# Trigoxyphins A−G: Diterpenes from *Trigonostemon xyphophylloides*

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Six new oxygenated daphnane-type diterpenoids, trigoxyphins A−F (**1**−**6**), a phenanthrene-type diterpenoid, trigoxyphin G (**7**), and two known compounds were isolated from twigs of *Trigonostemon xyphophylloides*. Their structures were established using spectroscopic methods. Compounds **1** and **2** exhibited strong cytotoxic activity against HL60 ($IC_{50}$: 0.27 and 0.49 $\mu$M) and A549 ($IC_{50}$: 7.5 and 4.9 $\mu$M) tumor cell lines, respectively.

The genus *Trigonostemon* (Euphorbiaceae) grows mainly in tropical and subtropical regions of Asia.[1] Previous chemical investigations on this plant genus afforded a number of structurally interesting diterpenoids, some of which showed a variety of biological activities.[2,3] In this study, six new highly oxygenated daphnane-type diterpenoids (**1**−**6**), a phenanthrene-type diterpenoid (**7**), and the known diterpenoids 3,4-*seco*-sonderianol[4] and 1,2-dihydroheudelotinol[5] were isolated from twigs of *Trigonostemon xyphophylloides* (Croiz.) L. K. Dai et T. L. Wu (Euphorbiaceae). We present herein the isolation, structural elucidation, and cytotoxic activity of these compounds.

## Results and Discussion

Compound **1** was obtained as a white powder. The HRESIMS displayed a sodiated molecular ion at *m*/*z* 609.2110 $[M + Na]^+$ (calcd for $C_{34}H_{34}O_9Na$, 609.2095), which is consistent with a molecular formula of $C_{34}H_{34}O_9$, requiring 18 double-bond equivalents. IR showed absorption bands at 3433 (OH), 1716 (carbonyl), and 1630 (aromatic) $cm^{-1}$. The $^{13}C$ NMR (Table 1) resolved 34 carbon resonances comprising four methyls, one olefinic methylene, 18 methines (four oxygenated and 11 olefinic ones), and 11 quaternary carbons (one ketone, one ester, four olefinic, one orthoester, and four oxygenated ones), as classified by the chemical shifts and HSQC spectrum. In addition, two monosubstituted benzene rings and a trisubstituted epoxide ($\delta_H$ 3.32, s; $\delta_C$ 59.7 and 67.3) were further distinguished by NMR analysis (Tables 1 and 2). Proton resonances at $\delta$ 3.79 (s, 1H) and 3.92 (brs, 1H), showing no correlation with any carbons in the HSQC spectrum, were assigned to the exchangeable protons of two OH groups. The aforementioned facts suggested that compound **1** was a daphnane-type diterpenoid.[6] The structure of **1** was further demonstrated by analysis of 2D NMR spectra, especially HMBC (Figure S1a, Supporting Information). The A, B, and C rings of **1** were readily constructed by comparison of the NMR data with those of known analogues[6] and by analysis of its HMBC spectrum. In particular, the HMBC correlations from H-1 ($\delta_H$ 7.65, brs, 1H) to C-3 ($\delta_C$ 209.5), C-4 ($\delta_C$ 72.4), C-10 ($\delta_C$ 48.0), and C-19 ($\delta_C$ 9.9) indicated the presence of an $\alpha,\beta$-unsaturation ketone in the A ring. The chemical shifts of C-6 at $\delta_C$ 59.7 and C-7 at $\delta_C$ 67.3 featured a typical trisubstituted 6,7-epoxide, which was confirmed by the multiple HMBC correlations from H-7 to C-6, C-8, C-9, and C-20 and from $H_3$-20 to C-5 and C-6. Two OH protons at $\delta$ 3.79 (s, 1H) and 3.92 (brs, 1H) correlating with C-4 ($\delta_C$ 72.4) and C-5 ($\delta_C$ 72.6) indicated the presence of C-4-OH and C-5-OH, respectively. Attachment of a benzoyloxy group at C-12 ($\delta_C$ 71.8) was indicated by the HMBC correlation from H-12 at $\delta_H$ 5.52 (d, $J$ = 7.9 Hz) to the C-1″ within the benzoyl group. The remaining three oxygenated carbons were assigned to C-9 ($\delta_C$ 80.7), C-13 ($\delta_C$ 87.0), and C-14 ($\delta_C$ 82.1) by the HMBC correlations of $H_3$-18/C-9, H-8/C-9, H-11/C-9, H-14/C-9, H-12/C-13, H-11/C-13, $H_3$-17/C-13, $H_2$-16/C-13, and H-12/C-14, suggesting the presence of a 9,13,14-orthobenzoate, which was confirmed by HMBC correlations from H-14 and H-3′ (or H-7′) to C-1′ ($\delta_C$ 118.3).

