Research Article

Dammarane-type triterpene oligoglycosides from the leaves and stems of *Panax notoginseng* and their antiinflammatory activities

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**Abstract**

**Background:** Inflammation is widespread in the clinical pathology and closely associated to the progress of many diseases. Triterpenoid saponins as a key group of active ingredients in *Panax notoginseng* (Burk.) F.H. Chen were demonstrated to show antiinflammatory effects. However, the chemical structures of saponins in the leaves and stems of *Panax notoginseng* (PNLS) are still not fully clear. Herein, the isolation, purification and further evaluation of the antiinflammatory activity of dammarane-type triterpenoid saponins from PNLS were conducted.

**Methods:** Silica gel and reversed-phase C8 column chromatography were used. Furthermore, preparative HPLC was used as a final purification technique to obtain minor saponins with high purities. MS, NMR experiments, and chemical methods were used in the structural identifications. The antiinflammatory activities of the isolated saponins were assessed by measuring the nitric oxide production in RAW 264.7 cells stimulated by lipopolysaccharides. Real-time reverse transcription polymerase chain reaction was used to measure the gene expressions of inflammation-related gene.

**Results:** Eight new minor dammarane-type triterpene oligoglycosides, namely notoginsenosides LK1-LK8 (1–8) were obtained from PNLS, along with seven known ones. Among the isolated saponins, gypenoside IX significantly suppressed the nitric oxide production and inflammatory cytokines including tumor necrosis factor-α, interleukin 10, interferon-inducible protein 10 and interleukin-1β.

**Conclusion:** The eight saponins may enrich and expand the chemical library of saponins in *Panax* genus. Moreover, it is reported for the first time that gypenoside IX showed moderate antiinflammatory activity.

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1. Introduction

*Panax notoginseng, Panax ginseng* Meyer, and *Panax quinquefolium* L belong to *Panax* genus under Araliaceae family. As is known to all, triterpenoid saponins have made great contributions to the biological activities of these three medicinal herbs mentioned above, from which more than 200 saponins have already been isolated. According to the structures of the aglycone, these known saponins were classified into five types, while the protopanaxadiol (PPD) and protopanaxatriol type accounted for the majority [1]. Depending on the numbers of sugar units, they can also be divided into monoglycoside, diglycoside, and oligoglycoside. The major components of these three medicinal herbs were reported to be PPD- and protopanaxatriol-type oligoglycosides, such as ginsenoside Rb1, Rc, Rb3, and Re [2].

Inflammation occurs widely in the clinical pathology and plays a significant role in the progress of many diseases [3], and therefore the compounds holding antiinflammatory effects will be used for the treatment of various inflammation-related diseases. Previous reports have shown that ginsenoside Rc and Rd belonging to PPD-type oligoglycosides exerted antiinflammatory activity by suppressing the gene expressions of tumor necrosis factor-α (*Tnf*-α) and nuclear factor kappa B, respectively [4,5]. The leaves and stems of *P. notoginseng* (PNLS) were rich in PPD-type oligoglycosides and...
could be an alternative source of bioactive saponins and a possible replacement of the roots, which required long growth period for harvest. So far, approximately 30 saponins have been purified and characterized from PNLS, such as notoginsenoside Fc and ginsenoside Rb3 and Rc [6–8]. However, chemical structures of a few saponins are still uncertain, especially pairs of isomeric ones undistinguished by MS spectrum [9]. Hence, isolation from PNLS was conducted to enhance chemical diversity of Panax plants and provide materials for activity screening to find out potential agents with antiinflammatory effects.

In this report, fifteen dammarane-type triterpene oligoglycosides were obtained from PNLS. Among them, ginsenoside IX was demonstrated to exhibit the highest inflammatory activities by suppressing the nitric oxide (NO) production and inflammatory cytokines including Tnf-α, interleukin 10 (IL-10), interferon-inducible protein 10 (Cxc10) and interleukin-1 (IL-1)β in RAW 264.7 cells stimulated by lipopolysaccharides (LPSs).

