Four New Cycloartane Glycosides from *Aquilegia vulgaris*

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Four new cycloartane glycosides, named aquilegiosides G—J, were isolated from the dried aerial parts of *Aquilegia vulgaris*. Their structures were determined by spectroscopic analysis and chemical evidence.

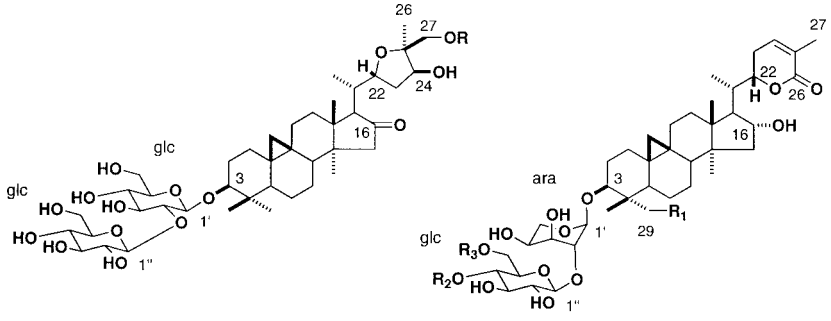
Key words *Aquilegia vulgaris*; cycloartane glycoside; aquilegioside; Ranunculaceae

Aquilegia vulgaris L. (Japanese name, seiyouodamaki) is cultivated as a garden plant. We previously reported on the structural elucidation of six new cycloartane glycosides, aquilegiosides A and B[1] from *A. flabellata* SIEB. *et* ZUCC. var. *flabellata* (Japanese name, odamaki) and aquilegiosides A, B, C, D, E and F[2] from *A. vulgaris*. In a continuing study on the glycosidic constituents, we have now isolated four new cycloartane glycosides, aquilegiosides G (**1**), H (**2**), I (**3**) and J (**4**) from the dried aerial parts of *A. vulgaris*. This paper describes the structural elucidation of the new cycloartane glycosides based on extensive two dimensional (2D) NMR spectroscopic analysis and hydrolysis.

Results and Discussion

The methanolic extract of the air-dried aerial parts of *A. vulgaris* was partitioned into a chloroform–water solvent system. The water-soluble portion was separated by MCI gel CHP20P, octadecyl silica gel (ODS) and silica gel column chromatographies and finally HPLC to give four aquilegiosides (**1**—**4**).

