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1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one, a new cytotoxic compound from *Dendrobium chrysotoxum*

Yegao Chen [a,*], Yupeng Li [a], Chen Qing [b,*], Yanli Zhang [b], Liqin Wang [a], Ying Liu [a]

[a] *Department of Chemistry, Yunnan Normal University, Kunming 650092, China*
[b] *Yunnan Key Laboratory of Pharmacology for Natural Products Research, Kunming Medical College, Kunming 650031, China*

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Abstract

A new compound, 1,4,5-trihydroxy-7-methoxy-9*H*-fluoren-9-one, has been isolated together with two known fluorenones, dendroflorin and denchrysan A, from the whole plant of *Dendrobium chrysotoxum*, a plant of *Dendrobium* genus, used as a health-food. The structure of the fluorenones has been determined on the basis of spectroscopic studies. The isolated compounds were evaluated *in vitro* for their inhibitory ability against the growth of human leukaemia cell lines K562 and HL-60, human lung adenocarcinoma A549, human hepatoma BEL-7402 and human stomach cancer SGC-7901. All three fluorenones displayed selective cytotoxicity against BEL-7402 with IC_{50} values of 1.49, 0.97 and 1.38 μg/ml, respectively.
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Keywords: *Dendrobium chrysotoxum*; Orchidaceae; Fluorenones; 1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one; Cytotoxicity

1. Introduction

"Shi-hu", an important Chinese herb prepared from the dried stems of *Dendrobium* species (Orchidaceae) is used as a health-food (Bao, Shun, & Chen, 2001). *Dendrobium chrysotoxum* Lindl is distributed in India, Nepal, Thailand, Laos, Vietnam and Yunnan in south-western China (Delectis Florae Reipublicae Popularis Sinicae Agendae, Academiae Sinicae Edita, 1999). 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 (Gong et al., 2006; Ma, Wang, Xu, & Xu, 1998; Ma, Xu, Xu, Wang, & Kickuchi, 1996; Ma, Xu, Xu, Wang, & Kickuchi, 1994; Yang et al., 2004; Yang, Chou, Wang, Hu, & Xu, 2004; Yang et al., 2001; Yang, Wang, Xu, & Hu, 2002). An EtOH extract and some of its compounds, such as erianin, chrysotoxine and confusarin were found to possess antitumour activity (Gong et al., 2004a; Gong et al., 2004b; Li, Wang, & Liu, 2001; Ma, Xu, & Xu, 1994; Ng, Liu, & Wang, 2000; Wang et al., 1997). In the course of our search for new bioactive natural products from medicinal plants in Yunnan, China, we investigated the plant and isolated a new fluorenone together with the known dendroflorin and denchrysan A (see Fig. 1). The isolation, structure elucidation and evaluation for cytotoxic activity of these three compounds are reported herein.

2. Materials and methods

2.1. General methods

MS were determined on an API Qstar Pulsar LC/TOF mass spectrometer (Applied Biosystems, Foster City, CA). NMR spectra were measured on a Bruker DRX-500 spectrometer with TMS as internal standard. Silica gel (200–300 mesh, Qingdao Marine Chemical Co., Qingdao City, China) and Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemical Co. Ltd., Uppsala, Sweden)

* Corresponding authors. Tel.: +86 8715516063; fax: +86 8715516061.
E-mail addresses: ygchen48@gmail.com (Y. Chen), qingchenhh@yeah.net (C. Qing).

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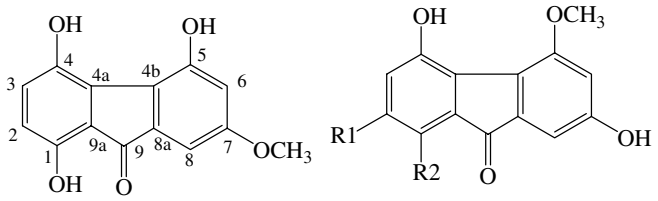

Fig. 1. Structures of constituents isolated from *D. chrysotoxum*.

were used for column chromatography and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co.). Solvents were of industrial purity and distilled prior to use. MTT [3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide] was purchased from Sigma–Aldrich (St. Louis, MO).

2.2. Plant material

The whole plant of *D. chrysotoxum* was collected from Simao County of Yunnan Province, China in February, 2005 and identified by Dr. Hong Yu, School of Life Science, Yunnan University, where a voucher specimen (No. 0502014) is deposited.

