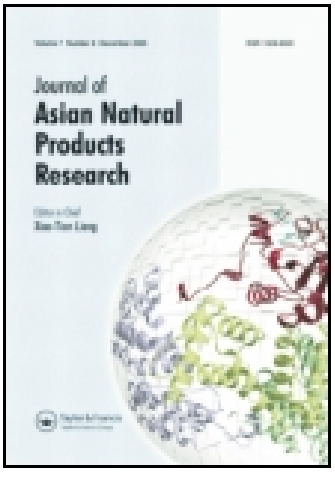

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Two new furostanol saponins from the fibrous root of *Ophiopogon japonicus*

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Two new furostanol saponins ophiopogonins J (**1**) and K (**2**) were isolated from the fibrous roots of *Ophiopogon japonicus*. The structures of **1** and **2** were established as (25*R*)-26-*O*-[(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-14-hydroxy-furost-5,20(22)-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**1**), and (25*R*)-26-*O*-[(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-furost-5,20(22)-diene 3-*O*-α-L-rhamnopyranosyl-(1 → 2)[(β-D-xylopyranosyl-(1 → 4)-β-D-glucopyranoside)] (**2**) on the basis of spectroscopic means including HRESIMS, 1D, and 2D NMR experiments.

Keywords: *Ophiopogon japonicus*; furostanol saponin; Liliaceae

1. Introduction

Ophiopogon japonicus (Thunb.) Ker-Gawl (Liliaceae), which is known as 'Maidong' in China, is widely used in traditional Chinese medicine for curing cardiovascular and cerebrovascular diseases as expectorant, antitussive, and tonic agent, as well as treating acute and chronic inflammatory diseases including pharyngitis, bronchitis, pneumonia, angina, cough, etc [1–3]. Chemical studies have shown that this plant includes saponins, homoisoflavonoids, flavonoids, amides, and terpenoids [4–12] from *O. japonicus*. Our further phytochemical investigation on fibrous roots of *O. japonicus* led to the isolation of two new furostanol saponins ophiopogonins J (**1**) and K (**2**) (Figure 1). Herein, we report the isolation and structural determination of the new constituents.

2. Results and discussion

Compound **1** was obtained as white amorphous powder. Its specific rotation $[a]_D^{20}$ (*c* 0.12, DMSO) was −83.0. The HRESIMS of **1** showed pseudo-molecular ion peaks at *m/z* 1085.5156 $[M + Na]^+$, 1061.5232 $[M-H]^-$, which suggested the molecular formula of $C_{51}H_{82}O_{23}$.

The ^{1}H NMR spectrum of **1** showed three proton singlets at δ_H 0.96 (s, Me-18), 1.13 (s, Me-19), and 1.69 (s, Me-21), indicating the presence of two angular methyl groups and one tertiary methyl group; a three-proton doublet at δ_H 1.07 (d, $J = 6.5$ Hz, Me-27), assignable to one secondary methyl group. Furthermore, signals for an olefinic proton at δ_H 5.37 (br. s), four anomeric protons at δ_H 5.01 (d, $J = 7.0$ Hz), 6.37 (br. d, $J = 1.0$ Hz), 4.86 (d, $J = 8.0$ Hz), 5.29 (d, $J = 8.0$ Hz), and the methyl group of a 6-deoxyhexopyr-

