Development of Bioactive Functions in Hydrangeae Dulcis Folium. VI.[1] Syntheses of Thunberginols A and F and Their 3′-Deoxy-Derivatives Using Regiospecific Lactonization of Stilbene Carboxylic Acid: Structures and Inhibitory Activity on Histamine Release of Hydramacrophyllols A and B

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Lactonization reaction of 2-carboxystilbene mediated by copper(II) chloride proceeded regiospecifically to give the five-membered lactone, while the bromolactonizations using *N*-bromosuccinimide and anodic oxidation were found to furnish the six-membered lactone. Using these regiospecific lactonization reactions as a key step, antiallergic and antimicrobial isocoumarins and the benzylidenephthalides thunberginols A and F and their 3′-deoxyanalogs were synthesized from phyllodulcin and hydrangenol.

Two phthalides called hydramacrophyllols A and B were isolated from Hydrangeae Dulcis Folium and their stereostructures were determined on the basis of physicochemical and chemical evidence, which included the syntheses of hydramacrophyllols A and B from hydrangenol by the application of the lactonization method using copper(II) chloride. In addition, hydramacrophyllols A and B were found to exhibit an inhibitory effect on the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction.

Key words regiospecific lactonization; thunberginol; hydramacrophyllol; benzylidenephthalide synthesis; isocoumarin synthesis; histamine release inhibitor

As a part of our characterization studies on the bioactive constituents of natural medicines,[2] we have so far isolated two isocoumarins [thunberginols A (**1**) and B],[3] three dihydroisocoumarins (thunberginols C, D, and E),[1,4] a benzylidenephthalide [thunberginol F (**2**)],[3] three dihydroisocoumarin glucosides (thunberginol G 3′-*O*-glucoside, (−)-hydrangenol glucoside, and (+)-hydrangenol glucoside),[1,4] and two phthalides [hydramacrophyllols A (**3**) and B (**4**)][5] as antiallergic and antimicrobial principles from Hydrangeae Dulcis Folium together with the known compounds hydrangenol (**5**), phyllodulcin (**6**), and hydrangeaic acid (**7**).[6] Furthermore, on the basis of detailed pharmaceutical assessment, isocoumarin and benzylidenephthalide were found to show much more potent antiallergic activity than the dihydrocoumarin or stilbene carboxylic acid. Particularly, **1** and **2** exhibited more potent inhibitory activity against type I allergy than the commercial antiallergic agents amlexanox, tranilast, and oxatomide in *in vitro* and *in vivo* bioassay and **1** was also expected to provide inhibition against type IV allergy.[7]

In order to obtain an adeqate amount of **1** and **2** to conduct various *in vivo* bioassays of antiallergic activity, we developed an efficient method for transforming dihydroisocoumarin into benzylidenephthalide and isocoumarin using regiospecific lactonization reactions of 2-carboxystilbene.[5] This paper presents a full account of the syntheses of thunberginols A (**1**) and F (**2**), 3′-deoxythunberginols A (**11**) and F (**15**) from phyllodulcin (**6**) and hydrangenol (**5**) by regioselective lactonization of 2-carboxystilbenes (**8**, **18**) using copper(II) chloride ($CuCl_2$), *N*-bromosuccinimide (NBS), and anodic oxidation. We also describe the structure determinations of hydramacrophyllols A (**3**) and B (**4**) including syntheses of **3** and **4** from **5** using copper(II) chloride lactonization method and the inhibitory activity of **3** and **4** on the histamine release on rat peritoneal exudate cells induced by antigen–antibody reaction.

Syntheses of 3′-Deoxythunberginols A (11) and F (15) from Hydrangenol (5) The starting material, hydrangenol (**5**), was isolated from Hydrangeae Dulcis Folium

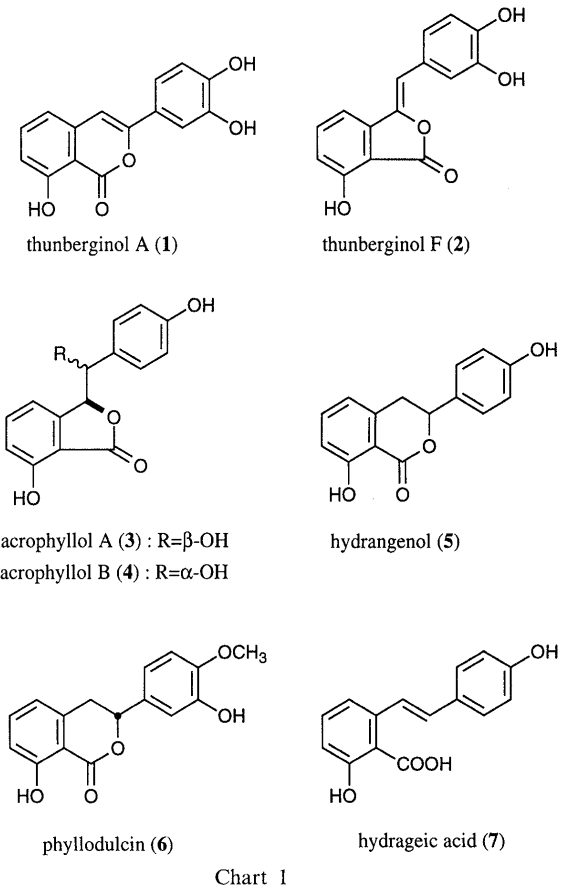

Chart I

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as a major constituent (2.35% yield from the natural medicine).[3] A new isocoumarin, 3′-deoxythunberginol A (**11**), was first synthesized from dihydroisocoumarin **5** using dehydrogenation reaction. Namely, protection of the hydroxyl groups in **5** with *tert*-butyldimethylsilyl (TBDMS) chloride and imidazole gave the 8, 4′-di-TBDMS ether, which was subjected to dehydrogenation reaction with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) followed by deprotection of TBDMS group with tetra-*n*-butylammonium fluoride (*n*-Bu_4NF) to give **11** (89.9% yield from **5**). 3′-Deoxythunberginol A (**11**) was obtained as pale yellow prisms of mp 200—202 °C and the molecular formula $C_{15}H_{10}O_4$ was confirmed by the molecular ion peak in EI-MS and high-resolution MS measurement. From the UV spectrum of **11**, it was presumed to possess a 3-phenylisocoumarin skeleton having a chelated 8-hydroxyl group[3] [absorption maxima (ε) in ethanol at 263 (20000), 310 (24000), and 366 nm (23000)]. The IR spectrum of **11** showed absorption bands due to hydroxyl, chelated δ-lactone, and aromatic ring at 3412, 1676, and 1518 cm^{-1}. The ^{1}H-NMR ($CDCl_3$) spectrum of **11** showed the presence of a conjugated olefin function [δ 6.82 (1H, s, 4-H)] together with disubstituted and trisubstituted benzene rings. Comparison of the spectral data for **11** with those for thunberginols A (**1**) and B[3] led us to confirm the structure of **11**.

Though a few methods have been reported for the construction of a benzylidenephthalide skeleton,[8] they seem to be impractical for synthesizing the large amount when yield is considerared.[9] In order to develop new transformation reaction from dihydroisocoumarin to benzylidenephthalide, we examined several lactonization reactions of 2-carboxystilbene prepared from dihydroisocoumarin. Alkaline treatment of **5** yielded hydrangeaic acid (**7**), which was converted to the 3,4′-dimethyl-2-carboxystilbene (**8**)[10] by methylation with methyl iodide and potassium carbonate followed by alkaline hydrolysis with 10% aqueous potassium hydroxide.

Bromolactonization of **8** with NBS in dimethylformamide (DMF) afforded only **9** as the lactone product in 43.2% yield. By electrochemical bromolactonization of **8** under constant current electrolysis using Pt electrode in the presence of phenylselenide [$(PhSe)_2$] and tetraethylammonium bromide (Et_4NBr),[11] **9** was also obtained in 17.4% yield. The bromolactone (**9**), obtained as colorless needles of mp 150—154 °C, showed a pair of isotope ion peaks at *m/z* 362 and 364 due to the molecular ion (M^+). The high-resolution MS measurement of **9** revealed the molecular formula to be $C_{17}H_{15}BrO_4$ and its IR spectrum showed an absorption band assignable to carbonyl group in a six-membered lactone at 1736 cm^{-1}. The ^{1}H-NMR ($CDCl_3$) spectrum of **9** showed signals due to a methine proton bearing a bromine at δ 5.46 (d, J=4.0 Hz) and another one adjacent to lactone–oxygen at δ 5.77 (d, J=4.0 Hz). In the difference nuclear Overhauser effect (NOE) experiment of **9**, NOE enhancements were observed between the 3-proton and 2′,6′-protons and between the 4-proton and 5-proton. On the basis of the above evidence and examination of the addition mechanism of bromolactonization reaction induced by NBS, the structure of **9** was deduced. Treatment of **9** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) furnished the isocoumarin methyl ether (**10**),[12] which was deprotected by boron tribromide (BBr_3) to give 3′-deoxythunberginol A (**11**).

