


Article

Study on Chemical Constituents of *Panax notoginseng* Leaves

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Abstract: *Panax notoginseng* (Burk.) F. H. is a genuine medicinal material in Yunnan Province. As accessories, *P. notoginseng* leaves mainly contain protopanaxadiol saponins. The preliminary findings have indicated that *P. notoginseng* leaves contribute to its significant pharmacological effects and have been administrated to tranquilize and treat cancer and nerve injury. Saponins from *P. notoginseng* leaves were isolated and purified by different chromatographic methods, and the structures of 1–22 were elucidated mainly through comprehensive analyses of spectroscopic data. Moreover, the SH-SY5Y cells protection bioactivities of all isolated compounds were tested by establishing L-glutamate models for nerve cell injury. As a result, twenty-two saponins, including eight dammarane saponins, namely notoginsenosides SL₁–SL₈ (1–8), were identified as new compounds, together with fourteen known compounds, namely notoginsenoside NL-A₃ (9), ginsenoside Rc (10), gypenoside IX (11), gypenoside XVII (12), notoginsenoside Fc (13), quinquenoside L₃ (14), notoginsenoside NL-B₁ (15), notoginsenoside NL-C₂ (16), notoginsenoside NL-H₂ (17), notoginsenoside NL-H₁ (18), vinoxenoside R₁₃ (19), ginsenoside II (20), majoroside F₄ (21), and notoginsenoside LK₄ (22). Among them, notoginsenoside SL₁ (1), notoginsenoside SL₃ (3), notoginsenoside NL-A₃ (9), and ginsenoside Rc (10) showed slight protective effects against L-glutamate-induced nerve cell injury (30 μM).

Keywords: *Panax notoginseng* leaves; protopanaxadiol saponins; SH-SY5Y cells; nerve injury



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1. Introduction

Panax notoginseng (Burk.) F. H. Chen, also called “Sanqi” in Chinese, which belongs to the *Panax* genus, family Araliaceae [1]. *P. notoginseng* is a valuable traditional Chinese medical herb, which has also been used as medicines for a long time, such as Yun-Nan-Bai-Yao, Xuesaitong capsules, and Xuesaitong injections [2]. As a genuine medicinal material in Yunnan, *P. notoginseng* is cultivated extensively in Wenshan on account of its unique geological and climatic conditions. Its roots have been widely used as tonic and main components in a great deal of compound preparations of Chinese medicine. *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 and other overseas countries [3]. As resource accessories, few studies have been published on the leaves of *P. notoginseng* in the early stage [4]. However, *P. notoginseng* leaves play vital roles in medicinal and edible value. Modern pharmacological studies reveal that *P. notoginseng* leaves have shown remarkable effects as promising tranquilization [5], antidepressant [6,7], antioxidant [8], and anticancer treatments [9–11], as well as have shown multiple benefits on the blood system, cardiovascular system [12], nervous system [13], and metabolic system. Chinese patent medicine “QiyeshenAnPian” takes *P. notoginseng* leaves as raw material, and it has remarkable effects on invigorating qi, tranquilization, stimulating blood circulation, relieving pain, etc. Approximately 3 Mt *P. notoginseng* stem-leaves are produced in China annually, while they are used for forage or discarded in the local environment [14]. Therefore, the studies of *P. notoginseng* leaves have become a hot topic nowadays.

New research has revealed that *P. notoginseng* leaves have been recognized as a rich source of dammarane triterpenoid saponins, flavonoids, polysaccharides, peptides, polyacetylenes, and fatty acids. Among them, saponins as major active ingredients are mainly protopanaxadiol saponins [15]. Cao et al. [12] identified 226 saponins from *P. notoginseng* leaves by online two-dimensional liquid chromatography. Li et al. [16] extracted *P. notoginseng* leaves with ethanol, then isolated and identified 16 monomer compounds from ethanol extract, including ginsenosides Rg₁, Rd, Re, Rb₁, Rb₃, Rg₃, Rh₂, F₂, Rc, notoginsenoside Fd, ginsenoside Mx, gypenoside Mx, and notoginsenoside R₁. Ruan et al. [17] isolated and identified 11 saponins with anti-inflammatory activity from *P. notoginseng* leaves: notoginsenosides NL-E₁-NL-E₄, NL-F₁, NL-F₂, NL-G₂, NL-H₁-NL-H₃, and all of those are 20 (S)-protopanaxadiol saponins. Yang et al. [18] isolated two new dammarane-type triterpenoids from the stems and leaves of *P. notoginseng*, namely notoginsenoside SY1 and notoginsenoside SY2.

In this paper, dammarane triterpenoid saponins from *P. notoginseng* leaves were isolated and identified. Moreover, the neuroprotective effect of saponins was tested in SH-SY5Y cells induced by L-glutamate.

2. Results and Discussion

The extract of *P. notoginseng* leaves was isolated by silica gel, Sephadex LH-20, ODS, and preparative high-performance liquid chromatography (pre-HPLC), and eight new dammarane-type triterpenoid saponins, notoginsenosides SL₁-SL₈ (1–8) were yielded (shown in Figure 1).

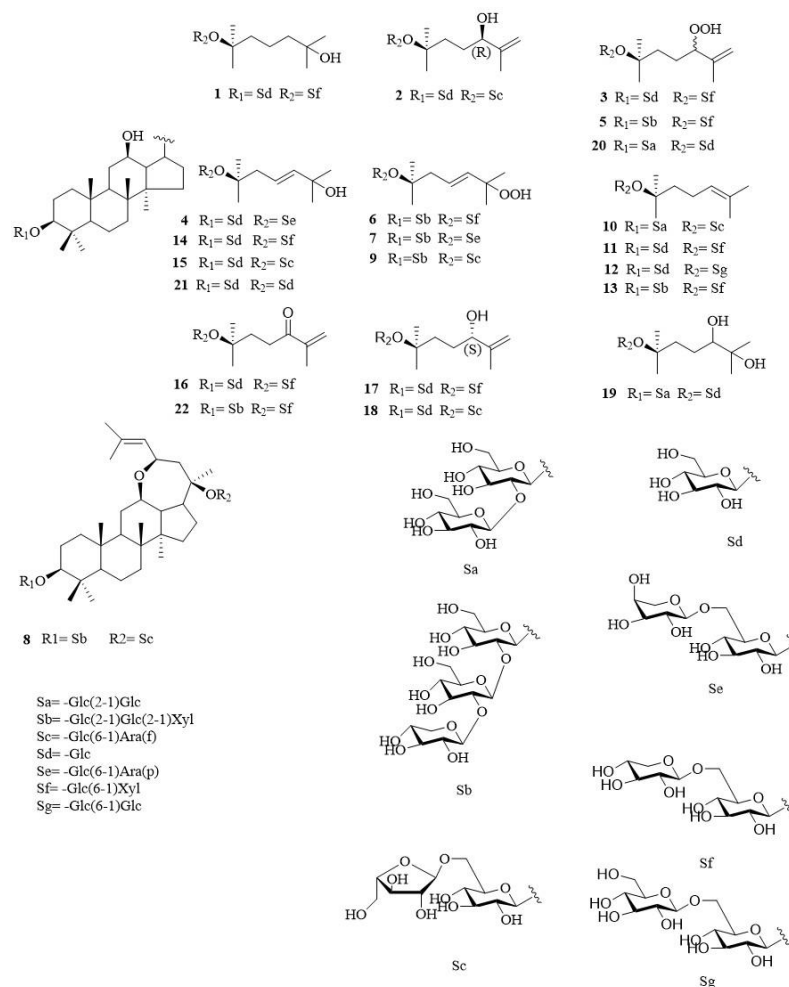


Figure 1. The structures of 1–22.

2.1. Structural Elucidation

Compound **1** (4.0 mg): a white amorphous powder. The molecular formula was assigned as $C_{47}H_{82}O_{18}$ by positive HR-ESI-MS spectrum at m/z 957.5231 $[M + Na]^+$ (calcd. For. $C_{47}H_{82}O_{18}Na$: 957.5542). $[\alpha]_D^{23} + 7.00$ (c 0.19, MeOH). The IR spectrum illustrated the presence of hydroxyl (3416 cm^{-1}). In the $^1\text{H-NMR}$ spectrum (shown in Table 1), eight methyl groups δ_H : 0.90 (3H, s), 0.81 (3H, s), 1.61 (3H, s), 1.59 (3H, s), 1.53 (3H, s), 1.30 (3H, s), 0.99 (3H, s), and 1.00 (3H, s), were perceived in the high field. In addition, four characteristic signals δ_H : 3.43 (1H, dd, $J = 4.8, 10.8$ Hz), 3.65 (1H, m), 1.34 (1H, m), and 1.46 (1H, m) were presented. The $^{13}\text{C-NMR}$ spectrum indicated 47 carbon signals (shown in Table 1), which included δ_C as: 88.6, 18.2, 70.2 and 83.0. The above indicated compound **1** was a protopanaxadiol saponin substituted by sugars at C-3 and C-20. A series of carbon signals (δ_C : 70.5, 39.8, 23.6, 27.5, 26.4, 25.2, 24.8) and hydrogen signals (δ_H : 1.93, 2.45, 1.81, 2.05) showed that compound **1** is a saponin without a double bond at C-24/25, and δ_C : 70.5 of which was protons bearing an oxygenated quaternary carbon at C-25. The HMBC correlations from H_2 -23/ H_3 -26/ H_3 -27 to C-24, and H_3 -26/ H_3 -27 to C-25 (shown in Supplementary Materials), indicated that a hydroxyl was located at C-25. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of the side chain was similar to 20(*R*)-Dammarane-3 β , 12 β , 20, 25-tetrol [19], who displays no double carbon signal, and it showed a hydroxyl in this constituent (δ_C : 70.5).

Table 1. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound **1** (δ in ppm).

