Note

Novel Dammarane-Type Triterpene Saponins from Panax ginseng Root

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Four phytochemical constituents were isolated from Panax ginseng root by repeated column chromatography (CC), medium pressure liquid chromatography (MPLC), high-speed counter current chromatography (HSCCC), and semi-preparative HPLC. Their structures were elucidated as the dammarane-type triterpene saponins ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) based on spectral data. Compounds 1–4 from P. ginseng root were new compounds from nature. They showed good hydroxyl radical scavenging activity and anti-bacterial activity against Escherichia coli and Staphylococcus aureus. However, they did not show any anti-inflammatory activity. In addition, they inhibited the growth of adenocarcinoma gastric stomach cells. Among them, ginsenoside-Rs11 (3) showed the best anti-oxidative, anti-bacterial, and anti-cancer activities.

Key words Panax ginseng; white ginseng; dammarane; ginsenoside-Rs11; adenocarcinoma gastric stomach

Panax ginseng (Araliaceae), a traditional oriental herbal medicine, has been used for thousands of years for treating various diseases in East Asian countries. P. ginseng has attracted significant interest because of its diverse pharmacological and therapeutic effects on the central nervous system, cardiovascular system, and immune-modulating functions. It is used for diabetes, as an anti-oxidant, anti-hypotensive, anti-tumor agent, anti-cancer agent to increase cognitive abilities, as a sedative, and as an analgesic. A number of dammarane-type triterpene oligoglycosides with anti-cancer and anti-arrhythmia properties have been reported.

The root of ginseng has a critical role in traditional medicine, and its principal constituents have been characterized as dammarane-type triterpene saponins. The majorities of these compounds are protopanaxadiols (PPD) and protopanaxatriol (PPT), which are aglycones of dammarane-type triterpenes. In PPDs, the sugar moieties are attached to the ring of the triterpene dammarane at the three-position (as in ginsenosides-Rg3, -Rd, -Re, and -Rb2), while in PPTs, the sugar moieties are attached at the six-position (as in ginsenosides-Rgl, -Re, and -Rg2).

As part of an ongoing study into the chemical and biological activities of ginseng and ginsenosides, we describe the isolation, identification, and biological activities of dammarane-type triterpene saponins from the dried root of P. ginseng. New compounds were purified by repeated column chromatography (CC), medium pressure liquid chromatography (MPLC), high-speed counter current chromatography (HSCCC), and semi-preparative HPLC (Semi-prep-HPLC).

Results and Discussion

Identification of Compounds 1–4 The n-butanol (n-BuOH)-soluble fraction of the ethanol (EtOH) extract from P. ginseng root was chromatographed by CC, MPLC, HSCCC, and Semi-prep-HPLC to yield compounds 1–4 (Fig. 1). Compounds 1–4 from P. ginseng root were isolated for the first time from nature.

Compound 1 was obtained as a white amorphous powder. The IR spectrum suggested the presence of hydroxyl groups (3365 cm−1) and an α,β-unsaturated carbonyl (1631 cm−1).

Its UV spectrum in methanol (MeOH) showed absorption maxima at 268, 293, and 394 nm. A molecular ion peak was measured at m/z 969 [M+Na]+ in the positive FAB-MS, which corresponds to a molecular formula of C42H62O14 by high resolution (HR)-FAB-MS (m/z 969.5415 [M+Na]+, Calcd for 969.5399). The 1H- and 13C-NMR data from 1 are shown in Table 1. The 1H-NMR spectrum showed one olefinic (δ 5.26), three anomic (δ 5.01, 5.19, and 6.48), and a methyl proton signal (δ 1.61) of 1-l-rhamnopyranoside. The configuration of the anomic positions were determined to be the α and β form based on the coupling constants for the anomic proton signals in the 1H-NMR spectrum of 1. In the 13C-NMR spectrum, the chemical shifts of three anomic carbons were observed at δ 98.8, 102.4 and 106.5 with a double bond between C-24 and C-25 (δ 126.4 and 131.5). Acidic hydrolysis of 1 yielded D-glucopyranoside and l-rhamnopyranoside.

