A new triterpenoid compound from stems and leaves of American ginseng
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A new dammarane-type triterpenoid compound was isolated from stems and leaves of American ginseng. The structure of the new sapogenin was elucidated by the combined analysis of NMR and HR-ESI-MS as dammar-20S, 25S-epoxy-3β, 12β, 26-triol (1). Compound 1 showed cytotoxic effect on human SM7721 and human Hela cells in vitro.

**Keywords:** stems and leaves of American ginseng; dammarane-type triterpene; dammar-20S; 25S-epoxy-3β; 12β; 26-triol; cytotoxic effect

1. Introduction
Ginseng roots are key components of internationally popular herbal medicines and dietary supplements. Two ginseng species, Asian ginseng (Panax ginseng C.A. Mey.) and American ginseng (Panax quinquefolius L.), are extensively used in traditional Chinese medicine (TCM) as a tonic, an adaptogen and a remedy for an extensive array of ailments. However, these species have different TCM ‘natures’. Asian ginseng is considered to be stimulating and invigorate yang, whereas American ginseng is considered to be calming and nourishing yin (Dharmananda 2002). In addition, Compared with Asian ginseng, there are some distinct active ingredients such as extracts of Asian ginseng roots had higher Rg1:Rb1 ratios than American ginseng and demonstrated that Rg1 dominance leads to angiogenesis, whereas Rb1 dominance promotes the opposite effect by limiting growth of cancer cells. (Schlag & McIntosh 2013). It is reported that American ginseng showed many medical effects such as anti-virus (Li et al. 1992), anti-cancer (Ma et al. 2008), neuroprotection (Lian et al. 2005), anti-obesity (Liu et al. 2008, 2010) and anti-diabetes mellitus (Xie et al. 2004). The pharmacological effects of American ginseng have been attributed primarily to ginsenosides, which are triterpenoid saponin glycosides (dammarenene-type saponins) (Attele et al. 1999). More than 27 putative ginsenosides have been isolated from ginseng roots and are classified into two main groups: the glycosides of 20(S)-protopanaxadiol (20(S)-dammar-24-ene-3β, 12β, 20S-triol) (Rb1, Rb2, Rc, Rd, Rg3 and Rh2) and those of 20(S)-protopanaxatriol (6α-hydroxy-20(S)-protopanaxadiol) (Re, Rf, Rg1, Rg2, Rh1 and R1) (Attele et al. 1999; Awang 2000; Shibata 2001). The main ginsenosides isolated from American ginseng

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(Rb1, Re, Rd, Re and Rg1) typically account for greater than 70% of the total ginsenosides (Court et al. 1996; Wills et al. 2002; Assinewe et al. 2003). However, the total contents of 12 ginsenosides (Rg1, Re, F11, Rf, Rg2, Rh1, Rb1, Rc, Rb2, Rb3, Rd, Rh2) in the different parts of 5-year-old American ginseng follow this order: leaf > root-hair > rhizome > root > stem (Qu et al. 2009). Moreover, the levels of ginsenoside Rg1, Rb3 and Rd in the leaves are dramatically higher than in the other parts of American ginseng (Zhang et al. 2008). Studies on the chemical constituents of American ginseng are numerous. However, research on the domestic stems and leaves of American ginseng saponins cultivation is still not deep enough, to further elucidate the chemical composition of the domestic ginseng stems and biological activity. To this end, the chemical composition and biological activity of the domestic American ginseng stems and leaves saponins were studied. A new dammarane-type triterpene isolated from the stems and leaves of American ginseng is reported in this paper. This paper describes the isolation and the structural elucidation of the new triterpene by chemical and spectroscopic methods (1D and 2D NMR, MS). The structure of this new compound was determined as dammar-20S, 25S-epoxy-3β, 12β, 26-triol (1) (Figure 1). In the MTT assays, cisplatin was chosen as the positive control, meanwhile, the biological activities of compound 1 and cisplatin were compared (Figure 6). The new compound showed significant cytotoxic activity against SM7721 and Hela cell lines, with the IC_{50} values of 56 and 63 μg/mL, respectively, which implied that compound 1 had moderate cytotoxic activity against cancer cells.

