Six new dammarane-type triterpene saponins from *Panax ginseng* flower buds and their cytotoxicity

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1. Introduction

*Panax ginseng* Meyer (Araliaceae) is a precious botanical drug that has been used as a general tonic in eastern countries for more than two thousand years. It is famous for the efficacy in reinforcing vital energy, invigorating spleen for benefiting the lung, and recuperating from diseases. Ginseng saponins (ginsenosides), the major biologically active components of *P. ginseng*, were contributed to its various pharmacological effects and have been administrated to treat cancer [1,2], diabetes [3], cardiovascular disorders [4], aging [5], and so on. Based on the diverse structure differentiation of the sapogenins, the known ginsenosides can be further classified into six different subtypes: the protopanaxadiol (PPD) type, protopanaxatriol type, octillol type, oleanolic acid type, C17 side chain–varied type, and miscellaneous triterpenes. The PPD, protopanaxatriol, oleanolic acid, and octillol types are the four most common subtypes among the saponins from ginseng plant [6,7].

Recent studies have been focusing on the synthesis of more active PPD derivatives from dammarane-type triterpene saponins, conversion products using artificial gastric juice, acidic and alkaline hydrolysis from total ginseng saponins, and extraction of malonylginsenosides, ginsenosides under room temperature. The new products obtained have showed promising antidiabetic and antitumor properties [8–13]. Researchers never stop to report new compounds related to ginsenosides [14,15]. In view of the wide range of

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A B S T R A C T

Background: *Panax ginseng* has been used for a variety of medical purposes in eastern countries for more than two thousand years. From the extensive experiences accumulated in its long medication use history and the substantial strong evidence in modern research studies, we know that ginseng has various pharmacological activities, such as antitumor, antidiabetic, antioxidant, and cardiovascular system—protective effects. The active chemical constituents of ginseng, ginsenosides, are rich in structural diversity and exhibit a wide range of biological activities.

Methods: Ginsenoside constituents from *P. ginseng* flower buds were isolated and purified by various chromatographic methods, and their structures were identified by spectroscopic analysis and comparison with the reported data. The 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide method was used to test their cytotoxic effects on three human cancer cell lines.

Results: Six ginsenosides, namely 6′-malonyl formyl ginsenoside F₁ (1), 3β-acetoxyl ginsenoside F₁ (2), ginsenoside Rb₂ (6), ginsenoside Rb₃ (7), 7β-hydroxyl ginsenoside Rd (8) and ginsenoside Rb₁ (10) were isolated and elucidated as new compounds, together with four known compounds (3–5 and 9). In addition, the cytotoxicity of these isolated compounds was shown as half inhibitory concentration values, a tentative structure–activity relationship was also discussed based on the results of our bioassay.

Conclusion: The study of chemical constituents was useful for the quality control of *P. ginseng* flower buds. The study on antitumor activities showed that new Compound 1 exhibited moderate cytotoxic activities against HL-60, MGC80-3 and Hep-G2 with half inhibitory concentration values of 16.74, 29.51 and 20.48 μM, respectively.

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potent activities, ginseng (ginsenosides) can be regarded as the source of active lead compounds research.

Owing to our continued interests in the chemical diversity and structure–bioactivity relationship of ginsenosides [16–21], we investigated the flower buds of P. ginseng and obtained 6 new ginsenosides, 1, 2, 6–8, and 10, and 4 known ginsenosides. Herein, we report the isolation and structure elucidation of these new ginsenosides, along with their antiproliferative activities against three human cancer cell lines (HL-60, MGC80-3, and Hep-G2).

2. Materials and methods

2.1. Plant materials

The flower buds of P. ginseng were collected in Tonghua city, Jilin province of China in July 2014 and identified by one of the authors (Xiao-Jie Gong). A voucher specimen (No.2014003) of this plant was deposited at our laboratory.

