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Leucophyllidine, a Cytotoxic Bisindole Alkaloid Constituted From the Union of an Eburnan and a New Vinylquinoline Alkaloid

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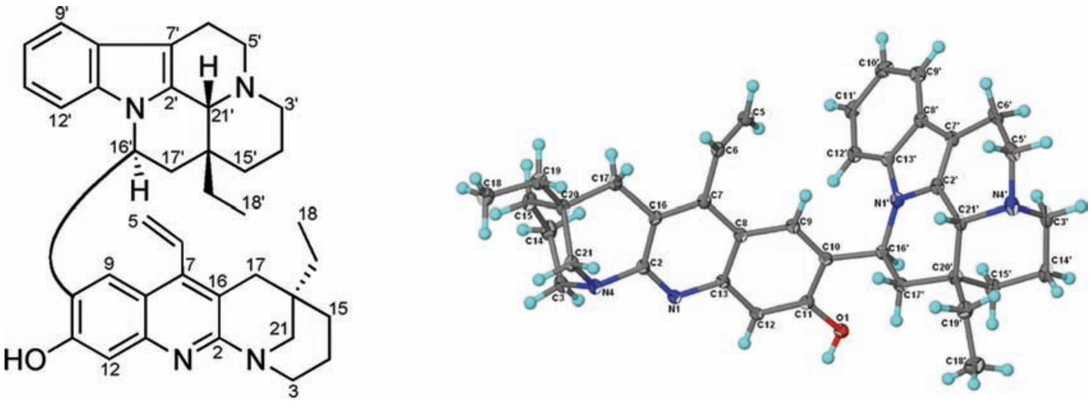
ABSTRACT



A cytotoxic bisindole alkaloid possessing an unprecedented structure constituted from the union of an eburnan half and a novel vinylquinoline alkaloid has been isolated from *Leuconotis griffithii*. The structure was established by analysis of the spectroscopic data and confirmed by X-ray diffraction analysis. A possible biogenetic pathway to the novel quinolinic coupling partner is presented from an *Aspidosperma* precursor.

Plants of the genus *Leuconotis* (Apocynaceae) are usually woody climbers and occur in Indonesia and Peninsular Malaysia.[1] The genus comprises a small group of ten species, of which three (*L. griffithii*, *L. maingayi*, and *L. eugenifolia*) are found in Peninsular Malaysia.[1–3] Previous studies of the Malayan *L. griffithii* and *L. eugenifolia* have provided in addition to the ring-opened alkaloids leuconolam and rhazinilam and their derivatives and various strychnan, kopsan, and eburnan derivatives,[4,5] while several yohimbines and the pentacyclic diazaspiro alkaloid, leuconoxine, were subsequently reported from *L. eugenifolia* occurring in Indonesia.[6] The alkaloidal composition bears a striking similarity to

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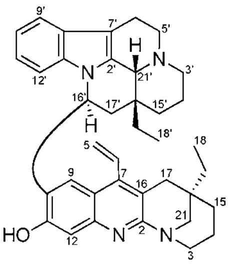
plants of the genus *Kopsia*.[7] In continuation of our studies of biologically active alkaloids from Malaysian Apocynaceae,[7–26] we undertook an investigation of the alkaloids of *Leuconotis* and reported the isolation of the novel tetracyclic ring-opened oxindole alkaloid, leucolusine from *L. griffithii*.[27] We now report the isolation, structure, and biological activity of a novel bisindole alkaloid, leucophyllidine **1**, from the stem-bark extract of the same plant.[28]



1

Leucophyllidine **1** was isolated as a minor alkaloid (yield, ca. 7.10 mg kg^{-1}) from the EtOH extract of the stem-bark of *L. griffithii*. It was obtained after repeated chromatographic fractionation of the basic fraction from the EtOH extract and subsequent crystallization from Et_2O−EtOAc, as pale yellowish crystals, mp 215−217 °C, $[\alpha]_D$ −138 (*c* 0.18, $CHCl_3$). The UV spectrum (230, 288, and 354 nm) indicated a composite chromophore from the superposition of indole and quinoline moieties,[29] while the IR spectrum showed the presence of only one functionality, viz., an absorption due to OH at 3376 cm^{-1}. The EI mass spectrum of **1** showed an M$^+$ at *m*/*z* 572 (base peak), which was analyzed for $C_{38}H_{44}N_4O$ (corresponding to a DBE value of 19), with other prominent fragment peaks due to loss of ethyl and C_4H_8N at *m*/*z* 543 and 502, respectively.[30]

