

Trigochilides A and B, Two Highly Modified Daphnane-Type Diterpenoids from *Trigonostemon chinensis*

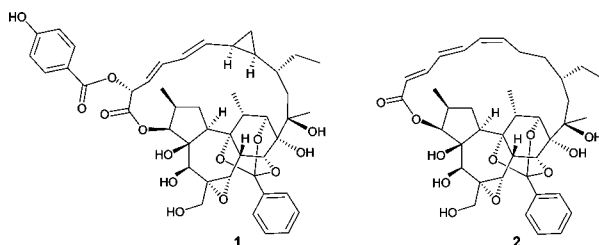
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ABSTRACT



Trigochilides A (1) and B (2), two highly modified daphnane-type diterpenoids with 12-carbon-containing polyketide appendages at C-16 forming a macro-lactone with C-3, were isolated from the twigs and leaves of *Trigonostemon chinensis*. Their structures were elucidated by spectroscopic analysis. Trigochilides A (1) showed modest cytotoxicity against two tumor cell lines.

The genus *Trigonostemon* (Euphorbiaceae) comprising ca. 50 species grows mainly in tropical and subtropical regions of Asia.¹ Previous chemical studies on this genus have led to the isolation of an array of structurally interesting compounds, including the modified daphnane-type diterpenoids,^{2,3} a flavonoidal indole alkaloid,⁴ diterpenoids,⁵ and phenanthrenes.⁶ The modified daphnane-type diterpenoids have been found to possess antiflea insecticide,^{2,3a}

cytotoxic,^{3b} and acaricidal^{3c} activities. In this study, two highly modified daphnane-type diterpenoids, trigochilides A (1) and B (2) with 12-carbon-containing polyketide appendages, which were directly attached at C-16 and formed a macrolactone between C-1' and C-3, were isolated from the twigs and leaves of *Trigonostemon chinensis* Merr. We report herein the isolation, structural elucidation, and cytotoxic activities of these compounds.

The air-dried powder (6.0 kg) of leaves and twigs of *T. chinensis* was extracted three times with 95% EtOH at room temperature to give a crude extract (314 g), which was partitioned between EtOAc and water to obtain the EtOAc-soluble fraction (91 g). The EtOAc-soluble fraction was separated on a column of MCI gel (CHP20P, 75–150 μ M) eluted with MeOH–H₂O (from 4/6 to 9/1, v/v) in gradient to afford fractions 1–7. Fraction 5 (5.5 g) was extensively

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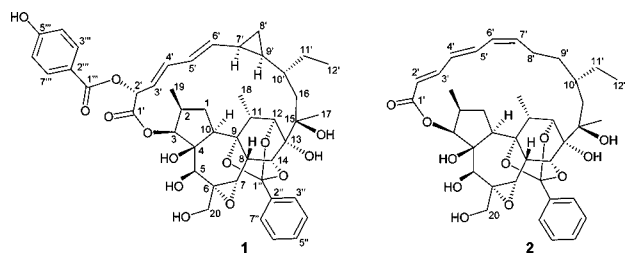
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Table 1. ^1H and ^{13}C NMR Spectroscopic Data of Trigochilides A (**1**) and B (**2**) (in CDCl_3)

