Labdane Diterpenes from Aster spathulifolius and Their Cytotoxic Effects on Human Cancer Cell Lines

Sung Ok Lee,† Sang Zin Choi,† Sang Un Choi,‡ Kang Choon Lee,† Young Won Chin,§ Jinwoong Kim,§ Young Choong Kim,§ and Kang Ro Lee*.†

College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea, Korea Research Institute of Chemical Technology, Sung Ok Lee,† Sang Zin Choi,† Sang Un Choi,‡ Kang Choon Lee,† Young Won Chin,§ Jinwoong Kim,§ College of Pharmacy, Sungkyunkwan University, Suwon 440-746, Korea, Korea Research Institute of Chemical Technology, Sung Ok Lee,† Sang Zin Choi,† Sang Un Choi,‡ Kang Choon Lee,† Young Won Chin,§ Jinwoong Kim,§

Received March 17, 2005

Three new labdane diterpenes (1–3), together with eight known diterpenoids, were isolated from a methanol extract of the aerial parts of Aster spathulifolius. The structures of 1–3 were determined as (13R)-labda-7,14-diene 13-O-β-D-(4′-O-acetyl)fucopyranoside (1), (13R)-labda-7,14-diene 13-O-β-D-(3′-O-acetyl)fucopyranoside (2), and (13R)-labda-14(15)-ene-8,13-diol 13-O-β-D-fucopyranoside (3), on the basis of spectroscopic and chemical methods. Compounds 1, 2, and four of the known compounds exhibited generally nonspecific cytotoxicity against human A549, SK-OV-3, SK-MEL-2, XF498, and HCT15 tumor cells.

Aster spathulifolius Maxim. (Asteraceae) is a perennial herb distributed along the eastern and southern coasts of South Korea, and its aerial parts have been used to treat asthma and diuresis in Korean traditional medicine.1,2 We have investigated the secondary metabolites produced by plants belonging to the genus Aster and have reported cytotoxic diterpenoids and sesquiterpene peroxides from Aster oharai and A. scaber, respectively.3,4 In the present study, three new diterpenoid glycosides (1–3) and eight known substances were isolated from methanol extracts of aerial parts of A. spathulifolius and their cytotoxicities against five human tumor cell lines were evaluated. The eight known compounds 7α-hydroxymanool,5 labda-7,14-dien-13-ol (4),6 (13R)-labda-7,14-diene 13-O-α-L-3′-O-acetyl)-6′-deoxydipyranyloside (5),7,9 (13R)-labda-7,14-diene 13-O-α-L-5′-O-acetyl)-6′-deoxydipyranyloside (6),7,8,9 epi-manoyl oxide 18-oic acid,12 (13R)-labda-7,14-dien-13-oic acid (7),3,7,8 13-hydroxymanool,5 labda-7,14-diene 13-O-α-D-fucopyranoside9 were identified by comparing their physical and spectroscopic data with those reported in the literature.

Results and Discussion

Compound 1 was obtained as a colorless oil, and its molecular formula was assigned as C_{28}H_{46}O_{6} on the basis of the sodiated molecular ion peak [M + Na]^+ at m/z 501.3187 in the HRFABMS. The IR spectrum showed the presence of hydroxyl and ester groups at 3446 and 1743 cm⁻¹, respectively. The 1H and 13C NMR spectra of 1 were similar to those of a known diterpene glycoside, (13R)-labda-7,14-diene 13-O-β-D-fucopyranoside (7), except for the presence of signals for an acetyl group at δH 2.16 (3H, s) and δC 20.8 and 171.4 in the 1H and 13C NMR spectra of 1. The H-4 (δ 5.15) and C-4 (δ 74.3) signals of 1 appeared more downfield than those of 7 (H-4′, δ 3.71; C-4′, δ 72.4). This supported the presence of an acetyl group at C-4′ in 1. The glycosyl and acetyl linkages were also confirmed by the HMBC data, which showed correlations of H-1 (δ 72.4) to C-13 (δ 81.1) and H-4′ (δ 5.15) to an acetyl carbon (δ 171.4), respectively (Figure 1). Alkaline hydrolysis of 1 afforded 7, which was identified by its 1H NMR spectrum as well as by direct comparison by co-TLC (Rf 0.26, n-hexane–EtOAc, 1:1) using an authentic sample. Acid hydrolysis of 7 yielded the aglycon, labda-7,14-dien-13-ol (4),6 and a sugar. The sugar was identified by a GC analysis as an acetylated derivative as well as by co-TLC using an authentic fucose (Rf 0.61, CHCl₃–MeOH–H₂O, 9:5:0.5). Treatment of a sugar from the acid hydrolysis of 7 with L-cysteine methyl ester and N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) afforded the trimethylsilyl ether of the methyl 2-(fucotetrahydroxybutyl)-thiazolidine-4(R)-carboxylate, which enabled the L- and D-monosaccharide derivatives to be readily separated from each other in a GC capillary column.15 This experiment

