A new 3,4-seco-oleanane-type triterpenoid with an unusual enedione moiety from Hypericum ascyron

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**A B S T R A C T**

A novel 3,4-seco-oleanane-type triterpenoid named 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid (1), bearing an unusual enedione moiety, was isolated from the aerial parts of Hypericum ascyron, together with a known feiedelane-type triterpenoid friedelin (2). The structure of 1 with absolute configuration was elucidated on the basis of spectroscopic methods and a single-crystal X-ray diffraction analysis.

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1. Introduction

The genus Hypericum, including about 400 species, is widely distributed in the whole world \cite{1}. Some plants of the genus have been used as traditional Chinese medicine for a long time, such as Hypericum perforatum (St. John’s wort), Hypericum wightianum, Hypericum japonicum, and Hypericum ascyron \cite{1}. Phytochemical investigations on plants of this genus have revealed polycyclic polyprenylated acylphloroglucinols (PPAPs) as their main secondary metabolites \cite{2}, which are a class of natural products possessing intriguing structures and diverse biological activities \cite{2}. Beside PPAPs, dianthrone derivatives \cite{3}, rottlerin-type compounds \cite{4–6}, and xanthones \cite{7,8} are also reported from this genus.

In the course of our continuous program for bioactive metabolites from the genus Hypericum \cite{9,10}, the aerial parts of H. ascyron, collected from Dabie Mountain areas, were phytochemically investigated, which led to the isolation of a new (3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid (1), and a known triterpenoids (friedelin, 2) (Fig. 1). To the best of our knowledge, triterpenoids, with only a few examples, were rarely reported from this genus \cite{11–13}. In addition, this is the first time to report 3,4-seco-oleanane-type and friedelan-type triterpenoids from the genus Hypericum. In this paper, we report the isolation and structure elucidation of compounds 1 and 2, as well as the cytotoxic evaluation of 1 against five human cancer cell lines.

2. Experimental

2.1. General

Optical rotations were determined with a Perkin-Elmer 241 polarimeter. UV and FT-IR spectra were measured using a Varian Cary 50 and a Bruker Vertex 70, respectively. NMR
The aerial parts of *H. ascron* (50 kg) were extracted with 95% ethanol (200 L, four times). After concentration, the extract was suspended in H$_2$O and partitioned with petroleum ether and chloroform, successively. The petroleum ether-soluble extract (780 g) was separated by a silica gel column chromatography (CC, 5 kg, 20 × 120 cm; petroleum ether–acetone, 50:1 → 0:100) to furnish seven fractions (1–7). Fr. 6 (32 g) was decolorized on MCI gel CC, eluting with 90% MeOH in H$_2$O, and then subjected to a silica gel CC (petroleum ether–acetone 30:1 → 0:1) to give five subfractions (Fr.6.1–Fr.6.5). Fr.6.4 was chromatographed on Sephadex LH-20 (CH$_3$OH) to afford four additional fractions Fr.6.4.1–Fr.6.4.4. Subfraction Fr.6.4.2 was then separated by ODS eluting with CH$_3$OH in H$_2$O from 50% to 100% to yield Fr.6.4.2.1–Fr.6.4.2.6. Fr.6.4.2.4 was purified with repeated semipreparative HPLC (80% CH$_3$OH and 75% CH$_3$CN) to give compound 1 (2 mg). The crystals of Fr. 7 were filtered and recrystallized to give compound 2 (14 mg).

$$\text{3.4} \text{ seco} – \text{ olean} – 13(18) \leftarrow \text{ cembr–12,19} \leftarrow \text{ dione} – 3 \leftarrow \text{ 13–ocicaridin}$$

Colorless crystal (CH$_3$OH); [α]$^20_D$ = 50.4 (c = 0.09, CH$_3$OH); UV (CH$_3$OH) $\lambda_{max}$ (log $\varepsilon$) = 203 (3.70) and 257 (3.64) nm; IR $\nu_{max}$ = 3432, 1738, 1687, 1667, 1470, 1458, 1389, and 1172 cm$^{-1}$; HREIMS [M + Na]$^+$ m/z 493.3237 (calcld for C$_{30}$H$_{48}$O$_{12}$Na, 493.3294); for $^1$H NMR (400 MHz) and $^{13}$C NMR (100 MHz) data see Table 1; crystallographic data for the structure of 1 have been deposited in the Cambridge Crystallographic Data Centre (CCDC deposition number: 981814).

