Antiparasitic Alkaloids from *Psychotria klugii*

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Psychotria klugii yielded two new benzoquinolizidine alkaloids, klugine (**1**) and 7′-*O*-demethylisocephaeline (**2**), together with the previously known cephaeline (**3**), isocephaeline (**4**), and 7-*O*-methylipecoside (**5**). The structures and stereochemistry of **1** and **2** were determined by 1D and 2D NMR data and circular dichroism experiments. Cephaeline (**3**) demonstrated potent in vitro antileishmanial activity against *Leishmania donavani* (IC_{50} 0.03 μg/mL) and was >20- and >5-fold more potent than pentamidine and amphotericin B, respectively, while klugine (**1**) (IC_{50} 0.40 μg/mL) and isocephaeline (**4**) (IC_{50} 0.45 μg/mL) were <13- and <15-fold less potent than **3**. In addition, emetine (**6**) (IC_{50} 0.03 μg/mL) was found to be as equally potent as **3**, but was >12-fold more toxic than **3** against VERO cells (IC_{50} 0.42 vs 5.3 μg/mL). Alkaloids **1** and **3** exhibited potent antimalarial activity against *Plasmodium falciparum* clones W2 and D6 (IC_{50} 27.7−46.3 ng/mL). Compound **3** was cytotoxic to SK-MEL, KB, BT-549, and SK-OV-3 human cancer cells, while **1** was inactive.

The genus *Psychotria* (Rubiaceae) comprises about 1200 species distributed in tropical regions worldwide.[1] In tropical America, including Central America and the West Indies, the center of distribution is the Amazon basin, but the genus is represented also in northeastern Brazil, the Brazilian "planalto", and part of the Andes. Chemical investigations of various *Psychotria* species have revealed the presence of alkaloids, including pyrrolidinoindolines (polyindolines), harman derivatives, and quinolines.[2−5] Some of these pyrrolidinoindoline dimers have exhibited functional antagonism of somatostatin (SRIH) and platelet activation.[1,6] In addition, antimalarial benzoquinones and antitumor triterpenoids were reported from *P. camponutans* and *P. serpens*, respectively.[7,8] *Psychotria klugii* Standl.,[9] a native Amazonian shrub collected in Loreto, Peru, has not previously been subjected to biological or chemical analysis. An EtOH extract of the whole plant showed sufficient initial in vitro antileishmanial and antimalarial activities to warrant bioassay-guided fractionation. This led to the isolation of two potent antiparasitic alkaloids, klugine (**1**) and cephaeline (**3**),[10] together with 7′-*O*-demethylisocephaeline (**2**), isocephaeline (**4**),[10] and 7-*O*-methylipecoside (**5**).[11,12]

Results and Discussion

Acid−base extraction of the EtOH extract of *P. klugii* resulted in the localization of the antiparasitic activities in the alkaloidal fraction. Column chromatography followed by centrifugal preparative thin-layer chromatography of the ether fraction containing the crude alkaloids resulted in the isolation of compounds **1**−**5**, of which **3**−**5** were identified by comparison of their physical and spectral data with those previously reported.[10−12]

