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Studies on *Hydrangea* Species. I.[1] Phenolic Components of *Hydrangea serrata* Seringe var. *thunbergii* Sugimoto

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Phenolic components in the plant were investigated to give phyllodulcin, phyllodulcin-8-0-β-D-glucoside, hydrangenol, umbelliferone, *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, methyl chlorogenate, chlorogenic acid, kaempferol, quercetin, isoquercitrin, rutin and three new components, hydrangea glucosides A,B, and C. On the basis of chemical and spectral data hydrangea glucosides A(I), B(II), and C(III) were characterized to 2-[β-(4′-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid-6-0-β-D-glucopyranoside, 2-[β-(4′-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid-β-O-β-D-glucoside and *p*-hydroxybenzaldehyde-O-β-D-glucopyranoside, respectively.

As a sweetening component in *Hydrangea serrata* Seringe var. *thunbergii* Sugimoto (Japanese name: Amacha) phyllodulcin was isolated and identified by Asahina and Asano[3a] and its absolute configuration was determined by Arakawa[4] to 3(*R*)-(3′-hydroxy-4′-methoxyphenyl)-8-hydroxydihydroisocoumarin. Recently, Kimura, *et al.*,[5] reported the isolation of phyllodulcin-8-O-β-D-glucoside from the fresh leaves and suggested that phyllodulcin occurred as phyllodulcin-8-O-β-D-glucoside in the plant.

For the purpose of studying on biosynthesis of phyllodulcin and its related compounds the investigation of phenolic constituents connected with phyllodulcin in the fresh leaves was of importance. The work reported in this paper deals with the isolation of phenolic components and the structural elucidation of new compounds, hydrangea glucosides A, B, and C.

Isolation

The concentrated methanol extract of fresh leaves was suspended in water and fractionated to the water soluble portion and the benzene soluble portion. The water soluble portion was extracted with butanol and the solvent was evaporated to dryness. The residue was subjected to chromatography on silica gel column using benzene–methanol (99:1 to 85:15 v/v) as the solvent and the following components were isolated: *p*-hydroxybenzoic acid, protocatechuic acid, gallic acid, chlorogenic acid, methyl chlorogenate, phyllodulcin-8-O-β-D-glucoside, hydrangea glucosides A, B, and C, kaempferol, quercetin, isoquercitrin and rutin. After alkaline hydrolysis of benzene soluble portion an unsaponifiable fraction was excluded by extracting with ether and the alkaline layer was acidified and extracted with ether. The ether extract was chromatographed on silica gel column using benzene as the solvent and phyllodulcin, hydrangenol and umbelliferone were isolated. The known

1) This work was presented at The 89th Annual Meeting of Pharmaceutical Society of Japan, Nagoya, April 1969.
2) Location: *Katakasu, Fukuoka.*
3) *a*) Y. Asahina and J. Asano, *Yakugaku Zasshi*, **51**, 595, 749 (1931); *b*) Y. Asahina, *ibid.*, No. 330, 881 (1909); *c*) Y. Asahina and J. Asano, *ibid.*, **50**, 580, 573 (1930).
4) H. Arakawa and M. Nakazaki, *Chem. & Ind.*, **1959**, 671; H. Arakawa, *Bull. Chem. Soc. Japan*, **33**, 200 (1960).
5) Y. Kimura, M. Takido, T. Takata and T. Kuriyama, Abstracts of Papers, The Annual Meeting of The Japanese Society of Pharmacognosy, Gifu, Oct. 1967, p. 49.