The relative configuration of **1** was fixed by a ROESY experiment (Figure S1b, Supporting Information). The ROESY cross-peaks of OH-4/H-8, H-11 and OH-5, H-8/H-12, H-11/H-8 and H-12, $H_3$-17/H-12, H-7/H-14, and OH-5/$H_3$-20 indicated that OH-4, OH-5, H-7, H-8, H-11, H-12, H-14, and Me-20 were cofacial and randomly assigned in a $\beta$-configuration. In consequence, the ROESY correlation between H-5 and H-10 suggested that they were α-oriented. The ROESY correlation between H-14 and $H_3$-17 revealed that the 9,13,14-orthobenzoate was α-directed. Thus, the structure of compound **1** was elucidated to be as shown.

Compound **2** had the molecular formula $C_{34}H_{36}O_9$. IR absorptions at 3448, 1747, and 1705 $cm^{-1}$ revealed the presence of OH and carbonyl groups. The $^1H$ and $^{13}C$ NMR data of **2** (Tables 1 and 2) showed many similarities to those of **1**, indicating that they were structural analogues. As compared with compound **1**, the main differences were due to the presence of one more methine ($\delta_H$ 2.37, m, 1H; $\delta_C$ 44.3) and one more methylene ($\delta_H$ 1.42, m and 2.37, m, each 1H; $\delta_C$ 32.5) in the A ring of **2**, with the concomitant absence of proton and carbon resonances of the $\Delta^1$ double bond in **1**, clearly indicating that the $\Delta^1$ double bond of **1** was saturated in **2**. This was supported by the upfield shift of $H_3$-19 ($\delta_H$ 1.07, d, $J$ = 6.1 Hz) and the downfield shift of C-3 ($\delta_C$ 218.8). The HMBC correlations (Figure S2a, Supporting Information) from $H_3$-19 to C-1, C-2, and C-3 and from $H_2$-1 to C-3 further validated the above conclusion. Two OH groups [$\delta_H$ 2.93 (s) and $\delta_H$ 2.69 (d, $J$ = 9.1 Hz)] were assigned to C-4 and C-5 by the HMBC correlations of OH-4/C-4 and OH-5/C-5, respectively. A benzoyloxy group was located at C-12 by the key HMBC from H-12 to the carbonyl of this group.

The relative configuration of **2** was completed by analysis of the ROESY spectrum (Figure S2b, Supporting Information), in which the correlations of H-10/H-5 and H-10/H-2 showed that H-10, H-5, and H-2 were cofacial, indicating that H-2 at the newly formed C-2 stereocenter was α-oriented. The structure of **2** was thus assigned as depicted.

Compound **3** gave a molecular formula of $C_{36}H_{38}O_{10}$, as determined by HREIMS. Comparison of the $^1H$ and $^{13}C$ NMR of **3** (Tables 1 and 2) with those of **2** suggested that **3** differed from **2** only by the presence of one additional acetyl group. This was supported by the fact that compound **3** showed 42 mass units more than that of **2**. In the $^1H$ NMR spectrum of **3**, H-5 at $\delta_H$ 5.28 was downfield shifted $\Delta\delta$ 1.47 as compared with that of **2**, indicating

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**Table 1.** $^{13}$C NMR Data ($\delta$) for Compounds **1**−**7**

