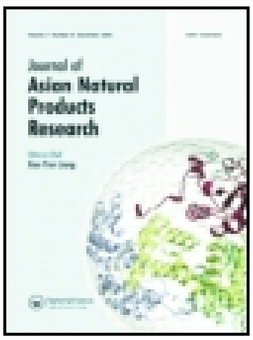

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Steroidal saponins from the rhizomes of *Polygonatum prattii*

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Seven steroidal saponins including two new furostanol glycosides were isolated from the rhizomes of *Polygonatum prattii* collected from Panzhihua, Sichuan province of China. The new compounds were determined as 26-*O*-β-D-glucopyranosyl-(25*R*)-3β,22ξ-dihydroxy-furost-5-en-7-one (pratioside G) and 26-*O*-β-D-glucopyranosyl-(25*R*)-22ξ-hydroxy-furost-5-en-3β-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (pratioside H), on the basis of detailed spectroscopic and chemical analysis.

Keywords: Convallariaceae; *Polygonatum prattii*; steroidal saponins; pratioside G; pratioside H; polymorphism

1. Introduction

Polygonatum prattii Baker (Convallariaceae), with a Chinese name "Kangding Yuzhu," is a polymorphic species mainly distributed in the Western Sichuan and the Northwestern Yunnan province of China [1]. The fresh rhizomes are used as substitutes of "Yuzhu" in Chinese Pharmacopoeia by local people for the treatment of lung disease, palpitations, diabetes, and upset stomach [2–3].

It is well known that taxonomy of genus *Polygonatum* is difficult due to the complex features between genus caused by the overlap in geographical distribution, conspicuous intersectional transition of interspecific and intraspecific morphological characteristic, and complicated variation. However, plant secondary metabolites showed important physiological, ecological, and morphological significance. In our previous work [4], the correlations between the molecular evolution of steroidal saponins as the main secondary metabolites in the genus and the morphological aspects were discussed. In which, six new steroidal saponins, pratiosides A, B, C, D_1, E_1, and F_1, were also identified from the rhizomes of *P. prattii* collected from Yungui Plateau (Chuxiong, Yunnan province, China) [5]. As a continuing research on molecular diversity of steroidal glycosides and to search for chemical support of polymorphism in *P. prattii*, the phytochemical study on the rhizomes of the same species collected from dry-hot valley region (Panzhihua, Sichuan province, China) was conducted to obtain seven steroidal saponins including two new furostanol glycosides.

2. Results and discussion

Five known compounds were obtained and identified as funkioside B (**3**) [6], protoaspidistrin (**4**) [7], aspidistrin (**5**) [7], dioscin (**6**) [8], and Tu_2 [9] (Figure 1),

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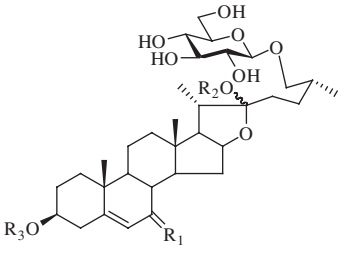

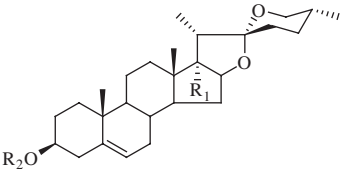

	R_1	R_2	R_3	
1	O	H	H	
2	H_2	H	Gal(4-1)Glc I[(3-1)Xyl](2-1)Glc II(2-1)Glc III	
3	H_2	CH_3	H	
4	H_2	CH_3	Gal(4-1)Glc[(3-1)Xyl](2-1)Glc	

	R_1	R_2
5	H	Gal(4-1)Glc[(3-1)Xyl](2-1)Glc
6	H	Glc[(2-1)Rha](4-1)Rha
7	OH	Glc[(2-1)Rha](4-1)Rha(4-1)Rha

Figure 1. Steroidal saponins **1**–**7** isolated from *Polygonatum prattii*.

respectively, on the basis of their physical and spectroscopic data and by comparison with reference values.

Both new compounds **1** and **2** were furostane steroidal saponins showing positive reaction to Ehrlich's reagent, supported by the typical quaternary carbon signals belonging to the characteristic C-22 of furostanol skeleton at δ 110.7 and δ 110.9, respectively [10–11].

