Study on the Constituents of *Desmodium styracifolium*[1)]

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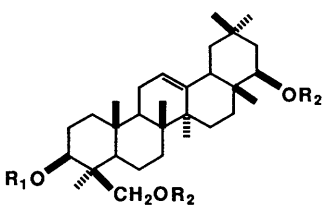
Two triterpenoid saponins (1 and 2) were isolated from Desmodii Herba [the dried whole plants of *Desmodium styracifolium* (OSBECK) MERR. (Leguminosae)] and their chemical structures were characterized as soyasaponin I and a new saponin, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]soyasapogenol E, respectively, by chemical and spectroscopic means.

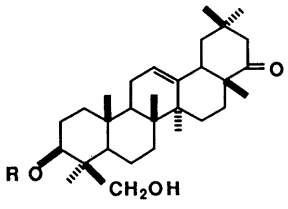
Keywords *Desmodium styracifolium*; Desmodii Herba; Leguminosae; cholestylithiasis; urolithiasis; triterpenoid; saponin; oleanane glycoside; soyasaponin I; glucuronide

Desmodii Herba,[2)] the whole plants of *Desmodium styracifolium* (OSBECK) MERR. (Leguminosae), has been used as a specific medicine to treat cholestylithiasis or urolithiasis in southern China.[3)] As a part of our studies on the ingredients of the leguminous plants and in order to identify the effective substance(s) against urolithiasis, we have investigated the constituents of this crude drug. We obtained two triterpenoid saponins **1** and **2** and determined their structures by means of spectroscopic analyses and chemical degradations.

Compound **1**, white crystalline powder, mp 238—242 °C, $[\alpha]_D$ −8.1° [dimethyl sulfoxide (DMSO)], showed a cluster ion $(M+1)^+$ at *m/z* 943 originating from the molecule in the fast atom bombardment mass spectrum (FAB-MS) and absorptions due to the hydroxyl group (3400 cm^{-1}) and carboxylate (1610 cm^{-1}) in the infrared (IR) spectrum. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1** exhibited signals characteristic of a triterpenoid glycoside, so **1** was hydrolyzed with 1 N HCl–MeOH to afford a sapogenol (**3**) identical with soyasapogenol B[4)] and methyl glycosides of rhamnose and galactose. From this result, **1** was considered to be soyasaponin I, which had been obtained from the seeds of *Glycine max* MERRILL by Kitagawa *et al.*[4)] However, it is unsafe to reach a conclusion solely from the ^{13}C-NMR spectrum. Furthermore, the sugar moiety of the methyl ester (**13**) of a new saponin (**2**) as described later showed a ^{13}C-signal pattern superimposable on that of the methyl ester (**4**) of **1**. Therefore, according to the usual chemical approach for saponins, the structure of the sugar part in **1** was unambiguously determined as described below. Since a uronic acid moiety was supposed to be present in the sugar part of **1** from the evidence of the ^{13}C-NMR and IR spectra (carboxylate group: ν_{max}^{KBr} 1610 cm^{-1} in **1**; δ_C 170.3 ppm in **4**), **1** was first treated with 0.5 N HCl–MeOH for 15 min at 70 °C to give two main methyl ester compounds **4** and **5**. They were then reduced with $NaBH_4$ in MeOH to afford **6** and **7**, respectively, which were successively derived into the corresponding permethyl ethers **8** and **9** by Hakomori's method.[5)] Compound **8**, colorless needles, mp 212—213 °C, $[\alpha]_D$ +7.9° ($CHCl_3$), showed three anomeric proton signals at δ 4.19 (1H, d, J=7.7 Hz), 4.67 (1H, d, J=7.7 Hz) and 5.25 (1H, d, J=1.5 Hz) in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. On acid hydrolysis with 1 N HCl–MeOH, **8** gave a mixture of methylosides of sugars and a methylated sapogenol (**10**). They were separated by silica gel column chromatography with *n*-hexane–acetone (4:1) as the eluent to provide, together with **10**, methyl 2,3,4-tri-*O*-methyl-β-L-rhamnopyranoside, methyl 3,4,6-tri-*O*-methyl-α-D-galactopyranoside and methyl 3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoside, which were shown to be identical with authentic specimens by thin layer chromatography (TLC), gas liquid chromatography (GLC) and ^{1}H-NMR spectroscopy. The aglycone derivative (**10**), colorless needles, mp 190—193 °C, $[\alpha]_D$ +88.7° ($CHCl_3$), FAB-MS *m/z*: 487 $(M+H)^+$, showed two methoxy groups at δ 3.28 and 3.31 in its ^{1}H-NMR spectrum. Moreover, the acetate (**11**) of **10**, colorless needles, mp 75—79 °C, $[\alpha]_D$ +60.2° ($CHCl_3$), electron impact mass spectrum (EI-MS) (*m/z*) 528 (M^+), exhibited a signal (1H, dd, J=4.9, 11.8 Hz) at δ 4.55 assignable to H-3 along with two methoxyl signals (δ 3.28 and 3.29) in the ^{1}H-NMR spectrum. Therefore, **11** was represented as 3-*O*-acetylsoyasapogenol B 22,24-di-*O*-methyl ether. On the other hand, the permethyl ether (**9**) was also hydrolyzed with 1 N HCl–MeOH to afford methyl 3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoside and methyl 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside (by GLC) besides **10**. From the above accumulated evidence, the structure of **6** could be represented as 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucopyranosyl]soyasapogenol B. Meanwhile, the peracetate (**12**) of **1** showed peaks at *m/z* 273 and 561 originating from the peracetylated terminal rhamnosyl and rhamnosyl-hexosyl cations in the EI-MS. The occurrence of the methyl ester of glucuronic acid (δ 105.4, 78.1, 76.4, 74.3, 77.6 and 170.3) in **4** was substantiated by the ^{1}H-NMR spectrum, so it was conceivable that the glucuronate in **4** was converted into glucose in **6** by $NaBH_4$ reduction. Consequently, the structure of **1** was concluded to be 3-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]soyasapogenol B, being identical with soyasaponin I.[4)]

