Phytochemistry xxx (2017) 1–8

Contents lists available at ScienceDirect

Phytochemistry

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

Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*

Lun Wang [a, b], Xi-Lan Jiang [b], Wen-Ming Zhang [c], Fu Li [a, b, *], Ashfaq-Ahmad Khan [e], Xin Liu [d], Kai Yu [c], Ming-Kui Wang [a, b, **]

[a] School of Pharmacy and Bioengineering, Chengdu University, Chengdu, 610106, China
[b] Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization and Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, 610041, China
[c] School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, 610013, China
[d] Beijing Entry-Exit Inspection and Quarantine Bureau, Beijing, 100026, China
[e] Department of Chemistry, Women University of Azad Jammu and Kashmir, Bagh, 12500, Pakistan

ARTICLE INFO

Article history:
Received 15 October 2016
Received in revised form
4 January 2017
Accepted 12 January 2017
Available online xxx

Keywords:
Ophiopogon japonicus
Liliaceae
C_{29}-steroidal saponins
C_{27}-steroidal saponins
Cytotoxic activity

ABSTRACT

Phytochemical investigation of the tubers of *Ophiopogon japonicus* led to the isolation of five previously undescribed steroidal saponins, ophiojaponins A–E, together with twelve known ones. The structures of these isolated compounds were elucidated by detailed spectroscopic analyses and chemical methods. Ophiojaponins A–C are rare naturally occurring C_{29} steroidal glycosides possessing a homo-cholestane skeleton with an aromatized ring E. Ruscogenin 1-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-sulfo-*β*-D-fucopyranosido-3-*O*-*β*-D-glucopyranoside was isolated as single component and its full spectroscopic data was reported for the first time. The isolated steroidal saponins were evaluated for their cytotoxicities against two human tumor cell lines MG-63 and SNU387. Among them, five known spirostane-type glycosides showed cytotoxic activity against both MG-63 and SNU387 cell lines with IC_{50} values ranging from 0.76 to 27.0 μM.

1. Introduction

Ophiopogon japonicus (Thunb.) Ker.-Gawl. (family Liliaceae) is an evergreen perennial, widely distributed in Southeast Asia, especially in the mainland China. Its tubers have been used for thousands of years as a traditional Chinese medicine for the treatment of acute and chronic inflammation and cardiovascular diseases (Jiangsu College of New Medicine, 1986; Xiao, 2002; Kou et al., 2005). Additionally, the tubers of *O. japonicus* are edible and are widely consumed in China, with effects on reducing blood sugar, blood pressure and improving immunity. Previous phytochemical studies of the tubers derived from *O. japonicus* resulted in the isolation and structure elucidation of C_{27} steroidal saponins (Watanabe et al., 1977; Dai et al., 2005; Xu et al., 2008; Zhou et al., 2008; Duan et al., 2010; Zhang et al., 2012; Li et al., 2013; Kang et al., 2013; Ye et al., 2013; Qi et al., 2015; Yan et al., 2016), homoisoflavonoids (Chang et al., 2002; Anh et al., 2003; Zhou et al., 2013), terpenoids (Adinolfi et al., 1990; Cheng et al., 2004; Liu et al., 2016) and amides (Nakanishi and Kameda, 1987). In recent years, steroidal saponins have attracted much more scientific attention because of their structural diversity and significant bioactivities, including antitumor (Sparg et al., 2004), anti-inflammatory (Lee et al., 2014), and hypoglycemic (Liu et al., 2012) activities as well as therapeutic potential for cardiovascular diseases (Vasanthi et al., 2012). As a part of a program to search for bioactive steroidal saponins from traditional Chinese medicines, a methanol extract of the tubers of *O. japonicus* was investigated. This procedure led to the isolation of five previously undescribed steroidal saponins, named ophiojaponins A–E (**1**–**5**), together with twelve known steroidal saponins (**6**–**17**) (Fig. 1). Among the identified compounds, the aglycones of compounds **2** and **3** were reported for the first time. In this paper, we describe the isolation and structural elucidation of these steroidal saponins, along with the evaluation of the cytotoxic activities of the 17 compounds against two human tumor cell lines, MG-63

* Corresponding author. School of Pharmacy and Bioengineering, Chengdu University, Chengdu, 610106, China.
** Corresponding author. School of Pharmacy and Bioengineering, Chengdu University, Chengdu, 610106, China.
E-mail addresses: lifu@cib.ac.cn (F. Li), wangmk@cib.ac.cn (M.-K. Wang).

http://dx.doi.org/10.1016/j.phytochem.2017.01.006
0031-9422/

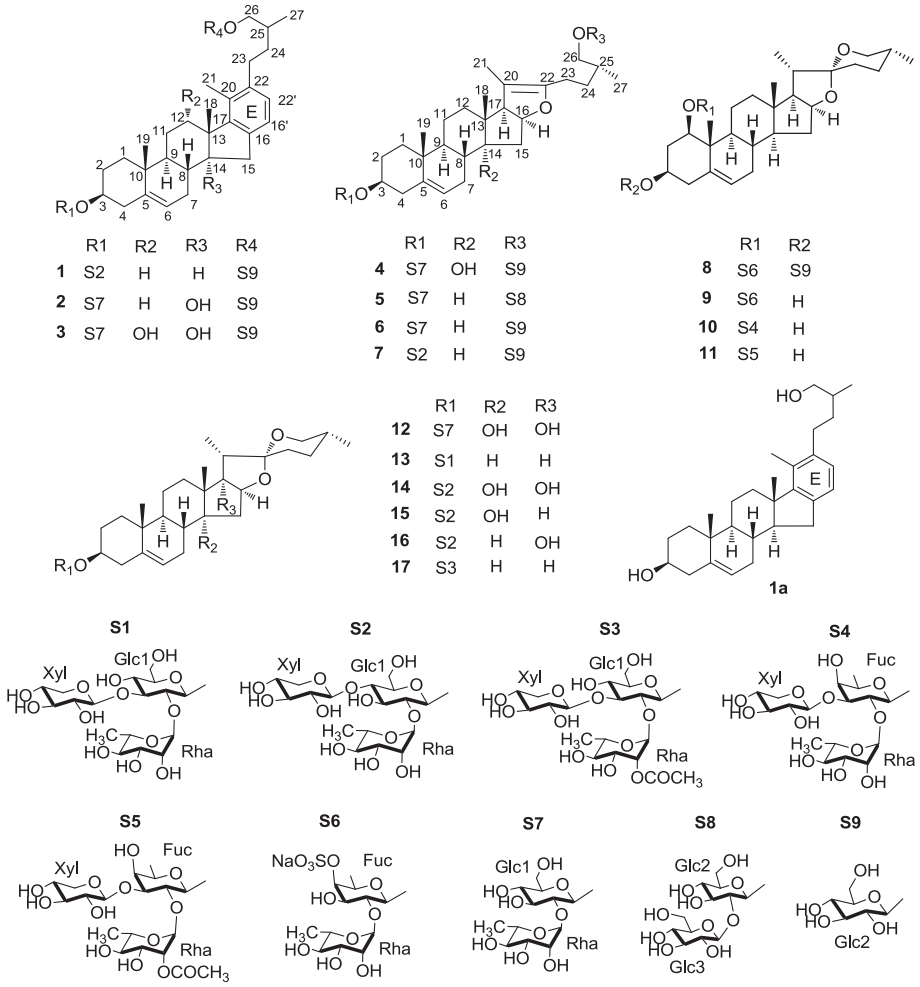

Fig. 1. Structures of compounds **1**–**17**.

and SNU387.