Aquilegioside G (**1**) was obtained as a white powder, $[\alpha]_D$ −30.8° (MeOH). In the negative-ion FAB-MS of **1**, a quasi-molecular ion peak was observed at *m/z* 811 $[M-H]^-$, while its positive-ion FAB-MS showed a quasi-molecular ion peak at *m/z* 835 $[M+Na]^+$. The positive high-resolution (HR)-FAB-MS showed a clustered molecular ion at *m/z* 835.4446 $[C_{42}H_{68}O_{15}Na]^+$. The ^{1}H-NMR spectrum displayed one cyclopropane methylene at δ 0.21 (d, J=3.7 Hz) and 0.49 (d, J=3.7 Hz), five quaternary methyls at δ 0.95, 1.15, 1.18, 1.35 and 1.48, a secondary methyl at δ 1.27 (J=6.7 Hz) and two anomeric protons at δ 4.97 (d, J=7.3 Hz) and 5.41 (d, J=7.3 Hz). The ^{1}H-NMR spectrum of **1** was similar to that of aquilegioside F.[2] In the ^{13}C-NMR spectrum of **1**, the chemical shifts of the aglycon moiety, except for the signals owing to the side-chain and the D-ring, and the sugar moiety showed coincidence with those of aquilegioside F. The NMR data could be assigned with the aid of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation spectroscopy (HMBC) experiments. A sequence of connectives through a secondary methyl proton at δ 1.27, a methine proton at δ 2.24 (m), an oxygen-bearing methine proton at δ 5.10 (br t, J=6.7 Hz), methylene protons at δ 2.18 (1H, ddd, J=3.8, 6.7, 12.8 Hz) and 2.45 (1H, ddd, J=6.7, 6.7, 12.8 Hz) and an oxygen-bearing methine proton at δ 4.53 (overlapped with signals due to the sugar moiety), in turn, was observed in the ^{1}H–^{1}H COSY, and their signals could be assigned to the H_3-21, H-20, H-22, H_2-23 and H-24, respectively. Further, the H-20 coupled with a methine proton at δ 2.86 (d, J=9.2 Hz, H-17). The HMBC led to the terminal structure on the side chain. The quaternary methyl proton at δ 1.48 showed correlation peaks with the carbon signals at δ 67.1 (CH_2, C-27), 78.7 (CH, C-24) and 83.6 (C, C-25). Further, a cross-peak between δ 5.10 (H-22) and δ 83.6 (C-25) resulted in the five-membered epoxy ring between C-22 and C-25 (Fig. 1). Furthermore, the long-range correlations between the H-17 and the carbon signals at δ 19.1 (CH_3, C-18), 36.1 (CH, C-20), 45.5 (C, C-14) and 219.0 (C, C-16) and the methylene protons (H-15) at δ 2.04 (1H, d, J=18.3 Hz) and 2.10 (1H, d, J=18.3 Hz) and the carbon signals at δ 19.9 (CH_3, C-28), 45.5 (C, C-14) and 219.0 (C, C-16) indicated the presence of a C-16 carbonyl group. The C-16 carbonyl group was supported by the coupling pattern with the H-17 signal (d, J=9.2 Hz). The nuclear Overhauser



	R
aquilegioside G (**1**) :	H
aquilegioside H (**2**) :	glc

	R_1	R_2	R_3
aquilegioside I (**3**) :	H	H	glc
aquilegioside J (**4**) :	OH	glc	H

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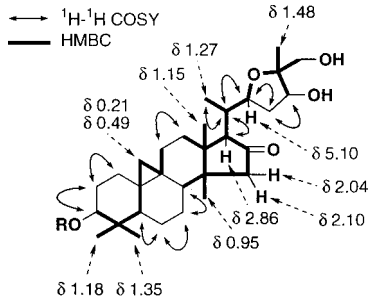

