Saponins from *Polygala japonica* and Their Effects on a Forced Swimming Test in Mice

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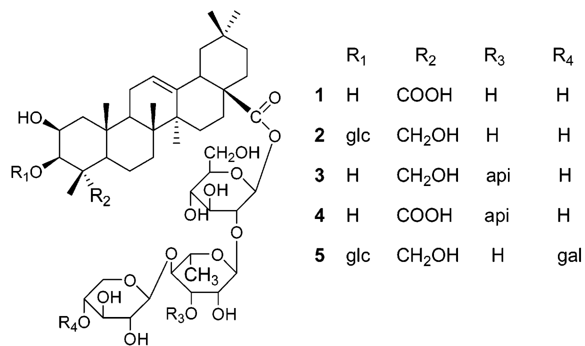
Five new triterpenoid saponins, polygalasaponins E (**1**), F (**2**), G (**3**), H (**4**), and J (**5**), along with eight known ones (**6**−**13**), were isolated from the aerial parts of *Polygala japonica*. Their structures were established by chemical and spectroscopic means. Forced swimming tests on mice showed that saponins **1** and **4** significantly reduce the immobility status by 58.1% and 51.3% at a dosage of 100 mg/kg administrated orally once daily for 5 days, respectively.

Polygala japonica HOUTT. (Polygalaceae) has been a folk medicine herb used as expectorant, anti-inflammatory, antibacterial, ataractic, and antidepressant agents in the south of China.[1] Previous studies indicated that *P. japonica* is a saponin-rich plant.[2−5] Also, many natural-occurring saponins were reported to possess strong antidepressant activity.[6−8] Five new triterpenoid saponins, named polygalasaponins E−H (**1**−**4**) and J (**5**), together with eight known ones, were isolated from the *n*-butanol fraction of *P. japonica*. Compounds **1** and **4** showed moderate antidepressant effects at a dosage of 100 mg/kg in the forced swimming tests (FSTs) on mice. This paper describes the isolation and identification of the new compounds and the evaluation of antidepressant activity.



	R_1	R_2	R_3	R_4
1	H	COOH	H	H
2	glc	CH_2OH	H	H
3	H	CH_2OH	api	H
4	H	COOH	api	H
5	glc	CH_2OH	H	gal

Results and Discussion

The 70% EtOH extract of the aerial parts of *P. japonica* was partitioned into petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH fractions. The *n*-BuOH fraction was repeatedly chromatographed to yield saponins **1**−**13**. Compounds **6**−**13** were identified as 2β,27-dihydroxy-23-carboxyoleanolic acid 3-*O*-β-D-glucopyranoside (**6**), polygalasaponin D (**7**), polygalasaponin II (**8**), polygalasaponin III (**9**), polygalasaponin VII (**10**), polygalasaponin XXI (**11**), polygalasaponin V (**12**), and polygalasaponin X (**13**), respectively, by comparison of their physical properties and spectroscopic data with literature values.[2,4,9,10]

Polygalasaponin E (**1**) revealed an $[M + Na]^+$ ion at *m/z* 965 and an $[M + K]^+$ ion at *m/z* 981 in the ESIMS, which were consistent with the molecular formula $C_{47}H_{74}O_{19}$. Acid hydrolysis of **1** afforded aglycone and glucose, rhamnose, and xylose, and their absolute configuration was determined to be D-glucose, D-xylose, and L-rhamose by GC analysis of chiral derivatives of sugars in the acid hydrolysate (see Experimental Section). The aglycone of **1** was identified as medicagenic acid by comparing its retention time with that of an authentic sample on HPLC.

The ^1H NMR data (Table 2) of **1** showed three anomeric protons at $\delta = 6.41$ (1H, s), 6.21 (1H, d, $J = 8.0$ Hz), and 5.05 (1H, d, $J = 8.0$ Hz), indicating the α-, β-, and β-configurations of the anomeric protons of rhamnose, glucose, and xylose, respectively. On the basis of the combined analysis of HMQC, $^1H-^1H$ COSY, HMBC, and TOCSY spectra, all proton and carbon signals of these sugars were assigned. The HMBC spectrum data elucidated the sugar sequence as xylopyranosyl-(1→4)-rhamnopyranosyl-(1→2)-glucopyranosyl as supported by the HMBC cross-peaks of H-1 ($\delta = 6.41$, s) of rhamnose with C-2 ($\delta = 76.2$) of glucose, and H-1 ($\delta = 5.05$, 1H, d, $J = 8.0$ Hz) of xylose with C-4 ($\delta = 84.9$) of rhamnose. The sugar chain was attached to C-28 of medicagenic acid by the observation of a HMBC correlation between C-28 ($\delta = 176.1$) of aglycone and H-1 ($\delta = 6.21$) of glucose. Thus, the structure of polygalajaponica E was identified as medicagenic acid 28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester.

