Two new dammarane-type triterpene sapogenins were isolated from the Chinese red ginseng. The new sapogenins were named as 24,26-dihydroxy-panaxdiol (1) and 24-hydroxy-panaxdiol (2). Their structures were elucidated by the combined analysis of NMR and mass spectrometry as 20(S),25(R)-epoxydammarane-3β,12β,24β,26-tetraol (1) and 20(S),25-epoxydammarane-3β,12β,24α-triol (2). The complete signal assignments of the two compounds were carried out by 2D NMR spectral and NOE differential spectroscopy analysis.

**Keywords:** dammarane-type triterpene sapogenin; Chinese red ginseng; 20(S),25(R)-epoxydammarane-3β,12β,24β,26-tetraol; 20(S),25-epoxydammarane-3β,12β,24α-triol

1. Introduction

Ginseng belongs to the Araliaceae family and its root has been used as a traditional medicine in Asian countries for over 2000 years (Coon & Ernst 2002). Ginseng has two preparations, and one is air-dried white ginseng and another one is steamed and sun-dried red ginseng. It contains various active components including ginsenosides, polysaccharides, peptides, polyacetylenic alcohols and fatty acids (An et al. 2011). The red ginseng is made from a ginseng plant going through an intensive process of cleaning, steaming and drying. Heat treatment of ginseng leads to chemical changes of ginsenosides that improve the health benefits of ginseng by inactivating catabolic enzymes and releasing the antioxidant substances from the ginseng. The steamed ginseng has a stiff texture, which not only protects the effective ingredients but also produces new constituents (Kim et al. 2000). Major ginsenosides of red ginseng saponin extract include less polar red ginseng-unique saponins Rg3, Rk1 and Rg5 in a HPLC analysis (Kim et al. 2008).
As supplementation with red ginseng is believed to improve health, numerous studies have been conducted to validate its beneficial effects. They demonstrate that red ginseng has anti-inflammatory (Jung et al. 2012), antidiabetic (Kim et al. 2011), anticancer (Lee et al. 2011) and arthritis-ameliorating effects (Jhun et al. 2014). At present, Chinese red ginseng and Korean ginseng are both commonly found in a market. There is no significant difference between the quality of Chinese red ginseng and Korean red ginseng according to reports. The contents of ginseng saponins and ginsenoside Rg1, Re, and Rb1 in Tongrentang red ginseng (Chinese red ginseng) are not lower than those in Korean red ginseng (Wu et al. 2007). The Chinese red ginseng is mainly produced in Jilin province. Reported herein are the isolation and the structural elucidation of the two new dammarane-type sapogenins, 1 and 2 from Chinese red ginseng (Panax ginseng C. A. Meyer) by Meyer chemical and spectroscopic methods (1D and 2D NMR, MS). The structure of 1 was determined as 20(S),25(R)-epoxydammarane-3β,12β,24β,26-tetraol and the structure of 2 was determined as 20(S),25-epoxydammarane-3β,12β,24α-triol.

2. Results and discussion

Repeated column chromatography of the EtOH extract of the Chinese red ginseng led to the isolation of new dammarane-type sapogenins 1 and 2 (Figure 1).

Compound 1 was obtained as a white amorphous solid (MeOH). The molecular formula was determined as C_{30}H_{52}O_{5} by NMR spectra and HR-ESI-MS at m/z 493.3853 [M + H]^+ (calcld. for 493.3875).

The $^1$H NMR spectra of 1 displayed seven methyl signals at δ 0.83 (3H, s), δ 0.91 (3H, s), δ 1.01 (3H, s), δ 1.04 (3H, s), δ 1.21 (3H, s), δ 1.34 (3H, s) and δ 1.43 (3H, s). The $^{13}$C NMR spectrum of 1 revealed 30 signals, including ten methylene [one of them bearing an oxygen atom (δ 68.73)], seven methine [three of them bearing an oxygen atom (δ 68.18, 71.05 and 78.35)], six quaternary [two of them bearing an oxygen atom (δ 78.09 and 78.09)] and seven methyl carbons. The chemical shifts of 1 showed resemblance with those of protopanaxadiol (Zhao et al. 1996) except the signals of the side chain. Furthermore, compared with 20(S)-panaxadiol (Duc et al. 1994), whose structure is dammar-20S,25-epoxydammarane-3β,6α,12β,24α-tetrol, a 20S,25-epoxy group was deduced to exist in compound 1. It can be summarised that the difference of 20 (S) and 20 (R) in dammarane-type saponin could be observed from the carbon signal of C-21 (S: δ 27; R: δ 20) (Fujita et al. 1995). In the $^{13}$C NMR spectrum of compound 1, the chemical shift of C-21 at δ 27.4 showed that the configuration of C-20 was S-form. These data can be

