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Chemical components from *Metapanax delavayi* leaves and their anti-BHP activities *in vitro*

Chongzhi Sun[a], Yang Wu[a], Bei Jiang[b], Ying Peng[a], Mengyue Wang[a], Jiaxun Li[c], Xiaobo Li[a,*]

[a] School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, PR China
[b] Institute of Materia Medica, College of Pharmacy and Chemistry, Dali University, Dali, 671000, PR China
[c] Lanping County Bureau of Agriculture, Lanping, 671400, PR China

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ABSTRACT

Two previously undescribed oleanane-type triterpene saponins named liangwanosides III–IV, and one undescribed eudesmane glycoside named liangwanoside A were obtained from the leaves of *Metapanax delavayi*, a Chinese folk medicine especially for tea used in Yunnan, together with four known compounds. The structures of the undescribed compounds were determined by detailed spectroscopic (1D/2D NMR), HR-ESI-MS data analysis and chemical evidence. The activity against human benign prostate hyperplasia was evaluated with BPH-1 cell line. Most of the isolated compounds showed moderate inhibitory activity against BPH-1 cells at 100 and 50 μM *in vitro*.

1. Introduction

Metapanax delavayi (Franch.) J. Wen & Frodin (Araliaceae), known as “liang wang cha” in China, is mainly distributed in the northern region of Vietnam, central and western regions of China (Committee, 2007). According to local ethnic minorities lived in Yunnan Province of China, the tender leaf and stem of *M. delavayi* has been used as food (Li et al., 2015), which has an efficacy to treat prostatitis (Zhou et al., 2017). The stem bark of *M. delavayi* has been used in Chinese folk medicine, which possesses the effects of clearing heat, anti-inflammation, expectorant, relieving cough and asthma (Zhou et al., 2017). The phytochemical studies on *M. delavayi* led to the isolation and identification of triterpenoid saponins (Kasai et al., 1987), polysaccharides (Miu, 2005), and several other components (William et al., 2008; Yang et al., 2014), which exhibited diverse biological activities, such as analgesia (Ge et al., 2000), antimalarial (Liu et al., 2015) and antioxidative activities (Miu, 2005). In order to clarify the effective chemical ingredients, the isolation and structural elucidation of aqueous extract of *M. delavayi* leaves were carried out in the present study. Three undescribed compounds (**1**-**3**) with four known compounds (**4**–**7**) were obtained. The inhibitory activities against BPH-1 cells of compounds **1–6** and the aglycone of the triterpene saponins named serratagenic acid (**8**) were evaluated.

2. Results and discussion

The aqueous extract from leaves of *M. delavayi* was firstly passed through a D101 macroporous resin column, then subjected to silica gel, Sephadex LH-20 column chromatography (CC) and RP-HPLC to yield three undescribed compounds, liangwanoside III (**1**), liangwanoside IV (**2**), liangwanoside A (**3**), and four known compounds, 3-*O*-*α*-L-arabinopyranoside-3*β*-hydroxyolean-12-ene-28,29-dioic acid-28-*O*-[*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranosyl] ester (**4**) (Zhang et al., 2018), liangwanoside II (**5**) (Kasai et al., 1987), serratagenic acid-3-*O*-*α*-L-arabinopyranoside (**6**) (Yu et al., 1995), byzantionoside B (**7**) (Matsunami et al., 2010). Serratagenic acid (**8**) (Kasai et al., 1987), the aglycone of the oleanane-type triterpenoid saponin, was obtained by acid hydrolysis method (See Fig. 1).

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{48}H_{76}O_{19}$, evidenced from the HR-ESI-MS data (*m/z* 955.49050 [M – H] $^-$, calcd. for 955.49026). The ^{1}H NMR spectrum (Table 1) of **1** clearly indicated the presence of six methyl protons (δ_H 1.47, 1.23, 1.24, 1.15, 1.05, and 0.95, each 3H, s), which were labeled as methyls C-30, 23, 27, 26, 24, and 25, respectively, and the characteristic proton signal of an olefinic proton (H-12, δ_H 5.51) corresponded to the carbon signal at δ_C 124.0 (C-12) in the HMQC spectrum (Supporting Information S3). A total of 48 carbon resonances appeared in the ^{13}C NMR spectrum (Table 1), of which 30 were ascribed to a triterpene skeleton, including the signals of two carbonyl carbons (C-28, δc 176.8; C-29, δc 181.4), and the remaining

* Corresponding author.
E-mail address: xbli@sjtu.edu.cn (X. Li).

