Further Alkaloids from the Leaves of *Trigonostemon lii*

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Four new indole alkaloids, trigonoliimines D – G (**1** – **4**, resp.), were isolated from *Trigonostemon lii*. Their structures were elucidated by spectroscopic methods, including extensive 1D- and 2D-NMR experiments.

Introduction. – 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 led to the isolation of a number of structurally interesting compounds, such as modified diterpenoids [2], flavonoidal indole alkaloids [3], diterpenoids, and phenanthrenes [4]. In 2010, we reported three novel indole alkaloids (trigonoliimines A – C) with unique ring systems [3c], which have attracted the interest of the chemists to attempt at the total synthesis [5]. In the current investigation, four new β-carboline alkaloids, trigonoliimines D – G (**1** – **4**, resp.), were isolated from *Trigonostemon lii* (*Fig. 1.*) Herein, we present the isolation, structure elucidation, and biological evaluation of the new alkaloids.

Results and Discussion. – Trigonoliimine D (**1**) was obtained as optically inactive, light-yellow gum, and its molecular formula was deduced as $C_{22}H_{17}N_3O_4$ from HR-ESI-MS (*m*/*z* 388.1281 ($[M+H]^+$, $C_{22}H_{18}N_3O_4^+$; calc. 388.1297), requiring 16 degrees of unsaturation. IR Absorptions indicated the presence of NH or OH (3426 cm^{-1}) and C=O (1634 cm^{-1}) groups, and aromatic-ring moieties (1609, 1549, and 1522 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) exhibited signals of two MeO groups (δ(H) 3.81 (*s*) and 3.83 (*s*)), two aromatic *ABX* spin systems (8.04 (*d*, *J* = 8.6), 6.77 (br. *d*, *J* = 8.6), and 7.21 (br. *s*); and 8.40 (*d*, *J* = 8.8), 6.89 (br. *d*, *J* = 8.8), and 7.02 (br. *s*)), two isolated aromatic H-atoms (7.49 (*s*) and 9.49 (*s*)), two aromatic NH groups (11.42 (*s*), 11.96 (*s*)), and an aromatic OH group (10.30 (br. *s*)). The ^{13}C-NMR spectrum (*Table 1*) accounted for all 22 C-atom signals, classified as eight sp^2 CH groups, twelve sp^2 C_q-atoms (including one C=O group), and two MeO groups by DEPT experiment. Comparison of the ^{1}H- and ^{13}C-NMR data of **1** with those of trigonostemine B [3e] revealed that **1** is closely related to trigonostemine B, except for the presence of an additional phenolic OH group (10.30 (br. *s*)) in **1**. HMBCs of the phenolic OH group with C(4) (δ(C) 102.1) and of the aromatic H-atom H–C(4) (δ(H) 7.49) with C(3) (δ(C) 154.8), C(4a) (132.2),

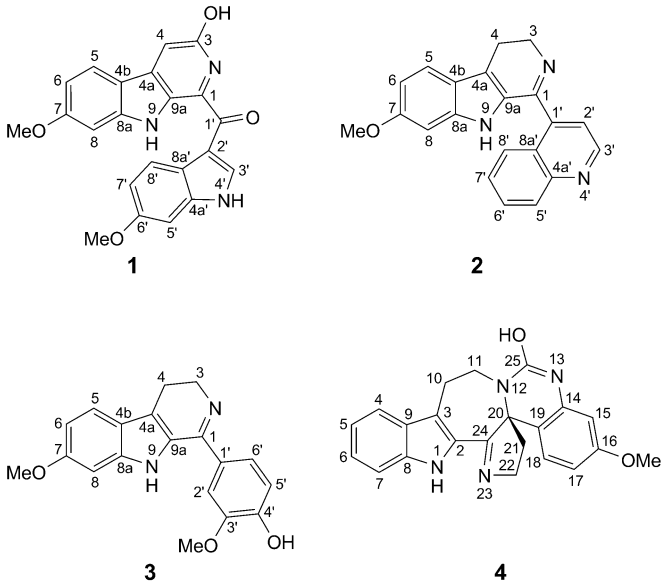

Fig. 1. *Structures of* **1**–**4**

C(4b) (113.5), and C(9a) (132.8) clearly confirmed that **1** is 3-hydroxytrigonostemine B (*Fig. 1*).

