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A New Steroidal Glycoside from *Ophiopogon japonicus* (Thunb.) Ker-Gawl.

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Abstract: To study the chemical constituents from traditional Chinese herb *Ophiopogon japonicus* (Thunb.) Ker-Gawl., a new steroidal glycoside, named ophiopojaponin C (**1**), together with two known ones, was isolated by column chromatography. Spectroscopic and chemical evidence revealed the structures to be ophiopogenin 3-O-[α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→4)-β-*D*-glucopyranoside (**1**), diosgenin 3-O-[2-O-acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-glucopyranoside (**2**), and ruscogenin 1-O-[2-O-acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-fucopyranoside (**3**).

Key words: C_{27} steroidal glycosides; Liliaceae; *Ophiopogon japonicus*.

The tuber of *Ophiopogon japonicus* (Thunb.) Ker-Gawl. has been recorded as having various functions, such as against cardiovascular diseases and as an antibacterial, and has been used as a potent drug to treat different diseases, especially heart diseases (Jiangsu New Medical College 1977). Since the first steriodal glycoside was isolated from the plant by Japanese scholars (Tada and Shoji 1972), considerable attention has been paid to investigations of the chemical components of *O. japonicus* in recent decades. Steroidal glycosides as the major glycosides with the aglycones of ruscogenin and diosgenin in this plant have been reported (Tada *et al.* 1973; Nohara *et al.* 1975; Nakanishi and Kameda 1987; Adinolfi *et al.* 1990; Branke and Hashilinger 1995; Chen *et al.* 2000). Because of our interest in the development of the "Sheng Mai San" injection, which is composed of *Panax ginseng* C. A. Meyer, *O. japonicus*, and *Schisandra chinensis* (Turcz.) Baill., during our reinvestigation of *O. japonicus* collected in Sichuan Province, three compounds, namely ophiopogenin 3-O-[α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→4)-β-*D*-glucopyranoside (**1**), diosgenin 3-*O*-[2-O-acetyl-α-*L*-rhamnopy-ranosyl(1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-glucopyranoside (**2**), and ruscogenin 1-O-[2-O- acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl (1→3)-β-*D*-fucopyranoside (**3**) were isolated from an ethanolic extract of the tuber. Compound **1** was new, whereas compounds **2** and **3** were known ones.

1 Results and Discussion

Compound **1** was obtained as colorless needles, with a melting point (mp) of 215–217 °C, and its molecular formula $C_{46}H_{72}O_{18}$ was determined from the quasi-molecular ion peak at *m/z* 885 $[(C_{44}H_{70}O_{18})\text{-}H]^-$ in its negative FAB mass spectrum and the ^{13}C-NMR (DEPT) spectrum, which was supported by its HR-ESI observed at *m/z* 885.446 8 (calculated 885.448 3, $C_{44}H_{70}O_{18}$-H). The IR spectrum of compound **1** showed the characteristic absorptions of 25 (*R*)-spirosteroid at 980, 920, 897, and 867 cm^{-1} (intensity: 897 > 920). Comparison of the ^{13}C-NMR spectrum with that of pennogenin showed that the aglycone had one quarternary carbon more and one tertiary carbon less than pennogenin. The

