Five New Triterpene Glycosides from Russell Lupine[1)]

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In a continuing study on the ingredients of Lupinus genus, we examined the oligoglycoside constituents of the Russell lupine (*L. polyphyllus* × *L. arboreus* hybrid). Five new oleanene glycosides, called Lupinosides PA_{1-5} (1—5), together with three known ones were isolated. Their structures of 1—5 were determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 21-*O*-β-D-xylopyranoside (1), 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 21-*O*-β-D-xylopyranoside (2), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A 21-*O*-β-D-xylopyranoside (3), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-α-L-rhamnopyranoside (4), and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (5), respectively.

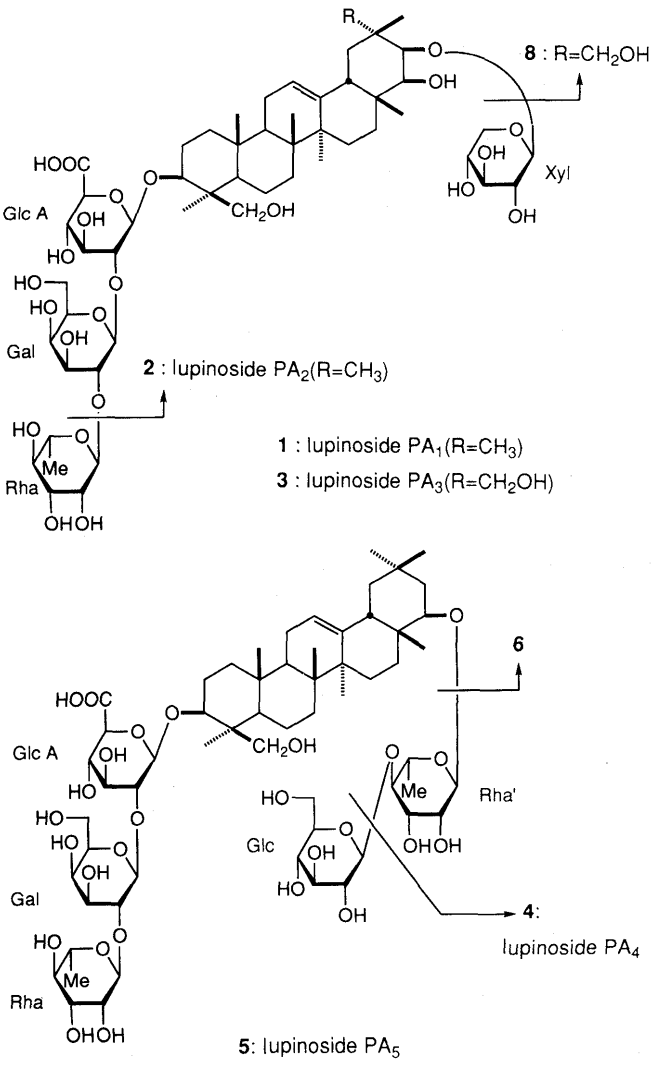
Keywords oleanene glycoside; lupinoside; Leguminosae; Russell lupine; *Lupinus polyphyllus* × *L. arboreus*

Among a couple of hundred species belonging to the genus *Lupinus* (Leguminosae), Russell lupine is one of the most cultivated in the world. The Russell lupine is the hybrid of *L. polyphyllus* and *L. arboreus*, and is a historically improved species in horticulture.[2)]

This genus contains many flavonoids as well as lupine alkaloids. We previously reported the isolation of nine isoflavonoids including three new ones from the roots of *L. luteus* and *L. polyphyllus* × *L. arboreus*.[3)] However, there has been no report on the isolation of triterpene glycoside. In a continuing study on the ingredients of Lupinus genus, we have found and isolated eight oleanene glycosides from the roots of *L. polyphyllus* × *L. arboreus*. This paper deals with the structural elucidation of these saponins.

The crude saponin fraction (fr. 4)[3)] (Fig. 1) was separated by normal and reversed phase column chromatographies to yield compounds **1**—**8** (Fig. 1). Compounds **6**—**8** were identified as soysaponin I (**6**),[4)] dehydrosoyasaponin I (**7**),[5)] kudzusaponin A_3 (**8**)[6)] by comparison with the proton (^{1}H)- and carbon-13 (^{13}C)-NMR spectral data (Tables I, II).

