ABSOLUTE STEREOSTRUCTURES OF 3*S*-PHYLLODULCIN, 3*R*- AND 3*S*-PHYLLODULCIN GLYCOSIDES, AND 3*R*- AND 3*S*-THUNBERGINOL H GLYCOSIDES FROM THE LEAVES OF *HYDRANGEA MACROPHYLLA* SERINGE VAR. *THUNBERGII* MAKINO[1]

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Abstract — A new dihydroisocoumarin, 3*S*-phyllodulcin, and nine new dihydroisocoumarin glycosides, 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucosides, 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides, 3*R*- and 3*S*-thunberginol I 4'-*O*-glucosides, 3*R*- and 3*S*-hydrangenol 4'-*O*-apiosylglucosides, and thunberginol I 8-*O*-glucoside, were isolated from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO. The absolute stereostructures of 3*S*-phyllodulcin, 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucosides, and 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides were elucidated on the chemical and physicochemical evidence. Phyllodulcin from the unprocessed leaves was found to be *ca*. a 5 : 1 enantiomer mixture at the 3-position.

Hydrangeae Dulcis Folium (Japanese name "Amacha"), which is a rare natural medicine indigenous to Japan, is prepared from the leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae) *via* several processing such as crumpling, fermentation, and drying. This natural medicine is listed in the Japanese Pharmacopoeia XIII, and extensively used in confectionery, drinks, and foods as an oral refrigerant and sweeting. Previously, we have found that the methanolic extract of this natural medicine exhibited potent antiallergic, antiulcer, antibacterial, antioxidative, and cholagoic activities.[2] As the antiallergic and antimicrobial principles of Hydrangeae Dulcis Folium, the processed leaves of *H. macrophylla* var. *thunbergii*, we have reported the isolation and structure elucidation of two isocoumarins (thunberginols A and B),[3] three dihydroisocoumarins (thunberginols C, D, and E),[4] a benzylidene-phthalide (thunberginol F),[3] three dihydroisocoumarin glucosides [thunberginol G 3'-*O*-glucoside, (+)- and (-)-hydrangenol 4'-*O*-glucosides],[4] and two phthalides (hydramacrophyllols A and B).[5] By use of the HPLC quantitative analysis method, the chemical processing of this natural medicine was clarified together with the distribution in the plant and seasonal fluctuation of the major constituents.[6]
Furthermore, we have reported two secoiridoid glucoside complexes called hydramacrosides A and B with histamine release inhibitory activity from the unprocessed leaves of *H. macrophylla* var. *thunbergii*.[7] As a continuation of this study, we have isolated phyllodulcin and new dihydroisocoumarin glycosides called 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucosides (**1**, **2**), 3*R*- and 3*S*-thunberginol H 8-*O*-glucosides (**3**, **4**), 3*R*- and 3*S*-thunberginol I 4'-*O*-glucosides, 3*R*- and 3*S*-hydrangenol 4'-*O*-apiosylglucosides, and thunberginol I 8-*O*-glucoside from the unprocessed leaves together with many known compounds. In addition, phyllodulcin was found to be an enantiomer mixture of *ca*. a 5 : 1 ratio at the 3-position. This paper deals with the isolation of nine new dihydroisocoumarin glycosides and 3*S*-phyllodulcin (**5**) from the leaves of *H. macrophylla* var. *thunbergii* and structure elucidation of **1**-**5**.

