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Diterpenoids and phenanthrenones from the leaves and stems of *Strophioblachia fimbricalyx*

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ABSTRACT

Five new compounds, namely fimbricalyxoids A–D (**1**–**4**) and 13-*O*-methylfimbricalyx B (**5**), together with five known compounds (**6**–**10**) were isolated from the leaves and stems of *Strophioblachia fimbricalyx*. Fimbricalyxoid A (**1**), a cleistanthane diterpenoid, possesses a rare 3,20-oxybridge, while fimbricalyxoids C–D (**3**–**4**) are the first *seco*-ring-A pentanorditerpenoids. Their structures and absolute configuration were established based on NMR and MS data and ECD calculation (**1**). Compounds **1**, **6**, and **7** exhibited cytotoxicity against five human tumor cell lines with IC_{50} values in the range of 1.4–8.2 μM.

The wide distribution and exposure of the Euphorbiaceae family to various environmental conditions induce its species to produce a large variety of secondary metabolites for survival/defense and make them a fertile source for drug discovery.[1] Wide ethnomedicinal uses have been recorded for its species in the treatment of diseases such as gonorrhea, arthritis, asthma, cancers and warts,[2] and fascinating structurally diversified isoprenoid natural products as well as a wide range of biological activities have been reported.[2,3] Euphorbiaceous diterpenoids, in particular, are among the most complex isoprenoid natural products with pronounced biological properties in this family and have attracted increasing interests from both phytochemists and organic synthetic chemists.[2,4–6] *Strophioblachia* is a small genus of Euphorbiaceae and only two species are distributed in eastern Asian.[7] *Strophioblachia fimbricalyx* is distributed widely in the south of Hainan, Guangxi and Yunnan provinces, China. Its roots are commonly used by the ethnic Thai people to treat migraine, fever, and cancer.[8,9] Previous phytochemical studies showed the presence of megastigmane glucosides, flavone glycosides, phenanthrenone, and phenanthropolones, and some of these compounds have been demonstrated to be antiplasmodial and cytotoxic agents.[8–11] In the search for biologically active components from *S. fimbricalyx*, the leaves and stems were investigated. As a result, four new diterpenoids and one phenanthrenone, along with five known compounds were isolated. Their structures were established by extensive spectroscopic analysis and the cytotoxic activities of all compounds against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) were evaluated using the MTS method. Herein we reported the extraction, isolation, structural elucidation, and cytotoxicity of these compounds (Fig. 1).

Compound **1** was obtained as a white powder. $[\alpha]^{25}_{D}$ 13 (*c* 0.02, MeOH). Its molecular formula, $C_{20}H_{26}O_3$, was established by the HRESIMS peak at *m*/*z* 337.1775 $[M+Na]^+$ (calcd for $C_{20}H_{26}O_3Na$, 337.1774), indicating 8 degrees of unsaturation. The IR spectrum showed the presence of hydroxy (3404 cm^{-1}) and conjugated aromatic (1588 cm^{-1}) and olefinic (1629 cm^{-1}) groups. ^1H NMR (Table 1) showed, in addition to signals for a terminal vinyl [δ_H 6.60 (dd, J = 17.9, 11.5 Hz; H-15), δ_H 5.55 (d, J = 11.5 Hz; H-16a), and δ_H 5.16 (d, J = 17.9 Hz; H-16b)], easily recognized resonances for three methyls (δ_H 1.03, 1.11, and 2.17; all s) and an aromatic proton (δ_H 6.68, s). The DEPT and HSQC spectra revealed 20 carbon signals assignable to three methyls, six methylenes (one sp^2), three methines (two sp^2), and eight quaternary carbons (five sp^2). The aforementioned data indicated four carbon–carbon double bonds and accounted for four of the eight degrees of unsaturation. The

