Tetrahedron 66 (2010) 5065–5070

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Trigochinins D–I: six new daphnane-type diterpenoids from *Trigonostemon chinensis*

Hua-Dong Chen [†], Sheng-Ping Yang [†], Xiu-Feng He, Hong-Bing Liu, Jian Ding, Jian-Min Yue [*]

State Key Laboratory of Drug Research, Institute of Materia Medica, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, PR China

ARTICLE INFO

Article history:
Received 7 April 2010
Received in revised form 24 April 2010
Accepted 27 April 2010
Available online 20 May 2010

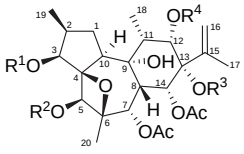
Keywords:
Daphnane-type diterpenoid
Trigochinins D–I
Absolute configuration
Cytotoxic activity

ABSTRACT

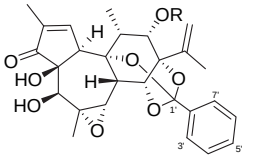
Six new highly oxygenated daphnane-type diterpenoids, trigochinins D–I (**1**–**6**), were isolated from the twigs and leaves of *Trigonostemon chinensis*. Their structures with the absolute configuration were established on the basis of spectroscopic method and CD analysis. Trigochinins D–F (**1**–**3**) possessed a rare 4,6-oxetane moiety in this compound class. Trigochinins E and F exhibited significant inhibiting activity against HL-60 tumor cell line.

1. Introduction

The genus *Trigonostemon* (Euphorbiaceae) comprising of ca. 50 species grows mainly in the tropical and subtropical regions of Asia.[1] Previously chemical investigations on this genus have led to the isolation of a number of structurally diverse diterpenoids,[2–6] and flavonoidal indole alkaloid.[7] The modified daphnane-type diterpenoids have been found to possess anti-flea insecticidal,[2,3a] cytotoxic,[3b] and acaricidal[3c] activities. In our recent study, two highly modified daphnane-type diterpenoids and three daphnane-type diterpenoids were isolated from the twigs and leaves of *Trigonostemon chinensis* Merr.[8,9] As the continuation, six new highly oxygenated daphnane-type diterpenoids, trigochinins D–I (**1**–**6**), were isolated from the same plant. Their structures with the absolute configuration were established on the basis of spectroscopic method and CD analysis. Trigochinins D–F (**1**–**3**) possessed a rare 4,6-oxetane moiety in the family of daphnane-type diterpenoids, and trigochinins G–I (**4**–**6**) featured either a 9,12,14- or a 9,13,14-orthoester group. Cytotoxic evaluation of these compounds showed that trigochinins E and F exhibited significant activity against HL-60 tumor cell line with the IC_{50} values of 8.1 and 6.4 μM, respectively. We present herein the isolation, structural elucidation, and cytotoxic tests of these compounds.



	R^1	R^2	R^3	R^4
1	H	Bz	Ac	Bz
2	Ac	Bz	Ac	Bz
3	Ac	Ac	Bz	Ac



	R
4	$COCH_2CH(CH_3)_2$
5	COC_6H_4(4-OH)
6	COC_6H_3(3-OCH_3)(4-OH)

2. Results and discussion

2.1. Structural elucidation

Trigochinin D (**1**), obtained as a white powder, had a molecular formula of $C_{40}H_{46}O_{13}$ as determined by the HRESIMS ion at *m*/*z* 757.2817 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836) with 18 degrees of unsaturation. The IR absorptions implied the presence of hydroxyls (3565, 3444 cm^{-1}) and ester carbonyl groups (1761, 1724 cm^{-1}). In accordance with its molecular formula, all the 40 carbons were well resolved as 40 carbon resonances in the ^{13}C NMR spectrum (Table 3), and were further classified by DEPT experiments as 7 methyls, 2 methylenes (1 olefinic), 19 methines (5 oxygenated and 10 olefinic ones), and 12 quaternary carbons (5 ester carbonyls, 4 oxygenated, and 3 olefinics). In addition, two tertiary methyls at δ_H 1.98 (s, 3H), and 1.33 (s, 3H), two secondary methyls at δ_H 1.19 (d, 6.4 Hz, 3H), and 0.94 (d, 6.7 Hz, 3H), a terminal double bond at δ_H 5.41 (s, 1H), and 5.43 (s, 1H), three acetyl groups, and two

* Corresponding author. Tel./fax: +86 21 50806718; e-mail address: jmyue@mail.shcnc.ac.cn (J.-M. Yue).
† These authors contributed equally.

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.04.118

benzoyl groups were distinguished by NMR spectra (Tables 1 and 3). The aforementioned evidence indicated that it is a daphnane-type diterpenoid.[9] Comparison of the ^{1}H and ^{13}C NMR data of **1** (Tables 1 and 3) with those of trigochinin A[9] indicated that their structures are closely related, the differences were likely aroused from the location of the different acyloxy groups. In the HMBC spectrum (Fig. 1a), two hydroxyls resonated at δ 2.80 (d, 10.4 Hz, 1H) and 3.61 (s, 1H) were located at C-3 and C-9 on the basis of the key HMBC correlations of OH-3/C-3, and OH-9/C-9, respectively; two benzoyloxy groups were placed on C-5 and C-12 by the HMBC correlations from H-5 and H-12 to the each corresponding benzoyl carbonyl, respectively; two acetoxy groups were attached to C-7 and C-14 by the HMBC correlations from H-7 and H-14 to the each corresponding acetyl carbonyl; the remaining one acetoxy group was only assignable to the leftover oxygenated carbon C-13 (δ 80.7), although no direct HMBC correlation was available.