Therefore, the anomic carbon signals indicated two β-d-glucopyranosyl and one α-l-rhamnopyranosyl moieties. The two β-d-glucopyranosyl anemic proton signals were confirmed to be linked at the C-6 and C-20 positions by long-range heteronuclear multiple bond connectivity (HMBCC) correlations between the proton signal at δ 5.01 (H-1’) and the carbon signal at δ 78.8 (C-6), and the proton signal at δ 5.19 (H-1”) and the carbon signal at δ 83.9 (C-20), respectively. A significant downfield shift for the C-2” (δ 79.0) was observed for the inner β-d-glucopyranosyl moiety at C-20 of the aglycone, which showed that the C-1” (δ 6.48) in the terminal α-l-rhamnopyranosyl moiety is linked to the inner β-d-glucopyranosyl moiety at C-20. Based on this data, the structure of 1 was identified as ginsenoside-Rg18.

Compound 2 was obtained as a white amorphous powder. Its IR spectrum suggested the presence of hydroxyl groups (3365 cm−1) and an α,β-unsaturated carbonyl (1631 cm−1). Its UV spectrum in MeOH showed absorption maxima at 268, 293, and 394 nm. A molecular ion peak was measured at m/z 1027 [M+Na]+ in the positive FAB-MS, which corre-
The structure of the aglycone, which showed that the C-1\textbeta-D-glucopyranosyl moiety at C-3 of the aglycone, which showed (3365 cm\(^{-1}\)) and an \(\alpha,\beta\)-unsaturated carbonyl (1631 cm\(^{-1}\)). Its UV spectrum in MeOH showed absorption maxima at 268, 293, and 394 nm. A molecular ion peak was measured at m/z 1143.5922 [M+Na]\(^\dagger\), Calcd for 1143.5927). The \(\textsuperscript{1}H\)- and \(\textsuperscript{13}C\)-NMR data from 3 were shown in Table 1. The \(\textsuperscript{1}H\)-NMR spectrum showed one olefinic (\(\delta\) 5.30), three anomeric (\(\delta\) 5.00, 5.01, and 5.18), and an acetyl methyl proton signal (\(\delta\) 2.05). The configuration of the anomeric positions were determined to be the \(\beta\) form based on the coupling constants of the anomeric proton signals in the \(\textsuperscript{1}H\)-NMR spectrum of 2. In the \(\textsuperscript{13}C\)-NMR spectrum, the chemical shifts of three anomeric carbons were observed at \(\delta\) 98.8, 106.3, and 106.4. Acidic hydrolysis of 2 yielded \(\beta\)-glucopyranoside. Therefore, the anomeric carbon signals indicated three \(\beta\)-\(\beta\)-glycopyranosyl moieties. The two \(\beta\)-\(\beta\)-glycopyranosyl anomeric proton signals were confirmed to be linked at the C-6 and C-20 positions by long-range HMBC correlations between the proton signal at \(\delta\) 5.00 (H-1\textbeta) and carbon signal at \(\delta\) 79.3 (C-6), and the proton signal at \(\delta\) 5.18 (H-1\textalpha) and carbon signal at \(\delta\) 83.9 (C-20), respectively. A significant downfield shift for the C-2\textalpha (\(\delta\) 83.9) was observed for the inner \(\beta\)-\(\beta\)-D-glucopyranosyl moiety at C-6 of the aglycone, which showed that the C-1\textalpha (\(\delta\) 5.01) in the terminal \(\beta\)-\(\beta\)-glycopyranosyl moiety is linked to the inner \(\beta\)-\(\beta\)-glycopyranosyl moiety at C-6. In the \(\textsuperscript{1}H\)-NMR spectrum of 2, the downfield shift of two hydroxymethylene proton signals (\(\delta\) 5.05 and 4.62) was attributed to acetylation of the hydroxyl group. These signals also showed a correlation with C-5\textbeta (\(\delta\) 75.9), thus the acetyl group was placed at the primary hydroxyl group of the sugar moiety (C-6\textalpha). The NMR assignments of compound 2 were very similar to 6-acetyl-Rg1, previously isolated from red ginseng, except for the position of the \(\beta\)-\(\beta\)-glycopyranosyl group at the C-4 hydroxyl of the sugar moiety. Based on this data, the structure of 2 was identified as 6-acetyl ginsenoside-Rg3.