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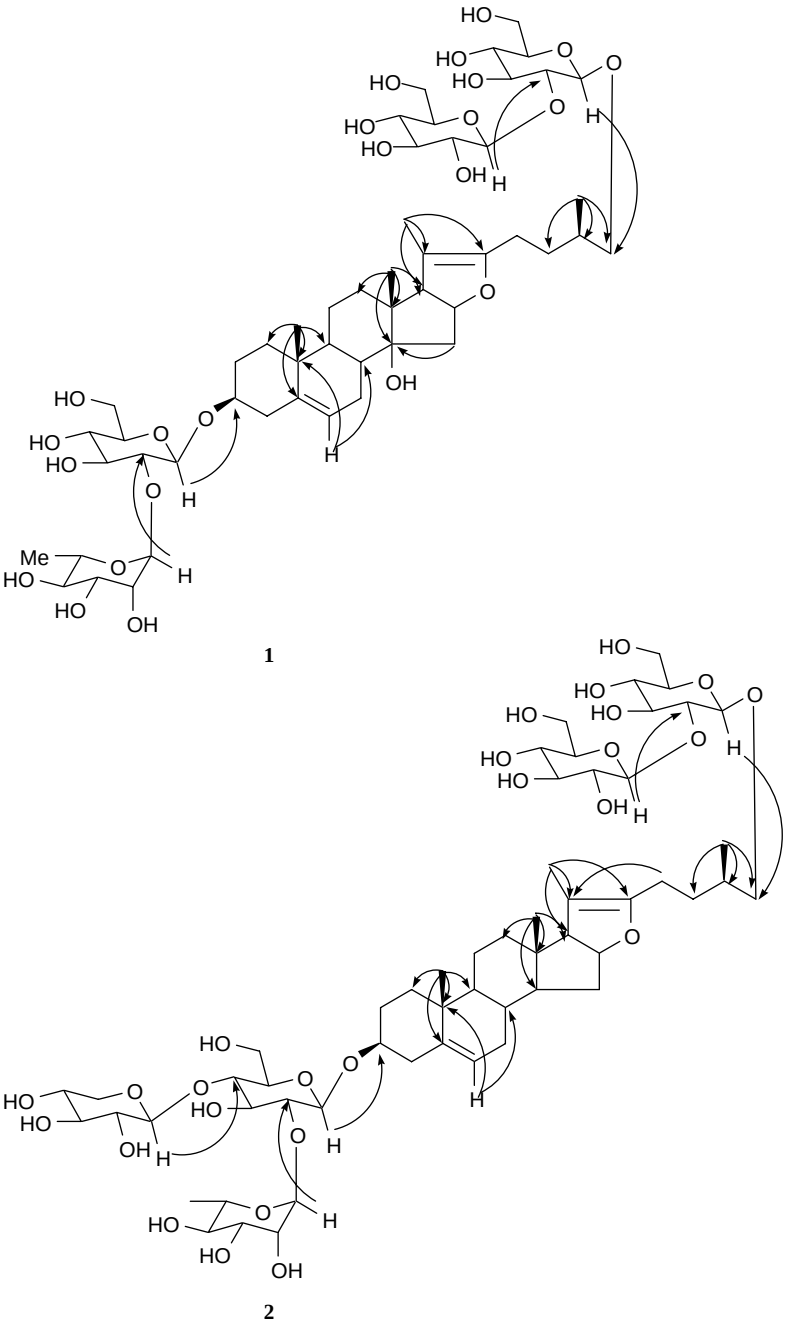

Figure 1. Structures and key HMBC correlations of compounds **1** and **2**.

anosyl moiety at δ_H 1.76 (d, $J = 6.0$ Hz) were observed. The ^{13}C NMR spectrum showed 51 carbon signals in which the signals at δ_C 140.8 (C-5), 122.3 (C-6), 152.3 (C-22), 103.9 (C-20), 61.6 (C-17), 19.4 (C-19) and 17.2 (C-27) were assigned readily. The ^{13}C NMR spectral data of aglycone were closely related to those of

pseudoprotodioscin [13,14], except for the differences at C-12 (−7.8 ppm), C-13 (+4.5 ppm), C-14 (+29.9 ppm), and C-15 (+6.6 ppm) in aglycone of **1**, which are suggestive of one hydroxy linking to C-14. The existence of 14-hydroxy group was further determined by long-range correlations between H-15 (δ_H 2.45) and C-14 (δ_C 84.8), and H-18 (δ_H 0.96) and C-14 (δ_C 84.8) in the HMBC experiment. The aglycone's 25*R* configuration was deduced from the difference in chemical shifts of the geminal protons 2H-26 ($\delta_a - \delta_b = 0.44 < 0.48$) [15]. Thus, the aglycone of **1** was identified as (25*R*)-3β,14,26-triol-furost-5,20(22)-diene.