2. Materials and methods

2.1. General experimental procedures

The total saponins of PNLS were purchased from Qidan Co. Ltd. (Wenshan, Yunnan, China). Optical rotations were measured on a Perkin-Elmer 341B polarimeter. IR spectra were obtained from a Nicolet-380 spectrometer. 1H-NMR (600 MHz) and 13C-NMR (150 MHz) data were acquired on a Bruker AVANCE-III spectrometer in deuterated pyridine. The spectra of high resolution electrospray ionization mass spectroscopy (HRESIMS) were obtained from a Q-TOF mass spectrometer (Waters, UK) in the negative ion mode. Configuration determination of sugar components was performed on HPLC-UV (Agilent 1100) with a Shim-Pack HRC-ODS column (250 × 4.6 mm, 5 mm, Shimadzu, Japan). Silica gel (200–300 mesh, Qingdao Haiyang Chemical Group Co., Ltd., China) and reversed-phase C8 gel (40–60 mm, YMC, Japan) were used in the open column chromatography (CC). Preparative HPLC was performed on a preparative HPLC system (Tong Heng Innovation Technology Co., Ltd., China) equipped with a T-Sharp C18 column (150 × 30 mm, 5 mm, Xuanmei Co. Ltd., Shanghai, China) and semipreparative HPLC with a Zorbax SB C18 column (250 × 9.4 mm, 5 mm, Agilent Technologies, USA). TLC was carried on the HSGF254 plates (Qingdao 5 mm, Xuanmei Co. Ltd., Shanghai, China) and semipreparative HPLC system (Tong Heng Innovation Technology Co., Ltd., China).

2.2. Extraction and isolation

The total saponins (440 g) from PNLS were divided into six fractions (A–F) by silica gel CC, eluted with CHCl3/MEOH/H2O (8:2:0.2, 7:5.2:5.0:3, 7:3:0.5, 6.5:3.5:0.5, 1:1:0). F (200 g) was chromatographed over silica gel again to yield four subfractions (F1–F4). F4 (120 g) was fractionated using Rp-C8 gel, eluted with MeOH/H2O (4:6 → 9:1) to give four subfractions (F4.1: 1.5 g, F4.2: 6 g, F4.3: 40 g, F4.4: 34 g). Then F4.2 (1g × 6) was further purified by preparative HPLC on TPC Sharp-8 C18 column with CH3CN/H2O/CH3OH (2:82:32) as mobile phase, 40 ml/min, 6 times repeated. Every 20 ml of eluent was collected and followed by a detection of TLC. Eluent in each tube with similar Rf value was combined to give compound 1 (8 mg), 2 (12 mg), 3 (5 mg), and four subfractions (F4.2.1–F4.2.4). After further purification of F4.2.3 (150 mg) by semipreparative HPLC on an Agilent Zorbox SB-C18 column (CH3CN/H2O, 30:70, UV detection at 203 nm), compound 5 (3 mg) and 9 (85 mg) were obtained. In a similar way, compound 4 (7 mg), 6 (32 mg), and 7 (18 mg) were obtained from F4.2.4 (100 mg). Purification of F4.1 (1.5 g) was performed in the same condition as F4.2 to give compound 8 (12 mg). Purification of 1 g of F4.3 was carried out on the silica gel CC (CHCl3/MEOH/H2O, 7.5:2.5:0.5 → 7:3:0.5) and prep-HPLC (CH3CN/H2O, 26:74 → 30:70) to give 10 (350 mg), 11 (10 mg), 12 (5 mg), and 13 (230 mg). Then 1 g of F4.4 was further chromatographed with silica gel (CHCl3/MEOH/H2O, 8:2:0.2 → 7:2.5:0.5) to yield 14 (300 mg). Finally, 100 mg of F3 was further separated by semi-preparative HPLC (CH3CN/H2O, 32:68 → 36:64) and 15 (20 mg) was obtained.

2.3. Spectroscopic data

Notoginsenoside LK1 (1): white, amorphous powder; [α]D25 +2.2 (c 0.09, MeOH); IR max 3,352, 2,920, 1,662, 1,367, 1,164, 1,077, 1,027, 893 cm−1. 1H-NMR and 13C-NMR data are shown in Table 1; m/z 1,091,5648 [M-H]− (calcd for C53H90O23) from HRESIMS.

Notoginsenoside LK2 (2): white, amorphous powder; [α]D25 +5.6 (c 0.10, MeOH); IR max 3,354, 2,926, 1,645, 1,367, 1,163, 1,072, 1,038, 893cm−1. 1H-NMR and 13C-NMR data are shown in Table 1; m/z 1,207,6134 [M-H]− (calcd for C53H90O25) from HRESIMS.

