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

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Abstract

Two new triterpene glycosides, coryternic acid 3-*O*-*β*-D-glucuronopyranoside (**1**) and coryternic acid 3-*O*-*β*-D-glucuronopyranoside-6′-*O*-methyl ester (**2**), were isolated from a MeOH extract of the tubers of *Corydalis ternata*. Acidic hydrolysis of **1** and **2** yielded a new triterpene as their aglycone, coryternic acid (**3**). The structures of these new compounds were determined through spectral analysis, including extensive 2D-NMR data. In this study we reported that triterpenoids were first isolated from the genus *Corydalis*. Compound **2** exhibited significant cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} = 15.16, 17.07, 13.32, and 11.95 μM, respectively) and significantly reduced NO production in lipopolysaccharide (LPS)-activated microglia/BV-2 cells with an IC_{50} value of 16.2 μM without cell toxicity.

Key words

Corydalis ternate · Papaveraceae · triterpenes · cytotoxicity · neuroinflammation

Abbreviations

SRB: sulforhodamine B
LPS: lipopolysaccharide
NO: nitric oxide

Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

The tubers of *Corydalis ternata* (Papaveraceae) have been used in the traditional Korean medicine for the treatment of spasms and gastric ulcers [1]. *C. ternata*, well known as *Corydalis* tuber in Korea, contains alkaloids such as berberine, coptisine, and protopine as its main chemical constituents [1, 2]. Among them, protopine was reported to decrease the glutamate level and increase the glutamate dehydrogenase (GDH) activity in the brains of rats [2]. In continuation of the search for bioactive constituents from Korean medicinal plant sources, we investigated a MeOH extract of the tubers of *C. ternata* for its cytotoxic potential based on the fact that the extract showed considerable cytotoxicity against four human cancer cell lines in screening procedures [3]. We had reported the isolation of the cytotoxic alkaloids from the tubers of *C. ternata*, recently [3]. In the process of our continuing efforts to study this source, we further isolated two new triterpene glycosides (**1**–**2**), in addition to the identification of a new triterpene as their aglycone (**3**), from the MeOH extract of this plant (**Fig. 1**) and evaluated the cytotoxicities of **1**–**3**. Moreover, we also tested the inhibitory activities of **1**–**3** on neuroinflammation in the LPS-activated microglia/BV-2 cell line.

Compound **1** was obtained as a white powder. The molecular formula was established as $C_{37}H_{56}O_{11}$ from the $[M + H]^+$ peak at *m*/*z* 677.3904 (calcd. for $C_{37}H_{57}O_{11}$: 677.3901) in the HR-FAB-MS. The IR spectrum indicated that **1** possesses hydroxy (3383 cm^{-1}), carbonyl (1666 cm^{-1}), and C=C double bond (1642 cm^{-1}) functional groups. The ^1H and ^{13}C NMR spectra of **1** (**Tables 1** and **2**) were similar to those of serratagenic acid [4], except for the presence of an additional OCH_3 group [δ_H 3.70; δ_C 50.9] and one set of resonances attributable to a *β*-glucuronopyranosyl unit [δ_H 4.38 (d, J = 7.5 Hz); δ_C 171.2, 105.5, 76.3, 76.3, 73.9, and 71.8] in **1** [5, 6]. Comparison of the ^{13}C NMR data of **1** with that of serratagenic acid showed the downfield shift of C-3 (+ 11.5) and upfield shift of C-2 (− 2.5) in **1**, indicating glycosylation at C-3. This linkage was confirmed by the HMBC correlation between H-1′ (δ_H 4.38) and C-3 (δ_C 89.6). HMBC correlations of H-30 (δ_H 1.13)/C-29 (δ_C 177.3) and *O*-CH_3 (δ_H 3.70)/C-29 (δ_C 177.3) indicated that the OCH_3 group was located at C-29, and the presence of a carboxyl group at C-28 was verified by the HMBC correlation of H-18 (δ_H 2.70)/C-28 (δ_C 179.8) (**Fig. 2**). The relative configuration of **1** was confirmed to be identical to that of serratagenic acid in the NOESY spectrum. The *β*-orientation of OH-3 was deduced from the correlations of H-3/H_3-23 and H-3/H-5 in the NOSEY spectrum, and the methyl ester group at C-29 was reconfirmed by the NOSEY correlations of H-18/H_3-30 and *O*-CH_3/H_3-27 (**Fig. 2**). Acidic hydrolysis of **1** yielded the aglycone **3** and D-glucuronic acid. Extensive studies of the 1D and 2D NMR spectra of **3** led to the identification of a new triterpene as the aglycone, 3*β*-hydroxy-olean-12-ene-28,29-dioic acid 29-methyl ester (**3**, cory-

