Studies on the Constituents of *Polygala japonica* HOUTT. I. Structures of Polygalasaponins I—X

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Ten new oleanane-type saponins, polygalasaponins I—X, along with two known saponins, bayogenin-3-*O*-β-D-glucopyranoside and lobatoside B were isolated from the aerial part of *Polygala japonica* HOUTT. The structures of these compounds were established on the basis of spectroscopic and chemical evidence.

Keywords *Polygala japonica*; Polygalaceae; polygalasaponin; oleanane-type saponin; bayogenin

Polygala japonica HOUTT. is widely distributed in southern China, and is used as an expectorant, an anti-inflammatory agent for pharyngitis and an antibacterial agent.[1] Fang and Yin[2,3] reported the isolation and structure determination of three saponins whose aglycone was 2α,3α,24-trihydroxyolean-12-en-28-oic acid from this plant. During the course of further investigation, we obtained ten new saponins whose aglycone was bayogenin, designated as polygalasaponins I—X (**1**—**10**). This paper deals with the isolation and structural elucidation of these saponins.

A 70% aqueous ethanol extract of the aerial part of *P. japonica* HOUTT. was passed through a porous polymer gel Mitsubishi Diaion HP-20 column and the adsorbed materials were eluted with 50% aqueous methanol and methanol, successively. The methanol eluate was chromatographed on silica gel and octadecyl silica (ODS) columns, followed by repeated semi-preparative HPLC on a reversed phase column [ODS, phenylalkyl (PhA)]. We isolated twelve saponins, the structures of ten of these were established and the two other were identified as bayogenin-3-*O*-β-D-glucopyranoside (**11**) and lobatoside B (**12**) by comparison of the spectral data with reported data.[4,5] New saponins (**1**—**10**) afforded bayogenin (**13**) as an aglycone on acid hydrolysis.

Polygalasaponin I (**1**) revealed a $[M+Na]^+$ ion peak at *m/z* 835 in the FAB-MS and elemental analysis data was consistent with $C_{42}H_{68}O_{15}$. On acid hydrolysis, **1** afforded D-glucose as a sugar moiety. The ^{1}H-NMR spectrum of **1** showed two anomeric proton signals at δ 5.12 (d, $J=8$ Hz) and 6.29 (d, $J=8$ Hz). Sugar proton signals (Tables I, II) were assigned by detailed proton spin decoupling experiment, ^{1}H–^{1}H correlation spectroscopy (COSY) and ^{13}C–^{1}H COSY spectra, while the ^{13}C-NMR spectrum of **1** exhibited two anomeric carbon signals at δ 95.8 and 105.7. Comparing the ^{13}C-NMR spectrum of **1** with that of bayogenin (**13**), glycosylation shifts at C-2 (−1.0 ppm), C-3 (+9.9 ppm) and C-28 (−3.7 ppm) of the aglycone indicated that **1** was a 3,28-bisdesmoside of bayogenin (**13**). The anomeric configurations of two glucoses were both determined to be β from the *J* values of their anomeric proton signals. Therefore, polygalasaponin I was characterized as 3-*O*-β-D-glucopyranosyl bayogenin 28-*O*-β-D-glucopyranosyl ester.

Polygalasaponin II (**2**) showed a $[M+Na]^+$ ion peak at *m/z* 982 in the FAB-MS. Combined with the result of elemental analysis, its molecular formula was deduced as $C_{48}H_{78}O_{19}$. Upon acid hydrolysis, **2** gave D-glucose and L-rhamnose as a sugar moiety. The ^{1}H-NMR spectrum of **2** showed three anomeric proton signals at δ 5.11 (d, $J=8$ Hz), 6.17 (d, $J=8$ Hz) and 6.53 (br s). The ^{13}C-NMR spectrum of **2** showed three anomeric carbon signals at δ 94.9, 101.5 and 105.7. Glycosylation shifts in the aglycone moiety indicated that **2** was a 3,28-bisdesmoside. Since the anomeric carbon signal (δ 94.7) due to glucose showed an ester-type glycoside linkage, one glucose was found to be linked at C-28 of bayogenin. Nuclear Overhauser effects (NOE)s were observed at H-3 [δ 4.27