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chemical shift of C-14 was downfield shifted to δ 86.6, which suggested that C-14 was substituted by a hydroxyl group. The ^{13}C-NMR spectral data (Table 1) of the aglycone moiety of **1** were similar to those of ophiopogenin (Agrawal *et al.* 1995), so the aglycone of **1** was deduced to be ophiopogenin, the structure of which is spirost-5-ene-3,14,17-triol (3β,14α,17α,25*R*). On complete acid hydrolysis of **1**, glucose, rhamnose, and xylose were determined by TLC and PC comparison with authentic samples. The ^{1}H-NMR spectrum of **1**, demonstrating the coupling constants of the anomeric proton signals at δ 4.92 (1H, d, J = 7.0 Hz), δ 5.01 (1H, d, J = 7.7 Hz), and δ 6.22 (1H, brs), indicated two β-linkages and one α-linkage in the sugar chain. Accordingly, the negative ion FAB mass spectrum displayed a quasi-molecular ion at *m/z* 885 $[M–H]^-$, together with fragment ion peaks at *m/z* 753 $[M–H–132]^-$, 739 $[M–H–146]^-$, and 445 $[M–H–146–132–162]^-$, which suggested that **1** contained one rhamnose and one xylose as terminal sugars, one glucose as an inner sugar in the sugar chain, and ophiopogenin as aglycone. This was further confirmed by two-dimensional NMR. From the HMQC and ^{1}H-^{1}H COSY spectra, the chemical shifts of the sugar moieties were assigned (Table 1). In the HMBC spectrum (Fig. 1), the long-range correlations between C-3 (δ 78.4) of the aglycone and H-1 (δ 4.92) of glucose, C-2 (77.8) of glucose and H-1 (δ 6.22) of rhamnose, and C-4 (81.7) of glucose and H-1 (δ 5.01) of xylose showed that the glucosyl unit was linked to C-3 of the aglycone, the rhamnosyl unit was linked to C-2 of glucose, and the xylosyl unit was linked to C-4 of glucose. Thus, the structure of **1** was identified as ophiopogenin 3-O-[α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→4)-β-*D*-glucopyranoside, named ophiopojaponin C.

The known compounds diosgenin 3-O-[2-O-acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl (1→3)-β-*D*-glucopyranoside (**2**; Watanabe *et al.* 1977) and ruscogenin 1-O-[2-O- acetyl-α-*L*-rhamnopyranosyl (1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-fucopyranoside (**3**; Yang *et al.* 1987; Yu and Xu 1988) were identified by direct comparison with the published data.

2 Experimental

2.1 General experimental procedures

Melting points were determined on an XRC-1 micromelting apparatus (Sichuan University, Sichuan, China) and are uncorrected. Optical rotation was measured with a JASCO-20C polarimeter (JASCO, Japan) at room temperature. The IR spectra were measured on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, CA, USA). NMR spectra were run on Bruker AM-400 MHz and DRX-500 MHz spectrometers

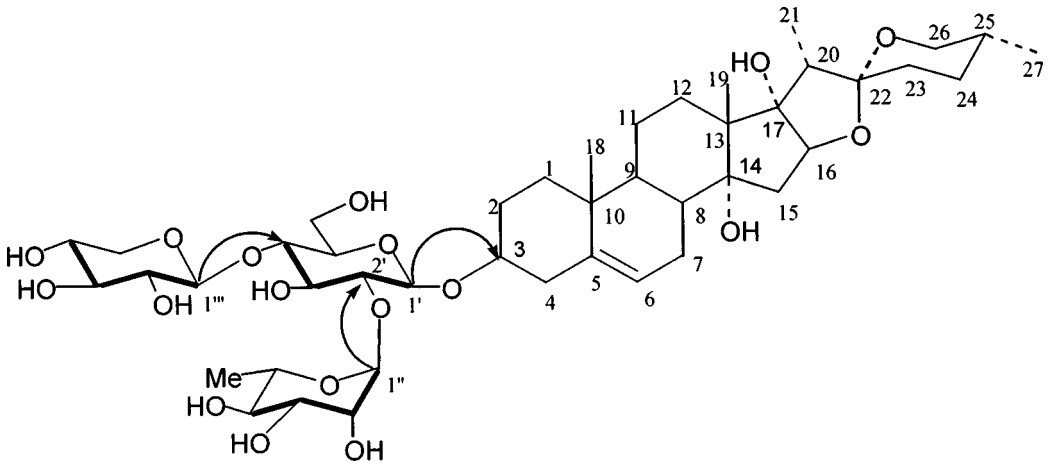

Fig. 1. The structure and key HMBC correlations of compound **1**.