On the other hand, lactonization of **8** with $CuCl_2$ was found to give desired five-membered lactones, which would be precursors of benzylidenephthalide. Namely, heating under reflux of **8** with $CuCl_2$ in methanol facilitated the lactonization to provide **12** and **13** in 63.8% and 15.3% yields, respectively. The five-membered lactones, **12** and **13**, obtained as colorless needles of mp 180—183 °C and 184—185 °C, respectively, were found to have the same molecular formula $C_{18}H_{18}O_5$ from their positive-mode FAB-MS [*m/z* 315 $(M+H)^+$] and by high-resolution FAB-MS measurement. In the IR spectra of **12** and **13**, a carbonyl absorption band was observed at 1767 cm^{-1} suggesting the presence of five-membered lactone. The ^{1}H-NMR ($CDCl_3$) spectrum of **12** showed signals assignable to a methine proton bearing a methoxyl group [δ 3.32 (3H, s), 4.44 (1H, d, J=5.9 Hz, 8-H)] and a methine proton linking to the lactone–oxygen [δ 5.55 (1H, d, J=5.9 Hz, 3-H)]. In the ^{13}C–^{1}H long-range coupling (COLOC) spectrum, long-range correlations were observed between the 3α-C and 3-H and between the 1′-C and 8-H. Furthermore, the difference NOE experiment of **12** exhibited enhancements between the following protons: 3-H and 4-H, 8-H and 2′, 6′-H. Finally, the relative configuration of **12** was established by X-ray crystallographic analysis.[5,13] The ^{1}H-NMR spectrum of **13** showed signals due to two methine protons bearing a methoxyl group [δ 3.29 (3H, s), 4.41 (1H, d, J=5.3 Hz, 8-H)] and a lactone–oxygen [δ 5.45 (1H, d, J=5.3 Hz, 3-H)], and similarity in the spectral properties of **12** and **13** showed them to be stereoisomers.

The treatment of a mixture of **12** and **13** with *p*-toluenesulfonic acid (*p*-TsOH) in benzene furnished the benzylidenephthalide **14** in 90.0% yield. By demethylation of **14** with BBr_3 in dichloromethane, 3′-deoxythunberginol F (**15**) was obtained as pale yellow needles of mp 193—195 °C in 72.0% yield. The MS and high-resolution MS measurement of **15** revealed its molecular formula to be $C_{15}H_{10}O_5$, and the UV spectrum suggested the benzylidenephthalide skeleton[3] at 231 (ε 13000), 306 (ε 8700), 319 (ε 9600), and 371 nm (ε 18000). The IR spectrum of **15** showed absorption bands due to hydroxyl, chelated γ-lactone, and aromatic ring moieties at 3400, 3295, 1753, and 1605 cm^{-1}, while the ^{1}H-NMR (CD_3OD) spectrum showed the presence of a conjugated olefin [δ 6.52 (1H, s, 8-H)]. Finally, comparison of the physicochemical data for **15** with those for thunberginol F (**2**) led us to identify the structure of **15**.

The five-membered lactones, **12** and **13**, were found to be similarly prepared from **8** in 46.9% and 11.2% yields, respectively, in the lactonization reaction with copper(II) bromide ($CuBr_2$) in the place of $CuCl_2$, while no reaction was observed when copper(I) chloride (CuCl) was used. It therefore seems that the lactonization involves an oxidation process. Although the precise mechanism of the five-membered lactonization reaction using $CuCl_2$ is not yet understood, we have deduced a plausible reaction route as shown in Chart 3. Thus, after chelation of copper(II)

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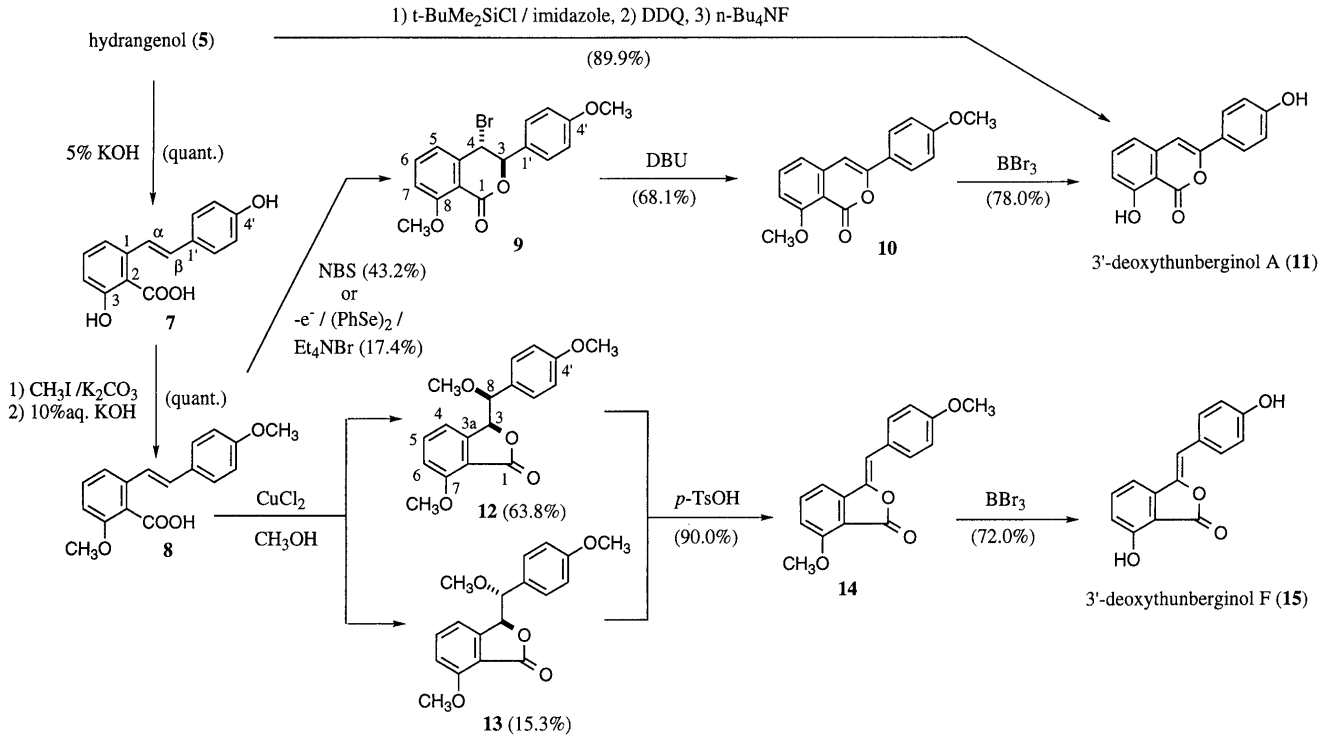

Chart 2

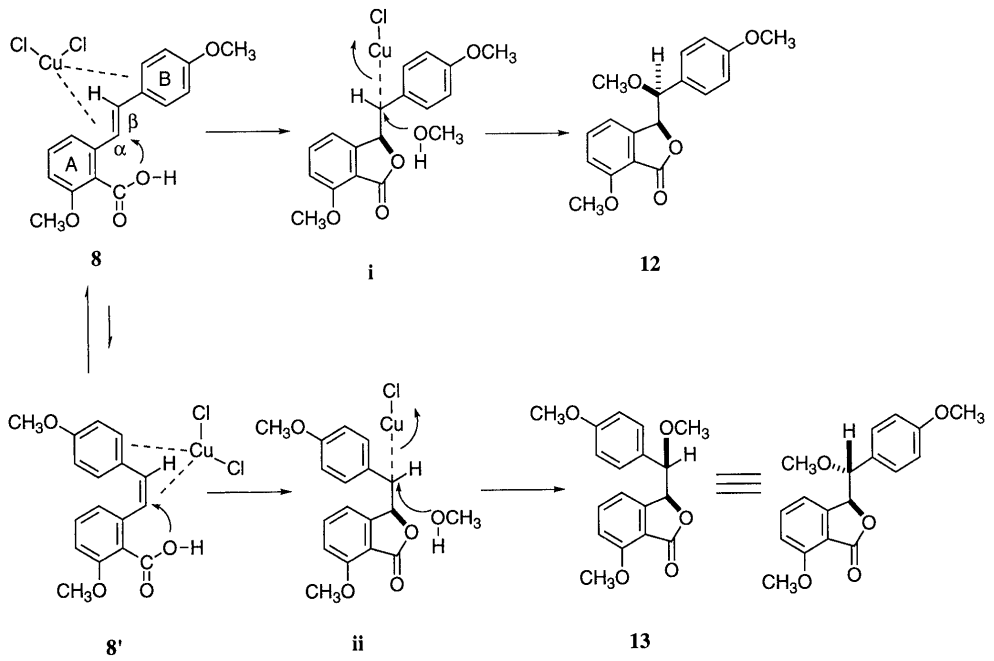

Chart 3

between the olefin and the B-benzene ring in 2-carboxystilbene (**8**), nucleophilic attack by a carboxyl group initiated on the α-carbon and the five-membered lactone intermediate (i) would be generated regiospecifically in the lactonization. Nucleophilic substitution of methanol to i constituent with reductive elimination in *SN*2 mode of copper would yield the 3,8-*syn*-methoxylactone (**12**). The 3,8-*anti*-methoxylactone (**13**) was presumed to be similarly formed *via* the reaction intermediate (ii) from (*Z*)-2-carboxystilbene (**8'**), which was readily given by isomerization of **8** in the reaction procedure.[14]

