Indole and β-Carboline Alkaloids from *Geissospermum sericeum*

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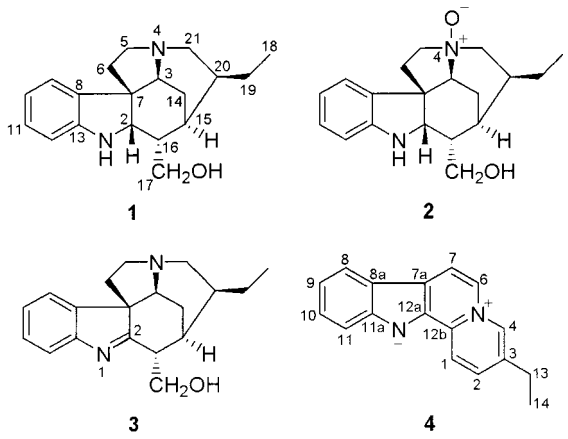
The indole alkaloid geissoschizoline (**1**) and two new derivatives, geissoschizoline N[4]-oxide (**2**) and 1,2-dehydrogeissoschizoline (**3**), were obtained from the bark of *Geissospermum sericeum* together with the β-carboline alkaloid flavopereirine (**4**). The in vitro antiplasmodial activity of these compounds was evaluated in chloroquine-resistant (K1) and chloroquine-sensitive (T9-96) *Plasmodium falciparum*. Their cytotoxicity was determined in a human (KB) cell line.

Geissospermum (Apocynaceae) is a small genus of Amazonian trees native to northern South America from Guyana to Brazil.[1] Several species are recognized locally as having antimalarial properties, including *G. laeve* (Vell.) Miers, *G. sericeum* (Sagot) Benth. & Hook.f., and *G. vellosii* Allemão.[2] These are usually taken as bark decoctions prepared in water (Brazil, Guyana, Surinam) or alcohol (French Guiana).[2] In our continuing investigation of plants used for the treatment of malaria[3] we report the isolation of three indole alkaloids and one β-carboline alkaloid from the bark of *G. sericeum*. The antiplasmodial activity of these compounds was assessed against both chloroquine-resistant (K1) and chloroquine-sensitive (T9-96) *Plasmodium falciparum*, and their cytotoxicity evaluated in a human (KB) cell line.

Fractions from a MeOH−H_2O (9:1) extract of the bark of *G. sericeum* selected on the basis of their antiplasmodial activity yielded four alkaloids on successive purification by VLC and preparative TLC. The similarity of the ^{1}H and ^{13}C NMR spectra of **1**−**3** indicated that these compounds were closely related. The molecular formula of **1** was determined to be $C_{19}H_{26}N_2O$ by HRMS. Of the 19 resonances observed in the ^{13}C NMR spectrum of **1**, three arose from quaternary carbon atoms, nine from methine, six from methylene, and one from a methyl carbon atom, according to DEPT experiments. The aromatic proton resonances in the ^{1}H NMR spectrum, their corresponding ^{13}C NMR resonance assignments (HSQC), and their long-range correlations to neighboring carbon atoms (HMBC) were typical of those of an indole alkaloid.[4] Furthermore, a key $^3J(^1H,^{13}C)$ correlation from H-9 (δ 7.04) to a quaternary carbon atom at δ 53.3 (C-7) observed in the HMBC spectrum indicated that **1** is a dihydroindole derivative with an extended fused ring system. The presence of characteristic resonances for CH_2OH (δ_H 3.67 and 3.74; δ_C 66.2) and Et groups (δ_H 0.92 (3H) and 1.21 (2H); δ_C 11.6 and 24.3, respectively) was also noted. The molecular structure of **1** was obtained by comprehensive analysis of two-dimensional NMR data, and long-range correlations obtained by HMBC are summarized in Table 1S (Supporting Information). From these data **1** was identified as the indole alkaloid geissoschizoline. An $[\alpha]_D$ value of +8° (*c* 3.97, MeOH) was measured for **1** and compared with the literature value of +32° (*c* 1, EtOH) obtained under different solution conditions.[5] A complete set of ^{1}H and ^{13}C NMR resonance assignments for **1** are given in Tables 1 and 2, respectively, as these have not been published previously.



