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Grevillosides A–F: Glucosides of 5-alkylresorcinol derivatives from leaves of *Grevillea robusta*

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ABSTRACT

From a MeOH extract of leaves of *Grevillea robusta*, seven compounds (**1–7**) were isolated. One known compound (**7**) was identified with a benzyl glucoside, icariside F_2. The structures of the six of these, named grevillosides A–F (**1–6**), were elucidated on detailed inspection of one- and two-dimensional NMR spectroscopic data as glucosides of 5-alkylresorcinols.

1. Introduction

Most of the 1200 species of the 55 genera in Proteaceae grow in Australia and South Africa, and only one species, *Helicia cochinchinensis*, grows naturally in Japan. Phytochemical investigation of *H. cochinchinensis* led to the isolation of 5-*O*-β-D-glucopyranosides of flavanones (Morimura et al., 2006). *Grevillea robusta* A. Cunn., belonging to Proteaceae, originates from subtropical areas of eastern Australia and has been planted in Japan. It is an evergreen tree between 20 m and 35 m in height with dark green delicately dented bipinnatifid leaves reminiscent of fronds. The leaves are 15–30 cm long with grey-white or rusty undersides. Phytochemical investigation of the same plant, collected in Egypt, has been reported and several phenolic glucosides were isolated (Ahmed et al., 2000). Cytotoxic 5-alkylresorcinol metabolites were also isolated from the title plant (Chuang and Wu, 2007) and a MeOH extract of its timber exhibited potent leishmanicidal activity (Takahashi et al., 2004). 5-Alkylresorcinols (Kozubeck and Tyman, 1999) are found in a mushroom, *Merulius incarnatus* (Jin and Zjawiony, 2006), a Malagasy Myrsinaceous plant, *Oncostemon bojerianum* (Chaturvedula et al., 2002), *Lysimachia japonica* (Primulaceae) (Arisawa et al., 1989), and some cereals (Ross et al., 2003; Chen et al., 2004). The cytotoxicities of 5-alkylresorcinols have also been discussed (Arisawa et al., 1989; Chaturvedula et al., 2002; Chuang and Wu, 2007). In our continuing studies on Okinawan plants, the chemical constituents of *G. robusta*, collected in Okinawa, were investigated.

2. Results and discussion

Air-dried leaves of *G. robusta* were extracted with MeOH three times and the concentrated MeOH extract was partitioned with solvents of increasing polarity. The 1-BuOH-soluble fraction was separated by means of various chromatographic procedures including CC on a highly-porous synthetic resin (Diaion HP-20), and then normal silica gel and reversed-phase (ODS) CC, droplet counter-current chromatography (DCCC), and HPLC to afford seven compounds (**1–7**). The details and yields are given in the Section 4. The structure of a known benzyl glycoside (**7**) was identified as icariside F_2 by comparison with data, reported in the literature (Miyase et al., 1988).

Grevilloside A (**1**), $[\alpha]_D$ −60.7, was isolated as an amorphous powder and its elemental composition was determined to be $C_{17}H_{24}O_8$ from the observation of a quasi-molecular ion peak ([M+Na]) on HR-ESI-mass-spectrometry. The IR spectrum exhibited absorption bands for hydroxyl groups (3396 cm^{-1}) and an aromatic ring (1614 cm^{-1} and 1507 cm^{-1}), and the UV spectrum also indicated the presence of an aromatic ring. In the ^1H NMR spectrum, three aromatic protons were observed, which were coupled to each other with coupling constants of 2 Hz. The ^{13}C NMR spectrum, together with the DEPT experiment results, indicated the presence of three aromatic signals each with a proton and another three each without a proton. Two of the aromatic resonances, without a proton were highly deshielded, indicating that they probably carried hydroxyl groups. The remaining signals in the ^{13}C NMR spectrum consisted of six assignable to β-glucopyranose, and one double bond, two methylenes and one oxymethylene. A sequential ^1H–^1H COSY correlation from H_2-11 (δ_H 3.59) to H_2-7

