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Protein tyrosine phosphatase 1B inhibitory triterpene glycosides from *Mussaenda pilosissima* Valeton

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ABSTRACT

Three new triterpene glycosides, mussapilosides A–C (**1**-**3**) together with six known triterpene glycosides, cincholic acid-28-*O*-*β*-D-glucopyranosyl ester (**4**), mussaendoside S (**5**), quinovic acid 28-*O*-*β*-D-glucopyranosyl ester (**6**), glycoside A (**7**), glycoside B (**8**), and quinovic acid 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-glucopyranoside (**9**) were isolated from the leaves of *Mussaenda pilosissima*. Their chemical structures were elucidated on the basis of extensive spectroscopic methods, including 1D, 2D NMR, and HR ESI MS spectra. All these compounds were examined for their inhibitory activity against PTP1B. The results indicated that compounds **2**, **6**, and **8** exhibited potent inhibitory effects with IC_{50} values of 3.4 ± 0.2, 4.2 ± 0.2, and 6.4 ± 0.2 μM, respectively. Compound **5** showed the moderate inhibitory effect with IC_{50} value of 63.0 ± 3.2 μM.

1. Introduction

Protein tyrosine phosphatase 1B (PTP1B) is a key regulator of insulin-receptor activity. Increased expression of PTP1B in adipose tissue and muscle is related to insulin resistance (Wu et al., 2001), while a decrease in PTP1B could increase insulin sensitivity (Nguyen et al., 2018). The possibility of several substrate receptor tyrosine kinases (RTKs) for PTP1B beyond insulin-receptor kinase raise concerns about the viability of PTP1B as a drug target.

The *Mussaenda* genus (Rubiaceae) has been used as oriental medicine for the treatment of sore throat and stomach illness (Chi, 1999). *Mussaenda pilosissima* Valeton has been used in traditional medicine for the treatment of kidney disease (Hien and Xuyen, 2011). However, the study on chemical constituent and biological effects from *M. pilosissima* have not been reported yet. This paper reports the isolation, structural elucidation, and PTP1B inhibitory activity of three new and six known triterpenoid glycosides from *M. pilosissima*.

2. Results and discussion

Compound **1** was isolated as a white amorphous powder and its molecular formula was determined as $C_{41}H_{66}O_{13}$ by the HR ESI MS ion peak at *m/z* 789.4400 $[M + Na]^+$ (Calcd. for $[C_{41}H_{66}O_{13}Na]^+$, 789.4396). The ^1H NMR spectrum of **1** (in CD_3OD) showed six tertiary methyl signals at δ_H 0.86, 0.89, 0.93, 0.95, 0.96, and 1.06 (each 3H, s); two anomeric protons at δ_H 4.33 (1H, d, J = 8.0 Hz) and 5.40 (1H, d, J = 8.0 Hz) suggested the presence of two sugar units. The ^{13}C NMR and DEPT spectra (Table 1) revealed the carbon signals of one carbonyl at δ_C 178.2, two quaternary olefinic carbons at δ_C 131.2 and 137.9, five non-protonated carbons at δ_C 31.4, 38.2, 38.9, 40.3, and 46.6, four methines at δ_C 40.2, 57.1, 57.7, and 90.9, eleven methylenes at δ_C 18.8, 19.5, 21.5, 24.4, 27.2, 32.0, 32.8, 35.1, 39.5, 40.7, and 42.4, and six methyl carbons at δ_C 16.7, 17.1, 21.0, 25.0, 28.4, and 33.0, assigned to 27-noroleanane-*type* triterpene aglycone; ten methines at δ_C 71.2, 71.7, 74.0, 75.7, 77.7, 78.3, 78.4, 78.7, 95.7, and 106.7 and two methylenes at δ_C 62.5 and 62.8, assigned to two sugar units. Analysis of the NMR data suggested that structure of **1** was similar to that of pyrocincholic acid 3*β*-*O*-*β*-D-quinovopyranosyl-28-[*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl] ester (Um et al., 2001) except for the difference in the sugar moieties at C-3 and C-28. The HMBC correlations (Fig. 2) between H-15 (δ_H 2.06 and 2.22) and C-13 (δ_C 131.2); between H-16 (δ_H 1.78 and 1.98)/H-18 (δ_H 2.43)/H-26 (δ_H 0.95) and C-14 (δ_C 137.9) confirmed the position of the double bond at C-13/C-14. The HMBC correlations between H-23 (δ_H 1.06)/H-24 (δ_H 0.86) and C-3 (δ_C 90.9)/C-4

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Table 1
^{1}H and ^{13}C NMR spectroscopic data for compounds **1** - **3** in CD_3OD.

