Steroidal Glycosides from the Fruits of *Solanum viarum*

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Three new steroidal glycosides, named solaviasides A, B, and C, have been isolated from the fruits of *Solanum viarum* Dunal (syn. *S. khasianum* var. *chatterjeeanum*, Solanaceae), along with seven known ones. Their chemical structures were determined on the basis of spectroscopic data and chemical evidence.

Key words *Solanum viarum*; Solanaceae; steroidal glycoside; solaviaside

Solanum viarum Dunal (syn. *S. khasianum* var. *chatterjeeanum*, Solanaceae) is a major source of steroidal raw material, and four glycosides of solasodine and diosgenin are reported to occur in its fruits and roots, respectively.[1)] As part of a continuing study of the steroidal constituents of solanaceous plants,[2)] we now describe the isolation and structural elucidation of three new steroidal glycosides and seven known steroidal glycosides from the fruits of *S. viarum*.

The MeOH extract of the fruits of *S. viarum* was successively subjected to Diaion HP20, silica gel, Sephadex LH-20, and Chromatorex octadecyl silica (ODS) column chromatography as well as HPLC on ODS, to afford ten steroidal glycosides (**1**—**10**).

Compounds **4**—**10** were identified as P-d [pregna-5,16-dien-3β-ol-20-one 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside] (**4**),[3,4)] solamargine (**5**),[4,5)] solasonine (**6**),[5)] indioside C (**7**),[6)] protodioscin (**8**),[4,7)] anguivioside XV (**9**),[8,9)] and aculeatiside A (**10**),[10)] respectively, based on comparison with their physical and spectral data with authentic samples or those already reported (Fig. 1).

Compound **1**, named solaviaside A, was obtained as an amorphous powder and exhibited an $[M+Na]^+$ ion peak at *m*/*z* 1057 in positive FAB-MS. The molecular formula of **1** was determined to be $C_{51}H_{86}O_{21}$ by high resolution (HR)-positive FAB-MS. The ^{1}H-NMR spectrum of **1** showed signals corresponding to two tertiary methyl groups (δ 1.08, 0.73); four secondary methyl groups [δ 1.78 (d, J=6.0 Hz), 1.63 (d, J=6.5 Hz), 1.19 (d, J=6.5 Hz), 1.05 (d, J=6.0 Hz)], two of which were assignable to H_3-6 of rhamnosyl groups; one olefinic proton [δ 5.36 (d, J=4.5 Hz)]; and four monosaccharide groups. The ^{13}C-NMR spectrum of **1**, which contained signals assignable to two olefinic carbons (δ 140.8, 122.0) and four anomeric carbons (δ 104.9, 102.9, 102.0, 100.3), displayed 51 carbon signals. These ^{1}H- and ^{13}C-NMR signals were assigned in detail with the help of ^{1}H–^{1}H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra, and the planar structure of **1** was determined (Fig. 2). The ^{13}C-NMR data of the A and B rings of the aglycone moiety and the sugar moiety were considerably similar to those of **8**, and nuclear Overhauser effect (NOE) correlations were observed between H-14 and H-17, H_3-18 and H-20, and H_3-18 and H_3-21 in the nuclear Overhauser ef-