| carbon | **1**[a] | **2**[a] | **3**[a] | **4**[a] | **5**[a] | **6**[a] | **7**[b] |
|---|---|---|---|---|---|---|---|
| 1 | 159.9 | 32.5 | 32.8 | 34.6 | 34.7 | 35.7 | 121.1 |
| 2 | 137.1 | 44.3 | 44.2 | 35.9 | 36.0 | 36.2 | 201.6 |
| 3 | 209.5 | 218.8 | 214.8 | 80.6 | 80.7 | 79.3 | 81.4 |
| 4 | 72.4 | 77.8 | 74.6 | 83.2 | 83.3 | 83.8 | 42.9 |
| 5 | 72.6 | 75.1 | 71.7 | 78.5 | 78.5 | 78.7 | 48.0 |
| 6 | 59.7 | 60.5 | 58.8 | 85.4 | 85.5 | 85.1 | 39.2 |
| 7 | 67.3 | 70.1 | 67.2 | 75.6 | 75.6 | 73.4 | 198.3 |
| 8 | 35.3 | 35.6 | 35.6 | 35.8 | 35.1 | 33.7 | 126.2 |
| 9 | 80.7 | 81.0 | 80.6 | 76.6 | 76.7 | 80.3 | 138.6 |
| 10 | 48.0 | 42.0 | 42.5 | 51.3 | 51.4 | 51.9 | 155.1 |
| 11 | 39.1 | 39.2 | 39.1 | 38.0 | 38.1 | 40.4 | 111.5 |
| 12 | 71.8 | 72.1 | 72.1 | 83.3 | 78.4 | 68.5 | 163.3 |
| 13 | 87.0 | 86.6 | 86.7 | 70.4 | 78.5 | 88.5 | 131.6 |
| 14 | 82.1 | 82.3 | 82.4 | 79.9 | 78.1 | 81.5 | 131.3 |
| 15 | 142.0 | 142.0 | 142.1 | 142.1 | 138.8 | 142.1 | 16.7 |
| 16 | 113.3 | 113.3 | 113.3 | 115.2 | 118.7 | 113.5 | 14.8 |
| 17 | 19.5 | 19.4 | 19.4 | 18.0 | 18.3 | 19.1 | 24.9 |
| 18 | 11.3 | 11.8 | 11.8 | 17.6 | 17.5 | 11.1 | |
| 19 | 9.9 | 12.0 | 11.7 | 13.0 | 13.0 | 13.0 | |
| 20 | 21.4 | 22.7 | 21.3 | 21.9 | 21.9 | 21.7 | |
| 1′ | 118.3 | 118.3 | 118.4 | 107.4 | 107.2 | 117.3 | |
| 2′ | 135.3 | 135.4 | 135.5 | 138.5 | 138.7 | 135.7 | |
| 3′ | 128.0 | 128.0 | 128.1 | 125.1 | 125.1 | 125.9 | |
| 4′ | 126.2 | 126.1 | 126.2 | 128.0 | 127.9 | 127.9 | |
| 5′ | 129.6 | 129.4 | 129.5 | 129.4 | 129.2 | 129.5 | |
| 6′ | 126.2 | 126.1 | 126.2 | 128.0 | 127.9 | 127.9 | |
| 7′ | 128.0 | 128.0 | 128.1 | 125.1 | 125.1 | 125.9 | |
| 1″ | 165.8 | 166.0 | 165.9 | 165.1 | 164.9 | 165.1 | |
| 2″ | 129.5 | 129.5 | 129.6 | 131.0 | 131.1 | 130.9 | |
| 3″ | 129.8 | 129.8 | 129.8 | 129.7 | 129.7 | 129.5 | |
| 4″ | 128.5 | 128.5 | 128.5 | 128.5 | 128.4 | 128.7 | |
| 5″ | 133.3 | 133.3 | 133.3 | 133.2 | 133.2 | 133.3 | |
| 6″ | 128.5 | 128.5 | 128.5 | 128.5 | 128.4 | 128.7 | |
| 7″ | 129.8 | 129.8 | 129.8 | 129.7 | 129.7 | 129.5 | |
| 3-OAc | | | | 171.4, 21.0 | 171.4, 21.0 | 170.1, 21.0 | |
| 5-OAc | | | 169.6, 20.6 | 171.2, 20.8 | 171.2, 20.8 | 171.0, 20.9 | |
| 7-OAc | | | | 169.4, 21.0 | 169.4, 21.0 | 169.7, 21.3 | |
| 13-OAc | | | | | 169.7, 22.6 | | |

[a] Data were measured in $CDCl_3$ at 100 MHz; chemical shift values are in $\delta$ (ppm) from TMS. [b] Data were measured in $CD_3OD$ at 100 MHz.

that an acetoxy group was located at C-5 ($\delta_C$ 71.7) of **3** instead of the OH of **2**, which was confirmed by the HMBC correlation from H-5 to the acetyl carbonyl ($\delta_C$ 169.6). The structure of **3** was confirmed by analysis of HMBC and ROESY spectra (Supporting Information).