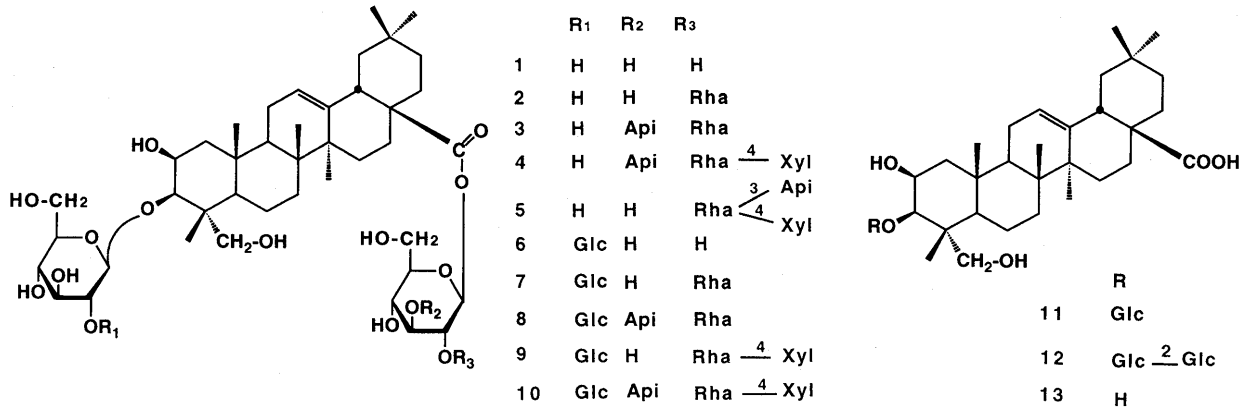

Chart 1

TABLE I. ^{1}H-NMR Spectral Data of Compounds **1**—**12** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**
Aglycone					
2	4.78 (1H, m)	4.77 (1H, m)	4.78 (1H, m)	4.79 (1H, m)	4.78 (1H, m)
3	4.29 (1H, d, J=3 Hz)	4.27 (1H, d, J=3 Hz)	4.29 (1H, d, J=3 Hz)	4.32 (1H, d, J=3 Hz)	4.29 (1H, d, J=3 Hz)
12	5.44 (1H, t-like)	5.47 (1H, t-like)	5.47 (1H, t-like)	5.47 (1H, t-like)	5.45 (1H, t-like)
18	3.18 (1H, dd, J=14, 4 Hz)	3.11 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.11 (1H, dd, J=14, 4 Hz)
23	3.66 (1H, d, J=11 Hz)	3.60 (1H, d, J=11 Hz)	3.61 (1H, d, J=11 Hz)	3.74 (1H, d, J=11 Hz)	3.75 (1H, d, J=11 Hz)
	4.30 (1H, d, J=11 Hz)	4.30 (1H, d, J=11 Hz)	4.31 (1H, d, J=11 Hz)	4.36 (1H, d, J=11 Hz)	4.32 (1H, d, J=11 Hz)
24	1.33 (3H, s)	1.30 (3H, s)	1.31 (3H, s)	1.32 (3H, s)	1.35 (3H, s)
25	1.56 (3H, s)	1.56 (3H, s)	1.58 (3H, s)	1.56 (3H, s)	1.56 (3H, s)
26	1.16 (3H, s)	1.15 (3H, s)	1.15 (3H, s)	1.16 (3H, s)	1.17 (3H, s)
27	1.22 (3H, s)	1.23 (3H, s)	1.22 (3H, s)	1.21 (3H, s)	1.23 (3H, s)
29	0.89 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.84 (3H, s)	0.88 (3H, s)
30	0.88 (3H, s)	0.81 (3H, s)	0.83 (3H, s)	0.82 (3H, s)	0.85 (3H, s)
C-3 sugar					
Glc-1 linner	5.12 (1H, d, J=8 Hz)	5.11 (1H, d, J=8 Hz)	5.13 (1H, d, J=8 Hz)	5.17 (1H, d, J=8 Hz)	5.13 (1H, d, J=8 Hz)
2	3.98 (1H, t, J=8.5 Hz)	4.00 (1H, t, J=8.5 Hz)	4.01 (1H, t, J=8.5 Hz)	4.03 (1H, t, J=8.5 Hz)	4.01 (1H, t, J=8.5 Hz)
3	4.12 (1H, t, J=8.5 Hz)	4.13 (1H, t, J=8.5 Hz)	4.14 (1H, t, J=8.5 Hz)	4.18 (1H, t, J=8.5 Hz)	4.14 (1H, t, J=8.5 Hz)
4	4.18 (1H, t, J=9 Hz)	4.18 (1H, t, J=9 Hz)	4.20 (1H, t, J=9 Hz)	4.24 (1H, t, J=9 Hz)	4.20 (1H, t, J=9 Hz)
5	3.88 (1H, m)	3.88 (1H, m)	3.88 (1H, m)	3.91 (1H, m)	3.88 (1H, m)
6	4.29 (1H, dd, J=12, 5 Hz)	4.30[a]	4.31[a]	4.36[a]	4.32[a]
	4.43 (1H, br d, J=12 Hz)	4.43[a]	4.44 (1H, dd, J=12, 2 Hz)	4.42[a]	4.44 (1H, dd, J=12, 2 Hz)

