Two New Cycloartane Glycosides, Thalictosides A and C from *Thalictrum thunbergii* D.C.

Hitoshi YOSHIMITSU,[a] Kazuhiro HAYASHI,[b] Kazushi SHINGU,[b] Junei KINJO,[b] Shoji YAHARA,[b] Kimiko NAKANO,[c] Kotaro MURAKAMI,[c] Toshiaki TOMIMATSU,[c] and Toshihiro NOHARA*,[b]

Faculty of Engineering, Kyushu Kyoritsu University,[a] 1–8 Jiyugaoka Yahata-nishi-ku, Kitakyushu 807, Japan, Faculty of Pharmaceutical Sciences, Kumamoto University,[b] 5–1 Oe-honmachi, Kumamoto 862, Japan, and Faculty of Pharmaceutical Sciences, Tokushima University,[c] Shomachi 1–78, Tokushima 770, Japan. *Received March 18, 1992*

Two new cycloartane glycosides, thalictosides A (1) and C (2), were isolated from the aerial part of *Thalictrum thunbergii* D.C. They were constituted of a new triterpenoidal sapogenol named thalictogenin a, that is, 3β,22*S*,26-trihydroxycycloartan-24-ene. Their structures were characterized as 3-*O*-β-D-quinovopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl thalictogenin a (1) and 3-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-β-D-fucopyranosyl thalictogenin a (2) on the bases of the chemical evidence and spectroscopic means.

Keywords *Thalictrum thunbergii*; Ranunculaceae; cycloartane glycoside; thalictoside; thalictogenin a

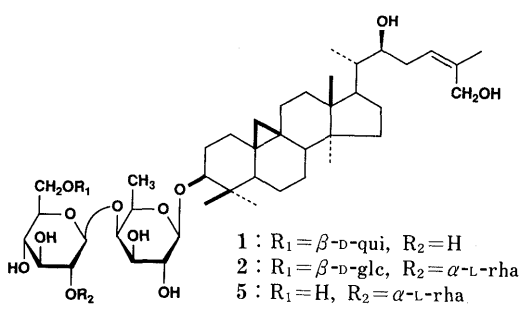
Thalictum thunbergii D.C. grows wild in Japan and its whole plants have been used as a folk medicine for treating stomachache in the region of Nagano prefecture. As regards its constituents, alkaloids, such as berberine, takatonine, magnoflorine and thalicberberine have been well known. However, no investigation of the neutral constituent except for the flavonoids[1] has so far been reported. Our investigation of this plant has resulted in the isolation of two triterpenoidal constituents and their structural characterization by chemical and spectroscopic means.

The methanolic extract of the aerial parts of the title plant cultivated in the Botanical Garden of Tokushima University was partitioned between an organic mixture of 1-BuOH and AcOEt, and water. The organic soluble fraction was subjected to silica gel, Sephadex LH-20 and octadecyl silica (ODS) column chromatographies, while the aqueous layer was subjected to Diaion HP-20P, silica gel and ODS column chromatographies to afford two triterpenoidal glycosides, designated as thalictosides A (**1**) and C (**2**).

Both **1** and **2** on acid hydrolysis with 1 N hydrogen chloride in MeOH afforded a sapogenol (**3**), designated as thalictogenin a, colorless needles, mp 202—204 °C, $[\alpha]_D$ +30.2° (pyridine), as a major sapogenol, together with unidentified artificial minor sapogenols derived from the genuine sapogenol. In addition, D-fucose, D-quinovose and D-glucose in the hydrolysate of **1** were detected by gas-liquid chromatographic analysis (GLC) as their trimethylsilyl ethers of their methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates.[2]

On the other hand, D-glucose, L-rhamnose and D-fucose were detected in the hydrolysate of **2**.

