Four New Oleanene Glycosides from Sophorae Subprostratae Radix. III[1)]

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In a previous paper, we reported the isolation and structure determination of three new oleanene glycosides, subprosides I, II and III, together with four known glycosides, kudzusaponin A_3, abrisaponin I, soyasaponin II and dehydrosoyasaponin I from *Sophora subprostrata* CHUX *et* T. CHEN (Leguminosae). In a continuing study on this crude drug, we report the characterization of four more new minor glycosides, subprosides IV (1), V (2), VI (3) and VII (4). Their chemical structures could be represented as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl abrisapogenol D 30-*O*-β-D-glucopyranoside (1), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl wistariasapo-genol B 30-*O*-β-D-glucopyranoside (2), 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A 21-*O*-α-L-rhamnopyranoside (3) and 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside (4) on the basis of chemical and physiochemical evidence.

Keywords Sophorae subprostratae Radix; *Sophora subprostrata*; Leguminosae; oleanene glycoside; kudzusapogenol A; abrisapogenol D; wistariasapogenol B; soyasapogenol B; subproside

In a previous paper, we reported the structure characterizations of three new oleanene glycosides, named subprosides I, II and III, together with four known glycosides, kudzusaponin A_3, abrisaponin I, soyasaponin II and dehydrosoyasaponin I from Sophorae Subprostratae Radix.[2)] As part of a continuing study on this crude drug, we are now dealing with the structure determinations of four new oleanene glycosides, subprosides IV (**1**), V (**2**), VI (**3**) and VII (**4**).

The methanol extract of dried roots (10 kg) was fractionated by using various column chromatographies of Diaion HP-20, Bondapak C_{18} and silica gel, followed by methylation with CH_2N_2 to provide glycosides **1a**—**4a** as methyl ester forms.

Subproside IV methyl ester (**1a**) was obtained as a white powder, $[\alpha]_D$ −4.6° (MeOH). On methanolysis, **1a** provided methyl glycosides of rhamnose, glucose and galactose as sugar moiety, as well as an aglycone, which was identified as abrisapogenol D on thin layer chromatography (TLC). The high resolution fast atom bombardment mass spectrum (HR FAB-MS) of **1a** showed a quasi-molecular ion peak at *m/z* 1141.5773 $[M+Na]^+$, indicating a molecular formula $C_{55}H_{90}O_{23}$. The negative FAB-MS gave peaks due to $[M+p$-nitrobenzylalcohol (NBA)$]^-$ at *m/z* 1272, $[M-H]^-$ at *m/z* 1117 and $[M-H-$rha-2 × hex-uronic acid (UA)$]^-$ at *m/z* 457. In addition, the electron impact (EI)-MS of its peracetate exhibited fragment ion peaks at *m/z* 561 $[$(rha-hex-)$Ac_6]^+$, 331 $[$(terminal hex-)$Ac_4]^+$ and 273 $[$(terminal rha-)$Ac_3]^+$. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1a** displayed signals due to seven tertiary methyl groups at δ 0.85, 0.99, 1.18, 1.20, 1.24, 1.25, 1.42, one secondary methyl group at δ 1.77 (3H, d, J=6.2 Hz) and four anomeric proton signals at δ 4.26 (1H, d, J=7.7 Hz), 4.96 (1H, d, J=7.7 Hz), 5.75 (1H, d, J=7.7 Hz) and 6.34 (1H, br s). In the ^{13}C-NMR spectrum (Tables I and II) of **1a**, signals of one ester carbonyl carbon (δ 170.4), two olefinic carbons (δ 122.9 and 144.0) and four anomeric carbons (δ 102.1, 102.8, 105.3 and 105.9) were observed. The carbon signals due to the aglycone moieties were almost identical with those of abrisapogenol D[3)] except for displacement of signals at C-2 (−1.7 ppm), C-3 (+11.9 ppm), C-4 (−1.5 ppm), C-20 (−0.8 ppm) and C-30 (+7.8 ppm). On the other hand, signals due to the sugar moieties were superimposable on those of subproside III.[2)] It suggested that **1a** possessed the same sugar moiety as that of subproside III. Therefore, **1a** was regarded as a 3,30-di-*O*-bisdesmoside of abrisapogenol D. Determination of D or L of each sugar configuration was achieved by gas liquid chromatography (GLC) analysis.[4)] That is, **1a** was at first reduced with $NaBH_4$ and subjected to hydrolysis with 1 N HCl water–dioxane (1 : 1) to give the sugar components, which were subsequently converted to the corresponding trimethylsilyl (TMS) ethers of methyl 2-(polyhydroxyalkyl)-thiazolidine-4(*R*)-carboxylates followed by GLC check.[4)] The sugar moieties was revealed to be composed of D-glucose, D-galactose and L-rhamnose. Therefore, the structure of **1** could be represented as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl abrisapogenol D 30-*O*-β-D-glucopyranoside.

