Three New Glycosides from the Leaves of *Hydrangea macrophylla* subsp. *serrata* (Thunb.) Makino[1)]

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Three new glycosides, 7-deoxyloganic acid β-D-glucopyranosyl ester (1), (3*R*)-hydrangenol 8,4′-di-*O*-β-D-glucopyranoside (2), and (6*R*,7*E*,9*R*)-megastigma-4,7-dien-3-one 9,13-di-*O*-β-D-glucopyranoside (3), have been isolated from the leaves of *Hydrangea macrophylla* subsp. *serrata* (Thunb.) Makino (Saxifragaceae). The structures of 1—3 were elucidated on the basis of spectral data and chemical evidence.

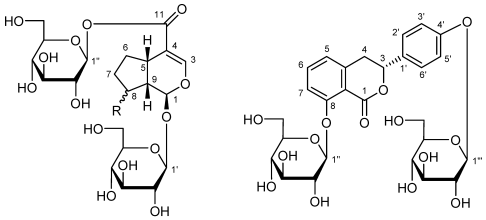
Key words *Hydrangea macrophylla* subsp. *serrata*; Saxifragaceae; iridoid glycoside; dihydroisocoumarin glycoside; megastigmane glycoside

Plants of the genus *Hydrangea* (Saxifragaceae) are known to contain secoiridoid glycosides[2—5)] and dihydroisocoumarins,[6,7)] and some of them possess antiallergic and antimicrobial activities.[5,7)] The constituents of the leaves of *H. macrophylla* subsp. *serrata* (Thunb.) Makino (*yama-ajisai* in Japanese) have been previously investigated and shown to contain dihydroisocoumarin glycosides.[8)] In previous papers, we reported on the isolation of secoiridoid glycosides from the leaves of the plant.[1,9)] Here, we report the isolation and structure elucidation of three new glycosides, 7-deoxyloganic acid β-D-glucopyranosyl ester (**1**), (3*R*)-hydrangenol 8,4′-di-*O*-β-D-glucopyranoside (**2**), and (6*R*,7*E*,9*R*)-megastigma-4,7-dien-3-one 9,13-di-*O*-β-D-glucopyranoside (**3**) from the leaves of the plant.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ −37.8° (MeOH). The molecular formula was determined to be $C_{22}H_{34}O_{14}$ by high-resolution (HR)-FAB-MS. Acid hydrolysis of **1** gave D-glucose, which was identified by its retention time and optical rotation using chiral detection by HPLC analysis. The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of asystasioside A (**4**)[10)] except for some signals surrounding C-8. The ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) and ^{1}H-detected heteronuclear multiple bond connectivity (HMBC) data provided evidence of the same planar structure for **1** as that of **4**. The difference between **1** and **4** was traced to differences in the stereochemistry of the methyl group at C-8. In the difference nuclear Overhauser effect (NOE) experiment, irradiation at δ_H 1.76 (H-9) caused NOE enhancement in the signal of the methyl group at C-8, and the configuration of the methyl group at C-8 was determined to be β. Thus, compound **1** was a C-8 epimer of **4**. On the basis of this evidence, the structure of **1** was determined to be 7-deoxyloganic acid β-D-glucopyranosyl ester.

Compound **2** was isolated as colorless needles, mp 205 °C, $[\alpha]_D$ −121.1° (MeOH). The molecular formula was determined to be $C_{27}H_{32}O_{14}$ by HR-FAB-MS. The ^{13}C-NMR spectrum of **2** was similar to that of hydrangenol (**5**),[8)] except for the presence of two hexosyl moieties [δ_C 62.5 (C-6‴), 62.6 (C-6″), 71.2 (C-4‴), 71.4 (C-4″), 74.7 (C-2‴), 74.9 (C-2″), 77.8 (C-3‴), 78.0 (C-3″), 78.2 (C-5‴), 78.5 (C-5″), 102.2 (C-1‴), 103.2 (C-1″)]. Acid hydrolysis of **2** gave only D-glucose in the above manner. In the ^{1}H-NMR spectrum of **2**, two anomeric proton signals [δ_H 4.92 (1H, d, J=7.6 Hz, H-1‴), 4.95 (1H, d, J=7.8 Hz, H-1″)] were recognized. The coupling constants of two anomeric protons indicated that the glycosyl linkages are of β-configuration. The nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum determined the positions of the β-D-glucopyranosyl groups to be C-8 and C-4′, by showing correlations between H-1″ and H-7, H-1‴ and H-3′, and H-1‴ and H-5′. The absolute configuration of **2** was determined from circular dichroism (CD) spectrum, which showed a Cotton curve [$\Delta\varepsilon$ (nm): −5.25 (205.5), −2.33 (212.9 sh), +7.75 (241.0), +4.14 (252.9 sh)] characteristic of the (3*R*)-dihydroisocoumarin.[11)] Therefore, the structure of **2** was determined to be (3*R*)-hydrangenol 8,4′-di-*O*-β-D-glucopyranoside.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ +89.3° (MeOH). The molecular formula was determined to be $C_{25}H_{40}O_{13}$ by HR-FAB-MS. The ^{13}C-NMR spectrum of **3** was similar to that of inamoside (**6**),[12)] except for the presence of an additional hexosyl moiety [δ_C 62.8 (C-6″), 71.6 (C-4″), 75.0 (C-2″), 78.0 (C-5″), 78.1 (C-3″), 103.5 (C-1″)] and differences in the chemical shifts at C-5 [δ_C 163.5 (−5.4)] and C-13 [δ_C 71.1 (+6.2)] due to glycosylation.[13)] Acid hydrolysis of **3** gave only D-glucose in the above manner. In the ^{1}H-NMR spectrum, two anomeric proton signals



