Phytochemistry 71 (2010) 1020–1024

Contents lists available at ScienceDirect

Phytochemistry

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

Dolabellane diterpenoids from *Aglaia odorata*

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ARTICLE INFO

Article history:
Received 21 November 2008
Received in revised form 27 January 2010
Available online 24 March 2010

Keywords:
Aglaia odorata
Meliaceae
Dolabellane diterpenoids
X-ray
Cytotoxicity

ABSTRACT

Dolabellane diterpenoids, (1*R*,3*E*,7*E*,10*S*,11*S*,12*R*)-dolabella-3,7-dien-10,18-diol (**1**), (1*R*,3*S*,7*E*,11*S*,12*R*)-dolabella-4(16),7-dien-3,18-diol (**2**), (1*R*,7*E*,11*S*,12*R*)-18-hydroxydolabella-4(16),7-dien-3-one (**3**), (1*R*,3*S*,4*S*,7*E*,11*S*,12*R*)-3,4-epoxydolabella-7-en-18-ol (**4**), and (1*R*,3*R*,7*E*,11*S*,12*R*)-dolabella-4(16),7,18-trien-3-ol (**5**), were obtained from the ornamental plant *Aglaia odorata*. Their structures were characterized on the basis of spectroscopic analyses and further confirmed by X-ray diffraction. Compounds **1** and **5** showed weak cytotoxicity against the human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, and lung cancer A-549 cells.

1. Introduction

Dolabellane diterpenoids have a 5,11-bicyclic skeleton, the first example of which was originally obtained from the digestive gland of the sea hare *Dolabella california* in the 1970s (Ireland and Faulkner, 1976; Ireland and Faulkner, 1977). Later studies of marine algae (Amico et al., 1980; Koenig et al., 1991), orals (Duh et al., 2001; Rodriguez et al., 1995; Wang et al., 2006), and sponges (Lu and Faulkner, 1998; Xiang and Chang, 2006) also indicated that these were rich sources of this class of natural products. Terrestrial plants, such as liverworts (Hepaticae), were also found to contain this type of diterpenoid (Asakawa et al., 1990; Huneck et al., 1986; Matsuo et al., 1988; Matsuo et al., 1984; Tringali et al., 1984). Additionally, dolabellanes and their glucosides were recently isolated from higher plants such as *Chrozophora oblique* (Mohamed et al., 1994), *Aglaia odorata* (Cai et al., 2005), cumin (Morikawa et al., 2004b), and *Nigella sativa* (Morikawa et al., 2004a). Moreover, dolabellanes were established to be metabolites of the fungi *Stachybotrys chartarum* and *Hericium ramasoum* (Hinkley et al., 2000; Saito et al., 1998). Members of the dolabellane family also have significant biological properties, including cytotoxic, antibacterial, antifungal, antiviral, molluscicidal, and phytotoxic activities (Piattelli et al., 1995), and these broad biological activities have attracted many chemical synthesis studies (Baldwin and Whitby, 2003; Williams David et al., 2007; Williams and Coleman, 1995; Williams et al., 1993; Williams and Heidebrecht, 2003; Zhu et al., 2001).

The genus *Aglaia* was reported to be the main source of the unique natural products, the aglains and the rocaglamides (Brader et al., 1998). Previous research indicated that three dolabellanes could be obtained from the whole plant of *A. odorata* cultivated in a greenhouse (Cai et al., 2005). However, another possibility was that they might have been isolated from plant material (bark) contaminated by liverworts. Accordingly, we examined the chemical constituents of the leaves of *A. odorata* collected from five different places, and found the three dolabellanes in all samples. Furthermore, some unidentified dolabellanes in the sample from Xiamen, China, initiated us to perform the isolation, structural elucidation, and cytotoxic activity bioassay studies. As a result, five new dolabellanes, (1*R*,3*E*,7*E*,10*S*,11*S*,12*R*)-dolabella-3,7-dien-10,18-diol (**1**), (1*R*,3*S*,7*E*,11*S*,12*R*)-dolabella-4(16),7-dien-3,18-diol (**2**), (1*R*,7*E*,11*S*,12*R*)-18-hydroxydolabella-4(16),7-dien-3-one (**3**), (1*R*,3*S*,4*S*,7*E*,11*S*,12*R*)-3,4-epoxydolabella-7-en-18-ol (**4**), (1*R*,3*R*,7*E*,11*S*,12*R*)-dolabella-4(16),7,18-trien-3-ol (**5**) together with three known compounds, (1*R*,3*E*,7*E*,11*S*,12*R*)-18-hydroxydolabella-3,7-diene (**6**), (1*R*,3*R*,7*E*,11*S*,12*R*)-dolabella-4(16),7-dien-3,18-diol (**7**), and (1*R*,3*E*,7*R*,11*S*,12*R*)-dolabella-3,8(17)-dien-7,18-diol (**8**) (Cai et al., 2005) were obtained (Fig. 1), of which compounds **1** and **5** showed weak cytotoxic activity against HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), and A-549 (lung cancer) cells.