Compound **4** had the molecular formula $C_{40}H_{46}O_{13}$, as determined by the HRESIMS, with 18 degrees of unsaturation. IR absorptions implied the presence of OH (3433 cm$^{-1}$) and ester carbonyl groups (1734, 1714 cm$^{-1}$). In accordance with its molecular formula, all 40 carbons were well resolved in the $^{13}$C NMR spectrum (Table 1) and were classified by DEPT experiments as seven methyl, two methylene (one olefinic), 19 methine (five oxygenated and 10 olefinic), and 12 quaternary carbons (four ester carbonyls, four oxygenated, one orthoester, and three olefinic). In addition, two tertiary methyl [$\delta_H$ 1.67 (s, 3H) and $\delta_H$ 1.47 (s, 3H)], two secondary methyl [$\delta_H$ 1.55 (d, $J$ = 6.9 Hz, 3H) and $\delta_H$ 0.99 (d, $J$ = 6.5 Hz, 3H)], a terminal double bond [$\delta_H$ 4.59 and $\delta_H$ 4.75 (each s, 1H)], three acetyl, an orthobenzoate, and a benzoyl group were distinguished by analysis of the NMR data (Tables 1 and 2). Resonances at $\delta_H$ 3.76 (s, 1H) and $\delta_H$ 2.43 (s, 1H), which did not correlate with any carbons in the HSQC spectrum, were attributable to OH groups. Analysis of the 1D and 2D NMR spectra indicated a daphnane-type diterpenoid structure for **4**.[6] In the HMBC spectrum (Figure S3a, Supporting Information), OAc groups were placed on C-3, C-5, and C-7 by correlations from H-3, H-5, and H-7 to the corresponding carbonyl. Hydroxy groups, resonating at $\delta_H$ 3.76 and $\delta_H$ 2.43, were assigned to C-13 and C-4 by the key correlations of OH-13/C-12 ($\delta_C$ 83.3) and OH-4/C-4 ($\delta_C$ 83.2) and C-5 ($\delta_C$ 78.5), respectively. HMBC correlations from H-8, H-11, H-12, H-14, and $H_3$-18 to C-9 ($\delta_C$ 76.6), from $H_3$-18 and H-14 to C-12 ($\delta_C$ 83.3), and from H-7 and H-8 to C-14 ($\delta_C$ 79.9) suggested the presence of an 9,12,14-orthobenzoate, which was confirmed by the key HMBC correlations from H-12, H-14, and H-3′ (H-7′) to C-1′ ($\delta_C$ 107.4). Thus, the remaining oxygenated quaternary carbon [$\delta_C$ 85.4] was tentatively assigned to C-6, bearing the only remaining benzoyloxy group by the HMBC correlations from H-7, H-8, and $H_3$-20 to C-6.

The relative configuration of **4** was mainly established by a ROESY experiment (Figure S3b, Supporting Information). Correlations of H-8/H-7, H-14, and $H_3$-17; H-11/H-8, H-12, H-16, and H-17; H-10/H-1, H-2, and H-5; H-2/H-3 and H-5; and H-5/$H_3$-20 indicated that **4** had the same relative configuration as **1**−**3** in the diterpenoid core, which was supported by the similar coupling patterns in their $^1$H NMR spectra. ROESY correlations of H-14/$H_3$-17 and $H_2$-16/H-11 and H-12 revealed that the C-15−C-17 moiety at C-13 was $\beta$-oriented and the 9,12,14-orthobenzoate was $\alpha$-directed. Therefore, compound **4** was established as depicted.

Compound **5** ($C_{42}H_{48}O_{14}$) had 42 mass units more than **4**. Comparison of the $^1$H and $^{13}$C NMR data of **5** (Tables 1 and 2) with those of **4** revealed that the only difference was the presence of one more acetyl group in **5**. In the $^{13}$C NMR spectrum of **5**, C-13 was shifted downfield $\Delta\delta$ 8.1, with concomitant absence of the OH-13 proton resonance observed in **4**, indicating that the acetoxy was located at C-13 ($\delta_C$ 78.5). As a result, both C-12 ($\delta_C$ 78.4) and C-14 ($\delta_C$ 78.1) of **5** were shifted upfield ($\Delta\delta$ 4.9 and $\Delta\delta$ 1.8, respectively) as compared with those of **4**, due largely to the $\gamma$-gauche effects from OAc-13. The structure of **5** was supported by the HMBC spectrum (Supporting Information). Therefore, the structure of **5** was established as shown.

Compound **6** had the molecular formula $C_{40}H_{46}O_{13}$, suggesting that it was an isomer of **4**. The NMR spectra of **6** and **4** showed