2. Results and discussion

2.1. Structural elucidation

The tubers of *O. japonicas* were extracted with hot MeOH. The extract was partitioned with $CHCl_3$ and *n*-BuOH, successively. The *n*-BuOH-soluble portion was subjected to macroporous resin (D101), silica gel and RP-C_{18} silica gel column chromatography (CC), and to reversed-phase preparative HPLC, giving compounds **1**–**17**. Compounds **6**–**17** were identified as 26-*O*-*β*-D-glucopyranosyl-3*β*,26-diol-25(R)-furost-5,20(22)-dien 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*O*-*β*-D-glucopyranoside (**6**) (Ali et al., 2013), pallidiflosides A (**7**) (Shen et al., 2011), ruscogenin 1-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-sulfo-*β*-D-fucopyranosido-3-*O*-*β*-D-glucopyranoside (**8**), ruscogenin 1-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-4-*O*-sulfo-*β*-D-fucopyranoside (**9**) (Watanabe et al., 1984), ophiopogonin D (**10**) (Dai et al., 2001), ophiopogonin C (**11**) (Watanabe et al., 1977), (25R)-spirost-5-ene-3*β*,14*α*,17*α*-triol-3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-*β*-D-glucopyranoside (**12**) (Chen et al., 2000), ophiopogonin D′ (**13**) (Watanabe et al., 1977), ophiopojaponin C (**14**) (Dai et al., 2005), 14-hydroxydiosgenin 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 4)]-*β*-D-glucopyranoside (**15**) (Wang et al., 2008a,b), pennogenin 3-*O*-*α*-L-rhamnopyranosyl-(1 → 2)-[*β*-D-xylopyranosyl-(1 → 4)]-*β*-D-glucopyranoside (**16**) (Wang et al., 2008a,b), and diosgenin 3-*O*-[2-*O*-acetyl-*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-xylopyranosyl-(1 → 3)-*β*-D-glucopyranoside (**17**) (Dai et al., 2005), respectively, based on mass spectrometric and NMR data compared with those reported.

Compound **1** was obtained as a white amorphous powder with the molecular formula $C_{52}H_{80}O_{20}$, as established by HRESIMS at *m/z* 1047.5157 $[M+Na]^+$ (calcd for $C_{52}H_{80}O_{20}Na$, 1047.5135) and ^{13}C NMR (DEPT) spectrum, requiring 13 degrees of unsaturation. The IR spectrum showed absorption for hydroxy (3434 cm^{-1}), methyl (2932 cm^{-1}) and olefinic (1634 cm^{-1}) groups. The ^1H NMR (Tables 1 and 2) spectrum of **1** contained signals for four characteristic methyl groups at δ_H 0.94 (3H, s), 1.05 (3H, d, $J = 6.7$ Hz), 1.11 (3H, s) and 2.32 (3H, s), an olefinic proton at δ_H 5.35 (1H, br s), a pair of *ortho*-coupled aromatic protons at δ_H 7.03 (1H, d, $J = 7.5$ Hz) and δ_H 7.11 (1H, d, $J = 7.5$ Hz), as well as signals for four anomeric proton signals at δ_H 4.85 (1H, d, $J = 7.8$ Hz), 4.97 (1H, d, $J = 7.3$ Hz), 5.02 (1H, d, $J = 7.7$ Hz) and 6.25 (1H, s). The ^{13}C NMR (Tables 1 and 2), DEPT and HSQC spectra showed 52 carbon resonances, of which four were attributed to anomeric carbons (δ_C 100.1, 102.0, 104.9 and 105.7), two were attributed to a trisubstituted double bond (δ_C 141.0 and 121.8) and six were attributed to a tetrasubstituted benzene ring (δ_C 122.9, 127.5, 131.3, 139.8, 140.7 and 151.8). The above NMR data coupled with literature references (Tagawa et al., 2003; Xiao et al., 2010) indicated the presence of a homo-aro-cholestane glycoside skeleton. Acid hydrolysis of **1** with 1 M HCl (dioxane–H_2O = 1:1) gave **1a** as the aglycone, and D-glucose, L-

Please cite this article in press as: Wang, L., et al., Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.006

Table 1
^{1}H and ^{13}C NMR data for the aglycone moieties of compounds **1**–**5** and **8** (δ in ppm, J in Hz).

Position	1[a]		2[a]		3[a]		4[a]		5[b]		8[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1β, 1α	0.98 m, 1.75 m[c]	37.4	1.09 m, 1.83 m	37.7	1.09 m, 1.81 m	37.5	1.04 m, 1.80 m[c]	37.8	0.96 m, 1.73 m	37.6	3.60 m	83.6
2β, 2α	1.89 m, 2.11 m	30.2	1.92 m, 2.14 m	30.3	1.90 m, 2.19 m	30.2	1.89 m, 2.11 m	30.2	1.88 m[c], 2.13 m	30.2	2.32 m, 2.66 m[c]	35.3
3	3.87 m	78.2	3.89 m	78.2	3.90 m	78.2	3.89 m	78.2	3.89 m	77.9	3.95 m	75.1
4β	2.73 m	39.0	2.80 m	39.1	2.81 m	39.1	2.76 m	39.0	2.74 m	39.0	2.48 m	39.7
4α	2.80 m		2.88 m[c]		2.88 m		2.82 m		2.79 m		2.65 m[c]	
5		141.0		140.7		140.4		140.4		140.8		138.4
6	5.35 br s	121.8	5.48 br s	122.5	5.49 br s	122.5	5.40 br s	122.3	5.29 br s	121.8	5.49 d (5.5)	125.6
7α	2.42 m	32.4	1.97 m	26.8	1.99 m	26.2	1.86 m[c]	26.8	1.48 m[c]	32.4	1.41 m[c]	32.0
7β	2.62 m[c]		2.64 m		2.66 m		2.48 m		1.86 m[c]		1.80 m	
8	1.63 m	31.0	2.04 m[c]	34.2	1.94 m	34.1	2.00 m	35.0	1.46 m[c]	31.4	1.48 m[c]	33.0
9	1.00 m	50.5	2.04 m[c]	43.4	2.21 m	40.1	1.81 m[c]	43.6	0.88 m	50.3	1.40 m[c]	50.4
10		37.1		37.5		37.0		37.4		37.1		42.9
11β	1.59 m[c]	21.3	1.69 m	20.2	2.08 m	30.1	1.57 m	20.5	1.39 m	21.3	1.49 m[c]	23.9
11α					2.11 m[c]				1.43 m		2.83 m	
12α	1.60 m[c]	37.0	2.78 m	29.7	4.90 br s	74.3	1.50 m[c]	31.8	1.14 m	39.7	1.27 m	39.9
12β	1.74 m[c]		2.17 m				2.34 m		1.74 m		1.49 m[c]	
13		47.2		53.3		55.5		47.8		43.4		40.1
14	1,51 m	57.7		83.4		85.9		84.8	0.86 m	55.0	1.12 m	57.0
15a,	2.63 m[c]	31.3	2.88 d (15.1)[c]	41.4	2.86 d (14.9)	41.4	1.94 m	42.4	1.45 m[c]	34.5	1.48 m[c]	32.4
15b	2.67 m		2.94 d (15.1)		2.90 d (14.9)		2.46 m		1.96 m		1.99 m	
16		140.7		139.3		140.2	5.28 m	85.2	4.85 m	84.5	4.49 m	81.1
17		151.8		150.8		148.8	3.37 d (9.7)	61.6	2.46 d (10.1)	64.5	1.79 m	62.9
18	0.94 s	16.6	1,13 s	21.2	1,08 s	22.7	0.96 s	17.7	0.74 s	14.1	0.82 s	16.7
19	1.11 s	19.4	1.19 s	19.2	1.17 s	19.4	1.14 s	19.4	1.05 s	19.4	1.26 s	14.7
20		131.3		132.2		132.5		104.0		103.5	1.89 m	42.0
21	2.32 s	14.7	2.29 s	14.5	2.61 s	14.1	1.68 s	11.9	1.66 s	11.9	1.05 d (6.9)	14.9
22		139.8		140.0		139.7		152.2		152.5		109.2
23a, 23b	1.99 m[c]	32.0	2.61 m	31.3	2.67 m	31.4	2.25 m	23.8	2.24 m	23.8	1.61 m, 1.68 m	31.8
24a, 24b	1.44 m, 1.83 m	35.5	1.38 m, 1.74 m	35.4	1.39 m, 1.75 m	35.4	1.51 m[c], 1.86 m[c]	31.4	1.48 m[c], 1.92 m	31.4	1.53 m[c]	29.3
25	1.99 m[c]	34.1	1.95 m	34.4	2.10 m[c]	34.5	1.98 m	33.5	1.96 m	33.6	1.53 m[c]	30.6
26a	3.63 dd (9.4, 6.1)	75.0	3.63 dd (9.5, 6.1)	75.0	3.60 dd (9.6, 6.0)	75.0	3.63 dd (9.5, 5.6)	75.0	3.55 dd (8.9, 5.3)	74.8	3.47 m	66.8
26b	3.96 m		3.96 m		3.96 m		3.95 m		3.90 m		3.55 m	
27	1.05 d (6.7)	17.4	1.04 d (6.7)	17.4	1.01 d (6.7)	17.3	1.03 d (6.6)	17.3	1.06 d (7.3)	17.2	0.66 d (5.3)	17.3
16′	7.11 d (7.5)	122.9	7.10 d (7.5)	124.0	7.14 d (7.5)	123.7						
22′	7.03 d (7.5)	127.5	6.97 d (7.5)	127.3	7.02 d (7.5)	127.4						