Lupinoside PA_1 (**1**), a white powder, $[\alpha]_D$ −15.3° (MeOH), showed a peak at *m/z* 1089 due to $[M-H]^-$ in the negative FAB-MS. The exact mass measurement under high resolution (HR) conditions showed the composition $C_{53}H_{86}NaO_{23}$ at *m/z* 1113.5471 $[M+Na]^+$. The sapogenol obtained by acid hydrolysis of **1** was identified with soyasapogenol A (**1a**)[7)] on thin layer chromatography (TLC). The monosaccharide mixture revealed the presence of glucuronic acid, galactose, xylose, and rhamnose. Their absolute configurations were determined to be D-form except for rhamnose (L-form), according to the procedure developed by Hara *et al.*[8)] Meanwhile, enzymatic hydrolysis of **1** with glycyrrhizinic acid hydrolase (GH)[9)] yielded a prosapogenin (**1b**), a white powder, $[\alpha]_D$ +70.2° (pyridine). Compound **1b** showed a peak at *m/z* 605 due to $[M-H]^-$ in the negative FAB-MS, indicating it to be a soyasapogenol A glycoside with a pentosyl unit. In the ^{1}H- and ^{13}C-NMR (Table II) spectrum of **1b**, signals of the sugar part were assignable to the terminal β-D-xylopyranosyl moiety, and the C-3 signal of the aglycone was in accord with that of **1a** (Table I). Moreover, a downfield shift (+11.3 ppm) at C-21 and an upfield shift (−1.2 ppm) at C-22 due to glycosylation were observed.[10)]



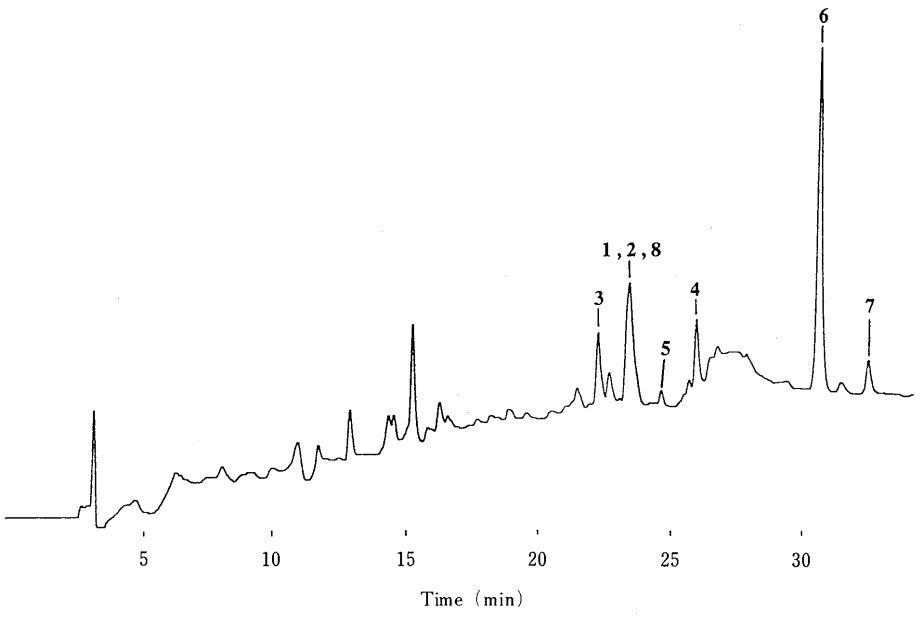


Fig. 1. HPLC Patterns of Crude Saponin Fraction (Fr. 4)

HPLC conditions: Column, Nova-Pak C_{18} (4 μm, 8 × 100 mm) with Radial-Pak RCM 8 × 10 module, elution in a program, solvent A, H_2O: TFA = 100 : 0.05 (v/v); solvent B, CH_3CN: H_2O: TFA = 60 : 40 : 0.05 (v/v). Program, 0→83% solvent B (30 min), 83% solvent B (5 min), 83→100% solvent B (5 min), 100% solvent B (5 min). Column tempreature, 40 °C, flow rate, 1 ml/min, detection, UV at 205 nm.

TABLE I. ^{13}C-NMR Data for Compounds **1**—**8**, **1a**, **b** and **4a** (Aglycone Moieties)