NO.	δ_C^b	δ_H^b (J in Hz)	NO.	δ_C^b	δ_H^b (J in Hz)
1	38.9	0.73 m, 1.54 m	3-O-Glc		
2	26.5	1.80 m, 2.20 m	1'	106.8	4.97 (d, $J = 7.8$ Hz)
3	88.6	3.43 (dd, $J = 4.8, 10.8$ Hz)	2'	75.6	4.08 m
4	39.4	/	3'	78.7	4.19 m
5	56.1	0.70 (d, $J = 11.4$ Hz)	4'	71.6	4.24 m
6	18.2	1.34 m, 1.46 m	5'	78.2	4.04 m
7	34.8	1.46 m ^a , 1.18 (d, $J = 9.6$ Hz)	6'	62.8	4.44 (dd, $J = 6.0, 12.0$ Hz)
8	39.7	/	20-O-Glc		
9	49.8	1.36 m	1''	97.9	5.12 (d, $J = 7.8$ Hz)
10	37.3	/	2''	74.8	3.93 m
11	30.7	1.51 m ^a , 1.97 m ^a	3''	78.5	4.25 m
12	70.2	3.65 m ^a	4''	71.3	4.20 m
13	49.4	2.00 m	5''	76.6	4.14 m
14	51.2	/	6''	69.4	4.31 m, 4.71 (d, $J = 11.4$ Hz)
15	30.3	0.95 m, 1.83 m	Xyl		
16	26.1	1.30 m, 1.51 m	1'''	105.5	4.97 (d, $J = 7.2$ Hz)
17	51.6	1.42 m	2'''	74.7	4.02 m ^a
18	15.8	0.90 s	3'''	77.8	4.13 m ^a
19	16.1	0.81 s	4'''	70.9	4.14 m ^a
20	83.0	/	5'''	66.8	3.96 (t, $J = 10.2$ Hz), 4.30 m
21	23.6	1.61 s			
22	39.8	2.80 (dd, $J = 7.8, 13.8$ Hz)			
23	26.4	1.93 m, 2.45 m			
24	27.5	1.81 m, 2.05 m			
25	70.5	/			
26	24.8	1.59 s			
27	25.2	1.53 s			
28	25.9	1.30 s			
29	16.6	0.99 s			
30	17.0	1.00 s			

^a: Overlapped signals, ^b: C_5D_5N , s: singlet, d: doublet, t: triplet, m: multiplet, ': the first sugar, '': the second sugar, ''': the third sugar, /: no hydrogen.

Three anomeric carbon resonances (δ_C : 106.8 (Glc H-1'), 97.9 (Glc C-1''), 105.5 (Xyl C-1''')) of sugars were observed in the $^{13}\text{C-NMR}$ spectrum. Besides, three hydrogen signals

(δ_{H} : 4.97 (d, $J = 7.8$ Hz, Glc H-1'), 5.12 (d, $J = 7.8$ Hz, Glc H-1''), 4.97 (d, $J = 7.2$ Hz, Xyl H-1''') of anomeric carbons were present from the $^1\text{H-NMR}$ spectrum, and all were β configurations. The HMBC spectrum showed the correlations from Glc H-1' (δ_{H} : 4.97) to C-3 (δ_{C} : 88.6), Glc H-1'' (δ_{H} : 5.12) to C-20 (δ_{C} : 83.0), and Xyl H-1''' (δ_{H} : 4.97) to C-6'' (δ_{C} : 69.4) (shown in Figure 2), respectively, from which indicated Glc C-1' was connected with C-3, and Glc C-6'' was connected with Xyl C-1''', finally Glc C-1'' was connected with C-20. Multiple methods were applied to determine the configurations of sugars, such as hydrolysis, derivatization, and GC analysis. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with gypenoside IX [20], and the structure of this compound was then determined and named as notoginsenoside SL₁.

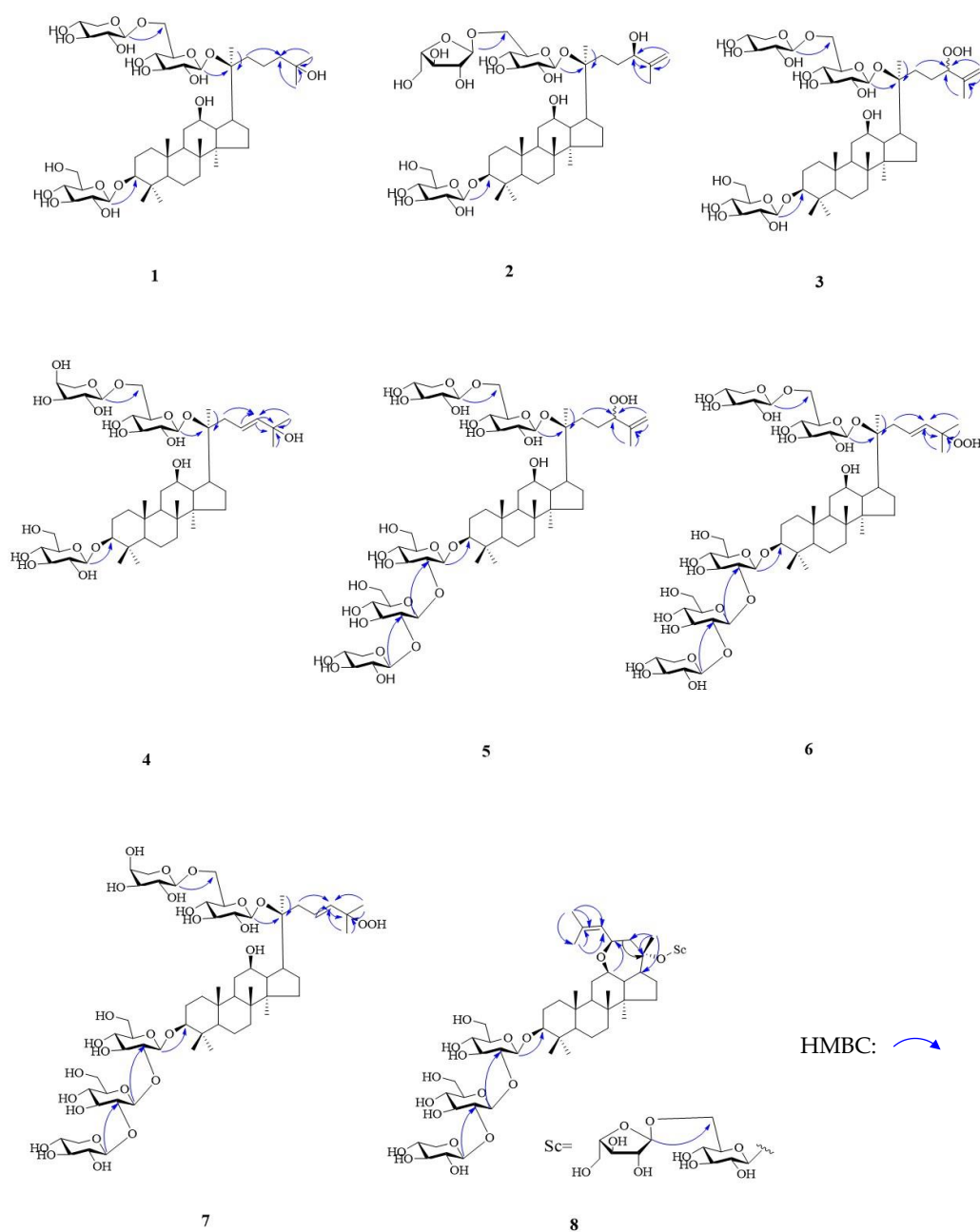


Figure 2. The key HMBC correlations of compounds 1–8.

- Compound 2 (24.0 mg) was obtained as a white amorphous powder. Its molecular formula was determined as $\text{C}_{47}\text{H}_{80}\text{O}_{18}$, evidenced by positive HR-ESI-MS data m/z 955.5245 $[\text{M} + \text{Na}]^+$ (calcd. For. $\text{C}_{47}\text{H}_{80}\text{O}_{18}\text{Na}$, 955.5232). $[\alpha]_{\text{D}}^{23} - 1.46$ (c 0.20, MeOH). As shown in

its IR spectrum, the signals of hydroxyl (3406 cm^{-1}) and double bond (1230 cm^{-1}) were significant. In the $^1\text{H-NMR}$ spectrum (shown in Table 2), seven methyl groups (δ_{H} : 0.93, 0.79, 1.63, 1.91, 1.31, 0.96 and 0.94, each 3H, s) were revealed. Moreover, signals [δ_{H} : 3.37 (1H, dd, $J = 4.8, 10.8\text{ Hz}$), 3.12 (1H, m), 1.36 (1H, m), 1.47 (1H, m)] were presented in the $^1\text{H-NMR}$ spectrum. In the $^{13}\text{C-NMR}$ spectrum (shown in Table 2), 47 carbon signals were revealed, including δ_{C} : 88.6, 18.2, 70.1, and 83.2. Compound 2 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 indicating from above. A set of carbon signals (δ_{C} : 150.0, 110.3, 22.3, 32.6, 76.6, 30.6, 17.8) and hydrogen signals (δ_{H} : 4.95, 5.26, 2.54, 2.24, 4.47, 4.95, 5.26, 1.91) indicated a hydroxyl was connected with C-24, and this compound was a saponin with a changeable side chain. Furthermore, the HMBC correlations from $\text{H}_3\text{-27}/\text{H}_2\text{-26}/\text{H}_1\text{-24}$ to C-25, and $\text{H}_2\text{-22}/\text{H}_2\text{-26}/\text{H}_3\text{-27}$ to C-24 showed the existence of a double bond between C-25 and C-26, and a hydroxyl at C-24 (shown in Supplementary Materials). The absolute configuration of the hydroxyl at C-24 was determined by its chemical shift of $^{13}\text{C-NMR}$. The chemical shift of a relatively low field corresponded to the *R* configuration of C-24, and a relatively high field corresponded to the *S* configuration of C-24. As shown in the $^{13}\text{C-NMR}$ spectrum, a hydroxyl existed in the relatively low field (δ_{C} : 76.6), and indicated the configuration of C-24 was *R*. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of side chain was similar to majoroside F₁ [21].

