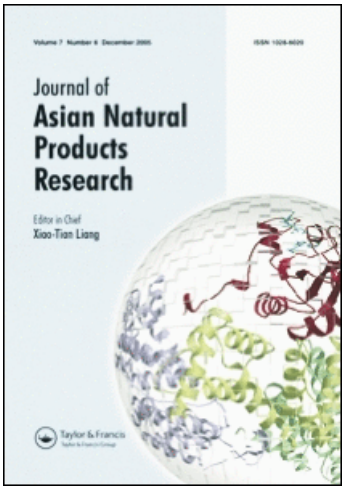

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ORIGINAL ARTICLE

Two new eudesmanolides from *Inula racemosa*

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Two new eudesmane-type sesquiterpene lactones were isolated from the roots of *Inula racemosa* and their structures were elucidated as 3β-hydroxy-11α,13-dihydroalantolactone (**1**) and 11α-hydroxy-eudesm-5-en-8β,12-olide (**2**). Their cytotoxic activities against five human cancer cell lines had been tested and compound **2** exhibited weak cytotoxic activity against BEL-7402 and HCT-8 cell lines. The anti-inflammatory activities were also tested, but neither of them showed any activities.

Keywords: Compositae; *Inula racemosa*; eudesmanolide; cytotoxic activity; anti-inflammation

1. Introduction

There are about 100 species of the genus *Inula* widely found in Europe, Asia, and Africa, mostly in the Mediterranean, with more than 20 species being distributed in China. Many of them have long been used in Chinese folk medicine, and most frequently employed for their peptic, relieving phlegm, detumescence, anti-inflammatory, and vermifuge properties [1]. *Inula racemosa* is a traditional Chinese medicine, which has been used as an antimicrobial agent for nearly a thousand years. In order to learn more about the structures and biological activities among the sesquiterpene lactones of *I. racemosa*, we now report on the isolation and structural determination of two new eudesmane-type sesquiterpene lactones **1** and **2** (Figure 1).

2. Results and discussion

Compound **1** was obtained as a colorless oil, $[\alpha]_D^{20}$ −48.4 (0.14, $CHCl_3$). Its molecular formula was determined as $C_{15}H_{22}O_3$ by HR-ESI-MS, giving a quasi-molecular ion peak at *m/z* 251.1639 $[M + H]^+$. The IR spectrum showed absorption bands of hydroxyl (3297 cm^{-1}) and γ-lactone (1752 cm^{-1}) groups. The ^1H NMR spectrum (Table 1) showed the signals of the following protons: one olefinic proton at δ_H 5.26 (1H, d, $J = 2.8$ Hz, H-6), two oxygenated methines at δ_H 4.74 (1H, m, H-8), 3.74 (1H, m, H-3), two methyl doublets at δ_H 1.24 (3H, d, $J = 7.2$ Hz, H-13), 1.11 (3H, d, $J = 7.2$ Hz, H-15), and one methyl singlet at δ_H 1.22 (3H, s, H-14). Its ^{13}C NMR (DEPT) spectrum revealed the presence of 15 carbon signals consisting of three quaternary, six methine, three methylene, and three methyl carbons, which gave

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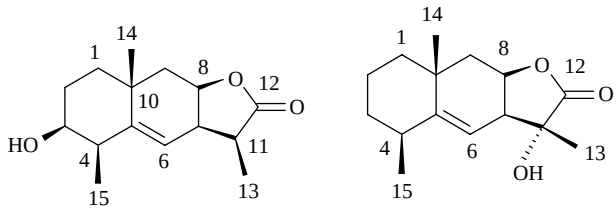


1 **2**

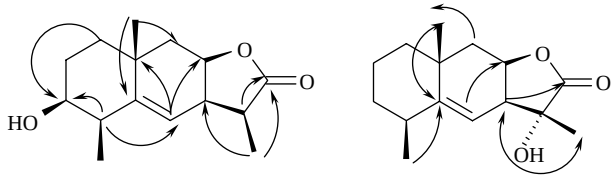
Figure 1. Structures of compounds **1** and **2**.