Syntheses of Thunberginols A (1) and F (2) from Phyllodulcin (6) As an extension of this synthetic study, we carried out the syntheses of thunberginols A (**1**) and F (**2**), which were isolated from Hydrangeae Dulcis Folium in poor yields (0.0086% and 0.0028%, respectively), from a major principle of this natural medicine, phyllodulcin (**6**, 1.99% yield). Thunberginol A (**1**) was synthesized from **6** in 86.0% yield by the following procedures: 1) demethylation of **6** with BBr_3, 2) *tert*-butyldimethylsilylation of the hydroxyl groups, 3) dehydrogenation with DDQ, and 4) deprotection with *n*-Bu_4NF. To synthesize thunberginol F (**2**), the lactonization methods of the stilbene carboxylic acid from phyllodulcin (**6**) were

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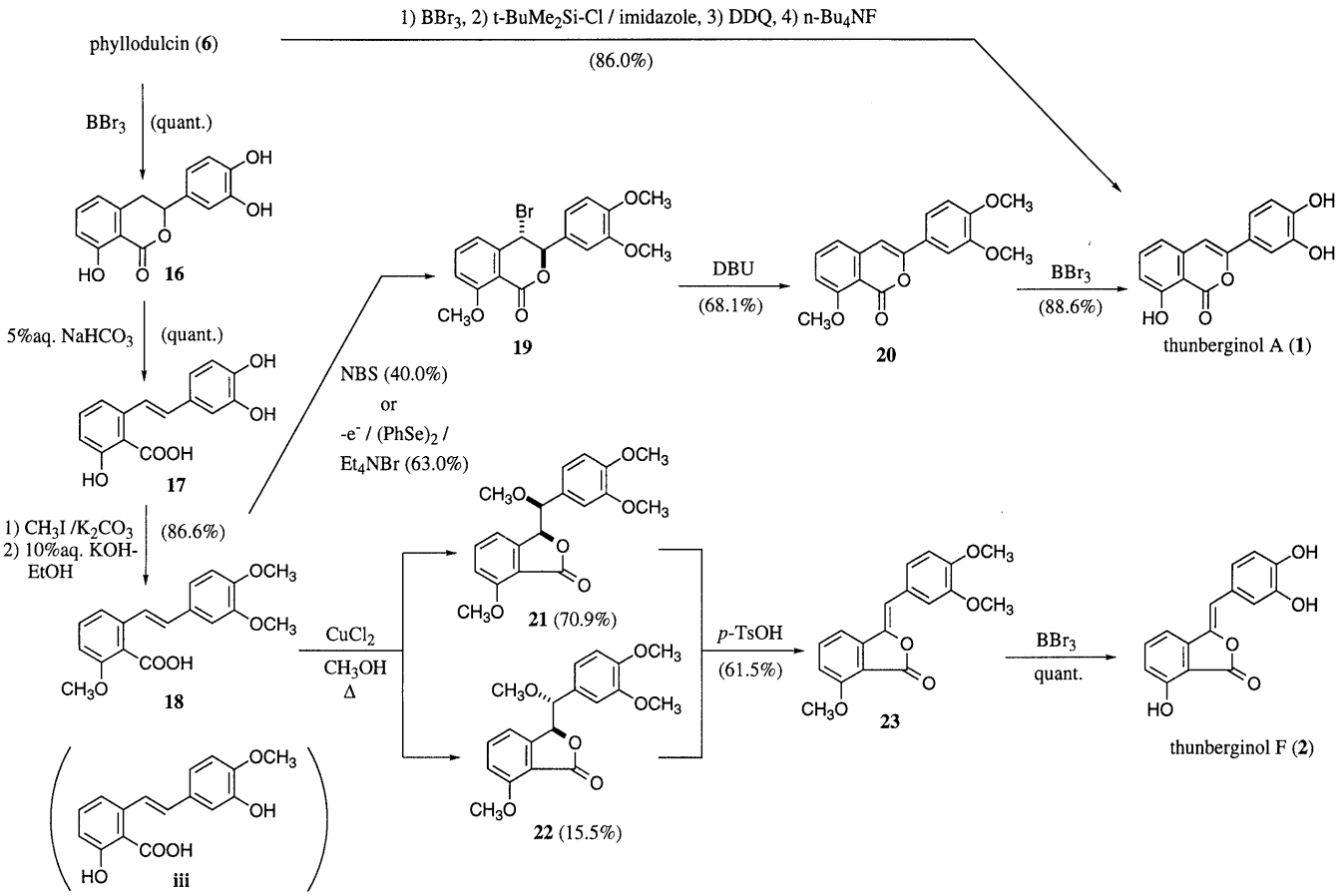

Chart 4

examined. We first attempted an application of the lactonization reaction for the stilbene derivative (iii). However, in spite of the treatment of **6** under various alkaline conditions, iii could not obtained. In contrast, thunberginol G (**16**), which was derived from **6** by BBr_3 treatment but changed to the racemate,[1,4] was found to readily afford the stilbene derivative (**17**) by weak alkaline treatment with 5% aqueous sodium bicarbonate ($NaHCO_3$). Methylation of **17** with CH_3I/K_2CO_3 followed by alkaline hydrolysis furnished 8,3′,4′-trimethylstilbene 2-carboxylic acid (**18**). Bromolactonization of **18** with NBS or anodic oxidation furnished the six-membered lactone (**19**) in 40.0% and 63.0% yields, respectively. The structure of **19** was deduced by comparison of the spectral data for **19** with those for **9**. Trimethylthunberginol A (**20**) was synthesized from **19** by DBU dehydrogenation reaction in 68.1% yield, and then **20** was demethylated with BBr_3 to give thunberginol A (**1**) in 88.6% yield.

Treatment of **18** with $CuCl_2$ and methanol under reflux provided the five-membered lactones, **21** and **22**, in 70.9% and 15.5% yield, respectively. The structures of **21** and **22** were characterized on the basis of chemical and physicochemical evidence[11] including the comparison of their spectral data with those of **12** and **13**. The lactonization reaction of **18** with $CuBr_2$ furnished **21** and **22** in 60.5% and 12.8% yield, respectively. A mixture of **21** and **22** was derived to **23** by the *p*-TsOH treatment in 61.5% yield and finally, demethylation of **23** quantitatively furnished thunberginol F (**2**).

Structures and Syntheses of Hydramacrophyllols A (3) and B (4) We earlier[3] reported the isolation of two phthalides called hydramacrophyllols A (**3**) and B (**4**) from the ethyl acetate-soluble fraction of Hydrangeae Dulcis Folium. Hydramacrophyllol A (**3**) was obtained as a white powder and showed slight optical activity of $[\alpha]_D^{25}$ $-5.9°$ (EtOH). In the negative-mode FAB-MS of **3**, a quasimolecular ion peak was observed at *m/z* 271 $(M-H)^-$ and high-resolution MS analysis revealed the molecular formula of **3** to be $C_{15}H_{12}O_5$. The IR spectrum of **3** showed absorption bands due to hydroxyl, chelated γ-lactone, and aromatic ring at 3440, 1742, and 1617 cm^{-1}, while its UV spectrum showed absorption maxima at 225 (ε 17000) and 301 nm (ε 4800). The ^{1}H-NMR (acetone-d_6) of **3** showed signals assignable to a di-substituted benzene ring [δ 6.77 (2H, d, J=8.6 Hz, 3′, 5′-H), 7.20 (2H, d, J=8.6 Hz, 2′, 6′-H)], a trisubstituted benzene ring [δ 6.71 (1H, d, J=7.3 Hz, 4-H), 6.87 (1H, d, J=7.6 Hz, 6-H), 7.47 (1H, dd, J=7.3, 7.6 Hz, 5-H)] and two methines bearing a hydroxyl group [δ 5.00 (1H, d, J=4.8 Hz, 8-H)] and a lactone-oxygen function [δ 5.65 (1H, d, J=4.8 Hz, 3-H)]. In the difference NOE experiment of **3**, NOE enhancements were observed between the 3-proton and 4-proton and between the 8-proton and 2′, 6′-protons. Finally, methylation of **3** with CH_3I and sodium hydride (NaH) provided **12** in 82.6% yield, which was found to be identical with spectral data except for the optical rotation. On the basis of these findings, the structure of **3** was determined.

