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Six new dammarane-type triterpenes from acidic hydrolysate of the stems-leaves of *Panax ginseng* and their inhibitory–activities against three human cancer cell lines

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ABSTRACT

Six new dammarane-type triterpenes, namely 3β,6α,12β-trihydroxy-20*S*,24*S*-epoxydammar-25-ene (**1**), 3β-acetoxy-6α,12β,25-trihydroxy-24,25-dihydrodammar-(*E*)-20(22)-ene (**2**), 3β,6α,12β-trihydroxy-20*S*,24*R*-epoxydammar-25-ene (**3**), 6α-acetoxy-3β,12β,20*R*-trihydroxydammar-25-ene (**4**), 6α-acetoxy-3β,12β,20*R*-trihydroxydammar-24-ene (**5**), and 12β-acetoxy-3β,6α,25-trihydroxy-24,25-dihydrodammar-(*E*)-20(22)-ene (**6**), together with thirteen known compounds (**7–19**) were isolated from the acidic hydrolysate of the stems-leaves of *Panax ginseng*. Their chemical structures were elucidated by considerable spectroscopic analyses and comparison with the reported data. All 19 compounds were evaluated for their cytotoxicties against three human cancer cell lines, HL-60, NCI-N87 and Hep-G2. Compound **11** exhibited significant inhibitory activity in a concentration-dependent manner against HL-60 and Hep-G2 with the IC_{50} values of 4.21 and 6.69 μM, respectively. Its activity was stronger than that of the positive control vinorelbine, of which the IC_{50} values against HL-60 and Hep-G2 were 11.47 and 23.12 μM, respectively. Compounds **4**, **7**, **8**, **12–14**, and **19** showed moderate cytotoxic activities at the concentrations of 1–200 μM against the three human cancer cell lines. The preliminary structure to activity relationship was also discussed based on the experimental data obtained. Complete side chain, the configuration of C-20, the substitution of hydroxyl group of C-25 and C-3 were important factors influencing the cytotoxicities. The results may also provide useful data for researching new anti-tumor agents.

1. Introduction

Panax ginseng C. A. Mey., a well-known herbal medicine, has been widely used worldwide and is also increasingly used as a general health tonic in many countries. There is much evidence showing that ginsenosides, the major active components of *P. ginseng* (Li and Yang, 2012a; Wang et al., 2013a; Yang et al., 2013), have various biomedical efficacies such as anti-stress, anti-fatigue, anti-aging, anti-mutagenic, anti-cancer, anti-diabetes, anti-oxidant, anti-inflammation, neurovascular modulatory, and hepato-protective effects. Our previous reports on developing new drugs for the prevention and/or treatment of chronic heart failure suggested that ginsenoside-Rg_2, one of the main constituents of total saponins from the stems-leaves of *P. ginseng* (GTSSL), could be used to prevent and treat cardiovascular disease (Tian et al., 2003; Lu et al., 2004) and was extensively metabolized *in vivo* (Gui et al., 2007; Yang et al., 2009a,b). It was also suggested that GTSSL composition (Li et al., 2012a) was different from the saponins from the roots and rhizomes of *P. ginseng*. Many studies demonstrated that saponins from the roots and rhizomes of *P. ginseng* can be hydrolyzed to form their metabolites including protopanaxadiol (PPD) and protopanaxatriol (PPT), which were more easily absorbed into the body (Lee et al., 2009) and display more potent activities than their original saponins (Wakabayashi et al., 1998; Chen et al., 2013). This reminds us that the hydrolysates of saponins, i.e., the aglycones, may be better utilized by human body and exhibit stronger bioactivities than the nonhydrolyzed saponins. Thus, the present study focused on the isolation and purification of bioactive constituents from the acid hydrolysis of GTSSL and the evaluation of their cytotoxicity against three human cancer cell lines (HL-60, NCI-N87 and Hep-G2) in order to clarify whether GTSSL can be hydrolyzed under acid condition and to

