Studies on the Constituents of *Polygala japonica* HOUTT. III. Structures of Polygalasaponins XX—XXVII

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Eight new oleanane-type saponins, polygalasaponins XX—XXVII, along with a known saponin, hederagenin 3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranoside, were isolated from the aerial part of *Polygala japonica*. The structures of these compounds were established on the basis of spectroscopic and chemical evidence.

Key words *Polygala japonica*; Polygalaceae; polygalasaponin; medicagenic acid

We previously reported[1,2] the isolation and structural elucidation of triterpenoid glycosides, called polygalasaponins I—XIX, isolated from the aerial part of *Polygala japonica* HOUTT. In a continuing investigation of oligoglycosidic constituents, we identified eight new saponins designated as polygalasaponins XX—XXVII (**1**—**8**), together with a known saponin, hederagenin 3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranoside (**9**).[3] This paper deals with the isolation and structural elucidation of these saponins.

The fractions B, E and P, which were obtained by a porous polymer gel (Diaion HP-20) column and SiO_2 column from a 70% aqueous ethanolic extract of the aerial part of *P. japonica* HOUTT.,[1] were subjected to octadecyl silica (ODS) and phenylalkyl silica (PhA) columns, respectively, to afford nine saponins (**1**—**9**).

Polygalasaponin XX (**1**) showed a quasi-molecular ion peak $[M+Na]^+$ at *m/z* 819 in the FAB-MS, and elemental analysis data was consistent with the formula $C_{42}H_{68}O_{14}$. The ^{1}H-NMR spectrum of **1** exhibited signals of seven singlet methyls at δ 0.96, 1.02, 1.05, 1.31, 1.35, 1.45 and 1.45, one tri-substituted olefinic proton at δ 5.49, and two anomeric protons at δ 4.93 (d, $J=8$ Hz) and 5.31 (d, $J=8$ Hz). The ^{13}C-NMR spectrum showed two anomeric carbons at δ 104.4 and 106.0, a pair of olefinic carbons at δ 122.7 and 144.9, and one carboxyl carbon signal at δ 180.2. On acid hydrolysis, **1** afforded an aglycone (**1a**) and D-glucose as a sugar moiety. Compound **1a** was identified as 2*β*,3*β*-dihydroxy-olean-12-en-28-oic acid by comparison of the ^{1}H- and ^{13}C-NMR data with reported data.[4] Sugar proton and carbon signals in the NMR spectra were assigned by ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{13}C–^{1}H COSY (Tables 1, 2 and 3). Comparing the ^{13}C-NMR spectrum of **1** with that of **1a**, glycosylation shifts at C-2 (−1.3 ppm) and C-3 (+11.1 ppm) of the aglycone moiety indicated that **1** was a 3-monodesmoside of **1a**. The sugar linkages were determined by the nuclear Overhauser effect (NOE) difference and a heteronuclear multiple bond coherence (HMBC) spectrum. When the signals at δ 4.93 and 5.31 (H-1 of each glucose) were irradiated, NOEs were observed at the signals due to H-3 [δ 3.30 (d, $J=3$ Hz)] of the aglycone moiety and H-2 [δ 4.10 (dd, $J=8.5$, 8 Hz)] of the glucose attached at C-3 of the aglycone moiety, respectively. In the HMBC spectrum, long-range couplings ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 4.93 and the carbon signal at δ 89.5 due to C-3 of the aglycone, and between the anomeric proton signal at δ 5.31 and the carbon signal at δ 83.2 due to C-2 of the inner glucose. The anomeric configurations of two glucoses were both determined to be *β* from the $^3J_{H-H}$ values of their anomeric proton signals. From these data, the structure of polygalasaponin XX was elucidated as 2*β*-hydroxy-3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranosyl olean-12-en-28-oic acid.

Polygalasaponin XXI (**2**) showed a $[M+Na]^+$ ion peak at *m/z* 1128 in the FAB-MS. Combined with the result of elemental analysis, its molecular formula was deduced to be $C_{53}H_{84}O_{24}$. Compound **2** furnished D-glucose, L-rhamnose and D-xylose as sugar moieties, and an aglycone (**2a**). Compound **2a** was methylated with diazomethane to give **2b**. Compound **2b** showed a $[M+Na]^+$ ion peak at *m/z* 553 in the FAB-MS. The ^{1}H-NMR spectrum of **2b** exhibited signals of six singlet methyls at δ 0.72, 0.90, 1.00, 1.12, 1.25, 1.36, two carbomethoxyls at δ 3.62 and 3.72, two carbinyls at δ 3.99 (d, $J=4$ Hz) and 4.17 (m), and one trisubstituted olefinic proton at δ 5.29 (t-like). By comparison of ^{1}H- and ^{13}C-NMR data with the reported data,[5] compound **2b** was identified as medicagenic acid dimethyl ester. We therefore assumed that **2a** was medicagenic acid. In the ^{1}H- and ^{13}C-NMR spectra, compound **2** exhibited four anomeric proton and carbon signals at δ 5.04 (d, $J=8$ Hz), 5.08 (d, $J=8$ Hz), 6.19 (d, $J=8$ Hz), 6.39 (d, $J=1.5$ Hz); and 94.8, 101.4, 105.4, 107.6, respectively. Sugar proton and carbon signals in the NMR spectra were assigned by ^{1}H–^{1}H COSY, homonuclear Hartmann–Hahn (HOHAHA) spectrum and ^{13}C–^{1}H COSY. We used the NOE difference and HMBC spectrum to determine the binding sites of each sugar. In the HMBC spectrum, long-range couplings ($^3J_{HCOC}$) were observed between the anomeric proton signal at δ 5.08 (H-1 of glucose) and the carbon signal at δ 86.2 due to C-3 of the aglycone, between the anomeric proton signal at δ 6.19 (H-1 of glucose) and the carbon signal at δ 176.5 due to C-28 of the aglycone, between the anomeric proton signal at δ 6.39 (H-1 of rhamnose) and the carbon signal at δ 76.7 due to C-2 of the ester-linked glucose, and between the anomeric proton signal at δ 5.04 (H-1 of xylose) and the carbon signal at δ 85.3 due to C-4 of rhamnose. The anomeric configurations of D-glucose and D-xylose were all determined to be *β* from the $^3J_{H-H}$ value of the anomeric

