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Bisleucocurine A, a novel bisindole alkaloid from *Leuconotis griffithii*

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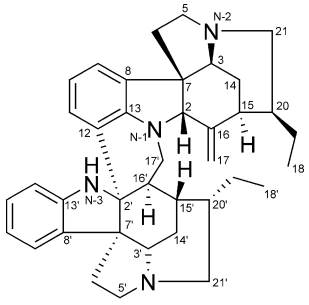
ABSTRACT

A novel bisindole alkaloid, bisleucocurine A (**1**), consisting of two strychnan skeletons with an N-1–C-17′ and a C-12–C-2′ bridges, was isolated from the leaves of *Leuconotis griffithii* and the structure was elucidated on the basis of spectroscopic data. Bisleucocurine A (**1**) showed cytotoxicity against various human cancer cell lines.

Leuconotis griffithii (Retz.) Gardner ex Thwaites is a member of the Apocynaceae family in Malaysia and Indonesia.[1] The species of *Leuconotis* have been known to produce monoterpene indole alkaloids,[2,3] whose skeletons are similar to those found in *Alstonia* and *Kopsia* species.[4] The species of *Alstonia*, *Kopsia*, *Hunteria*, *Tabernaemontana*, and *Leuconotis* have been known to produce various alkaloids depending on the area where the plants were distributed. Recently, we isolated new bisindole alkaloids such as 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] alasmontamine A consisting of a bisvobtusine-type skeleton from *Tabernaemontana elegans*,[7] and bisleuconothine A consisting of an eburnane–aspidosperma-type skeleton from the barks of *L. griffithii*.[8] In our continuing search for structurally and biogenetically interesting alkaloids from tropical plants, bisleucocurine A, a novel bisindole alkaloid consisting of two strychnan skeletons, has been isolated from the leaves of *L. griffithii* together with anhydropereirine.[9] In this Letter, we describe the isolation and structure elucidation of **1**.

Bisleucocurine A (**1**),[10,11] yellowish amorphous solid, $[\alpha]_D^{22}$ +284 (*c* 0.2, MeOH), showed molecular formula, $C_{38}H_{46}N_4$, which was determined by HRESIMS [*m*/*z* 559.3799 $(M+H)^+$, Δ +0.4 mmu]. IR absorption band was characteristic of amino or hydroxyl (3430 cm^{-1}) group. ^1H and ^{13}C NMR data (Table 1) suggested the presence of eleven sp^3 methylenes, eight sp^3 methines, two methyls, three sp^3 quaternary carbons, one sp^2 methylene, seven sp^2 methines, and six sp^2 quaternary carbons. Among them, five sp^3 methylenes (δ_C 48.6; δ_H 2.94 and 3.08, δ_C 50.4; δ_H 2.63 and 3.08, δ_C 50.5; δ_H 2.42 and 2.88, δ_C 55.6; δ_H 3.13 and 3.29, and δ_C 56.3; δ_H 3.17 and 3.40), three sp^3 methines (δ_C 67.8; δ_H 3.19, δ_C 71.4; δ_H 3.22, and δ_C 77.0; δ_H 3.79), and one sp^3 quaternary carbon (δ_C 70.3) were attached to a nitrogen atom.



bisleucocurine A (**1**)

The gross structure of **1** was deduced from extensive analyses of the two-dimensional NMR data, including the ^1H–^1H COSY, HSQC, and HMBC spectra in CD_3OD (Fig. 1). The ^1H–^1H COSY and HSQC spectra revealed connectivities of seven partial structures **a** (C-5–C-6), **b** (C-9–C-11), **c** (C-3, C-14–C-15), **d** (C-18–C-21), **e** (C-5′–C-6′), **f** (C-9′–C-12′), and **g** (C-3′, C-14′–C-21′), as shown in Figure 1. These partial structures were classified into two units A and B.