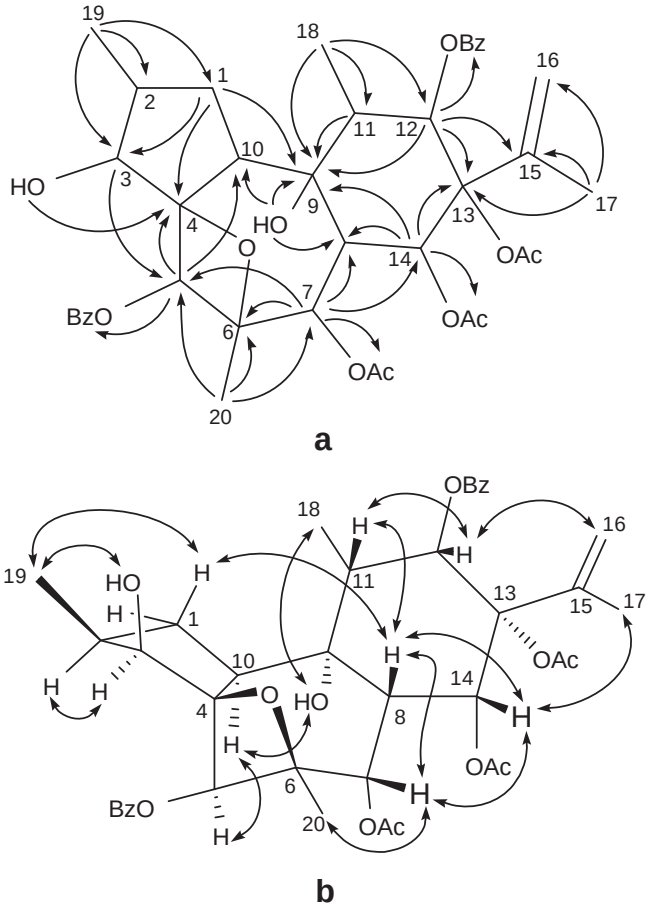


Figure 1. (a) Selected HMBC (H→C), and (b) ROESY (H↔H) correlations of **1**.

The relative configuration and conformation of **1** was mainly established by comparison with trigochinin A[9] and a ROESY experiment (Fig. 1b), in which the correlations of H-1β/H-8, H-1β/H_3-19, H_3-19/OH-3, H-8/H-7, H-11 and H-14, and H-11/H-12 indicated that they were co-facial toward β-faces. In consequence, the ROESY correlations of H-5/H-10, and H-10/OH-9 suggested that they were α-oriented. The strong ROESY correlations of H_3-17/H-14 and H_2-16/H-12 suggested that C-15 occupied the axial orientation at C-13 was in a β-direction. Thus, the structure of compound **1** was established.

Trigochinin E (**2**) possessed a molecular formula of $C_{42}H_{48}O_{14}$ as determined by HRESIMS. Analysis of the ^{1}H and ^{13}C NMR data of **2** showed that it was likely an acetylated derivative of **1**, and this was supported by the presence of 42 mass units more in the molecular formula than that of **1**. Direct comparison with compound **1**, the H-3 of **2** at δ 5.22 (d, 10.1 Hz, 1H) was downfield shifted ca. $\Delta\delta$ 1.04 due to the acetylation effect, indicating that **2** is definitely the 3-acetyl derivative of **1**. The structure of **2** was finally confirmed by 2D NMR experiments, including HSQC, HMBC, and ROESY spectra (Supplementary data).

Trigochinin F (**3**) gave a molecular formula of $C_{37}H_{46}O_{14}$ as determined by the HRESIMS at m/z 737.2801 $[M+Na]^{+}$ (calcd for $C_{37}H_{46}O_{14}Na$, 737.2785). The ^{1}H and ^{13}C NMR data of **3** (Tables 1 and 3) showed high similarity to those of **1**, and the differences were the acylation patterns. One hydroxyl group resonated at 3.44 (s, 1H) was assigned to C-9 by the HMBCs from OH-9 to C-9 (δ 76.7), C-8 (δ 39.1), and C-10 (δ 49.6). Five acetoxy groups were located at C-3, C-5, C-7, C-12, and C-14 by the HMBCs from H-3, H-5, H-7, H-12, and H-14 to the each corresponding carbonyl of the acetyl, respectively. The remaining benzoyl group was only assignable to the oxygenated C-13 (δ 81.7). The relative configuration of **3** was assigned to be the same as that of **1** by comparing their NMR data and ROESY experiment (Supplementary data). Therefore, the structure of **3** was established as depicted.

Table 1
^{1}H NMR data of compounds **1**–**3** (in $CDCl_3$)[a,b,c]

