J Ginseng Res 44 (2020) 673–679

Contents lists available at ScienceDirect

Journal of Ginseng Research

journal homepage: http://www.ginsengres.org

Research Article

New dammarane-type triterpenoid saponins from *Panax notoginseng* saponins

Qian Li [1,2,3], Mingrui Yuan [1,2,3], Xiaohui Li [1,2,3], Jinyu Li [1,2,3], Ming Xu [1,2,3], Di Wei [1,2,3], Desong Wu [1,2,3], Jinfu Wan [1,2,3], Shuangxi Mei [1,2,3], Tao Cui [1,2,3], Jingkun Wang [1,2,3,*], Zhaoyun Zhu [1,2,3,**]

[1] *Yunnan Institute of Materia Medica, Kunming, China*
[2] *Innovation and R&D Center, Yunnan Bai Yao Group, Kunming, China*
[3] *Yunnan Province Company Key Laboratory for TCM and Ethnic Drug of New Drug Creation, Kunming, China*

ARTICLE INFO

Article history:
Received 27 January 2018
Received in Revised form
4 December 2018
Accepted 6 December 2018
Available online 13 December 2018

Keywords:
Cytotoxicity
Fingerprint
New dammarane-type triterpenoid saponins
Panax Notoginseng saponins

ABSTRACT

Background: *Panax notoginseng* saponin (PNS) is the extraction from the roots and rhizomes of *Panax notoginseng* (Burk.) F. H. Chen. PNS is the main bioactive component of Xuesaitong, Xueshuantong, and other Chinese patent medicines, which are all bestselling prescriptions in China to treat cardiocerebrovascular diseases. Notoginsenoside R_1 and ginsenoside Rg_1, Rd, Re, and Rb_1 are the principal effective constituents of PNS, but a systematic research on the rare saponin compositions has not been conducted.
Objective: The objective of this study was to conduct a systematic chemical study on PNS and establish the HPLC fingerprint of PNS to provide scientific evidence in quality control. In addition, the cytotoxicity of the new compounds was tested.
Methods: Pure saponins from PNS were isolated by means of many chromatographic methods, and their structures were determined by extensive analyses of NMR and HR-ESI-MS studies. The fingerprint was established by HPLC-UV method. The cytotoxicity of the compounds was tested by 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide assay.
Results and Conclusion: Three new triterpenoid saponins (**1**–**3**) together with 25 known rare saponins (**4**–**28**) were isolated from PNS, except for the five main compounds (notoginsenoside R_1 and ginsenoside Rg_1, Rd, Re, and Rb_1). In addition, the HPLC fingerprint of PNS was established, and the peaks of the isolated compounds were marked. The study of chemical constituents and fingerprint was useful for the quality control of PNS. The study on antitumor activities showed that new Compound **2** exhibited significant inhibitory activity against the tested cell lines.

1. Introduction

Panax notoginseng (Burk.) F.H. Chen (*P. notoginseng*), commonly called “Sanqi” or “Tianqi” in Chinese is a species of the genus *Panax*, family Araliaceae [1]. *P. notoginseng* is one of the most widely used Chinese herbal drugs for the treatment of cardiovascular diseases, such as occlusive vasculitis, coronary diseases, atherosclerosis, and cerebral infarction in China, Korea, and Russia for a long time [2]. There are about 200 chemical compositions that have been isolated from *P. notoginseng*, including saponins, flavonoids, and cyclopeptides [3]. Dammarane triterpenoidal saponins are the major bioactive ingredients of *P. notoginseng* [4].

Panax notoginseng saponin (PNS) are developed into the traditional Chinese medicine agents with the trademarks of Xuesaitong injection, Xueshuantong injection, and Xuesaitong tablet, which are all bestselling prescriptions used for treatment of cardiovascular and cerebrovascular diseases in China [5,6]. Notoginsenoside R_1 and ginsenoside Rg_1, Rd, Re, and Rb_1 are regarded as the main active constituents [7,8]. But, there is a lack of references about the extraction and structure identification of the rare ginsenosides in PNS. As the study on microconstituents is important to control the quality and safety of clinical medication, we investigated the rare content in the PNS; three new and 25 known dammarane-type triterpenoids were isolated and identified (Fig. 1). We also

* Corresponding author. Yunnan Institute of Materia Medica, Kunming 650111, China.
** Corresponding author. Yunnan Institute of Materia Medica, Kunming 650111, China.
E-mail addresses: wjkyimm1@163.com (J. Wang), zzy1370@163.com (Z. Zhu).

https://doi.org/10.1016/j.jgr.2018.12.001
p1226-8453 e2093-4947/$ – see front matter

Table 1
^{1}H [δ in ppm, multiplicity (J in Hz)] and ^{13}C NMR (δ in ppm) spectroscopic data of Compounds **1**–**3**.[1)]