Compound **2**, white crystalline powder, mp 272—280 °C, $[\alpha]_D$ −43.2° (pyridine), showed hydroxyl group (3420 cm^{-1}), carbonyl group (1700 cm^{-1}) and carboxylate (1620 cm^{-1}) absorptions in the IR spectrum. It was treated with 0.2 N HCl–MeOH for 1 h at 70 °C to give the corresponding methyl ester (**13**), colorless needles, mp 229—231 °C, $[\alpha]_D$ −8.2° (pyridine), which showed IR absorptions at 3420 cm^{-1} (OH) and 1750 (ester) and 1702 (carbonyl), and





	R_1	R_2		R
1	-glc UA^2-gal^2-rha	H	**2**	-glc UA^2-gal^2-rha
3	-H	H	**13**	-glc UA Me^2-gal^2-rha
4	-glc UA Me^2-gal^2-rha	H	**14**	-H
5	-glc UA Me^2-gal	H		
6	-glc^2-gal^2-rha	H		
7	-glc^2-gal	H		
10	-H	Me		
11	-Ac	Me		
16	-Ac	Ac		

Chart 1

a cluster ion ($M^+ + 1$) originating from the molecular ion at *m/z* 955 in the FAB-MS. On acid hydrolysis with 1 N HCl–MeOH, **2** liberated a sapogenol (**14**), colorless needles, mp 252—257 °C, $[\alpha]_D$ +29.7° ($CHCl_3$). The EI-MS of **14** exhibited a molecular peak at *m/z* 456 ($C_{30}H_{48}O_3$), a base peak at *m/z* 232 ($C_{16}H_{24}O$) and a peak at 224 ($C_{14}H_{24}O_2$), the latter two of which were generated by retro Diels–Alder fission[6] at the C-ring on the oleanene skeleton. This observation in the EI-MS suggested that **14** possesses two hydroxyl groups on the A/B rings, and one carbonyl group on the D/E rings. Compound **14** was then reduced with $NaBH_4$ in MeOH to yield two products, which were separated by silica gel column chromatography using *n*-hexane–acetone (4:1) as the eluent to provide **3** and **15**. They were successively acetylated to give compounds **16** and **17**. The ^{1}H-NMR spectrum of the former was identical with that of soyasapogenol B 3*β*,22*β*,24-triacetate. Compound **17** was shown to be 3*β*,22*α*,24-trihydroxyolean-12-ene triacetate by the ^{1}H-NMR spectrum (H-22*β*: 1H, dd, *J*=5.1, 10.8 Hz, *δ* 4.75). Therefore, the structure of **14** could be characterized as 3*β*,24-dihydroxyolean-12-en-22-one, namely it was regarded as identical with soyasapogenol E,[4] which had been obtained from the hydrolysate of the crude saponin of *Glycine max* by Kitagawa *et al.*[4] The structure of the sugar moiety was deduced from the ^{13}C-NMR spectrum. The chemical shifts due to the sugar moiety were superimposable on those of **4**, and therefore the sugar structure of **2** was concluded to be the same as that of **4**. Consequently, the structure of **2** was determined to be 3-*O*-[α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl]soyasapogenol E.

