Anti-inflammatory and Cytotoxic Triterpenes from the Rot Roots of *Panax notoginseng*

Jia-Huan Shang1,2 · Guo-Wei Xu1,2 · Hong-Tao Zhu1,3 · Dong Wang1,3 · Chong-Ren Yang1 · Ying-Jun Zhang1,3

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Abstract

Four new protopanaxatriol-type triterpenes (1–2) and glucosides (3–4), were isolated from the rot roots of *Panax notoginseng* (Burk.) Chen, along with four known ones (5–8). Their structures were elucidated on the basis of extensive spectroscopic analysis (HRESIMS, NMR, UV, IR, and OR) and acidic hydrolysis. The possible transformation pathway of these compounds were also speculated from ginsenoside Rg1. Compound 1, with a unique α,β-unsaturated ketene in its side chain, showed significant inhibitory effects against NO production on Murine macrophage cells (IC50 = 4.12 ± 0.20 μM) and comparable cytotoxicities against five human cancer cell lines (myeloid leukemia HL-60, lung cancer A-549 cells, hepatocellular carcinoma SMMC7721, breast cancer MCF-7, and colon cancer SW480) to positive control, cisplatin (DDP).

Graphical Abstract

Keywords *Panax notoginseng* · Rot root · Triterpenes and saponins · Inhibition on NO production · Cytotoxicity

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1 State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People’s Republic of China

2 University of Chinese Academy of Sciences, Beijing 100049, People’s Republic of China

3 Yunnan Key Laboratory of Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People’s Republic of China

*Ying-Jun Zhang*
zhangyj@mail.kib.ac.cn
1 Introduction

Panax notoginseng (Burk.) Chen (Araliaceae), a well-known member called Sanqi or Tianqi in Ginseng family for the treatment of cardiovascular diseases, has been domesticated and cultivated for more than 400 years in the southwest of China [1]. It is now used as one of the major raw materials for many traditional Chinese medicinal preparations, such as Pien Tze Huang, Yunnan Baiyao, Sanqi oral liquid, etc. [2]. Due to the increasing demands from medicinal industry and herbal market, the plantation of Sanqi has been enlarged sharply in recent decades. However, it is susceptible to various diseases during the plantation, because of the sensitive property to environmental factors. Among which, root rot caused mainly by microbial infections is the main destructive disease [3–5]. So far, more than 200 chemical compounds including triterpenoid saponins, flavonoids, amino acids, and so on, have been reported from the roots, stems, leaves, flower heads, fruits, and fruit pedicels, and ginsenosides are found to be the dominant active principles [6]. Previous HPLC-HRMS study revealed that the oxidation levels of constituents in roots of P. notoginseng are significantly increased after being infected by root rot diseases [7]. In order to clarify the chemical composition and explore the possible transformation mechanism in the rot roots of P. notoginseng, the minor chemical constituents of the rot root were studied in detail firstly. This led to the identification of four new (1–4) and four known (5–8) triterpenes and saponins (Fig. 1), and their possible transformation pathway were speculated in this paper. All the isolates were evaluated for their anti-inflammatory activity (inhibition NO production) on Murine macrophage cells and cytotoxicities against five human cancer cell lines.

2 Results and discussion

The air-dried rot roots of P. notoginseng were crushed into small grains and extracted with MeOH. Further repeated column chromatography (CC) over macroporous resin D101, silica gel and RP-18, followed by semi-preparative HPLC, yielded eight dammarane type triterpenes. Four of them (1–4) including two saponins (3–4) are new compounds.

Compound 1 was obtained as white amorphous powder. Its molecular formula C30H50O5 was determined by the positive-mode HRESIMS (m/z 513.3551 [M+Na]+), corresponding to six degrees of unsaturation. The IR spectrum indicated the presence of hydroxy groups (3391 cm−1), keto (1738 cm −1), double bond (1674 cm −1) and methyls (2954 cm−1). The UV spectrum presented a weak absorption at 254 nm (K-band) indicating the existence of a conjugated α,β-unsaturated ketene in this structure. The 13C NMR spectrum (Table 1) showed the presence of 30 carbon resonances, assignable to seven methyls, nine methylenes with a vinyl (δC 124.9), seven methines with three oxygen-bearing ones (δC 68.1, 71.6 and 78.9), and seven quaternary carbons, including one oxygenated carbon (δC 72.9), one olefinic carbon (δC 145.2) and one ketone (δC 203.2). The 1H NMR spectrum (Table 2) displayed seven singlet or doublet (JH=8.5 Hz, J=10.8 Hz) protons at δH 1.01, 1.10, 1.38, 1.47, 1.89 and 2.02 (each 3H, s), two olefinic protons at δH 5.66 and 6.06 (each 1H, s), three oxygen methines at δH 3.54 (d, J=10.8 Hz), 4.43 (t, J=8.5 Hz), and 3.95 (t, J=10.8 Hz), and four active hydrogen protons at δH 5.32, 5.79, 7.02, 7.39 (each 1H, s).