The electron impact mass spectrometry (EI-MS) of **3** showed a molecular ion $[M]^+$ peak at *m/z* 458 along with other fragment peaks. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **3** showed an AB quartet signal (J=3.7 Hz) at δ 0.33 and 0.57, as characteristic of a cyclopropane methylene, a singlet vinylic methyl signal on an olefinic linkage at δ 2.04, four singlet methyl signals at δ 0.93, 1.09, 1.13, 1.25, and one doublet methyl signal (J=6.6 Hz) at δ 1.19. Besides them, the ^{1}H-NMR spectrum revealed the presence of two secondary hydroxyl groups [1H, dd, J=11.4, 2.0 Hz at δ 3.56 and 1H, br s, at δ 4.06] and one olefinic proton at δ 5.71 (1H, t, J=7.1 Hz). Another AB quartet signal at δ 4.50 and 4.57 (J=11.9 Hz) was assignable to a hydroxymethyl group. Based on the above evidence, **3** was considered to be a cycloartane derivative. Moreover, it became clear that both the hydroxymethyl and the methyl groups attached to the olefinic carbon and located at the terminal of the side chain, and that an olefinic proton at C-24 correlated to the methylene at C-23, which in turn coupled with a hydroxy methine proton at C-22 based on the proton–proton correlation spectroscopy (^{1}H–^{1}H COSY) and ^{1}H–carbon-13 (^{13}C) long range COSY of **3**. Furthermore, a comparative study of the ^{13}C-NMR spectrum of **3** with that of 3β,26-dihydroxycycloart-24-ene[3] showed a low field shift (+40 ppm) of the C-22 signal in **3**, indicating that one of the C-22 protons was substituted with a hydroxyl group. In addition, observation of the nuclear Overhauser effect (NOE) experiment between the olefinic methyl proton at C-27 and an olefinic proton at C-24 revealed that their correlation was *cis*. The chemical shifts, except for the signals due to the side chain moiety and the C-17 on the D-ring, showed coincidence with that of 3β,26-dihydroxycycloart-24-ene in the ^{13}C-NMR spectrum of **3**. Furthermore, configuration at C-22 was determined to be *S* by the Moscher's method[4] after acetylation of the hydroxymethyl group at C-26. The differences of the respective chemical shifts between, [(+)-α-methoxy-α-(trifluoromethyl) phenylacetate (MTPA) and (−)-MTPA of 26-*O*-acetyl **3**] on CH_3-21, H-24 and CH_3-27 were +0.04, −0.03 and −0.02 ppm, respectively. Thus, the structure of thalictogenin a (**3**) was determined to be 3β,22*S*,26-trihydroxycycloart-24-ene.



1 : R_1=β-D-qui, R_2=H
2 : R_1=β-D-glc, R_2=α-L-rha
5 : R_1=H, R_2=α-L-rha

Thalictoside A (**1**), an amorphous powder, $[\alpha]_D^{25}$ −1.3° (pyridine), displayed peaks at *m/z* 1065 $[M+NBA]^-$, 911 $[M-H]^-$ in the negative fast atom bombardment mass spectra (neg. FAB-MS). The ^{1}H-NMR spectrum showed

TABLE I. ^{13}C-NMR Data for **1**—**6** (Pyridine-d_5)

	a)	1	2	3	4	5	6
Sapogenol 1	32.0	32.2	32.0	32.4	31.8	32.2	31.9
2	30.4	30.0	29.7	31.3	29.0	29.7	29.1
3	78.8	88.6	89.1	80.0	89.7	89.5	88.8
4	40.5	41.3	41.1	41.1	40.6	41.4	40.6
5	47.2	47.7	47.5	47.5	47.4	47.8	47.8
6	21.1	21.2	20.6	21.5	20.9	21.1	20.9
7	28.2	28.0	27.8	28.0	27.8	28.0	27.9
8	48.0	48.0	47.8	48.2	47.9	47.9	47.3
9	20.0	20.1	19.8	20.0	20.5	20.0	20.6
10	26.1	26.3	26.1	26.6	25.2	26.3	25.1
11	26.1	26.2	26.0	26.3	25.9	26.2	25.9
12	35.6	35.9	35.6	35.9	35.4	35.9	35.4
13	45.4	45.4	45.2	45.4	45.2	45.5	45.2
14	48.8	49.1	48.9	49.1	48.8	49.1	48.9
15	33.0	33.4	33.2	33.4	32.9	33.4	32.9
16	26.5	26.7	26.5	26.7	26.3	26.7	26.4
17	52.3	49.1	48.9	49.1	48.7	49.1	48.7
18	18.1	19.6	19.4	19.6	19.3	19.6	19.3
19	29.9	29.7	29.5	30.0	29.7	29.7	29.7
20	36.0	41.7	41.4	41.7	38.9	41.7	38.9
21	18.3	12.1	11.9	12.1	12.1	12.1	12.4
22	36.0	72.7	72.6	72.7	75.7	72.8	75.7
23	25.1	34.9	34.6	34.8	30.8	34.9	30.9
24	127.0	125.2	124.9	125.2	125.8	125.2	125.9
25	134.4	137.7	137.4	137.7	132.3	137.8	132.3
26	69.0	61.1	60.8	61.1	62.9	61.1	63.0
27	13.7	22.3	22.0	22.2	21.5	22.3	21.5
28	19.4	18.4	18.1	18.4	17.8	18.3	17.8
29	25.1	25.8	25.6	26.2	25.2	25.8	25.1
30	14.0	15.4	15.1	14.9	14.9	15.3	14.9
Fucose 1		106.9	107.0		102.8	107.5	103.2
2		73.5	74.9		69.8	73.0	71.2
3		75.7	75.6		73.6	76.0	70.2
4		82.9	77.8		76.9	78.5	73.6
5		70.4	72.7		69.7	72.0	70.0
6		17.9	17.8		16.8	17.9	16.6
Glucose 1		106.6	102.7		101.2	103.3	100.7
2		75.8	78.5		71.5	79.0	75.0
3		78.9	78.2		72.8	78.0	75.1
4		71.4	71.4		69.2	70.6	69.0
5		77.4	76.6		73.1	78.2	71.8
6		69.9	69.8		67.8	63.1	62.0
Quinovose 1		105.3			100.2		
2		75.4			71.4		
3		77.9			72.8		
4		76.9			73.2		
5		72.9			70.0		
6		18.6			17.3		
Rhamnose 1			101.7			102.0	97.4
2			72.1			72.5	73.3
3			72.3			72.7	69.1
4			74.1			74.6	70.6
5			70.4			70.1	67.3
6			18.5			18.9	17.5
Glucose 1			105.3				
2			75.6				
3			78.2				
4			71.7				
5			78.2				
6			62.5				

