Dysoxydensins A–G, Seven New Clerodane Diterpenoids from *Dysoxylum densiflorum*

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Abstract

Seven new clerodane diterpenoids, dysoxydensins A–G (**1**–**7**), together with six known clerodanes, were isolated from *Dysoxylum densiflorum*. The structures of all the compounds were elucidated by extensive spectroscopic analysis. These compounds were evaluated for their cytotoxic activities against five human cancer cell lines, and compounds **2, 3**, and **5** showed moderate cytotoxic activities.

Supporting information available online at http://www.thieme-connect.de/products

Introduction

Plants of the genus *Dysoxylum* (Meliaceae), which is naturally distributed in India and southeast Asia, are rich sources of structurally diverse and biologically prospective diterpenes [1–4]. A phytochemical investigation of diterpenes exhibited various bioactivities, such as antibacterial [5], antileishmanial [6], antineoplastic [7–11], anti-inflammatory [12], antitubercular [13], antidepressant [14], antiarrythmic [15], and so on. To better understand the distribution of diterpenoids in the genus *Dysoxylum*, the chemical components of *Dysoxylum densiflorum* (Blume) Miq. were investigated. As a result, thirteen clerodane diterpenoids, including seven new compounds, dysoxydensins A–G (**1**–**7**) (**Fig. 1**), were isolated from the EtOAc extracts of *D. densiflorum*. Their structures were elucidated by extensive spectroscopic analysis. The six known diterpenoids were determined as rosestachone [16], roseostachenone [16], clerod-14-ene-3α,4β,13ξ-triol [17], clerod-14-ene-3α-acetyl-4β,13ξ-triol [17], (3α,4β,13*E*)-neoclerod-13-ene-3,4,15-triol [18], and 15-hydroxy-3-cleroden-2-one [19]. All the compounds were evaluated for their cytotoxic activities against human HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines, using the MTT method [20]. Herein, this paper reports the isolation, structural elucidation, and cytotoxic activities of the new diterpenoids.

* These authors contributed equally to this work.

Results and Discussion

Dysoxydensin A (**1**) showed a molecular formula of $C_{20}H_{34}O_3$ by HREIMS (*m/z* 322.2509 $[M]^+$), indicating four degrees of unsaturation. The IR spectrum revealed absorption bands for a hydroxyl group (3428 cm^{-1}), a carbonyl group (1701 cm^{-1}), and an olefinic bond (1635 cm^{-1}). The ^1H, ^{13}C NMR and DEPT spectra of **1** (**Tables 1** and **2**) exhibited 20 carbon resonances, assigned to one ketonic carbonyl [δ_C 212.9 (s)], one vinylic group [δ_C 111.3 (t), 147.0 (d)] with corresponding protons at δ_H 5.19 (dd, J = 17.3, 1.5 Hz), 4.96 (dd, J = 10.7, 1.5 Hz), and 5.93 (dd, J = 17.3, 10.7 Hz), five methyls with four corresponding singlet methyl protons at δ_H 1.24, 1.06, 0.76, and 0.74, and a secondary methyl proton at δ_H 0.79 (d, J = 6.3 Hz), six methylenes, two methines, and four quaternary carbons (two oxygenated). Besides one ketonic carbonyl and a C-C double bond, the degrees of unsaturation required two rings to meet the molecular formula. Close similarities of the chemical shifts and coupling constants of **1** with the known compound clerod-14-ene-3α,4β,13ξ-triol [17] revealed that **1** possessed a clerodane diterpenoid skeleton (**Tables 1** and **2**). The difference was the presence of a ketonic carbonyl (δ_C 212.9, C-3) in **1** instead of one oxymethine at δ_C 76.2 (C-3) in clerod-14-ene-3α,4β,13ξ-triol, which was further confirmed by the HMBC correlations of δ_H 1.54 (H-1b), 2.98 (H-2a), 2.03 (H-2b), and 1.06 (H_3-18) with δ_C 212.9 (C-3) (**Fig. 2**).

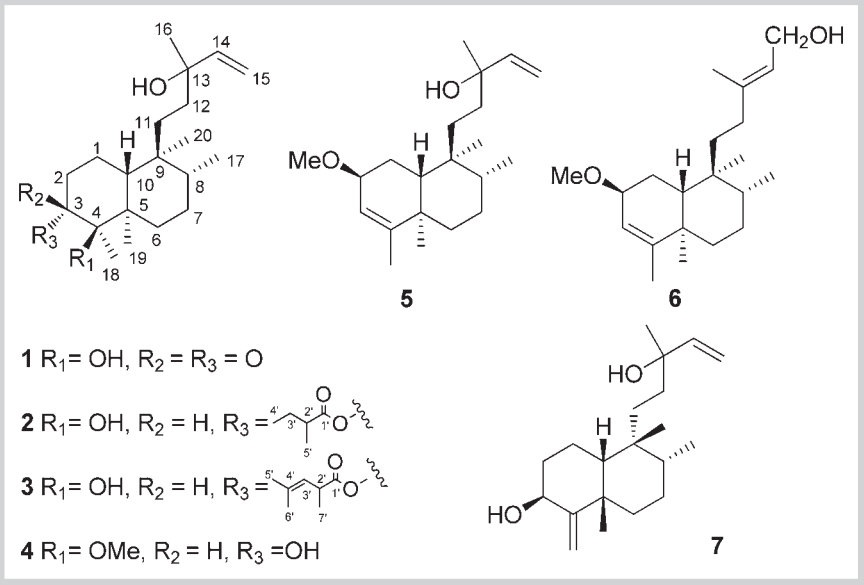

Fig. 1 Structures of dysoxydensins A–G (**1**–**7**).

