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

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Received July 1, 1992

From the bark of *Robinia pseudo-acacia* L., six new triterpene glycosides, Robiniosides E—J, were isolated and their structures were elucidated as abrisapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside (1), abrisapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranoside (2), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol B 22-*O*-α-L-rhamnopyranoside (3), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol B 22-*O*-α-L-rhamnopyranoside (4), 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol E 30-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (5) and 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol E 30-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside (6), respectively.

Keywords *Robinia pseudo-acacia*; Leguminosae; abrisapogenol; robinioside; triterpene glycoside

We previously reported the isolation of robiniosides A—D and sophoraflavoside II from the bark of *Robinia pseudo-acacia*.[2)] In a continuing investigation on the oligoglycosidic constituents, we obtained six more triterpenoidal glycosides, robiniosides E–J (**1**—**6**). This paper deals with the isolation and structural elucidation of these new compounds (**1**—**6**).

The methanol extract of the bark of *Robinia pseudo-acacia* L. was partitioned between *n*-hexane and 80% MeOH, and then the MeOH extract was further partitioned with 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 two oleanene glycoside methyl esters (**1a** and **2a**). On the other hand, the aqueous layer was concentrated and subjected to MCI gel CHP 20P column chromatography eluting with dil. MeOH gradiently. Triterpenoid fractions were collected and evaporated to give a residue which was treated with CH_2N_2 after passing through Amberlite IR-120B, then separated by normal and reversed phase column chromatographies to yield four oleanene glycoside methyl esters (**3a**—**6a**).

Robinioside E (**1**) was obtained as the corresponding methyl ester (**1a**), a white powder, $[\alpha]_D$ −12.2° (MeOH). Acid hydrolysis of **1a** with 2 N HCl–MeOH provided a sapogenol (**7**), colorless needles, mp 281—283 °C, $[\alpha]_D$ +26.4° (pyridine), which showed a molecular ion peak at *m/z* 474 and other characteristic peaks at 250 $[D/E\ ring]^+$ and 224 $[A/B\ ring]^+$ due to retro Diels–Alder fission in the electron impacting mass spectrum (EI-MS). The tetraacetate (**7a**) of **7**, colorless plates, mp 157—158 °C, $[\alpha]_D$ +59.3° ($CHCl_3$), displayed signals at δ 5.29 (1H, br t), 4.71 (1H, t, J=3.7 Hz), 4.59 (1H, dd, J=10.6, 5.5 Hz), 4.37, 4.14 (2H, ABq, J=11.7 Hz), 3.74 and 3.67 (2H, ABq, J=10.6 Hz) in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, which was identical with that of abrisapogenol B tetraacetate obtained from *Abrus cantoniensis*.[3)] Robinioside E methyl ester (**1a**) showed a peak at *m/z* 971 due to $[M-H]^-$ in the negative fast atom bombardment mass spectrum (FAB-MS). Three anomeric proton signals were observed at δ 6.31 (1H, br s), 5.79 (1H, d, J=7.7 Hz) and 4.93 (1H, d, J=7.7 Hz) in the ^{1}H-NMR spectrum of **1a**. A comparative study of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **1a** with that of sophoraflavoside II[2)] led to the identification in the sugar moiety, that is, suggesting the occurrence of an 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 moiety showed a downfield shift by 11.1 ppm at the C-3 in comparison with that of abrisapogenol B, to which the sugar part was attached. From the above evidence, the structure of **1** could be represented as abrisapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranoside.

Robinioside F methyl ester (**2a**), a white powder, $[\alpha]_D$ −11.2° (MeOH), on acid hydrolysis afforded abrisapogenol B (**7**) as sapogenol, which was identified by thin layer chromatography (TLC) and ^{1}H-NMR spectrum. The negative FAB-MS gave peaks at *m/z* 971 and 473 due to $[M-H]^-$ and $[aglycone\ moiety]^-$, respectively. However, in the ^{13}C-NMR spectrum of **2a**, signals caused by the sapogenol moiety suggested the glycosidic linkage is located at the C-3-OH of abrisapogenol B as well as **1a**, and other signals derived from the sugar residue were assignable to the *O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl residue, which was substantiated by the observation of three anomeric proton signals at 6.43 (1H, br s), 5.88 (1H, d, J=7.7 Hz) and 4.97 (1H, d, J=7.7 Hz) in the ^{1}H-NMR spectrum of **2a**. Therefore, the structure of robinioside F (**2**) was characterized as abrisapogenol B 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranoside.

