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A NEW 9,10-DIHYDROPHENANTHRENE GLYCOSIDE FROM *Dendrobium primulinum*

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*A new 9,10-dihydrophenanthrene glycoside named 2,4,5,9*S*-tetrahydroxy-9,10-dihydrophenanthrene 4*-O-β-*D-glucopyranoside (**1**), together with 11 known compounds, was isolated from* Dendrobium primulinum. *Their structures were determined by analysis of spectroscopic data.*

Keywords: *Dendrobium primulinum*, 9,10-dihydrophenanthrene glycoside, 2,4,5,9*S*-tetrahydroxy-9,10-dihydrophenanthrene 4-*O*-*β*-D-glucopyranoside.

Dendrobium, one of the largest genera in the Orchidaceae family with more than 1100 species identified, is widely distributed throughout Asia, Europe, and Australia [1]. There are 75 species and two varieties of *Dendrobium* in China [2], and the stems of many species are used as "Shi-Hu" in traditional Chinese medicines to nourish the stomach, promote the production of body fluid, and reduce fever. *Dendrobium primulinum* Lindl. is distributed in southwest of China, India, Nepal, Burma, Laos, Vietnam, and Thailand and is widely used as a medicinal *Dendrobium* plant in "Dai Medicine." Previous phytochemical studies on *D. primulinum* have revealed the presence of a series of compounds such as alkaloids, nucleosides, and flavonoids [3, 4]. Our further investigation on this plant species led to the isolation of a novel 9,10-dihydrophenanthrene glycoside (**1**) and 11 known compounds **2–12**.

Compound **1** was obtained as a white amorphous powder from MeOH with $[\alpha]_D^{25}$ –27° (*c* 0.3; MeOH). The molecular formula of $C_{20}H_{22}O_9$ was deduced from the positive HR-ESI-MS *m/z* 429.1160 $[M + Na]^+$ (calcd 429.1162) and its NMR data (Table 1), indicating 10 degrees of unsaturation. The negative-ion ESI-MS^n data showed *m/z* 243 $[M - H - 162]^-$, suggesting the presence of one hexose. Its UV maxima absorptions at 211.2, 270.2, and 296.4 nm were similar to those of 9,10-dihydrophenanthrene derivatives [5]. The IR spectrum showed absorption bands for hydroxyl (3396 cm^{-1}) and aromatic (1618, 1451 cm^{-1}) groups. The aromatic region of the ^1H NMR spectrum showed three adjacent aromatic protons at δ 7.14 (1H, t, J = 7.8 Hz), 7.05 (1H, d, J = 7.8 Hz), and 6.84 (1H, d, J = 7.8 Hz) and a pair of *meta*-coupled protons at δ 6.62 (1H, d, J = 1.8 Hz) and 6.50 (1H, d, J = 1.8 Hz), indicating a 1,2,3-trisubstituted benzene ring and a 1,3,4,5-tetrasubstituted benzene ring, respectively. In the aliphatic region, two methylene protons at δ 2.72 (1H, dd, J = 14.4, 3.6 Hz) and 2.62 (1H, dd, J = 14.4, 9.6 Hz) and one oxygenated methine proton at δ 4.48 (1H, dd, J = 9.6, 3.6 Hz) were attributed to a $-OCHCH_2$ group by ^1H–^1H COSY correlations, and one anomeric signal at δ 5.13 (1H, d, J = 7.8 Hz) and six protons in the range 3.23–3.72 were due to a sugar residue. The ^{13}C NMR and DEPT spectrum confirmed the presence of 12 aromatic carbons (three bearing oxygen atoms), one oxygenated methine carbon at δ 67.7, one methylene carbon at δ 39.7, together with six hexose carbons. In addition, based on the IR absorption at 3396 $cm^{-1}$and its molecular formula and oxygenated C-atoms (δ 157.7, 153.1, 153.0), three hydroxyl groups were assumed. Acid hydrolysis of **1** afforded D-glucose, which was identified by GC analysis, and the J value of the anomeric proton δ 5.13 (1H, d, J = 7.8 Hz) indicated a *β*-configuration. In the HMBC spectrum, the anomeric proton (H-1′) showed correlation with C-4, indicating the attachment of *β*-D-glucose to C-4. The NOESY correlations between H-1′ to H-3 and H-5′ to H-3 confirmed the location of glucose (Fig. 1).

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TABLE 1. ^{1}H (600 MHz), ^{13}C NMR (150 MHz) Data, and HMBC Correlations of Compound **1** (DMSO-d_6+ CD_3OD, δ, ppm, J/Hz)

C atom	δ_H	δ_C	HMBC
1	6.50 (1H, d, J = 1.8)	110.4	C-2, C-3, C-4a, C-10
2	–	157.7	
3	6.62 (1H, d, J = 1.8)	100.7	C-2, C-4, C-4a
4	–	153.1	
4a	–	112.8	
4b	–	120.0	
5	–	153.0	
6	6.84 (1H, d, J = 7.8)	118.2	C-4b, C-5
7	7.14 (1H, t, J = 7.8)	127.4	C-5, C-6, C-8a
8	7.05 (1H, d, J = 7.8)	117.1	
8a	–	142.9	
9	4.48 (1H, dd, J = 9.6, 3.6)	67.7	
10	2.72 (1H, dd, J = 14.4, 3.6), 2.62 (1H, dd, J = 14.4, 9.6)	39.7	C-4a, C-8a, C-9, C-10a
10a	–	139.2	
1′	5.13 (1H, d, J = 7.8)	99.6	C-4
2′	3.28 (1H, m)	73.4	C-1′, C-3′
3′	3.34 (1H, m)	76.8	C-2′, C-4′
4′	3.23 (1H, m)	69.7	C-3′, C-6′
5′	3.39 (1H, m)	77.6	C-6′
6′	3.72 (1H, m) 3.52 (1H, m)	60.9	C-4′, C-5′

