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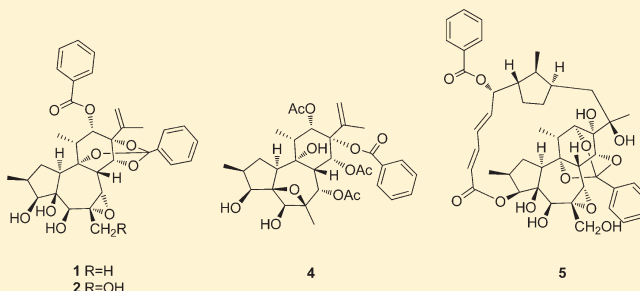
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Trigonosins A–F, Daphnane Diterpenoids from *Trigonostemon thyrsoides*Shi-Fei Li,^{†,‡} Ying-Tong Di,[†] Shun-Lin Li,[†] Yu Zhang,[†] Fu-Mei Yang,[§] Qian-Yun Sun,[§] James Mpetga Simo,[†] Hong-Ping He,^{*,†} and Xiao-Jiang Hao^{*,†}[†]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, People's Republic of China[‡]Graduate School of Chinese Academy of Sciences, Beijing 100039, People's Republic of China[§]The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Science, Guiyang 550002, People's Republic of China

S Supporting Information

ABSTRACT: Phytochemical study of the roots of *Trigonostemon thyrsoides* led to the isolation of four new oxygenated daphnane-type diterpenoids, trigonosins A–D (1–4), and two new modified daphnanes, trigonosins E and F (5 and 6). The structures and relative configurations were elucidated on the basis of extensive spectroscopic analysis, including 1D and 2D NMR experiments. All compounds isolated were evaluated for their cytotoxicity against HL-60, A549, and MCF-7 human cancer cell lines.



Daphnane diterpenoids are characteristic constituents of the Euphorbiaceae and Thymelaeaceae plant families¹ and show various biological effects, such as neurotrophic,² antihyperglycemic,³ antifertility,⁴ and pesticide activities.⁵ Plants of the genus *Trigonostemon* (Euphorbiaceae) are usually bushes or small trees and occur in tropical and subtropical areas. The genus comprises a small group of 10 species in mainland China, of which four (*T. thyrsoides*, *T. huangmosu*, *T. lii*, and *T. filipes*) are found in Yunnan Province. As a continuation of phytochemical studies on this genus,⁶ four highly oxygenated daphnane-type diterpenoids, trigonosins A–D (1–4), and two new modified daphnanes, trigonosins E and F (5 and 6), along with four known diterpenoids, trigoxyphins A (8) and B (7)⁷ and rediocides C (9)⁸ and A (10),⁹ were isolated from the roots of *T. thyrsoides*. All compounds were tested for their cytotoxicities against the HL-60, A549, and MCF-7 human cancer cell lines. Herein, we report the isolation and structural elucidation of these compounds, as well as their cytotoxicities.

Compound 1 was obtained as a white, amorphous powder. Its molecular formula was determined as C₃₄H₃₈O₉ by HRESIMS at *m/z* 613.2400 [M+Na]⁺ (calcd for C₃₄H₃₈O₉Na, 613.2413). The IR spectrum of 1 showed absorption bands at 3429 (hydroxy), 1709 (carbonyl), and 1642 and 1452 (aromatic) cm⁻¹. In the ¹³C NMR spectrum (Table 1), 34 carbon signals were resolved comprising four methyls, two methylenes, 19 methines (five oxygenated and 10 olefinic ones), and nine

