

## Notes

### Chemical Constituents of the Rhizome of *Eleutherine bulbosa* and Their Inhibitory Effect on the Pro-Inflammatory Cytokines Production in Lipopolysaccharide-Stimulated Bone Marrow-derived Dendritic Cells

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Inflammatory responses, initiated by the invasion of pathogens or by tissue injury caused by free radicals, are a series of vascular and cellular reactions. Some important chemical mediators of inflammation are interleukin (IL)-1, -6, -12, and tumor necrosis factor- $\alpha$ , prostaglandins, etc... IL-6 has pro- and anti-inflammatory properties. IL-6 is involved not only in the activation of the immune system but also in regenerative process as well as in the regulation of metabolism.<sup>1</sup> The IL-12 family of cytokines is key players in the regulation of T cell responses. IL-12 has both early pro-inflammatory and late anti-inflammatory effects.<sup>2</sup> In addition to these, TNF- $\alpha$  is a well-characterized pro-inflammatory cytokine released primarily from monocytes and macrophages upon invasion of the host by a wide variety of pathogens. It plays a crucial role in host defense and in the inflammatory response. Although it has numerous beneficial roles in immune regulation, it has also been implicated in the pathogenesis of both acute and chronic inflammatory disease.<sup>3</sup>

Since ancient times, traditional medicines and phyto-pharmaceuticals have been used for the treatment of inflammatory and other disorders. Natural products offer great hope in the identification of bioactive compounds and their development into drugs for the treatments of inflammatory diseases. One of the well-known drugs, aspirin was discovered based on the known analgesic and antipyretic properties of the bark of willow-tree since 400 BC.<sup>4</sup> Recently, we have focused on a number of medicinal plants with anti-inflammatory activities and found some of them to possess the anti-inflammatory active compounds such as *Acanthopanax koreanum*<sup>5</sup> and *Hedychium coronarium*.<sup>6</sup>

*Eleutherine bulbosa* (Miller) Urb. is an herbal medicinal plant from Iridaceae family. This plant is used in oriental

medicine for the treatment of diseases such as heart failure, cancer, intestinal disorders, skin disease, and infertility.<sup>7</sup> Previous phytochemical investigation of *E. bulbosa* has resulted in the identification of some aromatic compounds and their glycosides such as eleutherinone, eleutherine, isoeleutherine, eleutherol,<sup>8</sup> (*R*)-4-hydroxyeleutherin, eleuthone, isoeleuthoside C, eleutherinol 8-O- $\beta$ -D-glucoside.<sup>9</sup>

In the course of screening of medicinal plants for anti-inflammatory activities, we found the methanol extract of the rhizome of *E. bulbosa* potently inhibit the lipopolysaccharide (LPS)-stimulated productions of IL-12 p40 and IL-6 cytokines in bone marrow-derived dendritic cells (DCs) with IC<sub>50</sub> values of 0.1  $\pm$  0.05 and 16.2  $\pm$  0.3  $\mu$ g/mL, respectively (Table 2). SB203580, an inhibitor of cytokine suppressive binding protein/p38 kinase, was used as a positive control. SB203580 inhibited IL-12 p40 and IL-6 production with IC<sub>50</sub> values of 2.5  $\pm$  0.1 and 1.7  $\pm$  0.2  $\mu$ g/mL, respectively. The methanol extract of the *E. bulbosa* rhizome was then fractionated with chloroform, ethyl acetate, and water. From these fractions and using combined chromatographic separations, one new and fourteen known compounds were isolated.

