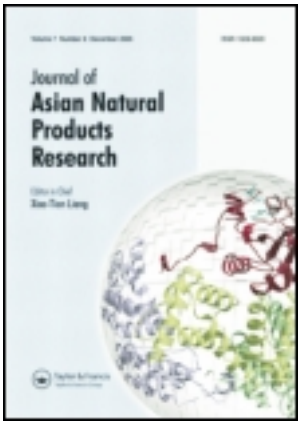

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Three new steroidal saponins from *Fritillaria pallidiflora*

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Three new steroidal saponins, pallidiflosides A (**1**), B (**2**), and C (**3**), have been isolated from the dry bulbs of *Fritillaria pallidiflora* Schrenk. Their structures were elucidated as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5,20(22)-dien-3β,26-diol-3-*O*-β-D-xylopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside (**1**); 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxyl-20,22-seco-25(*R*)-furost-5-en-20,22-dione-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranoside (**2**); and (25*R*)-spirost-5-ene-3β,17α-diol-3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside (**3**) by spectroscopic techniques and chemical means.

Keywords: *Fritillaria pallidiflora*; steroidal saponins; NMR

1. Introduction

Fritillaria pallidiflora Schrenk is one of the well-known traditional Chinese medicines. It belongs to the *Fritillaria* genus of Liliaceae family and is mainly distributed in Xinjiang Province of China. The dry bulbs of *F. pallidiflora* have been used as antitussive, antiasthmatic, and expectorant agents for hundreds of years [1]. The alkaloids and saponins are the main effective ingredients in the *Fritillaria* genus of Liliaceae family [2]. However, a literature survey concerning the secondary metabolites of *F. pallidiflora* showed that no systematic chemical work on the saponin constituents of the plant had been carried out. Therefore, a phytochemical investigation on the saponin constituents of the dry bulbs of *F. pallidiflora* was carried out. As a result, three new steroidal saponins, pallidiflosides A (**1**), B (**2**), and C (**3**) (see Figure 1), were obtained. Herein, we report their isolation and structural elucidation.

2. Results and discussion

Compound **1** was obtained as a white amorphous solid with $[\alpha]_D^{20}$-56.1 (MeOH, c 0.052) and gave positive results with Liebermann–Burchard and Ehrlich reagent tests. The IR spectrum showed absorption bands for hydroxyl (3364 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). Its molecular formula $C_{50}H_{80}O_{21}$ was deduced from HR-ESI-MS at *m/z* 1015.5105 $[M - H]^-$. The ESI-MS in positive-ion mode showed ion peaks at *m/z* 1039 $[M + Na]^+$, 907 $[(M + Na^+)\text{-}132]^+$, 893 $[(M + Na^+)\text{-}146]^+$, 877 $[(M + Na^+)\text{-}162]^+$, 761 $[(M + Na^+)\text{-}132\text{-}146]^+$, and 599 $[(M + Na^+)\text{-}132\text{-}146\text{-}162]^+$. The ^1H NMR spectrum of **1** showed three methyl singlets at δ 0.74, 1.04, and 1.66; one methyl doublet at δ 1.03 (d, $J = 6.5$ Hz); one

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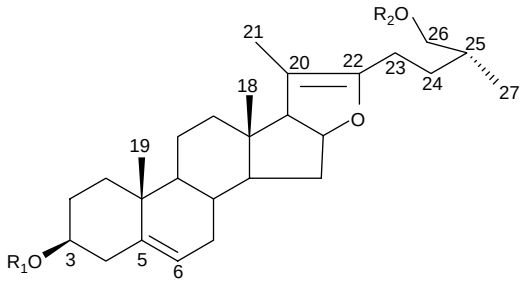

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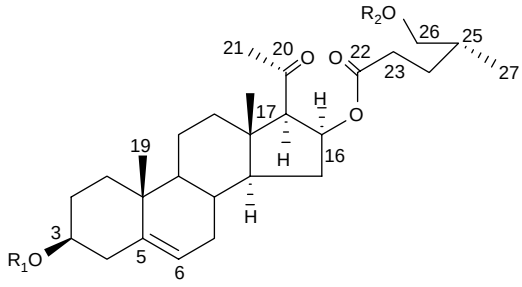