Table 1 ^{1}H NMR data of **1**–**7** in acetone-d_6 at 400 MHz (δ in ppm and J in Hz).

No.	1	2	3	4	5	6	7
1a	1.82 m	1.31 m	1.44 m	1.55 m	1.82 brd (13.7)	1.85 m	1.78 m
1b	1.54 m	1.24 m	1.35 m	1.23 m	1.46 m	1.50 m	1.41 m
2a	2.98 m	1.90 m	2.04 m	1.80 m	3.55 brs	3.56 brs	1.91 dd (13.4, 2.6)
2b	2.03 m	1.44 dd (14.2, 2.4)	1.58 m	1.57 m			1.47 m
3		4.58 brs	4.75 brs	3.81 brs	5.35 d (2.9)	5.35 brs	4.23 brs
6a	1.86 ddd (12.6, 12.6, 5.1	1.69 m	1.80 m	1.75 m	1.68 brd (12.2)	1.70 brd (12.5)	1.63 m
6b	1.29 m	1.19 m	1.30 m	1.18 m	1.15 m	1.17 m	1.42 m
7a	1.43 m	1.32 m	1.44 m	1.38 m	1.46 m	1.47 m	1.51 m
7b	1.38 m	1.20 m	1.31 m	1.24 m	1.38 m	1.42 m	1.38 m
8	1.49 m	1.31 m	1.42 m	1.37 m	1.46 m	1.53 m	1.41 m
10	2.48 dd (12.8, 2.8)	1.84 dd (12.0, 2.1)	1.95 dd (12.2, 2.0)	1.91 dd (12.2, 2.0)	1.55 brd (12.8)	1.58 m	1.04 dd (12.3, 2.0)
11a	1.46 m	1.31 m	1.40 m	1.35 m	1.39 m	1.48 m	1.36 m
11b	1.39 m	1.23 m	1.33 m	1.28 m	1.36 m	1.36 m	1.33 m
12a	1.47 m	1.31 m	1.42 m	1.38 m	1.52 m	2.05 m	1.35 m
12b	1.39 m	1.23 m	1.36 m	1.33 m	1.32 m	1.80 m	1.24 m
14	5.93 dd (17.3, 10.7)	5.78 dd (17.3, 10.7)	5.90 dd (17.3, 10.7)	5.89 dd (17.3, 10.7)	5.91 dd (17.3, 10.7)	5.37 brs	5.88 dd (17.3, 10.7)
15a	5.19 dd (17.3, 1.5)	5.06 dd (17.3, 1.8)	5.18 dd (17.3, 1.8)	5.17 dd (17.3, 1.6)	5.20 d (17.3)		5.17 dd (17.3, 1.7)
15b	4.96 dd (10.7, 1.5)	4.83 dd (10.7, 1.8)	4.95 dd (10.7, 1.8)	4.95 dd (10.7, 1.6)	4.95 d (10.7)	4.05 brs	4.95 dd (10.7, 1.7)
16	1.24 s	1.10 s	1.22 s	1.21 s	1.20 s	1.64 s	1.20 s
17	0.79 d (6.3)	0.65 d (6.1)	0.76 d (6.0)	0.73 d (6.0)	0.78 d (5.2)	0.81 d (6.2)	0.78 d (6.3)
18	1.06 s	0.96 s	1.07 m	1.13 s	1.60 s	1.61 s	4.73, 4.67 s
19	0.76 s	0.99 s	1.10 s	1.13 s	0.94 s	0.95 s	1.23 s
20	0.74 s	0.60 s	0.72 s	0.70 s	0.72 s	0.74 s	0.75 s
2′		2.24 dq (14.0, 7.0)	2.42 dt (13.8, 6.8)				
3′a		1.55 m	5.70 brs				
3′b		1.36 m					
4′		0.77 t (7.4)					
5′		1.01 d (7.0)	1.07 s				
6′			1.05 s				
7′			2.12 brs				
OMe				3.14 s	3.28 s	3.28 s	

The side chain at C-9 was assigned as an equatorial bond to reduce steric hindrance. Then the coupling constants (J = 12.8, 2.8 Hz) positioned H-10 at the axial bond, which was assumed to be temporarily in the β position. In the ROESY spectrum of **1**,

Table 2 ^{13}C NMR data of **1**-**7** in acetone-d_6 at 150 MHz (δ in ppm and J in Hz).