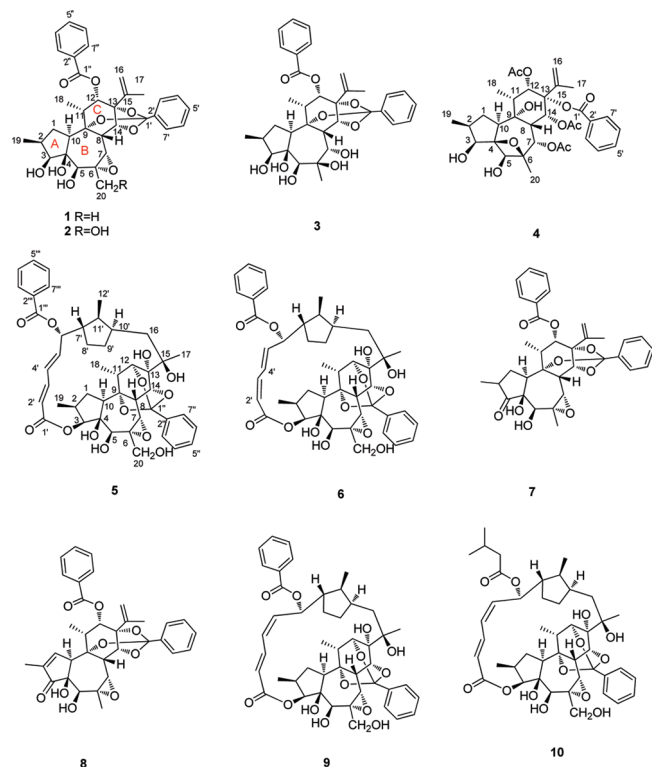
quaternary carbons (one ester, three olefinic, one ortho-ester, and four oxygenated ones) as classified by their chemical shifts and from the HSQC spectrum. In addition, two monosubstituted benzene rings and a trisubstituted epoxide (δ_H 3.29, s; δ_C 60.3 and 67.0) moiety were distinguished by NMR analysis (Tables 1 and 2). Proton resonances at δ_H 3.13 (d, *J* = 9.2 Hz, 1H), 3.59 (brs, 1H), and 4.50 (brs, 1H), showing no correlations with any carbons in the HSQC spectrum, were assigned to the exchangeable protons of three OH groups. Detailed comparison of the NMR data of 1 with those of the known compound trigoxyphin B (7) suggested that 1 differs from 7 only by the presence of an additional oxygenated methine at δ_C (78.2) instead of a ketone group (δ_C 209.5) at C-3 in the latter. HMBC correlations of H-3 to C-2, C-4, C-5, and C-10 and those of OH-3 to C-3 indicated the oxygenated methine to be located at C-3 (Figure 1). The relative configuration of 1 was established by a ROESY experiment (Figure 2) in which the correlations of H-10/H-5 and H-5/H-3 indicated that H-3, H-5, and H-10 are cofacial and H-3 at the newly formed C-3 stereocenter is α-oriented. The relative configurations of the other chiral centers in compound 1 were assigned as being identical to

Special Issue: Special Issue in Honor of Koji Nakanishi

Received: September 13, 2010

Published: December 30, 2010

those of **7**, by comparing their NMR data and from the ROESY experiment.



Compound **2** gave a molecular formula of $C_{34}H_{38}O_{10}$, as determined by the HRESIMS, which was 16 mass units greater than **1**. Many similarities between the 1H and ^{13}C NMR data of **2** (Tables 1 and 2) and those of **1** suggested that they are structural analogues. As compared with compound **1**, the main differences were due to the presence of a hydroxymethyl group (δ_C 65.5) in **2**, with absence of a methyl group in the latter. The hydroxymethyl group was placed at C-20 by the HMBC correlations of H_2 -20 to C-5, C-6, and C-7. Therefore, the structure of **2** was established as shown.

Compound **3** had the molecular formula $C_{34}H_{40}O_{10}$ as determined by the HRESIMS at m/z 631.2515 $[M + Na]^+$ (calcd for $C_{34}H_{40}O_{10}Na$, 631.2519.1242) with 18 mass units more than **1**. Comparison of the 1H and ^{13}C NMR data of **3** (Tables 1 and 2) with those of **1** revealed that a main difference was that C-6 and C-7 were shifted downfield about $\Delta\delta$ 16.3 and 14.6, respectively, as compared with those of **1**. These observations suggested that 6,7-dihydroxy groups in **3** replace the 6,7-epoxide moiety in **1**. HMBC correlations of OH-7 to C-7 and H_3 -20 to C-6 and C-7 confirmed the suggestion. The ROESY correlations of H-10/H-2, H-5, and OH-7 indicated that H-2, H-5, OH-7, and H-10 are cofacial and assigned as α -configuration. The Me-20 was deduced also to be α -oriented by the ROESY correlations between H-5 and Me-20. Therefore, the structure of compound **3** was determined as shown.

