Ingol and Ingenol Diterpenes from the Aerial Parts of *Euphorbia royleana* and Their Antiangiogenic Activities

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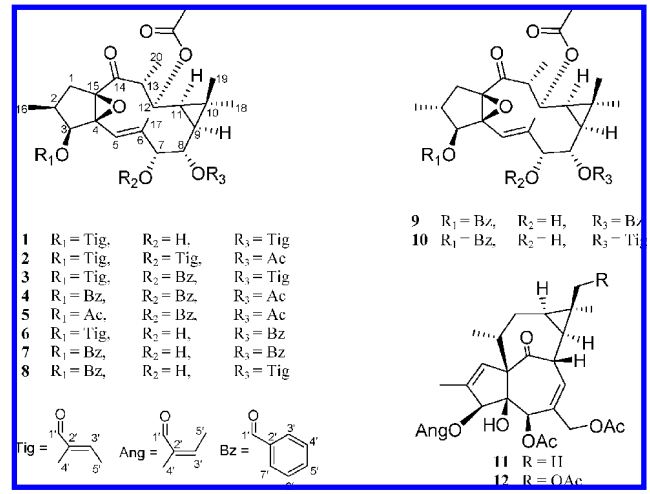
Received December 24, 2008

Ten new ingol lathyrane-type diterpenes (**1**−**10**) and two known ingenol derivatives (**11** and **12**) were isolated from the aerial parts of *Euphorbia royleana*. The structures of **1**−**10** were elucidated on the basis of spectroscopic methods including 2D NMR analysis, and the structure of compound **1** was confirmed by single-crystal X-ray crystallography. Antiangiogenic effects of all compounds except for **5** were tested using a zebrafish model, with compounds **11** and **12** being active in this bioassay.

Euphorbia is the largest genus of the family Euphorbiaceae, with there being over 1000 species worldwide and about 80 species in mainland China. According to a literature survey, diterpenoids are one of the main types of chemical components of *Euphorbia*,[1] and some of these exhibit cytotoxic activity,[2,3] antiviral activity,[4] and a survival effect on TrkA fibroblasts.[5] *Euphorbia royleana* Boiss. is a common thorny succulent species distributed in southwestern mainland China, which has been used traditionally as a fencing plant by local villagers in dry and hot valleys. Previous chemical studies have shown that the main chemical constituents of this species are triterpenoids and diterpenoids.[6−10] Recent bioactivity investigations on *E. royleana* have shown anti-inflammatory,[11] piscicidal,[12] and antiacetylcholinesterase[12] activities, as well as immunosuppressive effects.[13] Aiming to search for potential bioactive constituents, we have investigated the aerial parts of *E. royleana*. Ten new ingol esters, **1**−**10**, together with two known ingenol esters, ingenol 3-angelate 5,20-diacetate (**11**)[14] and 5,17,20-triacetyl-3-*O*[(*Z*)-2-methyl-2-butenoyl]-17-hydroxyingenol (**12**),[15,16] were isolated. This paper describes the separation and structure elucidation of these new ingol esters and reports the antiangiogenic activities of compounds **1**−**4** and **6**−**12** using a zebrafish model.



Results and Discussion

Compound **1** was obtained as colorless crystals from methanol, with mp 96−97 °C and $[\alpha]^{27}_{D}$ +14.8 (*c* 0.2, MeOH). Its HRESIMS indicated the molecular formula $C_{32}H_{44}O_9$, as determined by the molecular ion peak at *m/z* 595.2896 $[M + Na]^+$ (calcd 595.2883), corresponding to 11 degrees of unsaturation. IR absorption bands at 3506, 2937, 1716, and 1652 cm^{-1} implied the presence of hydroxy, methylene, carbonyl, and olefinic groups, respectively. The ^{13}C NMR spectrum displayed 32 carbon signals, which could be assigned to a diterpenoid with one acetyl (δ_C 170.5, 21.0) and two tiglyl groups (one at δ_C 167.5, 138.3, 128.3, 14.5, 12.0, and the other at δ_C 167.4, 138.2, 128.0, 14.5, 12.0) (Tables 1 and 3). The diterpene part was found to possess five methyls (two secondary, two tertiary, and one vinylic), nine methines (four oxygenated and an olefinic carbon), four quaternary carbons (two oxygenated), one carbonyl, and one methylene. On considering that lathyrane-type diterpenes are major chemical constituents of this plant, we compared the 1D NMR data of **1** with those of the known compound 8-*O*-benzoylingol 3,12-diacetate.[17] The ^1H and ^{13}C NMR data of these two compounds were very close, except for the different types of ester residues. The two tiglyl groups were located in **1** at C-3 and C-8 by HMBC correlations from H-3 (δ_H 5.27, d, J = 8.4 Hz) and H-8 (δ_H 4.56, dd, J = 1.5, 10.6 Hz) to the tiglyl carbonyls (δ_C 167.5 and 167.4), respectively. The HMBC correlation between H-12 (δ_H 4.87, dd, J = 3.9, 11.0 Hz) and the acetyl carbonyl (δ_C 170.5) was used to establish the location of the latter group at C-12 (Figure 1).

