Steroids 77 (2012) 1298–1305

Contents lists available at SciVerse ScienceDirect

Steroids

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

Steroidal saponins from the tuber of *Ophiopogon japonicus*

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ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 8 April 2012
Accepted 11 April 2012
Available online 25 April 2012

Keywords:
Ophiopogon japonicus
Steroidal saponins
Ophiopogonins H–O
Structural identification

ABSTRACT

Eight novel steroidal saponins, ophiopogonins H–O (**1**–**8**), along with seven known steroidal saponins (**9**–**15**) were isolated from the tubers of *Ophiopogon japonicus*. The structures of these new compounds were determined by detailed spectroscopic analysis, including extensive 1D and 2D NMR data, and the analysis of hydrolytic reaction products. For the first time, rare furostanol saponins with disaccharide moiety linked at position C-26 of the aglycone were reported to be isolated from a natural source.

1. Introduction

Ophiopogon japonicus Ker-Gawl. (Liliaceae) is an evergreen perennial that is widely used in traditional Chinese medicine. Recent pharmacological studies indicated that steroidal saponins from this plant possess broad biological activities such as inhibitory activities against cardiac ischemia, hypoxia and myocardial infarction [1–3]. A survey of the literature showed that a series of steroidal saponins were isolated from *O. japonicus*, but furostanol saponins from this plant have rarely been reported [4–9]. In the search for novel and bioactive steroidal saponins from *O. japonicus*, a further phytochemical analysis has been carried out on the fresh tubers of *O. japonicus*, with particular attention on the furostanol saponins. This paper reports the isolation and structural elucidation of seven new furostanol saponins (**1**–**7**), a novel spirostanol saponin (**8**), and seven known spirostanol saponins (**9**–**15**) (Fig. 1). Their structures were determined by the analysis of 1D and 2D NMR data, HRMS data, physical data, and chemical methods. The chemical structures of the compounds with the disaccharide moiety linked at position C-26 of the aglycone are rare in natural products.

2. Experimental

2.1. General methods

The optical rotations of the isolated compounds were measured with a Perkin-Elmer 343 polarimeter. The 1D and 2D NMR experiments were performed on a Varian ^{UNITY}INOVA 600 spectrometer at 27 °C. The HRESIMS and HRMALDIMS were performed using on a 9.4 T Q-FT-MS Apex Qe (Bruker Co., Billerica, MA, USA). FABMS was recorded on a Micromass Zabspec. Thin-layer chromatography (TLC) was performed on plates precoated with silica GF254 (Qingdao Marine Chemistry Factory, China). Silica-gel (Qingdao Marine Chemistry Factory, China), 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 with the following components: analytical columns, Megres RP-18 column (4.6 × 250 mm, ODS, 5 μm, Hanbon Co. Ltd., China) and Venusil XBP C18 column (4.6 × 250 mm, ODS, 5 μm, Agela Technologies, China); preparative column, a Hanbon lichrospher C_{18} (5 μm, 10.0 × 250 mm, Hanbon Sci. & Tech, Huaian, China); and detector, Alltech ELSD 2000 (evaporative light-scattering detector). Agilent Technologies 6890 gas chromatograph; 5973 mass spectrograph detector; HP-5 capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent, Santa Clara, CA, USA). Hexamethyldisilane and trimethylchlorosilane were purchased from Sigma–Aldrich (St. Louis, MO, USA).

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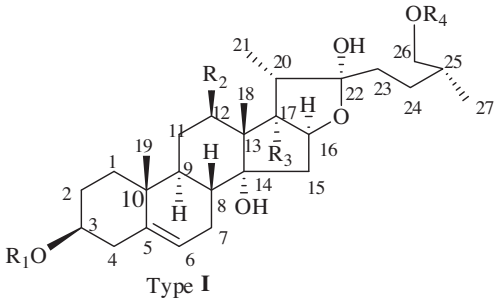
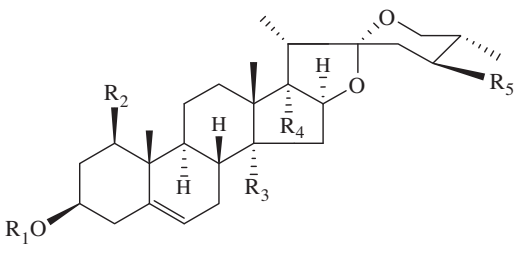
http://dx.doi.org/10.1016/j.steroids.2012.04.015





