Fitoterapia 104 (2015) 50–54

Contents lists available at ScienceDirect

Fitoterapia

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

Brasilane sesquiterpenoids and alkane derivatives from cultures of the basidiomycete *Coltricia sideroides*

Dong-Bao Hu [a,b], Shen Zhang [a,b], Jiang-Bo He [a,b], Ze-Jun Dong [a], Zheng-Hui Li [a], Tao Feng [a,*], Ji-Kai Liu [a,*]

[a] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form 9 May 2015
Accepted 14 May 2015
Available online 16 May 2015

Keywords:
Coltricia sideroides
Brasilane-type sesquiterpenoids
Alkane derivatives
Cytotoxicities

ABSTRACT

Three new brasilane-type sesquiterpenoids, brasilanes A–C (**1**–**3**), together with two new alkane derivatives, colisiderin A (**4**) and 7(*E*),9(*E*)-undecandiene-2,4,5-triol (**5**), were isolated from cultures of the basidiomycete *Coltricia sideroides*. Their structures were elucidated by NMR and MS data analyses. The absolute configuration of **4** was determined by TDDFT ECD calculations while brasilane-type sesquiterpenoids were isolated from cultures of mushroom for the first time. Compounds **2** and **4** showed weak cytotoxicities against HL-60 and SW480, respectively.

1. Introduction

Our recent investigations on the secondary metabolites of higher fungi have obtained more and more sesquiterpenoids with structure diversity. For instance, conosilane A is an unprecedented sesquiterpenoid skeleton with a four-ring system from cultures of *Conocybe siliginea* [1], while trefolane A is another sesquiterpenoid with a new skeleton from cultures of *Tremella foliacea* [2]. The fungus *Coltricia sideroides* is one of the polypore species, which belongs to the wood-decaying fungi family. However, there was not any report about chemical or biological activity studies on this fungus yet. Therefore, we interestedly investigated on the chemical constituent of cultures of *C. sideroides*, searching for new natural products. As a result, three new brasilane-type sesquiterpenoids (**1**–**3**), together with two new alkane derivatives, colisiderin A (**4**) and 7(*E*),9(*E*)-undecandiene-2,4,5-triol (**5**), were isolated from cultures of mushroom *C. sideroides* (Scheme 1). To date, only five brasilane derivatives have been isolated from the liverwort *Conocephalum conicum* [3] and the endophytic fungus *Xylaria* sp. NCY2 [4], while only four halogenated brasilane derivatives were reported, which were isolated from the red alga *Laurencia obtuse* [5,6]. Compounds **1**–**5** were evaluated for their cytotoxicities against five human cancer cell lines.

2. Experimental section

2.1. General experimental procedures

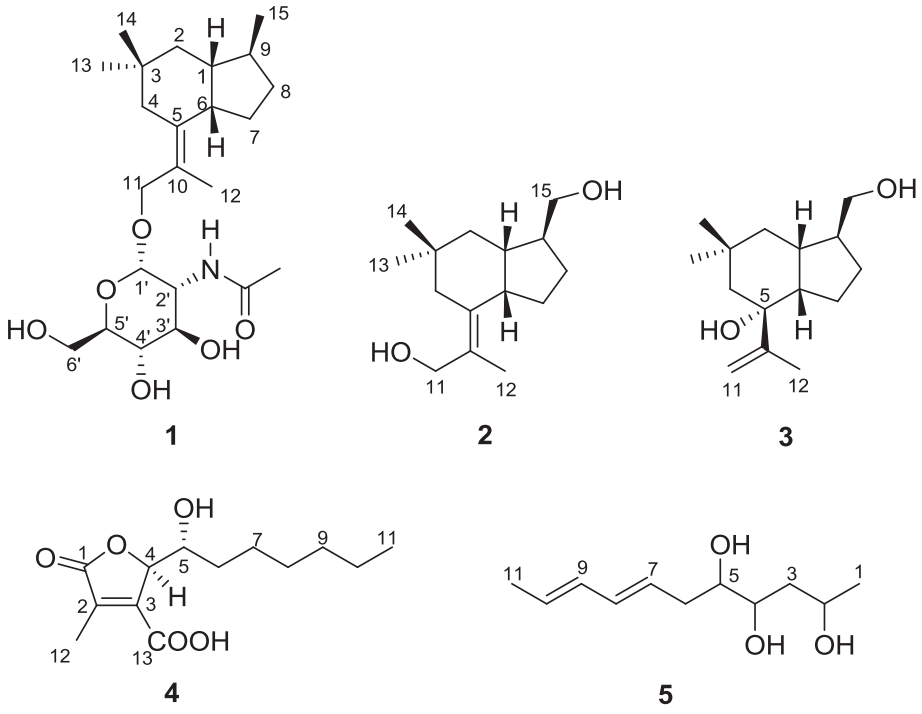
Optical rotations were measured on a Jasco-P-1020 polarimeter. IR spectra were obtained by using a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. NMR spectra were acquired with instruments of Avance III 600 and AM-400. HREIMS was measured on a waters autoSpec Premier P776 instrument. Silica gel (300–400 mesh, Qingdao Marine Chemical Inc., China) and Sephadex LH-20 (Amersham Biosciences, Sweden) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating silica gel plates immersed in vanillin-H_2SO_4 in EtOH, in combination with Agilent 1200 series HPLC system (Eclipse XDB-C18 column, 5 μm, 4.6 × 150 mm).

