FULL PAPER

New Compounds from the Roots and Stems of *Trigonostemon lii* and Their Cytotoxic Activities

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A new carboline alkaloid, Trifiline D (**1**) and a new degraded diterpenoid, Trigoxyphin X (**4**) were isolated from the roots and stems of *Trigonostemon lii*. Their structures were elucidated by extensive spectroscopic analyses including 1D- and 2D-NMR techniques. Compound **1** exhibited weak inhibitory activity against MCF-7, A-549, MGC-803, and COLO-205 with IC_{50} values ranging from 27.4 to 35.4 μM.

Keywords: *Trigonostemon lii*, Carboline alkaloids, Degraded diterpenoids.

Introduction

Trigonostemon includes about 50 species, belongs to the family of Euphorbiaceae, most of them are distributed in tropical and subtropical regions of Asia, and ten species being endemic to South China [1]. It has attracted considerable attention as rich source of new diterpenoids [2 – 7], alkaloids [8 – 13], phenanthrenes [14][15], and other kinds of compounds [16 – 19], with diverse structures and significant biological activities. In our continuing investigation on the chemical constitutes from *Trigonostemon lii* Y.T.Chang, a new carboline alkaloid, trifiline D (**1**), and a new degraded diterpenoid, trigoxyphin X (**4**), together with three known compounds were isolated from this plant (*Fig. 1*). This article presents the isolation and structure elucidation of these compounds using detailed spectroscopic analyses, including 1D- and 2D-NMR techniques. In addition, the cytotoxicity activities against five human cancer cell lines are also described.

Results and Discussion

Trifiline D (**1**) was obtained as light yellow amorphous powder with optical activity. The molecular formula of **1** was established as $C_{24}H_{25}N_3O_2$ by positive-mode HR-ESI-MS (*m/z* 388.2021 $[M + H]^+$, $C_{24}H_{26}N_3O_2^+$, calc. 388.2020), with 14 degrees of unsaturation. The IR spectrum of **1** indicated the presence of a NH or OH group (3426 cm^{-1}) and typical absorptions for aromatic-ring moieties (1631, 1574, and 1465 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table*) exhibited signals of two MeO groups (δ(H) 3.78 (*s*) and 3.78 (*s*)) and two broad NH

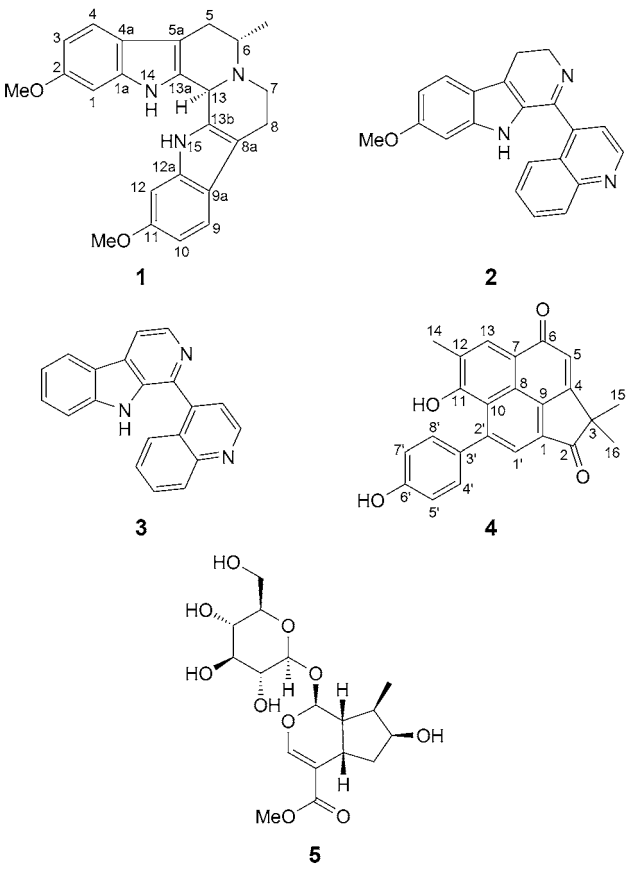

Fig. 1. Structures of compounds **1** – **5**.

