Diosgenin Glucuronides from *Solanum lyratum* and their Cytotoxicity against Tumor Cell Lines

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Bioassay-directed fractionation of the cytotoxicity active fraction of the whole plant from *Solanum lyratum* led to the isolation of a new steroidal saponin, diosgenin 3-0- β-d-glucopyranosiduronic acid methyl ester (2), as well as four known compounds, diosgenin (1), diosgenin 3-0- β-d-glucopyranosiduronic acid (3), diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-d-glucopyranosiduronic acid (4), diosgenin 3-O-α-L-rhamnopyranosyl-(1→2)-β-d-glucuroniduronic acid methyl ester (5). The structures of the isolated compounds were elucidated on the basis of their spectral data and chemical evidences. Compound 1 was isolated for the first time from this plant, and compound 3 was isolated as a new natural product. Cytotoxic activities of the isolated compounds were evaluated and the cytotoxicities of compounds 2–5 reported for the first time.

Key words: *Solanum lyratum*, Cytotoxicity, Diosgenin Glucuronides

Introduction

The whole plants of *Solanum lyratum* Thunb. (Solanaceae) are called “Baimaoteng” in Chinese medicine. The aerial part of *Solanum lyratum* had traditionally been used in therapies against cancer, malaria, jaundice, edema, hepatitis etc. (Shim et al., 1995). Previously, it was reported that the aqueous extract of *Solanum lyratum* has cytotoxic activity against HL60 cell lines (Shi and Yan, 2002) and the EtOAC extract has apoptosis-inducing activity in Bel-7404 cell lines (Shan et al., 2001). Many steroidal glucuronides and steroidal alkaloid glycosides had been isolated from *Solanum lyratum*. Some of them have inhibitory activity for cancer cell growth (Murakami et al., 1985; Yahara et al., 1985; Yung et al., 1997). However, bioactivity components responsible for its anticancer activity have not been well understood.

To systematically evaluate its potential anticancer activity, the bioactivity ingredients were isolated from the whole plant of *Solanum lyratum* by activity-guided fractionation. The structures of the isolated compounds were elucidated on the basis of their spectral data and chemical evidences, and their cytotoxic activities were determined in this study.

Materials and Methods

**General**

The NMR spectra were recorded with a Bruker-ARX 300 or 600 spectrometer operating at 300 or 600 MHz for 1H and 75 or 150 MHz for 13C NMR, respectively. Chemical shifts were reported in ppm on the δ scale with TMS as the internal standard. ESI-MS were recorded on a Finnigan LCQ LC-MS analyzer. The IR absorption spectra were recorded in KBr discs using a Bruker IFS-55 infrared spectrometer. Column chromatography was performed with silica gel (Qingdao Haiyang Chemical Group Co. Ltd, Qingdao, People’s Republic of China) and Sephadex LH-20 (25–100 µm, Pharmacia). TLC was performed on precoated Si gel 60 F254 plates (0.2 mm thick, Merck) and spots were detected by spraying with 10% ethanolic H2SO4 reagent.
Plant

The whole plants of Solanum lyratum were purchased from ShangHai XuHui Chinese Herbal Medicine Factory (Shanghai, China) in January 2003. This plant was identified by Professor Qi-Shi Sun, College of Chinese Traditional Medicine, Shenyang Pharmaceutical University.

Extraction and isolation

The air-dried aerial parts of Solanum lyratum (15 kg) were extracted with 95% EtOH. The EtOH solutions were combined, filtered and evaporated under vacuum. The dry EtOH extract (705 g, 4.7%) was further processed by successive solvent partitioning to give petroleum ether (705 g, 4.7%) was further processed by successive solvent partitioning to give petroleum ether (705 g, 4.7%), EtOAc (135 g), n-BuOH (150 g) and aqueous (210 g) fractions, respectively. Each fraction was evaluated for the antiproliferative activity on the tumor cell lines. Consequently, it was shown that the activity resided predominantly in the EtOAc fraction (Table I). Thus, the EtOAc fraction was subjected to column chromatography on silica gel. Using petroleum ether/acetone (100:0 ~ 1:2) to offer compound 1 (36 mg). Fraction E14-09 was separated by silica gel using CHCl3/MeOH (1:1 ~ 0:1) and recrystallization to give compound 2 (40 mg). Fraction E14-09 was chromatographed on a silica gel column using CHCl3/MeOH (20:1 ~ 0:1) and a Sephadex LH20 column using CHCl3/MeOH (20:1 ~ 0:1) to separate fraction E14 and 0.79 (3H, s, 18-CH3), 0.97 (3H, d, J = 4.6 Hz, 21-CH3), 1.03 (3H, s, 19-CH3). – 13C NMR (75 MHz, CD3OD): δ (ppm) = 37.2 (C-1), 31.6 (C-2), 71.7 (C-3), 42.3 (C-4), 140.8 (C-5), 121.4 (C-6), 32.0 (C-7), 31.4 (C-8), 50.0 (C-9), 36.6 (C-10), 20.8 (C-11), 39.8 (C-12), 40.2 (C-13), 56.5 (C-14), 31.8 (C-15), 80.8 (C-16), 62.1 (C-17), 16.3 (C-18), 19.4 (C-19), 41.6 (C-20), 14.5 (C-21), 109.3 (C-22), 31.4 (C-23), 28.8 (C-24), 30.3 (C-25), 66.8 (C-26), 17.1 (C-27).