No.	1	2	3
1	1.17 (m)	1.31 (m)	1.26 (m)
	1.94 (m)	2.03 (m)	1.97 (m)
2	2.23 (m)	2.44 (m)	2.35 (m)
3	4.18 (t, 10.4)	5.22 (d, 10.1)	5.22 (d, 10.3)
5	6.33 (s)	6.31 (s)	6.03 (s)
7	5.67 (d, 4.1)	5.67 (d, 4.1)	5.63 (d, 4.1)
8	2.94 (dd, 4.1, 1.6)	3.02 (dd, 4.1, 1.6)	2.90 (dd, 4.1, 1.4)
10	2.15 (m)	2.21 (m)	2.08 (m)
11	2.17 (m)	2.24 (m)	2.18 (m)
12	6.22 (dd, 3.7, 1.1)	6.23 (dd, 3.3, 1.6)	6.35 (dd, 4.2, 1.4)
14	6.00 (br s)	6.00 (br s)	6.07 (br s)
16	5.43 (s)	5.43 (s)	5.46 (s)
	5.41 (s)	5.40 (s)	5.41 (s)
17	1.98 (s)	1.97 (s)	1.93 (s)
18	1.19 (d, 6.4)	1.21 (d, 6.4)	1.14 (d, 6.9)
19	0.94 (d, 6.7)	0.86 (d, 6.9)	0.87 (d, 7.2)
20	1.33 (s)	1.38 (s)	1.25 (s)
3′	8.13 (dd, 7.9, 1.1)	7.97 (dd, 8.1, 1.2)	7.87 (dd, 8.3, 1.4)
4′	7.48 (m)	7.45 (m)	7.40 (m)
5′	7.60 (m)	7.59 (m)	7.53 (m)
6′	7.48 (m)	7.45 (m)	7.40 (m)
7′	8.13 (dd, 8.1, 1.1)	7.97 (dd, 8.1, 1.2)	7.87 (dd, 8.3, 1.4)
3″	8.07 (dd, 8.1, 1.2)	8.07 (dd, 8.9, 1.9)	
4″	7.45 (m)	7.61 (m)	
5″	7.57 (m)	7.48 (m)	
6″	7.45 (m)	7.61 (m)	
7″	8.07 (dd, 8.1, 1.2)	8.07 (dd, 8.9, 1.9)	
OH-3	2.80 (d, 10.4)		
OH-9	3.61 (s)	3.63 (s)	3.44 (s)
3-OAc		2.05 (s)	2.04 (s)
5-OAc			2.17 (s)
7-OAc	2.15 (s)	2.14 (s)	2.08 (s)
12-OAc			1.93 (s)
13-OAc	1.76 (s)	1.76 (s)	
14-OAc	1.91 (s)	1.90 (s)	2.11 (s)

[a] Data were recorded at 400 MHz, chemical shifts are in parts per million, and the coupling constants J are in hertz (in parentheses).
[b] 3′–7′ stand for the proton numbers of the benzoyloxy at C-5 for **1** and **2**, and the benzoyloxy at C-13 for **3**.
[c] 3″–7″ stand for the proton numbers of the benzoyloxy at C-12 for **1** and **2**.

Trigochinin G (**4**) was obtained as a white powder. The HRESIMS displayed a sodiated molecular ion at m/z 589.2429 $[M+Na]^{+}$ (calcd for $C_{32}H_{38}O_9Na$, 589.2414), which is consistent with a molecular formula of $C_{32}H_{38}O_9$ requiring 14 double-bond equivalents. The IR absorption bands at 3466, 1709, and 1691 cm^{-1} indicated the presence of hydroxyl and ester functionalities. The ^{13}C NMR (Table 2) resolved 32 carbon resonances comprising with 6 methyls, 2

Table 2
^{1}H NMR data of compounds **4**–**6** (in $CDCl_3$)[a,b]

No.	4	5	6
1	7.64 (br s)	7.65 (br s)	7.65 (br s)
5	4.04 (br s)	4.04 (br s)	4.05 (br s)
7	3.28 (s)	3.32 (s)	3.31 (s)
8	3.30 (d, 2.6)	3.38 (d, 2.3)	3.39 (d, 2.7)
10	4.04 (br s)	4.04 (br s)	4.05 (br s)
11	3.02 (m)	3.15 (m)	3.16 (m)
12	5.33 (d, 8.3)	5.47 (d, 8.2)	5.44 (d, 8.0)
14	4.63 (d, 2.6)	4.68 (d, 2.3)	4.69 (d, 2.7)
16	5.20 (br s)	5.29 (br s)	5.31 (br s)
	5.03 (br s)	5.04 (br s)	5.05 (br s)
17	1.78 (s)	1.79 (s)	1.80 (s)
18	1.10 (d, 7.2)	1.12 (d, 7.1)	1.13 (d, 7.0)
19	1.79 (s)	1.76 (s)	1.77 (s)
20	1.46 (s)	1.47 (s)	1.47 (s)
3′	7.77 (m)	7.81 (m)	7.81 (m)
4′	7.40 (m)	7.41 (m)	7.41 (m)
5′	7.38 (m)	7.39 (m)	7.40 (m)
6′	7.40 (m)	7.41 (m)	7.41 (m)
7′	7.77 (m)	7.81 (m)	7.81 (m)
2″	2.24 (m)		
3″	3.02 (m)	7.93 (m)	7.54 (d, 2.1)
4″	0.95 (d, 6.6)	6.82 (m)	
5″	0.95 (d, 6.6)		
6″		6.82 (m)	6.92 (d, 8.2)
7″		7.93 (m)	7.65 (dd, 8.2, 2.1)
4-OH	3.75 (s)	3.79, (s)	3.76 (s)
5-OH	3.90 (d, 2.7)	3.91 (d, 2.7)	3.90 (d, 3.0)
13-OH			
5″-OH		6.28 (br s)	6.05 (br s)
4″-OCH_3			3.91 (s)

[a] Data were recorded at 400 MHz, chemical shifts are in parts per million, and the coupling constants *J* are in hertz (in parentheses).
[b] 2″–7″ stand for the proton numbers of the acyloxy at C-12 for **4**–**6**.