Table 2. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound 2 (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	38.9	0.76 m, 1.52 m	3-O-Glc		
2	26.5	1.82 m, 2.28 m	1'	106.8	4.97 (d, $J = 7.8\text{ Hz}$)
3	88.6	3.37 (dd, $J = 4.8, 10.8\text{ Hz}$)	2'	76.0	4.08 m ^a
4	39.4	/	3'	78.9	4.21 m ^a
5	56.1	0.72 m	4'	71.8	3.99 m
6	18.2	1.36 m, 1.47 m	5'	78.2	4.05 m
7	34.8	1.20 m, 1.48 m	6'	62.8	4.44 (dd, $J = 4.8, 10.8\text{ Hz}$)
8	39.7	/	20-O-Glc		
9	49.9	1.37 m	1''	97.9	5.17 (d, $J = 7.7\text{ Hz}$)
10	36.7	/	2''	75.1	3.97 m
11	30.1	1.40 m, 1.99 m ^a	3''	78.8	4.30 m
12	70.1	3.12 m	4''	71.9	4.22 m
13	49.2	2.05 m	5''	76.4	4.04 m
14	51.2	/	6''	68.3	4.09 m, 4.36 m
15	30.5	0.97 m, 1.45 m	Ara(f)		
16	26.4	1.38 m, 2.24 m	1'''	109.8	4.69 br.s
17	51.7	2.54 m	2'''	83.4	4.13 m ^a
18	15.6	0.93 s	3'''	78.7	4.16 m ^a
19	16.0	0.79 s	4'''	85.9	4.06 m ^a
20	83.2	/	5'''	62.7	3.71 (d, $J = 10.2\text{ Hz}$), 4.30 m
21	22.3	1.63 s			
22	32.6	2.54 m, 2.24 (dd, $J = 9.8, 15.2\text{ Hz}$)			
23	30.6	1.53 m, 2.23 m			
24	76.6	4.47 m			
25	150.0	/			
26	110.3	4.95 br.s, 5.26 br.s			
27	17.8	1.91 s			
28	27.9	1.31 s			
29	16.6	0.96 s			
30	17.0	0.94 s			

^a: Overlapped signals, ^b: $\text{C}_5\text{D}_5\text{N}$, s: singlet, d: doublet, m: multiplet, br.s: broad singlet, ': the first sugar, '': the second sugar, ''': the third sugar, /: no hydrogen.

Three anomeric carbon signals (δ_{C} : 106.8 (Glc C-1'), 97.9 (Glc C-1''), 109.8 (Ara(f) C-1''')) and hydrogen signals [δ_{H} : 4.97 (d, $J = 7.8\text{ Hz}$, Glc H-1'), 5.17 (d, $J = 7.7\text{ Hz}$, Glc H-1''), 4.69 (br.s, Ara (f) H-1''')] were observed, and it was revealed that the configurations of two

glucoses were β , and that arabinose (f) was α . In the HMBC spectrum, the correlations from Glc H-1' (δ_{H} : 4.97) to C-3 (δ_{C} : 88.6), Glc H-1'' (δ_{H} : 5.17) to C-20 (δ_{C} : 83.2), and Ara (f) H-1''' (δ_{H} : 4.69) to C-6'' (δ_{C} : 68.3), respectively, indicated that Glc C-1' was connected with C-3, and Glc C-6'' was connected with Ara (f) C-1''', and finally, Glc C-1'' was connected with C-20 (shown in Figure 2). Three glycosides were β -D-glucoses and α -L-arabinose determined by hydrolysis, derivatization, and GC analysis. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with notoginsenoside Fe [22]. Consequently, the structure of compound 2 was determined and named as notoginsenoside SL₂.

- Compound 3 (40.5 mg): white amorphous powder. The molecular formula of 3 was deduced to be C₄₇H₈₀O₁₉ by positive HR-ESI-MS data at m/z 971.5255 [M + Na]⁺ (calculated for C₄₇H₈₀O₁₉Na, 971.5242). $[\alpha]_{\text{D}}^{23} + 7.89$ (c 0.18, MeOH). The IR absorptions revealed the existence of hydroxyl (3421 cm⁻¹) and double bond (1261 cm⁻¹). In the $^1\text{H-NMR}$ spectrum (shown in Table 3), seven angular methyl groups (δ_{H} : 0.97, 0.81, 1.63, 1.96, 1.32, 1.01 and 0.98, each 3H, s), and two hydrogen signals of oxymethine [δ_{H} : 3.38 (1H, dd, $J = 4.2$ Hz, 11.6 Hz), 4.18 (1H, m)] were illustrated. The $^{13}\text{C-NMR}$ spectrum indicated 47 carbon signals (shown in Table 3), including three characteristic carbon signals (δ_{C} : 88.6, 18.2, and 83.0). Compound 3 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 from above. The $^1\text{H-NMR}$ signals [δ_{H} : 1.97, 2.23 (m, H₂-23), 4.80 (m, H-24), 5.09, 5.27 (br.s, H₂-26)] and $^{13}\text{C-NMR}$ signals (δ_{C} : 145.9, 113.3, 22.2, 23.6, 89.8, 26.0, 17.3) indicated that C-24 of this compound was substituted, and its lateral chain was changed. Combining the $^{13}\text{C-NMR}$ (δ_{C} : 89.8) with the data of MS, it revealed that C-24 of this compound was replaced by hydroxyperoxy. Besides, the HMBC correlations from H₂-26/H₃-27 to C-25, and H₂-23/H₂-26/H₃-27 to C-24 verified that an alkene proton signal existed between C-25 and C-26 (shown in Supplementary Materials). Furthermore, a hydroxyperoxy existed at C-24. As for the configuration of hydroxyperoxy at C-24, it was necessary to convert hydroxyperoxy into hydroxyl. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of side chain was similar to ginsenoside II [23].

Anomeric carbon signals [δ_{C} : 106.8 (Glc C-1'), 97.9 (Glc C-1''), 105.5 (Xyl C-1''')] and hydrogen signals [δ_{H} : 4.97 (d, $J = 7.6$ Hz, Glc H-1'), 5.12 (br.s, Glc H-1''), 4.97 (d, $J = 7.2$ Hz, Xyl H-1''')] were observed and revealed the configurations of two glucoses were β . In the HMBC spectrum, Glc H-1' (δ_{H} : 4.97) was correlated with C-3 (δ_{C} : 88.6), Glc H-1'' (δ_{H} : 5.12) was correlated with C-20 (δ_{C} : 83.0), and Xyl H-1'' (δ_{H} : 4.97) was correlated with C-6'' (δ_{C} : 68.3) (shown in Figure 2), respectively, from which it was indicated that Glc C-1' was connected with C-3, and Glc C-6'' was connected with Xyl C-1''', finally Glc C-1'' was connected with C-20. Three glycosides were β -D-glucoses and β -D-xylose, determined by hydrolysis, derivatization, and GC analysis. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with gypenoside IX [20]. Consequently, the structure of compound 3 was determined and named as notoginsenoside SL₃.

- Compound 4 (3.0 mg) was obtained as a white amorphous powder. Its molecular formula was determined as C₄₇H₈₀O₁₈, evidenced by positive HR-ESI-MS data (m/z 955.5247 [M + Na]⁺, calculated for C₄₇H₈₀O₁₈Na, 955.5641). $[\alpha]_{\text{D}}^{23} + 19.20$ (c 0.15, MeOH). The IR absorptions revealed the existence of hydroxyl (3433 cm⁻¹) and double bond (1260 cm⁻¹). The $^1\text{H-NMR}$ showed eight angular methyl groups (δ_{H} : 0.87, 0.95, 1.48, 1.59, 1.53, 1.30, 0.99, and 1.02, each 3H, s) and four characteristic hydrogen signals [3.43 (1H, dd, $J = 4.8$ Hz, 10.8 Hz), 3.65 (1H, m), 1.34, 1.47 (2H, m)] (shown in Table 4). In its $^{13}\text{C-NMR}$ spectrum, 47 carbon signals were indicated, including three characteristic carbon signals (δ_{C} : 89.2, 18.5, 83.8) (shown in Table 4). Compound 4 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 indicating from above. The $^1\text{H-NMR}$ signals [δ_{H} : 6.09 (d, $J = 15.5$ Hz, H₁-24), 6.23 (ddd, $J = 5.8, 8.5, 15.5$ Hz, H₁-23)] and $^{13}\text{C-NMR}$ signals (δ_{C} : 122.7, 142.2, 70.0, 17.7, 17.8) indicated that C-25 of this compound was substituted, and its lateral chain was changed. Furthermore, its $^{13}\text{C-NMR}$ indicated a quaternary carbon replaced by hydroxyl at C-25. The HMBC correlations from H₁-23/H₁-24/H₃-26/H₃-27 to C-25 and H₂-22/H₁-23/H₃-26/H₃-27 to C-24 indicated that an alkene proton signal existed

between C-23 and C-24 (shown in Supplementary Materials), and a hydroxyl at C-25. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of the side chain was similar to quinquenoside L3 [24].

Anomeric carbon signals [δ_{C} : 106.8 (Glc C-1'), 97.9 (Glc C-1''), 104.6 (Ara (p) C-1''')] and hydrogen signals [δ_{H} : 4.93 (d, $J = 7.8$ Hz, Glc H-1'), 5.14 (d, $J = 7.8$ Hz, Glc H-1''), 5.00 (d, $J = 6.0$ Hz, Ara (p) C-1''')] were observed, and revealed that the configurations of two glucoses were β and the configuration of an arabinose was α . Besides, the carbon signals of sugars [104.6 (Ara (p) C-1'''), 72.1 (Ara (p) C-2'''), 74.1 (Ara (p) C-3'''), 68.5 (Ara (p) C-4'''), and 65.6 (Ara (p) C-5''')] revealed that this arabinose was a pyranose. In the HMBC spectrum, Glc H-1' (δ_{H} : 4.93) was correlated with C-3 (δ_{C} : 89.2). Glc H-1'' (δ_{H} : 5.14) was correlated with C-20 (δ_{C} : 83.8), and Ara (p) H-1''' (δ_{H} : 5.00) was correlated with Glc C-6'' (δ_{C} : 68.3) (shown in Figure 2), respectively. It was indicated Glc C-1' was connected with C-3, and Glc C-6'' was connected with Ara (p) C-1''', and finally, Glc C-1'' was connected with C-20. The configurations of glycosides were β -D-glucoses and α -L-arabinose determined by similar methods above. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with ginsenoside Rd₂. Then, the structure of compound 4 was elucidated and named as notoginsenoside SL₄.