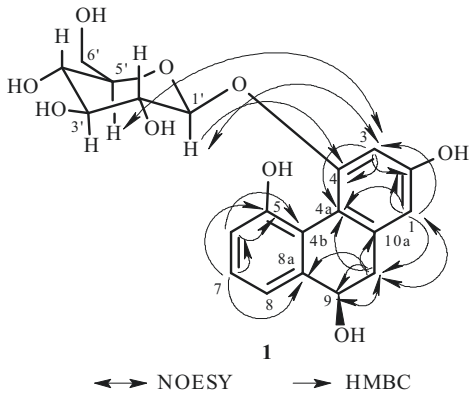

Fig. 1. Structure of compound **1** and its HMBC and NOESY correlations.

The CD spectrum of **1** showed a negative Cotton effect at 233 nm and a positive Cotton effects at 268 nm, which were in agreement with that of (+)-(9*S*)-hydroxy-9,10-dihydrophenanthrene [6]. Therefore, the structure of compound **1** was deduced as 2,4,5,9*S*-tetrahydroxy-9,10-dihydrophenanthrene 4-*O*-*β*-D-glucopyranoside.

The known compounds were identified as 2,4,7-trihydroxy-9,10-dihydrophenanthrene (**2**) [7], denthyrsinol (**3**) [8], moscatin (**4**) [9], moscatilin (**5**) [10], gigantol (**6**) [11], batatasin III (**7**) [12], tristin (**8**) [13], 3,4′,5-trihydroxybibenzyl (**9**) [14], 3,6,9-trihydroxy-3,4-dihydroanthracen-1(2*H*)-one (**10**) [15], *β*-sitosterol (**11**) [16], and *β*-daucosterol (**12**) [17] by comparing their spectroscopic data with literature data.

All the above compounds were isolated from this plant for the first time.

EXPERIMENTAL

General Procedures. Optical rotations were taken on a PerkinElmer 341 polarimeter. IR spectra were carried out on a Nicolet-Nexus 670 infrared spectrophotometer with KBr pellet. NMR spectra were run on a Bruker AV-600 NMR spectrometer

using TMS as an internal standard. ESI-MS and HR-ESI-MS were recorded on a Waters Micromass Q-TOF micro mass spectrometer. The CD spectra were obtained on a Chirascan spectrometer. Column chromatography was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), RP-18 silicagel (ODS, 50 μm, YMC), and Sephadex LH-20 (Pharmacia).

Plant Material. The whole plant of *D. primulinum* was collected in Longling, Yunnan Province, China, in November 2012, and identified by Prof. Xinjia Ming, Chongqing Academy of Chinese Materia Medica. A voucher specimen (No. 20121101) was deposited at the Shanghai Institute of Pharmaceutical Industry, Shanghai, China.

Extraction and Isolation. The air-dried and powdered stems of *D. primulinum* (4 kg) were extracted with 95% ethanol (four times, each 40 L) at room temperature. Evaporation of the solvent under reduced pressure afforded a brown crude extract (300 g). The extract was suspended in water and then partitioned with petroleum ether (60–90°C), EtOAc, and *n*-BuOH successively to obtain four fractions. The EtOAc fraction (40 g) was subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH (100:1, 50:1, 30:1, 15:1, 10:1, 0:1) to yield six fractions (Fr. 1–Fr. 6). Fraction 2 (11.8 g) was isolated by silica gel column chromatography with CH_2Cl_2–acetone (40:1–10:1) to give six subfractions (Fr. 2.1–Fr. 2.6). Fraction 2.2 (3.0 g) was rechromatographed on silica gel column with petroleum ether–acetone (5:1–3:1) and then purified over a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) and recrystallized (petroleum ether–acetone) to afford compounds **4** (112 mg), **5** (210 mg), **6** (738 mg), and **11** (80 mg). Fraction 2.4 (4.2 g) was further subjected to silica gel column chromatography and eluted with petroleum ether–EtOAc (3:1–1:1) and then purified over a Sephadex LH-20 column with CH_2Cl_2–MeOH (1:1) and preparative TLC to obtain compounds **3** (9 mg) and **8** (170 mg). By the same method, compound **7** (103 mg) was obtained from Fr. 3 (4.2 g), compounds **2** (30 mg) and **9** (20 mg) were obtained from Fr. 4 (4.5 g), and compound **10** (6 mg) was obtained from Fr. 5 (3.3 g). The *n*-BuOH fraction (42 g) was subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH–H_2O (30:1:0, 15:1:0, 10:1:0, 4:1:0.1, 7:3:0.5, 0:1:0) to yield six fractions (Fr. I–VI). Fraction III (7.4 g) was subjected to Sephadex LH-20 column chromatography and eluted with EtOH and recrystallized (MeOH) to give compound **12** (19 mg). Fraction IV (10.5 g) was subjected to ODS column chromatography and eluted with a step gradient of MeOH–H_2O (1:9–6:4) to yield nine subfractions (Fr. IV.1–Fr. IV.9). Fraction IV. 5 was purified over a Sephadex LH-20 column with EtOH and silica gel column chromatography with CH_2Cl_2–MeOH–H_2O (6:1:0.1) to obtain compound **1** (43 mg).

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