2. Results and discussion

Repeated column chromatography (CC) of the EtOH extract of the stems and leaves of American ginseng led to the isolation of the new dammarane-type triterpene. Compound 1 was obtained as a white amorphous solid (ethyl acetate). The molecular formula was determined as C_{30}H_{52}O_{4} by NMR spectra and HR-ESI-MS at m/z 477.3944 [M + H]^+. The IR spectrum of 1 showed absorption bands at ν_{max} 3363, 2948, 2875, 1451, 1385 and 1375 cm^{-1}, due to hydroxy groups, methyl groups and methylene groups. The 1H NMR spectrum of 1 displayed seven methyl signals at δ1.63 (3H, s), 1.38 (3H, s), 1.23 (3H, s), 1.05 (3H, s), 1.03 (3H, s), 0.91 (3H, s)
and 0.90 (3H, s). Compared with panaxadiol (Liu et al. 2011), the \(^1\)H NMR spectrum also displayed the disappearance of H-26 at \(\delta\) 1.18–1.26 and the appearance of H-26 at \(\delta\) 3.92 (1H, d, \(J = 11.5\) Hz) and \(\delta\) 3.90 (1H, d, \(J = 11.5\) Hz) in compound 1. The \(^{13}\)C NMR spectrum of 1 revealed 30 signals, revealing 11 methylene (one of them bearing an oxygen atom (\(\delta\) 67.11)), 6 methine [two of them bearing an oxygen atom (\(\delta\) 78.06 and 70.36)], 6 quaternary [two of them showing an oxygen atom (\(\delta\) 77.87 and 77.09)] and 7 methyl carbons. The chemical shifts of 1 showed resemblance with those of protopanaxdiol (Zhao et al. 1996) except for the signals of the side-chain. On further comparison with 20(S)-panaxadiol (Fujita et al. 1995), whose structure is dammar-20S, 25-epoxy-3β, 12β-diol, a 20S, 25-epoxy group was deduced to exist in compound 1. In the HMBC spectrum of 1 (Figure 2), the correlations between H-27 with C-24, C-25 and C-26, H-26 with C-24, C-25 and C-27 were observed. Combined with the 1H NMR spectrum, the location of a hydroxy was determined to be at C-26. HMQC and HMBC experiments also showed correlations between H-21 with C-16, C-17 and C-20; H-17 with C-12, C-15 and C-20; H-24 with C-23, C-25, C-26 and C-27. By analysis of two-dimensional NMR spectra, the proton and carbon signals of 1 were assigned. The important and diagnostic NOEs observed in the NOE differential spectrum of 1 are illustrated in Figure 3. The \(S\)-configuration of C-20 and the \(S\)-configuration of C-25 were confirmed by observation of the NOE between (i) H-21 and H-17, (ii) H-26 and H-21 (Figure 3). The absolute configuration of C-20 and C-25 was determined to be \(S\) by the single-crystal X-ray diffraction experiment performed on a clear columnar crystal recrystallised from CH\(_3\)CN (Figure 4). According to the above structural information, the structure of 1 was determined to be dammar-20S, 25S-epoxy-3β, 12β, 26-triol.

