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New triterpenoid saponins from the roots of *Platycodon grandiflorum*

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Abstract—Bioassay-directed fractionation of the antiviral active fraction of the roots of *Platycodon grandiflorum* leads to the isolation of three new triterpenoid saponins, platycosides G1–G3 (**1**–**3**), as well as two known saponins, platycodin D3 (**4**), and platycoside E (**5**). The structures of the new compounds were elucidated on the basis of their spectral data and chemical evidences. The isolated saponins were tested for their antiviral activities against respiratory syncytial virus (RSV), herpes simplex type 1 virus (HSV-1) and influenza type A virus (Flu A). Compound **4** showed weak anti-RSV activity.

1. Introduction

The roots of *Platycodon grandiflorum* (Jacq.) A. DC. (Campanulaceae) have been used in antiphlogistic, antitussive and expectorant as a traditional Chinese medicine,[1] and it contains the abundance of saponins. Since Oshika first studied its saponin in 1918,[2] over 20 triterpenoid saponins have been reported.[3–10] Some of them showed anti-inflammatory, antitumor and immunomodulatory activities.[11–13] During our investigation of antiviral constituents from the traditional Chinese medicine,[14,15] the MeOH extract of the roots of *P. grandiflorum* exhibited the inhibitory effect against respiratory syncytial virus (RSV) (IC_{50} 44.1 μg/ml). The constituents of active MeOH extract were isolated by the bioassay-directed fractionation, and purified to yield three new trace triterpenoid saponins, platycosides G1–G3 (**1**–**3**), with two known platycodin D3 (**4**) and platycoside E (**5**) by preparative HPLC. In this paper, we report their structural determination and antiviral activities against HSV-1, RSV and Flu A.

2. Results and discussion

Platycoside G1 (**1**) was obtained as an amorphous powder, $[\alpha]_D^{20} = -11.2$ ($c = 0.32$, MeOH) The ^{13}C NMR spectrum of **1** showed signals corresponding to 64 carbons, which were sorted by DEPT experiments into six methyl, 15 methene, 35 methine, and eight quaternary carbons. Its molecular formula of $C_{64}H_{104}O_{34}$ was determined by HRFABMS. The spectral features and physicochemical properties revealed **1** to be a triterpenoid saponin. The IR spectrum exhibited absorptions at 3400 cm^{-1} (OH), 1742 cm^{-1} (ester carbonyl), and 1644 cm^{-1} (double bond). The five tertiary methyl groups (δ 1.01, 1.08, 1.12, 1.38, and 1.68) and one olefinic proton (δ 5.63, br s) were observed in the ^1H NMR spectrum. The ^{13}C NMR spectrum showed five sp^3 carbons at δ 17.4, 18.8, 24.4, 26.7, and 33.0, and two sp^2 olefinic carbons at δ 122.8 and 144.0, and five oxygenated methene and methine carbons at δ 68.6, 88.5, 73.7, 63.1 and 67.1 (see Table 2). The information of ^1H NMR spectrum coupled with ^{13}C NMR spectrum indicated that **1** possessed a 2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acidic aglycon.[9] The chemical shifts of C-3 (δ 88.5) and C-28 (δ 175.7) revealed that **1** was a bisdesmosidic glycoside. Thirty-four of 64 carbons were assigned to the oligosaccharide moieties. The ^1H and ^{13}C NMR spectra of **1** exhibited six sugar anomeric protons at δ 4.83 (d, J= 7.3 Hz), 4.74 (d, J=8.2 Hz), 5.03 (d, J=7.7 Hz), 6.43 (br s), 5.76 (br s), and 5.15 (δ, J=7.5 Hz) and carbons at δ 105.8, 104.7, 105.4, 93.3, 100.8, and 106.5 (see Tables 1 and 3). The monosaccharides were identified as arabinose, rhamnose, xylose, and glucose by TLC and a combination of DEPT, TOCSY, HMQC, and HMBC experiments, respectively. Comparing with NMR spectrum of platycoside E (**5**), the absence of apiose in **1** was also disclosed. Saponins **1** and **5** were further completely acid hydrolyzed in the parallel condition. The results exhibited that the