Compound **1** was obtained as a pale yellow powder. Its basic ion peak at *m/z* 419 [M-H]<sup>-</sup> was observed on negative-ion ESI-MS, and HR-ESI-MS analysis revealed the molecular formula to be C<sub>21</sub>H<sub>24</sub>O<sub>9</sub>, with a cluster ion peak at *m/z* 419.1338 [M-H]<sup>-</sup> (calcd for C<sub>21</sub>H<sub>23</sub>O<sub>9</sub>, 419.1342). The <sup>1</sup>H-NMR spectrum of **1** (in CD<sub>3</sub>OD) showed the following signals: a tertiary methyl group at  $\delta$ <sub>H</sub> 2.45, a secondary methyl group at  $\delta$ <sub>H</sub> 1.46 (d, *J* = 6.1 Hz), three singlet aromatic protons at  $\delta$ <sub>H</sub> 6.45, 6.67, and 6.85, and an anomeric proton at  $\delta$ <sub>H</sub> 4.93 (Table 1). The <sup>13</sup>C-NMR and DEPT data of **1** reveal-

**Table 1.** The NMR spectroscopic data for compound **1**

Pos.	<b>1</b>	
	$\delta_C^{a,b}$	$\delta_H^{a,c}$ (mult., <i>J</i> in Hz)
Aglycone		
1a	163.4	–
2	78.0	4.87 (ddq, 4.0, 6.1, 12.0)
3	45.9	2.57 (dd, 4.0, 16.8), 2.62 (dd, 12.0, 16.8)
4	194.3	–
4a	114.6	–
5	137.6	–
6	124.2	6.85 (s)
6a	141.1	–
7	103.6	6.67 (s)
8	161.4	–
9	103.5	6.45 (s)
10	158.7	–
10a	109.9	–
2-Me	20.7	1.46 (d, 6.1)
5-Me	23.3	2.45 (s)
8-O-Glc		
1'	101.7	4.93 (d, 7.2)
2'	74.8	3.40*
3'	78.0	3.47*
4'	71.4	3.31 (d, 8.5)
5'	78.3	3.42*
6'	62.5	3.62 (dd, 5.6, 12.2), 3.83 (dd, 2.2, 12.2)

<sup>a</sup>recorded in CD<sub>3</sub>OD. <sup>b</sup>400 MHz. <sup>c</sup>100 MHz. \*overlapped signals

ed 21 carbon signals, 15 of which were assigned to be a dihydronaphthopyrone moiety and the remaining 6 assigned to a monosaccharide moiety. The aglycone of **1** was concluded to be dihydroeleutherinol (**1a**).<sup>10</sup> The NMR data of **1** were similar to those of eleutherinoside A (**3**)<sup>11</sup> except for the disappearance of a double bond in the  $\gamma$ -pyrone ring. The HMBC correlations from H-2 ( $\delta_H$  4.87) to C-1a ( $\delta_C$  163.4), C-3 ( $\delta_C$  45.9), C-4 ( $\delta_C$  194.3), and 2-Me ( $\delta_C$  20.7); from H-3 ( $\delta_H$  2.57 and 2.62) to C-2 ( $\delta_C$  78.0), C-4 ( $\delta_C$  194.3), and 2-Me ( $\delta_C$  20.7) (see Figure 2) suggested that the methyl and carbonyl groups were at C-2 and C-4 of the dihydropyrone ring, respectively. On the other hand, HMBC correlations between 5-Me ( $\delta_H$  2.45) and C-4a ( $\delta_C$  114.6), C-5 ( $\delta_C$  137.6), and C-6 ( $\delta_C$  124.2), between H-7 ( $\delta_H$  6.67) and C-6 ( $\delta_C$  124.2), C-8 ( $\delta_C$  161.4), C-9 ( $\delta_C$  103.5), and C-10a ( $\delta_C$  109.9), between H-9 ( $\delta_H$  6.45) and C-7 ( $\delta_C$  103.6), C-8 ( $\delta_C$  161.4), C-10 ( $\delta_C$  158.7), and C-10a ( $\delta_C$  109.9) were observed. These confirmed that one methyl and two hydroxyl groups were at C-5, C-8, and C-10, respectively. Acid hydrolysis of **1** revealed D-glucose and aglycone **1a**. Moreover, the position of glucose at C-8 was confirmed by HMBC correlations between H-1' glc ( $\delta_H$  4.93) and C-8 ( $\delta_C$  161.4). The CD spectrum of **1** showed a negative Cotton effect around 319 nm (See Supporting Information), similarly to those of (2*S*)-5-hydroxy-6,8-dimethoxy-2-methyl-4*H*-2,3-dihydronaphtho[2,3-*b*]-pyran-4-one,<sup>12</sup> suggested the configuration at C-2 to be *S*. In addition, the aglycone **1a** can be determined by comparing the optical rotation of **1a** with those of