Robinioside G methyl ester (**3a**), a white powder, $[\alpha]_D$ −27.5° (MeOH), on acid hydrolysis provided a sapogenol which was identical with abrisapogenol B (**7**) in respect to the TLC and ^{1}H-NMR spectrum. A comparative study of the ^{1}H- and ^{13}C-NMR spectra of **3a** with those of **1a** led to identification at the sugar moiety, suggesting the occurrence of an α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl moiety and a terminal α-L-rhamnopyranosyl moiety. This fact was supported by the appearance of a $[M-H]^-$ peak at

m/z 1117 which was 146 mass units higher than that of **1a** in the negative FAB-MS of **3a**. Enzymatic hydrolysis with glycyrrhizinic acid hydrolase[4] of a mixture of **3** and **4**, occurring during the course of the separation procedure, yielded a sole product **8**, a white powder, $[\alpha]_D$ +19.6° (MeOH), which gave peaks at m/z 619 and 473 due to $[M-H]^-$ and $[M-Rha-H]^-$, respectively, in negative FAB-MS. It showed a lack of the rhamnosyl-galactosyl-glucuronosyl group in **3**. Meanwhile, the ^{1}H-NMR signals at δ 5.49 (1H, br s), 5.32 (1H, br s), 1.71 (1H, d, J=5.5 Hz) and 1.55, 1.23, 1.22, 1.09, 1.02 and 0.97 (each 3H, s) in **8** were also in a good agreement with the above deduced structure. Furthermore, in the ^{13}C-NMR spectrum of **8**, signals caused by the sugar part could be assigned to one mole of α-L-rhamnopyranosyl moiety, and other signals due to the sapogenol part revealed that the C-3-OH was free and that the sugar moiety linked to the C-22-OH of sapogenol [glycosylation shifts δ (ppm): C-21, 30.7 (−6.6); C-22, 79.6 (+4.0)] in comparison with those of abrisapogenol B.[3] Consequently, the full structure of robinioside G (**3**) was elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol B 22-*O*-α-L-rhamnopyranoside.

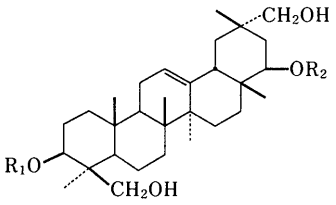
Robinioside H methyl ester (**4a**), a white powder, $[\alpha]_D$ −28.4° (MeOH), on acid hydrolysis afforded abrisapogenol B (**7**) as sapogenol. Four anomeric proton signals were observed at δ 6.38 (1H, br s), 5.84 (1H, d, J=7.7 Hz), 5.48 (1H, br s) and 4.94 (1H, d, J=7.7 Hz) together with signals due to two secondary methyl groups at δ 1.76 (1H, d, J=6.2 Hz) and 1.70 (1H, d, J=5.5 Hz) in the ^{1}H-NMR spectrum of **4a**. The ^{13}C-NMR spectrum of **4a** suggested the appearance of a terminal α-L-rhamnopyranosyl group at C-22-OH and an α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-6-*O*-methyl-β-D-glucuronopyranosyl moiety at C-3-OH of sapogenol by comparing it with those of **2a** and **3a**. This was supported by the $[M-H]^-$ peak at m/z 1117 in the negative FAB-MS of **4a**. Therefore, the full structure of robinioside H (**4**) could be elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucuronopyranosyl abrisapogenol B 22-*O*-α-L-rhamnopyranoside.