Keywords: Platycosides G1–G3; Triterpenoid saponin; *Platycodon grandiflorum*; Campanulaceae; Antiviral activity.
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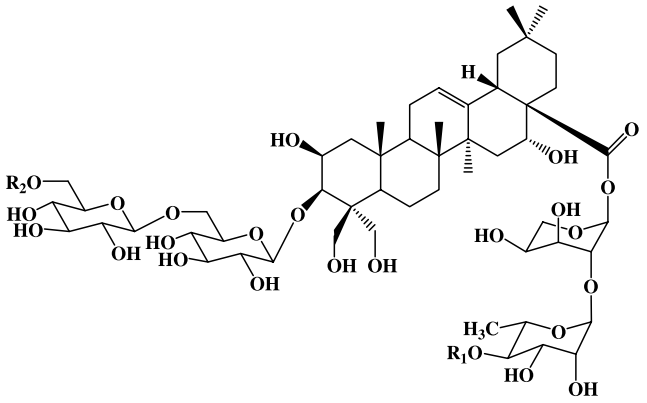
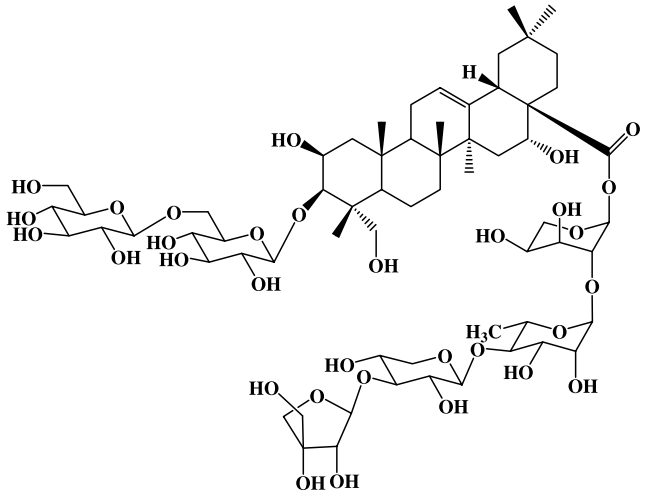


	R_1	R_2
1	− xyl	− glc
2	H	− glc
4	− xyl (3⟶1) api	H
5	− xyl (3⟶1) api	− glc



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sapogenin of **1** was identical with that of **5**, and the monosaccharides were confirmed. Their absolute configurations were determined to be L-rabinose, L-rhamnose, D-xylose, and D-glucose (ratio 1:1:1:3) by GLC analysis of the trimethylsilyl (TMSi) derivatives, respectively.[16] All the monosaccharides of **1** were in pyranose forms, as determined by their ^{1}H and ^{13}C NMR data and 2D NMR experiments. The β-anomeric configurations of the glucose and xylose units were determined by their $^3J_{H1,H2}$ coupling constants (7.3–8.2 Hz), and arabinose and rhamnose were determined as the α-configurations by the broad singlet of their anomeric protons. The sequence of the glycan part connected to C-3 of the aglycon was deduced from the following HMBC correlations: H-1‴ (δ 5.03) of terminal glucose with C-6″ (δ 70.1) of centre glucose, H-1″ (δ 4.74) of centre glucose with C-6′ (δ 70.6) of inner glucose, H-1′ (δ 4.83) of inner glucose with C-3 (δ 88.5) of aglycon (see Fig. 1). The second bisdesmosidic part at C-28 was established by the following HMBC information: the correlations between H-1 (δ 5.15) of xylose and C-4 (δ 83.3), H-1 (δ 5.76) of rhamnose and C-2 (δ 74.5) of arabinose, H-1 (δ 6.43) of arabinose and C-28 (δ 175.7) of the aglycon (see Fig. 1). On the basis of all the foregoing evidence, platycoside G1 (**1**) was elucidated as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxyolean-12-ene-28-oic acid 28-*O*-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl (1→2)-α-L-arabinopyranoside.