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

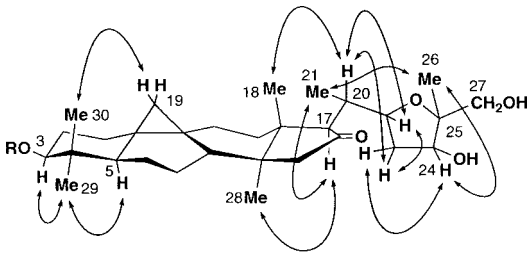

Fig. 2. NOEDS and NOESY Correlations of **1**

effect (NOE) correlations, H-18/H-20, H-20/H-22 and H-23β, H-22/H-23β, H-28/H-17, H-17/H-21, H-21/H-26, H-26/H-24 and H-24/H-23α in the nuclear Overhauser and exchange spectroscopy (NOESY) and nuclear Overhauser effect difference spectrum (NOEDS) suggested 20*S*, 22*R*, 24*S* and 25*S* configurations (Fig. 2).[3] Furthermore, the configuration of the C-3 hydroxyl group was determined to be β-configuration from the fact that NOE was observed between H-3 and H-29, while it was not observed between H-3 and H-30, which showed NOE with H-19 (Fig. 2). On acid hydrolysis, **1** afforded D-glucose, the structure of which was confirmed by the ^{1}H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis, together with several unidentified artificial sapogenols. The anomeric centers of the glucose moieties were determined to be β-configuration from the large $^3J_{H1-H2}$ values. The $^4C_1$-conformation of glucose was shown by comparison of the carbon resonances for monosaccharide. In the HMBC, the anomeric proton signals at δ 4.97 (H-1′) and 5.41 (H-1″) showed long-range correlations with the carbon signals at δ 88.6 (C-3) and 83.4 (C-2′), respectively. From the evidence presented above, the structure of **1** was concluded to be (20*S*,22*R*,24*S*,25*S*)-22,25-epoxy-3β,24,27-trihydroxy-cycloartan-16-one 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Aquilegioside H (**2**) was obtained as a white powder, $[\alpha]_D$ −32.4° (MeOH). The ^{1}H-NMR spectrum displayed one cyclopropane methylene at δ 0.23 (d, J=3.7 Hz) and 0.51 (d, J=3.7 Hz), five quaternary methyls at δ 0.96, 1.15, 1.18, 1.35 and 1.45, a secondary methyl at δ 1.28 (J=6.1 Hz) and three anomeric protons at δ 4.96 (d, J=7.9 Hz), 4.98 (d, J=7.9 Hz) and 5.39 (d, J=7.3 Hz). The ^{1}H-NMR spectrum of **2** was identical with that of **1** with the exception of the H-23, H-24, H-26, H-27 and an additional anomeric proton signals. Furthermore, a comparison of the ^{13}C-NMR spectrum of **2** with that of **1** showed the signal due to C-27, which was shifted remarkably downfield by 7.9 ppm, and additional six carbon signals (105.9, 78.7, 78.4, 75.1, 71.5, 62.6). In the NOESY and NOEDS, the NOE correlations due to the aglycon moiety of **2** showed the same result as that of **1**. Meanwhile, the negative-ion FAB-MS of **2** showed a quasi-molecular ion peak due to $[M-H]^-$ at *m/z* 974, which was higher by 162 mass units than that of **1**. On acid hydrolysis, **2** afforded D-glucose, together with several unidentified artificial sapogenols. The foregoing evidence indicated the presence of an additional glucosyl unit in **2**, which was linked to the C-27 hydroxyl group of an aglycon moiety. The NMR data of **2** showed signals due to a trisaccharide moiety consisting of three glucopyranosyl moieties. The anomeric proton signals at δ 4.96 (H-1′), 4.98 (H-1‴) and 5.39 (H-1″) showed long-range correlations with the carbon signals at δ 88.7 (C-3), 75.0 (C-27) and 83.5 (C-2′), respectively. These long-range correlations showed that the disaccharide and monosaccharide moieties were linked to the C-3 and C-27 hydroxyl groups of an aglycon moiety, respectively. The full structure of **2** was formulated as 27-*O*-β-D-glucopyranosyl (20*S*,22*R*,24*S*,25*S*)-22,25-epoxy-3β,24,27-trihydroxy-cycloartan-16-one 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranoside.

