Research article

Complete $^1$H-NMR and $^{13}$C-NMR spectral assignment of five malonyl ginsenosides from the fresh flower buds of *Panax ginseng*

Yu-Shuai Wang $^1$, Yin-Ping Jin $^1$, Wei Gao $^1$, Sheng-Yuan Xiao $^{1,2}$, Yu-Wei Zhang $^1$, Pei-He Zheng $^1$, Jia Wang $^1$, Jun-Xia Liu $^1$, Cheng-He Sun $^1$, Ying-Ping Wang $^{1,*}$

$^1$Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences, Changchun, China
$^2$College of Life Science of Beijing Institute of Technology, Beijing, China

Abstract

Background: Ginsenosides are the major effective ingredients responsible for the pharmacological effects of ginseng. Malonyl ginsenosides are natural ginsenosides that contain a malonyl group attached to a glucose unit of the corresponding neutral ginsenosides.

Methods: Medium-pressure liquid chromatography and semipreparative high-performance liquid chromatography were used to isolate purified compounds and their structures determined by extensive one-dimensional- and two-dimensional nuclear magnetic resonance (NMR) experiments.

Results: A new saponin, namely malonyl-ginsenoside Re, was isolated from the fresh flower buds of *Panax ginseng*, along with malonyl-ginsenosides Rb$_1$, Rb$_2$, Rc, Rd. Some assignments for previously published $^1$H- and $^{13}$C-NMR spectra were found to be inaccurate.

Conclusion: This study reports the complete NMR assignment of malonyl-ginsenoside Re, Rb$_1$, Rb$_2$, Rc, and Rd for the first time.

Copyright © 2015, The Korean Society of Ginseng. Published by Elsevier. All rights reserved.

1. Introduction

*Panax ginseng* Meyer is one of the most widespread traditional drugs used in China for thousands of years to produce various pharmacological and biological effects. The most important components contributing to its multiple medicinal properties are the ginsenosides, a group of triterpenoid saponins. Up to now, >150 ginsenosides have been isolated from *Panax* species [1]. Among these known compounds, malonyl ginsenosides (M-Rs; e.g., m-Rb$_1$, m-Rb$_2$, m-Rc, and m-Rd) are natural ginsenosides that exist in both fresh and air-dried ginseng and contain a malonyl residue attached at the 6-position of a glucosyl unit of the corresponding neutral ginsenoside [2,3]. Malonyl ginsenosides are considered an important form of ginsenoside in white ginseng, however, they are unstable and readily demalonylated or decarboxylated to their respective counterparts or acetylates by treatment with hot water or hot methanol [3–6]. Because malonyl ginsenosides are thermally unstable, their monomeric compounds are hard to obtain, although up to 20 malonyl ginsenosides have been detected by liquid chromatography/quadropole time-of-flight mass spectrometry [7]. Only six malonyl ginsenosides have been isolated and characterized [8–11]. Kitagawa et al [8] and Yamaguchi et al [9] reported the presence of malonyl ginsenosides Rb$_6$, Rb$_2$, Rc, and Rd in both *P. ginseng* and *P. quinquefolius* [8,9]. Sun et al [10] and Ruan et al [11] isolated malonyl notoginsenoside R4 and malonyl-ginsenoside R3 from the fresh roots of *P. ginseng*, respectively [10,11]. All previously isolated malonyl ginsenosides were derived from protopanaxadiol (PPT)-type ginsenosides [12].

In this study, we isolated five malonyl ginsenosides from the flower buds of *P. ginseng* and malonyl-ginsenoside Re (M-Re) was obtained as a PPT-type malonyl ginsenoside for the first time. Identification and characterization of ginsenosides are usually conducted using nuclear magnetic resonance (NMR) analyses, but several imperfections and/or inaccuracies existed in the published NMR data of malonyl ginsenosides given the lack of two-dimensional (2D) NMR techniques at the time of characterization. Here, with the help of modern 2D NMR techniques including...
correlation spectroscopy, rotating frame nuclear Overhauser effect spectroscopy (ROESY), heteronuclear single-quantum coherence (HSQC), and heteronuclear multiple-band coherence (HMBC) experiments, complete NMR assignments of malonyl-ginsenosides Rb₁, Rb₂, Rc, Rd, and Re were determined for the first time.