Robinioside I methyl ester (**5a**), a white powder, $[\alpha]_D$ −14.3° (MeOH), on acid hydrolysis yielded a sapogenol (**9**), colorless needles, mp 276—278 °C, $[\alpha]_D$ +83.2° (MeOH), which exhibited a molecular ion peak at m/z 474 and other characteristic peaks at 250 $[D/E\ ring]^+$ and 206 $[A/B\ ring\text{-}H_2O]^+$ due to retro Diels–Alder fission in the EI-MS. Tetraacetate (**9a**) of **9**, colorless plates, mp 278—280 °C, $[\alpha]_D$ +72.3° ($CHCl_3$), showed signals at δ 5.27 (1H, br t), 4.66 (1H, t, J=3.3 Hz), 4.59 (1H, dd, J=10.6, 5.1 Hz), 4.37, 4.14 (2H, ABq, J=11.7 Hz), 4.11 and 3.99 (2H, ABq, J=10.6 Hz) in the ^{1}H-NMR spectrum, which was identical with that of abrisapogenol E tetraacetate obtained from *Abrus cantoniensis*.[3] On partial acid hydrolysis, **5a** provided a prosapogenin **10a**, a white powder, $[\alpha]_D$ −13.7° (MeOH), which showed a peak at m/z 1133 due to $[M-H]^-$ in the negative FAB-MS. Therefore, the prosapogenin (**10a**) was characterized as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol E 30-*O*-β-D-glucopyranoside, identical with subproside V isolated from *Sophora subprostrata*,[5] according to the ^{1}H- and ^{13}C-NMR spec-

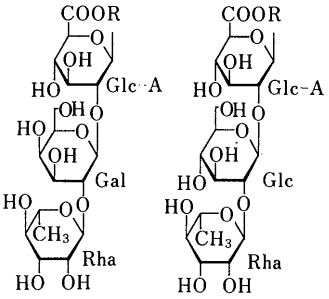
TABLE I. ^{13}C-NMR Spectral Data for **1a**—**6a**, **8** and **10a** (Pyridine-d_5)

	1a	**2a**	**3a**	**4a**	**8**	**5a**	**6a**	**10a**
C- 1	38.5	38.4	38.4	38.5	38.8	38.6	38.5	38.5
C- 2	26.4[a]	26.3[a]	26.5[a]	26.2[a]	28.3	26.4[a]	26.3[a]	26.4[a]
C- 3	91.2	91.6	91.1	91.6	80.0	91.3	91.6	91.3
C- 4	43.8	43.6	43.7	43.7	43.1	43.9	43.6	43.8
C- 5	56.0	56.2	55.9	56.2	56.2	56.1	56.1	56.0
C- 6	18.4	18.5	18.3	18.5	19.0	18.5	18.5	18.5
C- 7	33.2	33.2	33.0	33.1	33.0	33.1	33.0	33.1
C- 8	39.8	39.8	39.9	39.9	40.0	40.0	39.9	40.0
C- 9	47.8	47.7	47.6	47.7	48.0	47.8	47.6	47.7
C-10	36.4	36.4	36.3	36.4	36.9	36.5	36.3	36.4
C-11	24.0	24.0	23.9	24.0	24.0	24.0	24.0	24.0
C-12	122.3	122.3	122.4	122.5	122.5	123.0	122.7	122.7
C-13	144.9	144.9	144.4	144.5	144.4	144.4	144.3	144.4
C-14	42.3	42.3	41.9	42.1	42.0	42.2	42.1	42.2
C-15	26.6[a]	26.5[a]	26.7[a]	26.6[a]	26.2	26.7[a]	26.5[a]	26.6[a]
C-16	28.8	28.8	28.2	28.4	28.3	28.2	28.1	28.1
C-17	38.2	38.2	37.6	37.7	37.7	37.9	37.8	37.8
C-18	44.7	44.7	44.4	44.6	44.5	44.4	44.4	44.4
C-19	41.4	41.4	40.8	41.0	41.0	42.2	42.1	42.2
C-20	36.4	36.4	35.9	36.1	36.0	35.0	34.9	35.0
C-21	37.2	37.2	30.6	30.8	30.7	37.1	36.9	37.1
C-22	75.6	75.6	79.6	79.8	79.6	75.4	75.2	75.4
C-23	22.9	22.7	22.8	22.7	23.6	23.0	22.7	23.0
C-24	63.5	63.3	63.4	63.3	64.5	63.6	63.3	63.5
C-25	15.8	15.6	15.7	15.7	16.2	15.8	15.6	15.7
C-26	16.9	17.0	16.7	16.9	16.9	16.9	16.9	16.8
C-27	25.5	25.5	25.7	25.8	25.7	26.0	25.9	26.0
C-28	21.1	21.1	21.4	21.5	21.4	21.2	20.9	21.3
C-29	73.0	73.0	72.7	72.8	72.7	29.1	28.9	29.1
C-30	24.4	24.4	23.4	23.6	23.4	77.8	77.7	77.9
Glc A								
1	105.4	105.1	105.3	105.2		105.5	105.1	105.4
2	78.2	78.2[b]	78.0	78.2[b]		78.3	78.1[b]	78.1[b]
3	76.8[b]	76.6	76.8[b]	76.7		76.9[b]	76.5	76.9[c]
4	73.5	73.4	73.4	73.4		73.6	73.3	73.6
5	77.6	77.6	77.5	77.6		77.7	77.7	77.7
6	170.3	170.3	170.2	170.3		170.4	170.2	170.3
COOMe	52.0	52.1	52.0	52.1		52.1	52.1	52.0
Gal								
1	101.7		101.6			101.8		101.7
2	76.5[b]		76.4[b]			76.6[b]		76.6[c]
3	76.4[b]		76.3[b]			76.5[b]		76.4[c]
4	71.1		71.0			71.2		71.4
5	76.5[b]		76.4[b]			76.6[b]		76.5[c]
6	61.5		61.4			61.6		61.5
Glc								
1		102.0		101.9			101.8	
2		79.1		79.1			79.0	
3		78.0[b]		78.0[b]			77.9[b]	
4		69.7		69.7			69.6	
5		78.3[b]		78.3[b]			78.1[b]	
6		61.2		61.3			61.2	
Rha								
1	102.3	101.9	102.2	101.9		102.4	101.8	102.4
2	72.3[c]	72.2[c]	72.2[c]	72.3[c]		72.4[c]	72.1[c]	72.3[c]
3	72.7[c]	72.6[c]	72.5[c]	72.6[c]		72.8[c]	72.5[c]	72.7[c]
4	74.3	74.3	74.2	74.3		74.3	74.1	74.3
5	69.3	69.4	69.2	69.4		69.4	69.3	69.3
6	18.9	18.9	18.8	18.9		19.0	18.8	18.9
Rha′								
1			98.2	98.3	98.3			
2			72.7[c]	72.7[c]	72.6[a]			
3			72.9[c]	73.0[c]	73.0[a]			
4			73.7	73.9	73.8			
5			70.2	70.3	70.2			
6			18.4	18.5	18.5			
Glc′								
1						105.6	105.4	105.8
2						75.5	75.4	75.5
3						78.7	78.5[b]	78.6[b]
4						71.8	71.6	71.7
5						77.2	77.0	78.4[b]
6						68.9	68.7	62.7
Api								
1						111.1	110.9	
2						77.8	77.5	
3						80.4	80.4	
4						65.7	65.5	
5						75.0	74.9	

