Chemistry of Natural Compounds, Vol. 46, No. 5, 2010

CHEMICAL CONSTITUENTS OF *Bulbophyllum odoratissimum*

Ye-Gao Chen,[1,2*] Yu-Peng Li,[1] Jun-Ju Xu,[1] Hong Yu,[3] and Yong Zhao[1]

UDC 547.972

Plants of *Bulbophyllum* genus (Orchidaceae) have been used in China as a substitute for "Shi-hu," an important Chinese herb prepared from the dried stems of *Dendrobium* species and as a precious health food and nutrient [1]. *Bulbophyllum odoratissimum* (J. E. Smith) Lindl. is a plant widely distributed in China, Nepal, Sikkim, Bhutan, India, Burma, Thailand, Laos, and Vietnam and used in folk medicine to treat tuberculosis, chronic inflammation, and fracture [2]. Previous investigations on the constituents of *B. odoratissimum* have revealed the presence of phenanthrene, lignan, flavonoids, bibenzyls, bulbophyllispiradienone and its derivatives, phenolic glycosides, aldehydes, and acids [3–5]. During the search for bioactive compounds from medicinal plants in Yunnan of China, we isolated ten cytotoxic phenolics, including phenanthrene, 9,10-dihydrophenanthrenes, and bibenzyls from *B. odoratissimum* [6, 7]. To find new active principles from *B. odoratissimum*, we reinvestigated the plant.

B. odoratissimum was collected from Simao County of Yunnan Province, China in February, 2004. The air-dried powdered whole plant of *B. odoratissimum* (20 kg) was exhaustively extracted with 95% EtOH at room temperature, and the EtOH extract was evaporated in vacuo to yield a dark brown residue (1 kg). H_2O (2.5 L) was added to the residue, and the resulting solution was extracted with petroleum ether, EtOAc, and *n*-BuOH successively (four times, each 1.5 L). The petroleum ether extract (116 g) was separated on a silica gel column, eluting with petroleum ether containing increasing amounts of acetone to obtain seven fractions from which compounds **1**–**11** were obtained. Fraction 3 (25 g) was subjected to repeated column chromatography (silica gel, petroleum ether–acetone 20:1) and preparative TLC (petroleum ether–acetone 10:1) to yield **11** (40 mg). The EtOAc extract (350 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of acetone to obtain six fractions. Fraction 1 (30 g) was subjected to repeated column chromatography (silica gel, petroleum ether–acetone 40:1) to yield **9** (1.0 g) and **10** (1.5 g). Fraction 3 (77 g) was separated to two subfractions by silica gel column chromatography (petroleum ether–acetone 4:1, 7:3). The second subfraction (47 g) was subjected to repeated column chromatography, first on silica gel ($CHCl_3$–acetone 80:1) and then on Sephadex LH-20 (MeOH–H_2O 9:1) to obtain **2** (15 mg), **3** (22 mg), **4** (15 mg), **5** (10 mg), **6** (5 mg), **7** (10 mg), **8** (5 mg), and **12** (20 mg). Fraction 4 (67 g) was subjected to repeated column chromatography (silica gel, $CHCl_3$–MeOH 10:1), and then purified by chromatography over Sephadex LH-20 (MeOH–H_2O 9:1) to yield **1** (20 mg).

Compound **1**, $C_{30}H_{26}O_6$, is a colorless powder. The mass spectrum exhibited peaks for ions at *m/z* 482 [M^+, 100%], 241, 197, 181, 157. The PMR spectrum displayed characteristic signals of dimeric 9,10-dihydrophenanthrenes. The ^{13}C NMR and DEPT spectra showed the signals of 30 carbons. Based on NMR and mass spectral data, **1** contains two similar 9,10-dihydrophenanthrene monomers. Comparison of the spectral data with those reported in literature indentified **1** as flavanthrin [8].