1					2				
no.	δ_{H} (mult, J , Hz) ^a	δ_{C} ^b	δ_{H} (mult, J , Hz) ^a	δ_{C} ^b	no.	δ_{H} (mult, J , Hz) ^a	δ_{C} ^b	δ_{H} (mult, J , Hz) ^a	δ_{C} ^b
1	1.89, m	34.8	2.19, m	35.6	7'	1.51, m	17.6	5.78, m	138.8
	1.72, m		1.88, m						
2	1.55, m	35.3	1.68, m	35.6	8'	α : 1.20, m	16.8	2.45, m	23.0
						β : 0.52, br d (5.2)		1.95, m	
3	4.58, br s	84.0	4.93, br d (3.0)	81.1	9'	1.00, m	28.3	α : 1.52, m	33.7
								β : 1.40, m	
4		81.2		82.9	10'	1.05, m	34.8	1.38, m	33.1
5	3.82, br s	72.3	3.87, br s	72.6	11'	1.46, m	30.8	1.38, m	29.1
						1.75, m		1.28, m	
6		60.7		60.3	12'	1.00, t (6.9)	11.7	0.88, t (6.9)	11.8
7	3.30, br s	65.1	3.36, br s	64.3	1''		108.4		108.4
8	4.31, br s	34.6	4.25, br s	35.3	2''		138.6		138.8
9		77.0		77.6	3''	7.67, m	125.1	7.71, m	125.1
10	2.94, dd (12.9, 6.0)	46.8	2.93, dd (13.3, 6.4)	47.6	4''	7.35, m	128.1	7.38, m	128.1
11	2.55, q (6.3)	37.0	3.36, m	37.2	5''	7.35, m	129.4	7.36, m	129.3
12	3.97, br s	82.7	4.18, br s	83.1	6''	7.35, m	128.1	7.38, m	128.1
13		71.1		71.4	7''	7.67, m	125.1	7.71, m	125.1
14	4.25, br s	80.0	4.22, br s	81.1	1'''		165.2		
15		76.0		76.0	2'''		121.1		
16	2.15, dd (14.3, 8.6)	41.4	1.91, m	37.7	3'''	7.96, d (8.2)	132.3		
	1.54, m		1.64, m						
17	1.21, s	20.8	1.28, s	22.2	4'''	6.83, d (8.2)	115.7		
18	1.46, d (6.3)	18.4	1.60, d (6.9)	18.8	5'''		160.9		
19	1.00, d (6.7)	13.0	1.01, d (6.4)	13.3	6'''	6.83, d (8.2)	115.7		
20	3.72, br d (7.2)	65.9	3.74, m	63.0	7'''	7.96, d (8.2)	132.3		
	3.64, br d (7.2)		3.62, m						
1'		171.1		169.1	4-OH	2.80, s			
2'	5.91, d (6.7)	69.3	5.78, d (15.4)	119.1	5-OH	4.00, br s			
3'	5.97, dd (15.1, 6.7)	122.3	7.47, dd (15.4, 11.6)	148.0	13-OH	3.71, s		3.01, br s	
4'	6.53, dd (15.1, 10.9)	133.4	6.31, dd (14.6, 11.6)	128.3	15-OH	1.62, br s		3.82, br s	
5'	6.23, dd (15.0, 10.9)	129.8	6.87, dd (14.6, 11.2)	137.6	20-OH	2.51, s			
6'	5.55, dd (15.0, 9.9)	138.9	6.15, t (11.2)	128.1					

^a Recorded at 400 MHz. ^b Recorded at 100 MHz

separated over columns of silica gel and RP-18 silica gel to obtain compounds **1** (9 mg) and **2** (5 mg).



Trigochilide A (**1**)⁷ was obtained as a white amorphous powder. The HRESIMS displayed a sodiated molecular ion at m/z 853.3411 [$\text{M} + \text{Na}$]⁺ (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{14}\text{Na}$, 853.3411) corresponding to the molecular formula of $\text{C}_{46}\text{H}_{54}\text{O}_{14}$ requiring 20 double-bond equivalents. The IR absorption bands at 3454, 1716, and 1693 cm^{-1} indicated the presence of hydroxyl and carbonyl functionalities. In accordance with the molecular formula, 46 carbon resonances were resolved in the ^{13}C NMR spectrum (Table 1), and were further classified by DEPT experiments as four methyls, five

methylenes, 26 methines (six oxygenated and 13 olefinic ones), and 11 quaternary carbons (five oxygenated, one orthoester, two esters, and three olefinics). In addition, one tertiary methyl (δ_{H} 1.21, 3H, s), two secondary methyls (δ_{H} 1.46, 3H, d, $J = 6.3$ Hz; δ_{H} 1.00, 3H, d, $J = 6.7$ Hz), one primary methyl (δ_{H} 1.12, 3H, t, $J = 6.9$ Hz), one *p*-hydroxybenzoyl group, and one monosubstituted benzene ring were distinguished by analysis of the NMR data (Table 1). The aforementioned data suggested that compound **1** featured a scaffold of modified daphnane-type diterpenoid orthoester.^{3,4} A detailed account of the structural elucidation of **1** is presented below.