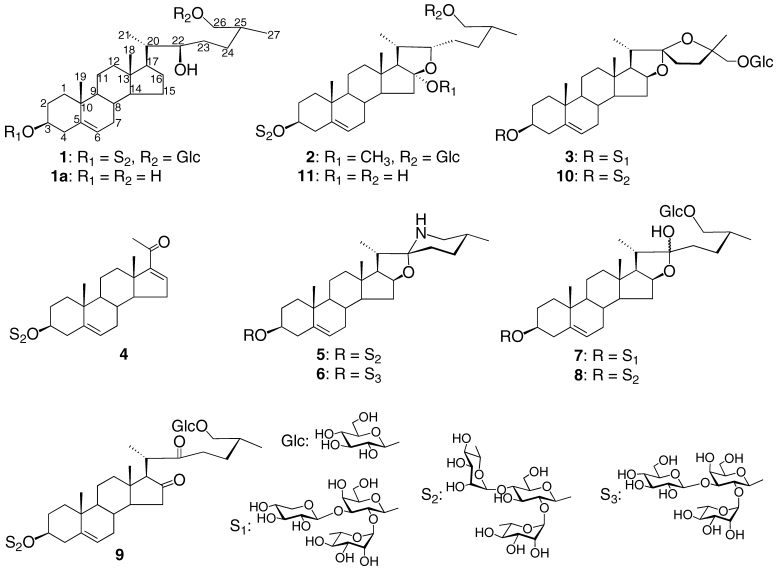

Fig. 1. Structures of **1**—**10**

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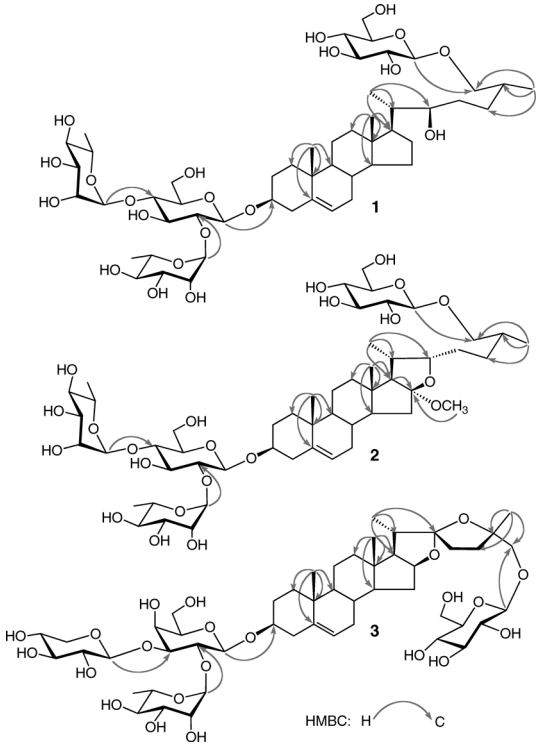

Fig. 2. ^{1}H–^{13}C Long-Range Correlations Observed for **1**—**3** in the HMBC Spectra (in Pyridine-d_5, 500 MHz)

fect spectroscopy (NOESY) spectrum of **1**.

On acidic hydrolysis, **1** afforded D-glucose and L-rhamnose along with a sapogenol (**1a**), whose ^{1}H-NMR data were more similar to those of (25*S*)-cholest-5-ene-3β,22β,26-triol rather than those of its 22-epimer, except that the signals corresponded to H_2-26.[11] Further, Agrawal has reported that the difference [$\Delta\delta$ (δHa-26$-\delta$Hb-26)] among the ^{1}H-NMR chemical shifts for geminal protons of the glycosyloxy methylene group of furostane-type glycosides reflects the orientation of the 27-methyl group, and the difference ($\Delta\delta$) is usually greater than 0.57 ppm in 25*S* compounds and less than 0.48 ppm in 25*R* compounds.[12] Although **1** is a cholestane-type glycoside, the structure of its side chain moiety (C-23—C-27) is the same as those of furostane-type glycosides. Therefore, we tried to determine the configuration at C-25 of **1** by applying this empirical rule.[12] The chemical shifts of signals due to H_2-26 in the ^{1}H-NMR spectrum of **1** were similar to those observed for 25*R* furostane-type glycosides, and their difference ($\Delta\delta$) was 0.32 ppm. From the above evidence, the configuration at C-25 was deduced to be *R*. Thus, **1** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-25*R*-cholest-5-ene-3β,22β,26-triol 26-*O*-β-D-glucopyranoside.