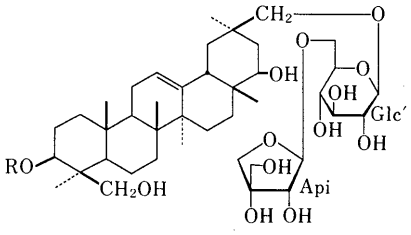
a—*c*) In each vertical column may be interchanged.



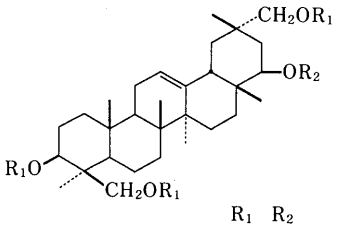
		R_1	R
robinioside E	(1)	S_1	H
	1a	S_2	H
robinioside F	(2)	S_3	H
	2a	S_4	H
robinioside G	(3)	S_1	Rha′
	3a	S_2	Rha′
robinioside H	(4)	S_3	Rha′
	4a	S_4	Rha′



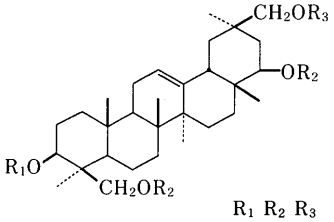
S_1 : R=H S_3 : R=H
S_2 : R=CH_3 S_4 : R=CH_3



