

Two New Cycloartane Glycosides from the Underground Parts of *Aquilegia vulgaris*

Hitoshi YOSHIMITSU,^{*,a} Makiko NISHIDA,^b and Toshihiro NOHARA^a

^a Faculty of Pharmaceutical Sciences, Sojo University; 4-22-1 Ikeda, Kumamoto 862-0082, Japan; and ^b Faculty of Home Economics, Kyushu Women's University; 1-1 Jiyugaoka, Yahatanishi-ku, Kitakyushu 807-8586, Japan.

Received July 22, 2008; accepted August 21, 2008; published online September 2, 2008

Two new cycloartane glycosides, named aquilegiosides K and L, have been isolated from the dried underground parts of *Aquilegia vulgaris*. Their structures were determined by two dimensional (2D) NMR spectroscopic analysis and chemical evidence.

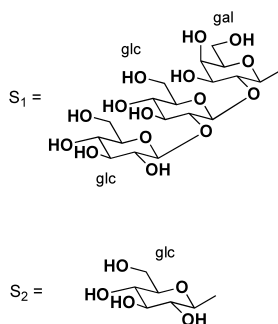
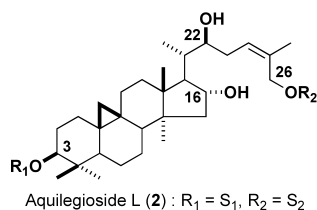
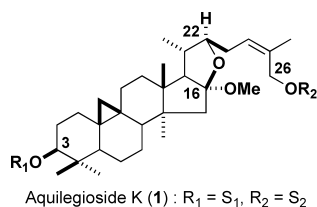
Key words *Aquilegia vulgaris*; cycloartane glycoside; aquilegioside; Ranunculaceae

Aquilegia vulgaris L. (Japanese name, seiyoudamaki) is cultivated as a garden plant. Previously, we have reported on the isolation and structural elucidation of eight cycloartane glycosides, aquilegiosides C, D, E, F, G, H, I, and J from the aerial parts of *A. vulgaris*.^{1,2)} During our investigation on the chemical constituents in the Ranunculaceous plant,^{3,4)} we have now isolated two new cycloartane glycosides, aquilegiosides K (**1**) and L (**2**), from the underground parts of *A. vulgaris*. In this paper, we describe the isolation and structural elucidation based on two dimensional (2D) NMR spectroscopic analysis and hydrolysis.

Results and Discussion

The methanolic extract of the air-dried underground parts of *A. vulgaris* was subjected to MCI gel CHP20P, octadecyl silica gel (ODS) and silica gel column chromatographies and finally HPLC to give aquilegiosides K (**1**) and L (**2**).

Aquilegioside K (**1**) was obtained as an amorphous powder. The molecular formula of **1**, C₅₅H₉₀O₂₄, was established by the high-resolution (HR)-ESI-MS [*m/z* 1157.5752, (M+Na)⁺]. The ¹H-NMR spectrum contained signals for a cyclopropane methylene proton at δ 0.06 (1H, d, *J*=4.0 Hz) and 0.42 (1H, d, *J*=4.0 Hz), five quaternary methyl protons at δ 1.06, 1.08, 1.14, 1.27 and 1.94, a secondary methyl proton at δ 1.16 (d, *J*=6.9 Hz), a methoxy proton at δ 3.34, four anomeric protons at δ 4.83 (d, *J*=7.4 Hz), 4.91 (d, *J*=7.4 Hz), 5.31 (d, *J*=7.5 Hz), and 5.44 (d, *J*=7.5 Hz), and an olefinic proton at δ 5.70 (1H, dd, *J*=6.3, 6.8 Hz). The ¹H-NMR spectrum of **1** was similar to that of aquilegioside C.¹⁾