The molecular formula of **1** was assigned as $C_{33}H_{52}O_{10}$ on the basis of the ^{13}C NMR data (Table 1) and negative mode ion HRESI-MS at *m/z* 607.3486 $[M - H]^-$. The negative ion FABMS showing a fragment ion peak at *m/z* 445 $[M - 162 - H]^-$, together with only one anomeric proton signal (δ 4.81, *d*, $J = 7.7$ Hz) in the ^1H NMR spectrum, indicated the existence of one hexosyl sugar unit in **1**. Six carbon signals at δ 105.0, 75.2, 78.6, 71.7, 78.5, 62.8 arising from one glucosyl unit were observed in the ^{13}C NMR spectrum, and its linkage to C-26-OH was further determined by the correlation between the only anomeric proton at δ 4.81 with C-26 (δ 75.3) in HMBC spectrum. The ^{13}C NMR spectral data of **1** were very similar to those of funkioside B [26-*O*-β-D-glucopyranosyl-(25*R*)-3β, 22ξ-dihydroxy-furost-5-ene] [6], except for the B-ring data. In the ^{13}C NMR spectrum of **1**, one carbonyl unit at δ

Table 1. ^{13}C NMR spectroscopic data of compounds **1**–**2** (in pyridine-d_5)

No.	1	2	No.	2
1	36.8	37.6	Gal-1	102.9
2	30.0	30.3	2	73.3
3	70.1	78.4	3	75.0
4	42.9	39.4	4	79.9
5	166.7	141.2	5	76.3
6	125.8	121.8	6	60.8
7	201.3	32.5	Glc I-1	104.7
8	45.1	31.8	2	81.4
9	50.0	50.5	3	87.0
10	38.7	37.2	4	70.9
11	21.2	21.3	5	77.9
12	39.0	40.1	6	63.1
13	41.6	40.8	Glc II-1	104.9
14	50.1	56.7	2	81.5
15	34.7	32.6	3	77.7
16	81.3	81.3	4	71.9
17	63.0	63.9	5	78.7
18	16.6	16.6	6	62.6
19	17.5	19.6	Glc III-1	105.0
20	40.7	41.0	2	74.8
21	16.6	16.6	3	76.9
22	110.7	110.9	4	70.5
23	32.1	30.3	5	76.6
24	28.4	28.5	6	62.2
25	34.3	34.4	Xyl-1	105.1
26	75.3	75.4	2	75.7
27	17.2	17.6	3	78.8
26-Glc-1	105.0	105.0	4	71.2
2	75.2	75.2	5	67.4
3	78.6	78.6		
4	71.7	71.9		
5	78.5	78.5		
6	62.8	63.0		

201.3 and a pair of olefinic carbon signals at δ 166.7 (C) and 125.8 (CH) were observed. The IR spectrum also displayed two absorption bands at 1675 and 1635 cm^{-1} due to α, β-unsaturated ketone [12]. Thus, one ketone at C-7 and the double bond between C-5 and C-6 were deduced. These were further confirmed by the HMBC correlations of H-4 (δ 2.62, 2.71) with C-5 (δ 166.7), C-10 (δ 38.7), C-6 (δ 125.8), and C-3 (δ 70.1), the olefinic proton at δ 5.85 (s, H-6) with C-4 (δ 42.9), C-10 (δ 38.7), and C-8 (δ 45.1), and H-8 (δ 2.46) with C-7 (δ 201.3) and C-10 (Figure 2). The 25*R* configuration depended on the chemical shifts of H_2-26, and H_3-27 [13]. Therefore, compound **1** was determined to be 26-*O*-β-D-glucopyranosyl-(25*R*)-3β,22ξ-dihydroxy-furost-5-en-7-one, and named pratioside G.