Acid hydrolysis of **1** gave glucose and rhamnose (3:1) as carbohydrate moieties. The downfield shifts for C-3 and C-26 of the aglycone (δ_C 77.9 and 74.7) allowed the deduction that C-3 and C-26 were the glycosyl sites. The sugar sequence of rhamnosyl-(1 → 2)-glucosyl and its linkage at C-3 were determined by gHMBC correlations between H-1″ (δ_H 6.37) and C-2′ (δ_C 77.7) and between H-1′ (δ_H 5.01) and C-3 (δ_C 77.9). The sugar sequence of glucosyl-(1 → 2)-glucosyl and its linkage at C-26 were ascertained by long-range correlations between H-1‴ (δ_H 5.29) and C-2‴ (δ_C 84.1) and between H−1‴ (δ_H 4.86) and C-26 (δ_C 74.7). The proton and carbon signals of the sugar moieties were assigned by gHMQC, gHMBC, gCOSY analyses and by comparison of the ^{13}C NMR data with those in the literature [9]. The β-configuration of glucose was determined by large *J* values of the anomeric-H-atom signals at δ_H 5.01 (d, $J = 7.0$ Hz, H−1′), 4.86 (d, $J = 8.0$ Hz, H−1‴), 5.29 (d, $J = 8.0$ Hz, H−1⁗), while the rhamnose had an α-configuration evidenced by carbon signals of δ_C 72.8 (C-3 of Rha) and δ_C 69.5 (C-5 of Rha) [16]. This way, the structure of **1** was determined as (25*R*)-26-*O*-[(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-14-hydroxy-furost-5,20(22)-diene 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside.

Compound **2** was obtained as white amorphous powder, with $[a]_D^{20}$ −72.1. Its ESIMS showed the pseudo-molecular ion peaks at *m/z* 1201 $[M + Na]^+$, 1177 $[M-H]^-$, and the corresponding fragments at *m/z* 1069 $[M + Na\text{-}132]^+$, 1045 $[M-H-132]^-$, 1015 $[M-H-162]^-$, 883 $[M-H-132-162]^-$, and 733 $[M-H-132-162-146]^-$. The molecular formula was assigned as $C_{56}H_{90}O_{26}$ on the basis of HRESIMS at *m/z* 1179.5756 $[M + H]^+$, together with its ^{1}H and ^{13}C NMR spectral data.

For the aglycon moiety, the ^{1}H NMR spectrum of **2** revealed the presence of four typical steroid methyl groups at δ_H 0.74 (s, 3H), 1.04 (s, 3H), 1.66 (s, 3H), and 1.06 (3H, d, $J = 6.5$ Hz), and an olefinic proton at δ_H 5.27 (1H, br. s). The ^{1}H and ^{13}C NMR spectral data of aglycon were basically consistent with those of pseudoprotodiosgenin [13] and its structure was further determined on the basis of extensive gHMQC, gHMBC, and gCOSY spectral data. The ^{13}C NMR spectral data of sugar moieties were closely identical to those of compound **1** except the additional signals at δ_C 105.8, 75.0, 78.4, 70.8, 67.4. Acid hydrolysis of **2** produced glucose, rhamnose, and xylose in the ratio of 3:1:1. The sugar sequence of rhamnosyl-(1 → 2)-[xylosyl-(1 → 4)]-glucosyl and its linkage at C-3 were identified by gHMBC correlations between H-1″ (δ_H 6.25) and C-2′ (δ_C 77.5), and between H-1‴ (δ_H 5.02) and C-4′ (δ_C 81.5), and between H-1′ (δ_H 4.96) and C-3 (δ_C 78.0). The β-configuration of glucose and the α-configuration of rhamnose were determined according to the method of compound **1**. Therefore, the structure of **2** was established as (25*R*)-26-*O*-[(β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-furost-5,20(22)-diene 3-*O*-α-L-rhamnopyranosyl-(1 → 2) [(β-D-xylopyranosyl-(1 → 4))]-β-D-glucopyranoside.

