

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 (IC₅₀ = 15.16, 17.07, 13.32, and 11.95 μ M, respectively) and significantly reduced NO production in lipopolysaccharide (LPS)-activated microglia/BV-2 cells with an IC₅₀ value of 16.2 μ M without cell toxicity.

Key words

Corydalis ternata · 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 proto-pine as its main chemical constituents [1,2]. Among them, proto-pine 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 C₃₇H₅₆O₁₁ from the [M + H]⁺ peak at *m/z* 677.3904 (calcd. for C₃₇H₅₆O₁₁: 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 ¹³C NMR spectra of **1** (● Tables 1 and 2) were similar to those of serratagenic acid [4], except for the presence of an additional OCH₃ 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 ¹³C 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-CH₃ (δ_{H} 3.70)/C-29 (δ_{C} 177.3) indicated that the OCH₃ 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/H₃-23 and H-3/H-5 in the NOESY spectrum, and the methyl ester group at C-29 was reconfirmed by the NOESY correlations of H-18/H₃-30 and O-CH₃/H₃-27 (● 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-

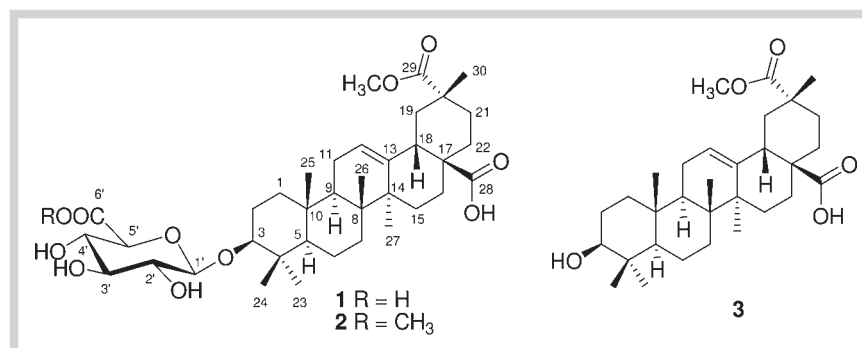


Fig. 1 Chemical structures of compounds **1–3**.

Table 1 ^1H 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 ^1H NMR run at 500 MHz in CD₃OD. Chemical shifts are given in δ values. Proton coupling constants (J) in Hz are given in parentheses

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

Compound **2** was obtained as a white powder with the molecular formula C₃₈H₅₈O₁₁ based on the $[\text{M} + \text{Na}]^+$ peak at m/z 713.3884 (calcd. for C₃₈H₅₈NaO₁₁: 713.3877) in the HR-FAB-MS. The ^1H and ^{13}C NMR spectra of **2** (● Tables 1 and 2) were very similar to those of **1**. The ^1H 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-CH₃ (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 ^1H 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 $3\beta\text{-O-(6'-O-methyl-}\beta\text{-D-glucuronopyranosyl)-olean-12-ene-28,29-dioic acid 29-methyl ester}$ (coryternic acid $3\text{-O-}\beta\text{-D-glucuronopyranoside-6'-O-methyl ester}$). 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

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 CD₃OD. Chemical shifts are given in δ values

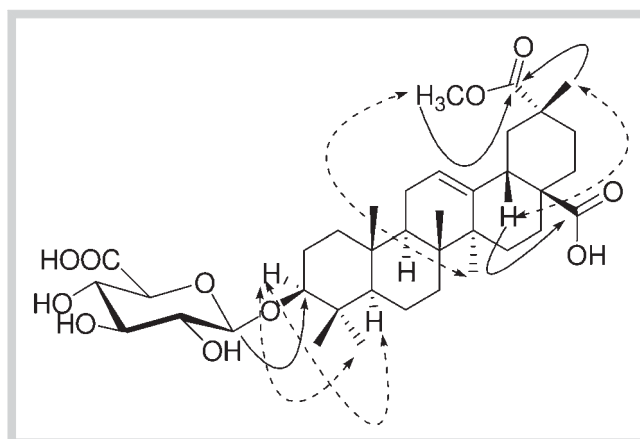
**Fig. 2** Key HMBC (→) and NOESY (↔) correlations of **1**.

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 IC₅₀ 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 (IC₅₀ = 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 ni-

tric oxide (NO) production in LPS-activated BV-2 cells. As shown in Fig. 3, compound 2 effectively inhibited the NO production with an IC₅₀ 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; [α]_D²⁵: +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 C₃₇H₅₇O₁₁: 677.3901). ¹H NMR, see Table 1 and ¹³C NMR, see Table 2.

Coryternic acid 3-O-β-D-glucuronopyranoside-6'-O-methyl ester (2): white powder; mp. 217–218 °C; [α]_D²⁵: +64.2 (c 0.07, MeOH); IR (KBr): ν_{max} = 3385, 2947, 2835, 1667, 1641, 1453, 1027 cm⁻¹;