2.2. Fungal material

Fruiting bodies of *C. sideroides* were collected at Fengkai, Guangdong Province, China in 2010 and identified by Prof. Yu-Cheng Dai (Beijing University). The voucher specimen (no. HFG20100917) was deposited at herbarium of Kunming Institute of Botany. Culture medium: glucose (5%), pork peptone (0.15%), yeast (0.5%), KH_2PO_4 (0.05%), and $MgSO_4$ (0.05%). Culture conditions: dark culture at 24 °C and 150 rpm for twenty-five days.

* Corresponding authors.
E-mail addresses: fengtao@mail.kib.ac.cn (T. Feng), jkliu@mail.kib.ac.cn (J.-K. Liu).

http://dx.doi.org/10.1016/j.fitote.2015.05.009
0367-326X/



Scheme 1. Structures of compounds **1**–**5**.

2.3. Extraction and isolation

The culture broth (20 L) was concentrated under vacuum, extracted three times with EtOAc. The organic layer was evaporated in vacuum to give a crude extract (23 g), which was separated firstly by Sephadex LH-20 (MeOH) column chromatography to remove impurities, then separated by reversed-phased C18 column (MeOH/H_2O, from 20:80 to 100:0) to give fractions A–D. Fraction A (1.9 g) was separated by Sephadex LH-20 (MeOH) column chromatography to afford sub-fraction A_1 (56.3 mg), which was further purified by Sephadex LH-20 (Me_2CO) to yield **1** (9.1 mg, concentration in extract (C/E) = 3.9×10^{-2}%). Fraction B (729.3 mg) was separated by Sephadex LH-20 (MeOH) column chromatography to afford sub-fractions B_1 and B_2. The sub-fraction B_1 (102.5 mg) was further purified by silica gel (petroleum ether–Me_2CO, 10:1) to yield **2** (2.0 mg, C/E = 8.7×10^{-3}%) and **4** (11.1 mg, C/E = 4.8×10^{-2}%). The sub-fraction B_2 (28.9 mg) was further purified by HPLC (MeCN–H_2O, from 60:40 to 70:30 in 20 min, retention time = 13.3 min) to obtain **3** (2.2 mg, C/E = 9.6×10^{-3}%). Fraction C (975.9 mg) was separated by Sephadex LH-20 (MeOH) column chromatography to afford sub-fraction C_1 (247.1 mg), which was further purified by silica gel (petroleum ether–Me_2CO, 4:1) to give **5** (53.0 mg, C/E = 2.3×10^{-1}%).