Table 3. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound 3 (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	38.9	0.76 m, 1.52 m	3-O-Glc		
2	26.5	1.82 m, 2.28 m	1'	106.8	4.97 (d, $J = 7.6$ Hz)
3	88.6	3.38 (dd, $J = 4.2, 11.6$ Hz)	2'	75.6	4.08 m ^a
4	39.4	/	3'	78.7	4.19 m ^a
5	56.1	0.73 (d, $J = 12.4$ Hz)	4'	71.6	4.24 m
6	18.2	1.36 m, 1.47 m	5'	78.2	4.04 m
7	34.8	1.18 m	6'	62.8	4.44 m, 4.64 (d, $J = 12.4$ Hz)
8	39.8	/	20-O-Glc		
9	50.0	1.37 m	1''	97.9	5.12 br.s
10	37.3	/	2''	75.1	3.93 m
11	30.7	1.51 m ^a , 1.99 m ^a	3''	78.8	4.25 m
12	66.9	4.18 m ^a	4''	71.9	4.20 m ^a
13	49.3	2.02 m	5''	76.4	4.14 m ^a
14	51.2	/	6''	68.3	4.10 m, 4.31 m
15	30.3	0.97 m ^a , 1.45 m ^a	Xyl		
16	26.1	1.30 m ^a , 2.25 m ^a	1'''	105.5	4.97 (d, $J = 7.2$ Hz)
17	51.6	1.42 m	2'''	74.7	4.02 m
18	15.8	0.97 s	3'''	77.8	4.13 m
19	16.1	0.81 s	4'''	70.9	4.14 m
20	83.0	/	5'''	66.8	3.96 (t, $J = 10.2$ Hz), 4.30 m
21	23.6	1.63 s			
22	22.2	1.64 m ^a			
23	26.0	1.97 m ^a , 2.23 m ^a			
24	89.8	4.80 m			
25	145.9	/			
26	113.3	5.09 br.s, 5.27 br.s			
27	17.3	1.96 s			
28	27.9	1.32 s			
29	16.6	1.01 s			
30	17.0	0.98 s			

^a: Overlapped signals, ^b: C₅D₅N, s: singlet, d: doublet, t: triplet, m: multiplet, br.s: broad singlet, ': the first sugar, '': the second sugar, ''': the third sugar, /: no hydrogen.

Table 4. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound 4 (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	39.1	0.73 m, 1.52 m	3-O-Glc		
2	26.8	1.34 m, 1.98 m	1'	106.8	4.93 (d, $J = 7.8$ Hz)
3	89.2	3.43 (dd, $J = 4.8, 10.8$ Hz)	2'	75.6	4.08 m ^a
4	39.7	/	3'	78.7	4.21 m ^a
5	56.4	0.66 m	4'	71.6	3.99 m
6	18.5	1.34 m, 1.47 m	5'	78.2	4.05 m
7	35.1	1.19 m ^a	6'	62.8	4.44 m
8	40.1	/	20-O-Glc		
9	50.1	1.35 m	1''	97.9	5.14 (d, $J = 7.8$ Hz)
10	36.9	/	2''	75.1	3.93 m
11	30.8	1.54 m ^a , 1.99 m ^a	3''	78.8	4.35 m
12	70.6	3.65 m ^a	4''	71.9	3.96 m
13	49.5	4.94 m	5''	76.4	4.04 m
14	51.5	/	6''	68.3	4.15 m, 4.25 m
15	30.5	0.97 m ^a , 1.45 m ^a	Ara (p)		
16	26.4	1.38 m ^a , 2.25 m ^a	1'''	104.6	5.00 (d, $J = 6.0$ Hz)
17	52.4	3.16 m	2'''	72.1	4.46 m
18	16.6	0.87 s	3'''	74.1	4.22 m
19	16.3	0.95 s	4'''	68.5	4.37 m
20	83.8	/	5'''	65.6	3.79 m, 4.30 m
21	23.3	1.48 s			
22	39.6	2.24 (dd, $J = 16.0, 9.6$ Hz)			
23	122.7	6.23 (ddd, $J = 5.8, 8.5, 15.5$ Hz)			
24	142.2	6.09 (d, $J = 15.5$ Hz)			
25	70.0	/			
26	17.7	1.59 s			
27	17.8	1.53 s			
28	27.9	1.30 s			
29	28.1	0.99 s			
30	17.2	1.02 s			

^a: Overlapped signals, ^b: $\text{C}_5\text{D}_5\text{N}$, s: singlet, d: doublet, m: multiple, '': the first sugar, ''': the second sugar, ''': the third sugar, /: no hydrogen.

• Compound 5 (57.1 mg) was obtained as a white amorphous powder. The molecular formula was deduced to be $\text{C}_{58}\text{H}_{98}\text{O}_{28}$ by positive HR-ESI-MS data at m/z 1265.6144 $[\text{M} + \text{Na}]^+$ (calculated for $\text{C}_{58}\text{H}_{98}\text{O}_{28}\text{Na}$, 1265.6142). $[\alpha]_{\text{D}}^{23} + 10.67$ (c 0.15, MeOH). The existence of hydroxyl (3417 cm^{-1}) and double bond (1258 cm^{-1}) was revealed from its IR spectrum. In its $^1\text{H-NMR}$ spectrum (shown in Table 5), seven angular methyl groups (δ_{H} : 0.96, 0.80, 1.64, 1.96, 1.28, 1.11 and 0.95, each 3H, s), and four characteristic hydrogen signals [δ_{H} : 3.30 (dd, $J = 3.9, 11.5$ Hz, 1H), 1.34 (m, 1H), 1.47 (m, 1H), 4.18 (m, 1H)] were indicated. The $^{13}\text{C-NMR}$ spectrum revealed 58 carbon signals (shown in Table 5), including four characteristic carbon signals (δ_{C} : 88.6, 18.2, 69.9, and 83.2). Compound 5 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 from above. The $^1\text{H-NMR}$ signals [δ_{H} : 1.97, 2.20 (m, H₂-23), 4.80 (t, $J = 6.7$ Hz, H-24), 5.09, 5.28 (br.s, H₂-26)], and $^{13}\text{C-NMR}$ signals (δ_{C} : 146.1, 113.1, 32.7, 90.0, 26.4, 17.3) indicated that C-24 of this compound was substituted and a double bond was revealed between C-25 and C-26. Combining the $^{13}\text{C-NMR}$ (δ_{C} : 90.0) with the data of MS, it revealed that C-24 of this compound was replaced by hydroxyperoxy. Besides, the HMBC correlations from H₂-23/H₂-26/H₃-27 to C-24, and H₂-26/H₃-27 to C-25 verified that an alkene proton signal existed between C-25 and C-26 (shown in Supplementary Materials), and a hydroxyperoxy existed at C-24. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of the side chain was similar to ginsenoside II [23].

Table 5. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound **5** (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	39.0	0.74 m, 1.52 m	3-O-Glc		
2	26.6	1.34 m, 1.82 m	1'	104.6	4.95 (d, $J = 7.7$ Hz)
3	88.6	3.30 (dd, $J = 3.9, 11.5$ Hz)	2'	82.3	4.13 m ^a
4	39.5	/	3'	78.5	4.37 m ^a
5	56.1	0.68 (d, $J = 11.9$ Hz)	4'	71.4	4.11 m ^a
6	18.2	1.34 m, 1.47 m	5'	78.1	3.99 m ^a
7	34.9	1.20 (d, $J = 11.9$ Hz)	6'	62.7	4.39 m ^a
8	39.8	/	-O-Glc		
9	50.0	1.35 m	1''	103.0	5.54 (d, $J = 6.8$ Hz)
10	36.7	/	2''	84.2	4.23 m ^a
11	30.7	1.51 m ^a , 1.89 m ^a	3''	77.7	4.31 m ^a
12	69.9	4.18 m ^a	4''	71.1	4.24 m ^a
13	49.3	2.00 m	5''	77.6	4.16 m ^a
14	51.1	/	6''	62.5	4.03 m, 4.39 m ^a
15	30.4	0.96 m ^a , 1.42 m ^a	-Xyl		
16	26.4	1.34 m ^a , 2.30 m ^a	1'''	106.2	5.44 (d, $J = 6.8$ Hz)
17	51.2	2.57 m	2'''	75.8	4.13 m ^a
18	15.8	0.96 s	3'''	78.5	4.37 m ^a
19	16.1	0.80 s	4'''	70.9	4.11 m ^a
20	83.2	/	5'''	67.2	3.71 (d, $J = 10.2$ Hz)
21	22.1	1.64 s	20-O-Glc		
22	32.7	1.90 m, 2.58 m	1''''	97.8	5.12 br.s
23	26.4	1.97 m ^a , 2.20 m ^a	2''''	74.7	3.88 m ^a
24	90.0	4.80 (t, $J = 6.7$ Hz)	3''''	78.1	4.17 m ^a
25	146.1	/	4''''	71.5	4.24 m ^a
26	113.1	5.09 br.s, 5.28 br.s	5''''	76.6	3.99 m ^a
27	17.3	1.96 s	6''''	69.9	4.30 m ^a , 4.76 (d, $J = 10.0$ Hz)
28	27.8	1.28 s	-Xyl		
29	16.4	1.11 s	1'''''	105.5	5.01 (d, $J = 7.4$ Hz)
30	17.2	0.95 s	2'''''	74.6	4.06 m ^a
			3'''''	78.4	4.37 m ^a
			4'''''	70.5	4.17 m ^a
			5'''''	66.8	3.76 (t, $J = 10.2$ Hz), 4.33 m ^a

^a: Overlapped signals, ^b: $\text{C}_5\text{D}_5\text{N}$, s: singlet, d: doublet, t: triplet, m: multiplet, br.s: broad singlet, '': the first sugar, ''': the second sugar, ''': the third sugar, ''': the fourth sugar, ''': the fifth sugar, /: no hydrogen.

Anomeric carbon signals [δ_{C} : 104.6 (Glc C-1'), 103.0 (Glc C-1''), 106.2 (Xyl C-1'''), 97.8 (Glc H-1'''), 105.5 (Xyl C-1''')] and hydrogen signals [(δ_{H} : 4.95 (d, $J = 7.7$ Hz, Glc H-1'), 5.54 (d, $J = 6.8$ Hz, Glc H-1''), 5.44 (d, $J = 6.8$ Hz, Xyl H-1'''), 5.12 (br.s, Glc H-1'''), and 5.01 (d, $J = 7.4$ Hz, Xyl H-1''')] were observed and revealed that the configurations of three glucoses were β . In the HMBC spectrum, Glc H-1' (δ_{H} : 4.95) was correlated with C-3 (δ_{C} 88.6), Glc H-1'' (δ_{H} : 5.54) was correlated with Glc C-2' (δ_{C} : 82.3), Xyl H-1''' (δ_{H} : 5.44) was correlated with C-2'' (δ_{C} : 84.2), Glc H-1'''' (δ_{H} : 5.12) was correlated with C-20 (δ_{C} : 83.2), and Xyl H-1''''' (δ_{H} 5.01) was correlated with Glc C-6'''' (δ_{C} : 69.9) (shown in Figure 2), respectively. From this it was indicated that Glc C-1' was connected with C-3, Glc C-1'' was connected with Glc C-2', Glc C-1''' was connected with Xyl C-2'', and Glc C-1'''' was connected with C-20, and finally Xyl C-1''''' was connected with Glc C-6'''''. The glycosides were β -D-glucoses and β -D-xyloses, which were determined by hydrolysis, derivatization, and GC analysis. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with notoginsenoside Fc [25]. Then, the structure of compound **5** was determined and named as notoginsenoside SL₅.