Position	1		2		3	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	39.9	1.70 (1H, m)	39.7	1.65 (1H, m)	40.0	1.71 (1H, m)
		1.05 (1H, m)		1.02 (1H, m)		1.05 (1H, m)
2	28.4	1.94 (1H, m)	28.2	1.91 (1H, m)	28.4	1.95 (1H, m)
		1.87 (1H, m)		1.84 (1H, m)		1.88 (1H, m)
3	79.0	3.55 (1H, m)	78.7	3.52 (1H, m)	79.0	3.55 (1H, m)
4	40.9		40.7		40.9	
5	61.8	1.46 (1H, m)	61.7	1.43 (1H, m)	61.9	1.46 (1H, m)
6	80.5	4.46 (1H, m)	80.2	4.45 (1H, m)	80.6	4.45 (1H, m)
7	45.8	2.59 (1H, m)	45.6	2.57 (1H, m)	45.8	2.55 (1H, m)
		1.98 (1H, m)		1.94 (1H, m)		1.96 (1H, m)
8	41.7		41.5		41.7	
9	51.1	1.59 (1H, m)	50.9	1.59 (1H, m)	51.1	1.58 (1H, m)
10	40.1		40.0		40.2	
11	32.7	1.50 (2H, m)	32.8	1.88 (1H, m)	31.5	1.98 (1H, m)
				1.52 (1H, m)		1.57 (1H, m)
12	80.2	4.29 (1H, m)	80.0	4.25 (1H, m)	80.2	4.28 (1H, m)
13	59.1	2.13 (1H, m)	55.4	2.29 (1H, m)	52.8	2.16 (1H, m)
14	52.2		51.7		51.6	
15	33.4	1.92 (1H, m)	32.9	1.52 (1H, m)	33.0	1.76 (1H, m)
		1.26 (1H, m)		1.22 (1H, m)		1.16 (1H, m)
16	34.7	2.16 (1H, m)	35.9	2.21 (1H, m)	35.1	2.18 (1H, m)
		1.60 (1H, m)		1.43 (1H, m)		2.05 (1H, m)
17	40.2	3.05 (1H, m)	38.4	2.91 (1H, m)	48.7	2.29 (1H, m)
18	17.8	1.25 (3H, s)	17.6	1.28 (3H, s)	17.8	1.24 (3H, s)
19	18.2	1.04 (3H, s)	18.1	1.03 (3H, s)	18.2	1.05 (3H, s)
20	160.2		156.3		156.5	
21	111.2	5.31 (2H, d, J = 12.5)	113.9	5.29 (2H, d, J = 12.5)	108.4	5.15 (1H, s)
						4.97 (1H, s)
22	77.0	4.52 (1H, m)	91.3	4.71 (1H, t, J = 7.1)	33.1	2.77 (1H, m)
						2.51 (1H, m)
23	36.3	2.73 (1H, m)	31.3	2.60 (1H, m)	33.2	2.06 (1H, m)
		2.54 (1H, m)		2.32 (1H, m)		2.46 (1H, m)
24	122.4	5.42 (1H, t, J = 6.9)	121.1	5.34 (1H, t, J = 6.9)	75.5	4.46 (1H, m)
25	132.8		133.4		150.0	
26	26.4	1.71 (3H, s)	26.1	1.65 (3H, s)	110.5	5.29 (1H, s)
						4.96 (1H, s)
27	18.6	1.65 (3H, s)	18.3	1.59 (3H, s)	18.7	1.91 (3H, s)
28	32.2	2.10 (3H, s)	32.0	2.08 (3H, s)	32.2	2.10 (3H, s)
29	16.8	1.63 (3H, s)	16.7	1.60 (3H, s)	16.8	1.63 (3H, s)
30	17.1	0.88 (3H, s)	16.9	0.82 (3H, s)	17.2	0.82 (3H, s)
6-O-sugar						
1	106.5	5.07 (1H, d, J = 8.0)	106.3	5.04 (1H, d, J = 7.8)	106.4	5.05 (1H, d, J = 7.8)
2	73.0	3.99 (1H, m)	73.2	3.93 (1H, m)	73.0	3.91 (1H, m)
3	78.7	3.99 (1H, m)	78.5	3.96 (1H, m)	78.6	3.98 (1H, m)
4	72.3	4.26 (1H, m)	72.1	4.22 (1H, m)	72.3	4.27 (1H, m)
5	75.9	4.13 (1H, m)	75.7	4.10 (1H, m)	75.9	4.13 (1H, m)
6	63.5	4.57 (1H, m)	63.4	4.54 (1H, m)	63.5	4.54 (1H, m)
		4.41 (1H, m)		4.36 (1H, m)		4.44 (1H, m)

NMR, nuclear magnetic resonance; s, singlet; d,doublet; t, triplet; m, multiplet
[1)] Measured in pyridine-d_5, 500 MHz for ^{1}H, 125 MHz for ^{13}C, The assignment was based on DEPT, correalation spectroscopy (COSY), HSQC, and HMBC experiments.

established the HPLC fingerprint and marked the compounds isolated. In addition, we tested the cytotoxic activity of the three new compounds against three human cancer cell lines.

2. Materials and methods

2.1. General experimental procedures

UV spectra: Shimadzu UV-2401A spectrophotometer (Shimadzu Instruments Co., Ltd, Tokyo, Japan). IR spectra: Nicolet FT-IR-360 spectrometer (Thermo Nicolet, Inc., Waltham, MA, USA). NMR spectra: Bruker ARX-400 spectrometers (Bruker Ltd, Karlsruhe, Germany). High resolution electrospray ionization mass spectrum (HR-ESI-MS) were taken using, Agilent G6230 instruments (Agilent Technologies, Santa Clara, CA, USA). Preparative HPLC was performed on an Agilent 1100 apparatus equipped with a Rheodyne injector and with UV detectors using a Thermo C_{18} column (10 × 250 mm, 5 μm). Column chromatography was performed using silica (200–300 mesh and 100–200 mesh; Qingdao Marine Chemical, Inc., Qingdao, People's Republic of China) and octadecylsilyl (ODS) (50 μm; YMC Co., Kyoto, Japan). The reagents were of HPLC grade or analytical grade (Sinopharm Chemical Reagent Co., Ltd, Beijing, China).

2.2. Plant material

The PNS was provided by Yunnan Baiyao Group Co., Ltd.

2.3. Extraction and isolation

The PNS (2 kg) was separated by silica gel column using a gradient of CH_2Cl_2/CH_3OH (100:1→50:1→25:1→10:1→5:1→2:1→1:1, v/v) to obtain eight fractions (Fr.1–Fr.8). Fr.1 (10 g) was chromatographed subsequently over silica gel chromatography with $CHCl_3$-MeOH (30:1→20:1→10:1, v/v) to get five major