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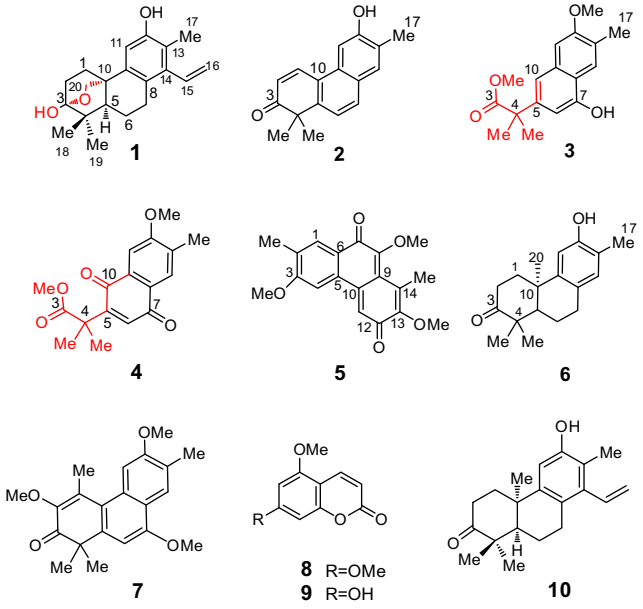

Figure 1. Structures of isolated compounds **1**–**10**.

remaining four required compound **1** to be tetracyclic. A comparison of the ^{1}H and ^{13}C data of **1** (Table 1) with those of sonderianol[12] indicated that they shared a similar skeleton, with the marked differences being the replacement of signals for a carbonyl and a methyl by those for an oxygenated quaternary carbon (δ_C 99.5) and an oxymethylene [δ_H 4.01 (d, J = 9.2 Hz); 4.12 (d, J = 9.2 Hz); δ_C 72.7]. It was likely that the original carbonyl was transformed to a hemiketal or a ketal group, while one of the methyls in sonderianol was oxidized to an oxymethylene. Besides, an additional ring should be present between the newly generated groups since both compounds shared the same degree of unsaturation. Detailed analysis of the 2D NMR spectra further revealed the detail of the structure for **1**. The HMBC correlations (Fig. 2) from H-15 to C-13 (δ_C 120.8), C-14 (δ_C 139.5) and C-8 (δ_C 127.8), as well as those from H-11 to C-12 (δ_C 152.3), C-13 (δ_C 120.8) and C-9, not only confirmed the presence of an oxyphenyl conjugated with a terminal vinyl group in the skeleton, but also located the oxygen-bearing substituent and the vinyl group at C-12 and C-14, respectively. The methyl at δ_H 2.17 (s) and δ_C 13.1 was located at C-13 on the basis of its HMBC correlations from Me-17 to C-13 (δ_C 120.8), C-12 (δ_C 152.3), and C-14 (δ_C 139.5) (Fig. 2). The two oxymethylene protons [δ_H 4.01 (d, J = 9.2 Hz); 4.12 (d, J = 9.2 Hz, δ_C 72.7); H_2-20)] showed HMBC correlations with the hemiketal carbon (δ_C 99.5, C-3), suggesting the presence of an ether linkage between C-20 and C-3. The ROESY correlations of H-5/H_2-20 and H_3-19 suggested these protons and the 3,20-oxy-bridge to be α-oriented. The absolute configuration of compound **1** was determined by quantum chemical TDDFT calculation of its theoretical ECD spectrum. In the 200–500 nm region, both the experimental and theoretical ECD spectra of **1** showed first negative and second double positive Cotton effects. Therefore, qualitative analysis of the calculated and experimental ECD spectra allowed the assignments of the absolute configuration of **1** as 3*R*,5*R*,10*S*. (Fig. 3 and Supplementary data S1-10, S1-11). The

Table 1
^{1}H and ^{13}C NMR spectra data of compounds **1**–**4** (δ in ppm, J in Hz)

