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HUNCANITERINE A, A NEW BISINDOLE ALKALOID FROM *HUNTERIA ZEYLANICA*

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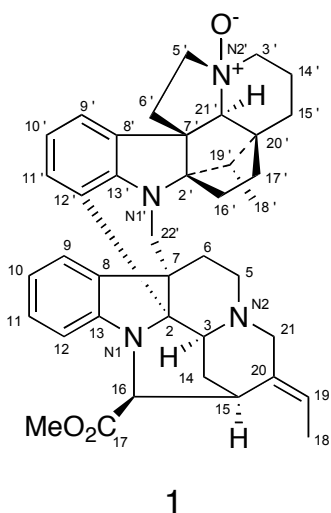
Abstract – A new bisindole alkaloid, huncaniterine A (**1**) consisting of corynantheine-type and aspidospermane-type skeletons, has been isolated from the bark of *Hunteria zeylanica* (Apocynaceae) and the structure was elucidated on the basis of spectroscopic data. Huncaniterine A (**1**) exhibited a vasorelaxant activity on isolated rat aorta ring.

INTRODUCTION

Hunteria zeylanica is a member of the Apocynaceae family in Malaysia, found mostly in Pahang and Selangor.¹ Traditionally the latex has been used for smearing on the sores caused by yaws.² The bark and leaves of *Hunteria zeylanica* have been known to produce various alkaloids depending on the area where the plants were distributed.³ Some pharmacological actions such as antinociceptive, antipyretic, and anti-inflammatory actions have also been reported recently.⁴ In our search for structurally and biogenetically interesting alkaloids from tropical plants in Malaysia, huncaniterine A (**1**), a new bisindole alkaloid with an N-oxide moiety consisting of corynantheine-type and aspidosperma-type skeletons, has been isolated from the bark of *Hunteria zeylanica* collected in Kampung Padang, Malaysia. In this paper we describe the isolation and structure elucidation of **1**, which exhibited a moderate vasorelaxant activity on isolated rat aorta ring.

The bark of *Hunteria zeylanica* was extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with sat. Na₂CO₃,

were extracted with CHCl_3 . CHCl_3 -soluble materials were subjected to an LH-20 column ($\text{CHCl}_3/\text{MeOH}$, 1:1) followed by an amino silica gel column (hexane/EtOAc, 1:0 $\rightarrow$ 0:1 and then $\text{CHCl}_3/\text{MeOH}$, 1:0 $\rightarrow$ 0:1) to give huncaniterine A (**1**, 55 mg, 0.006% yield) together with a known alkaloid, 19'S-pleiomutinine.⁵



RESULTS AND DISCUSSION

Huncaniterine A (**1**) showed the pseudomolecular ion peak at m/z 631 ($\text{M}+\text{H}$)⁺ in the FABMS, and the molecular formula, $\text{C}_{40}\text{H}_{46}\text{N}_4\text{O}_3$, was established by HRFABMS [m/z 631.3616, ($\text{M}+\text{H}$)⁺, Δ -3.2 mmu]. IR absorptions implied the presence of an ester carbonyl (1750 cm^{-1}) functionality. Analysis of the ^1H and ^{13}C NMR data (Table 1) and the HMQC spectrum of **1** revealed the presence of five sp^3 quaternary carbons, five sp^3 methines, twelve sp^3 methylenes, three methyls, eight sp^2 methines, and seven sp^2 quaternary carbons. Among them, four sp^3 methylenes (δ_{C} 48.1; δ_{H} 2.87 and 3.17; δ_{C} 53.7; δ_{H} 3.07 and 4.48; δ_{C} 65.3; δ_{H} 3.92; δ_{C} 68.8; δ_{H} 3.79 and 4.09), three sp^3 methines (δ_{C} 54.0; δ_{H} 3.13; δ_{C} 87.7; δ_{H} 3.92; δ_{C} 57.2; δ_{H} 5.06), and four quaternary carbons (δ_{C} 67.3, 79.6, 146.9, and 148.4) were ascribed to those bearing a nitrogen atom.

The gross structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the ^1H - ^1H COSY, HOHAHA, HSQC, HSQC-HOHAHA, and HMBC spectra in CDCl_3 (Figure 1). The ^1H - ^1H COSY, HOHAHA, and HSQC-HOHAHA spectra revealed connectivities of nine partial structures **a** (C-5 ~ C-6), **b** (C-3, C-14 ~ C-16, C-20 ~ C-21), **c** (C-18 ~ C-19), **d** (C-9 ~ C-12), **e** (C-5' ~ C-6'), **f** (C-3', C-14' ~ C-15'), **g** (C-16' ~ C-17'), **h** (C-18' ~ C-19'), and **i** (C-9' ~ C-11') as shown in Figure 1. These partial structures were classed into two units A and B.