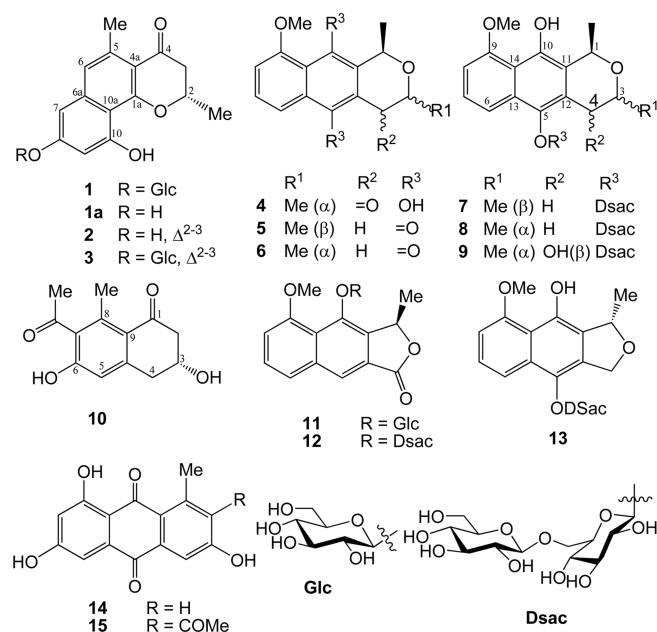
**Table 2.** Anti-inflammatory effects of compounds on LPS-stimulated bone marrow-derived dendritic cells

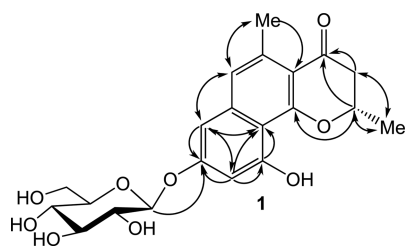
	IC <sub>50</sub>		
	IL-12 p40 ( $\mu$ g/mL)	IL-6 ( $\mu$ g/mL)	TNF- $\alpha$ ( $\mu$ g/mL)
Methanol extract	0.1 $\pm$ 0.05	16.2 $\pm$ 0.3	> 50
SB203580 <sup>a</sup>	2.5 $\pm$ 0.1	1.7 $\pm$ 0.2	3.6 $\pm$ 0.2
Compounds	IL-12 p40 ( $\mu$ M)	IL-6 ( $\mu$ M)	TNF- $\alpha$ ( $\mu$ M)
<b>1</b>	1.0 $\pm$ 0.1	5.0 $\pm$ 0.2	> 50
<b>4</b>	5.0 $\pm$ 0.4	8.7 $\pm$ 0.3	61.2 $\pm$ 1.5
<b>5</b>	0.1 $\pm$ 0.08	1.7 $\pm$ 0.1	39.6 $\pm$ 2.0
<b>6</b>	0.2 $\pm$ 0.1	2.6 $\pm$ 0.4	> 50
SB203580 <sup>a</sup>	5.2 $\pm$ 0.1	3.5 $\pm$ 0.2	7.5 $\pm$ 0.2

<sup>a</sup>Positive control. Data is presented as the mean  $\pm$  S.D. Samples run in triplicate.

series of 2-methylchroman-4-one as well as the optical rotation of (*R*) dihydroeleutherinol ( $[\alpha]_D^{25} = +8.8$ ).<sup>13</sup> So, the optical rotation of **1a** ( $[\alpha]_D^{25} = -38.3$ ) suggested a stereochemistry at C-2 to be *S* by comparing the optical rotation of (*S*) 5,7-dihydroxy-2-methylchroman-4-one ( $[\alpha]_D = -58.6$ )<sup>14</sup> and (*R*) 7-methoxy-2-methylchroman-4-one ( $[\alpha]_D = +53.2$ ).<sup>14</sup> To the best of our knowledge, aglycone **1a** was with *S* configuration was reported for the first time. Consequently, the structure of **1** was determined to be (2*S*) dihydroeleutherinol-8-*O*- $\beta$ -D-glucopyranoside.

