HETEROCYCLES, Vol. 65, No. 5, 2005, pp. 1197 - 1203
Received, 17th January, 2005, Accepted, 7th March, 2005, Published online, 7th March, 2005

TWO NEW STEROID SAPONINS FROM *PARIS POLYPHYLLA*

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Abstract –Two new steroid saponins **(1-2)**, together with five known compounds **(3-7)**, were isolated from the underground part of *Paris polyphylla*. They also showed moderate cytotoxic activities against the liver cancer cell line of BEL-7402 *in vitro* as antineoplastic agents.

INTRODUCTION

'Chonglou' as a famous folk medicinal herb in the south of China has been used not only an anti-biotic and anti-inflammatory drug but also to treat injuries from fractures, parotitis, mastitis and snake bite as well as to stop bleeding.[1] Many steroidal saponins have been isolated from this genus[2] and *Dioscorea*,[3] some of which have antineoplastic activity.[4] And recently, one synthetic steroidal saponin, pamaqueside, was found to be a good inhibitor of cholesterol absorption.[5] In recent study, we have isolated seven steriodal saponins from the title plant, comprising two new compounds **(1** and **2)** and five known compounds. The structural elucidation of **(1)** and **(2)** was accomplished mainly on the basis of the interpretation of 2D NMR spectral data, including HMBC, HMQC, and chemical degradation. We also have tested the bioassay in order to find if these compounds have antineoplastic activity.

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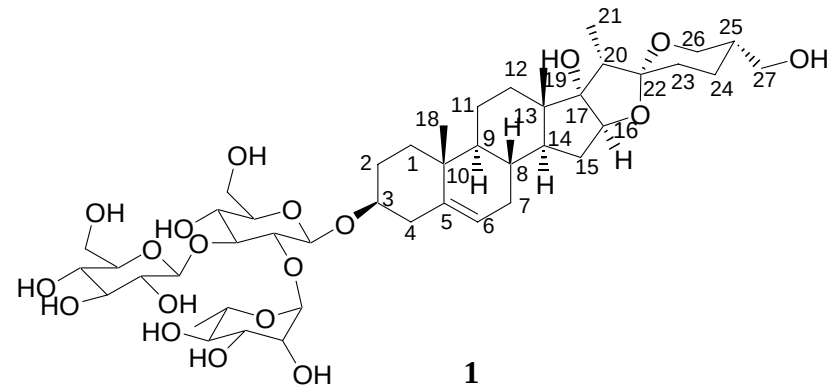

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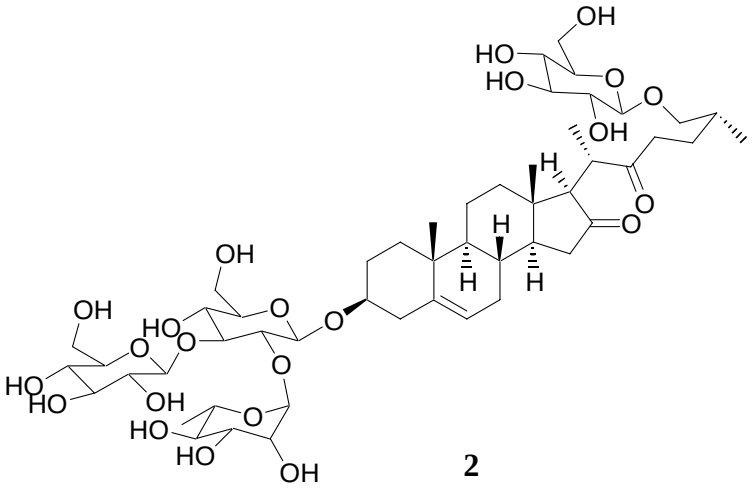

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Figure 1. Structures of compounds **(1)** and **(2)**

RESULTS AND DISCUSSION

The known compounds, 3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranosyl diosgenin **(3)**,[6] 3-*O*-[*α*-L-rhamnopyranosyl(1→2)]-[*α*-L-arabinofuranosyl(1→4)]-*β*-D-glucopyranosyl pennogenin **(4)**,[7] 3-*O*-[*α*-L-rhamnopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl pennogenin **(5)**,[8] 3-*O*-[*α*-L-rhamnopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→4)]-*β*-D-glucopyranosyl pennogenin **(6)**,[9] 3-*O*-[*α*-L-rhamnopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl 26-*O*-*β*-D-glucopyranosyl 22-methoxyl diosgenin **(7)**[4] were identified by comparison of their spectral data with those described in the literature.

