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Biguaiascorzolides A and B: Two novel dimeric guaianolides with a rare skeleton, from *Scorzonera austriaca*

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ABSTRACT

The root of *Scorzonera austriaca* has been used in indigenous cuisines as a delicious food and in the Tibetan traditional medicine in northwestern China. Two novel dimeric guaianolides linked by a carbon–carbon bond with a rare carbon skeleton, termed biguaiascorzolides A (**1**) and B (**2**), respectively, have been isolated from roots of *S. austriaca*. Acetylation of **1** gave **1a**. The structures of **1**, **1a** and **2** were characterised by HR-ESI-MS, EI-MS, UV, IR, and 1D- and 2D-NMR techniques (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC, and NOESY experiments). The cytotoxicity of **1a** was assayed against selected cancer cell lines, including the human erythroleukaemia adriamycin-resistant subline (K562/ADM) and human stomach carcinoma (MGC-803) cell lines. Compound **1a** exhibits a moderate activity against K562/ADM cell lines (IC_{50} 39.8 μm) and is inactive towards MGC-803 cell lines.

1. Introduction

The genus *Scorzonera* (Asteraceae) occurs mainly in the drought areas of Europe and Asia. It consists of 28 European species distributing all over the continent and 23 species distributing in China. Some *Scorzonera* spices have been used as delicious asparagus-like vegetables and folk-medicinal plants in Europe and Asia (Zidorn, Ellmerer, Sturm, & Stuppner, 2003). The topical plant, *Scorzonera austriaca* Willd, occurs in the northwestern mountainous regions of China. Its root has been used in indigenous cuisines as a delicious food and in Tibetan traditional medicine for the treatment of many diseases, such as fever, carbuncle, inflammation and mastitis (Gao, 1987).

Previous phytochemical investigations on the members of *Scorzonera* have resulted in the identification of sesquiterpenes and sesquiterpene lactones from *S. hispanica* and *S. austriaca* (Bryanskii, Tolstikhina, Zinchenko, & Semenov, 1992; Li, Wu, Shi, & Zhu, 2004; Zidorn, Ellmerer-Müller, & Stuppner, 2000). Among them, a substance with a high immunomodulating activity was isolated from *S. hispanica* and has been submitted for a patent (Semenov, Tolstikhina, Bryanskii, & Khobrakova, 2002). Yet, the biological activities of *S. austriaca* have not been investigated. Sesquiterpene lactones are known as characteristic constituents of the Asteraceae family (Zdero & Bohlmann, 1990). Some of them have biological and pharmacological activities, such as antimicrobial, antitumoural, cytotoxic and anti-inflammatory activities. They also exhibit effects on the central nervous and cardiovascular systems, and an allergenic potency (Fang et al., 2005; Scotti, Fernandes, Ferreira, & Emerenciano, 2007a). It is noteworthy that the medical sesquiterpenolides, artemisinin and its derivative artesunate, have been used as effective and economic anti-malarial drugs, and have antitumour activity both *in vitro* and *in vivo*. They may become novel anticancer drugs (Efferth, Dunstan, Sauerbrey, Miyachi, & Chitambar, 2001). Furthermore, a sesquiterpene lactone parthenolide from the traditional herbal medicine, Feverfew, is well-known to have anti-inflammatory activity and as in NF-κB inhibitor in the treatment of breast and pancreatic cancers, and is currently under clinical evaluation (Hehner, Hofmann, Droge, & Schmitz, 1999). Therefore, the topic of sesquiterpenolides, involving their structures, biological properties, structure–activity relationships and natural sources of intermediates used for synthesising valuable compounds, has attracted the attention of chemists. (Manzano, Guerra, Moreno-Dorado, Jorge, & Massanet, 2006; Scotti et al., 2007b)

The investigation of bioactive sesquiterpenoids from Asteraceae has been one of our research subjects (Chen, Zhu, Shen, & Jia, 1996; Li et al., 2004; Yang, Zhu, & Jia, 2003; Zhu, Yang, & Jia, 1999; Zhu, Zhao, Huang, & Wu, 2008; Zhu, Zhu, & Jia, 2000). In this work we will present our new results for two novel dimeric sesquiterpenolides from *S. austriaca*, termed biguaiascorzolides A (**1**) and B (**2**), and the acetylated compound (**1a**), which have a rare carbon skeleton linked by a carbon–carbon bond. Their structures were characterised by HR-ESI-MS, FAB-MS, EI-MS, UV, IR, and 1D- and

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2D-NMR techniques (^{1}H and ^{13}C NMR, ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments). In addition, the cytotoxicity of **1a** was evaluated against selected cancer cell lines including the human erythroleukaemia adriamycin-resistant subline (K562/ADM) and human stomach carcinoma (MGC-803) cell lines.