Brasilane A (**1**): white, amorphous solid, $[\alpha]^{15}_D$ + 99.0 (*c* 0.21 MeOH); IR (KBr) ν_{max} 3439, 2953, 2926, 2869, 1641,1384, 1024 cm^{-1}; ^1H NMR and ^{13}C NMR data for glucoside moiety, δ_H 4.76 (1H, d, *J* = 3.6 Hz, H-1′), 3.85 (1H, dd, *J* = 3.6, 2.4 Hz, H-2′), 3.66 (1H, dd, *J* = 2.3, 1.8 Hz, H-3′), 3.36 (1H, t, *J* = 9.3 Hz, H-4′), 3.64–3.60 (1H, m, H-5′), 3.81 (1H, dd, *J* = 8.6, 2.2 Hz, H-6′a), 3.71 (1H, dd, *J* = 12.9, 4.2 Hz, H-6′b), 1.96 (3H, s, CH_3CO); δ_c 173.5 (CH_3CO), 22.6 (CH_3CO), 96.6 (C-1′), 55.5 (C-2′), 72.6 (C-3′), 72.4 (C-4′), 73.8 (C-5′), 62.7 (C-6′); ^1H NMR and ^{13}C NMR data for aglycone moiety, see Tables 2 and 1, respectively; HREIMS *m/z* 425.2786 $[M]^+$ (calcd for $C_{23}H_{39}NO_6$, 425.2777).

Brasilane B (**2**): colorless oils, $[\alpha]^{15}_D$ − 10.8 (*c* 0.38 MeOH); IR (KBr) ν_{max} 3427, 2952, 2922, 2870, 2853, 1631,1465, 1383, 1364, 1254, 1030, 999.8 cm^{-1}; ^1H NMR data (see Table 2); ^{13}C NMR data (see Table 1); HREIMS *m/z* 238.1929 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

Brasilane C (**3**): colorless oils, $[\alpha]^{15}_D$ + 15.1 (*c* 0.24 MeOH); IR (KBr) ν_{max} 3428, 2952, 2925, 2855, 1638,1461, 1380, 1262, 1098, 1026, 803 cm^{-1}; ^1H NMR data (see Table 2); ^{13}C NMR data (see Table 1); HREIMS *m/z* 238.1938 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

Colisiderin A (**4**): white, amorphous solid, $[\alpha]^{13}_D$ − 47.5 (*c* 0.11 MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.27), 200 (4.09) nm; IR (KBr) ν_{max} 3451, 2958, 2929, 2859, 1740, 1636,1424, 1414 cm^{-1}; ^1H NMR data (see Table 2); ^{13}C NMR data (see Table 1); HREIMS *m/z* 256.1311 $[M]^+$ (calcd for $C_{13}H_{20}O_5$, 256.1311).

7(*E*),9(*E*)-Undecandiene-2,4,5-triol (**5**): white, amorphous solid, UV (MeOH) λ_{max} (log ε) 227 (4.52), 195 (4.09) nm; IR (KBr) ν_{max} 3419, 3018, 2964, 2930, 2857, 1630,1444, 1412, 1377, 1344, 1309, 1108, 1052, 987 cm^{-1}; ^1H NMR data (see Table 2); ^{13}C NMR data (see Table 1); HREIMS *m/z* 200.1418 $[M]^+$ (calcd for $C_{11}H_{20}O_3$, 200.1412).

Table 1
^{13}C NMR data for compounds **1**–**5** (δ in ppm).

No.	**1**[a] (aglycone) methanol-d_4	**2**[b] $CDCl_3$	**3**[b] $CDCl_3$	**4**[a] DMSO-d_6	**5**[a] $CDCl_3$
1	46.8 d	44.8 d	37.9 d	173.4 s	23.5 q
2	42.0 t	41.0 t	41.2 t	134.9 s	65.1 d
3	34.2 s	33.6 s	32.7 s	148.4 s	39.2 t
4	45.2 t	44.3 t	49.1 t	83.7 d	70.9 d
5	140.4 s	137.1 s	77.3 s	68.2 d	74.1 d
6	49.2 d	49.7 d	48.5 d	33.8 t	35.2 t
7	32.3 t	31.4 t	23.7 t	25.5 t	127.0 d
8	34.5 t	28.5 t	26.7 t	28.6 t	133.6 d
9	33.1 d	40.1 d	42.4 d	31.2 t	131.2 d
10	124.6 s	127.1 s	152.2 s	22.0 t	128.3 d
11	70.0 t	65.6 t	109.5 t	13.9 q	18.0 q
12	18.0 q	18.1 q	20.1 q	10.3 q	
13	32.8 q	32.5 q	34.7 q	163.7 s	
14	26.6 q	26.1 q	28.0 q		
15	18.4 q	65.4 t	65.1 t		

[a] Data were recorded at 100 MHz.
[b] Data were recorded at 150 MHz.

