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Constituents of *Trigonostemon heterophyllus*

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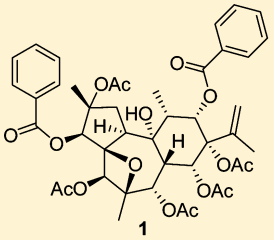
Supporting Information

ABSTRACT: Six new diterpenoids, **1**–**6**, a new prenylated sesquiterpenoid, **7**, and six known compounds were isolated from *Trigonostemon heterophyllus*. The structures of **1**–**7** were elucidated on the basis of NMR and MS analysis. The daphnane diterpenoids trigoheterins A (**1**) and B (**2**) possess a rare 4,6-oxetane moiety within this compound class, and trigoheteric acid methyl ester (**6**) is the first example of a 15,16-dinor-3,4-*seco*-cleistanthane. Trigoheterin E (**5**) exhibited cytotoxic activity against the HL-60 (IC_{50} 1.8 μM) and A-549 (IC_{50} 10.0 μM) human cancer cell lines.



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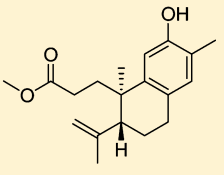

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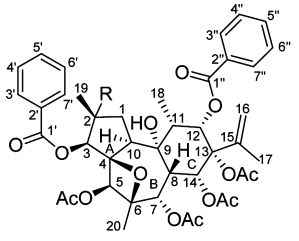
The genus *Trigonostemon* (Euphorbiaceae) in the People's Republic of China comprises 10 species growing mainly in the southern regions.[1] Previous chemical investigations on this genus in our group have afforded an array of structurally and biologically interesting diterpenoids, which showed potent antimicrobial,[2] cytotoxic,[3−6] and Met tyrosine kinase inhibitory activities.[7] A chemical study on *Trigonostemon heterophyllus* Merr., a shrub native to Hainan Province, has not been documented hitherto. In the current study, 13 compounds, including five new daphnane diterpenoids (**1**–**5**), a new 3,4-seco-cleistanthane dinorditerpenoid (**6**), a new prenylated bisabolane sesquiterpenoid (**7**), and six known diterpenoid analogues, were isolated from the twigs of *T. heterophyllus*. Trigoheterins A (**1**) and B (**2**) possess a rare 4,6-oxetane moiety in the family of daphnane diterpenoids,[4,7] and trigoheteric acid methyl ester (**6**) features the first 15,16-dinor-3,4-*seco*-cleistanthane skeleton.[2,8] The cytotoxic activities of **1**–**7** against two human cancer cell lines were evaluated. Herein, we present the isolation, structural elucidation, and cytotoxic evaluation of these isolates.



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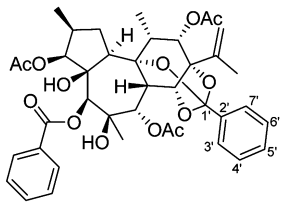
2 R = H



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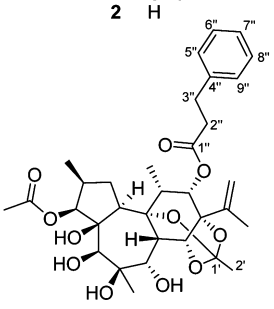

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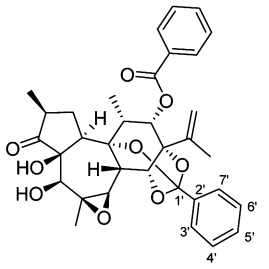

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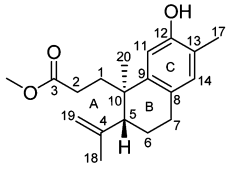

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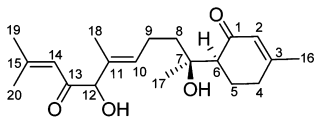