The relative configuration of **1** was established by a ROESY NMR experiment (Figure 2) and confirmed by single-crystal X-ray crystallography (Figure 3). ROESY correlations of Me-19 with H-8 and H-12 showed that H-8 and H-12 are located on the same side of the molecule as Me-19. They were further determined to be β-oriented by an X-ray diffraction experiment, and α-orientations were determined for the tiglyl group at C-8 and the acetyl group at C-12. The epoxide ring, Me-16, and tiglyl group on C-3 were also determined as β-oriented by X-ray diffraction. Moreover, the $J_{2,3}$ values of **1** were 8.4 Hz, consistent with a *cis*-configuration.[14] Thus, compound **1** was determined as 12-*O*-acetylingol 3,8-ditiglate.

Compound **2** was isolated as a white powder with $[\alpha]^{27}_{D}$ +21.4 (*c* 0.4, MeOH). Its molecular formula was determined as $C_{34}H_{46}O_{10}$ by HRESIMS (*m/z* 637.2978 $[M + Na]^+$). The ^1H and ^{13}C NMR data of **2** were very similar to those of **1**, except for the signals of an additional acetyl group. This showed that **2** is a tetrasubstituted

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10.1021/np800816n CCC: $40.75
Published on Web 05/13/2009

Table 1. ^{1}H NMR Asignments of Compounds **1**−**5**[a]

position	**1**	**2**	**3**	**4**	**5**
1α	2.84 (dd, 9.2, 14.9)	2.78 (dd, 9.2, 14.9)	2.81 (dd, 9.1, 14.9)	2.88 (dd, 9.1, 14.9)	2.79 (dd, 9.1, 15.0)
1β	1.69 (d, 14.9)	1.67 (d, 14.9)	1.69 (d, 14.9)	1.76 (d, 14.9)	1.69 (d, 15.0)
2	2.60 (m)	2.50 (m)	2.53 (m)	2.66 (m)	2.50 (m)
3	5.27 (d, 8.4)	5.21 (d, 8.5)	5.18 (d, 8.4)	5.40 (d, 8.5)	5.17 (d, 8.5)
5	5.82 (br s)	5.57 (br s)	5.73 (br s)	5.82 (br s)	5.71 (br s)
7	4.27 (br s)	5.22 (br s)	5.46 (br s)	5.48 (br s)	5.46 (br s)
8	4.56 (dd, 1.5, 10.6)	4.56 (dd, 1.5, 10.5)	4.74 (dd, 1.5, 10.8)	4.68 (dd, 1.5, 10.9)	4.68 (dd, 1.7, 10.7)
9	1.45 (t, 10.6)	1.21 (t, 10.5)	1.44 (t, 10.8)	1.39 (t, 10.9)	1.39 (t, 10.7)
11	1.14 (overlap)	1.12 (dd, 9.0, 11.0)	1.22 (dd, 9.0, 11.0)	1.24 (dd, 9.0, 11.0)	1.21 (dd, 9.0, 11.0)
12	4.87 (dd, 3.9, 11.0)	4.85 (dd, 3.9, 11.0)	4.93 (dd, 3.9, 11.0)	4.94 (dd, 3.9, 10.9)	4.92 (dd, 3.9, 11.0)
13	2.93 (m)	2.92 (m)	3.02 (m)	3.04 (m)	2.98 (m)
16	0.89 (3H, d, 7.4)	0.88 (3H, d, 7.5)	0.90 (3H, d, 7.4)	0.98 (3H, d, 7.4)	0.91 (3H, d, 7.4)
17	2.07 (3H, s)	2.08 (3H, s)	2.20 (3H, s)	2.21 (3H, s)	2.18 (3H, s)
18	1.08 (3H, s)	1.07 (3H, s)	1.13 (3H, s)	1.13 (3H, s)	1.13 (3H, s)
19	0.83 (3H, s)	0.82 (3H, s)	0.84 (3H, s)	0.88 (3H, s)	0.88 (3H, s)
20	1.07 (3H, d, 7.2)	1.04 (3H, d, 7.3)	1.10 (3H, d, 7.3)	1.13 (3H, d, 6.8)	1.09 (3H, d, 7.3)
OAc	2.10 (3H, s)	2.07 (3H, s)	2.11 (3H, s)	2.12 (3H, s)	2.12 (3H, s)
		1.97 (3H, s)		2.00 (3H, s)	2.16 (3H, s)
					2.02 (3H, s)
OTigl	6.90 (m)	6.82 (2H, m)	6.81 (2H, m)		
	1.84 (6H, s)	1.80 (6H, s)	1.78 (6H, s)		
	1.80 (6H, d, 7.0)	1.77 (6H, d, 6.5)	1.75 (6H, d, 7.5)		
OBz			7.80 (2H, d, 7.4)	7.98 (4H, d, 7.9)	8.04 (2H, d, 7.1)
			7.58 (t, 7.5)	7.58 (2H, t, 7.4)	7.61 (t, 7.4)
			7.47 (2H, t, 7.4)	7.45 (4H, t, 7.4)	7.48 (2H, t, 7.8)

[a] Spectra were recorded in $CDCl_3$, and chemical shifts (δ) are in ppm with J values in Hz.

