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Research article

New dammarane-type triterpenoids from the leaves of *Panax notoginseng* and their protein tyrosine phosphatase 1B inhibitory activity

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ABSTRACT

Background: *Panax notoginseng* has been used as a general tonic agent to invigorate human body for millennia in China and continued to be used until present.
Methods: Some chromatographic methods were performed to isolate pure triterpenoids, and their structures were determined by nuclear magnetic resonance (NMR) experiments. Anti-diabetes activities of isolated compounds were evaluated through their inhibitory activity of protein tyrosine phosphatase 1B (PTP1B) enzyme.
Results and Conclusion: Three new dammarane-type triterpenoids, notoginsenoside-LX (1), notoginsenoside-LY (2), and notoginsenoside-FZ (3) together with eighteen known compounds were isolated from the *Panax notoginseng* leaves. The structure-activity relationship of the compounds with dammarane-type triterpenoids and their PTP1B inhibitory activity were also reported. Results showed that compounds 2, 15, 20, and 21 can significantly inhibit the enzyme activity of PTP1B in a dose-dependent manner, with inhibitory concentration 50 (IC_{50}) values of 29.08 μM, 21.27 μM, 28.12 μM, and 26.59 μM, respectively. The results suggested that *Panax notoginseng* leaves might have potential as a new therapeutic agent for the treatment of diabetes.

1. Introduction

Panax notoginseng (Chinese ginseng) or "sanqi" is a functional food in China [1]. Based on the United States (US) Dietary Supplement Health and Education Act (DSHEA) of 1994, notoginseng tea or capsules are being sold as over-the-counter dietary supplements in the US health food market [2]. *P. notoginseng* has been used for many years because of its beneficial anti-inflammatory and blood circulation properties [3,4]. *P. notoginseng* also possesses several interesting pharmacological activities, such as anti-aging, anti-tumor, immunostimulating, and radioresistance activities [5–8]. *P. notoginseng* belongs to the same genus as Korean ginseng (*Panax ginseng* Meyer) and American ginseng (*Panax quinquefolius* L.), and their main components are similar.

Dammarane triterpene saponins are the major bioactive ingredients of *P. notoginseng.* To date, more than 60 dammarane-type triterpenoids have been obtained from *P. notoginseng* [9]. The main constituents of these dammarane-type triterpenoids are ginsenosides that contain an aglycone with a dammarane skeleton. In continuing the search for the minor bioactive constituents from *P. notoginseng*, the leaves of this plant were chemically investigated.

Protein tyrosine phosphatase 1B (PTP1B) is a major non-transmembrane phosphotyrosine phosphatase in classical insulin-targeted tissues. PTP1B overexpression can inhibit the increased expression of insulin in insulin-resistant states [10]. A previous report suggested that PTP1B can be used to treat obesity and type-2 diabetes mellitus [11].

In the present study, 21 dammarane-type triterpenes (3 new and 18 known ones) were isolated from the leaves of *P. notoginseng*. Besides the isolation and structure elucidation of the new compounds, the inhibitory effects of all compounds on PTP1B activity were evaluated. The current data suggest that some compounds can be developed as antidiabetic agents in future translational studies.

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2. Materials and methods

2.1. General experimental procedure

Column chromatography (cc): silica gel (SiO_2: 300–400 mesh, Qingdao Marine Chemical Group Co., Qingdao, China); macroporous resin D 101 (Tianjin Chemical Co., Tianjin, China); RP C18 silica gel (300–400 mesh, Agela Technologies Co., Tianjin, China); Sephadex LH-20 (Pharmacia Co., Peapack, USA). Optical rotations were measured on a Perkin-Elmer 241MC polarimeter (Perkin-Elmer Co., Waltham, USA) using methanol (Concord Technology Co., Tianjin, China) as the solvent. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker ARX-600 (Bruker Co., Karlsruhe, Germany) (^{1}H, 600 MHz; ^{13}C, 150 MHz) spectrometer in C_5D_5N with tetramethylsilane as internal standard. Infrared (IR) spectra on a Bruker Inter-Frame Space (IFS)-55 infrared spectrophotometer (Bruker Co., Karlsruhe, Germany) were recorded in Potassium bromide (KBr) disks. High-resolution electrospray ionization mass spectra (HRESIMS) were recorded on an Agilent 1100 LC-MSD (Mass Spectrometer Detector) TOF (time-of-flight) system (Agilent Technologies, Inc., Santa Clara, USA) [ionization mode, positive; nebulizing gas (N_2) pressure, 35 psi; drying gas (N_2) flow, 12 L/min; temp, 325°C; capillary voltage, 3,000 V; fragmentor voltage, 225 V]. Gas chromatography (GC) was performed on the Agilent technologies 6890N apparatus (Agilent Technologies, Inc., Santa Clara, USA) with an OV-17 column (30 m × 0.32 mm). The column temperature was programmed from 80°C to 280°C at a rate of 10°C/min. Nitrogen was used as the carrier gas at 1.5 mL/min. The injector and detector temperature was at 280°C and the injection volume was 1 μL with the split ratio being 10:1. All chemicals and solvents were analytical or high performance liquid chromatography (HPLC) grade and purchased from Lab Co. Ltd. (Lab Science and Trade Co., Ltd, Shenyang, China). Reversed-phase preparative HPLC was carried out on an octadecyl silica column [YMC-Pack Octadecylsilyl (ODS) A (YMC Co., Kyoto, Japan) (250 mm × 10 mm, 5 μm)] at 25°C at a flow rate of 3.0 mL/min with the eluent MeOH/H_2O 66:34 (HPLC system I), 70:30 (HPLC system II), 75:25 (HPLC system III), 80:20 (HPLC system IV), 82:18 (HPLC system V), or 9:1 (HPLC system VI). Ultraviolet (UV) spectrophotometric detection was carried out at 203 nm.

