Studies on the Constituents of *Polygala japonica* HOUTT. II.[1] Structures of Polygalasaponins XI—XIX

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Nine new oleanane-type saponins polygalasaponins XI—XIX were isolated from the aerial part of *Polygala japonica*. The structures of these compounds were established on basis of spectroscopic and chemical evidence.

Key words *Polygala japonica*; Polygalaceae; polygalasaponin; polygalagenin; bayogenin

We previously reported[1] the isolation and structural elucidation of bayogenin glycosides, called polygalasaponins I—X isolated from the aerial part of *Polygala japonica* HOUTT. In a continuing investigation on the oligoglycosidic constituents, we identified nine new saponins designated as polygalasaponins XI—XIX (**1**—**9**). This paper deals with the isolation and structural elucidation of these saponins.

The fractions E, K, N, O and P, which were obtained by a porous polymer gel Mitsubishi 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) column, respectively, to afford nine new polygalasaponins, XI—XIX.

Polygalasaponin XI (**1**) revealed a quasi-molecular ion peak $[M+Na]^+$ at m/z 1160[2] in the FAB-MS, and elemental analysis data was consistent with the formula $C_{54}H_{88}O_{25}$. On acid hydrolysis, **1** afforded bayogenin (**1a**) as an aglycone, and only D-glucose as a sugar moiety. In the ^{1}H- and ^{13}C-NMR spectra, **1** exhibited four anomeric proton and carbon signals at δ 5.10 (d, $J=8$ Hz), 5.36 (d, $J=8$ Hz), 5.69 (d, $J=8$ Hz), 6.18 (d, $J=8$ Hz); 93.7, 103.0, 104.7, 105.8. On comparison of the ^{13}C-NMR spectrum of **1** with that of bayogenin (**1a**),[3] glycosylation shifts at C-2 (−1.3 ppm), C-3 (+9.8 ppm) and C-28 (−3.7 ppm) of the aglycone moiety indicated that **1** was a 3,28-bisdesmoside of bayogenin (**1a**). Sugar proton signals in the ^{1}H-NMR spectrum were assigned by detailed proton spin decoupling experiments starting from irradiation at each anomeric proton signal. The sugar linkages were decided by the nuclear Overhauser effect (NOE) method. When the signals at δ 5.10, 5.36, 5.69 (H-1 of each glucose) were irradiated, NOEs were observed at the signals due to H-3 of the aglycone moiety, H-2 of glucose attached at C-3 of the aglycone moiety and H-2 of glucose linked at C-28 of the aglycone moiety, respectively. Based on the foregoing evidence, the chemical structure of polygalasaponin XI has been concluded to be 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-bayogenin 28-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl ester.

Polygalasaponin XII (**2**) showed a $[M+Na]^+$ ion peak at m/z 833 in the FAB-MS. Combined with the result of elemental analysis, its molecular formula was deduced as $C_{42}H_{66}O_{15}$. Upon acid hydrolysis, **2** furnished D-glucose and compound **2a**. Compound **2a** revealed a $[M+Na]^+$ ion peak at m/z 509 in the FAB-MS. The ^{1}H- and ^{13}C-NMR chemical shifts of **2a** were similar to those of bayogenin (**1a**) except for the appearance of an aldehyde [^{1}H-NMR: δ_H 9.65 (1H, s); ^{13}C-NMR: δ_C 207.3] instead of one hydroxymethyl group in **1a**. When **2a** was reduced with $NaBH_4$, the reductant was identified as bayogenin (**1a**). Thus **2a** was characterized as 2β,3β-dihydroxy-23-oxo-olean-12-en-28-oic acid (called polygalagenin). The ^{1}H- and ^{13}C-NMR analysis including two dimensional techniques, *i.e.* ^{1}H–^{1}H correlation spectroscopy (COSY), ^{13}C–^{1}H COSY, and the difference NOE spectra, led us to presume that **2** was a polygalagenin glycoside containing only D-glucopyranose. The ^{1}H- and ^{13}C-NMR spectra of **2** exhibited two anomeric proton and carbon signals at δ 4.89 (d, $J=8$ Hz), 6.29 (d, $J=8$ Hz); 95.8, 104.7. Glycosylation shifts in the aglycone moiety suggested that **2** was a 3,28-bisdesmoside of polygalagenin (**2a**). When **2** was reduced with $NaBH_4$, the reductant was identified as polygalasaponin I (**1b**) by direct comparison of the spectral data with those of **1b**.[1] From these data, the structure of polygalasaponin XII was elucidated as 3-*O*-β-D-glucopyranosyl-polygalagenin 28-*O*-β-D-glucopyranosyl ester.

