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FLAVONOID DIGLYCOSIDES FROM *Dendrobium officinale* LEAVES AND THEIR TYROSINASE INHIBITORY ACTIVITY

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Four flavonoid C-*glycosides, including two new diglycosides, dendrodiglycosides A (**1**) and B (**2**), along with known isoviolanthin (**3**) and apigenin 6*-C-β-*D-glucopyranosyl-(1→2)*-α-*L-arabinopyranoside (**4**), were isolated from the leaves of* Dendrobium officinale *(Orchidaceae). Their structures were elucidated by NMR and MS. Apigenin 6*-C-β-*D-glucopyranosyl-(1→2)*-α-*L-arabinopyranoside exhibited an inhibitory effect against tyrosinase with an IC_{50} value of 146.5 μg/mL.*

Keywords: Orchidaceae, *Dendrobium officinale*, flavonoid diglycosides, *C*-glycosides, tyrosinase inhibitory activity.

The dry stems of the orchidaceous plant *Dendrobium officinale* Kimura & Migo are a famous traditional Chinese medicine [1]. The leaves of the plant are used as food material in China [2]. Flavonoid diglycosides are major constituents of *D. officinale* leaves [2]. However, these compounds, which are usually detected by liquid chromatography-mass spectrometry (LC-MS) [3], are very difficult to isolate and purify. To better identify the beneficial flavonoids of *D. officinale* [1], we conducted a phytochemical study of its leaves, which led to the isolation of four flavonoid *C*-glycosides, including two new diglycosides, dendrodiglycosides A (**1**) and B (**2**). We report here their molecular structure and bioassay results.

Dendrodiglycoside A (**1**) has the molecular formula $C_{26}H_{28}O_{14}$ by ^{13}C NMR (Table 1) and HR-ESI-MS showing an ion peak at *m/z* 563.1404 $[M - H]^-$ (calcd for $C_{26}H_{27}O_{14}$, 563.1406). Its ^{1}H and ^{13}C NMR data in DMSO-d_6 (Table 1) indicated the presence of a *p*-disubstituted phenyl ring [δ_H 7.86 (2H, br.d, J = 8.7 Hz, H-2′, 6′), 6.90 (2H, br.d, J = 8.7 Hz, H-3′, 5′)], a *C-β*-arabinofuranosyl group [δ_H 5.39 (1H, d, J = 5.7 Hz, H-1″); δ_C 75.3 (C-1″), 86.2 (C-2″), 76.5 (C-3″), 85.1 (C-4″), 61.3 (C-5″)], and an *O-β*-glucopyranosyl group [δ_H 4.37 (1H, d, J = 7.8 Hz, H-1‴); δ_C 101.5 (C-1‴), 73.6 (C-2‴), 76.7 (C-3‴), 70.0 (C-4‴), 76.6 (C-5‴), 60.8 (C-6‴)] [4–7]. Usually, the $J_{1,2}$, $J_{2,3}$, and $J_{3,4}$ values of *β*-L-arabinofuranosyl moieties are larger than those of *α*-L-arabinofuranosyl moieties (Table 2). For example, $J_{1,2}$ values of aconicarmichoside D with an *β*-L-arabinofuranosyl moiety and aconicarmichoside C with an *α*-L-arabinofuranosyl moiety are 4.8 and 0 Hz, respectively [5]. In contrast, $J_{1,2}$ values of compounds **1** and **2** were 5.7 and 2.3 Hz (Tables 1 and 3), respectively. Additionally, the anomeric carbon of the *β*-L-arabinofuranosyl group appears at a higher field than that in the *α*-L-arabinofuranosyl group. For example, the chemical shifts of C-1′ in aconicarmichoside D and aconicarmichoside C are 103.6 and 109.5 ppm [5] respectively. In contrast, the chemical shifts of C-1″ in **1** and **2** were 75.3 and 77.9 ppm (Tables 1 and 3) respectively. Therefore, a *β*-arabinofuranosyl moiety was suggested to be presented in **1**. By comparing its NMR data with those of apigenin 6-*C*-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside (**4**) [8], compound **1** should also be an apigenin *C*-glycoside with two sugars.

