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Research Article

New hydroperoxylated and 20,24-epoxylated dammarane triterpenes from the rot roots of *Panax notoginseng*

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ABSTRACT

Background: Root rot is a serious destructive disease of *Panax notoginseng*, a famous cultivated araliaceous herb called Sanqi or Tianqi in Southwest China.
Methods: The chemical substances of Sanqi rot roots were explored by chromatographic techniques. MS, 1D/2D-NMR, and single crystal X-ray diffraction were applied to determine the structures. Murine macrophage RAW264.7 and five human cancer cell lines were used separately for evaluating the anti-inflammatory and cytotoxic activities.
Results and Conclusion: Thirty dammarane-type triterpenes and saponins were isolated from the rot roots of *P. notoginseng*. Among them, seven triterpenes, namely, 20(*S*)-dammar-25-ene-24(*S*)-hydroperoxyl-3β,6α,12β,20-tetrol (**1**), 20(*S*)-dammar-3-oxo-23-ene-25-hydroperoxyl-6α,12β,20-triol (**2**), 20(*S*)-dammar-12-oxo-23-ene-25-hydroperoxyl-3β,6α,20-triol (**3**), 20(*S*)-dammar-3-oxo-23-ene-25-hydroperoxyl-12β,20-diol (**4**), 20(*S*),24(*R*)-epoxy-3,4-*seco*-dammar-25-hydroxy-12-one-3-oic acid (**5**), 20(*S*),24(*R*)-epoxy-3,4-*seco*-dammar-25-hydroxy-12-one-3-oic acid methyl ester (**6**), and 6α-hydroxy-22,23,24,25,26,27-hexanordammar-3,12,20-trione (**7**), are new compounds. In addition, 12 known ones (**12**–**16** and **19**–**25**) were reported in Sanqi for the first time. The new Compound **1** showed comparable antiinflammatory activity on inhibition of NO production to the positive control, whereas the known compounds **9**, **12**, **13**, and **16** displayed moderate cytotoxicities against five human cancer cell lines. The results will provide scientific basis for understanding the chemical constituents of Sanqi rot roots and new candidates for searching antiinflammatory and antitumor agents.

1. Introduction

Panax notoginseng (Burk.) F. H. Chen has been domesticated and cultivated in Southwestern China for more than 400 years. Sanqi or Tianqi is known as a famous araliaceous traditional Chinese medicinal (TCM) herb , and its roots have been used widely as a tonic and hemostatic agent and one of the major ingredients of Danshen dripping pill, a traditional Chinese medicinal preparation for treating common cardiovascular diseases in the world, e.g., China, Korea, and Russia [1]. Owing to its sensitivity to sunlight, heat, and humidity, the plantation of Sanqi is restricted to Southwest China, and the root is susceptible to various diseases [2], particularly, root rot disease, caused mainly by infection by plant pathogens; this is not only the most serious destructive disease of Sanqi in its cultivated regions of China but also a big problem affecting the industrialization of this precious medicinal material because it may cause the loss of production accounting for an average of 20%, even up to 70% in severe situation [3,4].

To date, more than 200 saponins, flavonoids, polyacetylenes, cyclopeptides, and amino acids have been identified in Sanqi. Among which, dammarane triterpenoid saponins are the major and bioactive secondary metabolites in the roots [5], with ginsenosides Rb1, Re, Rd, and Rg1 and notoginsenoside R1 as the five defining saponins. Our previous work on steam-processed Sanqi roots

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resulted in a series of new transformed saponins with promoting effects on the differentiation of PC12 cells [6,7]. Moreover, high performance liquid chromatography/electrospray ionization quadruple time-of-flight mass spectrometry (HPLC–QTOF/MS) comparison showed that the oxidation level of the chemical constituents in Sanqi roots significantly increased after being infected by the root rot disease [8]. To search for new bioactive components and to clarify the discrepancy and their transformation mechanisms in the rot roots of *P. notoginseng*, a detailed chemical investigation was carried out. This led to the isolation of 30 dammarane triterpenes (Fig. 1), including four saponins (**10**, **15**, **18**, and **20**) and 10 hydroperoxylated (**1**–**4** and **13**–**18**) and seven 20,24-epoxylated (**5**–**6** and **26**–**30**) analogs. Of them, four hydroperoxylated (**1**–**4**) and two 20,24-epoxylated (**5**–**6**) derivatives are new compounds, together with **7,** who is losing of the side chain from C-22 to C-27.

The 20(*S*)-protopanaxadiol (PPD), being reported with stronger cytotoxicity on leukemia THP-1 cells [9], has been developed as an anticancer drug candidate [10]. Moreover, compound K (CK) and ginsenosides Rh1 and Rg1 were reported to inhibit the NO production in RAW264.7 cells [11,12]. Herein, all isolates were assayed for the antiinflammatory and cytotoxic activities, which might provide new candidates for searching antiinflammatory and antitumor agents and basic theory for the scientific utilization of rot roots of *P. notoginseng*.

2. Materials and methods

2.1. General experimental procedures

Optical rotations, IR, and UV spectra were recorded on a JASCO P-1020 polarimeter (Tokyo, Japan), Hercules Bio-Rad FTS-135 series spectrometer (CA, USA), and Shimadzu UV-2401 PC ultraviolet–visible spectrophotometer (Tokyo, Japan), respectively. Electrospray ionization mass spectra (ESI-MS) and high-resolution electrospray ionization mass spectra (HR-ESI-MS) were carried out on an Agilent G6230 TOF MS spectrometer. 1D- and 2D-NMR spectra were recorded on a Bruker DRX-500, 600 or 800 spectrometer (Karlsruhe, Germany). The X-ray diffraction instrument (Bruker APEX DUO, Karlsruhe, Germany) was used for characterizing the structures of the crystal. Macroporous resin D101 and silica gel (200–300 mesh) were used for column chromatography (CC), and TLC analysis with silica gel GF254 plates were visualized by heating after spraying with 10% H_2SO_4/EtOH. All CC and TLC materials were purchased from Qingdao Haiyang Chemical Co. Ltd., China. Reverse-phase semipreparative HPLC (Capcell Pak MGII C_{18} column, 5 μm, 250 mm × 10 mm, Tokyo, Japan) was performed on a Hanbon series (Jiangsu Hanbon Science & Technology Co., China) at 25 °C with a flowing rate of 3.0 mL/min.