Trigonoliimine E (**2**) was isolated as light-yellow solid. Its molecular formula was deduced as $C_{21}H_{17}N_3O$ from HR-ESI-MS (*m*/*z* 328.1443 ($[M+H]^+$, $C_{21}H_{18}N_3O^+$; calc. 328.1449), implying 15 degrees of unsaturation. All 21 C-atoms were resolved in the ^{13}C-NMR and DEPT spectra (*Table 1*) as signals corresponding to nine sp^2 C_q-atoms, nine sp^2 CH groups, two sp^3 CH_2 groups, and one MeO group. The NMR data of **2** (*Table 1*) closely resembled those of trigonostemonine C [3b] except that in **2** a $-CH_2-CH_2-$ unit (δ(C) 49.5, δ(H) 4.08; and δ(C) 20.5, δ(H) 3.07) replaced the $-CH=CH-$ group in the already known metabolite. The HMBCs of CH_2(3) with C(4a) (δ(C) 119.9), C(4) (20.5), and C(1) (160.4), and of CH_2(4) with C(4a), C(9a), and C(3), in combination with a ^{1}H,^{1}H-COSY correlation between CH_2(3) and CH_2(4) evidenced that the two CH_2 groups mentioned above are CH_2(3) and CH_2(4). Thus, the structure of **2** was assigned as depicted in *Fig. 1*.

Trigonoliimine F (**3**) was isolated as red solid. The positive-ion-mode HR-ESI-MS (*m*/*z* 323.1396 ($[M+H]^+$; calc. 323.1395)) provided the molecular formula $C_{19}H_{18}N_2O_3$, indicating twelve degrees of unsaturation. The ^{1}H-NMR spectrum (*Table 1*) indicated the presence of two MeO groups (δ(H) 3.89 and 3.96), two aromatic *ABX* spin systems (7.65 (*d*, *J* = 8.9), 6.88 (*dd*, *J* = 2.1, 8.9), and 6.92 (*d*, *J* = 2.1); and 7.41 (*s*), 7.02 (*d*, *J* = 8.2), and 7.44 (*d*, *J* = 8.2)), and a *N*CH_2CH_2 unit (3.28 (*t*, *J* = 8.0) and 3.94 (*t*, *J* = 8.0)). By comparison of the ^{13}C-NMR data of **3** (δ(C) 162.4 (C(1)), 43.2 (C(3)), 20.1 (C(4)), 128.2 (C(4a)), 121.0 (C(4b)), 123.5 (C(5)), 116.0 (C(6)), 163.1 (C(7)), 94.6 (C(8)), 145.1 (C(8a)), and 125.7 (C(9a))) with those of **2**, **3** should also possess the same moiety of 3,4-dihydro-7-methoxy-β-carboline as **2**, which

Table 1. *^{1}H- and ^{13}C-NMR Data* (400 and 100 MHz, resp.) *of* **1**–**3**. δ in ppm, J in Hz.

