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Four new sesquiterpene derivatives from *Dendrobium findlayanum*

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[ABSTRACT] Three new sesquiterpene glycosides with alloaromadendrane and ylangene-derived type aglycones, named dendrofindlayanosides A−C (**1**−**3**), one new cyclopacamphane type sesquiterpene named dendrofindlayanobilin A (**4**), together with five known compounds have been isolated from stems of *Dendrobium findlayanum*. Their structures were determined on the basis of spectroscopic and chemical methods.

[KEY WORDS] *Dendrobium findlayanum*; Sesquiterpene derivatives; Alloaromadendrane; Cyclopacamphane

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Introduction

The *Dendrobium* genus is one of the largest in Orchidaceae, containing more than 1500 species, and widely distributed in throughout Asia, Europe and Australia [1]. There are more than 100 *Dendrobium* species in China, and the fresh or dried stems of many species have been used both as medicine for the treatment of diabetes, fever, chronic atrophic gastritis, and skin aging diseases, as well as a high-quality health food now. The main chemical components of *Dendrobium* are alkaloids, phenolic compounds, sesquiterpenoids and polysaccharides with antioxidant, anti-inflammatory and anti-tumor effects etc. [2-7]. Our previous study on *D. findlayanum* which grown in the southwest of China and used as one of the most common source for Shi-Hu, has led to the isolation of four new seco-dendrobines, findlayines A−D and four new phenolic compounds with anti-inflammatory, anti-tumor and antioxidant activities [8]. In order to supply more evidence to the efficacy and safety of *D. findlayanum* in clinical applications, we investigated chemical constituents of the stems of *D. findlayanum* and led to the isolation of four new sesquiterpene derivatives, dendrofindlayanosides A−C (**1**−**3**), dendrofindlayanobilin A (**4**), and five known compounds. This paper will describe the isolation, structural elucidation and the immunomodulatory bioassay *in vitro* of all isolates.

Results and Discussion

Compound **1** was obtained as colorless oil. A molecular formula of $C_{21}H_{34}O_8$ was established by the HRESI-MS $[M + Na]^+$ *m/z* 437.2135 (Calcd. for 437.2146). The presence of hydroxyl (3426 cm^{-1}) was deduced from the IR spectrum. In the ^{13}C NMR spectrum (Table 2), 21 carbon signals were observed, constituted by two methyls, seven methylenes, ten methines, and two quaternary carbons. On enzymatic hydrolysis of **1**, glucose was obtained as the only sugar component. The ^1H NMR spectrum of **1** revealed only one anomeric proton signal at δ_H 4.24 (1H, d, J = 7.8 Hz). Therefore, the glucose unit was assigned in

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Dedicated to Professor SUN Han-Dong on the Occasion of His 80th Birthday

the *β* configuration. And acid hydrolysis of **1** furnished glc, which was detected by TLC comparison with authentic sample. Elucidation of ^{1}H-^{1}H COSY, HSQC and HMBC spectra of **1** revealed the planar structure of its aglycone was similar to that of dendroside C [9], except for the loss of a hydroxy methyl group (δ_C 72.2, t), along with the appearance of aldehyde group (δ_C 205.7, d), which was evidenced by the aldehyde proton (δ_H 8.68, s) and the HMBC cross-peaks from H-6 (δ_H 1.18, d) , H-7 (δ_H 1.70, d) and H-12 (δ_H 1.22, s) to C-13 (δ_C 205.7, d) (Fig. 2).

