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Lignan Glycosides and Phenolic Compound Glycosides from the Branches of *Tabebuia chrysotricha*

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Abstract

From the branches of *Tabebuia chrysotricha* (Bignoniaceae), two lignan glycosides (1, 2) and two phenolic compound glycosides (3, 4) were isolated, along with 15 known compounds (5 - 19). The structures of the new compounds were elucidated on the basis of spectroscopic evidence and those of the known compounds were identified by comparison of spectroscopic data with those reported in the literature. The DPPH radical-scavenging activity was assayed for the compounds isolated, the aryltetralin-type lignans showing moderate activity.

Keywords

***Tabebuia chrysotricha*, Bignoniaceae, Lignan Glycoside, Phenolic Glycoside, DPPH Radical-Scavenging Activity**

1. Introduction

Tabebuia chrysotricha (Martius ex De Candolle) Standley (Bignoniaceae), known as the golden trumpet tree, is a tall deciduous tree of 5 - 8 m in height that is native to Brazil and Colombia. It has showy bright yellow, trumpet-shaped flowers from March to April in Okinawa as well as in many nations; it is planted as a roadside tree for ornamental purposes. Many phenolic glycosides and iridoid glucosides have been isolated from related species—*T. impetiginosa* [1]-[3] and *T. avellanedae* [4]. It has been reported that compounds isolated from *T. avellanedae* showed fairly strong nitric oxide production inhibitory activity [4], and a bark extract of *T. avella-*

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nedae exhibited antiulcergenic activity [5]. Phytochemical and analgesic investigation of *T. chrysotricha* has been reported, naphthoquinones being found to be active compounds [6]. Phytochemical investigation of a polar fraction of a leaf extract of *T. chrysotricha* afforded two new lignan glycosides (**1** and **2**) and two new phenolic compound glycosides (**3** and **4**) along with 15 known compounds (**5** - **19**). The structures of the known compounds were spectroscopically identified as (+)-lyoniresinols 3a-*O*-*β*-D-glucopyranoside (**5**) [7] and 3a-*O*-(2''-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (**6**) [8], (–)-isolariciresinol 3a-*O*-*β*-D-glucopyranoside (**7**) [9], 3,4-dimethoxyphenol *O*-(6'-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside 5''-*O*-4'''-hydroxybenzoate (**8**) [1], 4-hydroxyphenethyl alcohol 7-*O*-(6'-*O*-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside 5''-*O*-4'''-hydroxybenzoate (**9**) [4], 5''-*O*-3''',4'''-dimethoxybenzoate (**10**) [1], 5''-*O*-3''',4''',5'''-trimethoxybenzoate (**11**) [1], 5''-*O*-ferulate (**12**) [10] and 5''-*O*-3''',4'''-dimethoxycinnamate (**13**) [10], 6-*O*-4''-hydroxybenzoylleonuride (**14**) [11], 6-*O*-vanilloylleonuride (**15**) [11], derwentioside B (**16**) [12], catalposide (**17**) [13], amphicoside (**18**) [14], and 6-*O*-veratrylcatalposide (**19**) [15]. The DPPH-scavenging activity of all compounds isolated was assayed. Among them, some lignan glycosides showed moderate activity.

2. Results and Discussion

Finely cut branches of *T. chrysotricha* were extracted with MeOH. The MeOH extract was partitioned with solvents, and the 1-BuOH-soluble fraction was separated by various kinds of column chromatography (CC) to afford four new compounds (**1** - **4**), and 15 known ones. This paper deals with structure elucidation of the new compounds and the DPPH radical-scavenging property of the isolated compounds was assayed.

