A New ent-Pimarane-Type Diterpenoid Glycoside from *Siegesbeckia pubescens*

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**Abstract:** A new ent-pimarane-type diterpenoid glucoside, along with eight known same skeleton type were isolated from the ethanol extract of *Siegesbeckia pubescens* Makino by means of various chromatographic techniques (silica gel, RP-8, Sephadex LH-20, Pre-HPLC). Their structures were elucidated on the basis of spectroscopic analyses and the new one identified as ent-15-methylene-2α,16,19-trihydroxy-pimar-8(14)-ene-19-O-β-D-glucopyranoside.

**Keywords:** *Siegesbeckia pubescens*; ent-pimarane-type diterpenoid; pubeside F. © 2018 ACG Publications. All rights reserved.

1. Introduction

The genus *Siegesbeckia* is a small member of Compositae family and only comprises four species, which distributed in tropical, subtropical, and temperate parts of the world [1]. Three species are found in China and have used as “Xi-Xian” included in Chinese Pharmacopoeia for their antirheumatic, lubricate joints and detoxifying properties[2]. Bioactivity studies on extracts or pure components have exhibited multiple positive effects, including antithrombotic, anti-inflammatory, antiallergic, immune-suppressive and so on [3-6]. *Siegesbeicia pubescens* Makino, an annual herb plant, is widely growing in the Midlands and the North of China. Previous investigation on *S. pubescens*, ent-kaurane and ent-pimarane diterpenoids were the main compositions of the plant and exhibited antithrombotic activity[5,7-8]. In the present study, we report the isolation and structure elucidation of a new ent-pimarane diterpenoid, together with eight known ones from the BuOH part of the enthanol extract.

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2. Materials and Methods

2.1. Material

The aerial of *S. pubescens* Makino was collected from Yuexi County, Anhui Province, China, in October 2009. It was identified by Dr. Qing-Shan Yang, Anhui University of Chinese Medicine. A voucher specimen (XF 201301) was deposited at the Laboratory of Phytochemistry, Anhui University of Chinese Medicine.

Optical rotation was recorded on a Jasco P-1020 automatic digital polarimeter. UV spectrum was measured on a Shimadzu UV-2401PC spectrophotometer. IR spectrum was obtained on a Bruker Tensor 27 FT -IR spectrometer with KBr pellet. NMR spectra were recorded on Bruker DRX-400 instruments with TMS as the internal standard. The chemical shifts were given in δ (ppm) scale with 7Li as the reference signal. ESI-MS and HR-ESI-MS spectra were acquired on API QSTAR Pulsar i mass spectrometer. Silica gel (200–300 mesh); and Sephadex LH-20 were used for column chromatography (CC). Preparative HPLC was performed on Waters Auto Purification 2545 system equipped with a Shimadzu ODS-18, 9.4 mm × 250 mm column. Fractions monitored by TLC, and spots were visualized by spraying with 10% H2SO4 in EtOH, followed by heating.

2.2. Extraction and Isolation

The air-dried and powdered aerial of *S. pubescens* Makino (10.7 kg) was diacolated with 95% ethanol (100 L) and 70% ethanol (30 L) at room temperature. The ethanol extract concentrated in vacuo to give a green crude extract, which was suspended in H2O and partitioned successively with petroleum ether (PE), EtOAc and n-BuOH. The n-BuOH part (264.2 g) was chromatographed on silica gel column (2.0 kg, 9.0 × 60 cm) eluting with a CH2Cl2-MeOH gradient system (95:5, 90:10, 85:15, 80:20, 70:30 each 20 L, v/v) to afford fraction Fr. 1–Fr. 6. Each Fraction was decolorized using MCI gel CHP 20P (0.8 L, 4.0 × 80 cm), eluted with 80% MeOH-H2O, and then subjected to Sephadex LH-20 (80 g, 2.0 × 150 cm) eluting with MeOH to yield sub-fractions. Fr. 2-2 (1.8 g) was separated on silica gel column, eluted with CH2Cl2-MeOH (92: 8) to give 7 (83 mg), the rest mix ingredient was purified by preparative HPLC using 35% MeOH-H2O detected at 215 nm to provide 7 (25 mg) and 8 (54 mg). Fr. 4-2 (8.3 g) was chromatographed on silica gel column eluted with CH2Cl2-MeOH (90: 10) to yield 9 (1.26 g). Fr. 4-3 (0.83 g) was subjected to Rp-18 column eluted with 60%MeOH-H2O, and positive Fr. 4-3-2 (30.6 mg ) was purified by preparative HPLC using 45% MeOH-H2O and provided 2 (7.3 mg) and 3 (11.6 mg). Fr. 4-4 (1.31 g) was subjected to silica gel column eluted with CH2Cl2-MeOH (90: 10) to provide 4 (12.8 mg). Fr. 4-4 (0.83 g) was subjected to silica gel CC eluted with CH2Cl2-MeOH (85: 15) to obtain 5 (31.8 mg). Fr. 5.2 (1.48 g) was applied an RP-18 column and isocratic elution (60 % MeOH- H2O) to yield Fr. 5.2.2, which further purified by preparative HPLC (40 % MeOH- H2O) to afford 6 (8 mg). Compound 1 (13 mg) was isolated from Fr.5.3 using repeated silica gel CC with CH2Cl2-MeOH (85: 15) and preparative HPLC with 45 % MeOH- H2O.

