



New antiplasmodial indole alkaloids from *Hunteria zeylanica*

Alfarius E. Nugroho^a, Masatomo Sugai^a, Yusuke Hirasawa^a, Takahiro Hosoya^a, Khalijah Awang^b, A. Hamid A. Hadi^b, Wiwied Ekasari^c, Aty Widyawaruyanti^c, Hiroshi Morita^{a,*}

^a Faculty of Pharmaceutical Sciences, Hoshi University, Ebara 2-4-41 Shinagawa-ku, Tokyo 142-8501, Japan

^b Department of Chemistry, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

^c Faculty of Pharmacy, Airlangga University, Jalan Dharmawangsa Dalam, Surabaya 60286, Indonesia

ARTICLE INFO

Article history:

Received 2 March 2011

Revised 24 March 2011

Accepted 29 March 2011

Available online 5 April 2011

Keywords:

Indole alkaloid

Bisnicalaterine D

Nicalaterine A

Hunteria zeylanica

Antiplasmodial activity

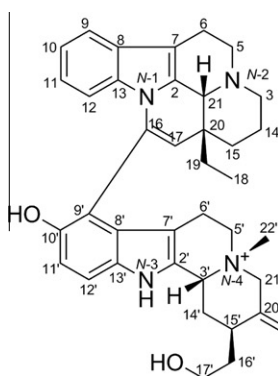
ABSTRACT

Two new indole alkaloids, bisnicalaterine D (**1**), consisting of an eburnane and a corynanthe type of skeletons, and nicalaterine A (**2**) were isolated from the bark of *Hunteria zeylanica*. Their structures were elucidated by various spectroscopic data such as NMR and CD spectra. A series of bisnicalaterines and nicalaterine A showed potent antiplasmodial activity against *Plasmodium falciparum* 3D7.

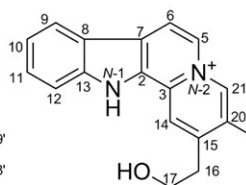
© 2011 Elsevier Ltd. All rights reserved.

In our search for new bioactive alkaloids from tropical plants in Malaysia and Indonesia, we have previously reported a series of cassiarins with potent antiplasmodial activity.^{1,2} *Hunteria zeylanica* (Retz.) Gardner ex Thwaites is a member of the Apocynaceae family in Malaysia, found mostly in Pahang and Selangor,³ and the bark and leaves have been known to produce various skeletal alkaloids depending on the area where the plants were distributed.^{4–9} In our

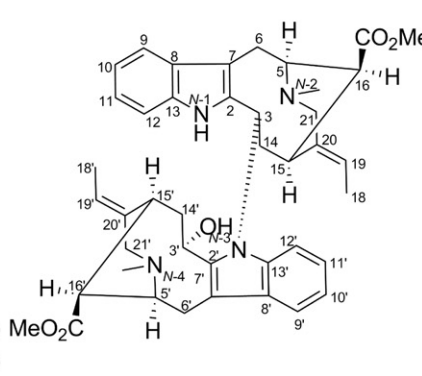
previous paper,^{10,11} we have reported the isolation of new bisindole alkaloids, bisnicalaterines A–C (**3–5**) from *H. zeylanica*. In this Letter, we report the isolation and structure elucidation of bisnicalaterine D (**1**), a new bisindole alkaloid consisting of an eburnane and a corynanthe type of skeletons, and nicalaterine A (**2**) as well as the antimalarial activity of **1–5** against *Plasmodium falciparum* 3D7.



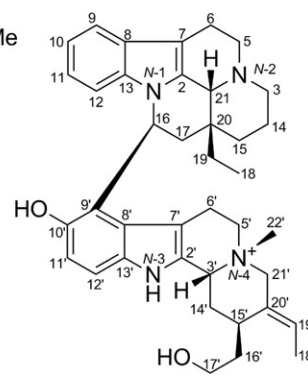
bisnicalaterine D (**1**:S_a)



nicalaterine A (**2**)



bisnicalaterine A (**3**:R_a)



bisnicalaterine B (**4**:S_a)
bisnicalaterine C (**5**:R_a)

* Corresponding author.

