Triterpene Glycosides from the Bark of *Robinia pseudo-acacia* L. I[1)]

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From the bark of *Robinia pseudo-acacia* L., five new triterpene glycosides, robiniosides A—D (3, 5—7) and compound III (4), were isolated and their structures were elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl 3β,22β-dihydroxyolean-12-en-29-oic acid (3), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl 3β,22β,24-trihydroxyolean-12-en-29-oic acid (4), whose sapogenol was unambiguously characterized and designated as oxytrogenin, 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl oxytrogenin (5), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-rhamnopyranoside (6), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-rhamnopyranoside (7), respectively, together with two known triterpene glycosides, kaikasaponin III (1) and 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl 3β,22β-dihydroxyolean-12-en-29-oic acid (2).

Keywords *Robinia pseudo-acacia*; Leguminosae; sophoradiol; 3β,22β-dihydroxyolean-12-en-29-oic acid; oxytrogenin; kaikasaponin III; robinioside; oleanene glycoside

Our recent investigation on the triterpene glycosides in the Leguminosae plants led to the isolation of many oleanene glycosides which possessed the peculiarities as follows: 1) carrying a methyl group at C-28 and oxygen function group(s) on the E-ring of the oleanene skeleton; 2) having a glucuronic acid residue in the sugar moiety; 3) expected to be effective for hepatic injury. In a continuing study of this project, we have examined the bark of *Robinia pseudo-acacia* L. (Leguminosae) to obtain five new triterpene glycosides, robiniosides A—D (**3**, **5**—**7**) and compound III (**4**), together with two known glycosides (**1** and **2**).

The methanol extract of the bark of *Robinia pseudo-acacia* L. was partitioned between *n*-hexane and 80% MeOH, and the MeOH extract was further partitioned between 1-BuOH and water. Removal of the solvent of the organic layer gave a residue, which was methylated with CH_2N_2 and separated by normal and reversed phase column chromatographies to yield three oleanene glycoside methyl esters (**1a**—**3a**). On the other hand, the aqueous layer was concentrated and subjected to the MCI gel CHP 20P column chromatography eluting with H_2O, subsequently with dil. MeOH, gradiently. The triterpene fractions were collected and evaporated to give a residue which was methylated with CH_2N_2 after treatment with Amberlite IR-120B and separated by normal and reversed phase column chromatographies to provide four oleanene glycoside methyl esters (**4a**—**7a**).

Compounds I methyl ester (**1a**) and II methyl ester (**2a**) were identified as the methyl esters of kaikasaponin III[2)] and 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl 3β,22β-dihydroxyolean-12-en-29-oic acid,[3)] respectively, by comparing the negative fast atom bombardment mass spectra (FAB-MS), and the proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra with those published earlier.

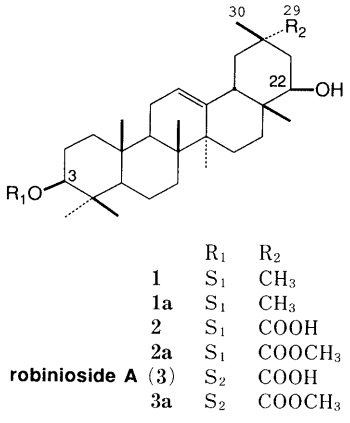
Robinioside A methyl ester (**3a**), a white powder, $[\alpha]_D$ −48.3° (MeOH), showed a quasi-molecular ion at *m/z* 1007.5195 $[M+Na]^+$ ($C_{50}H_{80}O_{19}Na$) in the high resolution fast atom bombardment mass spectrum (HR FAB-MS). Acid hydrolysis of **3a** with 2N HCl–MeOH provided a sapogenol (**9a**), colorless needles, identical with the authentic specimen of 3β,22β-dihydroxyolean-12-en-29-oic acid methyl ester in respect to thin layer chromatography (TLC), $[\alpha]_D$, electron impact mass spectrum (EI-MS) and ^{1}H-NMR spectrum.[3)] The ^{1}H-NMR spectrum of **3a** displayed three anomeric proton signals at δ 6.45 (1H, br s), 5.86 (1H, d, *J*=7.3 Hz) and 5.01 (1H, d, *J*=7.3 Hz), together with one olefinic proton signal at δ 5.35 (1H, br s), two methoxyl signals at δ 3.74 and 3.67 (each 3H, s), one secondary methyl proton signal at δ 1.81 (3H, d, *J*=6.2 Hz) and seven tertiary methyl proton signals at δ 1.68, 1.37, 1.24, 1.24, 1.12, 1.00 and 0.86 (each 3H, s). In the ^{13}C-NMR spectrum of **3a** as listed in Table I, signals due to the sapogenol moiety indicated that the glycosidic linkage located at the C-3 hydroxyl group as well as that of **2a**. Analysis of other signals due to the sugar moiety led to assigning the sugar structure as α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosiduronic acid. Therefore, the structure of robinioside A (**3**) was characterized as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl 3β,22β-dihydroxyolean-12-en-29-oic acid.