The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, with the exception of a subset of resonances for which significant chemical shift changes were recorded. In particular, those of C-3, C-5, and C-21 showed downfield shifts of +13.0, +14.8, and +13.3 ppm, respectively. Smaller upfield shifts were noted for C-6 (−5.1 ppm), C-7 (−3.5 ppm), C-14 (−3.7 ppm), and C-20 (−4.1 ppm). The pattern of long-range connectivities observed for **2** by HMBC was identical to that of **1**, although some correlations were lost due to the selective broadening of the ^{1}H resonances of H-3, 5-CH_2, and 21-CH_2. HRMS of **2** provided a molecular formula of $C_{19}H_{26}N_2O_2$ and confirmed that **2** differs from **1** only in the incorporation of an additional oxygen atom into the structure of **2**. This oxygen was located at N-4 based on the large downfield chemical shifts observed for its neighboring carbon atoms compared with those of **1**, a feature that is characteristic of N-oxides.[6] Compound **2** was therefore identified as geissoschizoline N[4]-oxide.

The molecular formula of $C_{19}H_{24}N_2O$ determined for **3** by HRMS suggested that it might be a geissoschizoline derivative with one additional degree of unsaturation. Inspection of the ^{1}H NMR spectrum of **3** showed that the 1H doublet corresponding to H-2 in **1** and **2** was absent and that the aromatic resonances of H-9 to H-12 were all shifted downfield. In the ^{13}C NMR spectrum the methine resonance corresponding to C-2 in **1** and **2** was replaced by a quaternary carbon resonance at δ 192.9 in **3**. The

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Table 1. ^{1}H NMR Data for Compounds **1**−**3** in $CDCl_3$ (500 MHz, 30 °C, δ in ppm, J in Hz)

proton	**1**	**2**	**3**
2	4.00 (d, 5.5)	3.90 (d, 4.9)	
3	2.93 (br s)	3.39 (br s)	3.78 (br s)
5	2.95 (m)	3.64 (2H, m)	3.17 (m)
	3.09 (m)		3.27 (m)
6	2.27 (2H, m)	2.05 (m)	1.94 (dd, 13.7, 5.9)
		2.49 (dd, 14.4, 7.3)	2.76 (ddd, 13.6, 12.1, 6.9)
9	7.04 (d, 7.6)	7.04 (d, 7.3)	7.33 (d, 7.3)
10	6.76 (td, 7.6, 0.9)	6.69 (t, 7.3)	7.21 (td, 7.5, 0.9)
11	7.02 (td, 7.6, 0.9)	6.99 (td, 7.3, 0.8)	7.31 (td, 7.6, 0.9)
12	6.59 (d, 7.6)	6.52 (d, 7.7)	7.53 (d, 7.7)
14	1.63 (m)	1.94 (m)	1.08 (ddd, 13.8, 4.3, 2.3)
	2.20 (ddd, 13.4, 4.0, 2.4)	2.17 (br d, 11.6)	1.65 (m)
15	1.40 (br s)	1.36 (br s)	1.96 (br s)
16	1.87 (m)	1.93 (m)	2.88 (m)
17	3.67 (dd, 10.4, 5.5)	3.47 (dd, 10.8, 5.3)	3.87 (dd, 11.2, 7.4)
	3.74 ('t', 10.4)	3.73 ('t', 10.9)	3.94 (dd, 11.2, 4.0)
18	0.92 (t, 7.4)	0.84 (t, 7.3)	0.98 (t, 7.4)
19	1.21 (2H, m)	1.12 (m)	1.36 (2H, m)
		1.25 (m)	
20	1.57 (m)	2.05 (m)	1.76 (m)
21	2.31 (m)	3.27 (2H, m)	2.48 ('t', 12.2)
	2.86 (dd, 11.6, 4.6)		3.19 (m)

Table 2. ^{13}C NMR Data for Compounds **1**−**3** in $CDCl_3$ (125 MHz, 30 °C, δ in ppm)

carbon	**1**[a]	**2**	**3**
2	65.7	63.6	192.9
3	67.7	80.7	69.8
5	55.8	70.6	57.4
6	39.9	34.8	33.4
7	53.3	49.8	66.0
8	137.7	134.6	144.3
9	122.5	122.0	121.0
10	120.0	119.2	125.7
11	128.0	128.6	127.8
12	109.8	109.7	120.0
13	149.9	149.8	153.6
14	26.2	22.5	28.6
15	28.6	27.2	36.0
16	34.5	33.7	38.2
17	66.2	64.6	64.5
18	11.6	11.2	11.5
19	24.3	22.2	24.6
20	42.3	38.2	41.5
21	50.2	63.5	51.5

[a] δ values obtained from indirectly detected data sets.

assignment of this resonance to C-2 of **3** was confirmed by $^3J(^1H,^{13}C)$ long-range correlations from 6-CH_2 and 17-CH_2 and a weaker $^2J(^1H,^{13}C)$ correlation from H-16. Compound **3** was therefore identified as 1,2-dehydrogeissoschizoline. ^{1}H and ^{13}C NMR resonance assignments for **2** and **3** are listed in Tables 1 and 2, respectively.