In unit A, the presence of an indoline ring (C-2, C-7–C-13, and N-1) and the connectivity of partial structure **a** and the indoline ring were revealed by the HMBC of H-9 to C-7 (δ_C 53.6) and C-13 (δ_C 147.2), H-10 to C-8 (δ_C 135.6) and C-12 (δ_C 124.6), H-11 to C-13, and H_2-6 to C-2 (δ_C 77.0), C-7, and C-8. HMBC of H-21b to C-3 (δ_C 67.8) and C-5 (δ_C 55.6), H-5a to C-3 established the connections among C-3, C-5, and C-21 through a nitrogen atom (N-2). HMBC cross peaks of H_2-19 to C-15 (δ_C 33.8) suggested the linkage between C-15 and C-20. The connections among C-2, C-15, and C-17 through C-16 (δ_C 140.9) were deduced from the HMBC of H_2-17 to C-2, C-15, and C-16. And finally the HMBC cross peak of H-2 to

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Table 1
^{1}H and ^{13}C NMR data of bisleucocurine A (**1**) in CD_3OD at 300 K

	Unit A				Unit B		
	δ_H	δ_C	HMBC		δ_H	δ_C	HMBC
2	3.79 (1H, s)	77.0	6, 17	2′		70.3	11, 6′, 16′, 17′
3	3.19 (1H, m)	67.8	2, 5a, 21b	3′	3.22 (1H, m)	71.4	5′a, 6′
5a	3.13 (1H, m)	55.6	6, 21b	5′a	3.17 (1H, m)	56.3	3′, 6′, 21′
5b	3.29 (1H, m)			5′b	3.40 (1H, m)		
6	2.37 (2H, m)	39.8	2	6′	2.50 (2H, m)	33.5	
7		53.6	2, 5a, 6, 9	7′		56.3	3′, 5′a, 6′, 9′, 16′
8		135.6	6, 10	8′		138.1	6′, 10′, 12′
9	6.96 (1H, d, 7.2)	122.3	11	9′	7.20 (1H, d, 7.4)	121.7	11′
10	6.34 (1H, dd, 7.6, 7.2)	119.1		10′	6.79 (1H, dd, 7.4, 7.4)	120.8	
11	6.57 (1H, d, 7.6)	123.5	9	11′	7.00 (1H, dd, 7.7, 7.4)	129.4	9′
12		124.6	10	12′	6.47 (1H, d, 7.7)	111.8	10′
13		147.2	9, 11	13′		149.0	9′, 11′
14a	1.86 (1H, br d, 13.7)	27.2		14′a	1.62 (1H, br d, 13.9)	27.0	
14b	2.42 (1H, m)			14′b	2.87 (1H, br d, 13.9)		
15	2.72 (1H, br s)	33.8	2, 17, 19, 21	15′	1.78 (1H, m)	31.1	16′
16		140.9	17	16′	2.07 (1H, m)	34.7	15′, 17′
17	5.25 (2H, s)	120.6	2	17′a	2.94 (1H, t, 11.3)	48.6	2
				17′b	3.08 (1H, d, 11.3)		
18	1.05 (3H, t, 7.3)	11.6	19, 20	18′	0.99 (3H, t, 7.3)	11.7	
19a	1.32 (1H, dq, 15.0, 7.3)	24.8	18	19′a	1.22 (1H, dq, 15.0, 7.3)	24.6	
19b	1.50 (1H, dq, 15.0, 7.3)			19′b	1.30 (1H, dq, 15.0, 7.3)		
20	1.73 (1H, m)	44.2	14b, 18, 19, 21	20′	1.74 (1H, m)	43.7	14′b
21a	2.42 (1H, m)	50.5	5b, 19	21′a	2.63 (1H, t, 12.7)	50.4	3′, 5′b
21b	2.88 (1H, m)			21′b	3.08 (1H, m)		

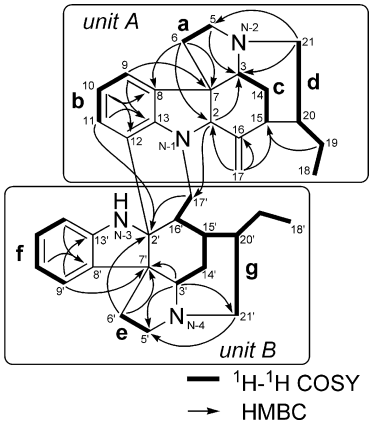


Figure 1. Selected 2D NMR correlations for bisleucocurine A (**1**).