The two of six unsaturated degrees were from the carbonyl and double bond. Above analysis displayed 1 to be a tetra cyclic triterpene. The NMR data of 1 were similar to those of 20(S)-protopanaxatriol (PPT) [8], except for an additional ketone (δC 203.2), the shielding of C-25 (δC 145.2 vs δC 150.4 in PPT), and the appearance of a terminal vinyl at C-25 (δC 124.9). This indicated 1 to be a PPT-type triterpene with an α,β-unsaturated ketone (C-24) on the side chain. The 1H–1H COSY correlations from H2-22 (δH 3.31, 3.02) to H2-23 (δH 2.39, 2.05) combined with the HMBC spectrum of H-22/H-23/H2-26 (δH 6.06, 5.66)/H-27 (δH 1.89) to C-24 (δC 72.9), H-26/H-27 to C-25 (δC 145.2), H-17 (δH 2.37)/H-21 (δH 1.38)/H-22/H-23 to C-20 (δC 72.9), and of an active hydrogen signals (20-OH, δH 7.02) to C-20/C-21 (δC 27.5) (Fig. 2), respectively, further verified the structure of 1.

Fig. 1 Compounds 1–8 isolated from the rot roots of P. notoginseng
The 20S configuration in 1 was constructed by comparison of the chemical shifts of C-21 (δC 27.5) and C-17 (δC 55.2) with those of 20R configuration [C-21 (δC 22.8) and C-17 (δC 50.7)] [9, 10]. Therefore, compound 1 was identified as 3β,6α,12β,20(S)-tetrahydroxydammar-24-one-25-ene.

Compound 2 presented a molecular formula C30H48O5 with seven degrees of unsaturation as deduced from HRESIMS experiment (m/z 511.3399 [M+Na]+). The 13C NMR and DEPT spectra showed six more carbon resonances than those of 1. Accordingly, compound 2 was determined to be 6α,20(S),24(R)-tri hydroxydammar-3,12-dione-25-ene.

Compound 3 was found to have a molecular formula of C36H60O11 on basis of the positive-mode HRESIMS experiment (m/z 691.4024 [M+Na]+). The 13C NMR and DEPT spectra showed six more carbon resonances than those of 1, suggesting the existence of a hexosyl unit (δC 98.8 d, 79.4 d, 75.8 d, 72.1 d, 63.5 t). So far, all hexosyl units in ginsenosides isolated from Panax species is only glucosyl (Glc) [14]. Further acid hydrolysis of 3 followed by GC analysis of the corresponding trimethylsilylated t-cysteine adduct characterized a glucopyranosyl moiety in compound 3. The 1H-NMR spectrum of 3 showed an anomeric proton signal of the β-D-glucopyranosyl moiety at δH 5.26 (d, J = 7.7 Hz), an active hydrogen at δH 14.37 (s) and two olefinic protons in a disubstituted E-double bond at δH 6.08 (d, J = 15.7 Hz) and δH 6.21 (m). The 13C and 1H NMR data of 3 were similar to those of a ginsenoside-Rh6 [15], except for the signals of C1–C5 at ring A. The very down field chemical shift at δC 219.1 suggested that 3 should be 3-oxidized ginsenoside-Rh6, as the hydroxy group at C-3 (δC 78.6) in ginsenoside-Rh6 was transferred to a ketone group. This

