Steroidal Saponins from *Hemerocallis fulva* var. *kwanso*

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Two steroidal saponins, hemeroside A and B, were isolated from the aerial part of *Hemerocallis fulva* var. *kwanso* for the first time. The structures of these compounds were established as 24*S*-hydroxy-neotokorogenin 1-*O*-α-L-arabinopyranosyl 24-*O*-β-D-glucopyranoside (1) and isorhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (2) through NMR experiments.

Key words *Hemerocallis fulva* var. *kwanso*; Liliaceae; steroidal saponin; hemeroside A; hemeroside B

The genus *Hemerocallis* of Liliaceae is commonly called day-lily, and is a garden plant the breed of which has been improved. This genus is distributed widely from southern Europe to the temperate zone of Asia. The roots of *Hemerocallis fulva* var. *kwanso* REGEL have been used as a diuretic, anastaltic and antiphlogistic agent in traditional Chinese medicine,[1)] and they show an activity to extend sleep.[2)] In Okinawa, Japan, people eat the young leaves which emerge from in spring.[2)] In the course of extensive studies on the plant constituents which react with Ehrlich's reagent, we have reported the isolation of some unique amino acid derivatives, fulvanines A—E from *H. fulva* var. *kwanso*.[3)] We now report the isolatation of two new steroidal saponins, called hemeroside A (**1**) and B (**2**), from the aerial parts of the plant. This is the first example of the isolation of steroidal saponins from *Hemerocallis* genus.

Results and Discussion

A methanol extract of the aerial parts of *Hemerocallis fulva* var. *kwanso* was fractionated using adsorption resin (Diaion HP-20) chromatography. Further separation of the resulting fractions by a combination of silica gel, reversed-phase silica gel (octadecyl silica (ODS)) and molecular filter resin (Sephadex LH-20) column chromatography led to the isolation of compounds **1** and **2**.

Hemeroside A (**1**) was obtained as a white powder, $[\alpha]_D$ −17.6°. The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3400 cm^{-1}), spiroketal (997, 949>898, 843 cm^{-1}) groups.[4)] It showed a quasi-molecular ion peak of $[M+Na]^+$ at *m*/*z* 781, and a fragment ion peak due to $[M-C_5H_9O_4]^+$ at *m*/*z* 625 in the positive ion FAB-MS. The high resolution (HR) FAB-MS analysis gave an $[M+Na]^+$ ion at *m*/*z* 781.4001, which corresponds to the composition $C_{38}H_{62}O_{15}Na$. The ^{1}H-NMR spectrum displayed signals due to four steroidal methyls at δ 0.77, 1.10, 1.32 and 1.39, in which two methyl signals (δ 1.10, 1.32) are doublets, eight methylene protons and twelve methine protons, including four methine protons attached to the carbons bearing a hydroxyl group at δ 4.11 (dd, J=2.0, 9.2 Hz), 4.23 (d, J=2.0 Hz), 4.60 (ddd, J=4.0, 9.2, 11.5 Hz), and 4.81 (ddd, J=4.0, 5.0, 9.0 Hz) for the aglycone, and signals for two anomeric protons at δ 5.03 (J=7.5 Hz) and 5.12 (J=7.3 Hz). All 38 carbons appeared in the ^{13}C-NMR spectrum of **1** (Tables 1, 2). Thus carbohydrate moieties consisted of hexose and pentose. Each carbon signal was assigned by C–H shift correlation spectroscopy (COSY) and distortionless enhancement by polarization transfer (DEPT). Furthermore, the ^{13}C-NMR spectrum exhibited seven carbon signals attached to an oxygen atom in the aglycone moiety at δ 64.3, 71.8, 73.0, 75.1, 81.6, 89.7, and 111.3, and two anomeric carbon signals at δ 101.3 and 108.1. Acid hydrolysis of **1** with 1 M hydrochloric acid in dioxane–H_2O (1 : 1, v/v) gave D-glucose and L-arabinose as the carbohydrate moieties. The ^{13}C-NMR spectrum of **1** was quite similar to that of neotokoronin[5)] ex-