The FAB-MS and elemental analysis of polygalasaponin XIII (**3**) gave the molecular formula $C_{42}H_{66}O_{15}$. The ^{1}H- and ^{13}C-NMR spectra of **3** indicated that **3** has two anomeric protons and carbons: one aldehyde [δ_H 9.79 (1H, s); δ_C 208.1], six methyl [δ_H 0.96 (s), 0.98 (s), 1.01 (s), 1.27 (s), 1.41 (s), 1.70 (s); δ_C 11.7, 16.8, 17.5, 23.9, 26.3, 33.4] and one carboxyl group (δ_C 180.2). Their ^{13}C-chemical shifts were like those of lobatoside B (**1c**) except for the presence of an aldehyde and loss of hydroxymethyl group. When **3** was reduced with $NaBH_4$, the reductant was identified as lobatoside B by direct comparison of the spectral data with those of **1c**.[1,3] Compound **3** liberated D-glucose as a sugar moiety, and polygalagenin (**2a**) as an aglycone on acid hydrolysis. Therefore, polygalasaponin XIII was determined to be 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin.

Polygalasaponins XIV (**4**), $C_{48}H_{76}O_{20}$ and XV (**5**), $C_{54}H_{86}O_{25}$ furnished D-glucose as a sugar moiety in addition to polygalagenin (**2a**) as an aglycone. In the NMR spectra, **4** showed three anomeric proton and carbon signals, **5** exhibited four anomeric proton and carbon signals. Glycosylation shifts in the aglycone moiety

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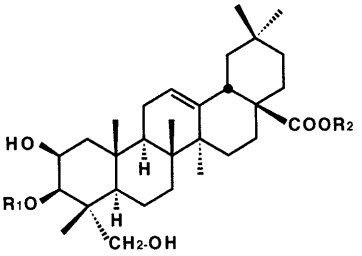
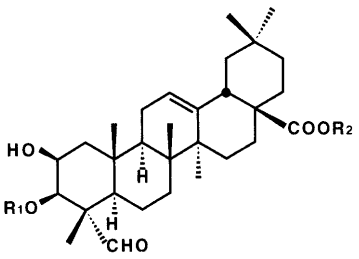


	R_1	R_2
1	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Glc
1a	H	H
1b	Glc	Glc
1c	Glc $\overset{2}{-}$ Glc	H



	R_1	R_2
2	Glc	Glc
2a	H	H
3	Glc $\overset{2}{-}$ Glc	H
4	Glc $\overset{2}{-}$ Glc	Glc
5	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Glc
6	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Glc $\overset{4}{-}$ Xyl
7	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Rha
8	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Rha $\overset{4}{-}$ Xyl
9	Glc $\overset{2}{-}$ Glc	Glc $\overset{2}{-}$ Rha $\overset{4}{-}$ Xyl, Glc $\overset{3}{-}$ Api

Chart 1

indicated that **4** and **5** were both 3,28-bisdesmoside of polygalagenin. The sugar linkages were decided by the NOE method. In the difference NOE spectra of **4**, when the signals at δ 4.93, 5.21 (H-1 of each glucose) were irradiated, NOEs were observed at δ 4.00 (d, $J=3$ Hz, H-3 of aglycone) and 4.02 (t, $J=8.5$ Hz, H-2 of glucose attached at C-3 of aglycone), respectively. NOEs were observed at H-3 of the aglycone, H-2 of glucose bound at C-3 of the aglycone and H-2 of glucose to be linked at C-28 of the aglycone on irradiation at δ 4.92, 5.22 and 5.68 in the difference NOE spectra of **5**. Based on the above evidence, the structures of polygalasaponins XIV and XV were characterized as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin 28-*O*-β-D-glucopyranosyl ester and 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin 28-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl ester.