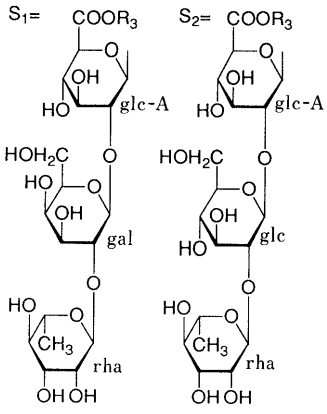
Acid hydrolysis of compound III and robinioside B methyl esters (**4a** and **5a**) afforded the same aglycone (**10a**), colorless needles, mp 252—254 °C, $[\alpha]_D$ +40.8° (MeOH), which showed the absorptions at 3436 cm^{-1} (OH) and 1716 cm^{-1} (ester carbonyl) in the infrared (IR) spectrum, and the molecular ion peak at *m/z* 502 and other characteristic peaks at *m/z* 278 $[D/E\ ring]^+$ and 224 $[A/B\ ring]^+$ due to retro Diels–Alder fission in the EI-MS. These peaks suggested the presence of one methoxycarbonyl group and one hydroxyl group in the D/E ring and two hydroxyl groups in the A/B ring by comparing with those of **9a**. The ^{1}H-NMR spectrum of **10a** displayed one olefinic proton at δ 5.38 (1H, br t), hydroxymethyl protons at δ 4.52, 3.72 (1H each, d, *J*=10.6 Hz), two methine protons at δ 3.88 (1H, br t), 3.69 (1H, br s), one methoxyl group at δ 3.66 (3H, s) and six tertiary methyl groups at δ 1.68, 1.55, 1.24, 1.22, 1.02 and 0.96 (each 3H, s). The ^{13}C-NMR signals, exhibiting one methoxycarbonyl group [δ 179.1 (s), 51.8 (d)], two olefinic carbons [δ 144.0 (s), 123.3 (d)] and three oxygenated carbons [δ 80.1 (d), 75.0 (d) and 64.6 (t)], were also in good agreement with the above deduced structure. The triacetate (**10b**) of **10a** showed the molecular ion peak at *m/z* 628

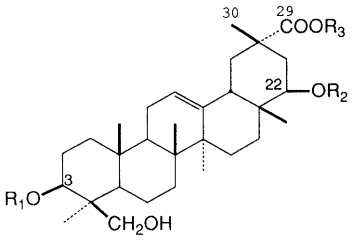
higher by 126 mass units than that of **10a** in the EI-MS, which also suggested that **10a** possessed three hydroxyl groups. Reduction of **10a** with lithium triethylborohydride in tetrahydrofuran (THF) yielded a product **10c**, colorless needles, which exhibited a molecular ion peak at *m/z* 474 lower by 28 mass units than that of **10a** in the EI-MS. The peracetate (**10d**) of **10c** was identical with that of abrisapogenol B obtained from *Abrus cantoniensis*.[4] The sapogenol **10a** was saponified with alkaline to yield the genuine sapogenol (**10**), *m/z* 488 $[M]^+$, showed the IR absorptions at 3456 cm^{-1} due to the hydroxyl groups and 1710 cm^{-1} due to the carboxyl group. Based on the above data, the structure of **10** was thus elucidated as 3β,22β,24-trihydroxyolean-12-en-29-oic acid and named as oxytrogenin which was the sapogenol of saponin-2 isolated by Jia *et al.* from *Oxytropis glabra*,[5] but no datum about the sapogenol was described. This sapogenol was also obtained as the aglycone of the glycosides from *Sophora flavescens* by Ding *et al.*[6] Compound IIIa (**4a**), a white powder, $[\alpha]_D$ $-24.9°$ (MeOH), showed a peak at *m/z* 1023.5132 due to $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$) in the HR FAB-MS. Three anomeric proton signals were observed at δ 6.31 (1H, br s), 5.67 (1H, d, $J=7.7$ Hz) and 4.93 (1H, d, $J=7.3$ Hz), together with signals due to one olefinic proton at δ 5.34 (1H, br s), two methoxyl groups at δ 3.76 and 3.66 (each 3H, s), one secondary methyl group at δ 1.77 (3H, d, $J=6.2$ Hz) and six tertiary methyl groups at δ 1.68, 1.43, 1.25, 1.23, 0.95 and 0.72 (each 3H, s) in the ^{1}H-NMR spectrum of **4a**. A comparative study of the ^{13}C-NMR spectrum of **4a** with those of **1a** and **2a** led to the identification of the sugar moiety, that is, suggesting it is to be the α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl residue. On the other hand, the ^{13}C-NMR signals due to the sapogenol part displayed the glycosylation shifts at C-2 (−2.2 ppm), C-3 (+11.1 ppm) and C-4 (−0.6 ppm) in comparison with those of **10a**, which indicated **4a** was a 3-*O*-monodesmoside. From the above evidence, the structure of **4** could be represented as oxytrogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside.