The molecular formula, $C_{27}H_{36}N_2O_5$, for **1** was established by HRESIMS. The UV spectrum demonstrated chromophores typical of the benzoquinolizidine (ipecac) alkaloids,[10] and its carbon skeleton was suggested from the NMR data (Table 1).[10,13,14] The ^{13}C NMR spectrum of **1** was found to be similar to those reported for (−)-6′-*O*-benzyl-9-demethylcephaeline,[14] except for differences associated with the presence of two hydroxyl groups at C-1′ (δ_C 79.5) and C-6′ (δ_C 146.4) and the absence of a benzyl group at C-6′. Furthermore, a close comparison of the ^{13}C NMR spectrum of **1** with those of other benzoquinolizidine derivatives,[10,14] such as cephaeline (**3**), isocephaeline (**4**), emetine (**6**), (−)-6′-*O*-benzyl-9-demethylcephaeline,[14] and 7′-*O*-demethylcephaeline, led to the conclusion that **1** is 1′-hydroxy-9-demethylcephaeline, which has not been reported previously as a natural product. Since the ^1H NMR data of cephaeline (**3**) were assigned unambiguously[10] and confirmed during the present investigation, the placement of the tertiary hydroxyl group at C-1′ was postulated, as the two multiplet signals due to the methylene group at C-14 in cephaeline (**3**)[15] (δ 2.10 and 1.54, 2 × m, H-14a and H-14b) were replaced in **1** by an ABC system as two doublets of doublets centered at δ 2.60 (dd, J = 3.1, 14.5 Hz, H-14a) and 1.15 (dd, J = 8.9, 14.5 Hz, H-14b). A ^1H−^1H COSY 2D NMR experiment indicated the presence of a $-CH-CH_2-CH(-CH_2)-CH(-CH_2)-CH_2-CH_3$ spin system in **1**. This allowed the assignment, using the HMQC experiment, of the following ^{13}C substructure: δ_{C-11b} 63.8, δ_{C-1} 40.6, δ_{C-2} 37.8, δ_{C-14} 37.0, δ_{C-3} 42.5, δ_{C-4} 62.2, δ_{C-12} 24.4, δ_{C-13} 11.5. The two methylene fragments of δ_{H-6} 3.09, 2.99 and δ_{H-7} 3.07, 2.65 were established by COSY correlations between these protons. The attachment of this fragment to δ_{C-7a} 127.8 was supported by an HMBC correlation from δ_{C-8} 116.2 to the H-7 protons. The HMBC correlation from δ_{C-6} 53.3 to δ_{H-11b} 3.17 confirmed the bonding through N-5 to close the ring B. The HMBC between δ_{C-11b} 63.8 and δ_{H-4} 2.12 and 3.08 confirms the closure of ring C. Also, the attachment of C-11b to C-11a was supported by the HMBC correlation from δ_{C-11} 109.7 to δ_{H-11b} 3.17. The ^{13}C NMR assignments of the two tetrasubstituted aromatic rings with OMe groups at C-10 and C-7′ and OH groups at C-9 and C-6′ positions, as observed in 9-demethylcephaeline,[10] were established by HMBC and NOESY correlations. The ^{13}C resonances at δ 147.8 and 147.6 (C-10/C-7′) were not distinguishable in the HMBC experiment, so the assignment of these two signals is left ambiguous. The same is true for the hydroxylated

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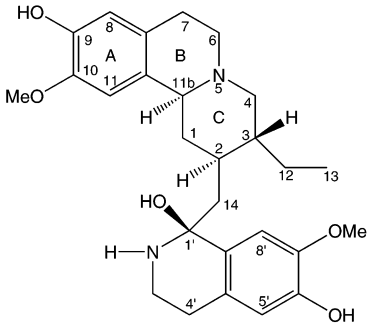

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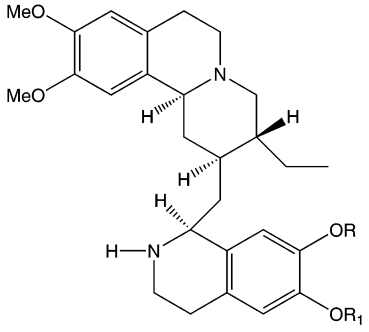

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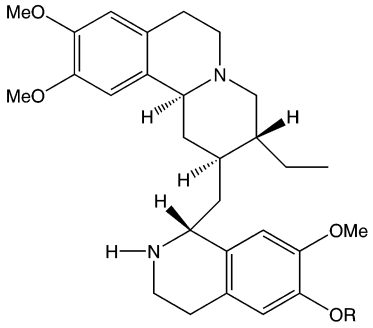

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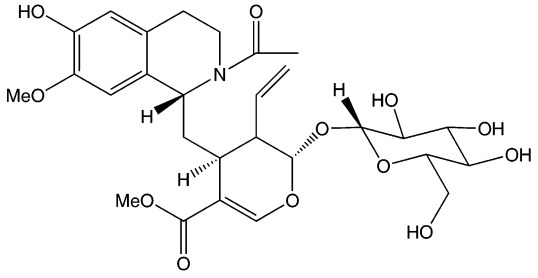

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aromatic carbons at δ 146.5 and 146.4 (C-9/C-6′). The placement of the methoxy groups was established by a ^3J HMBC correlation between δ_{C-10} 147.8 and δ 3.79 (OMe-10) and a NOESY correlation between δ 3.79 and 6.79 (H-11). The H-11 signal was distinguished from that of H-8 by an HMBC correlation from C-11 to H-11b. The placement of the second methoxy group at C-7′ was established analogously by the HMBC correlation between $\delta_{C-7'}$ 147.6 and δ 3.78 (OMe-7′) and a NOESY correlation between δ 3.78 and 6.68 (H-8′). H-8′ was distinguished from H-5′ by an HMBC correlation between $\delta_{C-1'}$ 79.5 and H-8′. The two methylene fragments of H-4′ (δ 2.74, 2.78) and H-3′ (δ 3.27, 3.03) were established by COSY correlations between these protons. The attachment of this fragment to $\delta_{C-4a'}$ 127.7 is supported by an HMBC correlation from $\delta_{C-5'}$ 116.4 to the H-4′ protons. On the basis of the foregoing data, the gross structure of **1** was established as shown.