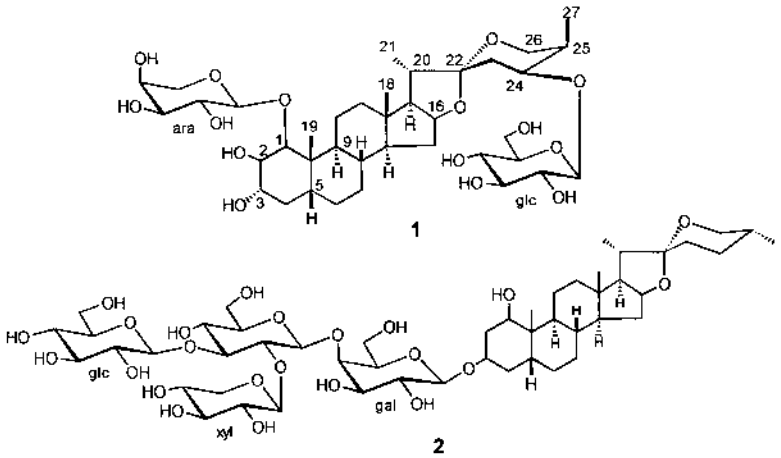

Chart 1. Chemical Structures of **1** and **2**

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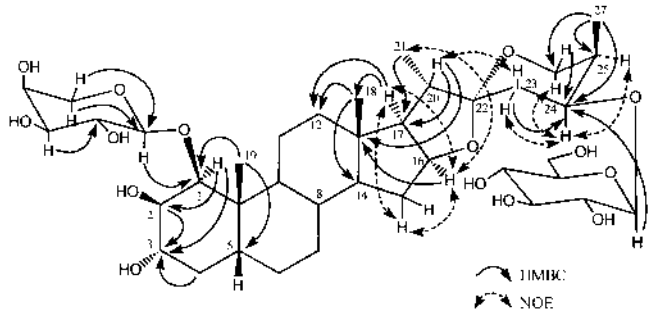

Fig. 1. HMBC and NOESY Correlations for **1**

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data[a] for Aglycone Parts of Compounds **1** and **2**

	1		**2**	
	H[b]	C	H[b]	C
1	4.23 (d, 2.0)	89.7	3.93 (dd, 4.0, 5.0)	74.7
2ax	4.11 (dd, 2.0, 9.2)	75.1	1.40 (ddd, 4.0, 4.0, 13.0)	30.4
2eq			1.86 (br dd, 5.0, 13.0)	
3	4.60 (ddd, 4.0, 9.2, 11.5)	71.8	3.95 (m)	77.4
4ax	2.22 (ddd, 10.0, 11.5, 11.5)	35.1	1.39 (ddd, 4.0, 8.0, 13.0)	35.2
4eq	1.86 (ddd, 4.0, 4.0, 11.5)		1.87 (ddd, 4.0, 13.0, 13.0)	
5	2.06 (m)	36.4	2.10 (ddd, 6.0, 8.0, 13.0)	29.2
6ax	1.26 (m)	36.1	1.58 (m)	27.7
6eq	1.71 (m)		1.71 (ddd, 4.0, 8.0, 10.0)	
7ax	1.06 (m)	26.4	1.33 (m)	26.2
7eq	1.26 (m)		1.61 (m)	
8	1.58 (br dd, 4.0, 10.0)	35.6	1.86 (m)	37.9
9	1.44 (br dd, 4.0, 10.0)	42.1	2.07 (m)	36.1
10		41.6		40.9
11	1.39 (m)	21.1	1.63 (m)	29.9
			2.10 (m)	
12ax	0.95 (m)	40.0	1.79 (ddd, 4.0, 12.0, 12.0)	35.9
12eq	1.62 (br dt, 2.0, 10.0)		1.54 (m)	
13		40.6		41.0
14	0.95 (m)	56.2	2.05 (m)	49.3
15a	1.31 (ddd, 4.0, 6.0, 11.0)	32.0	1.49 (ddd, 6.5, 11.5, 13.5)	32.1
15b	1.96 (ddd, 5.0, 6.0, 11.0)		2.03 (ddd, 4.0, 6.5, 13.5)	
16	4.53 (ddd, 6.0, 6.0, 8.6)	81.6	4.56 (m)	81.3
17	1.76 (dd, 6.5, 8.6)	62.4	1.91 (dd, 6.5, 8.0)	63.1
18	0.77 (s)	16.6	0.92 (s)	15.9
19	1.39 (s)	19.2	0.85 (s)	14.3
20	1.95 (dd, 6.5, 7.0)	42.5	1.98 (dd, 6.5, 13.0)	42.0
21	1.10 (d, 7.0)	14.7	1.11 (d, 7.0)	15.0
22		111.3		109.2
23ax	2.12 (dd, 9.0, 11.0)	34.2	1.67 (m)	31.9
23eq	2.11 (dd, 5.0, 11.0)			
24	4.81 (ddd, 4.0, 5.0, 9.0)	73.0	1.17 (ddd, 4.0, 13.0, 13.0)	29.3
24			1.61 (dd, 4.0, 13.0)	
25	2.27 (ddt, 2.0, 4.0, 6.0)	31.8	1.56 (m)	30.6
26ax	3.52 (dd, 2.0, 11.0)	64.3	3.49 (t, 10.0)	66.9
26eq	3.95 (dd, 2.0, 11.0)		3.55 (dd, 4.0, 10.)	
27	1.32 (d, 6.0)	9.9	0.69 (d, 6.0)	17.3