The ^{1}H NMR spectrum (Table 1) showed signals due to six aromatic hydrogens, four of which correspond to the four contiguous hydrogens of an indole unit as indicated from the COSY spectrum. The remaining two aromatic hydrogens appeared as two singlets at δ 7.77 and 7.54. The ^{1}H NMR spectrum also revealed the presence of two ethyl and one vinyl side chain, two isolated methylenes, and one isolated methine. The ^{13}C NMR spectrum (Table 1) accounted for all 38 carbon resonances comprising 2 methyls, 14 methylenes, 9 methines, and 13 quaternary carbons. Examination of the NMR chemical shifts, as well as the COSY and HETCOR data, revealed partial structures (unsubstituted indole, NCH_2CH_2, $NCH_2CH_2CH_2$, $NCHCH_2$, NCH, and CH_3CH_2) which indicated that one unit of the bisindole corresponds to an eburnan moiety.[9] This attribution was readily confirmed by examination of the ^{13}C NMR spectrum as well as the HMBC spectrum, where the signals corresponding to an eburnan half (eburnamine) were clearly evident.[5,31] The signal due to H-16′ was observed as a one-H multiplet at δ 5.77, suggesting branching of the bisindole from this carbon,[9] a conclusion which was supported by the observed H-9 to C-16′ three-bond correlation seen in the HMBC spectrum of **1** (vide infra). Since the eburnan alkaloids present in this plant such as (−)-eburnamine, (+)-isoeburnamine, (+)-eburnamonine, and (+)-eburnamenine all belong to the 20β,21β-enantiomeric group, the eburnan half in leucophyllidine is in all probability derived from (−)-eburnamine.[8,9,32,33] The attachment of the other unit at C-16′ is likely to be β, in analogy to the other *Aspidosperma-eburnea* bisindoles with branching from the eburnan C-16′.[9,33]

The other unit constituting the bisindole, after discounting the eburnan half, incorporates an aromatic ring substituted at C-10 and C-11, from the observation of two aromatic singlets at δ 7.54 and 7.77. Other fragments associated with this monomeric unit include an ethyl (δ 7.1, 35.0) and a vinyl (δ 123.1, 131.1) side chain. The OH group indicated in the IR spectrum constitutes one of the aromatic substituents, which is consistent with the observed shift of this carbon at δ 155.8, while the adjacent aromatic carbon (δ 118.7) represents the site of eburnan substitution. The carbon shifts of the aromatic unit can be assigned based on the HMBC spectrum. The three-bond correlation from the aromatic signal at δ_H 7.54 to the eburnan C-16′ (δ 49.7) and to another aromatic carbon at δ 143.9 (C-7) allowed the assignment of this signal to H-9 and that at δ 7.77 to H-12. The correlation from H-9 to the signal at δ 155.8 establishes hydroxy substitution to be at C-11 and hence eburnan substitution at

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Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1**[a]

position	δ_C	δ_H	position	δ_C	δ_H
2	160.7		2′	133.6	
3	55.6	3.12 t (11) 3.80 m	3′	44.3	2.41 m 2.62 m
5	123.1	4.84 d (18) 5.32 d (12)	5′	50.6	3.34 m 3.34 m
6	131.1	6.50 dd (18, 12)	6′	17.1	2.62 m 2.99 m
7	143.9		7′	104.5	
8	130.9		8′	128.3	
9	123.5	7.54 s	9′	117.7	7.48 d (7.3)
10	118.7		10′	118.9	7.02 t (7.3)
11	155.8		11′	120.2	6.85 t (7.3)
12	110.1	7.77 s	12′	112.4	6.71 d (7.3)
13	147.2		13′	136.1	
14	19.4	1.24 m 1.36 m	14′	20.4	1.36 m 1.80 m
15	36.0	1.45 m 1.69 m	15′	24.4	1.20 m 1.36 m
16	120.9		16′	49.7	5.77 m
17	36.4	2.39 m 2.58 m	17′	42.0	1.58 m 2.56 m
18	7.1	0.89 t (7)	18′	7.5	0.77 t (7)
19	35.0	1.27 m 1.27 m	19′	28.6	1.45 m 2.06 m
20	30.5		20′	34.7	
21	57.3	2.88 d (12) 2.99 d (12)	21′	59.3	4.05 s

[a] $CDCl_3$, 400 MHz.