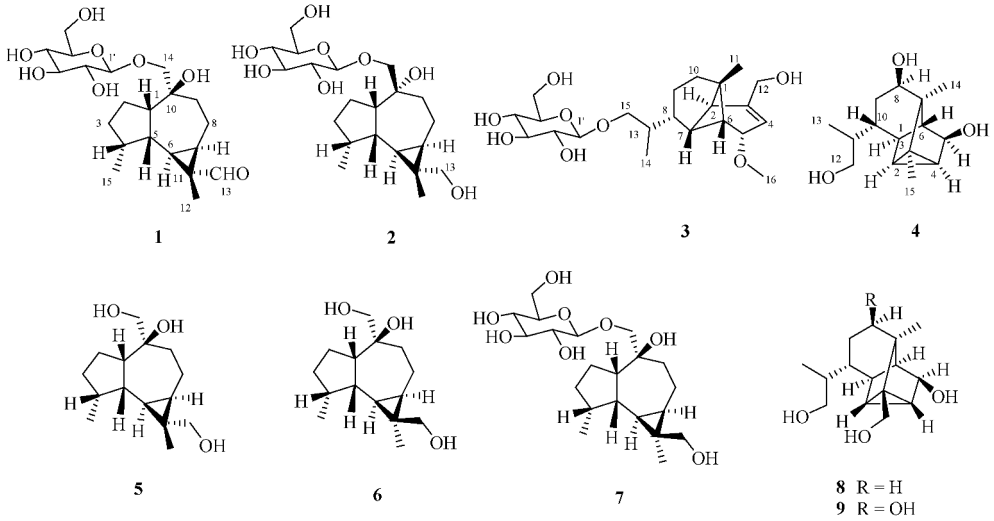

Fig. 1 Chemical structures of the compounds 1–9

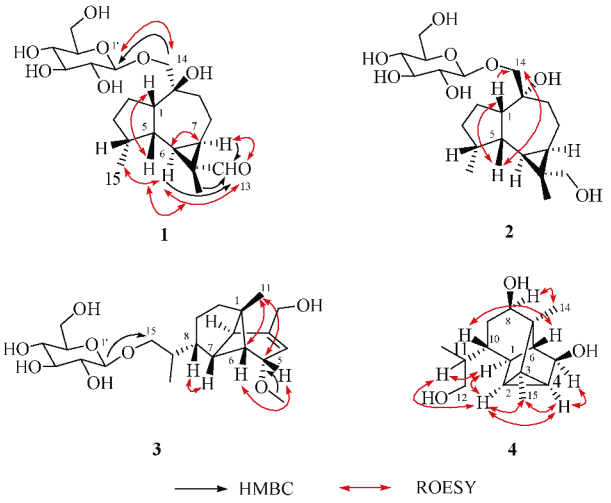

Fig. 2 Selected HMBC and ROESY correlations of compounds 1–4

Further analysis of ROESY correlation signals of H-6 and H-7, H-13, H-15; H-7 and H-13; H-5 and H-12; and H-1 and H-5, H-4 in the ROESY spectrum of **1** enabled establishment of the relative configure of aglycone of **1**. The glucose unit was further determined to connect to C-14 according to the ROESY correlation of H-14 and $H_{glc\text{-}1}$, and also on the HMBC correlation from H-14 to $C_{glc\text{-}1}$ (Fig. 2). Thus, the structure of **1** was assigned as 13-aldehyde-10*β*, 14-trihydroxyalloaromadendrane 14-*O*-*β*-D-glucopyranoside. Compound **1** is a new sesquiterpene glycoside and has been given the trival name dendronobiloside A.

Compound **2** had molecular formula $C_{21}H_{36}O_8$, as deduced from HRESI-MS $[M + Na]^+$ *m/z* 439.2302 (Calcd. for 439.2302) and NMR spectra (Tables 1 and 2), indicating four degrees of unsaturation. Its IR spectrum displayed the presence of hydroxyl group (3431 cm^{-1}). Enzymatic hydrolysis of **2** yielded glucose as its sugar component. In the ^{1}H NMR spectrum of **2**, one anomeric proton signal was found at δ_H 4.26 (1H, d, J = 7.8 Hz), so the glucose unit should link to the aglycone in the *β* configuration. And acid hydrolysis of **1** furnished glc, which was detected by TLC comparison with authentic sample. Comparing the NMR data (Tables 1 and 2) with those of dendroside C [9] indicated that the aglycone of two compounds had the same planar structure. The difference was the *β* configuration of C-14 in compound **2** instead of the *α* configuration in dendroside C [9], which was confirmed by the ROESY correlations from H-1 (δ_H 2.20, t) to H-5 (δ_H 1.68, m) and H-14 (δ_H 3.83, d; 3.50, d), and from H-5 to H-14 (Fig. 2). Accordingly, compound **2** was determined structurally as 10*α*, 13, 14-trihydroxyalloaromadendrane 14-*O*-*β*-D-glucopyranoside. Since this is a new sesquiterpene glycoside, it has been assigned the trival name dendrofindlayanoside B.