[a] Recorded at 600 (150) MHz in pyridine-d_5.
[b] Recorded at 400 (100) MHz in pyridine-d_5.
[c] Overlapped with other signals.

rhamnose and D-xylose as the carbohydrate moieties. Aglycone **1a** was identified as homo-aro-cholest-5-ene-3β,26-diol (Tagawa et al., 2003; Xiao et al., 2009) from its spectroscopic data. The absolute configurations of the sugars were determined on the basis of the HPLC analysis of their derivatives (section 3.4). The β-configurations of glucose and xylose were assigned based on the large coupling constants ($^{3}J_{1,2}$ > 7.0 Hz) of their anomeric protons. The carbon signals for δ_C 72.8 (C-3 of Rha) and δ_C 69.5 (C-5 of Rha) provided the evidence for the α-configuration of rhamnose (Agrawal, 1992). The downfield shifts for C-3 and C-26 of the aglycone (δ_C 78.2 and 75.0) allowed the deduction that C-3 and C-26 were both glycosylated. The sequence of the trisaccharide chain rhamnosyl-(1 → 2)-[xylosyl-(1 → 4)]-glucosyl at C-3 was deduced from the HMBC correlations (Fig. 2) between δ_H 6.25 (H-1 of Rha) and δ_C 77.3 (C-2 of Glc1), δ_H 5.02 (H-1 of Xyl) and δ_C 81.5 (C-4 of Glc1), and δ_H 4.97 (H-1 of Glc1) and δ_C 78.2 (C-3). The second glucose residue was determined to be linked to C-26 by observation of an HMBC correlation between δ_H 4.85 (H-1 of Glc2) and δ_C 75.0 (C-26). Thus, the structure of **1** was determined to be 3β-*O*-α-L-rhamnopyranosyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranosyl homo-aro-cholest-5-ene-26-*O*-β-D-glucopyranoside, and named ophiojaponin A.

Compound **2** was isolated as a white amorphous powder. The positive HRESIMS showed an $[M+Na]^+$ ion at *m/z* 931.4674, corresponding to the molecular formula $C_{47}H_{72}O_{17}$. In the ^{1}H NMR spectrum (Tables 1 and 2), four methyl group signals, two *ortho*-coupled aromatic proton signals and one olefinic proton signal, together with three anomeric proton signals were observed. The ^{13}C NMR (Tables 1 and 2), DEPT and HSQC spectra exhibited 47 carbon signals. These data allowed to assign compound **2** to the same saponin family of **1**. Comparison of the NMR data of aglycone of **2** with those of **1** indicated that these two compounds shared a similar aglycone except for the main differences at C-12 (−7.3 ppm), C-13 (+6.1 ppm), C-14 (+25.7 ppm), C-15(+10.1 ppm), suggesting the presence of one hydroxyl group at C-14 in **2** instead of one hydrogen at C-14 in **1**. This was further confirmed by long-range correlations between C-14 (δ_C 83.4) and H-18 (δ_H 1.13), H-15a (δ_H 2.88) and H-15b (δ_H 2.94) in the HMBC experiment. The significant downfield shifts of the H-9, H-12α protons, and the upfield shifts of the C-9, C-12 could be explained by the 1,3-diaxial interactions and γ-effects with the C-14α hydroxy group. Thus, the aglycone of **2** was identified as homo-aro-cholest-5-ene-3β,14α,26-triol, which was an unknown steroidal aglycone.

Acid hydrolysis of **2** gave D-glucose and L-rhamnose. A comparison of the ^{13}C-NMR data attributed to the sugar residues of **2** with those of **1** indicated that the only difference of the sugar parts of these two compounds was the absence of β-D-xylopyranosyl in **2**. In the HMBC spectrum, the long-range correlations between δ_H 4.85 (H-1 of Glc2) and δ_C 75.0 (C-26), δ_H 6.25 (H-1 of Rha) and δ_C 77.3 (C-2 of Glc1), and δ_H 4.97 (H-1 of Glc1) and δ_C 78.2 (C-3) further confirmed the sugar sequence and linkage sites. Therefore, the structure of **2** was elucidated as 3β-*O*-α-L-rhamnopyranosyl-

Please cite this article in press as: Wang, L., et al., Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.006

Table 2
^{1}H and ^{13}C NMR data for the sugar moieties of compounds **1**–**5** and **8** (δ in ppm, J in Hz).