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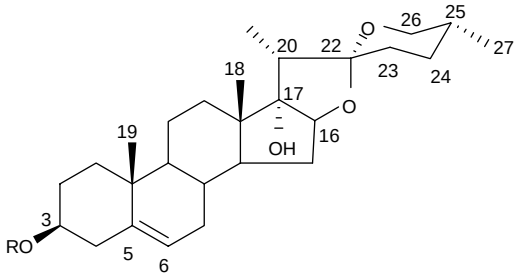

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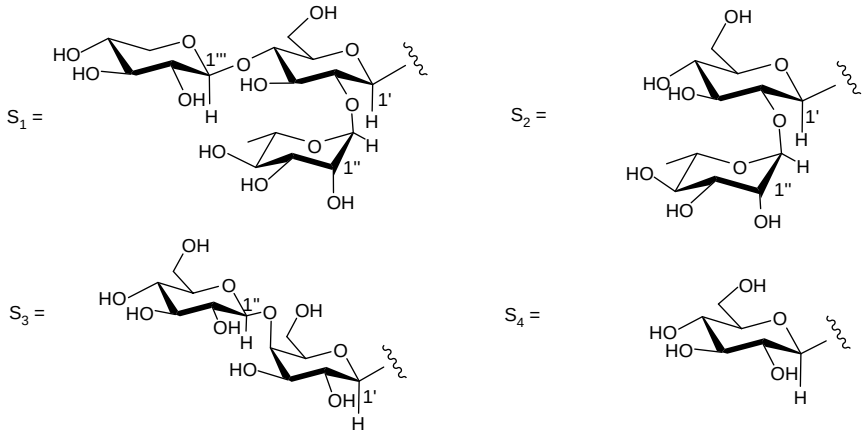

Figure 1. The structures of compounds **1**–**3**.

olefinic proton at δ 5.30 (d, $J = 4.0$ Hz); four anomeric proton signals at δ 4.96 (d, $J = 7.2$ Hz), 6.33 (s), 5.06 (d, $J = 7.0$ Hz), and 4.89 (d, $J = 7.8$ Hz). The ^{13}C NMR spectrum of **1** revealed characteristic signals for two pairs of olefinic carbons at δ 140.8 and 121.9 and δ 103.6 and 152.4; four anomeric carbons of sugars at δ 100.0, 102.0, 105.9, and 105.0. The above evidences indicated that **1** was a furostanol saponin with four sugar units. Comparison of the NMR spectral data of **1** (Table 1) with the literature data [3] allowed the identification of the aglycone as the previously reported furost-5,20(22)-dien-3β,26-diol. The geminal proton resonances of H_2-26 appeared at δ 3.64 (H_a-26) and 3.94 (H_b-26), which showed the Δab of δH_a-δH_b as 0.30 ppm (< 0.48 ppm), indicating that the configuration of C-25 was *R* [4]. The HMBC spectrum (Figure 2) of **1** showed correlations between the proton signal at δ 1.66 (H-21) and the carbon signals at δ 64.5 (C-17), 103.6 (C-20), and 152.4 (C-22); between the proton signal at δ 1.04 (H-19) and the carbon signals at δ 140.8 (C-5), 50.3 (C-9), and 37.1 (C-10). The above evidences convinced the presence of two double bonds at C-5 (6) and C-20 (22). Acid hydrolysis of **1** afforded L-rhamnose, D-xylose, and D-glucose in a ratio of 1:1:2 on the basis of gas chromatography (GC) analysis. The β-anomeric configurations for both glucose and xylose were judged from their coupling

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of the aglycone moieties of compounds **1** and **2** in pyridine-d_5.