	6	**7**	**8**	**9**	**10**
Aglycone					
2	4.71 (1H, m)	4.72 (1H, m)	4.73 (1H, m)	4.73 (1H, m)	4.73 (1H, m)
3	4.15 (1H, d, J=3 Hz)	4.13 (1H, d, J=3 Hz)	4.14 (1H, d, J=3 Hz)	4.16 (1H, d, J=3 Hz)	4.15 (1H, d, J=3 Hz)
12	5.41 (1H, t-like)	5.46 (1H, t-like)	5.47 (1H, t-like)	5.45 (1H, t-like)	5.45 (1H, t-like)
18	3.16 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.10 (1H, dd, J=14, 4 Hz)	3.09 (1H, dd, J=14, 4 Hz)
23	3.69 (1H, d, J=11 Hz)	3.62 (1H, d, J=11 Hz)	3.65 (1H, d, J=11 Hz)	3.75 (1H, d, J=11 Hz)	3.75 (1H, d, J=11 Hz)
	4.31 (1H, d, J=11 Hz)	4.37 (1H, d, J=11 Hz)	4.31 (1H, d, J=11 Hz)	4.29 (1H, d, J=11 Hz)	4.30 (1H, d, J=11 Hz)
24	1.40 (3H, s)	1.37 (3H, s)	1.39 (3H, s)	1.38 (3H, s)	1.38 (3H, s)
25	1.53 (3H, s)	1.55 (3H, s)	1.57 (3H, s)	1.53 (3H, s)	1.53 (3H, s)
26	1.14 (3H, s)	1.14 (3H, s)	1.15 (3H, s)	1.16 (3H, s)	1.14 (3H, s)
27	1.18 (3H, s)	1.21 (3H, s)	1.20 (3H, s)	1.21 (3H, s)	1.20 (3H, s)
29	0.87 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.84 (3H, s)	0.85 (3H, s)
30	0.86 (3H, s)	0.81 (3H, s)	0.82 (3H, s)	0.83 (3H, s)	0.83 (3H, s)
C-3 sugar					
Glc-1 inner	5.06 (1H, d, J=8 Hz)	5.06 (1H, d, J=8 Hz)	5.07 (1H, d, J=8 Hz)	5.07 (1H, d, J=8 Hz)	5.08 (1H, d, J=8 Hz)
2	4.08 (1H, t, J=8.5 Hz)	4.08 (1H, t, J=8.5 Hz)	4.10 (1H, t, J=8.5 Hz)	4.09 (1H, t, J=8.5 Hz)	4.09 (1H, t, J=8.5 Hz)
3	4.19 (1H, t, J=9 Hz)	4.19 (1H, t, J=9 Hz)	4.21 (1H, t, J=9 Hz)	4.21 (1H, t, J=9 Hz)	4.22 (1H, t, J=9 Hz)
4	4.10 (1H, t, J=9 Hz)	4.10 (1H, t, J=9 Hz)	4.13 (1H, t, J=9 Hz)	4.12 (1H, t, J=9 Hz)	4.12 (1H, t, J=9 Hz)
5	3.80 (1H, m)	3.81 (1H, m)	3.82 (1H, m)	3.82 (1H, m)	3.82 (1H, m)
6	4.23[a]	4.23 (1H, dd, J=12, 5 Hz)	4.23[a]	4.25[a]	4.23[a]
	4.39[a]	4.41[a]	4.39[a]	4.41 (1H, dd, J=12, 2 Hz)	4.41 (1H, dd, J=12, 2 Hz)
Glc-1 terminal	5.32 (1H, d, J=8 Hz)	5.32 (1H, d, J=8 Hz)	5.34 (1H, d, J=8 Hz)	5.33 (1H, d, J=8 Hz)	5.33 (1H, d, J=8 Hz)
2	4.06 (1H, t, J=8.5 Hz)	4.07 (1H, t, J=8.5 Hz)	4.09[a]	4.08 (1H, t, J=8.5 Hz)	4.08[a]
3	4.17 (1H, t, J=9 Hz)	4.17 (1H, t, J=9 Hz)	4.19 (1H, t, J=9 Hz)	4.18 (1H, t, J=9 Hz)	4.20 (1H, t, J=9 Hz)
4	4.19 (1H, t, J=9 Hz)	4.24 (1H, t, J=9 Hz)	4.23[a]	4.22 (1H, t, J=9 Hz)	4.23[a]
5	3.89 (1H, m)	3.90 (1H, m)	3.91 (1H, m)	3.90 (1H, m)	3.91 (1H, m)
6	4.37[a]	4.39[a]	4.39[a]	4.38[a]	4.39[a]
	4.47 (1H, dd, J=12, 2 Hz)	4.48 (1H, dd, J=12, 2 Hz)	4.50 (1H, dd, J=12, 2 Hz)	4.50 (1H, dd, J=12, 2 Hz)	4.50 (1H, dd, J=12, 2 Hz)

Recorded at 500 MHz. *a*) Overlapping with other signals.

(d, J=3 Hz)] of the aglycone moiety on irradiation at H-1 [δ 5.11 (d, J=8 Hz)] of glucose and at H-2 (δ 4.43) of glucose to be linked at C-28 of bayogenin on irradiation at H-1 [δ 6.53 (br s)] of rhamnose in the difference NOE spectrum. Based upon the above evidence, the structure of polygalasaponin II was elucidated as 3-*O*-β-D-glucopyranosyl bayogenin 28-*O*-[α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester.

Polygalasaponin III (**3**), $C_{53}H_{86}O_{23}$, showed four anomeric proton signals at δ 5.13 (d, J=8 Hz), 5.81 (d, J=3 Hz), 6.03 (br s) and 6.18 (d, J=7 Hz) in the ^{1}H-NMR spectrum. On acid hydrolysis, **3** afforded D-glucose, L-rhamnose and D-apiose as a sugar moiety. The NOE experiment irradiating at each anomeric proton signal and the hetero nuclear multiple bond coherence (HMBC) spectrum showed the connections of individual monosaccharides (Chart 2). So, the structure of polygalasaponin III was determined to be 3-*O*-β-D-glucopyranosyl bayogenin 28-*O*-{α-L-rhamnopyranosyl(1→2)-[β-D-apiofuranosyl(1→3)]-β-D-glucopyranosyl} ester.