a) 3β, 26-Dihydroxycycloart-24-ene.[3]

three doublet signals with $J=7.3$, 7.7, and 7.7 Hz at δ 4.72, 5.03, and 5.16, respectively, ascribable to the anomeric protons. To confirm the structure of the oligosaccharide moiety, **1** was converted into the corresponding peracetate to afford **1**-peracetate (**4**), an amorphous powder, $[\alpha]_D^{26}$ $+6.1°$ ($CHCl_3$), which showed fragment ion peaks in EI-MS at m/z 273, 561 and 791 originating from the peracetylated terminal deoxyhexose, deoxyhexosyl hexose and deoxyhexosyl-hexostyl-deoxyhexose, respectively, in the EI-MS. In the ^{1}H-NMR spectrum of **4**, three doublet signals ascribable to the anomeric protons appeared at δ 4.41, 4.49, and 4.51 ($J=7.7$, 9.2, and 8.4 Hz, respectively). Moreover, the presence of acetyl fucopyranosyl, glucopyranosyl and quinovopyranosyl units were substantiated based on the detailed analyses of the ^{1}H–^{1}H COSY. In addition, the long range ^{1}H–^{13}C COSY was observed between an anomeric carbon of the peracetyl quinovopyranose and H_2-6 of the triacetyl glucopyranose, and between an anomeric carbon of the triacetyl glucopyranose and H-4 of the diacetyl fucopyranose. NOE was detected between H-3 in the aglycone and the anomeric proton of the diacetyl fucopyranose. Based on the above data, **1** was formulated as 3-*O*-β-D-quinovopyranosyl-(1→6)-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside.