Compound 3 was isolated as a white amorphous powder. The IR spectrum suggested the presence of hydroxyl groups (3365 cm\(^{-1}\)) and an \(\alpha,\beta\)-unsaturated carbonyl (1631 cm\(^{-1}\)). Its UV spectrum in MeOH showed absorption maxima at 268, 293, and 394 nm. A molecular ion peak was measured at m/z 1143.5922 [M+Na]\(^\dagger\), Calcd for 1143.5927). The \(\textsuperscript{1}H\)- and \(\textsuperscript{13}C\)-NMR data from 3 were shown in Table 1. The \(\textsuperscript{1}H\)-NMR spectrum showed one olefinic (\(\delta\) 5.44), four anomeric (\(\delta\) 4.93, 5.21, 5.40 and 5.67), and an acetyl methyl proton signal (\(\delta\) 2.06). The configuration of the anomeric positions were determined to be the \(\alpha\) and \(\beta\) form based on the coupling constants of the anomeric proton signals in the \(\textsuperscript{1}H\)-NMR spectrum of 3. In the \(\textsuperscript{13}C\)-NMR spectrum, the chemical shifts of four anomeric carbons were observed at \(\delta\) 98.8, 105.6, 106.8, and 110.6. Acidic hydrolysis of 3 afforded \(\delta\)-glucopyranoside and l-arabinofuranosyl moieties. A significant downfield shift for the C-2\textbeta (\(\delta\) 83.9) was observed for the inner \(\beta\)-\(\beta\)-glycopyranosyl and l-arabinofuranosyl moieties. A significant downfield shift for the C-2\textbeta (\(\delta\) 83.9) was observed for the inner \(\beta\)-\(\beta\)-glycopyranosyl and l-arabinofuranosyl moieties. A significant downfield shift for the C-2\textbeta (\(\delta\) 83.9) was observed for the inner \(\beta\)-\(\beta\)-glycopyranosyl moieties.
Table 1. $^1$H- and $^{13}$C-NMR Data for the Aglycone and Sugar Moieties of Compounds 1–4 (500 MHz, Pyridine-$d_5$)

| No. | $\delta$ (H) | $\delta$ (C) |
|-----|--------------|--------------|
| 1   | 0.74, 1.51   | 39.9         |
| 2   | 1.81, 2.15   | 28.4         |
| 3   | 3.52         | 79.8         |
| 4   | —            | 41.6         |
| 5   | 1.45         | 61.9         |
| 6   | 4.63         | 78.8         |
| 7   | 1.95, 2.54   | 45.6         |
| 8   | —            | 41.7         |
| 9   | 1.56         | 51.9         |
| 10  | —            | 39.9         |
| 11  | 1.41, 1.95   | 31.4         |
| 12  | 3.95         | 70.8         |
| 13  | 1.99         | 50.5         |
| 14  | —            | 51.9         |
| 15  | 1.16, 1.72   | 31.4         |
| 16  | 1.42, 1.85   | 23.8         |
| 17  | 2.45         | 52.2         |
| 18  | 1.05         | 18.3         |
| 19  | 0.92         | 18.1         |
| 20  | —            | 83.9         |
| 21  | 1.58         | 19.3         |
| 22  | 1.79, 2.33   | 36.5         |
| 23  | 2.24, 2.51   | 22.9         |
| 24  | 5.26         | 126.4        |
| 25  | —            | 131.5        |
| 26  | 1.59         | 26.2         |
| 27  | 1.55         | 18.0         |
| 28  | 2.10         | 32.2         |
| 29  | 1.64         | 18.2         |
| 30  | 0.87         | 18.2         |

COCO3 — — 171.6 — —
3-O-glcc-1' — — 2.05 21.5 2.06 21.4
2' — — — — 4.93 105.6
3' — — — — 4.17 83.9
4' — — — — 4.23 78.8
5' — — — — 3.99 72.1
6' — — — — 4.09 75.6
2'-O-glcc-1'' — — — — 4.94, 4.81 65.3
2'' — — — — 5.40 106.8
2'' — — — — 4.15 77.6
3'' — — — — 4.17 78.6
4'' — — — — 4.31 72.2
5'' — — — — 4.01 79.7
6'' — — — — 4.49, 4.23 63.3
6-O-glcc-1' 5.01 106.5 5.00 106.3 — — — — 5.25 102.4
2' 4.10 75.7 4.11 75.6 — — 4.00 79.8
3' 4.22 78.8 4.21 78.8 — — 4.21 79.7
4' 4.24 72.4 4.25 76.0 — — 4.18 71.9
5' 4.01 78.6 4.02 78.6 — — 3.98 78.6
6' 4.50, 4.34 63.6 4.35, 4.23 63.3 — — 4.50, 4.38 63.6
2'-O-rha-1'' — — — — 6.49 102.4
2'' — — — — 4.51 72.8
3'' — — — — 4.67 72.9
4'' — — — — 4.22 74.7
5'' — — — — 4.93 70.5
6'' — — — — 1.77 19.2
2'-O-glcc-1'' — — — — 5.01 106.4 — — — —
2'' — — — — 4.19 75.6
Table 1. Continued