Polygalasaponin IV (**4**) revealed a $[M+Na]^+$ ion peak at m/z 1246 in the FAB-MS and elemental analysis data was consistent with $C_{58}H_{94}O_{27}$. Compound **4** gave D-glucose, D-xylose, D-apiose and L-rhamnose as a sugar moiety on acid hydrolysis. In the NMR spectra, **4** exhibited five anomeric proton and carbon signals at δ 5.08 (d, J=7 Hz), 5.17 (d, J=8 Hz), 5.82 (d, J=3 Hz), 6.03 (br s), 6.23 (d, J=7 Hz); 94.1, 101.4, 105.8, 107.5, 111.0. The sugar linkages were determined by means of NOE with irradiation at each anomeric proton signal. Therefore, the

TABLE II. ^{1}H-NMR Spectral Data of Compounds **1**—**10** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**
C-28 sugar					
Glc- 1	6.29 (1H, d, J=8 Hz)	6.17 (1H, d, J=8 Hz)	6.18 (1H, d, J=7 Hz)	6.23 (1H, d, J=7 Hz)	6.22 (1H, d, J=7 Hz)
2	4.16 (1H, t, J=8.5 Hz)	4.43[a]	4.33 (1H, t, J=8 Hz)	4.34 (1H, t, J=8 Hz)	4.26 (1H, t, J=8 Hz)
3	4.25 (1H, t, J=9 Hz)	4.29[a]	4.14 (1H, t, J=8.5 Hz)	4.16 (1H, t, J=8.5 Hz)	4.29 (1H, t, J=8.5 Hz)
4	4.29 (1H, t, J=9 Hz)	4.30[a]	4.26[a]	4.26[a]	4.27[a]
5	3.99 (1H, m)	3.96 (1H, m)	3.93 (1H, m)	3.96 (1H, m)	3.96 (1H, m)
6	4.37 (1H, dd, J=12, 5 Hz)	4.30[a]	4.26[a]	4.34[a]	4.34[a]
	4.42 (1H, br d, J=12 Hz)	4.38 (1H, br d, J=12 Hz)	4.30[a]	4.48 (1H, br d, J=12 Hz)	4.39 (1H, dd, J=12, 2 Hz)
Rha-1		6.53 (1H, br s)	6.03 (1H, br s)	6.03 (1H, br s)	6.20 (1H, br s)
2		4.77 (1H, br s)	4.69 (1H, br s)	4.70 (1H, br s)	5.03 (1H, br s)
3		4.54[a]	4.47 (1H, dd, J=9.5, 3 Hz)	4.62 (1H, dd, J=9.5, 3 Hz)	4.66 (1H, dd, J=9.5, 3 Hz)
4		4.29[a]	4.26 (1H, t, J=9.5 Hz)	4.35 (1H, t, J=9.5 Hz)	4.52 (1H, t, J=9.5 Hz)
5		4.52 (1H, m)	4.40 (1H, m)	4.42 (1H, m)	4.48 (1H, m)
6		1.74 (3H, d, J=6.5 Hz)	1.72 (3H, d, J=6.5 Hz)	1.81 (3H, d, J=6.5 Hz)	1.78 (3H, d, J=6.5 Hz)
Api- 1			5.81 (1H, d, J=3 Hz)	5.82 (1H, d, J=3 Hz)	6.05 (1H, d, J=3 Hz)
2			4.76 (1H, d, J=3 Hz)	4.79 (1H, d, J=3 Hz)	4.78 (1H, d, J=3 Hz)
4			4.09 (1H, d, J=11 Hz)	4.12 (1H, d, J=11 Hz)	4.03 (1H, d, J=11 Hz)
			4.10 (1H, d, J=11 Hz)	4.14 (1H, d, J=11 Hz)	4.06 (1H, d, J=11 Hz)
5			4.29 (1H, d, J=9.5 Hz)	4.30 (1H, d, J=9.5 Hz)	4.18 (1H, d, J=9.5 Hz)
			4.65 (1H, d, J=9.5 Hz)	4.68 (1H, d, J=9.5 Hz)	4.58 (1H, d, J=9.5 Hz)
Xyl- 1				5.08 (1H, d, J=7 Hz)	5.32 (1H, d, J=7 Hz)
2				4.09[a]	3.99 (1H, t, J=8.5 Hz)
3				4.07[a]	4.09 (1H, t, J=8.5 Hz)
4				4.18[a]	4.17[a]
5				3.53 (1H, t, J=11 Hz)	3.51 (1H, t, J=11 Hz)
				4.24[a]	4.23[a]