2. Materials and methods

2.1. General procedures

Optical rotations were measured on a Perkin–Elmer Model 341 polarimeter. IR spectra were recorded on a Nicolet 170SX FT-IR instrument using KBr discs over the range 400–4000 cm^{-1}. NMR spectra were obtained on a Bruker AM-400 and a Varian Mercury-300/400BB NMR spectrometer with TMS as an internal standard in $CDCl_3$. HR-ESI-MS were obtained on a Bruker Daltonics APEX-II 47e spectrometer. EI-MS and FAB-MS were measured on an HP5988a GC/MS and a VG-ZAB-HS at 70ev. UV detection was measured on a Shimadzu UV-260 spectrophotometer. Analytical and preparative thin-layer chromatography (TLC) were performed on silica gel plates (GF_{254} 10–40 μm, Qingdao Marine Chemical Factory). Analytical TLC was provided to follow the separation and check the purity of isolated compounds. Spots on the plates were observed under UV light and visualised by spraying them with 5% H_2SO_4 in EtOH (v/v), followed by heating. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical Co.).

2.2. Plant material

The whole plant of *S. austriaca* was collected in mountainous areas at altitudes between 2500 and 3000 m in Qinghai Province of China and was identified by Prof. Fu-Jia Zhang, School of Life Science, Lanzhou University. A voucher specimen (No. Sa19990801) has been deposited in the State Key Laboratory of Applied Organic Chemistry, Lanzhou University, China.

2.3. Extraction and isolation

The air-dried roots of *S. austriaca* (3.5 kg) were powdered and extracted with acetone (20 l) four times at room temperature, each extraction lasted five days. The extracts were concentrated under reduced pressure and filtered. The filtrate was then evaporated to yield crude extract of acetone (182 g). The extract was chromatographed on silica gel columns using a stepwise solvent gradient method with petroleum ether (60–90 °C)/acetone and finally washed with MeOH. The fraction (6:1 petroleum ether/acetone) was subjected to further chromatographic separation with petroleum ether/EtOAc, then $CHCl_3$/acetone, to yield pure **2** (3 mg). The other fraction (3:1 petroleum ether/acetone) was subjected to chromatographic separation with petroleum ether/EtOAc, then $CHCl_3$/Me_2CO and further isolated by preparative TLC to afford **1** (20 mg).

2.4. Acetylation of compound *1*

Compound **1** (18 mg) was acetylated with a 1:1 mixture of Ac_2O and pyridine. The solution was allowed to stand overnight at room temperature. After the usual work-up, pure **1a** (10 mg) was obtained.

2.5. Cytotoxic assay

The cytotoxicity was determined by the [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] (MTT) colorimetric assay, as previously reported (Hussain, Nouri, & Oliver, 1993): a compound under study was dissolved in DMSO to make a stock solution, which was diluted to a working solution before use (the final DMSO concentration was 0.1% v/v). Logarithmically growing K562/ADM cells, containing 2×10^8 cells/l and BGC-823 cells containing 1×10^8 cells/l, respectively, were seeded in 96-well flat-microtitre plates (NUNC, Nunclon, Nagle, Denmark). After 24 h of incubation, the cells were treated with **1a** at various concentrations (0, 10, 50, 100 and 200 mg/l) and with carboplatin at a concentration of 100 mg/l as a positive control and then grown in a humidified atmosphere at 37 °C in 5% CO_2 for 72 h. Parallel controls consisted of deionized H_2O with the same DMSO concentration. Three replicate wells were used at each point in the experiments. 10 μl of MTT solution (5 mg/ml in PBS) were added to each well and incubated for 4 h before termination of culture. Hundred microlitre extraction buffer (10% SDS with 0.01 M HCl) were then added to each well. After an overnight incubation at 37 °C, to dissolve the crystal product, the absorbance was detected at a wavelength of 570 nm, using a microplate reader (Power Wave X200, Bio-TEK, USA) to calculate the inhibition rate (IR) for the cell proliferation and, accordingly, the 50% growth-inhibitory concentration (IC_{50}) value. The cytotoxicity was calculated as cytotoxicity IR (%) = [(A_{570} of untreated cells − A_{570} of treated cells)/A_{570} of untreated cells] × 100%.

