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Cytotoxic germacrane-type sesquiterpene lactones from the whole plant of *Inula cappa*

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ABSTRACT

Phytochemical investigation on the whole plant of *Inula cappa* led to the isolation of two new germacrane-type sesquiterpene lactones, ineupatolides D and E (**1** and **2**), together with three known analogs. The structures of the new compounds were established by extensive analysis of 1D and 2D NMR spectra, as well as MS data. Their absolute configurations were determined by CD spectra. All compounds showed moderate inhibitory effects on A431, A549, BGC-823, HL-60, HT-29, and MCF-7 cancer cell lines with IC_{50} values ranging from 2.1 to 36.3 μM.

1. Introduction

The genus *Inula* (Asteraceae) comprises more than one hundred species [1]. Sesquiterpenoids were considered as the characteristic constituents of this genus with diverse bioactivities such as cytotoxic, anti-inflammatory, and antifungal activities [2]. *Inula cappa*, a medicinal herb known as “Yang er ju” in Chinese, is widely distributed in the southern part of China. Its whole plant or roots have long been used for the treatment of rheumatoid arthritis, malaria, dysentery, and hepatitis [3]. Previous investigations of this plant reported the isolation of germacrane-type sesquiterpene lactones [4,5], along with inositol derivatives [6–8], flavonoids [9], and phenolic glycosides [10,11]. The highly oxygenated germacrane-type sesquiterpene lactones showed potent cytotoxic activity [5,12,13].

As part of our continuing search for bioactive constituents with novel structures from natural resources, a detailed investigation of *I. cappa* was carried out, resulting the isolation of two new highly oxygenated germacrane-type sesquiterpene lactones, ineupatolides D and E (**1** and **2**), and three known analogs (**3**–**5**,Fig. 1). This paper describes the isolation and structure elucidation of these isolates, and their cytotoxic activity against six human tumor cell lines as well.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder. Its molecular formula was determined as $C_{26}H_{36}O_9$ by HR-ESIMS (m/z 515.2253 for $C_{26}H_{36}O_9Na$, calcd. m/z 515.2257), and implied an unsaturation equivalence of nine (see Supporting information). The IR spectrum suggests the presences of hydroxy (3439 cm^{-1}), carbonyl (1716 cm^{-1}), and double bond (1645 cm^{-1}) groups. The ^{13}C and DEPT NMR spectra gave 26 resonances ascribed to 6 methyls, 4 methylenes, 8 methines, and 8 quaternary carbons (Table 1). Among them, three ester carboxyls (δ_C 165.0, 167.8 and 169.7), and one exocyclic olefinic group (δ_C 126.1 and 134.1), were identified. The ^1H NMR spectrum displayed `signals for six methyls (δ_H 1.02, 1.07, 1.40, 1.94, 1.95, 2.12), two olefinic protons (δ_H 5.71, m; 6.10, m), and two exocyclic olefinic protons (δ_H 6.22, d, $J = 3.3$ Hz; 5.66, d, $J = 3.3$ Hz) (Table 1). In the HMBC spectrum (Fig. 2), both

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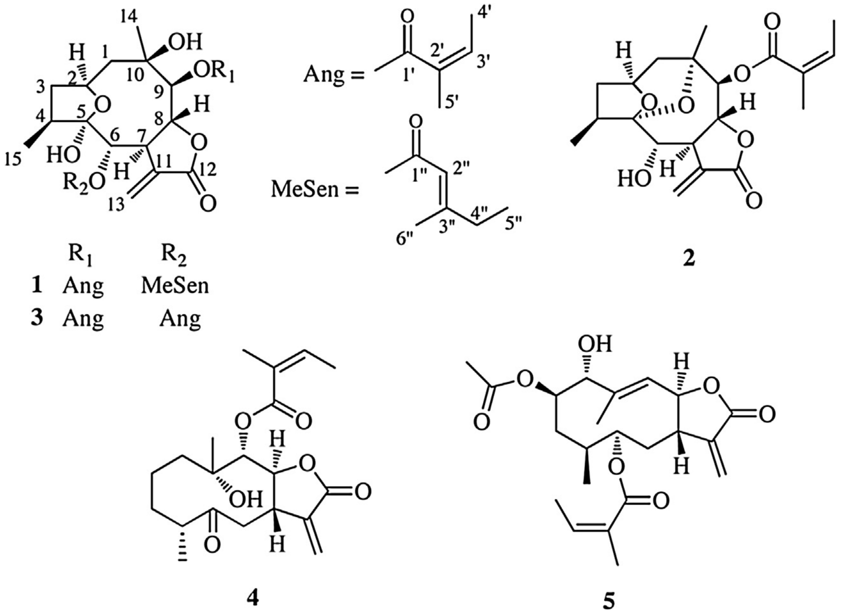

Fig. 1. Structures of compounds **1**–**5**.