| No. | 1       | 2       | 3       | 4       |
|-----|---------|---------|---------|---------|
|     | δ_H    | δ_C    | δ_H    | δ_C    | δ_H    | δ_C    | δ_H    | δ_C    |
| 3°  | —       | —       | 4.49   | 79.3   | —       | —       | —       | —       |
| 4°  | —       | —       | 4.11   | 72.4   | —       | —       | —       | —       |
| 5°  | —       | —       | 3.99   | 78.8   | —       | —       | —       | —       |
| 6°  | —       | —       | 4.49, 4.27 | 63.6   | —       | —       | —       | —       |
| 20-O-glc-1° | 5.19 | 98.8   | —       | —       | 5.21   | 98.8   | 5.19   | 98.8   |
| 2°  | 3.99   | 79.0   | —       | —       | 3.98   | 75.6   | 3.98   | 74.7   |
| 3°  | 4.20   | 79.6   | —       | —       | 4.19   | 78.7   | 4.22   | 79.2   |
| 4°  | 4.20   | 72.0   | —       | —       | 4.22   | 72.6   | 4.20   | 72.1   |
| 5°  | 4.00   | 76.0   | —       | —       | 4.09   | 78.8   | 3.97   | 78.8   |
| 6°  | 4.37, 4.21 | 63.3 | —       | —       | 4.29, 4.19 | 69.0 | 4.36, 4.23 | 63.4 |
| 20-O-glc-1° | —       | —       | 5.18   | 98.8   | —       | —       | —       | —       |
| 2°  | —       | —       | 4.08   | 75.7   | 3.98   | 75.6   | 3.98   | 74.7   |
| 3°  | —       | —       | 4.20   | 79.7   | 4.19   | 78.7   | 4.22   | 79.2   |
| 4°  | —       | —       | 4.23   | 72.0   | 4.22   | 72.6   | 4.20   | 72.1   |
| 5°  | —       | —       | 4.05   | 75.9   | 4.09   | 78.8   | 3.97   | 78.8   |
| 6°  | —       | —       | 5.05, 4.62 | 65.8   | 4.29, 4.19 | 69.0 | 4.36, 4.23 | 63.4 |
| 2°-O-rha-1° | 6.48 | 102.4  | —       | —       | —       | —       | —       | —       |
| 2°  | 4.50   | 72.8   | —       | —       | —       | —       | —       | —       |
| 3°  | 4.37   | 73.1   | —       | —       | —       | —       | —       | —       |
| 4°  | 4.33   | 74.7   | —       | —       | —       | —       | —       | —       |
| 5°  | 4.35   | 70.1   | —       | —       | —       | —       | —       | —       |
| 6°  | 1.61   | 19.3   | —       | —       | —       | —       | —       | —       |
| 6°-O-ara(f)-1° | —       | —       | —       | —       | 5.67   | 110.6  | —       | —       |
| 2°  | —       | —       | —       | —       | 4.21   | 84.8   | —       | —       |
| 3°  | —       | —       | —       | —       | 4.78   | 79.7   | —       | —       |
| 4°  | —       | —       | —       | —       | 4.78   | 86.5   | —       | —       |
| 5°  | —       | —       | —       | —       | 4.22, 4.46 | 63.2 | —       | —       |

Chemical shifts are reported in parts per million (δ).

etγ,20,21) Based on this data, the structure of 3 was identified as ginsenoside-Rs11.