Friedelin

$$\text{3.4} \text{ seco} – \text{ olean} – 13(18) \leftarrow \text{ cembr–12,19} \leftarrow \text{ dione} – 3 \leftarrow \text{ 13–ocicaridin}$$

2.4. X-ray crystallographic analysis of compound 1

Crystal data for compound 1: C$_{30}$H$_{48}$O$_{12}$, $M = 470.67$, monoclinic, $a = 9.3962$ (2), $b = 12.5915$ (3), $c = 11.2401$ (3), $\alpha = 90.00^\circ$, $\beta = 93.6990$ (10)$^\circ$, $\gamma = 90.00^\circ$, $V = 1327.07$ (6) Å$^3$, $T = 100$ (2) K, space group P2$_1$, $Z = 2$, $\mu$ (CuK$_\alpha$) = 0.594 mm$^{-1}$, 12007 reflections measured, 4399 independent reflections ($R_{int} = 0.0444$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$). The final $R_I$ values were 0.0454 ($R > 2\sigma (I)$).

Flack parameter = 0.04 (19). The Hooft parameter is 0.06 (6) for 1983 Bijvoet pairs.

2.2. Plant material

The aerial parts of *H. ascron* were collected from Dabie Mountain areas, Hubei Province, China, in October 2012, and identified by Professor Jianping Wang. A voucher specimen (ID 20121012) has been deposited with Herbarium of Materia Medica, Faculty of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, Wuhan, China.
Compound 1 was isolated as colorless crystals. The molecular formula was assigned as C_{30}H_{48}O_{4} based on the pseudomolecular ion peak at m/z 493.3237 ([M + Na]^{+}, calcd for C_{30}H_{48}O_{4}Na, 493.3294) in the HRESIMS, indicating eight degrees of unsaturation. The IR spectrum showed absorption bands at 1738, 1687, and 1667 cm\(^{-1}\), suggesting the presence of carboxy and carbonyl groups. The \(^1\)H NMR spectrum (Table 1) exhibited eight methyl signals at 0.82 (3H, d, J = 6.8 Hz), 0.90 (3H, s), 0.92 (3H, d, J = 7.0 Hz), 0.98 (3H, s), 1.01 (3H, s), 1.08 (3H, s), 1.12 (3H, s), and 1.26 (3H, s). The \(^1\)C NMR spectrum (Fig. 2) and DEPT spectra displayed 30 carbon signals assignable to eight methyls, nine methylenes, three methines, five nonoxygenated quaternary carbons, two carbonyls (\(\delta_c\) 205.4 and 211.3), a carbonyl (\(\delta_c\) 176.8), and two quaternary olefinic carbons (\(\delta_c\) 145.1 and 148.2). Since four degrees of unsaturation were occupied by the carbonyl, the carboxy, and the olefinic groups, compound 1 was deduced to be a triterpenoid possessing a tetracyclic nucleus.

Detailed analyses of the \(^1\)H–\(^1\)H COSY spectrum and trace HMBC correlations (Fig. 2) confirmed the presence of cyclic rings B to E of an oleane-type triterpenoid. The HMBC correlations from CH\(_2\)-29, CH\(_2\)-30, and H-21 to C-19, from CH\(_2\)-28 and H-22 to C-18, from CH\(_2\)-27 and H-11 to C-13, and from H-11 to C-12 revealed the location of the two carbonyls at C-12 and C-19, and double bond between C-13 and C-18, which formed an unusual enedione moiety as the known compound olean-13(18)-ene-12,19-dione-3-oic acid, which is the first 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid, and which is the first 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid, which is the first 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid, which is the first 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid [14]. The appearance of a 3,4-seco-type A ring was deduced to be the same as 3,4-seco-olean-18-en-3,28-dioic acid [15] by \(^1\)H–\(^1\)H COSY and HMBC correlations as follows: \(^1\)H–\(^1\)H COSY peaks of CH\(_2\)-23/H-4, CH\(_2\)-24/H-4, H-4/H-5, and H-1/H-2; HMBC correlations from CH\(_2\)-23 and CH\(_2\)-24 to C-5, H-2 to C-3, and CH\(_2\)-25 to C-1, C-5, and C-10. Thus, the planar structure of compound 1 was elucidated.