Compound **4** was obtained as a white powder. ESIMS analysis of **4** produced a pseudomolecular ion at m/z 653 $[M + Na]^+$, and positive HRESIMS gave a molecular formula of $C_{33}H_{42}O_{12}$ from the ion at m/z 653.2560 $[M + Na]^+$ (calcd for $C_{33}H_{42}O_{12}Na$, 653.2573), with 13 degrees of unsaturation. The IR spectrum showed the presence of hydroxy groups

Table 1. ^{13}C NMR [δ_C (pattern)] Data for Compounds **1**–**4** in $CDCl_3$

carbon	1 ^a	2 ^a	3 ^a	4 ^b
1	34.3	34.3	34.7	34.9
2	36.6	36.7	36.4	31.2
3	78.2	78.0	76.7	73.1
4	80.0	80.0	84.4	93.8
5	74.6	73.0	75.7	71.9
6	60.3	61.2	76.6	86.7
7	67.0	63.3	81.6	79.1
8	35.2	35.2	34.8	39.3
9	81.1	81.2	81.9	76.9
10	48.5	48.5	51.2	48.0
11	39.2	39.3	39.2	40.0
12	72.6	72.4	72.4	72.5
13	86.4	86.6	87.3	81.9
14	82.3	82.3	84.3	75.4
15	142.2	142.1	142.0	139.2
16	113.1	113.2	113.1	119.5
17	19.4	19.4	19.4	20.0
18	11.7	11.7	11.6	11.4
19	13.0	12.9	13.1	15.6
20	22.6	65.5	25.5	18.8
1'	118.2	118.2	118.0	164.0
2'	135.6	135.6	135.1	129.8
3'/7'	126.1	126.1	126.0	129.4
4'/6'	127.9	128.0	128.1	128.5
5'	129.3	129.4	129.7	133.1
1''	166.4	166.2	166.1	
2''	129.4	129.5	129.4	
3''/7''	129.8	129.7	129.8	
4''/6''	128.5	128.5	128.5	
5''	133.4	133.3	133.3	
OAc-7				169.8, 21.3
OAc-12				169.3, 20.8
OAc-14				169.0, 21.5

^a Measured at 100 MHz. ^b Measured at 125 MHz.

(3567, 3511, and 3459 cm^{-1}) and an ester carbonyl (1755 and 1728 cm^{-1}). In accordance with the molecular formula, 33 carbon resonances were resolved in the ^{13}C NMR spectrum (Table 1) and were further classified by DEPT experiments as seven methyls, two methylenes, 14 methines (five oxygenated and five olefinic), and 10 quaternary carbons (four ester carbonyls, four oxygenated and two olefinic carbons). Three proton resonances at δ_H 2.84 (d, J = 10.7 Hz, 1H), 3.38 (brs, 1H), and 3.88 (d, J = 10.9 Hz, 1H), which did not correlate with any carbons in the HSQC spectrum, were attributable to the presence of hydroxy groups. Detailed comparison of the 1H and ^{13}C NMR data of **4** with those of the known daphnane-type diterpenoid trigochinin A¹⁰ revealed that the only difference between them was that the benzoyloxy group at C-5 in trigochinin A was replaced by a hydroxy group in **4**. This was confirmed by the 1H – 1H COSY (Figure 1) correlation of OH-5 (δ_H 3.88, d, J = 10.9 Hz) to H-5 (δ_H 5.08, d, J = 10.9 Hz) and the HMBC correlations of OH-5 to C-4 (δ_C 93.8) and C-5 (δ_C 71.9). The relative configuration of **4** was elucidated by a ROESY experiment as shown in Figure 2, and the absolute configuration of **4** was