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Table 1
^{1}H and ^{13}C NMR data of compounds **1–2** in C_5D_5N (δ in ppm, J in Hz).[a]

No.	1		2	
	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.57, m[b]	39.4	1.56, m[b]	39.4
	1.01, m[b]		1.01, m[b]	
2	1.87, m[b]	28.6	1.87, m[b]	28.6
	1.84, m[b]		1.83, m[b]	
3	3.44, dd (11.2, 5.0)	78.5	3.44, m[b]	78.5
4		39.8		39.8
5	0.84, d (11.3)	56.2	0.84, d (11.6)	56.2
6	1.55, m[b]	19.3	1.54, m[b]	19.3
	1.40, m[b]		1.37, m[b]	
7	1.45, m[b]	33.6	1.46, m[b]	33.6
	1.36, m[b]		1.36, m[b]	
8		40.4		40.4
9	1.66, dd (10.8, 7.1)	48.6	1.66, dd (10.8, 7.1)	48.5
10		37.9		37.8
11	1.96, m[b]	24.3	1.96, m[b]	24.3
	1.95, m[b]		1.95, m[b]	
12	5.51, s	124.0	5.52, s	124.0
13		144.1		144.1
14		42.8		42.8
15	2.35, t (12.1)	28.7	2.35, t (12.1)	28.7
	1.18, m		1.17, m	
16	2.22, m[b]	24.0	2.22, m[b]	23.9
	2.03, m[b]		2.03, m[b]	
17		47.5		47.4
18	3.34, dd (14.4, 4.8)	41.3	3.36, dd (14.1, 4.7)	41.3
19	2.56, t (13.9)	41.3	2.56, t (13.9)	41.3
	1.92, m[b]		1.92, m[b]	
20		42.6		42.6
21	2.23, m[b]	29.6	2.23, m[b]	29.5
	1.76, d (13.0)		1.77, d (13.3)	
22	1.99, m[b]	32.2	2.03, m[b]	32.1
	1.91, m[b]		1.94, m[b]	
23-CH_3	1.23, s	29.2	1.23, s	29.2
24-CH_3	1.05, s	17.0	1.04, s	17.0
25-CH_3	0.95, s	16.1	0.96, s	16.1
26-CH_3	1.15, s	18.0	1.16, s	18.0
27-CH_3	1.24, s	26.5	1.24, s	26.5
28		176.8		176.8
29		181.4		181.3
30-CH_3	1.47, s	20.4	1.47, s	20.4
28-Sugar Glc-1	6.30, d (8.2)	96.2	6.31, d (7.9)	96.2
2	4.17, m[b]	74.5	4.17, m	74.3
3	4.26, m[b]	79.3	4.24, m[b]	79.0
4	4.38, m[b]	71.3	4.25, m[b]	72.0
5	4.18, m[b]	77.7	4.24, m[b]	79.2
6	4.72, m[b]	69.8	4.76, d (10.7)	69.9
	4.37, m[b]		4.39, m[b]	
Glc-1′	4.99, m[b]	105.5	5.07, d (7.7)	105.8
2′	3.98, t (8.4)	75.8	4.04, t (8.0)	75.6
3′	3.69, d (8.6)	77.0	3.92, m	78.9
4′	4.45, t (9.4)	78.6	4.38, m[b]	71.4
5′	4.14, m[b]	78.5	4.15, m[b]	78.5
6′	4.22, m[b]	61.7	4.51, d (11.7)	63.1
	4.12, m[b]		4.38, m[b]	
Rha-1	5.90, s	103.2		
2	4.59, dd (9.3, 3.3)	73.3		
3	4.71, m[b]	73.1		
4	4.36, m[b]	74.3		
5	5.01, m[b]	70.8		
6-CH_3	1.72, d (6.2)	19.0		

[a] ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz in C_5D_5N.
[b] Signals overlapped.