C-10 (δ 118.7). The remaining correlations from H-9 and H-12 allowed the assignment of the other aromatic carbons (Figure 1). The low-field resonance observed at δ 160.7 indicated the presence of an imine function,[18,22,34] and this, with the two remaining quaternary aromatic signals at δ 120.9 and 143.9, can be attributed to C-2, C-16, and C-7, respectively, of a quinoline chromophore.[22] Attachment of the vinyl side chain is to C-7 due to the observed three-bond correlation from the vinylic H-5 to C-7.

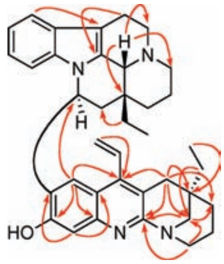

Figure 1. Selected HMBCs of **1**.

The NMR data also showed the presence of an isolated methylene and an isolated aminomethylene, in addition to an ethyl side chain attached to a quaternary carbon. The three-bond correlations observed from H-17 to C-21, H-21 to C-2, and the two bond correlation from H-17 to C-20 allowed the methylene, ethyl-substituted quaternary carbon, and the aminomethylene fragments to be linked to form a third ring fused to the vinylquinoline unit, constituting a tetrahydrobenzo[*b*][1,8]naphthyridine ring system.[35] This leaves a three-carbon fragment to complete assembly of the molecule. The COSY spectrum showed the presence of an NCH_2CH_2 fragment. This partial structure should in fact be part of the NC(3)−C(14)−C(15) fragment, but extensive overlap allowed only the NC(3)−C(14) fragment to be discerned. The presence of the C-15 methylenes as part of the NC(3)−C(14)−C(15) fragment linked from C-15 to C-20 can however be deduced indirectly from the correlations observed from H-21 and H-17 to C-15. The observed correlation from H-3 to C-2 allowed the linking of this fragment from C-3 to N-4 to complete assembly of the fourth ring. The resulting tetracyclic quinolinic structure unraveled corresponds to a new alkaloid skeleton. A search of the literature, however, revealed that a vinyl-substituted quinoline alkaloid different from the quinolinic coupling partner in **1**, named strictigine **2**, was isolated from *Rhazya stricta*.[36]

Although the structure proposed for strictigine was different from that of the vinylquinoline moiety of leucophyllidine **1**, the NMR data of the nonaromatic part of the molecule were virtually similar to those of the quinolinic coupling partner in **1**. A closer examination of the NMR data of strictigine revealed, in addition to a major incorrect assignment of the δ 37.0 carbon shift and the absence of the C-7 signal, an excessive number of ^4J and ^5J HMBCs, which in combination led to the wrong structural assignment (Figure 2).

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(37) The crystals of **1** are monoclinic, belonging to space group *P*21, with $a = 12.7151(3)$ Å, $b = 8.5294(3)$ Å, $c = 17.2463(5)$ Å, $a = 90.00°$, $b = 108.811(2)°$, $g = 90.00(10)°$, $V = 1770.50(9)$ Å^3, $D_x = 1.240$ mg m^{-3}, and $Z = 2$. The structure was solved by direct methods and refined by the least squares method. The final *R*-factor was 0.0489.

