Three New Oleanene Glycosides from *Sophora flavescens*[1)]

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Three new oleanene glycosides, sophoraflavosides II—IV (2—4) were isolated together with sophoraflavoside I (1) as the corresponding methyl ester forms from Sophorae Radix, the fresh roots of *Sophora flavescens* AITON (Leguminosae). Their structures have been elucidated as oxytrogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranoside (2), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-arabinopyranoside (3) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (4), along with unambiguous characterization as 3β,22β,24-trihydroxyolean-12-en-29-oic acid for their sapogenol, named oxytrogenin (5) on the bases of chemical reactions and spectral analyses.

Keywords *Sophora flavescens*; Leguminosae; sophoraflavoside; oxytrogenin; 3β,22β,24-trihydroxyolean-12-en-29-oic acid; bisdesmoside; oleanene glycoside

Sophorae Radix is the dried root of *Sophora flavescens* AITON (Leguminosae) distributed widely in China, mainly at Shangxi, Hubei, Henan and Hebei, It has been used as a traditional Chinese medicine for removing heat and damp, killing parasites and causing diuresis.[2)] As its chemical constituents, alkaloids and flavonoids have so far been reported,[2)] Kitagawa, *et al.* reported the isolation and structure determination of a soyasapogenol B glycoside, sophoraflavoside I (**1**)[3)] from the commercial Sophora Radix imported from Korea. As a part of our chemical studies on the triterpene glycosides in leguminous plants, we have investigated the triterpene glycosidic constituents of the fresh roots of *Sophora flavescens*. This paper deals with the structure elucidation of three new oleanene glycosides, named sophoraflavoside II—IV (**2—4**).

The methanolic extract of the fresh root of *Sophora flavescens* was partitioned between 1-BuOH and water. The organic layer was evaporated and shaken with 1-BuOH–AcOEt–H_2O (1 : 4 : 5). The aqueous layer was concentrated and subjected to Diaion HP-20P chromatography eluting with water and subsequently with MeOH. The MeOH eluate was evaporated to give a residue which was separated by using various column chromatographies of Sephadex LH-20, Bondapak C_{18}, and silica gel after treatment with Amberlite IR-120B and methylation with diazomethane (CH_2N_2) during the separation procedure to provide glycosides, sophoraflavoside I—IV methyl esters (**1a—4a**), in 60.6, 62.5, 194 and 16.4 mg yields, respectively.

Glycoside **1** was identified as sophoraflavoside I by comparison of proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) data with those of the reported values.[3)]

Sophoraflavoside II (**2**) was obtained as a methyl ester **2a**, a white powder, $[\alpha]_D$ −17.2° (MeOH). On methanolysis, **2a** furnished a sapogenol, **5a**, colorless plates, mp 253—255 °C, $[\alpha]_D$ +43.2° ($CHCl_3$), which showed a molecular ion at *m/z* 502 and prominent fragment ion peaks at *m/z* 278 derived from the D/E ring and 224 from the A/B ring *via* retro Diels–Alder fission[4)] in the electron impact mass spectrum (EI-MS), indicating that **5a** should possess two hydroxyl groups at A, B-ring, and one hydroxyl and one methoxycarbonyl groups at C, D-ring on the olean-12-en skeleton. The ^{13}C-NMR spectrum of **5a** revealed the presence of a total of thirty carbon signals, in which the signals due to three oxygenated carbons [δ 64.6 (t), 75.0 (d) and 80.0 (d)], one trisubstituted double bond [δ 123.2 (d) and 144.0 (s)] and one methoxycarbonyl group [δ 51.7 (s) and 179.1 (s)], also supporting **5a** to be an olean-12-en derivative having three hydroxyl groups. The ^{1}H-NMR spectrum of **5a** showed signals due to one hydroxymethyl group at δ 3.73, 4.54 (1H each, d, J = 11.0 Hz), one oxygenated methine proton at δ 3.90 (1H, t, J = 3.0 Hz) and one olefinic proton at δ 5.39 (1H, t, J = 3.5 Hz), assignable to H_2-24, H-22α and H-12, respectively. The triacetate (**6a**) of **5a**, colorless plates, *m/z* 628, displayed signals due to two methine protons adjacent to the acetoxyl group at δ 4.75 (1H, t, J = 3.5 Hz), 4.58 (1H, dd, J = 5.1, 10.2 Hz), and one acetoxymethyl group at δ 4.14, 4.37 (1H each, d, J = 11.5 Hz), which could be assigned to H-22α, H-3α and H_2-24, respectively.

In order to characterize a genuine sapogenol, **5a** was converted to sapogenol **5**, *m/z* 488, colorless needles. The ^{1}H-NMR spectrum of **5** was almost similar with that of **5a** except for the disappearance of a signal due to the methoxyl group at δ 3.66 ppm.

