Chemical Components of *Dendrobium polyanthum*

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A new tetrahydroanthracene, 3,6,9-trihydroxy-3,4-dihydroanthracen-1(2*H*)-one (**1**), six phenolics, moscatilin (**2**), gigantol (**3**), batatasin (**4**), moscatin (**5**), 9,10-dihydromoscatin (**6**), 10-dihydrophenanthrene-2,4,7-triol (**7**), and a sesquiterpenoid, corchoionoside C (**8**), together with two sterols *β*-sitosterol (**9**) and daucosterol (**10**), were isolated from the stems of *Dendrobium polyanthum*. Compounds **1** and **2** were assessed for cytotoxic activity against two human tumor cell lines (A549 and HL-60).

Key Words: *Dendrobium polyanthum*, Orchidaceae, Tetrahedroanthracene, Bibenzyl, Phenanthrene

Introduction

The stems of several *Dendrobium* species (Orchidaceae) are used as "Shi-Hu" in traditional Chinese medicines to nourish the stomach, promote the production of body fluid and reduce fever.[1,2] Previous studies on chemical constituents of the genus led to the isolation of a series of diverse compounds, including alkaloids, fluorenones, sesquiterpenoids, bibenzyls and phenanthrenes. Some of these compounds were found to possess antitumour activities.[3] There are about 80 *Dendrobium* species distributed over China.[4] A few of them were investigated in this laboratory from 2004 and led to isolation of some new phenolic compounds.[5,6] *Dendrobium polyanthum* is widely distributed in south-west of China, India, Nepal, Sikkim, Burma, Laos, Vietnam and Thailand,[4] and to the best of our knowledge, only two alkaloids, hygrine and dendroprimine, have been identified from it by GC-MS method.[7,8] In the course of our effort to find biologically active compounds from this species, a new tetrahydroanthracen, (*S*)-3,6,9-trihydroxy-3,4-dihydroanthracen-1(2*H*)-one (**1**), together with nine known compounds (**2-10**) were isolated from this plant (Fig. 1). All of these compounds were identified for the first time from *D. polyanthum* in this report. Moreover this is the first time for the isolation of tetrahedroanthracene homologue and corchoionoside C (**8**) from the genus *Dendrobium*.

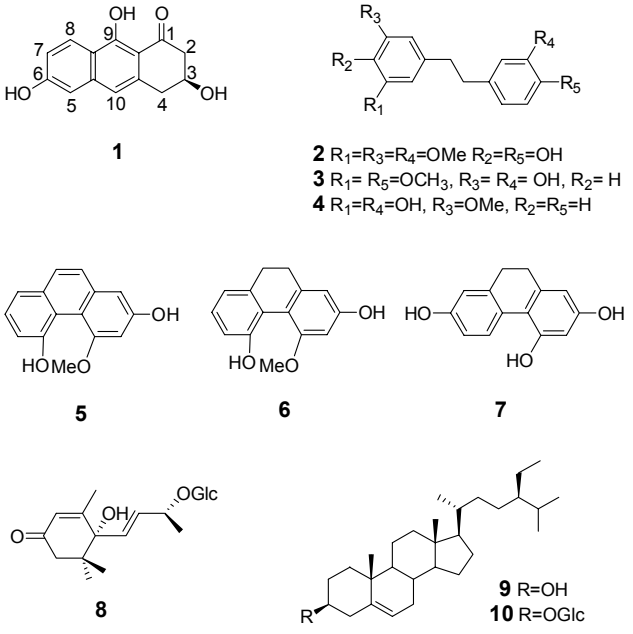

Figure 1. Structures of compounds **1-10** from the stems of *D. primulinum*.