2.2. Plant material

P. notoginseng leaves were from the Yunnan province of the People's Republic of China and identified by Professor Jincai Lu of Shenyang Pharmaceutical University.

2.3. Extraction and isolation

Air-dried *P. notoginseng* leaves (35 kg) were extracted with 70% ethanol (2 × 350 L) and then evaporated under vacuum at 30°C. Ethanol extracts (1.6 kg) were applied on a macroporous resin column (10.5 kg) preconditioned with distilled water. Elution began with water to remove impurities and then with 70% ethanol (100 L) to isolate the saponin fraction, which was dried with a spray dryer to yield the total saponins (1 kg).

The total saponin (1 kg) was fractionated by silica gel column (300 mm × 1,600 mm, 30 kg) using a gradient of CH_2Cl_2/CH_3OH (7:1 350 L–4:1 350 L–3:1 350 L) and CH_3OH (300 L) to obtain 10 fractions, A–J. Fraction A (18 g) was subjected to chromatography on silica gel (70 mm × 800 mm, 400 g) and then eluted with ligarine and acetone in increasing polarity to yield 10 fractions, A1–A10, compounds 15 (20 mg), 16 (10 mg), and 17 (20 mg). Fractions B (20 g), C (25 g), D (23 g), and E (13 g) were subjected to chromatography on silica gel (70 mm × 800 mm, 500 g) and then eluted with methanol and dichloromethane in increasing polarity to yield seven fractions, B1–B7, eight fractions, C1–C8, 10 fractions, D1–D10, and five fractions, E1–E5. Fraction C4 (100 mg) was passed over a Sephadex LH-20 column (30 mm × 800 mm, 80 g) and then eluted with MeOH (500 mL) to obtain compound 20 (15 mg). Fraction E3 (1 g) was subjected to chromatography on ODS (30 mm × 150 mm, 50 g) and then eluted successively with solvents of decreasing polarity (MeOH/H_2O, 4:6 600 mL–6:4 600 mL–7:3 600 mL–9:1 600 mL–1:0 600 mL) to yield seven fractions, E3-1–E3-7. Compound 21 (8 mg) was obtained as granulated crystal from E3-6. Fractions F (18 g), G (15 g), H (20 g), and I (20 g) were subjected to chromatography on ODS (50 mm × 250 mm, 250 g) and then eluted successively with solvents of decreasing polarity (MeOH/H_2O, 3:7 3 L–5:5 3 L–7:3 3 L–9:1 3 L–1:0 3 L) to yield 11 fractions, F1–F11, eight fractions, G1–G8, seven fractions, H1–H7, and eight fractions, I1–I8. Compound 1 (10 mg) was obtained as granulated crystal from F-5. Isolation of the following 15 compounds was performed by preparative HPLC: compounds 2 (18 mg; t_R 75.0 min), 12 (30 mg; t_R 38.9 min), 13 (20 mg; t_R 46.8 min), and 14 (60 mg; t_R 53.5 min) were isolated from fraction D3 (500 mg) by HPLC system I; compound 18 (4 mg; t_R 41.8 min) was isolated from fraction A2 (60 mg) by HPLC system VI; compounds 3 (15 mg; t_R 70.3 min), 4 (15 mg; t_R 45.8 min), 5 (14 mg; t_R 58.9 min), and 6 (14 mg; t_R 65.9 min) were isolated from fraction I4 (200 mg) by HPLC system II; compounds 7 (40 mg; t_R 33.5 min) and 8 (60 mg; t_R 49.6 min) were isolated from fraction H5 (500 mg) by HPLC system III; compounds 9 (8 mg; t_R 17.5 min), 10 (70 mg; t_R 26.5 min), and 11 (65 mg; t_R 33.7 min) were isolated from fraction G6 (1 g) by HPLC system IV; compound 19 (6 mg; t_R 122.9 min) was isolated from fraction B2 (40 mg) by HPLC system V.