methylenes (1 olefinic), 14 methines (4 oxygenated and 6 olefinic ones), and 10 quaternary carbons (1 ketone, 1 ester, 3 olefinics, 1 orthoester, and 4 oxygenated ones) as classified by chemical shifts and HSQC spectrum. In addition, a mono-substituted benzene ring, a 3-methylbutanoate and a trisubstituted epoxide (δ_H 3.28, s; δ_C 59.6 and 67.3) were further distinguished by analysis of its NMR data (Tables 2 and 3). Two proton resonances at δ 3.75 (s, 1H) and 3.90 (d, 2.7 Hz, 1H) showing no correlation with any carbons in the HSQC spectrum were assigned to two hydroxyl protons. The aforementioned data suggested that compound **4** is also a daphnane-typed diterpenoid.[10] The structure of **4** was further demonstrated by analysis of 2D NMR spectra, especially HMBC (Fig. 2a). The A, B, and C rings of **4** were readily constructed by comparison of the NMR data with those of known daphnane-typed diterpenoid,[10] and in combination with the analysis of its HMBC spectrum. In particular, the HMBC correlations from H-1 (δ_H 7.64, br s, 1H) to C-3 (δ_C 209.5), C-4 (δ 72.4), and C-10 (δ 47.9) indicated the presence of an α,β-unsaturation ketone in the A-ring; the Me-19 (δ 9.9) was located at C-2 by the HMBC correlations from Me-19 to C-1, C-2, and C-3. The chemical shifts of C-6 at δ_C 59.6 and C-7 at δ_C 67.3 revealed the presence of a trisubstituted 6,7-epoxide, which was confirmed by the HMBC correlations from H_3-20 to C-5, C-6, and C-7, and from H-7 to C-5, C-8, and C-14. The attachment of the 3-methylbutanoate group at C-12 (δ_C 70.9) was achieved by the HMBC correlation from H-12 at δ_H 5.33 (d, 8.3 Hz) to the carbon carbonyl C-1″ of this group. Two hydroxyl groups resonated at δ 3.75 (s, 1H) and 3.90 (d, 2.7 Hz, 1H) showing HMBC correlations with C-4 (δ_C 72.4) and C-5 (δ_C 72.6) were placed at C-4 and C-5, respectively. The remaining three oxygenated carbons were assigned to C-9 (δ_C 80.7), C-13 (δ_C 86.8), and C-14 (δ_C 82.2) by the HMBC correlations of H_3-18/C-9, H-8/C-9, H-14/C-9, H-12/C-13, H-12/C-14, and H-7/C-14, suggesting the presence of a 9,13,14-orthobenzoate, which was confirmed by the HMBC correlations from H-14, and the aromatic proton H-3′ (H-7′) to the orthoester carbon C-1′ (δ_C 118.2).

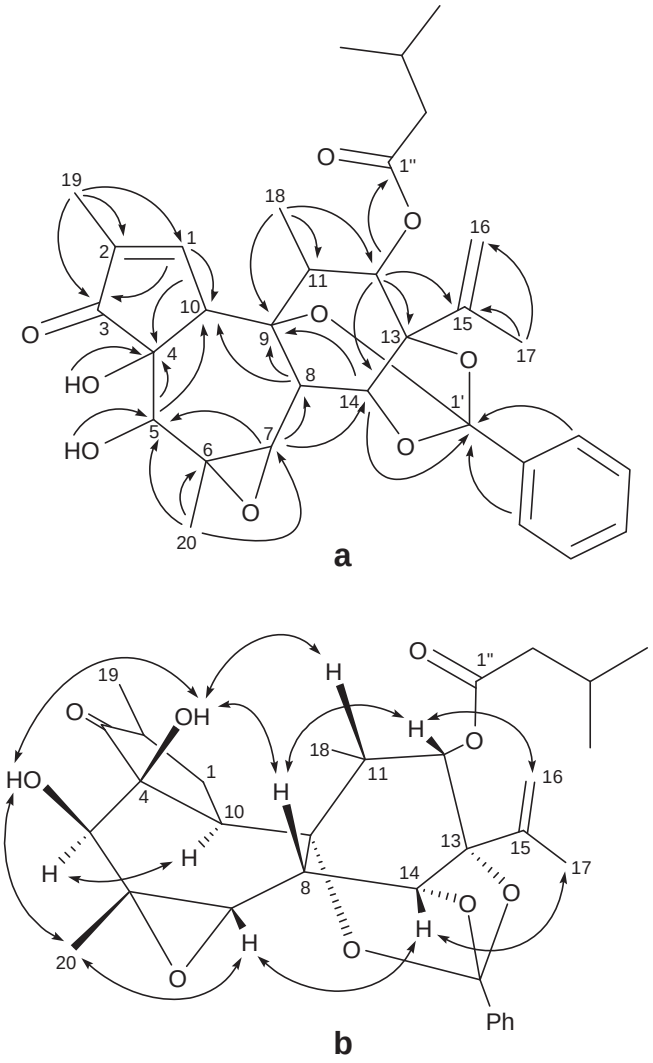


Figure 2. (a) Selected HMBC (H→C), and (b) ROESY (H↔H) correlations of **4**.

The relative configuration of **4** was fixed by a performance of ROESY experiment (Fig. 2b). The ROESY cross-peaks of OH-4/H-11, H-8 and OH-5, OH-5/H_3-20, H_3-20/H-7, H-7/H-14, and H-8/H-12 indicated that OH-4, OH-5, H-7, H-8, H-11, H-12, H-14, and CH_3-20 were co-facial and randomly assigned in a β-configuration. In consequence, the ROESY correlation between H-5 and H-10 suggested that they were α-oriented. The ROESY correlations of H-14/H_3-17, H-12/H_2-16, and H-8/H-11 revealed that the 9,13,14-orthobenzoate was also α-oriented. Thus, the structure of trigochinin G (**4**) was elucidated.

