Medicinal Flowers. XXXX.[1] Structures of Dihydroisocoumarin Glycosides and Inhibitory Effects on Aldose Reducatase from the Flowers of *Hydrangea macrophylla* var. *thunbergii*

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Six dihydroisocoumarin glycosides, florahydrosides I and II, thunberginol G 8-*O*-*β*-D-glucopyranoside, thunberginol C 8-*O*-*β*-D-glucopyranoside, 4-hydroxythunberginol G 3′-*O*-*β*-D-glucopyranoside, and thunberginol D 3′-*O*-*β*-D-glucopyranoside, have been isolated from the flowers of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae) together with 20 known compounds. The chemical structures of the new compounds were elucidated on the basis of chemical and physicochemical evidence. Among the constituents, acylated quinic acid analog, neochlorogenic acid, was shown to substantially inhibit aldose reductase [IC_{50}=5.6 μM]. In addition, the inhibitory effects on aldose reductase of several caffeoylquinic acid analogs were examined for structure–activity relationship study. As the results, 4,5-*O*-*trans*-*p*-dicaffeoyl-D-quinic acid was found to exhibit a potent inhibitory effect [IC_{50}=0.29 μM].

Key words florahydroside; *Hydrangea macrophylla* var. *thunbergii*; medicinal flower; dihydroisocoumarin glycoside; aldose reductase; acylated quinic acid analog

The Saxifragaceae plant, *Hydrangea* (*H.*) *macrophylla* SERINGE var. *thunbergii* MAKINO, is native to Japan. The processed leaves of this plant (Hydrangea Dulcis Folium) are currently used as a natural medicine for an oral refrigerant and as a sweetener for diabetic patients. In addition, these preparations are listed in the Japanese Pharmacopoeia. Previously, we reported the isolation and structure determination of many chemical constituents with anti-diabetic, anti-allergic, and anti-bacterial activities from the processed leaves and the dried leaves of this plant.[2–6] However, a full chemical analysis of the flowers from this plant has not yet been performed. In the course of our chemical and pharmacological studies on Hydrangea plants[2–8] and medicinal flowers,[9–14] we recently reported the isolation and structure elucidation of secoiridoid glycosides named hydrangeamines A and B with a pyridine ring from the flowers of *H. macrophylla* var. *thunbergii* cultivated in Nagano prefecture, Japan.[15] As a continuing study, six dihydroisocoumarin glycosides, florahydrosides I (**1**) and II (**2**), thunberginol G 8-*O*-*β*-D-glucopyranoside (**3**), thunberginol C 8-*O*-*β*-D-glucopyranoside (**4**), 4-hydroxythunberginol G 3′-*O*-*β*-D-glucopyranoside (**5**), and thunberginol D 3′-*O*-*β*-D-glucopyranoside (**6**), were isolated from the flowers of *H. macrophylla* var. *thunbergii* together with 20 known compounds including five acylated quinic acid analogs. Furthermore, the inhibitory effects of the isolated compounds and its related compounds on aldose reductase were investigated. This paper deals with the structure elucidation of the new dihydroisocoumarin glycosides (**1**–**6**) and the inhibitory effects of the constituents and their related compounds, acylated quinic acid analogs, on aldose reductase.

A methanol (MeOH) extract (32.2% from the flowers of *H. macrophylla* var. *thunbergii* cultivated in Nagano province) was partitioned between EtOAc–H_2O (1 : 1) to furnish an EtOAc-soluble fraction (8.8%) and an aqueous layer. The aqueous layer was further extracted with 1-butanol (1-BuOH) to give a 1-BuOH (12.5%) and a H_2O (11.0%) soluble fraction. The EtOAc- and 1-BuOH-soluble fractions were subjected to normal-phase and reversed-phase silica gel column chromatography and repeated HPLC to give florahydrosides I (**1**, 0.068%) and II (**2**, 0.014%), thunberginol G 8-*O*-*β*-D-glucopyranoside (**3**, 0.035%), thunberginol C 8-*O*-*β*-D-glucopyranoside (**4**, 0.045%), 4-hydroxythunberginol G 3′-*O*-*β*-D-glucopyranoside (**5**, 0.020%), and thunberginol D 3′-*O*-*β*-D-glucopyranoside (**6**, 0.027%) together with (3*R*)-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**7**, 0.034%),[16] (3*S*)-phyllodulcin 8-*O*-*β*-D-glucopyranoside (**8**, 0.0059%),[16] (+)-hydrangenol 4′-*O*-*β*-D-glucopyranoside (**9**, 0.0040%),[17] (3*R*)-thunberginol I 4′-*O*-*β*-D-glucopyranoside (**10**, 0.056%),[18] (+)-3-(4-methoxyphenyl)-8-hydroxy-3,4-dihydroisocoumarin (**11**, hydrangenol monomethyl ether, 0.011%),[19] phyllodulcin (**12**, 0.19%),[20,21] hydrangenol 8-*O*-*β*-D-glucopyranoside (**13**, 2.87%),[16,18] thunberginol G 3′-*O*-*β*-D-glucopyranoside (**14**, 0.16%),[16,22] hydrangenol (**15**, 4.79%),[23,24] thunberginol G (**16**, 0.067%),[5,25] neochlorogenic acid (**17**, 0.075%),[26,27] 3-*O*-*trans*-*p*-coumaroyl-D-quinic acid (**18**, 0.27%),[28,29] 3-*O*-*cis*-*p*-coumaroyl-D-quinic acid (**19**, 0.029%),[29] chlorogenic acid (**20**, 0.054%),[30] chlorogenic acid methyl ester (**21**, 0.049%),[9,31] taxiphyllin (**22**, 0.076%),[8,32,33] umbelliferone glucoside (**23**, 0.11%),[34,35] *α*-morroniside (**24**, 0.021%),[36] *trans*-*p*-coumaric acid (0.013%),[30,37] and shikimic acid (0.36%)[38] (Fig. 1).

