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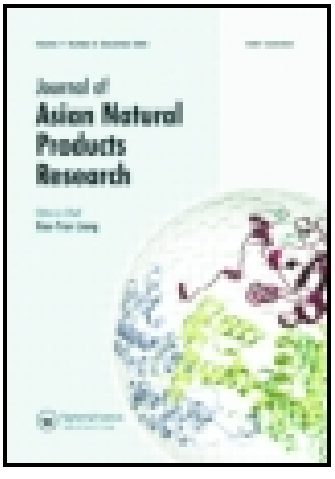

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Studies on chemical constituents of Ophiopogon japonicus

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Published online: 01 Aug 2014.

To cite this article: Yue Liu, Ling-Zhi Meng, Sheng-Xu Xie, Tun-Hai Xu, Lian-kun Sun, Tong-Hua Liu, Ya-Juan Xu & Dong-Ming Xu (2014): Studies on chemical constituents of Ophiopogon japonicus, Journal of Asian Natural Products Research, DOI: 10.1080/10286020.2014.935348

To link to this article: http://dx.doi.org/10.1080/10286020.2014.935348

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Journal of Asian Natural Products Research, 2014
http://dx.doi.org/10.1080/10286020.2014.935348

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Studies on chemical constituents of *Ophiopogon japonicus*

Yue Liu[a], Ling-Zhi Meng[a], Sheng-Xu Xie[a], Tun-Hai Xu[b]*, Lian-kun Sun[c], Tong-Hua Liu[b], Ya-Juan Xu[a]* and Dong-Ming Xu[a]

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(*Received 6 August 2013; final version received 12 June 2014*)

Two new and six known steroidal glucosides were isolated from the tuber of *Ophiopogon japonicus*. The new steroidal glucosides were established as (20*R*,25*R*)-26-*O*-β-D-glucopyranosyl-3β,26-dihydroxycholest-5-en-16,22-dioxo-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**1**) and 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5-en-3β,14α,17α,22α,26-pentaol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**3**) on the basis of spectroscopic data as well as chemical evidence.

Keywords: *Ophiopogon japonicus*; Liliaceae; steroidal glucoside

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1. Introduction

The tuber of *Ophiopogon japonicus* (L.f.) Ker-Gawl., "maidong," is a traditional Chinese medicine and has various medical functions for curing cardiovascular diseases and bacterial infections. Phytochemical studies on this plant were reported previously [1]. In the previous papers, we reported the isolation of steroidal glucosides from the tuber of *O. japonicus* (L.f.) Ker-Gawl [2,3]. Further examination led to isolation of two new glucosides (compounds **1** and **3**) together with six known steroidal glucosides. Their structures were determined by combination of chemical methods with the analysis of NMR, HR-MS, and physical data.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was assigned as $C_{45}H_{72}O_{18}$ on the positive ion HR-ESI-MS ($[M + Na]^+$, *m/z* 923.4583). ESI-MS showed fragment ion peaks at *m/z* 899 $[M-H]^-$, 881 $[M-H-H_2O]^-$, and 735 $[M-H-18-146]^-$ in negative ion mode and those at *m/z* 923 $[M + Na]^+$, 761 $[M + Na-162]^+$, 777 $[M + Na-146]^+$, and 615 $[M + Na-146-162]^+$ in positive ion mode, suggesting the presence of two hexoses and one deoxyhexose moiety. The ^1H NMR spectrum of **1** showed diagnostic proton signals of four methyl groups at δ 0.63 (3H, s), 0.92 (3H, s), 0.86 (3H, d, $J = 6.0$ Hz), and 1.39 (3H, d, $J = 6.6$ Hz), one oxymethine at δ 4.17 (1H, m), one oxymethylene at δ 3.45 (1H, dd, $J = 6.0$, 9.6 Hz) and 3.91 (1H, m), one olefinic group at δ 5.18 (1H, br s), and three anomeric protons at δ 4.72 (1H, d, $J = 7.8$ Hz), 6.27 (1H, br s), and 4.93 (1H, d, $J = 7.2$ Hz), and a pentosyl H-6 signal at δ 1.66 (3H, d, $J = 6.0$ Hz). In ^{13}C NMR spectrum of **1**, characteristic carbon signals of four methyl groups at δ 14.3, 19.4, 16.3, and 17.5, three anomeric carbons at δ 100.5, 102.2, and 105.1, olefinic carbon signals at δ 141.1 and