Compound 4 was obtained as a white amorphous powder. The IR spectrum suggested the presence of hydroxyl groups (3365 cm⁻¹) and an αβ-unsaturated carbonyl (1631 cm⁻¹). Its UV spectrum in MeOH showed absorption maxima at 268, 293, and 394 nm. A molecular ion peak was measured at m/z 985 [M+Na]+ in the positive FAB-MS, which corresponds to a molecular formula of C48H34O10 by HR-FAB-MS (m/z 985.5373 [M+Na]+, Calcd for 985.5348). The 1H- and 13C-NMR data from 4 are shown in Table 1. The 1H-NMR spectrum showed two olefinic (δ 6.16 and 6.26), three anomeric (δ 5.19, 5.25, and 6.49), and a methyl proton signal (δ 1.77) of l-rhamnopyranoside. The configuration of the anomeric positions were determined to be the α and β form based on the coupling constants of the anomeric proton signals in the 1H-NMR spectrum of 4. In the 13C-NMR spectrum, the chemical shifts of three anomeric carbons were observed at δ 98.8, 102.4, and 102.4 (overlap). In the HMBC spectrum, correlations were observed between the proton signal at δ 5.70 (H-22) and the carbon signals at δ 40.2 (C-24) and 81.9 (C-25), and the proton signal at δ 6.26 (H-23) and the carbon signals at δ 40.2 (C-24), 81.9 (C-25), 25.8 (C-26), and 25.9 (C-27), respectively. Up to this point it could be concluded that the double bond might be located between C-22 and C-23.22,23) Acidic hydrolysis of 4 yielded β-glucopyranoside and l-rhamnopyranoside. Therefore, the anomeric carbon signals indicated two β-D-glucopyranosyl and one α-L-rhamnopyranosyl moieties. The two β-D-glucopyranosyl anomeric proton signals were confirmed to be linked at the C-6 and C-20 positions by long-range HMBC correlations between the proton signal at δ 5.25 (H-1') and carbon signal at δ 80.1 (C-6), and the proton signal at δ 5.19 (H-1") and the carbon signal at δ 83.7 (C-20), respectively. A significant downfield shift for the C-2' (δ 79.8) was observed for the inner β-D-glucopyranosyl moiety at C-6 of the aglycone, which showed that the C-1" (δ 6.49) of the terminal α-L-rhamnopyranosyl moiety is linked to the inner β-D-glucopyranosyl moiety at C-6. Based on this data, the structure of 4 was identified as ginsenoside-Re7.

Biological Activities of Compounds 1–4 The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical contains an odd electron that is responsible for its absorbance at 540 nm, and for the visible deep purple color. A higher % in our assay indicates better scavenging activity or anti-oxidant potential. As shown in Table 2, ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) had weak DPPH radical-scavenging activity. The ·OH scavenging activity of ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) was greater than 85% at a concentration of 50μM. Ginsenoside-Rs11 (3) in particular had a scavenging activity of greater than 90% under the same conditions. These results suggest that ginsenoside-Rs11 (3) may be an effective scavenger of ·OH radicals. The anti-bacterial activities of ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) in Escherichia (E.) coli, Staphylo-
of the RAW264.7 cells with ginsenoside-Rg18 (the generation of nitric oxide (NO). Secretion of NO from the inflammatory mediators LPS and IFN-γ 99.74%, respectively. RAW264.7 cells were incubated with control group, which were measured at approximately 100 and difference in cell viability between the normal group and the Re7 (inflammatory activity. 4). This result indicates that the saponins did not show anti-
tive only in live cells. Cells were incubated for 1 d and then treated with ginsenoside-Rg18 (-4), and ginsenoside-Re7 (4) at 50 µM, which all caused some inhibition of gastric cancer cell growth. In particular, ginsenoside-Rs11 (3) had the greatest cancer cell growth inhibition (55.06%) of the various extracts. Ginsenoside-Rg18 (1) had the smallest effect, inhibiting the AGS cell growth rate by 38.01%. These experiments show that ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) have a significant anti-proliferative effect on AGS cells (Table 5).

As part of an ongoing study into the chemical and biological activities of ginseng and ginsenosides, we describe the isolation, identification, and biological activities of dammarane-
coccus (S.) aureus, and Helicobacter (H.) pylori are shown in Table 3. Ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) inhibited the growth of E. coli and S. aureus at a concentration of 500 µM. In particular, ginsenoside-Rs11 (3) showed good antibacterial activity against E. coli.

The anti-inflammatory activity was investigated using RAW264.7 cells stimulated by lipopolysaccharide (LPS)/inter-
teron (IFN-γ). As shown in Table 4, there was no significant difference in cell viability between the normal group and the control group, which were measured at approximately 100 and 99.74%, respectively. RAW264.7 cells were incubated with the inflammatory mediators LPS and IFN-γ, which induced the generation of nitric oxide (NO). Secretion of NO from RAW264.7 macrophage cells in the normal group decreased 52.91% compared to the control group (100%). Treatment of the RAW264.7 cells with ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) did not lead to a decrease in NO production (Table 4). This result indicates that the saponins did not show anti-
flammatory activity.