Geissoschizoline has been reported previously as a constituent of *G. vellosii*,[7] but is also known as an acid hydrolysis product (2 M HCl) of the bisindole alkaloid geissospermine,[5] a constituent of the bark of several species of *Geissospermum*.[8] LC-MS analysis of the MeOH−H_2O (9:1) extract of *G. sericeum* bark was carried out to determine whether geissoschizoline was produced only as an artifact of the isolation procedure used here, as this incorporated a standard acid workup step with 0.5 M HCl (Experimental Section). The results confirmed that geissoschizoline was present as a significant component of the original extract. The derivatives 1,2-dehydrogeissoschizoline (**3**) and geissoschizoline N^4-oxide (**2**) were also detected in this analysis, but as minor components. The fact that a relatively large amount of the N^4-oxide was isolated relative to the dehydro derivative (Experimental Section) suggests that the concentration of the former may have increased as a result of the procedures used. Neither geissoschizoline N^4-oxide (**2**) nor 1,2-dehydrogeissoschizoline (**3**) has been reported previously as a natural product, although the latter can be obtained synthetically by oxidation of geissoschizoline with $KMnO_4$.[5]

Table 3. In Vitro Antiplasmodial Activity (K1 and T9-96 *P. falciparum*) and Cytotoxicity (KB Cell Line) of Alkaloids from *Geissospermum sericeum*

	IC_{50} (μM)[a]		
sample tested	K1	T9-96	KB
crude extract	1.78 (0.047)	nt	nt
1	>40	>40	>40
2	>40	>40	>40
3	27.26 (10.9)	35.37 (2.36)	>40
4	11.53 (0.54)	1.83 (0.10)	10.7 (1.45)
chloroquine	0.32 (0.076)	0.03 (0.004)	20.4 (6.89)

[a] Values quoted for the crude extract are in μg/mL. Numbers in parentheses are ± confidence limits; nt = not tested; number of replicates = 3.

The ^{1}H NMR spectrum of **4** acquired in DMSO-d_6 consisted principally of aromatic proton resonances together with those for an Et group attached to a quaternary carbon (δ 1.37, 3H, t, J = 7.5 Hz and 2.93, 2H, q, J = 7.5 Hz). The structural relationships among the aromatic protons and the location of the Et substituent were determined by site selective excitation of ^{1}H resonances in 1D ROE experiments using the XSROESY pulse sequence.[9] These through-space connectivities allowed the structure of **4** to be identified as that of the known β-carboline alkaloid, flavopereirine. This was supported by HRMS data, which gave a molecular formula for **4** of $C_{17}H_{14}N_2$, as expected. The ^{1}H NMR assignments obtained for **4** were also in agreement with a set of values published previously for a synthetic sample of flavopereirine.[10] This compound has been found naturally as a constituent of the bark of *G. laeve*, *Strychnos longicaudata*, and *S. melinoniana*.[8]

Bioactivity data for **1**−**4** and the MeOH−H_2O (9:1) extract of the bark of *G. sericeum* are summarized in Table 3. Of the four test compounds, the β-carboline alkaloid flavopereirine (**4**) had the most antiplasmodial activity against K1 and T9-96 strains of *P. falciparum* (IC_{50} = 11.53 and 1.83 μM, respectively). None of the compounds were as active as the positive control, chloroquine. The activity of flavopereirine was affected by the resistance phenotype