		R
robinioside I	(5)	S_1
	5a	S_2
robinioside J	(6)	S_3
	6a	S_4



		R_1	R_2
abrisapogenol B	(7)	H	H
	7a	Ac	Ac
	8	H	Rha′



		R_1	R_2	R_3
abrisapogenol E	(9)	H	H	H
	9a	Ac	Ac	Ac
	10a	S_2	H	Glc′

tral data. Furthermore, the negative FAB-MS of **5a** gave a peak at *m/z* 1265 due to $[M-H]^-$, which was higher by 132 mass units than that of **10a**. A comparative study of the ^{13}C-NMR spectrum of **5a** with that of **10a** indicated the presence of one mole of an apiosyl group[6] [δ (ppm): C-1, 111.1 (d); C-2, 77.8 (d); C-3, 80.4 (s); C-4, 65.7 (t); C-5, 75.0 (t)] in **5a**, which was linked to the C-6 hydroxyl group of the C-30 glucopyranosyl moiety according to the glycosylation shifts [δ (ppm): C-5, 77.0 (−1.2); C-6, 68.9 (+6.2)]. The ^{1}H-NMR spectrum of **5a** also supported the above proposed structure by the appearance of five anomeric proton signals at δ6.22 (1H, br s), 5.74 (1H, d, J=2.2 Hz), 5.71 (1H, d, J=7.7 Hz), 4.89 (1H, d, J=7.7 Hz) and 4.58 (1H, d, J=7.7 Hz). Based on the above evidence, the full structure of robinioside I (**5**) could be represented as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-galactopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol E 30-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

Robinioside J methyl ester (**6a**), a white powder, $[\alpha]_D$ −22.1° (MeOH), showed a peak at *m/z* 1265 due to $[M-H]^-$ in the negative FAB-MS. Acid hydrolysis of **6a** furnished a sapogenol (**9**) identical with abrisapogenol E in respect to the TLC and ^{1}H-NMR spectrum. According to the ^{13}C-NMR spectrum, signals caused by the sapogenol part showed good coincidence with those of **5a**, and the remaining signals derived from the sugar moiety disclosed the presence of an *O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl group at C-3-OH and an *O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranosyl group at C-30-OH in **6a** in comparison with those of **2a** and **5a**, respectively. Consequently, the structure of robinioside J (**6**) was elucidated as 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl(1→2)-β-D-glucuronopyranosyl abrisapogenol E 30-*O*-β-D-apiofuranosyl(1→6)-β-D-glucopyranoside.

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. Thin layer chromatography was performed on a precoated Kieselgel 60 F_{254} plate (0.2 mm Merck) and detection was achieved by spraying with 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. The triterpene fraction (9.8 g) was treated with excess diazomethane after being passed through an Amberlite IR-120B column, followed by column chromatography on Bondapak C_{18} column chromatography eluted with 50% MeOH→MeOH to provide robinioside E and F methyl esters, **1a** (68 mg) and **2a** (54 mg), respectively. The aqueous layer was concentrated and subjected to MCI gel CHP 20P column chromatography with H_2O and MeOH. The triterpene part was collected and evaporated to a residue, which was methylated with CH_2N_2 after treatment with an Amberlite IR-120B column and separation by normal and reversed phase column chromatographies to yield the robinioside G—J methyl esters, **3a** (87 mg), **4a** (48 mg), **5a** (156 mg) and **6a** (102 mg), respectively.

Robinioside E Methyl Ester (1a) A white powder, $[\alpha]_D^{26}$ −12.2° (c=0.92, MeOH). *Anal.* Calcd for $C_{49}H_{80}O_{19}\cdot 3H_2O$: C, 57.31; H, 8.38. Found: C, 57.50; H, 8.23. Negative FAB-MS *m/z*: 971 $[M-H]^-$, 825 $[M-Rha-H]^-$, 473 $[M-Glc\ A(Me)-Gal-Rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.72, 0.97, 1.27, 1.29, 1.42, 1.46 (each 3H, s, Me×6), 1.76 (1H, d, J=6.2 Hz, Rha Me-6), 3.77 (3H, s, COOMe), 4.93 (1H, d, J=7.7 Hz, Glc A H-1), 5.36 (1H, br s, H-12), 5.79 (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 1a A solution of **1a** (40 mg) in 2N HCl–MeOH (2 ml) was heated at 90 °C for 2 h. The precipitate was collected by filtration and recrystallized with MeOH to yield **7** (11 mg), colorless