In the ¹³C-NMR (Table 1) spectrum of **1**, the chemical shifts showed coincidence with those of aquilegioside C, with the exception of the signals owing to the terminal on the side chain of the aglycon and sugar moieties. The structural assignment was achieved by a ¹H-¹H correlation spectroscopy (COSY), total correlation spectroscopy (TOCSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiments. A sequence of the connectives through a secondary methyl proton at δ 1.16, a methine proton at δ 2.37 (m), an oxygen-bearing methine proton at δ 4.42 (ddd, *J*=4.6, 4.6, 8.1 Hz), a methylene proton at δ 2.43 (1H, ddd, *J*=4.6, 6.8, 13.8 Hz) and 2.54 (1H, ddd, *J*=6.3, 8.1, 13.8 Hz), and an olefinic proton at δ

Table 1. ¹³C-NMR Chemical Shifts of **1** and **2**

	1	2		1	2
1	31.8	31.8	3-O-	gal	gal
2	29.6	29.7	1'	105.1	105.0
3	88.6	88.7	2'	79.9	79.9
4	41.1	41.1	3'	74.8	74.8
5	47.2	47.3	4'	69.9	69.9
6	20.6	20.8	5'	76.3	76.3
7	26.0	26.1	6'	62.4	62.4
8	47.4	47.8		glc	glc
9	19.2	19.2	1''	102.6	102.6
10	26.3	26.1	2''	85.2	85.2
11	26.6	26.5	3''	78.1	78.0
12	31.3	32.9	4''	71.1	71.1
13	44.1	46.6	5''	77.5	77.5
14	49.1	47.6	6''	62.1	62.1
15	45.1	47.1		glc	glc
16	119.4	75.5	1'''	105.8	105.8
17	69.1	58.2	2'''	76.1	76.0
18	19.2	19.5	3'''	77.3	77.3
19	30.2	29.7	4'''	70.8	70.8
20	34.6	39.0	5'''	78.7	78.7
21	17.6	12.6	6'''	61.7	61.8
22	86.6	73.2	26-O-	glc	glc
23	30.8	33.1	1''''	102.4	102.5
24	128.3	128.3	2''''	74.7	74.7
25	132.8	132.8	3''''	78.1	78.0
26	67.1	67.2	4''''	71.3	71.3
27	21.8	21.9	5''''	78.1	78.0
28	19.2	20.3	6''''	62.4	62.3
29	25.5	25.4			
30	15.1	15.2			
OMe	49.9				

a) Measured in pyridine-d₅.

5.70, in turn, was observed in the ^1H - ^1H COSY experiment, and their signals could be assigned to the 21- H_3 , 20-H, 22-H, 23- H_2 , and 24-H, respectively. In the HMBC experiment, the long-range correlations between the quaternary methyl proton at δ 1.94 and the carbon signals at δ 67.1 (CH_2), 128.3 (CH, 24-C), and 132.8 (C) indicated that both the quaternary methyl and hydroxy-methyl groups attached to the olefinic carbon and located at the terminal on the side chain. In addition, the long-range correlation cross-peaks between the methine proton at δ 2.39 (br s) and the carbon signals at δ 17.6 (CH_3 , 21-C), 19.2 (CH_3 , 18-C), 86.6 (CH, 22-C), and 119.4 (C); the oxygen-bearing methine proton at δ 4.42 (22-H) and δ 119.4 (C); the methoxy proton at δ 3.34 and δ 119.4 (C) resulted in the five-membered acetal ring which is constructed by an ether bond between 16-C and 22-C (Fig. 1). The 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 (3-H and 29- H_3 ; 8-H and 18- H_3 ; 8-H and 19- H_2 ; 15 α -H and 28- H_3 ; 15 α -H and OMe; 17-H and 21- H_3 ; 17-H and 22-H; 17-H and 28- H_3 ; 18- H_3 and 19- H_2 ; 18- H_3 and 20-H; 19- H_2 and 30- H_3 ; 21- H_3 and OMe; 21- H_3 and 22-H; H-24 and 27- H_3 ; 28- H_3 and OMe) (Fig. 2). This result suggested 3*S*, 16*S*, 17*R*, 20*S*, and 22*R* configurations and 24*Z*. **1** was hydrolyzed with 1 M hydrochloric acid to afford D-glucopyranose and D-galactopyranose, whose structures were confirmed by the ^1H -NMR data and an optical rotation using a chiral detection in the HPLC analysis. The aglycon decomposed under an acid condition. The anomeric centers of the D-glucopyranosyl and D-galactopyranosyl units were determined to be both β -configurations from the $^3J_{\text{H1-H2}}$ value. In the HMBC experiment, long-range correlations were observed between the anomeric proton (δ 4.83) of terminal D-glucopyranosyl unit and the 26-C (δ 67.1), the anomeric proton (δ 4.91) of 2-substituted D-galactopyranosyl unit and the 3-C (δ 88.6), the anomeric proton (δ 5.31) of terminal D-glucopyranosyl unit and the 2-C (δ 85.2) of 2-substituted D-glucopyranosyl unit, and the anomeric proton (δ 5.44) of 2-substituted D-glucopyranosyl unit and the 2-C (δ 79.9) of 2-substituted D-galactopyranosyl unit. Meanwhile, the presence of a 2-substituted β -D-galactopyranosyl ($^4\text{C}_1$) unit, a 2-substituted β -D-glucopyranosyl ($^4\text{C}_1$) unit, and two terminal β -D-glucopyranosyl units was shown by comparison of the carbon chemical shifts for each monosaccharide (Fig. 3). Therefore, the structure of **1** was elucidated to be 26-*O*- β -D-glucopyranosyl (16*S*,20*S*,22*R*,24*Z*)-16 β ,22-epoxy-16 α -methoxy-cycloart-24-en-3 β ,26-diol 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranoside.