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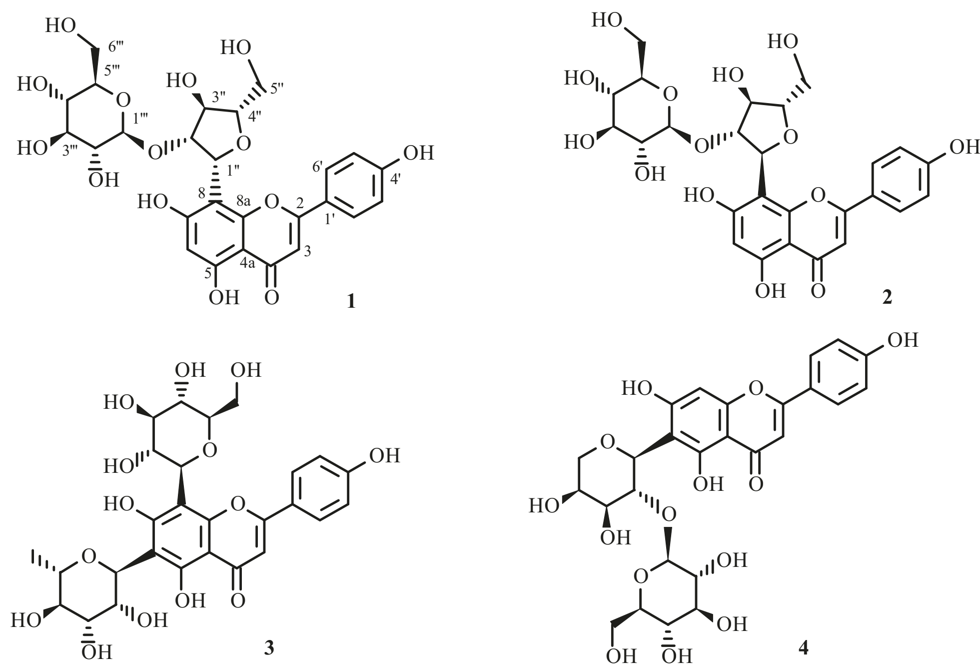

Based on 2D NMR correlations (Fig. 1), the apigenin moiety was confirmed. In its HMBC spectrum, correlations from H-1″ to C-7 and C-8a, as well as from H-2″ to C-8 were observed (Fig. 1), implying that the *C*-*β*-arabinofuranosyl group was located at C-8; correlations from H-2″ to C-1‴, as well as from H-1‴ to C-2″, indicated that the *O*-*β*-glucopyranosyl group was linked to the 2-OH of arabinose. Because the D-glucosyl and L-arabinosyl groups were previously identified in *D. officinale* [2], we suspect that the sugars identified in the study were also D-glucose and L-arabinose. We hydrolyzed compound **4** to yield D-glucose. Accordingly, compound **1** was elucidated to be apigenin 8-*C*-*β*-D-glucopyranosyl-(1→2)-*β*-L-arabinofuranoside, named dendrodiglycoside A.

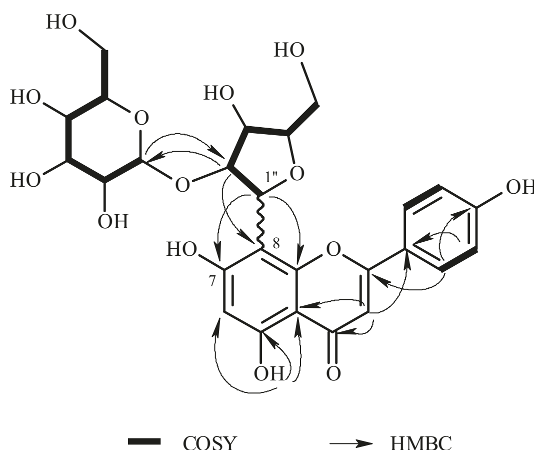

Fig. 1. Key ^{1}H–^{1}H COSY and HMBC correlations for **1** and **2** in DMSO-d_6.

According to its HR-ESI-MS demonstrating an ion peak at *m/z* 563.1403 $[M – H]^-$ (calcd for $C_{26}H_{27}O_{14}$, 563.1406) and ^{13}C NMR data (Table 3), the molecular formula of compound **2** was determined to be $C_{26}H_{28}O_{14}$, indicating that compound **2** is an isomer of compound **1**. The ^{1}H and ^{13}C NMR data in DMSO-d_6 (Table 3) indicated the presence of a *p*-disubstituted phenyl ring [δ_H 7.75 (2H, br.d, J = 8.6 Hz, H-2′, 6′), 6.87 (2H, br.d, J = 8.6 Hz, H-3′, 5′)], a *C*-*α*-arabinofuranosyl group [δ_H 5.33 (1H, d, J = 2.3 Hz, H-1″); δ_C 77.9 (C-1″), 87.8 (C-2″), 77.0 (C-3″), 87.3 (C-4″), 61.0 (C-5″)] (Table 2) [5–7, 9], and an *O*-*β*-glucopyranosyl group [δ_H 4.30 (1H, d, J = 7.8 Hz, H-1‴); δ_C 101.2 (C-1‴), 73.7 (C-2‴), 76.7 (C-3‴), 70.0 (C-4‴), 76.8 (C-5‴), 60.8 (C-6‴)]. By comparing its NMR data with those of compound **1**, compound **2** was determined to also be an apigenin *C*-glycoside with two sugars, with a difference in the C-1 configuration in the arabinofuranosyl fragment.

