Oleanene-Type Triterpene Glycosides from Puerariae Radix. II.[1] Isolation of Saponins and the Application of Tandem Mass Spectrometry to Their Structure Determination

Tomonori ARAO,[a] Junei KINJO,[a] Toshihiro NOHARA,*,[a] and Ryuichi ISOBE[b]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] 5–1 Oe-honmachi, Kumamoto 862, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,[b] 3–1–1 Maidashi, Higashi-ku, Fukuoka 812, Japan.
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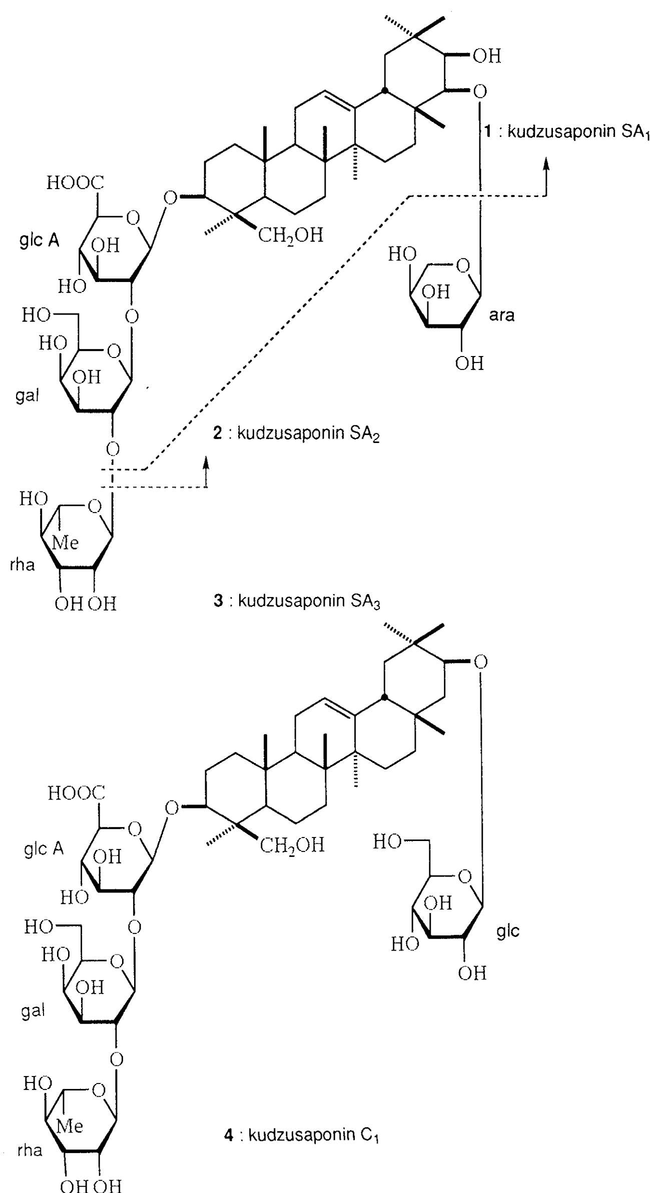
A continuing study of the ingredients of Puerariae Radix, the roots of *Pueraria lobata* (WILLD.) OHWI, which is one of the most important crude drugs, has resulted in the first isolation of four new oleanene-type triterpene glycosides, named kudzusaponins SA_1, SA_2, SA_3 and C_1 (1—4). Their structures were determined to be 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A (1), 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside (2), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside (3) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol C 21-*O*-β-D-glucopyranoside (4), respectively. The usefulness of tandem mass spectrometry in the structural determination of oleanene-type triterpene bisdesmosides is also discussed.

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. Studies on the chemical constituents of this plant have been carried out by many investigators, and various isoflavonoids have been found.[2] During the course of our study of the constituents of leguminous plants, we had found the existence of triterpenoidal saponins in this plant and elucidated the structures of their sapogenols along with many aromatic compounds.[3] As a continuing study on the ingredients of *P. lobata*, we have isolated four triterpenoidal saponins from the fresh roots of this plant. This paper deals with the structural elucidation of these saponins and the application of fast atom bombardment mass spectrometry (FAB-MS) in combination with tandem mass spectrometry (MS/MS) to this structural determination.

