Oleanene-Type Triterpene Glycosides from Puerariae Radix. III.[1] Three New Saponins from *Pueraria thomsonii*

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From the root of *Pueraria thomsonii* (Leguminosae), three new oleanane-type triterpene glycosides, named kudzusaponin B_1, acetyl-kaikasaponin III and acetyl-soyasaponin I were isolated, together with soyasaponin I (4) and subproside V (5). Their structures were determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol B (1), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl sophoradiol 22-*O*-acetate (2) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-acetate (3), respectively.

Key words *Pueraria thomsonii*; Leguminosae; triterpene; saponin; kudzusaponin

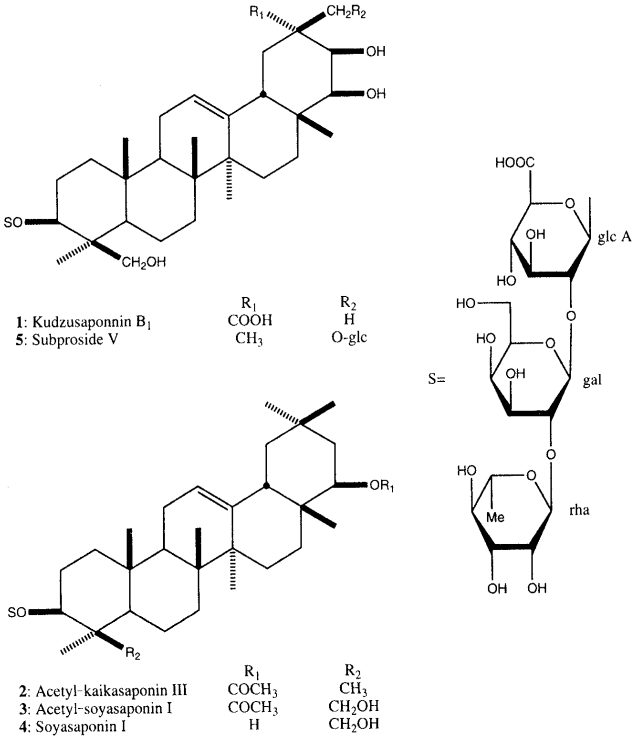
Puerariae Radix, the root of *Pueraria lobata* OHWI or *Pueraria thomsonii* BENTHUM, is one of the most important Oriental crude drugs used as a perspiration, antipyretic and antispasmodic agent.[2] During the course of our studies on the constituents of these plants,[3] we had reported four new oleanene-type triterpene saponins from *P. lobata*.[4] As a continuing study on the ingredients of Puerariae Radix, we have examined the triterpenoidal constituents in the root of *P. thomsonii*. This paper deals with the isolation and structural elucidation of five triterpenoidal saponins.

The methanolic extract of the root of *P. thomsonii* was partitioned between 1-BuOH and water. The 1-BuOH layer was concentrated and subjected to normal- and reversed-phase column chromatography to yield compounds **1**—**5**. Compounds **4** and **5** were identified as soyasaponin I[5] and subproside V,[6] respectively, by comparison of the ^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2).

Kudzusaponin B_1 (**1**) showed an $[M+Na]^+$ ion at *m/z* 1011 in the positive ion FAB mass spectrum, which corresponds to the composition $C_{48}H_{76}NaO_{21}$ ($[M+Na]^+$) given by the exact mass measurement under high resolution (HR). The occurrence of six tertiary methyl signals in the ^{1}H-NMR spectrum suggested **1** to be an oleanane derivative. The sapogenol obtained by acid hydrolysis of **1** was identified as kudzusapogenol B (**1a**)[3a] on TLC. A monosaccharide mixture revealed the presence of glucuronic acid, galactose 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.*[7] The ^{13}C-NMR signals of the sugar part of **1** were identical with those of the β-fabatriosyl moiety[8] of **4** (Table 2), while the signals due to the aglycone part (Table 1) were in good accordance with those of **1a** except for C-2 and -3, which were shifted downfield due to glycosylation.[9] Therefore, the structure of **1** was elucidated to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol B. This is the first isolation of a saponin having kudzusapogenol B as an aglycone.

