CHEMICAL CONSTITUENTS OF *Dendrobium chrysotoxum*

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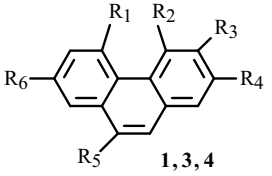
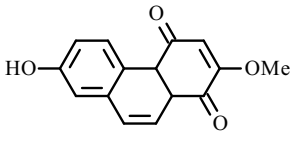
The stems of several *Dendrobium* species (Orchidaceae) are used in traditional Chinese medicine as a tonic to nourish the stomach, promote the production of body fluid, and reduce fever [1]. *D. chrysotoxum* Lindl. is distributed in India, Nepal, Thailand, Laos, Vietnam, and Yunnan of southwestern China [2]. Previous investigations on the constituents from *D. chrysotoxum* have isolated a series of aromatic compounds such as bibenzyls, phenanthrenes, 9,10-dihydrophenanthrenes, fluorenones, and simple aromatic acids and esters [3–10]. The EtOH extract and some of the compounds such as erianin, chrysotoxine, and confusarin were found to possess antitumor activity [11–15]. To find of further antitumor principles from *D. chrysotoxum*, we investigated the plant.

Dendrobium chrysotoxum was collected from Simao County of Yunnan, China in February, 2005. The air-dried whole plants (0.5 kg) were chopped and exhaustively extracted with 95% EtOH. The EtOH extract (22 g) was applied to a silica gel column, eluting with petroleum ether containing increasing amounts of EtOAc to offer five fractions (A–E). Fraction B (5 g) was subjected to repeated column chromatography (silica gel, petroleum ether–EtOAc 4:1; then Sephadex LH-20, MeOH–H_2O 9:1) to afford **1** (15 mg), **3** (9 mg), **4** (4 mg), **5** (5 mg), **6** (4 mg), **8** (7 mg), and **10** (25 mg). Fraction C (5 g) was chromatographed over silica gel ($CHCl_3$–Me_2CO 20:1) and then purified by chromatography over Sephadex LH-20 (MeOH–H_2O 9:1) to furnish **7** (3 mg) and **11** (6 mg). Fraction D (4 g) was separated on silica gel chromatography ($CHCl_3$–MeOH 15:1) and then purified by chromatography over Sephadex LH-20 (MeOH–H_2O 9:1) to yield **2** (8 mg) and **9** (6 mg).



1, 3, 4



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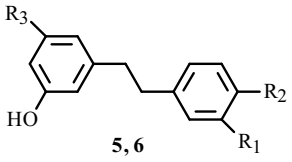

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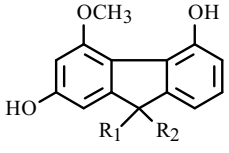

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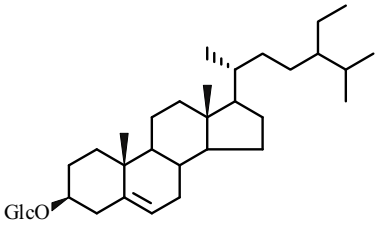

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1: $R_1 = R_4 = OH$, $R_2 = R_5 = OMe$, $R_3 = R_6 = H$; **3:** $R_1 = R_4 = OH$, $R_2 = OMe$, $R_3 = R_5 = R_6 = H$
4: $R_1 = R_5 = H$, $R_2 = R_4 = OMe$, $R_3 = R_6 = OH$; **5:** $R_1 = OH$, $R_2 = H$, $R_3 = OMe$
6: $R_1 = R_3 = OMe$, $R_2 = OH$; **7:** $R_1 = H$, $R_2 = OH$; **8:** $R_1R_2 = O$

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Compound **1**, $C_{16}H_{14}O_4$, white amorphous powder. The mass spectrum exhibited peaks for ions at *m/z* 270 (M^+, 100), 255, 227, 212, 184, and 139. The PMR spectrum [$(CD_3)_2CO$, δ, ppm, J/Hz] displayed signals of six aromatic protons at 7.94 (1H, dd, J = 6.8, 1.2, H-8), 7.47 (1H, dd, J = 6.8, 6.8, H-7), 7.15 (1H, dd, J = 6.8, 1.2, H-6), 7.03 (1H, d, J = 2.2, H-1), 6.85 (1H, d, J = 2.2, H-3), and 6.95 (1H, s, H-10), and two methoxyl groups [4.17 (3H, s, 4-OMe), and 4.04 (3H, s, 9-OMe)]. The ^{13}C NMR and DEPT spectra [$(CD_3)_2CO$, δ, ppm] had signals at 158.1 (C-4), 156.8 (C-2), 155.6 (C-9), 155.4 (C-9),138.3 (C-10a), 129.6 (C-8a), 127.4 (C-7), 121.4 (C-4b), 118.0 (C-6), 114.8 (C-8), 110.5 (C-4a), 107.5 (C-1), 103.3 (C-10), 101.0 (C-3), 59.0 (4-OCH_3), and 56.4 (9-OCH_3). Based on NMR (^1H NMR, ^{13}C NMR, C-H COSY, HMBC, HSQC, and NOESY) and mass spectral data, **1** contains two methoxyls and two hydroxyls. By comparison of the spectral data with those reported in the literature, **1** was identified as 4,9-dimethoxyphenanthrene-2,5-diol [16].