**Table 2.** $^1$H NMR Data for Compounds **1**−**7**

| proton | **1**[a] | **2**[a] | **3**[a] | **4**[a] | **5**[a] | **6**[a] | **7**[b] |
|---|---|---|---|---|---|---|---|
| 1 | 7.65 (brs) | 1.42 (m) | 1.42 (m) | 2.03 (m) | 2.07 (m) | 1.88 (m) | 8.71 (d, 2.4) |
| | | 2.37 (m) | 2.36 (m) | 2.19 (m) | 2.20 (m) | 2.13 (m) | |
| 2 | | 2.37 (m) | 2.46 (m) | 1.85 (m) | 1.89 (m) | 1.96 (m) | |
| 3 | | | | 4.76 (s) | 4.77 (d, 4.7) | 4.95 (d, 5.9) | 4.14 (s) |
| 5 | 4.06 (brs) | 3.81 (d, 9.1) | 5.28 (s) | 4.94 (s) | 4.94 (s) | 5.02 (s) | 3.23 (m) |
| 6α | | | | | | | 2.77 (dd, 15.8, 5.1) |
| 6β | | | | | | | 2.64 (dd, 15.8, 13.6) |
| 7 | 3.32 (s) | 3.46 (s) | 3.32 (s) | 6.65 (s) | 6.60 (s) | 6.76 (s) | |
| 8 | 3.40 (d, 1.6) | 3.26 (d, 2.7) | 3.38 (d, 2.8) | 2.84 (s) | 2.76 (s) | 2.65 (d, 2.2) | |
| 10 | 4.06 (brs) | 3.16 (dd, 13.0, 5.3) | 3.31 (dd, 12.9, 5.4) | 2.91 (dd, 13.2, 5.9) | 2.95 (dd, 13.2, 6.2) | 2.88 (dd, 12.9, 4.9) | |
| 11 | 3.18 (m) | 3.04 (m) | 3.07 (m) | 2.55 (q, 6.9) | 2.58 (q, 6.8) | 3.10 (q, 7.2) | 7.16 (s) |
| 12 | 5.52 (d, 7.9) | 5.49 (d, 7.7) | 5.50 (d, 7.6) | 3.95 (d, 1.5) | 4.84 (d, 1.4) | 3.95 (m) | |
| 14 | 4.70 (d, 1.3) | 4.68 (d, 2.7) | 4.68 (d, 2.8) | 4.44 (d, 2.4) | 4.62 (d, 2.7) | 4.55 (d, 2.4) | 7.77 (s) |
| 15 | | | | | | | 2.23 (s) |
| 16 | 5.31 (brs) | 5.29 (s) | 5.30 (s) | 4.59 (s) | 4.67 (s) | 4.98 (s) | 0.84 (s) |
| | 5.05 (brs) | 5.05 (s) | 5.05 (s) | 4.75 (s) | 4.98 (s) | 5.07 (s) | |
| 17 | 1.81 (s) | 1.81 (s) | 1.80 (s) | 1.47 (s) | 1.40 (s) | 1.64 (s) | 1.23 (s) |
| 18 | 1.14 (d, 6.9) | 1.22 (d, 6.8) | 1.19 (d, 6.8) | 1.55 (d, 6.9) | 1.51 (d, 6.8) | 1.29 (d, 7.2) | |
| 19 | 1.77 (s) | 1.07 (d, 6.1) | 1.05 (d, 6.4) | 0.99 (d, 6.5) | 1.01 (d, 6.6) | 0.98 (d, 6.5) | |
| 20 | 1.48 (s) | 1.49 (s) | 1.31 (s) | 1.67 (s) | 1.67 (s) | 1.59 (s) | |
| 3′ | 7.82 (m) | 7.81 (m) | 7.80 (m) | 7.63 (m) | 7.62 (m) | 7.65 (m) | |
| 4′ | 7.45 (m) | 7.41 (m) | 7.41 (m) | 7.38 (m) | 7.35 (m) | 7.37 (m) | |
| 5′ | 7.42 (m) | 7.40 (m) | 7.40 (m) | 7.38 (m) | 7.35 (m) | 7.37 (m) | |
| 6′ | 7.45 (m) | 7.41 (m) | 7.41 (m) | 7.38 (m) | 7.35 (m) | 7.37 (m) | |
| 7′ | 7.82 (m) | 7.81 (m) | 7.80 (m) | 7.63 (m) | 7.62 (m) | 7.65 (m) | |
| 3″ | 8.05 (brd, 7.8) | 8.06 (dd, 8.4, 1.2) | 8.05 (dd, 8.3, 1.2) | 7.96 (dd, 8.2, 1.2) | 7.94 (dd, 8.6, 1.4) | 8.02 (dd, 8.2, 1.3) | |
| 4″ | 7.44 (m) | 7.44 (m) | 7.44 (m) | 7.48 (m) | 7.46 (m) | 7.48 (m) | |
| 5″ | 7.57 (m) | 7.57 (m) | 7.57 (m) | 7.62 (m) | 7.60 (m) | 7.61 (m) | |
| 6″ | 7.44 (m) | 7.44 (m) | 7.44 (m) | 7.48 (m) | 7.46 (m) | 7.48 (m) | |
| 7″ | 8.05 (brd, 7.8) | 8.06 (dd, 8.4, 1.2) | 8.05 (dd, 8.3, 1.2) | 7.96 (dd, 8.2, 1.2) | 7.94 (dd, 8.6, 1.4) | 8.02 (dd, 8.2, 1.3) | |
| 4-OH | 3.79 (s) | 2.93 (s) | 3.09 (s) | 2.43 (s) | 2.43 (s) | 2.61 (s) | |
| 5-OH | 3.92 (brs) | 2.69 (d, 9.1) | | | | | |
| 13- OH | | | | 3.76 (s) | | | |
| 3-OAc | | | | 2.22 (s) | 2.21 (s) | 2.21 (s) | |
| 5-OAc | | | 2.18 (s) | 2.21 (s) | 2.21 (s) | 2.18 (s) | |
| 7-OAc | | | | 1.84 (s) | 1.80 (s) | 1.77 (s) | |
| 13-OAc | | | | | 2.01 (s) | | |