Table 1. ^{1}H NMR spectroscopic data (δ) for the sugar moieties of **1–3** (400 MHz in pyridine-d_5)

H	**1**	**2**	**3**
Glucose (inner)			
1′	4.83 d (7.3)	4.80 d (7.6)	4.79 d (7.2)
2′	4.07 t (7.3)	4.00 t (7.6)	4.03–4.06 m
3′	4.02–4.04 m	3.95–3.96 m	
4′	3.82–3.90 m	3.87–3.87 m	3.88–3.96 m
5′	4.11 dd (3.8, 7.5)	4.10 dd (3.3, 7.4)	4.08–4.11 m
6′	5.01 br d (7.5)	4.80 br d (7.1)	5.01–5.15 m
	3.82–3.90 m	3.73–3.75 m	3.88–3.94 m
Glucose (centre)			
1″	4.74 d (8.2)	4.69 d (7.2)	
2″	4.02–4.04 m	3.95–3.96 m	
3″, 4″, 5″	4.18–4.21 m	4.08–4.16 m	
6″	4.74 br d (8.2)	4.71 br d (7.1)	
	3.82–3.90 m	3.73–3.75 m	
Glucose (terminal)			
1‴	5.03 d (7.7)	4.99 d (8.0)	4.92 d (7.6)
2‴	4.01 t (7.7)	3.95–3.96 m	3.88–3.94 m
3‴, 4‴	4.18–4.21 m	4.08–4.16 m	4.15–4.17 m
5‴	4.02–4.04 m	3.95–3.96 m	3.88–3.96 m
6‴	4.65 br d (8.0)	4.60 br d (6.8)	4.65 br d (7.7)
	3.82–3.90 m	3.73–3.75 m	3.88–3.94 m
Arabinose			
1	6.43 br s	6.45 br s	6.39 br s
2	4.48–4.51 m	4.38–4.45 m	4.65–4.70 m
3	4.82–3.90 m	3.87–3.87 m	3.88–3.94 m
4	4.36–4.38 m	4.38–4.45 m	4.23–4.28 m
5	4.46–4.48 m	4.51–4.55 m	4.40–4.45 m
	4.82–3.90 m	4.38–4.45 m	4.23–4.28 m
Rhamnose			
1	5.76 br s	5.73 br s	5.65 br s
2, 3	4.02–4.04 m	4.38–4.45 m	3.88–3.94 m
4, 5	4.36–4.38 m	4.22–4.28 m	4.23–4.28 m
6	1.71 d (5.2)	1.61 d (6.0)	1.63 (overlap)
Xylose			
1	5.15 d (7.5)		5.15 d (7.6)
2	4.02–4.04 m		4.03–4.06 m
3	4.82–3.90 m		3.88–3.94 m
4	4.18–4.21 m		4.15–4.17 m
5	3.48 t (10.1)		3.52 t (9.6)
Apiose			
1			6.26 br s
2			5.65 br s
3			—
4			4.15–4.17 m
			4.88 d (7.8)
5			4.23–4.28 m

The assignments are based upon DEPT, HMQC, and HMBC experiments. Overlapped signals are labeled with multiplicity (m). Coupling constants (*J* values in Hz) are shown in parentheses.

Platycoside G2 (**2**) was analysed to have the molecular formula of $C_{59}H_{96}O_{30}$ by its HRFAB mass spectrum and ^{13}C and DEPT NMR data. The ^{1}H and ^{13}C NMR signals of **2** were assigned by DEPT, TOCSY, HMQC, and HMBC

