Research article

Glycosyl glycerides from hydroponic Panax ginseng inhibited NO production in lipopolysaccharide-stimulated RAW264.7 cells

Byeong-Ju Cha 1, Ji-Hae Park 1, Sabina Shrestha 1, Nam-In Baek 1, Sang Min Lee 1, Tae Hoon Lee 1, Jiyoung Kim 1, Geum-Soog Kim 2, Seung-Yu Kim 2, Dae-Young Lee 2, *

1 Graduate School of Biotechnology, Kyung Hee University, Yongin-si, Gyeonggi-do, Korea
2 Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, Rural Development Administration, Eumseong, Chungbuk, Korea

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A B S T R A C T

Background: Although the aerial parts of hydroponic Panax ginseng are reported to contain higher contents of total ginsenosides than those of roots, the isolation and identification of active metabolites from the aerial parts of hydroponic P. ginseng have not been carried out so far.

Methods: The aerial parts of hydroponic P. ginseng were applied on repeated silica gel and octadecylsilane columns to yield four glycosyl glycerides (Compounds 1-4), which were identified based on nuclear magnetic resonance, infrared, fast atom bombardment mass spectrometry, and gas chromatography/mass spectrometry data. Compounds 1-4 were evaluated for inhibition activity on NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 cells.

Results and conclusion: The glycosyl glycerides were identified to be (2S)-3-O-β-D-galactopyranosyl-sn-glycerol (1), (2S)-1-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol (2), (2S)-1-O-linolenoyl-2-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol (3), and (2S)-1-O-linoleoyl-2-O-linoleoyl-3-O-β-D-galactopyranosyl-sn-glycerol (4). Compounds 1 and 2 showed moderate inhibition activity on NO production in LPS-stimulated RAW264.7 cells [half maximal inhibitory concentration (IC50) 63.8 ± 6.4μM and 59.4 ± 6.8μM, respectively] without cytotoxicity at concentrations < 100μM, whereas Compounds 3 and 4 showed good inhibition effect [IC50; 7.7 ± 0.6μM and 8.0 ± 0.9μM, respectively] without cytotoxicity at concentrations < 20μM. All isolated compounds showed reduced messenger RNA (mRNA) expression of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α in LPS-induced macrophage cells with strong inhibition of mRNA activity observed for Compounds 3 and 4.

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1. Introduction

Panax ginseng Meyer is a famous traditional medicinal plant belonging to the Araliaceae family. The genus name Panax originates from the word panacea, which means “a remedy for all diseases.” The 4–6-year-old roots of this perennial herbaceous plant are mainly used for medicinal purposes. P. ginseng leaves are palmate, and the flowers bloom in June. Ginseng has primarily been cultivated in the forest areas of East Asia including Korea, China, Russia, and Japan. Traditionally, P. ginseng is cultivated in soil, and numerous pharmacological and phytochemical studies of the extracts or compounds from soil-grown plants were conducted. P. ginseng contains ginsenosides, polyacetylenes, sugars, and some essential oils [1,2] used for enhancement of immunocompetence, nutritional fortification, improvement of liver function, and their anticancer, antioxidant, and antidiabetic effects [3–7]. More than 70 kinds of saponins have been isolated from P. ginseng. There is a growing interest in using safe, high-quality agricultural products, leading to hydroponic cultivation of ginseng using high-tech culture facilities. Hydroponic cultivation of ginseng takes much less...
time than soil cultivation and is accomplished in just 3–4 months in a moisture-, light-, and temperature-controlled environment without pesticide treatment. Hydroponically cultivated ginseng is mainly used in fresh and high-quality ginseng products [8,9]. The aerial parts of hydroponic P. ginseng are reported to contain higher anti-inflammatory [15–17] activities. Therefore, this study describes the isolation of four glycolipids (Compounds 1–4) from the hydroponic P. ginseng, and evaluation of their anti-inflammatory activities on NO production in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells.

2. Materials and methods

2.1. Aerial parts of hydroponic P. ginseng

Aerial parts of hydroponic P. ginseng cultivated for 4 months in an aeroponic system were obtained from the Department of Herbal Crop Research, National Institute of Horticultural and Herbal Science, RDA, Eumseong, Korea.