Compound **2**, named solaviaside B, was obtained as an amorphous powder. The molecular formula of **2** was determined to be $C_{52}H_{86}O_{22}$ by HR-positive FAB-MS. In the ^{1}H-NMR spectrum, signals corresponding to two tertiary methyl groups (δ 1.05, 0.84), two secondary methyl groups [δ 1.08 (d, J=7.5 Hz), 1.05 (d, J=7.0 Hz)], one methoxyl group (δ 3.36), one olefinic proton [δ 5.30 (br d, J=4.5 Hz)], and four monosaccharide groups similar to those of **1** were observed. The ^{13}C-NMR spectrum of **2** exhibited 52 carbon signals, including those corresponding to two olefinic carbons (δ 140.9, 121.7), one acetal carbon (δ 119.1), four anomeric carbons (δ 104.9, 103.0, 102.0, 100.4), and one methoxyl carbon (δ 50.4). These signals were assigned with the help of 2D-NMR techniques as done for **1**, and the planar structure of **2** was elucidated (Fig. 2). In the ^{13}C-NMR spectrum of **2**, compared with that of **1**, the signals corresponding to the A and B rings of the aglycone moiety and the sugar moiety were almost superimposable; in addition, the chemical shift difference ($\Delta\delta$: 0.32 ppm) of the signals corresponding to H_2-26 was similar to that observed for **1**. Further, key NOE correlations were observed between Hα-15 and the methoxyl group, Hβ-15 and H_3-18, H_3-18 and H-20, and H_3-18 and H-22 in the NOESY spectrum of **2** (Fig. 3). Thus, **2** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranosyl-22*S*,25*R*-furost-5-ene-16α-methoxy-3β,26-diol 26-*O*-β-D-glucopyranoside. Previously, an analogous compound, tribol (**11**), to **2** was isolated as natural product.[13] Therefore, the mehoxyl group at C-16 in **2** might be artificially formed *via* reaction of hemiketal group with MeOH during the extraction and/or isolation procedures.

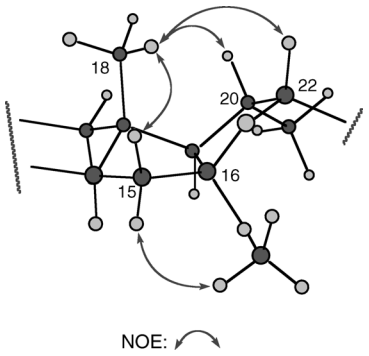

Fig. 3. Selected NOE Correlations Observed for **2** in the NOESY Spectrum (in Pyridine-d_5, 500 MHz)

Compound **3**, named solaviaside C, was obtained as an amorphous powder, and its positive FAB-MS showed an $[M+Na]^+$ ion peak at *m/z* 1055. The molecular formula of **3** was determined to be $C_{50}H_{80}O_{22}$ by HR-positive FAB-MS. The ^{1}H-NMR spectrum of **3** showed signals corresponding to three tertiary methyl groups (δ 1.40, 1.05, 0.80), two secondary methyl groups [δ 1.67 (d, J=6.0 Hz), 1.08 (d, J=6.5 Hz)], one olefinic proton [δ 5.32 (br s)], and four anomeric protons [δ 6.22 (br s), 5.01 (d, J=7.5 Hz), 4.96 (d, J=7.5 Hz), 4.94 (d, J=7.5 Hz)]. The ^{13}C-NMR spectrum of **3** consisted of 50 carbon signals, including two corresponding to olefinic carbons (δ 140.8, 121.6), one to an acetal carbon (δ 120.1), and four to anomeric carbons (δ 106.5, 105.2, 102.0, 100.4). These NMR signals were assigned in detail by using the 2D-NMR spectra. The assigned ^{13}C-NMR data of the aglycone moiety and the sugar moiety were considerably similar to those of **10** and **7**, respectively. In addition, **3** afforded D-glucose, D-galactose, D-xylose, L-rhamnose, and isonuatigenin[10,14] on acidic hydrolysis. On the basis of these data, **3** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-β-D-galactopyranosyl-nuatigenin 26-*O*-β-D-glucopyranoside.