2.2. Plant material

Rot roots of *Panax notoginseng*, identified by Prof. C.R. Yang of Kunming Institute of Botany (KIB), Chinese Academy of Sciences, were collected in Wenshan District, Yunnan Province, China, on February 2017. A voucher specimen (KIB-Z-2017001) was deposited in the State Key Laboratory of Phytochemistry and Plant Resource in West China of KIB, Chinese Academy of Sciences.

2.3. Extraction and isolation

The fresh rot roots (70 kg) were cut from the healthy part, air-dried under room temperature, and extracted with MeOH under reflux (× 3). The MeOH extract (4.0 kg) was loaded to a macroporous resin D101 column, eluting with H_2O to remove the saccharide portion and then with MeOH to give the total saponin fraction (Fr.) (2.7 kg). Further silica gel CC (250 × 30 cm) with $CHCl_3$-MeOH (7:3) of the MeOH fraction gave Fr. A–Fr. C. TLC and HPLC analysis showed Fr. C (220.8 g) containing mainly notoginsenoside R1 and ginsenosides Rg1, Re, Rd, and Rb1.

Fr. B (62.1 g) was fractionated by CC of RP-18 (MeOH-H_2O, 1:9 to 9:1) to yield eight subfractions (Fr. B1–B8). Fr. B1 (1.5 g) was subjected to silica gel ($CHCl_3$-MeOH, 10:1) and RP-18 (MeOH-H_2O, 1:1 to 9:1), followed by semipreparative HPLC (MeCN-H_2O, 2:8) to yield **2** (2.2 mg), **3** (3.1 mg), and **20** (12 mg). Fr. B3 (10 g) was chromatographed over silica gel CC ($CHCl_3$-MeOH, 200:1 to 95:5) and semipreparative HPLC (MeCN-H_2O, 35:65) to yield **1** (10 mg), **9** (100 mg), **11** (65 mg), **17** (22 mg), **18** (3 mg), and **24** (15 mg). Compounds **10** (120 mg) and **15** (13 mg) from Fr. B2 (2.0 g), and **7** (18 mg) and **19** (120 mg) from Fr. B4 (1.1 g), were obtained by silica gel CC, eluting with $CHCl_3$-MeOH (100:1 to 10:1, 200:1 to 95:5). Subfractions of Frs. B5.1–5.5, Frs. B6.1–6.5, and Frs. B7.1–7.5 were yielded separately by silica gel CC ($CHCl_3$-MeOH, 200:1 to 50:1) from Fr. B5 (1.5 g), Fr. B6 (1.0 g), and Fr. B7 (1.2 g). Furthermore, RP-18 CC (MeOH-H_2O, 9:1 to 20:1 and 50:50) gave **8** (80 mg) from Fr. B5.2 (230 mg) and **6** (2.3 mg) and **12** (30 mg) from Fr. B7.3 (80 mg). Semipreparative HPLC (MeCN-H_2O, 43:57) resulted in **4** (1.0 mg), **13** (19 mg), **14** (11 mg), **16** (36 mg), and **23** (8.2 mg) from Fr. B5.5, and **5** (2.0 mg), **26** (2.5 mg), **28** (4.0 mg), and **29** (2.3 mg) from Fr. B7.4 (250 mg). Crystal **30** (116 mg) was obtained from Fr. B6.3 (370 mg) by recrystallization in $CHCl_3$-MeOH (1:1). Compounds **22** (9.0 mg) and **27** (100 mg) from Fr. B6.4 (210 mg), and **21** (32 mg) and **25** (18 mg) from Fr. B6.5 (70 mg) were obtained by semipreparative HPLC (MeCN-H_2O, 33:67 and 52:48).

2.4. 20(S)-dammar-25-ene-24(S)-hydroperoxyl-3β,6α,12β,20-tetrol (*1*)

White amorphous powder; [*a*]25 D +26.8 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.16) nm; IR (KBr) ν_{max} 3416, 2961, 2932, 2876, 1648, 1631, 1465, 1451, 1384 cm^{-1}; ^1H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS *m/z* 531.3659 $[M+Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662).

2.5. 20(S)-dammar-3-oxo-23-ene-25-hydroperoxyl-6α,12β,20-triol (*2*)

White amorphous powder; [*a*]25 D +85.8 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.14) nm; IR (KBr) ν_{max} 3423, 2966, 2940, 2875, 1693, 1631, 1383; ^1H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS *m/z* 529.3500 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3500).

2.6. 20(S)-dammar-12-oxo-23-ene-25-hydroperoxyl-3β,6α,20-triol (*3*)

White amorphous powder; [*a*]25 D +41.1 (*c* 0.34, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.17) nm; IR (KBr) ν_{max} 3431, 2971, 2932, 1698, 1630, 1425 cm^{-1}; ^1H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS *m/z* 529.3502 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3505).

2.7. 20(S)-dammar-3-oxo-23-ene-25-hydroperoxyl-12β,20-diol (*4*)

White amorphous powder; [*a*]25 D +19.2 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.27) nm; IR (KBr) ν_{max} 3416, 2960, 2934, 2873, 1705, 1630, 1384 cm^{-1}; ^1H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS *m/z* 513.3552 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_5Na$, 513.3550).