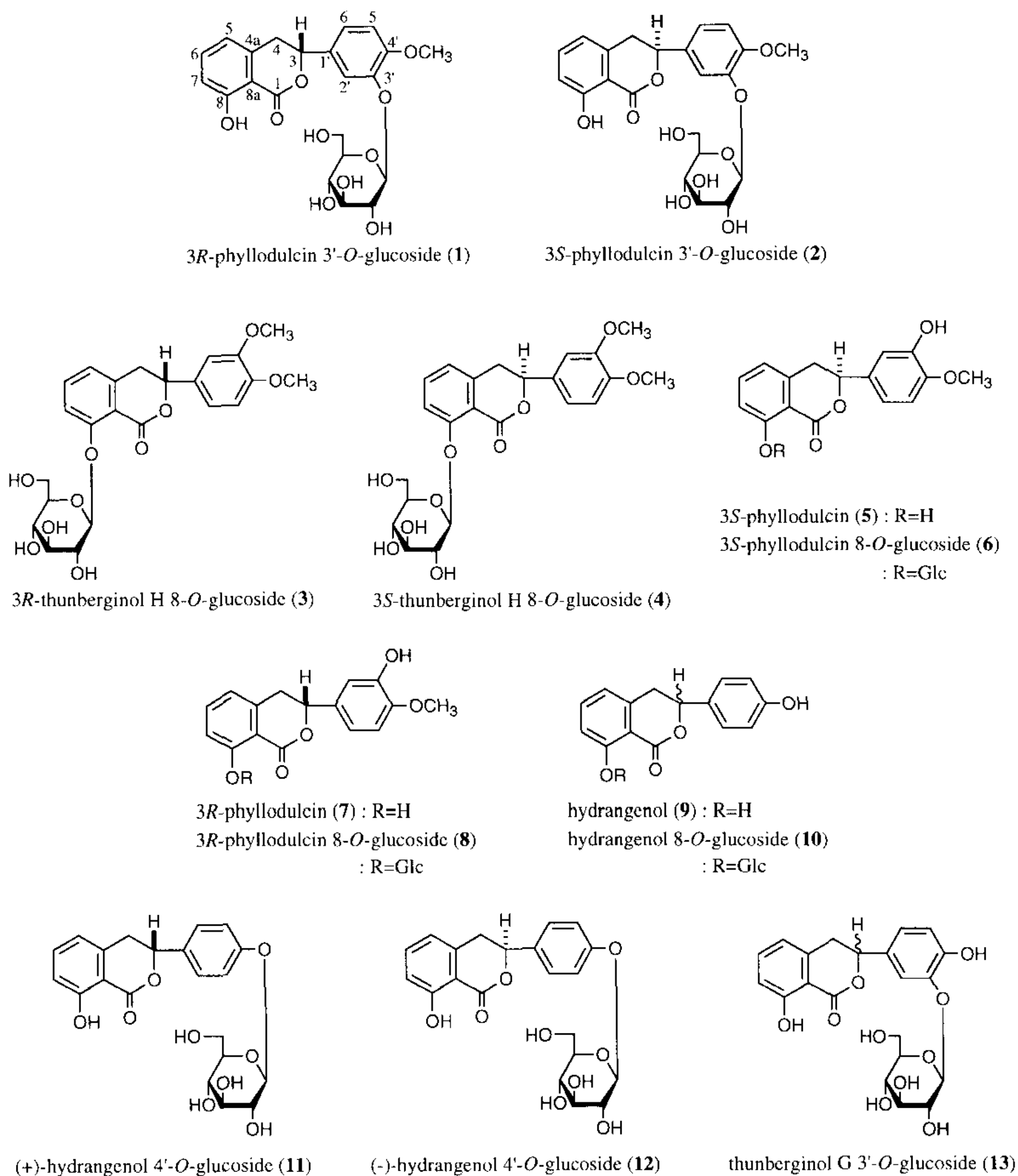
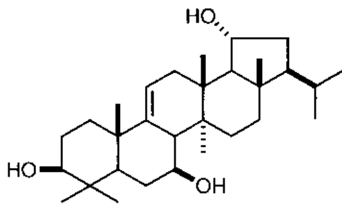
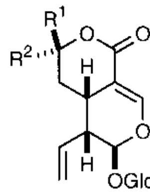


Figure 1

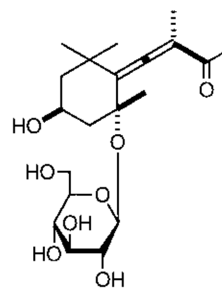
The methanolic extract from the leaves of *H. macrophylla* var. *thunbergii* cultivated in Nagano Prefecture was partitioned into a mixture of ethyl acetate and water to furnish the ethyl acetate-soluble fraction and water phase. The water phase was further extracted with 1-butanol to give the 1-butanol-soluble fraction and water-soluble fraction. The 1-butanol-soluble fraction was subjected to silica gel column chromatography and repeated HPLC (YMC-Pack R&D D-ODS-A) to afford 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucoside mixture (**1**, **2**), 3*R*- and 3*S*-thunberginol H 8-*O*-glucoside mixture (**3**, **4**), 3*R*- and 3*S*-



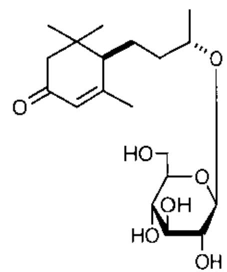
rubiarbonol B (**14**)



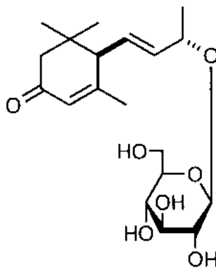
vogeloside (**15**)
: R^1= OCH_3, R^2= H
epi-vogeloside (**16**)
: R^1= H, R^2= OCH_3



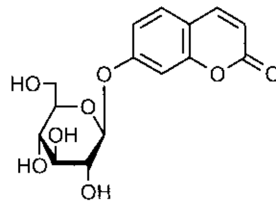
citroside A (**17**)



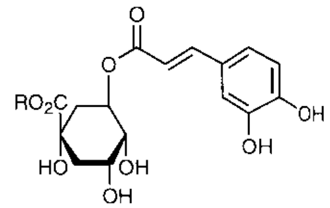
blumenol C glucoside (**18**)



