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Bisleuconothine A, an eburnane–aspidosperma bisindole alkaloid from *Leuconotis griffithii*

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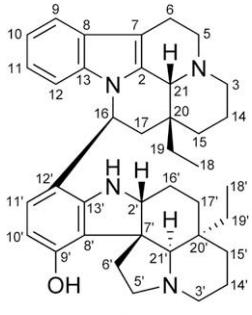
ABSTRACT

A new bisindole alkaloid, bisleuconothine A (**1**) consisting of an eburnane–aspidosperma type skeleton, was isolated from the bark of *Leuconotis griffithii*. The structure including absolute stereochemistry was elucidated on the basis of 2D NMR data and X-ray analysis. Bisleuconothine A (**1**) showed cell growth inhibitory activity against various human cancer cell lines.

Leuconotis griffithii Hk.f is a member of the Apocynaceae family in Malaysia and Indonesia.[1] Some monoterpene indole alkaloids such as leuconolam and rhazinilam, whose skeletons are similar to those found in *Alstonia* and *Kopsia* species, have been isolated from *Leuconotis* species.[2] Recently, a new type of bisindole alkaloid, leucophyllidine,[3] and a tetracyclic ring-opened oxindole alkaloid, leucolusine,[4] has also been isolated from *L. griffithii*. The plants belonging to Apocynaceae such as *Alstonia*, *Kopsia*, *Hunteria*, and *Tabernaemontana* genus have been known to produce various alkaloids depending on the area where the plants were distributed. Recently, we isolated a new bisindole alkaloid, bisnicalaterine A consisting of two vobasine type skeletons from *Hunteria zeylanica*,[5] biscarpamontamine A consisting of an aspidosperma–iboga type skeleton from *Tabernaemontana sphaerocarpa*,[6] and alasmontamine A consisting of bis vobtusine type skeletons from *T. elegans*.[7] In our search for structurally and biogenetically interesting alkaloids from tropical plants in Malaysia, bisleuconothine A (**1**), a new bisindole alkaloid consisting of an eburnane–aspidosperma type skeleton, was isolated from the bark of *L. griffithii*. In this Letter, we describe the isolation and structure elucidation of **1**, showing cell growth inhibitory activity against various human cancer cell lines.

Our screening study on cell growth inhibitory against various human cancer cell lines in traditional medicines discovered that a methanol extract of the bark of *L. griffithii* (Apocynaceae) showed effective cell growth inhibitory activity. Our efforts on identifying active compounds that target human cancer cells resulted in the isolation of a new bisindole alkaloid, bisleuconothine A (**1**) from the bark of *L. griffithii*. This Letter describes structure elucidation and cell growth inhibitory activity of bisleuconothine A (**1**).



1

The bark of *L. griffithii* (216 g) was extracted with MeOH, and the extract (17 g) was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with saturated Na_2CO_3 aq to pH 10 and extracted with $CHCl_3$ to give an alkaloidal fraction (1.88 g). The alkaloidal fraction was subjected to a Sephadex LH-20 column, and the fractions containing the dimers were further separated using an amino silica gel column (*n*-hexane/EtOAc, 1:0 → 0:1; $CHCl_3$/MeOH, 1:0 → 0:1) to give bisleuconothine

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A (**1**, 288 mg, 0.13%) together with (+)-eburnamenine (7 mg, 0.0007%).[8]

Bisleuconothine A (**1**),[9] colorless needles, $[\alpha]_D^{23}$ +43 (*c* 0.2, MeOH), showed molecular formula, $C_{38}H_{48}N_4O$, which was determined by HRESIMS [*m/z* 577.3904 $(M+H)^+$, Δ −0.3 mmu]. IR absorption band was characteristic of amino or hydroxyl group (3430 cm^{-1}). ^1H and ^{13}C NMR data (Table 1) suggested the presence of 15 sp^3 methylenes, 4 sp^3 methines, 2 methyls, 3 sp^3 quaternary carbons, 6 sp^2 methines, and 8 sp^2 quaternary carbons. Among them, 4 sp^3 methylenes (δ_C 45.2; δ_H 2.48 and 2.55, δ_C 51.7; δ_H 3.26 and 3.27, δ_C 55.1; δ_H 2.00 and 3.02, and δ_C 53.9; δ_H 2.31 and 3.06), 4 sp^3 methines (δ_C 51.3; δ_H 5.06, δ_C 60.4; δ_H 4.02, δ_C 65.7; δ_H 3.46, and δ_C 70.4; δ_H 3.05), and 3 sp^2 quaternary carbons (δ_C 133.8, δ_C 137.8, and δ_C 149.2) were attached to the nitrogen atom.

