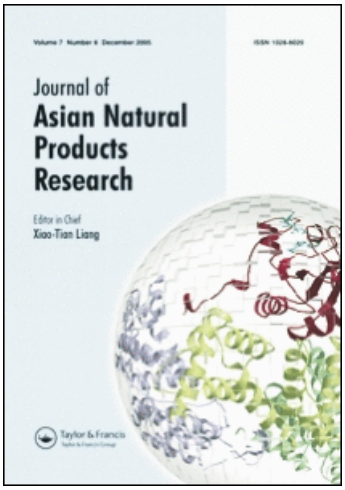

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ORIGINAL ARTICLE

Three new dammarane-type triterpene saponins from the leaves of *Panax ginseng* C.A. Meyer

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Three new dammarane-type triterpene ginsenosides, together with six known ginsenosides, were isolated from the leaves of *Panax ginseng* C.A. Meyer. The new saponins were named as ginsenoside Rh_{11}, ginsenoside Rh_{12}, and ginsenoside Rh_{13}. Their structures were elucidated as (20*S*)-3β,6α,12β,20-tetrahydroxydammara-25-ene-24-one 20-*O*-β-D-glucopyranoside (**1**), (20*S*)-3β,12β,20,24,25-pentahydroxydammarane 20-*O*-β-D-glucopyranoside (**2**), and (20*S*,23*E*)-3β,12β,20,25-tetrahydroxydammara-23-ene 20-*O*-β-D-glucopyranoside (**3**) on the basis of 1D and 2D NMR experiments and mass spectra. The known ginsenosides were identified as ginsenoside M_{7cd}, ginsenoside Rg_6, ginsenoside Rb_3, gypenoside XVII, gypenoside IX, and 20-(*E*)-ginsenoside F_4.

Keywords: *Panax ginseng* C.A. Meyer; leaves; dammarane-type triterpenoid; saponins

1. Introduction

As a typical herbal medicine, *Panax ginseng* C.A. Meyer (*P. ginseng*) has been widely used as a dietary supplement to improve health and vitality in many countries. A number of studies investigated ginseng's immunomodulatory effects, cancer chemopreventive action, and anticancer activity [1–3]. The roots of this plant possessed the main position in the traditional Chinese medicine and the principal constituents in the roots have been characterized as dammarane-type triterpenoid saponins [4–6]. Researchers reported that some dammarane-type triterpenoid saponins were isolated and identified from the leaves of *P. ginseng* [7–11]. However, the other saponins were yet left uncharacterized from the leaves. As a further systematic study on chemical constituents from the leaves, three new saponins, along with 21 known ones, were isolated from the leaves. Among the known compounds, ginsenoside M_{7cd}, ginsenoside Rg_6, ginsenoside Rb_3, 20-(*E*)-ginsenoside F_4, gypenoside-XVII, and gypenoside-IX were first isolated from the leaves of *P. ginseng*. This paper mainly describes the isolation and structural elucidation of three new ginsenosides (Figure 1).

2. Results and discussion

Two kilograms of the air-dried leaves of *P. ginseng* were extracted three times with water under heating. The extracts were