	1a[7b]	**1b**	**1**	**2**	**3**	**4**	**4b**	**5**	**6**	**7**	**8**
C-1	38.9	38.8	38.5	38.6	38.4	38.4	38.8	38.3	38.5	38.3	38.4
C-2	28.4	28.3	26.6	26.4	26.4	25.9	28.2	25.9	26.3	27.1	26.5
C-3	80.1	80.1	91.2	90.8	91.0	91.0	80.0	90.9	91.1	91.0	90.9
C-4	43.2	43.1	43.8	43.7	43.7	43.7	43.1	43.6	43.7	43.7	43.7
C-5	56.3	56.2	56.2	56.1	55.8	55.8	56.2	55.7	55.9	55.8	55.8
C-6	19.1	19.0	18.5	18.7	18.3	18.3	19.0	18.2	18.4	18.2	18.3
C-7	33.2	33.1	32.9	32.9	32.8	32.9	33.3	32.8	33.1	32.8	32.7
C-8	40.3	40.2	40.1	40.2	40.0	39.8	40.0	39.7	39.8	39.6	40.0
C-9	48.1	48.0	47.7	47.7	47.6	47.5	48.0	47.4	47.7	47.6	47.6
C-10	37.0	36.9	36.6	36.6	36.3	36.2	36.9	36.1	36.3	36.2	36.2
C-11	24.2	24.1	24.1	24.0	24.0	23.8	24.0	23.7	23.9	23.8	23.9
C-12	122.5	122.5	122.5	122.6	122.4	122.3	122.5	122.3	122.2	122.6	122.3
C-13	144.5	144.4	144.4	144.5	144.4	144.2	144.2	144.1	144.7	141.6	144.5
C-14	42.1	42.0	42.0	42.0	41.9	41.8	42.0	41.7	42.2	41.8	41.8
C-15	26.6	26.4	26.6	26.4	26.5	26.4	26.1	26.3	26.5	26.4	26.5
C-16	27.5	27.6	27.5	27.5	27.4	28.1	28.3	27.9	28.5	27.1	27.2
C-17	39.2	39.0	39.0	39.1	39.0	37.3	37.4	37.2	37.8	47.6	38.9
C-18	44.0	43.7	43.7	43.8	43.0	44.9	45.1	44.7	45.1	47.4	43.0
C-19	47.3	47.4	47.4	47.4	41.4	46.2	46.3	46.0	46.6	46.4	40.9
C-20	36.6	36.6	36.4	36.4	41.1	30.2	30.4	30.1	30.7	33.9	40.9
C-21	74.6	85.9	85.7	85.8	81.4	35.4	35.5	35.2	42.1	50.7	70.1
C-22	79.6	78.4	78.2	77.9	78.4	79.2	79.4	79.4	75.4	215.6	79.6
C-23	23.6	23.5	22.9	22.7	22.8	22.8	23.5	22.6	22.8	22.7	22.8
C-24	64.6	64.5	63.5	63.4	63.5	63.4	64.5	63.2	63.5	63.4	63.4
C-25	16.2	16.1	15.7	15.7	15.6	15.6	16.2	15.5	15.7	15.5	15.6
C-26	17.0	16.9	16.8	16.9	16.7	16.6	16.9	16.5	16.8	16.5	16.7
C-27	26.7	26.6	26.6	26.6	26.5	25.7	25.8	25.6	25.5	25.2	26.5
C-28	22.3	22.1	22.1	22.1	22.0	27.5	27.7	27.4	28.5	25.0	22.1
C-29	31.5	31.2	31.2	31.3	70.4	32.8	32.9	32.7	33.2	31.7	71.3
C-30	21.3	22.1	22.1	22.1	18.1	21.3	21.4	21.3	21.0	20.7	17.3

Chemical shifts (δ: ppm) were measured in pyridine-d_5.

Therefore, the structure of **1b** was identified as 21-*O*-β-D-xylopyranosyl soyasapogenol A. Since the signals ascribable to sugar moiety linked at C-3 were superimposable on those of **6**—**8** in the ^{13}C-NMR of **1**, the full structure of **1** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 21-*O*-β-D-xylopyranoside.

Lupinoside PA_2 (**2**), a white powder, $[\alpha]_D$ −1.6° (pyridine), furnished **1a**, D-glucuronic acid, D-galactose

TABLE II. ^{13}C-NMR Data for Compounds **1**—**8**, **1b**, and **4b** (Sugar Moieties)

	1b	**1**	**2**	**3**	**4**	**4b**	**5**	**6**	**7**	**8**
Glc A-1		105.4	104.8	105.3	105.2		105.1	105.3	105.3	105.2
A-2		78.2[c]	80.3	78.4[c]	78.1		78.0[c]	78.2	78.1	78.2
A-3		76.5[a]	77.0[a]	76.5[a]	76.4[a]		76.3[a]	76.5[a]	76.3[a]	76.4[a]
A-4		73.8	73.3	73.8	73.6[c]		73.4	73.7	73.6	73.7
A-5		77.7	77.9	77.6	77.6		77.9[c]	77.7	77.6	77.6
A-6		172.9	173.2	172.7	172.6		172.3	172.2	172.2	172.4
Gal-1		101.7	104.8	101.6	101.5		101.4	101.6	101.5	101.6
2		76.6[a]	72.8	76.5[a]	77.4[a]		77.2[a]	76.5[a]	76.4[a]	76.5[a]
3		76.4[a]	75.1	76.3[a]	76.2[a]		76.1[a]	76.3[a]	76.2[a]	76.2[a]
4		71.0	70.6[b]	71.0	70.9		71.0	71.0	70.9	71.0
5		77.5[a]	77.3[a]	77.5[a]	77.4[a]		77.5[a]	77.5[a]	77.5[a]	77.5[a]
6		61.5	62.1	61.5	61.3		61.2	61.4	61.3	61.4
Rha-1		102.3		102.3	102.2		102.1	102.3	102.2	102.2
2		72.3[b]		72.3[b]	72.2[b]		72.3[b]	72.2[b]	72.2[b]	72.2[b]
3		72.6[b]		72.7[b]	72.4[b]		72.4[b]	72.6[b]	72.5[b]	72.6[b]
4		74.3		74.3	74.2		74.0	74.2	74.2	74.3
5		69.3		69.3	69.1		68.9	69.8	69.1	69.2
6		18.9		18.9	18.7		18.6	18.8	18.7	18.8
Xyl-1	107.0	107.0	106.8	106.4						
2	75.3	75.2	75.1	75.3						
3	78.2	78.2[c]	78.3	78.3[c]						
4	71.0	71.0	70.8[b]	71.0						
5	67.1	67.1	66.9	67.2						
Rha′-1					97.9	98.1	97.5			
2					72.5[b]	72.6[b]	72.0[b]			
3					72.8[b]	73.0[b]	70.7			
4					73.6[c]	73.8	84.5			
5					70.1	70.2	68.3			
6					18.4	18.5	18.1			
Glc-1							106.3			
2							76.0			
3							78.0[c]			
4							71.7			
5							78.0[c]			
6							62.2			

