Pregnane- and Furostane-Type Oligoglycosides from the Seeds of *Allium tuberosum*

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Two furostane-type steroidal oligoglycosides (1, 2), together with a new pregnane-type oligoglycoside (3), were obtained from the seeds of *Allium tuberosum* ROTTLER. On the basis of spectroscopic analysis, the structures of three new oligoglycosides (1—3) were elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-3β,22ξ,26-trihydroxyl-5α-furostane 3-*O*-β-chacotrioside, 26-*O*-β-D-glucopyranosyl-(25*S*)-3β,5β,6α,22ξ,26-pentahydroxyl-5β-furostane 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, and 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl 3β,5β,6α,16β-tetrahydroxypregnane 16-(5-*O*-β-D-glucopyranoyl-4(*S*)-methyl-5-hydroxypentanoic acid) ester, respectively.

Key words *Allium tuberosum*; pregnane; steroidal oligoglycoside; furostane; Liliaceae; β-chacotriose

Our studies on the constituents of *Solanum*-genera plants have resulted in the isolation of numerous steroidal glycosides: spirostane, furostane, solasodane and solanidane glycosides, some of which were found to exhibit cytotoxic[1] and anti-HSV-1[2] activities. As a further extension of these studies, we had begun to investigate the constituents of *Allium tuberosum*, isolated four spirostanol-type steroids and reported these in a preceding paper.[3] Recently, S.-M. Sang,[4] D.-Q. Yu,[5] and A. Lao[6—8] also reported spirostanol and furostanol oligoglycosides from the same plant. However, no compound has been tested for cytotoxic activity except for tuberoside M, which showed an inhibitory effect on the growth of HL-60 human promyelocytic leukemia cells with an IC_{50} value of 6.8 μg/ml.[4] In a continuing study on the constituents of the seeds of the title plant, we have isolated two new furostane-type oligoglycosides (**1**, **2**), together with a novel pregnane-type steroidal oligoglycoside (**3**). Herein we describe their structural characterization and cytotoxic activity against human lung cancer (PC-12) and human colon cancer (HCT-116) cell lines.

The methanolic extract (92.5 g) of the seeds (2.1 kg) of *A. tuberosum* was partitioned between hexane and 80% MeOH. The lower fraction was subjected to Diaion HP-20 chromatography and octadecylsilanized (ODS) silica gel column chromatography to provide a saponin fraction, which was further separated by silica gel column chromatography, and finally preparative HPLC to furnish compounds **1** (10.5 mg), **2** (20.6 mg), **3** (8.3 mg), and protodioscin (10.6 mg).

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ −45.4° (pyridine), showing a positive reaction to the Ehrlich reagent.[9] It suggested **1** to be a furostane-type oligoglycoside. In the positive FAB-MS, it showed a quasi-molecular ion peak of $[M+Na]^+$ at *m/z* 1073, and a fragment ion peak due to $[M+H-H_2O]^+$ at *m/z* 1033. The high-resolution FAB-MS gave an $[M+Na]^+$ ion at *m/z* 1073.5509, corresponding to the composition $C_{51}H_{86}NaO_{22}$, which was also deduced from the analysis of ^{13}C-NMR and the distortionless enhancement by polarization transfer (DEPT) spectral data. The ^{1}H-NMR spectrum in pyridine-d_5 displayed signals due to four steroidal methyl groups at δ 0.81 and 1.08 (each 3H, s), 1.06, 1.17 (each 3H, d, *J*=6.7 Hz), two secondary methyl groups of deoxyhexopyranoses at δ 1.63 and 1.68 (each 3H, d, *J*=6.1 Hz), and four anomeric protons at δ 4.92 (1H, d, *J*=7.3 Hz), 5.00 (1H, d, *J*=7.9 Hz), 5.82 (1H, br s) and 6.38 (1H, br s). Acid hydrolysis of **1** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and L-rhamnose, in a ratio of 1 : 1 as carbohydrate components by GLC analysis after conversion to the thiazolidine derivatives,[10] while the aglycone was identified as (25*R*)-spirostan-3β-ol (tigogenin) by comparison with an authentic sample.[11,12] In the ^{13}C-NMR spectrum, signals due to the sugar moieties were assigned to be an α-L-rhamnopyranosy-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranose (β-chacotriose)[13] and a terminal β-D-glucopy-

