Development of Bioactive Functions in Hydrangeae Dulcis Folium. V.[1] On the Antiallergic and Antimicrobial Principles of Hydrangeae Dulcis Folium. (2). Thunberginols C, D, and E, Thunberginol G 3′-*O*-Glucoside, (−)-Hydrangenol 4′-*O*-Glucoside, and (+)-Hydrangenol 4′-*O*-Glucoside

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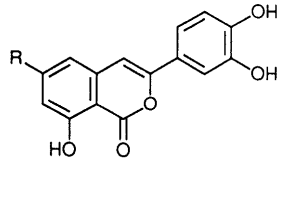
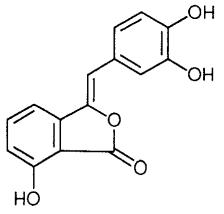
Following the characterization of thunberginols A, B, and F, six bioactive principles, thunberginols C, D, and E, thunberginol G 3′-*O*-glucoside, (−)-hydrangenol 4′-*O*-glucoside, and (+)-hydrangenol 4′-*O*-glucoside, were isolated from Hydrangeae Dulcis Folium, the processed leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO, together with four kaempferol and quercetin oligoglycosides. Their chemical structures have been determined on the basis of chemical and physicochemical evidence. Thunberginols C, D, E, and G and (−)-hydrangenol 4′-*O*-glucoside showed antiallergic activity in the *in vitro* bioassay using the Schultz–Dale reaction. These components also exhibited inhibitory activities on the histamine release from rat mast cells and on the histamine-induced contraction in isolated guinea pig tracheal chain. In addition, thunberginols C, D, E, and G showed antimicrobial activities against oral bacteria.

Key words Hydrangeae Dulcis Folium; *Hydrangea macrophylla* var. *thunbergii;* thunberginol; dihydroisocoumarin glucoside; antiallergic activity; oral bacteria antimicrobial activity

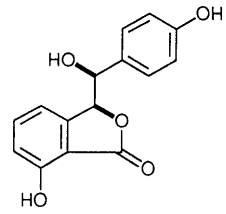
As a part of our characterization studies on the bioactive constituents of natural medicines,[2] we have been investigating new functional components of Hydrangeae Dulcis Folium, the processed leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae).[1,3] We have so far isolated eight antiallergic and antimicrobial principles named thunberginols A (**1**),[4] B (**2**),[4] C (**13**),[5] D (**14**),[5] E (**15**),[5] and F (**3**)[4] and hydramacrophyllols A (**4**)[6] and B (**5**)[6] from the less polar fraction (the chloroform and ethyl acetate-soluble portions) of Hydrangeae Dulcis Folium, together with various known compounds such as phyllodulcin (**8**), hydrangenol (**6**), and hydrangeaic acid (**12**), and we have reported the structures of **1**, **2**, and **3**.[4] Furthermore, inhibitory activities of **1** for type I, II, III, and IV allergy were examined in detail. We found that **1** showed more potent inhibitory activity against type I allergy than commercial antiallergic agents such as amlexanox, tranilast, and oxatomide in the *in vitro* and *in vivo* bioassay, and **1** was also expected to show inhibitory activity against type IV allergy.[7] In continuing studies to find antiallergic and antimicrobial principles of Hydrangeae Dulcis Folium, we isolated thunberginol G 3′-*O*-glucoside (**17**), (−)-hydrangenol 4′-*O*-glucoside (**18**), and (+)-hydrangenol 4′-*O*-glucoside (**19**), together with four known flavonol glycosides from the glycoside fraction (the 1-butanol-



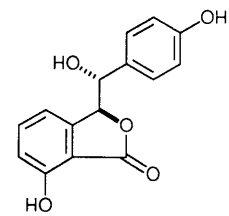
thunberginol A (**1**) : R=H
thunberginol B (**2**) : R=OH



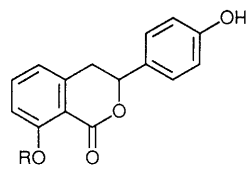
thunberginol F (**3**)



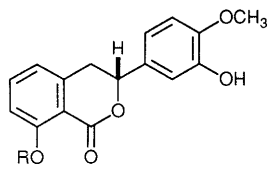
hydramacrophyllol A (**4**)



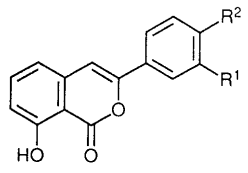
hydramacrophyllol B (**5**)



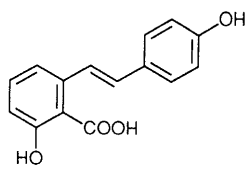
hydrangenol (**6**) : R=H
7 : R=β-D-Glu.



phyllodulcin (**8**) : R=H
9 : R=β-D-Glu.



dehydrohydrangenol (**10**) : R^1=H, R^2=OH
dehydrophyllodulcin (**11**) : R^1=OH, R^2=OCH_3



hydrangeaic acid (**12**)

Chart 1

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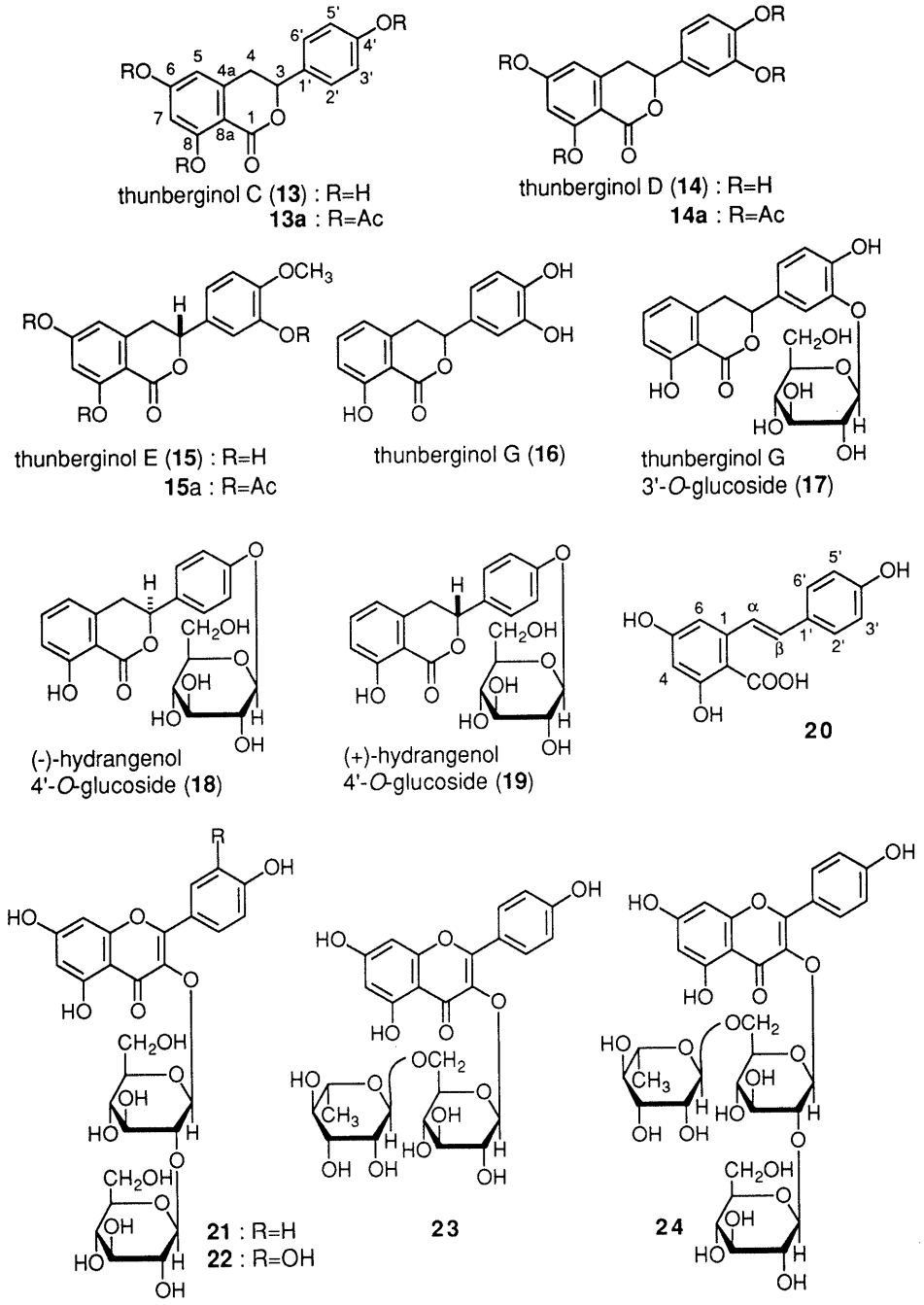