2.2. Reagents and instruments

Kieselgel 60 and LiChroprep RP-18 resins were used for column chromatography (Merck, Darmstadt, Germany). Kieselgel 60 F254 (Merck) and RP-18 F1545 (Merck) were used as solid phases for TLC plates. The TLC plates were divided into one fraction (Ve/Vt = 0.38–0.55, TLC Rf = 0.50 (RP-18 F1545; acetone–acetonitrile–H2O = 4:3:1)) and eight fractions (Ve/Vt = 0.50–0.58) to yield eight fractions (HPE15-12 to HPE15-12-8) including compounds 1–4. Fraction HPE15-12-1 (142.7 mg, MeOH–H2O = 4:1, 900 mL) was further fractionated to yield seven fractions (HPE15-18-1 to HPE15-18-7) including 25) a-3-oilinolenoyl-3-0-β-d-galactopyranosyl-sn-glycerol [3].

2.3. Isolation of glycosyl glycerides

The dried and powdered aerial parts of hydroponic P. ginseng (6.27 kg) were extracted with 80% MeOH (30 L × 3) at room temperature for 24 h. The extracts were filtered through a filter paper and evaporated under reduced pressure at 45°C to yield 1.4 kg of extract. The extract was poured into H2O (3 L) and then extracted with ethyl acetate (EtOAc; 3 L × 3) and n-butanol (n-BuOH; 2.6 L × 3) successively. Each layer was concentrated under reduced pressure to obtain EtOAc (75 g), n-BuOH (470 g), and H2O (855 g) fractions. The EtOAc fraction (75 g) was applied on a silica gel column (φ 14 × 16 cm) and eluted with CHCl3–MeOH (30:1, 60 L) and CHCl3–MeOH–H2O (15:3:1, 136 L) to obtain 24 fractions (HPE1 to HPE24). Fraction HPE9 (9.28 g, Ve/Vt = 0.10–0.16, where Ve refers to the volume of eluent for the corresponding fraction and Vt represents the total elution volume) was applied on a silica gel column (φ 7 × 15 cm) using n-hexane–EtOAc (1:2, 2.8 L) as eluent to obtain 13 fractions (HPE9-1 to HPE9-13). Fraction HPE9-10 (4.47 g, Ve/Vt = 0.24–0.98) was further fractionated on an octadecyl silica gel (octadesilcylane or ODS) column (φ 4.5 × 5 cm, MeOH–H2O = 15:1, 41 mL) to produce nine fractions (HPE9-10 to HPE9-10-9) including 2(S)-1-O-linoyleoyl-2-O-linoyleoyl-3-0-β-d-galactopyranosyl-sn-glycerol [4], HPE9-10-4, 141.6 mg; Ve/Vt = 0.24–0.29, TLC Rf = 0.25 (RP-18 F1545, MeOH–H2O = 50:1). Rf = 0.50 (Kieselgel 60 F254, n-hexane–EtOAc = 1:30). Fraction HPE9-10-2 (3.14 g, Ve/Vt = 0.06–0.14) was further fractionated on the ODS column (φ 4 × 6 cm, acetone–acetonitrile–H2O = 2:1:1, 3.6 L) to yield 10 fractions (HPE9-10-2–1 to HPE9-10-2–10) including (25)-1-O-linoyleoyl-2-O-linoyleoyl-3-0-β-d-galactopyranosyl-sn-glycerol [5].

25) a-3-oilinolenoyl-3-0-β-d-galactopyranosyl-sn-glycerol [1]. HPE-15-12-6, 29.4 mg, Ve/Vt = 0.14–0.28, TLC Rf = 0.30 (RP-18 F1545, MeOH–H2O = 4:1). Rf = 0.50 (Kieselgel 60 F254, CHCl3–MeOH–H2O = 10:3:1). Fraction HPE15-18 (142.7 mg, Ve/Vt = 0.50–0.58) was further fractionated on the ODS column (φ 4 × 8 cm, MeOH–H2O = 4:1, 900 mL) to yield seven fractions (HPE15-18-1 to HPE15-18-7) including (25)-a-3-oilinolenoyl-3-0-β-d-galactopyranosyl-sn-glycerol [2].