Position[a])	**1**		**2**		**3**	
	δ(H)[b])	δ(C)[b])	δ(H)[c])	δ(C)[c])	δ(H)[c])	δ(C)[c])
1		135.9		160.4		162.4
3		154.8	4.08 (*t*, *J* = 8.4)	49.5	3.94 (*t*, *J* = 8.0)	43.2
4	7.49 (*s*)	102.1	3.07 (*t*, *J* = 8.4)	20.5	3.28 (*t*, *J* = 8.0)	20.1
4a		132.2		119.9		128.2
4b		113.5		120.7		121.0
5	8.04 (*d*, *J* = 8.6)	122.9	7.52 (*d*, *J* = 8.8)	121.9	7.65 (*d*, *J* = 8.9)	123.5
6	6.77 (br. *d*, *J* = 8.6)	108.6	6.76 (*dd*, *J* = 8.8, 2.0)	112.9	6.88 (*dd*, *J* = 2.1, 8.9)	116.0
7		160.9		160.4		163.1
8	7.21 (br. *s*)	95.7	6.69 (*d*, *J* = 2.0)	95.1	6.92 (*d*, *J* = 2.1)	94.6
8a		144.9		140.8		145.1
9a		132.8		127.5		125.7
1′		186.6		149.2		120.7
2′		114.5	7.59 (*d*, *J* = 4.4)	121.9	7.41 (*s*)	113.5
3′	9.49 (*s*)	137.4	8.98 (*d*, *J* = 4.4)	151.1		150.4
4′						156.5
4a′		136.8		145.3		
5′	7.02 (br. *s*)	95.3	8.14 (*d*, *J* = 8.8)	129.7	7.02 (*d*, *J* = 8.2)	117.7
6′		156.3	7.80–7.82 (*m*)	131.5	7.44 (*d*, *J* = 8.2)	126.6
7′	6.89 (br. *d*, *J* = 8.8)	111.4	7.56–7.78 (*m*)	128.7		
8′	8.40 (*d*, *J* = 8.8)	122.5	7.79 (*d*, *J* = 8.8)	126.6		
8a′		121.4		127.5		
*Me*O–C(7)	3.83 (*s*)	55.3	3.75 (*s*)	55.8	3.89 (*s*)	56.1
*Me*O–C(3′)					3.96 (*s*)	56.5
*Me*O–C(6′)	3.81 (*s*)	55.3				
H–N(9)	11.42 (*s*)					
H–N(4′)	11.96 (*s*)					
HO–C(3)	10.30 (br. *s*)					

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

displays eight degrees of unsaturation. Moreover, the remaining four degrees of unsaturation should arise from a 1,3,4-trisubstituted phenyl moiety in **3**. Data from 2D-NMR spectra further supported this substitution pattern, as shown in *Fig. 2*. The HMBC cross-peaks of MeO at δ(H) 3.96 with C(3′), H–C(2′)/C(3′), H–C(5′)/C(3′) and C(4′), and H–C(6′)/C(4′) indicated that the MeO and phenolic OH groups were located at C(3′) and at C(4′), respectively, which was further confirmed by the ROESY correlations MeO (δ(H) 3.96)/H–C(2′) and H–C(5′)/H–C(6′). The HMBCs of CH_2(3) with C(1), and H–C(6′) with C(1) indicated that the trisubstituted phenyl moiety and the β-carboline moiety are linked *via* C(1)–C(1′) bond. Thus, the structure of **3** was assigned as shown in *Fig. 1*.

Trigonoliimine G (**4**) was obtained as optically active, light-yellow gum. Its molecular formula was deduced as $C_{22}H_{20}N_4O_2$ from positive-ion HR-ESI-MS (*m*/*z* 373.1672 ([$M + H$]$^+$, $C_{22}H_{21}N_4O_2^+$; calc. 373.1665)); it contained one more O-atom than trigonoliimine A [3c]. The 1D-NMR data of both compounds (*Table 2*) matched closely, except that one sp^2 C_q-atom (C(25) at δ(C) 157.0)) in **4** replaced one sp^2 CH

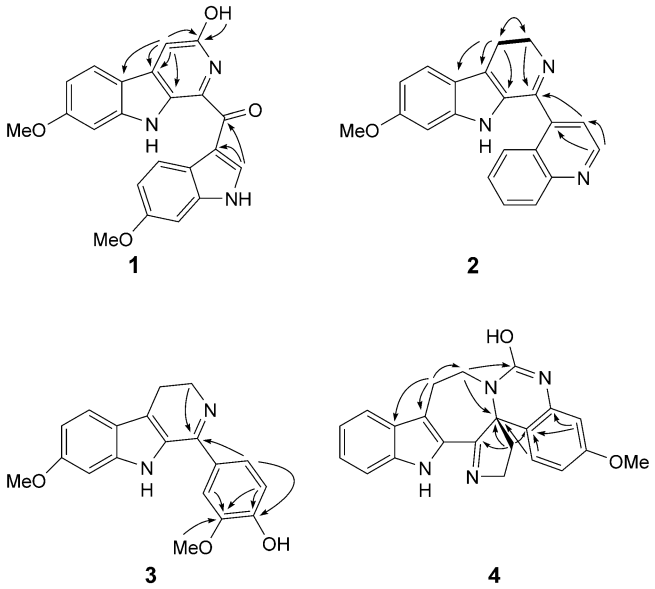

Fig. 2. *Selected* $^{1}H,^{1}H$*-COSY* (—) *and HMB* (H → C) *correlations of* **1**–**4**

Table 2. ^{1}H*- and* ^{13}C*-NMR Data* (500 and 125 MHz, resp.) *of* **4**. δ in ppm, J in Hz.