A methanolic extract of the fresh root of *P. lobata* was partitioned between 1-BuOH and water. Both layers were concentrated and subjected to polystyrene gel column chromatography. Each crude saponin fraction was subjected to normal and reversed phase column chromatography to yield compounds **1**—**4**.

Kudzusaponin SA_1 (**1**) showed a $[M-H]^-$ ion at *m/z* 811 in the negative ion FAB mass spectrum, which corresponds to the composition $C_{42}H_{67}O_{15}$ from the exact mass measurement under high resolution (HR) conditions. The sapogenol obtained by acid hydrolysis of **1** was identified as soyasapogenol A (**1a**)[3a,4] on TLC. The monosaccharide mixture revealed the presence of glucuronic acid and galactose. Their absolute configurations were determined to be the D-form according to a procedure developed by Hara *et al.*[5] In the ^{13}C-NMR spectrum of **1** (Tables 1 and 2), signals of the sugar part were identical with those of kaikasaponin I,[6b] and those of the aglycone part were in accordance with **1a** except for C-2 and -3 which were shifted downfield due to glycosylation.[7]



* To whom correspondence should be addressed.

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

	1a[3a]	**1**	**2**	**3**	**4a**[3a]	**4**
C-1	38.9	38.6	38.5	38.6	38.9	38.5
C-2	28.4	26.5	26.5	26.7	28.4	26.6
C-3	80.1	90.7	90.9	91.2	80.1	91.1
C-4	43.2	43.8	43.6	43.9	43.2	43.8
C-5	56.3	56.0	56.1	56.0	56.3	56.0
C-6	19.1	18.6	18.6	18.5	19.1	18.4
C-7	33.2	32.9	32.8	32.8	33.3	33.0
C-8	40.3	40.1	40.3	40.2	40.1	39.9
C-9	48.1	47.7	47.6	47.8	48.1	47.7
C-10	37.0	36.6	36.7	36.9	37.0	36.4
C-11	24.2	24.1	24.0	24.1	24.1	24.0
C-12	122.5	122.5	122.5	122.5	122.7	123.0
C-13	144.5	144.5	144.2	144.4	144.3	144.2
C-14	42.1	42.0	41.8	41.8	41.9	41.8
C-15	26.6	26.5	26.4	26.7	26.5	26.4
C-16	27.5	27.4	27.7	27.9	28.6	28.3
C-17	39.2	39.2	39.2	39.3	35.1	35.1
C-18	44.0	43.9	44.4	44.4	47.2	46.8
C-19	47.3	47.2	47.2	47.2	46.5	44.9
C-20	36.6	36.4	36.3	36.4	36.9	37.1
C-21	74.6	74.7	73.1	72.5	72.8	83.7
C-22	79.6	79.6	92.3	92.7	47.7	44.9
C-23	23.6	22.7	23.1	23.1	23.5	23.0
C-24	64.6	63.5	63.3	63.6	64.5	63.6
C-25	16.2	15.7	15.6	15.8	16.2	15.7
C-26	17.0	16.8	16.7	16.7	16.9	16.8
C-27	26.7	26.7	26.7	26.7	26.0	26.0
C-28	22.3	22.3	22.7	23.0	28.7	28.4
C-29	31.5	31.6	31.4	31.5	29.9	29.6
C-30	21.3	21.4	21.3	21.4	17.7	18.4

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

Table 2. ^{13}C-NMR Data for Compounds **1**—**4** (Sugar Moieties)

