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

Masayuki YOSHIKAWA,* Emiko UCHIDA, Nobuyasu CHATANI, Nobutoshi MURAKAMI, and Johji YAMAHARA

Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607, Japan

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; benzyldenephthalide; 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 reaction³⁾ 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-H₂O), C₁₅H₁₀O₅, UV[EtOH, nm(log ε)]: 223(4.0), 347(4.1),

365(4.0); (EtOH+AlCl₃): 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 Ac₂O 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 isocoumarins⁸ 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 ¹³C 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-H₂O), C₁₅H₁₀O₆, UV[EtOH, nm(log ε)]: 235(4.1), 268(4.1), 324(4.3), 350(4.2); (EtOH+AlCl₃): 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 2¹⁰ 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 ¹³C 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), C₁₅H₁₀O₅, 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 ¹³C 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 ¹³C NMR signals of 3 could be analyzed completely by use of ¹H-¹H-COSY, ¹H-¹³C COSY and long range ¹H-¹³C 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 derivatives¹³ 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.

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)].

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 (1)	10 ⁻⁵	50.5± 2.9**	14.6±11.7
	3x10 ⁻⁵	100.0**	24.1± 2.7*
	10 ⁻⁴	100.0**	93.4± 4.6**
Thunberginol B (2)	10 ⁻⁵	49.8± 2.4**	16.5± 5.9
	3x10 ⁻⁵	95.7± 2.0**	13.7± 7.4
	10 ⁻⁴	100.0**	65.2± 6.9**
Thunberginol F (3)	10 ⁻⁵	53.3±18.7	12.1± 4.0
	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).

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.