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(δ_H 3.30–3.45) established the alkyl chain to be as shown in Fig. 1 (Fig. 2). The coupling constants (11 Hz) of two olefinic protons indicated the geometry of the double bond to be of the *cis* form. A HMBC correlation from H-1′ (δ_H 4.84) to C-1 (δ_C 160.3) confirmed that the β-glucopyranosyl moiety was on the phenolic hydroxyl group and this was further supported by the difference NOE experiment, in which irradiation of the anomeric proton caused significant enhancement of two aromatic protons (δ_H 6.45 and 6.39) (Fig. 2). HMBC correlations between H-2 (δ_H 6.39) and C-4 and C-6 (δ_C 110.6 and 109.2), and H-7 (δ_H 3.30–3.45) and C-4 and C-6 established the structure, as shown in Fig. 1. The absolute configuration of glucose was determined to be of the D-series on HPLC analysis of the hydrolyzate of **1** using an optical rotation detector.

Grevilloside B (**2**), $[\alpha]_D$ −37.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{17}H_{26}O_8$ by HR-ESI-MS. In the ^{13}C NMR spectrum, the aromatic substitution pattern was established to be the same as that of grevilloside A (**1**), and the HMBC spectrum also showed the same correlation between the anomeric proton (δ_H 4.86) and the highly deshielded carbon atom (δ_C 160.0). The alkyl chain consisted of four methylenes and one oxymethylene. Thus, the structure of grevilloside B (**2**) was elucidated to be a dihydro derivative of grevilloside A (**1**), as shown in Fig. 1.

Grevilloside C (**3**), $[\alpha]_D$ −49.6, was also isolated as an amorphous powder and its elemental composition was determined by HR-ESI-MS to be $C_{17}H_{24}O_9$, which is two hydrogen atom less and one oxygen atom more than in the case of grevilloside B (**2**). In the IR spectrum, absorption for the carboxyl moiety was observed at 1713 cm^{-1}. The ^{13}C NMR spectrum showed that **3** was an analogous compound to **2**, and the oxymethylene signal observed in that of **2** was replaced by a carbonyl carbon signal. Therefore, grevilloside C (**3**) was elucidated to be the 11-carboxylic acid derivative of grevilloside B (**2**).

Grevilloside D (**4**), $[\alpha]_D$ −27.6, and grevilloside E (**5**), $[\alpha]_D$ −42.1, were isolated as amorphous powders, and their elemental compositions were determined to be $C_{16}H_{24}O_8$ and $C_{16}H_{22}O_9$, respectively, by HR-ESI-MS. The NMR spectroscopic data showed that these were analogous compounds to grevillosides B (**2**) and C (**3**), respectively, and together with the data obtained on mass-spectrometry,

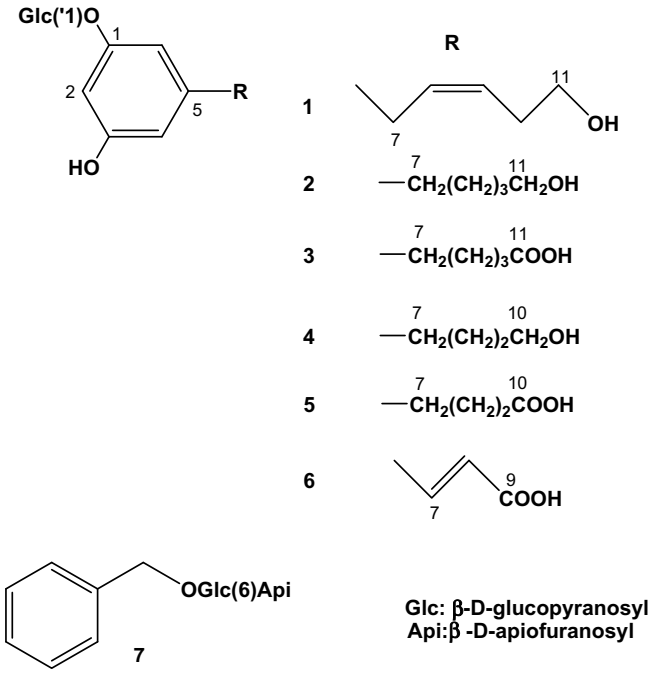

Fig. 1. Structures.