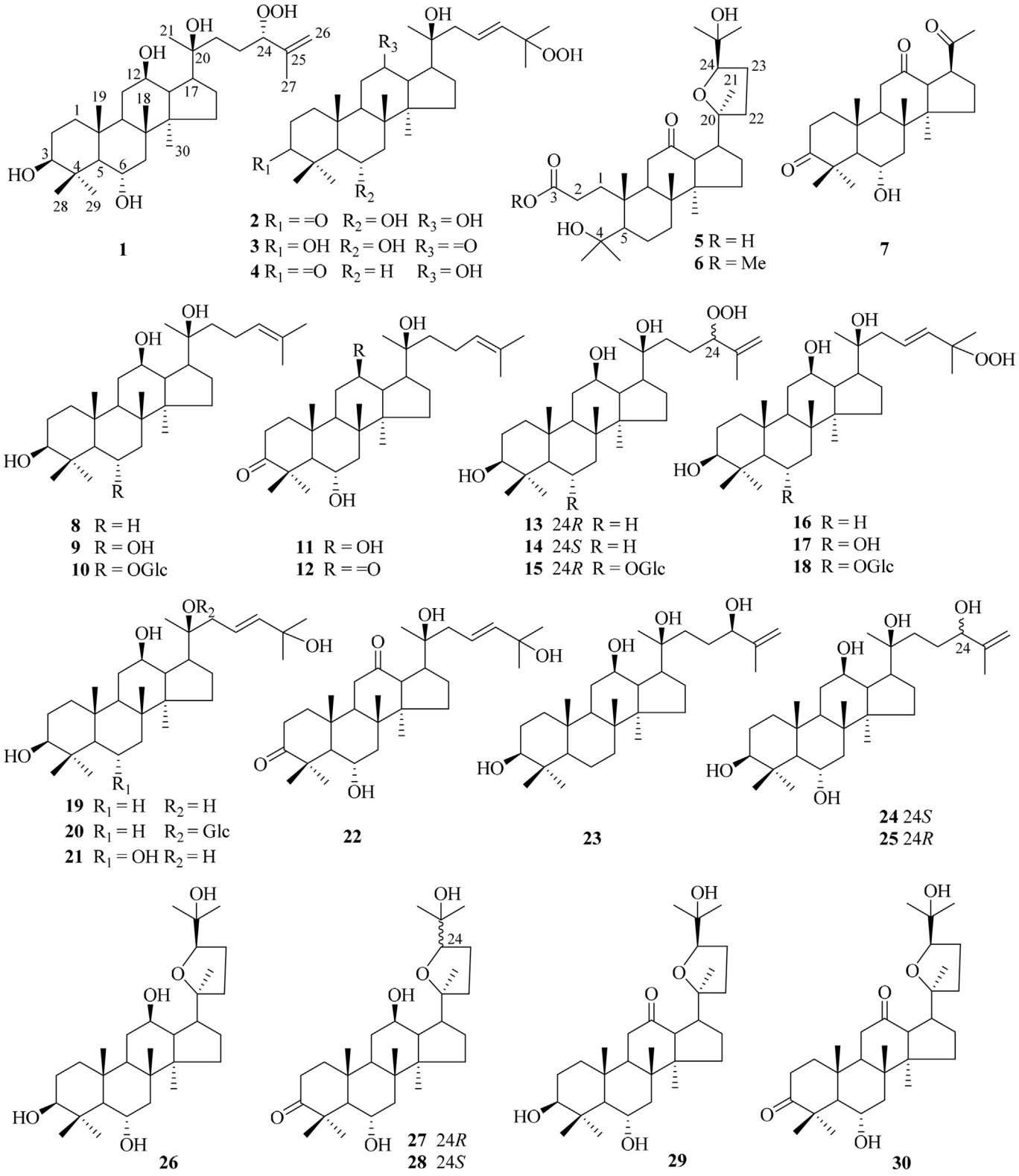

Fig. 1. Compounds **1**–**30** from the rot roots of *P. notoginseng*. (Glc, *β*-D-glucopyranosyl).

*2.8. 20(S),24(R)-epoxy-3,4-seco-dammar-25-hydroxy-12-one-3-oic acid (**5**)*

Colorless columnar crystal; [*a*]25 D +46.5 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.20) nm; IR (KBr) ν_{max} 3439, 2879, 1707, 1634, 1384 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS *m*/*z* 529.3504 $[M+Na]^{+}$ (calcd for $C_{30}H_{50}O_6Na$, 529.3500).

Table 1
^{1}H NMR (600 MHz, 800 MHz, and 500 MHz in C_5D_5N) data for compounds **1**–**7** (δ in ppm, J in hertz)