Compound **3**, $C_{15}H_{14}O_3$, is a colorless powder. The mass spectrum exhibited peaks for ions at *m/z* 242 [M^+, 100%], 227, 181, 121. The PMR spectrum (CD_3OD) displayed characteristic signals of 9,10-dihydrophenanthrenes, showing resonance for a 1,2,4-trisubstitued benzene ring. The ^{13}C NMR and DEPT spectra (CD_3OD), showed signals of 15 carbons. Based on NMR and mass spectral data, **3** contains two hydroxyls. Comparison of the spectral data with those reported in the literature identified **3** as lusianthridin [9].

1) Department of Chemistry, fax: +86 871 5516061, e-mail: ygchen48@gmail.com; 2) Engineering Research Center of Sustainable Development and Utilization of Biomass Energy, Ministry of Education, Yunnan Normal University, Kunming 650092, China; 3) School of Life Science, Yunnan University, Kunming 650031, China. Published in Khimiya Prirodnykh Soedinenii, No. 5, pp. 664–665, September–October, 2010. Original article submitted May 5, 2009.

0009-3130/10/4605-0787

Compound **4**, $C_{16}H_{12}O_5$, is reddish powder. The PMR spectrum (CD_3OD, δ, ppm, J/Hz) showed characteristic signals of phenanthroquinone, showing resonances for a 1,2,4,5-tetrasubstitued benzene ring [9.50 (1H, s, H-5), 7.60 (1H, s, H-8)], a pair of *ortho*-coupled aromatic protons [7.98 and 8.18 (each 1H, d, J = 8.4, H-9 and H-10)], a single aromatic proton [6.34 (1H, s, H-3)], and two methoxyl groups [3.74 (3H, s, MeO-2), 3.99 (3H, s, MeO-6)]. The ^{13}C NMR and DEPT spectra (CD_3OD, ppm) showed signals of 16 carbons, including two carbonyls [190.3 (s, C-4) and 182.5 (s, C-1)]. Based on NMR data, **4** contains two carbonyl groups characteristic of a 1,4-quinone unit. Comparison of the spectral data with those reported in the literature identified **4** as dioscoreanone [10].

Compound **5**, $C_{15}H_{12}O_4$, is reddish powder. The mass spectrum exhibited peaks for ions at *m/z* (%): 256 [M^+, 100], 241, 213, 171, 115, 69. The PMR spectrum (C_5D_5N, δ, ppm, J/Hz) displayed characteristic signals of 9,10-dihydrophenanthroquinone, showing resonances for a 1,2,4-trisubstitued benzene ring [8.42 (1H, d, J = 8.6, H-5), 7.16 (1H, d, J = 8.6, H-6), 7.04 (1H, s, H-8)], a single aromatic proton [6.05 (1H, s, H-3)] and a methoxyl group [3.66 (3H, s, MeO-2)], and two methylenes [2.66 (2H, m, 9-CH_2) 2.62 (2H, m, 10-CH_2)]. The ^{13}C NMR and DEPT spectra (C_5D_5N, δ, ppm) showed signals of 15 carbons, including two carbonyls [188.5 (s, C-4) and 182.0 (s, C-1)]. Based on NMR and MS data, **5** contains two carbonyl groups characteristic of a 1,4-quinone unit. Comparison of the spectral data with those reported in the literature identified **5** as ephemeranthoquinone [11].

Compound **7**, $C_{16}H_{18}O_4$, is colorless gum. The mass spectrum showed peaks for ions at *m/z* 274 [M^+], 168, 167 (100%), 137, 123, 107, and 77. The PMR spectrum indicated characteristic signals of bibenzyls. The ^{13}C NMR and DEPT spectra showed signals of 16 carbons. Based on NMR and mass spectral data, **7** contains two methoxyls and two hydroxyls. The mass spectral fragmentation is consistent with one hydroxyl on a benzene ring, and two methoxyls and one hydroxyl on another benzene ring. Comparison of the spectral data with those reported in the literature identified **7** as aloifol I [12].

Compound **8**, $C_{17}H_{19}O_5$, is colorless gum. The mass spectrum exhibited peaks for ions at *m/z* 304 [M^+], 168, 167 (100%), 137, 77. The PMR spectrum also displayed characteristic signals of bibenzyls. The ^{13}C NMR and DEPT spectra showed signals of 17 carbons. Based on NMR and mass spectral data, **8** contains three methoxyls and two hydroxyls. The mass spectral fragmentation is consistent with one hydroxyl and one methoxyl on a benzene ring, and two methoxyls and one hydroxyl on another benzene ring. Comparison of the spectral data with those reported in the literature identified **8** as cannabistilbene II [13].

