Five New Triterpene Saponins, Polygalasaponins XXVIII—XXXII from the Root of *Polygala japonica* HOUTT.

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Five new oleanane-type saponins, polygalasaponins XXVIII—XXXII, along with one known saponin, polygalasaponin XXIV, and one known acylated sucrose, tenuifoliside C, were isolated from the root of *Polygala japonica*. The structures of these new compounds were elucidated as 3-*O*-*β*-D-glucopyranosyl presenegenin 28-*O*-*β*-D-xylopyranosyl (1→4)-*α*-L-rhamnopyranosyl (1→2)-*β*-D-fucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl presenegenin 28-*O*-*β*-D-galactopyranosyl (1→5)-*β*-D-apiofuranosyl (1→4)-*β*-D-xylopyranosyl (1→4)-*α*-L-rhamnopyranosyl (1→2)-*β*-D-fucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl presenegenin 28-*O*-*β*-D-galactopyranosyl (1→4)-*β*-D-xylopyranosyl (1→4)-*α*-L-rhamnopyranosyl (1→2)-[4-*O*-*p*-methoxycinnamoyl]-[*β*-D-glucopyranosyl (1→3)]-*β*-D-fucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl presenegenin 28-*O*-*α*-L-arabinopyranosyl (1→3)-*β*-D-xylopyranosyl (1→4)-[*β*-D-apiofuranosyl (1→3)]-*α*-L-rhamnopyranosyl (1→2)-[4-*O*-3,4,5-trimethoxycinnamoyl]-*β*-D-fucopyranosyl ester, 3-*O*-*β*-D-glucopyranosyl presenegenin 28-*O*-*α*-L-arabinopyranosyl (1→3)-*β*-D-xylopyranosyl (1→4)-[*β*-D-apiofuranosyl (1→3)]-*α*-L-rhamnopyranosyl (1→2)-[4-*O*-*p*-methoxycinnamoyl]-[*α*-L-rhamnopyranosyl (1→3)]-*β*-D-fucopyranosyl ester, respectively, on the basis of spectroscopic and chemical evidence.

Key words *Polygala japonica*; polygalasaponin; presenegenin; senegenic acid; oleanane-type saponin; Polygalaceae

We previously reported[1–3] the isolation and structural elucidation of 27 new triterpenoid glycosides called polygalasaponins I—XXVII together with three known saponins, 3-*O*-*β*-D-glucopyranosyl bayogenin, lobatoside B, 3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranosyl hederagenin, isolated from the aerial part of *Polygala japonica* HOUTT. (Polygalaceae). We continued our investigation of the constituents of this plant, and isolated five new saponins (**1**—**5**), one known saponin, polygalasaponin XXIV (**6**),[3] and one known acylated sucrose, tenuifoliside C (**7**),[4] from the root of this plant. Compounds **6** and **7** were identified by comparison of the ^{1}H- and ^{13}C-NMR data with reported data, respectively.

A 70% methanolic aqueous extract of the root of *P. japonica* HOUTT. was passed through a porous polymer gel Mitsubishi Diaion HP-20 column and the adsorbed materials were eluted with 50% and 70% aqueous methanol and methanol, successively. The 70% methanol and methanol eluate were chromatographed on a silica gel column followed by repeated semi-preparative HPLC on a reversed phase column [ODS, phenylalkyl (PhA-T-5)] to give **1**—**7**. On acid hydrolysis, saponins **1**—**6** afforded senegenic acid (**1a**) which was well known as an artifact aglycone of presenegenin (**1b**) glycosides.[5–7] We therefore assumed that saponins **1**—**6** were presenegenin glycosides.