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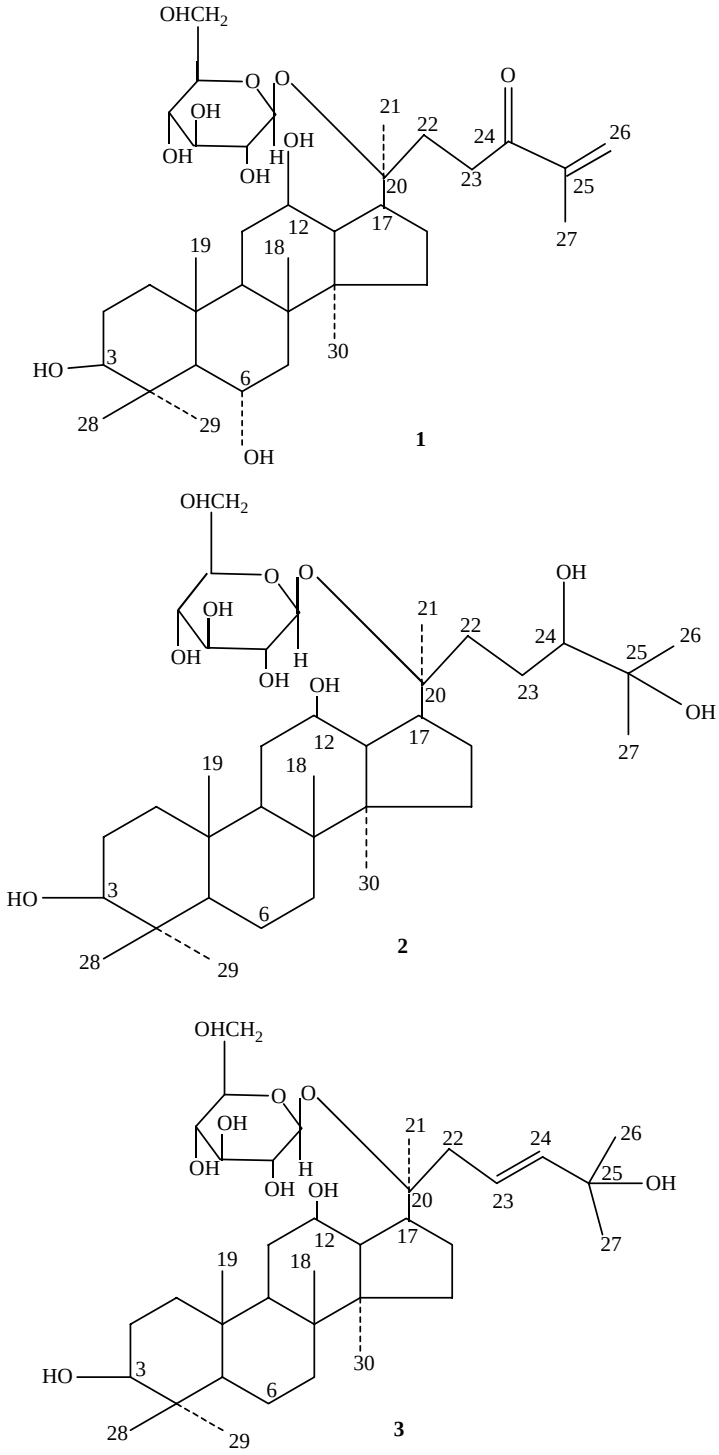


Figure 1. Structures of compounds **1**–**3**.

combined and then separated by a macroporous absorption resin (AB-8) column to give 85% ethanol eluates which upon drying afforded the total saponins (230 g). This fraction was subjected to normal-phase and reversed-phase silica gel column chromatography and final HPLC to afford ginsenoside Rh_{11} (**1**, 3.5 mg), ginsenoside Rh_{12} (**2**, 5.0 mg), ginsenoside Rh_{13} (**3**, 3.2 mg), ginsenoside M_{7cd} [12] (**4**, 68.0 mg), ginsenoside Rg_6 [13] (**5**, 71.0 mg), 20-(*E*)-ginsenoside F_4 [14] (**6**, 16.0 mg), gypenoside-XVII [15] (**7**, 13.0 mg), ginsenoside Rb_3 [16] (**8**, 5.6 mg), gypenoside-IX [17] (**9**, 7.0 mg), majoroside F_4 [18] (**10**, 145.0 mg), ginsenoside F_1 [19] (**11**, 100.0 mg), ginsenoside F_2 [20] (**12**, 56.3 mg), ginsenoside F_3 [20] (**13**, 125.0 mg), ginsenoside F_5 [21] (**14**, 100.5 mg), ginsenoside Rd_2 [22] (**15**, 16.0 mg), notoginsenoside Fe [22] (**16**, 48.0 mg), 20-(*S*)-ginsenoside Rh_1 [23] (**17**, 20.0 mg), ginsenoside Re [16] (**18**, 235.0 mg), 20-(*R*)-ginsenoside Rg_2 [24] (**19**, 15.0 mg), 20-(*S*)-ginsenoside Rg_2 [24] (**20**, 18.5 mg), ginsenoside Rb_1 [16] (**21**, 49.3 mg), ginsenoside Rb_2 [16] (**22**, 206.2 mg), ginsenoside Rc [16] (**23**, 52.0 mg), and ginsenoside Rd [16] (**24**, 607.0 mg).

