THUNBERGINOLS A, B, AND F, NEW ANTIALLERGIC AND ANTIMICROBIAL PRINCIPLES FROM HYDRANGEAE DULCIS FOLIUM

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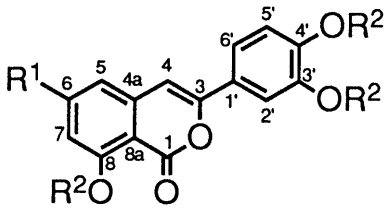
Six new antiallergic and antimicrobial principles, thunberginols A, B, C, D, E, and F, were isolated from Hydrangeae Dulcis Folium, the fermented and dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO. The chemical structures of thunberginols A, B, and F have been determined on the basis of chemical and physicochemical evidence. Thunberginols A, B, and F showed more potent antiallergic activity than phyllodulcin, hydrangenol, and AA-861 in the *in vitro* test using the Schults-Dale reaction in sensitized guinea pig bronchial muscle. Thunberginols A, B, and F also exhibited antimicrobial activity against oral bacteria.

KEYWORDS Hydrangeae Dulcis Folium; *Hydrangea macrophylla* SERINGE var. *thunbergii*; Saxifragaceae; thunberginol A; thunberginol B; thunberginol F; isocoumarin; benzylidenephthalide; antiallergic activity; antimicrobial activity

Hydrangeae Dulcis Folium (Amacha in Japanese), the fermented and dried leaves of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae), has been used as an oral refrigerant and a sweetening. In regard to the bioactive constituent of this crude drug, phyllodulcin(**4**) has been shown to be a sweet and antifungal principle.[1] As a part of our studies on biologically active constituents of crude drugs,[2] we have isolated six new antiallergic and antimicrobial principles named thunberginols A(**1**), B(**2**), C, D, E, and F(**3**) from Hydrangeae Dulcis Folium cultivated and processed in Nagano Prefecture. This paper deals with the structure elucidations of thunberginols A(**1**), B(**2**), and F(**3**) .

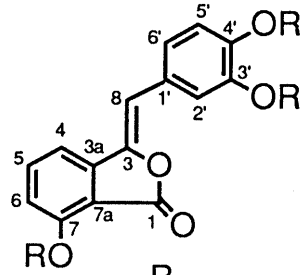
The MeOH extract of Hydrangeae Dulcis Folium showed strong antiallergic activity on the *in vivo* passive cutaneous anaphylaxis(PCA) test and the *in vitro* test using the Schults-Dale reaction[3] in sensitized guinea pig bronchial muscle. The MeOH extract of the Folium was partitioned into AcOEt and water, and the AcOEt soluble portion was subjected to column chromatography with ordinary phase and reversed phase silica gel and Sephadex LH-20 by monitoring the antiallergic activity on the *in vitro* bioassay. Six new active principles, thunberginols A(**1**, 0.0014% from the crude drug), B(**2**, 0.0016%), C(0.0010%),[4] D(0.0012%),[4] and E(0.0040%),[4] and F(**3**, 0.0016%), have so far been isolated from the AcOEt soluble portion together with phyllodulcin(**4**, 1.99%), hydrangenol(**5**, 2.35%), phyllodulcin monomethyl ether(0.0004%), umbelliferone(1.41%), hydrangeic acid(0.0024%), dihydroresveratrol(**6**, 0.0008%),[5] isoarborinol(**7**, 0.0026%),[5] and rubiarbonol B(**8**, 0.0022%).[5]

Thunberginol A(**1**), yellow prisms, mp 240°C(MeOH-H_2O), $C_{15}H_{10}O_5$, UV[EtOH, nm(log ε)]: 223(4.0), 347(4.1),

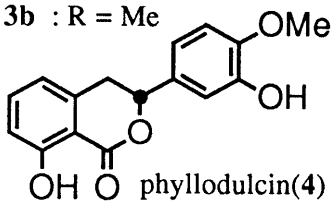


thunberginol A(**1**)
: $R^1 = R^2 = H$
1a : $R^1 = H$, $R^2 = Ac$
1b : $R^1 = H$, $R^2 = Me$

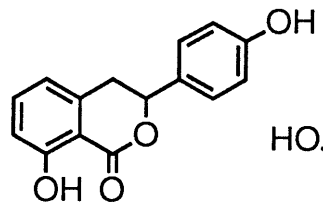
thunberginol B(**2**)
: $R^1 = OH$, $R^2 = H$
2a : $R^1 = OAc$, $R^2 = Ac$