Thalictoside C (**2**), an amorphous powder, $[\alpha]_D^{26}$ $-23.5°$ (pyridine), exhibited a $[M-H]^-$ peak at m/z 1073 in the neg. FAB-MS, indicating **2** to be a tetraglycoside. In the ^{1}H-NMR spectrum, three doublet signals at δ 4.63, 5.06, and 5.45 ($J=7.7$, 7.7, and 7.0 Hz, respectively), and a broad singlet signal at δ 6.24, ascribable to the anomeric protons were observed. To verify the structure of the oligosaccaride moiety, **2** was hydrolysed with a crude enzyme from *Turbo cornutus* to yield glucose and a prosapogenin (**5**) as an amorphous powder, $[\alpha]_D^{26}$ $-11.7°$ (MeOH). In the ^{1}H-NMR spectrum of **5**, two doublet signals at δ 4.71 and 5.62 ($J=7.7$ and 7.0 Hz, respectively), and a broad singlet signal at δ 6.36 ascribable to the anomeric protons, were detected. Moreover, acetylation of **5** provided a peracetate (**6**), as an amorphous powder, $[\alpha]_D^{25}$ $+10.5°$ ($CHCl_3$), whose EI-MS showed the same fragment ion peaks as those of **4**, indicating that the sugar moiety of **2** was composed of deoxyhexosyl-hexosyl-deoxyhexose. The ^{1}H-NMR spectrum of **6** displayed two doublet signals at δ 4.47 ($J=7.7$ Hz) and 4.54 ($J=7.0$ Hz), and a broad singlet signal at δ 5.02, ascribable to the anomeric protons. Moreover, as a component sugar, fucose, glucose and rhamnose were proved based on the detailed analysis of the ^{1}H–^{1}H COSY spectrum and the NOEs in **6**, which were observed between the anomeric proton of the triacetyl rhamnopyranosyl moiety and H-2 of the triacetyl glucopyranosyl moiety, between the anomeric proton of the triacetyl glucopyranosyl residue and the H-4 of the diacetylfucopyranosyl moiety, and between the anomeric proton of the diacetylfucopyranosyl moiety and H-3 of the aglycone part. From the above data, the structure of **5** was concluded to be thalictogenin a 3-*O*-β-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside.

On the other hand, a comparative study of the ^{13}C-NMR spectrum of **2** with that of **5** showed the presence of the additional terminal glucopyranosyl unit, and its linkage was considered to be the C_6-hydroxyl group of the inner glucopyranosyl moiety, since a glycosylation shift was observed on the corresponding carbon by +7.8 ppm. Based on the above evidence, the structure of **2** was established as thalictogenin a 3-*O*-β-D-glucopyranosyl-(1→6)-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→4)-β-D-fucopyranoside.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic digital polarimeter. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-GX 400 NMR spectrometer and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. FAB-MS and EI-MS were measured with a JEOL DX-303 HF spectrometer and taken in a 3-nitrobenzylalcohol matrix. Thin layer chromatography was performed on precoated Kiesel gel 60 F_{245} (Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography was carried out on Kiesel gel (230—400 mesh, Merk), Sephdex LH-20 (Pharmacia Fine Chem. Co., Ltd.), ODS (PrePak-500/C_{18}, Waters) and Diaion HP-20P (Mitsubishi Chemical Ind.). Gas chromatographic (GC) analysis was performed with an HP-5890A equipped with an OV-1 column (30 m × 0.32 mm, Ohio Valley Specially Chemical).

Extraction and Separation The fresh aerial parts (3.6 kg) of *Thalictrum thumbergii* D.C. harvested at the Botanical Garden of Tokushima University in May, 1991 were extracted with MeOH and the extract was partitioned between an organic mixture of 1-BuOH and AcOEt (1 : 4, v/v), and H_2O (1 : 1, v/v). The organic fraction (50.3 g) was subjected to silica gel column chromatography using $CHCl_3$–MeOH–H_2O (1 : 0 : 0→30 : 1 : 0→10 : 1 : 0→9 : 2 : 0.2→8 : 2 : 0.2→7 : 3 : 0.5, v/v) to afford four fractions (fr. 1—4). Fraction 2 (200 mg) was further purified by Sephadex LH-20 chromatography with MeOH–H_2O (4 : 1, v/v), followed by ODS column chromatography with MeOH–H_2O (60→65→70%) to afford thalictoside A (**1**) (110 mg). Fraction 3 (840 mg) was subjected to ODS column chromatography with MeOH–H_2O (60→63→66%) to provide thalictoside C (**2**) (346 mg). On the other hand, the aqueous layer (154.9 g) was subjected to Diaion HP-20P column chromatography with MeOH–H_2O (0→30→50→70→90%) to yield four fractions (fr. 1—4). Fraction 3 was purified by silica gel chromatography with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v) followed by ODS chromatography with MeOH–H_2O (63→66%) to give thalictoside C (**2**) (146 mg).

