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# Two novel furostanol saponins from *Ophiopogon japonicus*

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Two novel furostanol saponins were isolated from the fresh tubers of *Ophiopogon japonicus*. Comprehensive spectroscopic analysis allowed the chemical structures of the compounds to be assigned as (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-22α-hydroxyfurost-5-ene-3-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**1**, ophiopogonin F) and (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl)]-22α-hydroxyfurost-5-ene-3-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside (**2**, ophiopogonin G). The rare furostanol saponins with two glucosyl residues at C-26 position were isolated from the natural source for the first time.

**Keywords:** *Ophiopogon japonicus*; furostanol saponins; ophiopogonin F; ophiopogonin G

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## 1. Introduction

*Ophiopogon japonicus* Ker-Gawl. (Liliaceae) is an evergreen perennial, widely used as a traditional Chinese medicine. It was known to treat cardiovascular diseases and exhibit activity against bacteria. Although a number of steroidal saponins were isolated from *O. japonicus* [1–6], study on furostanol saponins from the fresh tubers of *O. japonicus* has rarely been carried out. Therefore, a further phytochemical analysis has been carried out on the fresh tubers of *O. japonicus* with particular attention to the furostanol saponins. This study led to the isolation of two new compounds **1** (ophiopogonin F) and **2** (ophiopogonin G) (Figure 1). The chemical structures of the compounds with two glucosyl residues at C-26 are rare in natural products. The structural determination of the two new compounds was carried out on the basis of extensive spectroscopic analysis, including 1D NMR ($^1$H and $^{13}$C NMR) and 2D NMR (COSY, HSQC, HMBC, and HSQC-TOCSY), and the results of acid hydrolysis.

## 2. Results and discussion

Compound **1** was obtained as a white amorphous powder. It showed positive Liebermann–Burchard and Ehrlich reagent tests. The molecular formula was assigned as $C_{56}H_{92}O_{27}$ on the basis of the negative-ion HR-ESI-MS at *m/z* 1195.5776 $[M-H]^-$, together with its $^1$H and $^{13}$C NMR spectral data (Table 1). FAB-MS showed the ion peaks at *m/z* 1179.5 $[M+H-H_2O]^+$, 1017.4 $[M+H-H_2O\text{-}162]^+$, 855.3 $[M+H-H_2O\text{-}162\text{-}162]^+$, 709.2 $[M+H-H_2O\text{-}162\text{-}162\text{-}146]^+$, 577.2 $[M+H-H_2O$-162-

