PERGAMON

Phytochemistry 51 (1999) 449–452

PHYTOCHEMISTRY

Cycloartane-type glycosides from *Aquilegia flabellata*

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Received 12 May 1998; received in revised form 28 August 1998

Abstract

Two new cycloartane-type glycosides, named aquilegiosides A and B, were isolated from the dried aerial parts of *Aquilegia flabellata* Sieb.et Zucc.var *flabellata* (Ranunculaceae). Their chemical structures have been characterized as 22*S*-3β,16α,29-trihydroxy-cycloart-24-
en-26,22-olide 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside and 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside, by chemical and spectroscopic evidence.

Keywords: *Aquilegia flabellata*; Ranunculaceae; Cycloartane glycoside; Aquilegioside

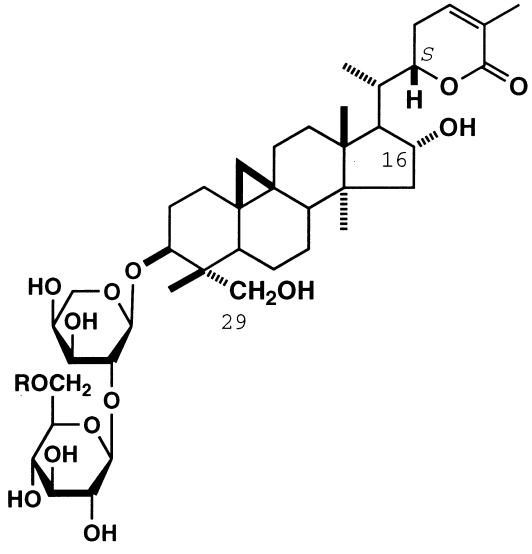
1. Introduction

Aquilegia flabellata Sieb.et Zucc.var *flabellata* (Japanese name, odamaki) is cultivated as a garden plant. This plant has not previously been for the subject of phytochemical analysis. As part of our continuing investigation on the chemical constituents in the Ranunculaceous plants (Yoshimitsu et al., 1992; Yoshimitsu, Hayashi, Kumabe, & Nohara, 1995), this paper deals with structural elucidations of two novel glycosides, named aquilegiosides A (**1**) and B (**2**).

2. Results and discussion

The methanol extract of *A. flabellata* was partitioned between ethyl acetate and water. Subsequent MCI gel CHP-20P column chromatography of the water soluble portion provided the glycosidic constituents, which were further purified by using a combination of silica gel and ODS column chromatographies to furnish two new glycosides, designated aquilegiosides A (**1**) and B (**2**).

Aquilegioside A (**1**) obtained as a white powder, $[\alpha]_D$ +10.8° (MeOH), showed a clustered molecular ion at *m/z* 965.4722 $[C_{47}H_{74}O_{19}Na]^+$ in the HR FAB-MS. The ^{1}H NMR spectrum displayed signals due to one cyclopropane methylene at δ 0.33 and 0.55, three *tertiary* methyls at δ 1.06, 1.11 and 1.20, one *secondary* methyl at δ 1.12 (*J* = 6.1 Hz), one olefinic methyl at δ



	R
1	glc
2	H

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0031-9422/99/$ - see front matter
PII: S0031-9422(98)00735-3

