Dihydroisocoumarin Constituents from the Leaves of *Hydrangea macrophylla* var. *thunbergii* (2).[1]: Absolute Stereostructures of Hydrangenol, Thunberginol I, and Phyllodulcin Glycosides and Isomerization Reaction at the 3-Positions of Phyllodulcin and Its Glycosides

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Following the characterization of 3*S*-phyllodulcin, 3*R*- and 3*S*-phyllodulcin 3′-*O*-glucosides, and 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides, six new dihydroisocoumarin glycosides, 3*R*- and 3*S*-hydrangenol 4′-*O*-apiosylglucosides, 3*R*- and 3*S*-thunberginol I 4′-*O*-glucosides, thunberginol I 8-*O*-glucoside, and 3*S*-phyllodulcin 8-*O*-glucoside, were isolated from the dried leaves of *Hydrangea macrophylla* var. *thunbergii* and their structures were determined on the basis of chemical and physicochemical evidence. In addition, isomerization reaction at the 3-positions of phyllodulcin and its glycosides was examined.

Key words thunberginol I glycoside; hydrangenol glycoside; *Hydrangea macrophylla* var. *thunbergii*; dihydroisocoumarin glycoside; dihydroisocoumarin 3-isomerization; Hydrangeae Dulcis Folium

In the course of our studies on bioactive constituents of natural medicines[2] and medicinal foodstuffs,[3] we have reported the isolation and structure elucidation of antiallergic and antimicrobial principles such as thunberginols A,[4] B,[4] C,[5] D,[5] E,[5] and F,[4] thunberginol G 3′-*O*-glucoside,[5] and hydramacrophyllols A[6] and B[6] from Hydrangeae Dulcis Folium, the processed leaves of *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (Saxifragaceae). We have found two secoiridoid glucoside complexes, hydramacrosides A[7] and B,[7] and four dihydroisocoumarin glycosides,[1] 3*R*- and 3*S*-phyllodulcin 3′-*O*-glucosides, 3*R*- and 3*S*- thunberginol H 8-*O*-glucosides, from the dried leaves of this plant. Furthermore, phyllodulcin from the dried leaves was found to be *ca*. 5 : 1 enantiomer mixture at the 3-position.[1] As a continuation of this study, we have isolated six new dihydroisocoumarin glycosides called 3*R*- and 3*S*-hydrangenol 4′-*O*-apiosylglucosides (**1**, **2**), 3*R*- and 3*S*-thunberginol I 4′-*O*-glucosides (**3**, **4**), thunberginol I 8-*O*-glucosides (**5**), and 3*S*-phyllodulcin 8-*O*-glucoside (**6**). This paper deals with the structure elucidation of these six new dihydroisocoumarin glycosides (**1**—**6**) and the isomerization reaction at the 3-positions of phyllodulcin and its glycosides.