Figure 1. The structures of compounds 1 and 2.
accommodated on the dammar-20S,25-epoxy-3β,12β-triol triterpene having three secondary hydroxyls and one primary hydroxyl. The location of this functional group at C-24 and C-26 was determined by the heteronuclear multiple-bond connectivity (HMBC) spectrum of 1. In the HMBC spectrum of 1, the correlations between H-26, H-27 with C-24 and H-27, H-24 with C-26 were observed. Combined with 1H NMR spectrum, and compared with the results from the report (Duc et al. 1994), the location of two hydroxyls was determined to be at C-24 and C-26. HMQC and HMBC experiments showed the correlation between H-21 (δ 1.34) with C-17 (δ 51.72), C-20 (δ 78.09) and C-22 (δ 24.01).

The stereochemistry of the side chain was finally determined by nuclear overhauser effect (NOE) differential spectroscopy and 2D NMR spectra. On irradiating the signal of the 21-methyl protons (δ 1.34), NOEs were observed at the signals of the H-27-methyl protons (δ 4.01) protons. In addition, the irradiation at the frequency of H-24 gave rise to NOE at the signal of the 27-methyl protons. All the above data pointed to the structure of 1 as 20(S),25(R)-epoxydammarane-3β,12β,24α-tetraol (Figure 1). Compound 1 was a minor glycoside in the red Panax ginseng C. A. Meyer.

Compound 2 was obtained as a white amorphous solid (MeOH). The molecular formula was determined as C30H52O4 by NMR spectra and HR-ESI-MS at m/z 477.3923 [M + H]+ (calcd. for 477.3956).

The 13C NMR spectrum of 2 revealed 30 signals. The aglycone of compound 2 revealed nine methylenes, seven methines [three of them bearing an oxygen atom (δ 70.63, 74.53 and 78.02)], six quaternary [two of them bearing an oxygen atoms (δ 78.13 and 78.59)] and eight methyl carbons. Compared with compound 1, the 1H NMR spectrum displayed the disappearance of H-26 at δ 3.90, 4.34 and the appearance of H-24 at δ 3.87 in compound 2. The spectral data of compound 2 showed resemblance with those of compound 1 except the side chain, therefore it also had a 20S,25-epoxy group in compound 2. The location of this functional group at C-24 was determined by the HMBC spectrum of 2, which showed long-range correlations between H-24 and C-25, C-26 and C-27 (δ 78.59, 23.28 and 30.12, respectively).

The stereochemistry of the side chain was finally determined by NOE differential spectroscopy and 2D NMR spectra. On irradiating the signal of the 21-methyl protons (δ 1.35), NOEs were observed at the signals of the H-26-methyl protons (δ 1.53) and the H-17 (δ 2.21) protons, while the irradiation of the signal of the 27-methyl protons showed NOEs at the signals of the H-24 (δ 3.87) and H-26 protons. In addition, the irradiation at the frequency of H-24 gave rise to NOE at the signal of the 27-methyl protons. Compared with the report (Duc et al. 1994), all the data pointed to the structure of 2 as 20(S),25-epoxydammarane-3β,12β,24α-triol (Figure 1). Compound 2 was also a minor glycoside in the red Panax ginseng C. A. Meyer.

3. Experimental
3.1. General experimental procedures
NMR spectra were measured at 500 MHz for 1H NMR, 125.8 MHz for 13C NMR and 500 MHz for HMBC HMQC and NOE on a Bruker Avance-500 spectrometer (Karlsruhe, Germany). NMR spectra were measured in pyridine-d5 using TMS as an internal standard (Cambridge Isotope Laboratories, Inc., Andover, MA, USA). HRESI-MS spectra were recorded using Ionspec 7.0 TFT-ICR-MS (IonSpec Corporation, Lake Forest, CA, USA). Chemical shifts (δ) were expressed in ppm. Preparative HPLC was carried out on a Shodex R1-201H Refractive Index Detector (Hangzhou Kexiao Chemical Equipment Co., Hangzhou, China) and SunFire Prep C18 Column (10 μm, 10 mm × 150 mm; Waters, Taunton, MA, USA), 1525 Binary HPLC pump (Waters). Silica gel H (200–300 mesh; Qingdao Marine Chemical Inc., Qingdao, China)
was used in column chromatography. Also, silica gel G plates (Qingdao Marine Chemical Inc.) were used in thin-layer chromatography.

