Triterpenoid Glycosides from Sophorae Subprostratae Radix[1)]

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Three new oleanene glycosides named subprosides I—III (1—3) were isolated as the corresponding methly ester forms together with four known glucuronide saponins, soyasaponin II (4), dehydrosoyasaponin I (5), kudzusaponin A_3 (6) and abrisaponin I (7), from Sophorae Subprostratae Radix, the roots of *Sophora subprostrata* CHUX *et* T. CHEN (Leguminosae). The structures of subprosides I—III were characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl (1→2)-β-D-glucuronopyranosyl-abrisapogenol C (1), 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl (1→2)-β-D-glucuronopyranosyl-kudzusapogenol A (2) and 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl-wistariasapogenol A 30-*O*-β-D-glucopyranoside (3) by the chemical and spectral evidence.

Keywords Sophorae Subprostratae Radix; *Sophora subprostrata*; Leguminosae; kudzusapogenol A; abrisapogenol C; wistariasapogenol A; oleanene glycoside; subproside; glucuronide

Sophorae Subprostratae Radix is the dried root of *Sophora subprostrata* CHUX *et* T. CHEN (Leguminosae) found in Southern China and has been used as a traditional Chinese medicine for lowering fever, reducing swelling and soothing sore throat.[2)] Anti-tumor activity was also reported.[2)] As its chemical constituents, several alkaloids, matrine, oxymatrine, anagyrine and methylcytisine, and some flavonoids; sophoranone, sophoradin, sophoranochromene and sophoradochromene, have been isolated.[2)] Sakamoto *et al.* recently reported the isolation and structure determination of several triterpenoid glycosides.[3)] In a preceding paper,[4)] we also reported the occurrence of sixteen kinds of oleanene sapogenols including four new ones in the methanolysate of the methanolic extract of this plant. This paper deals with the structure elucidation of three new oleanene glycosides called subprosides I—III (**1**—**3**).

The methanol extract of the root of Sophorae Subprostratae Radix was separated using various column chromatographies of Diaion HP-20, Sephadex LH-20 and Bondapak C_{18}, and silica gel after methylation with diazomethane during the separation procedure to provide glycosides **1a**—**7a**. Glycosides **4**—**7** were identified as soyasaponin II,[5)] dehydrosoyasaponin I,[6)] kudzusaponin A_3,[7)] and abrisaponin I,[8)] respectively, by comparison of their physical and spectral data with those of the reported values.

Subproside I methyl ester (**1a**), a white powder, $[\alpha]_D$ −3.3° (MeOH), on methanolysis, furnished a sapogenol identical with abrisapogenol C (**1b**)[8)] when compared with an authentic sample on thin layer chromatography (TLC). The high resolution fast atom bombardment mass spectrum (HR-FAB-MS) of **1a** showed a quasi-molecular ion at *m/z* 995.5211 $[M+Na]^+$($C_{49}H_{80}O_{19}Na$). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1a** disclosed three anomeric proton signals at δ 5.01 (1H, d, J=7.7 Hz), 5.71 (1H, d, J=7.7 Hz) and 6.39 (1H, br s) together with seven methyl signals. The negative FAB-MS gave peaks due to $[M-H]^-$ at *m/z* 971, [M − H − methylpentose]$^-$ at *m/z* 825 and [M − H − methylpentose − hexose − methoxycarbonylpentose]$^-$ at *m/z* 473. In addition, the electron impacting mass spectrum (EI-MS) of its acetate exhibited fragment ion peaks at *m/z* 561 [(methylpentosyl-hexosyl-)Ac_6]$^+$ and 273 [(terminal methylpentosyl-)Ac_3]$^+$, suggesting that **1a** possessed a methylpentosyl-hexosyl moiety as terminal carbohydrate residue. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table I) of **1a** showed thirty signals due to the sapogenol part which were almost identical with those of **1b**[8)] except for that of C-3, where downfield shift due to glycosylation[9)] (+11.9 ppm) was observed, indicating that **1a** is a 3-*O*-monodesmoside. Moreover, **1a** exhibited nineteen signals due to the sugar moiety, including three anomeric carbons at δ 101.8, 102.4 and 105.1 and one ester carbonyl carbon