Polygalasaponin F (**2**) showed an $[M + Na]^+$ ion and an $[M + K]^+$ ion at *m/z* 1113 and 1129, respectively. Combined with the ^1H and ^{13}C NMR spectrum (Tables 1, 2) of **2**, its molecular formula was deduced to be $C_{53}H_{86}O_{23}$. Acid hydrolysis of **2** and GC analysis of chiral derivatives of sugars in the acid hydrolysate afforded aglycone, D-glucose, L-rhamnose, and D-xylose. The aglycone of **2** was determined to be bayogenin on the basis of the analysis of ^1H and ^{13}C NMR data.

The ^1H and ^{13}C NMR spectrum of **2** showed four anomeric protons at $\delta = 6.50$ (1H, d, $J = 1.0$ Hz), 6.20 (1H, d, $J = 8.0$ Hz), 5.15 (1H, d, $J = 7.0$ Hz), and 5.06 (1H, d, $J = 8.0$ Hz) and four corresponding anomeric carbons at $\delta = 94.3$, 101.1, 105.3, and 107.4. A glucose was linked to C-3 of the aglycone on the basis of the HMBC correlation of its anomeric proton with C-3 of the aglycone. Three additional monosaccharide moieties were identical to that of **1** by detailed analysis of the HMBC correlations, and this glycosidic moiety was attached to C-28 of bayogenin. Therefore, the structure of polygalasaponin F was elucidated as 3-*O*-β-D-glucopyranosyl bayogenin 28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl] ester.

Polygalasaponin G (**3**) has the molecular formula $C_{52}H_{84}O_{22}$, deduced from the ESIMS and ^1H and ^{13}C NMR spectra. The NMR data (Tables 1, 2) of **3** showed great similarity with those of the aglycone moiety of **1**, implying that the compounds carry the same aglycone. Acid hydrolysis of **3** and GC analysis of chiral derivatives of sugars in the acid hydrolysate afforded D-glucose, L-rhamnose, D-xylose, and D-apiose. The ^1H NMR spectrum revealed four anomeric protons at $\delta = 6.24$ (1H, d, $J = 1.0$ Hz), 6.22 (1H, d, $J = 8.0$ Hz), 6.09 (1H, d, $J = 5.0$ Hz), and 5.34 (1H, d, $J = 8.0$ Hz). All sugar signals were assigned on the basis of HMQC, $^1H-^1H$ COSY, and TOCSY spectra. The anomeric configurations of those

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Table 1. ^{13}C NMR Data of Compounds **1**−**5** in Pyridine-d_5 (125 MHz)

