Steroids 77 (2012) 1242–1248

Contents lists available at SciVerse ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Steroidal saponins with antimicrobial activity from stems and leaves of *Paris polyphylla* var. *yunnanensis*

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ARTICLE INFO

Article history:
Received 9 May 2012
Received in revised form 5 July 2012
Accepted 13 July 2012
Available online 28 July 2012

Keywords:
Paris polyphylla var. *yunnanensis*
Trilliliaceae
Steroidal saponins
Chonglouosides SL-1–SL-6

ABSTRACT

Rhizoma Paridis, the root of *Paris polyphylla* var. *yunnanensis* (Trilliaceae), is a Chinese traditional medicine, which resources become less and less. However, the aerial parts of this herb, which can regenerate every year, were discarded. In order to expand the resources, detailed chemical investigation on the stems and leaves of *Paris polyphylla* var. *yunnanensis* led to isolation of one sapogenin and 24 steroidal saponins (**1**–**25**), including 6 new glycosides, named chonglouosides SL-1–SL-6 (**1**–**6**). Their structures were elucidated on the basis of detailed analyses of their 1D and 2D NMR spectra and acid hydrolysis. Among them, compounds **3** and **4** are the first 23,27-dihydroxydiosgenin saponin having a sugar chain attached to C-23 or C-27, while compound **6** is the first 27-hydroxyruscogenin glycoside bearing 1, 27-di-*O*-sugar chains. The known compounds **10**, **12**, **14**, **19**, **20**, **22**, and **25** were isolated from the genus *Paris* for the first time. Antimicrobial testing activities of the selected compounds showed that compound **2**, **3**, **6**, **8**, **9**, **11**, **13**, **17**, **18**, **21**, and **24** were active against *Propionibacterium acnes* with MIC values of 62.5, 62.5, 3.9, 16.5, 17.2, 7.8, 39.0, 17.2, 31.3, 62.5, and 31.3 μg/ml, respectively.

1. Introduction

The genus *Paris* (Trilliliaceae) contains 24 plant species throughout the world, which were widely distributed in regions of the Eurasian continent from temperate zone to the tropics. Rhizoma Paridis ("Chong-lou" in Chinses), the dried rhizomes of *Paris polyphylla* var. *yunnanensis*, is a Chinese traditional medicine, which mainly grows in southwest China and has been widely used to treat tumor, hemostatic, antimicrobial agent, and analgesics [1]. Several groups have investigated the chemical constituents the rhizomes of *P. polyphylla* var. *yunnanensis* and isolated more than 30 steroidal saponins [2–8]. Recently, a total of 98 ingredients were identified in the crude extract from the species by UPLC/Q-TOF MS [9]. These saponins have various activities, such as cytotoxicity [8], potential anti-cancer agent to kill hepatocarcinoma cells with multi-drug resistance [10], protective effects on ethanol- or indomethacin-induced gastric mucosal lesions in rats [4], platelet agonist [11], and contractile agonist for the uterus [12]. Total steroidal saponins, as a patient medicine named 'Gong-xue-ning', have achieved reliable curative effects for abnormal uterine bleeding [13].

Because of slow growth and excessive excavation for many years, the wild populations of this herb have greatly declined and are in the edge of distinction [14]. At the same time, the aerial parts of this herb, which can regenerate every year, were discarded. Our group reported two sapogenins, eight steroidal saponins and two keampferol glycosides from the seeds, leaves, and stems of *P. polyphylla* var. *yunnanensis* in the early 1990s [15–19]. In order to broaden the resources available, there is urgent need to make a systematic phytochemical study on the arerial parts of the titled species. As a result, six new spirostanol saponins (**1**–**6**), together with one known sapogenin and 18 known steroidal saponins were isolated from the stems and leaves of *P. polyphylla* var. *yunnanensis*. Compounds **3** and **4** are the first 23, 27-dihydroxydiosgenin saponins having a sugar chain attached to C-23 or C-27, while compound **6** is the first 27-hydroxyruscogenin glycoside bearing 1, 27-di-*O*-sugar chains. Some of the isolates were evaluated for their antimicrobial activities against *Propionibacterium acnes*. Herein, we report the isolation, structural elucidation, and antimicrobial activities of these steroidal saponins.

2. Experimental

2.1. General methods

Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV spectra were measured using a Shimadzu UV-2401 PC spectraphotometer. IR spectra were obtained on Bruker Tensor-27 infrared spectrophotometer with KBr pellets. ESI-MS

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0039-128X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2012.07.007

spectra were recorded on a Bruker HTC/Esquire spectrometer, HRESIMS spectra were recorded on an API Qstar Pulsar instrument. NMR experiments were performed on Bruker AM-400, DRX-500, and Avance III 600 instruments with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. Column chromatography (CC) was performed on D101 macroporous resin column (40–60 mesh, Tianjin Pesticide Co., China), silica gel (200–300 and 300–400 mesh, Qingdao Marine Chemical Co., China), Rp-18 (40–63 μm, Merck), and Sephadex LH-20 (GE Healthcare, Sweden). TLC was performed on $HSGF_{254}$ (0.2 mm, Qingdao Marine Chemical Co., China) or Rp-18 F_{254} (0.25 mm, Merck). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 in EtOH. GC analysis was performed on a HP5890 gas chromatograph equipped with an H_2 flame ionization detector. Semi-preparative HPLC was run on Agilent 1100 liquid chromatograph with diode array detector (DAD), Zorbax-SB-C18 column (5 μm; 25 cm × 9.4 mm i.d.).

2.2. Plant material

The stems and leaves of *Paris polyphylla* var. *yunnanensis* were collected in September 2006 from Chengjiang County, Yunnan Province, PR China. The plant material was authenticated by one of the authors, Prof. Chang-Xiang Chen. A voucher specimen (No. HY0006) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China.