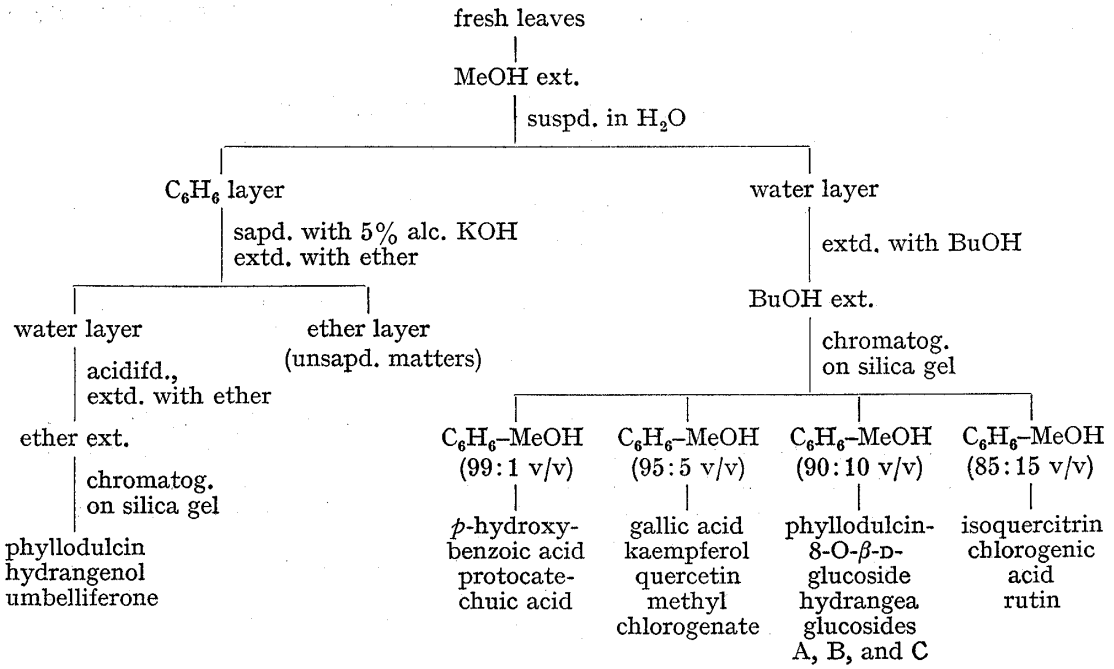

Chart 1. Isolation of Phenolic Compounds

compounds isolated in the procedure were identified by mixed melting point, cochromatography and by comparison of spectral data with those of the authentic sample.

Hydrangea Glucoside A (I)——Upon acid and enzymic hydrolysis (I), mp 180—182°, $C_{21}H_{24}O_{10}$, $[\alpha]_D^{19}$—40°, yielded D-glucose and hydrangenol as aglycon. I was assumed the glucoside of hydrangenol. However, I was soluble in sodium bicarbonate solution at room temperature, showing an acidic character and the infrared (IR) spectral bands at 2600, 1720 cm^{-1} revealed the presence of carboxyl group in this glucoside. I gave hexaacetate (I-a), mp 269—271°, $C_{33}H_{36}O_{16}$, on acetylation with Ac_2O–AcONa or Ac_2O–pyridine. The nuclear magnetic resonance (NMR) spectrum of I-a[6] indicated the signal corresponding to a phenol acetate at 2.30 ppm, an alcoholic acetate at 2.15 ppm and glucose tetraacetate at 2.00—2.10 ppm. Additionally, the NMR spectrum of I-a showed ABX-type signals of bibenzyl at 2.98 and 3.30 ppm. From the spectral data I-a differed from hydrangenol-8-O-β-D-glucosdie pentaacetate,[7] mp 246—248°, $C_{31}H_{32}O_{14}$, and I was characterized to the glucoside of hydrangenol whose lactone ring opened.

To clarify the site of glucose to the aglycon the methylation followed by acid hydrolysis was carried out. I was methylated by Hakomori's procedure[8] to give hexamethylate I-b, $C_{27}H_{34}O_9$, which exhibited a methoxycarbonyl (1740 cm^{-1}) in IR spectrum and a methoxycarbonyl (3.90 ppm), a phenolic methoxy (3.75 ppm), four alcoholic methoxy signals (3.35—3.62 ppm) and an anomeric proton of the sugar moiety at 4.85 ppm as a doublet (J=6 Hz) in NMR spectrum. I-b was subjected to methanolysis to give a phenolic substance I-c, mp 45°, $C_{17}H_{16}O_4$, showing an unstable purple coloration by $FeCl_3$ reagent.[3-c] I-c showed an associated phenol with carbonyl group at 12.8 ppm[9] in NMR spectrum and the carbonyl

6) NMR spectra of hydrangenol, 4′-acetylhydrangenol and 4′,8-diacetylhydrangenol-Hydrangenol (C_5D_5N): 2.94 (1H, d.d, J=17,5), 3.32 (1H, d.d, J=17,11), 5.52 (1H, d.d, J=11,5); 4′-acetylhydrangenol: 2.30 (3H, s, 4′Ac), 3.02 (1H, d.d, J=17,5), 3.32 (1H, d.d, J=17,11), 5.55 (1H, d.d, J=11,5), 6.70—7.45 (7H, aromatic), 10.88 (1H, s, OH); 4′,8-diacetylhydrangenol: 2.30 (3H, s, 4′Ac), 2.36 (3H, s, 8-Ac), 3.02 (1H, d.d, J=17,5), 3.38 (1H, d.d, J=17,11), 5.50 (1H, d.d, J=11,5), 7.02—7.58 (7H, aromatic). Abbreviation: s=singlet, d=doublet. d,d=doublet of doublet, m=multiplet

7) Y. Ueno, *Yakugaku Zasshi*, **57**, 602 (1937).

8) S. Hakomori, *J. Biochem.* (Tokyo), **55**, 205 (1964).

9) M. Sasaki, Y. Kaneko, K. Oshita, H. Takamatsu, Y. Asano and T. Yokotsuka, *Agr. Biol. Chem.*, **34**, 1296 (1970).

group at 1660 cm^{-1} [10] in IR spectrum and was identified with 2-methoxy carbonyl-3-hydroxy-4'-methoxystilbene, mp 45°, which was synthesized by partial methylation of hydrangeaic acid[3b] with diazomethane.

