Two Labdane Diterpene and Megastigmane Glycosides from *Aquilegia hybrida*

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Two new labdane diterpene glycosides, named aquosides A and B, and a new megastigmane glycoside, named aquoside C, have been isolated from the air-dried aerial parts of *Aquilegia hybrida* together with three known compounds comprising a phenolic glycoside and two flavone C-glycosides. Their structures were determined on the basis of spectroscopic data and chemical evidence.

Key words *Aquilegia hybrida*; labdane diterpene glycoside; megastigmane glycoside; aquoside; Ranunculaceae

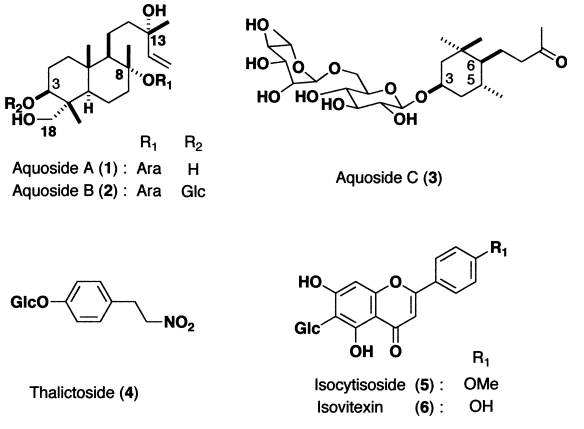
Aquilegia hybrida hort. (Japanese name, seiyouodamaki) is cultivated as a garden plant. Previously, we have reported on the isolation and structural elucidation of ten new cycloartane glycosides, aquilegiosides A and B from *A. flabellata* SIEB. *et* ZUCC. var. *flabellata*[1] (Japanese name, odamaki) and aquilegiosides A, B, C, D, E, F, G, H, I, and J from *A. vulgaris* L.[2,3] (Japanese name, seiyouodamaki). A search of the literature showed that no chemical investigation has been done on *A. hybrida*.

During our investigation on the chemical constituents in the Ranunculaceous plant,[4,5] we have now isolated two new labdane diterpene glycosides, aquosides A (**1**) and B (**2**), and a new megastigmane glycoside, aquoside C (**3**), from the air-dried aerial parts of *A. hybrida* together with three known compounds (**4**—**6**). In this paper, we describe the isolation and stereostructure elucidation based on 2D NMR spectroscopic analysis and hydrolysis.

Results and Discussion

The methanolic extract of the air-dried aerial parts of *A. hybrida* was partitioned into an ethyl acetate–water solvent system. The water-soluble portion was subjected to MCI gel CHP20P and silica gel column chromatographies and finally HPLC to give aquosides A (**1**), B (**2**), C (**3**), and thalictoside[6] (**4**); the insoluble portion was subjected to silica gel column chromatography to give isocytisoside[7] (**5**) and isovitexin[8] (**6**).

Aquoside A (**1**) was obtained as an amorphous powder. The molecular formula of **1**, $C_{25}H_{44}O_8$, was established by the high-resolution (HR)-ESI-MS [*m*/*z* 495.2972, $(M+Na)^+$]. The ^{1}H-NMR (pyridine-d_5, Table 1) spectrum contained signals for four quaternary methyl protons at δ 0.86, 0.94, 1.37, and 1.46, an anomeric proton at δ 4.87 (d, J= 6.3 Hz), and three olefinic protons at δ 5.09 (1H, dd, J= 1.7, 10.9 Hz), 5.54 (1H, dd, J=1.7, 17.2 Hz), and 6.25 (1H, dd, J=10.9, 17.2 Hz). Three signals at δ 5.09, 5.54, and 6.25 were due to a vinyl group. **1** was hydrolyzed with 2 M hydrochloric acid to afford L-arabinopyranose, the structure of which was confirmed by the ^{1}H-NMR coupling pattern and optical rotation using chiral detection in the HPLC analysis. The aglycon decomposed under an acid condition. The ^{13}C-NMR (pyridine-d_5, Table 1) spectrum gave twenty carbon signals due to the aglycon moiety together with an L-arabinopyranosyl unit (δ 97.6, 72.8, 74.5, 69.0, 65.7). The structural assignment was achieved by the ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments. The ^{1}H–^{1}H COSY and HMBC experiments led us to the plane structure of **1** as labda-14-en-3,8,13,18-tetraol 8-*O*-L-arabinopyranoside (Fig. 1). The anomeric center of the L-arabinopyranosyl unit was determined to be α-configuration from the $^3J_{H1-H2}$ value. The presence of an α-L-arabinopyranosyl ($^4C_1$) unit was shown by comparison of the carbon chemical shifts for monosaccharide. The relative stereostructure of the aglycon moiety was characterized by a nuclear Overhauser and exchange spectroscopy (NOESY) experiment, which showed nuclear Overhauser effect (NOE) correlations between the following proton pairs (3α-H and 5α-H; 5α-H and 9α-H; 5α-H and 18-H_3; 11-H and 17-H_3; 17-H_3 and 20-H_3; 19-H_3 and 20-H_3) (Fig. 2). Meanwhile, the B ring and the side chain of the aglycon moiety were superimposable on that of sclareol[9] (13*S*-type) or 13-*epi*-sclareol[9] (13*R*-type). A comparative study of the ^{13}C-NMR ($CDCl_3$, Table 1) spectrum of **1** with that of sclareol (13*S*-type) revealed identical signals due to C-13, C-14, C-15, and C-16 on the side chain. Therefore, the absolute configuration at the 13-position was assigned as *S*. The structure of **1** was elucidated except for the absolute configurations at the 3-, 5-, and 8—10- positions.