of K1, as indicated by a value of 6.3 for IC_{50}(K1)/IC_{50}(T9-96). Flavopereirine also showed moderate cytotoxic activity (IC_{50} = 10.7 μM) with essentially no selectivity of action against K1. Although the antiplasmodial activity of flavopereirine has not been tested previously, Wright et al. obtained an IC_{50} value of 3.02 μM for the related derivative 5,6-dihydroflavopereirine against K1 *P. falciparum*.[11] The cytotoxicity of flavopereirine has been noted previously because of its ability to selectively inhibit the synthesis of cancer cell DNA compared to that of healthy cells.[12] In contrast, both geissoschizoline (**1**) and its N^4-oxide (**2**) lacked antiplasmodial activity and cytotoxicity at 40 μM. The 1,2-dehydro derivative (**3**) showed some antiplasmodial activity against both K1 and T9-96 strains of *P. falciparum*. A degree of selectivity was indicated by the fact that it showed less than 50% inhibition of KB cell-dependent MTT reduction at 40 μM. Overall, the antiplasmodial activities of **1**−**4** did not account completely for the activity shown by the crude extract (Table 3). This suggests that some of the other components present in the alkaloid-rich fractions obtained from *G. sericeum* may have appreciable activity. The antiplasmodial activity demonstrated in vitro for extracts of *G. sericeum* and some of its constituent alkaloids appears to confirm earlier reports[2] describing the traditional use of this plant in the treatment of malaria.

Experimental Section

General Experimental Procedures. Specific optical rotation was determined using an AA-5 polarimeter (Optical Activity Ltd.) at 20 °C. UV−vis spectra were recorded on a Shimadzu UV-1601 spectrophotometer. ^{1}H and ^{13}C NMR spectra were acquired on either Varian 500 MHz or JEOL 270 MHz instruments, and standard pulse sequences and parameters were used for the experiments. All chemical shift values (δ) are given in ppm. Spectra were referenced to residual solvent signals with resonances at $\delta_{H/C}$ 7.25/77.0 ($CDCl_3$) and 2.50/39.5 (DMSO-d_6), relative to TMS. HRESIMS were determined using a Micromass LC TOF mass spectrometer calibrated with a PEG ammonium calibration solution. LC-MS data were recorded using a quadrupole ion-trap mass spectrometer (ThermoFinnigan, LCQ Classic) with an APCI source. Chromatographic separation of alkaloids in the MeOH−H_2O (9:1) bark extract of *G. sericeum* was achieved using a 150 mm × 4.6 mm i.d., 4 μm Phenomenex Polar RP column and a 1 mL/min linear gradient of 25−100% MeOH in 1% aqueous HOAc over 25 min. Survey scans were made in the range *m/z* 125−1200, and the most intense ion from each survey scan was subjected to MS−MS analysis using a collision energy of 45% and an isolation width of 3 amu. Alkaloids **1**−**4** were identified in the analysis by using retention time data and product ion spectra obtained from the isolated compounds.

Plant Material. Bark material of *G. sericeum* was collected by Dr. William Milliken from the State of Roraima, Brazil, as part of a study into the traditional use of antimalarial plants in northern Amazonia. The plant was authenticated at the Royal Botanic Gardens, Kew, U.K. A voucher specimen has been deposited at the Herbarium, Royal Botanic Gardens, Kew (Milliken 2239).

Biological Assays. In vitro antiplasmodial activity against chloroquine-resistant (K1) and chloroquine-sensitive (T9-96) *P. falciparum* was assessed as described previously.[3] Evaluation of cytotoxicity was carried out in vitro using a human cell line (KB).[13] Cell viability was assessed using a modified version of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.[14] Stock solutions (8 μM in DMSO) were diluted in RPMI 1640 medium (Sigma) as required and dispensed as 50 μL aliquots on a 96-well microtiter plate. A 50 μL aliquot of cell suspension (0.5×10^6 viable cells/mL in 20% FCS in RPMI 1640 medium) was added to each well before incubation of the plate for 48 h at 37 °C. A 10 μL aliquot of 5 mg/mL MTT in RPMI 1640 medium was then added to each well and the plate incubated for a further 15 min. Cell supernatants were discarded and the adhered cells on the bottom of each well solubilized in 175 μL of DMSO followed by 25 μL of Sørensen's glycine buffer, pH 10.5. The OD of each well was measured at 570 nm with a microplate reader (Molecular Devices Inc.), and IC_{50} values were calculated using Excel 5 (Microsoft) with XL fit Version 1.02 "add-in" (ID Business Solutions Ltd., U.K.). Chloroquine was used as a positive control in all three bioassays.

