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Two New Steroidal Glycosides from *Ophiopogon japonicus*

Hao Fu DAI[1], Jun ZHOU[1]*, Zhong Tao DING[2], Jiang XIONG[1], Ning Hua TAN[1]

[1]Laboratory of Phytochemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204
[2]Department of Chemistry, Yunnan University, Kunming 650091

Abstract: Two new C_{27} steroidal glycosides, named ophiopojaponin A (**1**) and B (**2**), were isolated from the tubers of famous traditional Chinese herb–*Ophiopogon japonicus*. The spectroscopic and chemical evidences revealed their structures to be Pennogenin 3-O-[2'-O-acetyl-α-L-rhamnopyranosyl (1→2)]-β-D-xylopyranosyl (1→3)-β-D-glucopyranoside (**1**) and 26-O-β-D-glucopyranosyl-(22ξ, 25R)-3β, 14α, 22ξ, 26-tetrahydroxyfurost-5-ene 3-O-α-L-rhamnopyranosyl (1→2)-β-D-glucopyranoside (**2**), respectively.

Keywords: Liliaceae, *Ophiopogon jappnicus*, C_{27} steroidal glycosides, ophiopojaponin A and B.

The tuber of *Ophiopogon japonicus* Ker-Gawl. is recorded to have various functions, such as against cardiovascular diseases and anti-bacteria, and used as a potent drug to treat different diseases, especially heart diseases[1]. Since the first steriodal glycoside was isolated from the plant by Japanese scholars[2], much attention has been paid to the studies 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[3]. Because of our interest in development of "Sheng Mai San" injection which is composed of *Panax ginseng*, *O. Japonicus* and *Schisandra chinensis*, during our reinvestigation of *O. Japonicus* collected in Sichuan province, two new steroidal glycosides named ophiopojaponin A (**1**) and B (**2**) (**Figure 1**) were isolated and their structures were elucidated by spectral means, especially 1D and 2D NMR spectroscopy.

Compound **1**, colorless needles, mp: 230~232^{0}C, $[\alpha]_D^{21}$ =-45.7 (c 0.35, MeOH), its molecular formula $C_{46}H_{72}O_{18}$ was determined from the quasi-molecular ion peak at *m/z* 911 $[(C_{46}H_{72}O_{18})\text{-}H]^-$ in its negative FAB mass spectrum and the ^{13}C NMR (DEPT) spectrum. The IR spectrum of compound **1** showed the characteristic absorptions of 25 (R)-spirosteroid at 980, 930, 910 and 870 cm^{-1}. Comparison of the ^{13}C NMR spectrum with that of diosgenin showed that the aglycone had one quarternary carbon more and one tertiary carbon less than diosgenin. The chemical shift of C-17 was downfield shifted to δ 90.2, which suggested that C-17 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 pennogenin[4]**,** so the aglycone of **1** was deduced to be pennogenin, whose structure is

spirost-5-ene-3, 17-diol (3β, 17α, 25R). 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 4.90 (1H, d, 7.2 Hz), δ 5.02 (1H, d, 7.7 Hz), δ 6.13 (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* 911[M-H]$^-$, together with fragment ion peaks at *m/z* 869 [M-43]$^-$, 723 [M-43-146]$^-$, 737 [M-43-132]$^-$, 429 [M-43-146-132-162]$^-$, which suggested that **1** contained one rhamnose and one xylose as terminal sugars, and one glucose as inner sugar in the sugar chain, an acetyl group, and pennogein. This was further confirmed by 2D NMR. From the HMQC and ^{1}H-^{1}H COSY spectra, the chemical shifts of the sugar moieties were assigned respectively (**Tables 1** and **2**). In the HMBC spectrum, the long-range correlations between C-3 (δ 78.4) of the aglycone and H-1 (δ 4.90) of glucose , carbonyl carbon (δ 177.6) of acetyl and H-2 (δ 6.05) of rhamnose, C-2 (77.1) of glucose and H-1 (δ 6.13) of rhamnose, C-3 (81.5) of glucose and H-1 (δ 5.02) of xylose showed that the glucosyl unit was linked to C-3 of the aglycone, O-COCH$_3$ linked to C-2 of rhamnose, 2-O-acetyl-rhamnosyl unit linked to C-2 of glucose, xylosyl unit linked to C-3 of glucose. Thus, the structure of **1** was identified as pennogenin 3-O-[2'-O-acetyl-α-L-rhamnopyranosyl (1→2)]-β-D-xylopyranosyl (1→3)-β-D-glucopyranoside, named ophiopojaponin A.