Aquoside B (**2**) was obtained as an amorphous powder. The HR-ESI-MS of **2** showed a peak at *m*/*z* 657.3490 corresponding to the molecular formula $[C_{31}H_{54}O_{13}Na]^+$ (Calcd for 657.3462). The ^{1}H-NMR spectrum (pyridine-d_5, Table 1)

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Table 1. ^{1}H- and ^{13}C-NMR Chemical Shifts of **1** and **2**

	δ_H			δ_C		
	1[a]	**1**[b]	**2**[a]	**1**[a]	**1**[b]	**2**[a]
1a	1.05 dt (3.5, 13.2)	1.03 m	10.5 dt (3.4, 13.5)	38.5	37.7	38.5
1b	1.74[c]	1.57[c]	1.71[c]			
2a	1.84 m	1.57[c]	1.89 m	27.5	25.8	25.7
2b	1.89[c]	1.57[c]	2.22 m			
3	4.10 dd (5.2, 11.5)	3.53 dd (8.1, 9.2)	4.25[c]	73.1	74.3	81.7
4				42.9	41.7	43.5
5	1.55[c]	1.05 br d (11.5)	1.78 br d (12.0)	48.1	48.2	47.5
6a	1.28[c]	1.23[c]	1.29 m	20.4	19.4	20.3
6b	1.75[c]	1.45[c]	1.82 m			
7a	1.89[c]	1.42[c]	1.96[c]	40.0	39.2	40.0
7b	2.11[c]	1.87 m	2.12[c]			
8				81.5	82.1	81.6
9	1.28[c]	1.16[c]	1.33 br s	59.9	59.5	59.9
10				39.8	38.7	39.0
11a	1.55[c]	1.22[c]	1.56 m	20.0	19.0	19.7
11b	1.99 tt (3.5, 12.6)	1.59[c]	2.02 m			
12a	1.68 dt (4.6, 12.6)	1.52 m	1.91[c]	46.3	44.3	46.3
12b	2.13[c]	1.60[c]	2.12[c]			
13				73.1	73.1	73.2
14	6.25 dd (10.9, 17.2)	5.83 dd (10.9, 17.2)	6.23 dd (10.9, 17.1)	147.5	146.0	147.6
15a	5.09 d (10.9)	4.91 d (10.9)	5.14 d (10.9)	111.0	110.7	111.0
15b	5.54 d (17.2)	5.11 d (17.2)	5.61 d (17.1)			
16	1.46 s	1.17 s	1.49 s	28.4	25.2	28.4
17	1.37 s	1.16 s	1.41 s	22.0	20.9	22.1
18a	3.60 d (10.9)	3.25 d (10.9)	3.66 d (10.7)	67.6	68.9	64.6
18b	4.09 d (10.9)	3.48 d (10.9)	4.35 d (10.7)			
19	0.94 s	0.67 s	0.92 s	12.8	11.3	13.3
20	0.86 s	0.80 s	0.86 s	16.4	15.9	16.6
Ara						
1	4.87 d (6.3)	4.33 d (6.9)	4.89 d (6.4)	97.6	96.1	97.6
2	4.30 dd (6.3, 8.6)	3.41 dd (6.9, 8.6)	4.32 dd (6.4, 8.6)	72.8	71.1	72.9
3	4.17 dd (3.5, 8.6)	3.48 dd (2.9, 8.6)	4.18 dd (3.5, 8.6)	74.5	72.8	74.5
4	4.28 ddd (1.8, 3.5, 4.0)	3.78 ddd (1.7, 2.9, 3.5)	4.30 br s	69.0	67.6	69.0
5a	3.69 dd (1.8, 12.1)	3.42 dd (1.7, 12.6)	3.70 dd (1.8, 12.1)	65.7	64.8	65.7
5b	4.21 dd (4.0, 12.1	3.80 dd (3.5, 12.6)	4.25[c]			
Glc						
1			5.16 d (8.0)			105.8
2			4.03 dd (8.0, 8.6)			75.8
3			4.19 dd (8.6, 8.6)			78.7
4			4.24 dd (8.6, 8.6)			71.6
5			3.93 ddd (2.3, 5.2, 8.6)			78.4
6a			4.41 dd (5.2, 11.5)			62.8
6b			4.53 dd (2.3, 11.5)			