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
1	37.6	0.98 m, 1.77 m	37.5	0.92 m, 1.71 o
2	30.2	1.94 o, 2.16 m	30.1	1.85 o, 2.11o
3	78.4	3.89 m	78.0	3.89 m
4	39.0	2.78 m, 2.85 m	39.0	2.75 d (12.6), 2.83 m
5	140.8		141.0	
6	121.9	5.30 d (4.0)	121.6	5.30 d (4.5)
7	32.4	1.52 o, 1.88 m	31.9	1.85 o
8	31.5	1.52 o	31.0	1.52 m
9	50.3	0.92 m	50.4	0.88 m
10	37.1		37.1	
11	21.3	1.45 m, 1.75 m	20.7	1.37 m, 1.47 m
12	39.7	1.16 m, 1.79 m	38.2	1.07 m, 2.15 o
13	43.4		42.3	
14	54.9	0.88 m	54.1	0.82 m
15	34.5	1.50 o, 2.13 td (7.8, 13.6)	35.5	1.31 td (3.9, 12.8), 2.43 o
16	84.5	4.79 d (7.8)	74.2	5.64 m
17	64.5	2.46 d (9.9)	66.7	2.47 d (7.8)
18	14.1	0.74 s	13.8	1.21 s
19	19.5	1.04 s	19.5	1.05 s
20	103.6		205.6	
21	11.9	1.66 s	30.5	2.12 s
22	152.4		173.3	
23	23.7	2.23 m	32.3	2.42 o
24	31.5	1.49 m, 1.85 m	29.0	1.53 m, 1.94 m
25	33.5	1.97 o	33.5	1.83 o
26	75.0	3.64 dd (9.5, 6.0), 3.94 m	74.8	3.50 dd (6.0, 9.3), 3.89 m
27	17.4	1.03 d (6.5)	17.0	0.90 d (6.5)

Note: Overlapped signals are indicated by 'o.'