Kieselgel 60 F254 and CHCl3–MeOH–H2O = 10:3:1) was applied on a silica gel column (φ 7 × 15 cm) using n-hexane–EtOAc (1:2, 28 L) as eluent to obtain 13 fractions (HPE9-1 to HPE9-13). Fraction HPE9-10 (4.47 g, Ve/Vt = 0.24–0.98) was further fractionated on an octadecyl silica gel (octadesilcylane or ODS) column (φ 4.5 × 5 cm, MeOH–H2O = 15:1, 41 mL) to produce nine fractions (HPE9-10 to HPE9-10-9) including 2(S)-1-O-linoyleoyl-2-O-linoyleoyl-3-0-β-d-galactopyranosyl-sn-glycerol [4], HPE9-10-4, 141.6 mg; Ve/Vt = 0.24–0.29, TLC Rf = 0.25 (RP-18 F1545, MeOH–H2O = 50:1). Rf = 0.50 (Kieselgel 60 F254, n-hexane–EtOAc = 1:30). Fraction HPE9-10-2 (3.14 g, Ve/Vt = 0.06–0.14) was further fractionated on the ODS column (φ 4 × 6 cm, acetone–acetonitrile–H2O = 2:1:1, 3.6 L) to yield 10 fractions (HPE9-10-2–1 to HPE9-10-2–10) including (25)-1-O-linoyleoyl-2-O-linoyleoyl-3-0-β-d-galactopyranosyl-sn-glycerol [5].
2.4. Alkaline hydrolysis and methylation of compounds 1–4

Each compound (1.5 mg) was dissolved in 0.5 mL of 1M KOH/MeOH and heated at 80°C in a heating block for 30 min. After cooling in an icebox, 0.5 mL of 14% methanol BF₃ (Aldrich Chemistry, St. Louis, MO, USA) was added and the mixture was heated at 80°C in the heating block for 30 min. After cooling, 1 mL of n-hexane high performance liquid chromatography (HPLC) grade and 0.5 mL of H₂O (HPLC grade) were added to the reaction mixture. The supernatants were collected and a small amount of 70% w/v Na₂SO₄ was added to remove the H₂O. The solutions were filtered using a syringe filter (0.2 μm, 13 mm) and stored at –4°C until GC/MS analysis.

2.5. GC/MS analysis

A DB-5 column (0.25-μm film thickness × 0.25 mm diameter × 30 m length) was used for the GC/MS experiment. Helium was used as the carrier gas at a flow rate of 23.3 mL/min. The oven temperature was programmed as follows: 80°C for 2 min, increased to 320°C at a rate of 15°C/min and held for 10 min. The injector and detector temperatures were set at 280°C and 250°C, respectively. Sample solutions (1 μL) were injected into the GC column with a 10:1 split ratio. Detection was performed by electron ionization (70 eV) and quadrupole mass spectrometry. Fatty acids were identified by comparing their mass spectra with those of a library (Wiley Library, version 2008; John Wiley & Sons Inc., Hoboken, NJ, USA).

2.6. Cell culture

Murine macrophage RAW264.7 cells (Korea Cell Line Bank, Seoul, Korea) were cultured at 37°C in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum, 2mM glutamate, 100 units/mL of penicillin, and 100 μg/mL of streptomycin (WEL-CENE Inc, Seoul, Korea) in a humidified incubator at 5% CO₂.

2.7. Measurements of nitric oxide

The amount of NO was calculated by measuring the amount of nitrite, an oxidized product, in the cell culture supernatants as previously explained [18]. RAW264.7 cells were seeded in 96-well culture plates at a density of 1 × 10⁴ cells/well and incubated for 12–18 h. After discarding the growth medium, cells were stimulated with 1 μg/mL LPS (Sigma-Aldrich Co., St. Louis, MO, USA) in the presence of various concentrations of each compound in a serum-free medium for 20 h. Next, 100 μL of cell culture supernatant was mixed with 100 μL of Griess reagent (Sigma-Aldrich Co.) in a new 96-well plate, followed by spectrophotometric measurement at 550 nm according to the manufacturer’s instructions (BioTek Instruments, Inc., Winooski, VT, USA). Nitrite concentrations were determined by comparison with a sodium nitrite standard curve.