Polygalasaponin XVI (**6**) afforded D-glucose and D-xylose on acid hydrolysis. Its molecular formula is $C_{59}H_{94}O_{29}$ from the FAB-MS and elemental analysis. The ^{1}H- and ^{13}C-NMR spectra of **6** disclosed five anomeric proton and carbon signals at δ 4.91 (d, $J=8$ Hz), 5.06 (d, $J=7.5$ Hz), 5.22 (d, $J=8$ Hz), 5.63 (d, $J=8$ Hz), 6.17 (d, $J=8$ Hz); 93.8, 101.8, 104.6, 105.8, 105.9, indicating them to be a 3,28-bisdesmoside. The ^{1}H- and ^{13}C-chemical shifts of **6** were similar to those of **5** except for the signals due to the terminal xylose moiety. To investigate the binding sites of five monosaccharides, we employed a difference NOE spectra. When the signals at δ 4.91, 5.22, 5.63 (H-1 of each glucose) and 5.06 (H-1 of xylose) were irradiated, NOEs were observed at signals due to H-3 of the aglycone, H-2 of glucose attached at C-3 of the aglycone, H-2 of glucose linked at C-28 of the aglycone, and H-4 of glucose bound at C-2 of ester-linked glucose, respectively. The structure of polygalasaponin XVI was thus deduced as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin 28-*O*-β-D-xylopyranosyl(1→4)-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl ester.

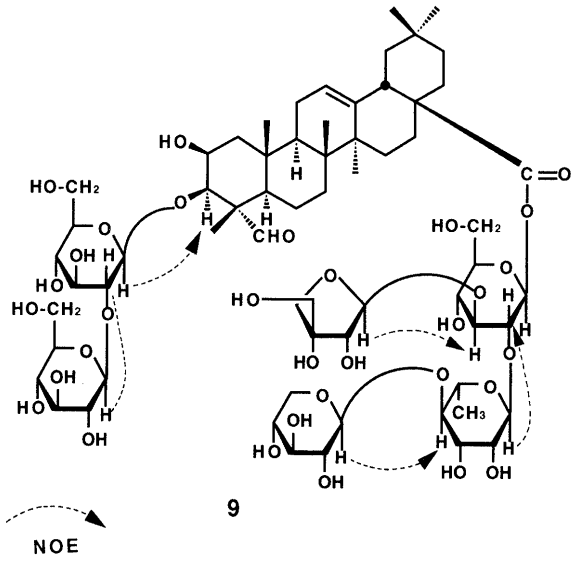


Chart 2

The ^{1}H-NMR spectrum of polygalasaponin XVII (**7**), $C_{54}H_{86}O_{24}$, showed four anomeric proton signals at δ 4.92 (d, $J=8$ Hz), 5.21 (d, $J=8$ Hz), 6.17 (d, $J=8$ Hz) and 6.61 (br s). On acid hydrolysis, **7** afforded D-glucose and L-rhamnose as sugar moieties. The sugar linkages were determined by means of NOE with irradiation at each anomeric proton signal. Therefore, the structure of polygalasaponin XVII was elucidated as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin 28-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester.

The elemental composition of polygalasaponin XVIII (**8**) was proved to be $C_{59}H_{94}O_{28}$ by FAB-MS and