Thalictoside A (1) An amorphous powder, $[\alpha]_D^{25}$ −1.3° (c=0.25, pyridine). Neg. FAB-MS (m/z): 1065 $[M+NBA]^-$, 911 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.27, 0.52 (each 1H, ABq, J=3.7 Hz, CH_2-19), 0.91, 1.05, 1.05 (each 3H, s, Me-18, Me-30, Me-28), 1.18 (3H, d, J=6.6 Hz, Me-21), 1.34, 2.03 (each, 3H, s, Me-29, Me-27), 1.60 (3H, d, J=5.5 Hz, qui Me-6), 1.74 (3H, d, J=6.7 Hz, fuc Me-6), 3.47 (1H, dd, J=11.6, 4.2 Hz, H-3), 4.48, 4.45 (each 1H, ABq, J=12.1 Hz, CH_2-26), 4.72 (1H, d, J=7.3 Hz, fuc-1), 5.03 (1H, d, J=7.7 Hz, glc H-1), 5.16 (1H, d, J=7.7 Hz, qui H-1), 5.69 (1H, dd, J=7.0, 7.3 Hz, H-24).

Acid Hydrolysis of 1 and 2 A solution of **2** (200 mg) in 10 ml of 1 N HCl–MeOH was heated on a water bath for 8 h. The reaction mixture was concentrated under reduced pressure and then subjected to Diaion HP-20P column chromatography eluted with water followed by MeOH. The MeOH eluate (122 mg) was subjected to silica gel column chromatography with $CHCl_3$–MeOH (100 : 1→50 : 1, v/v) to yield thalictogenin a (**3**), colorless needles (38 mg), mp 202—204 °C, $[\alpha]_D^{19}$ +30.2° (c=0.50, pyridine).

Compound **1** was hydrolysed in the same manner as for **2** to give the same sapogenol.

Thalictoside C (2) An amorphous powder, $[\alpha]_D^{26}$ −23.5° (c=0.48, pyridine). Neg. FAB-MS (m/z) 1073 $[M-H]^-$, 911 $[M-\text{hexose}-H]^-$, 765 $[M-\text{hexose}-\text{deoxyhexose}-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.22, 0.47 (each 1H, ABq, J=3.4 Hz, CH_2-19), 0.90, 0.99, 1.03 (each 3H, s, Me-18, Me-30, Me-28), 1.15 (3H, d, J=6.6 Hz, Me-21), 1.30, 2.01 (each 3H, s, Me-29, Me-27), 1.65 (3H, d, J=6.2 Hz, fuc Me-6), 1.72 (3H, d, J=5.9 Hz, rha Me-6), 3.42 (1H, dd, J=4.0, 11.4 Hz, H-3), 4.46, 4.54 (each 1H, ABq, J=12.1 Hz, CH_2-26), 4.63 (1H, d, J=7.7 Hz, fuc H-1), 5.06 (1H, d, J=7.7 Hz, glc H-1), 5.45 (1H, d, J=7.0 Hz, glc H-1), 5.66 (1H, dd, J=7.0, 7.3 Hz, H-24), 6.24 (1H, br s, rha H-1).

Partial Acetylation of 3 A mixture of **3** (38 mg) in $CHCl_3$ (2 ml) and Ac_2O (2 ml) was allowed to stand for 5 h at room temperature. The solvent was removed with a stream of nitrogen and the residue was subjected to silica gel column chromatography with n-hexane–acetone (10 : 1, v/v) to provice 26-O-acetate of **3** (15 mg).

26-*O*-Acetyl 3,22-(+)- and (−)-MTPA of 3 (+)-MTP acetic acid (9.2 mg) and N,N'-dicyclohexylcarbodiimide (DCC) (13.2 mg) were dissolved in anhydrous ether (1 ml) and the solution was stirred for 30 min at room temperature. To the above solution was added 26-O-acetyl-**3** (5 mg) in a small amount of ether and 4-dimethylaminopyridine (DMAP) (2.7 mg). The reaction mixture was allowed to stand with stirring overnight at room temperature, the solvent removed by drying in a stream of nitrogen and the residue was purified by silica gel column chromatography with n-hexane–AcOEt (6 : 1→4 : 1, v/v) to furnish 26-O-acetyl-3,22-(+)-MTPA of **3**. 26-O-Acetyl 3,22-(−)-MTPA (1.8 mg) of **3** was also prepared in the same method as above. 26-O-Acetyl-3,22-(+)-MTPA, a white powder, $[\alpha]_D^{25}$ +10.9° (c=0.13, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.33, 0.57 (each 1H, ABq, J=3.7 Hz, CH_2-19), 0.92 (3H, d, J=7.0 Hz, CH_3-21), 1.74 (3H, s, CH_3-27), 2.05 (3H, s, 26-O-Ac), 4.47, 4.60 (each 1H, ABq, J=12.3 Hz, CH_2-26), 5.31 (1H, dd, J=7.3, 7.0 Hz, H-24). 26-O-Acetyl-3,22-(−)-MTPA, a white powder, $[\alpha]_D^{25}$ +57.3° (c=0.38, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.34, 0.57 (each 1H, ABq, J=3.9 Hz, CH_2-19), 0.88 (3H, d, J=6.2 Hz, CH_3-21), 1.76 (3H, s, CH_3-27), 2.06 (3H, s, 26-O-Ac), 4.52, 4.46 (each 1H, ABq, J=12.1 Hz, CH_2-26), 5.34 (1H, dd, J=7.3, 7.0 Hz, H-24).