2.8. Viability of RAW264.7 macrophage cells

RAW264.7 cells were seeded in 96-well cell culture plates at a density of 1 × 10⁴ cells/well and incubated for 12–18 h. After discarding the growth medium, cells were treated with various concentrations of each compound in a serum-free medium for 20 h. After treatment, 10 μL of 10 μg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (Sigma-Aldrich Co.) solution was added to each well (except for the blank well), and the sample was reincubated in an incubator (at 37°C, 5% CO₂) in darkness. After 30–60 min, culture supernatants were removed, and 100 μL dimethyl sulfoxide (Sigma-Aldrich Co.) was added to completely dissolve formazan crystals. The absorbance was read at 550 nm with a spectrophotometer.

2.9. Reverse transcriptase-polymerase chain reaction analysis

RAW264.7 macrophages were treated with various concentrations of samples with 1 μg/mL of LPS for 24 h. Total RNA was prepared from RAW264.7 cells using a TRIzol Reagent kit (Invitrogen, Carlsbad, CA, USA). The total RNA (5 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Thermo Scientific, Pittsburgh, PA, USA). The following primers were used for polymerase chain reaction amplification: interleukin-1β (IL-1β): 5‘-TTC ACA GAG GAT ACC ACT CC-3’ (sense) and 5‘-GAA GCT GTG GCA GCT ATT GTC T-3’ (antisense); IL-6: 5‘-
GAG GAT ACC ACT CCC AAC AG-3′ (sense) and 5′-TTC ACA GAG GAT
ACC ACT CC-3′ (antisense); tumor necrosis factor-α (TNF-α); 5′-ATG
AGC ACA GAA AGC ATG ATC-3′ (sense) and 5′-TAC AGG CTT GTC ACT
CGA ATT-3′ (antisense); and glyceraldehyde 3-phosphate dehydro-
egenase (GAPDH): 5′-CGA CTT CAA CAG CAA CTC CCA CTC TTC C-
3′ (sense) and 5′-TCG GTG GTC CAG GGT TTC TTA CTC CTT-3′
(antisense). GAPDH messenger RNA (mRNA) levels were used as
internal controls.

2.10. Statistical analysis

Unless otherwise stated, all experiments were performed with
triplicate samples and repeated at least three times. The data were
presented as means ± standard deviation, and statistical compar-
isons between groups were performed using a one-way analysis of
variance test followed by a Student t test using SigmaPlot software
Ver.11 (San Joe, California, USA).