2.3. Extraction and isolation

The dried aerial parts of *P. polyphylla* var. *yunnanensis* (16 kg) were crushed and extracted three times with 75% EtOH (60 L × 3) under reflux for a total of 6 h and the combined extract was concentrated under reduced pressure to afford a residue. The residue was dissolved in H_2O and passed through a D101 macroporous resin column, eluting with H_2O and 80% EtOH, successively. Evaporated 80% EtOH fraction (crude saponin-rich mixture, 1.24 kg) was subjected to a silica gel column chromatography ($CHCl_3$–MeOH, 1:0 → 0:1, v/v) to give eight fractions [Fr.1 (8.5 g), Fr.2 (5.3 g), Fr.3 (50.0 g), Fr.4 (6.7 g), Fr.5 (482.0 g), Fr.6 (35.6 g), Fr.7 (384.0 g), Fr.8 (18.7 g)] on the basis of TLC plate analysis. Fraction 2 (1.1 g) was fractionated by Sephadex LH-20 column chromatography ($CHCl_3$–MeOH, 1:1, v/v) to give two fractions: Fr.2-1 (486 mg) and Fr.2-2 (300 mg). Fr.2-1 was purified by silica gel column chromatography (petroleum ether-EtOAc, 1:1 → 1:5) and further crystallized to afford **7** (80 mg). Fr.2-2 was separated by a Rp-18 column chromatography (MeOH–H_2O, 50:50 → 100:0) and further purified by semi-prep. HPLC (MeCN–H_2O, 55:45 → 60:40, v/v) to give **1** (8 mg, t_R = 33.4 min) and **8** (25 mg, t_R = 35.6 min). Fraction 3 (10.0 g) was separated by an Rp-18 column chromatography (MeOH–H_2O, 45:55 → 100:0, v/v) to give three fractions: Fr.3-1 (3.5 g), Fr.3-2 (2.4 g), and Fr.3-3 (1.8 g). Fr.3-1 (1.5 g) was crystallized to afford **9** (500 mg) and the mother liquor was further purified by HPLC (MeCN–H_2O, 50:50 → 55:45, v/v) to give **10** (12 mg, t_R = 32.5 min). Fr.3-2 (500 mg) was separated by semi-prep. HPLC (MeCN–H_2O, 40:60 → 50:50, v/v) to give **11** (35 mg, t_R = 32.1 min), **12** (42 mg, t_R = 33.6 min), and **13** (18 mg, t_R = 30.9 min). Fr.3-3 was repeatedly purified by a column chromatography ($CHCl_3$–MeOH–H_2O, 100:10:1 → 70:30:5, v/v) and an Rp-18 column chromatography (MeOH–H_2O, 45:55 → 80:20, v/v) to give **19** (128 mg) and **20** (20 mg). Fr.4 was isolated with Rp-18 column chromatography (MeOH–H_2O, 45:55 → 80:20, v/v) to give two fractions: Fr.4-1 (2.8 g) and Fr.4-2 (2.0 g). Fr.4-1 (80 mg) and Fr.4-2 (100 mg) were further purified by semi-prep. HPLC (MeCN–H_2O, 35:65 → 40:60 and 25:75 → 35:65, v/v) to give **2** (30 mg, t_R = 26.9 min), **21** (45 mg, t_R = 30.9 min) and **22** (**20**, 10 mg, t_R = 28.4 min), respectively. Fr.5 (300 g) was separated by a silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 80:20:2 → 70:30:5, v/v) and further crystallized to give **14** (80 g). Fr.6 (35.6 g) was separated by an Rp-18 silica gel column chromatography (MeOH–H_2O, 40:60 → 75:25, v/v) to obtain four fractions: Fr.6-1 (20.0 g), Fr.6-2 (4.6 g), Fr.6-3 (1.0 g), and Fr.6-4 (0.8 g). Fr.6-1 (100 mg) was subjected to a silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 80:20:2 → 70:30:5, v/v) and further purified by semi-prep. HPLC (MeCN–H_2O, (42:58 → 55:45, v/v) to afford **17** (50 mg, t_R = 27.7 min). Fr.6-2 (180 mg) was separated by semi-prep. HPLC (MeCN–H_2O, 40:60 → 55:45, v/v) to afford **18** (48 mg, t_R = 26.8 min), **15** (36 mg, t_R = 28.1 min) and **5** (7 mg, t_R = 30.2 min). Fr.6-3 was isolated with an Rp-18 column chromatography (MeOH–H_2O, 40:60 → 80:20, v/v) and further crystallized to give **16** (148 mg). Fr.6-4 was isolated by semi-prep. HPLC (MeCN–H_2O, 25:75 → 30:70, v/v) to afford **6** (21 mg, t_R = 31.6 min). Fr.7 (150 g) was separated first by a silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 80:20:2 → 70:30:5, v/v) and further crystallized to give **24** (46 g). Fr.8 was separated by a silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 80:20:2 → 60:40:6, v/v) to give three fractions: Fr.8-1 (5.2 g), Fr.8-2 (4.4 g) and Fr.8-3 (0.6 g). Fr.8-1 (2.0 g) and Fr.8-2 (2.0 g) were separated repeatedly by a silica gel column chromatography ($CHCl_3$–MeOH–H_2O, 75:25:4 → 60:40:6, v/v) and an Rp-18 silica gel column chromatography (MeOH–H_2O (40:60 → 80:20, v/v) to afford **23** (268 mg) and **25** (48 mg). Fr.8-3 (200 mg) was separated by semi-prep. HPLC (MeCN–H_2O, 25:75 → 30:70, v/v) to afford **3** (115 mg, t_R = 30.0 min) and **4** (9 mg, t_R = 32.4 min).

2.3.1. Chonglouoside SL-1 (**1**)

White, amorphous powder; $[\alpha]_D^{15}$ − 32.7 (*c* 0.11, MeOH); ESI: *m/z* 615 $[M+Na]^+$; HRESI–MS: *m/z* 615.4394 $[M+Na]^+$ (Calcd. for $C_{33}H_{52}O_9Na$, 615.3509); IR (KBr) ν_{max} (cm^{-1}): 3425, 2950, 2929, 2872, 1634, 1455, 1378, 1242, 1079, 1051, 982, 953, 921, 899, 866 (intensity: 899 > 921); ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.2. Chonglouoside SL-2 (**2**)

White amorphous powder; $[\alpha]_D^{16}$ − 106.2 (*c* 0.11, MeOH); ESI: *m/z* 777 $[M+Na]^+$; HRESI–MS: *m/z* 777.4032 $[M+Na]^+$ (Calcd. for 777.4037, $C_{39}H_{62}O_{14}Na$); IR (KBr) ν_{max} (cm^{-1}): 3441, 3416, 2971, 2929, 2895, 1651, 1545, 1453, 1371, 1140, 1106, 1087, 1034, 1002, 984, 965, 939, 906, 837, 816; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.3. Chonglouoside SL-3 (**3**)

