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NOTE

Gallates of isoorientin and (2*S*)-1,2-propanediol glucoside from the leaves of *Schoepfia jasminodora*

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Abstract From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *Schoepfia jasminodora* collected from Okinawa Island, two new galloyl esters and 15 known compounds were isolated. The structures of the new compounds were elucidated by spectroscopic analysis to be isoorientin 4″-*O*-gallate and (2*S*)-1,2-propanediol 1-*O*-*β*-D-glucopyranoside 6′-*O*-gallate. The compounds possessing galloyl or cathechol moieties showed equal radical scavenging activity.

Keywords *Schoepfia jasminodora* · Olacaceae · *C*-Glucosyl flavone · (2*S*)-1,2-Propanediol · Radical scavenging activity

Introduction

About 30 species of the genus *Schoepfia*, belonging to the family Olacaceae, are distributed in Asia and America, with only one species, *S. jasminodora*, growing wild in southern Japan (Kagoshima to Okinawa Island), but the population is small [1]. *S. jasminodora* is a deciduous tree and grows to about 10 m in height. Its trivial name, "boroboronoki", is based on the brittleness of its twigs. Only the Olacaceous plant, Brazilian *Ptychoepetalum olacoides*, is well known as Muira Puama and is used as an invigorating drug [2]. However, the clinical use of *S. jasminodora* and its chemical components has not yet been reported. From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *S. jasminodora*, two gallates of isoorientin (**1**) and (2*S*)-1,2-propnanediol 1-*O*-*β*-D-glucopyranoside (**2**) were isolated along with two flavone *C*-glucopyranosides, isoorientin (**3**) [3] and orientin (**4**) [4], three flavonoid *β*-D-glucopyranosides, apigenin and luteolin 7-*O*-*β*-D-glucopyranosides (**5**, **6**) [5, 6] and quercetin 3-*O*-*β*-D-glucopyranoside (**7**) [7], two chalcone *C*-glucopyranosides, schoepfin C (**8**) [8] and 2′,4,4′-trihydroxy-3′-*C*-*β*-glucopyranosyl chalcone (**9**) [9], four megastigmane glucosides, corchoionoside C (**10**) [10], linarionoside A (**11**) [11], staphylionoisde D (**12**) [12] and (3*S*,5*R*,6*R*,7*E*,9*S*)-7-megastigmene-3,5,6,9-tetrol 3-*O*-*β*-D-glucopyranoside (**13**) [13], an iridoid glucoside, 6*β*-hydroxyloganin (**14**) [14], and three quinic acid acyl esters, quinic acid 5-*O*-*p*-hydroxybenzoate (**15**) [15] and 5-*O*-*p*-*Z*-coumarate (**16**) [16], and chlorogenic acid (**17**) [17, 18]. This paper deals with the isolation and structure elucidation of the new compounds. The radical scavenging activity of some of the isolated compounds was also evaluated.

Results and discussion

From the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *S. jasminodora*, two new gallates of isoorientin (**1**) and (2*S*)-1,2-propnanediol 1-*O*-*β*-D-glucopyranoside (**2**) were isolated by a combination of several chromatographic techniques (Fig. 1) (see "Experimental").

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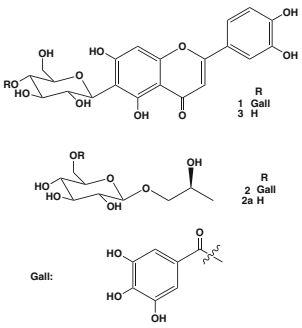
Fig. 1 Structures of new compounds isolated