Results and Discussion

Compound **1** was obtained as a brown amorphous powder, its molecular formula was assigned as $C_{14}H_{12}O_4$ from its HR-ESIMS (m/z = 267.0639 $[M + Na]^+$), indicating 9 degrees of unsaturation. The IR spectrum exhibited hydroxyl group (3394 cm^{-1}, brs; 3296 cm^{-1}, brs), carbonyl bond (1640 cm^{-1}), and aromatic moieties (1592, 1534, 1484 cm^{-1}). The ^{13}C NMR spectrum of **1** revealed fourteen carbon atoms, including two methylene, five methine, and seven quaternary carbons. Three aromatic atoms at δ_H = 8.44 (1H, d, J = 2.6, H-5), 7.73 (1H, dd, J = 9.3, 2.6, H-7) and 10.20 (1H, d, J = 9.3, H-8) in the ^1H NMR spectrum indicated a 1,2,4-substituted aromatic ring. The signal at δ_H = 6.99 (1H, s, H-10) correlated with three carbons at δ_C = 105.9 (C-5), 128.6 (C-8a) and 120.4 (C-9a) in the HMBC spectrum (Fig. 2) indicating a naphthalene skeleton. The cross-peaks [H-2 / H-3, H-3 / H-4] in the COSY spectrum indicated a partial structure $-CH_2CHOHCH_2-$. The partial structure linked with the carbonyl bond (C-1) and the naphthalene framework (C-9a, C-4a) and formed a hexatomic ring by the correlations [H-3 /

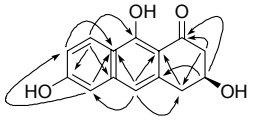

Figure 2. Key HMBC correlations of (*S*) 3,6,9-trihydroxy-3,4-dihydroanthracen-1(2*H*)-one (**1**).

C-1, C-4a; H-4 / C-4a, C-9a, C-10] in the HMBC spectrum. Compared the optical rotation (+21.6) of **1** with (*R*) aloesaponol II (– 43)[9,10] and (*S*) scytalone (+32),[11,12] it can be established that the compound probably has the *S* absolute configuration. Thus, compound **1** was identified as (*S*) 3,6,9-trihydroxy-3,4-dihydroanthracen-1(2*H*)-one.

The nine known compounds were identified on the basis of spectroscopic analysis and comparing spectra data with literature as moscatilin (**2**),[13] gigantol (**3**),[14] batatasin (**4**),[15,16,17] moscatin (**5**),[18] 9,10-dihydro-moscatin (**6**),[17] 10-dihydrophenanthrene-2,4,7-triol (**7**),[17,19] corchoionoside C (**8**)[20,21] and by comparing R_f values with authentic samples.[5,6] Some phenolics from Dendrobium species have been reported the antitumor activity ever by some researcher,[3,22,23] thus, compounds **1**-**2** were assessed for cytotoxic activity against two human tumor cell lines (A549 and HL-60). Compound **2** has a week effect for inhibition ratio of 61.9% at the concentration up to10^{-5} mol/L and compound **1** has no effect.

Experimental

General procedures. Melting points were measured on a XRC-1 micro-melting point apparatus (Beijing, China) and were uncorrected. Optical rotations were measured with a Horiba SEAP-300 spectropolarimeter. UV spectra were measured on a Hitachi UV-3210 spectrophotometer (Shanghai, China). IR spectra were measured with a Bio-Rad FTS-135 IR spectrometer (Richmond, CA) with KBr pellets. FABMS was obtained on a VG Auto Spec-3000 mass spectrometer (VG, Manchester, England), and HR-ESIMS was recorded with an API QSTAR Pulsar 1 spectrometer. 1D and 2D NMR spectra were recorded on Bruker AM-400 MHz and DRX-500 spectrometers (Karlsruhe, Germany), with chemical shifts (δ) in ppm relative to trimethylsilane (TMS) as internal standard and coupling constants in hertz (Hz). Column chromatography was carried out on silica gel (200 ~ 300 mesh) and TLC was carried out on plates precoated with silica gel (10 ~ 40 μm, Qindao Marine Chemical Ltd., Qingdao, PRC). Sephadex LH-20 was purchased from Amersham Biosciences.

Table 1. ^{1}H and ^{13}C NMR assignments and two-dimensional NMR correlations of **1**

Position	δ_H (mult, *J* in Hz)	δ_C (mult)	HMBC (H-C)
1		197.8 (s)	
2-α	3.31 (1H, dd, *J* = 15.8, 3.6)	51.1 (t)	1, 3, 4
2-β	3.11 (1H, dd, *J* = 15.8, 9.0)		
3	4.62 (1H, m)	66.4 (d)	1, 4a
4-α	3.41 (1H, dd, *J* = 16.5, 3.7)	41.6 (t)	2, 3, 4a, 10, 9a
4-β	3.27 (1H, dd, *J* = 16.5, 8.0)		
4a		144.5 (s)	
5	8.44 (1H, d, *J* = 2.6)	105.9 (d)	6, 7, 8a
6		156.4 (s)	
7	7.73 (1H, dd, *J* = 9.3, 2.6)	121.8 (d)	5, 8a
8	10.20 (1H, d, *J* = 9.3)	129.4 (d)	10a, 6, 9
8a		128.6 (s)	
9		159.3 (s)	
9a		120.4 (s)	
10	6.99 (1H, s)	110.1 (d)	4, 5, 8a, 9, 9a
10a		127.7 (s)	

Spectra were recorded in pyridine-D_5, chemical shifts (δ) are in ppm.