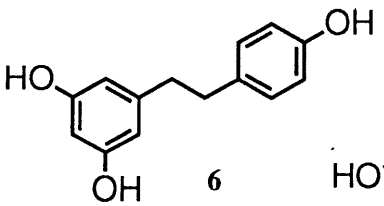
thunberginol F(**3**)
: R = H
3a : R = Ac
3b : R = Me



phyllodulcin(**4**)



hydrangenol(**5**)



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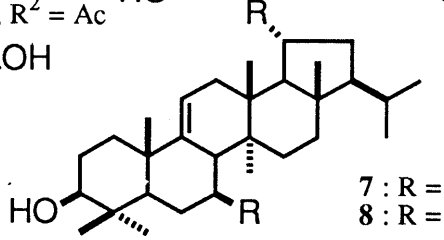

7 : R = H
8 : R = OH

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365(4.0); (EtOH+$AlCl_3$): 224, 333, 407 nm, IR(KBr, cm^{-1}): 3436, 1669, 1611, 1526, 1221, EI-MS(m/z, %): 270(M^+, 100), 242(45), furnished the triacetate(**1a**)[6] by acetylation with Ac_2O in pyridine, while methylation of **1** with diazomethane in MeOH-ether afforded the trimethyl ether(**1b**).[7] Comparisons of IR and UV data for **1**, **1a**, and **1b** with those for known isocoumarins[8] led us to presume the 3-phenylisocoumarin structure in **1**. The ^1H NMR data of **1** showed the presence of two trisubstituted benzene rings [δ 7.69(dd, J=8, 8Hz, 6-H), 7.10(br.d, J=8Hz, 5-H), 6.93(br.d, J=8Hz, 7-H); δ 7.30(d, J=2Hz, 2'-H), 7.24(dd, J=2, 8Hz, 6'-H), 6.88(d, J=8Hz, 5'-H)], a conjugated olefin function [δ 7.23(s, 4-H)] and a chelated hydroxyl group[δ 10.87(br s)]. Based on the above-mentioned evidence and examination of ^{13}C NMR data of **1** (Table II), the structure of thunberginol A has been proposed as **1**. Finally, the chemical structure of **1** was determined by chemical correlation with phyllodulcin(**4**). Thus, diazomethane methylation of **4** and subsequent DDQ oxidation in benzene provided thunberginol A trimethyl ether(**1b**) in 70% yield.

Thunberginol B(**2**), pale yellow needles, mp 244°C(MeOH-H_2O), $C_{15}H_{10}O_6$, UV[EtOH, nm(log ε)]: 235(4.1), 268(4.1), 324(4.3), 350(4.2); (EtOH+$AlCl_3$): 235, 275, 332, 380 nm, IR(KBr, cm^{-1}): 3374, 3171, 1676, 1620, 1528, 1250, EI-MS(m/z, %): 286(M^+, 100), 258(53), furnished the tetraacetate(**2a**)[9] by ordinary acetylation. The ^1H NMR spectrum of **2**[10] was fairly similar to that of **1**, but it lacked the 6-H signal indicative of a tetrasubstituted ring in **2**. These physicochemical properties and comparison of ^{13}C NMR data for **2** with those for **1** have led us to formulate thunberginol B as **2**.

Thunberginol F(**3**), yellow needles, mp 242-243°C(MeOH), $C_{15}H_{10}O_5$, UV[EtOH, nm(log ε)]: 225(4.1), 290(4.4), 381(4.0), EI-MS(m/z, %): 270(M^+, 100), showed hydroxyl, chelated γ- lactone, and aromatic ring absorptions in its IR spectrum (3272, 1738, 1605, 1468 cm^{-1}). The ^1H and ^{13}C NMR (Table I) data of **3** showed the presence of two trisubstituted benzene rings[δ 7.58(dd, J=8, 8Hz, 5-H), 7.41(br.d, J=8Hz, 4-H), 6.91(br d, J=8Hz, 6-H), 7.38(d, J=2Hz, 2'-H),7.04(dd, J=2, 8Hz, 6'-H), 6.79(d, J=8Hz, 5'-H)], a conjugated olefin [δ 6.61(s, 8-H)] and a weakly chelated hydroxyl group [δ 9.27(br s)]. The ^1H and ^{13}C NMR signals of **3** could be analyzed completely by use of ^1H-^1H-COSY, ^1H-^{13}C COSY and long range ^1H-^{13}C COSY(J_{CH}=10Hz, 5Hz) experiments. Ordinary acetylation of **3** provided the triacetate(**3a**),[11] while the trimethyl ether(**3b**)[12] was obtained by diazomethane methylation. Comparisons of spectral data for **3**, **3a**, and **3b** with those for various phthalide derivatives[13] showed the structure **3** to be 7, 3', 4'-trihydroxybenzylidenephthalide. The geometry of benzylidene side chain in **3** was confirmed by NOE experiments of **3b**. Namely, the NOE correlations were observed in the pairs of protons of **3b** [4-H&8-H; 8-H&2'-H; 8-H&6'-H; 6-H&7-Me(δ 3.93); 2'-H&3'-Me(δ 3.97); 4'-OMe(δ 4.03)&5'-H] in its NOESY and difference NOE spectra. Consequently, the structure of thunberginol F was shown to be **3**.