No.	1	2	3	4	5	6	7
1	23.1 t	17.5 t	17.6 t	16.9 t	24.2 t	24.1 t	17.3 t
2	37.5 t	28.1 t	28.2 t	31.4 t	74.9 d	74.9 d	36.1 t
3	212.9 s	77.5 d	76.7 d	70.2 d	121.4 d	121.5 d	74.3 d
4	81.0 s	75.3 s	75.5 s	80.4 s	149.5 s	149.2 s	162.2 s
5	44.8 s	42.1 s	42.2 s	43.2 s	39.4 s	39.4 s	40.6 s
6	32.1 t	32.7 t	32.7 t	32.8 t	37.1 t	37.0 t	39.1 t
7	27.8 t	27.5 t	27.5 t	27.6 t	28.1 t	28.1 t	27.8 t
8	36.8 d	36.8 d	36.7 d	36.8 d	36.9 d	36.8 d	37.2 d
9	39.2 s	38.9 s	38.9 s	38.9 s	38.6 s	38.9 s	39.5 s
10	39.2 d	40.7 d	40.7 d	40.6 d	42.0 d	42.1 d	49.7 d
11	33.1 t	33.1 t	33.1 t	33.2 t	32.2 t	37.5 t	32.7 t
12	36.1 t	36.3 t	36.3 t	36.3 t	35.3 t	32.8 t	36.1 t
13	72.9 s	73.0 s	73.0 s	73.0 s	72.9 s	138.5 s	72.9 s
14	147.0 d	147.0 d	147.0 d	147.0 d	147.3 d	125.5 d	146.9 d
15	111.3 t	111.3 t	111.2 t	111.2 t	111.0 t	59.2 t	111.3 t
16	28.3 q	28.4 q	28.4 q	28.3 q	28.5 q	16.5 q	28.4 q
17	16.0 q	16.3 q	16.3 q	16.4 q	16.1 q	16.1 q	16.3 q
18	16.6 q	21.1 q	21.1 q	18.2 q	18.1 q	18.1 q	108.1 t
19	16.6 q	17.5 q	17.4 q	14.3 q	18.7 q	18.7 q	23.0 q
20	18.7 q	18.9 q	18.9 q	19.2 q	18.8 q	18.7 q	18.9 q
1′		175.5 s	166.1 s				
2′		42.4 d	38.7 d				
3′		27.3 t	114.8 d				
4′		11.9 q	166.4 s				
5′		17.0 q	21.1 q				
6′			21.0 q				
7′			15.9 q				
OMe				49.8 q	56.2 q	56.3 q	

correlations of δ_H 2.48 (H-10)/4.33 (4-OH), δ_H 2.48 (H-10)/2.98 (H-2a), and δ_H 4.33 (4-OH)/1.82 (H-1a) suggested that they were all located on the same side. The methyl protons δ_H 0.76 (H_3-19) did not show ROESY correlations to any of the above protons, but showed correlations to δ_H 1.06 (H_3-18) and 1.54 (H-1b), which placed them on the other side (**Fig. 2**). Meanwhile, ROESY correlations of δ_H 2.48 (H-10)/1.46, 1.39 (2H, H-11) and δ_H 2.48 (H-10)/1.49 (H-8) placed two methyls (Me-17 and 20) at the α position. Therefore, compound **1** was established as clerod-3-oxo-14-ene-4β,13ξ-diol and is shown in **Fig. 1**.

Dysoxydensin B (2) was assigned the molecular formula $C_{25}H_{44}O_4$ by its HREIMS at *m/z* 408.3232 $[M]^+$, which assumed that a five carbon substituent group appeared, by comparison of its ^1H and ^{13}C NMR spectral data (**Tables 1** and **2**) with those of **1**. A 2-methyl-butyryl group was suggested to be substituent at C-3 by comparison with data in the literature [21–23]. The assumption was supported by HMBC correlations of δ_H 4.58 (H-3) with δ_C 175.5 (C-1′), of δ_H 1.01 (H_3-5′) and 1.55 (H-3′a) with δ_C 175.5 (C-1′), of δ_H 1.01 (H_3-5′) with δ_C 27.3 (C-3′), and of δ_H 0.77 (H_3-4′) with δ_C 42.4 (C-2′). Moreover, the ROESY correlation of δ_H 1.84 (H-10)/4.58 (H-3) and 3.50 (4-OH) of δ_H 0.96 (H_3-18)/0.99 (H_3-19) showed that H-10, H-3, and 4-OH were on the same side, while the CH_3-18 and CH_3-19 were on the opposite side. Other parts of **2** were identical to those of **1**, which was supported by HMBC, HMQC, and ROESY spectra. Hence, **2** was elucidated as clerod-14-ene-3α-(2-methyl-butyryl)-4β,13ξ-diol (**Fig. 1**).