18 were ascribed to three sugar moieties. The above spectroscopic data indicated that **1** was a Δ^{12}-oleanane-type triterpenoid saponin (Zhao et al., 2014). The HMBC correlations of H-18 (δ_H 3.34) to C-28 (δ_C 176.8) and CH_3-30 (δ_H 1.47, 3H, s) to C-29 (δ_C 181.4) confirmed the locations of carboxyl group at C-28 and C-29, respectively.

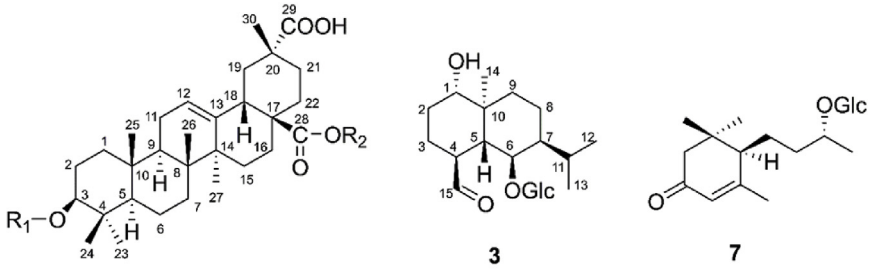
The NOESY spectrum of **1** (Supporting Information S6) showed NOE enhancement between δ_H 1.47 (3H, s, CH_3-30) and δ_H 3.34 (dd, 1H, J = 14.4, 4.8 Hz, H-18), indicating that the relative stereochemistry of this methyl group was β-orientation. Detailed analysis of the 1D and 2D NMR data (Table 1 and Supporting Information S1–S6) revealed that the aglycone of **1** was serratagenic acid which was reconfirmed by the NOSEY correlations of H-3/CH_3-23, H-3/H-5, H-5/H-9, H-9/CH_3-27, CH_3-25/CH_3-26, H-18/CH_3-30 and H-18/CH_3-26 (Kim et al., 2011). The structure of **1** was similar to Liangwanoside II (Kasai et al., 1987), with the difference of a reductional arabinopyranosyl group.

The ^{1}H NMR spectrum showed three anomeric protons for three sugar moieties that resonated at δ_H 6.30, 4.99 and 5.90. The ^{13}C NMR spectrum of **1** displayed the presence of three sugar moieties including two glucopyranosyls (δ_C 96.2, 74.5, 79.3, 71.3, 77.7, 69.8), (δ_C 105.5, 75.8, 77.0, 78.6, 78.5, 61.7) and one rhamnopyranosyl (δ_C 103.2, 73.3, 73.1, 74.3, 70.8, 19.0). After acid hydrolysis, the sugar units were confirmed to be D-glucose and L-rhamnoside, which were identified by GC-MS analysis. The orientation of the two glucopyranosyl residues were deduced to be β, according to the large coupling constant of the anomeric proton (Glc-1H, δ_H 6.30, d, J = 8.2 Hz) and the chemical shifts of Glc C-1′–C-6′ (δ_C 105.5, 75.8, 77.0, 78.6, 78.5, 61.7), (Ye et al., 2001), respectively. The anomeric proton (δ_H 5.90) of the Rha unit showed a strong HMBC correlation with the carbons at positions Rha-3 (δ_C 73.1) and Rha-5 (δ_C 70.8), which indicated an α-orientation (Mair et al., 2018).

As shown in Fig. 2, the HMBC correlation between Glc-1H (δ_H 6.30) and the carbonyl carbon at δ_C 176.8 indicated that the glucopyranosyl group was posited at C-28. The HMBC cross-peaks of Glc-1′H (δ_H 4.99) to Glc-6C (δ_C 69.8) and Rha-1H (δ_H 5.90) to Glc-4′C (δ_C 78.6) confirmed the sequence of sugar moieties at C-28 was α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl. Thus, the structure of **1** was elucidated as 3β-hydroxyolean-12-ene-28,29-dioic acid-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester and named liangwanoside III.