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**Table 1** 13C (150 MHz) NMR data of 1–4 in pyridine-d₅ (δ in ppm)

| No. | 1     | 2     | 3     | 4     | No. | 1     | 2     | 3     | 4     |
|-----|-------|-------|-------|-------|-----|-------|-------|-------|-------|
| 1   | 39.8  | 39.7  | 40.3  | 40.5  | 19  | 17.9  | 17.9  | 17.5  | 18.7  |
| 2   | 28.6  | 33.5  | 33.8  | 33.7  | 20  | 72.9  | 73.7  | 83.5  | 73.5  |
| 3   | 78.9  | 218.7 | 219.1 | 219.0 | 21  | 27.5  | 27.4  | 23.7  | 27.5  |
| 4   | 40.8  | 48.1  | 48.1  | 48.6  | 22  | 33.2  | 38.9  | 40.3  | 36.3  |
| 5   | 62.2  | 59.1  | 59.5  | 58.4  | 23  | 30.5  | 31.4  | 127.0 | 23.5  |
| 6   | 68.1  | 67.2  | 67.3  | 79.8  | 24  | 203.2 | 76.5  | 138.6 | 126.8 |
| 7   | 48.0  | 45.1  | 45.8  | 43.8  | 25  | 145.2 | 150.4 | 81.8  | 131.3 |
| 8   | 41.6  | 41.6  | 41.0  | 40.7  | 26  | 124.9 | 110.8 | 25.6  | 26.3  |
| 9   | 50.6  | 53.5  | 49.0  | 48.8  | 27  | 18.3  | 18.4  | 25.9  | 18.2  |
| 10  | 39.8  | 38.6  | 38.6  | 38.9  | 28  | 32.4  | 32.4  | 32.6  | 32.3  |
| 11  | 32.6  | 40.6  | 32.2  | 33.3  | 29  | 17.0  | 20.3  | 20.5  | 20.2  |
| 12  | 71.6  | 211.4 | 70.7  | 71.2  | 30  | 71.5  | 17.4  | 16.7  | 17.1  |
| 13  | 48.6  | 56.5  | 49.8  | 49.3  | 1’  | 98.8  | 105.8 |
| 14  | 52.1  | 56.3  | 51.9  | 52.2  | 2’  | 75.8  | 75.9  |
| 15  | 31.8  | 32.6  | 31.1  | 31.7  | 3’  | 79.4  | 80.1  |
| 16  | 27.3  | 24.9  | 26.8  | 27.3  | 4’  | 72.1  | 72.4  |
| 17  | 55.2  | 44.4  | 52.5  | 55.1  | 5’  | 78.8  | 78.6  |
| 18  | 18.0  | 16.3  | 18.2  | 16.5  | 6’  | 63.5  | 63.6  |
was further verified by the HMBC correlations from H-2-2 (δ_H 2.82, 2.32), H-28 (δ_H 1.68) and H-29 (δ_H 1.73) to C-3 (δ_C 219.1). Moreover, on the basis of the correlations of δ_H 14.37 (–OOH) with C-25 (δ_C 81.8), H-1 (δ_H 5.26) with C-20 (δ_C 83.5) in HMBC spectrum and H-1 with H-21 (δ_H 1.62, s)/H2-22 (δ_H 3.08 dd, J = 14.1, 6.1 Hz; δ_H 2.76 m)/H-23 (δ_H 6.21, m) in ROESY spectrum, the positions of peroxy and glucosyl groups were confirmed to be linked with C-25 and C-20, respectively. Therefore, compound 3 was assigned as 3-oxo-20(S)-ginsenoside-Rh6.

Compound 4 was an amorphous white powder and had a molecular formula C_{36}H_{60}O_{9}, as deduced from HRESIMS experiment (m/z 659.4126 [M+Na]^+). An β-D-glucopyranosyl unit (δ_C 105.8 d, 80.1 d, 78.6 d, 75.9 d, 72.4 d, 69.3 d, 66.9 d, 66.8 d).
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The anomeric proton at $\delta_H 5.04$ (d, $J = 7.8$ Hz) and confirmed by GC analysis after acidic hydrolysis. Furthermore, the HMBC correlations from H-5/H-7/H-1' to C-6 ($\delta_C 79.8$) indicated the glucosyl group was substituted at C-6. The $^1$H and $^{13}$C NMR data of 4 were in good agreement with those of 20(S)-ginsenoside-Rh1 [16], except for signals of ring A, which showed a lower field quaternary carbon at $\delta_C 219.0$. Similar to those of 2 and 3, H$_2$-2 ($\delta_H 2.83, 2.31$), CH$_3$-28 ($\delta_H 1.79, s$) and CH$_3$-29 ($\delta_H 1.89, s$) displayed HMBC correlations with $\delta_C 219.0$, indicating that 4 was an oxidized ginsenoside-Rh1 at C-3. The absolute configuration of C-20 ($\delta_C 73.5$) was identified as 20S when compared its surrounding carbon signals [C-17 ($\delta_C 55.1$), C-21 ($\delta_C 27.5$) and C-22 ($\delta_C 36.3$)] with that in 3-oxo-20(R)-ginsenoside-Rh1 [C-17 ($\delta_C 50.9$), C-21 ($\delta_C 23.2$), and C-22 ($\delta_C 43.6$)] [17]. Accordingly, compound 4 was identified as 3-oxo-20(S)-ginsenoside-Rh1.