In unit A, the connectivity of partial structures **a** and **b** revealed by the ^1H - ^1H COSY and HOHAHA spectra were analyzed by the HMBC spectrum. HMBC correlations from H_2 -5 to C-3 (δ_{C} 54.0), H-3 to C-21 (δ_{C} 53.7), and H_2 -21 to C-5 (δ_{C} 48.1) established the connections among C-3, C-5, and C-21 through

a nitrogen atom (N2). HMBC cross peaks of H-19 to C-21 and H₃-18 to C-20 (δ_{C} 136.8) indicated the presence of ethylidene group at C-20, constructing the connectivity of partial structures **b** and **c** in unit A. Another partial structure **d** and the presence of methyl carbonate at C-16 were analyzed by the HMBC correlations as shown in Figure 1. These data suggested that unit A possessed corynantheine-type skeleton such as dihydropleiocarpamine.⁶ In unit B, connectivities of partial structures **e**, **f**, **g**, **h**, and **i** were deduced from long-range ¹H-¹³C correlations (Figure 1) showing that part B possessed an aspidospermane-type indole alkaloid framework. HMBC cross-peaks of H₂-6' to C-2' (δ_{C} 79.6) and C-21' (δ_{C} 87.7), H-21' to C-7' (δ_{C} 57.2), and H-19' to C-2' (δ_{C} 79.6), C-7', and C-16' (δ_{C} 21.8) revealed the connectivity among partial structures **e**, **g**, and **h** through C-7' and C-21'. In addition, HMBC cross peaks of H₂-15' to C-17' (δ_{C} 32.9), H-21' to C-15' (δ_{C} 25.5), H-19' to C-17' and C-20' (δ_{C} 45.3) suggested the linkage among partial structures **f**, **g**, and **h** through C-20'. Detailed analyses of the ¹³C chemical shifts (δ 65.3, 68.8, and 87.7, respectively) of C-3', C-5', and C-21' indicated the presence of an N-oxide functionality in unit B. These data implied unit B possessed an aspidospermane-type skeleton such as tubuxenine.⁷ Finally, the linkage between units A and B through the C-N methylene bridge (C-22') and that of C-2 to C-12' were provided by HMBC correlations of H₂-22' to C-2 (δ_{C} 67.3), C-7 (δ_{C} 46.1), C-8 (δ_{C} 135.2), and C-13' (δ_{C} 148.4), and H-11' to C-2, respectively. Thus, the gross structure of huncaniterine A (**1**) was assigned as shown in Figure 1.

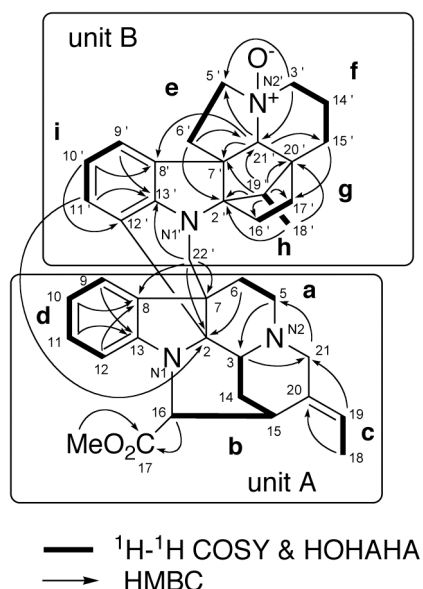


Figure 1. Selected 2D NMR correlations for huncaniterine A (**1**).

The relative stereochemistry of each monoterpeneindole unit in **1** was assigned by NOESY correlations as shown in computer-generated 3D drawing (Figure 2). The NOESY correlations of H-6 and H-19 to H-21, and H-15 to H₃-18 in unit A and H-21' to H-9' and H₃-18', and H-5' to H-14' and H-17' in unit B

suggested that these hydrogens have the same spatial orientation. Furthermore, cross-peaks between H-22' and H-6, H-9, and H-16' confirmed the stereochemistry of the dimeric linkage with the N-oxide moiety as shown in Figure 2. The structure of **1** was also confirmed by chemical conversion to 19'S-pleiomutinine by use of sodium hydrogen sulfite in methanol.