- Compound **6** (10.0 mg) was a white amorphous powder. The molecular formula of **6** was deduced to be $\text{C}_{58}\text{H}_{98}\text{O}_{28}$ by positive HR-ESI-MS data at m/z 1265.6149 [$\text{M} + \text{Na}$]⁺ (calculated for $\text{C}_{58}\text{H}_{98}\text{O}_{28}\text{Na}$, 1265.6142). [$\alpha_{\text{D}}^{23} + 7.45$ (c 0.15, MeOH)]. The IR absorptions revealed the existence of hydroxyl (3415 cm^{-1}) and double bond (1258 cm^{-1}). In the $^1\text{H-NMR}$

NMR spectrum (shown in Table 6), eight angular methyl groups (δ_{H} : 1.01, 0.83, 1.63, 1.62, 1.62, 1.29, 1.12, and 0.92, each 3H, s) were shown. Four characteristic hydrogen signals [δ_{H} : 3.30 (dd-like, 1H), 1.38 (m, 1H), 1.54 (m, 1H), 4.07 (m, 1H)] were revealed. The ^{13}C -NMR spectrum indicated 58 carbon signals, including four characteristic carbon signals (δ_{C} : 88.6, 18.2, 70.2, and 83.0) (shown in Table 6). Compound 6 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 from above. According to HSQC, H-23 (δ_{H} : 6.20, m) was correlated with C-23 (δ_{C} : 126.5), and H-24 (δ_{H} : 6.15, br.s) was correlated with C-24 (δ_{C} : 137.8), which indicated that a double bond existed between C-23 and C-24. Combining MS spectrum with carbon spectrum (δ_{C} : 81.1), a hydroperoxyl was presented at C-25. Furthermore, the HMBC correlations from H₃-26/H₃-27/H₁-24/H₁-23 to C-25 and H₃-27/H₃-26/H₁-23/H₂-22 to C-24 verified that an alkene proton signal existed between C-23 and C-24, and a hydroxyperoxy existed at C-25 (shown in Supplementary Materials). The ^1H -NMR and ^{13}C -NMR data of the side chain was similar to notoginsenoside E [26].

Table 6. ^1H -NMR (600 MHz) and ^{13}C -NMR (150 MHz) data of compound 6 (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	38.9	0.74 m, 1.52 m	3-O-Glc		
2	26.5	1.33 m, 1.81 m	1'	104.6	4.96 (d, $J = 6.2$ Hz)
3	88.6	3.30 (dd-like)	2'	82.7	4.15 m ^a
4	39.5	/	3'	77.7	4.39 m ^a
5	56.1	0.69 (d, $J = 11.7$ Hz)	4'	71.4	4.14 m ^a
6	18.2	1.38 m, 1.54 m ^a	5'	77.9	3.99 m ^a
7	34.8	1.21 (d, $J = 11.0$ Hz)	6'	62.7	4.51 m ^a
8	39.8	/	-O-Glc		
9	49.9	1.38 (d, $J = 11.7$ Hz)	1''	102.9	5.54 (dd-like)
10	36.7	/	2''	84.3	4.24 m ^a
11	30.7	1.54 m ^a , 1.90 m ^a	3''	78.1	4.31 m ^a
12	70.2	4.07 m ^a	4''	70.9	4.24 m ^a
13	49.4	2.02 m ^a	5''	77.6	4.16 m ^a
14	51.2	/	6''	62.5	4.03 m, 4.39 m ^a
15	30.3	0.98 m ^a , 1.44 m ^a	-Xyl		
16	26.1	1.46 m ^a , 2.30 m ^a	1'''	106.2	5.45 (d, $J = 6.0$ Hz)
17	51.6	2.47 m	2'''	75.8	4.14 m ^a
18	15.8	1.01 s	3'''	78.5	4.39 m ^a
19	16.1	0.83 s	4'''	71.3	4.14 m ^a
20	83.0	/	5'''	67.2	3.72 m ^a
21	23.0	1.63 s	20-O-Glc		
22	39.8	2.85 m, 3.13 m ^a	1''''	98.0	5.20 br.s
23	126.5	6.20 m ^a	2''''	74.8	3.95 m ^a
24	137.8	6.15 br.s	3''''	78.1	4.21 m ^a
25	81.1	/	4''''	71.5	4.24 m ^a
26	24.8	1.62 s	5''''	76.6	3.99 m ^a
27	25.2	1.62 s	6''''	69.7	4.35 m ^a , 4.76 (d, $J = 11.2$ Hz)
28	27.8	1.29 s	-Xyl		
29	16.3	1.12 s	1'''''	105.4	5.00 (d, $J = 6.8$ Hz)
30	17.0	0.92 s	2'''''	74.7	4.05 m ^a
			3'''''	78.8	4.39 m ^a
			4'''''	70.5	4.17 m ^a
			5'''''	66.8	3.72 m ^a , 4.34 m ^a

^a: Overlapped signals, ^b: C₅D₅N, s: singlet, d: doublet, m: multiplet, br.s: broad singlet, ': the first sugar, '': the second sugar, ''': the third sugar, ''': the fourth sugar, ''': the fifth sugar, /: no hydrogen.

Anomeric carbon signals [δ_{C} : 104.6 (Glc C-1'), 102.9 (Glc C-1''), 106.2 (Xyl C-1'''), 98.0 (Glc C-1'''), 105.4 (Xyl C-1''')] and hydrogen signals [δ_{H} : 4.96 (d, $J = 6.2$ Hz, Glc H-1'), 5.54 (d-like, Glc H-1''), 5.45 (d, $J = 6.0$ Hz, Xyl H-1'''), 5.20 (br.s, Glc H-1'''), 5.00 (d, $J = 6.8$ Hz, Xyl H-1''')] were observed, and the configurations of five glycosyl signals were all β . In the HMBC spectrum, the correlations from Glc H-1' (δ_{H} : 4.96) to C-3 (δ_{C} : 88.6), Glc

H-1'' (δ_{H} : 5.54) to Glc C-2' (δ_{C} : 82.7), Xyl H-1''' (δ_{H} : 5.45) to Glc C-2'' (δ_{H} : 84.3), Glc H-1'''' (δ_{H} : 5.20) to C-20 (δ_{C} : 83.0), and Xyl H-1''''' (δ_{H} : 5.00) to Glc C-6'''' (δ_{C} : 69.7) (shown in Figure 2), respectively, from which indicated Glc C-1' was connected with C-3, Glc C-1'' was connected with Glc C-2', Glc C-1''' was connected with Xyl C-2'', and Glc C-1'''' was connected with C-20, finally Xyl C-1''''' was connected with Glc C-6'''''. Five glycosides were determined as β -D-glucoses and β -D-xyloses by same methods above. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with notoginsenoside Fc [27]. Finally, the structure of compound 6 was elucidated and named as notoginsenoside SL₆.

- Compound 7 (24.6 mg) was obtained as a white amorphous powder. The molecular formula of 7 was deduced to be C₅₈H₉₈O₂₈ by positive HR-ESI-MS data at m/z 1265.6146 [M + Na]⁺ (calculated for C₅₈H₉₈O₂₈Na, 1265.6142). $[\alpha]_{\text{D}}^{24} + 4.40$ (c 0.18, MeOH). The IR absorptions revealed the existence of hydroxyl (3425 cm⁻¹) and double bond (1257 cm⁻¹). In its $^1\text{H-NMR}$ spectrum (shown in Table 7), there were eight angular methyl groups (δ_{H} : 0.79, 0.93, 1.63, 1.61, 1.61, 1.27, 1.14, and 0.94, each 3H, s). Four characteristic hydrogen signals [δ_{H} : 3.30 (dd, $J = 4.2, 11.4$ Hz, 1H), 1.37 (m, 1H), 1.55 (m, 1H), 4.13 (m, 1H)] were revealed. The $^{13}\text{C-NMR}$ spectrum indicated 58 carbon signals, including four characteristic carbon signals (δ_{C} : 88.6, 18.2, 70.5 and 83.3) (shown in Table 7). Compound 7 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 from above. According to the hydrogen spectrum [δ_{H} : 5.20 (m, H-23), 6.15 (br.s, H-24)] and carbon spectrum [δ_{C} : 126.2 (C-23), 137.8 (C-24)], a double bond existed between C-23 and C-24. In combining MS spectrum with carbon spectrum (δ_{C} : 81.1), a hydroxyperoxy was presented at C-25. Furthermore, the HMBC correlations from H₃-26/H₃-27/H₁-24/H₁-23 to C-25, and H₃-27/H₃-26/H₁-23/H₂-22 to C-24 verified that an alkene proton signal existed between C-23 and C-24 (shown in Supplementary Materials), and a hydroxyperoxy existed at C-25. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of side chain was similar to notoginsenoside E [26].

Anomeric carbon signals (δ_{C} : 104.6 (Glc C-1'), 102.9 (Glc C-1''), 106.2 (Xyl C-1'''), 98.0 (Glc C-1''''), 104.1 (Ara (p) C-1''''')) and hydrogen signals [δ_{H} : 4.95 (d, $J = 6.2$ Hz, Glc H-1'), 5.52 (d, $J = 7.8$ Hz, Glc H-1''), 5.43 (d, $J = 6.6$ Hz, Xyl H-1'''), 5.20 (br.s, Glc H-1''''), 5.00 (d, $J = 6.8$ Hz, Ara (p) H-1''''')] were observed, what were the signals of five glycosyl and the configurations of glucoses were β and arabinose was α . In the HMBC spectrum, Glc H-1' (δ_{H} : 4.95) was correlated with C-3 (δ_{C} : 88.6). Glc H-1'' (δ_{H} : 5.52) was correlated with Glc C-2' (δ_{C} : 82.7), Xyl H-1''' (δ_{H} : 5.43) was correlated with Glc C-2'' (δ_{H} : 84.2), Glc H-1'''' (δ_{H} : 5.20) was correlated with C-20 (δ_{C} : 83.3), and Ara (p) H-1'''' (δ_{H} : 5.00) was correlated with Glc C-6'''' (δ_{C} : 68.7) (shown in Figure 2), respectively, from which indicated Glc C-1' was connected with C-3, Glc C-1'' was connected with Glc C-2', Glc C-1''' was connected with Xyl C-2'', and Glc C-1'''' was connected with C-20, and finally, Ara (p) C-1'''' was connected with Glc C-6'''''. Five glycosides were β -D-glucoses, β -D-xylose and α -L-arabinose, which was determined by same methods above. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with notoginsenoside Fz [4]. Accordingly, the structure of compound 7 was determined and named as notoginsenoside SL₇.

