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Three New Indole Alkaloids from *Trigonostemon lii*

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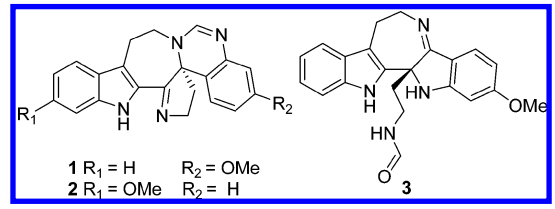
ABSTRACT



Three unprecedented indole alkaloids, trigonoliimines A−C (1−3) with a unique polycyclic system, were isolated from the leaves of *Trigonostemon lii* Y. T. Chang. The structures of 1−3 were determined by the spectroscopic, computational, and CD exciton chirality approaches. Trigonoliimine A showed modest anti-HIV-1 activity (EC_{50} = 0.95 μg/mL, TI = 7.9).

The genus *Trigonostemon* (Euphorbiaceae) comprising ca. 50 species is widely distributed in India, Malaysia, and middle Asia.[1] Previous chemical investigations on this genus have involved an array of structurally interesting compounds such as modified daphnane-type diterpenoids,[2] alkaloids,[3] diterpenoids, and phenanthrenes.[4] In this study, three novel indole alkaloids, trigonoliimines A−C (**1**−**3**), with unprecedented polycyclic skeletons, were isolated from the extract of the leaves of *Trigonostemon. lii* Y. T. Chang collected in Yunnan Province of China. Here, we report the isolation, structural elucidation, and anti-HIV-1 activity of compounds **1**−**3**.

The powdered leaves (12.0 kg) of *T. lii* were percolated three times with 95% EtOH to give a crude extract (1.0 kg). The extract was suspended in 1.5 L of water and then partitioned with petroleum ether, EtOAc, and *n*-BuOH

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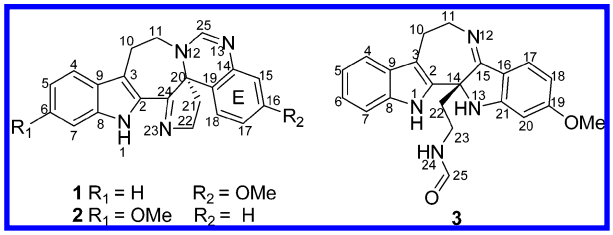
successively. The *n*-BuOH-soluble fraction (250 g) was subjected to RP-18 column eluted with 10%−100% MeOH. The 70% MeOH fraction was further chromatographed over Sephadex LH-20 gel, followed by semipreparative HPLC [MeOH/H_2O (containing 0.1% Et_2NH), 70/30] to give trigonoliimines A (**1**, 5.3 mg), B (**2**, 3.1 mg), and C (**3**, 15.7 mg).



Trigonoliimine A (**1**)[5] was obtained as a light yellow gum. The molecular formula of **1** was established as $C_{22}H_{20}N_4O$ by positive HRESIMS data (*m*/*z* 357.1709 $[M + H]^+$, calcd 357.1715), with 15 degrees of unsaturation. The UV absorptions at 205 and 306 nm suggested the indole moiety, whereas the IR spectrum showed bands at 3425 and 1627 cm^{-1}, suggesting the presence of *N*H and imine functionalities, respectively. The ^1H NMR spectrum of **1** (Table 1) indicated the presence of a methoxyl group (δ_H 3.65) and one 1,2-disubstituted aromatic ring [δ_H 7.44 (d, J = 7.5 Hz, H-4), 6.99 (t, J = 7.5 Hz, H-5), 7.15 (t, J = 7.5 Hz, H-6) and 7.32 (d, J = 7.5 Hz, H-7)]. Proton resonance at δ_H 11.50 was attributable to an indolic nitrogen proton. Two sets of units *N*CH_2CH_2 in the high field of ^1H NMR were deduced from the $^1H-^1H$ COSY and HSQC spectra. These deductions were further confirmed by the ^{13}C NMR, which also revealed an additional one benzene moiety, two imine groups, a fully substituted double bond, as well as one sp^3 quaternary carbon. The aforementioned groups represented nine degrees of unsaturation. Thus, there must be six rings in the structure.