a) Measured in pyridine-d_5. *b*) Measured in $CDCl_3$. *c*) Overlapped.

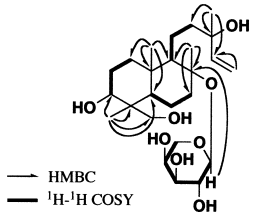

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations of **1**

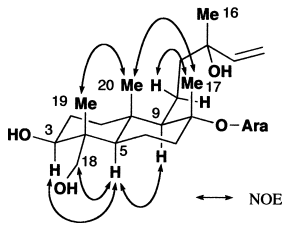

Fig. 2. NOE Correlations of **1**

of **2** was identical with that of **1**, with the exception of an additional anomeric proton [δ 5.16 (d, J=8.0 Hz)]. A comparison of the ^{13}C-NMR (pyridine-d_5, Table 1) spectrum of **2** with that of **1** showed the signal due to C-3, which was shifted remarkably downfield by 8.1 ppm, and additional six carbon signals (δ 105.8, 78.7, 78.4, 75.8, 71.6, 62.8). On acid hydrolysis, **2** afforded L-arabinopyranose and D-glucopyranose. The anomeric center of the D-glucopyranosyl unit was determined to be β-configuration from the $^{3}J_{H1-H2}$ value. The presence of a β-D-glucopyranosyl ($^{4}C_{1}$) unit was shown by comparison of the carbon chemical shifts for monosaccharide. The ^{1}H–^{1}H COSY and HMBC experiments led us to the same plane structure as that of **1** except that the β-D-glucopyranosyl unit linked the hydroxyl group at C-3. The fore-

going evidence indicated that the 8-position of **2** and **1** showed the selfsame absolute configuration, because glycosylation shifts at C-7 (β-C), C-8 (α-C), and C-9 (β'-C) were caused by α-L-arabinopyranose.[10] In a NOESY experiment, NOE due to the aglycon moiety of **2** showed the same correlations as that of **1**. This result suggested that the aglycon moiety of **2** had the same absolute configuration as **1** had. Meanwhile, a comparative study of the ^{13}C-NMR (pyridine-d_5, Table 1) spectrum of **2** with that of **1** revealed that the 2-position (β-C) and 4-position (β'-C) of the former were shifted to a higher field by 1.8 ppm and to a lower field by 0.6 ppm, respectively, to the latter (glycosylation shifts[11,12]). Consequently, the 3-position of **2** determined *S* configuration. The absolute stereostructure was elucidated to be 3-*O*-β-D-glucopyranosyl (3*S*,5*R*,8*R*,9*R*,10*S*,13*S*)-labda-14-en-3,8,13,18-tetraol 8-*O*-α-L-arabinopyranoside.