C		**1**		**2**		**3**
	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)	δ_C	δ_H (mult, *J* in Hz)
Aglycon						
1	39.5	0.96 (m)/1.76 (m)	40.0	1.00 (m)/1.67 (m)	39.9	1.04 (m)/1.67 (m)
2	27.2	1.73 (m)/1.96 (m)	27.1	1.72 (m)/1.85 (m)	27.2	1.73 (m)/1.96 (m)
3	90.9	3.19 (dd, 12.0, 4.5)	90.6	3.11 (dd, 12.0, 4.5)	91.4	3.17 (dd, 12.0, 4.5)
4	40.3	–	40.1	–	40.4	–
5	57.1	0.81 (br d, 11.5)	57.1	0.75 (br d, 12.0)	56.9	0.77 (br d, 11.5)
6	19.5	1.45 (m)/1.60 (m)	19.3	1.36 (m)/1.53 (m)	19.3	1.35 (m)/1.55 (m)
7	40.7	1.15 (m)/1.84 (m)	37.8	1.25 (m)/1.64 (m)	37.5	1.21 (m)/1.64 (m)
8	38.2	–	40.7	–	40.5	–
9	57.7	1.00 (m)	48.4	2.11 (dd, 5.5, 11.0)	48.4	2.18 (dd, 5.5, 11.0)
10	38.9	–	37.9	–	38.0	–
11	18.8	1.51 (m)/1.62 (m)	24.0	1.92 (m)/1.96 (m)	24.0	1.91 (m)/1.96 (m)
12	32.8	1.87 (m)/2.24 (m)	127.8	5.68 (br s)	127.0	5.64 (br s)
13	131.2	–	137.5	–	138.5	–
14	137.9	–	57.3	–	57.5	–
15	21.5	2.06 (m)/2.22 (m)	25.7	1.67 (m)/2.08 (m)	25.8	1.65 (m)/2.05 (m)
16	24.4	1.78 (m)/1.98 (m)	25.1	1.74 (m)/2.09 (m)	25.3	1.64 (m)/2.06 (m)
17	46.6	–	48.5	–	48.5	–
18	40.2	2.43 (dd, 12.0, 3.0)	44.6	2.92 (dd, 14.0, 3.5)	44.9	2.92 (br d, 12.0)
19	42.4	1.09 (m)/1.53 (m)	44.6	1.15 (m)/1.43 (m)	44.8	1.14 (m)/1.44 (m)
20	31.4	–	31.6	–	31.0	–
21	35.1	1.25 (m)/1.44 (m)	34.8	1.20 (m)/1.35 (m)	34.9	1.17 (m)/1.33 (m)
22	32.0	1.59 (m)/1.85 (m)	32.7	1.57 (m)/1.76 (m)	33.5	1.50 (m)/1.76 (m)
23	28.4	1.06 (s)	28.5	1.02 (s)	28.4	1.06 (s)
24	16.7	0.86 (s)	17.0	0.85 (s)	17.0	0.86 (s)
25	17.1	0.89 (s)	16.9	0.99 (s)	16.8	0.99 (s)
26	21.0	0.95 (s)	19.0	0.90 (s)	19.0	0.91 (s)
27			182.5	–	182.7	
28	178.2	–	178.0	–	179.1	
29	33.0	0.93 (s)	33.6	0.89 (s)	33.7	0.89 (s)
30	25.0	0.96 (s)	24.0	0.94 (s)	24.1	0.95 (s)
	3-*O*-glc		3-*O*-ara		3-*O*-glc	
1′	106.7	4.33 (d, 8.0)	107.1	4.28 (d, 6.5)	105.4	4.44 (d, 7.0)
2′	75.7	3.20 (dd, 9.0, 8.0)	72.8	3.58 (m)	81.1	3.57 (m)
3′	78.3	3.35 (m)	74.3	3.52 (m)	78.5	3.56 (m)
4′	71.7	3.30 (m)	69.5	3.82 (br s)	71.6	3.30 (m)
5′	77.7	3.27 (m)	66.4	3.54 (dd, 12.0, 5.0)	77.3	3.27 (m)
				3.85 (dd, 12.0, 2.5)		
6′	62.8	3.69 (dd, 12.0, 6.0)			62.8	3.67 (dd, 12.0, 6.0)
		3.85 (dd, 12.0, 2.0)				3.87 (dd, 12.0, 2.0)
	28-*O*-glc		28-*O*-glc		2′-*O*-glc	
1″	95.7	5.40 (d, 8.0)	95.7	5.42 (d, 8.0)	104.5	4.68 (d, 8.0)
2″	74.0	3.33 (dd, 9.0, 8.0)	74.0	3.34 (dd, 9.0, 8.0)	76.3	3.23 (dd, 9.0, 8.0)
3″	78.4	3.41 (t, 9.0)	78.3	3.42 (t, 9.0)	77.7	3.36 (t, 9.0)
4″	71.2	3.38 (m)	71.2	3.38 (m)	71.9	3.23 (m)
5″	78.7	3.36 (m)	78.7	3.37 (m)	78.3	3.25 (m)
6″	62.5	3.70 (dd, `	62.5	3.69 (dd, 12.0, 6.0)	63.1	3.63 (dd, 12.0, 6.0)
		3.82 (br d, 12.0, 2.0)		3.85 (dd, 12.0, 2.0)		3.83 (dd, 12.0, 2.0)