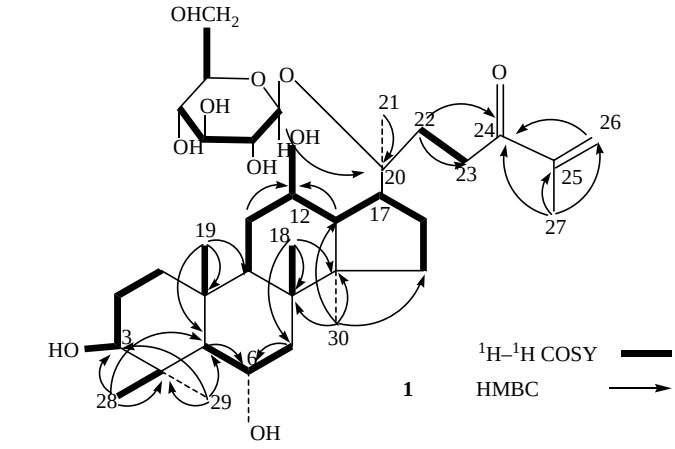
Ginsenoside Rh_{11} (**1**) was isolated as a white powder with positive optical rotation ($[\alpha]_D^{22.8}$ + 55.8 in MeOH). The molecular formula, $C_{36}H_{60}O_{10}$, was established on the basis of the quasi-molecular ion peak in the high-resolution (HR)-ESI-MS at *m/z* 675.40582 $[M + Na]^+$. The IR spectrum of **1** showed strong absorption bands at 3377 and 1073 cm^{-1}, suggestive of the glycosidic structure, and the bands at 1696 and 1647 cm^{-1} due to a double bond and carbonyl. Furthermore, the carbonyl absorption maximum was observed at 221.44 nm in its UV spectrum. Acid hydrolysis of **1** with methanol hydrochloric acid [MeOH:HCl (1:1)] liberated a D-glucose, which was identified by TLC comparison with an authentic sample, showing **1** was a glucoside. The ^1H, ^{13}C NMR (Table 1), and distortionless enhancement by polarization transfer (DEPT) spectra proposed **1** to be composed of a glucopyranosyl moiety and an aglycone part having four hydroxyl groups, one carbonyl carbon, and one C=C double bond. The configuration of

Table 1. ^{13}C NMR spectral data for ginsenosides Rh_{11} (**1**), Rh_{12} (**2**), and Rh_{13} (**3**).

C	1	2	3	C	1	2	3
C-1	39.5	39.5	39.5	C-19	17.6	16.1	16.2
C-2	28.2	28.4	28.4	C-20	83.2	84.0	83.4
C-3	79.5	79.1	79.1	C-21	22.0	22.8	23.1
C-4	40.5	39.7	39.7	C-22	32.9	33.7	39.6
C-5	61.9	56.5	56.5	C-23	30.0	26.6	123.0
C-6	67.8	18.9	18.9	C-24	202.5	79.6	142.2
C-7	47.6	35.3	35.3	C-25	144.6	73.1	70.1
C-8	41.3	40.2	40.2	C-26	125.1	27.1	30.8
C-9	50.0	50.4	50.3	C-27	17.7	25.4	31.0
C-10	39.5	37.5	37.5	C-28	32.2	28.8	28.8
C-11	31.1	31.0	30.8	C-29	16.7	16.7	16.5
C-12	70.3	70.5	70.6	C-30	18.0	17.4	17.3
C-13	49.3	49.4	49.7	C-1′	98.2	98.4	98.4
C-14	51.5	51.7	51.7	C-2′	75.2	75.4	75.4
C-15	30.9	31.3	31.2	C-3′	78.6	78.3	78.5
C-16	26.8	26.9	26.6	C-4′	71.8	72.0	71.8
C-17	52.2	52.8	52.5	C-5′	78.4	78.2	78.2
C-18	17.5	16.5	16.5	C-6′	63.1	63.3	63.1