Aquoside C (**3**) showed a clustered molecular ion at *m/z* 543.2817 $[C_{25}H_{44}O_{11}Na]^+$ in the HR-ESI-MS. The ^{1}H-NMR (CD_3OD) spectrum showed three quaternary methyl groups (δ 0.88, 0.99, 2.13), a secondary methyl group [δ 0.92 (d, J=6.9 Hz)], and two anomeric protons [δ 4.26 (d, J=7.5 Hz), 4.73 (d, J=1.7 Hz)]. Acid hydrolysis of **3** gave L-rhamnopyronose and D-glucopyronose, together with several unidentified artificial aglycons. The anomeric configuration of L-rhamonopyranose could not be deduced from the $^3J_{H1-H2}$ value. However, the ^{13}C shifts of the L-rhamonopyranose were superimposable on those of methyl α-L-rhamonopyranoside. Therefore, a terminal α-L-rhamonopyranosyl unit showed $^1C_4$ conformation. The ^{1}H–^{1}H COSY and HMBC experiments indicated that the plane structure of **3** was identical with that of sedumoside G[13] (Fig. 3). However, a comparison of the ^{13}C-NMR (CD_3OD) spectrum of **3** with that of sedumoside G revealed different signals on the fringe of the 3-position. Meanwhile, the relative stereostructure of the aglycon moiety was characterized by a NOESY and nuclear Overhauser effect difference spectroscopy (NOEDS) experiment, which showed NOE correlations between the following proton pairs (2β-H and 11-H$_3$; 2α-H and 3α-H; 3α-H and 4α-H; 4a-H and 13-H$_3$; 5β-H and 11-H$_3$; 6α-H and 12-H$_3$; 6α-H and 13-H$_3$; 7-H and 11-H$_3$) (Fig. 4). These NOE correlations suggested the configuration of the 3-hydroxyl as β. Consequently, **3** was elucidated to be isosarmentol G 3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside.

The plant of *A. hybrida* has not previously been the subject of phytochemical analysis. In regard to the chemical constituents of the genus *Aquilegia* plants, ten cycloartan-type triterpene glycosides have been elucidated from *A. flabellate* and *A. vulgaris*. Two labdane-type diterpene glycosides and megastigmane-type glycoside were isolated from the genus *Aquilegia* plants for the first time.

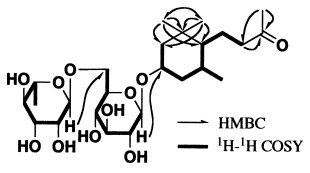

Fig. 3. ^{1}H–^{1}H COSY and HMBC Correlations of **3**

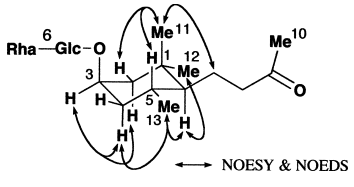

Fig. 4. NOE Correlations of **3**

Experimental

General Procedure Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter. The NMR spectra were measured with a JEOL ECA 500 NMR spectrometer. Chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The HR-ESI-MS was recorded with a JEOL JMS-T100LP spectrometer. HPLC was carried out using the TSK gel-120A (7.8 mm i.d.×300 mm) 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) and MCI gel CHP20P (Mitsubishi Chemical Ind.)

Plant Material The plant seeds defined as those of *Aquilegia hybrida* hort. were provided by Sakata Seed Corp., Kanagawa, Japan. They were cultivated at the Botanical Garden of Kumamoto University.

Extraction and Isolation The air-dried aerial part of *Aquilegia hybrida* (896 g) was extracted with MeOH at room temperature for one month. The MeOH extract (97 g) was partitioned between ethyl acetate-soluble (11 g), water-soluble (79 g), and insoluble (7 g) portions. The insoluble portion was subjected to silica gel column chromatography [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, v/v/v)] to furnish isocytisoside (**5**, 4.6 g) and isovitexin (**6**, 80 mg). The water-soluble portion (79 g) was subjected to MCI gel CHP20P column chromatography [MeOH–H_2O (10 : 90→30 : 70→50 : 50→70 : 30, v/v)→MeOH] to afford four fractions [Fractions 1 (2.8 g), 2 (375 mg), 3 (523 mg), and 4 (1.0 g)]. Fraction 1 (2.8 g) was further separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, v/v/v)], followed by HPLC [MeOH–H_2O (15 : 85, v/v)] to furnish thalictoside (**4**, 430 mg). Fraction 2 (375 mg) was further separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v/v)], followed by HPLC [MeOH–H_2O (50 : 50, v/v)] to furnish aquoside C (**3**, 11 mg). Fraction 3 (523 mg) was further separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (6 : 4 : 0.5, v/v/v)], followed by HPLC [MeOH–H_2O (50 : 50, v/v)] to furnish aquoside B (**2**, 7 mg). Fraction 4 (1.0 g) was further separated by silica gel column chromatography [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v/v)], followed by HPLC [MeOH–H_2O (50 : 50, v/v)] to furnish aquoside A (**1**, 13 mg).