2. Materials and methods

2.1. General experimental procedure

Medium-pressure liquid chromatography (MPLC) purifications were carried out on a Yamaazen YFLC-AL-580 instrument (Yamaazen Co., Osaka, Japan) equipped with silica gel columns (Hi-Flash technology) and products were eluted with a step-wise gradient at a flow rate of 1.0 mL/min using solvent A (water containing 0.005% ammonium hydroxide and 0.02% ammonium acetate) and solvent B (acetonitrile). The elution rate using solvent B was 17.5% for 0–4 min, 17.5–28.9% for 4–9 min, 28.9–40% for 9–19 min, and 40% for 19–24 min.

The ¹H-, ¹³C-, and ²D-NMR spectra were measured using a Bruker AV600 NMR spectrometer (Bruker Co., Karlsruhe, Germany; 600 MHz for ¹H and 150 MHz for ¹³C) with tetramethylsilane as an internal standard. Chemical shifts (δ) are expressed in ppm, with the coupling constants (J) reported in Hertz (Hz). The electrospray ionization mass spectrometry (ESI-MS) and high-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were recorded using an Agilent 1200 HPLC with a 6300 Ion-trap liquid chromatography/mass spectrophotometry (LC/MS) system (Agilent Co., Santa Clara, CA, USA). Thin-layer chromatography (TLC) was performed using a silica gel 60 RP-18 F254S and Kieselgel 60 plates (Merck, Darmstadt, Germany).

2.2. Plant material

The fresh flower buds of P. ginseng were collected from Fu-Song, Jilin, China, in May 2014, and authenticated by one of the authors, Professor Shi-quan Xu. A voucher of the specimen collected (ZYC-RS-20131008) was deposited in the conditions of −20°C at the Institute of Special Wild Economic Animals and Plants, Chinese Academy of Agricultural Sciences.

2.3. Extraction and isolation

The fresh flower buds of P. ginseng (2.0 kg) were extracted five times with 80% methanol, a 6× dilution of the extracting solution was subjected to a nanofiltration membrane (ESNA1-K1-8040, Hydranautics Corporation, USA) to eliminate most of the pigment, and the filtrate (96.8 g) subjected to column chromatography on a porous polymer polystyrene resin (AB-8). After washing the column with eight column volumes of distilled water, elution was carried out with 30% and 60% aqueous ethanol, and finally with 100% ethanol. The fraction eluted with 30% ethanol (8.9 g) was loaded onto a MPLC system eluted with CH₂Cl₂-MeOH-H₂O (5:1; 0.1–4:1; 0.1–3:1; 0.1–1:1) to yield six fractions (AG1–6). Fraction AG4 (2.2 g) was further separated using semipreparative reversed-phase HPLC and eluted with CH₃CN-H₂O (1:4) at 3 mL/min to yield malonyl-ginsenoside Re (30 mg; tR 25.2 min). The fraction eluted with 60% ethanol (48.0 g) was processed on a MPLC system using a linear gradient elution (7 mL/min) of 25–45% methanol in CH₂Cl₂ for 250 min in order to collect fraction BG1–9. M-Rb₁, M-Rb₂, M-Rc, and M-Rd were primarily distributed within fraction BG8 through analysis by LC/MS. Fraction BG8 (7.8 g) was then applied to semipreparative reversed-phase HPLC using a linear gradient elution (3 mL/min) of 29–34% acetonitrile in water for 50 min to yield M-Rb₁ (21 mg; tR 19.7 min), M-Rb₂ (18 mg; tR 24.0 min), M-Rc (22 mg; tR 29.8 min), and M-Rd (27 mg; tR 43.9 min; Fig. 1).

2.4. Characterization of compounds 1–5

Compound 1 was obtained as a white amorphous powder and gave peaks at m/z 1031.4 [M+H]⁺, 987.6 [M–H–CO₂]⁻, 945.4 [M–CO₂H₂COO]⁻, 927.8 [M–CO₂H₂COOH–H₂O]⁻, 783.7 [M–CO₂H₂COOH–glu]⁻, 637.5 [M–CO₂H₂COOH–rha–glu]⁻, and 475.3 [M–CO₂H₂COOH–rha–glu]⁻ in negative-mode ESI-MS, indicating its molecular weight to be 1032. HRESIMS: m/z 1055.5391 [M+Na]⁺ (calculated for C₅₁H₈₄NaO₂₁, 1055.5397). IR max was 3408, 2932, 1731, 1636, 1599, 1454, 1385, 1075, and 1050 cm⁻¹. Libermann–Buchard and Molish reactions were positive. Eight methyl groups and six quaternary carbons were identified in the analysis of the NMR spectrum (Tables 1 and 2). Molish reaction was used to test the existence of saccharides, test of Libermann-Buchard for steroids or tripterpenes.

