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Phenylpropanoid and Iridoid Glucosides from the Whole Plant of *Hemiphragma heterophyllum* and Their alpha-Glucosidase Inhibitory Activities

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ABSTRACT

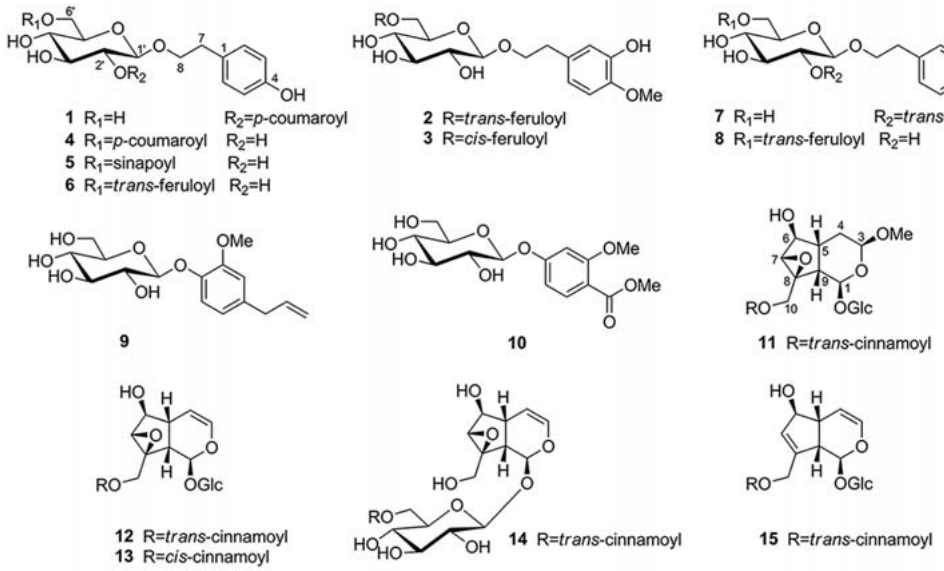
Three phenylpropanoid glucosides (**1**–**3**) and one iridoid glucoside (**11**), together with eleven known glucosides, were isolated from the ethanol extract of the whole plant of *Hemiphragma heterophyllum*. Their structures were elucidated by means of 1D and 2D NMR spectroscopy, HRMS, and chemical methods. All compounds except **11** and **13**–**15** showed varying degrees of α-glucosidase inhibitory activity. Compounds **5**, **9**, and **12** were marginally active in the bioassay, while compounds **1**–**4**, **6**–**8**, and **10** exhibited appreciable inhibitory activity with an IC_{50} value of 33.6 ~ 83.1 μM, which was much lower than that of the positive control acarbose (IC_{50} = 310.8 μM).

Introduction

Hemiphragma heterophyllum Wall. (Scrophulariaceae) is distributed mainly in the rocky mountains of Yunnan province in China [1]. The herb is used by Yi people as folk medicine for "dispelling wind", "eliminating dampness", "removing blood stasis", and relieving pain [2, 3]. Previous chemical investigations focused on the aerial parts and revealed the presence of a series of monoterpene glucosides, phenylpropanoid glucosides, and flavonoid glucosides [4–9]. These compounds were shown to possess diverse activities, such as fungistatic [10], antioxidative [11, 12], neuroprotective [13], cytotoxic [14], and anti-inflammatory [15] properties. As part of our ongoing research program to find structurally diverse and biologically active compounds from medicinal plants, we investigated the whole plant of *H. heterophyllum*. The ethanolic extract showed a modest α-glucosidase inhibitory effect (IC_{50} = 491.6 μg/mL). Fractionation of this extract afforded three new phenylpropanoid glucosides, heterophosides A–C (**1**–**3**), and one iridoid glucoside, piscroside D (**11**), together with eleven known glucosides (▶ **Fig. 1**). The isolated compounds were assayed for their α-glucosidase inhibitory activity using *p*-nitrophenyl-α-D-glucopyranoside (PNPG) as a substrate.

Results and Discussion

Compound **1** was isolated as an off-white amorphous powder and had the molecular formula $C_{23}H_{26}O_9$ as indicated by the quasi-molecular ion in the HRESIMS at *m/z* 469.1470 $[M + Na]^+$ (calcd.



▶ **Fig. 1** Structures of compounds **1**–**15**.