TABLE I. ^{13}C-NMR Chemical Shifts of Sapogenol Moieties of Subprosides **1a**, **2a** and **3a**, Prosapogenin **3c** and Sapogenols **1b**, **2b** and **3b** (Pyridine-d_5)

	1a	1b	2a	2b	3a	3b	3c
C-1	38.6	39.0	38.5	38.9	38.3	38.8	38.4
2	26.2	28.0	26.4	28.4	26.4	28.4	26.6
3	89.8	77.9	90.9	80.1	91.1	80.0	91.2
4	38.8	39.3	43.8	43.2	43.6	43.2	43.8
5	55.5	55.6	55.8	56.3	55.8	56.3	56.0
6	18.3	18.7	18.4	19.1	18.2	19.0	18.4
7	32.6	32.8	32.7	33.2	32.8	33.3	33.0
8	40.0	40.2	40.0	40.3	39.5	39.8	39.7
9	47.6	47.9	47.6	48.1	47.4	47.9	47.6
10	36.6	37.1	36.3	37.0	36.2	37.0	36.4
11	23.7	23.8	23.9	24.1	23.7	24.0	23.9
12	122.3	122.5	122.3	122.5	123.8	123.9	123.8
13	144.5	144.6	144.5	144.6	141.6	142.1	142.2
14	41.8	42.0	41.8	42.0	41.7	42.0	42.8
15	26.4	26.5	26.5	26.6	25.0	25.5	25.4
16	27.1	27.3	27.2	27.4	26.8	27.3	27.3
17	39.4	39.3	38.9	39.0	47.5	47.7	47.7
18	43.0	43.2	43.1	43.2	46.5	47.4	47.3
19	40.8	41.0	41.0	41.1	42.3	42.9	42.8
20	40.0	40.2	41.0	41.0	37.7	38.9	38.8
21	70.3	70.3	70.4	70.5	46.6	47.0	47.0
22	79.6	79.7	79.6	79.7	216.0	216.0	216.0
23	28.1	28.6	22.7	23.5	22.7	23.5	23.0
24	15.4	15.7	63.3	64.5	63.2	64.5	63.5
25	16.5	16.5	15.6	16.2	15.5	16.2	15.7
26	16.8	17.4	16.7	17.0	16.5	16.8	16.7
27	26.5	26.6	26.5	26.7	25.0	25.3	25.3
28	22.1	22.2	22.1	22.3	20.9	21.3	21.2
29	71.2	71.5	71.3	71.7	26.9	26.9	26.9
30	17.3	17.0	17.3	17.5	75.4	68.2	68.2

TABLE II. ^{13}C-NMR Chemical Shifts of Sugar Moieties of Subprosides **1a**, **2a** and **3a** and Prosapogenin **3c** (Pyridine-d_5)

	1a	**2a**	**3a**	**3c**
Glc UA				
1	105.1	105.3	105.2	105.4
2	78.7[a]	77.7[a]	78.3[a]	78.2
3	76.5[b]	76.4[b]	76.2[b]	76.4[a]
4	74.1	74.2	74.3	74.3
5	78.2[a]	77.5[a]	77.8[c]	77.7
6	170.5	170.2	170.1	170.4
COOMe	51.8	52.0	52.0	52.1
Gal				
1	101.8		101.4	101.7
2	76.3[b]		77.4[c]	76.9[a]
3	76.0[b]		76.2[b]	76.5[a]
4	70.1		71.4	71.1
5	76.3[b]		76.7[b]	76.6[a]
6	61.8		61.3	61.5
Rham				
1	102.4	102.3	102.1	102.1
2	72.1[c]	72.2[c]	72.1[d]	72.1[b]
3	72.4[c]	72.5[c]	72.4[d]	72.4[b]
4	73.0	73.5	73.3	73.6
5	69.4	69.2	69.0	69.0
6	18.7	18.7	18.7	18.7
Ara (p)				
1		101.7		
2		76.8[b]		
3		75.6		
4		70.1		
5		66.8		
Glc				
1			104.8	
2			74.8	
3			78.3[a]	
4			70.8	
5			78.1[a]	
6			62.5	