Compound **3** was isolated as colorless oil. The molecular formula, $C_{22}H_{36}O_8$, was established by HRESIMS $[M + Na]^+$ *m/z* 451.2301 (Calcd. for 451.2302). The presence of hydroxyl groups (3450 cm^{-1}) was deduced from the IR spectrum. In the ^{13}C NMR spectrum, 21 carbon signals including three methyls, five methylenes, eleven methines, and two quaternary carbons were observed (Table 2). On enzymatic hydrolysis of **3**, glucose was obtained as the only sugar component. The ^{1}H NMR spectrum of **3** revealed only one anomeric

proton signal at δ_H 4.21 (1H, d, J = 7.8 Hz) (Table 1). Therefore, the glucose unit was assigned in the β configuration. And acid hydrolysis of **1** furnished glc, which was detected by TLC comparison with authentic sample.

Comparing the NMR data (Tables 1 and 2) with those of philippinlin B [10] indicated the two compounds were similar, and the difference was the methoxy attaching to C-5 in compound **3** replaced the hydroxyl in philippinlin B [10], and the additional glucose moiety attached to C-15 (δ_C 74.2, t) in compound **3**, which was confirmed by the HMBC cross-peaks from H-OMe (δ_H 3.38, s) to C-5 (δ_C 80.9, d), and from H-1′(δ_H 4.21, d) to C-15 (Fig. 2). Thus, the 2D structure of **3** was defined.

The relative configurations of the six chiral centers at C-1, C-2, C-5, C-6, C-7 and C-8 in **3** were similar with those in philippinlin B [10], confirmed by the ROESY correlation signals of H-5 and H-6, H3-11; H-6 and H3-11; and H-7 and H-8 in the ROESY spectrum of **3** (Fig. 2). Assuming the β-orientation of H-5, both H-6 and H3-11 should be positioned on the β face. Thus, the relative structure of compound **3** was determined, named dendrofindlayanoside C.

Compound **4** was isolated as colorless oil. The molecular formula, $C_{15}H_{24}O_3$, was established by HRESI-MS $[M + Na]^+$ *m/z* 275.1615 (Calcd. for 275.1618). The absorption band at 3435 cm^{-1} in IR spectrum indicated the presence of hydroxyl groups. The ^{13}C NMR spectrum of **4** showed 15 carbon signals belonging to three methyls, two methylenes, eight methines and two quaternary carbons. A tetracyclic system skeleton was deduced to exist in the structure of **4** according to its unsaturation degree. Comparing the NMR data with those of dendrobane A [9] indicated that the two compounds have the same planar structure, which was supported by the ^1H-^1H COSY correlations of H-2/H-4, H-8/H-9/H-10, H-5/H-6/H-1, H-12/H-11/H-13, and was further established by the HMBC correlation signals at H_3-15/C-2, -4, -7; H-10/C-2, -6, -8, -13; C-3/H-1, -5, -6, -14; C-7/H-5, -9, -15 (Fig. 2).