Extraction and Isolation. Soxhlet extraction of powdered bark of *G. sericeum* (39 g) in MeOH−H_2O (9:1) gave an extract yielding 7.1 g of residue after rotary evaporation. This was suspended in 500 mL of 0.5 M HCl and left to stand overnight before filtration into a separating funnel and sequential solvent partition by Et_2O, $CHCl_3$, and EtOAc (each 2 × 500 mL) to give fractions A−C, respectively. The aqueous layer was made alkaline (to pH ~10) by gradual addition of anhydrous Na_2CO_3 and partitioned sequentially with equal volumes (3 × 500 mL) of Et_2O, $CHCl_3$, and EtOAc to give fractions D−F, respectively. All fractions were evaporated to dryness in vacuo and tested for antiplasmodial activity. Fractions D−F had IC_{50} values of 10.15, 2.21, and 2.47 μg/mL, respectively, against chloroquine-resistant (K1) *P. falciparum*. They also exhibited similar TLC profiles and were combined, prior to VLC fractionation over a 15 μm Si column (4 × 4 cm) and sequential elution with $CHCl_3$−MeOH (40 × 25−30 mL). VLC fractions 27−40 (790 mg) were pooled on the basis of their similar TLC profiles and positive reaction with Dragendorff's reagent to give fraction IV, which was then separated by PTLC (× 3) with Et_2NH−toluene (1:9) to give fractions IV.1 (baseline, 245 mg), IV.2 (R_f = 0.32, 185 mg), and IV.3 (R_f = 0.50, 30 mg). Further fractionation of IV.1 by PTLC (3 plates) with MeOH−Et_2NH−toluene (1:1:8) yielded **1** (R_f = 0.35, 102.8 mg). Similarly IV.2 subjected to PTLC (× 2) with the same solvent system yielded **2** (R_f = 0.08, 54.9 mg) and **4** (R_f = 0.41, 3.2 mg), while IV.3 yielded **3** (R_f = 0.73, 12.2 mg) under the same conditions (PTLC × 1). The purified compounds **1**−**4** gave positive reactions with Dragendorff's reagent.

Geissoschizoline (1): $[\alpha]^{20}_D$ +8° (*c* 3.97, MeOH); UV (MeOH) λ_{max} 246, 300 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; APCI-MS *m/z* 299 $[M + H]^+$; HRESIMS *m/z* 299.2118 $[M + H]^+$ (calcd for $C_{19}H_{27}N_2O$, 299.2123).

Geissoschizoline N^4-oxide (2): $[\alpha]^{20}_D$ +31° (*c* 0.64, MeOH); UV (MeOH) λ_{max} 245, 300 nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; APCI-MS *m/z* 315 $[M + H]^+$; HRESIMS *m/z* 315.2072 $[M + H]^+$ (calcd for $C_{19}H_{27}N_2O_2$, 315.2073).

1,2-Dehydrogeissoschizoline (3): $[\alpha]^{20}_D$ −235° (*c* 0.49, MeOH); UV (MeOH) λ_{max} 255sh nm; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2; APCI-MS *m/z* 297 $[M + H]^+$; HRESIMS *m/z* 297.1956 $[M + H]^+$ (calcd for $C_{19}H_{25}N_2O$, 297.1967).

Flavopereirine (4): UV (MeOH) λ_{max} 235sh, 247sh, 291, 322sh, 347, 388 nm; ^{1}H NMR (DMSO-d_6, 500 MHz, 37 °C) δ 9.30 (1H, s, H-4), 9.02 (1H, d, J = 8.7 Hz, H-1), 8.93 (1H, d, J = 6.7 Hz, H-6), 8.75 (1H, d, J = 6.7 Hz, H-7), 8.40 (1H, d, J = 8.1 Hz, H-8), 8.32 (1H, d, J = 8.7 Hz, H-2), 7.86 (1H, d, J = 8.1 Hz, H-11), 7.67 (1H, td, J = 8.1, 1.0 Hz, H-10), 7.40 (1H, t, J = 7.7 Hz, H-9), 2.93 (2H, q, J = 7.5 Hz, 13-CH_2), 1.37 (3H, t, J = 7.5 Hz, 14-CH_3); ROE connectivities; H-2↔13-CH_2, H-4↔13-CH_2, H-4↔H-6, H-7↔H-8; APCI-MS *m/z* 247 $[M + H]^+$; HRESIMS *m/z* 247.1231 $[M + H]^+$ (calcd for $C_{17}H_{15}N_2$, 247.1235).

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Supporting Information Available: Long-range $^{1}H-^{13}C$ correlations obtained by HMBC for **1** in $CDCl_3$ (Table 1S). This material is available free of charge via the Internet at http://pubs.acs.org.

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