



Chlorantholides A–F, eudesmane-type sesquiterpene lactones from *Chloranthus elatior*

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ARTICLE INFO

Article history:

Received 16 June 2011

Received in revised form 4 December 2011

Accepted 10 February 2012

Available online 2 March 2012

Keywords:

Chloranthaceae

Chloranthus elatior

Eudesmanolide

Sesquiterpene

CD exciton chirality method

Structure revision

ABSTRACT

Six eudesmane-type sesquiterpene lactones, named chlorantholides A–F, were isolated from the ethanol extract of *Chloranthus elatior* (Chloranthaceae) together with 12 known compounds. Their structures were elucidated on the basis of extensive spectroscopic analysis, and their absolute configurations were studied by the CD exciton chirality method. The structure of a recently reported eudesmanolide from *Chloranthus anhuiensis*: 8 β -hydroxy-1-oxoeudesma-3,7(11)-dien-12,8-olide, was also revised as 8 β -hydroxy-2-oxoeudesma-3,7(11)-dien-12,8-olide (chlorantholide D).

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1. Introduction

The genus *Chloranthus* (Chloranthaceae) is a rich source of bioactive sesquiterpenoids, and a series of eudesmanolides, lindenanolides, and unique dimeric lindenanes have been isolated from *Chloranthus* spp. as their characteristic secondary metabolites (Kawabata et al., 1984, 1995; Li et al., 2008; Uchida et al., 1980; Wang et al., 2008; Xiao et al., 2010; Xu et al., 2007, 2010; Yuan et al., 2008). As part of BioBioPha to assemble a large-scale natural product library which is very valuable in the discovery of new drug leads from nature (Wang et al., 2009, 2010a,b), the phytochemical investigation on the whole plants of *Chloranthus elatior* afforded six new eudesman-12,8-olides, named chlorantholides A–F (**1**–**6**, Fig. 1), along with 12 known compounds, shizukolidol (**7**) (Kawabata et al., 1984), 4 α ,8 β -dihydroxyeudesm-7(11)-en-12,8-olide (**8**) (Xiao et al., 2010), 15-nor-14-oxolabda-8(17),12E-dien-18-oic acid (**9**) (Sultan et al., 2008; Xiao et al., 2010), 4-epicommunic acid (**10**) (Bohlmann et al., 1980; Xiao et al., 2010), 13-hydroxy-labda-8(17),14-dien-18-oic acid (**11**) (Hieda et al., 1983), 14,15-dinor-13-oxolabda-8(17),11E-dien-18-oic acid (**12**) (Mendes et al., 2005), aromadendrane-4 β ,10 α -diol (**13**) (Goldsby and Burke,

1987), bornyl *p*-coumarate (**14**) (Han and Huang, 1993), erythro-1-(3,4-dimethoxyphenyl)propane-1,2-diol (**15**) (Takeshita and Sato, 1989), β -hydroxypropiovanillone (**16**) (Lin et al., 1994), flavokawain A (**17**) (Detsi et al., 2009), and 7,4'-di-O-methylnaringenin (**18**) (Vasconcelos et al., 1998). This paper describes the structure elucidation of these new eudesmanolides and structure revision of a recently published eudesmanolide.

2. Results and discussion

Compound **1**, obtained as amorphous powder, had a molecular formula of C₁₅H₁₆O₃ based on the positive high resolution electrospray ionization mass spectrum (HRESIMS), showing a quasi-molecular ion peak at *m/z* 245.1174 (calcd for C₁₅H₁₇O₃, 245.1177). The ¹H NMR spectrum (Table 1) showed two olefinic protons at δ_H 6.00 (br s) and 5.59 (s), and three methyl signals at δ_H 2.04 (dd, *J* = 1.4, 1.3 Hz), 1.94 (d, *J* = 1.8 Hz) and 1.08 (s). The ¹³C NMR spectrum (Table 2) indicated 15 carbon resonances, including an α,β -unsaturated ketone carbonyl at δ_C 196.8 (s), an α,β -unsaturated ester carbonyl at δ_C 170.6 (s), and six olefinic carbons at δ_C 159.3 (s), 148.3 (s), 146.3 (s), 127.9 (d), 122.1 (s), and 116.1 (d). The above NMR spectroscopic data and the degrees of unsaturation suggested that this compound was a sesquiterpene lactone containing three rings. Considering its biological source, it should be an eudesm-7(11)-en-12,8-olide derivative (Kawabata et al., 1984; Xiao et al., 2010; Xu et al., 2010). The heteronuclear multiple bond connectivity (HMBC) correlations from the protons