Structures of New Dihydroisocoumarin Glycosides (1–6) Florahydrosides I (**1**) and II (**2**) were isolated as a white amorphous powder with negative optical rotation (**1**: $[\alpha]_D^{20}$ −8.0°, **2**: $[\alpha]_D^{25}$ −21.0°, in MeOH). Their IR spectra showed absorption bands due to hydroxy (**1** and **2**: 3400 cm^{-1}), lactone (**1**: 1684 cm^{-1}, **2**: 1686 cm^{-1}), aromatic ring (**1**: 1610, 1508 cm^{-1}, **2**: 1618, 1510 cm^{-1}), and ether functions (**1**: 1071 cm^{-1}, **2**: 1073 cm^{-1}). In the FAB-MS spectra of **1** and **2**, the common quasimolecular ion peak was observed at *m*/*z* 487 ($[M+Na]^+$) and the molecular formula $C_{22}H_{24}O_{11}$ was determined by high-resolution (HR) MS measurement. Acid hydrolysis of **1** and **2** with 5% aqueous H_2SO_4 liberated D-glucose, which was iden-

The authors declare no conflict of interest.

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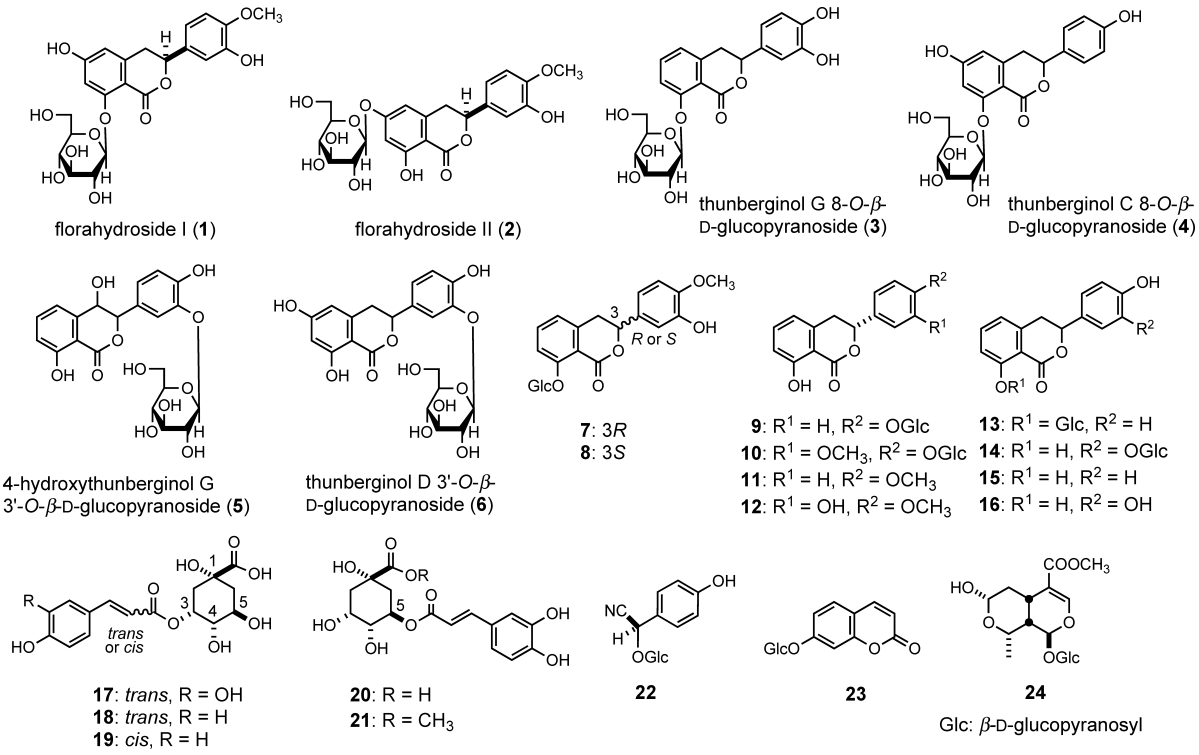


Fig. 1. Structures of Constituents from the Flower of *Hydrangea macrophylla*

tified by HPLC analysis using an optical rotation detector. The ^{1}H-NMR (methanol-d_4) and ^{13}C-NMR (Table 1) spectra of **1**, which were assigned by various NMR experiments,[39] showed signals assignable to a dihydroisocoumarin moiety [δ 2.93 (dd, J=16.5, 2.1 Hz, H-4a), 3.14 (dd, J=16.5, 12.4 Hz, H-4b), 5.29 (dd, J=12.4, 2.1 Hz, H-3), 6.45 (brs, H-5), 6.78 (brs, H-7)], ABX-type aromatic ring [δ 6.90 (1H, dd, J=8.2, 1.4 Hz, H-6′), 6.92 (1H, d, J=8.2 Hz, H-5′), 6.94 (1H, d, J=1.4 Hz, H-2′)], a methoxy group [δ 3.85 (s, OCH_3)], and a β-D-glucopyranosyl moiety [δ 4.81 (1H, d, J=7.6 Hz, H-1′)]. The proton and carbon signals of the 3-phenyldihydroisocoumarin part in the ^{1}H- and ^{13}C-NMR spectra of **1** were superimposable on those of (3*R*)-thunberginol E [(3*R*)-3,4-dihydro-6,8-dihydroxy-3-(3-hydroxy-4-methoxyphenyl)isocoumarin],[22] except for the signals around the 8-position, while the proton and carbon signals due to the dihydroisocoumarin part including glycoside moiety were very similar to those of (3*S*)-scorzocreticoside I [(3*S*)-3,4-dihydro-6,8-dihydroxy-3-(4-methoxyphenyl)isocoumarin 8-*O*-β-D-glucopyranoside].[40] As shown in Fig. 2, the double quantum filter correlation spectroscopy (DQF-COSY) experiment of **1** indicated the presence of certain structures (highlighted by bold lines). Heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments revealed long-range correlations between the following protons and carbons: H-3 and C-4a, 1′, 6′; H-4 and C-5, 8a; H-5 and C-4, 8a; H-7 and C-6, 8, 8a; H-2′ and C-4′; H-5′ and C-1′; H-6′ and C-3, 1′, 2′, 4′; H-1′ and C-8; $OC\underline{H}_3$ and C-4′. In addition, the position of the glucoside linkage was confirmed by a nuclear Overhauser effect spectroscopy (NOESY) experiment, which was showed NOE correlations between H-7 and H-1′. Next, the absolute configuration at the 3-position of **1** was identified by circular dichroism (CD) spectrum. Namely, **1** showed a positive Cotton effect at 236 nm ($\Delta\varepsilon$ +8.62) and a negative Cotton effect at 255 nm ($\Delta\varepsilon$ −0.79) for (3*S*)-dihydroisocoumarin.[18] On the basis of all this evidence, the chemical structure of florahydroside I (**1**) was determined to be (3*S*)-thunberginol E 8-*O*-β-D-glucopyranoside.