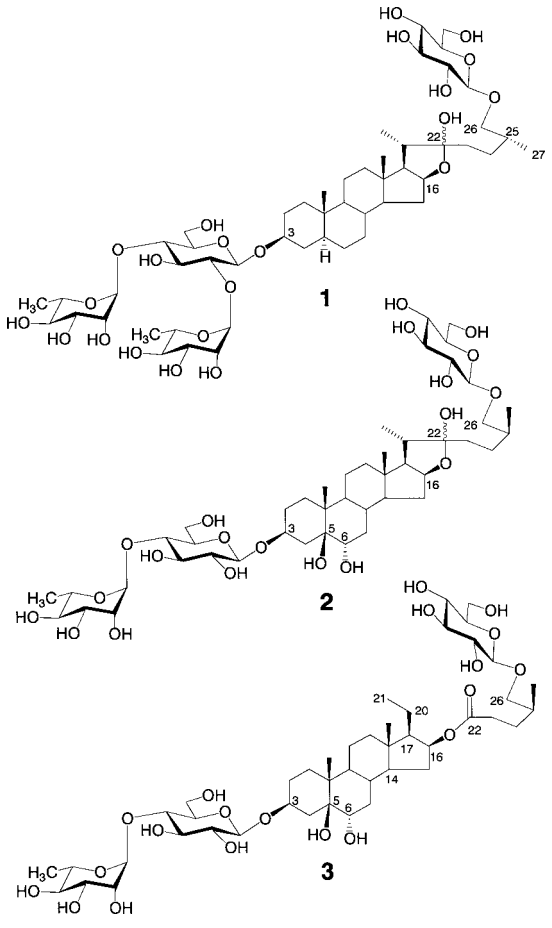

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Table 1. ^{13}C-NMR Data for **1**, **2**, **3**, and Protodioscin in Pyridine-d_5

	1	**1a**	**2**	**3**	Protodioscin		**1**	**1a**	**2**	**3**	Protodioscin
C- 1	37.2	37.2	35.8	35.8	37.5	glc-1	99.8	99.8	101.9	101.9	100.3
- 2	31.0	30.0	29.1	28.9	30.2	-2	78.8	78.8	74.8	74.8	78.1
- 3	77.2	77.1	79.2	79.2	78.5	-3	76.9	76.9	76.7	76.7	76.9
- 4	34.5	34.4	35.0	35.1	39.0	-4	77.9	77.9	78.5	78.5	77.9
- 5	44.6	44.6	73.0	73.0	140.8	-5	78.1	78.1	77.5	77.5	77.8
- 6	29.0	28.9	66.1	66.1	121.8	-6	61.4	61.4	61.1	61.1	61.3
- 7	32.4	32.4	35.5	35.5	32.2						
- 8	35.2	35.2	34.6	34.4	31.7	rha-1	102.1	102.1			102.0
- 9	54.4	54.4	44.6	45.0	50.4	(1→2)-2	72.4	72.4			72.5
-10	35.9	35.9	43.0	43.1	37.1	-3	72.7	72.7			72.7
-11	21.2	21.3	21.8	21.5	21.0	-4	73.9	73.9			74.1
-12	40.0	40.1	40.1	38.2	39.9	-5	69.5	69.5			69.5
-13	41.1	40.7	40.9	42.2	40.8	-6	18.6	18.6			18.6
-14	56.4	56.4	56.3	53.8	56.6						
-15	32.1	32.1	32.4	35.3	32.3	rha-1	102.9	102.9	102.7	102.7	102.9
-16	81.1	81.1	81.2	74.5	81.1	(1→4)-2	72.5	72.5	72.5	72.5	72.5
-17	63.9	63.0	63.7	57.1	63.8	-3	72.8	72.8	72.7	72.7	72.8
-18	16.5	16.6	16.6	13.5	16.3	-4	74.1	74.0	73.9	73.9	73.9
-19	12.4	12.3	17.5	17.5	19.4	-5	70.4	70.4	70.4	70.4	70.4
-20	40.7	42.0	40.6	17.2	40.7	-6	18.5	18.5	18.5	18.5	18.5
-21	16.4	15.0	16.4	13.2	16.4						
-22	110.6	109.2	110.6	173.2	110.7	26-*O*-glc-1	105.1		105.1	105.0	105.1
-23	29.9	31.8	37.1	32.4	30.0	-2	75.2		75.1	75.1	75.2
-24	28.3	29.2	28.3	29.2	28.3	-3	78.5		78.5	78.6	78.4
-25	34.4	30.6	34.4	33.6	34.4	-4	71.7		71.7	71.7	71.7
-26	75.0	66.8	75.3	74.7	75.0	-5	78.6		78.4	78.4	78.3
-27	17.5	17.3	17.5	16.9	17.5	-6	62.9		62.8	62.9	62.9