Besides, the relative configuration of compound **4** was elucidated by a ROESY experiment and by comparison of the NMR data with those of dendrobane A [9]. The relative configuration was similar with that of dendrobane A [9], except for the β-orientation of H-6 in compound **4** instead of the α-orientation in dendrobane A [9], which was supported by the upfield shift of C-6 (δ_C 47.0, d) in compound **4**, and the disappearance of the ROSEY correlations of H-6 with H-8 and H-4. The relative configuration of compound **4** was further proved by the ROESY correlation signals between H-2 and H-1, H-4, H-11, H-15; H-4 and H-5, H-15; H-6 and H-10; H-1 and H-11, H-12; and H-8 and H-14 (Fig. 2). Thus, the relative structure of compound **4** was determined, named dendrofindlayanobilin A.

Five known sesquiterpenes including, 10β, 12, 14-tridroxyaromadendrane (**5**) [11], 10β, 13, 14-tridroxyaromadendrane (**6**) [12], dendroside A (**7**) [12], dendronobilin I (**8**) [13] and dendronobilin N (**9**) [14] were identified by comparison of their NMR data with those in the literatures. The immunomodulatory bioassay *in vitro* of isolates was evaluated, but all compounds were inactive. Compound **3**, one ylangene-derived sesquiterpene, was reported for the first time from the genus *Dendrobium*. From the results of our experiment, it could be dedicated that just like *D. nobile*, the main compositions of *D. findlayanum* are sesquiterpenes or its alkaloids.

Experimental

General

Optical rotations were obtained on a Jasco P-1020 digital polarimeter (Horiba, Tokyo, Japan). UV spectra were taken on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were obtained on a Bruker Tensor 27 infrared spectrophotometer (Bruker Optics GmbH, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on Bruker AM-400, DRX-500 or Av III-600 instruments with TMS as the internal standard (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ (ppm) scale with reference to the solvent signal. Mass spectra were recorded on an API QSTAR time-of-flight spectrometer (MDS Sciqaszex, Concord, Ontario, Canada) or LCMS-IT-TOF (Shimadzu, Kyoto, Japan) spectrometer. Semi-preparative HPLC was performed on Agilent 1100 liquid chromatography with a ZORBAX SB-C_{18} (5 μm, 9.4 mm × 250 mm) column (Agilent, USA) at a flow rate of 3.0 mL·min^{-1}. Column chromatography was performed on silica gel (200−300 mesh, Qingdao Marine Chemical Inc., Qingdao, China). Lichroprep RP-18 gel (40−63 μm, Merck, Darmstadt, Germany), MCI gel CHP-20P (75−150 μm, Mitsubishi Chemical Corp., Tokyo, Japan), Sephadex LH-20 (20−150 μm, Amersham Biosciences, Uppsala, Sweden). Fractions were monitored by TLC, and spots were visualized by UV light (254 nm) and sprayed with 5% H_2SO_4 in ethanol, followed by heating.

Plant material

The stems of *Dendrobium findlayanum* Par. et Rchb. f. were collected in April 2013 from the Wenshan County, Yunnan Province, China, and identified by Prof. YU Hong, Yunnan University, Yunnan Province, China. A voucher specimen (KIB Zsh-13) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The dried and powdered stems of *D. findlayanum* (5.0 kg) were extracted with 95% aqueous EtOH (4 × 15 L, 2 days each) at room temperature and filtered. The filtrate was evaporated under reduced pressure and further fractionated between EtOAc and H_2O and between *n*-BuOH and H_2O, successively.

The *n*-BuOH part (100 g) was chromatographed on Si CC (1000 g), eluted with $CHCl_3$−MeOH (95 : 5, 90 : 10, 80 : 20, 60 : 40), to afford four sub-fractions (A−D). Fraction A (15 g) was separated by repeated Si CC ($CHCl_3$−MeOH, 60 : 1, 40 : 1, 20 : 1) and Sephadex LH-20 column (MeOH) to yield compounds **4** (10 mg) and **8** (4 mg). Fraction B (8 g)

was performed on a MCI CHP-20P gel CC (160 g) and eluted with MeOH−H_2O (10 : 90, 30 : 70, 60 : 40, 80 : 20) to give four fractions (B1−B3). Fraction B2 (0.5 g) was performed on Si CC (5 g), eluted with $CHCl_3$−MeOH (29 : 1), and further purified on a Sephadex LH-20 column ($CHCl_3$−MeOH, 1 : 1) to yield compounds **5** (6 mg), **6** (5 mg) and **9** (5 mg).