2. Results and discussion

Compound **1** had a molecular formula $C_{20}H_{34}O_2$ by high resolution ESI-MS at *m/z* 329.2462 (calcd. for $C_{20}H_{34}O_2Na$, 329.2456) in combination with ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2). Its IR spectrum implied the presence of hydroxyls (3423,

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doi:10.1016/j.phytochem.2010.03.005

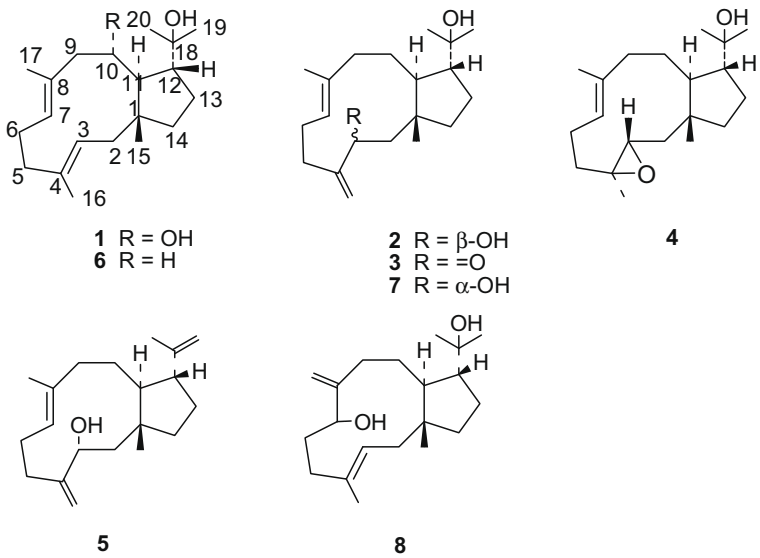

Fig. 1. Dolabellanes (**1**–**8**) isolated from leaves of *A. odorata*.

3351 cm^{-1}) and double bonds (1638, 1628 cm^{-1}). The ^{13}C NMR and DEPT spectra displayed five methyls (δ_C 32.5, 23.6, 23.5, 17.6, 16.4), six methylenes (δ_C 50.0, 39.0, 38.1, 37.8, 26.0, 25.4), three sp^3 methines (δ_C 68.2, 49.1, 47.3), two sp^3 quaternary carbons (δ_C 45.4, 73.1), and two trisubstituted double bonds (δ_C 134.4, 132.8, 126.0, 124.2) consistent with two protons at δ_H 4.94 (1H, t, J = 8.0 Hz) and 4.79 (1H, t, J = 5.5 Hz) in the ^1H NMR spectrum. The ^1H- and ^{13}C NMR spectroscopic patterns of **1** showed similarities to those of the known compound **6** (Cai et al., 2005) with an additional oxymethine (68.2 ppm) in **1**. Through analysis of its molecular formula, an additional hydroxyl was proposed for **1**. Moreover, its location was assigned to C-10 on the basis of the HMBC spectrum of **1**, in which correlations between δ_H 3.50 (H-10, d, J = 10.0 Hz) with δ_C 45.4 (C-1), δ_C 47.3 (C-11) and δ_C 134.4 (C-8), and between δ_C 68.2 (C-10) with δ_H 2.70 (1H, dd, J = 11.5, 10.0 Hz, H-9), δ_H 2.10 (1H, d, J = 11.5 Hz, H-9)], and δ_H 2.46 (1H, q, J = 11.0 Hz, H-12) were observed.