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RESULTS AND DISCUSSION

Compound **1** was obtained as a white solid, and its molecular formula was established as $C_{44}H_{50}O_{16}$, on the basis of the HRESIMS requiring 20 degrees of unsaturation. The IR spectrum showed the presence of OH (3446 cm^{-1}) and carbonyl (1753 and 1730 cm^{-1}) groups. All 44 carbon resonances were well-resolved in the ^{13}C NMR spectrum (Table 1) and were further classified by DEPT experiments as nine methyls, two methylenes (one olefinic), 18 methines (five oxygenated and 10 olefinic), and 15 quaternary carbons (seven ester carbonyls, five oxygenated, and three olefinic). A methyl doublet (δ_H 1.21, 3H, d, 6.8), three methyl singlets (δ_H 1.16, 1.51, and 1.98, each 3H, s), a terminal double bond (δ_H 5.42 and 5.43, each 1H, s), five acetyl groups, and two benzoyl groups were evident from the NMR data (Tables 1 and 2). These functionalities accounted for 16 out of the 20 degrees of unsaturation, and the remaining four required that **1** is tetracyclic. A ^1H NMR resonance at δ_H 3.58 (about 1H, s), which did not correlate with any carbons in the HSQC spectrum, was attributed to an OH group. Analysis of the 1D- and 2D-NMR spectra of **1**, especially the HMBC spectrum

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Table 1. ^{13}C NMR Spectroscopic Data of Compounds 1−7[a]

carbon	1	2	3	4	5	6	7
1	39.6	34.9	36.2	35.2	33.1	34.4	203.6
2	85.3	31.0	35.8	35.7	42.8	29.5	127.3
3	77.3	73.1	78.1	80.4	220.6	175.3	163.9
4	91.0	91.2	84.8	85.0	75.0	146.6	31.5
5	72.8	73.3	77.5	75.9	72.8	46.9	25.0
6	84.3	84.0	75.2	76.2	59.6	24.8	55.1
7	79.2	79.3	78.1	80.8	67.1	29.4	74.4
8	39.1	39.1	33.8	34.2	35.4	128.6	36.2
9	76.8	76.7	80.3	80.8	81.1	141.5	22.3
10	47.2	49.3	52.5	51.5	44.3	40.7	133.0
11	39.7	40.0	39.4	38.6	39.3	112.4	132.8
12	73.4	73.3	71.8	71.5	72.2	152.5	83.3
13	80.6	80.6	86.7	86.6	86.5	122.0	199.2
14	74.9	75.1	82.5	84.1	82.4	131.2	118.8
15	140.0	140.1	141.7	142.0	142.0		159.9
16	119.3	119.2	113.1	112.7	113.3		24.2
17	20.1	20.1	19.4	19.0	19.4	15.4	25.7
18	11.6	11.8	11.8	11.4	11.8	22.7	10.8
19	23.0	16.2	12.9	13.0	12.5	114.2	28.0
20	19.7	19.8	25.1	26.4	21.7	28.0	21.3
1′	164.7	165.6	117.5	119.1	118.2		
2′	130.1	130.1	135.4	21.5	135.5		
3′	129.7	129.6	125.9		128.0		
4′	128.6	128.6	127.9		126.1		
5′	133.3	133.2	129.5		129.5		
6′	128.6	128.6	127.9		126.1		
7′	129.7	129.6	125.9		128.0		
1″	165.7	165.7	166.2	172.29	165.8		
2″	129.9	129.8	129.3	35.4	129.6		
3″	129.6	129.5	129.9	30.7	129.8		
4″	128.6	128.5	128.4	140.1	128.5		
5″	133.4	133.3	133.5	128.2	133.3		
6″	128.6	128.5	128.4	128.5	128.5		
7″	129.6	129.5	129.9	126.3	129.8		
8″				128.5			
9″				128.2			
OAc-2	170.4						
	22.0						
OAc-3			168.2	172.32			
			20.4	20.9			
OAc-5	170.1	170.2					
	21.2	20.1					
OAc-7	169.9	169.9	170.3				
	21.3	21.3	21.4				
OAc-12			170.7				
			20.7				
OAc-13	167.9	167.9					
	20.4	21.2					
OAc-14	168.7	168.8					
	21.4	21.4					
OMe						51.7	