The molecular formula of $C_{27}H_{46}O_4$ for dysoxydensin C (**3**) was determined by HREIMS (*m/z* 434.3389 $[M]^+$). The ^1H and ^{13}C NMR spectral data of **3** were closely related to those of **2** (**Tables 1** and **2**), except for the presence of a seven carbon substituent group in **3** at C-3, which was consistent with the molecular formula of **3**. 2,4-Dimethyl-3-ene-valeryl was proposed by HMBC correlations of δ_H 4.75 (H-3) with δ_C 166.1 (C-1′), of δ_H 2.12 (H_3-7′) and 5.70 (H-3′a) with δ_C 166.1 (C-1′), of δ_H 2.12 (H_3-7′) with δ_C 114.8 (C-3′), and of δ_H 1.07 (H_3-5′), 1.05 (H_3-6′) with δ_C 166.4 (C-4′). Moreover, HSQC, HMBC, and ROESY spectral data supported that other parts of **2** and **3** were identical. Thus, **3** was deduced as clerod-14-ene-3α-(2,4-dimethyl-3-ene-valeryl)-4β,13ξ-diol (**Fig. 1**).

Dysoxydensin D (**4**) gave its molecular formula as $C_{21}H_{38}O_3$ by HREIMS at *m/z* 338.2821 $[M]^+$. Analysis of the ^1H and ^{13}C NMR spectral data of **4** showed close similarities with those of **1** (**Tables 1** and **2**), with the exception of a reduction at C-3 (δ_C 70.2, δ_H 3.81) and methoxylation at C-4 (δ_C 49.8, δ_H 3.14) in **4**. The assignment was also supported by HMBC correlations of δ_H 1.55 (H-1a), 1.80 (H-2a), and 1.13 (H_3-18) with δ_C 70.2 (C-3), of δ_H 3.58 (3-OH) with δ_C 31.4 (C-2), 70.2 (C-3), and 80.4 (C-4), and of δ_H 3.14 (OMe) with δ_C 80.4 (C-4). H-3 was positioned at the equatorial bond by an NOE correlation of δ_H 3.81 (H-3)/1.80, 1.57 (2H, H-2) in the ROESY spectrum of **4**, which was the α orientation of 3-OH. Meanwhile, ROESY correlations of δ_H 3.58 (3-OH)/1.57 (H-2b) and δ_H 1.57 (H-2b)/1.13 (H_3-18) further supported both 3-OH and CH_3-18 at the α position. Thus, **4** was established as clerod-4β-methoxyl-14-ene-3α,13ξ-diol by HSQC, HMBC, and ROESY spectral data (**Fig. 1**).

Dysoxydensin E (**5**) had the molecular formula of $C_{21}H_{36}O_2$ by its HREIMS at *m/z* 320.2708 $[M]^+$. The ^1H and ^{13}C NMR spectral data of **5** (**Tables 1** and **2**) displayed similarities with those of roseostachenone [16]. Instead of a ketonic carbonyl at C-2 in roseostachenone, an oxymethine [δ_C 74.9 (d), δ_H 3.55 (brs)] and a methoxyl group [δ_C 56.2 (s), δ_H 3.28 (s)] were present in **5**. The HMBC correlations of δ_H 3.55 (H-2) with δ_C 42.0 (C-10), 121.4 (C-3), and

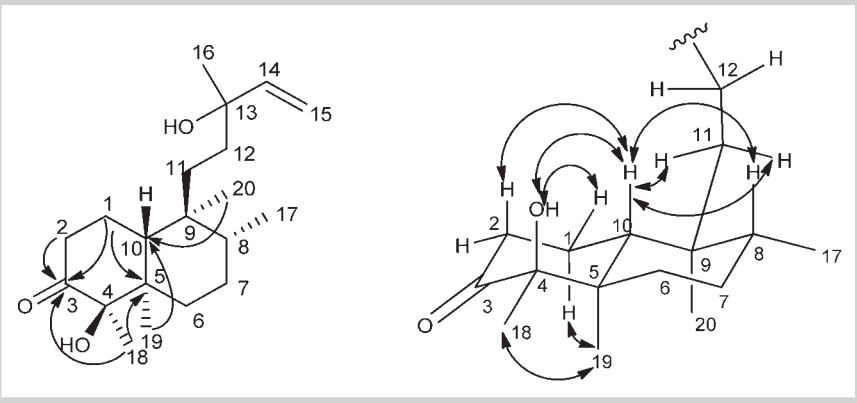

Fig. 2 Key HMBC (→) and ROESY (↔) correlations of **1**.

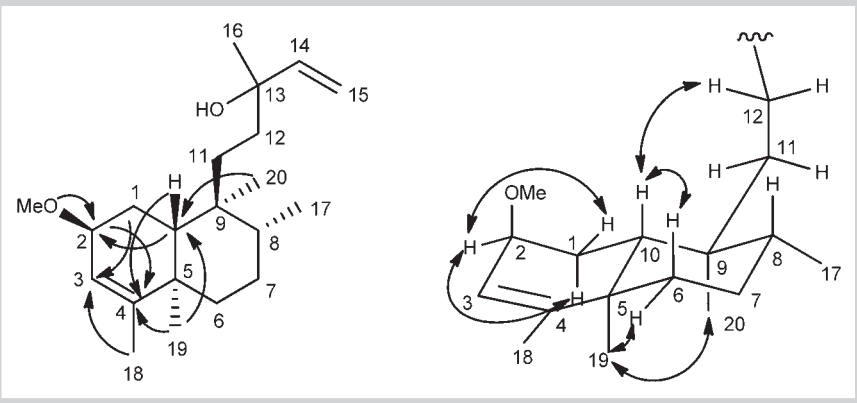

Fig. 3 Key HMBC (→) and ROESY (↔) correlations of **5**.