Table 1. ^{1}H-NMR Spectral Data of Compounds **1**—**9** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**
Aglycone					
2	4.73 (1H, m)	4.73 (1H, m)	4.64 (1H, m)	4.64 (1H, m)	4.63 (1H, m)
3	4.17 (1H, d, J=3 Hz)	4.12 (1H, d, J=3 Hz)	4.00 (1H, d, J=3 Hz)	4.00 (1H, d, J=3 Hz)	3.96 (1H, d, J=3 Hz)
12	5.44 (1H, t-like)	5.43 (1H, t-like)	5.46 (1H, t-like)	5.42 (1H, t-like)	5.42 (1H, t-like)
18	3.13	3.18	3.27	3.18	3.14
	(1H, dd, J=14, 4 Hz)	(1H, dd, J=14, 4 Hz)	(1H, dd, J=14, 4 Hz)	(1H, dd, J=14, 4 Hz)	(1H, dd, J=14, 4 Hz)
23	3.63 (1H, d, J=11 Hz)	9.72 (1H, s)	9.79 (1H, s)	9.78 (1H, s)	9.76 (1H, s)
	4.29 (1H, d, J=11 Hz)				
24	1.35 (3H, s)	1.68 (3H, s)	1.70 (3H, s)	1.71 (3H, s)	1.65 (3H, s)
25	1.48 (3H, s)	1.48 (3H, s)	1.41 (3H, s)	1.44 (3H, s)	1.37 (3H, s)
26	1.08 (3H, s)	1.10 (3H, s)	1.01 (3H, s)	1.09 (3H, s)	1.01 (3H, s)
27	1.18 (3H, s)	1.24 (3H, s)	1.27 (3H, s)	1.24 (3H, s)	1.23 (3H, s)
29	0.86 (3H, s)	0.92 (3H, s)	0.98 (3H, s)	0.92 (3H, s)	0.90 (3H, s)
30	0.86 (3H, s)	0.89 (3H, s)	0.96 (3H, s)	0.89 (3H, s)	0.88 (3H, s)
C-3 sugar					
Glc-1 (inn.)	5.10 (1H, d, J=8 Hz)	4.89 (1H, d, J=8 Hz)	4.92 (1H, d, J=8 Hz)	4.93 (1H, d, J=8 Hz)	4.92 (1H, d, J=8 Hz)
2	4.11 (1H, t, J=8.5 Hz)	3.87 (1H, t, J=8.5 Hz)	4.00 (1H, t, J=8.5 Hz)	4.02 (1H, t, J=8.5 Hz)	4.03 (1H, t, J=8.5 Hz)
Glc-1 (ter.)	5.36 (1H, d, J=8 Hz)		5.19 (1H, d, J=8 Hz)	5.21 (1H, d, J=8 Hz)	5.22 (1H, d, J=8 Hz)
C-28 sugar					
Glc-1 (inn.)	6.18 (1H, d, J=8 Hz)	6.29 (1H, d, J=8 Hz)		6.29 (1H, d, J=8 Hz)	6.18 (1H, d, J=8 Hz)
2	4.44 (1H, t, J=8.5 Hz)				4.45 (1H, t, J=8.5 Hz)
3					4.27 (1H, t, J=9 Hz)
Glc-1 (ter.)	5.69 (1H, d, J=8 Hz)				5.68 (1H, d, J=8 Hz)

	6	**7**	**8**	**9**
Aglycone				
2	4.63 (1H, m)	4.65 (1H, m)	4.65 (1H, m)	4.64 (1H, m)
3	3.97 (1H, d, J=3 Hz)	3.96 (1H, d, J=3 Hz)	4.01 (1H, d, J=3 Hz)	4.01 (1H, d, J=3 Hz)
12	5.42 (1H, t-like)	5.46 (1H, t-like)	5.45 (1H, t-like)	5.44 (1H, t-like)
18	3.13 (1H, dd, J=14, 4 Hz)	3.14 (1H, dd, J=14, 4 Hz)	3.11 (1H, dd, J=14, 4 Hz)	3.09 (1H, dd, J=14, 4 Hz)
23	9.74 (1H, s)	9.71 (1H, s)	9.75 (1H, s)	9.77 (1H, s)
24	1.67 (3H, s)	1.67 (3H, s)	1.70 (3H, s)	1.70 (3H, s)
25	1.38 (3H, s)	1.44 (3H, s)	1.43 (3H, s)	1.43 (3H, s)
26	1.01 (3H, s)	1.08 (3H, s)	1.09 (3H, s)	1.07 (3H, s)
27	1.24 (3H, s)	1.25 (3H, s)	1.27 (3H, s)	1.25 (3H, s)
29	0.91 (3H, s)	0.90 (3H, s)	0.88 (3H, s)	0.88 (3H, s)
30	0.89 (3H, s)	0.82 (3H, s)	0.85 (3H, s)	0.86 (3H, s)
C-3 sugar				
Glc-1 (inn.)	4.91 (1H, d, J=8 Hz)	4.92 (1H, d, J=8 Hz)	4.91 (1H, d, J=8 Hz)	4.91 (1H, d, J=8 Hz)
2	4.02 (1H, t, J=8.5 Hz)	4.02 (1H, t, J=8.5 Hz)	4.01 (1H, t, J=8.5 Hz)	3.99 (1H, t, J=8.5 Hz)
Glc-1 (ter.)	5.22 (1H, d, J=8 Hz),	5.21 (1H, d, J=8 Hz)	5.21 (1H, d, J=8 Hz)	5.20 (1H, d, J=8 Hz)
C-28 sugar				
Glc-1 (inn.)	6.17 (1H, d, J=8 Hz)	6.17 (1H, d, J=8 Hz)	6.18 (1H, d, J=8 Hz)	6.22 (1H, d, J=7 Hz)
2	4.39 (1H, t, J=8.5 Hz)	4.48 (1H, t, J=8.5 Hz)	4.39 (1H, t, J=8 Hz)	4.31 (1H, t, J=8 Hz)
3	4.31 (1H, t, J=9 Hz)	4.21 (1H, t, J=9 Hz)	4.28 (1H, t, J=9 Hz)	4.15 (1H, t, J=8.5 Hz)
Glc-1 (ter.)	5.63 (1H, d, J=8 Hz)			
4	4.23 (1H, t, J=9 Hz)			
Rha-1		6.61 (1H, br s)	6.45 (1H, br s)	5.97 (1H, br s)
4			4.34 (1H, t, J=9.5 Hz)	4.30 (1H, t, J=9.5 Hz)
Xyl-1	5.06 (1H, d, J=7.5 Hz)		5.04 (1H, d, J=7.5 Hz)	5.05 (1H, d, J=7 Hz)
Api-1				5.79 (1H, d, J=3 Hz)