The known compounds were characterized as eleutherinol (**2**),<sup>9</sup> eleutherinoside A (**3**),<sup>11</sup> (–)-hongconin (**4**),<sup>15</sup> eleutherin (**5**),<sup>16</sup> isoeleutherin (**6**),<sup>17</sup> eleuthoside C (**7**),<sup>16</sup> eleutherinoside C (**8**),<sup>18</sup> eleutherinoside B (**9**),<sup>18</sup> (*R*)-7-acetyl-3,6-dihydroxy-8-methyltetralone (**10**),<sup>19</sup> eleuthoside A (**11**),<sup>16</sup> eleuthoside B (**12**),<sup>16</sup> eleutherinoside D (**13**),<sup>18</sup> 3,6,8-trihydroxy-1-methyl-anthraquinone (**14**),<sup>20</sup> and 2-acetyl-3,6,8-trihydroxy-1-methyl-

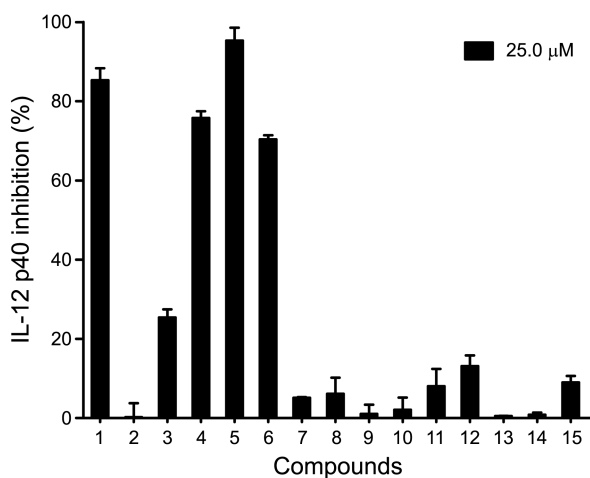
**Figure 1.** Structures of compounds **1-15** from the rhizome of *E. bulbosa*.



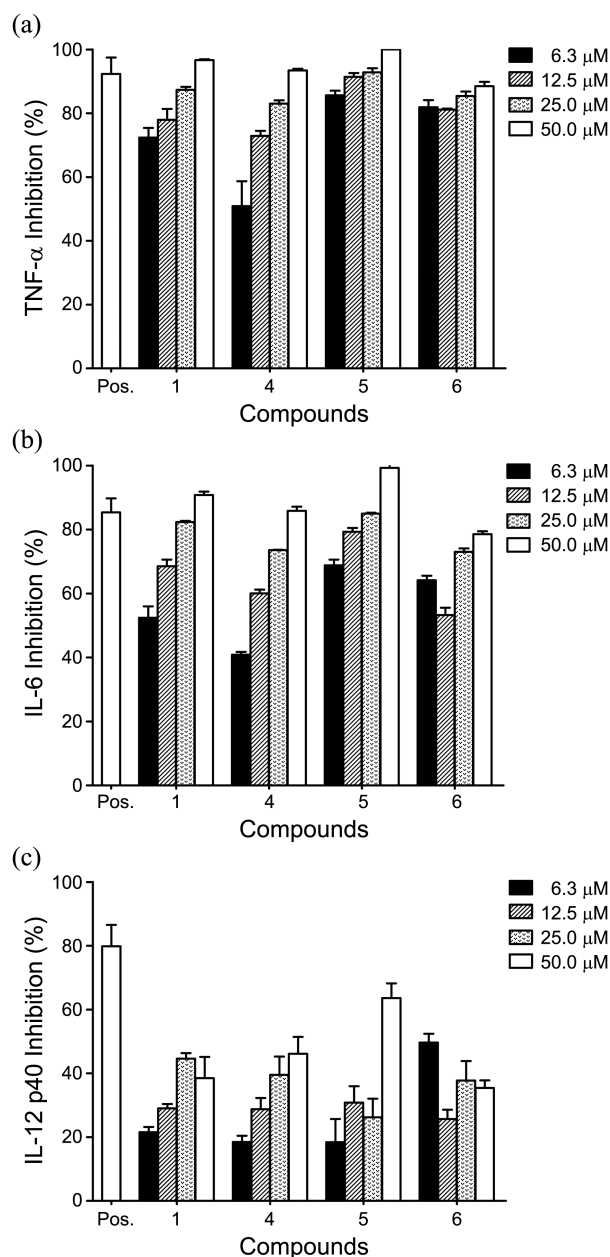
**Figure 2.** The important HMBC correlations for compound **1**.

