

Structure Elucidation of A New Iridoid from *Artemisia integrifolia*

Qinghu Wang^{✉*}, Junsheng Hao[✉], Yanhua Xu[✉] and Yiping Bao[✉]

College of Traditional Mongolian Medicine, Inner Mongolia University for Nationalities,
Tongliao 028000, Inner Mongolia, China

(Received January 04, 2018; Revised February 22, 2018; Accepted March 03, 2018)

Abstract: The ether extract of *Artemisia integrifolia* was separated by chromatography and afforded a new iridoid, arteintegin A (**6**), together with five known compounds, namely chamazulene (**1**), acetylenes (E)-**2**, (E)-**3**, (Z)-**4**, eugenol (**5**). The structure elucidation of the new compound was carried out by 1D (¹H NMR and ¹³C NMR) and 2D-NMR (COSY, HSQC and NOESY) spectral analysis.

Keywords: Iridoid; *Artemisia integrifolia*; NMR; HR-ESI-MS. 2018 ACG Publications. All rights reserved.

1. Introduction

Artemisia integrifolia L, belonging to the family Compositae, is distributed throughout Inner Mongolia [1]. It is characteristic cuisine of Daur in Hulunbeier of Inner Mongolia, and is considered to be the green vegetables for possessing rich in nutrition and heat-clearing and detoxicating [2]. In Mongolian medicine, *Artemisia integrifolia* is cold in property, bitter with a flavor. It has been used as a folk medicine to treat cardiovascular disease and liver diseases [2]. The ethanol extract of *Artemisia integrifolia* possess very good *in vitro* superoxide, hydroxyl and nitric oxide radical scavenging, and lipid peroxidation inhibiting activities [3]. The phytochemical screening of the plant extract showed the presence of flavonoids, coumarin, amino acids and proteins [4]. Recently, we carried out a systematic chemical study on the ether extract from the aerial parts of *Artemisia integrifolia*, which resulted in the isolation of a new iridoid with a new carbon skeleton. Here, we report the structural characterization of the new compound by spectral analysis.

2. Materials and Methods

2.1. Instrumentation and Reagents

The IR spectra were recored in KBr discs on a Thermo Nicolet 200 double beam spectrophotometer. The HR-ESI-MS spectra were measured on a Waters Xevo G2-S QT (Waters, USA). NMR spectra were measured in DMSO-d₆ using a Bruker AV-500 MHz spectrometer (Bruker, Germany) with tetramethylsilane (TMS) as the internal reference, and chemical shifts are expressed in δ (ppm). Column chromatography was performed by using silica gel (200-300 mesh, Marine Chemical

* Corresponding author: E-Mail: wqh196812@163.com

Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Fractions were monitored by TLC (silica gel GF₂₅₄ 10-40 μ m, Marine Chemical Factory, Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH.

2.2. Plant Materials

The aerial parts of *Artemisia integrifolia* were collected in Hailaer (126°04' E and 48°20' N), Hulunbeier, Inner Mongolia of China, in April 2015, and identified by Prof. Buhebateer (Inner Mongolia University for Nationalities). The aerial part of *Artemisia integrifolia* L. were placed in the shade to dry. A voucher (NO. 20150410) has been deposited in a warehouse (The storage temperature was 10 – 30°C, and the relative humidity (RH) was 30–50%, and 24 hours was dark) in the School of Traditional Mongolian Medicine of Inner Mongolia University for Nationalities.

2.3. Extraction

The air-dried and powdered aerial parts of *Artemisia integrifolia* L. (650 g) were extracted twice with ether (3.0 L) at room temperature for 24 h. Evaporation of the solvent under reduced pressure delivered the ether extract (198 g). The extract was isolated by column chromatography on silica gel and gradiently eluted with petroleum ether (P.E.)-CH₃COCH₃ (80:1 to 5:1) to give 4 fractions (Fractions 1-4). Fraction 1 [231 mg, (P.E.-CH₃COCH₃ (80:1) elute] was subjected to silica gel column chromatography using P.E.-CH₃COCH₃ (80:1) to give **1** (12 mg); Fraction 2 [3.5 g, (P.E.-CH₃COCH₃ (60:1) elute] was exposed to silica gel column chromatography using P.E.-CH₃COCH₃ with increasing polarity (60: 1 to 20: 1) to give **2** (21 mg), **3** (45 mg) and **4** (18 mg); Fraction 3 [480 mg, (P.E.-CH₃COCH₃ (20:1) elute] was subjected to silica gel column chromatography using P.E.-CH₃COCH₃ (30:1) to give **5** (32 mg); Fraction 4 [173 mg, CHCl₃-CH₃COCH₃ (5:1) elute] was further chromatographed on Sephadex LH-20 column eluting with CH₃OH, and then separated by semi-preparative HPLC (CH₃OH-H₂O, 35:65) yielding **6** (15 mg, t_r 10.2 min).