![Figure 1. Chemical Structures of compounds 1-9](image-url)
Figure 2. Key $^1$H-$^1$H COSY and HMBC correlations of compound 1

Figure 3. Key ROESY corrections of compound 1

2.3. Spectroscopic Data

*Pubeside F (I):* White amorphous powder; $[\alpha]_{D}^{20.0} = -32.20$ (c 0.001, MeOH); UV (MeOH): $\lambda_{\text{max}}$ (log $\varepsilon$) = 204 (3.75) nm; IR (KBr): $\nu_{\text{max}} = 3416, 2924, 2850, 1645, 1597, 1464, 1375, 1080$ cm$^{-1}$; $^1$H-NMR and $^{13}$C-NMR (MeOD, 400/100 MHz) see Table 1; HR-ESI-MS calcd for C$_{26}$H$_{44}$O$_8$Na [M + Na]$^+$ 507.2934, found 507.2926.

2.4. Acid Hydrolysis

Compound I (3 mg) were individually refluxed with 5 % HCl in MeOH (5 mL) for 4 hours. The solution was diluted with H$_2$O (5 mL) and extracted with EtOAc (10 mL) for 3 times. The aqueous layer was neutralized with NaHCO$_3$ and concentrated in vacumn to give a residue. The residue was purified by RP-18 column, eluted with 20% MeOH-H$_2$O. The sugar unit was identified as D-glucose on the basis of TLC and optical rotation ([$\alpha$]$_D^{18.3}$: +40.0 (c 0.05, MeOH)) [9,10].

3. Results and Discussion

3.1. Structure Elucidation

Compound I was obtained as a white amorphous power. Its molecular formula was determined to be C$_{26}$H$_{44}$O$_8$ with five degrees of unsaturation on the basis of the HR-ESIMS (positive ion): $m/z$ 507.2926 [M + Na]$^+$ (calcd. for C$_{26}$H$_{44}$O$_8$Na) and the $^{13}$C NMR data (Table1). The IR spectrum showed the presence of hydroxyl (3416 cm$^{-1}$) and double bond (1645 cm$^{-1}$) functionalities. The $^1$H NMR spectrum of I exhibited three methyl singlet signals at $\delta_H$ 0.85, 0.94, 1.09; three oxygenated-
methylene groups \([\delta_H 4.04, 3.31 (1H each, d, 11.6 Hz), 3.61 (2H, m) and 3.87 (1H, dd, 12.0, 6.2 Hz), 3.71 (1H, d, 12.0, 4.8 Hz)]\) signals; one olefin proton \([\delta_H 5.24 (s)\), and an anomic proton \([\delta_H 4.20 (d, J = 7.6 Hz)]\) signals. The \(^{13}\)C NMR spectrum of 1 displayed 26 carbon resonances, according to three methyl, nine methylene, four methine, four quaternary carbons, and a glucopyranosyl moiety. The NMR characters of 1 were similar to those of ent-2\(\alpha\),15,16,19-tetrahydroxypimar-8(14)-en-19-O-\(\beta\)-glucopyranoside\([11]\) except for the side chain in position C-13. The HMBC cross-peaks (Figure 2) from \(\delta_H 0.94 (H-17)\) to C-12, C-13, C-14 and C-15 together with the COSY correlations of H-15/H-16 indicated the carbon signal \(\delta_C 44.5 (t)\) should be connect to C-13. In addition, the HMBC cross-peaks from the anomic proton \(\delta_H 4.20\) to C-19, and the coupling constant \((J = 7.6 Hz)\) indicated that sugar moieties was attached to C-19 via a \(\beta\)-linkage. Furthermore, the key expected correlations were observed as follows: from \(\delta_H 0.85 (20\text{-Me})\) to C-1, C-5, C-9 and C-10, from \(\delta_H 1.09 (18\text{-Me})\) to C-3, C-4, C-5 and C-19 in the HMBC spectrum, and of H-1/H-2/H-3, H-5/H-6/H-7, H-9/H-11/H-12 in the \(^{1}\text{H}\) \(^{13}\)C COSY spectrum. Based on the above evidences, the planar structure of 1 was established.

