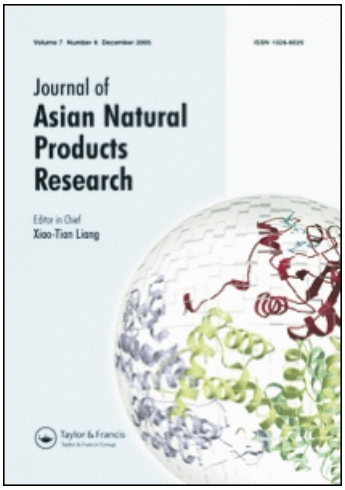

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A novel steroidal glycoside, ophiofurospiside A from *Ophiopogon japonicus* (Thunb.) Ker-Gawl

Tun-Hai Xu[a], Ya-Juan Xu[b]*, Sheng-Xu Xie[b], Hong-Feng Zhao[b], Dong Han[b], Yu Li[a], Jian-zhao Niu[a] and Dong-Ming Xu[b]

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A new furospirostanol saponin, ophiofurospiside A (**1**), was isolated together with the known steroidal glycosides 2, 3, and 4 from the tubers of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. Using chemical and spectral analyses (IR, MS, 1D NMR, and 2D NMR), the structure of **1** was established as 26-*O*-β-D-glucopyranosyl-(22S, 25R)-furospirost-5-ene-3β, 17α, 26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-[β-D-xylopyranosyl-(1 → 4)]-glucopyranoside (**1**). Three known steroidal saponins **2**–**4** were identified on the basis of spectroscopic data.

Keywords: liliaceae; *Ophiopogon japonicus* (L.f) Ker-Gawl; furospirostanol saponin; ophiofurospiside A

1. Introduction

The tuber of *Ophiopogon japonicus* (L.f) Ker-Gawl is a Chinese traditional medicine named "Maidong". The tuber was recorded to have various medical functions for curing cardiovascular diseases and bacterial infections, especially heart diseases. Phytochemical studies on this plant were reported previously.[1] In the search for new and bioactive components from Chinese traditional medicine, we investigated the tubers of *O. japonicus*. In the present paper, we report the isolation and structure elucidation of a new furospirostanol saponin, namely ophiofurospiside A (**1**) together with three known steroidal glycosides 2, 3, and 4 using 1D, 2D NMR techniques, ESI-MS analysis, as well as chemical methods.

2. Results and discussion

Ophiofurospiside A (**1**) was isolated as a white amorphous powder. **1** exhibited the molecular formula $C_{50}H_{80}O_{23}$ by its HRMS analysis. The ESI-MS of **1** showed a quasi-molecular ion peak at *m/z* 1047 $[M - H]^-$. Other significant ion peaks visible at *m/z* 915 $[M - 132\text{-}H]^-$, 753 $[M - 132\text{-}162\text{-}H]^-$, 769 $[M - 132\text{-}146\text{-}H]^-$, and 590 $[M - 132\text{-}146\text{-}162\text{-}OH{-}H]^-$ suggested the presence of pentose, hexoses, and deoxyhexose. Complete hydrolysis of **1** with HCl yielded glucose, rhamnose, and xylose by comparing with the authentic samples on high-performance TLC (HPTLC). The ^1H NMR spectrum of **1** showed diagnostic signals of three tertiary methyl groups at δ 0.92 (3H, s), 1.06 (3H, s), 1.35 (3H, s) and two secondary

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Table 1. ^{13}C NMR spectral data of compound **1**. (δ_C, 125 MHz, C_5D_5N).