Compound **1**, $[\alpha]_D^{24}$–27.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{31}H_{42}O_{15}$ by high-resolution (HR)-electrospray ionization (ESI)-mass spectrometry (MS). The IR spectrum exhibited absorption bands arising from a glycosidic nature (3365 cm^{-1}), aromatic ring(s) (1603 and 1513 cm^{-1}), and phenolic (1271 cm^{-1}) and alcoholic C-O stretchings (1075 and 1028 cm^{-1}). The presence of aromatic ring(s) was supported by the UV absorption maxima. In the ^{1}H-NMR spectrum, there were two singlet aromatic signals, three aromatic signals coupled in an ABX system, two methoxy signals, probably two anomeric proton signals [δ_H 5.40 (1H, d, *J* = 2.1 Hz, H-1''') and 4.19 (1H, d, *J* = 7.8 Hz, H-1'')], and two sets of isolated methylene signals [δ_H 3.97 (1H, d, *J* = 9.7 Hz, H-4'''a) and 3.67 (1H, d, *J* = 9.7 Hz, H-4'''b), and 3.57 (1H, d, *J* = 11.5 Hz, H-5'''a) and 3.51 (1H, d, *J* = 11.5 Hz, H-5'''b)]. The anomeric carbon signal at δ_C 111.5 with δ_H 5.40 was characteristic of apiofuranose, and the presence of D-apiose and D-glucose was detected on the sugar analysis of a hydrolyzate. A total 12 aromatic carbon signals were observed in the ^{13}C-NMR spectrum, together with one methylene, three methine and two oxygenated methylene carbon signals (**Table 1**), which were similar to those of an aryltetralin-type lignan, (–)-isolariciresinol 3a-*O*-*β*-D-glucopyranoside (**7**) [9], co-occurring in this plant. Sugar linkages were identified by detailed analysis of the heteronuclear multiple bond correlation (HMBC) spectrum, in which H-1''' was correlated to C-2'' (δ_C 80.3), and H-1'' to C-3a (δ_C 69.4), and the modes of sugar linkages were determined to be *β* from the coupling constants of H-1'' and H-1'''. The coupling constants of H-4 and H-3 showed that the three substituents at C-2, C-3 and C-4 were in equatorial orientations, and the negative Cotton effect at 292 nm and the positive ones at 273 and 239 nm observed in the circular dichroism (CD) spectrum indicated compound **1** has the 2*R*, 3*R* and 4*S* configurations [16]. Therefore, the structure of **1** was elucidated to be as shown in **Figure 1**.

Compound **2**, $[\alpha]_D^{24}$–26.7, was isolated as an amorphous powder and its elemental composition was found to be $C_{32}H_{44}O_{15}$. Spectroscopic data indicated that compound **2** was an analogous compound to **1**, and NMR data (**Table 1**) suggested the presence of a methoxy group with six protons and that the aromatic ring (C-1' to C-6') was symmetrically substituted. The cotton effects in the CD spectrum were similar to those of **1** and thus the structure of **2** was elucidated to be as shown in **Figure 1**.

Compound **3**, $[\alpha]_D^{24}$–75.0, was isolated as an amorphous powder and its elemental composition was determined to be $C_{27}H_{34}O_{15}$ by HR-ESI-MS. The IR spectrum exhibited absorption bands assignable to hydroxy groups (3384 cm^{-1}), an ester linkage (1709 cm^{-1}), and aromatic rings (1599 and 1511 cm^{-1}). In the ^{1}H-NMR spectrum, two anomeric proton signals [δ_H 5.01 (1H, d, *J* = 2.0 Hz, H-1'') and 4.78 (1H, d, *J* = 8.0 Hz, H-1')] were observed similarly to in the afore mentioned compounds (**1** and **2**), along with one singlet aromatic signal with two protons and two doublet signals coupled in an AA'BB' system. Two methoxy signals with 6 and 3 protons, respectively, were also observed. Therefore, both aromatic rings were symmetrically substituted. HPLC sugar analysis of a hydrolyzate revealed the presence of D-apiose and D-glucose. In the ^{1}H and ^{13}C NMR spec-

Table 1. ^{13}C-NMR spectrscopic data for compounds 1 and 2 (100 MHz, CD_3OD).

C	Compounds	
	1	**2**
1	33.9	33.9
2	39.3	39.2
2a	65.8	65.2
3	46.0	45.9
3a	69.4	69.3
4	47.8	48.0
5	117.5	117.4
6	145.9	145.1
7	147.2	147.2
8	112.6	112.6
9	129.3	129.2
10	134.5	134.3
1'	138.9	137.8
2'	114.7	108.1
3'	149.0	149.3
4'	145.9	135.0
5'	116.2	149.3
6'	123.2	108.1
1"	104.3	104.5
2"	80.3	80.4
3"	77.8	77.8
4"	71.8	71.8
5"	78.3	78.3
6"	62.8	62.8
1"'	111.5	111.6
2"'	78.4	78.2
3"'	80.4	80.3
4"'	75.3	75.3
5"'	65.8	65.8
7-CH_3O-	56.5	56.5
3'-CH_3O-	56.7	57.0
5'-CH_3O-		57.0

tra, H-5", C-6' and C-5" signals were obviously shifted downfield, when compared to the corresponding signals of **1** and **2** (Table 2). The HMBC correlations from H_2-2"', 6"' to C-4" (δ_C 163.7) and C-7"', and H_2-5" to C-7"' established the site of esterification of *p*-hydroxybenzoic acid, and those from H-1' to C-1 and H-2 to C-3 together with the chemical shift [δ_C 61.3 (3H)] of one of the methoxy groups confirmed the structure of the aglycone moiety to be that of a 3,4,5-trimethoxyphenol (Figure 2). The site of the glycosidic linkage was confirmed by HMBC correlation from H-1"' and C-5". Therefore, the structure of **3** was determined to be as shown in Figure 1.