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Table. ^{1}H- and ^{13}C-NMR (600 and 150 MHz, resp.) data of compounds **1** and **4**. δ in ppm, J in Hz

Position[a])	**1**		Position[a])	**4**	
	δ(H)[b])	δ(C)[b])		δ(H)[c])	δ(C)[c])
1	6.92 (*dd*, *J* = 8.5, 2.0)	95.6 (*d*)	1		132.0 (*s*)
1a		138.5 (*s*)	2		206.9 (*s*)
2		156.9 (*s*)	3		49.5 (*s*)
3	6.69 (*d*, *J* = 2.0)	109.3 (*d*)	4		157.3 (*s*)
4	7.32 (*d*, *J* = 8.5)	119.1 (*d*)	5	6.82 (*s*)	119.2 (*d*)
4a		122.4 (*s*)	6		176.1 (*s*)
5	3.39 – 3.37 (*m*), 2.68 – 2.66 (*m*)	28.3 (*t*)	7		126.0 (*s*)
5a		107.2 (*s*)	8		125.8 (*s*)
6	3.79 – 3.81 (*m*)	58.2 (*d*)	9		141.3 (*s*)
7	3.63 – 3.64 (*m*), 2.51 (*dd*, *J* = 11.4, 3.7)	49.7 (*t*)	10		129.6 (*s*)
8	2.86 – 2.88 (*m*), 2.73 – 2.76 (*m*)	23.2 (*t*)	11		159.1 (*s*)
8a		108.2 (*s*)	12		131.5 (*s*)
9	7.29 (*d*, *J* = 8.5)	118.8 (*d*)	13	8.28 (*s*)	136.1 (*d*)
9a		122.4 (*s*)	14	2.37 (*s*)	17.4 (*q*)
10	6.68 (*d*, *J* = 2.0)	109.2 (*d*)	15	1.45 (*s*)	24.3 (*q*)
11		156.9 (*s*)	16	1.45 (*s*)	24.3 (*q*)
12	6.89 (*dd*, *J* = 8.5, 2.0)	95.5 (*d*)	1′	8.49 (*s*)	131.3 (*d*)
12a		138.3 (*s*)	2′		137.7 (*s*)
13	3.75 – 3.76 (*m*)	56.5 (*d*)	3′		138.4 (*s*)
13a		136.5 (*s*)	4′, 8′	7.51 (*d*, *J* = 8.5)	132.1 (*d*)
13b		135.8 (*s*)	5′, 7′	6.89 (*d*, *J* = 8.5)	116.0 (*d*)
14-NH	9.85 (br. *s*)		6′		159.1 (*s*)
15-NH	9.91 (br. *s*)				
2-MeO	3.78 (*s*)	55.7 (*q*)			
11-MeO	3.78 (*s*)	55.7 (*q*)			
6-Me	1.59 (*d*, *J* = 6.2)	20.4 (*q*)			

[a]) Atom numbering as indicated in *Fig. 1*. [b]) Recorded in (D_6)acetone. [c]) Recorded in CD_3OD.

