Note

Nitrile-Containing Phenolic Glucosides from the Leaves of *Glochidion acuminatum*

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From the EtOAc-soluble fraction of a MeOH extract of the leaves of *Glochidion acuminatum*, six new compounds along with five known ones were isolated. The structures of the new compounds were elucidated to be two gallates, a *p*-hydroxybenzoate and an (*S*)-2-(4-hydroxycyclohex-1-en-1-yl)acetate of a nitrile-containing phenolic glucoside, methyl 2-(2-hydroxyphenyl)acetate *β*-D-glucopyranoside, and (*S*)-methyl 2-[4-sulfooxycyclohex-1-en-1-yl]acetate on the basis of spectroscopic evidence.

Key words *Glochidion acuminatum*; Euphorbiaceae; 2-(2,4-dihydroxyphenyl)acetonitrile; gallate; sulfate

In previous works, a novel dimeric butenolide[1] and C-8 compounds[2] were isolated from the 1-BuOH-soluble fraction of a methanol (MeOH) extract of leaves of *Glochidion acuminatum* Müller-Argoviensis. It is known that the larvae of the *Epicephala* moth feed on the fruit of *G. acuminatum*.[3] From the EtOAc-soluble fraction of a MeOH extract of the leaves of *G. acuminatum*, two gallic acid (**1**, **2**), one *p*-hydroxybenzoic acid (**3**), and one (*S*)-1-(4-hydroxycyclohex-1-en-1-yl)acetic acid ester (**4**) of a nitrile-containing phenolic glucoside, a methyl 2-(2-hydroxyphenyl)acetate *β*-D-glucopyranoside (**5**), and a (*S*)-methyl 2-[4-sulfooxycyclohex-1-en-1-yl]acetate (**6**), along with five known compounds, (+)-menisdaurilide (**7**),[4] (+)-aquilegiolide (**8**),[4] (−)-loliolide (**9**),[5] (+)-epigallocatechin 3-*O*-gallate (**10**),[6] and phloretin 4′-*O*-*β*-D-glucopyranoside (**11**),[7] were isolated (Fig. 1).

Compounds **1**–**6** were isolated using various separation methods, *i.e.* normal and reversed-phase silica gel column chromatography, HPLC and droplet counter-current chromatography. The structures of the new compounds were elucidated on the basis of spectroscopic evidence and those of the known compounds were identified by comparison with reported spectral data in the literature.

Compound **1**, $[\alpha]_D$ −45.1, was isolated as an amorphous powder and its elemental composition was determined to be $C_{21}H_{21}NO_{11}$ by observation of a quasi-molecular ion peak in high-resolution (HR) electrospray-ionization (ESI) MS. The IR spectrum exhibited absorptions due to hydroxy groups (3323 cm^{-1}), a triple bond (2260 cm^{-1}), an ester carbonyl group (1699 cm^{-1}), an aromatic ring (1614 and 1511 cm^{-1}), and phenolic and aliphatic C–O bonds (1239 and 1073 cm^{-1}), respectively, and the presence of the aromatic ring was also supported by the UV absorption band at 278 nm. The ^{1}H-NMR spectral data comprised three aromatic protons [δ_H 6.51 (dd, J=8, 2 Hz), 6.69 (d, J=2 Hz) and 7.12 (d, J=8 Hz)], coupled in an ABX system, one singlet aromatic signal for two protons (δ_H 7.08), isolated methylene protons [δ_H 3.77 (d, J=11 Hz) and 3.78 (d, J=11 Hz)] and an anomeric proton [δ_H 4.87 (d, J=7 Hz)]. The ^{13}C-NMR spectrum exhibited five typical signals [δ_C 110.4×2 (d), 121.4 (s), 139.9 (s), 146.5×2 (s), and 168.4 (s)] assignable to a gallic acid moiety (Table 1), and acid hydrolysis of **1** liberated D-glucose as a sugar component. Of the remaining eight carbon signals, δ_C 120.1 (s) was assigned as that of a nitrile functional group with a typical IR absorption band at 2260 cm^{-1}, and six sp^2 signals as those of a trisubstituted benzene ring (Table 1). The heteronuclear multiple bond correlation (HMBC) spectroscopy (Fig. 2) between methylene protons (δ_H 3.77 and 3.78) and the nitrile carbon, C-2 and C-6 [with δ_H 7.12 (d, J=8 Hz)], H-5 and C-1 and 3, and H-6 and C-2 and 4, established the structure of the aglycone to be as shown in Fig. 1. This substitution arrangement is rarely found in nature. Further correlations, the anomeric proton with C-2 and H_2-6′ with the carbonyl carbon of the galloyl moiety, were also observed in the HMBC spectrum (Fig. 2). Mild alkaline hydrolysis of **1** with $NaOCH_3$ in MeOH gave a deacylated derivative, 2-(2,4-dihydroxyphenyl)acetonitrile 2-*O*-*β*-D-glucopyranoside (**1a**) (=**12** in Fig. 1). Therefore, the structure of **1** was elucidated to be 2-(2,4-dihydroxyphenyl)acetonitrile 2-*O*-*β*-D-glucopyranoside 6′-*O*-gallate, as shown in Fig. 1. 2-(2,4-Dihydroxyphenyl)acetonitrile 2-*O*-*β*-D-glucopyranoside (**1a**=**12**) was first isolated in 1994 from *Ehretia philippinensis*, as ehretioside B,[8] while in 2005, Su *et al.* claimed the first isolation of **12** from the roots of *Semiaquilegia adoxoides* (Ranunculaceae) as a novel cyanogenic glucoside.[9] However, compound **12**, isolated by Su *et al.* is ehretioside B (**1a**) and it is not cyanogenic.