Subproside V methyl ester (**2a**) was obtained as a white powder, $[\alpha]_D$ −14.7° (MeOH). The HR FAB-MS of **2a** showed a quasi-molecular ion peak at *m/z* 1157.5720 $[M+Na]^+$, indicating a molecular formula $C_{55}H_{90}O_{24}$. The negative FAB-MS showed an $[M-H]^-$ ion at *m/z* 1133, suggesting the occurrence of four sugars in **2a**. On methanolysis, **2a** provided a sapogenol, which was identified with wistariasapogenol B and was also identical with the reductive product of wistariasapogenol A, as well as methyl glycosides of glucose, galactose and rhamnose. The ^{1}H-NMR spectrum of **2a** showed four anomeric proton signals at δ 4.26 (1H, d, J=7.0 Hz), 4.96 (1H, d, J=7.3 Hz), 5.80 (1H, d, J=7.7 Hz) and 6.30 (1H, br s). Six tertiary methyl signals (0.69, 0.94, 1.20, 1.23, 1.27 and 1.44) in the aglycone part were observed, suggesting that **2a** had one less methyl group than **1a**. In the ^{13}C-NMR spectrum of **2a**, signals due to C-3 and C-30 in the aglycone moieties (Table I) exhibited downfield shifts (+11.1 and +7.6 ppm, respectively) in comparison with those of wistariasapogenol B.[5)] On the other hand, signals due to the sugar moieties were almost the same as those of **1a**, so that **2a** was also estimated as a 3,30-di-*O*-bisdesmoside. On the basis of the above results, the structure of **2** was determined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→

Table I. ^{13}C-NMR Chemical Shifts of Sapogenol Moieties of **1a**—**4a**, Prosapogenin **4c** and Aglycone **3b** (Pyridine-d_5)

	1a	2a	3a	3b	4a	4c
C- 1	38.8	38.5	38.5	38.9	38.5	38.6
C- 2	26.4	26.4	26.6	28.4	25.7	25.9
C- 3	90.0	91.3	91.2	80.1	91.0	91.0
C- 4	37.9	43.8	43.8	43.2	43.9	43.8
C- 5	55.9	56.0	56.0	56.3	56.0	56.0
C- 6	18.5	18.5	18.4	19.1	18.4	18.4
C- 7	33.0	33.0	32.8	33.2	33.4	33.2
C- 8	40.1	40.0	40.1	40.3	39.5	39.7
C- 9	47.9	47.7	47.7	48.1	47.7	47.7
C-10	36.9	36.4	36.4	37.0	36.4	36.4
C-11	23.8	24.0	24.0	24.1	23.8	23.9
C-12	122.9	122.7	122.5	122.5	122.7	122.4
C-13	144.0	144.6	144.2	144.6	143.8	144.2
C-14	42.3	42.2	41.8	42.0	42.4	42.1
C-15	26.4	26.6	26.6	26.6	26.5	26.7
C-16	28.2	28.7	26.6	27.3	28.7	28.6
C-17	37.9	37.8	39.2	39.1	37.6	37.4
C-18	44.5	44.4	43.8	43.2	46.1	45.7
C-19	42.3	42.2	41.0	41.1	46.3	46.3
C-20	35.1	35.0	40.9	41.0	30.3	30.5
C-21	37.2	37.1	74.3	70.4	36.7	37.4
C-22	75.5	75.4	78.2	79.8	81.8	82.4
C-23	28.5	23.0	22.9	23.5	22.8	22.8
C-24	16.8	63.5	63.5	64.6	63.3	63.3
C-25	15.7	15.7	15.7	16.2	15.8	15.7
C-26	17.1	16.9	16.7	17.0	16.9	16.8
C-27	26.1	26.0	26.6	26.6	25.5	25.2
C-28	21.3	21.2	22.9	22.3	29.0	28.6
C-29	28.5	29.1	71.8	71.7	31.3	32.3
C-30	78.0	77.9	17.6	17.5	21.4	21.0