The crystal data are as follows: C\(_{30}\)H\(_{52}\)O\(_4\); Mr = 476.72, dimensions 0.42 mm \(\times\) 0.38 mm \(\times\) 0.26 mm, monoclinic, P2\(_1\), \(a = 6.3782(8)\) Å, \(b = 11.2461(15)\) Å, \(c = 18.615(3)\) Å,
\( \alpha = 90.00^\circ, V = 1320.68(51)\, \text{Å}^3, Z = 2, D_{\text{calc}} = 1.156\, \text{g/cm}^3, F_{000} = 528. \) Data collection was performed on a SMART CCD using graphite-monochromated radiation (\( \lambda = 0.71073\, \text{Å} \)); 5181 unique reflections were collected to \( \theta_{\max} = 22.302^\circ \), in which 4261 reflections were observed \([F_2 > 2\sigma (F_2)]\). The structure was solved by direct methods [SHELXS-97(Sheldrick, 2008)] and refined by full-matrix least-squares on \( F^2 \). In the structure refinements, non-hydrogen atoms were refined anisotropically. All hydrogen atoms were placed on geometrically ideal positions by the ‘ride on’ method. The final indices were \( R = 0.0666, R_W = 0.1237 \) and \( S = 0.1496 \). Based on the above evidence, the structure of 1 could be characterised as dammar-20\( S \), 25\( \beta \)-epoxy-3\( \beta \), 12\( \beta \), 26-triol. Compound 1 is a minor glycoside in stems and leaves of American ginseng. Compound 1 showed cytotoxic effect on human SM7721 and human Hela cells in vitro (Figure 5). From the comparison of the bioactivity of compound 1 and cisplatin, we can see that the IC\(_{50}\) value of compound 1 is larger than that of cisplatin. Even so, compound 1 showed cytotoxic effect on SM7721 and Hela cells in vitro. Especially for the SM7721 cells, the IC\(_{50}\) value of the new compound is closer to that of cisplatin. Therefore, compound 1 showed better cytotoxic effect on human SM7721 cells.

3. Experimental

3.1. General experimental procedures

IR spectra were taken on an AVATAR 330 FT infrared spectrophotometer. NMR spectra were measured at 500 MHz for \(^1\)H NMR, 125.8 MHz for \(^{13}\)C NMR and 500 MHz for HMBC and

![Figure 4. Crystal structure of compound 1.](image)

![Figure 5. Inhibition of the growth of four cell lines by compound 1.](image)
HMQC on a Bruker Avance-500 spectrometer (Karlsruhe, Germany). NMR spectra were measured in pyridine-$d_5$ using TMS as the internal standard (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). HR-ESI-MS were recorded using Ionspec 7.0 TFT-ICR-MS (IonSpec Corporation, Lake Forest, CA, USA). Chemical shifts ($\delta$) are expressed in ppm. Preparative HPLC was carried out on a Shodex R1-201H Refractive Index Detector and SunFire Prep C18 Column (10 $\mu$m, 10 mm × 150 mm), 510 BINARY HPLC PUMP (Waters). Silica gel H (200–300 mesh; Qingdao Marine Chemical, Inc., Qingdao, China) was used in CC. Also, silica gel G plates (Qingdao Marine Chemical, Inc.) were used in thin layer chromatography.

3.2. Plant material
The stems and leaves of American ginseng were provided by the Jilin TongBao Chinese traditional medicine science & technology development Co., Ltd. A voucher specimen (No. 20110922) has been deposited at the Institute of Frontier Medical Science, Jilin University, China.