position	1[a]		2[a]		3[a]		4[a]		5[b]		8[a]	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
Glc1-1	4.97 d (7.3)	100.1	5.03 d (7.1)	100.3	5.03 d (7.1)	100.3	5.00 d (7.3)	100.2	5.10 d (7.1)	100.3	5.06 d (7.7)	102.6
2	4.22 m[c]	77.3	4.25 m[c]	78.0	4.25 m[c]	77.9	4.24 m[c]	77.8	4.27 m[c]	77.8	4.00 m	75.1
3	4.20 m[c]	77.6	4.27 m[c]	79.6	4.27 m[c]	79.6	4.25 m[c]	79.6	4.28 m[c]	79.7	4.29 m[c]	78.4
4	4.18 m	81.5	4.15 m	71.8	4.14 m	71.9	4.14 m	71.8	4.18 m[c]	71.8	4.27 m[c]	71.5
5	3.84 m	76.2	3.89 m	77.9	3.89 m	77.9	3.88 m	77.9	3.89 m[c]	77.9	3.94 m	78.4
6	4.52 m	61.7	4.35 m[c]	62.7	4.35 m[c]	62.7	4.35 m[c]	62.6	4.33 m[c]	62.7	4.37 dd (11.9, 4.9)	62.5
	4.45 br. d (11.8)		4.50 br. d (11.8)		4.50 br. d (11.8)		4.49 br. d (11.8)		4.49 br. d (11.8)		4.50 m	
Fuc-1											4.44 d (7.7)	99.9
2											4.42 m	75.6
3											4.11 dd (9.0, 3.5)	76.0
4											5.05 m	78.7
5											3.63 m	70.4
6											1.63 d (6.2)	17.3
Rha-1	6.25 br s	102.0	6.36 br s	102.1	6.36 br s	102.1	6.34 br s	102.0	6.38 br s	102.1	6.26 br s	101.5
2	4.79 br s	72.5	4.80 br s	72.6	4.80 br s	72.5	4.79 br s	72.5	4.79 br s	72.6	4.74 br s	72.3
3	4.60 dd (9.4, 3.2)	72.8	4.64 dd (9.2, 3.2)	72.9	4.64 dd (9.2, 3.2)	72.8	4.61 dd (9.2, 3.3)	72.8	4.62 dd (9.2, 3.3)	72.8	4.60 dd (9.4, 3.3)	72.4
4	4.35 m	74.1	4.36 m[c]	74.2	4.36 m[c]	74.2	4.33 m[c]	74.1	4.34 m[c]	74.2	4.28 m[c]	74.1
5	4.93 m	69.5	5.00 m	69.5	5.00 m	69.5	4.97 m	69.5	5.00 m	69.5	4.78 m	69.4
6	1.78 d (6.2)	18.7	1.79 d (6.2)	18.7	1.78 d (6.1)	18.7	1.77 d (6.2)	18.6	1.77 d (6.2)	18.7	1.71 d (6.2)	19.0
Xyl-1	5.02 d (7.7)	105.7										
2	3.99 m[c]	74.9										
3	4.10 dd (8.9, 8.7)	78.3										
4	4.15 m	70.8										
5	3.67 dd (10.8, 10.8)	67.3										
	4.27 m											
Glc2-1	4.85 d (7.8)	104.9	4.85 d (7.8)	104.9	4.84 d (7.7)	104.9	4.83 d (7.8)	104.8	4.87 d (7.6)	103.2		
2	4.05 m	75.2	4.05 m	75.2	4.04 m	75.2	4.02 m	75.1	4.15 m	84.1		
3	4.20 m[c]	78.6	4.25 m[c]	78.6	4.26 m[c]	78.6	4.24 m[c]	78.5	4.29 m[c]	78.0		
4	4.23 m[c]	71.8	4.22 m	71.8	4.22 m	71.8	4.21 m	71.7	4.19 m[c]	71.3		
5	3.97 m[c]	78.5	3.95 m	78.5	3.96 m	78.5	3.94 m	78.4	3.88 m[c]	78.3		
6	4.39 m[c]	62.9	4.39 m[c]	62.9	4.39 m[c]	62.9	4.37 m[c]	62.8	4.33 m[c]	62.5		
	4.57 br. d (11.7)		4.57 br. d (11.8)		4.57 br. d (11.8)		4.57 br. d (11.7)		4.49 m[c]			
Glc3-1									5.30 d (7.7)	106.5		
2									4.10 m	77.1		
3									4.28 m[c]	78.3		
4									4.30 m[c]	71.4		
5									3.90 m[c]	78.6		
6									4.31 m, 4.49 m[c]	62.5		

[a] Recorded at 600 (150) MHz in pyridine-d_5.
[b] Recorded at 400 (100) MHz in pyridine-d_5.
[c] Overlapped with other signals.

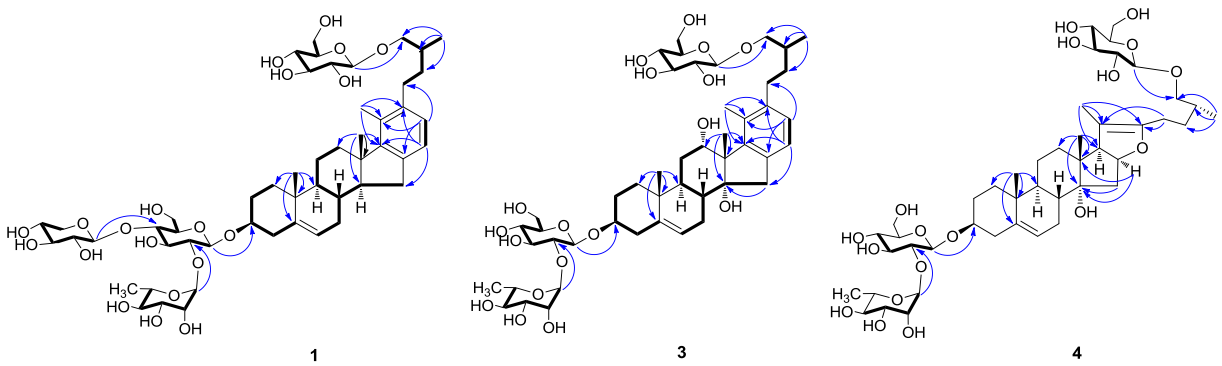

Fig. 2. Key ^{1}H-^{1}H COSY (bold) and HMBC (arrow) correlations of compounds **1**, **3** and **4**.

(1 → 2)-β-D-glucopyranosyl homo-aro-14α-hydroxy-cholest-5-ene-26-*O*-β-D-glucopyranoside, and named ophiojaponin B.

Compound **3** had a molecular formula $C_{47}H_{72}O_{18}$ as determined by HRESIMS (947.4623 $[M+Na]^+$, calcd for $C_{47}H_{72}O_{18}Na$, 947.4611). Its molecular weight was larger than that of **2** by one oxygen atom. Comparison of the ^{1}H and ^{13}C NMR data of **3** with those of **2** indicated that the structure of **3** is similar to that of **2**, with an additional hydroxy group at aglycone. The additional hydroxy group substituent at C-12 position was confirmed by the HMBC correlations (Fig. 2) between H-18 (δ_H 1.08) and C-12 (δ_C 74.3), H-12 (δ_H 4.90) and C-13 (δ_C 55.5), and H-12 (δ_H 4.90) and C-14 (δ_C 85.9). The α configuration of the hydroxy group at C-12 of the aglycone was determined by the NOESY correlations between H-12 (δ_H 4.90) and H-18 (δ_H 1.08). Thus, the structure of the aglycone moiety of **3** was identified as homo-aro-cholest-5-ene-3β,12α,14α,26-tetrol, a previously undescribed steroidal sapogenin. Consequently, the

Please cite this article in press as: Wang, L., et al., Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.006

structure of **3** was assigned as 3β-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranosyl homo-aro-12α,14α-dihydroxy-cholest-5-ene-26-*O*-β-D-glucopyranoside, and named ophiojaponin C.

