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TWO NEW EUDESMANES FROM *Inula helenium*

Yong-Ming Zhao,[1] Yu-jin Wang,[2] Mei Dong,[3]
Man-Li Zhang,[1] Chang-Hong Huo,[1*]
Yu-Cheng Gu,[1,4] and Qing-Wen Shi[1*]

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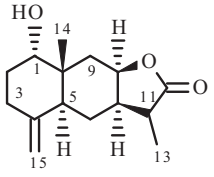
*Two new eudesmanes, 1α-hydroxy-11,13-dihydroisoalantolactone (**1**), 3α-hydroxy-11,13-dihydroalantolactone (**2**), and one known compound were isolated from the roots of* Inula helenium*. Their structures were established on the basis of spectral evidence.*

Keywords: *Inula helenium*, Compositae, sesquiterpene lactones, eudesmane.

Inula helenium is a widely occurring perennial herb in Europe and East Asia. Its roots have been traditionally used as an expectorant, antitussive, diaphoretic and bactericidal agent in folk medicine [1]. As the principal and characteristic constituents, seven sesquiterpene lactones, including one germacrane (4β,5α-epoxy-1(10),11(13)-germacradien-8,12-olide), one elemane (igalane) and five eudesmanes (alantolactone, isoalantolactone, 11α,13-dihydroalantolactone, 11α,13-dihydroisoalantolactone, 5-epoxyalantolactone), were obtained from the roots of *I. helenium*, which exhibited strong activities against three tumor cell lines (MK-1, HeLa, and $B_{16}F_{10}$) suggesting that the 11,13-dehydrolactone moiety of these sequiterpenes contributed to the antiproliferative activitiy [2]. Isocostunolide is another sesquiterpene lactone isolated from the same part of this plant that could effectively induce cytotoxicity in three cancer cell lines (A2058, HT-29, and HepG2) [3]. One thymol derivative, 10-isobutyryloxy-8,9-epoxythymol isobutyrate, was also reported to be a major constituent of *I. helenium*, which showed moderate antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa,* and *Candida albicans* [4].

Our continuing investigation of bioactive metabolites from this medicinal plant led to isolation of two new eudesmanes **1** and **2**, together with one known compound, namely macrophyllilactone E (**3**). Their structures were elucidated by spectral data. Macrophyllilactone E (**3**) is reported for the first time here from *I. helenium*, and the confusion in ^{13}C NMR data previously reported [5] for this compound is clarified here.



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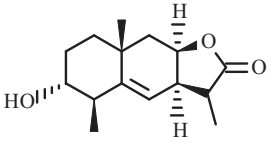

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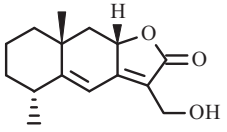

3

1) School of Pharmaceutical Sciences, Hebei Medical University, Shijiazhuang 050017, P. R. China, e-mail: rainbowhuo@hebmu.edu.cn; shiqingwen@hebmu.edu.cn; 2) Department of Forensic Medicine, Shanxi Medical University, 030001, Taiyuan, Shanxi Province, P. R. China; 3) Department of Forensic Medicine, Hebei Medical University, 050017, Shijiazhuang, Hebei Province, P. R. China; 4) Syngenta Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom. Published in Khimiya Prirodnykh Soedinenii, No. 3, pp. 315–317, May–June, 2010. Original article submitted December 29, 2008.