1.82, three anomeric protons at δ 5.08 (d, $J=7.9$ Hz), 5.19 (d, $J=7.9$ Hz) and 5.24 (d, $J=5.5$ Hz) and one olefinic proton at δ 6.46 (1H, d, $J=6.1$ Hz). These spectral data indicated that **1** was a cycloartane triglycoside derivative. In the HMBC experiment, long-range correlations were observed between the olefinic methyl protons (δ 1.82) and two olefinic carbons (δ 128.0 and 140.4); an ester carbonyl carbon (δ 166.4). In addition, a sequence of correlations through an olefinic proton at δ 6.46, two methylene-protons at δ 2.02 and 2.59 and an oxygen-bearing methine proton at δ 5.44, in turn, were observed in the ^{1}H–^{1}H-COSY spectrum. Furthermore, a HMBC was observed between the oxygen-bearing methine proton (δ 5.44) and one secondary methyl carbon (C-21, δ 13.3). Successive ^{1}H–^{1}H correlations through the secondary methyl protons (δ 1.12), a methine proton at δ 1.70, a methine proton at δ 2.47 and a hydroxy methine proton at δ 4.25. From these above evidences, it became clear that the olefinic methyl and the ester carbonyl carbon were at C-27 and C-26, respectively and that two oxygen-bearing methine protons (δ 5.44 and 4.25) could be assigned to H-22 and H-16. Moreover, the presence of the downfield-shifted oxygen-bearing methine proton (H-22, δ 5.44) and the ester carbonyl carbon (δ 166.4) suggested that the side chain contained an α,β-unsaturated six-membered lactone. **1** showed a strong negative Cotton effect at 255 nm (θ-7620) in its CD spectrum, thus it was assigned an *S*-configuration at C-22 (Liu, & Huang, 1983). The H-16 signal was assigned from the coupling constant with the H-17 signal. The coupling constant ($J_{17\alpha,16}=6.1$ Hz) between the H-16 and the H-17 suggested the configuration of the 16-hydroxyl as α (Cheung, & Nelson, 1989; Xu, Xu, & Li, 1992; Ponglux et al., 1992). Also, with regard to the rings A, B and C, a comparative study on the ^{13}C NMR spectrum of **1** with those of thalictoside A and C (Yoshimitsu et al., 1992) demonstrated C-3 and C-29 to be substituted by a hydroxy group. Furthermore, the configuration of the 3-hydoroxy group was determined to be β from the fact that a NOE was observed between H-3 (δ 4.35) and H-29 (δ 3.79), while not observed between H-3 and H-30 (δ 1.11), which showed NOE with H_2-19 (δ 0.33 and 0.55). On acid hydrolysis, **1** afforded glucose and arabinose. Meanwhile, the negative FAB-MS of **1** gave a $[M\text{-}H]^-$ ion peak at *m/z* 941 along with 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))$^-$. These evidences suggested that its sugar moiety was composed of a glucosyl–glucosyl–arabinosyl unit. The ^{1}H NMR and ^{13}C NMR spectra of the sugar moiety were assigned by the ^{1}H–^{1}H-COSY, HMQC and HMBC techniques. In addition, the HMBC were observed between the anomeric proton (δ 5.24 (d, $J=5.5$ Hz)) of arabinose and the C-3 (δ 81.6) of the aglycone, the anomeric proton (δ 5.19 (d, $J=7.9$ Hz)) of inner glucose and the C-2 (δ 80.1) of arabinose and the anomeric proton (δ 5.08 (d, $J=7.9$ Hz)) of terminal glucose and the C-6 of inner glucose. Consequently, the structure of **1** was determined to be 22*S*-3β,16α,29-trihydroxy-cycloart-24-en-26,22-olide 3-*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside.

Aquilegioside B (**2**) obtained as a white powder, $[\alpha]_D$ +13.2° (MeOH), showed a clustered molecular ion at *m/z* 803.4196 $[C_{44}H_{64}O_{14}Na]^+$ in the HR FAB-MS. The ^{1}H NMR spectrum displayed signals due to an AB quartet at δ 0.32 and 0.56, three tertiary methyls at δ 1.07, 1.12 and 1.22, one secondary methyl at δ 1.12 ($J=6.1$ Hz), one olefinic methyl at δ 1.82, two anomeric protons at δ 5.21 (d, $J=7.3$ Hz) and 5.27 (d, $J=5.5$ Hz) and one olefinic proton at δ 6.45 (1H, d, $J=6.1$ Hz). In the ^{13}C NMR spectrum of **2**, the signal due to the aglycone moiety were also in good agreement with those of **1**, although the signals due to the sugar moiety were not identical. On acid hydrolysis, **2** afforded glucose and arabinose. Meanwhile, the negative FAB-MS of **2** gave a $[M\text{-}H]^-$ ion peak at *m/z* 779, which was lower by 162 mass units than that of **1**, along with fragment peaks at *m/z* 617 (*m/z* 779–162 (hexose unit))$^-$ and 485 (*m/z* 617–132 (pentose unit))$^-$. These evidences suggested that its sugar moiety was composed of a glucosyl–arabinosyl unit. The ^{1}H NMR and ^{13}C NMR spectra of the sugar moiety were assigned by the ^{1}H–^{1}H-COSY, HMQC and HMBC techniques. In addition, HMBC's were observed between the anomeric proton (δ 5.27 (d, $J=5.5$ Hz)) of arabinose and the C-3 (δ 82.1) of aglycone and the anomeric proton (δ 5.21 (d, $J=7.3$ Hz)) of glucose and the C-2 (δ 80.4) of arabinose. Consequently, the structure of **2** was determined to be 22*S*-3β,16α,29-trihydroxy-cycloart-24-en-26,22-olide 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside.