Table 2. ^{1}H NMR Assignments of Compounds **6**−**10**[a]

position	**6**	**7**	**8**	**9**	**10**
1α	2.86 (dd, 9.3, 15.0)	2.92 (dd, 9.2,15.0)	2.90 (dd, 9.3, 15.0)	2.19 (2H, m)	2.18 (2H, m)
1β	1.71 (d, 15.0)	1.77 (d, 15.0)	1.75 (d, 15.0)		
2	2.60 (m)	2.70 (m)	2.70 (m)	2.05 (overlap)	2.05 (m)
3	5.29 (d, 8.4)	5.50 (d, 8.4)	5.48 (d, 8.4)	5.37 (d, 8.2)	5.36 (d, 8.2)
5	5.87 (br s)	5.92 (br s)	5.87 (br s)	5.96 (br s)	5.90 (br s)
7	4.38 (br s)	4.36 (br s)	4.25 (br s)	4.30 (br s)	4.19 (br s)
8	4.77 (dd, 1.5, 10.3)	4.78 (dd, 1.5, 10.5)	4.57 (dd, 1.5, 10.5)	4.75 (dd, 1.5, 10.3)	4.55 (dd, 1.5, 10.4)
9	1.56 (t, 10.3)	1.56 (overlap)	1.46 (t, 10.5)	1.56 (overlap)	1.48 (t, 10.4)
11	1.18 (overlap)	1.20 (overlap)	1.15 (overlap)	1.20 (overlap)	1.13 (overlap)
12	4.92 (dd, 3.9, 11.0)	4.94 (dd, 3.8, 11.0)	4.89 (dd, 3.9, 11.0)	4.93 (dd, 3.9, 11.0)	4.88 (dd, 3.9, 10.7)
13	2.98 (m)	3.00 (m)	2.96 (m)	2.98 (m)	2.95 (m)
16	0.91 (3H, d, 7.4)	0.99 (3H, d, 7.5)	0.97 (3H, d, 7.4)	1.17 (overlap)	1.13 (3H, d, 7.4)
17	2.12 (3H, s)	2.16 (3H, s)	2.12 (3H, s)	2.06 (3H, s)	2.02 (3H, s)
18	1.14 (3H, s)	1.14 (3H, s)	1.09 (3H, s)	1.14 (overlap)	1.09 (3H, s)
19	0.85 (3H, s)	0.84 (3H, s)	0.83 (3H, s)	0.85 (3H, s)	0.82 (3H, s)
20	1.10 (3H, d, 7.3)	1.12 (3H, d, 7.4)	1.10 (3H, d, 7.7)	1.10 (overlap)	1.08 (3H, d, 7.7)
OAc	2.10 (3H, s)	2.10 (3H, s)	2.12 (3H, s)	2.10 (3H, s)	2.11 (3H, s)
OTigl	6.92 (m)		6.89 (m)		6.88 (m)
	1.84 (3H, s)		1.83 (3H, s)		1.82 (3H, s)
	1.80 (3H, d, 7.2)		1.80 (3H, d, 7.3)		1.79 (3H, d, 7.1)
OBz	8.05 (2H, d, 7.6)	8.05 (4H, d, 7.3)	8.05 (2H, d, 7.6)	8.05 (4H, d, 7.4)	8.05 (2H, d, 7.1)
	7.59 (t, 7.4)	7.58 (2H, m)	7.58 (t, 7.4)	7.58 (2H, t, 7.4)	7.58 (t, 7.4)
	7.46 (2H, t, 7.7)	7.46 (4H, m)	7.46 (2H, t, 7.7)	7.46 (4H, t, 7.4)	7.45 (2H, t, 7.9)

[a] Spectra were recorded in $CDCl_3$, and chemical shifts (δ) are in ppm with J values in Hz.

ingol ester bearing two acetyls and two tiglyl groups. The two acetyl groups were located at C-8 and C-12 by the HMBC correlations from H-8 (δ_H 4.56, dd, J = 1.5, 10.5 Hz) and H-12 (δ_H 4.85, dd, J = 3.9, 11.0 Hz) to two acetyl carbonyls (δ_C 170.3 and 170.2). The HMBC correlations between H-3 (δ_H 5.21, d, J = 8.5 Hz) and H-7 (δ_H 5.22, br s) and the carbonyls of the two tiglyl groups (δ_C 167.3 and 167.1) supported these tiglyl groups being placed at C-3 and C-7 (Figure 1).

The relative configuration of **2** was determined by analysis of ROESY correlations and comparison with **1**. The chemical shifts of H-1α (2.78, d, 14.9 Hz) and H-1β (1.69, dd, 9.2, 14.9 Hz) were similar to compound **1** (Table 1). A ROESY correlation of H-3 and H-5 of **2** showed H-3 to be α-oriented. Other chiral centers were deduced to be the same as those of **1** by the comparison of the chemical shifts and analysis of the ROESY spectrum. Therefore, compound **2** was established as 8,12-*O*-diacetylingol 3,7-ditiglate.