Table 1. ^1H and ^{13}C NMR Data of Trigonoliimine A (**1**) and ^{13}C NMR Data of Trigonoliimine B (**2**)

	1			**2**
no.	δ_C[a]	δ_H[b]	δ_C[c]	δ_C[c]
1 (*N*H)	-	11.50 (s)	-	-
2	127.9	-	126.5	125.1
3	115.6	-	117.4	117.7
4	119.1	7.44 (d, 7.5)	119.0	119.3
5	119.1	6.99 (t,7.5)	119.6	110.0
6	123.4	7.15 (t, 7.5)	124.3	157.6
7	111.7	7.32 (d,7.5)	111.4	93.4
8	136.5	-	136.8	137.4
9	127.1	-	127.2	121.0
10α	29.1	3.06 (m)	29.4	29.0
10β		2.95 (m)		
11α	46.6	4.00 (br d, 14.5)	47.9	47.5
11β		3.74 (t, 12.5)		
14	143.0	-	141.0	139.6
15	109.2	6.55 (overlapped)	108.6	123.4
16	159.6	-	160.1	128.2
17	110.3	6.54 (overlapped)	111.4	124.8
18	123.2	6.53 (overlapped)	123.2	121.8
19	115.0	-	113.7	121.3
20	76.5	-	77.2	76.5
21α	40.6	2.05 (m)	40.4	39.8
21β		2.14 (m)		
22α	56.2	3.55 (m)	56.0	55.2
22β		4.10 (dd, 8.5, 16.0)		
24	166.4	-	167.4	166.6
25	150.2	7.48 (s)	149.9	149.0
OMe	55.1	3.65 (3H, s)	55.0	54.6

[a] Measured in DMSO-d_6 at 125 MHz. [b] Measured in DMSO-d_6 at 500 MHz. [c] Measured in $CDCl_3/CD_3OD$ (3:1) at 125 MHz.

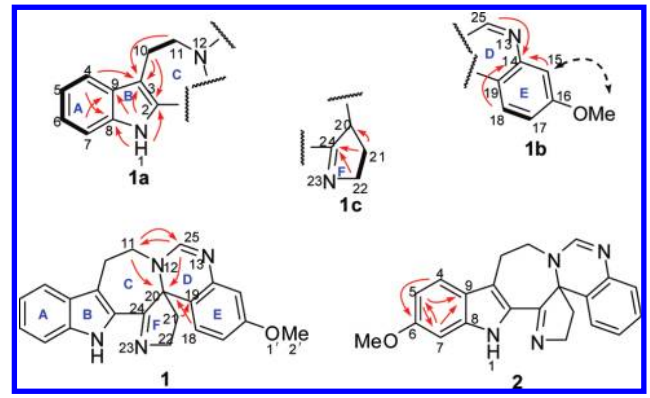


Figure 1. Partial fragments (**1a**−**1c**), $^1H-^1H$ COSY (−), key HMBC (→) correlations of **1** and **2**, and key ROESY (←−→) correlation of **1**.

Comprehensive analysis of the 1D and 2D NMR spectra, especially HMBC (Figure 1), elucidated that compound **1** featured a unique ring system. A detailed account of the structural elucidation of **1** is presented below. The observed HMBC correlations of the indolic nitrogen proton (*N*H-1) to C-2, C-3, C-8, and C-9, H-4 to C-3 and C-8, and H-7 to C-9 were consistent with a 2,3-disubstituted indole moiety. In addition, one of the ethanamine fragments attached to C-3 was confirmed by the HMBC correlations from H_2-10 to C-3 and C-2 and H-11β to C-3. The above data established the partial structure (**1a**, Figure 1).

Moiety **1b** contained the 1,2,4-trisubstituted benzene ring fragment, the imine group, and the methoxyl group. HMBC correlations (in $CDCl_3/CD_3OD$ (3:1), Supporting Information) of H-15 and H-18 to C-14 (δ_C 143.0) and of H-17 to C-19 indicated that the imine group attached at C-14 by the C−N bond. The ROESY correlations (in $CDCl_3/CD_3OD$ (3:1), Supporting Information) of H-15 with the OCH_3 indicated that the methoxy group was linked at C-16.

