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Ophiopojaponin D, a New Phenylpropanoid Glycoside from *Ophiopogon japonicus* Ker-Gawl

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A new phenolic glycoside, ophiopojaponin D (**1**), together with two known compounds, was isolated from the tubers of a famous traditional Chinese herb-*Ophiopogon japonicus* Ker-Gawl. The spectroscopic and chemical data revealed their structures to be 3-tetradecyloxy-4-hydroxy-allylbenzene-4-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**1**), 3, 4-dihydroxy-allylbenzene-4-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (**2**) and L-pyroglutamic acid (**3**).

Key words: *Ophiopogon japonicus*, Phenolic glycoside, Ophiopojaponin D

INTRODUCTION

The tuber of *Ophiopogon japonicus* Ker-Gawl. has been reported to possess various functions, such as anti-cardiovascular diseases and anti-bacterial activities, and is used as a potent drug for the treatment of different diseases, especially heart diseases (Jiangsu New Medical College, 1977). Since the isolation of the first steroidal glycoside by Japanese scholars (Tada *et al.*, 1972), much attention has been paid to studies on the chemical components of *O. japonicus* over recent decades. To date, besides borneol and its glycosides (Adinolfi *et al.*, 1990), homoisoflavonoids (Tada *et al.*, 1980), and steroidal glycosides, as major glycosides, as well as the aglycones, ruscogenin and diosgenin, have been isolated from the *O. japonicus* plants (Nakanishi *et al.*, 1987; Tada *et al.*, 1973; Branke *et al.*, 1995; Chen *et al.*, 2000; Nohara *et al.*, 1975). Two new C_{27} steroidal glycosides were reported in our earlier paper (Dai *et al.*, 2000). In our continuous studies on the tuber of *O. japonicus*, a new phenolic glycoside, ophiopojaponin D (**1**), together with two known compounds were isolated, and their structures elucidated using chemical and spectral means, especially 1D and 2D NMR spectroscopy.

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MATERIALS AND METHODS

General procedure

The NMR spectra were run on Bruker AM-400 MHz and DRX-500 MHz spectrometers, using TMS as an internal standard. The FAB-MS spectra were measured with a VG Auto Spectrometer. The IR spectra were measured on a Bio-Tad FTS 135 instrument, as KBr pellets. The UV spectra were measured on a Shimadzu UV-210A spectrometer. Melting point determinations were obtained on a Kofler hot stage apparatus, and are reported uncorrected. Optical rotation was measured at room temperature using a J-20C polarimeter. Column chromatography was performed with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Lichprep Lobar Rp-18 gel (Merck, 310 mm×25 mm) columns. TLC was performed with silica gel 60 F_{254}, (Merck), and developed by spraying with 10% H_2SO_4 followed by heating.

Plants material

The tubers of *Ophiopogon japonicus* used in this research were collected in Sichuan province, China during 1999. The voucher specimen (KZ 8635) was deposited at the Kunming Institute of Botany, the Chinese Academy of Sciences.

Extraction and isolation

The tubers of *O. Japonicus* (10 kg) were extracted three times with hot 95% EtOH. After removal of the solvent by evaporation, the residue was dissolved in water and

subjected to chromatography on D-101 resin, with H_2O and MeOH as the eluents, to afford two fractions. The MeOH eluent was concentrated *in vacuo*, and 25 g of crude glycosides obtained, which was subjected to chromatography on silica gel, with $CHCl_3$-MeOH-H_2O (4:1:0.1) as eluent, yielding eight fractions (Fr1-Fr8). Fraction 5 (3.0 g) was subjected to chromatography on silica gel, with $CHCl_3$-MeOH-H_2O (7:1:0.1) as eluent, and to further chromatography on a reversed-phase silica gel (Rp-18) column, with MeOH-H_2O (6:4 to 7:3) as eluent, to afford compounds **1** (10 mg) and **2** (21 mg). Fraction 6 (6.0 g) was subjected to chromatography on a Sephadex LH-20 column, with EtOH:H_2O (7:3) as eluent, and yielded four subfractions. Subfraction 2 (1.2 g) was subjected to further chromatography on a silica gel column, with $CHCl_3$-MeOH-H_2O (6:1:0.1) as eluent, to afford compound **3** (200 mg).