	1	**2**	**3**	**4**
Glc A				
1	105.4	104.4	105.5	105.4
2	80.9	80.9	78.5	78.7[a]
3	75.5[a]	75.4[a]	75.8[a]	76.4[b]
4	73.7	73.5	73.9	73.8
5	78.2[b]	78.0[b]	77.9	77.7
6	172.4	177.1	172.4	172.3
Gal				
1	105.0	104.3	102.5	102.4
2	73.0	73.5	77.7[a]	77.7[b]
3	77.8[b]	75.7[b]	76.5[a]	75.8[b]
4	71.1	69.9	71.2	71.1
5	77.3[a]	77.9[a]	76.7[a]	76.6[b]
6	62.6	61.6	61.6	61.6
Rha				
1			101.8	101.7
2			72.5[c]	72.4[c]
3			72.8[c]	72.8[c]
4			74.4	74.4
5			69.5	69.4
6			19.0	18.9
Ara				
1		108.4	108.8	
2		73.1	73.9	
3		74.9	75.4	
4		69.6	70.0	
5		67.4	67.7	
Glc				
1				106.6
2				75.8
3				78.1[a]
4				71.9
5				78.4[a]
6				63.0

a—c) In each vertical column, these may be interchanged.

Therefore, the structure of **1** was elucidated to be 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A.

Kudzusaponin SA$_2$ (**2**) showed a $[M-H]^-$ ion at *m/z* 943 in the negative ion FAB mass spectrum, $C_{47}H_{75}O_{19}$ ($[M-H]^-$) by HR/FAB-MS. The components obtained by acid hydrolysis of **3** were identified as **1a**, D-glucuronic acid, D-galactose and L-arabinose in the same manner as above. In the ^{13}C-NMR spectrum of **2**, the signals for the sugar moiety were superimposable on those of **1**, except for those of the additional α-L-arabinopyranosyl moiety.[8] On the other hand, the C-3 and -22 signals of **2** appeared at a much lower field than those of **1a**, due to glycosylation. Therefore, **2** was concluded to be 3-*O*-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside, *i.e.*, desglucosyl soyasaponin A$_2$.[8a]

Kudzusaponin SA$_3$ (**3**) showed a $[M-H]^-$ ion at *m/z* 1089 in the negative ion FAB mass spectrum. The HR/FAB-MS of **3** showed the composition $C_{53}H_{85}O_{23}$ ($[M-H]^-$), which is more than that of **2** by a deoxyhexosyl moiety. By acid hydrolysis, the sapogenol was identified with **1a** and the component sugars were identified to be D-glucuronic acid, D-galactose, L-rhamnose and L-arabinose. Comparative analysis of the ^{13}C-NMR spectra of **3** and **2** showed that signals ascribable to an aglycone moiety and an arabinopyranosyl group at C-21 were in good agreement with each other. Since the remaining signals were identical with the sugar signals for soyasaponin I,[9b] the structure of **3** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol A 22-*O*-α-L-arabinopyranoside.

Kudzusaponin C$_1$ (**4**) showed a $[M-H]^-$ ion at *m/z* 1103 in the negative ion FAB mass spectrum. The molecular formula was determined to be $C_{54}H_{88}O_{23}Na$ ($[M+Na]^+$) by HR/FAB-MS. The sapogenol and sugars obtained by acid hydrolysis of **4** were identified as kudzusapogenol C (**4a**),[3a] D-glucuronic acid, D-galactose, L-rhamnose and D-glucose. On comparative analysis of the ^{13}C-NMR spectra of **4** and **3**, the signals due to the sugar moiety at C-3 were superimposable upon each other. Furthermore, the remaining sugar signals were identical with a β-D-glucopyranosyl residue. Since the C-21 signal of **4** was observed in a lower field than that of **4a** due to glycosylation, the structure of 4 was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol C 21-*O*-β-D-glucopyranoside.

