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C_{22}-steroidal lactone glycosides from stems and leaves of *Paris polyphylla* var. *yunnanensis*

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ABSTRACT

Further phytochemical investigation on the stems and leaves of *Paris polyphylla* var. *yunnanensis* has led to the isolation of three C_{22}-steroidal lactone glycosides. Two of these are new compounds, designated as chonglouoside SL-7 (**1**) and chonglouoside SL-8 (**2**). Their structures were elucidated on the basis of extensive spectroscopic analysis, as well as comparison with the reported spectroscopic data. This is the first report of C_{22}-steroidal lactone glycosides isolated from the *Paris* genus. Compounds **1** and **3** showed moderate antimicrobic activity against *Propionibacterium acnes* with MIC values of 31.3 and 3.9 μg/mL, respectively.

1. Introduction

Paris polyphylla var. *yunnanensis* (Trililiaceae) is mainly distributed in southwestern China, especially in the Yunnan, Sichuan, and Guizhou Provinces [1]. Its rhizome is a Traditional Chinese Medicine (TCM) and used for the treatments of furuncle, abscess, sore throat, snake bite, injuries from falls, and convulsion [2]. It is also an important ingredient of some Chinese patent medicines, such as "Gongxuening Capsules", "Jidesheng Sheyao Tablet", "Biyan Qingdu Keli", etc. Steroidal saponins are believed to be the main active ingredients in this species and showed antitumor, platelet agonist, and contractile agonist for the uterus [3–8]. However, the resources of this herb have greatly declined and are in the edge of distinction because its rhizomes grow very slowly and have been excessively collected for many years [9]. In recent years, the farmers have been cultivating *P. polyphylla* var. *yunnanensis* on a large scale in Yunnan Province for solving the shortage of the resources. But the stems and leaves of this herb with a huge amount of annually renewable resources were discarded. In order to take full advantage of the waste resources, we have recently investigated chemical constituents of the stems and leaves of *P. polyphylla* var. *yunnanensis* and this has led to the isolation of one sapogenin and 24 steroidal saponins with antimicrobic activity [10]. Continuing examination of the extract has resulted in the characterization of three C_{22}-steroidal lactone glycosides (**1**–**3**) (Fig. 1). Among them, the first two are new and named chonglouoside SL-7 (**1**) and chonglouoside SL-8 (**2**), while the third compound was identified as dumoside by the comparison of the observed spectroscopic data with those in the literature [11]. To the best of our knowledge, this is the first report of C_{22}-steroidal lactone glycosides isolated from the *Paris* genus. This paper describes the isolation, structural elucidation, and antimicrobic activity of the three compounds.

2. Experiment part

2.1. General experimental procedures

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 recorded on a Bruker Tensor-27 infrared spectrophotometer with KBr pellets. NMR spectra were performed on Bruker AM-400 and Avance III 600 instruments with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESI-MS spectra were recorded on a Bruker HTC/Esquire spectrometer. HR-ESI-MS

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spectra were recorded on an API Qstar Pulsar instrument. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), D101 macroporous resin column (40–60 mesh, Tianjin Pesticide Co., China), Rp-18 (40–63 μm, Merk). Fractions were monitored by TLC (GF254, Qingdao Marine Chemical Ltd., Qingdao, China), and by heating silica gel plates sprayed with 10% H_2SO_4 in ethanol. 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.). GC analysis was performed on a HP5890 gas chromatograph equipped with an H_2 flame ionization detector.

2.2. Plant material

The stems and leaves of *P. polyphylla* var. *yunnanensis* were collected in September 2006 from Chengjiang County, Yunnan Province, China, and identified 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, Kunming Institute of Botany, Chinese Academy of Sciences.

2.3. Extraction and isolation

The air-dried aerial parts of *P. polyphylla* var. *yunnanensis* (16 kg) were crushed and extracted with 60 L of 75% for three times under reflux for a total of 6 h, and then combined extract was concentrated under reduced pressure to afford a residue. The residue was dissolved in H_2O and passed through a D-101 macroporous resin, eluting with H_2O, 80% EtOH and 95% EtOH, successively. The evaporated 80% EtOH fraction (1.24 kg) was chromatographed on silica gel column eluting with $CHCl_3$–MeOH (1:0 → 0:1) to get fractions 1–8. Fr. 4 (6.7 g) was subjected to column chromatography on Rp-18 gel (MPLC, MeOH–H_2O 4.5:5.5 → 4:1) to afford two subfractions (Fr. 4—1 and Fr. 4—2). Fr. 4—1 was further purified by semi-preparative HPLC (MeCN–H_2O 35:65 → 40:60 v/v; flow rate: 3 mL/min) to yield **2** (10 mg) and **3** (26 mg). Fr. 4–2 was further purified by semi-preparative HPLC (MeCN–H_2O 25:75 → 30:70 v/v; flow rate: 3 mL/min) to obtain **1** (35 mg).