No.	1	2	3	4[a]	5	6	7[b]
1	1.07 m	1.61 m	0.96 m	1.33 m	1.95 m	1.82 m	1.48 m
	1.70 m	1.76 m	1.44 m	1.79 m	3.20 m	3.11 m	2.25 m
2	1.88 m, 1.95 m	2.31 m, 2.84 m	1.87 m	1.51 m, 2.46 m	2.56 m, 3.02 m	2.40 m, 2.88 m	2.27 m, 2.77 m
3	3.56 dt (11.8,5.1)		3.51 dt (11.5,4.6)				
5	1.26 d (10.4)	1.96 d (10.6)	1.25 d (10.5)	1.38 m	1.69 m	1.62 m	1.91 m
6	4.44 m	4.25 m	4.47 m	1.42 m, 1.50 m	1.68 m	1.62 m	4.25 m
7	1.91 m, 1.99 m	1.92 m	1.97 m	1.28 m, 2.51 m	1.30 m, 1.48 m	1.29 m, 1.46 m	1.86 m
9	1.62 m	1.71 m	1.91 m	1.55 m	2.20 m	2.13 m	1.90 m
11	1.60 m, 2.17 m	1.61 m, 2.12 m	2.37 m, 2.40 m	1.62 m, 2.07 m	2.42 m, 2.59 m	2.40 m, 2.47 m	1.89 m, 2.24 m
12	3.95 m	3.95 m		3.93 m			
13	2.08 t (10.6)	2.08 m	3.37 d (9.7)	2.08 m	3.24 d (9.0)	3.23 d (9.4)	3.27 d (12.0)
15	1.04 m, 1.57 m	1.08 m, 1.63 m	1.18 m, 1.91 m	1.08 m, 1.64 m	1.16 m, 1.79 m	1.16 m, 1.80 m	1.13 m, 1.72 m
16	1.37 m, 1.86 m	1.48 m, 1.88 m	1.90 m, 2.09 m	1.49 m, 1.91 m	1.82 m	1.82 m	1.71 m, 1.97 m
17	2.36 dt (18.0,7.2)	2.40 dt (18.0,7.2)	2.74 m	2.39 dt (18.0,7.2)	2.78 dd (16.2,7.2)	2.78 m	3.37 m
18	1.13 s	1.00 s	1.32 s	1.09 s	1.26 s	1.24 s	1.13 s
19	1.03 s	0.88 s	0.91 s	0.94 s	1.19 s	1.15 s	0.78 s
21	1.42 s	1.45 s	1.46 s	1.47 s	1.25 s	1.25 s	2.22 s
22	1.75 td (13.0,4.9)	2.47 dd (13.8,4.8)	2.48 dd (13.2,4.8)	2.49 m	1.59 m	1.58 m	
	2.28 td (13.1,4.0)	2.81 m	2.59 dd (13.5,5.9)	2.82 dd (13.6,5.5)	1.95 m	1.94 m	
23	2.48 m	6.28 m	6.16 m	6.29 m	1.98 m, 2.05 m	1.95 m, 2.04 m	
24	4.81 t (6.8)	6.09 d (16.0)	6.06 d (15.8)	6.09 d (15.9)	3.97 t (7.1)	3.98 t (7.3)	
26	5.12 s, 5.29 s	1.59 s	1.55 s	1.60 s	1.45 s	1.45 s	
27	1.94 s	1.59 s	1.55 s	1.60 s	1.40 s	1.40 s	
28	2.03 s	1.70 s	2.00 s	1.17 s	1.50 s	1.48 s	1.68 s
29	1.48 s	1.73 s	1.46 s	1.08 s	1.46 s	1.42 s	1.64 s
30	0.97 s	1.34 s	1.02 s	0.95 s	0.85 s	0.85 s	0.82 s
31						3.54 s	

d: doublet; m: multiplet; s: singlet; t: triplet
[a] 800 MHz.
[b] 500 MHz.

Table 2
^{13}C NMR (150 MHz, 200 MHz, and 125 MHz in C_5D_5N) data for compounds **1**–**7** (δ in ppm)

No.	1	2	3	4[a]	5	6	7[b]
1	39.8	40.4	39.4	40.2	36.0	35.6	39.2
2	28.6	33.8	28.4	34.7	30.0	29.5	33.1
3	78.9	219.2	78.6	216.9	177.4	175.2	218.2
4	40.8	48.2	40.8	47.9	75.2	75.1	47.7
5	62.2	59.5	62.0	55.7	52.8	52.8	58.5
6	68.2	67.3	68.1	20.4	23.4	23.2	66.6
7	48.0	45.9	47.2	34.9	34.3	34.3	44.4
8	41.6	41.0	42.1	40.4	40.9	40.9	40.8
9	50.6	49.3	54.4	50.1	47.5	47.4	52.0
10	39.8	38.6	40.0	37.4	42.3	42.2	38.1
11	32.6	33.3	40.5	32.9	40.3	40.2	39.1
12	71.5	71.3	212.3	71.3	210.9	210.9	209.0
13	48.7	49.1	56.7	49.5	57.8	57.8	57.5
14	52.1	52.2	56.0	52.2	56.7	56.7	54.4
15	31.9	31.7	32.3	31.7	32.9	32.8	31.5
16	27.4	27.2	25.0	27.2	25.7	25.7	25.5
17	55.2	54.5	44.8	54.6	43.6	43.6	47.5
18	17.9	17.4	17.9	16.0	15.9	15.9	15.7
19	18.1	18.4	17.8	17.4	21.4	21.3	17.3
20	73.3	73.8	74.0	73.8	85.9	85.8	209.9
21	27.7	28.2	27.6	28.2	25.8	25.8	29.9
22	32.4	40.8	46.0	40.8	36.0	36.0	
23	26.8	127.7	127.2	127.7	27.4	27.4	
24	90.6	138.1	138.7	138.1	85.1	85.1	
25	146.7	81.8	81.7	81.8	71.6	71.6	
26	113.9	25.6	25.5	25.8	27.0	27.0	
27	17.8	25.8	25.7	25.6	27.6	27.5	
28	32.5	32.6	32.3	27.3	34.6	34.8	19.9
29	17.0	20.5	16.9	21.6	28.7	28.5	32.0
30	17.5	16.6	17.7	16.5	17.2	17.1	16.8
31						51.7	

[a] 200 MHz.
[b] 125 MHz.

2.9. 20(S),24(R)-epoxy-3,4-seco-dammar-25-hydroxy-12-one-3-oic acid methyl ester (**6**)

White amorphous powder; [a]25 D +38.8 (c 0.17, MeOH); UV (MeOH) λ_{max} (log ε) 202 (0.20) nm; IR (KBr) ν_{max} 3440, 2969, 1733, 1708, 1630, 1383 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS m/z 543.3667 [M+Na]$^+$ (calcd for $C_{31}H_{52}O_6Na$, 543.3662).

2.10. 6α-hydroxy-22,23,24,25,26,27-hexanordammar-3,12,20-trione (**7**)

Colorless needle crystal; [a]25 D +183.2 (c 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 203 (0.18), 224 (0.14) nm; IR (KBr) ν_{max} 3516, 3436, 2974, 2957, 2876, 1701, 1355 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Tables 1 and 2; HR-ESI-MS m/z 411.2503 [M+Na]$^+$ (calcd for $C_{24}H_{36}O_4Na$, 411.2506).

2.11. Inhibition of NO production in RAW264.7 macrophages

The procedure was carried out as reported previously [13].

2.12. Cytotoxic assay

The procedure was carried out as reported previously [14].