3*R*- and 3*S*- Hydrangenol 4′-*O*-Apiosylglucosides 3*R*-hydrangenol 4′-*O*-apiosylglucoside (**1**) was isolated as a white powder of negative optical rotation ($[\alpha]_D^{25}$ −23.9°). The IR spectrum of **1** showed absorption bands ascribable to hydroxyl, chelated δ-lactone, and aromatic ring at 3570, 1670, and 1618 cm^{-1}, while absorption maxima ($\log \varepsilon$) were observed at 245 (3.2) and 314 (2.9) nm. In the positive-ion FAB-MS of **1**, a quasimolecular ion peak was observed at *m*/*z* 573 $(M+Na)^+$ and the molecular formula $C_{26}H_{30}O_{13}$ of **1** was confirmed by high-resolution MS measurement of the quasimolecular ion peak. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **1** showed signals assignable to a hydrangenol moiety [δ 5.73 (dd, *J*=3.1, 12.1 Hz, 3-H), 6.87 (d, *J*=6.9 Hz, 5-H), 6.90 (d, *J*=8.1 Hz, 7-H), 7.53 (dd, *J*=6.9, 8.1 Hz, 6-H); δ 7.09 (d, *J*=8.6 Hz, 3′, 5′-H), 7.47 (d, *J*=8.6 Hz, 2′, 6′-H), 10.92 (1H, br s, 8-OH)], a β-D-glucopyranosyl moiety [δ 4.85 (d, *J*=7.5 Hz, 1″-H)], and β-D-apiofuranosyl moiety [δ 4.83 (d, *J*=3.0 Hz, 1‴-H)]. Comparison of the NMR data for **1** with those of hydrangenol (**7**)[5] and 3*R*-hydrangenol 4′-*O*-glucoside (**9**)[5] allowed us to presume the structure of **1** to be the β-D-apiofuranoside of **9**. The position of the apioside linkage was characterized by a nuclear Overhauser effect (NOE) spectroscopy (NOESY) experiment on **1**, which showed NOE correlations between the anomeric proton (1‴-H) of the β-D-apiofuranosyl moiety and the 6″-protons of the β-D-glucopyranosyl moiety. Furthermore, a glycosidation shift was observed around the 6″-carbon in the ^{13}C-NMR data of **1**. The absolute configuration at the 3-position in **1** was identified by the circular dichroism (CD) spectrum, which showed the characteristic CD curve for 3*R*-dihydroisocoumarin[8] ($[\theta]_{257}$+2200, $[\theta]_{226}$−3000, $[\theta]_{221}$−5800). Acid hydrolysis of **1** with 5% aqueous sulfuric acid–dioxane (1 : 1) liberated D-apiose and D-glucose, which were identified by GLC analysis of the trimethylsilyl thiazolide derivatives.[9] Finally, partial methanolysis of **1** with 3% hydrogen chloride in dry methanol furnished 3*R*-hydrangenol 4′-*O*-glucosides (**9**).[5] On the basis of this evidence, the structure of **1** was determined to be 3*R*-hydrangenol 4′-*O*-β-D-apiofuranosyl (1→6)-β-D-glucopyranoside.

3*S*-Hydrangenol 4′-*O*-apiosylglucoside (**2**) was also isolated as a white powder of negative optical rotation ($[\alpha]_D^{25}$ −88.6°). The molecular formula $C_{26}H_{30}O_{13}$, which was the same as that of **1**, was confirmed from the quasimolecular ion peak at *m*/*z* 573 $(M+Na)^+$ in the positive-ion FAB-MS and by high-resolution MS measurement. The UV and IR spectra of **2** were found to be very similar to those of **1**. Comparison of the ^{1}H- and ^{13}C-NMR (Table 1) for **2** with those for **1** led us to presume the structure of **2** as the 3-epimer of **1**. The CD spectrum of **2** showed the characteristic pattern of the 3*S*-configuration[8] ($[\theta]_{258}$−4800, $[\theta]_{239}$+3200, $[\theta]_{226}$−1700). Acid hydrolysis of **2** liberated D-glucose and D-apiose,[9] while treatment of **2** with 3% hydrogen chloride

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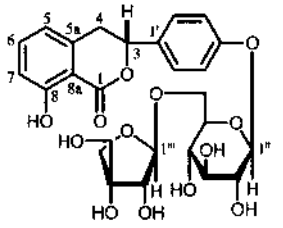
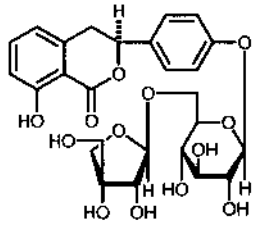


3*R*-hydrangenol 4'-*O*-apiosylglucoside (**1**)



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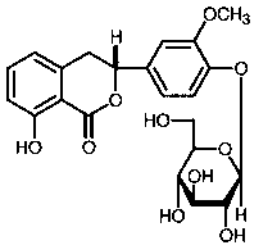
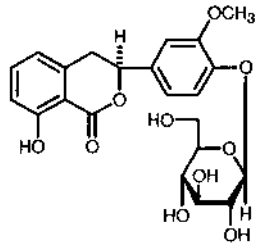


3*R*-thunberginol I 4'-*O*-glucoside (**3**)