Consequently, the site of sugar linkage in (I) must be the phenol at C-6 and hydrangea glucoside A (I) is defined as 2-[β-(4'-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid 6-O-β-D-glucopyranoside.

Hydrangea Glucoside B (II)——II, mp 214—216°, $[\alpha]_D^{15}$ −30°, $C_{21}H_{24}O_{10}$, was soluble in sodium bicarbonate solution at room temperature and showed a stable purple coloration for $FeCl_3$ reagent.[3c] II was suggested to have an orthophenol carboxylic acid character by IR spectrum (1690 cm^{-1}).[10] From the spectral data of NMR at 2.98, 3.15, 5.68 ppm corresponding to ABX type proton of bibenzyl, ultraviolet (UV) absorption at 303 mμ and of mass fragment pattern, II was assigned to be a substance like I. Acid hydrolysis of II afforded hydrangenol, together with II-a, mp 220—221°, $C_{14}H_{14}O_3$ and D-glucose. By the deficiency of a stable purple coloration for $FeCl_3$ reagent and of the IR band at 1690 cm^{-1}, II-a was characterized to the decarboxylated phenol. Methylation of II-a by Hakomori's method gave II-c, mp 101—102°, which was identical with 3,4'-dimethoxystilbene synthesized by the decarboxylation of hydrangeaic acid[3b] followed by methylation. When hydrolyzed with β-glucosidase (II) gave hydrangenol, II-a and II-b on thin-layer chromatography (TLC). By a stable coloring for $FeCl_3$ reagent and by cochromatography with an authentic sample II-b was deduced to 2-[β-(4'-hydroxy phenyl) - β - hydroxyethyl] - 6 - hydroxy benzoic acid. In order to determine the site of glucose in II the acetylation and the methylation of II were carried out. Hexaacetate, oily substance II-d, $C_{33}H_{36}O_{16}\cdot 1\frac{1}{2}H_2O$, showed two signals corresponding to phenol acetate at 2.30 ppm in addition to glucose tetraacetate at 2.08 ppm in NMR spectrum. II was methylated by Hakomori's method to give heptamethylate, oily substance II-e, $C_{28}H_{38}O_{10}\cdot \frac{1}{2}H_2O$. II-e showed the absorption at 280, 285 mμ in UV spectrum and indicated two methoxy groups of phenol at 3.80 ppm, a methoxycarbonyl group at 3.90 ppm and tetramethoxy signals of sugar moiety at 3.38—3.64 ppm in NMR spectrum. Accordingly, II-e was confirmed not to be a stilbene derivative as I-b.

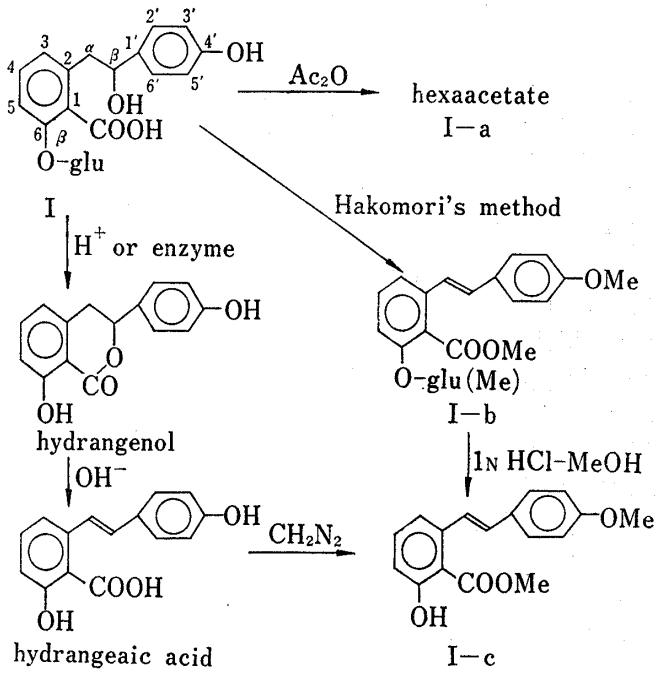

Chart 2

On the basis of chemical and the spectral data it was concluded that D-glucose attached to an alcoholic function in II-b. The structure of hydrangea glucoside B (II), therefore, was established to 2-[β-(4'-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid-β-O-β-D-glucoside.

Hydrangea Glucoside C (III)——III, mp 168—170°, $[\alpha]_D^{16}$ −81.3°, $C_{13}H_{16}O_7\cdot H_2O$, presented the carbonyl band at 1660 cm^{-1} in IR spectrum and showed the positive coloring test for 2,4-dinitrophenylhydrazine solution. Hydrolysis of III with β-glucosidase afforded an

10) M. Yamato, *Yakugaku Zasshi*, **79**, 1069 (1959); W.J. McGahren and L.A. Mitscher., *J. Org. Chem.*, **33**, 1577 (1968).