Chart 2

soluble portion) of this crude drug.

In this paper, we present a full account of the structure elucidation of thunberginols C (**13**), D (**14**), and E (**15**), thunberginol G 3′-*O*-glucoside (**17**), (−)-hydrangenol 4′-*O*-glucoside (**18**), and (+)-hydrangenol 4′-*O*-glucoside (**19**). In addition, we describe the antiallergic and antimicrobial activities of thunberginols C (**13**), D (**14**), E (**15**), and G (**16**), (−)-hydrangenol 4′-*O*-glucoside (**18**), and various related compounds.[8]

The methanolic extract of Hydrangeae Dulcis Folium was separated into the chloroform-soluble portion, ethyl acetate-soluble portion, and 1-butanol-soluble portion. From the chloroform and ethyl acetate-soluble portions, thunberginols (**1**, **2**, **3**, **13**, **14**, **15**) and hydramacrophyllols (**4**, **5**) were isolated as described in our previous papers.[4] The 1-butanol-soluble portion was subjected to ordinary-phase and reversed-phase silica gel column chromatography and finally Sephadex LH-20 column chromatography to afford thunberginol G 3′-*O*-glucoside (**17**, 0.0005% from the crude drug), (−)-hydrangenol 4′-*O*-glucoside (**18**, 0.0004%), and (+)-hydrangenol 4′-*O*-glucoside (**19**, 0.0002%) together with kaempferol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside[9] (**21**, 0.95%), quercetin 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside[10] (**22**, 0.20%), kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside[10] (**23**, 0.15%), and kaempferol 3-*O*-*β*-D-glucopyranosyl(1→2)[*α*-L-rhamnopyranosyl(1→6)]*β*-D-glucopyranoside[11] (**24**, 0.48%).

Chemical Structures of Thunberginols C (13), D (14), and E (15), Thunberginol G 3′-*O*-Glucoside (17), (−)-Hydrangenol 4′-*O*-Glucoside (18), and (+)-Hydrangenol 4′-*O*-Glucoside (19) Thunberginol C (**13**) was isolated as

colorless needles of mp 197—198 °C and it was found to be a racemate by specific rotation measurement. Thunberginol C (**13**) was more polar than hydrangenol (**6**) in TLC examination and its molecular formula $C_{15}H_{12}O_5$ was confirmed from the molecular ion peak at *m/z* 272 (M^+) in the electron impact mass spectrum (EI-MS) of **13** and by high-resolution MS measurement. The IR spectrum of **13** showed absorption bands due to phenolic hydroxyl, chelated δ-lactone, and aromatic ring at 3357—2500 (broad), 1649, 1630, 1522, and 1055 cm^{-1}. In the UV spectrum of **13**, it showed absorption maxima (log ε) in ethanol solution at 218 (3.8), 271 (4.1), and 301 (4.4) nm and in ethanol solution containing aluminium chloride at 229, 285, and 334 nm. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra of **13**, which were completely assigned on the basis of various NMR experiments,[12] showed signals assignable to a disubstituted benzene ring [δ 6.80 (d, *J*=9 Hz, 3′,5′-H), 7.31 (d, *J*=9 Hz, 2′,6′-H)], a tetrasubstituted benzene ring [δ 6.30 (d, *J*=2 Hz, 5-H), 6.22 (d, *J*=2Hz, 7-H)], and a chelated δ-lactone [δ 5.54 (dd, *J*=3, 12 Hz, 3-H), 3.03 (dd, *J*=3, 17 Hz), 3.24 (dd, *J*=12, 17 Hz)(4-H_2), 11.10 (s, 8-OH)].

Acetylation of **13** with acetic anhydride in pyridine at room temperature provided the triacetate (**13a**), while the stilbene derivative (**20**) was obtained by alkaline treatment of **13** with 0.5% potassium hydroxide. On the basis of those findings and comparison of the physical data (UV, ^{1}H- and ^{13}C-NMR spectra) for **13** with those for hydrangenol (**6**), the structure of thunberginol C was determined to be 3-(4′-hydroxyphenyl)-6,8-dihydroxydihydroisocoumarin (**13**).

Thunberginol D (**14**), isolated as colorless needles of mp 199—200 °C, was also shown to be a racemate from the specific rotation ($[\alpha]_D^{25}$ ±0°). The UV spectrum of **14** showed absorption maxima (log ε) at 229 (4.0), 272 (4.1), and 297 (4.4) nm. The IR spectrum of **14**, which was very similar to that of **13**, showed absorption bands due to phenolic hydroxyl, chelated δ-lactone, and aromatic ring. In the positive-mode FAB-MS of **14**, a quasimolecular ion peak was observed at *m/z* 289 $(M+H)^+$ and the molecular formula was determined to be $C_{15}H_{12}O_6$ by high-resolution MS measurement. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[12] of **14** showed the presence of a trisubstituted benzene ring [δ 6.75 (2H, br s, 2′,6′-H), 6.87 (br s, 5′-H)], a tetrasubstituted benzene ring [δ 6.29 (d, *J*=2 Hz, 5-H), 6.22 (d, *J*=2 Hz, 7-H)], and a chelated δ-lactone [δ 5.49 (dd, *J*=3, 12 Hz, 3-H), 3.03 (dd, *J*=3, 17 Hz), 3.21 (dd, *J*=12, 17 Hz) (4-H_2), 11.10 (br s, 8-OH)]. Ordinary acetylation of **14** furnished the tetraacetate (**14a**). Comparison of the ^{13}C-NMR data for **14** and **14a** with those for **6**, **13**, and **13a** led us to confirm the structure of thunberginol D as 3-(3′,4′-dihydroxyphenyl)-6,8-dihydroxydihydroisocoumarin (**14**).