2.4. Notoginsenoside-LX (1)

(20*S*,23*R*)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 3-*O*-β-D-glucopyranoside-20-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-LX): white amorphous powder; [α]20 D = −20.8 (*c* = 0.30, MeOH); IR ν_{max} 3425, 2930, 1637, 1452, 1384, 1079, 620 cm^{-1}; Libermann-Burchard and Molish reactions were positive; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 937.5097 $[M+Na]^+$ (calculated for $C_{47}H_{78}O_{17}Na$, 937.5137).

2.5. Notoginsenoside-LY (2)

(20*S*,23*R*)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 20-*O*-α-L-arabinofuranosyl -(1→6)-β-D-glucopyranoside (notoginsenoside-LY): white granulated crystal; [α]20 D = −11.4 (*c* = 0.45, MeOH); IR ν_{max} 3419, 2942, 1637, 1452, 1384, 1043, 621 cm^{-1}; Libermann-Burchard and Molish reactions were positive; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 775.4577 (calculated for $C_{41}H_{68}O_{12}Na$, 775.4608).

2.6. Notoginsenoside-F_Z (3)

20(*S*)-protopanaxadiol 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl -(1→2)-β-D-glucopyranoside-20-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-F_Z): white granulated crystal; [α]20 D = −12.2 (*c* = 0.44, MeOH); IR ν_{max} 3426, 2924, 1650, 1458, 1384, 668 cm^{-1}; Libermann-Burchard and Molish reactions were positive; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 1233.6235 (calculated for $C_{58}H_{98}O_{26}Na$, 1233.6244).

2.7. Acid hydrolysis of 1, 2, and 3

Solutions of compounds 1, 2, and 3 (5 mg each) in 2M HCl/MeOH (4:1) (8 mL) were stirred at 90°C for 2 hours. After cooling, each

Table 1
^{1}H and ^{13}C NMR (600 MHz, 150 MHz in C_5D_5N) data for 1–3[1)]

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.3	0.95 m	39.5	0.96 m	39.1	0.73 m
		1.49 m		1.62 m		1.51 m
2	26.6	1.81 m	28.1	1.32 m	26.7	1.34 m
		2.22 m		1.84 m		1.80 m
3	88.6	3.36 dd (12.0, 4.8)	77.8	3.40 dd (10.8, 5.4)	88.7	3.28 dd (11.4, 4.2)
4	39.6		39.4		39.6	
5	56.2	0.68 m	56.2	0.78 m	56.2	0.66 m
6	18.3	1.36 m	18.6	1.42 m	18.3	1.35 m
		1.44 m		1.52 m		1.48 m
7	35.1	1.17 m	35.1	1.21 m	35.0	1.19 m
		1.37 m		1.41 m		1.45 m
8	39.7		39.7		39.9	
9	50.5	1.46 m	50.5	1.52 m	50.1	1.35 m
10	37.0		37.4		36.8	
11	29.9	1.32 m	30.0	1.38 m	30.6	1.54 m
		1.90 m		1.98 m		1.96 m
12	79.6	3.66 m	79.6	3.63 m	70.6	4.15 m
13	49.7	1.58 m	49.7	1.60 m	49.4	1.97 m
14	51.2		51.2		51.3	
15	32.4	1.13 m	32.4	1.13 m	30.7	0.97 m
		1.47 m		1.49 m		1.54 m
16	25.5	2.08 m	25.4	2.12 m	26.5	1.80 m
		2.34 m		2.33 m		2.18 m
17	46.4	3.19 ddd (12.9, 8. 7, 4.6)	46.4	3.16 ddd (12.9, 8.7, 4.4)	51.5	2.57 m
18	15.4	0.80 s	15.4	0.87 s	15.9	0.78 s
19	16.4	0.92 s	16.4	0.95 s	16.2	0.93 s
20	81.9		81.9		83.4	
21	24.8	1.48 s	24.5	1.48 s	22.2	1.63 s
22	51.9	2.24 dd (16.0, 9.6)	51.9	2.24 dd (16.0, 9.6)	36.1	1.82 m
		2.84 br d (16.0)		2.85 br d (16.0)		2.39 m
23	72.6	4.82 br dd (17.4, 7.8)	72.6	4.83 br dd (17.4, 7.8)	23.1	2.38 m
						2.57 m
24	129.1	5.52 d (7.8)	129.1	5.52 d (7.8)	125.8	5.31 m
25	131.2		131.1		131.0	
26	25.6	1.82 s	25.6	1.81 s	25.7	1.61 s
27	18.9	1.65 s	18.8	1.65 s	17.8	1.64 s
28	28.0	1.29 s	28.5	1.20 s	28.0	1.26 s
29	16.7	0.99 s	16.2	1.02 s	16.6	1.09 s
30	16.9	1.15 s	16.9	1.10 s	17.3	0.94 s
3-O-sugar						
Glc						
G1	106.0	4.94 d (7.8)			104.7	4.93 d (7.8)
G2	75.7	4.03 m			82.9	4.10 m
G3	78.7	4.22 m			77.7	4.13 m
G4	72.2	4.03 m			71.7	4.23 m
G5	78.3	4.03 m			77.7	3.85 m
G6	63.0	4.62 dd (12.0, 2.4)			62.9	4.35 m
		4.41 dd (11.4, 5.4)				4.57 br d (12.0)
Glc′						
G′1					103.1	5.52 d (7.8)
G′2					84.3	4.20 m
G′3					78.2	3.95 m
G′4					71.6	4.06 m
G′5					77.9	4.29 m
G′6					62.6	4.36 m
						4.47 m
Xyl						
X1					106.3	5.43 d (6.6)
X2					75.9	4.46 m
X3					79.2	4.18 m
X4					70.0	4.15 m
X5					67.3	3.68 m
						4.30 m
20-O-sugars						
Glc″						
G″1	99.2	5.11 d (7.8)	99.1	5.10 d (7.8)	98.0	5.14 d (7.8)
G″2	75.2	3.95 dd (7.9, 7.9)	75.1	3.95 dd (7.9, 7.9)	74.8	3.93 m
G″3	78.8	4.26 m	78.7	4.20 m	78.6	4.35 m
G″4	71.8	4.22 m	72.1	4.21 m	71.0	3.96 m
G″5	76.5	4.12 m	76.4	4.11 m	76.7	4.04 m
G″6	69.0	4.80 m	68.9	4.80 m	69.1	4.25 m
		4.10 m		4.10 m		4.70 m
Ara(f)						
A1	110.3	5.69 d (1.8)	110.2	5.68 d (1.8)		
A2	83.4	4.95 m	83.3	4.94 m		
A3	78.7	4.85 m	78.8	4.86 m		
A4	85.8	4.75 m	85.8	4.76 m		
A5	62.5	4.34 dd (12.0, 2.4)	62.4	4.34 dd (12.0, 2.4)		
		4.23 m		4.23 m		
Ara(p)						
A1					104.6	5.00 d (6.0)
A2					72.1	4.46 m
A3					74.1	4.22 m
A4					68.5	4.37 m
A5					65.6	3.79 m
						4.30 m