2.6. Characteristic data of compounds

Compound **1**: colourless gum, EIMS *m/z* (%): 494 (3) $[M]^+$, 476 (8) $[M-18]^+$, 403 (32), 169 (98), 161 (32), 155 (39), 91 (100), 79 (50), 55 (61) and 43 (30).^{1}H and ^{13}C NMR (see Table 1).

Compound **1a**: $C_{32}H_{40}O_7$; colourless gum, $[\alpha]_D^{20}$ + 31 (*c* 0.814, CH_3OH); IR (KBr) ν_{max} 3385, 2930, 1772, 1737, 1642, 1237, 1178, 1028, 991, 913 and 731 cm^{-1}; UV (CH_3OH) λ (logε) 210 (4.2) nm; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 537.2845 (calcd. for $C_{32}H_{40}O_7$ + H, 537.2847), 554.3121 $[M + Na]^+$.

Compound **2**: colourless gum, $[\alpha]_D^{20}$ − 15 (*c* 0.36, $CHCl_3$); IR (KBr) ν_{max} 3425, 2918, 1770, 1730, 1634, 1456, 1375, 1109, 1066, 913 and 739 cm^{-1}; UV (CH_3OH) λ (logε) 210.40 (4.2) nm; ^{1}H and ^{13}C NMR data (see Tables 1 and 2); HRESIMS *m/z* 495.2718 (calcd. for $C_{30}H_{38}O_6$ + H, 495.2741) and 554.3121 $[M + Na]^+$. FABMS (ZAB-HS): 495.5 $[M + H]^+$; EIMS *m/z* (%): 494 (5) $[M]^+$, 161 (20), 105 (29), 91 (64), 95 (30), 79 (50), 69 (39), 55 (100), 57 (50) and 43 (78).

Table 1
^{13}C NMR and DEPT data for **1**, **1a** and **2** in $CDCl_3$.

Position	1[a]	1a[a]	2[b]	Position	1[a]	1a[a]	2[b]
1	39.7 d	39.9 d	39.7 d	1′	39.9 d	41.0 d	44.9 d
2	44.8 t	45.0 t	45.2 t	2′	26.6 t	26.4 t	25.1 t
3	219.3 s	219.2 s	219.3 s	3′	36.4 t	36.2 t	29.7 t
4	51.2 d	51.6 d	51.6 d	4′	143.7 s	144.0 s	148.0 s
5	47.4 d	46.9 d	45.8 d	5′	53.3 d	53.5 d	51.7 d
6	88.2 d	88.4 d	88.9 d	6′	84.6 d	84.3 d	85.9 d
7	48.6 d	48.5 d	48.0 d	7′	45.0 d	45.7 d	42.9 d
8	32.8 t	32.9 t	33.1 t	8′	38.8 t	37.2 t	46.4 t
9	39.6 t	38.7 t	39.0 t	9′	73.7 d	75.2 d	211.3 s
10	151.7 s	149.4 s	149.6 s	10′	149.3 s	147.7 s	47.8 d
11	41.9 d	41.9 d	41.8 d	11′	41.6 d	41.6 d	41.6 d
12	178.5 s	178.3 s	177.9 s	12′	178.6 s	178.4 s	178.5 s
13	13.2 q	13.2 q	13.0 q	13′	12.8 q	12.8 q	12.6 q
14	112.2 t	112.5 t	112.3 t	14′	112.7 t	113.7 t	15.6 q
15	26.9 t	26.5. t	26.0 t	15′	126.1 d	126.1 d	125.8 d
				OAc		170.0 s	
						21.2 q	

[a] Recorded at 100.32 MHz.
[b] Recorded at 75.32 MHz.

Table 2
^{1}H NMR data for **1**, **1a** and **2** in $CDCl_3$, δ_H mult. (*J* in Hz)*.