Table II. ^{13}C-NMR Chemical Shifts of Sugar Moieties of **1a**—**4a** and Prosapogenin **4c** (Pyridine-d_5)

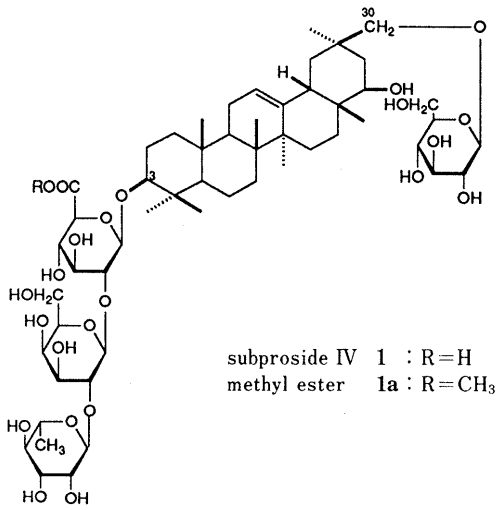
	1a	2a	3a	4a	4c
Glc UA 1	105.3	105.4	105.4	105.3	105.4
Glc UA 2	79.1	78.2[a]	77.7	78.0[a]	78.2[a]
Glc UA 3	76.4[b]	76.4[b]	76.4[b]	76.3[b]	76.4[b]
Glc UA 4	74.4	74.3	74.1	74.2	74.2
Glc UA 5	76.8[b]	76.8[b]	76.5[b]	77.5[c]	77.5[c]
Glc UA 6	170.4	170.4	170.4	170.2	170.3
COOMe	52.0	52.0	52.1	52.0	52.0
Gal 1	102.1	101.7	101.7	101.7	101.7
Gal 2	78.6[a]	77.7[c]	76.9[b]	77.0[c]	77.8[c]
Gal 3	76.3[b]	76.4[b]	76.4[b]	76.6[b]	75.6
Gal 4	71.6	71.6	71.1	71.6	71.9
Gal 5	76.6[b]	76.6[b]	76.6[b]	76.8[b]	76.8[b]
Gal 6	62.0	61.5	61.5	62.6	62.9
Rham 1	102.8	102.4	102.4	102.3	102.5
Rham 2	72.4[d]	72.3[d]	72.4[d]	72.3[d]	72.3[d]
Rham 3	72.7[d]	72.7[d]	72.8[d]	72.6[d]	72.6[d]
Rham 4	73.2	73.6	73.6	73.6	73.6
Rham 5	69.5	69.3	69.4	69.3	69.2
Rham 6	19.0	18.9	18.9	18.8	18.8
Rham′ 1			102.4		
Rham′ 2			71.6		
Rham′ 3			72.2[d]		
Rham′ 4			73.1		
Rham′ 5			70.6		
Rham′ 6			18.7		
Xyl (p) 1				100.3	102.3
Xyl (p) 2				83.5	75.1
Xyl (p) 3				77.8[c]	78.7[a]
Xyl (p) 4				71.5	70.4
Xyl (p) 5				66.8	66.8
Glc 1	105.9	105.8		106.1	
Glc 2	74.4	75.5		75.7	
Glc 3	78.7[a]	78.7[a]		78.4[a]	
Glc 4	70.6	71.1		70.4	
Glc 5	78.6[a]	78.5[a]		77.9[c]	
Glc 6	62.8	62.7		62.7	

