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Two unusual dendrobine-type alkaloids from *Dendrobium findlayanum*

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ABSTRACT

Two unusual dendrobine-type alkaloids, findlayines E and F (**1**, **2**), along with five known dendrobine-type alkaloids (**3**–**7**), were isolated from the stems of *Dendrobium findlayanum* Par. et Rchb. f. Compound **1** is the first example of dendrobine-type alkaloids with a 2-ethoxy-2-oxoethyl group attaching to the C-2, and compound **2** is a nor-dendrobine-type alkaloid, featuring a 5-decarboxylated structure. The structures of compounds **1** and **2** were elucidated by means of extensive spectroscopic analyses, and their absolute configuration were confirmed by electronic circular dichroism (ECD) calculations. All isolates were evaluated for their cytotoxicity against HL-60, SMMC-7721, A-549 and MCF-7 human cancer cells.

1. Introduction

Dendrobium is a large and diverse genus of Orchidaceae with approximately 1500 species distributed throughout the world. There are more than 80 *Dendrobium* species in China, and the fresh or dried stems of many species have been used both as medicine for the treatment of chronic atrophic gastritis, diabetes, fever, and skin aging diseases, as well as a high-quality health food now. The main chemical components of *Dendrobium* are alkaloids, phenolics, and polysaccharides with anti-inflammatory, antitumor, and antioxidant effects [1–4].

The plant *D. findlayanum* Par. et Rchb. f. is a perennial herb belonging to the genus *Dendrobium* and mainly distributed in southern China. Flower of the plant is similar with that of *D. nobile* and stem of the plant was usually used as one of the common source of Shihu for the usage of nourishing the stomach, promoting secretion of saliva and reducing fever. Previous phytochemical studies of this plant resulted in the isolation of sesquiterpenes, phenolics and dendrobine-type alkaloids [5,6], of which dendrobine-type alkaloids are the characteristic chemical components of *Dendrobium* and their distinct structures of which have attracted the attention of several research groups to get these alkaloids synthetically [7–10].

To enrich the dendrobine-type alkaloids of *Dendrobium* and seek structurally interesting dendrobine-type alkaloids, we investigated the chemical constituents of the stems of *D. findlayanum* and have found a series of new dendrobine-type alkaloids and bioactive phenolics [11]. Further examination of its stems herein led to isolation of two unusual dendrobine-type alkaloids, findlayines E and F (**1**, **2**), along with five known dendrobine-type alkaloids including dendrofindline A (**3**) [12], 6-hydroxy-dendroxine (**4**) [5], nobiline (**5**) [13], dihydronobilonine (**6**) [14] and mubironine B (**7**) [15]. Compound **1** is the first example of dendrobine-type alkaloids with a 2-ethoxy-2-oxoethyl group attaching to the C-2, and compound **2** is a nor-dendrobine-type alkaloid, featuring a 5-decarboxylated structure. The structures of compounds **1** and **2** were elucidated by means of extensive spectroscopic analyses, and their absolute configurations were confirmed by electronic circular dichroism (ECD) calculations. All isolates were evaluated cytotoxicity activities against human cancer cell lines of HL-60, SMMC-7721, A-549, and MCF-7 by the MTS method [16], but no activity was noted. In this paper, the isolation, structure elucidation and cytotoxicity evaluation are reported.

2. Experimental

2.1. 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). CD spectra were recorded with an Applied Photophysics Chirascan spectrometer (Agilent, America). IR spectra were obtained on a Bruker Tensor 27

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infrared spectrophotometer (Bruker Optics GmbH, Ettlingen, Germany) with KBr pellets. NMR spectra were recorded on Bruker 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 × 250 mm) column (Agilent, USA) at a flow rate of 3.0 mL/min. 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 (210 and 254 nm) and sprayed with 5% H_2SO_4 in ethanol, followed by heating.

2.2. Plant material

The stems of *D. findlayanum* (cultivated) were collected in April 2013 from Wenshan county, Yunnan Province, PR China, and identified by Prof. Hong Yu. A voucher specimen (No. KIBZsh-13) was deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The sun-dried and powdered stems of *D. findlayanum* (5 kg) were extracted with 95% ethanol (15 L × 4, 2 days each time) 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 EtOAc fraction (150 g) was subjected to silica gel column chromatography (Si CC, 1500 g), eluting with $CHCl_3$-MeOH (100:0, 69:1, 29:1, 9:1, 4:1, *v/v*), to afford five fractions A–E.