HMBC, heteronuclear multiple bond correlation; HSQC, heteronuclear single quantum correlation; NMR, nuclear magnetic resonance. m, multiplet; dd, double doublet; ddd, double double doublet; s, singlet; br d, broad doublet; br dd, broad double doublet.
[1)] The assignments were based on HSQC and HMBC experiments.

reaction mixture was diluted to 30 mL with water and then extracted with CH_2Cl_2 (30 mL × 3). The aqueous layer was neutralized with 1M KOH. After concentration, the residue was examined by thin layer chromatography (TLC; *n*-BuOH/H_2O/HOAc 3:2:1) and compared with authentic samples [12]. The retention factor (Rf) values of glucose, arabinose, and xylose were 0.38, 0.43, and 0.51, respectively.

2.8. Determination of sugar components

Monosaccharide subunits were obtained as described above. The residue was dissolved in pyridine (0.5 mL) and then added to trimethylchlorosilane (0.2 mL) and hexamethyldisilazane (0.5 mL). The mixture was stirred at 20°C for 15 minutes. The mixture was then extracted with CH_2Cl_2 (2 mL) following the addition of H_2O (2 mL). The CH_2Cl_2 layer was examined by GC [12].

2.9. Inhibitory activity against PTP1B assay

The assay buffer (pH 7.4), consisting of 1 mM ethylene diamine tetra acetic acid (EDTA), 50 mM 3,3-dimethyl glutarate, 5 mM glutathione, and 0.5% fetal calf serum (FCS) (not heat inactivated) was adjusted to an ionic strength of 0.15M by the addition of NaCl [13]. Compounds (final concentration ranging from 0 μM to 200 μM) were added to the assay buffers containing PTP1B. The reaction mixtures were allowed to stand at 37°C for 5 minutes following the addition of the compounds. The reaction was started by the addition of *p*-nitrophenyl phosphate and incubated for another 30 min, and followed by the addition of 5 μL 0.5M NaOH solution to terminate the reaction. The absorbance at 405 nm was recorded using a microplate absorbance reader to test the enzyme activity.

