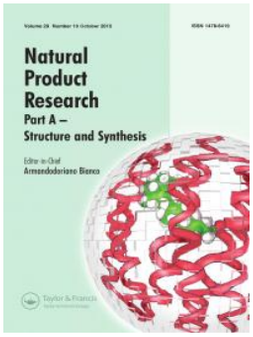

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Three new phenanthrenone constituents from *Trigonostemon lii*

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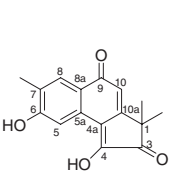

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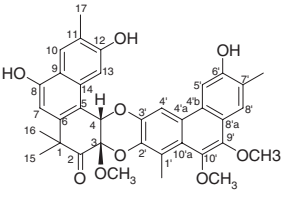

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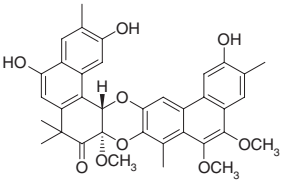

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Three new phenanthrenone constituents, trigoxyphins U–W (**1**, **7** and **9**), together with eight known ones, trigoxyphin M (**2**), 6,9-*O*-dimethyltrigonostemone (**3**), trigonstemone (**4**), thrigonosomone B (**5**), trigonochinene E (**6**), actephiiol A (**8**), epiactephilol A (**10**) and neoboutomannin (**11**), were obtained from the methanol extract of the leaves and stems of *Trigonostemon lii*. The structures of the new metabolites were elucidated by analysing the spectroscopic data (1D NMR, 2D NMR, HR-ESI-MS and IR). Compounds **1**–**6** were evaluated for their cytotoxic activities on five human tumour cell lines by using the MTT method, and compound **1** exhibited inhibitory activity against HL-60, SMMC-7721, A-549, MCF-7 and SW480 with IC_{50} values ranging from 3.77 to 14.51 μM.

Keywords: Euphorbiaceae; *Trigonostemon lii*; phenanthrenone; trigoxyphins U–W; cytotoxic activity

1. Introduction

Plants of genus *Trigonostemon* containing about 50 species (Euphorbiaceae) are usually bushes or small trees, distributed in tropical and subtropical areas (Kiu et al. 1997). Most of them were used by the natives as herbal medicine to treat some diseases such as asthma, diarrhoea and methysis (Sakata et al. 1971; Carney et al. 1999; He et al. 2000). Previous phytochemical studies on Trigonostemon *filipes*, Trigonostemon *lii*, Trigonostemon *thyrsoideum* and Trigonostemon *howii* resulted in the isolation of modified daphnane-type diterpenoids, diterpenoids, phenanthrenes and indole alkaloids by our group (Hu et al. 2009; Tan et al. 2010; Li et al. 2011, 2012; Tang et al. 2012). In the continuing research for bioactive secondary metabolites from the plants of this genus, the MeOH extract of *T. lii* was subjected to chromatographic procedures to yield a new degraded diterpenoid, trigoxyphin U (**1**), two new phenanthrenone dimers, trigoxyphin V (**7**) and trigoxyphin W (**9**) and eight known phenanthrenones (**2**–**6**, **8**, **10**

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and **11**). This article focuses on the isolation, structural elucidation and cytotoxic activities of these compounds.