Trigochinins H (**5**) and I (**6**) were obtained as white amorphous powders, and were determined to have molecular formulas of $C_{34}H_{34}O_{10}$ and $C_{35}H_{36}O_{11}$ by HREIMS, respectively. Comparison of the NMR and MS data of compounds **5** and **6** with those of **4** showed that they are structural congeners, and only differ in the nature of the C-12 acyloxy groups (Tables 2 and 3). The presence of a 4-hydroxybenzoyl group [δ_H 7.93 (m, 2H), and 6.82 (m, 2H); δ_C 165.5, 160.0, 132.2 (2C), 121.7, and 115.3 (2C)] in **5**, and a 3-methoxy-4-hydroxybenzoyl group [δ_H 7.65 (dd, 8.2, 2.1 Hz, 1H), 7.54 (d, 2.1 Hz, 1H), 6.92 (d, 8.2 Hz, 1H), and 3.91 (s, 3H); δ_C 165.6, 150.4, 146.2, 124.6, 121.5, 114.2, 111.8, and 56.0] in **6** was evidenced by the NMR data, respectively. The acyloxy groups were attached to the C-12 of each compound by the key HMBC from H-12 to the corresponding carbonyl of acyloxy group, respectively (Supplementary data). The structures of trigochinins H (**5**) and I (**6**) were thus assigned.

Table 3
^{13}C NMR data of compounds **1**–**6** (in $CDCl_3$)[a,b,c]

No.	1	2	3	4	5	6
1	35.0	34.7	34.7	160.0	160.4	160.0
2	32.7	31.0	30.9	137.0	137.1	137.1
3	72.1	73.8	72.6	209.5	209.6	209.6
4	92.7	91.0	91.0	72.4	72.4	72.4
5	73.6	73.9	73.1	72.6	72.6	72.6
6	84.8	84.0	83.7	59.6	59.8	59.7
7	78.9	79.3	79.2	67.3	67.4	67.3
8	39.2	39.2	39.1	35.2	35.3	35.3
9	76.7	76.8	76.7	80.7	80.7	80.7
10	49.5	49.6	49.6	47.9	48.0	48.0
11	40.0	40.0	40.3	38.9	39.2	39.2
12	73.5	73.5	72.6	70.9	71.5	71.7
13	80.7	80.6	81.7	86.8	87.1	87.1
14	75.1	75.1	75.2	82.2	82.2	82.0
15	140.1	140.1	139.1	142.1	142.1	142.2
16	119.2	119.2	119.7	113.2	113.2	113.1
17	20.1	20.1	19.6	19.5	19.5	19.5
18	11.8	11.8	11.6	11.2	11.2	11.2
19	15.4	15.8	15.8	9.9	9.9	9.9
20	19.6	19.8	20.0	21.4	21.4	21.4
1′	166.0	165.9	164.0	118.2	118.2	118.2
2′	129.8	129.9	129.9	135.2	135.3	135.3
3′	129.8	129.6	129.5	128.0	128.0	128.0
4′	128.6	128.6	128.5	126.2	126.2	126.2
5′	133.3	133.3	133.2	129.6	129.6	129.6
6′	128.6	128.6	128.5	126.2	126.2	126.2
7′	129.8	129.6	129.5	128.0	128.0	128.0
1″	165.7	165.7		172.4	165.5	165.6
2″	129.4	129.5		43.1	121.7	121.5
3″	129.5	129.5		25.5	132.2	111.8
4″	128.5	128.5		22.4	115.3	146.2
5″	133.3	133.3		22.4	160.0	150.4
6″	128.5	128.5			115.3	114.2
7″	129.5	129.5			132.2	124.6
OMe						56.0
3-OAc		170.3, 20.7	170.1, 20.5			
5-OAc			170.2, 20.8			
7-OAc	170.0, 21.3	170.0, 21.4	170.0, 21.2			
12-OAc			169.3, 20.8			
13-OAc	167.9, 21.3	167.9, 21.3				
14-OAc	168.7, 21.4	168.7, 21.4	169.0, 21.5			

[a] Data were recorded at 100 MHz, chemical shifts (δ) are in part per million; the full assignments were achieved by 2D NMR spectra.
[b] 1′–7′ stand for the carbon numbers of the benzoyloxy at C-5 for **1** and **2**, the benzoyloxy at C-13 for **3**, and the 9,13,14-orthobenzoate for **4**–**6**.
[c] 1″–7″ stand for the carbon numbers of the benzoyloxy at C-12 for **1** and **2**, and the acyloxy at C-12 for **4**–**6**.

The absolute configuration of trigochinins D–I (**1**–**6**) was determined by applying the CD exciton chirality method.[11] The first positive Cotton effects of compounds **1** at 244 nm were distinguished, which are considered to be aroused from the exciton coupling between the benzoate moiety at C-12 and the Δ^{15} double bond. The coupling two chromophores in compound **1** being in a clockwise manner allowed the assignment of its absolute configuration as depicted (Fig. 3). The CD split manner of compound **2** was very similar to that of **1**, indicating that the absolution configuration of **2** was assignable as shown (Fig. 3). The CD split manner of **3**, which centered at the UV maximum (ca. λ_{max}=231 nm) of a benzoate,[12] was recognized to be the exciton coupling between the benzoate moiety at C-13 and the Δ^{15} double bond, indicating a positive chirality for **3**. The two chromophores were arranged in a clockwise manner to assign the absolute configuration of **3** as depicted (Fig. 3). The CD spectrum of **4** showed the first negative Cotton effect at λ_{max}=241 nm (Fig. 4) corresponding to the exciton coupling between the α,β-unsaturated ketone (UV λ_{max}=234 nm)[14] and the Δ^{15} double bond.[8] The counterclockwise manner of two chromophores in space thus defined the absolute configuration of **4** as depicted. Similarly, the first negative Cotton effect at λ 255 nm of **5** coming from the exciton split between two chromophores of the α,β-unsaturated ketone[14] and the *p*-hydroxybenzoate;[13] and the first negative Cotton effect at λ 258 nm of **6** arousing from the exciton coupling between two chromophores of the α,β-unsaturated ketone[14] and the 4-hydroxy-3-methoxy-benzoate,[13] indicated that compounds **5** and **6** shared the same absolute configuration as **4** (Fig. 4).