To the best of our knowledge, **1**—**3** are new compounds, and the isolation of **4**—**10** from *S. viarum* is described here for the first time.

Experimental

All instruments and materials used were the same as those cited in a previous report,[15] unless otherwise specified.

Plant Material The fruits of *S. viarum* were collected in the Medical Plant Garden of Kumamoto University, Kumamoto Prefecture, Japan, in December 2003, and identified by Professor Toshihiro Nohara, Faculty of Pharmaceutical Sciences, Sojo University.

Extraction and Isolation The fresh fruits of *S. viarum* (3.05 kg) were extracted with MeOH at room temperature, and the solvent was removed under reduced pressure to give a syrup (192.8 g). The MeOH extract was chromatographed over Diaion HP20 column (H_2O, MeOH, 50% aceone, acetone) to afford fractions (frs.) 1—4. Fraction 2 (38.0 g) was subjected to silica gel column chromatography (CC) [Merck. Art. 7734, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 6 : 4 : 1, 0 : 1 : 0)] to afford frs. 2.1—2.7. Chromatography of fr. 2.4 over Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) produced frs. 2.4.1—2.4.5 and **5** (6569 mg). Fraction 2.4.2 (1735 mg) was successively subjected to Chromatorex ODS CC (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC (column, COSMOSIL 5C18 AR-II, Nacalai Tesque, Inc., 20 mm×250 mm; solvent, 80% MeOH) to afford fr. 2.4.2.1 (16 mg), **3** (8 mg) and **5** (14 mg). Fraction 2.4.4 (1239 mg) was chromatographed over Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to produce frs. 2.4.4.1—2.4.4.5 and **5** (69 mg). Fractions 2.4.4.1 (47 mg), 2.4.4.3 (107 mg), and 2.4.4.4 (494 mg) were each subjected to HPLC (70% MeOH) under the conditions similar to those used for fr. 2.4.2 to afford **2** (14 mg) from fr. 2.4.4.1, fr. 2.4.4.3.1 from fr. 2.4.4.3, and **10** (18 mg) and **8** (19 mg) from fr. 2.4.4.4. Chromatography of fr. 2.4.4.3.1 (65 mg) over silica gel column [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 0 : 1 : 0)] produced **4** (25 mg). Fraction 2.5 (15.6 g) was subjected to Chromatorex ODS CC (65% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to afford frs. 2.5.1—2.5.5. Fraction 2.5.1 (11.04 g) was successively subjected to Chromatorex ODS CC (50% MeOH, 60% MeOH, 70% MeOH), silica gel CC [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)], and HPLC under the same conditions as those used for fr. 2.4.4.1 to afford fr. 2.5.1.1 and fr. 2.5.1.2 (25 mg). Fraction 2.5.1.1 (270 mg) was chromatographed over Chromatorex ODS column (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) to produce frs. 2.5.1.1.1—2.5.1.1.3. HPLC (fr. 2.5.1.1.1, 80% MeOH; fr. 2.5.1.1.3, 75% MeOH) of fr. 2.5.1.1.1 (127 mg) and fr. 2.5.1.1.3 (124 mg) under the conditions similar to those used for fr. 2.4.4.1 yielded **6** (43 mg) from fr. 2.5.1.1.1 and **9** (45 mg) from fr. 2.5.1.1.3. Fraction 2.5.2 (3.1 g) was subjected to silica gel CC [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] to afford frs. 2.5.2.1—2.5.2.6. Fractions 2.5.2.3 (150 mg) and 2.5.2.5 (1945 mg) were each successively subjected to Chromatorex ODS CC (50% MeOH, 60% MeOH, 65% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH) and HPLC (fr. 2.5.2.3, 75% MeOH; fr. 2.5.2.5, 65% MeOH) under the conditions similar to those used for fr. 2.4.4.1 to produce **5** (106 mg) from fr. 2.5.2.3 and **7** (94 mg) from fr. 2.5.2.5. Fraction 2.5.3 (1.6 g) was successively subjected to silica gel CC [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (10 : 2 : 0.1, 8 : 2 : 0.2, 7 : 3 : 0.5, 6 : 4 : 1, 0 : 1 : 0)] and HPLC under the same conditions as those used for fr. 2.5.2.3 to afford **6** (84 mg). Fraction 2.5.5 (3.0 g) was successively subjected to silica gel CC [Merck. Art. 9385, $CHCl_3$–MeOH–H_2O (14 : 2 : 0.1, 10 : 2 : 0.1, 8 : 2 : 0.2, 6 : 4 : 1, 0 : 1 : 0)], Chromatorex ODS CC (60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH), and HPLC under the same conditions as those used for fr. 2.4.4.1 to afford **1** (29 mg), **10** (18 mg), and **3** (72 mg).