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

(Figure S1, Supporting Information), indicated the presence of the typical A, B, and C rings of a daphnane diterpenoid.[9,10] In the HMBC spectrum of **1** (Figure S1, Supporting Information), the OH group resonating at δ_H 3.58 was assigned to C-9 for the key correlation between OH-9 and C-9 (δ_C 76.8). Two benzoyloxy groups were placed at C-3 (δ_C 77.3) and C-12 (δ_C 73.4) on the basis of HMBC correlations from H-3 and H-12 to each of the corresponding carbonyls of the benzoyl groups, respectively. Three acetoxy groups were located at C-5 (δ_C 72.8), C-7 (δ_C 79.2), and C-14 (δ_C 74.9) from the HMBC correlations of H-5, H-7, and H-14 to each of the carbonyls of these acetyls, respectively. The remaining one degree of unsaturation accounted for an oxetane ring. The downfield-shifted C-4 (δ_C 91.0) and C-6 (δ_C 84.3) signals suggested the formation of this ring between C-4 and C-6.[9] This conclusion was also supported by comparing their NMR data (Tables 1 and 2) with those of trigochinins A and B.[7] The HMBC correlations from H-12, H-14, H_2-16, and H_3-17 to C-13 (δ_C 80.6) supported the placement of an acetoxy group at C-13. The remaining oxygenated quaternary carbon at δ_C 85.3 was only assignable to C-2, bearing the last acetoxy group, as judged from the HMBC correlations from H_2-1, H-3, and H_3-19 to C-2 (Figure S1, Supporting Information). As a result, the proton signal of Me-19 in **1** was observed as a singlet, and the carbon signals of C-1, C-3, and C-19 of **1** shifted downfield by 5.1, 3.5, and 7.2 ppm, respectively, as compared to those of trigochinin B,[7] due largely to the strong inductive effect from OAc-2. Thus, the planar structure of **1** was determined.

The relative configuration of **1** was defined by comparing its NMR data with those of trigochinins A and B,[7] as well as the analysis of its ROESY spectrum (Figure S1, Supporting Information), in which the correlations of H-1β/H-8, H-1β/H_3-19, H-8/H-7, H-8/H-11, H-8/H-14, H-7/H-14, H-12/H-11, H-12/H_2-16, H-8/H_3-17, and H-14/H_3-17 indicated that they are all cofacial toward the β-face. As a consequence, the ROESY correlations of H-3/H-10, H-5/H-10, OH-9/H-5, OH-9/H-10, and OH-9/H_3-18 suggested that they are α-oriented. The configuration of the oxetane moiety was assigned as being identical with that of trigochinins A and B[7] by comparison of their relevant NMR data. Therefore, the structure of trigoheterin A (**1**) was assigned as depicted.

The HRESIMS of compound **2** gave a sodiated molecular ion peak at m/z 799.2909 $[M + Na]^+$, corresponding to a molecular formula of $C_{42}H_{48}O_{14}$. The ^{1}H NMR spectrum of **2** was very similar to that of **1** (Table 2), except for the absence of the proton signal of the C-2 acetate group and the upfield-shifted proton resonances of H-3 at δ_H 5.46 (d, 10.4) and Me-19 at δ_H 0.94 (3H, d, 7.3) in **2**. Furthermore, in the ^{13}C NMR spectrum of **2** (Table 1), the C-2 signal at δ_C 31.0 was observed as a methine resonance. The above analysis indicated that **2** is the 2-deacetoxy derivative of **1**. This deduction was supported by its molecular weight, showing 58 mass units less than that of **1**, and confirmed by the HMBC spectrum (Supporting Information). The ROESY correlations of H-10/H-2, H-2/H-1α, and H-2/H-3 showed that H-2 is α-oriented (Supporting Information). The other stereocenters in **2** were assigned as being identical with those in **1** by comparing their NMR data (Tables 1 and 2), as well as from the analysis of its ROESY spectrum (Supporting Information). Thus, the structure of **2** (trigoheterin B) was determined as shown.

The molecular formula of **3** was defined as $C_{40}H_{46}O_{13}$ from the sodiated molecular ion peak at m/z 757.2831 $[M + Na]^+$ in the HRESIMS. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) revealed that **3** is also a daphnane diterpenoid and shares the same C ring as trigohownin D,[6] on the basis of the analysis of its HMBC spectrum (Figure S1, Supporting Information). The main differences were the substitution patterns of the A and B rings. The proton resonances at δ_H 3.48 and 4.63 (each 1H, s),

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Table 2. ^{1}H NMR Spectroscopic Data of Compounds 1−7[a]