469.1475). The IR spectrum indicated the presence of hydroxyl (3434 cm^{-1}) and double bond functionalities (1638 and 1443 cm^{-1}). The ^1H NMR spectrum exhibited characteristic signals for aromatic rings (two AA'BB' systems at the δ_H 6.62–7.51 region), *trans*-olefinic protons (AB system, δ_H 7.66 and 6.34, both *d*, J_{AB} = 16.0 Hz), and an ethyl residue (δ_H 2.73, 2H, *t*, *J* = 6.7 Hz; δ_H 3.66, 1H, *m* and 4.05, 1H, *dd*, *J* = 15.6, 6.7 Hz) at the benzylic position. Comparing the ^1H and ^{13}C NMR data (▶ **Table 1**) with those of osmanthuside A suggested that compound **1** consisted of a *p*-coumaroyl group, a *p*-substituted phenylethyl alcohol moiety, and a glucose residue [16]. A doublet proton resonance at δ_H 4.53 (*d*, *J* = 8.0 Hz) from the anomeric proton of glucose and the corresponding carbon resonance at δ_C 102.4 (C-1′) revealed the *β*-configuration of the latter. Acid hydrolysis of **1** afforded D-glucose, which was identified by TLC comparison with an authentic sample and measurement of the optical rotation value. The *p*-coumaroyl moiety was located at C-2′ of the glucose on the basis of the strong deshielding of the H-2′ signals of the glucose unit (δ_H 4.84, 1H, *m*) and the HMBC correlations (▶ **Fig. 2**) from H-2′ to C-9″ (δ_C 168.9). Compound **1** differs in this way from osmanthuside A in that the *p*-coumaroyl residue is located at C-6′ [16]. Finally, the structure of heterophoside A (**1**) was established as 2-(4-hydroxyphenyl)ethyl 2′-*O*-*trans*-*p*-coumaroyl-*β*-D-glucopyranoside.

Compound **2** was obtained as an off-white amorphous powder. In the positive ion HRESIMS, a quasi-molecular ion peak at *m/z* 529.1685 $[M + Na]^+$ was observed, which was consistent with the molecular formula $C_{25}H_{30}O_{11}$. The IR spectrum indicated the existence of hydroxyl (3435 cm^{-1}) and double bond functionalities (1651 and 1444 cm^{-1}). The ^1H NMR spectrum of **2** displayed aromatic protons at δ_H 6.59–7.12 (two ABX systems) and *trans*-olefinic protons at δ_H 7.62 and 6.38, both *d*, J_{AB} = 15.9 Hz (AB system). The NMR data (▶ **Table 1**) of **2** were very similar to that of plantainoside E, except for the sugar region [17]. The major difference between these two compounds was the presence of signals for only one glucose moiety in the NMR spectra of **2**. Based on HMBC correlations from H-1′ (δ_H 4.35) of glucose to C-8 (δ_C 72.4) of the 3,4-disubstituted phenylethyl unit, and from H_2-6′ (δ_H 4.53, 4.40) of glucose to C-9″ (δ_C 169.2) of the feruloyl group, compound **2** was determined as 2-(3-hydroxy-4-methoxyphenyl) ethyl 6′-*O*-*trans*-feruloyl-*β*-D-glucopyranoside, named as heterophoside B.

Compound **3** was also obtained as an off-white amorphous powder. The quasi-molecular ion at *m/z* 529.1685 $[M + Na]^+$ in the positive HRESIMS was consistent with the molecular formula $C_{25}H_{30}O_{11}$. Careful analysis of the 1D and 2D NMR data (▶ **Table 1**) from **3** and **2** revealed that they were almost identical to each other, except for the olefinic proton resonance of the feruloyl moiety. The coupling constant of $J_{H\text{-}7'',H\text{-}8''}$ = 12.8 Hz in **3** versus $J_{H\text{-}7'',H\text{-}8''}$ = 15.9 Hz in **2** suggested the presence of *cis*-olefinic protons in **3**. On the basis of these data, the structure of heterophoside C (**3**) was elucidated as 2-(3-hydroxy-4-methoxyphenyl)ethyl 6′-*O*-*cis*-feruloyl-*β*-D-glucopyranoside.

