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

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Abstract

Two new triterpene glycosides, coryteneric acid 3-O-β-D-glucuronopyranoside (1) and coryteneric acid 3-O-β-D-glucuronopyranosylide-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 µM, respectively) and significantly reduced NO production in lipopolysaccharide (LPS)-activated microglia/BV-2 cell line.

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 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 C37H56O11 from the [M + H]+ peak at m/z 677.3904 (calcd. for C37H57O11: 677.3901) in the HR-ESI-MS. The IR spectrum indicated that 1 possesses hydroxy (3383 cm−1), carboxyl (1666 cm−1), and C=C double bond (1642 cm−1) functional groups. The 1H and 13C NMR spectra of 1 (Figures 1 and 2) were similar to those of serratagenic acid [4], except for the presence of an additional OCH3 group [δH 3.70; δC 50.9] and one set of resonances attributable to a β-glucuronopyranosyl unit [δH 4.38 (d, J = 7.5 Hz); δC 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-28 (−2.5) in 1, indicating glycosylation at C-3. This linkage was confirmed by the HMBC correlation between H-1′ (δH 4.38) and C-3 (δC 89.6). HMBC correlations of H-30 (δH 1.13)/C-29 (δC 177.3) and O-CH3 (δH 3.70)/C-29 (δC 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 (δH 2.70)/C-28 (δC 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 NOSEY spectrum, and the methyl ester group at C-29 was reconfirmed by the NOESY correlation 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, 3β-hydroxy-olean-12-ene-28,29-dioic acid 29-methyl ester (3, cory-
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 method [6]. The results confirmed that compounds 1–3 exhibited cytotoxic activities against the tested cell lines, with compound 1 displaying the highest cytotoxicity against all the cell lines tested. The relative configuration of compound 2 was confirmed to be identical to that of 1 in the NOESY spectrum. Thus, 2 was characterized as 3β-O-(6′-O-methyl-β-D-glucuronopyranosyl)-olean-12-ene-28,29-dioic acid 29-methyl ester (coryternic acid 3′-β-glucuronopyranoside). 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 were evaluated against the A549, SK-OV-3, SK-MEL-2, and HCT15 human cancer cell lines. The results indicated that all the compounds exhibited cytotoxic activities against the tested cell lines, with compound 1 displaying the highest cytotoxicity against all the cell lines tested. The relative configuration of compound 2 was confirmed to be identical to that of 1 in the NOESY spectrum. Thus, 2 was characterized as 3β-O-(6′-O-methyl-β-D-glucuronopyranosyl)-olean-12-ene-28,29-dioic acid 29-methyl ester (coryternic acid 3′-β-glucuronopyranoside). To the best of our knowledge, this is the first study to report triterpenoids being isolated from the genus Corydalis.
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 nitric 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.

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>A549 (µM)</th>
<th>SK-OV-3</th>
<th>SK-MEL-2</th>
<th>HCT-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;30.0</td>
<td>&gt;30.0</td>
<td>19.02</td>
<td>20.83</td>
</tr>
<tr>
<td>2</td>
<td>15.16</td>
<td>17.07</td>
<td>13.32</td>
<td>11.95</td>
</tr>
<tr>
<td>3</td>
<td>&gt;30.0</td>
<td>&gt;30.0</td>
<td>28.71</td>
<td>28.64</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*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. Doxorubicin as a positive control.

### 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): νmax = 3383, 2947, 2835, 1666, 1642, 1544, 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): νmax = 3385, 2947, 2835, 1667, 1453, 1027 cm⁻¹;
**FAB-MS**: m/z = 713 [M + Na]+; HR-FAB-MS: m/z = 713.3884 [M + Na]+ (calcd. for C38H58NaO11: 713.3877). 

**1H NMR**, see Table 1 and **13C NMR**, see Table 2.

**Coryternic acid (3)**: white powder; mp. 292–293 °C; [α]D25: +81.0 (c 0.30, MeOH); IR (KBr): νmax = 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 C31H49O5: 501.3580). 

**1H NMR**, see Table 1 and **13C 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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**References**

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