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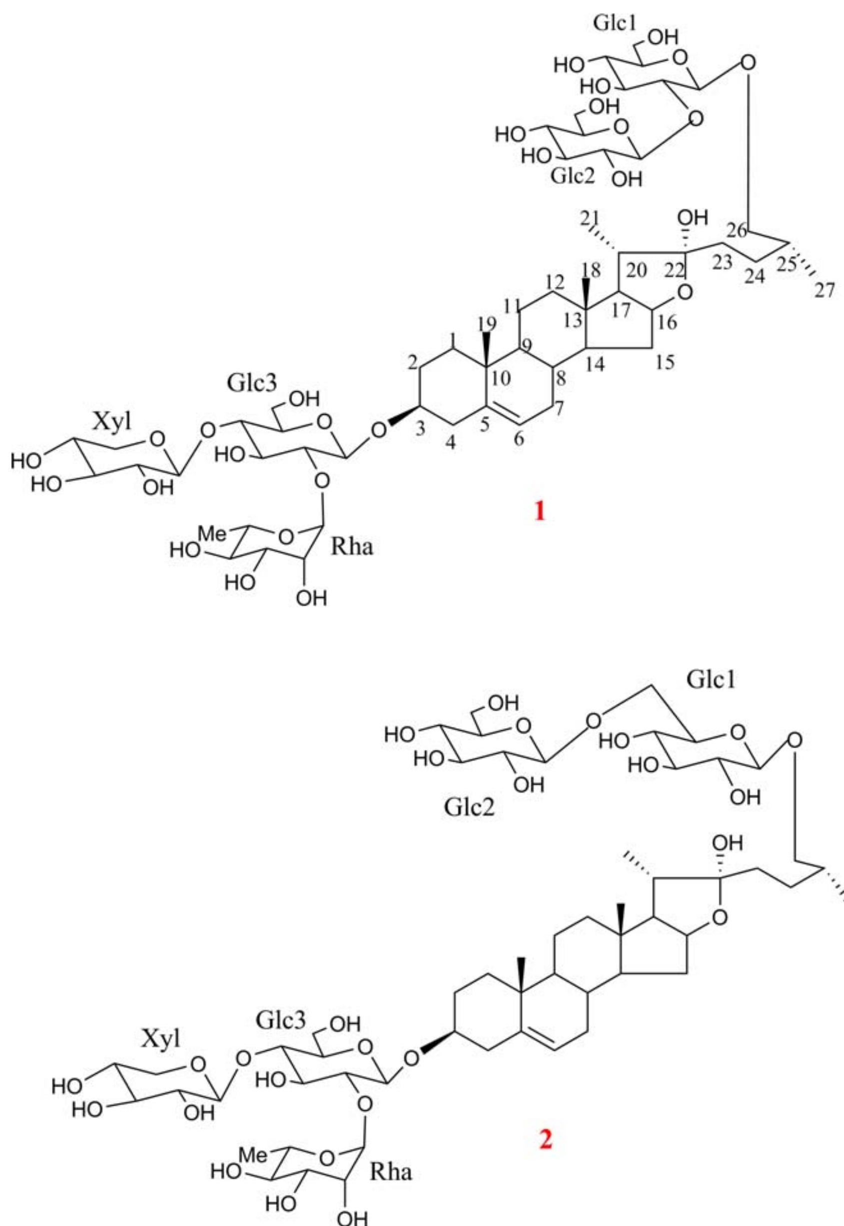


Figure 1. Structures of compounds **1** and **2**.

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162-146-132]$^+$, and 415.2 [M + H−$H_2O$-162-162-146-132-162]$^+$, attributable to the sequential losses of a pentose, a deoxyhexose, and three hexose residues.

The $^1$H NMR spectrum of **1** showed two methyl singlets at $\delta$ 0.91 (3H, s) and 1.03 (3H, s), three methyl doublets at $\delta$ 1.06 (3H, d, $J = 6.6$ Hz), 1.32 (3H, d, $J = 6.8$ Hz), and 1.77 (3H, d, $J = 6.2$ Hz), five anomeric proton signals at $\delta$ 4.97 (1H, d, $J = 7.8$ Hz), 5.02 (1H, d, $J = 7.7$ Hz), 4.84 (1H, d, $J = 7.8$ Hz), 5.28 (1H, d, $J = 7.8$ Hz), and 6.26 (1H, s), and an olefinic proton signal at $\delta$ 5.26 (1H, br s,

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** ($\delta$ in pyridine-$d_5$).