Compound **11** was isolated as an off-white amorphous powder and had the molecular formula $C_{25}H_{32}O_{12}$ as indicated by the quasi-molecular ion at *m/z* 559.1591 $[M + Cl]^-$ in HRESIMS. The ^1H and ^{13}C NMR spectra (▶ **Table 2**) revealed the presence of a C_9-type iridoid aglycone, a sugar residue, and a *trans*-cinnamoyl moiety, thus indicating that compound **11** was an analog of piscroside B with the same molecular formula [18]. Comparison of their NMR data revealed that C-10 at δ_C 61.5 in piscroside B was downfield shifted to δ_C 64.6 in **11**, while H-6′a at δ_H 4.44 and H-6′b at δ_H 4.53 in piscroside B were upfield shifted to δ_H 3.94 and 3.68 in **11**. This suggested that the esterification position of the *trans*-cinnamoyl residue was changed from C-6′ in piscroside B to C-10 in **11**. The HMBC correlation of H_2-10/C-9″ and ^1H-^1H COSY correlations of H-7″/H-8″, H-2″/H-3″, H-6″/H-5″, and H-3″/H-4″/

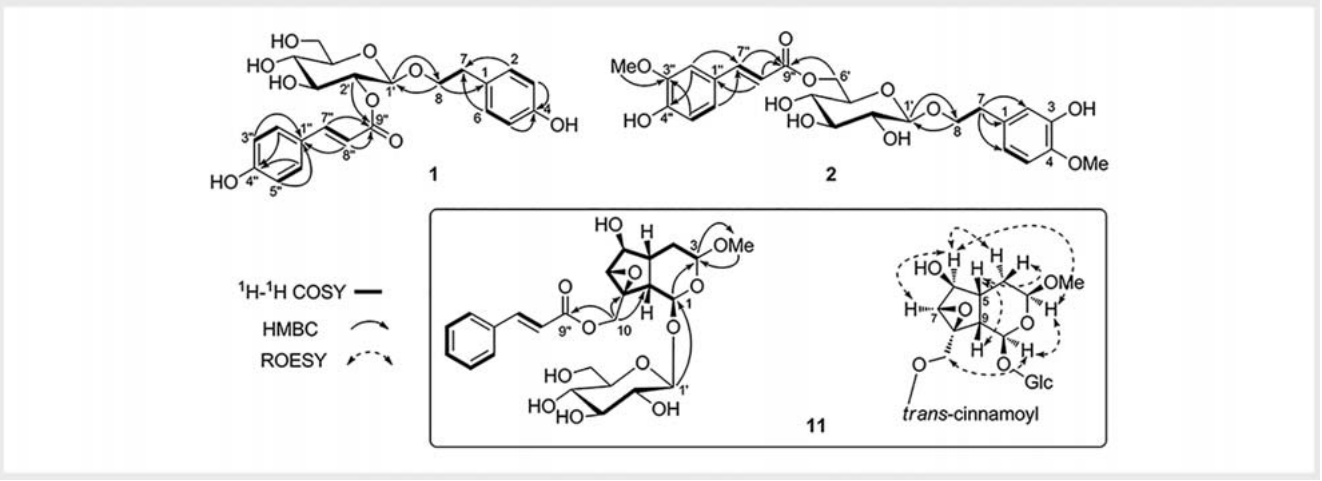
▶ **Table 1** ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectroscopic data of compounds **1–3** (δ in ppm, J in Hz) in CD_3OD.