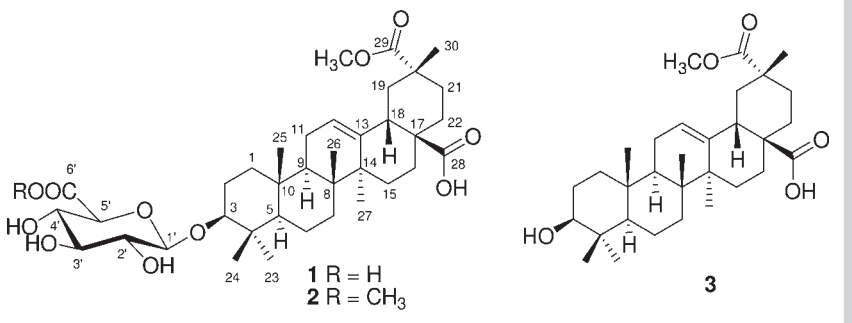

Fig. 1 Chemical structures of compounds **1**–**3**.

Table 1 ^{1}H NMR spectral data of compounds **1–3**[a].

H	1	2	3
1	1.48 m, 1.34 m	1.47 m, 1.33 m	1.48 m, 1.30 m
2	1.75 m, 1.45 m	1.77 m, 1.45 m	1.76 m, 1.40 m
3	3.17 dd (11.5, 3.5)	3.17 dd (12.0, 3.5)	3.15 dd (11.5, 5.0)
5	0.80 m	0.81 m	0.75 m
6	1.55 m, 1.36 m	1.57 m, 1.34 m	1.58 m, 1.34 m
7	1.62 m, 1.36 m	1.62 m, 1.33 m	1.63 m, 1.34 m
9	1.61 m	1.61 m	1.62 m
11	1.92 m, 1.69 m	1.91 m, 1.67 m	1.98 m, 1.69 m
12	5.30 br t (1.5)	5.29 br t (1.5)	5.30 br t (3.5)
15	1.57 m, 1.13 m	1.58 m, 1.13 m	1.59 m, 1.11 m
16	1.63 m, 1.33 m	1.63 m, 1.32 m	1.64 m, 1.32 m
18	2.70 dd (13.5, 3.5)	2.70 dd (13.5, 3.5)	2.70 dd (14.0, 4.0)
19	1.88 m, 1.63 m	1.89 m, 1.63 m	1.92 m, 1.64 m
21	1.92 m, 1.66 m	1.91 m, 1.65 m	1.95 m, 1.66 m
22	1.87 m, 1.61 m	1.88 m, 1.61 m	1.90 m, 1.63 m
23	1.05 s	1.04 s	0.97 s
24	0.85 s	0.84 s	0.80 s
25	0.95 s	0.94 s	0.94 s
26	0.80 s	0.80 s	0.77 s
27	1.17 s	1.17 s	1.16 s
30	1.13 s	1.13 s	1.13 s
29-OCH_3	3.70 s	3.69 s	3.69 s
GluA1′	4.38 d (7.5)	4.38 d (8.0)	
2′	3.24 t (8.5)	3.24 t (9.0)	
3′	3.37 t (9.0)	3.34 t (9.0)	
4′	3.51 t (9.0)	3.51 t (9.0)	
5′	3.77 br d (8.5)	3.82 d (9.5)	
6′-OCH_3		3.77 s	

[a] ^{1}H NMR run at 500 MHz in CD_3OD. Chemical shifts are given in δ values. Proton coupling constants (*J*) in Hz are given in parentheses

Table 2 ^{13}C NMR spectral data of compounds **1–3**[a].

