Structural Elucidation of a Coumarin with New Skeleton from *Artemisia ordosica*

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Abstract: A new coumarin, named as arteordocoumarin A (1), together with eight known compounds (2-9) were isolated from the CHCl₃ extract of *Artemisia ordosica* (*A. ordosica*). The structures of 1 was elucidated by spectroscopic methods, including UV, IR, HR-ESI-MS and extensive 1D and 2D NMR techniques.

Keywords: Arteordocoumarin A; *Artemisia ordosica*; NMR.

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1. Introduction

*A. ordosica*, Asteraceae family, is one of the main arido-active shrubs growing in the arid and semi-arid areas of the north China including Inner Mongolia, Ningxia, Gansu and Shanxi [1, 2]. The aerial parts of A. ordosica is utilized as a folk medicine for expelling rheumatism, clearing heat, and dispelling swelling [3]. Sterols [4], coumarins [5], terpenoids [6], flavonoids [7, 8] and acetylenes [3] were isolated previously from this plant. However, the secondary metabolites from *Artemisia ordosica* often differ when grown in different ecological environments. In order to continue our research on the bioactive secondary metabolites from *Artemisia ordosica* collected in Tongliao of Inner Mongolia, China, we now describe the isolation and structure elucidation of a new coumarin compound, together with eight known ones.

2. Materials and Methods

2.1. Instrumentation and Reagents

A Shimadzu UV-2201 spectrometer (Shimadzu, Japan) was used to record the UV spectra. The IR spectra were recorded in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer (Shimadzu, Japan). A Waters Xevo G2-S QT (Waters, USA) was used to measure the HR-ESI-MS spectra. NMR spectra were measured on a Bruker AV–500 spectrometer (Bruker, Germany) with tetramethylsilane (TMS) as the internal reference, and chemical shifts are expressed in δ (ppm). Column

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chromatography was performed by using silica gel (200-300 mesh, Marine Chemical Factory, Qingdao, China).

2.2. Plant Materials

The aerial parts of *A. ordosica* were collected in Tongliao, Inner Mongolia of China, in June 2017, and identified by Prof. Buhebateer (Inner Mongolia University for Nationalities). A voucher (ref. no. 20170612) has been deposited at the Herbarium of college of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

2.3. Extraction

Ground-dried aerial parts of *A. ordosica* (2.0 kg) were extracted with CHCl₃ (25 L) under reflux after extraction with 10 L petroleum ether. Evaporation of the solvent under reduced pressure delivered the CHCl₃ extract (150.0 g). The CHCl₃ extract was fractionated by column chromatography on silica gel and gradually eluted with petroleum ether-CH₂COCH₃ (60:1 to 20:1) to give 3 fractions (Fr. 1-3). Fr. 1 (320 mg) was separated by TLC (cyclohexane-ethyl acetate, 10:1) yielding 1 (17 mg), 2 (11 mg) and 3 (15 mg); Fr. 2 (400.0 mg) was further eluted on a Sephadex LH-20 column with MeOH:CHCl₃ (v/v: 1:1) and then separated by TLC (cyclohexane-ethyl acetate, 7:1) yielding 4 (13 mg), 5 (9 mg), 6 (15 mg) and 7 (18 mg). Fr. 3 (330 mg) was separated by TLC (cyclohexane-ethyl acetate, 3:1) yielding 8 (16 mg) and 9 (21 mg).

*Arteordocoumarin A* (1): White needle; ¹H-NMR (500MHz, DMSO-d₆) and ¹³C-NMR (125MHz, DMSO-d₆) spectral data see Table 1; HR-ESI-MS at m/z 209.0467 [M-H]⁻ (caled for C₁₀H₁₅O₅, 209.0450).

6,7-dimethoxycoumarin (2) [9]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 6.31 (1H, d, J = 9.5 Hz, H-3), 7.65 (1H, d, J = 9.5 Hz, H-4), 6.86 (1H, s, H-5), 6.88 (1H, s, H-8), 3.94 (3H, s, 6-OCH₃), 3.97 (3H, s, 7-OCH₃); ¹³C-NMR (125MHz, CDCl₃) δC: 161.5 (C-2), 113.6 (C-3), 143.3 (C-4), 107.9 (C-5), 146.3 (C-6), 152.8 (C-7), 100.0 (C-8), 150.0 (C-9), 111.4 (C-10), 56.4 (O-CH₃), 56.3 (7-OCH₃).

