Fitoterapia 103 (2015) 97–105

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Minor dehydrogenated and cleavaged dammarane-type saponins from the steamed roots of *Panax notoginseng*

Cheng-Zhen Gu [a,b], Jun-Jiang Lv [a], Xiao-Xia Zhang [a], Hui Yan [a], Hong-Tao Zhu [a], Huai-Rong Luo [a], Dong Wang [a], Chong-Ren Yang [a], Min Xu [a,*], Ying-Jun Zhang [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People's Republic of China
[b] University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

ARTICLE INFO

Article history:
Received 16 January 2015
Accepted in revised form 9 March 2015
Accepted 14 March 2015
Available online 19 March 2015

Keywords:
Panax notoginseng
Triterpenoid saponins
PC12 cells

ABSTRACT

Nine new minor dehydrogenated and cleavaged dammarane-type triterpenoid saponins, namely notoginsenosides ST6-ST14 (**1**–**9**) were isolated from the steamed roots of *Panax notoginseng*, together with 14 known ones. Among them, **5–7** and **21–22** were protopanaxadiol type and the left 18 compounds, including **1–4**, **8–20**, and **23** were protopanaxatriol type saponins. Their structures were identified by extensive analysis of MS, 1D and 2D NMR spectra, and acidic hydrolysis. Resulted from the side chain cleavage, the new saponins **1** and **2** featured in a ketone group at C-25, and **3–5** had an aldehyde unit at C-23. The known saponins **12**, **16** and **18** displayed the enhancing potential of neurite outgrowth of NGF-mediated PC12 cells at a concentration of 10 μM, while **20** exhibited acetyl cholinesterase inhibitory activity, with IC_{50} value of 13.97 μM.

1. Introduction

Panax notoginseng, a famous herbal medicine from ancient China, has been used traditionally for the treatment of various diseases for thousands of years [1–6]. The roots have been used traditionally in both raw and processed forms, due to their different therapeutical functions. The raw ones have been used to treat various internal or external hemorrhage, disperse bruises, eliminate blood stasis, improve blood circulation, reduce swelling and pain, as well as to disperse blood clots, due to its hemostatic property and effects on cardiovascular diseases. While, the steamed form has been used as a tonic to nourish blood and increase production of various blood cells in anemic conditions [7,8]. Dammarane-type triterpenoid saponins were found to be the main chemical constituents and bioactive principles in *P. notoginseng* [9–12]. Previous study proved that the biological activities of saponins were related with their structures [13,14]. Notoginsenoside R_1, ginsenosides Rg_1, Re, Rb_1 and Rd are the main saponins in the raw roots. Ginsenosides 20(*R*/*S*)-Rh_1, Rk_3, Rh_4, 20(*S*/*R*)-Rg_3, Rk_1, and Rg_5, the minor or even trace components in the raw roots, are found to be the main constituents of the processed roots [15,16].

Previously, we reported 20 dammarane-type triterpenoids from the steaming processed roots of *P. notoginseng*, in addition to the eight major saponins [15]. All of them were minor or even trace components in the raw roots, and notoginsenosides ST-1–ST-5 were new saponins. Notoginsenoside ST-4 was also found to be a promising agent for herpes simplex virus infection [17]. Inspired by the above study that a rich chemical diversity of saponins in the processed materials with various promising bioactivities, we have commenced a program on investigating the minor new saponins from the steamed roots of *P. notoginseng*. This led to the isolation of nine new compounds, namely notoginsenosides ST6–ST14 (**1**–**9**), along with 14 known saponins. Their structures were determined by extensive spectroscopic analysis and acidic hydrolysis. Moreover, the isolated compounds were tested for their effects on the neurite outgrowth of NGF-mediated PC12 cells, and

* Corresponding authors. Tel./fax: +86 871 6522 3235.
E-mail addresses: minxu@mail.kib.ac.cn (M. Xu), zhangyj@mail.kib.ac.cn (Y.-J. Zhang).

http://dx.doi.org/10.1016/j.fitote.2015.03.014
0367-326X/

their cytotoxic and acetyl cholinesterase inhibitory activities. Herein, we describe the results obtained in the study.

2. Experimental

2.1. General experiment procedures

Optical rotations were performed on a P-1020 polarimeter (JASCO, Tokyo, Japan). UV spectrum was recorded on a Shimadzu UV-2401A spectrophotometer. IR spectrum was measured on a Bruker Tensor 27 spectrometer with KBr pellets. 1D and 2D spectra were run on Bruker DRX-400, 500, AVANCE III-600 and spectrometers operating at 400, 500 and 600 MHz for ^{1}H, and 100, 125 and 150 MHz for ^{13}C, respectively. Coupling constants are expressed in Hertz and chemical shifts are given on ppm scale with solvents as internal standard. ESI-MS and HRESIMS were measured at Bruker HCT/Esquire and Agilent G6230. The apparatus of HPLC was an Agilent 1260 with DAD detector. Semi-preparative HPLC was performed on an Agilent 1260 liquid chromatography with a 5 μm Thermo BDS HYPERSIL-C_{18} column (10 × 250 mm). Column chromatography (CC) was performed with Diaion 101 resin (Shandong Lukang Pharmaceutical Co., Ltd., China), Diaion HP20SS (Mitsubishi Chemical Co., Tokyo, Japan), silica gel (200–300 mesh) (Qingdao Marine Chemical and Industrial Factory, China), MCI-gel CHP20P (75-100 μm) (Mitsubishi Chemical Co., Ltd., Japan), RP-8 or RP-18 gel (40–60 μm) (Merck, Darmstadt, Germany). Fractions were monitored by TLC and spots were visualized by heating the silica gel plates sprayed with 10% sulphuric ethanol solution. *S*-Acetylthiocholine iodide, 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), and acetyl cholinesterase derived from human erythrocytes were purchased from Sigma Chemical.

2.2. Plant material

Air-dried roots of *P. notoginseng* were collected from Wenshan County, Yunnan Province, China, in 2011. The raw notoginseng was crushed into small grains and then steamed at high temperature (120 °C) and pressure (0.12 MPa) for 12 h, yielding the steamed notoginseng.

2.3. Extraction and isolation

The steamed notoginseng (15 kg) was extracted with methanol/H_2O (80:20, v/v, 3 × 3 h) under reflux. The extract (3 kg) was subjected to a Diaion 101 column chromatography (250 × 30 cm), eluting with water to remove saccharides, and then with methanol to afford the total saponin fraction (2 kg) [16]. The total saponin fraction was fractionated on a silica gel column (250 × 30 cm), eluting with $CHCl_3$–MeOH–H_2O (85:15:1–75:25:2), to afford eight fractions (Fr. A–H). Fr. B and D–G were further subjected to CC of RP-18 (MeOH–H_2O, 50:50 to 90:10) to yield sub-fractions B1–B5 from Fr. B (200 g), D1–D5 from Fr. D (150 g), E1–E5 from Fr. E (50 g), F1–F5 from Fr. F (40 g), and G1–G4 from Fr. G (30 g), respectively.

Fr. B4 (60 mg) and Fr. D3 (245 mg) were purified by semi-preparative HPLC (MeOH–H_2O, 62:38 to 67:23, and 43:57 to 48:52, resp.) to give **6** (9 mg) and **7** (4 mg) from Fr. B4, and **17** (8 mg), **18** (15 mg), **21** (7 mg), **22** (28 mg), and **23** (35 mg) from Fr. D3, resp.