Aquilegioside I (**3**) was obtained as a white powder, $[\alpha]_D$ −1.9° (MeOH). In the positive-ion FAB-MS of **3**, a quasi-molecular ion peak was observed at *m/z* 949 $[M+Na]^+$. The positive HR-FAB-MS showed a clustered molecular ion at *m/z* 949.4765 $[C_{47}H_{74}O_{18}Na]^+$. The ^{1}H-NMR spectrum displayed one cyclopropane methylene at δ 0.27 (d, J=3.7 Hz) and 0.51 (d, J=3.7 Hz), four quaternary methyls at δ 1.05, 1.14, 1.25 and 1.29, a secondary methyl at δ 1.12 (J=6.7 Hz), an olefinic methyl at δ 1.82, three anomeric protons at δ 5.03 (d, J=5.5 Hz), 5.12 (d, J=7.3 Hz) and 5.18 (d, J=7.9 Hz) and an olefinic proton at δ 6.46 (1H, d, J=6.1 Hz). The ^{1}H-NMR spectrum of **3** was identical with that of aquilegioside A[1] with the exception of the H-3, which was shifted to upper field by 0.92 ppm, and an additional quaternary methyl signal. In the ^{13}C-NMR spectrum of **3**, the signals due to the aglycon moiety except for the A-ring and the sugar moiety were in good agreement with those of aquilegioside A. The above data suggested that **3** lost the C-29 hydroxyl group from aquilegioside A. The configuration of the C-3 hydroxyl group bearing a sugar moiety was determined to be β-configuration from the coupling pattern and constant of the H-3 (1H, dd, J=4.3, 11.6 Hz). On acid hydrolysis, **3** afforded D-glucose and L-arabinose, together with several unidentified artificial sapogenols. Furthermore, the negative-ion FAB-MS of **3** gave a $[M-H]^-$ ion peak at *m/z* 925 along with fragment peaks at *m/z* 763 [*m/z* 925−162 (hexose unit)]$^-$, 601 [*m/z* 763−162 (hexose unit)]$^-$ and 469 [*m/z* 601−132 (pentose unit)]$^-$. The foregoing evidence suggested that its sugar moiety was composed of a glycosyl-glycosyl-arabinosyl unit. The anomeric center of the arabinose moiety was determined to be α-configuration from the $^3J_{H1-H2}$ value and the carbon chemical shifts. The signals due to C-3, C-4 and C-5 of the arabinose moiety were shifted to upper field by 1.1, 0.9 and 1.9, respectively, as compared with those of the arabinose moiety in 5α-cholestan-3β-ol 3-*O*-α-L-arabinopyranoside, which indicated the predominance of the $^1C_4$-conformation.[4] In the HMBC, the anomeric proton signals at δ 5.03 (H-1′), 5.12 (H-1‴) and 5.18 (H-1″) showed long-range correlations with the carbon signals at δ 88.8 (C-3) 70.0 (C-6″) and 80.0 (C-2′), respectively. Accord-

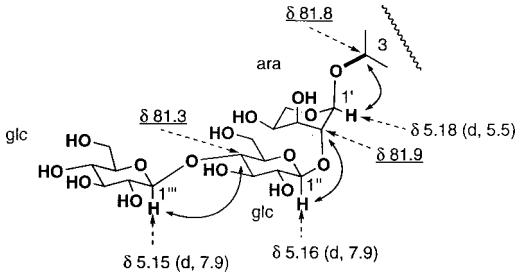

Fig. 3. Long-Range Correlation of the Saccharide Moieties of **4**

J values (Hz) in the ^{1}H-NMR spectra are given in parentheses. Underlined values indicate ^{13}C-NMR chemical shifts.

ingly, the structure of **3** was determined to be 22*S*-3β,16α-dihydroxy-cycloart-24-en-26,22-olide 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside.

Aquilegioside J (**4**) was obtained as a white powder, $[\alpha]_D$ +15.2° (MeOH) and showed the same ion peak at *m/z* 941 due to $[M-H]^-$ as that of aquilegioside A in the negative-ion FAB-MS.[1] The ^{1}H-NMR spectrum displayed one cyclopropane methylene at δ 0.32 (d, *J*=3.7 Hz) and 0.55 (d, *J*=3.7 Hz), three quaternary methyls at δ 1.07, 1.11 and 1.22, a secondary methyl at δ 1.12 (*J*=6.1 Hz), an olefinic methyl at δ 1.82, three anomeric protons at δ 5.15 (d, *J*=7.9 Hz), 5.16 (d, *J*=7.9 Hz) and 5.18 (d, *J*=5.5 Hz) and an olefinic proton at δ 6.45 (1H, d, *J*=6.1 Hz). The ^{1}H-NMR spectrum of **4** was essentially analogous with that of aquilegioside A. In the ^{1}H- and ^{13}C-NMR data of **4**, the signals due to the aglycon moiety were in good agreement with those of aquilegioside A, although the signals due to the sugar moiety were not identical. On acid hydrolysis, **4** afforded D-glucose and L-arabinose, together with several unidentified artificial sapogenols. Furthermore, the negative-ion FAB-MS of **4** gave fragment peaks at *m/z* 779 [*m/z* 941−162 (hexose unit)]$^-$, 617 [*m/z* 779−162 (hexose unit)]$^-$ and 485 [*m/z* 617−132 (pentose unit)]$^-$. The foregoing evidence suggested a glycosyl-glycosyl-arabinosyl sugar unit. The $^1C_4$-conformation of the arabinose moiety was determined by the same procedure as in **3**. The HMBC correlation from each anomeric proton across the glycosidic bond to the carbon of another substituted monosaccharide or the aglycon is shown in Fig. 3. Thus, the structure of **4** was determined to be 22*S*-3β,16α-dihydroxy-cycloart-24-en-26,22-olide 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside.