Polygalasaponin XXVIII (**1**) showed a $[M-H]^-$ ion

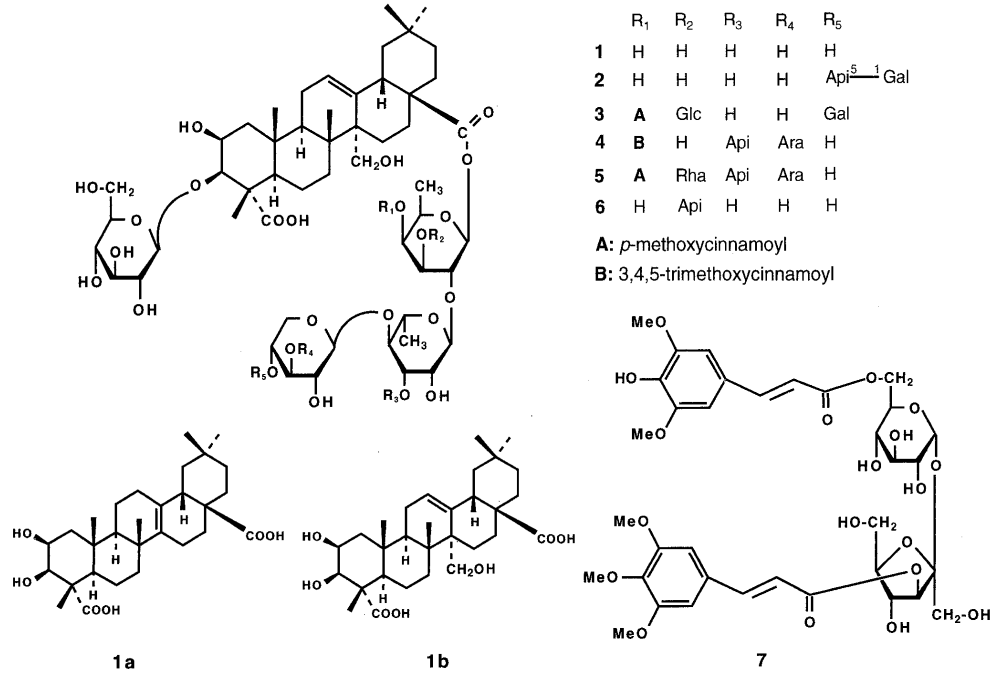

Chart 1

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Table 1. ^{1}H-NMR Data of Compounds **1**—**5** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**
Aglycone					
2	4.71 (1H, m)	4.71 (1H, m)	4.70 (1H, m)	4.68 (1H, m)	4.66 (1H, m)
3	4.60 (1H, d, J=3 Hz)	4.58 (1H, d, J=3 Hz)	4.59 (1H, d, J=3 Hz)	4.61 (1H, d, J=3 Hz)	4.59 (1H, d, J=3 Hz)
12	5.79 (1H, t-like)	5.80 (1H, t-like)	5.84 (1H, t-like)	5.78 (1H, t-like)	5.88 (1H, t-like)
18	3.22 (1H, dd, J=14, 4 Hz)	3.22 (1H, dd, J=14, 4 Hz)	3.23 (1H, dd, J=14, 4 Hz)	3.23 (1H, dd, J=14, 4 Hz)	3.26 (1H, dd, J=14, 4 Hz)
24	1.95 (3H, s)	1.94 (3H, s)	1.95 (3H, s)	1.97 (3H, s)	1.94 (3H, s)
25	1.57 (3H, s)	1.53 (3H, s)	1.52 (3H, s)	1.55 (3H, s)	1.57 (3H, s)
26	1.13 (3H, s)	1.12 (3H, s)	1.14 (3H, s)	1.10 (3H, s)	1.14 (3H, s)
27	3.79 (1H, d, J=12 Hz)	3.78 (1H, d, J=12 Hz)	3.82 (1H, d, J=12 Hz)	3.80[a]	3.83 (1H, d, J=12 Hz)
	4.05 (1H, d, J=12 Hz)	4.06 (1H, d, J=12 Hz)	4.06 (1H, d, J=12 Hz)	4.06 (1H, d, J=12 Hz)	4.06[a]
29	0.79 (3H, s)	0.79 (3H, s)	0.80 (3H, s)	0.80 (3H, s)	0.80 (3H, s)
30	0.94 (3H, s)	0.92 (3H, s)	0.93 (3H, s)	0.93 (3H, s)	1.04 (3H, s)
C-3 sugar					
Glc-1	5.06 (1H, d, J=8 Hz)	5.03 (1H, d, J=8 Hz)	5.03 (1H, d, J=8 Hz)	5.05 (1H, d, J=8 Hz)	5.04 (1H, d, J=8 Hz)
2	3.92[a]	3.90[a]	3.91[a]	3.92[a]	3.89[a]
3	4.15[a]	4.14[a]	4.14[a]	4.15[a]	4.15[a]
4	4.16[a]	4.14[a]	4.15[a]	4.16[a]	4.16[a]
5	3.91[a]	3.91[a]	3.92[a]	3.89[a]	3.89[a]
6	4.26 (1H, dd, J=12, 5 Hz)	4.27[a]	4.29[a]	4.29 (1H, dd, J=12, 5 Hz)	4.28[a]
	4.45 (1H, dd, J=12, 2 Hz)	4.47 (1H, dd, J=12, 2 Hz)	4.45[a]	4.45[a]	4.45[a]
C-28 sugar					
Fuc-1	6.06 (1H, d, J=8 Hz)	6.05 (1H, d, J=8 Hz)	6.16 (1H, d, J=8 Hz)	6.14 (1H, d, J=8 Hz)	6.08 (1H, d, J=8 Hz)
2	4.64 (1H, t, J=8.5 Hz)	4.62 (1H, t, J=8.5 Hz)	4.77 (1H, t, J=8.5 Hz)	4.69 (1H, t, J=8.5 Hz)	4.64 (1H, t, J=8.5 Hz)
3	4.18[a]	4.14[a]	4.50[a]	4.49[a]	4.44[a]
4	3.95[a]	3.94[a]	6.07 (1H, d, J=3 Hz)	5.76 (1H, d, J=3 Hz)	5.87 (1H, d, J=3.5 Hz)
5	3.90[a]	3.89[a]	4.10[a]	4.18[a]	4.15[a]
6	1.50 (3H, d, J=6 Hz)	1.48 (3H, d, J=6 Hz)	1.28 (3H, d, J=6 Hz)	1.39 (3H, d, J=6 Hz)	1.33 (3H, d, J=6 Hz)
Rha-1 (F-2)	6.40 (1H, br s)	6.34 (1H, br s)	6.44 (1H, br s)	6.29 (1H, br s)	5.81 (1H, br s)
2	4.79 (1H, br s)	4.78 (1H, br s)	4.83 (1H, br s)	5.01 (1H, br s)	4.77 (1H, br s)