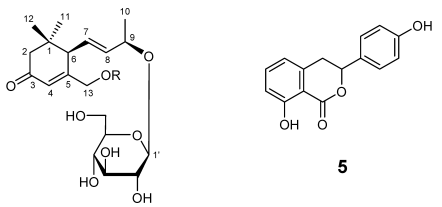


Chart 1

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[δ_H 4.27 (1H, d, J=7.7 Hz, H-1″), 4.35 (1H, d, J=8.1 Hz, H-1′)] were recognized. The coupling constants of two anomeric protons indicated that the glycosyl linkages are of β-configuration. These indicated that the additional β-D-glucopyranosyl moiety in **3** is attached to the hydroxyl group at C-13 in **6**. The absolute configurations at C-6 and C-9 were determined as follows. In the CD spectrum, the observed data [$\Delta\varepsilon$ (nm): +10.8 (244.4), −0.55 (324.4)] agreed well to those of **6**,[12] and therefore, *R* configuration at C-6 was deduced. Calis *et al.* reported that ^{13}C-NMR is of particular diagnostic value in assigning the absolute configuration at C-9 of megastigma-4,7-dien-3-one 9-*O*-glycosides, with δ_C values of *ca.* 74 and *ca.* 77 for the corresponding 9*S* and 9*R* forms, respectively.[14] Thus, the 9*R* configuration of **3** resulted from the ^{13}C-NMR data which showed C-9 at δ_C 78.0. Consequently, the structure of **3** was determined to be (6*R*,7*E*,9*R*)-megastigma-4,7-dien-3-one 9,13-di-*O*-β-D-glucopyranoside.

Experimental

General Procedures Melting points were determined with a Yanagimoto micromelting apparatus and are uncorrected. Optical rotations were determined using a JASCO DIP-360 digital polarimeter. CD spectra were measured on a JASCO J-720 spectropolarimeter. UV spectra were recorded with a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as internal standard. The HR-FAB-MS were recorded on a JEOL JMS-700 mass spectrometer. Column chromatography was carried out on a Kieselgel 60 (230—400 mesh, Merck) and Diaion HP-20 (Mitsubishi-Chemical). HPLC was performed by using a system comprised of CCPS pump (Tosoh), a UV-8020 detector (Tosoh), and a JASCO OR-2090 plus chiral detector.

Plant Material The leaves of *Hydrangea macrophylla* subsp. *serrata* were collected in the Aizu region in Fukushima Prefecture, Japan, in August of 2003.

Extraction and Isolation The leaves of *H. macrophylla* subsp. *serrata* (1.2 kg) were extracted with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure. The MeOH extract (110 g) was suspended in water, and this suspension was extracted with $CHCl_3$, AcOEt, *n*-BuOH and H_2O. The H_2O soluble fraction was passed through a Diaion HP-20 column, and adsorbed material was eluted with H_2O and MeOH. The MeOH elute fraction was concentrated. The residue (8.0 g) was chromatographed on a silica gel column using $CHCl_3$–MeOH–H_2O (30 : 10 : 1), and the elute was separated into 10 fractions. Fraction 7 was purified by preparative HPLC [column, TSKgel ODS-120T (7.8 mm i.d.×30 cm, Tosoh); mobile phase, MeOH–H_2O (1 : 4); flow rate, 1.0 ml/min; UV detector, 225 nm] to give **1** (2.2 mg). Fraction 8 was purified by preparative HPLC [column, Cosmosil 5SL (10 mm i.d.×25 cm, Nacalai Tesque); mobile phase, CH_2Cl_2–MeOH–H_2O (50 : 10 : 1); flow rate, 1.0 ml/min; UV detector, 240 nm] to give **2** (6.1 mg) and **3** (1.2 mg).