	6	**7**	**8**	**9**	**10**
C-28 sugar					
Glc- 1	6.28 (1H, d, J=8 Hz)	6.16 (1H, d, J=8 Hz)	6.18 (1H, d, J=7 Hz)	6.18 (1H, d, J=8 Hz)	6.19 (1H, d, J=7 Hz)
2	4.15 (1H, t, J=8.5 Hz)	4.44 (1H, t, J=8.5 Hz)	4.33 (1H, t, J=8 Hz)	4.38 (1H, t, J=8.5 Hz)	4.30 (1H, t, J=8 Hz)
3	4.23 (1H, t, J=9 Hz)	4.29 (1H, t, J=9 Hz)	4.15 (1H, t, J=8.5 Hz)	4.28 (1H, t, J=9 Hz)	4.13 (1H, t, J=8.5 Hz)
4	4.30 (1H, t, J=9 Hz)	4.32 (1H, t, J=9 Hz)	4.26[a]	4.29[a]	4.26[a]
5	3.99 (1H, m)	3.96 (1H, m)	3.93 (1H, m)	3.95 (1H, m)	3.93 (1H, m)
6	4.34[a]	4.31[a]	4.23[a]	4.31[a]	4.26[a]
	4.41 (1H, dd, J=12, 2 Hz)	4.37 (1H, dd, J=12, 2 Hz)	4.28[a]	4.38[a]	4.31[a]
Rha-1		6.55 (1H, br s)	6.05 (1H, br s)	6.45 (1H, br s)	5.98 (1H, br s)
2		4.77 (1H, br s)	4.69 (1H, br s)	4.83 (1H, br s)	4.69 (1H, br s)
3		4.52 (1H, dd, J=9.5, 3 Hz)	4.47 (1H, dd, J=9.5, 3 Hz)	4.70 (1H, dd, J=9.5, 3 Hz)	4.60 (1H, dd, J=9.5, 3 Hz)
4		4.29[a]	4.26[a]	4.35 (1H, t, J=9.5 Hz)	4.30 (1H, t, J=9.5 Hz)
5		4.51 (1H, m)	4.41 (1H, m)	4.53 (1H, m)	4.39 (1H, m)
6		1.74 (3H, d, J=6.5 Hz)	1.72 (3H, d, J=6.5 Hz)	1.81 (3H, d, J=6.5 Hz)	1.79 (3H, d, J=6.5 Hz)
Api- 1			5.81 (1H, d, J=3 Hz)		5.80 (1H, d, J=3 Hz)
2			4.76 (1H, d, J=3 Hz)		4.76 (1H, d, J=3 Hz)
4			4.08 (1H, d, J=11 Hz)		4.08[a]
			4.11 (1H, d, J=11 Hz)		4.11[a]
5			4.29 (1H, d, J=9.5 Hz)		4.30[a]
			4.65 (1H, d, J=9.5 Hz)		4.66 (1H, d, J=9.5 Hz)
Xyl- 1				5.05 (1H, d, J=7 Hz)	5.05 (1H, d, J=7 Hz)
2				4.07[a]	4.08[a]
3				4.06[a]	4.05[a]
4				4.16[a]	4.16[a]
5				3.53 (1H, t, J=11 Hz)	3.51 (1H, t, J=11 Hz)
				4.22[a]	4.23[a]

Recorded at 500 MHz. *a*) Overlapping with other signals.

structure of polygalasaponin IV was elucidated as 3-*O*-*β*-D-glucopyranosyl bayogenin 28-*O*-{*β*-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl(1→2)-[*β*-D-apiofuranosyl-(1→3)]-*β*-D-glucopyranosyl} ester.

Polygalasaponin V (**5**) afforded D-glucose, D-xylose, D-apiose and L-rhamnose on acid hydrolysis. Its molecular formula is $C_{58}H_{94}O_{27}$ from the FAB-MS and elemental analysis. The ^{1}H-NMR spectrum of **5** disclosed five anomeric proton signals at δ 5.13 (d, J=8 Hz), 5.32 (d, J=7 Hz), 6.05 (d, J=3 Hz), 6.20 (br s) and 6.22 (d, J=7 Hz). The binding sites of each monosaccharide were determined by the NOE method. When the signals at δ 5.13 (H-1 of glucose), 5.32 (H-1 of xylose), 6.05 (H-1 of apiose) and 6.20 (H-1 of rhamnose) were irradiated, NOEs were observed at signals due to H-3 of an aglycone, H-4 of rhamnose, H-3 of rhamnose and H-2 of ester-linked glucose, respectively. From these data, the structure of polygalasaponin V was determined to be 3-*O*-*β*-D-glucopyranosyl bayogenin 28-*O*-{*β*-D-xylopyranosyl(1→4)-[*β*-D-apiofuranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl} ester.

Polygalasaponin VI (**6**) showed a $[M+Na]^+$ ion peak at m/z 997 in the FAB-MS and had a molecular formula $C_{48}H_{78}O_{20}$ by elemental analysis. On acid hydrolysis, **6** afforded D-glucose. In the ^{1}H-NMR spectrum, **6** showed three anomeric proton signals at δ 5.06 (d, J=8 Hz), 5.32

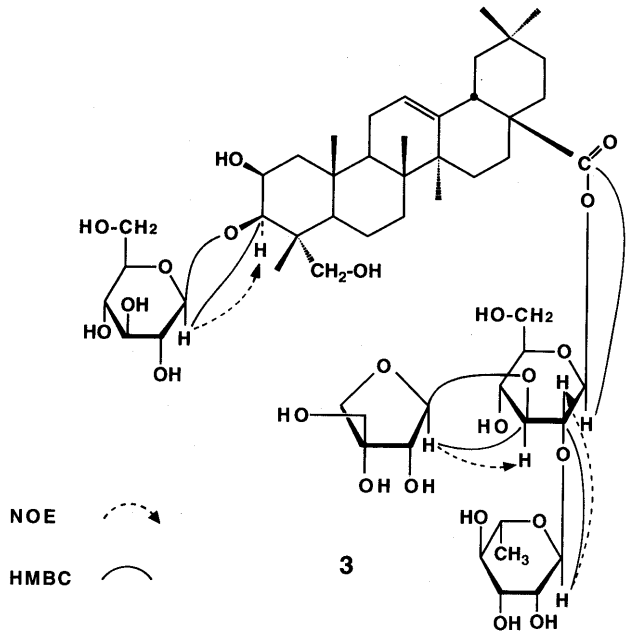

Chart 2

(d, $J=8$ Hz) and 6.28 (d, $J=8$ Hz). The ^{13}C-NMR spectrum of **6** exhibited three anomeric carbon signals, and glycosylation shifts at C-2 (−1.3 ppm), C-3 (+9.9 ppm) and C-28 (−3.7 ppm) of aglycone indicated that **6** was a 3,28-bisdesmoside. NOEs were observed at H-3 (δ 4.15) of the aglycone and H-2 [δ 4.08 (t, $J=8.5$ Hz)] of glucose attached at C-3 of the aglycone, with irradiation at anomeric proton signals δ 5.06 (d, $J=8$ Hz) and 5.32 (d, $J=8$ Hz), respectively. From these results, the structure of polygalasaponin VI was elucidated as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]bayogenin 28-*O*-β-D-glucopyranosyl ester.