On the other hand, the ^{1}H-NMR (methanol-d_4) and ^{13}C-NMR (Table 1) spectra of **2**,[39] showed signals assignable to thunberinol E with a β-D-glucopyranosyl moiety as well as **1**. In addition, on the basis of the comparison of the NMR data for **2** with those of **1** and the DQF-COSY and HMBC experiments on **2** (Fig. 2), the position of the glucoside linkage on **2** was determined to be the 6-position. Next, the absolute configuration at the 3-position of **2** was found to be *S* by the CD spectrum as well as **1**. On the basis of all this evidence, the chemical structure of florahydroside II (**2**) was determined to be (3*S*)-thunberginol E 6-*O*-β-D-glucopyranoside.

Thunberginol G 8-*O*-β-D-glucopyranoside (**3**) and thunberginol C 8-*O*-β-D-glucopyranoside (**4**), which were obtained as a white amorphous powder and a mixture of diastereoisomers, showed absorption bands due to hydroxy, lactone, aromatic ring, and ether functions in their IR spectra. In the FAB-MS spectra of **3** and **4**, the common quasimolecular ion peak was observed at m/z 457 ($[M+Na]^+$) and the molecular formula $C_{21}H_{22}O_{10}$ was determined by HR-MS measurement. Acid hydrolysis of **3** and **4** liberated D-glucose, which was identified by HPLC analysis using an optical rotation detector. The ^{1}H-NMR (methanol-d_4) and ^{13}C-NMR (Table 1) spectra of **3**, which were assigned by various NMR experiments, revealed the products to be a *ca.* 2 : 1 mixture of two diastereoisomers. Namely, the ^{1}H-NMR showed double signals assignable to a dihydroisocoumarin moiety {[major diastereoisomer]: δ 3.09 (dd, J=16.5, 2.8 Hz, H-4a), 3.28 (m, H-4b), 5.36 (dd, J=11.7, 2.8 Hz, H-3), 7.10 (brd, J=8.2 Hz, H-5), 7.40 (brd, J=8.2 Hz, H-7), 7.40 (dd, J=8.2, 8.2 Hz, H-6), [minor diastereoisomer]: δ 3.16 (0.33H, dd, J=16.5, 2.8 Hz, H-4a), 3.28 (1H, m, H-4b), 5.41 (0.33H, dd, J=11.7, 2.8 Hz, H-3), 7.04

Table 1. ^{13}C-NMR (125 MHz) Spectroscopic Data for Compounds **1**–**6** in Methanol-d_4

Position	1	2	3	4	5	6
1	166.4	171.4	166.0/165.4	166.7/166.7	171.8/171.8	171.7/171.7
3	80.9	82.0	81.8/81.0	81.1/80.5	85.1/84.7	81.8/81.9
4	37.2	35.9	37.1/36.7	37.3/36.9	75.7/75.3	35.7/35.7
4a	145.5	143.4	143.7/143.2	145.5/145.2	150.2/150.3	143.6/143.7
5	110.3	108.3	123.2/122.7	110.7/110.2	116.8/116.8	108.0/108.0
6	165.4	165.2	136.6/136.3	166.2/166.2	137.0/137.1	166.4/166.4
7	105.3	103.6	118.2/117.0	105.6/104.6	115.6/115.7	102.2/102.2
8	163.2	165.2	160.8/160.3	163.3/162.8	158.0/158.0	165.6/165.7
8a	107.2	104.1	116.0/115.8	106.5/106.5	113.5/113.6	101.7/101.7
1′	132.7	132.6	131.1/131.3	130.8/130.9	132.1/131.9	131.5/131.6
2′	114.6	114.5	114.8/114.6	129.1/128.9	118.3/117.4	117.2/117.4
3′	147.7	147.7	146.6/146.5	116.3/116.3	146.5/146.4	146.7/146.7
4′	149.4	149.5	147.1/146.8	159.1/158.9	148.5/148.1	148.8/148.9
5′	112.5	112.5	116.3/116.3	116.3/116.3	116.6/116.4	117.0/117.1
6′	119.1	119.0	119.4/119.1	129.1/128.9	124.0/123.9	123.0/123.2
OCH_3	56.4	56.4	—	—	—	—
Glu-1″	105.1	101.4	105.1/103.1	105.2/103.3	104.5/104.1	104.0/104.3
2″	74.9	74.7	74.7/75.0	74.9/74.5	74.9/74.8	74.9/74.9
3″	77.1	77.9	77.2/77.8	77.2/77.7	77.7/77.6	77.6/77.6
4″	71.1	71.2	71.3/71.2	71.2/71.1	71.3/71.3	71.4/71.5
5″	78.7	78.4	78.8/78.5	78.7/78.4	78.2/78.4	78.3/78.4
6″	62.5	62.4	62.6/62.5	62.5/62.5	62.4/62.5	62.5/62.5

Compounds **3**–**6** were obtained as 3-epimeric mixtures.