Chonglouoside SL-7 (**1**): white amorphous powder; $[\alpha]_D^{23} = -150.8$ (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3441, 2936, 1765, 1632, 1452, 1384, 1203, 1095, 1067, 1037, 915, 839, and 810 cm^{-1}; negative ion ESI-MS *m/z* 813 $[M-H]^-$; HRESI-MS *m/z* 813.3931 $[M-H]^-$ (calcd for $C_{40}H_{61}O_{17}$, 813.3908); ^1H and ^{13}C NMR data see Table 1.

Chonglouoside SL-8 (**2**): white amorphous powder; $[\alpha]_D^{17} = -134.3$ (*c* 0.1, MeOH); IR (KBr) ν_{max}: 3425, 2934, 1757, 1633, 1453, 1382, 1285, 1136, 1040, 913, 814, and 608 cm^{-1}; positive ion ESI-MS *m/z* 819 $[M+Na]^+$; HRESI-MS *m/z* 795.3807 $[M-H]^-$ (calcd for $C_{40}H_{59}O_{16}$, 795.3803); ^1H and ^{13}C NMR see Table 1.

2.4. Acidic hydrolysis of **1** and **2**, and GC analysis

Compounds **1** and **2** (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; and split ratio: 1/50. Peaks of the hydrolysate were detected by comparison with retention times of authentic samples of D-glucose and L-rhamnose after treatment with trimethyl–chlorosilane (TMCS) in pyridine. The absolute configurations of the sugar residues were determined to be L-rhamnose (t_R 15.43 min) and D-glucose (t_R 19.01 min).

2.5. Antimicrobial assays

The antimicrobial assay was carried out as described in the literature [10]. Each experiment was repeated three times. Erythromycin was used as a positive control. MIC was defined

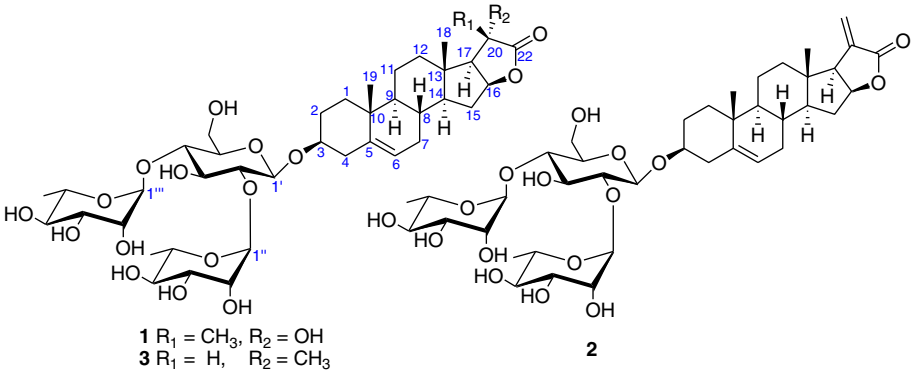

Fig. 1. Chemical structures of compounds **1**–**3**.

Table 1
^{1}H NMR and ^{13}C NMR data for compounds **1** and **2** in C_5D_5N[a].