Compound **2** was isolated as a white amorphous powder. The HR-ESI-MS of **2** showed a major ion peak at m/z 809.43258 $[M - H]^-$ (calcd. 809.43235), supporting the molecular formula of $C_{42}H_{66}O_{15}$. A comparison of the 1D and 2D NMR spectra (Table 1 and Supporting Information S8–S14) similar to **1** revealed that the aglycone of **2** was also serratagenic acid, with the difference of a reductional rhamnopyranosyl group. In the ^{1}H NMR spectrum (Table 1), signals for two anomeric protons at δ_H 6.31 (1H, J = 7.9 Hz) and δ_H 5.07 (1H, J = 7.7 Hz) were diagnostic for the presence of two sugar residues. The 2D NMR data confirmed the presence of two glucopyranosyl units (δ_C 96.2, 74.3, 79.0, 72.0, 79.2, 69.9; δ_C 105.8, 75.6, 78.9, 71.4, 78.5, 63.1). Acid hydrolysis of **2** afforded D-glucose by GC-MS analysis. The coupling constants (Glc-1H, δ_H 6.31, d, J = 7.9 Hz; Glc-1′H, δ_H 5.07, d, J = 7.7 Hz) confirmed the β-glycosidic linkages for two glucose units (Liang et al., 2011). The HMBC correlations (Fig. 2 and Supporting Information S11) between the Glc-1H (δ_H 6.31) and the aglycone C-28 (δ_C 176.8), as well as the Glc-1′H (δ_H 5.07) and Glc-6C (δ_C 69.9), indicated the sugar residues at C-28 were determined to be β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl. Consequently, **2** was identified as 3β-hydroxyolean-12-ene-28,29-dioic acid-28-*O*-[β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester and named liangwanoside IV.

Compound **3** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{21}H_{36}O_8$ on the HR-ESI-MS $[M - H + HCOOH]^-$ m/z 461.23970 (calcd. 461.23867). The IR spectrum featured absorption bands at 3151 and 1762 cm^{-1} which were characteristic of hydroxy group and carbonyl group, respectively. The ^{1}H NMR spectrum of **3** (Table 2) indicated the presence of three methyl singlets (δ_H 0.98, 1.41, 1.15), two protons attached to oxygenated carbons at δ_H 3.55 and δ_H 4.48, one anomeric proton at δ_H 4.97, and one aldehyde proton at δ_H 9.73. In the ^{13}C NMR and HSQC spectra of **3**, 21 carbon signals were detected. These signals resulted from three methyls, four methylenes, six methines (including two oxygenated), a quaternary carbon, and an aldehyde group. Moreover, one set of proton signals at δ_H 3.89–4.97, and their corresponding carbons resonating at



1 R_1 = H R_2 = β-D-Glcp-β-D-Glcp-α-L-Rhap
2 R_1 = H R_2 = β-D-Glcp-β-D-Glcp
4 R_1 = α-L-Arap R_2 = β-D-Glcp-β-D-Glcp
5 R_1 = α-L-Arap R_2 = β-D-Glcp-β-D-Glcp-α-L-Rhap
6 R_1 = α-L-Arap R_2 = H
8 R_1 = H R_2 = H

Fig. 1. Structures of compounds **1–7** isolated from the extract of *M. delavayi*, and serratagenic acid (**8**).

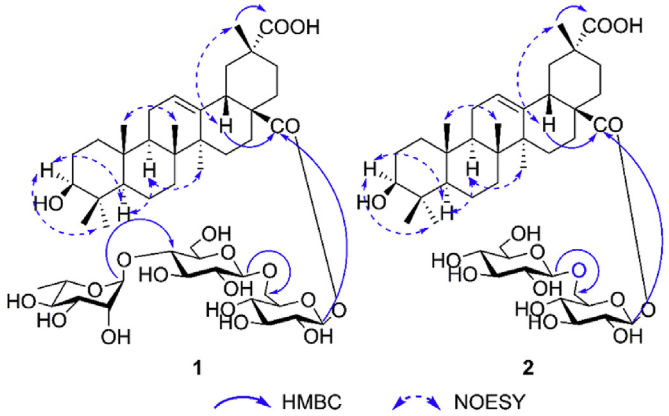


Fig. 2. Key HMBC and NOESY correlations for compounds **1–2**.

δ_C 63.3, 71.6, 74.9, 78.2, 79.1 and 102.3, suggested the presence of a hexose residue.