3. Results and discussion

3.1. Structure elucidation of compounds 1–3

Compound 1 was obtained as white amorphous powder. The molecular formula of 1 was deduced to be $C_{47}H_{78}O_{17}$ by positive

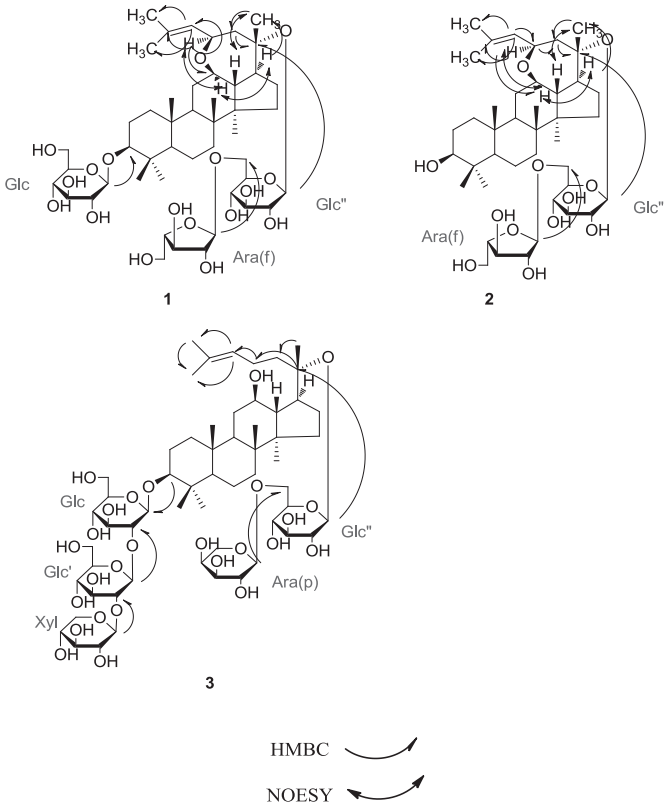

Fig. 1. The important HMBC and NOESY correlations of compounds **1**–**3**. HMBC, heteronuclear multiple bond correlation; NOESY, nuclear Overhauser effect spectroscopy.

mass spectrometry (HRESIMS) data at *m/z* 937.5097 $[M+Na]^+$ (calculated for $C_{47}H_{78}O_{17}Na$, 937.5137). The IR spectrum showed absorption bands for hydroxyl (3425 cm^{-1}), olefinic carbons (1637 cm^{-1}), and ether moiety (1079 cm^{-1}). The ^{13}C NMR (Table 1) showed 47 carbon signals. The distortionless enhancement by polarization transfer (DEPT) spectrum exhibited eight methyls, 11 methylenes, 22 methines, and six quaternary carbons. Eight signals of the aglycone moiety were assigned to methyl carbons at [C-18 (δ_c 15.4), C-19 (δ_c 16.4), C-21 (δ_c 24.8), C-26 (δ_c 25.6), C-27 (δ_c 18.9), C-28 (δ_c 28.0), C-29 (δ_c 16.7), C-30 (δ_c 16.9)]. Four oxygen substituted carbons were observed at C-23 (δ_c 72.6), C-12 (δ_c 79.6), C-20 (δ_c 81.9), and C-3 (δ_c 88.6); a pair of olefinic carbons were detected at C-24 (δ_c 129.1) and C-25 (δ_c 131.2). This data, in combination with the proton NMR signals, eight methyl groups at [δ 0.80 (3H, s), 0.92 (3H, s), 0.99 (3H, s), 1.15 (3H, s), 1.29 (3H, s), 1.48 (3H, s), 1.65 (3H, s), 1.82 (3H, s)], three oxygen substituted protons at H-3 (δ_H 3.36 1H, dd, J = 12, 4.8 Hz), H-12 (δ_H 3.66, 1H, m), H-23 (δ_H 4.82, 1H, br dd, J = 17.4, 7.8 Hz), and one olefin proton at H-24 (δ_H 5.52, 1H, d, J = 7.8 Hz), suggested that 1 was a dammarane-type triterpene. The ^1H and ^{13}C signals were fully assigned according to heteronuclear single quantum correlation (HSQC) spectra (Table 1). The NMR data for the side-chain moiety of 1 were almost indistinguishable from those of ginsenosides Rh18 [14]. Otherwise, its NMR spectra were closely similar to those of notoginsenoside Fe [15], except the presence of the ether linkage between C-12 and C-23. In the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 1), the presence of long-range correlations between the proton signal at H-23 (δ_H 4.82, 1H, br dd, J = 17.4, 7.8 Hz) and carbon signals at C-12 (δ_c 79.6), C-24 (δ_c 129.1), and C-25 (δ_c 131.2) indicated the presence of the ether linkage between C-12 and C-23. Moreover, key correlation peaks were observed between the proton signal at H-1_{Glc} (δ_H 4.94, 1H, d, J = 7.8 Hz) and the carbon resonance signal at C-3 (δ_c 88.6), H-$1_{Glc''}$ (δ_H 5.11, 1H, d, J = 7.8 Hz) and C-20 (δ_c 81.9), H-1_{Ara} (δ_H 5.69, 1H, d, J = 1.8 Hz), and C-$6_{Glc''}$ (δ_c 69.0), which indicated that the C-1_{Glc}, C-$1_{Glc''}$, and C-1_{Ara} were linked to C-3, C-20 of the aglycone, and C-$6_{Glc''}$, respectively. Furthermore, the stereochemistry of 1 was confirmed by the nuclear Overhauser effect spectroscopy (NOESY) spectrum (Fig. 1), and the correlation between the proton signals at H-23 (δ_H 4.82, 1H, br dd, J = 17.4, 7.8 Hz) and H-12 (δ_H 3.66, 1H, m), H-12 (δ_H 3.66, 1H, m) and H-17 (δ_H 3.19, ddd, J = 12.9, 8.7, 4.6 Hz,), H-13 (δ_H 1.58, 1H, m) and H-21 (δ_H 1.48, 3H, s) indicated the structure of 1 as in Fig. 2. The following abbreviations are used: m = multiplet, dd = double doublet, ddd = double double doublet, s = singlet, br d = broad doublet, br dd = broad double doublet.