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121.5, and two keto functions at δ 216.9 and 212.6 were observed. The ^{1}H and ^{13}C NMR spectral data (Table 1) of **1** are assigned unequivocally according to its $^{1}H-^{1}H$ COSY, HMQC, and HMBC analysis. By comparing ^{1}H and ^{13}C NMR data of compound **1** with those of compound **2** (Figure 1) which was identified as (20*S*,25*R*)-26-*O*-β-D-glucopyranosyl-3β,26-dihydroxycholesten-5-en-16,22-dioxo-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside on the basis of its identical spectral evidences as the reference [4], except for almost identical data of sugar moieties and most of aglycone units, obvious differences lied in the spectral data of C-12, C-13, C-14, C-16, C-17, C-18, C-20, C-21, and C-22 in ^{13}C NMR spectrum, and H-17, H-18, H-20, and H-21 in ^{1}H NMR spectrum. Significant upfield shifts of C-12, C-14, C-16, C-17, and C-22 carbon signals by Δδ −1.0, −0.8, −0.9, −2.4, and −0.5 ppm, respectively; downfield shifts of C-13, C-18, C-20, and C-21 carbon signals by Δδ 0.7, 1.3, 0.3, and 0.6; characteristic proton signals at δ 2.33 (1H, d, $J = 8.4$ Hz, H-17), 0.63 (3H, s, H-18), 2.71 (1H, m, H-20), and 1.39 (3H, $J = 6.6$ Hz, H-21) in **1**; and those at δ 2.62 (1H, d, $J = 10.2$ Hz, H-17), 0.56 (3H, s, H-18), 2.81 (1H, m, H-20), and 0.92 (3H, $J = 6.6$ Hz, H-21) in **2** inferred that compound **1** was the C-17 or C-20 epimer of compound **2**. In NOESY spectrum, no correlation between H-18 (δ 0.63) and H-17 (δ 2.33) and a weak correlation between H-17 and H-9 (δ 0.83) were observed, inferring the α-orientation of H-17. The correlations in NOESY spectrum between H-18 (δ 0.63) and H-21 (δ 1.39), H-17 (δ 2.33) and H-20 (δ 2.71) indicated the configuration of C-20 in **1** was *R*. For CD spectra (Figure 2), compound **2** had a negative Cotton effect at 292.8 nm and compound **1** had a more stronger negative Cotton effect at 297.4 nm. In accordance with the octant rule of octahydro-2*H*-inden-2-one [5,6], negative Cotton effect of cyclopentanone carbonyl will be observed for 17-α-H (*R*), while its negative Cotton effect will be significantly weakened for 17-β-H (*S*) (Figure 3). Thus, it was inferred that C-17 was *R*-configuration (17-α-H) in compounds **1** and **2**. Compound **1** was the C-20*R* epimer of compound **2**. Besides, C-25 configuration of **1** was deduced to be *R* based on the difference of chemical shifts ($\Delta\delta_{ab} = \delta H_a - \delta H_b$) of the geminal protons at H-26 ($\delta H_a - \delta H_b = 0.46 < 0.48$). It had been described that $\Delta\delta_{ab}$ is usually > 0.57 ppm in 25*S* compounds and < 0.48 in 25*R* compounds [7–9].