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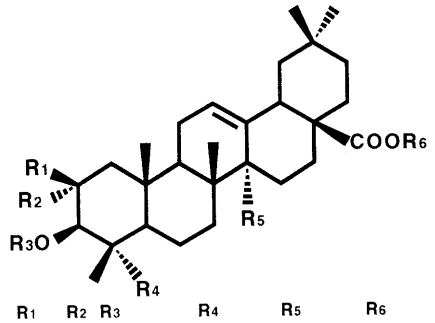

	R1	R2	R3	R4	R5	R6
1	OH	H	Glc $\overset{2}{-}$Glc	CH_3	CH_3	H
1a	OH	H	H	CH_3	CH_3	H
2	OH	H	Glc	COOH	CH_3	Glc$\overset{2}{-}$Rha$\overset{4}{-}$Xyl
2a	OH	H	H	COOH	CH_3	H
2b	OH	H	H	$COOCH_3$	CH_3	CH_3
3	OH	H	Glc	COOH	CH_3	Glc$\overset{2}{-}$Rha$\overset{4}{-}$Xyl, Api^{3}
4	O=		Glc	COOH	CH_3	Glc$\overset{2}{-}$Rha$\overset{4}{-}$Xyl
4a	O=		H	COOH	CH_3	H
5	OH	H	Glc	COOH	CH_2-OH	Fuc$\overset{2}{-}$Rha$\overset{4}{-}$Xyl, Api^{3}
5b	OH	H	H	COOH	CH_2-OH	H
9	H	H	Glc $\overset{2}{-}$Glc	CH_2-OH	CH_3	H

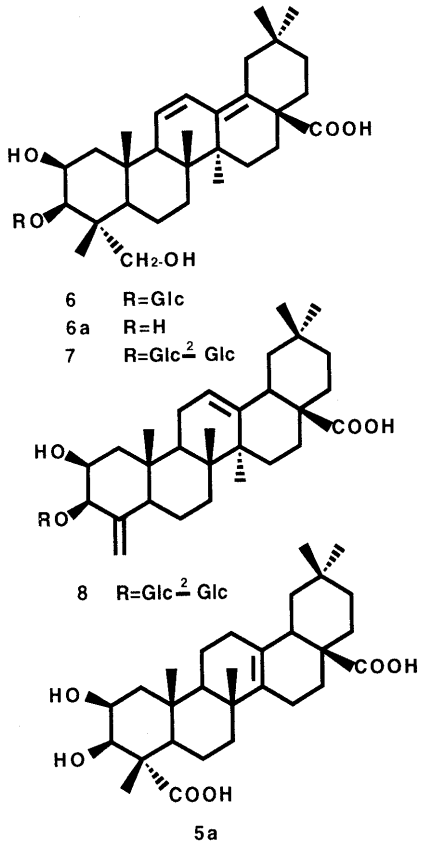

Chart 1

proton signals, and that of L-rhamnose was determined to be α by comparison of the ^{13}C chemical shifts of C-3 and C-5 of rhamnose.[6] Based on the foregoing evidence, the chemical structure of polygalasaponin XXI has been concluded to be 3-*O*-β-D-glucopyranosy medicagenic acid 28-*O*-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl ester.

The FAB-MS and elemental analysis of polygalasaponin XXII (**3**) gave the molecular formula $C_{58}H_{92}O_{28}$. Compound **3** afforded D-glucose, L-rhamnose, D-apiose, D-xylose and medicagenic acid (**2a**) on acid hydrolysis. The ^1H- and ^{13}C-NMR spectra of **3** were similar to those of **2**, except for the presence of signals due to the apiose moiety. We therefore assumed that **3** was a 3, 28-bisdesmoside of medicagenic acid. The sugar linkages were determined by NOE difference and HMBC spectrum (see Chart 2) after assignment of all proton signals due to a sugar moiety (see Table 1). In the difference NOE spectra of **3**, when the signals at δ 5.08 (H-1 of glucose), 5.06 (H-1 of xylose), 5.92 (H-1 of rhamnose) and 5.79 (H-1 of apiose) were irradiated, NOEs were observed at δ 4.69 (H-3 of the aglycone), 4.31 (H-4 of rhamnose), 4.15 and 4.29 (H-2, H-3 of ester-linked glucose), respectively. The anomeric configuration of D-glucose and D-xylose were all determined to be β from the $^3J_{H-H}$ value of the anomeric proton signals, and those of L-rhamnose and D-apiose were determined to be α and β, respectively, by comparison of the ^{13}C chemical shifts of C-3 and C-5 of rhamnose[6] and C-2 of apiose.[7] So, the structure of polygalasaponin XXII was determined to be 3-*O*-β-D-glucopyranosyl medicagenic acid 28-*O*-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-[β-D-apiofuranosyl (1→3)]-β-D-glucopyranosyl ester.