Table 2. ^1H NMR [δ_{H} (mult. J (Hz))] Data for Compounds 1–4 in CDCl_3

position	1 ^a	2 ^a	3 ^a	4 ^b
1 β	1.81 (overlapped)	1.82 (m)	1.93 (m)	1.96 (m)
1 α	1.49 (overlapped)	1.52 (m)	1.60 (m)	1.09 (t, 11.3)
2	1.64 (m)	1.66 (m)	1.71 (m)	2.26 (m)
3	3.84 (m)	3.89 (m)	4.29 (brs)	4.18 (t, 10.7)
5	3.54 (d, 7.6)	3.78 (d, 4.0)	3.36 (d, 8.0)	5.08 (d, 10.9)
7	3.29 (brs)	3.49 (brs)	4.21 (brs)	5.69 (d, 4.3)
8	3.30 (d, 2.0)	3.32 (d, 2.0)	2.80 (brs)	2.80 (d, 3.2)
10	2.85 (dd, 13.3, 5.6)	2.78 (dd, 13.5, 5.7)	2.75 (dd, 13.0, 5.0)	2.00 (m)
11	3.00 (m)	3.00 (t, 7.2)	3.24 (t, 6.8)	2.16 (m)
12	5.51 (d, 7.6)	5.50 (d, 7.2)	5.50 (d, 7.6)	6.36 (d, 3.2)
14	4.66 (d, 2.1)	4.68 (d, 2.0)	4.72 (d, 1.8)	6.03 (brs)
16b	5.30 (brs)	5.29 (brs)	5.28 (brs)	5.47 (brs)
16a	5.04 (brs)	5.04 (brs)	5.05 (brs)	5.42 (brs)
17	1.80 (brs)	1.79 (brs)	1.80 (brs)	1.95 (brs)
18	1.18 (d, 6.6)	1.20 (d, 6.7)	1.21 (d, 6.6)	1.13 (d, 6.6)
19	1.03 (d, 6.6)	1.04 (d, 6.2)	1.04 (d, 6.6)	0.92 (d, 7.0)
20a	1.48 (brs)	3.91 (m)	1.51 (brs)	1.27 (brs)
20b		3.85 (m)		
3' / 7'	7.79 (dd, 6.0, 2.5)	7.77 (m)	7.73 (m)	7.59 (d, 7.6)
4' / 6'	7.38 (m)	7.39 (m)	7.41 (m)	7.40 (t, 7.6)
5'	7.38 (overlapped)	7.39 (overlapped)	7.41 (overlapped)	7.54 (t, 7.6)
3'' / 7''	8.05 (d, 7.8)	8.05 (d, 7.5)	8.05 (d, 7.6)	
4'' / 6''	7.45 (t, 7.8)	7.44 (t, 7.5)	7.43 (t, 7.6)	
5''	7.58 (t, 7.8)	7.58 (t, 7.5)	7.57 (t, 7.6)	
OH-3	3.59 (brs)			2.84 (d, 10.7)
OH-4	4.50 (brs)		4.52 (brs)	
OH-5	3.13 (d, 9.2)			3.88 (d, 10.9)
OH-7			3.45 (brs)	
OH-9				3.38 (brs)
OAc-7				2.05 (brs)
OAc-12				1.92 (brs)
OAc-14				2.12 (brs)

^a Measured at 400 MHz. ^b Measured at 500 MHz.

determined as identical with trigochinin A by the closely similar patterns of Cotton effects in the CD spectra (see Supporting Information). Therefore, the structure of compound **4** was determined as shown.

Compound **5** was isolated as a white powder. ESIMS analysis of **5** demonstrated pseudomolecular ions at m/z 815.0 $[\text{M} + \text{H}]^+$ and 837.4 $[\text{M} + \text{Na}]^+$. In the HRESIMS a sodiated molecular ion occurred at m/z 837.3469 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{13}\text{Na}$, 837.3462), consistent with a molecular formula of $\text{C}_{46}\text{H}_{54}\text{O}_{13}$. The IR spectrum showed absorption bands at 3440 (OH), 1711 (carbonyl), and 1630 and 1451 (aromatic) cm^{-1} . Analysis of the ^{13}C NMR data (Table 3) including the DEPT spectrum of **5** revealed 46 carbon resonances comprising two ester-type carbonyls, two phenyl groups, four olefinic methines, an orthoester carbon, five oxygen-bearing quaternary carbons, six oxygenated methines, one oxygenated methylene, six aliphatic methines, five other methylenes, and four methyl groups. Comparison of the ^1H and ^{13}C NMR data of **5** with those of rediocide C (**9**)⁸ suggested that they are similar compounds and share the same modified daphnane skeleton, except that the olefinic signals due to C-3' at δ_{C} 138.9 and C-4' at δ_{C} 130.3 in **9**