Aquilegioside L (**2**) was obtained as an amorphous powder. The HR-ESI-MS of **2** showed a peak at m/z 1145.5751 corresponding to the molecular formula $[\text{C}_{34}\text{H}_{90}\text{O}_{24}\text{Na}]^+$ (Calcd for 1145.5720). The ^1H -NMR spectrum of **2** was similar to that of **1** except for the disappearance of a methoxy proton. In the ^{13}C -NMR (Table 1) spectrum of **2**, the chemical shifts showed coincidence with those of **1**, with the exception of the signals owing to the side-chain and D-ring of the aglycon moiety. In the ^1H - ^1H COSY experiment, a sequence of the connectives through an olefinic proton at δ 5.74 (t, $J=6.9$ Hz), a methylene proton at δ 2.41 (ddd, $J=5.7, 6.9, 13.8$ Hz) and 2.72 (ddd, $J=6.9, 8.0, 13.8$ Hz), and

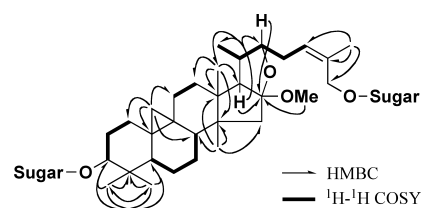


Fig. 1. ^1H - ^1H COSY and HMBC Correlations of **1**

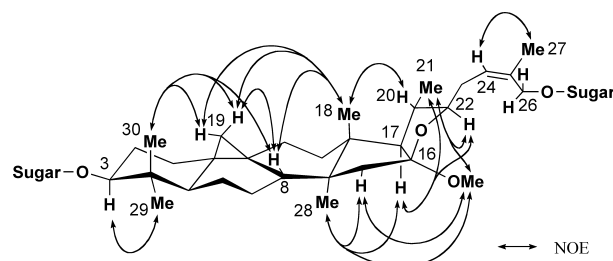


Fig. 2. NOE Correlations of **1**

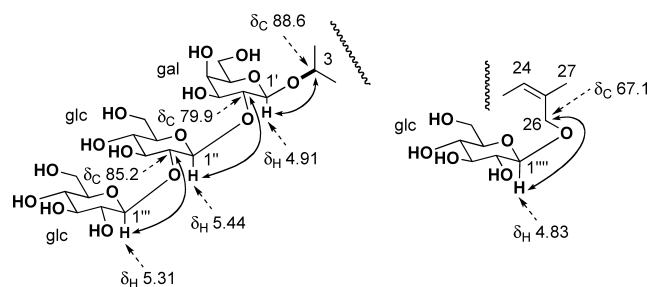


Fig. 3. ^1H - ^{13}C Long-Range Correlation of the Saccharide Moieties of **1**

an oxygen-bearing methine proton at δ 4.34 (m), in turn, was observed, and their signals could be assigned to the 24- H , 23- H_2 , and 22- H , respectively. The long-range correlations between the quaternary methyl proton at δ 1.89 and the carbon signals at δ 67.2 (CH_2), 128.3 (CH, 24-C), and 132.8 (C) indicated that both the quaternary methyl and hydroxy-methyl groups attached to the olefinic carbon and located at the terminal on the side chain. In addition, the long-range correlation cross-peaks between a secondary methyl proton at δ 1.10 (d, $J=6.3$ Hz), and the carbon signals at δ 39.0 (CH), 58.2 (CH), and 73.2 (CH, 22-C) were observed in the HMBC experiment. Furthermore, a sequence of the connectives through a secondary methyl proton at δ 1.10 (d, $J=6.3$ Hz), a methine proton at δ 1.84 (m), a methine proton at δ 2.28 (dd, $J=6.7, 10.3$ Hz), an oxygen-bearing methine proton at δ 4.45 (m), and a methylene proton at δ 1.67 (overlapping) and 1.87 (overlapping) was observed, and their signals could be assigned to the 21- H_3 , 20-H, 17-H, 16-H, and 15- H_2 , respectively. In a NOESY experiment, NOE correlations between the following proton pairs (3-H and 29- H_3 ; 8-H and 18- H_3 ; 8-H and 19- H_2 ; 16-H and 18- H_3 ; 17-H and 21- H_3 ; 17-H and 28- H_3 ; 18- H_3 and 19- H_2 ; 18- H_3 and 20-H; 19- H_2 and 30- H_3 ; 24-H and 27- H_3) were suggested 3*S*, 16*R*, 17*R*, and 20*S* configurations and 24*Z*. Meanwhile, the configuration of the 22-position was determined by a comparison of the ^{13}C -NMR spectrum of **2** with that of the known compounds (22*S*-type: squarroside I⁵) and 22*R*-type: cyclopassi-