Compound **1** was shown to be effective against urolithiasis by pharmacological testing; this result will be reported elsewhere.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-360 digital polarimeter (cell length, 0.5 dm). EI-MS were recorded with a JEOL JMS-01SG spectrometer. ^{1}H (400 MHz)- and ^{13}C (67.5 MHz)-NMR spectra were measured with JEOL GX-400 spectrometers. IR spectra were taken with a Hitachi IR spectrometer, model 270—30. Column chromatography was carried out with Sephadex LH-20 (25—100 μm, Pharmacia Fine Chem. Co., Ltd.), Kieselgel 60 (70—230 mesh, Merck). TLC was conducted on precoated Kiselgel 60 F_{254} plates (0.2 mm, Merck) with $CHCl_2$–MeOH–H_2O (7:3:0.5); spots were located by ultraviolet (UV) illumination and by the use of 10% H_2SO_4 sprays. For GLC, a Shimadzu GC-3BF apparatus was used with 3% 1,4-butanediol succinate; 3 mm × 2 m glass column; column temperature, 140—160 °C; carrier gas, N_2. GC-MS, OV-17 (3%); 1 m glass column; column temperature, 130—190 °C (increasing temperature at 3 °C/min); carrier gas (20 ml/min); reagent gas, isobutane; standard, methyl 4,6-di-*O*-acetyl-2,3-di-*O*-methyl-*β*-D-glucopyranoside.

Extraction and Isolation The whole plants of *Desmodium styracifolium* (1 kg), Desmodii Herba, were extracted with refluxing MeOH. Removal of the solvent under reduced pressure gave the MeOH extract (50 g), which was partitioned into *n*-BuOH–H_2O. Removal of the solvent from the organic phase gave the *n*-BuOH extract. Separation of the *n*-BuOH extract by Sephadex LH-20 column chromatography (MeOH) furnished a triterpenoid fraction (21.6 g) and a flavonoid fraction (9.73 g). The triterpenoid fraction was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 9:2:0.1→8:2:0.2→7:3:0.5) to give compounds **1** (870 mg) and **2** (57 mg). Compound **1**: White crystalline powder, mp 238—242 °C, $[\alpha]_D^{21}$ −8.1° (*c*=0.71, DMSO), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1610 (carboxylate). FAB-MS *m/z*: 943 $(M+H)^+$. ^{13}C-NMR (pyridine-d_5) *δ*: 38.6, 26.5, 90.8, 43.9, 56.0, 18.8, 33.3, 40.0, 47.8, 36.4, 24.1, 123.0, 144.9, 42.4, 26.5, 28.7, 38.0, 45.4, 46.8, 30.9, 42.3, 75.6, 22.9, 63.5, 15.8, 17.0, 25.7, 28.7, 33.3, 21.1 (C_1-C_{30}), 104.7, 78.0, 76.1, 74.4, 76.6 (glc UA C_1-C_5), 101.7, 76.6, 76.1, 71.1, 76.6, 61.7 (gal C_1-C_6), 101.7, 72.3, 72.3, 74.4, 69.1, 18.8 (rha C_1-C_6). Compound **2**, white crystalline powder, mp 272—280 °C, $[\alpha]_D^{24}$ −43.2° (*c*=0.50, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1700 (carbonyl), 1620 (carboxylate).