7-Deoxyloganic Acid β-D-Glucopyranosyl Ester (**1**): Amorphous powder. $[\alpha]_D^{25}$ −37.8° (c=0.138, MeOH). UV λ_{max} (MeOH) nm (log ε): 238 (4.0). HR-FAB-MS m/z: 545.1857 ([M+Na]$^+$, Calcd for $C_{22}H_{34}O_{14}Na$: 545.1847). ^{1}H-NMR (400 MHz, CD_3OD) δ: 1.10 (3H, d, J=7.0 Hz, H_3-10), 1.20 (1H, m, H-7β), 1.44 (1H, m, H-6α), 1.76 (1H, m, H-9), 1.87 (1H, m, H-7α), 1.98 (1H, m, H-8), 2.22 (1H, m, H-6β), 2.90 (1H, m, H-5), 3.20 (1H, dd, J=9.2, 8.0 Hz, H-2′), 3.65—3.70 (2H, m, H-6′a, H-6″a), 3.84 (1H, dd, J=11.7, 2.2 Hz, H-6′b), 3.89 (1H, dd, J=11.7, 2.0 Hz, H-6″b), 4.67 (1H, d, J=8.0 Hz, H-1′), 5.26 (1H, d, J=5.5 Hz, H-1), 5.51 (1H, d, J=8.1 Hz, H-1″), 7.51 (1H, d, J=1.1 Hz, H-3). ^{13}C-NMR (100 MHz, CD_3OD) δ: 20.8 (C-10), 33.2 (C-6), 34.2 (C-7), 35.1 (C-5), 36.5 (C-8), 49.6 (C-9), 62.3 (C-6″), 62.8 (C-6′), 71.1 (C-4″), 71.6 (C-4′), 74.0 (C-2″), 74.8 (C-2′), 78.1 (C-3′), 78.4 (C-3″), 78.8 (C-5′, C-5″), 95.4 (C-1″), 98.0 (C-1), 100.2 (C-1′), 112.3 (C-4), 154.2 (C-3), 167.7 (C-11).

(3*R*)-Hydrangenol 8,4′-Di-*O*-β-D-glucopyranoside (**2**): Colorless needles (from MeOH), mp 205 °C. $[\alpha]_D^{25}$ −121.1° (c=0.180, MeOH). UV λ_{max} (MeOH) nm (log ε): 245 (3.2), 314 (2.9). CD (c=3.07×10^{-5} M, MeOH) $\Delta\varepsilon$ (nm): −5.25 (205.5), −2.33 (212.9 sh), +7.75 (241.0), +4.14 (252.9 sh). HR-FAB-MS m/z: 581.1868 ([M+H]$^+$, Calcd for $C_{27}H_{33}O_{14}$: 581.1870). ^{1}H-NMR (400 MHz, CD_3OD) δ: 3.22 (1H, dd, J=16.6, 3.4 Hz, H-4a), 3.26—3.36 (1H, m, H-4b), 3.63—3.72 (2H, m, H-6″a, H-6‴a), 3.91 (2H, dd, J=12.2, 1.8 Hz, H-6″b, H-6‴b), 4.92 (1H, d, J=7.6 Hz, H-1‴), 4.95 (1H, d, J=7.8 Hz, H-1″), 5.53 (1H, dd, J=10.5, 3.4 Hz, H-3), 7.04 (1H, d, J=7.6 Hz, H-5), 7.12 (2H, d, J=8.8 Hz, H-3′, H-5′), 7.30 (1H, d, J=8.5 Hz, H-7), 7.40 (2H, d, J=8.8 Hz, H-2′, H-6′), 7.54 (1H, dd, J=8.5, 7.6 Hz, H-6). ^{13}C-NMR (100 MHz, CD_3OD) δ: 36.6 (C-4), 62.5 (C-6‴), 62.6 (C-6″), 71.2 (C-4‴), 71.4 (C-4″), 74.7 (C-2‴), 74.9 (C-2″), 77.8 (C-3‴), 78.0 (C-3″), 78.2 (C-5‴), 78.5 (C-5″), 80.6 (C-3), 102.2 (C-1‴), 103.2 (C-1″), 115.8 (C-8a), 117.1 (C-5), 117.8 (C-3′, C-5′), 122.7 (C-7), 128.8 (C-2′, C-6′), 133.8 (C-1′), 136.4 (C-6), 143.1 (C-4a), 159.3 (C-4′), 160.4 (C-8), 165.2 (C-1).