resonances of one ester carbonyl at δ_C 179.0 (C), one trisubstituted olefin at δ_C 149.3 (C) and 117.8 (CH), and two oxygenated carbons at δ_C 76.5 (CH) and 72.7 (CH). All of these proposed that **1** was an eudesmane-type sesquiterpene with one hydroxyl group and one double bond, similar to that of 11α,13-dihydroalantolactone [2], except that one more hydroxyl group was present in **1**. The location of the hydroxyl group was determined to be at C-3, based on HMBC (Figure 2) correlations between H-4 and C-3 (δ_C 72.7); H-15 (δ_H 1.11, 3H, d, $J = 7.2$ Hz) and C-3 (δ_C 72.7), and in the HSQC spectrum, the correlation between the proton at δ_H 3.74 and the carbon at δ_C 72.7 was observed, all of which suggested that the hydroxyl group was connected to C-3 (δ_C 72.7). CH_3-13, 14, 15 were in β-orientation which were confirmed in 11α,13-dihydroalantolactone [2], so H-4 was in α-orientation. In addition, NOE enhancement between H-3 (δ_H 3.74) and H-4 (δ_H 2.66) was observed, all of which indicated that 3-OH should be in β-configuration. Also, in the NOESY spectrum of **1**, H-8 correlated with H-7 and H-11. The coupling constant ($J_{H\text{-}7,H\text{-}11} = 7.2$ Hz) between H-7 and H-11 was also consistent with a *cis*-orientation. On the basis of the above analysis, **1** was determined as 3β-hydroxy-11α,13-dihydroalantolactone (Figure 1).

Compound **2** was obtained as a yellow oil, $[\alpha]_D^{20}$ −4.80 (0.23, $CHCl_3$). Its HR-ESI-MS provided a quasi-molecular ion peak at *m/z* 251.1639 $[M + H]^+$, suggesting that the molecular formula was $C_{15}H_{22}O_3$. The IR spectrum showed absorption bands of hydroxyl (3295 cm^{-1})and γ-lactone (1764 cm^{-1}) groups. The ^1H and ^{13}C NMR spectra (Table 1) showed three methyls, one secondary [δ_H 1.11 (3H, d, $J = 7.6$ Hz), δ_C 22.9] and two tertiary [δ_H 1.20 (s) and 1.43 (s), δ_C 28.6 and 20.8], one oxygenated methine [δ_H 5.06 (m), δ_C 76.8], and one oxygenated quaternary carbon [δ_C 77.4]. The above information suggested that compound **2** also possessed the eudesmanolide skeleton, which was a structural isomer of compound **1**. The obvious difference is that 3β-hydroxy in **1** was absent in compound **2**, and one oxygenated quaternary carbon [δ_C 77.4] was present in its structure. In the ^1H NMR spectrum, one of the methyl doublets [δ_H 1.24 (3H, d, $J = 7.2$ Hz)] in **1** became a singlet [δ_H 1.43 (3H, s)] in compound **2**, and in the ^{13}C NMR spectrum, C-13 shifted downfield to 20.8, all of which indicated that the hydroxyl group was assigned to C-11. The location of the hydroxyl group was also confirmed by HMBC (Figure 2) correlations between 13-CH_3 and C-12, C-7 and C-11, H-8 and C-11. The NOE correlation of 13-CH_3 with 14β-CH_3 was observed, indicating that 13-CH_3 was in β-configuration, and thus the 11-OH was in α-configuration. Therefore, compound **2** was determined to have the structure as shown in Figure 1. The derivative of alantolactone with 11-OH was rare in natural products, compound **2** was the second one, and the first one was 2α,11α-dihydroxy-11,13-dihydroalantolactone, which was isolated from the aerial parts of *Ondetia linearis* [3].

Table 1. ^{1}H and ^{13}C NMR spectral data for compounds **1** and **2** (300 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, $CDCl_3$, J in Hz, δ in ppm).