E-mail address: moritah@hoshi.ac.jp (H. Morita).

The bark of *H. zeylanica*¹² was extracted with MeOH, and part (33 g) of the extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na₂CO₃ (aq) to pH 10 and extracted with CHCl₃ and *n*-BuOH subsequently. The *n*-BuOH fraction was subjected to an HP-20 column (H₂O/MeOH 0:1 to 1:0), and the 80% MeOH fraction (3.5 g) was further separated by using a Sephadex LH-20 column. Fractions containing **1** was then separated by a silica gel column (CHCl₃/MeOH, 9:1 to 0:1), followed by an ODS Sep-Pak (MeOH/H₂O 1:9 to 1:0) to give bisnicalaterine D (**1**, 8.1 mg, 0.003%), while purification of fractions containing **2** by an amino silica gel column (CHCl₃/MeOH, 9:1 to 0:1) and a silica gel column (CHCl₃/MeOH, 8:2) yielded nicalaterine A (**2**, 2.0 mg, 0.0008%).

Bisnicalaterine D (**1**),¹³ a yellowish amorphous solid, [α]_D²⁰ –72 (c 1.0, MeOH), showed a molecular formula, C₃₉H₄₇N₄O₂, which was determined by HRESIMS [*m/z* 603.3689 (M)⁺, Δ –0.5 mmu]. IR absorption band (3430 cm^{–1}) was characteristic of amino or

hydroxyl group. ¹H and ¹³C NMR data (Table 1) suggested the presence of one sp³ quaternary carbon, 12 sp³ methylenes, three sp³ methines, three methyls, eight sp² methines, and 12 sp² quaternary carbons. The ¹³C NMR spectrum of **1** is very similar to those of bisnicalaterines B (**4**).¹¹ Compared to spectroscopic data of **4**, **1** has two less sp³ carbons, two additional sp² carbons (δ_c 131.9 and δ_c 117.7) and also 2 amu smaller which suggests the existence of an additional double bond in **1**. The existence of a double bond between C-16 and C-17 was confirmed by the HMBC correlations of H-17 to C-16, C-19, C-20, C-21 and C-9', H₃-18 to C-20, and H-21 to C-15. Further analysis of the two-dimensional NMR data (¹H–¹H COSY, HMQC, and HMBC spectra) revealed the gross structure of **1** as shown in Figure 1.

The stereochemistry of each monoterpeneindole unit in **1** was assigned by NOESY correlations as shown in computer-generated 3D drawing (Fig. 2). In unit A, the NOESY correlations of H₂-19/H-21 suggested that H-21 and an ethyl group (C-18–C-19) were β -oriented. While in unit B, the NOESY correlations of H-3'/H₃-22', H-21'b, and H₂-16', H₃-22'/H-21'b and H-21'b/H-16'b suggested that H-3', C-16' and C-22' were β -oriented, while the correlations of H-19'/H-21'a and H-15'/H₃-18' established the *E*-configuration of the ethylidene side chain. Thus the relative stereochemistry of units A and B was assigned as shown in Figure 2.

In the case of bisnicalaterines B (**4**) and C (**5**), there were two possible conformations around C-16–C-9' bond, the twisted and extended conformations.¹¹ In bisnicalaterine D (**1**), the NOESY correlation of H-6'b/H-21 and the highly shifted chemical shift (δ_H 1.53 and 2.50, respectively) of H-6'a and H₃-22' at ammonium nitrogen atom suggested that **1** possessed the twisted conformation observed in bisnicalaterine B (**4**).¹¹

The absolute structure of **1** was deduced by comparing its CD spectrum to that of bisnicalaterine B (**4**). The CD spectrum¹³ of **1** showed a similar CD pattern to that of **4**,¹¹ thus the absolute structure of **1** was determined to be of 20*R*, 21*R*, 4'*R*, 3'*R*, 15'*R*.