Compound **2**, $[\alpha]_D$ −5.12, was isolated as an amorphous powder and its elemental composition was determined to be $C_{28}H_{25}NO_{15}$ by HR-ESI-MS. The IR and UV spectra were similar to those of **1**, and the NMR spectra also showed close resemblance with those of **1**. In the ^{1}H-NMR spectrum, two two-proton singlet signals (δ_H 7.10 and 7.17) were observed in the aromatic region and five sets of signals assignable to a gallic acid moiety were observed in the ^{13}C-NMR spectrum. The elemental composition of **2** coincided with the presence of one more galloyl unit in **2** than in **1**. In the NMR spectra, H-3′ was obviously shifted downfield (δ_H 5.26), and C-3′ shifted downfield by 1.0 ppm, and both C-2′ and C-4′ shifted upfield by 1.5 ppm (Table 1), when these signals were compared with

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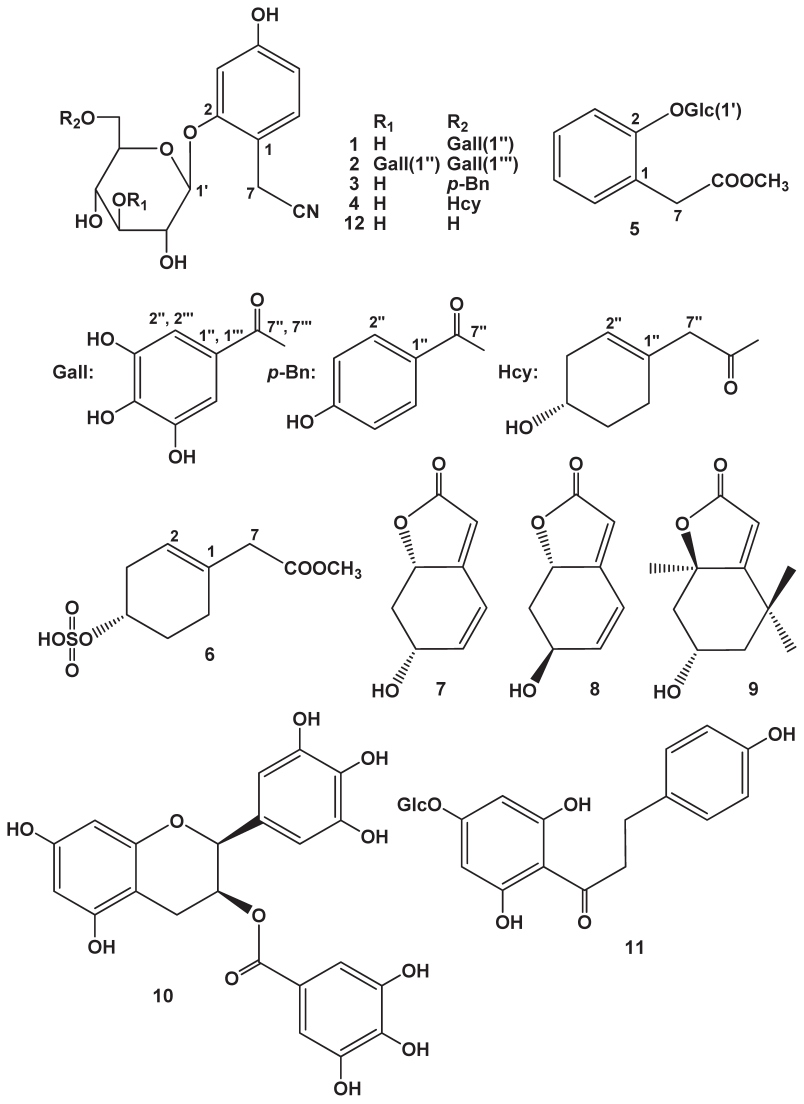