![Figure 1. Key HMBC (the upper part) and NOESY (the lower part) correlations for compounds 1 and 3.](image-url)
The absolute configuration of C-13 of the d-fucopyranose unit in the sugar moiety. The signals in the sugar unit of compound (1H, br dq, $J(z, 3.0, 10.0)$) showed a correlation between H-3 and the anomeric proton in the $^1{H}$ NMR spectrum. The absolute configuration of C-13 of 1 was assigned as (R)-labda-7,14-diene by comparing the optical rotation data of (13R)-labda-7,14-dien-13-ol ([$\alpha$]$_D$ = -1.8°) and (13S)-labda-7,14-dien-13-ol ([$\alpha$]$_D$ = +20.0°). The optical rotation of the aglycon ([$\alpha$]$_D$ = -2.3°) obtained from acid hydrolysis with I was almost the same as that of (13R)-labda-7,14-dien-13-ol. Furthermore, the optical rotation ([H, $J(z, 3.0, 10.0)$]) of I was in accordance with that of 7 ([H, $J(z, -58.4°)$]). The NOESY spectrum of 1 showed corresponding NOE correlations of the stereoisomer of 7 (Figure 1). Therefore, the structure of compound 1 was determined as (13R)-labda-7,14-diene 13-O-($\beta$)-d-(3′′-O-acetyl)fucopyranoside.

Compound 2 was obtained as a colorless oil, and its molecular formula was determined as C$_{28}$H$_{46}$O$_6$ by the basis of the quasimolecular ion peak [M + Na]$^+$ at $m/z$ 501.3190 in the HRFABMS. The IR and NMR spectra of 2 were almost the same as those of 1. The only difference was the position of the acetyl group, whose location was determined to be at C-3′′ by a comparison with the NMR data of the d-fucopyranose unit in 7. The H-3′′ ($J(z, 4.84$) and C-3′′ ($J(75.7$) signals in 2 appeared more downfield than those of 7 (H-3′, $J(3.58$; C-3′, $J(71.3)). The HMBC spectrum of 2 showed a correlation between H-3′′ ($J(4.84$) and an acetyl carbon ($J(170.6$). Alkaline hydrolysis of 2 afforded (13R)-labda-7,14-diene 13-O-($\beta$)-d-fucopyranoside, which was identified from its $^1{H}$ NMR spectrum as well as by direct comparison by co-TLC (RI 0.26, n-hexane—EtOAc, 1:1) with 7. Analysis of its $^1{H}$—H COSY, HMBC, and HMBM spectra allowed the assignment of all $^1{H}$ and $^{13}$C NMR signals for 2 (Table 1). Therefore, the structure of 2 was determined as (13R)-labda-7,14-diene 13-O-($\beta$)-d-(3′′-O-acetyl)fucopyranoside.