| | **1** | | **2** | |
|---|---|---|---|---|
| Position | $\delta_C$ | $\delta_H$, $J$ (Hz) | $\delta_C$ | $\delta_H$, $J$ (Hz) |
| 1 | 37.5 | 0.97 m, 1.73 o[a] | 37.5 | 0.97 o, 1.73 o |
| 2 | 30.2 | 1.86 o, 2.10 m | 30.2 | 1.87 m, 2.10 o |
| 3 | 78.3 | 3.87 o | 78.4 | 3.86 m |
| 4 | 39.0 | 2.70 m, 2.75 m | 39.0 | 2.74 m, 2.69 m |
| 5 | 140.8 | | 140.8 | |
| 6 | 121.9 | 5.26 br s | 121.9 | 5.27 br s |
| 7 | 32.4 | 1.48 o, 1.84 o | 32.5 | 1.46 o, 1.85 o |
| 8 | 31.7 | 1.56 m | 31.7 | 1.56 m |
| 9 | 50.4 | 0.88 m | 50.4 | 0.87 o |
| 10 | 37.1 | | 37.2 | |
| 11 | 21.2 | 1.41–1.43 o | 21.1 | 1.43–1.45 o |
| 12 | 40.0 | 1.10 m, 1.75 o | 40.0 | 1.10 m, 1.72 o |
| 13 | 40.8 | | 40.8 | |
| 14 | 56.7 | 1.06 o | 56.6 | 1.06 m |
| 15 | 32.5 | 1.48 o, 2.02 m | 32.4 | 1.46 o, 2.02 o |
| 16 | 81.2 | 4.94 o | 81.1 | 4.94 o |
| 17 | 63.8 | 1.91 dd (6.6, 7.8) | 63.9 | 1.92 o |
| 18 | 16.6 | 0.91 s | 16.5 | 0.88 s |
| 19 | 19.4 | 1.03 s | 19.4 | 1.04 s |
| 20 | 40.9 | 2.23 p (6.8) | 40.7 | 2.23 p (6.6) |
| 21 | 16.5 | 1.32 d (6.8) | 16.5 | 1.33 d (6.6) |
| 22 | 110.7 | | 110.7 | |
| 23 | 37.3 | 2.02 o | 37.2 | 2.03 o, 2.04 o |
| 24 | 28.2 | 1.64 m, 2.01 o | 28.4 | 1.68 m, 2.04 o |
| 25 | 34.4 | 1.97 m | 34.3 | 1.92 o |
| 26 | 75.2 | 3.59 dd (5.4, 9.2), 3.87 o | 75.2 | 3.56 dd (6.0, 9.4), 4.01 o |
| 27 | 17.6 | 1.06 d (6.6) | 17.5 | 0.98 d (6.7) |
| Sugar part | | | | |
| 3-*O*-Glc3 | | | 3-*O*-Glc3 | |
| 1 | 100.1 | 4.97 d (7.8) | 100.1 | 4.96 d (7.8) |
| 2 | 77.5 | 4.21 m | 77.5 | 4.22 o |
| 3 | 77.3 | 4.23 m | 77.3 | 4.20 o |
| 4 | 81.6 | 4.20 m | 81.6 | 4.19 o |
| 5 | 76.3 | 3.84 m | 76.3 | 3.83 m |
| 6 | 61.7 | 4.44 br d (12.6), 4.51 o | 61.7 | 4.43 br d (9.9), 4.51 o |
| Rha-(1 → 2) | | | Rha-(1 → 2) | |
| 1 | 102.0 | 6.26 br s | 102.0 | 6.26 br s |
| 2 | 72.5 | 4.79 d (1.5) | 72.5 | 4.79 d (1.7) |
| 3 | 72.8 | 4.60 dd (3.1, 9.2) | 72.8 | 4.60 dd (3.2, 9.2) |
| 4 | 74.2 | 4.34 o | 74.2 | 4.35 o |
| 5 | 69.6 | 4.94 o | 69.6 | 4.93 o |
| 6 | 18.7 | 1.77 d (6.2) | 18.7 | 1.77 d (6.2) |
| Xyl-(1 → 4) | | | Xyl-(1 → 4) | |
| 1 | 105.8 | 5.02 d (7.7) | 105.8 | 5.02 d (7.7) |
| 2 | 75.2 | 3.97 m | 75.0 | 3.97 o |
| 3 | 78.4 | 4.10 o | 78.4 | 4.11 dd (8.2, 9.0) |
| 4 | 70.8 | 4.16 o | 70.8 | 4.17 o |
| 5 | 67.4 | 3.67 dd (10.6, 10.9), 4.26 o | 67.4 | 3.67 dd (10.7, 10.9), 4.26 dd (5.1, 10.9) |