To assess the cytotoxic activity, we used the 3-(4,5-di-
methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method to test whether these extracts moderated the growth of adenocarcinoma gastric stomach (AGS) cells. Cell viability was measured by detecting the purple formazan metabolized in MTT by mitochondrial dehydrogenases, which are active only in live cells. Cells were incubated for 1 d and then treated with ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) at 50 µM, which all caused some inhibition of gastric cancer cell growth. In particular, ginsenoside-Rs11 (3) had the greatest activity against E. coli, S. aureus, and H. pylori. To assess the anti-inflammatory activity, we used RAW264.7 cells stimulated by LPS/IFN-γ. As shown in Table 4, there was no significant difference in cell viability between the normal group and the control group, which were measured at approximately 100 and 99.74%, respectively. RAW264.7 cells were incubated with the inflammatory mediators LPS and IFN-γ, which induced the generation of nitric oxide (NO). Secretion of NO from RAW264.7 macrophage cells in the normal group decreased 52.91% compared to the control group (100%). Treatment of the RAW264.7 cells with ginsenoside-Rg18 (1), 6-acetyl ginsenoside-Rg3 (2), ginsenoside-Rs11 (3), and ginsenoside-Re7 (4) did not lead to a decrease in NO production (Table 4). This result indicates that the saponins did not show anti-inflammatory activity.

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glutaric acid (TMG), and dimethyl sulfoxide (DMSO) were purchased from J.T. Baker (NJ, U.S.A.). The Griess reagent, MTT, 3,3-tetramethylene blue dye in water (7 L) and partitioned successively with ethyl acetate and hexane to afford a brown residue (139 g). The residue was dissolved in CHCl₃ (3 L) and concentrated to reflux, and the extracts were combined and concentrated to 100 mL by heating to 100°C. CC was conducted with a LiChroprep RP-18 (40–63 μm, Merck Co., Germany). An MPLC system (Biotage, Uppsala, Sweden) equipped with cartridges (KP-SIL, 39×225 mm) was used. A HSCCC system (Tauto Biotech Co., Ltd., Shanghai, China) was equipped with three preparative coils (i.d. 1.5 mm, total volume is 300 mL, and a 20 mL sample loop). HPLC chromatograms were recorded on a Waters 1525 Binary HPLC Pump (Milford) equipped with a Waters 2489 UV/Vis detector (Milford). Semiprep-HPLC was carried out on an Agilent series 1260 separation system (Santa Clara, CA, U.S.A.) with a fraction collector. A 1260 Sugar Determinations were carried out with a HP 5890 series 2 gas chromatograph (Santa Clara, CA, U.S.A.) [column temperature: 230°C; detector temperature: 200°C; He gas flow rate: 1 mL/min]. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was obtained from Sigma Chemical Co. (MO, U.S.A.), and hydrogen peroxide (H₂O₂) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). AGS and RAW264.7 cells were obtained from the Korea Cell Line Bank (KCLB, Seoul, Korea). Dulbecco’s modified Eagle’s medium (DMEM), Roswell Park Memorial Institute 1640 (RPMI-1640), fetal bovine serum (FBS), and penicillin/streptomycin were obtained from Welgene (Daegu, Republic of Korea). The lipopolysaccharide (LPS) used in this study was from Sigma Chemical Co. (MO, U.S.A.), and interferon-gamma (IFN-γ) was from Pepro Tech (NJ, U.S.A.). The Griess reagent, MTT, 3,3-tetramethylbenzidine (TMB), and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co.