Fr. E2 (27 g) was applied to RP-18 CC (MeOH–H_2O, 43:57), followed with recrystallization (MeOH–H_2O 35:65) to yield **8** (2.0 g). Fr. E4 (186 mg), F4 (136 mg) F5 (51 mg), G2 (161 mg), and G4 (56 mg) were purified by semi-preparative HPLC with CH_3CN–H_2O (35:65 to 40:60, 22:78 to 30:70, 25:75 to 32:68, 18:81 to 22:78, and 20:80 to 22:78, resp.) as mobile phase, to afford **5** (3 mg), **19** (51 mg), and **20** (42 mg) from Fr. E4, **11** (8 mg), **12** (6 mg), **13** (5 mg), **14** (7 mg), and **9** (3 mg) from Fr. F4, **1** (11 mg) and **2** (5 mg) from Fr. F5, **3** (20 mg), **4** (23 mg), and **10** (25 mg) from Fr. G2, and **15** (11 mg), and **16** (7 mg) from Fr. G4, respectively.

2.4. Spectroscopic data

2.4.1. Notoginsenoside ST6 (**1**)

Amorphous powder; $[\alpha]_D^{20}$ +11.7 (*c* 1.00, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.48) and 201 (2.99) nm; IR (KBr) ν_{max} 3425, 2956, 2931, 2876, 1618, 1583, 1383, 1269, 1074, 1032, 579, and 532 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; ESI-MS (positive ion mode) *m/z* 643 $[M + Na]^+$; and HRESIMS (positive ion mode) *m/z* 621.3998 $[M + H]^+$ (calcd $C_{35}H_{57}O_9$, 621.3997).

2.4.2. Notoginsenoside ST7 (**2**)

Amorphous powder; $[\alpha]_D^{20}$ +1.2 (*c* 0.92, MeOH); UV (MeOH) λ_{max} (log ε) 300 (3.43), and 201 (2.95) nm; IR (KBr) ν_{max} 3426, 2956, 2931, 2876, 1618, 1582, 1384, 1268, 1384, 1156, 1074, 1032, 927, 574, and 530 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; ESI-MS (positive ion mode) *m/z* 643 $[M + Na]^+$; and HRESIMS (positive ion mode) *m/z* 621.4003 $[M + H]^+$ (calcd for $C_{35}H_{57}O_9$, 621.3997).

2.4.3. Notoginsenoside ST8 (**3**)

Amorphous powder; $[\alpha]_D^{20}$ + 24.3 (*c* 1.85, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.34) nm; IR (KBr) ν_{max} 3425, 2955, 2935, 2876, 1647, 1450, 1368, 1154, 1074, 1032, 613, and 531 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; ESI-MS (positive ion mode) *m/z* 563 $[M - OH^-]^+$; and HRESIMS (positive ion mode) *m/z* 603.3507 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_9Na$, 603.3504).

2.4.4. Notoginsenoside ST9 (**4**)

Amorphous powder; $[\alpha]_D^{20}$ + 9.0 (*c* 1.02, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.19) nm; IR (KBr) ν_{max} 3424, 2955, 2927, 2873, 2855, 1647, 1462, 1383, 1154, 1074, 1034, 636, and 533 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; ESI-MS (positive ion mode) *m/z* 563 $[M - OH^-]^+$; and HRESIMS (positive ion mode) *m/z* 603.3505 $[M + Na]^+$ (calcd for $C_{32}H_{52}O_9Na$, 603.3504).

2.4.5. Notoginsenoside ST10 (**5**)

Amorphous powder; $[\alpha]_D^{20}$ −1.5 (*c* 0.78, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.35) nm; IR (KBr) ν_{max} 3425, 2943, 2929, 2877, 2858, 1641, 1384, 1160, 1077, 1073, 895, 621, and 577 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 2; ESI-MS (negative ion mode) *m/z* 761 $[M + Cl]^-$; and HRESIMS (positive ion mode) *m/z* 749.4088 $[M + Na]^+$ (calcd for $C_{38}H_{62}O_{13}Na$, 749.4083).

Table 1
^{13}C and ^{1}H NMR data of compounds **1**, **2**, and **9** (Record at 600 MHz for ^{1}H and 150 MHz for ^{13}C in pyridine-d_5, δ in ppm).

No.	**1**		**2**		**9**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.5 t	1.02, 1.68 m	39.9 t	1.02, 1.68 m	40.0 t	1.03, 1.64 m
2	27.9 t	1.87, 1.94 m	28.4 t	1.87, 1.95 m	28.3 t	1.82, 1.94 m
3	78.6 d	3.55 m	78.9 d	3.55 m	79.0 d	3.54 m
4	40.4 s		40.8 s		40.8 s	
5	61.5 d	1.45 d (11.4)	61.8 d	1.46 d (10.8)	61.8 d	1.45 d (10.8)
6	80.0 d	4.46 m	80.5 d	4.47 m	80.6 d	4.42 m
7	45.1 t	1.98 m	45.7 t	1.98 m	45.7 t	1.93 m
		2.56 br d (12.6)		2.57 dd (3, 12.6)		2.55 br d (12.0)
8	41.1 s		41.8 s		41.1 s	
9	50.7 d	1.57 m	51.1 d	1.58 m	50.6 d	1.58 m
10	39.7 s		40.1 s		39.8 s	
11	33.0 t	1.49, 2.06 m	33.6 t	1.49, 2.05 m	32.2 t	1.46, 2.06 m
12	71.7 d	3.92 m	72.0 d	3.92 m	71.4 d	3.92 m
13	52.1 d	2.12 m	51.6 d	2.12 m	50.4 d	1.97 m
14	51.2 s		51.1 s		52.3 s	
15	32.7 t	1.18, 1.75 m	33.2 t	1.19, 1.75 m	32.0 t	1.15, 1.62 m
16	29.7 t	1.42, 1.75 m	30.1 t	1.42, 1.90 m	30.4 t	1.25, 1.64 m
17	51.2 d	2.83 m	51.1 d	2.83 m	53.8 d	2.34 m
18	17.4 q	1.27 s	17.8 q	1.26 s	17.7 q	1.18 s
19	17.7 q	1.06 s	18.2 q	1.07 s	18.0 q	0.98 s
20	156.8 s		157.5 s		74.6 s	
21	14.8 q	2.07 s	21.3 q	2.10 s	30.1 q	1.57 s
22	123.7 d	6.27 d (11.4)	123.7 d	6.27 d (11.4)	136.8 d	6.14 d (15.0)
23	140.5 d	7.67 dd (11.4, 15.6)	140.8 d	7.66 dd (11.4, 15.6)	126.6 d	7.01 dd (15.0, 10.8)
24	128.5 d	6.18 d (15.6)	128.6 d	6.20 d (15.6)	126.6 d	6.10 d (10.8)
25	198.1 s		198.4 s		133.9 s	
26	27.1 q	2.26 s	28.1 q	2.21 s	26.3 q	1.69 s
27					18.6 q	1.61 s
28	31.7 q	2.10 s	32.1 q	2.11 s	32.1 q	2.10 s
29	16.4 q	1.65 s	16.8 q	1.65 s	16.8 q	1.62 s
30	16.8 q	0.86 s	17.1 q	0.85 s	17.5 q	0.81 s
1′	106.0 d	5.07 d (7.8)	106.5 d	5.08 d (7.8)	106.6 d	5.05 d (7.8)
2′	75.3 d	4.14 t (7.2)	75.9 d	4.14 t (7.8)	75.9 d	4.13 m
3′	79.7 d	4.30 t (8.4)	80.2 d	4.31 t (9.0)	80.1 d	4.29 m
4′	71.9 d	4.26 t (8.4)	72.2 d	4.27 t (9.0)	72.2 d	4.27 m
5′	78.2 d	4.00 m	78.7 d	4.00 m	78.7 d	3.98 m
6′	63.1 t	4.41 dd (5.4, 11.4)	63.5 t	4.41 dd (5.4, 11.4)	63.4 t	4.41 m
		4.58 br d (11.4)		4.58 dd (2.4, 12.0)		4.56 m

