same way.

Portion C (12 g) was sectioned into seven fractions (C1-C7) by MCI CC (acetone/H_2O, v/v, 3:7–8:2, each 800 mL). Compound **9** (10 mg) was yielded from fraction C2 (188 mg) by using PTLC (CH_2Cl_2/acetone, v/v, 10:1). And portion F (3.5 g) was subjected to MCI CC (acetone/H_2O, v/v, 2:8–8:2, each 200 mL) to afford five fractions (F1-F5). Fraction F2 (183 mg) was subjected to PTLC (CH_2Cl_2/acetone, v/v, 13:1; CH_2Cl_2/MeOH, v/v, 20:1) to give **14** (36 mg). Compound **13** (28 mg) was obtained from fraction F3 (910 mg) by Sephadex LH-20 CC (MeOH) and PTLC (PE/EtOAc, v/v, 3:2).

The structures of isolates (Fig. 1) were elucidated by combination of spectroscopic methods (MS, ^1H, ^{13}C NMR and 2D NMR) and comparison with the literature. They were identified as pinosylvin monomethyl ether (**1**)(Ngo and Brown, 1998),

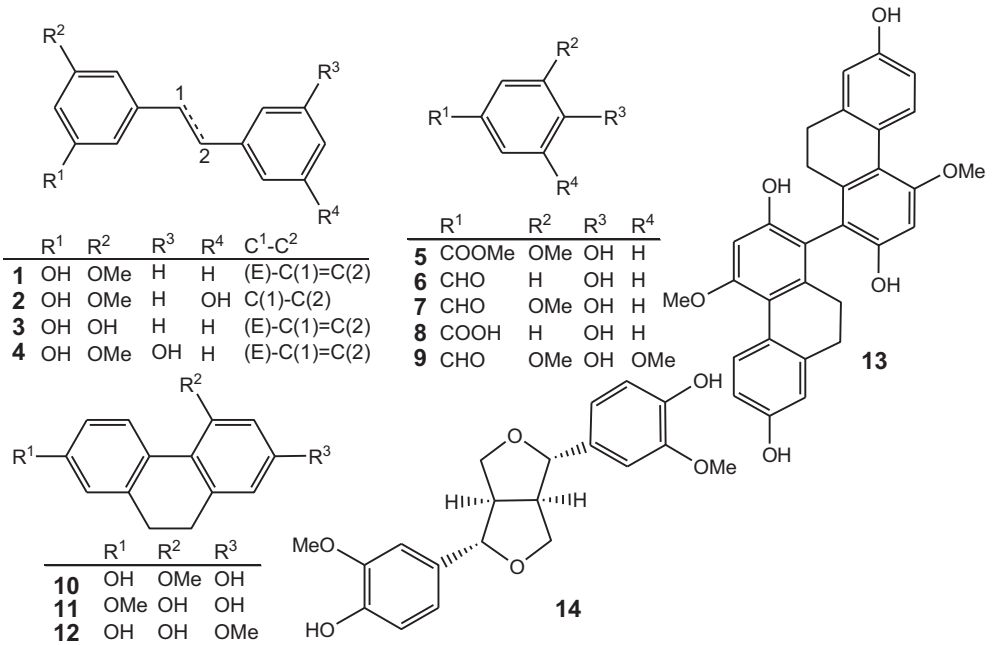

Fig. 1. Structures of compounds **1-14**.