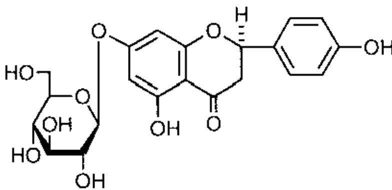
(6*R*,7*E*,9*R*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucoside (**19**)



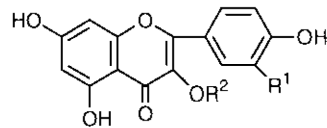
umbelliferone 7-*O*-glucoside (**20**)



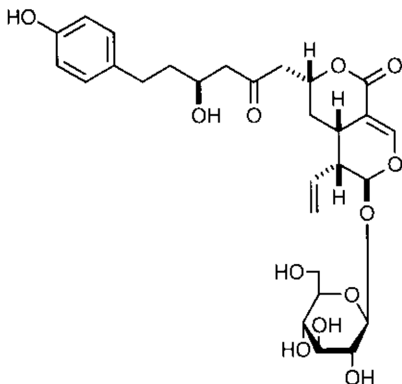
chlorogenic acid (**21**) : R=H
methyl chlorogenate (**22**) : R=CH_3



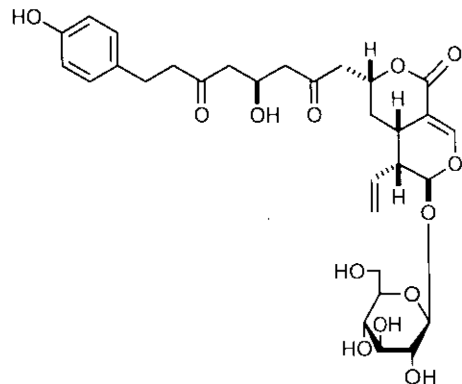
prunin (**23**)



24 : R^1=OH, R^2=Glc $\overset{2}{—}$ Glc
25 : R^1=H, R^2=Glc $\overset{2}{—}$ Glc
|6
Rha
26 : R^1=H, R^2=Glc $\overset{2}{—}$ Glc



hydramacroside A (**27**)



hydramacroside B (**28**)

Figure 2

thunberginol I 4'-*O*-glucoside mixture, 3R- and 3*S*-hydrangenol 4'-*O*-apiosylglucoside mixture, and thunberginol I 8-*O*-glucoside (0.0012%) together with vogeloside[8] (**15**, 0.0009%), *epi*-vogeloside[8] (**16**, 0.0011%), umbelliferone 7-*O*-glucoside[9] (**20**, 0.0012%), 3*R*- and 3*S*-phyllodulcin 8-*O*-glucoside mixture[6] (**6**, **8**, 0.55%), blumenol C glucoside[10] (**18**, 0.0037%), (6*R*,7*E*,9*R*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucoside[11] (**19**, 0.0012%), (+)- and (-)-hydrangenol 4'-*O*-glucoside mixture[4] (**11**, **12**, 0.0003%), thunberginol G 3'-*O*-glucoside[4] (**13**, 0.0004%), hydrangenol 8-*O*-glucoside[6] (**10**, 1.40%), citroside A[12] (**17**, 0.0008%), prunin[13] (**23**, 0.0008%), chlorogenic acid[14] (**21**, 0.014%), methyl chlorogenate[14] (**22**, 0.024%), quercetin 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside[15] (**24**, 0020%), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→6)]-β-D-glucopyranoside[16] (**25**, 0.0031%), kaempferol 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranoside[17] (**26**, 0.0058%), and hydramacrosides A[7] (**27**, 0.0016%) and B[7] (**28**, 0.0018%). Each enantiomeric mixture was successfully separated by chiral column HPLC (Ceramospher Chiral RU-1) to give 3*R*-phyllodulcin 3'-*O*-glucoside (**1**, 0.0003%), 3*S*-phyllodulcin 3'-*O*-glucoside (**2**, 0.0002%), 3*R*-thunberginol H 8-*O*-glucoside (**3**, 0.0012%), 3*S*-thunberginol H 8-*O*-glucoside (**4**, 0.0018%), 3*R*-thunberginol I 4'-*O*-glucoside (0.0002%), 3*S*-thunberginol I 4'-*O*-glucoside (0.0001%), 3*R*-hydrangenol 4'-*O*-apiosylglucoside (0.0001%), and 3*S*-hydrangenol 4'-*O*-apiosylglucoside (0.0003%).

The ethyl acetate-soluble fraction was subjected to silica gel and ODS column chromatography to yield 3*R*- and 3*S*-phyllodulcin mixture, hydrangenol (**9**, 0.015%), and rubiarbonol B[18] (**14**, 0.0022%). 3*R*- and 3*S*-phyllodulcin mixture was further purified by chiral column HPLC (Ceramospher Chiral RU-1) to give 3*R*- (**7**, 0.0084%) and 3*S*-enantiomers (**5**, 0.0017%).

