Oleanene-Type Triterpene Glycosides from Puerariae Radix. IV.[1] Six New Saponins from *Pueraria lobata*

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From Puerariae Radix, the root of *Pueraria lobata* (Leguminosae), six new oleanene-type triterpene glycosides, called kudzusaponins A_1 (1), A_2 (2), A_4 (3), A_5 (4), SA_4 (5), and SB_1 (6) were isolated together with kudzusaponin A_3 (7), soyasaponins SA_3 (8), and I (9). The structures of 1—6 were determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A 22-*O*-β-D-xylopyranoside, 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A, 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A, 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A, 3-*O*-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside, and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl (β-fabatriosyl) soyasapogenol B 22-*O*-α-L-arabinopyranoside, respectively.

Key words *Pueraria lobata*; Leguminosae; oleanene-type triterpene bisdesmoside; triterpenoidal saponin; kudzusaponin; tandem mass spectrometry

Puerariae Radix, the root of *Pueraria lobata* (WILLD.) OHWI, is one of the most important oriental crude drugs used as a perspiration, antipyretic, and antispasmodic agent. In the preceding paper,[2] we reported the isolation of four new triterpenoidal saponins from Puerariae Radix and elucidated their structures. In addition, we described the usefulness of tandem mass spectrometry in the structural determination of oleanene-type triterpene bisdesmosides. In our continuing study on the ingredients of *P. lobata*, we have now obtained six new triterpenoidal saponins and three known ones from the fresh root of this plant. This paper deals with the isolation and the structural elucidation of these saponins.

Fresh root of *P. lobata* was extracted with MeOH once at room temperature, and the extract was partitioned between AcOEt and 40% MeOH. The 40% MeOH layer was concentrated and subjected to normal and reversed phase column chromatography to yield compounds **1**—**9**. Compounds **7**, **8**, and **9** were identified as kudzusaponin A_3,[3] soyasaponin A_3,[4] and soyasaponin I[5] by comparison with the ^{1}H- and ^{13}C-NMR data (Tables 1 and 2), respectively.

Kudzusaponin A_1 (**1**) showed an $[M+Na]^+$ ion peak at *m/z* 1099 in the positive ion FAB mass spectrum, which corresponds to a composition $C_{52}H_{84}NaO_{23}$ ($[M+Na]^+$) given by the exact mass measurement under high resolution (HR) conditions. The sapogenol obtained by acid hydrolysis of **1** was identified as kudzusapogenol A (**1a**)[6] on TLC. A monosaccharide mixture revealed the presence of glucuronic acid (glc A), arabinose (ara), rhamnose (rha), and xylose (xyl). Their absolute configurations were determined to be D-glc A, D-xyl, L-ara, and L-rha according to a procedure developed by Hara *et al.*[7] The ^{13}C-NMR spectrum of **1** was superimposable on those of subproside II (**1b**)[3] except for the additional xylopyranosyl signals and C-22 signal which was shifted (+13.5 ppm) by glycosylation[8] (Tables 1 and 2). Since the peak of $[D/E\ ring+xyl+Na]^+$ ion derived *via retro* Diels–Alder fission[9] appeared at *m/z* 421 in the tandem mass spectrometry (MS/MS) of an $[M+Na]^+$ ion,[2] the structure of **1** was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-arabinopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A 22-*O*-β-D-xylopyranoside.

Kudzusaponin A_2 (**2**) showed an $[M+Na]^+$ ion peak ($C_{42}H_{68}NaO_{16}$) at *m/z* 851 in the positive ion FAB mass spectrum. The components obtained by acid hydrolysis of **2** were identified as **1a**, D-glc A, and D-galactose (gal) in the same manner as above. The ^{13}C-NMR spectrum of the sugar part of **2** was superimposable on those of soyasaponin III (**2a**).[5a,10] The ^{13}C-NMR signals of the aglycone part of **2** were in good accordance with those of **1a** except for C-3, which showed a downfield shift (+10.5 ppm) due to glycosylation. Therefore, **2** was concluded to be 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A.

Kudzusaponin A_4 (**3**) showed an $[M+Na]^+$ ion peak ($C_{42}H_{68}NaO_{16}$) at *m/z* 851 in the positive ion FAB mass spectrum. The components obtained by acid hydrolysis of **3** were identified as **1a**, D-glc A, and D-glucose (glc) in the same manner as above. Although the ^{13}C-NMR signals of **3** were very close to those of **2**, the signals due to one terminal glucopyranosyl unit appeared instead of the signals due to the galactopyranosyl unit. Therefore, **3** was concluded to be 3-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A.

