Supporting Information

New Triterpenoids from the Tubers of Corydalis ternata: Structural Elucidation and Bioactivity Evaluation

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**Materials and Methods**

**General experimental procedures**

Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Jasco P-1020 polarimeter (Jasco). IR spectra were recorded on a Bruker IFS-66/S FT-IR spectrometer (Bruker). Fast-atom bombardment (FAB) and HR-FAB mass spectra were obtained on a JEOL JMS700 mass spectrometer (JEOL). Nuclear magnetic resonance (NMR) spectra, including $^1$H-$^1$H COSY, HMQC, HMBC, and NOESY experiments, were recorded on a Varian UNITY INOVA 500 NMR spectrometer (Varian) operating at 500 MHz ($^1$H) and 125 MHz ($^{13}$C), with chemical shifts given in ppm ($\delta$). Preparative high performance liquid chromatography (HPLC) used a Gilson 306 pump (Gilson) with a Shodex refractive index detector (Shodex). Silica gel 60 (Merck, 70-230 mesh and 230-400 mesh) and RP-C$_{18}$ silica gel (Merck, 230-400 mesh) were used for column chromatography. The packing material for molecular sieve column chromatography was Sephadex LH-20 (Pharmacia). Merck precoated Silica gel F$_{254}$ plates and RP-18 F$_{254s}$ plates (Merck) were used for TLC. Spots were detected on TLC under UV light or by heating after spraying with 10% H$_2$SO$_4$ in C$_2$H$_5$OH (v/v).

**Plant material**

The tubers of *C. ternata* were collected in Jinju, Gyeongsangnam-do, Korea, in May, 2009, and the plant was identified by one of the authors (J.H.L.). A voucher specimen (SKKU 2009-5) has been deposited in the herbarium of the School of Pharmacy, Sungkyunkwan University, Suwon, Korea.

**Extraction and isolation**

The tubers of *C. ternata* (11 kg) were dried, powdered, and extracted with 80% aqueous MeOH two times (2 × 48 h) at room temperature, and filtered. The filtrate was evaporated
under vacuum to obtain a MeOH extract (750 g), 250 g of which was suspended in distilled H₂O (7.2 L), and then partitioned with CHCl₃ after pre-treatment with 1 N of hydrochloric acid (HCl) and partitioned with CHCl₃ after pre-treatment with 1 N of ammonium hydroxide (NH₄OH) successively, yielding CH-fraction (14.5 g) and CN-fraction (3 g), respectively. The CH-fraction (14.5 g) was separated on a silica gel column (230-400 mesh, 450 g, 6x90 cm) using a solvent gradient of CHCl₃-MeOH (10:1, 5:1, and 1:1, 1 L of each solvent), to yield seven fractions (CH1 – CH7). Fraction CH3 (250 mg) was separated further on Sephadex LH-20 (100 g, 3x90 cm) using a solvent system of CH₂Cl₂-MeOH (1:1, 500 mL) to afford six sub-fractions (CH31 – CH36). The sub-fraction CH34 (150 mg) was purified further by semi-preparative HPLC, using a solvent system of MeOH-H₂O (3:1) over 30 min at a flow rate of 2.0 mL/min (Econosil RP-18 10μ column; 250 × 10 mm; Shodex refractive index detector) to give 1 (45 mg, Rₜ = 15.5 min) and 2 (5 mg, Rₜ = 17.0 min).

**Acidic hydrolysis of 1-2**

Compound 1 (17 mg) was hydrolyzed in 5% HCl solution (MeOH-H₂O, 2:8) under reflux for 3 h. After cooling, the reaction mixture was partitioned with EtOAc and washed with H₂O, and the EtOAc fraction (9 mg) was evaporated in vacuo and chromatographed over a silica gel Waters Sep-Pak Vac 6 cc (CHCl₃-MeOH, 18:1) to give the aglycone, coryternic acid 3 (7 mg). After neutralization of the aqueous fraction by passage through an Amberlite IRA-67 column, the fraction was repeatedly evaporated in vacuo to give a sugar fraction (8 mg). The sugar of the fraction was analyzed by silica gel TLC and developed with EtOAc-MeOH-H₂O-AcOH (13:6:3:3). The Rₜ value (0.44) of the product was identical to that of D-glucuronic acid (authentic sample). Finally, the measurement of optical rotation of glucuronic acid obtained from 1 confirmed the sugar to be D-configuration; D-glucuronic acid, [α]²⁵D : +8.5 (c 0.1, H₂O) [6, 7]. In the same manner, acidic hydrolysis of 2 (3 mg) was carried out. After
separation from the sugar fraction (1.5 mg), 6′-O-methyl-β-D-glucuronopyranoside for 2 was identified by its $^1$H NMR data [8] and optical rotation value; $[\alpha]_{25}^{25}D : +9.7 (c 0.05, H_2O)$ [6, 7].

**Cytotoxicity testing**

A sulforhodamine B (SRB) bioassay was used to determine the cytotoxicity of each compound against four cultured human cancer cell lines. The assays were performed at the Korea Research Institute of Chemical Technology. The cell lines used were A549 (non-small cell lung carcinoma), SK-OV-3 (ovary malignant ascites), SK-MEL-2 (skin melanoma), and HCT-15 (colon adenocarcinoma). Doxorubicin (purity ≥98%, Sigma) was used as a positive control.

**Measurement of cell viability and NO production**

Dulbecco’s modified Eagle medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin (PS) were purchased from Invitrogen. Lipopolysaccharide (LPS) and N-monomethyl L-arginine (NMMA) were obtained from Sigma Chemical Company.

Cells were maintained in DMEM supplemented with 5% FBS and 1% PS. To measure NO production, BV-2 cells were plated into a 96-well plate ($3 \times 10^4$ cells/well) and treated with 100 ng/mL of LPS in the presence or absence of compounds 1-3 for 24 h. Nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulfanilamide, 0.1% N-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using a microplate reader. Sodium nitrite was used as a standard to calculate NO$^2-$ concentration. N-monomethyl-L-arginine (NMMA, purity ≥98%; Sigma), a well known NOS inhibitor, was used as a positive control. Cell viability was measured by a 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay. After incubation with compounds for 24 h, the medium was removed.
Cells were incubated with MTT (0.1 mg/mL) for 1 h at 37 °C. Absorbance was measured at 540 nm using a microplate reader.
NMR spectra of compounds 1-3

Fig. 1S $^1$H NMR spectrum of 1 (CD$_3$OD, 500 MHz).

Fig. 2S $^{13}$C NMR spectrum of 1 (CD$_3$OD, 125 MHz).
Fig. 3S DEPT spectrum of 1.

Fig. 4S HMQC spectrum of 1.
Fig. 5S HMBC spectrum of 1.

Fig. 6S NOESY spectrum of 1.
Fig. 7S $^1$H NMR spectrum of 2 (CD$_3$OD, 500 MHz).

Fig. 8S $^{13}$C NMR spectrum of 2 (CD$_3$OD, 125 MHz).
Fig. 9S DEPT spectrum of 2.

Fig. 10S HMQC spectrum of 2.
Fig. 11S HMBC spectrum of 2.

Fig. 12S NOESY spectrum of 2.
**Fig. 13S** $^1$H NMR spectrum of 3 (CD$_3$OD, 500 MHz).

**Fig. 14S** $^{13}$C NMR spectrum of 3 (CD$_3$OD, 125 MHz).