Assignments were done by HSQC, HMBC, and COSY experiments. Glc, glucopyranosyl; Ara, arabinopyranosyl.

(δ_C 40.3)/C-5 (δ_C 57.1) suggested the position of an oxygenated group at C-3. The multiplicity of H-3 [(δ_H 3.19 (dd, J = 12.0, 4.5 Hz)] suggested the configuration of H-3 as α (*axial* orientation), which was further confirmed by the NOESY observation between H-3 (δ_H 3.19) and H-5 (δ_H 0.81)/H-23 (δ_H 1.06). The D-glucose moiety in **1** was identified by acid hydrolysis (identified as TMS derivatives by GC−MS). In addition, the large coupling constants of glc H-1′ and glc H-2′ as well as those of glc H-1″ and glc H-2″, J = 8.0 Hz, confirmed the presence of the sugar moiety as β-D-glucopyranosyl. The position of monosaccharide, β-D-glucopyranosyl at C-3 and C-28 was proven by HMBC correlations between glc H-1′ (δ_H 4.33) and C-3 (δ_C 90.9), and between glc H-1″ (δ_H 5.40) and C-28 (δ_C 178.2). Consequently, the structure of **1** was elucidated as 3-*O*-β-D-glucopyranosyl pyrocincholic acid 28-*O*-β-D-glucopyranosyl ester, an undescribed compound and named as mussapiloside A.

