Tetrahedron Letters 50 (2009) 2917–2919

Contents lists available at ScienceDirect

Tetrahedron Letters

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

New phenanthrenes from Trigonostemon lii Y.T. Chang

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ARTICLE INFO

Article history:
Received 22 January 2009
Revised 22 March 2009
Accepted 27 March 2009
Available online 1 April 2009

ABSTRACT

Three new phenanthrenes, thrigonosomone A (**1**), 6,9-*O*-dedimethyltrigonostemone (**2**), and thrigonosomone B (**3**) were isolated from *Trigonostemon lii*. Their structures were elucidated by spectroscopic methods. Thrigonosomone A (**1**) possessed a novel seven-membered cyclic anhydride moiety, and its relative configuration was determined by chemical computation and ROESY experiment. The cytotoxicity of compounds **1**–**3** was also evaluated.

The genus *Trigonostemon* belongs to Euphorbiaceae comprising about 50 species, mainly distributed in tropical and subtropical Asia.[1] Previous studies on the chemical constituents and their activities from two species of this genus have led to the isolation of several classes of compounds including diterpenoid,[2–6] phenanthrenone,[7] and flavonoidal indole alkaloid.[8] Among them, Daphnane diterpenoids have been found to possess potent activity against fleas.

Trigonostemon lii Y. T. Chang is a shrub or small tree endemic to South China. So far, there has been no report on the chemical constitute from this species. In our continuing investigation on novel and potentially bioactive secondary metabolites from Euphorbiaceae,[9–11] three new phenanthrenes, thrigonosomone A (**1**), 6,9-*O*-dedimethyltrigonostemone (**2**), and thrigonosomone B (**3**) were isolated from the roots and stems of *T. lii*. Compound **1,** featuring an unusually seven-membered cyclic anhydride moiety, is the first of naturally occurring highly oxygenated and seven-membered O-heterocycle. Herein we describe the isolation and structure elucidation and cytotoxic activities of three new compounds.

Roots and stems of *T. lii*, collected in Xishuangbanna of Yunnan Province, PR China, were extracted with 95% EtOH, and the extract was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc extract (100 g) was further separated by a series of column chromatography including silica gel, sephadex LH-20, and reversed-phase silica gel columns to afford compounds **1** (4.6 mg), **2** (60 mg), and **3** (4.2 mg).

Compound **1** was obtained as a yellow solid, α_D^{24} −50.79 (*c* 0.11, $CHCl_3$), and was analyzed for a molecular formula of $C_{18}H_{18}O_6$ by HRESIMS ($[M+Na]^+$ at *m/z* 353.1009, calcd 353.1001), corresponding to 10 degrees of unsaturation. The IR absorption spectrum suggested the presence of hydroxyl (3423 cm^{-1}), aromatic (1636, 1433 cm^{-1}), and ester carbonyl (1748 cm^{-1}) groups. The 1D and 2D NMR spectra (Table 1) displayed 18 carbon signals consisting of one *O*-methyl group, three methyl groups, four methines, eight quaternary carbons, and two ester carbonyls (δ_C 177.9, 170.7). Among them, three aromatic methines (δ_C 105.2, δ_H 7.28; δ_C 124.5, δ_H 7.95, and δ_C 102.8, δ_H 6.59) and seven aromatic quaternary carbons composed of a five-substituted naphthyl moiety (Fig. 1). Since two ester carbonyl groups and the naphthyl accounted for 9 of 10 unsaturations, compound **1** was inferred to possess the other ring. The other ring was deduced as a seven-membered O-heterocycle fragment based on the HMBC correlations (Fig. 2) from a *gem*-dimethyl group C-1-Me_2 (δ_H 1.68, 1.63) to C-10a (δ_C 138.4), C-2 (δ_C 177.9) and C-1 (δ_C 41.6), and from H-4 (δ_H 6.41) to C-2 (δ_C 177.9), C-3 (δ_C 170.7) and C-10a (δ_C 138.4). The connectivity of the seven-membered ring to naphthyl was furnished by HMBC correlations from H-4 to C-5a, and from H-10 to C-4a and C-1. The HMBC correlation between *O*-methyl group and C-4, and ROESY correlation between H-4 and H-5 indicated that the *O*-methyl group was placed at C-4. The substituents of naphthyl were assigned by the HMBC and ROESY experiments. The aromatic methyl group was placed at C-7 by its HMBC correlations with C-6, C-7, and C-8 and ROESY correlation with H-8. The ^1H NMR spectrum contained only 16 proton resonances, and two remaining protons in the molecule were attributed to the exchangeable protons of two OH groups. Accordingly, the two hydroxy groups were placed at C-6 and C-9, which were supported by the HMBC correlations of H-8/C-9, H-10/C-9, and H-8/C-6, and ROE correlation of H-4/H-5. Finally, the planar structure of **1** was established as shown in Figure 2.