anthraquinone (**15**).<sup>21</sup> They were elucidated on the basis of spectral data and chemical evidence, which were in good agreement with those reported in the literature (see Figure 1).

Continuing with our interest in the evaluation of the *anti*-inflammatory plant and to search novel *anti*-inflammatory agent, we have evaluated the effects of compounds from *E. bulbosa* in the inflammatory response by bone marrow-derived dendritic cells. We first used a colorimetric MTT assay to confirm that these compounds have no or little effect on the cell viability (data not shown). None of them exhibited cytotoxic activity. Upon LPS treatment, dendritic cells (DCs) are known to secrete pro-inflammatory cytokine, including IL-6, IL-12 p40, and TNF- $\alpha$ . In our experiments, dendritic cells were incubated in 48-well plates at a density of  $2 \times 10^5$  cells/mL, and then treated for 1 h with the compounds at the concentration of 25  $\mu$ M, and then stimulated with LPS (10 ng/mL) (see Figure 3).<sup>22</sup> One new, **1**, and three known compounds, **4**, **5**, and **6** showed potent inhibitory activities at the concentration of 25  $\mu$ M. All these active compounds were chosen for further tested at the concentrations of 6.3 to 50.0  $\mu$ M (see Figure 4). Positive control, SB203580, inhibited IL-12 p40, IL-6, and TNF- $\alpha$  production with IC<sub>50</sub> values of  $5.2 \pm 0.1$ ,  $3.5 \pm 0.1$ , and  $7.5 \pm 0.2$   $\mu$ M, respectively (Table 2). Of these compounds, compounds **1**, **4**, **5**, and **6** inhibited potent activity of LPS-stimulated IL-12 p40 production reducing the levels of this cytokine with IC<sub>50</sub> values ranging from  $0.1 \pm 0.08$  to  $5.0 \pm 0.4$   $\mu$ M. Compounds



**Figure 3.** Effect of compounds **1-15** on IL-12 p40 production by LPS-stimulated BMDCs at the concentration of 25.0  $\mu$ M. The data were presented as inhibition rate (%) compared to the value of vehicle-treated DCs. SB203580 was used as positive control (Pos.).



**Figure 4.** Effects of compounds **1**, **4**, **5**, and **6** on IL-12 p40 (a), IL-6 (b), and TNF- $\alpha$  (c) productions by LPS-stimulated BMDCs at the concentrations of 6.3, 12.5, 25.0, and 50.0  $\mu$ M. The data were presented as inhibition rate (%) compared to the value of vehicle-treated DCs. SB203580 was used as positive control (Pos.).