	1	2	3	4	5	6	7
position	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)	(mult., *J* in Hz)
1	1.78, m	1.37, m	1.73, m	1.61, m	1.62, m	2.10, 2H, m	
	2.43, dd (13.3, 6.3)	2.06, m	2.14, m	1.88, m	2.41, m		
2		2.50, m	2.02, m	1.79, m	2.27, m	2.01, m	5.86, s
						2.29, m	
3	5.80, s	5.46, d (10.4)	5.14, d (5.3)	5.14, d (5.8)			
4							2.34, 2H, m
5	6.12, s	6.08, s	5.16, s	3.40, s	3.88, s	2.38, dd (11.8, 2.0)	2.06, m
							1.75, m
6						1.76, m	2.38, m
						1.89, m	
7	5.62, d (4.1)	5.64, d (3.9)	5.58, s	4.09, s	3.31, s	2.70, 2H, m	
8	2.93, dd (4.1, 2.1)	3.00, dd (3.9, 2.1)	2.94, d (2.2)	2.52, d (2.5)	3.29, d (2.7)		1.49, m
							1.65, m
9							2.13, m
							2.30, m
10	2.73, dd (13.7, 6.3)	2.19, m	2.77, dd (13.5, 5.1)	2.63, dd (13.7, 5.1)	3.15, m		5.62, t (7.0)
11	2.12, m	2.24, m	3.20, m	3.04, m	3.17, m	6.76, s	
12	6.21, br d (3.0)	6.24, br d (3.7)	5.30, d (7.8)	5.17, d (7.8)	5.51, d (7.6)		4.41, d (4.0)
14	6.01, br s	5.99, br s	4.64, d (2.2)	4.39, d (2.5)	4.87, d (2.7)	6.80, s	6.12, d (1.0)
16	5.42, s	5.40, s	4.99, s	4.93, t (1.5)	5.06, s		1.97, 3H, s
	5.43, s	5.43, s	5.11, s	5.07, s	5.30, s		
17	1.98, 3H, s	1.97, 3H, s	1.77, 3H, s	1.59, 3H, s	1.81, 3H, s	2.20, 3H, s	1.21, 3H, s
18	1.21, 3H, d (6.8)	1.21, 3H, d (6.6)	1.23, 3H, d (6.8)	1.04, 3H, d (6.8)	1.23, 3H, d (6.5)	1.80, 3H, s	1.43, 3H, s
19	1.51, 3H, s	0.94, 3H, d (7.3)	0.89, 3H, d (6.7)	0.98, 3H, d (6.7)	1.12, 3H, d (6.6)	4.71, s	1.92, 3H, s
						4.96, s	
20	1.16, 3H, s	1.25, 3H, s	1.14, 3H, s	1.49, 3H, s	1.46, 3H, s	1.19, 3H, s	2.21, 3H, s
2′				1.67, 3H, s			
3′	8.19, d (7.3)	8.17, dd (8.5, 1.1)	7.68, m		7.81, m		
4′	7.50, m	7.48, m	7.37, m		7.40, m		
5′	7.61, m	7.59, m	7.38, m		7.41, m		
6′	7.50, m	7.48, m	7.37, m		7.40, m		
7′	8.19, d (7.3)	8.17, dd (8.5, 1.1)	7.68, m		7.81, m		
2″				2.70, 2H, m			
3″	8.07, d (7.3)	8.07, dd (8.6, 1.4)	8.06, m	2.95, 2H, m	8.06, d (7.3)		
4″	7.50, m	7.48, m	7.46, m		7.41, m		
5″	7.61, m	7.59, m	7.60, m	7.19, m	7.58, m		
6″	7.50, m	7.48, m	7.46, m	7.27, m	7.41, m		
7″	8.07, d (7.3)	8.07, dd (8.6, 1.4)	8.06, m	7.20, m	8.06, d (7.3)		
8‴				7.27, m			
9‴				7.19, m			
OH-4			3.48, s	3.56, s	3.60, s		
OH-6			4.63, s				
OH-7				3.35, s			5.26, s
OH-9	3.58, s	3.58, s					
OH-12						6.26, br s	4.17, d (4.0)
OAc-2	2.03, 3H, s						
OAc-3			1.98, 3H, s	2.17, 3H, s			
OAc-5	1.76, 3H, s	1.78, 3H, s					
OAc-7	2.08, 3H, s	2.08, 3H, s	1.77, 3H, s				
OAc-12			2.11, 3H, s				
OAc-13	1.76, 3H, s	1.76, 3H, s					
OAc-14	1.89, 3H, s	1.90, 3H, s					
OMe						3.63, 3H, s	