The absolute configuration of compound **1** was resolved using optical rotation and circular dichroism experiments. A comparison of the CD spectra of **1** with cephaeline (**3**) and isocephaeline (**4**),[10] as well as emetine (**6**) and isoemetine,[16] was consistent with **1** being an analogue of the cephaeline/emetine series of compounds with *R* absolute configuration at C-1′. On the basis of this assumption the spatial orientation of the relevant protons of **1** was confirmed from the NOESY spectrum (Figure 1), which showed correlations between H-2 (δ 1.59), H-1α (δ 2.07), and H-11b (δ 3.17), indicating their *cis* (α-face) disposition. On the other hand, NOESY cross-peaks were observed between H-1β (δ 1.57), H-3 (δ 1.43), and H-14a (δ 2.60), thereby confirming the β-orientation of H-3. As a result, the C-14 methylene group could be placed on the β-face of the molecule. The NOESY spectrum also showed correlations between H-11b, δ 3.08 (H-4α), and H-12 (δ 1.69, 1.13), indicating their *cis* (α-face) disposition. The NOESY spectrum of **3** showed similar correlations as observed for **1**, in addition to the correlation between H-8 (δ 6.59) and H-1′ (δ 4.11).

Compound **1** exhibited a positive Cotton effect in the 215−230 nm region and a negative Cotton effect at λ 291 nm, respectively, in its CD spectrum. The relevance of these Cotton effects to the issue of absolute configuration at the C-11b and C-1′ benzylic stereocenters is explicable in terms of the aromatic quadrant rule.[17] When viewed along the C-9−C-11a−C-11b axis, N-5 and the C-4 methylene are in the positive lower right quadrant, and the C-1 methylene and C-2 methine are in the negative left lower quadrant for the *S* absolute configuration at C-11b shown in structure **1**. The contribution of these substituents to the observed Cotton effects will thus largely cancel each other out. However, when viewed along the C-6′−C-8′a−C-1′ axis, the C-3 methylene group is located in the positive left upper quadrant and the C-14 methylene group and all of its substituent tricyclic ring system are in the positive right lower quadrant. Collectively these quadrant projections then explain the high-amplitude positive Cotton effect for the $A_{1g} \rightarrow B_{1u}$ aromatic transition at 217 nm, and hence the *S* absolute configuration of compound **1** at C-1′. Since the signs of the quadrants are reversed when considering the ca. 290 nm ($A_{1g} \rightarrow B_{2u}$) transition because of the antipodal natures of "normal" aromatic ellipticities,[17] the negative Cotton effect at 291 nm in the CD spectrum of **1** was also readily explicable. Cephaeline (**3**) and emetine (**6**)[16] with their C-1′ *R* absolute configuration showed similar Cotton effects in the corresponding regions of their CD spectra. Compound **1** thus possesses the same spatial arrangement of substituents at C-1′ as cephaeline (**3**) and emetine (**6**),[16] but opposite absolute configuration due to a change of substituent priorities at this stereocenter. Collectively, the CD and NOESY data then permitted assignment of 2*R*,3*R*,-11b*S*,1′*S* absolute configuration to compound **1**.

The ^{1}H and ^{13}C NMR spectral data (Table 1) of **2**, $C_{27}H_{36}N_2O_4$, were generally similar to those observed for cephaeline (**3**) and isocephaeline (**4**), except for the presence of a hydroxyl group at C-7′, instead of an *O*-methyl functionality. In addition, the close comparison of the NMR and CD spectra of **2** with those of **1**, **3**, and **4** suggested that **2** is the 7′-*O*-demethyl derivative of isocephaeline. The

