Phytochemistry Letters 21 (2017) 273–277

Contents lists available at ScienceDirect

Phytochemistry Letters

journal homepage: www.elsevier.com/locate/phytol

New *ent*-kaurane and *ent*-pimarane diterpenoids from *Siegesbeckia pubescens*

Qing-Qing Yuan[a,b], Wei-Bin Song[a], Wen-Qiong Wang[a], Jiao-Jiao Wei[a,b], Jun Li[a,b], Yi Liu[a,b], Li-Jiang Xuan[a,*]

[a] State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 501 Haike Road, Shanghai, 201203, PR China
[b] University of Chinese Academy of Sciences, No.19A Yuquan Road, Beijing, 100049, PR China

ARTICLE INFO

Keywords:
Siegesbeckia pubescens
Asteraceae
ent-Kauran diterpenoids
ent-Pimarane diterpenoids
Cytotoxic activity

ABSTRACT

Phytochemical investigation of the aerial parts of *Siegesbeckia pubescens* afforded two new *ent*-kaurane diterpenoids (**1-2**), together with sixteen known *ent*-kaurane and *ent*-pimarane diterpenoids (**3–18**). Their structures were elucidated on the basis of extensive spectroscopic methods The absolute configurations of **1–2** and **12** were determined by single-crystal X-ray diffraction analyses. All compounds were evaluated for their cytotoxic activities against two human cancer cell lines A375 and HCT-116.

1. Introduction

The genus *Siegesbeckia* belonging to the Asteraceae family, are widely distributed in tropical and temperate regions of the world. There are three species of the genus growing in china and the aerial parts have been used traditionally to treat arthritis, hypertention, malaria, neurasthenia and snakebite (Xiong et al., 1992). Previous studies focused on *Siegesbeckia* species revealed the presence of a series of *ent*-kaurane and *ent*-pimarane diterpenoids (Giang et al., 2005; Kim et al., 1979; Liu et al., 2012; Liu and Roder, 1991; Wang et al., 2009, 2010; Wang and Hu, 2006; Xiang et al., 2004; Xiong et al., 2001). These diterpenoids have been reported to possess diverse activities, such as cytotoxic (Wang et al., 2010), anti-inflammatory (Park et al., 2007; Wang et al., 2011, 2014), and PTP1 B inhibitory (Kim et al., 2006) properties. As a part of our ongoing research program to discover structurally diverse and biologically active diterpenoids from traditional medicinal plants, we investigated the aerial parts of *Siegesbeckia pubescens*. Two new *ent*-kaurane diterpenoids (**1–2**) and sixteen known diterpenoids (**3–18**) were identified. The diterpenoids were tested for their cytotoxic activities against the A375 and HCT-116 cell lines.

2. Results and discussion

The 70% acetone extract of *S. pubescens* was evaporated under reduced pressure and partitioned between $CHCl_3$ and water. The $CHCl_3$ and water-soluble layers were separated using repeated column chromatography to yield two new *ent*-kaurane diterpenoids (**1–2**) (Fig. 1) and sixteen known compounds (**3–18**) (Supplementary data).

Compound **1** possessed a molecular formula of $C_{24}H_{38}O_5$ based on its ^{13}C NMR data and the HRESIMS ion at *m/z* 429.2610 ($[M + Na]^+$, calcd 429.2611), indicating six indices of hydrogen deficiency. The ^1H NMR data (Table 1) showed two tertiary methyl groups (δ_H 1.19 and 1.36) and one doublet (J = 7.0 Hz) of dimethyl protons at δ_H 1.17 due to the isobutyryloxyl group. The ^{13}C NMR data (Table 1) exhibited the presence of 24 carbon signals, which were classified by HSQC and DEPT experiments as four methyls, ten methylenes, four methines and six quaternary carbons. Comparison of the NMR data of 1 and those of *ent*-18-acetoxy-16α-hydroxy-17-isobutyryloxykauran-19-oic acid (Wang et al., 2010) indicated the absence of one acetoxy group at C-18 in **1**. The HMBC correlations of Me-18 (δ_H 1.36) with C-3 (δ_C 39.1), C-4 (δ_C 44.3), and C-19 (δ_C 180.6) indicated that the methyl group (δ_H 1.36 and δ_C 29.8) was attached to C-4. In the NOESY spectrum, the key correlations of Me-18/H-5, H-5/H-9, H-9/H-15β, H-15β/H_2-17 and Me-20/H_2-14 showed that Me-18, H-5, H-9 and H_2-17 were cofacial and were randomly assigned as β-oriented, whereas Me-20 was α-oriented (Fig. 2). The absolute configuration of **1** was determined as (4*R*,5*S*,8*S*,9*R*,10*S*,13*R*,16*R*) by single-crystal X-ray diffraction analysis [absolute structure parameter: 0.03 (7)] (Fig. 3). Accordingly, compound **1** was unambiguously established as (4*R*,5*S*,8*S*,9*R*,10*S*,13*R*,16*R*)-16-hydroxy-17-isobutyryloxykauran-19-oic acid.