Sapogenol **5a** was reduced by lithium triethylborohydride in tetrahydrofuran (THF) to afford a reduced product **7**, colorless plates. The EI-MS of **7** gave a molecular ion peak at *m/z* 474. Sapogenol **7** was transformed to the corresponding acetate to distinctly confirm its structure. The tetraacetate (**8**) of **7**, colorless needles, displayed signals due to two acetoxymethylenes (δ 3.68, 3.74, d, J = 10.8 Hz and 4.15, 4.37, d, J = 11.7 Hz), two methines (4.59, dd, J = 5.7, 10.4 Hz and 4.71, br s) adjacent to the acetoxyl group, which was the same with those of abrisapogenol B tetraacetate isolated from *Abrus cantoniensis*.[5)] From the above evidence, the structure of **5** could be characterized as 3β,22β,24-trihydroxyolean-12-en-29-oic acid, which was assumed to be identical with the sapogenol of saponin-2 obtained by Jia *et al.* from *Oxytropis glabra*.[6)] However, they didn't obtain the sapogenol and describe the data. Therefore, we named it oxytrogenin. This sapogenol was simultaneously obtained from the hydrolysate of *Robinia pseudo-acacia*.[7)]

The high resolution fast atom bombardment (HR FAB)-

MS of **2a** showed a quasi-molecular ion at *m/z* 1023.5141 $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$). The ^{1}H-NMR spectrum of **2a** disclosed three anomeric proton signals at δ4.94 (d, J=7.6 Hz), 5.79 (1H, d, J=7.3 Hz) and 6.37 (1H, br s) together with six methyl signals. The negative FAB-MS gave peaks due to $[M-H]^-$ at *m/z* 999, $[M-H-$rhamnose (rha)$]^-$ at *m/z* 853 and $[M-H-$rha$-$hexose (hex)$-$glucuronic acid methyl ester (UA)$]^-$ at *m/z* 501, suggesting that **2a** possessed a methylpentosyl moiety as a terminal carbohydrate residue. The ^{13}C-NMR spectrum (Table I) of **2a** showed thirty signals due to the sapogenol part whose chemical shifts were almost identical with those of **5a** except for that of C-2, C-3 and C-4, where respective shifts, -1.7, $+11.2$ and -0.7 ppm were observed due to glycosylation shifts,[8] indicating that **2a** was a 3-*O*-monodesmoside. Moreover, **2a** exhibited nineteen signals due to the sugar moiety (Table II), including three anomeric carbons at δ101.7, 102.4 and 105.4, and one ester carbonyl carbon at δ170.4. The signals due to C-2 of glucuronic acid at δ78.3 and C-2 of galactose at δ76.9 indicated that the saccharide possessed glycosidic linkages at the C-2 hydroxyl of galactopyranosyl and glucuronopyranosyl moieties. A comparative study of the ^{13}C-NMR spectral data (Table II) due to the sugar moiety of **2a** with those of soyasaponin I methyl ester obtained from *Sophora flavescens*[3] also supported the above result. Consequently, the structure of **2** was characterized as oxytrogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranoside. This glycoside was identical with compound III obtained from *Robinia pseudo-acacia* at the same time in our laboratory.[7]

Sophoraflavoside III methyl ester (**3a**), a white powder, $[\alpha]_D$ $-23.0°$ (MeOH), on methanolysis, provided oxytrogenin (**5a**). The HR FAB-MS of **3a** showed a quasi-molecular ion peak at *m/z* 1155.5563 $[M+Na]^+$, indicating a molecular formula $C_{55}H_{88}O_{24}$. The negative FAB-MS showed a cluster ion due to $[M+NBA]^-$ at *m/z* 1286. In addition, the EI-MS of its peracetate suggested the presence of four sugars including a terminal rhamnose and a terminal pentose in **3a**. The ^{13}C-NMR spectrum (Tables I and II) of **3a**, also displayed four anomeric carbons (δ101.5, 102.2, 102.2 and 105.3). The carbon signals due to the aglycone moiety were almost identical with those of **5a** except for displacement of signals at C-2 (-2.7 ppm), C-3 ($+11.1$ ppm), C-4 (-0.5 ppm), C-21 (-5.0 ppm) and C-22 ($+6.2$ ppm). Therefore, **3a** was regarded as a 3,22-di-*O*-bisdesmoside of oxytrogenin. The sugars obtained from **3a** were converted to the trimethylsilyl ethers and then were subjected to gas liquid chromatography (GLC) analysis to give peaks due to arabinose, rhamnose and galactose. Determination of D or L of each sugar configuration was achieved by the GLC analysis.[9] The **3a** was subjected to hydrolysis with 1 N HCl H_2O–dioxane (1 : 1) to give the sugar components, which were subsequently converted to the corresponding trimethylsilyl (TMS) ethers of methyl 2-(polyhydroxyalkyl)-