the anomeric position was determined to be β on the basis of the coupling constant of the anomeric proton signal in the ^{1}H NMR spectrum of **1** [δ 5.03 (1H, d, $J = 7.8$ Hz, H-1′)]. The signals due to the β-D-glucopyranosyl moiety were observed in the ^{13}C NMR spectrum of **1** at δ 98.2, 75.2, 78.6, 71.8, 78.4, and 63.1. The signal of C-6 at δ 67.8 suggested that the aglycone of **1** was a protopanaxatriol-type one with variations in its side chain. The proton and carbon signals of **1** in the ^{1}H and ^{13}C NMR spectra resembled those of ginsenoside-M_{7cd} [12], except for the signals of the carbons in the side chain (C-22–C-27). The chemical shift of C-24 at δ 202.5 suggested that **1** was a derivative of ginsenoside-M_{7cd} with a carbonyl in C-24, which made two olefinic carbon signals change to δ 144.6 and 125.1. As shown in Figure 2, the ^{1}H–^{1}H correlation spectroscopy (COSY) experiment on **1**, indicated the presence of partial structures, shown by bold lines, and in the heteronuclear multiple-bond correlation (HMBC) experiment, long-range correlations were observed between the following protons and carbons: H-18 and C-7, 8, 14; H-19 and C-5, 9, 10; H-21 and C-17, 20, 22; H-22 and C-23, 24; H-26 and C-24, 25; H-27 and C-24, 25. The β-D-glucopyranosyl was suggested to link to C-20 of the aglycone by the elucidation of the HMBC spectrum which showed a long-range correlation between the protons at δ 5.03 and C-20 at δ 83.2. On the basis of the above evidence, compound **1** was established as (20*S*)-3β,6α,12β,20-tetrahydroxydammara-25-ene-24-one 20-*O*-β-D-glucopyranoside and named as ginsenoside Rh_{11}.

Ginsenoside Rh_{12} (**2**) was also obtained as a amorphous powder with positive optical rotation ($[\alpha]_D^{23.1}$ + 13.5 in MeOH). The molecular formula, $C_{36}H_{64}O_{10}$, was established on the basis of the quasi-molecular ion peak in the HR-ESI-MS at *m/z* 679.4391 $[M + Na]^+$. The IR spectrum of **2** showed absorption bands at 3369, 2947, 1386, and 1076 cm^{-1} assignable to hydroxyl, methyl, methylene, and ether functions. Acid hydrolysis of **2** with methanol hydrochloric acid [MeOH:HCl (1:1)] liberated a D-glucose, which was identified by TLC comparison with an authentic sample, showing that **2** was a glucoside. The ^{1}H, ^{13}C NMR (Table 1), and DEPT spectra proposed **2** to be composed of a glucopyranosyl moiety and an aglycone part having five hydroxyl groups. The configuration of the anomeric position was determined to be β on the basis of the coupling constant of the anomeric proton signal in the ^{1}H NMR spectrum of **2** [δ 5.11 (1H, d, $J = 7.2$ Hz, H-1′)]. The signals due to the β-D-glucopyranosyl moiety were observed in the ^{13}C NMR spectrum at δ 98.4, 75.4, 78.3, 72.0, 78.2, and 63.3. The proton and carbon signals of **2** in the ^{1}H and ^{13}C NMR spectra were similar to those of ginsenoside Rg_7 [7], except for the signals due to the side chain part (C-23–C-27). Furthermore, compared to ginsenoside Rg_7, the remarkable upfield shift (about 10 ppm) of C-3 was observed, indicating that compound **2** possessed one hydroxyl group at C-3, not a glucose group. In addition, there was no characteristic feature about double bond carbon signals. So, the coupling system of the side chain was established by comprehensive analyses of 2D NMR spectra. In the ^{1}H–^{1}H COSY spectrum of **2**, two geminal proton signals at δ 1.62 and 1.72 (H-23) correlated not only with the proton signal at δ 3.75 (1H, t, $J = 10.2$ Hz, H-24), but also with other two geminal proton signals at δ 1.87 and 2.34 (H-22). In the HMBC spectrum of **2**, long-range correlations were observed between the proton signal at δ 3.75 (H-24) and the carbon signals at δ 27.1, 25.4 (C-26, 27), 33.7 (C-22), and 73.1 (C-25), and between the methyl proton signal at δ 1.54 (H-21) and carbon signals at δ 52.8 (C-17) and 33.7 (C-22). From these observations, it could be deduced that the two hydroxyl groups might be located at C-24 and C-25.