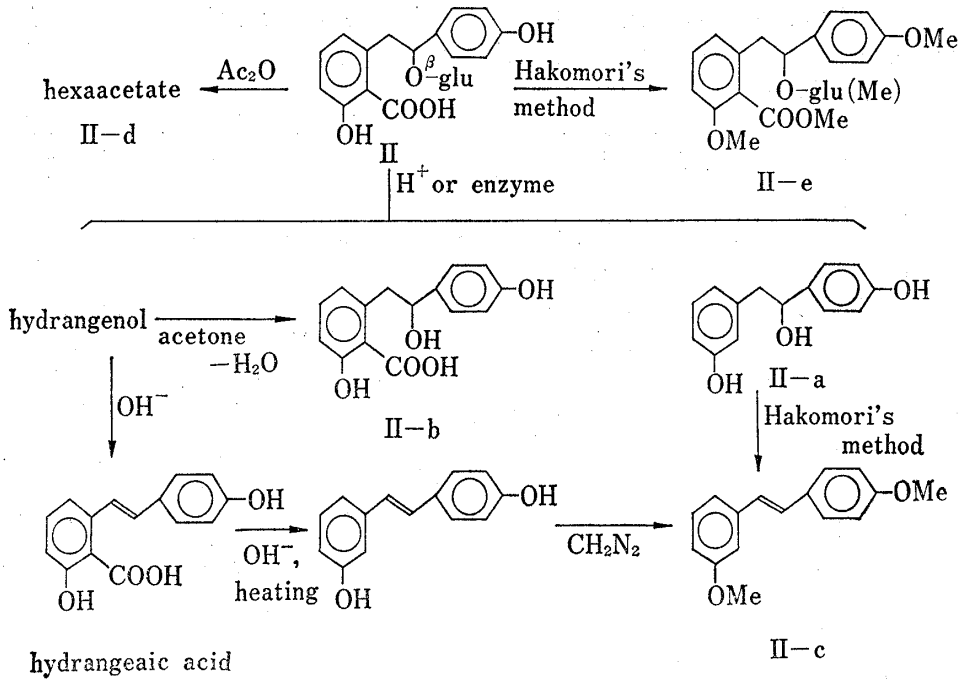

Chart 3

aglycon III-a, mp 112—114°, $C_7H_6O_2$ and D-glucose. As III-a revealed the carbonyl band at 1670 cm^{-1} in IR spectrum and signals of an aldehyde at 9.90 ppm, a phenol at 6.40 ppm and AB type four protons at 6.95 and 7.80 ppm in NMR spectrum, III-a was deduced to *p*-hydroxybenzaldehyde. III-a was identified by mixed melting point and by comparison of IR and NMR spectra with those of an authentic sample.

Consequently, III was proved to the glucoside of *p*-hydroxybenzaldehyde. The structure of III was confirmed by comparison of its acetate III-c with tetraacetate of *p*-hydroxybenzaldehyde-O-β-D-glucoside as follow. By standing α-D-tetraacetylbromoglucose,[11] mp 88—89°, $[\alpha]_D^{15}$199.5°, with sodium salt of *p*-hydroxybenzaldehyde in acetone for 24 hr at room temperature, *p*-hydroxybenzaldehyde-O-β-D-glucopyranoside tetraacetate III-b, mp 143—145°, $C_{21}H_{24}O_{11}\cdot\frac{1}{2}H_2O$, $[\alpha]_D^{15}$—26° was synthesized in the yield of 25% by the conventional method.[12] Acetate of III, III-c, mp 143—145°, $[\alpha]_D^{15}$—22° was identical with III-b by mixed melting point and by comparison of IR spectrum.

The presence of hydrangea glucosides A and B, the prototype of natural occurring dihydroisocoumarin, together with phyllodulcin-8-O-β-D-glucoside in the plant is of biogenetical significance. Further studies on biosynthesis of phyllodulcin are in progress.