The ring F (fragment **1c**, Figure 1) consisting of the *N*CH_2CH_2 moiety (C-21−C-22), C-20, C-24, and *N*-23 was

(5) Trigonoliimine A (**1**): a light yellow gum; $[\alpha]^{10}_D$ = + 13.3 (*c* 0.3, $CHCl_3$); CD ($CHCl_3$) 222 nm ($\Delta\varepsilon$ + 9.7), 225 nm ($\Delta\varepsilon$ − 2.5); UV (MeOH) λ_{max} (log ε) 205 nm (4.5), 306 nm (4.1); IR (KBr) υ_{max} 3425, 2960, 2925, 1627, and 1452 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 357 $[M + H]^+$; HRESIMS *m*/*z* 357.1709 $[M + H]^+$ (calcd for $C_{22}H_{20}N_4O$, 357.1715).

really established by the HMBC correlations of H_2-21 to C-20 and H-21α and H_2-22 to C-24, respectively. Furthermore, the key HMBC correlations of H-25 and H-11α to C-20, H-25 to C-11, and H-11*β* to C-25 supported the connectivity of C-20 to the two moieties **1a** and **1b** through heteroatom *N*-12. HMBC crosspeaks of H_2-21 to C-19 and H-18 to C-20 further connected C-19 to C-20, and the remaining C-2 therefore must be attached to C-24 forming an azepane agreement with the molecular weight. Thus, the gross structure of trigonoliimine A was unambiguously assigned as **1** with an unusual fused-hexacyclic ring system (two five-, three six-, and one seven-membered rings).

To determine its absolute configuation, chemical computation was employed. First, A SPARTAN 08 search of conformations of compound **1** was performed, which identified two stable conformations, I and II, only with different rotated angle of the OCH_3 around the C-16−O-1′ single bond. Reoptimization of the structure of the stable conformations was carried out at HF/6-31G* and then B3LYP/6-31G(2d, p) levels in Gaussian 03. Harmonic vibrational frequencies of each conformation were then calculated using B3LYP/6-31G (2d, p) to confirm their stability. Then, the potential energy surface (PES) was scanned, which started from the optimized geometry of conformation I and varied the dihedral angle C15C16O1′C2′. The results exhibited only two minima and suggested that the occurrence of the comparatively rapid interconversion of I and II was a logical process in solution at room temperature. Relative and free energy and equilibrium populations at room temperature of the two conformations were further calculated and listed in Table 2. Lastly, the "self-consistent reaction field" method (SCRF) was employed to perform the OR calculation of the two major conformers of compound **1** in chloroform at the B3LYP-SCRF/6-311++G (d, 2p) level.[6] As shown in Table 2, the computed optical rotation value (+18.1) of **1** is matched up to the experimental value (+13.3) for the *S* enantiomer, thus tentatively assigning the 20*S* configuration.

Table 2. Computed Energy, Populations, and Optical Rotation for Trigonoliimine A (**1**)

	Trigonoliimine A		
conformation	I (1 *S*)	II (2 *S*)	exptl
relative energy [kcal mol^{-1}]	0.00	0.12	
equilibrium populations (%)	53.4	46.6	
$[\alpha]_D$	−23.88	+ 62.24	
sum of $[\alpha]_D$		+ 18.11	+ 13.3

The molecular formula of **2**[7] was indicated to be $C_{22}H_{20}N_4O$ by positive HRESIMS (*m/z* 357.1718 $[M + H]^+$, (calcd: 357.1715)), which was the same as that of **1**. Comparison of the 1D NMR spectropic data of **2** with that of **1** showed a resemblence. All these suggested that **2** should be an isomer of **1**, which was further confirmed by 2D NMR expriments. Specifically, the HMBC correlations of H-7 to C-5 and C-9, H-5 to C-7 and C-9, and H-4 to C-6 indicated that the OMe group was linked to C-6, as shown in Figure 1.

