Supporting Information

Taraxasterane-Type Triterpene and Neolignans from *Geum japonicum* Thunb.

var. *chinense* F. Bolle

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Experimental Section

Extraction and isolation
Assay for inhibitory activity against LPS-induced NO production in RAW264.7 macrophages
Extraction and isolation

Powdered air-dried whole plants of *G. japonicum* Thunb. var. *chinense* (15.0 kg) were percolated with 95% EtOH at room temperature (45L × 5 h × 3). The combined filtrates were concentrated under *vacuo* to afford a residue extract (2.0 kg), which was suspended in water (4.0 L) and partitioned with petroleum ether (PE, 60–90 ºC, 30 L), *CH₂Cl₂* (45 L), EtOAc (40 L), and *n*-BuOH (45 L), successfully. The PE extract (158.2 g) was submitted to column chromatography using silica gel (100–200 mesh, 1.5 kg, 10 × 100 cm) and eluting with a step gradient of PE-EtOAc (100:0, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, each 20 L) to give 15 fractions (Fr.1–Fr.15). The white deposition of Fr.4 (50:1, 5.3 g) in PE was further filtered to yield *n*-tritriacontanol ([41], 38.0 mg). Fr.6 (20:1, 9.0 g) was chromatographed on silica gel (200–300 mesh, 180 g, 6 × 30 cm) eluting with PE-EtOAc (30:1), followed by Sephadex LH-20 (3 × 100 cm) eluting with *CH₂Cl₂*-MeOH (1:1) to give 26 (8.0 mg) and β-sitosterol ([18], 88.3 mg). Fr.7 (20:1, 17.3 g) and Fr.8 (10:1, 20.0 g) were subjected to MCI gel column (4 × 40 cm) eluting with 80% MeOH to give subfractions Fr.7A and Fr.8A, respectively. Fr.7A (5.1 g) was chromatographed on silica gel (200–300 mesh, 300 g, 6 × 30 cm) with *CH₂Cl₂*-MeOH (1:1), followed by Sephadex LH-20 (3 × 100 cm) to yield ursolic acid ([43], 4.5 mg), and α-tocoquinone ([44], 152.3 mg). Fr.8A (9.3 g) was submitted to silica gel column (200–300 mesh, 180 g, 6 × 30 cm) with PE-EtOAc (15:1) to give palmitic acid ([39], 53.0 mg) and linoleic acid ([40], 17.2 mg). The *CH₂Cl₂* extract (135.7 g) was submitted to column chromatography using silica gel (100–200 mesh, 1.2 kg, 10 × 90 cm) and eluting with a step gradient of *CH₂Cl₂*-MeOH (100:0, 100:1, 50:1, 20:1, 10:1, 5:1, 2:1, 1:1, each 20 L) to give ten fractions (Fr.16–Fr.25). Fr.16 (100:0, 0.6 g) was chromatographed over Sephadex LH-20 (3 × 100 cm) eluting with *CH₂Cl₂*-MeOH (1:1) to yield 20β,28-epoxy-28-hydroxy-taraxasteran-3β-ol ([1], 3.0 mg), (9Z,11E)-13-hydroxy-9,11-octadecadienoic acid ethyl ester ([43], 4.5 mg), and α-tocoquinone ([44], 152.3 mg). Fr.7A (5.1 g) was chromatographed over Sephadex LH-20 (3 × 100 cm) eluting with *CH₂Cl₂*-MeOH (1:1) to yield 20β,28-epoxy-28-hydroxy-taraxasteran-3β-ol ([1], 3.0 mg), (9Z,11E)-13-hydroxy-9,11-octadecadienoic acid ethyl ester ([43], 4.5 mg), and α-tocoquinone ([44], 152.3 mg). 