Hydramacrophyllol B (**4**), obtained in the form of a white powder, showed no optical activity. The proton

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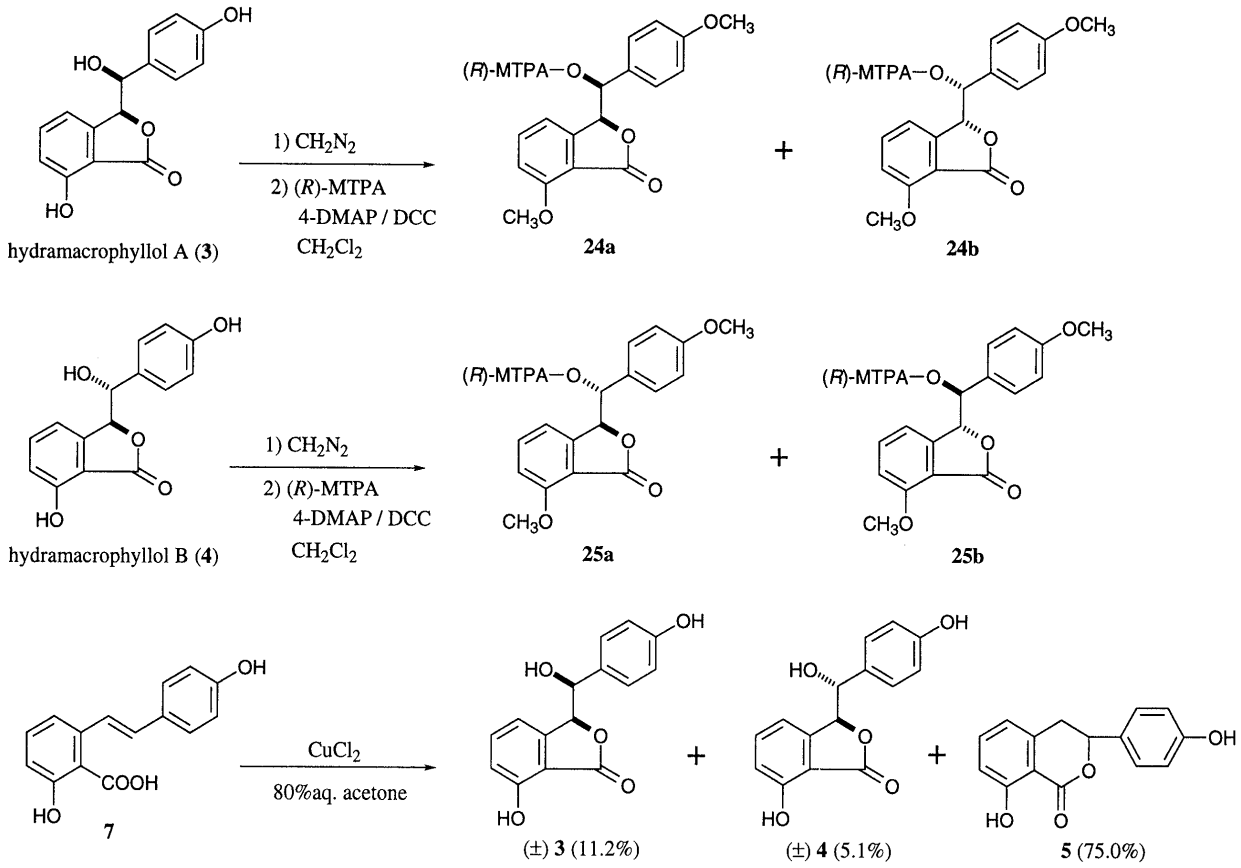

Chart 5

Table 1. Effect of Hydramacropyllols A (3) and B (4) on the Histamine Release from Rat Peritoneal Exudate Cells Induced by Antigen–Antibody Reaction

	Conc. (M)	n	Inhibition of histamine release (%)
Hydramacrophyllol A (3)	10^{-6}	4	4.5±4.2
	10^{-5}	4	15.8±5.2
	10^{-4}	4	42.2±2.9
Hydramacrophyllol B (4)	10^{-6}	4	5.0±4.4
	10^{-5}	4	10.5±1.7
	10^{-4}	4	46.8±1.3
Amlexanox	10^{-6}	4	−4.2±1.6
	10^{-5}	4	11.8±7.4
	10^{-4}	4	61.2±3.1
Tranilast	10^{-5}	4	0.7±2.1
	10^{-4}	4	8.7±3.1

Each value represents the mean with S.E. Peritoneal exudate cells were incubated with test samples for 15 min.

signals in the ^{1}H-NMR spectrum of **4** were fairly similar to those of **3** except for the coupling constant between the two oxymethine protons. The methylation of **4** with CH_3I/NaH furnished **13** in 80.2% yield, so that the structure of hydramacrophyllol B was determined to be **3**.

The optical purity of **3** and **4** was determined by HPLC analysis of the corresponding (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetate [(*R*)-MTPA ester, **24**, **25**], which was prepared by methylation with diazomethane (CH_2N_2) followed by esterification with (*R*)-α-methoxy-α-trifluorophenylacetic acid in the presence of dicyclohexylcarbodiimide (DCC) and 4-methoxyaminopyridine (DMAP). The HPLC chromatogram of **24** showed two peaks in a ratio of 68 : 32, while two peaks with nearly equal area were observed in the chromatogram of **25**.

Finally, racemic **3** and **4** were directly synthesized from hydrangeaic acid (**7**). Namely, treatment of **7** with $CuCl_2$ in 80% aqueous acetone gave **3** and **4** in 11.2% and 5.1% yield, respectively, together with hydrangenol (**5**, 75.0%), which was easily derived from **15** by preferential acidic lactonization under this reaction condition.

Inhibitory Effect of Hydramacrophyllols A (3) and B (4) on the Histamine Release As a part of our characterization studies on the antiallergic components of Hydrangeae Dulcis Folium, the inhibitory effect of hydramacrophyllols A (**3**) and B (**4**) was examined. As shown in Table 1, **3** and **4** were found to inhibit the histamine release from rat peritoneal exudate cells induced by antigen–antibody reaction in a concentration dependent manner (10^{-6}—10^{-4} M). Their inhibitory activities seemed to correspond to that of the commercial antiallergic agent amlexanox, while another antiallergic agent, tranilast, was found to lack the activity.

Experimental

The instruments used for obtaining physical data and experimental conditions for chromatography were the same as described previously.[1]

Conversion from Hydrangenol (5) to 3′-Deoxythunberginol A (11) A solution of **5** (100 mg, 0.39 mmol) in DMF (2.0 ml) was treated with TBDMS chloride (236 mg, 1.5 mmol) and imidazole (160 mg, 2.4 mmol), and the whole mixture was stirred at room temperature (25 °C) for 4 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with 5% aqueous HCl and saturated brine, and then dried over $MgSO_4$ powder. After removal of the desiccant by filtration, the filtrate was evaporated under reduced pressure to give a residue, which was purified by silica gel

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column chromatography [24 g, *n*-hexane–AcOEt (10 : 1)] to yield the di-TBDMS derivative (240 mg). A solution of the di-TBDMS derivative (220 mg, 0.46 mmol) in dry benzene (5 ml) was treated with DDQ (2.1 mg, 0.01 mmol) and the whole solution was heated under reflux for 5 h. After cooling, the reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $NaHCO_3$ and saturated brine, then dried over $MgSO_4$ powder and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [13 g, *n*-hexane–AcOEt (50 : 1)] to afford the dehydrogenation product (195.2 mg, 89.9%). A solution of the dehydrogenation product (21 mg, 0.04 mmol) in *n*-Bu_4NF–THF (1.0 M solution, 0.26 ml) was stirred at room temperature (25 °C) for 5 min. After removal of the solvent from the reaction solution under reduced pressure, the residue was chromatographed on a silica gel column [1.0 g, $CHCl_3$–AcOEt (15 : 1)] to provide **11** (11 mg, quant.).

3′-Deoxythunberginol A (**11**): Pale yellow prisms from EtOH–AcOEt, mp 200—202 °C. High-resolution EI-MS Calcd for $C_{15}H_{10}O_4$ (M^+): 254.0578. Found: 254.0561. UV [EtOH, nm (ε)]: 263 (20000), 310 (24000), 366 (23000). IR (KBr) cm^{-1}: 3412, 1676, 1518. ^{1}H-NMR (CD_3OD) δ: 6.82 (1H, s, 4-H), 6.91 (2H, d, J=8.9 Hz, 3′, 5′-H), 6.92 (1H, d, J=8.3 Hz, 7-H), 6.94 (1H, d, J=7.9 Hz, 5-H), 7.59 (1H, dd, J=7.9, 8.3 Hz, 6-H), 7.71 (2H, d, J=8.9 Hz, 2′, 6′-H). EI-MS *m/z* (%): 254 (M^+, 100).

Alkaline Treatment of Hydrangenol (5) Giving Hydrangeaic Acid (7) A solution of **5** (2.0 g, 7.8 mmol) in MeOH (26 ml) was treated with 5% aqueous KOH (26 ml) and the whole mixture was heated under reflux for 1.5 h. After cooling, the reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Evaporation of the solvent from the filtrate under reduced pressure furnished **7** (2.0 g, quant.), which was identified with an authentic sample[3] by TLC, IR, ^{1}H-NMR, and ^{13}C-NMR spectra comparisons.