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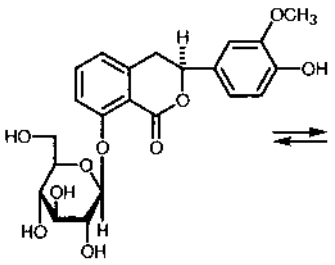
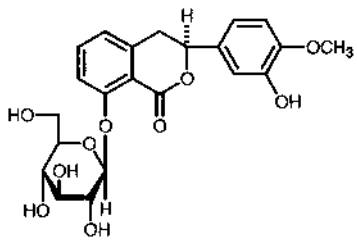


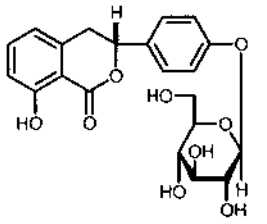
thunberginol I 8-*O*-glucoside (**5**)



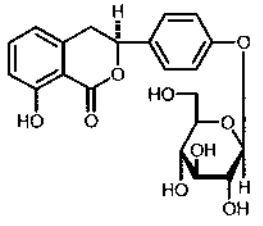
3*S*-phyllodulcin 8-*O*-glucoside (**6**)

Chart 1

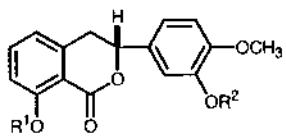
hydrangenol (**7**) : R=H
hydrangenol 8-*O*-glucoside (**8**) : R=Glc



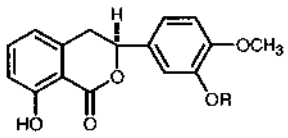
3*R*-hydrangenol 4'-*O*-glucoside (**9**)



3*S*-hydrangenol 4'-*O*-glucoside (**10**)



3*R*-phyllodulcin (**11**) : R^1=R^2=H
3*R*-phyllodulcin 8-*O*-glucoside (**12**) : R^1=Glc, R^2=H
3*R*-phyllodulcin 3'-*O*-glucoside (**13**) : R^1=H, R^2=Glc



3*S*-phyllodulcin (**14**) : R=H
3*S*-phyllodulcin 3'-*O*-glucoside (**15**) : R=Glc

Glc : β-D-glucopylanosyl

Chart 2

Table 1. ^{13}C-NMR Data of **1**, **2**, **3**, **4** and **5**

	1	2	3	4	5
C-1	169.1	169.1	169.1	169.0	161.2
C-3	80.0	80.1	80.2	80.2	78.3
C-4	33.4	33.6	33.5	33.6	35.1
C-4a	140.4	140.4	140.4	140.4	141.6
C-5	118.3	118.3	118.2	118.1	120.7
C-6	136.3	136.3	136.3	136.2	134.5
C-7	115.5	115.4	115.4	115.5	128.3
C-8	160.9	160.9	161.0	161.0	158.4
C-8a	108.4	108.4	108.3	108.4	114.3
C-1′	131.4	131.5	131.7	131.7	131.3
C-2′	128.0	128.0	114.9	114.9	111.9
C-3′	116.1	116.1	146.6	146.6	146.4
C-4′	157.6	157.5	148.8	148.8	147.7
C-5′	116.1	116.1	111.0	111.1	117.5
C-6′	128.0	128.0	119.0	119.0	113.9
3′-OCH_3			55.6	55.6	
4′-OCH_3					55.7
Glc-1″	100.3	100.2	99.7	99.7	100.6
2″	73.1	73.1	73.0	73.1	73.2
3″	76.4	75.6	76.9	76.9	77.2
4″	69.9	69.9	69.5	69.5	69.7
5″	75.7	75.8	76.7	76.7	76.6
6″	67.7	67.7	60.5	60.5	60.7
Api-1‴	109.2	109.2			
2‴	78.6	78.5			
3‴	78.6	78.5			
4‴	75.5	75.5			
5‴	62.9	62.9			

The spectra were taken with DMSO-d_6.

in dry methanol liberated 3*S*-hydrangenol 4′-*O*-glucoside (**10**). Consequently, the structure of 3*S*-hydrangenol 4′-*O*-apiosylglucoside (**2**) was characterized as shown.