Compound **2** had a molecular formula $C_{62}H_{102}O_{33}$, as deduced by the HRESIMS at *m/z* 1373.6257 $[M - H]^-$. The FABMS (negative ion mode) showed the molecular ion peak at *m/z* 1374 $[M]^-$ and fragment ion peaks at *m/z* 1242 $[M\text{-}132]^-$, 1212 $[M\text{-}162]^-$, 1080 $[M\text{-}132\text{-}162]^-$, 1050 $[M\text{-}162\text{-}162]^-$, 918 $[M\text{-}132\text{-}162\text{-}162]^-$, 755 $[M\text{-}132\text{-}162\text{-}162\text{-}162\text{-}H]^-$, 431 $[M\text{-}132\text{-}162\text{-}162\text{-}162\text{-}162\text{-}162\text{-}H]^-$, suggesting the existence of one terminal pentosyl and five hexosyl units. These were coincident with the observation of six anomeric proton signals at δ 4.73 (*d*, $J = 7.7$ Hz), 4.87 (*d*, $J = 7.8$ Hz), 5.14 (*d*, $J = 7.6$ Hz), 5.15 (*d*, $J = 7.4$ Hz), 5.25 (*d*, $J = 7.8$ Hz), and 5.55 (*d*, $J = 7.2$ Hz) in the ^{1}H NMR spectrum. All β-configuration of sugar units were reflected by their *J* values. Comparing ^{13}C NMR spectral data of **2** with those of protoaspidistrin (26-*O*-β-D-glucopyranosyl-(25*R*)-22ξ-hydroxy-furost-5-en-3β-*O*-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, **4**) [7] revealed that **2** had one more glucosyl unit (δ 104.9, 81.5, 77.7, 71.9, 78.7, 62.6) than **4**. The linkage of the additional glucosyl unit was determined by HMBC experiment (Figure 2), in which correlations of the anomeric proton at δ 5.14 (Glc II H-1) with the carbon at δ 81.4 (Glc I C-2) and the terminal glucosyl anomeric proton at δ 5.55 (Glc III H-1) with the additional glucosyl C-2 (δ 81.5, Glc II) were observed. Furthermore, other HMBC correlations of Gal H-1 at δ 4.87 with C-3 at δ 78.4, Glc I H-1 at δ 5.15 with Gal C-4 at δ 79.9, and Xyl H-1 at δ 5.25 with Glc I C-3 at δ 87.0 confirmed the sugar linkages in **2**. The configuration of 25*R* in **2** was also identified according to reference [13]. The absolute configurations of monosaccharides were determined as D-galactose, D-glucose, and D-xylose, on the basis of acid hydrolysis and GC analysis of the corresponding trimethylsilylated L-cysteine adducts [14]. Accordingly, pratioside H (**2**) was deduced to be 26-*O*-β-D-glucopyranosyl-(25*R*)-

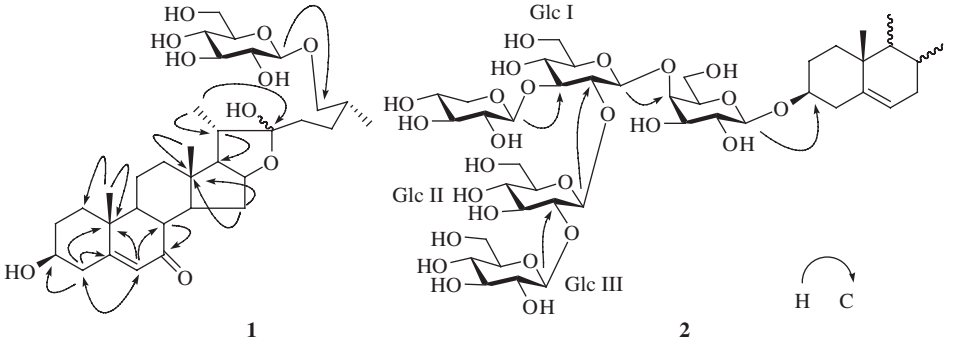

Figure 2. Important HMBC correlations of compounds **1** and **2**.

22ξ-hydroxy-furost-5-en-3β-*O*-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside.