Table 1. ^{1}H and ^{13}C NMR Data for Compounds **1** and **2**

	1			**2**		
position	$^{1}H^{a}$	$^{13}C^{b}$	HMBC	$^{1}H^{a}$	$^{13}C^{b}$	HMBC
1	2.07 m, 1.57 m	40.6 t		2.71 m, 1.30 m	36.9 t[b]	
2	1.59 m	37.8 d	H-4	1.68 m	37.1 d	H-4
3	1.43 m	42.5 t	H-13	1.53 m	41.3 t	H-13
4	3.08 m, 2.12 m	62.2 t	H-2, H-11b H-12	3.18 m, 2.32 m	61.6 t	H-2, H-11b
6	3.09 m, 2.99 m	53.3 t	H-11b	3.20 m, 2.69 m	51.9 t	H-11b
7	3.07 m, 2.65 m	29.3 t	H-8	3.07 m, 2.95 m	25.3 t	H-8
7a		127.8 s	H-11, H-11b H-6		126.9 s	H-11, H-11b
8	6.52 s	116.2 d	H-7	6.77 s	112.1 d	H-7
9		146.5 s[c]	H-11		146.8 s	H-11 OMe-9
10		147.8 s[d]	H-8 OMe-10		148.0 s	H-8 OMe-10
11	6.79 s	109.7 d	H-11b	6.76 s	109.0 d	H-11b
11a		129.7 s	H-7, H-8		127.9 s	H-7, H-8
11b	3.17 brd (10.6)	63.8 d	H-4, H-6 H-11	3.47 brd (10.9)	62.7 d	H-2, H-11
12	1.69 m, 1.13 m	24.4 t		1.67 m, 1.10 m	23.3 t	
13	0.95 t (7.7)	11.5 q		0.87 t (7.5)	10.1 q	
14a	2.60 dd (3.1, 14.6)	37.0 t	H-3, OH-1′	2.35 m	38.0 t	H-3
14b	1.15 dd (8.9, 14.6)			1.65 m		H-1′
1′		79.5 s	H-8′	4.52 m	53.6 s	H-2, H-8′
3′	3.27 m, 3.03 m	41.0 t		3.52 m, 3.32 m	39.5 t	
4′	2.74 m, 2.78 m	28.5 t	H-5′	3.04 m, 2.67 m	27.6 t	H-5′
4a′		127.7 s	H-8′		123.2 s	H-8′
5′	6.55 s	116.4 d	H-4′	6.44 s	115.2 d	H-4′
6′		146.4 s[c]	H-8′		145.6 s	H-8′
7′		147.6 s[d]	H-5′		145.8 s	H-5′
8′	6.68 s	110.0 d		6.76 s	113.2 d	
8a′		129.7 s	H-4′, H-5′		126.0 s	H-4′, H-5′
OMe	3.79 s	56.8 q		3.82 s	55.4 q	
	3.78 s	56.6 q		3.80 s	55.8 q	
NH	7.86 s					

[a] Coupling constants (*J* values in Hz) are in parentheses. [b] Multiplicities of carbon signals were determined by DEPT (135°) experiments. [c,d] Interchangeable signals.

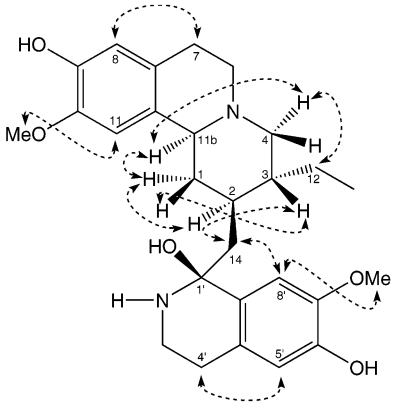

Figure 1. NOESY correlations of **1**.