NOE correlations between H-12 (δ_H 2.46, 1H, q, J = 11.0 Hz) and H-10 and H-15 (δ_H 1.00, 3H, s) placed those protons on the same side of the molecule; and as well as correlations between H-7 and H-9, and between H-3 and H-5 (δ_H 2.15, 1H, m; 1.99, 1H, m) suggested an *E*-geometry for the two double bonds C-3/4 and C-7/8. The relative structure was further supported X-ray diffraction (Fig. 2). Since the optical rotation of **6** {α_D^{23} −46.2 (c 0.25, $CHCl_3$)} was similar in magnitude, but opposite sign to that of a literature compound (its enantiomorph) (Amico et al., 1981), the absolute configuration of C-1, 11 and 12 of **6** was determined to be 1*R*, 11*S* and 12*R*, respectively. Because of its co-occurrence in the plant as well as the negative optical rotations for all eight compounds, compounds **1**–**8** were regarded as having the same configuration. Thus compound **1** is elucidated to be (1*R*,3*E*,7*E*,10*S*,11*S*,12*R*)-dolabella-3,7-dien-10,18-diol.

Table 1
^{13}C NMR spectroscopic data for **1**–**5** in $CDCl_3$ (δ in ppm).[a]

No.	1	2	3	4	5
1	45.4 s	44.8 s	44.9 s	44.3 s	43.4 s
2	38.1 t	43.8 t	45.0 t	42.0 t	44.2 t
3	124.2 d	71.2 d	204.1 s	64.5 d	71.6 d
4	132.8 s	157.9 s	153.1 s	63.1 s	157.2 s
5	39.0 t	37.4 t	32.1 t	38.3 t	36.4 t
6	25.4 t	30.4 t	29.6 t	25.0 t	29.9 t
7	126.0 d	125.3 d	126.6 d	126.6 d	125.0 d
8	134.4 s	135.7 s	135.5 s	134.6 s	135.5 s
9	50.0 t	40.1 t	40.2 t	39.5 t	39.6 t
10	68.2 d	27.7 t	28.6 t	33.2 t	27.6 t
11	47.3 d	44.6 d	42.5 d	41.8 d	44.2 d
12	49.1 d	57.0 d	58.6 d	61.6 d	54.5 d
13	26.0 t	25.9 t	25.6 t	26.9 t	25.7 t
14	37.8 t	36.0 t	37.2 t	45.3 t	35.6 t
15	23.5 q	23.7 q	24.5 q	23.8 q	24.2 q
16	16.4 q	111.8 t	119.1 t	17.4 q	112.0 s
17	17.6 q	16.3 q	15.7 q	16.9 q	16.3 q
18	73.1 s	74.3 s	73.8 s	72.5 s	147.9 s
19	23.6 q	25.9 q	26.3 q	28.6 q	110.7 t
20	32.5 q	30.0 q	30.0 q	31.3 q	18.4 q

[a] Compounds **1**, **2**, and **4** were measured on a Bruker DRX-500 MHz, **3** and **5** on a Bruker AM-400 MHz; assignments were made by COSY, HMQC, and HMBC data.

Compound **2** was found to have the molecular formula $C_{20}H_{34}O_2$ as evidenced by high resolution ESI-MS [m/z 329.2450 $(M+Na)^+$ (calcd. for $C_{20}H_{34}O_2Na$, 329.2456)]. Its IR spectrum indicated the presence of hydroxyls (3330 cm^{-1}) and double bonds (1646 cm^{-1}). The ^{13}C NMR and DEPT spectra showed four methyls (δ_C 30.0, 25.9, 23.7, 16.3), seven sp^3 methylenes (δ_C 43.8, 40.1, 37.4, 36.0, 30.4, 27.7, 25.9), three sp^3 methines (δ_C 71.2, 57.0, 44.6), two sp^3 quaternary carbons (δ_C 44.8, 74.3), two double bonds [δ_C 125.3 (d), 135.7 (s); 111.8 (t), 157.9 (s)] corresponding to a trisubstituted double bond proton at δ_H 5.22 (1H, dd, J = 9.5, 3.5 Hz, H-7) and vinyl protons at δ_H 4.95 (1H, s) and 5.08 (1H, s) in the HSQC spectrum. Its ^1H NMR spectra was similar to those of compound **7** except that the signal at δ_H 4.44 (1H, dd, J = 8.0, 0.9 Hz) in **7** (Cai et al., 2005) shifted to δ_H 3.97 (1H, dd, J = 8.5, 6.0 Hz) in **2**, which suggested the only difference between **2** and **7** was the epimeric configuration of 3-OH. In the ROESY spectrum of **2**, a strong NOE response between δ_H 0.79 (Me-15) and one proton of H-2 (δ_H 1.40, 1H, m) established the latter as β. On the other hand, the other proton H_α-2 (δ_H 1.57) showed a strong NOE interaction with H-3, further supporting the α-orientation of H-3 (Fig 1). Thus **2** was identified as (1*R*,3*S*,7*E*,11*S*,12*R*)-dolabella-4(16),7-dien-3,18-diol.