3. Results and discussion

Detection of Compound 1 (pale yellow wax) involved spraying
the plate with 10% sulfuric acid followed by heating. Formation of a
dark purple color confirms the presence of Compound 1. The
molecular weight was determined to be 486 from the molecule ion
peak m/z 487 [M+H]+ in the positive FAB/MS. Compound 1 showed
absorbance bands due to the hydroxyl (3,364 cm−1), carbonyl
(1,732 cm−1), and double bond (1,610 cm−1) groups in the IR
spectrum. The 1H-NMR spectrum showed six olefinic proton signals
at δH 5.37–5.46, a terminal methyl proton signal at δH 0.88, and
several unsaturated fatty acid with three double bonds. A hemiacetal
proton signal at δH 4.83 (J = 7.6 Hz) and several oxygenated methine
and methylene proton signals at δH 4.00–4.50 were also observed as
the signals of a sugar moiety. The proton signals of an oxygenated
methine at δH 4.42 (H-2), and two oxygenated methylenes at δH
4.48 (H-1), δH 4.32 (H-3a), and δH 4.05 (H-3b) due to a glycerol
moiety were also observed. Based on these results, Compound 1
was assumed to be a monoglycosyl monoglyceride. The 13C-NMR
spectrum showed the carbon signals of a hexose at δC 105.8, 77.0,
75.2, 72.5, 70.1, and 62.3, which were identified as those of a β-
galactopyranose from the chemical shifts. In addition, an
oxygenated methine carbon signal at δC 72.2 and two oxygenated meth-
ylene carbon signals at δC 69.0 and 66.5 were confirmed as the
signals of a glycerol moiety. An ester carbonyl (δC 173.4), six olefin
methine (δC 127.5, 128.2, 128.5, 128.6, 130.1 × 2, 132.1), a terminal
methyl (δC 14.3), and eight methylene (δC 20.7, 25.0, 25.9, 25.7, 27.2,
28.9, 29.4, 34.2) carbon signals were observed indicating that
Compound 1 contains a hexadecatrienoic acid as a fatty acid moi-
ety. The fatty acid methyl ester obtained by alkaline hydrolysis,
methyl esterification, and solvent fractionation appeared as a clear
peak at 13′86” on the GC/MS spectrum, which was identified as
7(Z),10(Z),13(Z)-hexadecatrienoic acid methyl ester by comparing
the mass spectrum of the peak with that of the library (Wiley Lib-
ary, version 2008). In the gradient heteronuclear multiple-bond
correlation gHMBC (Gradient Heteronuclear Multiple Bond Corre-
lation) spectrum, long-range correlations were observed between
the oxygenated methylene proton signal of glycerol H-1 (δH 0.466)
and the ester carbonyl carbon signal C-1’ (δC 173.4), and between
the anomeric proton signal H-1 (δH 4.83) and the oxygenated methine
methine carbon signal of glycerol C-3 (δC 72.2), indicating that the
fatty acid and the galactose moieties were linked to the hydroxys of
C-1 and C-3 of glycerol, respectively. Therefore, the chemical
structure of Compound 1 was determined to be (2S)-1-0-
7(Z),10(Z),13(Z)-hexadecatrienoyl-3-O-β-D-galactopyranosyl-sn-
glycerol, named as panaxcerol A (Fig. 1) [19].

Detection of Compound 2 involved spraying the plate with 10%
sulfuric acid followed by heating. Formation of a dark purple color
confirms the presence of Compound 2. The molecular weight was
determined to be 514 [M]+ from the molecule ion peak m/z 515
[M+H]+ in the positive FAB/MS. Compound 2 showed absorbance
bands due to the hydroxyl (3,364 cm−1), carbonyl (1,730 cm−1),
and double bond (1,585 cm−1) groups in the IR spectrum. The 1H-NMR
and 13C NMR spectra of Compound 2 were very similar to that of
Compound 1 with the exception of the number of methylene units.
The 1H-NMR showed six olefinic proton signals at δH 5.39–5.46,
a terminal methyl proton signal at δH 0.90, and several methylene
proton signals at δH 1.19–2.89 due to an unsaturated fatty acid with
three double bonds. A hemiacetal proton signal at δH 4.82 (d, J
= 7.6 Hz), several oxygenated methine and methylene proton
signals at δH 4.01–4.52 due to a sugar moiety, an oxygenated
methine proton signal at δH 4.43 (H-2), and two oxygenated methylene
proton signals at δH 4.48 (H-1), δH 4.32 (H-3a), and δH 4.05 (H-3b)
due to a glycerol moiety were observed. The 13C-NMR spectrum showed hexose carbon signals at δC 105.8, 77.0, 75.2, 72.5,
69.0, and 62.3 owing to a β-galactopyranosyl derivative and three
unsaturated oxygen carbon signals at δC 72.1, 70.1, and 66.5 of a glycerol,
indicating that Compound 2 was a monogalactosyl mono-
acylglyceride. An ester carbonyl (δC 173.5), six olefin methine (δC
127.5, 128.0, 128.6, 130.5 × 2, 132.0), a terminal methyl (δC
14.5), and 10 methylene (δC 20.8, 25.1, 25.8, 25.9, 27.4, 29.2,
29.3, 29.8, 34.2) carbon signals were observed indicating that
the fatty acid was an octadecatrienoic acid. The alkaline hydrolysis,
methyl esterification, and solvent partition of Compound 2 gave the
fatty acid methyl ester, which was identified to be 9(Z),12(Z),15(Z)-
octadecatrienoic acid (methyl linolenate, RT (Retention Time) = 15′20″) by GC/MS analysis. In the gHMBC spectrum, long-
range correlations were observed between the oxygenated methy-
lene proton signal of the glycerol H-1 (δH 4.51) and the ester carbonyl carbon signal C-1’ (δC 173.5), and between the anomeric
proton signal H-1’ (δH 4.82) and the oxygenated methylene carbon
signal of the glycerol C-3 (δC 72.1). These indicated that the fatty
acid and the galactose moieties were connected to the hydroxys of
C-1 and C-3 of the glycerol, respectively. Consequently, Compound
2 was identified to be (2S)-1-O-9(Z),12(Z),15(Z)-octadecatrienoyl-3-
O-β-D-galactopyranosyl-sn-glycerol, named as panaxcerol B (Fig. 1)
[20].