[a] Data were measured in $CDCl_3$ at 400 MHz; chemical shifts are expressed in $\delta$ (ppm); the coupling constants ($J$) are given in parentheses (Hz). [b] Data were measured in $CD_3OD$ at 400 MHz.

many similarities (Tables 1 and 2), and the differences occurred mainly in the C ring, which was likely due to the location of the orthoester group. In the HMBC spectrum (Supporting Information), one OH group [$\delta$ 2.61 (s, 1H)] was located at C-4 by the key HMBC correlation between OH-4 and C-4; acetoxy groups were attached to C-3, C-5, and C-7 by the HMBC correlations from H-3, H-5, and H-7 to the corresponding acetyl carbonyl; a benzoyloxy group was assigned to C-6 ($\delta_C$ 85.1) by its chemical shift and the HMBC correlations from H-7, H-8, and $H_3$-20 to C-6. An orthobenzoate group was assigned to C-9 ($\delta_C$ 80.3), C-13 ($\delta_C$ 88.5), and C-14 ($\delta_C$ 81.5) by their chemical shifts and confirmed by the key HMBC correlations from H-14 and H-3′(H-7′) to C-1′. The remaining OH group was then located at C-12 ($\delta_C$ 68.5) by the chemical shift and the HMBC correlations of H-11/C-12, Me-18/C-12, and H-14/C-12. The relative configuration of **6** was assigned as depicted by the coupling patterns and comparison with compounds **1**−**5**. Thus, the structure of compound **6** was established.

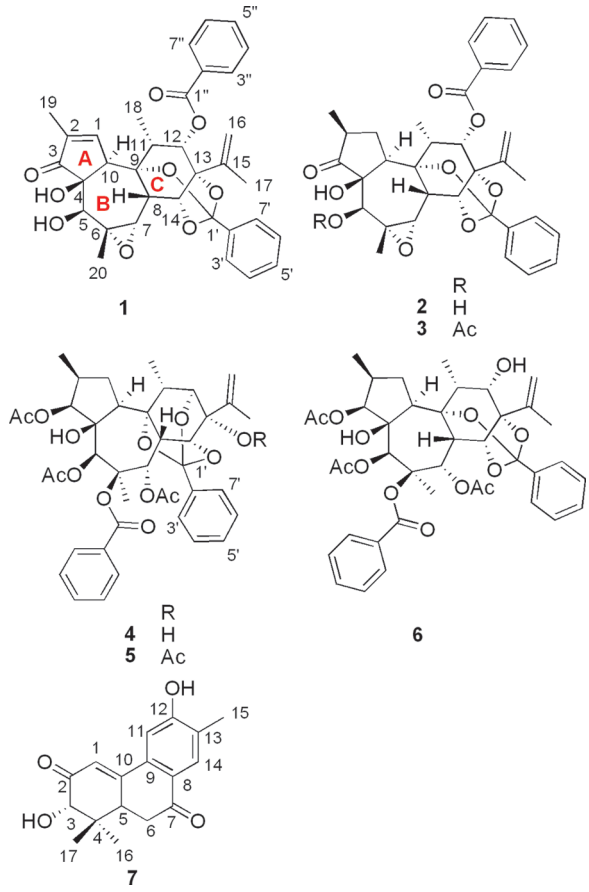
Compounds **1**−**6** are a group of daphnane-type diterpenoids bearing either an 9,12,14-orthobenzoate or an 9,13,14-orthobenzoate group and were named trigoxyphins A−F, respectively.

Compound **7** displayed a sodiated molecular ion at *m/z* 309.1109 $[M + Na]^+$ (calcd for $C_{17}H_{18}O_4Na$, 309.1097), consistent with a molecular formula of $C_{17}H_{18}O_4$, requiring nine double-bond equivalents. The IR spectrum had absorption bands at 3493, 3346 (OH), and 1664 $cm^{-1}$ (ketone group conjugated with multiple double bonds).[7] The $^{13}$C NMR (Table 1) resolved 17 resonances assigned to three methyl, one methylene, five methine (one oxygenated and three olefinic), and eight quaternary carbons (two keto and five olefinic). The data suggested that **7** was a phenanthrenoid-type diterpenoid.[7] Analysis of its $^1$H and $^{13}$C NMR spectra indicated that it was a congener of domohinone,[7] with the only difference being an OH-12 in **7** instead of a MeO-12 in domohinone[7] (Tables 1 and 2). This assignment was confirmed by the HMBC spectrum, and the relative configuration of **7** was assigned by the ROESY spectrum (Figures S4a and S4b, Supporting Information). Compound **7** was named trigoxyphin G.