Table 1. ^{13}C-NMR Data for **1**—**4** (Pyridine-d_5)

	1	**2**	**3**	**4**
C-1	31.9	31.9	32.1	32.2
2	29.9	29.9	29.9	29.4
3	88.6	88.7	88.8	81.8
4	41.3	41.3	41.4	45.6
5	47.4	47.4	47.5	40.9
6	20.9	20.9	21.2	21.0
7	26.1	26.2	26.4	26.3
8	47.1	47.2	48.1	48.2
9	19.2	19.2	19.5	19.4
10	26.6	26.7	26.0	26.1
11	26.4	26.4	26.6	26.6
12	31.5	31.6	33.0	33.0
13	42.3	42.3	46.8	46.8
14	45.5	45.6	47.7	47.7
15	50.8	50.9	48.7	48.7
16	219.0	219.0	77.2	77.2
17	58.8	59.0	57.5	57.6
18	19.1	19.2	19.0	19.0
19	30.0	30.0	30.1	30.3
20	36.1	36.4	39.7	39.7
21	12.0	12.2	13.3	13.3
22	76.6	77.5	79.6	79.6
23	39.5	38.8	28.3	28.3
24	78.7	77.9	140.4	140.4
25	83.6	84.1	128.0	128.0
26	22.5	22.5	166.4	166.4
27	67.1	75.0	17.2	17.2
28	19.9	19.9	20.5	20.5
29	25.7	25.8	26.0	64.1
30	15.3	15.4	15.5	11.9
	glc	glc	ara	ara
C-1′	104.9	104.8	104.5	103.7
2′	83.4	83.5	80.0	81.9
3′	78.4	78.5	73.1	73.8
4′	71.6	71.6	68.1	78.5
5′	78.0	78.0	64.6	65.4
6′	62.8	62.8		
	glc	glc	glc	glc
C-1″	106.0	106.1	105.4	105.7
2″	77.1	77.1	76.0	75.9
3″	78.2	78.2	78.3	76.4
4″	71.7	71.7	71.8	81.3
5″	78.0	78.0	77.0	76.5
6″	62.8	62.8	70.0	62.1
		glc	glc	glc
C-1‴		105.9	105.5	105.3
2‴		75.1	75.3	74.8
3‴		78.7	78.3	78.3
4‴		71.5	71.7	71.6
5‴		78.4	78.4	78.5
6‴		62.6	62.9	62.5

Experimental

Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL alpha 500 NMR spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS were recorded with a JEOL DX-303 HF spectrometer. The HR-FAB-MS were recorded with a JEOL HX-110 spectrometer. HPLC was carried out using a TSK gel-120A (7.8 mm i.d.×30 cm) column with a Tosoh CCPM pump, Tosoh RI-8010 detector and JASCO OR-2090 detector. TLC was performed on pre-coated Kieselgel 60 F_{254} (Merck), and detection was achieved by spraying with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kieselgel (230—400 mesh, Merck), ODS (PrePAK-500/C_{18}, Waters) and MCI gel CHP20P (Mitsubishi Chemical Ind.).