OMe functions at the C-9 and C-10 positions were established by ^{3}J HMBC correlations between δ_{C-9} 146.8, δ 6.76 (H-11) and δ 3.82 (OMe-9), and δ_{C-10} 148.0, δ 6.77 (H-8) and 3.80 (OMe-10), as well as by NOESY correlations between δ 3.82 (OMe-9) and H-8, and δ 3.80 (OMe-10) and H-11. C-11 was distinguished from C-8 by the HMBC correlations between δ_{C-11} 109.0 and δ 3.47 (H-11b), and δ_{C-8} 112.1 and δ 3.07 (H-7). In addition, the HMBC spectrum showed correlations between δ 6.44 (H-5′), $\delta_{C-7'}$ 145.8, and $\delta_{C-8a'}$ 126.0, and δ 6.76 (H-8′), $\delta_{C-1'}$ 53.6, $\delta_{C-4a'}$ 123.2, and $\delta_{C-6'}$ 145.6, confirming the placement of the hydroxyl groups at C-6′ and C-7′. The configuration of **2** was assigned by comparison of NOESY and CD spectra with those observed for **4**. The CD spectrum of **2** showed a positive Cotton effect at λ 288 nm in MeOH, similar to those observed for isocephaeline (**4**) and also explicable in terms of the aromatic quadrant rule, suggesting 2*S*,3*R*,11b*S*,1′*S* absolute configuration for 7′-*O*-demethylisocephaeline (**2**). On the basis of these observations, the NOESY spectrum confirmed the spatial orientation of the relevant protons by showing correlations between H-2 (δ 1.68), H-11b (δ 3.47), and H-1α (δ 2.71), as well as between H-11b, δ 3.18 (H-4α) and H-12 (δ 1.67. 1.10), suggesting they are on the same face (α) of the molecule. On the other hand, the NOESY spectrum showed correlations between H-1β (δ 1.30) and H-3 (δ 1.53), thereby confirming the β-orientation of H-3. In addition, the NOESY spectrum also showed cross-peaks between H-8′ (δ 6.76), H-1′ (δ 4.52), and H-14a (δ 2.35), which was found to be in agreement with those observed for isocephaeline (**4**).

Antileishmanial activity evaluation of the isolated compounds revealed that alkaloids **1**, **3**, and **4** showed strong activity against *L. donavani* promastigotes (Table 2). Among these, cephaeline (**3**) was the most potent (IC_{50} 0.03 μg/mL) and was >20- and >5-fold more active than the antileishmanial agents pentamidine and amphotericin B, respectively (IC_{50} 0.7 and 0.17 μg/mL). On the other hand, emetine (**6**) displayed potent activity against *L. donavani* (IC_{50} 0.03 μg/mL), but was more toxic than cephaeline against VERO cells (IC_{50} 0.42 vs 5.3 μg/mL). In addition, klugine (**1**) and isocephaeline (**4**) also demonstrated strong activities, with IC_{50} values of 0.40 and 0.45 μg/mL, respectively, against *L. donavani*, and were devoid of significant toxicity against VERO cells. Antileishmanial activity for cephaeline (**3**) and isocephaeline (**4**) has not been reported previously, but emetine (**6**) and dehydroemetine were found to be highly active, but toxic, for the treatment of cutaneous leishmaniasis caused by *L. major*.[18,19] Because of the potent activity against *L. donavani* and the relatively low toxicity toward VERO and HL-60 cell lines (vide infra), compounds

Table 2. Antiparasitic Activities of Compound **1**−**4** and **6**[a]

	L. donovani (μg/mL)		*P. falciparum* (ng/mL)				VERO (μg/mL)
			D6[b]		W2[c]		
extract/compound	IC_{50}	IC_{90}	IC_{50}	SI[d]	IC_{50}	SI[d]	IC_{50}
P. klugii (EtOH extract)	3.3 ± 0.8	>50	600 ± 53.33	>79	940 ± 106.67	>51	NC
1	0.40 ± 0.02	2.5 ± 1	37.7 ± 1.77	>265	46.3 ± 0.44	>217	NC
2	7.5 ± 0.27	19 ± 0.67	1500 ± 66.67	>7	3067 ± 311	>3	NC
3	0.03 ± 0	1.5 ± 0.3	38 ± 0.67	132	27.7 ± 5.11	193	5.3 ± 1.1
4	0.45 ± 0.02	0.98 ± 0.15	186.7 ± 17.7	>54	290 ± 6.67	>35	NC
6	0.03 ± 0	0.13 ± 0	72.7 ± 24.89	6.0	78 ± 16	5.0	0.42 ± 0.01
chloroquine	NT	NT	18 ± 2	>556	176.7 ± 25.6	>56	NC
artemisinin	NT	NT	14.3 ± 1.8	>699	8.5 ± 1.5	>1176	NC
pentamidine	0.7 ± 0.1	2.4 ± 0.3	NT		NT		NT
amphotericin B	0.17 ± 0.01	0.28 ± 0.02	NT		NT		NT

[a] The IC_{50} and IC_{90} values are expressed as mean ± SEM of three determinations. [b]Chloroquine-sensitive clone. [c] Chloroquine-resistant clone. [d] Selectivity index = IC_{50} VERO cells/IC_{50} *P. falciparum*. NA= not active. NC= not cytotoxic (up to the maximum dose tested; 10 μg/mL for pure compounds and 47.6 μg/mL for crude extracts). NT= not tested.