Compound 2 was obtained as a white amorphous powder. The molecular formula was determined as C₅₆H₉₂NaO₂₅ based on HRESIMS data at m/z 1217.5921 [M+Na]⁺ (calculated for C₅₆H₉₂NaO₂₅, 1217.5915). The ¹H- and ¹³C-NMR spectra of compounds 1–5 were recorded using Agilent 500 MHz and 125 MHz spectrometers, respectively, with tetramethylsilane as an internal standard. The chemical shifts (δ) are expressed in ppm, with the coupling constants (J) reported in Hertz (Hz). The electrospray ionization mass spectrometry (ESI-MS) and high-resolution electrospray ionization mass spectrometry (HRESIMS) spectra were recorded using an Agilent 1200 HPLC with a 6300 Ion-trap liquid chromatography/mass spectrophotometry (LC/MS) system (Agilent Co., Santa Clara, CA, USA). Thin-layer chromatography (TLC) was performed using a silica gel 60 RP-18 F254S and Kieselgel 60 plates (Merck, Darmstadt, Germany).

2.4. Characterization of compounds 1–5

Compound 1 was obtained as a white amorphous powder and gave peaks at m/z 1031.4 [M+H]⁺, 987.6 [M–H–CO₂]⁻, 945.4 [M–CO₂H₂COO]⁻, 927.8 [M–CO₂H₂COOH–H₂O]⁻, 783.7 [M–CO₂H₂COOH–glu]⁻, 637.5 [M–CO₂H₂COOH–rha–glu]⁻, and 475.3 [M–CO₂H₂COOH–rha–2glu]⁻ in negative-mode ESI-MS, indicating its molecular weight to be 1032. HRESIMS: m/z 1055.5391 [M+Na]⁺ (calculated for C₅₁H₈₄NaO₂₁, 1055.5397). IR max was 3408, 2932, 1731, 1636, 1599, 1454, 1385, 1075, and 1050 cm⁻¹. Libermann–Buchard and Molish reactions were positive. Eight methyl groups and six quaternary carbons were identified in the analysis of the NMR spectrum (Tables 1 and 2). Molish reaction was used to test the existence of saccharides, test of Libermann-Buchard for steroids or tripterpenes.

Compound 2 was obtained as a white amorphous powder. The molecular formula was determined as C₅₆H₉₂NaO₂₅ based on HRESIMS data at m/z 1217.5921 [M+Na]⁺ (calculated for C₅₆H₉₂NaO₂₅,
Table 1
The 13C-NMR data of compounds 1–5 (150 MHz, pyridine-d5, δC)

| Compound | 1 (δC) | 2 (δC) | 3 (δC) | 4 (δC) | 5 (δC) |
|----------|--------|--------|--------|--------|--------|
| C2       | 131.96 | 131.62 | 131.54 | 131.54 | 131.23 |
| C3       | 131.62 | 131.54 | 131.54 | 131.54 | 131.23 |
| C4       | 131.54 | 131.54 | 131.54 | 131.54 | 131.23 |
| C5       | 131.23 | 131.23 | 131.23 | 131.23 | 131.23 |

C, carbon; NMR, nuclear magnetic resonance.

1217.5925). Negative-mode ESI-MS (m/z) readings: 1193.4 [M-H]-, 1149.5 [M-H-CO2]-, 1107.4 [M-COCH2COOH], 1089.5 [M-COCH2COOH-H2O], 945.5 [M-COCH2COOH-glu], 783.2 [M-COCH2COOH-2glu], 621.1 [M-COCH2COOH-3glu], and 493.3 [M-COCH2COOH-4glu]-. IR (max.): 3383, 2937, 1724, 1638, 1454, 1383, and 1076. Libermann-Burchard and Molish reactions were positive. The 13C-NMR spectrum and 1H-NMR data are shown in Tables 1 and 2. The IR spectrum showed absorption bands for hydroxyl (3383 cm⁻¹), carbonyl (1724 cm⁻¹), double bond (1638 cm⁻¹), methyl (1383 cm⁻¹) and ether moiety (1076 cm⁻¹).