3	4.69[a]	4.64 (1H, dd, J=9.5, 3 Hz)	4.65 (1H, dd, J=9.5, 3 Hz)	4.59 (1H, dd, J=9.5, 3 Hz)	4.41[a]
4	4.31 (1H, t, J=9.5 Hz)	4.21 (1H, t, J=9.5 Hz)	4.26 (1H, t, J=9.5 Hz)	4.50[a]	4.44[a]
5	4.46[a]	4.42[a]	4.49[a]	4.50[a]	4.28[a]
6	1.67 (3H, d, J=6 Hz)	1.62 (3H, d, J=6 Hz)	1.75 (3H, d, J=6 Hz)	1.75 (3H, d, J=6 Hz)	1.68 (3H, d, J=6 Hz)
Rha-1 (F-3)					5.56 (1H, br s)
2					4.78[a]
3					4.41[a]
4					4.29[a]
5					4.48[a]
6					1.75 (3H, d, J=6 Hz)
Glc-1			5.10 (1H, d, J=8 Hz)		
2			3.94 (1H, t, J=8.5 Hz)		
3			4.11[a]		
4			4.12[a]		
5			3.90[a]		
6			4.28[a]		
			4.45[a]		
Xyl-1	5.40 (1H, d, J=7 Hz)	4.85 (1H, d, J=7.5 Hz)	4.97 (1H, d, J=7.5 Hz)	5.30 (1H, d, J=7.5 Hz)	5.26 (1H, d, J=7.5 Hz)
2	4.02[a]	4.03[a]	4.02[a]	3.98 (1H, t, J=8.5 Hz)	3.99[a]
3	4.02[a]	4.03[a]	4.03[a]	4.13 (1H, t, J=9.5 Hz)	4.14[a]
4	4.14[a]	4.26 (1H, t, J=8.5 Hz)	4.28[a]	4.04[a]	4.04[a]
5	3.50 (1H, t, J=11 Hz)	3.33 (1H, t, J=11 Hz)	3.45 (1H, t, J=11 Hz)	3.42 (1H, t, J=11 Hz)	3.41 (1H, t, J=11 Hz)
	4.23[a]	4.34[a]	4.28[a]	4.19[a]	4.20[a]
Api-1		6.34 (1H, br s)		6.01 (1H, d, J=4 Hz)	6.09 (1H, d, J=3 Hz)
2		4.39 (1H, br s)		4.80 (1H, d, J=4 Hz)	4.79[a]
4		4.17[a]		4.18[a]	4.30[a]
		5.03 (1H, d, J=9.5 Hz)		4.52[a]	4.49[a]
5		4.18 (1H, d, J=12 Hz)		4.05[a]	4.03[a]
		4.63 (1H, d, J=12 Hz)		4.05[a]	4.03[a]
Ara-1				5.15 (1H, d, J=7 Hz)	5.16 (1H, d, J=7 Hz)
2				4.47[a]	4.46[a]
3				4.06[a]	4.07[a]
4				4.20[a]	4.22[a]
5				3.61 (1H, br d, J=12 Hz)	3.62 (1H, br d, J=12 Hz)
				4.22[a]	4.24[a]
Gal-1		4.82 (1H, d, J=8 Hz)	4.95 (1H, d, J=8 Hz)		
2		4.38[a]	4.45[a]		
3		4.36[a]	4.10[a]		
4		4.48 (1H, d, J=3 Hz)	4.47 (1H, d, J=3 Hz)		
5		4.00[a]	4.08[a]		

Table 1. (continued)

	1	2	3	4	5
6		4.36 (1H, dd, J=12, 5 Hz)	4.35 (1H, dd, J=12, 5 Hz)		
		4.42 (1H, dd, J=12, 2 Hz)	4.42[a]		
Cinn-2			7.38 (1H, d, J=8.5 Hz)	6.85 (1H, s)	7.41 (1H, d, J=8.5 Hz)
3			6.96 (1H, d, J=8.5 Hz)		6.98 (1H, d, J=8.5 Hz)
5			6.96 (1H, d, J=8.5 Hz)		6.98 (1H, d, J=8.5 Hz)
6			7.38 (1H, d, J=8.5 Hz)	6.85 (1H, s)	7.41 (1H, d, J=8.5 Hz)
7			7.89 (1H, d, J=16 Hz)	7.95 (1H, d, J=16 Hz)	7.94 (1H, d, J=16 Hz)
8			6.52 (1H, d, J=16 Hz)	6.62 (1H, d, J=16 Hz)	6.56 (1H, d, J=16 Hz)
OMe			3.67 (3H, s)	3.81 (6H, s)	3.64 (3H, s)
				3.90 (3H, s)	

Recorded at 400 MHz at 35 °C. Assignments were based on ^{1}H–^{1}H COSY, HOHAHA, difference NOE and detailed proton spin decoupling experiments. *a*) Overlapping with other signals.