The gross structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the ^1H–^1H COSY, HMQC, and HMBC spectra in CD_3OD (Fig. 1). The ^1H–^1H COSY and HMQC spectra revealed connectivities of ten partial structures **a** (C-5–C-6), **b** (C-9–C-12), **c** (C-16–C-17), **d** (C-3, C-14–C-15), **e** (C-18–C-19), **f** (C-5′–C-6′), **g** (C-10′–C-11′), **h** (C-2′, C-16′–C-17′), **i** (C-18′–C-19′), and **j** (C-3′, C-14′–C-15′) as shown in Figure 1. These partial structures were classed into two parts A and B.

In part A, the connectivity of partial structure **a** and an indole ring (C-2, C-7–C-13, and *N*) was revealed by the HMBC correlations of H-9 to C-7 (δ_C 105.4) and H_2-6 to C-2 (δ_C 133.8) and C-8 (δ_C 129.3). HMBC correlations of H_3-18 to C-20 (δ_C 35.9), H-21 to C-7 and C-19 (δ_C 29.5), and H_2-19 to C-15 (δ_C 25.2) and C-17 (δ_C 43.1) established the connections among C-15, C-17, C-19, and C-21 through C-20 and the connection between C-21 and C-2. HMBC cross-peaks of H_2-3 and H-21 to C-5 (δ_C 51.7) suggested the connection among C-3 (δ_C 45.2), C-5, and C-21 (δ_C 60.4) through a nitrogen atom. The ^1H and ^{13}C NMR chemical shifts of C-16 (δ_C 51.3; δ_H 5.06) were characteristic of an eburnane skeleton.[3]

In part B, the presence of an indoline ring (C-2′, C-7′–C-13′, and *N*) and the connections among C-2′, C-6′, C-8′, and C-21′ through C-7′ were revealed by the HMBC correlations of H-6′a to C-2′ (δ_C 65.7) and C-7′ (δ_C 54.2), H-6′a, H-10′, and H-21′ to C-8′ (δ_C 121.2), H-10′ to C-12′ (δ_C 117.0), and H-11′ to C-9′ (δ_C 154.4) and C-13′ (δ_C 149.2). HMBC cross-peaks of H-3′a to C-5′ and C-21′ established the connections among C-3′, C-5′, and C-21′ through a nitrogen atom. The connections among C-15′, C-17′, C-19′, and C-21′ through C-20′ were deduced by HMBC correlations of H-19′b to C-15′, H-19′a to C-17′ and C-21′, and H_3-18′ to C-20′. These data implied part B possessed an aspidosperma skeleton. Finally, the linkage between C-16 in part A and C-12′ in part B was provided by HMBC correlations of H-16 to C-11′, C-12′, and C-13′. Thus, the gross structure of bisleuconothine A (**1**) was assigned to be a new bisindole alkaloid connecting between C-16 of an eburnane and C-12′ of an aspidosperma skeletons as shown in Figure 1.

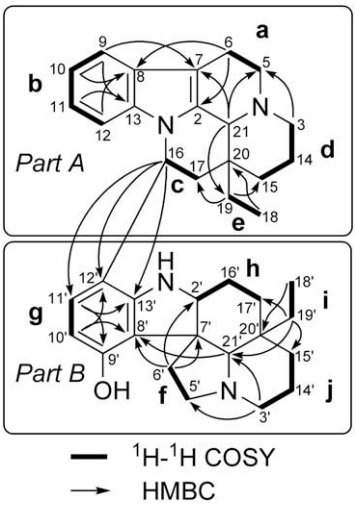


Figure 1. Selected 2D NMR correlations for bisleuconothine A (**1**).