3*R*- and 3*S*-Thunberginol I 4′-*O*-Glucosides 3*R*- and 3*S*-thunberginol I 4′-*O*-glucosides (**3**, **4**) were isolated as white powders of positive and negative optical rotations ($[\alpha]_D^{25}$ +15.5°, −11.9°), respectively. Their IR spectra were similar to each other and showed absorption bands due to hydroxyl, chelated δ-lactone, and aromatic ring. The UV spectra of **3** and **4** showed absorption maxima suggestive of the dihydroisocoumarin structure. The compounds (**3**, **4**) were found to have the same molecular formula $C_{22}H_{24}O_{10}$, which was identified from the quasimolecular ion peaks at *m*/*z* 449 $(M+H)^+$ and *m*/*z* 471 $(M+Na)^+$ in their positive-ion FAB-MS and by high-resolution MS analysis. Acid hydrolysis of **3** and **4** liberated D-glucose.[9]

The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **3** showed the presence of two trisubstituted benzene rings [δ 6.87 (d, J=7.3 Hz, 5-H), 6.90 (d, J=8.1 Hz, 7-H), 7.53 (dd, J=7.3, 8.1 Hz, 6-H); δ 7.01 (dd, J=1.7, 8.5 Hz, 6′-H), 7.12 (d, J=8.5 Hz, 5′-H), 7.18 (d, J=1.7 Hz, 2′-H)], a chelated 8-hydroxyl and δ-lactone [δ 5.71 (dd, J=3.0, 11.9 Hz, 3-H), 10.91 (br s, 8-OH)], a methoxyl group [δ 3.80 (s, 3′-OCH_3)], and a β-D-glucopyranosyl moiety [δ 4.94 (d, J=7.2 Hz, 1″-H)]. The positions of the glucoside linkage and the methoxyl group were elucidated by the NOESY experiment, in which NOE correlations were observed between the anomeric proton (1″-H) of the β-D-glucopyranosyl moiety and the 5′-proton and between the methoxyl protons and the 2′-proton. On the basis of this evidence and examination of the CD spectrum of **3**, which showed the characteristic CD curve for the 3*R*-dihydroisocoumarin[8] ($[\theta]_{256}$+10000, $[\theta]_{240}$ −5000), the structure of 3*R*-thunberginol I 4′-*O*-glucoside was determined to be 3*R*-(3′-*O*-methoxy-4′-*O*-β-D-glucopyranosylphenyl)-8-hydroxydihydroisocoumarin (**3**).

The ^{1}H- and ^{13}C-NMR (Table 1) spectra of **4** were found to be superimposable on those of **3** and the NOESY experiment on **4** showed the same NOE correlations as those of **3**. This evidence revealed that **4** was the epimer of **3** at the 3-position. The CD spectrum of **4** showed the characteristic CD curve for the 3*S*-dihydroisocoumarin (**8**) ($[\theta]_{255}$−8300, $[\theta]_{240}$ +4800) and consequently, the structure of 3*S*-thunberginol I 4′-*O*-glucoside (**4**) was determined as shown.

Thunberginol I and 3*S*-Phyllodulcin 8-*O*-Glucosides Thunberginol I 8-*O*-glucoside (**5**) was obtained as a white powder and shown to be a 3-epimeric mixture by examination of the ^{1}H- and ^{13}C-NMR spectra. Since the 3-position in **5** showed tautomer-like behavior during the separation procedure, we could not separate the 3-epimers of **5**. The IR spectrum of **5** showed absorption bands ascribable to hydroxyl, δ-lactone, and benzene ring at 3325, 1713, and 1601 cm^{-1}, while its UV spectrum showed absorption maxima ($\log \varepsilon$) at 226 (4.1) and 287 (3.6) nm, suggestive of the dihydroisocoumarin structure. The molecular formula, $C_{22}H_{24}O_{10}$, which was the same as that of **3** or **4**, was determined from the positive-ion FAB-MS [*m*/*z* 471 $(M+Na)^+$] and by high-resolution MS measurement. On acid hydrolysis of **5**, D-glucose was detected.[9] The ^{1}H-NMR spectrum of **5** showed two pairing signals due to a thunberginol I moiety and a β-D-glucopyranosyl moiety. In the NOESY experiment on **5**, NOE correlations were observed between the anomeric proton (1″-H) and the 7-proton and between the methoxyl protons and the 2′-proton. Consequently, the structure of thunberginol I 8-*O*-glucoside (**5**) was characterized as shown.