were shifted downfield to δ_{C} 145.8 and δ_{C} 135.3 in **5**, respectively. The geometry of the $2'\Delta$ and $4'\Delta$ olefins in **5** was established as *E* and *E*, respectively, on the basis of large ($J_{\text{H}2',\text{H}3'} = 15.4$ Hz and $J_{\text{H}4',\text{H}5'} = 14.8$ Hz) coupling constants between the olefinic protons, and confirmed by the ROESY correlations of H-2' / H-4' and H-3' / H-5' (Figure 2). The relative configuration of **5** was elucidated by the measurement of J couplings and the ROESY spectrum as being the same as those of rediocide C.⁸ Therefore, the structure of compound **5** was determined as shown.

The positive HRESIMS of **6** showed a sodiated ion peak at m/z 837.3468 $[\text{M} + \text{Na}]^+$ ascribable to the molecular formula $\text{C}_{46}\text{H}_{54}\text{O}_{13}$ (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{13}\text{Na}$, 837.3462). The NMR data (Table 3) of **6** were closely similar to those of **5**, except that the olefinic signals due to C-2' at δ_{C} 122.7, C-3' at δ_{C} 145.8, and C-4' at δ_{C} 135.3 in **5** were shifted upfield to δ_{C} 119.2, δ_{C} 141.8, and δ_{C} 130.4 in **6**, respectively; in addition the olefinic signal due to C-5' (δ_{C} 135.7) in **5** was shifted downfield to δ_{C} 141.0 in **6**. The geometry of the $2'\Delta$ and $4'\Delta$ olefins in **6** was established as *Z* and *E*, respectively, on the basis of the small ($J_{\text{H}2',\text{H}3'} = 11.9$ Hz) and the large ($J_{\text{H}2',\text{H}3'} = 15.0$ Hz) coupling constants between the

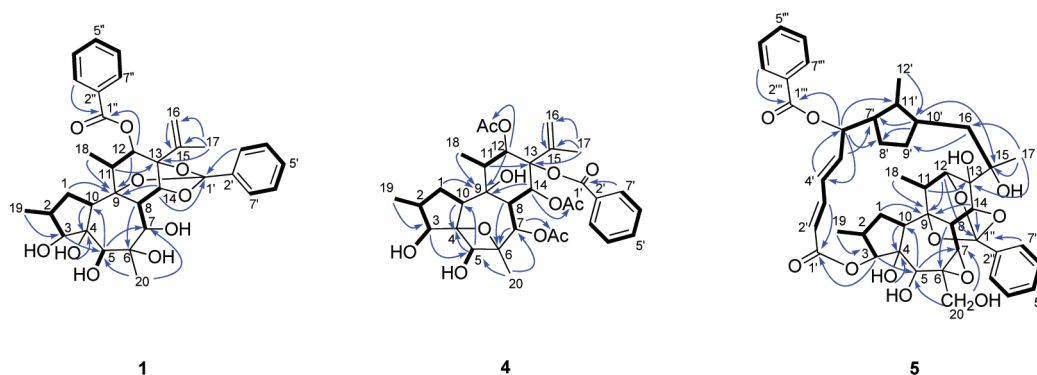


Figure 1. Key HMBC (arrows) and COSY (bold) correlations for **1**, **4**, and **5**.