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**5** in Pyridine-d_5

	1	2	3	4	5
1	44.3	44.2	44.3	44.2	44.2
2	70.3	70.3	70.3	70.3	70.3
3	86.0	86.1	86.0	85.8	86.1
4	52.9	52.9	52.9	52.9	52.9
5	52.5	52.5	52.5	52.7	52.5
6	21.4	21.4	21.6	21.2	21.2
7	33.6	33.6	33.6	33.9	34.0
8	41.2	41.2	41.2	41.2	41.2
9	49.4	49.3	49.3	49.3	49.3
10	37.1	37.0	37.0	37.0	37.1
11	23.6	23.5	23.6	23.6	23.6
12	127.9	127.9	127.8	127.9	127.8
13	138.9	138.9	138.9	139.0	139.0
14	48.0	48.1	48.0	48.0	48.0
15	24.6	24.5	24.5	24.5	24.6
16	24.1	24.0	24.0	24.0	24.1
17	46.9	46.9	47.1	47.0	47.0
18	42.0	42.0	42.1	42.0	42.1
19	45.4	45.3	45.4	45.4	45.6
20	30.8	30.8	30.8	30.8	30.9
21	33.8	33.9	33.9	33.7	34.0
22	32.4	32.4	32.4	32.4	32.2
23	180.8	180.8	180.8	180.8	180.8
24	14.2	14.2	14.2	14.3	14.3
25	17.5	17.5	17.5	17.5	17.6
26	18.8	18.5	18.8	19.0	19.2
27	64.5	64.5	64.4	64.5	64.5
28	176.7	176.7	176.7	176.6	176.5
29	33.1	33.1	33.0	33.1	33.1
30	24.1	24.0	24.0	24.0	24.1
Cinn.					
1			127.4	130.4	127.5
2			130.5	106.5	130.5
3			114.8	141.2	114.8
4			162.1	154.1	162.0
5			114.8	141.2	114.8
6			130.5	106.5	130.5
7			145.8	145.9	145.6
8			116.0	117.8	115.8
9			167.9	167.6	167.2
OMe			55.4	56.4	55.4
				56.4	
				60.7	
C-3 sugar					
Glc-1	105.4	105.4	105.4	105.4	105.4
2	75.3	75.3	75.3	75.3	75.3
3	78.4	78.3	78.3	78.3	78.3
4	71.6	71.6	71.6	71.6	71.6
5	78.4	78.3	78.3	78.3	78.3
6	62.7	62.8	62.7	62.7	62.8

Table 2. (continued)

	1	2	3	4	5
C-28 sugar					
Fuc-1	94.8	94.8	94.6	94.6	95.0
2	74.0	74.5	73.3	75.8	76.8
3	76.7	76.6	83.3	74.2	79.9
4	73.2	73.2	74.3	74.6	73.4
5	72.5	72.4	70.8	70.8	70.8
6	16.9	16.9	16.7	16.7	16.9
Rha-1 (F-1)	101.2	101.4	101.6	102.3	102.2
2	71.8	71.9	71.9	71.7	71.7
3	72.5	72.4	72.3	82.3	82.6
4	85.1	85.1	84.9	78.5	78.1
5	68.3	68.3	68.5	68.4	68.8
6	18.6	18.8	18.8	18.9	18.7
Rha-1 (F-3)					104.9
2					72.2
3					72.8
4					73.7
5					70.9
6					18.7
Glc-1			105.8		
2			75.1		
3			78.3		
4			71.2		
5			78.3		
6			62.8		
Xyl-1	107.4	107.2	106.9	104.8	104.8
2	76.2	75.2	75.6	75.0	74.6
3	78.8	76.8	76.6	86.3	85.9
4	70.9	78.1	78.2	69.3	69.3
5	67.5	64.7	65.0	66.6	66.5
Api-1		109.6		111.8	111.9
2		77.8		78.1	77.8
3		81.2		79.7	80.0
4		76.1		74.2	74.4
5		67.5		64.8	64.3
Gal-1		103.1	104.5		
2		72.3	71.7		
3		73.3	75.0		
4		70.1	70.1		
5		77.0	77.3		
6		62.1	62.2		
Ara-1				105.6	105.5
2				72.7	72.5
3				74.8	74.3
4				69.2	69.2
5				67.2	67.1

Recorded at 100 MHz at 35 °C. Assignments of carbon signals of the sugar moiety were based on the HSQC spectrum.