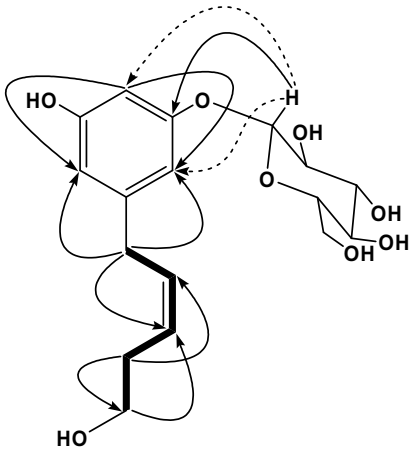

Fig. 2. Diagnostic two-dimensional NMR correlations (bold line: COSY, and plain curves: HMBC from H to C) and the results of the difference NOE experiments (dashed curves) for **1**.

the structures of grevilloside D (**4**) and grevilloside E (**5**) were elucidated to be one methylene less forms of grevillosides B (**2**) and C (**3**), respectively.

Grevilloside F (**6**), $[\alpha]_D$ −58.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{15}H_{18}O_9$ by HR-ESI-MS. The NMR spectra indicated that the aromatic moiety was the same as that of the aforementioned compounds. However, the side chain consisted of a double bond (δ_H 6.39 and 7.54) and a carboxylic acid moiety. The geometry of the double bond was determined to be *trans* from the coupling constants (J = 16 Hz) of the olefinic protons in the ^1H NMR spectrum. Therefore, the structure of grevilloside F (**6**) was elucidated to be as shown in Fig. 1.

3. Conclusion

From leaves of *G. robusta*, six new 5-alkylresorcinol glucosides were isolated. As far as we know, this is the first example of the isolation of glycosidic forms of these alkylresorcinol. Biological evaluation of the isolated compounds is in progress.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-1030 polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/VIS spectrophotometers, respectively. ^1H 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. Positive-ion HR-MS was performed with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-TOF-MS.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecyl silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan) [Φ = 50 mm, L = 25 cm, linear gradient: MeOH–H_2O (1:9, 1 L) → (1:1, 1 L), fractions of 10 g being collected], respectively. The droplet counter-current chromatography (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns (Φ = 2 mm, L = 40 cm), and the lower and upper layers of a solvent mixture of $CHCl_3$–MeOH–H_2O-*n*-PrOH (9:12:8:2) were 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 was performed

on an ODS column (Inertsil; GL Science, Tokyo, Japan; Φ = 6 mm, L = 25 cm), and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor.

4.2. Plant material

Leaves of *G. robusta* A. Cunn. (Proteaceae) were collected in Okinawa, Japan, in August 2005, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (05-GR-Okinawa-0629).

4.3. Extraction and isolation

Dried leaves of *G. robusta* (6.35 kg) were extracted three times with MeOH (30 L) at 25 °C for one week and then concentrated to 3 L *in vacuo*. The extract was washed with *n*-hexane (3 L, 32.6 g) and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in H_2O (3 L) and then extracted with EtOAc (3 L) to give an EtOAc-soluble fraction (160 g). The aqueous-layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (405 g), and the remaining aqueous-layer was concentrated to furnish a water-soluble fraction (475 g).