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

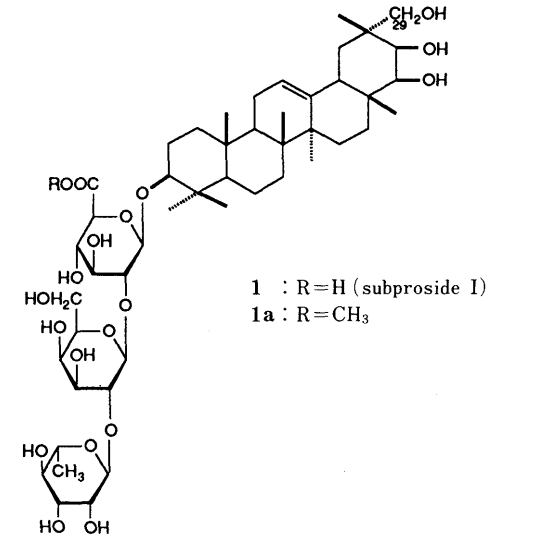
at δ 170.5. In the ^{1}H–^{1}H correlation spectroscopy (COSY) NMR spectrum of the acetate, all signals due to the sugar moiety could be assigned as shown in the Experimental section, suggesting the presence of an oligosaccharide moiety, namely, a rhamnosyl-galactosyl-glucuronic acid methyl ester residue. The appearances of signals due to two H-2 of both galactopyranosyl and glucuronopyranosyl moieties were observed in higher fields at δ 4.04 (1H, dd, J=7.3, 9.9 Hz) and 3.81 (1H, t, J=7.7 Hz), respectively, than those of protons adjacent to the acetoxyl groups, indicating that the above two component saccharides possessed glycosidic linkages at C-2-OH of galactopyranose and glucuronopyranose. A comparative study of the spectral data due to the sugar moiety of **1a** with those of soyasaponin I methyl ester and abrisaponin I methyl ester obtained from *Abrus cantoniensis*[8] also supported the above result. Consequently, the structure of **1** was characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl-abrisapogenol C.

Subproside II methyl ester (**2a**), a white powder, $[\alpha]_D$ −7.8° (MeOH), on methanolysis, yielded a sapogenol identical with kudzusapogenol A (**2b**) obtained from *Pueraria lobata*.[10] The HR-FAB-MS of **2a** showed a quasi-molecular ion at m/z 981.5048 $[M+Na]^+$ ($C_{48}H_{78}O_{19}Na$) and positive FAB-MS spectrum exhibited peaks due to $[M+K]^+$ at m/z 997, $[M+Na]^+$ at m/z 981, $[M+H]^+$ at m/z 959, $[M+H-$methylpentose$]^+$ at m/z 813, $[M+H-$methylpentose$-$pentose$]^+$ at m/z 681. The ^{1}H-NMR spectrum of **2a** showed three anomeric proton signals at δ 4.91 (1H, d, J=7.7 Hz), 5.46 (1H, d, J=7.3 Hz) and 6.10 (1H, br s) along with six methyl signals. The above evidence indicated that the sugar residue of **2a** was constituted of methylpentosyl-pentosyl-methoxycarbonylpentose. The EI-MS of its acetate showed fragment ion peaks at m/z 763 [(methylpentosyl-pentosyl-6-*O*-methyl glucuronosyl-)Ac_7]$^+$, 489 [(methylpentosyl-pentosyl-)Ac_5]$^+$ and 273 [(terminal methylpentosyl-)Ac_3]$^+$, confirming that sugar moiety was in a linear chain form. A comparative study of the ^{13}C-NMR data of **2a** with those of kudzusapogenol A led to assignment of its structure as a kudzusapogenol 3-*O*-monodesmoside. Furthermore, on the basis of a detailed ^{1}H–^{1}H COSY NMR study of the acetate, all signals due to the sugar moiety could be assigned as shown in the Experimental section. The respective coupling constants determined component saccharides to be a rhamnosyl-arabinosyl-glucuronic acid methyl ester residue, and appearances of signals at higher field at δ 3.84 (1H, dd, J=7.7, 9.9 Hz) and 3.97 (1H, dd, J=7.0, 9.1 Hz) due to two H-2 on arabinopyranosyl and glucuronopyranosyl moieties than those of protons adjacent to the acetoxyl groups also revealed that the respective saccharide component possessed sugar linkage at C-2-OH. Furthermore, the ^{13}C-NMR signals due to two C-2 of both glucuronic acid and arabinose shifted toward downfield, δ 77.7 and 76.8 ppm, respectively. The result of the ^{13}C-NMR spectrum was consistent with the evidence of the ^{1}H-NMR spectrum. Therefore, the structure of **2** could be represented as 3-*O*-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl(1→2)-β-D-glucuronopyranosyl-kudzusapogenol A.