floic acid D⁶). The 22-positions of squarroside I and cyclopassifloic acid D were resonated in the range at δ 72.7 and 69.7, respectively. Therefore, the absolute configuration at the 22-position was assigned as *S*, because the 22-position of **2** showed a chemical shift at δ 73.2. On acid hydrolysis, **2** afforded D-glucopyranose and D-galactopyranose. The ¹H-¹H COSY, TOCSY and HMBC experiments led us to the same structure of the sugar moiety as that of **1**. Consequently, the structure of **2** was elucidated to be 26-*O*- β -D-glucopyranosyl (2*S*,2*Z*)-cycloart-24-en-3 β ,16 α ,26-triol 3-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-galactopyranoside.

In regard to the chemical constituents of the genus *Aquilegia* plants, ten cycloartan-type triterpene glycosides have been elucidated from *A. flabellate*⁷⁾ and *A. vulgaris*,^{1,2)} and two labdane-type diterpene glycosides and a megastigmane-type glycoside have been isolated from *A. hybrida*.⁸⁾ A cycloartane glycoside possessing a five-membered acetal ring, which is constructed by an ether bond between 16-C and 22-C, has been isolated only from the *A. vulgaris*. It is curious that 2*S*-type and 2*R*-type were isolated from the aerial and underground parts, respectively.

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. The chemical shifts (δ) are reported in parts per million (ppm) and *J* values in Hz, using pyridine-*d*₅ for ¹H-NMR (7.20 ppm) and ¹³C-NMR (123.5 ppm) as an internal standard. The HR-ESI-MS was recorded with a JEOL JMS-T100LP spectrometer. HPLC was carried out using the Mightysil RP-18 (10.0 mm i.d. \times 250 mm, Kanto Chemical Co., Ltd., Tokyo, Japan); column with a Tosoh CCPM pump, Tosoh RI-8010 detector, and JASCO OR-2090 detector. TLC was performed on pre-coated Kieselgel 60 F₂₅₄ (Merck Ltd., Tokyo, Japan), and detection was achieved by spraying with 10% H₂SO₄ followed by heating. Column chromatography was carried out on Kieselgel (230–400 mesh, Merck Ltd., Tokyo, Japan) and MCI gel CHP20P (Mitsubishi Chemical Co., Tokyo, Japan).