2.2. Apparatus and chemicals

Optical rotation values were measured using a P–2000 digital polarimeter (Jasco, Tokyo, Japan). IR spectra were recorded on a Nicolet 550 FT–IR spectrophotometer (Nicolet Instrument, Inc., Madison, USA) using KBr pellets. NMR spectra were measured using a DRX 500 MHz NMR spectrometer (Bruker Corporation, Bremen, Germany) and LC MS spectra were recorded using a QTOF 6540 mass spectrometer (Agilent Technologies) using a Kaseisorb 5 column (5 μm, 21.4 × 100 mm; Kasei Kogyo Co. Ltd, Tokyo, Japan). Analytical-grade reagents of CHCl3/MeOH/H2O (gradient, 100:10:1 to 10:10:1) were used for purification of subfraction E5-D. The acid hydrolysis of new compounds and sugar determination were based on our previously reported method by HPLC analysis [16]. All the glucose residues from those 6 new compounds showed the retention time of 12.6 min, which was the same as D-glucose [16]. In addition, the rhamnosyl moiety in 6, the xylosyl moiety in 2, and the arabinosyl moiety in 8, were detected by one of the authors (Jinmei Liu) using a Milli-Q system (Billerica, USA) in our laboratory.

2.3. Extraction and isolation

The extraction of P. ginseng flower buds (41 kg) was performed with 70% EtOH (3 × 16 L) under reflux. The extract was concentrated on a rotary evaporator to give a dark brown residue (892 g), and then the residue was dissolved in H2O (4 L) and subjected to liquid–liquid partitioning using n–hexane (3 × 12 L) and EtOAc (3 × 12 L) to provide the n-hexane (200 g), EtOAc (31 g), and water-soluble (645 g) fractions. The EtOAc fraction was separated on silica gel and eluted with CHCl3/MeOH/H2O (gradient, 100:10:1 to 10:10:1) to yield fractions E1–E9. Fraction E5 (2.8 g) was chromatographed on an MCI gel column using 75% EtOH to yield 6 subfractions E5A–E5F, and the subfraction E5E (62 mg) was purified by semipreparative HPLC with 80% MeOH to afford 1 (11.4 mg). Similarly, Fraction E6 (900 mg) was separated by 80% EtOH to yield 5 subfractions E6A–E6E, and the subfraction E6D (41 mg) was purified with 75% MeOH to afford 2 (10.2 mg). Fraction E7 (4.8 g) was separated by a gradient of EtOH/H2O (60% to 100%), and 3 (524 mg) was afforded with 80% EtOH. The water fraction (200 g) was further fractionated on a Diaion HP-20 resin column, eluting with EtOH/H2O (gradient, 0:100:0) to yield 5 subfractions W1–W5. Fraction W3 (26 g) was separated on an MCI gel column eluting with EtOH/H2O (gradient, 40:60 to 100:0), producing 11 subfractions W3A–W3K. And, the subfraction W3B (6 g) was separated by MCI gel column eluting with 70% EtOH, giving two subfractions W3B-1 and W3B-2. Semipreparative HPLC was used to purify subfraction W3B-2 (911 mg) to obtain 4 (25.4 mg) and 5 (661 mg). Subfraction W3D (3.2 g) was separated by combining silica gel (CHCl3/MeOH/H2O 6:1:0.1 to 2:1:0.1) and MCI gel (75% EtOH) column. After the MCI gel fractionation, four subfractions W3D-1 to W3D-4 were obtained. Subfraction W3D-2 (77 mg) and W3D-4 (43 mg) were all further purified by semipreparative HPLC with 35% MeCN to afford 6 (8.6 mg), 7 (9.2 mg), and 8 (7.5 mg). Subfraction W3H (14 mg) was separated by silica gel (CHCl3/MeOH/H2O 5:1:0.1) repeatedly to afford 9 (282 mg). Fraction W4 (21 g) was separated by MCI gel column eluting with 80% EtOH, giving 6 subfractions W4A–W4F. Further purification of subfraction W4D (92 mg) by semipreparative HPLC with 35% MeCN afforded 10 (12.5 mg).