Position[a])	δ(H)[b])	δ(C)[b])	Position[a])	δ(H)[b])	δ(C)[b])
2		125.5	15	6.67 (*d*, *J* = 2.5)	107.9
3		116.0	16		159.7
4	7.50 (*d*, *J* = 8.0)	117.7	17	7.42 (*d*, *J* = 8.0)	112.5
5	7.07 (*t*, *J* = 8.0)	118.5	18	6.71 (*d*, *J* = 8.5)	122.8
6	7.24 (*t*, *J* = 8.0)	122.9	19		112.9
7	7.42 (*d*, *J* = 8.0)	112.7	20		78.7
8		136.5	21	2.42–2.45 (*m*), 2.36–2.39 (*m*)	38.9
9		126.4	22	4.22–4.26 (*m*), 3.66–3.69 (*m*)	55.1
10	3.18–3.21 (*m*)	26.7	24		167.1
11	4.39–4.42 (*m*), 3.86–3.89 (*m*)	45.0	25		157.0
14		139.1	MeO	3.75 (*s*)	53.8

[a]) Atom numbering as indicated in *Fig. 1.* [b]) Recorded in $CDCl_3/CD_3OD$ 3 : 1.

group in the latter, evidencing that **4** had the same skeleton as trigonoliimine A, but with one additional OH group. The OH group should be at C(25), which was confirmed by the HMBC of H–C(11a) with C(25). Thus, the gross planar structure of trigonoliimine G (**4**) was established as shown. Considering the low optical rotation values ($[\alpha]_D^{10} = +7.2$), **4** should have been isolated with a low level of (*R*)-enantiomeric excess [5].

All isolated compounds were evaluated for their cytotoxicities against the acute myelogenous leukemia (HL-60) or human lung cancer (A-549) cell lines; however, none of them exhibited a significant bioactivity ($IC_{50} > 40$ μM).

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Experimental Part

General. Column chromatography (CC): silica gel (SiO_2; 200–300 mesh, 10–40 μm; *Qingdao Haiyang Chemical Factory*, P. R. China), C_{18} reversed-phase (RP) SiO_2 (40–63 μm; *Daiso Co.*, Japan), and *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Sweden). HPLC: *Agilent-1100* series; *Zorbax SB-C18* (250 × 9.4 mm); DAD detector. Optical rotations: *Horiba SEPA-300* polarimeter or *JASCO DIP-370* digital polarimeter. UV Spectra: *Shimadzu UV-2401PC* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Bio-Rad FTS-135* spectrometer; KBr pellets; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR spectra: *DRX-500 NMR* or *Bruker AM-400* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS and HR-ESI-MS: *VG Auto Spec 3000* spectrometer; in m/z.

Plant Material. The whole leaves of *T. lii*, collected in Xishuangbanna of Yunnan Province, P. R. China, in October 2010, were identified by Prof. *Jing-Yun Cui*, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences.

Extraction and Isolation. 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 H_2O and then partitioned with petroleum ether (PE), AcOEt, and BuOH, successively. The AcOEt fraction (100 g) was submitted to CC (SiO_2; $CHCl_3$/MeOH 1:0–0:1), from which one fraction eluted with $CHCl_3$/MeOH 10:1 was further submitted to CC (SiO_2; $CHCl_3$/MeOH 15:1; and *RP-18*; 70% MeOH) to afford compounds **1** (30 mg), **2** (18 mg), and **3** (17 mg). The BuOH-soluble fraction (250 g) was subjected to CC (*RP-18*; 10–100% MeOH). The 60%-MeOH fraction was further subjected to CC (*Sephadex LH-20*), followed by semi-prep. HPLC (MeOH/H_2O (containing 0.1% Et_2NH) 60:40) to give **4** (2.0 mg).

