Bioorganic & Medicinal Chemistry Letters 24 (2014) 4203–4208

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Triterpene glycosides from red ginseng marc and their anti-inflammatory activities

Ill-Min Chung [a], Young-Ock Kim [b], Mohammed Ali [c], Seung-Hyun Kim [a], Inmyoung Park [d], Eun-Hye Kim [a], Ye-Sul Yang [a], Hye-Ran Park [e], Eun-Suk Son [e], Ateeque Ahmad [a,*]

[a] Department of Applied Bioscience, College of Life and Environmental Sciences, Konkuk University, Seoul 143-701, South Korea
[b] Department of Medicinal Crop Research Institute, National Institute of Horticultural and Herbal Science, RDA, Eumseong 369-873, South Korea
[c] Faculty of Pharmacy, Hamdard University, New Delhi 110062, India
[d] Department of Microbiology, College of Natural Sciences, Pusan National University, Busan 609-735, South Korea
[e] Department of Applied Biochemistry, College of Biomedical and Health Science, Konkuk University, Chungju 380-701, South Korea

ARTICLE INFO

Article history:
Received 28 January 2014
Revised 24 June 2014
Accepted 15 July 2014
Available online 22 July 2014

Keywords:
Panax ginseng
Araliaceae
Triterpene glycosides
Antiinflammatory activity

ABSTRACT

Three new triterpene glycosides ursan-3β,19α,22β-triol-3-*O*-β-D-glucopyranosyl (2′→1″)-β-D-glucopyranoside (**1**), ursan-3α,11β-diol-3-*O*-α-D-glucopyranosyl-(6′→1″)-α-D-glucopyranosyl-(6″→1‴)-α-D-glucopyranosyl-(6‴→1⁗)-α-D-glucopyranoside (**2**) and lanost-5,24-dien-3β-ol-3-*O*-β-D-glucopyranosyl-(6′→1″)-β-D-glucopyranosyl-(6″→1‴)-β-D-glucopyranoside (**3**), together with one known compound were isolated and identified from the marc of red ginseng. Their structures were elucidated by spectroscopic data analysis. Compounds (**1**–**3**) were investigated for anti-inflammatory effects using the RAW 264.7 macrophage cell line. In the cell proliferation assay, lipopolysaccharide stimulation decreased cell proliferation of RAW 264.7 macrophage cells, but the suppression of cell proliferation was significantly protected by treatment with compounds **2** and **3**. Compounds **2** and **3** had a suppressive effect on the production of nitric oxide (NO), and they inhibited mRNA expression of proinflammatory mediators such as inducible nitric oxide synthase, and cyclooxygenase-2, and proinflammatory cytokines such as two interleukins and tumor necrosis factor-α. These findings suggest that compounds **2** and **3** have potential anti-inflammatory activities.

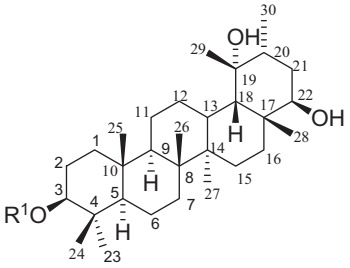
Panax ginseng C. A. Meyer (Araliaceae), is one of the best known traditional Oriental medicines, and it has been investigated extensively in the search for bioactive compounds.[1] The roots of *P. ginseng* are used in traditional medicine in Japan, mainland China and Korea and are known to produce several bioactive compounds. The glycosidic constituents, which are the principal ingredients of ginseng, have been the subject of many investigations and various ginsenosides have been characterized.[2–8] Among these, dammarane-type triterpene oligoglycosides have been identified as the principal components of white ginseng.[9–11] Of the two kinds of ginseng, white ginseng is air-dried, and red ginseng is produced by steaming raw ginseng at 98–100 °C for 2–3 h. Differences in biological activities and chemical constituents of the red and white forms of ginseng have been reported.[12]

Inflammation, a physiological response to infection, is closely associated with the release of proinflammatory mediators such as inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2), and proinflammatory cytokines such as interleukins (ILs) and Tumor necrosis factor (TNF-α).[13] Activation of macrophages plays an central role in the initiation of the inflammatory response[14] by the production of cytokines, IL-1β, TNF-α, granulocyte/macrophage colony stimulating factor (GM-CSF), nitric oxide (NO), COX-2 and other inflammatory mediators.[15] Due to their highly reproducible response to lipopolysaccharide (LPS), the RAW 264.7 mouse macrophage cell line is used widely for inflammation studies.