Methylation of 7 Followed by Alkaline Hydrolysis Giving 8 A solution of **7** (1.9 g, 7.3 mmol) in DMF (15 ml) was treated with CH_3I (9.2 ml, 150 mmol) and K_2CO_3 powder (5.0 g, 36 mmol), and the whole mixture was stirred at room temperature (25 °C) for 4 h in the dark. The reaction mixture was poured into saturated brine and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $Na_2S_2O_3$ and brine, then dried over $MgSO_4$ powder and filtered. Evaporation of the filtrate under reduced pressure yielded a residue, which was purified by silica gel column chromatography [100 g, *n*-hexane–AcOEt (3 : 1)] to give the trimethyl derivative (2.2 g, quant.). The trimethyl derivative (2.2 g, 7.3 mmol) was dissolved in 10% KOH–EtOH (20 ml) and the solution was heated under reflux for 16 h. After cooling, the reaction solution was neutralized with Amberlite IRC-76 (H^+ form) and then filtered to remove the resin. Removal of the solvent from the filtrate under reduced pressure provided the methylated stilbene carboxylic acid (**8**, 2.1 g, quant.), which was identified by comparison of the physical data with reported values.[10]

Bromolactonization of 8 with NBS Giving 9 A solution of **8** (11 mg, 0.04 mmol) in DMF (0.5 ml) was treated with NBS (14 mg, 0.08 mmol) and the whole mixture was stirred at room temperature (25 °C) for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with aqueous saturated $Na_2S_2O_3$ and brine, then dried over $MgSO_4$ and filtered. Removal of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (1 : 1)] to give the six-membered bromolactone (**9**, 6.1 mg, 43.2%).

9: Colorless needles from EtOH–isopropyl ether, mp 150—154 °C. High-resolution EI-MS Calcd for $C_{17}H_{15}{}^{79}BrO_4$ (M^+): 362.0154. Found: 362.0158. UV [EtOH, nm (ε)]: 315 (7600). IR (KBr) cm^{-1}: 1736, 1597, 1076. ^{1}H-NMR ($CDCl_3$) δ: 3.76, 3.97 (3H each, both s, OCH_3 × 2), 5.46 (1H, d, J=4.0 Hz, 4-H), 5.77 (1H, d, J=4.0 Hz, 3-H), 6.82 (2H, d, J=8.9 Hz, 3′, 5′-H), 7.00 (1H, d, J=8.6 Hz, 5-H), 7.01 (1H, d, J=7.6 Hz, 7-H), 7.17 (2H, d, J=8.9 Hz, 2′, 6′-H), 7.50 (1H, dd, J=7.6, 8.6 Hz, 6-H). EI-MS *m/z* (%): 364 (M^+, 3.6), 362 (M^+, 3.6), 226 (100).

Bromolactonization of 8 by Anodic Oxidation Giving 9 A solution of **8** (30 mg, 0.07 mmol) in CH_3CN–H_2O (3 : 1, 13 ml) containing Et_4NBr (75 mg, 0.36 mmol) and $(PhSe)_2$ (29 mg, 0.09 mmol) was subjected to constant current electrolysis for 1 h (Pt electrode, 6.5 mA/cm^2, 0 °C). The reaction solution was poured into ice-water and the whole mixture was extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by preparative TLC [SiO_2, *n*-hexane–AcOEt (1 : 3)] to give **9** (5.0 mg, 17.4%), which was identified with an authentic sample as described above by TLC, IR, and ^{1}H-NMR spectra comparisons.

Treatment of 9 with DBU Giving 10 A solution of **9** (3.4 mg, 0.01 mmol) in dry benzene (1.0 ml) was treated with DBU (15 ml, 0.10 mmol) and the whole solution was heated under reflux for 2 h. After cooling, the reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine and then dried over $MgSO_4$. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (1 : 1)] to give **10** (1.8 mg, 68.1%), which was identified by comparison of the physical data with reported values.[12]

Demethylation of 10 with BBr_3 Giving 3′-Deoxythunberginol A (11) A solution of **10** (20.0 mg, 0.006 mmol) in BBr_3–CH_2Cl_2 (1.0 M solution, 0.40 mmol) was stirred at room temperature (25 °C) for 30 min. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and dried over $MgSO_4$. After removal of the solvent from the filtrate under reduced pressure, the residue was purified by silica gel column chromatography [20 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower phase)] to afford **11** (14 mg, 78.0%), which was identified with an authentic sample obtained from **5** by TLC, IR, and ^{1}H-NMR spectra comparisons.

Lactonization of 8 with $CuCl_2$ Giving the Five-Membered Lactones (12, 13) A solution of **8** (1.0 g, 3.2 mmol) in MeOH (10 ml) was treated with $CuCl_2$ (2.1 g, 16 mmol) and the whole mixture was heated under reflux for 2 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was washed with brine and then dried over $MgSO_4$. Removal of the solvent from the filtrate under reduced pressure furnished a mixture of **12** and **13** (0.92 g, 83.3%). This mixture (100 mg) was subjected to HPLC [column: Develosil 100-5 (10 i.d. × 250 mm), mobile phase; CH_2Cl_2–AcOEt (10 : 1), flow rate: 4.0 ml/min, detection: UV 254 nm] separation to give **12** (77 mg, 63.8%) and **13** (18.3 mg, 15.3%).

12: Colorless needles from EtOH, mp 180—183 °C. High-resolution positive-mode FAB-MS Calcd for $C_{18}H_{19}O_5$ $(M+H)^+$: 315.1233. Found: 315.1204. UV [EtOH, nm (ε)]: 228 (18000), 283 (3700), 299 (5400). IR (KBr) cm^{-1}: 1767, 1611, 1034. ^{1}H-NMR ($CDCl_3$) δ: 3.32, 3.79, 3.92 (3H each, all s, OCH_3 × 3), 4.44 (1H, d, J=5.9 Hz, 8-H), 5.55 (1H, d, J=5.9 Hz, 3-H), 6.57 (1H, d, J=7.6 Hz, 6-H), 6.78 (2H, d, J=8.9 Hz, 3′, 5′-H), 6.83 (1H, d, J=8.3 Hz, 4-H), 7.08 (2H, d, J=8.9 Hz, 2′, 6′-H), 7.44 (1H, dd, J=7.6, 8.3 Hz, 5-H). Positive-mode FAB-MS *m/z*: 315 $(M+H)^+$.

13: Colorless needles from EtOH, mp 184—185 °C. High-resolution positive-mode FAB-MS Calcd for $C_{18}H_{19}O_5$ $(M+H)^+$: 315.1233. Found: 315.1210. UV [EtOH, nm (ε)]: 229 (18000), 285 (3700), 300 (5400). IR (KBr) cm^{-1}: 1767, 1611, 1034. ^{1}H-NMR ($CDCl_3$) δ: 3.29, 3.81, 3.95 (3H each, all s, OCH_3 × 3), 4.41 (1H, d, J=5.3 Hz, 8-H), 5.45 (1H, d, J=5.3 Hz, 3-H), 6.87 (1H, d, J=8.1 Hz, 6-H), 6.89 (2H, d, J=8.9 Hz, 3′, 5′-H), 6.90 (1H, d, J=8.1 Hz, 4-H), 7.23 (2H, d, J=8.9 Hz, 2′, 6′-H), 7.53 (1H, dd, J=8.1, 8.1 Hz, 5-H). Positive-mode FAB-MS m/z: 315 $(M+H)^+$.

Treatment of 12 and 13 with *p*-TsOH Giving 14 A solution of the five-membered lactone mixture (**12** and **13**, 500 mg) in dry benzene (3.0 ml) was treated with *p*-TsOH (5.0 mg) and the whole solution was heated under reflux for 6 h. After cooling, the reaction solution was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner yielded a product, which was purified by silica gel column chromatography [30 g, *n*-hexane–AcOEt–CH_2Cl_2 (2 : 1 : 1)] to give **14** (400 mg, 90.0%).

14: Pale yellow needles from isopropyl ether–acetone, mp 188—190 °C. High-resolution EI-MS Calcd for $C_{17}H_{14}O_4$ (M^+): 282.0890. Found: 282.0863. UV [EtOH, nm (ε)]: 231 (17000), 304 (9100), 318 (9400), 368 (21000). IR (KBr) cm^{-1}: 1771, 1603. ^{1}H-NMR ($CDCl_3$) δ: 3.85, 4.02 (3H each, both s, OCH_3 × 2), 6.35 (1H, s, 8-H), 6.91 (1H, d, J=7.9 Hz, 6-H), 6.93 (2H, d, J=8.9 Hz, 3′, 5′-H), 7.29 (1H, d, J=7.9 Hz, 4-H), 7.63 (1H, dd, J=7.9, 7.9 Hz, 5-H), 7.80 (2H, d, J=8.9 Hz, 2′, 6′-H). EI-MS *m/z* (%): 282 (M^+, 100).

Demethylation of 14 with BBr_3 Giving 3′-Deoxythunberginol F (15) A solution of **14** (200 mg, 0.70 mmol) in $(CH_2)_2Cl_2$ (5.0 ml) was treated with BBr_3–CH_2Cl_2 solution (1.0 M solution, 7.0 ml) and the whole solution was heated under reflux for 7 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. Work-up

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of the AcOEt extract as described above furnished a product, which was separated by silica gel column chromatography [10 g, $CHCl_3$–MeOH (10 : 1)] to give **15** (130 mg, 72.0%).

