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Neuroprotective triterpene saponins from the leaves of *Panax notoginseng*

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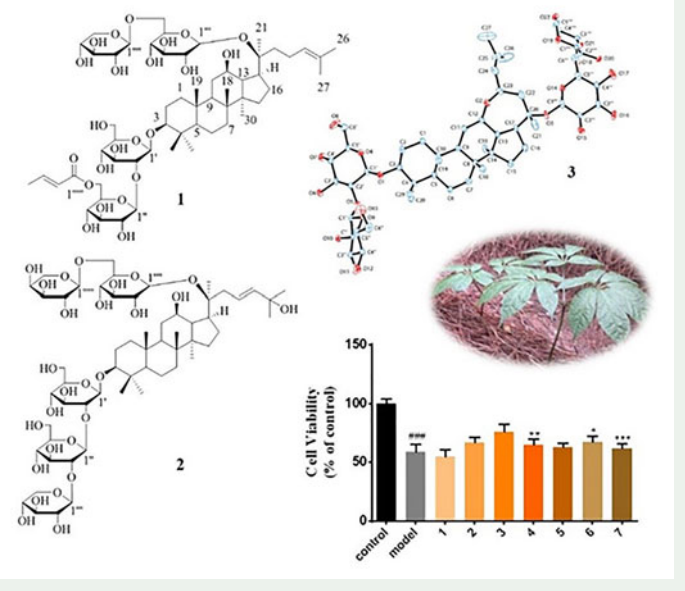
ABSTRACT

Two new triterpene saponins, namely notoginsenoside Ng5 (**1**) and notoginsenoside Ng6 (**2**) were isolated from the leaves of *Panax notoginseng*, along with five known ones. Their structures were determined by chemical methods, NMR and X-ray experiments. The absolute configuration of compound **3** with four sugar units was confirmed by single crystal X-ray analysis. Compounds **2–4** and **6** inhibited PC12 cell damage induced by serum deprivation, and increased cell viability from 58.7 ± 6.7% to 66.7 ± 4.5%, 76.1 ± 6.1%, 64.7 ± 5.2% and 67.2 ± 5.0% at 10 μM, respectively.



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1. Introduction

Panax notoginseng, also referred to as 'Chinese ginseng', is a perennial herb with dark green leaves widely cultivated in Southwestern China. To date, more than 200 chemical compounds have been isolated from this plant and its endophytic fungi, most of them are the triterpene saponins (Wang et al. 2016; Jin et al. 2017). Recent studies have shown that *Panax notoginseng* (*P. notoginseng*) saponins possessed various biological effects, including neuroprotection (Liu et al. 2017), anti-cancer (Gu et al. 2017), anti-depression (Zhang et al. 2016), anti-inflammation (Li et al. 2019) and inhibiting vascular smooth muscle cell proliferation (Fang et al. 2018), etc. After firstly reporting the isolation of three novel tricyclic tetranordammarane saponins from the *P. notoginseng* leaves (Liu et al. 2018), we continue with our investigation to further enrich the diversity of bioactive saponins of this plant. Herein, we present the discovery of seven dammarane-type saponins, including two new saponins, termed notoginsenoside Ng5 (**1**) and notoginsenoside Ng6 (**2**), and five known saponins (**3–7**) from the ethanolic and water extracts of this herb. As an important source of neuroprotective constituents, plants are receiving much attention from science world (Carito et al. 2014; Les et al. 2017; Venditti and Bianco 2019). In this study, we evaluated, in the serum deprivation-induced PC12 cell damage model, the neuroprotective effects of compounds **1–7** at 10 μM. Owing to the difficulty obtaining high quality crystal, single crystal of saponins were seldom reported. Here, for the first time, a single crystal of compound **3** was reported to confirm the absolute configuration of 12(*R*),23(*R*)-epoxy dammarane-type saponin.