Ophiopojaponin D (1)

White amorphous powder, m.p. 174~176°C; $[\alpha]_D^{21}$ -67.3° (c 0.6, MeOH); HRFAB-MS: *m/z* 653.3948 (calcd. for $C_{35}H_{57}O_{11}$, 653.3901); IRν_{max}^{KBr} (cm^{-1}): 3395, 1640, 1598, 1510, 1435, 1275, 1237, 1213, 1068, 915; UVλ_{max} nm (MeOH): 205, 216, 278; ^{1}H-NMR (400 MHz, pyridine-d_5), ^{13}C-NMR (100 MHz, pyridine-d_5): Table I.

3,4-Dihydroxy-allylbenzene-4-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside (2)

White amorphous powder, m.p. 186~188°C; FAB-MS: *m/z* 447 $[M-H]^-$; IRν_{max}^{KBr} (cm^{-1}): 3400, 1640, 1600, 1515; 1437, 1275, 1240, 1215, 1070, 920; UVλ_{max} nm (MeOH): 204, 215, 278; ^{1}H-NMR (400 MHz, pyridine-d_5): δ 1.60 (1H, d, *J* = 6.1 Hz, H-6"), 3.21 (2H, d, *J* = 6.7 Hz, H-7), 5.28 (1H, d, *J* = 7.4 Hz, H-1'), 5.52 (1H, brs, H-1"), 6.72 (1H, dd, *J* = 8.2, 2.0 Hz, H-6), 7.07 (1H, d, *J* = 2.0 Hz, H-2), 7.58 (1H, d, *J* = 8.2 Hz, H-5); ^{13}C-NMR (100 MHz, pyridine-d_5): δ 137.1 (C-1), 117.6 (C-2), 149.6 (C-3), 145.1(C-4), 121.3 (C-5), 120.2 (C-6), 40.0 (C-7), 138.2 (C-8), 115.6 (C-9), 105.9 (C-1'), 74.9 (C-2'), 77.6 (C-3'), 71.6 (C-4'), 78.4 (C-5'), 68.0 (C-6'), 102.8 (C-1"), 72.3 (C-2"), 72.9 (C-3"), 74.2 (C-4"), 69.9 (C-5"), 18.7 (C-6").

L-Pyroglutamic acid (3)

Colorless needles, m.p. 162~164°C; $[\alpha]_D^{21}$ -15.2 (c 1.5, H_2O); EIMS: *m/z* 129 $[M]^+$, 101, 56; IRν_{max}^{KBr} (cm^{-1}): 3404, 2886, 2725, 2514, 1721, 1650, 1233; UVλ_{max} nm (MeOH): 202; ^{1}H-NMR (400 MHz, CD_3OD): d 4.24 (1H, dd, *J* = 4.0, 8.0 Hz, H-2), 2.48 (1H, m, H-3), 2.15 (1H, m, H-3), 2.32 (2H, m, H-4); ^{13}C-NMR (100 MHz, CD_3OD): d 181.0 (C-6), 175.7 (C-5), 57.0 (C-2), 30.3 (C-3), 28.0 (C-4).

Acid hydrolysis of 1 and 2

A solution of **1** (5 mg) in 10 mL HCl-MeOH (2 mol/L) was refluxed at 100°C for 3 h, and then neutralized with sat. $Ba(OH)_2$ aq. The MeOH was removed by evaporation *in vacuo* followed by partitioning between H_2O and $CHCl_3$. Glucose and rhamnose in the water layer were revealed by TLC ($CHCl_3$-MeOH-H_2O, 7:3:0.5) and PC (Paper Chromatography) (*n*-BuOH-CH_3COOH-H_2O, 4:1:5) analyses. Compound **2** was acid-hydrolyzed, using the same method described above, and yielded glucose and rhamnose from the water layer.

RESULTS AND DISCUSSION

Compound **1** was obtained as white amorphous powder. The HRFAB-MS (calcd. for $C_{35}H_{57}O_{11}$, 653.3901) of **1** gave a $[M-H]^-$ ion peak at *m/z* 653.3948, corresponding to the molecular formula $C_{35}H_{58}O_{11}$. TLC of the mild acid hydrolyzed **1** revealed the presence of glucose and rhamnose. By means of ^{1}H-, ^{13}C-, and DEPT-NMR spectra, combined with the molecular formula, compound **1** was readily deduced to contain a benzene ring, an allyl group, a tetradecyloxy group, and rhamnose and glucose moieties. In addition, the coupling constants of two anomeric proton signals at δ 5.25 (1H, d, *J* = 7.7 Hz) and 5.50 (1H, brs) suggested the linkages of the glucose and rhamnose were of the β and α forms, respectively. The proton signals at δ 7.05 (1H, d, *J* = 2.0 Hz), 7.57 (1H, d, *J* = 8.1 Hz), and 6.73 (1H, dd, *J* = 2.0, 8.1 Hz) were typically characteristics of a 1, 3, 4-trisubstituted phenyl. The carbon signals at δ 39.8 (CH_2), 115.7 (CH_2), and 138.5 (CH) indicated the presence of an allyl group. The carbon signals at δ 148.1 and 146.7, of two quaternary carbons, suggested the presence of two oxygen substitutions on the benzene ring. With the aid of HMQC, ^{1}H-^{1}H COSY and HMBC spectra, the chemical shifts of all the carbons

