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Bipleiophylline, an Unprecedented Cytotoxic Bisindole Alkaloid Constituted from the Bridging of Two Indole Moieties by an Aromatic Spacer Unit

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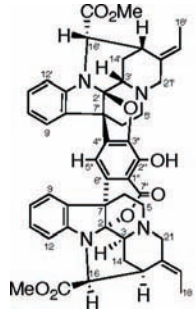
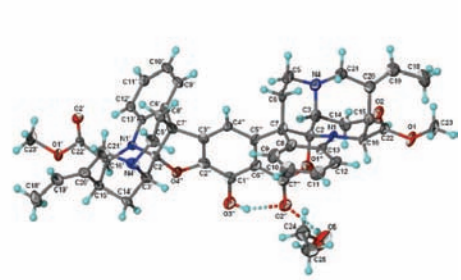
ABSTRACT





A cytotoxic bisindole alkaloid possessing an unprecedented structure in which two indole moieties are bridged by an aromatic spacer unit has been isolated from *Alstonia angustifolia*. The structure was established by analysis of the spectroscopic data and confirmed by X-ray diffraction analysis. A possible biogenetic pathway from pyrocatechuic acid and pleiocarpamine is presented.

The genus *Alstonia* (Apocynaceae) represents a rich source of both indole and bisindole alkaloids. A noteworthy feature of the alkaloids of *Alstonia* is the preponderance of the macroline unit which abounds in the alkaloids found in plants of the genus.[1−13] A number of the *Alstonia* bisindoles are known for displaying significant *in vitro* antiamoebic activity against *P. falciparum* (the causative agent of Malaria) as well as cytotoxic activity against several human cancer cell lines, with the noticeable feature that the bioactive bisindole alkaloids invariably displayed greater potency compared to the monomeric *Alstonia* alkaloids.[3,14−18] Several new indole alkaloids have also been recently reported from the Chinese *A. scholaris*,[19,20] while a novel indole alkaloid, actinophyllic acid from an Australian *Alstonia* (*A. actinophylla*), was

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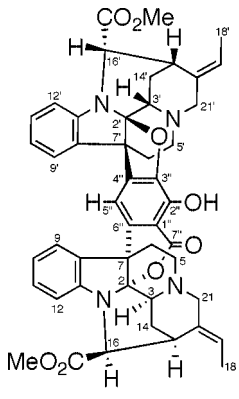
Table 1. ^{1}H and ^{13}C NMR Spectral Data of **1**[a]

position	δ_C	δ_H	position	δ_C	δ_H	position	δ_C	δ_H
2	106.6		2′	103.2		1″	107.7	
3	51.1	3.35 m	3′	51.7	3.30 m	2″	147.7	
5a	48.2	3.09 m	5a′	47.8	2.91 m	3″	146.9	
5b		3.09 m	5b′		2.91 m			
6β	24.3	2.34 m	6a′	28.3	2.50 m	4″	130.7	
6α		2.58 m	6b′		2.50 m			
7	54.8		7′	49.2		5″	110.7	7.38 s
8	132.2		8′	134.4		6″	137.6	
9	122.6	6.93 dd (8, 1)	9′	122.3	6.76 dd (8, 1)	7″	168.1	
10	120.9	6.84 td (8, 1)	10′	120.9	6.81 td (8, 1)	2″-OH		11.31 s
11	127.9[b]	7.09 td (8, 1)[b]	11′	128.0[b]	7.11 td (8, 1)[b]			
12	110.5[c]	6.37 br d (8)[c]	12′	110.4[c]	6.35 br d (8)[c]			
13	144.6		13′	145.2				
14α	26.7	2.19 d (14)	14α′	26.3	1.88 d (14)			
14β		2.78 dt (14, 3)	14β′		2.73 dt (14, 3)			
15	31.3[d]	3.48 m[d]	15′	31.2[d]	3.45 m[d]			
16	57.7	4.79 d (4)	16′	57.3	4.68 d (4)			
17	169.2[e]		17′	169.6[e]				
18	12.5[f]	1.64 dd (7, 2)[f]	18′	12.3[f]	1.63 dd (7, 2)[f]			
19	122.6	5.56 q (7)	19′	120.4	5.49 q (7)			
20	132.4		20′	133.6				
21α	51.6	3.16 d (13)	21α′	52.7	3.15 d (13)			
21β		4.31 d (13)	21β′		4.17 d (13)			
CO_2Me	52.2	3.76 s	CO_2Me'	52.2	3.75 s			