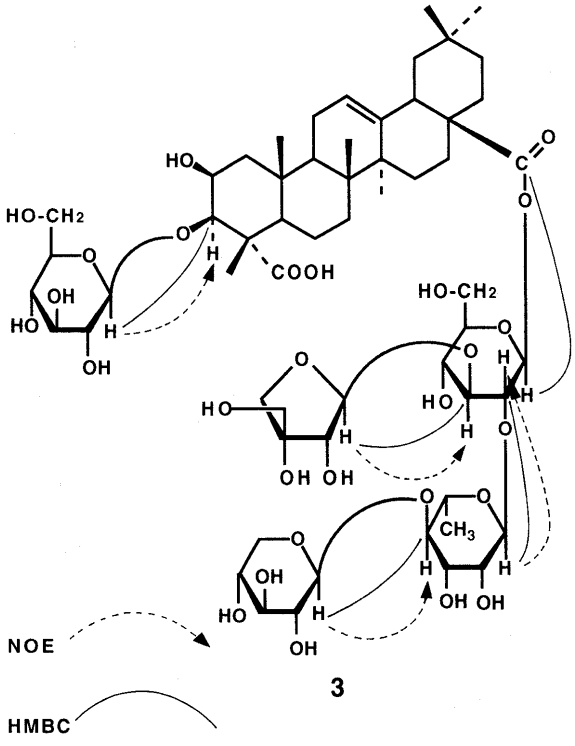

Chart 2

Polygalasaponin XXIII (**4**) showed a $[M+Na]^+$ ion peak at *m/z* 1126 in the FAB-MS. Combined with the result of elemental analysis, its molecular formula was deduced as $C_{53}H_{82}O_{24}$. Upon acid hydrolysis, compound **4** furnished D-glucose, L-rhamnose, D-xylose, and aglycone

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**8** in C_5D_5N

	1	2	3	4
Aglycon				
2	4.63 (1H, m)	4.79 (1H, m)	4.79 (1H, m)	
3	3.30 (1H, d, J=3 Hz)	4.69 (1H, d, J=4 Hz)	4.69 (1H, d, J=4 Hz)	5.74 (1H, s)
12	5.49 (1H, t-like)	5.45 (1H, t-like)	5.45 (1H, t-like)	5.41 (1H, t-like)
18	3.30[a)]	3.12 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.11 (1H, dd, J=14, 4 Hz)
23	1.35 (3H, s)			
24	1.45 (3H, s)	1.94 (3H, s)	1.95 (3H, s)	1.51 (3H, s)
25	1.45 (3H, s)	1.54 (3H, s)	1.59 (3H, s)	0.97 (3H, s)
26	1.05 (3H, s)	1.14 (3H, s)	1.12 (3H, s)	1.05 (3H, s)
27	1.31 (3H, s)	1.24 (3H, s)	1.22 (3H, s)	1.24 (3H, s)
29	0.96 (3H, s)	0.85 (3H, s)	0.85 (3H, s)	0.85 (3H, s)
30	1.02 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.85 (3H, s)
C-3 sugar				
Glc-1 (inn.)	4.93 (1H, d, J=8 Hz)	5.08 (1H, d, J=8 Hz)	5.08 (1H, d, J=8 Hz)	5.13 (1H, d, J=7.5 Hz)
2	4.10 (1H, dd, J=8, 8.5 Hz)	3.93 (1H, d, J=8.5 Hz)	3.93 (1H, t, J=8 Hz)	4.03[a)]
3	4.27[a)]	4.14[a)]	4.14[a)]	4.10 (1H, t, J=9 Hz)
4	4.12[a)]	4.15[a)]	4.15[a)]	4.14 (1H, t, J=9 Hz)
5	3.91 (1H, m)	3.91 (1H, m)	3.92 (1H, m)	3.83 (1H, m)
6	4.25[a)]	4.28[a)]	4.28[a)]	4.25[a)]
	4.46[a)]	4.46 (1H, dd, J=12, 2.5 Hz)	4.46 (1H, dd, J=12, 2 Hz)	4.39[a)]
Glc-1 (ter.)	5.31 (1H, d, J=8 Hz)			
2	4.09 (1H, dd, J=8, 8.5 Hz)			
3	4.20[a)]			
4	4.28[a)]			
5	3.91 (1H, m)			
6	4.42[a)]			
	4.47[a)]			
C-28 sugar				
Glc-1		6.19 (1H, d, J=8 Hz)	6.23 (1H, d, J=8 Hz)	6.19 (1H, d, J=6.5 Hz)
2		4.36[a)]	4.29[a)]	4.35[a)]
3		4.27[a)]	4.15[a)]	4.27[a)]
4		4.28[a)]	4.26[a)]	4.28[a)]
5		3.96 (1H, m)	3.94 (1H, m)	3.95 (1H, m)
6		4.32[a)]	4.29[a)]	4.26[a)]
		4.38 (1H, dd, J=12, 2.5 Hz)	4.30[a)]	4.34[a)]
Fuc-1				
2				
3				
4				
5				
6				
Rha-1		6.39 (1H, d, J=1.5 Hz)	5.92 (1H, br s)	6.40 (1H, br s)
2		4.81 (1H, dd, J=3, 1.5 Hz)	4.67[a)]	4.81 (1H, br s)
3		4.69 (1H, dd, J=9, 3 Hz)	4.59 (1H, dd, J=9, 3 Hz)	4.67 (1H, dd, J=9, 3 Hz)
4		4.34[a)]	4.31[a)]	4.33[a)]
5		4.51 (1H, m)	4.36 (1H, m)	4.49 (1H, m)
6		1.79 (3H, d, J=6 Hz)	1.76 (3H, d, J=6 Hz)	1.78 (3H, d, J=6 Hz)
Api-1			5.79 (1H, d, J=2.5 Hz)	
2			4.74 (1H, d, J=2.5 Hz)	
4			4.28[a)]	
			4.66 (1H, d, J=9.5 Hz)	
5			4.09 (1H, d, J=11 Hz)	
			4.12 (1H, d, J=11 Hz)	
Xyl-1		5.04 (1H, d, J=8 Hz)	5.06 (1H, d, J=8 Hz)	5.04 (1H, d, J=7 Hz)
2		4.05[a)]	4.03[a)]	4.05[a)]
3		4.05[a)]	4.03[a)]	4.06[a)]
4		4.18[a)]	4.17[a)]	4.19[a)]
5		3.53 (1H, t, J=11 Hz)	3.50 (1H, t, J=11 Hz)	3.53 (1H, t, J=11 Hz)
		4.24[a)]	4.24[a)]	4.25[a)]