needles, mp 281—283 °C, $[\alpha]_D^{26}$ +26.4° (c=0.81, pyridine). EI-MS m/z: 474 $[M]^+$, 456 $[M-H_2O]^+$, 443 $[M-CH_2OH]^+$, 425 $[M-CH_2OH-H_2O]^+$, 407 $[M-CH_2OH-2H_2O]^+$, 250 [D/E ring]$^+$, 224 [A/B ring]$^+$, 219 [D/E ring-CH_2OH]$^+$, 206 [A/B ring-H_2O]$^+$, 175 [A/B ring-CH_2OH-H_2O]$^+$. ^{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 (3H, brs, H-3, H_2-29), 3.73, 4.54 (2H, ABq, J=11.7 Hz, H_2-24), 5.41 (1H, brs, 12-H), identified with abrisapogenol B.

A solution of **7** (8 mg) in Ac_2O–pyridine (1:1, 2 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 tetraacetate **7a** (6 mg) as colorless plates (MeOH), mp 157—158 °C, $[\alpha]_D^{26}$ +59.3° (c=0.24, $CHCl_3$). ^{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), 3.67, 3.74 (2H, ABq, J=10.6 Hz, H_2-29), 4.14, 4.37 (2H, ABq, J=11.7 Hz, H_2-24), 4.59 (1H, dd, J=5.5, 10.6 Hz, H-3), 4.71 (1H, t, J=3.7 Hz, H-22), 5.29 (1H, brt, H-12), identified with abrisapogenol B tetraacetate.

Robinioside F (2) Methyl Ester (2a) A white powder, $[\alpha]_D^{26}$ −11.2° (c=1.14, MeOH). *Anal.* Calcd for $C_{49}H_{80}O_{19}\cdot 4H_2O$: C, 56.32; H, 8.43. Found: C, 56.56; H, 8.33. Negative FAB-MS m/z: 971 $[M-H]^-$, 825 $[M-Rha-H]^-$, 473 $[M-Glc\ A\ (Me)-Glc-Rha-H]^-$. ^{1}H-NMR (pyridine-$d_5$) δ: 0.70, 0.97, 1.26, 1.27, 1.46, 1.47 (each 3H, s, Me×6), 1.79 (1H, d, J=5.5 Hz, Rha Me-6), 3.78 (3H, s, COOMe), 4.97 (1H, d, J=7.7 Hz, Glc A H-1), 5.36 (1H, brs, H-12), 5.88 (1H, d, J=7.7 Hz, Glc H-1), 6.43 (1H, brs, Rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 2a A solution of **2a** (18 mg) in 2 N HCl–MeOH (2 ml) was treated in the same way as **1a** to afford a sapogenol which was identical with abrisapogenol B (**7**) in respect to the TLC and ^{1}H-NMR spectrum.

Robinioside G (3) Methyl Ester (3a) A white powder, $[\alpha]_D^{26}$ −27.5° (c=1.20, MeOH). *Anal.* Calcd for $C_{55}H_{90}O_{23}\cdot 3\ 1/2H_2O$: C, 55.88; H, 8.21. Found: C, 55.69; H, 8.08. Negative FAB-MS m/z: 1117 $[M-H]^-$, 971 $[M-Rha-H]^-$, 809 $[M-Gal-Rha-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.72, 0.95, 1.07, 1.19, 1.24, 1.40 (each 3H, s, Me×6), 1.70 (1H, d, J=5.5 Hz, Rha′ Me-6), 1.73 (1H, d, J=6.2 Hz, Rha Me-6), 3.75 (3H, s, COOMe), 5.26 (1H, brs, H-12), 5.48 (1H, brs, Rha′ H-1), 5.75 (1H, d, J=7.3 Hz, Gal H-1), 6.31 (1H, brs, Rha H-1), Glc A H-1; hidden by H_2O signal. ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 3a A solution of **3a** (16 mg) in 2 N HCl–MeOH (2 ml) was hydrolyzed as described for **1a** to provide a sapogenol which was identical with abrisapogenol B (**7**) in respect to the TLC and ^{1}H-NMR spectrum.