**Extraction and Isolation**

The dried and powdered root of *P. ginseng* (7.0 kg) was extracted with EtOH (3×21 L), heated to reflux, and the extracts were combined and concentrated to afford a brown residue (139 g). The residue was dissolved in water (7 L) and partitioned successively with n-hexane (3×7 L), CHCl₃ (3×7 L), EtOAc (3×7 L), and n-BuOH (3×7 L) to give the n-hexane-soluble (50 g), chloroform (CHCl₃)-soluble (11 g), EtOAc-soluble (11 g), and n-BuOH-soluble (50 g) fractions. The n-BuOH extract (50 g) was separated by MPLC using CHCl₃–MeOH as the mobile phase (gradient: 100:0→0:100, v/v). Thirteen fractions were obtained by combing those with similar RF on TLC behavior (1→13). Fraction 4 (8 g, Vₑ/Vᵢ=0.15) was repeatedly chromatographed by MPLC using CHCl₃–MeOH (gradient: 100:0→0:100, v/v) as the mobile phase (total volume=1254 mL) to obtain 7 fractions (WGB 4.1–4.7). The combined fractions (WGB 4.5, 4.6 and 4.7; 3 g, Vₑ/Vᵢ=0.42) were separated by MPLC using CHCl₃–MeOH (gradient: 90:10→0:100, v/v) as the mobile phase (total volume=748 mL) to obtain 6 fractions (WGB 4.5.1–4.5.6). WGB 4.5.2 (920 mg, Vₑ/Vᵢ=0.12) was separated by MPLC using CHCl₃–MeOH (gradient: 75:25→100:0, v/v) as the mobile phase (total volume=2948 mL) to obtain 6 fractions (WGB 4.5.2.1–4.5.2.6). WGB 4.5.2.4 (320 mg, Vₑ/Vᵢ=0.09) was separated on a LiChroprep RP18 column (φ1.0×32 cm) using MeOH–water (gradient: 1:3→1:0, v/v) as the mobile phase (total volume=400 mL) to obtain 3 fractions (WGB 4.5.2.4.1–4.5.2.4.3). WGB 4.5.2.4.2 (290 mg, Vₑ/Vᵢ=0.01) was separated by HSCCC using EtOAc–n-BuOH–water (4:1:1:6; v/v/v) as the mobile phase to afford 3 additional fractions (WGB 4.5.2.4.2.1–4.5.2.4.2.3). WGB 4.5.2.4.2.3 was further separated by Semiprep-HPLC using ACN/water (gradient: 20:80→0:100, v/v) as the mobile phase to afford 5 additional fractions (WGB 4.5.2.4.2.3.1–4.5.2.4.2.3.5) including compound 1 (65 mg, WGB 4.5.2.4.2.3.3). Fraction 5 (8 g, Vₑ/Vᵢ=0.17) was repeatedly chromatographed by MPLC using CHCl₃–MeOH (gradient: 100:0→0:100, v/v) as the mobile phase (total volume=1932 mL) to obtain 9 fractions (WGB 5.1–5.9). WGB 5.5 (120 mg, Vₑ/Vᵢ=0.07) was separated by MPLC using CHCl₃–MeOH (gradient: 100:0→0:100, v/v) as the mobile phase (total volume=882 mL) to obtain 5 fractions (WGB 5.5.1–5.5.5). WGB 5.5.2 (10 mg, Vₑ/Vᵢ=0.10) was separated by a LiChroprep RP18 column (φ1.0×32 cm) using MeOH–water (gradient: 1:3→1:0, v/v) as the mobile phase (total volume=400 mL) to obtain 10 fractions (WGB 5.5.2.1–5.5.2.10) including compound 2 (27 mg, WGB 5.5.2.5). WGB 5.8 (320 mg, Vₑ/Vᵢ=0.01) was separated by MPLC using CHCl₃–MeOH (gradient: 80:20→0:100, v/v) as the mobile phase (total volume=3600 mL) to obtain 7 fractions (WGB 5.8.1–5.8.7). WGB 5.8.5 (55 mg, Vₑ/Vᵢ=0.02) was separated by MPLC using CHCl₃–MeOH (gradient: 80:20→0:100, v/v) as the mobile phase (total volume=2,100 mL) to obtain 10 fractions (WGB 5.8.5.1–5.8.5.10). WGB 5.8.5.3 (10 mg, Vₑ/Vᵢ=0.08) was separated with a LiChroprep RP18 column (φ1.0×32 cm) using MeOH–water (gradient: 1:3→1:0, v/v) as the mobile phase (total volume=500 mL) to obtain 10 fractions (WGB 5.8.5.3.1–5.8.5.3.10) including compound 3 (26 mg, WGB 5.8.5.3.8). WGB 5.8.6.6 (61 mg, Vₑ/Vᵢ=0.02) was separated by MPLC using CHCl₃–MeOH (gradient: 80:20→0:100, v/v) as the mobile phase (total volume=2100 mL) to obtain 10 fractions (WGB 5.8.6.1.1–5.8.6.1.10). WGB 5.8.6.4.4 (40 mg, Vₑ/Vᵢ=0.03) was separated with a LiChroprep RP18 column (φ1.0×32 cm) using MeOH–water (gradient: 1:3→1:0, v/v) as the mobile phase (total volume=680 mL) to obtain 7 fractions (WGB 5.8.6.4.1–5.8.6.4.7). WGB 5.8.6.4.2 (21 mg, Vₑ/Vᵢ=0.09) was further separated by Semiprep-HPLC using ACN–water (gradient: 20:80→0:100, v/v) to afford 6 additional fractions (WGB 5.8.6.4.2.1–5.8.6.4.2.6) including compound 4 (33 mg, WGB 5.8.6.4.2.1).
by filtration, and the filtrate was concentrated under reduced pressure. The residue was compared with standard sugars by cellulose TLC (pyridine–EtOAc–H2O–acetic acid, 36:36:7:21, v/v/v/v), and the sugars were identified as 1-rhamnopyranoside and α-glucopyranoside.