Trigonoliimine D (=*(3-Hydroxy-7-methoxy-9*H-*β-carbolin-1-yl)(6-methoxy-1*H-*indol-3-yl)methanone*; **1**). Light-yellow gum. $[\alpha]_D^{19} = 0$ ($c = 0.30$, MeOH). UV (MeOH): 214 (4.3), 295 (3.9), 417 (3.7). IR: 3426, 2961, 1634, 1609, 1549, 1522, 1438. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 388.1281 ($[M+H]^+$, $C_{22}H_{18}N_3O_4^+$; calc. 388.1297).

Trigonoliimine E (=*4,9-Dihydro-7-methoxy-1-(quinolin-4-yl)-3*H-*β-carboline*; **2**). Light-yellow solid. $[\alpha]_D^{19} = 0$ ($c = 0.38$, MeOH). UV (MeOH): 206 (4.3), 319 (3.7), 330 (3.7). IR: 3421, 1600, 1549, 1522. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 328.1443 ($[M+H]^+$, $C_{21}H_{18}N_3O^+$; calc. 328.1449).

Trigonoliimine F (=*4-(4,9-Dihydro-7-methoxy-3*H-*β-carbolin-1-yl)-2-methoxyphenol*; **3**). Red solid. $[\alpha]_D^{19} = 0$ ($c = 0.34$, MeOH). UV (MeOH): 206 (3.7), 253 (3.2), 358 (3.1), 448 (3.3). IR: 3418, 1598, 1530, 1520. ^{1}H- and ^{13}C-NMR: see *Table 1*. HR-ESI-MS: 323.1396 ($[M+H]^+$, $C_{19}H_{19}N_2O_3^+$; calc. 323.1395).

Trigonoliimine G (=*(17a*R*)-9,14,16,17-Tetrahydro-3-methoxy-8*H-*indolo[2′,3′:4,5]pyrrolo-[3′,2′:2,3]azepino[1,2-*c*]quinazolin-6-ol*; **4**). Light-yellow gum. $[\alpha]_D^{10} = +7.2$ ($c = 0.14$, MeOH). UV (MeOH): 210 (4.5), 315 (4.2). IR: 3441, 2956, 2930, 1619, 1452. ^{1}H- and ^{13}C-NMR: see *Table 2*. HR-ESI-MS: 373.1672 ($[M+H]^+$, $C_{22}H_{21}N_4O_2^+$; calc. 373.1665).

MTT Cytotoxicity Assay. Human myeloid leukemia HL-60 and lung cancer A-549 cells were cultured in RPMI-1640 containing 10% fetal bovine serum, 100 U ml^{-1} penicillin, and 100 μg ml^{-1} streptomycin sulfate. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay. Cells were maintained at 37° in air with 5% CO_2. Hundred μl of adherent cells were seeded at the initial density of 1 × 105 cells/ml in 96-well tissue culture plates. After incubation for 24 h at 37°, each tumor cancer cell line was exposed to the test compounds dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8.0, and 40 μM in triplicates for 48 h, with cisplatin as positive control. After compounds treatment, cell viability was detected, and the cell growth curve was plotted. The IC_{50} values (concentration in μM required to inhibit cell viability by 50%) were calculated by the method of *Reed* and *Muench* [6].

REFERENCES

[1] S. K. Chen, B. Y. Chen, H. Li, in 'Flora of China (*Zhongguo Zhiwu Zhi*)', Science Press, Beijing, 1997, Vol. 44(2), p. 162.