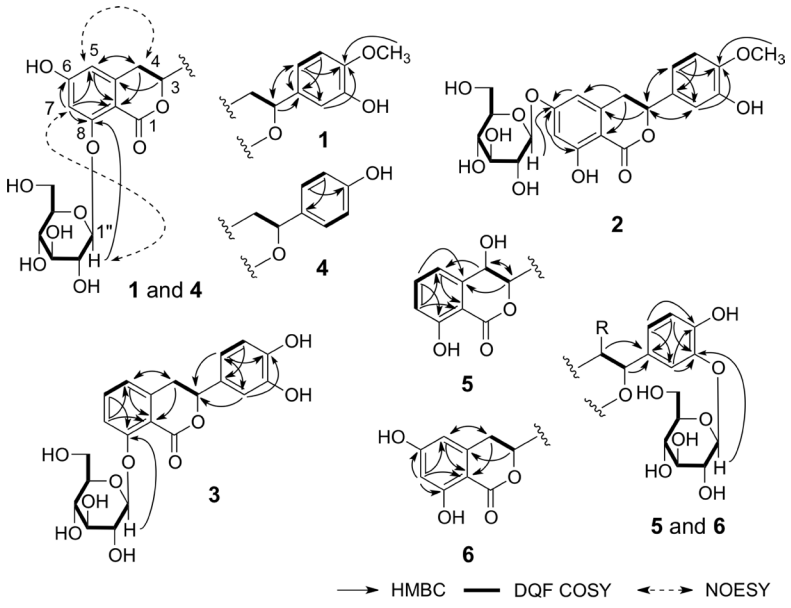


Fig. 2. Important 2D-NMR Correlations of Compounds **1**–**6**

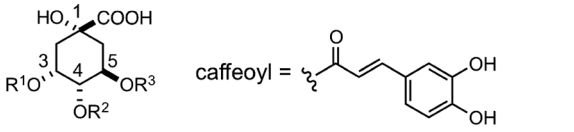
(0.33H, br d, J=7.6 Hz, H-5), 7.29 (0.33H, br d, J=7.6 Hz, H-7), 7.53 (0.33H, dd, J=7.6, 7.6 Hz, H-6)}, an ABX-type aromatic ring {[major diastereoisomer]: δ 6.79 (d, J=8.5 Hz, H-5′), 6.82 (dd, J=8.5, 2.0 Hz, H-6′), 6.93 (d, J=2.0 Hz, H-2′), [minor diastereoisomer]: δ 6.79 (0.33H, m, H-5′), 6.82 (0.33H, m, H-6′), 6.88 (0.33H, br s, H-2′)} and a β-D-glucopyranosyl moiety {[major diastereoisomer]: δ 4.90 (d, J=7.6 Hz, H-1′), [minor diastereoisomer]: δ 4.94 (d, J=8.3 Hz, H-1′)}. The proton and carbon signals of the aglycon part in the ^{1}H- and ^{13}C-NMR spectra of **3** were superimposable on those of thunberginol G (**16**),[5,25] except for the signals around the 8-position, while the proton and carbon signals due to the dihydroisocoumarin part including glycoside moiety were very similar to those of **7**,[16] **8**,[16] and **13**.[16,18] As shown in Fig. 2, the HMBC experiments revealed long-range correlations between the following protons and carbons: H-4 and C-5, 8a; H-5 and C-4, 8a; H-6 and C-8; H-7 and C-5, 8a; H-2′ and C-3, 4′; H-5′ and C-1′; H-6′ and C-3, 2′, 4′; H-1″ and C-8. Furthermore, the 3-position of dihydroisocoumarins with the 4′-hydroxyphenyl group such as hydrangenol (**15**), thunberginols B, C, and G (**16**), were reported to display tautomer-like behavior.[18] Therefore, **3** was displayed to be a 3-epimeric mixture of thunberginol G glycoside. On the basis of all this evidence, the chemical structure of **3** was determined to be as shown.

On the other hand, the ^{1}H-NMR (methanol-d_4) and ^{13}C-NMR (Table 1) spectra of **4** revealed the products to be a *ca.* 7:3 mixture of two diastereoisomers. The proton and carbon signals of the dihydroisocoumarin part in the ^{1}H- and ^{13}C-NMR spectra of **4** were superimposable on those of **1**, while the proton and carbon signals due to the 3-phenyl part were very similar to those of hydrangenol 8-*O*-*β*-D-glucopyranoside (**13**).[16,18] On the basis of the DQF-COSY and HMBC experiments (Fig. 1), the structure of **4** was determined to be thunberginol C[17,20,22] with glucoside. Next, the position of the glucoside linkage in **4** was confirmed based on HMBC and NOESY experiments, which was showed long-range correlations between H-1″ and C-8 and NOE correlations between H-7 and H-1″, respectively. Consequently, the chemical structure of **4** was determined to be as shown.

4-Hydroxythunberginol G 3′-*O*-*β*-D-glucopyranoside (**5**) and thunberginol D 3′-*O*-*β*-D-glucopyranoside (**6**), which were obtained as a mixture of diastereoisomers, showed absorption bands due to hydroxy, lactone, aromatic ring, and ether functions in their IR spectra. The common molecular formula ($C_{21}H_{22}O_{11}$) of **5** and **6** was determined from the quasimolecular ion peak (*m/z* 473 $[M+Na]^+$) in the positive-ion FAB-MS and by HR-MS measurement. Acid hydrolysis of **5** and **6** liberated D-glucose. The ^{1}H-NMR (methanol-d_4) and ^{13}C-NMR (Table 1) spectra of **5**,[39] showed signals assignable to a dihydroisocoumarin moiety {*ca.* 11:9 mixture of two diastereoisomers (3-epimeric mixture), [major diastereoisomer]: δ 4.81 (d, J=5.5 Hz, H-4), 5.60 (d, J=5.5 Hz, H-3), 6.53 (brd, J=7.9 Hz, H-7), 6.80 (brd, J=7.9 Hz, H-5), 7.40 (dd, J=7.9, 7.9 Hz, H-6), [minor diastereoisomer]: δ 4.91 (d, J=5.5 Hz, H-4), 5.61 (d, J=5.5 Hz, H-3), 6.82 (brd, J=7.9 Hz, H-5), 7.45 (dd, J=7.9, 7.9 Hz, H-6), 6.74 (overlap, H-7); ABX-type aromatic ring, and a *β*-D-glucopyranosyl moiety. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **5** were superimposable on those of thunberginol G 3′-*O*-*β*-D-glucopyranoside (**14**),[16,22] except for the signals around the 4-position. The structure of dihydroisocoumarin moiety including the two hydroxy groups on **5** was confirmed based on DQF and HMBC experiments, which was showed long-range correlations between the following protons and carbons: H-3 and C-4; H-4 and C-3, 5; H-5 and C-8a; H-6 and C-8; H-7 and C-8a. On the basis of all this evidence, the chemical structure of **5** was determined to be as shown.[41]