Compound **4**, $[\alpha]_D^{24}$–31.9, was also isolated as an amorphous powder and its elemental composition was found to be $C_{28}H_{36}O_{14}$. The IR and UV spectra showed high similarity to those of **3**. The ^{1}H NMR spectrum exhibited three aromatic protons in an ABX coupling system, four protons in an AA'BB' coupling system and two methoxy signals. Two anomeric protons [δ_H 5.44 (1H, d, J = 1.4 Hz, H-1") and 4.30 (1H, d, J = 7.7 Hz, H-1')]

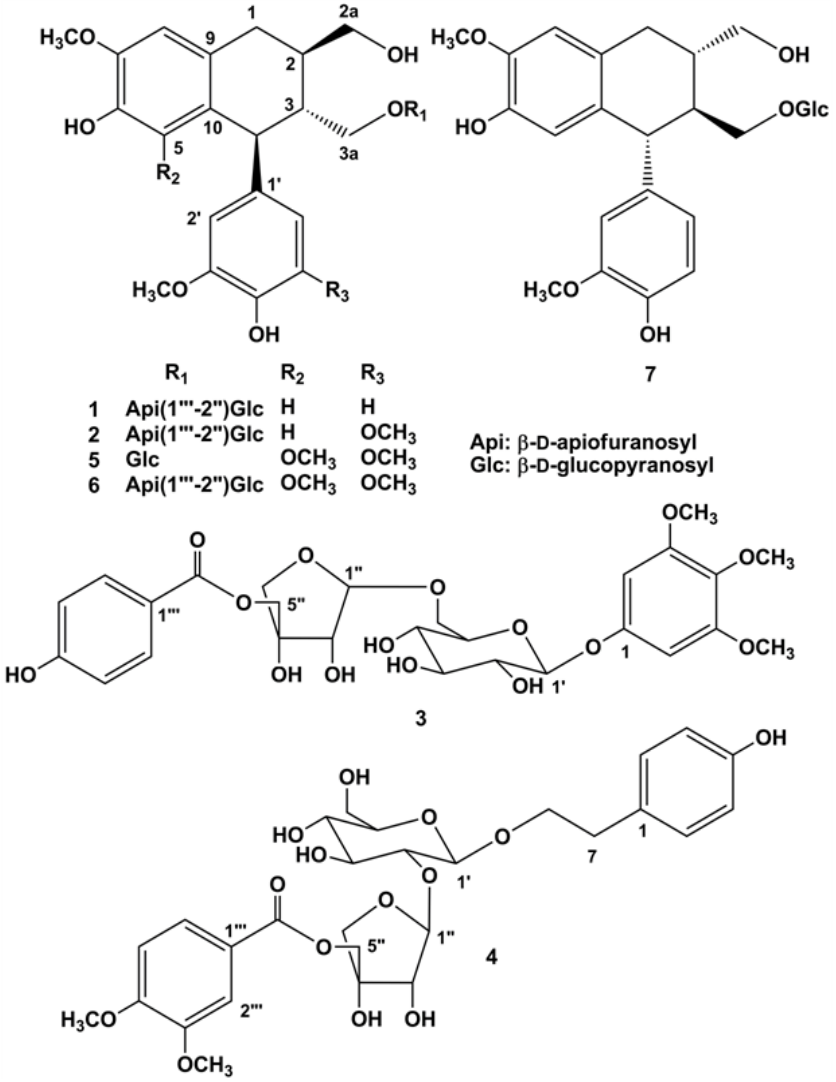

Figure 1. Structures of compounds isolated.

were also similarly observed to in the afore mentioned compounds, one for apiofuranose and the other for glucopyranose. The presence of these sugars was confirmed by HPLC analysis of a hydrolyzate. In the ^{13}C-NMR spectrum, six signals assignable to a trisubstituted aromatic ring, four signals for a symmetrically substituted aromatic ring, and two signals for methylene and oxygenated methylene were also observed. The HMBC correlation cross peaks between H-6''' [δ_H 7.56 (1H, dd, J = 8.0, 2.0 Hz)] and C-7''' (δ_C 167.8), and H_2-5'' and C-7''' established the site of esterification, and those between H-2''' and C-4''' together with δ_H 3.87 (3H, s) and C-4''', and H-5''' and C-3''' together with δ_H 3.83 (3H, s) and C-3''' confirmed the structure of the acyl moiety to be that of a 3,4-dimethoxybenzoate (**Figure 3**). Further HMBC correlations between H-1' and C-8 (δ_C 71.8), H_2-8 and C-1, H_2-7 and C-2 (and 6), and H-2 (and 6) and H-4 (δ_C 156.7) substantiated the position of the glycosidic linkage and the structure of the aglycone to be that of a *p*-hydroxyphenethyl alcohol. The linkage positions of two sugars were also established by the HMBC correlation between H-1'' and C-2' (δ_C 79.0). Therefore, the structure of **4** was assigned as shown in **Figure 1**.