The FAB-MS and elemental analysis of polygalasaponin VII (**7**) gave the molecular formula $C_{54}H_{88}O_{24}$. Compound **7** showed the presence of four anomeric proton and carbon signals [δ 5.06 (d, $J=8$ Hz), 5.32 (d, $J=8$ Hz), 6.16 (d, $J=8$ Hz), 6.55 (br s); 94.9, 101.5, 103.0, 105.9] in the NMR spectra. On acid hydrolysis, **7** gave D-glucose and L-rhamnose as a sugar moiety. The sugar linkages were decided by the NOE method. When the signals at δ 5.06, 5.32 (H-1 of each glucose) and 6.55 (H-1 of rhamnose) were irradiated, NOEs were observed at signals due to H-3 of bayogenin, H-2 of glucose attached at C-3 of bayogenin and H-2 of glucose linked at C-28 of bayogenin, respectively. The structure of polygalasaponin VII was thus characterized as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]bayogenin 28-*O*-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl] ester.

Polygalasaponin VIII (**8**) revealed a $[M+Na]^+$ ion peak at *m/z* 1276 in the FAB-MS and elemental analysis data was consistent with $C_{59}H_{96}O_{28}$. On acid hydrolysis, **8** afforded D-glucose, L-rhamnose and D-apiose as a sugar moiety. In the NMR spectra, **8** exhibited five anomeric proton and carbon signals at δ 5.07 (d, $J=8$ Hz), 5.34 (d, $J=8$ Hz), 5.81 (d, $J=3$ Hz), 6.05 (br s), 6.18 (d, $J=7$ Hz); 94.3, 101.7, 102.9, 105.9, 111.2, indicating that **8** was a 3,28-bisdesmoside. The ^{1}H- and ^{13}C-NMR chemical shifts of **8** were similar to those of **3** except for the signals due to the terminal glucose moiety. To investigate the binding sites of five monosaccharides, we employed a difference NOE. When the signals at δ 5.07, 5.34 (H-1 of each glucose), 5.81 (H-1 of apiose) and 6.05 (H-1 of rhamnose) were irradiated, NOEs were observed at δ 4.14 (d, $J=3$ Hz, H-3 of bayogenin), 4.10 (t, $J=8$ Hz, H-2 of glucose attached at C-3 of bayogenin), and 4.15 and 4.33 (H-3, H-2 of ester-linked glucose), respectively. Consequently, the structure of polygalasaponin VIII was deduced as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]bayogenin 28-*O*-{β-D-apiofuranosyl(1→3)-[α-L-rhamnopyranosyl(1→2)]-β-D-glucopyranosyl} ester.

Polygalasaponin IX (**9**), $C_{59}H_{96}O_{28}$, was similar to that of **7** except for a terminal xylose moiety in the ^{1}H- and ^{13}C-NMR spectra. Acid hydrolysis liberated D-glucose, L-rhamnose and D-xylose. The sugar linkages were determined by the NOE method. When the signals at δ 5.07, 5.33 (H-1 of each glucose), 6.45 (H-1 of rhamnose) and 5.05 (H-1 of xylose) were irradiated, NOEs were observed at signals due to H-3 of bayogenin, H-2 of glucose attached at C-3 of bayogenin, H-2 of ester-linked glucose and H-4 of rhamnose, respectively. The structure of polygalasaponin IX was then determined to be 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]bayogenin 28-*O*-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester.

Polygalasaponin X (**10**) yielded D-glucose, D-apiose, L-rhamnose and D-xylose on acid hydrolysis. In the NMR spectra, **10** showed six anomeric proton and carbon signals at δ 5.05 (d, $J=7$ Hz), 5.08 (d, $J=8$ Hz), 5.33 (d, $J=8$ Hz), 5.80 (d, $J=3$ Hz), 5.98 (br s), 6.19 (d, $J=7$ Hz); 94.2, 101.5, 103.0, 105.8, 107.4, 111.0. Glycosylation shifts at C-2 (−1.2 ppm), C-3 (+10.0 ppm) and C-28 (−3.9 ppm) of the aglycone suggested that **10** was a 3,28-bisdesmoside of bayogenin, since the anomeric proton signal (δ 6.19, d, $J=7$ Hz) and carbon signal (δ 94.2) due to glucose suggested that this glucose is attached at C-28 of bayogenin. The binding sites of five other monosaccharides were determined by the NOE method. When the signals at δ 5.08, 5.33 (H-1 of each glucose), 5.98 (H-1 of rhamnose), 5.80 (H-1 of apiose) and 5.05 (H-1 of xylose) were irradiated, NOEs were observed at signals due to H-3 of the aglycone, H-2 of glucose bound at C-3 of the aglycone, H-2 and H-3 of ester-linked glucose and H-4 of rhamnose, respectively. From these data, the structure of polygalasaponin X was elucidated as 3-*O*-[β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl]bayogenin 28-*O*-{β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl (1→2)-[β-D-apiofuranosyl(1→3)]-β-D-glucopyranosyl} ester.