Compound **4** was isolated as a white amorphous powder. The molecular formula of **4** was determined to be $C_{45}H_{72}O_{18}$ based on the pseudomolecular ion peak at *m/z* 923.4619 $[M+Na]^+$ in its positive HRESIMS. The IR spectrum showed the absorption bands for hydroxyl (3412 cm^{-1}), methyl (2931 cm^{-1}) and olefinic (1641 cm^{-1}) groups. The ^1H NMR spectrum (Tables 1 and 2) of **4** showed three singlet methyl signals at δ_H 0.96 (3H, s, CH_3-18), 1.14 (3H, s, CH_3-19) and 1.68 (3H, s, CH_3-21); two doublet methyl signals at δ_H 1.03 (3H, d, $J = 6.6$ Hz, CH_3-27) and 1.77 (3H, d, $J = 6.2$ Hz, CH_3-Rha). Furthermore, signals for an olefinic proton at δ_H 5.40 (br s, H-6), three anomeric protons at δ_H 4.83 (1H, d, $J = 7.8$ Hz), 5.00 (1H, d, $J = 7.3$ Hz) and 6.34 (1H, s) were observed. The ^{13}C NMR spectrum (Tables 1 and 2) displayed 45 carbon signals, in which the characteristic carbon signals at δ_C 140.4 (C-5), 122.3 (C-6), 104.0 (C-20), 152.2 (C-22), 85.2 (C-16) and 75.0 (C-26) were assigned readily. These data indicated that **4** is a furostanol saponin with a $\Delta^{20,22}$ double bond (Mimaki et al., 1996). Comparison of the NMR data of **4** with those of ophiopogonin J (Kang et al., 2013) suggested that they shared the same aglycone and sugar chain linked to C-3 of the aglycone. The HMBC correlation (Fig. 2) between δ_H 4.83 (H-1 of Glc2) and δ_C 75.0 (C-26) indicated the existence of a glucose unit linked to C-26 of the aglycone moiety. The C-25 configuration was deduced to be *R* based on the difference of chemical shifts ($\Delta_{ab} = \Delta_a - \Delta_b$) of the geminal protons at H_2-26 ($\Delta_{ab} = 0.32 < 0.48$ ppm) (Agrawal, 2004). Acid hydrolysis of **4** gave D-glucose and L-rhamnose. The β-configuration of glucose was determined by large *J* values of the anomeric protons while the rhamnose had an α-configuration evidenced by carbon signals of C-3 and C-5 of rhamnose. Thus, the structure of **4** was determined as (25R)-26-*O*-β-D-glucopyranosyl-14α-hydroxy-furost-5,20(22)-diene 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside, and named ophiojaponin D.

Compound **5** was obtained as white amorphous powder. Its molecular formula was determined as $C_{51}H_{82}O_{22}$ by HRESIMS. The ^1H NMR spectrum (Tables 1 and 2) of **5** exhibited three tertiary methyls at δ_H 0.74 (s, CH_3-18), 1.05 (s, CH_3-19) and 1.66 (s, CH_3-21), two secondary methyls at δ_H 1.06 (d, $J = 7.3$ Hz, CH_3-27) and 1.77 (d, $J = 6.2$ Hz, CH_3-Rha), an olefinic proton at δ_H 5.29 (br s, H-6), together with four anomeric protons at δ_H 4.87 (1H, d, $J = 7.6$ Hz), 5.10 (1H, d, $J = 7.1$ Hz), 5.30 (1H, d, $J = 7.7$ Hz) and 6.38 (1H, s). The ^{13}C NMR spectrum (Tables 1 and 2) of **5** showed the characteristic carbon resonances at δ_C 140.8 (C-5), 121.8 (C-6), 103.5 (C-20), 152.5 (C-22), 84.5 (C-16) and 74.8 (C-26). These data revealed that **5** is also a furostanol saponin with a $\Delta^{20,22}$ double bond. A detailed comparison of the NMR data of **5** with those of **6** and **7** indicated that they shared the same aglycone. The ^{13}C NMR spectral data of the sugar moieties of **5** were closely identical to those of **6** except the presence of an additional β-glucopyranosyl unit at C-2 of Glc2 unit. This was further confirmed by the HMBC corrections of δ_H 5.30 (H-1 of Glc3) with δ_C 84.1 (C-2 of Glc2) and δ_H 4.87 (H-1 of Glc2) with δ_C 74.8 (C-26). Thus, ophiojaponin E (**5**) was determined to be (25R)-26-*O*-β-D-glucopyranosyl-furost-5,20(22)-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **8** had a molecular formula $C_{45}H_{72}O_{20}S$, as determined by HRESIMS (*m/z*: 963.4260 $[M-H]^-$ and 965.4412 $[M+H]^+$). On the basis of 1D, 2D NMR and mass spectrometry data, the structure of the **8** was elucidated as ruscogenin 1-*O*-α-L-rhamnopyranosyl-(1 → 2)-4-*O*-sulfo-β-D-fucopyranosido-3-*O*-β-D-glucopyranoside, previously isolated from O. *jaburan* and was reported in the form of a mixture (Watanabe et al., 1984). In this paper, **8** was isolated and identified for the first time as a single component, and its full spectroscopic data were also reported.

2.2. Cytotoxic activity

The isolated compounds **1**–**17** were tested for their cytotoxicity against MG-63 and SNU387 human cancer cell lines using the MTT assay, with cisplatin as the positive control (Table 3). Compounds **11**, **13** and **15**–**17** showed cytotoxic activity against both MG-63 and SNU387 cell lines with IC_{50} values ranging from 0.76 to 27.0 μM. Compound **10** was only cytotoxic to MG-63 cells. Compound **13** was a derivative of **17** without the acetyl group at C-2 of the α-L-rhamnopyranosyl moiety and the cytotoxicity of **13** was less potent than that of **17**. According to the cytotoxic activities of **13**–**16**, it can be concluded that the presence of a hydroxyl group at C-14 or C-17 of the aglycone reduced their cytotoxicities. Compound **14**, possessing hydroxyl groups at both C-14 and C-17 position, displayed no cytotoxicity.

2.3. Conclusion

Phytochemical investigation of the tubers of *O. japonicus* resulted in the isolation and characterisation of five previously undescribed steroidal saponins (**1**–**5**), including three C_{29} steroidal glycosides possessing a homo-aro-cholestane skeleton (**1**–**3**) and two furostanol saponins (**4**–**5**), together with twelve known ones (**6**–**17**), including two furostanol saponins and ten spirostanol saponins. Among these known compounds, **8** was isolated and identified for the first time as a single component. All the isolates were evaluated for cytotoxic activities against MG-63 and SNU387 cell lines, and the spirostanol saponins **11**, **13** and **15**–**17** showed cytotoxic activity against both MG-63 and SNU387 cells. Spirostanol glycoside **17** was the most cytotoxic to MG-63 and SNU387 cells with IC_{50} values of 1.90 μM and 0.76 μM, respectively.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a Perkin-Elmer 341 polarimeter at room temperature. IR (KBr-disks) spectra were recorded by a Perkin-Elmer 1725X-FT spectrometer. NMR spectra were recorded on Bruker Avance 600 and Bruker Avance III 400 spectrometers in pyridine-d_5, with TMS as the internal standard. HR-ESI-MS were recorded on a LTQ Orbitrap XL mass spectrometer. Analytical HPLC was carried out on Prominence LC-20AT with a model SPD-M20A detector and Ultimate ® C_{18} column (250 mm × 4.60 mm, 5 μm). Preparative HPLC was carried out on P3000 with a UV3000 detector (Beijing ChuangXinTongHeng Science and Technology Co., Ltd) and Ultimate ® C_{18} column (250 mm × 21.2 mm, 5 μm; 250 mm × 30 mm, 10 μm, respectively).

Table 3
Cytotoxic activities of the isolated compounds **1**–**17**, and cisplatin against MG-63 and SNU387 cells.