The HMBC correlations of (Fig. 3) H-12, H-13 to a quaternary carbon at δ_C 26.0 (C-11) and H-13 to C-12 showed that C-12 and C-13 were respectively connected with C-11. The HMBC correlation between H-14 and C-10 supported that C-14 was attached to C-10, which was further confirmed by the HMBC correlations from H-14 to C-1, C-5, and C-9. ^{1}H-^{1}H COSY correlation of H-15 to H-4 indicated that C-15 was connected with C-4, which was confirmed by the HMBC correlations of H-15 to C-3 and C-5. The ^{1}H-^{1}H COSY correlations of H-5 to H-6, H-6 to H-7 and the HMBC correlations of H-6 and H-7 to C-11 identified that C-6 was connected between C-5 and C-7. The HMBC correlations of H-7 to C-12 and C-13 and the ^{1}H-^{1}H COSY correlation of H-11 to H-7 indicated C-11 was attached to C-7. C-1 was attached to C-2 based on the ^{1}H-^{1}H COSY correlations from H-1 to H-2 then to H-3. It was also indicated that the glycosidic site was attached to C-6 by the correlation of HMBC from H-1′ to C-6. All the information mentioned above indicated that **3** had the same scaffold as 1α,6β-dihydroxy-5,10-bis-epi-eudesm-15-carboxaldehyde-6-*O*-β-D-glucopyranoside (Hao et al., 2015), but C-11 of **3** (δ_C 26.0) was linked with a proton (δ_H 2.29), not a hydroxyl group.

The relative configurations of **3** were deduced from the analysis of coupling constant and NOESY spectrum (Fig. 3). The coupling constant of H-6 ($J_{6,7}$ = 4.4 Hz, $J_{5,6}$ = 11.5 Hz), and the interaction of H-6 with H-7 and H-14 suggested that H-6, 7 and 14 were in α-orientations, H-5 was in β-orientation (Xu et al., 2010). H-14 interaction with H-4 suggested α-orientation of H-4. H-5 interaction with H-1 and H-15 indicated that H-1 and H-15 were in β-orientations. The coupling constant of anomeric proton (δ_H 4.97, J = 7.7 Hz) and chemical shift of carbons (C-1′, δ_C 102.3; C-2′, δ_C 74.9; C-3′, δ_C 79.1; C-4′, δ_C 71.6; C-5′, δ_C 78.2;

Table 2
^{1}H and ^{13}C NMR data of compound **3** in C_5D_5N (δ in ppm, J in Hz).[a]

No.	δ_H (J in Hz)	δ_C
1	3.55, dd (11.5, 4.3)	78.2
2	1.88, m	29.7
	1.68, qd (13.2, 4.3)	
3	1.54, m	25.9
	1.44, m[b]	
4	2.65, m	48.1
5	2.15, t (11.5)	45.7
6	4.48, dd (11.5, 4.4)	78.4
7	2.01, m[b]	43.0
8	1.81, m	23.8
	1.64, tt (14.4, 4.4)	
9	1.99, m[b]	33.3
	1.46, m[b]	
10		42.0
11	2.29, dq (13.9, 6.8)	26.0
12-CH_3	0.98, d (6.8)	23.3
13-CH_3	1.41, d (6.8)	24.5
14-CH_3	1.15, s	12.1
15	9.73, d (4.5)	203.7
1′	4.97, d (7.7)	102.3
2′	4.04, t (8.4)	74.9
3′	4.20, t (9.1)	79.1
4′	4.30, t (9.1)	71.6
5′	3.89, m	78.2
6′	4.50, dd (11.5, 2.4)	63.3
	4.42, dd (11.5, 4.5)	
1-OH	6.06, s	
2′-OH	6.96, brs	
3′-OH	7.13, brs[b]	
4′-OH	7.25, brs[b]	
6′-OH	5.53, brs	

[a] ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz in C_5D_5N.
[b] Signals overlapped.

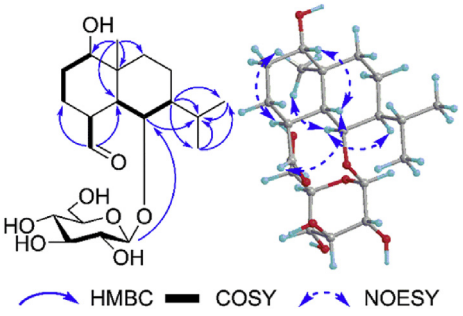


Fig. 3. Key COSY, HMBC and NOESY correlations for compound **3**.