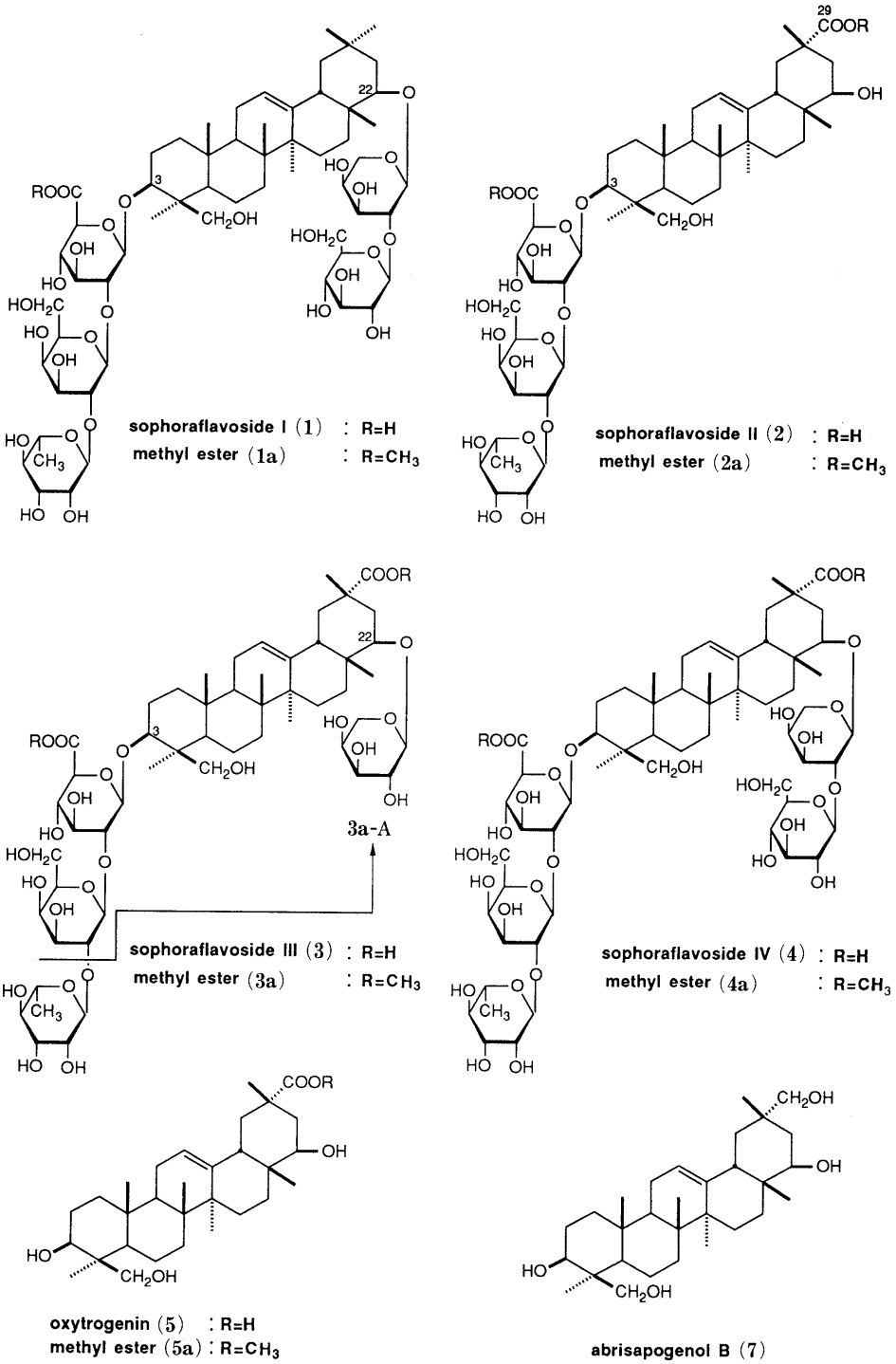
Table I. ^{13}C-NMR Chemical Shifts for Sapogenol Moieties of **1a**—**4a** and Prosapogenin **3a**-A, and of Sapogenol **5a** (Pyridine-d_5)