No.	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	131.1		132.7		133.0	
2	131.1	7.00 d (7.8)	117.1	6.71 s	117.2	6.72 s
3	116.2	6.63 d (7.8)	147.3		147.3	
4	156.7		147.5		147.5	
5	116.2	6.63 d (7.8)	112.8	6.65 d (8.1)	115.2	6.67 m
6	131.1	7.00 d (7.8)	121.2	6.60 d (8.1)	121.3	6.65 m
7	36.4	2.73 t (6.7)	36.8	2.81 t (7.5)	36.8	2.81 m
8	72.0	4.05 dd (15.6, 6.7)	72.4	3.95 dd (16.7, 8.1)	72.3	3.94 m
		3.66 m		3.76 m		3.73 m
OMe			56.5	3.74 s	56.5	3.78 s
1′	102.4	4.53 d (8.0)	104.6	4.35 d (7.9)	104.6	4.32 d (7.6)
2′	75.3	4.84 m	75.1	3.27 t (8.1)	75.2	3.23 t (8.3)
3′	76.3	3.61 m	78.0	3.43 m	78.1	3.37 m
4′	71.8	3.43 t (9.1)	72.0	3.40 m	72.0	3.34 m
5′	78.2	3.35 m	75.4	3.57 m	75.5	3.53 m
6′	62.7	3.92 d (11.8)	64.8	4.53 d (11.7)	64.6	4.52 d (11.9)
		3.73 dd (11.8, 5.3)		4.40 dd (11.7, 6.0)		4.37 dd (11.9, 5.9)
1″	127.3		127.7		128.1	
2″	131.4	7.50 d (8.0)	111.7	7.12 s	113.0	7.16 s
3″	117.1	6.85 d (8.0)	149.4		147.6	
4″	161.5		150.7		148.5	
5″	117.1	6.85 d (8.0)	116.6	6.81 d (8.1)	116.3	6.80 d (8.1)
6″	131.4	7.50 d (8.0)	124.4	7.00 d (8.1)	127.0	7.15 m
7″	146.9	7.66 d (16.0)	147.2	7.62 d (15.9)	145.9	6.89 d (12.8)
8″	115.4	6.34 d (16.0)	115.3	6.38 d (15.9)	115.9	5.85 d (12.8)
9″	168.9		169.2		168.3	
OMe			56.5	3.84 s	56.6	3.89 s

H-5″ provided additional evidence (▶ **Fig. 2**). It should be mentioned that all naturally occurring 7,8-epoxy-iridoids exhibit a β-epoxide function according to literature data [19–21]. The ROESY spectrum (▶ **Fig. 2**) displayed correlations between α-oriented H-7 (δ_H 3.50) and H-6 (δ_H 4.14), and also between H-6 and H-3 (δ_H 4.67), H-1 (δ_H 5.11), H-4 (δ_H 1.91), indicating the coaxial α-orientation of H-6, H-3, H-1, and H-4 (▶ **Fig. 2**). The large coupling constant for H-1α (d, J = 8.6 Hz) and H-9 (d, J = 8.1 Hz), suggested that they were *trans*-diaxial oriented, thus positioning H-9 in β-orientation [22]. Proton H-9 showed a strong ROESY correlation with H-5 (δ_H 2.11), consistent with the large coupling constant between H-6α (d, J = 9.1 Hz) and H-5 (m), which confirmed the *cis*-junction of the iridoid skeleton. Although no correlation was observed between the β-oriented methoxyl group (δ_H 3.52) and H-9β (δ_H 2.49) or H-5β (δ_H 2.11), its β-orientation was indirectly inferred from the ROESY correlations and J-couplings of the iridoid ring. Thus, compound **11**, named piscroside D, was determined to be 10-O-*trans*-cinnamoyl-3β-methoxy-3,4-dihydrocatalpol.

In addition, 11 known compounds, eutigoside A (**4**) [23], 2-(4-hydroxyphenyl)ethyl 6′-O-sinapoyl-β-D-glucopyranoside (**5**) [24, 25], grayanoside A (**6**) [26], bacopaside B (**7**) [27], osmanthuside E (**8**) [28], citrusin C (**9**) [29], 3-methoxyl-4-O-β-D-glucopyranosyloxy-benzoic acid methyl ester (**10**) [30], globularin (**12**) [31], globularicisin (**13**) [31], picroside I (**14**) [32], and *iso*-scrophulariosid (**15**) [33], were identified by comparing their NMR data with literature values (▶ **Fig. 1**).

The isolated compounds were tested for their α-glucosidase inhibitory activities with acarbose as the positive control. All compounds except **11** and **13–15** showed varying degrees of activity (▶ **Table 3**). Compounds **5, 9**, and **12** were marginally active in the bioassay, while compounds **1–4, 6–8**, and **10** exhibited appreciable inhibition of α-glucosidase. Despite the limited number of compounds tested here, a preliminary analysis of the structure-activity relationship for α-glucosidase inhibitory activity was possible. The activity of compound **1** was very similar to that of compound **4**, indicating that changes in the glucose-coumaroyl connectivity from C-2′ to C-6′ has little impact on α-glucosidase in-



▶ **Fig. 2** Key 2D NMR correlations of compounds **1**, **2**, and **11**.

▶ **Table 2** ^{13}C (100 MHz) and ^{1}H (400 MHz) NMR spectroscopic data of compound **11** in CD_3OD (δ in ppm, J in Hz).