Aquoside A (**1**): Amorphous powder; $[\alpha]_D$=−6.1° (c=0.63, MeOH); HR-ESI-MS *m/z* 495.2972 (M+Na; Calcd for $C_{25}H_{44}O_8Na$, 495.2934); ^{1}H- and ^{13}C-NMR data (Table 1).

Aquoside B (**2**): Amorphous powder; $[\alpha]_D$=−8.5° (c=0.56, MeOH); HR-ESI-MS *m/z* 657.3490 (M+Na; Calcd for $C_{31}H_{54}O_{13}Na$, 657.3462); ^{1}H- and ^{13}C-NMR data (Table 1).

Aquoside C (**3**): Amorphous powder; $[\alpha]_D$=−36.8° (c=0.55, MeOH); HR-ESI-MS *m/z* 543.2817 (M+Na; Calcd for $C_{25}H_{44}O_{11}Na$, 543.2781); ^{1}H-NMR (CD_3OD) δ: 0.65 (1H, ddd, J=2.3, 5.7, 8.6 Hz, 6-H), 0.88 (3H, s, 12-H$_3$), 0.92 (3H, d, J=6.9 Hz, 13-H$_3$), 0.99 (3H, s, 11-H$_3$), 1.12 (1H, ddd, J=2.9, 11.5, 14.3 Hz, 4β-H), 1.25 (3H, d, J=6.3 Hz, rha 6-H$_3$), 1.35 (1H, br dd, J=4.1, 13.7 Hz, 2α-H), 1.38 (1H, m, 7-H), 1.74 (1H, m, 7-H), 1.80 (2H, overlapped, 2β-H, 5β-H), 1.90 (1H, ddd, J=3.1, 5.4, 14.3 Hz, 4α-H), 2.13 (3H, s, 10-H$_3$), 2.46 (1H, ddd, J=5.8, 10.9, 16.6 Hz, 8-H), 2.62 (1H, ddd, J=5.2, 11.5, 16.6 Hz, 8-H), 3.13 (1H, dd, J=7.5, 9.2 Hz, glc 2-H), 3.25 (1H, dd, J=9.2, 9.2 Hz, glc 4-H), 3.33 (1H, dd, J=9.2, 9.2 Hz, glc 3-H), 3.36 (2H, overlapped, glc 5-H, rha 4-H), 3.58 (1H, dd, J=6.3, 11.5 Hz, glc 6-H), 3.65 (1H, m, rha 5-H), 3.67 (1H, dd, J=3.5, 9.8 Hz, rha 3-H), 3.83 (1H, dd, J=1.7, 3.5 Hz, rha 2-H), 3.93 (1H, m, 3-H), 3.96 (1H, dd, J=1.2, 11.5 Hz, glc 6-H), 4.26 (1H, d, J=7.5 Hz, glc 1-H), 4.73 (1H, d, J=1.7 Hz, rha 1-H). ^{13}C-NMR (CD_3OD) δ: 35.5 (1-C), 46.8 (2-C), 76.5 (3-C), 40.8 (4-C), 30.2 (5-C), 54.2 (6-C), 24.2 (7-C), 46.5 (8-C), 212.9 (9-C), 30.0 (10-C), 23.4 (11-C), 32.1 (12-C), 21.3 (13-C), 103.2 (glc 1-C), 75.3 (glc 2-C), 78.3 (glc 3-C), 71.9 (glc 4-C), 76.8 (glc 5-C), 68.5 (glc 6-C), 102.4 (rha 1-C), 72.2 (rha 2-C), 72.4 (rha 3-C), 74.0 (rha 4-C), 69.9 (rha 5-C), 18.2 (rha 6-C).

Sugar Analysis A solution of each compound (**1**, **2**, or **3**) (1 mg) in 2 M 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 μl). 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, optical rotation (OR). The t_R (min) of the sugars was as follows. **1**: L-arabinose 6.2 (+), **2**: L-arabinose 6.2 (+), D-glucose 7.4 (+), **3**: L-rhamnose 4.7 (−), D-glucose 7.4 (+). [reference: L-rhamnose 4.7 (negative optical rotation: −), L-arabiose 6.2 (positive optical rotation: +), D-glucose 7.4 (positive optical rotation: +)].

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