Compound 3 was obtained as a white amorphous powder. The molecular formula was determined as C56H92NaO25, based on HRE-MS data at m/z 1187.5826 [M+Na]+ (calculated for C56H92NaO25, 1187.5820). Negative-mode ESI-MS (m/z) readings: 1163.4 [M-H]-, 1119.2 [M-H-CO2]-, 1077.1 [M-COCH2COOH], 1059.1 [M-COCH2COOH-H2O]-, 945.2 [M-COCH2COOH-Ara(p)]-.

Please cite this article in press as: Wang Y-S, et al., Complete 1H-NMR and 13C-NMR spectral assignment of five malonyl ginsenosides from the fresh flower buds of Panax ginseng, Journal of Ginseng Research (2015), http://dx.doi.org/10.1016/j.jgr.2015.08.003.
The IR spectrum showed absorption bands for hydroxyl (3392 cm⁻¹), carbonyl (1728 cm⁻¹), and ether moiety (1076 cm⁻¹). Molish reaction was used to prove the existence of saccharides, and test of Libermann-Burchard for steroids or triterpenes.

Compound 4 was obtained as a white amorphous powder. The molecular formula was determined as C₅₂H₉₂O₂₄Na₂ by HREIMS at m/z 1187.5820 [M+Na]⁺ (calculated for C₅₂H₉₂O₂₄Na₂ 1187.5820). Negative-mode ESI-MS (m/z) readings: 1163.4 [M-H]⁻, 1191.7 [M-H-CO₂H]⁻, 1077.4 [M-COCH₂COOH]⁻, 1059.3 [M-COCH₂COOH-H₂O]⁻, 945.2 [M-COCH₂COOH-Ara[α]]⁻, 783.3 [M-COCH₂COOH-Ara[α]-f]-, 621.2 [M-COCH₂COOH-Ara[α]-2glu]⁻, and 459.1 [M-COCH₂COOH-Ara[α]-3glu]⁻. IR νmax: 3387, 2942, 1636, 1452, 1388, and 1076. Libermann-Burchard and Molish reactions were positive. The IR spectrum showed absorption bands for hydroxyl (3387 cm⁻¹), carbonyl (1728 cm⁻¹), and ether moiety (1076 cm⁻¹). Molish reaction was used to prove the existence of saccharides, and test of Libermann-Burchard for steroids or triterpenes.

Table 2
The ¹H-NMR data of compounds 1–5 (600 MHz, pyridine-d₅, δ, J in Hz)

| H      | Compound 1 | Compound 2 | Compound 3 | Compound 4 | Compound 5 |
|--------|------------|------------|------------|------------|------------|
| 1      | 0.93, 1.63 | 0.73, 1.51 | 0.71, 1.52 | 0.70, 1.50 | 0.71, 1.49 |
| 2      | 1.74, 1.83 | 1.80, 2.16 | 1.81, 2.16 | 1.81, 2.16 | 1.79, 2.13 |
| 3      | 3.43 (dd, 4.6, 11.5) | 3.24 (dd, 4.3, 11.8) | 3.23 (dd, 4.3, 11.8) | 3.22 (dd, 4.3, 11.7) | 3.23 (dd, 4.3, 11.5) |
| 4      | 1.30 (d, 10.7) | 0.67 | 0.66 | 0.65 | 0.65 |
| 5      | 4.65 | 1.47, 1.36 | 1.34, 1.46 | 1.36, 1.34 | 1.47, 1.36 |
| 6      | 4.51 | 1.13, 1.45 | 1.20, 1.45 | 1.14, 1.43 | 1.14, 1.43 |
| 7      | 1.96, 2.22 | 1.33 | 1.32 | 1.33 | 1.34 |
| 8      | 1.48 | 1.13, 1.99 | 1.52, 1.95 | 1.48, 1.95 | 1.52, 1.94 |
| 9      | 1.47, 2.02 | 4.30 | 4.11 | 4.16 | 4.10 |
| 10     | 4.11 | 4.10 | 1.97 | 1.95 | 1.94 |
| 11     | 1.92 | 1.97 | 1.94 | 1.97 | 1.95 |
| 12     | 0.84, 1.44 | 0.96, 1.53 | 0.96, 1.52 | 0.96, 1.34 | 0.97, 1.53 |
| 13     | 1.21, 1.74 | 1.36, 1.81 | 1.34, 1.80 | 1.34, 1.80 | 1.34, 1.79 |
| 14     | 2.46 | 2.56 | 2.54 | 2.52 | 2.52 |
| 15     | 1.15 (s) | 0.94 (s) | 0.93 (s) | 0.92 (s) | 0.93 (s) |
| 16     | 0.93 (s) | 0.81 (s) | 0.79 (s) | 0.79 (s) | 0.79 (s) |
| 17     | 1.54 (s) | 1.58 (s) | 1.59 (s) | 1.61 (s) | 1.59 (s) |
| 18     | 1.73, 2.32 | 1.81, 2.37 | 1.80, 2.35 | 1.80, 2.34 | 1.80, 2.34 |
| 19     | 2.30, 2.49 | 2.37, 2.55 | 2.35, 2.54 | 2.34, 2.53 | 2.22, 2.46 |
| 20     | 5.28 (t-like) | 5.29 (t-like) | 5.28 (t-like) | 5.28 (t-like) | 5.21 (t, 6.9) |
| 21     | 1.62 (s) | 1.63 (s) | 1.63 (s) | 1.63 (s) | 1.57 (s) |
| 22     | 1.63 (s) | 1.63 (s) | 1.63 (s) | 1.64 (s) | 1.59 (s) |
| 23     | 1.62 (s) | 1.61 (s) | 1.61 (s) | 1.61 (s) | 1.57 (s) |
| 24     | 1.62 (s) | 1.61 (s) | 1.61 (s) | 1.61 (s) | 1.57 (s) |
| 25     | 0.93 (s) | 0.92 (s) | 0.91 (s) | 0.92 (s) | 0.91 (s) |