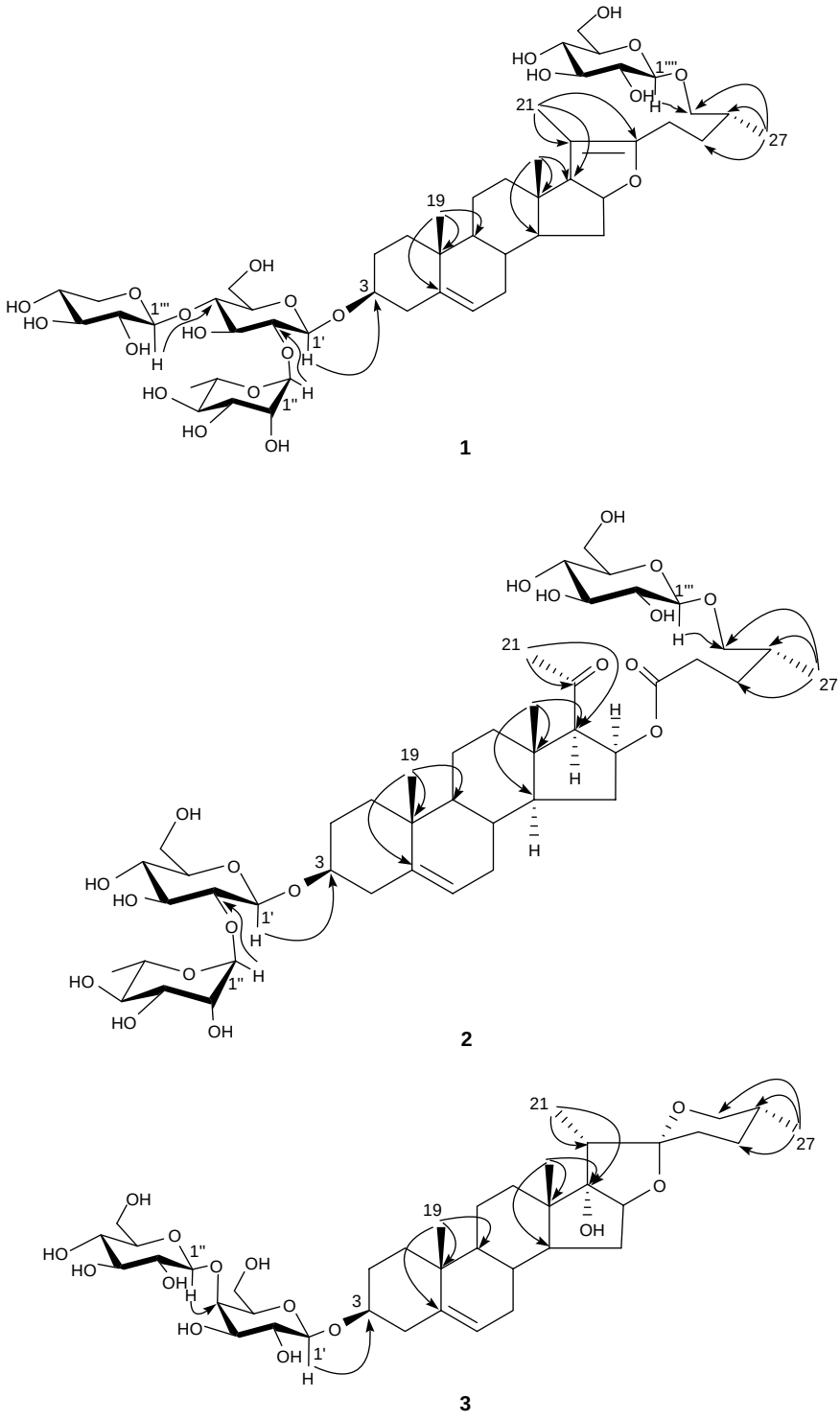

Figure 2. Key HMBC correlations of compounds **1**–**3**.

Table 2. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of the sugar moieties of compounds **1** and **2** in pyridine-d_5.

	1		**2**	
No.	δ_C	δ_H	δ_C	δ_H
(3-*O*)-β-D-Glc	100.0	4.96 d (7.2)	100.4	5.03 d (7.8)
2′	77.5	4.23 dd (7.2, 9.1)	79.7	4.26 dd (7.8, 9.0)
3′	77.4	4.23 m	78.4	4.21 dd (9.0, 9.0)
4′	81.5	4.19 m	71.7	4.14 o
5′	76.3	3.87 m	77.9	3.88 o
6′	61.8	4.36 m, 4.49 d (12.5)	62.5	4.29 m, 4.51 d (10.2)
(Glc^2)-α-L-Rha	102.0	6.33 s	102.1	6.38 s
2″	72.6	4.82 d (3.5)	72.6	4.81 brs
3″	72.9	4.64 dd (3.5, 9.4)	72.9	4.62 dd (3.3, 9.3)
4″	74.2	4.42 m	74.0	4.35 o
5″	69.6	4.95 o	69.5	4.97 o
6″	18.7	1.81 d (6.1)	18.7	1.77 d (6.1)
(Glc^4)-β-D-Xyl	105.9	5.06 d (7.0)		
2‴	75.0	4.03 dd (7.0, 9.0)		
3‴	78.7	4.10 m		
4‴	70.8	4.15 m		
5‴	67.4	3.69 d (10.6), 4.29 d (10.6)		
(26-*O*)-β-D-Glc	105.0	4.89 d (7.8)	105.0	4.78 d (7.5)
2⁗	75.3	4.03 m	75.2	4.03 dd (7.5, 9.0)
3⁗	78.6	4.26 m	78.6	4.24 m
4⁗	71.7	4.25 m	71.5	4.20 m
5⁗	78.4	3.95 d (9.0)	78.6	3.92 m
6⁗	62.9	4.43 m, 4.54 d (11.5)	62.9	4.32 m, 4.52 d (11.4)

Note: Overlapped signals are indicated by 'o.'

constants ($^3J_{H1,H2} > 7.0$) [5]. The α-anomeric configuration for the rhamnose was determined by its C-5″ data (δ 69.6) [6]. The HMBC spectrum (Figure 2) showed correlations between the anomeric proton signal H-1′ at δ 4.96 and the carbon signal C-3 at δ 78.4, between H-1″ at δ 6.33 and C-2′ at δ 77.5, between H-1‴ at δ 5.06 and C-4′ at δ 81.5, and between H-1⁗ at δ 4.89 and C-26 at δ 75.0. According to the accumulated evidence above, the structure of **1** was characterized as 26-*O*-β-D-glucopyranosyl-(25*R*)-furost-5,20(22)-dien-3β,26-diol-3-*O*-β-D-xylopyranosyl(1 → 4)-[α-L-rhamnopyranosyl(1 → 2)]-β-D-glucopyranoside, named as pallidifloside A (**1**).