C-3, suggesting the linkage of C-2 and C-3 through C-7, completed the structure of unit A, a strychnan alkaloid as in anhydropereirine.[9]

The chemical shift of the remaining carbons suggested that unit B had a similar structure to unit A. The presence of an indoline ring (C-2′, C-7′–C-13′, and N-3) was revealed by the HMBC of H-6′ and H-9′ to C-7′ (δ_C 56.3) and H-6′ to C-2′ (δ_C 70.3). HMBC of H-3′ to C-5′ (δ_C 56.3) and C-21′ (δ_C 50.4), H-21′a to C-5′ established the connections among C-3′, C-5′, and C-21′ through a nitrogen atom (N-4). The connection of partial structure **g** to the indoline ring through C-3′ and C-16′ was deduced from the HMBC of H-3′ to C-7′ and H_2-17′ to C-2′. Hence, unit B was also revealed to consist of a strychnan skeleton. Finally, the linkages between units A and B from C-12 to C-2′ and N-1 to C-17′ were provided by the HMBC of H-2 to C-17′ and H-11 to C-2′. Thus, the gross structure of bisleucocurine A (**1**) was assigned to be a novel bisindole alkaloid consisting of two strychnan units connected between C-12 and C-2′, and between N-1 and C-17′ as shown in Figure 1. The stereochemistry of each monoterpene indole unit in **1** was assigned by NOESY

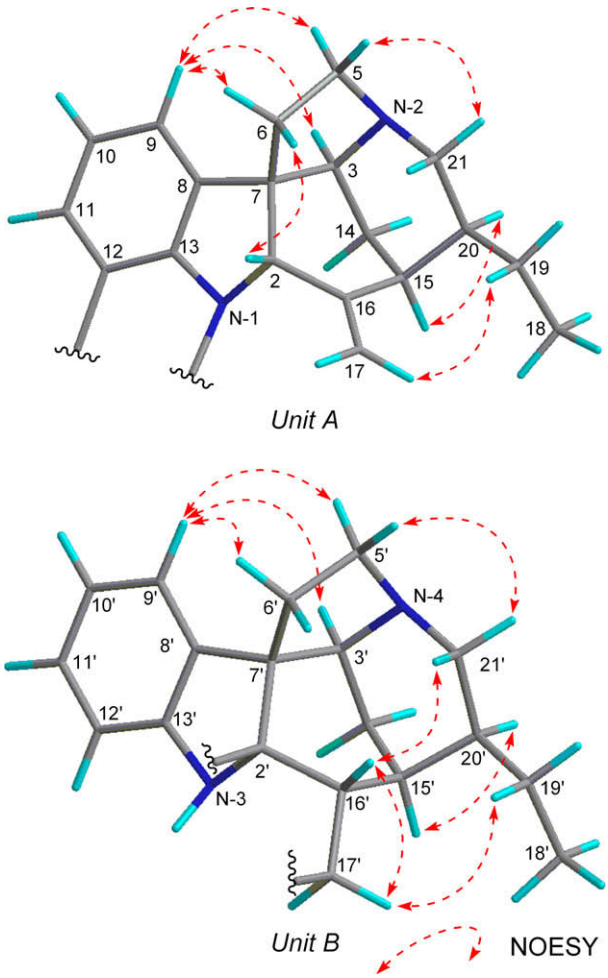


Figure 2. Selected NOESY correlations for bieleucocurine A (**1**).

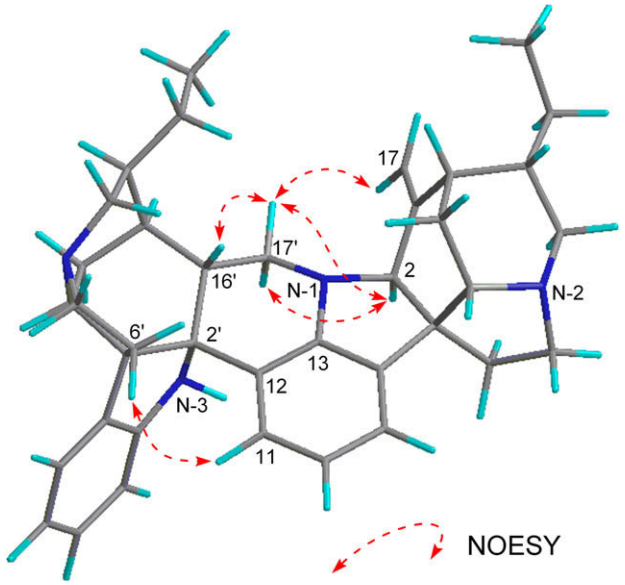


Figure 3. Stereostructure of bisleucocurine A (**1**) with selected NOESY correlations.

correlations as shown in computer-generated 3D drawing (Fig. 2).