**brs, broad singlet; dd, double doublet; H, hydrogen; m, multiplet; NMR, nuclear magnetic resonance; s, singlet; t, triplet; t-like, triplet-like.**
COCH₂COOH-2glu]⁻, and 459.0 [M-COCH₂COOH-3glu]⁻. IR νmax: 3392, 2944, 1731, 1634, 1453, 1388, and 1076. Libermann-Burchard and Molish reactions were positive. Molish reaction was used to prove the existence of saccharides, and test of Libermann-Burchard for steroids or triterpenes.

2.5. Acid hydrolysis of compound 1

Compound 1 (5.0 mg) was hydrolyzed with 3.0N HCl (5 mL) at 100°C for 2 h. The reaction mixture was extracted with chloroform to afford the aglycone, and the aqueous layer was repeatedly evaporated to dryness with methanol until neutral. The sample was then analyzed by TLC over a silica gel with n-BuOH-AcOH-H₂O (9:4:2) as the developing solvent. The sample spots were detected by spraying diphenylamine-aniline-phosphoric acid reagent (2% aniline in acetone; 2% diphenyl in acetone: 85% phosphoric acid = 5:5:1) and heating at 100°C [13]. The chromatogenic agent was used to react with monosaccharides and appear coloration through heating. β-D-Glucose and α-L-rhamnose were used as authentic samples. Furthermore, the aqueous layer residues mentioned above were dissolved in anhydrous pyridine (2 mL) and stirred with L-cysteine methyl (1.5 mg) for 1 h at 60°C, then 1.2 mL of hexamethyldisilazane:trimethylchlorosiane (3:1) was added and the mixture was stirred at 60°C for another 30 min. The precipitate was centrifuged and the supernatant dried under N₂ steam at room temperature [14]. The residue was partitioned between hexane and water, and the hexane layer was analyzed by GC. Identification of β-D-glucose and α-L-rhamnose was carried out for compound 1, giving peaks at 9.90 min and 17.08 min, respectively.

3. Results

Compound 1 was obtained as a white amorphous powder. The molecular formula was determined as C₅₁H₈₄O₂₁ based on HRE-SIMS data at m/z 1055.5391 [M⁺+Na]⁺ (calculated for C₅₁H₈₄NaO₂₁, 1055.5397). The IR spectrum showed absorption bands for hydroxyl (3408 cm⁻¹), carbonyl (1731 cm⁻¹) and methyl (1385 cm⁻¹) groups, as well as double bond (1636 cm⁻¹), and ether moieties (1075 cm⁻¹). The ¹H-NMR spectrum (Table 2) showed eight methyl signals at C-M1 (δc 169.308) and C-M3 (δc 171.075). These data suggest that compound 1 was a dammarane-type triterpene glycoside with a double bond and a malonyl group [15–18]. The chemical structure of compound 1 was further elucidated by a HMBC (Figure 2) experiment in which correlations were observed between H-1’ (δH 5.22, d, J = 6.8 Hz) and the carbon resonance signal at C-6 (δc 75.122), H-1” (δH 6.46, brs) and C-2’ (δc 79.083), and H-1”’ (δH 5.04, d, J = 7.7 Hz) and C-20 (δc 83.925), which indicated that the C-1-Glc, C-1’-Glc, and C-1Rha were linked to C-6, C-2’, and C-20, respectively. The malonyl group was assigned to the C20-glc-C-6” position based on the correlations of C20-glc-H-6” with C-M1 and C-4”, as shown in Figure 3.