ranose (Table 1). In addition, enzymatic hydrolysis of **1** by β-glucosidase provided a corresponding spirostanol glycoside **1a**. Thus, the structure of **1** was determined to be 26-*O*-β-D-glucopyranosyl-(25*R*)-3β,22ξ,26-trihydroxyl-5α-furostane 3-*O*-β-chacotrioside. This compound was a new furostane type oligoglycoside in our literature survey.

Compound **2** was isolated as an amorphous powder, $[\alpha]_D$ −53.2° (pyridine). It reacted positively to the Ehrlich reagent on TLC. It exhibited a *quasi*-molecular ion peak of $[M+Na]^+$ at *m/z* 959 in the positive FAB-MS. The high-resolution FAB-MS gave an $[M+Na]^+$ ion at *m/z* 959.4833, corresponding to the composition $C_{45}H_{76}NaO_{20}$, which was also supported by analysis of the ^{13}C-NMR and DEPT spectral data. The ^{1}H-NMR spectrum in pyridine-d_5 displayed signals due to four steroidal methyl groups at δ 0.88 and 1.15 (each 3H, s), 1.04 and 1.33 (each 3H, d, J=6.7 Hz), one secondary methyl group of deoxyhexopyranose at δ 1.71 (3H, d, J=6.1 Hz), and three anomeric protons at δ 4.82 (1H, d, J=7.9 Hz), 5.03 (1H, d, J=7.9 Hz), and 5.87 (1H, s). Acid hydrolysis of **2** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and L-rhamnose, in a ratio of 2 : 1 by GLC analysis as described above. Enzymatic hydrolysis of **2** by β-glucosidase gave the corresponding spirostanol oligoglycoside (**2a**), which was identified as (25*S*)-3β,5β,6α-trihydroxyspirostane 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside, obtained from the same plant material as reported in the previous paper,[3] by comparing their ^{1}H-, ^{13}C-NMR and MS data. Thus, the structure of **2** was determined to be 26-*O*-β-D-glucopyranosyl-(25*S*)-3β,5β,6α,22ξ,26-pentahydroxyl-5β-furostane 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranoside. This compound was a new furostane-type oligoglycoside.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −28.4° (MeOH), showing a *quasi*-molecular ion peak due to $[M+Na]^+$ at *m/z* 959 and a fragment ion peak due to $[M+Na-\text{deoxyhexopyranose}]^+$ at *m/z* 813 in the positive FAB-MS. The high-resolution FAB-MS gave an $[M+Na]^+$ ion at *m/z* 959.4794, which corresponds to the composition $C_{45}H_{76}NaO_{20}$. Acid hydrolysis of **3** with 1 M HCl in dioxane–H_2O (1 : 1) afforded D-glucose and L-rhamnose, in a ratio of 2 : 1 by GLC analysis as described above. The ^{1}H-NMR spectrum displayed signals due to two tertiary methyl groups at δ 0.76 and 1.17 (each 3H, s), one secondary methyl group at δ 0.99 (3H, d, J=6.7 Hz), one terminal methyl group of an alkane at δ 0.91 (3H, t, J=7.3 Hz), one secondary methyl group of deoxyhexopyranose at δ 1.71 (3H, d, J=6.1 Hz), and three anomeric protons at δ 4.81 (1H, d, J=7.9 Hz), 5.00 (1H, d, J=7.9 Hz), and 5.88 (1H, br s). The carbon signals that arose from the A and B rings on the aglycone and sugar moieties attached at C-3 and C-26 in **3** were superimposable on those of **2** (Table 1). It was suggested that **3** had 26-*O*-β-D-glucopyranose and 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranose as the carbohydrate moieties, and 3β,5β,6α,26-tetrahydroxy-steroidal derivatives as an aglycone part. On the other hand, an ester carbonyl carbon signal appeared at δ 173.2 and a new methylene carbon signal was observed at δ 17.2 instead of an acetal carbon signal at C-22 (*ca.* 110 ppm). To determine the structure of the aglycone part, ^{1}H–^{1}H COSY, heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond correlation (HMBC) experiments were carried out for assignment of the respective proton and carbon signals as shown in Fig. 1. The methyl signal at δ 0.91 (3H, t, J=7.2 Hz) was correlated to the carbon signals at C-17 (δ 57.1) and δ 17.2 in the HMBC, so that the methyl proton signal was assigned to H_3-21 and the methylene carbon signal at δ 17.2 was located to C-20.

