Phytochemistry Letters 18 (2016) 1–4

Contents lists available at ScienceDirect

Phytochemistry Letters

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

Short communication

New monoterpenes from stalks and infructescence of *Sibiraea leavigata*

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ARTICLE INFO

Article history:
Received 13 May 2016
Received in revised form 16 August 2016
Accepted 26 August 2016
Available online xxx

Keywords:
Monoterpenes
Sibiraea leavigata
Cytotoxic activity

ABSTRACT

Chemical investigation of the ethanol extract of the stalks and infructescence of *Sibiraea leavigata* led to the isolation of two new monoterpenes named (4*R*)-2-(2-hydroxy-4-methyl-3-pentenyl)furan-2(5*H*)-one (**1**) and (2*R*,4*R*)-2-(2-hydroxyethyl)-4-(2-methyl-1-propenyl)furan-5*H*-2-one (**2**) along with eight known phenylpropanoids (**3–10**). Their structures were established on the basis of the interpretation of spectroscopic data and electronic circular dichroism (ECD) calculations. In addition, all of these isolates were evaluated for their cytotoxic activity. The results showed that compound **3** displayed moderate cytotoxicity with IC_{50} values ranging from 10.8 to 49.2 $\mu g\,mL^{-1}$ against five cell lines. While **1** showed selective promotion effects on proliferation of gastric cancer MGC803 and RSC96 cell lines.

1. Introduction

Liu-cha (Salix tea) is a traditional Tibetan medicine mainly used to cure pyrexia and plague by folks, and the leaf of which is the same as Salix leaf, so it was named as Liu-cha (Salix tea). Two plants from the genus *Sibiraea*, *Sibiraea leavigata* and *Sibiraea angustata*, were recorded as the original materials of Liu-cha (Duan et al., 2010; Wu et al., 2012). Most phytochemical and bioactive researches on Liu-cha were focused on *S. angustata*, however rare study was carried out on *S. leavigata*. Terpenoids were reported as the major components of *S. angustata* (Ito et al., 2009; Wang et al., 2013; Li et al., 2010). Biological researches showed that extracts of *S. angustata* exhibited hypolipidemic and antitumor activities (Xie et al., 2014), and improved liver function in rats with nonalcoholic fatty liver disease (Ma et al., 2010).

Sibiraea laevigata (L.) Maxim, a shrub up to 1.5 m height, is mainly growing in the open forests, slopes, meadows, and stream sides in S Gansu (Min Xian, Xigu), E Qinghai (Haiyan Xian, Xining Shi), E Xizang (Suo Xian) of China and also distributes in Kazakhstan, Russia (Siberia) and SE Europe (Bosnia, Croatia) (Editorial Committee of Chinese flora, 1974). The stalks and infructescence of *S. leavigata* have been utilized medicinally as qi-regulating drug and dispelling the wind heat in traditional Tibetan medicine (Duan et al., 2010). Chemical and pharmacological studies on the stalks and infructescence of *S. leavigata* have been poorly conducted. As a part of our research for new bioactive secondary metabolites from Tibetan medicine, two new monoterpenes, (4*R*)-2-(2-hydroxy-4-methyl-3-pentenyl)furan-2(5*H*)-one (**1**) and (2*R*,4*R*)-2-(2-hydroxyethyl)-4-(2-methyl-1-propenyl) furan-5*H*-2-one (**2**), were isolated from the stalks and infructescence of *S. leavigata*, together with eight known phenylpropanoids. The structures of new compounds with their absolute stereochemistry were elucidated on basis of comprehensive spectroscopic analysis and computational method. All of the isolates were tested for their cytotoxic activity against four tumour cell lines and RSC96 cell line. The results obtained are discussed herein.

2. Results and discussion

Repeated column chromatography over silica gel, preparative and semi-preparative HPLC of the ethanol extract from the stalks and infructescence of *S. leavigata*, led to the isolation of two new monoterpenes, named (4*R*)-2-(2-hydroxy-4-methyl-3-pentenyl)

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http://dx.doi.org/10.1016/j.phytol.2016.08.016
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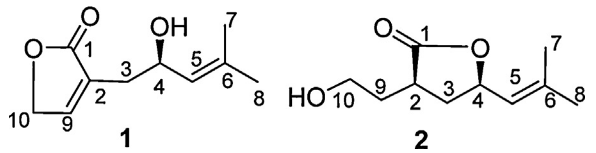

Fig. 1. Chemical structures of compounds **1** and **2** from stalks and infructescence of *S. leavigata.*