On the other hand, the proton and carbon signals of the aglycon part in the ^{1}H- and ^{13}C-NMR spectra of **6** were superimposable on those of thunberginol D,[20,22] except for the signals around the 3′-position, while the proton and carbon signals due to the 3-benzene ring including a glucoside moiety were very similar to those of **5**. In addition, on the basis of the DQF-COSY and HMBC experiments (Fig. 1), the chemical structure of **6** were determined to be as shown.

Inhibitory Effect on Rat Lens Aldose Reductase Aldose reductase, a key enzyme in the polyol pathway, has been reported to catalyze the reduction of glucose to sorbitol. Sorbitol does not readily diffuse across cell membranes, and the intracellular accumulation of sorbitol has been implicated in the chronic complications of diabetes such as cataract. Previously, we have reported that various constituents such as acylated quinic acids, flavonoids, and terpenoids from natural medicines and medicinal foods inhibited aldose reductase inhibitory effect.[1,9,42–47] In the present study, the inhibitory effects on aldose reductase of the isolated constituents from the flowers of *H. macrophylla* var. *thunbergii* were examined. Among the constituents, neochlorogenic acid (**17**) inhibited aldose reductase [IC_{50}=5.6 μM]. In addition, chlorogenic acid methyl ester (**21**) showed the inhibitory effect [IC_{50}=2.9 μM] in agreement with the previous study.[44] On the other hand, dihydroisocoumarin glycosides (**1**–**10**, **13**, **14**) and dihydroisocoumarins (**11**, **12**) lacked the inhibitory effects [IC_{50}>100 μM]. Hydrangenol (**15**), thunberginol G (**16**), taxiphyllin (**22**), and umbelliferone glucoside (**23**) exhibited moderate inhibitory effects [IC_{50}=48–69 μM]. Next, the inhibitory effects on aldose reductase of caffeoylquinic acid analogs **25**–**28**, which were previously obtained from *Ilex paraguariensis*,[48,49] were examined for the structure–activity relationship study (Fig. 3, Table 2). The inhibitory effect of D-quinic acid with *trans-p*-caffeoyl group at the 5-position [chlorogenic acid (**20**, reference compound,[50] IC_{50}=0.41 μM)] was stronger than those of D-quinic acids with *trans-p*-caffeoyl group at the 3 or 4-positions [**17** or 4-*O*-*trans-p*-caffeoyl-D-quinic acid (**25**, IC_{50}=11.8 μM)]. In addition, quinic acids with two caffeoyl groups [3,4-di-*O*-*trans*-caffeoyl-D-quinic acid (**26**, IC_{50}=0.34 μM), 3,5-di-*O*-*trans*-caffeoyl-D-quinic acid (**27**, IC_{50}=0.31 μM), and 4,5-di-*O*-*trans*-caffeoyl-D-quinic acid (**28**, IC_{50}=0.29 μM)] exhibited potent inhibitory effects. Those biological effects were equal to or stronger than that of a reference compound, chlorogenic acid (**20**). Further study for the development of acylated quinic acid analogs as potent anti-cataract agents are expected.



25: R^1 = R^3 = H, R^2 = caffeoyl **27**: R^1 = R^3 = caffeoyl, R^2 = H
26: R^1 = R^2 = caffeoyl, R^3 = H **28**: R^2 = R^3 = caffeoyl, R^1 = H

Fig. 3. Structures of Caffeoylquinic Acid Analogs **25**–**28**

Experimental

General Experimental Procedures The following instruments were used to obtain physical data: specific rotations, a Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, a Shimadzu FTIR-8100 spectrometer; CD spectra, a JASCO J-720WI spectrometer; EI-MS and HR-EI-MS, JEOL JMS-GCMATE mass spectrometer; FAB-MS and HR-FAB-MS, a JEOL JMS-SX 102A mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA600 (600 MHz) spectrometers; ^{13}C-NMR spectra, JEOL JNM-ECA600 (150 MHz) spectrometers with tetramethylsilane as an internal standard; and HPLC, a Shimadzu RID-6A refractive index and SPD-10A*vp* UV-VIS detectors. YMC-Pack ODS-A (YMC), COSMOSIL-5C_{18}-PAQ (Nacalai Tesque) and COSMOSIL-Cholester (Nacalai Tesque) {[250×4.6 mm i.d. (5 μm) for analytical purposes] and [250×20 mm i.d. (5 μm) for preparative purposes]} columns were used. The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography (CC), Silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh); reverse-phase silica gel CC, Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, precoated TLC plates with Silica gel 60F_{254} (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F_{254S} (Merck, 0.25 mm) (reverse phase); reversed-phase HPTLC, precoated

Table 2. Inhibitory Effects on Aldose Reductase of Compounds from the Flowers of *H. macrophylla* var. *thunbergii* and Caffeoylquinic Acid Analogs

Sample	IC_{50} (μM)
Hydrangenol (**15**)	47.8
Thunberginol G (**16**)	58.3
Neochlorogenic acid (**17**)	5.6
3-*O*-*trans*-Coumaroyl-D-quinic acid (**18**)	11.5
3-*O*-*cis*-Coumaroyl-D-quinic acid (**19**)	55.2
Taxiphyllin (**22**)	54.1
Umbelliferone glucoside (**23**)	68.6
4-*O*-*trans*-Caffeoyl-D-quinic acid (**25**)	11.8
3,4-Di-*O*-*trans*-caffeoyl-D-quinic acid (**26**)	0.34
3,5-Di-*O*-*trans*-caffeoyl-D-quinic acid (**27**)	0.31
4,5-Di-*O*-*trans*-caffeoyl-D-quinic acid (**28**)	0.29
Chlorogenic acid methyl ester (**21**)[44]	2.4
Chlorogenic acid (**20**) (positive control)[50]	0.41

Each value represents the mean of 2–4 experiments. The IC_{50} values of compounds **1**–**14**, and **24** were less than 100 μM.