	R_1	R_2	R_3	R_4
1	S_1	H	OH	S_5
2	S_1	H	H	S_7
3	S_1	H	H	S_6
4	S_2	H	OH	S_6
5	S_1	OH	H	S_7
6	S_1	OH	OH	S_7
7	S_1	H	H	S_5

	R_1	R_2	R_3	R_4	R_5
8	S_1	H	OH	OH	OS_7
9	S_1	H	OH	H	H
10	S_1	H	H	H	H
11	H	OS_3	H	H	H
12	S_2	H	OH	OH	H
13	S_2	H	OH	H	H
14	S_1	H	OH	OH	H
15	S_4	H	H	H	H

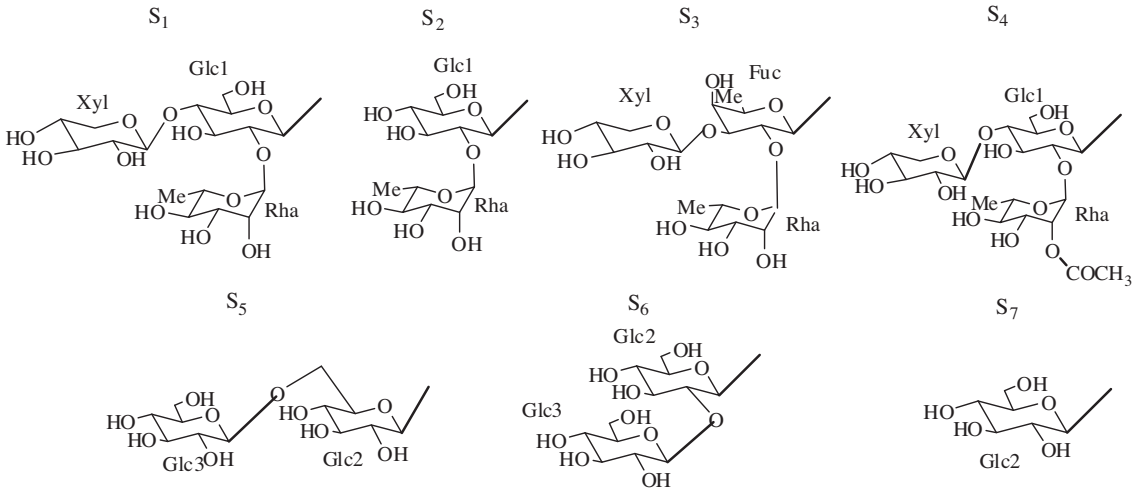


Fig. 1. Steroidal saponins 1–15 isolated from the tuber of *O. japonicus*.

2.2. Plant material

The 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) was deposited at the Herbarium of Beijing Institute of Radiation Medicine, Beijing, China.

2.3. Extraction and isolation

The tubers of *O. japonicus* (56 kg) were refluxed twice with EtOH–H_2O (60:40, 300 L, each for 2 h). 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 (20:80, 50:50 and 90:10; 60,000 mL each), to yield three fractions (A–C).

Fr. B (220 g) was chromatographed on a macroporous resin SP825 (15 × 100 cm) and eluted with a gradient mixture of EtOH-H_2O (20:80, 30:70, 40:60, 55:45 and 80:20; 20,000 mL of each) to provide twelve fractions (B_1–B_{12}). Fr. B_4 (15 g) was separated chromatographically on a silica-gel column (10 × 30 cm) with a gradient mixture of $CHCl_3$–MeOH–H_2O (70:26:6, 70:30:6, 65:36:10, 60:40:12 and 60:45:12, lower phase) as eluent, and a total of 380 tubes were collected. Tubes 111–150 were purified using an ODS silica-gel column (3 × 50 cm; MeCN-H_2O, 15:85 and 17:83)