Four known compounds were identified as 6α,20(S),24(S)-trihydroxydammar-3,12-dione-25-ene (5) [11], ginsenoside-F$_1$ (6) [18], 12-oxo-20(S)-protopanaxatriol (7) [19], 6α,12β-dihydroxydammar-3-one-20(S)-O-$\beta$-d-glucopyranoside (8) [20], respectively, by comparison of their spectroscopic data with literature reported values. Related substrate specificity test probed that the glucoside bond of ginsenoside Rg$_1$ can be selectively hydrolyzed by $\beta$-d-glucosidase and thus produced rare ginsenoside F$_1$ [21]. Accordingly, $\beta$-d-glucosidase is widespread in fungi [22] and bacteria [23], and the oxidative stress response in this herb after infected by the root rot decease might play the main role for the oxidation of hydroxyl group at C-3, C-12, or the side chain. As all the eight compounds are PPT-type triterpenes, we speculated that they might be transformed from ginsenoside Rg$_1$, the most abundant PPT-type saponin in the root of *P. notoginseng*, and the possible transformation pathway of these eight compounds were deduced as Fig. 3.

Compound 1 is a triterpene aglycone with a terminal $\alpha,\beta$-unsaturated ketene on the side chain, whose diglycoside and triglycoside in flower buds of *P. notoginseng* and tetragnoside in Xueshantong Injection were reported previously by LC–MS(Q-TOF) analysis [24, 25]. In the present study, compound 1 was isolated and identified with other three new compounds (2–4) and four known ones from the rot roots of *P. notoginseng* for the first time. Compounds 2 and 5 were a pair of diastereoisomers and separated simultaneously by semi-preparative HPLC.

Since the glycosides in *Panax* species were reported to have strong anti-inflammatory and anti-tumor activities [24–30], all the isolates were evaluated for their inhibitory effects against NO production on Murine macrophage cell line and cytotoxicities against five human cancer cells.

Fig. 2 Key $^1$H-$^1$H COSY and $^1$H-$^{13}$C HMBC correlations of compounds 1–4.

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(myeloid leukemia HL-60, lung cancer A-549 cells, hepatocellular carcinoma SMMC7721, breast cancer MCF-7, and colon cancer SW480). Only compound 1 displayed significant inhibition of NO production with IC$_{50}$ value of 4.12 ± 0.2 μM (L-NMMA: IC$_{50}$ = 34.32 ± 1.20 μM) and obvious cytotoxicities against all the five human cancer cell lines, comparable to the positive control, DDP (Table 3). At primary concentration of 50 μM for NO inhibition and 40 μM for cytotoxicity, the other compounds 2–8 displayed no obvious anti-inflammatory and cytotoxic activities.

3 Experimental

3.1 General Experimental Procedures

Optical rotations were measured with a P-1020 polarimeter (JASCO, Tokyo, Japan). IR spectra were measured on Bio-Rad FTS-135 series spectrometer or NICOLLET iS10 with OMNIC 9.8.372 software. UV spectra were recorded on a Shimadzu UV2401A ultra-violet-visible spectrophotometer. HRESIMS spectra were run on an API QSTAR.
Table 3 Cytotoxicities of compound I against five human cancer cell lines

| Cancer cells                     | IC\textsubscript{50 ± SD} (μM) | I     | DDP   | Taxol |
|----------------------------------|---------------------------------|-------|-------|-------|
| Myeloid leukemia HL-60           | 19.12 ± 0.21                    | 2.87 ± 0.08 | < 0.008 |
| Lung cancer A-549                | 16.30 ± 0.21                    | 20.62 ± 2.70 | < 0.008 |
| Hepatocellular carcinoma SMMC7721 | 16.69 ± 0.33                    | 17.40 ± 0.51 | < 0.008 |
| Breast cancer MCF-7              | 16.78 ± 0.32                    | 14.96 ± 0.45 | < 0.008 |
| Colon cancer SW480               | 13.58 ± 0.71                    | 14.34 ± 1.15 | < 0.008 |