The proton signal at δ 5.41 was assigned to H-16, which was shifted downfield (+0.47 ppm) compared with that of **2**. This fact suggested the ester carbonyl group (δ 173.2) was attached to the C-16 position. The terminal glucosyl H-1 (δ 4.81) was correlated to C-26 (δ 74.7), and the H_3-27 methyl signal [δ 0.99 (d, J=6.7 Hz)] was correlated to C-26 (δ 74.7), C-25 (δ 33.6), and C-24 (29.2) in the HMBC. The H_2-24 protons (δ 1.62, 2.01, each 1H, m) were correlated to the H_2-23 proton (δ 2.45, 2H, m) in the ^{1}H–^{1}H COSY, and the H_2-23 and H-16 protons were correlated to the C-22 ester carbonyl carbon (δ 173.2) in the HMBC. Therefore, the side chain sequence was determined to be 5-*O*-β-D-glucopyranoyl-4-methyl-5-hydroxypentanoic acid. In a biosynthetic pathway, Compound **3** might be derived from **2** in the plant body as follows: (1) dehydrogenation of the C-22 hydroxy group to a pseudo-form, (2) oxidative cleavage of the C-20—22 bond like Marker degradation, and (3) reduction of the C-20 carbonyl to afford compound **3**. In this pathway, the stereochemistry at C-25 in **2** and **3** was kept as *S* configuration.

The hydroxy group of H-6 (δ 4.03, br d, J=11.8 Hz) was assigned a β configuration due to the correlations of H-6 with H-7β (δ 2.29) and H-8 (δ 2.12) in the NOESY spectrum (Fig. 2). The A/B ring was assigned a *cis* configuration due to the correlations of H-9 (δ 1.21) with H-2α (δ 1.39) and 4α (δ 1.58) in the NOESY spectrum (Fig. 2). In addition, the hydroxy group of H-16 (δ 5.41) was assigned a β configuration due to correlations of H-16 with H-17 (δ 1.22) and H-17 with H-14 (δ 0.74) in the NOESY spectrum. The small $W_{1/2}$ value of the H-3 proton at δ 4.62 (1H, br s, $W_{1/2}$=7.6 Hz) suggested it to be a 3β axial hydroxyl group.

Thus, the structure of **3** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl 3β,5β,6α,16β-tetrahydroxypregnane 16-(5-*O*-β-D-glucopyranoyl-4(*S*)-methyl-5-hydroxypentanoic acid) ester.