a) Measured in 600 MHz for ^{1}H, and 150 MHz for ^{13}C, in pyridine-d_5. *b*) Coupling constants (*J*) in Hz are given in parentheses.

cept for the chemical shifts of carbons in ring F and the additional signals of glucose. The hydroxyl group in ring F was located at C-24, which was supported by the spin-coupling system through the ^{1}H–^{1}H COSY spectrum and the heteronuclear multiple bond connectivity (HMBC) correlation from H-23 (δ 2.11, 2.12) and H-27 (δ 1.32) to C-24 (δ 73.0). The *S* configuration of the hydroxyl group at C-24 was shown by nuclear Overhauser enhancement spectroscopy (NOESY)

Table 2. ^{1}H- and ^{13}C-NMR Spectral Data[a] for Sugar Parts of Compounds **1** and **2**

	1		**2**	
	H[b]	C	H[b]	C
gal-1			4.85 (d, 7.5)	102.4
2			4.39 (dd, 7.5, 9.0)	73.2
3			4.09 (overlap)	75.6
4			4.58 (m)	79.9
5			3.91 (m)	76.3
6			4.16 (dd, 9.0, 10.5)	60.6
			4.65 (dd, 9.0, 10.5)	
glc-1	5.03 (d, 7.5)	101.3	5.17 (d, 7.5)	105.2
2	4.05 (dd, 7.5, 8.3)	75.3	4.41 (dd, 7.5, 9.0)	81.4
3	4.26 (dd, 8.3, 9.3)	78.7	4.17 (dd, 9.0, 9.0)	86.8
4	4.30 (dd, 8.5, 9.3)	71.6	3.82 (dd, 9.0, 10.0)	70.5
5	3.92 (ddd, 2.3, 5.0, 8.5)	78.4	3.87 (ddd, 2.5, 8.0, 10.0)	77.6
6	4.38 (dd, 5.0, 12.0)	62.6	4.03 (dd, 8.0, 12.0)	63.0
	4.47 (dd, 2.3, 12.0)		4.50 (dd, 2.5, 12.0)	
glc-1′			5.23 (d, 7.5)	105.0
2′			3.96 (dd, 7.5, 9.0)	75.3
3′			4.09 (dd, 9.0, 9.0)	78.7
4′			4.21 (t, 9.0)	71.1
5′			4.10 (overlap)	77.8
6′			4.36 (dd, 5.0, 12.0)	62.5
			4.58 (dd, 2.5, 12.0)	
xyl-1			5.56 (d, 7.5)	104.9
2			4.11 (dd, 5.0, 7.5)	75.1
3			3.91 (ddd, 2.5, 5.0, 10.)	78.8
4			4.11 (overlap)	70.8
5			3.67 (dd, 10.0, 11.0)	67.4
			4.22 (dd, 5.0, 11.0)	
ara-1	5.12 (d, 7.3)	108.1		
2	4.52 (dd, 7.3,8.9)	74.1		
3	4.15 (dd, 3.6, 8.9)	75.4		
4	4.25 (ddd, 1.5, 2.2, 3.6)	69.9		
5	3.81 (dd, 1.5, 12.1)	67.7		
	4.35 (dd, 2.2, 12.1)			

a) Measured in 600 MHz for ^{1}H, and 150 MHz for ^{13}C, in pyridine-d_5. *b*) Coupling constants (*J*) in Hz are given in parentheses.