Fraction C (21 g) was loaded on a RP-18 column (MeOH-H_2O, 30:70, 50:50, 70:30, 90:10) to give four sub-fractions C1 − C4. Fraction C2 (350 mg) was purified by chromatography over silica gel CC (3.5 g) using petroleum ether-acetone (9:1) as the eluent and followed by Sephadex-LH-20 column ($CHCl_3$-MeOH, 1:1) to give compound **1** (25 mg). Fraction C3 (500 mg) was purified by chromatography over silica gel CC (5 g) and eluted with petroleum ether-acetone (gradient system: 12:1–1:1), and then further purified by preparative HPLC (MeOH-H_2O, 39:61) to afford compound **2** (5 mg, t_R = 47 min).

Fraction D (15 g) was separated on silica gel CC (150 g) and eluted with petroleum ether-acetone (5:1) to obtain sub-fractions D1–D4. Fraction D2 (600 mg) was separated on an RP-18 column (MeOH-H_2O gradient system: 20–90%) to give five sub-fractions D2–1 − D2–5. After repeated CC on silica gel, eluting with $CHCl_3$–MeOH (gradient system: 30:1–1:1), compounds **3** (6 mg) and **4** (7 mg) precipitated from fraction D2–2. Compound **5** (10 mg) was obtained from fraction D3 (200 mg) by silica gel CC (2 g) and eluted with $CHCl_3$-MeOH (9:1). Fraction D4 (350 mg) was purified on a Sephadex LH-20 column (MeOH) to yield compounds **6** (8 mg) and **7** (15 mg).

2.4. Spectroscopic data

Findlayine E **(1)**: colorless oil; $[\alpha]_D^{23}$ –45.7 (*c* 0.35, MeOH); UV (MeOH) λ_{max} (log ε) 246 (3.33), 194 (1.79) nm; ECD (c 0.70, MeOH) λ (Δε) 239 (+4.38), 270(−1.30); IR (KBr) ν_{max} 3437, 2957, 2873, 1730, 1679, 1632, 1460, 1382, 1271, 1235, 1189, 1080, 1029 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive-ion ESIMS: *m/z* 364 $[M + H]^+$; positive-ion HRESIMS: $[M + H]^+$ *m/z* 364.2123 (calcd for $C_{20}H_{30}NO_5$ 364.2118).

Findlayine F **(2)**: colorless oil; $[\alpha]_D^{25}$ − 53.2 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 268 (3.70), 203 (1.96) nm; ECD (c 0.26, MeOH) λ (Δε) 204 (+14.99), 270 (−4.22); IR (KBr) ν_{max} 3437, 2925, 2854, 1633, 1427, 1093 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; positive-ion ESIMS: *m/z* 256 $[M + Na]^+$; positive-ion HRESIMS: $[M + Na]^+$ *m/z* 256.1313 (calcd for 256.1308).

Table 1
^1H (600 MHz) and ^{13}C (150 MHz) NMR data of compounds **1** and **2** in $CDCl_3$, δ in ppm, *J* in Hz.

Position	1		2	
	δ_H	δ_C, Type	δ_H	δ_C, Type
1		55.1 (s)		71.8 (s)
2		72.1 (s)		174.7 (s)
3		203.1 (s)		185.2 (s)
4		143.1 (s)		146.2 (s)
5		145.4 (s)	6.68 (s)	146.9 (d)
6	2.58 (m)	49.1 (d)		82.8 (s)
7	2.13 (m)	36.7 (t)	2.04 (m)	41.2 (t)
	1.52 (m)		1.72 (m)	
8	1.80 (m)	31.1 (t)	2.29 (m)	32.2 (t)
	1.37 (m)		1.60 (m)	
9	2.32 (m)	54.7 (d)	2.55 (t, 8.4)	46.7 (d)
10	1.24 (s)	25.9 (q)	1.25 (s)	15.2 (q)
11	3.14 (m)	50.3 (t)	4.31 (dd, 18.0, 7.2)	69.6 (t)
	2.35 (m)		4.02 (dd, 18.0, 1.2)	
12	2.82 (m)	31.9 (d)	2.97 (m)	26.0 (d)
13	1.06 (d, 6.6)	20.6 (q)	1.05 (d, 6.6)	21.5 (q)
14	1.22 (d, 6.6)	21.9 (q)	1.10 (d, 6.6)	21.9 (q)
15		170.1(s)		
16	3.83 (s)	52.6 (q)		
17	2.62 (s)	38.8 (t)		
18		172.3 (s)		
19	4.09 (m)	61.7 (t)		
20	1.24 (m)	14.4 (q)		