The stereochemistry of each monoterpene indole unit in **1** was assigned by NOESY correlations as shown in computer-generated 3D drawing (Fig. 2). In part A, the NOESY correlations of H-15a/

Table 1
^1H and ^{13}C NMR data of bisleuconothine A (**1**) in $CDCl_3/CD_3OD$ (1:2) at 300 K[a]

	[δ_H (*J*, Hz)]	[δ_c]	HMBC		[δ_H (*J*, Hz)]	[δ_c]	HMBC
2		133.8	6, 21	2′	3.46 (1H, m)	65.7	6′a
3a	2.48 (1H, dd, 11.7, 11.4)	45.2	5, 14b, 15b, 21	3′a	2.00 (1H, dd, 11.3, 11.3)	55.1	5′a, 15′
3b	2.55 (1H, br d, 11.4)			3′b	3.02 (1H, m)		
5a	3.26 (1H, m)	51.7	3, 6b, 21	5′a	2.31 (1H, ddd, 8.7, 8.7, 8.7)	53.9	3′a, 21′
5b	3.27 (1H, m)			5′b	3.06 (1H, m)		
6a	2.59 (1H, br d, 15.2)	17.7	5	6′a	1.76 (1H, m)	36.9	5′a
6b	2.99 (1H, m)			6′b	2.17 (1H, m)		
7		105.4	5, 6, 9, 21	7′		54.2	6′a, 10′
8		129.3		8′		121.2	6′a, 10′, 21′
9	7.36 (1H, d, 7.7)	118.4	11	9′		154.4	11′
10	6.92 (1H, dd, 7.7, 7.5)	119.9	12	10′	6.17 (1H, br d, 7.0)	109.3	
11	6.76 (1H, dd, 7.5, 7.4)	121.1	9	11′	6.65 (1H, d, 7.0)	127.4	16
12	6.59 (1H, br d, 7.4)	112.6	10, 11	12′		117.0	16, 10′
13		137.8	9, 11	13′		149.2	11′
14a	1.41 (1H, br d, 18.0)	21.3	3, 15b	14′a	1.51 (1H, m)	22.4	3′b
14b	1.76 (1H, m)			14′b	1.73 (1H, m)		
15a	1.20 (1H, m)	25.2	14a, 17a, 19a, 21	15′a	1.15 (1H, m)	35.4	3′, 19′b
15b	1.44 (1H, br d, 15.8)			15′b	1.65 (1H, br d, 13.4)		
16	5.06 (1H, d, 7.2)	51.3		16′a	1.58 (1H, ddd, 11.7, 11.7, 11.7)	29.5	
				16′b	1.80 (1H, m)		
17a	1.82 (1H, m)	43.1	19	17′a	1.15 (1H, m)	23.3	19′a, 21′
17b	2.18 (1H, m)			17′b	1.90 (1H, m)		
18	0.90 (3H, t, 7.4)	7.8	19	18′	0.76 (3H, t, 7.4)	7.0	19′
19a	1.51 (1H, m)	29.5	15, 18, 21	19′a	1.21 (1H, m)	29.0	18′, 21′
19b	2.08 (1H, m)			19′b	1.87 (1H, m)		
20		35.9	18, 19, 21	20′		36.4	15′, 18′, 19′a
21	4.02 (1H, s)	60.4	3, 5, 15b, 19	21′	3.05 (1H, br s)	70.4	3′, 5′b, 15′b, 17′a, 19′b

[a] δ in ppm.