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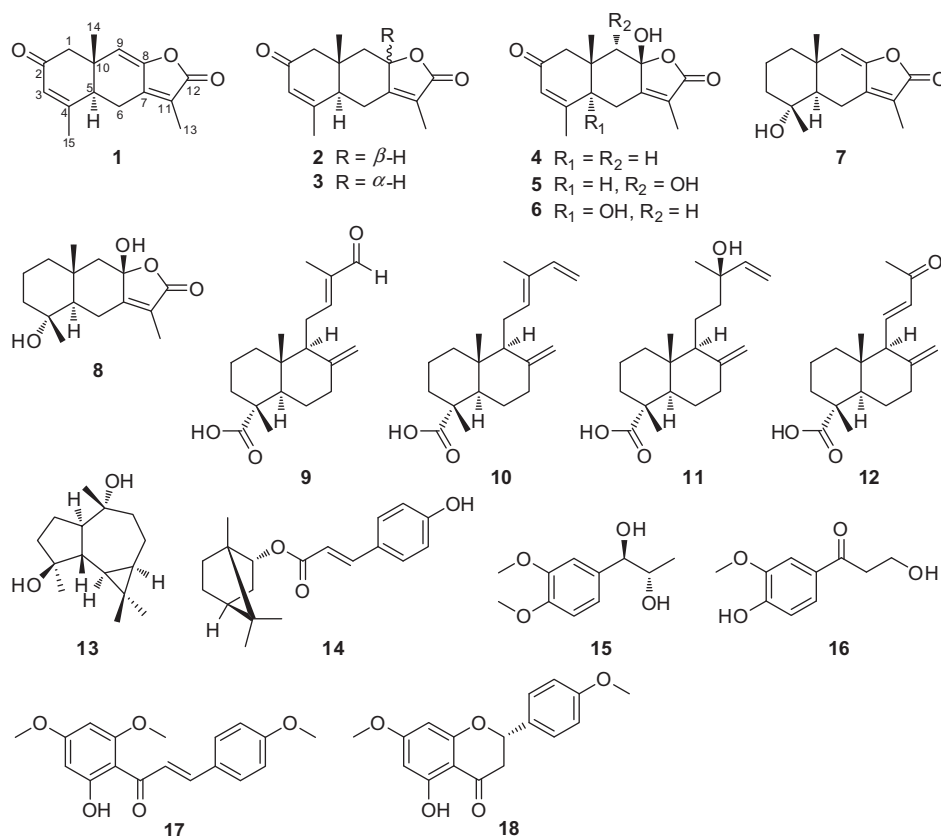
Fig. 1. Eudesmanolides and other constituents from *Chloranthus elatior*.

Table 1

¹H NMR spectroscopic data of chlorantholides A–C (1–3).

No.	1 ^a	2 ^b	3 ^b
1	2.47 (d, 16.3)	2.15 (H _β , d, 16.1)	2.15 (H _β , d, 15.5)
	2.51 (d, 16.3)	2.32 (H _α , d, 16.1)	2.44 (H _α , d, 15.5)
3	6.00 (br s)	5.84 (br s)	5.83 (br s)
5	2.94 (br d, 13.6)	2.54 (br d, 13.3)	3.08 (ddq, 12.6, 6.4, 1.0)
6	2.51 (H _β , ddq, 16.9, 13.6, 1.8)	2.33 (H _β , ddq, 13.9, 13.3, 1.3)	2.65 (H _β , dd, 18.2, 12.6)
	3.07 (H _α , dd, 16.9, 4.2)	3.12 (H _α , dd, 13.9, 4.0)	2.99 (H _α , ddq, 18.2, 6.4, 1.9)
8		5.01 (br dd, 11.6, 6.3)	5.25 (ddq, 10.7, 7.0, 1.8)
9	5.59 (s)	1.24 (H _α , dd, 11.8, 11.6)	1.30 (H _β , dd, 13.5, 7.0)
		2.20 (H _β , dd, 11.8, 6.3)	2.32 (H _α , dd, 13.5, 10.7)
13	1.94 (d, 1.8)	1.78 (dd, 1.5, 1.3)	1.73 (dd, 1.9, 1.8)
14	1.08 (s)	0.96 (s)	0.67 (s)
15	2.04 (dd, 1.4, 1.3)	1.99 (dd, 1.4, 1.2)	1.96 (dd, 1.5, 1.0)