Chamazulene (1): Blue liquid; ¹H-NMR (500MHz, CDCl₃) δ_{H} : 7.24 (1H, d, $J = 3.5$ Hz, H-1), 7.64 (1H, d, $J = 3.5$ Hz, H-2), 8.18 (1H, s, H-4), 7.41 (1H, d, $J = 9.5$ Hz, H-6), 7.01 (1H, d, $J = 9.5$ Hz, H-7), 2.68 (3H, s, H-11), 2.88 (2H, q, $J = 7.5$ Hz, H-12), 1.36 (3H, d, $J = 7.5$ Hz, H-13), 2.85 (3H, s, H-14); ¹³C-NMR (125MHz, CDCl₃) δ_{C} : 112.8 (C-1), 136.1 (C-2), 125.4 (C-3), 134.8 (C-4), 135.8 (C-5), 136.5 (C-6), 124.9 (C-7), 127.3 (C-8), 144.2 (C-9), 125.4 (C-10), 12.9 (C-11), 33.9 (C-12), 17.5 (C-13), 24.2 (C-14).

Acetylene-2: Yellowish solid; ¹H-NMR (500MHz, CDCl₃) δ_{H} : 1.97 (3H, s, H-1), 4.98 (1H, s, H-6), 6.68 (1H, d, $J = 6.0$ Hz, H-8), 6.22 (1H, dd, $J = 6.0, 1.5$ Hz, H-9), 2.07-1.60 (4H, m, H-11, 12), 4.15-3.87 (2H, m, H-13); ¹³C-NMR (125MHz, CDCl₃) δ_{C} : 4.61 (C-1), 79.6 (C-2), 65.1 (C-3), 76.4 (C-4), 71.6 (C-5), 79.8 (C-6), 168.8 (C-7), 125.7 (C-8), 136.1 (C-9), 120.8 (C-10), 35.5 (C-11), 24.4 (C-12), 69.3 (C-13).

Acetylene-3: Yellowish solid; ¹H-NMR (500MHz, CDCl₃) δ_{H} : 1.99 (3H, s, H-1), 4.97 (1H, s, H-6), 6.65 (1H, d, $J = 6.0$ Hz, H-8), 6.22 (1H, dd, $J = 6.0, 1.0$ Hz, H-9), 1.90-1.60 (6H, m, H-11, 12, 13), 3.98 (1H, td, $J = 11.5, 3.0$ Hz, Ha-14) 4, 3.84 (1H, dd, $J = 11.5, 4.0$ Hz, Hb-14); ¹³C-NMR (125MHz, CDCl₃) δ_{C} : 4.69 (C-1), 79.8 (C-2), 65.0 (C-3), 76.2 (C-4), 71.6 (C-5), 79.6 (C-6), 169.8 (C-7), 125.1 (C-8), 138.4 (C-9), 112.7 (C-10), 32.5 (C-11), 24.4 (C-12), 19.2 (C-13), 64.3 (C-14).

Acetylene-4: Yellowish solid; ¹H-NMR (500MHz, CDCl₃) δ_{H} : 2.03 (3H, s, H-1), 4.62 (1H, s, H-6), 6.22 (1H, d, $J = 5.5$ Hz, H-8), 6.19 (1H, d, $J = 5.5$ Hz, H-9), 1.81-1.66 (6H, m, H-11, 12, 13), 4.14 (1H, td, $J = 11.5, 3.0$ Hz, Ha-14) 4, 3.85 (1H, dd, $J = 11.5, 4.0$ Hz, Hb-14); ¹³C-NMR (125MHz,

CDCl₃) δ_C : 4.81 (C-1), 80.5 (C-2), 65.2 (C-3), 78.7 (C-4), 70.8 (C-5), 78.7 (C-6), 168.0 (C-7), 126.7 (C-8), 137.9 (C-9), 112.9 (C-10), 32.6 (C-11), 24.4 (C-12), 19.1 (C-13), 64.3(C-14).