Table 3. Cytotoxic Activities of Compounds **1**, **3**, and **4**[a]

	IC_{50} (μg/mL)			
compound	SK-MEL[b]	KB[c]	BT-549[d]	SK-OV-3[e]
1	>10	>10	>10	>10
3	0.25 ± 0.07	0.33 ± 0.08	0.86 ± 0.09	0.18 ± 0.01
4	>10	>10	>10	>10
doxorubicin	1.57 ± 0.11	1.7 ± 0.13	1.0 ± 0.13	1.53 ± 0.04

[a] The IC_{50} values are expressed as mean ± SEM of three determinations. [b] SK-MEL: human malignant melanoma. [c] KB: human epidermal carcinoma. [d] BT-549: human ductal carcinoma. [e] SK-OV-3: human ovary carcinoma.

Table 4. Cell Proliferation, Aggregation, and Adhesion Activities of Compounds **1**, **3**, and **6**[a]

compound	cell aggregation MIC (μg/mL) (A)	cell proliferation IC_{50} (μg/mL) (B)	specific index (B)/(A)	cell adhesion IC_{50} (μg/mL) (C)	specific index (B)/(C)
1	20.8 ± 0.65	25.0 ± 1.49	1.20	NA	
3	6.9 ± 0.67	14.9 ± 0.90	2.17	>10	
6	0.26 ± 0.06	0.38 ± 0.08	1.53	>50	
cytochalasin	2.5 ± 0.43	>30	13.0	5.5 ± 1.23	>5.5

[a] Assay system for inhibitors of LFA-1/ICAM-1-mediated aggregation combined with XTT assay as a primary assay. Following LFA-1/ICAM-1-mediated adhesion, assay was performed with HL-60 cells and CHO−ICAM-1 cells as a secondary assay. The IC_{50}/MIC values are expressed as mean ± SEM of three determinations.

1, **3**, and **4** warrant further investigation as potential antileishmanial drugs.

Antimalarial evaluation (Table 2) revealed that alkaloids **1** and **3** were equally potent against the chloroquine-resistant *P. falciparum* clone W2 (IC_{50} 46.3 and 27.7 ng/mL) and the chloroquine-sensitive clone D6 (IC_{50} 37.7 and 38 ng/mL, respectively), while **4** was weakly active and **2** was inactive against both these clones. In contrast, emetine (**6**) displayed strong activity against the W2 and D6 clones (**6**) (IC_{50} 78 and 72.7 ng/mL), but was >12-fold more toxic than **3** against VERO cells. Cephaeline (**3**) was reported to inhibit in vivo parasitemia against *P. berghei* at a dose of 6 mg/kg/day.[20] When tested for in vitro cytotoxic activity against the SK-MEL, KB, BT-549, and SK-OV-3 human cancer cell lines, cephaeline (**3**) was more potent than doxorubicin (IC_{50} values of 0.25, 0.33, 0.86, and 0.18 μg/mL; vs 1.57, 1.7, 1.0, and 1.3 μg/mL, respectively), while klugine (**1**) and isocephaeline (**4**) were devoid of cytotoxic activity against these cell lines. Alkaloids **1**, **2**, and **6** were also evaluated for their in vitro effects on lymphocyte-associated antigen-1 (LFA-1:CD11a/CD18)/intercellular adhesion molecule-1 (ICAM-1:CD54) mediated aggregation and adhesion using HL-60 and HeLa cell lines (Table 4).[21−23] Emetine (**6**) strongly inhibited cell aggregation (MIC 0.26 μg/mL, as compared to cephaeline (**3**) and cytochalasin B, which exhibited MIC values of 6.9 and 2.5 μg/mL, respectively), but was inactive in the cell adhesion assay (IC_{50} >50 μg/mL, vs 5.5 μg/mL for cytochalasin B). On the contrary, **1** was inactive in the primary cell aggregation assay, while cephaeline (**3**) and emetine (**6**) were >2- and >78-fold more cytotoxic than cytochalasin B, respectively (as determined by XTT assay).