^a Measured in CDCl₃ (δ_H 7.26 ppm).^b Measured in DMSO-*d*₆ (δ_H 2.49 ppm).

at δ_H 2.04 (dd, *J* = 1.4, 1.3 Hz, Me-15) to the carbons at δ_C 127.9 (d, C-3), 159.3 (s, C-4) and 45.8 (d, C-5), and from the protons at δ_H 1.08 (s, Me-14) to the carbons at δ_C 51.4 (t, C-1), 45.8 (d, C-5) and 116.1 (d, C-9) were observed, establishing the presence of two double bonds at C-3 and C-8. The observable HMBC correlations from the protons at δ_H 2.47, 2.51 (each d, *J* = 16.3 Hz, H-1) to the carbons at δ_C 196.8 (s, C-2), 45.8 (d, C-5), 116.1 (d, C-9) and 19.4 (q, C-14) were indicative of a ketone group at C-2. Moreover, the correlations from the olefinic proton at δ_H 5.59 (s, H-9) to the carbons at δ_C 51.4 (t, C-1), 45.8 (d, C-5), 146.3 (s, C-7) and 148.3 (s, C-8) further verified the above deduction. Stereochemically, no variable chiral carbon appeared in the eudesmanolide. Accordingly, the structure of **1** was elucidated as 2-oxoeudesma-3,7(11),8-trien-12,8-olide, named chlorantholide A.

Compound **2**, obtained as amorphous powder, had the molecular formula C₁₅H₁₈O₃ according to its positive HRESIMS at *m/z* 247.1339 (calcd for C₁₅H₁₉O₃, 247.1334). The NMR spectroscopic data (Tables 1 and 2) were similar to those of chlorantholide A (**1**), and the major difference was that the ¹³C NMR spectrum (Table 2) only displayed four olefinic carbons, and an oxygenated methine signal (δ_H 5.01, δ_C 77.2) was newly detected, which hinted that **2** should be 8,9-dihydrochlorantholide A. The inference was confirmed by the following HMBC correlations: from the proton at δ_H 5.01 (br dd, *J* = 11.6, 6.3 Hz, H-8) to the carbons at δ_C 162.6 (s, C-7), 45.2 (t, C-9), 119.8 (s, C-11) and 174.1 (s, C-12), and from the protons at δ_H 0.96 (s, Me-14) to the carbons at δ_C 52.4 (t, C-1), 46.8 (d, C-5) and 45.2 (t, C-9). The strong rotating frame Overhauser enhancement spectroscopy (ROESY) correlations of H-8 ↔ Me-

Table 2¹³C NMR spectroscopic data of chlorantholides A–F (1–6).

No.	1 ^a	2 ^b	3 ^b	4 ^a	4 ^b	5 ^b	6 ^b
1	51.4 (t)	52.4 (t)	52.8 (t)	53.7 (t)	53.2 (t)	49.4 (t)	47.6 (t)
2	196.8 (s)	197.5 (s)	197.4 (s)	198.0 (s)	197.6 (s)	198.6 (s)	197.9 (s)
3	127.9 (d)	126.0 (d)	125.9 (d)	127.3 (d)	125.9 (d)	125.3 (d)	126.3 (d)
4	159.3 (s)	161.7 (s)	161.1 (s)	159.8 (s)	161.8 (s)	161.6 (s)	161.3 (s)
5	45.8 (d)	46.8 (d)	41.0 (d)	49.2 (d)	48.2 (d)	42.0 (d)	74.3 (s)
6	22.1 (t)	24.5 (t)	24.3 (t)	23.7 (t)	23.2 (t)	23.0 (t)	30.1 (t)
7	146.3 (s)	162.6 (s)	162.6 (s)	159.0 (s)	160.1 (s)	157.1 (s)	158.4 (s)
8	148.3 (s)	77.2 (d)	76.7 (d)	102.9 (s)	103.3 (s)	104.5 (s)	103.0 (s)
9	116.1 (d)	45.2 (t)	42.4 (t)	49.8 (t)	49.0 (t)	76.1 (d)	44.0 (t)
10	39.1 (s)	38.2 (s)	36.3 (s)	38.3 (s)	38.1 (s)	42.9 (s)	40.7 (s)
11	122.1 (s)	119.8 (s)	120.4 (s)	123.3 (s)	121.7 (s)	123.6 (s)	124.4 (s)
12	170.6 (s)	174.1 (s)	174.2 (s)	172.0 (s)	171.8 (s)	172.2 (s)	171.8 (s)
13	8.6 (q)	8.2 (q)	8.3 (q)	8.4 (q)	8.2 (q)	8.2 (q)	8.1 (q)
14	19.4 (q)	16.7 (q)	21.1 (q)	17.4 (q)	17.4 (q)	16.9 (q)	22.9 (q)
15	21.7 (q)	21.6 (q)	21.4 (q)	22.1 (q)	21.8 (q)	21.9 (q)	19.4 (q)