To further determine relative configuration of C-4 on the seven-membered ring of compound **1**, ROESY experiment and computer-

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doi:10.1016/j.tetlet.2009.03.186

Table 1
^{1}H [δ_H (*J*, Hz)] NMR data at 400 MHz and ^{13}C [δ_C] NMR data at 100 MHz of thrigonosomone A (**1**) at 300 K

No.	^{13}C (δ_C) [a]	^{1}H (δ_H) [a]
1	41.6 s	
2	177.9 s	
3	170.7 s	
4	76.8 d	6.41, s
4a	110.7 s	
5	105.2 d	7.28, s
5a	130.9 s	
6	156.4 s	
7	127.0 s	
8	124.5 d	7.95, s
8a	119.2 s	
9	155.0 s	
10	102.8 d	6.59, s
10a	138.4 s	
1-Me	29.7 q	1.68, s
1-Me	29.3 q	1.63, s
7-Me	16.8 q	2.36, s
OMe	53.2 q	3.68, s

[a] Measured in $CDCl_3$–CD_3OD (1:2).

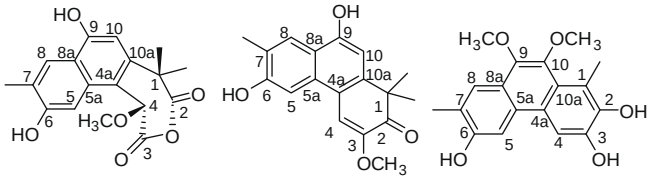


Figure 1. Structures of compounds **1**–**3**.

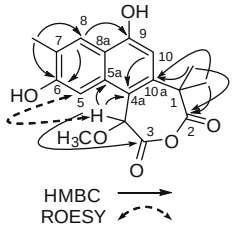


Figure 2. Key HMBC and ROESY correlations of **1**.

generated molecular modeling using DFT calculations at the B3LYP/6-31G+(d) level (GAUSSIAN 03, D01) were employed.[12] Ester carbonyl group at C-3 was arbitrarily assigned downside of plane of naphthalene moiety. The calculation showed two low-energy isomers of **1** (Fig. 3) which were distinguished as *r* and *s* according to orientation difference of OCH_3 at C-4. The calculated distance between H-4 and H-5 is 3.72 Å in *r*-isomer and is 1.85 Å in *s*-isomer, respectively. With consideration of well-defined interaction observed for this pair of protons in ROESY spectrum, relative configuration of **1** was assigned to be *s*-isomer as shown in Figure 1.

Compound **2** was isolated as deep yellow needles, mp 164–165 °C, and the molecular formula was established as $C_{18}H_{18}O_4$, as determined by positive HRESIMS ([M+H]$^+$ at *m/z* 299.1287, calcd 299.1283), indicating 10° of unsaturation. The IR absorption spectrum suggested the presence of hydroxyl (3395 cm^{-1}), aromatic (1630, 1460 cm^{-1}), and α,β-unsaturated ketone (1670 cm^{-1}) groups. The ^{1}H and ^{13}C NMR data of **2** (Table 2) were closely related to those of trigonostemone,[7] except for the loss of two *O*-methyl group signals. The hydroxyl groups are assigned at C-6 and C-9, respectively, due to HMBC correlations of H-5/C-6, CH_3-C-7/C-6, H-8/C-6, H-8/C-9, and H-10/C-9, and ROE correlation of H-4/H-5(Fig. 4A). Therefore, compound **2** was assigned as 6,9-*O*-dedimethyltrigonostemone.