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

which did not correlate with any carbons in the HSQC spectrum, were assignable to OH groups. In the HMBC spectrum of **3** (Figure S1, Supporting Information), two OH groups at δ_H 3.48 and 4.63 were assigned to C-4 (δ_C 84.8) and C-6 (δ_C 75.2) by the key correlations of OH-4/C-4 and OH-6/C-6, respectively. Two acetoxy groups were readily placed at C-3 (δ_C 78.1) and C-7 (δ_C 78.1) by the HMBC correlations from H-3 and H-7 to each of the corresponding carbonyls of the

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acetyls, respectively. The only benzoyloxy group was located at C-5 (δ_C 77.5) by the HMBC correlation from H-5 to its carbonyl at δ_C 166.2 (Figure S1, Supporting Information). The relative configuration of **3** was assigned as being identical with that of trigohownin D[6] by comparing their NMR data and by the analysis of its ROESY spectrum (Figure S1, Supporting Information). Thus, the structure of **3** (trigoheterin C) was established as shown.

Compound **4** gave a molecular formula of $C_{33}H_{44}O_{11}$, as established by the HRESIMS at m/z 639.2750 $[M + Na]^+$. Analysis of the ^1H and ^{13}C NMR spectra (Tables 1 and 2) suggested the compound to be a daphnane diterpenoid bearing orthoacetate, phenylpropionyl, and acetyl groups. Five oxygenated methines and four oxygenated quaternary carbons were also observed in its ^{13}C NMR spectrum. The ^1H NMR resonances at δ_H 3.35 and 3.56, which showed no correlations with any carbons in the HSQC spectrum, were assigned to OH groups. In the HMBC spectrum of **4** (Supporting Information), two OH groups resonating at δ_H 3.35 and 3.56 (each 1H, s) were assigned to C-7 and C-4 from the key correlations of OH-7/C-7 (δ_C 80.8) and OH-4/C-4 (δ_C 85.0), respectively. The presence of a 9,13,14-orthoacetate group was determined by the typical chemical shifts of C-1′, C-9, C-13, and C-14 at δ_C 119.1, 80.8, 86.6, and 84.1,[5,6,11] respectively, and was confirmed by the HMBC correlations from H-14 and H_3-2′ to C-1′ (Supporting Information). The HMBC correlations from H-3 and H-12 to the overlapped two carbonyls at δ_C 172.3 allowed only the attachment of the acetoxy and phenylpropionyloxy groups at C-3 (δ_C 80.4) and C-12 (δ_C 71.5) indistinguishably. However, the key ROESY correlation between H_2-2″ and H_3-18 (Figure S1, Supporting Information) was indicative of the linkage of the phenylpropionyloxy group at C-12, which is close sterically to Me-18, and so the acetoxy group was placed at C-3. The remaining oxygenated methine was located at C-5 (δ_C 75.9), bearing an OH group, from the HMBC correlations of H-3, H-7, and H-10/C-5 and H-5/C-4. Consequently, the last oxygenated quaternary carbon was assignable only to C-6 (δ_C 76.2), again bearing an OH group, from the HMBC correlations from H-7, H-8, and H_3-20 to C-6. The relative configuration of **4** was assigned as being identical with that of **3** by comparing their NMR data and by analysis of its ROESY spectrum (Figure S1, Supporting Information), in which the correlation between OH-4 and H-8 showed that OH-4 is β-oriented. Therefore, the structure of **4** (trigoheterin D) was elucidated as shown.

Compound **5** gave a molecular formula of $C_{34}H_{36}O_9$ as determined by HRESIMS at m/z 611.2281 $[M + Na]^+$. The ^1H and ^{13}C NMR data (Tables 1 and 2) of **5** revealed the features of a daphnane diterpenoid orthoester, and orthobenzoate, benzoyloxy, carbonyl, and trisubstituted epoxide functionalities were recognized. Four oxygenated methines and four oxygenated quaternary carbons were also distinguished from the NMR data. The ^1H and ^{13}C NMR data of **5** resembled closely those of trigoxyphin B,[5] except for some small variation of the chemical shifts. Interpretation of the 1D- and 2D-NMR spectra of **5**, especially the HMBC spectrum (Supporting Information), showed that it shares the same planar structure as trigoxyphin B,[5] indicating that **5** is a stereoisomer of trigoxyphin B. The relative configuration of **5** was defined by analysis of its ROESY spectrum (Figure S1, Supporting Information), in which the correlations of H-1β/H-8, H-8/OH-4, H-8/H-11, H-8/H-14, H-8/H-12, and H-12/H-11 indicated that they are β-oriented. As a consequence, the ROESY correlations of H-2/H-10, H-10/H-5, H-5/H-7, H-5/H_3-20, and H-7/H_3-20 suggested that they were α-oriented and that the 6,7-epoxide is β-oriented. The strong ROESY correlations of H-12/H-16b, H-14/H-16b, H-12/H_3-17, and H-14/H_3-17 revealed that the 9,13,14-orthobenzoate group is α-directed. Thus, the structure of **5** (trigoheterin E) was elucidated as the 6,7-epimer of trigoxyphin B.[5]