Compound **3** showed a molecular formula of $C_{39}H_{48}O_{10}$ as assigned by HRESIMS (m/z 699.3135 $[M + Na]^+$). Analysis of its ^{1}H and ^{13}C NMR spectra indicated that the structure of **3** is closely related to that of **1** (Tables 1 and 3). The only difference was the presence of an additional benzoyl group in **3** (δ_H 7.45−7.82, 5H; δ_C 165.0, 133.1, 130.0, 2 × 129.5, 2 × 128.5). A HMBC correlation of H-7 (δ_H 5.46, br s) with the carbonyl (δ_C 165.0) showed the benzoyl group to be located at C-7, which was supported by the downfield chemical shift of H-7 from δ_H 4.27 in **1** to δ_H 5.46 in **3**. All the chiral centers were deduced to be the same as those of **1** by comparison of the chemical shifts and analysis of the ROESY spectrum. Thus, compound **3** was identified as 12-*O*-acetyl-7-*O*-benzoylingol 3,8-ditiglate.

Compound **4** was isolated as a white powder and exhibited a quasimolecular ion peak at m/z 681.2666 $[M + Na]^+$ in the HRESIMS, appropriate for a molecular formula of $C_{38}H_{42}O_{10}$. The

Table 3. ^{13}C NMR Assignments of **1**−**10**[a]

position	1	2	3	4	5	6	7	8	9	10
1	31.9 t	31.6 t	31.7 t	31.8 t	31.6 t	31.9 t	32.0 t	31.9 t	32.0 t	32.6 t
2	30.0 d	29.7 d	29.7 d	30.0 d	29.5 d	30.1 d	30.2 d	30.0 d	33.0 d	31.0 d
3	77.0 d	76.7 d	76.9 d	77.6 d	76.9 d	77.4 d	78.0 d	78.0 d	82.2 d	81.9 d
4	73.9 s	73.5 s	73.5 s	73.5 s	73.3 s	74.0 s	73.9 s	73.9 s	72.3 s	71.9 s
5	116.7 d	117.1 d	117.3 d	117.5 d	117.2 d	117.0 d	116.9 d	116.6 d	117.1 d	116.5 d
6	141.4 s	139.6 s	139.6 s	139.5 s	139.5 s	141.4 s	141.7 s	141.7 s	142.1 s	141.8 s
7	76.3 d	76.2 d	76.8 d	76.6 d	76.9 d	76.4 d	76.4 d	76.3 d	76.6 d	76.2 d
8	74.2 d	71.7 d	71.7 d	71.9 d	71.8 d	75.0 d	74.9 d	74.2 d	75.1 d	74.0 d
9	23.5 d	24.8 d	25.2 d	24.9 d	24.9 d	23.6 d	23.6 d	23.5 d	23.9 d	23.6 d
10	19.1 s	19.2 s	19.3 s	19.4 s	19.4 s	19.3 s	19.3 s	19.1 s	19.7 s	19.1 s
11	30.9 d	30.6 d	30.8 d	30.7 d	30.7 d	31.0 d	31.0 d	30.9 d	31.4 d	31.7 d
12	70.8 d	70.5 d	70.7 d	70.6 d	70.6 d	70.8 d	70.8 d	70.8 d	71.2 d	70.9 d
13	43.3 d	43.1 d	43.3 d	43.3 d	43.2 d	43.4 d	43.4 d	43.3 d	43.9 d	43.4 d
14	207.7 s	207.6 s	207.7 s	207.6 s	207.6 s	207.6 s	207.5 s	207.7 s	207.8 s	207.3 s
15	71.4 s	71.1 s	71.2 s	71.4 s	71.2 s	71.5 s	71.6 s	71.5 s	71.1 s	70.7 s
16	17.1 q	16.9 q	16.9 q	17.1 q	16.9 q	17.1 q	17.2 q	17.2 q	16.9 q	16.4 q
17	17.4 q	17.4 q	17.5 q	17.7 q	17.6 q	17.4 q	17.5 q	17.5 q	17.8 q	17.3 q
18	29.1 q	29.1 q	29.2 q	29.2 q	29.2 q	29.2 q	29.2 q	29.1 q	29.6 q	29.1 q
19	16.4 q	16.0 q	16.1 q	16.1 q	16.1 q	16.4 q	16.4 q	16.3 q	16.8 q	16.4 q
20	13.3 q	13.2 q	13.3 q	13.4 q	13.4 q	13.3 q	13.4 q	13.4 q	13.8 q	13.3 q
OAc	170.5 s	170.1 s, 170.2 s	170.3 s	170.5 s 170.4 s	170.5 s 170.5 s 170.4 s	170.4 s	170.4 s	170.5 s	170.9 s	170.4 s
	21.0 q	20.9 q 20.9 a	20.9 q	21.0 q 21.0 q	21.0 q 21.0 q 20.9 q	21.0 q	21.0 q	21.1 q	21.4 q	21.0 q
OTig	167.5 s	167.4 s	167.3 s			167.5 s		167.4 s		167.4 s
	167.4 s	166.4 s	167.1 s							
	138.3 d	138.2 d	138.2 d			138.3 d		138.2 d		138.0 d
	138.2 d	137.8 d	137.9 d							
	128.3 s	128.4 s	128.1 s			128.1 s		128.3 s		128.4 s
	128.0 s	127.8 s	127.8 s							
	14.5 q	14.4 q	14.4 q			14.5 q		14.5 q		14.4 q
	14.5 q	14.4 q	14.4 q							
	12.0 q	12.1 q	11.9 q			12.0 q		12.0 q		12.0 q
	12.0 q	11.9 q	11.9 q							
OBz			165.0 s	166.0 s 165.0 s	165.2 s	166.1 s	2 × 166.0 s	166.0 s	166.6 s 166.5 s	166.2 s
			133.1 d	133.3 d 133.2 d	133.3 d	133.3 d	2 × 133.3 d	133.3 d	133.8 d 133.7 d	133.3 d
			130.0 s	130.0 s 129.7 s	129.9 s	129.9 s	2 × 129.9 s	129.7 s	2 × 130.2 s	129.7 s
			2 × 129.5 d	2 × 129.6 d 2 × 129.4 d	2 × 129.6 d	2 × 129.7 d	2 × 129.7 d	2 × 129.5 d	2 × 130.1 d	2 × 129.7 d
			2 × 128.5 d	2 × 128.5 d 2 × 128.4 d	2 × 128.6 d	2 × 128.5 d	2 × 128.5 d	2 × 128.5 d	2 × 128.9 d	2 × 128.5 d