3*S*-Phyllodulcin 8-*O*-glucoside (**6**), also isolated as a white powder of negative optical rotation ($[\alpha]_D^{25}$ −121.3°), showed absorption bands due to hydroxyl, δ-lactone, aromatic ring in the IR spectrum. The molecular formula $C_{22}H_{24}O_{10}$ of **6** was determined from the quasimolecular ion peak at *m*/*z* 449 $(M+H)^+$ and by high-resolution MS measurement. The ^{1}H-NMR (DMSO-d_6) spectrum of **6** showed signals assignable to a phyllodulcin moiety [δ 3.76 (s, 4′-OCH_3), 5.39 (dd, J=3.0, 10.9 Hz, 3-H), 6.85 (dd, J=1.8, 8.4 Hz, 6′-H), 6.91 (d, J=1.8 Hz, 2′-H), 6.93 (d, J=8.4 Hz, 5′-H), 7.02 (d, J=7.4 Hz, 5-H), 7.18 (d, J=8.4 Hz, 7-H), 7.52 (dd, J=7.4, 8.4 Hz, 6-H)] and a β-D-glucopyranosyl moiety [δ 4.94 (d, J=7.2 Hz, 1″-H)]. In the CD spectrum of **6**, the CD curve due to 3*S*-dihydroisocoumarin[8] was observed. Finally, as described later, treatment of 3*R*-phyllodulcin 8-*O*-glucoside (**12**)[10] with 50% aqueous methanol under reflux furnished a mixture of **6** and **12**. On the basis of this evidence, the structure of 3*S*-phyllodulcin 8-*O*-glucoside (**6**) was determined as shown.

Isomerization Reaction at the 3-Position of Phyllodulcin and Its Glycosides The 3-position of dihydroisocoumarin having the 4′-hydroxyl group was known to show tautomer-like behavior and this type dihydroisocoumarin existed as a racemic mixture [e.g. hydrangenol (**7**)]. Furthermore, the TLC and HPLC chromatograms of hydrangenol 8-*O*-glucoside (**8**) showed two spots or peaks in *ca*. 1 : 1 ratio, but these could not be separated because of the tautomer-like behavior as previously described.[5,11] On the other hand, the

3-position of phyllodulcin having the 4′-methoxyl group was known to be stable and the absolute configuration at the 3-position of phyllodulcin was reported to be *R*.[12]

We recently found that phyllodulcin from the dried leaves of *Hydrangea macrophylla* var. *thunbergii* was an enantiomer mixture of *ca*. 5 : 1 ratio at the 3-position, and 3*S*-phyllodulcin (**14**) was isolated using chiral HPLC.[1] In order to examine the stability of the 3-position in dihydroisocoumarin having the 4′-methoxyl group, 3*R*- and 3*S*-phyllodulcin (**11**, **14**) and their glucosides (**6**, **12**, **13**, **15**) were treated under extraction conditions of the leaves. After preliminary examination, 3*R*-phyllodulcin (**11**) was found to convert 3*S*-phyllodulcin (**14**) under reflux for 3 h in the aqueous-methanol solution (1 : 1) and provided a mixture of **11** and **14** in *ca*. 1 : 0.6 ratio, while **14** also yielded a mixture of **11** and **14** in *ca*. 1.3 : 1 ratio under the same conditions. By the same treatment, 3*R*-phyllodulcin 8-*O*-glucoside (**12**) and the 3′-*O*-glucoside (**13**) furnished *ca*. 0.1 : 1 mixture of **6** and **12** and *ca*. 1 : 0.01 mixture of **13** and **15**, whereas 3*S*-phyllodulcin 8-*O*-glucoside (**6**) and the 3′-*O*-glucoside (**15**) yielded *ca*. 1 : 1.5 mixture of **6** and **12** and *ca*. 0.01 : 1 mixture of **13** and **15**, respectively. This evidence revealed that the 3-positions of phyllodulcins (**11**, **14**) and their glucosides (**6**, **12**, **13**, **15**) also had a tautomer-like behavior under the reflux condition of their aqueous-methanol solution.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.[1]