Fig. 1. Chemical structure of Compounds 1–4 isolated from the aerial parts of hydroponic Panax ginseng.
Detection of Compound 3 involved spraying the plate with 10% sulfuric acid followed by heating. Formation of a dark brown color confirms the presence of Compound 3. The molecular weight was determined to be 774 from the molecule ion peak m/z 775 [M+H]+ in the positive FAB/MS. Compound 3 showed absorbance bands due to the hydroxyl (3,399 cm⁻¹), carbonyl (1,737 cm⁻¹), and double bond (1,590 cm⁻¹) groups in the IR spectrum. ¹H-NMR and ¹³C-NMR spectra of Compound 3 were similar to those of Compound 2, with the exception of the integration value of the fatty acid moiety. Two ester carbonyl (δC 172.6, 172.8), 12 olefin methine (δC 127.2 × 2, 127.7 × 2, 128.2 × 2, 128.7 × 2, 128.8 × 2, 130.2 × 2, 131.7 × 2, δH 5.37–5.49), two terminal methyl (δC 14.3 × 2, δH 0.92), and 20 methylene (δC...
20.7 × 2, 25.1 × 2, 25.8 × 2, 25.9 × 2, 27.4 × 2, 29.2 × 2, 29.3 × 2, 29.4 × 2, 29.8 × 2, 34.1, 34.4, δH 1.15–2.91) carbon signals were observed, indicating Compound 3 to be a monogalactosyl diacylglyceride including two octadecatrienoic acids as fatty acid moieties. The observation of oxygenated methylene (δH 4.47, 4.64) and oxygenated methine (δH 5.61) proton signals in the lower magnetic field compared with those of Compound 2 confirmed Compound 3 to have two ester bonds at C-1 and C-2. In the gHMBC spectrum, glycerol proton signals from oxygenated methylenes, H-1a and 1b (δH 4.47, 4.64), and oxygenated methine, H-2 (δH 5.61), showed correlations with two ester carbonyl carbon C-1” and C-1’’ (δC 172.6, 172.8) signals of fatty acids. The anomeric proton signal (δH 4.64) and the oxygenated methylene carbon signal (δC 67.7) of glycerol showed correlation with each other, indicating the two fatty acids and the galactose to be linked to the hydroxys of C-1 (δC 62.9), C-2 (δC 70.6), and C-3 (δC 67.7) of glycerol, respectively. The fatty acid methyl ester obtained by chemical reaction was identified to be 9(Z),12(Z),15(Z)-octadecatrienoic acid methyl ester (methyl linolenate, RT = 15’20) by the GC/MS analysis. Based on the aforementioned data, Compound 3 was identified as a monogalactosyl diacylglyceride, (2S)-1-O-linolenoyl-2-O-linolenoyl-3-O-β-D-galactopyranosyl-sn-glycerol, and the compound was named panaxcerol C (Fig. 1) [21].