fractions (Fr.1-1—Fr.1-5) based on thin-layer chromatography (TLC) analysis. Fr.1-2 was purified by ODS eluted with MeOH-H_2O (40:60→55:45→70:30, v/v) to provide Compounds **2** (5.1 mg) and **4** (75.5 mg). Fr.1-3 was then separated into three major fractions (Fr.1-3a—Fr.1-3c) by silica gel chromatography with $CHCl_3$-MeOH (15:1 and 13:1, v/v) as eluent. Fr.1-3a and Fr.1-3b were further separated by preparative HPLC (p-HPLC) eluting with MeCN-H_2O 28:72 to yield Compounds **3** (10.2 mg) and **16** (25.3 mg), while **25** (3.0 mg) and **8** (4.5 mg) were prepared from Fr.1-3c with MeCN-H_2O (29:71, v/v) as a solvent system. Fr.2 (18.8 g) was subjected to chromatography on ODS gel to afford 10 factions (Fr.2-1—Fr.2-10). Fr.2-3 was subjected to chromatography on silica gel to yield Compound **6** (7.7 mg). Compounds **7** (5.3 mg) and **14** (3.6 mg) were isolated from Fr.2-6 by p-HPLC with MeCN-H_2O (30:70, v/v), and Compounds **18** (9.8 mg) and **20** (5.6 mg) were isolated from Fr.2-7 by p-HPLC with MeCN-H_2O (28:72, v/v). Fr.2-8 was chromatographed over silica gel to obtain 10 major fractions with $CHCl_3$-MeOH (50:1 to 5:1, v/v) as eluent (Fr.2-8-1—Fr.2-8-10). Fr.2-8-5 was separated subsequently by ODS chromatography eluting with MeOH-H_2O (40:60→55:45→70:30, v/v) to afford **19** (2.3 mg). Fr.2-4 was fractionated by ODS eluted with MeOH-H_2O (40:60→60:40→80:20, v/v) to afford five major fractions (Fr.2-4-1—Fr.2-4-5). The analysis of a combined fraction of Fr.2-4-1—Fr.2-4-3 was performed by p-HPLC. Compound **27** (9.5 mg) was isolated from Fr.2-4-1 by HPLC system of MeCN-H_2O (28:72, v/v). Compounds **10** (8.5 mg) and **15** (3.3 mg) were prepared from Fr.2-4-2 with MeCN-H_2O (20:80, v/v) as a solvent system, whereas **9** (11.2 mg) and **12** (3.5 mg) were obtained from Fr.2-4-2 with MeCN-H_2O (37:63, v/v) as a solvent system. Compounds **11** (3.8 mg) and **28** (2.2 mg) were got from Fr.2-4-3 by HPLC system of MeCN-H_2O (43:57 and 35:65, v/v). Fr.5 (23.5 g) was subjected to chromatography on ODS gel to provide ten factions (Fr.5-1—Fr.5-10). Compound **5** (1115.8 mg) was purified by recrystallizing from Fr.5-1. The analysis of other compounds isolated from Fr.5 was performed by p-HPLC: Compound **22** (mg) was purified from Fr.5-2, and Compounds **17** (51.1 mg) and **21** (12.7 mg) were isolated from Fr.5-3 by HPLC system of MeCN-H_2O (30:70, v/v). Compound **13** (13.4 mg) were isolated from Fr.5-5 HPLC system of MeCN-H_2O (34:66, v/v). Compounds **1** (4.3 mg) and **26** (8.5 mg) were prepared from Fr.5-7 with MeCN-H_2O (27:73, v/v) as a solvent system, whereas **23** (36.7 mg) and **24** (8.9 mg) were prepared from Fr.5-8 with MeCN-H_2O (37:63, v/v) as a solvent system.

2.4. Notoginsenoside Ab_1 (**1**)

3β,6α,12β,22S-tetrahydroxy-dammar-20(21),24-diene-6-O-β-D-glucopyranoside: white amorphous powder; [α]20D : +10.5, (c = 0.20, MeOH); IR ν_{max} 3420, 2931, 1634, 1454, 1384, 1074, 1032 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HR-ESI-MS *m/z* 659.4130 $[M+Na]^+$ (calculated for $C_{36}H_{60}O_9Na$, 659.4135).

2.5. Notoginsenoside Ab_2 (**2**)

22S-hydroperoxyl-3β,6α,12β-trihydroxy-dammar-20(21),24-diene-6-O-β-D-glucopyranoside: white amorphous powder; [α]20D : +11.6, (c = 0.18, MeOH); IR ν_{max} 3422, 2933, 1637, 1452, 1384, 1075, 1031 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HR-ESI-MS 675.4077 $[M + Na]^+$ (calcd. for $C_{36}H_{60}O_{10}Na$ 675.4084).

2.6. Notoginsenoside Ab_3 (**3**)

3β,6α,12β,24R-tetrahydroxy-dammar-20(21),25-diene-6-O-β-D-glucopyranoside: white amorphous powder; [α]20D : +3.4, (c = 0.25, MeOH); IR ν_{max} 3416, 2941, 1636, 1452, 1386, 1163, 1076, 1041 cm^{-1}; ^1H and ^{13}C NMR: see Table 1; HR-ESI-MS 659.4132 $[M+Na]^+$ (calcd. For. $C_{36}H_{60}O_9Na$ 659.4135).

2.7. Acid hydrolysis and HPLC analysis

The absolute configurations of the sugar moieties in Compounds **1**—**3** were determined by the method of literature reported [9]. Compounds **1**—**3** (2.0 mg/sample) were refluxed with 10 mL of 60% aqueous dioxane with 5% HCl for 2 h. The reaction mixture was evaporated under vacuum and then suspended in H_2O and extracted with $CHCl_3$. After drying in vacuum, the residue of aqueous layer was melted in 0.2 mL of C_5H_5N with 2 mg of L-cysteine methyl ester hydrochloride followed by warming at 60°C for 1 h. After that, 5 mL of o-tolylisothiocyanate is added and warmed up at 60°C for another hour. The reaction mixture was analyzed directly by reversed-phase HPLC on a Thermo C_{18} column (250 × 4.6 mm, 5 μm), with 20% CH_3CN at a flow rate of 1.0 mL/min at 30°C, and the detection wavelength was 254 nm. The analysis of standard monosaccharide, D-glucose, followed the same procedure. The value of t_R of the standard monosaccharide derivatives was 17.8 min, and the derivatives of **1**—**3** gave peaks at t_R 17.7—17.9 min, respectively.

2.8. Computational studies

Conformational searches were performed with Gaussian 09W program (Gaussian Inc., USA). The geometry of each conformer in the energy window of the conformational search was optimized with Gaussian 09W in vacuum, at the B3LYP-6-31g (d,p) level. Imaginary vibrational frequency of each conformer was checked, and no such frequency indicates true energy minima. Isotropic magnetic shielding was calculated with the GIAO (gauge-independent atomic orbital) method at the B3LYP/6-31G (d, P) level by using Gaussian 09W [10,11].

2.9. Fingerprint analysis

Chromatographic conditions: Waters 1525 HPLC system (Waters Corp., Milford, Massachusetts, USA); Chromatographic column: VP ODS C_{18} (250 mm × 4.6 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA); volume flow: 1.0 mL/min; column temperature: 30°C; detection wavelength: 203 nm; injection volume: 10 μL. The samples were eluted with the mixture of Solvent A (water) and Solvent B (acetonitrile). The elution rate using Solvent B was 20—45% for 0—60 min.

Preparation of samples: Accurately weighed 25 mg of powder sample was diluted with 10 mL of 70% methanol. Before injection, the samples were filtered through a 0.45-μm membrane filter.

2.10. Cell line

HepG-2 (human hepatic cancer cell line), NCI-H460 (human lung cancer cell), and MCF-7 (human breast cancer cell) were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The HepG-2 and NCI-H460 cancer cells were maintained in Roswell Park Memorial Institute 1640 medium, and MCF-7 cancer cells were maintained in high-glucose Dulbecco's minimum essential medium, supplemented with 10% fetal bovine serum. The cells grew in a 5% CO_2 incubator at 37°C. The cells were routinely digested and passaged every 3 days.