Compound **(1)** was obtained as a white amorphous powder. The molecular formula was determined as $C_{45}H_{72}O_{19}$ from the positive HR-ESI-MS at *m*/*z* 917.4741 for the $[M+H]^+$ ion (calcd for $C_{45}H_{73}O_{19}$, 917.4746 $[M+H]^+$). The ^{13}C and DEPT NMR spectra gave 45 signals, of which 18 were assigned to the sugar moiety and 27 to a steroidal aglycon. The ^1H NMR spectrum of **1** was very similar to that of pennogenin,[7] but the singlet ascribable to the C_{27}-CH_3 was missing signals corresponding to a primary hydroxylgroup. The ^{13}C NMR spectrum of **1** confirmed this by the presence of signal at δ 64.7 and the signals due to C_{24}, C_{25}, C_{26} were shifted by –5.0, +8.3, –2.7 ppm respectively. So we determined its aglycon as 27-hydroxylpennogenin.[10]

Acid hydrolysis of **1** yielded D-glucose and L-rhamnose in the ratio of 2:1 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The chemical shifts, the signal multiplicities, the absolute values of the coupling constants, and their magnitude in the ^{1}H NMR spectra, as well as the ^{13}C NMR spectral data, indicated a β-configuration for the glucosyl units [δ 4.78 (1H, d, J = 8.2 Hz, H-1 of glc1); δ 105.1 (C-1 of glc1)], and an α-configuration for the rhamnosyl unit [δ 6.43 (1H, br s, H-1 of rha); δ 101.9 (C-1 of rha)]. The ^{13}C NMR spectral data allowed the assignment of the pyranose forms of D-glucose and L-rhamnose. All ^{1}H and ^{13}C NMR signals of the three sugar unit in **1** were assigned using HMQC, HMBC spectra. The linkage sites and sequences of the three saccharides and of the aglycon were deduced from a HMBC and ESI-MS experiment. HMBC cross peaks were observed between H-1 of one glucose (glc1) and C-3 of the aglycon, H-1 of the rhamnose and C-2 of glc1, H-1 of the glucose (glc2) and C-3 of the glc1 (Table 1). Thus, the structure of **1** was elucidated as 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl-27-hydroxylpennogenin.

Compound **(2)** was isolated as an white amorphous powder. The molecular weight was determined from the negative HR-ESI-MS at *m/z* 1061.5165 for the $[M-H]^-$ ion (calcd for $C_{51}H_{81}O_{23}$, 1061.5168 $[M-H]^-$). The ^{13}C and DEPT NMR spectra gave 51 signals, of which 24 were assigned to the sugar moiety and 27 to a steroidal aglycon. In the aglycon, two quaternary carbons displayed in downfield (δ 217.5, 213.2), indicated the presentation of two carbonyl groups. The ^{13}C NMR signals due to the aglycon were similar to 3β,26-dihydroxycholest-5-ene-16,22-dione, kryptogenin,[3,11] except for those of C-26 and C-3. Hydrolysis of **2** yielded D-glucose and L-rhamnose in the ratio of 3:1 by GC analysis of the leucine derivatives of the component monosaccharides compared with the leucine derivatives of the standard sugars. The linkage sites and sequences of the three saccharides, and of the aglycon were also deduced from the HMBC (Table 1) and ESI-MS experiment. Therefore, the structure of **2** was determined as 3-*O*-[α-L-rhamnopyranosyl(1→2)]-[β-D-glucopyranosyl(1→3)]-β-D-glucopyranosyl 26-*O*-β-D-glucopyranosyl cholest-5-ene-16,22-dione.

EXPERIMENTAL

General Experimental Procedures. Optical rotations were measured in MeOH with a Perkin-Elmer model 341 polarimeter. NMR spectra were obtained on a Bruker AMX-500 spectrometer in C_5D_5N solution. ESI-MS was run on a Bruker Esquire 3000 plus spectrometer in MeOH and HR-ESI-MS was run on a Bruker Atex III spectrometer in MeOH, respectively. GC: Shimadzu GC-MS-QP5050A; db-1 column, 0.25 mm i.d. × 30 m; column temperature, 200 °C; injection temperature, 250 °C; carrier gas N_2 at flow rate of 32.2 mL/min; detector, EI-MS.

Plant Material. The underground parts of *Paris polypholla* was collected in Guangxi Province, People's republic of China in April, 2004. A voucher specimen of the plant (No. 2004003) was identified by Mr.

Jin-Gui Shen and deposited at the herbarium of National Center for Drug Screening, Shanghai, People's Republic of China.