Compound **2**, colorless needles, mp: 198~201^{0}C, $[\alpha]_D^{23}$=-42.6 (C 0.16, C_5H_5N) , has the composition $C_{45}H_{74}O_{19}$, determined from its FABMS which showed a quasimolecular ion peak at *m/z* 917 [M-H]$^-$ and ^{13}C NMR (DEPT) data. Compound **2** showed strong absorptions of a vinyl group (1620 cm^{-1}) and a hydroxyl group (3400 cm^{-1}) but no absorptions of a spirosteroid in the IR spectrum, and was positive to Ehrlich reagent reaction, which suggested it may be a furostanol glycoside. The aglycone of compound **2** could be proved to be 3β, 14α, 22ξ, 26-tetrahydroxy- (25R)-5-furostene by direct comparison of the ^{1}H and ^{13}C NMR spetra (**Tables 1** and **2**) with those of the reference[5]. Complete acid hydrolysis of **2** yielded an aglycone and sugar residues consisting of glucose and rhamnose. The ^{1}H NMR spectrum of **2** demonstrating the coupling constants of the anomeric proton signals at δ 4.81 (1H, d, 7.8 Hz), δ 5.01 (1H, d, 6.8 Hz), δ 6.38 (1H, brs) indicated two β-linkages and one α-linkage in the sugar chains. Accordingly, the negative ion FAB mass spectrum displayed the fragment ion peaks at *m/z* 771 [M-146]$^-$, 755 [M-162]$^-$, 609 [M-146-162]$^-$, which suggested that **2** contained one rhamnose and one glucose as terminal sugars, and one glucose as inner sugar in the sugar chain. This was further confirmed by 2D NMR. In HMBC spectrum, the long-range correlations between C-3 (δ 78.6) of the aglycone and H-1 (δ 5.01) of glucose, C-2 (δ 79.7) of glucose and H-1 (δ 5.01) of glucose, C-2 (δ 79.7) of glucose and H-1 (δ 6.38) of rhamnose, C-26 (75.3) of aglycone and H-1 (δ 4.81) of glucose were observed, which indicated that two glucosyl units were linked to C-26, C-3 of the aglycone respectively, and the rhamnosyl unit was linked to C-2 of glucose. From the above evidence, the structure of compound 2 was assigned to be 26-O-β-D-glucopyranosyl-(22ξ, 25R) -3β, 14α, 22ξ, 26-tetrahydroxyfurost-5-ene

3-O-α-L-rhamnopyranosyl (1→2)-β-D-glucopy-ranoside, named ophiopojaponin B.

Figure 1. The long-range ^{1}H-^{13}C correlations for Compounds **1** and **2**

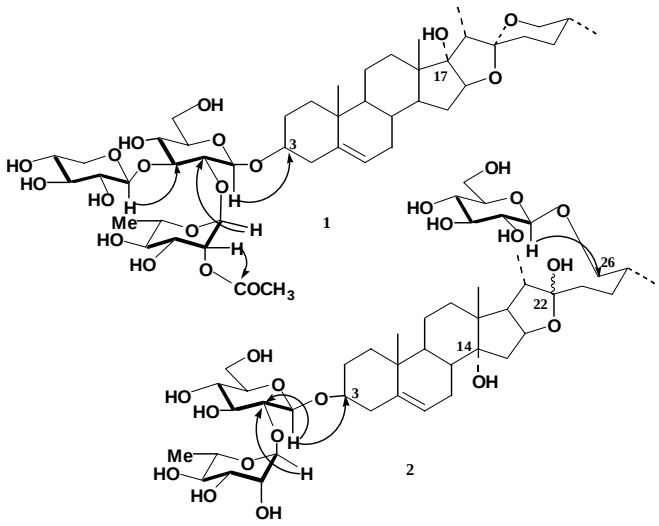

Table 1 ^{13}C NMR (100 MHz) data of **1** and **2** (δ in ppm)

The aglycones moieties			The sugar moieties		
Position	**1**	**2**	Position	**1**	**2**
1	37.6	37.9	3-O-Glc-1	100.1	100.4
2	30.2	30.2	2	77.1	79.7
3	78.4	78.6	3	81.5	78.0
4	39.1	40.2	4	70.8	71.9
5	140.9	140.5	5	78.5	78.6
6	121.9	122.4	6	61.8	62.8
7	32.1	26.8	Rha-1	98.8	102.1
8	32.4	34.4	2	74.0	72.6
9	50.4	43.8	3	70.5	72.9
10	37.2	37.6	4	74.2	74.2
11	21.0	20.5	5	69.6	69.5
12	32.1	37.3	6	18.6	18.7
13	45.2	45.5	$\underline{C}H_3CO$	21.0	
14	53.1	86.5	$CH_3\underline{C}O$	177.6	
15	32.5	39.1	Xyl-1	105.8	
16	90.2	81.6	2	75.0	
17	90.2	60.7	3	77.3	
18	17.2	20.2	4	70.8	
19	19.5	19.4	5	67.4	
20	44.9	40.9	26-O-Glc-1		104.9
21	9.6	16.7	2		75.3
22	110.0	111.1	3		78.4
23	31.7	32.2	4		71.9
24	28.9	28.5	5		78.2
25	30.5	35.7	6		62.8
26	66.8	75.3			
27	17.3	17.6			

The data were measured in pyridine-d_5 with reference to TMS.

Table 2 ^{1}H NMR (400 MHz) data of **1** and **2** (δ in ppm, J in Hz)

Position	**1**	**2**	Position	**1**	**2**
H-27	0.66 (d, 5.4)	0.98 (d, 6.6)	RhaH-1"	6.13 (brs)	6.38 (brs)
H-18	0.95 (s)	1.02 (s)	H-2"	6.05 (m)	4.63 (m)
H-19	1.07 (s)	1.13 (s)	H-4"	4.25 (m)	4.36 (m)
H-21	1.22 (d, 7.1)	1.35 (d, 6.8)	H-6"	1.78 (d, 6.6)	1.77 (d, 6.1)
H-26	3.50 (m)	3.64 (m)	H-Ac	1.99 (s)	
H-3	3.80 (m)	3.94 (m)	XylH-1"'	5.02 (d, 7.7)	
H-6	5.30 (m)	5.36 (m)	H-2"'	4.01 (m)	
H-16	4.45 (m)	5.43 (m)	H-3"'	4.17 (m)	
3-OGlcH-1'	4.90 (d, 7.2)	5.01 (d, 6.8)	26-OGlcH-1		4.81 (d, 7.8)
H-2'	4.17 (m)	4.38 (m)	H-2		4.03 (m)
H-3'	4.17 (m)	4.23 (m)	H-3		4.16 (m)

The data were measured in pyridine-d_5 with reference to TMS.

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