TABLE 1. ^{1}H (500 MHz) and ^{13}C (126 MHz) NMR Data for Dendrodiglycoside A (**1**) (δ, ppm, J/Hz)

C atom	DMSO-d$_6$		Methanol-d$_4$	
	δ_H	δ_C	δ_H	δ_C
2	–	162.7 (C)	–	165.9 (C)
3	6.63 (s)	102.6 (CH)	6.56 (s)	103.6 (CH)
4	–	181.1 (C)	–	183.7 (C)
4a	–	101.5 (C)	–	104.2 (C)
5	–	160.6 (C)	–	Disappeared
6	5.95 (br.s)	100.5[a]	Disappeared	Disappeared
7	–	168.0 (C)	–	168.2 (C)
8	–	105.1 (C)	–	105.8 (C)
8a	–	155.8 (C)	–	157.7 (C)
1′	–	121.9 (C)	–	123.6 (C)
2′, 6′	7.86 (br.d, J = 8.7)	128.4 (CH)	7.87 (d, J = 8.7)	129.7 (CH)
3′, 5′	6.90 (br.d, J = 8.7)	116.0 (CH)	6.92 (d, J = 8.7)	117.0 (CH)
4′	–	160.8 (C)	–	162.6 (C)
1″	5.39 (d, J = 5.7)	75.3 (CH)	5.70 (d, J = 6.2)	77.5 (CH)
2″	4.78 (overlapped)	86.2 (CH)	4.98 (dd, J = 6.2, 5.0)	88.3 (CH)
3″	4.14 (t, J = 4.5)	76.5 (CH)	4.42 (t, J = 5.0)	77.5 (CH)
4″	3.91 (m)	85.1 (CH)	4.21 (m)	85.6 (CH)
5″	3.53 (m)	61.3 (CH_2)	3.80 (dd, J = 12.2, 4.3) 3.77 (dd, J = 12.2, 5.4)	62.3 (CH_2)
1‴	4.37 (d, J = 7.8)	101.5 (CH)	4.49 (d, J = 7.8)	103.5 (CH)
2‴	2.89 (m)	73.6 (CH)	3.18 (m)	75.2 (CH)
3‴	3.09 (br.t, J = 9.0)	76.7 (CH)	3.31 (overlapped)	77.9 (CH)
4‴	2.97 (ddd, J = 9.5, 9.0, 4.1)	70.0 (CH)	3.19 (m)	71.5 (CH)
5‴	2.89 (m)	76.6 (CH)	3.06 (m)	77.5 (CH)
6‴	3.36 (overlapped) 3.21 (dd, J = 11.6, 5.0)	60.8 (CH_2)	3.47 (dd, J = 11.7, 2.7) 3.37 (dd, J = 11.7, 4.9)	62.5 (CH_2)
5-OH	13.09 (s)			
5″-OH	4.62 (t, J = 4.7)			
2‴-OH	–			
3‴-OH	4.88 (d, J = 3.5)			
4‴-OH	4.78 (overlapped)			

[a]Detected by HSQC and HMBC.

The apigenin moiety in **2** was deduced by its 2D NMR correlations (Fig. 1). HMBC correlations from H-1″ to C-7 and C-8a and from H-2″ to C-8 were observed (Fig. 1), indicating that the *C*-*α*-arabinofuranosyl group was located at C-8; HMBC correlations from H-2″ to C-1‴ and from H-1‴ to C-2″ indicated that the *O*-*β*-glucopyranosyl group was located at 2″-OH. Thus, compound **2** was elucidated to be apigenin 8-*C*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinofuranoside and named dendrodiglycoside B.

The NMR data of compounds **1** and **2** in methanol-d$_4$ are also presented in the paper (Tables 1 and 3). Some NMR signals of the aglycones disappeared. However, the signals of sugar moieties may be useful as a reference.