Run	^{13}C NMR, δ						
1	37.4	15	32.1	*C-3*			
2	32.2	16	89.9	glc′ 1′	99.9	xyl 1‴	105.7
3	78.4	17	89.6	2′	77.5	2‴	75.3
4	41.4	18	16.9	3′	76.1	3‴	78.3
5	140.9	19	19.4	4′	81.4	4‴	70.7
6	121.8	20	38.8	5′	77.4	5‴	62.3
7	32.0	21	9.9	6′	62.6	*C-26*	
8	32.1	22	120.4	rha 1″	101.9	glc 1	105.5
9	50.1	23	32.2	2″	72.4	2	74.8
10	37.0	24	33.6	3″	72.7	3	78.5
11	20.8	25	84.2	4″	74.0	4	71.5
12	33.6	26	77.2	5″	69.5	5	78.3
13	45.0	27	24.3	6″	18.6	6	61.5
14	52.7						

methyl groups at δ 1.15 (3H, d, $J = 7.3$ Hz), 1.75 (3H, d, $J = 6.5$ Hz), an olefinic proton at δ 5.25 (s) and four doublets of anomeric protons at δ 4.93 (1H, d, $J = 7.8$ Hz) 6.25 (1H, br s) 5.01 (1H, d, $J = 7.5$ Hz) 4.91 (1H, d, $J = 7.5$ Hz). The ^{13}C NMR spectrum (Table 1) of **1** showed the signals of angular methyl groups at δ 16.9, 19.4, 9.9, and 24.3, olefinic carbons at δ 121.8 and 140.9, and anomeric carbons at δ 99.9, 101.9, 105.7, and 105.5. In addition, resonances for the quaternary C-22 and C-25 are observed at δ 120.4 and 84.2, respectively, showing that the sapogenin has a "furanose" F-ring, suggesting **1** to be a furospirostanol tetraglycoside.[2,3]

Based on the ESI-MS, the molecular weight of the aglycon moiety was 446, which is 16 more than that of nuatigenin,[4] suggesting that the hydrogen atom at C-17 in nuatigenin was substituted by a hydroxyl in **1**. Moreover, the ^{13}C NMR spectral data of the aglycone of **1** (Table 1) were almost consistent with those of nuatigenin,[4] except that the signals of C-17 (δ 89.6), C-16 (δ 90.0), and C-13 (δ 45.1) were shifted downfield compared with those C-17 at δ 62.2, C-16 at δ 81.1, and C-13 at δ 40.0 of nuatigenin.[4] In addition, the C-21 signal of **1** appears at δ 9.87 due to steric interaction with the 17α-hydroxyl group.[2] This conclusion was also supported by the HMBC correlations from two angular methyl groups CH_3-18 and CH_3-21 to C-17. Consequently, the aglycone of **1** could be determined as (22S, 25R)-furospirost-5-en-3β, 17α, 26-triol.

As described above, the sugar moiety of **1** consisted of glucose, rhamnose, and xylose. The coupling constants of the anomeric protons revealed the β configurations for glucoses and xylose and α configurations for rhamnose.[7,8] The positions of the sugar residues in **1** were defined unambiguously by HMBC experiment (Figure 1). An HMBC correlation between H-1′ (δ 4.93) of glucose′ and C-3 (δ 78.5) of the aglycone indicated that glucose′ was linked to C-3 of the aglycone a correlation between H-1″ (δ 6.25) of rhamnose and C-2′ (δ 77.5) of glucose′ indicated that rhamnose was linked to C-2′ of the glucose′ a correlation between H-1″(δ 5.01) of xylose and C-4′ (δ 81.4) of glucose′ indicated that xylose was linked to C-4′ of the glucose′. These assignments showed that the trisaccharide moiety, 3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-[β-D-xylopyranosyl-(1 → 4)]-glucopyranoside, was linked to the C-3 of the aglycone. Additionally, an HMBC correlation between H-1 (δ 4.91) of glucose and C-26 (δ 77.2) of the aglycone indicated that the glucose was linked to C-26 of the aglycone. On the basis of these evidences, the structure of **1** was elucidated as 26-*O*-β-D-glucopyranosyl-(22S, 25R)-furospirost-5-ene-3β, 17α, 26-triol-3-*O*-[α-L-rhamnopyranosyl-(1 → 2)]-[β-D-xylopyranosyl-(1 → 4)]-glucopyranoside.