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Table 1 – *continued*

| Position | 1 $\delta_C$ | 1 $\delta_H$, $J$ (Hz) | 2 $\delta_C$ | 2 $\delta_H$, $J$ (Hz) |
|---|---|---|---|---|
| 26-*O*-Glc1 | | | 26-*O*-Glc1 | |
| 1 | 103.2 | 4.84 d (7.8) | 104.9 | 4.73 d (7.8) |
| 2 | 84.3 | 4.13 o | 75.1 | 3.95 m |
| 3 | 78.0 | 4.31 o | 78.5 | 4.17 o |
| 4 | 71.4 | 4.19 o | 71.6 | 4.15 o |
| 5 | 78.3 | 3.87 o | 77.3 | 4.05 o |
| 6 | 62.6 | 4.34 o, 4.49 o | 70.2 | 4.33 o, 4.82 br d (9.6) |
| Glc2-(1 → 2) | | | Glc2-(1 → 6) | |
| 1 | 106.6 | 5.28 d (7.8) | 105.5 | 5.10 d (7.8) |
| 2 | 77.0 | 4.10 o | 75.4 | 4.04 o |
| 3 | 78.1 | 4.23 o | 78.6 | 4.23 o |
| 4 | 71.5 | 4.29 o | 71.7 | 4.24 o |
| 5 | 78.7 | 3.95 m | 78.4 | 3.92 m |
| 6 | 62.7 | 4.41 dd (3.6, 12.6), 4.49 o | 62.8 | 4.37 dd (5.0, 12.6), 4.50 o |

Note: [a]Overlapped with other signals.

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H-6). The $^{13}$C NMR spectrum showed 56 carbon signals, in which the characteristic carbon signals at $\delta$ 140.8 (C-5), 121.9 (C-6), 19.4 (C-19), and 110.7 (C-22) were assigned readily. These data indicated that compound **1** is a furostanol saponin with five sugar moieties and $\Delta^{5(6)}$ [7,8]. The α-configuration of C-22 hydroxyl group of the aglycone moiety was deduced from the hemiketal carbon signal at $\delta$ 110.7, about 3–4 ppm higher than that of the β-configuration [9,10]. The chemical shift difference between the two protons of 2H-26 ($\Delta ab = 0.28 < 0.48$) demonstrated the 25*R* configuration of **1** [11,12]. The $^{1}$H and $^{13}$C NMR spectral data of the aglycone were closely related to those of the protodioscin [8,13]. Thus, the aglycone of **1** was identified as (25*R*)-3β,22α,26-trihydroxyfurost-5-ene.

As for the sugar moiety, acid hydrolysis of **1** with 1 M HCl in dioxane–$H_2O$ (1:1) yielded glucose, rhamnose, and xylose as the sugar components. The $^{13}$C NMR spectrum indicated the presence of five anomeric carbon signals at $\delta$ 100.1, 102.0, 105.8, 103.2, and 106.6, which showed correlations with their corresponding anomeric proton signals at $\delta$ 4.97, 6.26, 5.02, 4.84, and 5.28, respectively, in the HSQC spectrum. The severe overlapping protons of the sugars were solved using the HSQC-TOCSY spectrum. The anomeric proton signal at $\delta$ 4.84 (H-1 of Glc1) showed correlations with the carbons at $\delta$ 84.3, 78.3, 78.0, 71.4, and 62.6, and the signal at $\delta$ 103.2 (C-1 of Glc1) showed correlations with proton signals at $\delta$ 4.84, 4.31, 4.19, 4.13, and 3.87 in the HSQC-TOCSY spectrum. Combined use of COSY and HSQC experiments established the resonance sequence of Glc1 (Table 1). All the resonances of Glc2, Glc3, Rha, and Xyl of **1** were assigned (Table 1) by the same method. The large coupling constant ($J_{Glc1} = 7.8$, $J_{Glc2} = 7.8$, $J_{Glc3} = 7.8$, and $J_{Xyl} = 7.7$) indicated the β-configuration of the four sugars [14–16]. The carbon signals for C-3 ($\delta$ 72.8) and C-5 ($\delta$ 69.6) gave evidence for α-configuration of Rha [14]. The sugar sequences of Glc3, Rha, Xyl, and its linkage to C-3 of the aglycone were ascertained by long-range corre-

lations between H-1 of Rha and C-2 of Glc3, H-1 of Xyl and C-4 of Glc3, and H-1 of Glc3 and C-3 of aglycone in the HMBC spectrum. On the other hand, the HMBC cross-peaks of H-1 of Glc1 with the carbon signal at $\delta$ 75.2 (C-26 of aglycone), H-1 of Glc2 with the carbon signal at $\delta$ 84.3 (C-2 of Glc1), allowed us to identify C-26 as the linkage site of two glucosyl residues (Figure 2). Thus, the structure of **1** was determined to be (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranosyl)]-22α-hydroxyfurost-5-ene-3-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, named ophiopogonin F.