(6*R*,7*E*,9*R*)-Megastigma-4,7-dien-3-one 9,13-Di-*O*-β-D-glucopyranoside (**3**): Amorphous powder. $[\alpha]_D^{25}$ +89.3° (c=0.032, MeOH). UV λ_{max} (MeOH) nm (log ε): 232 (4.0). CD (c=5.31×10^{-5} M, MeOH) $\Delta\varepsilon$ (nm): +10.8 (244.4), −0.55 (324.4). HR-FAB-MS m/z: 571.2372 ([M+Na]$^+$, Calcd for $C_{25}H_{40}O_{13}Na$: 571.2367). ^{1}H-NMR (600 MHz, CD_3OD) δ: 1.02 (3H, s, H_3-11), 1.03 (3H, s, H_3-12), 1.29 (3H, d, J=6.3 Hz, H_3-10), 2.08 (1H, d, J=16.8 Hz, H-2α), 2.52 (1H, d, J=16.8 Hz, H-2β), 2.77 (1H, d, J=8.8 Hz, H-6), 3.18 (1H, dd, J=9.2, 8.8 Hz, H-2′), 3.24 (1H, dd, J=9.2, 8.0 Hz, H-2″), 3.65 (1H, dd, J=11.7, 3.3 Hz, H-6′a), 3.66 (1H, dd, J=12.1, 3.3 Hz, H-6″a), 3.83 (1H, dd, J=11.7, 2.2 Hz, H-6′b), 3.87 (1H, dd, J=12.1, 2.2 Hz, H-6″b), 4.27 (1H, d, J=7.7 Hz, H-1″), 4.32 (1H, dd, J=16.5, 1.8 Hz, H-13a), 4.35 (1H, d, J=8.1 Hz, H-1′), 4.40 (1H, br quintet, J=6.3 Hz, H-9), 4.46 (1H, dd, J=16.5, 1.8 Hz, H-13b), 5.70 (1H, dd, J=15.4, 6.3 Hz, H-8), 5.80 (1H, dd, J=15.4, 8.8 Hz, H-7), 6.25 (1H, s, H-4). ^{13}C-NMR (150 MHz, CD_3OD) δ: 21.1 (C-10), 27.9 (C-11, C-12), 37.1 (C-1), 49.0 (C-2), 52.3 (C-6), 62.8 (C-6″), 63.6 (C-6′), 71.1 (C-13), 71.6 (C-4″), 72.6 (C-4′), 75.0 (C-2″), 75.3 (C-2′), 78.0 (C-9, C-5″), 78.1 (C-3″), 78.7 (C-5′), 78.9 (C-3′), 103.5 (C-1′, C-1″), 124.2 (C-4), 128.6 (C-8), 138.9 (C-7), 163.5 (C-5), 202.9 (C-3).

Acid Hydrolysis of 1—3 Each of the compounds, **1**—**3** (*ca.* 0.3 mg), was refluxed with 5% HCl for 5 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The solution was concentrated *in vacuo* and dried to give a sugar fraction. The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex SUGAR KS-801 (8.0 mm i.d.×30 cm, Showa Denko; column temperature, 60 °C; mobile phase, H_2O; flow rate, 1.0 ml/min; chiral detection. Identification of D-glucose present in the sugar fraction was carried out by the comparison of its retention time and optical rotation with that of authentic sample; t_R (min) 7.5 (D-glucose, positive optical rotation).

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References and Notes

1) Part III in a series of studies on the constituents of the genus *Hydrangea* plants; Part II: Kanno M., Kakuda R., Kikuchi M., *J. Tohoku Pharmaceutical University*, **54**, 57—61 (2007).
2) Inouye H., Takeda Y., Uesato S., Uobe K., Hashimoto T., *Tetrahedron Lett.*, **21**, 1059—1062 (1980).
3) Uesato S., Hashimoto T., Takeda Y., Uobe K., Inouye H., *Chem. Pharm. Bull.*, **29**, 3421—3424 (1981).
4) Uesato S., Takeda Y., Hashimoto T., Uobe K., Inouye H., *Helv. Chim. Acta*, **67**, 2111—2127 (1984).
5) Yoshikawa M., Ueda T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **42**, 1691—1693 (1994).
6) Suzuki H., Ikeda T., Matsumoto T., Noguchi M., *Agric. Biol. Chem.*, **41**, 1815—1817 (1977).
7) 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).
8) Hashimoto T., Tori M., Asakawa Y., *Phytochemistry*, **26**, 3323—3330 (1987).
9) Sakai H., Kakuda R., Yaoita Y., Kikuchi M., *J. Nat. Med.*, **61**, 226—228 (2007).
10) Demuth H., Jensen S. R., Nielsen B. J., *Phytochemistry*, **28**, 3361—3364 (1989).
11) 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).
12) Aimi N., Hoshino H., Nishimura M., Sakai S.-I., Haginiwa J., *Tetrahedron Lett.*, **31**, 5169—5172 (1990).
13) Kasai R., Okihara M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **1977**, 175—178 (1977).
14) Calis I., Kuruuzum-Uz A., Lorenzetto P. A., Ruedi P., *Phytochemistry*, **59**, 451—457 (2002).