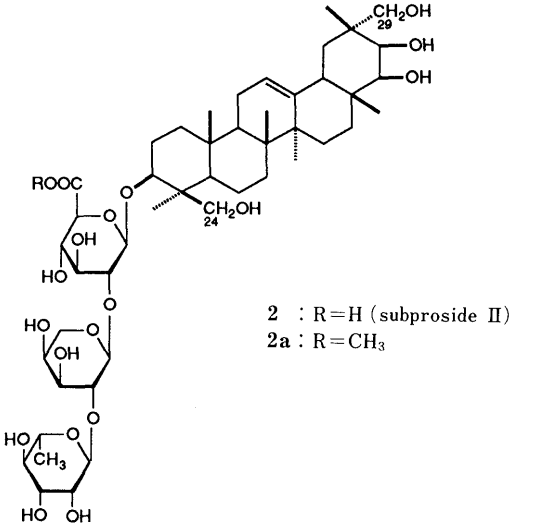
Subproside III methyl ester (**3a**), a white powder, $[\alpha]_D$ −6.3° (MeOH), on methanolysis, provided wistariasapogenol A (**3b**)[11] as sapogenol. The HR-FAB-MS showed a quasi-molecular ion at m/z 1155.5569 $[M+Na]^+$ ($C_{55}H_{88}O_{24}Na$) and negative FAB-MS spectrum showed peaks due to $[M]^-$ at m/z 1132, $[M-H]^-$ at m/z 1131 and $[M-H-$methylpentose$]^-$ at m/z 985 in **3a**, suggesting the occurrence of four sugars including a terminal methylpentose in **3a**. The ^{1}H-NMR spectrum of **3a** showed four anomeric proton signals at δ 4.30 (1H, d, J=7.0 Hz), 4.74 (1H, d, J=7.7 Hz), 5.68 (1H, d, J=7.3 Hz) and 6.71 (1H, br s) along with six methyl group signals. On enzymatic hydrolysis, **3a** provided a prosapogenin (**3c**) as a white powder, whose negative FAB-MS spectrum gave peaks due to $[M+p$-nitrobenzylalcohol (NBA)$]^-$ at m/z 1123 and $[M-H]^-$ at m/z 969. In the ^{13}C-NMR spectrum of **3c**, a signal at C-3 in the sapogenol part (Table I) showed a downfield shift (+11.1 ppm) in comparison with that of **3b**, and signals due to the sugar moieties (Table II) were superimposable with those of **1a**, so that it was deduced that **3c** is a 3-*O*-monodesmoside of wistariasapogenol A having the same sugar moiety as **1a**. A comparative study of the ^{13}C-NMR spectrum of **3a** with that of **3c** suggested that one additional glucosyl moietyl was attached in **3a**. In addition, a downfield shift by +7.2 ppm at C-30 in **3a** indicated that **3a** was a 3,30-di-*O*-bisdesmoside. Thus, the structure of **3** was eatablished as 3-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl]-wistariasapogenol A 30-*O*-β-D-glucopyrano-

side.

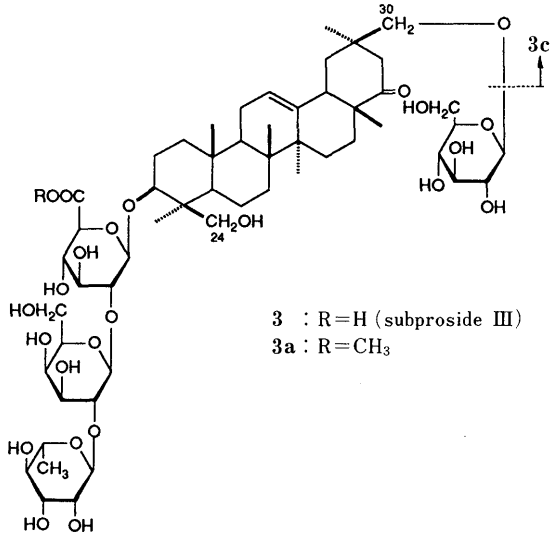
Soyasapogenol type glycosides so far obtained from the leguminous plants generally contained 3-*O*- and 22-*O*-glycosidic linkages, so that subproside III is the first example of bisdesmoside having a glycosidic linkage on the C-30 hydroxymethyl group. Now, determination of the other glycosides from *S. subprostrata* is in progress.