The sugar moieties of 1 were determined to be D-glucose (Glc) and L-arabinose (Ara) [t_R (min): 26.60 and 6.24] by GC. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition. Retention times were consistent. Three anomeric protons were observed at δ 4.94 (1H, d, J = 7.8 Hz), 5.11 (1H, d, J = 7.8 Hz), and 5.69 (1H, d, J = 1.8 Hz). On the basis of HSQC, HMBC, NOESY correlations, and chemical reactions, two β-D-glucopyranose (δ 4.94 and 5.11) (Glc and Glc″) and one α-L-arabinofuranosyl (δ 5.69; Ara) were identified.

On the basis of the above analyses, compound 1 could be deduced to be (20*S*,23*R*)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 3-*O*-β-D-glucopyranoside-20-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-LX).

Compound 2 was obtained as white granulated crystal and assigned the molecular formula $C_{41}H_{68}O_{12}$, as determined from its $[M+Na]^+$ ion at *m/z* 775.4577 (calculated for $C_{41}H_{68}O_{12}Na$, 775.4608) in the HRESIMS. Its IR spectrum also exhibited strong absorption bands at 3419 cm^{-1}, 1637 cm^{-1}, and 1043 cm^{-1}. The NMR data (Table 1) of 2 were closely similar to those of 1. Compared with 1, the shift to upfield of C-3 [−11 parts per million (ppm)] and shift to downfield of C-2 (+2 ppm) indicated that C-3 of 2 was linked to hydroxyl. Comparison of the NMR data (Table 1) of 1 with those of 2 suggested that the only difference was the presence of one additional glucopyranosyl group in 1, and this was supported by the presence of more units of 162 in the molecular formula of 1 than that of 2. Besides, the NMR spectra of 2 were closely similar to those of ginsenoside Mc [16], except the presence of the ether linkage between C-12 and C-23. All the proton and carbon signals for compound 2 were assigned using 2D NMR spectra (Fig. 1). Therefore, compound 2 could be established to be (20*S*,23*R*)-3β-hydroxy-12β,23-epoxy-dammar-24-ene 20-*O*-α-L-arabinofuranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-LY).

Compound 3 was obtained as white amorphous powder. It was determined to have a molecular formula of $C_{58}H_{98}O_{26}$ based on a $[M+Na]^+$ ion at *m/z* 1233.6235 (calculated for $C_{58}H_{98}O_{26}Na$, 1233.6244) in the HRESIMS. The IR spectrum showed absorption bands for hydroxyl (3426 cm^{-1}) and olefinic carbons (1650 cm^{-1}). The ^1H NMR spectrum (Table 1) showed eight methyl groups [δ 0.78 (3H, s), 0.93 (3H, s), 1.63 (3H, s), 1.61 (3H, s), 1.64 (3H, s), 1.26 (3H, s), 1.09 (3H, s), 0.94 (3H, s)], one olefinic proton [δ 5.31 (1H, m)], two oxygen substituted protons [δ 3.28 (1H, dd, J = 11.4, 4.2 Hz), 3.62 (1H, m)], and five anomeric protons [δ 4.93 (1H, d, J = 7.8 Hz), 5.52 (1H, d, J = 7.8 Hz), 5.43 (1H, d, J = 6.6 Hz), 5.14 (1H, d, J = 7.8 Hz), 5.00 (1H, d, J = 6.0 Hz)]. The ^{13}C NMR (Table 1) showed 58 carbon signals including a pair of olefinic carbons at C-24 (δ_c 125.8) and C-25 (δ_c 131.0). The chemical structure of 3 was further elucidated by a HMBC (Fig. 1) experiment, in which the following correlations were observed from H-3 (δ_H 3.28, 1H, dd, J = 11.4, 4.2 Hz) to C-1_{Glc} (δ_c 104.7); H-$1_{Glc'}$ (δ_H 5.52, 1H, d, J = 7.8 Hz) to C-2_{Glc} (δ_c 82.9); H-1_{xyl} (δ_H 5.43, 1H, d, J = 6.6 Hz) to C-$2_{Glc'}$ (δ_c 84.3); H-$1_{Glc''}$ (δ_H 5.14, 1H, d, J = 7.8 Hz) to C-20 (δ_c 83.4); and H-1_{Ara} (δ_H 5.00, 1H, d, J = 6.0 Hz) to C-$6_{Glc''}$ (δ_c 69.1). The NMR data for the tetracyclic part