*singlet*s (δ(H) 9.85 (1 H, br. *s*) and 9.91 (1 H, br. *s*)). The ^{13}C-NMR spectrum accounted for all 24 C-atom resonances comprising three Me groups (two MeO groups), three sp^3 CH_2 groups, eight CH groups (including six aromatic CH groups), and 10 sp^2 quaternary C-atoms. Two aromatic *AMX* spin systems at (δ(H) (7.32 (*d*, *J* = 8.5 Hz), 7.29 (*d*, *J* = 8.5 Hz), 6.92 (*dd*, *J* = 8.5, 2.0 Hz), 6.89 (*dd*, *J* = 8.5, 2.0 Hz), 6.69 (*d*, *J* = 2.0 Hz), and 6.68 (*d*, *J* = 2.0 Hz)) were observed on the basis of analysis NMR data. In the HMBC spectrum, two NH *singlet*s showed correlations to C(1a), C(4a), C(5a), and C(13a), and C(8a), C(9a), C(12a), and C(13b), respectively, suggesting that two indole units were determined. Careful comparison of its NMR spectra (*Table*) with those of trifiline A [13] revealed that compound **1** was closely related to it, with a MeCH moiety at C/H 58.2 and 20.4/3.79 – 3.81 (*m*) and 1.59 (*d*, *J* = 6.2) in **1** instead of one CH_2 group in the latter. The ^{1}H,^{1}H-COSY correlations of CH_2(5) (δ(H) (3.37 – 3.39 (*m*) and 2.66 – 2.68 (*m*))/H–C(6) (δ(H) (3.79 – 3.81 (m))/Me–C(6) (δ(H) (1.59 (*d*, *J* = 6.2)) and the key HMBC correlations of H–C(6) to C(7) (δ(C) (49.7 (*t*))/C(13) (δ(C) (56.5 (*d*)) and H(5) to C(4a) (δ(C) (122.4 (*s*)), C(13a) (δ(C) (136.5 (*s*)) and C(5a) (δ(C) (107.2 (*s*)) also supported this difference in **1** (*Fig. 2*). The relative configuration of **1** was constructed by analysis of key correlations observed in the ROESY NMR spectrum. The ROE correlations of H–C(13) (δ(H) (3.75 – 3.76 (*m*))/Me–C(6) (*Fig. 3*) indicated that H–C (13) and Me–C(6) were on the same side, and arbitrarily assigned as α-orientation. Therefore, the relative configuration of **1** was elucidated as shown.

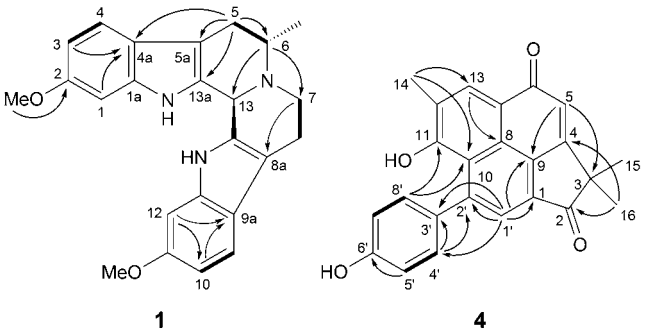

Fig. 2. Selected ^{1}H,^{1}H-COSY (▬) and HMBC (H → C) data of compounds **1** and **4**.

Trigoxyphin X (**4**) was isolated as dull red powder. Its molecular formula was deduced as $C_{24}H_{18}O_4$ from HR-ESI-MS (*m/z* 371.1280 $[M + H]^+$, $C_{24}H_{19}O_4^+$; calc. 371.1278), implying 16 degrees of unsaturation. IR spectrum indicated the presence of OH groups (3431 cm^{-1}), C=O groups (1720 and 1639 cm^{-1}) and aromatic functionalities (1631, 1567, and 1462 cm^{-1}). The ^{1}H-NMR

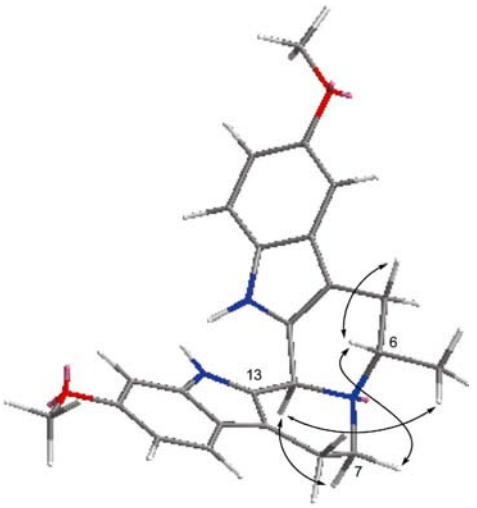

Fig. 3. Key NOESY correlations of compound **1**.