Detection of Compound 4 involved spraying the plate with 10% sulfuric acid followed by heating. Formation of a dark brown color
confirms the presence of Compound 4. The molecular weight was determined to be 778 from the molecule ion peaks at m/z 779 [M+H]+ in the positive FAB/MS. Compound 4 exhibited absorbance bands due to the hydroxyl (3,417 cm⁻¹), carbonyl (1,736 cm⁻¹), and double bond (1,595 cm⁻¹) groups in the IR spectrum. ¹H-NMR and ¹³C-NMR spectra of Compound 4 were similar to those of Compound 3, with the exception of fatty acids moieties. Compound 4 showed eight olefinic methine signals (δc 128.4 × 2, 128.7 × 2, 130.4 × 2, 130.5 × 2, δH 5.40–5.50) instead of the 12 olefinic methine signals of Compound 3. Therefore, both the fatty acids of Compound 4 were identified as octadecadienoic acid. The fatty acid methyl ester obtained by chemical reaction was identified as 9(Z), 12(Z)-octadecadienoic acid methyl ester (methyl linoleate, RT = 14′50″) by the GC/MS analysis. Based on these results, the chemical structure of Compound 4 was determined to be 2(5′)-1,0-linoleoyl-2′-0-linoleoyl-3′-0-β-D-galactopyranosyl-sn-glycerol, named panaxerol D (Fig. 1) [21].

In this study, four glycosyl glycerides were isolated from the aerial parts of hydroponic Panax ginseng and their structures were identified. The isolated glycosyl glycerides were evaluated for potential inhibition of NO production in LPS-stimulated RAW264.7 macrophage cells (Fig. 2). Compounds 1 and 2 showed half maximal inhibitory concentration (IC₅₀) values of 63.8 ± 6.4μM and 59.4 ± 6.8μM and lethal concentration (LD₅₀) values >100μM, respectively (Table 1). Compounds 3 and 4 showed IC₅₀ values of 7.7 ± 0.6μM and 8.0 ± 0.9μM and LD₅₀ values >20μM and >20μM, respectively (Table 1). Compounds 3 and 4 exhibited a greater effect than 1′-N′-monomethyl arginine, a well-known inhibitor (IC₅₀: 25.5μM). Compounds 3 and 4 also exhibited a greater effect than the naturally derived active compounds, muqobilinol (IC₅₀: 23.8μM), sigmostepeillin A (IC₅₀: 9.9μM), and ginsenoside Rh2 (IC₅₀ >50μM) from a marine sponge (Latrunculia sp.) and Panax ginseng. [22,23]. Compounds 3 and 4 have two fatty acids in the molecule, whereas Compounds 1 and 2 have one fatty acid. This molecular structure is responsible for the decrease in the polarity of Compounds 3 and 4 compared with that of Compounds 1 and 2. Because of this variation, the permeability of Compounds 3 and 4 to the cell membrane is increased and the activity or cytotoxicity to the cells is also increased. Compounds 1 and 2 had moderate inhibition on NO production in LPS-stimulated RAW264.7 cells (IC₅₀: 63.8 ± 6.4μM and 59.4 ± 6.8μM) without cytotoxicity at concentrations lower than 100μM, whereas Compounds 3 and 4 showed good inhibition (IC₅₀: 7.7 ± 0.6μM and 8.0 ± 0.9μM) without cytotoxicity at concentrations <20μM (Table 1). Proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α, are also induced as part of the inflammatory process in LPS-stimulated RAW264.7 cells. Therefore, the effects of Compounds 1–4 were investigated on the expressions of IL-1β, IL-6, and TNF-α in RAW264.7 macrophage cells treated with 1 μg/mL LPS for 24 h in the presence of various concentrations (50μM, 100μM or 10μM, 20μM) of each compounds. The anti-inflammatory activities of Compounds 1–4 were evaluated, and the results indicated that Compounds 3 and 4 inhibited the expressions of IL-1β, IL-6, and TNF-α mRNA in a concentration-dependent manner in LPS-stimulated cells, without affecting the expression of the control gene GAPDH (Fig. 3).

Our results suggest that Compounds 1–4 from the hydroponic Panax ginseng may be used as potential anti-inflammatory agents in the skin drugs or functional cosmetics industry.

Conflicts of interest

All contributing authors declare no conflicts of interest.

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