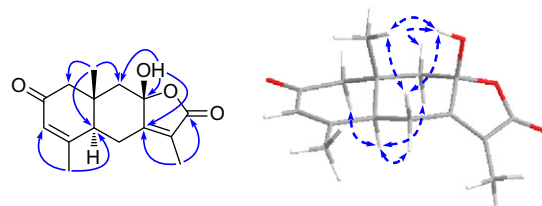
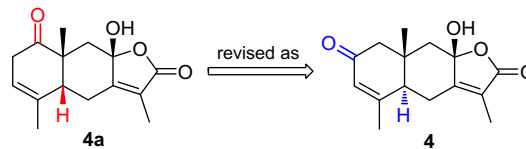
^a Measured in CDCl₃ (δ_C 77.0 ppm).^b Measured in DMSO-*d*₆ (δ_C 39.5 ppm).**Table 3**¹H NMR spectroscopic data of chlorantholides D–F (4–6) in DMSO-*d*₆ (δ_H 2.49 ppm).

No.	4	5	6
1	2.11 (H _β , d, 15.9)	1.90 (H _β , d, 16.1)	1.90 (H _β , d, 16.6)
	2.27 (H _α , d, 15.9)	2.69 (H _α , d, 16.1)	2.56 (H _α , d, 16.6)
3	5.82 (br s)	5.77 (br s)	5.75 (br s)
5	2.56 (br d, 13.1)	2.73 (br d, 13.1)	
6	2.27 (H _β , ddq, 13.2, 13.1, 1.2)	2.25 (H _β , ddq, 13.2, 13.1, 0.9)	2.62 (H _β , d, 13.6)
	3.01 (H _α , dd, 13.2, 3.4)	2.94 (H _α , dd, 13.2, 3.6)	2.87 (H _α , d, 13.6)
9	1.62 (H _α , d, 13.2)	3.44 (d, 5.4)	1.88 (H _β , d, 12.9)
	2.12 (H _β , d, 13.2)		1.92 (H _α , d, 12.9)
13	1.78 (d, 1.2)	1.78 (d, 0.9)	1.74 (s)
14	1.02 (s)	1.05 (s)	1.15 (s)
15	2.00 (dd, 1.4, 1.3)	1.99 (dd, 1.4, 1.3)	2.03 (br s)
5-OH			5.41 (s)
8-OH	7.30 (s)	7.44 (s)	7.23 (s)
9-OH		5.72 (d, 5.4)	

14, H-8 ↔ H-9 β , H-8 ↔ H-6 β , H-5 ↔ H-6 α , and H-5 ↔ H-9 α indicated a β -orientation of H-8. As a result, structure **2** was established as 2-oxoeudesma-3,7(11)-dien-12,8 α -olide and was named chlorantholide B.

Compounds **3** and **2** shared the same molecular formula C₁₅H₁₈O₃ according to its positive HRESIMS at *m/z* 247.1327 (calcd for C₁₅H₁₉O₃, 247.1334). The NMR spectroscopic data (Tables 1 and 2) were very similar to those of **2**, and the study on the HMBC spectrum allowed us to deduce that **3** should be an 8-epimer. The deduction was confirmed by the ROESY correlations of H-8 ↔ H-5, H-8 ↔ H-6 α , H-8 ↔ H-9 α , Me-14 ↔ H-6 β , and Me-14 ↔ H-9 β . Thereupon, structure **3** was established as 2-oxoeudesma-3,7(11)-dien-12,8 β -olide, named chlorantholide C.

