ORGANIC LETTERS

2013
Vol. 15, No. 7
1492–1495

Two New Types of Bisindole Alkaloid from *Trigonostemon lutescens*

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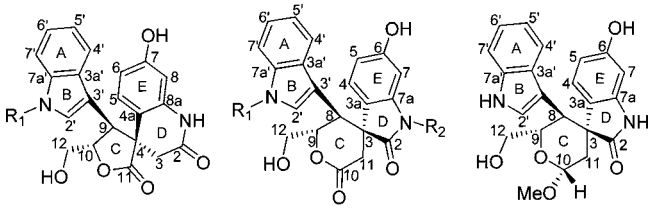
Received January 29, 2013

ABSTRACT



1 R_1 = H
2 R_1 = CH_2OCH_3

3 R_1 = H R_2 = CH_2OH
4 R_1 = R_2 = H
5 R_1 = CH_2OCH_3 R_2 = H

6

Six unprecedented bisindole alkaloids, trigolutesins A and B (1−2) with a unique polycyclic skeleton and trigolutes A−D (3−6) with another polycyclic skeleton, were isolated from the twigs of *Trigonostemon lutescens*. Their structures and relative configurations were elucidated by spectroscopic data and single-crystal X-ray diffraction crystallography. Trigolutesin A (1) showed weak AChE inhibitory activity.

The genus *Trigonostemon* (Euphorbiaceae) comprising ca. 50 species grows mainly in the tropical and subtropical regions of Asia.[1] Plants of the genus are used as traditional medicine to cure asthma, poisonous snake bites, and food poisoning in Thai folk medicine.[2] Previous chemical studies on this genus have led to the isolation of numbers of structurally interesting compounds such as modified diterpenoids,[3,4] flavonoidal indole alkaloids,[5] diterpenoids, and phenanthrenes.[6] So far, there has been no report on the chemical constituents of *Trigonostemon lutescens*.[7] In this paper, we describe the isolation and structural elucidation of compounds **1**−**6**, six novel bisindole alkaloids, from the EtOH extract of the twigs of *Trigonostemon lutescens* collected in the Guangxi Zhuang Autonomous Region of China by extensive spectroscopic analysis [one-dimensional (1D) and two-dimensional (2D) NMR] and X-ray crystallography.

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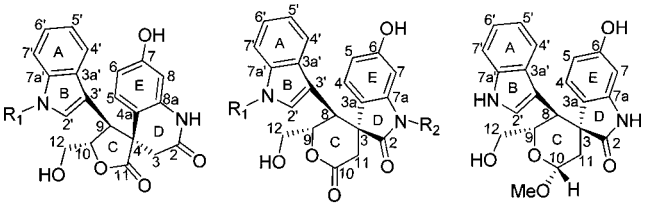
(7) **Trigolutesin A (1)**: colorless crystal; mp 326−327 °C; $[\alpha]^{30.5}_{D}$ = −41.0 (*c* 0.19, MeOH); CD (MeOH) 225 nm (Δε +30.0), 280 nm (Δε +2.3); UV (MeOH) λ_{max} (log ε) 195 nm (5.0), 197 nm (5.0), 218 nm (3.2); IR (KBr) ν_{max} 3566, 3275, 2924, 1771, 1651, 669 cm^{-1}; ^1H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS *m*/*z* 401.1 $[M + Na]^+$; HREIMS *m*/*z* 378.1221 $[M]^+$ (calcd for $C_{21}H_{18}N_2O_5$, 378.1216). CCDC deposit no. 914982.

10.1021/ol4002619
Published on Web 03/12/2013

Herein we present the isolation, structural elucidation, and biological evaluation of these compounds.

The air-dried powder of twigs of *Trigonostemon lutescens* (15.7 kg) was extracted three times with 95% EtOH at room temperature, and the solvent was evaporated in vacuo. The extract was suspended in H_2O and then partitioned with petroleum ether, EtOAc, and *n*-BuOH, successively. The EtOAc-soluble fraction (52.4 g) was subjected to column chromatography (CC) on silica gel eluted with $CHCl_3$/MeOH (100:0 to 1:1) as eluent. Combining the fractions by TLC monitoring gave 10 fractions (Fr.1−Fr.10). Fraction 7 (6.6 g) was repeatedly purified by silica gel and RP-18 silica gel to afford **1** (10.4 mg), **2** (10.0 mg), **3** (3.0 mg), **4** (11.0 mg), **5** (2.2 mg), and **6** (19.4 mg).