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investigate the biological activities of the resultant products so as to discover drug candidates.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. The molecular formula was deduced to be $C_{30}H_{50}O_4$ on the basis of quasi-molecular ion peak in positive HR–ESI–MS at *m/z* 949.7491 $[2M+H]^+$ (calcd for 949.7485). Its IR spectrum displayed strong absorption bands at 3384, 1650 and 1079 cm^{-1}. The ^{13}C NMR spectrum (Table 1) and HSQC experiments of **1** showed 30 carbons including seven methyls at δc 16.8, 17.6, 18.1, 18.4, 18.6, 29.2, and 32.3, nine methylenes at δc 28.5, 28.9, 30.8, 32.7, 32.9, 32.9, 40.8, 47.9, and 110.7 (a terminal double bond CH_2 carbon), eight methines at δc 49.5, 49.8, 50.6, 62.3, 68.1, 71.1, 78.8, and 84.1, and six quaternary carbons at δc 39.7, 39.8, 41.5, 52.5, 87.5, and 146.2, which were consistent with the skeleton of ocotillol-type triterpenes (Morita et al., 1982; Tanaka et al., 1985; Yang et al., 1991). The ^1H and ^{13}C NMR spectra (Table 1) of **1** gave a signal pattern similar to that of protopanaxatriol oxide II (3β,6α,12β,25-tetrahydroxy-20*S*,24*S*-epoxydammarane) (Morita et al., 1982) except for the isopropanol unit. A set of new signals due to a propene unit [δ_H 4.79 (1H, br s), 5.13 (1H, br s), 1.64 (3H, s); δ_C 110.7 (CH_2), 146.2 (C), 18.6 (CH_3)] in place of the isopropanol unit of protopanaxatriol oxide II were observed. These data as well as 2D NMR spectra suggested that **1** was a dehydration derivative of protopanaxatriol oxide II. The orientation of the 21-CH_3 group was determined by NOESY, in which the proton signal of 21-CH_3 group was correlated with signal of 30-CH_3 group (α-orientation), suggesting that the 21-CH_3 group was α-orientation. On the other hand, the β-orientation of H-24 was confirmed by the observation of NOESY cross-peak between δ_H 4.56 (1H, dd, *J* = 5.6, 11.0 Hz) and 1.14 (3H, s, 18-CH_3). Based on the above evidences, the chemical structure of **1** was determined to be 3β,6α,12β-trihydroxy-20*S*,24*S*-epoxydammar-25-ene (Fig. 1).

Table 1
^1H and ^{13}C NMR spectral data for **1** and **3** (in pyr-d_5,δ in ppm, *J* in Hz)[a].

C No.	**1**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	0.99 (m); 1.50 (m)	40.8	0.99 (m); 1.49 (m)	39.6
2	1.94 (m); 1.82 (m)	28.9	1.52 (m); 1.88 (m)	28.5
3	3.52 (m)	78.8	3.51 (m)	78.7
4		39.8		40.7
5	1.24 (m)	62.3	1.21 d (10.5)	62.2
6	4.42 (m)	68.1	4.41 (m)	68.1
7	1.94 (m); 1.90 (m)	47.9	1.94 (m); 1.89 (m)	47.8
8		41.5		41.4
9	1.62 (m)	50.6	1.59 (m)	50.5
10		39.7		39.8
11	1.41 (m); 1.68 (m)	32.9	1.33 (m); 1.89 (m)	33.2
12	3.76 (m)	71.1	3.73 (m)	70.9
13	1.82 (m)	49.8	1.59 (m)	49.7
14		52.5		52.4
15	1.20 (m); 1.62 (m)	32.7	1.22 (m); 1.60 (m)	32.9
16	1.40 (m); 1.83 (m)	28.5	1.49 (m); 1.89 (m)	28.8
17	2.26 (m)	49.5	2.23 (m)	49.4
18	1.14 (s)	17.6	1.13 (s)	17.6
19	1.02 (s)	18.1	1.00 (s)	18.0
20		87.5		86.7
21	1.21 (s)	29.2	1.26 (s)	26.9
22	2.16 (m); 1.51 (m)	32.9	2.11 (m); 1.62 (m)	32.6
23	1.80 (m); 1.41 (m)	30.8	1.66 (m); 1.21 (m)	30.2
24	4.56 (dd, *J* = 5.6, 11.0)	84.1	4.41 (m)	81.2
25			146.2	144.8
26	4.79(br s);5.13(br s)	110.7	4.90(br s); 5.13(br s)	112.9
27	1.64 (s)	18.6	1.84 (s)	19.3
28	1.99 (s)	32.3	1.96 (s)	32.3
29	1.44 (s)	16.8	1.42 (s)	16.8
30	0.92 (s)	18.4	0.92 (s)	18.4

[a] All the signals were assigned by 1D and 2D NMR spectra.