	1a	**2a**	**3a**	**3a**-A	**4a**	**5a**
C- 1	38.6 (t)	38.6	38.4	38.6	38.6	38.9
C- 2	26.1 (t)	26.7	25.7	25.9	25.9	28.4
C- 3	91.3 (d)	91.2	91.1	90.8	91.3	80.0
C- 4	43.9 (s)	43.9	43.7	43.8	43.9	43.2
C- 5	56.1 (d)	56.0	55.8	56.1	56.1	56.3
C- 6	18.5 (t)	18.5	18.3	18.6	18.4	19.1
C- 7	33.2 (t)	33.2	33.0	33.2	33.3	33.5
C- 8	39.8 (s)	39.9	39.5	39.7	39.7	40.0
C- 9	47.8 (d)	47.8	47.5	47.7	47.7	48.0
C-10	36.4 (s)	36.5	36.2	36.4	36.5	37.0
C-11	22.8 (t)	24.1	23.8	24.0	24.0	24.1
C-12	122.7 (d)	123.2	123.4	123.8	123.6	123.2
C-13	144.2 (s)	144.0	143.2	143.3	143.3	144.0
C-14	42.2 (s)	42.3	42.1	42.3	42.2	42.3
C-15	26.7 (t)	26.3	26.5	26.6	26.7	26.3
C-16	28.7 (t)	28.6	28.6	28.8	28.8	28.6
C-17	37.5 (s)	37.8	37.1	37.3	37.4	37.8
C-18	45.5 (d)	44.2	44.5	44.7	44.6	44.2
C-19	46.4 (t)	41.0	40.8	41.0	41.0	41.0
C-20	30.5 (s)	42.7	42.1	42.3	42.2	42.6
C-21	35.7 (t)	37.3	32.3	32.5	31.5	37.3
C-22	80.4 (d)	75.0	81.2	81.4	80.7	75.0
C-23	23.0 (q)	23.0	22.8	22.7	23.0	23.5
C-24	63.6 (t)	63.6	63.3	63.5	63.4	64.6
C-25	15.8 (q)	15.8	15.6	15.7	15.8	16.2
C-26	16.9 (q)	16.9	16.7	16.9	16.9	17.0
C-27	25.4 (q)	25.5	24.9	24.9	25.1	25.5
C-28	28.4 (q)	21.0	20.5	20.7	20.6	21.0
C-29	32.4 (q)	179.1 (s)	178.6	178.8	178.8	179.1
C-30	21.1 (q)	24.4	24.2	24.4	24.3	24.4
OCH_3	52.1 (q)	51.8	51.7	51.8	51.8	51.7

Table II. ^{13}C-NMR Chemical Shifts for Sugar Moieties of **1a**—**4a** and Prosapogenin **3a**-A (Pyridine-d_5)

		1a	**2a**	**3a**	**3a**-A	**4a**
Glc						
UA	1	105.5	105.4	105.3	105.4	105.5
	2	78.2[a]	78.3	77.9[a]	80.7	78.2[a]
	3	76.5[b]	76.4[a]	76.4[b]	77.3[a]	76.5[b]
	4	74.3	74.3	74.4	73.6	74.4
	5	77.6	77.9	77.5[a]	77.9[a]	77.7
	6	170.4	170.4	170.2	170.4	170.4
COOMe		52.1	52.1	52.0	52.1	52.1
Gal						
	1	101.7	101.7	101.5	105.0	101.8
	2	76.9[b]	76.9[a]	76.7[b]	72.7[b]	76.9[b]
	3	76.4[b]	76.5[a]	76.3[b]	75.5	76.0[b]
	4	71.2[c]	71.2	70.9	71.7	71.2[c]
	5	76.4[b]	76.6[a]	76.3[b]	77.0[a]	76.6[b]
	6	61.6	61.6	61.4	62.7	61.6
Rham						
	1	102.2	102.4	102.2		102.4
	2	72.4[d]	72.4[b]	72.1[c]		72.4[d]
	3	72.8[d]	72.8[b]	72.2[c]		72.7[d]
	4	73.6	73.6	73.4		73.6
	5	69.3	69.4	69.1		69.4
	6	18.9	19.0	18.7		19.0
Ara (p)						
	1	97.7		102.2	102.4	98.6
	2	80.0		72.5[c]	72.4[b]	80.0
	3	71.5[c]		74.1	74.6	71.6[c]
	4	66.6		69.0	69.2	67.4
	5	62.5		66.3	66.6	62.7
Glc						
	1	105.7				105.9
	2	75.7				75.9
	3	78.4[a]				78.4[a]
	4	71.5				72.2
	5	78.1[a]				78.2[a]
	6	62.6				62.7

a—*d*) In each vertical column may be interchanged.



thiazolidine-4(*R*)-carboxylates followed by GLC analysis. The sugar moiety was revealed to be composed of D-galactose, L-arabinose and L-rhamnose. On partial acid hydrolysis, **3a** provided two main prosapogenins, **3a**-A and **3a**-B. The latter was identified as **2a** and the former exhibited three anomeric carbons at δ 102.4, 105.0 and 105.4. The signals due to terminal rhamnose disappeared in the ^{13}C-NMR spectrum (Table II) of **3a**-A. From the carbon chemical shifts of the terminal arabinosyl moiety and the coupling constants of anomeric protons at δ 4.85 (d, J=6.6 Hz) for **3a** and 4.88 (d, J=6.6 Hz) for **3a**-A in the NMR spectra, the arabinopyranosyl moieties in **3a** and **3a**-A were concluded to be $^4C_1$ conformation with α-L-configuration which was different from those of **1a** and **4a**. Therefore, the structure of **3** could be represented as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-arabinopyranoside.