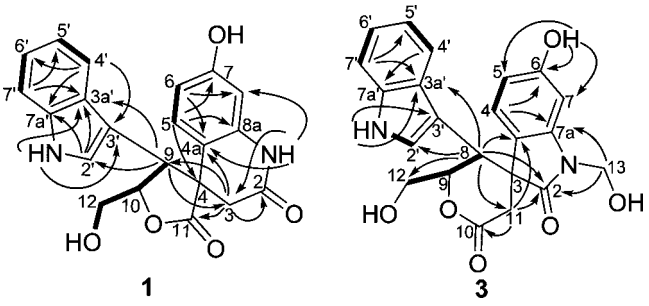
1 R_1 = H
2 R_1 = CH_2OCH_3

3 R_1 = H R_2 = CH_2OH
4 R_1 = R_2 = H
5 R_1 = CH_2OCH_3 R_2 = H

6

Trigolutesin A (**1**)[7] was isolated as a colorless crystal (CH_3OH). Its molecular formula, $C_{21}H_{18}N_2O_5$, with 14 degrees of unsaturation, was established on the basis of the HREIMS at *m*/*z* 378.1221 $[M]^+$. ^{13}C NMR and DEPT revealed 21 carbons due to eight sp^2 quaternary carbons, eight sp^2 methines, one sp^3 quaternary carbon, two sp^3 methines, and two sp^3 methylenes. The ^1H NMR signals at δ_H 7.30 (1H, d, J = 8.0), 6.80 (1H, t, J = 7.4), 6.90 (1H, t, J = 7.2), and 7.13 (1H, d, J = 8.1) implied the presence of a 1, 2-disubstituted benzene ring moiety in the structure. Furthermore, the ^1H NMR peaks at δ_H 5.99 (1H, d, J = 2.4), 6.40 (1H, dd, J = 2.4, 8.4), and 6.78 (1H, d, J = 8.4) revealed the presence of a 1,2,4-trisubstituted benzene ring moiety. Besides the above signals, δ_H 9.49 (1H, s, *N*H-1), 10.72 (1H, d, J = 2.0 *N*H-1′), 9.60 (1H, br s, OH-7), and 5.11 (1H, t, J = 5.5 OH-12) were also found in the ^1H NMR spectrum.

The observed HMBC correlations (Figure 1) of the proton *N*H-1′ to C-3′ and C-3a′, H-2′ to C-3a′ and C-7a′, H-4′ to C-3′, C-6′, and C-7a′, and H-7′ to C-5′ and C-3a′ were consistent with a 3′-substituted indole moiety. The above data established the partial structure (the rings of A and B). The ring D consisting of the *N*H-C=O moiety (C-2, C-3, C-4, C-4a, and C-8a) was established by the HMBC correlations of the nitrogen proton (*N*H-1) to C-3, C-4a and C-8, H-3 to C-2, C-9 and C-4a, and H-5 to C-4, respectively. Thus, the partial unit of D and E rings contained the 1, 2, 4-trisubstituted benzene ring fragment, the hydroxyl, and the carbonyl group (*N*H-C=O) were deduced. The $^1H-^1H$ COSY revealed a fragment of H-10 to H-9 and H-12. The connection of these units was further indicated by HMBC correlations from H-9 to C-3, C-3a′, and C-2′, from H-3 to C-11. The five-membered C ring was



1 **3**

Figure 1. Key COSY (—) and HMBC (→) correlations of **1** and **3**.

deduced according to the molecular formula and further elucidated by the X-ray diffraction. The relative configuration of **1**[7] was assigned by the X-ray diffraction (Figure 2). Thus, the structure of **1** was elucidated as shown and named trigolutesin A.

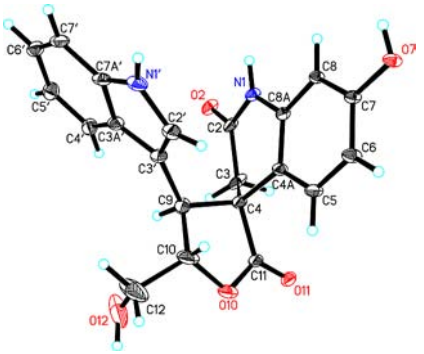


Figure 2. Single-crystal X-ray structure of **1**.