Compound **2** was isolated as a white amorphous solid with positive Liebermann–Burchard and Ehrlich reagent tests. Its molecular formula was determined to be $C_{56}H_{92}O_{27}$ by the negative-ion HR-ESI-MS at *m/z* 1195.5775 $[M-H]^-$. FAB-MS showed the ion peaks at *m/z* 1179.5 $[M+H-H_2O]^+$, 1033.4 $[M+H-H_2O\text{-}146]^+$, 901.3 $[M+H-H_2O\text{-}146\text{-}132]^+$, 739.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162]^+$, 577.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162\text{-}162]^+$, and 415.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162\text{-}162\text{-}162]^+$, suggesting that **2** contained a pentose, a deoxyhexose, and three hexose residues. Acid hydrolysis of **2** with 1 M HCl in dioxane–$H_2O$ (1:1) gave glucose, rhamnose, and xylose.

The $^1$H and $^{13}$C NMR spectral data of the aglycone were closely identical to those of compound **1**. Thus, the aglycone of **1** was identified as (25*R*)-3β,22α,26-trihydroxyfurost-5-ene [8,13]. However, comparing the $^{13}$C NMR spectral data of **2** with those of **1**, the evident differences were recognized in the signals at $\delta$ 104.9 (C-1 of Glc1), 105.5 (C-1 of Glc2), and 70.2 (C-6 of Glc1). These data indicated that the sugar chain of C-26 was different

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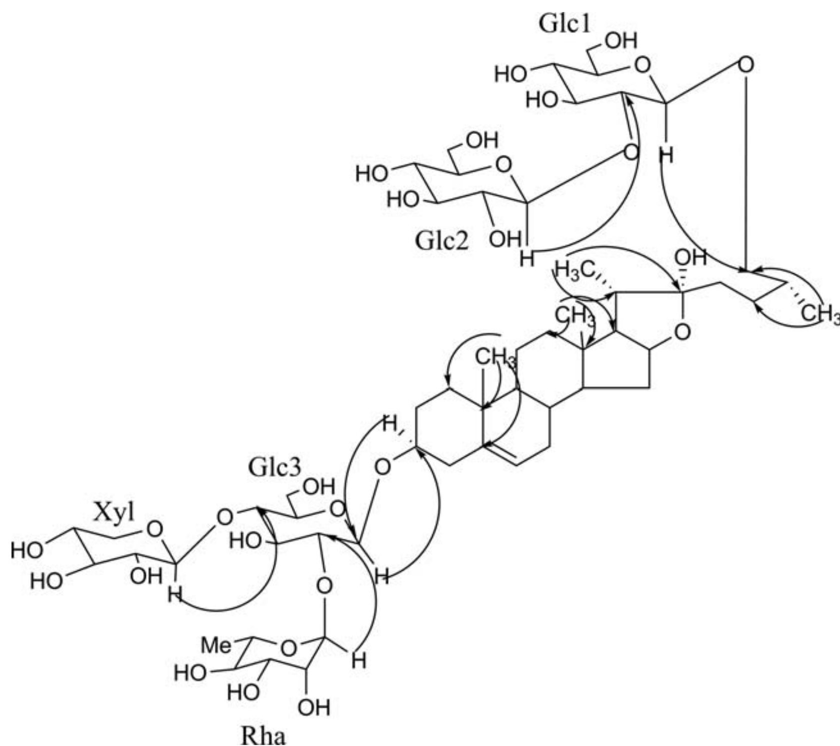


Figure 2. Key HMBC correlations for compound **1**.

