THUNBERGINOLS C, D, AND E, NEW ANTIALLERGIC AND ANTIMICROBIAL DIHYDROISOCOUMARINS, AND THUNBERGINOL G 3'-O-GLUCOSIDE AND (-)-HYDRANGENOL 4'-O-GLUCOSIDE, NEW DIHYDROISOCOUMARIN GLYCOSIDES, FROM HYDRANGEAE DULCIS FOLIUM

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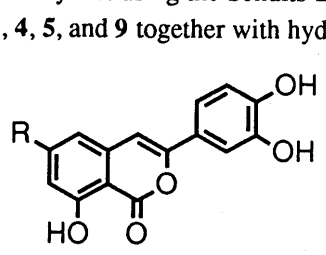
New antiallergic and antimicrobial dihydroisocoumarins, thunberginols C, D, and E, were isolated from Hydrangeae Dulcis Folium, the fermented and dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO, together with new dihydroisocoumarin glycosides, thunberginol G 3'-O-glucoside and (-)-hydrangenol 4'-O-glucoside. Their chemical structures have been determined on the basis of chemical and physicochemical evidence. Thunberginols C, D, E, G, and (-)-hydrangenol 4'-O-glucoside showed antiallergic activity in the *in vitro* bioassay using the Schults-Dale reaction in sensitized guinea pig bronchial muscle, and they also exhibited antimicrobial activity against oral bacteria.

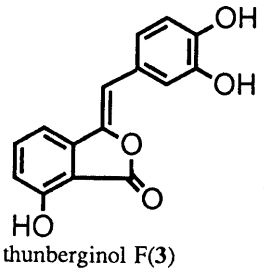
KEYWORDS Hydrangeae Dulcis Folium; *Hydrangea macrophylla* var. *thunbergii*; thunberginol C; thunberginol D; thunberginol E; thunberginol G 3'-O-glucoside; (-)-hydrangenol 4'-O-glucoside; antiallergic activity; antimicrobial activity; dihydroisocoumarin

In the previous paper,[1] we have reported the isolation of six antiallergic principles named thunberginols A, B, C, D, E, and F from Hydrangeae Dulcis Folium, the fermented and dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae) which is used as an oral refrigerant and a sweetener, and described the chemical structures of thunberginols A(**1**), B(**2**), and F(**3**). In a continuing study, we isolated new dihydroisocoumarin glycosides, thunberginol G 3'-O-glucoside(**7**) and (-)-hydrangenol 4'-O-glucoside(**11**) from the water-soluble portion of the same Hydrangeae Dulcis Folium. This paper deals with the structure elucidation of thunberginols C(**4**), D(**5**), and E(**9**), and thunberginol G 3'-O-glucoside(**7**) and (-)-hydrangenol 4'-O-glucoside(**11**).

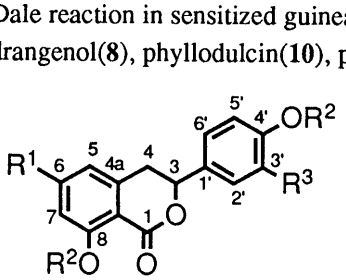
The MeOH extract of the Folium was partitioned into AcOEt and water to furnish the AcOEt soluble portion and the water soluble portion as described previously.[1] Repeated separation of the AcOEt soluble portion monitoring with the antiallergic activity test using the Schults-Dale reaction in sensitized guinea pig bronchial muscle[2] afforded new active constituents **1**, **2**, **3**, **4**, **5**, and **9** together with hydrangenol(**8**), phyllodulcin(**10**), phyllodulcin monomethyl ether, hydrangeic acid,



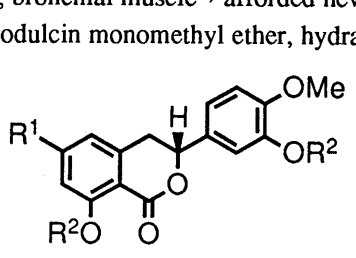
thunberginol A(**1**)
: R = H
thunberginol B(**2**)
: R = OH