Compound	IC_{50} (μM)[a]		Compound	IC_{50} (μM)[a]	
	MG-63	SNU387		MG-63	SNU387
1	>40	>40	**10**	15.56	>40
2	>40	>40	**11**	19.76	15.51
3	>40	>40	**12**	>40	>40
4	>40	>40	**13**	3.09	3.63
5	>40	>40	**14**	>40	>40
6	>40	>40	**15**	22.51	27.01
7	>40	>40	**16**	6.38	10.60
8	>40	>40	**17**	1.90	0.76
9	>40	>40	cisplatin[b]	11.31	5.59

[a] Compounds with $IC_{50} > 40$ μM were considered inactive.
[b] Positive control.

Please cite this article in press as: Wang, L., et al., Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.006

Silica gel (200–300 mesh, Qingdao Marine Chemical Co., People's Republic of China) and silica gel 60 (40–63 μm, Merck, Germany), RP-C_{18} (20–40 μm, Welch materials, Inc., USA) were used for column chromatography (CC). TLC was performed on precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd), and detection was achieved by 5% H_2SO_4-EtOH for saponins.

3.2. Plant material

The tubers of *Ophiopogon japonicus* (Thunb.) Ker.-Gawl. (Liliaceae) were purchased from Sichuan Neautus Traditional Chinese Medicine Co., Ltd, People's Republic of China, which were collected from Huayuan town, Santai county, Mianyang city, Sichuan province, People's Republic of China, in April 2013. The plant identification was verified by Prof. Wei-Kai Bao of Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (CIB-A-418) has been deposited at the Laboratory of Phytochemistry, Chengdu Institute of Biology, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried and powdered tubers of *O. japonicus* (48 kg) were extracted with MeOH at 50 °C three times (200 L, 1 d, each). After removal of the solvent by evaporation, the residue was suspended in H_2O and then extracted with $CHCl_3$ (3 × 6 L) and n-BuOH (3 × 6 L), successively. The n-BuOH extract (1150 g) was subjected to an D101 macroporous resin column, eluted with MeOH–H_2O (0:100, 30:70, 50:50, 90:10, v/v) to afford three fractions, Fr.1–Fr.3. Fraction 3 (154 g) was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (50:1:0, 20:1:0, 10:1:0.1, 5:1:0.1, 4:1:0.1, 3:1:0.1, 5:2:0.1, 2:1:0.1, 1:1:0.1) to give twelve fractions, Fr.3a–Fr.3l. Fr.3d (11 g) was subjected to RP-C_{18} CC, eluting with MeOH–H_2O (from 60% to 90%) to obtain subfractions 3d1 to 3d7. Subfraction 3d7 (4.1 g) was purified by preparative HPLC (MeOH–H_2O, 86:14) to afford **17** (180 mg). Fr.3e (10 g) was applied to a RP-C_{18} column eluted with MeOH–H_2O (from 60% to 90%) to give nine subfractions, 3e1–3e9. Subfraction 3e7 (0.4 g) was separated by silica gel CC with $CHCl_3$–MeOH–H_2O (10:1:0.1) to yield **11** (120 mg). Fr.3f (10.5 g) was subjected to a RP-C_{18} column eluting with MeOH–H_2O (from 60% to 90%) to produce nine subfractions 3f1–3f9. Subfraction 3f4 (1.5 g) was further purified by the preparative HPLC using MeOH–H_2O (72:28) to yield **12** (620 mg), Subfraction 3f8 (1.2 g) was purified by the preparative HPLC using MeOH–H_2O (86:14) to give **13** (0.8 g). Fr.3g (15.1 g) was separated by RP-C_{18} CC in elution with gradient solvent system (from 60% to 90%) to give seven subfractions (3g1–3g7). Subfraction 3g4 (1.4 g) was further separated by preparative HPLC eluted with MeOH–H_2O (75:25) to afford **14** (105 mg). Compounds **15** (240 mg) and **16** (150 mg) were obtained by using preparative HPLC with MeOH–H_2O (75:25) from subfraction 3g5. Subfraction 3g7 (3.8 g) was purified by the preparative HPLC using MeOH–H_2O (83:17) to yield **10** (1.8 g). Fr.3i (19.5 g) was chromatographed on a RP-C_{18} column eluting with MeOH–H_2O (from 50% to 80%) to give subfraction 3i1 to 3i8. Subfraction 3i4 (3.6 g) were further separated by repeated preparative HPLC eluted with MeCN–H_2O (30:70) to obtain **3** (58 mg) and **4** (67 mg). Subfraction 3i5 (2.8 g) were separated by repeated preparative HPLC eluted with MeCN–H_2O (30:70) to give pure **2** (65 mg). Compounds **1** (55 mg), **6** (72 mg), **7** (34 mg) and **9** (50 mg) were obtained from subfraction 3i6 by the preparative HPLC using MeCN–H_2O (30:70). Fr.3j (22.4 g) was submitted to a RP-C_{18} column eluted with MeOH–H_2O (from 50% to 80%) to afford six subfractions, 3j1–3j6. Subfraction 3j4 was separated by silica gel CC with $CHCl_3$–MeOH–H_2O (4:1:0.1) to yield **5** (60 mg). Fr.3l (8.4 g) was separated initially by RP-C_{18} CC, eluted with MeOH–H_2O (from 40% to 70%), to afford four subfractions (3l1–3l4). Compound **8** (185 mg) was isolated from subfraction 3l3 by silica gel CC with $CHCl_3$–MeOH–H_2O (5:2:0.1).

3.3.1. Ophiojaponin A (**1**)

White amorphous powder; $[\alpha]_D^{20}$ −65.3 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3434, 2931, 1634, 1041 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 1047.5157 $[M+Na]^+$ (calcd for $C_{52}H_{80}O_{20}Na$, 1047.5135).

3.3.2. Homo-aro-cholest-5-ene-3β,26-diol (**1a**)

Amorphous solid; $[\alpha]_D^{20}$ −31.8 (*c* 0.11, $CHCl_3$); IR (KBr) ν_{max} 3427, 2922, 1644, 1456, 1059 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ_H: 5.40 (1H, br s, H-6), 1.03 (3H, s, CH_3-18), 1.08 (3H, s, CH_3-19), 2.29 (3H, s, CH_3-21), 1.02 (3H, d, J = 6.7 Hz, CH_3-27), 6.98(1H, d, J = 7.4 Hz, H-16′), 6.92(1H, d, J = 7.4 Hz, H-22′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ_C: 37.3 (C-1), 32.4 (C-2), 71.9 (C-3), 42.5 (C-4), 141.2 (C-5), 121.6 (C-6), 32.1 (C-7), 30.9 (C-8), 50.4 (C-9), 36.8 (C-10), 21.3 (C-11), 36.9 (C-12), 47.2 (C-13), 57.6 (C-14), 31.8 (C-15), 140.8 (C-16), 151.7 (C-17), 16.5 (C-18), 19.5 (C-19), 131.3 (C-20), 14.7 (C-21), 139.4 (C-22), 31.2 (C-23), 34.7 (C-24), 36.2 (C-25), 68.4 (C-26), 16.8 (C-27), 122.6 (C-16′), 127.0 (C-22′). HRESIMS *m/z* 445.3079 $[M+Na]^+$ (calcd for $C_{29}H_{42}O_2Na$, 445.3077).

3.3.3. Ophiojaponin B (**2**)

White amorphous powder; $[\alpha]_D^{20}$ −61.6 (*c* 0.06, MeOH); IR (KBr) ν_{max} 3429, 2932, 1633, 1035 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 931.4674 $[M+Na]^+$ (calcd for $C_{47}H_{72}O_{17}Na$, 931.4662).