2.3. Extraction and isolation

The dried powdered whole plant of *D. chrysotoxum* (0.5 kg) was extracted with 95% ethanol (five volumes, each 5 l) at room temperature. The ethanol extract was evaporated *in vacuo*, to yield a dark brown residue (22 g, 4.40%), and directly applied to a silica gel column, eluting with petroleum ether containing increasing amounts of ethyl acetate to obtain 5 fractions. Fr. 2 (5 g) was subjected to repeated column chromatography, first on silica gel (petroleum ether:ethyl acetate, 4:1) and then on Sephadex LH-20 (methanol:water, 9:1) to afford **1** (8 mg, 0.0016%). Fr. 3 (5 g) was chromatographed over silica gel (chloroform:acetone, 20:1), and then purified by chromatography over Sephadex LH-20 (methanol:water, 9:1) to furnish **2** (5 mg, 0.001%) and **3** (6 mg, 0.0012%).

1,4,5-Trihydroxy-7-methoxy-9*H*-fluoren-9-one (**1**) was obtained as a reddish powder. EI-MS: *m/z* (%) 258 (M^+, 100), 243 (99), 215 (20), 187 (9), 129 (5); ^{1}H NMR (DMSO-d_6, 500 MHz): δ 8.62 (1H, s, 4-OH), 6.82 (1H, d, $J = 8.9$, H-3), 6.67 (1H, s, H-6), 6.65 (1H, s, H-8), 6.60 (1H, d, $J = 8.9$, H-2), 3.99 (3H, s, 7-OCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 191.4 (C-9), 160.2 (C-5), 152.9 (C-7), 151.4 (C-1), 143.3 (C-4), 136.8 (C-8a), 127.6 (C-3), 125.2 (C-4a), 120.1 (C-4b), 119.9 (C-2), 117.3 (C-9a), 105.3 (C-6), 104.7 (C-8), 57.4 (7-OCH_3); HRESI-MS: *m/z* 259.0630 $[M+H]^+$, molecular weight of $C_{14}H_{11}O_5$ is 259.0606.

Dendroflorin (**2**) was obtained as a reddish powder. EI-MS: *m/z* 258 (M^+), 243, 215, 187, 129; ^{1}H NMR [$(CD_3)_2CO$, 500 MHz]: δ 6.86 (1H, d, $J = 8.8$, H-3), 6.60 (1H, d, $J = 8.8$, H-2), 6.74 (1H, s, H-6), 6.78 (1H, s, H-8), 4.10 (3H, s, 5-OCH_3); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz]: δ 194.0 (C-9), 160.0 (C-7), 152.8 (C-5), 151.6 (C-1), 143.8 (C-4), 136.1 (C-8a), 128.3 (C-3), 123.1 (C-4a), 121.0 (C-4b), 118.4 (C-2), 116.2 (C-9a), 104.8 (C-6), 104.3 (C-8), 56.1 (5-OCH_3).

Denchrysan A (**3**) was obtained as a reddish powder. EI-MS: *m/z* 258 (M^+), 243, 215; ^{1}H NMR [$(CD_3)_2CO$, 500 MHz]: δ 6.77 (1H, d, $J = 1.6$, H-1), 6.75 (1H, d, $J = 1.6$, H-3), 6.65 (1H, d, $J = 2.2$, H-8), 6.40 (1H, d, $J = 2.2$, H-6), 4.10 (3H, s, 5-OCH_3); ^{13}C NMR [$(CD_3)_2CO$, 125 MHz]: δ 193.8 (C-9), 160.5 (C-7), 160.2 (C-2), 153.0 (C-5), 153.0 (C-4), 137.5 (C-8a), 137.5 (C-9a), 124.1 (C-4b), 120.4 (C-4a), 110.4 (C-3), 104.8 (C-6), 106.8 (C-6), 106.5 (C-8), 105.8 (C-1), 57.9 (5-OCH_3).

2.4. Cell growth inhibition assay

Growth inhibition of sample on tumour cells was measured by microculture tetrazolium (MTT) assay, with minor modification (Alley et al., 1988; Mosmman, 1983; Zhou, Yue, Han, & Yang, 1993). Briefly, adherent cells were seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition, while suspended cells were seeded just before drug addition. The cell densities were selected, based on the results of preliminary tests, to maintain the control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the optical density and the number of viable cells. Each tumour cell line was exposed to sample at 0.01, 0.1, 1.0, 10 and 100 μM concentrations for different periods (adherent cells 72 h, suspended cells 48 h) and each concentration was tested in triplicate. At the end of exposure, 20 μl of 5 g/l MTT was added to each well and the plates were incubated for 4 h at 37 °C, then triplex solution (10% SDS/5% isobutanol/0.012 M HCl) was added and the plates were incubated for 12–20 h at 37 °C. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media and DMSO control wells, in which sample was absent, were included in all the experiments, in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula:

$$\text{Growth inhibition } (\%) = (\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}/\text{OD}_{\text{control}}) \times 100\%$$

The cytotoxicity of samples on tumour cells were expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells), which were calculated by LOGIT method.