3.3. Extraction and isolation
The stems and leaves of American ginseng (2.5 kg) were extracted with 70% EtOH (50 L × 3) and the EtOH soluble fraction was concentrated. The residue (175 g) was then subjected to silica gel CC eluting with CHCl$_3$–MeOH mixture to give 68 fractions. Fractions 56–60 (120 mg) were combined and then subjected to preparative RP-HPLC with MeOH–H$_2$O (76:24) as mobile phase to obtain compound 1 (8 mg, 0.004%). Compound 1 (8 mg): white amorphous solid (ethyl acetate); positive HR-ESI-MS showed a quasimolecular ion at $m/z$ 477.3944 [M+H]$^+$ (calcd for C$_{30}$H$_{53}$O$_4$, 477.3938). IR (KBr) $\nu_{\text{max}}$: 3363 (C=O), 2948, 2875 (C–CH$_3$), 1385, 1375 (C–CH$_3$) and 1451 (C–CH$_2$) cm$^{-1}$; $^1$H NMR (500 MHz, pyr-$d_5$): $\delta$ 1.64 (H-1e), 0.90 (H-1a), 1.25 (H-2a), 1.16 (H-2e), 3.42 (H-3), 0.80 (H-5), 1.54 (H-6e), 1.44 (H-6a), 1.44 (H-7e), 1.23 (H-7a), 1.47 (H-9), 2.00 (H-11e), 1.02 (H-11a), 0.75 (H-12, td, J = 10.5 and 4.5 Hz), 1.93 (H-13), 1.47 (H-15e), 1.02 (H-15a), 1.66 (H-16e), 1.50 (H-16a), 2.16 (H-17), 1.05 (H-18), 0.91 (H-19), 1.38 (H-21), 1.46 (H-22e), 1.38 (H-22a), 1.65 (H-23e), 1.05 (H-23a), 1.92 (H-24e), 1.47 (H-24a), 0.91 (H-26e, dd, J = 11.5 Hz), 0.896 (H-26a, dd, J = 11.5 Hz), 1.63 (H-27), 1.23 (H-28), 1.03 (H-29) and 0.90 (H-30). $^{13}$C NMR (125.8 MHz, pyr-$d_5$): $\delta$ 39.3 (C-1), 328.4 (C-2), 878.06 (C-3), 399.95 (C-4), 856.2 (C-5), 851.6 (C-6), 835.0 (C-7), 393.8 (C-8), 850.4 (C-9), 837.2 (C-10), 832.1 (C-11), 870.4 (C-12), 849.1 (C-13), 852.1 (C-14), 831.9 (C-15), 827.4 (C-16), 852.0 (C-17), 816.0 (C-18), 817.0 (C-19), 814.0 (C-20), 850.0 (C-21), 854.0 (C-22), 850.0 (C-23), 836.0 (C-24), 850.0 (C-25), 850.0 (C-26), 850.0 (C-27), 850.0 (C-28), 850.0 (C-29), 850.0 (C-30).

Figure 6. Comparison of the biological activity of compound 1 and cisplatin.
δ16.4(C-19), δ77.9 (C-20), δ27.2(C-21), δ27.3(C-22), δ15.6(C-23), δ31.9(C-24), δ77.1(C-25), δ67.1(C-26), δ26.9(C-27), δ28.1(C-28), δ16.1(C-29) and δ17.9(C-30).

3.4. In vitro cytotoxicity bioassay
In the cytotoxicity assay, human SM7721 and human Hela cells were used as the target cells according to the method reported (Jin et al. 2008). For drug exposure experiments, after contact of the cells with the drug for 24 h, 10 μL of 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide (MTT) solution (5 mg/mL) was added to each well, and the tumour cells were incubated at 37°C with foetal calf serum in a humidified atmosphere of 5% CO₂ for 4 h. At the end of the incubation period, the growth medium was removed and replaced with 150 μL DMSO at room temperature. After agitating for 10 min, the absorbance was determined at 492 nm on a Bio-Rad (model 550) microplate reader to calculate the 50% inhibition concentration (IC₅₀). DMSO and MTT were purchased from Sigma Chemical (Sigma Chemical Co., Saint Louis, MO, USA).

4. Conclusion
In this paper, a new sapogenin was isolated from the stems and leaves of American ginseng and identified as dammar-20S,25S-epoxy-3β,12β, 26-triol by the combination analysis of NMR and HR-ESI-MS, which showed good cytotoxic activity against SM7721 and Hela cell lines. The result of this experiment will be of benefit to explore a new drug against cancer.

Supplementary material
Supplementary material relating to this paper is available online, alongside Figures S1–S15.

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Disclosure statement
No potential conflict of interest was reported by the authors.

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