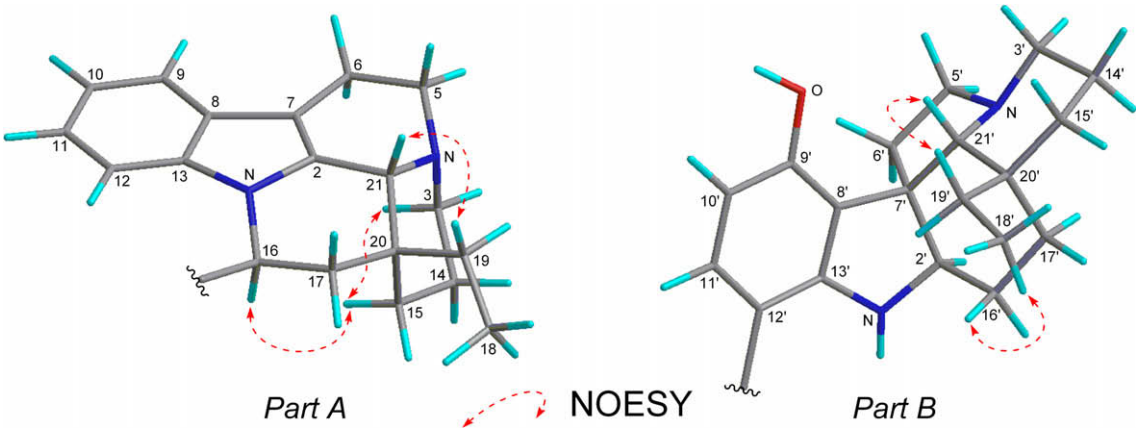


Figure 2. Selected NOESY correlations for parts A and B in bisleuconothine A (**1**).

H-3a and H-16 and H-19a/H-21 suggested that C-15 and H-16 were α-oriented, and H-21 and an ethyl group (C-18–C-19) were β-oriented, respectively. While in unit B, the NOESY correlations of H-19′a/H-21′ and H-16′a/H_3-18′ suggested that H-16′a, C-19′, and H-21′ were β-oriented. The configuration of H-2′ was elucidated to be α-oriented by large ^3J coupling constant (11.7 Hz) of H-2′ and H-16′a as shown in Table 1. Thus the relative stereochemistry of parts A and B was assigned as shown in Figure 2.

Total structure of **1** including absolute stereochemistry was assigned by X-ray analysis for the crystal of **1** obtained from MeOH/H_2O (Fig. 3).[10] The absolute configurations were assigned through the Flack parameter, $\chi = -0.00(13)$.[11]

A plausible biogenetic pathway for bisleuconothine A (**1**) is proposed as shown in Scheme 1. Biogenetically, bisleuconothine A (**1**) might be derived by intermolecular coupling between an iminium carbon (C-16) in an eburnane type skeleton (**A**) and C-12 in an aspidosperma type skeleton (**B**).

Bisleuconothine A (**1**) showed a cell growth inhibitory activity against four human cancer cell lines, HL60, HCT116, MCF7, and A549 (IC_{50} values 11.0, 5.7, 9.2, and 7.0 μM, respectively). To determine the effect of **1** for cell growth inhibitory activity against HCT116, cell cycle analysis was carried out. After treatment of **1** for 24 h, **1** induced G1 phase arrest in HCT116 in dose-dependent manner (68.3%, 0 μM; 72.0%, 5 μM; 75.1%, 10 μM; 79.5%, 20 μM).

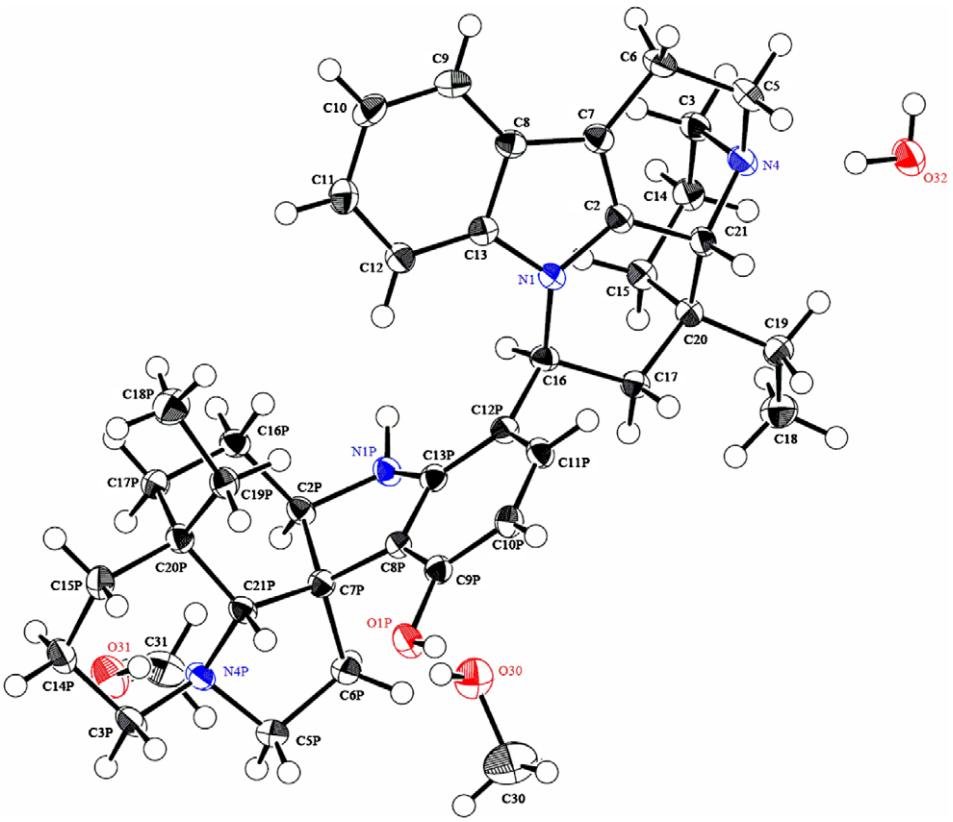
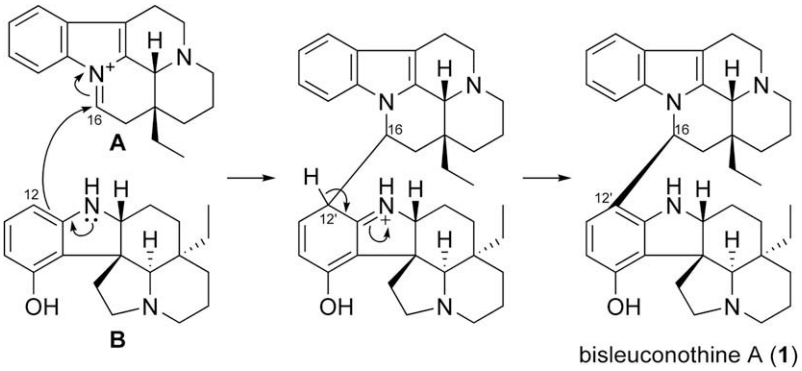