No.	**1**[b]	**1a**[a]	**2**[b]	No.	**1**[b]	**1a**[a]	**2**[b]
1α	3.03 m	3.07 dd (8.1, 8.2)	3.11 dd (8.0, 6.4, 6.4)	1′α	3.46 m	3.26 dd (7.7, 7.4)	3.33 m
2a	2.53 m	2.56 m	2.65 ddd (10.2, 6.4, 5.2)	2′a	1.89 m	1.80 m	1.82 m
2b	2.47 m	2.46 m	2.48 d (6.0)	2′b	2.10 m	2.09 m	2.06 m
3	–	–	–	3′a	2.48 m	2.47 m	2.32–2.28 m
				3′b	2.47 m	2.46 m	2.36–2.62 m
4β	2.38 m	2.37 ddd (9.8, 5.0, 4.8)	2.38 ddd (10, 5.6, 4.8)	4′	–	–	–
5α	2.53 m	2.63 ddd (9.1, 8.9, 8.1)	2.83 brdd (9.0, 8.8)	5′α	2.95 m	2.97 brt (9.3)	2.91 brdd (11.2, 8.8)
6β	3.93 dd (9.6,8.7)	3.93 dd (9.0,8.9)	3.78 t (10.0)	6′β	4.04 dd (9.9, 9.6)	3.96 dd (8.3, 9.3)	3.93 dd (8.8, 9.2)
7α	2.16 m	2.17 m	2.23 m	7′α	2.17 m	2.16 m	2.26 m
8a	2.27 m	2.26 m	2.18 m	8′a	2.35 m	2.27 m	2.30–2.15 m
8b	1.35 m	1.35 ddd (16.0, 11.1, 4.9)	1.35 ddd (12.4, 12.0, 5.2)	8′b	1.47 m	1.56 ddd (13.8,10.9,3.1)	2.30–2.15 m
9a	2.53 m	2.55 dd (15.2, 5.0, 2.5)	2.57 ddd (10.8, 4.4)	9′β	4.59 brd (6.0)	5.53 dd (4.2, 3.8)	–
9b	2.14 m	2.13 m	2.14 m				
10	–	–	–	10′	–	–	2.20–2.07 m
11	2.20 m	2.20 m	2.21 m	11′	2.20 m	2.19 m	2.19 m
12	–	–	–	12′	–	–	–
13α	1.25 d (6.3)	1.25 d (6.3)	1.26 d (7.3)	13′α	1.20 d (6.9)	1.20 d (6.3)	1.26 d (7.3)
14a	4.89 s	4.96 s	4.96 s	14′a	5.02 s	5.10 s	0.87 d (6.4)
14b	4.66 s	4.64 s	4.63 s	14′b	4.95 s	4.98 s	
15a	2.16 m	2.15 m	2.15 m	15′	5.60 s	5.58 brs	5.52 s
15b	1.90 m	1.91 m	1.91 ddd (13.6, 5.2, 4.8)				
				OAC		2.12 s	

[a] Recorded at 400.16 MHz.
[b] Recorded at 300.32 MHz.
* The assignment of **1a** was performed by ^{1}H-^{1}H COSY, HMQC, HMBC and NOESY experiments and that of **2** by ^{1}H-^{1}H COSY and HMBC experiments.

3. Results and discussion

3.1. Phytochemical investigation

Compound **1** was obtained as a colourless gum. Its EI-MS data provided a molecular ion peak at *m/z* 494 $[M]^+$ and suggested its molecular formula as $C_{30}H_{38}O_6$ and 12 degrees of unsaturation. ^{13}C NMR and DEPT spectra of **1** displayed 30 carbon signals assigned to two methyl, nine methylene, 13 methine and six quaternary carbons (Table 1). One ketone carbonyl (δ_C 210.0) and three *exo*-olefin signals (δ_C 151.7, 143.7, 149.3, 126.1, 112.7 and 112.2) were evident. Two lactone carbonyl carbons (δ_C 178.6 and 178.5) and two carbons bearing oxygen (δ_C 88.2 and 84.6) were assigned to two γ-lactones, respectively. A signal for carbon bearing oxygen at δ_C 73.7 was attributable to a hydroxyl group. Furthermore, the ^{1}H NMR spectrum of **1a** showed three *exo*-olefin methylenes (δ_H 4.89, 4.66, 5.02, 4.95 and 5.60) and two doublet methyl signals at δ_H 1.25 (3H, J = 6.3 Hz) and 1.20 (3H, J = 6.3 Hz), which are characteristic of methyl protons of the α-methyl-γ-lactone groups commonly in guaianolide sesquiterpene lactones (Kumari, Masilamani, Ganesh, & Aravind, 2003; Macias, Varela, Torres, & Molinillo, 1993). All these observations suggest that **1** is a dimeric guaianolide sesquiterpene with a hydroxyl group (Kumari et al., 2003; Macias et al., 1993). In order for the site of dimer connection and the position of hydroxyl to be confirmed, compound **1** was acetylated to **1a**.