Sophoraflavoside IV (**4**) was obtained as a methyl ester **4a**, a white powder, $[\alpha]_D$ −18.4° (MeOH). The HR FAB-MS of **4a** showed a quasi-molecular ion peak at m/z 1317.6080 $[M+Na]^+$, indicating a molecular formula $C_{61}H_{98}O_{29}$. Methanolysis of **4a** afforded the sapogenol **5a**. The sugar moiety was transformed to the trimethylsilyl ethers and was subjected to the GLC analysis. The peaks due to arabinose, rhamnose, glucose and galactose were

observed. The negative FAB-MS showed peaks due to $[M-H]^-$ at *m/z* 1293, $[M-H-rha]^-$ at *m/z* 1147 and $[M-H-rha-hex]^-$ at *m/z* 985. The EI-MS of the peracetate revealed fragment ion peaks at *m/z* 561 [(rha-hex-)Ac_6]$^+$, 547 [(hex-pen-)Ac_6]$^+$, 331 [(terminal hex-)-Ac_4]$^+$ and 273 [(terminal rha-)Ac_3]$^+$, indicating the occurrence of five sugars in which rhamnose and a hexose located at the terminal site. The ^{13}C-NMR spectrum of **4a** exhibited signals assignable to five anomeric carbons [δ 98.6, 101.8, 102.4, 105.5 and 105.9], indicating that **4a** was a pentaglycoside of oxytrogenin. Absolute configuration of the respective sugar in **4a** was determined by GLC analysis of its TMS derivative, indicating the sugar moiety was D-galactose, D-glucose, L-rhamnose and L-arabinose. On comparison of the ^{13}C-NMR data for **4a** (Tables I and II) with those of sophoraflavoside I (**1a**), signals due to the sugar moiety were almost superimposable on those of **1a**, suggesting that both glycosides possessed the same sugar moiety. Based on the above evidence, the structure of **4** was determined as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside.

Experimental

Optical rotations were measured on 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 as an internal standard. The FAB-(NBA as a matrix) and EI-MS were recorded with a JEOL DX-300 spectrometer and HR FAB-MS was measured with a JEOL HX-110. GLC analysis was performed on a HP-5890A gas chromatograph with an H_2 flame ionization detector, the column was OV-1 (0.32 mm × 30 m), condition-1: column temperature (temp.): 230 °C; detection temp.: 270 °C; injection temp.: 270 °C; carrier gas: He (2.2 kg/cm^2), condition-2: column temperature (temp.): 160 °C; detection temp.: 200°C; injection temp.: 200°C; carrier gas: He (2.2 kg/cm^2). Column chromatography was carried out with Diaion HP 20P (Mitsubishi Chem. Ind. Co. Ltd.), Bondapak C_{18} (37—75 μ, Waters Associates, Inc.) and Kieselgel 60 (70—230 and 230—400 mesh, Merck). TLC was conducted on a precoated Kieselgel 60 F_{254} plate (0.2 mm, Merck), and detection was achieved by spraying it with 10% H_2SO_4 followed by heating.

Extraction and Separation The fresh roots (3.9 kg) of *Sophora flavescens* (Leguminosae) collected in Kumamoto prefecture were extracted with MeOH and the extract (328 g) was partitioned between 1-BuOH and water. The 1-BuOH layer was concentrated and partitioned with 1-BuOH–AcOEt–water (1:4:5). The water layer was evaporated *in vacuo* to remove the organic solvent and the resulting residue was chromatographed on Diaion CHP-20P with water and MeOH. The MeOH eluate was fractionated by Bondapak C_{18} eluted with 20→50% MeOH, gradiently. Each fraction (fr.) was passed through the Amberlite IR-120B and methylated with CH_2N_2, respectively, to give four fractions. The first fr. (2.05 g) was further separated by using Sephadex LH-20 with 50% MeOH and Bondapak C_{18} with 50→55% MeOH to give sophoraflavoside IV methyl ester (**4a**) (16.4 mg). The second fr. (1.56 g) was also chromatographed by silica gel with $CHCl_3$–MeOH–water (C:M:W) = 8:2:0.2 and Bondapak C_{18} with 50→60% MeOH to afford sophoraflavoside III methyl ester (**3a**) (194.0 mg). The third and fourth fr. were respectively separated by various column chromatographies of Bondapak C_{18} with 50→60% MeOH, Si gel with C:M:W = 9:1:0.1→8:2:0.2, sophoraflavoside II methyl ester (**2a**) (62.5 mg) from fr. III and sophoraflavoside I methyl ester (**1a**) (60.6 mg) from fr. IV were obtained.