3.2. Plant material

The Chinese red ginseng was provided by the Jilin HuangFeng group Co., Ltd (No. 110820) had been deposited at the Institute of Frontier Medical Science, Jilin University, China.

3.3. Extraction and isolation

The Chinese red ginseng (2.5 kg) was extracted with 85% EtOH (10 L × 3) and the EtOH-soluble fraction was concentrated. The residue (86 g) was subjected to macro-reticular absorption resin (D101) and eluted with H2O (20 L) and 95% EtOH (40 L). The EtOH fraction (62 g) was then subjected to silica gel CC eluting with CHCl3–MeOH mixture to give 164 fractions. Fractions 62–75 (63 mg) were combined and then subjected to preparative RP-HPLC with MeCN–MeOH–H2O (52:20:28) as mobile phase to obtain compounds 1 (9 mg, 0.001%) and 2 (13 mg, 0.001%).

3.3.1. Compound 1 (9 mg)

White amorphous power (MeOH); positive HR-ESI-MS showed a quasi-molecular ion at m/z 493.3853 [M + H]+ (calcd. for 493.3875). 1H NMR (500 MHz, pyr-d5): δ1.50(H-1e, m), δ6.95 (H-1a, m), δ1.95(H-2a, m), δ1.61(H-2e, m), δ3.42(H-3, dd, J = 10.3 Hz, 5.7 Hz), δ0.81(H-5, m), δ1.54(H-6e, m), δ1.22(H-6a, m), δ1.39(H-7e, m), δ1.25(H-7a, m), δ1.54(H-9, m), δ2.11(H-11e, m), δ1.24(H-11a, m), δ1.76(H-12, s), δ2.21(H-13, m), δ1.54(H-15e, m), δ0.99(H-15a, m), δ1.61(H-16e, m), δ1.50(H-16a, m), δ2.21(H-17, m), δ1.04(H-18, s), δ0.83(H-19, s), δ1.34(H-21, s), δ2.11(H-22e, m), δ1.95(H-22a, m), δ2.43(H-23e, m), δ1.25(H-23a, m), δ4.01(H-24, s), δ4.34 (H-26e, d, J = 10.8 Hz), δ3.90(H-26a, d, J = 11.1 Hz), δ1.43(H-27, s), δ1.21(H-28, s), δ1.01 (H-29, s) and δ0.91(H-30, s).

13C NMR (125.8 MHz, pyr-d5): δ39.55(C-1), δ28.32(C-2), δ78.35(C-3), δ39.60(C-4), δ56.55 (C-5), δ18.86(C-6), δ35.24(C-7), δ40.21(C-8), δ50.99(C-9), δ37.47(C-10), δ32.72(C-11), δ71.05 (C-12), δ49.11(C-13), δ52.42(C-14), δ32.64(C-15), δ27.48(C-16), δ51.72(C-17), δ15.80(C-18), δ16.62(C-19), δ78.09(C-20), δ27.40(C-21), δ27.40(C-22), δ21.47(C-23), δ68.18(C-24), δ78.09 (C-25), δ68.73(C-26), δ23.67(C-27), δ28.67(C-28), δ16.24(C-29) and δ18.8(C-30).

3.3.2. Compound 2 (13 mg)

White amorphous solid (MeOH); positive HR-ESI-MS showed a quasi-molecular ion at m/z 477.3923 [M + H]+ (calcd. for 477.3956). 1H NMR (500 MHz, pyr-d5): δ1.70(H-1e, m), δ6.97 (H-1a, m), δ1.94(H-2a, m), δ1.82(H-2e, m), δ3.43(H-3, dd, J = 10.9 Hz, 5.3 Hz), δ0.83(H-5, m), δ1.56(H-6e, m), δ1.48(H-6a, m), δ1.48(H-7e, m), δ1.24(H-7a, m), δ1.50(H-9, m), δ2.10(H-11e, m), δ1.45(H-11a, m), δ3.76(H-12, td, J = 10.1 Hz, 4.9 Hz), δ1.93(H-13, m), δ1.48(H-15e, m), δ1.01(H-15a, m), δ1.82(H-16e, m), δ1.26(H-16a, m), δ2.21(H-17, m), δ1.02(H-18, s), δ0.89(H-19, s), δ1.35(H-21, s), δ1.95(H-22e, m), δ1.59(H-22a, m), δ2.01(H-23e, m), δ1.94(H-23a, m), δ3.87(H-24, dd, J = 10.6 Hz, 4.9 Hz), δ1.53(H-26, s), δ1.68(H-27, s), δ1.23(H-28, s), δ1.03(H-29, s) and δ0.92(H-30, s).

