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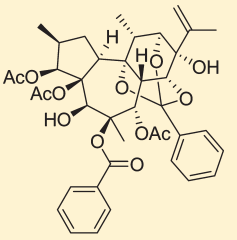
Daphnane-Type Diterpenoids from *Trigonostemon howii*

Shi-Hui Dong, Chuan-Rui Zhang, Cheng-Hui Xu, Jian Ding, and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zu Chong Zhi Road, Shanghai, 201203, People's Republic of China

Supporting Information

ABSTRACT: Nine new daphnane-type diterpenoids (**1**−**9**), named trigohownins A−I, and four known analogues were isolated from *Trigonostemon howii*. Their structures were elucidated on the basis of extensive NMR and MS analyses. Trigohownins A (**1**) and D (**4**) exhibited moderate cytotoxic activity against the HL-60 tumor cell line, with IC_{50} values of 17.0 and 9.3 μM, respectively.



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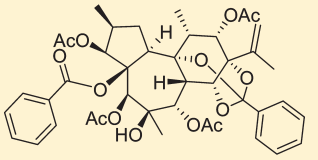

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The genus *Trigonostemon* (Euphorbiaceae) consists of about 50 species that grow mainly in tropical and subtropical areas of Asia.[1] Previous chemical investigations on the members of this genus focused on *T. reidioides*,[2] *T. chinensis*,[3,4] *T. lii*,[5] *T. thyrsoideum*,[6] and *T. xyphophylloides*,[7] resulting in the isolation of structurally diverse compounds such as daphnane-type diterpenoids,[4,6,7] indole alkaloids,[5] and phenanthrenes.[2] The daphnane-type diterpenoids are an important compound class isolated from this genus, and they are reported to have a variety of biological activities such as inhibition against MET tyrosine kinase activity,[4] inhibition against HIV-1-induced cytopathic effect,[6] and cytotoxic activity.[4,6−12] A chemical investigation of *Trigonostemon howii* Merr. et Chun (Euphorbiaceae), a shrub native to Hainan Province of China, has not been documented previously. In the current study, nine new daphnane-type diterpenoids (**1**−**9**) and four known analogues were isolated from the twigs of *T. howii*. The cytotoxic activities of compounds **1**−**9** against tumor cell lines HL-60 and A-549 were evaluated. Herein we present the isolation, structure elucidation, and cytotoxic evaluation of these isolates.

RESULTS AND DISCUSSION

Compound **1**, a white solid, gave a molecular formula of $C_{40}H_{46}O_{13}$, as established on the basis of the HRESIMS spectrum, requiring 18 degrees of unsaturation. Its IR spectrum showed the presence of OH (3396 cm^{-1}) and carbonyl (1759, 1741, and 1703 cm^{-1}) groups. All 40 carbon resonances were well resolved in the ^{13}C NMR spectrum (Table 1) and were further classified by DEPT experiments as seven methyls, two methylenes (one olefinic), 19 methines (five oxygenated and 10 olefinic), and 12 quaternary carbons (four ester carbonyls, one orthoester, and four oxygenated and three olefinic carbons). Two methyl doublets (δ_H 0.96, 3H, d, 6.7 and 1.75, 3H, d, 6.9), two methyl singlets (δ_H 1.59 and 1.67, each 3H, s), a terminal double bond (δ_H 4.53 and 4.56, each 1H, s), three acetyl groups, an orthobenzoate group, and a benzoyl group were evident from the ^1H NMR data (Table 2). This accounted for 15 of the 18 degrees of unsaturation, and the remaining three required that **1** was tricyclic. Proton resonances at δ_H 3.69, s and 6.15, d, 11.2, which did not correlate with any carbons in the HSQC spectrum, were attributed to OH groups. Analysis of the 1D and 2D NMR spectra of **1**, especially the HMBC spectrum (Figure S1, Supporting Information), indicated the typical A, B, and C rings of daphnane-type diterpenoids.[13,14] In the HMBC spectrum of **1** (Figure S1, Supporting Information), OH groups resonating at δ_H 3.69 and 6.15 were assigned to C-13 and C-5 by the key correlations between 13-OH and C-13 (δ_C 70.0) and between 5-OH and C-5 (δ_C 78.6), respectively. Acetoxyl groups were placed at C-3 (δ_C 76.7) and C-7 (δ_C 74.8) on the basis of HMBC correlations from H-3 and H-7 to each of the corresponding carbonyls of the acetyls, respectively; attachment of the orthobenzoate group at C-9, C-12, and C-14 was determined by HMBC correlations from H-12, H-14, and H-3′ (or H-7′) to C-1′ and from H-12, H-14, and H-11 to C-9, which was supported by the chemical shifts of C-1′, C-9, C-12, and C-14 at δ_C 107.5, 76.6, 83.1, and 79.9, respectively.[7,9] The only benzoyloxy group was attached to C-6 (δ_C 85.7) by comparing its chemical shift with that in trigoxyphins D−F.[7] The remaining oxygenated quaternary carbon at δ_C 95.5 was only assignable to C-4 bearing the remaining acetoxyl group by the HMBC correlations from 5-OH, H-1, and H-10 to C-4 (Figure S1, Supporting Information). As a result, the C-4 signal of **1** shifted downfield severely as compared to its known analogues with a 4-OH.[4,6,7,14] Thus, the planar structure of **1** was determined.