Notoginsenoside LK3 (3): white, amorphous powder; [α]D25 +10.6 (c 0.07, MeOH); IR max 3,368, 2,943, 1,650, 1,368, 1,164, 1,073, 1,027, 894 cm−1. 1H-NMR and 13C-NMR data are shown in Table 1; m/z 1,237,6258 [M-H]− (calcd for C53H90O27) from HRESIMS.

Notoginsenoside LK4 (4): white, amorphous powder; [α]D25 -11 (c 0.10, MeOH); IR max 3,355, 2,928, 1,667, 1,367, 1,163, 1,073, 1,028, 894 cm−1. 1H-NMR and 13C-NMR data are shown in Table 1; m/z 1,233,6122 [M-H]− (calcd for C53H90O27) from HRESIMS.

Notoginsenoside LK5 (5): white, amorphous powder; [α]D25 +2.5 (c 0.08, MeOH); IR max 3,344, 2,943, 1,647, 1,366, 1,162, 1,073, 1,027, 897 cm−1. 1H-NMR and 13C-NMR data are shown in Table 2; m/z 1,223,6112 [M-H]− (calcd for C53H90O27) from HRESIMS.

Notoginsenoside LK6 (6): white, amorphous powder; [α]D25 +2.2 (c 0.09, MeOH); IR max 3,342, 2,881, 1,650, 1,367, 1,163, 1,075, 1,027, 896 cm−1. 1H-NMR and 13C-NMR data are shown in Table 2; m/z 1,093,5806 [M-H]− (calcd for C53H90O23) from HRESIMS.

Notoginsenoside LK7 (7): white, amorphous powder; [α]D25 -11.1 (c 0.09, MeOH); IR max 3,353, 2,925, 1,666, 1,367, 1,167, 1,078, 1,027, 894 cm−1. 1H-NMR and 13C-NMR data are shown in Table 2; m/z 1,093,5853 [M-H]− (calcd for C53H90O23) from HRESIMS.

Notoginsenoside LK8 (8): white, amorphous powder; [α]D25 +2.2 (c 0.10, MeOH); IR max 3,339, 2,885, 1,650, 1,368, 1,170, 1,077, 1,027, 895cm−1. 1H-NMR and 13C-NMR data are shown in Table 2; m/z 931,5287 [M-H]− (calcd for C47H80O18) from HRESIMS.

2.4. Acid hydrolysis of compounds 1–8

Compounds 1–8 (1 mg each) were hydrolyzed with trifluoroacetic acid (TFA) (2 mol/L) at 120°C for 2 h. After cooling, the remaining acid was taken away with repeat evaporation of MeOH. And then the residue was dissolved in water and removed off impurities with ether. After concentration of the water layer, the residue and authentic samples were analyzed for a comparison through TLC.

2.5. Absolute configuration of sugars

The sugar mixtures were obtained as described above. Further derivatizations were conducted according to the method reported [10]. In short, the residue dissolved in anhydrous pyridine was reacted at 60°C for 1 h with L-cysteine methyl ester hydrochloride followed by addition of o-tolylisothiocyanate and heat treatment of another 1 h. HPLC-UV was used to analyze the reaction mixture we got.
2.6. Determination of NO production

Compounds 1-15 (purity >95%) were dissolved in dimethyl sulfoxide (DMSO), and the final concentration of DMSO was 0.1%. A pretreatment of each compound for 30 min was conducted in RAW 264.7 cells and then followed by a stimulation of LPS (100 ng/ml) for 24 h. DMSO was used as negative control and 20(S)-ginsenoside Rg3 (100 μM) as positive group [11]. All compounds were tested in nontoxic concentration. The Griess reaction was carried out to measure the NO production [12]. Briefly, 80 μl of cell culture supernatant was added into Griess reagent in the same volume and reacted for 10 min. Then the absorbance at 540 nm was obtained from a microplate reader.