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

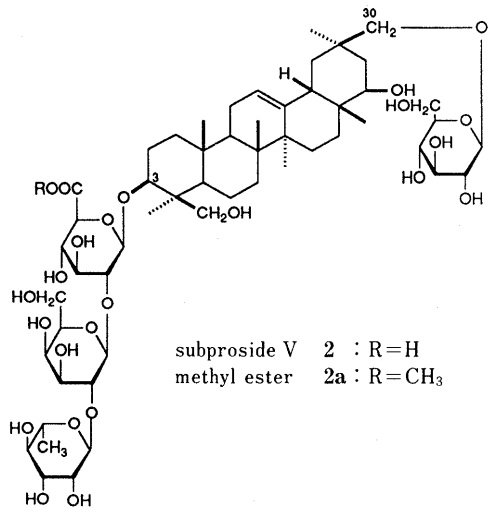
2)-β-D-glucuronopyranosyl wistariasapogenol B 30-*O*-β-D-glucopyranoside.

Subproside VI methyl ester (**3a**), a white powder, $[\alpha]_D$ −16.4° (MeOH), on methanolysis, **3a** yielded a sapogenol (**3b**), which was identical with kudzusapogenol A by comparing the ^{1}H- and ^{13}C-NMR spectra,[6] and methyl galactoside and methyl rhamnoside. The HR FAB-MS of **3a** showed a quasi-molecular ion peak at *m/z* 1157.5712 $[M+Na]^+$ ($C_{55}H_{90}NaO_{24}$). In the ^{1}H-NMR spectrum of **3a**, four anomeric proton signals at δ 4.94 (1H, d, *J*=7.0 Hz), 5.80 (1H, d, *J*=7.3 Hz), 6.07 (1H, br s) and 6.31 (1H, br s) along with two secondary methyl group signals of the rhamnosyl moieties at δ 1.73 (3H, d, *J*=6.2 Hz) and 1.78 (3H, d, *J*=6.2 Hz) were observed, indicating the **3a** contained 2 mol of rhamnosyl residues. The negative FAB-MS disclosed peaks due to $[M+NBA]^-$, $[M-H]^-$, $[M-H-rha]^-$ and $[M-H-rha-hex]^-$ at *m/z* 1288, 1133, 987 and 825, respectively, which indicated that **3a** was a tetraglycoside composed of 1 mol uronic acid, hexose and 2 mol of rhamnose. The EI-MS of the peracetyl derivative of **3a** exhibited peaks at *m/z* 872 [(hex-UA-retro Diels–Alder fission (RDA) of A/B ring-)Ac_7]$^+$, 622 [(rha-RDA of D/E ring-)Ac_5]$^+$, 562 [(rha-RDA of D/E ring-)Ac_4]$^+$, 561 [(rha-hex-)Ac_6]$^+$ and 273 [(terminal rha-)Ac_3]$^+$, so that **3a** included two terminal rhamnosyl moieties, one of which should be attached to the hydroxyl group on the E ring. A comparative study of the ^{13}C-NMR spectrum (Tables I and II) in **3a** with that of kudzusaponin A_3[2] showed that **3a** should possess one more additional molecule of the rhamnosyl moiety. Moreover, a downfield shift (+4.2 ppm) at C-21, an upfield shift at C-22 (−1.4 ppm) and slight displacements at C-17 by +0.3 ppm, at C-29 by +0.5 ppm and at C-30 by +0.3 ppm were observed. However, all the other carbon signals were the same as those of kudzusaponin A_3. Therefore, the structure of **3** could be defined to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl kudzusapogenol A 21-*O*-α-L-rhamnopyranoside.