3. Results and discussion

3.1. Structure elucidation of compounds **1**–**7**

Compound **1** was presented as white amorphous powder with a molecular formula $C_{30}H_{52}O_6$ based on HR-ESI-MS at m/z 531.3659

$[M+Na]^+$ (calcd for $C_{30}H_{52}O_6Na$, 531.3662). The IR absorptions revealed the existence of hydroxyl (3416 cm^{-1}) and double (1631 cm^{-1}) bond. In ^1H NMR spectrum (Table 1), seven singlet methyl (δ_H 2.03, 1.94, 1.48, 1.42, 1.13, 1.03, and 0.97, each 3H, s), two olefinic (δ_H 5.29 and 5.12, each 1H, s), four oxymethine (δ_H 4.81 [t, J = 6.8 Hz], and 4.44, 3.95, and 3.56 [each 1H, m]), and five oxygen-bearing (δ_H 12.94, 7.35, 7.09 [each 1H, s], 5.83 [1H, d, J = 5.5 Hz], and 5.36 [1H, d, J = 6.7 Hz]) proton signals were observed. The ^{13}C NMR spectrum showed 30 carbon resonances assignable to seven methyls, nine methylenes with a vinyl (δ_C 113.9), eight methines with four oxygen-bearing ones (δ_C 90.6, 78.9, 71.5, and 68.2), and six quaternary carbons with an olefinic (δ_C 146.7) and an oxygen-bearing (δ_C 73.3) ones. The NMR data were similar to those of 25,26-en-24(*S*)-hydroperoxyl-20(*S*)-PPD (**14**) [15]. However, the C-6 methylene (δ_C 19.2) in **14** was replaced by an oxymethine (δ_C 68.3, δ_H 4.44), along with the shielding of C-5 ($\Delta\delta_C$ +5.4 ppm), C-7 ($\Delta\delta_C$ +4.3 ppm), and C-8 ($\Delta\delta_C$ +3.6 ppm) in **1**, indicating that **1** was a protopanaxatriol (PPT)-type peroxide analog. The ^1H-^1H homonuclear chemical shift correlation spectroscopy. (COSY) of **1** (Fig. 2) indicated the presence of four fragments: -CH-CH(O)-CH_2-, -CH-CH_2-CH(O)-CH-CH-, and two -CH_2-CH_2-CH(O)-. In the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 2) of **1**, both H_3-28 (δ_H 2.03) and H_3-29 (δ_H 1.48) were correlated with C-3 (δ_C 78.9)/C-4 (δ_C 40.8)/C-5 (δ_C 62.2), whereas another two methyls of H_3-18 (δ_H 1.13) and H_3-19 (δ_H 1.03) were correlated withC-7 (δ_C 48.0)/C-8 (δ_C 41.6)/C-9 (δ_C 50.6)/C-14 (δ_C 52.1) and C-1 (δ_C 39.8)/C-5/C-9/C-10 (δ_C 39.8), respectively. Moreover, four hydroxyl protons of 3-OH (δ_H 5.83), 6-OH (δ_H 5.36), 12-OH (δ_H 7.35), and 20-OH (δ_H 7.09) were correlated with C-2 (δ_C 28.6)/C-3/C-4, C-5/C-6 (δ_C 68.2)/C-7, C-11 (δ_C 32.6)/C-12 (δ_C 71.5)/C-13 (δ_C 48.7), and C-17 (δ_C 55.2)/C-20 (δ_C 73.3)/C-21 (δ_C 27.7)/C-22 (δ_C 32.4), respectively, furnishing the typical PPT core. The position of peroxy group was confirmed to be linked to C-24 from the HMBC correlations of δ_H 12.94 with C-24 (δ_C 90.6), combined with the ^1H-^1H COSY correlation of 24-OOH with H-24 (δ_H 4.81).

The absolute configuration of Compound **1** was determined on the basis of ^{13}C NMR, nuclear overhauser effect spectroscopy (ROESY), optional rotation, CD spectra, and biogenetic consideration. Dammarane-type triterpenes derived from the all-chair formed epoxy−squalene requires the *trans*fused rings A/B, B/C, and C/D and β-configurations of H-13, CH_3-18, CH_3-19, and the side chain at C-17 on its tetracyclic skeleton [16]. The 3β-OH in **1** was firstly identified by the chemical shift of C-3 (δ_C 78.9), which was *ca.* 2.8 ppm upfield shifted, related to those of the 3α-OH derivatives [17]. In the ROESY spectrum of **1** (Fig. 2), H-6 (δ_H 4.44) and H-12 (δ_H 3.95) were correlated with the two β-methyls of H_3-18 and H_3-19, and H-9 (δ_H 1.62), H-17 (δ_H 2.36), and α-methyl of H_3-30, respectively, indicating the 6α and 12β hydroxy groups of **1**. Moreover, compared with the 20*R* configuration (C-17 [δ_C 50.7] and C-21 [δ_C 22.8]), the obviously deshielded C-17 (δ_C 55.2) and C-21 (δ_C 27.7) revealed the 20*S* configuration in **1** [17,18]. The 24*S* configuration for **1** was determined by comparing the $[\alpha]_D$ value with that of **14** and the negative Cotton effect at 216 nm for both **1** and **14** (**S2**). On the basis of the aforementioned evidence, Compound **1** was deduced to be 20(*S*)-dammar-25-ene-24(*S*)-hydroperoxyl-3β,6α,12β,20-tetrol.