a—*c*) Assignments in each vertical column may be interchanged.

and D-xylose in the same manner as above. In the HR/positive FAB-MS, **2** showed a peak at *m/z* 943 due to $[M-H]^-$ in the negative FAB-MS, and at *m/z* 967.4880 $[M+Na]^+$ ($C_{47}H_{76}NaO_{19}$). These data suggested that the structure of **2** was a desrhamnosyl compound of **1**. In the ^{13}C-NMR spectrum of **2** (Tables I, II), the terminal rhamnosyl signals disappeared and the signals for aglycone and 21-*O*-xylopyranosyl moiety were superimposable on those of **1**. The remaining sugar signals were identical with those of kaikasaponin I.[11] Therefore, **2** was concluded to be the desrhamnosyl compound of **1**, that is, 3-*O*-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl soyasapogenol A 21-*O*-*β*-D-xylopyranoside.

Lupinoside PA_3 (**3**), a white powder, $[\alpha]_D$ −12.9° (MeOH), showed a peak at *m/z* 1105 due to $[M-H]^-$ in the negative FAB-MS, and at *m/z* 1129.5409 $[M+Na]^+$ ($C_{53}H_{86}NaO_{24}$) in the HR/positive FAB-MS. The sapogenol obtained by acid hydrolysis of **3** was identified with kudzusapogenol A (**3a**)[7b] on TLC. The component sugars were determined to be D-glucuronic acid, D-galactose, D-xylose and L-rhamnose. In the ^{13}C-NMR spectrum of **3**, the signals for the sugar region were superimposable on those of **1**. On the comparative analysis of the ^{13}C-NMR spectra of **3** and **8**, the C-21 signal of **3** appeared at a much lower field than that of **8** due to glycosylation. Therefore, the structure of **3** was established as 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl kudzusapogenol A 21-*O*-*β*-D-xylopyranoside.

Lupinoside PA_4 (**4**), a white powder, $[\alpha]_D$ −21.9° (MeOH), gave soyasapogenol B (**4a**),[7a] D-glucuronic acid, D-galactose and L-rhamnose by acid hydrolysis. In the negative and HR/positive FAB-MS, **5** showed a peak at *m/z* 1087 $[M-H]^-$ and at *m/z* 1089.5839 $[M+H]^+$ ($C_{54}H_{89}O_{22}$), respectively. These MS appeared to a greater extent than those of **6** by a deoxyhexsoyl moiety. The enzymatic hydrolysis with GH afforded a prosapogenin (**4b**), a white powder, $[\alpha]_D$ +27.9° (pyridine), negative FAB-MS: *m/z* 603 $[M-H]^-$. Since the signals due to a rhamnopyranosyl unit which was attached to C-22 of 4a were observed in the ^{13}C-NMR spectrum of **4b**, the structure of **4b** was characterized as 22-*O*-*α*-L-rhamnopyranosyl soyasapogenol B. Comparison with the ^{13}C-NMR data for **4** and **6** showed the signals ascribable to sugar moiety linked at C-3 were superimposable on those of **6**. Consequently, the structure of **4** was elucidated as 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl-(1→2)-*β*-D-glucuronopyranosyl soyasapogenol B 22-*O*-*α*-L-rhamnopyranoside.