Robinioside B (**5**) methyl ester (**5a**), a white powder, $[\alpha]_D$ $-27.5°$ (MeOH), showed a peak at *m/z* 1023.5134 due to $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$) in the HR FAB-MS. However, in the ^{13}C-NMR spectrum of **5a**, signals caused from the sapogenol moiety suggested the glycosidic linkage to be at the C-3 hydroxyl group of oxytrogenin as well as that of **4a**. On the other hand, the remaining signals derived from the sugar residue were superimposable on those of **3a**. From the above evidence, the structure of **5** was characterized as oxytrogenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranoside.

Robinioside C (**6**) methyl ester (**6a**), a white powder, $[\alpha]_D$ $-35.2°$ (MeOH), on acid hydrolysis provided the oxytrogenin methyl ester (**10a**) as sapogenol. The HR FAB-MS of **6a** showed a peak due to $[M+Na]^+$ ($C_{56}H_{90}O_{24}Na$) at *m/z* 1169.5726. A comparative study of the ^{1}H- and ^{13}C-NMR spectra of **6a** with those of **4a** led to the identification of the sugar moiety, that is, suggesting the presence of an α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl group and a terminal α-L-rhamnopyranosyl group. The negative FAB-MS of **6a** gave a $[M-H]^-$ peak at *m/z* 1145 higher by 146 mass units than that of **4a**, which also supported the above deduced structure. Enzymatic hydrolysis of **6** with glycyrrhizinic acid hydrolase (GH)[7] yielded a product (**11**), which was subsequently treated with diazomethane to afford the corresponding methyl ester (**11a**), a white powder, $[\alpha]_D$ $-83.3°$ (MeOH). Compound **11a** showed the peaks at *m/z* 647 due to $[M-H]^-$ and 501 due to $[M-rha-H]^-$ in the negative FAB-MS, which indicated no existence of the

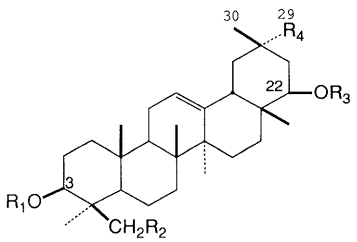






		R_1	R_2	R_3
	1	S_1	CH_3	H
	1a	S_1	CH_3	CH_3
	2	S_1	COOH	H
	2a	S_1	$COOCH_3$	CH_3
robinioside A	(3)	S_2	COOH	H
	3a	S_2	$COOCH_3$	CH_3

		R_1	R_2	R_3
compound III	(4)	S_1	H	H
	4a	S_1	H	CH_3
robinioside B	(5)	S_2	H	H
	5a	S_2	H	CH_3
robinioside C	(6)	S_1	Rha′	H
	6a	S_1	Rha′	CH_3
robinioside D	(7)	S_2	Rha′	H
	7a	S_2	Rha′	CH_3



	R_1	R_2	R_3	R_4
8a	H	H	H	CH_3
9a	H	H	H	$COOCH_3$
10	H	OH	H	COOH
10a	H	OH	H	$COOCH_3$
10b	Ac	OAc	Ac	$COOCH_3$
10c	H	OH	H	CH_2OH
10d	Ac	OAc	Ac	CH_2OAc
11	H	OH	Rha	COOH
11a	H	OH	Rha	$COOCH_3$

TABLE I. ^{13}C-NMR Spectral Data for **1a**—**7a**, **10**, **10a** and **11a** (Pyridine-d_5)