The relative configuration of **1** was defined by analysis of its ROESY spectrum (Figure S1, Supporting Information). The

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Table 1. ^{13}C NMR Spectroscopic Data (δ) of Compounds 1−9[a]

carbon	1	2	3	4	5	6	7	8	9
1	35.0	34.5	34.8	35.3	36.2	35.6	35.4	35.4	35.6
2	37.8	36.9	35.8	37.0	35.7	36.0	35.9	35.9	35.6
3	76.7	77.3	80.6	76.2	78.1	79.2	78.8	78.7	78.2
4	95.5	91.0	83.6	91.2	84.9	83.4	83.2	83.1	84.3
5	78.6	77.5	78.1	77.5	77.7	79.2	79.2	79.0	77.8
6	85.7	74.5	87.1	74.6	75.3	84.7	84.7	84.8	75.4
7	74.8	79.7	79.1	76.9	78.3	76.0	73.8	73.7	78.5
8	35.7	35.9	36.0	34.5	33.6	40.1	39.4	39.6	39.2
9	76.6	76.4	78.2	80.1	79.6	76.9	76.0	76.2	76.2
10	54.6	53.7	51.0	54.9	52.7	54.5	55.0	54.8	55.9
11	37.9	37.5	37.6	38.6	39.6	39.1	37.4	37.2	37.5
12	83.1	82.9	83.3	70.9	72.6	74.2	71.5	71.0	71.6
13	70.0	70.0	70.1	86.9	86.6	75.9	77.2	77.2	76.4
14	79.9	80.3	81.8	81.9	82.7	73.8	72.3	73.7	74.0
15	142.4	141.5	141.8	141.8	142.0	144.9	144.6	144.0	144.6
16	114.9	115.8	115.4	113.1	112.8	113.6	112.9	113.4	113.1
17	17.8	18.2	17.9	19.2	19.2	18.7	18.1	18.0	18.9
18	17.8	17.4	17.6	11.9	11.6	12.3	10.9	11.0	10.6
19	13.0	13.1	13.0	12.9	12.9	13.0	13.0	13.0	13.0
20	21.5	26.8	22.4	26.8	25.2	22.1	22.2	22.0	25.5
1′	107.5	107.4	108.0	117.1	166.1	165.4	165.0	165.0	166.2
2′	138.2	138.3	138.0	135.1	129.5	129.8	129.8	130.2	129.2
3′	125.1	125.0	125.0	125.9	129.8	129.8	129.8	129.7	129.9
4′	128.0	128.0	128.2	127.8	128.5	128.5	128.7	128.6	128.5
5′	129.6	129.5	129.7	129.5	133.4	133.4	133.3	133.3	133.6
6′	128.0	128.0	128.2	127.8	128.5	128.5	128.7	128.6	128.5
7′	125.1	125.0	125.0	125.9	129.8	129.8	129.8	129.7	129.9
1″	169.2	166.2	165.3	165.9	166.3	166.1	165.9	165.6	166.4
2″	130.1	131.8	131.3	130.8	129.6	130.7	131.0	131.1	129.6
3″	129.4	128.8	129.6	129.3	129.9	129.7	129.7	129.4	129.8
4″	128.7	128.4	128.5	128.8	128.5	128.4	128.4	128.3	128.5
5″	133.8	132.4	133.1	132.6	133.5	133.0	133.2	132.9	133.3
6″	128.7	128.4	128.5	128.8	128.5	128.4	128.4	128.3	128.5
7″	129.4	128.8	129.6	129.3	129.9	129.7	129.7	129.4	129.8
1‴					118.8				
2‴					21.7				
OAc-3	169.1	169.2	171.4	169.5	168.1	169.9	169.6	169.6	168.2
	21.9	20.5	21.0	20.5	20.3	20.9	20.9	20.8	20.3
OAc-4	168.9, 20.3								
OAc-5		171.2	171.1	171.3		171.1	171.1	171.1	
		20.9	20.9	20.8		21.0	20.9	20.9	
OAc-7	169.5	170.0		170.3	170.0	171.9	168.6	168.1	168.7
	21.0	21.0		21.2	21.6	21.7	21.4	20.9	21.6
OAc-12				170.0					
				20.5					
OAc-13								170.4	
								20.7	
OAc-14							169.9		170.2
							20.9		21.0

[a] Data were measured in $CDCl_3$ at 100 MHz.