A portion (237 g) of the 1-BuOH-soluble fraction was applied to a Diaion HP-20 column (Φ = 75 mm, L = 50 cm) using H_2O–MeOH (4:1, 8 L), (2:3, 8 L), (3:2, 8 L), and (1:4, 8 L), and MeOH (6 L), 1 l fractions being collected. The residue (19.9 g in fractions 4–6) of the MeOH–H_2O (1:4, v/v) eluent was subjected to silica gel (450 g) CC, with elution with $CHCl_3$ (3 L) and $CHCl_3$–MeOH [(49:1, 3 L), (24:1, 3 L), (23:2, 3 L), (9:1, 3 L), (7:1, 3 L), (17:3, 3 L), (4:1, 3 L), (3:1, 3 L), and (3:2, 3 L)], 500 mL fractions being collected. Combined fractions 46–52 (1.20 g) of the 20–25% MeOH in H_2O (v/v) eluate were separated by ODS open CC to give **6** (187 mg).

The residue (45.1 g) of fractions 7–12, obtained on Diaion HP-20 CC, was subjected to silica gel (450 g) CC, with elution with $CHCl_3$ (4.5 L) and $CHCl_3$–MeOH [(49:1, 4.5 L), (24:1, 4.5 L), (23:2, 4.5 L), (9:1, 4.5 L), (7:1, 4.5 L), (17:3, 4.5 L), (4:1, 4.5 L), (3:1, 4.5 L), and (3:2, 4.5 L)], 500 mL fractions being collected. An aliquot (1.76 g) of combined fractions 61–66 (2.61 g) of the 12.5–15% MeOH in H_2O (v/v) eluate was separated by ODS open CC gave two residues in fractions 88–99 (204 mg) and fractions 100–119 (548 mg), followed by DCCC. The residue (27.2 mg) of fractions 20–24 from DCCC was purified by preparative HPLC (MeOH–H_2O, 1:3) to give **1** (7.9 mg) from the peak at 15 min. From the residue (38.5 mg) of fractions 25–28 from DCCC, a further amount of **1** (3.7 mg) was obtained by the same method. The residue (548 mg) of fractions 100–119, obtained on ODS open CC, was separated by DCCC (152 mg in fractions 25–35), and then purified by preparative HPLC (MeOH–H_2O, 1:4) to give **7** (27.0 mg) and **2** (21.3 mg) from the peaks at 35 min and 43 min, respectively. An aliquot (1.85 g) of the residue (2.01 g) of fractions 67–73, obtained on silica gel CC, was subjected to ODS open CC gave two fractions, 273 mg in fractions 70–88 and 485 mg in fractions 100–115. The former was the separated by DCCC to afford 130 mg of the residue of fractions 12–18, which was then purified by repeated HPLC (MeOH–H_2O, 1:4) to furnish **4** (1.9 mg) from the peak at 13 min. From the latter, **3** (175 mg) was isolated by DCCC from fractions 28–35. The residue (3.85 g) of fractions 74–87, obtained on silica gel CC, was subjected to ODS open CC to give **5** (41.7 mg) in fractions 70–88.

4.4. Characterization data

4.4.1. Grevilloside A (*1*)

Amorphous powder; $[\alpha]_D^{31}$ −60.7 (c = 0.53, MeOH); IR ν_{max} (film) cm^{-1}: 3396, 2924, 1614, 1507, 1455, 1171, 1075; UV λ_{max} (MeOH) nm (log ε): 277 (3.21), 228 (3.55); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.45 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.39 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.33 (1H, *dd*, *J* = 2, 2 Hz, H-4), 5.62 (1H, *dtt*, *J* = 11, 7, 1 Hz, H-8), 5.52 (1H, *dtt*, *J* = 11, 7, 2 Hz, H-9), 4.84 (1H, *d*, *J* = 8 Hz, H-1′), 3.89 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.70 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.59 (2H, *t*, *J* = 7 Hz, H-11), 3.43 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.40 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 3.39 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.43–3.47 (1H, *m*, H-5′), 3.30–3.45 (2H, *m*, H_2-7), 2.38 (2H, *ttd*, *J* = 7, 7, 1 Hz, H_2-10); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 379.1361 $[M+Na]^+$ (calc. for $C_{17}H_{24}O_8Na$, 379.1363).

