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

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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

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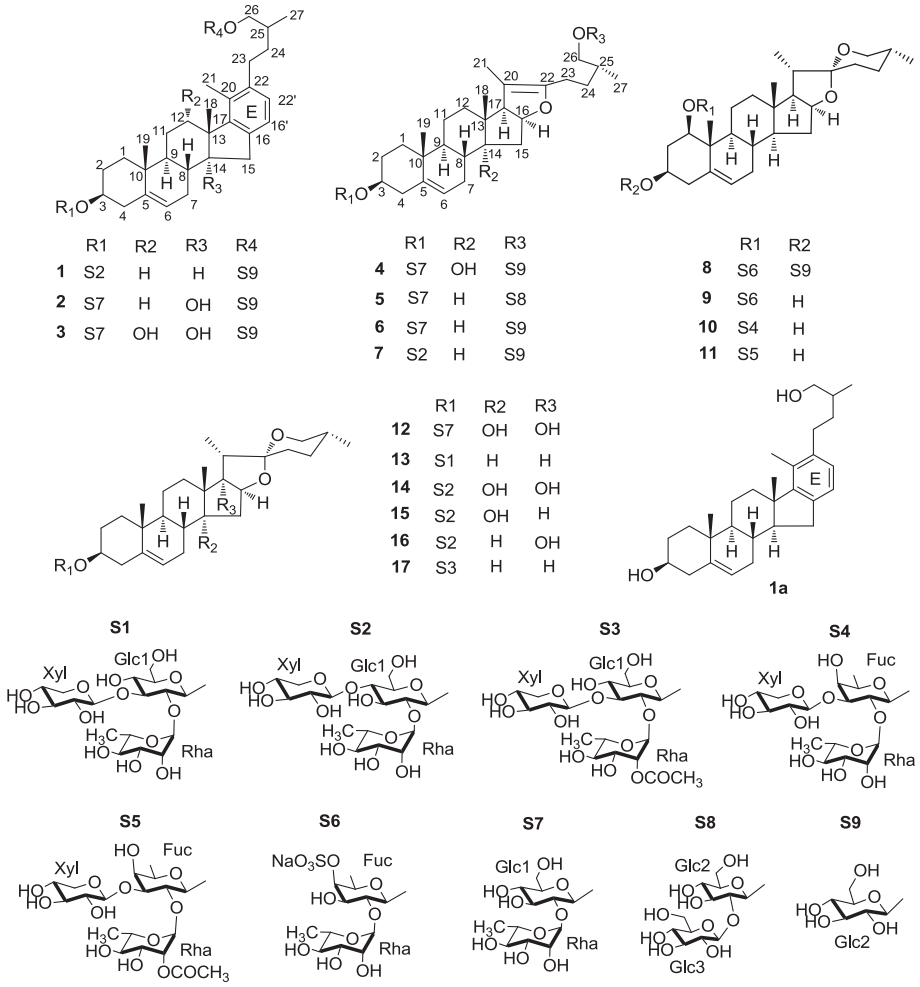

Fig. 1. Structures of compounds **1**–**17**.

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-

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