Lupinoside PA_5 (**5**), a white powder, $[\alpha]_D$ −23.0°

(MeOH) furnished D-glucose in addition to the same components as **4** under acid hydrolysis. In the FAB-MS, **5** showed a peak at *m/z* 1249 $[M-H]^-$, and at *m/z* 1273.6189 $[M+Na]^+$ ($C_{60}H_{98}NaO_{27}$), appeared in more quantity than those of **4** by a hexsoyl moiety. A comparative study of the ^{13}C-NMR spectrum of **5** and **4** showed that the signals ascribable to β-D-glucopyranosyl moiety were added to those of **4** (Tables I, II). Moreover, a downfield shift at C-4, and upfield shifts at C-3 and C-5 of the rhamnosyl unit were observed, indicating the β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl unit. Since the enzymatic hydrolysate of **5** by GH was more polar than **4b** by TLC, the hydrolysate of **5** was concluded to be 22-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl soyasapogenol B. Therefore, the full structure of **5** was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside.

Experimental

The seeds were purchased from Sakata No Tane Co., Ltd. The instruments and reagents used in this study were the same as described in an earlier paper.[12] The optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. IR spectra were recorded with a JEOL FT-IR spectrometer, JIR-6500W. ^1H- 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 EI- and FAB-MS were measured with a JEOL DX-300 spectrometer. HR FAB-MS were measured with a JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI. TLC was performed on precoated Kieselgel 60 F_{254} plates (Merck). GC was performed by a Hewlett Packard HP5890A. The GC conditions were as follows: column, Ohio Valley OV-1 (0.5 μ film bonded, 0.32 × 30 m); column oven temperature, 230 °C; injection port temperature, 270 °C; detection temperature, 270 °C, carrier gas, He (1.8 kg/cm²). Column chromatography was carried out on Kieselgel 60 (70—230 mesh, and 230—400 mesh, Merck), Sephadex LH-20 (Pharmacia), Bondapak C_{18} (Waters) Chromatorex ODS-DU 3050MT (Fuji Silysia) and MCI gel CHP 20P (Mitsubishi Chemical. Ind.). HPLC was carried out on a system of a pump: CCPM (Toso), UV detector: UV-970 (JASCO) and a column heater: U-620 (Sugai).

Extraction and Isolation Fresh roots (4.56 kg) of *L. polyphyllus* × *L. arboreus* were cultivated in the medicinal garden of our department, and extracted with MeOH twice under reflux. The extract (257 g) was partitioned with EtOAc and 80% MeOH. The 80% MeOH portion (208 g) was subjected to Bondapak C_{18} column chromatography using 0%→100% MeOH to give fractions 1 to 4. Fraction 4 (9.1 g) was further separated by MCI gel CHP 20P (0%→100% MeOH), Sephadex LH-20 (0%→100% MeOH) and silica gel ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2→7 : 3 : 0.5) to provide compounds **1** (0.0024%, from the fresh roots), **2** (0.0009%), **3** (0.0035%), **4** (0.0032%), **5** (0.0013%), **6** (0.029%), **7** (0.0013%) and **8** (0.0026%).

Compound 1 (Lupinoside PA_1) A white amorphous powder, $[\alpha]_D^{25}$ −15.3° (*c* = 0.52, MeOH). IR (KBr): 3405 (ν_{O-H}), 1730 ($\nu_{C=O}$) cm^{-1}. HR FAB-MS *m/z* 1113.5471 $[M+Na]^+$ (Calcd for $C_{53}H_{86}NaO_{23}$: 1113.5457). Negative FAB-MS: *m/z* 1089 $[M-H]^-$, 943 $[M-H-Rha]^-$, 781 $[M-H-Rha-Gal]^-$, 605 $[M-H-Rha-Gal-Glc\ A]^-$. ^1H-NMR (in pyridine-d_5): 0.68, 0.92, 1.29, 1.33, 1.37, 1.44, 1.49 (each 3H, s, *tert*-Me × 7), 1.80 (3H, d, *J* = 5.6 Hz, Rha H-6), 5.27 (1H, s, H-12), 6.25 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Identification of Sapogenol and Sugars for 1 A sample of **1** was hydrolyzed in 2 N HCl/H_2O at 80 °C for 2 h. After filtration of the mixture, the precipitate was identified as soyasapogenol A (**1a**) by TLC. *Rf*: 0.37 ($CHCl_3$: MeOH = 19 : 1), 0.28 (*n*-hexane : acetone = 2 : 1). The filrate was neutralized with 2 N KOH/H_2O. The sugar mixture was subjected to TLC analysis [HPTLC, Kieselgel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1, *Rf*: 0.51 (rhamnose), 0.43 (xylose), 0.25 (galactose), 0.08 (glucuronic acid); $CHCl_3$–MeOH–acetone–H_2O, 3 : 3 : 3 : 1, *Rf*: 0.70 (rhamnose), 0.59 (xylose), 0.37 (galactose), 0.14 (glucuronic acid)]