Experimental

Melting points were determined on a Kofler block and are uncorrected. IR spectra were obtained with a KOKEN DS-301 and UV spectra were recorded with a Shimadzu SV-50A. NMR spectra were taken with a Nihondenshi C-60H in $CDCl_3$ unless otherwise specified. Chemical shifts were expressed in ppm from Me_4Si as internal reference and coupling constants(J) in cps. Mass spectral data were determined on a JEOL-01 double forcus high resolution spectrometer. TLC were performed on silica gel G(Kiesel gel G, Merck), polyamide(Wako, B-10) and cellulose powder(MN-Cellulose powder 300) employing the following solvent system: TLC (1): C_6H_6–MeOH–AcOH (45–8–4 v/v), C_6H_6–isoPrOH–AcOH (45–8–4 v/v) on silica gel for phenols and phenolic acids, TLC (2): AcOEt–MeCOEt–HCOOH–H_2O (5–3–1–1 v/v), AcOEt–MeCOEt–HCOOH–H_2O–C_6H_6 (4–3–1–1–2 v/v) on silica gel or polyamide for glycosides of phenol and phenol carboxylic acids, TLC (3): 30% AcOH, BuOH–AcOH–H_2O (4–1–5 v/v) on cellulose powder for flavonoids. 1% $FeCl_3$, 1% 2,4-dinitrophenylhydrazine in dil. HCl and 10% H_2SO_4 (spraying followed by heating) were used as

11) P.G. Scheuer and F. Smith, *J. Am. Chem. Soc.*, **76**, 3224 (1954).
12) J. Conichie and G.A. Levvy "Method in Carbohydrate Chemistry," Vol. II, ed., by R.L. Whistler and M.L. Wolfrom, Academic press, New York 1963, p. 335.

coloring reagents. Paper chromatography (PPC) for sugar was run on Toyo Roshi No. 50 using the upper layer of BuOH–AcOH–H_2O (4–1–5 v/v) and BuOH–Pyridine–H_2O (6:4:3 v/v) by ascending method with double development technique and a coloring reagent was aniline hydrogenphthalate.

Isolation (Chart 1)——The fresh leaves of the plant (7 kg) collected in Fukuoka (May, 1968 and 1970) were extracted with MeOH at room temperature. MeOH extract was concentrated and evaporated to dryness *in vacuo*. The residue (490 g) was suspended in water and fractionated to the water soluble portion and the benzene soluble portion. The water soluble portion was extracted with BuOH and the solvent was evaporated to dryness. The residue (58 g) was subjected to chromatography on silica gel column using C_6H_6–MeOH (99:1 to 85:15 v/v) as solvent and phenolic compounds were isolated. The C_6H_6 soluble matters (98 g) were refluxed with 5% alc. KOH (700 ml) for 1 hr and the solvent was evaporated. The alkaline layer was extracted with ether and the solvent was evaporated to give unsaponifiable matters (6.3 g). The alkaline layer was acidified with 10% HCl and extracted with ether. The ether extract (50 g) was subjected to chromatography on silica gel column using C_6H_6 as the solvent to give phyllodulcin (22 g), hydrangenol (2 g) and umbelliferone. The known compounds isolated and their derivatives were identified with the authentic sample by mixed melting point, cochromatography and by comparison of spectral data.

Hydrangea Glucoside A(I)——Colorless needles (from MeOH–H_2O) (4.2 g), mp 180—182°, $[\alpha]_D^{19}$ −40° (EtOH, c=0.1). *Anal.* Calcd. for $C_{21}H_{24}O_{10}$: C, 57.79; H, 5.54. Found: C, 57.43; H, 5.69. IR ν_{max}^{KBr} cm^{-1}: 2600 (OH), 1720 (C=O), 1610 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 286, 304 (3.77, 3.81).

Acid Hydrolysis of (I)——I (100 mg) was treated with 5% H_2SO_4 (5 ml) on a steam bath for 1 hr to yield precipitate. It was collected by filtration, washed with water and recrystallized from MeOH–H_2O to give colorless needles (66 mg), mp 180—181°, identified with an authentic sample of hydrangenol, mp 176°, IR ν_{max}^{KBr} cm^{-1}: 3320 (OH), 1670 (C=O), 1620 (C=C). UV λ_{max}^{EtOH} mμ (log ε): 285, 315 (3.63, 3.91), by mixed melting point and by comparison of IR spectrum. The filtrate was neutralized with barium carbonate filtrated and concentrated *in vacuo*. After removal of salt the filtrate was evaporated *in vacuo* to give syrup and it was examined by PPC to give D-glucose.

Enzymic hydrolysis of (I)——I (20 mg) suspended in water (10 ml) was stirred with β-glucosidase (Sigma Chemical Co., U.S.A.) (20 mg) for 5 days at 30°. The reaction mixture was extracted with EtOAc, evaporated and the residue was recrystallized from MeOH–H_2O to give hydrangenol.

Acetylation of (I)——I (1.4 g) in pyridine (5 ml) was acetylated with Ac_2O (10 ml) for 5 days at room temperature, and treated with ice water to give precipitate. It was collected by filtration, washed with water and recrystallized from MeOH–$CHCl_3$ to give colorless fine needles (I-a) (1.6 g), mp 269—271° (decomp.). *Anal.* Calcd. for $C_{33}H_{36}O_{16}$: C, 57.56; H, 5.27. Found: C, 57.96; H, 5.29. Mass spectrum m/e: 644 (M^+ -CO_2), 331 (glucose tetraacetate). IR ν_{max}^{KBr} cm^{-1}: 3440 (OH), 1758 (C=O), 1610 (C=C). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 296 (3.41). NMR 2.00—2.10 (12H, sugar, 4×Ac), 2.15 (3H, s, alc. Ac), 2.30 (3H, s, 4′-Ac), 2.98 (1H, d.d, J=15,5), 3.30 (1H, d.d, J=15,9), 4.30—5.50 (6H, m, sugar), 7.00—7.48 (7H, aromatic).