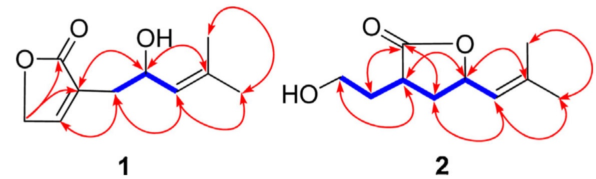

Fig. 2. Selected HMBC (H → C) and COSY (H—H) correlations of compounds **1** and **2**.

furan-2(5*H*)-one (**1**) and (2*R*,4*R*)-2-(2-hydroxyethyl)-4-(2-methyl-1-propenyl)furan-5*H*-2-one (**2**), (Fig. 1) along with eight known phenylpropanoids (**3**–**10**).

(4*R*)-2-(2-Hydroxy-4-methyl-3-pentenyl)furan-2(5*H*)-one (**1**), obtained as a colorless oil, possessed a molecular formula $C_{10}H_{14}O_3$ with four double-bond equivalents (DBEs) as determined by the positive ESIMS (*m/z* 183 $[M+H]^+$, 205 $[M+Na]^+$ and 387 $[2M+Na]^+$) and HRESIMS (*m/z* 387.1782 $[2M+Na]^+$, calcd. 387.1778). The IR spectrum showed the presence of hydroxyl (3422 cm^{-1}), alkyl (2926 and 2856 cm^{-1}) groups, ester (1749 and 1206 cm^{-1}) group, and double bonds (1629 cm^{-1}). The ^{13}C NMR (Table 1) and HSQC spectra displayed 10 carbon signals, arising from one carbonyl (δ_C 177.4), two double bonds (δ_C 136.6, 128.5, 131.6 and 150.0), two methyls (δ_C 25.9 and 18.2), two methylenes, including one oxygen-bearing one (δ_C 72.1), and one oxygen-bearing methine (δ_C 67.2). The ^1H NMR data (Table 1) showed typical resonances for two methyl groups (δ_H 1.72 and 1.67, each 3H, s) and two olefinic protons. The aforementioned data indicated that **1** could be a monoterpene lactone (Ito et al., 2009; Li et al., 2010). In the ^1H-^1H COSY spectrum of **1**, two proton spin systems, H-3/H-4/H-5 and H-9/H-10 were observed (Fig. 2). In HMBC spectrum, the correlations from H-3 [δ_H 2.50, ddd (*J* = 14.4, 7.5, 1.3 Hz) and 2.37, m] and H-9 (δ_H 7.41, m) to the carbonyl C-1 (δ_C 177.4), from H-4 [δ_H 4.58, dd (*J* = 14.7, 7.5 Hz)] to C-6 (δ_C 136.6), from H-7 (δ_H 1.72, s) and H-8 (δ_H 1.67, s) to C-5 (δ_C 128.5), and from H-10 [4.82, dd (*J* = 3.1, 1.5)] to C-1 (δ_C 177.4) were observed (Fig. 2). Thus, the planar structure of **1** was established as shown in Fig. 2. The absolute configuration of **1** was determined by ECD calculation. The calculated ECD spectrum was consistent with the experimental curve (Fig. 4), which suggested that the absolute configuration of compound **1** was 4*R* (Fig. 4).