Position	1		2		3		4	
	δ_H[a]	δ_C[b]	δ_H[a]	δ_C[b]	δ_H[c]	δ_C[d]	δ_H[e]	δ_C[f]
1a	2. 41[g]	35.3	8.22 d (10.1)	139.7				
1b	1.67[g]							
2a	2.26 dt (17.8, 6.1)	29.6	6.31 d (10.1)	123.7				
2b	1.91 t (12.2)							
3		99.5		205.3		179.3		176.0
4		40.5		48.1		47.9		44.4
5	1.60[g]	48.0		147.1		143.8		153.5
6a	1.85 d (10.3)	20.9	7.40 d (8.6)	121.9	7.16 br s	115.1	6.77	133.3
6b	1.60[g]							
7a	2.84 d (17.0)	29.1	7.75 d (8.6)	130.2		154.2		185.0
7b	2.40[g]							
8		127.8		127.9		120.3		125.1
9		137.0		130.9		136.5		132.5
10		36.5		121.6	6.62 d (1.6)	105.8		184.6
11	6.68 s	112.0	7.53 s	104.2	7.08 s	105.7	7.43 s	107.0
12		152.3		154.6		158.9		162.5
13		120.8		126.7		127.9		133.8
14		139.5	7.61 s	130.1	7.83 s	124.0	7.83 s	128.7
15	6.60 dd (17.9, 11.5)	135.4						
16a	5.55 d (11.5)	120.2						
16b	5.16 d (17.9)							
17	2.17 s	13.1	2.44 s	16.4	2.33 s	17.3	2.31 s	16.7
18	1.11 s	27.2	1.52 s	28.0	1.60 s[g]	27.1[g]	1.49 s[g]	25.2[g]
19	1.03 s	18.1	1.52 s	28.0	1.60 s[g]	27.1[g]	1.49 s[g]	25.2[g]
20a	4.01 d (9.2)	72.7						
20b	4.12 d (9,2)							
OMe-3					3.61 s	52.8	3.67 s	52.5
Ome-12					3.88 s	55.9	4.00 s	56.1

[a] Measured in $CDCl_3$ at 600 MHz.
[b] Measured in $CDCl_3$ at 150 MHz.
[c] Measured in CD_3OD at 600 MHz.
[d] Measured in CD_3OD at 150 MHz.
[e] Measured in $CDCl_3$ at 800 MHz.
[f] Measured in $CDCl_3$ at 200 MHz.
[g] Overlapped by other signal.

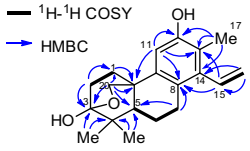

Figure 2. ^{1}H–^{1}H COSY (bold bond) and selected HMBC correlation of **1**.

structure of compound **1** was thus established as depicted and named fimbricalyxoid A (Fig. 1).

Compound **2** was obtained as a white powder with a molecular formula of $C_{17}H_{16}O_2$ on the basis of HRESIMS at *m/z* 253.1224 [M+H]$^+$ (calcd for $C_{17}H_{17}O_2$, 253.1229), showing two mass units less than that of flueggrene A.[13] The IR absorptions at 3381 and 1709 cm^{-1} indicated the presence of hydroxy and carbonyl functionalities, respectively. The ^{1}H NMR data of **2** (Table 1) showed signals assignable to six aromatic protons [(δ_H 8.22, d, *J* = 10.1 Hz (H-1); 6.31, d, *J* = 10.1 Hz (H-2); 7.40, d, *J* = 8.6 Hz (H-6); 7.75, d, *J* = 8.6 Hz (H-7); 7.53, s (H-11); 7.61, s (H-14)] and three methyls (δ_H 2.44, s; 1.52, s; 1.52, s), while in the ^{13}C NMR (with DEPT) spectrum, all 17 carbons were well-resolved and were classified as three methyls, six methines, and seven quaternary carbons. The NMR data of compound **2** were very similar to those of flueggrene A,[13] and the differences were, except for the upfield shift of the carbonyl signal ($\Delta\delta_C$ −9.7), the presence of signals for a $\Delta^{1,2}$ double bond [(δ_C 139.7 (C-1), 123.7 (C-2); and δ_H 8.22 d, *J* = 10.1 Hz (H-1), 6.31, d, *J* = 10.1 Hz (H-2)] and the absence of signals for two methylenes [(δ_H 3.38, t, *J* = 6.8 Hz, δ_C 25.4 (CH_2-1); δ_H 2.84, t, *J* = 6.8 Hz, δ_C 36.4 (CH_2-2)] in compound **2**.[13] These observations suggested **2** to be the 1, 2-didehydro derivative of flueggrene A, which was further supported by its 2D NMR (HSQC, ^{1}H–^{1}H COSY and HMBC) data (Figs. 4 and S2). Compound **2** was therefore structurally established and named fimbricalyxoid B.