TLC plates with Silica gel RP-18 WF_{254S} (Merck, 0.25 mm). Detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material Flowers of *Hydrangea macrophylla* SERINGE var. *thunbergii* MAKINO (Saxifragaceae), which were cultivated in Nagano prefecture, Japan, in 2010, were purchased from Kurohime Medical Herb Tea Co., Ltd. (Nagano, Japan). A voucher of the plant is on file in our laboratory (KPU Medicinal Flower-2010-HM).

Extraction and Isolation Dry flowers of *H. macrophylla* var. *thunbergii* (647 g) were extracted three times with MeOH for 3 h under reflux. Evaporation of the solvent under reduced pressure provided a MeOH extract (209 g, 32.2%). A part of the MeOH extract (198 g) was partitioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (53.9 g, 8.8%) and aqueous phase, which was extracted with 1-BuOH to give 1-BuOH- (76.9 g, 12.5%) and H_2O- (67.5 g, 11.0%) soluble fractions as reported previously.[15] A part of the 1-BuOH-soluble fraction (72.5 g) was subjected to normal-phase silica gel CC [500 g, $CHCl_3$–MeOH–H_2O (20 : 3 : 1→10 : 3 : 1→7 : 3 : 1→6 : 4 : 1)→MeOH] to give 2 fractions [Fr. B1 (44.2 g) and Fr. B2 (16.4 g)]. Fraction B1 (42.6 g) was subjected to reversed-phase silica gel CC [740 g, MeOH–H_2O (10 : 90→20 : 80→30 : 70→40 : 60→50 : 50→MeOH)] to give 10 fractions [Fr. B1-1 (2.23 g), Fr. B1-2, Fr. B1-3, Fr. B1-4 (4.69 g), Fr. B1-5 (3.54 g), Fr. B1-6 (6.23 g), Fr. B1-7, Fr. B1-8 (6.11 g), Fr. B1-9 (0.92 g), Fr. B1-10]. A part of Fraction B1-1 was separated by HPLC [MeOH–H_2O (20 : 80, COSMOSIL-5C_{18}-PAQ)] to give shikimic acid (413 mg). A part of Fr. B1-3 was purified by HPLC {MeOH–H_2O ([1] 30 : 70, YMC-Pack ODS-A), [2] 20 : 80, COSMOSIL-Cholester} to give neochlorogenic acid (**17**, 23 mg), 3-*O*-*trans*-*p*-coumaroyl-D-quinic acid (**18**, 70 mg), 3-*O*-*cis*-*p*-coumaroyl-D-quinic acid (**19**, 8.8 mg), chlorogenic acid (**20**, 17 mg), taxiphyllin (**22**, 24 mg), umbelliferone glucoside (**23**, 14 mg), and α-morroniside (**24**, 6.4 mg). A part of Fr. B1-4 was purified by HPLC {MeOH–H_2O ([1] 40 : 60, YMC-Pack ODS-A), [2] 30 : 70, COSMOSIL-Cholester} to give hydrangenol 8-*O*-β-D-glucopyranoside (**13**, 197 mg) and chlorogenic acid methyl ester (**21**, 5.1 mg). A part of Fr. B1-5 was purified by HPLC {MeOH–H_2O ([1] 45 : 55, YMC-Pack ODS-A), [2] 40 : 60, COSMOSIL-Cholester} to give 4-hydroxythunberginol G 3′-*O*-β-D-glucopyranoside (**5**, 5.8 mg), hydrangenol 8-*O*-β-D-glucopyranoside (**13**, 42 mg), and *trans*-*p*-coumaric acid (3.7 mg). A part of Fr. B1-6 was purified by HPLC {MeOH–H_2O ([1] 40 : 60, YMC-Pack ODS-A), [2] 40 : 60, COSMOSIL-Cholester} to give thunberginol G 8-*O*-β-D-glucopyranoside (**3**, 4.1 mg), thunberginol C 8-*O*-β-D-glucopyranoside (**4**, 5.3 mg), and (3*R*)-phyllodulcin 8-*O*-β-D-glucopyranoside (**7**, 4.0 mg). A part of Fr. B1-8 was purified by HPLC {MeOH–H_2O ([1] 40 : 60, YMC-Pack ODS-A), [2] 35 : 65, COSMOSIL-Cholester} to give florahydroside I (**1**, 36 mg), florahydroside II (**2**, 6.3 mg), thunberginol D 3′-*O*-β-D-glucopyranoside (**6**, 14 mg), (3*S*)-phyllodulcin 8-*O*-β-D-glucopyranoside (**8**, 3.2 mg), (+)-hydrangenol 4′-*O*-β-D-glucopyranoside (**9**, 2.1 mg), and thunberginol G 3′-*O*-β-D-glucopyranoside (**14**, 102 mg). A part of Fr. B1-9 was purified by HPLC [MeOH–H_2O (45 : 55, YMC-Pack ODS-A)] to give (3*R*)-thunberginol I 4′-*O*-β-D-glucopyranoside (**10**, 34 mg). A part of the EtOAc-soluble fraction (20.0 g) was subjected to normal-phase silica gel CC [300 g, *n*-hexane–EtOAc (10 : 1→5 : 1→2 : 1, v/v)→$CHCl_3$–MeOH (20 : 1→10 : 1)→MeOH] to give 10 fractions [Fr. E1-1, Fr. E1-2, Fr. E1-3, Fr. E1-4 (2.67 g), Fr. E1-5, Fr. E1-6 (0.75 g), Fr. E1-7, Fr. E1-8, Fr. E1-9, Fr. E1-10]. A part of Fr. E1-4 was purified by HPLC [MeOH–H_2O (60 : 40, YMC-Pack ODS-A)] to give hydrangenol (**15**, 921 mg). A part of Fr. E1-6 was purified by HPLC [MeOH–H_2O (60 : 40, YMC-Pack ODS-A)] to give phyllodulcin (**12**, 39 mg), hydrangenol (**15**, 37 mg), and thunberginol G (**16**, 13 mg).