Data expressed as mean ± SD (n = 3)

Polar-1 spectrometer. NMR spectra were measured in pyridine-\(d_6\) and recorded on a Bruker DRX-600 spectrometer, using TMS as an internal standard. Chemical shifts were reported in units of δ (ppm) and coupling constants (\(J\)) were expressed in Hz. Column chromatography (CC) were carried out over macro-porous resin D101 (Cangzhou Baogeng Co. Ltd., China), silica gel (200–300 mesh, Qingdao Haiyang Chemical Co. Ltd., China), RP-C18 gel (40–60/μm, Merck, Darmstadt, Germany). A Hanbon series (Hanbon Sci & Tech) were used for semi-preparative HPLC with a Capcell Pak MGII C18 column (5 μm, 250 mm × 10 mm).

3.2 Plant Material

The rot roots of \textit{P. notoginseng} (Burk.) F. H. Chen were collected in Wenshan County, Yunnan Province, People’s Republic of China, in February 2017, and identified by Prof. C. R. Yang from Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS). A voucher specimen (KIB-Z-2017001) was deposited in the State Key Laboratory of Phytochemistry and Plant Resource in West China of KIB, CAS.

3.3 Extraction and Isolation

The fresh rot roots of \textit{P. notoginseng} (70 kg) were cut from the healthy part, air-dried under room temperature, and then extracted with CH\(_2\)OH–H\(_2\)O three times. After removal of the organic solvent, the methanol extract (4.0 kg) was subjected to macro-porous resin D101 CC (250 × 30 cm) eluting with H\(_2\)O to remove most of the saccharides and then washing with MeOH to give a crude saponins fraction (2.7 kg), which was further fractionated on a silica gel CC (250 × 30 cm), eluting with CHCl\(_3\)–CH\(_3\)OH (7:3), to afford three fractions (Fr. A–C). TLC and HPLC analysis showed Fr. A (220.8 g) containing mainly notoginsenoside R\(_1\) and ginsenosides R\(_g1\), Re, Rd and Rb\(_1\).

Fr. C (220.8 g) was applied to RP-18 CC, eluting with CH\(_2\)OH–H\(_2\)O (1:9 to 9:1), to yield six sub-fractions (Fr. C1–C6). Fr. C2 (1.2 g) was column chromatographed over silica gel CC, eluting with CHCl\(_3\)–CH\(_2\)OH (10:1) to give eight sub-fractions (Frs. C1.1–C1.8). Fr. C1.4 (37 mg) and Fr. C1.5 (21 mg) were separately purified with semi-preparative HPLC (CH\(_3\)CN–H\(_2\)O, 35:65) to yield 7 (2.9 mg) and 8 (10.0 mg), 4 (6.2 mg) and 6 (20 mg). Fr. C1.6 (21 mg) was purified with semi-preparative HPLC (CH\(_3\)CN–H\(_2\)O, 35:65) to yield 3 (4.2 mg). Fr. C3 (4 g) was subjected to a silica gel CC eluting with CHCl\(_3\)–CH\(_2\)OH (200:1 to 95:5), followed with semi-preparative HPLC (CH\(_3\)CN–H\(_2\)O, 31:69) to yield 1 (3.2 mg). Fr. C5 (1.1 g) was subjected to a silica gel CC eluting with CHCl\(_3\)–CH\(_2\)OH (200:1 to 10:1), followed with semi-preparative HPLC (CH\(_3\)CN–H\(_2\)O, 44:56) to yield 5 (2.8 mg) and 2 (2.5 mg).

3.3.1 \(\beta\)\textsubscript{6,6a,12β,20(S)-Tetrahydroxydammar-24-one-25-ene (1)}

White amorphous powder; \([\alpha]\)\textsubscript{D}\(^25\) + 24.83 (c 0.06, MeOH); IR (KBr) \(\nu\)\textsubscript{max}: 3391, 2954, 2924, 2853, 1738, 1674, 1463, 1414, 1031; UV (MeOH) \(\lambda\)\textsubscript{max} (log \(\varepsilon\)): 254 (0.10), 196 (0.35) nm; \(1^1\)C and \(1^1\)H NMR data: see Tables 1 and 2; HRESIMS (pos.): \(m/z\) 513.3551 [M+Na]\(^+\), (calcd. for C\(_{30}\)H\(_{48}\)O\(_5\)Na, 513.3550).