peak at m/z 1103.5270 in the high-resolution (HR) negative FAB-MS, suggesting the formula of **1** to be $C_{53}H_{84}O_{24}$. On acid hydrolysis, **1** afforded D-glucose, D-fucose, L-rhamnose and D-xylose as a sugar moiety. The ^{1}H-NMR spectrum suggested the presence of five singlet methyls (δ 0.79, 0.94, 1.13, 1.57, 1.95), a pair of hydroxymethyls [δ 3.79 (br d, J=12 Hz), 4.05 (d, J=12 Hz)], a trisubstituted olefinic proton [δ 5.79 (t-like)] in the aglycone moiety and four anomeric proton signals [δ 5.04 (d, J=7 Hz), 5.06 (d, J=8 Hz), 6.06 (d, J=8 Hz), 6.40 (br s)]. Sugar proton and carbon signals in the NMR spectra (Tables 1, 2) were assigned by ^{1}H–^{1}H correlation spectroscopy (COSY) and a heteronuclear single quantum coherence (HSQC) spectrum. The sugar linkages were decided by a nuclear Overhauser effect (NOE) difference and a heteronuclear multiple bond coherence (HMBC) spectrum. In the HMBC spectrum, long-range couplings ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 5.06 (H-1 of glucose) and the carbon signal at δ 86.0 due to the C-3 of the aglycone, between the anomeric proton signal at δ 6.06 (H-1 of fucose) and the carbon signal at δ 176.7 due to the C-28 of the aglycone, between the anomeric proton signal at δ 6.40 (H-1 of rhamnose) and the carbon signal at δ 74.0 due to the C-2 of fucose, and between the anomeric proton signal at δ 5.04 (H-1 of xylose) and the carbon signal at δ 85.1 due to the C-4 of rhamnose. When the signals at δ 5.06 (H-1 of glucose), 6.40 (H-1 of rhamnose) and 5.04 (H-1 of xylose) were irradiated, NOEs were observed at the signals of H-3 [δ 4.60 (d, J=3 Hz)] of aglycone moiety, H-2 [δ 4.64 (t, J=8.5 Hz)] of fucose, and H-4 [δ 4.31 (t, J=9.5 Hz)] of rhamnose, respectively. From these data, the structure of polygalasaponin XXVIII was elucidated as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl (1→2)-β-D-fucopyranosyl ester.

Polygalasaponin XXIX (**2**) showed a $[M+Na]^+$ ion peak at m/z 1422 in the FAB-MS. Combined with the results of elemental analysis, its molecular formula was deduced as $C_{64}H_{102}O_{33}$. Upon acid hydrolysis, **2** gave D-glucose, D-fucose, L-rhamnose, D-apiose, D-xylose and D-galactose to be a sugar moiety. The ^{1}H-NMR spectrum of **2** showed six anomeric proton signals at δ 4.82 (d, J=8 Hz), 4.85 (d, J=7.5 Hz), 5.03 (d, J=8 Hz), 6.05 (d, J=8 Hz), 6.34 (br s) and 6.34 (br s). Sugar proton signals were assigned by homonuclear Hartmann–Hahn (HOHAHA) spectrum and detailed proton spin decoupling experiments starting from irradiation at each anomeric proton signal. Apiose proton signals were further assigned by ^{1}H–^{1}H COSY and NOE spectrum. The ^{13}C chemical shifts of **2** were similar to those of **1** except for the presence of signals due to the apiose and galactose moieties. The apiose and galactose linkage were decided by the NOE and HMBC spectrum. When the signal at δ 6.34 (H-1 of Api) was irradiated, the NOE was observed at the signal due to the H-4 [δ 4.26 (t, J=8.5 Hz)] of xylose. In the HMBC spectrum, long-range couplings ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 4.82 (H-1 of Gal) and the carbon signal at δ 67.5 due to the C-5 of apiose, between the anomeric proton signal at δ 6.34 (H-1 of Api) and the carbon signal at δ 78.1 due to the C-4 of xylose, and between the anomeric carbon signal at δ 109.6 (C-1 of Api) and the proton signal at δ 4.26 (t, J=8.5 Hz) due to the H-4 of xylose. So, the structure of polygalasaponin XXIX was determined to be 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-galactopyranosyl (1→5)-β-D-apiofuranosyl (1→4)-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-fucopyranosyl ester.