The molecular formula $C_{32}H_{40}O_7$ of **1a** was deduced from the HR-ESI-MS detection (at *m/z* 537.2845, requires 537.2847 $[M + H]^+$). The presence of γ-lactone, carbonyl and double band was indicated by IR bands at 1772, 1737 and 1641 cm^{-1}, respectively. The ^{13}C NMR spectrum of **1a** displayed 32 carbon signals, which were assigned, using HMQC, HMBC and DEPT experiments, to seven quaternary, 13 methine, nine methylene and three methyl carbon atoms. Actually, ^{1}H and ^{13}C NMR spectra of **1a** are very similar to that of **1** (Table 1), except that the peak of oxymethine of **1a**, ranging from δ_C 73.7 to 75.2, locates downfield. In addition, the methyl protons of acetyl signals (δ_C 170.0 *s* and 21.2 *q*) gave rise to a singlet peak at 2.12 in its ^{1}H NMR. ^{1}H-^{1}H COSY and HMBC correlation experiments established the complete planar structure of **1a** and clearly indicated that **1a** was a dimeric molecule with two guaian-12,6-olide units (Fig. 2). The HMBC spectrum of **1a** showed the key correlations of a guaianolide unit with a carbonyl group and an exocyclic double bond, δ_C 219.3 (C-3)/H-1, H-2a, H-2b, H-4, H-15b, 10(14); δ_C 26.5 (C-15)/H-4, H-5, H-15′; and δ_C 149.4 (C-10)/H-14a, H-14b, H-9a, H-9b, H-1, H-2b, H-5. They allowed us to assign **1a** as containing a ketone group at C-3, a methylene group at C-4, and an exocyclic double bond between C-10 and C-14, respectively. Moreover, the key correlations of another guaianolide unit with an acetyl group and two exocyclic double bonds, δ_C 170.0 (C-1″)/H-2″, H-9′; δ_C 144.0 (C-4′)/H-15′, H-5′, H-3a′, H-3b′, H-1′, H-15a, H-15b; δ_C 147.7 (C-10′)/H-14a′, H-14b′, H-1′, H-9′, H-8a′, indicated that an acetyl group was located at C-9′ and two terminal double bonds located between C-4′ and C-15′, and between C-10′ and C-14′, respectively. ^{1}H and ^{13}C NMR data for two subunits are similar to the literature data for dihydroestafiatone (Kumari et al., 2003) and annuolide D (Macias et al., 1993), respectively. A linkage between two units was determined by the HMBC experiment, which showed the correlations of H-15b/C-4′, C-3, C-4, C-5; C-15′/H-15a, H-5′, H-3′a, H-3′b, H-1′; and H-15′/C-15, C-3′, C-4′, C-5′. They indicated that **1a** had a carbon–carbon linkage between C-15 and C-15′.

The relative stereochemistry of **1a** was mainly determined from coupling constants and NOESY correlations. The coupling constants for H-5 ($J_{1,5}$ = 8.1 Hz) and H-5′ ($J_{1',5'}$ = 7.7 Hz) clearly indicated that both the AB and A′B′ adopt a *cis* ring fusion structure. The coupling constants for H-6 ($J_{5,6}$ = 8.9, $J_{6,7}$ = 9.0 Hz) and H-6′ ($J_{5',6'}$ = 8.3, $J_{6',7'}$ = 9.3 Hz) indicated the existence of two *trans*-fused α-methyl-γ-lactone rings in **1a**. The orientation of H-6 and H-11 was determined to be β and that of H-1 H-5, H-7, CH_2-15 and Me-13 were determined to be α by NOESY correlations (H-6/H-4, H-11; H-5/H-1; H-13/H-7, H-5) and by the coupling constant of H-6 to H-7. Meanwhile, H-1′, 9′-OAc and 13′-Me were also assigned to be in an α-configuration by the strong correlations in NOESY (H-5′/H-1′, H-7′; H-13′/H-7′; H-6′/H-9′; H-11′/H-6′). Hence, the structure of **1a** was elucidated unambiguously, as shown in Fig. 1. The acetylation does not change the orientation of 9′-OH in **1**. Therefore, compound **1** is a novel dimeric guaianolide and is given a trivial name of biguaiascorzolide A.

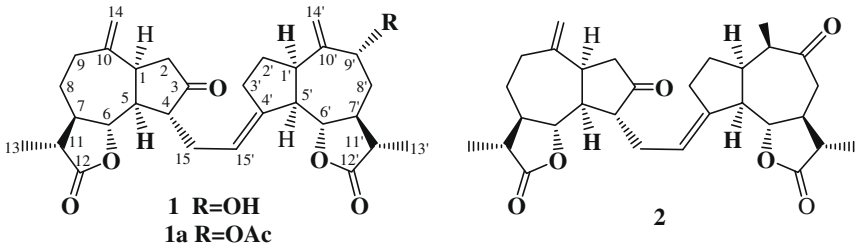

Fig. 1. Structures of dimeric guaianolides **1**, **1a** and **2**.