In unit A, the NOESY correlations of H-2/H-6, H-3/H-9, and H-17/H-19 suggested that C-6 and H-2 were β-oriented, and H-3 and H-20 were α-oriented. On the other hand, the relative configurations at C-3′, 7′, 15′, and 20′ in unit B were the same as the corresponding configurations of unit A and the β-configuration of H-16′ was deduced from NOESY correlation of H-16′/H-21′a (Fig. 2).

The total relative structure of **1** including an intermediate 6-membered ring (N-1, C-12, C-13, C-2′, C-16′, and C-17′) was then deduced from the NOESY correlations of H-2/H_2-17′, H-11/H-6′, and H-16′/H-17′b and a large ^3J coupling constant between H-16′ and H-17′a (11.3 Hz) as shown in Figure 3. The difference in the cyclization pattern caused a considerable change in the total conformation of **1** compared to those of toxiferine I.[12] The calabash-curare alkaloids have a symmetrical conformation,[13] while **1** does not.

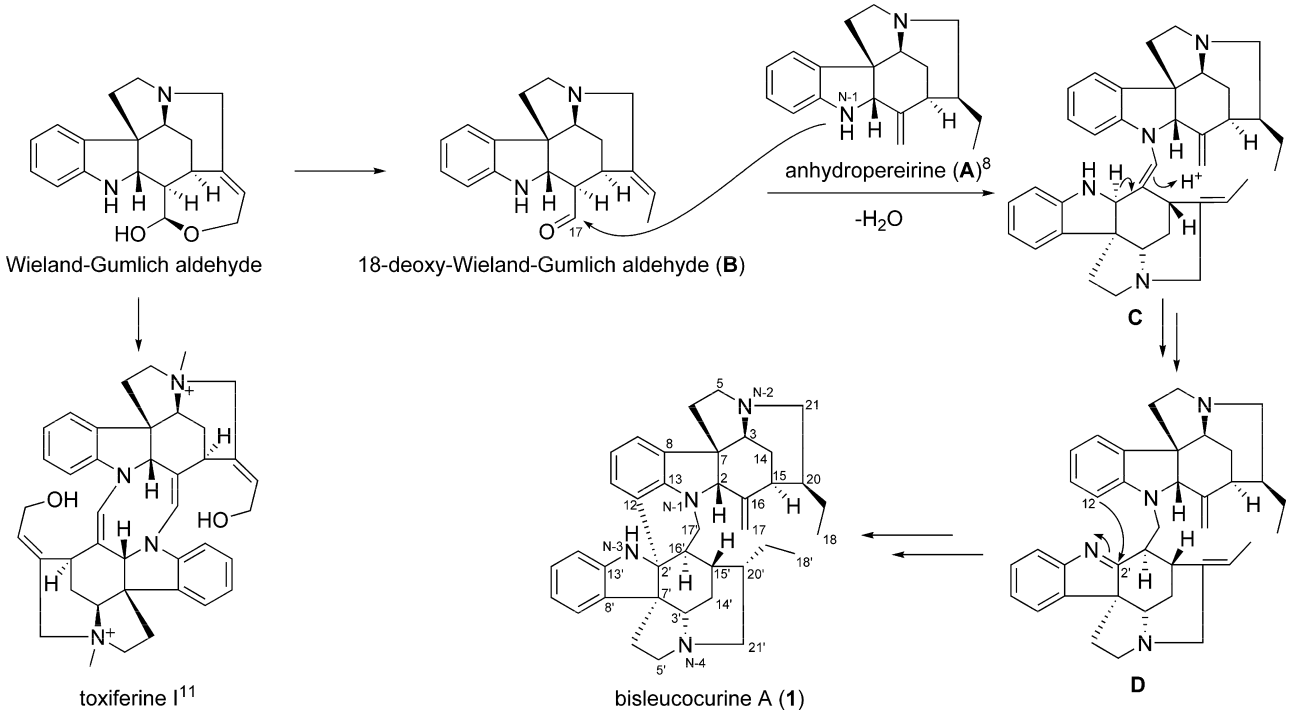
The absolute structure of bisleucocurine A (**1**) was assigned on the basis of comparison between the calculated and the experimental CD spectra.[14] The calculated CD spectrum of the isomer with 2*S*,3*S*,7*R*,15*S*,20*S*,2′*S*,3′*S*,7′*R*,15′*S*,16′*R*,20′*S* configurations showed the same CE pattern compared to that of **1** (positive CEs above 230 nm and a negative CE around 220 nm). Hence, the absolute stereostructure of bisleucocurine A (**1**) was proposed as shown in Figure 3.