Eugenol (5): White oily liquid; ¹H-NMR (500MHz, CDCl₃) δ_H : 6.70 (1H, s, H-2), 6.87 (1H, d, *J* = 8.5 Hz, H-5), 6.71 (1H, d, *J* = 8.5 Hz, H-6), 3.35 (2H, d, *J* = 8.5 Hz, H-7), 5.97 (1H, m, H-8), 5.10 (1H, m, Ha-9), 5.08 (1H, m, Hb-9), 3.90 (3H, s); ¹³C-NMR (125MHz, CDCl₃) δ_C : 131.9 (C-1), 111.1 (C-2), 146.4 (C-3), 144.2 (C-4), 114.2 (C-5), 121.2 (C-6), 39.9 (C-7), 137.8 (C-8), 115.5 (C-9), -OCH₃ (55.9).

Arteintegin A (6): White needle; ¹H-NMR (500MHz, DMSO-d₆) and ¹³C-NMR (125MHz, DMSO-d₆) spectral data see Table 1; HR-ESI-MS at *m/z* 137.0980 [M-H]⁻ (calcd for C₉H₁₃O₅, 137.0966).

3. Results and Discussion

The ether extract from the aerial parts of *Artemisia integrifolia* L was separated by chromatography and afforded a new iridoid, arteintegin A (**6**), together with five known compounds, namely chamazulene (**1**), acetylenes (E)-**2**, (E)-**3**, (Z)-**4**, eugenol (**5**) (Figure 1). The structures of the known compounds were identified by comparing their spectroscopic data with those reported in the literature [5-8].

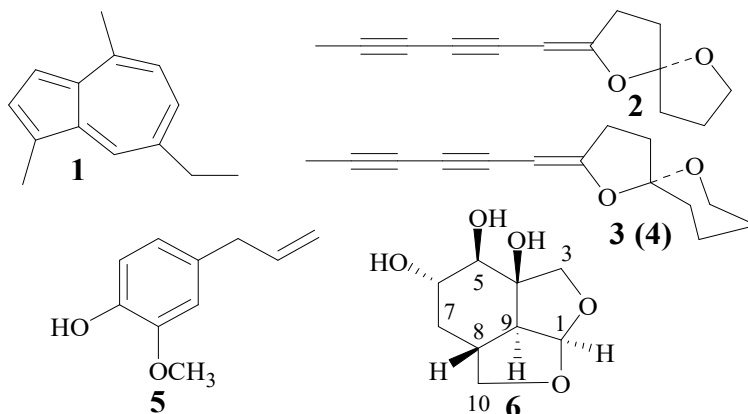
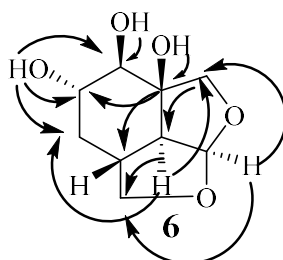


Figure 1. Structure of compounds 1-6

Compound **6** was obtained as a white needle, mp 129-131 °C; IR (KBr) ν_{\max} (cm⁻¹): 3439 cm⁻¹. The molecular formula was determined to be C₉H₁₄O₅ by HR-ESI-MS at *m/z* 137.0980 [M-H]⁻ (calcd for C₉H₁₃O₅, 137.0966). The ¹H NMR and ¹³C NMR spectra (Table 1) of **6** exhibited the presence of an acetal moiety [δ_H 5.33 (1H, d, *J* = 5.5 Hz, H-1); δ_C 100.0 (C-1)], two secondary hydroxyl groups [δ_H 4.08 (1H, d, *J* = 5.5 Hz, H-5), 3.83 (1H, m, H-6); δ_C 72.7 (C-5), 74.8 (C-6)] and a tertiary group [δ_C 83.8 (C-4)] which were unambiguously confirmed by the HSQC and DEPT experiments. In the HMBC spectrum (Figure 2), the signal of H-1 was correlated with the carbon signals at δ_C 71.3 (C-3), 83.8 (C-4), 35.3 (C-8), 44.5 (C-9) and 77.8 (C-10). Likewise, the HMBC correlations from δ_H 4.59 (4-OH) to δ_C 71.3 (C-3), 83.8 (C-4), 72.7 (C-5) and 44.5 (C-9); δ_H 4.78 (5-OH) to δ_C 83.8 (C-4), 72.7 (C-5) and 74.8 (C-6); δ_H 4.85 (6-OH) to δ_C 72.7 (C-5), 74.8 (C-6) and 20.6 (C-7) revealed that the three hydroxyl groups were linked to the C-4, C-5 and C-6, respectively.