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Fr.17 (100:1, 4.4 g) was chromatographed on silica gel (200–300 mesh, 90 g, 6 × 15 cm) eluting with *CH₂Cl₂*-MeOH (100:1), followed by Sephadex LH-20 (3 × 100 cm) with *CH₂Cl₂*-MeOH (1:1) to afford ursolic acid (7, 5.0 mg), 3′-O-methyl-3,4-methylenedioxy ellagic acid ([36], 398.0 mg), and loliolide ([15], 60.0 mg). Fr.18 (50:1, 10.1 g) was chromatographed on silica gel (200–300 mesh, 200 g, 6 × 35 cm) eluting with *CH₂Cl₂*-MeOH (50:1), followed by Sephadex LH-20 (3 × 100 cm, *CH₂Cl₂*-MeOH, 1:1) and RP-HPLC (20% MeOH, 70% MeOH) to give 1β,2α,19α-trihydroxy-3-oxo-12-ursen-28-oic acid ([13], 65.0 mg), isololiolide ([16], 3.0 mg), *p*-hydroxycinnamic acid ([27], 22.0 mg), 3-O-trans-p-coumaroyl tormentic acid ([9], 8.0 mg). The pigment of Fr.19 (20:1, 19.8 g) was removed by MCI gel column (4 × 40 cm) eluting with 80% MeOH, then followed by silica gel column (200–300 mesh, 200 g, 6 × 35 cm) with *CH₂Cl₂*-MeOH (30:1) and Sephadex LH-20 (3 × 100 cm) with *CH₂Cl₂*-MeOH (1:1) to give 3,3′-di-O-methyllellagic acid ([34], 7.0 mg), *p*-hydroxybenzoic acid ([30], 17.6 mg), progallin A ([32], 140.0 mg), and querectin ([22], 2.1 mg). Fr.20 (10:1, 14.6 g) was chromatographed on silica gel (200–300 mesh, 300 g, 6 × 70 cm) eluting with *CH₂Cl₂*-MeOH (10:1) to give tormentic acid ([8], 314.0 mg) and daucosterol ([19], 230.0 mg). Fr.21 (10:1, 39.9 g) was submitted to silica gel column (200–300 mesh, 800 g, 10 × 30 cm) eluting with *CH₂Cl₂*-MeOH (10:1) to give six subfractions (Fr.21A–Fr.21F). Fr.21D (0.1 g) was chromatographed over Sephadex LH-20 (1.5 × 120 cm, *CH₂Cl₂*-MeOH, 1:1), followed by RP-HPLC (55% MeOH) to give tiliroside ([24], 28.8 mg, *t* _R_ 36.7 min) and cis-tiliroside ([25], 2.0 mg, *t* _R_ 38.1 min). Fr.21E (0.4 g) was chromatographed on silica gel column (200–300 mesh, 8 g, 4 × 10 cm) with *CH₂Cl₂*-MeOH (10:1) and Sephadex LH-20 (3 × 100 cm) with *CH₂Cl₂*-MeOH (1:1) to give 2α,3β,19α,23-tetrahydroxy-12-ursen-28-oic acid ([11], 0.2 g), arjunetin ([14], 28.4 mg), agrimonolide
6-O-β-D-glucoside (20), 13.9 mg), amburoside A (21, 2.6 mg), megastigman-7-ene-3,5,6,9-tetrol (17, 11.6 mg), and kaempferol 3-O-β-D-glucopyranoside (23, 3.0 mg). Fr.21F (235.0 mg) was chromatographed over Sephadex LH-20 (1.5 × 120 cm, CH2Cl2-MeOH, 1:1), followed by RP-HPLC (45% MeOH) to give compound 2 (8.4 mg, tR 26.6 min), compound 3 (3.0 mg, tR 29.7 min), 28-β-D-glucoside-2α,3β,19α-trihydroxy-12-ursen-28-oic acid (10, 30.0 mg, tR 45.1 min), 3,3′-di-O-methylellagic acid-4′-O-β-D-glucopyranoside (36, 3.0 mg, tR 34.7 min), and 3′-O-methyl-3,4-O-metheneellagic acid-4′-O-β-D-glucopyranoside (38, 4.2 mg, tR 39.5 min). The EtOAc extract (130.0 g) was submitted to column chromatography using Diaion HP-20 resin and eluting with a gradient of EtOH-H2O (6 × 50 cm, 90:10, 70:30, 50:50, 30:70, each 15 L) to give four fractions (Fr.26–Fr.29). Fr.28 (70:30, 3.0 g) was chromatographed over Sephadex LH-20 (3 × 100 cm) eluting with MeOH, followed by RP-HPLC (45% MeOH) to give compound 4 (7.7 mg, tR 32.1 min), cupressoside A (5, 11.2 mg, tR 33.6 min), compound 6 (4.2 mg, tR 35.2 min), and eLLagic acid (33, 38.3 mg, tR 44.0 min). Fr.27 (50:50, 28.0 g) was further purified to afford nigaichigoside F1 (12, 8.0 g). Fr.26 (30:70, 10.3 g) was chromatographed over Sephadex LH-20 (3 × 100 cm) with MeOH to afford gallic acid (31, 29.0 mg) and sanguin H-4 (38, 11.5 mg). The purities of all isolates (> 95.0%) were analyzed by HPLC.