Fig. 1. Structures of Compounds Isolated

those of **1**. In the HMBC spectrum, H-3′ showed a significant correlation peak with one of the carbonyl groups in the galloyl moiety and a H-1′–H-2′–H-3′–H contiguous relation was observed on ^{1}H–^{1}H correlation spectroscopy. Therefore, the structure of **2** was elucidated to be 2-(2,4-dihydroxyphenyl)-acetonitrile 2-*O*-*β*-D-glucopyranoside 3′,6′-*O*-digallate, as shown in Fig. 1.

Compound **3**, $[\alpha]_D$ −42.9, was isolated as an amorphous powder and it elemental composition was determined to be $C_{21}H_{21}NO_9$. Its IR spectrum also exhibited a characteristic absorption for a triple bond and its NMR spectra were similar to those of compound **1**. Since the aromatic proton signals of the acyl substituent appeared as AB doublets, 7.90 (2H, d, *J*=9 Hz, H-2″ and 6″) and 6.82 (2H, d, *J*=9 Hz, H-2″ and 5″), together with the presence of a highly deshielded carbon signal at δ_C 163.6, the galloyl group in **1** must be replaced by *p*-hydroxybenzoate. Therefore, the structure of **3** was elucidated to be 2-(2,4-dihydroxyphenyl)acetonitrile 2-*O*-*β*-D-glucopyranoside 6′-*O*-*p*-hydroxybezoate, as shown in Fig. 1.

Compound **4**, $[\alpha]_D$ −61.8, was isolated as an amorphous powder and its elemental composition was $C_{22}H_{27}NO_9$. Spectroscopic evidence revealed that compound **4** was a similar compound to **1**–**3**, the acyl substituent being different. As revealed by the NMR spectroscopic data, the acyl moiety comprised one trisubstituted double bond, and four methylenes, one oxygenated methine carbon and a carbonyl carbon. Judging from this elemental composition, one degree of unsaturation must be satisfied by the formation of a ring system in the acyl framework. The above evidence substantiates that the structure of the acyl group was 2-(4-hydroxycyclohex-1-en-1-yl)acetate, which is found as an acyl moiety in various compounds, isolated from the 1-BuOH-soluble fraction of the same MeOH extract of the title plant.[2] Since the absolute configuration at the 4-position of the methyl 2-(4-hydroxycyclohex-1-en-1-yl)acetate, isolated as an alkaline hydrolysis product from glochidacuminoside B, which was obtained from the same plant,[2] was determined by the modified Mosher's method, it must have the same *S* configuration. Therefore, the structure of **4** was elucidated to be 2-(2,4-dihydroxyphenyl)acetonitrile 2-*O*-*β*-D-glucopyranoside 6′-*O*-(*S*)-2-(4-hydroxycyclohex-1-en-1-yl)acetate, as shown in Fig. 1.

Compound **5**, $[\alpha]_D$ −38.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{15}H_{20}O_8$. In the ^{13}C-NMR spectrum, six typical signals were

Table 1. ^{13}C-NMR Spectra Data for Compounds **1**–**5** and **1a** (100 MHz, CD_3OD)