3*R*- and 3*S*-Phyllodulcin 3'-*O*-Glucosides

3*R*-Phyllodulcin 3'-*O*-glucoside (**1**) was obtained as a white powder and its IR spectrum showed absorption bands assignable to hydroxyl, chelated δ-lactone, and aromatic ring at 3570, 1650, and 1618 cm^{-1}. The UV spectrum of **1** showed absorption maxima (log ε) at 227 (3.6), 280 (3.0), and 312 (3.0) nm. In the positive-ion FAB-MS of **1**, quasimolecular ion peaks were observed at *m/z* 449 $(M+H)^+$ and *m/z* 471 $(M+Na)^+$ and its molecular formula $C_{22}H_{24}O_{10}$ was confirmed by high-resolution MS measurement of the quasimolecular ion peak $(M+H)^+$. The ^1H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned on the basis of various NMR experiment,[19] showed signals assignable to two trisubstituted benzene rings [δ 7.03 (d, *J*=8.6 Hz, 5'-H), 7.11 (dd, *J*=1.7, 8.6 Hz, 6'-H), 7.24 (d, *J*=1.7 Hz, 2'-H); δ 6.86 (d, *J*=8.4 Hz, 5-H), 6.89 (d, *J*=8.4 Hz, 7-H), 7.52 (dd, *J*=8.4, 8.4 Hz, 6-H)], a chelated 8-hydroxyl and δ-lactone [δ 5.68 (dd, *J*=3.0, 11.6 Hz, 3-H), 10.9 (br s, 8-OH)], a methoxyl group [δ 3.79 (s)], and a β-D-glucopyranosyl moiety [δ 4.93 (d, *J*=6.9 Hz, 1''-H)]. Comparison of the NMR data for **1** with those for phyllodulcin[3,20] and phyllodulcin 8-*O*-glucoside[4,20] allowed us to presume the structure of **1** to be the β-D-glucopyranoside of phyllodulcin. The positions of the glucoside linkage and methoxyl group were characterized by a NOESY experiment on **1**, in which NOE correlations were observed between the anomeric proton and the 2'-proton and between the methoxyl group and the 5'-proton. Finally, the absolute stereostructure of **1** was clarified by the circular dichroism (CD) spectrum, which showed the characteristic CD curve for 3*R*-dihydroisocoumarin[20] ($[\theta]_{258}$ +3200, $[\theta]_{240}$ -5800, $[\theta]_{224}$ -3800). Consequently, the structure of **1** was determined to be 3*R*-phyllodulcin 3'-*O*-β-D-glucopyranoside.

3*S*-Phyllodulcin 3'-*O*-glucoside (**2**) was also isolated as a white powder. The molecular formula $C_{22}H_{24}O_{10}$, which was the same as that of **1**, was confirmed by the quasimolecular ion peaks at *m/z* 449 $(M+H)^+$ and *m/z* 471 $(M+Na)^+$ and by high-resolution MS analysis. The UV and IR spectra of **2** were found to be very similar to those of **1**. Comparison of the ^1H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1)[19] for **2** with those for **1** led us to deduce the structure of **2** as the 3-epimer of **1**. The CD spectrum of **2** showed the characteristic pattern of the 3*S*-configuration[20] ($[\theta]_{271}$ -2900, $[\theta]_{260}$ -3100, $[\theta]_{242}$ +2100,

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

	1[a]	**2**[a]	**3**[b]	**4**[b]
C-1	169.0	169.0	165.8	165.2
3	80.0	80.2	81.5	80.9
4	33.3	33.6	37.1	36.7
4a	140.4	140.5	143.6	143.2
5	118.2	117.6	123.3	122.7
6	136.3	136.1	136.6	136.4
7	115.4	115.8	118.3	117.1
8	161.0	161.8	160.8	160.3
8a	108.4	108.4	116.0	115.7
1'	130.4	130.5	132.5	132.7
2'	114.1	113.6	111.5	111.4
3'	146.2	146.2	150.7	150.6
4'	149.1	149.1	151.0	150.8
5'	112.2	112.1	112.8	112.8
6'	120.1	120.4	120.4	120.3
3'-OCH_3			56.6	56.6
4'-OCH_3	55.6	55.6	56.5	56.5
Glc-1''	100.0	99.7	105.1	103.1
2''	73.1	73.1	75.0	74.7
3''	76.9	76.9	78.8	78.5
4''	69.5	69.6	71.3	71.2
5''	76.8	76.9	77.2	77.8
6''	60.4	60.4	62.7	62.6

The spectra were taken with DMSO-d_6[a] or CD_3OD.[b]

$[\theta]_{231}$ -3000, $[\theta]_{227}$ -2800). Finally, glycosidation of 3*R*- and 3*S*-phyllodulcin mixture from Hydrangeae Dulcis Folium with *O*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)trichloroacetimidate in the presence of boron trifluoride-etherate ($BF_3{\cdot}Et_2O$) followed by deacetylation with 5% aqueous potassium carbonate afforded a mixture of **1** and **2** in *ca.* a 2 : 1 ratio. On the basis of the above mentioned evidence, the absolute stereostructures of 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucosides were determined as shown.