spectrum, giving a cross peak between H-26ax and H-24ax and was confirmed from the coupling constants of H-23, H-24 and H-25. The locations of two carbohydrates were assigned to be 1-*O*-α-L-arabinopyranosyl and 24-*O*-β-D-glucopyranosyl by the correlations of the anomeric protons of D-glucose at δ 5.03 (J=7.5 Hz) and L-arabinose at δ 5.12 (J=7.3 Hz) to the carbinyl carbons at δ 73.0 (C-24) and 89.7 (C-1), respectively, in the HMBC spectrum (Fig. 1). Consequently, hemeroside A (**1**) was assigned as (24*S*,25*R*)-5β-spirostan-1β,2β,3α,24-tetraol 1-*O*-α-L-arabinopyranosyl 24-*O*-β-D-glucopyranoside.

Hemeroside B (**2**) was obtained as colorless needles, mp 287—290 °C, $[\alpha]_D$ −56.0°. In the positive ion FAB-MS, quasi-molecular ion peaks were observed at *m/z* 1073 $[M+Na]^+$ and *m/z* 1051 $[M+H]^+$, and fragment ion peaks were observed at *m/z* 922 $[M+Na-H_2O-\text{pentose}]^+$ and *m/z* 737 $[M-H_2O-\text{pentose}-\text{hexose}]^+$. The HR FAB-MS analysis revealed the molecular formula of **2** to be $C_{50}H_{82}O_{23}$. The IR spectrum of **2** showed absorption bands ascribable to hydroxyl (3400 cm^{-1}), spiroketal (982, 969<897, 866) groups. Acid hydrolysis of **2** gave D-galactose, D-glucose and D-xylose as the carbohydrate moieties, together with a known steroidal sapogenin, (25*R*)-5β-spirostan-1β,3β-diol, that is, isorhodeasapogenin.[6] The ^{1}H-NMR spectrum of **2** showed

signals for four methyls at δ 0.69 (d, J=6.0 Hz), 0.85 (s), 0.92 (s) and 1.11 (d, J=7.0 Hz), two methine protons attached to the carbons bearing a hydroxyl group at δ 3.93 (dd, J=4.0, 5.0 Hz) and 3.95 (m), and four anomeric protons at δ 4.85 (d, J=7.5 Hz), 5.17 (d, J=7.5 Hz), 5.23 (d, J=7.5 Hz) and 5.56 (d, J=7.5 Hz) (Tables 1, 2). Five carbon signals attached to the oxygen atom of agylcone moiety at δ 66.9, 74.7, 77.4, 81.3 and 109.2, and four anomeric carbon signals at δ 102.4, 104.9, 105.0 and 105.2 in the ^{13}C-NMR spectrum (Tables 1, 2) of **2**. The connectivities of the sugar parts were determined with the aid of an HMBC experiment. Long-range couplings were observed between a proton signal at δ 4.85 (gal-H-1) and a carbon signal at δ 77.4 (C-3), between a proton at δ 5.17 (glc-H-1) and a carbon signal at δ 79.9 (gal-C-4), between a proton signal at δ 5.23 (glc-H-1′) and a carbon signal at δ 86.8 (glc-C-3), and between a proton signal at δ 5.56 (xyl-H-1) and a carbon signal at δ 81.4 (glc-C-2), indicating glycosylation at C-3 with a glc(1′→3)-[xyl(1→2)]-glc(1→4)-gal moiety. Consequently, the structure of **2** was established as isorhodeasapogenin 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Experimental