The molecular formula of compound **6** was defined as $C_{19}H_{26}O_3$ by HRESIMS at m/z 325.1782 $[M + Na]^+$, requiring seven degrees of unsaturation. Its IR spectrum showed the presence of hydroxy (3433 cm^{-1}), carbonyl (1720 cm^{-1}), and benzene ring (1626, 1585, and 1507 cm^{-1}) functionalities. All 19 carbons were well-resolved in the ^{13}C NMR spectrum (Table 1) and were further classified by DEPT experiments as four methyls (one *O*-methyl), five methylenes (one olefinic), three methines (two olefinic), and seven quaternary carbons (one ester carbonyl and five olefinic). Three methyl singlets at δ_H 1.19, 1.80, and 2.20 (each 3H, s), an *O*-methyl group at δ_H 3.63 (3H, s), a terminal double bond (δ_H 4.71 and 4.96, each 1H, s; δ_C 114.2 and 146.6), an ester carbonyl group (δ_C 175.3), and a tetrasubstituted benzene ring were evident from the analysis of its NMR data (Tables 1 and 2). These functionalities accounted for six out of the seven degrees of unsaturation, indicating that **6** has a bicyclic core including the benzene ring. The aforementioned data strongly suggested that **6** is a dinorditerpenoid based on the carbon skeleton of 3,4-seco-sonderianol,[8] a coexisting major diterpenoid. Analysis of the 1D- and 2D-NMR spectra of **6**, especially the HMBC spectrum (Figure S2, Supporting Information), allowed the establishment of its planar structure. The HMBC correlations from H_2-1, H_2-2, and OCH_3 to the carbonyl at δ_C 175.3 showed a methoxycarbonyl group attached to C-2. The terminal $\Delta^{4(19)}$ double bond was assigned by the multiple HMBC correlations of H_3-18/C-4 and C-19, H-5/C-4 and C-19, and H_2-19/C-4, C-5, and C-18. An OH group was located at C-12 (δ_C 152.5) by its chemical shift and from the HMBC correlations between H-11 (δ_H 6.76), H-14 (δ_H 6.80), and H_3-17 (δ_H 2.20, 3H, s) to C-12. Thus, the planar structure of **6** was assigned as the 15,16-dinor derivative of 3,4-seco-sonderianol.[8]

The relative configuration of **6** was assigned by a ROESY experiment (Figure S2, Supporting Information), in which the correlation of H-6β/H-1a indicated that H-6β and the methyl propionate group took the 1,3-diaxial bonds of the ring B and were randomly assigned in a β-configuration. The ROESY correlations of H-5/H_2-6 and H-5/H_3-20 suggested that H-5 is β-oriented (Figure S2, Supporting Information). Thus, the structure of **6** (trigoheteric acid methyl ester) was determined as 15,16-dinor-3,4-seco-sonderianol. It is worth noting that compound **6** possesses the first reported 15,16-dinor-3,4-seco-cleistanthane skeleton.

Compound **7** gave a molecular formula of $C_{20}H_{30}O_4$, as assigned by the HRESIMS at m/z 357.2049 $[M + Na]^+$, requiring six degrees of unsaturation. The UV absorption band at 238 nm (log ε 4.16) implied the presence of an α,β-unsaturated carbonyl group. Its ^{13}C NMR spectrum (Table 1) resolved 20 carbon resonances comprising five methyls, four methylenes, five methines (three olefinic and an oxygenated one), and six quaternary carbons (two carbonyl, three olefinic, and one oxygenated). The three trisubstituted double bonds and two keto groups accounted for five out of the six degrees of unsaturation, and the one remaining thus required **7** to be monocyclic. The aforementioned data suggested that **7** is a prenylated bisabolane sesquiterpenoid.[12,13] This deduction was