Compound **2**, $C_{15}H_{10}O_4$, red amorphous powder. The mass spectrum exhibited peaks for ions at *m/z* 254 (M^+, 100), 225, 197, and 155. The PMR spectrum (DMSO-d_6, δ, ppm, J/Hz) displayed signals of six aromatic protons at 9.31 (1H, d, J = 9.2, H-5), 8.02 (1H, d, J = 8.5, H-9), 7.90 (1H, d, J = 8.5, H-10), 7.30 (1H, d, J = 9.2, H-6), 7.20 (1H, s, H-8), and 6.20 (1H, s, H-3), and a methoxyl group [3.74 (3H, s, 2-OMe)]. The ^{13}C NMR and DEPT spectra (DMSO-d_6, δ, ppm) had signals at 189.7 (C-4), 181.6 (C-1), 159.7 (C-2), 159.0 (C-7), 140.3 (C-8a), 133.6 (C-9), 131.0 (C-10), 129.6 (C-10a), 128.1 (C-4a), 124.7 (C-4b), 123.9 (C-6), 123.2 (C-5), 112.4 (C-3), 110.2 (C-8), and 57.7 (2-OCH_3). Based on NMR and mass spectral data, **2** contain a methoxyl, a hydroxyl, and two carbonyl groups. By comparison of the spectral data with those reported in the literature, **2** was identified as densiflorol B [17].

Compound **3**, $C_{15}H_{12}O_3$, white amorphous powder. The mass spectrum exhibited peaks for ions at *m/z* 240 (M^+, 100), 225, 197, and 139. The PMR spectrum [$(CD_3)_2CO$, δ, ppm, J/Hz] displayed signals of seven aromatic protons at 7.62 (1H, d, J = 8.8, H-9), 7.49 (1H, d, J = 8.8, H-10), 7.44 (1H, dd, J = 7.6, 7.6, H-7), 7.41 (1H, dd, J = 7.6, 2.0, H-6), 7.13 (1H, dd, J = 7.6, 2.0, H-8), 7.07 (1H, d, J = 2.4, H-1), and 7.00 (1H, d, J = 2.4, H-3), two hydroxyls [9.52 (1H, s, 5-OH) and 9.06 (1H, s, 2-OH)], and a methoxyl [4.16 (3H, s, 4-OMe)]. The ^{13}C NMR and DEPT spectra [$(CD_3)_2CO$, δ, ppm] had signals at 157.8 (C-4), 156.8 (C-2), 155.7 (C-5), 137.5 (C-10a), 135.4 (C-8a), 130.2 (C-7), 127.8 (C-9), 127.4 (C-10), 121.5 (C-8), 120.2 (C-4b), 117.4 (C-6), 114.0 (C-4a), 108.3 (C-1), 103.1 (C-3), and 59.1 (4-OMe). By comparison of the spectral data with those reported in the literature, **3** was identified as moscatin [18].

Compound **4**, $C_{16}H_{14}O_5$, light yellow gum. The mass spectrum exhibited peaks for ions at *m/z* 270 (M^+, 100), 255, 233, 212, 209, 184, 167, 156, 155, 139, 128, and 127. The PMR spectrum [$(CD_3)_2CO$, δ, ppm, J/Hz] displayed signals of six aromatic protons at 9.36 (1H, d, J = 9.2, H-5), 7.61 (1H, d, J = 8.8, H-10), 7.47 (1H, d, J = 8.8, H-9), 7.24 (1H, s, H-8), 7.22 (1H, s, H-1), and 7.20 (1H, d, J = 9.2, H-6), two hydroxyls [8.62 (1H, s, 7-OH) and 7.98 (1H, s, 3-OH)], and two methoxyls [3.97 (3H, s, 2-OCH_3) and 3.92 (3H, s, 4-OCH_3)]. The ^{13}C NMR and DEPT spectra [$(CD_3)_2CO$, δ, ppm] had signals at 155.9 (C-7), 148.4 (C-2), 145.3 (C-4), 141.1 (C-3), 135.0 (C-4a), 129.1 (C-5), 128.1 (C-10), 126.4 (C-8a), 125.3 (C-9), 124.0 (C-4b), 120.0 (C-10a), 117.4 (C-6), 112.2 (C-8), 106.0 (C-1), 59.6 (4-OCH_3), and 56.3 (2-OCH_3). Based on NMR and mass spectral data analysis, and comparison of the spectral data with those reported in the literature, **4** was identified as 3,7-dihydroxy-2,4-dimethoxyphenanthrene [17].