Among the compounds isolated, aryltetralin-type lignan glycosides showed moderate DPPH radical-scavenging activity. The IC_{50} values were as follows: **1**: 43.6 ± 0.9 μM, **2**: 35.2 ± 0.2 μM, **5**: 17.7 ± 0.2 μM, **6**: 30.6 ± 0.2 μM and **7**: 69.9 ± 0.3 μM; that of Trolox being 17.5 ± 0.5 μM. Compound **5** showed comparable activity to the positive control and as a general trend, an increase in the number of methoxy groups positively affected the activity.

Table 2. ^{13}C-NMR spectrscopic data for compounds 3 and 4 (100 MHz, CD_3OD).

C	Compounds	
	3	**4**
1	155.9	130.5
2,6	96.7	130.8
3,5	154.8	116.1
4	134.8	156.7
7		36.4
8		71.8
1'	103.2	103.1
2'	74.9	79.0
3'	77.9	78.1
4'	71.6	71.7
5'	77.0	77.8
6'	68.4	62.8
1"	110.5	110.1
2"	78.6	78.5
3"	79.0	79.2
4"	75.0	75.4
5"	67.5	68.7
1"'	122.0	123.5
2"'	133.0	113.9
3"'	116.2	150.3
4"'	163.7	155.1
5"'	116.2	112.1
6"'	133.0	125.2
7"'	167.9	167.8
3,5-CH_3O-	56.8	
4-CH_3O-	61.3	
3'-CH_3O-		56.5
4'-CH_3O-		56.6

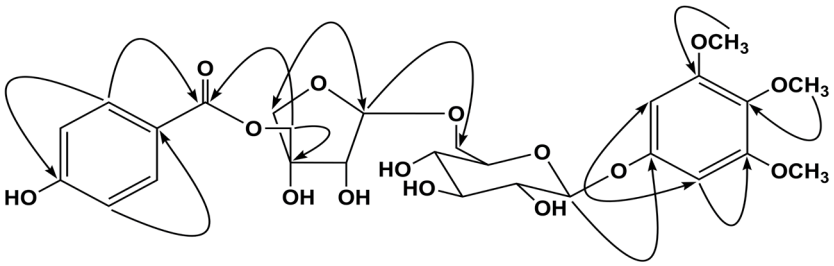

Figure 2. Important HMBC correlation of compound **3**.

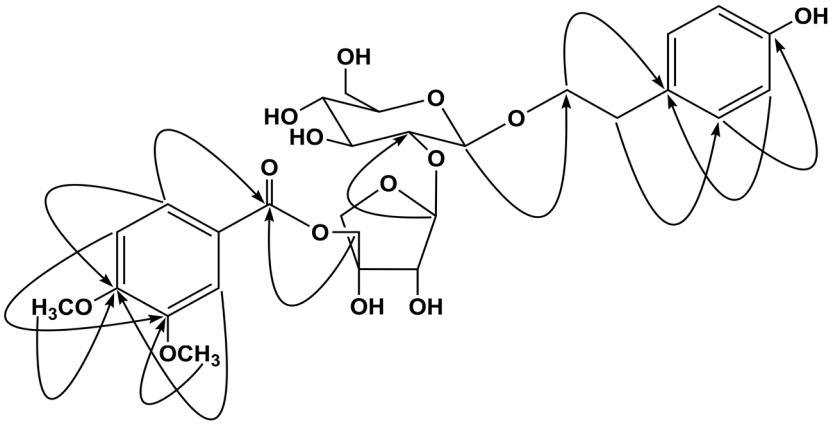

Figure 3. Important HMBC correlation of compound **4**.

3. Conclusion

From the branches of *T. chrysotricha* collected in the Okinawa Island, two new lignan glycosides and two new acylated phenolic glycosides were isolated. Their structures were elucidated by one- and two-dimensional NMR spectroscopy. The absolute structures of the lignans were determined by the results of CD spectral analyses. Among the compounds isolated, aryltetralin-type lignan glycosides (**1**, **2**, **5** - **7**) showed moderate DPPH radical-scavenging activity.