Extraction and Isolation. The dried and nubbly underground parts of *P. polypholla* (0.5 kg) were extracted successively with MeOH (3×2 L) at rt for 48 h. Removal of MeOH under reduced pressure left a pale yellow powder (20 g). The powder was subjected to macroporous resin to remove sugar. The residue was chromatographed on silica gel column ($CHCl_3$-MeOH-H_2O 6:1:0.1, 4:1:0.1 and 2:1:0.1), to yield three parts. Fraction A (1.0 g) was chromatographed by silica gel column ($CHCl_3$-MeOH-H_2O 6:1:0.1) and RP-18 flash column (20-45 um, Fuji Silysia Chemical Ltd., Fuji, Japan; MeOH-H_2O 4:1), to give **3** (50 mg). Fraction B (1.0 g) was passed through a Sephadex LH-20 column (25-100 um, Merck, Darmstadt, Germany; MeOH). Then, the subfraction was subjected to RP-18 flash column chromatography (MeOH-H_2O 1:1), to provide **1** (22 mg), **5** (40 mg). Fraction C (3.0 g) was subjected to Sephadex LH-20 (MeOH), MCI gel CHP 20P (75-150 um, Mitsubishi Kasei Industry Co., Ltd., Tokyo, Japan, water-acetone 4:1, 3:1, 2:1) column chromatography, RP-18 (MeOH-H_2O 1:1, 2:1) and silica gel column ($CHCl_3$-MeOH-H_2O 4:1:0.1, 2:1:0.1 and 1:1:0.1), to yield **2** (130 mg), **6** (100 mg), **4** (160 mg), **7** (13 mg).

3-*O*-[*α*-L-Rhamnopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl 27-hydroxyl-pennogenin (1). White amorphous powder; $[\alpha]^{23}_D$ –65.4° (*c* 0.47, MeOH); ^{1}H and ^{13}C NMR spectra, Table 1; HR-ESI-MS (*m*/*z*) 917.4741 (calcd for $C_{45}H_{73}O_{19}$ $[M+H]^+$, 917.4746); ESI-MS (*m*/*z*) 917 $[M+H]^+$, 899 $[M+H-H_2O]^+$, 737 $[M+H-H_2O\text{-Glc}]^+$, 591 $[M+H-H_2O\text{-Glc-Rha}]^+$, 429 $[M+H-H_2O\text{-Glc-2Rha}]^+$.

3-*O*-[*α*-L-Rhamnopyranosyl(1→2)]-[*β*-D-glucopyranosyl(1→3)]-*β*-D-glucopyranosyl-26-*O*-*β*-D-glucopyranosyl cholest-5-ene-16,22-dione (2). White amorphous powder; $[\alpha]^{25}_D$ –93.8° (*c* 0.49, MeOH); ^{1}H and ^{13}C NMR spectra, Table 1; HR-ESI-MS (*m*/*z*) 1061.5165 (calcd for $C_{51}H_{81}O_{23}$ $[M-H]^-$, 1061.5168); ESI-MS (*m*/*z*) 1061 $[M-H]^-$, 576 $[M-H-2Glc-Rha]^-$.

Acid Hydrolysis of Compounds (1) and **(2).**[12] Compounds **(1)** and **(2)** (4 mg each) in 10% HCl-dioxane (1:1, 1 mL) were heated at 80 °C for 4 h in a water bath. The reaction mixtures were neutralized with Ag_2CO_3, filtered, and then extracted with $CHCl_3$ (1 mL × 3). After concentration, each H_2O layer (monosaccharide part) was examined by TLC with $CHCl_3$-MeOH-H_2O (55:45:10) and compared with authentic samples.

Determination of Sugar Components.[12] The monosaccharide subunits were obtained by 10% HCl-dioxane (1:1, 1 mL) hydrolysis as described above. The sugar residues were then dissolved in 1 mL anhydrous pyridine under Ar, 2 mg of L-leucine methyl ester hydrochloride was added, and the mixture was warmed at 60 °C for 1 h. Then 2 mg of $NaBH_4$ were added, and the mixture was stirred for 1 h at ambient temperature. Then 0.2 mL of trimethylsilylation reagent trimethylchlorosilane was added and warming at 60 °C was continued for another 30 min. The leucine derivatives were subjected to GC