2.4. Acid hydrolysis of new compounds

The acid hydrolysis of new compounds and sugar determination were based on our previously reported method by HPLC analysis [16]. All the glucose residues from those 6 new compounds showed the retention time of 12.6 min, which was the same as D-glucose [16]. In addition, the rhamnosyl moiety in 6 and the arabinosyl moiety in 7 were α-configured (tR 9.6 and 10.7 min, respectively) [16,22], and the xylosyl moiety in 7 was β-configured (tR 9.2 min) [22], showing that the retention times were consistent with those of the authentic samples.

2.5. Cytotoxicity assay

HL-60 and MGC80-3 cells were cultured in RPMI 1640 medium, and Hep-G2 cells were cultured in Dulbecco’s modified Eagle’s medium, supplemented with 10% fetal bovine serum, 100 unit/mL penicillin plus 100 μg/mL streptomycin at 37°C in a humidified atmosphere of 5% CO2. The cytotoxicity assay was detected by one of the authors (Weizhi Li) using an MTT method [23]. MCF-7, Hep-G2, and HL-60 cells were seeded into 96-well plates in a density of 2 × 104, 1 × 105, and 0.5 × 105 cells/well in 100 μL, respectively. After 24 h of incubation at 37°C, cell counts were determined by MTT method at 1, 5, 100, 150, and 200 (μM) were added and incubated for 48 h. Continuously, each well was
treated with 20 μL of MIT solution (5 mg/mL), and the plates were incubated for another 4 h. Then, 100 μL of dissolving solution (containing 5% isopropanol, 10% sodium dodecyl sulfate, and 0.1% HCl, dissolved in water) was added to dissolve the formazan. The optical density was measured at 540 nm using a microplate reader.

Table 1

| No. | 1          | 2          |
|-----|------------|------------|
|     | δc (ppm)  | δh (ppm)  |
| 1   | 39.4       | 38.9       |
| 2   | 28.2       | 23.9       |
| 3   | 78.6       | 81.3       |
| 4   | 40.4       | 39.2       |
| 5   | 61.8       | 61.4       |
| 6   | 67.8       | 67.4       |
| 7   | 47.5       | 47.3       |
| 8   | 41.3       | 41.2       |
| 9   | 50.0       | 49.7       |
| 10  | 39.4       | 38.6       |
| 11  | 30.8       | 30.8       |
| 12  | 70.2       | 70.3       |
| 13  | 49.2       | 49.1       |
| 14  | 51.4       | 51.4       |
| 15  | 31.0       | 30.9       |
| 16  | 26.7       | 26.7       |
| 17  | 51.6       | 51.8       |
| 18  | 17.7       | 17.6       |
| 19  | 17.5       | 17.4       |
| 20  | 83.5       | 83.4       |
| 21  | 22.1       | 22.5       |
| 22  | 36.2       | 36.1       |
| 23  | 23.0       | 23.4       |
| 24  | 126.1      | 126.0      |
| 25  | 131.1      | 131.1      |
| 26  | 25.8       | 25.8       |
| 27  | 17.8       | 17.9       |
| 28  | 32.0       | 31.4       |
| 29  | 16.5       | 17.0       |
| 30  | 17.5       | 17.4       |
| 31  | 98.0       | 98.4       |
| 32  | 75.0       | 75.3       |
| 33  | 79.2       | 79.3       |
| 34  | 71.5       | 71.6       |
| 35  | 74.8       | 78.3       |
| 36  | 65.8       | 62.9       |
| 37  | 50.5       | 45.0       |
| 1'  | 167.0      | 170.9      |
| 2'  | 41.7       | 21.3       |
| 3'  | 167.3      | 21.1       |
| 4'  | 52.3       | 36.7       |

3. Results and discussion

3.1. Structure identification

Six new (1, 2, 6–8, and 10) and four known (3–5 and 9) dammarane-type saponins were isolated. The four known saponins were identified as ginsenosides F₁ (3) [24], Rc (4) [25], Re (5), and Rd (9) [19].