ROESY correlations of H-8/4-OAc, H-8/H-7, H-8/H-11, H-8/H-14, and H-12/H-11 indicated that 4-OAc, H-7, H-8, H-11, H-12, and H-14 were cofacial and randomly assigned in a β-orientation. In consequence, the ROESY cross-peaks of H-3/H-2, H-3/H-5, 5-OH/3-OAc, H-5/H-10, and H_3-20/H-5 revealed that H-3, H-5, H-10, and H_3-20 were α-oriented. ROESY correlations of

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Table 2. ^{1}H NMR Spectroscopic Data of Compounds 1−5[a]

	1	2	3	4	5
proton position	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)
1	2.13, m	2.11, m	1.98, m	1.98, m	1.64, m
	2.25, m	2.27, m	2.12, m	2.18, m	2.07, m
2	1.92, m	2.01, m	1.86, m	2.03, m	2.02, m
3	6.21, d (4.5)	5.48, d (4.1)	4.75, d (4.5)	5.49, d (4.1)	5.15, d (7.2)
5	3.50, d (11.2)	5.06, s	5.09, s	5.15, s	5.18, s
7	6.62, s	5.50, s	5.26, s	5.55, s	5.57, s
8	2.36, s	3.05, s	2.89, s	2.95, d (2.3)	2.90, d (2.0)
10	2.98, dd (13.3, 5.5)	3.01, dd (13.2, 6.0)	3.16, dd (13.4, 5.8)	3.03, dd (13.1, 5.3)	2.62, dd (13.5, 4.8)
11	2.70, m	2.49, m	2.49, m	3.40, m	3.23, m
12	3.99, br s	3.97, d (1.3)	4.00, d (1.4)	5.43, d (8.0)	5.41, d (7.6)
14	4.39, d (2.4)	4.50, d (2.3)	4.40, d (2.4)	4.62, d (2.3)	4.51, d (2.6)
16	4.56, s	4.78, s	4.72, s	5.12, s	5.18, s
	4.53, s	4.75, s	4.61, d (1.1)	4.99, s	4.99, s
17	1.59, 3H, s	1.70, 3H, s	1.46, 3H, s	1.75, 3H, s	1.74, 3H, s
18	1.75, 3H, d (6.9)	1.60, 3H, d (6.6)	1.53, 3H, d (6.8)	1.31, 3H, d (6.7)	1.14, 3H, d (7.0)
19	0.96, 3H, d (6.7)	0.94, 3H, d (6.4)	0.96, 3H, d (6.6)	0.94, 3H, d (6.2)	0.85, 3H, d (6.3)
20	1.67, 3H, s	1.14, 3H, s	1.82, 3H, s	1.12, 3H, s	1.15, 3H, s
3′	7.63, m	7.65, m	7.66, dd (7.7, 2.4)	7.68, m	8.07, m
4′	7.36, m	7.37, m	7.40, m	7.36, m	7.47, m
5′	7.37, m	7.38, m	7.41, m	7.35, m	7.60, m
6′	7.36, m	7.37, m	7.40, m	7.36, m	7.47, m
7′	7.63, m	7.65, m	7.66, dd (7.7, 2.4)	7.68, m	8.07, m
3″	7.88, br d (7.7)	7.77, br d (7.7)	7.94, dd (8.4, 1.4)	8.01, m	8.07, m
4″	7.49, t (7.7)	7.43, t (7.7)	7.46, m	7.53, m	7.47, m
5″	7.63, m	7.56, m	7.60, m	7.55, m	7.60, m
6″	7.49, t (7.6)	7.43, t (7.7)	7.46, m	7.53, m	7.47, m
7″	7.88, br d (7.7)	7.77, br d (7.7)	7.94, dd (8.4, 1.4)	8.01, m	8.07, m
2‴					1.67, 3H, s
OH-4			2.42, s		3.51, s
OH-5	6.15, d (11.2)				
OH-6					4.70, s
OH-13	3.69, s				
OAc-3	1.67, 3H, s	1.80, 3H, s	2.21, 3H, s	1.76, 3H, s	1.96, 3H, s
OAc-4	1.64, 3H, s				
OAc-5		2.26, 3H, s	2.19, 3H, s	2.26, 3H, s	
OAc-7	1.74, 3H, s	1.75, 3H, s		1.65, 3H, s	2.17, 3H, s
OAc-12				2.07, 3H, s	

[a] Data were measured in $CDCl_3$ at 400 MHz.

H-12/H_2-16 and H-12/H_3-17 suggested that the 9,12,14-orthobenzoate group was α-directed. Therefore, the structure of trigohownin A (**1**) was assigned as depicted.

Compound **2** (trigohownin B) had the same molecular formula, $C_{40}H_{46}O_{13}$, as **1**, as determined by the HRESIMS spectrum at *m*/*z* 757.2827 $[M + Na]^+$. The IR absorption bands showed the presence of OH (3537 and 3448 cm^{-1}) and carbonyl (1740 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed that **2** was also a daphnane-type diterpenoid and had the same C ring as **1** based on the analysis of its HMBC spectrum (Figure S1, Supporting Information). The main differences were the substitution patterns of the A and B rings. Three acetoxyl groups were readily placed at C-3 (δ_C 77.3), C-5 (δ_C 77.5), and C-7 (δ_C 79.7) on the basis of the key HMBC correlations from H-3, H-5, and H-7 to each of the corresponding carbonyl signals of the acetyls. The only benzoyloxy group was located at C-4 (δ_C 91.0) by its chemical shift and HMBC correlations from H-1 and H-10 to C-4. The remaining oxygenated quaternary carbon at δ_C 74.5 was thus assigned to C-6 bearing an OH group by HMBC correlations from H-5, H-7, and H_3-20 to C-6 (Figure S1, Supporting Information). As a consequence, the signals of C-4 and C-6 shifted upfield by 4.5 and 11.2 ppm relative to those of **1**, respectively, resulting from their different substitution patterns and the γ-gauche effects from 5-Ac, while the carbon signal of C-7 shifted downfield by 4.9 ppm as compared with that of **1** due largely to the absence of a γ-gauche effect from 6-Bz in **2**. The relative configuration of **2** was assigned to be identical with that of **1** by comparing