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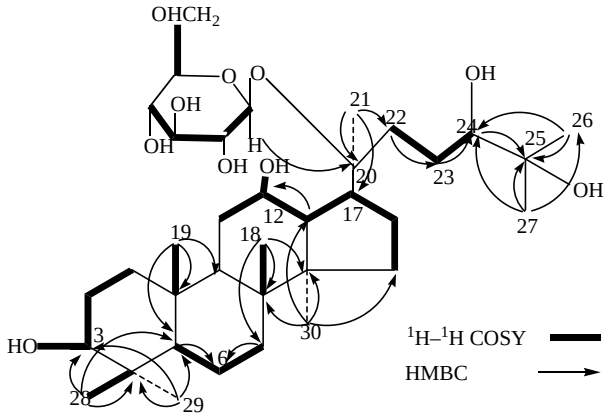

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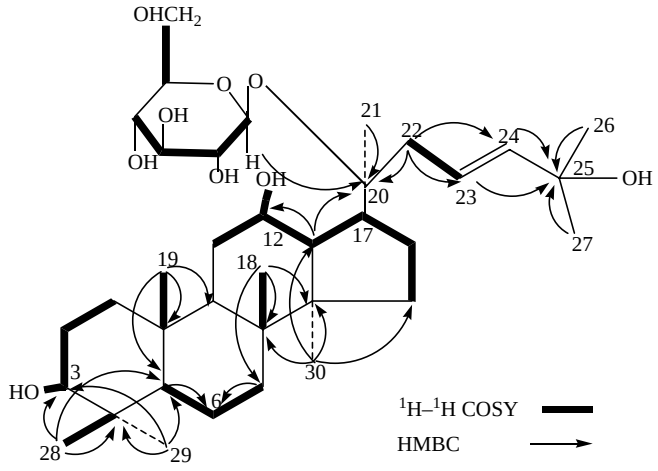

3

Figure 2. Significant $^{1}H-^{1}H$ COSY and HMBC correlations for compounds **1**–**3**.

The long-range correlation between the anomeric proton at δ 5.11 and C-20 at δ 84.0 in the HMBC spectrum showed that β-D-glucopyranosyl was linked to C-20 of the aglycone.

As shown in Figure 2, the $^1H-^1H$ COSY experiment on **2** indicated the presence of partial structures, shown by bold lines, and in the HMBC experiment, long-range correlations were observed. On the basis of these findings, the structure of compound **2** was elucidated as (20*S*)-3β,12β,20,24,25-pentahydroxydammarane 20-*O*-β-D-glucopyranoside and named as ginsenoside Rh_{12}.