As the main chemical constituents in *P. prattii*, steroidal saponins displayed significant differences in not only the sapogenins, but also the sugar linkage and sequence. Six saponins with only one form of sugar chain (galactopyranosyl-(4→1)-glucopyranosyl-(2→1)-glucopyranosyl) attached to C-3-OH, but five types of sapogenins including pennogenin, isonarthogenin, gentrogenin, isochiapagenin, and (25*R*)-spirost-3β,12α,17α-triol-5-ene were identified [5]. However, the present study on the same species from dry-hot valley region (Panzhihua, Sichuan province) led to the isolation of seven steroidal saponins involving only three sapogenins, diosgenin, pennogenin and (25*R*)-spirost-3β-ol-5-ene-7-one, while these saponins displayed diversity of sugar moieties. Two saponins have free C-3-OH, but C-26-OH is glycosylated with glucosyl units. Rhamnosyl and xylosyl moieties existed in the molecules, in addition to galactosyl and glucosyl unit, while glucosyl unit also appeared at the inner position linked to the aglycone directly. Two samples were all collected in October, and the results would provide chemical evidences for evolution of the morphologic diversity and the polymorphic of secondary metabolites in the title plant from different environments.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured on a HORIBA SEPA-3000 automatic digital polarimeter (JASCO, Tokyo, Japan). IR spectra were conducted on a Bio-Rad FTS-135 spectrometer (Bio-Rad, Hercules, California, America) with KBr pellets. NMR spectra were recorded in pyridine-d_5 on Bruker AM-400 and DRX-500 instrument (Bruker, Rheinstetten, Germany), with TMS as internal standard. VG AutoSpe 3000 (VG, Manchester, UK) and API Qstar Pulsar LC/TOF spectrometers (PE Biosystems, Waltham in Massachusetts, America) were the instruments for FABMS (negative ion mode) and HRESIMS (negative ion mode), respectively. GC analysis for the absolute configuration of monosaccharides was run on Agilent Technologies HP5890 gas chromatograph (Agilent, Santa Clara, America) with conditions as former work [14]. Silica gel (200–300 mesh and 10–40 μm, Qingdao Makall, China), MCI gel CHP 20P (Mitsubishi, Japan), Sephadex LH-20 (Pharmacia, Sweden), and reversed phase silica gel RP-8 (40–63 μm, Merck, Germany) were used for column chromatography (CC).

3.2. Plant material

The fresh rhizomes were collected from Panzhihua, Sichuan province, China, and identified by Prof. Heng Li of Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS). A voucher specimen is deposited at the Herbarium of KIB, CAS.

3.3. Extraction and isolation

The fresh rhizomes (30 kg) of *P. prattii* were extracted with MeOH under reflux for three times. After removal of the solvent *in vacuo*, the MeOH extract was suspended in H_2O and then partitioned with *n*-BuOH. The organic solution fraction (160 g) was applied to a silica gel (4 kg) column chromatography (CC), eluting with $CHCl_3$-MeOH-H_2O (7:2.5:0.4), to give five fractions (Frs. 1–5). Fr. 2 (14 g) was chromatographed over silica gel ($CHCl_3$-MeOH-H_2O, 8:2:0.2) and reversed-phase Rp-8 (MeOH–H_2O, 6:4–1:0) columns to afford **1** (4 mg) and **3** (45 mg). Fr. 3 (9.5 g) was subjected to silica gel CC eluting with $CHCl_3$–

MeOH–H_2O (7:3:0.5) to give **5** (60 mg). Fr.4 (6 g) was applied to silica gel ($CHCl_3$–MeOH–H_2O, 7:3:0.5–6:4:1) and MCI gel CHP-20P (MeOH–H_2O, 1:1–1:0) CC to afford **6** (6 mg) and **7** (12 mg). Fr. 5 (69 g) was separated by repeated CC over silica gel ($CHCl_3$–MeOH–H_2O, 6:4:1), RP-8 (40–80% MeOH), and Sephadex LH-20 (5–40% MeOH) to yield **2** (22 mg) and **4** (2 g).