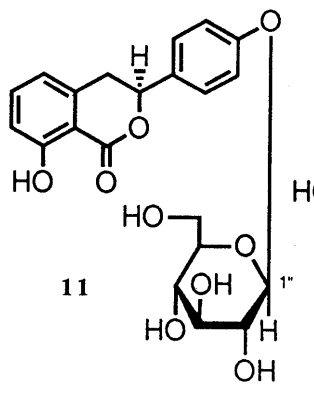


thunberginol F(**3**)



thunberginol C (**4**)
: R^1 = OH, R^2 = R^3 = H
4a : R^1 = OAc, R^2 = Ac, R^3 = H
thunberginol D (**5**)
: R^1 = R^3 = OH, R^2 = H
5a : R^1 = R^3 = OAc, R^2 = Ac
thunberginol G (**6**)
: R^1 = R^2 = H, R^3 = OH
7 : R^1 = R^2 = H, R^3 = O-β-D-Glu
hydrangenol (**8**)
: R^1 = R^2 = R^3 = H





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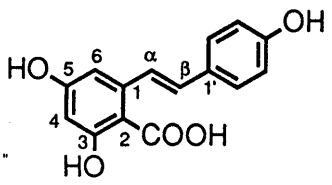
thunberginol E (**9**)
: R^1 = OH, R^2 = H
9a : R^1 = OAc, R^2 = Ac
phyllodulcin (**10**)
: R^1 = R^2 = H



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umbelliferone, dihydroresveratrol, isoarborinol, and rubiarbonol B.[1] The water-soluble portion, after repeated chromatographic purification with reversed phase silica gel and Sephadex LH-20, furnished **7**(0.0005% from the crude drug) and **11**(0.0006%) along with four flavonol glycosides, kaempferol 3-O-β-D-glucopyranosyl-(1-2)-β-D-glucopyranoside(0.95%), kaempferol 3-O-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside(0.15%), kaempferol 3-O-β-D-glucopyranosyl-(1-2)-[α-L-rhamnopyranosyl-(1-6)]-β-D-glucopyranoside(0.48%), and quercetin 3-O-β-D-glucopyranosyl-(1-2)-β-D-glucopyranoside(0.20%).

Thunberginol C(**4**), colorless needles, mp 197-198°C(MeOH), $[\alpha]_D$ ±0°(EtOH), $C_{15}H_{12}O_5$, UV[EtOH, nm(log ε)]: 218(3.8), 271(4.1), 301(4.4); (EtOH+$AlCl_3$, nm): 229, 285, 334; EI-MS(m/z, %): 272(M^+, 4), 228(100), was shown by its IR spectrum to have hydroxyl(3357, 1055 cm^{-1}), chelated δ-lactone(1649 cm^{-1}) and aromatic ring(1630, 1522 cm^{-1}) functions. The ^1H NMR spectrum of **4** showed signals assignable to a disubstituted benzene ring[δ 6.80(d, J=9Hz, 3', 5'-H), 7.31(d, J=9Hz, 2', 6'-H)], a tetrasubstituted benzene ring[δ 6.22(d, J=2Hz, 7-H), 6.30(d, J=2Hz, 5-H)]and a chelated δ-lactone[δ 5.54(dd, J=3, 12Hz, 3-H)], 3.03(dd, J=3, 17Hz), 3.24(dd, J=12, 17Hz)(4-H_2), 11.10(s, 5-OH)] groups. Acetylation of **4** with Ac_2O in pyridine provided the triacetate(**4a**),[3] while alkaline treatment of **4** with 0.5% KOH afforded the stilbene derivative(**12**).[4] This evidence and comparison of the ^{13}C NMR data for **4** with those for hydrangenol(**8**) led us to confirm the structure of thunberginol C as **4**.

Thunberginol D(**5**), colorless needles, mp 199-200°C(MeOH), $[\alpha]_D$ ±0°(EtOH), $C_{15}H_{12}O_6$, UV[EtOH, nm(log ε)]: 229 (4.0), 272(4.1), 297(4.4); IR(KBr, cm^{-1}): 3409, 1645, 1628, 1520, 1244, positive FAB-MS(m/z): 289$(M+H)^+$, provided the tetraacetate(**5a**)[5] by ordinary acetylation. The ^1H NMR spectra of **5**[6] and **5a** were very similar to those of **4** and **4a**, respectively, but they lacked the 3'-H signal indicative of a trisubstituted benzene ring in **5**. Based on this evidence and comparison of the ^{13}C NMR data for **5** and **5a** with those for **4**, **4a**, and **8**, the structure of thunberginol D has been determined as **5**.