Compound **2** possessed a molecular formula $C_{30}H_{50}O_6$, as deduced by the HR-ESI-MS at *m/z* 529.3500 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3500) and the distortionless enhancement by polarization transfer (DEPT) spectrum, corresponding to six degrees of unsaturation. The NMR data (Tables 1 and 2) of **2** showed some similarity to those of **1**. However, a ketone (δ_C 219.2), a *trans*disubstituted double bond (δ_C 138.1, δ_H 6.09 [d 16.0] and δ_C 127.7, δ_H 6.28 [m]), and an oxy-quaternary carbon (δ_C 81.8) that appeared in **2** were replaced by an oxymethine (C-3, δ_C 78.9), a terminal double bond between C-25 and C-26 (δ_C 146.7, 113.9), and a peroxylated methine of C-24 (δ_C 90.6) in **1**. These NMR features were in good agreement with those of **17** [19], except for an additional ketone in **2**, indicating **2** was a C-3-oxidized PPT-type peroxide analog. This deduction was verified by the HMBC correlations from H-2 (δ_H 2.84, 2.31), H-28 (δ_H 1.70), and H-29 (δ_H 1.73) to C-3 (δ_C 219.2), along with the ROESY correlations of H-6 (δ_H 4.25) with H-18 (δ_H 0.88) and H-19 (δ_H 1.00) and H-12 (δ_H 3.95) with H-9 (δ_H 1.71), H-17 (δ_H 2.40), and H-30 (δ_H 1.34). Thus, Compound **2** was characterized as 20(*S*)-dammar-3-oxo-23-ene-25-hydroperoxyl-6α,12β,20-triol.

Compound **3** had a molecular formula $C_{30}H_{50}O_6$, as determined by positive-mode HR-ESI-MS at *m/z* 529.3502 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3505). The ^1H and ^{13}C NMR data, indicating a ketone (δ_C 219.2) and a *trans*disubstituted double bond (δ_C 138.7, δ_H 6.06 [d 15.8], δ_C 127.2, and δ_H 6.16 [m]), were quite similar to those of **2**. The C-3 ketone (δ_C 219.2) and C-12 oxymethine (δ_C 71.3) in **2** were upfield and downfield shifted to δ_C 212.3 and δ_C 78.6 in **3**, respectively, indicating the location of the ketone group in **3** and **2** was different. The ^1H-^1H COSY correlations between H-3 (δ_H 3.51) and H-2 (δ_H 1.87)/3-OH (δ_H 5.85), and HMBC correlations from H-2/H-28 (δ_H 2.00)/H-29 (δ_H 1.46) to C-3 (δ_C 78.6) and from H_2-11 (δ_H 2.40, 2.37)/H-13 (δ_H 3.37)/H-17 (δ_H 2.74) to C-12 ketone (δ_C 212.3) revealed that **3** was a C-12-oxidized PPT-type peroxide analog. Compound **3** was therefore identified as 20(*S*)-dammar-12-oxo-23-ene-25-hydroperoxyl-3β,6α,20-triol.

Compound **4** showed a molecular formula $C_{30}H_{50}O_5$, based on HR-ESI-MS at *m/z* 513.3552 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_5Na$,

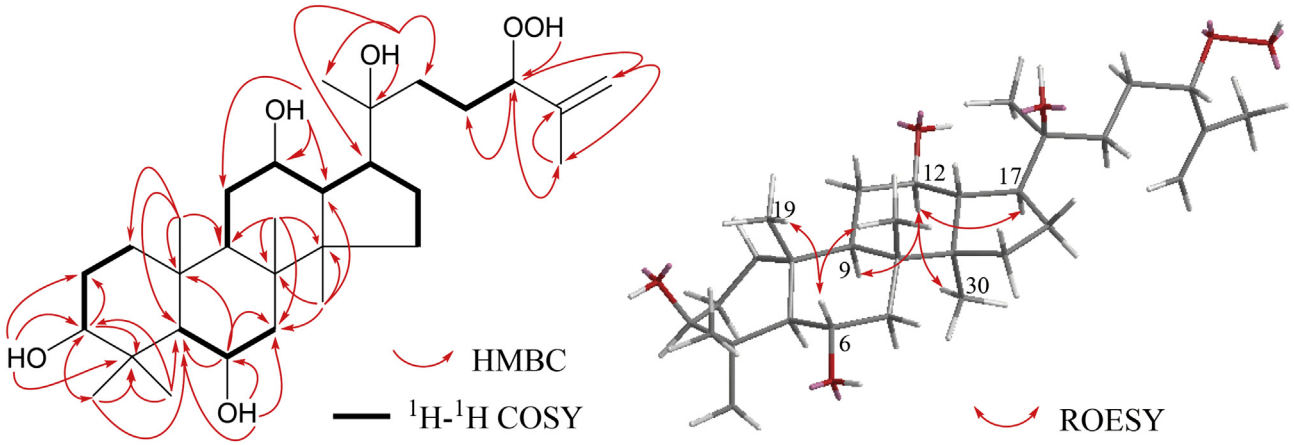

Fig. 2. Key ^1H-^1H COSY, HMBC, and ROESY correlations of Compound **1**.

513.3550). It also showed coherent NMR data (Tables 1 and 2) to **2**, excepting the appearance of a methylene (δ_C 20.4) in **4** instead of the oxymethine (δ_C 67.3 [C-6], δ_H 5.78 [6-OH]) in **2**. When taking the molecular weight (16 Da less than **2**) into consideration, the disappearance of a hydroxyl group at C-6 was reasonably deduced for **4**, suggesting **4** to be a C-3-oxidized PPD-type peroxide derivative. The ^{1}H-^{1}H COSY correlations of H_2-6 (δ_H 1.50, 1.42) with H-5 (δ_H 1.38)/H_2-7 (δ_H 2.51, 1.28) and H-12 (δ_H 3.93) with H-13 (δ_H 2.08) together with the HMBC correlations from H_2-6 to C-5 (δ_C 55.7)/C-6 (δ_C 20.4)/C-7 (δ_C 34.9) and Ha-2 (δ_H 2.46)/H-28 (δ_H 1.17)/H-29 (δ_H 1.08) to C-3 (δ_C 216.9) further confirmed the structure of **4**. Accordingly, Compound **4** was determined to be 20(*S*)-dammar-3-oxo-23-ene-25-hydroperoxyl-12β,20-diol.