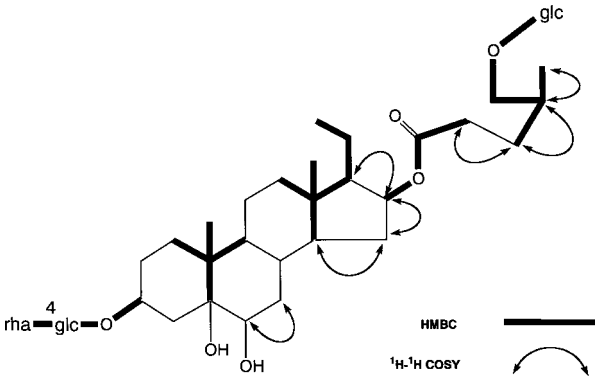

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations Observed for **3**

Compounds **1**—**3** and protodioscin were tested for cytotoxicity[1] against PC-12[14] and HCT-116[15] cell lines. Only protodioscin was effective against both cell lines [GI_{50}: 1.98 μM (PC-12), 3.78 μM (HCT-116)], while **1**—**3** showed no activity at less than 5 μM.

Work is in progress to use these oligoglycosides to study other activity such as anti-HSV-1 activity.

Experimental

The optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a JEOL α-500 spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as internal standard. Standard pulse sequences were employed for the DEPT, HMQC, and HMBC experiments. NOESY spectra were measured with mixing times of 600 ms. The FAB-MS were measured with a JEOL DX-300 and/or SX102A spectrometer. The HR-FAB-MS were measured with a JEOL DX-303 HF spectrometer and taken in a glycerol, triethylene glycol and *m*-nitrobenzyl alcohol matrix. GLC was performed on an HP5890A gas chromatograph with a flame ionization detector. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). Column chromatography was carried out on Kieselgel 60 (70—230 mesh and 230—400 mesh), Diaion HP-20 (Mitsubishi Chemical Ind.), Sephadex LH-20 (Pharmacia), and Chromatorex ODS-DU 3050MT (Fuji Silysia). HPLC separations were conducted on an ODS column (Cosmosil 5C18 MS, ϕ20×250 mm, 5 μm) using a TOSOH CCPS pump equipped with a differential refractometer (JASCO 830-RI). β-Glucosidase extracted from almonds was purchased from Sigma Chemical Co.

Extraction and Isolation The seeds (2.1 kg) of *Allium tuberosum*, which were purchased from Takii Seed Co. Ltd. in 1998, were ground down and extracted with MeOH twice under reflux. The combined extract was concentrated (92.5 g) and partitioned between hexane and 80% MeOH. The 80% MeOH fraction (56.0 g) was subjected to Diaion HP-20 column chromatography using H_2O and MeOH. The MeOH eluate (10.0 g) was separated by Chromatorex ODS (60% MeOH→MeOH) to give fractions I to IV. Fraction II (5.3 g) was further separated by silica gel column chromatography ($CHCl_3$: MeOH : H_2O=8 : 2 : 0.2→7 : 3 : 0.5) to give subfractions II-1—II-7. Fraction II-5 (284.4 mg) was subjected to silica gel column chromatography eluting with $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5), ODS silica gel with MeOH–H_2O (3 : 2), and finally preparative HPLC using MeOH–H_2O (3 : 2) to furnish **2** (20.6 mg) and **3** (8.3 mg). Fraction II-6 (568.8 mg) was subjected to preparative HPLC to afford **1** (10.5 mg) and protodioscin (10.6 mg).