Fraction C (18 g) was loaded on a RP-18 column (MeOH−H_2O gradient system: 10%−80%) to obtain fractions C1−C4. After repeated CC on silica gel (200−300 mesh), eluting with $CHCl_3$−MeOH (gradient system: 29 : 1−5 : 1), compounds **1** (5 mg), **2** (6 mg) and **7** (8 mg) precipitated from fraction C1. Fraction C2 (2 g) was performed on a MCI CHP-20P gel CC (40 g) and eluted with MeOH−H_2O (10 : 90, 30 : 70, 60 : 40, 80 : 20) to afford three sub-fractions (C2-1− C2-3). Fraction C2-2 was performed on Si CC, eluted with $CHCl_3$−MeOH (9 : 1), and further purified by preparative HPLC MeOH−H_2O (60 : 40) to afford compound **3** (4 mg, t_R = 56 min).

Spectroscopic data

Dendrofindlayanoside A: White amorphous powder, $C_{21}H_{34}O_8$; $[\alpha]_D^{20}$ −16.7 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 205 (2.01) nm; IR (KBr) ν_{max} 3426, 2924, 2872, 1684, 1631, 1451, 1419, 1384, 1078, 595, 473 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESI-MS: *m/z* 437 $[M + Na]^+$; positive-ion HRESI-MS $[M + Na]^+$ *m/z* 437.2135 (Calcd. for 437.2146).

Dendrofindlayanoside B: White amorphous powder, $C_{21}H_{36}O_8$; $[\alpha]_D^{20}$ −22.8 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.06) nm; IR (KBr) ν_{max} 3431, 2926, 2872, 1631, 1384, 1287, 1078, 1044, 879, 712, 581, 471 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESI-MS: *m/z* 439 $[M + Na]^+$; positive-ion HRESI-MS $[M + Na]^+$ *m/z* 439.2302 (Calcd. for 439.2302).

Dendrofindlayanoside C: White amorphous powder, $C_{22}H_{36}O_8$; $[\alpha]_D^{21}$ −28.2 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 203.8 (1.97) nm; IR (KBr) ν_{max} 3450, 2920, 2850, 1640, 1384, 1079, 470 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESI-MS *m/z* 451 $[M + Na]^+$; positive-ion HRESI-MS $[M + Na]^+$ *m/z* 451.2301 (Calcd. for 451.2302).

Table 1 ^1H NMR spectroscopic data (methanol-d_4, 600 MHz) for 1–4 (δ in ppm, *J* in Hz)