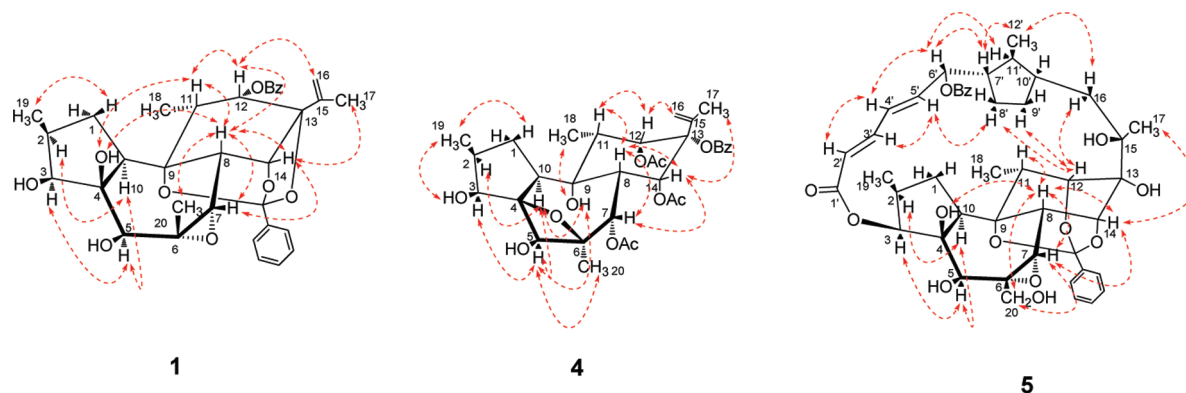


Figure 2. Key ROESY data of **1**, **4**, and **5**.

olefinic protons. These arguments were confirmed by ROESY correlations of H-2'/H-3' and H-4'/H-6'. The relative configuration of **6** was deduced from the ROESY spectrum as being the same as for **5**. Therefore, the structure of compound **6** was determined as shown.

Compounds **1–6** and the four known daphnane diterpenoids **7–10** isolated from the roots of *T. thyrsoideum* were tested for their *in vitro* cytotoxicities against the HL-60 and against A549 and MCF-7 human cell lines by the MTT and SRB methods, as described previously in the literature.^{11,12} However, all compounds did not show significant inhibitory activity against the tumor cells used ($IC_{50} > 10 \mu M$).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were obtained on a JASCO DIP-370 digital polarimeter. IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets, and UV data were measured using a UV-210A spectrometer. 1D and 2D NMR spectra were measured on Bruker AM-400, DRX-500, and AV-600 NMR spectrometers. ESIMS were recorded using a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer. Column chromatography was performed on Si gel H (10–40 μm ; Qingdao Marine Chemical Factory) and Sephadex LH-20 (40–70 μm , Amersham Pharmacia Biotech AB, Uppsala, Sweden). MPLC was performed on a Büchi Sepacore System (Büchi Labortechnik AG, Switzerland) and columns packed with Chromatorex C₁₈ (40–75 μm , Fuji Silysia Chemical Ltd., Japan). Preparative HPLC was performed by using an Agilent 1200 series system equipped with a Zorbax XDB-C18, 9.4 mm \times 150 mm, column.

Plant Material. The roots of *Trigonostemon thyrsoideum* were collected in Xishuangbanna in Yunnan Province, People's Republic of China, in July 2008, and identified by Mr. Xue-Dong, Li, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (KIB20080705) was deposited in the Herbarium of Kunming Institute of Botany.

Extraction and Isolation. Air-dried, powdered roots (5.0 kg) of *T. thyrsoideum* were extracted three times with MeOH at 50 °C. After removal of the solvent by evaporation, the residue was suspended in H₂O and partitioned with EtOAc. The EtOAc (50 g) fraction was subjected to silica gel column chromatography with a gradient elution system of petroleum ether–acetone (100:0–30:70) to obtain five fractions (A–E). Fraction C (1.2 g) was separated and purified by MPLC (MeOH–H₂O, 85:15) to yield six fractions (C1–C6). Fraction C2 was subjected to Sephadex LH-20 column chromatography (MeOH–H₂O, 10:1) to yield compound **4** (30 mg). Fraction C3 was purified using Sephadex LH-20 (CHCl₃–MeOH, 1:1) and then preparative HPLC (CH₃CN–H₂O, eluting from 55:45 for 20 min with a flow rate of 30 mL/min) to afford compounds **5** (10 mg), **6** (8.0 mg), **9** (6.0 mg), and **10** (8.0 mg). Using the same procedures, fraction C4 gave **1** (6.0 mg), **2** (4.0 mg), **3** (5.0 mg), **7** (6.0 mg), and **8** (5.5 mg).