Acidic hydrolyzation of **1** with mineral acid afforded rhamnose and glucose as the sugar components identified on TLC by comparison with authentic samples. The large coupling constants ($^{3}J_{1,2} > 7.0$ Hz) were consistent with the β-configuration of glucose and the splitting (br s) of anomeric proton signal inferred the α-configuration of rhamnose [10–12]. The 3,26-bisdesmoside structure of **1** was characterized by a HMBC experiment. Long-range correlations were observed between H-1 of Glc at δ 4.93 and C-3 of the aglycone at δ 78.0, between H-1 of Rha at δ 6.27 and C-2 of Glc at δ 79.8, and between H-1′ of Glu′ at δ 4.72 and C-26 of the aglycone at δ 75.3. Consequently, compound **1** was elucidated to be (20*R*,25*R*)-26-*O*-β-D-glucopyranosyl-3β,26-dihydroxycholest-5-en-16,22-dioxo-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

Compound **3** was obtained as a white amorphous powder. The molecular formula was assigned as $C_{45}H_{74}O_{20}$ on the basis of the positive-ion HR-ESI-MS peak at *m/z* 957.4692 $[M + Na]^+$. The negative ion ESI-MS showed fragment ion peaks at *m/z* 933 $[M-H]^-$, 915 $[M-H-H_2O]^-$, 777 $[M-H-18-162]^-$, and 463 $[M-H-162-146-162]^-$, and the positive ion ESI-MS showed fragment ion peaks at *m/z* 957 $[M + Na]^+$, 939 $[M + Na-H_2O]^+$, 921 $[M + Na-2H_2O]^+$, 775 $[M + Na-H_2O-$

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Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1–3** (600/150 MHz in pyridine-d_5; δ in ppm, J in Hz).

	δ_c			δ_H (J, Hz)		
No.	**1**	**2**	**3**	**1**	**2**	**3**
1	38.4	37.5	37.9	1.21 (m), 1.51 (m)	1.25 (m), 1.56 (m)	0.89 (m), 1.68 (m)
2	30.3	30.3	30.4	1.76 (m), 2.00–2.02 (m)	1.75 (m), 2.01–2.03 (m)	1.74 (m), 1.97 (m)
3	78.0	78.0	78.0	4.17 (m)	4.18 (m)	4.11-4.12 (m)
4	39.1	39.1	39.1	2.09 (dd, 18.0, 7.8), 2.63 (m)	2.03-2.04 (m), 2.62 (m)	1.66 (m), 2.64-2.72 (m)
5	141.1	141.1	140.4			
6	121.5	121.5	122.4	5.18 (br s)	5.17 (br s)	5.26 (br s)
7	32.1	32.1	26.3	1.39 (m), 1.70–1.71 (m)	1.38 (m), 1.74 (m)	1.68 (m), 2.36-–2.41 (m)
8	31.1	31.1	36.2	1.32 (m)	1.32 (m)	1.82 (m)
9	50.2	50.1	43.7	0.81–0.83 (m)	0.80–0.84 (m)	1.66 (m)
10	37.2	37.2	37.5			
11	20.9	20.8	20.2	1.21 (m), 1.32 (m)	1.25 (m), 1.34 (m)	0.92 (m), 1.52 (m)
12	39.3	40.3	26.8	2.48–2.53 (m), 2.71 (m)	2.53–2.58 (m), 2.70-2.72 (m)	1.26 (m), 1.74 (m)
13	42.5	41.8	48.6			
14	50.4	51.2	87.8	1.25 (m)	1.28 (m)	
15	39.0	38.8	40.4	1.76 (m), 1.24 (m)	1.75 (m), 1.24 (m)	2.43-2.48 (m), 1.74 (m)
16	216.9	217.8	90.9			4.99 (dd, 6.6, 7.2)
17	64.1	66.5	91.7	2.33 (d, 8.4)	2.62 (d, 10.2)	
18	14.3	13.0	20.9	0.63 (s)	0.56 (s)	1.02 (s)
19	19.4	19.5	19.5	0.92 (s)	0.94 (s)	1.02 (s)
20	44.1	43.8	44.0	2.71 (m)	2.81 (m)	2.50-2.53 (m)
21	16.3	15.7	10.8	1.39 (d, 6.6)	0.92 (d, 6.0)	1.29 (d, 7.2)
22	212.6	213.1	111.3			
23	37.3	37.3	37.2	1.57–1.60 (m), 2.62 (m)	1.57 (m), 2.63 (m)	1.68 (m), 1.96 (m)
24	28.2	27.9	28.2	1.51 (m), 1.83 (m)	1.56 (m), 1.83 (m)	1.69 (m), 1.74 (m)
25	33.6	33.6	34.4	1.83 (m)	1.88 (m)	1.82 (m)
26	75.3	75.4	75.4	3.45 (dd, 6.0, 9.6), 3.91 (m)	3.51 (m), 3.93 (m)	3.52 (dd, 6.0, 9.6), 3.91 (m)
27	17.5	17.6	17.6	0.86 (d, 6.0)	0.89 (d, 5.4)	0.90 (d, 6.6)
3-*O*-Glc						
1	100.5	100.5	100.4	4.93 (d, 7.2)	4.94 (d, 7.2)	4.92 (d, 7.2)