Assignment of methyl groups based on ^{13}C–^{1}H COSY measurement.

elemental analysis. On acid hydrolysis, **8** liberated D-glucose, L-rhamnose and D-xylose as sugar components. The ^{1}H- and ^{13}C-NMR spectra of **8** showed five anomeric proton and carbon signals. The ^{13}C-chemical shifts were like those of **7** except for the signals due to the terminal xylose moiety. The sugar linkages were determined by the NOE method. The structure of polygalasaponin XVIII was thus established as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranosyl-polygalagenin 28-*O*-*β*-D-xylopyranosyl-(1→4)-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl ester.

Polygalasaponin XIX (**9**) yielded D-glucose, D-apiose, L-rhamnose and D-xylose on acid hydrolysis. In the NMR spectra, **9** showed six anomeric proton and carbon signals at δ 4.91 (d, J=8 Hz), 5.05 (d, J=7 Hz), 5.20 (d, J=8 Hz), 5.79 (d, J=3 Hz), 5.97 (br s), 6.22 (d, J=7 Hz); 94.1, 101.3, 101.9, 106.0, 107.3, 110.9. Glycosylation shifts at C-2 (−2.0 ppm), C-3 (+10.4 ppm) and C-28 (−5.4 ppm) of the aglycone moiety suggested that **9** was a 3,28-bis-desmoside of polygalagenin (**2a**), since the anomeric proton signal at δ 6.22 (d, J=7 Hz) and anomeric carbon signal at δ 94.1 due to glucose suggested that this glucose

Table 2. ^{13}C-NMR Spectral Data of Compounds **1**—**9** in Pyridine-d_5

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
C-3 sugar									
Glc-1 (inn.)	103.0	104.7	101.8	101.8	101.8	101.8	101.8	102.0	101.9
2	83.6	75.0	83.2	83.2	83.1	83.2	83.3	83.2	83.3
3	78.1	78.5	78.0	78.2	78.2	78.3	78.2	78.0	78.0
4	71.1	71.6	71.2	71.1	71.1	71.2	71.2	71.1	71.1
5	78.1	78.5	78.2	78.3	78.2	78.3	78.2	78.3	78.3
6	62.5	62.7	62.5	62.5	62.5	62.7	62.5	62.5	62.5
Glc-1 (ter.)	105.8		106.0	106.0	105.9	105.9	106.0	106.0	106.0
2	76.8		77.0	77.0	77.0	77.0	77.0	76.9	77.0
3	78.4		78.5	78.0	77.9	78.0	78.0	78.4	78.2
4	71.4		71.3	71.3	71.3	71.3	71.4	71.3	71.3
5	78.4		78.3	78.5	78.4	78.3	78.5	78.6	78.5
6	62.6		62.7	62.7	62.7	62.7	62.7	62.7	62.7
C-28 sugar									
Glc-1 (inn.)	93.7	95.8		95.8	93.6	93.8	94.9	94.8	94.1
2	78.9	74.2		74.2	78.8	78.9	75.5	76.4	75.7
3	79.0	78.9		78.9	79.0	79.2	80.0	79.5	86.9
4	70.8	71.2		71.2	70.8	70.8	71.1	71.4	69.3
5	79.2	79.3		79.3	79.1	79.5	79.0	78.9	78.0
6	62.1	62.3		62.3	62.1	62.1	62.2	62.2	62.0
Glc-1 (ter.)	104.7				104.6	104.6			
2	76.0				75.9	74.9			
3	78.4				78.4	75.7			
4	73.0				72.8	82.7			
5	78.1				78.1	76.4			
6	64.0				63.9	63.0			
Rha-1							101.3	101.3	101.3
2							72.4	71.9	71.6
3							72.6	72.6	72.4
4							73.9	85.3	84.5
5							69.7	68.3	68.8
6							18.7	18.6	18.6
Xyl-1						105.8		107.6	107.3
2						76.4		76.3	76.2
3						78.5		78.7	78.7
4						70.9		70.9	70.9
5						67.4		67.5	67.5
Api-1									110.9
2									77.9
3									80.2
4									75.3
5									64.8