Table 1. NMR Spectral Data of Compounds **(1)** and **(2)** in C_5D_5N[a]

position	**1**			**2**		
	^{1}H	^{13}C	HMBC	^{1}H	^{13}C	HMBC
1	1.82 m	37.9		1.80 m	37.5	
2	1.94 m 2.08 m	30.4	1, 3	1.97 m 2.17 m	30.3	
3	3.82 m	78.0		3.88 m	78.0	1'
4	2.77 m	39.1	2, 3, 10	2.85 m	39.0	
5		141.1			141.2	
6	5.40 s	122.2	4, 5, 7, 8, 10	5.43 s	121.8	10
7	1.64 m 1.98 m	32.8		1.65 m 1.93 m	32.3	
8	1.64 m	32.7		1.65 m	31.3	
9	0.97 m	50.6	8, 10, 11	1.06 m	50.4	5, 10, 18, 19
10		37.5			37.4	
11	1.79 m 1.84 m	21.0	8, 9	1.53 m	21.1	
12	1.54 m	32.4	11, 13, 14, 18	1.70 m	38.6	
13		45.5			42.7	
14	2.08 m	53.4	7, 8, 13, 18	1.58 m	50.6	18
15	1.52 m, 2.20 m	32.2	14, 16, 17	2.36 d (11.5)	39.3	16
16	4.49 t (6.8)	90.4	13, 17, 20		217.5	
17		90.5		2.54 d (6.8)	64.2	13, 16, 18, 20, 21
18	0.98 s	17.4	11, 12, 13, 14, 16, 17	0.85 s	14.4	12, 13, 14, 17
19	1.10 s	19.8	1, 5, 9, 10, 18	1.11 s	19.7	5, 9, 10
20	2.25 q (7.2)	45.2	16, 17, 21, 22, 23	2.85 m	44.4	16, 17, 22
21	1.25 d (7.0)	10.2	16, 17, 20, 21	1.58 d (5.0)	16.4	17, 22
22		110.6			213.2	
23	2.20 m	32.2	22, 24	2.70 m, 2.85 m	39.3	22
24	1.79 m 1.83 m	23.9		2.10 m	28.4	
25	2.08 m	39.4		2.00 m	33.7	24, 26, 27
26	4.04 m 3.90 m	64.2	22, 24, 27	4.04 d (4.8)	75.3	25, 26, 1’’’’
27	3.65 dd (7.1, 10.5) 3.70 dd (4.9, 10.5)	64.7	24, 25, 26	1.06 d (4.4)	17.7	24, 25, 26
C-3-glc (glc1)				C-3-glc (glc1)		
1'	4.93 d (6.8)	100.3	3	4.97 m	100.2	3, 5'
2'	4.20 m	77.4	1', 1''	4.20 m	77.4	3'
3'	4.19 m	89.8	2', 1'''	4.22 m	89.5	2', 1'''
4'	4.06 m	70.0		4.00 m	69.9	
5'	3.90 m	78.1		4.22 m	78.0	
6'	4.28 m	62.9	5'	4.28 m	62.7	5'
Rha				Rha		
1''	6.35 br s	102.5	2', 5''	6.32 br s	102.4	2'', 5'', 2'
2''	4.28 m	72.8		4.59 m	73.0	
3''	4.46 m	73.1	4''	4.18 m	72.0	
4''	4.30 m	74.5	3'', 6''	4.36 m	74.2	2'', 3'', 5''
5''	4.05 m	70.0	4''	4.05 m	69.8	
6''	1.75 d (6.6)	18.7	4'', 5''	1.78 d (6.7)	18.9	4'', 5''
Glc (glc2)				Glc (glc2)		
1'''	5.10 d (7.7)	104.9	3', 5'''	5.11 m	104.7	3'
2'''	4.02 m	75.3	4'''	4.05 m	75.1	
3'''	4.05 m	79.0	4'''	4.20 m	78.0	
4'''	4.10 m	71.9		4.16 m	71.7	
5'''	3.85 m	78.2		4.03 m	78.6	
6'''	4.20 m	62.7	5'''	4.38 m	63.1	5'''
				C-26-glc (glc3)		
				4.87 d (7.2)	105.0	1'''', 5''''
				4.05 m	75.4	3''''
				4.03 m	78.6	
				4.90 m	72.6	3'''', 5''''
				4.22 m	78.8	
				4.20 m	62.7	5''''

[a] 500 MHz; ^{1}H NMR referenced to δ 7.58 and ^{13}C NMR to δ 135.9 (C_5D_5N); J values (Hz) in parentheses.

analysis to identify the sugars. Column temperature 200 °C; injection temperature 250 °C; carrier gas N_2 at flow rate of 32.2 mL/min; derivatives of D-glucose, and L-rhamnose: 13.95, and 8.87 min, respectively.

Bioassay. Antitumor activities were evaluated by SRB (sulforhodamine B) assay[13] using 5-FU as the positive control. All the isolated compounds **(1-7)** have been tested their cytotoxicity on BEL-7402 liver cancer cell line, compounds **(3-6)** were found to be active with an ED_{50} of 8.3±0.3 μM, 6.7±0.4 μM, 8.1±0.3 μM, and 3.4±0.2 μM, respectively.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Fund of China (30371679) and the support is gratefully acknowledged.

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