Thunberginol E (**15**) was isolated as colorless needles of mp 216—217 °C and it was found to be optically active ($[\alpha]_D^{25}$ +38.5°). In the UV spectrum of **15**, absorption maxima (log ε) were observed at 223 (4.1), 272 (4.3), and 304 (4.5) nm, and its IR spectrum showed absorption bands assignable to phenolic hydroxyl, chelated δ-lactone, and aromatic ring. The ^{1}H-NMR (DMSO-d_6) and ^{13}C-NMR (Table 1) spectra[12] of **15** showed signals assignable to a trisubstituted benzene ring [δ 6.86-6.96 (m, 2′,5′,6′-H)], a tetrasubstituted benzene ring [δ 6.29 (d, *J*=2 Hz, 5-H), 6.22 (d, *J*=2 Hz, 7-H)] and a chelated δ-lactone ring [δ 5.54 (dd, *J*=3, 12 Hz, 3-H), 3.05 (dd, *J*=3, 17 Hz), 3.22 (dd, *J*=12, 17 Hz) (4-H_2)], together with a methoxyl group [δ 3.78 (3H, s)]. By ordinary acetylation, the triacetate (**15a**) was obtained. Comparison of the ^{1}H-NMR and ^{13}C-NMR spectra[12] for **15** and **15a** with those for phyllodulcin (**8**) and its diacetate led us to presume that the structure of thunberginol E is 6-hydroxyphyllodulcin (**15**). The location of the methoxyl group in **15** was determined from a difference nuclear Overhauser effect (NOE) experiment on **15a**. Namely, NOE correlation was observed between the 5′-proton [δ 6.99 (d, *J*=8 Hz)] and the 4′-OCH_3 (δ 3.84). Finally, the absolute configuration of **15** was clarified by the circular dichroism (CD) spectrum, which showed the characteristic CD curve for 3*R*-dihydroisocoumarins[13] ($[\theta]_{301}$ −3000, $[\theta]_{279}$ +4200, $[\theta]_{250}$ +6800). Consequently, the absolute stereostructure of thunberginol E was determined to be 3(*R*)-3-(3′-hydroxy-4′-methoxyphenyl)-6,8-dihydroxydihydroisocoumarin (**15**).

Thunberginol G 3′-*O*-glucoside (**17**) was obtained as a white powder and it was shown to be a 3-epimeric mixture[14] by examination of the ^{1}H-NMR and ^{13}C-NMR spectra. The positive-mode FAB-MS of **17** showed a quasimolecular ion peak at *m/z* 457 $(M+Na)^+$. Methanolysis of **17** with 9% hydrogen chloride liberated thunberginol G (**16**) and methyl D-glucoside. Thunberginol G (**16**) was found to be identical with the demethyl derivative[15] of phyllodulcin (**8**), which was prepared by boron tribromide (BBr_3) treatment. Finally, methylation of **17** with diazomethane in methanol selectively furnished the 4′-*O*-methylated derivative, which was subsequently subjected to β-glucosidase hydrolysis to provide (±)-phyllodulcin. This chemical evidence and comparison of the ^{1}H-NMR and ^{13}C-NMR data for **17** with those for **16** led us to elucidate the 3′-*O*-β-D-glucopyranoside structure of thunberginol G [3-(3′,4′-dihydroxyphenyl)-8-hydroxydihydroisocoumarin, **17**].

(−)-Hydrangenol 4′-*O*-glucoside (**18**), isolated as a white powder of $[\alpha]_D^{25}$ −4.7°, showed absorption maxima (log ε) at 243 (3.4) and 314 (3.1) nm. The IR spectrum of **18** showed absorption bands at 3570, 1670, 1617, 1516, 1238, and 1076 cm^{-1} suggestive of a dihydroisocoumarin glycoside structure. In the positive-mode FAB-MS of **18**, quasimolecular ion peaks were observed at *m/z* 419 $(M+H)^+$ and 441 $(M+Na)^+$, and the molecular formula $C_{21}H_{22}O_9$ was determined by high-resolution MS measurement. Methanolysis of **18** liberated hydrangenol (**6**) and methyl D-glucoside. The position of the glucoside linkage in **18** was clarified by a difference NOE experiment on **18**, in which NOE correlations were observed between the anomeric proton [δ 4.90 (d, *J*=8 Hz)] and the 3′,5′-protons [δ 7.08 (2H, d, *J*=9 Hz)]. Finally, the absolute configuration of **18** was determined from CD data, which showed a Cotton curve ($[\theta]_{259}$ −4500, $[\theta]_{237}$ +3300, $[\theta]_{220}$ −1100) characteristic of the 3*S*-dihydroisocoumarin.[13] Consequently, the absolute stereostructure of (−)-hydrangenol 4′-*O*-glucoside (**18**) was elucidated to be as shown.

Table 1. ^{13}C-NMR Data for **6**, **8**, **13**, **13a**, **14**, **14a**, **15**, **15a**, **17**, **18**, and **19**[a]

	6	**8**	**13**	**13a**	**14**	**14a**	**15**	**15a**	**17**	**18**	**19**
1	170.8	169.4	169.4	161.1	169.4	160.9	169.5	161.2	164.0	169.0	169.0
3	81.8	80.3	79.7	78.6	79.7	78.1	79.7	78.4	83.2	79.8	79.9
4	35.0	33.7	33.6	36.2	33.7	36.2	33.9	36.1	36.2 (36.1)	33.6	33.5
4a	141.3	140.6	142.2	142.0	142.2	141.8	142.4	142.1	142.5 (142.4)	140.4	140.4
5	119.4	118.6	106.8	118.2	106.8	118.3	107.1	118.2	120.2	118.3	118.2
6	137.1	136.5	164.4	154.6	164.4	154.7	164.7	154.6	138.3	136.1	136.2
7	116.5	115.6	100.9	116.8	100.9	116.9	101.1	116.7	118.0 (117.9)	115.6	115.5
8	162.8	161.1	163.3	153.2	163.3	153.2	163.5	153.2	158.1	161.8	161.0
8a	109.4	108.6	100.3	115.3	100.3	115.3	100.4	115.4	110.3	108.4	108.4
1′	130.3	130.9	128.6	135.5	129.3	136.7	131.1	130.4	132.1 (132.0)	131.5	131.4
2′	129.0	112.1	128.0	127.3	114.1	121.3	112.1	118.2	118.3	127.9	127.9
3′	116.4	146.6	115.1	121.9	145.1	141.8	146.5	139.8	147.5	116.0	116.1
4′	158.8	148.1	157.6	150.8	145.6	142.2	148.0	151.4	149.7 (149.6)	157.5	157.5
5′	116.4	114.1	115.1	121.9	115.3	123.8	114.0	120.9	117.6	116.0	116.1
6′	129.0	117.7	128.0	127.3	117.7	124.1	117.6	124.7	124.1 (123.8)	127.9	127.9
OMe		55.8					55.8	56.0			
OAc				21.0		20.6		20.2			
				21.1		20.6		20.6			
				21.1		21.0		20.7			
				168.1		21.1		168.2			
				169.3		168.1		168.8			
				169.3		168.1		169.3			
						168.1					
						169.3					
Glu-1″									105.1 (104.8)	100.1	100.1
2″									75.6	73.1	73.1
3″									79.2 (79.1)	76.9	76.9
4″									72.2 (72.1)	69.6	69.6
5″									78.4	76.5	76.5
6″									63.3 (63.2)	60.6	60.6