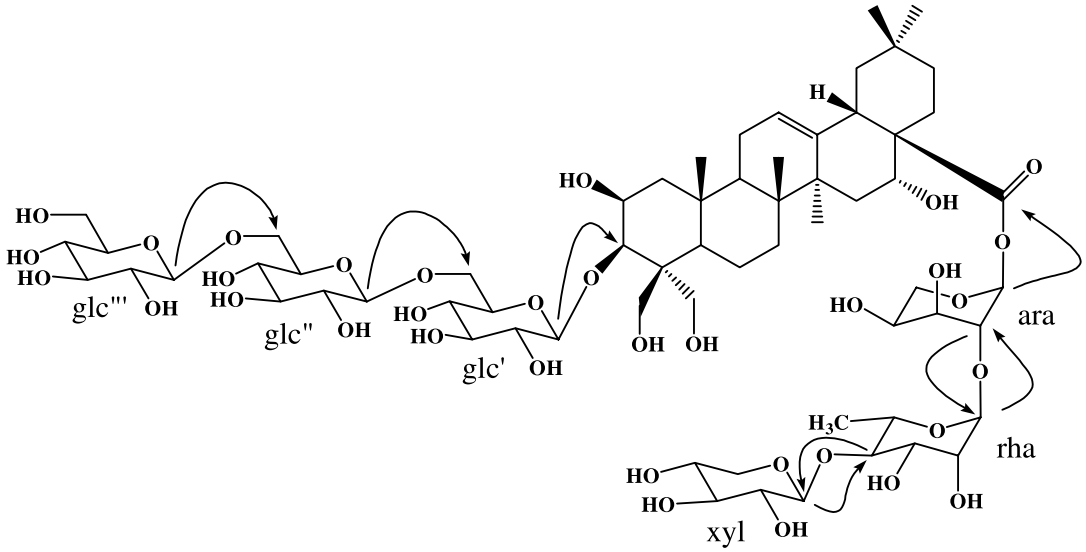

Figure 1. Key HMBC correlations for the sugar sequence of **1** (from H to C).

experiments. The comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of **1** clearly revealed that the sapogenin of **2** was in identical with that of **1**, and **2** were suggested to be a bisdesmosidic glycoside (see Tables 1–3). The anomeric proton and carbon signals of one xylosyl group in NMR spectra of **2** where disappeared, and the chemical shift of C-4 of rhamnose in **2** was shifted upfield from δ 83.3 to 74.0. By comparison with **1**, the configurations of the monosaccharides in **2** were assigned for β-glucose, α-arabiose, and α-rhamnose, respectively. The spectral data deduced that compound **2** was a dexylosyl platycoside G1. Comparing the spectral characteristic of **2** with **1**, the absolute configurations were supposed as L-rabinose, L-rhamnose, and D-glucose. On the basis of these results, platycoside G2 (**2**) was identified as 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-2β,3β,16α,23,24-pentahydroxy-olean-12-ene-28-oic acid 28-α-L-rhamnopyranosyl-(1→2)-α-L-arabino-pyranoside.

Table 2. ^{13}C NMR spectroscopic data (δ) for the aglycon moieties of **1**–**3** (100 MHz in pyridine-d_5)[a]

C	1	2	3
1	45.0	45.2	44.4
2	68.6	70.0	69.7
3	88.5	88.8	84.5
4	47.8	47.5	43.1
5	47.3	47.0	47.9
6	19.1	19.2	18.7
7	33.2	33.7	33.6
8	40.2	40.5	40.5
9	44.7	48.1	48.0
10	37.7	37.9	37.4
11	23.7	24.0	24.4
12	122.8	122.6	123.2
13	144.0	144.1	144.4
14	42.1	42.4	42.6
15	35.8	36.0	36.5
16	73.7	73.8	74.3
17	49.4	49.7	49.9
18	41.3	41.5	41.6
19	47.8	47.4	47.4
20	30.6	30.9	31.3
21	35.8	36.0	36.3
22	32.4	32.0	32.6
23	63.1	63.2	66.3
24	67.1	66.0	15.6
25	18.8	19.1	18.0
26	17.4	17.6	17.9
27	26.7	27.0	27.6
28	175.7	175.6	176.1
29	33.0	33.3	33.6
30	24.4	24.8	25.2

[a] Assignments are based on HMQC, TOCSY and HMBC experiments.

Platycoside G3 (**3**) has a molecular formula of $C_{63}H_{102}O_{32}$ determined by HRFAB mass spectrum. The ^{1}H and ^{13}C NMR signals of **3** were assigned by DEPT, TOCSY, HMQC, and HMBC experiments. Comparing the ^{1}H and ^{13}C NMR signals of **3** with those of **4**, the chemical shift assignable to H-24 of **3** in the aglycon moiety was shifted upfield to δ 1.45, and the C-24 to δ 15.6. Other NMR signals of **3** were similar to those of **4** (see Tables 1–3). The ^{1}H and ^{13}C NMR spectra suggested that the aglycon of **3** was polygalacic acid.[9] The HMBC experiment of **3** indicated that the sugar sequence was also a bisdesmosidic glycoside, and comparison of the NMR spectrum of **3** with that of **4** showed that their glycans were the same. On the basis of the above results, **3** was concluded to be 3-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-polygalacic acid 28-*O*-β-D-apiofuranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside.