4.4.2. Grevilloside B (*2*)

Amorphous powder; $[\alpha]_D^{31}$ −37.5 (c = 1.42, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2929, 1595, 1508, 1458, 1172, 1074; UV λ_{max} (MeOH) nm (log ε): 271 (3.13), 226 (3.43); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.44 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.40 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.33 (1H, *dd*, *J* = 2, 2 Hz, H-4), 4.86 (1H, *d*, *J* = 8 Hz, H-1′), 3.90 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.71 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.54 (2H, *t*, *J* = 8 Hz, H-11), 3.46 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.44-3.48 (2H, *m*, H-2′ and 5′), 3.42 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 2.51 (2H, *t*, *J* = 7 Hz, H_2-7), 1.61 (2H, *m*, H_2-8), 1.55 (2H, *m*, H_2-10), 1.37 (2H, *m*, H_2-9); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 381.1523 $[M+Na]^+$ (calc. for $C_{17}H_{26}O_8Na$, 381.1519).

4.4.3. Grevilloside C (*3*)

Amorphous powder; $[\alpha]_D^{31}$ −49.6 (c = 0.75, MeOH); IR ν_{max} (film) cm^{-1}: 3388, 2932, 1713, 1597, 1508, 1457, 1173, 1075; UV λ_{max} (MeOH) nm (log ε): 275 (3.27), 225 (3.52); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.44 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.39 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.31 (1H, *dd*, *J* = 2, 2 Hz, H-4), 4.85 (1H, *d*, *J* = 8 Hz, H-1′), 3.89 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.70 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.43–3.48 (1H, *m*, H-5′), 3.44 (1H, *dd*, *J* = 9, 8 Hz, H-2′), 3.42 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.40 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 2.52 (2H, *m*, H_2-7), 2.30 (2H, *m*, H_2-10), 1.60–1.64 (4H, *m*, H_2-8 and H_2-9); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 395.1318 $[M+Na]^+$ (calc. for $C_{17}H_{24}O_9Na$, 395.1312).

4.4.4. Grevilloside D (*4*)

Amorphous powder; $[\alpha]_D^{25}$ −27.6 (c = 0.13, MeOH); IR ν_{max} (film) cm^{-1}: 3366, 2923, 1596, 1508, 1456, 1299, 1171, 1073; UV λ_{max} (MeOH) nm (log ε): 223 (4.01), 273 (3.47); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.44 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.38 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.32 (1H, *dd*, *J* = 2, 2 Hz, H-4), 4.85 (1H, *d*, *J* = 8 Hz, H-

Table 1
^{13}C NMR spectroscopic data for grevillosides A–F (**1–6**) (100 MHz, CD_3OD)

	1	2	3	4	5	6
1	160.3	160.0	160.2	160.2	160.2	160.6
2	102.8	102.5	102.7	102.7	102.8	107.4
3	159.5	159.2	159.4	159.4	159.4	160.0
4	110.6	110.7	110.7	110.7	110.7	110.1
5	144.7	146.3	145.9	146.2	145.3	137.8
6	109.2	109.3	109.2	109.3	109.3	108.9
7	34.6	36.9	36.6	36.8	36.2	146.3
8	131.3	32.1	31.7	28.6	27.6	119.7
9	127.7	26.5	25.7	33.2	34.4	170.3
10	31.8	33.4	34.8	62.9	177.6	
11	62.8	63.0	177.6			
1′	102.3	102.2	102.3	102.3	102.3	102.4
2′	75.0	74.9	75.0	75.0	75.0	75.0
3′	78.2	78.0	78.1	78.2	78.1	78.3
4′	71.5	71.4	71.5	71.5	71.5	71.5
5′	78.1	78.0	78.1	78.2	78.1	78.1
6′	62.6	62.5	62.5	62.7	62.6	62.6

1′), 3.89 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.70 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.55 (2H, *t*, *J* = 7 Hz, H-11), 3.35–3.43 (4H, *m*, H-2′, 3′, 4′ and 5′), 2.53 (2H, *t*, *J* = 7 Hz, H_2-7), 1.65 (2H, *quintetd*, *J* = 7, 2 Hz, H_2-8), 1.54 (2H, *quintetd*, *J* = 7, 2 Hz, H_2-9); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 379.1365 $[M+Na]^+$ (calc. for $C_{16}H_{24}O_8Na$, 367.1363).