REFERENCES AND NOTES

- 1a) Y. Asahina and J. Asano, *Ber.*, **62**, 171(1929); b) H. Arakawa, *Bull. Chem. Soc. Jpn.*, **33**, 200(1960); c) K. Nozawa, M. Yamada, Y. Tsuda, K. Kawai, and S. Nakajima, *Chem. Pharm. Bull.*, **29**, 2689(1981).
- 2) M. Yoshikawa, E. Uchida, A. Kawaguchi, I. Kitagawa, and J. Yamahara, *Chem. Pharm. Bull.*, **40**, 2248(1992).
- 3a) 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. Snyder, *J. Pharm. Methods*, **26**, 277(1991).
- 4) M. Yoshikawa, E. Uchida, N. Chatani, H. Kobayashi, Y. Naitoh, Y. Okuno, H. Matsuda, N. Murakami, and J. Yamahara, *Chem. Pharm. Bull.*, in preparation.
- 5a) The chemical and physicochemical properties of **6**, **7**, and **8** were identical with those of dihydroresveratrol,^{b)} isoarborinol,^{c)} and rubiarbonol B,^{d)} which were isolated from the infected tuber of *Dioscorea dumetorum*, the whole plant of *Vandellia sesseliflora*, and the root of *Rubia oncotricha*, respectively; b) S. A. Adesanya, S. K. Ogundana, and M. F. Roberts, *Phytochemistry*, **28**, 773(1989); c) P. L. Majumder and A. Pal, *Indian J. Chem.*, **24B**, 614(1985); d) H. Itokawa, Y. Qiao, and K. Takeya, *Chem. Pharm. Bull.*, **38**, 1435(1990).
- 6) Thunberginol A triacetate(**1a**): white powder, $\text{C}_{21}\text{H}_{16}\text{O}_8$, UV[EtOH, nm(log ϵ): 237(4.4), 299(4.4), 346(4.6), IR(KBr, cm^{-1}): 1773, 1732, 1642, 1505, 1196, ^1H NMR(CDCl_3 , δ): 2.32, 2.33, 2.45(8, 3', 4'-OAc), 6.91(s, 4-H), 7.14(br d, J=8Hz, 7-H), 7.30(d, J=8Hz, 5'-H), 7.37(br d, J=8Hz, 5-H), 7.69(d, J=2Hz, 2'-H), 7.71(dd, J=8, 8Hz, 6-H), 7.73(dd, J=2, 8Hz, 6'-H), EI-MS(m/z , %): 396(M^+ , 11), 354(35), 312(42), 270(100).
- 7) Thunberginol A trimethyl ether(**1b**): white powder, $\text{C}_{18}\text{H}_{16}\text{O}_5$, UV[EtOH, nm(log ϵ): 236(4.5), 319(4.3), 363(4.4), IR(KBr, cm^{-1}): 1719, 1518, 1257, ^1H NMR(CDCl_3 , δ): 3.94, 4.00, 4.02(8, 3', 4'-OMe), 6.75(s, 4-H), 6.91(br d, J=8Hz, 7-H), 6.92(d, J=8Hz, 5'-H), 7.01(br d, J=8Hz, 5-H), 7.36(d, J=2Hz, 2'-H), 7.47(dd, J=2, 8Hz, 6'=H), 7.60(dd, J=8, 8Hz, 6-H), EI-MS(m/z , %): 312(M^+ , 100), 284(37).
- 8) a) R. Charubala, A. Guggisberg, M. Hesse, and H. Schmid, *Helv. Chim. Acta*, **57**, 1096(1974); b) T. R. Govindachari, P. C. Parthasarathy, H. K. Desai, and K. S. Ramachandran, *Indian J. Chem.*, **13**, 537(1975); c) M. Mizuno, S. Yoshida, M. Iinuma, T. Tanaka, F. A. Lang, and K. Goto, *Chem. Pharm. Bull.*, **38**, 2075(1990).
- 9) Thunberginol B tetraacetate(**2a**): white powder, $\text{C}_{23}\text{H}_{18}\text{O}_{10}$, UV[EtOH, nm(log ϵ): 251(4.5), 300(4.5), 342(4.7), IR(KBr, cm^{-1}): 1775, 1736, 1612, 1507, 1202, ^1H NMR(CDCl_3 , δ): 2.32, 2.33, 2.35, 2.44(6, 8, 3', 4'-OAc), 6.86(s, 4-H), 6.96(d, J=2Hz, 7-H), 7.20(d, J=2Hz, 5-H), 7.31(d, J=9Hz, 5'-H), 7.68(d, J=2Hz, 2'-H), 7.72(dd, J=2, 9Hz, 6'-H), EI-MS(m/z , %): 454(M^+ , 10), 412(28), 370(52), 328(100), 286(52).
- 10) The ^1H NMR (DMSO- d_6 , δ) of **2**: δ 6.31(d, J=2Hz, 5-H), 6.48(d, J=2Hz, 7-H), 6.85(d, J=8Hz, 5'-H), 7.11(s, 4-H), 7.22(dd, J=2, 8Hz, 6'-H), 7.27(d, J=2Hz, 2'=H), 10.94(br s, 8-OH).
- 11) Thunberginol F triacetate(**3a**): white powder, $\text{C}_{21}\text{H}_{16}\text{O}_8$, UV[EtOH, nm(log ϵ): 274(4.2), 345(4.5), IR(KBr, cm^{-1}): 1780, 1608, 1506, 1211, ^1H NMR (CDCl_3 , δ): 2.31, 2.34, 2.45(7, 3', 4'-OAc), 6.39(s, 8-H), 7.21(br d, J=8Hz, 6-H), 7.25(d, J=8Hz, 5'-H), 7.61-7.76(4H), EI-MS(m/z , %): 396(M^+ , 9), 354(28), 312(35), 270(100).
- 12) Thunberginol F trimethyl ether(**3b**): pale yellow amorphous powder, $\text{C}_{18}\text{H}_{16}\text{O}_5$, UV[EtOH, nm(log ϵ): 227(4.4), 273(4.7), 357(4.4), IR(KBr, cm^{-1}): 1773, 1601, 1518, 1256, ^1H NMR(CDCl_3 , δ): 3.93, 3.97, 4.03(7, 3', 4'-OMe), 6.34(s, 8-H), 6.89(d, J=9Hz, 5'-H), 6.91(br.d, J=8Hz, 6-H), 7.29(br d, J=8Hz, 4-H), 7.39(dd, J=2, 9Hz, 6'-H), 7.46(d, J=2Hz, 2'-H), 7.63(dd, J=8, 8Hz, 5-H). EI-MS(m/z , %): 312(M^+ , 100), 297(25).
- 13a) G. Blasko and M. Shamma, *Phytochemistry*, **40**, 1971(1984); b) E. Napolitano, G. Spinelli, R. Fiaschi, and A. Marsili, *J. Chem. Soc. Perkin trans. 1*, **1986**, 785.

(Received September 17, 1992)