Assignments based on ^{13}C–^{1}H COSY measurement.

was attached at C-28 of polygalagenin. The binding sites of five other monosaccharides were determined by the NOE method. When signals at δ4.91, 5.20 (H-1 of each glucose), 5.05 (H-1 of xylose), 5.79 (H-1 of apiose) and 5.97 (H-1 of rhamnose) were irradiated, NOEs were observed at δ4.01 (H-3 of the aglycone), 3.99 (H-2 of glucose attached at C-3 of the aglycone), 4.30 (H-4 of rhamnose), and 4.15, 4.31 (H-3, H-2 of glucose linked at C-28 of the aglycone), respectively. From these data, the structure of polygalasaponin XIX was thus established as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-polygalagenin 28-*O*-[β-D-apiofuranosyl(1→3)]-β-D-xylopyranosyl(1→4)-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester.

The anomeric configurations of glucose and xylose in these saponins were all determined to 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[4] and C-2 of apiose.[5]

Experimental

General Procedure The instruments used to obtain physical data and the experimental conditions for chromatography were the same as in our previous paper.[1]

Isolation of Polygalasaponins XI—XIX As was noted earlier,[1] the methanol eluate (80 g) was chromatographed on a silica gel column to give 19 fractions (frs. A—S). From frs. E (5.8 g), K (2.5 g), N (5.8 g), O (1.4 g) and P (12.9 g), compounds **1**—**9** were isolated by preparative and semi-preparative HPLC. **1** (72 mg), **2** (171 mg), **3** (1.16 g), **4** (94 mg), **5** (262 mg), **6** (27 mg), **7** (22 mg), **8** (35 mg), **9** (78 mg).

Polygalasaponin XI (**1**): Amorphous powder, $[\alpha]_D^{21}$ +30.6° (c=0.62, MeOH). *Anal.* Calcd for $C_{54}H_{88}O_{25}\cdot 7/2H_2O$: C, 54.03; H, 7.98. Found: C, 53.97; H, 8.24. FAB-MS m/z: 1160 $[M+Na]^+$. ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: δ (aglycone moiety C-1→C-30) 44.0, 70.3, 83.0, 42.3, 48.2, 18.1, 32.3, 40.1, 48.6, 36.9, 24.0, 122.8, 144.5, 42.7, 29.1, 23.2, 47.0, 41.9, 46.3, 30.8, 34.1, 33.2, 65.8, 14.8, 17.2, 17.6, 26.2, 176.5, 33.2, 23.8. Sugar moiety: shown in Table 2.

Polygalasaponin XII (**2**): Amorphous powder, $[\alpha]_D^{24}$ +39.6° (c=0.82, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 5/2H_2O$: C, 58.93; H, 8.36. Found: C, 58.73; H, 8.40. FAB-MS m/z: 833 $[M+Na]^+$. ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: δ (aglycone moiety C-1→C-30) 43.8, 69.3, 83.0, 54.6, 48.3, 20.4, 32.6, 40.4, 48.5, 36.3, 24.0, 123.1, 144.2, 42.4, 28.2, 23.4, 47.0, 41.8, 46.2, 30.8, 34.1, 32.6, 206.9, 11.8, 16.8, 17.6, 26.2, 176.4, 33.2, 23.7. The variation of each carbon signal in the aglycone moiety of

compounds **4**—**9** was less than 0.8 ppm, except the C-23 signal by comparison with that of compound **2**. The C-23 signal was observed at δ 206.9 ppm in the monoglucoside at C-3 of the aglycone (**2a**), while in the diglucoside it was observed at δ 208.0—208.3 ppm.