(*Continued*)

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Table 1 – *continued*

	δ_c			δ_H (*J*, Hz)		
No.	**1**	**2**	**3**	**1**	**2**	**3**
2	79.8	79.8	79.8	4.17 (m)	4.18 (m)	4.16 (m)
3	78.7	78.6	78.6	3.91 (m)	3.93 (m)	3.93 (m)
4	71.9	72.0	72.0	4.11 (m)	4.11 (m)	4.05 (m)
5	77.9	78.0	78.0	3.86 (m)	3.87 (m)	3.85 (m)
6	62.8	62.8	62.8	4.25 (m), 4.40 (br d, 11.4)	4.25 (m), 4.41 (br d, 10.2)	4.23 (m), 4.39 (br d, 11.4)
Rha						
1	102.2	102.2	102.2	6.27 (br s)	6.28 (br s)	6.27 (br s)
2	72.7	72.7	72.7	4.69 (br s)	4.69 (br s)	4.68 (br s)
3	73.0	73.0	73.0	4.52 (br d, 9.0)	4.52–4.53 (m)	4.51 (br d, 6.6)
4	74.3	74.3	74.3	4.22 (m)	4.22 (m)	4.24 (m)
5	69.6	69.6	69.6	4.86–4.90 (m)	4.88–4.90 (m)	4.87–4.92 (m)
6	18.8	18.8	18.8	1.66 (d, 6.0)	1.66 (d, 6.0)	1.67 (d, 6.0)
26-*O*-Glc						
1′	105.1	105.0	105.1	4.72 (d, 7.8)	4.74 (d, 7.2)	4.71 (d, 7.8)
2′	75.1	75.2	75.4	4.23 (m)	4.24 (m)	4.24 (m)
3′	78.8	78.8	78.8	4.14 (m)	4.15 (m)	4.16 (m)
4′	72.9	71.9	71.8	4.11 (m)	4.11 (m)	4.05 (m)
5′	78.4	78.4	78.4	3.85 (m)	3.85 (m)	3.83 (m)
6′	63.0	63.0	63.0	4.24 (m), 4.45 (br d, 11.4)	4.24 (m), 4.45 (br d, 10.8)	4.22 (m), 4.43 (br d, 11.4)

Note: Assignments based on ^{1}H–^{1}H COSY, HMQC, and HMBC experiments.

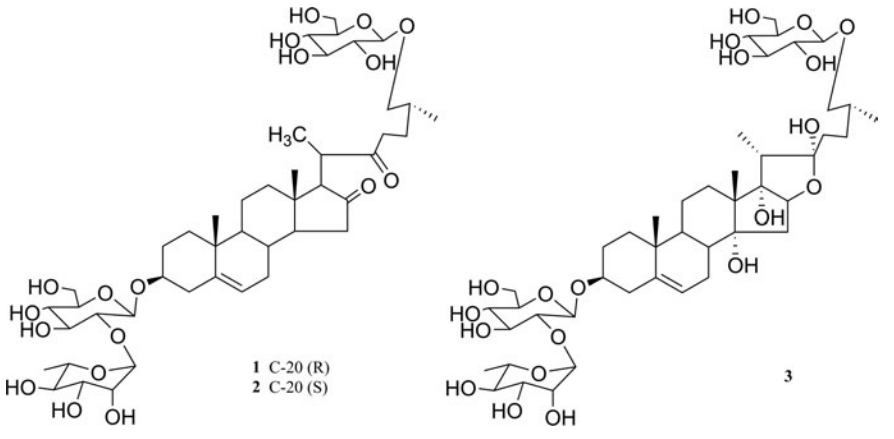

Figure 1. Structures of compounds **1–3**.