4. Experimental

4.1. Plant Material

Branches of *T. chrysotricha* were collected in Nakijin village, Nakagami County, Okinawa, Japan, in July2007, and a voucher specimen was deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University (07-TC-Okinawa-0710).

4.2. 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 spectrometer at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. CD spectra were measured with a JASCO J-720 spectropolarimeter. Positive-ion HR-ESI-MS was performed with a QSTAR XL NanoSpray™ System.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [Φ = 45 mm, L = 25 cm; H_2O-MeOH (9:1, 1 L) → (9:1, 1 L), linear gradient, 10 g fractions being collected]. The droplet counter-current chromatograph (DCCC) (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-*n*-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. High-performance liquid chromatography (HPLC) was performed on an ODS column (Inertsil; ODS-3, GL Science, Tokyo, Japan; Φ = 6 mm, L = 250 mm, 1 mL/min), and the eluate was monitored with a UV detector at 254 nm, and a refractive index monitor.

4.3. Extraction and Isolation

Branches of *T. chrysotricha* (13.5 kg) were extracted three times with MeOH (45 L × 3) at room temperature for one week and then the extract was concentrated to 3 L *in vacuo*. The concentrated extract was washed with *n*-hexane (3 L, 35.5 g), and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (3 L) and then extracted with EtOAc (3 L) to give 53.2 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (146 g), and the remaining water-layer was concentrated to furnish 28.9 g of a water-soluble fraction. The 1-BuOH-soluble fraction (145 g) was subjected to Diaion HP-20 CC (Φ = 50 mm, L = 50 cm), using H_2O-MeOH (4:1, 6 L), (3:2, 6 L), (2:3, 6 L), and (1:4, 6 L), and MeOH (6 L), 1 L fractions being collected. The residue (38.4 g) in fractions 10 - 15 was subjected to silica gel (1.50 kg) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (12 L), and $CHCl_3$-MeOH (49:1, 6 L), (24:1, 6 L), (23:2, 6 L), (9:1, 6 L), (17:3, 6 L), (4:1, 6 L), (3:1, 6 L), and (7:3, 6 L)], 500 mL fractions being collected. The residue (1.08 g) in fractions 30 - 36 was separated by ODS open CC and the residue (252 mg) in fractions 92 - 100 was subjected to DCCC. The residue (20.0 mg) in fractions 69 - 72 was finally purified by HPLC (H_2O-MeOH, 11:9) to afford 2.6 mg of **18** from the peak at 16 min. The residue (200 mg) in fractions 101 - 117 obtained on ODS open CC was subjected to DCCC, 6.9 mg of **3** being obtained in fractions 97 - 101. The residue (36.8 mg) in fractions 60 - 65 was purified by HPLC (H_2O-MeOH, 13:7) to give 3.0 mg of **9** and 3.0 mg of **14** from the peaks at 26 min and 37 min, respectively. The residue (17.0 mg) in fractions 66 - 77 was purified by HPLC (H_2O-MeOH, 13:7) to give a further amount (2.0 mg) of **18** and 6.0 mg of **15** from the peaks at 30 min and 40 min, respectively. The residue (51.0 mg) in fractions 118 - 133 obtained on ODS open CC was subjected to DCCC to yield 9.8 mg of **10** in fractions 109 - 133. An aliquot (2.00 g out of 7.08 g) of

fractions 37 - 42 obtained on silica gel CC was subjected to ODS open CC, and the residue (257 mg) in fractions 93 - 107 was purified by DCCC to give 6.9 mg of **5** in fractions 51 - 59. The residue (45.0 mg) in fractions 112 - 120 was purified by DCCC to obtain 3.8 mg of **9** in fractions 51 - 60. The residue (550 mg) in fractions 121 - 145 was subjected to DCCC, and then the residue (364 mg) in fractions 64 - 72 was purified by HPLC (H_2O-MeOH, 7:3) to afford 2.7 mg of **7**, 7.3 mg of **17** and 5.9 mg of **16** from the peaks at 23 min, 33 min and 35 min, respectively. The residue (25.0 mg) in fractions 85 - 90 was purified by HPLC (H_2O-MeOH, 61:39) to give 3.9 mg of **8** from the peak at 17 min. The residue (2.0 g out of 5.42 g) in fractions 43 - 49 obtained on silica gel CC was subjected to ODS open CC, and then the residue (256 mg) in fractions 51 - 58 was purified by DCCC to give a further amount (25.5 mg) of **5** in fractions 55 - 58. The residue (3.17 g) in fractions 50 - 59 obtained on silica gel CC was subjected to ODS open CC. The residue (259 mg) in fractions 55 - 71 was separated by DCCC, and then the residue (55.1 mg) in fractions 35 - 39 was purified by HPLC (H_2O-MeOH, 7:3) to give 5.9 mg of **1** from the peak at 17 min. The residue (30.9 mg) in fractions 40 - 41 was purified by HPLC (H_2O-MeOH, 13:7) to afford 8.0 mg of **2**, 5.5 mg of **6** and 3.1 mg of **1** from the peaks at 15 min, 17 min and 20 min, respectively. The residue (2.00 g) in fractions 60 - 67 obtained on silica gel CC was subjected to ODS open CC, and then the residue (200 mg) in fractions 86 - 106 was separated by DCCC. The residue (38.9 mg) in fractions 51 - 63 was purified by HPLC (H_2O-MeOH, 7:3) to give 4.5 mg of **2**, 5.4 mg of **6** and 2.0 mg of **1** from the peaks at 20 min, 22 min and 27 min, respectively.