No.	δ_C	δ_H
1	96.1	5.11 d (8.6)
3	100.2	4.67 d (8.9)
4α	29.8	1.91 br d (12.4)
4β		1.65 m
5	38.3	2.11 m
6	74.8	4.14 d (9.1)
7	62.5	3.50 br s
8	63.5	
9	43.4	2.49 t (8.1)
10	64.6	4.99 d (12.7)
		4.22 d (12.7)
OMe	56.6	3.52 s
1′	100.0	4.73 d (7.9)
2′	74.6	3.18 m
3′	77.9	3.37 m
4′	71.6	3.27 m
5′	78.5	3.30 m
6′	63.2	3.94 d (11.8)
		3.68 dd (11.8, 6.2)
1″	135.9	
2″, 6″	129.5	7.65 2H d (3.9)
3″, 5″	130.2	7.43 2H m
4″	131.8	7.43 m
7″	146.9	7.74 d (16.0)
8″	118.7	6.59 d (16.0)
9″	168.6	

▶ **Table 3** α-Glucosidase inhibitory activities of compounds **1**–**15**.

Name	IC_{50} (μM)[a]
1	33.9 (31.9–34.9)
2	88.6 (85.8–91.4)
3	50.6 (49.4–53.7)
4	33.6 (30.7–35.1)
5	208.8 (198.9–213.7)
6	46.7 (43.6–49.5)
7	48.9 (46.7–50.1)
8	41.2 (40.1–42.3)
9	109.0 (108.0–112.1)
10	83.1 (82.8–86.4)
11	> 400
12	335.2 (332.1–337.7)
13	> 400
14	> 400
15	> 400
Acarbose	310.8 (308.4–313.7)

[a] IC_{50} values represent the means of three parallel measurements

hibitory activity. This was corroborated by the similar potency also observed for compounds **7** and **8** with a feruloyl group. The activity of compound **2**, bearing a *trans*-feruloyl group at C-6′ of glucose, was lower than that of **3** with a *cis*-feruloyl group. Moreover, compound **6** showed lower activity than **4**, implying that the activity was weakened by the introduction of a methoxyl group at the C-3 position. Compound **8** was slightly more potent than compound **6**, which has a hydroxyl group at the 3′-position. More generally, the phenylpropanoid glucosides (**1**–**10**) showed stronger α-glucosidase inhibitory effects than the iridoid glucosides (**11**–**15**), which suggests that phenylpropanoid glucosides might play the main role in the α-glucosidase inhibitory activity of the ethanol extract of *H. heterophyllum*.

Material and Methods

General experimental procedures

Silica gel (100–200, 200–300 mesh; Qingdao Marine Chemical, Inc.), MCI gel (75–150 µm; Mitsubishi Chemical Corporation), RP-18 reverse-phase silica gel (40–63 µm; Merck), and Sephadex LH-20 were used for column chromatography (CC). Semipreparative HPLC was performed on an Agilent 1260 liquid chromatograph equipped with an Eclipse XDB-C_{18} (9.4 × 250 mm, 5 µm) column. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets. UV spectra were recorded using a Shimadzu UV-2401A spectrophotometer. Optical rotations were obtained on a Horiba SEPA-300 high sensitivity polarimeter. NMR spectra were recorded on Bruker AM-400 spectrometers with TMS as an internal standard. HRESIMS was measured using an Agilent 1290 UPLC/6540 Q-TOF instrument. The α-glucosidase from *Saccharomyces cerevisiae* from Sigma-Aldrich Trading Co. Ltd., acarbose from J&K China Chemical Ltd., and PNPG from Sigma-Aldrich Trading Co. Ltd. were used in the bioactivity assay.

Plant material

Aerial parts and roots of *H. heterophyllum* were collected in Chuxiong City, Yunnan province, P. R. China, in July 2016. The plant material was authenticated by Prof. Qing-Song Yang at the Yunnan Minzu University. A voucher specimen (No. 2016-07-23) was deposited at the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, School of Ethnomedicine and Ethnopharmacy, Yunnan Minzu University.

Extraction and isolation

The air-dried whole plants (20.0 kg) of *H. heterophyllum* were powdered and extracted three times with 95% EtOH (60 L) at room temperature for 48 h. The three extracts were combined and concentrated under reduced pressure to give a sticky residue (3.8 kg). The residue was suspended in water (4 L) and then partitioned sequentially with petroleum ether (6 × 5 L), EtOAc (6 × 5 L), and *n*-BuOH (6 × 5 L). The EtOAc-soluble fraction (2.0 kg) was fractionated in two portions on a silica gel column (100–200 mesh, 15 × 120 cm) eluted with a $CHCl_3$/MeOH gradient system (10 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 0 : 10, v/v; collection size: 1000 mL) to yield five fractions (Frs I~V) after TLC analysis.