All fractions and isolates, the MeOH extract of *P. grandiflorum* were tested for their antiviral activities against HSV-1, RSV and Flu A. All samples had no activity against HSV-1 and Flu A. The MeOH extract and fraction F02 showed moderate anti-RSV activity, and saponin **4** exhibited weak anti-RSV activity (see Table 4).

3. Experimental

3.1. General procedures

Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR absoption spectra were obtained with

Table 3. ^{13}C NMR Spectroscopic data (δ) for the sugar moieties of **1–3** (100 MHz in pyridine-d_5)[a]

C	1	2	3
Glucose (inner)			
1′	105.8	105.9	105.7
2′	74.8	74.7	75.5
3′	78.4	78.6	78.7
4′	72.0	72.5	72.2
5′	76.2	77.0	77.0
6′	70.6	70.1	70.8
Glucose (centre)			
1″	104.7	104.8	
2″	74.8	75.3	
3″	78.3	78.4	
4″	71.2	71.2	
5″	76.8	76.4	
6″	70.1	70.4	
Glucose (terminal)			
1‴	105.4	105.5	105.3
2‴	74.9	75.1	75.5
3‴	78.2	78.2	78.6
4‴	70.8	71.5	70.4
5‴	77.4	77.6	78.0
6‴	62.8	62.9	62.9
Arabinose			
1	93.3	93.4	93.7
2	74.5	75.1	75.5
3	71.7	70.7	71.8
4	66.0	65.5	66.3
5	62.8	62.6	63.2
Rhamnose			
1	100.8	101.4	101.4
2	70.9	72.2	72.3
3	72.4	72.3	73.0
4	83.3	74.0	83.9
5	68.3	70.1	68.9
6	18.1	18.6	18.7
Xylose			
1	106.5		106.8
2	75.7		75.6
3	78.0		85.0
4	69.8		70.1
5	67.1		67.2
Apiose			
1			111.4
2			78.3
3			80.7
4			75.5
5			65.6

[a] Assignments are based on HMQC, TOCSY and HMBC experiments.

Table 4. Anti-RSV activity of the triterpenoid saponins from *Platycodon grandiflorum*

Sample	RSV[a] IC_{50} (μg/mg)	CC_{50} (μg/mg)[b]	SI[c]
MeOH ext.	44.1	>200	4.5
Fraction F01	Inactive	>200	—
Fraction F02	25.0	>200	8.0
Fraction F03	Inactive	>200	—
1	Inactive	>200	—
2	Inactive	>200	—
3	Inactive	>200	—
4	200.0	>200	1.0
5	Inactive	>200	—
Ribavirin[d]	2.6	62.5	24.0

[a] IC_{50} is the concentration of the sample required to inhibit virus-induced CPE 50%.
[b] CC_{50} is the concentration of the 50% cytotoxic effect.
[c] SI is selectivity index = CC_{50}/IC_{50}.
[d] Ribavirin, an approved drug for the treatment of RSV infections in humans.