Kudzusaponin A_5 (**4**) showed an $[M+Na]^+$ ion peak ($C_{48}H_{78}NaO_{20}$) at *m/z* 997 in the positive ion FAB mass spectrum. The components obtained by acid hydrolysis of **4** were identified as **1a**, D-glc A, D-glc, and L-rha in the above manner. A comparative analysis of the ^{13}C-NMR of **4** and **3** showed that signals of **4** were superimposable on those of **3**, except for those due to the occurrence of one additional rhamnopyranosyl moiety and a 2-*O*-substituted glucopyranosyl moiety. Since signals resulting from the sugar moiety were consistent with those of robinioside F (**4a**),[11] **4** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-

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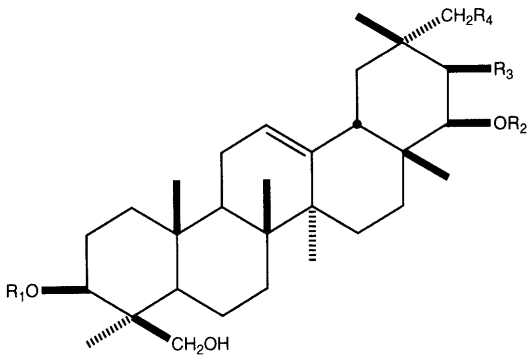
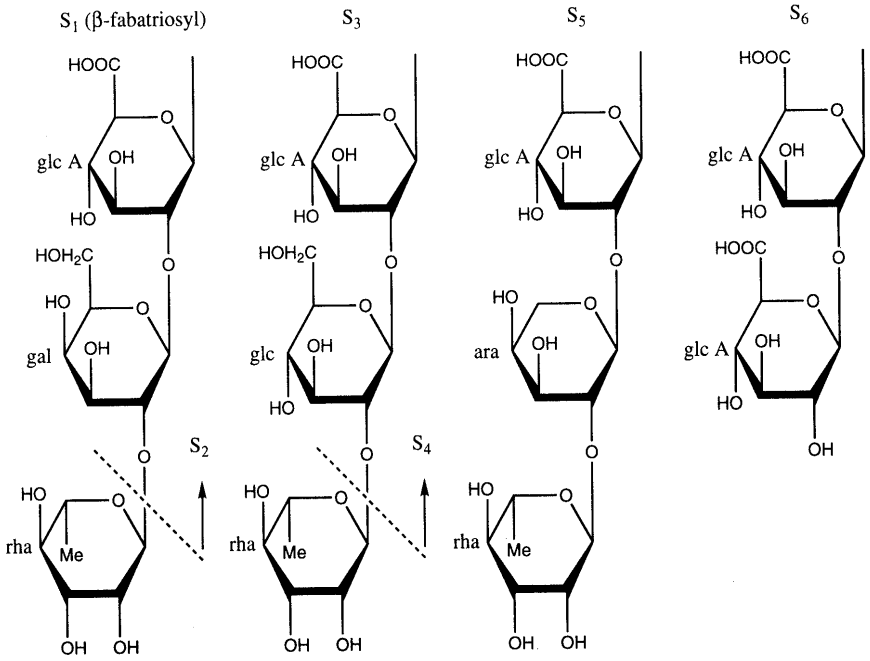


	R_1	R_2	R_3	R_4
1:kudzusaponin A_1	S_5	xyl	OH	OH
2:kudzusaponin A_2	S_2	H	OH	OH
3:kudzusaponin A_4	S_4	H	OH	OH
4:kudzusaponin A_5	S_3	H	OH	OH
5:kudzusaponin SA_4	S_6	ara	OH	H
6:kudzusaponin SB_1	S_1	ara	H	H
7:kudzusaponin A_3	S_1	H	OH	OH
8:soyasaponin A_3	S_1	H	OH	H
9:soyasaponin I	S_1	H	H	H



glucuronopyranosyl kudzusapogenol A.