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TABLE 1. NMR Data of **1** ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_H	δ_C	^1H–^1H COSY	C atom	δ_H	δ_C	^1H–^1H COSY
1	4.34 br.s	73.3	H-2a, 2b	8	4.49 br.s	77.8	H-7, 9a, 9b
2a	1.76 m	29.0	H-1, 2b	9a	2.17 (br.d, J = 15.3)	40.5	H-8, 9b
2b	1.42 m		H-1, 2a	9b	1.51 m		H-8, 9a
3a	1.71 m	35.8	H-3b	10		34.7	
3b	1.31 m		H-3a	11	2.82 m	41.2	H-7, 13
4		150.6		12		179.4	
5	2.37 m	41.8	H-6a, 6b, 15a, 15b	13	1.23 (d, J = 7.2)	9.3	H-11
6a	1.57 m	20.8	H-5, 7, 6b	14	0.81 s	17.0	
6b	1.18 m		H-5, 7, 6a	15a	5.01 br.s	109.8	H-5, 15b
7	2.42 m	40.3	H-6a, 6b, 8, 11	15b	4.64 br.s		H-5, 15a

TABLE 2. NMR Data of **2** ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_H	δ_C	^1H–^1H COSY	HMBC	NOESY
1α	1.17 m	39.8	H-1β, 2α, 2β	C-2, 3, 5, 9, 10	H-1β, 2β, 2α
1β	1.65 m		H-1α, 2α, 2β	C-2, 3, 5, 10	H-1α, 2β, 2α, 9β
2α	1.63 m	25.4	H-1α, 1β, 2β, 3	C-3, 10	H-1β, 1α, 2β, 3
2β	1.83 m		H-1α, 1β, 2α, 3	C-1, 3, 4, 10	H-1β, 1α, 2α, 3, 15
3	3.76 m	73.0	H-2, 4	C-2, 15	H-2α, 2β, 4
4	2.66 m	45.2	H-3, 15	C-2, 3, 5, 6, 10, 15	H-3, 6, 8, 15
5		149.5			
6	5.27 br.s	117.7	H-7	C-4, 5, 7, 8, 10, 11	H-4, 7, 9α, 13
7	3.03 m	38.6	H-6, 8, 11	C-5, 6, 11, 12	H-6, 8, 11
8	4.75 br.s	76.8	H-7, 9α, 9β	C-6, 10	H-4, 7, 9α, 9β
9α	2.18 (dd, J = 3.0, 15.0)	42.7	H-8, 9β	C-1, 5, 7, 8, 10	H-6, 8, 9β
9β	1.48 (dd, J = 2.4, 15.0)		H-8, 9α	C-1, 10, 14	H-1β, 8, 9α, 14
10		32.5			
11	2.91 m	40.2	H-7, 13	C-6, 7, 12, 13	H-7, 13
12		179.3			
13	1.25 (d, J = 8.0)	11.0	H-11	C-11, 12	H-6, 11
14	0.81 s	17.7		C-1	H-9β, 15
15	1.12 (d, J = 7.5)	15.6	H-4	C-3, 4, 5	H-2β, 4, 14

Compound **1** was obtained as colorless needles, and its molecular formula was established as $C_{15}H_{22}O_3$ (*m/z* 250.1585) by accurate mass spectral measurement (HR-EI-MS). The ^1H NMR spectrum (Table 1) showed the signals of two methyl groups at δ 1.23 and 0.81, and the characteristic exomethylene signals at δ 5.01 and 4.64. The ^1H and ^{13}C NMR data are similar to those of 11,13-dihydroisoalantolactone [6], but distinctly different at C-1. The signals at δ_H 4.34 (H-1) and δ_C 73.3 (C-1) indicated the presence of a hydroxy group at C-1. The orientation of 1-OH was presumed to be α according to the signal of H-1 as a broad singlet, indicating that the dihedral angles between H-1 and H-2a/H-2b were both about 60°. Therefore, compound **1** was assigned 1α-hydroxy-11,13-dihydroisoalantolactone, and the structure was further confirmed by ^1H–^1H COSY.