Isolation of Hydrangenol and Thunberginol I Glycosides (1—5) 3*R*- and 3*S*-Hydrangenol 4′-*O*-apiosylglucosides (**1**, **2**), 3*R*- and 3*S*-thunberginol I 4′-*O*-glucosides (**3**, **4**), and thunberginol I 8-*O*-glucoside (**5**) were isolated as described earlier.[1] 3*R*- and 3*S*-Phyllodulcin 8-*O*-glucoside mixture (38.6 mg)[1] was separated by chiral column HPLC [column: Ceramospher chiral RU-1 (250×10 mm i.d., Shiseido, Ltd.), solvent: MeOH, flow rate: 2 ml/min] to give **6** (8.5 mg) and **12** (30 mg).

3*R*-Hydrangenol 4′-*O*-Apiosylglucoside (**1**): A white powder, $[\alpha]_D^{25}$ −23.9° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{30}O_{13}Na$ $(M+Na)^+$: 573.1584. Found: 573.1600. CD $[\theta]_{25}$ (*c*=0.0025, MeOH, nm): −5800 (221), −3000 (226), +2200 (257). UV [λ_{max}^{MeOH} nm (log ε)]: 245 (3.2), 314 (2.9). IR (KBr, cm^{-1}): 3570, 1670, 1618, 1000. ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 4.83 (1H, d, *J*=3.0 Hz, 1‴-H), 4.85 (1H, d, *J*=7.5 Hz, 1″-H), 5.73 (1H, dd, *J*=3.1, 12.1 Hz, 3-H), 6.87 (1H, d, *J*=6.9 Hz, 5-H), 6.90 (1H, d, *J*=8.1 Hz, 7-H), 7.09 (2H, d, *J*=8.6 Hz, 3′, 5′-H), 7.47 (2H, d, *J*=8.6 Hz, 2′, 6′-H), 7.53 (1H, dd, *J*=6.9, 8.1 Hz, 6-H), 10.92 (1H, br s, 8-OH). ^{13}C-NMR (DMSO-d_6, 68 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m*/*z* 573 $(M+Na)^+$.

3*S*-Hydrangenol 4′-*O*-Apiosylglucoside (**2**): A white powder, $[\alpha]_D^{25}$ −88.6° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{26}H_{30}O_{13}Na$ $(M+Na)^+$: 573.1584. Found: 573.1596. CD $[\theta]_{25}$ [*c*=0.0025, MeOH, nm]: −1700 (226), +3200 (239), −4800 (258). UV [λ_{max}^{MeOH} nm (log ε)]: 242 (3.3), 314 (3.0). IR (KBr, cm^{-1}): 3570, 1670, 1617. ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 4.84 (1H, d, *J*=3.0 Hz, 1‴-H), 4.86 (1H, d, *J*=7.3 Hz, 1″-H), 5.72 (1H, dd, *J*=3.0, 12.2 Hz, 3-H), 6.88 (1H, d, *J*=7.4 Hz, 5-H), 6.91 (1H, d, *J*=7.9 Hz, 7-H), 7.09 (2H, d, *J*=8.9 Hz, 3′, 5′-H), 7.48 (2H, d, *J*=8.9 Hz, 2′, 6′-H), 7.53 (1H, dd, *J*=7.4, 7.9 Hz, 6-H), 10.89 (1H, br s, 8-OH). ^{13}C-NMR (DMSO-d_6, 68 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m*/*z* 573 $(M+Na)^+$.