Table 1. $^1$H NMR (500 MHz) and $^{13}$C NMR (125 MHz) Data of 1–3 in CDCl$_3$

<table>
<thead>
<tr>
<th>position</th>
<th>$\delta$$_H$ (mult., Hz)</th>
<th>$\delta$</th>
<th>$\delta$$_H$ (mult., Hz)</th>
<th>$\delta$</th>
<th>$\delta$$_H$ (mult., Hz)</th>
<th>$\delta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1α</td>
<td>0.95 (td, 3.5, 12.5)</td>
<td>39.3</td>
<td>0.93 (td, 3.5, 12.5)</td>
<td>39.3</td>
<td>0.94 (m)</td>
<td>39.4</td>
</tr>
<tr>
<td>1β</td>
<td>1.78 (br d, 12.5)</td>
<td>1.81 (br d, 12.5)</td>
<td>1.81 (br d, 12.5)</td>
<td>1.81 (br d, 12.5)</td>
<td>1.81 (br d, 12.5)</td>
<td>19.1</td>
</tr>
<tr>
<td>2</td>
<td>1.54 (m)</td>
<td>18.8</td>
<td>1.58 (m)</td>
<td>18.8</td>
<td>1.59 (m)</td>
<td>19.1</td>
</tr>
<tr>
<td>3α</td>
<td>1.16 (m)</td>
<td>42.3</td>
<td>1.17 (m)</td>
<td>42.2</td>
<td>1.13 (m)</td>
<td>42.2</td>
</tr>
<tr>
<td>3β</td>
<td>1.41 (m)</td>
<td>32.9</td>
<td>1.42 (m)</td>
<td>32.9</td>
<td>1.42 (m)</td>
<td>32.9</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>50.2</td>
<td></td>
<td>50.2</td>
<td></td>
<td>50.2</td>
</tr>
<tr>
<td>5α</td>
<td>1.95 (m)</td>
<td>23.8</td>
<td>1.96 (br d, 17.5)</td>
<td>23.8</td>
<td>1.98 (br d, 17.5)</td>
<td>22.3</td>
</tr>
<tr>
<td>6α</td>
<td>1.91 (m)</td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>43.6</td>
</tr>
<tr>
<td>7</td>
<td>5.39 (s)</td>
<td>122.3</td>
<td>5.39 (s)</td>
<td>122.3</td>
<td>1.74 (m)</td>
<td>43.6</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>135.3</td>
<td></td>
<td>135.3</td>
<td></td>
<td>74.6</td>
</tr>
<tr>
<td>9α</td>
<td>1.53 (m)</td>
<td>55.5</td>
<td>1.53 (m)</td>
<td>55.4</td>
<td>1.59 (m)</td>
<td>62.6</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>37.1</td>
<td></td>
<td>37.1</td>
<td></td>
<td>38.7</td>
</tr>
<tr>
<td>11</td>
<td>1.46 (m)</td>
<td>22.3</td>
<td>1.46 (m)</td>
<td>21.8</td>
<td>1.46 (m)</td>
<td>22.1</td>
</tr>
<tr>
<td>12</td>
<td>1.54 (m)</td>
<td>43.3</td>
<td>1.55 (m)</td>
<td>43.5</td>
<td>1.55 (m)</td>
<td>44.8</td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>1.84 (m)</td>
<td>82.4</td>
</tr>
<tr>
<td>14</td>
<td>6.00 (dd, 10.5, 17.5)</td>
<td>142.2</td>
<td>6.02 (dd, 10.5, 17.5)</td>
<td>142.2</td>
<td>5.92 (dd, 10.5)</td>
<td>146.8</td>
</tr>
<tr>
<td>15</td>
<td>5.19 (dd, 10.5, 17.5)</td>
<td>115.1</td>
<td>5.18 (dd, 10.5, 17.5)</td>
<td>115.1</td>
<td>5.20 (dd, 10.5, 17.5)</td>
<td>111.8</td>
</tr>
<tr>
<td>16</td>
<td>1.34 (s)</td>
<td>21.8</td>
<td>1.34 (s)</td>
<td>21.9</td>
<td>1.27 (s)</td>
<td>27.4</td>
</tr>
<tr>
<td>17</td>
<td>1.69 (s)</td>
<td>22.3</td>
<td>1.68 (s)</td>
<td>22.3</td>
<td>1.37 (s)</td>
<td>23.9</td>
</tr>
<tr>
<td>18</td>
<td>0.86 (s)</td>
<td>33.1</td>
<td>0.86 (s)</td>
<td>33.2</td>
<td>1.02 (s)</td>
<td>34.0</td>
</tr>
<tr>
<td>19</td>
<td>0.88 (s)</td>
<td>21.5</td>
<td>0.88 (s)</td>
<td>21.5</td>
<td>0.89 (s)</td>
<td>22.2</td>
</tr>
<tr>
<td>20</td>
<td>0.75 (s)</td>
<td>13.6</td>
<td>0.75 (s)</td>
<td>13.6</td>
<td>0.79 (s)</td>
<td>15.9</td>
</tr>
<tr>
<td>fucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1′</td>
<td>4.31 (d, 8.0)</td>
<td>97.4</td>
<td>4.36 (d, 8.0)</td>
<td>97.9</td>
<td>4.32 (d, 8.0)</td>
<td>97.6</td>
</tr>
<tr>
<td>2′</td>
<td>3.60 (d, 8.0, 10.0)</td>
<td>71.7</td>
<td>3.73 (d, 8.0, 10.0)</td>
<td>69.3</td>
<td>3.59 (d, 8.0, 10.0)</td>
<td>71.8</td>
</tr>
<tr>
<td>3′</td>
<td>3.76 (dd, 4.0, 10.0)</td>
<td>72.4</td>
<td>4.84 (dd, 3.0, 10.0)</td>
<td>75.7</td>
<td>3.54 (br d, 3.0, 10.0)</td>
<td>71.4</td>
</tr>
<tr>
<td>4′</td>
<td>5.15 (br d, 3.0)</td>
<td>74.3</td>
<td>3.81 (br d, 3.0)</td>
<td>70.3</td>
<td>3.63 (br d, 3.0)</td>
<td>72.6</td>
</tr>
<tr>
<td>5′</td>
<td>3.65 (dq, 1.0, 6.0)</td>
<td>69.2</td>
<td>3.61 (dq, 1.0, 6.0)</td>
<td>70.2</td>
<td>3.58 (br d, 1.0, 6.0)</td>
<td>70.9</td>
</tr>
<tr>
<td>6′</td>
<td>1.18 (d, 6.0)</td>
<td>16.5</td>
<td>1.18 (d, 6.0)</td>
<td>16.3</td>
<td>1.21 (d, 6.0)</td>
<td>16.6</td>
</tr>
<tr>
<td>COCH$_3$</td>
<td>2.16 (s)</td>
<td>20.8</td>
<td>2.17 (s)</td>
<td>21.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>171.4</td>
<td></td>
<td>170.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Assignments were based on $^1{H}$—$^1{H}$ COSY, HMQC, and HMBM experiments.
Table 2. Cytotoxicity of Compounds Isolated from Aster spathulifolius\(^a\)

