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Two New Steroidal Glycosides from *Scopolia japonica* MAXIM.[1)]

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Two new steroidal glycosides, scopolosides I (1) and II (2), together with funkioside D (3) were obtained from the methanolic extract of the fresh underground parts of *Scopolia japonica* MAXIM. (Solanaceae) collected in Hiroshima prefecture, and the chemical structures of 1 and 2 were characterized to be (25*R*)-spirost-5-ene-3β,15α,23α-triol, named scopologenin (4), 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and scopologenin 3-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, respectively, by spectroscopic and chemical evidence.

Keywords *Scopolia japonica*; Solanaceae; steroidal glycoside; scopoloside; scopologenin; spirostanol; funkioside D

Scopolia japonica MAXIM. (Solanaceae) grows wild in a mountainous district in Japan, and its underground parts are an important source of *Scopolia* extract or atropine, whose yield varies distinctly in growth locations. As a part of a series of studies on the steroidal components in the solanaceous plants, a survey of the ingredients of the title plant led us to the first recognition of the occurrence of steroidal glycosides. Thus, we tried to reveal a correlation of the productive amounts between the tropane alkaloid and steroidal components.

In this paper, we firstly deal with the isolation and chemical characterization of the steroidal glycosides.

The methanolic extract of the fresh underground parts of the plant was subjected to polystyrene gel, ODS and silica gel column chromatographies to afford two new steroidal glycosides, scopolosides I (**1**) and II (**2**), which were constituted of a new sapogenol, together with funkioside D (**3**),[2)] which was identified by proton (^{1}H) and carbon-13 (^{13}C)-nuclear magnetic resonance (NMR) and chemical evidence.

Scopoloside I (**1**), a white powder (mp 229—234 °C), $[\alpha]_D$ $-49.1°$ (MeOH), showed the molecular formula $C_{45}H_{72}O_{20}$ ($M^+ + Na$, *m/z* 955.4493 , require: 955.4515) in the high-resolution positive fast atom bombardment mass spectrum (FAB-MS) and exhibited characteristic absorption bands due to spiroketal at 920, 826 and 800 cm^{-1}, together with strong hydroxyl absorption at 3388 cm^{-1} in the infrared (IR) spectrum. The ^{1}H-NMR spectrum of **1** displayed two singlet signals at δ 0.88 and 1.11 and two doublet signals at δ 0.68 ($J=5.5$ Hz) and 1.20 ($J=7.3$ Hz) due to steroidal methyl signals, and three doublet signals at δ 4.89 ($J=7.9$ Hz), 5.12 ($J=7.9$ Hz) and 5.20 ($J=7.3$ Hz) attributable to the anomeric protons of the sugar residue. The above data suggested that **1** was a steroidal triglycoside.

On hydrolysis with 1 N HCl, **1** produced D-glucose, D-galactose and a new aglycone (**4**), named scopologenin.