Compound **3** was isolated as a white powder with a molecular formula of $C_{17}H_{20}O_4$ on the basis of its ^{13}C NMR data and the HRESIMS at *m/z* 289.1436 [M+H]$^+$ (calcd for $C_{17}H_{21}O_4$, 289.1440), corresponding to eight degrees of unsaturation. The IR spectrum suggested the presence of carbonyl (1706 cm^{-1}) and hydroxy (3438 cm^{-1}) functionalities. Its ^{1}H and ^{13}C NMR (with DEPT) data (Table 1) showed typical signals for a 2, 3, 5, 7- or 2, 3, 6, 8-tetrasubstituted naphthalene system [δ_H 6.62 (d, *J* = 1.6 Hz) and 7.16 (br s)

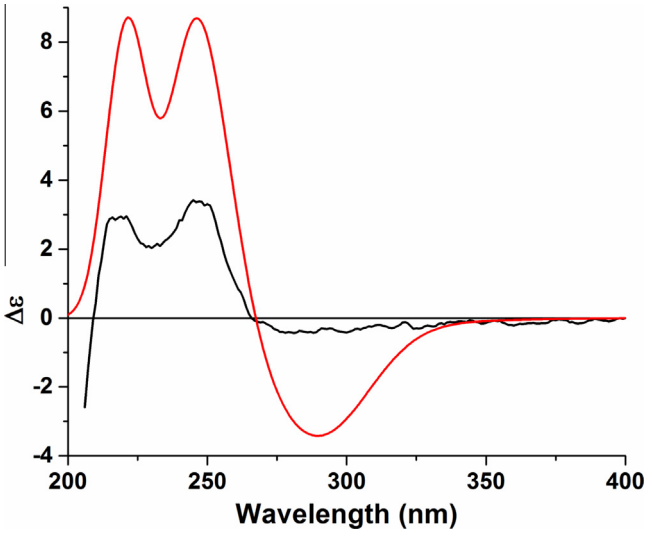

Figure 3. Experimental (black color) and calculated (red color) ECD spectra for compounds **1**.

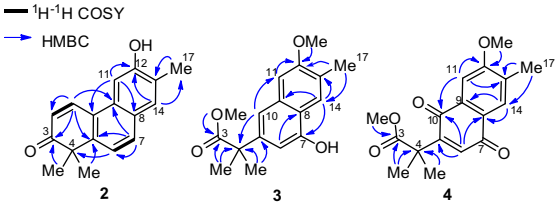

Figure 4. ^{1}H–^{1}H COSY (bold bond) and selected HMBC correlations of **2**, **3** and **4**.

for one ring and δ_H 7.08 (s) and 7.83 (s) for the other; δ_C 105.8, 115.1, 105.7, and 124.0, all aromatic carbons]. Two downfield carbon signals at δ_C 147.1 and 154.6 indicated a dioxysubstituted property of the naphthalene system. Besides, signals for a carboxylic/ester carbon [δ_C 179.3 (C-3)], two methoxyls [(δ_H 3.61, s, δ_C 52.8 (OMe-3); and δ_H 3.88, s, δ_C 55.9 (OMe-12)], three tertiary methyls [δ_H 1.60 (6H, s) and 2.33 (3H, s); δ_C 27.1 (2C) and 17.3 (CH_3-17, 18 and 19)], and one quaternary carbon (δ_C 47.9, C-4) were also observed. These observations, when compared with those of **2**, suggested that both compounds possessed similar naphthalene system and compound **3** seemed to be the 2,3:1,10-diseco derivative of **2** due to the lack of the $\Delta^{1,2}$ double bond or its counterpart in the structure. Analysis of the 2D NMR (HSQC, HMBC) data not only confirmed this conclusion, but also revealed the structural detail for **3** (Fig. 4). In particular, HMBC correlations from H_3-17 to C-12, C-14 and from H-14 to C-7 attached the two oxysubstituents to C-12 and C-7, while those from the methoxy group at δ_H 3.61 (s) to C-3 and from that at δ_H 3.88 (s) to C-12 located the two methoxy groups at C-3 and C-12, respectively. Correlations from the proton at δ_H 6.62 to C-4 and C-11 suggested that this proton was at C-10. Thus, the structure of **3** was elucidated as depicted, and this compound was named fimbricalyxoid C. Compound **3** was the first *seco*-A-ring pentanorditerpene.