3. Experimental

3.1. General procedures

Optical rotations were taken with a JASCO DIP-1000 automatic digital polarimeter and CD spectrum on a JASCO J-720 spectropolarimeter. The ^{1}H and ^{13}C NMR spectra were measured with a JEOL JNM-GX 400 and alpha 500 NMR spectrometer and chemical sifts are given on a δ(ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS was measured with a JEOL DX-303 HF spectrometer and taken in a 3-nitrobenzylalcohol matrix. The HR FAB-MS were recorded with a JEOL HX-110 spectrometer. HPLC was carried out by using a TSK gel-120T (7.8

mm i.d. × 30 cm) column with a Tosoh CCPM pump and Tosoh RI-8010 differential refractometer as detector. TLC was performed on precoated Kiesel gel 60 F_{245} (Merck) and detection was achieved by spraying them with 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kiesel gel (230–400 mesh, Merk) and MCI gel CHP-20P (Mitsubishi Chemical Ind.)

3.2. Extraction and isolation

The dried aerial parts (135 g) of *A. flabellata* Sieb.et Zucc.var *flabellata* harvested at Fukuoka were extracted with MeOH, and the extract was partitioned between ethyl acetate and H_2O (1:1). The H_2O soluble portion (26.4 g) was subjected to MCI gel CHP-20P CC with MeOH–H_2O (0→30→50→70→90→100%) to afford seven fractions (Fr.1–7). Fr.6 was further separated by silica gel CC with $CHCl_3$–MeOH–H_2O (14:6:1), followed by HPLC with MeOH–H_2O (3:1), to furnish aquilegioside A (**1**) (22 mg). Fr.7 was further separated by silica gel CC with $CHCl_3$–MeOH–H_2O (14:6:1), followed by HPLC with MeOH–H_2O (3:1), to furnish aquilegioside B (**2**) (4 mg).

3.3. Aquilegioside A (*1*)

Powder. $[\alpha]_D^{25}$ +10.8° (MeOH: *c* 0.22). Neg. FAB-MS (*m/z*): 941 $[M\text{-}H]^-$. HR FAB-MS (*m/z*): 965.47 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{19}Na$ 965.4722). ^1H NMR (pyridine-d_5) δ: 0.33, 0.55 (each 1H, d, $J=3.7$ Hz, H_2-19), 1.06, 1.11, 1.20 and 1.82 (each 3H, s, H_3-18, H_3-30, H_3-28 and H_3-27), 1.12 (3H, d, $J=6.1$ Hz, H_3-21), 1.70 (1H, m, H-20), 2.02 (1H, m, H-23), 2.47 (1H, dd, $J=6.1$, 11.0 Hz, H-17), 2.59 (1H, m, H-23), 3.68 (1H, br d, $J=9.8$ Hz, ara H-5), 3.79 (1H, br d, $J=9.8$ Hz, H-29), 3.95 (1H, m, inner glc H-5), 3.96 (1H, m, terminal glc H-5), 4.03 (1H, dd, $J=7.9$, 8.5 Hz, inner glc H-2), 4.05 (1H, dd, $J=7.9$, 8.5 Hz, terminal glc H-2), 4.13 (1H, t, $J=8.5$ Hz, inner glc H-3), 4.24 (1H, m, terminal glc H-3), 4.25 (1H, m, terminal glc H-4), 4.26 (1H, m, ara H-5), 4.29 (1H, m, ara H-3), 4.32(1H, m, ara H-4), 4.35 (1H, m, H-3), 4.36 (1H, m, terminal glc H-6), 4.40 (1H, m, inner glc H-6), 4.51 (1H, br d, $J=11.6$ Hz, terminal glc H-6), 4.68 (1H, dd, $J=5.5$, 7.3 Hz, ara H-2), 4.75 (1H, br d, $J=11.0$ Hz, inner glc H-6), 5.08 (1H, d, $J=7.9$ Hz, terminal glc H-1), 5.19 (1H, d, $J=7.9$ Hz, inner glc H-1), 5.24 (1H, d, $J=5.5$ Hz, ara H-1), 5.44 (1H, dd, $J=4.0$, 12.2 Hz, H-22), 6.45 (1H, d, $J=6.1$ Hz, H-24). ^{13}C NMR (pyridine-d_5): see Table 1. CD (MeOH: *c* 0.00346 M) $[\theta]$ (nm): −7620 (255) (negative max.).