Nicalaterine A (**2**),¹⁴ a yellowish amorphous solid, showed a molecular formula, C₁₉H₁₉N₂O, which was determined by HRESIMS [*m/z* 291.1496 (M)⁺, Δ –0.1 mmu]. IR absorption band (3430 cm^{–1}) was characteristic of amino or hydroxyl group. The UV spectrum suggested the presence of a highly conjugated ring system [λ_{max} 386 (ϵ 8000), 346 (8300), 291 (6900), 240 (16,100), and 235 (sh, 15,000)] as in flavopereirine.¹⁵ ¹H and ¹³C NMR data (Table 1) suggested the presence of three sp³ methylenes, one methyl, eight sp²

Table 1
¹H and ¹³C NMR data of bisnicalaterine D (**1**) and nicalaterine A (**2**) in CD₃OD at 300 K^a

Position	1	2
	[δ_H (J, Hz)]	[δ_c]
2		125.3
3a	3.37 (1H, m)	46.7
3b	3.37 (1H, m)	132.4
5a	3.87 (1H, m)	53.1
5b	3.97 (1H, m)	8.69 (1H, br s)
6a	3.13 (1H, m)	16.9
6b	3.30 (1H, m)	8.44 (1H, br s)
7		108.2
8		128.7
9	7.48 (1H, d, 7.6)	119.7
10	6.97 (1H, m)	121.7
11	6.69 (1H, t, 8.3)	124.2
12	5.83 (1H, d, 8.3)	113.4
13		136.2
14a	1.83 (1H, m)	19.4
14b	1.98 (1H, m)	8.58 (1H, s)
15a	1.68 (1H, m)	29.1
15b	1.86 (1H, m)	151.1
16		131.9
17	5.26 (1H, s)	117.7
18	1.11 (3H, t, 7.6)	8.8
19a	1.85 (1H, m)	27.9
19b	2.00 (1H, m)	2.98 (2H, q, 7.2)
20		39.2
21	5.22 (1H, s)	59.1
2'		131.3
3'	4.68 (1H, dd, 11.0, 3.5)	62.8
5a'	2.93 (1H, m)	52.2
5b'	3.76 (1H, m)	
6a'	1.53 (1H, m)	18.7
6b'	2.93 (1H, m)	
7'		102.6
8'		126.8
9'		112.1
10'		150.9
11'	6.96 (1H, m)	114.3
12'	7.39 (1H, d, 8.2)	114.8
13'		133.0
14a'	2.16 (1H, m)	36.1
14b'	2.25 (1H, m)	
15'	3.16 (1H, m)	30.4
16a'	1.86 (1H, m)	34.5
16b'	1.96 (1H, m)	
17a'	3.51 (1H, m)	60.1
17b'	3.62 (1H, m)	
18'	1.73 (3H, d, 6.9)	13.3
19'	5.79 (1H, q, 6.9)	132.1
20'		129.2
21a'	3.66 (1H, m)	68.1
21b'	4.18 (1H, br d, 13.8)	
22'	2.50 (3H, s)	48.6

^a δ In ppm.

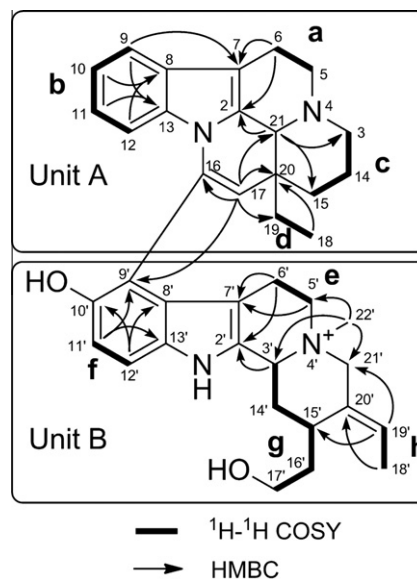


Figure 1. Selected 2D NMR correlations for bisnicalaterine D (**1**).