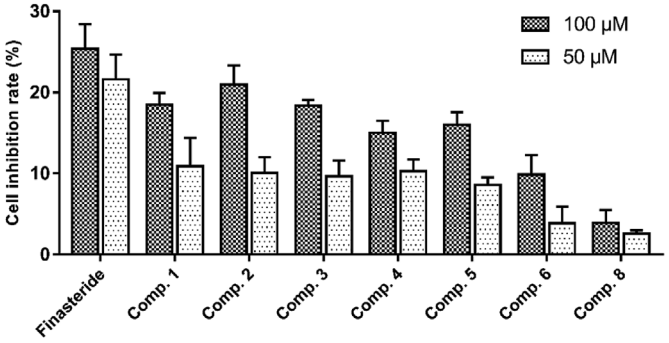


Fig. 4. The repressive effect of compounds **1–6** & **8** on the growth of BPH-1 cells (n = 3).

C-6′, δ_C 63.3) indicated the sugar moiety was *β*-glucose (Gorin and Mazurek, 1975). The *β*-D-glucose of the sugar moiety was determined by acid hydrolysis and comparison with an authentic sample by GC-MS. Thus, the structure of **3** was assigned as 5-epi-eudesm-15-carboxaldehyde-6-*O*-*β*-D-glucopyranoside-1*α*,6*β*-diol, named liangwanoside A.

Their inhibitory activities against BPH-1 cells of compounds **1–6**, **8** were studied *in vitro* (Fig. 4). In previous reports, compound **7** had effects of increasing alkaline phosphatase and inhibition of arachidonic acid release activity (Bukhari et al., 2015; Mao et al., 2014). As **7** was known compound and had a low content in *M. delavayi* leaves, we didn't investigate its activity on anti-BPH. The result showed that compounds **1–6** inhibited cell proliferation for 48 h, and **8** didn't exhibit inhibitory effect obviously. The inhibition rates of compound **1–6** were from 3.9% to 10.9% at 50 μM, and from 9.9% to 21.0% at 100 μM, respectively. It was reported that compound **4** possessed hepatoprotective effect (Zhang et al., 2018), and **5** showed the activity of IL-6 formation inhibition (Tsuji et al., 1997). In our present study, compounds **4–5** also exhibited the anti-BPH activity *in vivo*, their inhibitory rates were 15.0 ± 1.4%, 16.0 ± 1.5% at 100 μM, respectively. But the undescribed compounds **1–3** showed stronger inhibitory effect with inhibitory rates of 18.5 ± 2.4%, 21.0 ± 2.4% and 18.4 ± 0.7% at 100 μM, respectively. The absorption of glycosides was often involved metabolism such as deglycosylation to the aglycone after oral administration, and the prototype components as well as their aglycone could be absorbed into plasma (Zhou et al., 2018). In our study, the glycosides (**1–2**, **4–6**) show stronger inhibitory activity of anti-proliferation on BPH-1 cells than their aglycone (**8**). Their activities *in vivo* remain to be further investigated.

3. Conclusion

A total of seven compounds were isolated from the aqueous extract of *M. delavayi* leaves, including three undescribed and four known compounds. Compounds **1–2**, **4–6** were derivatives of serratagenic acid, **3** and **7** were sesquiterpenes. Compounds **1–6,** which were demonstrated to displayed moderate inhibitory activity against BPH-1 cells, would be developed to the precursors of novel anti-BPH drugs. Considering the large contents of the derivatives of serratagenic acid in *M. delavayi* leaves, they might be the major bioactive components against BPH *in vivo* and need to be further studied.