The anomeric configurations of glucose and xylose in these saponins were determined to all be β from the J value of the anomeric proton signals, and 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[6] and C-1 and C-2 of apiose.[7]

Experimental

General Procedure ^{1}H- and ^{13}C-NMR spectra were obtained with a JEOL GSX-500 FT NMR at 35 °C and chemical shifts were given in ppm with tetramethylsilane as an internal stantard. FAB-MS was recorded on a JEOL JMS-SX102 mass-spectrometer. Optical rotations were measured with a JASCO DIP-360 digital polarimeter. Gas chro-

TABLE III. ^{13}C-NMR Spectral Data of Sugar Moiety of Compounds **1**—**12** in Pyridine-d_5

C	1	2	3	4	5	6	7	8	9	10
C-3 sugar										
Glc- 1 inner	105.7	105.7	105.7	105.8	105.7	103.0	103.0	102.9	103.0	103.0
2	75.5	75.5	75.5	75.5	75.2	83.7	83.8	83.8	83.8	83.6
3	78.6	78.6	78.6	78.6	78.6	78.1	78.0	78.0	78.0	78.1
4	71.7	71.7	71.7	71.6	71.7	71.1	71.1	71.1	71.1	71.1
5	78.3	78.3	78.3	78.3	78.3	78.1	78.0	78.0	78.0	78.1
6	62.7	62.7	62.7	62.7	62.7	62.5	62.5	62.5	62.5	62.5
Glc 1 terminal						105.8	105.9	105.9	105.8	105.8
2						76.8	76.8	76.8	76.7	76.7
3						78.4	78.4	78.4	78.3	78.4
4						71.4	71.3	71.3	71.3	71.4
5						78.4	78.4	78.4	78.3	78.4
6						62.6	62.6	62.6	62.6	62.6
C-28 sugar										
Glc- 1	95.8	94.9	94.3	94.1	94.8	95.8	94.9	94.3	94.8	94.2
2	74.2	75.9	75.5	75.7	78.5	74.2	75.9	75.4	76.7	75.9
3	78.9	79.7	87.4	87.2	78.7	78.9	79.8	87.6	79.5	87.2
4	71.2	71.5	69.3	69.2	71.4	71.3	71.5	69.3	71.4	69.3
5	79.3	78.9	78.2	78.1	78.7	79.3	78.9	78.2	78.9	78.2
6	62.3	62.2	61.9	61.8	62.5	62.3	62.2	61.9	62.2	62.0
Rha-1		101.5	101.7	101.4	101.9		101.5	101.7	101.4	101.5
2		72.3	72.0	71.6	71.4		72.3	72.0	71.9	71.7
3		72.6	72.5	72.4	82.2		72.6	72.5	72.6	72.4
4		73.9	73.7	84.7	78.9		73.9	73.6	85.5	84.6
5		69.8	70.4	68.7	68.6		69.8	70.4	68.3	68.8
6		18.8	18.8	18.6	19.1		18.8	18.8	18.6	18.6
Api- 1			111.2	111.0	111.6			111.2		111.0
2			78.0	78.0	77.7			77.9		78.0
3			80.2	80.3	79.6			80.2		80.2
4			75.2	75.3	74.6			75.2		75.3
5			64.7	64.7	64.7			64.7		64.8
Xyl- 1				107.5	105.4				107.7	107.4
2				76.2	75.8				76.3	76.2
3				78.7	78.7				78.8	78.7
4				71.0	71.3				70.9	70.9
5				67.5	67.2				67.5	67.5

Recorded at 125.65 MHz at 35 °C.

matography (GC) was run on a Hitachi G-3000 gas chromatograph. Preparative and semi-preparative HPLC was carried out on a column of Develosil Lop-ODS (5 cm × 50 cm) and YMC D-ODS-7 (2 cm × 25 cm) or Develosil PhA-7 (2 cm × 25 cm), respectively.

Extraction and Isolation *Polygala japonica* HOUTT. was collected in Jiangxi, China in May 1993. The aerial part (dried, 5 kg) of *P. japonica* HOUTT. was extracted twice with 70% aqueous EtOH. The extract was passed through a porous polymer gel Mitsubishi Diaion HP-20 column after evaporation of EtOH. 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 with $CHCl_3$–MeOH–H_2O (85 : 14 : 1), increasing a portion of MeOH to give 19 fractions (frs. A—S). From frs. B, E and O, compounds **1**—**12** were isolated by preparative and semi-preparative HPLC. **1** (258 mg), **2** (105 mg), **3** (30 mg), **4** (36 mg), **5** (36 mg), **6** (329 mg), **7** (150 mg), **8** (135 mg), **9** (78 mg), **10** (48 mg), **11** (486 mg), **12** (831 mg).