2. Results and discussion

Compound **1** was isolated as a white powder, $[\alpha]_D^{25}$ –8.8 (*c* 0.10, CH_3OH). Its formula was established as $C_{57}H_{94}O_{23}$ by HR-ESI-MS data: *m/z* 1169.6059 $[M+Na]^+$; calcd for $C_{57}H_{94}O_{23}Na$, 1169.6078. The presence of hydroxyl, ester and olefin groups were deduced by the adsorption bands at 3360, 1712 and 1657 cm^{-1} in its IR spectrum. In ^{13}C NMR spectrum (Table S1, supplementary material), 57 carbon signals were observed, 30 of them were assigned to the aglycone, including eight methyl carbons (δ_C 16.0, 16.2, 16.5, 17.4, 17.9, 22.2, 25.8 and 28.0), three oxygen substituted carbons (δ_C 70.1, 83.5 and 89.2) and a pair of olefinic carbons (δ_C 125.9 and 131.0). According to the HSQC spectrum, the proton signals could be assigned to the above 30 carbons, indicating the sapogenin of **1** was 20(*S*)-protopanaxadiol (He et al. 2005). Four sugar units were suggested from four pair anomeric protons/carbons at δ_H 4.90 (1H, d, $J=7.2$ Hz, H-1′)/δ_C 104.9, δ_H 5.32 (1H, d, $J=7.8$ Hz, H-1″)/δ_C 106.2, δ_H 5.12 (1H, d, $J=7.2$ Hz, H-1‴)/δ_C 98.0 and δ_H 4.97 (1H, d, $J=7.2$ Hz, H-1⁗)/δ_C 105.7 in ^1H and ^{13}C NMR spectra (Table S1, supplementary material). Their absolute configurations were proved to be D-glucose and D-xylose by the acid hydrolysis and GC analysis of **1**. Besides, the positions and sequences of the sugar moieties were confirmed by HMBC spectrum according to the correlations (Figure S6, supplementary material) from H-1′ to C-3 (δ_C 89.2), H-1″ to C-2′ (δ_C 84.2), H-1‴ to C-20 (δ_C 83.5), and H-1⁗ to C-6‴ (δ_C 69.9). Furthermore, apart from 53 carbons due to the skeleton and sugars, the remaining signals of **1** were assigned to a crotonic group (δ_C 17.8, 123.2, 144.7 and 166.6) (Li

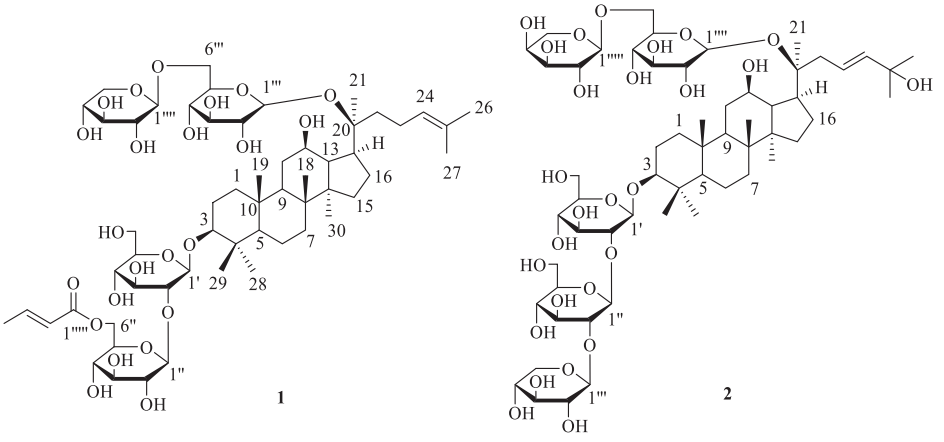

Figure 1. Structures of compounds **1** and **2**.

et al. 2018), and it was placed at C-6″ position of glucose according to the HMBC correlations from H-6″ [δ_H 4.99 (d, 11.4), 4.88 (m)] to C-1‴″ (δ_C 166.6). Consequently, the structure of compound **1** was deduced as 3-*O*-{6-*O*-[(*E*)-but-2-enoyl]-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl}-3β,12β,20(*S*)-trihydroxy-dammar-24-ene 20-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside Ng5) (Figure 1).