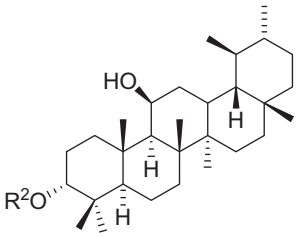
Excessive and uncontrolled production of inflammatory cytokines may lead to systemic complications such as microcirculatory dysfunction, tissue damage, and septic shock, which can exact a high mortality.[16] Over-production of NO has been related to the development of septic shock, neuropathological diseases, rheumatoid arthritis, and other autoimmune diseases.[17] It has been shown that NO can have proinflammatory (immunostimulatory, anti-apoptotic) or anti-inflammatory (immunosuppressive, pro-apoptotic) activities.[18] Herein, the isolation and structure elucidation of three new triterpene glycosides ursan-3β,19α,22β-triol-3-*O*-β-D-glucopyranosyl (2′→1″)-β-D-glucopyranoside (**1**), ursan-3α,

* Corresponding author. Tel.: +82 24503730; fax: +82 24467856.
E-mail address: ateeque97@gmail.com (A. Ahmad).

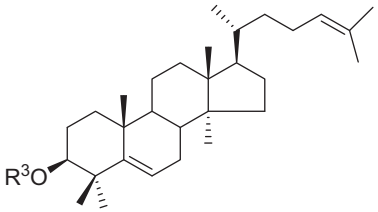
http://dx.doi.org/10.1016/j.bmcl.2014.07.042
0960-894X/



1, R^1 = -Glcβ(2'→1")Glcβ



2, R^2 = -Glcα(6'→1")Glcα(6"→1"')Glcα(6"'→1"")Glcα



3

3, R^3 = -Glcβ(6'→1")Glcβ(6"→1"')Glcβ

Figure 1. Isolated compounds **(1-3)** from *Panax ginseng*.

11β-diol-3-*O*-α-D-glucopyranosyl-(6′→1″)-α-D-glucopyranosyl-(6″→1‴)-α-D-glucopyranosyl-(6‴→1⁗)-α-D-glucopyranoside (**2**) and lanost-5,24-dien-3β-ol-3-*O*-β-D-glucopyranosyl-(6′→1″)-β-D-glucopyranosyl-(6″→1‴)-β-D-glucopyranoside (**3**) (Fig. 1) along with one known compound are reported. These compounds were evaluated for their anti-inflammatory activities using LPS-induced NO release in murine macrophage cells (RAW 264.7).

Compound (**1**)[19] was obtained as a yellow semi-solid. The IR absorption bands at 3490, 3365, and 3287 cm^{-1} indicated the presence of hydroxy groups. Its molecular ion peak at *m*/*z* 785 $[M+H]^+$ was determined on the basis of the FAB mass and ^{13}C NMR spectra, consistent with the molecular ion peak of a triterpeniod glycoside, indicating seven degrees of unsaturation. High-resolution ESIFTMS provided the exact mass of the protonated molecular ion (*m*/*z* 785.5088), from which the molecular composition $C_{42}H_{73}O_{13}$ was calculated. The ^1H NMR spectrum (Table 1) of **1** showed signals for two one-proton double doublets at δ_H 4.07 (dd, *J* = 9.0, 5.3 Hz), and 3.23 (dd, *J* = 4.2, 7.2 Hz) and these were assigned to the oxygenated methine H-3α, and H-22α protons, respectively. The sugar units in **1** were identified as β-glucopyranose by analysis of the coupling constants of the anomeric signals of the sugar protons as one-proton doublets at δ_H 4.36 (d, *J* = 8.9 Hz, H-1′), and 4.34 (d, *J* = 7.8 Hz, H-1″). The remaining sugar protons appeared as one-proton doublets at δ_H 3.62 (dd, *J* = 8.9, 8.4 Hz, H-2′) and 3.54 (dd, *J* = 7.8, 5.4 Hz, H-2″), and as one-proton multiplets that resonated between δ_H 4.02–3.35 (H-3′, H-4′, H-5′, H-3″, H-4″ and H-5″). The methylene protons in sugars appeared as double doublets at δ_H 3.21, 3.18 (dd, *J* = 7.8, 10.2 Hz), and 3.11, 3.09 (dd, *J* = 7.8, 7.8 Hz). Seven three-proton broad signals at δ_H 0.93, 0.95, 0.99, 1.01, 1.10, 1.21, 1.30 and one three proton doublet at δ_H 1.25 (d, *J* = 6.0 Hz) were due to tertiary methyls from C-23 to C-29 and secondary methyl (C-30), all attached to saturated carbons. The remaining methine and methylene protons resonated in the range δ_H 2.05–1.08. The ^{13}C NMR spectrum (Table 1) of **1** displayed 42 carbon signals, with 30 attributed to the aglycone part and 12 to disaccharide units (see Table 1). Important carbon signals appeared for anomeric carbons at δ_C 105.5 (C-1′) and 102.8 (C-1″), and the other sugar carbons resonated between δ_C 79.7 to 61.6, with oxygenated triterpeniod carbons observed at δ_C 80.8 (C-3), 71.6 (C-19) and 77.6 (C-22) and methyl carbons at δ_C 29.3 (C-23), 29.0 (C-30), 26.6 (C-28), while the other methyl carbons resonated between δ_C 22.3–16.0. The absence of a C-19 signal near δ_C 39.0 and a C-22 signal near δ_C 36.0 in the ^{13}C NMR spectrum indicated the presence of hydroxy groups on these carbons. The signal of H-2′ at δ_H 3.62 in the deshielded region of ^1H NMR spectrum and at δ_C 78.9 for C-2′ in the ^{13}C NMR spectrum suggested a (2→1) linkage of the sugar units. The absence of any signal beyond δ_H 4.36 in the ^1H NMR spectrum and δ_C 105.5 in the ^{13}C NMR spectrum supported the saturated nature of the molecule. The ^1H and ^{13}C NMR spectroscopic data of **1** were compared with the reported data for pentacyclic triterpenes.[20–24] The ^1H and ^{13}C NMR data of the sugars were also compared through computer-assisted structural analysis of polysaccharides units.[25]