Thunberginol E(**9**), colorless needles, mp 216-217°C(MeOH), $[\alpha]_D$ +38.5°(EtOH), $C_{16}H_{14}O_6$, UV[EtOH, nm(log ε)]: 223(4.1), 272(4.3), 304(4.5); IR(KBr, cm^{-1}): 3370, 1672, 1695, 1530, 1238, EI-MS(m/z, %): 302(M^+, 100), 284(14), 258 (52), afforded the triacetate (**9a**)[7] by acetylation. Detailed comparisons of the ^1H and ^{13}C NMR data for **9**[8] and **9a** with those for phyllodulcin(**10**) and its diacetate led us to presume the structure **9** to be 6-hydroxyphyllodulcin. The location of methoxyl group in **9a** was finally determined from its different NOE experiment which showed the NOE correlation between 5'-H[δ 6.99(d, J=8Hz)] and 4'-OMe(δ 3.84). The CD spectrum of **9** showed the characteristic curve for dihydroisocoumarins with 3R configuration($[\theta]_{301}$ -3000, $[\theta]_{279}$ +4200, $[\theta]_{250}$ -6800).[9] Consequently, the absolute structure of **9** was clarified as shown.

Thunberginol G 3'-O-glucoside(**7**), white powder, $[\alpha]_D$ -32.9°(EtOH), $C_{21}H_{22}O_{10}$, UV[EtOH, nm(log ε)]: 284(3.8), 315(3.9), IR(KBr, cm^{-1}): 3282, 1673, 1619, 1518, 1464, 1230, positive FAB-MS(m/z): 457$(M+Na)^+$, was isolated as 3-epimeric mixture.[10] Methanolysis of **7** with 9% HCl-MeOH liberated thunberginol G(**6**), white powder, $[\alpha]_D$ ±0°(EtOH), $C_{15}H_{12}O_5$, and methyl D-glucopyranoside. Thunberginol G(**6**) was shown to be identical in all respects with desmethylphyllodulcin[11] prepared from **10** by BBr_3 treatment. Partial methylation of **7** with diazomethane and subsequent β-glucosidase hydrolysis provided (±)-phyllodulcin. This chemical evidence and comparisons of the ^1H and ^{13}C NMR data for **7** with those for **6** and **8** led us to elucidate the 3'-O-glucoside structure of **7**.

Table I. ^{13}C NMR Data for **4**, **4a**, **5**, **5a**, **9**, **9a**, and **11**[c]

	4[a]	**4a**[b]	**5**[a]	**5a**[b]	**9**[a]	**9a**[b]	**11**[a]
1	169.4	161.1	169.4	160.9	169.5	161.2	169.3
3	79.7	78.6	79.7	78.1	79.7	78.4	80.2
4	33.6	36.2	33.7	36.2	33.9	36.1	33.6
4a	142.2	142.0	142.2	141.8	142.4	142.1	140.6
5	106.8	118.2	106.8	118.3	107.1	118.2	118.5
6	164.4	154.6	164.4	154.7	164.7	154.6	136.5
7	100.9	116.8	100.9	116.9	101.1	116.7	115.6
8	163.3	153.2	163.3	153.2	163.5	153.2	161.0
8a	100.3	115.3	100.3	115.3	100.4	115.4	108.5
1'	128.6	135.5	129.3	136.7	131.1	130.4	131.6
2'	128.0	127.3	114.1	121.3	112.1	118.2	128.1
3'	115.1	121.9	145.1	141.8	146.5	139.8	116.3
4'	157.6	150.8	145.6	142.2	148.0	151.4	157.7
5'	115.1	121.9	115.3	123.8	114.0	120.9	116.3
6'	128.0	127.3	117.7	124.1	117.6	124.7	128.1
OMe					55.8	56.0	

a, b) The ^{13}C NMR spectra were measured in a) DMSO-d_6 or b) $CDCl_3$.
c) The signal assignments were based on spectral analyses of ^1H-^{13}C COSY and COLOC experiments.

(-)-Hydrangenol 4'-O-glucoside(**11**),[12] white powder, $[\alpha]_D$ -20.0°(MeOH), $C_{21}H_{22}O_9$, UV[MeOH, nm(log ε)]: 245(3.9), 316(3.8), IR(KBr, cm^{-1}): 3500, 1669, 1615, 1516, 1238, 1076, positive FAB-MS(m/z): 441 $(M+Na)^+$, liberated hydrangenol(**8**) and methyl D-glucopyranoside by methanolysis. Selective glycosidation of **8** with O-(2, 3, 4, 6-tetra-O-acetyl-α-D-glucopyranosyl)trichloroacetimidate in the presence of BBr_3-Et_2O followed by deacetylation yielded the 3-epimeric mixture of **11**. Furthermore, the NOE correlations were observed between the anomeric

proton[δ 4.90(d, J=8Hz)] and 3', 5'-H [δ 7.08(d, J=9Hz)] in the different NOE experiment of **11**. Finally, absolute stereostructure of **11** was determined from the analysis of CD spectrum which showed the Cotton curve characteristic of 3S configuration($[\theta]_{275}$ -2090, $[\theta]_{265}$ -3760, $[\theta]_{236}$ +1880).[9] Consequently, the structure of (-)-hydrangenol 4'-O-glucoside(**11**) was elucidated as shown.