C	1	2	3
1	38.3 t	38.3 t	38.6 t
2	25.5 t	25.5 t	26.6 t
3	89.6 d	89.6 d	78.5 d
4	38.7 s	38.7 s	39.3 s
5	55.5 d	55.5 d	55.5 d
6	17.9 t	17.8 t	18.3 t
7	32.6 t	32.5 t	32.8 t
8	39.1 s	39.1 s	39.3 s
9	48.4 d	48.2 d	48.3 d
10	36.5 s	36.4 s	36.9 s
11	22.8 t	22.8 t	23.0 t
12	122.8 d	122.8 d	123.0 d
13	143.3 s	143.3 s	143.5 s
14	41.3 s	41.3 s	41.5 s
15	27.4 t	27.4 t	27.6 t
16	23.1 t	23.0 t	23.3 t
17	45.6 s	45.6 s	45.8 s
18	42.5 d	42.5 d	42.8 d
19	41.9 t	41.9 t	42.1 t
20	43.6 s	43.6 s	43.8 s
21	29.9 t	29.8 t	30.1 t
22	33.6 t	33.6 t	33.8 t
23	27.1 q	27.0 q	27.4 q
24	15.5 q	15.5 q	15.1 q
25	14.5 q	14.5 q	14.7 q
26	16.5 q	16.2 q	16.4 q
27	25.0 q	24.8 q	25.1 q
28	179.8 s	179.8 s	180.0 s
29	177.3 s	177.3 s	177.6 s
30	27.3 q	27.2 q	27.5 q
29-OCH_3	50.9 q	50.8 q	51.1 q
GluA1′	105.5 d	105.6 d	
2′	73.9 d	73.8 d	
3′	76.3 d	76.0 d	
4′	71.8 d	71.7 d	
5′	76.3 d	75.2 d	
6′	171.2 s	169.9 s	
6′-OCH_3		51.3 s	

[a] ^{13}C NMR run at 125 MHz in CD_3OD. Chemical shifts are given in δ values

ternic acid). D-Glucuronic acid was detected by TLC and identified based on its optical rotation value [6,7]. Thus, **1** was elucidated as 3*β*-*O*-(*β*-D-glucuronopyranosyl)-olean-12-ene-28,29-dioic acid 29-methyl ester (coryternic acid 3-*O*-*β*-D-glucuronopyranoside).

Compound **2** was obtained as a white powder with the molecular formula $C_{38}H_{58}O_{11}$ based on the $[M + Na]^+$ peak at *m*/*z* 713.3884 (calcd. for $C_{38}H_{58}NaO_{11}$: 713.3877) in the HR-FAB-MS. The ^{1}H and ^{13}C NMR spectra of **2** (**Tables 1** and **2**) were very similar to those of **1**. The ^{1}H NMR spectrum of **2** displayed the signal for an additional methoxy group (δ_H 3.77). The sugar carbon data (**Table 2**) revealed a noticeable difference in the δ value of C-6′ (−1.3) in comparison to the corresponding value in the sugar unit of **1**. This evidence, together with the observation of the HMBC correlation between *O*-CH_3 (δ_H 3.77) and C-6′ (δ_C 169.9), suggested that compound **2** contains a 6′-*O*-methyl-*β*-glucuronopyranoside [8]. Acidic hydrolysis and spectral analysis of **2** confirmed the presence of the 6′-*O*-methyl-*β*-D-glucuronopyranoside from the ^{1}H NMR coupling constant (*J* = 8.0 Hz) and its optical rotation value [6–8]. The relative configuration of **2** was confirmed to be identical to that of **1** in the NOESY spectrum. Thus, **2** was characterized as 3*β*-*O*-(6′-*O*-methyl-*β*-D-glucuronopyranosyl)-olean-12-ene-28,29-dioic acid 29-methyl ester (coryternic acid 3-*O*-*β*-D-glucuronopyranoside-6′-*O*-methyl ester). To the best of our knowledge, this is the first study to report triterpenoids being isolated from the genus *Corydalis*.