Acetyl-kaikasaponin III (**2**) showed an $[M+Na]^+$ ion ($C_{50}H_{80}NaO_{18}$) at *m/z* 991 in the positive ion FAB mass spectrum. The acid hydrolysate of **2** gave the same component sugars as those of **1**. A genuine sapogenol (**2a**) was obtained by enzymatic hydrolysis. In the ^{1}H-NMR of **2a**, an acetyl signal was observed at δ 2.03. Furthermore, the signal due to H-22 of **2a** showed a downfield shift (+1.21 ppm) in comparison with that of sophoradiol (**2b**).[10] Therefore, **2a** was concluded to be sophoradiol 22-*O*-acetate.[11] Since the ^{13}C-NMR signals of the sugar moiety of **2** were superimposable on those of kaikasaponin III (**2c**),[12b] **2** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl sophoradiol 22-*O*-acetate.

Acetyl-soyasaponin I (**3**) showed an $[M+Na]^+$ ion ($C_{50}H_{80}NaO_{19}$) at *m/z* 1007 in the positive ion FAB mass spectrum. On acid hydrolysis, **3** gave the same component sugars as those of **1** and **2**. The aglycone obtained by acid



	R_1	R_2
1: Kudzusaponnin B_1 / **5**: Subproside V	COOH / CH_3	H	O-glc
2: Acetyl-kaikasaponin III	$COCH_3$	CH_3
3: Acetyl-soyasaponin I	$COCH_3$	CH_2OH
4: Soyasaponin I	H	CH_2OH

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Table 1. ^{13}C-NMR Data for Compounds **1**—**5**, **1a**, **2a**, and **2c** (Aglycone Moieties)

C	1	1a	2	2a	2c	3	4	5
1	38.5	38.9	38.8	39.1	38.8	38.5	38.5	38.8
2	26.5	28.4	26.3	28.1	26.5	26.2	26.5	26.3
3	91.1	80.1	89.8	78.1	89.9	91.1	91.2	91.4
4	43.8	43.2	39.7	39.4	39.6	43.6	43.9	43.9
5	55.9	56.3	55.8	55.7	55.8	56.1	56.1	56.2
6	18.5	19.1	18.6	18.7	18.5	18.5	18.5	18.6
7	32.8	33.1	33.3	32.1	33.2	32.9	33.3	33.3
8	40.1	40.3	39.7	40.1	39.9	40.0	39.9	40.0
9	47.6	48.0	47.8	47.9	47.9	47.7	47.8	47.8
10	36.4	37.0	36.5	37.2	36.8	36.6	36.5	36.4
11	24.1	24.2	23.8	23.8	23.7	24.0	24.0	24.0
12	123.1	123.8	123.4	122.9	122.5	122.8	122.4	123.2
13	143.9	143.6	144.2	144.1	144.7	144.1	144.9	144.1
14	42.0	42.0	41.9	41.8	42.3	41.9	42.4	42.3
15	26.5	26.5	26.5	26.2	26.4	26.6	26.5	26.3
16	27.4	27.4	29.9	28.7	28.6	30.0	28.5	28.5
17	39.0	38.9	37.4	36.6	37.9	36.4	38.0	37.9
18	42.8	42.7	44.7	44.8	45.3	44.7	45.2	44.8
19	42.5	42.4	46.1	46.2	46.7	46.1	46.8	42.1
20	49.4	44.9	30.5	30.5	30.8	30.1	30.9	35.0
21	70.8	70.5	38.5	38.5	42.2	38.5	42.4	37.0
22	79.2	79.1	77.7	78.3	75.5	77.7	75.6	75.5
23	22.9	23.5	28.4	28.7	28.6	23.0	22.7	22.9
24	63.6	64.5	15.6	15.8	15.6	63.6	63.6	63.4
25	15.7	16.2	16.8	16.6	16.7	15.8	15.7	15.9
26	16.8	16.9	16.9	17.0	17.1	16.8	17.0	17.1
27	26.5	26.5	26.2	26.3	25.7	26.3	25.7	25.9
28	22.2	22.1	27.1	27.2	28.3	27.1	28.7	21.0
29	181.0	178.7	33.6	33.6	33.2	33.6	33.3	28.7
30	17.0	16.5	21.2	21.2	21.1	21.2	21.2	77.5
C=O			170.0	170.1		170.1		
Me			21.2	21.0		21.2		

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

Table 2. ^{13}C-NMR Data for Compounds **1**—**5** and **2c** (Sugar Moieties)