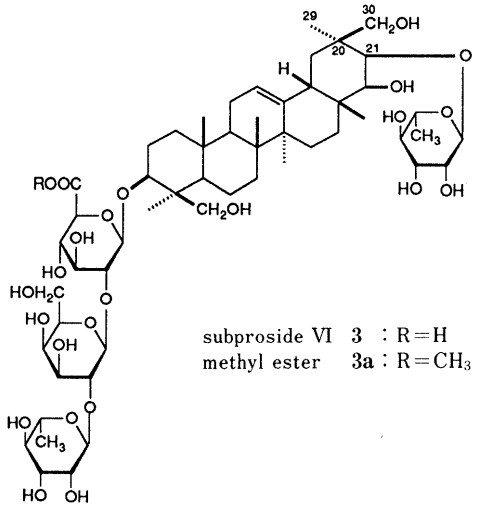
Subproside VII methyl ester (**4a**), a white powder, $[\alpha]_D$ −15.7° (MeOH), showed a quasi-molecular ion peak at *m/z* 1273.6185 $[M+Na]^+$ in the HR FAB-MS, indicating the molecular formula $C_{60}H_{98}O_{27}$. The methanolysis of **4a** afforded soyasapogenol B as a sapogenol, and methyl glycosides of rhamnose, galactose, glucose and xylose as sugar. The positive FAB-MS showed peaks due to $[M+NBA]^+$ at *m/z* 1403, $[M+H]^+$ at *m/z* 1251 and $[M+H-rha-2\times hex]^+$ at *m/z* 781 and $[M+H-rha-2\times hex\text{-}xyl\text{-}glc\ UA]^+$ at *m/z* 459 in **4a**, and the EI-MS of the peracetate of **4a** revealed fragment ion peaks at *m/z* 561 [(rha-hex-)Ac_6]$^+$, 547 [(hex-pen-)Ac_6]$^+$, 331 [(terminal hex-)Ac_4]$^+$ and 273 [(terminal rha-)Ac_3]$^+$, indicating the occurrence of five sugars in which rhamnose and glucose were located at the terminal site. The ^{1}H-NMR spectrum of **4a** showed five anomeric proton signals at δ 4.48 (1H, d, *J*=7.1 Hz), 4.95 (1H, d, *J*=7.7 Hz), 5.32 (1H, d, *J*=7.7 Hz), 5.57 (1H, d, *J*=7.3 Hz) and 6.23 (1H, br s) along



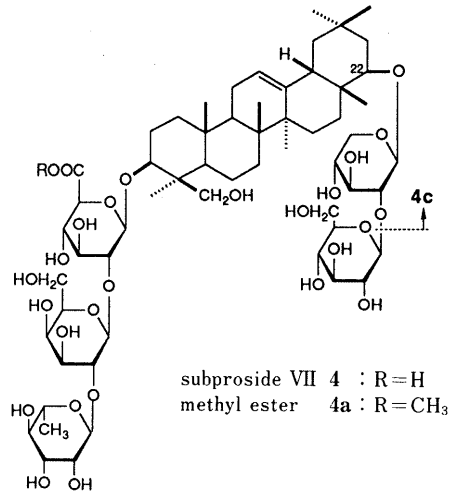
subproside IV 1 : R=H
methyl ester 1a : R=CH_3



subproside V 2 : R=H
methyl ester 2a : R=CH_3



subproside VI 3 : R=H
methyl ester 3a : R=CH_3



subproside VII 4 : R=H
methyl ester 4a : R=CH_3

Chart 1

with seven methyl group signals, and the ^{13}C-NMR spectrum of **4a** exhibited signals assignable to five anomeric carbons [δ 100.3, 101.7, 102.3, 105.3 and 106.1], indicating that **4a** was a pentaglycoside of soyasapogenol B. The D, L of respective sugars in **4a** was determined by GLC analysis of its TMS derivative. Comparison of the ^{13}C-NMR data for **4a** (Table I) with those of soyasaponin I methyl ester[7] and soyasapogenol B led us to presume that **4a** was a 3,22-di-*O*-bisdesmoside of soyasapogenol B. In order to elucidate the structures of carbohydrate moieties, we attempted enzymatic hydrolysis with β-glucosidase to provide a white powder, which showed $[M+NBA]^+$ at *m/z* 1241, $[M+H]^+$ at *m/z* 1089 and $[M+H-$rha-hex-pen-UA$]^+$ at *m/z* 459 in its FAB-MS. It showed the cleavage of 1 mol each of hexose, pentose, methylpentose and uronic acid in **4a**. In a comparative study of the ^{13}C-NMR spectra of **4c** and **4a**, signals due to the terminal glucosyl moiety disappeared in **4c** and signals of the pentosyl moiety in **4c** were unambiguously assigned to methyl D-xylopyranoside.[8] Therefore **4a** possessed the same sugar residue with soyasaponin I at C-3 and a β-D-glucopyranosyl (1→2)-β-D-xylopyranosyl residue at C-22. Based on the above evidence, the structure of **4** was determined as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl soyasapogenol B 22-*O*-β-D-glucopyranosyl-(1→2)-β-D-xylopyranoside.