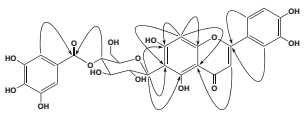
Isoorientin 4″-*O*-gallate (**1**), [α] +8.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{28}H_{24}O_{15}$ by high-resolution (HR)-electrospray ionization (ESI)-MS. The IR spectrum exhibited the presence of hydroxy groups (3340 cm^{-1}), a conjugated ester (1695 cm^{-1}), a carbonyl group (1650 cm^{-1}), and aromatic rings (1616 and 1491 cm^{-1}), as well as phenolic hydroxy groups (1234 cm^{-1}). Absorption bands in the UV spectrum also supported the presence of the aromatic rings (348 and 271 nm). In the ^{1}H-NMR spectrum, three aromatic proton signals in an ABX coupling system, two singlet signals for aromatic and olefinic protons, respectively, and one singlet aromatic signal for two protons were observed. A singlet signal for a strongly chelated proton at δ_H 13.63 was also observed. The ^{13}C-NMR spectrum exhibited two carbon signals, δ_C 108.5 (d) and 145.5 (s), which each obviously accounted for two carbon atoms and must represent a galloyl moiety together with the signals at δ_C 119.5, 138.4 and 165.3. The ^{1}H–^{1}H correlation and heteronuclear single quantum correlation spectra revealed the connectivity of all aliphatic methine [5.11 (1H, dd, $J = 10$, 10 Hz, H-4″), 4.99 (1H, d, $J = 10$ Hz, H-1″), 4.38 (1H, dd, $J = 10$, 10 Hz, H-2″), 3.79 (1H, dd, $J = 10$, 10 Hz, H-3″), 3.71 (1H, ddd, $J = 10$, 6, 2 Hz, H-5″)] and methylene [3.67 (1H, dd, $J = 12$, 2 Hz, H-6″a), 3.61 (1H, dd, $J = 12$, 6 Hz, H-6″b)] protons and corresponding carbons. All the aliphatic methines were in *trans* relationships from their coupling constants and the anomeric proton was on the relatively shielded carbon atom (δ_C 75.4). Thus the sugar moiety was expected to be *C*-glucoside. The 4″-proton was obviously shifted downfield (δ_H 5.11), whereas that of isoorientin was at around δ_H 3.40 [19]. From the nature of the remaining 15 carbon signals and high degree of unsaturation, **1** was expected to have a *C*-glycosyl flavonoid skeleton. The ^{13}C-NMR data for **1** were similar to those for isoorietin (**3**) (Table 1), and in the heteronuclear multiple bond correlation (HMBC) spectrum, the anomeric proton showed cross peaks with C-5, 6 and 7 (Fig. 2). The methine proton (H-4″) shifted downfield showed a cross peak with the carbonyl carbon (δ_C 168.0),

Table 1 ^{13}C-NMR spectroscopic data for **1** and isoorientin (**3**) (100 MHz, DMSO-d_6 or CD_3OD)

C	1	1[a]	3
2	163.7	166.3	163.6
3	102.8	104.0	102.7
4	181.8	184.0	181.8
5	160.8	162.3	160.6
6	108.8	109.0	108.8
7	163.4	164.9	163.1
8	93.5	95.2	93.4
9	156.3	158.8	156.1
10	103.3	105.3	103.4
1′	121.4	123.7	121.4
2′	113.3	114.3	113.3
3′	145.7	147.1	145.7
4′	149.7	151.1	149.6
5′	116.0	116.9	116.0
6′	118.9	120.4	118.9
1″	73.2	75.4	73.0
2″	70.3	73.1	70.5
3″	76.3	78.2	78.9
4″	71.9	72.8	70.2
5″	79.5	81.0	81.4
6″	61.2	62.8	61.4
1‴	119.5	121.4	
2‴,6‴	108.5	110.5	
5‴,3‴	145.5	146.6	
4‴	138.4	140.1	
7‴	165.3	168.0	

[a] Measured in CD_3OD

Fig. 2 Selected HMBC correlation of compound **1**



confirming that the position of esterification was the hydroxy group at the 4″-position, and in fact mild alkaline hydrolysis of **1** gave isoorientin (**3**) [3]. Therefore, the structure of **1** was elucidated to be as shown in Fig. 1.