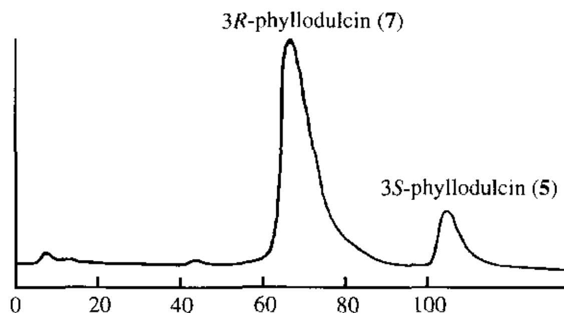
3*R*- and 3*S*-Thunberginol H 8-*O*-Glucosides

3*R*- and 3*S*-Thunberginol H 8-*O*-glucosides (**3**, **4**) were isolated as a white powder. Their IR spectra were similar to each other and showed absorption bands ascribable to hydroxyl, δ-lactone, and aromatic ring. The UV spectra of **3** and **4** showed absorption maxima suggestive of the dihydroisocoumarin structure. **3** and **4** were found to have the same molecular formula $C_{23}H_{26}O_{10}$, which was obtained from the quasi-molecular ion peak in their positive-ion FAB-MS at *m/z* 485 $(M+Na)^+$ and by high-resolution MS measurement. The ^{1}H-NMR (CD_3OD) and ^{13}C-NMR (Table 1) spectra[19] of **3** showed the presence of two trisubstituted benzene rings [δ 6.98 (d, *J*=8.3 Hz, 5'-H), 7.06 (dd, *J*=2.0, 8.3 Hz, 6'-H), 7.12 (d, *J*=2.0 Hz, 2'-H); δ 7.11 (d, *J*=7.6 Hz, 5-H), 7.41 (d, *J*=8.2 Hz, 7-H), 7.59 (dd, *J*=7.6, 8.2 Hz, 6-H)], δ-lactone [δ 5.46 (dd, *J*=2.6, 12.2 Hz, 3-H)], two methoxyl groups [δ 3.85 (s, 4'-OCH_3), 3.86 (s, 3'-OCH_3)], and a β-D-glucopyranosyl moiety [δ 4.91 (d, *J*=7.2 Hz, 1''-H)]. In the NOESY experiment on **3**, NOE correlations were observed between the anomeric proton and the 7-proton, between the 3'-methoxyl protons and the 2'-proton, and between the 4'-methoxyl protons and the 5'-proton. Diazomethane methylation of 3*R*-phyllodulcin 8-*O*-glucoside (**8**) furnished **3** in a 75% yield. On the basis of this evidence and examination of the CD spectrum of **3**, the structure of 3*R*-thunberginol H 8-*O*-glucoside (**3**) was elucidated as shown. The ^{1}H- and ^{13}C-NMR (Table 1) spectra[19] of **4** were found to resemble those of **3** and the NOESY experiment on **4** showed the same NOE correlations as those of **3**. This evidence led us to confirm the structure to be the epimer of **3** at the 3-position. The CD spectrum of **4** showed the characteristic CD curve for the 3*S*-dihydroisocoumarin[20] ($[\theta]_{295}$ -9000, $[\theta]_{260}$ -7900, $[\theta]_{241}$ +9900) and consequently, the structure of 3*S*-thunberginol H 8-*O*-glucoside (**4**) was determined as shown.

3*S*-Phyllodulcin

Although the 3-position of phyllodulcin was reported to be *R* configuration,[21] phyllodulcin obtained from the leaves of *H. macrophylla* var. *thunbergii* was found to be an enantiomeric mixture of *ca.* a 5 : 1 ratio at the 3-position by HPLC examination as shown in Figure 3.