Kudzusaponin SA_4 (**5**) showed an $[M+Na]^+$ ion peak ($C_{47}H_{74}NaO_{20}$) at *m/z* 981 in the positive ion FAB mass spectrum. The components obtained by acid hydrolysis of **5** were identified as soyasapogenol A (**5a**),[6] D-glc A, L-ara in the above manner. The ^{13}C-NMR signals for the sugar part were superimposable on those of glycyrrhizin (**5b**)[12] except for the appearance of additional arabinopyranosyl signals. In a comparative analysis of the ^{13}C-NMR of **5** and **5a**, the signals due to C-3 and C-22 of **5** were observed in a much lower field (+9.9 ppm and +13.0 ppm, respectively) due to glycosylation. Furthermore, the peak of [D/E ring+ara+Na]$^+$ ion derived *via retro* Diels–Alder fission appeared at *m/z* 405 in the MS/MS of an $[M+Na]^+$ ion. Therefore, **5** was concluded to be 3-*O*-β-D-glucuronopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside.

Kudzusaponin SB_1 (**6**) showed an $[M+Na]^+$ ion peak ($C_{47}H_{74}NaO_{20}$) at *m/z* 1073 in the positive ion FAB mass spectrum. The components obtained by acid hydrolysis of **6** were identified as soyasapogenol B (**6a**),[5a] D-glc A, D-gal, L-rha, and L-ara. The ^{13}C-NMR signals of **6** were superimposable on those of soyasaponin I (**9**) except for additional arabinopyranosyl signals as well as a downfield shift of the C-22 signal (+7.3 ppm) by glycosylation. Since the peak of [D/E ring+ara+Na]$^+$ ion derived *via retro* Diels–Alder fission appeared at *m/z* 389 in the MS/MS of an $[M+Na]^+$ ion, **6** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-α-L-arabinopyranoside.

The saponins of *P. lobata* are characterized by a great

Table 1. ^{13}C-NMR Data for Aglycone Moieties of **1**—**9**, **1a**, **1b**, and **5a**

	1	**1a**[6]	**1b**[3]	**2**	**3**	**4**	**5**	**5a**[6]	**6**	**7**	**8**	**9**
C-1	38.7	38.9	38.5	38.5	38.6	38.5	38.8	38.9	39.0	38.4	38.8	38.8
C-2	26.7	28.4	26.4	26.6	26.8	26.6	26.6	28.4	26.2	26.5	26.6	26.2
C-3	91.1	80.1	90.9	90.6	90.8	91.5	90.0	80.1	91.7	91.0	91.5	90.7
C-4	43.9	43.2	43.8	43.8	43.9	43.7	44.0	43.2	44.0	43.8	43.9	44.1
C-5	56.1	56.3	55.8	56.0	56.2	56.1	56.2	56.3	56.4	55.9	56.2	56.3
C-6	18.6	19.1	18.4	18.6	18.7	18.5	19.0	19.1	18.7	18.4	18.7	18.6
C-7	32.9	33.2	32.7	32.8	33.0	32.8	33.0	33.2	33.5	32.8	33.1	33.4
C-8	40.2	40.3	40.0	40.1	40.3	40.2	40.2	40.3	39.9	40.1	39.3	39.8
C-9	47.8	48.1	47.6	47.6	47.8	47.7	47.8	48.1	48.0	47.6	47.9	47.7
C-10	36.5	37.0	36.3	36.4	36.5	36.4	36.8	37.0	36.6	36.3	36.5	36.5
C-11	24.1	24.1	23.9	24.0	24.2	24.1	24.1	24.2	24.2	24.0	24.2	24.0
C-12	122.5	122.5	122.3	122.4	122.6	122.5	122.9	122.5	123.6	122.3	122.7	122.6
C-13	144.6	144.6	144.5	144.6	144.8	144.7	144.3	144.5	144.4	144.5	144.6	144.7
C-14	41.8	42.0	41.8	41.9	42.2	42.0	41.8	42.1	42.5	41.9	42.2	42.4
C-15	26.7	26.6	26.5	26.6	26.8	26.6	26.6	26.6	26.5	26.5	26.6	26.5
C-16	27.7	27.4	27.2	27.3	27.5	27.3	27.8	27.5	28.9	27.3	27.4	28.9
C-17	39.3	39.0	38.9	39.0	39.2	39.0	39.3	39.2	37.6	38.9	38.8	37.9
C-18	43.8	43.2	43.1	43.2	43.4	43.2	44.4	44.0	46.0	43.1	44.0	45.5
C-19	40.9	41.1	41.0	41.0	41.2	41.0	47.2	47.3	46.8	40.9	47.5	46.7
C-20	41.5	41.0	41.0	41.0	41.2	41.0	36.5	36.6	30.7	40.9	36.5	30.7
C-21	69.8	70.5	70.4	70.3	70.5	70.4	73.7	74.6	37.1	70.3	75.0	41.9
C-22	93.1	79.7	79.6	79.7	80.0	79.8	92.6	79.6	82.9	79.7	79.4	75.6
C-23	23.2	23.5	22.7	22.6	22.7	22.8	23.1	23.6	23.0	22.9	23.0	23.0
C-24	63.4	64.5	63.3	63.5	63.5	63.4	63.2	64.6	63.6	63.5	63.6	63.0
C-25	15.8	16.2	15.6	15.6	15.7	15.6	15.5	16.2	16.0	15.7	15.9	15.7
C-26	16.8	17.0	16.7	16.8	17.0	16.9	16.7	17.0	17.2	16.7	17.1	17.0
C-27	26.8	26.7	26.5	26.6	26.8	26.6	26.7	26.7	25.5	26.5	26.9	25.4
C-28	22.9	22.3	22.1	22.2	22.4	22.3	22.9	22.3	21.2	22.1	22.3	20.9
C-29	70.3	71.7	71.3	71.9	71.7	71.6	31.5	31.5	32.4	71.5	31.7	32.9
C-30	17.5	17.5	17.3	17.5	17.7	17.5	21.4	21.3	28.9	17.4	21.5	28.7