General Methods Optical rotations were determined on a Horiba digital polarimeter. IR spectra were measured with a Shimadzu FT-IR-8100 spectrometer. NMR spectra were recorded on a GE NMR OMEGA 600 instrument at 600 MHz (^{1}H) and 150 MHz (^{13}C) and on a Varian XL-300 instrument at 300 MHz (^{1}H) and 75 MHz (^{13}C) using tetramethylsilane (TMS) as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. The FAB-MS and HR positive FAB-MS (70 eV, 3-nitrobenzyl alcohol as a matrix) were measured on a JEOL JMS-SX 102AQQ mass spectrometer. Column chromatography was carried out on Silica gel 60 (120—230 μm, Merck), Lichroprep Rp-18 (ODS, 40—63 μm, Merck), Lichroprep Si 60 (40—60 μm, Merck), Diaion HP-20 (Nippon Rensui), and Sephadex LH-20 (Pharmacia).

Isolation of 1 and 2 The fresh aerial parts (2.5 kg) of *Hemerocallis fulva* var. *kwanso* collected at Shiga, Japan were extracted with methanol at room temperature for 1 week. The extract was concentrated and the residue (185 g) was subjected to chromatography on an adsorption resin (Diaion HP-20) column with water and then methanol. The water elution (65 g) was chromatographed on silica gel (80% ethanol) to yield the positive and negative fractions by Ehrlich's test. The positive fraction (20 g) was subjected to repeated column chromatography on silica gel, Sephadex LH-20 and reversed phase silica gel (ODS), successively, to give hemeroside A (25 mg) with oxypinnatanine.[7] The negative fraction was subjected to column chromatography on silica gel and ODS to give hemeroside B (15 mg).

1: A white powder (mp 120—125 °C), $[\alpha]_D^{26}$ −17.6° (c=0.9, MeOH). IR (KBr, cm^{-1}): 3400 (OH), 997, 949>898, 843 (spiroketal). ^{1}H- and ^{13}C-NMR (pyridine-d_5): see Table 1 and 2. HR-FAB-MS *m*/*z*: Calcd for $C_{38}H_{62}O_{15}Na$: 781.3987. Found: 781.4001 $(M+Na)^+$.

Acid Hydrolysis of 1 Compound **1** (1.5 mg) was heated in 1 ml 1 M HCl–50% dioxane at 90 °C for 2 h. Dioxane was removed and the solution extracted with EtOAc (1 ml×3 times). The aqueous layer was neutralized by passage through an ion-exchange resin (Amberlite IRA-400)column, concentrated and then the residue was analyzed by HPTLC [*n*-BuOH–AcOH–H_2O, 4 : 1 : 5; D-glucose, *Rf* 0.31; L-arabinose, *Rf* 0.40] and HPLC [column, CAPCELL PAK NH_2 (Shiseido); detector, Shodex RI SE-52; 85% CH_3CN, 1.0 ml/min; L-arabinose, t_R 5.0; D-glucose, t_R 11.0].

2: Colorless needles, mp 287—290 °C, $[\alpha]_D^{26}$ −56.0° (c=1.5, pyridine). IR (KBr, cm^{-1}): 3440 (OH), 982, 964, 922<897, 866 (spiroketal). ^{1}H- and ^{13}C-NMR (pyridine-d_5): see Table 1 and 2. HR-FAB-MS *m*/*z*: Calcd for $C_{50}H_{82}O_{23}Na$: 1073.5135. Found: 1073.5144 $(M+Na)^+$.

Acid Hydrolysis of 2 Compound **2** (10.5 mg) was hydrolyzed with 2% H_2SO_4 at 90 °C for 1.5 h. After cooling, the precipitate was filtered and washed with water. The residue was recrystallized with methanol to give an aglycone (3.8 mg) as colorless needles. The filtrate was subjected to neutralization as described for **1** to give a mixture of monosaccharides. The monosaccharides were analyzed by HPTLC [D-galactose, *Rf* 0.25; D-glucose, *Rf* 0.31; D-xylose, *Rf* 0.52] and HPLC [D-xylose, t_R 6.0; D-galactose, t_R 10.5; D-glucose, t_R 11.0].

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