2.5. The cytotoxicity assay

The human tumor cell lines HL-60, SMMC-7721, A-549, and MCF-7 were used, which were obtained from ATCC (Manassas, VA, USA). All the cells were cultured in RPMI-1640 or DMEM (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium (MTS) (Sigma, St. Louis, MO, USA) [17]. Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 1 × 10^5 cells/mL in 100 μL medium. Each tumor cell line was exposed to the test compound at a concentration of 25 μM in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After the incubation, MTS (100 μg) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were lysed with 100 μL of 20% SDS–50% DMF after removal of 100 μL medium. The optical density of the lysate was measured at 490 nm in a 96-well microtiter plate reader (Bio-Rad 680).

3. Results and discussion

Compound **1** had molecular formula $C_{20}H_{29}NO_5$, as deduced from HRESIMS $[M + H]^+$ *m/z* 364.2123 (calcd. for 364.2118) and NMR spectra (Table 1), indicating seven degrees of unsaturation. Its IR spectrum showed strong absorption bonds at 1730 and 1679 cm^{-1}, indicated the existence of α,β-unsaturated carbonyl groups.

The ^{13}C NMR and DEPT spectra (Table 1) exhibited 20 carbon signals, including five methyls (one O-CH_3), five methylenes, three

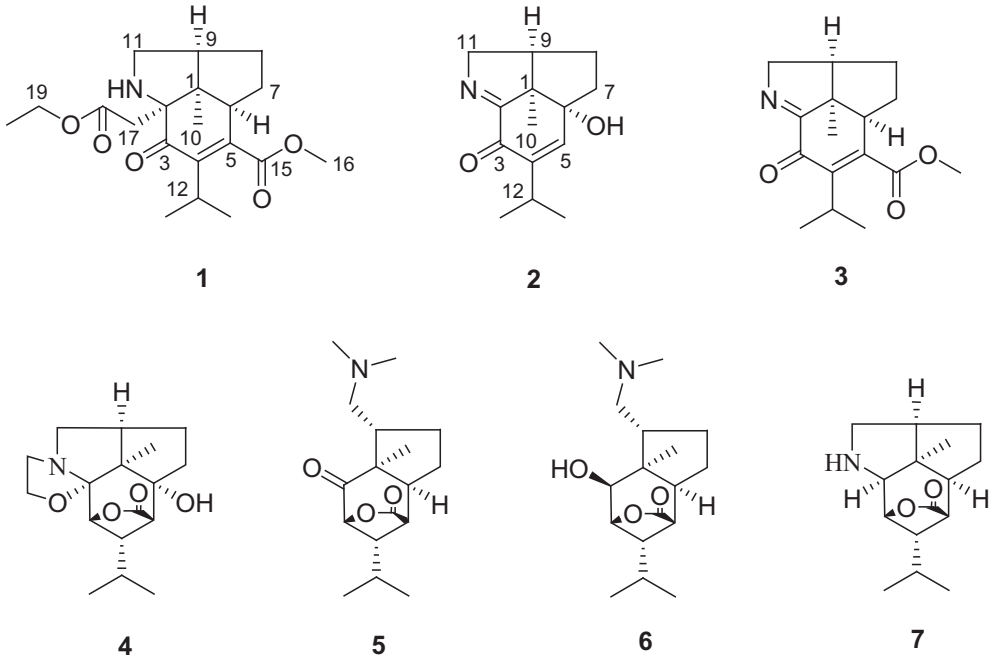

Fig. 1. Chemical structures of the compounds **1–7**.