3. Experimental

3.1 General experimental procedures

JASCO P-2000 Digital Polarimeters (Jasco Co., Limited, Tokyo, Japan) were used in Optical rotation values detection. IR spectra were obtained from a Nicolet 5700 spectrometer (Thermo Electron Scientific Instruments Co., Miami, FL, USA). NMR spectra were recorded on a UNITYINOVA 500 spectrometer (Varian Medical System Inc., Palo Alto, CA, USA). and a JNM-ECA 400 spectrometer (Jeol, Tokyo, Japan) with tetra-methyl silane as internal standard. The HRESIMS were carried out on Nano LC-Q-TOF (Q-Star, AB Scix, Vaughan, Canada) and APEX IV (7.0T) mass spectrometer (Bruker Co., Boston, MA, USA). HPLC was carried out on Agilent LC 1100 with an UV detector (Agilent Technologies, Santa Clara, CA, USA). GC analysis was performed on an HP6890 plus instrument (Agilent Technologies) equipped with an H2 flame ionzation detector. The GC analysis conditions were HP-5 quartz capillary column (30 m × 0.32 mm × 0.25 μm); column temperature, 140–240°C; programmed oven temperature increase, 10°C/min; carrier gas, N2 (1.5 ml/min); injector temperature, 240°C; detector temperature, 260°C; injection volume, 1 μl; split ratio, 1:50. Column chromatography (CC) was performed over silica gel (100–200 and 300–400 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China), Daion HP-20 polyporous resin (Mitsubishi Chemical Co., Tokyo, Japan), and Sephadex LH-20 gel (Amersham Biosciences AB, Upsala, Sweden).

3.2 Plant material

The fibrous roots of *Ophiopogon japonicus* were purchased from Anguo traditional Chinese medicine market, Hebei Province, China, in September 2005 and identified by Prof. Peng-Fei Tu. A voucher specimen (MD20050906) has been deposited in the herbarium of Peking University Modern Research Center for Traditional Chinese Medicine.

3.3 Extraction and isolation

The fibrous roots of *O. japonicus* (45 kg) were extracted with EtOH (70%) under reflux and then filtered by gauze. The extract was concentrated under reduced pressure and the residue was suspended in H_2O and subsequently extracted successively with petroleum ether (60 ~ 90°C), EtOAc, and *n*-BuOH. The *n*-BuOH soluble fraction was subjected to Diaion HP-20 resin CC eluted with 20% EtOH, 55% EtOH, and 80% EtOH in H_2O to afford three fractions (Fr. 1 ~ 3). Fr. 2 (100.0 g) was separated by silica gel CC (100–200 mesh) eluted with $CHCl_3$:MeOH (1:0, 100:1, 50:1, 30:1, 15:1, 10:1, 5:1, 2:1, 0:1) to give seven fractions (Fr. 2-1 ~ 7). Fr. 2–6 (25 g) was subjected to silica gel eluted with EtOAc:EtOH (20:1, 15:1, 10:1, 9:1, 8:1, 6:1, 4:1) to afford Fr. 2-6-1 ~ 7. Fr. 2-6-5 (5 g) was further separated on octa-decylsilyl silicon silica gel column by gradient MeOH–H_2O, and tubes (119–127) were purified on Sephadex LH-20 CC with MeOH as eluent to afford **1** (14 mg). Fr. 2-6-6 (3 g) was purified on Sephadex LH-20 CC with MeOH, and then by repeated semi-preparative HPLC (Zorbax XDB-C_{18} column, 9.4 mm × 250 mm, 5 μm, MeOH:H_2O (62:38), flow rate 2.0 ml/min, UV 203 nm) to yield **2** (29 mg).