	1	2	3	4	5		1	2	3	4	5
1	44.9	43.9	44.7	44.9	43.8	3		78.2			78.2
2	71.3	70.2	71.3	71.3	70.2	4		71.2			71.2
3	75.6	82.7	72.9	75.6	82.9	5		77.9			77.9
4	53.6	42.1	42.1	53.6	42.1	6		62.3			62.3
5	52.0	47.5	48.0	52.0	47.6	C-28 sugars					
6	21.4	17.8	18.1	21.3	17.9	Glu-1	94.5	94.3	94.4	94.5	94.3
7	32.0	31.9	32.0	32.0	31.9	2	76.2	76.2	77.9	78.0	76.2
8	40.1	39.7	39.8	40.1	39.7	3	79.0	79.2	78.4	78.3	79.2
9	48.5	48.2	48.3	48.5	48.2	4	71.0	70.9	71.0	71.0	70.9
10	36.7	36.6	36.9	36.6	36.6	5	78.4	78.5	78.4	78.3	78.5
11	23.7	23.7	23.7	23.7	23.7	6	61.8	61.7	62.0	62.1	61.9
12	122.5	122.5	122.6	122.5	122.5	Rha-1	101.0	101.1	101.5	101.4	101.1
23	143.8	143.8	143.8	143.7	143.8	2	71.4	71.5	71.0	71.0	71.3
14	42.1	42.4	42.0	42.0	42.4	3	72.2	72.3	81.8	81.9	72.2
15	28.1	28.2	27.9	27.7	28.3	4	84.9	85.2	78.6	78.4	85.5
16	23.2	23.0	23.2	23.2	23.0	5	68.1	67.9	68.2	68.3	67.8
17	46.8	46.8	46.7	46.7	46.8	6	18.3	18.2	18.7	18.7	18.2
18	41.8	41.6	41.6	41.7	41.6	Xyl-1	107.2	107.4	105.0	105.0	107.0
19	46.0	46.0	46.1	46.0	46.0	2	75.9	75.9	75.4	75.4	75.5
20	30.4	30.3	30.4	30.4	30.3	3	78.5	78.4	78.4	78.4	76.6
21	33.6	33.6	33.6	33.6	33.7	4	70.6	70.6	70.9	70.9	77.9
22	32.8	32.8	32.9	32.9	32.7	5	67.2	67.1	66.8	66.8	64.6
23	180.8	65.2	67.5	180.8	65.3	Api-1			111.2	111.3	
24	13.4	14.7	14.2	13.4	14.7	2			77.2	77.2	
25	16.8	17.0	17.0	16.8	16.9	3			79.2	79.2	
26	17.1	17.2	17.3	17.2	17.1	4			74.2	74.2	
27	25.7	25.6	25.7	25.7	25.7	5			64.2	64.2	
28	176.1	176.1	176.1	176.1	176.1	Gal-1					104.3
29	32.8	32.7	32.7	32.1	32.7	2					71.4
30	23.5	23.3	23.4	23.5	23.3	3					74.6
C-3 sugar						4					69.7
Glu-1		105.3			105.3	5					76.9
2		75.1			75.2	6					61.7

sugars were determined by their coupling constants. The linkage sites among monosaccharides were determined by the following HMBC correlations: H-1 ($\delta = 6.09$) of apiose and C-3 ($\delta = 81.8$) of rhamnose, H-1 ($\delta = 5.34$) of xylose and C-4 ($\delta = 78.6$) of rhamnose, H-1 ($\delta = 6.24$) of rhamnose and C-2 ($\delta = 77.9$) of glucose. The sugar chain was attached to C-28 of the aglycone via a HMBC correlation of H-1 of glucose and C-28 ($\delta = 176.1$) of bayogenin. On the basis of the above evidence, the structure of polygalasaponin G was elucidated as bayogenin 28-*O*-{β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl} ester.

Polygalasaponin H (**4**) has similar NMR data of the aglycone and sugar moieties compared to those of **1** except for a terminal apiose in the ^1H and ^{13}C NMR spectra (Tables 1, 2). This indicated that the two compounds share the same aglycone and similar sugar substitution. Acid hydrolysis of **4** and GC analysis of chiral derivatives of sugars in the acid hydrolysate afforded D-glucose, L-rhamnose, D-xylose, and D-apiose. The ^1H NMR spectrum of **4** showed four anomeric protons at $\delta = 6.26$ (1H, d, $J = 7.0$ Hz), 6.19 (1H, s), 6.09 (1H, d, $J = 5.0$ Hz), and 5.34 (1H, d, $J = 8.0$ Hz). The presence of a HMBC correlation between H-1 ($\delta = 6.09$) of apiose and C-3 ($\delta = 81.9$) of rhamnose implied that additional apiose was located at C-3 of rhamnose. Therefore, the structure of polygalasaponin H was established as medicagenic acid 28-*O*-{β-D-xylopyranosyl-(1→4)-[β-D-apiofuranosyl-(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl} ester.