3. Results and discussion

3.1. Phytochemical investigation

The 95% ethanol extract of *D. chrysotoxum* was subjected to a succession of chromatographic procedures,

including silica gel chromatography and gel permeation chromatography using Sephadex LH-20 to afford 3 isolates.

Compound **1** had the molecular formula $C_{14}H_{10}O_5$ by HRESI-MS (high resolution electrospray ionisation mass spectrometry) at *m/z* 259.0630 $[M+H]^+$(calcd for $C_{14}H_{11}O_5$, 259.0606). The ^1H NMR spectrum showed resonances for a 1,2,3,4-tetrasubstituted benzene moiety [δ 6.82 (1H, d, $J = 8.9$, H-3), 6.60 (1H, d, $J = 8.9$, H-2), a 1,2,3,5-tetrasubstituted benzene ring [δ 6.67 (1H, s, H-6), 6.65 (1H, s, H-8)], a hydroxyl group [δ 8.62 (1H, s, 4-OH)] and an aromatic methoxyl singlet at δ 3.99 (3H, s, 7-OCH_3). The ^{13}C NMR and DEPT (distortionless enhanced polarization transfer) spectra displayed twelve aromatic carbons, a methoxyl moiety and a ketone group [δ 191.4 (C-9)], indicating that **1** was a fluorenone (Ma et al., 1998; Yang et al., 2004). Correlations from 4-OH to C-4 (δ 143.3) and C-3 (δ 127.6), H-8 to C-9, and 7-OCH_3 to C-7 (δ 152.9) in the HMBC (heteronuclear multiple bond correlation) spectrum (see Fig. 2) suggested **1** was a 1,4,5,7-oxygenated substituted fluorenone, which was further confirmed by NOESY spectrum (see Fig. 3). Cross peaks among 7-OCH_3 to H-8 and H-6 in NOESY (nuclear Overhauser effect spectroscopy) spectrum explained hydroxyl groups were located at C-1, 4 and C-5, whereas the methoxy group was linked at C-7. Thus, the structure of **1** was assigned unambiguously as 1,4,5-trihydroxy-7-methoxy-9*H*-fluoren-9-one. It is a new compound, and the difference between the structure of **1** and the known dendroflorin (**2**) lies only in the position of the methoxyl groups (Yang et al., 2001). **2** was obtained also from this study, and easily dissolved in acetone, but **1** was insoluble in acetone.

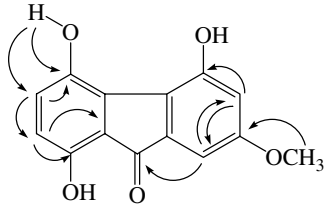

Fig. 2. HMBC correlations from H to C for **1**.

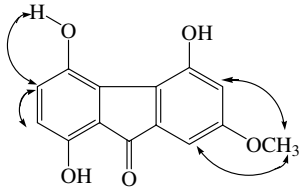

Fig. 3. NOE correlations for **1**.

On the basis of spectroscopic data analysis (NMR and MS) and comparison with reports in literature, compound **3** was identified to be denchrysan A (**3**) (Yang et al., 2004; Ye, Zhao, & Qin, 2003).

3.2. Cytotoxic activity

The isolated compounds were evaluated *in vitro* for their inhibitory ability against the growth of human leukaemia cell lines K562 and HL-60, human lung adenocarcinoma A549, human hepatoma BEL-7402 and human stomach cancer SGC-7901, using cisplatin as a positive control (Table 1). All three fluorenones displayed selective cytotoxicity against BEL-7402, with IC_{50} values of 1.49, 0.97 and 1.38 μg/ml, respectively. They were inactive or showed a weak activity against other cells. Dendroflorin (**2**) and nobilone, a fluorenone isolated from *D. nobile* was reported before to exhibited significant antioxidant activities and inhibitory effects on NO production (Zhang et al., 2007). The present study is the first time that cytotoxic activity was evaluated for fluorenones isolated from *Dendrobium* species.

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Table 1
Cytotoxicity of compounds **1**–**3** isolated from *D. chrysotoxum*

Compound	IC_{50} value (μg/ml)				
	K562[a]	HL-60[b]	A549[c]	BEL-7402[d]	SGC-7901[e]
1,4,5-Trihydroxy-7-methoxy-9-fluorenone (**1**)	32.18	10.39	18.40	1.49	15.48
Dendroflorin (**2**)	26.65	10–100	9.03	0.97	5.53
Denchrysan A (**3**)	52.28	16.66	13.34	1.38	12.34
Cisplatin	0.08	0.70	0.44	0.18	0.21

[a] Human leukaemia cell lines K562.
[b] Human leukaemia cell lines HL-60.
[c] Human lung adenocarcinoma A549.
[d] Human hepatoma BEL-7402.
[e] Human stomach cancer SGC-7901.

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