confirmed by its HMBC spectrum (Figure S2, Supporting Information), in which the carbonyl group at C-1 (δ_C 203.6), conjugated with the Δ^2 double bond (δ_H 5.86; δ_C 127.3 and 163.9), was assigned by the multiple HMBC correlations of H-2, H_2-5, and H-6/C-1, H-4/C-2 and C-3, and H_3-16/C-2 and C-3. The only oxygenated quaternary carbon at δ_C 74.4 was assigned to C-7, bearing an OH group, by the HMBC correlations from H-6, H_2-8, H_2-9, and H_3-17 to C-7. The trisubstituted Δ^{10} double bond (δ_H 5.62; δ_C 132.8 and 133.0) was determined by the HMBC correlations of H-10/C-8, C-9, C-12, and C-18, H_2-9/C-10 and C-11, H_3-18/C-10 and C-11, and H-12/C-10 and C-11, which also suggested an OH group could be placed at C-12 (δ_C 83.3). The remaining carbonyl group at C-13 (δ_C 199.2), conjugated with the Δ^{14} double bond (δ_H 6.12; δ_C 118.8 and 159.9), was assigned on the basis of the multiple HMBC correlations of H_3-19/C-13, C-14, and C-15, H_3-20/C-13, C-14, and C-15, H-14/C-13 and C-15, and H-12/C-13 (Figure S2, Supporting Information). The relative configuration of **7** was established mainly by comparing its NMR data with known analogues[12,13] and analysis of the ROESY spectrum, in which the correlation of H-6/H-5b (Figure S2, Supporting Information) suggested that H-6 is axial and was randomly assigned as being α-oriented. Consequently, the ROESY correlations of H_2-5/H_3-17 and H-6/H_3-17 (Figure S2, Supporting Information) suggested that OH-7 is β-oriented, on the basis of the prerequisite of the presence of an intramolecular hydrogen bond between OH-7 and the C-1 carbonyl.[14−16] This analysis is consistent with the configuration of the other analogues.[12,13,17] The *E* geometry of the Δ^{10} double bond was assigned by the ROESY correlations of H-10/H_2-8, H-10/H_2-9, and H_3-18/H_2-9 (Figure S2, Supporting Information). Further work toward the establishment of the absolute configuration at C-12 of **7** was not attempted due to the limited amount of sample available. Thus, the structure of **7** (trigohetone) was assigned as shown.

Six known diterpenoids, 3,4-seco-sonderianol,[8] 18-hydroxymanool,[18] 1,2-dihydroheudelotinol,[19] sandaracopimaradiene-1α,9α-diol,[20] and trigochinins B and E,[4,7] were also obtained from this study and were identified on the basis of their ^{1}H NMR, ^{13}C NMR, and ESIMS data and comparison with literature values.

Compounds **1**−**7** were evaluated for cytotoxic activity against two human tumor cell lines, HL-60 (premyelocytic leukemia) and A-549 (lung adenocarcinoma), using MTT[21] and SRB methods,[22] respectively. Trigoheterin E (**5**) exhibited moderate cytotoxic activity against both HL-60 and A-549 cell lines with IC_{50} values of 1.8 and 10.0 μM, respectively, while the other compounds were inactive (IC_{50} > 10.0 μM).

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on an SGW X-4 melting point 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. All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China).