*2.4.6. Notoginsenoside ST11 (**6**)*

Amorphous powder; $[\alpha]_D^{20}$ −8.4 (*c* 0.57, MeOH); IR (KBr) ν_{max} 3424, 2927, 2857, 2856, 1631, 1384, 1162, 1077, 1043, 895, 609, and 575 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; ESI-MS (positive ion mode) *m/z* 759 $[M + Na]^+$; and HRESIMS (positive ion mode) *m/z* 759.4657 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{11}Na$, 759.4654).

*2.4.7. Notoginsenoside ST12 (**7**)*

Amorphous powder; $[\alpha]_D^{20}$ −4.7 (*c* 0.53, MeOH); IR (KBr) ν_{max} 3424, 2928, 2875, 2857, 1632, 1384, 1162, 1077, 1043, 895, 611, and 575 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; ESI-MS (positive ion mode) *m/z* 759 $[M + Na]^+$; and HRESIMS (positive ion mode) *m/z* 759.4658 $[M + Na]^+$ (calcd for $C_{41}H_{68}O_{11}Na$, 759.4654).

*2.4.8. Notoginsenoside ST13 (**8**)*

Amorphous powder; $[\alpha]_D^{20}$ + 0.57 (*c* 1.16, MeOH); IR (KBr) ν_{max} 3425, 2963, 2934, 2876, 1631, 1462, 1384, 1152, 1075, 1029, 928, and 587 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 3; ESI-MS (positive ion mode) *m/z* 661 $[M + Na]^+$; and HRESIMS (positive ion mode) *m/z* 639.4471 $[M + H]^+$, (calcd for $C_{36}H_{63}O_9$, 639.4467).

*2.4.9. Notoginsenoside ST14 (**9**)*

Amorphous powder; $[\alpha]_D^{20}$ −1.8 (*c* 0.51, MeOH); IR (KBr) ν_{max} 3424, 2959, 2929, 2876, 2854, 1632, 1453, 1383, 1153, 1076, 1031, 929, 640, and 596 cm^{-1}; ^{1}H and ^{13}C NMR data see Table 1; ESI-MS (negative ion mode) *m/z* 671 $[M + Cl]^-$; and HRESIMS (positive ion mode) *m/z* 659.4133 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_9Na$, 659.4130).

2.5. Cytotoxic bioassay

As previously reported [18].

2.6. Neurite outgrowth-promoting assay

The neurotrophic activities of the test compounds were examined according to an assay using PC12 cells (Kunming Institute of Zoology, Chinese Academy of Science) as reported [19]. Briefly, PC12 cells were maintained in F12 medium supplemented with 12.5% horse serum (HS) (Hyclone), and 2.5% fetal bovine serum (FBS) (Hyclone), and incubated at 5% CO_2 and 37 °C. Test compounds were dissolved in DMSO. For the neurite outgrowth-promoting activity bioassay, PC12 cells were seeded at a density of 5×10^4 cells/mL in 48-well plate

Table 2
^{13}C and ^{1}H NMR data of compounds **3**–**5** (Record at 600 MHz for ^{1}H and 150 MHz for ^{13}C in pyridine-d_5, δ in ppm).

No.	**3**		**4**		**5**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.5 t	0.97, 1.64 m	39.5 t	1.01, 1.67 m	39.6 t	0.74, 1.49 m
2	27.9 t	1.85. 1.92 m	27.9 t	1.86, 1.94 m	27.2 t	1.83, 2.22 m
3	78.3 d	3.54 dd (4.8, 11.4)	78.5 d	3.55 dd (4.2, 11.4)	89.3 d	3.31 dd (4.2, 11.4)
4	40.4 s		40.4 s		40.1 s	
5	61.4 d	1.41 d (12.6)	61.4 d	1.45 d (10.8)	56.7 d	0.68 br d (11.4)
6	80.1 d	4.43 m	80.0 d	4.47 m	18.8 t	1.37, 1.50 m
7	45.4 t	1.93 m	45.4 t	1.97 m	35.8 t	1.22 m
		2.52 dd (3.6, 12.0)		2.57 dd (3, 12.6)		1.46 m
8	41.3 s		41.4 s		40.7 s	
9	50.7 d	1.53 m	50.7 d	1.58 m	51.3 d	1.39 m
10	39.7 s		39.7 s		37.4 s	
11	33.0 t	1.51, 2.00 m	33.2 t	1.50, 1.96 m	33.4 t	1.47, 1.95 m
12	71.5 d	3.87 m	71.5 d	3.94 m	71.9 d	3.90 m
13	52.6 d	2.11 m	50.7 d	2.19 m	53.4 d	2.12 m
14	51.3 s		51.2 s		51.7 s	
15	32.8 t	1.18, 1.73 m	32.8 t	1.23, 1.77 m	33.3 t	1.12, 1.70 m
16	30.0 t	1.40, 1.72 m	29.8 t	1.45, 1.81 m	30.4 t	1.50, 2.10 m
17	51.0 d	2.76 m	50.7 d	2.79 m	51.5 d	2.86 m
18	17.3 q	1.21 s	17.3 q	1.26 s	16.2 q	1.02 s
19	17.7 q	1.03 s	17.7 q	1.06 s	16.9 q	0.82 s
20	170.0 s		169.0 s		170.6 s	
21	15.3 q	2.30 s	21.3 q	2.10 s	15.8 q	2.33 s
22	127.4 d	6.24 d (8.4)	128.6 d	6.07 d (7.8)	127.9 d	6.29 d (8.4)
23	191.5 d	10.21 d (8.4)	190.4 d	10.46 d (7.8)	191.9 d	10.20 d (8.4)
28	31.7 q	2.08 s	31.7 q	2.12 s	28.5 q	1.32 s
29	16.3 q	1.61 s	16.3 q	1.65 s	17.0 q	1.13 s
30	16.7 q	0.79 s	16.7 q	0.89 s	17.3 q	0.96 s
1′	106.0 d	5.03 d (7.8)	106.0 d	5.07 d (7.8)	105.6 d	4.97 d (7.8)
2′	75.5 d	4.10 t (9.6)	75.4 d	4.14 t (8.4)	83.9 d	4.29 m
3′	79.7 d	4.28 t (10.8)	79.6 d	4.31 t (8.4)	78.8 d	4.36 m
4′	71.8 d	4.23 t (10.8)	71.9 d	4.26 t (9.6)	72.0 d	4.19 m
5′	78.1 d	3.97 m	78.2 d	4.00 m	78.8 d	3.97 m
6′	63.1 t	4.38 dd (6.6, 13.8)	63.1 t	4.41 dd (5.4,11.4)	63.2 t	4.39
		4.55 dd (1.8, 12.0)		4.58 dd (2.4, 11.4)		4.61 m
1″					106.5 d	5.42 d (7.2 Hz)
2″					77.6 d	4.18 m
3″					78.4 d	4.29 m
4″					72.0 d	4.40 m
5″					78.8 d	3.97 m
6″					63.1 t	4.39, 4.61 m