a) Compounds **6**, **8**, **13**, **14**, **15**, **17**, **18**, and **19** were measured in DMSO-d_6 and compounds **13a**, **14a**, and **15a** in $CDCl_3$.

(+)-Hydrangenol 4′-*O*-glucoside (**19**) was also isolated as a white powder of $[\alpha]_D^{25}$ +6.9°. The molecular formula $C_{21}H_{22}O_9$ of **19** was confirmed by the quasimolecular ion peaks at *m/z* 419 $(M+H)^+$ and 441 $(M+Na)^+$, and by high-resolution MS measurement. The IR and UV spectra of **19** were found to be superimposable on those of **18**. Comparison of the ^{1}H-NMR and ^{13}C-NMR data (Table 1) for **19** with those for **18** led us to deduce the structure of **19** as the 3-epimer of **18**. The CD spectrum of **19** showed the characteristic pattern of the 3*R* configuration.[13] Finally, the chemical structures of **18** and **19** were confirmed by the following chemical derivation from hydrangenol (**6**). Namely, glycosidation of **6** with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate[16] in the presence of boron trifluoride-etherate followed by deacetylation with 5% aqueous potassium carbonate afforded a mixture (*ca.* 1 : 1) of **18** and **19**. On the basis of the above-mentioned evidence, the absolute stereostructure of (+)-hydrangenol 4′-*O*-glucoside (**19**) was determined to be as shown.

Antiallergic Activities of Thunberginols C (13), D (14), E (15), and G (16), (−)-Hydrangenol 4′-*O*-Glucoside (18) and Related Compounds (7, 9, 10, 11, 12) Antiallergic activities of thunberginols C (**13**), D (**14**), E (**15**), and G (**16**), and (−)-hydrangenol 4′-*O*-glucoside (**18**) were examined by using the Schultz–Dale reaction in sensitized guinea pig bronchial muscle and a bioassay to test the inhibitory activity against histamine release from rat mast cells induced by compound 48/80 or calcium ionophore A-23187. Furthermore, in order to obtain the activity data of related compounds, two dihydroisocoumarin glucosides [hydrangenol 8-*O*-glucoside (**7**)[3a] and phyllodulcin 8-*O*-glucoside (**9**)[3a]] obtained from the fresh leaves of *Hydrangea macrophylla* var. *thunbergii*, two synthetic isocoumarins [dehydrohydrangenol (**10**)[6] and dehydrophyllodulcin (**11**)[6]], and a stilbene [hydrageaic acid (**12**)[4]] were also examined by the same bioassay.

As shown in Table 2, thunberginols (**13**, **14**, **15**, **16**) and isocoumarins (**10**, **11**) exhibited more potent inhibitory activities on the Schultz–Dale reaction than two commercial antiallergic agents, tranilast and disodium cromoglycate (DSCG). Two dihydroisocoumarin glycosides (**9**, **18**) and a stilbene derivative (**12**) also showed activity similar to that of tranilast. On the other hand, thunberginols D (**14**) and E (**15**) were found to exhibit inhibitory activity on histamine release from rat mast cells induced by compound 48/80, and thunberginol A (**13**) showed inhibitory activity on histamine release from mast cells induced by calcium ionophore A-23187.

Inhibitory activities of thunberginols C (**13**), D (**14**), E (**15**), and G (**16**) and (−)-hydrangenol 4′-*O*-glucoside (**18**) on histamine-induced contraction of tracheal chain isolated from sensitized guinea pigs are summarized in Table 3. Thunberginols C (**13**), D (**14**), and E (**15**) showed inhibitory activity, but their activities were weaker than that of diphenhydramine.

Antimicrobial Activities of Thunberginols (13, 14, 15, 16), Dihydroisocoumarin Glucosides (7, 9, 18), Hydrangenol (6), Phyllodulcin (8), and Flavonol Oligoglycosides (21, 22, 24) As shown in Table 4, we have examined antimicrobial

Table 2. Inhibitory Effects of Thunberginols C (**13**), D (**14**), E (**15**), and G (**16**), (−)-Hydrangenol 4′-*O*-Glucoside (**18**), and Related Compounds (**7**, **9**, **10**, **11**, **12**) on the Schultz–Dale (S.D.) Reaction in Sensitized Guinea Pig Tracheal Chain and the Histamine Release from Rat Mast Cells Induced by Compound 48/80 and Calcium Ionophore A-23187

	IC_{50} (M)		
	S.D.	Compound 48/80	A-23187
Thunberginol C (**13**)	2.5×10^{-5}	$>10^{-4}$	8.5×10^{-5} (72.8)
Thunberginol D (**14**)	3.3×10^{-5}	9.0×10^{-5} (84.8)	$>10^{-4}$
Thunberginol E (**15**)	3.7×10^{-5}	8.5×10^{-5} (97.9)	$>10^{-4}$
Thunberginol G (**16**)	3.8×10^{-5}	$>10^{-4}$	$>10^{-4}$
Hydrangenol 8-*O*-Glu (**7**)	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
Phyllodulcin 8-*O*-Glu (**9**)	5.0×10^{-5}	$>10^{-4}$	$>10^{-4}$
(−)-Hydrangenol 4′-*O*-Glu (**18**)	5.7×10^{-5}	$>10^{-4}$	$>10^{-4}$
Dehydrohydrangenol (**10**)	3.5×10^{-5}	$>10^{-4}$	$>10^{-4}$
Dehydrophyllodulcin (**11**)	4.3×10^{-5}	$>10^{-4}$	$>10^{-4}$
Hydrageaic acid (**12**)	5.2×10^{-5}	$>10^{-4}$	$>10^{-4}$
DSCG	$>10^{-4}$	$>10^{-4}$	$>10^{-4}$
Tranilast	4.7×10^{-5}	$>10^{-4}$ (25.7)	$>10^{-4}$

The values in parentheses denote the inhibition percentage of histamine release at 10^{-4} M.