Trigonosin A (1): white powder; $[\alpha]_D^{26} -83.1$ (c 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.67), 230 (4.55) nm; IR (KBr) ν_{max} 3429, 2955, 2881, 1709, 1642, 1452, 1355, 1317, 1283, 1180, 1111, 1090, 1026, 986, 907, 782, 714 cm^{-1} ; ¹H and ¹³C NMR data see Tables 2 and 1; ESIMS m/z 591.4 $[M + H]^+$, 613.4 $[M + Na]^+$; HRESIMS m/z 613.2400 $[M + Na]^+$ (calcd for C₃₄H₃₈O₉Na, 613.2413).

Trigonosin B (2): white powder; $[\alpha]_D^{26} -32.3$ (c 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.17), 230 (4.11) nm; IR (KBr) ν_{max} 3431, 2958, 2931, 2876, 1719, 1630, 1452, 1386, 1354, 1316, 1282, 1177, 1119, 1082, 1026, 988, 918, 758, 713 cm^{-1} ; ¹H and ¹³C NMR data see

Table 3. ^1H (400 MHz) and ^{13}C (100 MHz) NMR Data for Compounds 5 and 6 in CDCl_3

5		6		5		6	
position	δ_{H} [mult. J (Hz)]	δ_{C}	δ_{H} [mult. J (Hz)]	δ_{C}	position	δ_{H} [mult. J (Hz)]	δ_{C}
1 β	2.26 (m)	35.4	2.10 (m)	37.3	4'	6.73 (dd, 14.8, 11.3)	135.3
1 α	1.94 (m)		2.10 (overlapped)		5'	6.23 (dd, 14.8, 10.0)	135.7
2	1.72 (m)	35.2	1.75 (m)	35.7	6'	5.75 (dd, 10.0, 6.0)	76.1
3	4.89 (d, 3.7)	82.7	4.89 (d, 5.0)	82.8	7'	2.29 (m)	49.9
4		82.5		81.6	8' β	1.93 (m)	25.5
5	3.88 (brs)	72.5	4.07 (brs)	74.0	8' α	1.60 (m)	
6		60.1		60.4	9' β	1.44 (m)	32.1
7	3.31 (brs)	63.8	3.44 (brs)	64.9	9' α	1.74 (m)	
8	4.38 (brs)	35.6	4.75 (brs)	35.6	10'	1.59 (m)	40.6
9		77.7		78.0	11'	1.88 (m)	36.3
10	3.01 (dd, 13.5, 6.2)	47.3	2.85 (t, 10.5)	49.4	12'	0.84 (d, 7.0)	18.2
11	3.09 (d, 6.6)	37.1	2.82 (d, 5.6)	36.7	1''		108.2
12	3.90 (brs)	84.0	4.02 (brs)	84.3	2''		138.8
13		71.4		71.6	3''/7''	7.71 (dd, 8.0, 2.5)	125.0
14	4.16 (brs)	81.2	4.26 (br s)	80.4	4''/6''	7.38 (overlapped)	128.1
15		75.3		76.3	5''	7.36 (m)	129.3
16 β	1.52 (m)	36.0	1.51 (m)	37.0	1'''		165.7
16 α	2.42 (d, 15.4)		2.35 (d, 15.4)		2'''		130.3
17	1.32 (s)	28.3	1.43 (s)	28.7	3'''/7'''	8.00 (dd, 8.4, 1.1)	129.5
18	1.65 (d, 6.6)	18.8	1.76 (d, 6.4)	18.9	4'''/6'''	7.42 (t, 8.4)	128.3
19	1.05 (d, 6.6)	13.2	1.10 (d, 6.6)	13.1	5'''	7.55 (t, 8.4)	132.9
20b	3.77 (d, 12.7)	61.7	3.91 (d, 12.7)	65.3	OH-4	3.57 (brs)	2.93 (brs)
20a	3.59 (d, 12.7)		3.81 (d, 12.7)		OH-5		
1'		169.4		168.7	OH-13	3.84 (brs)	3.78 (brs)
2'	5.89 (d, 15.4)	122.7	5.72 (d, 11.9)	119.2	OH-15		3.68 (brs)
3'	7.38 (overlapped)	145.8	6.54 (t, 11.9)	141.8			

Tables 2 and 1; ESIMS m/z 607.4 $[\text{M} + \text{H}]^+$, 645.4 $[\text{M} + \text{K}]^+$; HRESIMS m/z 645.2108 $[\text{M} + \text{K}]^+$ (calcd for $\text{C}_{34}\text{H}_{38}\text{O}_{10}\text{K}$, 645.2102).