The ESIMS and HRESIMS of polygalasaponin J (**5**) gave the molecular formula $C_{59}H_{96}O_{28}$. The molecular weight of **5** was 162 mass units more than that of **2**, which implied an additional hexose unit in **5**. Acid hydrolysis of **5** and GC analysis of chiral derivatives of sugars in the acid hydrolysate yielded D-glucose, L-rhamnose, D-xylose, and D-galactose. Detailed comparison of the ^1H and ^{13}C NMR data (Tables 1, 2) implied that the structure of **5** was similar to **1** except for a terminal galactose moiety. Galactose was located at C-4 of xylose owing to the HMBC correlation between H-1 of galactose and C-4 of xylose. Thus, the structure of polygalasaponin J was identified as 3-*O*-β-D-glucopyranosyl bayogenin 28-*O*-[β-D-galactopyranosyl-(1→4)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranosyl] ester.

Compounds **1**, **4**, **6**, and **12** were tested for their antidepressant activity. The immobility time of forced swimming in mice exposed to compounds **1** and **4** (100 mg/kg) for 5 consecutive days was significantly reduced by 58.1% ($P < 0.01$) and 51.3% ($P < 0.05$) (Table 3), respectively, while the reduction of immobility time of forced swimming in mice exposed to compounds **6** and **12** was not observed (Table 3). This suggested that compounds **1** and **4** may be the antidepressant constituents of *P. japonica*.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 343 polarimeter. IR were recorded on a Bruker Vector22 spectrometer with KBr pellet. NMR spectra were operated on a Bruker DRX-500 spectrometer at 500 MHz for ^1H and 125 MHz for ^{13}C NMR. Chemical shifts were reported in ppm with TMS as internal standard. EIMS and HRESIMS were recorded on a Varian MAT-212 mass spectrometer and a Q-TOF micro mass spectrometer, respectively. Gas chromatography analysis was operated on an HP-5892 II with FID detector, and an HP-20M (Carbowx 20M) capillary column (25 m × 0.32 mm × 0.3 μm) was used. Semipreparative HPLC was performed on a Waters liquid chromatograph 510 instrument with a PDA UV detector at 212 nm using an ODS column (Kromasil, 5 μm, 300 × 10 mm). Column chromatography was performed on silica gel (200−300 mesh, Yantai, China), silica gel H (10−40 μm, Yantai, China), macroporous resin (AB-8, Tianjin, China), RP silica gel (ODS, 25−40 μm, Merck), and Sephadex LH-20 (Pharmacia). TLC analysis was run on $HSGF_{254}$ precoated silica gel plates (10−40 μm, Yantai, China).

Plant Material. *P. japonica* was purchased from the Market for Traditional Chinese Medicines of Bozhou, Anhui Province, China, and identified by Dr. Bao-Kang Huang of the Department of Pharmacognosy, School of Pharmacy, Second Military Medical University, Shanghai, China. A voucher specimen (No. 0211-1) is deposited at

Table 2. ^{1}H NMR Spectral Data for the Sugar Moieties of **1**−**5** in Pyridine-d_5 (500 MHz)