(**4a**). Compound **4a** revealed a $[M+Na]^+$ ion peak at m/z 523 in the FAB-MS. The ^{13}C-NMR chemical shifts of **4a** were similar to those of medicagenic acid (**2a**) except for the appearance of a carbonyl carbon (δ 210.5) instead of one carbinyl carbon at C-2 in medicagenic acid (**2a**). Thus, **4a** was presumed to be 3β-hydroxy-2-oxo-olean-12-en-23,28-dioic acid. In the ^{1}H-NMR spectrum, compound **4** showed four anomeric proton signals at δ 5.04 (d, J=7 Hz), 5.13 (d, J=7.5 Hz), 6.19 (d, J=6.5 Hz) and 6.40 (br s), and one carbinyl proton signal at δ 5.74 (s) due to H-3 of the aglycone. The ^{13}C-NMR chemical shifts of **4** were similar to those of **2** except for the appearance of a carbonyl carbon (δ 207.5) instead of one carbinyl carbon at C-2 of the aglycone moiety in **2**. In the HMBC spectrum, long-

Table 1. (continued)

	5	6	7	8
Aglycon				
2	4.70 (1H, m)	4.88 (1H, m)	4.83 (1H, m)	4.52 (1H, m)
3	4.58 (1H, d, J=3 Hz)	4.37 (1H, d, J=3 Hz)	4.26 (1H, d, J=3 Hz)	4.57 (1H, d, J=3 Hz)
11		5.91 (1H, br d, J=11 Hz)	5.89 (1H, d, J=11 Hz)	
12	5.80 (1H, t-like)	6.65 (1H, dd, J=11, 2.5 Hz)	6.64 (1H, br d, J=11 Hz)	5.46 (1H, t-like)
18	3.22 (1H, dd, J=14, 4 Hz)			3.29 (1H, dd, J=14, 4 Hz)
23		3.69 (1H, d, J=11 Hz)	3.74 (1H, d, J=11 Hz)	5.01 (br s)
		4.39 (1H, d, J=11 Hz)	4.39 (1H, d, J=11 Hz)	6.09 (br s)
24	1.94 (3H, s)	1.33 (3H, s)	1.41 (3H, s)	
25	1.54 (3H, s)	1.63 (3H, s)	1.61 (3H, s)	1.21 (3H, s)
26	1.12 (3H, s)	1.14 (3H, s)	1.13 (3H, s)	1.04 (3H, s)
27	3.78 (1H, br d, J=12 Hz)	1.07 (3H, s)	1.05 (3H, s)	1.29 (3H, s)
	4.06 (1H, br d, J=12 Hz)			
29	0.80 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	0.97 (3H, s)
30	0.94 (3H, s)	0.90 (3H, s)	0.90 (3H, s)	1.01 (3H, s)
C-3 sugar				
Glc-1 (inn.)	5.04 (1H, d, J=8 Hz)	5.18 (1H, d, J=8 Hz)	5.13 (1H, d, J=8 Hz)	5.17 (1H, d, J=8 Hz)
2	3.92[a]	4.03 (1H, t, J=8.5 Hz)	4.11 (1H, t, J=8.5 Hz)	4.11 (1H, dd, J=8, 8.5 Hz)
3	4.15[a]	4.16 (1H, t, J=8.5 Hz)	4.22 (1H, t, J=8.5 Hz)	4.32 (1H, t, J=8.5 Hz)
4	4.16[a]	4.21 (1H, t, J=8.5 Hz)	4.13 (1H, t, J=9 Hz)	4.20 (1H, t, J=9 Hz)
5	3.95 (1H, m)	3.91 (1H, m)	3.84 (1H, m)	3.92 (1H, m)
6	4.31[a]	4.33 (1H, dd, J=12, 5 Hz)	4.27[a]	4.50[a]
	4.46 (1H, dd, J=12, 2 Hz)	4.47 (1H, dd, J=12, 2 Hz)	4.44[a]	4.54 (1H, dd, J=12, 2 Hz)
Glc-1 (ter.)			5.34 (1H, d, J=8 Hz)	5.27 (1H, d, J=7.5 Hz)
2			4.09 (1H, t, J=8.5 Hz)	4.11 (1H, dd, J=7.5, 8.5 Hz)
3			4.19 (1H, t, J=8.5 Hz)	4.16 (1H, t, J=8.5 Hz)
4			4.23 (1H, t, J=9 Hz)	4.16 (1H, t, J=9 Hz)
5			3.92 (1H, m)	3.90 (1H, m)
6			4.39[a]	4.33 (1H, dd, J=12, 5 Hz)
			4.51 (1H, dd, J=12, 2 Hz)	4.46 (1H, dd, J=12, 2 Hz)
C-28 sugar				
Glc-1				
2				
3				
4				
5				
6				
Fuc-1	6.06 (1H, d, J=8 Hz)			
2	4.63 (1H, t, J=8.5 Hz)			
3	4.00[a]			
4	4.15[a]			
5	3.86[a]			
6	1.49 (3H, d, J=6 Hz)			
Rha-1	6.37 (1H, d, J=1.5 Hz)			
2	4.79[a]			
3	4.66 (1H, dd, J=9, 3 Hz)			
4	4.28 (1H, t, J=9 Hz)			
5	4.43 (1H, m)			
6	1.65 (3H, d, J=6 Hz)			
Api-1	6.14 (1H, d, J=2.5 Hz)			
2	4.79 (1H, d, J=2.5 Hz)			
4	4.29 (1H, d, J=9.5 Hz)			
	4.70 (1H, d, J=9.5 Hz)			
5	4.16[a]			
	4.16[a]			
Xyl-1	4.93 (1H, d, J=7.5 Hz)			
2	4.02[a]			
3	4.02[a]			
4	4.04[a]			
5	3.40 (1H, t, J=11 Hz)			
	4.18[a]			