Compound **4** had a molecular formula of C₁₅H₁₈O₄ based on the positive HRESIMS, showing a quasi-molecular ion peak at *m/z* 263.1283 (calcd for C₁₅H₁₉O₄, 263.1283). The ¹H NMR spectrum (Table 3) showed a low-field active hydrogen at δ_H 7.30 (s), an olefinic proton at δ_H 5.82 (br s), and three methyl signals at δ_H 2.00 (dd, *J* = 1.4, 1.3 Hz), 1.78 (d, *J* = 1.2 Hz) and 1.02 (s). Analysis of the ¹³C NMR spectrum (Table 2) also indicated 15 carbon resonances, including an α,β -unsaturated ketone carbonyl at δ_C 197.6 (s), an α,β -unsaturated lactone at δ_C 171.8 (s), four olefinic carbons at δ_C 161.8 (s), 160.1 (s), 125.9 (d) and 121.7 (s), a typical hemiketal carbon at δ_C 103.3 (s), as well as eight high-field carbons at δ_C 53.2 (t), 49.0 (t), 48.2 (d), 38.1 (s), 23.2 (t), 21.8 (q), 17.4 (q) and 8.2 (q). On the basis of these NMR data, a preliminary conclusion could be made that **4** should be 8-hydroxychlorantholide B. The HMBC correlations (Fig. 2) from the active proton at δ_H 7.30 (s, 8-OH) to the carbons at δ_C 160.1 (s, C-7), 103.3 (s, C-8) and 49.0 (t, C-9), were

**Fig. 2.** Significant HMBC and ROESY correlations of chlorantholide D (**4**).**Fig. 3.** Structure revision of **4a**.

observed, which confirmed the presence of a hydroxyl group at C-8. The detectable ROESY correlations (DMSO-*d*₆, Fig. 2) of 8-OH ↔ H-6 β , H-9 β and Me-14 suggested the β -orientation of C-8 hydroxy group. As a result, structure **4** was characterized as 8 β -hydroxy-2-oxoeudesma-3,7(11)-dien-12,8-olide, named chlorantholide D.

Recently, a new eudesmanolide (**4a**, Fig. 3) was isolated from *C. anhuiensis* (Xu et al., 2010). However, the authors proposed an incorrect structure, which should be revised as 8 β -hydroxy-2-ox-

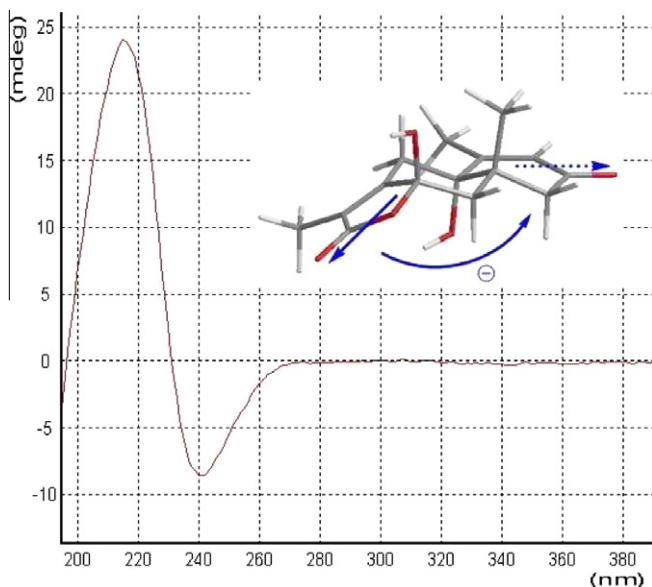


Fig. 4. CD spectrum of chlorantholide F (**6**) measured in MeOH, and the stereoview of **6**, arrows denote the electric transition dipole of the chromophores.

oeudesma-3,7(11)-dien-12,8-olide (chlorantholide D, **4**). This revision is based on the following evidence: (1) the signal at δ_C 197.9 (s) (Xu et al., 2010) is typical for an α,β -unsaturated ketone carbonyl, and a saturated one would be around 212 ppm (Yuan et al., 2008); (2) an HMBC experiment, which is indispensable for establishing a correct structure, was not applied in the structure determination of **4a**; (3) the ^{13}C NMR spectroscopic data (Xu et al., 2010) of **4a** are in accordance with those of **4** (Table 2); (4) the ROESY correlations (Fig. 2) of H-5 \leftrightarrow H-1 α , H-6 α and H-9 α were clearly detected in our current study, and all eudesmanolides from the genus possess 5 α H configuration without exception so far.