	1 (multi. *J* Hz)	**2** (multi. *J* Hz)	**3** (multi. *J* Hz)	**4** (multi. *J* Hz)	**5** (multi. *J* Hz)
C-3 sugar					
Glu-1		5.15(d, 7.0)			5.14(d, 8.0)
2		4.02(t, 8.0)			4.01(d, 8.0)
3		4.17			4.17
4		4.23			4.21
5		3.89(m)			3.90(m)
6		4.46(dd, 11.0, 2.0)			4.45
		4.32			4.32
C-28 sugars					
Glu-1	6.21(d, 8.0)	6.20(d, 8.0)	6.22(d, 8.0)	6.26(d, 7.0)	6.18(d, 8.0)
2	4.37(d, 8.0)	4.39	4.29(m)	4.30(dd, 7.0, 2.0)	4.07
3	4.30	4.28	4.23	4.23(m)	4.28
4	4.28	4.30	4.27(t,9.0)	4.29(m)	4.29
5	4.06	3.96(m)	3.97(m)	4.00	3.95
6	4.40(dd, 12.0, 2.0)	4.39	4.40(dd, 14.0, 2.0)	4.41(dd, 11.0, 2.0)	4.41(m)
	4.34	4.33	4.32(dd, 14.0, 4.0)	4.35(dd, 11.0, 5.0)	4.33(m)
Rha-1	6.41(s)	6.50(d, 1.0)	6.24(d, 1.0)	6.19(s)	6.45(s)
2	4.83(brs)	4.85(m)	5.04(t-like)	5.04(brs)	4.86(br)
3	4.71(dd, 8.0, 3.0)	4.73(dd, 10.0, 3.0)	4.64(dd, 8.0, 4.0)	4.67(dd, 8.0, 3.0)	4.70(dd, 10.0, 2.0)
4	4.35	4.37	4.52(t, 8.0)	4.55(t, 9.0)	4.29
5	4.52(dd, 6.0, 9.0)	4.53(m)	4.51(m)	4.52(m)	4.53(m)
6	1.79(d, 6.0)	1.83(d, 6.0)	1.79(d, 5.0)	1.78(d, 6.0)	1.78(d, 6.0)
Xyl-1	5.05(d, 8.0)	5.06(d, 8.0)	5.34(d, 8.0)	5.34(d, 8.0)	4.97(d, 8.0)
2	4.05	4.09(m)	4.00(m)	4.00	4.07
3	3.97(m)	4.09(m)	4.10(t, 8.0)	4.10(t, 8.0)	4.37
4	4.18(m)	4.19	4.16(m)	4.19	4.30
5	4.24(dd, 11.0, 5.0)	4.25(m)	4.21	4.20	4.32
	3.52(t, 10.0)	3.54(t, 11.0)	3.48(t, 10.0)	3.48(t, 10.0)	3.47(t, 13.0)
Api-1			6.09(d, 5.0)	6.09(d, 5.0)	
2			4.79(d, 4.0)	4.78(d, 4.0)	
4			4.59(d, 9.0)	4.59(d, 9.0)	
			4.19(d, 9.0)	4.19(d, 9.0)	
5			4.07(d, 11.0)	4.05(d, 12.0)	
			4.01(d, 11.0)	4.02(d, 12.0)	
Gal-1					4.90(d, 8.0)
2					4.36
3					4.10
4					4.46
5					4.11
6					4.37
					4.33

Table 3. Reduction of Floating Time of Forced Swimming in Mice Treated with Compounds **1**, **4**, **6**, and **12** (means ± SEM)[a]

		10 mg/kg		100 mg/kg	
	n	floating time (s)	reduction (%)	floating time (s)	reduction (%)
control	10	149.0 ± 38.20			
fluoxetine	10	73.5 ± 28.48	50.7**		
1	10	96.2 ± 72.13	35.4	62.5 ± 39.09	58.1**
4	10	80.7 ± 78.52	45.8	72.5 ± 30.10	51.3*
6	10	95.1 ± 68.62	36.2	122.5 ± 53.67	17.8
12	10	123.9 ± 77.03	16.8	124.3 ± 63.71	16.6

[a] *: $P < 0.05$, **: $P < 0.01$.

the Herbarium of the School of Pharmacy, Second Military Medical University, Shanghai, China.

Materials and Methods. The dried and cut aerial parts of *P. japonica* (7 kg) were refluxed 3 h with 70% EtOH. After removal of the solvent, the extract was partitioned with petroleum ether (1 L × 4), $CHCl_3$ (1 L × 4), EtOAc (1 L × 4), and water-saturated *n*-BuOH (1 L × 4), respectively. The *n*-BuOH extract was applied to macroporous resin (1000 g) column chromatography and washed with H_2O (5.0 L), 40% EtOH (10.0 L), 70% EtOH (5.0 L), and 95% EtOH (5.0 L) (v/v), respectively. The 40% EtOH fraction (82.3 g) was chromatographed on a silica gel column (8 × 80 cm, 500 g) with the gradient $CHCl_3$/CH_3OH (20:1, 3 L; 15:1, 2 L; 10:1, 2 L; 6:1, 2 L; 4:1, 2 L; 2:1, 2 L; 1:1, 2L) as eluents to afford 15 fractions, A−O (each about 1 L). Fraction C (3.4 g) was refractionated on a silica gel column eluting with EtOAc/CH_3OH (20:1), followed by Sephadex LH-20 chromatography (2.5 × 100 cm, 50 g) with $CHCl_3$/CH_3OH (1:1), to yield **6** (670 mg). Fractions F−I (23.4 g) were subjected to RP silica gel (ODS) column chromatography (5 × 40 cm, 200 g), eluting with the gradient H_2O/CH_3OH (100:0 → 0:100), and then purified by repeated RP silica gel (ODS) (CH_3CN/H_2O, 40:60), Sephadex LH-20 (CH_3OH), and semipreparative HPLC (CH_3CN/H_2O, 45:55, flow rate of 3 mL/min) column chromatography to give **2** (472 mg), **3** (203 mg), **1** (1680 mg), **7** (16 mg), **8** (20 mg), **9** (18 mg), and **10** (30 mg). Fractions J and K (5.3 g) were subjected to silica gel chromatography (4 × 40 cm, 50 g) and washed with $CHCl_3$/CH_3OH/H_2O (65:30:10, 1L) to yield **4** (1130 mg), then by semipreparative HPLC (CH_3CN/H_2O, 35:65, flow rate of 3 mL/min) to afford **4** (230 mg) and **11** (85 mg). Similarly, fractions L−N (13.6 g) were repeatedly chromatographed on RP silica gel (ODS) and semipreparative HPLC (CH_3CN/H_2O, 32:68, flow rate of 3 mL/min) to give **12** (1850 mg), **5** (27 mg), and **13** (18 mg).