3*S*-Phyllodulcin (**5**) was isolated as a white powder of $[\alpha]_D^{25}$ -62.8° (MeOH). The physical data of **5** such as UV, IR, and ^{1}H- and ^{13}C-NMR was completely identified with those of 3*R*-phyllodulcin (**7**). The CD spectrum of **5** showed the characteristic CD curve for 3*S*-dihydroisocoumarin[20] ($[\theta]_{313}$ -2900, $[\theta]_{256}$



Conditions for HPLC
chromatograph : Shimadzu LC-10AS
column : Ceramospher Chiral RU-1
(250 x 4.6 mm i.d.)
solvent : MeOH
flow rate : 1.0 mL/min
detector : Shimadzu SPD-10A
detection : UV 254 nm

Figure 3. HPLC Chromatogram of Phylloulcin from the Leaves of *Hydrangea macrophylla* var. *thunbergii*

-10800, $[\theta]_{240}$ +12700), so that the structure of 3*S*-phyllodulcin (**5**) was determined. 3*S*-Phyllodulcin (**5**) was also found to be present in the processed leaves of *H. macrophylla* var. *thunbergii* together with **7** by HPLC analysis. On the other hand, **5** was not obtained by the treatment of **7** with methanol under reflux or with silica gel in a chloroform-methanol-water mixture. This evidence allowed us to confirm 3*S*-phyllodulcin (**5**) as a genuine compound of *H. macrophylla* var. *thunbergii*.

EXPERIMENTAL

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described previously.[2]

Extraction and Isolation

The air-dried leaves (1.7 kg) of *H. macrophylla* var. *thunbergii* cultivated in Nagano Prefecture were extracted with MeOH (18 L x 3) under reflux and the solvent was evaporated from the extract under reduced pressure to give the MeOH extract (479 g). The MeOH extract was suspended in water and the suspension was extracted with AcOEt to give the AcOEt-soluble fraction (127 g) and the water phase. The water phase was further extracted with *n*-BuOH to yield the *n*-BuOH-soluble fraction (318 g) and the water-soluble fraction (34 g). The *n*-BuOH-soluble fraction (148 g) was subjected to silica gel column chromatography [BW-200 (Fuji Silysia Chemical Ltd., 3.0 kg), $CHCl_3$-MeOH-H_2O (26 : 6 : 0.7 → 10 : 3 : 1, lower layer → 65 : 35 : 10, lower layer → 6 : 4 : 1)] to give nine fractions [fr. 1 (1.1 g), fr. 2 (2.1 g), fr. 3 (1.9 g), fr. 4 (8.1 g), fr. 5 (7.8 g), fr. 6 (23.4 g), fr. 7 (5.3 g), fr. 8 (20.8 g), fr. 9 (24.5 g)]. Fraction 2 (537 mg) was purified by HPLC [YMC-Pack R & D ODS-5-A (250 x 20 mm i.d., YMC Co., Ltd.), MeOH-H_2O (1 : 1, v/v)] to furnish **20** (20.0 mg), **15** (15.0 mg), and **16** (18.5 mg) together with **1** and **2** mixture, **3** and **4** mixture, and 3*R*- and 3*S*-thunberginol I 4'-*O*-glucoside mixture. Each 3*R*- and 3*S*-mixture was separated by chiral column HPLC [Ceramospher Chiral RU-1 (Shiseido Ltd.), MeOH-H_2O (1 : 1, v/v)] to give **1** (4.6 mg) and **2** (3.0 mg), **3** (20.0 mg) and **4** (30.0 mg), and 3*R*-thunberginol I 4'-*O*-glucoside (2.8 mg) and 3*S*-thunberginol I 4'-*O*-glucoside (2.2 mg). Fraction 3 (1.0 g) was subjected to HPLC [MeOH-H_2O (1 : 1, v/v)] to yield **18** (63.4 mg), **19** (21.0 mg), and 3*R*- and 3*S*-phyllodulcin 8-*O*-glucoside mixture (**6** and **8**, 38.6 mg). Silica gel column chromatography [250 g, $CHCl_3$-MeOH (7 : 1 → 5 : 1 → 2 : 1) → MeOH] of fraction 7 (5.3 g) furnished five fractions [fr. 7-1 (103 mg), fr. 7-2 (938 mg), fr. 7-3 (2.8 g), fr. 7-4 (422 mg), fr. 7-5 (600 mg)]. Fraction 7-1 (103 mg) was purified by HPLC [MeOH-H_2O (4 : 6, v/v)] to give **23** (14.0 mg). By separation of fraction 7-2 (938 mg) with HPLC [MeOH-H_2O (4 : 6, v/v)], **10** (106.0 mg), **21** (238.0 mg), and **22** (102.0 mg) were isolated. Fraction 7-3 (100 mg) was purified by ODS column chromatography [Chromatorex ODS-5 (Fuji Silysia Chemical Ltd.), MeOH-H_2O (4 : 6, v/v)] to give **17** (14.0 mg). Fraction 7-4 (422 mg) was subjected to HPLC