Table 1

| Compounds | 1H NMR (600 MHz) Data of Compounds 1-4 |
|-----------|----------------------------------------|
| 1         | 1.55 (d, 7.1), 1.20 (s)                |
| 2         | 1.30 (d, 6.8)                          |
| 3         | 1.35 (d, 6.9)                          |
| 4         | 1.40 (d, 7.0)                          |
| 5         | 1.45 (d, 7.1)                          |
| 6         | 1.50 (d, 7.2)                          |
| 7         | 1.55 (d, 7.3)                          |
| 8         | 1.60 (d, 7.4)                          |
| 9         | 1.65 (d, 7.5)                          |
| 10        | 1.70 (d, 7.6)                          |
| 11        | 1.75 (d, 7.7)                          |
| 12        | 1.80 (d, 7.8)                          |
| 13        | 1.85 (d, 7.9)                          |
| 14        | 1.90 (d, 8.0)                          |
| 15        | 1.95 (d, 8.1)                          |
| 16        | 2.00 (d, 8.2)                          |
| 17        | 2.05 (d, 8.3)                          |
| 18        | 2.10 (d, 8.4)                          |
| 19        | 2.15 (d, 8.5)                          |
| 20        | 2.20 (d, 8.6)                          |
| 21        | 2.25 (d, 8.7)                          |
| 22        | 2.30 (d, 8.8)                          |
| 23        | 2.35 (d, 8.9)                          |
| 24        | 2.40 (d, 9.0)                          |
| 25        | 2.45 (d, 9.1)                          |
| 26        | 2.50 (d, 9.2)                          |
| 27        | 2.55 (d, 9.3)                          |
| 28        | 2.60 (d, 9.4)                          |
| 29        | 2.65 (d, 9.5)                          |
| 30        | 2.70 (d, 9.6)                          |
| 31        | 2.75 (d, 9.7)                          |
| 32        | 2.80 (d, 9.8)                          |
| 33        | 2.85 (d, 9.9)                          |
| 34        | 2.90 (d, 10.0)                         |
| 35        | 2.95 (d, 10.1)                         |
| 36        | 3.00 (d, 10.2)                         |
| 37        | 3.05 (d, 10.3)                         |
| 38        | 3.10 (d, 10.4)                         |
| 39        | 3.15 (d, 10.5)                         |
| 40        | 3.20 (d, 10.6)                         |
| 41        | 3.25 (d, 10.7)                         |
| 42        | 3.30 (d, 10.8)                         |
| 43        | 3.35 (d, 10.9)                         |
| 44        | 3.40 (d, 11.0)                         |
| 45        | 3.45 (d, 11.1)                         |
| 46        | 3.50 (d, 11.2)                         |
| 47        | 3.55 (d, 11.3)                         |
| 48        | 3.60 (d, 11.4)                         |
| 49        | 3.65 (d, 11.5)                         |
| 50        | 3.70 (d, 11.6)                         |

Ara, α-L-arabinofuranosyl or arabinopyranosyl; d, doublet; Glc, β-D-glucopyranosyl; m, multiplet; o, overlapped signals; s, singlet; t, triplet; Xyl, β-D-xylpyranosyl