Scopologenin (**4**), colorless needles, mp 245—247 °C, $[\alpha]_D$ $-51.6°$ ($CHCl_3$), showed the molecular formula $C_{27}H_{42}O_5$ (M^+, *m/z* 446.3036, require: 446.3032) in the high-resolution electron impact (EI)-MS. The ^{1}H-NMR spectrum of **4** showed an olefinic proton signal at δ 5.50 (d, $J=3.3$ Hz), together with four characteristic methyl signals at δ 0.67 (d, $J=5.9$ Hz), 1.04 (s), 1.15 (s) and 1.21 (d, $J=7.0$ Hz) which arose from the spirostanol derivative in the ^{1}H-NMR spectrum. In addition, six proton signals at δ 3.11 (m), 3.36 (t, $J=10.6$ Hz), 3.45 (m), 3.86 (m), 4.52 (br dd, $J=3.8$, 10.0 Hz) and 4.78 (dd, $J=3.8$, 9.3 Hz) adjacent to the oxygen bearing carbons were observed. On comparative study of the ^{13}C-NMR spectrum for **4** with that of diosgenin,[3)] that of **4** was similar to that of diosgenin, except for C-15, C-23 and their neighbouring carbons, thus suggesting that two hydroxyl groups were attached to C-15 and C-23 in **4**. The peracetate (**5**) of **4**, a white powder, showed a molecular ion $[M]^+$ at *m/z* 572 in the EI-MS. The ^{1}H-NMR spectrum of **5** exhibited three acetyl groups at δ 2.03 (s, 6H) and 2.07 (s, 3H), three protons at δ 4.59 (1H, m), 4.79 (1H, dd, $J=4.8$, 11.7 Hz) and 5.32 (1H, dd, $J=3.7$, 11.0 Hz) adjacent to the carbons attached to the acetoxyl group, an oxygenated methine at δ 4.25 (1H, dd, $J=3.7$, 8.8 Hz) and oxygenated methylene signals at δ 3.32 (1H, t, $J=10.7$ Hz) and 3.39 (1H, m), along with four methyl signals at δ 0.83 (d, $J=6.6$ Hz), 0.90 (s), 0.95 (d, $J=7.0$ Hz) and 1.03 (s). The above data suggested that **5** should be a triacetate. These signals could be assigned by proton–proton correlation spectroscopy (^{1}H–^{1}H COSY) observed between H_3-27 (δ 0.83 (d, $J=6.6$ Hz)) and H-25 (δ 1.85 (m)), H-25 and H_2-24 (δ 1.57 (m) and 1.87 (m)), H_2-24 and H-23 (δ 4.79 (dd, $J=4.7$, 11.7 Hz)), and between H-25 and H_2-26 (δ 3.32 (t, $J=10.7$ Hz) and 3.39 (m)) in **5**. Other correlations were also observed between H_3-21 (δ 0.95 (d, $J=7.0$ Hz)) and H-20 (δ 2.10 (m)), H-20 and H-17 (δ 1.89 (dd, $J=5.5$, 8.8 Hz)), H-17 and H-16 (δ 4.25 (dd, $J=3.7$, 8.8 Hz)), H-16 and H-15 (δ 5.32 (dd, $J=3.7$, 11.0 Hz)). Therefore, three acetoxyl groups were shown to attach to C-3, C-15 and C-23. As regards the configurations at C-15, C-23 and C-25, they were revealed to be 15α and 23α-acetoxy, and C-25 (*R*) orientations were revealed by observation of the nuclear Overhauser effect (NOE) experiments between H-15 and H_3-18, and between H-23 and H-25. Based on the above evidence, the structure of triacetate **5** was determined to be scopologenin 3β,15α,23α-triacetate. Consequently, the aglycone moiety, scopologenin (**4**), of **1** was characterized as (25*R*)-spirost-5-ene-3β,15α,23α-triol.

Next, scopoloside I (**1**) was partially hydrolyzed with 1 N H_2SO_4 in 50% EtOH to provide two prosapogenins, **6** and **7**, and the aglycone **4**. Prosapogenin **6**, an amorphous powder, $[\alpha]_D$ $-39.1°$ (MeOH), was regarded to be a monoglycoside since the ^{13}C-NMR spectrum of **6** showed one anomeric carbon signal at δ 103.2 (d), and a pseudo-molecular ion peak was detected at *m/z* 607 $[M-H]^-$ in the negative FAB-MS. The ^{13}C-NMR spectrum of **6** disclosed the presence of a β-galactopyranosyl residue