Bisleucocurine A (**1**) is the first example of a strychnan dimer with an N-1–C-17′ and a C-12–C-2′ bridge. Strychnan–strychnan bisindole alkaloids are a well-known class of alkaloid comprising more than 25 compounds.

About one third of the known strychnan–strychnan bisindoles possessed an N-1–C-17′ and a C-17–N-1′ double bridges, while the others possessed either an N-1–C-17′, a C-23–C-5′, a C-18–C-21′, or a C-19–C-10′ single bridge.[15] However, there are no examples with an N-1–C-17′ and a C-12–C-2′ double bridges as in bisleucocurine A (**1**).[16]

Bisleucocurine A (**1**) might be generated from the coupling reaction between two strychnan alkaloids as shown in Scheme 1. The intermolecular coupling reaction between N-1 of anhydropereirine (**A**) and an aldehyde group (C-17) of 18-deoxy-Wieland-Gumlich aldehyde (**B**) form an enamine (**C**) as in the case of calabash-curare alkaloids such as toxiferine I.[17] Unit B of the resulting dimer would form an imine (**D**) which undergoes a nucleophilic reaction with the benzene ring of unit A to form the C-12–C-2′ bridge, followed by reduction to give bisleucocurine A (**1**).

Bisleucocurine A (**1**) showed a cell growth inhibitory activity against four human cancer cell lines, HL60, HCT116, MCF7, and A549 (IC_{50} values 3.8, 23.6, 16.9, and 11.9 μM, respectively).



Scheme 1. Plausible biogenetic pathway to bisleucocurine A (**1**).

Acknowledgments

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10. The leaves of *L. griffithii* collected at Mersing, Malaysia, in 2007, were extracted with MeOH, and part (87 g) of the extract was treated with 3% tartaric acid (pH 2) and then partitioned with EtOAc. The aqueous layer was treated with satd aq Na_2CO_3 to pH 10 and extracted with $CHCl_3$ to give an alkaloidal fraction (1.8 g). The alkaloidal fraction was subjected to a Sephadex LH-20 column, and the fractions containing the dimer were further separated using silica gel column ($CHCl_3$/MeOH, 1:0→0:1), to give bisleucocurine A (**1**, 10 mg, 0.002%) together with anhydropereirine.[8]
11. Bisleucocurine A (**1**): yellowish amorphous solid; $[\alpha]_D^{22}$ +284 (*c* 0.2, MeOH); UV (MeOH) λ_{max} 204 (ε 37600), 240 (sh, 9800), 261 (8600) and 312 (6000) nm; CD (MeOH) λ_{max} 210 (Δ +13.13), 215 (Δ 0), 220 (Δ −13.34), 229 (Δ 0), 238 (Δ +9.55), 258 (Δ +26.31), 290 (Δ +4.24), 317 (Δ +12.06) nm; IR (KBr) ν_{max} 3430 cm^{-1}; ^1H and ^{13}C NMR (Table 1); ESIMS (pos.) *m*/*z* 559 $[M+H]^+$; HRESIMS *m*/*z* 559.3799 $[M+H]^+$, calcd for $C_{38}H_{47}N_4$, 559.3795.
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14. (a) CD calculation was performed by Turbomole 6.1 using RI-TD-DFT-BP86/aug-cc-pVDZ level of theory with COSMO solvation model (ε 33) on RI-DFT-BP86/SV(P) optimized geometries. For review and example of the combination of computational and experimental data to determine absolute configuration.; (b) Mukhopadhyay, P.; Wipf, P.; Beratan, D. N. *Acc. Chem. Res.* **2009**, *42*, 809–819; (c) Andrianasolo, H. E.; Haramaty, L.; Rosario-Passapera, R.; Bidle, K.; White, E.; Vetriani, C.; Falkowski, P.; Lutz, R. *J. Nat. Prod.* **2009**, *72*, 1216–1219.
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16. A referee pointed out bisleucocurine A (**1**) is a member of the well-know strychnan–strychnan class of bisindoles, however we think that **1** is an unprecedented strychnan dimer with an N-1–C-17′ and a C-12–C-2′ double bridges.
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