Compound **5**, a colorless columnar crystal, had a molecular formula $C_{30}H_{50}O_6$ determined by the *m/z* 529.3504 $[M+Na]^+$ (calcd for $C_{30}H_{50}O_6Na$, 529.3500). Its NMR spectra, combined with heteronuclear single quantum correlation (HSQC) spectra, showed 30 carbon resonances and were similar to those of an ocotillol-type triterpene, 20*S*,24*R*-epoxy-dammar-3β,6α,25-triol-12-one (**29**) [20], featuring a 20,24-epoxylated hydroxyisopropyl-tetrahydrofuran side chain at the C-20, except for the signals of A-ring. The carbonyl signal (δ_C 177.4) and an additional oxy-quaternary carbon at δ_C 75.2 suggested that the A-ring cleavage in **5** retained an open form with a carboxylic acid residue. This illation was confirmed by ^{1}H-^{1}H COSY correlations of H_2-1 with H-2 and H-6 with H-5/H-7, as well as the HMBC correlations of H-5/H-6/H-28/H-29 with C-4 and H-1/H-2 with C-3 (δ_C 177.4). So far, all ocotillol-type triterpenes isolated from *Panax* species possessed a 20*S* configuration [21], which could be recognized by the obvious differences for C-17, C-20, and C-21 between 20(*S*) and 20(*R*) configurations [22]. A clear triplet (J = 7.1 Hz) H-24 indicated the 24*R* configuration for **5**, when comparing with the 24*S* configuration (dd, J = 10.0, 5.5 Hz) [23]. Finally, the structure of **5** was unequivocally confirmed by the oak ridge thermal ellipsoid of plot (ORTEP) drawing of X-ray crystallography analysis (Fig. 3) to be 20(*S*),24(*R*)-epoxy-3,4-*seco*-dammar-25-hydroxy-12-one-3-oic acid.

Compound **6** had a molecular formula of $C_{31}H_{52}O_6$, as deduced by the HR-ESI-MS at *m/z* 543.3667 $[M+Na]^+$ (calcd for $C_{31}H_{52}O_6Na$, 543.3662). The ^{13}C NMR spectrum showing the existence of 31 carbons was closely similar to that of **5**, except for an additional methoxy carbon (δ_C 51.7, δ_H 3.54) and an upper-field shifted carbonyl carbon ($\Delta\delta_C$ −2.2 ppm) in **6**. These NMR characteristics suggested that the carboxylic acid in **5** was esterified to methyl ester in **6**. Furthermore, HMBC correlation from -OCH_3 (δ_H 3.54) to C-3 (δ_C 175.2) and of the same negative Cotton effects at 220 and 285 nm with those of **5** (**S3**) confirmed the structure of **6**. Hence, Compound **6** was elucidated to be 20(*S*),24(*R*)-epoxy-3,4-*seco*-dammar-25-hydroxy-12-one-3-oic acid methyl ester.

Compound **7** was isolated as colorless needle crystal and had a molecular formula of $C_{24}H_{36}O_4$, as determined by the HR-ESI-MS at *m/z* 411.2503 $[M + Na]^+$ (calcd for $C_{24}H_{36}O_4Na$, 411.2506) in the HR-ESI-MS spectrum, referring to seven degrees of unsaturation. IR absorptions for hydroxy (3516 cm^{-1}) and carbonyl (1701 cm^{-1}) groups were observed effortlessly. The ^{13}C NMR and DEPT spectra of **7**, showing the existence of six methyls, six methylenes, four methines with one oxymethine (δ_C 66.6), and seven quaternary carbons with three ketones (δ_C 218.2, 209.9, 209.0), were consistent with those of panaxadione (**30**) [24], except for the absence of the signals from the side chain (C-20 to C-27) and appearance of an additional ketone (δ_C 209.0). Considering the molecular weight, it can be inferred that the side chain of Compound **7** was lost and left with a methyl ketone structure. The above analysis was confirmed by the HMBC correlations of H-13/H-17/H-21 to C-20 (δ_C 209.9). The stereochemistry of C-17 was established as *S* configuration by the ROESY correlation of H-17 with H-30 first and X-ray crystallography analysis finally (Fig. 3). Consequently, Compound **7** was deduced to be 6α-hydroxy-22,23,24,25,26,27-hexanordammar-3,12,20-trione.

3.2. Structure identification of known compounds ***8*–*30***

Twenty-three known triterpenes and saponins were identified as 20(*S*)-PPD (**8**) [25], 20(*S*)-PPT (**9**), 20(*S*)-ginsenoside Rh1 (**10**) [26], 20(*S*)-dammar-3-oxo-24-ene-6α,12β,20-triol (**11**) [27], 6α,20(*S*)-dihydroxydammar-3,12-dione-24-ene (**12**) [28], 25,26-en-24(*R*)-hydroperoxyl-20(*S*)-PPD (**13**), 25,26-en-24(*S*)-hydroperoxyl-20(*S*)-PPD (**14**) [15], 20(*S*)-ginsenoside SL1 (**15**) [29], 23,24-

Table 3
Antiinflammatory activities (uM) of compounds **1**, **13**, and **14**

Compound	IC_{50} ± SD
1	42.47 ± 0.49
13	17.18 ± 0.35
14	25.87 ± 0.60
L-NMMA	39.26 ± 0.91

SD, standard deviation
Data are expressed as means ± SD (n = 3)

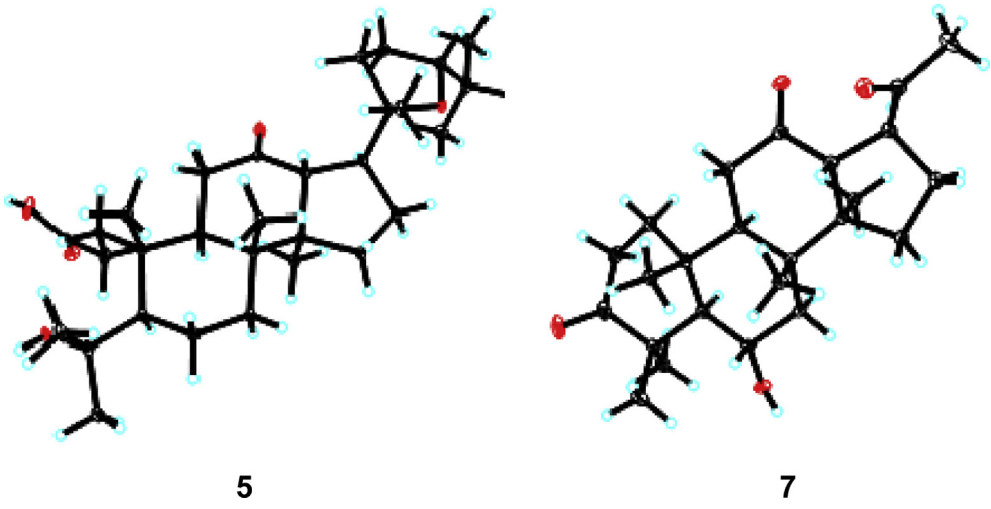

Fig. 3. ORTEP of **5** and **7** with thermal ellipsoids shown at 30% probability.