The molecular formula $C_{20}H_{32}O_2$ of compound **3** was determined by high resolution ESI-MS at m/z 327.2306 $[M+Na]^+$. The ^1H- and ^{13}C NMR spectra of **3** showed similarities to those of **2** except that the resonances for the oxymethine at C-3 in **2** were replaced by δ_C 204.1 (ketone function) in **3**. The ^{13}C NMR spectrum of **3** exhibited a downfield chemical shift signal for an olefinic carbon at δ_C 119.1 (C-16), suggesting the presence of a conjugated α,β-unsaturated ketone, which was consistent with the UV maximum absorption at 241 nm and IR bands (1678 and 1655 cm^{-1}). The

Table 2
^{1}H NMR spectroscopic data for **1**–**5** in $CDCl_3$; *J* in Hz and δ in ppm.[a]

C	1	2	3	4	5
2	2.02 (dd, 5.5, 16.0) 1.81 (1H, dd, 5.5, 16.0)	1.57 (1H, m) 1.40 (1H, m)	2.64 (1H, d, 16.8) 2.55 (1H, d, 16.8)	1.58 (2H, m)	1.70 (1H, m) 1.45 (1H, m)
3	4.79 (1H, t, 5.5)	3.97 (1H, dd, 8.5, 6.0)		2.96 (1H, dd, 10.5, 3.0)	4.04 (1H, d, 7.5)
5	2.15 (1H, m) 1.99 (1H, m)	2.43 (1H, m) 2.24 (1H, m)	2.88 (1H, m) 2.17 (1H, m)	2.33 (1H, m) 2.05 (1H, m)	2.40 (1H, m) 2.21 (1H, m)
6	2.25 (1H, m) 2.08 (1H, m)	2.28 (1H, m) 2.20 (1H, m)	2.20 (1H, m) 2.14 (1H, m)	2.38 (1H, m) 2.15 (1H, m)	2.23 (1H, m) 2.17 (1H, m)
7	4.94 (1H, t, 8.0)	5.22 (1H, dd, 9.5, 3.5)	5.00 (1H, dd, 9.0, 3.5)	5.10 (1H, d, 11.0)	5.17 (1H, dd, 9.5, 3.5)
9	2.70 (1H, dd, 11.5, 10.0) 2.10 (1H, d, 11.5)	2.15 (1H, m) 2.00 (1H, m)	2.08 (1H, m) 1.98 (1H, m)	2.23 (1H, m) 2.10 (1H, m)	1.99 (1H, m) 1.75 (1H, m)
10	3.50 (1H, d, 10.0)	1.43 (1H, m)	1.56 (1H, m) 1.42 (1H, m)	1.73 (1H, m) 1.64 (1H, m)	1.50 (1H, m) 1.26 (1H, m)
11	1.80 (1H, m)	1.59 (1H, m)	1.92 (1H, m)	1.65 (1H, m)	1.54 (1H, m)
12	2.46 (1H, q, 11.0)	1.90 (1H, m)	1.92 (1H, m)	1.68 (1H, m)	2.37 (1H, m)
13	1.81 (1H, m) 1.22 (1H, m)	1.85 (1H, m) 1.35 (1H, m)	1.75 (1H, m) 1.54 (1H, m)	1.62 (1H, m) 1.35 (1H, m)	1.77 (1H, m) 1.47 (1H, m)
14	1.59 (1H, m) 1.34 (1H, m)	1.80 (1H, m) 1.28 (1H, m)	1.33 (1H, m) 1.22 (1H, m)	1.58 (1H, m) 1.36 (1H, m)	2.01 (1H, m) 1.30 (1H, m)
15	1.00 (3H, s)	0.79 (3H, s)	0.88 (3H, s)	1.29 (3H, s)	0.79 (3H, s)
16	1.52 (3H, s)	5.08 (1H, s) 4.95 (1H, s)	5.52 (1H, s) 5.46 (1H, s)	1.30 (3H, s)	5.10 (1H, s) 4.95 (1H, s)
17	1.58 (3H, s)	1.56 (3H, s)	1.56 (3H, s)	1.71 (3H, s)	1.53 (3H, s)
19	1.29 (3H, s)	1.25 (3H, s)	1.26 (3H, s)	1.20 (3H, s)	4.73 (1H, s) 4.68 (1H, s)
20	1.29 (3H, s)	1.26 (3H, s)	1.26 (3H, s)	1.27 (3H, s)	1.74 (3H, s)