Compound **2** was isolated as a white amorphous powder. The HR ESI MS of **2** exhibited an ion peak at *m/z* 779.4224 $[M-H]^-$ corresponding to the molecular formula of $C_{41}H_{64}O_{14}$ (Calcd. for $[C_{41}H_{63}O_{14}]^-$, 779.4223). The ^{1}H NMR spectrum of **2** showed the proton signals of one olefinic proton at δ_H 5.68 (1H, br s), six tertiary methyl groups at δ_H 0.85, 0.89, 0.90, 0.94, 0.99, and 1.02 (each 3H, s) indicated the presence of an oleanane-triterpene-*type* aglycone; two anomeric protons at δ_H 4.28 (d, J = 6.5 Hz) and 5.42 (d, J = 8.0 Hz) suggested the presence of two sugar units. The ^{13}C NMR and DEPT spectra (Table 1) revealed the signals of 41 carbons, including 9 non-protonated carbons, 14 methines, 12 methylenes, and 6 methyl carbons. Analysis of ^{1}H and ^{13}C NMR data indicated that the structure of **2** was similar to that of 3-*O*-β-D-xylopyranosyl cincholic acid 28-*O*-β-D-glucopyranosyl ester (Kang et al., 2003) except for the difference of sugar moiety at C-3. The HMBC correlations (Fig. 2) between H-12 (δ_H 5.68) and C-9 (δ_C 48.4)/C-14 (δ_C 57.3)/C-18 (δ_C 44.6), and between H-18 (δ_H 2.92) and C-12 (δ_C 127.8)/C-13 (δ_C 137.5) confirmed the position of the double bond at C-12/C-13. The HMBC correlations between H-23 (δ_H 1.02)/H-24 (δ_H 0.85) and C-3 (δ_C 90.6) suggested the position of an oxygenated group at C-3. The α configuration of H-3 was proven by the NOESY correlation between H-3 (δ_H 3.11) and H-5 (δ_H 0.75)/H-23 (δ_H 1.02). The HMBC correlations from H-15 (δ_H 1.67 and 2.08) to C-27 (δ_C 182.5), and from H-16 (δ_H 1.74 and 2.09)/H-18 (δ_H 2.92) to C-

28 (δ_C 178.0) indicated the presence of two carboxyl groups at C-14 and C-17. The sugar moieties in compound **2** were determined to be D-glucose, and L-arabinose by acid hydrolysis (identified by TMS derivatives). Furthermore, the multiplicities of two anomeric protons in the ^{1}H NMR spectrum as ara H-1′ [δ_H 4.28 (d, J =6.5 Hz)] and glc H-1″ [5.42 (d, J =8.0 Hz)] confirmed the configurations of the sugar moieties as *α*-L-arabinopyranosyl and *β*-D-glucopyranosyl. The positions of *O*-*α*-L-arabinopyranosyl at C-3 and *O*-*β*-D-glucopyranosyl at C-28 of the aglycone was confirmed by HMBC correlations from ara H-1′ (δ_H 4.28) to C-3 (δ_C 90.6) and Glc H″-1 (δ_H 5.42) to C-28 (δ_C 178.0). Based on the above evidence, the structure of **2** was determined to be 3-*O*-*α*-L-arabinopyranosyl cincholic acid 28-*O*-*β*-D-glucopyranosyl ester, an undescribed compound and named as mussapiloside B.

The molecular formula of compound **3** was determined as $C_{42}H_{66}O_{15}$ by the HR ESI MS ion peak at *m/z* 809.4321 $[M-H]^-$ (Calcd. for $[C_{42}H_{65}O_{15}]^-$, 809.4323). Similar to compound **2**, the aglycone of **3** was found to be cincholic acid by the analysis of ^{1}H and ^{13}C NMR data. Acid hydrolysis of **3** gave D-glucose (identified as TMS derivatives by GCMS). In addition, the multiplicities of two anomeric protons [δ_H 4.44 (d, J =7.0 Hz) and 4.68 (d, J =8.0 Hz)] suggested the sugar moiety as *β*-D-glucopyranosyl. Moreover, the HMBC correlations from glc H-1″ (δ_H 4.68) to glc C-2′ (δ_C 81.1), from glc H-1′ (δ_H 4.44) to C-3 (δ_C 91.4) and the COSY correlations between glc H-1″ (δ_H 4.44) and glc H-2″ (δ_H 3.57) indicated the sugar linkages as *O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside which attached to C-3 of the aglycone. Thus, the structure of **3** was elucidated as cincholic acid 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, an undescribed compound and named as mussapiloside C.

The known compounds were characterized as cincholic acid-28-O-*β*-D-glucopyranosyl ester (**4**) (Zhang et al., 2005), mussaendoside S (**5**) (Weimin et al., 1995), quinovic acid 28-*O*-*β*-D-glucopyranosyl ester **(6)** (Kang et al., 2003), glycoside A **(7)** (P. Sahu et al., 2000), glycoside B **(8)** (P. Sahu et al., 1999), and quinovic acid 3-*O*-*β*-D-glucopyranosyl (1→2)-*β*-D-glucopyranoside **(9)** (Lamidi et al., 1995) by analysis of NMR and mass spectroscopic data and in comparison with the reported literature (Fig. 1).