4. Experimental

4.1. General experimental procedures

Specific rotations were determined on a JASCO P-2000 polarimeter (Hachioji, Tokyo, Japan). UV spectra were recorded using a UV-1102 UV–vis spectrophotometer (Techcomp, Shanghai, China). IR spectra were determined on an Equinox 55 FT-IR spectrometer (Bruker Optics Inc., MA, USA). NMR spectra were recorded on a Bruker Avance 600 MHz spectrometer (Bruker BioSpin Corp., MA, USA). HR-ESI-MS data were obtained on a Waters ACQUITY™ UPLC-Q-TOF-MS (Waters Corp., Milford, USA). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Qingdao, China), D101-type macroporous resin (Baoen Corp., Cangzhou, China), and Sephadex LH-20 (Pharmacia, Stockholm, Sweden) were used for CC. RP-HPLC was performed on a Shimadzu LC 2010 AHT instrument, using a YMC ODS-AQ column (20 × 250 mm, 5 μm, YMC, Kyoto, Japan). GC-MS was conducted on an Agilent 7890A-5975C instrument (Agilent Technologies, Inc., CA, USA). TLC was carried out with GF254 plates (Qingdao Haiyang Chemical Co., Qingdao, China). Spots were visualized by spraying with 10% H_2SO_4 in EtOH followed by heating. The analytically pure reagents were from Sinopharm Chemical Reagent Co., (Shanghai, China).

4.2. Plant materials

The leaves of *Metapanax delavayi* (Araliaceae) were collected from Lanping County, Yunnan Province, China (GPS coordinates: 26°27′34.64″N, 99°19′1.25″E), in October 2014, autumn, and identified by one of authors Prof. Xiaobo Li. A voucher specimen (20141001) was deposited at School of Pharmacy, Shanghai Jiao Tong University.

4.3. Extraction and isolation

Dried and powdered leaves of *M. delavayi* (1 kg) were extracted three times by water (3 × 10 L, 1 h each) under reflux. The combined extraction filtrated with eight layers of gauze, and then concentrated under reduced pressure to obtain a crude aqueous extract (380 g), which was suspended in H_2O and chromatographed over a D101 column. After eluting with H_2O, the D101 column was successively eluted with 10%, 20%, 30%, 40% and 95% EtOH, to afford six fractions (F1–F6). F3 (13.6 g) was subjected to silica gel CC (CH_2Cl_2/MeOH, 15:1–3:1, v/v) to afford eight fractions (F3-1–F3-8). F3-2 was then separated by silica gel CC eluted with CH_2Cl_2/MeOH (15:1–3:1), then purified by RP-HPLC using MeCN/H_2O (10% v/v, 2 mL/min) as mobile phase to yield compound **3** (95.1 mg, t_R = 17.9 min). F4 (20.7 g) was chromatographed by silica gel CC using CH_2Cl_2/MeOH/H_2O (4:1:0.1, v/v/v) as mobile phase, to yield 12 fractions. F4-1 to F4-12. F4-3 (63.3 mg) was separated by repeated Sephadex LH-20 and purified by RP-HPLC MeCN/H_2O (10% v/v, 2 mL/min, UV 210 nm) to give compound **7** (2.3 mg, t_R = 32.0 min). F5 (30.5 g) was separated by silica gel CC with CH_2Cl_2/MeOH/H_2O (4:1:0.1, v/v/v) as mobile phase, to yield 30 fractions, F5-1 to F5-30. F5-9 (72.6 mg) was separated via RP-HPLC (MeCN/H_2O, 20:80, v/v, 2 mL/min) to afford compound **4** (33.0 mg, t_R = 17.5 min). F5-8 (80.1 mg) was subsequently subjected to silica gel CC eluting with CH_2Cl_2/MeOH/H_2O (4:1:0.1, v/v), and purified by RP-HPLC using MeCN/H_2O (30% v/v, 2 mL/min, UV 210 nm) as mobile phase to yield compound **1** (34.5 mg, t_R = 13.5 min). F5-7 (63.3 mg) was repeated on a silica gel column, Sephadex LH-20 and RP-HPLC (MeCN/H_2O, 30:70, v/v, 2 mL/min) to yield compound **2** (27.0 mg, t_R = 23.5 min). F5-2 (47.6 mg) was subjected to passage over a silica gel column eluted with CH_2Cl_2–MeOH (8:1, v/v) to give 13 fractions. The eighth fraction (32.7 mg) was further purified over Sephadex LH-20 and RP-HPLC (MeCN/H_2O, 30:70, v/v, 2 mL/min, UV 210 nm) to give compound **6** (18.8 mg). F5-20 (11.3 g) was further separated by repeated Sephadex LH-20 eluted with MeOH to afford compound **5** (10.2 g). Compound 5 (300 mg) was hydrolyzed by heating in 2 M HCl (50 mL). The reaction mixture was partitioned between EtOAc (50 mL) and H_2O each, and the EtOAc extract was subjected to silica gel CC (CH_2Cl_2/MeOH/H_2O, 30:1:0.04, v/v/v) to afford compound **8** (63.2 mg), its identity was confirmed by comparison with published NMR spectroscopic data.