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

Extraction and Isolation. The powdered, air-dried twigs of *T. heterophyllus* (8.0 kg) were extracted three times with 95% EtOH (each 25 L, three days) at room temperature to give an ethanolic extract (250 g), which was partitioned between EtOAc and water to obtain an EtOAc-soluble fraction (48 g). The EtOAc-soluble fraction was subjected to MCI gel column chromatography, eluted with MeOH−H_2O (40:60 to 90:10), to produce fractions A−D. Fraction B (8 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 passage over 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 purified by a semipreparative HPLC using 65% MeCN in water as the mobile phase, to yield compounds **6** (15 mg), **7** (2.5 mg), and 3,4-seco-sonderianol (17 mg). Using the same purification procedures, fraction B2 gave 18-hydroxymanool (11 mg) and sandaracopimaradiene-1α,9α-diol (9 mg), while fraction B3 gave 1,2-dihydroheudelotinol (8 mg). Fraction C (10 g) was chromatographed over a silica gel column, eluted with petroleum ether−acetone (10:1 to 1:1), to afford four major subfractions, C1−C4. Fraction C2 was subjected to Sephadex LH-20 column chromatography, eluted with MeOH, to obtain the major portion, which was then purified by semipreparative HPLC with 60% MeCN in water as the mobile phase, to yield compounds **3** (15 mg) and **5** (8 mg). Fraction C3 was chromatographed over a silica gel column, eluted with $CHCl_3$−MeOH (100:1 to 10:1), to afford a major fraction, which was then purified by semipreparative HPLC with 60% MeCN in water as the mobile phase, to yield compound **4** (16 mg). Fraction C4 was chromatographed over a silica gel column, eluted with $CHCl_3$−MeOH (100:1 to 20:1), to afford three major fractions, C4a−C4c. Fraction C4a was purified by semipreparative HPLC, with the mobile phase 55% MeCN in water, to afford compound **2** (10 mg) and trigochinins B (8 mg) and E (12 mg). Fraction C4b was subjected to silica gel column chromatography, eluted with petroleum ether−EtOAc (4:1 to 1:1), to obtain compound **1** (3 mg).

*Trigoheterin A (**1**):* white solid; mp 260−261 °C; $[\alpha]^{23}_D$ +13.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.51) nm; IR (KBr) ν_{max} 3568, 3446, 2926, 1753, 1730, 1452, 1371, 1275, 1240, 1115, 1026, 714 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 857.2961 [M + Na]$^+$ (calcd for $C_{44}H_{50}NaO_{16}$, 857.2997).

*Trigoheterin B (**2**):* white solid; mp 251−253 °C; $[\alpha]^{23}_D$ +94.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.43) nm; IR (KBr) ν_{max} 3577, 2974, 2931, 1751, 1713, 1452, 1367, 1279, 1236, 1115, 714 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 799.2909 [M + Na]$^+$ (calcd for $C_{42}H_{48}NaO_{14}$, 799.2942).

*Trigoheterin C (**3**):* white solid; mp 225−227 °C; $[\alpha]^{23}_D$ +51.0 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.16) nm; IR (KBr) ν_{max} 3442, 2987, 1722, 1452, 1273, 1230, 1092, 1030, 712 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 757.2831 [M + Na]$^+$ (calcd for $C_{40}H_{46}NaO_{13}$, 757.2836).

*Trigoheterin D (**4**):* colorless solid; $[\alpha]^{23}_D$ +21.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.26) nm; IR (film) ν_{max} 3439, 2089, 1635, 696 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 639.2750 [M + Na]$^+$ (calcd for $C_{33}H_{44}NaO_{11}$, 639.2781).

*Trigoheterin E (**5**):* white solid; mp 242−243 °C; $[\alpha]^{23}_D$ +48.0 (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ε) 231 (4.13) nm; IR (KBr) ν_{max} 3440, 2976, 1743, 1697, 1452, 1356, 1283, 1082, 997, 719 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 611.2281 [M + Na]$^+$ (calcd for $C_{34}H_{36}NaO_9$, 611.2257).

*Trigoheteric acid methyl ester (**6**):* colorless oil; $[\alpha]^{23}_{D}$ −62.0 (*c* 0.28, MeOH); UV (MeOH) λ_{max} (log ε) 281 (3.68) nm; IR (film) ν_{max} 3433, 2940, 1738, 1720, 1626, 1507, 1204, 1021, 897 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 325.1782 $[M + Na]^+$ (calcd for $C_{19}H_{26}NaO_3$, 325.1780).

*Trigohetone (**7**):* colorless oil; $[\alpha]^{23}_{D}$ +108.0 (*c* 0.03, MeOH); UV (MeOH) λ_{max} (log ε) 238 (4.16) nm; IR (film) ν_{max} 3437, 2933, 2872, 1643, 1437, 1381, 1217, 1024 923, 594 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS *m*/*z* 357.2049 $[M + Na]^+$ (calcd for $C_{20}H_{30}NaO_4$, 357.2042).

Cytotoxicity Assay. Cytotoxic activities of compounds **1**−**7** against HL-60 and A-549 cells were evaluated by using the MTT and SRB assay, respectively.[21,22] The detailed procedures were reported in previous literature.[5,6]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

IR, HRESIMS, and 1D and 2D NMR spectra of compounds **1**−**7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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