1: Amorphous powder. $[\alpha]_D^{31}$ −90.3° (c=3.3, pyridine). Positive FAB-MS m/z: 1057 $[M+Na]^+$. HR-positive FAB-MS m/z: 1057.5630 $[M+Na]^+$ (Calcd for $C_{51}H_{86}O_{21}Na$: 1057.5559). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 6.38 (1H, s, H-1 of Rha), 5.85 (1H, s, H-1 of Rha′), 5.36 (1H, d, J=4.5 Hz, H-6 of Ag), 4.85 (1H, d, J=8.0 Hz, H-1 of Glc′), 4.68 (1H, br s, H-2 of Rha), 4.68 (1H, s, H-2 of Rha′), 4.96 (1H, d, J=7.5 Hz, H-1 of Glc), 4.85 (1H, d, J=7.5 Hz, H-1 of Glc′), 4.85 (1H, d, J=3.5 Hz, H-2 of Rha), 4.70 (1H, d, J=3.0 Hz, H-2 of Rha′), 4.64 (1H, dd, J=3.5, 9.5 Hz, H-3 of Rha), 4.58 (1H, dd, J=2.5, 12.0 Hz, Ha-6 of Glc′), 4.56 (1H, dd, J=3.0, 9.5 Hz, H-3 of Rha′), 4.35 (1H, dd, J=9.5, 9.5 Hz, H-4 of Rha′), 4.28 (1H, dd, J=8.5, 8.5 Hz, H-3 of Glc′), 4.10 (1H, dd, J=3.0,12.0 Hz, Hb-6 of Glc), 4.06 (1H, dd, J=8.0, 9.0 Hz, H-3 of Glc′), 3.97 (1H, dd, J=6.5, 9.0 Hz, Ha-26 of Ag), 3.65 (1H, dd, J=5.5, 9.0 Hz, Hb-26 of Ag), 2.82 (1H, br dd, J=3.0, 13.0 Hz, Ha-4 of Ag), 2.74 (1H, dd, J=13.0, 13.0 Hz, Hb-4 of Ag), 1.78 (3H, d, J=6.0 Hz, H_3-6 of Rha), 1.63 (3H, d, J=6.0 Hz, H_3-6 of Rha′), 1.19 (3H, d, J=6.5 Hz, H_3-21 of Ag), 1.08 (3H, s, H_3-19 of Ag), 1.05 (3H, d, J=6.5 Hz, H_3-27 of Ag), 0.73 (3H, s, H_3-18 of Ag). ^{13}C-NMR data: see Table 1.