Polygalasaponin XIII (**3**): Amorphous powder, $[\alpha]_D^{24}$ +69.6° (c=0.97, MeOH). *Anal.* Calcd for $C_{42}H_{66}O_{15}\cdot 3H_2O$: C, 58.32; H, 8.39. Found: C, 58.51; H, 8.42. FAB-MS *m/z*: 833 $[M+Na]^+$. ^{1}H-NMR: shown in Table 1. ^{13}C-NMR: δ (aglycone moiety C-1→C-30) 43.5, 69.0, 82.7, 54.3, 48.2, 20.3, 32.7, 40.2, 48.5, 36.4, 24.0, 122.4, 145.0, 42.4, 28.3, 23.7, 46.7, 42.1, 46.6, 31.0, 34.3, 33.3, 208.1, 11.7, 16.8, 17.5, 26.3, 180.2, 33.4, 23.9. Sugar moiety: shown in Table 2.

Polygalasaponin XIV (**4**): Amorphous powder, $[\alpha]_D^{24}$ +48.6° (c=1.12, MeOH). *Anal.* Calcd for $C_{48}H_{76}O_{20}\cdot 5/2H_2O$: C, 56.62; H, 8.02 Found: C, 56.68; H, 8.24. FAB-MS *m/z*: 995 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2

Polygalasaponin XV (**5**): Amorphous powder, $[\alpha]_D^{24}$ +15.5° (c=1.10, pyridine). *Anal.* Calcd for $C_{54}H_{86}O_{25}\cdot 3H_2O$: C, 54.53; H, 7.80. Found: C, 54.60; H, 7.83. FAB-MS *m/z*: 1158 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2.

Polygalasaponin XVI (**6**): Amorphous powder, $[\alpha]_D^{24}$ +31.9° (c=0.83, MeOH). *Anal.* Calcd for $C_{59}H_{94}O_{29}\cdot 6H_2O$: C, 51.52; H, 7.77. Found: C, 51.49; H, 7.97. FAB-MS *m/z*: 1290 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2.

Polygalasaponin XVII (**7**): Amorphous powder, $[\alpha]_D^{24}$ +12.0° (c=0.50, MeOH). *Anal.* Calcd for $C_{54}H_{86}O_{24}\cdot 13/2H_2O$: C, 52.46; H, 8.07. Found: C, 52.63; H, 7.85. FAB-MS *m/z*: 1142 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2.

Polygalasaponin XVIII (**8**): Amorphous powder, $[\alpha]_D^{24}$ +0.8° (c=1.19, pyridine). *Anal.* Calcd for $C_{59}H_{94}O_{28}\cdot 4H_2O$: C, 53.55; H, 7.77. Found: C, 53.61; H, 7.91. FAB-MS *m/z*: 1274 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2.

Polygalasaponin XIX (**9**): Amorphous powder, $[\alpha]_D^{24}$ −10.6° (c=0.71, pyridine). *Anal.* Calcd for $C_{64}H_{102}O_{32}\cdot 5H_2O$: C, 52.17; H, 7.66. Found: C, 52.19; H, 7.78. FAB-MS *m/z*: 1406 $[M+Na]^+$. ^{1}H- and ^{13}C-NMR: shown in Tables 1 and 2.