	1		**2**	
Position	δ_H	δ_C	δ_H	δ_C
1	1.67 (1H, m, H-1β) 1.18 (1H, dd, J = 14.8, 4.4, H-1α)	39.9	1.62 (1H, m, H-1β) 1.16 (1H, m, H-1α)	42.1
2	1.83 (1H, dddd, J = 14.8, 12.4, 4.4, 3.2, H-2β) 1.64 (1H, m, H-2α)	25.6	1.82 (1H, m, H-2β) 1.16 (1H, m, H-2α)	16.8
3	3.74 (1H, m, H-3α)	72.7	1.55 (2H, m, H-3)	32.8
4	2.66 (1H, t, J = 7.2, H-4α)	45.2	2.48 (1H, m, H-4α)	38.3
5	–	149.3	–	151.3
6	5.26 (1H, d, J = 2.8, H-6)	117.8	5.13 (1H, d, J = 3.6, H-6)	114.8
7	3.02 (1H, m, H-7α)	38.7	2.89 (1H, dd, J = 1.8, 5.7, H-7α)	45.8
8	4.74 (1H, m, H-8α)	76.5	5.06 (1H, m, H-8α)	76.8
9	2.13 (1H, dd, J = 15.0, 3.3, H-9β) 1.47 (1H, br d, J = 15.0, H-9α)	42.4	2.13 (1H, dd, J = 15.0, 3.3, H-9β) 1.51 (1H, br d, J = 15.0, H-9α)	42.6
10	–	32.3	–	32.8
11	2.90 (1H, t, J = 7.2, H-11α)	40.3	–	77.4
12	–	179.0	–	177.5
13	1.24 (3H, d, J = 7.2, H-13)	10.7	1.43 (3H, s, H-13)	20.8
14	1.22 (3H, s, H-14)	28.8	1.20 (3H, s, H-14)	28.6
15	1.11 (3H, d, J = 7.2, H-15)	15.6	1.11 (3H, d, J = 7.6, H-15)	22.9



1 2

Figure 2. Key HMBC correlations of compounds **1** and **2**.

The cytotoxicities of compounds **1** and **2** were evaluated *in vitro* using the MTT method [4]. Compound **1** displayed no cytotoxic activity against all five human cancer cell lines, and compound **2** exhibited only weak cytotoxic activity against BEL-7402 and HCT-8 human cancer cell lines with IC_{50} values of 9.6 and 9.2 μg/ml, respectively.

Compounds **1** and **2** were tested for their anti-inflammatory activities *in vitro* by an assay to release β-glucuronidase from rat PMNs induced by PAF. Compounds **1** and **2** showed inhibitory ratios of 31.0 and 28.1%, respectively, which indicated that these two compounds had no anti-inflammatory activities.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured by a Jasco P2000 polarimeter. IR spectra were carried out on a Nicolet IMPACT 400 spectrophotometer with KBr disks. ^{1}H NMR (300 MHz) spectra were recorded on a Mercury-300 spectrophotometer. ^{13}C NMR (100 MHz) and HMBC spectra were run on a Mercury-400 with TMS as the internal standard. HR-ESI-MS and ESI-MS were performed on an Agilent 1100 LC/MSD Trap-SL mass spectrometer. Silica gel (160–200 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Sephadex LH-20 (Pharmacia, Uppsala, Sweden), and RP-18 (Merck, 40–60 μm; Darmstadt, Germany) were used for column chromatography (CC) and silica gel GF-254 (Qingdao Marine Chemical Factory) was used for TLC. HPLC experiments were performed on a preparative YMC-Pack ODS-A column (10 μm, 250 × 20 mm i.d.; YMC, Kyoto, Japan) equipped with a Shimadzu SPD-6A UV spectrophotometric detector at 210 nm and a Thermo Consta Metric pumping system running with a flow rate of 4 ml/min.

3.2 Plant material

The roots of *I. racemosa* were collected in Tibet, China, in April 2008, and identified by Prof. Lin Ma, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College. A voucher specimen (No. 2240) is deposited at the Herbarium of the Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College.