<table>
<thead>
<tr>
<th>cell line</th>
<th>compound(^a)</th>
<th>A549</th>
<th>SK-OV-3</th>
<th>SK-MEL-2</th>
<th>XF498</th>
<th>HCT15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.9</td>
<td>5.8</td>
<td>3.4</td>
<td>8.9</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.8</td>
<td>3.8</td>
<td>3.5</td>
<td>5.1</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.8</td>
<td>3.4</td>
<td>3.5</td>
<td>3.8</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.9</td>
<td>5.7</td>
<td>4.2</td>
<td>8.9</td>
<td>3.2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td>3.6</td>
<td>3.5</td>
<td>4.2</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>etoposide</td>
<td>1.5</td>
<td>2.7</td>
<td>0.08</td>
<td>2.6</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>doxorubicin</td>
<td>0.012</td>
<td>0.12</td>
<td>0.003</td>
<td>0.01</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) EDT\(_0\) is defined as the concentration (\(\mu\)g/mL) causing a 50% inhibition of cell growth in vitro. \(^b\) All other known compounds obtained in this investigation exhibited EDT\(_0\) values of \(>5 \mu\)g/mL for all cell lines.

fucopyranose. Acidic hydrolysis\(^{14}\) of 3 yielded the aglycon, labda-14(15)-ene-8,13-diol, and a sugar. The aglycon was confirmed by comparison of the optical rotation, \(^1\)H NMR spectroscopic, and EIMS data with literature values.\(^{10,11,17}\) and the sugar was identified by co-TLC and by GC.\(^{15}\) The fucopyranose. Acidic hydrolysis\(^{14}\) of the isolated compounds was subjected to GC analysis: column DB-5 MS (15 m × 0.25 mm × 0.25 \(\mu\)m), detector MS (ionization EI), temperature 100 °C to 280 °C at 5 °C/min. The GC data were obtained on a Jeol JMS 700 mass spectrometer. The HRFABMS data were obtained on a JEOL JMS 700 mass spectrometer. The IR (neat) \(\nu\)max 3432, 2960, 1745, 1640, 1400 cm\(^{-1}\); \(^1\)H NMR and \(^{13}\)C NMR, see Table 1; HRFABMS m/z 510.3187 (calcd for C\(_{28}\)H\(_{46}\)O\(_6\)Na, 510.3192).