Table 1. ^{13}C-NMR Spectral Data for **1**—**3** (in Pyridine-d_5, 125 MHz)

	1	2	3		1	2	3
Ag-1	37.6	37.5	37.4	Glc-1	100.3	100.4	
2	30.2	30.2	30.0	2	77.9	77.9	
3	78.2	78.2	77.5	3	78.5	78.5	
4	39.0	39.0	38.7	4	78.8	78.9	
5	140.8	140.9	140.8	5	76.9	76.9	
6	122.0	121.7	121.6	6	61.4	61.4	
7	32.3	32.2	32.2	Gal-1			100.4
8	32.2	31.4	31.5	3			76.2
9	50.5	50.1	50.2	3			84.9
10	37.0	37.2	37.0	4			70.1
11	21.4	20.8	21.0	5			74.9
12	40.2	38.9	39.8	6			62.1
13	42.4	41.3	40.4	Rha-1	102.0	102.0	102.0
14	57.0	55.5	56.3	2	72.5	72.5	72.3
15	24.6	35.3	32.1	3	72.8	72.8	72.7
16	28.2	119.1	80.9	4	74.1	74.2	74.0
17	53.1	72.4	62.3	5	69.5	69.5	69.3
18	12.0	13.8	16.1	6	18.6	18.6	18.5
19	19.4	19.4	19.3	Rha′-1	102.9	103.0	
20	41.5	34.2	38.4	2	72.7	72.7	
21	12.5	17.3	15.0	3	72.5	72.5	
22	73.0	87.5	120.1	4	73.9	73.9	
23	33.8	30.0	33.1	5	70.4	70.5	
24	31.2	32.0	33.8	6	18.5	18.5	
25	34.4	34.2	83.8	Xyl-1			106.5
26	75.3	75.1	77.3	2			74.5
27	17.6	17.7	24.3	3			78.1
OCH_3		50.4		4			70.8
				5			66.9
				Glc′-1	104.9	104.9	105.2
				2	75.2	75.3	75.2
				3	78.6	78.7	78.3
				4	71.8	71.8	71.5
				5	77.8	78.0	78.2
				6	62.9	62.9	62.6

δ in ppm from tetramethylsilane (TMS). Glc, glucopyranosyl; Gal, galactopyranosyl; Rha, rhamnopyranosyl; Xyl, xylopyranosyl; Ag, aglycone moiety.

2: Amorphous powder. $[\alpha]_D^{31}$ −70.3° (c=0.9, pyridine). Positive FAB-MS m/z: 1085 $[M+Na]^+$. HR-positive FAB-MS m/z: 1085.5560 $[M+Na]^+$ (Calcd for $C_{52}H_{86}O_{22}Na$: 1085.5508). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 6.37 (1H, s, H-1 of Rha), 5.83 (1H, s, H-1 of Rha′), 5.30 (1H, br d, J=4.5 Hz, H-6 of Ag), 4.86 (1H, d, J=8.1 Hz, H-1 of Glc′), 4.81 (1H, d, J=3.5 Hz, H-2 of Rha), 4.67 (1H, J=3.5 Hz, H-2 of Rha′), 4.61 (1H, dd, J=3.5, 9.0 Hz, H-3 of Rha), 4.55 (1H, dd, J=2.5, 12.0 Hz, H-6 of Glc′), 4.52 (1H, dd, J=3.5, 9.0 Hz, H-3 of Rha′), 4.08 (1H, dd, J=3.5, 12.0 Hz, H-6 of Glc), 4.04 (1H, dd, J=8.0, 9.0 Hz, H-2 of Glc′), 3.98 (1H, dd, J=7.5, 9.5 Hz, Ha-26 of Ag), 3.86 (1H, m, H-3 of Ag), 3.68 (1H, dd, J=5.5, 9.0 Hz, Hb-26 of Ag), 3.36 (3H, s, OCH_3), 2.78 (1H, br dd, J=3.5, 13.5 Hz, Ha-4 of Ag), 2.71 (1H, br dd, J=13.5, 13.5 Hz, Hb-4 of Ag), 2.25 (1H, m, H-20 of Ag), 2.12 (1H, dd, J=6.5, 12.0 Hz, H-15 of Ag), 1.96 (1H, d, J=3.0 Hz, H-17 of Ag), 1.75 (3H, d, J=6.5 Hz, H_3-6 of Rha), 1.62 (3H, d, J=6.5 Hz, H_3-6 of Rha′), 1.08 (3H, d, J=7.5 Hz, H_3-21 of Ag), 1.05 (3H, d, J=7.0 Hz, H_3-27 of Ag), 1.05 (3H, s, H_3-19 of Ag), 0.84 (3H, s, H_3-18 of Ag). ^{13}C-NMR data: see Table 1.