spectrum (*Table*) of **4** revealed the presence of three Me *singlet*s (δ(H) 2.37 (*s*), 1.45 (*s*), and 1.45 (*s*)), three isolated aromatic H-atoms (6.82 (*s*), 8.28 (*s*), and 8.49 (*s*)), and a typical A_2B_2 spin system (δ(H) 7.51 (*d*, J = 8.5, H–C(3′)/C(5′)) and 6.89 (*d*, J = 8.5, H–C(2′)/C(6′))). The ^{13}C-NMR spectrum of **4** (*Table*) displayed 24 C-atom resonances comprising three Me groups (δ(C) 17.4 (C(14)), and 24.3 (C(15 and 16)), seven aromatic CH groups (δ(C) 119.2 (C(5)), 136.1 (C(13)), 131.3 (C(1′)), 132.1 (C(4′ and 8′)), and 116.0 (C(5′ and 7′)), and 14 quaternary C-atoms (two C=O groups (δ(C) 206.9 (C(2)) and 176.1 (C(6)), eleven olefinic and aromatic C-atoms (δ(C) 132.0 (C(1)), 157.3 (C(4)), 126.0 (C(7)), 125.8 (C(8)), 141.3 (C(9)), 129.6 (C(10)), 131.5 (C(12)), 137.7 (C(2′)), and 138.4 (C(3′), 159.1 (C(11 and 6′)), and one alkyl C-atom (δ(C) 49.5 (C(3)), all of which were assigned by the analysis of its phase-sensitive HSQC spectrum. Comparison of the NMR data of **4** to those of trigoxyphin Q [6], showed that they were closely related analogs featuring identical carbon frameworks. The main distinction was attributable to the presence of a trisubstituted olefin unit and a *p*-hydroxyphenyl group in the former, which replaced the MeCH moiety and 4-hydroxy-3,5-dimethoxyphenyl group in the latter. Further analysis of 2D-NMR data confirmed this conclusion. Moreover, HMBC data of H–C(1′) (δ(H) (8.49 (*s*))/C(1) (δ(C)) 132.0 (*s*)), C(2′) (δ(C) (137.7(*s*)), and C(9) (δ(C) 141.3 (*s*)) indicated that the olefin unit was located at C(1′) and C(2′). The cross peak of H–C(4′(8′)) (δ(H) (7.51 (*d*, J = 8.5)) to C(2′) and C(3′), and of H–C(1′) to C(2′), C(3′) (δ(C) (138.4 (*s*)), C(4′) (δ(C) 132.1 (*d*)) in HMBC spectrum showed the *p*-hydroxyphenyl bond C(2′). The structure of compound **4** was finally determined as shown (*Fig. 2*).

By comparison of the physical and spectral data with literature values, the three known compounds (**2**, **3**, and **5**) were identified, respectively, as trigonoliimine E [8] trigonostemonine C [12], and loganin [19].

Compounds **1** – **5** were tested for cytotoxicity against five human cancer cell lines (HeLa, MCF-7, A-549, MGC-803, and COLO-205) using the MTT method [19] and their cytotoxic activities were measured in parallel with doxorubicin as the positive control. Only compound **1** showed weak inhibitory activities against MCF-7, A-549, MGC-803, and COLO-205 with IC_{50} values of 28.6, 27.4, 34.6, and 35.4 μM, respectively, along with doxorubicin as a positive control of 0.85, 1.04, 0.57, and 1.21 μM, respectively.

