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

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A R T I C L E   I N F O

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

A B S T R A C T

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: 8β-hydroxy-1-oxoedusma-3,7(11)-diol-12,8-olide, was also revised as 8β-hydroxy-2-oxoedusma-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, linde-nanolides, 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), 4α,8β-dihydroxyeudesm-7(11)-en-12,8-olide (8) (Xiao et al., 2010), 15-nor-14-oxo-Δ1(7),12(17)enedioic acid (9) (Sultan et al., 2008; Xiao et al., 2010), 4-epi-4-hydroxy-1,8-nor-Δ1(7),12(17)-enedioic acid (10) (Bohmann et al., 1980; Xiao et al., 2010), 13-hydroxy-Δ1(7),14-enedioic acid (11) (Hieda et al., 1983), 14,15-dinor-13-oxo-Δ1(7),11(13)-enedioic acid (12) (Mendes et al., 2005), aroandrenedione-4β,10β-diol (13) (Goldsby and Burke, 1987), bornyl p-coumarate (14) (Han and Huang, 1993), erythro-1-[(3,4-dimethoxyphenyl)propiovanillone (15) (Takeshita and Sato, 1989), β-dihydroxypropiovanillone (16) (Lin et al., 1994), flavokawain A (17) (Detsi et al., 2009), and 7,4'-di-O-methylkaempferol (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 C_{15}H_{24}O_{3} based on the positive high resolution electrospray ionization mass spectrum (HRESIMS), showing a quasi-molecular ion peak at m/z 245.1174 (calcd for C_{15}H_{23}O_{3}, 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...
at δH 2.04 (dd, J = 1.4, 1.3 Hz, Me-15) to the carbons at δC 127.9 (d, C-12), 159.3 (s, C-4) and 45.8 (d, C-5), and from the protons at δH 1.08 (s, Me-14) to the carbons at δC 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 δH 2.47, 2.51 (each d, J = 16.3 Hz, H-1) to the carbons at δC 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 δH 5.59 (s, H-9) to the carbons at δC 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 (δH 5.01, δC 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 δH 5.01 (br dd, J = 11.6, 6.3 Hz, H-8) to the carbons at δC 162.6 (s, C-7), 119.8 (s, C-11) and 174.1 (s, C-12), and from the protons at δH 0.96 (s, Me-14) to the carbons at δC 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 ↔

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

<table>
<thead>
<tr>
<th>No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.47 (d, 16.3)</td>
<td>2.15 (Hα, d, 16.1)</td>
<td>2.15 (Hα, d, 15.5)</td>
</tr>
<tr>
<td>2</td>
<td>2.51 (d, 16.3)</td>
<td>2.32 (Hα, d, 16.1)</td>
<td>2.44 (Hα, d, 15.5)</td>
</tr>
<tr>
<td>3</td>
<td>6.00 (br s)</td>
<td>5.84 (br s)</td>
<td>5.83 (br s)</td>
</tr>
<tr>
<td>5</td>
<td>2.94 (br d, 13.6)</td>
<td>2.54 (br d, 13.3)</td>
<td>3.08 (dδδ, 12.6, 6.4, 1.0)</td>
</tr>
<tr>
<td>6</td>
<td>2.51 (Hα, ddq, 16.9, 13.6, 1.8)</td>
<td>2.33 (Hα, ddq, 13.9, 13.3, 1.3)</td>
<td>2.65 (Hα, dd, 18.2, 12.6)</td>
</tr>
<tr>
<td>8</td>
<td>3.07 (Hα, dd, 16.9, 4.2)</td>
<td>3.12 (Hα, dd, 13.9, 4.0)</td>
<td>2.99 (Hα, ddq, 18.2, 6.4, 1.9)</td>
</tr>
<tr>
<td>9</td>
<td>5.59 (s)</td>
<td>5.01 (br dd, 11.6, 6.3)</td>
<td>5.25 (dδδ, 16.7, 7.0, 1.8)</td>
</tr>
<tr>
<td>13</td>
<td>1.94 (d, 1.8)</td>
<td>1.78 (dd, 1.5, 1.3)</td>
<td>1.73 (dd, 1.9, 1.8)</td>
</tr>
<tr>
<td>14</td>
<td>1.08 (s)</td>
<td>0.96 (s)</td>
<td>0.67 (s)</td>
</tr>
<tr>
<td>15</td>
<td>2.04 (dd, 1.4, 1.3)</td>
<td>1.99 (dd, 1.4, 1.2)</td>
<td>1.96 (dd, 1.5, 1.0)</td>
</tr>
</tbody>
</table>

a Measured in CDCl3 (δH 7.26 ppm).
b Measured in DMSO-d6 (δH 2.49 ppm).
14, H-8 ↔ H-9β, H-8 ↔ H-6β, H-5 ↔ H-6α, and H-5 ↔ H-9α indicated a β-orientation of H-8. As a result, structure 2 was established as 2-oxoedusma-3,7(11)-dien-12,8β-olide and was named chlorantholide B.

Compounds 3 and 2 shared the same molecular formula C_{15}H_{19}O_{3} according to its positive HRESIMS at m/z 247.1327 (calcd for C_{15}H_{18}O_{3}, 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-9β, H-8↔H-6β, H-8↔H-9α, Me-14↔H-6β, and Me-14↔H-9β. Thereupon, structure 3 was established as 2-oxoedusma-3,7(11)-dien-12,8β-olide, named chlorantholide C.