In this study, the cytotoxicities of **1–3** against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines were evaluated using the sulforhodamine B (SRB) bioassay *in vitro* [9]. The results

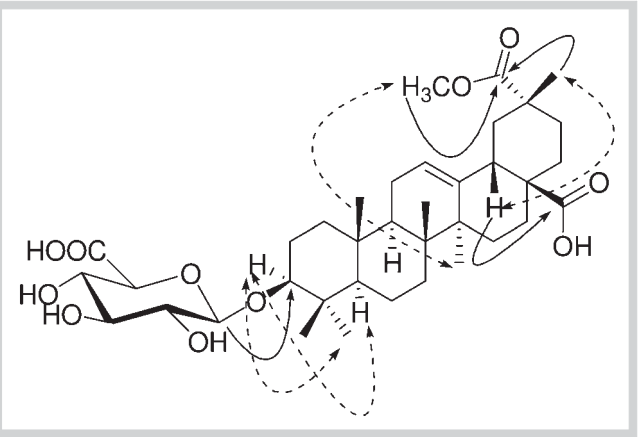

Fig. 2 Key HMBC (→) and NOESY (⇢) correlations of **1**.

Table 3 Cytotoxicity of compounds **1**–**3** against four cultured human cancer cell lines using the SRB assay *in vitro*.

Compound	IC_{50} (μM)[a]			
	A549	SK-OV-3	SK-MEL-2	HCT-15
1	> 30.0	> 30.0	19.02	20.83
2	15.16	17.07	13.32	11.95
3	> 30.0	> 30.0	28.71	28.64
Doxorubicin[b]	0.01	0.01	0.01	0.02

[a] IC_{50} value of compounds against each cancer cell line, which was defined as the concentration (μM) that caused 50% inhibition of cell growth *in vitro*. [b] Doxorubicin as a positive control

of this assay (**Table 3**) showed that compound **2** exhibited significant cytotoxicity against the A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines (IC_{50} = 15.16, 17.07, 13.32, and 11.95 μM, respectively). Neuroinflammation has been implicated in various neurodegenerative disorders such as Alzheimer's and Parkinson's disease [10–12]. The inhibitory effects of **1**–**3** on neuroinflammation were also evaluated by assessing cell viability and nitric oxide (NO) production in LPS-activated BV-2 cells. As shown in **Fig. 3**, compound **2** effectively inhibited the NO production with an IC_{50} value of 16.2 μM without significant cell toxicity. Compound **3** also reduced NO levels in the medium; however, it showed very minimal cell toxicity. Compound **2** might be a potential natural agent for the treatment of various tumors and for the improvement of neurodegenerative diseases through suppression of neuroinflammation in the brain.

Materials and Methods

Coryternic acid 3-O-β-D-glucuronopyranoside (**1**): white powder; mp. 214–215 °C; $[\alpha]_D^{25}$: + 58.3 (*c* 0.25, MeOH); IR (KBr): ν_{max} = 3383, 2947, 2835, 1666, 1642, 1454, 1026 cm^{-1}; FAB-MS: *m/z* = 677 $[M + H]^+$; HR-FAB-MS: *m/z* = 677.3904 $[M + H]^+$ (calcd. for $C_{37}H_{57}O_{11}$: 677.3901). ^1H NMR, see **Table 1** and ^{13}C NMR, see **Table 2**.

Coryternic acid 3-O-β-D-glucuronopyranoside-6′-O-methyl ester (**2**): white powder; mp. 217–218 °C; $[\alpha]_D^{25}$: + 64.2 (*c* 0.07, MeOH); IR (KBr): ν_{max} = 3385, 2947, 2835, 1667, 1641, 1453, 1027 cm^{-1};