Methanolysis of 1 A solution of **1** (2.4 mg) in 1 N HCl–MeOH (0.5 ml) was heated under reflux for 1.5 h. After neutralization with 3% KOH–MeOH solution, the reaction mixture was filtered to remove the inorganic material. Removal of the solvent from the filtrate gave the product, which was identified as soyasapogenol B (**3**), *Rf* 0.35 (solvent, *n*-hexane : acetone = 2:1) and methyl glycosides (*Rf* 0.53 and 0.24, respectively; solvent, $CHCl_3$: MeOH : acetone : H_2O = 3:3:3:1) of rhamnose and galactose (identified by TLC comparison with authentic specimens).

Partial Methanolysis of 1 A solution of **1** (500 mg) in 0.5 N HCl–MeOH (3 ml) was refluxed (at 70 °C) for 15 min. After neutralization with 3% KOH–MeOH solution, the reaction mixture was filtered to remove the inorganic materials. Removal of the solvent from the filtrate gave the products, which were purified by Sephadex LH-20 (MeOH) and silica gel ($CHCl_3$: MeOH : H_2O = 9:2:0.1) column chromatographies to give compounds **4** (92 mg) and **5** (78 mg). Compound **4**: White crystalline powder, mp 241—244 °C, $[\alpha]_D^{20}$ −8.1° (*c*=0.50, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1750 ($COOCH_3$). FAB-MS *m/z*: 957 $(M+H)^+$. ^{13}C-NMR (pyridine-d_5) *δ*: 38.6, 26.6, 91.3, 43.9, 56.1, 18.9, 33.2, 39.9, 47.8, 36.4, 24.0, 122.3, 144.8, 42.3, 26.4, 28.6, 37.9, 45.2, 46.8, 30.9, 42.3, 75.5, 23.0, 63.5, 15.8, 17.0, 25.7, 28.6, 33.2, 21.1, (C_1-C_{30}), 105.4, 78.1, 76.4, 74.3, 77.6, 170.3 (glc UA C_1-C_6), 101.7, 76.9, 76.4, 71.1, 76.4, 61.6 (gal C_1-C_6), 102.3, 72.3, 72.7, 73.6, 69.3, 18.9 (rha C_1-C_6). Compound **5**: White crystalline powder, mp 232—240 °C, $[\alpha]_D^{20}$ +26.1° (*c*=0.50, pyridine). FAB-MS *m/z*: 811 $(M+H)^+$. ^{13}C-NMR (pyridine-d_5) *δ*: 39.9, 28.9, 91.3, 44.1, 56.4, 19.2, 33.6, 40.2, 48.1, 36.8, 24.4, 122.7, 145.2, 42.6, 26.7, 28.9, 38.3, 45.6, 47.1, 31.2, 42.6, 75.8, 23.4, 63.9, 16.2, 17.3, 26.0, 28.9, 33.6, 21.5 (C_1-C_{30}), 105.4, 80.8, 76.9, 73.6, 77.9, 170.3 (glu UA C_1-C_6), 104.9, 72.6, 75.4, 70.5, 77.2, 62.6 (gal C_1-C_6).

$NaBH_4$ Reduction of 4 A solution of **4** (90 mg) was reduced with $NaBH_4$ (360 mg) in MeOH at room temperature for 24 h. The reaction mixture was then neutralized with AcOH and subjected to Sephadex LH-20 column chromatography in order to remove the resultant salts, affording the crude product, which was purified by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 8:2:0.2) to furnish the reduced product **6**, (56.9 mg). Compound **6**: Colorless needles, mp 253—256 °C, $[\alpha]_D^{23}$ −3.9° (*c*=0.55, pyridine). FAB-MS *m/z*: 951 $(M+Na)^+$, 929 $(M+H)^+$. ^{13}C-NMR (pyridine-d_5) *δ*: 38.6, 28.6, 90.8, 43.9, 56.2, 18.7, 33.3, 39.9, 47.8, 36.5, 24.0, 122.4, 144.8, 42.3, 26.6, 28.6, 38.0, 45.3, 46.8, 30.8, 42.3, 75.6, 22.7, 63.4, 15.7, 17.0, 25.7, 28.6, 33.3, 21.1, (C_1-C_{30}), 105.6, 79.4, 77.4, 71.5, 78.6, 62.3 (glc C_1-C_6), 102.7, 76.5, 74.6, 71.5, 76.9, 61.9 (gal C_1-C_6), 102.0, 72.4, 72.7, 73.0, 69.7, 18.9 (rha C_1-C_6).