[a] Compounds **1** and **3** were measured on a Bruker AM-400 MHz, **2**, **4** and **5** on a Bruker DRX-500 MHz.

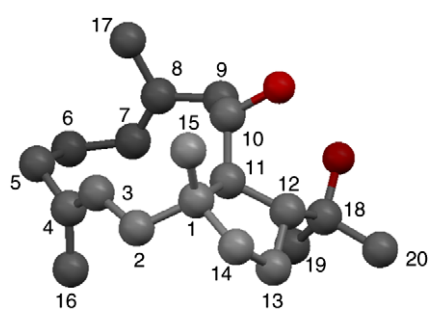

Fig. 2. X-ray crystal structure of **1**.

assumption was also supported by HMBC crosspeaks between δ_C 204.1 (C-3) with H-16 (δ_H 5.52 (1H, s) and 5.46 (1H, s)), H-5 (δ_H 2.88 (1H, m) and 2.17 (1H, m)), and H-2 (δ_H 2.64 (1H, m) and 2.55 (1H, m)). Therefore, compound **3** was elucidated to be (1*R*,7*E*,11*S*,12*R*)-18-hydroxydolabella-4(16),7-dien-3-one.

Compound **4** possessed a molecular formula of $C_{20}H_{34}O_2$ by high resolution ESI-MS at *m*/*z* 329.2451[M+Na]$^+$. The ^{1}H- and ^{13}C NMR spectra of **4** displayed similarities to those of **6**, except for the absence of a double bond. Instead, signals at δ_C 64.5 (d) and 63.1 (s) were present in **4**, suggesting the presence of a 3,4-epoxide. The suggestion was further supported by HMBC correlations of δ_H 2.96 (1H, dd, *J* = 10.5, 3.0 Hz, H-3) with δ_C 44.3 (C-1, s), 17.4 (q, C-16), and 63.1 (s, C-4), and the COSY correlation between δ_H 2.96 and δ_H 1.58 (2H, m, H-2). Two NOE correlations between H-3 and Me-15, and between H-11 (δ_H 1.65, m) and Me-16 indicated 3S and 4S configurations, and so compound **4** was identified as (1*R*,3*S*,4*S*,7*E*,11*S*,12*R*)-3,4-epoxydolabella-7-en-18-ol.

Compound **5** was found to have the molecular formula $C_{20}H_{32}O$ by high resolution ESI-MS at *m*/*z* 311.2347 [M+Na]$^+$. The ^{13}C NMR spectroscopic data of **5** were similar to those of known compounds **7** (Cai et al., 2005), with the exception of a vinyl substituent at (δ_C 147.9 and 110.7) in **5**, instead of the signals of a quaternary carbon (δ_C 73.0, C-18) and a methyl (C-19/20) in **7**. Detailed analysis of the HMBC spectrum led to the conclusion that 5 contained an anhydro structure. Thus **5** was (1*R*,3*R*,7*E*,11*S*,12*R*)-dolabella-4(16),7,18-trien-3-ol.

Table 3
Cytotoxicity of compounds **1** and **5**.[a]

Compound	HL-60	SMMC-7721	A-549	MCF-7	W480
1	13.47	15.79	16.56	>40	>40
5	16.60	26.08	33.61	>40	>40
Cisplatin	1.77	11.66	13.35	25.16	8.94

[a] Results are expressed as IC_{50} value in μm.