The residue (41.8 g) in fractions 16 - 21 obtained on Diaion HP-20 CC was subjected to silica gel (1.50 kg) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (12 L), and $CHCl_3$-MeOH (49:1, 6 L), (24:1, 6 L), (23:2, 6 L), (9:1, 6 L), (17:3, 6 L), (4:1, 6 L), (3:1, 6 L), and (7:3, 6 L)], 500 mL fractions being collected. The residue (2.45 g) in fractions 32 - 37 was separated by ODS open CC to give 45.0 mg of **19** in fractions 112 - 120. The residue (175 mg) in fractions 96 - 111 was purified by DCCC to yield 7.0 mg of **12** in fractions 118 - 125. The residue (2.50 g) in fractions 38 - 45 was separated by DCCC, and then the residue (46.7 mg) in fractions 59 - 72 was purified by HPLC (H_2O-MeOH, 11:9) to afford 9.3 mg of **13** from the peak at 13 min. The residue (70.0 mg) in fractions 92 - 97 and that (81.8 mg) in fractions 105 - 118 were purified by HPLC (H_2O-MeOH, 11:9) to give 7.2 mg of **4** from the peak at 12 min and 8.3 mg of **11** from the peak at 17 min, respectively.

4.4. Compound 1

Amorphous powder; $[\alpha]_D^{24}$–27.8 (*c* 0.43, MeOH); IR ν_{max} (film) cm^{-1}: 3365, 2923, 1603, 1513, 1461, 1426, 1271, 1075, 1028; UV λ_{max} (MeOH) nm (log ε): 331 (3.74), 277 (3.88), ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.84 (1H, d, *J* = 2.1 Hz, H-2'), 6.74 (1H, d, *J* = 8.1 Hz, H-5'), 6.62 (1H, dd, *J* = 8.1, 2.1 Hz, H-6'), 6.62 (1H, s, H-8), 6.19 (1H, s, H-5), 5.40 (1H, d, *J* = 2.1 Hz, H-1'''), 4.19 (1H, d, *J* = 7.8 Hz, H-1''), 4.13 (1H, d, *J* = 10.5 Hz, H-4), 4.05 (1H, dd, *J* = 10.5, 3.0 Hz, H-3aa), 4.03 (1H, d, *J* = 2.1 Hz, H-2'''), 3.97 (1H, d, *J* = 9.7 Hz, H-4'''a), 3.84 (1H, dd, *J* = 11.5, 4.0 Hz, H-2aa), 3.82 (3H, s, CH_3O- on C-7), 3.81 (3H, s, CH_3O- on C-3'), 3.79 (1H, dd, *J* = 12.0, 2.4 Hz, H-6''a), 3.75 (1H, dd, *J* = 11.5, 6.0 Hz, H-2ab), 3.67 (1H, d, *J* = 9.7 Hz, H-4'''b), 3.64 (1H, dd, *J* = 12.0, 5.2 Hz, H-6''b), 3.57 (1H, d, *J* = 11.5 Hz, H-5'''a), 3.51 (1H, d, *J* = 11.5 Hz, H-5'''b), 3.43 (1H, dd, J = 9.0, 8.6 Hz, H-3''), 3.32 (1H, m, H-2''), 3.25 (1H, br d, J = 8.8 Hz, H-4''), 3.20 (1H, dd, *J* = 10.5, 2.6 Hz, H-3ab), 3.18 (1H, ddd, J = 9.0, 5.7, 2.4 Hz, H-5''), 2.82 (2H, m, H_2-1), 2.11 (1H, m, H-2), 1.79 (1H, br dd, *J* = 10.5, 10.5 Hz, H-3); ^{13}C-NMR (100 MHz, CD_3OD): **Table 1**; CD $\Delta\varepsilon$ (nm) (*c* 4.4 × 10^{-5} M): –2.73 (292), +2.46 (273), +2.46 (239); HR-ESI-MS (positive-ion mode) *m/z*: 677.2410 $[M + Na]^+$ (Calcd for $C_{31}H_{42}O_{15}Na$: 677.2416).