(2*S*)-1,2-Propanediol 1-*O*-β-D-glucopyranoside 6′-*O*-gallate (**2**), [α] −15.3, was isolated as an amorphous powder and its elemental composition was determined to be $C_{16}H_{22}O_{11}$ by HR-ESI–MS. The IR spectrum exhibited absorption bands for hydroxy groups (3388 cm^{-1}), a conjugated ester (1698 cm^{-1}), and an aromatic ring (1610 and 1514 cm^{-1}), and the UV spectrum for an aromatic ring (271 nm). The NMR spectra indicated the presence of a galloyl moiety, and six of the carbon signals were assignable as those of a 6-substituted glucopyranosyl group.

Thus, the aglycone comprised only three carbons, methyl, and primary and secondary alcohols. The absolute configuration of the glucose obtained on acid hydrolysis was determined to be of the D-series and the mode of linkage was β, as judged from the coupling constant ($J = 8$ Hz) of the anomeric proton on ^{1}H-NMR spectroscopy. Since, in the HMBC spectrum, the anomeric proton (δ_H 4.23) showed a cross peak with the primary alcohol (δ_C 76.7) and H_2-6′ (δ_H 4.54 and 4.39) with the carbonyl carbon (δ_C 168.3), the structure of **2** was elucidated to be as shown in Fig. 1. Mild alkaline hydrolysis of **2** gave **2a** [20, 21], whose chemical shifts and coupling patterns as to H_2-1a and 1b (δ_H 3.86 and 3.37) protons were indistinguishable from those of **2c** on comparison with those of synthetically prepared (2*R*)- and (2*S*)-1,2-propanediol 1-*O*-β-D-glucopyranosides (**2b** and **2c**, respectively) (Table 2) [22]. Thus, the absolute configuration at the 2-position of **2** was determined to be *S*.

Antioxidant activity was examined for some of isolated compounds using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging system. As expected from their structures, the IC_{50} values of compounds that possessed galloyl or cathechol moieties were generally 50 μM; that of the positive control, Trolox, was 15.4 ± 0.3 μM. **1**: 43.3 ± 4.6 μM, **2**: 40.5 ± 3.2 μM, **3**: 43.4 ± 2.6 μM, **4**: 47.3 ± 0.8 μM, **6**: 45.2 ± 1.1 μM, **7**: 46.3 ± 2.3 μM, and **17**: 55.8 ± 9.7 μM. Compounds **1**, **2**, **3**, **4**, **6**, **7** and **17** showed comparable activity to those possessing galloyl and cathechol moieties and they were less active than the positive control.

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 and 100 MHz, with tetramethylsilane as an internal standard at 35°C. Positive-ion HR-ESI–MS was performed with an Applied Biosystems QSTAR XL NanoSprayTM System.

A highly porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto) [$\Phi = 50$ mm, $L = 25$ cm, linear gradient: MeOH–H_2O (1:9, 1.5 l) → (9:1, 1.5 l), fractions of 10 g being collected]. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($\Phi = 2$ 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; $\Phi = 6$ mm, $L = 250$ mm, 1.6 ml/min), and the eluate was monitored with a UV detector at 254 nm, and a refractive index monitor.

Table 2 NMR spectroscopic data for **2** and **2a** and reference compounds (2*R*)- and (2*S*)-1,2-propanediol 1-*O*-β-D-glucopyranosides (**2b** and **2c**, respectively) (C: 100 MHz; H: 400 MHz, CD_3OD)