1 : R=H (subproside I)
1a : R=CH_3



2 : R=H (subproside II)
2a : R=CH_3



3 : R=H (subproside III)
3a : R=CH_3

Formulae

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The ^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 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. Column chromatography was carried out with MCI-gel CHP 20P (75—150 μ, 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 dried roots (8 kg) of *Sophora subprostrata* (Leguminosae) were extracted with MeOH and the extract (923 g) was partitioned between 40% MeOH and AcOEt. The 40% MeOH layer was concentrated *in vacuo* to remove MeOH. The water layer was fractionated on Diaion HP-20 column chromatography eluted successively with H_2O, 50% MeOH, 80% MeOH and MeOH. The MeOH eluate portion (IV, 32 g) treated with ion exchange resin was separated by various column chromatographies of silica gel with $CHCl_3$–MeOH–H_2O (8:2:0.2 and 7:3:0.5), Sephadex LH-20 with MeOH and Bondapak C_{18} with 40—60% MeOH. The fractions IV_2 (920 mg) and IV_3 (890 mg) were respectively treated with excess diazomethane etherate followed by column chromatography over silica gel using $CHCl_3$–MeOH–H_2O (9:1:0.1→8:2:0.2) and further purified using Bondapak C_{18} column chromatography. From fr. IV_2, glycoside **1a** (10.6 mg) was obtained as a methyl ester. From fr. IV_3, **4a** (53.3 mg) and **5a** (51.3 mg) were obtained as methyl esters. The 80% MeOH eluate portion (III, 37.5 g), after treatment with ion exchange resin was also fractionated using Bondapak C_{18} eluted with 40%→60%MeOH to give three fractions III_1, III_2 (9.77 g) and III_3 (4.98 g). Each fracton was methylated with diazomethane etherate, then chromatographed on silica gel with the same solvent as above, and purified by Bondapak C_{18} column chromatography. Glycosides **1a** (99.5 mg) and **3a** (107.2 mg) from fr. III_3, and **6a** (80.6 mg) from fr. III_2 were obtained as methyl esters. Fraction II was chromatographed on Sephadex LH-20 using MeOH and MCI gel CHP-20P eluting with 0%→70% MeOH. Fractions II_4 (392 mg) and II_5 (219 mg) were separately methylated followed by silica gel column chromatography with $CHCl_3$–MeOH–H_2O (9:1:0.1) and (8:2:0.2). The glycosides **2a** (21 mg) and **7a** (5 mg) were obtained from fr. II_5, and **6a** (54 mg) was obtained from fr. II_4 as methyl esters.

Identification of Known Glycosides 4a Colorless needles, $[\alpha]_D^{24}$ −3.5° (c=0.60, MeOH). Negative FAB-MS (m/z): 925 [M−H]$^-$, 779 [M−H−rham]$^-$, 647 [M−H−rham−ara]$^-$, 457 [M−H−rham−ara−glc UA]$^-$. ^1H-NMR (pyridine-d_5) δ: 0.74, 0.97, 1.01, 1.23, 1.29, 1.30, 1.44 (each 3H, s, 7×Me), 1.76 (3H, d, J=6.2 Hz), 4.95 (1H, d, J=7.7 Hz), 5.58 (1H, d, J=7.3 Hz), 6.24 (1H, br s). ^{13}C-NMR (pyridine-d_5) δ: 38.7, 26.7, 91.2, 44.0, 56.1, 18.6, 33.3, 39.9, 47.8, 36.5, 24.0, 122.4, 144.8, 42.4, 26.4, 28.7, 38.0, 45.3, 46.8, 30.9, 42.4, 75.6, 23.0, 63.5, 15.8, 17.0, 25.7, 28.7, 33.3, 21.2 (C_{1-30}), 105.5, 77.6, 76.4, 73.7, 78.0, 170.4, 52.2 (UA C_{1-6} and Me), 101.7, 76.9, 75.8, 70.5, 67.0 (ara C_{1-5}), 102.5, 72.4, 72.8, 74.3, 69.4, 18.9 (rham C_{1-6}). Identified with soyasaponin II methyl ester.