Table 3. Inhibitory Effects of Thunberginols C (**13**), D (**14**), E (**15**), and G (**16**) and (−)-Hydrangenol 4′-*O*-Glucoside (**18**) on the Histamine (His)-Induced Contraction in Isolated Guinea Pig Tracheal Chain

Compounds	Conc. (M)	His (Inhibition %)
Thunberginol C (**13**)	10^{-5}	5.9± 11.7
	3×10^{-5}	11.3± 7.2
	10^{-4}	48.5± 3.2**
Thunberginol D (**14**)	10^{-5}	10.5± 3.4*
	3×10^{-5}	5.3± 5.9
	10^{-4}	25.6± 6.0*
Thunberginol E (**15**)	10^{-5}	13.2± 0.6*
	3×10^{-5}	20.8± 3.5*
	10^{-4}	37.1± 4.4**
Thunberginol G (**16**)	10^{-5}	1.9± 6.8
	3×10^{-5}	2.8± 2.6
	10^{-4}	0.0± 1.4
(−)-Hydrangenol 4′-*O*-glucoside (**18**)	10^{-5}	0.0
	3×10^{-5}	7.0± 2.0
	10^{-4}	5.0± 3.1
Diphenhydramine	10^{-5}	76.9± 2.5**

Each value represents the mean with standard error of 3—8 experiments (* $p<0.05$, ** $p<0.01$).

activities of thunberginols (**13**—**16**), dihydroisocoumarin glucosides (**7**, **9**, **18**), and flavonol oligoglycosides (**21**, **22**, **24**) against two oral bacteria, *Bacteroides melaninogenicus* and *Fusobacterium nucleatum*. Thunberginols C (**13**), D (**14**), and G (**16**) were found to exhibit antimicrobial activities similar to those of thunberginols A (**1**), B (**2**), and F (**3**) and hydrangenol (**6**).[4] Thunberginol E (**15**) and flavonols (kaempferol and quercetin) also showed antimicrobial activities, but their activities were weaker than that of **6**. On the other hand, hydrangenol glucosides (**7**, **18**), phyllodulcin glucoside (**9**), and flavonol oligoglycosides (**21**, **22**, **24**) were found to lack antimicrobial activities (minimum inhibitory concentration (*MIC*) >128 ppm). These results indicated that the antimicrobial activities of

Table 4. Antimicrobial Activities of Thunberginols (**13**, **14**, **15**, **16**), Dihydroisocoumarin Glucosides (**7**, **9**, **18**), Flavonol Glycosides (**21**, **22**, **24**) and Flavonols against Oral Bacteria (*MIC*, ppm)

	Bacteroides melaninogenicus	*Fusobacterium nucleatum*
Thunberginol C (**13**)	10	10
Thunberginol D (**14**)	10	10
Thunberginol E (**15**)	50	30
Thunberginol G (**16**)	20	20
Hydrangenol 8-*O*-glucoside (**7**)	>128	>128
Phyllodulcin 8-*O*-glucoside (**9**)	>128	>128
Hydrangenol 4′-*O*-glucoside (**18**)	>128	>128
21	>128	>128
22	>128	>128
24	>128	>128
Quercetin	16	>128
Kaempferol	32	>128

Table 5. Antimicrobial Activities of Phyllodulcin (**8**) and Hydrangenol (**6**) (*MIC*, ppm)

	Phyllodulcin (**8**)	Hydrangenol (**6**)
Periodontopathic bacteria		
Fusobacterium nucleatum	100	5
Bacteroides melaninogenicus	100	10
Porphyromonas gingivalis	50	10
Prerotella intermedia	50	10
Caprocytophaga sputigena	100	25
Haemophilus actinomycetemcomitans	150	10
Trichophyton mentagrophytes	100	>100
Candida albicans	>100	>100
Aspergillus fumigatus	>100	>100
Cryptococcus neoformans	100	100
Bacillus subtilis	>100	>100
Staphylococcus aureus	>100	>100
Streptococcus mutans	>100	>100
Streptococcus pyrogenes	>100	>100
Streptococcus faecalis	>100	>100
Escherichia coli	>100	>100
Klebsiella pneumoniae	>100	>100
Klebsiella oxytoca	>100	>100
Proteus vulgaris	>100	>100
Proteus mirabilis	>100	>100
Serratia marcescens	>100	>100
Enterobacter cloacae	>100	>100
Acinetobacter calcoaceticus	>100	>100
Pseudomonas aeruginosa	>100	>100

isocoumarin and flavonol were decreased remarkably by glycosidation and methylation of the hydroxy group. Furthermore, microbial activities of hydrangenol (**6**) and phyllodulcin (**8**) were examined in detail. As shown in Table 5, **6** exhibited antimicrobial activity for various periodontopathic bacteria (*MIC* 5—25 ppm) and **8** showed only weak activity, while **6** and **8** lacked activity towards eighteen other bacteria and fungi, including cariogenic bacteria and pathogenic fungi (*MIC*≧100).

Experimental

The following instruments were used to obtain physical data: melting points, Yanagimoto micro-melting point apparatus (values are uncorrected); specific rotations, Horiba SEPA-200 digital polarimeter ($l=5$ cm); UV spectra, Shimadzu UV-1200 spectrometer; CD spectra, Jasco J 500 C spectropolarimeter; IR spectra, Shimadzu FTIR-8100

spectrometer; EI-MS and high-resolution EI-MS, Hitachi M-80 mass spectrometer; FAB-MS and high-resolution FAB-MS, JMS-SX 102 mass spectrometer; ^{1}H-NMR spectra, JEOL EX-270 (270 MHz) spectrometer; ^{13}C NMR spectra, JEOL EX-270 (68 MHz) spectrometer with tetramethylsilane as an internal standard.

The following experimental conditions were used for chromatography: ordinary-phase column chromatography; Silica gel 60 (Merck, 70—230 mesh), reversed-phase column chromatography; Silica gel 60 silanized (Merck, 70—230 mesh); Sephadex LH-20 (Pharmacia) ; TLC, pre-coated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary-phase) and Silica gel RP-18 60F_{254} (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with Silica gel RP-18 60WF_{254S} (Merck, 0.25 mm) (reversed phase). Detection was done by spraying 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4, followed by heating.

Extraction and Isolation The MeOH extract (2.5 kg) obtained from Hydrangeae Dulcis Folium (the fermented and dried leaves, 12.3 kg) cultivated and processed in Nagano Prefecture in 1990, was suspended in water and the suspension was extracted successively with $CHCl_3$, AcOEt, and 1-BuOH to give the $CHCl_3$-soluble portion, AcOEt-soluble portion, and 1-BuOH-soluble portion (510 g).[4] The 1-BuOH-soluble portion (100 g) was subjected to silica gel column chromatography (2 kg, eluted with $CHCl_3$–MeOH–H_2O), reversed-phase silica gel column chromatography (H_2O–MeOH) and then Sephadex LH-20 column chromatography (MeOH) to give thunberginol G 3′-*O*-glucoside (**17**, 0.0005% from the crude drug), a mixture of (−) and (+)-hydrangenol 4′-*O*-glucosides (**18**+**19**, 0.0006%), kaempferol 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside (**21**, 0.95%), quercetin 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside (**22**, 0.20%), kaempferol 3-*O*-*α*-L-rhamnopyranosyl(1→6)-*β*-D-glucopyranoside (**23**, 0.15%), and kaempferol 3-*O*-*β*-D-glucopyranosyl(1→2)[*α*-L-rhamnopyranosyl(1→6)]-*β*-D-glucopyranoside (**24**, 0.48%). A mixture of (−) and (+)-hydrangenol 4′-*O*-glucosides was separated by HPLC [column: Ceramospher Chiral RU-1 (Shiseido Co., Ltd.), 10 × 250 mm i.d.; solvent: MeOH; flow rate, 2.0 ml/min] to give **18** (0.0004%) and **19** (0.0002%). Known flavonol glycosides (**21**, **22**, **23**, **24**) were identified by comparison of their physical data with reported values.[9-11]