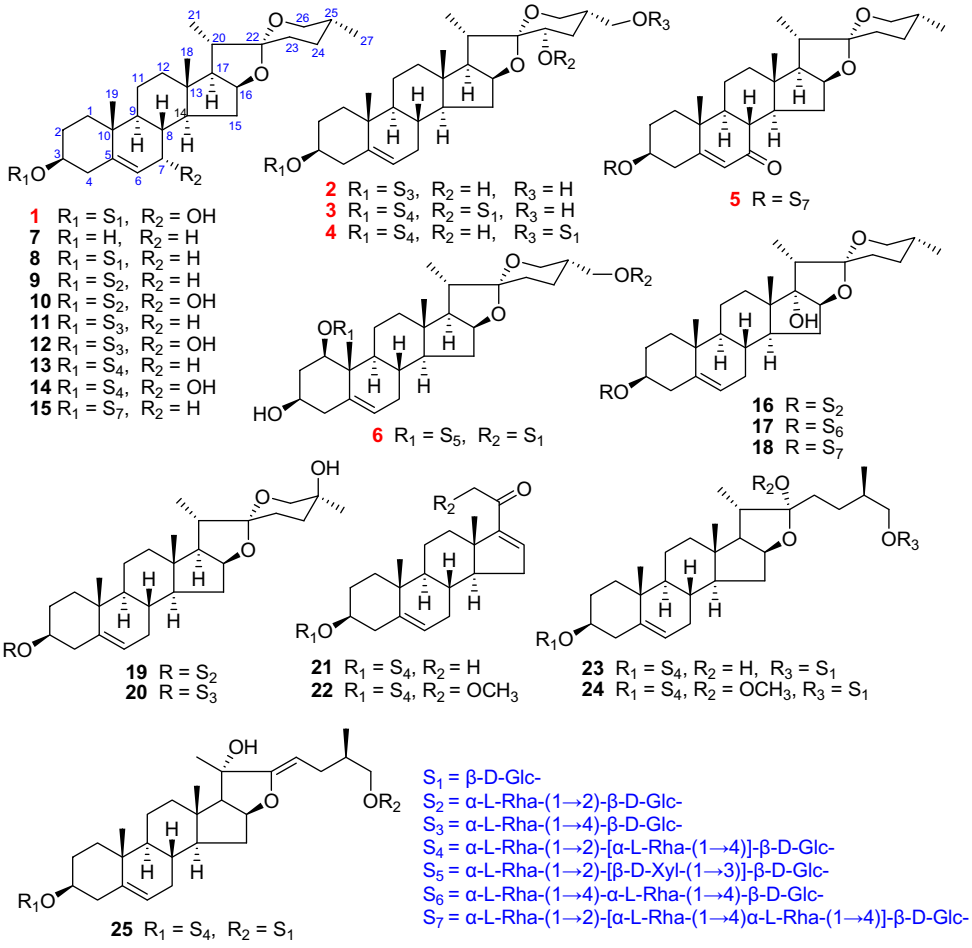
White amorphous powder: $[\alpha]_D^{15}$ − 37.4 (*c* 0.12, MeOH); ESI: *m/z* 1085 $[M+Na]^+$; HRESI–MS: *m/z* 1085.5147 $[M+Na]^+$ (Calcd. for 1085.5144, $C_{51}H_{82}O_{23}Na$); IR (KBr) ν_{max} (cm^{-1}): 3439, 3425, 2934, 2906, 1640, 1453, 1383, 1130, 1070, 1042, 993, 914, 837, 811; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.4. Chonglouoside SL-4 (**4**)

White amorphous powder; $[\alpha]_D^{20}$ − 90.2 (*c* 0.11, MeOH); ESI: *m/z* 1085 $[M+Na]^+$; HRESI–MS: *m/z* 1061.5188 $[M-H]^-$ (Calcd. for 1061.5168, $C_{51}H_{81}O_{23}$); IR (KBr) ν_{max} (cm^{-1}): 3425, 2935, 2905, 2849, 1641, 1454, 1383, 1129, 1100, 1068, 1042, 963, 904, 837, 812; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

2.3.5. Chonglouoside SL-5 (**5**)

White amorphous powder: $[\alpha]_D^{15}$ − 32.8 (*c* 0.12, MeOH); ESI: *m/z* 1051 $[M+Na]^+$; HRESI–MS: *m/z* 1051.5078 $[M+Na]^+$ (Calcd. for 1051.5089, $C_{51}H_{80}O_{21}Na$); UV (MeOH) λ_{max} (log ε) 238 (3.28) nm; IR (KBr) ν_{max} (cm^{-1}): 3427, 2931, 2875, 1658, 1456, 1384, 1242, 1132, 1051, 981, 916, 899, 834, 803 (intensity: 899 > 916); ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.



*2.3.6. Chonglouoside SL-6 (**6**)*

White amorphous powder; $[\alpha]_D^{23}$ − 93.7 (c 0.1, MeOH); ESI: *m/z* 1049 $[M+H]^+$; HRESI–MS: *m/z* 1047.5005 $[M-H]^-$ (Calcd. for 1047.5012, $H_{50}H_{79}O_{23}$); IR (KBr) ν_{max} (cm^{-1}): 3425, 2971, 2930, 2906, 1632, 1551, 1453, 1378, 1156, 1041, 985, 910, 875, 835; ^1H NMR data see Table 1; ^{13}C NMR data see Table 2.

*2.3.7. Acid hydrolysis of compounds **1**–**6** and GC analysis*

Compounds **1**–**6** (2 mg) were refluxed with 2 M HCl (1, 4 dioxane/H_2O 1:1, 2 ml) on water bath for 2 h. After cooling, the reaction mixture was extracted with $CHCl_3$ (3 × 5 ml). The aqueous layer was evaporated to dryness with MeOH until neutral. The dried residue was dissolved in 1 ml anhydrous pyridine and treated with L-cysteine methyl ester hydrochloride (1.5 mg) stirred at 60 °C for 1 h. Trimethylsilylimidazole (1.0 ml) was added to the reaction mixtures, and they were kept at 60 °C for 30 min. The supernatants (4 μl) were analyzed by GC, respectively, under the following conditions: H_2 flame ionization detector. Column: 30QC2/AC-5 quartz capillary column (30 m × 0.32 mm). Column temperature: 180–280 °C with the rate of 3 °C/min, and the carrier gas was N_2 (1 ml/min); injector temperature: 250 °C; split ratio: 1/50. The configurations of D-glucose, L-rhamnose, and D-xylose for compounds **1**–**6** were determined by comparison of the retentions times of the corresponding derivatives with those of standard D-glucose, L-rhamnose, and D-xylose giving a single peak at 19.01, 15.43, and 18.34 min, respectively.