Table I. ^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) data of **1** (*J* in Hz)

The aglycone moiety			The sugar moieties		
Position	δ_H	δ_C	Position	δ_H	δ_C
1		137.0	1"	5.25 (1H, d, *J*=7.7)	105.9
2	7.05 (1H, d, *J*=2.0)	117.6	2"	4.26 (1H, overlap)	75.0
3		149.8	3"	4.08 (1H, overlap)	77.6
4		145.1	4"	4.07 (1H, overlap)	71.5
5	7.57 (1H, d, *J* = 8.1)	121.0	5"	4.21 (1H, overlap)	78.4
6	6.73 (1H, dd, *J*=2.0, 8.1)	120.1	6"	4.21, 4.65 (2H, m)	68.0
7	3.23 (2H, d, *J* = 6.8)	40.0	1"'	5.50 (1H, brs)	102.6
8	5.90 (1H, m)	138.2	2"'	4.65 (1H, overlap)	72.3
9	4.99 (2H, m)	115.6	3"'	4.50 (1H, m)	72.8
1'	4.26 (2H, overlap)	64.9	4"'	4.26 (1H, overlap)	74.1
14'	0.82 (3H, t, *J*=7.0)	14.3	5"'	4.35 (1H, m)	69.9
			6"'	1.59 (3H, d, *J*=6.2)	18.7

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

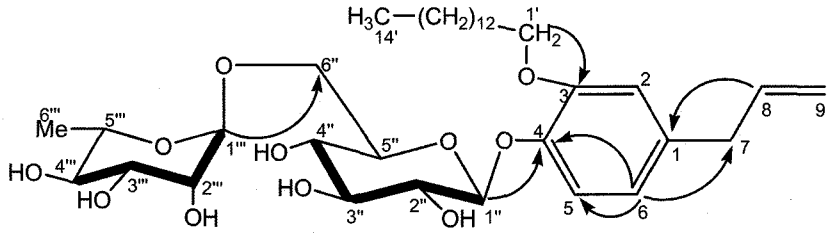

Fig. 1. The long-range ^{1}H-^{13}C correlations for compound **1**

and protons were assigned, as shown in Table I. The correlation of the H-1' (δ 4.26) and C-3 (δ 149.8) in the HMBC spectrum (Fig. 1) suggested that position 3 was substituted by a tetradecyloxy group. In the neg. FABMS spectrum, besides the quasi-molecular ion peaks at *m/z* 653 $[M-H]^-$, typical fragment ions at *m/z* 507 $[M\text{-rha}+H]^-$ and 345 $[M\text{-rha-glc}+H]^-$ were also observed. The glycosylation shift of C-6" (δ 68.0) due to glucose showed the linkage of rha (1→6) glc. The position of the sugar chain on the aglycone was determined by the correlation of H-1" (δ 5.25) and C-4 (δ 145.1) in the HMBC spectrum. Thus, compound **1** was identified as 3-tetradecyloxy-4-hydroxy-allylbenzene-4-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, and named ophiopojaponin D.

Compound **2** was obtained as white amorphous powder. The structure of **2** was identified as 3, 4-dihydroxy-allylbenzene-4-*O*-α-L-rhamnopyranosyl (1→6)-β-D-glucopyranoside, by comparison of the FAB-MS, ^{1}H-NMR, and ^{13}C-NMR spectral data with those data previously reported in the literature (Zhou *et al.*, 1999).

Compound **3** was obtained as colorless needles. The structure of **3** was determined to be L-pyroglutamic acid by comparison of the EIMS, ^{1}H-NMR, and ^{13}C-NMR spectral data with those data previously reported in the literature (Hao *et al.*, 2000).

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