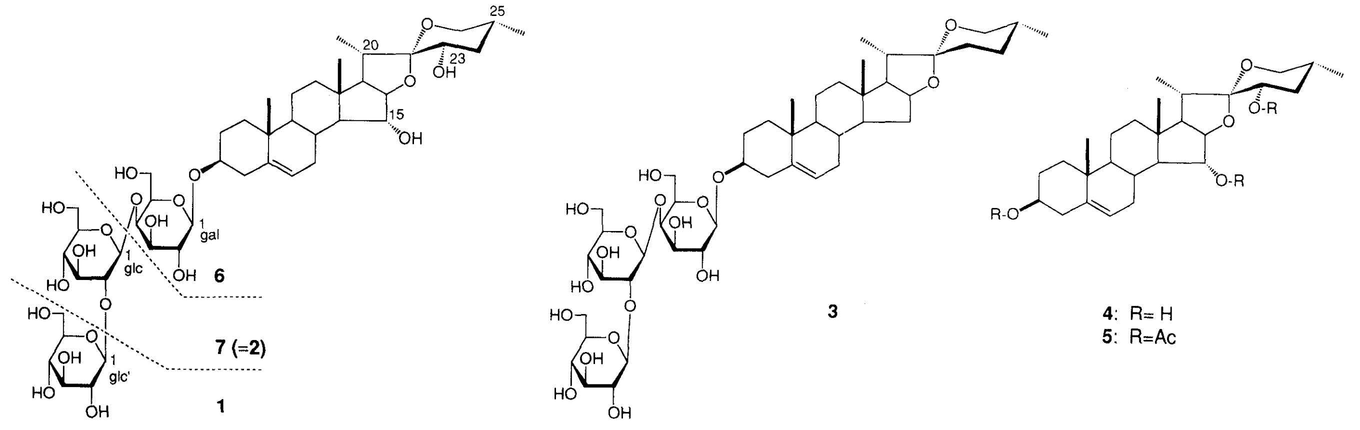

Fig. 1

Table I. ^{13}C NMR Data for **1**, **2**, **3**, **4**, **6** and **7** (in Pyridine-d_5)

	1	**4**	**6**	**7**	**2**	**3**
C- 1	37.5	37.9	37.6	37.6	37.5	37.5
C- 2	30.2	33.1	30.3	30.3	30.2	30.2
C- 3	78.1	71.3	78.1	78.1	77.9	78.2
C- 4	39.1	43.4	39.3	39.2	39.1	38.7
C- 5	140.1	141.2	140.2	140.2	140.1	140.1
C- 6	122.5	121.9	122.6	122.6	122.5	121.6
C- 7	32.9	32.6	33.1	33.0	33.0	32.3
C- 8	32.2	32.4	32.3	32.3	32.2	31.6
C- 9	50.2	50.5	50.3	50.3	50.2	50.3
C-10	36.9	37.0	37.0	37.0	36.9	37.0
C-11	20.9	21.1	21.0	21.0	20.9	21.2
C-12	40.7	40.9	40.8	40.8	40.7	39.2
C-13	40.7	40.8	40.8	40.8	40.7	41.3
C-14	59.9	60.1	60.1	60.0	60.0	56.7
C-15	79.0	79.2	79.1	79.1	79.0	32.3
C-16	91.2	91.4	91.4	91.3	91.3	82.3
C-17	60.7	61.0	60.9	60.8	60.7	60.8
C-18	14.7	14.8	14.8	14.8	14.7	16.7
C-19	19.4	19.6	19.4	19.4	19.3	19.3
C-20	35.7	35.8	35.8	35.8	35.7	44.7
C-21	16.8	16.9	16.9	16.9	16.8	14.7
C-22	111.4	111.5	111.4	111.4	111.4	111.8
C-23	67.3	67.4	67.4	67.3	67.3	30.0
C-24	38.6	38.8	38.8	38.7	38.7	28.9
C-25	31.6	31.7	35.8	31.6	31.6	31.7
C-26	65.9	65.9	66.0	65.9	65.8	65.9
C-27	17.8	18.0	17.9	17.9	17.8	16.9
gal-1	102.5		103.2	102.8	102.7	102.6
gal-2	73.2		72.7	73.5	73.4	73.3
gal-3	75.0		75.4	75.2	75.1	75.1
gal-4	80.9		70.4	80.0	79.9	81.1
gal-5	75.4		76.9	75.4	75.3	75.6
gal-6	60.3		62.6	60.9	60.8	60.5
glc -1	105.1			107.1	107.0	105.2
glc -2	86.0			75.9	75.8	86.1
glc -3	77.5			78.5	78.6	77.6
glc -4	70.2			72.3	72.2	70.3
glc -5	78.3			78.7	78.4	77.9
glc -6	61.4			63.1	63.0	61.6
glc'-1	106.8					106.9
glc'-2	76.6					76.7
glc'-3	78.8					78.9
glc'-4	71.7					71.8
glc'-5	78.1					77.9
glc'-6	63.1					63.2