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from that of **1**. In the HSQC-TOCSY spectrum, the anomeric proton signal at $\delta$ 4.73 showed correlations with carbon signals at $\delta$ 70.2, 71.6, 75.1, 77.3, and 78.6, and the anomeric proton signal at $\delta$ 5.10 showed correlations with carbon signals at $\delta$ 62.8, 71.7, 75.4, 78.4, and 78.6. Combined use of COSY and HSQC experiments established the resonance sequences of the two Glc (Table 1). In the HMBC spectrum, the long-range correlations between H-1 of Rha and C-2 of Glc3, H-1 of Xyl and C-4 of Glc3, H-1 of Glc2 and C-6 of Glc1, H-1 of Glc3 and C-3, and H-1 of Glc1 and C-26 indicated the sugar sequence and their linkages. Thus, the structure of **2** was elucidated as (25*R*)-26-[(*O*-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl)]-22 α-hydroxyfurost-5-ene-3-*O*-β-D-xylopyranosyl-(1 → 4)-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-β-D-glucopyranoside, named ophiopogonin G.

## 3. Experimental

### 3.1 General experimental procedures

Optical rotations were measured with a Perkin-Elmer 343 polarimeter. IR spectra were recorded on the Bio-Rad FTS-65A spectrometer. The NMR spectra were recorded with a Varian $^{UNITY}$*INOVA* 600 (599.8 MHz for $^1$H NMR and 150.8 MHz for $^{13}$C NMR), and the chemical shifts were given on the $\delta$ (ppm) scale with tetramethylsilane as an internal standard. The HR-ESI-MS was recorded on a 9.4 T Q-FT-MS Apex Qe (Bruker Co., Billerica, MA, USA). FAB-MS was recorded on a Micromass Zabspec. Macroporous resin SP825 (Mitsubishi Chemical, Kyoto, Japan) and ODS silica gel (120 Å, 50 μm; YMC, Kyoto, Japan) were used for chromatography. HPLC was performed using an Agilent 1100 system: an analytical column, ODS (5 μm, 4.6 × 250 mm; Grace, Deerfield, IL, USA); preparative column, a Hanbon lichrospher $C_{18}$ (5 μm, 10.0 × 250 mm; Hanbon Sci. & Tech, Huaian, China); detector, Agilent RID (refractive index detector); and Alltech ELSD 2000 (evaporative light-scattering detector). Gas chromatographic analysis was performed with an Agilent 6890 Series gas chromatograph equipped with an $H_2$ flame ionization detector. The column was an HP-5 capillary column (30 m × 0.25 mm × 0.25 μm; Agilent, Milford, MA, USA).

### 3.2 Plant material

The fresh tubers of *O. japonicus* were collected from the Mianyang region of Sichuan Province, China in April 2007. The plant was identified by Prof. Li-Juan Zhang (Tianjin University of Traditional Chinese Medicine), and a voucher specimen (No. 070403) has been deposited in the Herbarium of Beijing Institute of Radiation Medicine, Beijing, China.

### 3.3 Extraction and isolation

The fresh tubers of *O. japonicus* (56.0 kg) were refluxed twice with EtOH–$H_2O$ (3:2). The combined extract was concentrated under reduced pressure. The extract was separated chromatographically on macroporous resin SP825 (15 × 100 cm) and eluted with a gradient mixture of EtOH–$H_2O$ (1:5, 1:1, and 9:1; 60,000 ml each), to give three fractions (A–C). Fraction B (220 g) was chromatographed on macroporous resin SP825 (15 × 100 cm) and eluted with a gradient mixture of EtOH–$H_2O$ (2:8, 3:7, 4:6, 11:9, and 8:2; 20,000 ml of each) to give 12 fractions ($B_1$–$B_{12}$). A part of fraction $B_7$ (12 g) was chromatographed on an ODS silica gel column (6 × 50 cm; acetone–$H_2O$, 23:77; 60 ml each) and then subjected to the preparative HPLC (column: 10 × 250 mm, RP-18, 5 μm, flow rate: 4.5 ml/min) with acetone–$H_2O$ (30:70) to yield compounds **1** (62.8 mg) and **2** (10.4 mg).