Methylation of (I)——According to the Hakomori's method NaH (0.4 g) was mixed with dimethyl sulfoxide (DMSO) (4 ml) at room temperature for 0.5 hr and to his reagent the solution of (I) (0.4 g) in DMSO (4 ml) was added and the reaction mixture was kept for 1 hr at room temperature. Then CH_3I (2 ml) was added and the reaction mixture was allowed to stand for 1 hr at room temperature. After dilution with water the reaction mixture was extracted with $CHCl_3$ and $CHCl_3$ layer was washed with water, dried and evaporated to dryness. The oily residue was chromatographed on silica gel column using C_6H_6 and $CHCl_3$ as the solvent. The fraction eluted with $CHCl_3$ afforded oily substance (I-b) (0.3 g). *Anal.* Calcd. for $C_{27}H_{34}O_9$: C, 64.53; H, 6.82. Found: C, 64.82; H, 6.98. IR ν_{max}^{KBr} cm^{-1}: 1740 (C=O), 1610 (C=C), 1100 (CH_3O). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 306, 320 (4.1, 4.1). NMR: 3.35—3.62 (12H, sugar, 4×CH_3O), 3.75 (3H, s, CH_3O), 3.90 (3H, s, $COOCH_3$), 4.85 (1H, d, anomeric H, J=6), 6.62—7.35 (9H, aromatic).

Methanolysis of (I-b)——I-b (50 mg) was hydrolyzed with 1N HCl-MeOH (10 ml) for 2 hr under refluxing on water bath. The reaction mixture was diluted with water and extracted with ether. The ether layer was washed with water, dried and the solvent was evaporated to dryness. The residue was subjected to chromatography on silica gel column using hexane and hexane–C_6H_6 (1:1 v/v) as the solvent. The fraction eluted with hexane–C_6H_6 (1:1 v/v) was crystallized from MeOH to give colorless needles, mp 45°, (I-c). I-c was identical with 2-methoxycarbonyl-3-hydroxy-4′-methoxystilbene, mp 45°. *Anal.* Calcd. for $C_{17}H_{16}O_4$: C, 71.82; H, 5.67. Found: C, 71.82; H, 5.71. IR ν_{max}^{KBr} cm^{-1}: 3100 (OH), 1660 (C=O), 1600 (C=C). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 300, 338 (3.1, 3.2). NMR: 3.84 (3H, s, CH_3O), 3.90 (3H, s, $COOCH_3$), 6.65—7.60 (9H, aromatic), 12.8 (1H, s, OH). It was synthesized by partial methylation of hydrangeaic acid with diazomethane and was identified by mixed melting point and comparison of IR spectrum.

Hydrangea Glucoside B(II)——Colorless needles (from MeOH–H_2O) (0.42 g), mp 214—216°, $[\alpha]_D^{15}$ −30° (EtOH, c=0.1). *Anal.* Calcd. for $C_{21}H_{24}O_{10}$: C, 57.79; H, 5.54. Found: C, 57.95; H, 5.58. IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1690 (C=O). Mass Spectrum m/e: 436 (M^+), 256 (M^+-$C_6H_{11}O_6$: hydrangenol), 230 (M^+-$C_6H_{11}O_5$, -CO_2: II-a). UV λ_{max}^{EtOH} mμ (log ε): 290, 303 (3.68, sh.). NMR (C_5D_5N): 2.98 (1H, d.d, J=12,3), 3.15 (1H, d.d, J=12,9), 5.68 (1H, d.d, J=9,3), 3.50—4.50 (6H, m, sugar), 6.95 (2H, d.d, J=9,2), 7.40 (2H, d.d, J=9,2).

Acid Hydrolysis of (II)——II (50 mg) was treated with 5% H_2SO_4 (10 ml) on a steam bath for 1 hr. The reaction mixture diluted with water was extracted with EtOAc and the solvent was evaporated to dryness.

The residue was subjected to chromatography on silica gel column using C_6H_6 as the solvent to afford hydrangenol which was identical with an authentic sample by mixed melting point and cochromatography. The following fraction eluted with C_6H_6–MeOH (99:1 v/v) was recrystallized from C_6H_6–acetone to afford (II-a), mp 220—221°, $[\alpha]_D^{13}$ 45° (MeOH, c=0.05). *Anal.* Calcd. for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13. Found: C, 72.49; H, 6.16. Mass Spectrum m/e: 230 (M^+), 124 (HO-C_6H_4-$\overset{+}{C}$HOH), 108 (HO-C_6H_4-$\overset{+}{C}H_2$). IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1613 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 280 (2.71). The water layer was neutralized with barium carbonate and worked up as usual to give D-glucose by PPC.