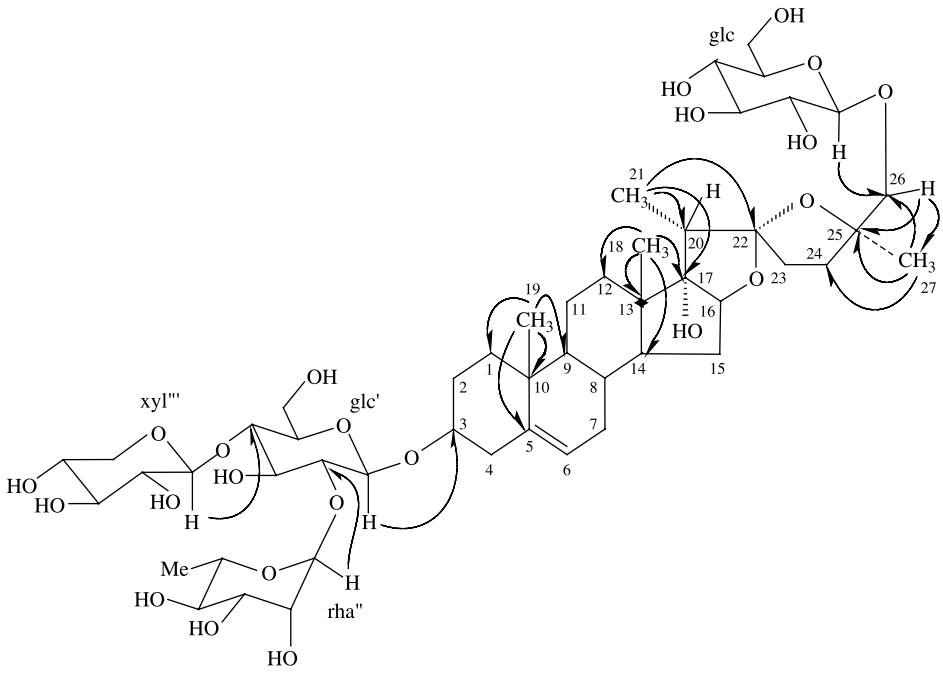

Figure 1. The key HMBC correlations of compound **1**.

Three known compounds **2**–**4** were identified as glycoside A (**2**),[5] glycoside D (**3**),[5] and ophiopogonin B (**4**)[6] by a comparison of their physical and spectroscopic data with those reported in the literature, respectively.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Kofler microscope apparatus and are uncorrected. The optical rotations were determined on WZZ-15 autopolarimeter. The IR spectra were measured on a Y-Zoom scroll Fourier transform infrared spectrometer with a KBr disc. The ESI-MS was recorded on LCQ-1700 ESI-MS instrument. The NMR spectra were obtained on a Bruker AM-500 instrument, using TMS as internal standard. HPLC was performed using an ODS column (Shimpark PREF-ODS, 250 × 4.6 mm). Column chromatography was performed on silica gel (200–300 mesh, Qingdao Oceanic Chemical Industry, Qingdao, China) and ODS reversed silica gel (25 × 2.5 cm, Nacalai Tesque, Kyoto, Japan). Macroporous resin D_{101} was made in Tianjin gel. TLC was conducted on silica gel 60 F_{254} (Merck, New Jersey, USA). Spots were detected after spraying with 10% H_2SO_4.

3.2 Plant material

The tubers of *O. japonicus* (L.f) Ker-Gawl were purchased from the company of Chinese Medicinal Materials in Changchun, Jilin Province, China, and identified by Prof. Minglu Deng, Changchun College of Traditional Chinese Medicine. A voucher specimen (145136) has been deposited in the Herbarium of Academy of Traditional Chinese Medicine and Material Medica of Jilin Province.