	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**10**	**10a**	**11a**
C- 1	38.9	38.7	38.7	38.5	38.4	38.4	38.3	38.9	38.9	38.8
C- 2	26.4[a)]	26.4[a)]	26.4[a)]	26.5[a)]	26.5[a)]	26.5	26.4	28.9	28.7	28.3
C- 3	90.0	89.8	89.8	91.2	91.6	91.1	91.5	80.1	80.1	80.0
C- 4	39.6	39.5	39.6	43.8	43.8	43.7	43.5	43.2	43.2	43.1
C- 5	55.9	55.7	55.7	56.0	56.1	55.8	56.0	56.3	56.3	56.2
C- 6	18.5	18.3	18.4	18.4	18.5	18.3	18.3	19.1	19.1	19.0
C- 7	33.1	33.0	33.0	33.1	33.1	33.0	32.9	33.6	33.5	33.3
C- 8	40.0	39.9	39.9	39.8	39.8	39.7	39.7	40.0	40.0	39.9
C- 9	47.9	47.7	47.8	47.7	47.6	47.5	47.5	48.4	48.1	47.9
C-10	36.8	36.7	36.8	36.4	36.4	36.3	36.2	37.0	37.0	36.9
C-11	23.8	23.7	23.8	24.0	24.0	23.8	23.8	24.1	24.1	24.0
C-12	122.5	123.2	123.3	123.1	123.1	123.3	123.3	123.2	123.3	123.4
C-13	144.8	143.9	143.9	144.0	144.0	144.2	143.3	144.3	144.0	143.3
C-14	42.4	42.2	42.2	42.2	42.2	41.9	41.9	42.4	42.3	42.0
C-15	26.3[a)]	26.2[a)]	26.2[a)]	26.2[a)]	26.2[a)]	25.8	25.8	26.4	26.3	25.9
C-16	28.7	28.7	28.5	28.5	28.5	28.3	28.2	28.4	28.4	28.3
C-17	37.9	37.7	37.8	37.8	37.8	37.2	37.2	38.0	37.9	37.3
C-18	45.3	44.1	44.1	44.1	44.1	44.0	44.0	44.7	44.3	44.2
C-19	46.7	40.8	40.9	40.9	40.9	40.5	40.5	41.6	41.0	40.7
C-20	30.8	42.5	42.6	42.6	42.6	42.0	42.0	42.6	42.7	42.1
C-21	42.4	37.1	37.2	37.1	37.2	30.9	30.9	37.8	37.3	31.0
C-22	75.5	74.9	75.0	74.9	74.9	79.1	79.1	75.3	75.0	79.2
C-23	28.6	28.3	28.3	22.9	22.6	22.8	22.6	23.6	23.6	23.6
C-24	15.7	15.6	15.6	63.4	63.3	63.4	63.2	64.6	64.6	64.5
C-25	16.8	16.7	16.7	15.8	15.6	15.6	15.5	16.3	16.3	16.2
C-26	17.2	17.0	17.0	16.9	16.9	16.7	16.7	17.1	17.1	16.7
C-27	25.7	25.5	25.5	25.5	25.5	25.3	25.3	25.5	25.6	25.5
C-28	28.4	20.8	20.9	20.9	20.9	20.9	20.9	21.0	21.0	21.0
C-29	33.3	179.0	179.1	179.1	179.1	178.4	178.4	181.5	179.1	178.4
C-30	21.1	24.3	24.4	24.3	24.3	23.4	23.5	25.0	24.5	23.4
COOMe		51.7	51.7	51.8	51.8	51.7	51.7		51.8	51.7
Glc-UA										
1	105.3	105.1	105.3	105.3	105.1	105.3	105.0			
2	79.0	78.9	78.5[b)]	78.0	78.1	77.9	78.1[a)]			
3	76.7[b)]	76.6[b)]	76.7	76.7[b)]	76.5	76.7[a)]	76.5			
4	73.1	73.0	73.2	73.4	73.3	73.4	73.3			
5	78.4	78.3	77.8	77.5	77.5	77.5	77.5			
6	170.6	170.6	170.6	170.2	170.2	170.2	170.2			
COOMe	51.9	51.9	51.9	52.1	52.0	52.0	52.0			
Gal										
1	101.9	101.9		101.6		101.5				
2	76.3[b)]	76.2[b)]		76.4[b)]		76.4[a)]				
3	76.1[b)]	76.0[b)]		76.3[b)]		76.3[a)]				
4	70.4	70.3		71.0		70.9				
5	76.4[b)]	76.3[b)]		76.4[b)]		76.4[a)]				
6	61.9	61.8		61.5		61.4				
Glc										
1			102.0		101.8		101.8			
2			79.4		79.0		78.9			
3			77.8		77.9		77.9			
4			69.4		69.6		69.5			
5			78.3[b)]		78.1		78.0[a)]			
6			62.3		61.6		61.2			
Rha										
1	102.7	102.6	102.0	102.2	101.8	102.2	101.8			
2	72.3[c)]	72.2[c)]	72.3[c)]	72.2[c)]	72.1[b)]	72.1[b)]	72.1[b)]			
3	72.6[c)]	72.5[c)]	72.7[c)]	72.5[c)]	72.5[b)]	72.4[b)]	72.5[b)]			
4	74.3	74.2	74.3	74.2	74.1	74.1	74.1			
5	69.3	69.3	69.4	69.2	69.2	69.1	69.2			
6	18.9	18.8	19.0	18.8	18.8	18.8	18.8			
Rha'										
1						98.3	98.4			98.5
2						72.5[b)]	72.5[b)]			72.5
3						72.8[b)]	72.8[b)]			72.9
4						73.6	73.6			73.7
5						70.2	70.3			70.3
6						18.3	18.4			18.4

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

rhamnosyl-galactosyl-glucuronosyl residue in **11a**. Furthermore, in the ^{13}C-NMR spectrum of **11a**, signals of the sugar part were assignable to the α-L-rhamnopyranosyl moiety, and C-3 chemical shift indicated that the C-3 hydroxyl group was free and the α-L-rhamnopyranosyl group linked to the C-22 hydroxyl group of the sapogenol [glycosylation shifts δ (ppm): C-17, 37.3 (−0.6); C-21, 31.0 (−6.3); C-22, 79.2 (+4.2)] in comparison with those of oxytrogenin methyl ester (**10a**). Consequently, the full structure of **6** was elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-rhamnopyranoside.