Table 2
^{1}H NMR data for compounds **1**–**5** (δ in ppm, J in Hz).

No.	**1**[a] (aglycone) methanol-d_4	**2**[b] $CDCl_3$	**3**[b] $CDCl_3$	**4**[a] DMSO-d_6	**5**[b] $CDCl_3$
1	1.69 m	1.70 m	2.03 m		1.23 d (6.3)
2	1.39 dd (12.9, 2.8)	1.51, dd (12.0, 5.4)	1.54 overlapped		4.12 m
	1.20 t (12.9)	1.14, dd (12.0, 7.1)	1.08 m		
3					1.67 m
					1.50 m
4	2.44 d (13.7)	2.31 d (13.7)	1.37 d (13.5)	5.04 br. s	3.89 m
	1.62 d (13.7)	1.56 d (13.7)	1.26 d (13.5)		
5				3.95 t (6.6)	3.60 m
6	2.04 m	1.86 m	1.54 overlapped	1.52 m	2.29 m
					2.19 m
7	2.15 m	2.08 m	1.43 m	1.41 m	5.53 dt (16.0, 7.3)
	1.62 m	1.60 m	1.14 m	1.25 br. d	
8	2.09 m	1.93 m	1.83 m	1.25 br. d	6.08 dd (16.0, 8.0)
	1.12 m	1.22 m	1.35 m		
9	1.99 m	2.03 m	2.14 m	1.25 br. d	6.02 ddd (14.4, 10.5, 1.3)
10				1.25 br. d	5.61 dq (14.4, 6.7)
11	4.18 d (11.1)	4.13 d (11.2)	4.95 s	0.85 d (6.8)	1.72 d (6.7)
	3.92 d (11.1)	3.90 d (11.2)	4.73 s		
12	1.84 s	1.82 s	1.69 d (0.8)	2.04 d (1.5)	
13	0.97 s	0.91 s	0.87 s		
14	0.83 s	0.78 s	1.06 s		
15	0.82 d (6.9)	3.61 dd (10.5, 6.5)	3.63 dd (10.2, 6.6)		
		3.33 dd (10.5, 7.3)	3.33 dd (10.2, 7.2)		

[a] Data were recorded at 400 MHz.
[b] Data were recorded at 600 MHz.

*2.3.1. Acid hydrolysis of **1** and identification of the sugar*

A mixture of **1** (4.0 mg) and 3 N HCl (10 mL) was heated at 10 °C for 3 h. After neutralizing with 1 N NaOH/H_2O, the solution was extracted with Et_2O (10 mL × 3) and the combined organic phase was evaporated to dryness under reduced pressure to yield aglycone (0.8 mg). The aqueous layer was evaporated to dryness under reduced pressure and subjected to silica gel CC to afford 2-amino-2-deoxy-D-glucose (1.3 mg). The optical rotation value was as follows: $[\alpha]_D^{25}$ + 63 (c 0.5, H_2O) (+70 in literature [7]).

2.4. Computational methods

All DFT and TDDFT calculations were carried out at 298 K in the gas phase with Gaussian 09 [8]. Conformational searches were carried out at the molecular mechanics level of theory employing MMFF force fields [9]. For compound **4**, the conformers with relative energy within 10 kcal/mol of the lowest-energy conformer were selected and further geometry optimized at the B3LYP/6-311++G(2d,p) level [10]. All the lowest-energy conformers, which correspond to 99% of the total Boltzmann distribution, were selected for ECD spectra calculation. The Boltzmann factor for each conformer was calculated based on Gibbs free energy [11]. Vibrational analysis at the B3LYP/6-311++G(2d,p) level of theory resulted in no imaginary frequencies, confirming the considered conformers as real minima. TDDFT was employed to calculate excitation energy (in nm) and rotatory strength R in dipole velocity form, at the B3LYP/6-311++G (2d,p) level [12].