Sophoraflavoside I Methyl Ester (1a) A white powder, $[\alpha]_D^{25}$ −31.0° (*c* = 0.42, MeOH). HR FAB-MS *m/z*: 1273.6187 $[M+Na]^+$ ($C_{60}H_{98}O_{27}Na$, Calcd for 1273.6193). Positive FAB-MS *m/z*: 1273 $[M+Na]^+$, 1127 $[M+Na-rha]^+$, 965 $[M+Na-rha-hex]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.91, 0.91, 1.16, 1.17, 1.22, 1.44 (each 3H, s, 7 × CH_3), 1.77 (3H, d, *J* = 6.2 Hz), 3.75 (3H, s), 4.95 (1H, d, *J* = 7.0 Hz, UA H-1), 5.09 (1H, d, *J* = 7.7 Hz, glc H-1), 5.18 (1H, br s, ara H-1), 5.28 (1H, br s, H-12), 5.77 (1H, d, *J* = 7.3 Hz, gal H-1), 6.29 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Sophoraflavoside II Methyl Ester (2a) A white powder, $[\alpha]_D^{22}$ −17.2° (*c* = 0.61, MeOH). HR FAB-MS *m/z*: 1023.5141 $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$, Calcd for 1023.5141). Negative FAB-MS *m/z*: 1154 $[M+NBA]^-$, 1000 $[M]^-$, 999 $[M-H]^-$, 853 $[M-H-rha]^-$, 501 $[M-H-rha-hex-UA]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.95, 1.23, 1.25, 1.42, 1.67 (each 3H, s, 6 × CH_3), 1.76 (3H, d, *J* = 5.9 Hz), 3.66 (3H, s), 3.76 (3H, s), 4.94 (1H, d, *J* = 7.6 Hz, UA H-1), 5.33 (1H, br s, H-12), 5.79 (1H, d, *J* = 7.3 Hz, gal H-1), 6.37 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 2a A solution of **2a** (60 mg) in 1 N HCl–MeOH was refluxed for 2 h and the reaction mixture was neutralized with 3% KOH–MeOH. The products were removed with organic solvent and partitioned with AcOEt. The AcOEt part (46.7 mg) was chromatographed over silica gel with *n*-hexane–acetone (2:1), and was recrystallized with MeOH and H_2O to provide a sapogenol (**5a**, 25 mg), colorless plates, mp 253—255 °C, $[\alpha]_D^{23}$ +43.2° (*c* = 0.25, $CHCl_3$). EI-MS *m/z* (relative intensity): 502 $[M]^+$(11), 471 $[M-OCH_3]^+$(6), 443 $[M-COOCH_3]^+$(5), 278 [D/E ring]$^+$(100), 263 [D/E ring−CH_3]$^+$(6), 260 [D/E ring−H_2O]$^+$(12), [D/E ring−OCH_3]$^+$(22), 224 [A/B ring]$^+$(13), 219 [D/E ring−$COOCH_3$]$^+$(39). ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.02, 1.22, 1.25, 1.57, 1.69 (each 3H, s, 6 × CH_3), 2.44 (1H, dd, *J* = 3.0, 13.6 Hz, H-21), 2.47 (1H, br d, *J* = 13.9 Hz, H-18), 2.59 (1H, t, *J* = 13.9 Hz, H-19), 3.66 (3H, s), 3.90 (1H, t, *J* = 3.0 Hz, H-22), 3.73, 4.54 (2H, ABq, *J* = 11.0 Hz, H_2-24), 5.39 (1H, t, *J* = 3.5 Hz, H-12). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acetylation of 5a A solution of **5a** (5 mg) in Ac_2O–pyridine (1:1) was kept at room temperature overnight. The reaction mixture was evaporated under N_2 gas and then chromatographed on silica gel using *n*-hexane–AcOEt (3:1), giving the peracetate **6a**, colorless needles, mp 213—215 °C (MeOH), $[\alpha]_D^{22}$ +45.1° (*c* = 0.10, $CHCl_3$). EI-MS *m/z* (relative intensity): 628 $[M]^+$(11), 568 $[M-AcOH]^+$(12), 509 $[M-COOCH_3-AcOH]^+$(4), 449 $[M-COOCH_3-2\times AcOH]^+$(3), 320 [D/E ring]$^+$(100), 308 [A/B ring]$^+$(13), 260 [D/E ring−AcOH]$^+$(39), 201 [D/E ring−AcOH−$COOCH_3$]$^+$(18). ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.97, 0.98, 1.03, 1.15, 1.33 (each 3H, s, 6 × CH_3), 2.04, 2.05, 2.07 (3 × OAc), 3.67 (3H, s), 4.14, 4.37 (2H, ABq, *J* = 11.5 Hz, H_2-24), 4.58 (1H, dd, *J* = 5.1, 10.2 Hz, H-3), 4.75 (1H, t, *J* = 3.5 Hz, H-22), 5.30 (1H, t, *J* = 3.5 Hz, H-12).