2. Results and discussion

2.1. *Structural elucidation*

The molecular formula of compound **1** was determined as $C_{16}H_{14}O_4$ with 10 degrees of unsaturation by analysis of the negative HR-ESI-MS data at *m/z* 269.0814 $[M - H]^-$ (calcd 269.0813). In the ^1H and ^{13}C NMR spectra of **1**, three methyls including a *gem*-dimethyl group (δ_H 1.33, 6H; δ_C 24.5, 2 × C) and a methyl group linked to an aromatic ring (δ_H 2.29, 3H, δ_C 16.7), three olefinic methines and ten quaternary carbons involving two carbonyls (δ_C 182.1 and 206.4), an oxygenated olefinic carbon (δ_C 164.7), an oxygenated aromatic carbon (δ_C 159.6) and a quaternary carbon (δ_C 42.3) were observed. Extensive analysis of HMBC and HSQC spectra data led to the establishment of two fragments **a** and **b**, which were deduced as follows in Figure S27 (Supplementary material). In the HMBC spectrum, the correlations of H-8 and H-10 with C-9 (δ_C 182.1) required that one carbonyl was assigned at C-9 by naphthoquinone former. The proton signal of the *gem*-dimethyl group showed HMBC correlations with another carbonyl (δ_C 206.4) to build fragment **b**. Long-range HMBC correlations from H-10 to C-1 and C-4 and H-5 to C-4 were observed suggesting that fragments **a** and **b** were connected as shown. Moreover, there should be presence of a hydroxyl located at C-4 to accord with the molecular formula in **1**. Thus, compound **1** was assigned and named trigoxyphin U.

The negative HR-ESI-MS of trigoxyphin V (**7**) displayed pseudo-molecular ion peak $[M - H]^-$ at *m/z* 609.2119 consistent with the molecular formula of $C_{36}H_{34}O_9$, indicated 20 degrees of unsaturation. The 1D and 2D NMR data showed that **7** was a highly substituted aromatic compound. Analysis of the ^{13}C NMR data led to the identification of a ketone moiety (δ_C 205.5), supported by a characteristic stretch in the IR spectrum at 1734 cm^{-1}. Further analysis of the 1D and 2D NMR data revealed the presence of six uncoupled aromatic protons (δ_C 103.7, 103.9, 105.9, 107.0, 123.3 and 124.1; δ_H 6.65, 7.28, 7.55, 7.76, 7.77 and 7.98), seven oxygenated aromatic carbons (δ_C 140.2, 140.7, 144.5, 145.0, 153.9, 154.5 and 155.8), two aromatic methoxy groups (δ_C 60.6 and 60.8; δ_H 3.87 and 4.00), a methyl ketal moiety (δ_C 96.5 and 51.8; δ_H 3.48), an oxymethine (δ_C 72.5; δ_H 5.54), three aromatic methyls (δ_C 16.4, 16.3 and 12.8; δ_H 2.36, 2.36 and 2.93) and an aliphatic *gem*-dimethyl group (δ_C 27.9 and 28.7; δ_H 1.61 and 1.41). Detailed comparison between the NMR and MS data of **7** and **8** implied that **7** was only different in the presence of an additional methoxy substituent at C-10′, suggested by the HMBC correlation of H_3-14′ to C-10′ and the ROESY correlations of H_3-11′/H_3-14′ and H_3-14′/ H_3-13′. Thus, the structure of **7** was established as shown in Figure 1.

Compound **9**, yellow solid, was isolated from the same sub-fraction with **7** by the semi-preparative HPLC technology. Its molecular formula was determined as $C_{36}H_{34}O_9$ by the negative HR-ESI-MS $[M - H]^-$ at *m/z* 609.2135 as the same as **7**. Comparative analysis of the ^1H and ^{13}C NMR data showed obvious similarity between **9** and **7**, with limited differences in the ^{13}C NMR chemical shift of C-2, C-1′, C-3′ and C-4′a and ^1H NMR chemical shifts of H-4′, H-5′ and H_3-11′. Analysis of the HMBC and ROESY data suggested that compound **9** contained the same two partial structures as **7**. Furthermore, it was found that two same peaks were observed in HPLC analysis of both compounds **7** and **9** after a certain period of time. This evidence meant that compounds **7** and **9** were transformed to each other in the solvent-like compounds **8** and **10** (Ovenden et al. 2001), which indicated that compounds **7** and **9** were the epimers of ketal moiety. In addition, the ROESY spectrum in **9** showed weaker correlation between H-4 and H_3-18 than in **7**, which suggested that **9** was the C-3 epimerisation of **7**. Thus, compound **9** was assigned and named trigoxyphin W.