The known compounds were identified to be isoviolanthin (**3**) [10] and apigenin 6-*C*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside (**4**) [8] by comparison of the obtained spectroscopic data with those published in the literature.

Compounds **1–4** were evaluated for tyrosinase inhibitory, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, protein tyrosine phosphatase-1 B (PTP1B) inhibitory, and *α*-glucosidase inhibitory activities. As shown in Tables **4** and **5**, compound **4** exhibited inhibitory activity against tyrosinase with an IC_{50} value of 146.5 μg/mL compared with the positive control phenylethyl resorcinol (IC_{50} of 0.05 μg/mL). Tyrosinase inhibitors can be used in the development of cosmetic skin-whitening agents [11].

Compound **3** exhibited weak antioxidant activity with a DPPH radical scavenging rate of 25.4% at a concentration of 100 μg/mL compared with the positive control Trolox (96.1% scavenging rate at 25 μg/mL).

TABLE 2. Coupling Constants of α-L-Arabinofuranosyl and β-L-Arabinofuranosyl Moieties in ^{1}H NMR of Some Known Compounds

Compound	Type	Solvent	$J_{1,2}$ (Hz)	$J_{2,3}$ (Hz)	$J_{3,4}$ (Hz)	Ref.
Aconicarmichoside C	α-L-Ara	Methanol-d_4	0	2.0	3.5	[5]
Aconicarmichoside D	β-L-Ara	Methanol-d_4	4.8	7.2	7.2	[5]
Methyl α-L-arabinofuranoside	α-L-Ara	D_2O	1.6	3.2	5.7	[6]
Methyl β-L-arabinofuranoside	β-L-Ara	D_2O	4.2	7.9	7.0	[6]
Methyl β-L-arabinofuranosyl-(1→2)-α-L-arabinofuranoside	α-L-Ara	Methanol-d_4	2.0	4.8	4.8	[7]
Methyl β-L-arabinofuranosyl-(1→2)-α-L-arabinofuranoside	β-L-Ara	Methanol-d_4	4.8	8.0	6.4	[7]

TABLE 3. ^{1}H and ^{13}C NMR Data for Dendrodiglycoside B (**2**) (δ, ppm, J/Hz)

C atom	DMSO-d_6		Methanol-d_4	
	δ_H (800 MHz)	δ_C (201 MHz)	δ_H (500 MHz)	δ_C (126 MHz)
2	–	160.5 (C)	–	165.0 (C)
3	6.40 (s)	101.9 (CH)	6.47 (s)	103.0 (CH)
4	–	179.5 (C)	–	183.0 (C)
4a	–	97.9 (C)	–	102.0 (C)
5	–	160.7 (C)	–	Disappeared
6	5.47 (s)	103.0 (CH)	Disappeared	Disappeared
7	–	175.8[a]	–	174.7 (C)
8	–	105.3 (C)	–	106.3 (C)
8a	–	156.1 (C)	–	158.1 (C)
1′	–	122.4[a]	–	123.6 (C)
2′, 6′	7.75 (br.d, J = 8.6)	127.6 (CH)	7.84 (br.d, J = 9.0)	129.4 (CH)
3′, 5′	6.87 (br.d, J = 8.6)	115.9 (CH)	6.91 (br.d, J = 9.0)	117.1 (CH)
4′	–	160.1 (C)	–	162.5 (C)
1″	5.33 (d, J = 2.3)	77.9 (CH)	5.71 (d, J = 4.3)	79.2 (CH)
2″	4.60 (br.d, J = 2.3)	87.8 (CH)	4.92 (overlapped)	88.8 (CH)
3″	4.03 (br.s)	77.0 (CH)	4.36 (dd, J = 2.7, 2.5)	78.0 (CH)
4″	3.80 (br.t, J = 6.5)	87.3 (CH)	4.17 (m)	87.4 (CH)
5″	3.53 (dd, J = 6.5, 6.0)	61.0 (CH_2)	3.80 (dd, J = 11.9, 6.2) 3.77 (dd, J = 11.9, 5.8)	62.1 (CH_2)
1‴	4.30 (d, J = 7.8)	101.2 (CH)	4.48 (d, J = 7.8)	103.2 (CH)
2‴	2.93 (m)	73.7 (CH)	3.20 (dd, J = 9.1, 7.8)	75.2 (CH)
3‴	3.14 (m)	76.7 (CH)	3.33 (t, J = 9.1)	78.0 (CH)
4‴	3.00 (m)	70.0 (CH)	3.23 (dd, J = 9.6, 9.1)	71.5 (CH)
5‴	3.00 (m)	76.8 (CH)	3.12 (m)	77.7 (CH)
6‴	3.51 (dd, J = 12.0, 1.4) 3.28 (dd, J = 12.0, 5.1)	60.8 (CH_2)	3.57 (dd, J = 11.9, 2.5) 3.46 (dd, J = 11.9, 4.9)	62.4 (CH_2)
5-OH	12.94 (s)			
5″-OH	4.47 (t, J = 6.0)			
2‴-OH	4.96 (d, J = 5.3)			
3‴-OH	4.89 (d, J = 4.7)			
4‴-OH	4.83 (d, J = 5.0)			