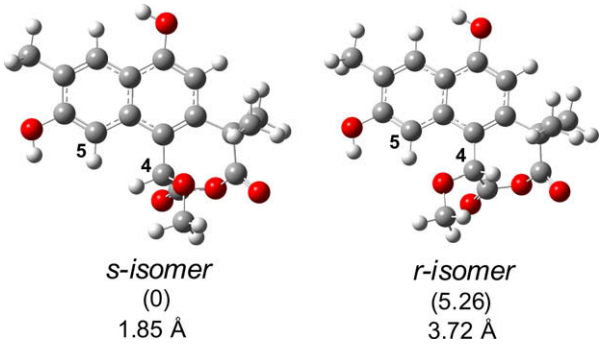


Figure 3. DFT-calculated two isomers (*s-isomer* and *r-isomer*) were found for **1**. Relative energies in kcal/mol of the two isomers are given in parentheses. Distance between H4 and H5 (in italic) is also given.

Table 2
^{1}H [δ_H (*J*, Hz)] NMR data at 400 MHz and ^{13}C [δ_C] NMR data at 100 MHz of compounds **2** and **3** at 300 K

No.	**2** [a]		**3** [b]	
	^{13}C (δ_C)	^{1}H (δ_H)	^{13}C (δ_C)	^{1}H (δ_H)
1	49.6 s		123.7 s	
2	201.3 s		145.2 s	
3	147.3 s		144.6 s	
4	114.6 d	7.39, s	105.3 d	7.81, s
4a	113.8 s		126.1 s	
5	104.2 d	7.41, s	106.6 d	7.78, s
5a	132.5 s		129.0 s	
6	156.3 s		155.0 s	
7	126.5 s		126.1 s	
8	124.7 d	7.95, s	124.0 d	7.81, s
8a	119.3 s		122.3 s	
9	154.4 s		145.0 s	
10	104.9 d	6.74, s	145.6 s	
10a	144.0 s		118.7 s	
1-Me	28.6 q	1.46, s	13.7 q	2.78, s
1-Me	28.6 q	1.46, s		
7-Me	16.8 q	2.36, s	16.7 q	2.38, s
OMe	55.6 q	3.85, s	60.8 q	3.96, s (C-9)
OMe			60.8 q	3.84, s (C-10)

[a] Measured in $CDCl_3$–CD_3OD (1:2).
[b] Measured in $(CD_3)_2CO$.

Compound **3** was isolated as a yellow solid, which gave a [M+H]$^+$ ion at *m/z* 315.1139 in HRESIMS consistent with a molecular formula $C_{18}H_{18}O_5$ (calcd 315.1133 for $C_{18}H_{19}O_5$), requiring 10 sites of unsaturation. The UV spectrum of **3** showed absorption at 202, 243, and 267 nm, indicating the presence of a conjugated system. The IR spectrum showed the presence of hydroxyl (3395 cm^{-1}) and aromatic (1636, 1460 cm^{-1}) groups. Analysis of ^{13}C NMR, DEPT, and HSQC data revealed that **3** contains eleven aromatic quaternary carbons (including six olefinic and five oxygenated), three olefinic methines, and four methyl groups (including