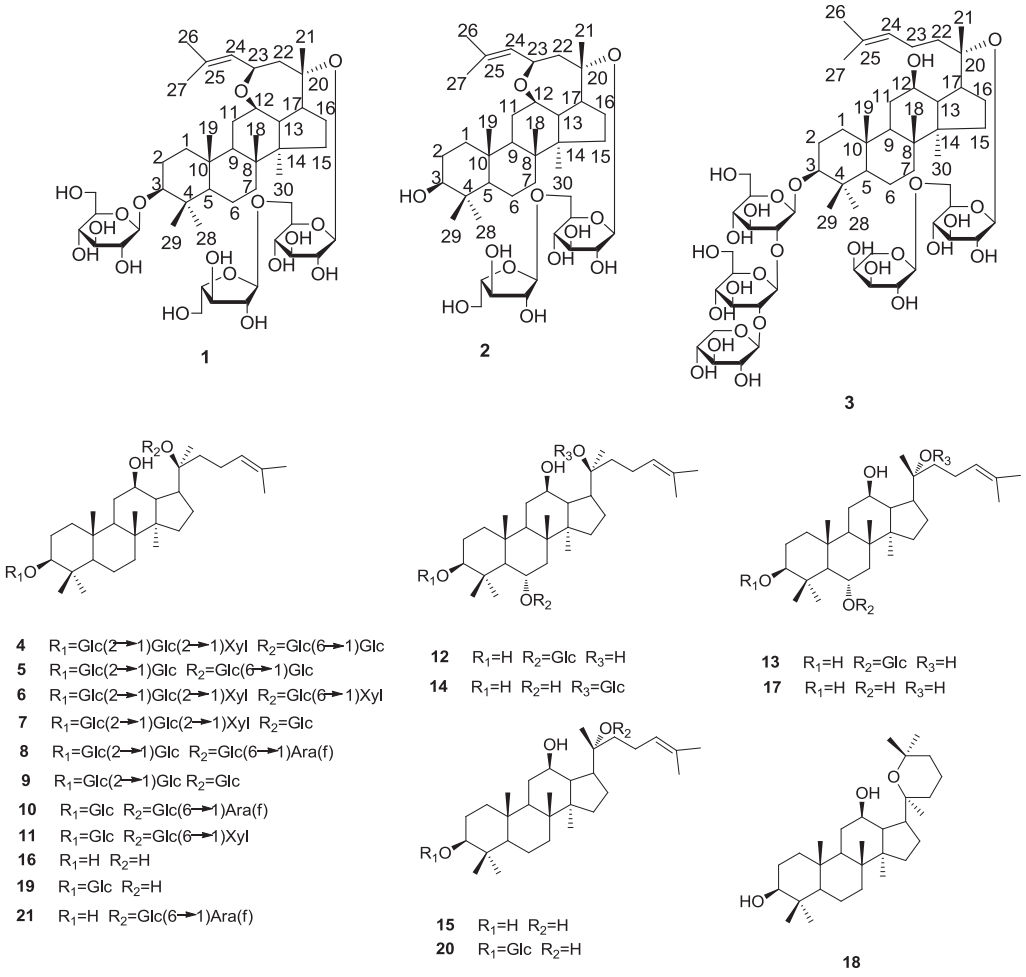

Fig. 2. Structures of compounds **1**–**21**. Ara(f), α-L-arabinofuranosyl; Ara(p), α-L-arabinopyranosyl; Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl.

of the aglycone and the glycosyl moieties linked to C-3 of aglycone were similar to those of notoginsenoside Fa [15], and glycosyl moieties linked to C-20 of aglycone were almost indistinguishable from those of ginsenoside Rb2 [17].