2.4. Antimicrobial assays

Propionibacterium acnes (NCTC 737) (Department of Dermatology, The Fourth Military Medical University) was incubated in brain heart infusion medium (BHI) with 1% glucose (Guangdong Huankai Microbial Sci & Tech. Co., Ltd.) at 37 °C for 72 h under anaerobic conditions and adjusted concentration by direct microscopic counts before the assay. The MIC (minimal inhibitory concentration) test was performed by the broth microdilution method [20]. The compounds were dissolved in DMSO and then added to bacteria suspension to obtain the final concentration of 5% (v/v) DMSO. Serial twofold dilutions from 1.000% – 0.008% (v/v) of the compounds were prepared and placed into a 96-well micro-titer plate. One hundred microliter of sample of each concentration were dispensed into the wells of a micro-titer plate. Each well was then inoculated with 100 μL of the bacterial suspension. The final concentration of the suspension was adjusted to 10^5 CFU/mL, and the plate was incubated under anaerobic condition at 37 °C for 24 h. After incubation, the wells were examined for growth of microorganisms and the MIC was determined. The MIC

is defined as the lowest concentration of the compounds at which the bacterium does not demonstrate visible growth. Each experiment was repeated three times. Erythromycin was used as a positive control. MIC was defined as the lowest concentration that inhibited visible growth and the MIC > 100 mg/mL was considered to be inactive.

3. Results and discussion

The EtOH extract of the stems and leaves of *P. polyphylla* var. *yunnanensis* was chromatographed repeatedly on D101 resin, silica gel, Rp-18, Sephadex LH-20, and semi-preparative HPLC column chromatography to yield six new spirostanol saponins, chonglouo-

Table 1
^{1}H NMR Data of Compounds **1**–**6** in C_5D_5N (δ in ppm, J in Hz, C_5D_5N).

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]	**6**[a]
1	1.62 m,	1.68 m,	1.69 m,	170 m,	1.71 m,	3.86 m
	0.91 m	0.94 m	0.95 m	0.96 m	0.95 m	
2	2.02 m,	2.05 m,	2.05 m,	2.05 m,	2.07 m,	2.68 m,
	1.69 m	1.68 m	1.84 m	1.84 m	1.84 m	2.45 m
3	3.84 m	3.86 m	3.85 m	3.86 m	3.90 m	3.79 m
4	2.77 m,	2.68 m,	2.75 m,	2.77 m,	2.94 m,	2.70 m,
	2.48 m	2.40 m	2.68 m	2.69 m	2.78 m	2.57 m
6	5.80 d (4.0)	5.28 d (4.3)	5.24 d (4.0)	5.28 d, (4.3)	5.75 br s	5.57 d (4.6)
7	3.98 m	2.01 m,	1.77 m,	1.83 m,		1.87 m,
		1.52 m	1.46 m	1.47 m		1.50 m
8	1.63 m	1.48 m	1.49 m	1.48 m	2.37 t (11.5)	1.57 m
9	1.57 m	0.87 m	0.87 m	0.87 m	1.37 m	1.65 m
11	1.47 m (2H)	1.39 m (2H)	1.39 m (2H)	1.41 m (2H)	1.43 m (2H)	2.89 m,
						1.64 m
12	1.72 m,	1.73 m,	1.70 m,	1.70 m,	1.62 m,	1.71 m,
	1.20 m	1.12 m	1.10 m	1.10 m	1.01 m	1.49 m
14	2.05 m	1.07 m	1.06 m	1.07 m	1.49 m	1.20 m
15	2.54 m,	2.01 m,	1.97 m,	2.03 m,	3.29 m,	2.00 m,
	1.58 m	1.81 m	1.49 m	1.49 m	1.75 m	1.44 m
16	4.62 m	4.65 m	4.62 m	4.60 m	4.64 m	4.40 m
17	1.88 m	1.89 m	1.90 t (7.8)	1.87 m	1.75 m	1.79 m
18	0.90 s	1.01 s	1.13 s	0.99 s	0.83 s	0.91 s
19	0.89 s	0.84 s	0.99 s	0.98 s	1.10 s	1.43 s
20	1.97 m	3.05 m	3.29 m	3.01 m	1.94 m	1.94 m
21	1.13 d (6.9)	1.21 d (7.0)	1.26 d (6.9)	1.15 d (6.8)	1.13 d (6.7)	1.08 d (6.7)
23	1.66 m,	3.95 m	4.19 m	3.85 m	1.65 m (2H)	1.64 m (2H)
	1.55 m					
24	1.53 m (2H)	2.30 m,	2.62 m,	2.17 m,	1.54 m (2H)	1.65 m (2H)
		2.03 m	2.16 m	1.90 m		
25	1.54 m	2.29 m	2.33 m	2.30 m	1.55 m	2.04 m
26	3.53 m,	4.10 m,	4.09 m,	4.02 m,	3.55 m,	4.04 m,
	3.45 m	3.90 m	3.90 m	3.71 t (11.1)	3.47 m	3.71 m
27	0.65 d (5.1)	3.75 m,	3.64 m,	3.95 m,	0.66 d (4.7)	3.91 m,
		3.71 m	3.60 m	3.47 t (8.9)		3.46 dd (12.0, 10.1)
	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	1-*O*-Glc
1′	5.00 d (7.7)	4.94 d (7.5)	4.93 d (7.6)	4.94 d (7.6)	4.93 d (7.4)	4.81 d (7.5)
2′	4.05 t (8.1)	3.99 m	4.20 m	4.21 m	4.16 m	4.18 m
3′	4.31 m	4.23 m	4.20 m	4.21 m	4.24 m	4.07 m
4′	4.28 m	4.45 t (9.3)	3.97 m	4.39 m	4.44 m	3.86 m
5′	4.00 m	3.72 m	3.63 m	3.63 m	3.64 m	3.81 m
6′	4.59 m,	4.25 m,	4.20 m,	4.20 m,	4.22 m,	4.50 m,
	4.42 m	4.14 m	4.09 m	4.08 m	4.06 m	4.21 m
		4′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha
1″		5.89 br s	6.40 br s	6.40 br s	6.42 br s	6.45 br s
2″		4.70 m	4.83 (m	4.83 m	4.88 m	4.79 m
3″		4.57 dd (9.2, 3.2)	4.62 (m)	4.63 m	4.62 m	4.60 m
4″		4.34 t (9.4)	4.33 (m)	4.33 m	4.38 m	4.33 m
5″		5.01 m	4.95 (m)	4.97 m	4.92 m	4.85 m
6″		1.71 d (6.1)	1.75 d (6.1)	1.75 d (6.2)	1.59 d (5.7)	1.77 d (5.8)
			4′-*O*-Rha	4′-*O*-Rha	4′-*O*-Rha	3′-*O*-Xyl
1‴			5.86 br s	5.86 br s	5.84 br s	4.95 d (7.5)
2‴			4.68 m	4.68 m	4.53 m	3.98 m
3‴			4.54 m	4.54 m	4.57 m	4.10 m
4‴			4.35 m	4.35 m	4.48 m	4.11 m
5‴			4.92 m	4.92 m	5.00 m	4.26 m, 3.71 m
6‴			1.62 d (6.1)	1.62 d (6.2)	1.59 d (5.7)	
			23-*O*-Glc	27-*O*-Glc	4″-*O*-Rha	27-*O*-Glc
1⁗			5.02 d (7.7)	4.78 d (7.7)	6.29 br s	4.78 d (7.5)
2⁗			4.01 m	4.02 m	4.94 m	4.03 m
3⁗			4.23 m	4.23 m	4.53 m	4.25 m
4⁗			4.23 m	4.24 m	4.33 m	4.23 m
5⁗			3.97 m	3.95 m	4.38 m	3.97 m
6⁗			4.48 m,	4.56 m,	1.75 d (5.9)	4.58 m,
			4.37 m	4.39 m		4.40 m