Compound **1**: An amorphous powder, $[\alpha]_D^{29}$ −45.4° (c=0.17, pyridine). Positive FAB-MS (m/z) 1073 $[M+Na]^+$, 1033 $[M+H-H_2O]^+$. HR-FAB-MS (m/z) 1073.5509 $[M+Na]^+$ (Calcd for $C_{51}H_{86}NaO_{22}$; 1073.5508). ^{1}H-NMR (pyridine-d_5) δ: 0.81 (3H, s, H_3-18), 0.85 (3H, s, H_3-19), 1.06 (3H, d, J=6.7 Hz, H_3-27), 1.17 (3H, d, J=6.7 Hz, H_3-21), 1.63 (3H, d, J=6.1 Hz, rha-H_3-6), 1.68 (3H, d, J=6.1 Hz, rha-H_3-6), 3.53 (1H, dd, J=6.7, 9.2 Hz, H-26a), 3.92 (1H, m, H-26b), 3.94 (1H, m, H-3), 4.92 (1H, d, J=7.3 Hz, 26-*O*-glc-H-1), 4.93 (1H, m, H-16), 5.00 (1H, d, J=7.9 Hz, glc-H-1), 5.82 (1H, s, rha-H-1), 6.38 (1H, s, rha-H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

Enzymatic Hydrolysis of 1 A mixture of **1** (6 mg) and β-glucosidase (10 mg; Sigma Co., EC 3.2.1.21 from almonds) in acetate buffer (1.0 ml; 100 mM, pH 4.0) was incubated at 37 °C for 3 d. The mixture was concentrated *in vacuo* to dryness and the residue was chromatographed over silica gel [$CHCl_3$–MeOH–H_2O (8 : 2 : 0.2)] to afford a spirostanol glycoside (**1a**; 3 mg) as an amorphous powder, $[\alpha]_D^{14}$ −45.7° (c=0.26, pyridine). Positive FAB-MS (m/z) 893 $[M+Na]^+$, 747 $[M+Na-deoxyhexopyranose]^+$. HR-

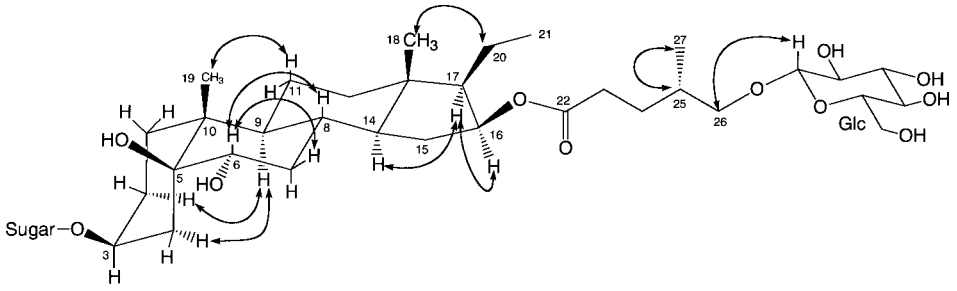

Fig. 2. Selected NOEs Observed for **3**

FAB-MS (*m/z*) 893.4924 $[M+Na]^+$ (Calcd for $C_{45}H_{74}NaO_{16}$; 893.4874). ^{1}H-NMR (pyridine-d_5) δ: 0.70 (3H, d, J=6.1 Hz, H_3-27), 0.83 (3H, s, H_3-18), 0.86 (3H, s, H_3-19), 1.14 (3H, d, J=7.3 Hz, H_3-21), 1.63 (3H, d, J=6.1 Hz, rha-H_3-6), 1.75 (3H, d, J=6.1 Hz, rha-H_3-6), 3.50 (1H, dd, J=10.4, 10.4 Hz, H-26a), 3.58 (1H, br d, J=10.4 Hz, H-26b), 4.98 (1H, d, J=7.3 Hz, glc-H-1), 5.85 (1H, s, rha-H-1), 6.36 (1H, s, rha-H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