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

variety of sugar chains linked at C-3. We have now obtained six sugar chains from this crude drug; this is very rare in leguminous saponins. For example, despite the isolation of twenty three saponins, *Abrus cantoniensis*[13] included only two sugar chains (β-fabatriose and β-abritetraose). All of these chains have glucuronic acid as the innermost sugar moiety. In the case of tri-glycoside, the terminal sugar is rhamnose. But there are four kinds of secondary sugar moieties. This wide variety of sugar chains made the isolation of saponins from *P. lobata* difficult.

Experimental

The fresh root of *Pueraria lobata* was collected in Kumamoto Prefecture. TLC was performed on pre-coated Kieselgel 60 F_{254} plates (Merck). 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.). The optical rotations were measured with a JASCO DIP-360 automatic digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured with JEOL JNM-EX 270 and JNM-GX 400 NMR spectrometers and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. All mass spectra were acquired with an SX/SX102A tandem mass spectrometer of BEBE geometry (JEOL), which was controlled by a DA-7000 data system (JEOL). Positive and negative ion FAB mass spectra were obtained using only the first spectrometer. The samples were diluted in pyridine at a concentration of 1 μg/μl. The solution (0.5 μl) was mixed with glycerol (0.5 μl) subjected to analysis. Ions were generated by bombardment with a neutral Xe atom operated at 5 kV. The mass range (m/z 1—2000) was scanned at 5-s under an ion source accelerating potential of 10 kV, and averaged intensities in decade scans were recorded. The molecular ions generated by FAB MS were selected as precursor ions, which were then collided with argon molecules in the third field-free region. The argon pressure was sufficient to attenuate the primary ion beam by 50%. The fragment ions were dispersed by the second spectrometer, and the spectra were recorded as collision-activated dissociation (CAD)[14] spectra.

Extraction and Isolation Fresh root (80 kg) of *P. lobata* was extracted with MeOH (100 l) once under reflux. The extract (4.4 kg) was partitioned with AcOEt and 40% MeOH. Removal of the solvent from each phase under reduced pressure gave the aqueous (2.9 kg) and AcOEt (1.5 kg) extracts. The aqueous extract was subjected to Diaion HP-20 column chromatography using H_2O and MeOH to give H_2O eluate (2.2 kg) and MeOH eluate (770 g). A part (120 g) of the MeOH eluate was subjected to Sephadex LH-20 column chromatography using MeOH to give crude saponin fraction. This fraction was further separated by MCI gel CHP 20P, Bondapak C_{18} (20%→100% MeOH), Chromatorex ODS (30%→100% MeOH), and silica gel (*n*-BuOH : AcOH : H_2O = 8 : 1 : 1), silica gel ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1) to provide compounds **1** (7 mg), **2** (6 mg), **3** (6 mg), **4** (26 mg), **5** (6 mg), **6** (6 mg), **7** (29 mg), **8** (7 mg), and **9** (120 mg).