Chlorantholide E (**5**), amorphous powder, possessed the molecular formula $\text{C}_{15}\text{H}_{18}\text{O}_5$, determined by the positive HRESIMS at m/z 279.1227 (calcd for $\text{C}_{15}\text{H}_{19}\text{O}_5$, 279.1232). The ^1H NMR spectrum (Table 3) displayed two active hydrogen protons at δ_H 7.44 (s) and 5.72 (d, $J = 5.4$ Hz), an olefinic proton at δ_H 5.77 (br s), an oxygenated methine proton at δ_H 3.44 (d, $J = 5.4$ Hz), and three methyl signals at δ_H 1.99 (dd, $J = 1.4, 1.3$ Hz), 1.78 (d, $J = 0.9$ Hz) and 1.05 (s). The ^{13}C NMR spectrum (Table 2) was generally similar to that of chlorantholide D (**4**), and an obvious difference was that an oxygen-bearing methine signal (δ_H 3.44, δ_C 76.1) replaced a methylene carbon in **4**, thus it was safe to draw the conclusion that **5** was a monohydroxylated derivative of chlorantholide D. According to the HMBC correlations from the protons at δ_H 1.05 (s, Me-14) to the carbons at δ_C 49.4 (t, C-1), 42.0 (d, C-5) and 76.1 (d), and from the active proton at δ_H 7.44 (s, 8-OH) to the carbons at δ_C 157.1 (s, C-7), 104.5 (s, C-8) and 76.1 (d), a hydroxy group was unambiguously positioned at C-9. The clear ROESY correlations of H-9 \leftrightarrow H-1 β , 8 β -OH and Me-14, and 9-OH \leftrightarrow H-1 α and H-5 allowed the assignment of the hydroxyl group as α -oriented. Thus compound **5** was determined as 8 β ,9 α -dihydroxy-2-oxoeudesma-3,7(11)-dien-12,8-olide.

Chlorantholide F (**6**) shared the same molecular formula $\text{C}_{15}\text{H}_{18}\text{O}_5$ with **5** on the basis of its positive HRESIMS at m/z 279.1236 (calcd for $\text{C}_{15}\text{H}_{19}\text{O}_5$, 279.1232). The ^1H NMR spectrum (Table 3) displayed two active hydrogen singlets at δ_H 7.23 and 5.41, an olefinic proton at δ_H 5.75 (br s), and three methyl singlets at δ_H 2.03, 1.74 and 1.15. The ^{13}C NMR spectrum (Table 2) was generally similar to that of chlorantholide D (**4**), nevertheless an oxygenated quaternary carbon (δ_C 74.3) displaced the sole high field methine carbon in **4**, which suggested that **6** was 5-hydroxychlor-

antholide D. This inference was further proved by analysis of the following HMBC correlations: from the protons at δ_H 2.03 (br s, Me-15) to the carbons at δ_C 126.3 (d, C-3) and 74.3 (s, C-5), and from the protons at δ_H 1.15 (s, Me-14) to the carbons at δ_C 47.6 (t, C-1), 74.3 (s, C-5) and 44.0 (t, C-9). The α -orientation of the hydroxy group at C-5 was based on the ROESY correlations of 5-OH \leftrightarrow H-1 α and H-6 α , and Me-14 \leftrightarrow H-1 β , H-6 β , 8 β -OH and H-9 β . Therefore, structure **6** was 5 α ,8 β -dihydroxy-2-oxoeudesma-3,7(11)-dien-12,8-olide.

The CD exciton chirality method was finally employed to establish the absolute configuration of chlorantholides as exemplified by the case of **6** (Koreeda et al., 1974). Its CD spectrum (Fig. 4) showed a split Cotton effect due to interaction between the conjugated enone and lactone chromophores. The negative first Cotton effect at λ_{max} 241 nm ($\Delta\epsilon$ -8.64) indicates a negative chirality between the two axes of electric transition moments (Fig. 4). Therefore, the absolute configuration of **6** was assigned as depicted in Fig. 1. In order to determine the optical purity, eudesmanolides **1–6** were analyzed by HPLC using a chiral column, and the results showed their chromatographic behavior appeared as a single peak without exception, as that of using an achiral one, which established that these eudesmanolides exhibited good optical activity.