[a] Spectra were recorded in $CDCl_3$, and chemical shifts (δ) are in ppm.

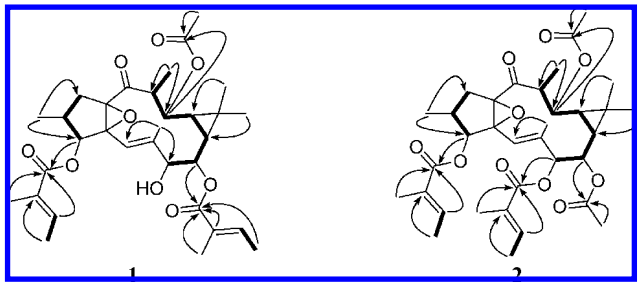

Figure 1. Key COSY (—) and HMBC (→) correlations of **1** and **2**.

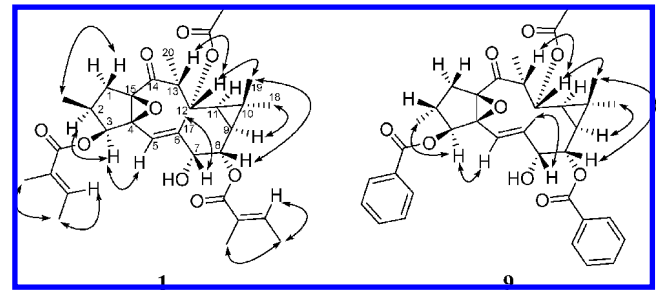

Figure 2. Selected ROESY correlations of **1** and **9**.

signals in the 1D NMR spectra of **4** (Tables 1 and 3) clearly indicated that the compound is an ingol tetraester bearing two acetyl and two benzoyl groups. Chemical shift considerations further showed that the ester moieties are located at the hydroxy groups bound to C-3, C-7, C-8, and C-12. Comparison of ^{1}H and ^{13}C NMR data of **4** with those of **2** suggested that the only difference was that the two tiglyl groups in **2** at C-3 and C-7 were replaced by two benzoyl groups in **4**, which was further confirmed by HMBC correlations of H-3 (δ_H 5.40, d, J = 8.5 Hz) and H-7 (δ_H 5.48, br s) with carbonyls of the two benzoyl groups (δ_C 166.0 and 165.0). Therefore, the structure of compound **4** was identified as 8,12-*O*-diacetylingol 3,7-dibenzoate.

The molecular formula of **5** was deduced as $C_{34}H_{42}O_9$ from its HRESIMS and ^{13}C NMR data. According to NMR evidence, compound **5** was found to be similar in structure to **4**. The only difference found was an acetyl group in **5** instead of a benzoyl group in **4**. A HMBC correlation of H-3 (δ_H 5.17, d, J = 8.5 Hz) with the carbonyl (δ_C 170.5) of the acetyl group suggested the latter group to be located at C-3. Accordingly, compound **5** was determined as 3,8,12-*O*-triacetylingol 7-benzoate.