Trigolutesin B (**2**)[8] gave the molecular formula $C_{23}H_{22}N_2O_6$ by positive HRESIMS. The NMR spectral data of **2** were almost identical with those of **1**. However, some additional peaks corresponding to a dimethyl ether group appeared. The dimethyl ether fragment attached to *N*-1′ was confirmed by the HMBC correlations from H-13 to C-2′ and C-7a′ and 3H (13-OMe) to C-13. The relative configuration of **2** was deduced to be the same as **1** by analysis of their ROESY spectra and comparison of NMR data, especially multiplicities of the proton signals to those

(8) **Trigolutesin B** (**2**): yellow amorphous solid; mp 166−179 °C; $[\alpha]^{30.5}_D$ = +13.0 (*c* 0.20, MeOH); CD (MeOH) 225 nm ($\Delta\varepsilon$ + 18.3), 277 nm ($\Delta\varepsilon$ + 2.0); UV (MeOH) λ_{max} (log ε) 194 nm (5.0), 195 nm (5.0), 200 nm (2.4), 218 nm (3.0); IR (KBr) ν_{max} 3566, 3217, 2924, 1944, 1918, 1685, 1651, 905 cm^{-1}; ^1H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS *m*/*z* 445.2 $[M + Na]^+$; HRESIMS (pos) *m*/*z* 423.1548 $[M + H]^+$ (calcd for $C_{23}H_{23}N_2O_6$, 423.1551).

(9) **Trigolute A** (**3**): colorless crystal; mp 202−204 °C; $[\alpha]^{30.5}_D$ = −10.2 (*c* 0.20, MeOH); CD (MeOH) 225 nm ($\Delta\varepsilon$ +27.2), 280 nm ($\Delta\varepsilon$ +2.2); UV (MeOH) λ_{max} (log ε) 194 nm (5.0), 198 nm (5.0), 217 nm (4.1), 220 nm (4.2), 223 nm (4.0); IR (KBr) ν_{max} 3440, 2925, 1631, 1117, 618 cm^{-1}; ^1H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS *m*/*z* 431.1 $[M + Na]^+$, HRESIMS (pos) *m*/*z* 409.1392 $[M + H]^+$ (calcd for $C_{22}H_{21}N_2O_6$, 409.1394). CCDC deposit no. 914983.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of Trigolutesin A (**1**) and Trigolute A (**3**) (in DMSO-d_6)

	1		**3**	
no.	δ_c[a]	δ_H[b] (J, Hz)	δ_c[c]	δ_H[d] (J, Hz)
1(*N*H)		9.39 (s)		
2	167.0		178.5	
3	39.2	2.90 (d,16.3,Ha) 2.56 (d,16.3,Hb)	49.9	
3a			121.0	
4	52.0		123.8	7.28 (d, 8.4)
4a	110.6			
5	126.8	6.78 (d, 8.4)	108.9	6.25 (overlap)
6	109.5	6.40 (dd, 2.4, 8.4)	157.4	
7	157.8		97.7	6.22 (overlap)
7a			142.6	
8	102.9	5.99 (d, 2.4)	36.6	4.04 (d, 11.3)
8a	139.8			
1′ (*N*H)		10.72 (d, 2.0)		10.81 (s)
2′	122.6	5.91 (d, 2.3)	122.0	6.90 (overlap)
3′	106.8		109.1	
3a′	127.7		127.6	
4′	118.0	7.30 (d, 8.0)	118.5	7.49(d, 8.0)
5′	118.5	6.80 (t, 7.4)	118.3	6.88 (overlap)
6′	121.0	6.90 (t, 7.2)	118.5	6.95 (overlap)
7′	111.2	7.13 (d, 8.1)	111.3	7.16 (d, 8.0)
7a′	135.3		135.3	
9	45.2	3.82 (d, 11.0)	81.9	5.12 (d, 11.4)
10	84.0	4.43 (m)	169.3	
11	177.8		38.4	3.17 (d, 17.0, Ha) 2.40 (d, 17.0, Hb)
12	60.3	3.34 (m)	61.8	3.37 (overlap, Ha) 3.17 (overlap, Hb)
13			62.5	4.96 (m, Ha) 4.84 (m, Hb)
6-OH				9.36 (s)
7-OH		9.50 (br s)		
12-OH		5.01 (t, 5.5)		4.92 (overlap)
13-OH				4.92 (overlap)

[a] Data recorded at 125 MHz. [b] Data recorded at 500 MHz. [c] Data recorded at 100 MHz. [d] Data recorded at 400 MHz.

of **1**. Finally, the structure of **2** was determined and named trigolutesin B.