2.11. Cell viability assay

The cells were plated in 96-well plates (1×10^4 cells/well) overnight, then **1**-**3** at various concentrations of 0.01, 0.1, 1, 10, and

100 μg/mL and the positive control cisplatin at concentrations of 0.5, 1, 2, 4, and 8 μg/mL were treated in the plates for 72 h. Subsequently, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide reagent (5 mg/mL) was added to each well for 4 h, and then 100 μL of triple liquid containing 10 mg SDS (sodium dodecyl sulfate), 1.2 μL of 36–37% concentrated hydrochloric acid, and 50 μL of isobutanol were added. After the coculture for 12 h, the reduction of cell viability was determined at 570 nm using a microplate reader (Bio-Rad, USA). The cell proliferation inhibition rate was calculated according to the following formula: Inhibition rate (%) = (Acontrol − Asample)/Acontrol × 100 [12].

3. Result and discussion

Compound **1** was obtained as white amorphous powder. The molecular formula of **1** was deduced to be $C_{36}H_{60}O_9$ by positive

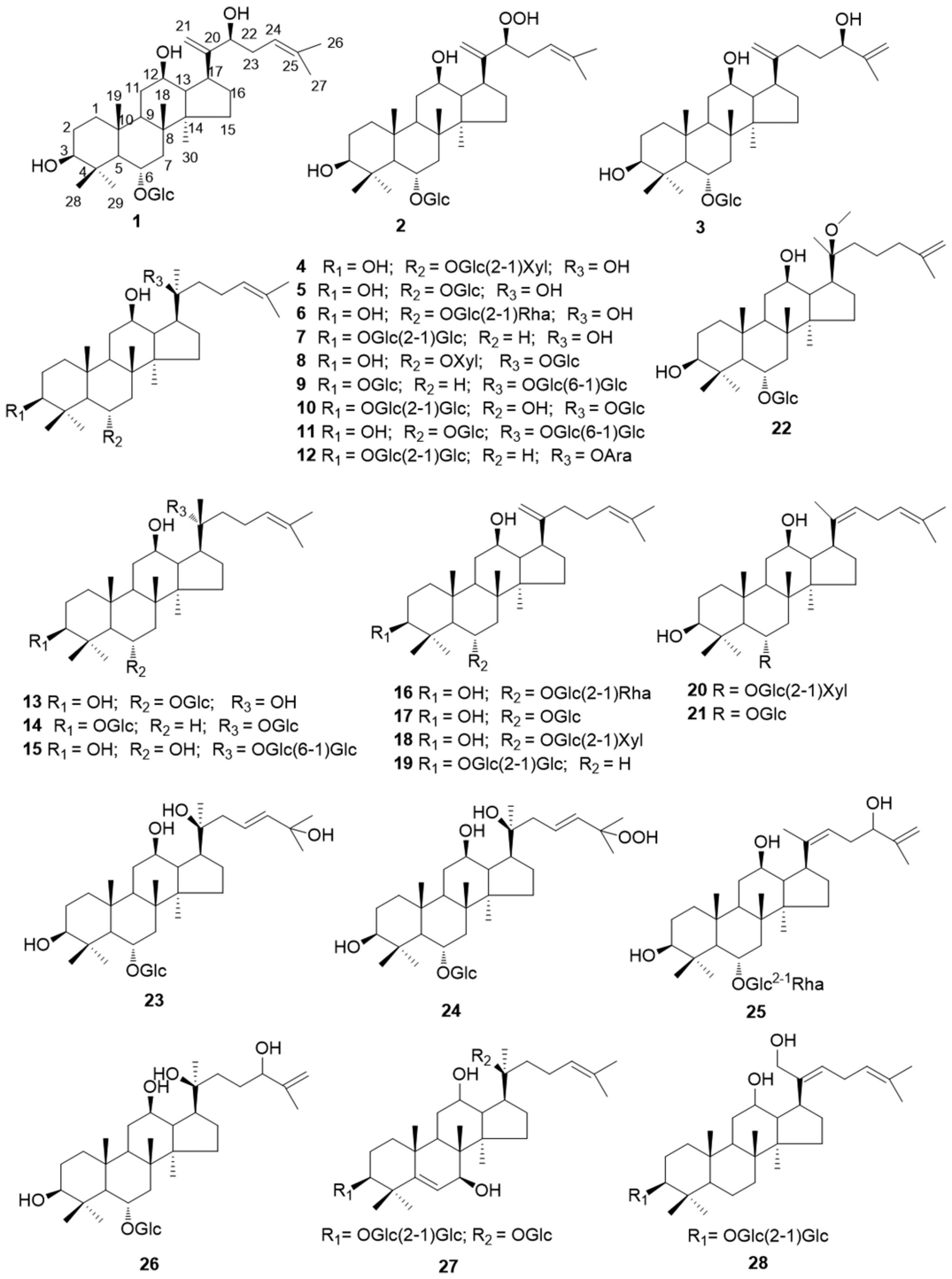

Fig. 1. The structure of the Compounds **1**–**28**. Glc, β-D-glucopyranosyl; Xyl, β-D-xylopyranosyl; Ara, α-L-arabinofuranosyl; Rha, α-L-rhamnopyranose.

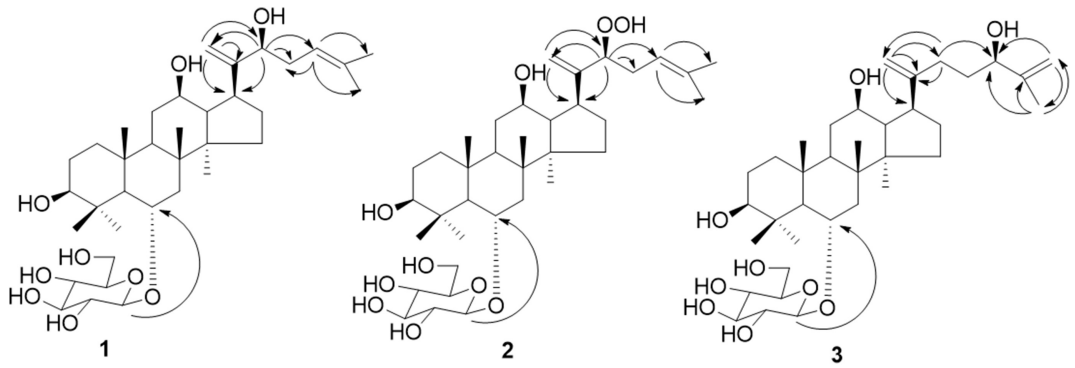

Fig. 2. The important HMBC correlations of Compounds **1**–**3**. HMBC, heteronuclear multiplebond correlation.