It is worthy to note that these four new bisdesmosides had another sugar linkage site at C-21, 22 or 30 in addition to the C-3 site of aglycone.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The ^{1}H- 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) were recorded with a JEOL DX-300 spectrometer. GLC analysis was performed on a HP-5890A gas chromatograph with an H_2 flame ionization detector; the column was OV-1 (0.32 mm × 30 m); conditions: column temperature (temp.): 230 °C; detect temp.: 270 °C; inject temp.: 270 °C; carrier gas: He_2 (2.75 kg/cm^2). 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 (10 kg) of *Sophora subprostrata* (Leguminosae) were extracted with MeOH, and the extract (888 g) was partitioned between 1-BuOH and water. The 1-BuOH layer was concentrated and partitioned with 1-BuOH–AcOEt–water (1 : 4 : 5).

The water layer was evaporated *in vacuo* to remove the organic solvent and chromatographed on Diaion CC with water and MeOH. The MeOH eluate was fractionated by using silica gel column chromatography eluted with $CHCl_3$–MeOH–water (7:3:0.5). The fraction, including the triterpene component, was combined and methylated with CH_2N_2 after being passed through Amberlite IR-120B. The crude saponin (1.43 g) was separated on silica gel with $CHCl_3$–MeOH–water (8:2:0.2) to give three fractions, the second (154.1 mg) of them was further purified by using Bondapak C_{18} with 30—55% MeOH to provide glycoside **3a** (12.1 mg) and **1a** (22.5 mg) as methyl esters. The third fraction (127.0 mg) was also purified by silica gel with 1-BuOH–AcOH–water (4:1:5) and Bondapak C_{18} with 40—50% MeOH to afford glycosides **2a** (56.9 mg) and **4a** (60.6 mg) as methyl ester forms.

Subproside IV Methyl Ester (1a) A white powder, *Rf* value: 0.27 ($CHCl_3$: MeOH : water = 7:3:0.5), $[\alpha]_D^{22}$ −4.6° (*c* = 0.24, MeOH). HR FAB-MS *m/z*: 1141.5773 $[M+Na]^+$ ($C_{55}H_{90}NaO_{23}$, Calcd for 1141.5772). Negative FAB-MS *m/z*: 1272 $[M+NBA]^-$, 1117 $[M-H]^-$, 457 $[M-H-\text{rha-2}\times\text{hex-UA}]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.85, 0.99, 1.18, 1.20, 1.24, 1.25, 1.42 (each 3H, s, 7 × CH_3), 1.77 (3H, d, *J* = 6.2 Hz), 4.26 (1H, d, *J* = 7.7 Hz), 4.96 (1H, d, *J* = 7.7 Hz), 5.75 (1H, d, *J* = 7.7 Hz), 6.34 (1H, br s). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 1a A solution of **1a** (3 mg) in 2 N HCl–MeOH was refluxed for 2 h and the reaction mixture was neutralized with 3% KOH–MeOH. The hydrolysate was checked on TLC: aglycone, *Rf*: 0.19 ($CHCl_3$: MeOH = 19:1).