C	**1**	**1a**	**1a**[a)]	**2**[b)]	**3**[c)]	**4**[c)]	**5**		**6**[c)]
1	113.2	112.5	111.1	113.2	112.7	112.7	125.7		
2	157.3	157.2	157.1	157.2	157.2	157.3	157.2		
3	105.5	104.8	104.8	105.6	105.0	105.5	116.8		
4	159.7	159.9	160.3	159.6	160.1	159.7	129.7		
5	111.3	110.8	110.5	111.5	110.9	111.3	123.6		
6	130.8	130.8	130.2	130.8	130.7	130.8	132.1		
7	18.2	18.1	17.9	18.2	18.2	18.2	36.6		
8	120.1	120.2	119.5	120.0	120.1	120.1	174.7		
1′	103.6	103.1	103.3	103.6	103.1	103.2	103.0		
2′	74.9	74.9	74.8	73.4	74.8	74.8	75.0		
3′	77.9	78.1	78.4	78.9	77.9	77.9	78.2		
4′	71.4	71.3	71.0	69.9	71.8	71.6	71.4		
5′	75.8	78.3	78.8	75.5	75.8	75.6	78.1		
6′	64.5	62.5	62.2	64.3	65.0	64.6	62.6		
1″	121.4			121.4[a]	122.1	132.3		1	132.4
2″	110.4			110.4[b]	133.1	124.2		2	123.6
3″	146.5			146.48[c]	116.3	35.2		3	32.8
4″	139.9			139.94[d]	163.6	64.4		4	75.1
5″	146.5			146.48[c]	116.3	32.0		5	29.4
6″	110.4			110.4[b]	133.1	28.1		6	27.3
7′	168.4			168.27[e]	168.2	43.4		7	43.3
8″						173.6		8	174.0
$-OCH_3$							52.5		52.3
1‴				121.8[a]					
2‴				110.5[b]					
3‴				146.53[c]					
4‴				139.86[d]					
5‴				146.53[c]					
6‴				110.5[b]					
7‴				168.29[e]					

a) Data for pyridine-d_5. *b*) Data with the same superscript may be interchanged. *c*) Data for 150 MHz.

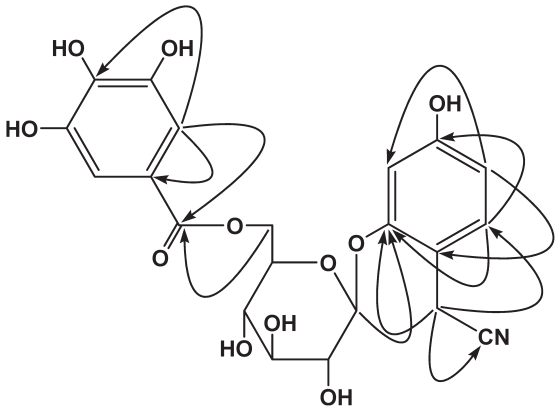

Fig. 2. Diagnostic HMBC Correlations for **1**

assigned as those of *β*-glucopyranoside,[2)] and the aglycone portion comprised a disubstituted benzene ring, and methylene and carbomethoxy carbons. The four aromatic protons were arranged in sequence and thus the structure of **5** was elucidated to be methyl 2-(2-hydroxyphenyl)acetate *β*-D-glucopyranoside, as shown in Fig. 1. From the 1-BuOH soluble fraction, a metal salt of 2-(2-hydroxyphenyl)acetic acid *β*-D-glucopyranoside was isolated,[2)] and its metal free form was also expected as a new compound. However, the metal-free form was first isolated by Zhang *et al.* from the roots of *Phyllanthus emblica*[10)] and its methyl ester (**5**) may be an artifact formed during the isolation procedures.

Compound **6**, $[\alpha]_D^{26}$ +3.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_9H_{14}O_6S$ by HR-ESI-MS in a negative-ion mode. NMR spectra were similar to those of the acyl moiety of compound **4** with a signal of methoxy group. In Table 1, the ^{13}C-NMR data for **6** are listed in the double prime section for comparison with those of the acyl region of **4**. The 4-position was obviously shifted downfield ($\Delta\delta$ +10.7), and the 3- and 5-positions upfield ($\Delta\delta$ −2.4 and −2.6, respectively). Therefore, the structure of **6** was expected to be the sulfuric acid ester of a methyl 2-(4-hydroxycyclohex-1-en-1-yl)acetate, as shown in Fig. 1. The absolute configuration at the 4-position was also expected to be *S* from the co-occurring compounds in *G. acuminatum*, although the optical rotation sign was opposite to that of (*S*)-methyl 2-(4-hydroxycyclohex-1-en-1-yl)acetate in MeOH, because sulfation had taken place on the hydroxy group, which is a crucial position for induction of optical activity.

Experimental

General Experimental Procedures Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM *α*-400 at 400 MHz and 100 MHz, respectively, or a Bruker Avance III at 600 MHz and 150 MHz, respectively, with tetramethylsilane

as an internal standard. HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSpray™ System.

Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and for octadecyl silica (ODS) open CC Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) was used. The droplet counter-current chromatograph (DCCC) (DCC-300 supplemented with extra 200 columns, Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ=2 mm, L=40 cm), the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O–1-PrOH (9 : 12 : 8 : 2) being used as the stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC (JASCO PU-980) was performed on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ=6 mm, L=250 mm, 1.6 mL/min), and the eluate was monitored with a UV detector (JASCO UV-975) at 254 nm, and a refractive index monitor (JASCO RI-930).

Plant Material Leaves of *G. acuminatum* Müller Argoviensis (Euphorbiaceae) were collected in Okinawa, Japan, in August 1995, and a voucher specimen was deposited in the Herbarium of the Department of Pharmacognosy, Graduate School of Biomedical Sciences, Hiroshima University (95-GA-Okinawa-0801). The plant was identified by Dr. Takakazu Shinzato of Subtropical Field Science Center, Faculty of Agriculture, Unuversity of the Ryukyus.

Extraction and Isolation The air-dried leaves of *G. acuminatum* (4.76 kg) were extracted three times with MeOH (30 L). The MeOH extract was concentrated to 3.0 L and then 150 mL of H_2O was added to make a 95% aqueous solution. This solution was washed with 3.0 L of *n*-hexane and then the methanolic layer was concentrated to a viscous gum (823 g). The gummy residue was suspended in 3.0 L of H_2O, and then extracted successively with 3.0 L each of EtOAc and 1-BuOH to afford 195 g and 288 g of EtOAc- and 1-BuOH-soluble fractions, respectively. The EtOAc-soluble fraction (194 g) was subjected to silica gel (900 g) CC with stepwise increases in the acetone contents in toluene {toluene 3 L, toluene–acetone [9 : 1 (3 L), 6 : 1 (3 L), 4 : 1 (3 L), 3 : 1 (3 L), 2 : 1 (3 L), 1 : 1 (3 L) and 1 : 2 (3 L]) and toluene–MeOH [5 : 1 (3 L), 4 : 1 (3 L), and 3 : 1 (3 L)], 500-mL fractions being collected. The residue (3.37 g) in fractions 23–25 was repeatedly subjected to silica gel (60 g) CC with stepwise increases in the acetone contents in $CHCl_3$ {$CHCl_3$ 500 mL, $CHCl_3$–actone [49 : 1 (500 mL), 24 : 1 (500 mL), 47 : 3 (500 mL), 23 : 2 (500 mL), 9 : 1 (500 mL), 4 : 1 (500 mL), and 7 : 3 (500 mL)] and MeOH (2 L)}, 5-g fractions being collected. The residue (747 mg) in fractions 66–82 was separated by ODS CC (Φ=2 cm, L=40 cm) with MeOH–H_2O [1 : 1 (200 mL), 3 : 2 (200 mL), 7 : 3 (200 mL) 4 : 1 (200 mL), and 9 : 1 (200 mL)], and then MeOH (200 mL), 5-g fractions being collected. The residue (123 mg) in fractions 13–30 was finally purified by HPLC (MeOH–H_2O, 7 : 13) to give 38.1 mg of **9** from the peak at 15.0 min.

The residue (35.7 g) in fractions 26–29 obtained on the first silica gel CC was repeatedly subjected to silica gel (900 g) CC with stepwise increases in the acetone contents in $CHCl_3$ {$CHCl_3$ 3 L, $CHCl_3$–acetone [19 : 1 (3 L), 9 : 1 (3 L), 17 : 3 (3 L), 4 : 1 (3 L), 7 : 3 (3 L), 3 : 2 (3 L), and; 1 : 1 (3 L)], and then $CHCl_3$–MeOH [3 : 1 (3 L), 2 : 1 (3 L), and 1 : 1 (3 L)]} and MeOH (3 L), 500-mL fractions being collected. The residue (2.53 g) in fractions 14–21 was separated by ODS CC (Φ=4.0 cm, L=22.5 cm) with a linear gradient [MeOH–H_2O (1 : 1, 1 L)→MeOH (1 L)→$CHCl_3$ (1 L)], 10-g fractions being collected. The residue (2.14 g) was finally purified by HPLC (MeOH–H_2O, 1 : 4) to yield 46.0 mg of **7** and 31.3 mg of **8** from the peaks at 6.0 min and 7.0 min, respectively. The residue (2.52 g out of 4.52 g) in fractions 22–31 was separated by ODS CC (Φ=4.0 cm, L=26.0 cm) with linear gradient [MeOH–H_2O (1 : 1, 1 L)→MeOH (1 L)→$CHCl_3$ (1 L)], 10-g fractions being collected. The residue (1.64 g) in fractions 6–15 was purified by silica gel CC (Φ=2.0 cm, L=23.0 cm) with a linear gradient from $CHCl_3$ (1 L) to $CHCl_3$–MeOH (9 : 1, 1 L), 15-g fractions being collected, to give 606 mg of **7** in fractions 4–27.