Trigolute A (**3**)[9] was isolated as a colorless crystal. Its positive HRESIMS at m/z 409.1392 $[M + H]^+$ (calcd for 409.1394) gave molecular formula $C_{22}H_{20}N_2O_6$, indicating 14 degrees of unsaturation. The IR absorptions implied the presence of hydroxyls (3440 cm^{-1}) and ester carbonyl groups (1631 cm^{-1}). The ^{1}H and ^{13}C NMR data of **3** clearly revealed that it shared the same 3′-substituted indole moiety and 1, 2, 4-trisubstituted aromatic ring as in compound **1**. The difference between **1** and **3** occurred in the rings of C and D. The six-membered ring C and the five-membered ring D were elucidated by the X-ray diffraction (Figure 3) and the 2D NMR data. The HMBC correlations of H-13 to C-2 and C-7a, H-8 to C-2, C-2′, C-3a, C-3a′, C-11 and C-12, and H-11 to C-2, C-3a and C-10, as well as the $^{1}H-^{1}H$ COSY correlations of H-8/H-9, H-9/H-12 confirmed the structure of rings C and D. The relative configuration of **3** was assigned by the X-ray diffraction. Thus, the structure of **3** was elucidated as shown in Figure 1 and named trigolutesin A.

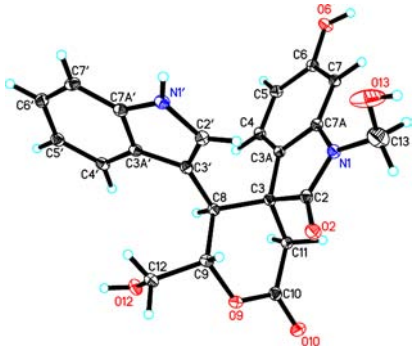


Figure 3. Single-crystal X-ray structure of **3**.

Trigolute B (**4**),[10] a yellow amorphous solid, was determined to have a molecular formula of $C_{21}H_{18}N_2O_5$ on the basis of its ^{13}C NMR (DEPT) spectrum and negative HRESIMS, which showed a quasimolecular ion peak at m/z 377.1129 $[M - H]^-$ (calcd for $C_{21}H_{17}N_2O_5$ 377.1143). NMR signals generally agreed with those of **3**, indicating that **4** was also an indole alkaloid, with an obvious difference of a missing hydroxymethyl. The position of nitrogen proton (δ_H 10.18) was established by the HMBC correlations of *N*H-1 to C-2, C-3, C-3a, and C-7a. All these suggested that **4** should be the same skeleton with **3**, which contained a six-membered ring C and a five-membered ring D. The relative configuration of **4** was deduced to be the same as **3** by analysis of their homologous relationship and comparison of NMR data, especially multiplicities of the proton signals to those of **3**. Finally, the structure of **4** was figured out and named trigolute B.

Trigolute C (**5**),[11] a colorless crystal, gave a molecular formula of $C_{23}H_{22}N_2O_6$ by HRESIMS ion at m/z 423.1545 $[M + H]^+$ (calcd for 423.1551). The NMR spectral data of **5** were almost identical with those of **4**; however, a group of additional peaks corresponding to a dimethyl ether appeared. The HMBC correlation from H-13 to C-7a′ and OMe-13, and OMe-13 to C-13 indicated that the dimethyl ether group was positioned at *N*-1′. The relative configuration of **5** was deduced to be the same as **3** and **4** by analysis of its ROESY spectra and comparison of their

(10) **Trigolute B** (**4**): yellow amorphous solid; mp 320−332 °C; $[\alpha]^{30.5}_D$ = +7.5 (*c* 0.18, MeOH); CD (MeOH) 218 nm ($\Delta\varepsilon$ + 14.0), 290 nm ($\Delta\varepsilon$ + 2.9); UV (MeOH) λ_{max} (log ε) 194 nm (5.0), 205nm (3.1), 214 nm (3.5); IR (KBr) ν_{max} 3565, 3337, 1771, 1650, 1422, 1337, 670 cm^{-1}; ^{1}H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS m/z 401.1 $[M + Na]^+$, HRESIMS (neg) m/z 377.1129 $[M - H]^-$ (calcd for $C_{21}H_{17}N_2O_5$, 377.1143).

(11) **Trigolute C** (**5**): colourless crystal; mp 194−196 °C; $[\alpha]^{30.5}_D$ = −5.5 (*c* 0.20, MeOH); CD (MeOH) 225 nm ($\Delta\varepsilon$ + 11.5), 290 nm ($\Delta\varepsilon$ + 2.8); UV (MeOH) λ_{max} (log ε) 193 nm (5.0), 194 nm (5.0), 198 nm (5.0), 217 nm (3.0); IR (KBr) ν_{max} 3566, 3120, 1771, 1686, 1426, 1339, 669 cm^{-1}; ^{1}H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS m/z 421.4 $[M - H]^-$, HRESIMS (pos) m/z 423.1545 $[M + H]^+$ (calcd for $C_{23}H_{23}N_2O_6$, 423.1551).