(Table I) as well as changes by −2.8, +6.8 and −4.1 ppm, respectively, the chemical shifts being assignable to C-2, C-3 and C-4 in the aglycone part by comparison with those of **4**. Thus, the structure of **6** was determined to be scopologenin 3-*O*-β-D-galactopyranoside. Another prosapogenin **7**, an amorphous powder, $[\alpha]_D$ −56.1° (MeOH), showed a pseudo-molecular ion peak at *m/z* 769 $[M-H]^-$ in the negative FAB-MS, suggesting **7** to be a diglycoside. The ^{13}C-NMR data for **7** exhibited the occurrence of a terminal β-glucopyranosyl residue and a β-galactopyranosyl residue shifted at C-4 (+9.6 ppm) by comparison with those of **6**, as shown in Table I. The ^{1}H- and ^{13}C-NMR spectral and MS data of **7** were in accordance with those of scopologenin II (**2**); thus, **7** was shown to be identical with **2**. The structure of **7** was thus characterized to be scopologenin 3-*O*-β-D-glucopylanosyl-(1→4)-β-D-galactopylanoside.

Moreover, the ^{13}C-NMR spectrum of **1** disclosed the presence of a terminal β-D-glucopyranosyl residue, a β-D-galactopyranosyl residue shifted at C-4 (+10.5 ppm) and β-D-glucopyranosyl residue shifted at C-2 (+10.1 ppm) by comparison with those of **6** and **7**.

Therefore, the structure of **1** was elucidated as (25*R*)-spirost-5-ene-3β,15α,23α-triol (scopologenin) 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken with a JASCO DIP-360 digital polarimeter (l=0.5). The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM GX-400 NMR spectrometer and chemical shifts are given in a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The FAB-MS and EI-MS spectra were measured with a JEOL JMS DX-303HF spectrometer. Thin layer chromatography (TLC) was performed on pre-coated Kieselgel 60 F_{245} (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography (CC) was carried out on Kieselgel 60 (270—400 mesh, Merck), Bondapak C_{18} (Waters Associates) and MCI gel CHP-20P (Mitsubishi Chemical Ind.). Gas chromatographic (GC) analysis was performed with a Hewlett-Packerd HP-5890A gas chromatograph equipped with an H_2 flame ionization detector; the column was silicone OV-1 (0.32 mm i.d. x 30 m, Ohio Valley Specialty Chemical); column bath temperature 230 °C, injection port temperature 270 °C, carrier gas (He) 30 ml/min.

Extraction and Separation The fresh underground parts (2.8 kg) of *Scopolia japonica* Maxim. harvested in Hiroshima prefecture, Japan in May 1988 were extracted with MeOH, and the concentrated extract (93.2 g) was partitioned between benzene and water. The aqueous layer was subjected to column chromatography over MCI gel CHP-20P with gradient mixtures of H_2O–MeOH, to provide seven fractions (frs. 1—7). Fraction 5 (1.02 g, 80% MeOH eluate) was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (7 : 2.8 : 0.3—7 : 3 : 0.5) to afford 12 fractions (frs.

8—19). Fraction 13 (240 mg) was precipitated with MeOH several times to give a white powder (**1**, 200 mg). Fraction 10 (41 mg) was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (8:2:0.2) to afford **2** (18 mg). Fraction 12 (29 mg) was subjected to Bondapak C_{18} CC with 50% dil. MeOH to provide **3** (5.2 mg).

Scopoloside I (1) A white powder, $[\alpha]_D^{22}$ −49.1° (c=0.50, MeOH), mp 229—234 °C. High-resolution positive FAB-MS m/z: 955.4493 ($[M+Na]^+$, $C_{45}H_{72}O_{20}Na$, require: 955.4515). Negative FAB-MS m/z: 931 $[M-H]^-$, 769 $[M-H-\text{hexose}]^-$, 607 $[M-H-2\times\text{hexose}]^-$. IR ν_{max}^{KBr} cm^{-1}: 3388 (OH), 920, 826, 800. ^{1}H-NMR (pyridine-d_5) δ: 0.68 (3H, d, J=5.5 Hz, H_3-27), 0.88 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 1.20 (3H, d, J=7.3 Hz, H_3-21), 3.26 (1H, t, J=10.3 Hz, H_{ax}-26), 3.46 (1H, m, H_{eq}-26), 4.89 (1H, d, J=7.9 Hz, gal H-1), 5.12 (1H, d, J=7.9 Hz, glc′ H-1), 5.20 (1H, d, J=7.3 Hz, glc H-1), 5.38 (1H, br s, H-6).