Compound **2** had a molecular formula of $C_{20}H_{32}O_4$, as deduced from the HRESIMS ion at *m/z* 359.2198 ($[M + Na]^+$, calcd 359.2193) and ^{13}C NMR data. The ^1H and ^{13}C NMR data (Table 1) of **2** was similar to those of 17-hydroxy-16α-*ent*-kauran-19-oic acid (Wu et al., 1996), except for the presence of an oxygenated methine (δ_C 77.5) in **2**. The ^1H–^1H COSY correlations from H-7 (δ_H 3.90) to H_2-6 (δ_H 2.47 and 2.59) and from H_2-6 to H-5 (δ_H 2.29) and the HMBC correlations from H-7 to C-5 (δ_C 47.9) and C-9 (δ_C 50.4) and from H-15 (δ_H 1.70) to C-7 (δ_C 77.5)

* Corresponding author.
E-mail address: ljxuan@simm.ac.cn (L.-J. Xuan).

http://dx.doi.org/10.1016/j.phytol.2017.07.003
Received 25 April 2017; Received in revised form 23 June 2017; Accepted 7 July 2017

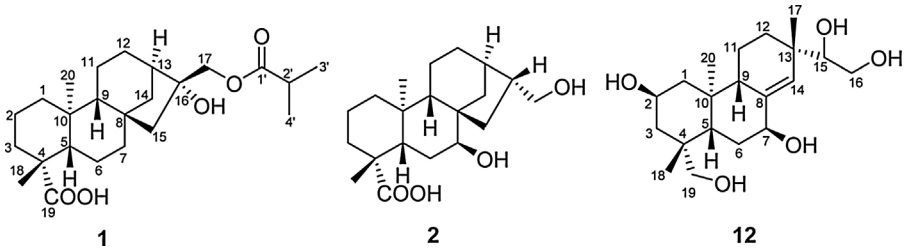

Fig. 1. Chemical structures of compounds **1**-**2** and **12**.

Table 1
^{1}H (500 MHz) and ^{13}C NMR (126 MHz) data for compounds **1**, **2** and **12**.

No.	1[a]		2[a]		12[b]	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1	1.85 m 0.86 td (13.5, 13.5, 4.3)	41.4	1.94 dt (13.3, 4.0, 4.0) 1.04 td (13.3, 13.3, 4.0)	41.7	0.89 m 1.80 dd (11.9, 3.3)	48.5
2	2.30 m 1.52 m	20.2	1.54 m 2.33 overlap	20.4	3.56 m	62.7
3	1.09 m 2.50 br d (13.0)	39.1	1.18 td (13.3, 13.2, 4.3), 2.43 m	39.3	0.71 m 2.04 m	44.8
4		44.3		44.0		39.1
5	1.12 m	57.3	2.29 m	47.9	1.58 m	46.8
6	2.22 m 2.08 m	23.3	2.59 m 2.47 m	31.6	1.65 dt (13.3, 2.4, 2.4) 1.34 m	29.6
7	1.76 m 1.52 m	43.0	3.90 dd (3.8, 2.1)	77.5	3.92 d (2.9)	71.1
8		45.5		49.9		139.5
9	1.05 m	56.5	1.84 m	50.4	2.04 m	45.8
10		40.4		40.2		39.4
11	1.61 overlap	19.2	1.65 m	19.4	1.40 m	17.8
12	1.49 m 1.63 m	27.1	1.49 m 1.65 m	33.0	0.77 overlap 1.88 dd (12.9, 4.0)	31.6
13	2.40 br s	46.7	2.43 m	39.3		37.1
14	2.04 m	38.0	1.30 m 1.87 m	37.2	5.32 s	132.3
15	1.77 m 1.87 m	54.3	1.70 dd (13.8, 5.2) 2.13 m	43.2	3.29 m	75.3
16		79.8	2.27 m	44.9	3.23 m 3.41 m	62.6
17	4.56 d (11.2) 4.66 d (11.2)	69.6	3.72 m	67.5	0.79 s	22.4
18	1.36 s	29.8	1.43 s	29.7	0.85 s	27.5
19		180.6		180.9	3.11 dd (10.6, 5.1) 3.46 m	63.6
20	1.19 s	16.4	1.26 s	16.5	0.66 s	15.9
1′		177.6				
2′	2.61 m	34.7				
3′	1.17 d (7.0)	19.5				
4′	1.17 d (7.0)	19.6				

[a] NMR data were recorded in pyridine-d_5.
[b] NMR data were recorded in DMSO-d_6.