The partial structure of the daphnane-type diterpenoid orthoester (Figure 1, component A) was readily established by the comprehensive analysis of the 1D and 2D NMR spectra, especially HMBC (Figure 1). Several structural

(7) **Trigochilide A (1)**: white amorphous powder; $[\alpha]_{\text{D}}^{25} +97$ (c 0.100, MeOH); UV (MeOH) λ_{max} (log ϵ) 256.6 (4.59) nm; IR (KBr) ν_{max} 3454, 2958, 2875, 1716, 1693, 1452, 1329, 1267, 1095, 1028, 712 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100 MHz) see Table 1; positive mode ESIMS m/z 831.0 [$\text{M} + \text{H}$]⁺; EIMS m/z 138 (56), 121 (92), 105 (100), 77 (63); HRESIMS m/z 853.3411 (calcd for $\text{C}_{46}\text{H}_{54}\text{O}_{14}\text{Na}$, 853.3411).

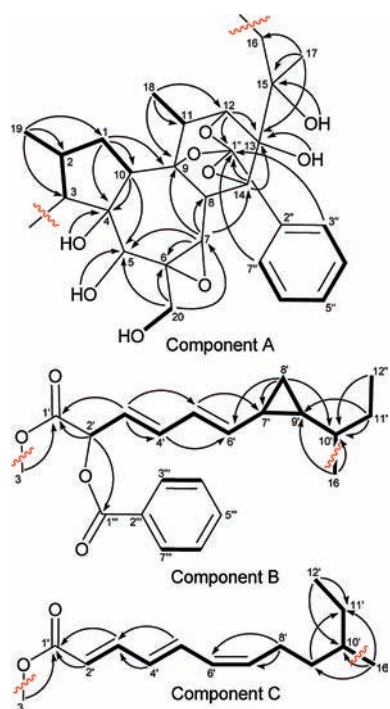


Figure 1. ^1H – ^1H COSY (–) and selected HMBC (→) correlations of component A of **1** and **2**, component B of **1**, and component C of **2**.

fragments (C-10–C-1–C-2–C-3/C-19, C-11–C-18, C-20–OH-20, and C-3''–C-7'') were established by the correlations observed in the ^1H – ^1H COSY spectrum (Figure 1, component A, bold lines). The connectivity of the structural fragments, the quaternary carbons, and the incorporated oxygen atoms was then achieved by the HMBC correlations. In particular, the presence of a 6,7-epoxide was revealed by the chemical shifts of C-6 at δ_{C} 60.7 and C-7 at δ_{C} 65.1 and confirmed by the mutual HMBC correlations from H-7 to C-5, C-6, C-8, and C-14. Two secondary methyls at δ_{H} 1.46 and 1.00 were assigned to Me-18 and Me-19 by the HMBC correlations from Me-18 to C-9, C-11 and C-12, and from Me-19 to C-1, C-2, and C-3, respectively. The only tertiary methyl at δ_{H} 1.21 (3H, s) was assigned to Me-17 on the basis of the HMBC correlations from Me-17 to C-13, C-15 and C-16. Four hydroxyls, whose protons resonated at δ_{H} 2.80 (s), 4.00 (br s), 3.71 (s), and 1.62 (br s) were distinguished by HSQC, were located at C-4, C-5, C-13, and C-15 by the corresponding HMBC correlation of each hydroxyl proton to their binding carbon, respectively. The proton of one hydroxyl resonating at δ_{H} 2.51 (s) was assigned to 20-OH by the correlation between 20-OH and H₂-20 in the ^1H – ^1H COSY spectrum. A typical orthoester carbon at δ_{C} 108.8 was attributable to the C-1'' of an orthobenzoate.^{3,4} The three remaining oxygenated carbons of C-9, C-12, and C-14 could thus be assigned to a 9, 12,14-orthobenzoate, and confirmed by the HMBC correlations from H-12, H-14, and H-3'' (or H-7'') to C-1''. The structure of component A was also confirmed by comparison of the ^{13}C NMR data of **1** with that of rediocide A,² which possessed the identical