Thunberginol C (**13**): Colorless needles, mp 197—198 °C (MeOH), $[\alpha]_D^{25}$ ±0° (*c*=0.1, EtOH). High-resolution EI-MS: Calcd for $C_{15}H_{12}O_5$ (M^+): 272.0708; Found: 272.0696. UV [EtOH, nm (log *ε*)]: 218 (3.8), 271 (4.1), 301 (4.4); (EtOH + $AlCl_3$, nm): 229, 285, 334. IR (KBr) cm^{-1}: 3357—2500 (br), 1649, 1630, 1522, 1055. ^{1}H-NMR (DMSO-d_6) *δ*: 3.03 (1H, dd, *J*=3, 17 Hz), 3.24 (1H, dd, *J*=12, 17 Hz) (4-H_2), 5.54 (1H, dd, *J*=3, 12 Hz, 3-H), 6.22 (1H, d, *J*=2 Hz, 7-H), 6.30 (1H, d, *J*=2 Hz, 5-H), 6.80 (2H, d, *J*=9 Hz, 3′,5′-H), 7.31 (2H, d, *J*=9 Hz, 2′,6′-H), 11.10 (s, 8-OH, D_2O exchangeable); ^{1}H-NMR (acetone-d_6) *δ*: 3.07 (1H, dd, *J*=3, 17 Hz), 3.30 (1H, dd, *J*=12, 17 Hz) (4-H_2), 5.56 (1H, dd, *J*=3, 12 Hz, 3-H), 6.30 (1H, d, *J*=2 Hz, 7-H), 6.36 (1H, d, *J*=2 Hz, 5-H), 6.90 (2H, d, *J*=9 Hz, 3′,5′-H), 7.39 (2H, d, *J*=9 Hz, 2′,6′-H). ^{13}C-NMR: given in Table 1. EI-MS (*m/z*, %) : 272 (M^+, 4), 228 (100).

Thunberginol D (**14**): Colorless needles, mp 199—200 °C (MeOH), $[\alpha]_D^{25}$ ±0° (*c*=0.1, EtOH). High-resolution positive-mode FAB-MS: Calcd for $C_{15}H_{13}O_6$ $(M+H)^+$: 289.0712; Found: 289.0681. UV [EtOH, nm (log *ε*)]: 229 (4.1), 272 (4.1), 297 (4.4). IR (KBr) cm^{-1}: 3409, 1645, 1628, 1520, 1244. ^{1}H-NMR (DMSO-d_6) *δ*: 3.03 (1H, dd, *J*=3, 17 Hz), 3.21 (1H, dd, *J*=12, 17 Hz) (4-H_2), 5.49 (1H, dd, *J*=3, 12 Hz, 3-H), 6.22 (1H, d, *J*=2 Hz, 7-H), 6.29 (1H, d, *J*=2 Hz, 5-H), 6.75 (2H, br s, 2′,6′-H), 6.87 (1H, br s, 5′-H), 11.10 (s, 8-OH); ^{1}H-NMR (CD_3OD) *δ*: 3.00 (1H, dd, *J*=3, 17 Hz), 3.21 (1H, dd, *J*=12, 17 Hz) (4-H_2), 5.41 (1H, dd, *J*=3, 12 Hz, 3-H), 6.22 (1H, d, *J*=2 Hz, 7-H), 6.25 (1H, d, *J*=2 Hz, 5-H), 6.78 (2H, s, 2′,6′-H), 6.91 (1H, s, 5′-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (*m/z*): 289 $(M+H)^+$.

Thunberginol E (**15**): Colorless needles, mp 216—217 °C (MeOH), $[\alpha]_D^{25}$ +38.5° (*c*=0.3, EtOH). High-resolution EI-MS: Calcd for $C_{16}H_{14}O_6$ (M^+): 302.0789; Found: 302.0772. CD (MeOH): $[\theta]_{301}$ −3000, $[\theta]_{279}$ +4200, $[\theta]_{250}$ +6800. UV [EtOH, nm (log *ε*)]: 223 (4.1), 272 (4.3), 304 (4.5). IR (KBr) cm^{-1}: 3370, 1672, 1695, 1530, 1238. ^{1}H-NMR (DMSO-d_6) *δ*: 3.05 (1H, dd, *J*=3, 17 Hz), 3.22 (1H, dd, *J*=12, 17 Hz) (4-H_2), 3.78 (3H, s, 4′-OCH_3), 5.54 (1H, dd, *J*=3, 12 Hz, 3-H), 6.22 (1H, d, *J*=2 Hz, 7-H), 6.29 (1H, d, *J*=2 Hz, 5-H), 6.86—6.96 (3H, m, 2′,5′, 6′-H). ^{13}C-NMR: given in Table 1. EI-MS (*m/z*, %): 302 (M^+, 100), 284 (14), 258 (52).

Thunberginol G 3′-*O*-Glucoside (**17**): A white powder. High-resolution positive-mode FAB-MS : Calcd for $C_{21}H_{22}NaO_{10}$ $(M+Na)^+$: 457.1116; Found: 457.1106. UV [EtOH, nm (log *ε*)]: 284 (3.8), 315 (3.9). IR (KBr) cm^{-1}: 3282, 1673, 1619, 1518, 1464, 1230. ^{1}H-NMR (DMSO-d_6) *δ*: 4.79 (*ca.* 1/2H, d, *J*=8 Hz, Glu-1), 4.78 (*ca.* 1/2H, d, *J*=8 Hz, Glu-1), 5.55 (1H, dd, *J*=2, 12 Hz, 3-H), 6.81 (1H, d, *J*=8 Hz, 5-H), 6.86 (*ca.* 1/2H, d, *J*=8 Hz, 5′-H), 6.87 (*ca.* 1/2H, d, *J*=8 Hz, 5′-H), 6.88 (1H, d, *J*=8 Hz, 7-H), 7.05 (*ca.* 1/2H, dd, *J*=2, 8 Hz, 6′-H), 7.07 (*ca.* 1/2H, dd, *J*=2, 8 Hz, 6′-H), 7.35 (*ca.* 1/2H, d, *J*=2 Hz, 2′-H), 7.37 (*ca.* 1/2H, d, *J*=2 Hz, 2′-H),7.46 (1H, t, *J*=8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (*m/z*): (glycerol matrix) 457 $(M+Na)^+$, (glycerol + LiCl matrix) 441 $(M+Li)^+$.