indicated that a hydroxy group was attached to C-7 (Fig. 2). The relative configuration of **2** was elucidated by analysis of its proton coupling constants and NOESY spectrum. The little coupling constant (J = 3.8, 2.1 Hz) of H-7 indicated that H-7 was in α-equatorial orientation. In the NOESY spectrum, the multiple correlations of H-7/H-14, Me-20/H-14, Me-18/H-5, H-5/H-9, H-9/H-15β, H-15α/H_2-17, and H-13/H_2-17 showed that Me-20 and H_2-17 were α-oriented, whereas Me-18, H-5 and H-9 were β-oriented (Fig. 2). The absolute configuration of **2** was assigned as (4*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,16*R*) by single-crystal X-ray diffraction analysis [absolute structure parameter: 0.06 (5)] (Fig. 4). Therefore, compound **2** was unambiguously determined as (4*R*,5*S*,7*S*,8*R*,9*S*,10*S*,13*R*,16*R*)-7,17-dihydroxykauran-19-oic acid. Compound **2** was previously obtained through chemical and microbiological syntheses (Croft et al., 1974). However, its ^{13}C NMR data and the absolute configuration were described here for the first time as a new natural product.

Compound **12** possessed a molecular formula of $C_{20}H_{34}O_5$ based on its HRESIMS ([M + Na]$^+$ *m/z* 377.2298, calcd 377.2298) and ^{13}C NMR data. The NMR data (Table 1) of **12** were compatible with the stucture of *ent*-2α,7α,15,16,19-pentahydroxypimar-8(14)-ene. The latter compound has earlier been reported as well as its C-7 epimer from *S. pubescens* (Wang et al., 2017), but their absolute configurations remain unsettled. Fortunately, we obtained the single crystal of compound **12** at room temperature from dimethyl sulfoxide. Single-crystal X-ray diffraction analysis was then performed to assign the absolute configuration of **12** as (2*S*,4*R*,5*S*,7*S*,9*S*,10*R*,13*S*,15*R*) (Fig. 5). Thus, compound **12** was unambiguously elucidated as (2*S*,4*R*,5*S*,7*S*,9*S*,10*R*,13*S*,15*R*)-2,7,15,16,19-pentahydroxypimar-8(14)-ene.

The known diterpenoids were identified by comparing their spectroscopic data with reported values as *ent*-17-isobutyryloxy-18-hydroxykauran-19-oic acid (**3**) (Wang et al., 2010), *ent*-18-acetoxy-17-isobutyryloxy-16β*H*-kauran-19-oic acid (**4**) (Wang et al., 2010), *ent*-16α,17,18-trihydroxykauran-19-oic acid (**5**) (Wang et al., 2010), *ent*-16α,17-dihydroxykauran-19-oic acid (**6**) (Yamasaki et al., 1976), *ent*-16α,17-dihydroxykauran-19-oic acid methyl ester (**7**) (Herz et al., 1983), *ent*-18-acetoxy-16α,17-dihydroxykauran-19-oic acid (**8**), *ent*-18-acetoxy-17-hydroxy-16β*H*-kauran-19-oic acid (**9**) (Wang et al., 2010), *ent*-17-methoxy-16α-kauran-19-oic acid (**10**) (Fu et al., 1997), *ent*-16α*H*-kauran-17,19-dioic acid (**11**) (Gao et al., 1985), kirenol (**13**) (Liu and Roder, 1991), isopropylidenkirenol (**14**) (Liu and Roder, 1991), darutigenol (**15**) (Barua et al., 1980), 15,16,17-trihydroxypimar-8(14)-ene (**16**) (Hanson and White, 1970), pubeside D (**17**) (Xiong et al., 2001), and *ent*-15,16-dihydroxypimar-8(14)-en-19-oic acid (**18**) (Lee et al., 2008).

All of the isolated diterpenoids were tested for their cytotoxic activities against the A375 (human melanoma) and HCT-116 (human colon carcinoma) cell lines using the MTT method (Mosmann, 1983). As shown in Table 2, compound **7** showed moderate cytotoxic activity against A375 and HCT-116 cells with the IC_{50} values of 6.8 and 10.2 μM. In addition, compounds **4**, **8** and **9** showed weak cytotoxicity against both two cell lines.

3. Experimental

3.1. General experimental procedures

Optical rotations were measured using a Perkin-Elmer 341 polarimeter. Melting points were measured using an SGM X-4 apparatus. IR spectra were recorded with a Perkin-Elmer 577 spectrophotometer. HRESIMS experiments were performed using an Agilent G6224A TOF mass spectrometer. NMR data were obtained on a Bruker AM-500 NMR spectrometer. X-ray crystal data were measured on a Bruker APEX-II CCD detector employing graphite monochromated Cu Kα radiation. Pre-coated silica gel GF254 plates (Qingdao Haiyang Chemical Co., Ltd) were used for TLC analyses. Materials for column chromatography (CC) were silica gel (200–300 mesh, Qingdao Haiyang Chemical Co., Ltd), Sephadex LH-20 (20–80 μm, Amersham Biosciences), and C_8 reversed-phase silica gel (20–45 μm; Fuji Silysia Chemical Ltd.). Semi-preparative HPLC was performed on an Agilent 1100 instrument equipped with a YMC-Pack ODS-A column (250 × 10 mm, S–5 μm).