2.5. Cytotoxicity assay

Five human cancer cell lines, human myeloidleukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxicity assay. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium) method in 96-well microplates [13]. Briefly, 100 μL of adherent cells was seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with an initial density of 1 × 105 cells/mL. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma, USA) as the positive controls (Table 3). After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [14].

3. Results and discussion

Compound **1** was obtained as white, amorphous solid. Its molecular formula was assigned as $C_{23}H_{39}NO_6$ on the basis of the HREIMS, which showed a molecular ion peak at m/z 425.2786 (calcd for $C_{23}H_{39}NO_6$, 425.2777), suggesting five degrees of unsaturation. The ^{1}H NMR (Table 2) and ^{13}C NMR data of **1** (Table 1) showed the presence of five methyls, six methylenes, eight methines, and four quaternary carbons. The ^{13}C characteristic NMR signals of δ_C 96.6 (C-1′, d), 55.5 (C-2′, d), 72.6 (C-3′, d), 72.4 (C-4′, d), 73.8 (C-5′, d), and 62.7 (C-6′, t), together with ^{1}H NMR signals of δ_H 4.76 (1H, d, J = 3.6 Hz, H-1′), 3.85 (1H, dd, J = 3.6, 2.4 Hz, H-2′), 3.66 (1H, dd, J = 2.3, 1.8 Hz, H-3′), 3.36 (1H, t, J = 9.3 Hz, H-4′), 3.64-3.60 (1H, m, H-5′), 3.81 (1H, dd, J = 8.6, 2.2 Hz, H-6′a), and 3.71 (1H, dd, J = 12.9, 4.2 Hz, H-6′b) indicated the presence of a glucoside moiety, similar to that reported in the literature [7]. However, in compound **1**, the glucoside moiety is a 2-acetamido-2-deoxy-glucoside according to the HMBC correlations from H-2′ to acetyl group [δ_C 173.5 (C═O), 22.6 (O═C–CH_3, q)]. In addition, two quaternary olefinic carbon signals at δ_C 140.4 (C-5) and 124.6 (C-10) suggested a double bond. Apart from these, the remaining two degrees of unsaturation in **1** suggested a two-ring sesquiterpenoid. Preliminary analyses of ^{1}H–^{1}H COSY and HMBC data (Fig. 1) suggested

Table 3
Cytotoxicities of compounds **2** and **4** (IC_{50}, μmol).

Entry	HL-60	SMMC-7721	A-549	MCF-7	SW480
2	23.4	>40	>40	>40	>40
4	>40	>40	>40	>40	34.3
Cisplatin	2.1	7.7	6.7	16.4	15.7

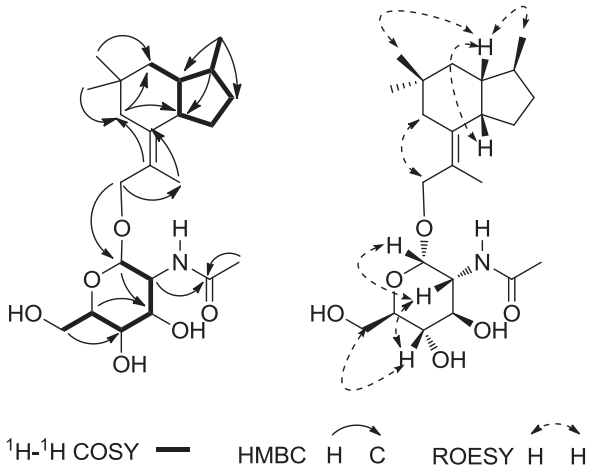


Fig. 1. Key 2D NMR correlations of compound **1**.