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

range coupling was observed between the singlet signal at δ 5.74 and the carbonyl carbon signal at δ 207.5. Therefore, the carbonyl carbon was located at C-2 of the aglycone. When **4** was reduced with $NaBH_4$, the reductant was identified as polygalasaponin XXI (**2**) by direct comparison of the spectral data with those of **2**. Therefore, polygalasaponin XXII was characterized as 3-*O*-β-D-glucopyranosyl 2-oxo-olean-12-en-23,28-dioic acid 28-*O*-β-D-

Table 2. ^{13}C-NMR Spectral Data of Aglycone Moiety of Compounds **1**—**8** in C_5D_5N

Carbon No.	1	2	3	4	5	6	7	8	4a	6a
1	44.0	44.3	44.3	54.7	44.2	43.8	43.6	44.9	53.8	44.6
2	70.2	70.3	70.3	207.5	70.3	70.6	70.5	70.9	210.5	71.6
3	89.5	86.2	86.1	85.3	86.0	83.0	82.8	81.4	81.4	73.1
4	38.9	52.9	52.9	58.2	52.9	42.7	42.7	146.5	58.6	42.6
5	56.3	52.5	52.5	52.4	52.5	47.5	47.9	51.5	51.6	47.9
6	18.4	21.4	21.3	21.0	21.4	18.1	18.2	21.4	21.1	18.4
7	33.3	32.4	32.3	32.3	33.6	33.3	33.6	31.9	32.7	33.3
8	39.9	40.5	40.4	40.0	41.2	41.3	41.3	40.0	39.8	41.3
9	48.5	48.8	48.8	48.0	49.3	55.5	55.5	46.5	48.0	55.5
10	37.0	36.9	36.9	43.3	37.0	36.8	36.8	40.0	43.5	37.0
11	24.0	24.1	24.0	23.8	23.5	127.4	127.4	24.8	23.8	127.4
12	122.7	122.8	122.9	122.1	127.9	126.0	126.0	122.7	121.9	125.9
13	144.9	144.1	144.1	144.2	138.8	136.7	136.7	145.1	145.0	136.7
14	42.4	42.5	42.4	42.4	48.0	43.0	42.9	42.4	42.3	42.7
15	28.3	28.4	28.3	28.5	24.5	36.3	36.3	28.2	28.3	36.3
16	23.8	23.5	23.4	23.4	24.1	25.7	25.7	23.8	23.7	25.7
17	46.7	47.1	47.1	47.1	46.9	48.7	48.8	46.1	46.6	48.7
18	42.1	42.2	42.1	42.1	42.0	133.2	133.3	42.2	42.0	133.2
19	46.6	46.4	46.3	46.3	45.3	41.0	41.0	46.8	46.5	41.0
20	31.0	30.8	30.8	30.8	30.8	32.8	32.8	31.0	31.0	32.8
21	34.3	34.0	34.0	34.0	33.8	37.5	37.5	34.2	34.3	37.5
22	33.4	33.1	33.1	32.8	32.4	32.7	32.7	33.2	33.2	32.7
23	29.8	180.8	180.7	180.8	180.8	65.4	65.6	107.3	180.0	67.5
24	18.5	14.2	14.2	13.6	14.2	14.5	14.3		13.3	14.0
25	16.5	17.0	17.0	16.7	17.5	20.1	20.0	15.7	16.8	20.0
26	17.5	17.5	17.5	17.1	18.6	17.1	17.2	17.5	17.0	17.1
27	26.3	26.1	26.1	26.0	64.5	20.2	20.2	26.3	26.1	20.2
28	180.2	176.5	176.2	176.5	176.7	178.9	179.0	180.2	180.1	179.0
29	33.3	33.1	33.1	33.1	33.1	32.4	32.5	33.3	33.3	32.4
30	23.8	23.8	23.8	23.8	24.1	24.5	24.5	23.8	23.7	24.4

Recorded at 100 MHz at 35 °C.

xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl ester.

Polygalasaponin XXIV (**5**), $C_{58}H_{92}O_{29}$, showed its $[M+Na]^+$ ion peak at *m/z* 1276 in the FAB-MS. The ^{1}H-NMR spectrum suggested the presence of five singlet methyls (δ 0.80, 0.94, 1.12, 1.54, 1.94), a pair of hydroxymethyl [δ 3.78 (br d, J=12 Hz), 4.06 (d, J=12 Hz)], a tri-substituted olefinic proton [δ 5.80 (t-like)] in the aglycone moiety, and five anomeric proton signals [δ 4.93 (d, J=7.5 Hz), 5.04 (d, J=8 Hz), 6.06 (d, J=8 Hz), 6.14 (d, J=2.5 Hz), 6.37 (d, J=1.5 Hz)]. On acid hydrolysis, compound **5** furnished **5a** and D-glucose, D-fucose, L-rhamnose, D-apiose and D-xylose. Compound **5a** revealed a $[M+Na]^+$ ion peak at *m/z* 511 in the FAB-MS. The ^{1}H-NMR spectrum of **5a** showed five singlet methyls at δ 1.00, 1.03, 1.07, 1.51, 2.00, and two carbinyl proton signals at δ 4.61 (m) and 4.72 (d, J=4 Hz). By comparison of the ^{1}H- and ^{13}C-NMR data with reported data,[8] compound **5a** was characterized as senegenic acid. Senegins which were isolated from *Polygala senega* and had presenegenin (**5b**) as a genuine aglycone, afforded senegenic acid (**5a**) by treatment with acid.[9] We therefore assumed that polygalasaponin XXIV was a presenegenin glycoside. By comparing the ^{13}C-NMR spectrum of the aglycone moiety of **5** and senegin III,[10] the genuine aglycone of **5** was determined to be presenegenin (2β,3β,27-trihydroxy-olean-12-en-23,28-dioic acid) (**5b**). To determine the binding sites of five monosaccharides, we used the NOE difference and HMBC spectrum after assignment of all the proton signals due to a sugar moiety. So, the structure of polygalasaponin XXIV was determined to be 3-*O*-β-D-glucopyranosyl presenegenin 28-*O*-β-D-xylopyranosyl (1→4)-α-L-rhamnopyranosyl (1→2)-[β-D-apiofuranosyl (1→3)]-β-D-fucopyranosyl ester.