Diosgenin 3-O-β-D-glucopyranosiduronic acid methyl ester (2): White amorphous powder. – IR: ν = 3329.7 (-OH), 2949.1, 1743.8 (-COO-), 1452.9 (>C=C<), 1381.8, 1170.0, 1051.7, 981.9, 921.7, 899.3 cm⁻¹. – Positive ESI-MS: m/z = 605.3 [M+H]+, 627.4 [M+Na]+; negative ESI-MS: m/z = 621.1 [M-H2O]-, 639.1 [M+Cl]-. – 1H NMR (600 MHz, DMSO-d6): δ (ppm) = 0.73 ± 2 (6H, br s, H-18, H-27), 0.90 (3H, d, J = 6.8 Hz, H-21), 0.96 (3H, s, H-19), 3.65 (3H, s, OCH3), 4.38 (1H, d, J = 7.79 Hz, H-1 of Glc), 5.34 (H, s, H-6). – 13C NMR (150 MHz, DMSO-d6): δ (ppm) = 36.8 (t, C-1), 31.1 (t, C-2), 77.5 (d, C-3), 38.3 (t, C-4), 140.4 (s, C-5), 121.2 (d, C-6), 31.6 (t, C-7), 29.3 (d, C-8), 49.6 (d, C-9), 36.4 (s, C-10), 20.5 (t, C-11), 39.3 (t, C-12), 39.6 (s, C-13), 55.8 (d, C-14), 31.6 (t, C-15), 80.3 (d, C-16), 61.9 (d, C-17), 16.1 (q, C-18), 19.2 (q, C-19), 41.2 (d, C-20), 14.8 (q, C-21), 108.5 (s, C-22), 31.0 (t, C-23), 28.6 (t, C-24), 29.9 (d, C-25), 66.0 (t, C-26), 17.2 (q, C-27), Glu 101.2 (d, C-1), 73.2 (d, C-2), 75.8 (d, C-3), 71.7 (d, C-4), 75.4 (d, C-5), 169.6 (s, C-6), 51.9 (3H, q, OCH3).

Diosgenin 3-O-β-D-glucopyranosiduronic acid (3): White amorphous powder. – Negative ESI-MS: m/z = 589.2 [M-H]-. – 1H NMR (600 MHz, DMSO-d6): δ (ppm) = 0.74 (3H, d, J = 4.3 Hz, H-27), 0.76 (3H, s, H-18), 0.91 (3H, s, H-19), 0.98 (3H, s, H-19), 4.19 (1H, d, J = 7.79 Hz, H-1 of Glu), 5.33 (H, s, H-6). – 13C NMR (150 MHz, DMSO-d6): δ (ppm) = 36.9 (C-1), 31.1 (C-2), 77.5 (C-3), 38.3 (C-4), 140.5 (C-5), 121.0 (C-6), 31.6 (C-7), 29.3 (C-8), 49.6 (C-9), 36.4 (C-10), 20.2 (C-11), 39.3 (C-12), 39.6 (C-13), 55.8 (C-14), 31.5 (C-15), 80.3 (C-16), 61.7 (C-17), 16.1 (C-18), 19.1 (C-19), 41.1 (C-20), 14.6 (C-21), 108.5 (C-22), 31.0 (C-23), 28.5 (C-24), 29.8 (C-25), 66.0 (C-26), 17.1 (C-27), Glu 101.7 (C-1), 73.4 (C-2), 76.8 (C-3), 72.2 (C-4), 76.6 (C-5), 172.5 (C-6).
Diosgenin 3-O-α-L-rhamnopyanosyl-(1→2)-β-D-glucuronidosiduronic acid (4): White amorphous powder. – Positive ESI-MS: m/z = 782.2 [M+2Na]⁺, 588.4 [M-rha], 413.2 [M-glu-rha]. – 1H NMR (600 MHz, DMSO-d₆): δ (ppm) = 0.72 (3H, d, J = 3.7 Hz, H-27), 0.73 (3H, s, H-18), 0.90 (3H, d, J = 6.84 Hz, H-21), 0.96 (3H, s, H-19), 1.08 (3H, d, J = 6.12 Hz, H-6 of Rha), 4.35 (1H, d, J = 7.80 Hz, H-1 of Glu), 5.03 (1H, s, H-1 of Rha), 5.33 (H, s, H-6). – 13C NMR (150 MHz, DMSO-d₆): δ (ppm) = 36.8 (C-1), 29.0 (C-2), 76.4 (C-3), 37.6 (C-4), 140.5 (C-5), 121.3 (C-6), 31.6 (C-7), 31.1 (C-8), 49.7 (C-9), 36.5 (C-10), 20.5 (C-11), 39.7 (C-12), 39.7 (C-13), 55.8 (C-14), 31.6 (C-15), 80.3 (C-16), 61.9 (C-17), 16.2 (C-18), 19.1 (C-19), 41.2 (C-20), 14.8 (C-21), 31.1 (C-22), 28.6 (C-23), 29.9 (C-24), 66.0 (C-26), 17.2 (C-27), Glu 97.9 (C-1), 78.0 (C-2), 78.0 (C-3), 71.9 (C-4), 73.3 (C-5), 172.9 (C-6), Rha 100.3 (C-1), 72.5 (C-2), 72.7 (C-3), 73.9 (C-4), 68.0 (C-5), 17.9 (C-6).

