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Highly Functionalized Daphnane Diterpenoids from *Trigonostemon thyrsoideum*

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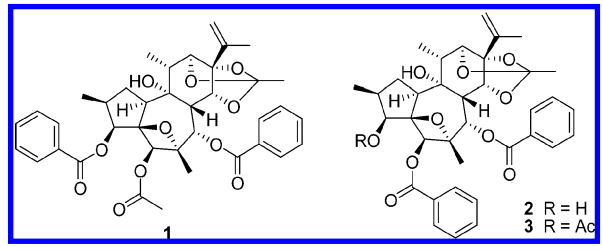
ABSTRACT



Trigonothyrins A−C (1−3), which are highly functionalized daphnane diterpenoids, were isolated from the stems of *Trigonostemon thyrsoideum*. Compounds 1−3 represent the first examples of daphnanes with an oxygen-bridged four-membered-ring system, and a linkage mode of 12,13,14-orthoester. Compound 3 was observed to inhibit HIV-1 induced cytopathic effects. The EC_{50} value was 2.19 μg/mL, and the therapeutic index (TI) was more than 90.

The genus *Trigonostemon* (Euphorbiaceae) is a prolific source of daphnane diterpenoids.[1] Previous investigations of *T. reidioides* and *T. chinensis* isolated modified daphnanes (rediocides A−G, trigochilides A and B),[2] 3,4-*seco*cleistanthanic diterpenes (trigonochinenes A−D), a highly aromatic tetranorditerpene (trigonochinene E),[3] and a phenanthrenone (trigonostemone),[4] together with a flavonoidal indole alkaloid (lotthanongine).[5]

Daphnane diterpenoids have been reported to possess various bioactivities, such as antiflea,[2a,b] acaricidal,[2c] antileukemic, neurotrophic effects,[6] and cytotoxicity.[2d,7] In this study, three new highly oxygenated daphnane diterpenoids trigonothyrins A−C (**1**−**3**) were isolated from the stems of the plant *T. thyrsoideum*. These compounds were evaluated

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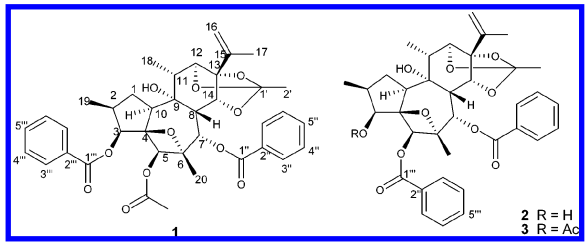
for their inhibitory activity against HIV-1, wherein compound **3** showed significant activity with an EC_{50} value of 2.19 μg/mL, and a TI value of more than 90. Herein, we report on the isolation, structural elucidation, and anti-HIV-1 activities of these compounds.

Air-dried *T. thyrsoideum* stems were powdered (8.0 kg), extracted with EtOAc (3 × 35 L, with each soaking for 7 days) at room temperature, and then filtered. The filtrate was concentrated in vacuo to give a residue (128 g), which was then subjected to silica gel column chromatography with a gradient elution of petroleum ether/acetone (100:0 to 1:2), and then methanol to obtain 10 fractions. Fraction 3 (2.1 g) eluted with petroleum ether/acetone (80:20) was repeatedly purified by MPLC (MeOH−H_2O), Sephadex LH-20 ($CHCl_3$−MeOH), and preparative HPLC (acetonitrile−H_2O) to afford compounds **1** (35 mg), **2** (3.7 mg), and **3** (4.0 mg).