Acetylation of 1 A solution of **1** (21.7 mg) in pyridine (3 ml) and Ac_2O (1.5 ml) was allowed to stand at room temperature overnight. The solvent was removed by drying with a stream of nitrogen and the residue was purified by silica gel column chromatography with benzene–acetone (9 : 1, v/v) to afford thalictoside A peracetate (**4**, 20.1 mg), a white powder, $[\alpha]_D^{25}$ +6.1° (c=1.18, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: sapogenol part, 0.33, 0.53 (each 1H, ABq, J=4.0 Hz, CH_2-19), 0.77, 0.86, 0.88 (each 3H, s, Me-30, Me-18, Me-29), 0.92 (3H, d, J=6.6 Hz, Me-21), 0.95, 1.75 (each 3H, s, Me-28, Me-27), 3.09 (1H, dd, J=11.7, 4.4 Hz, H-3), 4.53, 4.63 (each 1H, ABq, J=12.1 Hz, CH_2-26), 5.34 (1H, t, J=7.5 Hz, H-24); sugar part, 4.41 (1H, d, J=7.7 Hz, fuc H-1), 5.06 (1H, dd, J=10.3, 7.7 Hz, fuc H-2), 4.91 (1H, dd, J=10.3, 2.6 Hz, fuc H-3), 3.84 (1H, d, J=2.5 Hz, fuc H-4), 3.65 (1H, dd, J=10.6, 6.4 Hz, fuc H-5), 1.29 (3H, d, J=6.6 Hz, fuc Me-6), 4.49 (1H, d, J=9.2 Hz, glc H-1), 4.96 (1H, dd, J=9.5, 8.1 Hz, glc H-2), 5.20 (1H, t, J=9.5 Hz, glc H-3), 4.89 (1H, br t, J=9.5 Hz, glc H-4), 3.59 (1H, ddd, J=9.5, 6.6, 2.5 Hz, glc H-5), 3.61 (1H, dd, J=11.0, 6.6 Hz, glc H-6a), 3.78 (1H, br d, J=11.0 Hz, glc H′-6b), 4.51 (1H, d, J=8.4 Hz, qui H-1), 4.92 (1H, t, J=9.5 Hz, qui H-2), 5.12 (1H, t, J=9.5 Hz, qui H-3), 4.80 (1H, t, J=9.5 Hz, qui H-4), 3.53 (1H, dq, J=9.8, 6.2 Hz, qui H-5), 1.22 (3H, d, J=6.3 Hz, qui Me-6).

GLC Analysis of Sugar in 1 **1** (6 mg) was heated in 1 N HCl water–dioxane (1 : 1) at 90 °C for 2 h. The precipitate was removed by filtration and the filtrate was treated with Amberlite IRA-400 to give a sugar fraction for GLC. To the pyridine solution of sugars (1 mg/0.1 ml), pyridine solution of L-cysteine methyl ester hydrochloride (2 mg/0.1 ml) was added and warmed at 60 °C for 1 h. Pyridine was removed and the residue dried *in vacuo*. To the residue, TMS-imidazole (0.3 ml) was added and the mixture was heated at 60 °C for another 1 h. The reaction mixture was partitioned between n-hexane and water. The n-hexane solution was subjected to GLC. Three peaks were observed at t_R (min): 14.620 (D-quinovose), 15.839 (D-fucose) and 23.306 (D-glucose). The standard monosaccharides were subjected to the same reaction and GLC analysis was performed under the same condition. *Cf.* t_R (min): 15.453 (L-quinovose), 17.222 (L-fucose), 24.505 (L-glucose).