Florahydroside I (**1**): A white amorphous powder; $[\alpha]_D^{20}$ −8.0° (*c*=0.50, MeOH); IR (KBr): ν_{max} 3400, 1684, 1610, 1508, 1071 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 236 (+8.62), 255 (−0.79); ^{1}H-NMR (methanol-d_4, 600 MHz) δ 2.93 (dd, *J*=16.5, 2.1 Hz, H-4a), 3.14 (dd, *J*=16.5, 12.4 Hz, H-4b), 3.85 (s, OCH_3), 4.81 (d, *J*=7.6 Hz, H-1′), 5.29 (dd, *J*=12.4, 2.1 Hz, H-3), 6.45 (brs, H-5), 6.78 (brs, H-7), 6.90 (dd, *J*=8.2, 1.4 Hz, H-6′), 6.92 (d, *J*=8.2 Hz, H-5′), 6.94 (d, *J*=1.4 Hz, H-2′); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS: *m/z* 487 $[M+Na]^+$; HR-FAB-MS: *m/z* 487.1214 (Calcd for $C_{22}H_{24}O_{11}Na$ $[M+Na]^+$: *m/z* 487.1216).

Florahydroside II (**2**): A white amorphous powder; $[\alpha]_D^{25}$ −21.0° (*c*=0.50, MeOH); IR (KBr): ν_{max} 3400, 1686, 1618, 1510, 1073 cm^{-1}; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 231 (+3.88), 249 (−3.14); ^{1}H-NMR (methanol-d_4, 600 MHz) δ 3.01 (dd, *J*=16.5, 2.0 Hz, H-4a), 3.15 (dd, *J*=16.5, 12.4 Hz, H-4b), 3.77 (s, OCH_3), 4.92 (d, *J*=7.6 Hz, H-1′), 5.40 (dd, *J*=12.4, 2.0 Hz, H-3), 6.46 (brs, H-7), 6.47 (brs, H-5), 6.83 (d, *J*=8.2 Hz, H-5′), 6.85 (brd, *J*=8.2 Hz, H-6′), 6.85 (brs, H-2′); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS: *m/z* 487 $[M+Na]^+$; HR-FAB-MS: *m/z* 487.1220 (Calcd for $C_{22}H_{24}O_{11}Na$ $[M+Na]^+$: *m/z* 487.1216).

Thunberginol G 8-*O*-β-D-Glucopyranoside (**3**): A white amorphous powder; IR (KBr): ν_{max} 3400, 1684, 1618, 1509, 1070 cm^{-1}; ^{1}H-NMR (methanol-d_4, 600 MHz, *ca.* 2 : 1 mixture of two diastereoisomers): [major diastereoisomer] δ 3.09 (dd, *J*=16.5, 2.8 Hz, H-4a), 3.28 (m, H-4b), 4.90 (d, *J*=7.6 Hz, H-1′), 5.36 (dd, *J*=12.4, 2.8 Hz, H-3), 6.79 (d, *J*=8.5 Hz, H-5′), 6.82 (dd, *J*=8.5, 2.0 Hz, H-6′), 6.93 (d, *J*=2.0 Hz, H-2′), 7.10 (brd, *J*=8.2 Hz, H-5), 7.40 (brd, *J*=8.2 Hz, H-7), 7.40 (dd, *J*=8.2, 8.2 Hz, H-6), [minor diastereoisomer] δ 3.16 (dd, *J*=16.5, 2.8 Hz, H-4a), 3.28 (m, H-4b), 4.94 (d, *J*=8.3 Hz, H-1″), 5.41 (dd, *J*=10.4, 2.8 Hz, H-3), 6.79 (m, H-5′), 6.82 (m, H-6′), 6.88 (brs, H-2′), 7.04 (brd, *J*=7.6 Hz, H-5), 7.29 (brd, *J*=7.6 Hz, H-7), 7.53 (dd, *J*=7.6, 7.6 Hz, H-6); ^{13}C-NMR: given in Table

1; positive-ion FAB-MS: *m/z* 457 $[M+Na]^+$; HR-FAB-MS: *m/z* 457.1105 (Calcd for $C_{21}H_{22}O_{10}Na$ $[M+Na]^+$: *m/z* 457.1111).

Thunberginol C 8-*O*-*β*-D-Glucopyranoside (**4**): A white amorphous powder; IR (KBr): ν_{max} 3400, 1684, 1617, 1509, 1071 cm^{-1}; ^{1}H-NMR (methanol-d_4, 600 MHz, *ca.* 7 : 3 mixture of two diastereoisomers): [major diastereoisomer] δ 2.95 (dd, *J*=16.5, 2.0 Hz, H-4a), 3.22 (dd, *J*=16.5, 13.1 Hz, H-4b), 4.82 (d, *J*=6.8 Hz, H-1″), 5.34 (dd, *J*=13.1, 2.0 Hz, H-3), 6.80 (d, *J*=8.9 Hz, H-3′,5′), 7.31 (d, *J*=8.9 Hz, H-2′,6′), 6.43 (brs, H-5), 6.75 (brs, H-7), [minor diastereoisomer] δ 3.03 (dd, *J*=16.5, 2.0 Hz, H-4a), 3.24 (m, H-4b), 4.82 (d, *J*=6.8 Hz, H-1″), 5.41 (dd, *J*=11.0, 2.8 Hz, H-3), 6.78 (d, *J*=8.9 Hz, H-3′,5′), 7.27 (d, *J*=8.9 Hz, H-2′,6′), 6.40 (brs, H-5), 6.66 (brs, H-7); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS: *m/z* 457 $[M+Na]^+$; HR-FAB-MS: *m/z* 457.1107 (Calcd for $C_{21}H_{22}O_{10}Na$ $[M+Na]^+$: *m/z* 457.1111).