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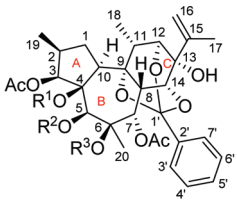
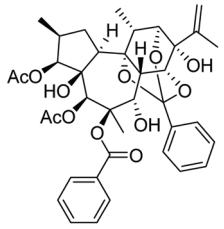
their NMR data and the analysis of its ROESY spectrum (S15, Supporting Information).



	R^1	R^2	R^3
1	Ac	H	Bz
2	Bz	Ac	H



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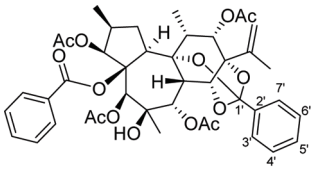

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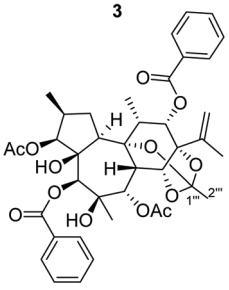

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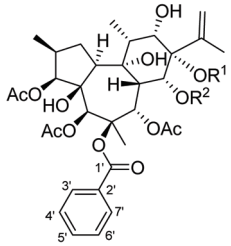
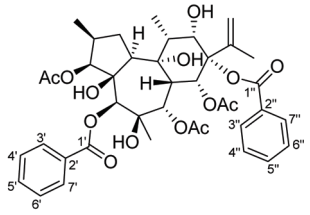


	R^1	R^2
6	Bz	H
7	Bz	Ac
8	Ac	Bz



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The HRESIMS spectrum of compound **3** gave a sodiated molecular ion peak at m/z 715.2740 $[M + Na]^+$ corresponding to the molecular formula $C_{38}H_{44}O_{12}$. The ^1H NMR spectrum of **3** (Table 2) was very similar to that of trigoxyphin D,[7] except for the presence of an obviously upfield-shifted H-7 at δ_H 5.26, s, instead of that of trigoxyphin D at δ_H 6.65, s, and with the concomitant absence of the proton signal of AcO-7 at δ_H 1.84 (s) in trigoxyphin D, indicating that **3** was the 7-deacetylated product of trigoxyphin D. This deduction was supported by its molecular weight, showing 42 mass units less than that of trigoxyphin D, and confirmed by the HMBC spectrum (Figure S1, Supporting Information). The relative configuration of **3** was assigned as being identical with that of trigoxyphin D[7] by its ROESY spectrum (S22, Supporting Information) and comparing their NMR patterns. Thus, the structure of **3** (trigohownin C) was determined as the 7-deacetate of trigoxyphin D.

The molecular formula of **4** was defined as $C_{42}H_{48}O_{14}$ by HRESIMS at m/z 799.2900 $[M + Na]^+$. Comparison of its 1D NMR data with those of **2** (Tables 1 and 2) suggested that they shared the same A and B rings, and this assignment was confirmed by the HMBC correlations (Figure S2, Supporting Information). According to the molecular formula and NMR data, an orthobenzoate group and an acetoxyl group were required in the C ring of **4** to satisfy its structure. Differing from **1**−**3**, the orthobenzoate group in **4** was attached to C-9 (δ_C 80.1), C-13 (δ_C 86.9), and C-14 (δ_C 81.9) by the typical quaternary carbon resonance C-1′ at δ_C 117.1 of the orthobenzoate group (Table 1),[7,9] which was confirmed by the HMBC correlation networks of H-14/C-1′, H_3-18 and H-7/C-9, H-7/C-14, and H-14 and H_3-17/C-13 (Figure S2, Supporting Information). The remaining acetoxyl group was placed at C-12 (δ_C 70.9) by the HMBC correlation from H-12 to the acetyl carbonyl (Figure S2, Supporting Information). The ROESY correlations of H-11/H-12, H-12/H_2-16, H-8/H-12, H-8/H-14, and H-7/H-14 indicated that H-12 and the C-15−C-17 moieties were β-oriented, and the 9,13,14-orthobenzoate group was α-oriented. The stereocenters in the A and B rings of **4** were identical to those of **2**, as assigned on the basis of the ROESY spectrum (Figure S2, Supporting Information). Thus, the structure of **4** (trigohownin D) was assigned.