Compound 1 was obtained as a white amorphous powder. Its molecular formula was determined as C₄₀H₆₀O₁₂, evidenced by the High-Resolution-Electron Spray Ionization-Mass Spectra data (HR-ESI-MS) data (m/z 761.4446) [M + Na]⁺, calculated for C₄₀H₆₀O₁₂Na, 761.4446). The absorption bands at 3321, 1651, and 1015 cm⁻¹ due to hydroxyl, ester, and olefin groups, respectively, were shown in its IR spectrum. The acidic hydrolysis of 1 gained α-glucose. One anomeric proton (δ 5.10) in the ¹H-NMR spectrum and one corresponding anomeric carbon were recorded at δ 98.0 in the ¹³C-NMR spectrum (Table 1). Furthermore, nine methyl singlets (δH 1.00, 1.03, 1.12, 1.47, 1.67, 1.70, 2.00, and 3.67) were observed in the ¹H-NMR spectrum, and δH 3.67 of which was protons bearing an oxygen function. Thirty carbon signals were assigned to the 20(S)-PPT sapogenin [19], and a set of signals showed a β-D-glucosyl moiety in the ¹³C-NMR spectrum. Compared with 3 [24], the presence of additional signals corresponding to two carbonyl carbons (δc 167.0 and 167.3), one methylene group [δc 41.7/δh 3.72, 3.71 (each 1H, br s)], and one methoxyl group [δH 5.23/δh 3.67 (3H, s), along with the Heteronuclear Multiple Bond Correlation (HMBC) cross-peaks of δH 5.05, 4.68/δc 167.0, δH 3.71/δH 167.0, δH 3.71/δc 167.0 and 3.67/δc 167.3 (Fig. 1), as well as a downfield shift for C-6 (δc 65.8) of the gluco- pyranosyl unit, revealed that there was a terminal methyl-esterified malonic acid group attached to C-6 of glucopyranosyl moiety in 1 [13,26,27]. On the basis of the aforementioned results, the structure of 1 was determined as 3β,6α,12β,20(S)-tetrahydroxy-dammare-24-ene-20-O-(6-α-malonyl methyl ester)-β-D-glucopyranoside and given the trivial name 6α-malonyl methyl ester ginsenoside F₁.

Compound 2 was isolated as a white amorphous powder with a molecular formula C₃₈H₆₄O₁₀Na, calculated for C₃₈H₆₄O₁₀Na, 703.4391). Its IR spectrum showed strong absorption bands at 3324 and 1012 cm⁻¹, suggesting an oligoglucosidic structure, together with absorption band at 1649 cm⁻¹ due to carbonyl group. The acid hydrolysis of 2 liberated α-glucose. The ¹H- and ¹³C-NMR spectra (Table 2) were very similar to those of 3 [24], except for the signals due to an acetoyl group at δc 21.3 and 170.9 and δH 2.11 (3H, s), which suggested that 2 was an acetyl derivative of 3 (Fig. 1). The acetoyl group was confirmed at C-3 position of aglycone moiety on the basis of an acetylation shift effect on C-3 around δc 81.3 and HMBC cross-peaks of H-3 [δH 4.76 (1H, t, J = 9.0 Hz)] to carbonyl carbon at δc 170.9 and H-2 [δH 2.11 (3H, s)] to C-3 (δc 81.3). Thus, the structure of 2 was determined as 6α,12β,20(S)-trihydroxy-3β-acetoxy-dammare-24-ene-20-O-β-D-glucopyranoside and named as 3β-acetoxyl ginsenoside F₁.