The FAB-MS and elemental analysis of polygalasaponin XXX (**3**), gave the molecular formula $C_{75}H_{112}O_{36}$. On acid hydrolysis, **3** afforded D-glucose, D-fucose, L-rhamnose, D-xylose, and D-galactose, while on alkaline hydrolysis, it gave *p*-methoxycinnamic acid. The ^{1}H-NMR spectrum of **3** exhibited six anomeric protons at δ 4.95 (d, J=8 Hz), 4.97 (d, J=7.5 Hz), 5.03 (d, J=8 Hz), 5.10 (d, J=8 Hz), 6.16 (d, J=8 Hz), 6.44 (br s), and *p*-methoxycinnamoyl signals at δ 3.67 (3H, s), 6.52 (1H, d, J=16 Hz), 6.96 (2H, d, J=8.5 Hz), 7.38 (2H, d, J=8.5 Hz), 7.89 (1H, d, J=16 Hz). The ^{13}C-NMR spectrum showed six anomeric carbon signals and *p*-methoxycinnamoyl carbon signals (see Table 2). Sugar linkages were decided by NOE and HMBC spectrum. When the signals at δ 5.03, 5.10 (H-1 of each Glc), 4.95 (H-1 of Gal), 4.97 (H-1 of Xyl) and 6.44 (H-1 of Rha) were irradiated, NOEs were observed at the signals due to the H-3 [δ 4.59 (d, J=3 Hz)] of the aglycone moiety, the H-3 [δ 4.50 (dd, J=9.5, 3 Hz)] of fucose, the H-4 [δ 4.28 (m)] of xylose, the H-4 [δ 4.26 (t, J=9.5 Hz)] of rhamnose and the H-2 [δ 4.77 (t, J=8.5 Hz)] of fucose, respectively. HMBC correlations were observed between the following carbons and protons in the oligosaccharide moieties of **3**: C-3 and H-1 of Glc, C-28 and H-1 of Fuc, C-2 of Fuc and H-1 of Rha, C-3 of Fuc and H-1 of Glc, C-4 of Rha and H-1 of Xyl, C-4 of Xyl and H-1 of Gal, and C-9 of *p*-methoxycinnamoyl and H-4 of Fuc. Based on the foregoing evidence, the structure of polygalasaponin XXX has been concluded to be 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-galactopyranosyl (1→4)-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-[4-*O*-*p*-methoxycinnamoyl]-[β-D-glucopyranosyl(1→3)]-β-D-fucopyranosyl ester.

Polygalasaponin XXXI (**4**) showed a $[M-H]^-$ ion peak at m/z 1587.6895 in the HR negative FAB-MS, suggesting the molecular formula of **4** to be $C_{75}H_{112}O_{36}$, which was the same as that of **3**. Compound **4** gave D-glucose, D-fucose, L-rhamnose, D-apiose, D-xylose, and L-arabinose on acid hydrolysis, while on alkaline hydrolysis, it furnished 3,4,5-trimethoxycinnamic acid. In the ^{1}H- and ^{13}C-NMR spectra, **4** showed six anomeric proton and carbon signals and a 3,4,5-trimethoxycinnamoyl signal (see Tables 1, 2). The NOE experiment, with irradiation at each anomeric proton signal, and the HMBC spectrum showed the connections between individual monosaccharide and 3,4,5-trimethoxycinnamic acid (see Chart 2). Therefore, the structure of polygalasaponin XXXI was elucidated as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-α-L-arabinopyranosyl(1→4)-β-D-xylopyranosyl (1→4)-[β-D-apiofuranosyl (1→3)]-α-L-rhamnopyranosyl (1→2)-[4-*O*-3,4,5-trimethoxycinnamoyl]-β-D-fucopyranosyl ester.

Polygalasaponin XXXII (**5**) afforded D-glucose, D-fucose, L-rhamnose, D-xylose, D-apiose and L-arabinose on acid hydrolysis, while on alkaline hydrolysis, it gave a *p*-methoxycinnamic acid. Its molecular formula is