$NaBH_4$ Reduction of 5 A solution of **5** (55 mg) was treated with $NaBH_4$ (200 mg) as described in the case of **4**. The purification of the product by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 9:2:0.1) furnished the reduction product **7**, (30 mg). Compound **7**: Colorless needles, mp 257—259 °C, $[\alpha]_D^{23}$ +42.3° (*c*=0.50, pyridine). ^{13}C-NMR (pyridine-d_5) *δ*: 38.7, 28.6, 90.6, 43.7, 56.2, 18.6, 33.3, 39.9, 47.8, 36.5, 24.0, 122.4, 144.9, 42.4, 26.4, 28.6, 38.0, 45.3, 46.8, 30.8, 42.4, 75.6, 22.8, 63.6, 15.7, 17.0, 25.7, 28.6, 33.3, 21.2 (C_1-C_{30}), 105.6, 81.9, 78.4, 71.2,

78.8, 62.8 (glc C_1-C_6), 104.7, 73.6, 75.4, 71.2, 77.1, 62.7 (gal C_1-C_6).

Complete Methylation of 6 A solution of **6** (10 ml) in DMSO (0.6 ml) was treated with dimsyl carbanion solution (1.5 ml) and the whole mixture was stirred at 70°C for 20 min. The reaction mixture was cooled, then methyl iodide (3 ml) was added, and the whole mixture was stirred at room temperature (20 °C) for 2 h, then poured into ice-water and extracted with diethyl ether. The product obtained by evaporation of the solvent under reduced pressure was purified by silica gel column chromatography (*n*-hexane : acetone = 3 : 1) to furnish the triglycosidic permethyl ether (**8**, 12 mg). Compound **8**: White crystalline powder, mp 212—213 °C, $[\alpha]_D^{21}$ +7.9° (*c* = 1.10, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.85, 0.90, 0.95, 1.00, 1.01, 1.09, 1.19, 1.24 (each 3H, s, 8 × Me), 3.28, 3.30, 3.37, 3.39, 3.46, 3.48, 3.51, 3.52, 3.67 (all s, 11 × OMe), 4.19 (1H, d, *J* = 7.7 Hz, glc H-1), 4.67 (1H, d, *J* = 7.7 Hz, gal H-1), 5.21 (1H, s, H-12), 5.25 (1H, d, *J* = 1.5 Hz, rha H-1).

Complete Methylation of 7 A solution of **7** (10 mg) in DMSO (0.6 ml) was methylated with dimsyl carbanion (1.5 ml) and methyl iodide (3 ml) as described for **6**. The purification of the product by silica gel column chromatography (*n*-hexane : acetone = 5 : 1) furnished the diglycosidic permethyl ether (**9**, 4.2 mg). Compound **9**: An amorphous powder, $[\alpha]_D^{21}$ +23.9° (*c* = 0.95, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.86, 0.90, 0.96, 1.00, 1.01, 1.09, 1.18 (each 3H, s, 7 × Me), 3.28, 3.29, 3.37, 3.39, 3.49, 3.51, 3.52, 3.60, 3.64 (each 3H, s, 9 × OMe), 4.26 (1H, d, *J* = 7.7 Hz, glc H-1), 4.64 (1H, d, *J* = 7.3 Hz, gal H-1), 5.22 (1H, s, H-12).