Plant Material The plant seeds defined as the seed of *A. vulgaris* were provided by Sakata Seed Corp., Kanagawa, Japan. The plant seeds were cultivated at the Botanical Garden of Kumamoto University.

Extraction and Isolation The dried aerial parts of *A. vulgaris* (1.3 kg) were extracted with MeOH at room temperature for six months, and the extract (308 g) was partitioned in chloroform and water (1 : 1). The water-soluble portion (221 g) was subjected to MCI gel CHP20P column chromatography with MeOH–H_2O (50→60→70→80→90%) to afford ten fractions (fr. 1—fr. 10). Fraction 3 (726 mg) was further separated by ODS column chromatography with MeOH–H_2O (40→50→60%) to afford two fractions (fr. 11—fr. 12). Fraction 11 was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (3 : 2), to furnish aquilegioside H (**2**) (10 mg). Fraction 6 (843 mg) was further separated by ODS column chromatography with MeOH–H_2O (40→50→60→70%) to afford three fractions (fr. 13—fr. 15). Fraction 13 was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (13 : 9), to furnish aquilegioside G (**1**) (7 mg). Fraction 8 (296 mg) was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (7 : 3), to furnish aquilegio-

side J (**4**) (6 mg). Fraction 9 (128 mg) was subjected to silica gel column chromatography with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), followed by HPLC with MeOH–H_2O (7 : 3), to furnish aquilegioside I (**3**) (6 mg).

Aquilegioside G (**1**): A white powder, $[\alpha]_D^{25}$ −30.8° (c=0.30, MeOH). Pos. FAB-MS (m/z): 835 $[M+Na]^+$. Neg. FAB-MS (m/z): 811 $[M-H]^-$. HR-FAB-MS (m/z): 835.4446 $[M+Na]^+$ (Calcd for $C_{42}H_{68}O_{15}Na$ 835.4456). ^{1}H-NMR (pyridine-d_5): δ: 0.21, 0.49 (each 1H, d, J=3.7 Hz, H_2-19), 0.68 (1H, H-6), 0.95 (3H, s, H_3-28), 1.00 (1H, H-7), 1.07 (3H, H-1, H-11, H-12), 1.15 (3H, s, H_3-18), 1.16 (1H, H-7), 1.18 (3H, s, H_3-30), 1.23 (1H, H-5), 1.27 (3H, d, J=6.7 Hz, H_3-21), 1.35 (3H, s, H_3-29), 1.40 (1H, H-1), 1.48 (3H, s, H_3-26), 1.51 (2H, H-6, H-8), 1.72 (1H, H-12), 1.89 (1H, H-2), 1.91 (1H, H-11), 2.04 (1H, d, J=18.3 Hz, H-15), 2.10 (1H, d, J=18.3 Hz, H-15), 2.18 (1H, ddd, J=3.8, 6.7, 12.8 Hz, H-23β), 2.24 (1H, m, H-20), 2.39 (1H, H-2), 2.45 (1H, ddd, J=6.7, 6.7, 12.8 Hz, H-23α), 2.86 (1H, d, J=9.2 Hz, H-17), 3.46 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.08 (1H, d, J=11.0 Hz, H-27), 4.29 (1H, d, J=11.0 Hz, H-27), 4.53 (1H, overlapped, H-24), 5.10 (1H, br t, J=6.7 Hz, H-22); glc-1′ to glc-6′, 4.97 (1H, d, J=7.3 Hz), 4.27 (1H, dd, J=7.3, 9.2 Hz), 4.32 (1H, dd, J=9.2, 9.2 Hz), 4.18 (1H, dd, J=9.2, 9.2 Hz), 3.92 (1H, m), 4.37 (1H, dd, J=4.9, 11.6 Hz), 4.55 (1H, br d, J=10.8 Hz); glc-1″ to glc-6″, 5.41 (1H, d, J=7.3 Hz), 4.15 (1H, dd, J=7.3, 9.2 Hz), 4.27 (1H, dd, J=9.2, 9.2 Hz), 4.34 (1H, dd, J=9.2, 9.2 Hz), 3.95 (1H, m), 4.46 (1H, dd, J=4.8, 11.6 Hz), 4.52 (1H, br d, J=10.6 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