[a] Measured at 100 MHz.
[b] Measured at 125 MHz. s, singlet; d, doublet; t, triplet; br, broad; m, multiplet.

Table 2
^{13}C NMR data of Compounds **1–6** (δ in ppm, C_5D_5N).

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]	**6**[a]
1	37.2 (t)	37.5 (t)	37.5 (t)	37.5 (t)	36.5 (t)	84.5 (d)
2	30.1 (t)	30.3 (t)	30.2 (t)	30.1 (t)	29.8 (t)	37.9 (t)
3	77.9 (d)	78.4 (d)	78.2 (d)	78.1 (d)	77.1 (d)	68.2 (d)
4	39.3 (t)	39.4 (t)	39.0 (t)	39.0 (t)	39.0 (t)	43.8 (t)
5	144.0 (s)	141.1 (s)	140.8 (s)	140.7 (s)	165.3 (s)	139.4 (s)
6	126.0 (d)	121.8 (d)	121.9 (d)	121.8 (d)	126.3 (d)	124.9 (d)
7	64.6 (d)	32.2 (t)	32.4 (t)	32.3 (t)	201.0 (s)	31.9 (t)
8	37.9 (d)	31.7 (d)	31.6 (d)	31.6 (d)	45.1 (d)	33.1 (d)
9	42.6 (d)	50.4 (d)	50.3 (d)	50.3 (d)	50.0 (d)	50.4 (d)
10	38.0 (s)	37.2 (s)	37.2 (s)	37.1 (s)	38.8 (s)	42.8 (s)
11	21.0 (t)	21.2 (t)	21.1 (t)	21.1 (t)	21.2 (t)	24.3 (t)
12	39.8 (t)	40.3 (t)	40.2 (t)	40.2 (t)	38.8 (t)	40.4 (t)
13	40.3 (s)	41.1 (s)	41.1 (s)	41.0 (s)	41.3 (s)	40.3 (s)
14	49.8 (d)	56.8 (d)	56.7 (d)	56.6 (d)	50.1 (d)	57.1 (d)
15	32.4 (t)	33.5 (t)	32.1 (t)	32.2 (t)	34.4 (t)	32.4 (t)
16	81.4 (d)	81.8 (d)	81.5 (d)	81.7 (d)	81.4 (d)	81.2 (d)
17	63.1 (d)	62.6 (d)	62.3 (d)	62.4 (d)	62.0 (d)	63.0 (d)
18	16.4 (q)	16.7 (q)	17.0 (q)	16.6 (q)	16.5 (q)	16.9 (q)
19	18.3 (q)	19.4 (q)	19.3 (q)	19.4 (q)	17.1 (q)	15.2 (q)
20	42.1 (d)	36.0 (d)	35.8 (d)	35.8 (d)	42.0 (d)	42.1 (d)
21	15.1 (q)	14.8 (q)	14.8 (q)	14.7 (q)	15.1 (q)	15.0 (q)
22	109.3 (s)	112.2 (s)	111.1 (s)	112.1 (s)	109.3 (s)	109.6 (s)
23	31.9 (t)	67.7 (d)	76.8 (d)	67.3 (d)	31.9 (t)	31.3 (t)
24	29.3 (t)	32.4 (t)	32.1 (t)	33.4 (t)	29.3 (t)	24.0 (t)
25	30.6 (d)	40.6 (d)	40.3 (d)	38.0 (d)	30.6 (d)	36.8 (d)
26	66.8 (t)	63.3 (t)	63.2 (t)	62.9 (t)	66.9 (t)	63.7 (t)
27	17.3 (q)$_3$	64.1 (t)	64.0 (t)	71.6 (t)	17.3 (q)	72.1 (t)
	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	3-*O*-Glc	1-*O*-Glc
1′	102.7 (d)	102.5 (d)	100.3 (d)	100.3 (d)	100.7 (d)	100.2 (d)
2′	75.4 (d)	75.6 (d)	78.4 (d)	78.5 (d)	77.7 (d)	76.3 (d)
3′	78.6 (d)	76.8 (d)	77.8 (d)	77.8 (d)	77.4 (d)	88.6 (d)
4′	71.8 (d)	78.6 (d)	78.0 (d)	78.0 (d)	77.5 (d)	70.2 (d)
5′	78.6 (d)	77.2 (d)	77.0 (d)	77.0 (d)	77.0 (d)	77.8 (d)
6′	63.0 (t)	61.7 (t)	61.4 (t)	61.3 (t)	61.4 (t)	63.6 (t)
		4′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha	2′-*O*-Rha
1″		102.8 (d)	102.0 (d)	102.1 (d)	102.0 (d)	101.8 (d)
2″		72.7 (d)	72.6 (d)	72.6 (d)	72.5 (d)	72.5 (d)
3″		72.9 (d)	72.8 (d)	72.8 (d)	72.8 (d)	72.5 (d)
4″		74.1 (d)	74.0 (d)	74.0 (d)	74.2 (d)	74.3 (d)
5″		70.4 (d)	69.5 (d)	69.6 (d)	69.5 (d)	69.6 (d)
6″		18.6 (q)	18.7 (q)	18.7 (q)	18.6 (q)	19.4 (q)
			4′-*O*-Rha	4′-*O*-Rha	4′-*O*-Rha	3′-*O*-Xyl
1‴			103.0 (d)	102.9 (d)	102.3 (d)	105.3 (d)
2‴			72.6 (d)	72.6 (d)	72.9 (d)	74.8 (d)
3‴			72.8 (d)	72.9 (d)	73.2 (d)	78.5 (d)
4‴			74.2 (d)	74.1 (d)	80.4 (d)	70.7 (d)
5‴			70.5 (d)	70.4 (d)	68.5 (d)	67.3 (t)
6‴			18.5 (q)	18.6 (q)	18.9 (q)	
			23-*O*-Glc	27-*O*-Glc	4″-*O*-Rha	27-*O*-Glc
1⁗			106.3 (d)	105.1 (d)	103.3 (d)	105.1 (d)
2⁗			75.4 (d)	75.2 (d)	72.6 (d)	75.3 (d)
3⁗			78.8 (d)	78.6 (d)	72.9 (d)	78.6 (d)
4⁗			71.7 (d)	71.6 (d)	74.1 (d)	71.8 (d)
5⁗			79.0 (d)	78.7 (d)	70.4 (d)	78.6 (d)
5⁗			62.8 (t)	62.8 (t)	18.4 (q)	62.9 (t)