149.5 (C-4), and of δ_H 3.28 (-OMe) and 1.55 (H-10) with δ_C 74.9 (C-2) also supported that the methoxyl group was at C-2. H-2 appeared as a broad singlet with NOE correlations to both protons of H-1 (δ_H 1.82, 1.46) (**Fig. 3**), which indicated a β orientation of 2-OMe. Other parts of **5** were identical to those of compounds **1**–**4**, supported by its 2D NMR spectra. Thus, **5** was established as clerod-2β-methoxyl-3(4),14-dien-13ξ-ol and is shown in **Fig. 1**. The chirality of C-13 is still unclear.

Dysoxydensin F (**6**) was assigned the molecular formula of $C_{21}H_{36}O_2$ by its HREIMS (*m/z* 320.2724 $[M]^+$). The ^1H and ^{13}C NMR spectral data of **6** (**Tables 1** and **2**) were closely related to those of **5**, except for the presence of a trisubstituted olefin [δ_C 125.5 (d), 138.5 (s), δ_H 5.37 (brs)] at $\Delta^{13/14}$, and a downfield methylene [δ_C 59.2 (t), δ_H 4.05 (brs)] at C-15 in **6**. An allylic alcohol moiety ($R_2C{=}CHCH_2OH$) as C-13/14/15/16 was indicated by HMBC correlations of δ_H 4.05 (H_2-15) with δ_C 138.5 (C-13) and of δ_H 2.05 (H-12a), 1.64 (H_3-16) with δ_C 125.5 (C-14). Furthermore, the NOE correlation of δ_H 4.05 (H-15)/1.64 (H_3-16) in the ROESY spectrum suggested an *E* configuration of $\Delta^{13/14}$. Other parts of **6** were identical to those of **5**, supported by its HSQC, HMBC, and ROESY spectra. Hence, **6** was deduced as clerod-2β-methoxyl-3(4),13*E*-dien-15ξ-ol (**Fig. 1**).

The molecular formula of dysoxydensin G (**7**) was assigned as $C_{20}H_{34}O_2$ by HREIMS (*m/z* 306.2552 $[M]^+$). Detailed analysis of the ^1H and ^{13}C NMR spectral data of **7** (**Tables 1** and **2**) assumed that it had a close structure to clerod-14-ene-3α,4β,13ξ-triol [17], differing by the presence of an exomethylene group [δ_C 108.1 (t), 162.2 (s)] at $\Delta^{4/18}$ in **7** instead of an sp^3 quaternary carbon at C-4 in clerod-14-ene-3α,4β,13ξ-triol. The assumption was supported by the HMBC correlations of δ_H 1.91 (H-2a), 1.04 (H-10), 4.73, 4.67 (H_2-18), and 1.23 (H_3-19) with δ_C 162.2 (C-4). H-10 was positioned at the axial bond by its coupling constants (J = 12.3, 2.0 Hz) and was assumed to have a β orientation. Unlike compounds **1**–**6**, NOE correlations of δ_H 1.04 (H-10)/1.41 (H-8) and 1.23 (H_3-19), and δ_H 1.23 (H_3-19)/3.62 (3-OH) and 0.75 (H_3-20) in the ROESY spectrum of **7**, assigned them all to be β-orientated (**Fig. 4**.). Thus, **7** was deduced as shown in **Fig. 1**.

All isolated clerodanes were evaluated for their cytotoxic activities against human HL-60, SMMC-7721, A-549, MCF-7, and SW480 cell lines, according to the MTS method [20]. The IC_{50} values were calculated by the Reed and Muench method [24, 25]. As a result, compounds **2, 3**, and **5** exhibited moderate cytotoxic activities compared with the positive control cisplatin (**Table 3**). All compounds with IC_{50} values > 40 µM were considered inactive.

Materials and Methods

General experimental procedures

Optical rotations were obtained with a Jasco P-1020 automatic digital polariscope. UV spectra were measured with a Shimadzu UV2401PC in MeOH solution. IR spectra were obtained on a Bruker tensor-27 infrared spectrophotometer using KBr pellets. 1D and 2D NMR spectra were recorded on a Bruker AM-400 and a DRX-500 NMR with TMS as the internal standard. Chemical shifts (δ) were expressed in ppm relative to TMS. HREIMS spectra were recorded on a Waters Auto Premier P776 spectrometer. Column chromatography (CC) was performed on silica gel (200–300 mesh, Qingdao Marine Chemical, Ltd.) and RP-18 gel (20–45 µm,