GLC Analysis of 1a To a solution of **1a** (3.1 mg) in MeOH was added $NaBH_4$ (*ca.* 5 mg), and the mixture was kept at room temperature for 30 min. The reaction mixture was worked up with Sephadex LH-20. The product was heated in 1 N HCl water–dioxane (1:1) at 90 °C for 2 h. The precipitate was removed by filtration and the supernatant was treated with Amberlite IRA-400 to give a sugar fraction. The pyridine solution of sugar (1 mg/100 μl) and the pyridine solution of L-cysteine methyl ester hydrochloride (2 mg/100 μl) were mixed and warmed at 60 °C for 1 h. The products' solvent was removed and its was dried *in vacuo*. The trimethylsilylation reagent TMS–imidazole (100 μl) was added and was heated at 60 °C for another 30 min. The reaction mixture was partitioned between *n*-hexane and water. The *n*-hexane solution was subjected to GLC. Three peaks were observed at t_R (min): 11.623 (L-rha), 17.124 (D-glc) and 18.172 (D-gal). The standard monosaccharides were subjected to the same reaction, and GLC analysis was performed under the same condition.

Acetylation of 1a A solution of **1a** (3 mg) in Ac_2O–pyridine (1:1) was kept at room temperature overnight. The reaction mixture was evaporated under N_2 gas and then chromatographed on silica gel using *n*-hexane–acetone (3:1), giving the peracetate, a white powder. EI-MS *m/z*: 561 (rha-hex-)$Ac_6]^+$, 331 (hex-)$Ac_4]^+$, 273 (rha-)$Ac_3]^+$.

Subproside V Methyl Ester (2a) A white powder, *Rf*: 0.27 ($CHCl_3$–MeOH–water = 7:3:0.5). $[\alpha]_D^{30}$ −14.7° (*c* = 0.32, MeOH). HR FAB-MS *m/z*: 1157.5720 $[M+Na]^+$ ($C_{55}H_{90}NaO_{24}$, Calcd for 1157.5721). Negative FAB-MS *m/z*: 1133 $[M-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.94, 1.20, 1.23, 1.27, 1.44 (each 3H, s, 6 × CH_3), 1.78 (3H, d, *J* = 6.2 Hz), 4.26 (1H, d, *J* = 7.0 Hz), 4.96 (1H, d, *J* = 7.3 Hz), 5.80 (1H, d, *J* = 7.7 Hz), 6.30 (1H, br s). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 2a A solution of **2a** (2 mg) in 2 N HCl–MeOH was refluxed for 2 h and the reaction mixture was neutralized with 3% KOH–MeOH. The hydrolysate was checked by TLC: aglycone, *Rf*: 0.17 ($CHCl_3$: MeOH = 19:1); sugars, *Rf*: 0.41 (gal), 0.45 (glc), 0.69 (rha) ($CHCl_3$: MeOH : AcOH : water = 3:2:3:0.5).

Subproside VI Methyl Ester (3a) A white powder, *Rf*: 0.27 ($CHCl_3$: MeOH : water = 7:3:0.5), $[\alpha]_D^{22}$ −16.4° (*c* = 0.43, MeOH). HR FAB-MS *m/z*: 1157.5712 $[M+Na]^+$ ($C_{55}H_{90}NaO_{24}$, Calcd for 1157.5721). Negative FAB-MS *m/z*: 1288 $[M+NBA]^-$, 1133 $[M-H]^-$, 987 $[M-H-\text{rha}]^-$, 825 $[M-H-\text{rha-hex}]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.97, 1.29, 1.30, 1.42, 1.44 (each 3H, s, 6 × CH_3), 1.73 (3H, d, *J* = 6.2 Hz), 1.78 (3H, d, *J* = 6.2 Hz), 4.94 (1H, d, *J* = 7.0 Hz), 5.80 (1H, d, *J* = 7.3 Hz), 6.07 (1H, br s), 6.31 (1H, br s). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 3a A solution of **3a** (12.6 mg) in 2 N HCl–MeOH (5 ml) was refluxed for 2 h. The reaction mixture was neutralized with 3% KOH–MeOH and partitioned between $CHCl_3$ and water. The $CHCl_3$ layer was evaporated and chromatographed on silica gel with $CHCl_3$–MeOH (30:1) to afford aglycone **3c** (11 mg), EI-MS *m/z*: 490 $[M]^+$, 224 [A/B ring from RDA]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.95, 1.03, 1.32, 1.33, 1.54, 1.55 (each 3H, s, 6 × CH_3), 3.72 (1H, br s, H-3), 3.88 (1H, d, *J* = 3.5 Hz, H-22), 3.67, 4.08 (2H, ABq, *J* = 10.1 Hz, H_2-29), 3.73, 4.54 (2H, ABq, *J* = 11.2 Hz, H_2-24), 4.52 (1H, d, *J* = 3.5 Hz, H-21), 5.40 (1H, br s, H-12). ^{13}C-NMR (pyridine-d_5): Table I. The water layer was neutralized with 3% KOH–MeOH and checked on TLC: sugars, *Rf*: 0.41 (gal), 0.69 (rha) ($CHCl_3$: MeOH : AcOH : water = 3:2:3:0.5).