Compound **5**, a white solid, had the molecular formula $C_{40}H_{46}O_{13}$, as determined by HRESIMS at m/z 757.2802 $[M + Na]^+$. Features of a daphnane-type diterpenoid orthoester for **5** were evident from its ^1H and ^{13}C NMR data (Tables 1 and 2), in which the functionalities of a terminal double bond, an orthoacetate group, two benzoyloxy groups, two acetoxyl groups, five oxygenated methines, and four oxygenated quaternary carbons were distinguishable. Proton resonances at δ_H 3.51, s, and 4.70, s, which did not correlate with any carbons in the HSQC spectrum, were attributed to OH groups. In the HMBC spectrum (Figure S2, Supporting Information) of **5**, the two OH groups, resonating at δ_H 3.51, s, and 4.70, s, were located at C-4 (δ_C 84.9) and C-6 (δ_C 75.3) by the key correlations of 4-OH/C-4 and 6-OH/C-6, respectively; benzoyloxy groups were placed at C-5 (δ_C 77.7) and C-12 (δ_C 72.6) according to the HMBC correlations from H-5 and H-12 to each of the corresponding benzoyl carbonyls, respectively; acetoxyl groups were located at C-3 (δ_C 78.1) and C-7 (δ_C 78.3) by the HMBC correlations from H-3 and H-7 to each of the corresponding carbonyl of the acetyls, respectively. An 9,13,14-orthoacetate group was assigned on the basis of the typical chemical shifts of C-1‴ (δ_C 118.8), C-9 (δ_C 79.6), C-13 (δ_C 86.6), and C-14 (δ_C 82.7)[7,9] and confirmed by the HMBC correlations of H-14/C-1‴ and C-9, H_2-16/C-13, and H-7/C-14 (Figure S2, Supporting Information). The relative configuration of **5** was assigned as depicted by the ROESY spectrum (S36, Supporting Information), as well as by comparing its NMR data with those of **4**. Therefore, the structure of **5** (trigohownin E) was elucidated as shown.

Compound **6** had the molecular formula $C_{40}H_{48}O_{14}$, as established by HRESIMS at m/z 775.2926 $[M + Na]^+$. Analysis of ^1H and ^{13}C NMR spectra of **6** (Tables 1 and 3) suggested a daphnane-type diterpenoid having three acetoxyl groups, two benzoyloxy groups, four methyl groups, five oxygenated methines, and four oxygenated quaternary carbons. Differing from **1**−**5**, the characteristic orthoester group was not observed in the 1D NMR spectra of **6** (Tables 1 and 3). In the HMBC spectrum of **6** (Figure S3, Supporting Information), a proton signal at δ_H 2.75, s, which did not correlate with any carbons in the HSQC spectrum, was assigned to the 4-OH by the correlation from 4-OH to C-4; acetoxyl groups were placed at C-3, C-5, and C-7, by correlations from H-3, H-5, and H-7 to each of the corresponding carbonyl signals of the acetyls. Two quaternary carbon signals, at δ_C 84.7 and 75.9, were assigned to C-6 and C-13, bearing benzoyloxy groups, by their chemical shifts[7] and HMBC correlations from H_3-20 and H-7 to C-6 and from H_2-16 and H_3-17 to C-13. A quaternary carbon signal at δ_C 76.9 was assigned to

Table 3. ^{1}H NMR Spectroscopic Data of Compounds 6−9[a]

	6	7	8	9
proton position	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)
1	1.77, m	1.69, m	1.77, m	1.67, m
	2.03, m	2.09, m	2.13, m	2.18, m
2	1.97, m	2.04, m	2.00, m	2.05, m
3	4.99, d (7.1)	5.02, d (7.1)	5.00, br s	5.14, d (7.2)
5	5.06, s	4.96, s	4.91, s	5.08, s
7	6.36, s	6.16, s	6.23, s	5.25, s
8	3.22, d (3.2)	3.60, d (6.2)	3.58, d (4.1)	3.57, d (6.7)
10	2.59, dd (13.2, 4.3)	2.50, dd (12.2, 3.0)	2.52, dd (10.3, 2.2)	2.40, dd (12.9, 4.4)
11	3.06, br s	3.16, br s	3.17, br s	3.14, br s
12	5.27, br s	5.10, br s	5.01, br s	5.25, br s
14	3.98, br s	5.39, d (6.2)	5.63, d (5.7)	5.64, d (6.2)
16	5.01, s	5.07, s	4.99, s	5.27, s
	4.60, s	4.78, s	4.75, s	4.95, s
17	1.23, 3H, s	1.33, 3H, s	1.38, 3H, s	1.78, 3H, s
18	1.11, 3H, d (7.0)	1.12, 3H, d (7.0)	1.15, 3H, d (7.1)	1.16, 3H, d (7.0)
19	0.91, 3H, d (6.4)	0.91, 3H, d (6.6)	0.94, 3H, d (6.8)	0.88, 3H, d (6.7)
20	1.65, 3H, s	1.63, 3H, s	1.56, 3H, s	1.09, 3H, s
3′	8.05, d (7.6)	8.12, d (7.4)	8.11, d (7.5)	8.05, m
4′	7.47, t (7.6)	7.49, t (7.4)	7.49, t (7.5)	7.46, m
5′	7.58, t (7.6)	7.57, t (7.4)	7.59, t (7.5)	7.59, m
6′	7.47, t (7.6)	7.49, t (7.4)	7.49, t (7.5)	7.46, m
7′	8.05, d (7.6)	8.12, d (7.4)	8.11, d (7.5)	8.05, m
3″	8.00, d (7.5)	8.01, d (7.4)	7.94, d (8.3)	8.05, m
4″	7.41, t (7.5)	7.42, t (7.4)	7.41, t (8.3)	7.46, m
5″	7.54, t (7.5)	7.57, t (7.4)	7.54, t (8.3)	7.59, m
6″	7.41, t (7.5)	7.42, t (7.4)	7.41, t (8.3)	7.46, m
7″	8.00, d (7.5)	8.01, d (7.4)	7.94, d (8.3)	8.05, m
OH-4	2.75, s		2.78, s	
OAc-3	2.23, 3H, s	2.22, 3H, s	2.21, 3H, s	1.95, 3H, s
OAc-5	2.13, 3H, s	2.11, 3H, s	2.09, 3H, s	
OAc-7	2.24, 3H, s	2.22, 3H, s	1.83, 3H, s	2.22, 3H, s
OAc-13			2.03, 3H, s	
OAc-14		2.00, 3H, s		2.01, 3H, s