The inhibitory activities of thunberginols C(**4**), D(**5**), E(**9**), G(**6**) and (-)-hydrangenol 4'-O-glucoside (**11**) on the Schults-Dale reaction and histamine-induced contraction are summarized in Table II. Compounds **4**, **5**, **6**, **9**, and **11** showed inhibitory activity comparable to AA-861[2b] in antigen-induced contraction of tracheal chain isolated from sensitized guinea pig, while they exhibited little inhibition for histamine-induced contraction. These findings showed that the antiallergic activity of **4**, **5**, **6**, **9**, and **11** concerned factors other than competition of histamine, as well as the antiallergic activity of **1**, **2**, and **3**.[1] Furthermore, **4**, **5**, **6**, **8**, **9**, **10**, and **11** showed antimicrobial activities against two oral bacteria [*Bacteroides melaninogenicus*; MIC: **4**(10ppm), **5**(10ppm), **6**(20ppm), **8**(10ppm), **9**(50ppm), **10**(100ppm), and **11**(100ppm); *Fusobacterium nucleatum*; MIC: **4**(10ppm), **5**(10ppm), **6**(20ppm), **8**(5ppm), **9**(30ppm), **10**(100ppm), and **11**(100ppm)].

Table II. Inhibitory Effects of Thunberginols C(**4**), D(**5**), E(**9**), G(**6**), and (-)-Hydrangenol 4'-O-glucoside(**11**) on the Schults-Dale (S.D.) Reaction and Histamine (His.)-Induced Contraction in Isolated Guinea Pig Tracheal Chain

Compounds	Conc. (M)	S.D. (Inhibition %)	His. (Inhibition %)
Thunberginol C (**4**)	10^{-5}	0.0± 0.0	5.9±11.7
	3×10^{-5}	57.2± 7.7	11.3± 7.2
	10^{-4}	100.0**	48.5± 3.2**
Thunberginol D (**5**)	10^{-5}	5.1± 6.3	10.5± 3.4*
	3×10^{-5}	44.1± 3.0*	5.3± 5.9
	10^{-4}	100.0**	25.6± 6.0*
Thunberginol E (**9**)	10^{-5}	0.0	13.2± 0.6*
	3×10^{-5}	34.1±16.1	20.8± 3.5*
	10^{-4}	100.0**	37.1± 4.4**
Thunberginol G (**6**)	10^{-5}	0.0	1.9± 6.8
	3×10^{-5}	30.5± 3.0*	2.8± 2.6
	10^{-4}	100.0**	0.0± 1.4
(-)-Hydrangenol 4'-O-glucoside (**11**)	10^{-5}	2.4± 1.7	0.0
	3×10^{-5}	22.4± 2.4	7.0± 2.0
	10^{-4}	92.5± 3.6*	5.0± 3.1**
AA-861	10^{-5}	0.0	
	3×10^{-5}	26.8±22.4	
	10^{-4}	100.0**	
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).