- Compound 8 (6.9 mg) was obtained as a white amorphous powder. The molecular formula of 8 was deduced to be C₅₈H₉₆O₂₆ by positive HR-ESI-MS data at m/z 1232.4306 [M + Na]⁺ (calculated for C₅₈H₉₆O₂₆Na, 1232.3532). $[\alpha]_{\text{D}}^{24} - 7.20$ (c 0.32, MeOH). The IR absorptions revealed the existence of hydroxyl (3412 cm⁻¹) and double bond (1265 cm⁻¹). In its $^1\text{H-NMR}$ spectrum (shown in Table 8), eight angular methyl groups (δ_{H} : 0.88, 0.92, 1.61, 1.65, 1.94, 1.27, 1.10, 0.79, each 3H, s) were shown, including two characteristic methyl groups linked to a sp² bond (δ_{H} : 1.65 and 1.94). The $^1\text{H-NMR}$ of 8 showed only an olefinic proton at δ_{H} 6.02 (d, $J = 7.6$ Hz, 1H). On the basis of four characteristic carbon signals (δ_{C} : 89.1, 18.5, 79.1 and 83.6), compound 8 was a protopanaxadiol saponin substituted by sugars at C-3 and C-20 from above. Furthermore, the HMBC correlations from H₂-22/H₁-23/H₃-26/H₃-27 to C-24 and H₁-25/H₃-26/H₃-27 to C-25 verified that an alkene proton signal existed between C-24 and C-25 (shown in Supplementary Materials). In addition, the HMBC correlations from H₁-12/H₂-22/H₁-24 to C-23 and the positive HR-ESI-MS data showed a molecule oxygen between C-12 and C-23. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of

the side chain was similar to quinquefoloside-Lb [28], whose $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data was assigned by comparing it with that in the literature.

Table 7. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound 7 (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	38.9	0.73 m, 1.52 m	3-O-Glc		
2	26.6	1.40 m, 1.95 m	1'	104.6	4.95 (d, $J = 6.2$ Hz)
3	88.6	3.30 (dd, $J = 4.2, 11.4$ Hz)	2'	82.7	4.13 m ^a
4	39.5	/	3'	77.6	4.20 m ^a
5	56.1	0.68 (d, $J = 11.3$ Hz)	4'	71.4	4.14 m
6	18.2	1.37 m, 1.55 m ^a	5'	77.6	3.90 m
7	34.9	1.20 (d, $J = 11.0$ Hz)	6'	62.7	4.39 m ^a , 4.60 m ^a
8	39.8	/	-O-Glc		
9	49.9	1.38 (d, $J = 11.7$ Hz)	1''	102.9	5.52 (d, $J = 7.8$ Hz)
10	36.7	/	2''	84.2	4.24 m ^a
11	30.7	1.53 m ^a , 1.92 m ^a	3''	78.1	3.95 m ^a
12	70.5	4.13 m ^a	4''	70.9	4.07 m ^a
13	49.4	2.00 m	5''	77.6	4.26 m ^a
14	51.2	/	6''	62.5	4.00 m, 4.37 m ^a
15	30.6	0.97 m ^a , 1.42 m ^a	-Xyl		
16	26.4	1.80 m ^a , 2.47 m ^a	1'''	106.2	5.43 (d, $J = 6.6$ Hz)
17	51.6	2.57 m ^a	2'''	75.8	4.12 m ^a
18	15.8	0.79 s	3'''	78.5	4.39 m ^a
19	16.1	0.93 s	4'''	71.3	4.12 m ^a
20	83.3	/	5'''	67.2	3.79 m ^a
21	22.4	1.63 s	-20-O-Glc		
22	39.6	1.85 m	1''''	98.0	5.20 br.s
23	126.2	5.20 m ^a	2''''	74.8	3.95 m ^a
24	137.8	6.15 br.s	3''''	78.5	4.33 m ^a
25	81.1	/	4''''	71.3	3.92 m ^a
26	24.8	1.61 s	5''''	76.4	3.99 m ^a
27	25.2	1.61 s	6''''	68.7	4.25 m ^a 4.76 (d, $J = 11.2$ Hz)
28	27.8	1.27 s	-Ara (p)		
29	16.3	1.14 s	1'''''	104.1	5.00 (d, $J = 6.8$ Hz)
30	17.0	0.94 s	2'''''	71.8	4.55 m ^a
			3'''''	73.9	4.23 m ^a
			4'''''	68.3	4.37 m ^a
			5'''''	65.1	3.79 m ^a , 4.31 m ^a

^a: Overlapped signals, ^b: C₅D₅N, s: singlet, d: doublet, m: multiplet, br.s: broad singlet, ': the first sugar, '': the second sugar, ''': the third sugar, ''': the fourth sugar, ''': the fifth sugar, /: no hydrogen.

Anomeric carbon signals (δ_{C} : 105.3 (Glc C-1'), 105.6 (Glc C-1''), 106.2 (Xyl C-1'''), 98.4 (Glc C-1'''), 110.3 (Ara (f) C-1'''')) and hydrogen signals [δ_{H} : 4.96 (d, $J = 8.3$ Hz, Glc H-1'), 5.40 (d, $J = 6.9$ Hz, Glc H-1''), 5.44 (d, $J = 6.6$ Hz, Xyl H-1'''), 5.18 (br.s, Glc H-1'''), 5.68 (d, $J = 6.8$ Hz, Ara (f) H-1'''')] were observed, which revealed five glycosyl signals existed and the configurations of glucoses were β and arabinose was α . In the HMBC spectrum, Glc H-1' (δ_{H} : 4.96) was correlated with C-3 (δ_{C} : 89.1). Glc H-1'' (δ_{H} : 5.40) was correlated with Glc C-2' (δ_{C} : 83.2), Xyl H-1''' (δ_{H} : 5.44) was correlated with Glc C-2'' (δ_{H} : 75.2), Glc H-1'''' (δ_{H} : 5.18) was correlated with C-20 (δ_{C} : 83.6), and Ara (f) H-1''''' (δ_{H} : 5.68) was correlated with Glc C-6'''' (δ_{C} : 67.2) (shown in Figure 2), respectively, from which it was indicated that Glc C-1' was connected with C-3, Glc C-1'' was connected with Glc C-2', Glc C-1''' was connected with Xyl C-2'', and Glc C-1'''' was connected with C-20, finally Ara (f) C-1''''' was connected with Glc C-6'''''. Five glycosides were β -D-glucoses, β -D-xylose and α -L-arabinose determining by same methods above. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ data of sugars was highly consistent with notoginsenoside NL-A₃ [29]. Consequently, the structure of compound 8 was elucidated and named as notoginsenoside SL₈.

Table 8. $^1\text{H-NMR}$ (600 MHz) and $^{13}\text{C-NMR}$ (150 MHz) data of compound **8** (δ in ppm).

NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)	NO.	$\delta_{\text{C}}^{\text{b}}$	$\delta_{\text{H}}^{\text{b}}$ (J in Hz)
1	38.9	0.86 m, 1.56 m	3-O-Glc		
2	26.7	1.76 m, 2.19 m	1'	105.3	4.96 (d, $J = 8.3$ Hz)
3	89.1	3.30 (dd, $J = 4.2, 11.4$ Hz)	2'	83.2	4.23 m ^a
4	39.1	/	3'	77.4	4.30 m ^a
5	56.5	0.61 (d, $J = 11.9$ Hz)	4'	71.4	4.24 m ^a
6	18.5	1.50 m, 1.40 m ^a	5'	78.1	3.94 m ^a
7	35.2	1.47 m, 1.15 m	6'	62.8	4.34 m ^a , 4.47 m ^a
8	40.1	/	-O-Glc		
9	50.2	1.51 (d, $J = 11.7$ Hz)	1''	105.6	5.40 (d, $J = 6.9$ Hz)
10	37.0	/	2''	75.2	4.08 m ^a
11	30.9	1.34 m ^a , 1.80 m ^a	3''	77.3	4.37 m ^a
12	79.1	3.72 m ^a	4''	71.7	4.13 m ^a
13	49.5	1.38 m ^a	5''	78.3	3.99 m ^a
14	51.6	/	6''	62.6	4.22 m, 4.47 m
15	31.1	1.15 m ^a , 1.43 m ^a	-Xyl		
16	26.9	2.16 m ^a , 2.24 m ^a	1'''	106.2	5.44 (d, $J = 6.6$ Hz)
17	49.8	3.61 m ^a	2'''	75.1	4.30 m ^a
18	15.9	0.88 s	3'''	78.5	4.37 m ^a
19	16.4	0.92 s	4'''	70.4	4.04 m ^a
20	83.6	/	5'''	65.8	3.75 m ^a
21	22.8	1.61 s	20-O-Glc		
22	52.7	2.16 m, 2.24 m	1''''	98.4	5.18 br.s
23	71.9	4.83 (t, $J = 8.2$ Hz)	2''''	74.8	3.95 m ^a
24	129.4	6.02 (d, $J = 7.6$ Hz)	3''''	78.5	4.33 m ^a
25	131.6	/	4''''	78.3	3.99 m ^a
26	29.7	1.65 s	5''''	71.3	4.70 m ^a
27	19.5	1.94 s	6''''	67.2	4.29 m ^a 4.76 (d, $J = 11.2$ Hz)
28	28.2	1.27 s	-Ara (f)		
29	16.7	1.10 s	1'''''	110.3	5.68 (d, $J = 6.8$ Hz)
30	17.4	0.79 s	2'''''	83.5	4.27 m ^a
			3'''''	78.8	4.41 m ^a
			4'''''	85.9	4.23 m ^a
			5'''''	62.9	4.30 m ^a , 4.40 m ^a

^a: Overlapped signals, ^b: C₅D₅N, s: singlet, d: doublet, t: triplet, m: multiplet, br.s: broad singlet, '': the first sugar, ''': the second sugar, ''': the third sugar, ''': the fourth sugar, ''': the fifth sugar, /: no hydrogen.