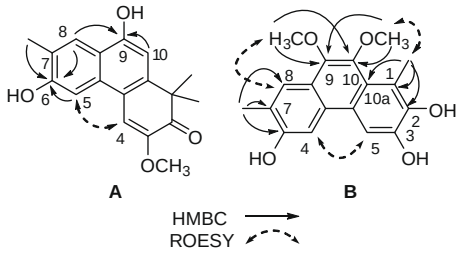
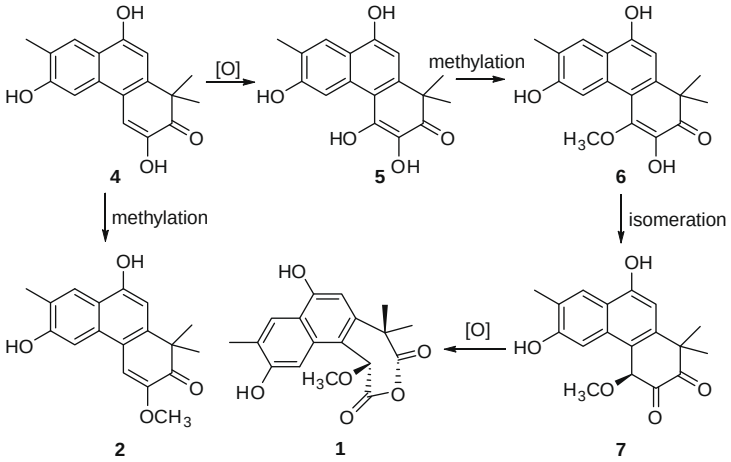


Figure 4. Key HMBC and ROESY correlations of compounds **2** and **3**.



Scheme 1. Biogenetic pathway proposed for compounds **1** and **2**.

two *O*-methyl groups). Apart from seven double bonds, the remaining elements of unsaturation in **3** were assumed to be a tricyclic skeleton. A phenanthrene skeleton was, therefore, deduced for compound **3** and its spectral parameters were consistent with those of similar phenanthrenes that were previously reported.[13] The relative positions of substituents on the phenanthrene nucleus were determined by a detailed analysis of 1D, 2D-NMR data (Fig. 4B). The ^{1}H NMR spectrum (Table 1) exhibited 15 proton signals for two methyl groups (δ_H 2.78 and 2.38), two *O*-methyl groups (δ_H 3.96 and 3.84), and three isolated olefinic proton signals (δ_H 7.81, 7.81, and 7.78), and the three remaining protons in the molecule were attributed to the exchangeable protons of three OH groups. In the HMBC spectrum, the proton signal of an aromatic methyl group (δ_H 2.78, s) showed correlation with C-10a, C-1, and C-2, and ROESY correlations were observed between the methyl and *O*-methyl group (δ_H 3.84, s) groups, indicating that the methyl group should be placed at C-1. Similarly, the HMBC correlations of another aromatic methyl group (δ_H 2.38, s) with C-6, C-7, and C-8, and the ROESY correlations were observed between methyl (δ_H 2.38, s) and H-8, indicating that this methyl group (δ_H 2.38) should be at C-7. The HMBC correlations from the proton signals of *O*-methyl groups at δ_H 3.96, 3.84 showed correlation with C-9 and C-10, respectively, and ROESY correlations were observed between *O*-methyl group (δ_H 3.96, s) and H-8, thus *O*-methyls (δ_H 3.96) should be placed at C-9, and *O*-methyl group (δ_H 3.84) should be placed at C-10, respectively. Finally, ROE correlation between H-4 and H-5 indicated that three hydroxyl groups were placed at C-2, C-3, and C-6. Thus, the structure of **3** was assigned as shown.

We proposed a possible biosynthetic pathway for compounds **1** and **2**, as shown in Scheme 1, in which **4** should be a common biogenetic precursor. From **4**, compound **1** is proposed to undergo oxidation and methylation, to construct intermediate **6**. Subsequent isomerization and oxidation would result in a ring-expended heptacyclic anhydride.

The cytotoxicity of compounds **1**–**3** against human leukemia HL-60 and human lung adenocarcinoma A549 cell lines was evaluated with MTT[14] and SRB[15] methods. Only compound **3** showed weak activity against HL-60 cells (IC_{50} = 17 μM; positive control compound etoposide, IC_{50} = 0.16 μM).

Acknowledgments

The authors are grateful to Professor Hua Peng, Kunming Institute of Botany, Chinese Academy of Sciences (CAS), for the identification of the plant material. This work was financially supported by grants from the Natural Science Foundation of China (1130801434), and the Ministry of Science and Technology (2009CB940900 and 2009CB522303).

A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.186.

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