Compound **2** was isolated as a white powder, $[\alpha]_D^{25}+27.8$ (*c* 0.10, CH_3OH). Its formula was established as $C_{58}H_{98}O_{27}$ by HR-ESI-MS data: *m/z* 1249.6182 $[M+Na]^+$; calcd for $C_{58}H_{98}O_{27}Na$, 1249.6188. The presence of hydroxyl and olefin groups were deduced by the adsorption bands at 3385 and 1647 cm^{-1} in its IR spectrum. Its ^1H NMR spectrum (Table S1, supplementary material) presented eight aglycone methyl singlets at δ_H 0.83, 0.89, 1.00, 1.11, 1.29, 1.57, 1.57 and 1.61, and two olefinic proton signals at δ_H 6.10 (1H, d, $J=15.6$ Hz) and 6.22 (1H, m). Two olefinic carbons (δ_C 122.7 and 142.4) and five anomeric carbons (δ_C 98.3, 103.2, 104.3, 104.8 and 106.4) were observed from the ^{13}C NMR spectrum (Table S1, supplementary material) of **2**. By comparing the ^{13}C- and ^1H-NMR data with references, the core of compound **2** was identical to the aglycone of notoginsenoside Fh5 (Liu et al. 2017). The absolute configurations of sugars were proved to be D-glucose, D-xylose and L-arabinose by the acid hydrolysis and GC experiments of **2**. Furthermore, the positions and sequences of the sugar moieties were established by the correlations from H-1′ (1H, 4.95, d, $J=6.3$ Hz) to C-3 (δ_C 88.9), H-1″ (1H, 5.54, d, $J=7.8$ Hz) to C-2′ (δ_C 83.0), H-1‴ (1H, 5.44, d, $J=6.3$ Hz) to C-2″ (δ_C 84.5), H-1⁗ (1H, 5.19, d, $J=7.8$ Hz) to C-20 (δ_C 83.4), and H-1⁗″ (1H, 5.01, d, $J=6.0$ Hz) to C-6⁗ (δ_C 69.1) in HMBC spectrum (Figure S15, supplementary material). Finally, in the view of the above evidences, compound **2** was established as 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl-3β,12β,20(*S*), 25-tetrahydroxydammar-23-ene 20-*O*-α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (notoginsenoside Ng6) (Figure 1).

Compound **3** was isolated as a colourless crystal (MeOH/H_2O, 1:1). Its formula was established as $C_{53}H_{88}O_{22}$ by HR-ESI-MS data: *m/z* 1075.5673 $[M-H]^-$; calcd for $C_{53}H_{87}O_{22}$, 1075.5694. Its ^1H NMR spectrum presented eight aglycone methyls from corresponding singlets at δ_H 0.82, 0.93, 1.08, 1.11, 1.28, 1.50, 1.68 and 1.82 and an olefinic proton from

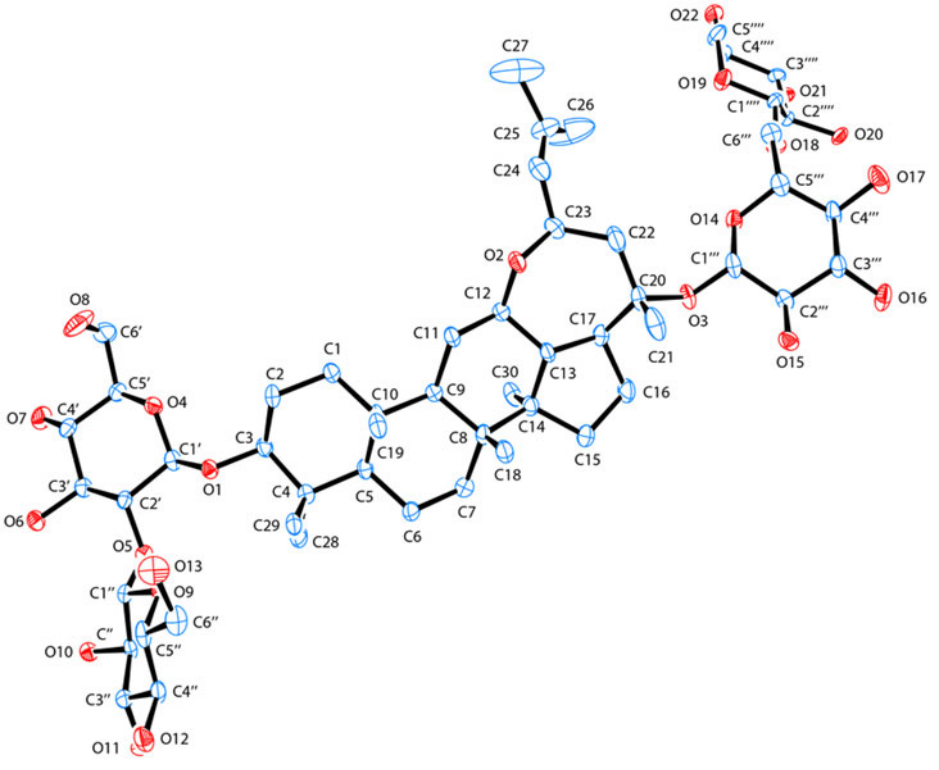

Figure 2. ORTEP drawings of compound **3**.

singlet at δ_H 5.54 (1H, d, $J = 7.2$ Hz). Its ^{13}C NMR spectrum presented two olefinic carbons (δ_C 129.2 and 131.1) and four anomeric carbons (δ_C 99.3, 105.1, 106.0 and 106.3). By comparing their NMR data with those of literature, compound **3** was determined as quinquefoloside-L_b (Jiang et al. 2008). Finally, according to the X-ray crystallography analysis [Cu Kα, Flack parameter: 0.03 (8)] of **3**, its absolute configuration was unquestionably confirmed, as depicted in Figure 2. Crystallographic data of **3** can be accessed *via* the Cambridge Crystallographic Data Centre (CCDC: 1897315).