Diosgenin 3-O-α-L-rhamnopyanosyl-(1→2)-β-D-glucuroniduronic acid methyl ester (5): White amorphous powder. – IR: ν̃ = 3319.3 (-OH), 2931.0, 1741.0 (-COOMe), 1452.1 (>C=C<), CO2 2931.0, 1741.0 (-).

Results and Discussion

The ethanol extract of Solanum lyratum had a high cytotoxic activity against A375-S2, SGC-7901 and Bel-7402 cells, a medium cytotoxic activity against HeLa and L929 cells and a low cytotoxic activity against MCF7 cells (Table I). Compared with the cytotoxic activity of the n-BuOH fraction,

Table I. The cytotoxic activity (IC₅₀, µg/ml) of extract/fraction against HeLa, A375-S2, SGC-7901, MCF7, L929, and Bel-7402 cell lines.

<table>
<thead>
<tr>
<th>Extract/fraction</th>
<th>HeLa</th>
<th>A375-S2</th>
<th>SGC-7901</th>
<th>Bel-7402</th>
<th>L929</th>
<th>MCF7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol extract</td>
<td>320.1</td>
<td>210.0</td>
<td>176.1</td>
<td>287.4</td>
<td>460.5</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>EtOAc fraction</td>
<td>20.9</td>
<td>9.7</td>
<td>65.4</td>
<td>106.2</td>
<td>76.5</td>
<td>129.2</td>
</tr>
<tr>
<td>n-BuOH fraction</td>
<td>64.6</td>
<td>31.7</td>
<td>114.8</td>
<td>180.2</td>
<td>357.7</td>
<td>266.3</td>
</tr>
<tr>
<td>Petroleum ether fraction</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>–</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>–</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
<td>&gt; 500</td>
</tr>
</tbody>
</table>

a IC₅₀ is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

Cell culture

HeLa (human uterine carcinoma) cell lines, A375-S2 (human malignant melanoma) cell lines, MCF7 (human breast cancer) cell lines, L929 (murine fibrosarcoma) cell lines, SGC-7901 (human gastric cancer) cell lines and Bel-7402 (human hepatocellular carcinoma) cell lines were obtained from American Type Culture Collection (ATCC, #CRL, 1872, MD, USA) and cultured in RPMI 1640 medium (GIBCO, USA) including l-glutamine with 10% FBS. All cells were maintained at 37 °C, 5% CO₂ in a humidified atmosphere incubator. The confluent cells were used for the cytotoxicity assay.

Cytotoxicity assay

The inhibition of the cellular growth was estimated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Mosmann (1983).