(−)-Hydrangenol 4′-*O*-Glucoside (**18**): A white powder, $[\alpha]_D^{25}$ −4.7° (*c*=0.1, MeOH). High-resolution positive-mode FAB-MS: Calcd for $C_{21}H_{23}O_9$ $(M+H)^+$: 419.1342; Found: 419.1355; Calcd for $C_{21}H_{22}NaO_9$ $(M+Na)^+$: 441.1162; Found: 441.1159. CD (MeOH): $[\theta]_{259}$ −4500, $[\theta]_{237}$ +3300, $[\theta]_{226}$ −1100. UV [MeOH, nm (log *ε*)]: 243 (3.4), 314 (3.1). IR (KBr) cm^{-1}: 3570, 1670, 1617. ^{1}H-NMR (DMSO-d_6) *δ*: 3.53 (2H, m, 4-H_2), 4.90 (1H, d, *J*=7 Hz, Glu-1), 5.71 (1H, dd, *J*=3, 12 Hz, 3-H), 6.81 (1H, d, *J*=7 Hz, 5-H), 6.86 (1H, d, *J*=8 Hz, 7-H), 7.08 (2H, d, *J*=9 Hz, 3′,5′-H), 7.45 (2H, d, *J*=9 Hz, 2′,6′-H), 7.49 (1H, dd, *J*=7, 8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (*m/z*): 419 $(M+H)^+$, 441 $(M+Na)^+$.

(+)-Hydrangenol 4′-*O*-Glucoside (**19**): A white powder, $[\alpha]_D^{25}$ +6.9° (*c*=0.1, MeOH). High-resolution positive-mode FAB-MS : Calcd for $C_{21}H_{23}O_9$ $(M+H)^+$: 419.1342; Found: 419.1360. CD (MeOH): $[\theta]_{262}$ +8600, $[\theta]_{221}$ −17000. UV [MeOH, nm (log *ε*)]: 243 (3.7), 314 (3.4). IR (KBr) cm^{-1}: 3570, 1670, 1617. ^{1}H-NMR (DMSO-d_6) *δ*: 3.53 (2H, m, 4-H_2), 4.90 (1H, d, *J*=7 Hz, Glu-1), 5.74 (1H, dd, *J*=3, 12 Hz, 3-H), 6.87 (1H, d, *J*=8 Hz, 5-H), 6.90 (1H, d, *J*=8 Hz, 7-H), 7.08 (2H, d, *J*=9 Hz, 3′,5′-H), 7.45 (2H, d, *J*=9 Hz, 2′,6′-H), 7.52 (1H, dd, *J*=8, 8 Hz, 6-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (*m/z*): 419 $(M+H)^+$, 441 $(M+Na)^+$.

Acetylation of Thunberginol C (13) A solution of **13** (10 mg) in pyridine (1.0 ml) was treated with Ac_2O (0.5 ml) and the reaction mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over Na_2SO_4, and filtered. After removal of the solvent under reduced pressure, a residue was purified by silica gel column (1 g, *n*-hexane : AcOEt = 1 : 1) to yield thunberginol C triacetate (**13a**, 10 mg, 68%).

Thunberginol C Triacetate (**13a**): A white powder. High-resolution EI-MS: Calcd for $C_{21}H_{18}O_8$ (M^+): 398.1001; Found: 398.1006. UV [EtOH, nm (log *ε*)]: 242 (4.4), 279 (4.8). IR (KBr) cm^{-1}: 1771, 1727, 1615, 1510, 1196. ^{1}H-NMR: ($CDCl_3$) *δ*: 2.31, 2.32, 2.38 (3H each, all s, OAc × 3), 3.10 (1H, dd, *J*=3, 17 Hz), 3.29 (1H, dd, *J*=12, 17 Hz) (4-H_2), 5.11 (1H, dd, *J*=3, 12 Hz, 3-H), 6.91 (1H, d, *J*=2 Hz, 7-H), 7.00 (1H, d, *J*=2 Hz, 5-H), 7.14 (2H, d, *J*=9 Hz, 3′,5′-H), 7.46 (2H, d, *J*=9 Hz, 2′,6′-H). ^{13}C-NMR: given in Table 1. EI-MS (*m/z*, %): 398 (M^+, 2), 356 (73), 314 (100), 254 (38).

Alkaline Treatment of 13 to Give 20 A solution of **13** (6.0 mg) in acetone (2.0 ml) was treated with 0.5% aqueous KOH (2.0 ml) and the reaction mixture was stirred at 60 °C for 30 min. After removal of the solvent from the reaction mixture under reduced pressure, the residue was purified on an LH-20 column (10 g, MeOH) to give the stilbene derivative (**20**, 2.3 mg, 38.3%).

20: A white powder. High-resolution positive FAB-MS Calcd for $C_{15}H_{13}O_5$ $(M+H)^+$: 273.1736; Found: 273.0785. UV [EtOH, nm (log *ε*)]: 298 (4.5), 242 (4.4). IR (KBr) cm^{-1}: 3240, 1615, 1574, 1462. ^{1}H-NMR (CD_3OD) *δ*: 6.18 (1H, d, *J*=2 Hz, 4-H), 6.54 (1H, d, *J*=2 Hz, 6-H), 6.74 (2H, d, *J*=9 Hz, 3′,5′-H), 6.76, 8.11 (1H each, both d, *J*=16 Hz, *α*,*β*-H), 7.36 (2H, d, *J*=9 Hz, 2′,6′-H). Positive-mode FAB-MS (*m/z*): 273 $(M+H)^+$.

Acetylation of Thunberginol D (14) A solution of **14** (10 mg) in pyridine (1.0 ml) was treated with Ac_2O (0.5 ml) and the reaction mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, then dried over Na_2SO_4, and filtered. Evaporation of the solvent from the filtrate under reduced pressure gave a residue, which was separated on a silica gel column (1 g, *n*-hexane : AcOEt = 1 : 1) to yield thunberginol D tetraacetate (**14a**, 9 mg, 60%).

Thunberginol D Tetraacetate (**14a**): A white powder, High-resolution positive FAB-MS: Calcd for $C_{23}H_{21}O_{10}$ $(M+H)^+$: 457.1135; Found: 457.1128. UV [EtOH, nm (log *ε*)]: 242 (4.5), 290 (5.1). IR (KBr) cm^{-1}:

1775, 1730, 1615, 1508, 1206. ^{1}H-NMR ($CDCl_3$) δ: 2.30 (6H), 2.32, 2.38 (3H each) (all s, OAc×4), 3.13 (1H, dd, J=3, 17 Hz), 3.28 (1H, dd, J=12, 17 Hz) (4-H_2), 5.51 (1H, dd, J=3, 12 Hz, 3-H), 6.92 (1H, d, J=2 Hz, 7-H), 7.00 (1H, d, J=2 Hz, 5-H), 7.24 (1H, d, J=9 Hz, 5′-H), 7.32 (1H, d, J=2 Hz, 2′-H), 7.33 (1H, dd, J=2, 9 Hz, 6′-H). ^{13}C-NMR: given in Table 1. Positive-mode FAB-MS (m/z): 457 $(M+H)^+$.