3.3.1 Ophiopogonin J (1)

White amorphous powder; $[a]_D^{20}$ −83.0 (*c* 0.123, DMSO); IR (KBr) ν_{max}: 3395, 2934, 1695, 1642, 1453, 1378, 1063,1043, 913, 893.5 cm^{-1}; ^1H NMR and ^{13}C NMR spectral data (in C_5D_5N) (see Table 1). HRESIMS: *m/z* 1085.5156 $[M + Na]^+$ (calcd for $C_{51}H_{82}O_{23}Na$, 1085.5145); 1061.5232 $[M-H]^-$ (calcd for $C_{51}H_{81}O_{23}$, 1061.5169).

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectral data of **1** in pyridine-d_5.

Position	δ_C	δ_H, *J* (Hz)	Position	δ_C	δ_H, *J* (Hz)
1	37.8	1.02 m, 1.79 m	27	17.2	1.07 d, (6.5)
2	30.2	1.90 m, 2.09 m	3-*O*-Glc 1′	100.2	5.01 d, (7.0)
3	77.9	3.89 m	2′	77.7	4.26 m
4	39.0	2.75 m, 2.80 m	3′	79.6	4.25 m
5	140.8		4′	71.8	4.18 m
6	122.3	5.37 br. S	5′	77.9	3.88 m
7	26.8	1.85 m, 2.45 m	6′	62.5	4.33 m, 4.49 m
8	35.0	2.01 m	Rha 1″	102.0	6.37 br. d, (1.0)
9	43.6	1.79 m	2″	72.6	4.78 m
10	37.4		3″	72.8	4.62 dd, (9.5, 3.5)
11	20.5	1.41 m, 1.57 m	4″	74.2	4.31 m
12	31.8	1.48 m, 2.32 m	5″	69.5	4.97 m
13	47.8		6″	18.7	1.76 d, (6.0)
14	84.8		Glc 1‴	103.2	4.86 d, (8.0)
15	42.4	2.01 m, 2.45 m	2‴	84.1	4.15 m
16	85.2	5. 30 m	3‴	77.7	4.29 m
17	61.6	3.38 d, (9.5)	4‴	71.3	4.17 m
18	17.7	0.96 s	5‴	78.3	3.86 m
19	19.4	1.13 s	6‴	62.6	4.33 m, 4.49 m
20	103.9		Glc 1⁗	106.3	5.29 d, (8.0)
21	12.0	1.69 s	2⁗	77.1	4.06 t, (9.5)
22	152.3		3⁗	77.7	4.26 m
23	23.9	2.25 m	4⁗	71.3	4.26 m
24	31.4	1.90 m, 2.10 m	5⁗	78.6	3.89 m
25	33.6	1.98 m	6⁗	62.5	4.31 m, 4.49 m
26	74.7	3.56 dd, (9.0, 6.0), 3.90 m			

3.3.2 Ophiopogonin I (2)

White amorphous powder; $[a]_D^{20}$ −72.1 (*c* 0.16, DMSO); IR (KBr) ν_{max}: 3400, 2932, 1698, 1643, 1441, 1370, 1134, 1076, 1038 cm^{-1}; ^{1}H NMR and ^{13}C NMR spectral data (in C_5D_5N) (see Table 2). ESIMS: *m/z* 1201 $[M + Na]^+$, 1177 $[M–H]^-$, 1069 $[M + Na\text{-}132]^+$, 1045 $[M–H\text{-}132]^-$, 1015 $[M–H\text{-}162]^-$, 883 $[M–H\text{-}132\text{-}162]^-$, 733 $[M–H\text{-}132\text{-}162\text{-}146]^-$. HRESIMS: *m/z* 1179.5756 $[M + H]^+$ (calcd for $C_{56}H_{91}O_{26}$, 1179.5799).

