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Trinorsesquiterpenoids from *Inula racemosa*

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ABSTRACT

Four trinorsesquiterpenoids (**1**–**4**) were isolated from the roots of *Inula racemosa* and the structures of two new compounds, (4*R*,5*S*,10*S*)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one (**1**) and (4*R*,5*R*,10*R*)-4,15-epoxy-11,12,13-trinoreudesman-8-one (**3**), were elucidated by extensive spectroscopic analysis. Furthermore, the structure of compound **2a** should be revised as (4*R*,5*R*,10*S*)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one (**2**) and compound **2** showed antiproliferative activity against A549, HepG2, and HT1080 cell lines with IC50 values of 3.71, 5.94, and 3.95 μg/mL, respectively.

1. Introduction

Inula racemosa Hook. f. is a traditional medicinal plant that has long been used in China, India and Europe. Previous phytochemical investigations into this plant have revealed the presence of sesquiterpenoids (Kaur and Kalsi, 1985; Kalsi et al., 1989; Goyal et al., 1990; Zhang et al., 2012) and we recently reported twenty-four sesquiterpene lactones isolated from *I. racemosa* (Ma et al., 2013). As part of our continuous interest in traditional Chinese herbal medicine, four trinorsesquiterpenoids (**1**–**4**) including two new trinorsesquiterpenoids **1** and **3** (Fig. 1) were isolated from the root of *I. racemosa*. In this paper, we report the structural elucidation of these compounds and their antiproliferative activities. Furthermore, the structure of compound **2a** (Fig. 1), previously isolated from *Pulicaria insignis* (Huang et al., 2010), should be revised as (4*R*,5*R*,10*S*)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one (**2**).

2. Results and discussion

Compound **1** was obtained as a colorless crystal after crystallization from $CHCl_3$. The HRESIMS spectrum displayed a quasi-molecular ion peak at *m*/*z* 212.1651 ($[M+NH_4]^+$) consistent with a molecular formula of $C_{12}H_{18}O_2$. The IR spectrum exhibited absorption bands at 3456, 1668, and 1618 cm^{-1}, suggesting the presence of hydroxy, carbonyl, and olefinic groups. The ^{13}C NMR data (Table 1) exhibited twelve carbon signals, which were assigned by a DEPT experiment as two methyls, four sp^3 methylenes, one sp^3 and two sp^2 methines, and two sp^3 and one sp^2 quaternary carbons. The ^1H and ^{13}C NMR data (Table 1) showed the appearance of an α,β-unsaturated carbonyl moiety [δ_C 199.5 (C-8), 127.3 (C-7), 156.1(C-6); δ_H 6.81 (d, *J* = 10 Hz, H-6), 5.91 (dd, *J* = 10, 1.2 Hz, H-7)]. The ^1H–^1H COSY spectrum (Fig. 2) identified two main substructures (H-1–H-2–H-3–H-4–H-15 and H-6–H-7). In the HMBC (Fig. 2), the correlations of H-7/C-9, H-6/C-8 and H-7/C-5 indicated that an α,β-unsaturated ketone was formed between C-6 and C-8. The correlations from H_3-14 to C-1, C-5, C-9 and C-10, and those from H_3-15 to C-3, C-4 and C-5 confirmed that CH_3-14, CH_3-15 and hydroxy were connected to C-10, C-4, and C-5 respectively. Thus, the planar structure of **1** was established as 5-hydroxy-11,12,13-trinoreudesm-6-en-8-one. The relative configuration of compound **1** was determined by X-ray diffraction analysis (Fig. 3). To determine its absolute configuration, the CD spectrum of 1 was measured in CH_3OH (Fig. 4). The CD spectrum of **1** showed a positive Cotton effect at 332 nm for the $n \rightarrow \pi^*$ transition and a negative Cotton effect at 250 nm for $\pi \rightarrow \pi^*$ transition. According to orbital helicity rule (Kirk, 1986), the absolute configurations of **1** were assigned as 4*R*,5*S*,10*S*. Consequently, the structure of compound **1** was determined as (4*R*,5*S*,10*S*)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one.

Compound **2** showed the same NMR spectral data as those reported by Huang et al. for **2a**. A comparison of the NMR data and optical rotation value of **2** with those of **2a** indicated that these two compounds were identical. The X-ray crystallographic analysis of

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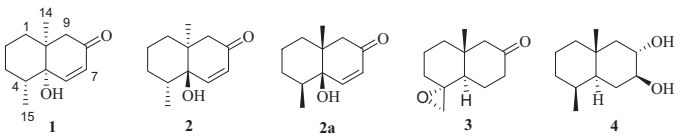

Fig. 1. The structures of related compounds.

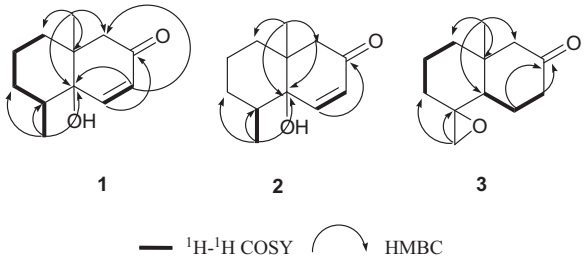

Fig. 2. Key 2D NMR correlations of compounds **1–3**.

this compound (Fig. 3) provided unequivocal evidence that its structure should be depicted as **2** instead of **2a**. The absolute configuration of **2** was determined to be 4*R*,5*R*,10*S*, deduced from the CD spectrum (Fig. 4) using Orbital helicity rule (Kirk, 1986), and it showed a negative Cotton effect at 332 nm due to the $n \rightarrow \pi^*$ transition and a positive Cotton effect at 243 nm due to $\pi \rightarrow \pi^*$ transition. Thus, the structure of compound **2a** should be revised as (4*R*,5*R*,10*S*)-5-hydroxy-11,12,13-trinoreudesm-6-en-8-one.