This is the first report on isolation and characterization of klugine (**1**) and 7′-*O*-demethylisocephaeline (**2**), as well as the first report on antileishmanial activity of **3** and **4**. The C-1′ epimer of **2**, 7′-*O*-demethylcephaeline, has been reported previously from *Cephaelis accuminata*,[10] while **3** and **4**, including **6**, were isolated previously from several species of the genus *Cephaelis*, including *C. ipecacuanha* (ipecac; syn. *Psychotria ipecacuanha*, family Rubiaceae).[10,13] However, emetine and cephaeline are the major constituents reported from *C. ipecacuanha*,[10] while klugine (**1**), cephaeline (**3**), and isocephaeline (**4**) are the main alkaloids of *P. klugii*.

Experimental Section

General Experimental Procedures. Melting points (uncorrected) were recorded on an Electrothermal 9100 instrument. Optical rotations were measured using a JASCO DIP-370 digital polarimeter in MeOH at ambient temperature. UV spectra were obtained in MeOH, using a Hewlett-Packard 8452A spectrophotometer. CD spectra were recorded on a JASCO J-715 spectrometer. IR spectra were taken as KBr disks on an Ati Mattson (Genesis Series) FTIR spectrophotometer. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$ or C_5D_5N, using TMS as internal standard. Multiplicity determinations (DEPT) and 2D NMR spectra (COSY, HMQC,

HMBC) were run using standard Bruker pulse programs. The mixing time for the NOESY experiments was 500 ms. The delay for long-range coupling in the HMBC (1/2*J*) was set to 63 ms. The HRMS were obtained by direct injection using a Bruker Bioapex-FTMS with electrospray ionization (ESI). TLC was carried out on Si gel F254 plates, with the solvent system CH_2Cl_2−MeOH−NH_3 (95:5:0.1). For flash column chromatography, Si gel G (J. T. Baker, 40 μm flash) was used, and CH_2Cl_2−MeOH−NH_3 mixtures as solvents. Centrifugal preparative TLC (using a Chromatotron instrument, Harrison Research Inc. Model 8924) was carried out on 4 mm Si gel GF Chromatotron rotors (Analtech, Inc.), with the solvent system $CHCl_3$−MeOH−NH_3 (95:5:0.1). The isolated compounds were visualized using short wave under UV light (254 nm), followed by spraying with Dragendorff's reagent.

Plant Material. The whole plant of *P. klugii* was collected from high forest over clay in June 1998 from Loreto (Maynas), Peru, and identified by Manuel Y. Rimachi. A voucher specimen (IBE 12273) has been deposited at the Herbarium of Mississippi State University.

Extraction and Isolation. The powdered air-dried stem bark (450 g) was extracted by percolation with 95% EtOH (6 L × 3), and the combined extracts were evaporated under reduced pressure and then freeze-dried (yield 25 g). Antiparasitic screening of the EtOH extract showed activity against *P. falciparum* clone D6 and *L. donavani* (IC_{50} 0.6 and 3 μg/mL, respectively). The crude EtOH extract (20 g) was dissolved in 0.1 N HCl (1 L, pH 4), partitioned with $CHCl_3$ (500 mL × 4), and the combined $CHCl_3$ fraction was put aside. The defatted aqueous acidic fraction was basified with NH_4OH (pH 11) and partitioned with diethyl ether (500 mL × 4). The combined ether layer was dried over anhydrous Na_2SO_4 and evaporated under a vacuum to yield 10 g of a crude alkaloidal mixture. This alkaloidal extract demonstrated antiparasitic activities against *P. falciparum* D6 and W2 clones and *L. donovani* (IC_{50} 0.073, 0.098, and <0.19 μg/mL, respectively).

The ether fraction (9 g) was flash chromatographed on silica gel (350 g), using $CHCl_3$ (1 L) as eluent, to afford **5** as plates (55 mg, mp 220−222 °C (recrystallized from MeOH); $[\alpha]_D$ −170° (*c* 0.1, MeOH); lit.[11] mp unreported; $[\alpha]_D$ −194°). Further elution with $CHCl_3$−MeOH−NH_3 (95:5:0.1) yielded **3** as amorphous granules (300 mg; $[\alpha]_D$ −51° (*c* 0.1, MeOH); lit.[13] $[\alpha]_D$ −43.4°), followed by mixture A (3.5 g). Mixture A was subjected to centrifugal preparative TLC (using a Chromatotron; 4 mm Si gel rotor, flow rate 4 mL min^{-1}), using $CHCl_3$−MeOH−NH_3 (95:5:0.1) as solvent, to afford **4** as an off-white solid (40 mg; mp 118−120 °C (recrystallized from MeOH); $[\alpha]_D$ −80° (*c* 0.1, MeOH); lit.[12] mp 108−116 °C; $[\alpha]_D$ −69.8°), followed by **1** and **2** (20 and 25 mg, respectively). The spectral data (UV, IR, NMR, and HRMS) of **3**−**5** were in agreement with those reported in the literature.[10−12]