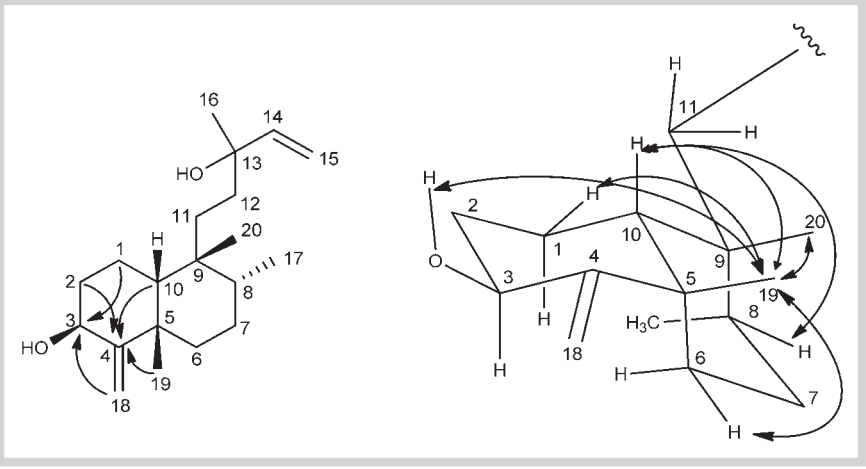

Fig. 4 Key HMBC (→) and ROESY (↔) correlations of **7**.

Entry	HL-60	SMMC-7721	A-549	MCF-7	SW480
2	15.1	19.6	17.9	16.0	15.5
3	17.0	15.5	11.9	14.2	16.6
5	23.7	27.5	31.7	> 40	> 40
Cisplatin	1.2	6.4	9.2	15.9	13.4

Table 3 Cytotoxic activities of compounds **2, 3**, and **5** (IC_{50}, μM).

and Fuji Silysia Chemical, Ltd.). HPLC was performed using Waters 600 pumps coupled with analytical and semipreparative Sunfire RP-18 columns (150 × 4.6 and 150 × 10 mm, respectively). The HPLC system employed a Waters 2996 photodiode array detector. Fractions were monitored by TLC (GF 254, Qingdao Haiyang Chemical Co., Ltd.), and spots were visualized by 10% H_2SO_4-ethanol reagent.

Plant material

The barks of *D. densiflorum* were collected from Xishuangbanna Autonomous Prefecture, Yunnan Province, People's Republic of China, and identified by Jingyun Cui of Xishuangbanna Botanic Garden. A voucher specimen (Cui 200811–18) has been deposited at the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and isolation

The air-dried bark (5 kg) of *D. densiflorum* was extracted with 90% MeOH (15 L × 3, 3 days) under room temperature. After removal of the solvent, the extract was suspended in H_2O and extracted three times with ethyl acetate. The EtOAc fraction (129 g) was subjected to CC on silica gel (200–300 mesh, 8 × 150 cm, 1.5 kg) and eluted with gradient mixtures of $CHCl_3$-Me_2CO (1 : 0, 20 : 1, 15 : 1, 10 : 1, 5 : 1, 1 : 1, 0 : 1, each 12 L). According to differences in composition monitored by TLC, five fractions were obtained. Fraction III (15.6 g) was separated by MPLC with RP-18 CC (4.9 × 46 cm, 450 g, MeOH-H_2O, 3 : 7–8 : 2, 30 L), followed by silica gel CC (200–300 mesh, 4 × 50 cm, 80 g, petroleum ether-EtOAc, 5 : 1–1 : 1, 7.5 L) to afford rosestachone (50 mg), roseostachenone (25 mg), and two subfractions, III-a and III-b. Subfraction III-a was chromatographed on silica gel CC (200–300 mesh, 1.0 × 20 cm, 7 g, $CHCl_3$-Me_2CO, 12 : 1–8 : 1, 0.5 L) to yield **5** (15 mg). Subfraction III-b was separated with silica gel CC (200–300 mesh, 1.0 × 25 cm, 10 g, petroleum ether-Me_2CO, 8 : 1–5 : 1, 0.7 L) to afford **1** (32 mg) and **6** (5 mg). Fraction IV (5.3 g) was subjected to MPLC with RP-18 CC (3 × 40 cm, 100 g, MeOH-H_2O, 3 : 7–7 : 3, 6 L) to afford clerod-14-ene-3α,4β,13ξ-triol (1428 mg) and a mixture. The mixture was later separated by silica gel CC (200–300 mesh, 1.5 × 30 cm, 25 g, petroleum ether-Me_2CO, 4 : 1–2 : 1, 1.2 L) to yield **7** (170 mg), (3α,4β,13*E*)-neoclerod-13-ene-3,4,15-triol (80 mg), and one subfraction. The subfraction was further purified by another silica gel CC (200–300 mesh, 1.0 × 20 cm, 8 g, petroleum ether-EtOAc, 4 : 1–2 : 1, 0.6 L) to afford **2** (46 mg). Fraction V (24 g) was isolated with MPLC RP-18 CC (4.9 × 46 cm, 450 g, MeOH-H_2O, 2 : 8–7 : 3, 40 L) to obtain subfractions V-a–Vc. Subfraction V-a was chromatographed on silica gel CC (200–300 mesh, 1.0 × 25 cm, 10 g, $CHCl_3$-Me_2CO, 8 : 1–5 : 1, 0.65 L) to yield 15-hydroxy-3-cleroden-2-one (35 mg), with MeOH-H_2O (4 : 6–6 : 4, 3 L) as the elution solvent. Subfraction V-c was separated with RP-18 CC (3 × 40 cm, 100 g) into clerod-14-ene-3α-acetyl-4β,13ξ-triol (12 mg) and two mixtures, one of which was further purified by a silica gel column (200–300 mesh, 1.5 × 30 cm, 25 g, petroleum ether-Me_2CO, 3 : 1–2 : 1, 0.6 L) to afford **3** (5 mg) and **4** (3 mg).