Compound **2** was obtained as a white amorphous solid with $[\alpha]_D^{20}$-59.2 (MeOH, c 0.052) and it gave positive results with Liebermann–Burchard and positive Ehrlich reagent tests. The IR spectrum showed absorption bands for hydroxyl (3363 cm^{-1}), carbonyl of ester (1745 cm^{-1}), and carbonyl of ketone (1713 cm^{-1}). Its molecular formula $C_{45}H_{72}O_{19}$ was deduced from the HR-ESI-MS at *m/z* 915.4598 $[M - H]^-$, and this was further supported by the ^{1}H NMR, ^{13}C NMR, and DEPT spectra (in pyridine-d_5; see Tables 1 and 2). The ESI-MS in positive-ion mode showed the ions at *m/z* 939 $[M + Na]^+$, 793 $[(M + Na^+)\text{-}146]^+$, 777 $[(M + Na^+)\text{-}162]^+$, and 631 $[(M + Na)^+\text{-}146\text{-}162]^+$. The ^{1}H NMR spectrum of **2** showed three methyl singlets at δ 1.21, 1.05, and 2.12; one methyl doublet at δ 0.90 (d, $J = 6.5$ Hz); one olefinic proton at δ 5.30 (d, $J = 4.5$ Hz); and three anomeric protons at δ 5.03 (d, $J = 7.8$ Hz), 6.38 (s), and 4.78 (d, $J = 7.5$ Hz). These data, combined with the analysis of its ^{13}C NMR spectrum (four methyl groups at δ 13.8, 19.5, 30.5, and 17.0, trisubstituted

double bonds at δ 141.0 and 121.6, an ester carbonyl at δ 173.3, a ketone carbonyl at δ 205.6, and three anomeric carbon signals at δ 100.4, 102.1, and 105.0), indicated that **2** was a furostanol saponin with three sugar units. Comparison of the NMR spectral data of **2** (Table 1) with the literature data [3] allowed the identification of the aglycone as the previously reported (3β,25*R*)-20,22-seco-25-furost-5-en-20,22-dione-3,26-diol. The HMBC correlations between the proton at δ 1.05 (H-19) and the carbons at δ 141.0 (C-5), 50.4 (C-9), and 37.1 (C-10), between the proton at δ 2.12 (H-21) and the carbons at δ 66.7 (C-17) and 205.6 (C-20), and no HMBC correlation between the proton at δ 2.12 (s, H-21) and the carbon at δ 173.3 (C-22) were observed, which convinced the presence of one double bond at C-5 (6) and the E ring of **2** was opened between C-20 and C-22. In the NOESY spectrum of **2**, the proton at δ 0.82 (m, H-14) showed the correlations with the protons at δ 5.64 (m, H-16) and δ 2.47 (d, $J = 7.8$ Hz, H-17), suggesting H-16 and H-17 were α-oriented. The HMBC and NOESY correlations mentioned above supported the conclusion, i.e. the aglycone of **2** was (3β, 25*R*)-20,22-seco-25-furost-5-en-20,22-dione-3,26-diol. Acid hydrolysis of **2** afforded L-rhamnose and D-glucose in a ratio of 1:2 on the basis of GC analysis. The β-anomeric configurations for two glucoses were judged from their coupling constants ($^3J_{H1,H2} > 7.0$) [5]. The α-anomeric configuration for the rhamnose was determined by its chemical shift of C-5″ at δ 69.5 [6]. The HMBC spectrum (Figure 2) showed correlations between H-1″ at δ 5.03 and C-3 at δ 78.0, between H-1″ at δ 6.38 and C-2′ at δ 79.7, and between H-1‴ at δ 4.78 and C-26 at δ 74.8. According to the above evidence, the structure of **2** was determined as 26-*O*-β-D-glucopyranosyl-3β,26-dihydroxyl-20,22-seco-25(*R*)-furost-5-en-20,22-dione-3-*O*-α-L-rhamnopyranosyl(1 → 2)-β-D-glucopyranoside, named as pallidifloside B (**2**).