3.3.1. *Pratioside G (**1**)*

White amorphous powder, $[\alpha]_D$ −94.9 (*c* 0.12, MeOH). IR υ_{max} (KBr) 3440 (OH), 1675 and 1635 (α, β-unsaturated ketone) cm^{-1}. ^1H NMR (pyridine-d_5, 500 MHz): δ 0.91 (*s*, CH_3-18), 0.96 (*d*, *J* = 6.7 Hz, CH_3-27), 1.10 (*s*, CH_3-19), 1.34 (*d*, *J* = 6.8 Hz, CH_3-21), 1.47-1.49 (2 H, *m*, H-9, 14), 1.91 (2 H, *m*, H-25, 17), 2.20-2.23 (*m*, H-20), 2.46 (*t*, *J* = 10.8 Hz, H-8), 2.62 (br *t*, *J* = 11.0 Hz, H-4a), 2.71 (br *d*, *J* = 11.0 Hz, H-4b), 3.29-3.31 (*m*, H-15), 3.58-3.60 (*m*, H-26a), 3.82-3.84 (*m*, H-Glc-5), 3.86-3.89 (*m*, H-3), 3.92–3.95 (*m*, H-26b,), 4.00-4.03 (*m*, H-Glc-2), 4.19 (*t*, *J* = 8.8 Hz, H-Glc-3), 5.85 (*s*, H-6), 4.81 (*d*, *J* = 7.7 Hz, H-Glc-1), 4.22 (H, *t*, *J* = 8.8 Hz, H-Glc-4), 4.39-4.41 (*m*, H-Glc-6a), 4.55 (br *d*, *J* = 8.6 Hz, H-Glc-6b). ^{13}C NMR (pyridine-d_5, 400 MHz) spectral data: see Table 1. FAB-MS (negative ion mode): *m/z* 607 $[M - H]^-$, 445 $[M - 162 - H]^-$. HRFAB-MS (negative ion mode): *m/z* 607.3486 $[M - H]^-$ (calculated for $C_{33}H_{51}O_{10}$, 607.3482).

3.3.2. *Pratioside H (**2**)*

White amorphous powder, $[\alpha]_D$ −53.0 (*c* 1.09, MeOH). ^1H NMR (pyridine-d_5, 500 MHz): δ 0.85 (*s*, CH_3-18), 0.86 (*s*, CH_3-19), 0.87 − 0.89 (*m*, H-9), 0.96 (*d*, *J* = 6.4 Hz, CH_3-27), 1.01-1.03 (*m*, H-14), 1.08 − 1.09 (*m*, H-12a), 1.32 (*d*, *J* = 6.3 Hz, CH_3-21), 1.47 − 1.49 (*m*, H-11), 1.64 − 1.67 (2 H, *m*, H-1a, 24a), 1.68 − 1.70 (*m*, H-23a), 1.71 (br *d*, *J* = 7.7 Hz, H-12b), 1.81 (br *d*, *J* = 14.5 Hz, H-7a), 1.91-1.92 (*m*, H-25), 2.00 − 2.03 (2 H, *m*, H-1b, 24b), 2.05 − 2.06 (*m*, H-23b), 2.20 − 2.22 (*m*, H-20), 2.39 (br *t*, *J* = 12.1 Hz, H-4a), 2.63 (br *d*, *J* = 12.1 Hz, H-4b), 3.56 − 3.60 (*m*, H-26), 4.73 (*d*, *J* = 7.7 Hz, H-26-Glc-1), 4.87 (*d*, *J* = 7.8 Hz, H-Gal-1), 4.92 (*q*, *J* = 7.2 Hz, H-16), 5.14 (*d*, *J* = 7.6 Hz, H-Glc II-1), 5.15 (*d*, *J* = 7.4 Hz, H-Glc I-1), 5.25 (*d*, *J* = 7.8 Hz, H-Xyl-1), 5.27 (br *s*, H-6), 5.55 (*d*, *J* = 7.2 Hz, H-Glc III-1). ^{13}C NMR (pyridine-d_5, 400 MHz) spectral data: see Table 1. FAB-MS (negative ion mode): *m/z* 1374 $[M]^-$, 1242 $[M - 132]^-$, 1212 $[M - 162]^-$, 1080 $[M - 132 - 162]^-$, 1050 $[M - 162 - 162]^-$, 918 $[M - 132 - 162 - 162]^-$, 755$[M - 132 - 162 - 162 - 162 - H]^-$, 431 $[M - 132 - 162 - 162 - 162 - 162 - 162 - H]^-$. HRESI-MS (negative ion mode): *m/z* 1373.6257 $[M - H]^-$ (calculated for $C_{62}H_{101}O_{33}$, 1373.6225).

3.4. *Acid hydrolysis of 2*

Compound **2** (10 mg) dissolved in 2 M HCl–dioxane (1:1, 4 ml) was hydrolyzed at 95°C for 6 h. After cooling to room temperature, 2 ml H_2O was added into the reaction mixture, then extracted with $CHCl_3$ (6 ml × 3). The $CHCl_3$ phase was identified as diosgenin by comparing with authentic sample on TLC. The aqueous layer was treated using the method as described in reference [14], and the absolute configurations of monosaccharides were determined to be D-galactose, D-glucose, and D-xylose respectively, on the basis of GC analysis of their derivatives.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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