Robinioside D (**7**) methyl ester (**7a**), a white powder, $[\alpha]_D$ −32.7° (MeOH), showed a peak due to $[M+Na]^+$ ($C_{56}H_{90}O_{24}Na$) at *m/z* 1169.5726 in the HR FAB-MS. Acid hydrolysis of **7a** afforded oxytrogenin methyl ester (**10a**) as sapogenol. Four anomeric proton signals were observed at δ 6.42 (1H, br s), 5.88 (1H, d, *J*=7.7 Hz), 5.49 (1H, br s) and 4.97 (1H, d, *J*=7.7 Hz), and together with the signals originated from two methoxyl protons at δ 3.76 and 3.66 (each 3H, s), two secondary methyl protons at δ 1.79 (3H, d, *J*=6.2 Hz) and 1.71 (3H, d, *J*=5.5 Hz) and six tertiary methyl protons at δ 1.47, 1.40, 1.18, 1.01, 0.92 and 0.71 (each 3H, s) in the ^{1}H-NMR spectrum of **7a**. The ^{13}C-NMR spectrum of **7a** suggested the appearance of a terminal α-L-rhamnopyranosyl group at the C-22 hydroxyl group and the α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl moiety at the C-3 hydroxyl group of sapogenol as compared with those of **6a**. The peak at *m/z* 1145 due to $[M-H]^-$ in the negative FAB-MS of **7a** was also in good agreement with the deduced structure. Therefore, the structure of **7** could be represented as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl oxytrogenin 22-*O*-α-L-rhamnopyranoside.

Experimental

Optical rotations were measured on a JASCO DIP-360 automatic digital polarimeter. The IR spectra were recorded with a Hitachi IR spectrometer, model 270-30. 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 (TMS) as an internal standard. The FAB- and EI-MS were recorded with a JEOL DX-303 HF spectrometer and taken in a glycerol matrix containing NaI, and HR FAB-MS spectra were measured with a JEOL HX-110 spectrometer. Thin layer chromatography was performed on a precoated Kieselgel 60 F_{254} plate (0.2 mm Merck) and detection was achieved by spraying 10% H_2SO_4 followed by heating. Column chromatography was carried out with Sephadex LH-20 (Pharmacia), MCI gel CHP 20P (Mitsubishi Kasei Corporation), Bondapak C_{18} (Waters Associates) and Kieselgel 60 (70—230 and 230—400 mesh, Merck).

Extraction and Separation The bark (9 kg) of *Robinia pseudo-acacia* L. collected in Kumamoto, Japan was extracted with MeOH and the extract (371 g) was partitioned between *n*-hexane and 80% MeOH. The 80% MeOH extract was further partitioned with 1-BuOH and water. The 1-BuOH soluble portion (58 g) was subjected to MCI gel CHP 20P column chromatography with water and 10% MeOH→MeOH to afford a number of fractions by TLC monitoring. The triterpene frs. (9.8 g) were respectively treated with excess diazomethane after passing through an Amberlite IR-120B column, followed by column chromatography on a Bondapak C_{18} column with 50% MeOH→MeOH to provide compounds **1a** (11 mg), **2a** (34 mg) and **3a** (25 mg). The aqueous layer was concentrated and subjected to the MCI gel CHP 20P column with H_2O→MeOH, and triterpene fractions were collected and evaporated into a residue which was methylated with CH_2N_2 after treatment of Amberlite IR-120B column and separated by normal and reversed phase column chromatographies to yield compounds **4a** (124 mg), **5a** (78 mg), **6a** (79 mg) and **7a** (54 mg), respectively.

Compound Ia (1a) (Kaikasaponin III Methyl Ester) A white powder, $[\alpha]_D$ −8.7° (*c*=0.60, MeOH). Negative FAB-MS *m/z*: 939 $[M-H]^-$, 793 $[M-rha-H]^-$, 401 $[M-glc\ UA(Me)-gal-rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.86, 0.98, 0.99, 1.11, 1.18, 1.23, 1.26, 1.33 (each 3H, s, Me×8), 1.67 (3H, d, *J*=6.2 Hz, rha Me-6), 3.25 (1H, dd, *J*=4.4, 11.7 Hz, H-3), 3.71 (3H, s, glc UA COOMe-6), 3.82 (1H, t, *J*=5.5 Hz, H-22), 4.92 (1H, d, *J*=7.3 Hz, glc UA H-1), 5.29 (1H, br s, H-12), 5.57 (1H, d, *J*=7.7 Hz, gal H-1), 6.18 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Hydrolysis of 1a A solution of **1a** (6 mg) in 2 N HCl–MeOH (2 ml) was heated at 90 °C for 1 h and then neutralized with 3% KOH–MeOH. The deposited salt was filtered off to give a solution which was evaporated to afford a residue. It was then partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract was chromatographed over silica gel column (*n*-hexane–acetone (8:1)) to provide the aglycone **8a**, colorless needles (MeOH), mp 228—230 °C, $[\alpha]_D$ +74.3° (*c*=0.28, $CHCl_3$). EI-MS *m/z*: 442 $[M]^+$, 234 [D/E ring]$^+$, 219, 189 [A/B ring−H_2O−H]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.79, 0.88, 0.91, 0.95, 1.00, 1.04, 1.12 (each 3H, s, Me×8), 3.22 (1H, dd, *J*=5.1, 11.0 Hz, H-3), 3.44 (1H, t, *J*=5.1 Hz, H-22), 5.25 (1H, br s, H-12).