Cytotoxic assay

Five human cancer cell lines, human myeloid leukemia HL-60, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW480 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 or DMEM medium, supplemented with 10% fetal bovine serum. The cytotoxic assay was performed according to the MTS [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulfopheny)-2H-tetrazolium] method [20]. Briefly, 100 μL of 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 an initial density of 5×10^3–1×10^4 cells/well. Each tumor cell line was exposed to the test compound at a concentration of 40 μm for the primary screening with 200 μL/well and coupling with three reduplicates at 37 °C. Five gradients of compound concentrations were tested in IC_{50} screenings. All compounds (purities > 90%) were evaluated for their cytotoxic activities using cisplatin (Sigma, > 98% purity) as a positive control. Forty-eight hours later, 20 μL MTS

reagent and 100 µL culture solution were added into each well after removing the supernatant. The reaction was extended for 1–4 hours. After compound treatment, cell viability was detected and the cell growth curve was graphed. IC_{50} values were calculated by the Reed and Muench method [24, 25].

Dysoxydensin A (**1**): a yellow amorphous powder; $[\alpha]_D^{19}$ – 6.5 [*c* 0.109, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3428, 2958, 2934, 1701, 1635, 1456, 1384, 1278, 1097, 921 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 322.2509 (calcd. for $C_{20}H_{34}O_3$ [M]$^+$, 322.2508).

Dysoxydensin B (**2**): a white amorphous powder; $[\alpha]_D^{19}$ – 61.0 [*c* 0.108, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3430, 2962, 2877, 1712, 1634, 1456, 1383, 1270, 1197, 1083 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 408.3232 (calcd. for $C_{25}H_{44}O_4$ [M]$^+$, 408.3240).

Dysoxydensin C (**3**): a white amorphous powder; $[\alpha]_D^{19}$ – 55.2 [*c* 0.108, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3429, 2960, 2873, 1695, 1635, 1456, 1386, 1229, 1171, 1017 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 434.3389 (calcd. for $C_{27}H_{46}O_4$ [M]$^+$, 434.3396).

Dysoxydensin D (**4**): yellow syrup; $[\alpha]_D^{19}$ – 33.6 [*c* 0.203, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3424, 2951, 2924, 2870, 1634, 1456, 1385, 1370, 1100, 1074, 1018, 975, 921 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 338.2821 (calcd. for $C_{21}H_{38}O_3$ [M]$^+$, 338.2821).

Dysoxydensin E (**5**): yellow syrup; $[\alpha]_D^{20}$ – 82.1 [*c* 0.137, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3441, 2963, 2927, 1640, 1452, 1382, 1081, 999, 917 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [see **Tables 1** and **2**, respectively; HREIMS *m/z* 320.2708 (calcd. for $C_{21}H_{36}O_2$ [M]$^+$, 320.2715).

Dysoxydensin F (**6**): yellow syrup; $[\alpha]_D^{19}$ – 73.6 [*c* 0.147, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3440, 2961, 2927, 1659, 1642, 1451, 1382, 1084, 1000, 984 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 320.2724 (calcd. for $C_{21}H_{36}O_2$ [M]$^+$, 320.2715).

Dysoxydensin G (**7**): yellow syrup; $[\alpha]_D^{20}$ – 17.2 [*c* 0.125, $(CH_3)_2CO$]; IR (KBr) ν_{max} 3430, 2950, 2923, 1633, 1454, 1384, 1107, 1048, 1020, 998, 978, 909 cm^{-1}; ^1H (400 MHz) and ^{13}C NMR (150 MHz) data [$(CD_3)_2CO$], see **Tables 1** and **2**, respectively; HREIMS *m/z* 306.2552 (calcd. for $C_{20}H_{34}O_2$ [M]$^+$, 306.2559).

Supporting information

1D and 2D NMR (HSQC, HMBC, ROESY) and MS spectra of dysoxydensins A–G (**1**–**7**) are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