Compound **2** was isolated as colorless needles. Its HR-EI-MS spectrum displayed a molecular ion at *m/z* 250.1575, indicating that **2** had the same molecular formula $C_{15}H_{22}O_3$ as compound **1**. The ^1H and ^{13}C NMR data of **2** (Table 2) are similar to that of alantolactone [7], but the characteristic doublet of exocyclic methylene was not observed for **2**; instead, a doublet methyl group at δ_H 1.12 (3H, d, J = 7.5 Hz) was observed. Combined with the chemical shift of C-12 (δ_C 179.3), this suggested that the five-memberd lactone ring in **2** is saturated. Furthermore, the ^{13}C signal at δ 73.0 ppm and the ^1H signal at δ_H 3.76 indicated the presence of a hydroxy group at C-3. NOESY correlations were observed between H-3 and H-2β, H-9β and H-1β/14, and H-14 and H-15, but no NOESY correlation was observed between H-3 and H-1α, which should be obvious if 3-OH was β-oriented, thus strongly indicating that this hydroxy group is α-oriented. Therefore, the structure of compound **2** was established as 3α-hydroxy-11,13-dihydroalantolactone, which was further confirmed by ^1H–^1H COSY and HMBC experiment.

TABLE 3. NMR Data of **3** ($CDCl_3$, δ, ppm, J/Hz)

Position	δ_H	δ_C	^1H–^1H COSY	HMBC
1a	1.58 m	39.7	H-1b	
1b	1.67 m		H-1a	C-9, 10, 14
2a	1.53 m	18.0	H-2b	
2b	1.95 m		H-2a	
3a	1.55 m	34.1	H-3b, 4	C-2, 15
3b	1.73 m		H-3a	C-2
4	2.78 m	40.6	H-3a, 15	C-2, 3, 5, 6, 10, 15
5		163.7		
6	6.36 s	112.6		C-4, 7, 8, 10, 11
7		158.9		
8	4.80 (dd, J = 13.0, 6.0)	76.5	H-9a, 9b	C-7, 9
9a	1.53 m	43.2	H-8, 9b	C-1, 7, 8, 10, 14
9b	2.17 (dd, J = 13.0, 6.0)		H-8, 9a	C-1, 5, 7, 8, 10
10		38.6		
11		118.2		
12		174.8		
13	4.44 s	55.4		C-7, 11, 12
14	1.27 s	29.5		C-1, 9, 10
15	1.28 (d, J = 7.5)	20.6	H-4	C-3, 4, 5

Compound **3**, macrophyllilactone E, was isolated as a colorless oil, which has been reported from *Inula macrophylla* [5]. With the aid of 2D NMR techniques, including ^1H–^1H COSY, HMQC, and HMBC spectra, exact ^1H and ^{13}C NMR assignments were achieved (Table 3). It revealed that the ^{13}C NMR data of several carbons had to be interchanged in the previous publication [5]. To the best of our knowledge, this eudesmane is isolated for the first time from *I. helenium*.

EXPERIMENTAL

General Procedures. NMR spectroscopic data were recorded on a Varian Inova 500NB NMR spectrometer with tetramethylsilane as internal standard. Mass spectra were measured with a VG-ZAB-HS mass spectrometer.

Plant Material and Extraction and Isolation. The roots of *I. helenium* were purchased at the Han Guo herbal material market, Hebei Province, in August 2004 and identified by Prof. Fengzhi Nie (Pharmacognosy Laboratory, School of Pharmaceutical Sciences, Hebei Medical University). A voucher specimen (No. 2005-06) has been deposited in the Herbarium of Natural Medicine, School of Pharmaceutical Sciences, Hebei Medical University. The air-dried roots of *I. helenium* (3.0 kg) were chipped and refluxed three times with 95% ethanol (3 × 4 L). The ethanol extract was evaporated under reduced pressure. After removing solvent, the residue was suspended in brine and partitioned sequentially with petroleum ether, dichloromethane, and ethyl acetate. The dichloromethane extract was applied to a silica gel column with CH_2Cl_2–Me_2CO step gradients (30:1 to 1:1) to yield 78 major fractions designated Fr. DA-1 to Fr. DA-78. Fraction DA-12 – Fr. DA-13 was subjected to preparative TLC [PE–Me_2CO (2:1)] to yield **1** and **2**. Fraction DA-16 was purified on preparative TLC [PE–EtOAc (3:2)] to afford **3**.

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