Trigoxyphins A−G (**1**−**7**) were tested for cytotoxic activity against the HL-60 (human premyelocytic leukemia) cell line using the MTT method[8] and against the BEL-7402 (human hepatocellular carcinoma) and A-549 (human lung adenocarcinoma) cell lines using the SRB method.[9] Adriamycin was the positive control ($IC_{50}$ 0.04 $\mu$M against HL60, $IC_{50}$ 0.17 $\mu$M against A549, and $IC_{50}$ 0.12 $\mu$M against BEL-7402). The tests showed that trigoxyphins A and B (**1** and **2**) exhibited strong activity against HL60 human leukemia cells, with $IC_{50}$ values of 0.27 and 0.49 $\mu$M, and moderate activity against A549 human lung adenocarcinoma cells, with $IC_{50}$ values of 7.5 and 4.9 $\mu$M, respectively; all of the compounds tested were inactive against BEL-7402 cells ($IC_{50}$ value > 10 $\mu$M was defined as inactive).

## Experimental Section

**General Experimental Procedures.** Melting points were measured on a SGW X-4 melting instrument and are uncorrected. Specific rotations were determined on a Perkin-Elmer 341 polarimeter. UV spectra were recorded on a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded on a Perkin-Elmer 577 spectrometer. NMR spectra were measured on a Bruker AM-400 spectrometer with TMS as internal standard. EIMS (70 eV) were done on a Finnigan MAT 95 mass spectrometer. ESIMS and HRESIMS were obtained on an Esquire 3000plus (Bruker Daltonics) and a Bruker Daltonics micrOTOFQII




mass spectrometer, respectively. Semipreparative HPLC was performed using a Waters 515 pump with a Waters 2487 detector (254 nm) and a YMC-Pack ODS-A column (250 × 10 mm, S-5 $\mu$m, 12 nm). Silica gel (200−300 mesh, Qingdao Haiyang Chemical Co., Ltd.) and $C_{18}$ reversed-phase silica gel (250 mesh, Merck) were used for column chromatography (CC). All solvents used were of analytical grade (Shanghai Chemical Reagents Company, Ltd.).

**Plant Material.** The twigs of *T. xyphophylloides* were collected from Sanya of 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 SMTX-2006-1Y) has been deposited at the Shanghai Institute of Materia Medica.

**Extraction and Isolation.** Air-dried powder of *T. xyphophylloides* twigs (8.5 kg) was extracted three times with 95% EtOH at room temperature to give an EtOH extract (230 g), which was partitioned between EtOAc and water to obtain the EtOAc-soluble fraction (60 g). The EtOAc-soluble fraction was separated on a column of MCI gel (MeOH/$H_2O$, 40/60 to 90/10, v/v) to afford fractions A−H. Fraction C (2.10 g) was chromatographed on a silica gel column eluted with petroleum ether/acetone (6:1 to 1:2, v/v) to afford major fractions C1−C4. Fraction C1 was purified by a semipreparative HPLC with 60% MeOH in $H_2O$ to yield compound **7** (15 mg). Using the same procedures, fraction C3 gave 1,2-dihydroheudelotinol (12 mg). Fraction E (12.3 g) was treated similarly to obtain 3,4-*seco*-sonderianol (30 mg). Fraction F (12.1 g) was chromatographed on a silica gel column eluted with petroleum ether/acetone (6:1 to 1:2, v/v) to afford subfractions F1−F4. Fraction F2 was purified by CC on C18 silica gel eluted with MeOH/$H_2O$ (60/40, v/v) to yield compounds **4** (8 mg), **5** (5 mg), and **6** (5 mg). CC of fraction G (10.0 g) on silica gel eluted with petroleum ether/acetone (4:1 to 1:2, v/v) afforded fractions G1−G6. Fraction G5 was separated by CC on reversed-phase C18 silica gel eluted with MeOH/$H_2O$ (6:4, v/v) to yield compounds **1** (10 mg), **2** (15 mg), and **3** (15 mg).

**Trigoxyphin A (1):** white powder; decomposed at 211 °C; $[\alpha]^{20}_D$ −37 (*c* 0.15, $CHCl_3$); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 232 (4.47) nm; IR (KBr) $\nu_{max}$ 3433, 2922, 1716, 1630, 1452, 1279, 1084, 1007 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 587 $[M + H]^+$, 609 $[M + Na]^+$, 1195 $[2 M + Na]^+$; HRESIMS *m/z* 609.2110 $[M + Na]^+$ (calcd for $C_{34}H_{34}O_9Na$, 609.2095).