4-Hydroxythunberginol G 3′-*O*-*β*-D-Glucopyranoside (**5**): A white amorphous powder; IR (KBr): ν_{max} 3400, 1682, 1619, 1508, 1073 cm^{-1}; ^{1}H-NMR [methanol-d_4, 600 MHz, *ca.* 11 : 9 mixture of two diastereoisomers (3-epimeric mixture)]: [major diastereoisomer] δ 4.69 (d, *J*=7.6 Hz, H-1″), 4.81 (d, *J*=5.5 Hz, H-4), 5.60 (d, *J*=5.5 Hz, H-3), 6.53 (brd, *J*=7.9 Hz, H-7), 6.76 (d, *J*=8.2 Hz, H-5′), 6.80 (brd, *J*=7.9 Hz, H-5), 6.88 (dd, *J*=8.2, 2.0 Hz, H-6′), 7.22 (d, *J*=2.0 Hz, H-2′), 7.40 (dd, *J*=7.9, 7.9 Hz, H-6), [minor diastereoisomer] δ 4.58 (d, *J*=7.6 Hz, H-1″), 4.91 (d, *J*=5.5 Hz, H-4), 5.61 (d, *J*=5.5 Hz, H-3), 6.74 (overlap, H-7), 6.74 (d, *J*=8.2 Hz, H-5′), 6.82 (brd, *J*=7.9 Hz, H-5), 6.86 (dd, *J*=8.2, 2.0 Hz, H-6′), 7.10 (d, *J*=2.0 Hz, H-2′), 7.45 (dd, *J*=7.9, 7.9 Hz, H-6); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS: *m/z* 473 $[M+Na]^+$; HR-FAB-MS: *m/z* 473.1056 (Calcd for $C_{21}H_{22}O_{11}Na$ $[M+Na]^+$: *m/z* 473.1060).

Thunberginol D 3′-*O*-*β*-D-Glucopyranoside (**6**): A white amorphous powder; IR (KBr): ν_{max} 3400, 1680, 1610, 1508, 1073 cm^{-1}; ^{1}H-NMR (methanol-d_4, 600 MHz, *ca.* 1 : 1 mixture of two diastereoisomers): [major diastereoisomer] δ 2.93 (dd, *J*=15.8, 3.4 Hz, H-4a), 3.17 (m, H-4b), 4.78 (d, *J*=7.6 Hz, H-1″), 5.47 (dd like, *J*=11.7, 3.4 Hz, H-3), 6.22 (brs, H-7), 6.26 (brs, H-5), 6.87 (d, *J*=8.2 Hz, H-5′), 7.05 (dd, *J*=8.2, 2.0 Hz, H-6′), 7.33 or 7.35 (d, *J*=2.0 Hz, H-2′), [minor diastereoisomer] δ 2.95 (dd, *J*=15.8, 3.4 Hz, H-4a), 3.17 (m, H-4b), 4.80 (d, *J*=8.3 Hz, H-1″), 5.47 (dd like, *J*=11.7, 3.4 Hz, H-3), 6.22 (brs, H-7), 6.26 (brs, H-5), 6.88 (d, *J*=8.2 Hz, H-5′), 7.07 (dd, *J*=8.2, 2.0 Hz, H-6′), 7.33 or 7.35 (d, *J*=2.0 Hz, H-2′); ^{13}C-NMR: given in Table 1; positive-ion FAB-MS: *m/z* 473 $[M+Na]^+$; HR-FAB-MS: *m/z* 473.1056 (Calcd for $C_{21}H_{22}O_{11}Na$ $[M+Na]^+$: *m/z* 473.1060).

Acid Hydrolysis of 1–6 Compounds **1–6** (1.0 mg each) were dissolved in 5% aqueous H_2SO_4–1,4-dioxane (1 : 1, v/v, 1.0 mL), and each solution was heated at 80°C for 1 h. After cooling, each reaction mixture was neutralized with Amberlite IRA-400 (OH^- form) and filtrated, and the solution was partitioned with EtOAc to give two layers. The aqueous layer was evaporated and then subjected to HPLC analysis to identify the D-glucose under the following conditions: HPLC column, Kaseisorb LC NH_2-60-5, 4.6 mm i.d.×250 mm; detection, optical rotation [Shodex OR-2 (Showa Denko Co., Ltd., Tokyo, Japan)]; mobile phase, MeCN–H_2O (85 : 15, v/v); flow rate, 0.50 mL/min; column temperature, room temperature. Identification of D-glucose was carried out by comparison of its retention time and optical rotation with that of an authentic sample [t_R: 18.9 min (positive optical rotation)].

Effects on Rat Lens Aldose Reductase The experiments were performed as described in our previous reports.[42] The supernatant fluid of rat lens homogenate was used as a crude enzyme. The enzyme suspension was diluted to produce *ca.* 10 nmoL/tube of nicotinamide adenine dinucleotide phosphate (NADP) in the following reaction. The incubation mixture contained phosphate buffer 135 mM (pH 7.0), Li_2SO_4 100 mM, reduced nicotinamide adenine dinucleotide phosphate (NADPH) 0.03 mM, DL-glyceraldehyde 1 mM as a substrate, and 100 μL of enzyme fraction, with 25 μL of sample solution, in a total volume of 0.5 mL. The reaction was initiated by the addition of NADPH at 30°C. After 30 min, the reaction was stopped by the addition of 150 μL of HCl 0.5 M. Then, 0.5 mL of NaOH 6 M containing imidazole 10 mM was added, and the solution was heated at 60°C for 20 min to convert NADP into a fluorescent product. Fluorescence was measured using a fluorescence microplate reader (FLUOstar OPTIMA, BMG Labtechnologies) at an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Each test sample was dissolved in DMSO. Measurements were performed in duplicate, and IC_{50} values were determined graphically.

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References and Note

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