The ¹H- and ¹³C-NMR spectroscopic data for compound 1 were similar to those of ginsenoside Re, except for the data attributed to a malonyl group (δH 3.75, δc 169.308, δc 44.401, δc 171.075). Other carbon shifts included an upfield shift of C-5” (δc 75.389) and a downfield shift of C-6” (δc 65.755), as compared to ginsenoside Re [15,16]. H-5” yielded a peak at 3.96, and H-6” at 4.98 and 4.71, based on the HSQC spectrum. Acid hydrolysis of compound 1 yielded ginsenoside Re. The absolute configurations of the sugar moieties were further determined to be β-D-glucose and α-L-rhamnose by chiral GC analysis. The relative configuration of 1 was established through analysis of the ROESY experiment. As shown in Figure 4, correlations of H-3 to H-28 and H-5 indicated β-orientation for the 3-OH group. H-17 showed ROESY correlations with H-30 and H-16a, therefore, the side chain at C-17 was β-oriented. ROESY correlation between H-17 and H-21 confirmed assignment of the C-20(S) configuration. Moreover, the chemical shifts of C-17, C-21, and C-22 were 51.873, 22.483, and 36.513, respectively, which corresponded to the NMR data of 20(S)-ginsenoside Re [15–17].

The structures of compounds 2–5 were identified based on their spectroscopy data and by comparison of their data with literature sources [8,18]. The NMR spectroscopic data of the malonyl group in the present study showed significant differences with values reported in the literature. The chemical shifts of the methylene between the two carboxyls of the malonyl group were ~44.176–44.801, which represented different values than those cited [8–11]. In the ¹H-NMR spectrum, the chemical shifts of H-5” and H-6” were ~3.98–4.03 and ~4.94–4.97, respectively, which are reported here for the first time.

By normal-phase silica gel TLC (n-BuOH-CH₃COOH-H₂O = 4:1:5), Rf values were 0.32 for M-Re (1), 0.17 for M-Rb1 (2), 0.18 for M-Rb2 (3), 0.20 for M-Rc (4), and 0.27 for M-Rd (5). Reverse-phase ODS TLC (MeOH-H₂O = 2:1) yielded Rf values of 0.72, 0.36, 0.30, 0.35, and 0.27, respectively. Each compound was light purple on TLC when sprayed with 10% H₂SO₄ in ethanol followed by heating at 105°C. HPLC retention times were 12.8 min for M-Re (1),...
Fig. 3. Key HMBC and 1H-1H COSY correlations for compound 1. COSY, correlated spectroscopy; HMBC, heteronuclear multiple bond correlation.

18.0 min for M-Rb₁ (2), 19.3 min for M-Rb₂ (3), 18.7 min for M-Rc (4), and 20.9 min for M-Rd (5).

4. Discussion

A phytochemical investigation of the fresh flowers of P. ginseng led to the isolation of a new saponin (20S)-Protopanaxatriol-6-[O-\(\alpha\)-L-rhamnopyranosyl-(1→2)-\(\beta\)-D-glucopyranosyl]-20-O-([6-O-malonyl]-\(\beta\)-D-glucopyranoside), along with malonyl-ginsenosides Rb₁, Rb₂, Rc, and Rd. The complete 1H-NMR data of the malonyl ginsenosides were assigned for the first time.

Malonyl ginsenosides are unstable, not readily available, making them more difficult to analyze by HPLC than their neutral counterparts, and not used as indices for evaluation and quality control of ginseng. However, they are reported to be present in significant quantities in ginseng species, so the conventional evaluation index may not comprehensively reflect all ginseng properties or processed products. This study reports a simple and efficient way to prepare malonyl ginsenosides, as well as their physicochemical and NMR data, which provided scientific basis for the preparation of the standard substance.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

We are grateful to Gao-Fei Hu (College of Science, Beijing University of Chemical Technology) for measuring the NMR spectra.