13C NMR (125.8 MHz, pyr-d5): δ39.58(C-1), δ28.37(C-2), δ78.02(C-3), δ39.64(C-4), δ56.55 (C-5), δ18.91(C-6), δ35.29(C-7), δ40.24(C-8), δ50.61(C-9), δ37.50(C-10), δ32.35(C-11), δ70.63(C-12), δ49.41(C-13), δ52.23(C-14), δ31.98(C-15), δ27.76(C-16), δ52.35(C-17), δ15.89 (C-18), δ16.64(C-19), δ78.13(C-20), δ26.51(C-21), δ28.23(C-22), δ26.01(C-23), δ74.53(C-24), δ78.59(C-25), δ23.28(C-26), δ30.12(C-27), δ28.74(C-28), δ16.33(C-29) and δ18.04(C-30).
4. Conclusion

In this article, two new dammarane-type triterpene sapogenins were isolated from the Chinese red ginseng and identified as 20(S),25(R)-epoxydammarane-3β,12β,24β,26-tetraol (1) and 20(S),25-epoxydammarane-3β,12β,24α-triol (2) by the combination analysis of NMR and HR-ESI-MS. The result of this experiment will help explore new drugs based on Chinese red ginseng.

Supplementary material

Supplementary material relating to this article is available online at http://dx.doi.org/10.1080/14786419.2015.1038538.

Acknowledgements

The authors are grateful to the Jilin HuangFeng group Co., Ltd for providing the Chinese red ginseng. They are also very grateful to Jianxi Song (Key Laboratory for Supramolecular Structure and Materials of Ministry of Education, Beihua University) for the measurement of NMR.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

An YE, Ahn SC, Yang DC, Park SJ, Kim BY, Baik MY. 2011. Chemical conversion of ginsenosides in puffed red ginseng. Lwt-Food Sci Technol. 44:370–374.

Coon JT, Ernst E. 2002. Panax ginseng: a systematic review of adverse effects and drug interactions. Drug Saf. 25:323–344.

Duc NM, Kasai R, Ohtani K, Ito A, Nham NT, Yamasaki K, Tanaka O. 1994. Saponins from Vietnamese ginseng, Panax vietnamensis Ha et Grushv. collected in central Vietnam. III. Chem Pharm Bull. 42:634–640.

Fujita S, Kasai R, Ohtani K, Yamasaki K, Chiu MH, Nie RL, Tanaka O. 1995. Dammarane glycosides from aerial part of Nealsomitira integripilola. Phytochemistry. 39:591–602.

Jhun J, Lee J, Byun JK, Kim EK, Woo JW, Lee JH, Kwok SK, Ju JH, Park KS, Kim HY, et al. 2014. Red ginseng extract ameliorates autoimmune arthritis via regulation of STAT3 pathway, Th17/Treg balance, and osteoclastogenesis in mice and human. Mediators Inflamm. 2014:351856.

Jung HJ, Choi H, Lim HW, Shin D, Kim H, Kwon B, Lee JE, Park EH, Lim CJ. 2012. Enhancement of anti-inflammatory and antinociceptive actions of red ginseng extract by fermentation. J Pharm Pharmacol. 64:756–762.

Kim HJ, Lee SG, Chae IG, Kim MJ, Im NK, Yu MH, Lee EJ, Lee IS. 2011. Antioxidant effects of fermented red ginseng extracts in streptozotocin-induced diabetic rats. J Ginseng Res. 35:129–137.

Kim J, Jeon M, Paeng KJ, Paeng IR. 2008. Competitive enzyme-linked immunosorbent assay for the determination of catecholamine, dopamine in serum. Anal Chim Acta. 619:87–93.

Kim WY, Kim JM, Han SB, Lee SK, Kim ND, Park MK, Kim CK, Park JH. 2000. Steaming of ginseng at high temperature enhances biological activity. J Nat Prod. 63:1702–1704.

Lee JI, Ha YW, Choi TW, Kim HJ, Kim SM, Jang HJ, Choi JH, Choi MH, Chung BC, Sethi G, et al. 2011. Cellular uptake of ginsenosides in Korean white ginseng and red ginseng and their apoptotic activities in human breast cancer cells. Planta Med. 77:133–140.

Wu JM, Lin HY, Zhao LH, Jia HT, Jia HK, Wang Y, Chen DW. 2007. Comparative study on quality of Tongrentang red ginseng and Korean red ginseng – determination of ginsenosides and polysaccharides. China J Chin Mater Med. 32:573–577.

Zhao P, Liu Y-Q, Yang C-R. 1996. Minor dammarane saponins from Panax notoginseng. Phytochemistry. 41:1419–1422.