3*R*-Thunberginol I 4′-*O*-Glucoside (**3**): A white powder, $[\alpha]_D^{25}$ +15.5° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{25}O_{10}$ $(M+H)^+$: 449.1448. Found: 449.1476. IR (KBr, cm^{-1}): 3410. 1665, 1619. CD $[\theta]_{25}$ (*c*=0.0025, MeOH, nm): −5000 (240), +10000 (256). UV [λ_{max}^{MeOH} nm (log ε)]: 280 (3.6), 313 (3.7). ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 3.80 (3H, s, 3′-OCH_3), 4.94 (1H, d, *J*=7.2 Hz, 1″-H), 5.71 (1H, dd, *J*=3.0, 11.9 Hz, 3-H), 6.87 (1H, d, *J*=7.3 Hz, 5-H), 6.90 (1H, d, *J*=8.1 Hz, 7-H), 7.01 (1H, dd, *J*=1.7, 8.5 Hz, 6′-H), 7.12 (1H, d, *J*=8.5 Hz, 5′-H), 7.18 (1H, d, *J*=1.7 Hz, 2′-H), 7.53 (1H, dd, *J*=7.3, 8.1 Hz, 6-H), 10.91 (1H, br s, 8-OH). ^{13}C-NMR (DMSO-d_6, 68 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m*/*z* 449 $(M+H)^+$, 471 $(M+Na)^+$.

3*S*-Thunberginol I 4′-*O*-Glucoside (**4**): A white powder, $[\alpha]_D^{25}$ −11.9° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{25}O_{10}$ $(M+H)^+$: 449.1448. Found: 449.1458. IR (KBr, cm^{-1}): 3410, 1669, 1618. CD $[\theta]_{25}$ (*c*=0.0025, MeOH, nm): +4800 (240), −8300 (255). UV [λ_{max}^{MeOH} nm (log ε)]: 280 (3.7), 313 (3.8). ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 3.80 (3H, s, 3′-OCH_3), 4.94 (1H, d, *J*=7.3 Hz, 1″-H), 5.70 (1H, dd, *J*=3.2, 12.1 Hz, 3-H), 6.87 (1H, d, *J*=7.5 Hz, 5-H), 6.90 (1H, d, *J*=8.3 Hz, 7-H), 7.02 (1H, dd, *J*=1.7, 8.6 Hz, 6′-H), 7.12 (1H, d, *J*=8.6 Hz, 5′-H), 7.17 (1H, d, *J*=1.7 Hz, 2′-H), 7.52 (1H, dd, *J*=7.5, 8.3 Hz, 6-H), 10.93 (1H, br s, 8-OH). ^{13}C-NMR (DMSO-d_6, 68 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m*/*z* 449 $(M+H)^+$, 471 $(M+Na)^+$.

Thunberginol I 8-*O*-Glucoside (**5**): A white powder, $[\alpha]_D^{25}$ −67.6° (*c*=0.5, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{24}O_{10}Na$ $(M+Na)^+$: 471.1267. Found: 471.1285. IR (KBr, cm^{-1}): 3325, 1713, 1601. CD $[\theta]_{25}$ (*c*=0.0025, MeOH, nm): +6500 (240), −4400 (262), −5700 (295). UV [λ_{max}^{MeOH} nm (log ε)]: 226 (4.1), 287(3.6). ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 3.77 (3H, s, 3′-OCH_3), 4.67, 4.95 (total 1H, both d, *J*=7.3 Hz, 1″-H), 5.39, 5.48 (total 1H, both dd, *J*=2.6, 10.6 Hz, 3-H), 6.79, 6.80 (total 1H, both d, *J*=8.6 Hz, 5′-H), 6.86, 7.33 (total 1H, both dd, *J*=2.0, 8.6 Hz, 6′-H), 6.92, 7.11 (total 1H, both d, *J*=2.0 Hz, 2′-H), 7.02, 7.11 (total 1H, both d, *J*=7.6 Hz, 5-H), 7.18, 7.31 (total 1H, both d, *J*=8.3 Hz, 7-H), 7.53, 7.61 (total 1H, both dd, *J*=7.6, 8.3 Hz, 6-H). ^{13}C-NMR (DMSO-d_6, 68 MHz) δc: given in Table 1. Positive-ion FAB-MS: *m*/*z* 471 $(M+Na)^+$.