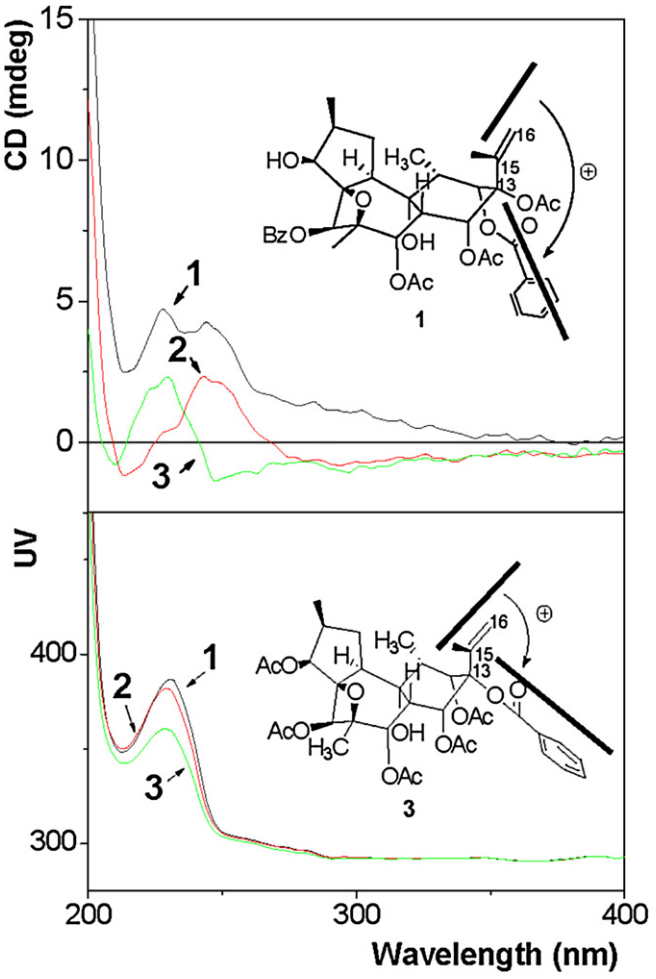


Figure 3. CD and UV spectra (in MeOH) of compounds **1**–**3**. Bold lines denote the electric transition dipole of the chromophores for **1** and **3**.

2.2. Biological evaluation of trigochinins D–I (1–6)

Trigochinins D–I (**1**–**6**) were tested for the anti-tumor activities against the HL-60 (human premyelocytic leukemia), BEL-7402 (human liver cancer), HMEC (human microvascular endothelial cell), A-549 (human lung adenocarcinoma), and P-388 (murine leukemia) cell lines by using either MTT[15] or SRB [16] methods, and with pseudolaric acid B[17] as the positive control (IC_{50}=4.2 μM against HL-60). Trigochinins E (**2**) and F (**3**) exhibited strong inhibition on HL-60 tumor cell lines with the IC_{50} values of 8.1 and 6.4 μM, respectively.

3. Experimental section

3.1. General experimental procedures

Optical rotations were determined on a Perkin–Elmer 341 polarimeter, and CD spectra were obtained on a Jasco 810 spectrometer. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin–Elmer 577 spectrometer with KBr disks. NMR spectra were acquired on

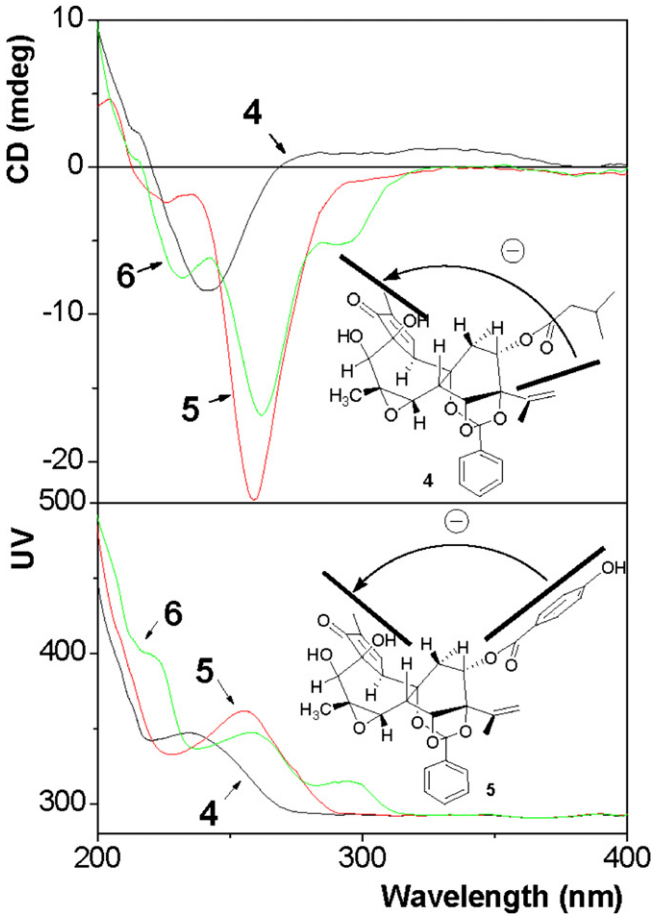


Figure 4. CD and UV spectra (in MeOH) of compounds **4**–**6**. Bold lines denote the electric transition dipole of the chromophores for **4** and **5**.