that compound **1** should be a brasilane-type sesquiterpenoid glycoside [15]. The double bond was located between C-5 and C-10 as supported by HMBC correlations from δ_H 1.84 (3H, s, H-12) to δ_C 124.6 (s, C-10) and from δ_H 2.44 (1H, d, J = 13.7 Hz, H-4a), 1.62 (1H, d, J = 13.7 Hz, H-4b) and δ_H 2.04 (1H, m, H-6) to δ_C 140.4 (s, C-5). In addition, the methyl of C-11 was oxygenated into an oxymethylene, while the glucosidic bond of C-1′–O–C-11 was also established by HMBC correlation from δ_H 4.76 (1H, d, J = 3.6 Hz, H-1′) to δ_C 70.0 (t, C-11). The relative stereochemistry of aglycone was established by an ROESY experiment (Fig. 1) with reference to those analogues reported previously [5,6]. The ROESY correlations of H-1/Me-14, H-1/Me-15, and H-1/H-6 suggested that H-1, H-6, C-14 and C-15 were in the same side [3], and they were arbitrarily assigned as being β oriented, while the ROESY correlation of H-4/H-11 suggested the olefinic double bond ($\Delta^{5,10}$) to be an *E* configuration. Based on the observed coupling constant ($J_{1',2'}$ = 3.6 Hz) of H-1′, the sugar was assumed to be an α-pyranose form. To clarify the configuration of the sugar, the compound was subjected to hydrolysis with 3 N HCl and the liberated D-glucosamine was identified by a comparison of its optical rotation value [$[\alpha]_D^{25} = +63$ (c 0.5, H_2O)] with that in literature (+70) [7]. Thus, the sugar part of this molecule was determined as 2-acetamido-2-deoxy-α-D-glucose. Therefore, compound **1** was established and named brasilane A.

Compound **2** was isolated as a colorless oil. The molecular formula was established to be $C_{15}H_{26}O_2$ on the basis of HREIMS at *m/z* 238.1929 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933). The ^{13}C NMR spectrum displayed fifteen carbon resonances with similarities to those data of the aglycone in compound **1** (Table 1) except that the methyl of C-15 in **1** was oxygenated into an oxymethylene (δ_C 65.4, t) in **2**, as supported by the HMBC correlation from δ_H 3.61 (1H, dd, J = 10.5, 6.5 Hz, H-15a) and 3.33 (1H, dd, J = 10.5, 7.3 Hz, H-15b) to δ_C 40.1 (d, C-9). Analyses of ROESY data suggested that the relative configuration of **2** was the same to that of **1**. Therefore, the structure of compound **2** was established and named brasilane B.

Compound **3** was isolated as a colorless oil, possessing a molecular formula $C_{15}H_{26}O_2$ as deduced from HREIMS at *m/z* 238.1938 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933). On the basis of extensive 2D NMR data analysis, we established that compound **3** was a derivative of compound **2**. However, a terminal double bond should be located between C-10 and C-11, and C-5 should be an oxygenated sp^3 quaternary carbon, which was suggested by the HMBC correlation from δ_H 1.69 (3H, d, J = 0.8 Hz, H-12) to δ_C 152.2 (s, C-10) and δ_C 109.5 (d, C-11) and from δ_H 1.37 (1H, d, J = 13.5 Hz, H-4a), 1.26 (1H, d, J = 14.4 Hz, H-4b), and 1.54 (overlapped, H-6) to δ_C 77.3 (s, C-5). The ROESY correlations of H-1/Me-14, H-1/CH_2-15, and H-1/H-6 suggested that H-1, H-6, Me-14 and CH_2-15 were in the same side, while the ROESY correlation of H-6/Me-12 suggested that the OH at C-5 should be in the other side. Thus, the structure of compound **3** was deduced as brasilane C.

Compound **4** was obtained as white, amorphous solid. HREIMS ion peak at *m/z* 256.1311 (calcd for $C_{13}H_{20}O_5$, 256.1311) gave the molecular formula $C_{13}H_{20}O_5$, suggesting four degrees of unsaturation. The ^{13}C NMR spectrum revealed two carbonyl carbons (δ_C 173.4 and 163.7) together with a fully substituted double bond (δ_C 134.9 and 148.4). The ^{1}H–^{1}H COSY spectrum revealed a spin system consistent with an *n*-heptyl chain (from C-5 to C-11) terminating in an oxymethine of C-5 (δ_H 3.95; δ_C 68.2), which had HMBC correlations extending to δ_C 83.7 (d, C-4) and δ_C 148.4 (s, C-3). The HMBC correlations from δ_H 5.04 (1H, br.s, H-4) to C-2 (δ_C 134.9), C-3 (δ_C 148.4) and C-1 (δ_C 173.4) constructed an α,β-unsaturated five-member lactone ring. In addition, a methyl group substituted at C-2 was supported by the HMBC correlations of C-12 (δ_H 2.04, δ_C 10.3) to C-1, C-2, and C-3, and a carboxyl substituted at C-3 was supported by the correlations of H-4 to C-13. These data suggested that compound **4** might be a lichesterinic acid derivative [15]. In order to determine the absolute configuration, TDDFT-ECD calculation protocol was pursued. As can be seen from the Fig. 2, ECD curves for the four possible stereostructures (4*R*/5*S*; 4*S*/5*R*; 4*R*/5*R*; 4*S*/5*S*) were calculated using the TD-DFT theory method. The calculated curves of 4*R*/5*S* were