REFERENCES AND NOTES

1) M. Yoshikawa, E. Uchida, N. Chatani, N. Murakami, and J. Yamahara, *Chem. Pharm. Bull.*, **40**, 3121(1992). 2)a) J. Yamahara, M. Kimata, T. Sawada, and H. Fujimura, *J. Med. Pharm. Soc. WAKAN-YAKU*, **3**, 153(1986); b) H. L. Cho, P. P. K. Ho, E. D. Mihelich, and D. W. Synder, *J. Pharm. Methods*, **26**, 277(1991). 3) Thunberginol C triacetate(**4a**): white powder, $C_{21}H_{18}O_8$, UV[EtOH, nm(log ε)]: 242(4.2), 279(4.8), IR(KBr, cm^{-1}): 1771, 1727, 1615, 1510, 1196, ^1H NMR($CDCl_3$, δ): 2.31, 2.32, 2.38(6, 8, 4'-OAc), 3.10(dd, J=3, 17Hz), 3.29(dd, J=12, 17Hz)(4-H_2), 5.11(dd, J=3, 12Hz, 3-H), 6.91(d, J=2Hz, 7-H), 7.00(d, J=2Hz, 5-H), 7.14(d, J=9Hz, 3', 5'-H), 7.46(d, J=9Hz, 2', 6'-H), EI-MS(m/z, %): 398(M^+, 2), 356(73), 314(100), 254(38). 4) **12**: white powder, $C_{15}H_{12}O_5$, UV[EtOH, nm(log ε]: 298(4.5), 242(4.4), IR(KBr, cm^{-1}): 3240, 1615, 1574, 1462, ^1H NMR(CD_3OD, δ): 6.18(d, J=2Hz, 4-H), 6.54(d, J=2Hz, 6-H), 6.74(d, J=9Hz, 3', 5'-H), 6.76, 8.11(both d, J=16Hz, α, β-H), 7.36(d, J=9Hz, 2', 6'-H), positive FAB-MS(m/z): 273$(M+H)^+$. 5) Thunberginol D tetraacetate(**5a**): white powder, $C_{23}H_{20}O_{10}$, UV[EtOH, nm(log ε)]: 242(4.5), 290(5.1), IR(KBr, cm^{-1}): 1775, 1730, 1615, 1508, 1206, ^1H NMR($CDCl_3$, δ): 2.30(6H), 2.32, 2.38(6, 8, 3', 4'-OAc), 3.13(dd, J=3, 17Hz), 3.28(dd, J=12, 17Hz)(4-H_2), 5.51(dd, J=3, 12Hz, 3-H), 6.92(d, J=2Hz, 7-H), 7.00(d, J=2Hz, 5-H), 7.24(d, J=9Hz, 5'-H), 7.32(d, J=2Hz, 2'-H), 7.33(dd, J=2, 9Hz, 6'-H), positive FAB-MS(m/z): 457$(M+H)^+$. 6) **5**: ^1H NMR(DMSO-d_6, δ): 3.03(dd, J=3,17Hz), 3.21(dd, J=12, 17Hz)(4-H_2), 5.49(dd, J=3, 12Hz, 3-H), 6.22(d, J=2Hz, 7-H), 6.29(d, J=2Hz, 5-H), 6.75(2H, br s, 2', 6'-H), 6.87(br s, 5'-H), 11.10(br s, 8-OH). 7) Thunberginol E triacetate(**9a**): white powder, $[\alpha]_D$ +69.7°(EtOH), $C_{22}H_{20}O_9$, UV[EtOH, nm(log ε)]: 226(4.5), 274(5.2), IR(KBr, cm^{-1}): 1771, 1727, 1615, 1516, 1202, ^1H NMR($CDCl_3$, δ): 2.32(6H), 2.37(6, 8, 3'-OAc), 3.09(dd, J=3, 17Hz), 3.29(dd, J=12, 17Hz)(4-H_2), 3.84(4'-OMe), 5.45(dd, J=3, 12Hz, 3-H), 6.91(d, J=2Hz, 7-H), 6.99(d, J=2Hz, 5-H), 6.99(d, J=8Hz, 5'-H), 7.14(d, J=2Hz, 2'-H), 7.27(dd, J=2, 8Hz, 6'-H), positive FAB-MS(m/z): 429$(M+H)^+$. 8) **9**: ^1H NMR(DMSO-d_6, δ): 3.05(dd, J=3, 17), 3.22(dd, J=12, 17)(4-H_2), 3.78(4'-OMe), 5.54(dd, J=3, 12Hz, 3-H), 6.22(d, J=2Hz, 7-H), 6.29(d, J=2Hz, 5-H), 6.86-6.96(3H, m, 2', 5', 6'-H). 9) T. Hashimoto, M. Tori, and Y. Asakawa, *Phytochemistry*, **26**, 3323(1987). 10) We have not succeeded in separating the isomeric mixture by HPLC so far. 11) M. Yamato, T. Kitamura, K. Hashigaki, Y. Kuwano, N. Yoshida, and T. Koyama, *Yakugaku Zasshi*, **92**, 367(1972). 12) **11**: ^1H NMR(DMSO-d_6, δ): 4.90(d, J=8Hz, 1"-H), 5.74(dd, J=3, 12Hz, 3-H), 6.88(d, J=8Hz, 5-H), 6.91(d, J=8Hz, 7-H), 7.08(d, J=9Hz, 3', 5'-H), 7.46(d, J=9Hz, 2', 6'-H), 7.53(dd, J=8, 8Hz, 6-H).

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