[a] Data were measured in $CDCl_3$ at 400 MHz.

C-9, bearing an OH group, by comparing its chemical shift with that of trigochinin A[4] and HMBC correlations from H-8, H-10, and H_3-18 to C-9. The remaining two oxygenated methines were assigned to C-12 (δ_C 74.2) and C-14 (δ_C 73.8), bearing OH groups, by the chemical shifts of relevant protons and carbons and the HMBC correlations of H-12 and H-14/C-9 and H-7/C-14. The relative configuration of **6** was established by a ROESY experiment (Figure S3, Supporting Information), as well as by analysis of its 1D NMR data (Tables 1 and 3). ROESY correlations of H-8/H-7, H-8/H-11, H-8/H-12, H-8/H-14, H-12/H_2-16, H_2-16/H_3-17, and H-14/H_3-17 indicated that H-8, H-11, H-12, H-14, and C-15−C-17 moieties were cofacial and randomly assigned in a β-configuration. The chemical shift of C-9 of **6** (Table 1) resembled that of trigochinins A, B, and D−F,[4,15] indicating that the 9-OH was α-oriented. The other stereocenters in the A and B rings of **6** were identical with those of **3** by comparing the NMR data of related protons and carbons. Thus, the structure of **6** (trigohownin F) was elucidated.

The molecular formula of compound **7** was defined as $C_{42}H_{50}O_{15}$ by HRESIMS. The ^{1}H NMR spectrum (Table 3) of **7** showed high similarities to that of **6**, and the major differences were that the proton signal of H-14 was severely downfield shifted at δ_H 5.39 compared with that of **6** at δ_H 3.98, and the occurrence of one more proton signal of an acetyl group at δ_H 2.00, s, in **7**, indicating that compound **7** was the 14-acetylated derivative of **6**. This deduction was supported by its molecular weight, 42 mass units more than **6**, and confirmed by its HMBC spectrum (S49, Supporting Information). The relative configuration of **7** was assigned as being identical with that of **6** by analysis of its ROESY spectrum (S50, Supporting Information) and the comparison of their NMR patterns. Thus, **7** (trigohownin G) was determined to be the 14-acetate of trigohownin F.

Compound **8** had the same molecular formula ($C_{42}H_{50}O_{15}$) as **7**. Comparison of its 1D NMR data with those of **7** (Tables 1 and 3) suggested that they had the same A and B rings, and this

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was confirmed by its HMBC spectrum (S56, Supporting Information). The main differences were the substitution patterns in the C ring. Attachment of a benzoyloxy group at C-14 (δ_C 73.7) was indicated by the key HMBC correlation between H-14 and the carbonyl carbon of the benzoyl group at δ_C 165.6. An oxygenated methine resonating at δ_C 71.0 was assigned to C-12, bearing an OH group, on the basis of HMBC correlations of H-12/C-13 and H_3-18/C-12. Finally, the remaining acetoxyl group was assignable to the oxygenated quaternary carbon C-13 (δ_C 77.2) by the HMBC correlations from H_2-16 and H_3-17 to C-13 (S56, Supporting Information). The relative configuration of **8** was identical to that of **7** by the analysis of its ROESY spectrum (S57, Supporting Information) and by comparing their NMR data. Thus, the structure of **8** (trigohownin H) was determined as shown.