Compound **2** was obtained as a white amorphous powder. HR–ESI–MS spectrum gave $[M+Cl]^-$ at *m/z* 553.3679 (calcd for 553.3665), supporting a molecular formula of $C_{32}H_{54}O_5$. Its IR spectrum showed strong absorption bands at 3435, 1643 and 1731 cm^{-1}, indicating hydroxyl, olefinic and ester carbonyl functional groups. Both the ^1H and ^{13}C NMR spectra (Table 2) of **2** closely resembled those of the known dammar-20(22)*E*,24-diene-3β,6α,12β-triol (Baek et al., 1996), except for signals due to the C-17 side chain and C-3. In the NMR spectra of **2**, a set of new signals assigned to an acetoxyl group were evident at δ_H 2.04 (3H, s) and δ_C 171.1 (C), 21.6 (CH_3). The acetoxyl group must be located at the C-3 position of **2** from HMBC cross-peaks between δ_H 4.71 (H-3) and δc 39.5 (C-4), 31.6 (C-28), 17.3 (C-29) and 171.1 (C=O) (Fig. 2) and the significantly downfield-shifted to δc 81.5 for C-3. Comparing the NMR signals of **2** with those of dammar-20(22)*E*,24-diene-3β,6α,12β-triol (Baek et al., 1996), in combination with HSQC and HMBC experiments, another significant difference was the lack of signals for a double bond in the C-17 side-chain, whereas signals assigned to an isopropanol unit appeared at δ_H 1.31 (6H, s) and δ_C 29.1 (CH_3), 30.2 (CH_3) and 69.9 (C–OH) were observed. The remaining stereochemistry with respect to the 20(22)-double bond of **2** was deduced to be identical to that of dammar-20(22)*E*,24-diene-3β,6α,12β-triol (Baek et al., 1996) from the agreement of the ^{13}C NMR chemical shifts of C-21 in both compounds, which the 21-CH_3 of the (*E*) structure was usually appeared at higher field around 13 ppm (Baek et al., 1996; Wang et al., 2004) whereas the 21-CH_3 of the (*Z*) structure was usually appeared at lower field around 20–30 ppm (Chen et al., 1987). This conclusion was also supported by no NOE enhancement between δ_H 1.77 (H_3-21) and δ_H 5.54 (H-22) as well as NOE enhancement between δ_H 1.77 (H_3-21) and δ_H 1.42 (H_a-23), 2.32 (H_b-23). Consequently, the chemical structure of **2** was characterized as 3β-acetoxy-6α,12β,25-trihydroxy-24,25-dihydrodammar-(*E*)-20(22)-ene (Fig. 1).

Compound **3** was isolated as a white amorphous powder and had the same formula as **1**, which was deduced from a quasi-molecular ion peak at *m/z* 971.3774 $[2M+Na]^+$ (calcd for 971.3782). Its IR spectrum also showed strong absorption bands at 3368, 1650 and 1078 cm^{-1}, due to hydroxyl, olefinic and ester carbonyl functional groups. The ^1H and ^{13}C NMR spectra (Table 1) of **3** closely resembled those of **1**. In the ^{13}C NMR spectra, the signal of C-24 was observed at δ_C 81.2 in **3**, whereas at δ_C 84.1 in **1**, suggesting that they are epimeric compounds. The H-24 and 21-CH_3 were α-oriented based on the NOESY correlation between H-24 (δ_H 4.41) and 21-CH_3 (δ_H 1.26), 21-CH_3 and 30-CH_3 (δ_H 0.92; α-orientation) (Fig. 2). Thus, the chemical structure of **3** was determined to be 3β,6α,12β-trihydroxy-20*S*,24*R*-epoxydammar-25-ene (Fig. 1).

Compound **4** was obtained as a white amorphous powder. HR–ESI–MS spectrum gave a quasi-molecular ion peak $[M+COOH]^-$ at *m/z* 563.3950 (calcd for 563.3953), corresponding to the molecular formula of $C_{32}H_{54}O_5$. Its IR spectrum exhibited strong absorption bands at 3433, 1650 and 1730 cm^{-1}. It showed an identical carbon skeleton as the known protopanaxatriol (**13** and **14**), with additional signals for an acetoxyl group (δ_H 2.07; δ_C 22.8 and 170.6). In the ^{13}C NMR spectra (Table 3), in combination with HSQC and HMBC experiments, the signal of C-6 was observed at δ_C 71.4 in **4**, whereas at δ_C 67.8 in **2**, suggesting that the acetoxyl group must be located at the C-6 position. This conclusion was also supported by HMBC correlation between H-6 (δ_H 5.64) and ester carbonyl group (δ_C 170.6). Comparing the NMR signals of **4** with those of protopanaxatriol (**13** and **14**), a 26-CH_3 signal (δ_H 2.00; δ_C 25.9) disappeared in protopanaxatriol (**13** and **14**) whereas a set of signal arose at δ_H 4.76 (1H, br s) and 4.81 (1H, br s) as well as δ_C