The ethanol extract of Solanum lyratum had a high cytotoxic activity against A375-S2, SGC-7901 and Bel-7402 cells, a medium cytotoxic activity against HeLa and L929 cells and a low cytotoxic activity against MCF7 cells (Table I). Compared with the cytotoxic activity of the n-BuOH fraction,
Table II. The cytotoxic activity (IC50, µg/ml) of fractions \(E_1\)–\(E_{15}\) against HeLa and A375-S2 cell lines.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>IC50 [µg/ml]a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HeLa</td>
</tr>
<tr>
<td>(E_1)</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>(E_2)</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>(E_3)</td>
<td>&gt; 100</td>
</tr>
<tr>
<td>(E_4)</td>
<td>49.7 ± 1.4</td>
</tr>
<tr>
<td>(E_5)</td>
<td>44.5 ± 0.9</td>
</tr>
<tr>
<td>(E_6)</td>
<td>29.7 ± 1.0</td>
</tr>
<tr>
<td>(E_7)</td>
<td>45.7 ± 0.9</td>
</tr>
<tr>
<td>(E_8)</td>
<td>41.7 ± 0.7</td>
</tr>
<tr>
<td>(E_9)</td>
<td>39.2 ± 1.1</td>
</tr>
<tr>
<td>(E_{10})</td>
<td>36.5 ± 1.2</td>
</tr>
<tr>
<td>(E_{11})</td>
<td>55.4 ± 0.6</td>
</tr>
<tr>
<td>(E_{12})</td>
<td>55.3 ± 0.9</td>
</tr>
<tr>
<td>(E_{13})</td>
<td>42.0 ± 0.8</td>
</tr>
<tr>
<td>(E_{14})</td>
<td>60.0 ± 1.0</td>
</tr>
<tr>
<td>(E_{15})</td>
<td>42.1 ± 1.8</td>
</tr>
</tbody>
</table>

a IC50 is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

the EtOAC fraction showed stronger cytotoxic activity. As for 15 fractions of the EtOAC fraction, fractions \(E_1\)–\(E_3\), which had low polarity, showed no cytotoxic activity, and fractions \(E_4\)–\(E_{15}\) exhibited significant dose-dependent cytotoxic activity for HeLa and A375-S2 cells (Table II).

Bioassay-directed fractionation of the EtOAC fraction from Solanum lyratum led to the isolation of five compounds. The structures (Fig. 1) were identified as diosgenin (1), diosgenin 3-\(\beta\)-d-glucopyranosiduronic acid methyl ester (2), diosgenin 3-\(\beta\)-d-glucopyranosiduronic acid (3), diosgenin 3-\(\alpha\)-l-rhamnopyranosyl(1→2)-\(\beta\)-d-glucopyranosiduronic acid (4), and diosgenin 3-\(\alpha\)-l-rhamnopyranosyl(1→2)-\(\beta\)-d-glucuroniduronic acid methyl ester (5).

**Fig. 1.** The structure of the isolated compounds: diosgenin (1), diosgenin 3-\(\beta\)-d-glucopyranosiduronic acid methyl ester (2), diosgenin 3-\(\beta\)-d-glucopyranosiduronic acid (3), diosgenin 3-\(\alpha\)-l-rhamnopyranosyl(1→2)-\(\beta\)-d-glucopyranosiduronic acid (4), and diosgenin 3-\(\alpha\)-l-rhamnopyranosyl(1→2)-\(\beta\)-d-glucuroniduronic acid methyl ester (5).

The IC50 values measured for the fractions are provided in Table II. The results show that the EtOAC fraction from Solanum lyratum exhibited significant cytotoxic activity against both cell lines. The cytotoxic activity can be quantified by the IC50 values, which represent the concentration that results in a 50% decrease in cell number. The values are expressed as the mean of three independent experiments.

- **Fraction E1** exhibited an IC50 value of >100 µg/ml for both HeLa and A375-S2 cells.
- **Fraction E2** showed an IC50 value of >100 µg/ml for both cell lines.
- **Fraction E3** also exhibited an IC50 value of >100 µg/ml for both cell lines.
- **Fraction E4** had an IC50 value of 49.7 ± 1.4 µg/ml for HeLa cells and 38.9 ± 1.2 µg/ml for A375-S2 cells.
- **Fraction E5** demonstrated an IC50 of 44.5 ± 0.9 µg/ml for HeLa cells and 29.8 ± 1.9 µg/ml for A375-S2 cells.
- **Fraction E6** exhibited an IC50 of 29.7 ± 1.0 µg/ml for HeLa cells and 15.0 ± 1.3 µg/ml for A375-S2 cells.
- **Fraction E7** had an IC50 of 45.7 ± 0.9 µg/ml for HeLa cells and 27.3 ± 0.8 µg/ml for A375-S2 cells.
- **Fraction E8** showed an IC50 of 41.7 ± 0.7 µg/ml for HeLa cells and 32.3 ± 1.1 µg/ml for A375-S2 cells.
- **Fraction E9** demonstrated an IC50 of 39.2 ± 1.1 µg/ml for HeLa cells and 16.5 ± 1.5 µg/ml for A375-S2 cells.
- **Fraction E10** exhibited an IC50 of 36.5 ± 1.2 µg/ml for HeLa cells and 43.7 ± 1.7 µg/ml for A375-S2 cells.
- **Fraction E11** had an IC50 of 55.4 ± 0.6 µg/ml for HeLa cells and 52.8 ± 0.9 µg/ml for A375-S2 cells.
- **Fraction E12** showed an IC50 of 55.3 ± 0.9 µg/ml for HeLa cells and 43.7 ± 1.0 µg/ml for A375-S2 cells.
- **Fraction E13** demonstrated an IC50 of 42.0 ± 0.8 µg/ml for HeLa cells and 28.1 ± 1.2 µg/ml for A375-S2 cells.
- **Fraction E14** exhibited an IC50 of 60.0 ± 1.0 µg/ml for HeLa cells and 42.3 ± 0.9 µg/ml for A375-S2 cells.
- **Fraction E15** showed an IC50 of 42.1 ± 1.8 µg/ml for HeLa cells and 30.3 ± 0.9 µg/ml for A375-S2 cells.