Compound **9** gave a molecular formula of $C_{40}H_{48}O_{14}$, as established by the HRESIMS at m/z 775.2906 $[M + Na]^+$. Analysis of the ^1H and ^{13}C NMR spectra of **9** (Tables 1 and 3) suggested a daphnane-type diterpenoid and showed the presence of three acetoxyl groups, two benzoyloxy groups, four methyl groups, five oxygenated methines, and four oxygenated quaternary carbons. In the HMBC spectrum of **9** (Figure S3, Supporting Information), acetoxyl groups were located at C-3 (δ_C 78.2), C-7 (δ_C 78.5), and C-14 (δ_C 74.0) by the correlations from H-3, H-7, and H-14 to each of the corresponding carbonyls of the acetyls. Attachment of a benzoyloxy group at C-5 (δ_C 77.8) was indicated by the key HMBC correlation between H-5 and the carbonyl carbon of the benzoyl group. The other benzoyloxy group was placed at C-13 (δ_C 76.4) by comparing its chemical shift with that of **7** and the HMBC correlations from H-8, H-12, and H-16a to C-13. An oxygenated methine was assigned to C-12 (δ_C 71.6), bearing an OH group, by the HMBC correlations of H-12/C-9 and C-13, and H_3-18/C-12. An oxygenated quaternary carbon at δ_C 75.4 was assigned to C-6, bearing an OH, by the HMBC correlations from H-7, H-8, and H_3-20 to C-6 (Figure S3, Supporting Information). The remaining two oxygenated quaternary carbons at δ_C 84.3 and 76.2 were respectively assigned to C-4 and C-9, bearing two OH groups, by comparing their chemical shifts with those of compounds **6**−**8** (Table 1). The relative configuration of **9** was established to be identical with those of **6**−**8** by the analysis of its ROESY spectrum (S64, Supporting Information) and comparing their NMR data. Thus, the structure of **9** (trigohownin I) was determined.

Four known analogues, trigoxyphins A and D−F,[7] were also obtained and were identified on the basis of ^1H NMR, ^{13}C NMR, and ESIMS data.

Compounds **1**−**9** were evaluated for cytotoxic activity against tumor cell lines HL-60 and A-549 using the MTT method[16] and SRB method,[17] respectively. Trigohownins A (**1**) and D (**4**) exhibited moderate cytotoxic activities against the HL-60 cell line, with IC_{50} values of 17.0 and 9.3 μM, respectively.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on an SGW X-4 melting instrument and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter at room temperature. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 IR spectrometer. NMR spectra were obtained on a Bruker AM-400 NMR spectrometer with TMS as internal standard. HRESIMS was carried out on a Bruker Daltonics micrOTOFQII mass spectrometer. Semipreparative HPLC was carried out on a Waters 515 pump with a Waters 2487 detector (254 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). Silica gel (300−400 mesh), C_{18} reversed-phase silica gel (250 mesh, Merck), Sephadex LH-20 (Amersham Biosciences), and MCI gel (CHP20P, 75−150 μM, Mitsubishi Chemical Industries, Ltd.) were used for column chromatography (CC), and precoated silica gel GF254 plates (Qingdao Marine Chemical Plant, Qingdao, P. R. of China) were used for TLC. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, P. R. China).

Plant Material. The twigs of *Trigonostemon howii* were collected in April 2007 from Sanya, Hainan Island, People's Republic of China, and authenticated by Professor S. M. Huang of the Department of Biology, Hainan University. A voucher specimen (accession number: SMTrho-2007-1Y) has been deposited at the Shanghai Institute of Materia Medica.

Extraction and Isolation. The air-dried powder of twigs of *T. howii* (2.5 kg) was extracted three times with 95% EtOH (each 10 L, for three days) at room temperature to give an ethanolic extract (260 g), which was partitioned between EtOAc and H_2O to obtain the EtOAc-soluble fraction (40 g). The EtOAc-soluble fraction was subjected to MCI gel CC, eluted with MeOH/H_2O (40/60 to 90/10), to produce fractions A−D. Fraction B (10 g) was chromatographed over a silica gel column, eluted with petroleum ether/acetone (10/1 to 1/1), to afford four major subfractions, B1−B4. Fraction B1 was subjected to a reversed-phase C_{18} silica gel column, eluted with MeOH/H_2O (50/50 to 80/20), to give two major fractions, B1a and B1b. Both of them were separated by semipreparative HPLC with 55% MeCN in H_2O, to yield compounds **8** (16 mg) and **9** (5 mg). Using the same purification procedures, fraction B3 gave compound **3** (10 mg) and trigoxyphin D (20 mg), while fraction B4 gave compound **1** (11 mg) and trigoxyphin F (18 mg). Fraction C (12 g) was chromatographed over a silica gel column, eluted with petroleum ether/acetone (10/1 to 1/1), to afford subfractions C1−C3. Fraction C1 was subjected to Sephadex LH-20 CC eluted with CH_3OH, and the major portion was further separated by semipreparative HPLC with 60% MeCN in H_2O to yield compounds **2** (8 mg), **6** (20 mg), and **7** (12 mg). Fraction C2 was subjected to silica gel CC, eluted with $CHCl_3$/MeOH (100/1 to 20/1), to afford four major fractions (C2a−C2d), each of which was then purified by semipreparative HPLC with 55% acetonitrile in water to yield compounds **4** (20 mg), **5** (4 mg), trigoxyphin A (17 mg), and trigoxyphin E (20 mg).