Kudzusaponin A_1 (1) A white amorphous powder, $[\alpha]_D^{25}$ +3.0° (c = 0.75, pyridine : H_2O = 1 : 1). HR positive ion FAB MS m/z: 1099.5298 (Calcd for $C_{52}H_{84}NaO_{23}$: 1099.5301). Positive ion FAB MS m/z: 1099 ($[M+Na]^+$), 645 ($[M+Na-rha-ara-glc\ A]^+$). MS/MS of m/z 1099 ($[M+Na]^+$), m/z: 953 ($[M+Na-rha]^+$), 821 ($[M+Na-rha-pentose]^+$), 645 ($[M+Na-rha-ara-glc\ A]^+$), 513 ($[M+Na-rha-ara-glc\ A\text{-}xyl]^+$), 421 ($[D/E\ ring+xyl+Na]^+$). ^{1}H-NMR (in pyridine-d_5): 0.68, 0.92, 1.37, 1.39, 1.41, 1.43 (each 3H, s, *tert*-Me × 6), 1.80 (3H, d, J = 6 Hz, rha H-6), 5.35 (1H, s, H-12), 6.17 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Sugars of 1 A small amount of **1** (1 mg) was hydrolyzed with 2 N HCl/H_2O and heated at 80 °C for 2 h. After the addition of $CHCl_3$, the organic layer was identified as kudzusapogenol A (**1a**) by TLC. Rfs, 0.26 ($CHCl_3$: MeOH = 19 : 1), 0.19 (hexane : acetone = 2 : 1). The aqueous layer was neutralized with 2 N KOH/H_2O. The sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$: MeOH : H_2O = 6 : 4 : 1, Rfs: 0.07 (glc A), 0.44 (ara), 0.58 (rha), and 0.42 (xyl).

Table 2. ^{13}C-NMR Data for Sugar Moieties of **1**—**9**, **1b**, **2a**, **4a** and **5b**

	1	**1b**[3]	**2**	**2a**[10]	**3**	**4**	**4a**[11]	**5**	**5b**[12]	**6**	**7**	**8**	**9**
glc A-1	105.5	105.3	104.9	105.3	105.3	105.2	105.1	104.7	104.6	104.8	105.3	105.0	104.9
2	77.7[a]	77.7	80.9	80.9	81.9	78.6[a]	78.2[a]	80.6	83.9	78.0	78.3	78.1	78.5
3	75.6[d]	76.4	77.7	77.2	77.9[a]	77.4[d]	76.6	75.5[a]	76.2	77.2[a]	76.6[a]	77.0[a]	76.8[a]
4	73.9[b]	74.2	73.6[b]	73.6	73.1	73.7[c]	73.4	72.9[c]	72.7	73.7[d]	73.7[d]	73.9[d]	73.7[d]
5	77.4[a]	77.5	78.2[a]	77.9	78.4[a]	77.8[b]	77.6	77.6[b]	77.1	77.2[a]	77.7[b]	78.1[b]	77.5[b]
6	172.4	170.2	172.4	170.3	172.7	172.5	170.3	172.5	172.1	176.2	171.1	176.2	173.2
glc A′-1								108.7	106.2				
2								75.7[a]	76.2				
3								77.8[b]	77.1				
4								72.9[c]	72.7				
5								77.6[b]	77.1				
6								172.2	171.7				
gal-1			104.9	104.9						102.0	101.6	101.8	101.9
2			73.0[b]	72.7						77.2[a]	77.5[b]	77.3[b]	77.6[b]
3			75.4[a]	75.4						76.6[b]	76.3[a]	75.8[a]	76.1[a]
4			71.1	71.0						71.0	71.0	71.0	70.9
5			77.2[a]	76.9						76.6[b]	76.5[a]	76.5[a]	76.2[a]
6			62.6	62.6						62.1	61.5	61.9	61.6
glc-1					104.9	102.0	102.0						
2					76.0	79.1	79.1						
3					78.6[a]	78.0[a]	78.0[a]						
4					70.0	69.7[b]	69.7						
5					78.4[a]	78.5[a]	78.3[a]						
6					61.7	61.3	61.2						
ara-1	102.0	101.7											
2	76.8[a]	76.8											
3	75.2[d]	75.6											
4	70.5[c]	70.1											
5	67.0[e]	66.8											
rha-1	102.4	102.3				102.0	101.9			102.0	102.3	102.1	102.1
2	72.3[f]	72.2				72.3[d]	72.2[b]			71.8[c]	72.3[c]	71.9[c]	71.9[c]
3	72.5[f]	72.5				72.7[d]	72.6[b]			72.0[c]	72.7[c]	72.0[c]	72.1[c]
4	73.8[b]	73.5				74.5[c]	74.3			73.9[d]	74.3[d]	73.8[d]	74.1[d]
5	69.4	69.2				69.4[b]	69.4			68.8	69.3	69.5	69.4
6	18.9	18.7				19.0	18.9			18.5	18.9	18.7	18.8
22-*O*-xyl-1	108.7												
2	74.3[b]												
3	78.0[a]												
4	70.5[c]												
5	67.7[e]												
22-*O*-ara-1								104.6		102.0			
2								73.7		73.7[d]			
3								75.2[a]		75.6			
4								69.8		69.6			
5								67.6		66.1			