The residue (52.5 g) in fractions 30–34 obtained on the first silica gel CC was repeatedly subjected to silica gel (750 g) CC with stepwise increases in the MeOH content in $CHCl_3$ {$CHCl_3$ 3 L, $CHCl_3$–MeOH [39 : 1 (3 L), 19 : 1 (3 L), 37 : 3 (3 L), 9 : 1 (3 L), 17 : 3, (3 L), 4 : 1 (3 L), 3 : 1 (3 L), 7 : 3 (3 L), and 3 : 2 (3 L)]} and $CHCl_3$–MeOH–H_2O (60 : 40 : 3, 3.12 L), 500-mL fractions being collected. The residue (6.30 g out of 7.51 g) in fractions 19–27 was separated by ODS CC (Φ=2.0 cm, L=22.5 cm) with 10% stepwise increases in the MeOH content in H_2O from 10% MeOH to 100% MeOH (400 mL each), 10-g fractions being collected. The residue (220 mg) in fractions 125–138 was separated by silica gel CC (40 g) with stepwise increases in the MeOH contents in $CHCl_3$ {$CHCl_3$ 200 mL, $CHCl_3$–MeOH [19 : 1 (200 mL), 9 : 1 (200 mL), 17 : 3, (200 mL), 4 : 1 (200 mL), 3 : 1 (200 mL), and 7 : 3 (200 mL)]}, 10-g fractions being collected. The residue (32.5 mg) in fractions 111–131 was purified by HPLC [MeOH–H_2O (1 : 1), 2.0 mL/min] to give 8.8 mg of **5** from the peak at 12.0 min. The residue (42.3 mg) in fractions 132–143 was purified by HPLC [MeOH–H_2O (1 : 1), 2.0 mL/min] to give 4.9 mg of **4** and 16.2 mg of **3** from the peaks at 12.2 min and 13.4 min, respectively. The residue (19.9 mg) in fractions 144–159 obtained on the second silica gel CC was separated by HPLC [MeOH–H_2O (9 : 11), 2.0 mL/min] to give 2.7 mg of **11** from the peak at 35.4 min.

The residue (4.66 g out of 6.78 g) in fractions 28–33 obtained on the second silica gel CC was separated by ODS CC (Φ=4.0 cm, L=24.5 cm) with a linear gradient [MeOH–H_2O (1 : 1, 1 L)→MeOH (1 L)→$CHCl_3$ (1 L)], 10-g fractions being collected. The residue (200 mg) in fractions 27–48 was purified by silica gel CC (35 g) with stepwise increases in the MeOH content in $CHCl_3$ {$CHCl_3$ 200 mL, $CHCl_3$–MeOH [19 : 1 (200 mL), 9 : 1 (200 mL), 17 : 3, (200 mL), 4 : 1 (200 mL), 3 : 1 (200 mL), and 7 : 3 (200 mL)]}, 10-g fractions being collected, to give 5306 mg of **6** in fractions 177–200. The residue (387 mg) in fractions 83–99 was subjected to DCCC to give 114 mg of **10** in fractions 13–17 and 210 mg of **1** in fractions 18–24. The residue (5.90 g) in fractions 34–42 obtained on the second silica gel CC was purified by ODS CC (Φ=4.0 cm, L=24.5 cm) with a linear gradient [MeOH–H_2O (1 : 1, 1 L)→MeOH (1 L)→$CHCl_3$ (1 L)], 10-g fractions being collected. The residue (40.3 mg out of 130 mg) in fractions 141–150 was purified by HPLC (MeOH–H_2O, 1 : 1) to give 24.3 mg of **2** from the peak at 34.0 min.