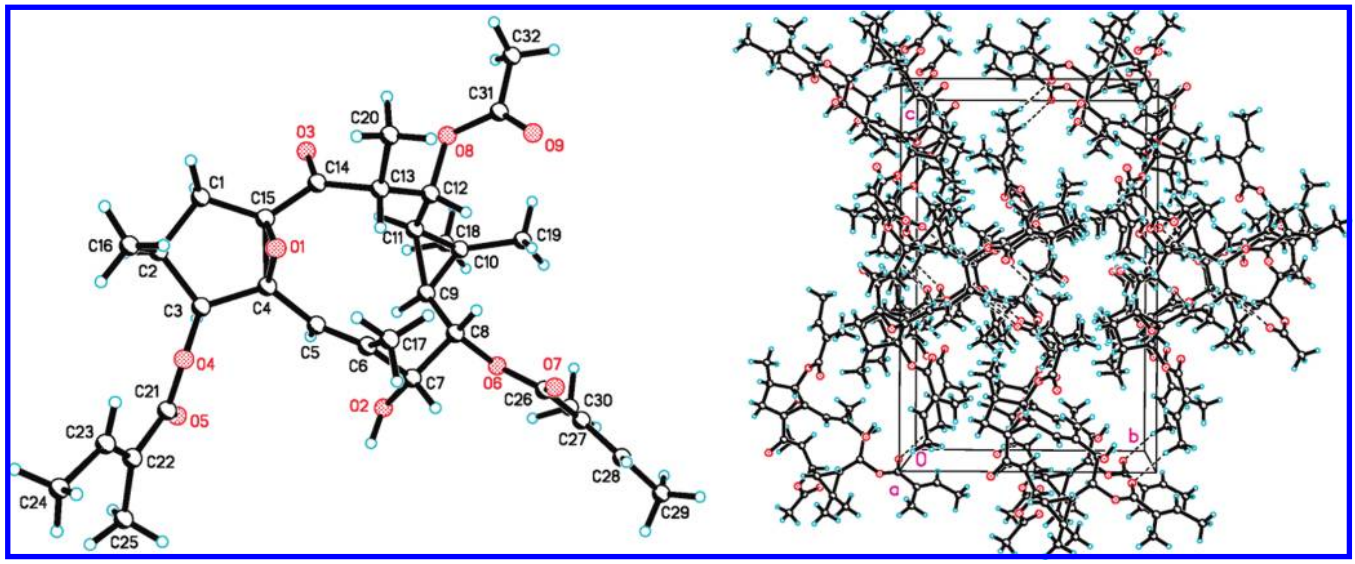

Figure 3. X-ray structure of **1** showing the relative configuration.

HRESIMS of **6** gave a molecular ion at *m/z* 617.2731 ([M + Na]$^+$), corresponding to the molecular formula $C_{34}H_{42}O_9$. A comparison of the ^{1}H and ^{13}C NMR data of compounds **6** and **1** suggested that a benzoyl group in **6** replaced a tiglyl group in **1**. The benzoyl group was determined to be located at C-8 by a HMBC correlation from H-8 (δ_H 4.77, dd, J = 1.5, 10.3 Hz) to the carbonyl of the benzoyl group (δ_C 166.1). Therefore, compound **6** was assigned as 12-*O*-acetyl-8-*O*-benzoylingol 3-tiglate.

The molecular formula of **7** was deduced as $C_{36}H_{40}O_9$ from its HRESIMS and ^{13}C NMR data. Analysis of the ^{1}H and ^{13}C NMR spectra showed compound **7** to be a diterpenoid with one acetyl and two benzoyl groups (Tables 2 and 3). A side-by-side comparison of 1D and 2D NMR data of **6** and **7** showed that a tiglyl group in **6** at C-3 was replaced by a benzoyl group in **7**. Thus, compound **7** was established as 12-*O*-acetylingol 3,8-dibenzoate.

Compound **8** gave the molecular formula $C_{34}H_{42}O_9$, as deduced by HRESIMS (*m/z* 617.2726 [M + Na]$^+$), the same as that of **6**. According to NMR evidence, compound **8** is very close to **6**. Chemical shifts and coupling constants determined were almost identical for both compounds (Tables 2 and 3). Analysis of the HMBC and ^{1}H−^{1}H COSY spectra of **8** showed a benzoyl group to be located at C-3 in **8** instead of a tiglyl group in **6** and a tiglyl group located at C-8 in **8** instead of a benzoyl group in **6**. Accordingly, compound **8** was elucidated as 12-*O*-acetyl-3-*O*-benzoylingol 8-tiglate.

Compound **9** was obtained as a white powder, with $[\alpha]^{27}_D$ + 17.7 (*c* 0.1, MeOH). Its molecular formula, $C_{36}H_{40}O_9$, was established by its HRESIMS at *m/z* 639.2564 [M + Na]$^+$ (calcd for $C_{36}H_{40}O_9Na$ 639.2570), indicating **9** to be an isomer of **7**. The only differences observed were that the chemical shift of H_2-1 moved to δ_H 2.19 in **9** from δ_H 2.92 and 1.77 in **7** and the signal of Me-16 shifted downfield to δ_H 1.17 in **9** from δ_H 0.99 in **7**. The strong ROESY correlations of H-3 and H-5 of **9** showed that H-3 is α-oriented, as in **7**. Thus, the chemical shift differences of H_2-1 in **9** with that of **7** can be rationalized by the change of configuration of C-2; that is, Me-16 is α-oriented in **9**. The above evidence showed compound **9** is an epimer of **7**. Thus, compound **9** was identified as 12-*O*-acetyl-2-*epi*-ingol 3,8-dibenzoate.