3. Concluding remarks

Six eudesmane sesquiterpene lactones, chlorantholides A–F (**1–6**), were isolated from the ethanol extract of *C. elatior* together with 12 known compounds, and the structure of a recently reported eudesmanolide from *C. anhuiensis*: 8 β -hydroxy-1-oxoeudesma-3,7(11)-dien-12,8-olide (**4a**), was revised as 8 β -hydroxy-2-oxoeudesma-3,7(11)-dien-12,8-olide (chlorantholide D, **4**). Current research shows eudesman-12,8-olides and labdan-18-oic acids were isolated as major secondary metabolites of *C. elatior*, unlike other species of this genus mainly abounding in lindenanes and dimeric ones, as well as labdan-19-oic acids (Kawabata et al., 1995; Li et al., 2008; Uchida et al., 1980; Wang et al., 2008; Xu et al., 2007, 2010; Yang et al., 2008; Zhang et al., 2010), which may be helpful in chemotaxonomical classifications. Since hydroxy proton signals are observable and often appeared as sharp peaks using $\text{DMSO}-d_6$ solvent, their HMBC and ROESY correlations can play a very important role in structure elucidation, especially for the determination of stereochemistry. For those terpenoids possessing two isolated but spatially close carbonyl-containing chromophores, the CD exciton chirality method is a highly effective way to determine absolute configuration using split-type Cotton effects.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a Jasco P-1020 (Jasco International Co., Ltd., Tokyo, Japan) automatic digital polarimeter. CD spectra were recorded on a Chirascan (Applied Photophysics, Leatherhead, Surrey, UK) circular dichroism spectrometer. IR spectra were obtained using a Bruker Tensor 27 FT-IR (Bruker Optics GmbH, Ettlingen, Germany) spectrometer with KBr pellets. NMR spectra were acquired on a Bruker Avance III 600 MHz (Bruker Bio-Spin GmbH, Rheinstetten, Germany) spectrometer with deuterated solvent as internal standard. ESIMS and HRESIMS were measured on an API QSTAR Pulsar i (MDS Sciex, Concord, Ontario, Canada) mass spectrometer. Silica gel 200–300 mesh (Qingdao Marine Chemical Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) were used for normal pressure column chromatography (CC). Fractions were monitored and analyzed by TLC, in combination with Agilent 1200 series HPLC system

equipped with an Extend-C18 column (5 μ m, 4.6 \times 150 mm). TCI Chiral MB-S column (5 μ m, 4.6 \times 250 mm, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan) was applied for determinations of enantiomeric purity using an Agilent 1200 series HPLC system, and eluted with *i*-PrOH: *n*-hexane (4:6, v/v) over 25.0 min at a flow rate of 1.0 ml/min (40 $^{\circ}$ C).

4.2. Plant material

Whole plants of *C. elatior* were collected in the Pu'er region of Yunnan Province, China, in April 2010, and identified by Mr. Yu Chen of Kunming Institute of Botany, CAS. A voucher specimen (No. BBP2011013CE) is deposited at BioBioPha.

4.3. Extraction and isolation

Dried and powdered whole plants (7.5 kg) of *C. elatior* were extracted with EtOH:H₂O (95:5, v/v) for 10 days at room temperature three times. The combined filtrates were concentrated *in vacuo* to afford a thick, dark extract (ca. 400 g), which was fractionalized by silica gel CC eluted with a gradient of increasing acetone in petroleum ether (80:20, 75:25, 70:30, 65:35, 60:40, 50:50, 40:60, 0:100) to yield fractions A–H, respectively. Fraction B was further isolated and purified by silica gel (CHCl₃/acetone, 100:1 \rightarrow 25:1) and Sephadex LH-20 (CHCl₃/MeOH, 1:1) to afford **1** (36 mg). Fraction C was subjected to silica gel CC using CHCl₃/MeOH (100:0 \rightarrow 50:1), and the fraction eluted by CHCl₃/MeOH 100:1 was further isolated and purified by preparative TLC (CHCl₃/MeOH, 50:1) and Sephadex LH-20 (CHCl₃/MeOH, 1:1) to afford **2** (45 mg). Fraction D was repeatedly subjected to silica gel CC using CHCl₃/MeOH (100:0 \rightarrow 30:1) to yield **3** (22 mg). Fraction E was separated by silica gel CC (CHCl₃/MeOH, 100:0 \rightarrow 30:1; CHCl₃/acetone, 50:1 \rightarrow 5:1) and Sephadex LH-20 (CHCl₃/MeOH, 1:1) to afford **4** (287 mg). Fraction F was subjected to silica gel CC using CHCl₃/MeOH (50:1 \rightarrow 20:1) to successively afford subfractions I and II. Subfraction I was further purified by preparative TLC (CHCl₃/MeOH, 15:1) and Sephadex LH-20 (CHCl₃/MeOH, 1:1) to afford **5** (48 mg), and compound **6** (139 mg) was acquired from subfraction II by the same techniques. The retention times (*t_R*) of **1**–**6** from analysis-type HPLC Extend-C18 column (20% \rightarrow 100% MeOH in H₂O over 10.0 min, 1.0 ml/min, 25 $^{\circ}$ C) were 7.4, 6.4, 6.3, 5.7, 5.5 and 4.3 min, respectively.