[a] $CDCl_3$, 400 MHz. [b] Assignments are interchangeable. [c] As above in b. [d] As above in b. [e] As above in b. [f] As above in b.

reported to be an effective inhibitor of carboxypeptidase U (CPU) and, hence, a potential facilitator of fibrinolysis.[21] In continuation of our own studies of biologically active alkaloids from Malaysian *Alstonia*,[4−9] we report herein the isolation and structure elucidation of the cytotoxic alkaloid, bipleiophylline, representing the first member of an unprecedented class of the bisindole alkaloids.[3,22]



1

Bipleiophylline, **1**, was isolated as a minor alkaloid (yield, ca. 2 mg kg^{-1}) from the EtOH extract of the stem bark of *A. angustifolia* Wall. It was obtained after repeated chromatographic fractionation of the basic fraction from the EtOH extract and subsequent crystallization from EtOH−$CHCl_3$ as pale reddish crystals, mp >220 °C dec, $[\alpha]_D$ +321 (*c* 0.14, $CHCl_3$). The UV spectrum (245, 281, and 337 nm) indicated a composite chromophore, with the two lower frequency bands resembling the spectrum of 2,7-dihydropleiocarpamine (254 and 295 nm),[23] while the IR spectrum showed bands at 3400, 1750, and 1668 cm^{-1}, suggesting the presence of OH, ester, and α,β-unsaturated lactone functionalities, respectively. The EI mass spectrum of **1** showed M^+ at *m*/*z* 794, which analyzed for $C_{47}H_{46}N_4O_8$ (corresponding to a DBE value of 27), with a prominent fragment peak due to loss of CO_2Me at *m*/*z* 735.[24] In addition, the presence of fragment ions at *m*/*z* 135 (base) and 107 are characteristic of dihydropleiocarpamine-type alkaloids.[23] The ^{13}C NMR spectrum (Table 1) accounted for all 47 carbon resonances

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and confirmed the presence of ester and/or lactone functionalities from the quaternary signals observed at δ 168.1, 169.2, and 169.6. The ^{1}H NMR spectrum (Table 1) showed signals due to eight aromatic hydrogens, corresponding to two unsubstituted indole moieties (δ 6.35−7.11), two methyl ester groups (δ_H 3.75, δ_C 52.2, 169.6; δ_H 3.76, δ_C 52.2, 169.2), two ethylidene side chains (two sets of methyl doublet of doublets at δ 1.63, 1.64; two one-H quartets at δ 5.49, 5.56), and an OH singlet at δ 11.31 which undergoes exchange with D_2O. The spectrum also showed the presence of a one-H aromatic singlet at δ 7.38, in addition to two well-resolved methine doublets at δ 4.68 and 4.79 (δ_C 57.3 and 57.7, respectively), each with a coupling constant of 4 Hz. The latter signals are reminiscent of the H-16 signals of pleiocarpamine (δ 5.21, d, 4 Hz; δ_C 61.6)[18] and of bisindoles incorporating pleiocarpamine units, such as, for example, villalstonine (δ 4.42, d, 4 Hz; δ_C 57.7),[13,25] and provided the first indication that **1** might be constituted from the union of two pleiocarpamine units. Closer examination of the ^{1}H and ^{13}C NMR data revealed not only a close correspondence of the signals with those of the pleiocarpamine half of villalstonine but also the presence of two sets of paired signals with similar chemical shifts. With the aid of COSY, HETCOR, and HMBC data, the paired signals could be distinguished to furnish two sets of signals corresponding to the two pleiocarpamine halves present.