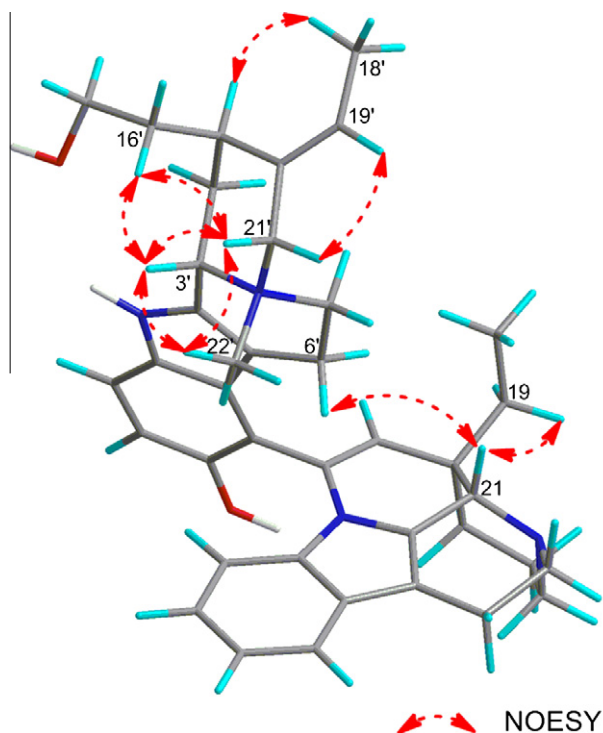


Figure 2. Selected NOESY correlations for bisnicalaterine D (1).

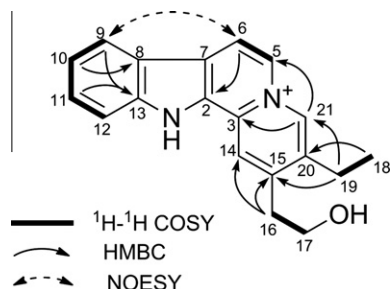


Figure 3. Selected 2D NMR correlations for nicalaterine A (2).

Table 2

Antiplasmodial activity of 1–5 against *P. falciparum* 3D7

	Antiplasmodial activity (IC ₅₀ , μM)	Cytotoxic activity ^a (IC ₅₀ , μM)	SI
1	>50	>50	—
2	0.11	>50	>450
3	4.36	16.2	3.7
4	1.13	>50	>44
5	0.05	>50	>1000

^a Against HL-60.

methines, and seven sp² quaternary carbons. The HMBC correlations of H₂–19 to C–15 and C–21, H₃–18 to C–20, and H₂–16 to C–15 and C–14 allowed the attachment of the ethyl side chain (C–18 and C–19) to C–20 and the 2-hydroxyethyl side chain to C–15. Further analysis of the two-dimensional NMR data (¹H–¹H COSY, HSQC, and HMBC spectra in CD₃OD) revealed the gross structure of **2** as shown in Figure 3.

Antimalarial activity^{16–18} for 1–5 against *P. falciparum* 3D7 was evaluated (Table 2). Nicalaterine A (**2**) and bisnicalaterine C (**5**)

showed potent antimalarial activity (IC₅₀ 0.11 and 0.05 μM, respectively) with a good selectivity (SI >450 and >1000, respectively). Bisnicalaterine C (**5**) with an extended conformation showed 20 times more effective than that of bisnicalaterine B (**4**) with a twisted conformation. On the other hand, bisnicalaterine D (**1**), which also possessed a twisted conformation, showed practically no antimalarial activity. It is interesting to note that the conformation around the C–16 – C–9' bond may play important roles to show antimalarial activity.

Acknowledgments

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, and a grant from the Open Research Center Project, and Takeda Science Foundation.