Assay for inhibitory activity against LPS-induced NO production in RAW264.7 macrophages

RAW264.7 macrophages grown on 100 mm culture dish were harvested and seeded in 96-well plates at 2 × 10^5 cells/well for NO production. The plates were pretreated with various concentrations of samples for 30 min and then incubated for 24 h with or without 1 μg/mL of LPS, and aminoguanidine (Sigma–Aldrich, purity ≥ 98.0%) was used as a positive control. Nitrite concentration in the culture supernatant was measured by the Griess reaction. Cell viability was measured by a MTT [3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide] assay (Sigma–Aldrich). Statistical calculations were carried out with the SPSS 13.0 for Windows software package. Results are expressed as the mean ± SEM of 3 independent experiments.
Legends for Figures and Tables

Fig. 1S Structures of the other known compounds from *G. japonicum* Thunb. var. *chinense* F. Bolle
Fig. 2S $^1$H NMR spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 3S $^{13}$C NMR spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 4S DEPT NMR spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 5S HSQC spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 6S $^1$H–$^1$H COSY spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 7S HMBC spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 8S NOESY spectrum of compound 1 (DMSO-$d_6$, 500 MHz)
Fig. 9S $^1$H NMR spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 10S $^{13}$C NMR spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 11S DEPT NMR spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 12S HSQC spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 13S $^1$H–$^1$H COSY spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 14S HMBC spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 15S NOESY spectrum of compound 2 (CD$_3$OD, 500 MHz)
Fig. 16S $^1$H NMR spectrum of compound 3 (CD$_3$OD, 500 MHz)
Fig. 17S $^{13}$C NMR spectrum of compound 3 (CD$_3$OD, 500 MHz)
Fig. 18S HSQC spectrum of compound 3 (CD$_3$OD, 500 MHz)
Fig. 19S HMBC spectrum of compound 3 (CD$_3$OD, 500 MHz)
Fig. 20S NOESY spectrum of compound 3 (CD$_3$OD, 500 MHz)
Fig. 21S CD curves of compounds 2 and 3

Table 1S Effect of 17 compounds against lipopolysaccharide-induced NO production in RAW264.7 macrophages (IC$_{50}$, $\mu$M)
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Fig. 18S HSQC spectrum of compound 3 (CD$_3$OD, 500 MHz).

Fig. 19S HMBC spectrum of compound 3 (CD$_3$OD, 500 MHz).
Fig. 20S NOESY spectrum of compound 3 (CD<sub>3</sub>OD, 500 MHz).

Fig. 21S CD curves of compounds 2 and 3. (The negative Cotton effect at 242.0 nm suggested an 8R configuration, while the positive Cotton effect at 235.5 nm indicated an 8S configuration.).
Table 1S  Effect of 17 compounds against lipopolysaccharide-induced NO production in RAW264.7 macrophages (IC\textsubscript{50} µM)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC\textsubscript{50} (a)</th>
<th>Compounds</th>
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\(a\) Data are expressed as mean ± SEM (n = 3).

\(b\) Positive control.