3-Deoxythunberginol F (**15**): Pale yellow needles from EtOH–AcOEt, mp 193—195 °C. High-resolution EI-MS Calcd for $C_{15}H_{10}O_4$ (M^+): 254.0578. Found: 254.0574. UV [EtOH, nm (ε)]: 231 (13000), 306 (8700), 319 (9600), 3700 (18000). IR (KBr) cm^{-1}: 3400, 3295, 1753, 1605. ^{1}H-NMR (CD_3OD) δ: 6.52 (1H, s, 8-H), 6.82 (2H, d, J=8.9 Hz, 3′, 5′-H), 6.86 (1H, d, J=8.2 Hz, 6-H), 7.32 (1H, d, J=7.6 Hz, 4-H), 7.56 (1H, dd, J=7.6, 8.2 Hz, 5-H), 7.69 (2H, d, J=8.9 Hz, 2′, 6′-H). EI-MS m/z (%): 254 (M^+, 100).

Lactonization of 8 with $CuBr_2$ Giving 12 and 13 A solution of **8** (30 mg, 0.11 mmol) in MeOH (3 ml) was treated with $CuBr_2$ (117 mg, 0.52 mmol) and the whole mixture was heated under reflux for 4 h. The reaction mixture was poured into ice-water and the whole was extracted with CH_2Cl_2. Work-up of the CH_2Cl_2 extract in the usual manner furnished a mixture of **12** and **13** (27.8 mg, 83.8%) which was subjected to HPLC (the same condition as described above) separation to yield **12** (13.0 mg, 46.9%) and **13** (3.1 mg, 11.2%). These were identified with authentic samples by TLC, HPLC, and ^{1}H-NMR spectrum comparisons.

Conversion from Phyllodulcin (6) to Thunberginol A (1) A solution of **6** (3.5 g, 12.2 mmol) in BBr_3–CH_2Cl_2 (1.0 M solution, 61 ml) was stirred at −15 °C for 2 h. The reaction solution was poured into ice-water and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed successively with aqueous saturated $Na_2S_2O_3$ and saturated brine, then dried with $MgSO_4$ powder. After removal of the desiccant by filtration, the filtrate was evaporated under reduced pressure to yield thunberginol G (**16**, 3.4 g, quant.), which was identified with an authentic sample[1] by TLC, IR, and ^{1}H-NMR spectra comparisons. A solution of **16** (1.2 g, 4.8 mmol) in DMF (5.0 ml) was treated with TBDMS chloride (5.4 g, 36 mmol) and imidazole (2.9 g, 43 mmol) and the whole solution was stirred at 40 °C for 1 h. The reaction solution was poured into ice-water and the whole solution was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the residue was purified by silica gel column chromatography [100 g, n-hexane–AcOEt (15 : 1)] to give the tri-TBDMS derivative (2.3 g, 84.8%): ^{1}H-NMR ($CDCl_3$) δ: 0.20 (18H, s, *Si*-CH_3 × 6), 1.00 (27H, s, *Si-tert*-butyl × 3), 3.00 (1H, d, J=16.1 Hz), 3.20 (1H, dd, J=9.6, 16.1 Hz) (4-H_2), 5.29 (1H, d, J=9.6 Hz, 3-H), 7.35 (1H, t-like, 6-H). A solution of the tri-TBDMS derivative (2.3 g, 3.7 mmol) in benzene (20 ml) was treated with DDQ (8.4 g, 37 mmol) and the whole mixture was heated under reflux for 10 h. After cooling, the reaction solution was poured into ice-water and the whole was extracted with AcOEt. The AcOEt extract was worked-up as described above for the dehydrogenation of **5** to yield a product, which was separated by silica gel column chromatography [120 g, n-hexane–AcOEt (30 : 1)] to give the dehydrogenation product (1.8 g, 86.0%): ^{1}H-NMR ($CDCl_3$) δ: 0.23 (18H, s, *Si*-CH_3 × 6), 1.08 (27H, s, *Si-tert*-butyl × 3), 6.63 (1H, s, 4-H), 6.83 (1H, d, J=7.3 Hz, 5-H), 6.86 (1H, d, J=8.6 Hz, 5′-H), 7.00 (1H, d, J=7.9 Hz, 7-H), 7.29 (1H, d, J=2.3 Hz, 2′-H), 7.35 (1H, dd, J=2.3, 8.6 Hz, 6′-H), 7.49 (1H, dd, J=7.3, 7.9 Hz, 6-H). A solution of the dehydrogenation product (1.6 g, 2.7 mmol) in n-Bu_4NF–THF (1.0 M solution, 14 ml) was stirred at room temperature (25 °C) for 5 min. After removal of the solvent from the reaction solution under reduced pressure, the residue was purified by silica gel column chromatography [50 g, benzene–AcOEt (7 : 1)] to give **1** (700.5 mg, quant.), which was identified with an authentic sample[3] by TLC, IR, and ^{1}H-NMR spectra comparisons.

Demethylation of Phyllodulcin (6) Followed by Alkaline Hydrolysis Giving 17 A solution of **16** (1.6 g, 5.8 mmol), which was obtained from **6** as described above, in MeOH (40 ml) was treated with 5% aqueous $NaHCO_3$ (40 ml) and the whole solution was stirred at room temperature (25 °C) for 1 h. The reaction mixture was neutralized with Dowex HCR-W2 (H^+ form) and the resin was removed by filtration. Removal of the solvent from the filtrate under reduced pressure afforded **17** (1.6 g, quant.): a yellow powder, UV [EtOH, nm (ε)]: 339 (15000). IR (KBr) cm^{-1}: 3389, 1644, 1595. ^{1}H-NMR (CD_3OD) δ: 6.73 (1H, d, J=8.2 Hz, 5′-H), 6.80 (1H, d, J=8.6 Hz, 6-H), 6.81 (1H, d, J=16.2 Hz, α-H), 6.83 (1H, dd, J=2.0, 8.2 Hz, 6′-H), 6.99 (1H, d, J=2.0 Hz, 2′-H), 7.13 (1H, d, J=7.3 Hz, 4-H), 7.32 (1H, dd, J=7.3, 8.6 Hz, 5-H), 7.51 (1H, d, J=16.2 Hz, β-H). EI-MS m/z (%): 236 ($M^+ - 2H_2O$, 3.2), 110 (100).

Methylation of 17 Followed by Alkaline Hydrolysis Giving 18 A solution of **17** (19 mg, 0.07 mmol) in DMF (1.0 ml) was treated with CH_3I (0.26 ml, 4.3 mmol) and K_2CO_3 (390 mg, 2.8 mmol) and the whole mixture was stirred at room temperature (25 °C) for 8 h. The reaction mixture was poured into saturated brine and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the residue was purified by silica gel column chromatography [3.0 g, n-hexane–AcOEt (2 : 1)] to yield the tetramethyl derivative (19 mg, 86.6%). The tetramethyl derivative (470 mg, 1.4 mmol) was dissolved in 10% KOH–EtOH solution (8.5 ml) and the solution was heated under reflux for 3.5 h. After cooling, the reaction solution was neutralized by Amberlite IRC-76 (H^+ form) and filtered. Removal of the solvent from the filtrate under reduced pressure furnished the methylated stilbene carboxylic acid (**18**, 450 mg, quant.)

18: A white powder. UV [EtOH, nm (ε)]: 302 (18000), 321 (22000). IR (KBr) cm^{-1}: 3415, 1719, 1583. ^{1}H-NMR (DMSO-d_6) δ: 3.66, 3.76, 3.80 (3H each, all s, OCH_3 × 3), 6.71 (1H, d, J=7.9 Hz, 6-H), 6.98 (1H, d, J=14.2 Hz, α-H), 6.98 (1H, dd, J=2.3, 7.6 Hz, 6′-H), 7.04 (1H, d, J=2.3 Hz, 2′-H), 7.03—7.07 (2H, m, 5′, 4-H), 7.06 (1H, d, J=14.2 Hz, β-H), 7.16 (1H, dd, J=7.3, 7.9 Hz, 5-H). Positive-mode FAB-MS m/z: 315 $(M+H)^+$.

Bromolactonization of 18 with NBS Giving 19 A solution of **18** (140 mg, 0.45 mmol) in DMF (3.0 ml) was treated with NBS (160 mg, 0.90 mmol) and the whole mixture was stirred at room temperature (25 °C) for 40 min. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a residue, which was purified by silica gel column chromatography [10 g, n-hexane–AcOEt (1 : 1)] to yield the bromolactone derivative (**19**, 69 mg, 40.0%).

19: Colorless needles from EtOH–isopropyl ether, mp 145—148 °C, $[\alpha]_D^{25}$ ±0° (EtOH). High-resolution EI-MS Calcd for $C_{18}H_{17}{}^{79}BrO_5$ (M^+): 392.0259; Found: 392.0258. UV [EtOH, nm (ε)]: 285 (4200), 309 (4800). IR (KBr) cm^{-1}: 1753, 1597. ^{1}H-NMR ($CDCl_3$) δ: 3.81, 3.82, 3.96 (3H each, all s, OCH_3 × 3), 5.49 (1H, d, J=4.0 Hz, 4-H), 5.76 (1H, d, J=4.0 Hz, 3-H), 6.76 (3H, br s, 2′, 5′, 6′-H), 7.00 (1H, d, J=8.6 Hz, 5-H), 7.01 (1H, d, J=7.6 Hz, 7-H), 7.50 (1H, dd, J=7.6, 8.6 Hz, 5-H). EI-MS m/z (%): 394 (M^+, 1.1), 392 (M^+, 1.1), 193 (100).