Acid Hydrolysis of 1, 2, and 3 Compound **1** (1 mg) was hydrolyzed with 1 M HCl in dioxane–H_2O (1 : 1) for 4 h at 80 °C. The reaction mixture was neutralized with 2 M NaOH in H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was concentrated to dryness *in vacuo* to give a residue, which was identified as tigogenin by silica gel TLC [*Rf* value: 0.50 (hexane–acetone=1 : 1)] comparing with an authentic sample. The aqueous layer was concentrated to dryness *in vacuo* to give a residue which was dissolved in dry pyridine, to which was added L-cysteine methyl ester hydrochloride.[10] The reaction mixture was heated for 2 h at 60 °C and concentrated to dryness by blowing N_2 gas. Trimethylsilylimidazole was added to the residue followed by heating for 1 h at 60 °C. The residue was extracted with hexane and H_2O, and the organic layer was analyzed by GLC; column: OV-17 (0.32 mm×30 m), detector: FID, column temp.: 230 °C, detector temp.: 270 °C, injector temp.: 270 °C, carrier gas: He (2.0 kg/cm^2). Peaks were observed at t_R (min): 11′78″ (D-Glc) and 8′12″ (L-Rha) in a ratio of 1 : 1. The standard monosaccharides were subjected to the same reaction and GLC analysis under the same conditions. Following this procedure, **2** and **3** (each 1 mg) were subjected to acid hydrolysis to yield D-glucose and L-rhamnose in a ratio of 2 : 1. In this procedure, the aglycones decomposed under acidic conditions.

Compound **2**: An amorphous powder, $[\alpha]_D^{29}$ −53.2° (c=0.20, pyridine). Positive FAB-MS (*m/z*) 959 $[M+Na]^+$. HR-FAB-MS (*m/z*) 959.4833 $[M+Na]^+$ (Calcd for $C_{45}H_{76}NaO_{20}$; 959.4828). ^{1}H-NMR (pyridine-d_5) δ: 0.88 (3H, s, H_3-18), 1.04 (3H, d, J=6.7 Hz, H_3-27), 1.15 (3H, s, H_3-19), 1.33 (3H, d, J=6.7 Hz, H_3-21), 1.71 (3H, d, J=6.1 Hz, rha-H_3-6), 3.49 (1H, dd, J=7.93, 7.94 Hz, H-26a),4.07 (1H, br d, J=10.8 Hz, H-6), 4.10 (1H, m, H-26b), 4.65 (1H, br s, $W_{1/2}$=7.3 Hz, H-3), 4.82 (1H, d, J=7.9 Hz, 26-*O*-glc-H-1), 4.97 (1H, m, H-16), 5.03 (1H, d, J=7.9 Hz, glc-H-1), 5.87 (1H, s, rha-H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

Compound **3**: An amorphous powder, $[\alpha]_D^{29}$ −28.4° (c=0.15, pyridine). Positive FAB-MS (*m/z*) 959 $[M+Na]^+$, 813 $[M+Na-$deoxyhexopyranose$]^+$. HR-FAB-MS (*m/z*) 959.4794 $[M+Na]^+$ (Calcd for $C_{45}H_{76}NaO_{20}$; 959.4828). ^{1}H-NMR (pyridine-d_5) δ: 0.74 (1H, m, H-14), 0.76 (3H, s, H_3-18), 0.87 (1H, m, H-20a), 0.91 (3H, t, J=7.3 Hz, H_3-21), 0.99 (3H, d, J=6.7 Hz, H_3-27), 1.17 (3H, s, H_3-19), 1.21 (1H, m, H-9), 1.22 (1H, m, H-17), 1.31 (1H, m, H-20b), 1.36 (1H, m, H-11β), 1.39 (1H, m, H-2α), 1.58 (1H, m, H-4α), 1.60 (1H, m, H-11α), 1.62 (1H, m, H-24a), 1.71 (3H, d, J=5.5 Hz, rha-H_3-6), 1.94 (1H, m, H-25), 2.01 (1H, m, H-24b), 2.12 (1H, m, H-8), 2.29 (1H, m, H-7β), 2.45 (2H, m, H_2-23), 3.50 (1H, dd, J=6.7, 8.6 Hz, H-26a), 4.02 (1H, m, H-26b), 4.03 (1H, br d J=11.8 Hz, H-6) 4.62 (1H, br s, $W_{1/2}$=7.6 Hz, H-3), 4.81 (1H, d, J=7.9 Hz, 26-*O*-glc-H-1), 5.00 (1H, d, J=7.9 Hz, glc-H-1), 5.41 (1H, m, H-16), 5.88 (1H, s, rha-H-1). ^{13}C-NMR (pyridine-d_5) δ: Table 1.

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