The ^1H–^1H COSY spectrum of **1** showed correlations of H-3 with H_2-2, Me-23 and H-1′; H-22 with H-20, H_2-21 and Me-28; and H-2′ with H-1′, H-3′ and H-1″, while the HMBC correlations (Fig. 2) of **1** exhibited correlations of C-3 with H-1′, H_2-2 and Me-23; C-19 with H-18, H-20 and Me-29; C-22 with H_2-21 and Me-28; and C-2′ with H-3′, H-1′ and H-1″. The HSQC spectrum of **1** showed a correlation of H-3 at δ_H 4.07 with C-3 at δ_C 80.8; H-22 at δ_H 3.23 with C-22 at δ_C 77.6; H-1′ at δ_H 4.36 with C-1′ at δ_C 105.5 and H-1″ at δ_H 4.34 with C-1″ at δ_C 102.8. The NOESY spectrum of **1** showed close similarities with the reported data of similar type of compounds.[22–24] The NOESY spectrum of showed cross peaks indicating their α-disposition. Other cross peaks in NOESY spectrum H-18 with H_3-29 and H-22 with H_3-28 also indicated β-orientation. The identity glucose units of **1** were determined by comparison of the ^{13}C NMR data (Table 1) with the corresponding monosaccharides,[26] which was further confirmed by the Co-Tlc comparison with an authentic sample of D-glucose. To determine the absolute configuration, **1** was subjected to acidic hydrolysis, followed by GC analysis in comparison with D-glucose, using a literature method.[27] On the basis of these evidence, the structure of **1** was established as ursan-3β,19α,22β-triol-3-*O*-β-D-glucopyranosyl (2′→1″)-β-D-glucopyranoside.

Compound (**2**)[28] was obtained as a yellow semi-solid mass, and, on the basis of the FAB mass and ^{13}C NMR spectra, the protonated molecular ion peak was determined at *m*/*z* 1093 $[M+H]^+$, consistent with being a pentacyclic triterpene tetraglucoside. High resolution ESIFTMS provided the exact mass of the protonated molecular ion (*m*/*z* 1093.6165; $C_{54}H_{93}O_{22}$). The ^1H NMR spectrum (Table 1) of **2** showed a one-proton double doublet signal at δ_H 3.55 (dd, *J* = 2.4, 5.4 Hz), and a one-proton triple doublet signal at δ_H 3.62 (ddd, *J* = 5.4, 7.8, 9.6 Hz), which were ascribed to the H-3β and H-11α carbinol protons, respectively. The sugar units in **2** were identified as α-glucopyranose by analysis of the coupling constants of the anomeric signals as four one-proton doublets at δ_H 4.33 (d, *J* = 5.4 Hz, H-1′), 4.42 (d, *J* = 6.0 Hz, H-1″), 4.66 (d, *J* = 3.5 Hz, H-1‴) and 4.70 (d, *J* = 3.1 Hz, H-1⁗). The remaining sugar protons appeared as multiplets and resonated between δ_H 4.20–3.31. The methylene protons in the sugars appeared as double doublets at δ_H 3.87, 3.85 (dd, *J* = 11.4, 10.2 Hz), 3.82, 3.80 (dd, *J* = 9.6, 9.6 Hz), 3.66, 3.63 (dd, *J* = 6.8, 7.8 Hz), and 3.26, 3.22 (dd, *J* = 9.0, 8.4 Hz).