3: Amorphous powder. $[\alpha]_D^{31}$ −73.2° (c=8.0, pyridine). Positive FAB-MS m/z: 1055 $[M+Na]^+$. HR-positive FAB-MS m/z: 1055.5056 $[M+Na]^+$ (Calcd for $C_{50}H_{80}O_{22}Na$: 1055.5039). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 6.22 (1H, s, H-1 of Rha), 5.32 (1H, br s, H-6 of Ag), 5.01 (1H, d, J=7.5 Hz, H-1 of Xyl), 4.96 (1H, d, J=7.5 Hz, H-1 of Gal), 4.94 (1H, d, J=7.5Hz, H-1 of Glc′), 4.90 (1H, dq, J=9.0, 6.0 Hz, H-5 of Rha), 4.86 (1H, br d, J=3.0 Hz, H-2 of Rha), 4.76 (1H, br d, J=2.0 Hz, H-4 of Gal), 4.57 (1H, dd, J=3.0, 9.0 Hz, H-3 of Rha), 4.52 (1H, dd, J=2.0, 11.5 Hz, Ha-6 of Glc′), 4.02 (1H, dd, J=8.0, 8.5 Hz, H-2 of Glc′), 3.89 (1H, d, J=9.5 Hz, Hb-27 of Ag), 3.62 (1H, dd, J=10.5, 10.5 Hz, Hb-5 of Xyl), 2.80 (1H, br dd, J=4.0, 12.5 Hz, Ha-4 of Ag), 2.73 (1H, br dd, J=12.5, 12.5 Hz, Hb-4 of Ag), 1.67 (3H, d, J=6.0 Hz, H_3-6 of Rha), 1.40 (3H, s, H_3-27 of Ag), 1.08 (3H, d, J=6.5 Hz, H_3-21 of Ag), 1.05 (3H, s, H_3-19 of Ag), 0.80 (3H, s, H_3-18 of

Ag). ^{13}C-NMR data: see Table 1.

Acidic Hydrolysis of 1—3 Compound **1** (11 mg) in 2 M HCl (2 ml) was heated at 95 °C for 2 h. The reaction mixture was diluted with H_2O (4 ml) and then extracted with BuOH (5 ml). The aqueous layer was neutralized with Amberlite MB-3 (Organo Co.) and then evaporated under reduced pressure to produce a monosaccharide fraction (4.0 mg), which was extracted with MeOH. The MeOH extract was analyzed by HPLC under the following conditions (condition 1): column, Shodex RS-Pac DC-613 (6.0 mm×150 mm, Showa Denko); solvent, CH_3CN–H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 70 °C; detector, JASCO OR-2090 plus; pump, JASCO PU-2080; and column oven, JASCO CO-2060. On the basis of the retention time (t_R) and optical activity of the monosaccharides, they were determined to be L-rhamnose [t_R (min) 4.3; optical activity, negative] and D-glucose [t_R (min) 6.6; optical activity, positive]. The BuOH extract was subjected to silica gel CC [Merck Art. 9385, hexane–acetone (10 : 1, 5 : 1, 2 : 1, 1 : 1)] to yield **1a** (3 mg).