Trigonosin C (3): white powder; $[\alpha]_{\text{D}}^{26} -11.1$ (c 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.47), 230 (4.36) nm; IR (KBr) ν_{max} 3431, 2958, 2876, 1720, 1629, 1452, 1387, 1350, 1317, 1281, 1177, 1120, 1075, 1027, 987, 925, 757, 711 cm^{-1} ; ^1H and ^{13}C NMR data see Tables 2 and 1; ESIMS m/z 609.4 $[\text{M} + \text{H}]^+$, 631.4 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 631.2515 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{34}\text{H}_{40}\text{O}_{10}\text{Na}$, 631.2519.1242).

Trigonosin D (4): white powder; $[\alpha]_{\text{D}}^{26} +0.8$ (c 0.30, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.13), 231 (4.11) nm; IR (KBr) ν_{max} 3567, 3511, 3459, 2992, 2963, 2934, 1755, 1728, 1452, 1377, 1272, 1237, 1134, 1111, 1076, 1024, 961, 772, 735, 717, 599 cm^{-1} ; ^1H and ^{13}C NMR data see Tables 2 and 1; ESIMS m/z 653 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 653.2560 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{33}\text{H}_{42}\text{O}_{12}\text{Na}$, 653.2573).

Trigonosin E (5): white powder; $[\alpha]_{\text{D}}^{26} -31.7$ (c 0.24, MeOH); UV (MeOH) λ_{max} (log ϵ) 193 (4.01), 202 (4.13), 255 (4.20) nm; IR (KBr) ν_{max} 3440, 2937, 1711, 1630, 1451, 1330, 1269, 1112, 1027, 993, 713 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; ESIMS m/z 815.0 $[\text{M} + \text{H}]^+$, 837.4 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 837.3469 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{13}\text{Na}$, 837.3462).

Trigonosin F (6): white powder; $[\alpha]_{\text{D}}^{26} +64.6$ (c 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.19), 255 (4.20) nm; IR (KBr) ν_{max} 3440, 2958, 1719, 1639, 1451, 1332, 1265, 1092, 1026, 713 cm^{-1} ; ^1H and ^{13}C NMR data see Table 3; ESIMS m/z 815.3 $[\text{M} + \text{H}]^+$, 837.5 $[\text{M} + \text{Na}]^+$; HRESIMS m/z 837.3468 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{13}\text{Na}$, 837.3462).

Cytotoxicity Assay. Cytotoxicity of compounds 1–10 against HL-60 (human promyelocytic leukemia) cells was measured by the

MTT method,¹² and that against A549 (human lung adenocarcinoma) and MCF-7 (human breast cancer) was determined by the SRB method. Adriamycin was used as the positive control (IC_{50} 0.13 μM against HL-60, IC_{50} 0.41 μM against A549, IC_{50} 0.15 μM against MCF-7). Cells were plated in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds for 72 h. Inhibition rates of cell proliferation after compound treatment were determined by the MTT and SRB methods.^{11,12}

■ ASSOCIATED CONTENT

Supporting Information. $[\alpha]$, MS, IR, UV, ^1H and ^{13}C NMR, and 2D NMR spectra of compounds 1–6 are available free of charge via the Internet at <http://pubs.acs.org>.

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■ ACKNOWLEDGMENT

This work was supported financially by the Ministry of Science and Technology of China (2009CB940900 and 2009CB522300), National Natural Science Funding of China (21072199), Natural Science Funding of Yunnan Province (2009CD112 and 21072199), Foundation of Chinese Academy

of Sciences to H P.H., and the Yong Academic and Technical Leader Raising Foundation of Yunnan Province (2010CI047).

■ DEDICATION

Dedicated to Dr. Koji Nakanishi of Columbia University for his pioneering work on bioactive natural products.

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