Ginsenoside Rh_{13} (**3**) was also obtained as an amorphous powder with positive optical rotation ($[\alpha]_D^{23.3}$ + 22.2 in MeOH). The molecular formula, $C_{36}H_{62}O_9$, was established on the basis of the quasi-molecular ion peak in the HR-ESI-MS at *m/z* 661.4263 $[M + Na]^+$. The IR spectrum of **3** showed absorption bands at 3402, 2937, 1387, and 1077 cm^{-1} assignable to hydroxyl, methyl, methylene, and ether functions. Acid hydrolysis of **3** with methanol hydrochloric acid [MeOH:HCl (1:1)] liberated a D-glucose, which was identified by TLC comparison with an authentic sample, indicating that **3** was a glucoside. The ^1H, ^{13}C NMR (Table 1), and DEPT (pyridine-d_5) spectra proposed **3** to be composed of a glucopyranosyl moiety and an aglycone part having four hydroxyl groups and one double bond. The configuration of the anomeric position was determined to be β on the basis of the coupling constant of the anomeric proton signal in the ^1H NMR spectrum of **3** [δ 5.08 (1H, d, J = 7.8 Hz, H-1′)]. The signals due to the β-D-glucopyranosyl moiety were observed in the ^{13}C NMR spectrum at δ 98.4, 75.4, 78.5, 71.8, 78.2, and 63.1. The proton and carbon signals of **3** in the ^1H and ^{13}C NMR spectra were identical with those of ginsenoside Rh_{12} (**2**), except for the signals due to the side chain part (C-23–C-27), which were similar to those of majoroside F_4 [18]. The β-D-glucopyranosyl was deduced to link to C-20 of the aglycone by the interpretation of the HMBC spectrum, which showed a long-range correlation between the anomeric proton at δ 5.08 and C-20 at δ 83.4. From the above results and detailed examination of the $^1H-^1H$ COSY and HMBC experiments (Figure 2), the structure of compound **3** was elucidated as (20*S*,23*E*)-3β,12β,20,25-tetrahydroxydammara-23-ene 20-*O*-β-D-glucopyranoside and named as ginsenoside Rh_{13}.

Known compounds (**4**–**24**) were identified by comparison of their physical data ($[\alpha]_D$, IR, ^1H and ^{13}C NMR) with reported values in the literature.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 digital polarimeter (l = 5 cm), the solvent used being MeOH. Melting points were determined on an MP-500D Yanaco micromelting point apparatus, and are uncorrected. IR spectra were measured on a Shimadzu FTIR-8100 spectrometer. UV spectra were obtained on a GBC Cintra 10e UV–vis spectrophotometer (Australia) equipped with quartz cells. HR mass spectrometry was performed using an Ion Spec Ultima 7.0T FTICR instrument (Ionspec, Lake Forest, CA, USA) with an ESI source in the positive ion mode. NMR spectra were obtained with a Bruker AV600 (600 MHz) spectrometer, using C_5D_5N as the solvent. Chemical shifts are given in δ (ppm) with tetramethylsilane (TMS) as an internal standard. HPLC was performed with an Agilent 1200 system using a YMC-Pack ODS-A column (250 × 4.6 mm i.d.). Preparative HPLC separations were carried out on a Shimadzu liquid chromatograph LC-8A, using a SPD-10Avp UV detector and YMC-Pack ODS-A column (250 × 20 mm i.d). Column chromatography was carried out on macroporous

absorption resin AB-8 (Tianjin Pesticide Manufacturer, Tianjin, China), silica gel (Qingdao Factory of Marine Chemical Industry, Qingdao, China), and ODS (Chromatorex, 100–200 mesh; Fuji Sylisia Chemical Ltd, Aichi, Japan). Spots were detected on the precoated TLC plates with silica gel $60F_{254}$ and RP-18_{254s} (0.25 mm; Merck Co., Dr Whitehouse Station, NJ, USA) by heating over 110°C after spraying with 10% H_2SO_4–EtOH solution.

3.2 Plant material

The leaves of *P. ginseng* were cultivated in Jilin Province, China. The botanical identification was undertaken by Prof. Minglu Deng (Changchun University of Chinese Medicine). A voucher specimen (No. 200609) has been deposited at the Department of Analytical Chemistry, College of Chemistry, Jilin University, China.

3.3 Extraction and isolation

The air-dried leaves (2 kg) were extracted three times with water under heating. The extracts were combined and subjected to a macroporous absorption resin AB-8 column by eluting with water to remove impurities, and then eluted with 85% EtOH. The eluate was collected and evaporated under vacuum to afford the crude saponin fraction (230 g), which was partitioned in an EtOAc–H_2O (1:1 v/v) mixture. Moreover, the aqueous phase was further extracted with *n*-BuOH. Removal of the solvent from the EtOAc-soluble, *n*-BuOH-soluble, and H_2O-soluble fractions under reduced pressure yielded 91, 106, and 33 g of the residue, respectively.