Methanolysis of 8 A solution of **8** (12 mg) in 1 N HCl–MeOH was heated under reflux for 2 h. The reaction mixture was neutralized with 3% KOH–MeOH solution and filtered. After evaporation of the solvent from the filtrate under reduced pressure, the residue was subjected to silica gel column chromatography (*n*-hexane : acetone = 4 : 1) to give a methylated sapogenol (**10**) and the methyl glycosidic components of the sugar moiety. Compound **10**: Colorless needles, mp 190—193 °C, $[\alpha]_D^{25}$ +88.7° (*c* = 0.50, $CHCl_3$). FAB-MS *m/z*: 487 $(M+H)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.89, 0.90, 0.92, 0.94, 1.00, 1.11, 1.22 (each 3H, s, 7 × Me), 3.28, 3.31 (each 3H, s, 2 × OMe), 3.20—3.28 (2H, m, H-3 and H-22), 3.21, 3.89 (2H, ABq, *J* = 9.2 Hz, H_2-24), 5.21 (1H, t, *J* = 3.7 Hz, H-12). The methylated sugar components were identified as follows. Methyl 3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoside, an oil, *Rf* 0.25 on TLC (solvent, *n*-hexane : acetone = 2 : 1), GLC, t_R 7.3 and 8.7 min. These methylated sugars were identical with those derived from the permethyl ether of prosapogenin A_D.[7] Moreover, the acetates of the above methylated sugars were identified by the gas chromatography–mass spectrum (GC-MS) pattern identification method.[8] Methyl 3,4,6-tri-*O*-methyl-α-galactopyranoside, oil, *Rf* 0.20 on TLC (solvent, *n*-hexane : acetone = 2 : 1), GLC, t_R 10.5 min. This methylated sugar was identical with the that derived from the permethyl ether of timosaponin A-III.[9] Methyl 2,3,4-tri-*O*-methyl-α-L-rhamnopyranoside, oil, *Rf* 0.53 on TLC (solvent, *n*-hexane : acetone = 2 : 1), GLC, t_R 2.2 min.

Methanolysis of 9 A solution of **9** (4.2 mg) in 1 N HCl–MeOH was refluxed for 1.5 h. The reaction mixture was worked up as described for the methanolysis of **8** and the resulting product was examined by TLC and GLC to identify **10**, methyl 3,4,6-tri-*O*-methyl-α- and -β-D-glucopyranoside, *Rf* 0.25 on TLC (solvent, *n*-hexane : acetone = 2 : 1), GLC, t_R 7.3 and 8.7 min, 2,3,4,6-tetra-*O*-methyl-α-D-galactopyranoside, *Rf* 0.37 on TLC (solvent, *n*-hexane : acetone = 2 : 1), GLC, t_R 10.5 min.

Acetylation of 10 A solution of **10** (8 mg) in Ac_2O–pyridine (1 : 1, 0.5 ml) was allowed to stand at room temperature (20 °C) for 12 h. The reaction mixture was evaporated under an N_2 atmosphere to give **11**. Compound **11**: mp 75—79 °C, $[\alpha]_D^{24}$ +60.2° (*c* = 0.50, $CHCl_3$). EI-MS *m/z*: 528 $(M)^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.85, 0.89, 0.96, 1.00, 1.10 (all s, 7 × Me), 2.05 (3H, s, OAc), 3.28, 3.29 (each 3H, s, 2 × OMe), 3.21, 3.89 (2H, ABq, *J* = 9.2 Hz, H_2-24), 4.55 (1H, dd, *J* = 4.9, 11.8 Hz, H-3), 5.22 (1H, br t, H-12).

Acetylation of 1 Compound **1** (10 mg) was acetylated in the same way as described above to give the acetate (**12**). Compound **12**: EI-MS *m/z*: 273 (terminal peracetylated methylpentosyl cation), 561 (terminal peracetylated methylpentosyl hexosyl cation).