Table 3. ^{1}H (500 MHz) and ^{13}C NMR (100 MHz) Data of Trigonoliimine C (**3**)

no.	δ_C[a]	δ_H[a]	δ_C[b]
1 (*N*H)	-	10.64 (s)	-
2	131.8	-	131.4
3	108.8	-	110.3
4	117.8	7.41 (d, 7.5)	118.5
5	118.6	6.98 (t, 7.5)	119.7
6	121.6	7.08 (t, 7.5)	122.7
7	110.9	7.36 (d, 7.5)	111.5
8	134.8	-	136.3
9	127.9	-	129.2
10α	23.3	3.05 (br d, 11.0)	24.3
10*β*		2.80 (t, 11.0)	
11α	46.5	4.24 (t, 12.0)	47.5
11*β*		3.99 (br d, 12.0)	
13 (*N*H)	-	6.83 (br s)	-
14	66.4	-	68.1
15	170.3	-	174.9
16	116.4	-	116.5
17	123.6	7.34 (d, 8.0)	125.2
18	105.7	6.26 (dd, 2.5, 8.0)	108.4
19	164.3	-	166.8
20	94.0	6.27 (d, 2.5)	95.3
21	156.8	-	159.0
22α	39.5	2.29 (m)	40.2
22*β*		2.51 (m)	
23	33.6	3.14 (2H, m)	34.9
24 (*N*H)	-	7.99 (br s)	-
25	161.1	7.93 (s)	163.4
OMe	55.3	3.77 (s)	55.8

[a] Measured in DMSO-d_6. [b] Measured in $CDCl_3/CD_3OD$ (3:1).

Trigonoliimine C (**3**)[8] was isolated as a light yellow gum. The positive HRESIMS at *m/z* 375.1813 $[M + H]^+$ gave molecular formula $C_{22}H_{22}N_4O_2$ (calcd: 375.1821), indicating 14 degrees of unsaturation. The ^{13}C NMR spectrum of compound **3** (Table 3) showed 22 well-resolved resonances, including sp^3 carbons (1 quaternary carbon, 4 methylenes, and 1 methoxyl) and sp^2 carbons (8 methines and 8 quaternary carbons). Among them, the carbon resonances at δ_C 170.3 and 161.1 were assigned to imine and formamide functionalities, respectively. In its ^{1}H NMR spectrum, only 19 protons showed HSQC correlations to these carbons.

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(7) Trigonoliimine B (**2**): a light yellow gum; $[\alpha]^{10}_D = +5.0$ (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 192 nm (4.2), 207 nm (4.4), 220 nm (4.4), 321 nm (4.0); IR (KBr) ν_{max} 3439, 2959, 2924, 1619, and 1452 cm^{-1}; ^{13}C NMR data (Table 1); ESIMS m/z 357 $[M + H]^+$; HRESIMS *m/z* 357.1718 $[M + H]^+$ (calcd for $C_{22}H_{20}N_4O$, 357.1715).

(8) Trigonoliimine C (**3**): a light yellow gum; $[\alpha]^{10}_D = -4.8$ (*c* 0.45, $CHCl_3$); CD ($CHCl_3$) 224 nm ($\Delta\varepsilon$ − 4.5), 228 nm ($\Delta\varepsilon$ + 8.4); UV (MeOH) λ_{max} (log ε) 224 nm (4.4), 253 nm (3.8); IR (KBr) υ_{max} 3440, 2961, 2926, 1622, and 1453 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 375 $[M + H]^+$; HRESIMS *m/z* 375.1813 $[M + H]^+$ (calcd for $C_{22}H_{22}N_4O_2$, 375.1821).

Besides, the remaining three [δ_H 10.64 (s, *N*H-1); 6.83 (br s, *N*H-13); and 7.99 (br s, *N*H-24)] were assigned to exchangable protons. 1D NMR and $^1H-^1H$ COSY further revealed signals for one 1,2-disubstituted aromatic ring, one 1,2,4-trisubstitued benzene ring, and two NCH_2CH_2 moieties. Apart from nine degrees of unsaturation occupied by sp^2 carbons, the remaining five degrees of unsaturation required **3** to have a pentacyclic core ring system.