**1**, **5**, and **6** also showed the potent inhibitory activity on the IL-6 production with IC<sub>50</sub> values ranging from  $1.7 \pm 0.1$  to  $5.0 \pm 0.2$   $\mu$ M. However, only two compounds **4** and **5** exhibited moderate inhibitory activity on the TNF- $\alpha$  production with IC<sub>50</sub> values of  $61.2 \pm 1.5$  and  $39.6 \pm 2.0$   $\mu$ M. (–) Hongconin (**4**), eleutherin (**5**), and isoeleutherin (**6**) isolated from *Eleutherine americana* also exhibited potent inhibitory activity on nitric oxide production LPS-activated mouse RAW 264.7 macrophage cell-line.<sup>13</sup> To the best of our knowledge, this is the first report on *anti*-inflammatory activities of *E. bulbosa* and its chemical components. Collectively, a new compound **1** as well as three known compounds, **4**, **5**

and **6** isolated from the rhizome of *E. bulbosa* inhibited the production of TNF- $\alpha$ , IL-6, and IL-12 p40 in LPS-stimulated DCs. Thus, the present study suggests that these compounds may have potent *anti*-inflammatory action.

### Experimental

**Plant Material.** The rhizome of *E. bulbosa* was collected in Tam Dao, Vinh Phuc province, Vietnam in June, 2011, and identified by Dr. Nguyen Quoc Binh, Museum of Natural, VAST, Vietnam. A voucher specimen (EB1106) was deposited at the Herbarium of Institute of Natural Products Chemistry.

**Dihydroeleutherinol-8-O- $\beta$ -D-glucopyranoside (1):** A pale yellow powder; mp 206-207 °C;  $[\alpha]_D^{25}$  -58.1 (MeOH,  $c = 0.3$ ); UV (MeOH)  $\lambda_{\max}$  (log  $\epsilon$ ) 223 (4.2), 261 (4.0); IR  $\nu_{\max}$  (KBr) 3495, 1640, 1610, 1233;  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR are given in Table 1; ESI-MS  $m/z$  419 [M-H] $^-$ ; HR-ESI-MS  $m/z$  455.1126 [M+Cl] $^-$  (Calcd for C<sub>21</sub>H<sub>24</sub>O<sub>9</sub>Cl, 455.1114),  $m/z$  419.1338 [M-H] $^-$  (Calcd for C<sub>21</sub>H<sub>23</sub>O<sub>9</sub>, 419.1348),  $m/z$  257.0818 [M-Glc] $^-$  (Calcd for C<sub>15</sub>H<sub>13</sub>O<sub>4</sub>, 257.0819); CD spectrum: see Supporting Information.

**Dihydroeleutherinol (1a):** A pale yellow powder;  $[\alpha]_D^{25}$  -38.3 (MeOH,  $c = 0.3$ );  $^1\text{H}$ -NMR (400 MHz, CD<sub>3</sub>OD)  $\delta_{\text{H}}$  4.88 (H-2), 2.72 (dd, 3.7, 16.8 (H<sub>a</sub>-3), 2.80 (dd, 12.0, 16.8 (H<sub>b</sub>-3), 6.90 (s, H-6), 6.48 (d, 2.2, H-7), 6.35 (d, 2.2, H-9), 1.60 (d, 6.1, 2-Me), and 2.59 (s, 5-Me);  $^{13}\text{C}$ -NMR (100 MHz, CD<sub>3</sub>OD)  $\delta_{\text{C}}$  163.8 (C-1a), 77.9 (C-2), 46.0 (C-3), 194.0 (C-4), 113.7 (C-4a), 137.3 (C-5), 123.3 (C-6), 141.7 (C-6a), 102.6 (C-7), 162.0 (C-8), 102.9 (C-9), 158.9 (C-10), 108.3 (C-10a), 20.8 (2-Me), and 23.4 (5-Me); HR-ESI-MS  $m/z$  257.0810 [M-H] $^-$  (Calcd for C<sub>15</sub>H<sub>13</sub>O<sub>4</sub>, 257.0819).

**Supporting Information.** General procedures, extraction, isolation, hydrolysis procedure, cell culture and measurement of cytokine production assays, and NMR and CD spectra of **1** and **1a** are available as Supporting Information.

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plant.

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