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Structure Elucidation of A New Iridoid from *Artemisia integrifolia*

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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 (^{1}H NMR and ^{13}C NMR) and 2D-NMR (COSY, HSQC and NOESY) spectral analysis.

Keywords: Iridoid; *Artemisia integrifolia;* NMR; HR-ESI-MS.

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-d_6 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

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Factory, Qingdao, China) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden). Fractions were monitored by TLC (silica gel GF_{254}10-40μm, Marine Chemical Factory, Qingdao, China), and spots were visualized by heating silica gel plates sprayed with 10% H_2SO_4 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.)-CH_3COCH_3 (80:1 to 5:1) to give 4 fractions (Fractions 1-4). Fraction 1 [231 mg, (P.E.-CH_3COCH_3 (80:1) elute] was subjected to silica gel column chromatography using P.E.-CH_3COCH_3 (80:1) to give **1** (12 mg); Fraction 2 [3.5 g, (P.E.-CH_3COCH_3 (60:1) elute] was exposed to silica gel column chromatography using P.E.-$CH_3COCH_3$with increasing polarity (60: 1to 20: 1) to give **2** (21 mg), **3** (45 mg) and **4** (18 mg); Fraction 3 [480 mg, (P.E.-CH_3COCH_3 (20:1) elute] was subjected to silica gel column chromatography using P.E.-CH_3COCH_3 (30:1) to give **5** (32 mg); Fraction 4 [173 mg, $CHCl_3$-CH_3COCH_3 (5:1) elute] was further chromatographed on Sephadex LH-20 column eluting with CH_3OH, and then separated by semi-preparative HPLC (CH_3OH-H_2O, 35:65) yielding **6** (15 mg, t_R 10.2 min).

*Chamazulene (**1**):* Blue liquid; ^{1}H-NMR (500MHz, $CDCl_3$) 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); ^{13}C-NMR (125MHz, $CDCl_3$) 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; ^{1}H-NMR (500MHz, $CDCl_3$) 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); ^{13}C-NMR (125MHz, $CDCl_3$) 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; ^{1}H-NMR (500MHz, $CDCl_3$) 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); ^{13}C-NMR (125MHz, $CDCl_3$) 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; ^{1}H-NMR (500MHz, $CDCl_3$) 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); ^{13}C-NMR (125MHz, $CDCl_3$) 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; ^{1}H-NMR (500MHz, $CDCl_3$) 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); ^{13}C-NMR (125MHz, $CDCl_3$) 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), -OCH_3 (55.9).

*Arteintegin A (**6**):* White needle; ^{1}H-NMR (500MHz, DMSO-d_6) and ^{13}C-NMR (125MHz, DMSO-d_6) spectral data see Table 1; HR-ESI-MS at *m/z* 137.0980 $[M-H]^-$ (calcd for $C_9H_{13}O_5$, 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].

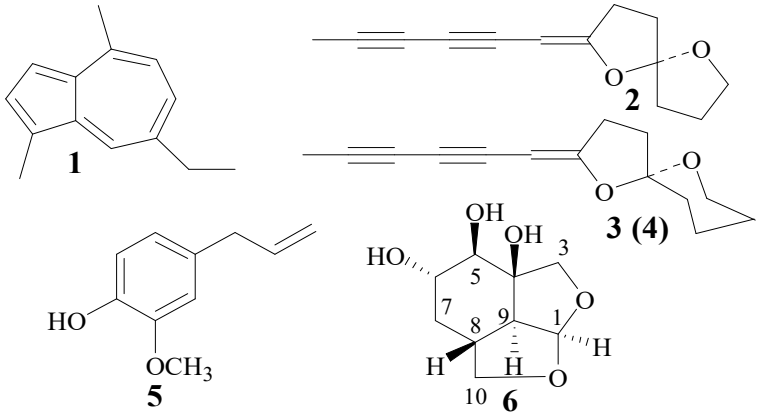

Figure 1. Structure of compounds **1**-**6**

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 $C_9H_{14}O_5$ by HR-ESI-MS at *m/z* 137.0980 $[M-H]^-$ (calcd for $C_9H_{13}O_5$, 137.0966). The ^{1}H NMR and ^{13}C 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 δc 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 δc 71.3 (C-3), 83.8 (C-4), 72.7 (C-5) and 44.5 (C-9); δ_H 4.78 (5-OH) to δc 83.8 (C-4), 72.7 (C-5) and 74.8 (C-6); δ_H 4.85 (6-OH) to δc 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.

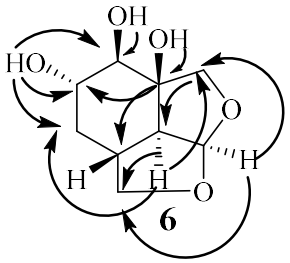

Figure 2. Selected HMBC correlations for **6**

Table 1. ^{1}H (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 ^{1}H-^{1}H 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**).

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Supporting Information

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

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