D, L Determination of Sugars A sample of **1** (3 mg) was methylated in ethereal CH_2N_2. To a solution of the methylated sample for **1** was added $NaBH_4$ (*ca.* 5 mg), and the mixture was kept at r.t. for 30 min. The reaction mixture was worked up with Sephadex LH-20. The product was heated in 2 N HCl/H_2O at 90 °C for 3 h. The precipitate was removed by filtration and the supernatant was treated with Amberlite IRA-400 to give a sugar fraction. This fraction was dissolved in pyridine (0.4 ml), then the mixture was added to a pyridine solution (0.4 ml) of L-cysteine methyl ester hydrochloride (0.09 mol/l) and warmed at 60 °C for 2 h. The mixture was evaporated under N_2 stream and dried *in vacuo*. The obtained syrup was trimethylsilylated with trimethylsilylimidazole (0.4 ml) at 60 °C for 1 h. After the addition of *n*-hexane (1 ml) and H_2O (1 ml), the *n*-hexane layer was taken off and checked by GC. The retention time (t_R) of the peaks was at 12.43 min (D-xylose), 15.61 min (L-rhamnose), 21.71 (D-glucose) and 23.36 min (D-galactose).

Enzymatic Hydrolysis of 1 To a solution of **1** (16 mg) in acetate buffer (pH 4.2, 30 ml) was added GH (100 μl) and the mixture was incubated at 37 °C for 2 d. When the hydrolysis had been completed, the hydrolysate was partitioned with 1-BuOH and H_2O. The 1-BuOH ext. was evaporated and purified over silica gel column chromatogaraphy with $CHCl_3$–MeOH–H_2O (1 : 0 : 0→9 : 1 : 0.1) to yield **1b** (3.3 mg), a white amorphous powder, $[\alpha]_D^{25}$ +70.2° (*c* = 0.33, pyridine). Negative FAB-MS: *m/z* 605 $[M-H]^-$, 473 $[M-H-Xyl]^-$. ^1H-NMR(in pyridine-d_5): 0.94, 1.00, 1.28, 1.32, 1.38, 1.52, 1.59 (each 3H, s, *tert*-Me × 7), 3.65(1H, dd, *J* = 11.4, 5.4 Hz, H-3), 3.73 (1H, d, *J* = 10.6 Hz, H-24_{ax}), 3.78(1H, t, *J* = 10.4 Hz, Xyl H-5_{ax}), 3.91, 4.07 (each 1H, d, *J* = 2.9, 3.1 Hz respectively, H-21, 22), 4.02 (1H, t, *J* = 8.1 Hz, Xyl H-2), 4.19 (1H, t, *J* = 8.4 Hz, Xyl H-3), 4.23 (1H, dd, *J* = 9.2, 4.4 Hz, Xyl H-4), 4.35 (1H, dd, *J* = 11.0, 4.8 Hz, Xyl H-5_{eq}), 4.53 (1H, d, *J* = 10.6 Hz, H-24_{eq}), 4.96 (1H, d, *J* = 7.7 Hz, Xyl H-1), 5.32 (1H, s, H-12). ^{13}C-NMR: Tables I and II.

Compound 2 (Lupinoside PA_2) A white amorphous powder, $[\alpha]_D^{25}$ −1.6° (*c* = 0.45, pyridine). IR (KBr): 3400 (ν_{O-H}), 1730 ($\nu_{C=O}$) cm^{-1}. HR FAB-MS *m/z* 967.4880 $[M+Na]^+$ (Calcd for $C_{47}H_{76}NaO_{19}$: 967.4878). Negative FAB-MS: *m/z* 943 $[M-H]^-$, 781 $[M-H-Gal]^-$. ^1H-NMR (in pyridine-d_5): 0.71, 0.91, 1.27, 1.29, 1.36, 1.37, 1.49 (each 3H, s, *tert*-Me × 7), 3.45 (1H, dd, *J* = 12.7, 4.2 Hz, H-3), 5.28 (1H, s, H-12). ^{13}C-NMR: Tables I and II.

Identification of Sapogenol and Sugars for 2 A sample of **2** was hydrolyzed in the above manner. The precipitate was identified as soyasapogenol A (**1a**) by TLC. *Rf*: 0.37 ($CHCl_3$: MeOH = 19 : 1), 0.28 (*n*-hexane : acetone = 2 : 1). After neutralization, the sugar mixture was subjected to TLC analysis [HPTLC, Kieselgel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1, *Rf*: 0.44 (xylose), 0.26 (galactose), 0.07 (glucuronic acid); $CHCl_3$–MeOH–acetone–H_2O, 3 : 3 : 3 : 1, *Rf*: 0.59 (xylose), 0.38 (galactose), 0.15 (glucuronic acid)]

D, L Determination of Sugars A sample of **2** (3 mg) was treated in the same manner as above. The derivatives were analyzed by GC. The t_R of the peaks was at 12.41 min (D-xylose), 21.89 (D-glucose) and 23.21 min (D-galactose).