NMR data, especially multiplicities of the proton signals to those of **4**. Finally, the structure of **5** was figured out and named trigolute C.

Trigolute D (**6**)[12] was obtained as a colorless crystal. The positive HRESIMS at m/z 395.1598 $[M + H]^+$ (calcd for 395.1601) gave a molecular formula $C_{22}H_{22}N_2O_5$, indicating 13 degrees of unsaturation. The NMR data of **6** were similar to those of **4**, except that a methoxy (δ_C 56.7) in **6** replaced the carbonyl (C-10) in **4**. This methine was assigned at C-10 by the HMBC correlations of the methoxy protons to C-10. The ROESY experiment showed that H-4 correlated with H-8 and H-11a, and H-10 correlated with H-9 and H-11b, which indicated that the H-9, H-10, and H-11b were in β-orientation, while H-8 and H-11a were in α-orientation (Figure 4). The relative configuration and conformation of **6** was therefore established as depicted.

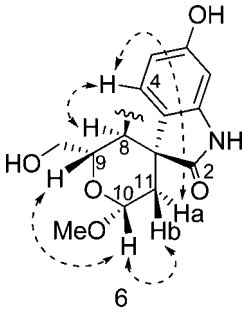
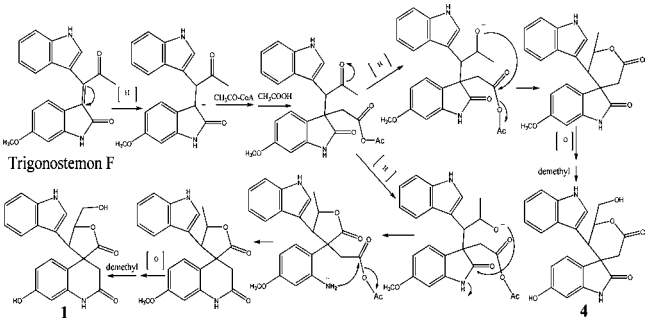


Figure 4. Key ROESY correlations (↔) of **6**.

The acetylcholinesterase inhibitory activities for compounds **1**–**6** were tested.[13] The purities of all compounds were more than 98% by HPLC analyses. Compound **1** showed weak inhibitory activity (percentage inhibition were 14.56%) at a concentration of 50 μg/mL, Meanwhile, the other compounds were inactive with inhibition ratios less than 10%.

Scheme 1. Possible Biogenetic Pathway of Compounds **1** and **4**



Some bisindole alkaloids have been previously isolated from genus *Trigonostemon*, but compounds **1**–**6** possessed different spiro skeleton from them.[14] A possible biogenetic pathway of compounds **1** and **4** was proposed as shown in Scheme 1. Trigonostemon F,[15] a major product isolated from *Trigonostemon chinensis*, was taken as the biogenetic precursor. A compound incorporating a similar spirolactone moiety, and possessing a 6/5/5/5/6 ring system related to the present alkaloids, has been previously encountered as an intermediate in the synthesis of the bioactive *Streptomyces* metabolites, maremycines A–D.[16]

Acknowledgment. This research was financially supported by the Special Fund for Agro-scientific Research in the Public Interest (201303117) and Fundamental Research Funds for Central Public Welfare Research Institutes (Chinese Academy of Tropical Agricultural Sciences, No. 1630052012014). We thank the members of the analytical group of the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, for the spectral measurements.

Supporting Information Available. ^{1}H and ^{13}C NMR data of compounds **1**–**6**, selected HMBC, ^{1}H–^{1}H COSY and ROESY correlations of **1** and **3**, ^{1}H, ^{13}C, and 2D NMR (HSQC, HMBC, COSY and ROESY), ESIMS and HRESIMS spectra of **1**–**6**, CD spectra of **1**–**6**, and X-ray crystallographic data for **1** and **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(12) **Trigolute D** (**6**): colorless crystal; mp 184–185 °C; $[\alpha]^{30.5}_D$ = −15.5 (*c* 0.20, MeOH); CD (MeOH) 219 nm ($\Delta\varepsilon$ + 26.9), 290 nm ($\Delta\varepsilon$ + 1.6); UV (MeOH) λ_{max} (log ε) 191 nm (5.0), 202 nm (2.3), 215 nm (2.8); IR (KBr) ν_{max} 3566, 3276, 1771, 1426, 1339, 669 cm^{-1}; ^{1}H and ^{13}C NMR data: see the Supporting Information, Tables 1 and 2; ESIMS m/z 393.3 $[M - H]^-$, HRESIMS (pos) m/z 395.1598 $[M + H]^+$ (calcd for $C_{22}H_{23}N_2O_5$, 395.1601).

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