Table I. Inhibitory Effects of Thunberginols A(**1**), B(**2**), F(**3**), Phyllodulcin(**4**), and Hydrangenol(**5**) 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 A	10^{-5}	50.5± 2.9**	14.6±11.7
(**1**)	$3x10^{-5}$	100.0**	24.1± 2.7*
	10^{-4}	100.0**	93.4± 4.6**
Thunberginol B	10^{-5}	49.8± 2.4**	16.5± 5.9
(**2**)	$3x10^{-5}$	95.7± 2.0**	13.7± 7.4
	10^{-4}	100.0**	65.2± 6.9**
Thunberginol F	10^{-5}	53.3±18.7	12.1± 4.0
(**3**)	$3x10^{-5}$	100.0**	12.0± 4.7
	10^{-4}	100.0**	43.2± 2.6**
Phyllodulcin(**4**)	10^{-5}	0.0	0.0
	$3x10^{-5}$	43.7±11.7*	4.2± 2.6
	10^{-4}	100.0**	38.2± 5.0**
Hydrangenol(**5**)	10^{-5}	0.0	0.0
	$3x10^{-5}$	18.1±10.3	0.0
	10^{-4}	100.0**	52.3± 0.5**
AA-861	10^{-5}	0.0	
	$3x10^{-5}$	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).

The inhibitory activities of thunberginols A(**1**), B(**2**), and F(**3**), phyllodulcin(**4**), and hydrangenol(**5**) on the Schults-Dale reaction and histamine-induced contraction were summarized in Table I. Among compounds tested, **1**, **2**, and **3** showed more potent inhibitory activity than **4**, **5**, and AA-861[3b] in antigen-induced contraction of tracheal chain isolated from sensitized guinea pig(Schults-Dale model), while they exhibited a little inhibition for histamine-induced contraction. This finding suggests that antiallergic activity of thunberginols concerns with factors other than competition of histamine. On the other hand, **1**, **2**, and **3** showed antimicrobial activities against two oral bacteria[*Bacteroides melaninogenicus*, MIC: **1**(5ppm), **2**(10ppm), and **3**(10ppm); *Fusobacterium nucleatum*, MIC: **1**(10ppm), **2**(10ppm), and **3**(10ppm)].

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Table II. The ^{13}C NMR Data for **1**, **1a**, **2**, **2a**, **3**, **3a**, and **3b** [a)]

	1	1a	2	2a		3	3a	3b
1	165.4	158.4	165.8	157.8	1	164.7	168.6	165.7
3	153.0	152.4	152.8	153.0	3	141.8	142.1[b)]	143.0[b)]
4	100.9	101.1	100.7	101.9	3a	142.5	142.2[b)]	143.2[b)]
4a	138.7	139.6	140.2	140.5	4	110.5	123.7[c)]	111.1[c)]
5	116.8	122.8	103.3	116.1	5	136.7	136.1	136.4
6	137.8	135.7	165.0	155.7	6	115.9	124.8	111.3[c)]
7	114.3	124.0[b)]	101.5	116.8	7	157.1	148.7	158.6
8	160.7	152.1	162.7	153.3	7a	108.1	117.4	107.1
8a	105.4	113.4	98.0	110.8	8	106.8	106.4	107.1
1'	122.5	130.3	122.5	130.0	1'	125.0	131.7	126.3
2'	112.4	120.6	112.3	120.8	2'	116.7	117.4	110.7
3'	145.9	142.5	145.7	142.5	3'	145.5	142.3[b)]	149.0
4'	148.0	143.6	147.8	143.8	4'	146.4	144.3	149.5
5'	116.2	124.1[b)]	116.1	124.1	5'	115.9	128.5	112.7
6'	117.2	123.4	117.1	123.5	6'	122.5	123.3[c)]	123.7

a) **1**, **2**, and **3** were measured in DMSO-d_6, while **1a**, **2a**, **3a**, and **3b** were in $CDCl_3$.

b), c) Assignments may be interchangeable within the same column.

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