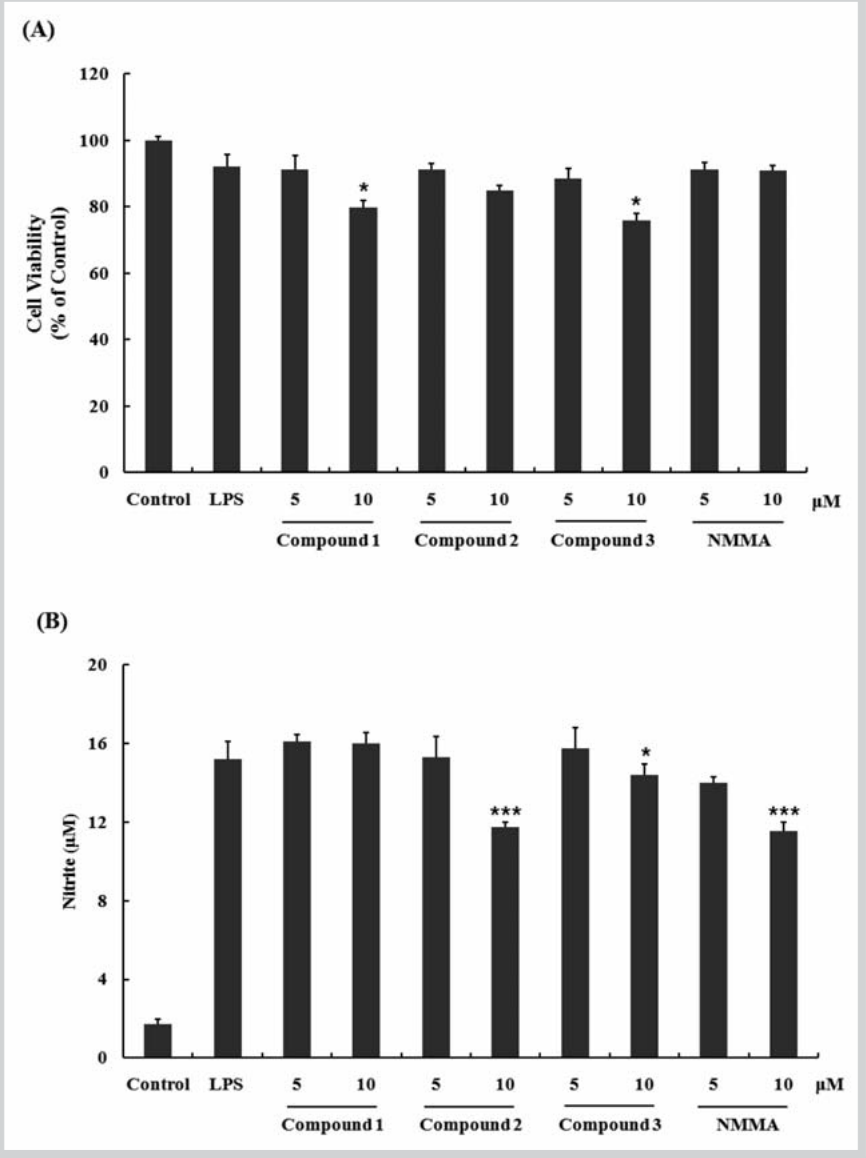

Fig. 3 The effect of compounds **1**–**3** on cell viability (**A**) and NO production (**B**) in LPS-activated BV-2 cells. Cell viability was detected by MTT assay, and NO production was measured by nitrite assay using a Griess reaction. NOS inhibitor, *N*-monomethyl-L-arginine (NMMA), was used as a positive control in this study. All data are presented as the mean ± SEM of three independent experiments. * $P < 0.05$ and *** $p < 0.001$ indicate significant differences compared to treatment with LPS alone.

FAB-MS: m/z = 713 $[M + Na]^+$; HR-FAB-MS: m/z = 713.3884 $[M + Na]^+$ (calcd. for $C_{38}H_{58}NaO_{11}$: 713.3877). ^{1}H NMR, see **Table 1** and ^{13}C NMR, see **Table 2**.

Coryternic acid (**3**): white powder; mp. 292–293 °C; $[\alpha]_D^{25}$: + 81.0 (*c* 0.30, MeOH); IR (KBr): ν_{max} = 3386, 2945, 2835, 1667, 1637, 1454, 1027 cm^{-1}; FAB-MS: m/z = 501 $[M + H]^+$; HR-FAB-MS: m/z = 501.3585 $[M + H]^+$ (calcd. for $C_{31}H_{49}O_5$: 501.3580). ^{1}H NMR, see **Table 1** and ^{13}C NMR, see **Table 2**.

A detailed description of the bioassays is available as Supporting Information. The positive controls, doxorubicin (purity ≥ 98 %) and *N*-monomethyl-L-arginine (NMMA, purity ≥ 98 %) were purchased from Sigma Corporation.

Supporting information

The spectral data of compounds **1**–**3**, the general experimental procedures, the isolation details, and details regarding the acidic hydrolysis of **1**–**2** and bioassays protocols are available as Supporting Information.

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