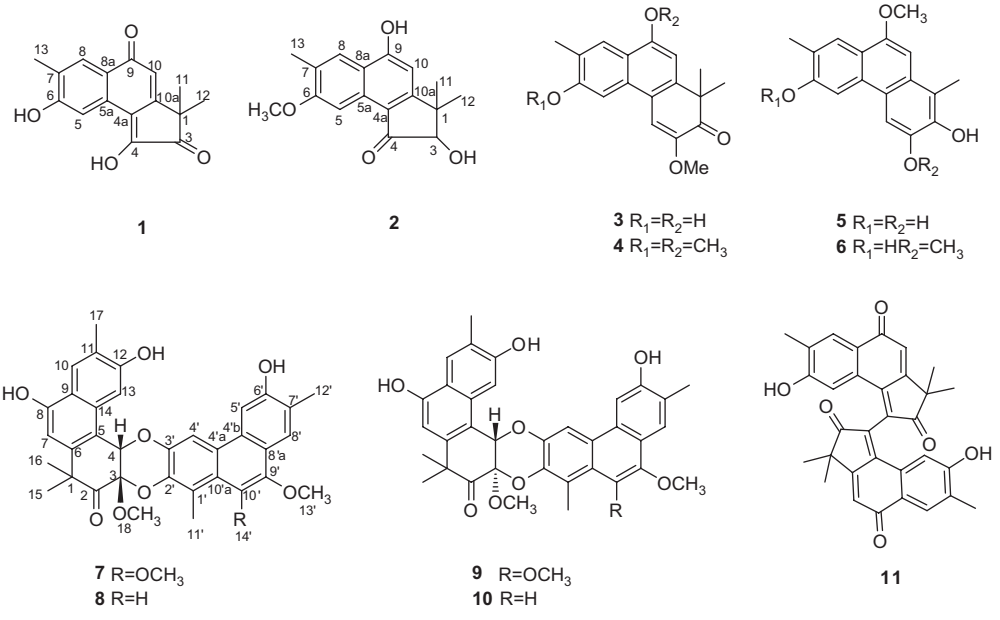

Figure 1. Phenanthrenone compounds from *T. lii.*

2.2. *Biological evaluation*

Compounds **1**–**6** were assayed for their cytotoxic activities on five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7 and SW480) by using the MTT method, and their cytotoxic activities were measured in parallel with determination of antitumour activity using cisplatin as the positive control. Only compound **1** exhibited potential cytotoxic activity against HL-60, SMMC-7721, A-549, MCF-7 and SW480 with IC_{50} values of 14.51, 12.16, 10.18, 3.77 and 4.92 μM.

3. Experimental section

3.1. *General experimental procedures*

Optical rotations were obtained on a JASCO DIP-370 digital polarimeter (JASCO, Tokyo, Japan). IR spectra were measured in a Bio-Rad FTS-135 spectrometer with KBr pellets (Bruker Optics, Ettlingen, Germany), and UV data were measured using a UV-210A spectrometer (Shimadzu, Kyoto, Japan). 1D and 2D NMR spectra were measured on Bruker AM-400, DRX-500 and AV-600 NMR spectrometers (Bruker Optics, Ettlingen, Germany), using TMS as an internal standard. ESI-MS were recorded using a Finnigan MAT 90 instrument and a VG Auto Spec-3000 spectrometer (Agilent, Santa Clara, CA, USA). Column chromatography was performed on Si gel H (10–40 μm; Qingdao Marine Chemical Factory, Qingdao, China) and Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden). MPLC was performed on Büchi Sepacore System (Büchi Labortechnik AG, Switzerland), and columns packed with Chromatorex C_{18} (40–75 μm, Fuji Silysia Chemical Ltd, Japan). Semi-preparative HPLC was performed by using an Agilent 1200 series system equipped with a Zorbax XDB-C18, 9.4 mm × 150 mm column.