a Bruker AM-400 spectrometer. EIMS and HREIMS (70 eV) were carried out on a Finnigan MAT 95 mass spectrometer. ESIMS and HRESIMS were obtained on an Esquire 3000plus (Bruker Daltonics) and a Waters-Micromass Q-TOF Ultima Global electrospray mass spectrometer, respectively. Silica gel (200–300 mesh) (Qingdao Haiyang Chemical Co. Ltd.), C18 reverse-phased silica gel (150–200 mesh, Merck), MCI gel (CHP20P, 75–150 μM, Mitsubishi Chemical Industries Ltd.), and Sephadex LH-20 gel (Amersham Biosciences) were used for column chromatography. All solvents used for chromatography were of analytical grade (Shanghai Chemical Plant, Shanghai, People's Republic of China).

3.2. Plant material

The plant material of *T. chinensis* was collected from Xishuangbanna, People's Republic of China in July, and was authenticated by Professor You-Kai Xu of Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (accession number TCh-2005-2Y) has been deposited in the Shanghai Institute of Materia Medica.

3.3. Extraction and isolation

The air-dried powders of leaves and twigs of *T. chinensis* (6 kg) were extracted three times with 95% EtOH at room temperature to give an ethanolic extract (314 g), which was partitioned between EtOAc and water to obtain an EtOAc soluble fraction E (91 g). The fraction E was separated over a column of MCI gel ($MeOH/H_2O$, 40/60 to 90/10, v/v) to afford six fractions 1a–1g. Fraction 1a (2.53 g) was separated over a reverse-phased silica gel column ($MeOH/H_2O$, 70/30 to 90/10, v/v) to afford three major fractions 1a1 (850 mg), 1a2 (316 mg), and 1a3 (278 mg). Fraction 1a1 was separated over a silica gel column eluted with petroleum ether/EtOAc (5/1, v/v) to give two major subfractions, and each of them was then purified on a column of reverse-phased C18 silica gel eluted with $MeOH/H_2O$ (75/35, v/v) to yield **1** (10 mg) and **2** (80 mg), respectively. Fraction 1a2 was treated with the procedure same as 1a1 to obtain compound **3**. Fraction 1d (1.00 g) was separated over a column of silica gel eluted with petroleum ether/EtOAc (4/1, v/v) to afford three major fractions 1d1 (200 mg), 1d2 (180 mg) and 1d3 (430 mg). Fractions 1d1–1d3 were purified separately over a column of reverse-phased C18 silica gel eluted with $MeOH/H_2O$ (7/3, v/v) to yield successively compounds **4** (51 mg), **5** (25 mg), and **6** (5 mg).

*3.3.1. Trigochinin D (**1**).* White powder; $[\alpha]_D^{21}$ +44.0 (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log ε) 229.8 (4.35) nm; IR (KBr) ν_{max} 3565, 3444, 2976, 2931, 1761, 1724, 1452, 1375, 1279, 1236, 1119, 1024, 716 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 1 and 3, respectively; positive mode ESIMS *m/z* 757.4 $[M+Na]^+$, 1491.7 $[2M+Na]^+$; negative mode ESIMS *m/z* 779.6 $[M+HCOO]^-$; EIMS *m/z* 675 (1), 613 (5), 443 (16), 105 (100), 77 (7); HRESIMS *m/z* 757.2817 $[M+Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2836).

*3.3.2. Trigochinin E (**2**).* White powder; $[\alpha]_D^{21}$ +73.0 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 231.0 (4.23) nm; IR (KBr) ν_{max} 3548, 3435, 2980, 2939, 1759, 1728, 1452, 1375, 1313, 1273, 1236, 1117, 1094, 1026, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 1 and 3, respectively; positive mode ESIMS *m/z* 799.5 $[M+Na]^+$, 1576.7 $[2M+Na]^+$; EIMS *m/z* 717 (2), 655 (17), 595 (8), 567 (8), 535 (16), 443 (61), 219 (25), 177 (30), 105 (100), 77 (29); HRESIMS *m/z* 799.2939 $[M+Na]^+$ (calcd for $C_{42}H_{48}O_{14}Na$, 799.2942).

*3.3.3. Trigochinin F (**3**).* White powder; $[\alpha]_D^{21}$ −7.0 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 230.8 (4.15) nm; IR (KBr) ν_{max} 3433, 2978, 2931, 1745, 1637, 1452, 1375, 1250, 1034, 714 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 1 and 3, respectively; positive mode ESIMS *m/z* 737.4 $[M+Na]^+$, 1452.4 $[2M+Na]^+$; EIMS *m/z* 697 (1), 655 (16), 381 (13), 177 (17), 105 (100), 77 (7); HRESIMS *m/z* 737.2801 $[M+Na]^+$ (calcd for $C_{37}H_{46}O_{14}Na$, 737.2785).

*3.3.4. Trigochinin G (**4**).* White powder; $[\alpha]_D^{21}$ −10.0 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 239.4 (3.94) nm; IR (KBr) ν_{max} 3466, 2960, 2926, 2874, 1709, 1691, 1632, 1452, 1358, 1254, 1194, 1086, 1003, 926, 756 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 2 ande 3, respectively; positive mode ESIMS *m/z* 567.3 $[M+H]^+$, 1155.5 $[2M+Na]^+$; EIMS *m/z* 566 (18), 281 (5), 189 (8), 105 (100), 77 (14), 55 (15); HRESIMS *m/z* 589.2429 $[M+Na]^+$ (calcd for $C_{32}H_{38}O_9Na$, 589.2414).