Polygalasaponins XXV (**6**), $C_{36}H_{56}O_{10}$, and XXVI (**7**), $C_{42}H_{66}O_{15}$, afforded D-glucose as a sugar moiety in addition to compound (**6a**) as an aglycone. Compound **6a** revealed a $[M+Na]^+$ ion peak at *m/z* 509 in the FAB-MS. The ^{13}C-NMR signals of **6a** were similar to those of 3β,23-dihydroxy-olean-11,13(18)-dien-28-oic acid except for a carbinyl carbon at C-2.[11] Thus compound **6a** was recognized as 2β,3β,23-trihydroxy-olean-11,13(18)-dien-28-oic acid. In the NMR spectra, compound **6** showed the presence of six singlet methyls (δ 0.90, 0.94, 1.07, 1.14, 1.33, 1.63), a pair of hydroxymethyls [δ 3.69 (d, J=11 Hz), 4.39 (d, J=11 Hz)], a pair of *cis*-olefinic proton signals [δ 5.91 (br d, J=11 Hz), 6.65 (dd, J=11, 2.5 Hz)] in the aglycone moiety, and one anomeric proton signal [δ 5.18 (d, J=8 Hz)]. The ^{13}C-NMR spectrum exhibited the presence of four olefinic carbons (δ 126.0, 127.4, 133.2, 136.7), one carboxyl carbon (δ 178.9), and one anomeric carbon (δ 105.8). From these NMR data, compound **6** was assumed to be a 2β,3β,23-trihydroxy-olean-11,13(18)-dien-28-oic acid monoglucoside. The binding site of this glucose was determined by HMBC spectrum. In the HMBC spectrum, long-range coupling ($^3J_{HCOC}$) was observed between the anomeric proton signal at δ 5.18 (d, J=8 Hz) and the carbon signal at δ 83.0 due to C-3 of

Table 3. ^{13}C-NMR Spectral Data of Sugar Moiety of Compounds **1**—**8** in C_5D_5N

	1	2	3	4	5	6	7	8
C-3 sugar								
Glc-1 (inn.)	104.4	105.4	105.4	103.9	105.3	105.8	103.2	101.0
2	83.2	75.3	75.3	75.0	75.3	75.5	83.7	84.6
3	78.3	78.4	78.4	78.6	78.4	78.6	78.1	78.4
4	71.5	71.6	71.6	71.5	71.6	71.7	71.1	71.6
5	78.2	78.4	78.4	78.1	78.4	78.3	78.1	77.9
6	62.6	62.7	62.8	62.7	62.8	62.8	62.5	62.6
Glc-1 (ter.)	106.0						105.9	106.8
2	77.1						76.8	76.5
3	78.1						78.4	78.6
4	71.8						71.5	71.4
5	78.0						78.3	78.2
6	62.8						62.7	62.8
C-28 sugar								
Glc-1		94.8	94.2	94.8				
2		76.7	76.0	76.8				
3		79.3	86.5	79.4				
4		71.4	69.3	71.4				
5		78.8	77.9	78.9				
6		62.3	62.0	62.3				
Fuc-1					94.8			
2					74.3			
3					85.2			
4					72.5			
5					73.2			
6					16.9			
Rha-1		101.4	101.4	101.4	101.3			
2		71.8	71.6	71.8	71.8			
3		72.5	72.4	72.6	72.4			
4		85.3	84.4	85.5	85.3			
5		68.5	68.9	68.4	68.3			
6		18.7	18.6	18.7	18.8			
Xyl-1		107.6	107.3	107.6	107.3			
2		76.3	76.1	76.3	76.7			
3		78.8	78.7	78.8	75.4			
4		71.0	71.0	70.9	69.3			
5		67.6	67.5	67.6	67.0			
Api-1			110.8		111.2			
2			78.0		77.8			
3			80.3		80.4			
4			75.3		75.2			
5			64.9		65.5			

Recorded at 100 MHz at 35 °C. Assignments based on ^{13}C–^{1}H COSY measurement.

the aglycone. Based on the foregoing evidence, the chemical structure of polygalasaponin XXV has been concluded to be 2β,23-dihydroxy-3-*O*-β-D-glucopyranosyl olean-11,13(18)-dien-28-oic acid. In the ^{1}H- and ^{13}C-NMR spectra, compound **7** exhibited two anomeric proton and carbon signals at δ 5.13 (d, J=8 Hz) and 5.34 (d, J=8 Hz); and 103.2 and 105.9, respectively. The ^{1}H and ^{13}C signals of **7** were similar to those of **6**, except that the presence of signals due to the terminal glucose moiety indicated that **7** was a 3-monodesmoside of 2β,3β,23-trihydroxy olean-11,13(18)-dien-28-oic acid. The sugar linkages were determined by HMBC spectrum after assignment of all proton signals due to a sugar moiety. In the HMBC spectrum, long-range couplings ($^{3}J_{HCOC}$) were observed between the anomeric proton signal at δ 5.13 (H-1 of inner glucose) and the carbon signal at δ 82.8 due to C-3 of the aglycone, and between the anomeric proton signal at δ 5.34 (H-1 of terminal glucose) and the carbon signal at δ 83.7 due to C-2 of inner glucose. The structure of polygalasaponin XXVI was thus established as 2β,23-dihydroxy-3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl olean-11,13(18)-dien-28-oic acid.