4.3.1. Liangwanoside III (1)

White powder; $[\alpha]_D^{25}$ –16.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.61) nm; IR (KBr) ν_{max} 3082, 2886, 1594, 1441, 1372, 1366,

1181, 1054 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) see Table 1. HR-ESI-MS *m/z* 955.49050 [M – H] $^-$ (calcd. for $C_{48}H_{75}O_{19}$, 955.49026).

4.3.2. Liangwanoside IV (2)

White powder; $[\alpha]_D^{25}$ –3.4 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.51) nm; IR (KBr) ν_{max} 3225, 2886, 1604, 1457, 1382, 1366, 1187, 1048 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) see Table 1. HR-ESI-MS *m/z* 809.43258 [M – H] $^-$ (calcd. for $C_{42}H_{65}O_{15}$, 809.43235).

4.3.3. Liangwanoside A (3)

White powder; $[\alpha]_D^{25}$ –5.8 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.23) nm; IR (KBr) ν_{max} 3151, 2802, 1762, 1727, 1403, 1276, 1054, 985, 869 cm^{-1}; ^1H NMR (C_5D_5N, 600 MHz) and ^{13}C NMR (C_5D_5N, 150 MHz) see Table 2. HR-ESI-MS *m/z* 461.23970 [M – H + HCOOH] $^-$ (calcd. for $C_{22}H_{37}O_{10}$, 461.23867).

4.4. Acid-hydrolysis and sugar analyses of the three undescribed compounds (1–3)

The procedures of the acid-hydrolysis and sugar analyses of the three undescribed compounds (**1–3**) were modified according to the published literature (Zong et al., 2015). Compounds **1–3** (2 mg) were dissolved in 2 M HCl (2 mL) and heated at 90 °C for 4 h, respectively. The reaction mixture was extracted with EtOAc. The aqueous layer was evaporated under vacuum, diluted repeatedly with H_2O, and evaporated in vacuo to furnish a neutral residue. The residue was dissolved in pyridine (2 mL), to which 2 mg of L-cysteine methyl ester hydrochloride was added. After heating at 60 °C for 2 h, the solvent was evaporated under N_2, and 0.2 mL trimethylsilylimidazole was added. The mixture was kept at 60 °C for another 2 h and partitioned between *n*-hexane and water. The organic layer centrifuged 20 minutes at 14000 rpm. The supernatant (1 μL) was directly analyzed by GC-MS using a HP-5 capillary column. The acetate derivatives of D-glucose, L-rhamnose, and L-arabinose had the following retention times, t_R (min): D-glucose (21.84), L-rhamnose (20.06), and L-arabinose (19.09). Identification of the sugar derivatives was based on a comparison of mass spectra with those of authentic samples.

4.5. BPH-1 cell survival test

The BPH-1 cells (Fenghui Biological Technology Co., Ltd., Hunan, China) were cultured in a RPMI 1640 medium supplemented with 20% FBS (Gibco, Melbourne, Australia), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were maintained in a humidified 5% CO_2, 95% air atmosphere at 37 °C for different periods of time and were then harvested for measurements. The BPH-1 cells were seeded in 96-well plates (5×10^3 cells/well) and allowed to adapt overnight, then treated with 100 and 50 μM of compounds **1–6**, **8** in the culture medium containing 20% FBS for 48 h. Finasteride was used as a positive control. The MTT solution (20 μL, 5 mg/mL) was added to each well, and the cells were incubated for another 4 h. The formazan crystals were dissolved in 150 μL of DMSO. Cell viability was assessed by measuring the absorbance at a 570 nm wavelength using a Thermo Varioskan microplate photometer (Thermo, Waltham, USA). The inhibition rate (%) = $[(A_{control} - A_{sample})/(A_{control} - A_{blank})] \times 100\%$. Experiments were performed in triplicate, and the values are the averages of three independent experiments.

Conflicts of interest

There are no conflicts to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.phytochem.2019.01.002.

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