coated with poly-L-lysine. After 24 h, the medium was changed to that containing 10 μM of each test compounds plus 5 ng/mL nerve growth factor (NGF) (sigma), or various concentrations of NGF (50 ng/mL for the positive control, 5 ng/mL for the negative control). The final concentration of DMSO was 0.05%, and the same concentration of DMSO was added into the negative control. After 72 h incubation, the neurite outgrowth was assessed under a phase contrast microscope. Neurite processes with a length equal to or greater than the diameter of the neuron cell body were scored as neurite bearing cells. The ratio of the neurite-bearing cells to total cells (with at least 100 cells examined/view area; 5 viewing area/well) was determined and expressed as a percentage.

2.7. Acetyl cholinesterase inhibitory activity

Acetyl cholinesterase (AChE) inhibitory activity of the compounds isolated was assayed by the spectrophotometric method developed by Ellman et al. [20], with slightly modification. Tacrine was used as positive control. A mixture (totally 200 μL) with phosphate buffer (pH 8.0), test compound (50 μM) dissolved in DMSO, and acetyl cholinesterase (0.02 U/mL) was incubated for 20 min (37 °C). Then, the mixture was initiated by the addition of 40 μL of solution containing DTNB (0.625 mM) and acetylthiocholine iodide (0.625 mM) for AChE assay. The hydrolysis of acetylthiocholineor was monitored at 405 nm every 30 s for 1 h. All the actions were performed in triplicate. The percentage inhibition was calculated as follows: % inhibition = (E − S) / E × 100 (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

2.8. Acidic hydrolysis of compounds

Compounds **1**, **5** and **6** (each 6 mg) were hydrolyzed in 2 M HCl (5 mL) at 65 °C for 6 h, resp. The reaction mixture was extracted with $CHCl_3$ for three times (3 × 5 mL). The aqueous layer was neutralized with 2 M NaOH. The monosaccharides were identified as glucose in **1** and **5**, and glucose and xylose in **6**, by co-TLC with authentic sugars, eluting with chloroform/*n*-butanol/methanol/acetic acid/water 17:10:6:2:3 (Rf = 0.35 for glucose, 0.46 for xylose). After evaporated to dryness a solution

Table 3
^{13}C and ^{1}H NMR data of compounds **6**–**8** (Record at 600 MHz for ^{1}H and 150 MHz for ^{13}C in pyridine-d_5, δ in ppm).

No.	**6**		**7**		**8**	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	39.7 t	0.76, 1.48 m	39.7 t	0.78, 1.50 m	39.7 t	1.06, 1.72 m
2	26.3 t	1.82, 2.21 m	27.4 t	1.85, 2.32 m	27.9 t	1.88, 1.95 m
3	89.4 d	3.31 dd (4.2, 11.4)	89.4 d	3.31 dd (5.4, 11.4)	78.6 d	3.56 m
4	40.2 s		40.2 s		40.4 s	
5	56.8 d	0.71 br d (11.4)	56.7 d	0.72 br d (11.4)	61.4 d	1.47 d (10.8)
6	18.9 t	1.43, 1.55 m	18.9 t	1.42, 1.55 m	80.0 d	4.47 m
7	35.8 t	1.23 m	35.8 t	1.24 m	45.3 t	1.98 m
		1.48 m		1.48 m		2.57 dd (3.0, 13.2)
8	40.7 s		40.6 s		41.3 s	
9	51.2 d	1.42 m	51.3 d	1.43 m	50.2 d	1.46 m
10	37.4 s		37.9 s		39.5 s	
11	32.7 t	1.44, 1.93 m	33.2 t	1.47, 1.96 m	32.6 t	1.48, 2.05 m
12	73.0 d	3.97 m	72.8 d	3.94 m	72.4 d	3.90 m
13	51.4 d	2.01 m	52.9 d	2.11 m	50.6 d	1.58 m
14	51.3 s		51.7 s		51.1 s	
15	33.1 t	1.01, 1.67 m	33.0 t	1.15, 1.72 m	32.6 t	1.17, 1.74 m
16	29.3 t	1.53, 1.95 m	31.2 t	1.59, 2.09 m	28.3 t	1.44, 1.73 m
17	50.9 d	2.82 m	48.7 d	2.86 m	50.6 d	2.09 m
18	16.7 q	1.03 s	16.2 q	1.03 s	17.4 q	1.26 s
19	17.0 q	0.83 s	16.9 q	0.84 s	17.7 q	1.07 s
20	140.0 s		155.9 s		139.1 s	
21	13.6 q	1.84 s	108.5 t	4.93, 5.18 br s	19.9 q	1.95 s
22	123.6 d	5.53 t (7.2)	34.2 t	2.32, 2.51 m	126.0 d	5.35 t (7.2)
23	27.4 t	1.44, 2.80 m	27.2 t	1.46, 2.22 m	23.2 t	2.51, 2.56 m
24	123.6 d	5.24 t (7.2)	125.8 d	5.31 t (7.2)	45.0 t	1.78, 1.83 m
25	131.7 s		131.6 s		69.6 s	
26	25.8 q	1.63 s	26.2 q	1.67 s	30.0 q	1.41 s
27	18.2 q	1.59 s	18.2 q	1.61 s	29.8 q	1.41 s
28	28.5 q	1.32 s	28.2 q	1.33 s	31.7 q	2.11 s
29	16.2 q	1.13 s	16.7 q	1.14 s	16.4 q	1.64 s
30	17.4 q	0.99 s	17.4 q	1.00 s	16.8 q	0.86 s
1′	105.5 d	4.96 d (7.8)	105.5 d	4.97 d (7.2)	106.0 d	5.08 d (7.8)
2′	84.5 d	4.20 m	84.5 d	4.21 m	75.5 d	4.14 t (7.2)
3′	78.9 d	4.35 m	78.9 d	4.36 m	79.6 d	4.30 m
4′	72.0 d	4.20 m	72.0 d	4.21 m	71.9 d	4.27 m
5′	78.7 d	3.97 m	78.1 d	3.98 m	78.1 d	3.99 m
6′	63.2 t	4.39	63.3 t	4.39	63.1 t	4.42 m
		4.61 m		4.61 m		4.57 br d (10.8)
1″	107.5 d	5.31 d (6.6)	107.5 d	5.32 d (7.2)		
2″	77.1 d	4.17 m	77.1 d	4.17 m		
3″	78.7 d	4.20 m	78.7 d	4.21 m		
4″	71.6 d	4.28 m	71.6 d	4.28 m		
5″	68.0 t	3.73, 4.43 m	68.0 t	3.74, 4.43 m		

of the monosaccharide mixture in pyridine (2 mL) was added to L-cysteine methyl ester hydrochloride (1.5 mg) and kept at 60 °C for 1 h. Trimethylsilylimidazole (1.5 mL) was then added to the reaction mixture at an ice-water bath and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, run on a Shimadzu GC-14C gas chromatograph equipped with a 30 m × 0.32 mm i.d. 30QC2/AC-5 quartz capillary column and an H_2 flame ionization detector with the following conditions: column temperature, 180–280 °C; programmed increase, 3 °C/min; carrier gas, N_2 (1 mL/min); injector and detector temperature, 250 °C; injection volume, 4 μL; and split ratio 1/50. The configuration of the sugar moiety was determined by comparing the retention time with the derivatives of the authentic samples. The retention times of D-/L-glucose and D-/L-xylose were 19.817/21.280 min and 14.726/15.371 min, resp. The glucose in **1**, **5** and **6**, and xylose in **6** were determined to be all D configurations.