Position	1	2	3	4
1	2.03 (m)	2.20 (t, 4.8)		1.78 (br s)
2	1.73 (m) 1.61 (m)	1.79 (m) 1.85 (m)	1.71 (d, 7.7)	0.86 (d, 5.4)
3	1.89 (m) 1.29 (m)	1.31 (m) 1.79 (m)		
4	2.05 (m)	2.04 (m)	5.61 (s)	1.06 (m)
5	2.05 (m)	1.68 (m)	3.96 (m)	3.76 (br s)
6	1.18 (m)	0.42 (t, 9.0)	2.39 (t, 3.0)	1.65 (br s)
7	1.70 (m)	0.88−0.92 (m)	1.93 (m)	
8	1.67−1.71 (m)	1.06 (m) 1.85 (m)	1.95 (m)	3.64 (br s)
9	1.84 (m) 1.78 (m)	1.83 (m)	1.69 (m)	1.56 (dd, 10.2, 3.0)
10			1.81 (m) 1.65 (m)	1.67 (d, 8.4)
11			0.83 (s)	1.44 (m)
12	1.22 (s)	1.07 (s)	3.96 (m) 3.91 (m)	3.57 (dd, 10.8, 4.2) 3.50 (dd, 10.8, 7.2)
13	8.68 (s)	3.53 (d, 10.8) 3.07 (d, 11.4)	1.72 (m)	0.92 (d, 6.6)
14	3.81 (m) 3.72 (m)	3.83 (d, 10.4) 3.50 (d, 10.4)	0.94 (d, 7.2)	1.27 (s)
15	0.85 (d, 6.6)	0.96 (d, 7.2)	3.76 (d, 7.2) 3.52 (d, 7.2)	1.08 (s)
1'	4.24 (d, 7.8)	4.26 (d, 7.8)	4.20 (d, 7.8)	
2'	3.22 (m)	3.15−3.22 (m)	3.14 (m)	
3'	3.27 (m)	3.26 (m)	3.33 (m)	
4'	3.27 (m)	3.27 (m)	3.27 (m)	
5'	3.35 (m)	3.34 (m)	3.23 (m)	
6'	3.87 (dd, 12.0, 1.8) 3.66 (dd, 12.0, 6.0)	3.86 (dd, 12.0, 1.8) 3.66 (dd, 12.0, 6.0)	3.83 (dd, 12.0, 1.8) 3.66 (dd, 12.0, 6.0)	
16			3.38(s)	

Table 2 ^{13}C NMR spectroscopic data (methanol-d_4, 150 MHz) for 1–4 (δ in ppm)

Position	1	2	3	4
1	54.2, CH	51.6, CH	49.1, C	42.5, CH
2	25.4, CH_2	24.9, CH_2	52.1, CH	22.2, CH
3	30.1, CH_2	30.3, CH_2	152.7, C	26.9, C
4	39.5, CH	40.3, CH	116.7, CH_2	27.5, CH
5	39.6, CH	40.4, CH	80.9, CH	79.4, CH
6	24.9, CH	20.4, CH	41.5, CH	47.0, CH
7	29.8, CH	25.3, CH	42.1, CH	49.7, C
8	17.9, CH_2	20.2, CH_2	40.9, CH	75.5, CH
9	31.7, CH_2	34.4, CH_2	23.5, CH_2	32.5, CH_2
10	76.4, C	76.8, C	37.5, CH_2	34.3, CH
11	37.6, C	25.7, C	19.5, CH_3	39.8, CH
12	8.2, CH_3	12.1, CH_3	65.5, CH_2	66.5, CH_2
13	205.7, CH	73.2, CH_2	38.8, CH	15.3, CH_3
14	79.3, CH_2	78.2, CH_2	14.1, CH_3	18.6, CH_3
15	16.3, CH_3	16.7, CH_3	74.2, CH_2	10.5, CH_3
1'	105.2, CH	105.2, CH	104.4, CH	
2'	75.2, CH	75.1, CH	75.0, CH	
3'	77.9, CH	77.9, CH	78.0, CH	
4'	71.6, CH	71.6, CH	71.6, CH	
5'	78.0, CH	78.0, CH	78.2, CH	
6'	62.7, CH_2	62.7, CH_2	62.7, CH_2	
16			56.6, CH_3	

Dendrofindlayanobilin A: colorless oil, $C_{15}H_{24}O_3$; $[\alpha]_D^{21}$ +0.50 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 201.6 (1.58) nm; IR (KBr) ν_{max} 3435, 2925, 2875, 1733, 1632, 1454, 1382, 1082, 1037, 565 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive-ion ESI-MS *m/z* 275 $[M + Na]^+$; positive-ion HRESI-MS $[M + Na]^+$ *m/z* 275.1615 (Calcd. for 275.1618).

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Appendix A. Supplementary data

1D and 2D NMR, HRESIMS, IR, UV and $[\alpha]_D$ spectra of compounds **1**−**4** are available as Supporting Information, which can be requested by sending E-mails to the corresponding author.

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