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

Determination of Absolute Configuration of Component Sugars of 1 A small amount of **1** (1 mg) was methylated with ethereal CH_2N_2. To a solution of the methylated sample of **1** was added $NaBH_4$, and the mixture was kept at room temperature for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was evaporated and heated in 2 N HCl/H_2O at 90 °C for 3 h. The precipitate was removed by filtration and the supernatant was neutralized with 2 N KOH/H_2O. The solvent was evaporated and dried *in vacuo*. The residue was suspended in MeOH and the precipitate was removed by filtration. The filtrate was evaporated and the residue was dissolved in pyridine (0.1 ml), then the solution was added to a pyridine solution (0.2 ml) of L-cysteine methyl ester hydrochloride (0.1 mol/l) and warmed at 60 °C for 2 h. The solvent was evaporated under N_2 stream and dried *in vacuo*. The remaining syrup was trimethylsilylated with trimethylsilylimidazole (0.1 ml) at 60 °C for 1 h. After the addition of hexane and H_2O, the hexane layer was taken out and checked by GC. The retention times (t_R) of the peaks were 13.2 min (D-glc), 7.5 min (L-ara), 9.0 min (L-rha), and 7.5 min (D-xyl).

Kudzusaponin A_2 (2) A white amorphous powder, $[\alpha]_D^{25}$ +5.0° (c=0.56, pyridine : H_2O=1 : 1). HR positive ion FAB MS m/z: 851.4401 (Calcd for $C_{42}H_{68}NaO_{16}$: 851.4405). Positive ion FAB MS m/z: 873 ($[M+2Na]^+$), 851 ($[M+Na]^+$), 711 ($[M+2Na-gal]^+$), 513 ($[M+Na-gal-glc\ A]^+$).

^{1}H-NMR (in pyridine-d_5): 0.73, 0.95, 1.31, 1.32, 1.35, 1.52 (each 3H, s, *tert*-Me×6), 4.96 (1H, d, J=7 Hz, glc A H-1), 5.34 (1H, s, H-12), 5.54 (1H, d, J=8 Hz, gal H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Component Sugars of 2 A small amount of **2** was treated in the manner described above. The aglycone was identified as kudzusapogenol A (**1a**). *Rf*s: 0.26 ($CHCl_3$: MeOH = 19 : 1), 0.19 (hexane : acetone = 2 : 1). The component sugars were identified as D-glc A and D-gal. *Rf*s in TLC analysis ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1): 0.07 (glc A), 0.28 (gal). The t_Rs of the peaks in GC analysis were 13.2 min (D-glc) and 14.0 min (D-gal).

Kudzusaponin A_4 (3) A white amorphous powder, $[\alpha]_D^{25}$ +9.5° (c=0.55, pyridine : H_2O=1 : 1). HR positive ion FAB MS m/z: 851.4411 (Calcd for $C_{42}H_{68}NaO_{16}$: 851.4405). Positive ion FAB MS m/z: 873 ($[M+2Na]^+$), 851 ($[M+Na]^+$), 711 ($[M+2Na-glc]^+$), 513 ($[M+Na-glc-glc\ A]^+$). Negative ion FAB MS m/z: 827 ($[M-H]^-$), 665 ($[M-H-glc]^-$).

^{1}H-NMR (in pyridine-d_5): 0.71, 0.95, 1.31, 1.32, 1.36, 1.52 (each 3H, s, *tert*-Me×6), 4.96 (1H, d, J=8 Hz, glc A H-1), 5.34 (1H, s, H-12), 5.61 (1H, d, J=7 Hz, glc H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Component Sugars of 3 A small amount of **3** was treated in the manner described above. The aglycone

was identified as kudzusapogenol A (**1a**). *Rf*s: 0.26 ($CHCl_3$: MeOH = 19 : 1), 0.19 (hexane : acetone = 2 : 1). The component sugars were identified as D-glc A and D-glc. *Rf*s in TLC analysis ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1): 0.07 (glc A), 0.33 (glc). The t_R of the peak in GC analysis was 13.2 min (D-glc).