Compound **3** was obtained as a white amorphous solid with $[\alpha]_D^{20}$-47.2 (MeOH, c 0.051) and gave results positive in Liebermann–Burchard and negative in Ehrlich reagent tests. The IR spectrum showed absorption bands for hydroxyl (3421 cm^{-1}) and a glycosidic linkage (1000–1100 cm^{-1}). Its molecular formula, $C_{39}H_{62}O_{14}$, was deduced from the HR-ESI-MS at *m/z* 753.4061 $[M - H]^-$. The ESI-MS in positive-ion mode showed the ions at *m/z* 777 $[M + Na]^+$, 615 $[(M + Na^+)\text{-}162]^+$, and 453 $[(M + Na^+)\text{-}162\text{-}162]^+$. The ^{1}H NMR spectrum of **3** showed two methyl singlets at δ 0.94 and 0.92; two methyl doublets at δ 1.25 (d, $J = 6.9$ Hz) and 0.69 (d, $J = 5.1$ Hz); one olefinic proton at δ 5.30 (d, $J = 4.5$ Hz); two anomeric proton signals at δ 4.90 (d, $J = 7.5$ Hz) and 5.33 (d, $J = 8.1$ Hz). The ^{13}C NMR spectrum of **3** revealed characteristic signals for one quaternary carbon signal at δ 109.5 and two olefinic carbon signals at δ 140.6 and 121.4 as well as two anomeric carbons of sugars at δ 102.6 and 106.8. The above evidences indicated that **3** was a spirostanol saponin with two sugar units. Comparing the ^{1}H and ^{13}C NMR spectral data of **3** (Table 3) with the literature data [7] allowed the identification of the aglycone as the previously reported spirost-5-ene-3β,17α-diol and the C-25*R* configuration was determined by the inspection of the ^{13}C NMR spectral data of C-27 (δ 17.0), C-26 (δ 66.7), C-25 (δ 30.0), and C-24 (δ 28.5) [6]. Acid hydrolysis of **3** afforded D-galactose and D-glucose in a ratio of 1:1 on the basis of GC analysis. The β-anomeric configurations for the glucose and galactose were judged from their coupling constants ($^3J_{H1,H2} > 7.0$) [5]. The HMBC spectrum (Figure 2) showed the correlation between H-1′ at δ 4.90 and C-3 at δ 77.7 and between H-1″ at δ 5.33 and C-4′ at δ 79.7. Thus, the structure of **3** was elucidated as (25*R*)-spirost-5-ene-3β,17α-diol-3-*O*-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside, named as pallidifloside C (**3**).

Table 3. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectral data of compound **3** in pyridine-d_5.

	3			**3**	
No.	δ_C	δ_H	No.	δ_C	δ_H
1	37.2	0.97 m, 1.72 m	(3-*O*)-β-D-Gal	102.6	4.90 d (7.5)
2	30.1	1.57 m, 2.07 m	2′	73.2	4.43 dd (7.5, 9.6)
3	77.7	3.82 m	3′	75.1	4.09 o
4	38.9	2.65 d (12.4), 2.71 m	4′	79.7	4.72 m
5	140.6		5′	75.8	4.10 o
6	121.4	5.30 d (4.5)	6′	60.7	4.23 o, 4.67 m
7	32.0	1.47 m, 1.90 m	(Gal^4)-β-D-Glc	106.8	5.33 d (8.1)
8	31.8	1.55 m	2″	74.9	4.19 dd (8.1, 8.4)
9	49.9	0.91 o	3″	78.5	4.32 m
10	36.7		4″	72.0	4.12 o
11	20.7	1.50 o	5″	78.2	3.92 m
12	32.2	1.89 m, 2.21 m	6″	62.8	4.22 m, 4.62 d (11.5)
13	44.9				
14	53.0	2.05 m			
15	32.9	1.51 m, 2.19 m			
16	89.7	4.49 t (7.5)			
17	89.8				
18	16.9	0.94 s			
19	19.1	0.92 s			
20	44.5	2.29 d (6.9)			
21	9.5	1.25 d (6.9)			
22	109.5				
23	32.4	1.52 o			
24	28.5	1.58 m			
25	30.0	1.33 m			
26	66.7	3.52 o, 3.95 m			
27	17.0	0.69 d (5.1)			

Note: Overlapped signals are indicated by 'o.'