This work was financially supported by the National Key Technology Research and Development Program of the Ministry of Science and Technology of China (no. 2011BAI03B01).

References

[1] Christensen LP. Ginsenosides: chemistry, biosynthesis, analysis, and potential health effect. Adv Food Nutr Res 2009;55:1–99.
[2] Sun GZ, Liu Z, Li XG, Zheng YN, Wang JY. Isolation and identification of two malonyl ginsenosides from the fresh root of Panax ginseng. Chin J Anal Chem 2005;33:1783–6.
[3] Wu W, Sun L, Zhang Z, Guo YY, Liu SY. Profiling and multivariate statistical analysis of Panax ginseng based on ultra-high-performance liquid chromatography coupled with quadrupole-time-of-flight mass spectrometry. J Pharmaceut Biomed 2015;107:141–50.
[4] Ren G, Chen F. Degradation of ginsenosides in American ginseng (Panax quinquefolium) extracts during microwave and conventional heating. J Agric Food Chem 1999;47:1501–5.
[5] Chu C, Xu SJ, Li XC, Yan JZ, Liu L. Profiling the ginsenosides of three ginseng products by LC-Q-TOF/MS. J Food Sci 2013;78:C653–9.
[6] Kitagawa I, Taniyama T, Yoshikawa M, Ikemichi Y, Nakagawa Y. Chemical studies on crude drug processing. VI Chemical Structures of Malonyl ginsenosides Rb₁, Rb₂, Rc, and Rd Isolated from the root of Panax ginseng C.A. Meyer. Chem Pharm Bull 1989;37:2961–70.
[7] Sun BS, Xu MY, Li Z, Wang YB, Sung CK. UPLC-Q-TOF-MS/MS analysis for steaming times-dependent profiling of steamed Panax quinquefolius and its ginsenosides transformations induced by repetitious steaming. J Ginseng Res 2012;36:277–90.
[8] Kitagawa I, Taniyama T, Hayashi T, Yoshikawa M. Malonyl ginsenosides Rb₁, Rb₂, Rc, and Rd, four new malonylated dammarane-type triterpenoligosaccharides from ginseng radix. Chem Pharm Bull 1983;31:3153–6.
[9] Yamaguchi H, Kasai R, Matsuura H, Tanaka O, Fuwa T. High-performance liquid chromatographic analysis of acidic saponins of ginseng and related plants. Chem Pharm Bull 1988;36:3468–73.
[10] Sun GZ, Li XC, Liu Z, Wang JY, Yang XW. Isolation and structure characterization of malonyl-notoginsenoside Rα from the root of Panax ginseng. Chin J Anal Chem 2007;28:1316–8.
[11] Ruan CC, Liu Z, Li X, Liu X, Wang LJ, Pan HY, Zheng YN, Sun GZ, Zhang YS, Zhang LX. Isolation and characterization of a new malonyl ginsenoside from the fresh root of Panax Ginseng. Molecules 2010;15:2319–25.
[12] Wan JY, Fan Y, Yu QT, Ge YZ, Yan CP, Aloga RN, Li P, Ma ZH, Qi LW. Integrated evaluation of malonyl ginsenosides, amino acids and polysaccharides in fresh and processed ginseng. J Pharmaceut Biomed 2015;107:89–97.
[13] Yan J, Guo XQ, Li XG, Wu XY, Gou XJ. TLC to quickly analyze monosaccharide composition of polysaccharide. Food Sci 2006;27:603–7.
[14] Hara S, Okabe H, Mihashi K. Gas-liquid chromatographic separation of aldose enantiomers as trimethylsilyl ethers of methyl 2-(phlydroxyalkyl)thiazolinedine-4- (R)-carboxylates. Chem Pharm Bull 1987;35:501–6.
[15] Peng RW, Li HZ, Chen JT, Wang DZ, He YN, Yang CR. Complete assignment of 1H and 13C NMR data for nine propanaxatriol glycosides. Mag Reson Chem 2002;40:483–8.
[16] Cho JG, In SJ, Jung YJ, Cha BJ, Lee DY, Kim YB, Yeom MH, Baek NI. Re-evaluation of the ginsenosides of three ginseng species. J Ginseng Res 2010;34:113–21.