[a] Measured at 100 MHz.
[b] Measured at 125 MHz.

sides SL-1–SL-6 (**1–6**), and 19 known compounds. The known compounds were identified as diosgenin (**7**) [1], polyphyllin A (**8**) [2], polyphyllin V (**9**) [2], sansevierin A (**10**) [21], progenin II (**11**) [22], diososeptemloside D (**12**) [23], dioscin (**13**) [2], diososeptemloside E (**14**) [23], paris saponin II (**15**) [2], polyphyllin VI (**16**) [3], pennogenin 3-*O*-α-L-rhamnopyranosyl(1 → 4)-α-L-rhamnopyranosyl(1 → 4)-β-D-glucopyranoside (**17**) [24], paris saponin VII (**18**) [3], isonuatigenin 3-*O*-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranoside (**19**) [25], diososeptemloside H (**20**) [23], hypoglaucin H (**21**) [16], 21-methoxyl pregna-5,16-dien-3β-ol-20-one 3-*O*-α-L-rhamnopyranosyl(1→2)-[α-L-rhamnopyranosyl(1→4)]-β-D-glucopyranoside (**22**) [26], protodioscin (**23**) [27], methylprotodioscin (**24**) [28], and 26-*O*-β-D-glucopyranosyl-3β,20α,26-triol-(25*R*)-5,22-dienofurostan 3-*O*-α-L-rhamnopyranosyl (1→2)-[α-L-rhamnopyranosyl(1 → 4)]-β-D-glucopyranoside (**25**) [29] on the basis of comparison of their MS and NMR spectra data with those reported in the literature. It is worthwhile to point out that compounds **10**, **12**, **14**, **19**, **20**, **22**, and **25** were isolated from the genus *Paris* for the first time.

Compound **1** was obtained as white amorphous powder. Its molecular formula $C_{33}H_{52}O_9$ was determined by its HRESI–MS at *m*/*z* 615.4394 $[M+Na]^+$ (Calcd. for 615.3509) and ^{13}C NMR data (Table 2), which indicates eight degrees of unsaturation. The ^{1}H NMR spectrum of **1** (Table 1) showed four methyl proton signals of a typical steroidal skeleton at δ_H 0.65 (3H, d, J = 5.1 Hz, Me-27), 0.89 (3H, s, Me-19), 0.90 (3H, s, Me-18), and 1.13 (3H, d, J = 6.9 Hz, Me-21), a hydroxymethyl group at δ_H 3.45 and 3.53 (1H each, m), and an olefinic proton at δ_H 5.80 (1H, d, J = 4.0 Hz), as well as an anomeric proton at δ_H 5.00 (1H, d, J = 7.7 Hz). The above ^{1}H NMR data, together with olefinic carbons signals at δ_C 144.0 (s, C-5) and 126.0 (d, C-6) and an acetalic carbon signal at δ_C 109.3 (s, C-22) in the ^{13}CNMR spectrum, suggested **1** to be a Δ^5-spirostanol skeleton in the aglycone. Further, the NMR spectroscopic data attributed to the aglycone of **1** were in good agreement with those of dioseptemloside D (**12**) [23]. Hence, the aglycone of **1** was identified as spirost-5-en-3β,7α-diol, as confirmed by the ^{1}H–^{1}H COSY, HMBC and ROESY correlations. The hydroxyl group was fixed at C-7 on the ground of ^{1}H–^{1}H correlation from the olefinic proton signal at δ_H 5.80 (H-6) to the oxymethine proton signal at δ_H 3.98 (H-7) and the HMBC correlations of H-6 (δ_H 5.80) and H-9 (δ_H 1.57) with C-7 (δ_H 64.6). The α-orientation of 7-OH was deduced from the resonance of C-7 (δ_C 64.6, d) [30] which could be further confirmed by ROESY correlations of H-7 with H-8, Hax-15, and Heq-15. The *R*-configuration of C-25 was deduced from the intensity of absorptions (899 > 921 cm^{-1}) in its IR spectrum [31] and the chemical shift of Me-27 at 0.65 ppm [32]. Acid hydrolysis of **1** yielded D-glucose as sugar residue, which was determined by GC analysis of their corresponding trimethylsilated L-cysteine adducts. The coupling constant ($^3J_{1,2}$ > 7.0 Hz) of the anomeric proton suggested that the glucopyranose was β-orientation. The HMBC correlation between δ_H 5.00 (H-1′) and δ_C 77.9 (C-3) revealed that the β-D-glucosyl unit was located at C-3 of the aglycone. Therefore, the structure of compound **1** was identified as (25*R*)-spirost-5-en-3β,7α-diol-3-*O*-β-D-glucopyranoside and named chonglouoside SL-1.

Compound **2** was isolated as white amorphous powder with a molecular formula of $C_{39}H_{62}O_{14}$ determined by positive-ion HRESI–MS (*m*/*z* 777.4032 $[M+Na]^+$, Calcd. for 777.4037) and ^{13}C NMR data (Table 2). Acid hydrolysis of **2** gave D-glucose and L-rhamnose as sugar residues, which were determined by GC analysis of their corresponding trimethylsilated L-cysteine adducts. The ^{1}H NMR and ^{13}C data (Tables 1 and 2) displayed three steroid methyl signals at δ_H 0.84 (3H, s, Me-19), 1.01 (3H, s, Me-18), and 1.21 (3H, d, J = 7.0 Hz, Me-21), two oxymethylene [δ_H 3.90, 4.10 (1H each, both m, H_2-26); δ_H 3.71, 3.75 (1H each, both m, H_2-27)], three oxymethines [δ_H 3.86 (m, H-3); δ_H 3.98 (1H, m, H-23); δ_H 4.65 (1H, m, H-16)], a trisubstituted olefin [δ_H 5.28 (1H, d, J = 4.5 Hz, H-6); δ_C 141.1 (s, C-5) and 121.8 (d, C-6)], an acetalic carbon signal at δ_C 112.2 (s, C-22) along with an anomeric proton for a glucose at δ_H 4.94 (d, J = 7.5 Hz, H-1′) and an anomeric proton for a rhamnose at δ_H 5.89 (br s, H-1″). Comparison of the ^{1}H and ^{13}C NMR spectra of **2** with those of borassoside B [33] showed their considerable structural similarity. The differences consisted only in the signals of one less rhamnopyranosyl unit disappearance in **2**. The β-configuration of glucopranosyl was determined on the coupling constant ($^3J_{1,2}$ > 7.0 Hz) of the anomeric proton, while the anomeric configuration of rhamnopyranosyl was defined as α-orientated on the basis of the chemical shift values of C-3″ (δ_C 72.9) and C-5″ (δ_C 70.4) with those of the corresponding carbons of methyl α- and