3.3 Extraction and isolation

The dried roots (10 kg) of *I. racemosa* were extracted with 95% EtOH (3 × 10 liters; 3 h) under reflux conditions. After evaporation of the solvents under reduced pressure, the residue (1045 g) was suspended in water (4 liters). This suspension was successively extracted with petroleum ether (PE, 60–90°C), EtOAc, and *n*-BuOH. The EtOAc fraction (95 g) was chromatographed on a silica gel column (160–200 mesh, 2500 g) eluted with a solvent system of $CHCl_3$–acetone (100:0–100:1–50:1–25:1–10:1–5:1–3:1–1:1, v/v). The fraction (1.67 g) of $CHCl_3$–acetone (100:1) was subjected to silica gel CC and eluted with PE–EtOAc (4:1–3:1–2:1–1:1, v/v) to give four fractions. Fraction 2 (145 mg) was subjected to RP-HPLC on an ODS

column with MeOH–H_2O (7:3) as the mobile phase and then purified on Sephadex LH-20 CC using PE–$CHCl_3$–MeOH (5:5:1) as the eluent to obtain compound **1** (10 mg). The PE fraction (195 g) was chromatographed on a silica gel column (160–200 mesh, 2000 g) eluted with PE–EtOAc (50:1–25:1–10:1–1:1, v/v). Fraction 4 (eluted with PE–EtOAc 1:1, 13 g) was continuously subjected to a silica gel CC, eluted with PE–EtOAc (10:1–5:1–3:1–1:1, v/v). Fraction 1 (386 mg) was subjected to RP-HPLC on an ODS column with MeOH–H_2O (7:3) as the mobile phase, and further purified on Sephadex LH-20 CC using PE–$CHCl_3$–MeOH (5:5:1) to obtain compound **2** (8 mg).

*3.3.1 3β-Hydroxy-11α,13-dihydroalantolactone (**1**)*

Colorless oil; $[\alpha]_D^{20}$ −48.4 (0.14, $CHCl_3$); IR (KBr) ν_{max}: 3297, 2930, 1752, 1375, and 1035 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS *m/z*: 251.4 $[M + H]^+$; HR-ESI-MS *m/z*: 251.1639 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1642).

*3.3.2 11α-Hydroxy-eudesm-5-en-8β,12-olide (**2**)*

Yellow oil; $[\alpha]_D^{20}$ −4.80 (0.23, $CHCl_3$); IR (KBr) ν_{max}: 3295, 2920, 1764, 1201, 1036 cm^{-1}; ^1H and ^{13}C NMR spectral data: see Table 1; ESI-MS *m/z*: 251.4 $[M + H]^+$; HR-ESI-MS *m/z*: 251.1647 $[M + H]^+$ (calcd for $C_{15}H_{23}O_3$, 251.1642).

3.4 Cytotoxic bioassays

Cytotoxicity was determined by the MTT method [4] using human cell lines A549 (human lung cancer), BEL-7402 (human liver cancer), BGC-823 (human stomach cancer), HCT-8 (human colon cancer), and A2780 (human ovarian cancer) grown in RPMI-1640 medium supplied with 10% fetal bovine serum. Cells in the logarithmic phase were cultured at a density of 10,000 cells/ml per well in a 96-well microtiter plate. Then, different concentrations of the test compounds dissolved in dimethyl sulfoxide (DMSO) were added to each well. Each concentration was tested in triplicate. After incubation at 37°C in 5% CO_2 for 96 h, 100 μl of MTT (0.4 mg/ml) was added to each well and incubated for another 4 h, and then the liquid in the wells was removed. DMSO (150 μl) was added to each well. The absorbance was recorded on a microplate reader (Bio-Rad model 550) at a wavelength of 540 nm. The IC_{50} value resulted from a 50% reduction of absorbance in the control assay which was treated with 2.5% DMSO alone. Taxol was used as a positive control.

3.5 Anti-inflammation bioassays

The anti-inflammatory activities were assayed by measuring the inhibition of the PAF-induced release of β-glucuronidase from rat PMNs *in vitro* as described previously [5]. The absorbance was read at 550 nm, and then the inhibitory ratio was calculated. Ginkgolide B (Sigma, St Louis, MO, USA; 98% pure) was used as a positive control.

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