All diterpenoids from *A. odorata* were tested for their ability to prevent the cytopathic effects of cancer in HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung cancer), MCF-7 (breast cancer), and W480 (colorectal cancer) cells, and their cytotoxicity was measured in parallel with the determination of antitumor activity using cisplatin as the positive control. Among these compounds, **1** and **5** showed anticancer activity against three cells (Table 3), while other compounds were noncytotoxic in all four cell lines (IC_{50} > 40 μm).

3. Concluding remarks

Instead of aglains and the rocaglamides, the typical chemical character of the genus *Aglaia* (Brader et al., 1998), dolabellanes are also common phytochemical constituents of cultivated species of *A. odorata*.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured with a Horiba SEPA-300 Digital Polarimeter. UV spectra were performed on a Shimadzu UV-2401PC spectrometer. IR (KBr) spectra were obtained on a Bruker Tensor-27 FT infrared spectrophotometer. 1D and 2D NMR spectra were recorded on AM-400 and DRX-500 MHz NMR spectrometers with TMS as the internal standard. EI-MS data were obtained on a Finnigan Trace DSQ spectrometer and high resolution ESI-MS data on a API Qstar Pulsar 1 spectrometer. Silica gel (200–300 mesh) for column chromatography (CC) and GFB_{254B} for TLC were obtained from Qingdao Marine Chemical Factory, Qingdao, China. Rp18 silica gel (45–60 μm) was bought from Merck Co., Germany. Spots on chromatograms were detected by spraying with 5% H_2SO_4 and heating.

4.2. Plant material

Leaves of *A. odorata* were collected in Xiamen Botanical Garden, Fujian Province, People's Republic of China, in April 2007. A voucher specimen (Luo 20070416) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

4.3. Extraction and isolation

Air-dried leaves (15.0 kg) were crushed and refluxed with EtOH–H_2O (9:1, V/V) (60 l × 3, each 2 h). After removal of the EtOH under reduced pressure, the viscous concentrate was partitioned with EtOAc and H_2O (4 × 15 l) to yield an EtOAc extract (350 g). The EtOAc extract was loaded onto a silica gel column (4.0 kg) and eluted with $CHCl_3$–MeOH (from 10:0 to 1:1) to give 10 fractions (A–J). Fr. B (24 g) was subjected to silica gel CC (500 g) using petroleum ether–acetone (19:1) as eluent to obtain two parts: B1 and B2. B1 (2.4 g) and B2 (6.4 g) were subjected to RP-18 silica gel CC (100 g) eluted with MeOH–H_2O (9:1) to yield compounds **5** (15 mg) and **6** (3.3 g), respectively. Fr. C (50 g) was separated using silica gel CC (800 g) and eluted with petroleum ether–acetone (from 19:1 to 9:1) to afford eight subfractions (C1–C8). Compound **4** (13 mg) was isolated from C4 (3 g) by RP-18 silica gel CC (100 g) using MeOH–H_2O (from 85:15) as the eluent. Fr. D (106 g) was subjected to silica gel CC (1.5 kg) and eluted with petroleum ether–acetone (from 9:1 to 4:1) to obtain eight subfractions (D1–D8). D3 (3.6 g) was purified by Sephadex LH-20 (100 g) eluted with MeOH to afford compound **2** (20 mg). Compound **1** (14 mg) was crystallized from Fr. D4 to Fr. D5 (9.3 g) was subjected to purification using a RP-18 gel column (100 g) eluted with MeOH–H_2O (from 4:1 to 9:1) to yield compound **7** (11 mg). Fr. E (90 g) was subjected to silica gel CC (1.2 g) and eluted with petroleum ether–acetone (from 4:1 to 7:3) to obtain five fractions (E1–E5). Fr. E3 and E4 were subjected to RP-18 gel CC (100 g) and eluted with MeOH–H_2O (3:1) to afford **3** (41 mg) and **8** (6 mg), respectively.