Fraction IV (545 g), with moderate α-glucosidase inhibitory activity (IC_{50} = 172.1 µg/mL), was fractionated by silica gel CC (200–300 mesh) with $CHCl_3$/MeOH (50 : 1, 30 : 1, 10 : 1, 5 : 5, 0 : 1, v/v) to provide subfractions IV_1~IV_7. Subfraction IV_1 (6.0 g) was subjected to silica gel CC (200–300 mesh, $CHCl_3$/MeOH, 50 : 1, v/v) to yield a fraction (310 mg), which was purified by semipreparative HPLC (MeOH/H_2O, 60 : 40, v/v, flow rate of 3 mL/min), to yield compound **11** (10.0 mg, t_R 23.7 min). Subfraction IV_2 (38.0 g) was chromatographed by MCI gel CC with MeOH/H_2O (0 : 100, 50 : 50, 70 : 30, 90 : 10, 100 : 0, v/v) to yield eight subfractions, $IV_{2.1}$~$IV_{2.9}$. Subfraction $IV_{2.2}$ (2.2 g) was purified by Sephadex LH-20 CC ($CHCl_3$/MeOH, 1 : 1, v/v) to furnish compounds **1** (9.5 mg), **7** (23.5 mg), and **9** (12.0 mg). Subfraction $IV_{2.3}$ (897 mg) was subjected to Sephadex LH-20 CC with MeOH to yield a fraction (58.2 mg), which was purified on a silica gel column (200–300 mesh, $CHCl_3$/MeOH, 50 : 1, v/v), to give compound **14** (16.0 mg). Subfraction $IV_{2.4}$ (300 mg) was subjected to Sephadex LH-20 CC ($CHCl_3$/MeOH, 1 : 1, v/v) to provide compound **5** (6.1 mg). Subfraction $IV_{2.5}$ (1.9 g) was separated by silica gel CC (200–300 mesh) with petroleum ether/acetone (5 : 1, v/v) to afford eight subfractions, $IV_{2.5.1}$~$IV_{2.5.6}$. Compounds **6** (5.0 mg, t_R 29.7 min) and **8** (7.0 mg, t_R 24.5 min) were obtained from subfraction $IV_{2.5.4}$ by semipreparative HPLC with MeOH/H_2O (43 : 57, v/v) at a flow rate of 3 mL/min. Subfraction $IV_{2.5.5}$ (185 mg) was purified by Sephadex LH-20 CC with MeOH to yield compound **4** (8.2 mg). Subfraction $IV_{2.6}$ (2.5 g) was purified by Sephadex LH-20 CC (MeOH), followed by semipreparative HPLC (MeOH/H_2O, 47 : 53, v/v, flow rate of 3 mL/min) to give compounds **2** (11.1 mg, t_R 29.1 min) and **3** (5.3 mg, t_R 30.6 min). Subfraction $IV_{2.7}$ (1.5 g) was subjected to RP-18 CC (MeOH/H_2O, 40 : 60, 60 : 40, 80 : 20, 100 : 0, v/v), followed by Sephadex LH-20 CC (MeOH) to yield compound **10** (8.9 mg). Subfraction IV_4 (496 mg) was subjected to Sephadex LH-20 CC with MeOH elution to yield a fraction (95.2 mg), which was purified on semipreparative HPLC with MeOH/H_2O (54 : 46, v/v) at a flow rate of 3 mL/min, to give compound **13** (16.0 mg, t_R 32.3 min). Subfraction IV_5 (30.5 g) was chromatographed on an MCI gel column with MeOH/H_2O (30 : 70, 60 : 40, 90 : 10, 100 : 0, v/v) to yield eight subfractions, $IV_{5.1}$~$IV_{5.9}$. Compound **15** (12.0 mg, t_R 35.6 min) was isolated from subfraction $IV_{5.5}$ (33.0 mg) by semipreparative HPLC with MeOH/H_2O (58 : 42, v/v) at a flow rate of 3 mL/min. Subfraction $IV_{5.6}$ (408 mg) was subjected to Sephadex LH-20 CC with MeOH to provide compound **12** (6.0 mg).