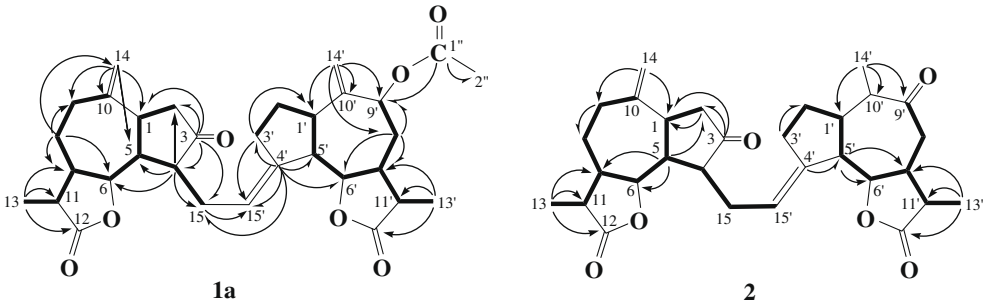

Fig. 2. Key COSY (bold), HMBC (H → C, C — H) correlations of **1a** and **2**.

Minor compound **2**, named biguaiascorzolide B, was obtained as a yellow gum with a molecular formula of $C_{30}H_{38}O_6$, determined by HR-ESI-MS at *m/z* 495.2718 (requires 495.2741 $[M + H]^+$). Its IR spectrum showed characteristic absorption bands at 3425 (hydroxyl), 1770 (γ-lactone), 1730 (ester carbonyl), and 1634 cm^{-1} (double bond). Its NMR spectra showed similarities of chemical shifts, coupling constants, and 2D-NMR correlations to **1a** (Tables 1 and 2, and Fig. 2). From these observations, compound **2** is assumed to have the same skeleton as **1a** and **1**. By comparing ^{13}C NMR data for **2** with those for **1**, it was found that the exocyclic double bond $\Delta^{10'(14')}$ in **1** was converted into a methine δ_C 47.8 (C-10′) and a methyl δC 15.6 (C-14′) in **2**, whereas the hydroxyl group at C-9′ in **1** was oxidised to a ketone functionality at δ_C 211.3 in **2**. A methyl group at C-10′ and a ketone at C-9′ were confirmed by the correlations between H-14′ at δ_H 0.87 and C-9′ at δ_C 211.3, as well as between C-10′ at δ_C 47.8 and C-1′ at δ_C 44.9 in the HMBC spectrum. The ^1H-^1H COSY spectrum manifested the correlation between H-15a at δ_H 2.15 and H-15′ at 5.58, indicating a linkage through C-15 and C-15′ between two units, and the correlation between H-1′ at δ_H 3.33 and H-14′ at 0.87, indicating the position of Me-14′. The relative stereochemistry of **2** was determined by comparing chemical shifts and coupling constants for **1a** and **2**. Me-14′ was assigned to be in a β-configuration from its ^1H NMR signals (δ_H 0.87, J = 6.4 Hz and δ_C 15.6) and by comparing those with literature data (Rodriguez & Boulanger, 1997; Zhang, Liao, & Yue, 2004). Consequently, the stereochemistry of **2** was elucidated, as shown in Fig. 1. It is also a novel dimeric guaianolide.

3.2. Anticancer activity

Many investigations have shown that guaianolides consist of a highly bioactive group of sesquiterpenes with a wide spectrum of biological activities, particularly antitumour and cytotoxic activities (Bruno et al., 2005). Some of them display strong activities against leukaemia, breast, colon, ovarian and lung cancers and are currently under clinical evaluation (Nosse, Chhor, Jeong, Bölhm, & Reiser, 2003). They are thought to have considerable potentials as anticancer drugs; e.g., cynaropicrin, isolated from *Saussurea lappa*, was reported to potently inhibit the proliferation of leucocyte cancer cell lines, and to possess immunomodulatory effects, immunosuppressive effects, and cytotoxic effects (Cho et al., 2004).