Acetylation of 3a A solution of **3a** (4 mg) was acetylated using the same method as for **1a** to afford the peracetate, EI-MS *m/z*: 872 [(hex-UA-RDA of A/B ring-)$Ac_7]^+$, 622 [(rha-RDA of C/D ring-)$Ac_5]^+$, 562 (rha-RDA of C/D ring-)$Ac_4]^+$, 561 [(rha-hex-)$Ac_6]^+$, 273 [(rha-)$Ac_3]^+$.

Subproside VII Methyl Ester (4a) A white powder, *Rf*: 0.22 ($CHCl_3$: MeOH : water = 7:3:0.5), $[\alpha]_D^{29}$ −15.7° (*c* = 0.41, MeOH). HR FAB-MS *m/z*: 1273.6185 $[M+Na]^+$ ($C_{60}H_{98}NaO_{27}$, Calcd for 1273.6194). Positive FAB-MS *m/z*: 1403 $[M+NBA]^+$, 1251 $[M+H]^+$, 781 $[M+H-\text{rha-2}\times\text{hex}]^+$, 459 $[M+H-\text{rha-2}\times\text{hex-pen-UA}]^+$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.73, 0.89, 0.90, 1.11, 1.17, 1.21, 1.43 (each 3H, s, 7 × CH_3), 1.76 (3H, d, *J* = 5.9 Hz), 4.48 (1H, d, *J* = 7.1 Hz), 4.95 (1H, d, *J* = 7.7 Hz), 5.32 (1H, d, *J* = 7.7 Hz), 5.57 (1H, d, *J* = 7.3 Hz), 6.23 (1H, br s). ^{13}C-NMR (pyridine-d_5): Tables I and II.

Acid Hydrolysis of 4a A solution of **4a** (3 mg) in 2 N HCl–MeOH was refluxed for 2 h and the reaction mixture was neutralized with 3% KOH–MeOH; the hydrolysate was checked using TLC: aglycone, *Rf*: 0.25 ($CHCl_3$: MeOH = 19:1).

GLC Analysis of 4a **4a** (3 mg) was reduced with $NaBH_4$, hydrolyzed with 1 N HCl water–dioxane, and then the TMS derivative was subjected to GLC analysis. t_R (min): 9.600 (D-xyl), 11.625 (L-rha), 17.108 (D-glc), 18.178 (D-gal).

Acetylation of 4a A solution of **4a** (3 mg) was acetylated using the same method as for **1a**; peracetate was obtained, EI-MS *m/z*: 561 [(rha-hex-)$Ac_6]^+$, 547 [(hex-pen-)$Ac_6]^+$, 331 [(hex-)$Ac_4]^+$, 273 [(rha-)$Ac_3]^+$.

Enzymic Hydrolysis of 4a A mixture of **4a** (20 mg) and glucosidase (20 mg) (*T. cornutus*, Seikagaku Kogyo Co., Ltd.) in an acetate buffer (pH 4.2, 4 ml) and dimethyl sulfoxide (1 ml) was incubated at 37 °C for one week. The reaction mixture was evaporated to dryness and dissolved with $CHCl_3$–MeOH. The filtrate was chromatographed over Si gel with $CHCl_3$–MeOH–water (8:2:0.2) to afford prosapogenin **4c** (12 mg) as a white powder. Negative FAB-MS *m/z*: 1241 $[M+NBA]^+$, 1089 $[M+H]^+$, 459 $[M+H-\text{rha-hex-pen-UA}]^+$. ^{13}C-NMR (pyridine-$d_5$): Tables I and II.

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