Acid Hydrolysis of 1 A solution of **1** (50 mg) in 3 ml of 1 N HCl was heated under reflux for 3 h. After removal of the solvent by N_2-blowing, the reaction mixture was diluted with water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water several times, dried over anhydrous $MgSO_4$, and subjected to silica gel CC with $CHCl_3$–MeOH (40:1) to yield an aglycone fraction. It was recrystallized from $CHCl_3$–MeOH (2:1) to give **4** (3.2 mg) as colorless needles, mp 245—247 °C, $[\alpha]_D^{28}$ −51.6° (c=0.71, $CHCl_3$). High-resolution EI-MS m/z: 446.3036 (M^+, $C_{27}H_{42}O_5$, require: 446.3032). ^{1}H-NMR (pyridine-d_5) δ: 0.67 (3H, d, J=5.9 Hz, H_3-27), 1.04 (3H, s, H_3-18), 1.15 (3H, s, H_3-19), 1.21 (3H, d, J=7.0 Hz, H_3-21), 3.11 (1H, m, H-23), 3.36 (1H, t, J=10.6 Hz, H-26), 3.45 (1H, m, H-26), 3.86 (1H, m, H-3), 4.52 (1H, br dd, J=3.8, 10.0 Hz, H-16), 4.78 (1H, dd, J=3.8, 9.3 Hz, H-15), 5.50 (1H, d, J=3.3 Hz, H-6).

Acetylation of 4 A mixture of **4** (3.2 mg) in pyridine (2 ml) and Ac_2O (1 ml) was left standing at room temperature overnight. After evaporation of pyridine and Ac_2O, the residue was subjected to silica gel CC with *n*-hexane–EtOAc (9:1) to afford the peracetate (**5**, 1.0 mg), a white powder, EI-MS m/z: 572 $[M]^+$, 530, 512, 487, 452, 445, 392. ^{1}H-NMR ($CDCl_3$) δ: 0.83 (3H, d, J=6.6 Hz, H_3-27), 0.90 (3H, s, H_3-18), 0.95 (3H, d, J=7.0 Hz, H_3-21), 1.03 (3H, s, H_3-19), 1.47 (1H, t, J=10.7 Hz, H-14), 1.57 (1H, m, H-24), 1.85 (1H, m, H-25), 1.87 (1H, m, H-24), 1.89 (1H, dd, J=5.5, 8.8 Hz, H-17), 2.03 (6H, s, 15, 23-O-Ac), 2.07 (3H, s, 3-O-Ac), 2.10 (1H, m, H-20), 3.32 (1H, t, J=10.7 Hz, H_{ax}-26), 3.39 (1H, m, H_{eq}-26), 4.25 (1H, dd, J=3.7, 8.8 Hz, H-16), 4.59 (1H, m, H-3), 4.79 (1H, dd, J=4.7, 11.7 Hz, H-23), 5.32 (1H, dd, J=3.7, 11.0 Hz, H-15), 5.32 (1H, br d, J=3.3 Hz, H-6).

Identification of Component Monosaccharides, 1, 2 and 3 Each compound (2 mg each) was heated in 1 N HCl water–dioxane (1:1, 0.5 ml) at 90 °C for 2 h. The precipitate was removed by filtration and the supernatant was treated with Amberlite IRA-400. One half of the neutralized solution was concentrated *in vacuo* to give a sugar fraction. The pyridine solution of the sugar fraction was derived into the trimethylsilyl ether of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(R)-carboxylate using the Mihashi *et al.* method,[4] and analyzed by GLC to detect two peaks at t_R (min): 16.6 (D-glucose), 17.7 (D-galactose). The standard monosaccharides were subjected to the same reaction, and GLC analysis was performed under the same condition. *c.f.* standard specimens, t_R (min): 17.4 (L-glucose), 16.6 (D-glucose), 18.8 (L-galactose), 17.7 (D-galactose).

The other half of the sugar solution furnished glucose (*Rf* 0.36) and galactose (*Rf* 0.27) detected by TLC impregnated with 0.5 M NaH_2PO_4 (solvent, 2-propanol–acetone–0.1 M lactic acid (2:2:1).[5]

The filtrate of **3** furnished diosgenin, which was detected by TLC.