Chemical shifts (δ) in ppm; coupling constants (J) in hertz
Table 2

| No. | δC | δH | δC | δH |
|-----|-----|-----|-----|-----|
| 1   | 39.6 | 1.54(o), 0.87(c,12.9) | 39.5 | 1.54(o), 0.73(c,12.0) |
| 2   | 27.2 | 2.21(m), 1.87(o) | 27.1 | 2.20(d,10.4), 1.84(o) |
| 3   | 89.3 | 3.29(m) | 89.3 | 3.27(m) |
| 4   | 40.1 | 40.0 | 40.0 | 40.1 |
| 5   | 56.8 | 0.69(d,12.4) | 56.7 | 0.67(d,12.0) |
| 6   | 18.8 | 1.47(o), 1.38(o) | 18.8 | 1.48(o), 1.37(o) |
| 7   | 35.5 | 1.46(o), 1.20(d,11.5) | 35.5 | 1.44(o), 1.19(d,11.2) |
| 8   | 40.4 | 40.3 | 40.3 | 40.3 |
| 9   | 50.6 | 1.38(o) | 50.5 | 1.38(o) |
| 10  | 37.3 | 37.2 | 37.2 | 37.3 |
| 11  | 31.2 | 1.54(o), 1.95(d,13.3) | 31.3 | 1.54(o), 1.98(o) |
| 12  | 70.5 | 4.20(o) | 70.6 | 4.14(o) |
| 13  | 49.9 | 2.03(t,10.6) | 49.8 | 2.06(o) |
| 14  | 51.8 | 51.8 | 51.8 | 52.1 |
| 15  | 31.1 | 1.54(o), 0.98(o) | 31.1 | 1.54(o), 0.97(o) |
| 16  | 27.0 | 1.87(o), 1.38(o) | 27.0 | 1.83(o), 1.37(o) |
| 17  | 52.5 | 2.60(m) | 52.2 | 2.56(m) |
| 18  | 16.3 | 0.92(s,3H) | 16.3 | 0.95(s,3H) |
| 19  | 16.7 | 0.82(s,3H) | 16.6 | 0.82(s,3H) |
| 20  | 83.6 | 83.8 | 83.9 | 73.5 |
| 21  | 22.1 | 1.58(s,3H) | 23.0 | 1.66(s,3H) |
| 22  | 33.1 | 3.19(m), 3.47(m) | 33.1 | 1.96(o), 2.64(m) |
| 23  | 30.1 | 2.75(m), 2.11(m) | 30.9 | 2.35(m), 2.06(o) |
| 24  | 203.0 | 76.4 | 4.47(o) | 76.5 | 4.46(o) |
| 25  | 144.7 | 149.4 | 149.4 | 149.4 |
| 26  | 125.8 | 6.37, 5.72s | 110.7 | 5.26, 4.94(o) |
| 27  | 18.2 | 1.84(s,3H) | 18.6 | 1.96(s,3H) |
| 28  | 28.5 | 1.28(s,3H) | 28.4 | 1.29(s,3H) |
| 29  | 17.0 | 1.12(s,3H) | 16.9 | 1.11(s,3H) |
| 30  | 17.8 | 0.97(s,3H) | 17.7 | 0.98(s,3H) |
| 31  | 105.1 | 4.94(d,8.8) | 105.5 | 4.94(d,7.1) |
| 32  | 83.4 | 4.10(o) | 83.9 | 4.26(o) |
| 33  | 78.6 | 4.25(o) | 78.7 | 4.26(o) |
| 34  | 71.7 | 4.26(o) | 72.0 | 4.36(o) |
| 35  | 78.2 | 3.87(o) | 78.4 | 3.94(o) |
| 36  | 63.4 | 4.37(o), 4.58(d,11.3) | 63.2 | 4.50(o), 4.59(d,11.0) |
| 37  | 103.6 | 5.51(d,7.5) | 106.5 | 5.39(d,7.8) |
| 38  | 84.9 | 4.22(o) | 77.5 | 4.17(o) |
| 39  | 78.6 | 4.30(o) | 78.3 | 3.94(o) |
| 40  | 71.5 | 4.24(o) | 72.0 | 4.36(o) |
| 41  | 78.3 | 3.96(o) | 78.7 | 3.43(o) |
| 42  | 63.2 | 4.49(d,10.7), 4.37(o) | 63.0 | 4.50(o), 4.36(o) |
| 43  | 106.8 | 5.43(d,6.3) | 106.3 | 5.42(d,6.3) |
| 44  | 76.3 | 4.13(o) | 76.4 | 4.13(o) |
| 45  | 79.0 | 4.36(o) | 79.0 | 4.37(o) |
| 46  | 71.5 | 4.17(o) | 71.8 | 4.14(o) |
| 47  | 67.8 | 4.31(o), 3.70(m) | 69.8 | 4.15(d,7.8) |
| 48  | 98.4 | 5.11(d,7.8) | 98.5 | 5.15(d,7.8) |
| 49  | 75.1 | 3.91(o) | 75.2 | 3.91(o) |
| 50  | 79.8 | 4.17(o) | 79.5 | 4.16(o) |
| 51  | 72.1 | 4.25(o) | 71.9 | 4.23(o) |
| 52  | 77.0 | 4.04(o) | 77.3 | 4.06(o) |
| 53  | 70.6 | 4.77(d,10.0), 4.27(o) | 70.3 | 4.77(d,10.4), 4.27(o) |
| 54  | 75.2 | 4.03(o) | 75.2 | 4.05(o) |
| 55  | 78.1 | 4.30(m) | 78.5 | 4.36(o) |
| 56  | 71.1 | 4.15(o) | 70.3 | 4.16(o) |
| 57  | 67.5 | 4.33(o), 3.70(m) | 67.3 | 3.77(t,9.7) |

Ara, α-L-arabinofuranosyl or arabinopyranosyl; d, doublet; Glc, β-D-glucopyranosyl; m, multiplet; o, overlapped signals; s, singlet; t, triplet; Xyl, β-D-xylpyranosyl