	1	2	2c	3	4	5
glc A-1	105.4	105.3	105.2	105.5	105.5	104.9
glc A-2	78.5	79.1	79.0	78.4	78.5	77.9
glc A-3	76.6[a]	76.2[a]	76.1[a]	76.6[a]	76.7[a]	76.8[a]
glc A-4	73.9	73.5	73.4	73.9	73.9	73.6
glc A-5	77.8[b]	77.4	77.2	77.7[b]	77.7[b]	77.2[b]
glc A-6	172.4	171.6	172.6	172.3	172.4	175.6
gal-1	101.7	102.0	101.9	101.7	101.7	101.7
gal-2	77.9[b]	78.8	78.5	77.7[b]	77.7[b]	77.8[b]
gal-3	76.5[a]	76.2[a]	76.0[a]	76.4[a]	76.5[a]	75.8
gal-4	71.1	70.5	70.3	71.1	71.1	70.9
gal-5	77.7[b]	76.6[a]	76.5[a]	76.6[a]	76.7[a]	76.4[a]
gal-6	61.5	61.9	61.8	61.6	61.6	61.8
rha-1	102.5	102.7	102.6	102.4	102.5	101.9
rha-2	72.4	72.4	72.2	72.4	72.4	71.9
rha-3	72.8	72.7	72.5	72.8	72.8	71.9
rha-4	74.4	74.3	74.2	74.4	74.4	73.8
rha-5	69.4	69.4	69.3	69.4	69.4	69.3
rha-6	19.0	18.9	18.5	19.0	19.0	18.6
glc-1						104.9
glc-2						74.9
glc-3						77.8[b]
glc-4						71.2
glc-5						77.8[b]
glc-6						62.3

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

hydrolysis of **3** was identified as soyasapogenol B 22-*O*-acetate on TLC. The ^{13}C-NMR signals of the sugar moiety of **3** and rings A—D of the aglycone moiety were superimposable on those of **4**, except for the ring E signals, which were in accordance with those of **2**. Therefore, **3** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-acetate.

Experimental

The root of *Pueraria thomsonii* was collected in the Hua-Nan region of China. TLC was performed on pre-coated Kieselgel 60F_{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 on a JASCO DIP-360 automatic digital polarimeter. ^{1}H- and ^{13}C-NMR spectra were measured on a JEOL JNM-EX 270 spectrometer and chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard. FAB-MS were measured on a JEOL DX-300 HF spectrometer. HR FAB-MS were measured on a JEOL SX102A mass spectrometer (JEOL), which was controlled by a JEOL DA-7000 data system.

Extraction and Isolation The root (20 kg) of *P. thomsonii* was extracted with MeOH (60 l) once under reflux. The extract (430 g) was partitioned between 1-BuOH and H_2O. Removal of the solvent from each phase under reduced pressure gave the aqueous (267 g) and 1-BuOH (121 g) extracts. The 1-BuOH extract was subjected to MCI gel CHP 20P column chromatography using 0%→100% MeOH to give fractions 1 to 4. Fractions 2 (9 g, 60% MeOH eluate) and 3 (15 g, 80% MeOH eluate) were further separated by Bondapak C_{18} (20%→100% MeOH), Chromatorex ODS (30%→100% MeOH), silica gel (1-BuOH : AcOH : H_2O = 8 : 1 : 1) and silica gel ($CHCl_3$: MeOH : H_2O + 0.1% AcOH = 7 : 3 : 0.5 + 0.1% AcOH—6 : 4 : 1 + 0.1% AcOH) to provide compounds **1** (7 mg), **2** (13 mg), **3** (4 mg), **4** (14 mg), and **5** (7 mg).

Kudzusaponin B_1 (1) A white amorphous powder, $[\alpha]_D^{25}$ −8.7° (c = 0.66, pyridine : H_2O = 1 : 1). HR positive ion FAB MS *m/z*: 1011.4824 (Calcd for $C_{48}H_{76}NaO_{21}$: 1011.4777). Positive ion FAB MS *m/z*: 1011 ($[M+Na]^+$), 989 ($[M+H]^+$), 865 ($[M+Na-rha]^+$), 843 ($[M+H-rha]^+$), 681 ($[M+H-rha-gal]^+$). Negative ion FAB MS *m/z*: 987 ($[M-H]^-$), 841 ($[M-H-rha]^-$), 679 ($[M-H-rha-gal]^-$), 503 ($[M-H-rha-gal-glc\ A]^-$). ^{1}H-NMR (in pyridine-d_5): 0.71, 0.96, 1.29, 1.29, 1.41, 1.96 (each 3H, s, *tert*-Me × 6), 1.72 (3H, d, J = 6 Hz, rha H-6), 5.33 (1H, br s, H-12), 5.68 (1H, d, J = 8 Hz, gal H-1), 6.17 (1H, br s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol of 1 A sample of **1** (1 mg) was hydrolyzed with 2 N HCl/MeOH (1 ml) and heated at 60 °C for 2 h. The MeOH was evaporated under a N_2 stream. The aglycone was extracted with $CHCl_3$ and identified as kudzusapogenol B methyl ester by TLC. *Rf*s, 0.35 ($CHCl_3$: MeOH = 19 : 1), 0.16 (*n*-hexane : acetone = 3 : 1).