146]$^+$, and 613 [M + Na−H_2O−146−162]$^+$, suggesting the presence of two hexoses and one deoxyhexose moieties. The ^{1}H NMR spectrum of **3** showed two methyl singlets at δ 1.02 (3H, s) and 1.02 (3H, s), three methyl doublets at δ 0.90 (3H, d, $J = 6.6$ Hz), 1.29 (3H, d, $J = 7.2$ Hz), and 1.67 (3H, d, $J = 6.0$ Hz), three anomeric proton signals at δ 4.92 (1H, d, $J = 7.2$ Hz, Glc-H-1), 6.27 (1H, br s), and 4.71 (1H, d, $J = 7.8$ Hz, Glc′-H-1), and an olefinic proton signal at δ 5.26 (1H, br s). These data indicated that compound **3** contains three sugar moieties. The ^{13}C NMR spectrum showed 45 carbon signals, in which the characteristic olefinic carbon signals at δ 140.4 and 122.4, hemiketal carbon signal at δ 111.3, and an oxymethylene carbon signal at δ 75.4

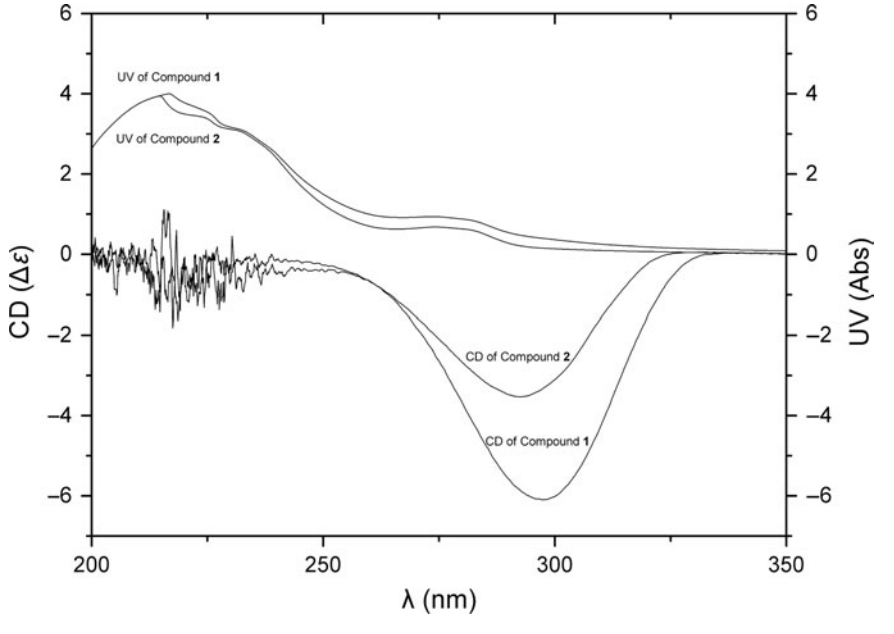

Figure 2. UV and CD spectra of compounds **1** and **2**.

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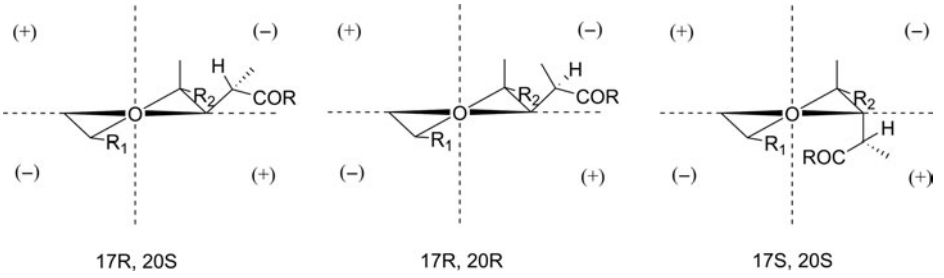

Figure 3. Analysis for CD spectra of compounds **1** and **2** in accordance with the octant rule (R, R_1, and R_2: other part of the molecules).