Compound **1**[8] was obtained as an amorphous powder. Its molecular formula was determined to be $C_{38}H_{42}O_{11}$ on the basis of positive HRESIMS, showing a quasi-molecular ion peak at *m/z* 697.2625 (calcd for $C_{38}H_{42}O_{11}Na$ 697.2624). The IR spectrum showed the absorption bands of hydroxy and carbonyl groups at 3540, 1748 cm^{-1}. In accordance with the molecular formula, 38 carbon resonances were resolved in the ^{13}C NMR spectrum (Table 1), and were further classified by DEPT experiments as six methyls, two methylenes, 19 methines, and 11 quaternary carbons. In combination with the ^1H NMR spectrum (Table 1), the functionalities of an isopropenyl (δ_C 136.8, s, C-15; 117.4, t, C-16; 19.3, q, C-17; δ_H 5.27, 5.07 each 1H, br s, H-16; 1.91, 3H, br s, Me-17), an acetoxy (δ_C 170.0, s; 20.4, q; δ_H 1.76, 3H, s), and two benzoyloxy groups were clearly distinguishable. The quaternary carbon at δ 118.9 was identified as a signal corresponding to an orthoester group, a structural moiety that occurs frequently in daphnane diterpenoids. The ^1H NMR spectrum also displayed two tertiary methyls (δ_H 1.35, 3H, s, Me-20; 1.27, 3H, s, Me-2′), two secondary methyls (δ_H 1.30, 3H, d, J = 7.1 Hz, Me-18; 0.94, 3H, d, J = 7.1 Hz, Me-19), and five oxygenated methines (δ_H 6.47, 1H, s, H-5; 5.86, 1H, d, J = 3.8 Hz, H-7; 5.45, 1H, d, J = 10.4 Hz, H-3; 4.46, 1H, br s, H-14; 4.03, 1H, br s, H-12). Considering its biological source, **1** should be a daphnane diterpenoid. The observable HMBC correlations from the proton at δ_H 5.45 (1H, d, J = 10.0 Hz, H-3) to the carbon at δ_C 165.7 (s, C-1‴), from δ_H 6.47 (1H, s, H-5) to δ_C 170.0 (s, 5-CH_3CO), and from δ_H 5.86 (1H, d, J = 3.8 Hz, H-7) to δ_C 166.5 (s, C-1″) indicate that two benzoyloxy groups are located at C-3 and C-7, while an acetoxy group is located at C-5. The HMBC correlations from the proton at δ_H 3.77 (1H, s, 9-OH) to C-9 and C-10 were observed, which indicate that a hydroxy group is attached at C-9. Until now, to the best of our knowledge, the orthoester moiety has only been observed at the C-ring in all previously reported daphnane diterpenoids. Therefore, carbons C-12, C-13, and C-14 should bear an orthoester moiety, which is a new linkage way in daphnanes. In view of the one unsettled degree of unsaturation and its molecular formula, an epoxy group must be embedded between the remaining oxygenated quaternary carbons of C-4 and C-6, wherein this conclusion is supported by the fact that two carbon signals at δ_C 92.1 (s, C-4) and 83.5 (s, C-6) in **1** are clearly shifted downfield in comparison to those of non-4,6-epoxy analogues.[2,7a] Therefore, the planar structure of **1** was defined.

The ROESY experiment (Figure 1) was applied to deduce the relative stereochemistry of **1**. Correlations of H-2/H-3, H-3/H-10, and H-5/H-10 were observed, indicating an α-orientation of these protons. As a consequence, the appearance of cross-peaks of H-7/H-8, H-7/H-14, H-7/Me-20, H-11/H-1β, and H-8/H-11 allowed these protons to be assigned as β-oriented. The correlations of H-8/H-14, H-11/H-12, H-11/H-16, and H-14/Me-17 revealed that the 12,13,14-orthoester is located at the α-face of the molecule. The correlations of O*H*-9/H-5 and O*H*-9/H-3″,7″ demonstrate an α-orientation of the hydroxy group at C-9. The A/B ring junction of all daphnanes isolated so far is a *trans* configuration, which has a β-oriented hydroxy group at C-4 based on the ROESY spectrum. From the same plant the daphnanes with 4β-OH were isolated, so the relative configuration at C-4 for **1** was also suggested to be β-oriented. On the basis of the above discussion and referring to published daphnane

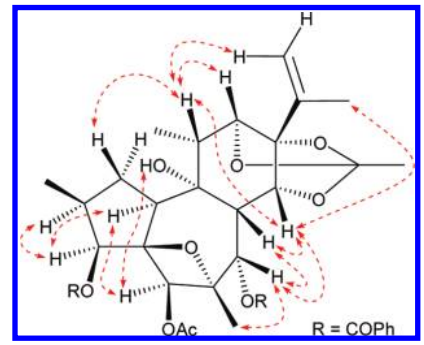


Figure 1. Important ROESY correlations of **1**.