*3.3.5. Trigochinin H (**5**).* White powder; $[\alpha]_D^{21}$ −96.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 263.2 (4.29) nm; IR (KBr) ν_{max} 3545, 3448, 1713, 1699, 1593, 1514, 1450, 1281, 1165, 1082, 1003, 922, 625 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 2 and 3, respectively; EIMS *m/z* 602 (5), 121 (90), 105 (100), 77 (21); HREIMS *m/z* 602.2160 $[M]^+$ (calcd for $C_{34}H_{34}O_{10}$, 602.2152).

*3.3.6. Trigochinin I (**6**).* White powder; $[\alpha]_D^{21}$ −70.0 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 256.2 (4.33) nm; IR (KBr) ν_{max} 3398, 2937, 1711, 1693, 1628, 1599, 1516, 1286, 1221, 1084, 1003, 760 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz) see Tables 2 and 3, respectively; EIMS *m/z* 632 (1) $[M]^+$, 602(8), 151 (16), 121 (100), 105 (83), 77 (11), 69 (3); HREIMS *m/z* 632.2265 $[M]^+$ (calcd for $C_{35}H_{36}O_{11}$, 632.2258).

Acknowledgements

Financial support of the National Natural Science Foundation of China (Grant No. 30630072; 30721005; 20932007) and National

Science & Technology Major Project 'Key New Drug Creation and Manufacturing Program' of China (Grant No. 2009ZX09301-001) is gratefully acknowledged. We thank Prof. Y.-K. Xu of Xishuangbanna Tropical Botanical Garden, CAS for the identification of this plant material.

Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.04.118. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Chen, S. K.; Chen, B. Y.; Li, H. *Flora of China (Zhongguo Zhiwu Zhi)*; Science: Beijing, 1997; Vol. 44, pp 162–170.
2. (a) Jayasuriya, H.; Zink, D. L.; Suresh, S. B.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Slayton, L.; Gregory, L.; Zakson-Aiken, M.; Shoop, W.; Singh, S. B. *J. Am. Chem. Soc.* **2000**, *122*, 4998; (b) Liao, S. G.; Chen, H. D.; Yue, J. M. *Chem. Rev.* **2009**, *109*, 1092.
3. (a) Jayasuriya, H.; Zink, D. L.; Borris, R. P.; Nanakorn, W.; Beck, H. T.; Balick, M. J.; Goetz, M. A.; Gregory, L.; Shoop, W.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 228; (b) Soonthornchareonnon, N.; Sakayarojkul, M.; Isaka, M.; Mahakittikun, V.; Chuakul, W.; Wongsinkongman, P. *Chem. Pharm. Bull.* **2005**, *53*, 241; (c) Tempeam, A.; Thasana, N.; Pavaro, C.; Chuakul, W.; Siripong, P.; Ruchirawat, S. *Chem. Pharm. Bull.* **2005**, *53*, 1321.
4. Yin, S.; Su, Z. S.; Zhou, Z. W.; Dong, L.; Yue, J. M. *J. Nat. Prod.* **2008**, *71*, 1414.
5. Zhang, L.; Luo, R. H.; Wang, F.; Jiang, M. Y.; Dong, Z. J.; Yang, L. M.; Zheng, Y. T.; Liu, J. K. *Org. Lett.* **2010**, *12*, 152.
6. (a) Kokpol, U.; Thebpatiphat, S.; Boonyaratavej, S.; Chedchuskulcai, V.; Ni, C. Z.; Clardy, J.; Chaichantipyuth, C.; Chittawong, V.; Miles, D. H. *J. Nat. Prod.* **1990**, *53*, 1148; (b) Hu, X. J.; Wang, Y. H.; Kong, L. Y.; He, H. P.; Gao, S.; Liu, H. Y.; Ding, J.; Xie, H.; Di, Y. T.; Hao, X. J. *Tetrahedron Lett.* **2009**, *50*, 2917.
7. Kanchanapoom, T.; Kasai, R.; Chumsri, P.; Kraisintud, K.; Yamasakia, K. *Tetrahedron Lett.* **2002**, *43*, 2941.
8. Chen, H. D.; He, X. F.; Wu, Y.; Yue, J. M. *Org. Lett.* **2009**, *11*, 4080.
9. Chen, H. D.; Yang, S. P.; He, X. F.; Ai, J.; Liu, Z. K.; Liu, H. B.; Geng, M. Y.; Yue, J. M. *Org. Lett.* **2010**, *12*, 1168.
10. Taninaka, H.; Takaishi, Y.; Honda, G.; Imakura, Y.; Sezik, E.; Yesilada, E. *Phytochemistry* **1999**, *53*, 1525; (b) Zhan, Z. J.; Fan, C. Q.; Ding, J.; Yue, J. M. *Bioorg. Med. Chem.* **2005**, *13*, 645.
11. Berova, N.; Nakanishi, K. In *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, K., Woody, R. W., Eds.; Wiley-VCH: New York, NY, 2000; pp 337–382.
12. Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590.
13. Pickett, L. W.; Muntz, M.; Mcpherson, E. M. *J. Am. Chem. Soc.* **1951**, *73*, 4862.
14. Koreeda, M.; Weiss, G.; Nakanishi, K. J. *J. Am. Chem. Soc.* **1973**, *95*, 239.
15. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
16. Skehan, P. A.; Storeng, R.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
17. Pan, D. J.; Li, Z. L.; Hu, C. Q.; Chen, K.; Chang, J. J.; Lee, K. H. *Planta Med.* **1990**, *56*, 383.