The PTP1B inhibitory activity of all isolated compounds (**1–9**) was evaluated (Quang et al., 2014). First, all compounds were screened at the concentration of 80 μM (Fig. 3). Of the isolates, compounds **2**, **5**, **6**, and **8** showed PTP1B inhibitory percentages over than 50 %. Thus, these compounds were further evaluated at the lower concentrations of 40.0, 10.0, 4.0, and 1.0 μM to get IC_{50} values. RK-682 was used as positive control, displayed the IC_{50} value of 4.47 ± 0.03 μM. Compounds **2**, **6**, and **8** exhibited potent inhibitory effects against the PTP1B enzyme with IC_{50} values of 3.4 ± 0.2, 4.2 ± 0.2, and 6.4 ± 0.2 μM, respectively. Compound **5** showed moderate PTP1B inhibitory activity with an IC_{50} value of 63.0 ± 3.2 μM. The structure-activity relationship of 27-carboxylicoleanane and ursane triterpene glycosides, suggested glucopyranosyl at C-28 plays an important role of PTP1B inhibitory activity. In our experimental conditions, compound **2** showed the most potent activity with an IC_{50} value of 3.4 ± 0.2 μM. Recent reports have shown the significant PTP1B effect of oleanane-*type* triterpenes from *Camellia japonica* (Uddin et al., 2014), uranes from *Rubus chingii* (Zhang et al., 2019). However, this is the first time that the effects of cincholic acid derivatives on PTP1B have been reported. Overall, these data suggest that triterpene glycosides from *M. pilosissima* may be powerful candidates in the development of PTP1B inhibitory agents for increased insulin sensitivity.

3. Experimental

3.1. General

All NMR spectra were recorded on a Bruker 500 MHz AVANCE. HR ESI MS were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. Optical rotations were determined on a Jasco DIP-370 digital polarimeter. HPLC was carried out using an AGILENT 1200 HPLC system. Column chromatography (CC) was performed on RP-18 resins (30–50 μm, Fuji Silysia Chemical Ltd.) or silica-gel (Kieselgel 60, 230–400 mesh, Merck). For thin layer chromatography (TLC), pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 $F_{254}S$ (0.25 mm, Merck) plates were used.

3.2. Plant material

The aerial parts of *Mussaenda pilosissima* Valeton. were collected at Vinh Phuc province, Viet Nam in February 2017 and identified by Dr. Nguyen The Cuong, Institute of Ecology and Biological Resources, VAST. A voucher specimen (NCCT-P69) was deposited at the Institute of Marine Biochemistry, VAST.

3.3. Extraction and isolation

The dried powder of the aerial parts of *M. pilosissima* Valeton. (4.2 kg) was sonicated 3 times with hot methanol. The extract was removed under reduced pressure to yield 360 g of a solid extract. The extract was suspended in water and successively partitioned with *n*-hexane, dichloromethane, and ethyl acetate giving *n*-hexane (MPA1, 56.8 g), dichloromethane (MPA2, 97.8 g), and ethyl acetate extracts (MPA3, 23 g) and water layer (MPA4). The MPA3 extract was chromatographed on a silica gel column eluted with dichloromethane: methanol (100:0 → 0:1, v/v) to give 8 sub-fractions, MPA3A-MPA3H. MPA3F was chromatographed on a RP-18 column eluting with methanol/water (1/1.5, v/v) to give eight smaller fractions, MPA3F1-MPA3F8. Compound **6** (17.0