Alkaline Hydrolysis of 5a A solution of **5a** (10 mg) in 3% KOH–MeOH was refluxed for 30 min. The mixture was neutralized with 1 N HCl–MeOH, the deposit salt was removed by Diaion chromatography with H_2O and chromatographed on silica gel column chromatography (C:M = 19:1) to afford the sapogenol **5** (5 mg), mp > 300 °C. EI-MS *m/z* (relative intensity): 488 $[M]^+$(6), 470 $[M-H_2O]^+$(3), 264 [D/E ring from RDA]$^+$(100), 246 [D/E ring from RDA−H_2O]$^+$(29), 224 [A/B ring from RDA]$^+$(26), 219 [D/E ring from RDA−COOH]$^+$(22). ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.04, 1.26, 1.29, 1.56, 1.86 (each 3H, s, 6 × CH_3), 2.55 (1H, dd, *J* = 3.1, 13.6 Hz, H-21), 2.67 (1H, br d, *J* = 13.8 Hz, H-18), 2.82 (1H, t, *J* = 13.8 Hz, H-19), 3.61 (1H, dd, *J* = 4.5, 11.7 Hz, H-3), 3.73, 4.54 (2H, ABq, *J* = 11.0 Hz, H_2-24), 4.03 (1H, br s, H-22), 5.43 (1H, br s, H-12).

Reduction of 5a Compound **5a** (20 mg) was dissolved in lithium triethylborohydride solution ($LiBEt_3H$/THF, 5 ml) and was kept at room temperature for 30 min. After decomposition of excess $LiBEt_3H$ with a small amount of AcOH, the solvent was removed and the reaction product was chromatographed by silica gel using *n*-hexane–acetone (2:1) and then crystallized with dil. MeOH to afford colorless plates **7** (7.2 mg), mp 278.5—280.5 °C. EI-MS *m/z* (relative intensity): 474 $[M]^+$(4), 456 $[M-H_2O]^+$(7), 443 $[M-CH_2OH]^+$(14), 250 [D/E ring]$^+$(75), 232 [D/E ring−H_2O]$^+$(37), 219 [D/E ring−CH_2OH]$^+$(100), 224 [A/B ring]$^+$(16). ^{1}H-NMR ($CDCl_3$) δ: 0.92, 0.97, 1.02, 1.14, 1.29, 1.37 (each 3H, s, 6 × CH_3), 1.98 (1H, t, *J* = 13.6, H-19$_{ax}$), 2.28 (1H, br d, *J* = 13.2 Hz, H-21$_{ax}$), 3.46, 4.34 (2H, ABq, *J* = 11.0 Hz, H_2-24), 3.67 (1H, dd, *J* = 3.1, 7.3 Hz, H-3), 5.30 (1H, br s, H-12).

Acetylation of 7 A solution of **7** (5 mg) in Ac_2O–pyridine (1:1) was acetylated by the usual method. The reaction mixture was evaporated and chromatographed on silica gel using *n*-hexane–acetone (5:1) to yield **8** (4 mg), colorless needles, mp 157—158 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.83, 0.97, 0.98, 1.03, 1.06, 1.13 (each 3H, s, 6 × CH_3), 2.04, 2.05, 2.07, 2.08 (4 × OAc), 3.68, 3.74 (2H, ABq, *J* = 10.8 Hz, H_2-29), 4.15, 4.37 (2H, ABq, *J* = 11.7 Hz, H_2-24), 4.59 (1H, dd, *J* = 5.7, 10.4 Hz, H-3), 4.71 (1H, br s, H-22), 5.29 (1H, br s, H-12).

Sophoraflavoside III Methyl Ester (3a) A white powder, $[\alpha]_D^{21}$ −23.0°

($c=0.58$, MeOH). HR FAB-MS m/z: 1155.5565 $[M+Na]^+$ ($C_{55}H_{88}O_{24}Na$, Calcd for 1155.5563). Negative FAB-MS m/z: 1286 $[M+NBA]^-$, 1132 $[M]^-$, 1131 $[M-H]^-$, 999 $[M-H-pen]^-$, 985 $[M-H-rha]^-$, 633 $[M-H-rha-hex-UA]^-$, 501 $[M-H-rha-hex-UA-pen]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.88, 1.13, 1.18, 1.41, 1.54 (each 3H, s, $6\times CH_3$), 1.76 (3H, d, $J=6.6$ Hz), 3.67, 3.75 (each 3H, s), 4.85 (1H, d, $J=6.6$ Hz, ara H-1), 4.95 (1H, d, $J=7.0$ Hz, UA H-1), 5.23 (1H, br s, H-12), 5.75 (1H, d, $J=7.3$ Hz, gal H-1), 6.26 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 3a A solution of **3a** (5 mg) in 1 N HCl–MeOH was refluxed for 2 h. The reaction mixture was neutralized with 3% KOH–MeOH and partitioned between $CHCl_3$ and water. The sapogenol of $CHCl_3$ layer was checked on TLC (*n*-hexane : acetone = 2 : 1). The water layer was concentrated and the residue was trimethylsilylated with trimethylsilyl (TMS)-imidazole, and checked by GLC on condition-2.