The ^{1}H and ^{13}C NMR spectra, however, showed the presence of residual signals after accounting for the two pleiocarpamine halves and subtracting the two monomeric units from the molecular formula yielded a fragment with partial formula $C_7H_2O_4$. Since an aromatic singlet and an OH signal were observed, and the ^{13}C NMR spectrum showed the presence of an additional seven downfield signals, corresponding to one sp^2 methine (δ 110.7), five quaternary carbons (δ 107.7, 130.7, 137.6, 146.9, and 147.7), and one ester or lactone carbonyl (δ 168.1), the additional presence of a highly substituted aromatic moiety was indicated. Comparison of the NMR data of **1** with those of other *Alstonia* bisindoles incorporating pleiocarpamine units showed that as in the other bisindoles, branching from the pleiocarpamine half in **1** is also from C-2 and C-7.[13,25−27] The downfield shift of C-2 and C-2′ at δ 106.6 and 103.2, respectively, indicated that they are linked to both a nitrogen and an oxygen atom, while the shift of C-7 and C-7′ at δ 54.8 and 49.2, respectively, suggested connection to carbon atoms (the bridging of C-2 by an oxygen and C-7 by a carbon atom is common and is exemplified by the bisindoles villalstonine,[25] pleiocorine,[26] and pleiocraline[27]). Based on HMBC data as well as comparison with the ^{13}C NMR data of pyrocatechuic acid (2,3-hydroxybenzoic acid) and its derivatives,[28] the mode of attachment of the two pleiocarpamine units to the aromatic spacer unit was unravelled.

The observed three-bond correlations from H-6 to C-6″, H-6′ to C-4″, and H-5″ to C-7, C-7′ in the HMBC spectrum, indicated that the aromatic spacer unit is branched from C-4″ and C-6″ to C-7′ and C-7 of the two pleiocarpamine units, respectively, in addition to defining the position of the lone aromatic H-5″ in relation to the attachment points of the pleiocarpamine halves on the aromatic moiety. The observed ^3J correlations from H-5″ to C-1″ (δ 107.7) and C-3″ (δ 146.9) indicated the presence of carboxyl and ether oxygen functions at the two meta carbons (relative to C-5″) of the aromatic unit. This leaves the phenolic OH at C-2″; its ready detection as a singlet at δ 11.31 is probably due to facile intramolecular hydrogen-bonding with the carbonyl oxygen of the proximate lactone function. This conclusion receives additional support from the observed correlations from the phenolic OH to C-1″, C-2″, and C-3″ in the HMBC spectrum. The evidence therefore is consistent with a structure in which the two pleiocarpamine halves are connected by an aromatic spacer unit via carboxyl and ether linkages to forge a δ-lactone and a dihydrofuran ring, respectively (Figure 1).

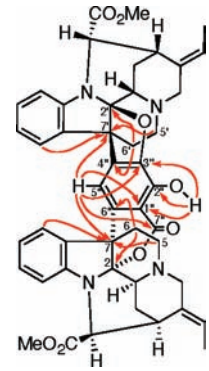

Figure 1. Selected HMBCs of **1**.

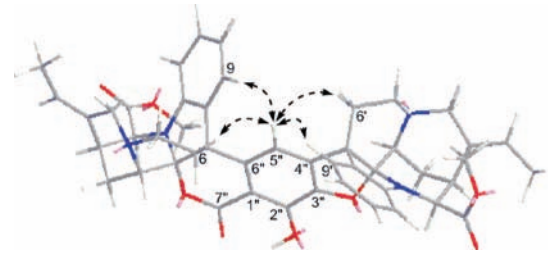

Figure 2. Selected NOEs of **1**.

An alternative structure based on 2,6-hydroxybenzoic acid as the spacer unit, in which both C-2 and C-2′ are bridged by ether oxygens resulting in incorporation of two dihydrofuran rings, can be ruled out as this would result in a symmetric bisindole which would in turn show NMR spectra exhibiting homotropic character which was not the case. Furthermore, such a structure would also not be in accord with the HMBC data. The overall data is therefore consistent with the proposed structure of bipleiophylline as shown in **1**. The structure is also in agreement with the NOE data