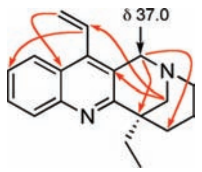


Figure 2. ^4J and ^5J HMBCs of strictigine **2**.[36]

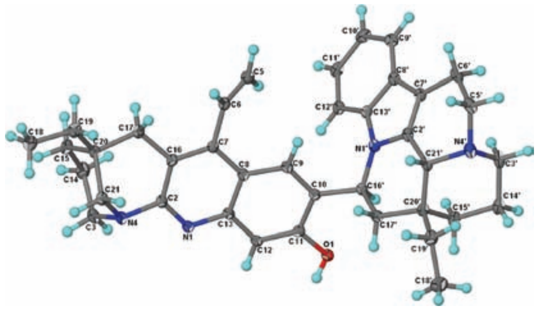
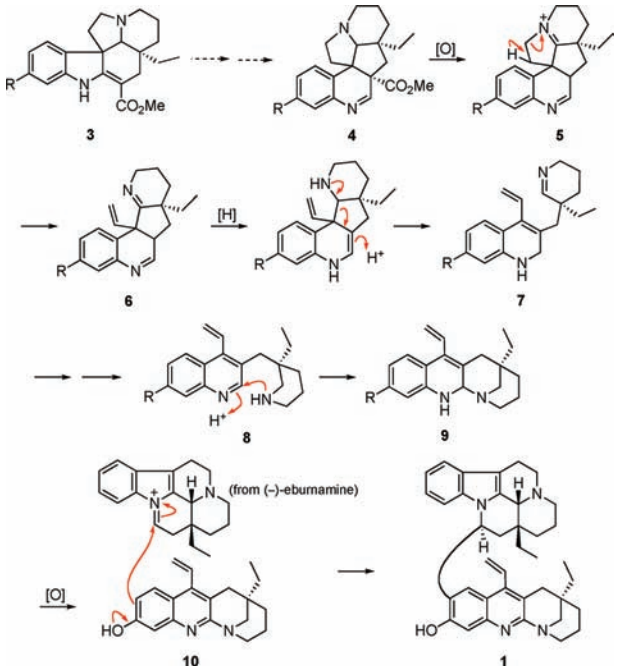


Figure 3. X-ray crystal structure of **1**. Thermal ellipsoids are shown at the 50% probability level. One molecule of EtOAc per molecule of **1** observed in the crystal lattice is omitted for clarity.

To secure unambiguous confirmation of the proposed structure of leucophyllidine, an X-ray diffraction analysis was carried out (Figure 3) which provided vindication of the structure we proposed for **1** based on analysis of the spectroscopic data.[37]

The structure of leucophyllidine is unprecedented on two counts. First, it represents the first member of a new structural subclass of the bisindole alkaloids based on the constituent monomeric halves.[9,38] Second, the vinylquinoline half is distinguished by a novel skeleton incorporating an unprecedented tetrahydrobenzo[*b*][1,8]naphthyridine chromophore. This intriguing skeleton raises an interesting question with regard to its biogenetic origin. Since the other alkaloids present in *Leuconotis* are all monoterpenoid indoles,[5–7,27] we postulated that **1** in all likelihood originates from an indole precursor. A possible pathway to **1** is presented in Scheme 1 from an *Aspidosperma* precursor (e.g., 11-hydroxyvincadifformine **3**).[39] Transformation of **3** to the *Melodinus*-type alkaloid **4** is well established.[40,41] Decarboxylation, followed by oxidation, gives the iminium ion **5**. Cleavage of the C(5)−N(4) bond (which has precedence in the thermally induced transformations of kopsinic acid and secodine derivatives[42]) furnishes the ring-E *seco*-alkaloid **6**. Reduction, followed in succession by a lone pair-assisted Grob-like fragmentation, leads to the vinyl-substituted dihydroquinoline derivative **7**, which on aromatization following reduction of the imine yields the vinylquinoline derivative **8**. Ring closure via attack of the piperidine NH onto the imine carbon yields the tetracyclic dihydroquinoline **9**, which on aromatization furnishes the vinylquinoline alkaloid incorporating a tetrahydrobenzo[*b*][1,8]naphthyridine core. Coupling of this novel vinylquinoline alkaloid **10** with (−)-eburnamine furnishes leucophyllidine. Leucophyllidine **1** showed pronounced in vitro cytotoxicity toward drug-sensitive as well as vincristine-resistant (VJ300) human KB cells, with IC_{50} values of 2.95 and 2.92 μg/mL (5.16 and 5.10 μM), respectively.[43]

Scheme 1. Possible Biogenetic Pathway to **1**



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Supporting Information Available: Isolation of **1**, 1D and 2D NMR spectra, and X-ray crystallographic data (CIF) of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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