[MeOH-H_2O (1 : 1, v/v)] followed by chiral column HPLC (MeOH) to afford 3*R*- (1.4 mg) and 3*S*-hydrangenol 4'-*O*-apiosylglucoside (4.8 mg), **27** (28.0 mg), **28** (30.0 mg), **11** (1.7 mg), and **12** (3.4 mg). HPLC [MeOH-H_2O (1 : 1, v/v)] of fraction 7-5 (108 mg) furnished **13** (6.2 mg) and thunberginol I 8-*O*-glucoside (19.9 mg). Fraction 8 (1.3 g) and 9 (3.1 g) were subjected to ODS column chromatography [MeOH-H_2O (35 : 65 → 7 : 3) → MeOH] followed by HPLC [MeOH-H_2O (4 : 6, v/v)] to afford **24** (348.0 mg from fraction 8), and **25** (53.0 mg) and **26** (98.0 mg, from fraction 9).
The AcOEt-soluble fraction (127 g) was purified by silica gel [$CHCl_3$-MeOH (30 : 1 → 10 : 1 → 5 : 1) → MeOH] and ODS column chromatography [MeOH-H_2O (8 : 2 → 9 : 1)] to give **9** (248.0 mg), **14** (37.4 mg), and 3*R*- and 3*S*-phyllodulcin mixture (172.0 mg). The 3*R*- and 3*S*-phyllodulcin mixture (15.0 mg) was separated by chiral column HPLC (MeOH) to furnish **5** (1.7 mg) and **7** (9.2 mg). The known compounds were identified by comparison of their physical data.

3*R*-Phyllodulcin 3'-*O*-Glucoside (**1**) : A white powder, $[\alpha]_D^{25}$ +26.9° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{25}O_{10}$ $(M+H)^+$: 449.1448. Found : 449.1475. UV λ_{max}^{MeOH} nm (log ε) : 227 (3.6), 280 (3.0), 312 (3.0). CD $[\theta]^{25}$ (MeOH, nm) : -3800 (224), -5800 (240), +3200 (258). IR (KBr) : 3570, 1650, 1618 cm^{-1}. ^1H-NMR (270 MHz, DMSO-d_6) δ: 3.79 (3H, s, 4'-OCH_3), 4.93 (1H, d, *J*=6.9 Hz, 1''-H), 5.68 (1H, dd, *J*=3.0, 11.6 Hz, 3-H), 6.86 (1H, d, *J*=8.4 Hz, 5-H), 6.89 (1H, d, *J*=8.4 Hz, 7-H), 7.03 (1H, d, *J*=8.6 Hz, 5'-H), 7.11 (1H, dd, *J*=1.7, 8.6 Hz, 6'-H), 7.24 (1H, d, *J*=1.7 Hz, 2'-H), 7.52 (1H, dd, *J*=8.4, 8.4 Hz, 6-H), 10.9 (1H, br s, 8-OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δc: given in Table 1. Positive-ion FAB-MS : *m/z* 449 $(M+H)^+$, 471 $(M+Na)^+$.
3*S*-Phyllodulcin 3'-*O*-Glucoside (**2**): A white powder, $[\alpha]_D^{25}$ +6.8° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{22}H_{25}O_{10}$ $(M+H)^+$: 449.1448. Found : 449.1468. UV λ_{max}^{MeOH} nm (log ε) : 230 (3.8), 277 (3.2), 312 (3.2). CD $[\theta]^{25}$ (MeOH, nm) : -2800 (227), -3000 (231), +2100 (242), -3100 (260), -2900 (271). IR (KBr) : 3570, 1655, 1618 cm^{-1}. ^1H-NMR (270 MHz, DMSO-d_6) δ: 3.78 (3H, s, 4'-OCH_3), 4.93 (1H, d, *J*=7.0 Hz, 1''-H), 5.64 (1H, dd, *J*=2.7, 11.9 Hz, 3-H), 6.79 (1H, d, *J*=7.8 Hz, 5-H), 6.85 (1H, d, *J*=8.1 Hz, 7-H), 7.02 (1H, d, *J*=8.6 Hz, 5'-H), 7.09 (1H, dd, *J*=1.7, 8.6 Hz, 6'-H), 7.25 (1H, d, *J*=1.7 Hz, 2'-H), 7.48 (1H, dd, *J*=7.8, 8.1 Hz, 6-H), 10.9 (1H, br s, 8-OH). ^{13}C-NMR (68 MHz, DMSO-d_6) δc: given in Table 1. Positive-ion FAB-MS : *m/z* 449 $(M+H)^+$, 471 $(M+Na)^+$.
3*R*-Thunberginol H 8-*O*-Glucoside (**3**) : A white powder, $[\alpha]_D^{25}$ +2.4° (*c*=0.4, MeOH). High-resolution positive-ion FAB-MS: Calcd for $C_{23}H_{26}O_{10}Na$ $(M+Na)^+$: 485.1424. Found : 485.1449. λ_{max}^{MeOH} nm (log ε) : 233 (4.1), 285 (3.6), 295 (3.6). CD $[\theta]^{25}$ (MeOH, nm) : +11000 (254), +7400 (296). IR (KBr) : 3400, 1709, 1603 cm^{-1}. ^1H-NMR (270 MHz, CD_3OD) δ: 3.85 (3H, s, 4'-OCH_3), 3.86 (3H, s, 3'-OCH_3), 4.91 (1H, d, *J*=7.2 Hz, 1''-H), 5.46 (1H, dd, *J*=2.6, 12.2 Hz, 3-H), 6.98 (1H, d, *J*=8.3 Hz, 5'-H), 7.06 (1H, dd, *J*=2.0, 8.3 Hz, 6'-H), 7.11 (1H, d, *J*=7.6 Hz, 5-H), 7.12 (1H, d, *J*=2.0 Hz, 2'-H), 7.41 (1H, d, *J*=8.2 Hz, 7-H), 7.59 (1H, dd, *J*=7.6, 8.2 Hz, 6-H). ^{13}C-NMR (68 MHz, CD_3OD) δc: given in Table 1. Positive-ion FAB-MS : *m/z* 485 $(M+Na)^+$.
3*S*-Thunberginol H 8-*O*-Glucoside (**4**) : A white powder, $[\alpha]_D^{25}$ -91.4° (*c*=0.1, MeOH). High-resolution positive-ion FAB-MS : Calcd for $C_{23}H_{26}O_{10}Na$ $(M+Na)^+$: 485.1424. Found : 485.1442. UV λ_{max}^{MeOH} nm (log ε) : 233 (4.1), 284 (3.6), 295 (3.5). CD $[\theta]^{25}$ (MeOH, nm) : +9900 (241), -7900 (260), -9000 (295). IR (KBr) : 3400, 1710, 1603 cm^{-1}. ^1H-NMR (270 MHz, CD_3OD) δ: 3.83 (3H, s, 4'-OCH_3), 3.84 (3H, s, 3'-OCH_3), 4.95 (1H, d, *J*=7.6 Hz, 1''-H), 5.51 (1H, dd, *J*=3.3, 10.5 Hz, 3-H), 6.95 (1H, d, *J*=8.3 Hz, 5'-H), 7.01 (1H, dd, *J*=1.9, 8.3 Hz, 6'-H), 7.05 (1H, d, *J*=7.6 Hz, 5-H), 7.08 (1H, d, *J*=1.9 Hz, 2'-H), 7.30 (1H, d, *J*=8.2 Hz, 7-H), 7.54 (1H, dd, *J*=7.6, 8.2 Hz, 6-H). ^{13}C-NMR (68 MHz, CD_3OD) δc: given in Table 1. Positive-ion FAB-MS : *m/z* 485 $(M+Na)^+$.
3*S*-Phyllodulcin (**5**) : A white powder, $[\alpha]_D^{25}$ (*c*=0.1, MeOH). CD $[\theta]^{25}$ (MeOH, nm) : +12700 (240), -10800 (256), -2900 (313).