	2		**2a**	**2b**	**2c**
	C	H	H	H	H
1	76.7	3.34 dd 10, 8	3.37 dd 10, 8	3.56 dd 10, 4	3.35 dd 10, 8
		3.77 dd 10, 3	3.86 dd 10, 3	3.71 dd 10, 7	3.86 dd 10, 3
2	67.7	3.93 dqd 8, 6, 3	3.95 dqd 8, 6, 3	3.94 dqd 7, 7, 4	3.95 dqd 8, 7, 2
3	19.1	1.09 d 6	1.13 d 6	1.15 d 7	1.13 d 7
1′	104.9	4.32 d 8	4.27 d 8	4.28 d 8	4.27 d 7
2′	75.2	3.28 dd 9, 8	3.21 dd 9, 8	3.21 dd 9, 8	3.21 dd 8, 7
3′	77.8	3.42 dd 9, 9	3.34 dd 9, 9	3.34 dd 9,7	3.34 dd 8, 8
4′	71.7	3.41 dd 9, 9	3.29 dd 9, 9	3.29 dd 7, 7	3.29 dd 8, 8
5′	75.5	3.56 ddd 9, 6, 2	3.29 ddd 9, 5, 2	3.29 ddd 7, 5, 2	3.29 ddd 8. 5, 2
6′	64.7	4.39 dd, 12, 6	3.67 dd 12, 5	3.66 dd 12, 5	3.66 dd 12, 5
		4.54 dd 12, 2	3.86 dd 12, 2	3.86 dd 12, 2	3.86 dd 12, 2
1″	121.5	–			
2″,6″	110.2	7.08 s			
3″,5″	146.5	–			
4″	139.8	–			
7″	168.3	–			

Plant material

Leaves of *S. jasminodora* were collected in Kunigami-son, Kunigami-gun, Okinawa, Japan in June 2005, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (05-SJ-Okinawa-0627).

Extraction and isolation

Air-dried leaves of *S. jasminodora* (5.66 kg) were extracted three times with MeOH (30 l × 3) at room temperature for one week and then concentrated to 3 l in vacuo. The concentrated extract was washed with *n*-hexane (3 l, 72.0 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 138 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 l) to give a 1-BuOH-soluble fraction (106 g), and the remaining water-layer was concentrated to furnish 358 g of a water-soluble fraction. The 1-BuOH-soluble fraction (106 g) was subjected to Diaion HP-20 CC ($\Phi = 55$ mm, $L = 45$ cm), using H_2O–MeOH (4:1, 3 l), (3:2, 3 l), (2:3, 3 l), and (1:4, 3 l), and MeOH (3 l), 500 ml fractions being collected. The residue (20.5 g) in fractions 4–6 from the 20% MeOH eluate was subjected to silica gel (450 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (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), (7:3, 3 l), and (3:2, 3 l)], 500 ml fractions being collected. The residue (1.44 g) in fractions 44–49 was separated by ODS open CC, followed by DCCC (114 mg in fractions 47–54) to give 73.6 mg of **13** in fractions 8–14. The residue (91.3 mg) in fractions 63–72 was purified by HPLC (MeOH–H_2O, 1:4) to yield 12.1 mg of **2** from the peak at 12 min.

The residue (8.1 g) in fractions 7–8 obtained on Diaion HP-20 CC was subjected to silica gel (350 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1.5 l), and $CHCl_3$–MeOH (97:3, 1.5 l), (19:1, 1.5 l), (23:2, 1.5 l), (9:1, 1.5 l), (7:1, 1.5 l), (17:3, 1.5 l), (4:1, 1.5 l), (3:1, 1.5 l), (7:3, 1.5 l), and (3:2, 1.5 l)], 250 ml fractions being collected. The residue (799 mg) in fractions 28–35 was separated by ODS open CC, followed by DCCC (102 mg in fractions 87–102) to give 26.6 mg of **10** in fractions 70–84. The residue (710 mg) in fractions 36–41 was separated by ODS open CC, followed by DCCC (142 mg in fractions 90–108) to afford 53.0 mg of **14** in fractions 50–59 and 9.7 mg of **12** in fractions 73–83. The residue (880 mg) in fractions 52–69 was separated by ODS open CC to give 38.4 mg of **3** in fractions 147–164 and 28.1 mg of **1** in fractions 194–204. The residue (820 mg) in fractions 70–78 was separated by ODS open CC to give 195 mg of **17** in fractions 60–88.