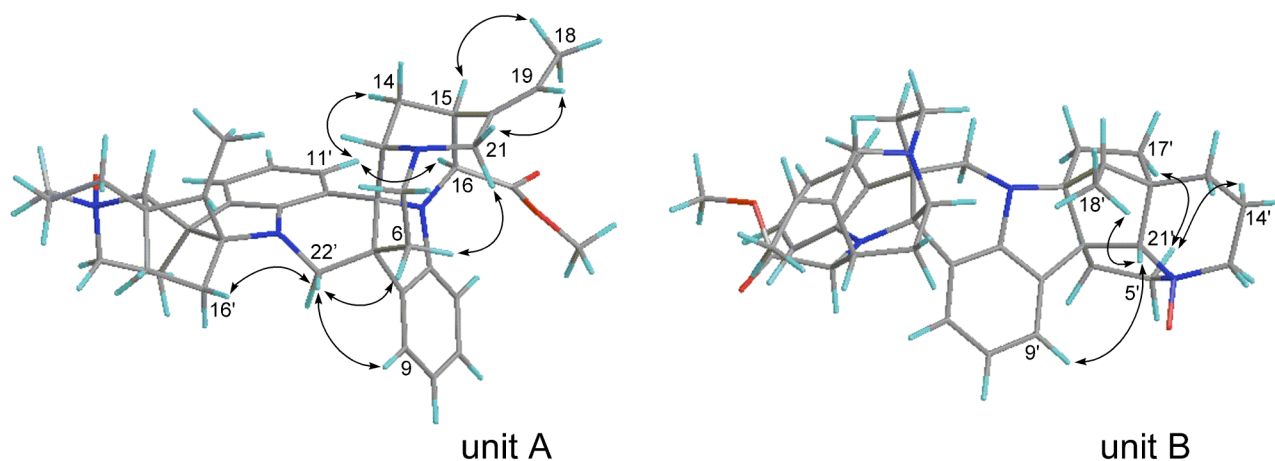


Figure 2. Selected NOESY correlations and relative configurations for huncaniterine A (**1**).

Huncaniterine A (**1**) was assayed for vasorelaxation effects on isolated rat aortic ring using a reported procedure.⁸ Huncaniterine A (**1**) (10^{-4} M) showed relaxation responses against norepinephrine (NE, 3×10^{-7} M) induced contraction of rat aorta strips with endothelium after achieving a maximal response (**1**, 50%).

EXPERIMENTAL

General Experimental Procedures ^1H and 2D NMR spectra were recorded on a 600 MHz spectrometers at 300K, while ^{13}C NMR spectra were measured on a 150 MHz spectrometer. NMR sample of huncaniterine A (**1**) was prepared by dissolving 5.0 mg in 200 μL of CDCl_3 in 5.0 mm cells (Shigemi Co. Ltd.), and chemical shifts were reported using residual CDCl_3 (δ_{H} 7.21 and δ_{C} 77.0) as an internal standard. Standard pulse sequences were employed for the 2D NMR experiments. ^1H - ^1H COSY, HOHAHA, and NOESY spectra were measured with spectral widths of both dimensions of 4800Hz, and 32 scans with two dummy scans were accumulated into 1K data points for each of 256 t_1 increments. NOESY and HOHAHA spectra in the phase sensitive mode were measured with a mixing time of 800 and 30 ms, respectively. For HSQC spectra in the phase sensitive mode and HMBC spectra, a total of 256 increments of 1K data points were collected. For HMBC spectra with Z-axis PFG, a 50 ms delay time was used for long-range C-H coupling. Zero-filling to 1K for F_1 and multiplication with squared cosine-bell windows shifted in both dimensions were performed prior to 2D Fourier transformation.

Table 1. ^1H [δ_{H} (J, Hz)] and ^{13}C NMR Data [δ_{C}] of huncaniterine A (**1**) in CDCl_3

Position	δ_{C}	δ_{H}
2	67.3	
3	54.0	3.13 (1H, m)
5a	48.1	2.87 (1H, brd, 13.1)
5b		3.17 (1H, m)
6a	27.3	1.12 (1H, m)
6b		2.44 (1H, m)
7	46.1	-
8	135.2	-
9	120.3	6.89 (1H, d, 7.5)
10	117.9	6.58 (1H, t, 7.5)
11	126.8	6.88 (1H, t, 7.5)
12	109.0	6.03 (1H, d, 7.5)
13	146.9	
14a	26.8	1.78 (1H, m)
14b		3.07 (1H, m)
15	30.7	3.54 (1H, m)
16	57.2	5.06 (1H, d, 4.4)
17	170.9	-
18	12.3	1.62 (3H, d, 6.5)
19	117.9	5.37 (1H, q, 6.5)
20	136.8	-
21a	53.7	3.07 (1H, m)
21b		4.48 (1H, brd, 12.3)
2'	79.6	
3'	65.3	3.92 (2H, m)
5'a	68.8	3.79 (1H, m)
5'b		4.09 (1H, m)
6'a	34.2	2.06 (1H, m)
6'b		2.33 (1H, m)
7'	57.2	
8'	131.7	
9'	122.7	7.13 (1H, d, 7.5)
10'	117.4	6.45 (1H, t, 7.5)
11'	129.9	7.17 (1H, d, 7.5)
12'	120.9	
13'	148.4	
14'a	20.1	1.91 (1H, m)
14'b		2.13 (1H, m)
15'a	25.5	1.53 (1H, m)
15'b		1.72 (1H, m)
16'a	21.8	1.53 (1H, m)
16'b		2.06 (1H, m)
17'a	32.9	1.53 (1H, m)
17'b		2.13 (1H, m)
18'	10.4	0.53 (3H, d, 7.3)
19'	50.9	2.22 (1H, q, 7.0)
20'	45.3	
21'	87.7	3.92 (1H, brs)
22'	41.2	3.57 and 3.63 (each 1H, m)
OCH_3	51.8	3.68 (3H, s)

Plant Material The barks of *Hunteria 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.