Compound 6 was isolated as a white amorphous powder with a molecular formula C₁₉H₃₂O₁₁Na determined by HR-ESI-MS spectrum (m/z 739.5289) [M + Na]⁺, calculated for C₁₉H₃₂O₁₁Na, 739.5289). Its IR spectrum showed absorption bands due to hydroxyl and olefin groups at 3412 and 1630 cm⁻¹. The ¹H-NMR spectrum of 6 (Table 2) displayed eight methyl singlets [δH 0.99, 1.10, 1.18, 1.36, 1.61, 1.62 (6H, 2H)], a methyldoublet [δH 1.77 (3H, d, J = 6.2 Hz)], an olefinic [δH 5.26 (1H, t, J = 7.5 Hz)], and three anomeric [δH 6.26 (1H, br s), 5.32 (1H, d, J = 5.0 Hz), 5.20 (1H, d, J = 8.0 Hz)]. In the ¹³C-NMR spectrum of 6, thirty carbon signals assigned to the aglycone and three sets of signals due to one β-D-glucopyranosyl, one α-arabinopyranosyl, and one α-rhamnopyranosyl groups were observed. The result of acidic hydrolysis of 6 was consistent with the ¹³C-NMR findings of sugar moiety type. Comparison of the ¹³C-NMR chemical shifts of the aglycone moiety in 6 with those of 20(S)-propanaxatriol and its glycosides [19] suggested that 6 (Fig. 1) was a 20(S)-PPT ginsenoside, which was confirmed by HMBC analysis. Furthermore, the HMBC correlation between anomeric proton of rhamnolysyl δH 6.26 (1H, br s) and δC 77.8 of C-2' arabinosyl showed that there was a sugar sequence of α-1-rhamnopyranosyl-(1→2)-α-1-arabinopyranosyl in 6. The ¹H and ¹³C-NMR data of the sugar moiety were identical to the reported data [28,29]. The linkage sites of the sugars and the aglycone were determined by HMBC correlations of H-1 [δH 5.32 (1H, d, J = 5.0 Hz)] to C-6 (δC 75.8) and H-1’ [δH 5.20 (1H, d, J = 8.0 Hz)] to C-20 (δC 83.3). Accordingly, the structure of 6 was determined as 6-O-[α-1-rhamnopyranosyl-(1→2)-α-1-arabinopyranosyl]-3β,6α,
12\(\beta,20(S)\)-tetrahydroxy-dammar-24-ene-20-\(\beta\)-d-glucopyranoside and given the trivial name ginsenoside Rh24 \[30\]. So far as we know, the sugars and their the sequence located at C-6 resulted in a new type of sugar chain among all the known ginsenosides in Panax genus plants\[6,7\].

Compound 7 was isolated as a white amorphous powder with a molecular formula \(\text{C}_{52}\text{H}_{88}\text{O}_{21}\) determined by HR-ESI-MS \((m/z 1071.5707 [M + Na]^+\), calcd for \(\text{C}_{52}\text{H}_{88}\text{O}_{21}\text{Na}, 1071.5716\)). \([\alpha]_D^{25} D + 48.2^c (c = 0.1, \text{MeOH}). Its IR spectrum showed absorption bands due to hydroxyl and olefin groups at 3389 and 1646 cm\(^{-1}\). The \(^{13}\text{C}\)-NMR spectrum gave 52 carbon signals, of which 22 were assigned to the sugar moiety and 30 to the 20\(\beta\)-PPD sapogenin \[19\]. The acidic hydrolysis of 7 obtained D-glucose, D-xylose, and L-arabinose. The \(^1\text{H}\)- and \(^{13}\text{C}\)-NMR data (Table 2) of 7 were very similar to those of 4 \[25\], except for a xylosyl instead of a glucosyl at C-2'. The xylosyl group at C-2' was further confirmed by the correlation observed between anomer proton of xylosyl \(\delta_{H} 5.27 (1\text{H}, d, J = 7.0\text{ Hz})\) and \(\delta_{C} 84.1\) of C-2' glucosyl that linked at C-3 of the aglycone in the HMBC experiment (Fig. 1). Thus, the structure of 7 was determined as 3-\(O\)-\[(\beta\-xylopyranosyl-(1\—2)-\(\beta\-d\)-glucopyranosyl)- 3\(\beta,12\beta,20(S)\)-trihydroxy-dammar-24-ene-20-\(\alpha\)-\L-arabinofuranosyl-(1\—6)-\(\beta\)-d-glucopyranoside and given the trivial name ginsenoside Rh25.