methyls at δ_H 1.94 and 1.95 showed correlations to the carbonyl carbon at δ_C 167.8 and two olefinic carbons at δ_C 127.2 and 139.3, indicating the existence of an angelate moiety. The ^1H–^1H correlations between H_3-5″ (δ_H 1.07, t, J = 7.4 Hz) and H_2-4″ (δ_H 2.19, m), and the HMBC correlation from the characteristic proton at δ_H 5.71 (1H, m) to C-4″ (δ_C 34.1) and C-6″ (δ_C 19.3), suggested the existence of a 4-methylsenecioyl residue. Apart from these two substituents, the left 15 carbons were indicative of the same sesquiterpene skeleton with that of dvaricin B, a known germacraenolide isolated from this plant [14]. The key HMBC correlations from H-6 (δ_H 5.09, d, J = 7.9 Hz) to the carbonyl carbon of the 4-methylsenecioyl group, and from H-9 (δ_H 5.24, d, J = 5.0 Hz) to the carbonyl carbon of the angelate group suggested that these two substituent groups were located at C-6 and C-9, respectively. The relative configuration was inferred from the ROESY experiment (Fig. 2). The observed correlations of H-6/H-8, H-6/Me-15, H-2/H-4, H-2/Me-14, Me-14/H-9, and H-9/H-7 indicated the orientation of H-2 (α), H-4 (α), H-6 (β), H-7 (α), H-8 (β), H-9 (α), Me-14 (α), and Me-15 (β), respectively. The absolute configuration of compound **1** was determined by CD spectrum (Fig. 2) based on the Geissman rule [15,16]. The negative Cotton effect at 259 nm for the $n\pi^*$ transition of α-methylene γ-lactone chromophore suggested C-7, C-8 *trans*-fusion of the γ-lactone with a 7*R* configuration. Additionally, the positive Cotton effect at 229 nm can result from the 4-methyl seneciolate chromophore at C-6 with *S*-configuration [16]. Therefore, compound **1** was finally proposed to be (2*S*,4*S*,5*S*,6*S*,7*R*,8*R*,9*S*,10*R*)-2,5-epoxy-5,10-dihydroxy-6-(4-methylsenecioyloxy)-9-angeloyloxy-germacran-8,12-olide, and named ineupatolide D.

Table 1
^1H (500 MHz, δ in ppm, J in Hz) and ^{13}C NMR (125 MHz, δ in ppm) data of compounds **1** and **2** in $CDCl_3$.

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	1.94[a]	48.0 t	2.76 (dd, 8.2, 13.5) 1.53 (dd, 13.5, 13.5)	46.9 t
2	4.37 (m)	72.0 d	4.56 (m, 1H)	71.3 d
3	H_a 1.47 (m) H_b 2.58 (m)	40.9 t	H_α 2.53 (m) H_β 1.27 (m)	39.0 t
4	2.34 (m)	45.0 d	2.26 (m)	36.0 d
5		106.0 s		106.2 s
6	5.09 (d, 7.9)	73.4 d	3.80 (t, 9.7)	69.2 d
7	3.93 (m)	45.8 d	3.21 (m)	50.9 d
8	4.75 (dd, 5.0, 4.9)	77.3 d	4.38 (t, 10.1)	76.9 d
9	5.24 (d, 5.0)	80.5 d	5.62 (d, 10.1)	79.4 d
10		73.3 s		72.6 s
11		134.1 s		138.4 s
12		169.7 s		169.5 s
13	6.22 d (d, 1H, 3.3) 5.66 d (d, 1H, 3.13)	126.1 t	6.21 (d, 3.1) 6.07 (d, 3.1)	121.0 t
14	1.40 (s)	25.7 q	1.20 (s)	24.8 q
15	1.02 (d, 7.1)	13.2 q	1.06 (d, 6.7)	13.3 q
	Ang		Ang	
1′		167.8 s		166.9 s
2′		127.2 s		127.5 s
3′	6.10 (m)	139.3 d	6.14 (m)	140.0 d
4′	1.95[a]	16.0 q	2.02 (d, 7.3)	16.0 q
5′	1.94[a]	20.8 q	1.92 (d, 1.5)	20.7 q
	MeSen			
1″		165.0 s		
2″	5.71 (m)	113.1 d		
3″		165.9 s		
4″	2.19 (m)	34.1 t		
5″	1.07 (t, 7.4)	12.1 q		
6″	2.12 (m)	19.3 q		
HO-6			1.76 (d, 9.7)	

[a] Overlapped with other signals, and the chemical shifts were determined by analysis of ^1H–^1H COSY, HSQC and HMBC spectra.

Compound **2**, obtained as a white amorphous powder, was given a molecular formula of $C_{20}H_{26}O_7$ by HRESIMS (m/z 401.1569 for $C_{20}H_{26}O_7Na$, calcd. m/z 401.1576), corresponding to an unsaturation equivalence of eight. Its ^1H and ^{13}C NMR data showed a high similarity to those of **1**, suggesting it as a germacrane-type sesquiterpene lactone with an angelate substituent group (Table 1). Two double bonds and two carbonyls in this molecule accounted for four of eight indices of hydrogen deficiency, thus the remaining four required **2** to be tetracyclic. The location of the angelate group at C-9 was inferred from the observed HMBC correlation from H-9 (δ_H 5.62) to the carbonyl carbon (δ_C 166.9) of the angelate group. The up-field chemical shift of H-6 (δ_H 3.65, t, J = 9.7), together with its correlation with HO-6 (δ_H 1.76, d, J = 9.7) in the ^1H–^1H COSY spectrum, revealed the presence of a hydroxy group at C-6. All the above evidences suggested that an additional oxygen bridge was formed between C-5 and C-10. Such a proposed structure explained well the high similarity of NMR data for compounds **1** and **2**, and was supported

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