Glycosidation of 3*R*- and 3*S*-Phyllodulcins (5, 7)

A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-D-glucopyranosyl)trichloroacetimidate (50 mg), phyllodulcin (**5** and **7** mixture from Hydrangeae Dulcis Folium, 345 mg), and Molecular Sieves-4A (500 mg) in CH_2Cl_2 (5.0 mL) was treated BF_3·etherate (44 μL) and the mixture was stirred at 20 °C under an N_2 atmosphere for 10 min. The reaction mixture was poured into ice-water and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with brine then dried over $MgSO_4$ and filtered. After evaporation of the solvent from the filtrate under reduced pressure, the residue was purified ODS column chromatography to give the glycosidation product (104 mg). A solution of the glycosidation product (70.0 mg) in MeOH (1.0 mL) was treated with 5% aqueous K_2CO_3 (1.0 mL) and the reaction mixture was stirred at 20 °C for 10 min. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [$CHCl_3$-MeOH-H_2O (10 : 3 : 1, lower layer)] to give a mixture of **1** and **2** (19.8 mg), which was identical with natural 3*R*- and 3*S*-phyllodulcin 3'-*O*-glucoside by chiral column HPLC (MeOH).

Diazomethane Methylation of 3*R*-Phyllodulcin 8-*O*-Glucoside (8)

A solution of **8** (20.0 mg) in MeOH (1.0 mL) was treated with 10% trimethylsilyldiazomethane (5.0 mL) and the reaction mixture was left standing at 20 °C for 15 min. After removal of the solvent under reduced pressure, the residue was purified by silica gel column chromatography [$CHCl_3$-MeOH (5 : 1)] to give **3** (15.5 mg), which was identified with an authentic 3*R*-thunberginol H 8-*O*-glucoside by chiral column HPLC (MeOH) and ^{1}H- and ^{13}C-NMR data comparisons.

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