[a]Detected by HMBC.

TABLE 4. Tyrosinase Inhibitory and DPPH Radical Scavenging Activities of Compounds **1–4**

Compound	Concentration, μg/mL	Tyrosinase inhibition activity		DPPH radical scavenging activity, %
		Inhibition rate, %	IC_{50}, μg/mL	
1	100	–8.6 ± 1.0	–	1.7 ± 7.6
2	100	8.7 ± 1.7	–	–7.2 ± 0.8
3	100	7.0 ± 1.9	–	25.4 ± 2.0
4	200	53.3 ± 0.0	146.5 ± 3.5	–
	100	46.4 ± 0.9		6.3 ± 0.5
	50	34.4 ± 0.4		–
	25	20.7 ± 1.5		–
	12.5	18.4 ± 1.8		–
Kojic acid[a]	5	56.2 ± 0.1	–	–
Phenylethyl resorcinol[a]	0.2	68.1 ± 1.2	0.05 ± 0.00	–
	0.1	60.9 ± 1.0		–
	0.05	51.7 ± 1.7		–
	0.025	36.5 ± 0.4		–
	0.0125	18.8 ± 1.5		–
Trolox[a]	25	–	–	96.1 ± 0.0

[a]Positive control; –: not tested.

TABLE 5. PTP1B Inhibitory and α-Glucosidase Inhibitory Activities of Compounds **1–4**

Compound	Concentration, μg/mL	PTP1B inhibition, %	α-Glucosidase inhibition, %
1	50	–6.9 ± 3.7	4.8 ± 2.5
2	50	6.7 ± 2.5	4.4 ± 3.8
3	50	2.6 ± 2.9	7.2 ± 2.2
4	50	8.0 ± 3.9	8.7 ± 1.9
Suramin (positive control)	29	88.6 ± 1.5	–
Quercetin (positive control)	3	–	63.5 ± 0.9

EXPERIMENTAL

General Experimental Procedures. Optical rotations were recorded using a JASCO P-1020 Polarimeter (Jasco Corp., Tokyo, Japan). Electronic circular dichroism (ECD) spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were collected using Bruker DRX-500 and Ascend^{TM} 800 III-600 spectrometers (Bruker Bio-Spin GmbH, Rheinstetten, Germany), with TMS as an internal standard. Electrospray ionization mass spectrometry (ESI-MS) analyses were performed on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). Silica gel G (80–100, 200–300, and 300–400 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), reverse phase (RP) C_{18} silica gel (40–75 μm, Fuji Silysia Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography, and silica gel GF_{254} (Qingdao Meigao Chemical Co., Ltd.) was used for preparative thin layer chromatography (TLC) as precoated plates. TLC spots were visualized under UV light at 254 nm by dipping them into 5% H_2SO_4 in alcohol followed by heating. Semipreparative high-performance liquid chromatography (HPLC) was performed with an Agilent 1200 series pump (Agilent Technologies, Santa Clara, CA, USA) equipped with a diode array detector, an Agilent Zorbax RX-C_8 (5 μm, ∅ 9.4 × 250 mm) column, and a chiral-phase CD-Ph column (5 μm, ∅ 4.6 × 250 mm; Shiseido, Japan).