3.3 Extraction and isolation

The dried tubers (5 kg) of *O. japonicus* were powdered and extracted three times with H_2O

at boiling. The extract was concentrated under reduced pressure to give the crude residue (156 g), which was chromatographed over a D_{101} macroporous resin column (10 × 80 cm), eluted successively with H_2O, 20% EtOH, and 40% EtOH. The 40% EtOH eluate was concentrated to dryness (13.5 g) and chromatographed over a silica gel column (200–300 mesh) eluted with $CHCl_3$:MeOH:H_2O (65:15:10–65:20:10) to give fractions A–E. Compound **2** (96 mg) was obtained from fraction A by HPLC. Fraction B was subjected to HPLC (column: 10 × 250 mm, RP-18, 10 μm, flow rate: 3.0 ml/min) with MeOH–H_2O (56:44) as eluent and afforded **1** (38 mg). Fraction C was subjected to HPLC with MeOH–H_2O (60:40) as eluent to afford **3** (18 mg) and **4** (20 mg).

3.3.1 *Ophiofurospiside A (**1**)*

Colourless amorphous powder, mp 220–222°C, $[\alpha]_D^{18}$ − 16 (c 0.36, MeOH). IR(KBr) ν_{max}: 3606, 2920, 1623, 1458, 1256, 1070, 1038 cm^{-1}. ^1H NMR (500 MHz, pyridine-d_5) δ: 0.92 (3H, s, CH_3-18), 1.06 (3H, s, CH_3-19), 1.35 (3H, s, CH_3-27), 1.15 (3H, d, J = 7.3 Hz, CH_3-21), 2.47 (1H, s, H-20β), 1.75 (3H, d, J = 6.5 Hz, rha-Me), 5.25 (1H, br, s, H-6), 4.93 (1H, d, J = 7.8 Hz, glc′-H-1′), 6.25 (1H, br s, rha-H-1″), 5.01(1H, d, J = 7.5 Hz, xyl-H-1‴), 4.91(1H, d, J = 7.5 Hz, glc-H-1). ^{13}C NMR (125 MHz, pyridine-d_5) spectral data are given in Table 1. HRMS *m/z*: 1047.5092 $[M - H]^-$ (calcd for $C_{50}H_{80}O_{23}$, 1048.5090). ESI-MS *m/z*: 1047 [M − H], 915 [M − 132-H], 753 [M − 132-162-H], 769 [M − 132-146-H], 590 [M − 132-146-162-OH—H]$^-$.

3.4 *Acid hydrolysis*

The saponin (10 mg) was heated with 2 M HCl–MeOH (10 ml) under reflux for 3 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The water layer was neutralized with Na_2CO_3, concentrated, and subjected to co-TLC analysis with authentic samples of D-glucose, D-xylose, and L-rhamnose, and developed with H_2O–MeOH–AcOH–EtOAc (15:15:20:65). Detection was carried out with aniline phthalate spray.

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References

[1]P.G. Xiao, *Modern Chinese Materia Medica* (Chemical Industry Press, Beijing, 2001), pp. 481–487.
[2]P.K. Agrawal, D.C. Jian, R.K. Gupta, and R.S. Thakur, *Phytochemistry* **24**, 2479 (1985).
[3]P.K. Agrawal, D.C. Jian, and A.K. Patheak, *Magn. Reson. Chem.* **33**, 923 (1995).
[4]R. Saijo, C. Fuke, K. Murakami, T. Nohara, and T. Tomimatsu, *Phytochemistry* **22**, 733 (1983).
[5]Y. Watanabe, S. Sanada, Y. Ida, and J. Shoji, *Chem. Pharm. Bull.* **31**, 1980 (1983).
[6]T. Asano, T. Murayama, Y. Hirai, and J. Shoji, *Chem. Pharm. Bull.* **41**, 566 (1993).
[7]P.K. Agrawal, *Phytochemistry* **31**, 3307 (1992).
[8]P.K. Agrawal, D.C. Jain, and R.K. Gupta, *Phytochemistry* **24**, 2479 (1985).