Compound IIa (2a) A white powder, $[\alpha]_D$ −9.9° (*c*=0.52, MeOH). Negative FAB-MS *m/z*: 983 $[M-H]^-$. 837 $[M-rha-H]^-$, 485 $[M-glc\ UA(Me)-gal-rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.87, 0.99, 1.16, 1.23, 1.23, 1.39, 1.68 (each 3H, s, Me×7), 1.75 (3H, d, *J*=6.2 Hz, rha Me-6), 3.30 (1H, m, H-3), 3.66, 3.73 (each 3H, s, COOMe×2), 5.02 (1H, d, *J*=7.3 Hz, glc UA H-1), 5.34 (1H, br s, H-12), 5.71 (1H, d, *J*=7.7 Hz, gal H-1), 6.32 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Robinioside A Methyl Ester (3a) A white powder, $[\alpha]_D$ −48.3° (*c*=1.00, MeOH). HR FAB-MS *m/z*: 1007.5195 $[M+Na]^+$ ($C_{50}H_{80}O_{19}Na$, Calcd for 1007.5193). Negative FAB-MS *m/z*: 983 $[M-H]^-$, 837 $[M-rha-H]^-$, 485 $[M-glc\ UA(Me)-glc-rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.86, 1.00, 1.12, 1.24, 1.24, 1.37, 1.68 (each 3H, s, Me×7), 1.81 (3H, d, *J*=6.2 Hz, rha Me-6), 3.30 (1H, m, H-3), 3.67, 3.74 (each 3H, s, COOMe), 5.01 (1H, d, *J*=7.3 Hz, glc UA H-1), 5.35 (1H, br s, H-12), 5.86 (1H, d, *J*=7.3 Hz, glc H-1), 6.45 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 3a A solution of **3a** (16 mg) in 2 N HCl–MeOH (3 ml) was heated at 90 °C for 2 h and then neutralized with 3% KOH–MeOH. The reaction mixture was filtered to give a solution which was evaporated to provide a residue, which was partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract was chromatographed over silica gel column (*n*-hexane–acetone (4:1)) to provide the aglycone **9a**, colorless needles (MeOH), mp 242—244 °C, $[\alpha]_D$ +59.5° (*c*=1.12, $CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3484 (OH), 1716 (COOMe). EI-MS *m/z*: 486 $[M]^+$, 468 $[M-H_2O]^+$, 453 $[M-H_2O-CH_3]^+$, 427 $[M-COOCH_3]^+$, 278 [D/E ring]$^+$, 231, 219, 207 [A/B ring]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.79, 0.89, 0.95, 0.98, 1.00, 1.13, 1.36 (each 3H, s, Me×7), 3.22 (1H, dd, *J*=4.8, 11.0 Hz, H-3), 3.54 (1H, t, *J*=3.3 Hz, H-22), 5.29 (1H, br t, H-12).

Compound III Methyl Ester (4a) A white powder, $[\alpha]_D$ −24.9° (*c*=0.61, MeOH). HR FAB-MS *m/z*: 1023.5132 $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$, Calcd for 1023.5142). Negative FAB-MS *m/z*: 999 $[M-1]^-$, 853 $[M-rha-H]^-$, 501 $[M-glc\ UA(Me)-gal-rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.72, 0.95, 1.23, 1.25, 1.43, 1.68 (each 3H, s, Me×6), 1.77 (3H, d, *J*=6.2 Hz, rha Me-6), 3.38 (1H, dd, *J*=4.4, 11.7 Hz, H-3), 3.66, 3.76 (each 3H, s, COOMe×2), 4.93 (1H, d, *J*=7.3 Hz, glc UA H-1), 5.34 (1H, br s, H-12), 5.67 (1H, d, *J*=7.7 Hz, gal H-1), 6.31 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 4a and 5a A solution of **4a** and **5a** (120 mg) in 2 N HCl–MeOH (5 ml) was refluxed at 90 °C for 2 h and then neutralized with 3% KOH–MeOH. The reaction mixture was filtered to give a solution which was concentrated and partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract was chromatographed over silica gel column ($CHCl_3$–MeOH (30:1)) to provide the same aglycone **10a** (48 mg), colorless needles, mp 252—254 °C, $[\alpha]_D$ +40.8° (*c*=0.42, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3436 (OH), 1716 (ester carbonyl). *Anal.* Calcd for $C_{31}H_{50}O_5 \cdot H_2O$: C, 72.06; H, 9.81. Found: C, 71.54; H, 10.00. EI-MS *m/z*: 502 $[M]^+$, 443 $[M-COOMe]^+$, 278 [D/E ring]$^+$, 224 [A/B ring]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.96, 1.02, 1.22, 1.24, 1.55, 1.68 (each 3H, s, Me×6), 3.66 (3H, s, COOMe), 3.88 (1H, br t, H-22), 3.72, 4.52 (2H, ABq, *J*=10.6 Hz, 24-H_2), 5.38 (1H, br t, H-12). ^{13}C-NMR (pyridine-d_5): Table I.