Compound 8 was obtained as a white amorphous powder. It exhibited the molecular formula \(\text{C}_{48}\text{H}_{82}\text{O}_{19}\) according to the HR-ESI-MS data \((m/z 985.5349 [M + Na]^+\), calcd for \(\text{C}_{48}\text{H}_{82}\text{O}_{19}\text{Na}, 985.5348\). \([\alpha]_D^{25} D + 51.5^c (c = 0.1, \text{MeOH}). Its IR spectrum showed absorption bands due to hydroxyl and olefin groups at 3397 and 1604 cm\(^{-1}\). Glucose was identified in the acid hydrolysates. Comparison of the \(^1\text{H}\)- and \(^{13}\text{C}\)-NMR data of 8 (Table 3) with those of 9 \[19\] revealed that some different signals due to the B-ring part of the sapogenin moiety was due to the presence of a hydroxyl group signals at \(\delta_{H} 4.02/\delta_{C} 74.6\). The long-range correlations between H-5 \([\delta_{H} 0.84 (1\text{H}, \text{br d, } J = 12.5\text{ Hz}), \text{H-6 [1.75 (1\text{H}, m), 1.93 (1\text{H, m})], H-18 [\delta_{H} 1.26 (3\text{H, s)}], and } \delta_{C} 74.6\text{ in the HMBC experiment (Fig. 1), as well as signals for } \text{C-6 and C-7, were shifted to lower field at } \delta_{C} 29.2\text{ and } \delta_{C} 74.6\text{ compared to those of 9 \[19\], indicating that the original methylene}}\)
Six new dammarane-type triterpene saponins

Table 2

| No. | δH | δC |
|-----|----|----|
| 1   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 2   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 3   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 4   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 5   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 6   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |

Table 3

| No. | δH | δC |
|-----|----|----|
| 1   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 2   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 3   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 4   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 5   | 16.8 4.71 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |
| 6   | 16.6 4.66 (1H, t, 7.0) | 64.4 4.75 (1H, dd, 11.5, 7.0) |

Note: δH and δC values are in ppm for 1H- and 13C-NMR data, respectively.

at C-7 was transformed to a hydroxyl group. In the rotating frame overhauser effect spectroscopy (ROESY) experiment, rotating frame overhauser effect (ROE) correlations were observed between H-7 [δH 4.01 (1H, m)], H-9 [δH 1.89 (1H, m)], and H-7 and H-30 [δH 1.17 (3H, s)] (Fig. 2). Thus, the hydroxyl at C-7 was β substituted. Consequently, the structure of 8 was determined as 3-O-[β-D- glucopyranopyranoyl-(1→2)-β-D-glucopyranopyranoyl]-3β,7β,12β,20S-tetrahydroxydammar-24-ene-20-O-[β-D-glucopyranoside and given the trivial name 7β-hydroxy ginsenoside Rd.

Compound 10 was obtained as a white amorphous powder. It possessed the molecular formula C52H86O19, which was determined by the HR-ESI-MS data (m/z 1037.5646 [M + Na]+, calc for C52H86O19Na, 1037.5641). [α]25 D = -40.4° (c = 0.1, MeOH). The IR spectrum of 10 showed absorption bands at 1728 and 1655 cm⁻¹ ascribed to an α,β-unsaturated ester and olefin and strong absorption bands at 3337 and 1073 cm⁻¹ suggestive of an oligoglycosidic structure. Glucose was identified in the acid hydrolyzates. The 1H- and 13C-NMR data (Table 3) of 10 were very similar to those of 9, except for the additional signals, i.e., a methyl group δH 1.77 (3H, s), a double bond δC 144.8, 123.2 (δH 7.07, 7.99), and a carbonyl carbon δC 166.4. Further analysis confirmed that these signals were assigned to a crotonic group. Based on the typical AB-system coupling constant (J = 15.5 Hz) of δH 7.07 and δH 5.99, the configuration of the crotonic unit was determined as (E). By comparing the 13C-NMR
Table 4  Cytotoxicity of Compounds 1–10 against three human cancer cell lines (IC₅₀, µM)