3.3.2 \(6α,20(S),24(\textit{R})\)-Trihydroxydammar-3,12-dione-25-ene (2)

White amorphous powder; \([\alpha]\)\textsubscript{D}\(^25\) + 94.95 (c 0.08, MeOH); IR (ATR) \(\nu\)\textsubscript{max}: 3478, 3452, 3405, 2956, 2916, 2880, 1690, 1111; UV (MeOH) \(\lambda\)\textsubscript{max} (log \(\varepsilon\)): 195 (0.35) nm; \(1^1\)C and \(1^1\)H NMR data: see Tables 1 and 2; HRESIMS (pos.): \(m/z\) 513.3999 [M+Na]\(^+\), (calcd. for C\(_{30}\)H\(_{48}\)O\(_5\)Na, 513.3999).

3.3.3 \(3\)-Oxo-20(S)-ginsenoside-R\(_b\)\(_9\) (3)

White amorphous powder; \([\alpha]\)\textsubscript{D}\(^25\) + 70.67 (c 0.12, MeOH); IR (ATR) \(\nu\)\textsubscript{max}: 3356, 2956, 2934, 2880, 1692, 1425, 1380, 1076, 1035; UV (MeOH) \(\lambda\)\textsubscript{max} (log \(\varepsilon\)): 195 (0.37) nm; \(1^1\)C and \(1^1\)H NMR data: see Tables 1 and 2; HRESIMS (pos.): \(m/z\) 691.4024 [M+Na]\(^+\), (calcd. for C\(_{36}\)H\(_{50}\)O\(_{11}\)Na, 691.4033).

3.3.4 \(3\)-Oxo-20(S)-ginsenoside Rh\(_b\) (4)

White amorphous powder; \([\alpha]\)\textsubscript{D}\(^25\) + 61.42 (c 0.13, MeOH); IR (KBr) \(\nu\)\textsubscript{max}: 3422, 2964, 2931, 2877, 1691, 1637, 1458, 1383, 1077, 1022; UV (MeOH) \(\lambda\)\textsubscript{max} (log \(\varepsilon\)): 196 (0.52), 254 (0.07) nm; \(1^1\)C and \(1^1\)H NMR data: see Tables 1 and 2; HRESIMS (pos.): \(m/z\) 659.4126 [M+Na]\(^+\), (calcd. for C\(_{36}\)H\(_{40}\)O\(_{11}\)Na, 659.4135).
Compounds 3 and 4 (each 2.0 mg) were hydrolyzed in 2 M HCl (3 mL) at 65 °C for 4 h, respectively. The reaction mixture was extracted with CH₂Cl₂, three times (3 × 3 mL). The aqueous layer was neutralized with 2 M NaOH and dried to produce a monosaccharide mixture. Next, l-cysteine methyl ester hydrochloride (1.0 mg) was added to a no aqueous pyridine solution of the sugar mixture (1.0 mL) and kept at 60 °C for 1 h. After this, trimethylsilylimidazole (1.0 mL) was added to the reaction mixture and kept at 60 °C for 30 min, followed the extraction with n-hexane (1 × 2 mL). The n-hexane layer was subjected to GC analysis, run on a Agilent Technologies HP5890 gas chromatograph, equipped with a HP-5 quartz capillary column (30 mm × 0.32 mm × 0.25 mm) and a H₂ flame ionization detector with the following conditions: column temperature, 180–280 °C; programmed increase, 3 °C/min; carrier gas, N₂ (1.5 mL/min); injector and detector temperature, 250 °C; injection volume, 2.0 μL; and split ratio 1/50. The configuration of the sugar moiety was determined by comparing the retention times of the derivatives of the authentic samples. The retention time with the derivatives of the authentic samples.