Enzymatic Hydrolysis of 3 and 4 To a solution of **3** and **4** (1:1, 104 mg) in acetate buffer (pH=4.2, 10 ml) was added glycyrrhizinic acid hydrolase (4 ml), and the mixture was incubated at 40 °C for 2 h. The reaction process was checked by TLC ($CHCl_3$–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 chromatography and eluted with H_2O and MeOH, followed by silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 10:1:0.1) to give a sole product **8** (32 mg), a white powder, $[\alpha]_D^{26}$ +19.6° (c=0.80, MeOH). Negative FAB-MS m/z: 619 $[M-H]^-$, 473 $[M-Rha-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.97, 1.02, 1.09, 1.22, 1.23, 1.55 (each 3H, s, Me×6), 1.71 (1H, d, J=5.5 Hz, Rha′ Me-6), 5.32 (1H, brs, H-12), 5.49 (1H, brs, Rha′ H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Robinioside H (4) Methyl Ester (4a) A white powder, $[\alpha]_D^{26}$ −28.4° (c=1.21, MeOH). *Anal.* Calcd for $C_{55}H_{90}O_{23}\cdot 3\ 1/2H_2O$: C, 55.88; H, 8.21. Found: C, 55.79; H, 8.01. Negative FAB-MS m/z: 1117 $[M-H]^-$, 971 $[M-Rha-H]^-$, 809 $[M-Glc-Rha-H]^-$. ^{1}H-NMR (pyridine-d_5) δ: 0.71, 0.95, 1.06, 1.19, 1.22, 1.44 (each 3H, s, Me×6), 1.70 (1H, d, J=5.5 Hz, Rha′ Me-6), 1.76 (1H, d, J=6.2 Hz, Rha Me-6), 3.76 (3H, s, COOMe), 4.94 (1H, d, J=7.7 Hz, Glc A H-1), 5.26 (1H, brs, H-12), 5.48 (1H, brs, Rha′ H-1), 5.84 (1H, d, J=7.7 Hz, Glc H-1), 6.38 (1H, brs, Rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 4a A solution of **4a** (14 mg) in 2 N HCl–MeOH (2 ml) was hydrolyzed as described for **1a** to afford a sapogenol which was identical with abrisapogenol B (**7**) by TLC and ^{1}H-NMR spectrum.

Robinioside I (5) Methyl Ester (5a) A white powder, $[\alpha]_D^{26}$ −14.3° (c=0.99, MeOH). *Anal.* Calcd for $C_{60}H_{98}O_{28}\cdot 2\ 1/2H_2O$: C, 54.92; H, 7.86. Found: C, 54.74; H, 7.65. Negative FAB-MS m/z: 1265 $[M-H]^-$, 1133 $[M-Api-H]^-$, 459. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.92, 1.12, 1.18, 1.24, 1.40 (each 3H, s, Me×6), 1.73 (1H, d, J=6.2 Hz, Rha Me-6), 3.75 (3H, s, COOMe), 4.58 (1H, d, J=7.7 Hz, Glc′ H-1), 4.89 (1H, d, J=7.7 Hz, Glc A H-1), 5.33 (1H, brs, H-12), 5.71 (1H, d, J=7.7 Hz, Gal H-1), 5.74 (1H, d, J=2.2 Hz, Api H-1), 6.22 (1H, brs, Rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 5a A solution of **5a** (50 mg) in 2 N HCl–MeOH (5 ml) was heated at 90 °C for 2 h and then neutralized with 3% KOH–MeOH. The reaction mixture was filtered to afford a solution which was concentrated and partitioned with $CHCl_3$–H_2O. The $CHCl_3$ extract was chromatographed over silica gel ($CHCl_3$–MeOH, 30:1) to provide **9** (15 mg), colorless needles (MeOH), mp 276—278 °C, $[\alpha]_D^{26}$ +83.2° (c=0.55, MeOH). EI-MS m/z: 474 $[M]^+$, 456 $[M-H_2O]^+$, 443 $[M-CH_2OH]^+$, 425 $[M-CH_2OH-H_2O]^+$, 407 $[M-CH_2OH-2H_2O]^+$, 250 [D/E ring]$^+$, 219 [D/E ring-CH_2OH]$^+$, 206 [A/B ring-H_2O]$^+$, 175 [A/B ring-CH_2OH-H_2O]$^+$. ^{1}H-NMR (pyridine-d_5) δ: 0.93, 1.00, 1.17, 1.23, 1.27, 1.56 (each 3H, s, Me×6), 2.59 (1H, brd, J=12.1 Hz, H-18), 3.64 (1H, dd, J=4.4, 11.7 Hz, H-3), 3.71, 4.51 (2H, ABq, J=10.6 Hz, H_2-24), 3.92 (2H, brs, H_2-30), 5.34 (1H, brs, H-12), identified with abrisapogenol E.