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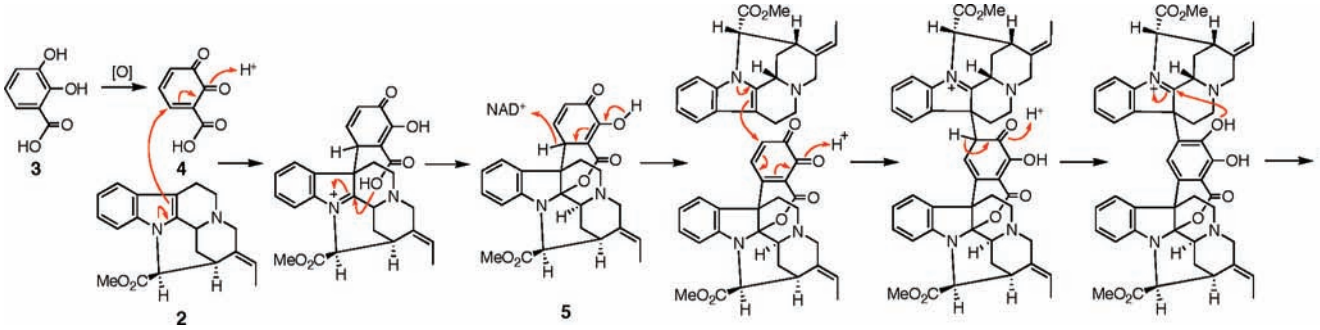
Scheme 1. Possible Biogenetic Pathway to **1**



which showed reciprocal NOEs between the aromatic H-5″ and H-6, H-6′, H-9, and H-9′ (Figure 2). Finally, in order to obtain unambiguous confirmation of the structure, X-ray diffraction analysis was carried out (Figure 3) which provided vindication of the structure deduced based on analysis of the spectroscopic data.[29]

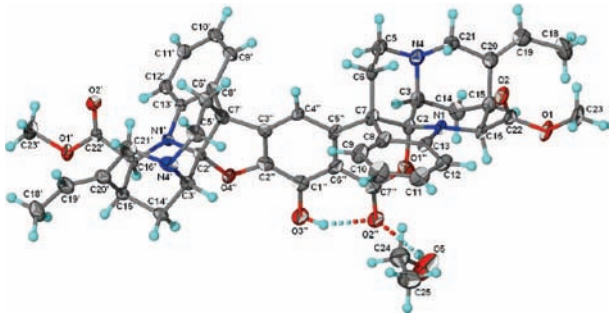


Figure 3. X-ray crystal structure of **1**.

The structure of bipleiophylline is unprecedented on two counts. First, it represents the first example of a bisindole constituted from the union of two pleiocarpamine halves.[3,22] Second, and more significantly, it represents the first example of a bisindole which is linked to two monomeric indole halves via an aromatic spacer unit.[3,22] A possible pathway to **1** from pleiocarpamine, **2**, and pyrocatechuic acid, **3**, is presented in Scheme 1. The sequence is initiated by conjugate addition of pleiocarpamine via its nucleophilic C-7 onto the oxidized *o*-quinone form of pyrocatechuic acid, **4**. Intramolecular capture of the resultant iminium ion by the carboxyl OH furnished the pleiocarpamine-pyrocatechuic acid adduct, **5**. Subsequent attack of a second pleiocarpamine, via 1,6-addition, followed by intramolecular capture of the iminium ion by phenolic OH furnishes **1**. Bipleiophylline **1** showed appreciable in vitro cytotoxicity toward drug-sensitive and vincristine resistant (VJ300) human KB as well as Jurkat cells, with IC_{50} values of 3.2, 2.0, and 3.7 μg/mL, respectively (vincristine was used as positive control).[30]

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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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(29) The crystals of **1** are triclinic, belonging to space group P1, with $a = 9.1602(10)$ Å, $b = 9.9973(2)$ Å, $c = 12.4147(2)$ Å, $\alpha = 79.1190(10)°$, $\beta = 81.7560(10)°$, $\gamma = 72.9240(10)°$, $V = 1062.55(3)$ Å^3, $D_x = 1.314$ Mg m^{-3}, and $Z = 1$. The structure was solved by direct methods and refined by the least-squares method. The final R-factor was 0.0569.

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