3. Results and discussion

The total saponin fraction of the steamed roots of *P. notoginseng* was applied to various column chromatographes (CC) over D-101 macro porous resin, silica gel, and Rp-18, followed with semi-preparation HPLC, to afford 23 minor dammarane type saponins (**1**–**23**). Compounds **1–4**, **8–20**, and **23** were protopanaxatriol type, and **5–7**, **21**, and **22** were protopanaxadiol type saponins. The known saponins **10**–**23** were identified as notoginsenoside R10 (**10**) [21], 20(*S*)-ginsenoside SG_2 (**11**) [22], 20(*R*)-ginsenoside SL_1 (**12**) [23], 20(*S*)-floralquinquenoside A (**13**) [24], 20(*R*)-ginsenoside SF (**14**) [25], 20(*S*)-(**15**) and 20(*R*)-(**16**) ginsenoside ST_2 [26], ginsenoside Rg_6 (**17**) [27], 3β,12β-dihydroxydammarane-(*E*)-20(22),24-diene-6-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside (**18**) [28], 20(*S*)-(**19**) and 20(*R*)-(**20**) notoginsenoside R_2 [28], 25-hydroxylginsenoside Rk_1 (**21**)

[29], 25-hydroxyl-(*E*)-20(22)-ene-ginsenoside Rg_3 (**22**) [30], and notoginsenoside T_5 (**23**) [31], respectively, by comparison of their spectroscopic data with literature values. Compounds **11–14** featuring in one peroxide group at C-24 (**11** and **12**) and C-25 (**13** and **14**) were isolated from the *P. notoginseng* for the first time. The nine new saponins were named as notoginsenosides ST6–ST14 (**1–9**).

Notoginsenoside ST6 (**1**) had a molecular formula $C_{35}H_{56}O_9$, as determined by the positive HRESIMS (*m/z* 621.3998 $[M + H]^+$, calcd 621.3997, $C_{35}H_{57}O_9$). In the ^1H and ^{13}C NMR spectra of **1** (Table 1), signals arising from a β-glucopyranosyl unit [anomeric proton at δ_H 5.07 (d, $J = 7.8$ Hz), and δ_C 106.0, 75.3, 79.7, 71.9, 78.2, 63.1] were observed, which was further confirmed to be D-glucosyl moiety on the basis of the acidic hydrolysis followed with GC analysis of its corresponding trimethylsilylated L-cysteine adduct. Besides the glucosyl signals, the left 29 carbon signals were observed, attributable to seven *tert*-methyls, six methylenes, 10 methines including three oxygenated (δ_C 78.6, 80.0, and 71.7) and three olefinic (δ_C 123.7, 140.5, and 128.5) ones, and six quaternary carbons including one olefinic (δ_C 156.8) and one carbonyl (δ_C 198.1) carbons. The ^1H NMR spectrum (Table 1) showed the existence of seven singlet methyls (δ_H 2.07 and δ_H 2.26), and three olefinic protons [δ_H 7.67 (1H, dd, $J = 11.4$, 15.6 Hz) and δ_H 6.18 (1H, d, $J = 15.6$ Hz)]. The above NMR characteristics of the aglycon part of **1** were comparable to those of 27-demethyl-(*E*,*E*)-20(22),23-dien-3β,6α,12β-trihydroxydammar-25-one [32], a protopanaxatriol triterpenoid, except for the downfield shift of the C-6 oxymethine (Δδ 11.9) in **1**. The aforementioned data suggested that the sugar moiety in **1** was located on C-6, which was confirmed by the HMBC correlation of the glucosyl H-1′ (δ_H 5.07) with the aglycon C-6 (δ_C 80.0) (Fig. S83). Therefore, the structure of notoginsenoside ST6 (**1**) was determined to be (3β,6α,12β,20*E*,23*E*)-3,6,12-trihydroxy-27-anordammar-20,23-diene-25-one-6-*O*-β-D-glucopyranoside.

Notoginsenoside ST7 (**2**) had a same molecular formula $C_{35}H_{56}O_9$ as **1**, as determined by the HRESIMS. The NMR data of **2** (Table 1) were quite similar to those of **1**. However, compared with **1**, the down-field shifts of C-20 (Δδ 0.9) and C-21 (Δδ 6.5) in **2** suggested that **2** was a stereo isomer of **1**. In ROESY spectrum of **2**, H-21 (δ_H 2.07) was correlated with H-22 (δ_H 6.27) (Fig. S83), indicating the configuration of double bond between C-20 and C-22 was *Z* form, rather than the *E* form in **1**. Thus, the structure of **2** was elucidated as (3β,6α,12β,20*Z*,23*E*)-3,6,12-trihydroxy-27-anordammar-20,23-diene-25-one-6-*O*-β-D-glucopyranoside.

The molecular formula of notoginsenosides ST8 (**3**) and ST9 (**4**) were both determined to be $C_{32}H_{52}O_9$, on the basis of the HRESIMS. The NMR data (Table 2) of **3** and **4** were similar to those of **1**, except for the signals arising from the side chain on C-17. Instead of a C-25 ketone signal in **1**, an aldehyde carbon (δ_C 191.5) appeared on the side chain of **3** and **4**. Moreover, compounds **3** and **4** lost three carbon signals, relative to **1**. The aldehyde group **3** and **4** were both assigned to be C-23, on the basis of the HMBC correlation of its corresponding proton [δ_H 10.21 (**3**), and 10.46 (**4**), H-23] with the olefinic carbon [δ_C 127.4 (**3**) and 128.6 (**4**), C-22].

Compared to those of compound **3**, the upper field chemical shift of C-20 (Δδ 1.0) and the lower field chemical shifts of C-21 (Δδ 6.0) and C-22 (Δδ 1.2) were observed for **4**, suggesting that **4** was a stereo isomer of **3**. The ROESY correlations of H-21 (δ_H 2.10) with H-22 (δ_H 6.07) in **4** revealed the double bond of C-20/C-22 in **4** was *Z* form (Fig. S83), which was different from the *E* form vinyl group in **3**. Accordingly, the structures of compounds **3** and **4** were determined to be (3β,6α,12β,20*E*)-(**3**) and (3β,6α,12β,20*Z*)-(**4**) 3,6,12-trihydroxy-24,25,26,27-tetranordammar-20(22)-ene-23-enal-6-*O*-β-D-glucopyranoside. Notoginsenosides ST8 (**3**) and ST9 (**4**) represent the first examples of a 24,25,26,27-tetranordammarane triterpenoid with an aldehyde group at C-23. They might be the oxidative cleavage derivatives of **1** and **2**, through the double bond between C-23 and C-24, respectively.