On the basis of anti-inflammatory and other activity of the genus reported previously, the cytotoxic activity of guaianolide lactones closely structurally related to our compounds (Zhang et al., 2006), and the high incidence of human leukaemia and stomach cancer in China, particularly in the countrysides of China, the cytotoxic activity of **1a** was evaluated against K562/ADM human erythroleukaemia adriamycin-resistant subline cell lines and MGC-803 human stomach carcinoma cell lines, using MTT colorimetric assay. Compound **1a** exhibits a moderate activity against K562/ADM cell lines (IC_{50} 39.8 μm) and is inactive for MGC-803 (IC_{50} > 100 μm). The results were obtained from the inhibition of **1a** of the viabilities of cancer cells after 72 h of incubation. They indicated that **1a** is more effective against K562/ADM cancer cells than MGC/ 803 gastric cancer cells as measured by growth inhibition and apoptosis assays at the same concentration of drug, which suggests that **1a** might possess different anticancer activities towards solid tumours and hemocytein tumours. The test shows that **1a** obviously inhibits the proliferation of K562/ADM cancer cells and induces an apoptosis in tumour cells. Thereby, the mechanism of action of **1a** on K562/ADM cancer cells is assumed to inhibit a variety of cellular functions which direct cells into apoptosis. Previous studies of natural guaianolide sesquiterpenes and synthetic analogues showed that the main determining factor responsible for the cytotoxicity of compounds under study was the existence of an α,β-unsaturated system (Nosse et al., 2003), which likely serves as an alkylating centre and can be a part of an ester, ketone, or lactone moiety. Besides, the double bonds (e.g., methylene groups at positions 10 or 3), substituents and their position are also important to the activity (Scotti et al., 2007a). The activity of **1a** may be related to the 6,12-lactonization, the double bond at the C-10 position and the presence of an acetyl group (or a hydroxyl group) in the molecule. The moderate activity of **1a** against K562/ADM cells may be due to the absence of an α-methylene bearing a γ-lactone moiety.

4. Conclusions

Except for the typical guaianolide framework, only a few dimeric types exist in nature. Most of them consist of two guaianolide units linked by a carbon–oxygen single bond. To our best knowledge, biguaiascorzolides A (**1**) and B (**2**) are two novel naturally occurring dimeric guaianolides with a rare carbon skeleton linked through a carbon–carbon bond and they are identified for the first time in this genus. Results of their bioassays indicate that bioactive molecules are characteristic of a guaianolide skeletal type structure. Although this work provides preliminary evidence for **1a** in the inhibition of some hemocytein tumour cells *in vitro*, further investigations of **1a** and its analogues on other hemocytein tumour cells are warranted. By elucidating how molecules interact, research in this area will provide the data needed to explore the relationship between anticancer activity and molecular structure. Studies should also be carried out to extend the anti-inflammation and anti-mastitis bioactivity evaluation in plants having inflammation and mastitis bioactivities and other pharmaceutical functions.

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References

Bruno, M., Rosselli, S., Maggio, A., Raccuglia, R. A., Bastow, K. F., & Lee, K.-H. (2005). Cytotoxic activity of some natural and synthetic guaianolides. *Journal of Natural Products, 68*(7), 1042–1046.

Bryanskii, O. V., Tolstikhina, V. V., Zinchenko, S. V., & Semenov, A. A. (1992). A sesquiterpene glucoside from cultivated cells. *Khimiya Prirodnykh Soedinenii, 28*(6), 640–645.

Chen, H., Zhu, Y., Shen, X.-M., & Jia, Z.-J. (1996). Four new sesquiterpene polyol esters from *Cremanthodium ellisii*. *Journal of Natural Products, 59*(12), 1117–1120.

Cho, J. Y., Kim, A. R., Jung, J. H., Chun, T., Rhee, M. H., & Yoo, E. S. (2004). Cytotoxic and pro-apoptotic activities of cynaropicrin, a sesquiterpene lactone, on the viability of leukocyte cancer cell lines. *European Journal of Pharmacology, 492*(2), 85–94.

Efferth, T., Dunstan, H., Sauerbrey, A., Miyachi, H., & Chitambar, C. R. (2001). The anti-malarial artesunate is also active against cancer. *International Journal of Oncology, 18*(4), 767–773.

Fang, F., Sang, S., Chen, K. Y., Gosslau, A., Ho, C.-T., & Rosen, R. T. (2005). Isolation and identification of cytotoxic compounds from Bay leaf (*Laurus nobilis*). *Food Chemistry, 93*(3), 497–501.

Gao, B. C. (1987). *The economic flora of Qinghai (p. 652)*. Xining, China: The Qinghai People Press.

Hehner, S. P., Hofmann, T. G., Droge, W., & Schmitz, M. L. (1999). The anti-inflammatory sesquiterpene lactone parthenolide inhibits NF-κB by targeting the IκB kinase complex. *Journal of Immunology, 163*(10), 5617–5623.

Hussain, R. F., Nouri, A. M. E., & Oliver, R. T. D. (1993). A new approach for measurement of cytotoxicity using colorimetric assay. *Journal of immunological methods, 160*(1), 89–96.