4.5. Compound 2

Amorphous powder, $[\alpha]_D^{24}$–26.7 (*c* 0.13, MeOH); ν_{max} (film) cm^{-1}: 3363, 2922, 1607, 1513, 1462, 1423, 1273, 1075, 1028; UV λ_{max} (MeOH) nm (log ε): 332 (3.63), 280 (3.86), 213 (4.23); ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.65 (1H, s, H-8), 6.54 (2H, s, H-2' and 6'), 6.21 (1H, s, H-5), 5.39 (1H, d, *J* = 2.2 Hz, H-1'''), 4.20(1H, d, *J* = 7.8 Hz, H-1''), 4.16 (1H, d, *J* = 10.5 Hz, H-4), 4.05 (1H, d, *J* = 2.2 Hz, H-2'''), 4.05 (1H, dd, *J* = 10.5, 3.0 Hz, H-3aa), 3.97 (1H, d, *J* = 9.7 Hz, H-4'''a), 3.83 (1H, dd, *J* = 11.5, 4.0 Hz, H-1aa), 3.81 (3H, s, CH_3O- on C-7), 3.80 (6H, s, CH_3O- on C-3' and 5'), 3.79 (1H, dd, *J* = 12.0, 2.4 Hz, H-6''a), 3.66 (1H, d, *J* = 9.7 Hz, H-4'''b), 3.63 (1H, dd, *J* = 12.0, 5.6 Hz, H-6''b), 3.76 (1H, dd, *J* = 11.5, 6.0 Hz, H-2ab), 3.57 (1H, d, *J* = 11.6 Hz, H-5'''a), 3.51 (1H, d, *J* = 11.6 Hz, H-5'''b), 3.43 (1H, dd, *J* = 9.0, 8.8 Hz, H-3''), 3.33 (1H, dd, *J* = 9.0, 7.8 Hz, H-2''), 3.26 (1H, br d, *J* = 8.8 Hz, H-4''), 3.17 (1H, ddd, *J* = 9.0, 5.6, 2.4 Hz, H-5''), 3.17 (1H, dd, *J* = 10.5, 2.5 Hz, H-3ab), 2.83 (2H, m, H_2-1), 2.10 (1H, m, H-2), 1.84 (1H, br dd, *J* = 10.5, 10.5 Hz, H-3); ^{13}C-NMR (100 MHz, CD_3OD): **Table 1**; CD

$\Delta\varepsilon$ (nm) (*c* 4.0 × 10^{-5} M): –3.61 (292), +2.56 (273), +2.00 (242); HR-ESI-MS (positive-ion mode) *m/z*: 707.2517 $[M + Na]^+$ (Calcd for $C_{32}H_{44}O_{15}Na$: 707.2522).

4.6. Compound 3

Amorphous powder, $[\alpha]_D^{24}$–75.0 (*c* 0.30, MeOH); IR ν_{max} (film) cm^{-1}: 3384, 1709, 1599, 1511, 1461, 1423, 1277, 1224, 1126, 1071, 1025; UV λ_{max} (MeOH) nm (log ε): 285 (3.57), 261 (3.86), 219 (4.07); ^{1}H-NMR (400 MHz, CD_3OD): 7.90 (2H, d, *J* = 8.6 Hz, H-2''' and 6'''), 6.82 (2H, d, *J* = 8.6 Hz, H-3''' and 5'''), 6.34 (2H, s, H-2 and 6), 5.01 (1H, d, *J* = 2.0 Hz, H-1''), 4.78 (1H, d, *J* = 8.0 Hz, H-1'), 4.34 (1H, d, *J* = 11.4 Hz, H-5''a), 4.30 (1H, d, *J* = 11.4 Hz, H-5''b), 4.07 (1H, d, *J* = 9.7 Hz, H-4''a), 4.06 (1H, dd, *J* = 11.5, 2.0 Hz, H-6'a), 3.95 (1H, d, *J* = 2.0 Hz, H-2''), 3.87 (1H, d, *J* = 9.7 Hz, H-4''b), 3.77 (6H, s, CH_3O- on C-3 and 5), 3.68 (3H, s, CH_3O- on C-4), 3.63 (1H, dd, *J* = 11.5, 6.3 Hz, H-6'b), 3.57 (1H, ddd, *J* = 9.0, 6.3, 2.0 Hz, H-5'), 3.45 (1H, br d, *J* = 8.0 Hz, H-3') 3.43 (H, brd, *J* = 8.0 Hz, H-2'), 4.34 (1H, m, H-4'); ^{13}C-NMR (100 MHz, CD_3OD): **Table 2**; HR-ESI-MS (positive-ion mode) *m/z*: 621.1792 $[M + Na]^+$ (Calcd for $C_{27}H_{34}O_{15}Na$: 621.1790).