3.4. Aquilegioside B (*2*)

Powder. $[\alpha]_D^{25}$ +13.2° (MeOH: *c* 0.38). Neg. FAB-MS (*m/z*): 779 $[M\text{-}H]^-$. HR FAB-MS (*m/z*): 803.42 $[M+Na]^+$ (Calcd for $C_{47}H_{74}O_{19}Na$ 803.4196). ^1H NMR (pyridine-d_5) δ: 0.32, 0.56 (each 1H, d, $J=3.7$ Hz, H_2-19), 1.07, 1.12, 1.22 and 1.82 (each 3H, s, H_3-18, H_3-30, H_3-28 and H_3-27), 1.12 (3H, d, $J=6.1$ Hz, H_3-21), 3.79 (1H, br d, $J=11.0$ Hz, ara H-5), 3.84 (1H, m, glc H-5), 4.10 (1H, dd, $J=7.3$, 7.9 Hz, glc H-2), 4.18 (1H, dd, $J=7.9$, 8.5 Hz, glc H-3), 4.25 (1H, m,

Table 1
^{13}C NMR data for **1** and **2** (δ, in pyridine-d_5)

	1	**2**
C-1	32.0	32.2
2	29.3	29.3
3	81.6	82.1
4	45.6	45.5
5	40.6	40.9
6	20.9	21.0
7	26.3	26.3
8	48.3	48.2
9	19.2	19.3
10	26.0	26.1
11	26.6	26.6
12	32.9	32.9
13	46.8	46.8
14	47.7	47.7
15	48.7	48.7
16	77.2	77.2
17	57.5	57.5
18	19.1	19.0
19	30.4	30.3
20	39.6	39.7
21	13.3	13.3
22	79.5	79.6
23	28.3	28.3
24	140.4	140.4
25	128.0	128.0
26	166.4	166.4
27	17.2	17.2
28	20.6	20.6
29	63.6	64.2
30	12.0	11.9
ara C-1	103.6	103.6
2	80.1	81.4
3	73.2	73.6
4	68.3	68.1
5	64.9	64.8
(Inner) glc C-1	105.1	106.0
2	75.9	76.2
3	78.2	78.4
4	71.7	71.4
5	76.9	78.3
6	69.8	62.5
(Terminal) glc C-1	105.3	
2	75.3	
3	78.2	
4	71.5	
5	78.4	
6	62.8	

glc H-4), 4.26 (1H, m, ara H-5), 4.30 (1H, m, ara H-3), 4.32 (1H, m, ara H-4), 4.39 (1H, m, glc H-6), 4.48 (1H, br d, $J=12.2$ Hz, glc H-6), 4.61 (1H, dd, $J=5.5$, 7.3 Hz, ara H-2), 5.21 (1H, d, $J=7.3$ Hz, glc H-1), 5.27 (1H, d, $J=5.5$ Hz, ara H-1), 5.45 (1H, dd, $J=4.0$, 12.2 Hz, H-22), 6.45 (1H, d, $J=6.1$ Hz, H-24). ^{13}C-NMR (pyridine-d_5): see Table 1.

3.5. Acid hydrolysis of compounds *1* and *2*

Each sample (1 mg) was refluxed with 1 N HCl water–dioxane (1:1) for 2 h on water bath. The precipitate was removed by filtration and the filtrate was treated with Amberlite IRA-400 to give a sugar fraction for TLC. Compounds **1** and **2** showed the presence of D-glucose and L-arabinose; identified on silica gel TLC (Solvent 1: 1-BuOH–HOAc–H_2O, 4:1:2 and solvent 2: $CHCl_3$–MeOH–H_2O, 25:17:3, Spray reagent: aniline phthalate) by comparing with authentic monosaccharides.

Acknowledgements

We are grateful to Professor H. Okabe and Dr. T. Nagao of Fukuoka University for their measurement of HR FAB-MS.

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