Compound **7**, $C_{14}H_{12}O_4$, red amorphous powder. The mass spectrum exhibited peaks for ions at *m/z* 244 $[M]^+$, 229, 227, 213, 184, 173, 155, 115, 92, and 77. The PMR spectrum [$(CD_3)_2CO$, δ, ppm, J/Hz] showed five aromatic protons at 7.10 (1H, dd, J = 7.8, 7.2, H-7), 7.06 (1H, d, J = 7.2, H-8), 6.86 (1H, d, J = 2.2, H-1), 6.75 (1H, d, J = 7.8, H-6), and 6.62 (1H, d, J = 2.2, H-3), two hydroxyls [9.11 (1H, s, 5-OH) and 4.66 (1H, d, J= 7.4, 9-OH)], a proton at 5.40 (1H, d, J = 7.4, H-9), and a methoxyl [4.13 (3H, s, 4-OCH_3)]. The ^{13}C NMR and DEPT spectra [$(CD_3)_2CO$, δ, ppm] had signals at 160.2 (C-2), 153.5 (C-4), 151.9 (C-9a), 151.3 (C-5), 149.0 (C-8a), 128.9 (C-7), 125.2 (C-4b), 119.5 (C-4a), 117.4 (C-8), 117.3 (C-6), 107.7 (C-1), 100.8 (C-3), 75.9 (C-9), and 57.6 (4-OCH_3). By comparison of the spectral data with those reported in the literature, **7** was identified as denchrysan B [19].

Compound **8**, $C_{14}H_{10}O_4$, red amorphous powder. The mass spectrum exhibited peaks for ions at *m/z* 242 $[M]^+$, 227, 199, 171, 155, 142, 126, 115, 101, and 89. The PMR spectrum (DMSO-d_6, ppm, δ, J/Hz) showed five aromatic protons at 7.12 (1H, dd, J = 7.7, 7.2, H-7), 7.07 (1H, d, J = 7.2, H-8), 6.96 (1H, d, J = 7.7, H-6), 6.73 (1H, s, H-3), and 6.72 (1H, s, H-1), and a methoxyl [4.02 (3H, s, 4-OCH_3)]. The ^{13}C NMR and DEPT spectra (DMSO-d_6, δ, ppm) had signals at 194.2 (C-9), 162.5 (C-2), 153.8 (C-5), 151.2 (C-4), 136.9 (C-8a), 135.8 (C-9a), 130.6 (C-6), 128.5 (C-4b), 125.7 (C-7), 121.2 (C-4a), 117.5 (C-8), 107.2 (C-1), 107.0 (C-3), and 58.4 (4-OCH_3). By comparison of the spectral data with those reported in the literature, **8** was identified as dengibsin [20].

Compound **9**, $C_8H_8O_4$, white amorphous powder. The PMR spectrum (CD_3OD, δ, ppm, J/Hz) showed three aromatic protons at 7.56 (1H, s, H-2), 7.53 (1H, d, J = 8.2, H-5), and 6.80 (1H, d, J = 8.2, H-6), and a methoxyl [3.88 (3H, s, 3-OCH_3)].

The ^{13}C NMR and DEPT spectra (CD_3OD, δ, ppm) had signals at 172.0 (C-7), 150.9 (C-4), 147.9 (C-3), 126.2 (C-1), 124.3 (C-2), 115.0 (C-6), 113.6 (C-5), and 55.9 (3-OCH_3). By comparison of the spectral data with those reported in the literature, **9** was identified as vanillic acid [21].

Compounds **5**, **6**, **10**, and **11** were identified as batatasin III, gigantol, stigmasterol and daucosterol respectively based on co-TLC and comparison of PMR, ^{13}C NMR, and EIMS with those of authentic samples [22]. Compound **1** was discovered in *Dendrobium* genus for the first time, and compounds **2**–**5** and **10** were originally isolated from *D. chrysotoxum*.

Compound **1** was evaluated *in vitro* for its inhibitory ability against the growth of human leukemia cell lines K562 and HL-60, human lung adenocarcinoma A549, human hepatoma BEL-7402, and human stomach cancer SGC-7901 by microculture tetrazolium (MTT) assay, with minor modification [23–25]. Result showed that it displayed cytotoxicity against K562, HL-60, A549, BEL-7402, and SGC-7901 with IC_{50} values of 45.64, 1~10, 8.65, 1.79, and 2.89 μg/mL respectively. It seems that **1** exhibited more selective cytotoxicity against BEL-7402 and SGC-7901.

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