2.2. Bioactivity Assays

In order to clarify the neuroprotective effect of saponins from *P. notoginseng* leaves, all the isolates were tested on L-glutamate-induced cellular damage in SH-SY5Y neuroblastoma cells by using MTT assays, and VPA was used as a positive control. The concentrations of L-glutamate and VPA were determined with gradient screening method. As a result, N-SL₁ (**1**), N-SL₃ (**3**), N-NL-A₂ (**9**), and G-Rc (**10**) displayed slight activities at 30 μM (Figure 3). Then, under this concentration, in vitro potential neuroprotective activities of those compounds were investigated.

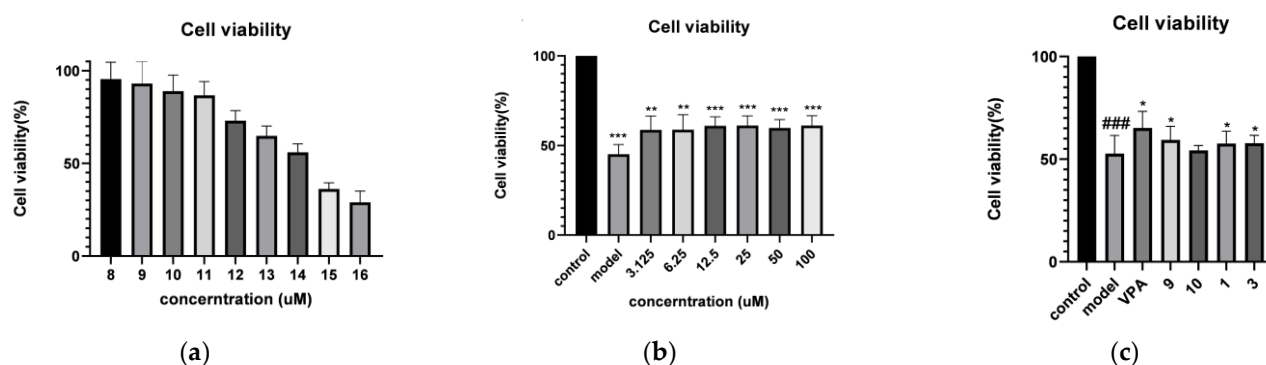


Figure 3. (a) Effect of L-glutamate pre-treatment (8, 9, 10, 11, 12, 13, 14, 15, 16 μM) on the viability of SH-SY5Y cells with glutamate-induced excitotoxicity. (b) Viability of SH-SY5Y cells pre-treated with VPA (3.125, 6.25, 12.5, 25, 50 μM) followed by exposure to 13 μM glutamate for 24 h. (c) Neuroprotective effects of compounds **1**, **3**, **9**, **10** at concentration at 30 μM in SH-SY5Y cells, respectively (** $p < 0.01$ and *** $p < 0.001$ vs. model group in **b**; ### $p < 0.001$ vs. control group, * $p < 0.05$ vs. model group in **c**).

2.3. Discussion

Studies have reported that protopanaxadiol saponins are the main active ingredient in *P. notoginseng* leaves. In recent years, more and more rare saponins with changed side chains in *P. notoginseng* leaves have been continuously discovered, and most of the rare saponins retain intact sugar chains, mainly characterized by their side chains.

As shown in results, 22 triterpene saponins from *P. notoginseng* leaves were isolated and identified. Eight saponins were identified as new compounds, and they all were saponins with changed side chains. Among them, compound **1**, **2**, **4** featured 25-hydroxyl, 25-ene-24-hydroxyl, and 23-ene-25-hydroxyl. Compounds **3** and **5** featured 25-ene-24 hydroxyperoxy. Compounds **6–7** featured 23-ene-25 hydroxyperoxy. Additionally, compound **8** featured an oxygen ring. These eight new compounds are all protopanaxadiol saponins, which further verifies the theory that protopanaxadiol saponins are mainly contained in *P. notoginseng* leaves. The rare variant ginsenosides in *P. notoginseng* leaves are more abundant than those in *P. notoginseng* roots. The growth environment of *P. notoginseng* leaves and *P. notoginseng* roots is different, which may be the main reason why *P. notoginseng* leaves are more abundant in rare variant ginsenosides. The Yunnan area, with a high-altitude and strong sunshine climate, provides strong ultraviolet environment, and the chlorophyll is used as a photosensitizer. Therefore, it is speculated that the main component saponins of *P. notoginseng* leaves may be oxidized due to strong ultraviolet environmental factors, and the structures of the side chains are changed.

The saponins in *P. notoginseng* have neuroprotective effects. Because the main active components of *P. notoginseng* leaves are also saponins, it is speculated that the saponins in *P. notoginseng* leaves also have neuroprotective activity. In this study, the neuroprotective activity of *P. notoginseng* leaves was described, and the study found that the monomeric compounds **1**, **3**, **9**, and **10** had neuroprotective activity (30 μM). Indeed, the investigation of *P. notoginseng* leaves will provide valuable information in understanding the chemical constituents of *P. notoginseng* leaves and searching new candidates for neuroprotection agents. The studies of the plant itself and the isolates are now in progress, which may provide the basic theory for following research.

3. Materials and Methods

3.1. Plant Materials

P. notoginseng plants were collected from a market. The voucher specimen was identified by associate Prof. Haizhou Li and Prof. Min Xu, and was kept in the Department of Pharmaceutical Chemistry and Biology's laboratory at Kunming University of Science and Technology (Kunming, China). Yunnan Weihe Pharmaceutical Co., Ltd. (Yuxi, China) was

entrusted to extract a sample with 60% ethanol, and made desugaring treatment with microporous adsorption resin which was then refined to get the total saponin of *P. notoginseng* leaves (Total yield was about 4%).

3.2. Experimental Instruments

The thin layer analysis was completed by three function ultraviolet analyzers (ZW-3, Jinan Sanquan Zhongshi Experimental Instrument Co., Ltd., Jinan, China). Respectively, optical rotations spectra were measured by JASCO DIP-370 (JASCO Co., Tokyo, Japan). The UV rotations spectra was measured by Shimadzu UV 2401 PC (Shimadzu, Japan). NMR analyses were carried out on a Bruker 600 MHz spectrometer (Bruker-Biospin group, Fällanden, Switzerland). HR-ESI-MS data was recorded on Waters AutoSpec Premier P776 (Agilent Technologies, Santa Clara, CA, USA). The HPLC-UV analyses were performed on a Waters 2695 series system (Waters Corporation, MA, USA) with an Agilent-C18 column (250 × 4.6 mm, 5 μm, CA, USA). A HBGK instrument equipped with a NU-3000 detector and a NP-7000C delivery system using an YMC-Pack ODS-A C18 column (250 mm × 10 mm, 5 μm) and a COSMOSIL Cholester (150 mm × 10 mm, 5 μm) was used for the semi-preparative HPLC. The TLC was performed using GF254 plates and HF-254 plates (Qingdao Marine Chemical Inc., Qingdao, China).

3.3. Extraction and Isolation

Dried parts of *P. notoginseng* (1.35 kg) leaves were dissolved with EtOH, then filtered and concentrated to a certain concentration to get a sample solution, which was subjected to a silica gel column with dichloromethane-methanol-water to afford Fr.1-Fr.14. Fr.5 (40.0 g) was separated by a silica gel column with the elution of dichloromethane-methanol-water, providing the subfractions Fr.5.1-Fr.5.5. Fr.5.3 (8.2 g) was subjected to a ODS column with methanol-water to afford Fr.5.3.1-Fr.5.3.5. Fr.5.3.2 was further purified by semi-preparative HPLC (YMC ODS-A, 29% MeCN-H₂O, $v = 10.0 \text{ mL}\cdot\text{min}^{-1}$) to attain **18** (100.9 mg) and **19** (9.5 mg). Fr.5.3.3 (200.5 mg) was subjected to an ODS column with methanol-water (25–35%) to afford Fr.5.3.1-Fr.5.3.5. Fr.5.3.3 was also purified by semi-preparative HPLC (COSMOSIL Cholester, 31% MeCN-H₂O, $v = 2.0 \text{ mL}\cdot\text{min}^{-1}$) to attain **10** (14.0 mg), **11** (11.6 mg), **12** (10.0 mg), **13** (12.0 mg), and **14** (43.0 mg). Fr.5.3.5 was purified by the same method to attain **1** (4.0 mg), **2** (24.0 mg), **3** (40.5 mg), **4** (3.0 mg), and **15** (128.2 mg).

Fr.7 (42.0 g) was applied on the column of ODS, eluting with 20–100% MeOH-H₂O, successively, providing Fr.7.1-Fr.7.6. The Fr.7.3 fraction was further separated by a silica gel column, eluting with dichloromethane-methanol-water in a gradient, and gave subfractions Fr.7.3.1-Fr.7.3.9. All parts were further separated by semi-preparative HPLC (COSMOSIL Cholester, MeCN-H₂O, $v = 2.0 \text{ mL}\cdot\text{min}^{-1}$) to attain **16** (34.2 mg), **17** (400.3 mg), **20** (43.2 mg), and **21** (7.0 mg). Fr.12 (12.8 g) was fractionated by ODS column (MeOH-H₂O, 20–100%) to yield seven subfractions (Fr.12.1-Fr.12.7) with similar methods. Fr.12.2 was subjected to silica gel (CHCl₃-MeOH-H₂O) to provide Fr.12.2.1-12.2.5. All parts were further separated by ODS column and semipreparative HPLC (COSMOSIL Cholester, MeCN-H₂O, $v = 2.0 \text{ mL}\cdot\text{min}^{-1}$) to yield **5** (57.1 mg), **6** (10.0 mg), **7** (24.6 mg), **8** (6.9 mg), **9** (10.3 mg), and **22** (17.2 mg).

3.3.1. Notoginsenoside SL₁ (**1**)

White amorphous powder; $[\alpha]_{\text{D}}^{23} + 7.00$ (c 0.19, MeOH); UV (MeOH) λ_{max} 192 (log ϵ) (5.56) nm; IR (KBr) ν_{max} 3416, 2878, 1388, 1078, 1038, and 579 cm⁻¹; ¹H and ¹³C-NMR data see Table S1 (Supplementary Materials); (+)-HRESIMS m/z 957.5231 [M + Na]⁺ (calcd. for C₄₇H₈₂O₁₈Na, 957.5542).