Compound 4 had a molecular formula of C_{15}H_{19}O_{3} based on the positive HRESIMS, showing a quasi–molecular ion peak at m/z 263.1283 (calcd for C_{15}H_{19}O_{3}, 263.1283). The 1H NMR spectrum (Table 3) showed a low-field active hydrogen at δH 7.30 (s), an olefinic proton at δH 5.82 (br s), and three methyl signals at δH 2.00 (dd, J = 1.4, 1.3 Hz), 1.78 (d, J = 1.4 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 δC 197.6 (s), an α,β-unsaturated lactone at δC 171.8 (s), four olefinic carbons at δC 161.8 (s), 160.1 (s), 125.9 (d) and 121.7 (s), a typical hemiketal carbon at δC 103.3 (s), as well as eight high-field carbons at δC 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 δH 7.30 (s, 8-OH) to the carbons at δC 160.1 (s, C-7), 103.3 (s, C-8) and 49.0 (t, C-9), were 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-6β, H-9β and Me-14 suggested the β-orientation of C-8 hydroxy group. As a result, structure 4 was characterized as 8β-hydroxy-2-oxoedusma-3,7(11)-dien-12,8β-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 8β-hydroxy-2-ox-
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 enones and lactone chromophores. The negative first Cotton effect at $\lambda_{\text{max}}$ 241 nm ($\Delta \varepsilon = -8.64$) 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. anhuicensis: 8β-hydroxy-1-oxyeudesma-3,7(11)-dien-12,8-olide (4a), was revised as 8β-hydroxy-2-oxyeudesma-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 indanenes 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 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 Biospin 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...
4.3. Extraction and isolation

Chen of Kunming Institute of Botany, CAS. A voucher specimen 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.

4.2. 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.

4.4. Chlorantholide A (1)

Amorphous powder; \( \phi = 5.0 \) (c 0.20, CHCl3); \( R_t = 0.70 \) (CHCl3:MeOH = 30:1); UV \( \lambda_{max} = 250, 276 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 1755, 1660, 1621, 1377, 1266, 1017; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 1 and 3; ESIMS (pos.) \( m/z \): 245 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 245.1177 (calcld for C\(_{15}\)H\(_{19}\)O\(_5\)).

4.5. Chlorantholide B (2)

Amorphous powder; \( \phi = 74.7 \) (c 0.19, CHCl3); \( R_t = 0.60 \) (CHCl3:MeOH = 30:1); UV \( \lambda_{max} = 225 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 1749, 1663, 1620, 1435, 1384, 1330, 1251, 1056, 1036; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 1 and 3; ESIMS (pos.) \( m/z \): 247 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 247.1339 (calcld for C\(_{15}\)H\(_{19}\)O\(_3\)).

4.6. Chlorantholide C (3)

Amorphous powder; \( \phi = 97.2 \) (c 0.20, CHCl3); \( R_t = 0.54 \) (CHCl3:MeOH = 30:1); UV \( \lambda_{max} = 233 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 1742, 1688, 1667, 1619, 1423, 1379, 1339, 1242, 1085, 1035; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 1 and 3; ESIMS (pos.) \( m/z \): 247 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 247.1327 (calcld for C\(_{15}\)H\(_{19}\)O\(_3\)).

4.7. Chlorantholide D (4)

Amorphous powder; \( \phi = 98.5 \) (c 0.19, CHCl3); \( R_t = 0.71 \) (CHCl3:MeOH = 15:1); UV \( \lambda_{max} = 239 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 3398, 1746, 1695, 1651, 1618, 1325, 1269, 1142, 949; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) \( m/z \): 263 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 263.1283 (calcld for C\(_{15}\)H\(_{19}\)O\(_4\)).

4.8. Chlorantholide E (5)

Amorphous powder; \( \phi = 90.8 \) (c 0.19, MeOH); \( R_t = 0.54 \) (CHCl3:MeOH = 15:1); CD (MeOH) \( \lambda_{max} \) (\( \Delta \)): 241 (-8.64), 215 (+23.9) nm; UV \( \lambda_{max} = 232 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 3418, 1761, 1702, 1656, 1438, 1325, 1270, 1136, 954; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) \( m/z \): 279 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 279.1227 (calcld for C\(_{15}\)H\(_{19}\)O\(_5\)).

4.9. Chlorantholide F (6)

Amorphous powder; \( \phi = 87.9 \) (c 0.19, MeOH); \( R_t = 0.34 \) (CHCl3:MeOH = 15:1); CD (MeOH) \( \lambda_{max} \) (\( \Delta \)): 241 (-8.64), 215 (+23.9) nm; UV \( \lambda_{max} = 232 \) nm; IR (KBr) \( \nu_{max} \) cm\(^{-1}\): 3418, 1761, 1702, 1656, 1438, 1325, 1270, 1136, 954; for \(^1\)H and \(^13\)C NMR spectroscopic data, see Tables 2 and 3; ESIMS (pos.) \( m/z \): 279 [M+H\(^+\)]; HRESIMS (pos.) \( m/z \): 279.1236 (calcld for C\(_{15}\)H\(_{19}\)O\(_5\)).

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


References