Position	1[b]		2[c]	
	δ_H, mult, (J in Hz)	δ_C, type	δ_H, (J in Hz)	δ_C, type
1a	1.71, m	37.5, CH_2	1.72, m	38.0, CH_2
1b	0.96, m		0.95, m	
2a	2.05, m	30.2, CH_2	2.07, m	30.6, CH_2
2b	1.81, m		1.85, m	
3	3.88, m	78.0, CH	3.88, m	78.4, CH
4a	2.79, m	38.9, CH_2	2.83, m	39.4, CH_2
4b	2.73, m		2.74, m	
5		140.8, C		141.3, C
6	5.30, d (5.4)	121.6, CH	5.30, d (4.7)	122.0, CH
7a	1.81, m	31.9, CH_2	1.82, m	32.6, CH_2
7b	1.47, m		1.46, m	
8	1.45, m	31.1, CH	1.41, m	32.0, CH
9	0.87, m	50.0, CH	0.89, m	50.6, CH
10		37.0, C		37.5, C
11a	1.40, m	20.4, CH_2	1.43, m	20.4, CH_2
11b	1.32, m		1.32, m	
12a	1.84, m	38.7, CH_2	1.70, m	38.5, CH_2
12b	1.16, m		1.16, m	
13		40.3, C		44.3, CH_2
14	0.97, m	55.9, CH	0.98, m	55.1, CH
15a	2.10, m	32.3, CH_2	2.16, m	33.8, CH_2
15b	1.49, m		1.50, m	
16	5.33, m	82.7, CH	4.86, m	82.7, CH
17	2.33, d (6.3)	64.3, CH	2.76, m	55.6, CH
18	0.85, s	13.7, CH_3	0.61, s	14.7, CH_3
19	1.01, s	19.4, CH_3	1.01, s	19.8, CH_3
20		74.4, C		138.1, C
21	1.82, s	20.0, CH_3	6.38, br s	122.5, CH_2
			5.55, br s	
22		179.1, C		171.9, C
Glc-1′	4.96, d (7.4)	100.3, CH	4.98, d (7.3)	100.7, CH
2′	4.22, m	78.0, CH	4.44, m	78.4, CH
3′	4.23, m	77.8, CH	4.25, m	78.2, CH
4′	4.42, m	78.5, CH	4.26, m	78.8, CH
5′	3.65, m	77.0, CH	3.67, m	77.5, CH
6′a	4.22, m	61.3, CH_2	4.22, m	61.7, CH_2
6′b	4.10, m		4.10, m	
Rha-1″	6.43, br s	102.1, CH	6.47, br s	102.6, CH
2″	4.85, br s	72.6, CH	4.90, m	73.1, CH
3″	4.65, m	72.8, CH	4.68, dd (9.2, 3.0)	73.3, CH
4″	4.39, m	74.2, CH	4.41, m	74.6, CH
5″	4.97, m	69.6, CH	5.01, m	70.1, CH
6″	1.77, d (6.2)	18.7, CH_3	1.79, d (6.2)	19.2, CH_3
Rha-1‴	5.88, br s	102.9, CH	5.92, br s	103.4, CH
2‴	4.70, br s	72.6, CH	4.73, m	73.1, CH
3‴	4.56, m	72.9, CH	4.60, dd (9.2, 3.0)	73.3, CH
4‴	4.37, m	74.0, CH	4.39, m	74.2, CH
5‴	4.99, m	70.4, CH	4.99, m	70.9, CH
6‴	1.64, d (6.2)	18.6, CH_3	1.66, d (6.2)	19.1, CH_3

[a] Assignments based on 2D NMR spectra.
[b] Recorded at 400 MHz.
[c] Recorded at 600 MHz.

as the lowest concentration that inhibited visible growth and the MIC > 100 mg/mL was considered to be inactive.

3. Results and discussion

Compound **1** was isolated as a white amorphous powder. Its molecular formula was assigned as $C_{40}H_{62}O_{17}$ on the basis of HRESI-MS peak at m/z 813.3931 $[M-H]^-$ (calcd. for $C_{40}H_{61}O_{17}$, 813.3908), indicating 10° of unsaturation. Its IR spectrum exhibited the absorption of hydroxyl (3441 cm^{-1}) and a five-membered lactone group at 1765 cm^{-1}. The ^{1}H NMR spectrum (Table 1) displayed the following representative signals: three tertiary methyl groups at δ_H 0.85 (s, Me-18), 1.01 (s, Me-19), 1.82 (s, Me-21), and one olefinic proton at δ_H 5.30 (d, J = 5.4 Hz, H-6), together with signals of three anomeric protons at δ_H 4.96 (d, J = 7.4 Hz, H-1′), 6.43 (br s, H-1″), and 5.88 (br s, H-1‴). The ^{13}C NMR spectrum showed 40 carbon signals, 22 of which were assigned to the aglycone moiety including those corresponding to one carbonyl carbon at δ_C 179.1 (C-22), two olefinic carbons at δ_C 140.8 (C-5) and 121.6 (C-6), an oxymethine carbon at δ_C 82.7 (C-16), and an oxygenated quaternary carbon at δ_C 74.4 (C-20), while the remaining were due to a three hexose units. The NMR signals of the aglycone of **1** were consistent with those of (20*S*)-hydroxyvespertilin [(20*S*)-3β, 16β, 20-trihydroxy-pregn-5-en-20-carboxylic acid (22, 16)-lactone] [12,13], which was confirmed by the ^{1}H-^{1}H COSY, HMBC and ROESY correlations (Fig. 2). The ^{1}H- and ^{13}C-NMR data of the C-1-C-11 of the aglycone moiety of **1** were superimposable on those of **3** [11], indicating that the hydroxyl group at C-3 was β-orientation. The hydroxyl group that was linked to C-20 could be deduced from the HMBC correlations of H-17 with C-20, and H-21 with C-20. The α-orientations of H-16, H-17, and OH-20 were deduced from ROESY correlations of H-14/H-16, H-16/H-17, and Me-18/Me-21. The sugar units were consisted in D-glucose and L-rhamnose on the basis of the results of the acidic hydrolysate and GC analysis and comparison with authentic standards. The β-configuration of glucopranosyl was determined on the coupling constant ($J_{1,2}$ > 7.0 Hz) of the anomeric proton [14], while the anomeric configuration of rhamnopyranosyls was defined as α-orientated on the basis of the chemical shift values of C-3″ (δ_C 72.8), C-5″ (δ_C 69.6), C-3‴ (δ_C 72.9), and C-5‴ (δ_C 70.4) with those of the corresponding carbons of methyl α- and β-rhamnopyranoside [15]. The sequence of the trisaccharide, which was the same as dumoside (**3**), was established from the HMBC correlations: H-1′ (δ_H 4.96) of Glc with C-3 (δ_C 78.0) of the aglycone, H-1″ (δ_H 6.43) of 2′-Rha with C-2′ (δ_C 78.0) of Glc, and H-1‴ (δ_H 5.88) of 4′-Rha with C-4′ (δ_C 78.5) of Glc. On the basis of the above evidence, the structure of **1** was elucidated as (20*S*)-3β, 16β, 20-trihydroxy-pregn-5-en-20-carboxylic acid (22, 16)-lactone-3-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, and named chonglouoside SL-7.