Plant Material. Leaves of cultivated *Dendrobium officinale* Kimura & Migo were collected in December 2019 in Yunnan, China. The plant was identified by Associate Professor Shu-Yun Li at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. PE1912) was deposited in the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. Dry leaves of *D. officinale* (9.0 kg) were ground into powder and then extracted with 90–95% EtOH (50 L × 4 h × 3) at 70°C. After filtration, the liquid was evaporated under reduced pressure to yield a crude extract (2.3 kg), which was suspended in water (1 L) and extracted with petroleum ether (3 L × 8). The aqueous phase was adjusted to pH 2–3 with 6 M HCl and then extracted with EtOAc (3 L × 5). The remaining aqueous phase was adjusted to pH 9–10 with 50% NaOH solution and then extracted with CH_2Cl_2 (2 L × 5). The remaining aqueous phase was adjusted to approximately pH 7 with 6 M HCl and then extracted with *n*-BuOH (2 L × 5) to yield the *n*-BuOH-soluble fraction (85.6 g).

The *n*-BuOH-soluble fraction was subjected to silica gel column chromatography and eluted with CH_2Cl_2–MeOH (20:1, 10:1, 5:1, 3:1, 2:1, 1:1, 0:1) to yield fractions A–F. Fraction D (CH_2Cl_2–MeOH, 3:1 eluting portion, 30.4 g) was loaded on an RP C_{18} column eluted with MeOH–H_2O (5:95→100:0) to yield fractions D1–D7. Fraction D5 (MeOH–H_2O, 20:80→50:50 eluting portion, 7.4 g) was subjected to silica gel column chromatography and eluted with EtOAc–acetone (15:1, 10:1, 5:1, 3:1, 2:1, 1:1) and MeOH to yield fractions D5.1–D5.5. Fraction D5.5 (EtOAc–Ac_2O, 10:1→5:1, 2.0 g) was isolated by Sephadex LH-20 column chromatography (MeOH) to yield the primary fraction D5.5.4 (420.9 mg). One-fifth of Fr. D5.5.4 was purified by semipreparative HPLC (CD-Ph column; MeOH–H_2O, 15:85, 1 mL/min) to yield compound **2** (3.9 mg, t_R = 71.122 min). Four-fifths of Fr. D5.5.4 was separated into Frs. D5.5.4.1–D5.5.4.5 by semipreparative HPLC (Zorbax RX-C_8 column; MeOH–H_2O, 25:75, 2 mL/min). Fraction D5.5.4.2 (95.2 mg, t_R of 11.720–17.187 min) was purified by semipreparative HPLC (CD-Ph column; MeOH–H_2O, 24:76, 1 mL/min) to yield a mixture (20.1 mg, t_R = 19.840 min) and compounds **1** (10.5 mg, t_R = 25.468 min) and **3** (43.9 mg, t_R = 43.347 min). The mixture (20.1 mg, t_R = 19.840 min) was isolated by semipreparative HPLC (CD-Ph column; MeCN–H_2O, 10:90, 1 mL/min) to yield compound **4** (12.8 mg, t_R = 26.607 min).

Dendrodiglycoside A (1), yellow solid, $[\alpha]_D^{20}$–4° (*c* 0.2, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 335 (4.21), 305 (4.20), 274 (4.24), 218 (4.49), 205 (4.46). ECD (*c* 0.015, MeOH): $\Delta\varepsilon_{305\ nm}$ +2.28, $\Delta\varepsilon_{272\ nm}$ –1.50, $\Delta\varepsilon_{250\ nm}$ –1.20, $\Delta\varepsilon_{238\ nm}$ –0.81, $\Delta\varepsilon_{216\ nm}$ +4.83. For ^1H and ^{13}C NMR data, see Table 1. ESI-MS *m/z* 563 $[M – H]^–$; HR-ESI-MS *m/z* 563.1404 $[M – H]^–$ (calcd for $C_{26}H_{27}O_{14}$, 563.1406).

Dendrodiglycoside B (2), yellow solid, $[\alpha]_D^{20}$–6° (*c* 0.1, MeOH). UV (MeOH, λ_{max}, nm) (log ε): 336 (3.87), 304 (3.88), 275 (3.91), 217 (4.18), 205 (4.17). ECD (*c* 0.034, MeOH): $\Delta\varepsilon_{305\ nm}$ +1.12, $\Delta\varepsilon_{275\ nm}$ –0.56, $\Delta\varepsilon_{236\ nm}$ –0.56, $\Delta\varepsilon_{216\ nm}$ +2.40. For ^1H and ^{13}C NMR data, see Table 3. ESI-MS *m/z* 587 $[M + Na]^+$; HR-ESI-MS *m/z* 563.1403 $[M – H]^–$ (calcd for $C_{26}H_{27}O_{14}$, 563.1406).