A solution of **9** (10 mg) in Ac_2O–pyridine (1:1, 1 ml) was allowed to stand at room temperature overnight. The reaction mixture was treated as described above for **7** to give the tetraacetate **9a** (8 mg), colorless plates (MeOH), mp 278—280 °C, $[\alpha]_D^{26}$ +72.3° (c=0.40, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 0.81, 0.94, 0.96, 0.98, 1.03, 1.15 (each 3H, s, Me×6), 2.00, 2.04, 2.05, 2.06 (each 3H, s, OAc×4), 2.20 (1H, dd, J=4.0, 13.6 Hz, H-18), 3.99, 4.11 (2H, ABq, J=10.6 Hz, H_2-30), 4.14, 4.37 (2H, ABq, J=11.7 Hz, H_2-24), 4.59 (1H, dd, J=10.6, 5.1 Hz, H-3), 4.66 (1H, t, J=3.3 Hz, H-22), 5.27 (1H, brt, H-12), identified with abrisapogenol B tetraacetate.

Partial Hydrolysis of 5a A solution of **5a** (100 mg) in 0.2 N HCl–MeOH (5 ml) was heated at 90 °C for 15 min and then neutralized with 3% KOH–MeOH. The reaction mixture was filtered to afford a solution which was evaporated to dryness and chromatographed over silica gel ($CHCl_3$–MeOH–H_2O, 8:2:0.2→7:3:0.5) to yield a main prosapogenin **10a** (14 mg), a white powder, $[\alpha]_D^{26}$ −13.7° (c=0.60, MeOH). Negative FAB-MS m/z: 1133 $[M-H]^-$, 987 $[M-Rha-H]^-$, 971 $[M-Glc-H]^-$, 473 [aglycone moiety]$^-$, 459. ^{1}H-NMR (pyridine-d_5) δ: 0.69, 0.94, 1.20, 1.23, 1.27, 1.44 (each 3H, s, Me×6), 1.77 (1H, d, J=6.2 Hz, Rha Me-6), 3.76 (3H, s, COOMe), 4.57 (1H, d, J=7.7 Hz, Glc′ H-1), 4.96 (1H, d, J=7.7 Hz, Glc A H-1), 5.36 (1H, brs, H-12), 5.80 (1H, d, J=7.7 Hz, Gal H-1), 6.30 (1H, brs, Rha H-1). ^{13}C-NMR (pyridine-d_5): Table I.

Robinioside J (6) Methyl Ester (6a) A white powder, $[\alpha]_D^{26}$ −22.1° (c=0.70, MeOH). *Anal.* Calcd for $C_{60}H_{98}O_{28}\cdot 3/2H_2O$: C, 55.68; H, 7.81. Found: C, 55.83; H, 7.62. Negative FAB-MS m/z: 1265 $[M-H]^-$, 1133 $[M-Api-H]^-$, 459. ^{1}H-NMR (pyridine-d_5) δ: 0.68, 0.93, 1.14, 1.20, 1.23, 1.46 (each 3H, s, Me×6), 1.78 (1H, d, J=6.2 Hz, Rha Me-6), 3.77 (3H, s, COOMe), 4.94 (1H, d, J=7.7 Hz, Glc A H-1), 5.34 (1H, brs, H-12), 5.79 (1H, d, J=2.2 Hz, Api H-1), 5.87 (1H, d, J=7.7 Hz, Glc H-1), 6.41 (1H, brs, Rha H-1), Glc′ H-1: hidden by other signals. ^{13}C-NMR (pyridine-d_5): Table I.

Acid Hydrolysis of 6a A solution of **6a** (9 mg) was hydrolyzed as described above to afford a sapogenol which was identical with abrisapogenol E (**9**) by TLC and ^{1}H-NMR spectrum.

Acknowledgements We are grateful to Mr. K. Mizutani of Maruzen Kasei Co., Ltd. for the supply of glycyrrhizinic acid hydrolase and to Mr. K. Takeda and Mr. T. Iriguchi of this Faculty for measurements of MS, ^{1}H- and ^{13}C-NMR spectra.

References and Notes

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