Figure 3. Molecular structure of bisleuconothine A (**1**) obtained by X-ray analysis. One H_2O and two MeOH molecules are contained in the crystal [Flack parameter: $\chi = -0.00(13)$].



Scheme 1. Plausible biogenetic path for bisleuconothine A (**1**).

Efforts are currently underway to elucidate the mode of action for the G1 phase arrest in HCT116 cells of bisleuconothine A (**1**).

Acknowledgments

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9. Bisleuconothine A (**1**): colorless needles; $[\alpha]_D^{23}$ +43 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 220 (ε 42000) and 290 (6900) nm; CD (MeOH) λ_{max} 214 (Δ 8.03), 219 (Δ 0), 224 (Δ −12.35), 237 (Δ 0), 244 (Δ 8.96), 262 (Δ 0), 278 (Δ −2.76), 291 (Δ 0), 297 (Δ 1.92) nm; IR (KBr) ν_{max} 3430, 1614, and 1456 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 577 $(M+H)^+$; HRESIMS *m*/*z* 577.3904 $(M+H)^+$ (calcd for $C_{38}H_{49}N_4O$, 577.3907).
10. All measurements were made on a Rigaku RAXIS RAPID imaging plate area detector with graphite monochromated Cu-Kα radiation. Crystal data of **1**: A colorless platelet crystal, dec. 191 °C, $C_{40}H_{58}N_4O_4$ (658.92), crystal dimensions 0.20 × 0.14 × 0.05 mm, space group $P2_12_12_1$ (#19), *a* = 11.9506 (2), *b* = 16.8306 (3), *c* = 18.0488 (3) Å, *V* = 3630.25 (12) $Å^3$, *Z* = 4, D_{calc} = 1.206 g/cm^3, Cu-Kα radiation (λ = 1.54187 Å), *T* = −180 (1) °C. Of the 42753 reflections that were collected, 6615 were unique (R_{int} = 0.027). The structure was solved by direct methods. R_1 = 0.0319 ($I > 2.00\sigma(I)$) and wR_2 = 0.0826. The absolute configuration was determined based on Flack parameter −0.00(13),[11] refined using 2906 Friedel pairs. All calculations were performed using the CrystalStructure crystallographic software package[12] except for refinement, which was performed using SHELXL-97.[13] The refined fractional atomic coordinates, bond lengths, bond angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). CCDC 754845 contains the supplementary crystallographic data for this Letter. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/deposit, or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).
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