Table 1 ^{13}C-NMR data for compound **1** in C_5D_5N (100 MHz)

Aglycone moieties				Sugar moieties			
C	**1**	**2**	**3**	C	**1**	**2**	**3**
1	37.9	37.7	84.7	1'	100.2	101.5	100.5
2	30.5	30.2	38.1	2'	77.8	76.4	73.8
3	78.4	78.4	68.4	3'	77.4	81.5	85.0
4	39.2	39.2	43.9	4'	81.7	71.5	71.1
5	140.5	140.4	139.6	5'	76.2	78.4	70.8
6	122.5	122.3	124.9	6'	61.9	61.9	17.2
7	26.8	32.6	32.5	1"	102.0	99.5	98.4
8	35.8	32.1	33.2	2"	72.5	75.2	74.3
9	42.2	50.7	50.8	3"	72.9	71.0	69.3
10	37.5	37.5	42.9	4"	74.2	71.9	72.5
11	20.5	21.5	24.1	5"	70.0	70.0	68.4
12	26.3	40.2	40.6	6"	18.7	18.3	18.9
13	48.5	40.8	40.4	1"'	105.8	105.8	105.9
14	86.6	57.0	57.3	2"'	75.0	76.4	74.9
15	40.0	32.4	32.2	3"'	78.4	77.4	78.0
16	90.6	81.5	81.3	4"'	70.9	72.4	71.1
17	91.3	63.2	63.2	5"'	67.4	67.5	67.2
18	20.2	16.9	17.0	CH_3COO		21.5	21.3
19	19.4	19.6	15.0	CH_3COO		171.2	171.1
20	45.2	42.3	42.1				
21	9.8	15.3	15.1				
22	109.7	109.8	109.4				
23	32.1	32.2	31.9				
24	29.5	29.5	29.4				
25	30.7	30.9	30.7				
26	67.0	67.2	67.0				
27	17.4	17.6	17.4				

(Bruker, Switzerland) using TMS as an internal. A VG Auto Spec-3000 spectrometer (Micromass, England) was used to record the FABMS spectrum. Silica gel (200–300 and 300–400 mesh, Marine Chemical Factory, Qingdao, China), and D-101 resin (Shanghai Yadong Heji Resin Inc., Shanghai, China) were used for column chromatography.

2.2 Plant materials

The plant used in the present study was collected in Sichuan Province in 1998, and was identified as *Ophiopogon japonicus* (Thunb.) Ker-Gawl. by one of the authors (Jun ZHOU). A voucher specimen was deposited in Kunming Institute of Botany, the Chinese Academy of Sciences.

2.3 Extraction and isolation

Tubers of *O. japonicus* (10 kg) was extracted with hot 95% ethanol (EtOH). After removal of the solvent by evaporation, the residue was dissolved in water and chromatographed on a D-101 resin with H_2O and methanol (MeOH) to give two fractions. The MeOH eluent as the main glycoside was concentrated in vacuum to obtain 25 g crude glycosides, which was

chromatographed on silica gel with $CHCl_3$: MeOH : H_2O (4 : 1 : 0.1) to give eight fractions. Fraction 3 (2.0 g) was chromatographed on silica gel with $CHCl_3$: MeOH : H_2O (7 : 1 : 0.1) to afford compounds **2** (130 mg) and **3** (6 mg). Fraction 5 (6.0 g) was chromatographed on silica gel with $CHCl_3$: MeOH : H_2O (4 : 1 : 0.1) to afford compound **1** (95 mg).