*Trigohownin A (**1**):* white solid; mp 147−148 °C; $[\alpha]^{24}_D$ +27.0 (*c* 0.06, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.17) nm; IR (KBr) ν_{max} 3396, 2974, 1759, 1741, 1703, 1452, 1369, 1281, 1223, 1030, 716 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 757.2825 $[M + Na]^+$ (calcd for $C_{40}H_{46}NaO_{13}$, 757.2836).

*Trigohownin B (**2**):* white solid; mp 151−153 °C; $[\alpha]^{24}_D$ +51.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 227 (4.03) nm; IR (KBr) ν_{max} 3537, 3448, 2962, 1740, 1452, 1371, 1229, 1124, 1030, 719 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 757.2827 $[M + Na]^+$ (calcd for $C_{40}H_{46}NaO_{13}$, 757.2836).

*Trigohownin C (**3**):* white solid; mp 125−127 °C; $[\alpha]^{24}_D$ −24.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.16) nm; IR (KBr) ν_{max} 3450, 2964, 1745, 1716, 1452, 1371, 1232, 1030, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 715.2740 $[M + Na]^+$ (calcd for $C_{38}H_{44}NaO_{12}$, 715.2730).

*Trigohownin D (**4**):* white solid; mp 230−231 °C; $[\alpha]^{24}_D$ +56.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.25) nm; IR (KBr) ν_{max} 3467, 2972, 1740, 1452, 1375, 1236, 1032, 908 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 799.2900 $[M + Na]^+$ (calcd for $C_{42}H_{48}NaO_{14}$, 799.2942).

*Trigohownin E (**5**):* white solid; mp 142−143 °C; $[\alpha]^{24}_D$ +7.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.30) nm; IR (KBr) ν_{max} 3437, 2970, 1759, 1720, 1452, 1400, 1271, 1230, 1070, 712 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS m/z 757.2802 $[M + Na]^+$ (calcd for $C_{40}H_{46}NaO_{13}$, 757.2836).

Trigohownin F (**6**): white solid; mp 139−140 °C; $[\alpha]^{24}_{D}$ −30.0 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.49) nm; IR (KBr) ν_{max} 3467, 2964, 1747, 1720, 1452, 1373, 1236, 1117, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 775.2926 $[M + Na]^+$ (calcd for $C_{40}H_{48}NaO_{14}$, 775.2942).

Trigohownin G (**7**): white solid; mp 142−143 °C; $[\alpha]^{24}_{D}$ −24.0 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.55) nm; IR (KBr) ν_{max} 3547, 3410, 2955, 1751, 1716, 1452, 1279, 1232, 1028, 721 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 817.3060 $[M + Na]^+$ (calcd for $C_{42}H_{50}NaO_{15}$, 817.3047).

Trigohownin H (**8**): white solid; mp 150−152 °C; $[\alpha]^{24}_{D}$ +17.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.52) nm; IR (KBr) ν_{max} 3446, 2966, 1736, 1452, 1373, 1236, 1034, 712 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 817.3042 $[M + Na]^+$ (calcd for $C_{42}H_{50}NaO_{15}$, 817.3047).

Trigohownin I (**9**): white solid; mp 138−140 °C; $[\alpha]^{24}_{D}$ +23.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.40) nm; IR (KBr) ν_{max} 3442, 2933, 1743, 1720, 1452, 1375, 1246, 1028, 714 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 3; HRESIMS *m*/*z* 775.2906 $[M + Na]^+$ (calcd for $C_{40}H_{48}NaO_{14}$, 775.2942).

Cytotoxic Assay. Cytotoxicity of compounds **1**−**9** against HL-60 cells was measured using the MTT method.[16] Briefly, cells in 100 μL of culture medium were plated in each well of 96-well plates (Falcon, CA). Cells were treated in triplicate with graded concentrations of compounds at 37 °C for 72 h. A 20 μL aliquot of MTT solution (5 mg/mL) was added directly to the appropriate wells. The cultures were incubated for 4 h, and then 100 μL of "triplex solution" (10% SDS/5% isobutanol/12 mM HCl) was added. The plates were incubated at 37 °C overnight and then measured using a plate reader at 570 nm (VERSA Max, Molecular Devices). The cytotoxic activities of **1**−**9** against A-549 cells were tested using the SRB assay.[17] In short, the cells were seeded in 96-well plates (Falcon, CA) and allowed to attach overnight. The cells were treated in triplicate with graded concentrations of compounds at 37 °C for 72 h and were then fixed with 10% trichloroacetic acid and incubated at 4 °C for 1 h. The culture plates were washed and dried, and SRB solution (0.4 wt %/vol in 1% acetic acid) was added; the plates were incubated for an additional 15 min. The culture plates were washed and dried again, the bound cell stains were solubilized with Tris buffer, and the optical density of each well was read using the plate reader at a wavelength of 515 nm. The results were all expressed as IC_{50} values as calculated by the Logit method.

■ ASSOCIATED CONTENT

Supporting Information. IR, HRESIMS, and 1D and 2D NMR spectra of compounds **1**−**9** are available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86-21-50806718. Fax: +86-21-50806718. E-mail: jmyue@mail.shcnc.ac.cn.

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