The *n*-BuOH-soluble phase was subjected to normal-phase silica gel column chromatography [AcOE–EtOH–H_2O (50:10:1 → 40:10:1 → 20:10:1 → 7:4:1, v/v/v) → EtOH] to give seven fractions. Fraction 2 (13 g) was separated by reversed-phase silica gel column chromatography, with CH_3OH–H_2O [(10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20, v/v) → CH_3OH] eluting to furnish five fractions. Fraction 2-2 (4 g) was separated by normal-phase silica gel column chromatography with $CHCl_3$–EtOAc (10:1) as the mobile phase and purified on preparative HPLC to yield compounds **22** (206.2 mg), **8** (5.6 mg), **17** (20.0 mg), and **20** (18.5 mg). Fraction 5 (20 g) was separated by reversed-phase silica gel column chromatography, eluted with CH_3OH–H_2O [(10:90 → 20:80 → 30:70 → 40:60 → 50:50 → 60:40 → 70:30 → 80:20, v/v) → CH_3OH] to furnish 14 fractions. Fraction 5-3 was purified by preparative HPLC [CH_3OH–H_2O (70:30, v/v)] to obtain compounds **14** (100.5 mg), **13** (125.0 mg), and **21** (49.3 mg). In the same manner, fraction 5-4 (2 g) was chromatographed to give compounds **5** (71.0 mg) and **6** (16.0 mg), fraction 5-5 (3 g) to give compounds **4** (68.0 mg), **7** (13.0 mg), and **9** (7.0 mg), fraction 5-7 (4 g) to give compounds **10** (145.0 mg), **18** (235.0 mg), and **19** (15.0 mg).

The EtOAc-soluble extract (91 g) was subjected to normal-phase silica gel column chromatography, with $CHCl_3$–EtOH–H_2O (100:10:1 → 80:10:1 → 60:10:1 → 40:10:1 → 20:10:1 → 10:10:1, v/v/v) as the eluent, followed by evaporation of the solvent under reduced pressure to collect nine fractions. Fraction 1 (9 g) was separated by using medium-pressure column chromatography and HPLC to give compounds **1** (3.5 mg), **15** (16.0 mg), and **12** (56.3 mg). Fraction 4 (5 g) was separated by reversed-phase silica gel column chromatography, and then each fraction was purified by preparative HPLC to give compounds **2** (5.0 mg) and **3** (3.2 mg). In the same manner, fraction 7 (18 g) was purified to give compounds **16** (48.0 mg), **23** (52.0 mg), and **24** (607.0 mg), and fraction 9 (1 g) to give compounds **11** (100.0 mg).

3.3.1 Ginsenoside Rh_{11} (1)

A white amorphous powder; mp 193–195°C; $[\alpha]_D^{22.8} + 55.8$ ($c = 6.00$, MeOH); IR (KBr) ν_{max}: 3377, 2937, 1696, 1647, 1384, 1073 cm^{-1}; UV λ_{max}^{MeOH}: 221.44 nm; ^1H NMR (600 MHz, C_5D_5N, J in Hz) δ (ppm): 0.84, 0.89, 0.94, 1.33, 1.43, 1.72, 1.86 (3H each, all s, H-19, 30, 18, 29, 21, 27, 28), 4.09 (1H, t, $J = 13.8$ Hz, H-3), 4.28 (1H, m, H-6), 4.03 (1H, m, H-12), 1.96 (2H, m, H-23), 6.08, 5.53 (1H, each, s, H-26), 3.23, 2.94 (1H, each, m, H-22), 1.08 (1H, d, $J = 10.2$ Hz, H-5), 5.03 (1H, d, $J = 7.8$ Hz, H-1′), 3.88 (1H, m, H-2′), 3.39 (1H, d, $J = 7.2$ Hz, H-3′), 4.04 (1H, m, H-4′), 3.73 (1H, m, H-5′), 4.38 (1H, d, $J = 11.4$ Hz, H-6′α), 4.19 (1H, dd, $J = 11.4$, 5.4 Hz, H-6′β); ^{13}C NMR spectral data: see Table 1; positive ion HR-ESI-MS: m/z 675.4058 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_{10}Na$, 675.4084).