2.4 Identification

Ophiopojaponin C (1) Colorless needles (MeOH), mp 215–217 °C; $[\alpha]_D^{12.3}$ –77.3° (*c* 0.51, MeOH), IR bands (KBr): 3 400, 2 941, 1 554, 1 376, 1 044, 980, 920, 897, 867 cm^{-1}; ^{1}H-NMR (C_5D_5N): δ 5.49 (1H, *brs*, H-6), 2.05 (1H, m, H-9), 4.79 (1H, m, H-16), 1.09 (3H, s, H-18), 1.07 (3H, s, H-19), 2.48 (1H, m, H-20), 1.26 (3H, d, *J* = 7.2 Hz, H-21), 3.50 (2H, m, H-26), 0. 66 (3H, d, *J* = 4.5 Hz, H-27), 4.92 (1H, d, *J* = 7.0 Hz, H-1'), 3.82 (1H, m, H-5'), 4.40, 4.48 (each 1H, d, *J* = 11.0 Hz, H-6'), 6.22 (1H, *brs*, H-1''), 4.78 (1H, m, H-2''), 4.58 (1H, m, H-3''), 4.37 (1H, t, *J* = 9.4 Hz, H-4''), 4.90 (1H, m, H-5''), 1.76 (3H, d, *J* = 6.2 Hz, H-6'), 5.01 (1H, d, *J* = 7.7 Hz, H-1'''), 3.97 (1H, t, *J* = 8.2 Hz, H-2'''), 4.11 (1H, t, *J* = 8.8 Hz, H-3'''), 4.15 (1H, m, H-4'''), 3.67 (1H, t, *J* = 8.8 Hz, H-5'''); ^{13}C-NMR (Table 1); HR-ESI *m/z* 885.446 8 (calculated 885.448 3, $C_{44}H_{70}O_{18}$-H); FAB-MS (negative) *m/z* 885 $[M–H]^–$, *m/z* 753 $[M–H–132]^–$, 739 $[M–H–146]^–$, 445 $[M–H–146–132–162]^–$.

Diosgenin 3-O-[2-O-acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-glucopyranoside (2) Colorless needles (MeOH), mp 242–244 °C; FAB-MS (negative) *m/z* IR bands (KBr): 3400, 1745, 978, 919, 896, 864 cm^{-1}; ^{1}H-NMR (C_5D_5N): d 0.70 (3H, d, *J* = 5.6 Hz, H-27), 0.89 (3H, s, H-18), 1.15 (3H, s, H-19), 1.16 (3H, d, *J* = 7.0 Hz, H-21), 1.50 (3H, d, *J* = 6.3 Hz, RhaH-6), 2.03 (3H, s, H-CH_3CO), 4.99 (1H, d, *J* = 7.8 Hz, GlcH-1), 5.03 (1H, d, *J* = 7.7 Hz, XylH-1), 6.26 (1H, br.s, Rha H-1); ^{13}C-NMR: see Table 1. The spectral data were similar to those of the reference (Watanabe *et al.* 1977).

Ruscogenin 1-*O*-[2-*O*-acetyl-α-*L*-rhamnopyranosyl(1→2)]-β-*D*-xylopyranosyl(1→3)-β-*D*-fucopyranoside (3) ^{1}H-NMR (C_5D_5N): δ 0.66 (3H, d, *J* = 4.5 Hz, H-27), 0.85 (3H, s, H-18), 0.86 (3H, s, H-19), 1.04 (3H, d, *J* = 6.5 Hz, H-21), 1.40 (3H, d, *J* = 5.5 Hz, Fuc H-6), 1.72 (3H, d, *J* = 5.8 Hz, Rha H-6), 1.96 (3H, s, H-CH_3CO), 4.95 (1H, d, *J* = 7.0 Hz, Xyl H-1), 5.57 (1H, d, *J* = 5.4 Hz, Fuc H-1), 6.07 (1H, brs, Rha H-1); ^{13}C-NMR: see Table 1. The spectral data were similar to those of the reference (Tada *et al.* 1973).

2.5 Acid hydrolysis of compound 1

A solution of compound **1** (4 mg) in 1 mL HCl–MeOH (2 mol/L) was refluxed at 100 °C for 3 h and then neutralized with saturated $Ba(OH)_2$ aq. The solution was partitioned with $CHCl_3$ after removal of MeOH. Glucose, rhamnose, and xylose in the water layer were revealed by TLC ($CHCl_3$: MeOH : H_2O 7 : 3 : 0.5) and PC (*n*-BuOH : CH_3COOH : H_2O 4 : 1 : 5) analyses.

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