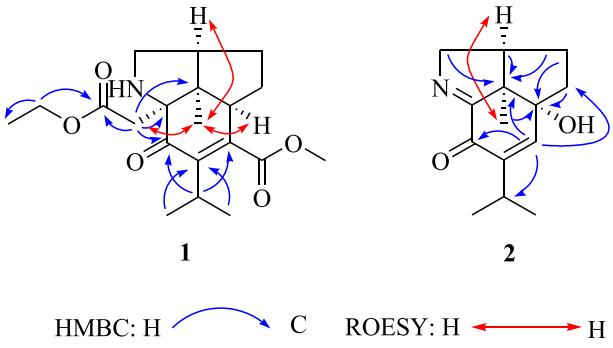

Fig. 2. Selected HMBC and ROESY correlations of compounds **1**, **2**.

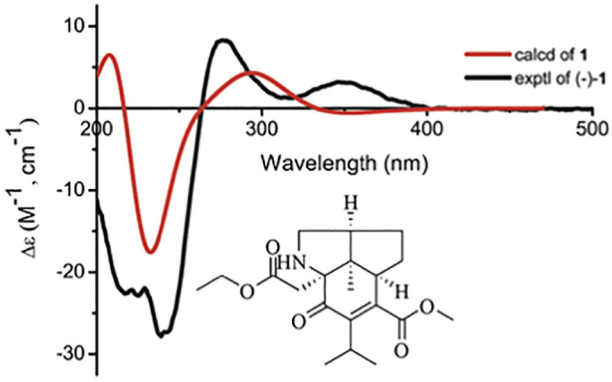

Fig. 3. Calculated and experimental ECD spectra of compound **1**.

methines, four quaternary carbons, and three carbonyl carbons. One carbonyl carbon (δ_C 203.1) and two quaternary olefinic carbons (δ_C 145.4, 143.1) in the ^{13}C NMR spectrum of **1** established an *α,β*-unsaturated carbonyl moiety (C-3-C-4-C-5), which was assigned by the HMBC correlations from H_2–17 (δ_H 2.62, s) and H-12 (δ_H 2.82, m) to C-3 (δ_C 203.1), H_3–13 (δ_H 1.06, d, J = 6.6 Hz) and H_3–14 (δ_H 1.22, d, J = 6.6 Hz) to C-4 (δ_C 143.1), and from H-12 and H-7 (δ_H 2.13, 1.52, m) to C-5 (δ_C 145.4). On the basis of the NMR data (Table 1), compound **1** was suggested to be a dendrobine-type alkaloid, which was supported by the HMBC correlations from H_2–7, H_2–8 (δ_H 1.80, 1.37, m), H_3–10 (δ_H 1.24, s) and H_2–11 (δ_H 3.14, 2.35, m) to C-1 (δ_C 55.1).

It could be inferred that the structure of **1** was similar with that of **3** [12], both as oxidized and substituted dendrobine-type alkaloid except for the 2-ethoxy-2-oxoethyl attaching to C-2 in **1**, replacing the imine at C-2 in **3**. It could be further confirmed by the molecular formula of $C_{20}H_{29}NO_5$, and the HMBC correlations from H_2–17 (δ_H 2.62, s) to C-1 (δ_C 55.1, s), C-2 (δ_C 72.1, s), C-3 (δ_C 203.1, s) and C-18 (δ_C 172.3, s), and from H_2–19 (δ_H 4.09, m) to C-18 and C-20 (δ_C 14.4, q), and the highfield shift of C-2 in **1**.

From the ROESY correlations of H_3-10/H-6, H_3-10/H-9, and H_3-10/H_2-17, it could be inferred that H_3-10, H-6, H-9, and H_2-17 of **1** were cofacial (Fig. 2), and the relative configuration of **1** was identical to that of findlayine D [10]. A dendrobine-type alkaloid with a 2-ethoxy-2-oxoethyl group attaching to C-2 is quite unusual, thus, electronic circular dichroism (ECD) calculations were used to assign the absolute configuration of compound **1** as (1*R*,2*S*,6*R*,9*S*) (Fig. 3), named as findlayine E.

