Supporting Information

Antiviral Triterpenoid Saponins from the Roots of *Ilex asprella*

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Extraction and isolation

Air-dried roots (3.0 kg) of *Ilex asprella* were ground and extracted under reflux with methanol (3 × 15 L, 2 h each). The MeOH extract was combined and concentrated under reduced pressure to afford a residue (177 g). The residue was absorbed on silica gel (200-300 mesh, 250 g) and fractionated by a silica gel column chromatography (CC) (1500 g, 100-200 mesh, 10 × 100 cm) eluting with CHCl₃-MeOH-H₂O (85:15:1, 80:20:2.5, 75:25:5, 70:30:5, 60:40:10, each 10 L) to afford five fractions (1-5). Fraction 1 (10 g) was chromatographed over a silica gel column (200-300 mesh, 5 × 80 cm) eluting with CHCl₃-MeOH-H₂O in a gradient system (90:10:0, 85:15:1, 80:20:2.5, 500 mL each) as guided by TLC analysis and further purified by MCI-gel CHP20P (75-100 μm, 2 × 60 cm) eluting with MeOH-H₂O (0:5, 5:95, 10:90, 15:85, 20:80, 200 mL each) and Sephadex LH-20 (2 × 60 cm) eluting with MeOH-H₂O (0:5, 5:95, 10:90, each 150 mL) to afford 6 (15 mg) and 4 (12 mg). Fraction 2 (25 g) was subjected to CC over silica gel (200-300 mesh, 5 × 80 cm) eluting with CHCl₃-MeOH-H₂O (85:15:1, 80:20:2.5, 75:25:5, 1 L each) and further purified by Rp-18 (40-60 μm, 1.5 × 50 cm) eluting with MeOH-H₂O (55:45, 60:40, 100 mL each) and Sephadex LH-20 eluting with MeOH-H₂O (15:85, 20:80, 100 mL each) to afford 2 (30 mg), 5 (3 mg), 3 (12 mg), 8 (2 mg), 9 (18 mg), 10 (12 mg), 11 (34 mg), and 12 (25 mg). Similarly, fraction 5 (50 g) was separated by silica gel CC (200-300 mesh, 8 × 75 cm) eluted by CHCl₃-MeOH-H₂O (85:15:1, 80:20:2.5, 75:25:5, 70:30:5, 60:40:10, 1.5 L each) and further purified by Rp-18 (40-60 μm, 1.5 × 50 cm) eluting with MeOH-H₂O (55:45, 60:40, 100 mL each), MCI-gel CHP20P (75-100 μm, 2 × 60 cm) eluting with MeOH-H₂O (35:65, 40:60, 100 mL each), and Sephadex LH-20 (2 × 60 cm) eluting
with MeOH-H$_2$O (30:70, 35:65, 15:85, 100 mL each) to afford compounds 1 (14 mg) and 7 (16 mg).

**Cell lines and biochemistry**

African green monkey kidney cells (Vero, ATCC CCL81), provided by Wuhan Institute of Virology, Chinese Academy of Sciences, were propagated in Dulbecco’s modified Eagle's medium (DMEM; Gibco) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco). The constituents of the maintenance medium were the same as those of the growth medium except that only 2% FBS was added. The cells were cultured at 37 °C in a humid atmosphere with 5% CO$_2$. HSV-1 strain F (ATCC VR-733), obtained from Hong Kong University, was propagated in Vero cells and stored at -80 °C until use.