5a A white powder, $[\alpha]_D^{25}$ −31.5° (c=0.55, MeOH). Negative FAB-MS (m/z): 953 [M−H]$^-$, 807 [M−H−rham]$^-$, 455 [M−H−rham−gal−glc UA]$^-$. ^1H-NMR (pyridine-d_5) δ: 0.71, 0.86, 0.87, 0.97, 1.18, 1.29, 1.45 (each 3H, s, 7×Me), 1.78 (3H, d, J=6.2 Hz), 4.98 (1H, d, J=7.5 Hz), 5.80 (1H, d, J=7.3 Hz), 6.31 (1H, br s). ^{13}C-NMR (pyridine-d_5) δ: 38.5, 25.3, 91.2, 43.8, 56.0, 18.4, 33.0, 39.7, 47.8, 36.4, 24.0, 123.9, 141.8, 41.9, 26.6, 27.3, 47.7, 47.6, 46.6, 34.1, 50.9, 215.6, 23.0, 63.5, 15.7, 16.6, 25.2, 20.9, 31.8, 23.0 (C_{1-30}), 105.4, 78.2, 76.9, 73.6, 77.7, 170.4, 52.1 (UA C_{1-6} and Me), 101.7, 76.6, 76.4, 71.1, 76.5, 61.5 (gal C_{1-6}), 102.4, 72.4, 72.8, 74.3, 69.4, 18.9 (rham C_{1-6}). Identified with dehydrosoyasaponin I methyl ester.

6a A white powder, $[\alpha]_D^{25}$ −6.3° (c=0.56, MeOH). Nagative FAB-MS (m/z): 1141 [M+NBA]$^-$, 987 [M−H]$^-$, 841 [M−H−rham]$^-$. ^1H-NMR (pyridine-d_5) δ: 0.69, 0.95, 1.31, 1.33, 1.40, 1.51 (each 3H, s, 6×Me), 1.77 (3H, d, J=5.9 Hz), 4.92 (1H, d, J=6.6 Hz), 5.76 (1H, d, J=7.3 Hz), 6.26 (1H, br s). ^{13}C-NMR (pyridine-d_5) δ: 38.4, 26.4, 91.1, 43.7, 55.8, 18.3, 32.7, 40.0, 47.6, 36.2, 23.9, 122.2, 144.5, 41.8, 26.5, 28.1, 38.9, 43.0, 40.8, 40.9, 70.1, 79.6, 22.8, 63.4, 15.6, 16.7, 26.5, 22.1, 71.3, 17.3 (C_{1-30}), 105.2, 78.0, 76.8, 73.4, 76.4, 170.2, 52.0 (UA C_{1-6} and Me), 101.5, 77.5, 76.2, 71.0, 76.3, 61.4 (gal C_{1-6}), 102.2, 72.2, 72.6, 74.2, 69.2, 18.8 (rham C_{1-6}). Identified with kudzusaponin A_3 methyl ester.

7a An amorphous powder, $[\alpha]_D^{25}$ 10.3° (c=0.56, MeOH). Positive

FAB-MS (*m/z*): 1021 $[M+Na]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.73, 0.87, 1.19, 1.25, 1.28, 1.45 (each 3H, s, 6 × Me), 1.73 (3H, d, J=5.9 Hz), 4.92 (1H, d, J=7.3 Hz), 5.68 (1H, d, J=7.3 Hz), 6.19 (1H, br s). ^{13}C-NMR (pyridine-d_5) δ: 38.4, 26.5, 91.2, 41.8, 56.0, 18.4, 33.0, 39.6, 48.4, 36.3, 23.9, 124.5, 141.4, 43.8, 26.1, 28.0, 47.3, 47.5, 43.8, 45.5, 46.6, 212.9, 22.9, 63.4, 15.6, 16.6, 25.1, 25.2, 21.1, 176.7, 51.9 (C_{1-30}, COOMe), 105.4, 78.1, 76.5, 73.5, 77.7, 170.3, 52.0 (UA C_{1-6} and Me), 101.7, 76.9, 76.4, 71.1, 76.4, 61.5 (gal C_{1-6}), 102.3, 72.3, 72.7, 74.3, 69.3, 18.9 (rham C_{1-6}). Identified with abrisaponin I methyl ester.