a Shimadzu IR-450 instrument as a film on KBr Disks. NMR spectra were obtained with a Bruker 400 spectrometer operating at 400 MHz for ^1H and 100 MHz for ^{13}C, respectively. Chemical shifts were reported in parts per million on the δ scale with TMS as the internal standard. FABMS were recorded on VG autospec 3000 system. Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, Qingdao, People's Republic of China), Diaion HP-20 (Mitsubishi Chemical, Japan), and ODS (100–200 mesh, Fuji Silysia Chemical Ltd, Japan). Reversed phase HPLC was carried out on a Agilent 1100 G1361A preparative pump, equipped with a Agilent 1100 G1315B Diode-array Detector, and Alltima C_{18} (250×22 mm, 10 μm), eluted with CH_3CN–H_2O (2:8) at a flow rate of 20 ml/min, with the UV detector set at 210 nm. GLC was carried out on a Shimadzu GC-7A, and column: Silicone OV-17 on Uniport HP 2%, 3 mm i.d.×2.1 m column; column temperature, initial temperature 140 °C for 16 min and rising 2 °C/min to final 170 °C; carrier gas, N_2, flow rate, 25 ml/min. TLC was performed on precoated Si gel 60 F_{254} plates (0.2 mm thick, Merck) with $CHCl_3$–MeOH–H_2O (7:3:1), $CHCl_3$–MeOH–H_2O–AcOH (7:3:1:1), $CHCl_3$–MeOH (9:1), and RP-18 F_{254} plates (0.2 mm thick, Merck) with MeOH–H_2O (6:4), and spots were detected by spraying with 10% ethanolic H_2SO_4 reagent.

3.2. Plant material

The roots of *Platycodon grandiflorum* (Jacq.) A. DC were collected in Hubei Province, PR China, in September 2003. The raw material was identified by Prof. Zhongzhen Zhao, Hong Kong Baptist University, Hong Kong, and a voucher specimen (CMED-0077-1) was deposited in the CMED-LAB of Hong Kong Jockey Club Institute of Chinese Medicine.

3.3. Extraction and isolation

The dried roots (933.8 g) were pulverized and extracted with MeOH for three times. The MeOH extract was concentrated in vacuum at 40 °C to give a residue (255.5 g), which was fractionated by CC on Diaion HP-20 eluting with H_2O 18 l, 50% MeOH 6 l, and MeOH 8 l, yield three fractions (F01-F03). Fraction F02 showed anti-RSV activity (see Table 4). This fraction was then subjected to silica gel, ODS CC, and purified by reversed phase HPLC to yield saponins **1** (123.5 mg, 0.013%), **2** (12.4 mg, 0.001%), **3** (18.0 mg, 0.002%), **4** (239.2 mg, 0.026%), and **5** (429.6 mg, 0.046%).

3.3.1. Platycoside G1 (1). White amorphous powder; $[\alpha]_D^{20} = -11.2$ ($c=0.3$, MeOH); IR (KBr) ν_{max}: 3400, 2920, 1742, 1644, 1040 cm^{-1}; ^1H NMR (pyridine-d_5, 400 MHz): aglycon δ 1.01 (3H, s, H-29), 1.08 (3H, s, H-26), 1.12 (3H, s, H-30), 1.34 (1H, br d, $J=8.6$ Hz, H-15a), 1.38 (3H, s, H-25), 1.41–1.44 (4H, m, H-6, 7a, 19a), 1.55–1.58 (1H, m, H-7b), 1.68 (3H, s, H-27), 1.71–1.81 (2H, m, H-1a, 15b), 1.91–2.01 (5H, m, H-1b, 5, 11, 9), 2.15 (1H, br t, $J=9.6$ Hz, H-22a), 2.24 (1H, br d, $J=10.0$ Hz, H-21a), 2.35 (2H, t, $J=9.6$ Hz, H-21b, 22b), 2.77 (1H, t, $J=13.3$ Hz, H-19b), 3.53 (1H, t, $J=13.3$ Hz, H-18), 3.82–3.90 (m, H-23a), 4.01–4.04 (m, H-24), 4.46–4.55 (m, H-2, 3),

4.66 (1H, d, $J=8.0$ Hz, H-23b), 5.26 (1H, br s, H-16), 5.63 (1H, br s, H-12); Other ^{1}H and ^{13}C NMR data: see Tables 1–3; FAB-MS *m/z*: 1417 $[M+H]^+$; HRFABMS *m/z*: 1417.6490 $[M+H]^+$ (Calcd for $C_{64}H_{105}O_{34}$ 1417.6487).