Chemical shifts (δ) in ppm; coupling constants (J) in hertz

2.7. Measurement of inflammation-related mRNA expressions of ginsenoside IX treated cells

RAW 246.7 cells were pretreated with ginsenoside IX (100 μM) or ginsenoside Rg3 (100 μM) for 30 min and then stimulated with LPS (100 ng/ml) for 4 h. Total RNA was extracted using RNA fast 200 (Fastagen, Shanghai, China) and reverse transcribed into cDNA with PrimeScript RT Master Mix (TaKaRa-Bio, Otsu, Japan), according to the manufacturer's instructions. The cDNA was amplified quantitatively using SYBR Premix Ex Taq (TaKaRa-Bio, Otsu, Japan). The sequences of the forward and reverse primers were 5'-CTCTTTTCATCTCCTGCTT-3' and 5'-GTGTCGTTTGTAGTGTAGGACG-3' for mouse Il-1α, 5'-CTCGTGAAGTGTAGTGTAG-3' and 5'-ACTGCTTCGCTTCTATTTTCT-3' for Il-1β, 5'-CCATGCGCCATCTCTAC-3' and 5'-CTCGTTTTCATCTCCTGCTT-3' for Cxcl10, and 5'-TGACTCTCCTGGC-CAAGACAG-3' and 5'-GGCAAGAGGAAACACAGCG-3' for Il-1β.
Real-time reverse transcription polymerase chain reaction was performed using an ABI ViiA 7 Real time PCR system (Applied Biosystems, CA, USA). The data was normalized to β-Actin mRNA.

2.8. Statistical analysis

Data from three independent experiments were presented as the mean ± standard deviation. Student’s t test and Prism Graphpad program were used in the data analysis and calculation, respectively.

3. Results

3.1. Structure elucidation of new compounds

Compound 1 had a molecular formula C_{53}H_{88}O_{23} deduced from m/z 1091.5648 (M-H)⁻ in the HRESIMS. The D-glucose and D-xylose were suggested from the acid hydrolysis of 1, and their relative configurations were determined as β because of signals at δ 4.94 (d, 7.2 Hz), 4.93 (d, 7.5 Hz), 5.12 (d, 8.0 Hz), and 5.40 (d, 7.5 Hz). Seven aglycone methyls were suggested from corresponding singlets at δ 0.8, 0.9, 1.0, 1.1, 1.3, 1.6, and 1.8 and olefinic protons from two singlets at δ 6.4 and 5.7 in the ¹H-NMR spectrum. In addition, a carbonyl group (δ 203.0) and a terminal double bond (δ 144.7 and 125.8) in the ¹³C-NMR spectrum were also observed. According to the above characteristic peaks, compound 1 was indicated to show the same aglycone as notoginsenoside B, especially the α, β-unsaturated ketone in the side chain [13]. Apart from 30 signals due to the skeleton, the remaining signals of compound 1 were assigned to sugar chain and identical to those of ginsenoside Rb3, especially four anomic carbon signals at δ 106.4, 106.4, 105.5, and 98.3 [14]. The positions of the functional groups in the side chain were further determined by observation of the correlations between H₂₋₋⁻ (δ 1.84) and C-24, C-25, and C-26 (δ 203.0, 144.7 and 125.8) and H₂₋₋ (δ 6.38 and 5.72) and C-24, C-25 and C-27 (δ 18.2) in the heteronuclear multiple bond correlation (HMBC) spectrum. The glycosylations were indicated to happen at C-3 and C-20 from the downfield signals at the corresponding position. Besides, the correlation between C-3 and H-1 from the glc, and C-20 and H-1 from the glc further corroborated the finding. Sugar sequences were indicated to be a 1→2 linkage type attaching to C-3 and 1→6 to C-20 from the correlations between signals at δ 83.8 and δ 4.93 and δ 70.5 and δ 5.40. Accordingly, compound 1 was identified as 3-O-[(β-D-glucopyranosyl)(1→2)-β-D-glucopyranosyl]-20-O-[(β-D-xylopyranosyl)(1→6)-β-D-glucopyranosyl]3β,12β,20(5)-trihydroxymembrane-25-en-24-one (Fig. 1).

Compound 2 was corresponded to a molecular formula of C_{58}H_{96}O_{26} determined from m/z 1207.6134 (M-H)⁻ in the HRESIMS. The absolute configurations of the glucose and xylose were proved.
to be D and arabinose to be L by acid hydrolysis of 2. The distinctive carbon signals at δ 143.0, 136.0, 127.8, and 115.3 suggested a conjugated double bond; the position of which was deduced from the correlations between H3-27 (δ 1.95) and C-24, C-25, and C-26 (δ 136.0, 143.0 and 115.3) and H2-26 (δ 5.04, 4.95) and C-24, C-25, and C-27 (δ 19.3) in the HMBC experiment. Furthermore, signals attributed to the aglycone particularly the above characteristic peaks were shown to be identical to those of quinquenoside L1 [15].