Meanwhile, the structural determination of bisdesmoside was performed by partial acid hydrolysis in the usual manner. Kitagawa *et al.* had developed chemical and physical degradation methods[10] by which the glucuronide linkage in oligoglycosides is selectively cleaved. Recently, Tanaka *et al.*[11a] and Okubo *et al.*[11b] have developed

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Table 3. Tandem Mass Spectral Data for **2**

	Precursor ions (m/z)		
	943 $[M-H]^-$	945 $[M+H]^+$	983 $[M+K]^+$
Daughter ions (m/z)			
[M−ara]	811 $[-H]^-$	813 $[+H]^+$	851 $[+K]^+$
[M−gal]	781 $[-H]^-$	783 $[+H]^+$	821 $[+K]^+$
[M−gal−glc A]	605 $[-H]^-$		645 $[+K]^+$
[D/E ring+ara]			421 $[+K]^+$

Table 4. Tandem Mass Spectral Data for **3**

	Precursor ions (m/z)		
	1089 $[M-H]^-$	1091 $[M+H]^+$	1113 $[M+Na]^+$
Daughter ions (m/z)			
[M−ara]	957 $[-H]^-$		
[M−rha]	943 $[-H]^-$	945 $[+H]^+$	967 $[+Na]^+$
[M−rha−gal]	781 $[-H]^-$		805 $[+Na]^+$
[M−rha−gal−ara]		783 $[+H]^+$	673 $[+Na]^+$
[M−rha−gal−glc A]	605 $[-H]^-$		629 $[+Na]^+$
[D/E ring+ara]			405 $[+Na]^+$

Table 5. Tandem Mass Spectral Data for **4**

	Precursor ions (m/z) 1127 $[M+Na]^+$
Daughter ions (m/z)	
[M−rha−gal−glc A]	643 $[+Na]^+$
[D/E ring+glc]	419 $[+Na]^+$

enzymatic methods. In our experiment, since the amounts of available compounds were limited, we tried a spectrometric method using MS/MS. The application of MS/MS to the structural determination of oleanene-type triterpene bisdesmoside, which has an ester glycosidic bond at C-28, was already characterized, and the selective cleavage of an ester glycosidic bond was observed.[12] In addition, ions due to the characteristic fragmentations originated *via* retro Diels–Alder fission[13] appeared. Therefore, it seemed that this technology was applicable for determining the structural information of non-ester type oleanene bisdesmosides (Me group at C-17) such as kudzusaponins $SA_{2,3}$ and C_1.

In the MS/MS of $[M-H]^-$, $[M+H]^+$ ions of **3**, the ions which originated from the cleavage of glycosidic bonds, were mostly observed (Table 3). In contrast, the MS/MS of an $[M+Na]^+$ ion generated various fragments of ions together with those given by glycosidic bond cleavage. Especially, the ion derived *via* retro Diels–Alder fission appeared at m/z 405. Furthermore, in the MS/MS of an $[M+K]^+$ ion of **2** and an $[M+Na]^+$ ion of **4** (Tables 4 and 5), similar ions were observed at m/z 421 and 419, respectively. Consequently, the MS/MS (positive ion mode with alkaline iodide) of oleanene-type triterpene bisdesmoside is assumed to be useful for its structural determination.

Experimental

The fresh roots of *Pueraria lobata* were 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, 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. ^1H- and ^{13}C-NMR spectra were measured with JEOL JNM-GX 270 and 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 $\mu g/\mu l$. The solution (0.5 μl) was mixed with glycerol (0.5 μl) and 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 the collision-activated dissociation (CAD)[14] spectra.

Extraction and Isolation Fresh roots (74.5 kg) of *P. lobata* were extracted with MeOH (100 l) once under reflux. The extract (4.2 kg) was partitioned with 1-BuOH and H_2O. Removal of the solvent from each phase under reduced pressure gave the aqueous (3.0 kg) and BuOH (1.2 kg) extracts. The aqueous extract (380 g) was subjected to MCI gel CHP 20P column chromatography using 0→100% MeOH to give fractions 1 to 6. Fraction 5 was further separated by Bondapak C_{18} (20→100% MeOH), Chromatorex ODS (30→100% MeOH) and silica gel (1-BuOH : AcOH : H_2O = 8 : 1 : 1) to provide compounds **2** (8 mg) and **3** (7 mg). Column chromatography of the 1-BuOH extract (849 g) over Diaion HP-20 (0→100% MeOH) furnished three fractions. Fraction 2 was further separated by MCI gel CHP 20P (0→100% MeOH), silica gel ($CHCl_3$: MeOH : 25%NH_4OH = 7 : 3 : 0.5→$CHCl_3$: MeOH : H_2O = 6 : 4 : 1), Bondapak C_{18} (0→100% MeOH), silica gel (1-BuOH : AcOH : H_2O = 8 : 1 : 1), and silica gel ($CHCl_3$: MeOH : H_2O = 8 : 2 : 0.2→6 : 4 : 1) to provide compounds **1** (6 mg) and **4** (7 mg).