Identification of the Sugars of 1—3 A sample of **1** (1 mg) was hydrolyzed with 2 N HCl/H_2O (1 ml) and heated at 80 °C for 2 h. After the partition between H_2O and $CHCl_3$, the aqueous layer was neutralized with 2 N KOH/H_2O. The sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60F_{254} (Merck Art 5554), $CHCl_3$: MeOH : H_2O = 6 : 4 : 1, *Rf*s: 0.09 (glucuronic acid), 0.28 (galactose), 0.56 (rhamnose). In the above manner, the sugars for **2** and **3** were also confirmed to be composed of the same units.

Determination of the Absolute Configuration of the Component Sugars of 1—3 A sample of **1** (1 mg) was methylated with ethereal CH_2N_2. To a solution of the methylated sample 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 partition between H_2O and $CHCl_3$. The H_2O layer was treated with 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 (0.1 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 removed and checked by GC. The retention times (t_R) of the peaks were 10.7 min (L-rhamnose), 15.6 min (D-glucose) and 16.6 min (D-galactose). In the above manner, the absolute configurations of component sugars for **2** and **3** were also the same form as those of **1**.

Acetyl-kaikasaponin III (2) A white amorphous powder, $[\alpha]_D^{25}$ +3.6° (c=0.37, pyridine). HR positive ion FAB MS m/z: 991.5225 (Calcd for $C_{50}H_{80}NaO_{18}$: 991.5242). Positive ion FAB MS m/z: 991 ($[M+Na]^+$). Negative ion FAB MS m/z: 967 ($[M-H]^-$), 821 ($[M-H-rha]^-$), 659 ($[M-H-rha-gal]^-$). ^{1}H-NMR (in pyridine-d_5): 0.85, 0.92, 0.94, 1.07, 1.19, 1.23, 1.42, 1.57, (each 3H, s, *tert*-Me×8), 1.77 (3H, d, J=6 Hz, rha H-6), 2.08 (3H, s, acetyl), 5.05 (1H, d, J=7 Hz, glc A H-1), 5.74 (1H, d, J=7 Hz, gal H-1), 6.34 (1H, s, rha-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol of 2 To a solution of **2** (3 mg) in acetate buffer (1 ml) was added glycyrrhizin hydrolase (100 μl), and the mixture was incubated at 40 °C for 2 d. The hydrolysate was filtered off to yield **2a** (0.5 mg). ^{1}H-NMR (in $CDCl_3$): 0.80, 0.83, 0.90, 0.95, 0.99, 1.01, 1.16, 1.26 (each 3H, s, *tert*-Me×8), 2.03 (3H, s, acetyl), 3.23 (1H, dd, J=5, 11 Hz, H-3), 4.65 (1H, m, H-22), 5.27 (1H, m, H-12).

Acetyl-soyasaponin I (3) A white amorphous powder, $[\alpha]_D^{25}$ −9.7° (c=0.95, pyridine). HR positive ion FAB MS m/z: 1007.5226 (Calcd for $C_{50}H_{80}O_{19}$: 1007.5192). Positive ion FAB MS m/z: 1007 ($[M+Na]^+$), 986 ($[M+H]^+$). Negative ion FAB MS m/z: 983 ($[M-H]^-$), 675 ($[M-H-rha-gal]^-$). ^{1}H-NMR (in pyridine-d_5): 0.70, 0.93, 1.07, 1.25, 1.25, 1.25, 1.43 (each 3H, s, *tert*-Me×7), 1.78 (3H, d, J=6 Hz, rha H-6), 2.08 (3H, s, acetyl), 5.27 (1H, s, H-12), 6.28 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Identification of the Sapogenol of 3 A sample of **3** (1 mg) was hydrolyzed with 2 N HCl/MeOH (1 ml) and heated at 60 °C for 2 h. The MeOH was evaporated under a N_2 stream. The aglycone was extracted with $CHCl_3$ and identified to be soyasapogenol B 22-*O*-acetate (**3a**) by TLC. *Rf*s, 0.44 ($CHCl_3$: MeOH = 19 : 1), 0.42 (*n*-hexane : acetone = 3 : 1).