Isoviolanthin (3), yellow solid, $[\alpha]_D^{24}$ –31° (*c* 0.1, MeOH). ^1H NMR (500 MHz, methanol-d_4, δ, ppm, J/Hz): 7.91 (2H, d, J = 8.4, H-2′, 6′), 6.88 (2H, d, J = 8.4, H-3′, 5′), 6.53 (1H, s, H-3), 5.14 (1H, s, H-1′), 5.02 (1H, d, J = 10.0, H-1′′′), 4.12 (1H, dd, J = 10.0, 9.5, H-2′′′), 4.05 (1H, br.s, H-2′), 3.95 (1H, br.d, J = 11.2, H-6′′′a), 3.78 (1H, dd, J = 11.2, 5.6, H-6′′′b), 3.67 (1H, dd, J = 9.4, 9.4, H-4′′′), 3.61 (1H, dd, J = 9.1, 3.1, H-3′), 3.54 (1H, m, H-3′′′), 3.53 (1H, m, H-4′), 3.47 (1H, m, H-5′′′), 3.44 (1H, m, H-5′), 1.39 (3H, d, J = 5.9, H_3-6′). ^{13}C NMR (126 MHz, methanol-d_4, δ, ppm): 183.9 (C, C-4), 166.5 (C, C-2), 164.8 (C, C-7), 162.9 (C, C-4′), 159.0 (C, C-5), 157.2 (C, C-8a), 130.1 (CH, C-2′, 6′), 123.2 (C, C-1′), 117.1 (CH, C-3′, 5′), 108.0 (C, C-6), 105.5 (C, C-8), 104.7 (C, C-4a), 103.3 (CH, C-3), 82.8 (CH, C-5′′′), 80.3 (CH, C-3′′′), 78.9 (CH, C-5′), 77.3 (CH, C-1′), 75.8 (CH, C-3′), 75.3 (CH, C-1′′′), 73.8 (CH, C-4′), 73.4 (CH, C-2′), 72.9 (CH, C-2′′′), 72.4 (CH, C-4′′′), 63.1 (CH_2, C-6′′′), 18.4 (CH_3, C-6′). ESI-MS *m/z*: 601 $[M + Na]^+$, 577 $[M – H]^–$.

Apigenin 6-*C*-*β*-D-glucopyranosyl-(1→2)-*α*-L-arabinopyranoside (4), yellow solid, $[\alpha]_D^{24}$ –1° (*c* 0.3, MeOH). ^1H NMR (500 MHz, methanol-d_4, δ, ppm, J/Hz): 7.83 (2H, br.d, J = 8.7, H-2′, 6′), 6.91 (2H, br.d, J = 8.7, H-3′, 5′), 6.56 (1H, s, H-3), 4.84 (1H, d, J = 9.9, H-1′), 4.36 (1H, d, J = 7.1, H-1′′′), 3.99 (1H, br.d, J = 11.9, H-5′a), 3.98 (1H, br.s, H-4′), 3.81 (1H, dd, J = 9.2, 2.6, H-3′), 3.71 (1H, d, J = 11.9, H-5′b), 3.36 (2H, br.s, H_2-6′′′), 3.25 (1H, dd, J = 9.1, 9.1, H-3′′′), 3.14 (1H, m, H-4′′′), 2.90 (1H, dt, J = 9.5, 3.5, H-5′′′). ^{13}C NMR (126 MHz, methanol-d_4, δ, ppm): 183.8 (C, C-4), 167.0 (C, C-7), 166.0 (C, C-2), 162.8 (C, C-5), 159.0 (C, C-8a), 129.4 (CH, C-2′, 6′), 123.2 (C, C-1′), 117.0 (CH, C-3′, 5′), 109.4 (C, C-6), 106.0 (CH, C-1′′′), 104.5 (C, C-4a), 103.7 (CH, C-3), 79.6 (CH, C-2′), 77.9 (CH, C-3′′′), 77.5 (CH, C-5′′′), 76.0 (CH, C-2′′′), 74.5 (CH, C-1′), 71.9 (CH_2, C-5′), 71.2 (CH, C-4′′′), 70.8 (CH, C-4′), 62.5 (CH_2, C-6′′′). ESI-MS *m/z*: 587 $[M + Na]^+$, 1151 $[2M + Na]^+$.