Polygalasaponin XXVII (**8**) afforded D-glucose on acid hydrolysis. Its molecular formula was determined to be $C_{41}H_{46}O_{14}$ from the FAB-MS and elemental analysis. The ^{1}H- and ^{13}C-NMR spectra of **8** disclosed the presence of five singlet methyls (δ 0.97, 1.01, 1.04, 1.21, 1.29), two carbinyls [δ 4.52 (m), 4.75 (d, J=3 Hz)], three olefinic protons [δ 5.01 (br s), 5.46 (t-like), 6.09 (br s)] in the aglycone moiety, two anomeric protons [δ 5.17 (d, J=8 Hz), 5.27 (d, J=7.5 Hz)] and four olefinic carbons (δ 107.3, 122.7, 145.1, 146.5), and one carboxyl carbon (δ 180.2). The ^{1}H and ^{13}C chemical shifts of **8** were similar to those of **1** except for the presence of an exomethylene group [δ_H 5.01 (br s), 6.09 (br s); δ_C 107.3, 146.5] instead of two methyl groups in **1**. The position of this exomethylene group was determined to be at C-4 by HMBC spectrum. In the HMBC spectrum, long-range couplings ($^{3}J_{HCOC}$) were observed between the exomethylene proton signals at δ 5.01 (br s), 6.09 (br s) and the carbon signals at δ 81.4 due to C-3 and at δ 51.5 due to C-5 of the aglycone. From these data, the structure of polygalasaponin XXVII was thus established as 2β-hydroxy-3-*O*-β-D-glucopyranosyl (1→2)-β-D-glucopyranosyl-24-norolean-4(23),12-dien-28-oic acid.

Experimental

General Procedure The instruments used to obtain the physical data and the experimental conditions for chromatography were the same, except that the NMR spectra were obtained with a JEOL GSX-500 spectrometer and a JEOL α-400 spectrometer, as in our previous paper.[1]

Isolation of Polygalasaponins XX—XXVII As was noted earlier,[1] the ethanolic extract was passed through a porous polymer gel Diaion HP-20 (Mitsubishikasei Co. Ltd.) column. After the content of the column was washed with water, the adsorbed materials were eluted with 50% aqueous methanol and methanol, successively. The methanol eluate (80 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (85 : 14 : 1), increasing a portion of MeOH to give 19 fractions (frs. A—S). From frs. B (1.8 g), E (5.8 g) and P (12.9 g), compounds **1**—**9** were isolated by preparative (column: Develosil Lop-ODS, 5 cm × 50 cm × 2, solvent: CH_3CN–H_2O system) and semi-preparative HPLC [column: Develosil PhA-7, 2 cm × 25 cm, solvent: CH_3CN–H_2O–TFA system (recycle)]. **1** (138 mg), **2** (176 mg), **3** (88 mg), **4** (64 mg), **5** (104 mg), **6** (94 mg), **7** (90 mg), **8** (45 mg), **9** (24 mg).

Polygalasaponin XX (**1**): Amorphous powder, $[\alpha]_D^{20}$ +33.5° (c=1.04, pyridine). *Anal.* Calcd for $C_{42}H_{68}O_{14}\cdot 5/2H_2O$: C, 59.91; H, 8.74. Found: C, 59.97; H, 8.65. FAB-MS m/z: 819 ([M+Na]$^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXI (**2**): Amorphous powder, $[\alpha]_D^{21}$ +31.3° (c=0.78, pyridine). *Anal.* Calcd for $C_{53}H_{84}O_{24}\cdot 15/2H_2O$: C, 51.32; H, 8.05. Found: C, 51.27; H, 7.94. FAB-MS m/z: 1128 ([M+Na]$^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXII (**3**): Amorphous powder, $[\alpha]_D^{20}$ −1.1° (c=0.92, pyridine). *Anal.* Calcd for $C_{58}H_{92}O_{28}\cdot 6H_2O$: C, 51.78; H, 7.79. Found: C, 51.83; H, 7.49. FAB-MS m/z: 1260 ([M+Na]$^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXIII (**4**): Amorphous powder, $[\alpha]_D^{20}$ +20.6° (c=0.85, pyridine). *Anal.* Calcd for $C_{53}H_{82}O_{24}\cdot 7H_2O$: C, 51.78; H, 7.87. Found: C, 51.95; H, 7.70. FAB-MS m/z: 1126 ([M+Na]$^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXIV (**5**): Amorphous powder, $[\alpha]_D^{20}$ +1.1° (c= 0.92, pyridine). *Anal.* Calcd for $C_{58}H_{92}O_{29}\cdot 9/2H_2O$: C, 52.20; H, 7.63. Found: C, 52.22; H, 7.66. FAB-MS m/z: 1276 ([M+Na]$^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXV (**6**): Amorphous powder, $[\alpha]_D^{21}$ −70.6° (c=0.86, pyridine). *Anal.* Calcd for $C_{36}H_{56}O_{10}\cdot 3H_2O$: C, 61.52; H, 8.89. Found: C, 61.68; H, 8.60. FAB-MS m/z: 671 ([M+Na]$^+$). ^{1}H-NMR: Table 1.