**Trigoxyphin B (2):** white powder; mp 277−278 °C; $[\alpha]^{21}_D$ +41 (*c* 0.19, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230.8 (4.12); IR (KBr) $\nu_{max}$ 3448, 2970, 1747, 1705, 1452, 1317, 1283, 1132, 1086, 1005, 714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 589.4 $[M + H]^+$, 611.4 $[M + Na]^+$, 1199.6 $[2 M + Na]^+$; EIMS *m/z* 535 (5), 381 (12), 177 (12), 105 (100), 77 (7); HRESIMS *m/z* 611.2252 $[M + Na]^+$ (calcd for $C_{34}H_{36}O_9Na$, 611.2257).

**Trigoxyphin C (3):** white powder; mp 230−232 °C; $[\alpha]^{21}_D$ +36 (*c* 0.12, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 230.0 (4.11); IR (KBr) $\nu_{max}$ 3433, 2972, 2931, 1747, 1724, 1629, 1371, 1358, 1281, 1227, 1082, 999, 922, 714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 631.3 $[M + H]^+$, 1283.4 $[2 M + Na]^+$; EIMS *m/z* 630 (2) $[M]^+$, 449 (4), 217 (16), 105 (100), 77 (76), 69 (25); HREIMS *m/z* 630.2475 $[M]^+$ (calcd for $C_{36}H_{38}O_{10}$, 630.2465).

**Trigoxyphin D (4):** white powder; mp 104−106 °C; $[\alpha]^{21}_D$ −14 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 231.0 (4.28); IR (KBr) $\nu_{max}$ 3433, 2933, 1734, 1714, 1632, 1454, 1383, 1229, 1032, 719 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 735 $[M + H]^+$, 757 $[M + Na]^+$; HRESIMS *m/z* 757.2865 $[M + Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2831).

**Trigoxyphin E (5):** white powder; mp 136−138 °C; $[\alpha]^{21}_D$ −2 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 231 (4.19); IR (KBr) $\nu_{max}$ 3431, 2928, 1743, 1632, 1452, 1369, 1230, 1028, 714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 777 $[M + H]^+$, 799 $[M + Na]^+$; HRESIMS *m/z* 799.2917 $[M]^+$ (calcd for $C_{42}H_{48}O_{14}Na$, 799.2942).

**Trigoxyphin F (6):** white powder; mp 223−224 °C; $[\alpha]^{21}_D$ +56 (*c* 0.05, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 231 (4.25); IR (KBr) $\nu_{max}$ 3433, 2924, 1751, 1718, 1630, 1452, 1375, 1230, 1036, 714 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) and $^{13}C$ NMR ($CDCl_3$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 735 $[M + H]^+$, 757 $[M + Na]^+$; HRESIMS *m/z* 757.2825 $[M + Na]^+$ (calcd for $C_{40}H_{46}O_{13}Na$, 757.2831).

**Trigoxyphin G (7):** yellow powder; mp 186−188 °C; $[\alpha]^{21}_D$ −63 (*c* 0.15, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 317 (4.64), 276 (4.84); IR (KBr) $\nu_{max}$ 3493, 3346, 2966, 2926, 2850, 1664, 1593, 1572, 1504, 1302, 1111, 669 $cm^{-1}$; $^1H$ NMR ($CD_3OD$, 400 MHz) and $^{13}C$ NMR ($CD_3OD$, 100 MHz), Tables 1 and 2; positive mode ESIMS *m/z* 287 $[M + H]^+$; HRESIMS *m/z* 309.1109 $[M + Na]^+$ (calcd for $C_{17}H_{18}O_4Na$, 309.1097).

**Cytotoxicity Assay.** Cytotoxicity of compounds **1**−**7** against HL-60 cells was measured using the MTT method.[8] Briefly, cells in 100 $\mu$L 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 $\mu$L aliquot of MTT solution (5 mg/mL) was added directly to the appropriate wells. The cultures were incubated for 4 h, and then 100 $\mu$L 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**−**7** against A-549 and BEL-7402 cells were tested using the SRB assay.[9] In brief, 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 on the same plate reader at a wavelength of 515 nm. The results were all expressed in $IC_{50}$ as calculated by the Logit method.

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**Supporting Information Available:** Figures S1−S4, IR, MS, $^{1}H$ and $^{13}C$ NMR, and 2D NMR spectra of compounds **1**−**7**, and CD spectra of compounds **1**−**6** are available free of charge via the Internet at http://pubs.acs.org.

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