Subproside I Methyl Ester (1a) A white powder, $[\alpha]_D^{24}$ −3.3° (c=0.59, MeOH). HR FAB-MS (*m/z*): 995.5211 $[M+Na]^+$ ($C_{49}H_{80}O_{19}Na$, Calcd for 995.5195). Negative FAB-MS (*m/z*): 971 $[M-H]^-$, 825 $[M-H-rham]^-$, 473 $[M-H-rham-gal-glc\ UA]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.76, 0.96, 1.04, 1.28, 1.30, 1.40, 1.57 (3H, each, s, 7 × CH_3), 1.66 (3H, d, J=6.2 Hz, rham-CH_3), 5.01 (1H, d, J=7.3 Hz), 5.71 (1H, d, J=7.3 Hz), 6.39 (1H, br s). A small amount of **1a** on acid hydrolysis (1 N HCl–MeOH) gave a sapogenol identical with abrisapogenol C (**1b**) on TLC.

Acetylation of 1a A solution of **1a** (10 mg) in Ac_2O–pyridine (1 : 1) was heated at 80°C for 2 h on a water bath. The reaction mixture was evaporated under N_2 gas and then chromatographed on silica gel using *n*-hexane–acetone (3 : 1) giving 1-Ac, a white powder. EI-MS (*m/z*): 561 [(rham-gal)Ac_6], 273 [(terminal rham)Ac_3]. ^{1}H-NMR ($CDCl_3$) δ: 0.81, 0.87, 0.94, 0.97, 1.07, 1.10, 1.15 (each 3H, all s, Me × 7), 1.21 (3H, d, J=6.2 Hz), 1.97 1.98, 1.99, 2.01, 2.06, 2.07, 2.08, 2.09, 2.11, 2.14, 2.15 (OAc × 11), 3.09 (1H, dd, J=5.1, 10.9 Hz, H-3), 3.58 (1H, ABq, J=11.2 Hz, H-29), 3.81 (1H, d, J=7.7, UA H-2), 3.85 (1H, m, gal H-5), 3.86 (1H, ABq, J=11.2 Hz, H-29′), 4.04 (1H, dd, J=7.3, 9.9 Hz, gal H-2), 4.08 (1H, d, J=9.9 Hz, UA H-5), 4.12 (1H, m, rham H-5); 4.13 (1H, dd, J=3.8, 10.4 Hz, gal H-6), 4.45 (1H, d, J=7.3 Hz, UA H-1), 4.62 (1H, d, J=7.7 Hz, gal H-1), 4.93 (1H, dd, J=3.3, 10.3 Hz, gal H-3), 4.97 (1H, d, J=3.3 Hz, H-21), 4.99 (1H, d, J=1.5 Hz, rham H-1), 5.07 (1H, dd, J=1.5, 3.3 Hz, rham H-2), 5.08 (1H, t, J=10.2 Hz, rham H-4), 5.09 (1H, t, J=10.2 Hz, UA H-4), 5.17 (1H, d, J=3.7 Hz, H-22), 5.19 (1H, dd, J=3.3, 9.9 Hz, rham H-3), 5.21 (1H, t, J=9.5 Hz, UA H-3), 5.29 (1H, br t, H-12), 5.35 (1H, d, J=3.3 Hz, gal H-4).

Subproside II Methyl Ester (2a) A pale yellowish powder, $[\alpha]_D^{24}$ −7.8° (c=0.46, MeOH). HR-FAB-MS (*m/z*): 981.5048 $[M+Na]^+$ ($C_{48}H_{78}O_{19}Na$, Calcd for 981.5038). Positive FAB-MS (*m/z*): 997 $[M+K]^+$, 981 $[M+Na]^+$, 813 $[M+H-rham]^+$, 681 $[M+H-rham-ara]^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.75, 0.98, 1.28, 1.32, 1.41, 1.47 (3H each, s, 6 × CH_3), 1.72 (3H, d, J=6.2 Hz, rham-CH_3), 4.91 (1H, d, J=7.7 Hz), 5.46 (1H, d, J=7.3 Hz), 6.10 (1H, br s). Methanolysis of **2a** yielded a sapogenol identical with kudzusapogenol A (**2b**) on TLC.