3.3.2. Platycoside G2 (2). White amorphous powder; $[\alpha]_D^{20}=-50.6$ ($c=0.2$, MeOH); IR (KBr) ν_{max}: 3400, 2920, 1742, 1646, 1038 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz): aglycon δ 0.94 (3H, s, H-29), 1.01 (3H, s, H-26), 1.06 (3H, s, H-30), 1.24 (1H, br d, $J=8.0$ Hz, H-15a), 1.34 (3H, s, H-25), 1.39–1.50 (4H, m, H-6, 7a, 19a), 1.63 (3H, s, H-27), 1.68–1.71 (1H, m, H-7b), 1.86–1.94 (7H, m, H-1, 5, 11, 9), 2.11–2.16 (1H, m H-22a), 2.19–2.33 (3H, m, H-21, 22b), 2.72 (1H, t, $J=13.3$ Hz, H-19b), 3.52 (1H, t, $J=13.3$ Hz, H-18), 3.85–3.87 (m, H- 23a), 3.95–3.96 (m, H-24), 4.51–4.44 (m, H-2, 3), 4.60 (1H, d, $J=8.0$ Hz, H-23b), 5.18 (1H, br s, H-16), 5.56 (1H, br s, H-12); Other ^{1}H- and ^{13}C NMR data: see Tables 1–3; FAB-MS *m/z*: 1285 $[M+H]^+$; HRFABMS *m/z*: 1285.6069 $[M+H]^+$ (Calcd for $C_{59}H_{97}O_{30}$ 1285.6065).

3.3.3. Platycoside G3 (3). White amorphous powder, $[\alpha]_D^{20}=-108$ ($c=0.3$, MeOH); IR (KBr) ν_{max}: 3400, 2920, 1742, 1646, 1038 cm^{-1}; ^{1}H NMR (pyridine-d_5, 400 MHz): aglycon δ 0.91 (3H, s, H-29), 1.05 (3H, s, H-26), 1.06 (3H, s, H-30), 1.25 (3H, s, H-25), 1.27(1H, br d, $J=8.0$ Hz, H-15a), 1.45 (3H, s, H-24), 1.47–1.50 (5H, m, H-6, 7a, 19a), 1.63 (3H, s, H-27), 1.68–1.76 (1H, m, H-7b), 1.86–2.11 (7H, m, H-1, 5, 9, 11, 15b), 2.20–2.29 (4H, m H-21, 22), 2.67 (1H, t, $J=13.2$ Hz, H-19b), 3.52 (1H, t, $J=13.3$ Hz, H-18), 3.88–3.94 (m, H-23a), 4.40–4.45 (m, H-2,3), 4.65–4.70 (m, H-23b), 5.15 (1H, br s, H-16), 5.46 (1H, br s, H-12); Other ^{1}H and ^{13}C NMR data: see Tables 1–3; FAB-MS *m/z*: 1371 $[M+H]^+$; HRFABMS *m/z*: 1371.6429 $[M+H]^+$ (calcd for $C_{63}H_{103}O_{32}$ 1371.6432).

3.4. Acid hydrolysis of platycosides G1 (1) and E (5)

Saponins **1** (25 mg) and **5** (30 mg) were heated in 1.5 ml of 1 M HCl (dioxane–H_2O, 1:1) at 96 °C for 3 h in a water bath, respectively, Dioxane was removed and the solution was extracted with EtOAc (2 ml×3), and removed EtOAc. The aglycons of **1** and **5** were consistent by comparing with TLC (silica gel plate, $CHCl_3$–MeOH–H_2O–AcOH 7:3:1:1, visualization by H_2SO_4 spray and then heated). The aqueous solution of acid hydrolysis of **1** was neutralized by passing through an Amberlite MB-3 resin column eluted with water, then concentrated and dried, and finally treated with 1-(trimethylsilyl)-imidozole at room temperature for 4 h. After the excess reagent was decomposed with H_2O, the reaction product was extracted with *n*-hexane (3 ml×2). The TMSi derivatives of the monosaccharides were identified as D-glucose, L-arabinose, L-rhamnose and D-xylose (3:1:1:1) by GLC analysis with authentic monosaccharides.

3.5. Viruses and cells

RSV strain Long, HSV-1 (15577) strain, and Madin Darby canine kidney (MDCK) cells were obtained from American Type Culture Collection. Flu A (H3N2) strain was obtained from Guangzhou, PR China; cytotoxicity assay and cytopathic effect reduction assay are corresponded with the reported methods.[15]

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References and notes

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