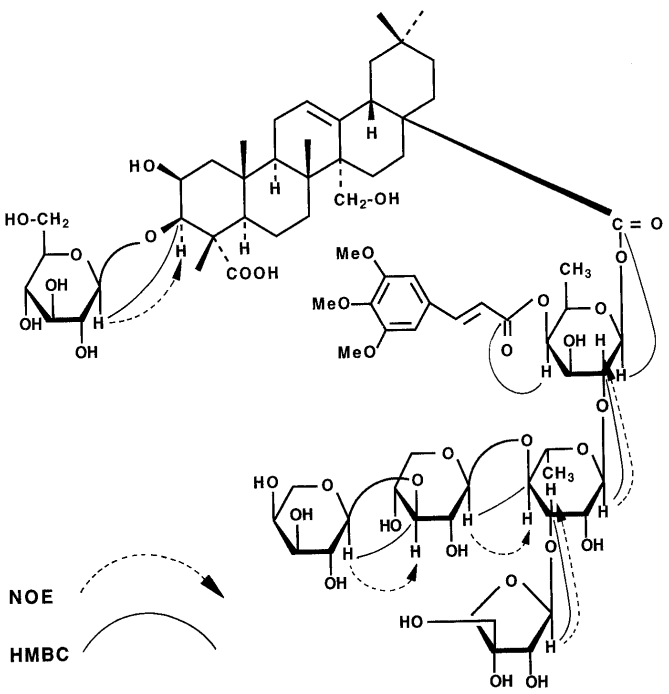

Chart 2. NOE and HMBC Correlations of **4**

$C_{79}H_{118}O_{38}$ from the FAB-MS and elemental analysis. The ^{1}H- and ^{13}C-NMR spectra of **5** disclosed seven anomeric proton and carbon signals at δ 5.04 (d, J=8 Hz), 5.16 (d, J=7.5 Hz), 5.26 (d, J=7.5 Hz), 5.56 (br s), 5.81 (br s), 6.08 (d, J=8 Hz), 6.09 (d, J=3 Hz), 95.0, 102.2, 104.8, 104.9, 105.4, 105.5, 111.9, and *p*-methoxycinnamoyl signals at δ 3.64 (3H, s), 6.56 (1H, d, J=16 Hz), 6.98 (2H, d, J=8.5 Hz), 7.41 (2H, d, J=8.5 Hz), 7.94 (1H, d, J=16 Hz), 55.4, 114.8 (2C), 115.8, 127.5, 130.5 (2C), 145.6, 167.2. The ^{1}H and ^{13}C chemical shifts of **5** were similar to those of **4** except for the presence of signals due to the terminal rhamnose moiety and the appearance of the *p*-methoxycinnamic acid instead of one 3,4,5-trimethoxycinnamic acid signal in **4**. The rhamnose and *p*-methoxycinnamic acid linkages were determined by the NOE and HMBC spectrum. When the signal at δ 5.56 (H-1 of terminal Rha) was irradiated, NOE was observed at signals due to the H-3 (δ 4.44) of fucose. In the HMBC spectrum, long-range couplings ($^3J_{HCOC}$) were observed between the proton signal at δ 5.87 (H-4 of Fuc) and the carbon signal at δ 167.2 due to the C-1 of the *p*-methoxycinnamic acid, and between the anomeric proton signal at δ 5.56 (H-1 of Rha) and the carbon signal at δ 79.9 due to the C-3 of fucose. From these data, the structure of polygalasaponin XXXII was thus established as 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-α-L-arabinopyranosyl (1→4)-β-D-xylopyranosyl (1→4)-[β-D-apiofuranosyl (1→3)]-α-L-rhamnopyranosyl (1→2)-[4-*O*-*p*-methoxycinnamoyl]-[α-L-rhamnopyranosyl (1→3)]-β-D-fucopyranosyl ester.

The anomeric configurations of glucose, fucose, xylose and galactose in these saponins were all determined to be β, and that of arabinose was determined to be α from the $^3J_{H1-H2}$ value of the anomeric proton signals, whereas those of rhamnose and apiose were determined to be α and β, respectively, by comparison of the ^{13}C-NMR data of C-3 and C-5 of rhamnose[8] and the C-1 and C-2 of apiose.[9]

Experimental

General Procedure ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL α-400 spectrometer at 35 °C and chemical shifts were given in ppm with tetramethylsilane as an internal stantard. FAB-MS were recorded on a JEOL JMS-SX102 mass-spectrometer and the mass number was counted fractions of 0.5 and over as a unit and eliminating the rest. High-resolution negative FAB-MS were recorded on a JEOL JMS-700 mass-spectrometer. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. Gas chromatography (GC) was run on a Hitachi G-3000 gas chromatograph. Semi-preparative HPLC was carried out on a column of Develosil ODS (2 cm × 25 cm) or Develosil PhA-T-5 (2 cm × 25 cm).

Extraction and Isolation *Polygala japonica* HOUTT. was collected in Jiangxi, China in May 1994, and dried roots (110 g) were extracted with 70% aqueous MeOH. The extract (12 g) was passed through a porous polymer gel Mitsubishi Diaion HP-20 column after the evaporation of MeOH. After the content of the column was washed with water, the adsorbed materials were eluted with 50% and 70% aqueous methanol and methanol, successively. The 70% aqueous methanol eluate (4.13 g) and methanol eluate (3.24 g) was chromatographed on a silica gel column, respectively, followed by repeated semi-preparative HPLC on a reversed phase column [ODS, phenylalkyl (PhA-T-5)] to give compounds **1**—**7**. **1** (120 mg), **2** (248 mg), **3** (20 mg), **4** (38 mg), **5** (36 mg), **6** (40 mg), **7** (130 mg).

Polygalasaponin XXVIII (**1**): Amorphous powder, $[\alpha]_D^{30}$ −1.0° (c=0.52, MeOH). Calcd for $C_{53}H_{83}O_{24}$ m/z: 1103.5275 ([M−H]$^-$). Found: HR-FAB-MS m/z: 1103.5270 ([M−H]$^-$). ^{1}H-NMR: shown in Table 1, ^{13}C-NMR: shown in Table 2.