Heterophoside A (***1***): Off-white amorphous powder; $[\alpha]_D^{25}$ − 36.8 (*c* 0.17, MeOH); UV (MeOH) λ_{max} (log ε): 212 (4.71), 225 (4.78), 313 (4.86) nm; IR (KBr) u_{max}: 3434, 2026, 1638, 1443, 1076, 859, 546 cm^{-1}; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see ▶ **Table 1**; HRESIMS *m/z*: 469.1470 $[M + Na]^+$ (calcd. for $C_{23}H_{26}NaO_9$, 469.1475).

Heterophoside B (***2***): Off-white amorphous powder; $[\alpha]_D^{25}$ − 34.2 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε): 201 (5.08), 288 (4.64), 326 (4.78) nm; IR (KBr) u_{max}: 3435, 2026, 1651, 1444, 1269, 1121, 1072, 859, 545 cm^{-1}; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see ▶ **Table 1**; HRESIMS *m/z*: 529.1685 $[M + Na]^+$ (calcd. for $C_{25}H_{30}NaO_{11}$, 529.1686).

Heterophoside C (***3***): Off-white amorphous powder; $[\alpha]_D^{25}$ − 23.5 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε): 204 (5.58), 288 (4.99), 326 (5.14) nm; IR (KBr) u_{max}: 3431, 2027, 1657, 1442, 1270, 1117, 857, 545 cm^{-1}; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see ▶ **Table 1**; HRESIMS *m/z*: 529.1685 $[M + Na]^+$ (calcd. for $C_{25}H_{30}NaO_{11}$, 529.1687).

Piscroside D (***11***): White amorphous powder; $[\alpha]_D^{25}$ − 48.3 (*c* 0.20, $CHCl_3$); UV (MeOH) λ_{max} (log ε): 283 (4.72) nm; IR (KBr) u_{max}: 3441, 2326, 1655, 1424, 1263, 1120, 1066, 863, 544 cm^{-1}; For ^1H NMR (CD_3OD, 400 MHz) and ^{13}C NMR (CD_3OD, 100 MHz) spectroscopic data, see ▶ **Table 2**; HRESIMS *m/z*: 559.1591 $[M + Cl]^-$ (calcd. for $C_{25}H_{32}ClO_{12}$, 559.1582).

α-Glucosidase inhibitory assay

The α-glucosidase inhibitory assay was carried out based on a previously described spectrophotometric method with slight modifications [34]. Acarbose was used as the positive control. Briefly, a total of 150 µL of reaction mixture containing 50 µL of 100 mM phosphate buffer (pH 6.8) and 10 µL of the sample at various concentrations was added to each well of a 96-well plate, followed by 20 µL of 0.2 U/mL α-glucosidase solution. The plate was incubated at 37 °C for 5 min, and then 20 µL of 2.5 mM PNPG substrate were added to the mixture to start the reaction. The reaction mixture was incubated at 37 °C for a further 15 min, and then 50 µL of 0.4 M Na_2CO_3 solution were added to terminate the reaction. The absorbance was immediately recorded at 400 nm using a microplate spectrophotometer (Multiskan Go; ThermoFisher). The IC_{50} values were calculated by log-linear regression using Microsoft Excel 2010 software.

Acid hydrolysis and identification of sugar

The glucose moiety was identified according to a previously described method with slight modifications [35]. Compounds **1** and **11** (each 7.0 mg) were separately dissolved in 6% HCl (3 mL) and heated at 90 °C for 2 h. After neutralization with 1.0 N NaOH, the reaction mixture was extracted with EtOAc to remove the aglycone. The aqueous phase was concentrated and the residue subjected to CC over silica gel with MeCN/H_2O (8 : 1, v/v) to yield glucose (R_f 0.34), which was identified by TLC comparison [CH_3Cl/MeOH (2 : 1, v/v), spraying with a 10% H_2SO_4 ethanol solution] with an authentic sample. The absolute configuration was determined by measurement of the optical rotation value. Hydrolysate of **1**: $[\alpha]_D^{20}$ + 48.9 (*c* 0.03, H_2O); hydrolysate of **11**: $[\alpha]_D^{20}$ + 46.3 (*c* 0.02, H_2O).

Supporting information

The IR, UV, HRMS, 1D, and 2D NMR spectra of the new compounds are available as Supporting Information.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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