Acetylation of Thunberginol E (15) A solution of **15** (10 mg) in pyridine (1.0 ml) was treated with Ac_2O (0.5 ml) and the reaction mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over Na_2SO_4, and filtered. After evaporation of the solvent from the filtrate under reduced pressure, the residue was chromatographed on a silica gel column (1 g, *n*-hexane : AcOEt = 1 : 1) to give thunberginol E triacetate (**15a**, 12 mg, 85%).

Thunberginol E Triacetate (**15a**): A white powder, $[\alpha]_D^{25}$ +69.7° (c=0.2, EtOH), High-resolution positive FAB-MS: Calcd for $C_{22}H_{21}O_9$ (M^+): 429.1185; Found: 429.1162. CD : $[\theta]_{301}$ −3000, $[\theta]_{279}$ +4200, $[\theta]_{250}$ +6800. UV [EtOH, nm (log ε)]: 226 (4.5), 274 (5.2). IR (KBr) cm^{-1}: 1771, 1727, 1615, 1516, 1202. ^{1}H-NMR ($CDCl_3$) δ: 2.32 (6H), 2.37 (3H)(all s, OAc×3), 3.09 (1H, dd, J=3, 17 Hz), 3.29 (1H, dd, J=12, 17 Hz) (4-H_2), 3.84 (3H, s, OCH_3), 5.45 (1H, dd, J=3, 12 Hz, 3-H), 6.91 (1H, d, J=2 Hz, 7-H), 6.99 (1H, d, J=2 Hz, 5-H), 6.99 (1H, d, J=8 Hz, 5′-H), 7.14 (1H, d, J=2 Hz, 2′-H), 7.27 (1H, dd, J=2, 8 Hz, 6′-H). ^{13}C-NMR: given in Table 1. Positive FAB-MS (m/z): 429 $(M+H)^+$.

Methanolysis of Thunberginol G 3′-*O*-Glucoside (17) A solution of **17** (1.5 mg) in 9% HCl–MeOH (1.0 ml) was stirred at 85 °C under an N_2 atmosphere for 20 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and filtered. After removal of the solvent from the filtrate under reduced pressure, the residue was separated on a silica gel column (1 g, $CHCl_3$: MeOH = 10 : 1) to afford thunberginol G (**16**, 1.0 mg) and methyl D-glucoside. A solution of methyl D-glucoside in pyridine (0.1 ml) was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA, 0.1 ml) for 1 h. The reaction solution was then subjected to GLC analysis to identify the trimethylsilyl (TMS) derivative of methyl D-glucoside; GLC conditions: CBRI-M25-0.25, 0.25 mm (i.d.) × 25 m capillary column, column temperature 140—280 °C, He flow rate 15 ml/min, t_R: 17.9 min, 18.3 min. Thunberginol G (**16**) was identified by TLC and ^{1}H-NMR data comparisons with an authentic demethyl derivative of **8**.

Demethylation of Phyllodulcin (8) with BBr_3 A solution of **8** (20 mg) in CH_2Cl_2 (0.9 ml) was treated with BBr_3 (33 μl) and the mixture was stirred at −15 °C for 30 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 aqueous saturated NaCl and dried over $MgSO_4$. After removal of the solvent from the CH_2Cl_2 extract under reduced pressure, the product was purified on a silica gel column [*n*-hexane : ether (1 : 2)] to give **16** (17 mg), which was identified by comparison of the physical data with reported values[14] and used as the authentic demethyl derivative of **8**.

Partial Methylation of Thunberginol G 3′-*O*-Glucoside (17) with Diazomethane Followed by Enzyme Hydrolysis A solution of **17** (11 mg) in MeOH (1.0 ml) was treated with 10% trimethylsilyldiazomethane (3.0 ml) and the reaction mixture was left standing at room temperature (20 °C) for 1 h. It was then concentrated to dryness under reduced pressure to yield the 4′-methyl derivative (13 mg). A solution of the 4′-methyl derivative (12 mg) and β-glucosidase (12.6 U/mg) in acetic acid buffer solution (pH 5, 5.0 ml) was stirred at 37 °C for 34 h. After removal of the solvent under reduced pressure, the residue was chromatographed on a silica gel column (0.8 g, *n*-hexane : AcOEt = 2 : 1) to yield (±)-phyllodulcin {2.6 mg, $[\alpha]_D^{25}$ ±0° (EtOH)}, which was shown to be identical with authentic phyllodulcin by TLC, IR, ^{1}H-NMR and ^{13}C-NMR spectral comparisons.

Methanolysis of (−)-Hydrangenol 4′-*O*-Glucoside (18) A solution of **18** (1.5 mg) in 9% HCl–MeOH (1.0 ml) was stirred at 85 °C under an N_2 atmosphere for 20 min. The reaction mixture was neutralized with Dowex 50W×8 (H^+ form) and filtered. The solvent was evaporated under reduced pressure. The residue was chromatographed on a silica gel column (1 g, $CHCl_3$: MeOH = 10 : 1) to yield hydrangenol (**6**, 1.0 mg) and methyl D-glucoside. Hydrangenol (**6**) was identified by TLC, ^{1}H-NMR and ^{13}C-NMR spectral comparisons with an authentic sample, and methyl D-glucoside was derived to the TMS derivative, which was identical with an authentic sample on GLC (the same condition as described above for methanolysis of **17**) analysis.

Glycosidation of Hydrangenol (6) with *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate Followed by Deacetylation A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate (1.54 g), hydrangenol (**6**, 200 mg), and Molecular Sieves-4A (400 mg) in CH_2Cl_2 (20.0 ml) was treated with BF_3–Et_2O (200 μl) and the mixture was stirred at room temperature (20 °C) under an N_2 atmosphere for 3 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ 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 (1.36 g) was purified on a silica gel column (60 g, benzene : AcOEt = 5 : 1) to give the 4′-glycosidation product of **6** (357.5 mg, 78.1%). A solution of the 4′-glycosidation product (20 mg) in MeOH (2.0 ml) was treated with 10% aqueous K_2CO_3 (2.0 ml) and the reaction mixture was stirred at room temperature (20 °C) for 10 min. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and filtered. The solvent was evaporated under reduced pressure to yield a mixture of (−) and (+)-hydrangenol 4′-*O*-glucosides [**18**:**19** (*ca.* 1 : 1)] (12.8 mg, 89.5%), which was identical with (−) and (+)-hydrangenol 4′-*O*-glucosides (**18**, **19**) obtained from Hydrangeae Dulcis Folium by HPLC (the same conditions as described above for the isolation of **18** and **19**).

Bioassay Tests for Antiallergic and Antimicrobial Activities The methods of bioassay testing were the same as those for thunberginols A (**1**), B (**2**), and F (**3**), and the extracts from Hydrangeae Dulcis Folium described in the previous papers.[3b,4]

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References and Notes

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