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### *3.3.1 Ophiopogonin F (**1**)*

A white amorphous powder. $[\alpha]_D^{20} = -59.1$ ($c = 0.110$, pyridine). $^1H$ and $^{13}C$ NMR spectral data: Table 1. IR (KBr) $\nu_{max}$: 3408 (OH), 2932 (CH), 1636 (C=C), 1375, 1072, 1042 cm$^{-1}$. HR-ESI-MS (neg.): *m/z* 1195.5776 $[M-H]^-$ (calcd for $C_{56}H_{91}O_{27}$, 1195.5753). FAB-MS (pos.): *m/z* 1179.5 $[M+H-H_2O]^+$, 1017.4 $[M+H-H_2O\text{-}162]^+$, 855.3 $[M+H-H_2O\text{-}162\text{-}162]^+$, 709.2 $[M+H-H_2O\text{-}162\text{-}162\text{-}146]^+$, 577.2 $[M+H-H_2O\text{-}162\text{-}162\text{-}146\text{-}132]^+$, and 415.2 $[M+H-H_2O\text{-}162\text{-}162\text{-}146\text{-}132\text{-}162]^+$.

### *3.3.2 Ophiopogonin G (**2**)*

A white amorphous powder. $[\alpha]_D^{20} = -57.5$ ($c = 0.080$, pyridine). $^1H$ and $^{13}C$ NMR spectral data: Table 1. IR (KBr) $\nu_{max}$: 3410 (OH), 2931 (CH), 1636 (C=C), 1375, 1042 cm$^{-1}$. HR-ESI-MS (neg.): *m/z* 1195.5775 $[M-H]^-$ (calcd for $C_{56}H_{91}O_{27}$, 1195.5753). FAB-MS (pos.): *m/z* 1179.5 $[M+H-H_2O]^+$, 1033.4 $[M+H-H_2O\text{-}146]^+$, 901.3 $[M+H-H_2O\text{-}146\text{-}132]^+$, 739.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162]^+$, 577.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162\text{-}162]^+$, and 415.2 $[M+H-H_2O\text{-}146\text{-}132\text{-}162\text{-}162\text{-}162]^+$.

## *3.4 Acid hydrolysis of compounds 1 and 2*

Compounds **1** and **2** (about 2.0 mg) were treated with 1 M HCl (dioxane–$H_2O$, 1:1, 2 ml) at 100°C for 1.5 h, respectively. The reaction mixture was neutralized with silver carbonate and the solvent was thoroughly driven out under $N_2$ gas overnight. The residue was extracted with $CHCl_3$ and $H_2O$. Then, in monosaccharide mixture, glucose, rhamnose, and xylose were detected by TLC analysis on a cellulose plate using *n*-BuOH–EtOAc–$C_5H_5N$–$H_2O$ (6:1:5:4) as development and aniline-*o*-phthalic acid as detection, comparing with the authentic samples: glucose ($R_f$ 0.46), xylose ($R_f$ 0.55), and rhamnose ($R_f$ 0.69). Furthermore, the sugar residue in pyridine (1 ml) was added to L-cysteine methyl ester hydrochloride (3.0 mg) and kept at 60°C for 1 h. Then, hexamethyldisilazane–trimethylchlorosilane (HMDS–TMCS; 0.6 ml) was added to the reaction mixture and kept at 60°C for 0.5 h. The supernatant (1.0 ml) was analyzed by GC under the following conditions: Agilent Technologies 6890 gas chromatograph was the equipment carrying an $H_2$ flame ionization detector and HP-5 capillary column (30 m × 0.25 mm × 0.25 μm). The conditions are as follows: column temperature: 180°C/250°C; programmed increase, 15°C/min; carrier gas: $N_2$ (1 ml/min); injection and detector temperature: 250°C; injection volume: 4.0 μl; split ratio: 1:50. The derivatives of D-glucose, D-xylose, and L-rhamnose were detected, $t_R$: 17.95 min (D-glucose derivative), 12.99 min (D-xylose derivative), and 14.53 min (L-rhamnose derivative).

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