3.4 Acid Hydrolysis of Compounds 3 and 4

Five human cancer cell lines, myeloid leukemia HL-60, lung cancer A-549 cells, hepatocellular carcinoma SMMC7721, breast cancer MCF-7, and colon cancer SW480, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium (HyClone, USA), supplemented with 10% fetal bovine serum (HyClone, USA). The cytotoxicity assay was performed according to the MTS [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium] method in 96-well microplates [33]. Briefly, adherent cells (100 μL) was seeded into each well of 96-well cell culture plates and allowed to adhere for 24 h before drug addition, while suspended cells were seeded just before drug addition, each tumor cell line was exposed to the test compound dissolved in DMSO in triplicates for 48 h at 37 °C, with DDP and Taxol (Sigma, USA) as positive controls. After the incubation, 20 μL MTS and 100 μL medium were added to each well after removal of 100 μL medium, and the incubation continued for 4 h at 37 °C. The optical density was measured at 492 nm using a Multiskan FC plate reader (Thermo Scientific, USA). Concentration of a compound inhibiting 50% of cell growth (IC₅₀) was calculated by the Reed and Muench method.

3.5 The Nitric Oxide Production in RAW264.7 Macrophages

As reported previously [31, 32], murine macrophage cell line RAW264.7 obtained from Cell Bank of Chinese Academy of Sciences (Beijing, People’s Republic of China), were seeded in 96-well cell culture plates (1.5 × 10⁵ cells/well) and treated with serial dilutions of the compounds with a maximum concentration of 50 μM in triplicate, followed by stimulation with 1 μg/mL LPS (Sigma, St. Louis, MO, USA) for 18 h. Nitric oxide production in the supernatant was assessed by Griess reagents (Reagent A & Reagent B, respectively, Sigma). The absorbance at 570 nm was measured with a microplate reader (Thermo, Waltham, MA, USA). NG-Methyl-l-arginine acetate salt (L-NMMA, Sigma), a well-known nitric oxide synthase (NOS) inhibitor, was used as a positive control. All the compounds were prepared as stock solutions in DMSO. The viability of RAW264.7 cells was evaluated by the MTS assay simultaneously to exclude the interference of the cytotoxicity of the test compounds. Concentration of a compound inhibiting 50% of cell growth (IC₅₀) was calculated by the Reed and Muench method.

3.6 Cytotoxic Assay

In summary, eight PPT-type triterpenes (1–2, 5, 8) and saponins (3–4, 6–7) were isolated from the rot roots of P. notoginseng for the first time. Among them, 1–4 are new compounds. Their structures were determined on the basis of the extensive spectroscopic analyses and acidic hydrolysis. Compound 1 with a novel terminal ketene at C-24 to C-26 showed significant cytotoxic and anti-inflammatory activities.

4 Conclusion

In summary, eight PPT-type triterpenes (1–2, 5, 8) and saponins (3–4, 6–7) were isolated from the rot roots of P. notoginseng for the first time. Among them, 1–4 are new compounds. Their structures were determined on the basis of the extensive spectroscopic analyses and acidic hydrolysis. Compound 1 with a novel terminal ketene at C-24 to C-26 showed significant cytotoxic and anti-inflammatory activities.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest.
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References