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were assigned to C-5, C-6, C-22, and C-26, respectively, by ^{1}H–^{1}H COSY, HSQC, and HMBC experiments. The carbon signals at δ 75.4 (C-26), 111.3 (C-22), 140.4 (C-5), and 122.4 (C-6) indicated that **3** is a furostanol saponin with Δ5 (6) [13–16].

In the HMBC spectrum, the proton signals at δ 1.29 (H-21), 1.02 (H-18), 2.51 (H-20), 2.46 (H-15), and 4.99 (H-16) showed the long-range correlations with the carbon signal at δ 91.7, and the proton signals at δ 2.46 (H-15) and δ 1.02 (H-18) showed the long-range correlations with the carbon signal at δ 87.8. Upon the combined analysis of these long-range correlations and the ^{1}H–^{1}H COSY and HSQC spectra of **3**, carbon signals at δ 91.8 and 88.1 could be assigned to C-17 and C-14, respectively. Comparison of the carbon signals of **3**, especially for those at δ 87.8 (C-14), 90.9 (C-16), and 91.7 (C-17), with those of cixi-ophiopogons A and B indicated the presence of α-orientations for C-14 and C-17 hydroxy groups [15,17]. The α-orientation of C-22 hydroxy group of the aglycone moiety was deduced from the hemiketal carbon signal at δ 111.3, approximately 3–4 ppm higher than that of β-configuration [18,19]. The chemical shift difference between two proton signals of H-26 ($\Delta\delta_{ab} = 0.39 < 0.48$) demonstrated the 25$R$-configuration of **3** [7–9]. Comparison of the NMR data of **3** with those of ophiopogonin H [20] also indicated that **3** contained the same aglycone as ophiopogonin H. Thus, the aglycone of **3** was identified as (25R)-furost-5-en-3β,14α,17α,22α,26-pentaol.

Acidic hydrolysis of **3** with mineral acid afforded glucose and rhamnose as the sugar components identified on TLC by comparison with authentic samples. The large coupling constants ($^3J_{1,2} > 7.0$ Hz) were consistent with the β-configuration of glucoses and the splitting (br s) of anomeric proton signal inferred the α-configuration of rhamnose [10–12]. The 3,26-bisdesmoside structure of **3** was characterized by a HMBC experiment, in which correlations were observed between H-1 of Glc at δ 4.92 and C-3 of the aglycone at δ 78.0, between H-1 of Rha at δ 6.27 and C-2 of Glc at δ 79.8, and between H-1′ of Glu′ at δ 4.71 and C-26 of the aglycone at δ 75.4. Consequently, compound **3** was determined to be 26-*O*-β-D-glucopyranosyl-(25R)-furost-5-en-3β,14α,17α,22α,26-pentaol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside.

The other compounds were identified as chonglouoside VI (**4**) [21], (25R)-5-en-spirost-3β,14α,17α-trihydroxy-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside (**5**) [22], 25R-dracaenoside F (**6**) [23], (25R)-spirost-5-en-3β,17α-dihydroxy-3-*O*-α-L-rhamnopyranosisyl-(1 → 2)-[β-D-xylopyranosyl-(1 → 4)]-β-D-glucopyranoside (**7**) [24], and ophiopojaponin C (**8**) [25], by comparison of their physical