| Compounds | HL-60 | MGC80-3 | Hep-G2 |
|-----------|-------|---------|--------|
| 1         | 16.74 ± 2.65 | 29.51 ± 0.82 | 20.48 ± 1.83 |
| 2         | 58.31 ± 3.32 | 42.18 ± 2.49 | 25.62 ± 0.87 |
| 3         | 65.36 ± 1.35 | 87.58 ± 1.76 | 82.14 ± 3.88 |
| 4         | >200          | >200      | >200   |
| 5         | >200          | >200      | >200   |
| 6         | 174.12 ± 1.93 | 168.14 ± 3.36 | >200   |
| 7         | >200          | >200      | >200   |
| 8         | 122.63 ± 2.46 | 158.83 ± 2.05 | >200   |
| 9         | >200          | >200      | >200   |
| 10        | 105.51 ± 4.84 | 82.37 ± 4.58 | 156.74 ± 2.61 |
| Vinorelbine | 9.36 ± 2.77   | 25.24 ± 3.18 | 18.86 ± 1.19 |

Data were expressed as mean ± SD (n – 3). Vinorelbine was used as a positive drug. IC₅₀, half inhibitory concentration; SD, standard deviation.

data with those of 9 [19], the terminal C-6’ resonance was deshielded, whereas that of C-5’ was shielded. The malonylation site at 6’-OH was confirmed by the HMBC cross-peaks of H-6’ [δH 4.75 (1H, dd, J = 11.5, 7.0 Hz) and δC 166.4 (Fig. 1)]. The structure of 10 was thus defined as 3-O-[6-O-{((E)-but-2-enoyl]-β-D-glucopyranoyl-(1→2)-β-D-glucopyranoyl}-3β,12β, 20(S)-trihydroxy-dammar-24-ene-20-O-β-D-glucopyranoside and given the trivial name ginsenoside Rh26. Its analogs with C-6’ crotonyl group have been isolated from the roots of *P. ginseng* and *P. quinquefolium* [26,31]. To the best of our knowledge, 10 that possesses crotonyl group is the first ginsenoside isolated from *P. ginseng* flower buds.

3.2. MTT assay

Ginsenosides have attracted increasing interest because of its promotion of tumor apoptosis and prevention of tumor invasion and metastasis [32–34]. To find more potent antitumor agents from *P. ginseng* flower buds, in this study, we evaluate the antitumor activity of the isolated compounds against three human cancer cell lines including human leukemia cell line HL-60, human gastric cancer cell line MGC80-3, and human hepatoma cell line Hep-G2 in an MTT assay, using vinorelbine as a positive control.

As can be seen from the data expressed in the IC₅₀ values in Table 4, Compound 7 did not affect the cell proliferation of all the three cell lines even at the highest test concentration of 200 µM, whereas the other new compounds displayed antiproliferative activity at wide different concentrations. The direct substitution of malonyl or acetyl at C-6’ or C-3 position to Compounds 1, 10, or 2 could increase the antiproliferative activity, by comparing Compounds 1 and 2 with 3, 9 and 10. The IC₅₀ value of Compound 1 was about threefold to fourfold higher than that of Compound 3 on the three cell lines, whereas Compound 10 showed minor cytotoxicity. Additional 7β-hydroxyl at C-7 position did not significantly increase antiproliferative potency, as shown in Compounds 8 and 9. Comparing with Compounds 1–3 with one sugar moiety, the antiproliferative effects of Compounds 4–10 with 3 or 4 sugar moieties were increased with the decreasing number of sugar moieties in a ginsenoside molecule, which was consistent with the literature [1]. And, the changes in the sugar type of the sugar moieties had a slight effect on the antitumor activity, which can be seen between Compounds 4 and 7 and between 5 and 6.

**Conflicts of interest**

The authors declare no conflict of interest.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2018.12.008.

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