**Figure 2.** Selected HMBC correlations for **6****Table 1.** ^1H (500 MHz) and ^{13}C -NMR (125MHz) data of compound **6** in DMSO-d_6

Position	Arteintegin A (6)	
	δ_{H} (ppm), J (Hz)	δ_{C} (ppm)
1	5.33 d (5.5)	100.0
3	4.42 d (10.0)	71.3
	3.45 d (10.0)	
4	—	83.8
5	4.08 d (10.0)	72.7
6	3.83 brd (10.0)	74.8
7	1.79 m	20.6
	1.68 brd (9.0)	
8	2.18 m	35.3
9	2.32 dd (5.5, 9.5)	44.5
10	3.88 dd (12.0, 2.0)	75.8
	3.55 dd (12.0, 5.5)	

The correlations observed in the HMBC spectrum further confirmed the structure of **6**, in which the correlations of H-5 (δ_{H} 4.08) with C-3 (δ_{C} 71.3), C-4 (δ_{C} 83.8), C-6 (δ_{C} 74.8), C-7 (δ_{C} 20.6), C-9 (δ_{C} 44.5) and H-10 (δ_{H} 3.88, 3.55) with C-1 (δ_{C} 100.0), C-7 (δ_{C} 20.6), C-8 (δ_{C} 35.3), C-9 (δ_{C} 44.5). The relative configuration of **6** was deduced by the NOESY experiments as follows and also by comparison of ^1H - ^1H coupling constants with those reported for related iridoids [9]. The NOESY interactions of H-1 with H-9, H-9 with H-5 indicated that H-1, H-5, H-9 were on the same side. Moreover, the NOESY interactions of H-8 with H-6, H-6 with 4-OH was observed. Thus, the structure of compound **6** was elucidated and named as arteintegin A (**6**).

Acknowledgments

This work was supported by the Science and Technology Major Project of Inner Mongolia Mongolian medicine in China (No. GCY20150712) and Graduate Research Innovation Projects of Inner Mongolia (No. B20161013601).

Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/RNP>

ORCID

Qinghu Wang: [0000-0002-4418-8661](https://orcid.org/0000-0002-4418-8661)

Junsheng Hao: [0000-0002-7733-133X](https://orcid.org/0000-0002-7733-133X)

Yanhua Xu: [0000-0001-5396-942X](https://orcid.org/0000-0001-5396-942X)

Yinping Bao: [0000-0003-1076-5472](https://orcid.org/0000-0003-1076-5472)

References

- [1] J. Zhang, S.J. Li, B.P. Xiang, and J. G. He (2008). Modulation effects of *Artemisia integrifolia* on immune function of mice, *Food. Sci.* **29**, 405-408.
- [2] Y. Tu, and X.H. Liu (2007). The Daur people eating-*Artemisia integrifolia*, *Chin. Folk Therap.* **15**, 10-11.
- [3] R. Liu, Q. Dong, and X.H. Wang (2010). Study on the antioxidant activity of flavonoid in *Artemisia selengensis*, *Sci. Tech. Food. Ind.* **31**, 319-321.
- [4] J.F. Hu, and X.Z. Feng (1999). New guaianolides from *Artemisia selengensis*, *J. Asian. Nat. Prod.* **1**, 169-176.
- [5] W.J. Zhang, C.X. You, K. Yang, Y. Su, Z.F. Geng, S.S. Du, C.F. Wang, Z.W. Deng, and Y.Y. Wang (2015). Bioactivity and chemical constituents of the essential oil from *Dendranthema indicum* (L.) Des Moul. Against two stored insects, *J. Oleo. Sci.* **64**, 553-560.
- [6] V. Martínez, O. Barberá, J. Sánchez-Parareda, and J. A. Marco (1987). Phenolic and acetylenic metabolites from *Artemisia assoana*, *Phytochemistry* **26**, 2619-2624.
- [7] L.F. Zan, T. Bau, H. Y. Bao, and Y. Li (2008). Chemical composition in fruiting body of *Cortinarius rufolivaceus*, *Mycosystema* **27**, 284-288.
- [8] Y. Nakamura, Y. Ohto, A. Murakami, S. Jiwajinda, and H. Ohigashi (1998). Isolation and identification of acetylenic spiroketal enol ethers from *Artemisia lactiflora* as inhibitors of superoxide generation induced by a tumor promoter in differentiated HL-60 cells, *J. Agr. Food Chem.* **46**, 5031-5036.
- [9] J.Q. Dai, Z.L. Liu, and L. Yang (2002). Non-glycosidic iridoids from *Cymbaria mongolica*, *Phytochemistry* **59**, 537-542

A C G
publications

© 2018 ACG Publications