Sugar chains were deduced to consist of β-D-glucopyranosyl, β-D-xyllopyranosyl, and α-L-arabinofuranosyl unit from the signals for five anomic carbons along with the coupling constants of the corresponding protons. The trisaccharide moiety was suggested to attach at C-3 and the disaccharide moiety at C-20 of the aglycone according to the sugar sequences of ginsenoside Fc (17). Finally, based on this elucidation along with two-dimensional spectra, such as heteronuclear single quantum correlation (HSQC) and HMBC, compound 3 was identified as 3-O-[β-D-glucopyranosyl(1→2)→β-D-glucopyranosyl]-20-O-[α-L-arabinofuranosyl(1→6)→β-D-glucopyranosyl]3β,12β,20(5) trihydroxydammar-23,25-diene. The distinctive signals agreed well with those of notoginsenoside LK4 (11), notoginsenoside Fc (10), and ginsenoside Rc (13) [8], ginsenoside Rb3 (14) [14] and gypenoside IX (15) [21] compared to the NMR and MS data reported.

3.2. Structure identification of known compounds

The known compounds 9–15 were identified as ginsenoside LXXI (9) [18], notoginsenoside Fc (10) [17], notoginsenoside FP2 (11) [16], notoginsenoside Fz (12) [8], ginsenoside Rc (13) [14], ginsenoside Rb3 (14) [14] and gypenoside IX (15) [21] compared to the NMR and MS data reported.

3.3. Antiinflammatory activity

Previous reports have revealed that saponins exerted antiinflammatory activity, and therefore all the isolated compounds were screened for antiinflammatory activity by measuring NO production of LPS-stimulated Raw 264.7 cells. As the results indicate (Fig. 2), only pretreatment of gypenoside IX at noncytotoxic concentration (100 μM) significantly decreased the NO production to 72.0% compared with the control group (100%), which indicated the antiinflammatory activity of gypenoside IX. Furthermore, the expressions of inflammation-related genes Tnf-a, Il-10, Cxcl10, and Il-1β were suppressed by gypenoside IX (Fig. 3). Taken together, gypenoside IX has the potential to be used as an antiinflammatory agent.

4. Discussion

PNLS were rich in dammarane-type saponins and could be considered as an alternative source of bioactive saponins. In the present study, eight new notoginsenosides bearing dehydrogenated or oxidized side chains along with seven known PPD-type saponins with common side chains have been isolated and identified from PNLS. Among them, compounds 1, 4, and 5 showed the terminal double bond and a hydroxyl group. The 13C-NMR spectrum of 6 matched well with those of gypenoside LXXI (9) [18], except for slight difference of the signals at C-24 and C-26 between 6 and 9. Specifically, signals went to high field by movement of δ 0.1 for C-24 and δ 0.5 for C-26 from 6 to 9 (6: δ 76.4 at C-24, δ 110.7 at C-26; 9: δ 76.3 at C-24, δ 110.3 at C-26), which suggested 6 and 9 were obtained as a pair of C-24 epimers. Furthermore, the configuration at C-24 in the 6 was identified to be S after a comparison of signals between 6 and reported C-24 epimers, such as vina-ginsenoside R9 and majoroside F1 [19]. Accordingly, compound 6 was deduced as 3-O-β-D-glucopyranosyl(1→2)→β-D-glucopyranosyl]-20-O-[β-D-xyllopyranosyl(1→6)→β-D-glucopyranosyl]3β,12β,20(5)- trihydroxydammar-24(R)-hydroxy-25-ene.
same α, β-unsaturated ketone as previously isolated saponins, such as notoginsenoside B from the dried root of *P. notoginseng*, ginsenoside III from the flower buds of *P. ginseng*, and vina-ginsenoside R20 from the roots of *Panax vietnamensis* Ha et Grushv. [13,22,23]. It has been indicated that this kind of side chain existed widely in genus *Panax*. Before our study, only quinquenaside L1 from the leaves and stems of *P. quinquefolium* was reported to bear a side chain with conjugated double bonds [15]. The discovery of 2 and 3 has greatly enhanced the chemical diversity of saponins of this type. In addition, this is the first report on a pair of C-24 epimers [notoginsenoside LK6 (6) and gypenoside LXXI (9)] from *P. notoginseng*, although this kind of C-24 epimers (vina-ginsenoside R9 and majoroside F1) has been previously isolated from *P. vietnamensis* [19]. In the previous study, 7 and 8 with undefined configurations at C-24 were proposed as floranotoginsenoside D and notoginsenoside F3, respectively [20,24]. Besides, only 1D NMR and HRESIMS without 2D NMR were listed for the identification of floranotoginsenoside D, and acid treatment was used to obtain notoginsenoside F3 from leaves of *P. notoginseng*. In this study, the configurations of 7 and 8 were determined by comparing their chemical shifts to those of the C-24 epimers (6 and 9). Moreover, the structure of 7 was completely characterized by chemical and spectroscopic methods, and 8 was formed naturally.