4.7. Compound 4

Amorphous powder, $[\alpha]_D^{24}$–31.9 (*c* 0.48, MeOH); IR ν_{max} (film) cm^{-1}: 3363, 1707, 1598, 1515, 1457, 1419, 1270, 1023; UV λ_{max} (MeOH) nm (log ε): 284 (3.86), 263 (3.97), 217 (4.22); ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.71 (1H, d. *J* = 2.0 Hz, H-2'''), 7.56 (1H, dd, *J* = 8.0, 2.0 Hz, H-6'''), 6.97 (1H, d, *J* = 8.0 Hz, H-5'''), 6.90 (2H, d, *J* = 8.0 Hz, H-2 and 6), 6.60 (2H, d, *J* = 8.0 Hz, H-3 and 5), 5.44 (1H, d, *J* = 1.4 Hz, H-1''), 4.43 (1H, d, *J* = 11.5 Hz, H-5''a), 4.34 (1H, d, *J* = 11.5 Hz, H-5''b), 4.30 (1H, d, *J* = 7.7 Hz, H-1'), 4.17 (1H, d, *J* = 9.7 Hz, H-4''a), 4.00 (1H, d, *J* = 1.4 Hz, H-2''), 3.95 (1H, ddd, *J* = 9.5, 8.0, 7.4 Hz, H-8a), 3.87 (3H, s, CH_3O- on C-4'''), 3.86 (1H, d, *J* = 9.6 Hz, H-4''b), 3.83 (3H, s, CH_3O- on C-3'''), 3.82 (1H, dd, *J* = 11.8, 2.0 Hz, H-6'a), 3.80 (1H, d, J = 9.7 Hz, H-4''b), 3.66 (1H, dd, *J* = 11.8, 5.4 Hz, H-6'b), 3.58 (1H, ddd, *J* = 9.5, 8.0, 7.4 Hz, H-8b), 3.57 (1H, dd, *J* = 9.0, 8.7 Hz, H-4'), 3.41 (1H, dd, *J* = 9.2, 7.7 Hz, H-2'), 3.39 (1H, dd, *J* = 9.2, 9.0 Hz, H-3'), 3.21 (1H, ddd, *J* = 9.0, 5.4, 2.0 Hz, H-5'), 2.71 (2H, t, *J* = 7.4 Hz, H_2-7); ^{13}C-NMR (100 MHz, CD_3OD): **Table 2**; HR-ESI-MS (positive-ion mode) *m/z*: 619.1998 $[M + Na]^+$ (Calcd for $C_{28}H_{36}O_{14}Na$: 619.1997).

4.8. Sugar Analysis

About 500 μg of each compound **1**, **2**, **3** and **4** was hydrolyzed with 1 M HCl (0.1 mL) at 90˚C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the water layers were analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH_2P-50 4E, CH_3CN-H_2O (4:1), 1 mL/min]. Their hydrolyzates gave peaks for D-apiose and D-glucose at 6.1 min and 19.4 min, respectively, with positive optical rotation signs. The peaks were identified by co-chromatography with authentic samples.

4.9. 2,2-Diphenyl-1-(2,4,6-trinitrophenyl)hydrazine (DPPH) Assay

The antioxidant activity was evaluated using the DPPH radical-scavenging system. On a 96-well plate, 2 μL-aliquots of DMSO solutions of the compounds were diluted with 98 μL of MeOH in triplicate. A 100 μL-aliquot of a methanolic solution of DPPH was added to each well to give a final concentration of 100 μM. The compounds were tested at final concentrations of 100, 50, 25 and 10 μM. Each mixture was incubated in the dark for 30 min at room temperature, followed by measurement of the absorbance at 515 nm using a Molecular Devices Versamax tunable microplate reader. DMSO was used as a negative control and Trolox as a positive control. Radical-scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

$$\%\text{Inhibition} = \left[\left(A_{control} - A_{test}\right)/A_{control}\right] \times 100$$

where $A_{control}$ is the absorbance of the control (DMSO) and A_{test} is the absorbance of the test compound.

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