The residue (28.5 g) in fractions 9–15 obtained on Diaion HP-20 CC was subjected to silica gel (600 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (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 (7:3, 3 l)], 500 ml fractions being collected. The residue (933 mg) in fractions 31–35 was separated by ODS open CC, followed by DCCC (117 mg in fractions 157–171) to give 58.5 mg of **11** in fractions 73–97. The residue (5.23 g) in fractions 45–51 was separated by ODS open CC to give 153 mg of **4** in fractions 118–125 and 75.4 mg of **6** in fractions 170–177. The residue (7.04 g) in fractions 52–59 was separated by ODS open CC to give 4.1 mg of **16** in fractions 65–81. ODS fractions 45–60 (30.1 mg) were combined and purified by HPLC (MeOH–H_2O, 1:9) to give 8.1 mg of **15** from the peak at 7 min.

The residue (7.22 g) in fraction 16–20 obtained on Diaion HP-20 CC was subjected to silica gel (180 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1.5 l), and $CHCl_3$–MeOH (97:3, 1.5 l), (19:1, 1.5 l), (23:2, 1.5 l), (9:1, 1.5 l), (7:1, 1.5 l), (17:3, 1.5 l), (4:1, 1.5 l), (3:1, 1.5 l), (7:3, 1.5 l), and (3:2, 1.5 l)], 250 ml fractions being collected. The residue (650 mg) in fractions 41–46 was separated by ODS open CC, followed by DCCC (160 mg in fraction 175–188) to give 26.2 mg of **7** in fractions 36–46 and 66.0 mg of **9** in fractions 47–55. Compound **5** (30.6 mg) was obtained from fractions 194–205 on ODS open CC as a precipitate.

The residue (20.9 g) in fractions 21–27 obtained on Diaion HP-20 CC was subjected to silica gel (500 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (3 l), and $CHCl_3$–MeOH (49:1, 3 l), (24:1, 3 l), (19:1, 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 (7:3, 3 l)], 500 ml fractions being collected. The residue (5.81 g) in fractions 25–31 was separated by ODS open CC to give 95.6 mg of **8** in fractions 211–223.

Isoorientin 4″-*O*-gallate (**1**). Amorphous powder, $[\alpha]_D^{23}$ +8.4 (*c* 0.95, MeOH). IR (film) cm^{-1}: 3340, 1695, 1650, 1616, 1491, 1446, 1348, 1234, 1115, 1039. UV (MeOH) nm (log ε): 348 (4.32), 271 (4.36), 217 (4.47). ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 13.63 (1H, s, 5-OH), 7.42 (1H, dd, $J = 8$, 2 Hz, H-6′), 7.40 (1H, d, $J = 2$ Hz, H-2′), 7.00 (2H, s, H-2‴ and 6‴), 6.90 (1H, d, $J = 8$ Hz, H-5′), 6.67 (1H, s, H-3), 6.51 (1H, s, H-8), 4.80 (1H, dd, $J = 10$, 10 Hz, H-4″), 4.69 (1H, d, $J = 10$ Hz, H-1″), 4.24 (1H, dd, $J = 10$, 10 Hz, H-2″), 3.51 (1H, dd, $J = 10$, 10 Hz, H-3″), 3.47 (1H, ddd, $J = 12$, 6, 2 Hz, H-5″), 3.41–3.28 (2H, m, H-6″a and 6″b). ^{1}H-NMR (400 MHz, CD_3OD) δ: 7.35 (1H, d, $J = 2$ Hz, H-2′), 7.34 (1H, dd, $J = 9$, 2 Hz, H-6′), 7.14 (2H, s, H-2‴ and 6‴), 6.89 (1H, d, $J = 9$ Hz, H-5′), 6.52 (1H, s, H-3), 6.48 (1H, s, H-8), 5.11 (1H, dd, $J = 10$, 10 Hz, H-4″), 4.99 (1H, d, $J = 10$ Hz, H-1″), 4.38 (1H, dd, $J = 10$, 10 Hz, H-2″), 3.79 (1H, dd, $J = 10$, 10 Hz, H-3″),

3.71 (1H, ddd, $J = 10$, 6, 2 Hz, H-5″), 3.67 (1H, dd, $J = 12$, 2 Hz, H-6″a), 3.61 (1H, dd, $J = 12$, 6 Hz, H-6″b). ^{13}C-NMR (100 MHz, DMSO-d_6): Table 1. ^{13}C-NMR (100 MHz, CD_3OD): Table 1. HR-ESI–MS (positive-ion mode) m/z 623.1007 $[M + Na]^+$ (Calcd for $C_{28}H_{24}O_{15}Na$: 623.1013).