3*S*-Phyllodulcin 8-*O*-Glucoside (**6**): A white powder, $[\alpha]_D^{25}$ −121.3° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{25}O_{10}$ $(M+H)^+$: 449.1448. Found: 449.1445. IR (KBr, cm^{-1}): 3422, 1709, 1603. CD $[\theta]_{25}$ (*c*=0.0025, MeOH, nm): +5000 (240), −8900 (258), −8100 (296). ^{1}H-NMR (DMSO-d_6, 270 MHz) δ: 3.76 (3H, s, 4′-OCH_3), 4.94 (1H, d, *J*=7.2 Hz, 1″-H), 5.39 (1H, dd, *J*=3.0, 10.9 Hz, 3-H), 6.85 (1H, dd, *J*=1.8, 8.4 Hz, 6′-H), 6.91 (1H, d, *J*=1.8 Hz, 2′-H), 6.93 (1H, d, *J*=8.4 Hz, 5′-H), 7.02 (1H, d, *J*=7.4 Hz, 5-H), 7.18 (1H, d, *J*=8.4 Hz, 7-H), 7.52 (1H, dd, *J*=7.4, 8.4 Hz, 6-H). Positive-ion FAB-MS: *m*/*z* 449 $(M+Na)^+$.

Acid Hydrolysis of 3*R*- and 3*S*-Hydrangenol 4′-*O*-Apiosylglucosides (1, 2), 3*R*- and 3*S*-Thunberginol I 4′-*O*-Glucosides (3, 4), and Thunberginol I 8-*O*-Glucoside (5) A solution of **1**—**5** (2 mg each) in 5% aqueous H_2SO_4–dioxane (1 : 1, v/v, 1 ml) was heated under reflux for 2 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered. After removal of the solvent *in vacuo* from the filtrate, the residue was passed through a Sep-Pak C18 cartridge with H_2O and MeOH. The H_2O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (2 mg) in pyridine (0.02 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (0.01 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives (**i**, **ii**) of D-glucose (from **1**, **2**, **3**, **4**, and **5**) and D-apiose (from **1** and **2**). GLC conditions: column, Supelco™-1, 0.25 mm (i.d.)×30 m; column temperature, 230 °C; t_R: **i**, 24.0 min, **ii**, 14.2 min.

Partial Methanolysis of 3*R*- and 3*S*-Hydrangenol 4′-*O*-Apiosylglucosides (1, 2) A solution of **1** or **2** (each 5.0 mg) in 3% HCl-dry MeOH (0.5 ml) was stirred at room temperature for 2 h. The reaction solution was neutralized with Amberlite IRA-400 (OH^- form) and the resin was filtered off. After removal of the solvent from the filtrate under reduced pressure the residue was purified by ODS silica gel column chromatography [MeOH–H_2O (1 : 1)] to give 3*R*- [**9** (3.0 mg) from **1**] or 3*S*-hydrangenol 4′-*O*-glucoside [**10** (2.9 mg) from **2**], which was identified with an authentic sample[1] by HPLC [Ceramospher Chiral RU-1 (250×4.6 mm i.d.), MeOH, 2.0 ml/min, t_R: **9**, 12.4 min; **10**, 27.2 min].

Isomerization Reaction of 3*R*- and 3*S*- Phyllodulcin (11, 14) and its Glucosides (6, 12, 13, 15) i) A solution of **6**, **11**—**15** (each 1.0 mg) in 50% aqueous MeOH (2 ml) was heated under reflux for 3 h. After removal of the solvent under reduced pressure, the residue was subjected to HPLC analysis [Ceramospher Chiral RU-1 (250×4.6 mm i.d.), MeOH, 2.0 ml/min] to determine the composition of the 3*R*- and 3*S*-isomers. The proportions of the isomers were characterized from comparison of the peak areas in HPLC analysis.

ii) A solution of **12** (35.0 mg) in 50% aqueous MeOH (10 ml) was heated under reflux for 3 h. After removal of the solvent under reduced pressure, the residue was subjected to HPLC [Ceramospher Chiral RU-1 (250×10 mm i.d.), MeOH, 2.0 ml/min, t_R: **6**, 26.0 min; **12**, 22.8 min] to give **6** (12.4 mg) and **12** (9.3 mg). **6** was identified with an authentic 3*S*-phyllodulcin 8-*O*-glucoside by CD and ^{1}H-NMR spectra.

References and Notes

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