3.3.2 Ginsenoside Rh_{12} (2)

A white amorphous powder; mp 176–178°C; $[\alpha]_D^{23.1} + 13.5$ ($c = 4.50$, MeOH); IR (KBr) ν_{max}: 3367, 2947, 1387, 1076 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N, J in Hz) δ (ppm): 0.77, 0.78, 0.82, 1.92, 1.11, 1.40, 1.45, 1.54 (3H each, all s, H-18, 19, 30, 29, 28, 27, 26, 21), 4.10 (1H, m, H-3), 3.91 (1H, m, H-12), 3.75 (1H, t, $J = 10.2$ Hz, H-24), 1.62 (1H, m, H-23α), 1.72 (1H, m, H-23β), 1.87 (1H, m, H-22α), 2.34 (1H, m, H-22β), 0.70 (1H, t, $J = 12.0$ Hz, H-5), 2.42 (1H, m, H-17), 1.92 (1H, m, H-13), 1.97 (1H, m, H-9), 5.11 (1H, d, $J = 7.2$ Hz, H-1′), 3.90 (1H, m, H-2′), 3.31 (1H, m, H-3′), 4.10 (1H, m, H-4′), 3.87 (1H, m, H-5′), 4.38 (1H, d, $J = 10.2$ Hz, H-6′α), 4.13 (1H, m, H-6′β); ^{13}C NMR spectral data: see Table 1; positive ion HR-ESI-MS: m/z 679.4391 $[M + Na]^+$ (calcd for $C_{36}H_{64}O_{10}Na$, 679.4397).

3.3.3 Ginsenoside Rh_{13} (3)

A white amorphous powder; mp 177–179°C; $[\alpha]_D^{23.3} + 22.2$ ($c = 6.10$, MeOH). IR (KBr) ν_{max}: 3402, 2937, 1387, 1077 cm^{-1}; ^1H NMR (600 MHz, C_5D_5N, J in Hz), δ (ppm): 0.76, 0.81, 0.93, 0.94, 1.12, 1.42, 1.43, 1.47 (3H each, all s, H-18, 19, 30, 29, 28, 26, 27, 21), 1.59 (1H, m, H-1α), 0.82 (1H, m, H-1β), 1.72 (2H, m, H-2), 4.08 (1H, t, $J = 9.0$ Hz, H-3), 0.70 (1H, d, $J = 2.4$ Hz, H-5), 1.17 (2H, t, $J = 7.2$ Hz, H-7), 1.36 (1H, m, H-9), 3.89 (1H, m, H-12), 1.90 (1H, t, $J = 10.8$ Hz, H-13), 0.84 (1H, m, H-15), 1.91 (1H, m, H-15), 1.80 (2H, m, H-16), 2.32 (1H, m, H-17), 2.92 (1H, d, $J = 2.4$ Hz, H-22α), 2.65 (1H, d, $J = 2.4$ Hz, H-22β), 6.18 (1H, m, H-23), 5.85 (1H, d, $J = 15.6$ Hz, H-24), 5.08 (1H, d, $J = 7.8$ Hz, H-1′), 3.90 (1H, m, H-2′), 3.31 (1H, m, H-3′), 3.94 (1H, m, H-4′), 3.86 (1H, m, H-5′), 4.38 (1H, d, $J = 9.6$ Hz, H-6′α), 4.14 (1H, dd, $J = 11.2$, 6.0 Hz, H-6′β); ^{13}C NMR spectral data: see Table 1; positive ion HR-ESI-MS: m/z 661.42632 $[M + Na]^+$ (calcd for $C_{36}H_{62}O_9Na$, 661.429155).

3.4 Acid hydrolysis of Rh_{11} (1), Rh_{12} (2) and Rh_{13} (3)

A solution of compounds **1**–**3** (2.5 mg) in MeOH:HCl (1:1) was placed in a capillary and the capillary was sealed. After heating at 80°C for 5 h, the solution was subjected to silica gel TLC, together with the standard samples, using n-BuOH–AcOH–H_2O (5:1:4, upper layer) and $CHCl_3$–MeOH–H_2O (16:8:1) as the developing solvents and using O-phthalic acid-aniline as the detection reagent. Only glucose was detected from compounds **1**–**3**.

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