The relative configuration of of 1 was established by a ROESY experiment (Figure 3). The correlations H-2 ↔ Me-20 indicated \(\beta\)-orientation of 2-OH, and H-19↔Me-20 revealed Me-18 adopted \(\beta\)-orientation. Therefore, the structure of compound 1 was identified as ent-15-methylene-2\(\alpha\),16,19-trihydroxy-pimar-8(14)-ene-19-O-\(\beta\)-D-glucopyranoside, and named pubeside F.

From the NMR and MS data and corresponding to those form literatures, the known ent-pimarane diterpenoids from the plant were identified as ent-2\(\alpha\),15R,16,19- tetrahydroxypimar-8(14)-ene (2)\([12]\), kirenol (3)\([13]\), ent-2-oxo-15,16,19-trihydroxypimar-8(14)-ene (4)\([8]\), pubeside D (5)\([8]\), ent-2\(\alpha\),15,16-trihydroxypimar-8(14)-en-19-oic acid (6)\([8]\), ent-16-O-acetoxy-2\(\alpha\),16,19-trihydroxypimar-8 (14)-ene (7) \([14]\), ent-15-O-acetoxy-2\(\alpha\),16, 19-trihydroxypimar-8(14)-ene (8)\([14]\) and darutoside (9)\([15]\).

### Table 1. \(^{1}\text{H}\) and \(^{13}\text{C}\) NMR data for compound 1

<table>
<thead>
<tr>
<th>Position</th>
<th>(\delta_C)</th>
<th>(\delta_H)</th>
<th>Position</th>
<th>(\delta_C)</th>
<th>(\delta_H)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>50.0 (t)</td>
<td>1.99, 1.04 (1H each, m)</td>
<td>14</td>
<td>132.6 (d)</td>
<td>5.24 (1H, s)</td>
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<tr>
<td>2</td>
<td>65.5 (d)</td>
<td>3.85 (1H, m)</td>
<td>15</td>
<td>44.5 (t)</td>
<td>1.64, 1.55 (1H each, m)</td>
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<tr>
<td>3</td>
<td>45.6 (t)</td>
<td>2.35, 0.86 (1H each, m)</td>
<td>16</td>
<td>60.0 (t)</td>
<td>3.61 (2H, m)</td>
</tr>
<tr>
<td>4</td>
<td>40.8 (s)</td>
<td></td>
<td>17</td>
<td>29.1 (q)</td>
<td>0.94 (3H, s)</td>
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<tr>
<td>5</td>
<td>56.7 (d)</td>
<td>1.18 (1H, m)</td>
<td>18</td>
<td>28.5 (q)</td>
<td>1.09 (3H, s)</td>
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<tr>
<td>6</td>
<td>23.5 (t)</td>
<td>1.73, 1.31 (1H each, m)</td>
<td>19</td>
<td>74.2 (t)</td>
<td>4.04, 3.31 (1H each, d, 11.6)</td>
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<tr>
<td>7</td>
<td>37.4 (t)</td>
<td>2.26, 2.04 (1H each, m)</td>
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<td>17.6 (q)</td>
<td>0.85 (3H, s)</td>
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<tr>
<td>8</td>
<td>137.3 (s)</td>
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<td>1’</td>
<td>105.1 (d)</td>
<td>4.20 (1H, d, 7.6)</td>
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<td>9</td>
<td>52.6 (d)</td>
<td>1.81 (1H, m)</td>
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<td>75.4 (d)</td>
<td>3.19 (1H, t, 8.4)</td>
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<td>10</td>
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<td>3’</td>
<td>78.4 (d)</td>
<td>3.35 (1H, m)</td>
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<tr>
<td>11</td>
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<td>3.28 (1H, m)</td>
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<tr>
<td>12</td>
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<td>5’</td>
<td>77.9 (d)</td>
<td>3.27 (1H, m)</td>
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<tr>
<td>13</td>
<td>34.0 (s)</td>
<td></td>
<td>6’</td>
<td>61.8 (t)</td>
<td>3.87 (1H, dd, 12.0, 6.2)</td>
</tr>
</tbody>
</table>

*400 MHz for \(^{1}\text{H}\) NMR and 100 MHz for \(^{13}\text{C}\) NMR in MeOD in ppm, \(J\) in Hz

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### Supporting Information

Supporting information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)
References