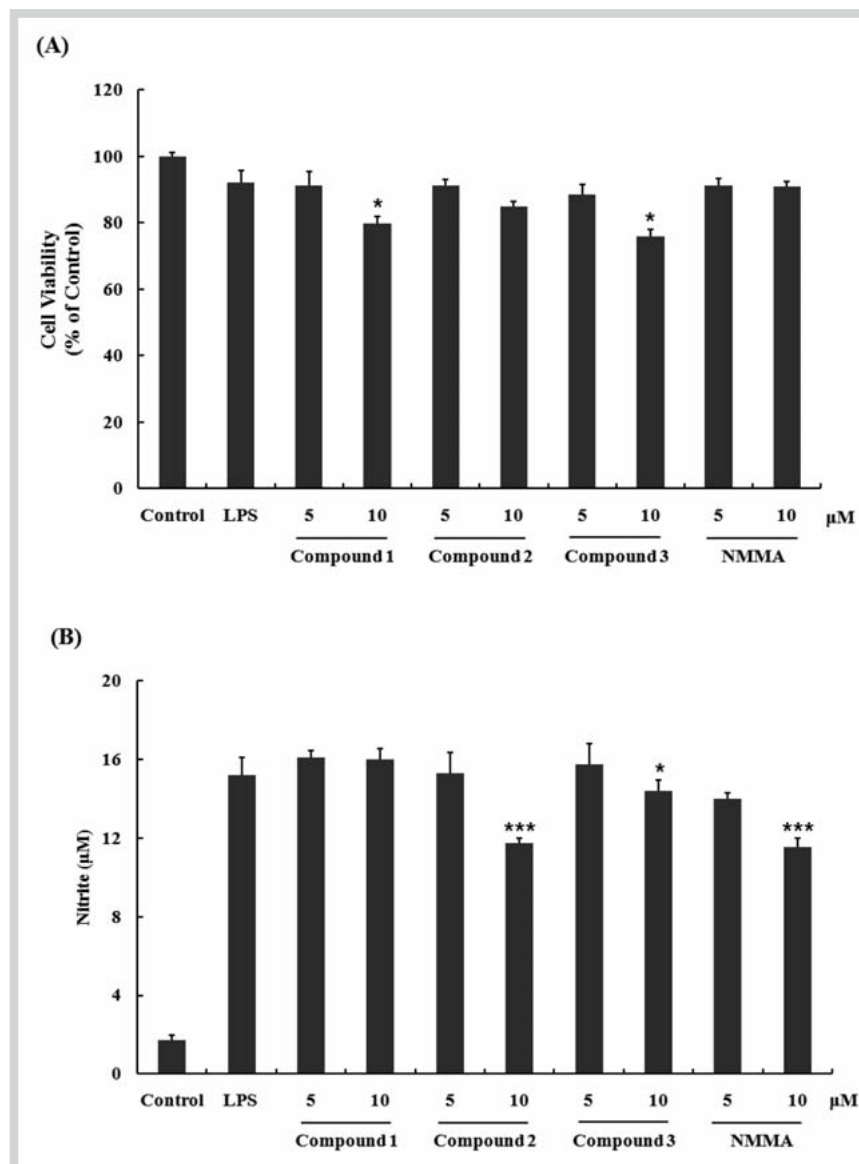


Fig. 3 The effect of compounds 1–3 on cell viability (A) and NO production (B) in LPS-activated BV-2 cells. Cell viability was detected by MTT assay, and NO production was measured by nitrite assay using a Griess reaction. NOS inhibitor, *N*-monomethyl-L-arginine (NMMA), was used as a positive control 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). 1H 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): $\nu_{max} = 3386, 2945, 2835, 1667, 1637, 1454, 1027$ 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). 1H 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 $\geq 98\%$) and *N*-monomethyl-L-arginine (NMMA, purity $\geq 98\%$) 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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References

- Lee HY, Kim CW. Isolation and quantitative determination of berberine and coptisine from tubers of *Corydalis ternata*. Korean J Pharmacognosy 1999; 30: 332–334
- Lee KH, Huh JW, Choi MM, Yoon SY, Yang SJ, Hong HN, Cho SW. Regulation of glutamate level in rat brain through activation of glutamate dehydrogenase by *Corydalis ternata*. Exp Mol Med 2005; 37: 371–377
- Kim KH, Lee IK, Piao CJ, Choi SU, Lee JH, Kim YS, Lee KR. Benzylisoquinoline alkaloids from the tubers of *Corydalis ternata* and their cytotoxicity. Bioorg Med Chem Lett 2010; 20: 4487–4490
- Yu SS, Yu DQ, Liang XT. Triterpenoid saponins from the bark of *Nothopanax davidii*. Phytochemistry 1995; 38: 695–698
- Lee KT, Choi J, Jung WT, Nam JH, Jung HJ, Park HJ. Structure of a new echinocystic acid bisdesmoside isolated from *Codonopsis lanceolata* roots and the cytotoxic activity of prosapogenins. J Agric Food Chem 2002; 50: 4190–4193
- Kim KH, Choi JW, Choi SU, Lee KR. Terpene glycosides and cytotoxic constituents from the seeds of *Amomum xanthioides*. Planta Med 2010; 76: 461–464
- Tapondjou AL, Miyamoto T, Lacaille-Dubois MA. Glucuronide triterpene saponins from *Bersama engleriana*. Phytochemistry 2006; 67: 2126–2132
- Melek FR, Miyase T, El-Gindi OD, Abdel-Khalik SM, Haggag MY. Saponins from *Fagonia mollis*. Phytochemistry 1996; 42: 1405–1407
- Skehan P, Stroheng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990; 82: 1107–1112
- McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Rev 1995; 21: 195–218
- Stoll G, Jander S, Schroeter M. Inflammation and glial responses in ischemic brain lesions. Prog Neurobiol 1998; 56: 149–171
- Kim YS, Joh TH. Microglia, major player in the brain inflammation: their roles in the pathogenesis of Parkinson's disease. Exp Mol Med 2006; 38: 333–347

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Bibliography

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