Compound **10** was isolated as a white powder, $[\alpha]^{27}_D$ +39.0 (*c* 0.1, MeOH). The molecular formula, $C_{34}H_{42}O_9$, was determined by its HRESIMS at *m/z* 617.2735 (calcd for $C_{34}H_{42}O_{12}Na$ 617.2726). It was shown to possess the same planar structure as compound **8** by analysis of its 1D and 2D NMR spectra. The ROESY correlation between H-3 and H-5 in **10** indicated H-3 to be α-oriented as in **8**. The chemical shift differences of H-1 and H-2 between **8** and **10** suggested the two compounds have a different configuration at C-2. Thus, compound **10** was determined to be an epimer of **8**, namely, 12-*O*-acetyl-3-*O*-benzoyl-2-*epi*-ingol 8-tiglate.

The antiangiogenic activities of compounds **1**−**4** and **6**−**12** were evaluated using a zebrafish model, in terms of the inhibition on the growth of intersegmental vessels, with PTK787 as positive control (IC_{50} 0.15 μg/mL).[18] The results showed that intersegmental vessels of embryos treated with compounds **11** and **12** were significantly less than that of the control (0.1% DMSO in sterile salt water). The inhibition ratios of compounds **11** and **12** were 100% and 87.5% at a concentration of 3 μg/mL, with IC_{50} values of 0.61 and 0.92 μg/mL, respectively. Accordingly, it was concluded that compounds **11** and **12** show antiangiogenic activity. The ingol esters (**1**−**10**) were all negative in the assay, with IC_{50} values more than 5 μg/mL (Table S1, Supporting Information).

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Horiba SEPA-300 polarimeter or a Perkin-Elmer model 241 polarimeter. UV data were obtained on a UV 210A spectrometer. IR spectra were recorded on a Bio-Rad FTS-135 spectrometer, using KBr pellets. 1D and 2D NMR experiments were performed on a Bruker AM-400 or DRX-500 spectrometer. Chemical shifts (δ) are expressed in ppm with reference to the solvent signals. Mass spectra were recorded on a VG Auto Spec-3000 or a Finnigan MAT 90 instrument. Column chromatography was performed either on silica gel (200−300 mesh, Qingdao Marine Chemical, Qingdao, People's Republic of China), silica gel H (10−40 μm, Qingdao Marine Chemical), or MCI gel CHP20P (75−150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on a Hewlett-Packard instrument (column: Zorbax SB-C18, 250 × 9.4 mm; DAD detector). Fractions were monitored by TLC, visualized by heating silica gel plates sprayed with 15% H_2SO_4 in EtOH.

Plant Material. The aerial parts of *E. royleana* were collected from Yuanjiang, Yunnan Province, People's Republic of China, in April 2008. A specimen (YangYP 20080504), identified by one of the authors (Y.-P.Y.), was deposited in the Herbarium of the Department of Taxonomy, Kunming Institute of Botany, Chinese Academy of Sciences.

Extraction and Isolation. The dry aerial parts of *E. royleana* (10 kg) were powdered and extracted with 70% aqueous acetone (3 × 20 L) for 24 h at room temperature. The solvent was concentrated in vacuo to give a crude extract. The extract was then dissolved in H_2O and partitioned with EtOAc. The EtOAc portion was subjected to column chromatography over MCI gel eluting with 95% EtOH and concentrated in vacuo. The residue (120 g) was then subjected to column chromatography over silica gel (200−300 mesh), eluting with petroleum

ether−Me_2CO (from 1:0 to 1:1), to give fractions A−H. Fraction A was subjected to column chromatography over silica gel eluting with petroleum ether−Me_2CO (100:1) to yield subfractions a−d. These subfractions were further purified by semipreparative HPLC (Agilent 1200 HPLC system; Zorbax SB-C18, 250 × 9.4 mm; DAD detector). Subfraction a yielded compounds **2** (10 mg), **3** (3 mg), and **11** (2 mg), eluted by 85% MeOH−H_2O. Compounds **4** (8 mg), **5** (1 mg), and **6** (2 mg) were obtained from subfraction b with 80% MeOH−H_2O. Compounds **7** (4 mg), **8** (6 mg), and **9** (2 mg) were furnished from subfraction c with 76% MeOH−H_2O. Finally, compounds **1** (15 mg), **10** (2 mg), and **12** (3 mg) were obtained from subfraction d with 73% MeOH−H_2O as eluant, respectively. All these compounds were detected at 202 nm.

Compound 1: colorless crystals from methanol; mp 96−97 °C; $[\alpha]_{27}^{D}$ +14.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 214 (4.14) nm; IR (KBr) ν_{max} 3506, 2971, 2937, 2878, 1716, 1652, 1441, 1375, 1258, 1131, 1078 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 595 $[M + Na]^+$; HRESIMS *m/z* 595.2896 (calcd for $C_{32}H_{44}O_9Na$, 595.2883).

Compound 2: white powder; $[\alpha]^{27}_D$ + 21.4 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 206 (4.10) nm; IR (KBr) ν_{max} 3436, 2956, 1739, 1715, 1653, 1443, 1374, 1242, 1020 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 637 $[M + Na]^+$; HRESIMS *m/z* 637.2978 (calcd for $C_{34}H_{46}O_{10}Na$, 637.2988).

Compound 3: white powder; $[\alpha]^{27}_D$ −26.6 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.26) nm; IR (KBr) ν_{max} 3437, 2934, 1732,1712, 1652, 1452, 1269, 1246, 1071 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 699 $[M + Na]^+$; HRESIMS *m/z* 699.3135 (calcd for $C_{39}H_{48}O_{10}Na$, 699.3145).