Plant material. The stems of *D. polyanthum* were collected in February 2006, from Xishuangbanna, Yunnan Province, China. It was identified by Prof. Yanhui Li (Kunming Institute of Botany). A voucher specimen (No. Zsh-3) was deposited at the state key laboratory of phytochemistry and plant resource in west China of Kunming Institute of Botany, Kunming, China.

Extraction and isolation. The air dried stems of the plant (3.0 kg) were powdered and extracted with 95% aqueous EtOH (12 L × 3) at reflux. The EtOH extract (36 L) was evaporated under reduced pressure and fractionated successively into EtOAc soluble (150 g) and *n*-BuOH soluble (80 g) fractions. A portion of EtOAc extract (140 g) was subjected to silica gel column chromatography (petroleum ether/Me_2CO, 8:1 → 7:3) to give ten fractions (A-J). Compared R_f values by TLC means with authentic sample, compound **9** was identified as the main constituent in the fraction A (5 g). Fraction C (12 g) was applied repeatedly to column chromatography over silica gel (petroleum ether/EtOAc, 8:1 → 7:3) and then Sephadex LH-20 ($CHCl_3$/CH_3OH, 1:1) to afford compounds **2** (30 mg), **3** (70 mg), **5** (44 mg) and **8** (20 mg). By the same methods, compound **4** (9 mg) was gotton from fraction D (7 g), compounds **6** (7 mg) and **7** (10 mg) were get from fraction F (17 g). A portion of *n*-BuOH extract (70 g) was subjected to silica gel column and eluted with $CHCl_3$/CH_3OH (10:1 → 10:3) to afford six fractions (I-VI). Fraction III (23 g) was applied to column chromatography repeatedly over silica gel ($CHCl_3$/CH_3OH, 15:1) and then Sephadex LH-20 (MeOH) to afford compounds **10** (50 mg) and **1** (45 mg).

(*S*) 3,6,9-Trihydroxy-3,4-dihydroanthracen-1(2*H*)-one (1): Brown amorphous powder ($(CH_3)_2CO$), m.p. 242 ~ 244 °C; [α] = + 21.6 (*c* 0.63, MeOH); UV/Vis (MeOH) λ_{max} (log ε_{max}): 357 nm (3.8), 322 nm (3.9), 255 nm (4.4), 223 nm (4.4), 201 nm (4.5); IR (KBr) ν_{max}: 3394, 3362, 2933, 1640, 1592, 1534, 1484, 1401, 1314, 1268, 1233, 1146, 1031, 926, 837, 657, 557 cm^{-1}; ^{1}H NMR (400 MHz, pyridine-D5) and ^{13}C NMR (100 MHz, pyridine-D5) data, see Table 1; FABMS (positive ion) *m/z* 245 (100) $[M+H]^+$; HR-ESIMS *m/z* 267.0639 $[M+Na]^+$ (calcd. 267.0633 for $C_{14}H_{12}O_4Na$).

Cytotoxic assay. Compound **1** and **2** was tested for its cytotoxic effects against human lung carcinoma A549 and human leukemia HL-60 cell lines using the sulforhodamine B (SRB)

Table 2. The cell growth inhibition rates of compounds **1**-**2**

cell strain	A549					HL-60				
concentration (mol/L)	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1	0	0	0	0	0	4.4	6.6	0	0	0
2	67.5	61.9	38.0	0	0	53.0	38.2	40.2	5.4	4.7
Etoposide	88.4	60.0	27.9	11.7	7.7	89.8	91.9	91.5	82.3	66.3

assay and the methyl-thiazol-tetrozolium (MTT) assay, respectively, etoposide was used as positive control. The cell growth inhibition rates as shown in Table 2.

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