3.2. *Plant material*

The leaves of *T. lii* were collected in Xishuangbanna, Yunnan Province, People's Republic of China, in November 2008, and the plant sample was identified by Prof. Shun-Cheng Zhang of

Xishuangbanna Institute of Botany, Chinese Academy of Sciences (CAS). The voucher specimen (KIB 08110211) has been deposited at the State Key Laboratory of Phyotochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS.

3.3. *Extraction and isolation*

The air-dried powder (12.0 kg) of leaves and stems of *T. lii* was extracted three times with MeOH at room temperature to give a crude extract, which was partitioned between EtOAc and H_2O. The EtOAc-soluble residue (200 g) was subjected to CC on silica gel using petroleum ether/acetone (10:1 to 1:2) to yield 10 fractions (F1–F10). Fraction F2 was separated using reversed-phase MPLC by a gradient of H_2O—MeOH and Sephadex LH-20($CHCl_3$—MeOH), to afford **11** (10 mg). Repeated column chromatography of fraction F3 over silica gel (petroleum ether—EtOAc, 1:1; $CHCl_3$—MeOH, 30:1) yielded **3** (15 mg), **4** (10 mg), **5** (8 mg) and **6** (10 mg). Fraction F4 was divided into five subfractions (A1–A5) by column chromatography over silica gel ($CHCl_3$—MeOH, from 30:1 to 1:1). Subfraction A1 was further purified by Sephadex LH-20 ($CHCl_3$—MeOH, 1:1) and semi-preparative HPLC to yield **7** (5 mg), **8** (6 mg), **9** (5 mg) and **10** (7 mg). Subfraction A2 was separated by Sephadex LH-20, eluting with MeOH and semi-preparative HPLC to yield **1** (50 mg) and **2** (10 mg).

Trigoxyphin U **(1):** pale yellow solid; UV (CH_3OH) λ_{max} (log ε) 202 (4.20), 219 (4.27), 247 (4.06), 292 (3.89), 305 (3.94) and 433 (4.22) nm; IR (KBr) ν_{max} 3426, 1742, 1658, 1626, 1573, 1476, 1399, 1344, 1297, 1225, 1164, 1113, 1057 and 686 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 8.40 (s, H-5), 8.00 (s, H-8), 6.72 (s, H-10), 2.29 (s, H-13) and 1.33 (s, H-11, 12); ^{13}C NMR (400 MHz, DMSO-d_6) δ:206.4 (s, C-3), 182.1 (s, C-9), 164.7 (s, C-4), 163.4 (s, C-10a), 159.6 (s, C-6), 131.5 (s, C-5a), 127.1 (s, C-7), 125.1 (d, C-8), 122.7 (s, C-4a), 117.7 (s, C-8a), 106.0 (d, C-5), 101.1 (s, C-10), 42.3 (s, C-1), 24.5 (q, C-11, 12) and 16.7 (q, C-13); positive ESI-MS *m/z* 293 $[M + Na]^+$, 563 $[2M + Na]^+$; negative HR-ESI-MS *m/z* 269.0814 $[M - H]^-$ (calcd for $C_{16}H_{13}O_4$, 269.0813).