Kudzusaponin A_5 (4) A white amorphous powder, $[\alpha]_D^{25}$ −7.5° (c = 0.67, pyridine : H_2O = 1 : 1). HR positive ion FAB MS *m/z*: 997.4977 (Calcd for $C_{48}H_{78}NaO_{20}$: 997.4984). Positive FAB MS *m/z*: 1019 ($[M+2Na]^+$), 997 ($[M+Na]^+$), 873 ($[M+2Na-rha]^+$), 711 ($[M+2Na-rha-glc]^+$), 513 ($[M+Na-rha-glc-glc\ A]^+$). Negative FAB MS *m/z*: 973 ($[M-H]^-$), 827 ($[M-H-rha]^-$), 665 ($[M-H-rha-gal]^-$), 665 ($[M-H-rha-gal-glc\ A]^-$). ^{1}H-NMR (in pyridine-d_5): 0.67, 0.95, 1.30, 1.31, 1.33, 1.51 (each 3H, s, *tert*-Me × 6), 1.79 (3H, d, J = 6 Hz, rha H-6), 4.98 (1H, d, J = 7 Hz, glc A H-1), 5.32 (1H, s, H-12), 5.86 (1H, d, J = 7 Hz, glc H-1), 6.39 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Component Sugars of 4 A small amount of **4** was treated in the manner described above. The aglycone was identified as kudzusapogenol A (**1a**). *Rf*s: 0.26 ($CHCl_3$: MeOH = 19 : 1), 0.19 (hexane : acetone = 2 : 1). The component sugars were identified as D-glc A, D-glc, and L-rha. *Rf*s in TLC analysis ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1): 0.07 (glc A), 0.33 (glc), 0.58 (rha). The t_Rs of the peaks in GC analysis were 9.0 min (L-rha) and 13.2 min (D-glc).

Kudzusaponin SA_4 (5) A white amorphous powder, $[\alpha]_D^{25}$ +5.2° (c = 0.60, pyridine : H_2O = 1 : 1). HR positive ion FAB MS *m/z*: 981.4700 (Calcd for $C_{47}H_{74}NaO_{20}$: 981.4671). Positive FAB MS *m/z*: 981 ($[M+Na]^+$), 805 ($[M+Na-glc\ A]^+$), 673 ($[M+Na-glc\ A-ara]^+$), 629 ($[M+Na-glc\ A-glc\ A]^+$). MS/MS of *m/z* 981 ($[M+Na]^+$), *m/z*: 849 ($[M+Na-ara]^+$), 805 ($[M+Na-glc\ A]^+$), 673 ($[M+Na-glc\ A-ara]^+$), 629 $[M+Na-glc\ A-glc\ A]^+$, 405 ([D/E ring + ara + Na]$^+$). ^{1}H-NMR (in pyridine-d_5): 0.76, 0.91, 1.23, 1.28, 1.35, 1.39, 1.43 (each 3H, s, *tert*-Me × 7), 4.87 (1H, d, J = 8 Hz, glc A H-1), 5.02 (1H, d, J = 7 Hz, glc A H-1), 5.33 (1H, s, H-12). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Component Sugars of 5 A small amount of **5** was treated in the manner described above. The aglycone was identified as soyasapogenol A (**5a**). *Rf*s: 0.40 ($CHCl_3$: MeOH = 19 : 1), 0.42 (hexane : acetone = 2 : 1). The component sugars were identified as D-glc A and L-ara. *Rf*s in TLC analysis ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1): 0.07 (glc A), 0.44 (ara). The t_Rs of the peaks in GC analysis were 7.5 min (L-ara) and 13.2 min (D-glc).