The relative configuration of 1 was primarily determined by comparison of its \(^1\)H and \(^13\)C NMR with the data of this compound in literature class and this assignment was consistent with biogenetic considerations. However, further solid evidence was necessary for its absolute configuration determination. Finally, a crystal suitable for X-ray diffraction experiment (Fig. 3) was obtained from CH\(_3\)OH, which enabled us to determine the structure and absolute configuration of compound 1 conclusively (Flack parameter = 0.04; Hooft parameter = 0.06). Herein, the structure of 1 was established as 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid, which is the first 3,4-seco-olean with an unusual enedione moiety.

The known triterpenoid, friedelin (2), was identified by comparison of its NMR data and optical rotation value with those reported in the literature [16].

Compound 1 was tested for its cytotoxic activity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480), and it showed no activity to all tested cancer cell lines up to a concentration of 40 μM.

**Table 1**

\(^1\)H and \(^13\)C NMR data for compound 1.\(^a\)

<table>
<thead>
<tr>
<th>No.</th>
<th>(\delta_H) (J in Hz)</th>
<th>(\delta_C)</th>
<th>No.</th>
<th>(\delta_H) (J in Hz)</th>
<th>(\delta_C)</th>
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<tbody>
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<td>1</td>
<td>1.73 (1H, m)</td>
<td>32.4 16</td>
<td>1</td>
<td>1.81 (1H, m)</td>
<td>36.7</td>
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<tr>
<td>2</td>
<td>1.65 (1H, m)</td>
<td>1.56 (1H, m)</td>
<td>2</td>
<td>2.19 (2H, m)</td>
<td>27.8 17</td>
</tr>
<tr>
<td>3</td>
<td>1.90 (1H, m)</td>
<td>25.3 19</td>
<td>4</td>
<td>4.74 (2H, t)</td>
<td>46.3</td>
</tr>
<tr>
<td>5</td>
<td>1.06 (1H, m)</td>
<td>18.0 21</td>
<td>6</td>
<td>1.46 (2H, m)</td>
<td>37.7 22</td>
</tr>
<tr>
<td>7</td>
<td>1.42 (1H, m)</td>
<td>32.7 22</td>
<td>8</td>
<td>41.2 23</td>
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<tr>
<td>9</td>
<td>1.87 (1H, m)</td>
<td>4.09 24</td>
<td>10</td>
<td>4.03 25</td>
<td>19.4</td>
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<tr>
<td>11</td>
<td>2.47 (1H, dd, 15.7, 13.3)</td>
<td>39.7 26</td>
<td>12</td>
<td>205.4 27</td>
<td>20.5</td>
</tr>
<tr>
<td>13</td>
<td>145.1 28</td>
<td>23.1</td>
<td>14</td>
<td>45.7 29</td>
<td>24.5</td>
</tr>
<tr>
<td>15</td>
<td>2.82 (1H, m)</td>
<td>25.0 30</td>
<td>1.19 (1H, dr, 13.7, 3.4)</td>
<td>24.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 400 MHz for \(^1\)H and 100 MHz for \(^13\)C, in CDCl\(_3\).

3. Results and discussion
4. Conclusions

As far as we known, triterpenoids were rarely reported from plants of this genus, with no more than ten examples, including lupine-type triterpenoids from *Hypericum balearicum* [11], lupine- and oleanane-type triterpenoids from *Hypericum geminiflorum* [12], and amyrin-type triterpenoids from *H. perfratum* [13]. Therefore, this is the first identification of triterpenoids with 3,4-seco-oleanane and friedelane skeletons from this genus. Moreover, 3,4-seco-olean-13(18)-ene-12,19-dione-3-oic acid (1) itself possesses unexpected functionalities of 3,4-seco A-ring and a rare enedione moiety, which make it to be a distinctive member of oleanane-type triterpenoids. It is notable that for most 3,4-seco-oleane-type triterpenoids, C-4 is an oxygenated quaternary carbon, and in the case of 1, it is an unusual methine. The interesting structural characteristics of 1 together with its exclusive origin of the genus *Hypericum* make it outstanding from the family of oleane-type triterpenoids.

Acknowledgments

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Appendix A supplementary data

Supplementary data associated with this article including HRESIMS, $^1$H and $^{13}$C NMR, IR, UV, and CD spectra can be found online at http://dx.doi.org/10.1016/j.fitote.2015.04.009.

References