3.4 Acid hydrolysis of compounds 1 and 2

A solution of compounds **1** and **2** (5 mg) was treated with 1 M HCl (dioxane:H_2O, 1:1, 4 ml) at 100°C for 1.5 h, respectively. After cooling, the reaction mixture was neutralized with Na_2CO_3, and then extracted with $CHCl_3$ (3 × 10 ml). The aqueous layer was concentrated to dryness. Then, 1 ml of pyridine and 2 mg of hydroxylamine hydrochloride were added to the dry residue, and the mixture was heated at 100°C for 1 h. After cooling, Ac_2O (1.5 ml) was added followed by heating at 100°C for 1 h and the mixture was evaporated to dryness under reduced pressure. The resulted residue was solved with $CHCl_3$ and analyzed on GC by comparing with aldononitrile peracetates of authentic samples. The D-glucose and L-rhamnose derivatives of **1** were detected with t_R values of 11.19 min and 8.59 min in the ratio of 3:1, and the derivatives of D-glucose, L-rhamnose, and D-xylose of **2** were detected with t_R values of 11.19, 8.59, and 8.59 min in the ratio of 3:1:1.

Table 2. ^{1}H NMR (400 MHZ) and ^{13}C NMR (100 MHZ) spectral data of **2** in pyridine-d_5.

Position	δ_C	δ_H, *J* (Hz)	Position	δ_C	δ_H, *J* (Hz)
1	37.6	0.97 m, 1.73 m	3-*O*-Glc 1′	100.1	4.96 d, (7.0)
2	30.2	1.85 m, 2.09 m	2′	77.5	4.22 m
3	78.0	3.88 m	3′	77.3	4.23 m
4	39.0	2.61 m, 2.67 m	4′	81.5	4.22 m
5	140.8		5′	76.3	3.84 m
6	121.8	5.27 br. s	6′	61.7	4.34 m, 4.45 m
7	32.5	1.47 m,1.85 m	Rha 1″	102.0	6.25 br. s
8	31.5	1.47 m	2″	72.5	4.77 m
9	50.4	0.88 m	3″	72.8	4.59 dd, (9.5, 3.5)
10	37.1		4″	74.1	4.32 m
11	21.3	1.38 m, 1.41 m	5″	69.6	4.91 m
12	39.7	1.12 m, 1.72 m	6″	18.7	1.76 d, (6.0)
13	43.5		Xyl 1‴	105.8	5.02 d, (7.0)
14	55.0	0.85 m	2‴	75.0	3.96 m
15	34.6	1.45 m,2.07 m	3‴	78.4	4.09 m
16	84.5	4.85 m	4‴	70.8	4.15 m
17	64.5	2.41 d, (6.0)	5‴	67.4	3.67 t, (8.0), 4.25 m
18	14.2	0.74 s	Glc 1⁗	103.3	4.86 d, (8.0)
19	19.5	1.04 s	2⁗	84.1	4.14 m
20	103.6		3⁗	78.0	4.32 m
21	11.9	1.66 s	4⁗	71.4	4.15 m
22	152.6		5⁗	78.3	3.86 m
23	23.9	2.19 m	6⁗	62.6	4.34 m, 4.45 m
24	31.5	1.48 m, 1.84 m	Glc 1⁗	106.6	5.29 d, (8.0)
25	33.7	1.92 m	2⁗	77.1	4.06 t, (8.0)
26	74.9	3.56 dd, (9.0, 6.0), 3.92 m	3⁗	78.2	4.25 m
27	17.3	1.06 d, (6.5)	4⁗	71.5	4.27 m
			5⁗	78.6	3.89 m
			6⁗	62.6	4.30 m, 4.49 m

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Note

1. These authors contributed equally to this work.

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