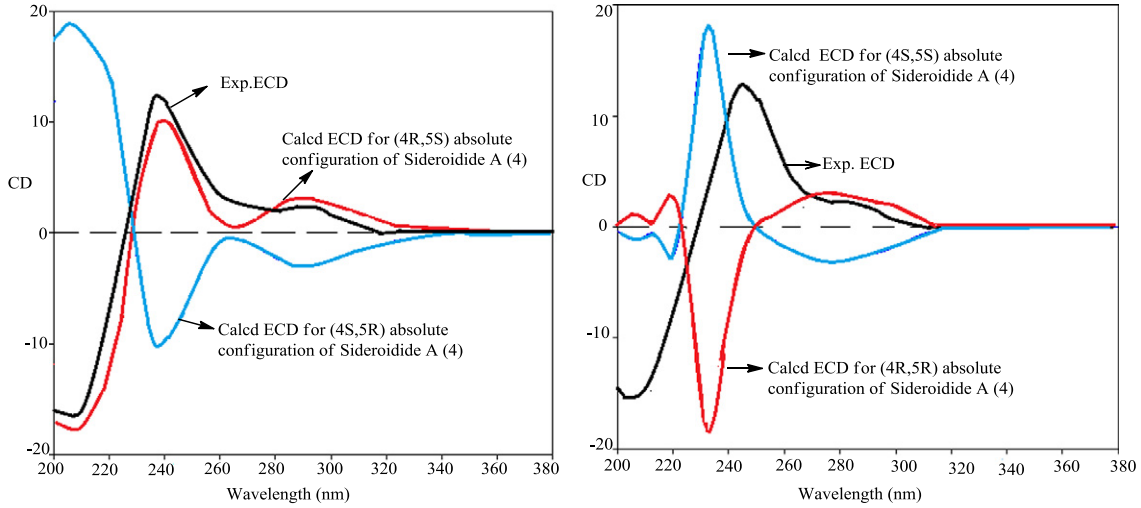


Fig. 2. Results of the TDDFT CD calculations and their comparison with the experimental CD spectrum of (4*R*,5*S*), (4*S*,4*R*), (4*S*,5*S*), and (4*R*,5*R*) configurations for colisiderin A (**4**).

in good agreement with the experimental CD spectrum. Thus, the absolute configurations of chiral carbons of **4** were established as 4*R* and 5*S*. Finally, compound **4** was identified and named colisiderin A.

Compound **5** was obtained as white, amorphous solid, the molecular formula $C_{11}H_{20}O_3$ was deduced from HREIMS *m/z* 200.1418 (calcd for $C_{11}H_{20}O_3$, 200.1412). The ^{13}C NMR data indicated the existence of two methyls, two olefinic double bonds, three oxymethines, as well as two methylenes. These data suggested that compound **5** might be an undecane derivative with two double bonds and three oxymethines. The ^1H–^1H COSY spectrum, as well as HMBC spectrum, suggested compound **5** to be 7,9-undecadiene-2,4,5-triol. The coupling constant of $J_{7,8} = 16.0$ Hz and $J_{9,10} = 14.4$ Hz suggested that two double bonds were *E*-form. However, the stereoconfigurations of three chiral centers could not be figured out currently. Compound **5** was elucidated as 7(*E*),9(*E*)-undecandiene-2,4,5-triol.