Acetylation of 10a A solution of **10a** (5 mg) in Ac_2O–pyridine (1:1, 1 ml) was allowed to stand at room temperature for 12 h. The reaction mixture was evaporated by blowing N_2 gas to give a residue which was purified by silica gel column chromatography (*n*-hexane–acetone (5:1)) to yield the peracetate **10b** (4 mg), colorless plates, mp 217—219 °C (MeOH),

$[\alpha]_D$ +59.7° (c=0.89, $CHCl_3$). EI-MS m/z: 628 $[M]^+$, 568 $[M-AcOH]^+$, 509 $[M-COOCH_3-AcOH]^+$, 449 $[M-COOCH_3-2\times AcOH]^+$, 320 [D/E ring]$^+$, 308 [A/B ring]$^+$. ^{1}H-NMR ($CDCl_3$) δ: 0.82, 0.97, 0.98, 1.03, 1.15, 1.33 (each 3H, s, Me×6), 2.04, 2.05, 2.06 (each 3H, s, OAc×3), 3.67 (3H, s, COOMe), 4.14, 4.37 (2H, ABq, J=11.7 Hz, 24-H_2), 4.59 (1H, dd, J=5.5, 10.6 Hz, 3-H), 4.75 (1H, br t, 22-H), 5.30 (1H, br t, 12-H).

Reduction of 10a Compound **10a** (14 mg) was dissolved in lithium triethylborohydride ($LiBEt_3H$)/THF (2 ml) and was allowed to stand at room temperature overnight. After removal of the solvent, the reaction product was purified by silica gel column chromatography (n-hexane–acetone (2 : 1)) to yield the reduction product **10c** (8 mg), colorless needles (MeOH–H_2O), mp 282—284 °C, $[\alpha]_D$ +25.3° (c=0.34, pyridine). EI-MS m/z: 474 $[M]^+$, 456 $[M-H_2O]^+$, 443 $[M-CH_2OH]^+$, 250 [D/E ring]$^+$, 224 [A/B ring]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.05, 1.27, 1.29, 1.49, 1.57 (each 3H, s, Me×6), 3.65 (2H, br s, 29-H_2), 3.73, 4.54 (2H, ABq, J=11.7 Hz, 24-H_2), 3.95 (1H, br t, 3-H), 5.41 (1H, br s, 12-H).

Acetylation of 10c A solution of **10c** (8 mg) in Ac_2O–pyridine (1 : 1, 1 ml) was allowed to stand at room temperature overnight. The reaction mixture was evaporated by blowing N_2 gas to give a residue which was purified by silica gel column chromatography (n-hexane–acetone (5 : 1)) to provide the peracetate **10d** (6 mg) as colorless plates, mp 156—158 °C, $[\alpha]_D$ +60.8° (c=0.44, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.83, 0.97, 0.98, 1.03, 1.06, 1.13 (each 3H, s, Me×6), 2.04, 2.05, 2.07, 2.08 (each 3H, s, OAc×4), 3.68, 3.73 (2H, ABq, J=10.6 Hz, 29-H_2), 4.13, 4.37 (2H, ABq, J=11.5 Hz, 24-H_2), 4.58 (1H, dd, J=5.5, 10.6 Hz, H-3), 4.71 (1H, br t, H-22), 5.28 (1H, br t, H-12).

Saponification of 10a A solution of **10a** (25 mg) in 3% KOH–dil. MeOH was refluxed at 90 °C for 3 h. The reaction mixture was neturalized with 1 N HCl–H_2O and subjected to MCI gel CHP 20P column eluting with water, and successively dil. MeOH, gradiently. The sapogenol fraction was evaporated and purified by silica gel column chromatography to afford the genuine sapogenol **10** (14 mg), white needles, mp >300 °C, $[\alpha]_D$ +54.8° (c=1.38, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3456 (OH), 1710 (COOH). EI-MS m/z: 488 $[M]^+$, 470 $[M-H_2O]^+$, 452 $[M-2H_2O]^+$, 443 $[M-COOH]^+$, 264 [D/E ring]$^+$, 246 [D/E ring$-H_2O$]$^+$, 224 [A/B ring]$^+$, 206 [A/B ring$-H_2O$]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.04, 1.26, 1.29, 1.57, 1.86 (each 3H, s, Me×6), 3.65 (1H, br d, J=11.7 Hz, H-3), 3.74, 4.54 (2H, ABq, J=11.3 Hz, 24-H_2), 4.04 (1H, br s, H-22), 5.43 (1H, br s, H-12). ^{13}C-NMR (pyridine-d_5): Table I.