Acid Hydrolysis of Polygalasaponin XII (2) 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 chromatographed on preparative TLC [Merck Kieselgel PF_{254}, $CHCl_3$–MeOH (9 : 1)], and recrystallized from MeOH to give polygalagenin (**2a**) (5.9 mg) as colorless needles, mp 296—298 °C (dec.), $[\alpha]_D^{21}$ +67.8° (c=0.59, MeOH). *Anal.* Calcd for $C_{30}H_{46}O_5$: C, 74.04; H, 9.53. Found : C, 73.89; H, 9.65. FAB-MS *m/z* 509 $[M+Na]^+$. ^{1}H-NMR (in pridine-d_5): δ 0.96 (3H, s, H_3-30), 1.02 (3H, s, H_3-29), 1.03 (3H, s, H_3-26), 1.31 (3H, s, H_3-27), 1.54 (3H, s, H_3-25), 1.72 (3H, s, H_3-24), 3.35 (1H, dd, J=14, 4 Hz, H-18), 4.07 (1H, d, J=3 Hz, H-3), 4.51 (1H, m, H-2), 5.49 (1H, t-like, H-12); ^{13}C-NMR: δ (C-1→C-30) 44.8, 71.1, 72.3, 55.5, 48.3, 21.0, 32.8, 40.2, 48.6, 36.4, 24.0, 121.9, 145.7, 42.5, 28.4, 24.0, 46.9, 42.4, 46.9, 31.1, 34.6, 33.4, 207.3, 11.0, 16.9, 17.6, 26.3, 181.6, 33.5, 24.0.

Reduction of Polygalagenin (2a) with $NaBH_4$ Compound **2a** (8 mg) was reduced with $NaBH_4$ (10 mg) in MeOH (1 ml) for 3 h at room temperature. The reaction mixture was diluted with H_2O, acidified with AcOH and extracted with EtOAc. The EtOAc layer was concentrated and the residue was purified by preparative TLC [Merck Kieselgel PF_{254}, $CHCl_3$–MeOH (9 : 1)] to give bayogenin (**1a**) (4 mg) that was identified by direct comparison of ^{1}H- and ^{13}C-NMR and $[\alpha]_D$ data with reported data.[3]

Acid Hydrolysis of Saponins 1—9 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[6] (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 polygalagenin (**2a**) from saponins **2**—**9** and bayogenin (**1a**) from saponin **1**. GC conditions: column, Supelco SPB™-1, 0.25 mm × 27 m; column temperature, 230 °C; carrier gas, N_2; t_R, D-apiose 9.80 min, L-apiose 9.11 min,[7] D-xylose 9.92 min, L-xylose 9.21 min, L-rhamnose 11.57 min, D-rhamnose 11.50 min,[7] D-glucose 16.81 min, L-glucose 16.36 min. D-Glucose was detected from **1**—**9**. L-Rhamnose was detected from **7**—**9**. D-Xylose was detected from **6**, **8**, **9**. D-Apiose was detected from **9**. HPLC conditions: column, Develosil PhA-7, 4.6 mm × 25 cm; solvent, MeOH–H_2O (80 : 20); flow rate, 1.0 ml/min; UV 205 nm; t_R, bayogenin 6.5 min, polygalagenin 9.5 min.

Reduction of Saponins 2 and 3 Each saponin (10 mg) in MeOH (1 ml) was reduced with $NaBH_4$ (5 mg) for 4 h at room temperature. After dilution with water, the reaction mixture was passed through a porous polymer gel Mitsubishi Diaion HP-20 column. After the content of the column was washed with water, the adsorbed material was eluted with MeOH to give polygalasaponin I (**1b**) (7 mg) and lobatoside B (**1c**) (8 mg), respectively, which were identified by direct comparison of ^{1}H- and ^{13}C-NMR data with reported data.[1,3]

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References and Notes

1) Zhang D., Miyase T., Kuroyanagi M., Umehara K., Ueno A., *Chem. Pharm. Bull.*, **43**, 115 (1995).
2) Mass number was counted by fractions of 0.5 and over as a unit and the rest was cut away.
3) Fujioka T., Iwamoto M., Iwase Y., Hachiyama S., Okabe H., Yamauchi T., Mihashi K., *Chem. Pharm. Bull.*, **37**, 2355 (1989).
4) Kasai R., Okihara M., Asakawa J., Mizutani K., Tanaka O., *Tetrahedron*, **35**, 1427 (1979).
5) Kitagawa I., Sakagami M., Hashiuchi M., Zhou J. L., Yoshikawa M., Ren J., *Chem. Pharm. Bull.*, **37**, 551 (1989).
6) Hara S., Okabe H., Mihashi K., *Chem. Pharm. Bull.*, **34**, 1843 (1986).
7) The t_R for L-apiose and D-rhamnose were obtained from their enantiomer (D-apiose + L-cysteine and L-rhamnose + L-cysteine, respectively)