3.3.4. Ophiojaponin C (**3**)

White amorphous powder; $[\alpha]_D^{20}$ −40.7 (*c* 0.05, MeOH); IR (KBr) ν_{max} 3419, 2930, 1636, 1044 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 947.4623 $[M+Na]^+$ (calcd for $C_{47}H_{72}O_{18}Na$, 947.4611).

3.3.5. Ophiojaponin D (**4**)

White amorphous powder; $[\alpha]_D^{20}$ −40.5 (*c* 0.07, MeOH); IR (KBr) ν_{max} 3412, 2931, 1641, 1043 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 923.4619 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{18}Na$, 923.4611).

3.3.6. Ophiojaponin E (**5**)

White amorphous powder; $[\alpha]_D^{20}$ −34.8 (*c* 0.08, MeOH); IR (KBr) ν_{max} 3413, 2932, 1642, 1075 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 1069.5204 $[M+Na]^+$ (calcd for $C_{51}H_{82}O_{22}Na$, 1069.5190).

3.3.7. Ruscogenin 1-O-α-L-rhamnopyranosyl-(1 → 2)-4-O-sulfo-β-D-fucopyranosido-3-O-β-D-glucopyranoside (**8**)

White amorphous powder; $[\alpha]_D^{20}$ −64.6 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3436, 2927, 1627, 1047 cm^{-1}; ^1H and ^{13}C NMR (pyridine-d_5) data for the aglycone moiety, see Table 1; ^1H and ^{13}C NMR (pyridine-d_5) data for the sugar moieties, see Table 2; HRESIMS *m/z* 963.4260 $[M-H]^-$ (calcd for $C_{45}H_{71}O_{20}S$, 963.4265) and *m/z* 965.4412 $[M+H]^+$ (calcd for $C_{45}H_{73}O_{20}S$, 965.4410).

3.4. Acid hydrolysis of the saponins and determination of absolute configurations of sugar moieties

Compound **1** (21 mg) was dissolved in 1 M HCl in dioxane–H_2O

(1:1; 20 ml) and heated at 95 °C for 2 h under an Ar atmosphere. The solutions were individually evaporated to remove dioxane and extracted with $CHCl_3$ (3 × 10 ml). The $CHCl_3$ extract was purified by chromatography on silica gel column eluted with $CHCl_3$–$(CH_3)_2CO$ (50:1) to yield aglycone **1a** (5 mg). The H_2O layer was evaporated under vacuum, diluted repeatedly with H_2O, and evaporated in vacuo to furnish a neutral residue. A portion of the residue (2 mg) was dissolved in pyridine (0.5 ml) containing L-cysteine methyl ester hydrochloride (2 mg) and heated at 60 °C for 1 h, phenylisothiocyanate (2 μl) was then added, and the mixture was heated at 60 °C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC (Tanaka et al., 2007) using a model LC-20AT pump, a SPD-M20A photodiode array detector, and a SIL-20AHT autosampler (Shimadzu, Japan). The column used was a 250 mm × 4.60 mm i.d., 5 μm, Ultimate ® C_{18} column (Welch materials, Inc., USA); mobile phase MeCN–H_2O (25:75, v/v) containing 50 mM H_3PO_4; detection UV (250 nm); flow rate 0.8 ml/min; column temperature 35 °C. From the acid hydrolysate of **1**, D-glucose, L-rhamnose and D-xylose were identified by comparison of the retention times of their derivatives with those of authentic sugars (Sigma–Aldrich) derivatized in the same way, which showed retention times of 23.90, 17.52 and 28.30 min, respectively. The constituent sugars of compounds **2**–**5** were identified by the same method as **1**, and D-glucose and L-rhamnose were detected in **2**–**5**.

3.5. Cytotoxicity assay

Cytotoxic activities of all isolated compounds against MG-63 (osteosarcoma) and SNU387 (hepatoma) cell lines (obtained from the Shanghai Cell Bank, Chinese Academy of Sciences) were evaluated by using the MTT method (Mosmann, 1983). Briefly, test cells were seeded in 96-well plates at a density of 5×10^3 cells per well in the medium. After being cultivated at 37 °C for 24 h in a 5% CO_2, cells were treated with tested compounds at different concentrations and incubated for 48 h. Then 100 μl of MTT solution (1 mg/ml) was added to each well and incubated for 4 h, after this, 100 μl of "triplex solution" (10% SDS/5% iBuOH/12 mM HCl) was added into each well, and the plate was allowed to stand overnight in the incubator. Absorbance was recorded on a microplate reader at 490 nm. Cell viability (%) was measured and cell growth curve was plotted. IC_{50} values were calculated by the Reed and Muench method (Reed and Muench, 1938).

Acknowledgments

We are very grateful to the Key Research Program of the Chinese Academy of Sciences, Grant No. KSZD-EW-Z-004 and the National Natural Science Foundation of China (No. 21561142003) for financial support.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.phytochem.2017.01.006.

References

Adinolfi, M., Parrilli, M., Zhu, Y.X., 1990. Terpenoid glycosides from *Ophiopogon japonicus* roots. Phytochemistry 29, 1696–1699.

Agrawal, P.K., 1992. NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides. Phytochemistry 31, 3307–3330.

Agrawal, P.K., 2004. NMR spectral investigations, part 51. Dependence of ^1H NMR chemical shifts of geminal protons of glycosyloxy methylene (H_2–26) on the orientation of the 27-methyl group of furostane-type steroidal saponins. Magn. Reson. Chem. 42, 990–993.

Ali, Z., Smillie, T.J., Khan, I.A., 2013. Cholestane steroid glycosides from the rhizomes of *Dioscorea villosa* (wild yam). Carbohyd. Res. 370, 86–91.

Anh, N.T.H., Van Sung, T., Porzel, A., Franke, K., Wessjohann, L.A., 2003. Homoisoflavonoids from *Ophiopogon japonicas* Ker-Gawler. Phytochemistry 62, 1153–1158.

Chang, J.M., Shen, C.C., Huang, Y.L., Chien, M.Y., Ou, J.C., Shieh, B.J., Chen, C.C., 2002. Five new homoisoflavonoids from the tuber of *Ophiopogon japonicus*. J. Nat. Prod. 65, 1731–1733.

Chen, J.J., Zhu, Z.L., Luo, S.D., 2000. Ci-xi-ophiopogon A and B, C_{27} steroiddal glycosides from *Ophiopogon japonicus*. Acta Bot. Yunnan 22, 97–102.

Cheng, Z.H., Wu, T., Bligh, S.W.A., Bashall, A., Yu, B.Y., 2004. Cis-eudesmane sesquiterpene glycosides from *Liriope muscari* and *Ophiopogon japonicus*. J. Nat. Prod. 67, 1761–1763.

Dai, H.F., Deng, S.M., Tan, N.H., Zhou, J., 2005. A new steroidal glycoside from *Ophiopogon japonicus* (Thunb.) Ker-Gawl. J. Integr. Plant Biol. 47, 1148–1152.

Dai, H.F., Zhou, J., Dai, Z.T., Zhu, Z.T., 2001. New steroidal glycosides from *Ophiopogon japonicus*. Acta Bot. Sin. 43, 97–100.

Duan, C.L., Li, Y.J., Li, P., Jiang, Y., Liu, J.X., Tu, P.F., 2010. Spirostanol saponins from the fibrous roots of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. Helv. Chim. Acta 93, 227–232.