(2*R*,4*R*)-2-(2-Hydroxyethyl)-4-(2-methyl-1-propenyl)furan-5*H*-2-one (**2**), a colorless oil, possessed a molecular formula $C_{10}H_{16}O_3$ with three unsaturation degrees as determined by the positive ESIMS (*m/z* 207 $[M+Na]^+$ and 391 $[2M+Na]^+$) and HRESIMS (*m/z* 391.2096 $[2M+Na]^+$, calcd. 391.2097). The IR spectrum showed the presence of hydroxyl (3421 cm^{-1}), alkyl (2970 and 2926 cm^{-1}) groups, ester (1763 and 1179 cm^{-1}) group, and double bonds (1630 cm^{-1}). The ^{13}C NMR (Table 1) and HSQC spectra displayed 10 carbon signals, arising from one carbonyl (δ_C 181.5), one double bond (δ_C 124.1 and 141.0), two methyls (δ_C 25.9 and 18.4), three methylenes (one oxygen-bearing methylene at δ_C 60.6), and two methines (one oxymethine at δ_C 77.5). The ^1H NMR data (Table 1) showed typical resonances for two methyls (δ_H 1.76 and 1.78, each 3H, s) and one olefinic proton. The aforementioned data indicated that **2** could also be a monoterpene lactone. In the ^1H-^1H COSY spectrum of **2**, the proton spin system H-2/H-3/H-4/H-5/H-9/H-10 (Fig. 2) could be constructed. In HMBC spectrum, based on the correlations from H-3 (δ_H 2.53, m and 1.64, m) and H-9 (δ_H 2.08, m and 1.59, m) to the carbonyl C-1 (δ_C 181.5), from H-4 (δ_H 5.18, m) to C-6 (δ_C 141.0), and from H-7 (δ_H 1.76, s) and H-8 (δ_H 1.78, s) to C-5 (δ_C 124.1), the monoterpene moiety can be constructed. The connectivity of lactone was determined through the deshielded chemical shifts of H-4 (δ_H 5.18) and C-4 (δ_C 77.5) (Ito et al., 2009; Wang et al., 2013; Li et al., 2010). Thus, the planar structure of compound **2** was established as shown in Fig. 2. In the NOE spectrum of **2** (Fig. 3), the observed cross-peak between H-2 and H-4 demonstrated that H-2 and H-4 located on the same face of the ring. Furthermore, the experimental ECD spectrum of **2** agreed well with that of the computational curve (Fig. 4), and the absolute configuration of **2** was determined to be 2*R*,4*R*. On the basis of the above evidences, the structure of **2** was deduced as shown in Fig. 1.

The known compounds were identified as 3-(3′,4′,5′-trimethoxyphenyl)-prop-2-en-1-al (**3**) (Joshi et al., 2005), (*E*)-3-(4-methoxyphenyl) acrylic acid (**4**) (Lorentzen et al., 2015), (*E*)-3-(3,4-dimethoxyphenyl) acrylic acid (**5**) (Lorentzen et al., 2015), (*E*)-ferulic acid (**6**) (Chen et al., 2015), caffeic acids (**7**) (Kumar et al., 2015), 3,4,5-trimethoxycinnamyl alcohol (**8**) (Sadik et al., 2003), dihydrosinapyl alcohol (**9**) (Lancefield et al., 2015), dihydroconiferylalcohol (**10**) (Huang et al., 2014) (Fig. 1), by comparison of their 1D spectroscopic data with those reported in the literature.

Since Liu-cha (Salix tea) is a traditional Tibetan medicine mainly used to cure pyrexia and plague by folks and has great potential to develop into a healthy beverage. All of the isolates were evaluated for their cytotoxic activity. The cytotoxic activity was tested using the MTT method (Chang et al., 2004). The results showed that compound **3** displayed moderate cytotoxicity with

Table 1
^1H and ^{13}C NMR data for compounds **1** and **2** (δ in ppm, in CD_3OD, 600 and 150 MHz, respectively).

No.	**1**		**2**	
	δ_C	δ_H, multi. (*J*, Hz)	δ_C	δ_H, multi. (*J*, Hz)
1	177.4, s		181.5, s	
2	131.6, s		39.5, t	2.86, m
3	34.5, t	2.50, ddd (14.4,.7.5,1.3) 2.37, m	37.2, t	2.53, m 1.64, m
4	67.2, d	4.58, dd (14.7, 7.5)	77.5, d	5.18, m
5	128.5, d	5.17, m	124.1, d	5.22, m
6	136.6, s		141.0, s	
7	25.9, q	1.72, s	18.4, q	1.76, s
8	18.2, q	1.67, s	25.9, q	1.78, s
9	150.0, d	7.41, m	34.2, t	2.08, m 1.59, m
10	72.1, t	4.82, dd (3.1, 1.5)	60.6, t	3.70, dt (12.1, 6.6) 3.62, m

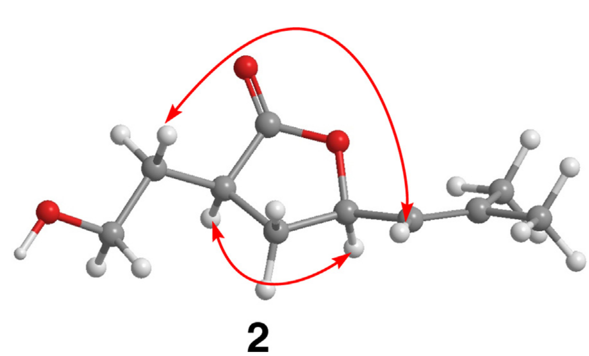

Fig. 3. Key NOESY (H↔H) correlations of compound **2**.