Extraction and Isolation The bark (1 kg) of *Hunteria zeylanica* was extracted with MeOH, and the extract (87 g) was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 10 with sat. Na_2CO_3 , were extracted with CHCl_3 . CHCl_3 -soluble materials (5.3 g) were subjected to an LH-20 column ($\text{CHCl}_3/\text{MeOH}$, 1:1) followed by an amino silica gel column ($\text{CHCl}_3/\text{MeOH}$, 1:0 $\rightarrow$ 0:1) to give huncaniterine A (**1**, 55 mg, 0.006% yield) together with 19'S-pleiomutinine.

Huncaniterine A (1): brownish solid; $[\alpha]_{\text{D}}^{23} +223^\circ$ (c 1.0, CHCl_3); UV (MeOH) λ_{max} 210 nm (ϵ 7300), 255 (ϵ 3000), and 310 (ϵ 1300); IR (KBr) ν_{max} 3400 (OH), 2940, 1750 (C=O), 1610, 1480, 1460 (aromatic ring), and 750 cm^{-1} ; HRESIMS m/z 631.3616 (M+H; calcd for $\text{C}_{40}\text{H}_{47}\text{N}_4\text{O}_3$, 631.3648). ESIMS m/z 631; ^1H -NMR and ^{13}C -NMR see Table 1.

19'S-Pleiomutinine: brownish solid; $[\alpha]_{\text{D}}^{23} +116^\circ$ (c 1.0, CHCl_3); IR (KBr) ν_{max} 2940, 1750 (C=O), 1730 (C=O), and 1610 cm^{-1} ; ESIMS m/z 615 (M+H). ^{13}C -NMR (CD_3OD) δ 67.3 (C-2), 53.9 (C-3), 27.0 (C-6), 46.0 (C-7), 135.6 (C-8), 120.5 (C-9), 118.1 (C-10), 126.3 (C-11), 109.0 (C-12), 146.5 (C-13), 30.5 (C-15), 57.7 (C-16), 170.2 (C-17), 11.2 (C-18), 117.8 (C-19), 136.3 (C-20), 53.5 (C-21), 80.1 (C-2'), 35.3 (C-6'), 57.7 (C-7'), 133.8 (C-8'), 121.1 (C-9'), 116.8 (C-10'), 129.6 (C-11'), 120.5 (C-12'), 149.0 (C-13'), 19.3 (C-14'), 26.2 (C-15'), 9.8 (C-18'), 51.2 (C-19'), 44.2 (C-20'), 72.6 (C-21'), 51.4 (OCH_3), and 40.7 (NCH_2).

Vasodilator Assay⁸ A male Wistar rat weighting 340 g was sacrificed by bleeding from carotid arteries under an anesthetization. A section of the thoracic aorta between the aortic arch and the diaphragm was removed and placed in oxygenated, modified Krebs-Henseleit solution (KHS: 118.0 mM NaCl, 4.7 mM KCl, 25.0 mM NaHCO_3 , 1.8 mM CaCl_2 , 1.2 mM NaH_2PO_4 , 1.2 mM MgSO_4 , and 11.0 mM glucose). The aorta was cleaned of loosely adhering fat and connective tissue and cut into ring preparations 3 mm in length. The tissue was placed in a well-oxygenated (95% O_2 , 5% CO_2) bath of 10 ml KHS solution at 37°C with one end connected to a tissue holder and the other to a force-displacement transducer (Nihon Kohden, TB-611T). The tissue was equilibrated for 60 min under a resting tension of 1.0 g. During this time the KHS in the tissue bath was replaced every 20 min.

After equilibration, each aortic ring was contracted by treatment with $3 \times 10^{-7}\text{ M}$ norepinephrine (NE). The presence of functional endothelial cells was confirmed by demonstrating relaxation to 10^{-5} M acetylcholine (Ach), and aortic ring in which 80% relaxation occurred, were regard as tissues with endothelium. When the NE-induced contraction reached plateau, huncaniterine A was added.

These animal experimental studies were conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, Hoshi University and under the supervision of the Committee on Animal Research of Hoshi University, which is accredited by the Ministry of Education, Science, Sports Culture, and Technology of Japan.

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