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

Extraction and Isolation The underground part of *Aquilegia vulgaris* (412 g) was extracted with MeOH at room temperature for one month. The MeOH extract (97 g) was subjected to MCI gel CHP20P column chromatography [MeOH-H₂O (10:90 \rightarrow 30:70 \rightarrow 40:60 \rightarrow 50:50 \rightarrow 60:40 \rightarrow 70:30 \rightarrow 80:20, v/v) \rightarrow MeOH] to afford four fractions [Fractions 1 (1.2 g), 2 (1.0 g), 3 (286 mg), and 4 (296 mg)]. Fraction 3 (286 mg) was further separated by ODS column chromatography [MeOH-H₂O (50:50 \rightarrow 60:40 \rightarrow 70:30, v/v)] and silica gel column chromatography [CHCl₃-MeOH-H₂O (6:4:0.5, v/v/v)], followed by HPLC [MeOH-H₂O (60:40, v/v)] to furnish aquilegioside L (**2**, 7 mg). Fraction 4 (296 mg) was further separated by ODS column chromatography [MeOH-H₂O (50:50 \rightarrow 60:40 \rightarrow 70:30, v/v)] and silica gel column chromatography [CHCl₃-MeOH-H₂O (6:4:0.5, v/v/v)], followed by HPLC [MeOH-H₂O (65:35, v/v)] to furnish aquilegioside K (**1**, 5 mg).

Aquilegioside K (**1**): Amorphous powder; [α]_D -9.4° (*c*=0.43, MeOH); HR-ESI-MS *m/z* 1157.5752 (M+Na; Calcd for C₅₃H₉₀O₂₄Na, 1157.5720); ¹H-NMR (pyridine-*d*₅) δ : 5.70 (1H, dd, *J*=6.3, 6.8 Hz, 24-H), 5.44 (1H, d, *J*=7.5 Hz, glc 1''-H), 5.31 (1H, d, *J*=7.5 Hz, glc 1'''-H), 4.91 (1H, d, *J*=7.4 Hz, gal 1'-H), 4.83 (1H, d, *J*=7.4 Hz, glc 1''''-H), 4.68 (1H, d, *J*=12.0 Hz, 26-H), 4.66 (1H, dd, *J*=7.4, 9.2 Hz, gal 2'-H), 4.52 (1H, dd, *J*=1.7, 11.5 Hz, glc 6''-H), 4.51 (1H, brs, gal 4'-H), 4.50 (overlapping, 26-H, glc 6'''-H), 4.47 (1H, dd, *J*=2.9, 11.5 Hz, gal 6'-H), 4.46 (1H, dd, *J*=3.4, 9.2 Hz, gal 3'-H), 4.42 (1H, ddd, *J*=4.6, 4.6, 8.1 Hz, 22-H), 4.38 (1H, dd, *J*=2.3, 11.5 Hz, glc 6''-H), 4.33 (1H, dd, *J*=5.7, 11.5 Hz, gal 6'-H), 4.31 (1H, dd, *J*=5.5, 11.5 Hz, glc 6'''-H), 4.27 (1H, dd, *J*=5.7, 11.5 Hz, glc 6''''-H), 4.24 (1H, dd, *J*=5.9, 11.5 Hz, glc 6''-H), 4.22 (1H, dd, *J*=9.2, 9.2 Hz, glc 3'''-H), 4.19 (1H, dd, *J*=9.2, 9.2 Hz, glc 3''-H), 4.16 (1H, dd, *J*=9.2, 9.2 Hz, glc 4''''-H), 4.14 (1H, dd, *J*=9.2, 9.2 Hz, glc 3''-H), 4.11 (1H, dd, *J*=7.5, 9.2 Hz, glc 2''-H), 4.10 (overlapping, gal 5'-H), 4.09 (1H, dd, *J*=9.2, 9.2 Hz,