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Experimental Part

General

Thin-layer chromatography (TLC): glass sheets precoated with silica gel *60 GF$_{254}$* (SiO_2; *Qingdao Marine Chemical Co., Ltd.*, Qingdao, P. R. China); visualized by UV 254 and 365 nm. Column chromatography (CC): SiO_2 (60 – 80, 100 – 200, and 300 – 400 mesh; *Qingdao Marine Chemical, Inc.*, P. R. China), *RP-18* silica gel (40 – 75 μm, *Fuji Silysia Chemical Ltd.*, Japan) and *Sephadex LH-20* (40 – 70 μm; *Amersham Pharmacia Biotech*, Sweden). Semi-prep. HPLC: *Agilent 1200* series system equipped with a diode array UV detector and *Zorbax SB C-18* (10 μm, 9.4 × 250 mm, flow rate 3 ml/min; *Agilent*, Santa Clara, CA, USA). Optical rotations: *JASCO DIP-370* digital polarimeter (*JASCO*, Tokyo, Japan). UV Spectra: *UV-210A* spectrometer (*Shimadzu*, Kyoto, Japan); λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrophotometer with KBr pellets (*Bruker Optics*, Ettlingen, Germany); $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *AV-600 NMR* and *AV-800 NMR* spectrometers (*Bruker Optics*); δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI- and HR-ESI-MS: *VG Auto spec-3000* spectrometer (*Agilent*); in *m*/*z*.

Plant Material

The dry roots and stems of *T. lii* Y.T.Chang were collected from Xishuangbanna, Yunnan Province, P. R. China, in October 2006. The plant was identified by Prof. *Hua Peng* and a voucher specimen (No. KIB 20061011) was deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation

The air-dried roots and stems of *T. lii* (7 kg) were powdered and extracted three times with 95% EtOH (16 l) under reflux for 3 × 4 h. The combined org. layers were evaporated under reduced pressure to give the crude MeOH extracts, which were suspended in H_2O (2 l), and then partitioned with petroleum ether (PE), AcOEt, and BuOH, successively. The AcOEt portion (100 g) was subjected to CC on SiO_2 (100 – 200 mesh, PE/acetone 50:1 – 0:1) to yield four fractions, *Frs. 1 – 4*. *Fr. 1* was subjected to CC on SiO_2 and *Sephadex LH-20* eluted with MeOH and then further purified by repeated CC on SiO_2 to obtain trifiline D (**1**; 2.6 mg). *Fr. 2* was also subjected to CC on SiO_2 and *Sephadex LH-20*, followed by prep. TLC with mobile phase of $CHCl_3$/acetone 7:3 to afford trigonoliimine E (**2**; 5.6 mg) and trigonostemonine C (**3**; 3.3 mg). *Fr. 3* was separated by *Sephadex LH-20*, eluting with MeOH and semi-prep. HPLC (MeOH/H_2O 45:55) to yield trigoxyphin X (**4**; 1.1 mg) and loganin (**5**; 3.6 mg).

Trifiline D (= **(6*S*,14b*S*)-5,8,9,14,14b,15-Hexahydro-2,12-dimethoxy-6-methyl-6*H*-diindolo[2,3-*a*:3′,2′-*h*]quinolizine**; **1**). Light yellow powder. $[\alpha]_D^{10} = -42.6$ (c = 0.36, MeOH). UV (MeOH): 217 (4.3), 332 (3.6), 383 (3.4). IR (KBr): 3426, 2925, 2851, 1631, 1574, 1500, 1465, 1322, 1281, 1156, 1031, 822, 584, 559. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 388.2021 ($[M + H]^+$, $C_{24}H_{26}N_3O_2^+$; calc. 388.2020).

Trigoxyphin X (= **7-Hydroxy-8-(4-hydroxyphenyl)-2,2,6-trimethyl-1*H*-cyclopenta[*cd*]phenalene-1,4(2*H*)-dione**; **4**). Dull red powder. $[\alpha]_D^{10} = -6.7$ (c = 0.50, MeOH). UV (MeOH): 209 (4.4), 227 (4.2), 288 (4.2), 308.5 (4.2), 368 (3.2), 546 (4.2). IR (KBr): 3431, 2955, 2924, 2853, 1720, 1639, 1631, 1567, 1462, 1330, 1268, 1176, 1106, 1046, 790, 582, 571. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS: 371.1280 ($[M + H]^+$, $C_{24}H_{19}O_4^+$; calc. 371.1278).

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