6,7,8-trimethoxycoumarin (3) [10]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 6.06 (1H, d, J = 9.0 Hz, H-3), 7.52 (1H, d, J = 9.0 Hz, H-4), 7.30 (1H, s, H-5), 3.88 (3H, s, 6-OCH₃), 3.86 (3H, s, 8-OCH₃), 3.82 (3H, s, 7-OCH₃); ¹³C-NMR (125MHz, CDCl₃) δC: 165.4 (C-2), 115.5 (C-3), 140.3 (C-4), 114.9 (C-5), 145.0 (C-6), 125.1 (C-7), 133.5 (C-8), 134.4 (C-9), 120.7 (C-10), 56.4 (6-OCH₃), 56.1 (8-OCH₃), 55.8 (7-OCH₃).

6-hydroxy-7-methoxycoumarin (4) [9]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 6.31 (1H, d, J = 9.5 Hz, H-3), 7.64 (1H, d, J = 9.5 Hz, H-4), 6.93 (1H, s, H-5), 6.85 (1H, s, H-8), 3.91 (3H, s, 7-OCH₃); ¹³C-NMR (125MHz, CDCl₃) δC: 161.6 (C-2), 113.5 (C-3), 143.5 (C-4), 108.3 (C-5), 147.3 (C-6), 152.0 (C-7), 103.0 (C-8), 153.0 (C-9), 113.4 (C-10), 56.5 (O-CH₃).

4-hydroxylacetonophene (5) [11]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 7.93 (2H, d, J = 8.5 Hz, H-2,6), 6.91 (2H, d, J = 8.5 Hz, H-3,5), 2.58 (3H, s, -CH₃); ¹³C-NMR (125MHz, CDCl₃) δC: 130.4 (C-1), 131.0 (C-2), 115.3 (C-3), 160.1 (C-4), 115.3 (C-5), 131.0 (C-6), 197.9 (C=O), 26.3 (-CH₃).

4-hydroxy-5-methoxylactophene (6) [11]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 7.59 (1H, d, J = 2.0 Hz, H-2), 6.98 (1H, d, J = 8.0 Hz, H-5), 7.62 (1H, d, J = 2.0 Hz, H-6), 3.90 (3H, s, -OCH₃), 2.51 (3H, s, -CH₃); ¹³C-NMR (125MHz, CDCl₃) δC: 129.9 (C-1), 111.1 (C-2), 147.0 (C-3), 146.8 (C-4), 114.4 (C-5), 126.9 (C-6), 190.3 (C=O), 56.8 (-OCH₃), 26.3 (-CH₃).

4-hydroxybenzaldehyde (7) [12]: White needle; ¹H-NMR (500MHz, CDCl₃) δH: 7.82 (2H, d, J = 8.5 Hz, H-2,6), 7.02 (2H, d, J = 8.5 Hz, H-3,5), 9.85 (1H, s, -CHO); ¹³C-NMR (125MHz, CDCl₃) δC: 139.3 (C-1), 132.5 (C-2), 116.0 (C-3), 162.0 (C-4), 116.0 (C-5), 132.5 (C-6), 191.3 (-CHO).
4-hydroxy-5-methoxybenzaldehyde (8) [13]: White needle; \(^1\)H-NMR (500MHz, CDCl\(_3\)) \(\delta\)H: 7.43 (1H, brs, H-2), 7.05 (1H, d, \(J = 8.5\) Hz, H-5), 7.45 (1H, brd, \(J = 8.5\) Hz, H-6), 9.83 (1H, s, -CHO), 3.99 (3H, s, -OCH\(_3\)); \(^1\)C-NMR (125MHz, CDCl\(_3\)) \(\delta\)C: 129.9 (C-1), 108.8 (C-2), 151.7 (C-3), 147.2 (C-4), 114.4 (C-5), 127.6 (C-6), 191.0 (C=O), 56.1 (-OCH\(_3\)).

4,5-dihydroxybenzaldehyde (9) [13]: \(^1\)H-NMR (500MHz, CDCl\(_3\)) \(\delta\)H: 7.44 (1H, d, \(J = 2.0\) Hz, H-2), 6.96 (1H, d, \(J = 8.0\) Hz, H-5), 7.46 (1H, brd, \(J = 8.0\), 2.0 Hz, H-6), 9.85 (1H, s, -CHO); \(^1\)C-NMR (125MHz, CDCl\(_3\)) \(\delta\)C: 129.8 (C-1), 108.9 (C-2), 146.8 (C-3), 146.2 (C-4), 114.3 (C-5), 127.7 (C-6), 191.0 (C=O).

3. Results and Discussion

From the CDCl\(_3\) of A. ordosica, six compounds were obtained using chromatographic methods (CC and TLC). On the basis of \(^1\)H, \(^1\)C NMR, COSY, HSQC, HMBC and HR-ESI-MS spectra, and modified Mosher’s method as well as by comparison with previous reports [9-13], compounds 1 was identified as a coumarin with new skeleton while the remaining eight compounds were found to be the known compounds, 6,7-dimethoxycoumarin (2), 6,7,8-trimethoxycoumarin (3), 6-hydroxy-7-methoxycoumarin (4), 4-hydroxylacetophenone (5), 4-hydroxy-5-methoxylacetophenone (6), 4-hydroxybenzaldehyde (7), 4-hydroxy-5-methoxybenzaldehyde (8) and 4,5-dihydroxybenzaldehyde (9) (Figure 1).