(13R)-Labda-7,14-diene 13-O-\(\beta\)-D-fucopyranoside (1): colorless oil; \([\alpha]_D\) \(^{19}\) = 63.3° (c 0.08, CHCl\(_3\)); IR (neat) \(\nu\)max 3446, 1743, 1645, 1366, 1237, 1041 cm\(^{-1}\); \(^1\)H NMR and \(^{13}\)C NMR, see Table 1; HRFABMS m/z 510.3187 (calcd for C\(_{28}\)H\(_{46}\)O\(_6\)Na, 510.3182).

(13R)-Labda-7,14-diene 13-O-\(\beta\)-D-fucopyranoside (2): colorless oil; \([\alpha]_D\) \(^{19}\) = 51.1° (c 0.04, CHCl\(_3\)); IR (neat) \(\nu\)max 3461, 1740, 1640, 1355, 1240, 1038 cm\(^{-1}\); \(^1\)H NMR and \(^{13}\)C NMR, see Table 1; HRFABMS m/z 510.3190 (calcd for C\(_{28}\)H\(_{46}\)O\(_6\)Na, 510.3192).

Alkaline Hydrolysis of Compounds 1 and 2. A solution of either compound 1 or 2 (each, 2 mg) in 10% dry NaO\(_{Me}\)–MeOH (1 mL) was stirred at 40 °C for 2 h. The reaction mixture was neutralized with 2 N HCl and partitioned between H\(_2\)O and n-hexane. The n-hexane layer was purified by silica gel column chromatography (n-hexane–EtOAc, 1:1) to afford (13R)-labda-7,14-diene 13-O-\(\beta\)-fucopyranoside (7) (each, 0.5 mg).

Acidic Hydrolysis. To a solution of each of compounds 3 (2 mg) and 7 (3 mg) in Me\(_2\)CO (3 mL) was added concentrated HCl (0.02 mL), and the mixture was stirred for 10 days at room temperature. Each reaction mixture was shaken with 5 mL of water–CHCl\(_3\). The aqueous layer was evaporated in vacuo, and subjected to silica gel column chromatography eluted with CHCl\(_3\)–MeOH–H\(_2\)O (50:10:1) to give the aglycons labda-14(15)-ene-8,13-diol and labda-7,14-dien-13-ol (4), respectively. The aqueous layer was concentrated to dryness in vacuo, separated over silica gel column chromatography eluted with CHCl\(_3\)–MeOH–H\(_2\)O (30:10:1), and then purified by a Sephadex LH-20 (MeOH) to afford a sugar.

Preparation of Trimethylsilyl Ether of the Methyl 2-(Fucotetrahydroxybutyl)thiazolidine-4(3H)-carboxylate. To a solution of the sugar (2.2 mg, 0.007 mol/L) in pyridine (2 mL) was added i-cysteine methyl ester hydrochloride (3.4 mg, 0.01 mol/L), and the mixture was stirred for 3 h at room temperature. Excess pyridine was removed with a vacuum pump and the reaction mixture dissolved in CH\(_2\)Cl\(_2\) (2 mg) and Et\(_3\)N (7 mg) and EIMS \(m/z\) 247.9958 (calcd for C\(_{28}\)H\(_{46}\)O\(_6\)Na, 247.9963).

Cytotoxicity Evaluation. Cytotoxicity testing was performed in vitro using the SRB (sulfrohamidine B) method\(^{18}\) against five human tumor cell lines, A549 (non-small-cell lung carcinoma), SK-OV-3 (adenocarcinoma, ovarian malignant ascites), SK-MEL-2 (malignant melanoma, metastasis to skin of thigh), XF498 (central nervous system tumor), and HCT15 (colon adenocarcinoma), at the Korea Research Institute of Chemical Technology. Etoposide and doxorubicin were used as positive controls.
Acknowledgment. This study was supported by 63 Research Fund, Sungkyunkwan University, 2003.

Supporting Information Available: 1H and 13C NMR data for compounds 1, 2, 3, and 7. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

2) Kim, T. J. Wild Flowers of Korea; Kugilmedia: Seoul, 1996; p 232.


NP058044E