mass spectrometry (HR-ESI-MS) data at *m/z* 659.4130 $[M+Na]^+$ (calculated for $C_{36}H_{60}O_9Na$, 659.4135). The ^{13}C NMR (Table 1) showed 36 carbon signals. The distortionless enhancement by polarization transfer (DEPT) spectrum exhibited 7 methyls, 9 methylenes, 14 methines, and 6 quaternary carbons signals. Four olefinic carbon signals at δ_C 160.2, 132.8, 122.4, and 111.2 ppm suggested two double bonds in the molecule. The ^1H NMR showed signals of seven methyl groups at δ_H 0.88 (3H, s), 1.04 (3H, s), 1.25 (3H, s), 1.63 (3H, s), 1.65 (3H, s), 1.71 (3H, s), and 2.10 (3H, s); four oxygen substituted protons at δ_H 3.55 (1H, m), 4.29 (1H, m), 4.46 (1H, m), and 4.52 (1H, m); and one anomeric proton at δ_H 5.07 (1H, d, J = 8.0). The ^1H and ^{13}C signals were fully assigned according to heteronuclear signal quantum correlation (HSQC) spectra (Table 1). Methylene carbon signal at δ_C 111.2 ppm showed correlation spots with protons at δ_H 5.18 (H-21) and 4.52 (H-21) ppm in HSQC spectrum. These two proton signals showed connections with carbon signals at δ_C 160.2 (C-20), 77.0 (C-22), and 40.2 (C-17) ppm in heteronuclear multiple bond correlation (HMBC) spectrum, and δ_H 4.52 (H-22) showed connection with δ_C 111.2 (C-20), 122.4 (C-24), and 40.2 (C-17) (Fig. 2). Thus, the signals at δ_C 160.2, 111.2, and 77.0 ppm were assigned to be the signals of C-20, C-21, and C-22, respectively. δ_H 5.42 (H-24) showed connections with δ_C 132.8 (C-25), 26.4 (C-26), and 18.6 (C-27). Therefore, it was concluded that the two double bonds were at △20(21) and △24(25). The signals of Compound **1** were quiet similar to those of ginsenoside Rk_3, except for the chemical shift of C-20, C-22, and C-23, which were at δ_C 160.2, 77.0, and 36.3 of Compound **1** but were at δ_C 155.4, 33.7, and 27.0 of ginsenoside Rk_3, respectively [13]. The shift to

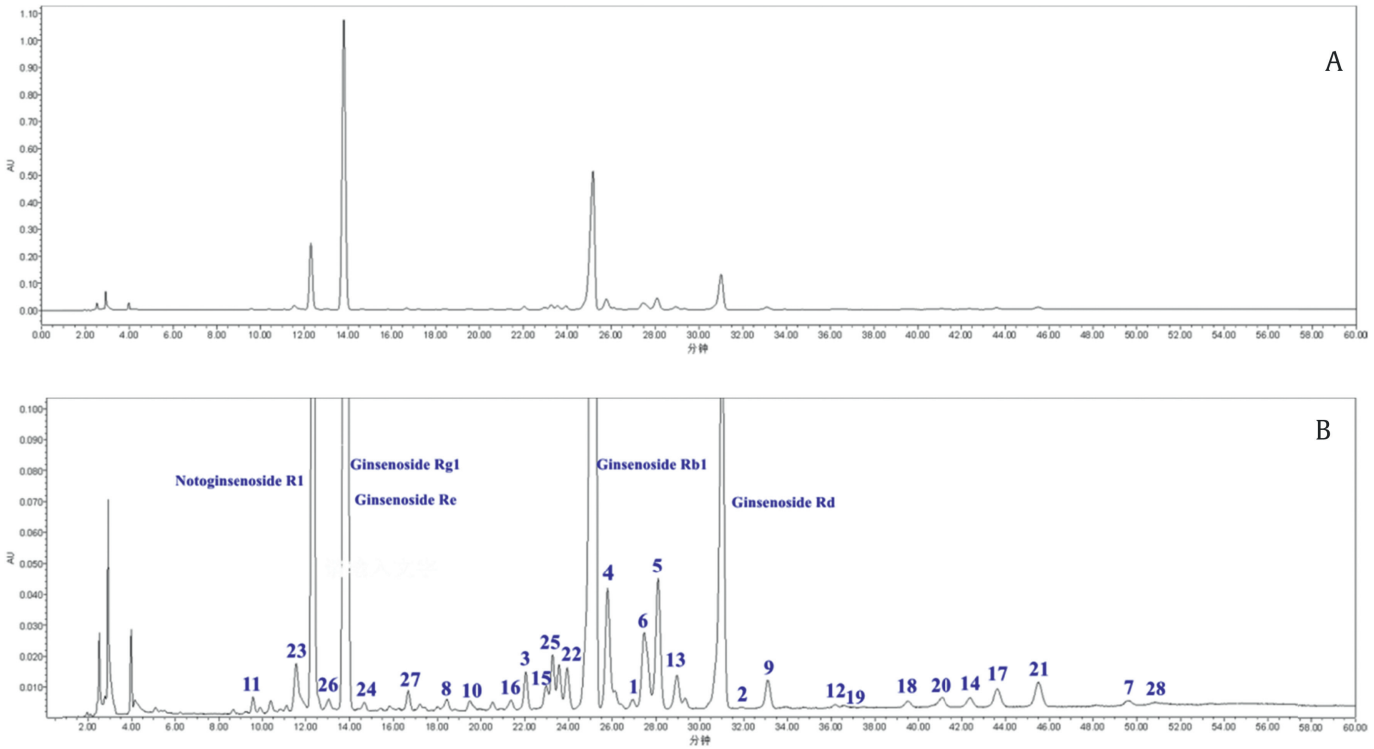

Fig. 3. (A) The HPLC fingerprint of PNS. (B) The magnified HPLC fingerprint of PNS with compounds isolated from PNS marked. (**1**, notoginsenoside Ab_1; **2**, notoginsenoside Ab_3; **3**, notoginsenoside Ab_3; **4**, notoginsenoside R_2; **5**, 20(S)-ginsenoside Rh_1; **6**, 20(S)-ginsenoside Rg_2; **7**, ginsenoside Rg_3; **8**, pseudoginsenoside RT_3; **9**, gypenoside-XVII; **10**, vina-ginsenoside-R_4; **11**, notoginsenoside R_3; **12**, quinquenoside L_{14}; **13**, 20(R)-ginsenoside Rh_1; **14**, ginsenoside F_2; **15**, ginsenoside U; **16**, ginsenoside Rg_6; **17**, ginsenoside Rk_3; **18**, notoginsenoside T_5; **19**, ginsenoside RK_1 **20**, ginsenoside Rg_4; **21**, ginsenoside-Rh_4; **22**, ginsenoside-Rh_5; **23**, 20(S)-ginsenoside-ST_2; **24**, floralquinquenoside A; **25**, ginsenoside-Rh_{14}; **26**, notopanaxoside A; **27**, notopanaxoside G; **28**, sanchirhinoside D). All the Compounds **1**-**28** isolated form PNS and the sample of PNS were analysis by HPLC at the same condition. In addition, **1**-**28** were marked on the HPLC spectrum of PNS.