Trigoxyphin V **(7)**: yellow solid; $[\alpha]_D^{27} = -174.2$ (*c* 0.27, MeOH); UV (MeOH) λ_{max} (log ϵ) 194 (4.6), 228 (4.7), 251 (4.8), 292 (4.5), 320 (4.2) nm; IR (KBr) ν_{max} 3427, 2976, 2936, 1734, 1633, 1601, 1495, 1451, 1413, 1274, 1244, 1144, 1056, 1030, 992, 889, 847 and 646 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-CD_3OD (1:1)) δ: 7.98 (s, H-10), 7.77 (s, H-8′), 7.76 (s, H-4′), 7.55 (s, H-5′), 7.28 (s, H-13), 6.65 (s, H-7), 5.54 (s, H-4), 4.00 (s, H-13′), 3.87 (s, H-14′), 3.48 (s, H-18), 2.93 (s, H-11′), 2.36 (s, H-12′, 17), 1.61 (s, H-16) and 1.41 (s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD (1:1)) δ: 205.5 (s, C-2), 155.8 (s, C-12), 154.5 (s, C-8), 153.9 (s, C-6′), 145.0 (s, C-9′), 144.5 (s, C-10′), 142.8 (s, C-6), 140.7 (s, C-2′), 140.2 (s, C-3′), 133.8 (s, C-14), 128.1 (s, C-4′b), 126.2 (s, C-11), 126.1 (s, C-7′), 124.2 (s, C-4′a), 124.1 (d, C-10), 123.8 (s, C-10′a), 123.3 (d, C-8′), 121.7 (s, C-8′a), 120.9 (s, C-1′), 118.9 (s, C-9), 113.8 (s, C-5), 107.0 (d, C-4′), 105.9 (d, C-5′), 103.9 (d, C-13), 103.7 (d, C-7), 96.5 (s, C-3), 72.5 (d, C-4), 60.8 (q, C-13′), 60.6 (q, C-14′), 51.8 (q, C-18), 47.4 (s, C-1), 28.7 (q, C-15), 27.9 (q, C-16), 16.4 (q, C-12′), 16.3 (q, C-17) and 12.8 (q, C-11′); positive ESI-MS *m/z*: 633 $[M + Na]^+$; negative HR-ESI-MS *m/z*:609.2119 $[M - H]^-$, $C_{36}H_{33}O_9$ (calcd 609.2124).

Trigoxyphin W **(9)**: yellow solid; $[\alpha]_D^{27} = -3.5$ (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ϵ) 196 (4.7), 227 (4.8), 251 (4.8), 291 (4.5) and 320 (4.3) nm; IR (KBr) ν_{max} 3439, 2977, 2936, 1734, 1633, 1601, 1495, 1451, 1413, 1276, 1245, 1144, 1060, 1033, 992, 887, 847 and 644 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$-CD_3OD (1:1)) δ: 8.01 (s, H-4′), 7.98 (s, H-10), 7.81 (s, H-8′), 7.68 (s, H-5′), 7.27 (s, H-13), 6.65 (s, H-7), 5.48 (s, H-4), 3.99 (s, H-13′), 3.81 (s, H-14′), 3.37 (s, H-18), 2.58 (s, H-11′), 2.42 (s, H-12′), 2.38 (s, H-17), 1.58 (s, H-16) and 1.42 (s, H-15); ^{13}C NMR (100 MHz, $CDCl_3$-CD_3OD (1:1)) δ: 206.6 (s, C-2), 155.8 (s, C-12), 154.4 (s, C-8), 153.8 (s, C-6′), 144.8 (s, C-9′), 144.5 (s, C-10′), 142.7 (s, C-6), 140.9 (s, C-2′), 140.8 (s, C-3′), 134.0 (s, C-14), 128.3 (s, C-4′b), 126.3 (s, C-11), 126.2 (s, C-7′), 124.7 (s, C-4′a), 124.1 (d, C-10), 123.5

(s, C-10′a), 123.3 (d, C-8′), 121.9 (s, C-8′a), 121.6 (s, C-1′), 119.0 (s, C-9), 114.0 (s, C-5), 106.9 (d, C-4′), 106.4 (d, C-5′), 103.9 (d, C-7, 13), 96.5 (s, C-3), 72.5 (d, C-4), 60.8 (q, C-13′, 14′), 51.8 (q, C-18), 47.5 (s, C-1), 29.0 (q, C-15), 28.0 (q, C-16), 16.5 (q, C-12′, 17) and 12.9 (q, C-11′); positive ESI-MS *m/z*: 633 $[M + Na]^+$; negative HR-ESI-MS *m/z*:609.2135 $[M - H]^-$, $C_{36}H_{33}O_9$ (calcd 609.2124).

3.4. *Cytotoxicity assay*

Assays were performed as described earlier (Li et al. 2011).

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S27.

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