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(8) Trigonothyrin A (**1**): amorphous powder, $[\alpha]^{25}_D$ +32.5 (*c* 0.34, MeOH); IR (KBr) ν_{max} 3540, 2974, 2932, 1748, 1720, 1452, 1384, 1278, 1113, 1070, 1027, 996, 867, 712 cm^{-1}; ^1H and ^{13}C NMR data: see Table 1; ESIMS *m/z* 697 $[M + Na]^+$, HRESIMS (pos.) *m/z* 697.2625 (calcd for $C_{38}H_{42}O_{11}Na$, 697.2624).

Table 1. NMR Spectroscopic Data for Trigonothyrins A−C (**1**−**3**) in $CDCl_3$

	1		2		3	
no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	1.32 m (H_β)	34.0 CH_2	1.12 m (H_β)	34.1 CH_2	1.25 m (H_β)	33.6 CH_2
	2.03 m (H_α)		1.91 m (H_α)		2.00 m (H_α)	
2	2.55 m	31.1 CH	2.23 m	32.8 CH	2.48 (1H, m)	30.8 CH
3	5.45 d (10.4)	73.5 CH	4.20 d (10.0)	72.4 CH	5.17 d (10.2)	74.5 CH
4		92.1 C		93.6 C		91.9 C
5	6.47 s	73.9 CH	6.67 s	74.4 CH	6.64 s	74.7 CH
6		83.5 C		84.4 C		83.5 C
7	5.86 d (3.8)	79.4 CH	5.89 d (3.9)	79.2 CH	5.88 d (3.7)	79.5 CH
8	2.86 br.d (3.8)	40.7 CH	2.80 dd (3.9, 1.2)	40.8 CH	2.85 dd (3.7, 1.2)	40.7 CH
9		76.1 C		76.0 C		76.0 C
10	2.27 dd (13.7, 6.0)	48.7 CH	2.24 m	48.8 CH	2.31 dd (13.9, 6.3)	48.7 CH
11	1.70 br q (7.1)	37.1 CH	1.71 br q (7.0)	37.1 CH	1.70 br q (7.0)	37.0 CH
12	4.03 br s	81.1 CH	4.03 br s	81.1 CH	4.03 br s	81.1 CH
13		86.2 C		86.2 C		86.2 C
14	4.46 br s	80.2 CH	4.47 br s	80.1 CH	4.46 br s	80.1 CH
15		136.8 C		136.8 C		136.7 C
16	5.07 br s	117.4 CH_2	5.07 br s	117.4 CH_2	5.06 br s	117.4 CH_2
	5.27 br s		5.27 br s		5.27 br s	
17	1.91 br s	19.3 CH_3	1.92 br s	19.4 CH_3	1.91 br s	19.4 CH_3
18	1.30 d (7.1)	13.1 CH_3	1.28 d (7.0)	13.1 CH_3	1.29 d (7.0)	13.1 CH_3
19	0.94 d (7.1)	16.5 CH_3	0.94 d (7.2)	15.8 CH_3	0.85 d (7.2)	16.3 CH_3
20	1.35 s	19.4 CH_3	1.45 s	19.4 CH_3	1.53 s	19.6 CH_3
1′		118.9 C		118.8 C		118.8 C
2′	1.27 s	15.6 CH_3	1.27 s	15.6 CH_3	1.27 s	15.6 CH_3
1″		166.5 C		166.6 C		166.6 C
2″		130.3 C		130.3 C		130.3 C
3″, 7″	8.09 d (7.7)	129.9 CH	8.14 m	129.9 CH	8.11 m	129.9 CH
4″, 6″	7.42 t (7.7)	128.2 CH	7.45 m	128.3 CH	7.43 m	128.3 CH
5″	7.53 t (7.7)	132.9 CH	7.55 m	132.9 CH	7.54 m	132.9 CH
1‴		165.7 C		165.8 C		165.7 C
2‴		130.3 C		130.3 C		130.3 C
3‴, 7‴	8.19 d (7.7)	129.7 CH	8.12 m	129.9 CH	7.99 m	129.5 CH
4‴, 6‴	7.48 t (7.7)	128.5 CH	7.43 m	128.4 CH	7.44 m	128.4 CH
5‴	7.60 t (7.7)	133.1 CH	7.55 m	133.2 CH	7.56 m	133.2 CH
5-CH_3CO		170.0 C				170.3 C
5-CH_3CO	1.76 s	20.4 CH_3			1.93 s	20.5 CH_3
9-OH	3.77 s		3.78 br s		3.79 br s	

diterpenoids,[2,7] the structure of **1** was finally assigned and named trigonothyrin A.