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter. UV spectra were obtained on a Shimadzu UV-2201 spectrophotometer. IR spectra were recorded on a Shimadzu IRPrestige-21 spectrophotometer. The NMR data were recorded on a Bruker AV-600 spectrometer (600 MHz) and a Varian INOVA-300 spectrophotometer (300 MHz for ^{1}H and 75 MHz for ^{13}C) in C_5D_5N. The ESI-MS data were obtained on a Finnigan LCQ mass spectrometer. The HR-ESI-MS data were obtained on a Waters LCT Premier XE time-of-flying mass spectrometer. GC analysis was performed on a Shimadzu GC-2010 gas chromatograph equipped with an H_2 flame ionization detector and a DB-5 quartz capillary column (30 m × 0.25 mm × 0.25 um). Chromatography was performed on silica gel (200–300 mesh; Qingdao Marine Chemical Group, Co., Qingdao, China) and preparative HPLC [Shimadzu-LC-8A pump, Shimadzu-SPD-20A UV spectrophotometric detector at 203 nm, Shimadzu Shim-pack PRC-ODS (10 μm, 20 × 250 mm; flow rate 12.0 ml/min)].

3.2 Plant material

The dry bulbs of *F. pallidiflora* Schrenk (2.0 kg) were collected in Yining, Xinjiang Province of China in June 2008, and identified by Dr Yong Tan of Shihezi University. A voucher specimen (no.

2008090158) has been deposited in Research Department of Natural Medicine, Shenyang Pharmaceutical University.

3.3 Extraction and isolation

The dry bulbs of *F. pallidiflora* (2.0 kg) were extracted with 70% EtOH (3 × 20 liters) for 3 h at 95°C. The extracts were combined and concentrated under reduced pressure to give the brown residue (98 g). The residue was suspended in water (2 liters) and then extracted with petroleum ether (2 liters), $CHCl_3$ (2 liters), EtOAc (2 liters), and *n*-BuOH (2 liters), respectively. The *n*-BuOH layer was concentrated under reduced pressure to give the brown extract (26.0), which was chromatographed on silica gel eluting with gradient $CHCl_3$/MeOH (100:1–100:100) to yield 10 fractions 1–10. Fraction 4 (1.2 g) was further purified by preparative HPLC (column: 20 × 250 mm, RP-18, 10 μm; flow rate: 12.0 ml/min) with MeOH–H_2O (75:25) as a mobile phase to afford compound **3** (18.2 mg; retention time 102.2 min). Fraction 6 (0.7 g) was further purified by preparative HPLC (column: 20 × 250 mm, RP-18, 10 μm; flow rate: 12.0 ml/min) with MeOH–H_2O (60:40) as a mobile phase to afford compound **1** (35.4 mg; retention time 42.2 min) and compound **2** (39.4 mg; retention time 13.6 min)

3.3.1 Pallidifloside A (1)

A white amorphous solid, positive Liebermann–Burchard and Ehrlich reagent tests; $[\alpha]_D^{20}$-56.1 (MeOH, c 0.052); UV (MeOH) λ_{max} (nm): 204.0; IR (KBR) ν_{max} (cm^{-1}): 3364, 2923, 1654, 1465, 1457, 1378, 1161, 1074, 1043, 913, and 839; ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2; ESI-MS: *m/z* 1039 $[M + Na]^+$, 907 $[(M + Na)\text{-}132]^+$, 893 $[(M + Na)\text{-}146]^+$, 877 $[(M + Na)\text{-}162]^+$, 761 $[(M + Na)\text{-}132\text{-}146]^+$, and 599 $[(M + Na)\text{-}132\text{-}146\text{-}162]^+$; HR-ESI-MS: *m/z* 1015.5105 $[M - H]^-$ (calculated for $C_{50}H_{79}O_{21}$, 1015.5114).