Enzymatic Hydrolysis of 2 Crude enzyme from *Turbo cornutus* (30 mg) in acetate buffer (pH 4.2, 10 ml) was added to a solution of **3** (30 mg) in dimethyl sulfoxide (DMSO) (7 ml). The solution was incubated at 37 °C for 5 d. The precipitate was collected by filtration. The aqueous filtrate was subjected to Diaion HP-20P column chroamtography with H_2O to remove DMSO and sugar, and then elution was followed by MeOH. The MeOH fraction was purified by column chromatography on silica gel with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, v/v) to furnish a prosapogenin (**5**, 7.8 mg), $[\alpha]_D^{26}$ −11.7° (c=0.70, MeOH). ^{1}H-NMR (pyridine-d_5) δ: 0.22, 0.47 (each 1H, ABq, J=4.03 Hz, CH_2-19), 0.88, 1.00, 1.03 (each 3H, s, Me-18, Me-30, Me-28), 1.17 (3H, d, J=7.6 Hz, Me-21), 1.32, 2.03 (each 3H,s, Me-29, Me-27), 1.56 (3H, d, J=6.2 Hz, fuc Me-6), 1.76 (3H, d, J=5.9 Hz, rha Me-6), 3.44 (1H, dd, J=4.0 Hz, H-3), 4.41, 4.56 (each 1H, ABq, J=12.0 Hz, CH_2-26), 4.71 (1H, d, J=7.7 Hz, fuc H-1), 5.62 (1H, d, J=7.0 Hz, glc H-1), 6.36 (1H, br s, rha H-1), 5.69 (1H, dd, J=7.32, 6.96 Hz, H-24).

Acetylation of 5 Compound **5** (7.8 mg) was acetylated in the same manner to yield a peracetate **6** (3 mg), $[\alpha]_D^{25}$ +10.5° (c=0.16 $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: sapogenol part, 0.32, 0.51 (each 1H, ABq, J=3.9 Hz, CH_2-19), 0.70, 0.85, 0.87 (each 3H, s, Me-30, Me-18, Me-29), 0.93 (3H, d, J=6.6 Hz, Me-21), 0.94, 1.75 (each 3H, s, Me-28, Me-27), 3.09 (1H, dd, J=12.4, 4.3 Hz, H-3), 4.53, 4.63 (each 1H, ABq, J=12.5 Hz, CH_2-26), 5.34 (1H, t, J=7.7 Hz, H-24); sugar part, 4.47 (1H, d, J=7.7 Hz, fuc H-1), 4.98 (1H, dd, J=9.0, 7.7 Hz, fuc H-2), 5.09 (1H, dd, J=9.0, 4.0 Hz, fuc H-3), 4.08 (1H, d, J=4.0 Hz, fuc H-4), 3.63 (1H, q, J=6.6 Hz, fuc H-5), 1.22 (3H, d, J=6.6 Hz, fuc Me-6), 4.54 (1H, d, J=7.0 Hz, glc-1), 3.81 (1H, dd, J=9.2, 7.0 Hz, glc H-2), 5.23 (1H, t, J=9.2 Hz, glc H-3), 4.96 (1H, t, J=9.2 Hz, glc H-4), 3.59 (1H, m, glc H-5), 4.12 (1H, dd, J=12.3, 5.5 Hz, glc H-6a), 4.18 (1H, dd, J=12.3, 3.0 Hz, glc H′-6b), 5.02 (1H, br s, rha

H-1), 5.11 (1H, m, rha H-2), 5.29 (1H, dd, $J=10.3$, 3.3 Hz, rha H-3), 5.10 (1H, t, $J=10.3$ Hz, rha H-4), 4.35 (1H, dq, $J=9.5$, 4.4 Hz, rha H-5), 1.26 (3H, d, $J=6.2$ Hz, rha Me-6).

GLC Analysis of Sugar in 2 The sugar components in **2** were checked by the same manner as in **1**. Three peaks were observed at t_R (min); 15.280 (L-rhamnose), 15.823 (D-fucose) and 23.376 (D-glucose) in the GLC analysis. *Cf.* t_R (min): 15.748 (D-rhamnose).

References and Notes

1) E. Shimizu, T. Tomimatsu, and T. Nohara, *Chem. Pharm. Bull.*, **32**, 5023 (1984).
2) S. Hara, H. Okabe, and K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986); *idem*, *ibid.*, **35**, 501 (1987).
3) V. Anjaneyulu, K. Harischandra Prasad, K. Ravi, and J. D. Connoll, *Phytochemistry*, **24**, 2359 (1985).
4) J. A. Dale and H. S. Moscher, *J. Am. Chem. Soc.*, **95**, 512 (1973).