Compound 3 (Lupinoside PA_3) A white amorphous powder, $[\alpha]_D^{25}$ −12.9° (*c* = 0.52, MeOH). IR (KBr): 3390 (ν_{O-H}), 1730 ($\nu_{C=O}$) cm^{-1}. HR FAB-MS *m/z* 1129.5409 $[M+Na]^+$ (Calcd for $C_{53}H_{86}NaO_{24}$: 1129.5407). Negative FAB-MS: *m/z* 1105 $[M-H]^-$, 959 $[M-H-Rha]^-$, 797 $[M-H-Rha-Gal]^-$, 621 $[M-H-Rha-Gal-Glc\ A]^-$. ^1H-NMR (in pyridine-d_5): 0.67, 0.92, 1.33, 1.36, 1.41, 1.54 (each 3H, s, *tert*-Me × 6), 1.79 (3H, d, *J* = 5.9 Hz, Rha H-6), 5.14 (1H, d, *J* = 7.3 Hz, Xyl H-1), 5.30 (1H, s, H-12), 6.24 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Identification of Sapogenol and Sugars for 3 A sample of **2** was hydrolyzed in the above manner. The precipitate was identified as kudzusapogenol A (**3a**) by TLC. *Rf*: 0.26 ($CHCl_3$: MeOH = 19 : 1), 0.17 (*n*-hexane : acetone = 2 : 1). After neutralization, the sugar mixture was subjected to TLC analysis [HPTLC, Kieselgel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1, *Rf*: 0.51 (rhamnose), 0.43 (xylose), 0.26 (galactose), 0.08 (glucuronic acid); $CHCl_3$–MeOH–acetone–H_2O, 3 : 3 : 3 : 1, *Rf*: 0.70 (rhamnose), 0.59 (xylose), 0.38 (galactose), 0.14 (glucuronic acid)].

D, L Determination of Sugars A sample of **3** (3 mg) was treated in the same manner. The derivatives were analyzed by GC. The t_R of the peaks was at 12.67 min (D-xylose), 15.67 (L-rhamnose), 21.73 (D-glucose) and 23.68 min (D-galactose).

Compound 4 (Lupinoside PA_4) A white amorphous powder, $[\alpha]_D^{25}$ −21.9° (*c* = 0.52, MeOH). IR (KBr): 3405 (ν_{O-H}), 1730 ($\nu_{C=O}$) cm^{-1}.

HR FAB-MS *m/z* 1089.5839 $[M+H]^+$ (Calcd for $C_{54}H_{89}O_{22}$: 1089.5845). Negative FAB-MS: *m/z* 1087 $[M-H]^-$, 941 $[M-H-Rha]^-$, 779 $[M-H-Rha-Gal]^-$, 603 $[M-H-Rha-Gal-Glc\ A]^-$. ^{1}H-NMR (in pyridine-d_5): 0.72, 0.86, 0.95, 1.02, 1.04, 1.25, 1.45 (each 3H, s, *tert*-Me × 7), 1.72, 1.78 (each 3H, d, J=5.5, 6.2 Hz respectively, Rha, Rha′ H-6), 3.40 (1H, dd, J=13.0, 6.5 Hz, H-3), 5.22 (1H, s, H-12), 5.40 (1H, s, Rha′ H-1), 6.24 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Identification of Sapogenol and Sugars for 4 A sample of **4** was hydrolyzed in the same manner. The precipitate was identified as soyasapogenol B (**4a**) by TLC. *Rf*: 0.33 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 2 : 1). After neutralization, the sugar mixture was subjected to TLC analysis [HPTLC, Kieselgel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1, *Rf*: 0.53 (rhamnose), 0.27 (galactose), 0.10 (glucuronic acid); $CHCl_3$–MeOH–acetone–H_2O, 3 : 3 : 3 : 1, *Rf*: 0.68 (rhamnose), 0.39 (galactose), 0.15 (glucuronic acid)].

D, L **Determination of Sugars** A sample of **4** (3mg) was treated in the same manner. The derivatives were analyzed by GC. The t_R of the peaks was at 15.89 (L-rhamnose), 21.91 (D-glucose) and 23.40 min (D-galactose).