Aquilegioside H (**2**): A white powder, $[\alpha]_D^{25}$ −32.4° (c=0.50, MeOH). Neg. FAB-MS (m/z): 973 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5): δ: 0.23, 0.51 (each 1H, d, J=3.7 Hz, H_2-19), 0.96 (3H, s, H_3-28), 1.15 (3H, s, H_3-18), 1.18 (3H, s, H_3-30), 1.28 (3H, d, J=6.1 Hz, H_3-21), 1.35 (3H, s, H_3-29), 1.45 (3H, s, H_3-26), 2.08 (1H, overlapped, H-23β), 2.25 (1H, m, H-20), 2.54 (1H, ddd, J=6.7, 6.7, 12.8 Hz, H-23α), 2.83 (1H, d, J=9.2 Hz, H-17), 3.46 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.26 (1H, overlapped, H-27), 4.39 (1H, overlapped, H-27), 4.45 (1H, overlapped, H-24), 5.10 (1H, br t, J=6.7 Hz, H-22); glc-1′ to glc-6′, 4.96 (1H, d, J=7.9 Hz), 4.25 (1H, overlapped), 4.34 (1H, dd, J=9.2, 9.2 Hz), 4.18 (1H, dd, J=9.2, 9.2 Hz), 3.92 (1H, m), 4.37 (1H, overlapped), 4.54 (1H, br d, J=10.9 Hz); glc-1″ to glc-6″, 5.39 (1H, d, J=7.3 Hz), 4.14 (1H, dd, J=7.3, 9.2 Hz), 4.25 (1H, overlapped), 4.32 (1H, dd, J=9.2, 9.2 Hz), 3.95 (1H, m), 4.47 (1H, overlapped), 4.52 (1H, br d, J=11.6 Hz); glc-1‴ to glc-6‴, 4.98 (1H, d, J=7.9 Hz), 4.03 (1H, dd, J=7.9, 9.2 Hz), 4.21 (1H, overlapped), 4.23 (1H, overlapped), 3.95 (1H, m), 4.37 (1H, overlapped), 4.52 (1H, br d, J=11.6 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

Aquilegioside I (**3**): A white powder, $[\alpha]_D^{25}$ −1.9° (c=0.30, MeOH). Pos. FAB-MS (m/z): 949 $[M+Na]^+$. Neg. FAB-MS (m/z): 925 $[M-H]^-$. HR-FAB-MS (m/z): 949.4765 $[M+Na]^+$ (Calcd for $C_{49}H_{80}O_{21}Na$ 949.4773). ^{1}H-NMR (pyridine-d_5): δ: 0.27, 0.51 (each 1H, d, J=3.7 Hz, H_2-19), 1.05 (3H, s, H_3-18), 1.12 (3H, d, J=6.7 Hz, H_3-21), 1.14 (3H, s, H_3-30), 1.25 (3H, s, H_3-28), 1.29 (3H, s, H_3-29), 1.78 (1H, m, H-20), 1.82 (3H, s, H_3-27), 2.02 (1H, overlapped, H-23), 2.49 (1H, dd, J=6.1, 10.3 Hz, H-17), 2.60 (1H, m, H-23), 3.41 (1H, dd, J=4.3, 11.6 Hz, H-3), 4.27 (1H, overlapped, H-16), 5.46 (1H, dd, J=4.0, 12.2 Hz, H-22), 6.46 (1H, d, J=6.1 Hz, H-24); ara-1′ to ara-5′, 5.03 (1H, d, J=5.5 Hz), 4.70 (1H, dd, J=6.1, 6.6 Hz), 4.39 (1H, overlapped), 4.39 (1H, overlapped), 3.77 (1H, br d, J=9.8 Hz), 4.28 (1H, overlapped); glc-1″ to glc-6″, 5.18 (1H, d, J=7.9 Hz), 4.04 (1H, dd, J=7.9, 8.5 Hz), 4.15 (1H, dd, J=8.5, 9.2 Hz), 4.22 (1H, dd, J=9.2, 9.2 Hz), 3.98 (1H, m), 4.44 (1H, dd, J=4.9, 11.6 Hz), 4.74 (1H, dd, J=3.0, 11.6 Hz); glc-1‴ to glc-6‴, 5.12 (1H, d, J=7.3 Hz), 4.05 (1H, dd, J=7.9, 8.5 Hz), 4.26 (1H, dd, J=8.5, 8.5 Hz), 4.30 (1H, dd, J=9.2, 9.2 Hz), 3.97 (1H, m), 4.37 (1H, overlapped), 4.54 (1H, dd, J=3.2, 11.6 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