^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXVI (**7**): Amorphous powder, $[\alpha]_D^{20}$ +52.1° (c=0.98, pyridine). *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 3H_2O$: C, 58.32; H, 8.40. Found: C, 58.62; H, 8.57. FAB-MS m/z: 833 ($[M+Na]^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Polygalasaponin XXVII (**8**): Amorphous powder, $[\alpha]_D^{25}$ +47.6° (c=1.23, MeOH). *Anal.* Calcd for $C_{41}H_{64}O_{14}\cdot 4H_2O$: C, 57.73; H, 8.51. Found: C, 57.98; H, 8.20. FAB-MS m/z: 803 ($[M+Na]^+$). ^{1}H-NMR: Table 1. ^{13}C-NMR: Tables 2 and 3.

Reduction of Polygalasaponin XXIII (4) with $NaBH_4$ Compound **4** (16 mg) was reduced with $NaBH_4$ (32 mg) in MeOH (1 ml) for 8 h at room temperature. After the reaction mixture was diluted with H_2O, it was then passed through a porous polymer gel Diaion HP-20 column. After the content of the column was washed with water, the adsorbed material was eluted with MeOH to give polygalasaponin XXI (**2**) (12 mg) that was identified by direct comparison of ^{1}H- and ^{13}C-NMR data with those of polygalasaponin XXI.

Acid Hydrolysis of Saponins 1—9 Compounds **1** (35 mg), **2** (24 mg), **4** (32 mg), **5** (30 mg) and **6** (31 mg) were refluxed with dioxane (4 ml) and 5% H_2SO_4 (2 ml) for 4 h, respectively. The reaction mixtures were diluted with H_2O and extracted with EtOAc. The EtOAc layer was concentrated to dryness. The residue was chromatographed on preparative TLC and semi-preparative HPLC: Compound **1** afforded 2β,3β-dihydroxy-olean-12-en-28-oic acid (**1a**) (5.8 mg), $[\alpha]_D^{20}$ +74.3° (c=0.58, pyridine), FAB-MS m/z 495 ($[M+Na]^+$) which was identified by comparison of the ^{1}H- and ^{13}C-NMR data with the reported data.[4] Compound **2** gave medicagenic acid (**2a**) (5.6 mg) as an aglycone, $[\alpha]_D^{28}$ +89.8° (c=0.56, MeOH), FAB-MS m/z 525 ($[M+Na]^+$). Compound **2a** was methylated with diazomethane to give medicagenic acid dimethyl ester (**2b**), which was identified by comparison of ^{1}H- and ^{13}C-NMR data with reported data.[5] Compound **4** yielded **4a** (11 mg) as an aglycone, $[\alpha]_D^{20}$ +76.1° (c=1.04, MeOH), FAB-MS m/z 523 ($[M+Na]^+$). ^{1}H-NMR (pyridine-d_5): δ 0.93 (3H, s, H_3-29), 0.97 (3H, s, H_3-30), 0.99 (3H, s, H_3-25), 1.00 (3H, s, H_3-26), 1.25 (3H, s, H_3-27), 1.51 (3H, s, H_3-24), 2.43 (1H, d, J=12 Hz, H-1), 2.57 (1H, d, J=12 Hz, H-1), 3.28 (1H, dd, J=14, 4 Hz, H-18), 5.46 (1H, s, H-3), 5.47 (1H, t-like, H-12). ^{13}C-NMR: shown in Table 2. Compound **5** gave senegenic acid (**5a**) (2.4 mg), $[\alpha]_D^{20}$ −14.6° (c=0.24, MeOH), FAB-MS m/z 511 ($[M+Na]^+$) which was identified by comparison of ^{1}H- and ^{13}C-NMR data with reported data.[6] Compound **6** furnished **6a** (6 mg) as an aglycone, $[\alpha]_D^{20}$ −89.6° (c=0.31, MeOH), FAB-MS m/z 509 ($[M+Na]^+$). ^{1}H-NMR (pyridine-d_5): δ 0.91 (3H, s, H_3-30), 0.95 (3H, s, H_3-29), 1.07 (3H, s, H_3-27), 1.17 (3H, s, H_3-26), 1.35 (3H, s, H_3-24), 1.62 (3H, s, H_3-25), 3.72 (1H, d, J=11 Hz, H-23), 4.18 (1H, d, J=11 Hz, H-23), 4.31 (1H, d, J=3 Hz, H-3), 4.60 (1H, m, H-2), 5.94 (1H, d, J=11 Hz, H-11), 6.67 (1H, dd, J=11, 2.5 Hz, H-12). ^{13}C-NMR: Table 2. Each saponin (2 mg) was heated at 100 °C with dioxane (0.05 ml) and 5% H_2SO_4 (0.05 ml) for 1 h. After dilution with water, the reaction mixture was extracted with EtOAc 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[12] (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 EtOAc layer was concentrated and subjected to HPLC to reveal a peak due to 2β,3β-dihydroxy-olean-12-en-28-oic acid (**1a**) from saponin **1**, medicagenic acid (**2a**) from saponins **2** and **3**, **4a** from saponin **4**, senegenic acid (**5a**) from saponin **5**, and **6a** from saponins **6** and **7**. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R (min): D-apiose 9.03, L-apiose 8.65,[13] D-xylose 9.46, L-xylose 8.73, L-rhamnose 11.00, D-rhamnose 11.02,[13] D-fucose 12.45, L-fucose 11.27, D-glucose 16.06, L-glucose 15.58. D-Glucose was detected from **1**—**9**. L-Rhamnose was detected from **2**—**5**. D-Xylose was detected from **2**—**5**. D-Apiose was detected from **3** and **5**. D-Fucose was detected from **5**. HPLC conditions: column, Develosil PhA-7, 4.6 mm × 25 cm; solvent, MeCN–H_2O–TFA (47.5 : 52.5 : 0.05); flow rate, 1.0 ml/min; UV 205 nm; t_R (min), 2β,3β-dihydroxy olean-12-en-28-oic acid 12.4, medicagenic acid 8.6, **4a** 10.7, senegenic acid 5.1 and **6a** 10.1.

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