Enzymatic Hydrolysis of 4 To a solution of **4** (23 mg) in acetate buffer (pH 4.2, 60 ml) was added GH (200 μl) and treated in the same manner as described for **1** to afford a prosapogenin, **4b** (8.0 mg), A white amorphous powder, $[\alpha]_D^{25}$ +27.9° (c=0.36, pyridine). Negative FAB-MS: *m/z* 603 $[M-H]^-$, 457 $[M-H-Rha]^-$. ^{1}H-NMR (in pyridine-d_5): 0.87, 0.97, 1.01, 1.04, 1.05, 1.21, 1.58 (each 3H, s, *tert*-Me × 7), 3.66 (1H, dd, J=10.5, 4.8 Hz, H-3), 3.74 (1H, d, J=11.0 Hz, H-24$_{ax}$), 4.34 (2H, m, Rha H-3, 5), 4.49—4.56 (3H, m, H-24$_{eq}$, Rha H-2, 4), 5.27 (1H, s, H-12), 5.41 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Compound 5 (Lupinoside PA$_5$) A white amorphous powder, $[\alpha]_D^{25}$ +23.0° (c=0.52, MeOH). IR (KBr): 3395 (ν_{O-H}), 1730 ($\nu_{C=O}$) cm^{-1}. HR FAB-MS: *m/z* 1273.6189 $[M+Na]^+$ (Calcd for $C_{60}H_{98}NaO_{27}$: 1273.6193). Negative FAB-MS: *m/z* 1249 $[M-H]^-$, 1103 $[M-H-Rha]^-$, 1087 $[M-H-Glc]^-$, 941 $[M-H-Rha-Gal]^-$, 765 $[M-H-Rha-Gal-Glc\ A]^-$. ^{1}H-NMR (in pyridine-d_5): 0.74, 0.85, 1.00, 1.01, 1.03, 1.26, 1.46 (each 3H, s, *tert*-Me × 7), 1.76, 1.80 (each 3H, d, J=6.2, 5.9 Hz respectively, Rha H-6 × 2), 3.42 (1H, br d, J=11.6 Hz, H-3), 5.27 (1H, s, H-12), 5.35 (1H, s, Rha′ H-1), 6.24 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Identification of Sapogenol and Sugars for 5 A sample of **5** was hydrolyzed in the above manner. The precipitate was identified as soyasapogenol B (**4a**) by TLC. *Rf*: 0.35 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 2 : 1). After neutralization, the sugar mixture was subjected to TLC analysis [HPTLC, Kieselgel 60 F_{254} (Merck Art 5628), $CHCl_3$–MeOH–H_2O, 6 : 4 : 1, *Rf*: 0.52 (rhamnose), 0.31 (glucose), 0.26 (galactose), 0.08(glucuronic acid); $CHCl_3$–MeOH–acetone–H_2O, 3 : 3 : 3 : 1, *Rf*: 0.70 (rhamnose), 0.37 (galactose), 0.14 (glucuronic acid)]

D, L **Determination of Sugars** A sample of **5** (3mg) was treated in the same manner. The derivatives were analyzed by GC. The t_R of the peaks was at 15.65 (L-rhamnose), 21.69 (D-glucose) and 23.38 min (D-galactose).

Identification of Prosapogenin for 5 A sample of **5** was hydrolyzed enzymatically in the same manner as described for **1**. The 1-BuOH ext. showed the prosapogenin for **5** on TLC. *Rf*, 0.37($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2), *cf.* **4a**, 0.67.

Compound 6 (Soyasaponin I) A white amorphous powder, $[\alpha]_D^{25}$ −5.9° (c=0.51, MeOH), ^{1}H-NMR (in pyridine-d_5): 0.71, 0.96, 1.00, 1.23, 1.29, 1.30, 1.45 (each 3H, s, *tert*-Me × 7), 1.79 (3H, d, J=5.9 Hz, Rha H-6), 3.40 (1H, dd, J=10.3, 4.8 Hz, H-3), 5.30 (1H, s, H-12), 6.29 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Compound 7 (Dehydrosoyasaponin I) A white amorphous powder, $[\alpha]_D^{25}$ −28.2° (c=0.48, MeOH). IR (KBr): 3400 (ν_{O-H}), 1735 ($\nu_{C=O}$) cm^{-1}. Negative FAB-MS: *m/z* 939 $[M+H]^-$, 793 $[M-H-Rha]^-$, 631 $[M-H-Rha-Gal]^-$. ^{1}H-NMR (in pyridine-d_5): 0.70, 0.85, 0.87, 0.96, 1.17, 1.31, 1.45 (each 3H, s, *tert*-Me × 7), 1.79 (3H, d, J=5.9 Hz, Rha H-6), 3.40 (1H, dd, J=10.3, 4.6 Hz, H-3), 5.24 (1H, s, H-12), 6.28 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

Compound 8 (Kudzusaponin A$_3$) A white amorphous powder, $[\alpha]_D^{25}$ −6.5° (c=0.50, MeOH). Positive FAB-MS: *m/z* 997 $[M+Na]^+$, 851 $[M+Na-Rha]^+$, 513 $[M+Na-Rha-Gal-Glc\ A]^+$. ^{1}H-NMR (in pyridine-d_5): 0.69, 0.95, 1.31, 1.35, 1.41, 1.51 (each 3H, s, *tert*-Me × 6), 1.78 (3H, d, J=6.2 Hz, Rha H-6), 5.33 (1H, s, H-12), 6.12 (1H, s, Rha H-1). ^{13}C-NMR: Tables I and II.

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References and Notes

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