Compound **2**,[9] an amorphous powder, was determined to have a molecular formula of $C_{36}H_{40}O_{10}$ on the basis of its ^{13}C NMR (DEPT) spectrum (Table 1) and positive HRESIMS, which showed a quasi-molecular ion peak at m/z 655.2508 (calcd for $C_{36}H_{40}O_{10}Na$ 655.2519). NMR signals (Table 1) generally agreed with those of **1**, indicating that **2** is also a daphnane, with an obvious difference of a missing acetoxy group. The proton signal at δ 4.20 (1H, d, $J = 10.0$ Hz) assigned to H-3 was remarkably shifted upfield in comparison to that of **1**, indicating that a hydroxy group is linked at C-3. HMBC correlations from the H-5 proton at δ 6.67 (1H, s) to the carbon at δ 165.8 (s, C-1‴), and from the H-7 proton at δ 5.89 (1H, d, $J = 3.9$ Hz) to δ_C 166.6 (s, C-1″) were observed, which allowed benzoyloxy groups to locate at C-5 and C-7, respectively. The gross structure of **2** was then characterized.

Compound **3**[10] gave a molecular formula of $C_{38}H_{42}O_{11}$ by the positive HRESIMS. The NMR spectral data (Table 1) of **3** were almost identical with those of **2**; however, an additional peak corresponding to an acetoxy group appeared. An esterification shift ($\Delta = 0.97$ ppm) of the H-3 proton was observed, in combination with the HMBC correlation from H-3 at δ 5.17 (1H, d, $J = 10.2$ Hz) to δ_C 170.3 (s), which indicated that the acetoxy group is positioned at C-3.

The relative configurations of **2** and **3** were deduced to be the same as **1** by analysis of their ROESY spectra and

(9) Trigonothyrin B (**2**): amorphous powder, $[\alpha]^{27}_D$ +14.8 (c 0.55, MeOH); IR (KBr) ν_{max} 3434, 2970, 2930, 1723, 1640, 1452, 1385, 1274, 1115, 1072, 1026, 991, 869, 713 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; ESIMS m/z 655 $[M + Na]^+$, HRESIMS (pos.) m/z 655.2508 (calcd for $C_{36}H_{40}O_{10}Na$, 655.2519).

(10) Trigonothyrin C (**3**): amorphous powder, $[\alpha]^{27}_D$ +24.7 (c 0.59, MeOH); IR (KBr) ν_{max} 3531, 3435, 2973, 2932, 1727, 1640, 1452, 1384, 1277, 1113, 1071, 1027, 992, 713 cm^{-1}; ^1H and ^{13}C NMR data: see Table 2; ESIMS m/z 697 $[M + Na]^+$, HRESIMS (pos.) m/z 697.2625 (calcd for $C_{38}H_{42}O_{11}Na$, 697.2624).

Table 2. Anti-HIV-1 Activities of Compounds **1**−**3** from *T. thyrsoideum*

compd	anti-HIV-1 activity EC_{50} (μg/mL)	cytotoxicity CC_{50} (μg/mL)	therapy index (TI) CC_{50}/EC_{50}
1	44.3	>200	>4.51
2	63.8	>200	>3.13
3	2.19	>200	>91.3
AZT	0.004	1390	347500

comparison of NMR data, especially multiplicities of the proton signals to those of **1**. Finally, the structures of **2** and **3** were figured out and named trigonothyrins B and C, respectively.

Compounds **1**−**3** were tested for inhibitory activity against HIV-1 (Supporting Information), and the results are summarized in Table 2. Compound **3** showed significant activity to prevent the cytopathic effects of HIV-1 in C8166 cells with an EC_{50} of 2.19 μg/mL, and a TI of more than 90. Considering that the structural variance of **2** and **3** only originated from an acetoxy group at C-3, the remarkable difference of their activity was definitely related to the acetoxy group, suggesting that the acetoxy group was necessary for activity.

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Supporting Information Available: Experiment procedures and NMR spectra of trigonothyrins A−C. This material is available free of charge via the Internet at http://pubs.acs.org.

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