**Determination of the Absolute Configuration of Sugars in Compounds 1–4**

Compounds 1–4 (each 10 mg) were treated as previously described. The dried sugar solution was dissolved in pyridine (0.1 mL), and added to a pyridine solution (0.1 mL) of l-cysteine methyl ester hydrochloride (2 mg) followed by warming to 60°C for 1 h. The solution was evaporated under a N2 stream and the residue was dried in vacuo. The residue was trimethylsilylated with TMS-HT (0.1 mL) at 37°C for 24 h, 100 µL of culture supernatant was allowed to react with 24 h, 100 µL of culture supernatant was allowed to react with 100 µL of Griess reagent and the mixture was incubated for 24 h at 37°C, and the medium containing MTT was removed. The incorporated formazan crystals in the viable cells were solubilized with DMSO (1 mL) and the absorbance of each well was read at 540 nm.27

**Measurement of Nitrite**

Nitric oxide (NO) production was assayed by measuring the accumulation of nitrite using a microplate assay method based on the Griess reaction.28 RAW264.7 cells were seeded in 96-well plates (5×104 cells·well−1) and LPS (1 µg·mL−1) and IFN-γ (10 ng·mL−1) were added. After incubating the samples for 24 h, 100 µL of culture supernatant was added to reagent with 100 µL of Griess reagent and the mixture was incubated at room temperature for 15 min. The optical density of the samples were measured at 540 nm using a microplate reader.29

**Statistical Analysis**

Results are expressed as the mean±standard deviation (S.D.). Statistical significance was determined by Duncan’s multiple range tests using SAS software (version 6.0, SAS Institute, Cary, NC, U.S.A.). Significance was set at p<0.05.

**Compound 1**: White amorphous powder, [α]D27 +2.9 (c=0.025, MeOH); UV (MeOH): λmax 265, 281, and 337 nm (logε: 3.30, 3.45, and 3.27); IR (KBr): vmax 3343 and 1072 (hydroxyl groups and the glycosidic linkage) cm−1; 1H- and 13C-NMR (500 MHz, pyridine-d5); Table 1.

**Compound 2**: White amorphous powder, [α]D27 +75.5 (c=0.025, MeOH); UV (MeOH): λmax 262 and 289 nm (logε: 3.20, and 2.60); IR (KBr): vmax 3340 and 1073 (hydroxyl groups and the glycosidic linkage) cm−1; 1H- and 13C-NMR (500 MHz, pyridine-d5); Table 1.

**Compound 3**: White amorphous powder, [α]D27 +11.7 (c=0.1, MeOH); UV (MeOH): λmax 261 and 281 nm (logε: 3.20 and 3.50); IR (KBr): vmax 3365 and 1071 (hydroxyl groups and the glycosidic linkage) cm−1; 1H- and 13C-NMR (500 MHz, pyridine-d5); Table 1.

**Compound 4**: White amorphous powder, [α]D27 +2.4 (c=0.025, MeOH); UV (MeOH): λmax 243, 281, and 301 nm (logε: 3.00, 2.60, and 1.90); IR (KBr): vmax 3341 and 1073 (hydroxyl groups and the glycosidic linkage) cm−1; 1H- and 13C-NMR (500 MHz, pyridine-d5); Table 1.

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**Conflict of Interest**

The authors declare no conflict of interest.

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