4.3.1. (1R,3E,7E,10S,11S,12R)-dolabella-3,7-dien-10,18-diol (*1*)

Colorless prism; $[\alpha_D^{22}]$-83.7 (*c* 0.21, MeOH); IR (KBr) υ_{max} 3423, 3351, 2927, 1638, 1628, 1449 cm^{-1}; positive ESI-MS *m/z*: 329 $[M+Na]^+$, high resolution ESI-MS *m/z*: 329.2462 [M+Na] (calcd. for $C_{36}H_{46}O_{12}Na$ 329.2456); for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.2. (1R,3S,7E,11S,12R)-dolabella-4(16),7-dien-3,18-diol (*2*)

White powder; $[\alpha_D^{22}]$-159.9 (*c* 0.24, MeOH); IR (KBr) υ_{max} 3330, 2960, 1646, 1452 cm^{-1}; high resolution ESI-MS *m/z*: 329.2450 $[M+Na]^+$ (calcd. for $C_{36}H_{46}O_{12}Na$ 329.2456); for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.3. (1R,7E,11S,12R)-18-hydroxydolabella-4(16),7-dien-3-one (*3*)

Colorless oil; $[\alpha_D^{22}]$-108.3 (*c* 1.10, MeOH); UV λ_{max}^{MeOH} nm (log ε): 241 (3.7); IR (KBr) υ_{max} 3398, 2942, 1678, 1655, 1445 cm^{-1}; high resolution ESI-MS *m/z*: 327.2306 $[M+Na]^+$ (calcd. for $C_{36}H_{46}O_{12}Na$ 327.2300); for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.4. (1R,3S,4S,7E,11S,12R)-3,4-epoxydolabella-7-en-18-ol (*4*)

Colorless oil; $[\alpha_D^{22}]$-69.6 (*c* 0.20, MeOH); IR (KBr) υ_{max} 3389, 2941, 1635, 1445 cm^{-1}; EI-MS *m/z*: 306 $[M]^+$ (3), high resolution ESI-MS *m/z*: 329.2451 $[M+Na]^+$ (calcd. for $C_{36}H_{46}O_{12}Na$ 329.2456); for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.5. (1R,3R,7E,11S,12R)-dolabella-4(16),7,18-trien-3-ol (*5*)

Colorless oil; $[\alpha_D^{22}]$-112.3 (*c* 0.26, MeOH); IR (KBr) υ_{max} 3409, 1655, 1644, 1442 cm^{-1}; EI-MS *m/z*: 288 $[M]^+$ (15), high resolution ESI-MS *m/z*: 311.2347 $[M+Na]^+$ (calcd. for $C_{36}H_{46}O_{12}Na$ 311.2350); for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2.

4.3.6. X-ray crystal structure of *1*

$C_{20}H_{34}O_2$, MW = 306.47; orthorhombic, space group $P2_12_12_1$; a = 9.4396(11) Å, b = 10.3295(12) Å, c = 19.135(2) Å. V = 1865.7(4) $Å^3$, Z = 4, D_{calc} = 1.087 g/cm^3. A colorless cube of dimensions 0.23 × 0.19 × 0.17 mm^3 was used for X-ray measurement on a Bruker APEX diffractometer with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation. Of the 14,925 reflections that were collected, 4446 were unique (R_{int} = 0.0501); equivalent reflections were merged. Data were collected and processed using CrystalClear (Rigaku). The structure was solved by direct methods and expanded using Fourier techniques. All calculations were performed using the CrystalStructure crystallographic software package except for refinement, which was performed using SHELXL-97 (Sheldrick, G.M., University of Gottingen, Gottingen, Germany, 1997). Crystallographic data for the structure of **6** have been deposited in the Cambridge Crystallographic Centre (deposition number is 668939). Copies of these data can be obtained, free of charge, on application to the CCDC via www.ccdc.cam.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).

4.4. Cytotoxic bioassays

The following human tumor cell lines were used: HL-60 (human myeloid leukemia), SMMC-7721 (hepatocellular carcinoma), A-549 (lung cancer), MCF-7 (breast cancer), and W480 (colorectal cancer). All cells were cultured in RPMI-1640 or DMEM medium (Hyclone, Logan, UT), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37 °C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates (Mosmann, 1983). Briefly, 100 μl adherent cells were 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 initial density of 1 × 105 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μm in triplicates for 48 h, with cisplatin (Sigma, St. Louis, MO) as positive control. After compound treatment, cell viability was detected and the cell growth curve was graphed. The IC_{50} value was calculated by the Reed and Muench method (Reed, 1938).

Acknowledgments

The authors are grateful to the National Natural Science Foundation of China (C30670214) and the Ministry of Science and Technology of PR China (2009CB522300, 2007AA021505) for partial financial support, and the members of the analytical group in the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, for the spectral measurements.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.phytochem.2010.03.005.

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