3.3.2. Notoginsenoside SL₂ (**2**)

White amorphous powder; $[\alpha]_{\text{D}}^{23} - 1.46$ (c 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 191 (3.95) nm; IR (KBr) ν_{max} 3406, 2878, 1645, 1265, 1230, 902, 847, 809 cm⁻¹; ¹H and ¹³C-

NMR data see Table S2; (+)-HR-ESI-MS m/z 955.5245 $[M + Na]^+$ (calcd. for $C_{47}H_{80}O_{18}Na$, 955.5232).

3.3.3. Notoginsenoside SL₃ (3)

White amorphous powder; $[\alpha]_D^{23} + 7.89$ (c 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 217 (3.65) nm; IR (KBr) ν_{max} 3421, 2879, 1630, 1261, 895 cm^{-1} ; 1H and ^{13}C -NMR data see Table S3; (+)-HR-ESI-MS m/z 971.5255 $[M + Na]^+$ (calcd. for $C_{47}H_{80}O_{19}Na$, 971.5242).

3.3.4. Notoginsenoside SL₄ (4)

White amorphous powder; $[\alpha]_D^{23} + 19.20$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 196 (3.25) nm; IR (KBr) ν_{max} 3433, 2876, 1634, 1260, 895 cm^{-1} ; 1H and ^{13}C -NMR data see Table S4; (+)-HR-ESI-MS m/z 955.5247 $[M + Na]^+$ (calcd. for $C_{47}H_{80}O_{18}Na$, 955.5647).

3.3.5. Notoginsenoside SL₅ (5)

White amorphous powder; $[\alpha]_D^{23} + 10.67$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.09); IR (KBr) ν_{max} 3417, 2926, 1635, 1453, 1312, 1258, 921, 846 cm^{-1} ; 1H and ^{13}C -NMR data see Table S5; (+)-HR-ESI-MS m/z 1265.6144 $[M + Na]^+$ (calcd. for $C_{58}H_{98}O_{28}Na$, 1265.6142).

3.3.6. Notoginsenoside SL₆ (6)

White amorphous powder; $[\alpha]_D^{23} + 7.5$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (3.77); IR (KBr) ν_{max} 3415, 2879, 1636, 1454, 1313, 1258, 1199, 1158, 922, 845 cm^{-1} ; 1H and ^{13}C -NMR data see Table S6; (+)-HR-ESI-MS m/z 1265.6149 $[M + Na]^+$ (calcd. for $C_{58}H_{98}O_{28}Na$, 1265.6142).

3.3.7. Notoginsenoside SL₇ (7)

White amorphous powder; $[\alpha]_D^{24} + 4.40$ (c 0.18, MeOH); UV (MeOH) λ_{max} (log ϵ) 201 (4.44) nm; IR (KBr) ν_{max} 3425, 2973, 1631, 1451, 1381, 1313, 1257, 881, 847 cm^{-1} ; 1H and ^{13}C -NMR data see Table S7; (+)-HR-ESI-MS m/z 1265.6146 $[M + Na]^+$ (calcd. for $C_{58}H_{98}O_{28}Na$, 1265.6142).

3.3.8. Notoginsenoside SL₈ (8)

White amorphous powder; $[\alpha]_D^{24} - 7.20$ (c 0.32, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (5.85) nm; IR (KBr) ν_{max} 3411, 2940, 1631, 1454, 1385, 1312, 1265, 894, 841 cm^{-1} ; 1H and ^{13}C -NMR data see Table S8; (+)-HR-ESI-MS m/z 1232.4306 $[M + Na]^+$ (calcd. for $C_{58}H_{96}O_{26}Na$, 1232.3532).

3.4. Hydrolysis of Sugar and Determination of Absolute Configuration

3.4.1. Acid Hydrolysis

Each compound (2.0 mg each) was dissolved in 2% HCl-dioxane (1:1) for a total of 4 mL solvent, and 80 °C under the condition responded for 5 h. After the reaction, the reactants were extracted with chloroform for 3 times (3×2 mL). Then, the water layer was neutralized with Amberlite IRA-401 and finally filtered and vacuum concentrated to obtain monosaccharide mixture.

3.4.2. Determination of Absolute Configuration

Monosaccharide mixture made the solvent in 2 mL pyridine and added L-Cysteine methyl ester hydrochloride (1.5 mg), then reacted at 60 °C for 1 h. Then, 1.5 mL N-(Trimethylsilyl) imidazole was added under the condition of an immediate ice-, and reacted at 60 °C for 30 min to obtain derivatives of monosaccharide. Next, monosaccharide derivatives were prepared by the same method. The derivative of monosaccharide and the standard were analyzed by GC. By comparing the retention time of monosaccharide derivatives of samples and standards, the types and absolute configuration of sugar in samples

were determined. The retention time of D/L-glucose, D/L-xylose, and D/L-arabinose is 19.817 min/21.223 min, 14.285 min/15.943 min, and 15.328 min/14.686 min.

3.5. Neuroprotective Effect

Glutamate (Glu) is the main transmitter of excitatory synapses in the central nervous system (CNS), and it plays an important role in excitatory of the dielectric synapse and flexibility of the synapse. Besides, it is pivotal in facilitating calcium transportation and the growth, differentiation, and recondition of intracerebral neurons [30]. Under normal circumstances, the release, ingestion, and reabsorption of glutamate are in dynamic equilibrium, whereas when glutamate is released or malabsorption occurs excessively, glutamate accumulates in the brain and causes the concentration ascension, resulting in a series of pathological reactions, finally leading to degeneration and necrosis of nerve cells [31]. Studies have confirmed that neurodegenerative diseases, such as Alzheimer disease (AD) and Parkinson disease (PD) are closely related to the neurotoxicity of glutamate [32]. Furthermore, it has been proposed that valproic acid (VPA), which is used in epileptic and bipolar disorders, may be protective against excitotoxicity insult [33]. The aerial part of *P. notoginseng* mainly contains protopanaxadiol saponins that mainly present the effect of central inhibition. Protopanaxadiol saponins are beneficial to tranquilizing, allaying excitement, and eliminating inflammation and analgesic effect. The protective effect of *P. notoginseng* leaves extract on glutamate induced SH-SY5Y cell injury was investigated in this paper.

3.5.1. Cell Culture

The study was performed using SH-SY5Y human neuroblastoma cells that were grown in Dulbecco's modified Eagle's medium with F12 (1:1) containing 10% fetal bovine serum (Dalian Meilun Biotech Co., Ltd., Dalian, China) and 0.1% penicillin-streptomycin solution. The cells were incubated at 37 °C with 5% CO₂. The SH-SY5Y cells were seeded into 96-well plates (1 × 10⁴ cells/well) and incubated in complete culture medium for 24 h prior to the addition of L-glutamate or VPA.

3.5.2. Treatment of Cell Damage by Glutamate

Cells were treated with 9 different concentrations of L-glutamate (8, 9, 10, 11, 12, 13, 14, 15, and 16 μM; Shanghai Titan Scientific Co., Ltd., Shanghai, China) to determine the glutamate toxicity in the cultured SH-SY5Y cells. The glutamate concentrations that caused a significant reduction in cell viability were determined by drawing dose-cell viability curves. The glutamate concentration of 13 μM caused a ~30% decrease in cell viability after 24 h, then used this for subsequent experiment. Cell viability was determined by MTT assays, as described below.

3.5.3. Screening of VPA Concentration

SH-SY5Y cells were treated with 6 different concentrations (3.125, 6.25, 12.5, 25, 50 and 100 μM) of VPA (Depakin, 400 mg/4 mL; lyophilized powder; Sanofi S.A., Paris, Britain) for 2 h prior to exposure to 13 μM glutamate. The effect of VPA treatment also was tested by MTT assays. The VPA concentrations caused a significant increase in cell viability determined by MTT assays. Finally, the VPA concentration of 13 μM was used as the optimum concentration.

3.5.4. Cell Viability Assay

The logarithmic phase cells were rinsed in phosphate-buffered saline (PBS) and digested with trypsin to make a single cell suspension. Then, the 96 well plate was made into a planked cell suspension and the cell concentration per well was 1 × 10⁴. Next, all models were randomly divided into three groups: the control group, model group (L-glutamate treated), positive control group (VPA treated), *P. notoginseng* leaves extract treated group, and 30 μM *P. notoginseng* leaves extract was added to administration group.

Finally, equivalent DEME culture medium was added to the control group and model group. Six multiple wells were set in each group and all cells were incubated for 15–20 h with 5% CO₂ at 37 °C. Then, the cell viability was evaluated by MTT assays.

3.5.5. MTT Assay

An MTT (Sigma-Aldrich; Merck KGaA) assay was applied to evaluate cell viability. After adding MTT solution (5 mg/mL) to each well, cells were incubated for 4 h with 5% CO₂ at 37 °C. Then, following the removal of the culture medium, 200 µL dimethyl sulfoxide was used to dissolve the formazan product. Finally, absorbance values were measured at 490 nm using a microplate reader (Tecan Trading Co., Ltd., Männedorf, Switzerland). Cell viability was calculated by considering the controls as 100%.

4. Conclusions

In conclusion, for the consideration of rational utilization of *P. notoginseng* resources, the chemical compositions of *P. notoginseng* leaves were studied mainly, especially rare saponins with altered side chains. Furthermore, their neuroprotective activities were further investigated. According to the results of the study, 22 saponins were isolated and purified, including 8 new compounds, which were all rare saponins with altered side chains. The neuroprotective activities of these saponins were determined by establishing a model of L-glutamate-induced nerve cell injury. The new compounds **1** and **3**, and known compounds **9** and **10** showed slight functions on neuroprotection. Our results not only increase the molecular diversity and bioactive diversity, but also provide a theoretical basis for promoting the rational utilization of *P. notoginseng* leaves. In addition, the study of *P. notoginseng* leaves can also promote the sustainable utilization of *P. notoginseng* medicinal resources and the economic development of the western region of China.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules28052194/s1>. Tables S1–S8: NMR data of Compounds 1–8, Figures S1–S72: key HMBC, HRESI, CD, IR and NMR spectra of Compounds 1–8.

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