References and notes

- Morita, H.; Oshimi, S.; Hirasawa, Y.; Koyama, K.; Honda, T.; Ekasari, W.; Indrayanto, G.; Zaini, N. C. *Org. Lett.* **2007**, 9, 3691.
- Oshimi, S.; Deguchi, J.; Hirasawa, Y.; Ekasari, W.; Widyawaruyanti, A.; Wahyuni, T. S.; Zaini, N. C.; Shiota, O.; Morita, H. *J. Nat. Prod.* **2009**, 72, 1899.
- Whitmore, T. C. In *Tree Flora of Malaya*; Whitmore, T. C., Ed.; Academic Press: London, 1972; 2, p 3.
- Lavaud, C.; Massiot, G.; Vercauteren, J.; Le Menoliev, L. *Phytochemistry* **1982**, 21, 445.
- Subhadhirasakul, S.; Aimi, N.; Takayama, H.; Ponglux, D.; Sakai, S. *Chem. Pharm. Bull.* **1994**, 42, 991.
- Subhadhirasakul, S.; Takayama, H.; Miyabe, Y.; Kitajima, M.; Ponglux, D.; Sakai, S.; Aimi, N. *Heterocycles* **1995**, 41, 2049.
- Subhadhirasakul, S.; Takayama, H.; Miyabe, Y.; Aimi, N.; Ponglux, D.; Sakai, S. *Chem. Pharm. Bull.* **1994**, 42, 2645.
- Takayama, H.; Subhadhirasakul, S.; Mizuki, J.; Kitajima, M.; Aimi, N.; Ponglux, D.; Sakai, S. *Chem. Pharm. Bull.* **1994**, 42, 1957.
- Takayama, H.; Subhadhirasakul, S.; Ohmori, O.; Kitajima, M.; Ponglux, D.; Aimi, N. *Heterocycles* **1998**, 47, 87.
- Nugroho, A. E.; Hirasawa, Y.; Kawahara, N.; Goda, Y.; Awang, K.; Hadi, A. H. A.; Morita, H. *J. Nat. Prod.* **2009**, 72, 1502.
- Hirasawa, Y.; Hara, M.; Nugroho, A. E.; Sugai, M.; Zaima, K.; Kawahara, N.; Goda, Y.; Awang, K.; Hadi, A. H. A.; Litaudon, M.; Morita, H. *J. Org. Chem.* **2010**, 75, 4218.
- Bark of *H. zeylanica* were collected in Kampung Padang, Malaysia, in 1994. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. A voucher specimen (Herbarium No. KL 4345) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.
- Bisnicalaterine D, yellowish amorphous solid, [α]_D²⁰ –72 (c 1.0, MeOH), UV (MeOH) λ_{max} 217 (ε 16800), 249 (6100), 277.5 (5300), 302 (4100); CD (MeOH) λ_{max} 205 (Δε 5.95), 218 (–6.09), 243 (6.54), 280 (–4.22), 365 (3.07); IR (KBr) ν_{max} 3430 cm^{–1}; ¹H and ¹³C NMR data see Table 1; EI-MS m/z 603 M⁺; HRESIMS [m/z 603.3689 (M)⁺, calcd for C₃₉H₄₇N₄O₂, 603.3694].
- Nicalaterine A (**2**), a yellowish amorphous solid, UV (MeOH) λ_{max}, 235 (sh, 15,000), 240 (16,100), 291 (6900), 346 (8300), and 386 (ε 8000); IR (KBr) ν_{max} 3430 cm^{–1}; ¹H and ¹³C NMR data see Table 1; EI-MS m/z 291 M⁺; HRESIMS [m/z 291.1496 (M)⁺, calcd for C₁₉H₁₉N₂O, 291.1497].
- Bejar, O.; Goutarel, R.; Junot, M. M.; Le Hir, A. *Compt. Rend.* **1957**, 244, 2066.
- Human malaria parasites were cultured according to the method of Trager and Jensen.¹⁷ The antimalarial activity of the isolated compounds was determined by the procedure described by Budimulja et al.¹⁸ In brief, stock solutions of the samples were prepared in DMSO (final DMSO concentrations of <0.5%) and were diluted to the required concentration with complete medium (RPMI 1640 supplemented with 10% human plasma, 25 mM HEPES and 25 mM NaHCO₃) until the final concentrations of samples in culture plate wells were 10; 1; 0.1; 0.01; 0.001 μ/mL. The malarial parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plates. Growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain. The antimalarial activity of each compound was expressed as an IC₅₀ value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control. The percentage of growth inhibition was expressed according to following equation: Growth inhibition % = 100 – [(test parasitaemia/control parasitemia) × 100]. Chloroquine: IC₅₀ 0.011 μM.
- Trager, W.; Jensen, J. B. *Science* **1976**, 193, 673.
- Budimulja, A. S.; Syafruddin, T. P.; Wilariat, P.; Marzuki, S. *Mol. Biochem. Parasitol.* **1997**, 84, 137.