Partial Hydrolysis of 1 A solution of **1** (57 mg) in 3 ml of 1 N H_2SO_4 in 50% EtOH was heated in a water bath at 90 °C for 40 min. The reaction mixture was suspended in water and the suspension was subjected to MCI gel CHP-20P and eluted with water. The fractions of the saponin and the sapogenol were obtained by elution with MeOH and then chromatographed on silica gel to yield the aglycone **4** (6.5 mg) and two prosapogenins **7** (10.3 mg) and **6** (3.5 mg) and unchanged **1** (11 mg). Prosapogenin **6**: A white amorphous powder, $[\alpha]_D^{22}$ −39.1° (c=0.23, MeOH). High-resolution positive FAB-MS m/z: 631.3458 ($[M+Na]^+$, $C_{33}H_{52}O_{10}Na$, require: 631.3453). Negative FAB-MS m/z: 607 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.68 (3H, d, J=5.9 Hz, H_3-27), 0.90 (3H, s, H_3-18), 1.12 (3H, s, H_3-19), 1.21 (3H, d, J=7.0 Hz, H_3-21), 3.36 (1H, t, J=10.6 Hz, H_{ax}-26), 3.47 (1H, m, H_{eq}-26), 4.97 (1H, d, J=7.7 Hz, gal H-1), 5.40 (1H, br s, H-6). Prosapogenin **7**: A white amorphous powder, $[\alpha]_D^{22}$ −56.1° (c=1.03, MeOH). Negative FAB-MS m/z: 769 $[M-H]^-$, 607. ^{1}H-NMR (pyridine-d_5) δ: 0.68 (3H, d, J=5.9 Hz, H_3-27), 0.88 (3H, s, H_3-18), 1.12 (3H, s, H_3-19), 1.20 (3H, d, J=7.0 Hz, H_3-21), 3.36 (1H, t, J=10.4 Hz, H_{ax}-26), 3.46 (1H, m, H_{eq}-26), 4.88 (1H, d, J=7.3 Hz, gal H-1), 5.30 (1H, d, J=7.7 Hz, glc H-1), 5.40 (1H, br s, H-6).

Scopoloside II (2) An amorphous powder, $[\alpha]_D^{23}$ −58.3° (c=0.49, MeOH). High-resolution positive FAB-MS m/z: 793.4033 ($[M+Na]^+$, $C_{39}H_{62}O_{15}Na$, require: 793.3986). Negative FAB-MS m/z: 769 $[M-H]^-$, 607 $[M-H-\text{hexose}]^-$. IR ν_{max}^{KBr} cm^{-1}: 3424 (OH). ^{1}H-NMR (pyridine-d_5) δ: 0.68 (3H, d, J=5.9 Hz, H_3-27), 0.88 (3H, s, H_3-18), 1.12 (3H, s, H_3-19), 1.20 (3H, d, J=7.0 Hz, H_3-21), 3.36 (1H, t, J=10.7 Hz, H_{ax}-26), 3.46 (1H, m, H_{eq}-26), 4.88 (1H, d, J=7.7 Hz), 5.29 (1H, d, J=8.0 Hz, glc H-1), 5.40 (1H, br s, H-6).

Funkioside D (3) A white amorphous powder, $[\alpha]_D^{24}$ −54.0° (c=0.63, MeOH). Negative FAB-MS m/z: 899 $[M-H]^-$, 739. ^{1}H-NMR (pyridine-d_5) δ: 0.71 (3H, d, J=5.5 Hz, H_3-27), 0.81 (3H, s, H_3-18), 1.11 (3H, s, H_3-19), 1.20 (3H, d, J=6.7 Hz, H_3-21), 3.45 (1H, m, H-26), 3.53 (1H, m, H-26), 4.89 (1H, d, J=7.7 Hz, gal H-1), 5.15 (1H, d, J=6.2 Hz, glc′ H-1), 5.22 (1H, d, J=5.5 Hz, glc H-1), 5.40 (1H, br s, H-6).

References and Notes

1) This work is Part XXV in the series of studies on the constituents of solanaceous plants.
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