Bromolactonization of 18 by Anodic Oxidation Giving 19 A solution of **18** (23 mg, 0.09 mmol) in CH_3CN–H_2O (3 : 1, 13 ml) containing Et_4NBr (100 mg, 0.48 mmol) and $(PhSe)_2$ (38 mg, 0.12 mmol) was subjected to constant current electrolysis for 1 h (Pt electrode, 6.3 mA/cm^2, 0 °C). The reaction solution was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the residue was separated by silica gel column chromatography [3.0 g, n-hexane–AcOEt (1 : 1)] to give **19** (18 mg, 63.0%), which was identified with an authentic sample obtained above by TLC, IR, and ^{1}H-NMR spectra comparisons.

Treatment of 19 with DBU Followed by Demethylation Giving Thunberginol A (1) A solution of **19** (5.0 mg, 0.01 mmol) in dry benzene (0.5 ml) was treated with DBU (19 ml, 0.13 mmol) and the whole mixture was heated under reflux for 2 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. After work-up of the AcOEt extract in the usual manner, the residue was purified by silica gel column chromatography [1.0 g, n-hexane–AcOEt (1 : 1)] to give thunberginol A trimethyl ether (**20**, 1.8 mg, 68.1%), which was identified with an authentic sample[3] by TLC, IR, and ^{1}H-NMR spectra comparisons. A solution of **20** (3.0 mg, 0.01 mmol) in BBr_3–CH_2Cl_2 (1.0 M solution, 0.05 ml) was stirred at room temperature (25 °C) for 1 h. Work-up of the reaction mixture followed by purification with silica gel column chromatography [1.0 g, $CHCl_3$–MeOH–H_2O (10 : 3 : 1, lower phase)] gave thunberginol A (**1**, 2.3 mg, 88.6%), which was identified with an authentic sample[3] by TLC, IR, and ^{1}H-NMR spectra comparisons.

Lactonization of 18 with $CuCl_2$ Giving the Five-Membered Lactone (21, 22) A solution of **18** (20 mg, 0.07 mmol) in MeOH (0.5 ml) was treated with $CuCl_2$ (60 mg, 0.44 mmol) and the whole mixture was heated under reflux for 2 h. The reaction solution was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above for **8** furnished a mixture of the five-membered lactones (**21** and **22**, 20 mg, 88.0%), which was subjected to HPLC (the same conditions as described above for **12** and **13**) separation to give **21** (16 mg, 70.9%) and **22** (3.4 mg, 15.5%).

21: Colorless needles from EtOH, mp 170—173 °C. High-resolution positive-mode FAB-MS: Calcd for $C_{19}H_{21}O_6$ $(M+H)^+$: 345.1338. Found: 345.1313. UV [EtOH, nm (ε)]: 233 (11000), 286 (3900), 300 (3700). IR (KBr) cm^{-1}: 1767, 1612. ^{1}H-NMR ($CDCl_3$) δ: 3.35, 3.71, 3.85,

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3.91 (3H each, all s, $OCH_3 \times 4$), 4.46 (1H, d, $J=5.9$ Hz, 8-H), 5.58 (1H, d, $J=5.9$ Hz, 3-H), 6.61 (1H, br s, 2′-H), 6.66 (1H, d, $J=8.2$ Hz, 6-H), 6.78 (2H, br s, 5′, 6′-H), 6.83 (1H, d, $J=8.2$ Hz, 4-H), 7.45 (1H, dd, $J=8.2$, 8.2 Hz, 5-H). Positive-mode FAB-MS *m/z*: 345 $(M+H)^+$.

22: Colorless needles from EtOH, mp 172—175 °C. High-resolution positive-mode FAB-MS Calcd for $C_{19}H_{21}O_6$ $(M+H)^+$: 345.1338. Found: 345.1330. UV [EtOH, nm (ε)]: 231 (6900), 286 (2700), 297 (2600). IR (KBr) cm^{-1}: 1767, 1612. ^{1}H-NMR ($CDCl_3$) δ: 3.32, 3.86, 3.88, 3.96 (3H each, all s, $OCH_3 \times 4$), 4.42 (1H, d, $J=5.3$ Hz, 8-H), 5.47 (1H, d, $J=5.3$ Hz, 3-H), 6.84 (1H each, both s, 5′, 6′-H), 6.80 (1H, br s, 2′-H), 6.86 (1H, d, $J=7.6$ Hz, 6-H), 6.89 (1H, d, $J=8.2$ Hz, 4-H), 7.52 (1H, dd, $J=7.6$, 8.2 Hz, 5-H). Positive-mode FAB-MS *m/z*: 345 $(M+H)^+$.

Lactonization of 18 with $CuBr_2$ Giving 21 and 22 A solution of **18** (30 mg, 0.096 mmol) in MeOH (3 ml) was treated with $CuBr_2$ (106 mg, 0.48 mmol) and the whole mixture was heated under reflux for 6 h. The reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above yielded a mixture of **21** and **22** (25.2 mg, 76.8%), which was subjected to HPLC (the same condition as described above) separation to give **21** (19.9 mg, 60.5%) and **22** (4.2 mg, 12.8%). Thus obtained **21** and **22** were identified with authentic samples by TLC, HPLC, and ^{1}H-NMR spectrum comparisons.

Treatment of 21 and 22 with *p*-TsOH Followed by Demethylation Giving Thunberginol F (2) A solution of a mixture of **21** and **22** (1.4 g, 4.2 mmol) in dry benzene (40 ml) was treated with *p*-TsOH (20 mg) and the whole mixture was heated under reflux for 12 h. After cooling, the reaction mixture was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract as described above for **14** gave a product, which was purified by silica gel column chromatography [50 g, *n*-hexane–AcOEt–CH_2Cl_2 (2 : 2 : 1)] to afford **23** (780 mg, 61.5%). Thunberginol F trimethyl ether (**23**) was identified with an authentic sample[3] by TLC, IR, and ^{1}H-NMR spectra comparisons. A solution of **23** (3.5 mg, 0.01 mmol) in $(CH_2)_2Cl_2$ (0.8 ml) was treated with BBr_3–CH_2Cl_2 (1.0 M solution, 0.15 ml) and the whole mixture was heated under reflux for 1 h. The reaction solution was treated with MeOH and then evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography [5.0 g, $CHCl_3$–MeOH (10 : 1)] to give thunberginol F (**2**, 3.6 mg, quant.), which was identified with an authentic sample[3] by TLC, IR, and ^{1}H-NMR spectra comparisons.

Isolation of Hydramacrophyllols A (3) and B (4) Hydramacrophyllols A (**3**, 0.00013%) and B (**4**, 0.00047%) were isolated from Hydrangeae Dulcis Folium as described.[3]

Hydramacrophyllol A (**3**): A white powder, $[\alpha]_D^{25}$ −5.9° ($c=0.34$, EtOH). High-resolution negative-mode FAB-MS Calcd for $C_{15}H_{11}O_5$ $(M-H)^-$: 271.0607. Found: 271.0612. UV [EtOH, nm (ε)]: 225 (17000), 301 (4800). IR (KBr) cm^{-1}: 3440, 1742, 1617. ^{1}H-NMR (acetone-d_6) δ: 5.00 (1H, d, $J=4.8$ Hz, 8-H), 5.65 (1H, d, $J=4.8$ Hz, 3-H), 6.71 (1H, d, $J=7.3$ Hz, 4-H), 6.77 (2H, d, $J=8.6$ Hz, 3′, 5′-H), 6.87 (1H, d, $J=7.6$ Hz, 6-H), 7.20 (2H, d, $J=8.6$ Hz, 2′, 6′-H), 7.47 (1H, dd $J=7.3$, 7.6 Hz, 5-H). Negative-mode FAB-MS *m/z*: 271 $(M-H)^-$.

Hydramacrophyllol B (**4**): A white powder, $[\alpha]_D^{25}$ ±0° ($c=0.13$, EtOH). High-resolution negative-mode FAB-MS Calcd for $C_{15}H_{11}O_5$ $(M-H)^-$: 271.0607. Found: 271.0615. UV [EtOH, nm (ε)]: 226 (15000), 300 (4700). IR (KBr) cm^{-1}: 3424, 3301, 1736, 1620. ^{1}H-NMR (acetone-d_6) δ: 5.12 (1H, d, $J=4.3$ Hz, 8-H), 5.67 (1H, d, $J=4.3$ Hz, 3-H), 6.63 (1H, d, $J=7.6$ Hz, 4-H), 6.81 (1H, d, $J=7.9$ Hz, 6-H), 6.88 (2H, d, $J=8.0$ Hz, 3′, 5′-H), 7.26 (2H, dd, $J=8.0$ Hz, 2′, 6′-H), 7.45 (1H, dd, $J=7.6$, 7.9 Hz, 5-H). Negative-mode FAB-MS *m/z*: 271 $(M-H)^-$.