Compound **2** was obtained as colorless oil with a molecular formula of $C_{14}H_{19}NO_2$, with 6 indices of hydrogen deficiency, based on the ion peak at *m/z* 256.1313 ($[M + Na]^+$, calcd. for 256.1308) in HRESIMS. The ^{13}C NMR and DEPT spectra (Table 1) of **2** contained 14 carbon resonances attributing to three methyls, three methylenes, three methines, and five quaternary carbons. The HMBC correlations from H-5 (δ_H 6.68, s) to C-1 (δ_C 71.8), C-3 (δ_C 185.2), C-7 (δ_C 41.2) and C-12 (δ_C 26.0), and from H_2-7 (δ_H 2.04, 1.72, m), H_2-8 (δ_H 2.29, 1.60, m), H_3-10 (δ_H 1.25, s) and H-11 (δ_H 4.01, dd, J = 18.0, 1.2 Hz) to C-1 indicated compound **2** was a dendrobine-type alkaloid.

On the basis of its NMR data (Table 1), it could be seen that the structure of **2** was similar with that of **3** except the key carbonyl carbon signal [12]. The difference between **2** and **3** is the hydroxyl attaching to C-6 (δ_C 82.8) in **2** but not the hydrogen [12], and **2** is a nor-dendrobine-type alkaloid, featuring a 5-decarboxylated structure, which can be confirmed by the HMBC correlation from H_2-7, H_2-8, H_3-10/C-6 (Figure 2), and the downfield shift of C-6 in **2**. Thus, the 2D structure of **2** was defined (Fig. 1).

In the ROESY spectrum, the cross-peaks of H_3-10 and H-9 were

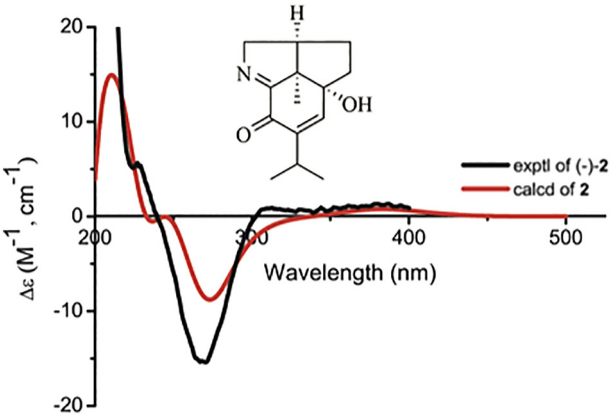

Fig. 4. Calculated and experimental ECD spectra of compound **2**.

observed (Fig. 2) and the interaction between H_3-10 and any of H_2-7 was absent (Fig. 2), assigning the alpha-orientation of 6-OH in **2**. Thus, compound **2** could be determined as an unusual nor-dendrobine-type alkaloid, featuring a 5-decarboxylated structure and then the absolute configuration was further defined as (1*R*,6*R*,9*S*) by electronic circular dichroism (ECD) calculations (Fig. 4), named findlayine F (**2**).

Dendrofindline A (**3**), 6-hydroxy-dendroxine (**4**), nobiline (**5**), dihydronobilonine (**6**) and mubironine B (**7**) were identified by comparison of their NMR data with those in the literatures.

Considering the EtOAc fraction of *D. findlayanum* in our previous study exhibited cytotoxic activity against the HL-60, SMMC-7721, A-549, and MCF-7 cell lines, with respective IC_{50} values of 49.4, 61.3, 96.8, and 90.1 μg/mL [11]. And combination treatment of dendrobine with cisplatin showed enhanced cytotoxicity and the induction of apoptosis involving pro-apoptotic proteins Bax and Bim [17]. Thus, all isolates were evaluated for their in vitro cytotoxicity against four human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7) according to a previously described procedure [16]. However no obviously cytotoxic activity was observed at the concentration of 40 μM. The main chemical components of *D. findlayanum* are dendrobine-type alkaloids and phenolics, being similar with those of *D. noble*, which provide some basis for the use of *D. findlayanum* as a substitute for *D. noble*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104607.

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