Polygalasaponin XXIX (**2**): Amorphous powder, $[\alpha]_D^{25}$ −11.2° (c=2.82, MeOH). *Anal.* Calcd for $C_{64}H_{102}O_{33}\cdot 7H_2O$: C, 50.39; H, 7.66. Found: C, 50.23; H, 7.41. FAB-MS m/z: 1422 [M+Na]$^+$. ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Polygalasaponin XXX (**3**): Amorphous powder, $[\alpha]_D^{30}$ −1.1° (c=0.46, MeOH). *Anal.* Calcd for $C_{75}H_{112}O_{36}\cdot 8H_2O$: C, 51.96; H, 7.44. Found: C, 52.17; H, 7.29. FAB-MS m/z: 1612 [M+Na]$^+$. UV λ_{max}^{MeOH} nm (log ε): 225 (4.08), 299 (4.23), 311 (4.29). ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Polygalasaponin XXXI (**4**): Amorphous powder, $[\alpha]_D^{30}$ −12.0° (c=0.25, MeOH). Calcd for $C_{75}H_{112}O_{36}$ m/z: 1587.6856 ([M−H]$^-$). Found: HR-FAB-MS m/z: 1587.6895 ([M−H]$^-$). UV λ_{max}^{MeOH} nm (log ε): 229 (4.15), 308 (4.12). ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Polygalasaponin XXXII (**5**): Amorphous powder, $[\alpha]_D^{26}$ −6.6° (c=0.48, MeOH). *Anal.* Calcd for $C_{79}H_{118}O_{38}\cdot 8H_2O$: C, 52.14; H, 7.42. Found: C, 52.18; H, 7.30. FAB-MS m/z: 1698 [M+Na]$^+$. UV λ_{max}^{MeOH} nm (log ε): 227 (4.08), 299 (4.24), 311 (4.30). ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: shown in Table 2.

Acid Hydrolysis of Saponins 1—6 Compound **2** (30 mg) was refluxed with dioxane (4 ml) and 5% H_2SO_4 (2 ml) for 4 h. The reaction mixture was diluted with H_2O and extracted with EtOAc. The EtOAc layer was concentrated to dryness. The residue was subjected to semi-preparative HPLC [Develosil ODS, MeCN–H_2O (45:55) +0.05% trifluoroacetic acid (TFA)] to give senegenic acid (**1a**) (2.4 mg), $[\alpha]_D^{30}$ +20.8° (c=0.24, MeOH). FAB-MS m/z: 511 [M+Na]$^+$, and this was identified by comparison of the ^{1}H- and ^{13}C-NMR data with reported data.[5]

Each saponin (2 mg) was heated with dioxane (0.05 ml) and 5% H_2SO_4 (0.05 ml) at 100 °C for 1 h. After dilution with water, the reaction mixtures were extracted with ethyl acetate twice and the water layer was passed through an Amberlite IRA-60E column. The water eluate was concentrated and the residue was treated with D-cysteine[10] (0.05 mg) in water (0.03 ml) and pyridine (0.015 ml) at 60 °C for 1 h with stirring. After the solvent was evaporated and the reaction mixture was dried, pyridine (0.015 ml), hexamethyldisilazane (0.015 ml) and trimethylsilylchloride (0.015 ml) were added to the residue. The reaction mixture was heated at 60 °C for 30 min. The supernatant was applied to GC. The ethyl acetate layer was concentrated and subjected to HPLC to reveal a peak due to senegenic acid (**1a**) from saponins **1**—**6**. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R: D-apiose 10.56 min, L-apiose 9.87 min,[11] D-xylose 10.96 min, L-xylose 10.12 min, D-arabinose 10.12 min, L-

arabinose 10.96 min, D-rhamnose 12.49 min,[11] L-rhamnose 12.56 min, D-fucose 13.47 min, L-fucose 12.60 min, D-glucose 18.34 min, L-glucose 17.83 min, D-galactose 20.16 min, L-galactose 18.84.[11] D-Glucose, D-fucose, L-rhamnose and D-xylose were detected from **1**—**6**. D-Apiose was detected from **2** and **4**—**6**. L-Arabinose was detected from **4** and **5**. D-Galactose was detected from **2** and **3**. HPLC conditions: column, Develosil PhA-T-5, 4.6 mm × 25 cm; solvent, MeCN–H_2O (40 : 60) + 0.05% TFA; flow rate, 1.0 ml/min; UV 205 nm; t_R, senegenic acid 13.2 min.

Alkaline Hydrolysis of 3—5 Each compound (2 mg) was treated with 5% NaOH aq (0.1 ml) for 4 h at room temperature, and the reaction mixture was passed through a column filled with Amberlite IR-120B. After the content of the column were washed with water, the adsorbed materials were eluted with methanol. The methanol eluate was concentrated and subjected to HPLC to reveal a peak due to *p*-methoxycinnamic acid from **3** and **5**, and 3,4,5-trimethoxycinnamic acid from **4**. HPLC conditions: column Develosil ODS-10, 4.6 mm × 25 cm; solvent, MeCN–H_2O (32.5 : 67.5) + 0.05% TFA; flow rate, 1.0 ml/min; UV 317 nm; t_R, 3,4,5-trimethoxycinnamic acid 8.7 min, *p*-methoxycinnamic acid 11.6 min.

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