downfield of C-20 (+4.8 ppm), C-22 (+43.3 ppm), and C-23 (+9.3 ppm) indicated that C-22 of **1** was linked to hydroxyl. In addition, the β configuration was prompted by the large coupling constant observed for the anomeric proton δ_H 5.07 (1H, d, $J = 8.0$). The absolute configurations of sugar was elucidated as D-glucose through acid hydrolysis and HPLC analysis. Moreover, the linkages between H-1" (δ_H 5.07) and C-6 (δ_C 80.5) were determined by HMBC correlations. The configuration of OH at C-6 was α based on the correlations between H-6 with H-18β, 19β in rotating frame overhauser effect (ROESY) spectrum. In addition, H-17 was deduced as α-forms by correlations between H-17 and Me-30 in the ROESY spectrum. The configuration of C-22 was identified by the comparison of the calculated and experimental chemical shifts of ^{13}C. The calculated chemical shifts of C-22 about (22*R*)-**1** and (22*S*)-**1** were δ_C 72.0 and δ_C 77.8, whereas the experimental result was δ_C 77.0. Therefore, the configuration of C-22 was identified as *S*. On the basis of the aforementioned analyses, Compound **1** could be deduced to be 3β,6α,12β,22*S*-tetrahydroxy-dammar-20(21),24-diene-6-O-β-D-glucopyranoside and named as notoginsenoside Ab_1.

Compound **2** was isolated as white amorphous powder. The HR-ESI-MS spectrum showed $[M+Na]^+$ at *m/z* 675.4077 (calcd. for $C_{36}H_{60}O_{10}Na$ 675.4084). It was proposed to possess a hydroperoxyl group due to positive response to N,N-dimethyl-p-phenylenediammonium dichloride reagent [14]. The ^{13}C NMR (Table 1) showed 36 carbon signals. The DEPT spectrum exhibited 7 methyls, 9 methylenes, 14 methines, and 6 quaternary carbons signals. The ^{1}H NMR showed signals of seven methyl groups at δ_H 0.82 (3H, s), 1.03 (3H, s), 1.28 (3H, s), 1.59 (3H, s), 1.60 (3H, s), 1.65 (3H, s), and 2.08 (3H, s). The signals of Compound **2** were quiet similar to those of Compound **1**, except for the chemical shift of C-20 (−3.9 ppm), C-22 (+14.3 ppm), and C-23 (−5 ppm), indicating the hydroperoxyl substitution of C-22. C-22 (δ_H 4.71, δ_C 91.3) bearing hydroperoxyl group was also supported by chemical shift comparison with those of related hydroperoxylated triterpenes [15–17]. In addition, the long-range correlations between H-21 and C-17, 22, H-22 and C-17, 21, 23, 24, H-24 and C-26, 27, in the HMBC spectrum further confirmed the structure (Fig. 2). The monosaccharide was determined to be D-glucose by HPLC analysis of chiral derivatives of sugars in the acid hydrolyzate. The β-anomeric configuration for the glucosyl unit was established from the coupling constant for the anomeric proton δ_H 5.04 (d, $J = 7.8$ Hz). Compared with the calculated chemical shifts of C-22 about (22*R*)-**2** (δ_C 82.8) and (22*S*)-**2** (δ_C 88.2), the configuration of C-22 in Compound **2** was identified as *S*. Compound **2** could be deduced to be 22S-hydroperoxyl-3β,6α,12β-trihydroxy-dammar-20(21),24-diene-6-O-β-D-glucopyranoside and named as notoginsenoside Ab_2.

Compound **3**, was obtained as white amorphous powder. The molecular formula was assigned as $C_{36}H_{60}O_9$ by the HR-ESI-MS spectrum at *m/z* 659.4132 $[M+Na]^+$ (calcd. For. $C_{36}H_{60}O_9Na$ 659.4135). The ^{13}C NMR (Table 1) showed 36 carbon signals, and the ^{1}H NMR showed six methyl groups signals. The signals were similar to those of ginsenoside-Rh_5 [18], except for the position of the double bond. The corrections between H-21 (δ_H 4.97, 5.15) and C-20, 17, 22 in HMBC spectrum (Fig. 2) demonstrated there is a double bond in C-20, 21. What is more, the chemical shift of C-20, C-21, and C-22 were at δ_C 156.5, 108.4, and 33.1 in Compound **3** but that of ginsenoside-Rh_5 were at δ_C 142.5, 13.2, and 122.2 in ginsenoside-Rh_5, further indicating that the double bond was in C-20, 21 instead of in C-20, 22. There was an additional β-D-glucose in Compound **3** based on the results of acid hydrolysis analysis and the coupling constant for the anomeric proton. The correlation of H-6/H-18β, H-6/H-19β in ROESY spectrum revealed the α-orientation of OH at C-6. The correlation between H-17 and Me-30 in ROESY spectrum indicated the α-forms of H-17. The configuration of C-24 was identified as *R* by the comparison of the calculated chemical shifts of (24*R*)-**3** (δ_C 72.3) and (24*S*)-**3** (δ_C 67.9) with experimental chemical shift of C-24 (δ_C 75.5). Compound **3** could be deduced to be 3β,6α,12β,24*R*-tetrahydroxy-dammar-20(21),25-diene-6-O-β-D-glucopyranoside and named as notoginsenoside Ab_3.

Table 2
IC_{50} values of the new Compounds (**1**–**3**) against HepG2, NCI-H460, and MCF-7 cells.