glc 4''-H), 4.07 (1H, dd, *J*=9.2, 9.2 Hz, glc 4''-H), 4.02 (1H, dd, *J*=7.5, 9.2 Hz, glc 2''-H), 4.01 (1H, dd, *J*=7.4, 9.2 Hz, glc 2'''-H), 3.93 (1H, m, glc 5''-H), 3.89 (1H, m, glc 5'''-H), 3.71 (1H, m, glc 5''''-H), 3.41 (1H, dd, *J*=4.0, 11.5 Hz, 3-H), 3.34 (3H, s, OMe), 2.54 (1H, ddd, *J*=6.3, 8.1, 13.8 Hz, 23-H), 2.43 (1H, ddd, *J*=4.6, 6.8, 13.8 Hz, 23-H), 2.39 (1H, brs, 17-H), 2.37 (1H, m, 20-H), 2.32 (1H, m, 2-H), 1.94 (3H, s, 27-H₃), 1.92 (1H, d, *J*=13.2 Hz, 15-H), 1.86 (overlapping, 11-H), 1.85 (overlapping, 2-H), 1.63 (1H, d, *J*=13.2 Hz, 15-H), 1.51 (1H, dd, *J*=5.2, 11.5 Hz, 8-H), 1.49 (1H, m, 12-H), 1.44 (1H, m, 6-H), 1.38 (overlapping, 12-H), 1.37 (overlapping, 1-H), 1.27 (3H, s, 29-H₃), 1.19 (overlapping, 7-H), 1.17 (overlapping, 5-H), 1.16 (3H, d, *J*=6.9 Hz, 21-H₃), 1.14 (3H, s, 18-H₃), 1.08 (3H, s, 30-H₃), 1.06 (3H, s, 28-H₃), 1.04 (overlapping, 1-H), 1.01 (overlapping, 11-H), 0.98 (1H, m, 7-H), 0.62 (1H, m, 6-H), 0.42 (1H, d, *J*=4.0 Hz, 19-H), 0.06 (1H, d, *J*=4.0 Hz, 19-H); ¹³C-NMR data (Table 1).

Aquilegioside L (**2**): Amorphous powder; [α]_D +3.5° (*c*=0.60, MeOH); HR-ESI-MS *m/z* 1145.5751 (M+Na; Calcd for C₅₄H₉₀O₂₄Na, 1145.5720); ¹H-NMR (pyridine-*d*₅) δ : 5.74 (1H, t, *J*=6.9 Hz, 24-H), 5.44 (1H, d, *J*=7.5 Hz, glc 1''-H), 5.31 (1H, d, *J*=8.0 Hz, glc 1'''-H), 4.91 (1H, d, *J*=8.0 Hz, gal 1'-H), 4.82 (1H, d, *J*=8.1 Hz, glc 1''''-H), 4.68 (1H, d, *J*=12.1 Hz, 26-H), 4.66 (1H, dd, *J*=8.0, 9.2 Hz, gal 2'-H), 4.53 (1H, dd, *J*=1.7, 11.5 Hz, glc 6''-H), 4.51 (1H, brs, gal 4'-H), 4.49 (overlapping, 26-H, gal 6''-H, glc 6'''-H), 4.47 (1H, dd, *J*=3.8, 9.2 Hz, gal 3'-H), 4.45 (1H, m, 16-H), 4.38 (1H, dd, *J*=2.9, 11.5 Hz, glc 6''-H), 4.34 (overlapping, H-22, gal 6'-H), 4.32 (1H, dd, *J*=5.7, 11.5 Hz, glc 6'''-H), 4.26 (1H, dd, *J*=5.7, 11.5 Hz, glc 6''''-H), 4.24 (1H, dd, *J*=5.2, 11.5 Hz, glc 6''-H), 4.19 (1H, dd, *J*=9.2, 9.2 Hz, glc 3'''-H), 4.17 (1H, dd, *J*=9.2, 9.2 Hz, glc 3''-H), 4.16 (1H, dd, *J*=9.2, 9.2 Hz, glc 4''''-H), 4.14 (1H, dd, *J*=9.2, 9.2 Hz, glc 3''-H), 4.11 (1H, dd, *J*=7.5, 9.2 Hz, glc 2''-H), 4.10 (1H, dd, *J*=9.2, 9.2 Hz, glc 4''-H), 4.09 (overlapping, gal 5'-H), 4.06 (1H, dd, *J*=9.2, 9.2 Hz, glc 4''-H), 4.01 (1H, dd, *J*=8.0, 9.2 Hz, glc 2''-H), 3.99 (1H, dd, *J*=8.1, 9.2 Hz, glc 2'''-H), 3.93 (1H, m, glc 5''-H), 3.89 (1H, m, glc 5'''-H), 3.71 (1H, m, glc 5''-H), 3.42 (1H, dd, *J*=4.6, 11.5 Hz, 3-H), 2.72 (1H, ddd, *J*=6.9, 8.0, 13.8 Hz, 23-H), 2.41 (1H, ddd, *J*=5.7, 6.9, 13.8 Hz, 23-H), 2.32 (1H, m, 2-H), 2.28 (1H, dd, *J*=6.7, 10.3 Hz, 17-H), 1.92 (overlapping, 11-H), 1.89 (3H, s, 27-H₃), 1.87 (overlapping, 2-H, 15-H), 1.84 (1H, m, 20-H), 1.67 (overlapping, 12-H, 15-H), 1.52 (1H, m, 12-H), 1.45 (1H, m, 6-H), 1.36 (overlapping, 1-H, 8-H), 1.27 (3H, s, 29-H₃), 1.22 (3H, s, 28-H₃), 1.19 (overlapping, 7-H), 1.17 (1H, dd, *J*=4.0, 12.0 Hz, 5-H), 1.11 (3H, s, 30-H₃), 1.10 (3H, d, *J*=6.3 Hz, 21-H₃), 1.04 (1H, m, 1-H), 1.00 (3H, s, 18-H₃), 0.99 (overlapping, 11-H), 0.96 (overlapping, 7-H), 0.64 (1H, m, 6-H), 0.39 (1H, d, *J*=3.5 Hz, 19-H), 0.11 (1H, d, *J*=3.5 Hz, 19-H); ¹³C-NMR data (Table 1).