Compounds **2**, **6**, and **9–12** were identified as 3,7-dihydroxy-2,4-dimethoxyphenanthrene [14], moscatilin [15], *bis* (2-ethylhexyl)phthalate [16], dibutylphthalate [16], *β*-sitosterol [17], and daucosterol [17], respectively, based on co-TLC and comparison of PMR, ^{13}C NMR, and EI-MS with those of authentic samples. Compounds **3**–**6** and **8**–**10** were discovered from the *Bulbophyllum* genus for the first time and the others were originally isolated from the plant.

ACKNOWLEDGMENT

This investigation was supported by a grant (2005DFA30670) for international collaborative research by the Ministry of Science and Technology of China, a grant (30860346) from Natural Science Foundation of China, and the program for New Century Excellent Talents in University (NCET-05-0824).

REFERENCES

1. X. S. Bao, Q. S. Shun, and L. Z. Chen, *The Medicinal Plants of Dendrobium (Shi-hu) in China*, A Coloured Atlas, Press of Fudan University and Press of Shanghai Medical University, Shanghai, 2001, p. 109.
2. Z. Y. Wu, *New Chinese Compendium of Materia Medica,* Shanghai Press of Science and Technology, Shanghai, **3**, 574 (1990).
3. J. G. Lin, W. G. Zhang, R. Zhao, Z. Y. Niu, K. Bao, D. L. Liu, N. L. Wang, and X. S. Yao, *Chin. Chem. Lett.*, **17**, 307 (2006).
4. D. L. Liu, F. G. Pang, X. Zhang, H. Gao, N. L. Wang, and X. S. Yao, *Acta Pharm. Sin.*, **41**, 738 (2006).
5. P. L. Majumder and R. C. Sen, *Phytochemistry*, **30**, 2092 (1991).
6. Y. G. Chen, J. J. Xu, H. Yu, C. Qing, Y. L. Zhang, L. Q. Wang, Y. Liu, and J. H. Wang, *Food Chem.*, **107**, 169 (2008).

7. Y. G. Chen, J. J. Xu, H. Yu, C. Qing, Y. L. Zhang, Y. Liu, and J. H. Wang, *J. Korean Chem. Soc.*, **51**, 352 (2007).
8. P. L. Majumder and S. Banerjee, *Tetrahedron*, **44**, 7303 (1988).
9. S. Takagi, M.Yamaki, and K. Inoue, *Phytochemistry*, **22**, 1011 (1983).
10. A. Itharat, A. Plubrukarn, P. Kongsaeree, T. Bui, N. Keawpradub, and P. J. Houghton, *Org. Lett*., **5**, 2879 (2003).
11. Y. Hernandez-Romero, J. I. Rojas, R. Castillo, A. Rojas, and R. Mata, *J. Nat. Prod*., **67**, 160 (2004).
12. R. K. Juneja, S. C. Sharma, and J. S. Tandon, *Phytochemistry*, **26**, 1123 (1987).
13. H. N. Elsohly, G. E. Ma, C. E. Turner, and M. A. ElSohly, *J. Nat. Prod*., **47**, 445 (1984).
14. C. Q. Fan, W. Wang, Y. P. Wang, G. W. Qin, and W. M. Zhao, *Phytochemistry*, **57**, 1255 (2001).
15. P. L. Majumder and R. C. Sen, *Phytochemistry*, **26**, 2121 (1987).
16. Y. G. Wang, C. B. Cui, B. Han, B. Cai, and Y. H. Pei, *Chin. J. Med. Chem*., **15**, 65 (2005).
17. G. W. Qin, A. I. Hamed, N. A. El-Emary, Y. G. Chen, L. Q. Wang, K. K. Cheung, and K. F. Cheng, *Planta Med*., **66**, 191 (2000).