Comp.	IC_{50} (μg/mL)[1)]		
	HepG2	NCI-H460	MCF-7
1	>100	>100	>100
2	4.49	8.06	7.38
3	>100	>100	>100
Cisplatin[2)]	0.97	1.34	2.66

Cell inhibition activity was determined by the MTT assay. MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide
[1)] IC_{50} is the concentration of the compound inhibiting 50% of cell proliferation.
[2)] Cisplatin was used as positive control.

The known compounds were identified as notoginsenoside R_2 (**4**) [19], 20(S)-ginsenoside Rh_1 (**5**) [20], 20(S)-ginsenoside Rg_2 (**6**) [21], ginsenoside Rg_3 (**7**) [22], pseudoginsenoside RT_3 (**8**) [23], gypenoside-XVII (**9**) [24], vina-ginsenoside-R_4 (**10**) [25], notoginsenoside R_3 (**11**) [26], quinquenoside L14 (**12**) [27], 20(R)-ginsenoside Rh_1 (**13**) [28], ginsenoside F_2 (**14**) [29], ginsenoside U (**15**) [30], ginsenoside Rg6 (**16**) [31], ginsenoside Rk_3 (**17**) [13], notoginsenoside T_5 (**18**) [32], ginsenoside Rk_1 (**19**) [33], ginsenoside Rg_4 (**20**) [34], ginsenoside-Rh_4 (**21**) [35], ginsenoside-Rh_5 (**22**) [18], 20(S)-ginsenoside-ST_2 (**23**) [36], floralquinquenoside A (**24**) [37], ginsenoside-Rh_{14} (**25**) [38], notopanaxoside A (**26**) [39], notopanaxoside G (**27**) [40], sanchirhinoside D (**28**) [41] by comparison of their data with the literature (Fig. 1).

As typical multiple-constituent and multiple-action traditional Chinese medicine, the quality is difficult to control, so a systematic study about the complicated ingredients of PNS is much essential. This is the first study to explore the rare content of PNS comprehensively. In the study, we isolated 3 new and 25 known dammarane-type triterpenes from PNS. In addition, a method of HPLC-UV was successfully applied to the determination of rare ginsenosides in PNS. As the Fig. 3 showed, we also marked the compounds isolated in the fingerprint. The results of this study are significant for quality improvement and evaluation of clinical medicines made of PNS.

The new Compounds (**1**–**3**) were evaluated for their cytotoxic activity against HepG2, NCI-H460, and MCF-7 cancer cell lines by using the modified 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide method, with cisplatin as the positive control (Table 2). New Compound **2** showed significant activity against HepG2, NCI-H460, and MCF-7 with IC_{50} 4.49, 8.06, and 7.38 μg/mL, respectively.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

Authors are glad to acknowledge the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences for the measurements of NMR, MS, HREIMS, and IR spectroscopic data. This work was supported by a grant from China national TCM standardization project (ZYBZH-Y-YN-44), Science and Technology Innovation Platform Construction Project (2015DC027), and Chemical Synthesis Platform Project (2016DC049) from Yunnan Provincial Science and Technology Department in China.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2018.12.001.