[2] H. Jayasuriya, D. L. Zink, S. B. Singh, R. P. Borris, W. Nanakorn, H. T. Beck, M. J. Balick, M. A. Goetz, L. Slayton, L. Gregory, M. Zakson-Aiken, W. Shoop, S. B. Singh, *J. Am. Chem. Soc.* **2000**, *122*, 4998; H. Jayasuriya, D. L. Zink, R. P. Borris, W. Nanakorn, H. T. Beck, M. J. Balick, M. A. Goetz, L. Gregory, W. L. Shoop, S. B. Singh, *J. Nat. Prod.* **2004**, *67*, 228; N. Soonthornchareonnon, M. Sakayarojkul, M. Isaka, V. Mahakittikun, W. Chuakul, P. Wongsinkongman, *Chem. Pharm. Bull.* **2005**, *53*, 241; A. Tempeam, N. Thasana, C. Pavaro, W. Chuakul, P. Siripong, S. Ruchirawat, *Chem. Pharm. Bull.* **2005**, *53*, 1321; H.-D. Chen, X.-F. He, J. Ai, M.-Y. Geng, J.-M. Yue, *Org. Lett.* **2009**, *11*, 4080; L. Zhang, R.-H. Luo, F. Wang, M.-Y. Jiang, Z.-J. Dong, L.-M. Yang, Y.-T. Zheng, J.-K. Liu, *Org. Lett.* **2010**, *12*, 152; S.-F. Li, Y.-T. Di, S.-L. Li, Y. Zhang, F.-M. Yang, Q.-Y. Sun, J. M. Simo, H.-P. He, X.-J. Hao, *J. Nat. Prod.* **2011**, *74*, 464.

[3] a) T. Kanchanapoom, R. Kasai, P. Chumsri, K. Kraisintu, K. Yamasaki, *Tetrahedron Lett.* **2002**, *43*, 2941; b) X.-J. Hu, Y.-T. Di, Y.-H. Wang, L.-Y. Kong, S. Gao, C.-S. Li, H.-Y. Liu, H.-P. He, J. Ding, H. Xie, X.-J. Hao, *Planta Med*. **2009**, *75*, 1157; c) C.-J. Tan, Y.-T. Di, Y.-H. Wang, Y. Zhang, Y.-K. Si, Q. Zhang, S. Gao, X.-J. Hu, X. Fang, S.-F. Li, X.-J. Hao, *Org. Lett.* **2010**, *12*, 2370; d) S.-F. Li, Y.-T. Di, H.-P. He, Y. Zhang, Y.-H. Wang, J.-L. Yin, C.-J. Tan, S.-L. Li, X.-J. Hao, *Tetrahedron Lett.* **2011**, *52*, 3186; e) S.-F. Li, Y. Zhang, Y. Li, X.-R. Li, L.-M. Kong, C.-J. Tan, S.-L. Li, Y.-T. Di, H.-P. He, X.-J. Hao, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 2296.

[4] U. Kokpol, S. Thebpatiphat, S. Boonyaratavej, V. Chedchuskulcai, C.-Z. Ni, J. Clardy, C. Chaichantipyuth, V. Chittawong, D. H. Miles, *J. Nat. Prod.* **1990**, *53*, 1148; S. Yin, Z.-S. Su, Z.-W. Zhou, L. Dong, J.-M. Yue, *J. Nat. Prod.* **2008**, *71*, 1414; X.-J. Hu, Y.-H. Wang, L.-Y. Kong, H.-P. He, S. Gao, H.-Y. Liu, J. Ding, H. Xie, Y.-T. Di, X.-J. Hao, *Tetrahedron Lett.* **2009**, *50*, 2917; H.-D. Chen, S.-P. Yang, X.-F. He, H.-B. Liu, J. Ding, J.-M. Yue, *Tetrahedron* **2010**, *66*, 5065; Q. Zhu, C.-P. Tang, C.-Q. Ke, X.-Q. Li, J. Liu, L.-S. Gan, H.-C. Weiss, E.-R. Gesing, Y. Ye, *J. Nat. Prod.* **2010**, *73*, 40.

[5] X.-B. Qi, H.-L. Bao, U. K. Tambar, *J. Am. Chem. Soc.* **2011**, *133*, 10050; S. Han, M. Movassaghi, *J. Am. Chem. Soc.* **2011**, *133*, 10768; S. Liu, X.-J. Hao, *Tetrahedron Lett.* **2011**, *52*, 5640; J. Qiu, J.-X. Zhang, S. Liu, X.-J. Hao, *Tetrahedron Lett.* **2013**, *54*, 300; B. Zhao, X.-Y. Hao, J.-X. Zhang, S. Liu, X.-J. Hao, *Org. Lett.* **2013**, *15*, 528.

[6] L. J. Reed, H. Muench, *Am. J. Hyg.* **1938**, *27*, 493.

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