Gypenoside IX was firstly isolated from *Gynostemma pentaphyllum* Makino and found in *P. notoginseng* and *P. ginseng* [25,26]. Although many reports demonstrated that *G. pentaphyllum* and gypenosides from this plant displayed antiinflammatory properties [27–29], there have been very few reports of gypenoside IX about antiinflammatory activity. Our data showed the moderate antiinflammatory effect of gypenoside IX, which could be served as an important pharmalogical active saponin in the genus of *Panax* or *Gynostemma*.

According to our data (Fig. 2), isolated saponins with four or five sugar units such as ginsenoside Rb3 (14) and 1–7 showed no effect on nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells. The data were presented as ratio [%] compared to the group of DMSO-treated cell (control group). 20(S)-Rg3 was used as positive control. Compounds, which showed less than 80% of NO production, can be regarded as the potential antiinflammatory inhibitors. Data from three independent experiments were expressed as the mean ± SD. **p < 0.01 and *p < 0.05 versus the control group. DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; Rg3, 20(S) ginsenoside Rg3; SD, standard deviation.

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Fig. 2. Effect of compounds 1–15 on nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells. The data were presented as ratio [%] compared to the group of DMSO-treated cell (control group). 20(S)-Rg3 was used as positive control. Compounds, which showed less than 80% of NO production, can be regarded as the potential antiinflammatory inhibitors. Data from three independent experiments were expressed as the mean ± SD. **p < 0.01 and *p < 0.05 versus the control group. DMSO, dimethyl sulfoxide; LPS, lipopolysaccharide; Rg3, 20(S) ginsenoside Rg3; SD, standard deviation.

Fig. 3. Effect of gypenoside IX. (A) On Tnf-α expression in the LPS-stimulated RAW 264.7 cells. (B) Il-10 expression in the LPS-stimulated RAW 264.7 cells. (C) Cxcl10 expression in the LPS-stimulated RAW 264.7 cells. (D) Il-1b expression in the LPS-stimulated RAW 264.7 cells. Data from six independent experiments were expressed as the mean ± SD. **p < 0.01 and *p < 0.05 versus the Model group. LPS (100 ng/ml) were used to build the model. Cxcl10, interferon-inducible protein 10; Il-10, interleukin 10; Il-1β, interleukin-1β; IX, gypenoside IX; LPS, lipopolysaccharide; Rg3, 20(S) ginsenoside Rg3; SD, standard deviation; Tnf-α, tumor necrosis factor-α.
on our experiment model. However, Ma et al [30] reported that ginsenoside Rb3 protected cardiomyocytes against ischemia-reperfusion injury by suppressing the nuclear factor kappa B pathway. Ginsenoside Rb1 with similar structure to ginsenoside Rb3 was reported to be metabolized after oral administration to compound K and then to present bioactivities on treating allergic inflammation [31]. Hence, it may be suggested that 1–7 might show effects by metabolizing to less polar saponins through orally administration. Thus, further pharmacological studies of these new compounds in vivo models should be processed.

In this study, we have isolated fifteen dammarane-type triterpene oligoglycosides especially eight new ones from the PNLS, which may enrich and expand the chemical library of saponins in Panax plants. Moreover, gypenoside IX as a PPD-type oligoglycoside was demonstrated to show antiinflammatory effect by suppressing the NO production and inflammation-related genes in LPS-stimulated RAW 264.7 cells. Further investigation is expected to increase insight into mechanisms of antiinflammation of gypenoside IX.

Conflicts of interest

All authors declare no conflicts of interest.

Acknowledgments

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