Table 3
Antimicrobial activities of compounds **2**, **3**, **6**, **8**–**11**, **13**–**15**, **17**–**21**, **23**, and **24**.

Compound	MIC (μg/ml)	Compound	MIC (μg/ml)
2	62.5	15	>100
3	62.5	17	17.2
6	3.9	18	31.3
8	16.5	19	>100
9	17.2	20	>100
10	>100	21	62.5
11	7.8	23	>100
13	39.0	24	31.3
14	>100	Erythromycin	0.0625

β-rhamnopyranoside [34]. The HMBC spectrum showed the long-range correlations from δ_H 4.94 (H-1′) to δ_C 78.4 (C-3) of the aglcone, and δ_H 5.89 (H-1″) to δ_C 78.6 (C-4′). The above data allowed the structural assignment of **2** as (23*S*,25*R*)-spirost-5-en-3β,23α,27-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranoside and named chonglouoside SL-2.

Compound **3** was also obtained as white amorphous powder, and its molecular formula deduced to be $C_{51}H_{82}O_{23}$ on the basis of the positive-ion HRESI–MS at *m*/*z* 1085.5147 $[M+Na]^+$ (Calcd. for 1051.5144) and ^{13}C NMR data (Table 2). The ^{13}C NMR spectroscopic data of **3** displayed the presence of an additional glucose (δ_C 106.3, 75.4, 78.8, 71.7, 79.0, and 62.8) and the shift downfield for C-23 (δ_C 67.6 → 76.8 ppm) when compared to those of borassoside B [33]. This suggested that the additional glucose unit was attached at C-23 of the aglycone. The HMBC correlation peak between the signals at δ_H 5.02 (H-1‴ of the additional glucose) and δ_C 76.8 (C-23 of the aglycone) further proved this assumptiom. In conclusion, the structure of **3** was elucidated as 23-*O*-β-D-glucopyranosyl-(23*S*,25*R*)-spirost-5-en-3β,23α,27-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucopyranoside and named chonglouoside SL-3.

Compound **4** had the same molecular formula as that of **3** on the basis of the negative-ion HRESI–MS at *m*/*z* 1061.5188 $[M-H]^-$ (Calcd. for 1061.5168) and ^{13}C NMR data (Table 2). The NMR data revealed that **4** should be a spirostanol glycoside similar to **3**. The major difference were the upfield shift of C-23 (δ_C 67.3) and the downfield shift of C-27 (δ_C 71.6) compared to **3** ($\Delta\delta$ −9.5 and +7.6, respectively). Therefore, it was supposed that the glucopyranosyl unit attached to C-23 in compound **3** was moved to C-27 in compound **4**, which was confirmed by the HMBC correlation of δ_H 4.78 (H-1:⁗) with δ_C 71.6 (C-27). Based on all the information above, the structure of **4** was established as 27-*O*-β-D-glucopyranosyl-(23*S*,25*R*)-spirost-5-en-3β,23α,27-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 4)]-β-D-glucoyranoside and named chonglouoside LS-4. It is noted that chonglouoside LS-3 (**3**) and chonglouoside SL-4 (**4**) were the first 23α,27-dihydroxydiosgenin saponin having a sugar chain attached to C-23 or C-27.

Compound **5** had a molecular formula of $C_{51}H_{80}O_{21}$ based on the positive-ion HRESI–MS at *m*/*z* 1051.5078 $[M+Na]^+$ (Calcd. for 1051.5089) and ^{13}C NMR data (Table 2). The two tertiary methyl proton groups at δ_H 0.83, 1.10 (3H each, s, Me-18 and 19), two secondary methyl protons at δ_H 0.66 (3H, d, *J* = 4.7 Hz, Me-27) and 1.13 (3H, d, *J* = 6.7 Hz, Me-21), and a trisubstituted olefinic proton at δ_H 5.75 (1H, s) (Table 1) were observed in the ^1H NMR spectrum coupled with information from the ^{13}C NMR spectrum (two angular methyl groups at δ_C 16.5 and 17.1, two secondary methyl groups at δ_C 17.3 and 15.1, and a trisubstituted double bond at δ_C 165.3 and 126.3, as well as one carbonyl signal at δ_C 201.0 (Table 2). The absorption bands in the IR spectrum at 1700 and 1658 cm^{-1}, and the UV maximum at 238 nm indicated the presence of an α, β-unsaturated ketone moiety in **5**. The above information suggested that the aglycone of **5** possessed a 7-oxospirost-5-en skeleton. After a comparison of the ^{13}C NMR signals of the aglycone moiety of **5** with those of kingianoside K [35] and an extensive HMQC and HMBC data analysis, the aglycone of **5** was identified as (25*R*)-7-oxospirost-5-en-3β-ol. Of the 51 carbon signals observed in the ^{13}C NMR spectrum of **5**, 27 were assigned to the aglycone part and the remaining 24 to the oligosaccharide moiety. Acid hydrolysis of **5** gave D-glucose and L-rhamnose by GC analysis compared with the authentic standards. Additionally, four anomeric proton signals at δ_H 4.93 (1H, d, *J* = 7.4 Hz), 5.84 (1H, br s), 6.29 (1H, br s), and 6.42 (1H, br s) and three methyl proton signals due to the rhamnosyl residues at δ_H 1.59 (3H, d, *J* = 5.7 Hz), 1.59 (3H, d, *J* = 5.7 Hz), and 1.75 (3H, d, *J* = 5.9 Hz) were observed in the ^1H NMR spectrum (Table 1) of **5**. Correspondingly, four anomeric-carbon signals at δ_C 100.7, 102.3, 103.3, and 102.0 were observed in the ^{13}C NMR spectrum. The sequence of the tetrasaccharide, which was the same as the known compounds **15** and **18**, was established from the further HMBC correlations: δ_H 4.93 (H-1′) with δ_C 77.1 (C-3), δ_H 6.42 (H-1″) with δ_C 77.7 (C-2′), δ_H 5.84 (H-1‴) with δ_C 77.5 (C-4′), and δ_H 6.29 (H-1⁗) with δ_C 80.4 (C-4‴). Thus, the structure of compound **5** was elucidated as (25*R*)-7-oxospirost-5-en-3β-ol-3-*O*-α-L-rhamnopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 4) [α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside and named chonglouoside SL-5.