*4.4.5. Grevilloside E (**5**)*

Amorphous powder; $[\alpha]_D^{31}$ −42.1 (*c* = 0.81, MeOH); IR ν_{max} (film) cm^{-1}: 3395, 2930, 1700, 1648, 1600, 1509, 1437, 1170, 1074; UV λ_{max} (MeOH) nm (log ε): 278 (3.41), 228 (3.55); ^{1}H NMR (CD_3OD, 400 MHz) δ: 6.45 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.40 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.32 (1H, *dd*, *J* = 2, 2 Hz, H-4), 4.86 (1H, *d*, *J* = 8 Hz, H-1′), 3.89 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.71 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.45 (1H, *dd*, J = 9, 8 Hz, H-2′), 3.44–3.48 (1H, *m*, H-5′), 3.44 (1H, *dd*, *J* = 9, 9 Hz, H-3′), 3.42 (1H, *dd*, *J* = 9, 9 Hz, H-4′), 2.54 (2H, *t*, *J* = 7 Hz, H_2-7), 2.28 (2H, *t*, *J* = 8 Hz, H_2-9), 1.87 (2H, *quintet*, *J* = 8 Hz, H_2-8); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 381.1160 $[M+Na]^+$ (calc. for $C_{16}H_{22}O_9Na$, 379.1156).

*4.4.6. Grevilloside F (**6**)*

Amorphous powder; $[\alpha]_D^{27}$ −58.9 (*c* = 0.52, MeOH); IR ν_{max} (film) cm^{-1}: 3367, 2931, 1637, 1593, 1508, 1451, 1396, 1333, 1276, 1175, 1074; UV λ_{max} (MeOH) nm (log ε): 277 (4.00), 222 (4.20); ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.54 (1H, *d*, *J* = 16 Hz, H-7), 6.84 (1H, *dd*, *J* = 2, 2 Hz, H-6), 6.68 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.62 (1H, *dd*, *J* = 2, 2 Hz, H-2), 6.39 (1H, *d*, *J* = 16 Hz, H-8), 4.84 (1H, *d*, *J* = 8 Hz, H-1′), 3.91 (1H, *dd*, *J* = 12, 2 Hz, H-6′*a*), 3.71 (1H, *dd*, *J* = 12, 5 Hz, H-6′*b*), 3.44–3.48 (3H, *m*, H-2′, 4′ and 5′), 3.41 (1H, *dd*, *J* = 9, 9 Hz, H-3′); ^{13}C NMR (CD_3OD, 100 MHz): Table 1; HR-ESI-TOF-MS (positive-ion mode) *m/z*: 365.0847 $[M+Na]^+$ (calc. for $C_{15}H_{18}O_9Na$, 365.0843).

4.4.7. Known compound isolated

Icariside F_2 (**7**), Amorphous powder, $[\alpha]_D^{29}$ −80.5 (*c* = 1.80, MeOH).

4.4.8. Analyses of the sugar moiety

About 500 μg each of grevillosides A-F (**1–6**) was hydrolyzed with 1 N HCl (0.1 mL) at 100 °C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL), and the aqueous layers were analyzed with a chiral detector (JASCO OR-2090plus) on an amino column [Asahipak NH2P-50 4E, CH_3CN–H_2O (4:1), 1 ml/min]. Hydrolyzates of grevillosides A-F (**1–6**) gave the peak for D-glucose at the retention time of 14.2 min (positive optical rotation sign). Peaks were identified by co-chromatography with authentic D-glucose.

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