Compound **1**: Off-white amorphous powder; $[\alpha]_D^{25}$ −45.1 (c=0.49, MeOH); IR ν_{max} (film) cm^{-1}: 3323, 2945, 2260, 1699, 1614, 1511, 1239, 1073, 1038; UV λ_{max} (MeOH) nm (logε): 278 (4.05), 216 (4.37); ^{1}H-NMR (400 MHz; CD_3OD) δ: 7.12 (1H, d, J=8 Hz, H-6), 7.08 (2H, s, H-2″ and 6″), 6.69 (1H, d, J=2 Hz, H-3), 6.51 (1H, dd, J=8, 2 Hz, H-5), 4.87 (1H, d,

J=7 Hz, H-1′), 4.58 (1H, dd, J=12, 2 Hz, H-6′a), 4.48 (1H, dd, J=12, 5 Hz, H-6′b), 3.78 (1H, d, J=11 Hz, H-7a), 3.77 (1H, d, J=11 Hz, H-7b), 3.54–3.51 (4H, m, H-2′, 3′, 4′, and 5′); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 486.1000 $[M+Na]^+$ (Calcd for $C_{21}H_{21}NO_{11}Na$: 486.1007).

Compound **2**: Off-white amorphous powder; $[\alpha]_D^{25}$ −5.1 (c=1.62, MeOH); IR ν_{max} (film) cm^{-1}: 3363, 2925, 2260, 1700, 1614, 1452, 1230, 1076, 1036; UV λ_{max} (MeOH) nm (log ε): 272 (4.17), 223 (4.29); ^{1}H-NMR (400 MHz; CD_3OD) δ: 7.14 (1H, d, J=8 Hz, H-6), 7.17 (2H, s, H-2″ and 6″, or H-2‴ and 6‴), 7.10 (2H, s, H-2‴ and 6‴, or H-2″ and 6″), 6.74 (1H, d, J=2 Hz, H-3), 6.53 (1H, dd, J=8, 2 Hz, H-5), 5.26 (1H, dd, J=9, 9 Hz, H-3′), 5.02 (1H, d, J=8 Hz, H-1′), 4.60 (1H, dd, J=12, 2 Hz, H-6′a), 4.50 (1H, dd, J=12, 5 Hz, H-6′b), 3.84–3.78 (4H, m, H-2′, 4′, and 5′), 3.77 (1H, d, J=11 Hz, H-7a), 3.76 (1H, d, J=11 Hz, H-7b); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 638.1105 $[M+Na]^+$ (Calcd for $C_{28}H_{25}NO_{15}Na$: 638.1116).

Compound **3**: Colorless amorphous powder; $[\alpha]_D^{26}$ −42.9 (c=1.08, MeOH); IR ν_{max} (film) cm^{-1}: 3332, 2959, 2267, 1726, 1605, 1511, 1234, 1071, 1013; UV λ_{max} (MeOH) nm (log ε): 259 (4.13), 215 (4.12); ^{1}H-NMR (600 MHz; CD_3OD) δ: 7.90 (2H, d, J=9 Hz, H-2″ and 6″), 7.13 (1H, d, J=8 Hz, H-6), 6.82 (2H, d, J=9 Hz, H-2″ and 5″), 6.72 (1H, d, J=2 Hz, H-3), 6.51 (1H, dd, J=8, 2 Hz, H-5), 4.91 (1H, d, J=7 Hz, H-1′), 4.69 (1H, dd, J=12, 2 Hz, H-6′a), 4.31 (1H, dd, J=12, 7 Hz, H-6′b), 3.80 (1H, d, J=18 Hz, H-7a), 3.75 (1H, d, J=18 Hz, H-7b), 3.74 (1H, m, H-5′), 3.54 (1H, dd, J=9, 7 Hz, H-2′), 3.51 (1H, dd, J=9, 9 Hz, H-3′), 3.46 (1H, dd, J=9, 9 Hz, H-4′); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 454.1108 $[M+Na]^+$ (Calcd for $C_{21}H_{21}NO_9Na$: 454.1109).