Notoginsenoside ST10 (**5**) had a molecular formula of $C_{38}H_{62}O_{13}$, as established by the HRESIMS (*m/z* 749.4088 $[M + Na]^+$, calcd 749.4083, $C_{38}H_{62}O_{13}Na$). The ^1H, ^{13}C NMR and ^1H-^1H COSY spectra (Fig. S83) of **5** (Table 2) revealed the existence of two β-glucopyranosyl units [anomeric signals at δ_H 4.97 (d, $J = 7.8$ Hz) and 5.42 (*d*, $J = 7.2$ Hz), and δ_C 105.6, 106.5], which were further confirmed to be both D configurations based on the acidic hydrolysis followed with GC analysis of its corresponding trimethylsilylated L-cysteine adduct. The NMR data belonged to the aglycon part of **5** were similar to those of **3**, except for the downfield shift of C-3 to δ_C 89.3 (Δδ 11.0), and the upfield shift of C-6 to δ_C 18.8 (Δδ 61.3). This indicated that compound **5** was a protopanaxadiol type saponin and its glycosylation occurred at the aglycon C-3 position. The construction was confirmed by the HMBC correlation of the inner glucosyl H-1′ (δ_H 4.97) with the aglycon C-3 (δ_C 89.3). Moreover, the terminal glucosyl moiety was linked to the inner glucosyl C-2′ based on the HMBC correlation from the terminal glucosyl H-1″ (δ_H 5.42) to the inner glucosyl C-2′ (δ_C 83.9) (Fig. S83). Other 2D NMR data determined the structure of notoginsenoside ST10 (**5**) to be (3β,12β,20*E*)-3,12-dihydroxy-24,25,26,27-tetranordammar-20(22)-ene-23-enal-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside.

The molecular formula of notoginsenoside ST11 (**6**) was assigned to be $C_{41}H_{68}O_{11}$, on the basis of the HRESIMS. The ^1H and ^{13}C NMR spectra (Table 3) of **6** were closely related to those of ginsenoside Rg5 [33], except that a terminal xylopyranosyl unit appeared in **6**, instead of the terminal glucopyranosyl unit in ginsenoside Rg5. Acidic hydrolysis followed with GC analysis confirmed the existence of D-glucosyl and D-xylosyl moieties in **6**. The large coupling constants [δ_H 4.96 (d, $J = 7.8$ Hz), 5.31 (*d*, $J = 6.6$ Hz)] of the anomeric protons indicated the β form for both sugar units. In the HMBC spectrum of **6** (Fig. S83), the correlation from the inner glucosyl H-1′ (δ_H 4.96) to the aglycon C-3 (δ_C 89.4), and the terminal xylosyl H-1″ (δ_H 5.31) to the inner glucosyl C-2′ (δ_C 84.5) revealed the sugar location and sequence of **6**. Other 2D NMR correlations confirmed the structure of **6** as shown in Fig. 1. Thus, the structure of notoginsenoside ST11 (**6**) was elucidated as (3β,12β,20*E*)-3,12-dihydroxydammar-20(22),24-diene-3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-glucopyranoside.

Notoginsenoside ST12 (**7**) had a molecular formula $C_{41}H_{68}O_{11}$, as elucidated from the HRESIMS. The NMR data (Table 3) of **7** were similar to those of **6**, except for the signals arising from the side chain. Instead of the trisubstituted double bond between C-20 and C-22 in **6**, a terminal double bond [δ_C 155.9 (C), 108.5 (CH_2)] appeared in **7**, which were assigned to between C-20 and C-21, due to the HMBC correlations of H-17 (δ_H 2.86) with C-20 (δ_C 155.9), C-21 (δ_C 108.5) and C-22 (δ_C 34.2), H-21 (δ_H 4.92, 5.18) with C-17 (δ_C 48.7) and C-22,