Acidic Hydrolysis of Compound 4. Compound **4** (10.4 mg, 0.0184 mmol) was dissolved in 2 mol/L HCl (3 mL) and stirred at 90°C for 4 h. After cooling, 3 mL of water was added to the reaction mixture, which was then dried under reduced pressure to yield a residue. The residue was purified by Sephadex LH-20 column chromatography (MeOH) to yield D-glucose (2.1 mg, 0.0117 mmol, 63% yield), identified by comparison with an authentic sample using TLC and its optical rotation value: $[\alpha]_D^{20}$+8° (*c* 0.2, MeOH).

Tyrosinase Inhibitory Activity Assay. This assay was performed according to a previously described procedure [12]. Tyrosinase activity was assayed spectrophotometrically using L-Dopa as the substrate. The assay medium, consisting of 40 μL mushroom tyrosinase (Sigma-Aldrich, St. Louis, MO, USA) solution (25 units/mL), 80 μL of phosphate-buffered saline

(1/15 mol/L, pH 6.8), and 40 μL sample solution containing 5 mM DMSO or 40 μL kojic acid (Sigma-Aldrich) or phenylethyl resorcinol (Aladdin Chemical Co. Ltd., Shanghai, China), was mixed in a 96-well microplate and preincubated at 35°C for 5 min. The reaction was then initiated by the addition of 40 μL L-Dopa solution (1.25 mM final concentration). After incubation for 5 min, the absorbance was measured at 490 nm in an ELISA reader. All samples were tested in triplicate. Kojic acid or phenylethyl resorcinol was used as a positive control. The percentage of tyrosinase inhibition was calculated as follows:

$$\% \text{ Inhibition} = (A - B)/A \times 100,$$

where A = absorbance at 490 nm without a test sample, and B = absorbance at 490 nm with a test sample. The IC_{50} (50% inventory concentration) was calculated using the Reed and Muench method [13].

Antioxidant Activity Assay. The DPPH radical scavenging activity assay was performed according to a previous method with some modifications [14]. Samples 30 μL in volume (100 μg/mL, dissolved in ethanol) and Trolox (25 μg/mL, dissolved in EtOH) were added to 270 μL of DPPH solution (100 μM, dissolved in EtOH). The reaction proceeded for 1 h at 30°C on a 96-well microplate. The absorbance was then read at 515 nm, and the percentage of total radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = (1 - A_1/A_0) \times 100\%,$$

where A_0 is the absorbance of DPPH without samples (control reaction) and A_1 is the absorbance of DPPH incubated with the samples. All tests were conducted in triplicate. Trolox (Sigma-Aldrich) was used as a positive control agent.

Protein Tyrosine Phosphatase-1 B Inhibitory Activity Assay. The PTP1B inhibitory activity assay was performed according to a previously described method with a PTP1B assay test kit (Millipore Co., MA, USA) [15]. The sample (10 μL, final concentration 50 μg/mL), PTP1B enzyme solution (5 μL, final concentration 0.025 μg/mL), buffer solution (35 μL), and substrate insulin receptor-5 (IR5, 50 μL, final concentration 60 μM) were added to a 96-well plate, mixed thoroughly, and then incubated at 30°C for 30 min. Next, the phosphate detection reagent Red Assay (25 μL) was added to terminate the enzymatic reaction. After incubation for 20 min at 30°C, the absorbance was measured at 620 nm using an ELISA reader. The formula for the inhibition rate of PTP1B activity was as follows:

$$\% \text{ Inhibition} = (1 - A_3/A_2) \times 100\%,$$

where A_2 is the absorbance at 620 nm without samples (control reaction) and A_3 is the absorbance at 620 nm with the samples. All tests were conducted in triplicate, and suramin (Sigma-Aldrich) was used as a positive control agent.

***α*-Glucosidase Inhibitory Activity Assay**. The *α*-glucosidase (Sigma-Aldrich) inhibitory activity assay was performed according to the previously described procedure with some modifications [15]. The sample (final concentration 50 μg/mL), *α*-glucosidase solution (final concentration 2.5 μg/mL), buffer solution, and substrate 4-nitrophenyl *α*-D-glucopyranoside (Sigma-Aldrich, final concentration 1 mM) were added into the 96-well plate and mixed thoroughly. After incubation for 50 min at 37°C, the absorbance was read at 405 nm. The inhibition rate of *α*-glucosidase activity was calculated using the following formula:

$$\% \text{ Inhibition} = (1 - A_5/A_4) \times 100\%,$$

where A_4 is the absorbance at 405 nm without samples (control reaction) and A_5 is the absorbance at 405 nm with the samples. All tests were conducted in triplicate, and quercetin (Sigma-Aldrich) was used as a positive control agent.

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