These results indicate that the EtOAC fraction from Solanum lyratum is cytotoxic against both cell lines. Further studies are needed to identify the active compounds and their potential therapeutic applications.
HMBC correlation (Fig. 2) was observed between a proton signal at $\delta$ 3.65 ($-\text{OCH}_3$) and a carbon signal at $\delta$ 169.6. All this confirmed the fact that compound 2 was diosgenin with a glucuronic methyl ester at C-3. From the above evidences, the structure of 2 was concluded to be diosgenin 3-O-$\beta$-d-glucopyranosiduronic acid methyl ester. It has not been reported yet, and it is a new compound.

Compound 3 was obtained as white amorphous powder. ESI-MS (negative mode) of compound 3 exhibited ions at $m/z$ 589.2 [M-H]$. Careful comparison of NMR spectral data of 3 with that of 2 showed that 3 has the same structure as 2, and that 3 differs structurally from 2 only by the elimination of $-\text{OCH}_3$ group signal in the sugar moiety. This assumption was confirmed by HMBC, HMQC and $^1$H,$^1$H-COSY experiments. From the above evidences, the structure of 3 was concluded to be diosgenin 3-O-$\beta$-d-glucopyranosiduronic acid. Compound 3 has been prepared by a standardized, simplified Koenig-Knorr synthesis (Schneider, 1971), and was isolated as a new natural product in the present study.

Compounds 1, 4 and 5 were identified by comparison of their spectral data with the literature data (Agrawal et al., 1985; Yahara et al., 1986).

The cytotoxic activity of the isolated compounds was determined against HeLa, A375-S2, SGC-7901 and Bel-7402 cells. The results are summarized in Table III. Compound 1 showed significant cytotoxic activities. It was reported that diosgenin induced apoptosis in HeLa cells via activation of the caspase pathway (Hou et al., 2004). Compounds 2 and 3 exhibited no cytotoxic activities. Compounds 4 and 5 exhibited moderated cytotoxic activities against HeLa, A375-S2, SGC-7901 and Bel-7402 cells in a concentration-dependent manner. Their cytotoxicity is reported for the first time.

**Acknowledgement**

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**Table III. The cytotoxic activity (IC$_{50}$, $\mu$g/ml) of the isolated compounds against HeLa, A375-S2, SGC-7901, and Bel-7402 cell lines.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>HeLa</th>
<th>A375-S2</th>
<th>SGC-7901</th>
<th>Bel-7402</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.4 ± 1.3</td>
<td>8.2 ± 0.6</td>
<td>21.3 ± 0.9</td>
<td>20.1 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>4</td>
<td>18.2 ± 1.6</td>
<td>12.2 ± 0.8</td>
<td>30.5 ± 0.8</td>
<td>35.6 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>19.8 ± 1.3</td>
<td>16.2 ± 0.6</td>
<td>37.2 ± 1.1</td>
<td>39.2 ± 0.9</td>
</tr>
<tr>
<td>5-Fu$^b$</td>
<td>17.5 ± 1.4</td>
<td>15.0 ± 0.7</td>
<td>3.9 ± 1.0</td>
<td>14.1 ± 0.4</td>
</tr>
</tbody>
</table>

$^a$ IC$_{50}$ is defined as the concentration which resulted in a 50% decrease in cell number. The values represent the mean of three independent experiments.

$^b$ 5-Fu (5-fluorouracil) was used as a positive control.