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and spectroscopic data with those reported in the literature, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on an X-6 microscope apparatus and are uncorrected (Beijing Fukai Instrument Co., Ltd, Beijing, China). Optical rotations were determined on a WZZ-2SS auto-polarimeter (Shanghai Jingke Scientific Instrument Co., Ltd, Shanghai, China). UV and CD spectra were obtained on a JASCO J-820 spectrometer (JASCO International Co. Ltd, Tokyo, Japan). NMR spectra were obtained on Bruker AV600 and AV400 instruments (Bruker Corporation, Rheinstetten, Switzerland), using tetramethylsilane as the internal standard. The HR-ESI-MS was recorded on an IonSpec HiResESI FTICR instrument (IonSpec Co., Lake Forest, CA, USA). ESI-MS were recorded on Finnigan Mat LCQ mass spectrometry (Thermo Fisher Scientific, Inc., West Palm Beach, FL, USA). High-performance liquid chromatography (HPLC) (Shimadzu Corporation, Kyoto, Japan) was carried out using an octadecylsilane-bonded silica gel column (Megres-C_{18}, 250 mm × 4.6 mm, 5 μm). Prep-HPLC (NP 7000, Hanbon Sci & Tech Co., Ltd, Huaian, Jiangsu, China) was carried out using an octadecylsilane-bonded silica gel column (Megres-C_{18}, 250 mm × 10 mm, 5 μm). Column chromatography was carried out on macroporous resin D_{101} made in Shandong Lu Neng Gel Co. (Shandong, China), silica gel (200–300 mesh, Qingdao Oceanic Chemical Industry, Qingdao, China), and reversed-phase silica gel (50 μm, YMC Co., Ltd, Kyoto, Japan). TLC was carried out on aluminum sheets (20 × 20 cm, RP-18F_{254S}, MERCK KGaA, Darmstadt, Germany).

3.2 Plant material

The tubers of *O. japonicus* were purchased from the Company of Chinese Medicinal Materials in Chengdu, Sichuan Province, China, and identified by Professor Minglu Deng, Changchun University of Chinese Medicine. A voucher specimen (No. 2009012) has been deposited in the Phytochemistry Laboratory of Jilin Academy of Chinese Medicine Sciences.

3.3 Extraction and isolation

The dried and powdered tubers of *O. japonicus* (10 kg) were exhaustively extracted with 70% EtOH. The extract was concentrated under vacuum to give a water solution which was then chromatographed on macroporous resin D_{21} (10 kg), eluting with water until the eluate was colorless and then with 80% EtOH (80 l). The 80% EtOH solution was concentrated and partitioned with EtOAc and *n*-BuOH, successively, to yield EtOAc extract (11 g) and *n*-BuOH layer extract (20 g). The *n*-BuOH extract was chromatographed on silica gel eluted with $CHCl_3$:MeOH (11:1). Fr. 189–Fr. 196 was separated by HPLC (*v*: 4.5 ml min^{-1}) with MeCN:H_2O (34:56) to yield **1** (17.8 mg, t_R: 15.465 min) and **2** (7.0 mg, t_R: 17.565 min). Fr. 291–Fr. 300 was separated by HPLC (*v*: 4.5 ml min^{-1}) with MeCN:H_2O (35:55) to yield **3** (70 mg, t_R: 7.782 min). The EtOAc extract was chromatographed on silica gel eluted with $CHCl_3$:MeOH (12:1) and ODS silica gel with MeOH–H_2O (8:2). Fr. 33–Fr. 50 was separated by HPLC (*v*: 4.5 ml min^{-1}) with MeOH:H_2O (78:22) to yield **4** (72 mg, t_R: 18.432 min), **5** (93 mg, t_R: 13.996 min), and **6** (98 mg, t_R: 20.132 min). Fr. 51–Fr. 61 was separated by HPLC (*v*: 4.0 ml min^{-1}) with MeOH:H_2O (78:22) to yield **7** (78 mg, t_R: 23.848 min). Fr. 83–Fr. 95 was separated by HPLC (*v*: 4.5 ml min^{-1}) with MeOH:H_2O (75:25) to yield **8** (13.2 mg, t_R: 20.198 min).

3.3.1 Compound **1**

Amorphous powder; m.p. 131.1–132.3°C; $[\alpha]_D^{27}$ −59.3 ($c = 0.30$, 30% aqueous

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solution of acetone); UV ($c = 7.11 \times 10^{-4}$ M, 70% aqueous solution of CH_3OH) λ_{max} (log ε): 216.7 (3.75), 274.0 (3.12) nm; CD ($c = 7.11 \times 10^{-4}$ M, 70% aqueous solution of CH_3OH): $\Delta\varepsilon_{297.4nm}$ −6.1. ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data are given in Table 1. ESI-MS: negative ion mode: *m/z* 899 $[M-H]^-$, 881 $[M-H-H_2O]^-$, 735 $[M-H-18-146]^-$; positive ion mode: *m/z* 923 $[M+Na]^+$, 761 $[M+Na-162]^+$, 777 $[M+Na-146]^+$, 615 $[M+Na-146-162]^+$. HR-ESI-MS: *m/z* 923.4583 $[M+Na]^+$ (calcd for $C_{45}H_{72}O_{18}Na$, 923.4616).