1. Y.J. Qiao, J.H. Shang, D. Wang, H.T. Zhu, C.R. Yang, Y.J. Zhang, Nat. Prod. Bioprospect. 8, 245–263 (2018)
2. P.G. Xia, S.C. Zhang, Z.S. Liang, Z.H. Qi, Chin. Tradit. Herb. Drugs. 45, 2564–2570 (2014)
3. N. Jiang, L.Y. Qin, Y.F. Ye, J. South. Agric. 42, 1070–1074 (2011)
4. X.T. Sun, L. Li, G.Q. Long, G.H. Zhang, Z.G. Meng, S.C. Yang, J.W. Chen, Chin. J. Ecol. 34, 885–893 (2015)
5. Z.S. Mao, Y.J. Long, S.S. Zhu, Z.J. Chen, F.G. Wei, Y.X. Zhu, X.H. He, Chin. Med. Mater. 36, 2051–2054 (2013)
6. T. Wang, R. Guo, G. Zhou, X. Zhou, Z. Kou, F. Sui, C. Li, L. Tang, Z. Wang, J. Ethnopharmacol. 188, 234–258 (2016)
7. R.Q. Tai, W.Z. Tan, E. Yang, Y. Feng, H.Z. Li, J. Kunming. Univ. Sci. Technol. (Nat. Sci) 40, 85–88 (2015)
8. S.R. Ko, K.J. Choi, K. Suzuki, Y. Suzuki, Chem. Pharm. Bull. 51, 404–408 (2003)
9. J. Asakawa, R. Kasai, K. Yamasaki, O. Tanaka, Tetrahedron 33, 1935–1939 (1997)
10. S. Fujita, R. Kasai, K. Ohtani, K. Yamasaki, M.H. Chiu, R.L. Nie, O. Tanaka, Phytochemistry 39, 591–602 (1995)
11. J.L. Yang, T.K.Q. Ha, B. Dhodary, K.H. Kim, J. Park, C.H. Lee, Y.C. Kim, W.K. Oh, J. Nat. Prod. 77, 1615–1623 (2014)
12. L.P. Christensen, Advances in Food and Nutrition Research (Elsevier, Cambridge, 2008), pp. 1–99
13. E. Bedir, N.J. Toyang, I.A. Khan, L.A. Walker, A.M. Clark, J. Nat. Prod. 64, 95–97 (2001)
14. L.W. Qi, C.Z. Wang, C.S. Yuan, Nat. Prod. Rep. 28, 467–495 (2011)
15. D.Y. Lee, B.J. Cha, Y.S. Lee, G.S. Kim, H.J. Noh, S.Y. Kim, H.C. Kang, H.K. Jin, N.I. Baek, Int. J. Mol. Sci. 16, 1677 (2015)
16. R.T. Teng, H.Z. Li, J.T. Chen, D.Z. Wang, Y.E. He, C.R. Yang, Magn. Reson. Chem. 40, 483–488 (2002)
17. L.B. Vinh, Y. Lee, Y.K. Han, J.S. Kan, J.U. Park, Y.R. Kim, S.Y. Yang, Y.H. Kim, Bioorg. Med. Chem. Lett. 27, 5149–5153 (2017)
18. Y.W. Zhang, Q.D. De, Y.J. Chen, X.S. Yao, Chin. Tradit. Herb. Drugs. 49, 442–446 (2001)
19. J. Zhang, H. Guo, Y. Tian, P. Liu, N. Li, J. Zhou, D. Guo, Phytochemistry 68, 2523–2530 (2007)
20. X. Liu, L.R. Qiao, D. Xie, J.G. Dai, J. Asian Nat. Prod. Res. 13, 652–658 (2011)
21. Q. Zhang, W.Q. Zhao, F. Meng, Y.X. Zhang, Chin. J. Antibiot. 37, 49–75 (2012)
22. M. Miyazawa, H. Ando, Y. Okuno, J. Mol. Catal B 27, 91–95 (2004)
23. Y.H. Pyo, T.C. Lee, Y.C. Lee, Food Res. Int. 38, 551–559 (2005)
24. W.Z. Yang, T. Bo, S. Ji, X. Qiao, D.A. Guo, M. Ye, Food Chem. 139, 762–769 (2013)
25. W.Z. Yang, J.X. Zhang, C.J. Yao, S. Qiu, M. Chen, H.Q. Pan, X.J. Shi, W.Y. Wu, D. Guo, J. Pharm. Biomed. Anal. 128, 322–332 (2016)
26. A. Rhule, S. Navarro, J.R. Smith, D.M. Shepherd, J. Ethnopharmacol. 106, 121–128 (2006)
27. S. Ahn, M.H. Siddiqi, H.Y. Noh, Y.J. Kim, Y.J. Kim, C.G. Jin, D.C. Yang, Sci. Bull. 60, 773–784 (2015)
28. N.W. He, Y. Zhao, L. Guo, J. Shang, X.B. Yang, J. Med. Food 15, 350–359 (2012)
29. D.F. Toh, D.N. Patel, E.C.Y. Chan, A. Teo, S.Y. Neo, H.L. Koh, Chin. Med. 6, 1–9 (2011)
30. P. Wang, J. Cui, X. Du, Q. Yang, C. Jia, M. Xiong, X. Yu, L. Li, W. Wang, Y. Chen, T. Zhang, J. Ethnopharmacol. 154, 663–671 (2014)
31. V.M. Dirsch, H. Stuppner, A.M. Vollmar, Planta Med. 64, 423–426 (1998)
32. D.W. Reif, S.A. Mccreedy, Arch. Biochem. Biophys. 320, 170–176 (1995)
33. K. Berg, L. Zhai, M. Chen, A. Kharazmi, T.C. Owen, Parasitol. Res. 80, 235–239 (1994)