Methylation of (II-a)——(II-a) (20 mg) was methylated by Hakomori's method as previously described and the product was subjected to chromatography on silica gel column using hexane and hexane–C_6H_6 (1:1 v/v) as the solvent to give (II-c). II-c was identified to 3,4′-dimethoxystilbene, mp 101—102°. Mass Spectrum: Calcd. for $C_{16}H_{16}O_2$: 240.115; Found: 240.118, IR ν_{max}^{KBr} cm^{-1}: 1600 (C=C), 1450 (CH_3O). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 305, 324 (3.55, 3.55). NMR: 3.80 (3H, s, CH_3O), 3.82 (3H, s, CH_3O), 6.84—7.80 (10H, aromatic). It was synthesized by the decarboxylation of hydrangeaic acid followed by methylation with diazomethane and was identified by comparison of IR spectrum.

Enzymic Hydrolysis of (II)——II (30 mg) suspended in water (30 ml) was stirred with β-glucosidase (30 mg) for 4 days at 28°. The reaction mixture was extracted with EtOAc and the solvent was evaporated to dryness. Along with hydrangenol and (II-a), (II-b) was detected on TLC. By coloring for $FeCl_3$ reagent and by cochromatography with an authentic sample (II-b) was deduced to 2-[β-(4′-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid.

Acetylation of (II)——II (50 mg) was acetylated with Ac_2O (5 ml), AcONa (50 mg) under refluxing for 2 hr. The reaction mixture was worked up as usual to give colorless oily substance (II-d) (58 mg). *Anal.* Calcd. for $C_{33}H_{36}O_{16}\cdot 1\frac{1}{2}H_2O$: C, 55.38; M, 5.49. Found: C, 55.44; H, 5.71. Mass Spectrum m/e: 644 (M^+-CO_2), 331 (glucose tetraacetate), 312 (M^+-CO_2,-glucose tetraacetate). IR ν_{max}^{KBr} cm^{-1}: 1760—1740 (C=O), 1600 (C=C). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 302 (3.36). NMR: 2.08 (12H, s, 4×Ac), 2.30 (6H, s, 2×Ac), 2.98 (1H, d.d, J=15,5), 3.03 (1H, d.d, J=15,9), 4.30—5.50 (6H, m, sugar), 6.55—7.25 (7H, aromatic).

Methylation of (II)——II (80 mg) dissolved in DMSO (8 ml) was methylated by Hakomori's method as previously described and oily product was subjected to chromatography on silica gel column using C_6H_6 and $CHCl_3$ as the solvent to afford oily substance, heptamethylate (II-e). *Anal.* Calcd. for $C_{28}H_{38}O_{10}\cdot \frac{1}{2}H_2O$: C, 61.86; H, 7.23. Found: C, 61.93; H, 7.09. IR ν_{max}^{KBr} cm^{-1}: 1740 (C=O), 1600 (C=C). UV $\lambda_{max}^{dioxane}$ mμ (log ε): 280, 285 (3.3, 3.0). NMR: 3.38 3.55, 3.63, 3.64 (3H, s, 4×CH_3O, resp.), 3.80 (6H, s, 2×CH_3O), 3.90 (3H, s, $COOCH_3$), 6.32—7.08 (7H, aromatic H).

Synthesis of 2-[β-(4′-Hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic Acid (II-b) and 3,4′-Dimethoxystilbene(II-c)——Hydrangenol (1 g) in 10% acetone–H_2O (300 ml) was refluxed for 10 hr on a steam bath. The reaction mixture was extracted with ether and the solvent was evaporated to dryness. The residue was subjected to chromatography on silica gel column using C_6H_6 and C_6H_6–MeOH (99:1 v/v) as the solvent to give the starting material, pale yellow hydrangeaic acid (0.5 g), mp 180—181°, IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1690 (C=O), 1600 (C=C). UV λ_{max}^{EtOH} mμ (log ε): 302, 326 (4.13, 4.06), and colorless needles, 2-[β-(4′-hydroxyphenyl)-β-hydroxyethyl]-6-hydroxybenzoic acid, mp 216—218°, $[\alpha]_D^{20}$ 20° (MeOH, c=0.05). *Anal.* Calcd. for $C_{15}H_{14}O_5$: C, 65.69; H, 5.15. Found: C, 65.76; H, 4.66. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1690 (C=O), 1620 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 286, 302 (sh., 3.0), indicating a stable purple coloration for $FeCl_3$ reagent. To the solution of hydrangeaic acid (0.1 g) dissolved in glycerol (3 ml) potassium hydroxide (0.1 g) was added and heated at 200° for 3 hr. The reaction mixture was diluted with water, extracted with ether and the solvent was evaporated to dryness. The residue was subjected to chromatography on silica gel column using $CHCl_3$ as the solvent to give colorless needles, 3,4′-dihydroxystilbene (40 mg), mp 210—212° (from C_6H_6). *Anal.* Calcd. for $C_{14}H_{12}O_2$: C, 79.22; H, 5.70. Found: C, 79.18; H, 5.83. IR ν_{max}^{KBr} cm^{-1}: 3300 (OH), 1610 (C=C). UV λ_{max}^{EtOH} mμ (log ε): 308, 326 (3.24, 3.24). 3,4′-Dihydroxystilbene (20 mg) was methylated with CH_2N_2 and the product was subjected to chromatography on silica gel column using C_6H_6 as the solvent to give colorless needles 3,4′-dimethoxystilbene.