Kumari, G. N. K., Masilamani, S., Ganesh, M. R., & Aravind, S. (2003). Microbial transformation of zaluzanin-D. *Phytochemistry, 62*(7), 1101–1104.

Li, J., Wu, Q.-X., Shi, Y.-P., & Zhu, Y. (2004). A new sesquiterpene lactone from *Scorzonera austriaca*. *Chinese Chemical Letters, 15*(11), 1309–1310.

Macias, F. A., Varela, R. M., Torres, A., & Molinillo, J. M. G. (1993). Potential allelopathic guaianolides from cultivar sunflower leaves, var SH-222. *Phytochemistry, 34*(3), 669–674.

Manzano, F. L., Guerra, F. M., Moreno-Dorado, F. J., Jorge, Z. D., & Massanet, G. M. (2006). Toward the synthesis of thapsigargin: Enantioselective synthesis of 7,11-dihydroxy-guaianolides. *Organic Letters, 8*(13), 2879–2882.

Nosse, B., Chhor, R. B., Jeong, W. B., Bölhm, C., & Reiser, O. (2003). Facile asymmetric synthesis of the core nuclei of xanthanolides, guaianolides, and eudesmanolides. *Organic Letters, 5*(6), 941–944.

Rodriguez, A. D., & Boulanger, A. (1997). New guaiane metabolites from the Caribbean Gorgonian coral, *Pseudopterogorgia americana*. *Journal of Natural Products, 60*(3), 207–211.

Scotti, M. T., Fernandes, M. B., Ferreira, M. J. P., & Emerenciano, V. P. (2007a). Quantitative structure–activity relationship of sesquiterpene lactones with cytotoxic activity. *Bioorganic and Medicinal Chemistry, 15*(8), 2927–2934.

Scotti, L., Scotti, M. T., Ishiki, H. M., Ferreira, M. J. P., Emerenciano, V. P., de S. Menezes, C. M., et al. (2007b). Quantitative elucidation of the structure–bitterness relationship of cynaropicrin and grosheimin derivatives. *Food Chemistry, 105*(1), 77–83.

Semenov, A.A., Tolstikhina, V.V., Bryanskii, O.V., & Khobrakova, V.B. (2002). *As Sibe Irkut Chem Inst (ASIR-S)*. Patent Number: RU2178709-C2.

Yang, C., Zhu, Y., & Jia, Z.-J. (2003). Sesquiterpene lactones and other constituents from the aerial parts of *Carpesium macrocephalum*. *Australian Journal of Chemistry, 56*(6), 621–624.

Zhang, H., Liao, Z.-X., & Yue, J.-M. (2004). Five new sesquiterpenoids from *Parasenecio petasitoides*. *Helvetica Chimica Acta, 87*(4), 976–982.

Zhang, S.-J., Zhao, M., Bai, L.-M., Hasegawa, T., Wang, J.-L., Wang, L. Y., et al. (2006). Bioactive Guaianolides from Siyekucai (*Ixeris chinensis*). *Journal of Natural Products, 69*(10), 1425–1428.

Zdero, C., & Bohlmann, F. (1990). Systematics and evolution within the *Compositae*, seen with the eyes of a chemist. *Plant Systematics and Evolution, 171*(1), 1–14.

Zidorn, C., Ellmerer-Müller, E. P., & Stuppner, H. (2000). Sesquiterpenoids from *Scorzonera hispanica*. *Pharmazie, 55*(7), 550–555.

Zidorn, C., Ellmerer, E. P., Sturm, S., & Stuppner, H. (2003). Tyrolobibenzyls E and F from *Scorzonera humilis* and distribution of caffeic acid derivatives, lignans and tyrolobibenzyls in European taxa of the subtribe *Scorzonerinae* (Lactuceae, Asteraceae). *Phytochemistry, 63*(1), 61–67.

Zhu, Y., Yang, L., & Jia, Z.-J. (1999). Novel highly oxygenated bisabolane sesquiterpenes from *Cremanthodium discoideum*. *Journal of Natural Products, 62*(11), 1479–1483.

Zhu, Y., Zhao, Y., Huang, G.-D., & Wu, W.-S. (2008). Four new compounds from Sinacalia tangutica. *Helvetica Chimica Acta, 91*(10), 1894–1901.

Zhu, Y., Zhu, Q.-X., & Jia, Z.-J. (2000). Epoxide sesquiterpenes and steroids from *Cremanthodium discoidem*. *Australian Journal of Chemistry, 53*(10), 831–834.