Compounds **1**–**5** were evaluated for their cytotoxicities against five human cancer cell lines (HL-60, SMMC-7712, A-549, MCF-7, and SW480) in vitro, with cisplatin as the positive control. Compounds **2** and **4** showed weak cytotoxicities against HL-60 and SW480, respectively (Table 3). Other compounds were inactive ($IC_{50} > 40$ μmol).

Conflict of interest

The manuscript titled "Brasilane sesquiterpenoids and alkane derivatives from cultures of the basidiomycete *Coltricia sideroides*" has been submitted to journal *Fitoterapia*. The authors declare that there is no conflict of interest, and do agree that the manuscript is to be published in *Fitoterapia*.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (81373289, U1132607), and the West Light Foundation of CAS (2013312D11016).

References

[1] X.Y. Yang, T. Feng, Z.H. Li, Y. Sheng, X. Yin, Y. Leng, et al., Conosilane A, an unprecedented sesquiterpene from the cultures of basidiomycete *Conocybe siliginea*, Org. Lett. 14 (2012) 5382–5384.
[2] J.H. Ding, T. Feng, Z.H. Li, X.Y. Yang, H. Guo, X. Yin, et al., Trefolane A, a sesquiterpenoid with a new skeleton from cultures of the basidiomycete *Tremella foliacea*, Org. Lett. 14 (2012) 4976–4978.
[3] S. Melching, W.A. Konig, Sesquiterpenes from the essential oil of the liverwort *Conocephalum conicum*, Phytochemistry 51 (1999) 517–523.
[4] Z.Y. Hu, Y.Y. Li, Y.J. Huang, W.J. Su, Y.M. Shen, Three new sesquiterpenoids from *Xylaria* sp. NCY2, Helv. Chim. Acta 91 (2008) 46–52.
[5] D. Iliopoulou, C. Vagias, D. Galanakis, D. Argyropoulos, V. Roussis, Brasilane-type sesquiterpenoids from *Laurencia obtuse*, Org. Lett. 4 (2002) 3263–3266.
[6] Z. Aydogmus, S. Imre, L. Ersoy, V. Wray, Halogenated secondary metabolites from *Laurencia obtusa*, Nat. Prod. Res. 18 (2004) 43–49.
[7] Y. Shiono, M. Kikuchi, T. Koseki, T. Murayama, E. Kwon, N. Aburai, et al., Isopimarane diterpene glycosides, isolated from endophytic fungus *Paraconiothyrium* sp. MY-42, Phytochemistry 72 (2011) 1400–1405.
[8] M.J., Frisch, G.W., Trucks, H.B., Schlegel, G.E., Scuseria, M.A., Robb, J.R., Cheeseman, et al. Gaussian, Inc., Wallingford CT, 2009.
[9] J.B. He, Y.N. Ji, D.B. Hu, S. Zhang, H. Yan, X.C. Liu, et al., Structure and absolute configuration of penicilliumine, a new alkaloid from *Penicillium commune* 366606, Tetrahedron Lett. 55 (2014) 2684–2686.
[10] Q.M. Li, J. Ren, B.D. Zhou, B. Bai, X.C. Liu, M.L. Wen, et al., Determining the absolute configuration of benzopyrenomycin by optical rotation, electronic circular dichroism, and population analysis of different conformations via DFT methods and experiments, Tetrahedron 69 (2013) 3067–3074.
[11] S. Zhang, D.B. Hu, J.B. He, K.Y. Guan, H.J. Zhu, A novel tetrahydroquinoline acid and a new racemic benzofuranone from *Capparis spinosa* L., a case study of absolute configuration determination using quantum methods, Tetrahedron 70 (2014) 869–873.
[12] B.D. Zhou, J. Ren, X.C. Liu, H.J. Zhu, Theoretical and experimental study of the absolute configuration of helical structure of (2*R*,3*S*)-Rubiginone A_2 analog, Tetrahedron 69 (2012) 1189–1194.
[13] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[14] L.J. Reed, H. Muench, A simple method of estimating fifty percent endpoints, Am. J. Hyg. 27 (1938) 493–497.
[15] M.M. Murta, M.B. de Azevedo, A.E. Greene, Synthesis and absolute stereochemistry of (−)-protolichesterinic acid, antitumor antibiotic lactone from *Cetraria islandica*, J. Org. Chem. 58 (1993) 7537–7541.