Compound **2** had a molecular formula of $C_{40}H_{60}O_{16}$ based on HREI-MS (m/z 795.3807 $[M-H]^-$, calcd 795.3803) and ^{13}C NMR spectrum (Table 1), which lacks that of compound **1** by a water molecule. The IR spectrum displayed an α, β-unsaturated γ-lactone group at 1757 and 1633 cm^{-1} as well as hydroxyl absorption at 3425 cm^{-1}. Comparison of NMR spectra indicated that **2** differed from **1** by the presence of an exocyclic double bond [δ_H 6.38 (1H, br s), 5.55 (1H, br s); δ_C 138.1 (s) and 122.5 (t)] instead of an oxygenated quaternary carbon and a methyl group in the latter. The HMBC correlations of *exo*-methylene protons (δ_H 6.38 and 5.55) with C-17 (δ_C 55.6), C-20 (δ_C 138.1) and C-22 (δ_C 171.9) hinted the location of *exo*-methylene at C-20 (Fig. 1). Therefore, the structure of **2** was determined as 3β, 16β-dihydroxy-pregn-5, 20-dien-carboxylic acid (22, 16)-lactone-3-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranoside, and named chonglouoside SL-8.

Compounds **1**–**3** are rare C-22 steroidal saponins, which aglycones contain a 20-carboxylic acid (22, 16)-lactone group. To

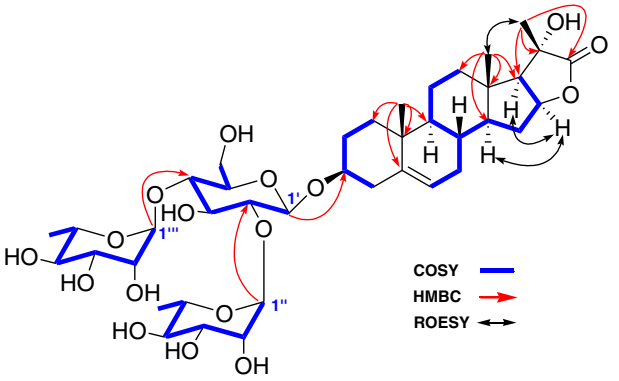

Fig. 2. Key 2D correlations of **1**.

the best of our knowledge, this is the first report of C_{22}-steroidal lactone glycosides isolated from the *Paris* genus. This type of compounds was previously only isolated from the *Asparagus dumosus* (Liliaceae) [11], *Solanum verspertulio* (Solanaceae) [12], *Solanum sodomaeum* (Solanaceae) [13], *Solanum hispidum* (Solanaceae) [16], *Dracaena cochinchinensis* (Agavaceae) [17], *Ypsilandra thibetica* (Liliaceae) [18], *Fritillaria pallidiflora* (Liliaceae) [19], and *Dioscorea spongiosa* (Dioscoreaceae) [20]. Compounds **1**–**3** were evaluated for their antimicrobic activity against *P. acnes*, in which erythromycin was used as a positive control (MIC value: 0.0625 μg/mL). The results revealed that **1** and **3** showed moderate inhibitory activity with the MIC values of 31.3 and 3.9 μg/mL, respectively.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.12.007.

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