(*S*)-1,2-Propanediol 1-*O*-*β*-D-glucopyranoside 6′-*O*-gallate (**2**). Amorphous powder, $[\alpha]_D^{23}$ −15.3 (*c* 0.81, MeOH). IR (film) cm^{-1}: 3388, 1698, 1652, 1610, 1514, 1452, 1341, 1231, 1073, 1039. UV (MeOH) nm (log ε): 271 (3.91), 217 (4.15). ^{1}H-NMR (400 MHz, CD_3OD): Table 2. ^{13}C-NMR (100 MHz, CD_3OD): Table 2. HR-ESI–MS (positive-ion mode) m/z 413.1054 $[M + Na]^+$ (Calcd for $C_{16}H_{22}O_{11}Na$: 413.1060).

Mild alkaline hydrolysis of **1**

To a solution of **1** (5.3 mg) in 0.9 ml of MeOH, 100 μl of 1 M NaOMe in MeOH was added, followed by stirring for 30 min at 25°C. The reaction mixture was neutralized with Amberlite IR-120B (H^+) and the hydrolyzed product was purified by prep. TLC [Merck 0.25 mm thickness, $CHCH_3$–MeOH–H_2O (15:6:1)] to give 1.22 mg of **1a**. The NMR spectra of **1a** [$[\alpha]_D^{23}$ −2.4 (*c* 0.12, DMSO)] were identical with those of isoorientin (**3**) [$[\alpha]_D^{25}$ −3.2 (*c* 0.31, DSMSO)], which co-occurs in this plant.

Mild alkaline hydrolysis of **2**

To a solution of **2** (6.0 mg) in 0.9 ml of MeOH, 100 μl of 1 M NaOMe in MeOH was added, followed by stirring for 30 min at 25°C. The reaction mixture was neutralized with Amberlite IR-120B (H^+) and the hydrolyzed product was purified by prep. TLC [Merck 0.25 mm thickness, $CHCH_3$–MeOH–H_2O (15:6:1)] to give 1.31 mg of **2a**. (*S*)-1,2-Propanediol 1-*O*-*β*-D-glucopyranoside (**2a**). Amorphous powder, $[\alpha]_D^{25}$ –4.4 (*c* 0.09, MeOH). ^{1}H-NMR (400 MHz, CD_3OD): Table 2. HR-ESI–MS (positive-ion mode) m/z 261.0948 $[M + Na]^+$ (Calcd for $C_9H_{18}O_7Na$: 261.0950).

Sugar analysis of **2**

Compound **2** (1.4 mg) was hydrolyzed with 1 N HCl (0.1 ml) at 90°C for 2 h. The reaction mixture was partitioned with an equal amount of EtOAc, and the water layer was analyzed with a chiral detector (JASCO OR-2090*plus*) on an amino column [Asahipak NH_2P-50 4E]. The hydrolyzate of **2** gave a peak for D-glucose at 11.8 min with a positive optical rotation sign [CH_3CN–H_2O (4:1), 1 ml/min]. The peak was identified by co-chromatography with authentic D-glucose.