Compound **3** was assigned a molecular formula of $C_{12}H_{18}O_2$ on the basis of HRESIMS (*m*/*z* 217.1199 [M+Na]$^+$). The IR spectrum suggested the presence of a carbonyl (1702 cm^{-1}) group. The NMR data (Table 1) showed the presence of a ketone (δ_C 210.7, C-8) and an oxirane [δ_C 58.9 (C-4), 50.9 (C-15); δ_H 2.75, (dd, *J* = 4.4, 2.0 Hz, H-15a), 2.57(d, *J* = 4.4 Hz, H-15b)]. A comparison of the NMR and IR data of **3** with those of **1** and **2** indicated that the three compounds were structurally similar, except for the absence of a doublet methyl and double bond in **1** and **2** and the presence of an oxirane moiety in **3**. The correlations from H_2-15 to C-3 and C-4 in HMBC (Fig. 2) suggested that the oxirane was at C-4 and C-15. The relative configuration of **3** was established by an X-ray diffraction analysis (Fig. 3). The absolute configuration of **3** was determined as 4*R*,5*R*,10*R* deduced from the CD spectrum (Fig. 4) using the octant rule (Kirk, 1986),and it showed a negative Cotton effect centering at 289 nm for $n \rightarrow \pi^*$ transition of the ketone moiety. Therefore, compound **3** was determined as (4*R*,5*R*,10*R*)-4,15-epoxy-11,12,13-trinoreudesman-8-one.

Additionally, the known compound (Xu and Shi, 2011) was identified as 11,12,13-trinoreudesm-5-en-7β,8α-diol (**4**).

All isolates (**1**–**4**) were evaluated for their antiproliferative activities against three human cancer cell lines (A549, HepG2, and HT1080) using the CCK-8 cell viability assay (Zhao et al., 2013). Compound **2** showed antiproliferative activity against A549, HepG2, and HT1080 cell lines with IC_{50} values of 3.71, 5.94, and 3.95 μg/mL, respectively. Unfortunately, compounds **1**, **3**, and **4** showed no such activity ($IC_{50} > 50$ μg/mL) against the tested cell lines.

Sesquiterpenoids with α,β-unsaturated carbonyl moiety generally show antiproliferative activities. Alkylation of biological nucleophiles by α,β-unsaturated carbonyl structures in a Michael-type addition is considered to be a general mechanism of action (Kupchanm et al., 1971). The antiproliferative activity of compound **2** was consistent with this conclusion. In contrast to its epimer (**2**), compound **1** showed no antiproliferative activity. These discrepancies might be caused by a different stereochemistry. Confirmed by X-ray analysis (Fig. 3), the two rings of compound **2** lay roughly in the same plane, whereas the two rings of compound **1** were almost perpendicular to each other, which might create a steric hindrance preventing compound **1** from reaching its target (Beekman et al., 1997). Thus, this hindered approach to a target molecule might be the reason for the decreased antiproliferative activity of compound **1**.

3. Materials and methods

3.1. General

Melting points were determined on an X-4 digital display micromelting point apparatus and were uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter with 1 dm cell. IR spectra were obtained on a Nicolet NEXUS 670 FT-IR spectrometer. CD spectra were recorded on an Olis DSM 1000 circular dichroism spectrophotometer. NMR spectra were recorded on a Bruker AVANCE III-400. Chemical shifts are given on the δ (ppm) scale using TMS as internal standard. HRESIMS was

Table 1
^{1}H (400 MHz) and ^{13}C NMR (100 MHz) data of compounds **1**–**3** in CDCl3 (δ in ppm and *J* in Hz).

Position	**1**		**2**		**3**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.24 m	34.8 CH_2	1.18 d (12.8)	33.0 CH_2	1.45 m	40.9 CH_2
	1.66 dd (13.2, 4.4)		1.90 m		1.53 m	
2	1.59 m	21.3 CH_2	1.74 m	15.9 CH_2	1.76 m	21.1 CH_2
	1.55 m		1.50 m		1.68 m	
3	1.46 m	29.4 CH_2	2.08 m	26.9 CH_2	1.95 m	35.3 CH_2
	1.39 m		1.45 m		1.37 m	
4	1.90 m	38.4 CH	1.98 m	38.5 CH		58.9 qC
5		75.3 qC		73.6 qC	2.12 dd (12.8, 3.2)	46.2 CH
6	6.81 d (10.0)	156.1 CH	6.76 d (10.0)	151.2 CH	1.89 m	21.2 CH_2
					1.30 m	
7	5.91 dd (10.4, 1.2)	127.3 CH	5.98 d (10.0)	30.3 CH	2.38 m	40.9 CH_2
					2.25 m	
8		199.5 qC		200.4 qC		210.7 qC
9	1.98 dd (17.2,1.2)	46.4 CH_2	1.93 d (16.4)	51.1 CH_2	2.26 d (13.6)	56.3 CH_2
	2.89 d (17.2)		2.92 d (16.4)		2.16 dd (14.0, 2.0)	
10		41.2 qC		40.3 qC		40.2 qC
14	1.01 s	23.7 CH_3	1.13 s	22.4 CH_3	0.86 s	18.1 CH_3
15	0.99 d (6.8)	15.7 CH_3	1.09 d (7.6)	16.7 CH_3	2.75 dd (4.4, 2.0)	50.9 CH_2
					2.25 d (4.4)	