Compound **4**: Colorless amorphous powder; $[\alpha]_D^{26}$ −61.8 (c=0.33, MeOH); IR ν_{max} (film) cm^{-1}: 3363, 2927, 2256, 1726, 1605, 1587, 1517, 1171, 1073, 1020; UV λ_{max} (MeOH) nm (log ε): 268 (4.90), 209 (4.30); ^{1}H-NMR (600 MHz; CD_3OD) δ: 7.15 (1H, d, J=8 Hz, H-6), 6.64 (1H, d, J=2 Hz, H-3), 6.51 (1H, dd, J=8, 2 Hz, H-5), 5.45 (1H, brs, H-2″), 4.81 (1H, d, J=8 Hz, H-1′), 4.44 (1H, dd, J=12, 2 Hz, H-6′a), 4.25 (1H, dd, J=12, 6 Hz, H-6′b), 3.80 (1H, m, H-4″), 3.77 (2H, m, H_2-7), 3.61 (1H, ddd, J=9, 6, 2 Hz, H-5′), 3.49 (1H, dd, J=9, 9 Hz, H-2′), 3.46 (1H, dd, J=9, 9 Hz, H-3′), 3.38 (1H, dd, J=9, 9 Hz, H-4′), 3.02 (2H, s, H_2-7″), 2.28 (1H, brd, J=17 Hz, H-3″a), 2.10 (2H, m, H_2-6″), 1.94 (1H, brd, J=17 Hz, H-3″b), 1.80 (1H, m, H-5″a), 1.55 (1H, m, H-5″b); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 472.1576 $[M+Na]^+$ (Calcd for $C_{22}H_{27}NO_9Na$: 472.1578).

Compound **5**: Colorless amorphous powder; $[\alpha]_D^{26}$ −38.0 (c=0.59, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2938, 2878, 1726, 1587, 1528, 1227, 1075, 1042, 1014; UV λ_{max} (MeOH) nm (log ε): 266 (2.96), 214 (3.83); ^{1}H-NMR (600 MHz; CD_3OD) δ: 7.24 (1H, ddd, J=7, 1, 1 Hz, H-4), 7.20–7.18 (2H, m, H-3 and 6), 6.99 (1H, ddd, J=7, 1, 1 Hz, H-5), 4.88 (1H, d, J=8 Hz, H-1′), 3.88 (1H, dd, J=12, 2 Hz, H-6′a), 3.69 (1H, dd, J=12, 5 Hz, H-6′b), 3.46–3.38 (4H, m, H-2′, 3′, 4′, and 5′), 3.67 (3H, s, –OCH_3); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) m/z: 351.1055 $[M+Na]^+$ (Calcd for $C_{15}H_{20}O_8Na$: 351.1050).

Compound **6**: Colorless amorphous powder; $[\alpha]_D^{26}$ +3.5 (c=1.36, MeOH); IR ν_{max} (film) cm^{-1}: 3423, 1731, 1718, 1218, 1069; ^{1}H-NMR (600 MHz; CD_3OD) δ: 5.45 (1H, brs, H-2), 3.80 (1H, m, H-4), 3.66 (3H, s, –OCH_3), 3.00 (2H, s, H_2-7), 2.48 (1H, brd, J=17 Hz, H-3a), 2.24 (1H, brd, J=17 Hz, H-3b), 2.19 (2H, m, H-6a), 2.14 (1H, m, H-6b), 2.01 (1H, dddd, J=9, 6, 3, 1 Hz, H-5a), 1.85 (1H, m, H-5b); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (negative-ion mode) m/z: 249.0434 $[M-H]^-$ (Calcd for $C_9H_{13}O_6S$: 249.0427).

Mild Alkaline Hydrolysis of 1 Compound **1** (16 mg) was treated in 1 mL of 0.1 M CH_3ONa in MeOH for 3 h at 35°C. The reaction mixture was diluted with 4 mL of H_2O and then neutralized with Amberlite IR-120B (H^+). The aqueous layer was extracted with $CHCl_3$ (4 mL) and then evaporated to dryness. The residue was dissolved in MeOH and the precipitate formed was removed by filtration to leave 10 mg of **1a** (=**12**). An expected compound, methyl gallate, was not present in the $CHCl_3$ layer. Probably it was decomposed in the basic media.

The NMR spectroscopic data for **1a** for pyridine-d_5 were identical with those of ehretioside B (**12**),[7] and those for CD_3OD with those of **12** isolated from *S. adoxoides*[8] (Table 1).

Sugar Analysis About 1.0 mg aliquots of compounds **1**–**5** were hydrolyzed with 1 M HCl (0.1 mL) at 90°C for 2 h. The reaction mixtures were then each washed with an equal amount of EtOAc and the aqueous layers were analyzed by HPLC, which gave a peak for D-glucose at 15.2 min with positive optical rotation signs. [Optical rotation detector: JASCO OR-2090*Plus*, column: Shodex Asahipak NH2P-50; Φ=4.5 mm, L=25 cm, solvent: CH_3CN–H_2O (4 : 1), 1 mL/min]. Peaks were identified by co-chromatography with authentic D-glucose.

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Conflict of Interest The authors declare no conflict of interest.

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