Antioxidant activity with the DPPH radical-scavenging system

Antioxidant activity was evaluated using a DPPH radical-scavenging system. In 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 50, 30, 10 and 5 μM. The mixtures were incubated in the dark for 30 min at room temperature, followed by measurement of the absorbance at 515 nm with 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:

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

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

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References

1. Hatusima S (1975) Flora of the ryukyus. Added and corrected. The Biological Society of Okinawa, Naha, p 241
2. Bucek EU, Fournier G, Dadoun H (1989) Volatile constituents of *Ptychoe petalum olacoides* root oil. Planta Med 53:231
3. Harborne JB, Mabry TJ (eds) (1982) The flavonoids: advances in research. Chapman & Hall, New York, p 74
4. Zhou X, Peng J, Fan G, Wu Y (2005) Isolation and purification of flavonoid glycosides from *Trollius ledebouri* using high-speed counter-current chromatography by stepwise increasing the flow-rate of the mobile phase. J Chromatogr A 1092:216–221
5. Harborne JB, Mabry TJ (eds) (1982) The flavonoids: advances in research. Chapman & Hall, New York, p 61
6. Harborne JB, Mabry TJ (eds) (1982) The flavonoids: advances in research. Chapman & Hall, New York, p 73
7. Kazuma K, Noda N, Suzuki M (2003) Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. Phytochemistry 62:229–237
8. Huang CF, Gan XW, Bai HY, Ma L, Hu LH (2008) Schoepfin A, B, C: three new chalcone *C*-glycosides from *Schoepfia chinensis*. Nat Prod Res 22:623–627
9. Ohashi H, Goto M, Imamura H (1977) A *C*-glucosylchalcone from the wood of *Cladrastis platycarpa*. Phytochemistry 16:1106–1107

10. Yoshikawa M, Shimada H, Saka M, Yoshimizu S, Yamahara J, Matsuda H (1997) Medicinal food stuffs. V. Moroheiya. (1): absolute stereo structures of corchoionosides A, B, and C, histamine release inhibitors from the leaves of Vietnamese *Corchorus olitorius* L. (Tiliaceae). Chem Pharm Bull 45:464–469
11. Otsuka H (1994) Linarionosides A–C and acyclic monoterpene diglucosides from *Linaria japonica*. Phytochemistry 37:461–465
12. Yu Q, Matsunami K, Otsuka H, Takeda Y (2005) Staphylionosides A–K: megastigmane glucosides from the leaves of *Staphylea bumalda* DC. Chem Pharm Bull 53:800–807
13. Otsuka H, Hirata E, Shinzato T, Takeda Y (2003) Stereochemistry of megastigmane glucosides from *Glochidion zeylanicum* and *Alangium premnifolium*. Phytochemistry 62:763–768
14. Jensen SR, Nielsen BJ (1982) Iridoid glucosides in fouquieriaceae. Phytochemistry 21:1623–1629
15. Kwak JH, Kim HJ, Lee KH, Kang SC, Zee OP (2009) Antioxidative iridoid glycosides and phenolic compounds from *Veronica peregrina*. Arch Pharm Res 32:207–213
16. Lu Y, Sun Y, Foo LY, McNabb WC, Mokan AL (2000) Phenolic glycosides of forage legume *Onobrychis viciifolia*. Phytochemistry 55:67–75
17. Cheminat A, Zawatzky R, Becker H, Brouillard R (1988) Caffeoyl conjugates from *Echinacea* species: structures and biological activity. Phytochemistry 27:2787–2794
18. Lin LC, Kuo YC, Chou CJ (1999) Immunomodulatory principles of *Dichrocephala bicolor*. J Nat Prod 62:405–408
19. Shitamoto J, Matsunami K, Otsuka H, Shinzato Y, Takeda Y (2010) Crotalionosides A-C, three new megastigmane glucosides, two new pterocarpan glucosides and a chalcone *C*-glucoside from the whole plants of *Crotalaria zanzibarica* Bentham. Chem Pharm Bull 58:1026–1032
20. Kitajima J, Ishikawa T, Tanaka Y (1998) Water-soluble constituents of fennel. I. Alkyl glycosides. Chem Pharm Bull 46:1643–1646
21. Ishikawa T, Kondo K, Kitajima J (2003) Water-soluble constituents of coriander. Chem Pharm Bull 51:32–39
22. Kijima H, Otsuka H, Ide T, Ogimi C, Hirata E, Takushi A, Takeda Y (1996) Glycosides of megastigmane and the simple alcohols from *Alangium premnifolium*. Phytochemistry 42:723–727