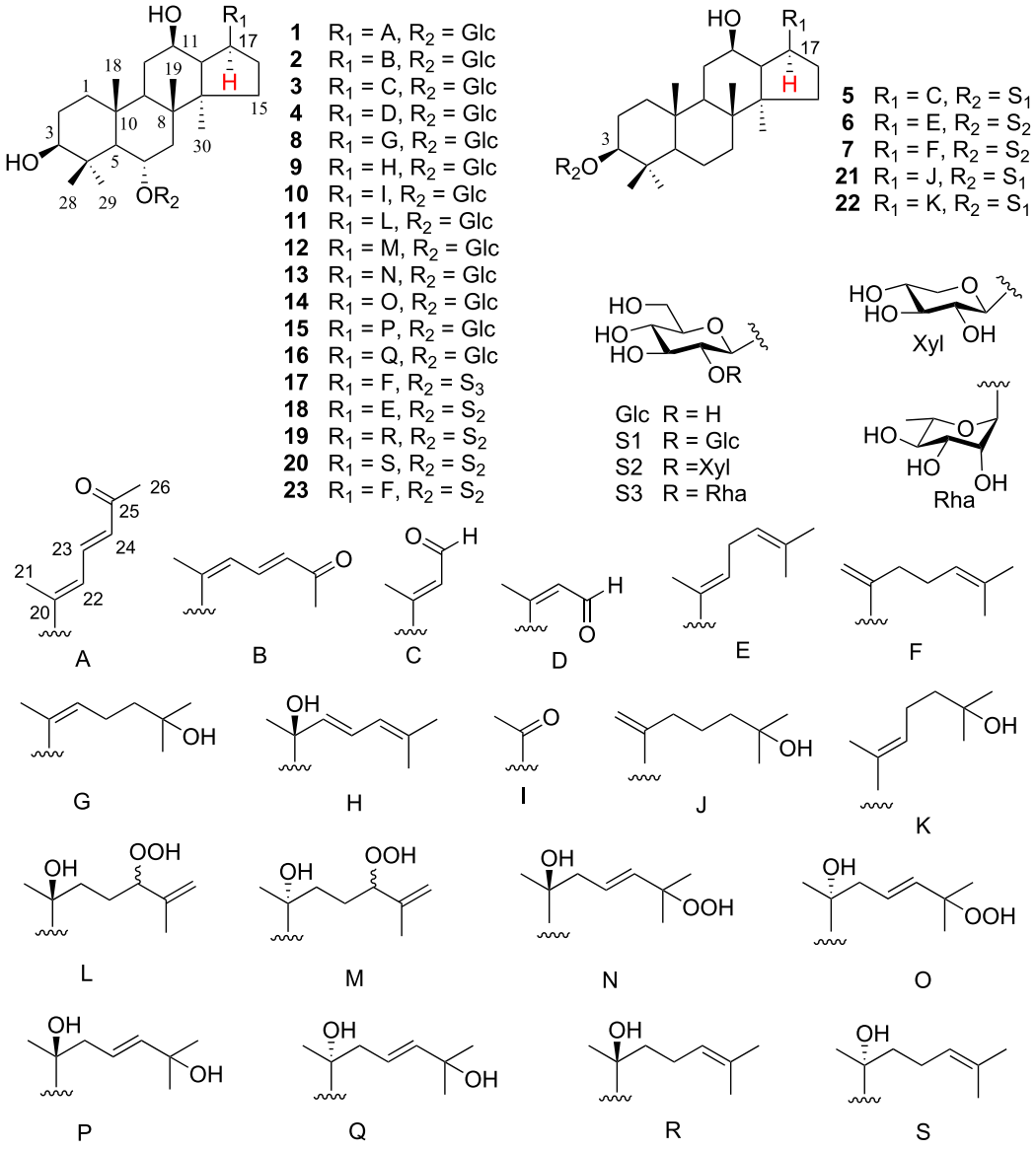

Fig. 1. The structures of compounds **1–23.**

along with H-22 (δ_H 2.32, 2.51) with C-20 and C-23 (δ_C 27.2) (Fig. S83). The other 2D NMR experiments confirmed the structure of notoginsenoside ST12 (**7**) to be (3*β*,12*β*)-3,12-dihydroxydammar-20(21), 24-diene-3-*O*-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucopyranoside.

The molecular formula of notoginsenoside ST13 (**8**) was identified as $C_{36}H_{60}O_9$, which was the same as that of sanchinoside B_1 [34], on the basis of the HRESIMS. The NMR data (Table 3) of **8** were also similar to those of sanchinoside B_1, except for the downfield shift of C-21 in **8** by 6.1 ppm. The 2D NMR experiments confirmed that both compounds had a same planar structure. However, in the ROESY spectrum of **8** (Fig. S83), the correlation between H-21 (δ_H 1.95) and H-22 (δ_H 5.35) revealed the configuration of double bond between C-20 and C-22 was *Z* form, which was opposite to the *E* form in sanchinoside B_1. Therefore, notoginsenoside ST13 (**8**) was elucidated as (3*β*, 6α, 12*β*, 20*Z*)-3, 6, 12, 25-tetrahydroxydammar-20(22)-ene-6-*O*-*β*-D-glucopyranoside.

Table 4
Activity of promoting differentiation of PC12 cells.

Samples	Differentiation rate (%)
Blank	0
NGF[a]	3.64
NGF[b]	21.72
Total saponins (50 μg/mL)	12.57
12	8.44
16	8.74
18	8.93

[a] Negative control, concentration of 5 ng/mL.
[b] Positive control, concentration of 50 ng/mL.

Notoginsenoside ST14 (**9**) possessed a molecular formula of $C_{36}H_{60}O_9$, as elucidated by the HRESIMS, corresponding to seven degrees of unsaturation. The NMR data (Table 1) of **9** were similar to those of notoginsenoside R_8 [35], except the appearance of a trisubstituted double bond [δ_C 126.6 (CH), 133.9 (C)] in **9**, instead of the one methylene signal due to C-24 (δ_C 40.2) and one quaternary carbon due to C-25 (δ_C 81.4) of the side chain in notoginsenoside R_8. The additional trisubstituted double bond was assignable to between C-24 and C-25, on the basis of the ^{1}H-^{1}H COSY correlation of H-23 (δ_H 7.01, *dd*, J = 10.8, 15.0 Hz) with both H-22 (δ_H 6.14, *d*, J = 15.0 Hz) and H-24 (δ_H 6.10, *d*, J = 10.8) (Fig. S83). Thus, the structure of notoginsenoside ST14 (**9**) was determined as (3*β*, 6α, 12*β*, 20*S*, 22*E*)-3, 6, 12, 20-tetra-hydroxydammar-22(23),24-diene-6-*O*-*β*-D-glucopyranoside.

The isolated saponins **1–23** from the steamed roots of *P. notoginseng* were evaluated for their neurite outgrowth-promoting effects, and the acetyl cholinesterase (AChE) inhibitory activities. As the results, the known saponins **12**, **16** and **18** displayed moderate enhancing potential of the neurite outgrowth of NGF-mediated PC12 cells at a concentration of 10 μM (Table 4), while **20** exhibited a moderate AChE inhibitory activity, with IC_{50} value of 13.97 μM, compared to 0.58 μM of the positive control (tacrine). Moreover, all the isolates showed no in vitro cytotoxicities against five human cancer cell lines (HL-60, SMMC-7712, A-549, MCF-7, and SW480), at a concentration of 40 μM.

In the present study, nine new minor saponins, namely notoginsenosides ST6-ST14 (**1–9**), bearing with a dehydrogenated or cleavaged side chain at C-17 were isolated from the processed roots of *P. notoginseng*, together with 14 known ones. The new saponins **3** and **4** are noteworthy in that they represent the first examples of a 24, 25, 26, 27-tetranordammarane triterpenoid with a aldehyde group at C-23. They might be the oxidative cleavage derivatives of **1** and **2**, through the double bond between C-23 and C-24, respectively. Moreover, 20(*S*)-ginsenoside SG_2 (**11**), 20(*R*)-ginsenoside SL_1 (**12**), 20(*S*)-floralquinquenoside A (**13**), and 20(*R*)-ginsenoside SF (**14**) were isolated from *P. notoginseng* for the first time. The possible transformation pathway of the isolates **1–5** and **11–16** was deduced as shown in Fig. S84. During the steaming process, hydrolysis of the terminal sugar moiety at C-6 of ginsenoside Re and notoginsenoside R_1 yielded ginsenoside Rg_1. Then, hydrolysis of glucosyl moiety at C-20 of Rg_1, Rb_1 and Rd occurred to give Rh_1 and Rg_3 and the following dehydration to give Rh_4, Rk_3, Rk_1, Rg_5, and intermediate X. Further cyclization, open-loop, dehydration and oxidative cleavage of Rg_5, Rh_4 and intermediate X gave rise to compounds **1–5**, while the oxidative and dehydration of Rh_1 gave compounds **11–16**.

The above data suggested that rich chemical diversity of saponins could be produced in the roots of *P. notoginseng*, due to the processing process. It is also noted that part of the saponins (**12**, **16**, **18**, and **20**) displayed moderate enhancing potential of the neurite outgrowth of NGF-mediated PC12 cells and AChE inhibitory activities, while all the isolates showed no in vitro cytotoxicities at a concentration of 40 μM. The results supported the traditional uses of notoginseng roots in raw or processed forms, due to their different therapeutical functions and principles. The present work may also bring out some ideas for the development of new processed strategies for traditional medicine.

Acknowledgment

The authors are grateful to the members of the analytical group at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for measuring the spectroscopic data. We also thank Prof. Yan Li's group (Kunming Institute of Botany, CAS) for the cytotoxicity assays. This work was supported by the Science and Technology Planning Project of Yunnan Province (2013FC008), the 973 Program of Ministry of Science and Technology of P. R. China (2011CB915503), the National Natural Science Foundation of China (No. 81173477), and the 12th Five Year National Science & Technology Supporting Program (2012BAI29B06).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2015.03.014.