Kudzusaponin SA_1 (1) A white amorphous powder, $[\alpha]_D^{25}$ +12.1° (c = 0.38, pyridine : H_2O = 1 : 1). HR negative ion FAB-MS m/z: 811.4481 ($C_{42}H_{67}O_{15}$, Calcd for 811.4480). Negative ion FAB-MS m/z: 811 $[M-H]^-$, 649$[M-H-gal]^-$, 473 $[M-H-gal-glc\ A]^-$. ^1H-NMR (in pyridine-d_5): 0.72, 0.94, 1.25, 1.27, 1.27, 1.36, 1.45 (each 3H, s, *tert*-Me × 7), 4.99 (1H, d, J = 7.3 Hz, glc A H-1), 5.57 (1H, d, J = 7.6 Hz, gal H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol and Sugars of 1 A small amount of **1** 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 to be soyasapogenol A (**1a**) by TLC. *Rf*s, 0.25 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 3 : 2). 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, *Rf*s: 0.12 (glucuronic acid), 0.35 (galactose)].

D, L Determination of Sugars of 1 A small amount of **1** 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 r.t. 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 hydrolysate was subjected to MCI gel CHP-20P and Amberlite IRA-400 to give a sugar fraction. This fraction 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 a 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 *n*-hexane and H_2O, the *n*-hexane layer was taken out and checked by GC. The retention times (t_R) of the peaks were 21.2 min (D-glucose) and 23.2 min (D-galactose).

Kudzusaponin SA_2 (2) A white amorphous powder, $[\alpha]_D^{25}$ +1.6° (c = 0.11, DMSO). HR negative ion FAB-MS m/z: 943.4903 (Calcd for $C_{47}H_{75}O_{19}$: 943.4902). Negative ion FAB-MS m/z: 943 $[M-H]^-$, 781 $[M-H-gal]^-$, 605 $[M-H-gal-glc\ A]^-$. Positive ion FAB-MS m/z: 983 $[M+K]^+$, 967 $[M+Na]^+$, 945 $[M+H]^+$. MS/MS: Table 3.

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^{1}H-NMR (in pyridine-d_5+D_2O): 0.67, 0.90, 1.24, 1.26, 1.33, 1.35, 1.38 (each 3H, s, *tert*-Me×7), 4.88 (1H, d, J=7.3 Hz, glc A H-1), 5.28 (1H, s, H-12), 5.52 (1H, d, J=7.3 Hz, gal H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol and Sugars of 2 A small amount of **2** was hydrolyzed in the same manner as described above. The aglycone was extracted with $CHCl_3$ and identified to be soyasapogenol A (**1a**) by TLC. *Rf*s, 0.25 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 3 : 2). 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, *Rf*s: 0.12 (glucuronic acid), 0.35 (galactose), 0.47 (arabinose)].

D, L Determination of Sugars of 2 A small amount of **2** was treated in the same manner as described above. The derivatives were analyzed by GC. The t_Rs of the peaks were 12.1 min (L-arabinose), 21.2 min (D-glucose) and 22.8 min (D-galactose).