Sugar Analysis A solution of each compound (**1** or **2**) (1.0 mg) in 2 M HCl/dioxane (1:1, 2 ml) was heated at 100 °C for 1 h. The reaction mixture was diluted with H₂O and evaporated to remove dioxane. The solution was neutralized with Amberlite MB-3 and passed through a SEP-PAK C₁₈ cartridge to give a sugar fraction. The sugar fraction was concentrated to dryness *in vacuo* to give a residue, which was dissolved in CH₃CN/H₂O (3:1, 250 μ l). The sugar fraction was analyzed by HPLC under the following conditions: column, Shodex RS-Pac DC-613 (6.0 mm i.d. \times 150 mm, Showa Denko, Tokyo, Japan); solvent, CH₃CN/H₂O (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**: D-glucose 7.4 (+), D-galactose 8.0 (+), **2**: D-glucose 7.4 (+), D-galactose 8.0 (+). [reference: D-glucose 7.4 (positive optical rotation: +), D-galactose 8.0 (positive optical rotation: +)].

References

- Nishida M., Yoshimitsu H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **51**, 683–687 (2003).
- Nishida M., Yoshimitsu H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **51**, 956–959 (2003).
- Yoshimitsu H., Nishida M., Nohara T., *Tetrahedron*, **57**, 10247–10252 (2001).
- Yoshimitsu H., Nishida M., Nohara T., *Chem. Pharm. Bull.*, **55**, 789–792 (2007).
- Yoshimitsu H., Nishida M., Qian Z.-A., Lei Z.-H., Nohara T., *Chem. Pharm. Bull.*, **48**, 828–831 (2000).
- Yoshikawa K., Katsuta S., Mizumori J., Arihara S., *J. Nat. Prod.*, **63**, 1229–1234 (2000).
- Yoshimitsu H., Nishida M., Hashimoto F., Nohara T., *Phytochemistry*, **51**, 449–452 (1999).
- Yoshimitsu H., Nishida M., Nohara T., *Chem. Pharm. Bull.*, **56**, 1009–1012 (2008).