Acetylation of 2a A solution of **2a** (10 mg) in Ac_2O–pyridine (1 : 1) was heated at 80°C for 2 h on a water bath. The reaction mixture was chromatographed on silica gel using *n*-hexane–acetone (3 : 1) giving 2-Ac, a white powder. EI-MS (*m/z*): 763 [(rham-ara-UA-)Ac_7], 489 [(rham-ara-)Ac_5], 273 [(terminal rham)Ac_3]. ^{1}H-NMR ($CDCl_3$) δ: 0.87, 0.94, 0.97, 1.07, 1.10, 1.15 (each 3H, all s, Me × 6), 1.21 (3H, d, J=6.2 Hz), 1.97, 1.98, 1.99, 2.01, 2.06, 2.07, 2.08, 2.09, 2.11, 2.14, 2.15 (OAc × 11), 3.21 (1H, dd, J=4.4, 11.7 Hz, H-3), 3.57 (1H, ABq, J=10.6 Hz, H-29), 3.58 (1H, m, ara H-5), 3.84 (1H, dd, J=7.7, 9.9 Hz, ara H-2), 3.85 (1H, ABq, J=10.6 Hz, H-29′), 3.97 (1H, dd, J=7.0, 9.1 Hz, UA H-2), 4.00 (1H, m, ara H-5′), 4.10 (1H, d, J=9.5 Hz, UA H-5), 4.12 (1H, m, rham H-5), 4.51 (1H, d, J=7.0 Hz, UA H-1), 4.55 (1H, d, J=7.7 Hz, ara H-1), 4.91 (1H, dd, J=3.3, 9.9 Hz, ara H-3), 4.97 (1H, d, J=3.3 Hz, H-21), 4.98 (1H, d, J=1.8 Hz, rham H-1), 5.08 (1H, t, J=9.9 Hz, rham H-4), 5.12 (1H, dd, J=1.8, 3.2 Hz, rham H-2), 5.13 (1H, m, UA H-4), 5.17 (1H, dd, J=3.2, 9.9 Hz, rham H-3), 5.19 (1H, m, UA H-3), 5.21 (1H, d, J=3.3 Hz, H-22), 5.24 (1H, m, ara H-4), 5.30 (1H, br t, H-12).

Subproside III Methyl Ester (3a) A white powder, $[\alpha]_D^{25}$ −6.3° (c=0.64, MeOH). HR-FAB-MS (*m/z*): 1155.5569 $[M+Na]^+$ ($C_{55}H_{88}O_{24}Na$, Calcd for 1155.5566). Negative FAB-MS (*m/z*): 1132 $[M]^-$, 1131 $[M-H]^-$, 985 $[M-H-rham]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.63, 0.78, 1.05, 1.08, 1.19, 1.36 (each 3H, s, 6 × CH_3), 1.77 (3H, d, J=6.2 Hz, rham-CH_3), 4.30 (1H, d, J=7.0 Hz), 4.74 (1H, d, J=7.7 Hz), 5.68 (1H, d, J=7.3 Hz), 6.17 (1H, br s). A solution of **3a** (37 mg) in 2 N HCl–MeOH (5 ml) was refluxed for 2 h and the reaction mixture was neutralized with 3% KOH–MeOH. The resultant salts were removed by passing through Sephadex LH-20 with MeOH. The products was chromatoraphed over silica gel with *n*-hexane–acetone (3 : 1) to afford sapogenin **3b** identical with wistariasapogenol A, a colorless prisms. EI-MS (*m/z*): 472 $[M]^+$, 248 (D/E ring part of the aglycone), 224 (A/B ring). ^{13}C-NMR (pyridine-d_5): (Table I).

Enzymic Hydrolysis of 3a A mixture of **3a** (26.8 mg) and glycosidase (10 mg) (*T. cornutus*, Seikagaku Kogyo Co., Ltd.) in acetate buffer (pH=4.2, 4 ml) and dimethylsulfoxide (1 ml) was incubated at 37°C overnight. The reaction mixture was evaporated to dryness and dissolved with $CHCl_3$–MeOH. The filtrate was chromatographed over silica gel with $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2) to afford prosapogenin **3c** (18 mg) as a white powder. Negative FAB-MS (*m/z*): 1123 $[M+NBA]^-$, 969 $[M-H]^-$.

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