4.4. Chlorantholide A (**1**)

Amorphous powder; $[\alpha]_D^{21} + 5.0$ (c 0.20, CHCl₃); *R_f* = 0.70 (CHCl₃:MeOH = 30:1); UV λ_{\max} : 250, 276 nm; IR (KBr) ν_{\max} cm⁻¹: 1755, 1660, 1621, 1377, 1266, 1017; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 3; ESIMS (pos.) *m/z*: 245 [M + H]⁺; HRESIMS (pos.) *m/z*: 245.1174 (calcd for C₁₅H₁₇O₃, 245.1177).

4.5. Chlorantholide B (**2**)

Amorphous powder; $[\alpha]_D^{21} + 74.7$ (c 0.19, CHCl₃); *R_f* = 0.60 (CHCl₃:MeOH = 30:1); UV λ_{\max} : 225 nm; IR (KBr) ν_{\max} cm⁻¹: 1749, 1663, 1620, 1435, 1330, 1251, 1096, 1036; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 3; ESIMS (pos.) *m/z*: 247 [M + H]⁺; HRESIMS (pos.) *m/z*: 247.1339 (calcd for C₁₅H₁₉O₃, 247.1334).

4.6. Chlorantholide C (**3**)

Amorphous powder; $[\alpha]_D^{21} - 97.2$ (c 0.20, CHCl₃); *R_f* = 0.54 (CHCl₃:MeOH = 30:1); UV λ_{\max} : 233 nm; IR (KBr) ν_{\max} cm⁻¹: 1742, 1688, 1667, 1619, 1423, 1379, 1339, 1242, 1089, 1035; for ¹H and ¹³C NMR spectroscopic data, see Tables 1 and 3; ESIMS

(pos.) *m/z*: 247 [M + H]⁺; HRESIMS (pos.) *m/z*: 247.1327 (calcd for C₁₅H₁₉O₃, 247.1334).

4.7. Chlorantholide D (**4**)

Amorphous powder; $[\alpha]_D^{21} + 98.5$ (c 0.19, CHCl₃); *R_f* = 0.71 (CHCl₃:MeOH = 15:1); UV λ_{\max} : 239 nm; IR (KBr) ν_{\max} cm⁻¹: 3398, 1746, 1695, 1651, 1618, 1325, 1269, 1142, 949; for ¹H and ¹³C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) *m/z*: 263 [M + H]⁺; HRESIMS (pos.) *m/z*: 263.1283 (calcd for C₁₅H₁₉O₄, 263.1283).

4.8. Chlorantholide E (**5**)

Amorphous powder; $[\alpha]_D^{22} + 90.8$ (c 0.19, MeOH); *R_f* = 0.54 (CHCl₃:MeOH = 15:1); UV λ_{\max} : 237 nm; IR (KBr) ν_{\max} cm⁻¹: 3416, 1751, 1687, 1650, 1613, 1379, 1164, 1104, 1020; for ¹H and ¹³C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) *m/z*: 279 [M + H]⁺; HRESIMS (pos.) *m/z*: 279.1227 (calcd for C₁₅H₁₉O₅, 279.1232).

4.9. Chlorantholide F (**6**)

Amorphous powder; $[\alpha]_D^{22} + 87.9$ (c 0.19, MeOH); *R_f* = 0.34 (CHCl₃:MeOH = 15:1); CD (MeOH) λ_{\max} ($\Delta\epsilon$): 241 (−8.64), 215 (+23.9) nm; UV λ_{\max} : 232 nm; IR (KBr) ν_{\max} cm⁻¹: 3418, 1761, 1702, 1656, 1438, 1325, 1270, 1136, 954; for ¹H and ¹³C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) *m/z*: 279 [M + H]⁺; HRESIMS (pos.) *m/z*: 279.1236 (calcd for C₁₅H₁₉O₅, 279.1232).

Acknowledgements

This work was financially supported by National Basic Research Program of China (973 Program) 2009CB522300, the “Western Light” Program of Chinese Academy of Sciences, and Natural Product Library Program of BioBioPha.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2012.02.008.

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