**Cytotoxicity assay**

The cytotoxicity of the test compounds on Vero cells was determined by an MTT assay. Vero cells in the logarithmic growth phase were trypsinized and diluted with medium to 2 × 105 cells/mL, and then 100 μL of this cell suspension solution was added to each well of a 96-well tissue culture plate. After a 24-h period of incubation at 37 °C in a humid atmosphere with 5% CO$_2$, the supernatant was removed. Then the test compounds were diluted with test medium to various concentrations (100 - 1.56 μmol/L), and 100 μL of this diluted solution was placed in the 96-well plate. After another 72 h of incubation, the MTT solution and DMSO were added. The optical
densities (OD) were measured with an enzyme immunoassay (EIA) reader (BIO-RAD 550) at a test wavelength of 570 nm and a reference wavelength of 630 nm. The toxicity concentrations of test compounds were investigated and recorded. Maximal nontoxic concentration was used for the HSV-1 inhibition assay. Aciclovir, a positive control purchased from Sigma (purity ≥ 99%), was dissolved in maintenance medium before use.

**Anti-HSV-1 assay**

Anti-HSV-1 activities of compounds 1-4, 6-7, and 9-12 were assayed with the plaque reduction assay, with aciclovir as the positive control. The Vero cells were seeded into a 96-well plate and allowed to form a monolayer. After removing the growth medium, 50 μL viral suspensions and the same volume of samples of different concentrations (samples were diluted with cell sustainable medium) were added to each well. Then each well was overlaid with medium containing 1% of methylcellulose, and the plate was incubated for 3 days. Thereafter, the cell monolayer was fixed and stained with formalin and crystal violet, respectively. The viral plaques were counted under a binocular microscope. The concentration reducing plaque formation by 100% relative to control was estimated from graphic plots and defined as 100% inhibitory concentration. Aciclovir, a positive control, was dissolved in maintenance medium before use.

**Acid hydrolysis of compounds 1 and 2**
Compounds 1 and 2 (each 5 mg) were hydrolyzed with 2 M HCl/dioxane (1:1, 4 mL) under reflux for 8 h, respectively. The reaction mixture was partitioned between H₂O and CHCl₃ (2 mL × 3). The aqueous layer was neutralized with 2 M NaOH and then dried to give a monosaccharide mixture. A solution of the sugar mixture in pyridine (2 mL) was added to L-cysteine methyl ester hydrochloride (about 1.5 mg) and kept at 60 °C for 1 h. Then trimethylsilylimidazole (about 1.5 mL) was added to the reaction mixture and kept at 60 °C for 30 min. The mixture was subjected to GC analysis, run on an HP5890 gas chromatograph (Agilent) with a quartz capillary column (30 mm × 0.32 mm × 0.25 μm): column temp 180-280 °C at 3 °C/min, carrier gas N₂ (1 mL/min), injector and detector temp 250 °C, split ratio 1:50. The configurations of D-glucose and D-xylose for compound 1 were determined by comparison of the retentions times of the corresponding derivatives with standard D-glucose and D-xylose, giving single peaks at 19.2 and 13.7 min, respectively. Compound 2 was determined by comparison of the retentions times of the corresponding derivatives with standard L-arabinose giving single peaks at 14.0 min. The configuration of the sugar moiety from compound 1 was D-glucose and D-xylose, and compound 2 was L-arabinose.
Fig. 1S $^1$H NMR spectrum of asprellanoside A (1) (CD$_3$OD, 400 MHz).
$^{13}$C NMR spectrum of asprellanoside A (1) (CD$_3$OD, 100 MHz).
Fig. 3S HSQC spectrum of asprellanoside A (1) in CD$_3$OD.
Fig. 4S HMBC spectrum of asprellanoside A (1) in CD$_3$OD.
Fig. 5S $^1$H-$^1$H COSY spectrum of asprellanoside A (1) in CD$_3$OD.
Fig. 6S $^1$H NMR spectrum of asprellanoside B (2) (C$_5$D$_5$N, 100 MHz).
Fig. 7S $^{13}$C NMR spectrum of asprellanoside B (2) (C$_5$D$_5$N, 100 MHz).
Fig. 8S HSQC spectrum of asprellanoside B (2) in C$_5$D$_5$N.
Fig. 9S HMBC spectrum of asprellanoside B (2) in C$_5$D$_5$N.
Fig. 10S $^{1}$H-$^{1}$H COSY spectrum of asprellanoside B (2) in C$_{5}$D$_{5}$N.