Interpretation of two-dimensional (2D) NMR data led to three partial moieties **a**−**c**. Unit **a** contained a 2,3-disubstituted 1*H*-indole ring on the basis of HMBC cross peaks of the exchangable proton at δ_H 10.64 (*N*H-1) to C-2, C-3, C-8, and C-9, H-4 to C-3 and C-8, and H-7 to C-9 (**3a**, Figure 2). In addition, the NCH_2CH_2 fragment attached to C-3 was confirmed by the HMBC correlations of H_2-10 to C-3 and C-2. The HMBC correlations of *N*H-13 to C-14, C-15 and C-16, H-20 to C-16 and C-21, and H-17 to C-15 confirmed the unit **b** (**3b**, Figure 2). The unit **c**, consisting of another NCH_2CH_2 moiety and the formamide group, was assigned as the *N*-ethylformamide fragment (**3c**, Figure 2) by the observed HMBC correlations of *N*H-24 to C-25 and H-25 to C-23.

The connectivity of these units was further indicated by HMBC expriments. The three-bond correlations from H-11α and H-17 to C-15 indicated the connection between fragments **3a** and **3b**. The connectivity of fragments **3a** and **3b** with **3c** was confirmed by the HMBC correlations of H-22β to C-2, H_2-22 and *N*H-13 to C-14, which also permit the seven-membered imine-containing rings. Thus, the gross structure of **3** was established as shown.

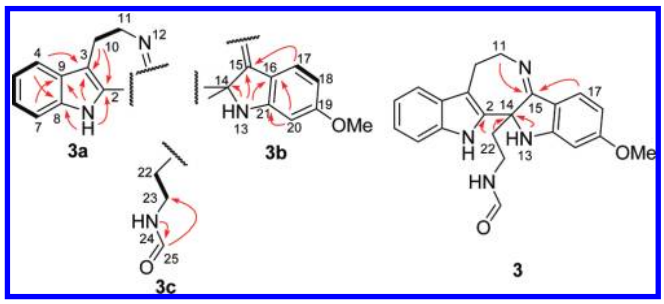

Figure 2. Partial fragments (**3a**−**3c**), $^1H-^1H$ COSY (−), and key HMBC (→) correlations of **3**.

The CD spectrum of **3** exhibited significantly positive cotton effects in 220−230 nm resulting from the exciton coupling between the two different chromophores of the indole moiety and the 6-methoxyindolin-3-imine moiety,[9] indicating that the transition dipole monents of the two chromophores were oriented in a clockwise manner (Figure 3). So, the absolute configuration of **3** was assigned as 14*R*.

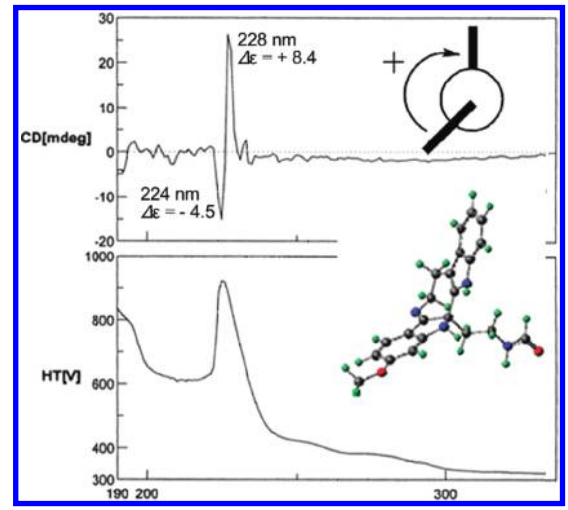

Figure 3. CD spectra of **3**. Bold lines denote the electric transition dipole of the chromophores for **3**.

The anti-HIV-1 activity of **1** and **3** was tested by a microtiter syncytium formation infectivity assay, with AZT (EC_{50} = 0.02 μg/mL, TI = 59924) as a positive control.[10] Compound **1** showed modest anti-HIV-1 activity (EC_{50} = 0.95 μg/mL, TI = 7.9).

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Supporting Information Available: General experimental procedures, optical rotation calculation, and 1D and 2D NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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