References

[1] Jiang KY, Qin ZN. Effects of *Panax notoginseng* saponins on post hypoxic cell damage of neurons in vitro. Acta Pharmacol Sin 1995;16:399–402.
[2] White CM, Fan C, Chow M. An evaluation of the haemostatic effect of externally applied notoginseng and notoginseng total saponins. J Clin Pharmacol 2000;40:1150–3.
[3] Yuan JQ, Guo WZ, Yang BJ. 116 cases of coronary angina pectoris treated with powder composed of radix ginseng, radix notoginseng and succinum. J Tradit Chin Med 1997;17:14–7.
[4] Matsuura H, Kasai R, Tanaka O, Saruwatari YI, Fuwa T, Zhou J. Further studies on dammarane–saponins of Sanchi-Ginseng. Chem Pharm Bull 1983;31:2281–7.
[5] Li SH, Chu Y. Anti-inflammatory effects of total saponins of *Panax notoginseng*. Acta Pharmacol Sin 1999;20:551–4.
[6] Komakine N, Okasaka M, Takaishi Y, Kawazoe K, Murakami K, Takaishi Y, et al. Phytochemical and analytical studies of *Panax notoginseng* (Burk.) F.H.Chen. J Nat Med 2006;60:135–7.
[7] The State Administration of Traditional Chinese Medicine, People's Republic of China. Zhong Hua Ben Cao Jin Xuan Ben, vol. 1. Shanghai: Shanghai Science and Technology Publishers; 1996. p. 34–8.
[8] The State Pharmacopoeia Commission, People,s Republic of China. Pharmacopoeia of the People's Republic of China, vol. 1. Beijing: Chemistry Industry Press; 2005. p. 10–1.
[9] Xiong ZG, Sun JJ. Effects of *Panax notoginseng* saponin Rb1 and Rg1 on myocardial action potential and slow inward current. Acta Pharmacol Sin 1989;10:520–52.
[10] Wu W, Zhang XM, Liu PM, Li JM, Wang JF. Effects of *Panax notoginseng* saponins Rg1 on cardiac electrophysiological properties and ventricular-fibrillation threshold in dogs. Acta Pharmacol Sin 1995;16:459–63.
[11] Han JA, Hu WY, Sun ZH. Effect of *Panax notoginseng* saponin on Ca^{2+}, CaM in craniocerebral injury. CJITWM 1999;4:227–9.
[12] Li XH, Dong ZR, Hao HL, Yang JC. Effect of *Panax notoginseng* saponin on procoagulant activity and differentiation induction in NB4 cells. CJITWM 2004;1:63–6.
[13] Sengupta S, Toh SA, Sellers LA, Skepper JN, Koolwijk P, Leung HW, et al. Modulating angiogenesis the yin and the yang in ginseng. Circulation 2004;110:1219–25.
[14] Sun HX, Yang ZG, Ye YP. Structure and biological activity of protopanaxatriol-type saponins from the roots of *Panax notoginseng*. Immunopharmacology 2006;6:14–5.
[15] Liao PY, Wang D, Zhang YJ, Yang CR. Dammarane-type glycosides from steamed notoginseng. J Agric Food Chem 2008;56:1751–6.
[16] Wang D, Liao PY, Zhu HT, Chen KK, Xu M, Zhang YJ, et al. The processing of *Panax notoginseng* and transformation of its saponin components. Food Chem 2012;132:1808–13.
[17] Pei Y, Du Q, Liao PY, Chen ZP, Wang D, Yang CR, et al. Notoginsenoside ST-4 inhibits virus penetration of herpes simplex virus in vitro. J Asian Nat Prod Res 2011;13(6):498–504.

[18] Lv JJ, Xu M, Wang D, Zhu HT, Yang CR, Wang YF, et al. Cytotoxic bisbenzylisoquinoline alkaloids from *Stephania epigaea*. J Nat Prod 2013; 76:926–32.
[19] Greene LA, Tischler AS. Proc Natl Acad Sci U S A 1976;73:2424–8.
[20] Ellman GL, Courtney KD, Andres VJ, Featherstone RM. Biochem Pharmacol 1961;7:88–95.
[21] Tung NH, Song GY, Kang HK, Kin YH. New dammarane saponins from the steamed ginseng leaves. Bull Korean Chem Soc 2010;31(7):2094–6.
[22] Tung NH, Yang SY, Kim JA, Song GY, Kin YH. Dammarane saponins from the black ginseng. Bull Korean Chem Soc 2010;31(11):3423–6.
[23] Tung NH, Song GY, Minh CV, Kiem PV, Jin LG, Boo HJ, et al. Steamed ginseng-leaf components enhance cytotoxic effects on human leukemia HL-60 cells. Chem Pharm Bull 2010;58(8):1111–5.
[24] Nakamura S, Sugimoto S, Matsuda H, Yoshikawa M. Medicinal flowers. XVII. New dammarane-type triterpene glycosides from flower buds of American ginseng, *Panax quinquefolium* L. Chem. Pharm Bull 2007;55(9): 1342–8.
[25] Tung NH, Cho K, Kim JA, Song GY, Kim YH. Dammarane-type triterpene glycosides from the steamed flower-buds of *Panax ginseng*. Bull Korean Chem Soc 2010;31(5):1381–4.
[26] Qu SJ, Tan JJ, Cai JG, Ling YP, Zhang FZ, Tan CH, et al. Minor dammarane saponins from the hongshen extract of Shenmai injection. J Asian Nat Prod Res 2011;13(2):178–81.
[27] Ryu JH, Park JH, Eun JH, Jung JH, Song DH. A dammarane glycoside from Korean red ginseng. Phytochemistry 1997;44(5):931–3.
[28] Chen GT, Yang M, Lu ZQ, Zhang JQ, Huang HL, Liang Y, et al. Microbial transformation of 20 (*S*)-protopanaxatriol-type saponins by *Absidia coerulea*. J Nat Prod 2007;70:1203–6.
[29] Wang JR, Yau LF, Zhang R, Xia Y, Ma J, Ho M, et al. Transformation of ginsenosides from notoginseng by artificial gastric juice can increase cytotoxicity toward cancer cells. J Agric Food Chem 2014;62:2558–73.
[30] Chen GT, Yang M, Song Y, Lu ZQ, Zhang JQ, Huang HL, et al. Microbial transformation of ginsenoside Rb1 by *Acremonium strictum*. Appl Microbiol Biotechnol 2008;77:1345–50.
[31] Teng RW, Li HZ, Wang DZ, Yang CR. Hydrolytic reaction of plant extracts to generate molecular diversity: new dammarane glycosides from the mild acid hydrolysate of root saponins of *Panax notoginseng*. Helv Chim Acta 2004;87:1270–8.
[32] Tran TL, Kim YR, Yang JL, Oh DR, Dao TT, Oh WK. Dammarane triterpenes from the leaves of *Panax notoginseng* enhance cellular immunity. Bioorg Med Chem 2014;22:499–504.
[33] Park IH, Kim NY, Han SB, Kim JM, Kwon SW, Kim HJ, et al. Three new dammarane glycosides from heat processed ginseng. Arch Pharm Res 2002;25:428–32.
[34] Wei JX, Wang LA, Du H, Li R. Isolation and identification of sanchinoside B1 and B2 from rootlets of *Panax notoginseng* (Burk.) F. H. Chen. Acta Pharmacol Sin 1985;20:288–93.
[35] Zhao P, Liu YQ, Yang CR. Minor dammarane saponins from *Panax notoginseng*. Phytochemistry 1996;41:1419–22.