References

[1] Park HJ, Kim DH, Park SJ, Kim JM, Ryu JH. Ginseng in traditional herbal prescriptions. J Ginseng Res 2012;36:225–41.
[2] Zhao GR, Xiang ZJ, Ye TX, Yuan YJ, Guo ZX. Antioxidant activities of Salvia miltiorrhiza and Panax notoginseng. Food Chem 2006;99:767–74.
[3] Wang T, Guo RX, Zhou GH, Zhou XD, Kou ZZ, Sui F, Li C, Tang LY, Wang ZJ. Traditional uses, botany, phytochemistry, pharmacology and toxicology of Panax notoginseng (Burk.) F.H. Chen: a review. J Ethnopharmacol 2016;188:234–58.
[4] Hong HD, Choi SY, Kim YC, Lee YC, Cho CW. Rapid determination of ginsenosides Rb 1, Rf, and Rg 1 in Korean ginseng using HPLC. J Ginseng Res 2009;33:8–12.
[5] Li Lan-Jie, Jin Yong-Ri, Wang Xiao-Zhong, Liu Ying, Wu Qian, Shi Xiao-Lei, Li Xu-Wen. Ionic liquid and aqueous two-phase extraction based on salting-out coupled with high-performance liquid chromatography for the determination of seven rare ginsenosides in Xue-Sai-Tong injection. J Sep Sci 2015;38:3055–62.
[6] Wang L, Li Z, Zhao X, Liu W, Liu Y, Yang J, Li X, Fan X, Cheng Y. A network study of Chinese medicine Xuesaitong injection to elucidate a complex mode of action with multicompound, multitarget, and multipathway, Evid. Based Complement. Alternat Med 2013;2013:652373.
[7] Liu SJ, Zhou SW. Panax notoginseng saponins attenuated cisplatin-induced nephrotoxicity. Acta Pharmacol Sin 2000;21:257–60.
[8] Ma LY, Xiao PG. Effects of Panax notoginsen Saponins on platelet aggregation in rats with middle cerebral artery occlusion or in vitro and on lipid fluidity of platelet membrane. Phytother Res 1998;12:138–40.
[9] Li XH, Li XH, Yao Q, Lu LH, Li YB, Wu DS, Fu DH, Mei SX, Cui T, Wang JK, et al. Iridoids from Phlomis likiangensis with a carbonate ester Substituent. Tetrahedron Lett 2017;58:3112–8.
[10] Previdi D, Nardini V, Rosa ME, Palaretti V, Silva GVJ, Donate PM. Stereochemical assignment of four diastereoisomers of a maculalactone derivative by computational NMR calculations. J Mol Struct 2019;1178:467–78.
[11] Liu DZ, Wang F, Liao TG, Tang JG, Steglich W, Zhu HJ, Liu JK. Vibralactone: a lipase inhibitor with an unusual fused β-lactone produced by cultures of the basidiomycete boreostereum vibrans. Org Lett 2006;8(25):5749–52. RGANIC.
[12] Mosmann TJ. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55–63.
[13] Park IH, Kim NY, Han SB, Kim JM, Kwon SW, Kim HJ, Park MK, Park JH. Three new dammarane glycosides from heat processed ginseng. Arch Pharm Res 2002;25(4):428–32.
[14] Knappe E, Peteri D. Thin-layer chromatographic identification of organic peroxides. Z Anal Chem 1962;190:386–9.
[15] Seikou N, Sachiko S, Hisashi, Masayuki Y. Medicinal flowers. XVII.[1)] new dammarane-type triterpene glycosides from flower buds of American ginseng, Panax quinquefolium L. Chem. Pharm. Bull. 2007;55(9):1342–8.
[16] Tung NH, Song GY, Minh CV, Kiem PV, Jin LG, Boo HJ, Kang HK, Kim YH. Steamed ginseng-leaf components enhance cytotoxic effects on human leukemia HL-60 cells. Chem Pharm Bull 2010;58(8):1111–5.
[17] Wang JR, Yamasaki Y, Tanaka T, Kouno I, Jiang ZH. Dammarane-type triterpene saponins from the flowers of Panax notoginseng. Molecules 2009;14:2087–94.
[18] Yang XW, Li KK, Zhou QL. 20(S)-Ginsenoside-Rf2, a novel triterpenoid saponin from stems and leaves of Panax ginseng. Chin Tradit Herb Drugs 2015;46:3137–45.
[19] Dou DQ, Ren J, Chen Y, Pei YP, Chen YJ. Study on the chemical constituents of the roots of commercial ginseng. China J Chin Mater Med 2003;28:522–4.
[20] Han BH, Park MH, Han YN, Woo LK, Sankawa U, Yahara S, Tanaka 0. Degradation of ginseng saponins under mild acidic conditions. Planta Med 1982;44:146–9.
[21] Yang XW, Li LY, Tian JM, Zhang ZW, Ye JM, Gu WF. Ginsenoside-Rg6, a novel triterpenoid saponin from the stem-leaves of *Panax ginseng* C. A. Mey. Chin Chem Lett 2000;11:909–12.
[22] Cheng LQ, Na JR, Bang MH, Kim MK, Yang DC. Conversion of major ginsenoside Rb1 to 20(S)-ginsenoside Rg3 by microbacterium sp. GS514. Phytochemistry 2008;69:218–24.
[23] Liu LM, Zhang XQ, Wang H, Ye WC, Zhao SX. Minor saponins constituents from Panax Notoginseng tap root. J Chin Pharmaceut Univ 2011;42:115–8.
[24] Besso HM, Kasai R, Wei JX, Wang JF, Saruwangtari YH, Fuwa T, Tanaka O. Further studies on dammarane-saponins of American ginseng, roots of Panax quinquefolium L. Chem Pharm Bull 1982;30:4534–8.
[25] Duc NM, Kasai R, Ohtani K, Ito A, Nham NT, Yamasaki K, Tanaka O. Saponins from Vietnamese Ginseng, Panax vietnamensis HA et GRUSHV. Collected in Central Vietnam. II. Chem Pharm Bull 1994;42:115–22.
[26] Yuan C, Xu FX, Huang XJ, Li SP, Zhang QW. A novel 12, 23-epoxy dammarane saponin from PanaPanax notoginseng. Chin J Nat Med 2015;13:303–6.
[27] Chen J, Zhao R, Zeng YM, Meng H, Zuo WJ, Li X, Wang JH. Three new triterpenoid saponins from the leaves and stems of Panax quinquefolium. J Asian Nat Prod Res 2009;11:195–201.
[28] Teng RW, Li HZ, Chen JT, Wang DZ, He YN, Yang CR. Complete assignment of ^{1}H and ^{13}C NMR data for nine protopanaxatriol glycosides. Mag Reson Chem 2002;40:483–8.
[29] Dou DQ, Wen Y, Pei YP, Yao XS, Chen YJ, Kawai H, Fukushima H. Ginsenoside-l a A Novel minor saponin from the leaves of Panax ginseng. Planta Med 1996;62:179–81.
[30] Han LF, Sakah KJ, Liu LL, Kojo A, Wang T, Zhang Y. Saponins from roots of Panax notoginseng. Chin Herb Med 2014;6:159–63.
[31] Ma LY, Yang XW. 20(R)-Ginsenoside-Rh19, a novel ginsenoside from alkaline hydrolysates of total saponins in stems-leaves of Panax ginseng. Chin Tradit Herb Drugs 2016;47:6–134.
[32] Song JP, Zeng J, Cui XM, Dai Y, Jiang ZY, Zhang XM, Zhou JM, Ma YB, Chen JJ. Studies on chemical constituents from rhizomes of Panax notoginseng (II). J Yunnan Univ 2007;29:287–90.
[33] Li KK, Yang XW. A new triterpene natural product from stems and leaves of Panax ginseng. Chin Tradit Herb Drugs 2015;46:169–73.
[34] Chen GT, Yang M, Lu ZQ, Zhang JQ, Huang HL, Liang Y, Guan SH, Song Y, Wu LJ, Guo DA. Microbial transformation of 20(S)-Protopanaxatriol-Type saponins by absidia coerulea. J Nat Prod 2007;70:1203–6.
[35] Liu LM, Zhang XQ, Wang H, Wen-cai YE, Shou-xun ZHAO. Minor saponins constituents from Panax Notoginseng tap root. J Chin Pharm Univ 2011;42:115–8.
[36] Qu SJ, Tan JJ, Cai JG, Ling YP, Zhang SF, Tan CH, Zhu DY. Minor dammarane saponins from the hongshen extract of Shenmai injection. J Asian Nat Prod Res 2011;13:178–81.
[37] Nakamura Seikou, Sugimoto Sachiko, Matsuda Hisashi, Yoshikawa Masayuki. Medicinal flowers. XVII.1) new dammarane-type triterpene glycosides from flower buds of American ginseng, Panax quinquefolium L. Chem Pharm Bull 2007;55(9):1342–8.
[38] Li KK, Yao CM, Yang XW. Four new dammarane-type triterpene saponins from the stems and leaves of Panax ginseng and their cytotoxicity on HL-60 cells. Planta Med 2012;78:189–92.
[39] Komakine N, Okasaka M, Takaishi Y, Kawazoe K, Murakami K. New dammarane-type saponin from roots of Panax notoginseng. J Nat Med 2006;60:135–7.
[40] Murakami T, Ueno T, Hirokawa N, Yashiro K, Murakami N, Yamahara J, Matsuda H, Saijoh R, Tanaka O. Bioactive saponins and glycosides. IX. Notoginseng (2) : structures of five new dammarane-type triterpene oligoglycosides, notoginsenosides-E, -G, -H, -I, and -J, and a novel acetylenic fatty acid glycoside, notoginsenic acid β-sophoroside, from the dried root of Panax notoginseng (BURK.) F. H. CHEN. Chem Pharm Bull 1997;45:1056–62.
[41] Sakah KJ, Wang T, Liu LL, Chen Y, Han LF, Zhang Y. Eight darmarane-type saponins isolated from the roots of Panax notoginseng. Acta Pharm Sin B 2013;3:381–4.