Hydrangea Glucoside C (III)——Colorless needles (from EtOH) (0.2 g), mp 168—170° (decomp.). *Anal.* Calcd. for $C_{13}H_{16}O_7\cdot H_2O$: C, 51.65; H, 6.00. Found: C, 51.20; H, 5.69. $[\alpha]_D^{16}$ −81.3° (MeOH, c=0.12). UV λ_{max}^{MeOH} mμ (log ε): 275, 282 (3.04, sh.). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1660 (C=O), 1610 (C=C). (III) showed reddish yellow for 2,4-dinitrophenylhydrazine reagent.

Enzymic Hydrolysis of (III)——III (50 mg) suspended in water (10 ml) was stirred with β-glucosidase (50 mg) for 19 hr at 27° and worked up as usual to give an aglycon, III-a (15 mg), mp 112—114° (from EtOH-C_6H_6). *Anal.* Calcd. for $C_7H_6O_2$: C, 68.84; H, 4.95. Found: C, 69.00; H, 5.22. IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1670 (C=O), 1600 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 285 (4.10). NMR: 6.40 (1H, m, OH, exchanged with D_2O), 6.95 (2H, d.d, J=9,2), 7.80 (2H, d.d, J=9,2), 9.90 (1H, s, CHO) and showed reddish yellow for 2,4-dinitrophenylhydrazine reagent. III-a was identified by mixed melting point and by comparison of IR and NMR spectra with those of the authentic p-hydroxybenzaldehyde. The water layer was concentrated *in vacuo* to afford syrup which was examined by PPC to give D-glucose.

Acetylation of (III)——III (0.01 g) in pyridine (1 ml) was acetylated with Ac_2O (2 ml) for 24 hr at room temperature and worked up as usual to give (III-c), mp 143—145° (from MeOH), $[\alpha]_D^{15}$ −22° ($CHCl_3$, c=

0.05). UV λ_{max}^{MeOH} mμ (log ε): 285 (3.46). IR ν_{max}^{KBr} cm^{-1}: 1760 (OAc), 1700 (CO).

Synthesis of *p*-Hydroxybenzaldehyde-O-*β*-D-glucopyranoside Tetraacetate(III-b)——To the solution of *p*-hydroxybenzaldehyde (1.7 g) dissolved in 4% NaOH (20 ml) a solution of *α*-D-tetraacetylbromoglucose, mp 88—89°, $[\alpha]_D^{15}$ 199.5° ($CHCl_3$, c=0.1) (4 g) in acetone (30 ml) was added and the reaction mixture was kept for 24 hr at room temperature. The solvent was evaporated *in vacuo* at 25—30° and the residue was diluted with water, extracted with $CHCl_3$ and evaporated to dryness *in vacuo*. The residue (4 g) was subjected to chromatography on silica gel column using C_6H_6 and C_6H_6–EtOAc as the solvent. The fraction eluted with C_6H_6–EtOAc (85:15 v/v) was crystallized from MeOH to give colorless needles (1.2 g), *p*-hydroxybenzaldehyde-O-*β*-D-glucopyranoside tetraacetate(III-b), mp 143—145°, $[\alpha]_D^{15}$ −26° ($CHCl_3$, c=0.3). *Anal.* Calcd. for $C_{21}H_{24}O_{11}\cdot\frac{1}{2}H_2O$; C, 54.66; H, 5.46. Found: C, 55.08; H, 5.17. UV λ_{max}^{MeOH} mμ (log ε): 284 (4.0). IR ν_{max}^{KBr} cm^{-1}: 1760 (AcO), 1700 (CO). NMR: 2.04 (12H, s, 4×Ac), 4.0—5.5 (7H, m, sugar), 7.10 (2H, d.d, J=9,2) 7.85 (2H, d.d, J=9,2) 9.90 (1H, s, CHO), indicating reddish yellow for 2,4-dinitrophenylhydrazine reagent. III-c was idnetical with III-b by mixed melting point and by comparison of UV and IR spectra.

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