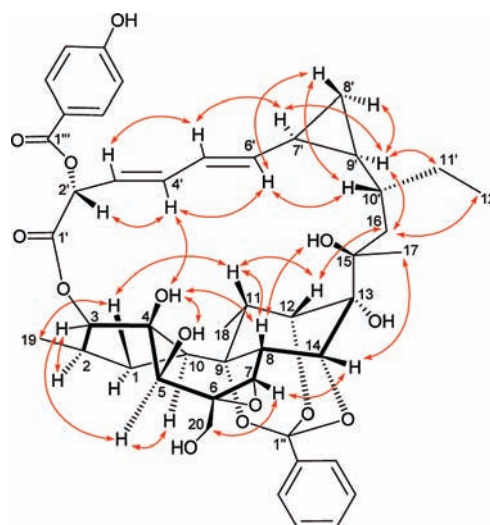


Figure 2. Key ROESY (↔) correlations of **1**.

core of a daphnane-type diterpenoid with **1** (see the Supporting Information).

Component B of a novel 12-carbon polyketide moiety was readily established by using a combination of 1D and 2D NMR techniques, especially ^1H – ^1H COSY and HMBC spectra. The main part of the polyketide (C-2'–C-12') was revealed by the correlations as observed in the ^1H – ^1H COSY spectrum (Figure 1, component B). The HMBC correlations from H-2' and H-3' to C-1' at δ_{C} 171.1 then connected C-1' and C-2'. The HMBC correlation between H-2' and C-1''' (δ_{C} 165.2) located the *p*-hydroxybenzoyl group at C-2'. The key correlation between H₂-16 and H-10' in the ^1H – ^1H COSY spectrum allowed the linkage of components A and B via the C-16 and C-10' bond, which was supported by the HMBC correlations from H₂-16 to C-9' and C-10'. The crucial HMBC correlation from H-3 to C-1' constructed the macro-lactone of **1** via an ester bond between the two components A and B. The planar structure of **1** was thus defined.

The relative configuration of **1** was established by ROESY experiment (Figure 2) and computer modeling. The ROESY cross-peaks of H-11/H-1 β , H-11/H-8, OH-4/H-8, OH-4/OH-5, H₂-20/H-7, H-7/H-14, and H-11/H-12 indicated that they were cofacial and were randomly assigned as β -oriented. In consequence, the ROESY correlations of H-5/H-10, H-5/H-3, and H-2/H-3 suggested that H-2, H-3, H-5, and H-10 were α -oriented. The mutual ROESY correlations of H-14/Me-17, H-12/H₂-16, and H-11/H-12 revealed that the 9,12,14-orthobenzoate was α -directed. The strong ROESY correlations of Me-17/H-14 and OH-15/H-8 suggested that C-15 occupied the axial orientation at C-13 and OH-15 took a β -direction. The geometry of the conjugated 3*E*,5*E*-diene was determined by the coupling constants ($J_{3',4'} = 15.1$ Hz, $J_{5',6'} = 15.0$ Hz), and was confirmed by the ROESY correlations (Figure 2). The mutual ROESY correlations of H-2'/H-4', H-4'/OH-4, H-4'/H-6', H-6'/H-8' β , H-6'/H-10', and H-8' β /H-10' indicated that they were in the same side, and were

β -directed. The ROESY correlations of H-5'/H-7', H-7'/H-9', H-8' α /H-9', H-9'/H₂-11, H-9'/H₂-16, and H₂-16/H₃-12' were consequently indicative of α -orientation for H-7', H-9' and the ethyl at C-10'. A computer modeled 3D structure of **1** was generated by using MM2 force field calculations for energy minimization with the molecular modeling program Chem3D Ultra 9.0. The relative configuration and the conformation of **1** assigned by its ROESY spectrum were compatible with those of **1** offered by computer modeling, in which the close contacts of atoms calculated in space were consistent with the ROESY correlations (Supporting Information). The structure of **1** was thus elucidated.