Following above procedure, **2** (5 mg) and **3** (15 mg) were each subjected to acid hydrolysis to yield monosaccharide fractions. The component monosaccharides were analyzed by HPLC (condition 1): L-rhamnose [t_R (min) 4.2; optical activity, negative] and D-glucose [t_R (min) 6.7; optical activity, positive] were detected from **2**; and L-rhamnose [t_R (min) 4.2; optical activity, negative], D-xylose [t_R (min) 5.1; optical activity, positive], D-glucose [t_R (min) 6.6; optical activity, positive], and D-galactose [t_R (min) 7.5; optical activity, positive] from **3**. The BuOH extract derived from **2** exhibited the presence of isonuatigenin[10] (t_R 24.79 min) on HPLC analysis [column, COSMOSIL 5C18 AR-II (4.6 mm×250 mm, Nacalai Tesque, Inc.); solvent, 85% MeOH; flow rate, 1.0 ml/min; column temperature, 40 °C; detector, SHIMADZU RID-10A; pump, SHIMADZU LC-10AD; and column oven, SHIMADZU CTO-6A]. However, the BuOH extract derived from **3** exhibited several spots by TLC, and the aglycone of **3** could not be obtained.

1a: Amorphous powder. $[\alpha]_D^{24}$ −11.2° (c=0.3, MeOH). ^{1}H-NMR (in pyridine-d_5, 500 MHz) δ: 5.42 (1H, d, J=4.5 Hz, H-6), 3.97 (1H, br dd, J=5.5, 8.0 Hz, H-22), 3.87 (1H, m, H-3), 3.82 (1H, dd, J=6.0, 10.5 Hz, Ha-26), 3.74 (1H, dd, J=6.0, 10.5 Hz, Hb-26), 1.20 (3H, d, J=6.5 Hz, H_3-21), 1.16 (3H, d, J=6.5 Hz, H_3-27), 1.08 (3H, s, H_3-19), 0.76 (3H, s, H_3-18).

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References

1) Patil S., Laloraya M. M., *Indian J. Chem.*, **23B**, 685—686 (1984).
2) Ono M., Shiono Y., Yanai Y., Fujiwara Y., Ikeda T., Nohara T., *Chem. Pharm. Bull.*, **56**, 1499—1501 (2008).
3) Hu K., Yao X. S., Dong A. J., Kobayashi H., Iwasaki S., Jing Y. K., *J. Nat. Prod.*, **62**, 299—301 (1999).
4) Ono M., Nishimura K., Suzuki K., Fukushima T., Igoshi K., Yoshimitsu H., Ikeda T., Nohara T., *Chem. Pharm. Bull.*, **54**, 230—233 (2006).
5) Usubillaga A., Aziz I., Tettamanzi M. C., Waibel R., Achenbach H., *Phytochemistry*, **44**, 537—543 (1997).
6) Yahara S., Nakamura T., Someya Y., Matsumoto T., Yamashita T., Nohara T., *Phytochemistry*, **43**, 1319—1323 (1996).
7) Ikeda T., Tsumagari H., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **52**, 142—145 (2004).
8) Honbu T., Ikeda T., Zhu X.-H., Yoshihara O., Okawa M., Nafady A. M., Nohara T., *J. Nat. Prod.*, **65**, 1918—1920 (2002).
9) Ono M., Yanai Y., Ikeda T., Okawa M., Nohara T., *Chem. Pharm. Bull.*, **51**, 1328—1331 (2003).
10) Saijo R., Fuke C., Murakami K., Nohara T., Tomimatsu T., *Phytochemistry*, **22**, 733—736 (1983).
11) Kaneko K., Tanaka M. W., Mitsuhashi H., *Phytochemistry*, **16**, 1247—1251(1977).
12) Agrawal P. K., *Steroids*, **70**, 715—724 (2005).
13) Conrad J., Dinchev D., Klaiber I., Mika S., Kostova I., Kraus W., *Fitoterapia*, **75**, 117—122 (2004).
14) Mimaki Y., Sashida Y., *Chem. Pharm. Bull.*, **38**, 3055—3059 (1990).
15) Ono M.,Mishima K., Yamasaki T., Masuoka C., Okawa M., Kinjo J., Ikeda T., Nohara T., *J. Nat. Med.*, **63**, 86—90 (2008).
16) Faini F., Torres R., Castillo M., *Phytochemistry*, **23**, 1301—1303 (1984).