Compound **6** gave a pseudo-molecular ion peak $[M-H]^-$ at *m*/*z* 1047.5005 (Calcd. for 1047.5012) in its negative-ion HRESI-MS. Combined with ^{13}C NMR data (Table 2), its molecular formula was defined as $C_{50}H_{80}O_{23}$. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **6** exhibited the presence of four sugar residues, clearly indicated by four anomeric carbon signals at δ_C 100.2, 101.8, 105.1, and 105.3, and four anomeric proton signals at δ_H 4.81 (1H, d, *J* = 7.5 Hz), 6.45 (1H, br s), 4.78 (1H, d, *J* = 7.5 Hz), and 4.95 (1H, d, *J* = 7.5 Hz). In addition, there were two tertiary methyl proton groups at δ_H 0.91, 1.43 (each 3H, s) and a secondary methyl proton at δ_H 1.08 (3H, d, *J* = 6.7 Hz), and a trisubstituted olefinic proton at δ_H 5.57 (1H, d, *J* = 4.6 Hz), and a dioxygenated quaternary carbon signal at δ_C 109.6 (s, C-22), which suggested that **6** possessed a Δ^5-spirostanol skeleton as an aglycone. Two more oxygen substituted carbon signals at δ_C 84.5 and 72.1 were observed in the ^{13}C NMR spectrum of **6**. Comparison of the NMR signals due to aglycone moiety with reported data suggested that the aglycone of **6** should be 27-hydroxyruscogenin [(25*R*)-spirost-5-en-1β,3β,27-triol] [36], and confirmed by 2D NMR experiments. The stereochemistry of the aglycone of **6** could confirmed by the ROESY experiments. The ROESY correlations of H-1(δ_H 3.86)/H-9 (δ_H 1.65), H-1 (δ_H 3.86)/H-3 (δ_H 3.79), H-14 (δ_H 1.20)/H-16 (δ_H 4.40), and H-14 (δ_H 1.20)/H-17 (δ_H 1.79) indicated that the hydroxyl groups at C-1 and C-3 were β-oriented and H-16 and H-17 were α-oriented. The identification of the sugar moieties was determined by acid hydrolysis of **6** and GC analysis with an authentic sample, which included one L-rhamnose, one D-xylose, and two D-glucoses. The ^1H NMR coupling constants ($^3J_{1,2}$ > 7.0 Hz) were consistent with β-configurations for xylose and glucose, while the α-configuration for the rhamnose was deduced by comparing the ^{13}C NMR spectroscopic data for C-3″ and C-5″ with those of those of the corresponding carbons of methyl α- and β-rhamnopyranoside. The linkage sites and sequences were determined by analyzing the 2D NMR spectrum of **6**. The HMBC spectrum showed the correlations of δ_H 4.81 (H-1′) with δ_C 84.5 (C-1) of the aglycone, δ_H 6.45 (H-1″) with δ_C 76.3 (C-2′), and δ_H 4.95 (H-1‴) with δ_C 88.6 (C-3″). As for another glycoside moiety, the correlations of the anomeric proton of δ_H 4.78 (H-1⁗) of glucose with δ_C 72.1 (C-27) of the aglycone was observed. From the above evidence, the structure of **6** could be formulated as 27-*O*-β-D-glucopyranosyl-(25*R*)-spirost-5-en-1β,3β,27-triol-1-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranoside and named chonglouoside SL-6. Although 27-hydroxyruscogenin [(25*R*)-spirost-5-en-1β,3β,27-

triol] was reported in 1975 [36], this is the first report for a 27-hydroxyruscogenin glycoside bearing 1, 27-di-*O*-sugar chains.

The saponins (**2**, **3**, **6**, **8**–**11**, **13**–**15**, **17**–**21**, **23**, and **24**) were evaluated for their antimicrobial activities against *Propionibacterium acnes*, in which erythromycin was used as a positive control. The results are shown in Table 3. Compounds **2**, **3**, **6**, **8**, **9**, **11**, **13**, **17**, **18**, **21**, and **24** exhibited inhibitory activities with MIC values in the range 3.9–62.5 μg/ml. Among these compounds, the new saponin chonglouoside SL-6 (**6**) containing a trisaccharide sugar moiety at C-1 showed the best activity with a MIC value of 3.9 μg/ml. Comparing the MIC values of polyphyllin V (**9**) with its hydroxylted derivatives (**10** and **19**), it was observed that the hydroxyl group at C-7 in **10** or the hydroxyl group at C-25 in **19** made the antimicrobial activities against *P. acnes* disappeared. The same phenomena were observed in dioscin (**13**) and its C-7 hydroxylted derivative (disoseptemloside E (**14**), as well as progenin II (**11**) and its C-25 hydroxylted derivative (disoseptemloside H, **20**). Furthermore, this assay demonstrated that introducing two hydroxyl groups at C-23 and C-27 of progenin II (**11**) or dioscin (**13**) considerable decreased its antimicrobial activity against *P. acnes* by comparison MIC values of chonglouoside SL-2 (**2**) and progenin II (**11**) or chonglouoside SL-3 (**3**) and dioscin (**13**).

This study demonstrated that the stems and leaves of *P. polyphylla* var. *yunnanensis* were rich in steroidal saponins and some of the isolates showed significant antimicrobial activity against *P. acnes*. Spirostanol saponins **8**, **9**, **16**, **13**, **15**, **18**, and **21**, which were isolated from the stems and leaves in the current study, have been reported in the rhizome of *P. polyphylla* var. *yunnanensis* [2–8]. The stems and leaves of *P. polyphylla* var. *yunnanensis* replacing the rhizomes still need further study.

Acknowledgement

We gratefully acknowledge financial support for this project from National Natural Science Funding of China (No. 31170333), the Young Academic and Technical Leader Raising Foundation of Yunnan Province (No. 2008PY066), and Scientific and Technological Projects of Yunnan Province (No. 2009AD013).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2012.07.007.

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