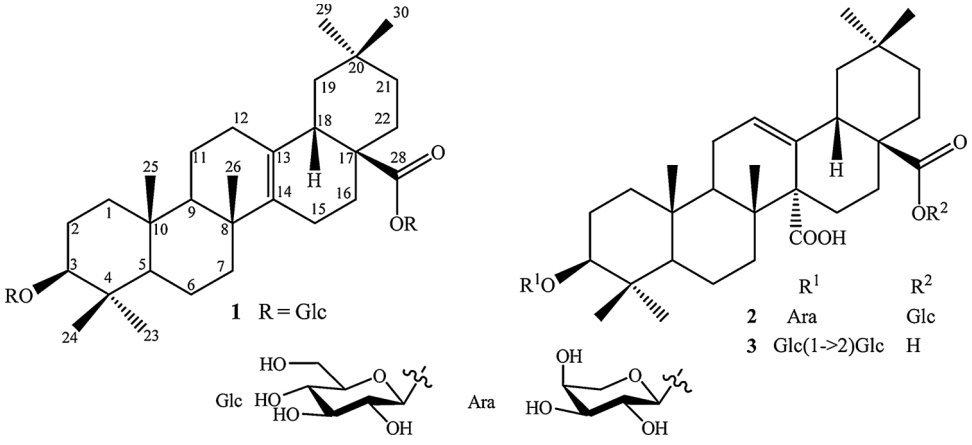

Fig. 1. Chemical structures of compounds **1—3**.

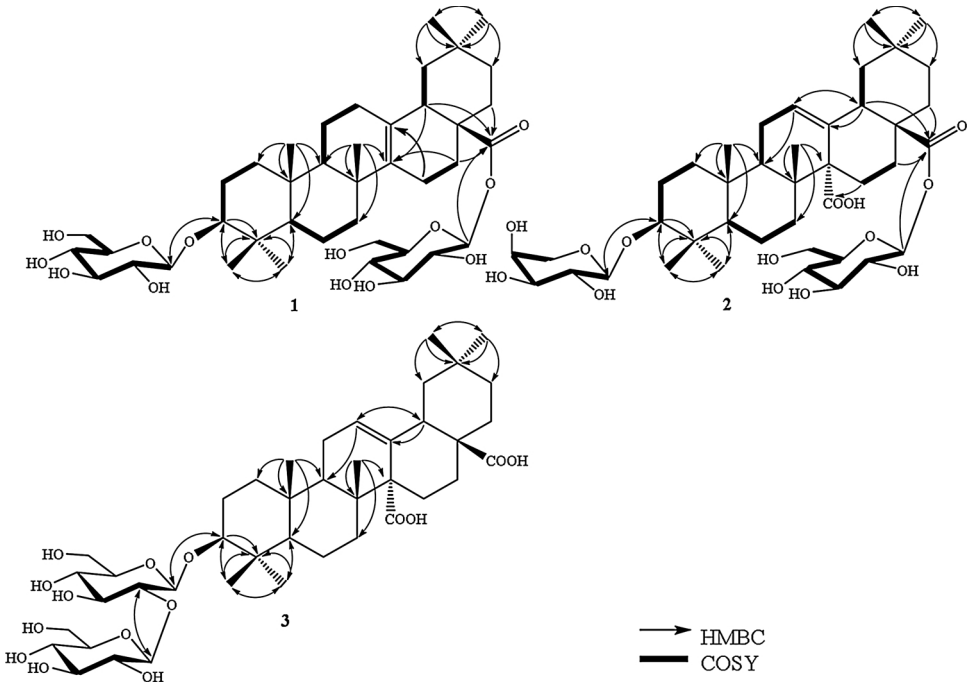

Fig. 2. The key HMBC and COSY correlations of compounds **1-3**.

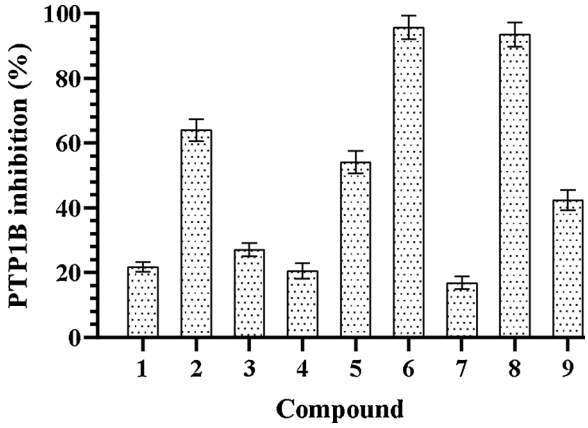

Fig. 3. PTP1B inhibition of compounds **1-9** at 80 μM.