Kudzusaponin SB_1 (6) A white amorphous powder, $[\alpha]_D^{25}$ −8.5° (c = 0.73, pyridine : H_2O = 1 : 1). HR negative ion FAB MS *m/z*: 1073.5531 (Calcd for $C_{53}H_{86}O_{22}$: 1073.5532). Negative ion FAB MS *m/z*: 1073 ($[M-H]^-$), 941 ($[M-H-ara]^-$), 927 ($[M-H-rha]^-$), 765 ($[M-H-rha-gal]^-$), 589 ($[M-H-rha-gal-glc\ A]^-$). Positive ion FAB MS *m/z*: 1097 ($[M+Na]^+$), 951 ($[M+Na-rha]^+$), 613 ($[M+Na-rha-gal-glc\ A]^+$). MS/MS of *m/z* 1073 ($[M-H]^-$), *m/z*: 927 ($[M-H-rha]^-$), 795 ($[M-H-rha-ara]^-$), 765 ($[M-H-rha-gal]^-$), 589 ($[M-H-rha-gal-glc\ A]^-$); MS/MS of *m/z* ($[M+Na]^+$), *m/z*: 965 ($[M+Na-ara]^+$), 951 ($[M+Na-rha]^+$), 789 ($[M+Na-rha-gal]^+$), 613 ($[M+Na-rha-gal-glc\ A]^+$), 389 ([D/E ring + Na + ara]$^+$). ^{1}H-NMR (in pyridine-d_5): 0.73, 0.84, 1.05, 1.11, 1.18, 1.18, 1.43 (each 3H, s, *tert*-Me × 7), 1.80 (3H, d, J = 6 Hz, rha H-6), 4.76 (1H, d, J = 5 Hz, ara H-1), 4.88 (1H, d, J = 8 Hz, glc A H-1), 5.17 (1H, s, H-12), 5.75 (1H, d, J = 8 Hz, gal H-1), 5.66 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Component Sugars of 6 A small amount of **6** was treated in the manner described above. The aglycone was identified as soyasapogenol B (**6a**). *Rf*s: 0.43 ($CHCl_3$: MeOH = 19 : 1), 0.45 (hexane : acetone = 2 : 1). The component sugars were identified as D-glc A, D-gal, L-rha, and L-ara. *Rf*s in TLC analysis ($CHCl_3$: MeOH : H_2O = 6 : 4 : 1): 0.07 (glc A), 0.28 (gal), 0.58 (rha), and 0.44 (ara). The t_Rs of the peaks in GC analysis were 7.5 min (L-ara), 9.0 min (L-rha), 13.2 min (D-glc), and 14.0 min (D-gal).

Kudzusaponin A_3 (7) A white amorphous powder, $[\alpha]_D^{25}$ −3.4° (c = 0.68, pyridine : H_2O = 1 : 1). HR positive ion FAB MS *m/z*: 998.4990 (Calcd for $C_{48}H_{78}NaO_{20}$: 997.4984). Positive ion. FAB MS *m/z*: 997 ($[M+Na]^+$), (975 $[M+H]^+$), 829 ($[M+H-rha]^+$), 667 ($[M+H-rha-gal]^+$). ^{1}H-NMR (in pyridine-d_5): 0.69, 0.95, 1.30, 1.30, 1.34, 1.50 (each 3H, s, *tert*-Me × 6), 1.77 (3H, d, J = 6 Hz, rha H-6), 4.96 (1H, d, J = 6 Hz, glc A H-1), 5.33 (1H, s, H-12), 5.78 (1H, d, J = 7 Hz, gal H-1), 6.27 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Soyasaponin A_3 (8) A white amorphous powder, $[\alpha]_D^{25}$ +0.7° (c = 0.42, pyridine : H_2O = 1 : 1). ^{1}H-NMR (in pyridine-d_5): 0.69, 0.89, 1.23, 1.23, 1.26, 1.37, 1.41 (each 3H, s, *tert*-Me × 7), 1.77 (3H, d, J = 6 Hz), 5.27 (1H, s, H-12), 5.49 (1H, d, J = 7, gal H-1), 5.96 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Soyasaponin I (9) A white amorphous powder, $[\alpha]_D^{25}$ −13.2° (c = 0.28, pyridine : H_2O = 1 : 1). ^{1}H-NMR (in pyridine-d_5): 0.73, 0.95, 1.00, 1.22, 1.30, 1.30, 1.54 (each 3H, s, *tert*-Me × 7), 1.83 (3H, d, J = 6 Hz), 5.30 (1H, s, H-12), 5.98 (1H, d, J = 7, gal H-1), 6.39 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

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References and Notes

1) Part I: see 6) in these references; Part II: see 2) in these references; Part III: see 15) in these references; this report corresponds to part LII in a series of studies on leguminous plants.
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