Jiangsu College of New Medicine, 1986. A Dictionary of the Trditional Chinese Medicines. Shanghai Science and Technology Press, Shanghai, p. 2082.

Kang, Z.Y., Zhang, M.J., Wang, J.X., Liu, J.X., Duan, C.L., Yu, D.Q., 2013. Two new furostanol saponins from the fibrous root of Ophiopogon japonicus. J. Asian Nat. Prod. Res. 15, 1230–1236.

Kou, J.P., Sun, Y., Lin, Y.W., Cheng, Z.H., Zheng, W., Yu, B.Y., Xu, Q., 2005. Anti-inflammatory activities of aqueous extract from Radix *ophiopogon japonicas* and its two constituents. Biol. Pharm. Bull. 28, 1234–1238.

Lee, C.L., Hwang, T.L., Yang, J.C., Cheng, H.T., He, W.J., Yen, C.T., Kuo, C.L., Chen, C.J., Chang, W.Y., Wu, Y.C., 2014. Anti-Inflammatory spirostanol and furostanol saponins from *Solanum macaonense*. J. Nat. Prod. 77, 1770–1783.

Li, N., Zhang, L., Zeng, K.W., Zhou, Y., Zhang, J.Y., Che, Y.Y., Tu, P.F., 2013. Cytotoxic steroidal saponins from *Ophiopogon japonicus*. Steroids 78, 1–7.

Liu, S.Q., Kang, L.P., Zhang, J., Liu, Y.X., Zhao, Y., Liu, B., Ma, B.P., 2016. New sesquiterpenoid glycoside and phenylpropanoid glycosides from the tuber of *Ophiopogon japonicus*. J. Asian Nat. Prod. Res. 18, 520–527.

Liu, Y.W., Zhu, X., Lu, Q., Wang, J.Y., Li, W., Wei, Y.Q., Yin, X.X., 2012. Total saponins from rhizome Anemarrhenae ameliorate diabetes-associated cognitive decline in rats: involvement of amyloid-beta decrease in brain. J. Ethnopharmacol. 139, 194–200.

Mimaki, Y., Takaashi, Y., Kuroda, M., Sashida, Y., Nikaido, T., 1996. Steroidal saponins from *Nolina recurvata* stems and their inhibitory activity on cyclic AMP phosphodiesterase. Phytochemistry 42, 1609–1615.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Nakanishi, H., Kameda, N., 1987. Studies on the component of Ophiopogonis tuber (China) II. Yakugaku Zasshi 107, 780–784.

Qi, J., Hu, Z.F., Zhou, Y.F., Hu, Y.J., Yu, B.Y., 2015. Steroidal sapogenins and glycosides from the fibrous roots of *Ophiopogon japonicus* and *Liriope spicata* var. prolifera with anti-inflammatory activity. Chem. Pharm. Bull. 63, 187–194.

Reed, L.J., Muench, H., 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27, 493–497.

Shen, S., Chen, C.J., Bu, R., Ga, L., Li, G.Y., Tan, Y., Li, X., Wang, J.H., 2011. Three new steroidal saponins from *Fritillaria pallidiflora*. J. Asian Nat. Prod. Res. 13, 1014–1022.

Sparg, S.G., Light, M.E., Staden, J., 2004. Biological activities and distribution of plant saponins. J. Ethnopharmacol. 94, 219–243.

Tagawa, C., Okawa, M., Ikeda, T., Yoshida, T., Nohara, T., 2003. Homo-cholestane glycosides from *Solanum aethiopicum*. Tetrahedron Lett. 44, 4839–4841.

Tanaka, T., Nakashima, T., Ueda, T., Tomii, K., Kouno, I., 2007. Facile discrimination of aldose enantiomers by reversed-phase HPLC. Chem. Pharm. Bull. 55, 899–901.

Vasanthi, H.R., ShriShriMal, N., Das, D.K., 2012. Phytochemicals from plants to combat cardiovascular disease. Curr. Med. Chem. 19, 2242–2251.

Wang, J.Z., Chen, X.B., Wang, F.P., 2008a. Structural elucidation of steroidal glycosides from *Ophiopogon japonicus*. Chin. J. Org. Chem. 28, 1620–1623.

Wang, J.Z., Ye, L.M., Chen, X.B., 2008b. A new C-27-steroidal glycoside from *Ophiopogon japonicus*. Chin. Chem. Lett. 19, 82–84.

Watanabe, Y., Sanada, S., Ida, Y., Shoji, J., 1984. Comparative studies on the constituents of Ophiopogonis tuber and its cogeners .3. Studies on the constituents of the subterranean part of *Ophiopogon ohwii* okuyama and *O. jaburan* (kunth) lodd. Chem. Pharm. Bull. 32, 3994–4002.

Watanabe, Y., Sanada, S., Tada, A., Shoji, J., 1977. Studies on the constituents of Ophiopogonis tuber. IV. On the structures of ophiopogonin A, B', C, C', D'. Chem. Pharm. Bull. 25, 3049–3056.

Xiao, C.M., Huang, J., Zhong, X.M., Tan, X.Y., Deng, P.C., 2009. Two new homo-aro-cholestane glycosides and a new cholestane glycoside from the roots and rhizomes of *Paris polyphylla* var. pseudothibetica. Helv. Chim. Acta 92, 2587–2595.

Xiao, C.M., Xi, Z., Xiao, Y.C., Ran, J., Yu, M., Huang, L., Huang, J., 2010. The NMR characteristic of a kind of novel homo-aro-cholestane aglycones. West China J. Pharm. Sci. 25, 722–725.

Xiao, P.G., 2002. Modern Chinese Materia Medica. Chemical Industry Press, Beijing, pp. 77–81.

Xu, Y.J., Xu, T.H., Hao, L.Z., Zhao, H.F., Xie, S.X., Si, Y.S., Han, D., Xu, D.M., 2008. Two new steroidal glucosides from *Ophiopogon japonicus* (L.f.) Ker-Gawl. Chin. Chem. Lett. 19, 825–828.

Yan, R.Y., Liu, Y.X., Kang, L.P., Zhao, Y., Sun, X.G., Zhang, J., Jia, D.X., Ma, B.P., 2016.

Steroidal saponins from the fresh tubers of *Ophiopogon japonicus*. Phytochem. Lett. 15, 72–80.
Ye, Y., Qu, Y., Tang, R.Q., Cao, S.N., Yang, W., Xiang, L., Qi, J.H., 2013. Three new neuritogenic steroidal saponins from *Ophiopogon japonicus* (Thunb.) Ker-Gawl. Steroids 78, 1171–1176.
Zhang, T., Kang, L.P., Yu, H.S., Liu, Y.X., Zhao, Y., Xiong, C.Q., Zhang, J., Zou, P., Song, X.B., Liu, C., Ma, B.P., 2012. Steroidal saponins from the tuber of *Ophiopogon japonicus*. Steroids 77, 1298–1305.
Zhou, C.X., Zou, L., Mo, J.X., Wang, X.Y., Yang, B., He, Q.J., Gan, L.S., 2013. Homoisoflavonoids from *Ophiopogon japonicus*. Helv. Chim. Acta 96, 1397–1405.
Zhou, Y.F., Qi, J., Zhu, D.N., Yu, B.Y., 2008. Two new steroidal glycosides from *Ophiopogon japonicus*. Chin. Chem. Lett. 19, 1086–1088.

Please cite this article in press as: Wang, L., et al., Homo-aro-cholestane, furostane and spirostane saponins from the tubers of *Ophiopogon japonicus*, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.006