Robinioside B (5) Methyl Ester (5a) A white powder, $[\alpha]_D$ −27.5° (c=1.49, MeOH). HR FAB-MS m/z: 1023.5134 $[M+Na]^+$ ($C_{50}H_{80}O_{20}Na$, Calcd for 1023.5142). Negative FAB-MS m/z: 999 $[M-H]^-$, 853 $[M-rha-H]^-$, 501 $[M-glc\ UA(Me)-glc-rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.69, 0.93, 1.20, 1.22, 1.41, 1.64 (each 3H, s, Me×6), 1.73 (3H, d, J=5.5 Hz, rha Me-6), 3.65, 3.76 (each 3H, s, COOMe×2), 4.91 (1H, d, J=7.7 Hz, glc UA H-1), 5.32 (1H, br s, H-12), 5.78 (1H, d, J=7.7 Hz, glc H-1), 6.42 (1H, br s, rha H-1). ^{13}C-NMR (pyridine-d_5): Table I. Acid hydrolysis of **5a** was accomplished together with **4a**.

Robinioside C (6) Methyl Ester (6a) A white powder, $[\alpha]_D$ −35.2° (c=0.98, MeOH). HR FAB-MS m/z: 1169.5726 $[M+Na]^+$ ($C_{56}H_{90}O_{24}Na$, Calcd for 1169.5721). Negative FAB-MS m/z: 1145 $[M-H]^-$, 999 $[M-rha-H]^-$, 501 $[M-glc\ UA(Me)-gal-2\times rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.73, 0.93, 1.01, 1.19, 1.39, 1.41 (each 3H, s, Me×6), 1.70 (3H, d, J=5.5 Hz, rha′ Me-6), 1.75 (3H, d, J=6.2 Hz, rha Me-6), 3.61, 3.75 (each 3H, s, COOMe×2), 4.93 (1H, d, J=7.3 Hz, glc UA H-1), 5.26 (1H, br s, H-12), 5.47 (1H, br s, rha′ H-1), 5.78 (1H, d, J=7.7 Hz, gal H-1), 6.31 (1H, br s, rha H-1).

Acid Hydrolysis of 6a A solution of **6a** (10 mg) in 2 N HCl–MeOH (5 ml) was refluxed at 90 °C for 2 h and then neutralized with 3% KOH–MeOH. The reaction mixture was filtered to give a solution which was concentrated and partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract was chromatographed over silica gel column ($CHCl_3$–MeOH (30 : 1)) to provide a sapogenol which was identical with oxytrogenin methyl ester (**10a**) with respect to TLC and ^{1}H-NMR spectrum.

Enzymatic Hydrolysis of 6 To a solution of **6** (50 mg) in acetate buffer (pH=4.2, 5 ml) was added glycyrrhizinic acid hydrolase (GH) (2 ml), and the mixture was incubated at 40 °C for 2 h. The reaction process was followed by TLC over silica gel ($CHCl_3$–MeOH–H_2O (7 : 3 : 0.5)) every 20 min. When the hydrolysis had been completed, the hydrolysate was subjected to MCI gel CHP 20P column using H_2O and MeOH to give a product **11**, which was treated with diazomethane followed by silica gel chromatography ($CHCl_3$–MeOH–H_2O (10 : 1 : 0.1)), the corresponding methyl ester **11a** (18 mg) was obtained as a white powder, $[\alpha]_D$ −83.3° (c=0.83, MeOH). Negative FAB-MS m/z: 647 $[M-H]^-$, 501 $[M-rha-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 0.99, 1.02, 1.16, 1.40, 1.55 (each 3H, s, Me×6), 1.70 (3H, d, J=5.5 Hz, rha′ Me-6), 3.62 (3H, s, COOMe), 3.72, 4.52 (2H, ABq, J=11.0 Hz, 24-H_2), 5.30 (1H, br s, H-12), 5.47 (1H, br s, rha′ H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Robinioside D (7) Methyl Ester (7a) A white powder, $[\alpha]_D$ −32.7° (c=1.17, MeOH). HR FAB-MS m/z: 1169.5726 $[M+Na]^+$ ($C_{56}H_{90}O_{24}Na$, Calcd for 1169.5721). Negative FAB-MS m/z: 1145 $[M-H]^-$, 999 $[M-rha-H]^-$, 501 $[M-glc\ UA(Me)-glc-2\times rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.71, 0.92, 1.01, 1.18, 1.40, 1.47 (each 3H, s, Me×6), 1.71 (3H, d, J=5.5 Hz, rha′ Me-6), 1.79 (3H, d, J=6.2 Hz, rha Me-6), 3.66, 3.76 (each 3H, s, COOMe), 4.97 (1H, d, J=7.7 Hz, glc UA H-1), 5.26 (1H, br s, H-12), 5.49 (1H, br s, rha′ H-1), 5.88 (1H, d, J=7.7 Hz, glc H-1), 6.42 (1H, br s, rha H-1).

Acid Hydrolysis of 7a A solution of **7a** (10 mg) was hydrolyzed in the same way as **6a** to afford a sapogenol which was identical with oxytrogenin methyl ester (**10a**) by TLC and ^{1}H-NMR spectrum.

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References and Notes

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