Compound 1: white amorphous solid; $[\alpha]^{20}_D$ −0.33 (*c* 0.12, MeOH); IR (KBr) ν_{max} 3422, 2932, 1733, 1699, 1652, 1075 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.48 (1H, t-like, H-12), 1.98 (3H, s, Me-24), 1.61 (3H, s, Me-25), 1.17 (3H, s, Me-26), 1.23 (3H, s, Me-27), 0.84 (3H, s, Me-29), 0.84 (3H, s, Me-30); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 1; ESIMS *m/z* 965 [M + Na]$^+$, 981 [M + K]$^+$; HRESIMS *m/z* 965.4734 [M + Na]$^+$ (calcd for $C_{47}H_{74}O_{19}Na$, 965.4722).

Compound 2: white amorphous solid; $[\alpha]^{20}_D$ 0.52 (*c* 0.23, MeOH); IR (KBr) ν_{max} 3421, 2932, 1733, 1652, 1076, 1043 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.46 (1H, t-like, H-12), 1.32 (3H, s, Me-24), 1.55 (3H, s, Me-25), 1.16 (3H, s, Me-26), 1.22 (3H, s, Me-27), 0.83 (3H, s, Me-29), 0.81 (3H, s, Me-30); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 1; ESIMS *m/z* 1113 [M + Na]$^+$, 1129 [M + K]$^+$; HRESIMS *m/z* 1113.5454 [M + Na]$^+$ (calcd for $C_{53}H_{86}O_{23}Na$, 1113.5458).

Compound 3: white amorphous solid; $[\alpha]^{20}_D$ −7.6 (*c* 0.075, MeOH); IR (KBr) ν_{max} 3427, 2943, 1750, 1636, 1049 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.45 (1H, t-like, H-12), 1.35 (3H, s, Me-24), 1.60 (3H, s, Me-25), 1.18 (3H, s, Me-26), 1.20 (3H, s, Me-27), 0.84 (3H, s, Me-29), 0.82 (3H, s, Me-30); ^{13}C NMR (pyridine-d_5, 125 MHz), see

Table 1; ESIMS *m/z* 1083 [M + Na]$^+$, 1099 [M + K]$^+$, 2143 [2M + Na]$^+$; HRESIMS *m/z* 1083.5361 [M + Na]$^+$ (calcd for $C_{52}H_{84}O_{22}Na$, 1083.5352).

Compound 4: white amorphous solid; $[\alpha]^{20}_D$ −2.4 (*c* 0.16, MeOH); IR (KBr) ν_{max} 3421, 2940, 1732, 1700, 1652, 1435, 1386, 1256, 1075, 1056 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.50 (1H, t-like, H-12), 2.03 (3H, s, Me-24), 1.64 (3H, s, Me-25), 1.18 (3H, s, Me-26), 1.22 (3H, s, Me-27), 0.89 (3H, s, Me-29), 0.83 (3H, s, Me-30); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 1; ESIMS *m/z* 1097 [M + Na]$^+$, 1113 [M + K]$^+$; HRESIMS *m/z* 1097.5142 [M + Na]$^+$ (calcd for $C_{52}H_{82}O_{23}$, 1097.5145).