Polygalasaponin I (**1**): Amorphous powder, $[\alpha]_D^{27}$ +25.7° (c=1.07, MeOH). *Anal.* Calcd for $C_{42}H_{68}O_{15}\cdot 5/2H_2O$: C, 58.79; H, 8.58. Found: C, 58.62; H, 8.47. FAB-MS m/z: 835 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR δ: aglycone moiety: 44.2 (C-1), 70.6 (C-2), 83.1 (C-3), 42.4 (C-4), 47.8 (C-5), 18.1 (C-6), 32.6 (C-7), 40.1 (C-8), 48.6 (C-9), 37.0 (C-10), 24.1 (C-11), 123.2 (C-12), 144.2 (C-13), 42.8 (C-14), 28.3 (C-15), 23.5 (C-16), 47.1 (C-17), 41.8 (C-18), 46.2 (C-19), 30.8 (C-20), 34.1 (C-21), 33.0 (C-22), 65.7 (C-23), 15.1 (C-24), 17.4 (C-25), 17.7 (C-26), 26.2 (C-27), 176.5 (C-28), 33.2 (C-29), 23.7 (C-30). ^{13}C-NMR data of aglycone moiety of **2**—**12** are nearly the same as this data; sugar moiety: shown in Table III.

Polygalasaponin II (**2**): Amorphous powder, $[\alpha]_D^{27}$ 0° (c=0.85, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{19}\cdot 4H_2O$: C, 55.91; H, 8.41. Found: C, 56.02; H, 8.50. FAB-MS m/z: 982 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin III (**3**): Amorphous powder, $[\alpha]_D^{27}$ −11.5° (c=0.39, MeOH). *Anal.* Calcd for $C_{53}H_{86}O_{23}\cdot 3H_2O$: C, 55.58; H, 8.10. Found: C, 55.71; H, 8.34. FAB-MS m/z: 1114 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin IV (**4**): Amorphous powder, $[\alpha]_D^{27}$ −10.8° (c=0.51, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{27}\cdot 11/2H_2O$: C, 52.72; H, 7.93. Found: C, 52.78; H, 8.00. FAB-MS m/z: 1246 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin V (**5**): Amorphous powder, $[\alpha]_D^{27}$ −16.7° (c=0.48, MeOH). *Anal.* Calcd for $C_{58}H_{94}O_{27}\cdot 5H_2O$: C, 53.04; H, 7.98. Found: C, 53.12; H, 7.85. FAB-MS m/z: 1246 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin VI (**6**): Amorphous powder, $[\alpha]_D^{27}$ +28.3° (c=1.15, MeOH). *Anal.* Calcd for $C_{48}H_{78}O_{20}\cdot H_2O$: C, 58.05; H, 8.12. Found: C, 57.85; H, 8.23. FAB-MS m/z: 997 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin VII (**7**): Amorphous powder, $[\alpha]_D^{27}$ +1.2° (c=0.84, MeOH). *Anal.* Calcd for $C_{54}H_{88}O_{24}\cdot H_2O$: C, 56.93; H, 7.96. Found: C, 57.13; H, 7.93. FAB-MS m/z: 1144 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin VIII (**8**): Amorphous powder, $[\alpha]_D^{25}$ +10.6° (c=1.04, pyridine). *Anal.* Calcd for $C_{59}H_{96}O_{28}\cdot 9/2H_2O$: C, 53.10; H, 7.93. Found: C, 53.20; H, 8.12. FAB-MS m/z: 1276 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin IX (**9**): Amorphous powder, $[\alpha]_D^{27}$ −1.3° (c=0.38, MeOH). *Anal.* Calcd for $C_{59}H_{96}O_{28}\cdot 7/2H_2O$: C, 53.83; H, 7.89. Found: C, 53.76; H, 8.14. FAB-MS m/z: 1276 $[M+Na]^+$. ^{1}H-NMR: shown in

Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Polygalasaponin X (**10**): Amorphous powder, $[\alpha]_D^{25}$ +17.2° (c=0.32, pyridine). *Anal.* Calcd for $C_{64}H_{104}O_{32}\cdot 11/2H_2O$: C, 51.78; H, 7.81. Found: C, 51.75; H, 7.77. FAB-MS m/z: 1408 $[M+Na]^+$. ^{1}H-NMR: shown in Tables I and II. ^{13}C-NMR sugar moiety: shown in Table III.

Acid Hydrolysis of Bayogenin-3-*O*-glucopyranoside (11) Compound **11** (100 mg) was refluxed with dioxane (8 ml) and 5% H_2SO_4 (2 ml) for 2 h. The reaction mixture was diluted with H_2O and extracted with ether. The ether layer was evaporated to dryness. The residue was recrystallized from MeOH–$CHCl_3$ to give bayogenin (**13**) (12 mg) as colorless needles, mp 320—322 °C (dec.), $[\alpha]_D^{27}$ +126.9° (c=0.67, pyridine) that was identified by comparison of ^{1}H- and ^{13}C-NMR data with reported data.[5]

Acid Hydrolysis of Saponins 1—12 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 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[8] (0.05 mg) in water (0.03 ml) and pyridine (0.015 ml) at 60 °C for 1 h with stirring. After the solution 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 bayogenin from every saponin. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R: D-apiose 10.3 min, L-apiose 9.7 min,[9] D-xylose 10.7 min, L-xylose 9.7 min, L-rhamnose 12.2 min, D-rhamnose 12.0 min,[9] D-glucose 17.8 min, L-glucose 17.2 min. D-Glucose was detected from **1**—**12**. L-Rhamnose was detected from **2**—**5**, **7**—**10**. D-Xylose was detected from **4**, **5**, **9** and **10**. D-Apiose was detected from **3**, **4**, **5**, **8** and **10**. HPLC conditions: column, Develosil R-ODS-7, 4.6 mm × 25 cm; solvent, MeCN–H_2O (55 : 45); flow rate, 1.0 ml/min; UV 205 nm; t_R, bayogenin 9.2 min.

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