Compound 4: white powder; $[\alpha]^{27}_D$ +52.2 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.09) nm; IR (KBr) ν_{max} 3436, 2956, 2939, 2880, 1730, 1603, 1452, 1371, 1273, 1241, 1113, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 681 $[M + Na]^+$; HRESIMS *m/z* 681.2666 (calcd for $C_{38}H_{42}O_{10}Na$, 681.2675).

Compound 5: white powder; $[\alpha]^{27}_D$ +42.2 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.02) nm; IR (KBr) ν_{max} 3436, 2956, 2939, 2880, 1730, 1603, 1452, 1371, 1273, 1241, 1113, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; positive ESIMS *m/z* 619 $[M + Na]^+$; HRESIMS *m/z* 619.2500 (calcd for $C_{34}H_{42}O_9Na$, 619.2519).

Compound 6: white powder; $[\alpha]^{27}_D$ +30.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.11) nm; IR (KBr) ν_{max} 3440, 2928, 1724, 1639, 1451, 1271, 1251, 1072 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 617 $[M + Na]^+$; HRESIMS *m/z* 617.2731 (calcd for $C_{34}H_{42}O_9Na$, 617.2726).

Compound 7: white powder; $[\alpha]^{27}_D$ +17.7 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.17) nm; IR (KBr) ν_{max} 3441, 2936, 2881, 1715, 1630, 1452, 1274, 1247, 1114 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 639 $[M + Na]^+$; HRESIMS *m/z* 639.2564 (calcd for $C_{36}H_{40}O_9Na$, 639.2570).

Compound 8: white powder; $[\alpha]^{27}_D$ +39.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.05) nm; IR (KBr) ν_{max} 3440, 2928, 1724, 1639, 1451, 1271, 1251, 1072 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 617 $[M + Na]^+$; HRESIMS *m/z* 617.2726 (calcd for $C_{34}H_{42}O_9Na$, 617.2726).

Compound 9: white powder; $[\alpha]^{26}_D$ +23.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.20) nm; IR (KBr) ν_{max} 3441, 2958, 2928, 1711, 1630, 1452, 1274, 1248, 1112 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 639 $[M + Na]^+$; HRESIMS *m/z* 639.2587 (calcd for $C_{36}H_{40}O_9Na$, 639.2587).

Compound 10: white powder; $[\alpha]^{27}_D$ +21.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 224 (4.00) nm; IR (KBr) ν_{max} 3441, 2928, 1727, 1709, 1639, 1629, 1451, 1271, 1251, 1024 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 2 and 3; positive ESIMS *m/z* 617 $[M + Na]^+$; HRESIMS *m/z* 617.2735 (calcd for $C_{34}H_{42}O_{12}Na$, 617.2726).

X-ray Crystallographic Data for 1. Formula: $C_{32}H_{44}O_9$; M_r = 572.67; monoclinic crystalline system; space group: $P2_12_12_1$; a = 11.2454(7) Å, b = 13.3881(9) Å, c = 20.6190(13) Å; V = 3104.3(3) $Å^3$; Z = 4; d = 1.225 g cm^{-3}; crystal dimensions 0.412 × 0.323 × 0.107 mm. The total number of independent reflections measured was 3605, of which 2894 were observed ($|F|^2 \geq 2\sigma|F|^2$). The final indices were R_1 = 0.0407, wR_2 = 0.0923, S = 0.957. Crystal structure measurements were made by using a Bruker Smart-Apex CCD diffractometer with graphite-monochromated Mo Kα radiation. The data were collected using the $\omega-2\theta$ scan technique to a maximum 2θ value of 50.0°. The crystal structures were solved by the direct method SHELX-97,[19] expanded by using difference Fourier techniques, and refined by the program and method NOMCSDP[20] and the full-matrix least-squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were included at their calculated positions. Crystallographic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 706245). Copies of these data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

Antiangiogenesis Assay.[18] Stock solutions (10 mg/mL) of all samples were prepared by dissolving the test compounds in 100% DMSO. These solutions were diluted in sterile salt water (5 mM NaCl, 0.17 mM KCl, 0.4 mM $CaCl_2$, 0.16 mM $MgSO_4$) to obtain solutions with the test compounds dissolved in 0.1% DMSO. These solutions were aliquoted into 96-well plates, and embryos at 24 hpf (hours postfertilization) were also transferred randomly into the above wells. After 24 h of treatment, the intersegmental vessels of embryos were visualized with green fluorescent protein labeling and endogenous alkaline phosphatase staining. The antiangiogenic activities of compounds were calculated from the inhibition ratio of angiogenesis. PTK787 was used as the positive control.

Acknowledgment. The work was supported by the Ford Foundation (1075-0729 and 1090-0448) and the West Light Foundation of Chinese Academy of Sciences (awarded to X.-L.L.).

Supporting Information Available: Antiangiogenic activity of compounds **1**−**4** and **6**−**12** (Table S1) and NMR spectra of compounds **1**−**10**. These materials are available free of charge via the Internet at http://pubs.acs.org.

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NP800816N