Compound 5: white amorphous solid; $[\alpha]^{20}_D$ 3.2 (*c* 0.29, MeOH); IR (KBr) ν_{max} 3420, 2933, 1733, 1733, 1652, 1074 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) δ 5.46 (1H, t-like, H-12), 1.28 (3H, s, Me-24), 1.51 (3H, s, Me-25), 1.11 (3H, s, Me-26), 1.24 (3H, s, Me-27), 0.85 (3H, s, Me-29), 0.80 (3H, s, Me-30); ^{13}C NMR (pyridine-d_5, 125 MHz), see Table 1; ESIMS *m/z* 1275 [M + Na]$^+$, 1291 [M + K]$^+$; HRESIMS *m/z* 1275.5988 (calcd for $C_{59}H_{96}O_{28}$, 1275.5986).

Acid Hydrolysis and GC Analysis of 1−5. Each compound (5 mg) was heated in 2.0 mol/L HCl/MeOH (1:1, v/v, 10 mL) at 90 °C for 4 h. The reaction mixture was evaporated to dryness and then partitioned between EtOAc and H_2O. The EtOAc extract was purified by chromatography on Sephadex LH-20 (2.5 × 100 cm, 50 g). Each aglycone was identified by comparing its retention time with that of an authentic sample on HPLC [Dikma Diamonsil ODS, 250 mm × 4.6 mm, 5 μm, $CH_3CN/H_2O/H_3PO_4$, 78:22:0.5, with flow rate of 1.0 mL/min]. The agylcones of **1** and **4** were determined to be medicagenic acid, while those of **2**, **3**, and **5** were bayogenin. The H_2O layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3) column and concentrated to yield a sugar residue. The residue was treated with D-cysteine (0.1 mg) in water (0.05 mL) and pyridine (0.03 mL) at 60 °C for 1 h with stirring. After the solvent was evaporated and the reaction mixture was dried, pyridine (0.03 mL), hexamethyldisilazane (0.03 mL), and trimethylsilyl chloride (0.03 mL) were added to the residue. The reaction mixture was heated at 60 °C for 30 min. The supernatant was applied to GC, and their retention times were compared with those of authentic monosaccharides.[2−5] D-Glucose (t_R 11.014 min), L-rhamnose (t_R 5.896 min), and D-xylose (t_R 4.102 min) were detected from **1**, **2**, **3**, **4**, and **5**. D-Apiose (t_R 4.892 min) was detected from **3** and **4**. D-Galactose (t_R 12.857 min) was detected from **5**.

Forced Swimming Test. Compounds **1**, **4**, **6**, and **12** were tested for antidepressant activity using the FST initially described by Porsolt.[11] Male ICR mice (18−22 g) from a breeding colony in the Experimental Animal Center of the Second Military Medical University, Shanghai, PR China, were housed with unlimited food and water in a room maintained with a 12:12 h light−dark cycle for a week before the experiment. The experiment was conducted in the light portion of the cycle, and all mice were gently handled daily. The tank (plexiglass cylinder, 20 cm tall and 14 cm in diameter) was filled with H_2O (24 °C) to a height of 10 cm. The FST was conducted in a quiet, darkened room lit by a 25-W red light. A camera system was used to observe and record the mouse's behavior. The mouse was dropped into the water 1 h after administration and observed for 6 min. All the substances were administered orally. The first 2 min the animal was allowed to adjust to the new conditions. In the next 4 min, the duration of the two types of motor activity, struggling and floating status, was recorded as immobility time with the observer unaware of administration. CMCNa (0.3%) solution and fluoxetine hydrochloride (10 mg/kg, Changzhou Huasheng Pharmaceuticals Co. Ltd.) were applied as normal and positive controls, respectively. Mice were treated with saponins (10g and 100 mg/kg) once daily for 5 consecutive days, while fluoxetine hydrochloride was applied only on the fifth day. The results of FST were analyzed by one-way ANOVA (SPSS) and followed Dunnett's t-tests. The data were presented as means ± SEM. All animal treatments were strictly in accordance with the National Institutes of Health Guide of the Care and Use of Laboratory Animals. The experiments were carried out under the approval of the Committee of Experimental Animal Administration of the University.

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