3.3.2 Pallidifloside B (2)

A white amorphous solid, positive Liebermann–Burchard and positive Ehrlich reagent tests; $[\alpha]_D^{20}$-59.2 (MeOH, c 0.052); UV (MeOH) λ_{max} (nm): 203.5, 238.5; IR (KBr) ν_{max} (cm^{-1}): 3363, 1745, 1713, 1652, 1074, 1040, 949, 912, and 840; ^1H and ^{13}C NMR spectral data are shown in Tables 1 and 2; ESI-MS: *m/z* 939 $[M + Na]^+$, 793 $[(M + Na)\text{-}146]^+$, 777 $[(M + Na)\text{-}162]^+$, 631 $[(M + Na)\text{-}146\text{-}162]^+$; HR-ESI-MS: *m/z* 915.4598 $[M - H]^-$ (calculated for $C_{45}H_{71}O_{19}$, 915.4590).

3.3.3 Pallidifloside C (3)

A white amorphous solid, positive Liebermann–Burchard and negative Ehrlich reagent tests; $[\alpha]_D^{20}$-47.2 (MeOH, c 0.051); UV (MeOH) λ_{max} (nm): 203.5; IR (KBr) ν_{max} (cm^{-1}): 3421, 2927, 1458, 1377, 1245, 1159, 1077, 918, and 893; ^1H and ^{13}C NMR spectral data are shown in Table 3; ESI-MS: *m/z* 777 $[M + Na]^+$, 615 $[(M + Na)\text{-}162]^+$, and 453 $[(M + Na)\text{-}162\text{-}162]^+$. HR-ESI-MS: *m/z* 753.4061 $[M - H]^-$ (calculated for $C_{39}H_{61}O_{14}$, 753.4061).

3.4 Acid hydrolysis of 1, 2, and 3

Compound **1** (2.0 mg) was hydrolyzed with 2 M HCl (5 ml), heated for 2 h at 95°C and extracted with $CHCl_3$ (3 × 5 ml). The aqueous layer was concentrated to dryness under a stream of nitrogen. The aqueous residue was redissolved in anhydrous pyridine (2 ml) and L-cysteine methyl ester hydrochloride (2.5 mg) was added to the solution of pyridine. The mixture was heated at 60°C for 2 h and acetic anhydride (2 ml) was added, followed by heating at 90°C for 0.5 h. The solution was

concentrated to dryness under a stream of nitrogen. The reaction product was subjected to GC analysis in *Shimadzu* GC-2010 (DB-5, 30 m × 0.25 mm, 0.25 μm) and FID detection. Column temperature: 120°C, carrier gas: N_2 (30 ml/min), split ratio: 10:1, injection temperature: 260°C, detector temperature: 260°C, and injection volume: 1 ul are the conditions for GC analysis. The absolute configurations of the monosaccharides of **1** were confirmed to be L-rhamnose ($t_R = 18.8$ min), D-glucose ($t_R = 21.2$ min), and D-xylose ($t_R = 16.9$ min) by comparison of the retention time of monosaccharide derivatives with those of standard sugars (the standard sugars were subjected to the same reaction): L-rhamnose ($t_R = 18.6$ min), D-glucose ($t_R = 21.4$ min), and D-xylose ($t_R = 16.8$ min).

Compound **2** (2.1 mg) was subjected to acid hydrolysis as described for **1** and the absolute configurations of monosaccharides of **2** were confirmed to be L-rhamnose ($t_R = 18.7$ min) and D-glucose ($t_R = 21.5$ min).

Compound **3** (2.0 mg) was subjected to acid hydrolysis as described for **1** and the absolute configurations of monosaccharides of **3** were confirmed to be D-galactose ($t_R = 21.9$ min) and D-glucose ($t_R = 21.3$ min).

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