References

1 *Aladesanmi AJ, Kelley CJ, Leary JD.* Two diterpenes and acetophenone from *Dysoxylum lenticellare.* Planta Med 1986; 52: 76
2 *Aladesanmi AJ.* The stem constituents of *Dysoxylum lenticellare.* Tetrahedron 1988; 44: 3749–3756
3 *Mulholland DA, Monkhe TV, Pegel KH, Taylor DAH.* Limonoids and diterpenoids from *Dysoxylum spectabile* (Meliaceae). Biochem Syst Eco 1999; 27: 313–315
4 *Luo XD, Wu SH, Ma YB, Wu DG. ent*-Pimarane derivatives from *Dysoxylum hainanense.* Phytochemistry 2001; 57: 131–134
5 *Climati E, Mastrogiovanni F, Valeri M, Salvini L, Bonechi C, Mamadalieva NZ, Egamberdieva D, Taddei AR, Tiezzi A.* Methyl carnosate, an antibacterial diterpene isolated from *Salvia officinalis* leaves. Nat Prod Commun 2013; 8: 429–430
6 *Santos AO, Izumi E, Ueda-Nakamura T, Dias-Filho BP, Veiga-Júnior VF, Nakamura CV.* Antileishmanial activity of diterpene acids in copaiba oil. Mem Inst Oswaldo Cruz 2013; 108: 59–64
7 *Tian W, Chen SY.* Recent advances in the molecular basis of anti-neoplastic mechanisms of oridonin. Chin J Integr Med 2013; 19: 315–320
8 *Fujioka T, Yamamoto M, Kashiwada Y, Fujii H, Mihashi K, Ikeshiro Y, Chen IS, Lee KH.* Novel cytotoxic diterpenes from the stem of *Dysoxylum kuskusense.* Bioorg Med Chem Lett 1998; 8: 3479–3482
9 *Duh CY, Wang SK, Chen IS.* Cytotoxic prenyleudesmane diterpenes from the fruits of *Dysoxylum kuskusense.* J Nat Prod 2000; 63: 1546–1547
10 *Nakanishi K, Doi M, Usami Y, Amagata T, Minoura K, Tanaka R, Numata A, Yamada T.* Anthcolorins A–F, novel cytotoxic metabolites from a sea urchin-derived *Aspergillus versicolor.* Tetrahedron 2013; 69: 4617–4623
11 *Wang WX, Zhu JJ, Zou Y, Hong ZL, Liu ST, Li M, Huang Y, Xiong J, Zhao Y, Yang GX, Xia G, Hu JF.* Trichotomone, a new cytotoxic dimeric abietane-derived diterpene from *Clerodendrum trichotomum.* Tetrahedron Lett 2013; 54: 2549–2552
12 *Xu Z, Yan S, Bi K, Han J, Chen Y, Wu Z, Liu H.* Isolation and identification of a new anti-inflammatory cyathane diterpenoid from the medicinal fungus *Cyathus hookeri* Berk. Fitoterapia 2013; 86: 159–162
13 *Tiwari N, Thakur J, Saikia D, Gupta MM.* Antitubercular diterpenoids from *Vitex trifolia.* Phytomedicine 2013; 20: 605–610
14 *Yang L, Qiao L, Ji C, Xie D, Gong NB, Lu Y, Zhang J, Dai J, Guo S.* Antidepressant abietane diterpenoids from Chinese eaglewood. J Nat Prod 2013; 76: 216–222
15 *Zainobiddinov AE, Khushmatov SS, Salimov BT, Usmanov PB.* Antiarrythmic and inotropic activity of diterpenoid alkaloids of atisine 15-acetoxyazomethine and atisine 15-hydroxyazomethine. O'zb Resp Fanlar Akad Ma'ruzalari 2012; 3: 57–60
16 *Fazio C, Passannanti S, Paternostro MP, Piozzi F.* Neo-clerodane diterpenoids from *Stachys rosea.* Phytochemistry 1992; 31: 3147–3149
17 *Meragelman KM, Espinar LA, Sosa VE, Uriburu ML, de la Fuente JR.* Terpenoid constituents of *Viguiera tucumanensis.* Phytochemistry 1996; 41: 499–502
18 *Yang SM, Wu SH, Qin XD, Luo XD, Wu DG.* Neoclerodane diterpenes from *Amoora stellato-squamosa.* Helv Chim Acta 2004; 87: 1279–1286
19 *Dos SAG, Perez CC, Tininis AG, Bolzani VS, Cavalheiro AJ.* Clerodane diterpenes from leaves of *Casearia sylvestris* Swartz. Quim Nova 2007; 30: 1100–1103
20 *Malich G, Markovic B, Winder C.* The sensitivity and specificity of the MTS tetrazolium assay for detecting the *in vitro* cytotoxicity of 20 chemicals using human cell lines. Toxicology 1997; 124: 179–192
21 *Prakash CVS, Hoch JM, Kingston DGI.* Structure and stereochemistry of new cytotoxic clerodane diterpenoids from the bark of *Casearia lucida* from the Madagascar rainforest. J Nat Prod 2002; 65: 100–107
22 *Shen YC, Wang CH, Cheng YB, Wang LT, Guh JH, Chien CT, Khalil AT.* New cytotoxic clerodane diterpenoids from the leaves and twigs of *Casearia membranacea.* J Nat Prod 2004; 67: 316–321
23 *Shen YC, Cheng YB, Ahmed AF, Lee CL, Chen SY, Chien CT, Kuo YH, Tzeng GL.* Cytotoxic clerodane diterpenoids from *Casearia membranacea.* J Nat Prod 2005; 68: 1665–1668
24 *Zamoiskii EA.* Evaluation of Reed-Muench method in determination of activity of biological preparations. Zh Mikrobiol Epidemiol Immunobiol 1956; 27: 77–83
25 *Stanic M.* A simplification of the estimation of the 50 percent endpoints according to the Reed and Muench method. Pathol Microbiol (Basel) 1963; 26: 298–302