Methyl Ester of 2 A solution of **2** (600 mg) in 0.2 N HCl–MeOH was heated under reflux for 15 min. The reaction mixture was worked up as described for **1**. The purification of the product by silica gel column chromatography ($CHCl_3$: MeOH : H_2O = 9 : 2 : 0.1) furnished the methyl ester (**13**). Compound **13**: Colorless needles, mp 229—231 °C, $[\alpha]_D^{21}$ −8.2° (pyridine). IR ν_{max}^{KBr} cm^{-1}: 3420 (OH), 1750 (ester), 1702 (carbonyl). FAB-MS *m/z*: 955 $(M+H)^+$. ^{13}C-NMR (pyridine-d_5) δ: 38.5, 27.3, 91.3, 43.2, 56.0, 18.9, 32.9, 39.8, 47.8, 36.4, 23.9, 122.9, 141.8, 41.9, 26.6, 27.3, 47.6, 47.6, 46.6, 32.0, 50.9, 215.6, 22.9, 63.5, 15.7, 16.7, 25.4, 31.8, 34.1, 20.9, (C_1-C_{30}), 105.5, 78.1, 76.5, 74.3, 77.6, 170.3 (glc UA C_1-C_6), 101.7, 76.8, 76.5, 71.1, 76.5, 61.6 (gal C_1-C_6), 102.4, 72.3, 72.7, 73.5, 69.4, 18.5 (rha C_1-C_6).

Methanolysis of 13 A solution of **13** (56 mg) in 1 N HCl–MeOH was refluxed (at 70 °C) for 1 h. The reaction mixture was worked up as described for the methanolysis of **1** and the resulting product was purified by silica gel column chromatography ($CHCl_3$) to furnish a sapogenol (**14**). Compound **14**: Colorless needles, mp 252—257 °C, $[\alpha]_D^{23}$ +29.7° (*c* = 0.50, $CHCl_3$). EI-MS *m/z*: 456, 232, 234. ^{1}H-NMR ($CDCl_3$) δ: 0.86, 0.90, 0.94, 0.99, 1.00, 1.22, 1.25 (each 3H, s, 7 × *tert*-Me), 3.45 (1H, dd, *J* = 4.4, 10.9 Hz, H-3), 3.35, 4.21 (2H, ABq, *J* = 11.0 Hz, H_2-24), 5.29 (1H, br t, *J* = 3.7 Hz, H-12).

$NaBH_4$ Reduction of 14 A solution of **14** (8 mg) in MeOH–$CHCl_3$ (1 : 1) was treated with a mixture of $NaBH_4$ (40 mg) in MeOH at room temperature for 1 h. Work-up as described above yielded two reduced products, **3** (1.9 mg) and **15** (0.2 mg) after silica gel column chromatography (solvent, *n*-hexane : acetone = 4 : 1).

Acetylation of 3 Compound **3** (1.9 mg) was acetylated in the usual manner to give the acetate (**16**) which was identical with soyasapogenol B 3β,22β,24-triacetate. Compound **16**: ^{1}H-NMR ($CDCl_3$) δ: 0.74, 0.83, 0.90, 0.91, 0.93, 0.96, 1.07 (each 3H, s, 7 × Me), 1.96, 1.97, 1.99 (each 3H, s, 3 × Ac), 4.08, 4.31 (2H, ABq, *J* = 11.7 Hz, H_2-24), 4.53 (1H, dd, *J* = 5.1 Hz, H-3), 4.57 (1H, t, *J* = 3.7 Hz, H-22), 5.19 (1H, br t, *J* = 3.3 Hz, H-12).

Acetylation of 15 Compound **15** (0.2 mg) was also acetylated to give **17**. Compound **17**: ^{1}H-NMR ($CDCl_3$) δ: 0.87, 0.92, 0.96, 0.98, 0.99, 1.03, 1.16 (each 3H, s, 7 × Me), 2.02, 2.04, 2.06 (each 3H, s, 3 × Ac), 4.15, 4.37 (2H, ABq, *J* = 11.7 Hz, H_2-24), 4.53 (1H, dd, *J* = 5.1 Hz, H-3), 4.75 (1H, dd, *J* = 5.1, 10.8 Hz, H-22), 5.22 (1H, br t, *J* = 3.3 Hz, H-12).

Acknowledgements We are grateful to Professor Dr. Kunihide Mihashi, Dr. Hikaru Okabe and Miss Sizuko Hachiyama of Fukuoka University for the identification of the methylated sugars (GC-MS pattern identification) and for valuable discussions.

References and Notes

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