The sugar moieties of 3 were determined to be D-glucose (Glc), D-xylose (Xyl), and L-arabinose (Ara) [t_R (min): 26.60, 8.86, and 6.23] by GC. The standard monosaccharides were subjected to the same reaction and GC analysis under the same condition. Retention times were consistent. Five anomeric protons were observed at δ 4.93 (1H, d, J = 7.8 Hz), 5.52 (1H, d, J = 7.8 Hz), 5.43 (1H, d, J = 6.6 Hz), 5.14 (1H, d, J = 7.8 Hz), and 5.00 (1H, d, J = 6.0 Hz). On the basis of HSQC, HMBC, NOESY correlations and chemical reactions, three β-D-glucopyranose (δ 4.93, 5.52, and 5.14) (Glc, Glc′, and Glc″), one α-L-arabinopyranosyl (δ 5.00) (Ara), and β-D-xylopyranosy (δ 5.43) (Xyl) were identified. The above evidence suggested that 3 possesses the structure of 20(*S*)-protopanaxadiol 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl -(1→2)-β-D-glucopyranoside-20-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside-F_Z).

3.2. Structure identification of known compounds

The known compounds were identified as notoginsenoside-Fa (4) [15], ginsenoside-Rb1 (5) [15], notoginsenoside-Fc (6) [15], vina-ginsenoside-R7 (7) [18], ginsenoside-Rc (8) [17], ginsenoside-Rd (9) [18], notoginsenoside-Fe (10) [15], gypenoside-IX (11) [15], 20(*S*)-ginsenoside-Rh1 (12) [19], 20(*R*)-ginsenoside-Rh1 (13) [19], ginsenoside-F1 (14) [20], 20(*R*)-protopanaxadiol (15) [21], 20(*S*)-protopanaxadiol (16) [21], protopanaxatriol (17) [22], panaxadiol (18) [21], 20(*S*)-ginsenoside-Rh2 (19) [23], 20(*R*)-ginsenoside-Rh2 (20) [23], and 20(*S*)-ginsenoside-Mc (21) [16] by NMR and mass spectrometric analyses and by comparison of obtained values with literature values of the corresponding compounds.

Table 2
Inhibitory effects of compounds 1–21 on PTP1B activity[1)]

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
1	77.24	12	>100
2	29.08	13	>100
3	>100	14	>100
4	>100	15	21.27
5	>100	16	57.14
6	>100	17	>100
7	>100	18	>100
8	>100	19	>100
9	>100	20	28.12
10	>100	21	26.59
11	>100	Na_3VO_4	21.04

IC_{50}, inhibitory concentration 50; PTP1B, protein tyrosine phosphatase 1B.
[1)] Na_3VO_4 was used as a positive control. The detailed methods for inhibitory activity against PTP1B are described in *Materials and methods*. Values are means of triplicate experiments.

3.3. PTP1B inhibitory activity

The current data (Table 2) suggest that protopanaxadiol (PPD)-type aglycones are more effective than dammarane triperpenoids having more than three sugars and that the presence of sugar moieties reduces the PTP1B inhibitory activity of the compounds. Compound 15 [20(*R*)-PPD] was more effective than compound 16 [20(*S*)-PPD] with inhibitory concentration 50 (IC_{50}) values of 21.27 μM and 57.14 μM, respectively, despite the fact that they differ from each other only by the absolute configuration of chiral carbon of C-20. Compound 20 [20(*R*)-ginsenoside-Rh2] was also more effective than compound 19 [20(*S*)-ginsenoside-Rh2]. These results suggest that 20(*R*)-PPD-type triterpenoids are more effective than 20(*S*)-PPD-type triterpenoids. The IC_{50} values of 12, 13, 14, and 17 showed that protopanaxatriol (PPT)-type triterpenoids exhibit no PTP1B inhibitory activity at all.

Ginsenosides possess antidiabetic activity, but their mechanisms are different. For example, ginsenoside Rb1 promotes adipogenesis through the regulation of peroxisome proliferator-activated receptor (PPAR)-γ and microRNA-27b, providing a good illustration to explain the antidiabetic effect of the ginsenoside [24]. The total saponins and ginsenoside Rb1 of ginseng stimulate the secretion of glucagon-like peptide-1 (GLP1) *in vivo* and *in vitro*, demonstrating an antidiabetic effect [25]. Ginsenoside Re reduces insulin resistance by activating the PPAR-γ pathway and inhibiting tumor necrosis factor (TNF)-α production [26]. However, the current study shows that the antidiabetic effects of *P. notoginseng* may be a result of the inhibitory activity of some ginsenosides against PTP1B.

In the present study, we isolated three new dammarane-type triterpenoids, elucidated as notoginsenoside-LX (1), notoginsenoside-LY (2), and notoginsenoside-F_Z (3), along with 18 known compounds from *P. notoginseng* leaves and all compounds were firstly evaluated for the inhibitory activity against PTP1B. Among the three new dammarane-type triperpenoids, compound 2 showed similar strong activity comparable to the known compounds 20 and 21. These results suggest that *P. notoginseng* leaves can be used in folk medicine for their antidiabetic property and that dammarane-type triterpenes enable this plant to be utilized for the treatment of diabetes.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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