The Chemical Synthesis of 3a To a tetrahydrofuran solution of soyasapogenol B (200 mg/3 ml) was added α,α-dimethoxytoluene (140 μl) and *p*-toluenesulfonic acid monohydrate (20 mg), and the mixture was kept at r.t. for 30 h. The reaction mixture was worked up with silica gel (*n*-hexane : AcOEt = 5 : 1) to obtain 3,24-*O*-benzylidene soyasapogenol B (166 mg). To a solution of 3,24-*O*-benzylidene soyasapogenol B (145 mg) in pyridine (2 ml) was added acetic anhydride (100 μl) and 4-dimethylaminopyridine (0.5 mg), and the mixture was kept at 50 °C for 2 h. The reaction mixture was partitioned between H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated to yield 3,24-*O*-benzylidene 22-*O*-acetyl soyasapogenol B. To a solution of 3,24-*O*-benzylidene 22-*O*-acetyl soyasapogenol B (5 mg) in acetic acid (400 μl) was added H_2O (100 μl), and the mixture was kept at 80 °C for 2 h. The reaction mixture was worked up with silica gel (*n*-hexane : AcOEt = 3 : 2) to obtain 22-*O*-acetyl soyasapogenol B (2 mg). ^{1}H-NMR (in pyridine-d_5): 0.92, 0.96, 0.96, 0.98, 1.09, 1.22, 1.57 (each 3H, s, *tert*-Me×7), 2.08 (3H, s, acetyl), 3.65 (1H, dd, J=5, 11 Hz, H-3), 3.72 (1H, d, J=11 Hz, H-24ax), 4.53 (1H, d, J=11 Hz, H-24eq), 4.88 (1H, m, H-22), 5.27 (1H, m, H-12). ^{13}C-NMR (in pyridine-d_5): 16.2 (C-25), 16.9 (C-26), 19.1 (C-6), 21.0 (C-30), 21.2 ($C\underline{H}_3CO$), 23.6 (C-23), 24.1 (C-11), 26.3 (C-2, 27), 27.2 (C-15, 28), 28.4 (C-16), 30.6 (C-20), 33.2 (C-7), 33.6 (C-29), 36.6 (C-17), 37.0 (C-10), 38.5 (C-21), 38.9 (C-1), 40.2 (C-8), 41.9 (C-14), 43.2 (C-4), 44.8 (C-18), 46.2 (C-19), 48.0 (C-9), 56.3 (C-5), 64.6 (C-24), 78.4 (C-22), 80.1 (C-3), 122.9 (C-12), 144.1 (C-13), 170.1 ($CH_3\underline{C}O$).

Soyasaponin I (4) A white amorphous powder, $[\alpha]_D^{25}$ −7.9° (c=1.46, pyridine). Negative ion FAB MS m/z: 941 ($[M-H]^-$), 796 ($[M-H-rha]^-$), 633 ($[M-H-rha-gal]^-$), 458 ($[M-H-rha-gal-glc A]^-$). ^{1}H-NMR (in pyridine-d_5 + D_2O): 0.71, 0.96, 1.00, 1.22, 1.29, 1.29, 1.44 (each 3H, s, *tert*-Me×7), 1.79 (3H, d, J=6 Hz, rha-6), 4.99 (1H, d, J=7 Hz, glc A H-1), 5.30 (1H, s, H-12), 5.80 (1H, d, J=8 Hz, gal H-1), 6.28 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

Subproside V (5) A white amorphous powder, $[\alpha]_D^{25}$ −6.6° (c=0.32, pyridine : H_2O = 1 : 1). Negative ion FAB MS m/z: 1119 ($[M-H]^-$), 973 ($[M-H-rha]^-$). ^{1}H-NMR (in pyridine-d_5 + D_2O): 0.68, 0.86, 0.86, 1.16, 1.20, 1.42 (each 3H, s, *tert*-Me×6), 1.79 (3H, d, J=6 Hz, rha-6), 5.33 (1H, s, H-12), 5.49 (1H, d, J=8 Hz, gal H-1), 5.97 (1H, s, rha H-1). ^{13}C-NMR: Tables 1 and 2.

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References and Notes

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