3.3.2 *Compound* **2**

UV ($c = 1.20 \times 10\text{-}^3$M, 70% aqueous solution of CH_3OH) λ_{max} (log ε): 214.7 (3.52), 274.1 (2.75) nm; CD (c 1.20×10^{-3}M, 70% aqueous solution of CH_3OH): $\Delta\varepsilon_{292.8nm}$ −3.5). ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data are given in Table 1.

3.3.3 *Compound* **3**

Amorphous powder; m.p. 179.1–180.0 °C; $[\alpha]_D^{27}$ −58.0 ($c = 0.10$, 30% aqueous solution of acetone). ^1H NMR (600 MHz, pyridine-d_5) and ^{13}C NMR (150 MHz, pyridine-d_5) spectral data are given in Table 1. ESI-MS: negative ion mode: *m/z* 933 $[M-H]^-$, 915 $[M-H-H_2O]^-$, 777 $[M-H-18-162]^-$, 463 $[M-H-162-146-162]^-$; positive ion mode: *m/z* 957 $[M+Na]^+$, 939 $[M+Na-H_2O]^+$, 921 $[M+Na-2H_2O]^+$, 775 $[M+Na-H_2O-146]^+$, 613 $[M+Na-H_2O-146-162]^+$. HR-ESI-MS: *m/z* 957.4692 $[M+Na]^+$ (calcd for $C_{45}H_{74}O_{20}Na$, 957.4671).

3.4 *Acid hydrolysis*

Compounds **1** and **3** (10 mg) were, respectively, dissolved in 1 mol/l HCl in MeOH:H_2O (1:1) and each refluxed for 2 h. Their reaction mixtures were neutralized with $NaHCO_3$ and extracted with $CHCl_3$ for three times. Each water phase was chromatographed on the silica gel HPTLC with the system of *n*-BuOH:*i*-PrOH:H_2O (10:5:4, homogenous), then the brown-colored spots were visualized by heating after spraying with phenylamine-ortho-benzene-dicarboxylic acid reagent. Glucose and rhamnose were detected by comparison with the authentic samples. Each remaining aqueous layer was desalted through 001 × 7 (732) resin column and concentrated to dryness. The residue was dissolved in pyridine (1 ml) and then L-cysteine methyl ester hydrochloride (2 mg) was added to the solution. The mixture was heated at 60°C for 2 h, and an equal volume of acetic anhydride was added, followed by heating at 90°C for 2 h. Then, the solution was concentrated to dryness and taken up in MeOH (0.5 ml), which was then analyzed by Shimadzu GC-2010 Plus gas chromatograph equipped with H_2 flame ionization detector (Shimadzu Corporation, Kyoto, Japan). DB-5 quartz capillary column: 30 m × 0.25 mm, 0.25 μm; column temperature: 160–280°C, programmed increase: 5°C/min; carrier gas N_2: 1.5 ml/min; injector and detector temperature: 280°C; injection volume: 1 μl; split ratio: 10:1. The monosaccharides of compounds were confirmed by comparison of the retention time of monosaccharides derivatives with those of standard sugars (the standard sugars were subjected to the same reaction; the retention times of the derivatives of L-rhamnose and D-glucose were 23.61 and 28.10 min, respectively).

Acknowledgments

The authors were financially supported by the International Science & Technology Cooperation Program of Commission and the Innovative Team of Beijing TCM University (no. 2011-CXTD-19) and the programs of Jilin Province Administration of Traditional Chinese Medicine (Nos. 2010-pt036; 2011-zd15; YJS-0212).

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