Compound **4** was isolated as a white powder with a molecular formula of $C_{17}H_{16}O_5$ on the basis of its ^{13}C NMR data and the HRESIMS at *m/z* 325.1049 [M+Na]$^+$ (calcd for $C_{17}H_{16}O_5Na$, 325.1046). The ^{1}H and ^{13}C NMR data of **4** (Table 1) were very similar to those of **3**. However, signals for C-7 (δ_C 154.2) and CH-10 (δ_H 6.62, d, *J* = 1.6 Hz; δ_C 105.8) in **3** were replaced by two carbonyl carbon signals at δ_C 185.0 (C-7) and 184.6 (C-10) in **4**, which suggested that **4** was a 7,10-diketone derivative of **3**. Further 2D NMR (HSQC and HMBC) spectral analysis confirmed this assignment (Fig. 4) and established the structure of **4** as shown (Fig. 1). Compound **4** was named fimbricalyxoid D.

Compound **5** was isolated as a red powder with a molecular formula of $C_{19}H_{18}O_5$ on the basis of its ^{13}C NMR data and the HRESIMS at *m/z* 349.1046 [M+Na]$^+$ (calcd for $C_{19}H_{18}O_5Na$, 349.1052). Its ^{1}H and ^{13}C NMR (Table 2) data were characteristic of a phenanthrenone and was very similar to those of fimbricalyx B,[9] with the only difference being the presence of signals for an additional

Table 2
^{1}H (600 MHz) and ^{13}C (125 MHz) NMR data of compound **5** in $CDCl_3$ (δ in ppm, *J* in Hz)

Position	δ_H	δ_C	Position	δ_H	δ_C
1	7.88 s	132.3	11	7.24 s	105.1
2		128.9	12		182.6
3		164.2	13		148.4
4	7.15 s	104.3	14		123.8
5		137.0	Me-2	2.23 s	16.0
6		124.2	Me-14	2.62 s	14.5
7		180.7	OMe-3	4.04 s	55.8
8		157.9	OMe-8	4.07 s	56.1
9		139.5	OMe-13	3.81 s	60.8
10		134.9			

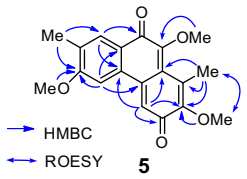

Figure 5. Key HMBC and ROESY correlations of **5**.

Table 3
Cytotoxic activity of compounds **1**, **6** and **7** (IC_{50}, μM)

Compound	HL-60	SMMC-7721	A-549	MCF-7	SW480
1	3.7	4.3	4.5	3.1	8.2
6	3.0	3.9	6.3	3.4	3.4
7	–	2.7	3.5	2.6	1.4
Cisplatin[a]	3.5	15.2	12.1	17.2	9.5

[a] Positive control.

methoxy group. Downfield shift of the signal for the oxidized quaternary carbon (C-13, δ_C from 145.5 to148.4) suggested that this methoxy group was located at C-13, which was further supported by the HMBC correlation from OCH_3-13 (δ_H 3.81,s) to C-13 (δ_C 148.4) and ROESY correlation from OCH_3-13 (δ_H 3.81, s) to CH_3-14 (δ_H 2.62 s) (Fig. 5). Thus, compound **5** was established as 13-*O*-methyl fimbricalyx B (Fig. 1).

Five known compounds were identified to be 12-hydroxy-13-methylpodocarpa-9,11,13-trien-3-one (**6**),[14] trigonostemon (**7**),[10] limettin (**8**),[15] 5-methoxy-7-hydroxycoumarin (**9**),[16] and sonderianol (**10**)[12] by comparing their spectroscopic data with those in the literature.

All compounds isolated were evaluated for their cytotoxic activity against human myeloid leukemia (HL-60), hepatocellular carcinoma (SMMC-7721), lung cancer (A-549), breast cancer (MCF-7), and colon cancer (SW480) cell lines with the MTT method.[17] Compounds **1**, **6** and **7** displayed cytotoxic activity with IC_{50} values ranging from 1.4 to 8.2 μM (for cisplatin, the value were 3.5 to 17.2 μM) against the five human tumor cell line (Table 3). Whereas, the other compounds were inactive (IC_{50} >40 μM) in the assay.

Acknowledgments

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Supplementary data

Supplementary data (general experimental procedures, NMR and HRESIMS, IR, UV, CD (**1**) spectra and ECD calculation (**1**) of new compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.04.034.

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