The remaining saponins were established as notoginsenoside LX (**4**) (Li et al. 2014), ginsenoside F_3 (**5**) (Li et al. 2016), notoginsenoside LK1 (**6**) (Li et al. 2019) and notoginsenoside Fc (**7**) (Yang et al. 1983). All the isolated compounds were tested for their neuroprotective effect on serum free induced PC12 cell. Compounds **2–4** and **6** were active against serum deficiency induced PC12 cell damage (Figure S1, supplementary material), and increased cell viability at 10 μM from 58.7 ± 6.7% to 66.7 ± 4.5%, 76.1 ± 6.1%, 64.7 ± 5.2% and 67.2 ± 5.0%, respectively.

3. Experimental section

3.1. Plant materials

Leaves of *P. notoginseng* were acquired in Wenshan, Yunnan province of China, in 2015, and authenticated by associate Prof. Lin Ma of our school. A sample (ID-22816) of *P. notoginseng* leaves has been deposited at our herbarium.

3.2. Instruments and chemicals

See SI-1 in supplementary material.

3.3. Extraction and isolation

See SI-2 in supplementary material. The air-dried leaves of *P. notoginseng* (25 kg) were successively extracted with ethyl alcohol and water. The ethanolic extract was applied to a diatomite column, a D101 column, silica gel column, MPLC system and p-HPLC to give compounds **3** (25 mg), **4** (18 mg), **5** (6 mg), **6** (10 mg), **7** (8 mg). And the water extract was subjected to a PRP-512B column, Sephadex LH-20 gel CC, MPLC system and p-HPLC to obtain compounds **1** (20 mg) and **2** (8 mg).

3.3.1. Notoginsenoside Ng5 (1)

White powder, $[\alpha]_D^{25}$–8.8 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 203 (3.22) nm; IR (microscope) ν_{max}: 3360, 2936, 1712, 1657, 1384, 1074 cm^{-1}; HRESIMS *m/z* 1169.6059 $[M+Na]^+$ (calcd for $C_{57}H_{94}O_{23}Na$, 1169.6078); ^{13}C NMR (150 MHz, C_5D_5N) and ^1H NMR (600 MHz, C_5D_5N) spectral data are listed in Table S1, supplementary material.

3.3.2. Notoginsenoside Ng6 (2)

White powder, $[\alpha]_D^{25}$+ 27.8 (*c* 0.10, CH_3OH); UV (CH_3OH) λ_{max} (log ε): 203 (3.25) nm; IR (microscope) ν_{max}: 3385, 2970, 2938, 1647, 1388, 1079, 1047 cm^{-1}; HRESIMS *m/z* 1249.6182 $[M+Na]^+$ (calcd for $C_{58}H_{98}O_{27}Na$, 1249.6188); ^{13}C NMR (150 MHz, C_5D_5N) and ^1H NMR (600 MHz, C_5D_5N) spectral data are listed in Table S1, supplementary material.

3.4. Acid hydrolysis of new saponins (1 and 2)

See SI-3 in supplementary material.

3.5. Absolute configuration of sugars

See SI-4 in supplementary material.

3.6. Neuroprotection bioassays

Neuroprotection bioassays were carried out as described by Li, Chen, et al (Li et al. 2011), and compounds **1–7** were tested for neuroprotective activity against serum deprivation induced PC12 cell by using MTT method. Results were expressed as the means ± SD.

4. Conclusions

In this chemical investigation of *P. notoginseng* leaves led to the discovery of seven triterpene saponins, including two new saponins (**1** and **2**), together with five known ones (**3 − 7**). The single crystal data of 12(*R*),23(*R*)-epoxy dammarane-type saponin (**3**)

was reported for the first time. Bioactive experiment results revealed that compounds **2–4** and **6** showed moderate neuroprotective effects on serum deficiency treated PC12 cell at 10 μM, but their structure–activity relationship still need to be explored.

Disclosure statement

All authors declare no conflicts of interest.

Funding

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