D, L Determination of Sugar A solution of **3a** (5 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 supernatant was treated with Amberlite IRA-400 to give a sugar fraction. The pyridine solutions of sugar (1 mg/100 μl) and L-cysteine methyl ester hydrochloride (2 mg/100 μl) were mixed and warmed at 60 °C for 1 h. After removal of the solvent, the product was dried *in vacuo*. The trimethylsilylation reagent, TMS-imidazole (100 μl) was added and heated at 60 °C for an another 30 min. The reaction mixture was partitioned between *n*-hexane and water. The *n*-hexane solution was subjected to GLC on condition-1. Three peaks were observed at t_R (min): 9.71 (L-ara), 11.74 (L-rha) and 18.39 (D-gal). The standard monosaccharides were subjected to the same reaction and GLC analysis was performed under the same condition, t_R (min): 10.49 (D-ara), 12.11 (D-rha) and 19.57 (L-gal).

Acetylation of 3a A solution of **3a** (4 mg) was acetylated in the usual manner to afford the peracetate, EI-MS m/z: 561 $[(rha\text{-}hex\text{-})Ac_6]^+$ and 273 $[(rha\text{-})Ac_3]^+$, 259 $[(pen\text{-})Ac_3]^+$.

Partial Acid Hydrolysis of 3a A solution of **3a** (100 mg) in 0.5 N HCl–MeOH was refluxed for 20 min. The reaction mixture was neutralized with 3% KOH–MeOH and evaporated to dryness to give a residue, which was chromatographed on silica gel with C : M : W = 9 : 1 : 0.1 to afford two prosapogenins, **3a**-A and **3a**-B. **3a**-B was identified as **2a**. **3a**-A, a white powder, ^{1}H-NMR (pyridine-d_5) δ: 0.73, 0.88, 1.14, 1.15, 1.35, 1.55 (each 3H, s, $6\times CH_3$), 3.67, 3.76 (each 3H, s), 4.88 (1H, d, $J=6.6$ Hz, ara H-1), 4.94 (1H, d, $J=7.0$ Hz, UA H-1), 5.24 (1H, br s, H-12), 5.56 (1H, d, $J=7.7$ Hz, gal H-1). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Sophoraflavoside IV Methyl Ester (4a) A white powder, $[\alpha]_D^{22}$ −18.4° ($c=0.50$, MeOH). HR FAB-MS m/z: 1317.6080 $[M+Na]^+$ ($C_{61}H_{98}O_{29}Na$, Calcd for 1317.6091). Negative FAB-MS m/z: 1293 $[M-H]^-$, 1147 $[M-H-rha]^-$, 985 $[M-H-rha-hex]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.71, 0.89, 1.18 (×2), 1.43, 1.54 (each 3H, s, $6\times CH_3$), 1.78 (3H, d, $J=6.2$ Hz), 3.64, 3.75 (each 3H, s), 4.97 (1H, d, $J=7.0$ Hz, UA H-1), 5.12 (1H, br s, ara H-1), 5.24 (1H, br s, H-12), 5.81 (1H, d, $J=7.3$ Hz, gal H-1), 6.31 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 4a A solution of **4a** (2 mg) in 1 N HCl–MeOH was refluxed for 2 h, and the reaction mixture was neutralized with 3% KOH–MeOH and partitioned between $CHCl_3$ and water. The organic layer containing the sapogenol was checked on TLC (*n*-hexane : acetone = 2 : 1). The aqueous layer was concentrated and the residue was trimethylsilylated with TMS-imidazole, and checked by GLC on condition-2.

D, L Determination of Sugar A solution of **4a** (3 mg) in 1 N HCl water–dioxane was refluxed and worked up in the usual manner, then the TMS derivative was subjected to GLC analysis on condition-1. t_R (min): 9.60 (L-ara), 11.61 (L-rha), 17.09 (D-glc), 18.17 (D-gal).

Acetylation of 4a A solution of **4a** (3 mg) was acetylated in the usual manner, to give the peracetate, EI-MS m/z: 561 $[(rha\text{-}hex\text{-})Ac_6]^+$, 547 $[(hex\text{-}pen\text{-})Ac_6]^+$, 331 $[(hex\text{-})Ac_4]^+$, 273 $[(rha\text{-})Ac_3]^+$.

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