(−)-Klugine (1): light brown granules; mp 288−290 °C (recrystallized from MeCN−CH_2Cl_2); $[\alpha]_D$ −66° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (4.35), 244 (3.90), 315 (3.75), 328 (3.80), 450 br (3.65) nm; CD (MeOH) λ_{max} ([θ]) 217 (+1.7 × 10^4), 291 (−2.3 × 10^3) nm; IR (KBr) ν_{max} 2985−2820, 1694, (C=O), 1670 (C=O), 1615, 1575, 1480, 1440, 1430, 1375, 1350, 1330, 1300, 1285, 1265, 1100 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 469.2682 $[M + H]^+$ (calcd for $C_{27}H_{36}N_2O_5$, 469.2696), 451.2572 $[M - OH]^+$ (calcd for $C_{27}H_{35}N_2O_4$, 451.2591), (100%).

(−)-7′-Demethylisocephaeline (2): light brown solid (MeCN−CH_2Cl_2); mp 214−216 °C; $[\alpha]_D$ −47° (*c* 1.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 204 (4.3), 242 (3.85), 282 sh (3.10), 305 (3.65), 316 (3.68), 460 (3.55) nm; CD (MeOH) λ_{max} ([θ]) 225 (−9.4 × 10^3), 288 (+1.6 × 10^3) nm; IR (KBr) ν_{max} 3411 (N−H), 2950, 2820, 1690 (C=O), 1670 (C=O), 1624, 1541, 1528, 1457, 1316, 1259, 1018 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 453.2741 $[M + H]^+$ (calcd for $C_{27}H_{36}N_2O_4$, 453.2749).

Antileishmanial Assay. A transgenic cell line of *L. donovani* promastigotes showing stable expression of luciferase was used as the test organism. Cells in 200 μL of growth medium (L-15 with 10% FCS) were plated at a density of 2 × 10^6 cells per mL in a clear 96-well microplate. Stock solutions of the standards and test compounds/extracts were prepared in DMSO. Culture medium without cells and the controls were incubated (at 26 °C for 72 h) simultaneously, in duplicate, at six concentrations of the test compounds. An aliquot of 50 μL was transferred from each well to a fresh opaque/black microplate, and 40 μL of Steadyglo reagent was added to each well. The plates were read immediately in a Polar Star galaxy microplate luminometer. IC_{50} and IC_{90} values were calculated from dose−response inhibition graphs. Pentamidine and amphotericin B were tested as standard antileishmanial agents.

Antimalarial/Parasite LDH Assay. The in vitro antimalarial assay procedure[24] utilized was an adaptation of the parasite lactate dehydrogenase (pLDH) assay developed by Makler et al.,[25,26] using a 96-well microplate assay protocol with two *P. falciparum* clones [Sierra Leone D6 (chloroquine-sensitive) and Indochina W2 (chloroquine-resistant)]. The primary screening involved determination of pLDH inhibition (percentage) of each sample tested at 15.9 and 1.59 μg/mL for extracts and pure compounds, respectively. The IC_{50} values were determined only for samples that inhibited parasite growth by >50% for one of the clones. The antimalarial agents chloroquine and artemisinin were used as positive controls, with DMSO as the negative (vehicle) control.

Cytotoxicity Assay. The in vitro cytotoxic activity was determined against four human cancer cell lines, SK-MEL, KB, BT-549, and SK-OV-3 (Table 3), obtained from the American Type Culture Collection (ATCC, Rockville, MD). For initial (primary) evaluation, extracts and fractions were screened at a single concentration (100 μg/mL). Follow-up secondary assays were then conducted at three concentrations (10, 3.3, and 1.1 μg/mL), using a culture-treated 96-well microplate. The level of toxicity of each sample was determined by measuring the effect on a fibroblast cell line from African green monkey kidney (VERO; nontransformed). For secondary assays, IC_{50} values were determined from logarithmic graphs of growth inhibition values. The cytotoxic agents doxorubicin and 5-fluorouracil were used as positive controls, while DMSO was used as the negative (vehicle) control.

Cell Aggregation Assay, **XTT Assay for Cytotoxicity, and Cell Adhesion Assay.** These assays were carried out as described previously.[27]

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