Trigochilide B (**2**)⁸ was isolated as a white amorphous powder, which was analyzed for the molecular formula of C₃₉H₅₀O₁₁ by HRESIMS at m/z 717.3257 [M + Na]⁺ (calculated for C₃₉H₅₀O₁₁Na, 717.3251). The ¹H and ¹³C NMR data (Table 1) of **2** clearly revealed that it shared the same component A as in compound **1**, the difference between **1** and **2** occurred in the 12-carbon-containing polyketide appendages. The presence of a linear conjugated triene moiety (C-2' to C-12') was clearly indicated by the ¹H–¹H COSY spectrum (Figure 1, component C). The HMBC correlations from H-2' and H-3' to C-1' (δ_C 169.1) placed the ester carbonyl at C-2' and allowed the full assignment of component C. The components A and C were finally assembled by a combination of ¹H–¹H COSY and HMBC data. The correlation between H₂-16 and H-10' in the ¹H–¹H COSY spectrum enabled the linkage of components A and C via the C-16–C-10' bond, which was supported by the HMBC correlations from H₂-16 to C-9', C-10' and C-11'. The key HMBC correlation between H-3 and C-1' led to the assignment of the point of attachment of the macrolactone ring via an ester bond between C-3 and C-1', which was further supported by the downfield shift of H-3 at δ 4.93 (br d, J = 3.0 Hz).

The relative configuration of **2** was elucidated by a ROESY spectroscopy (Figure 3) and comparison with compound **1**. The NMR data and ROESY spectrum confirmed that the relative configuration of component A of **2** was identical to that of **1**. The geometry of the conjugated triene of component C was determined as *2E,4E,6Z* by the coupling constants of 15.4, 14.6, and 11.2 Hz, respectively, and was confirmed by the ROESY correlations of H-2'/H-

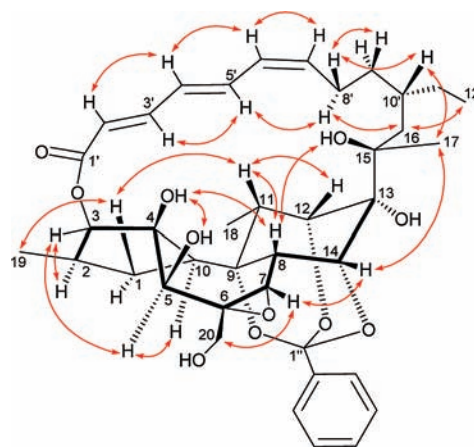


Figure 3. Key ROESY (\leftrightarrow) correlations of **2**.

4', H-4'/H-6', H-6'/H-7', H-3'/H-5', and H-5'/H-8 α . The presence of the conjugated triene rendered the macro-lactone ring conformationally well organized, and allowed one favorable conformation predominant in solution. The mutual ROESY correlations of Me-12'/H₂-16, H-10'/H-8' β , H-8' β /H-9' β , H-10'/H₃-17, and H-8' α /H₂-16 revealed that the ethyl group at C-10 was α -directed. The relative configuration and conformation of **2** was therefore established as depicted.

The cytotoxic activities of the trigochilides A (**1**) and B (**2**) were evaluated against two tumor cell lines according to the standard protocols (Supporting Information). Compound **1** showed modest cytotoxicity against HL-60 (human leukemia) and BEL-7402 (human hepatoma) with the IC₅₀ values of 3.68 and 8.22 μ M, respectively, whereas compound **2** only exhibited weak cytotoxicity against two tumor cell lines with IC₅₀ values of 33.35 and 54.85 μ M, respectively.

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Supporting Information Available: Experimental procedures, 1D and 2D NMR, EIMS, HRESIMS, IR spectra of trigochilides A (**1**) and B (**2**). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(8) **Trigochilide B (2):** white amorphous powder; [α]_D²⁵ –31 (c 0.165, MeOH); UV (MeOH) λ_{\max} (log ϵ) 302.2 (4.05) nm; IR (KBr) ν_{\max} 3435, 2962, 1699, 1646, 1452, 1331, 1271, 1086, 1028, 999, 756 cm^{–1}; ¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100 MHz) see Table 1; EIMS m/z 122 (89), 105 (100), 77 (57), 51 (16); HRESIMS m/z 717.3257 (calcd for C₃₉H₅₀O₁₁Na, 717.3251).