Table 4
Cytotoxicities (μM) of compounds **9**, **12**, **13**, and **16**

Compound	IC_{50} ± SD				
	Myeloid leukemia HL-60	Lung cancer A-549	Hepatocellular carcinoma SMMC7721	Breast cancer MCF-7	Colon cancer SW480
9	16.58 ± 0.34	34.38 ± 0.42	24.16 ± 0.67	17.06 ± 0.67	17.00 ± 0.37
12	27.37 ± 1.72	> 100	> 100	> 100	> 100
13	16.13 ± 0.42	30.86 ± 0.91	> 100	> 100	26.79 ± 0.31
16	12.37 ± 0.48	> 100	> 100	> 100	> 100
DDP	2.61 ± 0.07	17.80 ± 0.59	10.42 ± 0.44	19.44 ± 1.56	20.80 ± 1.04
Taxol	< 0.008	< 0.008	< 0.008	< 0.008	< 0.008

SD, standard deviation; DDP, cisplatin
Data are expressed as means ± SD (n = 3)

en-25-hydroperoxyl-20(*S*)-PPD (**16**) [15], 20(*S*)-dammar-23-ene-25-hydroperoxyl-3β,6α,12β,20-tetrol (**17**) [19], 20(*S*)-floralquinquenoside A (**18**) [30], 20(*S*)-dammar-23-ene-3β,12β,20,25-tetrol (**19**) [31], 20(*S*),23(*E*)-ginsenoside Rh13 (**20**) [32], 20(*S*)-dammar-23-ene-3β,6α,12β,20,25-pentanol (**21**) [33], 6α,20(*S*),25-trihydroxy-dammar-3,12-dione-23-ene (**22**) [28], 25,26-en-24(*R*)-hydroxyl-20(*S*)-PPD (**23**) [31], 20(*S*), 24(*S*)-dammar-25(26)-ene-3β,6α,12β,20,24-pentanol (**24**) [19], 20(*S*), 24(*R*)-dammar-25(26)-ene-3β,6α,12β,20,24-pentanol (**25**) [34], 20(*S*),24(*R*)-dammar-20,24-epoxy-3β,6α,12β,25-tetrol (**26**) [21], 20(*S*),24(*R*)-dammar-3-one-20,24-epoxy-6α,12β,25-triol (**27**) [35], 20(*S*), 24(*S*)-dammar-3-one-20,24-epoxy-6α,12β,25-triol (**28**), 20(*S*),24(*R*)-epoxy-dammar-3β,6α, 25-triol-12-one (**29**) [20], and panaxadione (**30**) [24] by comparing with the NMR and MS data with literature values. Among them, **10**, **15**, **18,** and **20** were saponins, and **12**–**16** and **19**–**25** were reported in Sanqi for the first time.

3.3. Antiinflammatory and cytotoxic activities

All isolates were evaluated for inhibition of NO production in murine macrophage cell line RAW264.7 (antiinflammatory) and cytotoxicity against five human cancer cell lines (breast cancer MCF-7, colon cancer SW480, hepatocellular carcinoma SMMC7721, lung cancer A-549, and myeloid leukemia HL-60).

As shown in Table 3, the new Compound **1** showed comparable antiinflammatory activity (half maximal inhibitory concentration IC_{50} = 42.47 ± 0.49 μM) to the positive control, L-NMMA (IC_{50} = 39.26 ± 0.91 μM), whereas **13** and **14** showed stronger inhibitory effects on NO production (IC_{50} = 17.18 ± 0.35 [**13**] and 25.87 ± 0.60 [**14**] μM). It was noted that the PPD analog showed stronger antiinflammatory activity than the PPT analog and triterpenes are superior to their saponins. Moreover, C-24-OOH is essential for the inhibition of NO production and 24*R* configuration is stronger than 24*S* configurations.

At a concentration of 40 μM, most isolates showed no cytotoxicities. Only compound **9** showed moderate cytotoxicities against all five cancer cells (Table 4). Moreover, **13** showed toxicities on HL-60, A-549, and SW480 cell lines, whereas **12** and **16** showed cytotoxicities against only HL-60 cells.

4. Conclusion

In summary, 30 dammarane-type triterpenes and saponins including 10 hydroperoxylated (**1**–**4** and **13**–**18**), seven 20,24-epoxylated (**5**–**6** and **26**–**30**), and one losing of the side chain (**7**) analogs were identified from the rot roots of *P. notoginseng*. Seven of them, **1**–**7**, are new compounds. Most compounds are sapogenins, and their oxidation level was increased, which was consistent with the analytic results in the previous report [8]. The new Compound **1** showed comparable antiinflammatory activity to the positive control, but weaker than the known triterpenes **13** and **14**. Moreover, Compounds **9**, **12**, **13**, and **16** showed moderate cytotoxicities against five human cancer cells. The investigation will provide valuable information in understanding the chemical constituents of Sanqi rot roots and searching new candidates for antiinflammatory and antitumor agents. Studies of the plant itself and the isolates against pathogenic microorganisms of the titled plant are now on progress, which may provide the basic theory for chemical and biological control of root rot disease of the titled herb.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Database: CCDC: 1862502, 1862503.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.01.008.

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