![Figure 1. Structures of compounds 1-9](image)

Compound 1 was obtained as a white needle, mp 137-139 °C; IR (KBr) \(\nu_{max}\) (cm\(^{-1}\)): 3312, 1683, 1643 and 1320 cm\(^{-1}\). The molecular formula was determined to be C\(_{10}\)H\(_8\)O\(_5\) by HR-ESI-MS exhibiting a pseudomolecular ion peak at \(m/z\) 209.0467 [M-H]\(^+\) (calcd for C\(_{10}\)H\(_8\)O\(_5\), 209.0450). In the \(^1\)H NMR spectrum (Table 1), two characteristic resonances for H-3 and H-4 of a coumarin at \(\delta_H\) 6.03 (1H, d, \(J = 10.0\) Hz, H-3) and 8.06 (1H, d, \(J = 10.0\) Hz, H-4). In addition, the signals at \(\delta_H\) 3.64 (3H, s) and 3.82 (3H, s) indicated the presence of two methoxy groups. The remaining signal at \(\delta_H\) 6.39 (1H, s) was assigned to H-9 compared with the data of H-25 (\(\delta_H\) 6.21) in fasciospongesides A [14], which was confirmed by the HMBC correlations (Figure 2) from \(\delta_H\) 6.39 (1H, s, H-9) to C-5 (\(\delta_C\) 104.8), C-6 (\(\delta_C\) 152.3) and C-7 (\(\delta_C\) 133.5).

![Figure 2. Selected HMBC correlations for 1](image)
Table 1. $^1$H (500 MHz) and $^{13}$C-NMR (125MHz) data of compound 1 in DMSO-$d_6$

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_H$ (ppm), $J$ (Hz)</th>
<th>$\delta_C$ (ppm)</th>
</tr>
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<tr>
<td>2</td>
<td>—</td>
<td>161.5</td>
</tr>
<tr>
<td>3</td>
<td>6.03 d (10.0)</td>
<td>109.1</td>
</tr>
<tr>
<td>4</td>
<td>8.09 d (10.0)</td>
<td>141.1</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>104.8</td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>152.3</td>
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<tr>
<td>7</td>
<td>—</td>
<td>133.5</td>
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<tr>
<td>8</td>
<td>—</td>
<td>157.0</td>
</tr>
<tr>
<td>9</td>
<td>6.39 s</td>
<td>89.7</td>
</tr>
<tr>
<td>7-OCH$_3$</td>
<td>3.64 s</td>
<td>60.6</td>
</tr>
<tr>
<td>8-OCH$_3$</td>
<td>3.82 s</td>
<td>56.5</td>
</tr>
</tbody>
</table>

The $^{13}$C NMR spectrum of 1 showed 10 carbon signals, of which 8 were assigned to the coumarin skeleton part and 2 to the two methoxy groups. In coumarin skeleton part, there were only 8 carbon signals ($\delta_C$ 161.5, 109.1, 141.8, 104.8, 152.3, 133.5, 157.0, 89.7), which was different from the 9 carbon signals of a usual coumarin skeleton [9, 10]. The benzene in a usual coumarin skeleton was substituted by the 1,3-diene cyclopentane in the new coumarin skeleton, in which the HMBC correlations from H-4 to C-2 ($\delta_C$ 161.5), C-6 ($\delta_C$ 152.3) and C-9 ($\delta_C$ 89.7), and H-3 to C-2 ($\delta_C$ 161.5) and C-5 ($\delta_C$ 104.8), H-9 to C-5 ($\delta_C$ 104.8), C-6 ($\delta_C$ 152.3) and C-7 ($\delta_C$ 133.5) were show. In addition, the HMBC correlations $\delta_H$ 3.64 (-OCH$_3$) to $\delta_C$ 133.5 (C-7) and 3.82 (-OCH$_3$) to $\delta_C$ 157.0 (C-8) revealed that the two methoxy groups were linked to the C-7 and C-8, respectively. The modified Mosher’s method was used to produce (R)- and (S)-MTPA esters (1a, 1b), and signals corresponding to H-3, H-4 and 8-OCH$_3$ were relatively deshielded in 1a compared to 1b, indicating that the absolute configuration of C-9 is S (Figure 3). Thus, the structure of compound 1 was elucidated and named as arteordocoumarin A.

Figure 3. Results with the modified Mosher’s method ($\Delta = \delta_S - \delta_R$) for compound 1

Acknowledgments

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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