Methylation of Hydramacrophyllol A (3) Giving 12 A solution of **3** (2.0 mg, 0.07 mmol) in DMF (0.5 ml) was treated with CH_3I (0.04 ml, 6.1 mmol) and NaH (11 mg, 0.44 mmol) and the whole mixture was stirred at room temperature (25 °C) for 2 h in the dark. The reaction mixture was poured into saturated brine and the whole was extracted with AcOEt. The AcOEt extract was worked-up as described above for **8** to give a product, which was purified by silica gel column chromatography [1.0 g, CH_2Cl_2–AcOEt (10 : 1)] to yield **12** (1.9 mg, 82.6%). **12** was identified with an authentic sample synthesized from **8** by TLC, IR, and ^{1}H-NMR spectra comparisons.

Methylation of Hydramacrophyllol B (4) Giving 13 A solution of **4** (4.0 mg, 0.14 mmol) in DMF (0.5 ml) was treated with CH_3I (0.08 ml, 12 mmol) and NaH (22 mg, 0.88 mmol) and the reaction mixture was stirred at room temperature (25 °C) for 2 h in the dark. The reaction mixture was poured into saturated brine and the whole was extracted with AcOEt. A residue, obtained after work-up as described above, was purified by silica gel column chromatography [1.0 g, CH_2Cl_2–AcOEt (10 : 1)] to give **13** (3.7 mg, 80.2%), which was identical with an authentic sample obtained from **8** by TLC, IR, and ^{1}H-NMR spectra comparisons.

Preparation of the MTPA Esters (24, 25) from Hydramacrophyllols A (3) and B (4) A solution of **3** (1.5 mg, 0.006 mmol) in MeOH (0.5 ml) was treated with CH_2N_2–ether (0.5 ml) until the yellow color persisted. The reaction mixture was left standing at room temperature (25 °C) for 1 h and then the solvent was evaporated under reduced pressure to furnish the dimethyl ether (1.6 mg, quant.). A solution of the dimethyl ether (1.0 mg, 0.003 mmol) was treated with (*R*)-MTPA (5.9 mg, 0.25 mmol), DCC (5.2 mg, 0.25 mmol), and 4-DMAP (1.9 mg, 0.15 mmol) and the whole mixture was stirred at room temperature (25 °C) for 1 h. The reaction mixture was poured into ice-water and the whole was extracted with CH_2Cl_2. The CH_2Cl_2 extract was successively washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, and then dried over $MgSO_4$ and filtered. Evaporation of the solvent from the filtrate under reduced pressure furnished a residue, which was purified by silica gel column chromatography [1.0 g, *n*-hexane–AcOEt (1 : 1)] to give a mixture of **24a** and **24b** (1.7 mg, quant.), colorless oil. ^{1}H-NMR ($CDCl_3$) δ: (the peaks due to major constituent) 3.47, 3.83, 3.97 (3H each, all s, $OCH_3 \times 3$), 5.58 (1H, d, $J=5.2$ Hz, 3-H), 6.26 (1H, d, $J=5.2$ Hz, 8-H), 6.81—7.49 (11H, m, aromatic H); (the peaks due to minor constituent) 3.39, 3.82, 3.94 (3H each, all s, $OCH_3 \times 3$), 5.61 (1H, d, $J=6.3$ Hz, 3-H), 6.03 (1H, d, $J=6.3$ Hz, 8-H), 6.81—7.49 (11H, m, aromatic H). The MTPA ester (a mixture of **25a** and **25b**) was also prepared from **4** (91.0 mg) by the same procedure as described above; colorless oil. ^{1}H-NMR ($CDCl_3$, δ): (the signals of one constituent) 3.44, 3.79, 3.92 (3H each, all s, $OCH_3 \times 3$), 5.66 (1H, d, $J=4.6$ Hz, 3-H), 6.20 (1H, d, $J=4.6$ Hz, 8-H), 6.63—7.56 (11H, m, aromatic H); (the signals of another constituent) 3.55, 3.77, 3.93 (3H each, all s, $OCH_3 \times 3$), 5.71 (1H, d, $J=3.6$ Hz, 3-H), 6.29 (1H, d, $J=3.6$ Hz, 8-H), 6.63—7.56 (11H, m, aromatic H).

Lactonization of Hydrangeaic Acid (7) Giving (±)-Hydramacrophyllols A (3) and B (4) A solution of **7** (80 mg, 0.32 mmol) in 80% aqueous acetone (8.0 ml) was treated with $CuCl_2$ (420 mg, 3.1 mmol) and the whole mixture was heated under reflux for 3 h. After cooling, it was poured into ice-water and the whole was extracted with AcOEt. Work-up of the AcOEt extract in the usual manner gave a product, which was purified by silica gel column chromatography to furnish a mixture of ±**3** and ±**4** and hydrangenol (**5**, 64.3 mg, 75.0%). A mixture of ±**3** and ±**4** was subjected to HPLC [Develosil (10 i.d. × 250 mm), 50% aqueous MeOH] to give ±**3** (9.6 mg, 11.2%) and ±**4** (4.3 mg, 5.1%), which were identical with authentic hydramacrophyllols A and B by TLC, HPLC, and ^{1}H-NMR data comparisons.

Bioassay Test for the Inhibitory Activity on the Histamine Release The methods of bioassay testing are completely the same as those for thunberginol A from Hydrangeae Dulcis Folium described previously.[3b,7b]

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References and Notes

1) Part V: Yoshikawa M., Matsuda H., Shimoda H., Shimada H., Harada E., Naitoh Y., Miki A., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 1440—1447 (1996).
2) Yoshikawa M., Yoshizumi S., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 492—499 (1996) and references cited therein.
3) *a*) Yoshikawa M., Uchida E., Chatani N., Murakami N., Yamahara J., *Chem. Pharm. Bull.*, **40**, 3121—3123 (1992); *b*) Yoshikawa M., Yoshizumi S., Murakami T., Matsuda H., Yamahara J., Murakami N., *ibid.*, **42**, 2225—2230 (1994).
4) Yoshikawa M., Uchida E., Chatani N., Kobayashi H., Naitoh Y., Okuno Y., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **40**, 3352—3354 (1992).
5) Yoshikawa M., Harada E., Yagi N., Okuno Y., Muraoka O., Aoyama H., Murakami N., *Chem. Pharm. Bull.*, **42**, 721—723 (1994).
6) *a*) Yoshikawa M., Chatani N., Harada E., Nishino Y., Yamahara J., Murakami N., *Yakugaku Zasshi*, **114**, 176—181 (1994); *b*) Yamahara J., Matsuda H., Shimoda H., Ishikawa H., Kawamori

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S., Wariishi N., Harada E., Murakami N., Yoshikawa M., *ibid.*, **114**, 401—413 (1994); *c*) Yoshikawa M., Ueda T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 1691—1693 (1994); *d*) Yamahara J., Miki A., Tsukamoto K., Murakami N., Yoshikawa M., *Natural Medicines*, **49**, 84—87 (1995).

7) *a*) Yoshikawa M., Harada E., Matsuda H., Chatani N., Yamahara J., Murakami N., 9th Symposium on the Development and Application of Naturally Occuring Drug Materials, Abstract of Papers, Shizuoka, July 1993, p. 45—48; *b*) Yamahara J., Matsuda H., Shimoda H., Wariishi N., Yagi N., Murakami N., Yoshikawa M., *Folia Pharmacol. Jpn.*, **105**, 365—379 (1995).

8) *a*) Castro C. E., Gaughan E. J., Owsley D. C., *J. Org. Chem.*, **31**, 4071—4078 (1966); *b*) Villemin O., Goussue D., *Heterocycles*, **29**, 1255—1261 (1989); *c*) Ohta S., Kamata Y., Inagaki T., Masuda S., Yamamoto S., Yamashita M., Kawasaki I., *Chem. Pharm. Bull.*, **41**, 1188—1190 (1993).

9) *a*) Several excellent synthetic methods of benzylidenephthalide and isocoumarin were recently reported[b]; *b*) Minami T., Nishimoto A., Nakamura Y., Hanaoka M., *Chem. Pharm. Bull.*, **42**, 1700—1702 (1994).

10) *a*) Torii S., Uneyama K., Ono M., *Tetrahedron Lett.*, **21**, 2653—2654 (1980); *b*) Yoshikawa M., Wang H. K., Tosirisuk V., Kitagawa I., *Chem. Pharm. Bull.*, **30**, 3057—3060 (1982).

11) Hashimoto T., Tori M., Asakawa Y., *Phytochemistry*, **27**, 109—113 (1988).

12) *a*) Huneck S., Schreiber K., *Phytochemistry*, **16**, 1013—1016 (1977); *b*) Nozawa K., Yamada M., Tsuda Y., Kawai K., Nakajima S., *Chem. Pharm. Bull.*, **29**, 2491—2495 (1981).

13) The X-ray crystrallographic data of **12** and **21** will be reported in detail elsewhere.

14) Although isolation of **8′** from the reaction mixture has not yet been accomplished, the comparison of **8** and **8′** in the mixture was confirmed by the ^{1}H-NMR (270 MHz, $CDCl_3$) analysis of the mixture.

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