Aquilegioside J (**4**): A white powder, $[\alpha]_D^{25}$ +15.2° (c=0.30, MeOH). Neg. FAB-MS (m/z): 941 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5): δ: 0.32, 0.55 (each 1H, d, J=3.7 Hz, H_2-19), 1.07 (3H, s, H_3-18), 1.12 (3H, d, J=6.1 Hz, H_3-21), 1.11 (3H, s, H_3-30), 1.22 (3H, s, H_3-28), 1.82 (3H, s, H_3-27), 2.01 (1H, overlapped, H-23), 2.47 (1H, dd, J=6.2, 11.2 Hz, H-17), 2.60 (1H, m, H-23), 4.25 (1H, overlapped, H-16), 4.30 (1H, overlapped, H-3), 5.44 (1H, dd, J=4.0, 12.1 Hz, H-22), 6.45 (1H, d, J=6.1 Hz, H-24); ara-1′ to ara-5′, 5.18 (1H, d, J=5.5 Hz), 4.54 (1H, overlapped), 4.23 (1H, br d, J=9.1 Hz), 4.31 (1H, overlapped), 3.67 (1H, br d, J=11.6 Hz), 4.26 (1H, br d, J=11.7 Hz); glc-1″ to glc-6″, 5.16 (1H, d, J=7.9 Hz), 4.07 (1H, dd, J=7.9, 8.5 Hz), 4.20 (1H, dd, J=8.5, 8.5 Hz), 4.27 (1H, overlapped), 3.81 (1H, m,), 4.42 (1H, br d, J=12.2 Hz), 4.55 (1H, br d, J=11.9 Hz); glc-1‴ to glc-6‴, 5.15 (1H, d, J=7.9 Hz), 4.07 (1H, dd, J=7.9, 8.5 Hz), 4.18 (1H, dd, J=8.5, 8.5 Hz), 4.18 (1H, dd, J=8.5, 8.5 Hz), 4.00 (1H, m), 4.29 (1H, overlapped), 4.54 (1H, br d, J=12.0 Hz). ^{13}C-NMR (pyridine-d_5): Table 1.

Sugar Analysis A solution of each compound (**1**, **2**, **3** or **4**) (1 mg) in 2 N HCl–dioxane (1 : 1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H_2O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C_{18} cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH_3CN–H_2O (3 : 1, 250 ml). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d.×150 mm, Showa Denko, Tokyo, Japan); solvent, CH_3CN–H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detection, refractive index (RI) and optical rotation (OR). t_R (min) of sugars were as follow. **1**: D-glucose 7.4 (+). **2**: D-glucose 7.4 (+). **3**: L-arabinose 6.2 (+), D-glucose 7.4 (+). **4**: L-arabinose 6.2 (+), D-glucose 7.4 (+). [reference: L-arabinose 6.2 (positive optical rotation: +), D-glucose 7.4 (positive optical rotation: +)].

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