mg) was yielded from MPA3F2 fraction using a silica gel column eluting with dichloromethane/acetone/water (1/2.5/0.15, v/v/v). The MPA3F4 fraction was chromatographed on a RP-18 column using acetone/water (1/1.3, v/v) as the eluent solvent to obtain compounds **4** (5.0 mg) and **5** (6.5 mg). MPA3F6 fraction was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/2.5/0.15, v/v/v) to give two fractions, MPA3F6A and MPA3F6B. Compounds **1** (2.2 mg) and **7** (30.0 mg) were obtained from MPA3F6A and MPA3F6B, respectively, using RP-18 column eluting with acetone/water (1/1.3, v/v). The MPA3F8 was separated on an RP-18 column eluting with acetone/water (1/1.3, v/v) to give two fractions, MPA3F8A and MPA3F8B. MPA3F8A was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/2.5/0.15, v/v/v) to yield compound **8** (10.0 mg). MPA3F8B was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/2.5/0.15, v/v/v) to yield compound **2** (4.3 mg). MPA3H was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/2.5/0.15, v/v/v) to give three smaller fractions, MPA3H1-MPA3H3. Compounds **3** (5.3 mg) and **9** (25.0 mg) were obtained from MPA3H2 and MPA3H3, respectively, using an RP-18 column with acetone/water (1/1.3, v/v) as an eluent.

3.3.1. Mussapiloside A (1)

White amorphous powder. $[\alpha]_D^{25}$ = -29.0° (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 789.4400 $[M + Na]^+$ (Calcd. for $[C_{41}H_{66}O_{13}Na]^+$, 789.4396). ^1H and ^{13}C NMR (CD_3OD): see Table 1.

3.3.2. Mussapiloside B (2)

White amorphous powder. $[\alpha]_D^{25}$ = +28.0° (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 779.4224 $[M-H]^-$ (Calcd. for $[C_{41}H_{63}O_{14}]^-$, 779.4223). ^1H and ^{13}C NMR (CD_3OD): see Table 1.

3.3.3. Mussapiloside C (3)

White amorphous powder. $[\alpha]_D^{25}$ = +37.0° (*c* 0.1 MeOH). HR-ESI-MS *m/z*: 809.4321 $[M-H]^-$ (Calcd. for $[C_{42}H_{65}O_{15}]^-$, 809.4323). ^1H and ^{13}C NMR (CD_3OD): see Table 1.

3.4. Acid hydrolysis

Each compound (**1—3**, 2.0 mg) was separately dissolved in 1.0 N HCl (dioxane—H_2O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. Acidic solution was neutralized with Ag_2CO_3 and the solvent thoroughly removed under a N_2 stream overnight. After extraction with $CHCl_3$, the aqueous layer was concentrated to dryness using N_2. The residue was dissolved in dry pyridine (0.1 mL), followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL). The reaction mixture was heated at 60 °C for 2 h. Trimethylsilylimidazole solution (0.1 mL) was then added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL each), and the organic layer was analyzed by gas chromatography (GC): column DB-5 (0.32 mm ID ×30 m length), detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2 mL/min). Under these conditions, the standard sugars gave peaks at t_R (min) 14.11 and 14.26 for D- and L-glucose, 9.82 and 15.24 for D- and L-arabinose, respectively. Peaks at t_R (min) 14.11 for D-glucose for **1** and **3**; 14.11 and 15.24 of D-glucose and L-arabinose, respectively for **2** were observed.

3.5. PTP1B assay procedures

PTP1B (human, recombinant) was purchased from ATGen Co., Ltd. (Gyeonggi-do, Korea). The enzyme activity was measured in a reaction mixture containing 1 mM *p*-nitrophenyl phosphate (*p*NPP) and PTP1B (0.05-0.1 μg) in 50 mM Bis-Tris, pH 6.0, 2 mM EDTA and 5 mM dithiothreitol (DTT). The reaction mixture was placed in a 37.5 °C incubator for 30 min, and the reaction was terminated by the addition of 10 N NaOH. The amount of produced *p*-nitrophenol was estimated by measuring the increase in absorbance at 405 nm. The non-enzymatic hydrolysis of 1 mM *p*NPP was corrected by measuring the increase in absorbance at 405 nm obtained in the absence of PTP1B enzyme.

Declaration of Competing Interest

The authors declared no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phytol.2020.01.023.

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