Kudzusaponin SA$_3$ (3) A white amorphous powder, $[\alpha]_D^{25}$ −2.8° (c=0.74, DMSO). HR negative ion FAB-MS *m/z*: 1089.5479 (Calcd for $C_{53}H_{85}O_{23}$: 1089.5481). Negative ion FAB-MS *m/z*: 1089 $[M-H]^-$, 943 $[M-H-rha]^-$, 781 $[M-H-rha-gal]^-$, 605 $[M-H-rha-gal-glc\ A]^-$. Positive ion FAB-MS *m/z*: 1113 $[M+Na]^+$, 1091 $[M+H^+]$. MS/MS: Table 4. ^{1}H-NMR (in pyridine-d_5+D_2O): 0.66, 0.89, 1.26, 1.31, 1.39, 1.43, 1.43 (each 3H, s, *tert*-Me×6), 1.80 (3H, d, J=6.2 Hz, rha H-6), 4.91 (1H, d, J=7.3 Hz, glc A H-1), 4.99 (1H, d, J=6.6 Hz, ara H-1), 5.32 (1H, s, H-12), 6.35 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of Sapogenol and Sugars of 3 A small amount of **3** was hydrolyzed in the same manner as described above. The aglycone was extracted with $CHCl_3$ and identified to be soyasapogenol A (**1a**) by TLC. *Rf*s, 0.25 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 3 : 2). 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, *Rf*s: 0.12 (glucuronic acid), 0.35 (galactose), 0.58 (rhamnose), 0.47 (arabinose)].

D, L Determination of Sugars of 3 A small amount of **3** was treated in the same manner as described above. The derivatives were analyzed by GC. The t_Rs of the peaks were 12.1 min (D-arabinose), 14.5 min (L-rhamnose), 21.2 min (D-glucose) and 22.8 min (D-galactose).

Kudzusaponin C$_1$ (4) A white amorphous powder, $[\alpha]_D^{25}$ −8.0° (c=0.73, pyridine : H_2O = 1 : 1). HR positive ion FAB-MS *m/z*: 1127.5614 (Calcd for $C_{54}H_{88}NaO_{23}$: 1127.5614). Negative ion FAB-MS *m/z*: 1103 $[M-H]^-$, 957 $[M-H-rha]^-$, 941 $[M-H-glc]^-$, 795 $[M-H-rha-gal\ or\ glc]^-$, 633 $[M-H-rha-gal-glc]^-$, 619 $[M-H-rha-gal-glc\ A]^-$. Positive ion FAB-MS *m/z*: 1127 $[M+Na]^+$, 981 $[M+Na-rha]^+$, 965 $[M+Na-glc]^+$, 819 $[M+Na-rha-gal\ or\ glc]^+$, 657 $[M+Na-rha-gal-glc]^+$, 643 $[M+Na-rha-gal-glcA]^+$. MS/MS: Table 5. ^{1}H-NMR (in pyridine-d_5+D_2O): 0.68, 0.83, 0.85, 1.12, 1.24, 1.36, 1.43 (each 3H, s, *tert*-Me×7), 1.78 (3H, d, J=6.2 Hz, rha-6), 4.88 (1H, d, J=7.8 Hz, glc A H-1), 5.20 (1H, s, H-12), 5.79 (1H, d, J=7.4 Hz, gal H-1), 6.28 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol and Sugars of 4 A small amount of **4** was hydrolyzed in the same manner as described above. The aglycone was extracted with $CHCl_3$ and identified to be kudzusapogenol C (**4a**) by TLC. *Rf*s, 0.25 ($CHCl_3$: MeOH = 19 : 1), 0.44 (*n*-hexane : acetone = 3 : 2). 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, *Rf*s: 0.12 (glucuronic acid), 0.35 (galactose), 0.58 (rhamnose), 0.40 (glucose).

D, L Determination of Sugars of 4 A small amount of **4** was treated in the same manner as described above. The derivatives were analyzed by GC. The t_Rs of the peaks were 14.5 min (L-rhamnose), 21.2 min (D-glucose) and 22.8 min (D-galactose).

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References and Notes

1) Part I: see 3*a*) in this references; this report corresponds to part XLIV in a series of studies on leguminous plants.
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