Ancistrobenomine A, the First Naphthylisoquinoline Oxygenated at Me-3, and Related 5,1′-Coupled Alkaloids, from the "New" Plant Species *Ancistrocladus benomensis*[1]

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Three new 5,1′-coupled naphthylisoquinoline alkaloids, ancistrobenomine A (**1**), 6-*O*-demethylancistrobenomine A (**2**), and 5′-*O*-demethylancistrocline (**3**), have been isolated from the stem bark of a botanically as yet undescribed highland liana *Ancistrocladus* sp., proposed to be named *"A. benomensis"* according to the region in Peninsular Malaysia where it has been discovered on the mountain of Gunung Benom. Two of the compounds possess an unprecedented structure with a novel hydroxymethylene group at C-3 of the fully dehydrogenated isoquinoline moiety. The structural elucidation was achieved by chemical, spectroscopic, and chiroptical methods. As typical of the so-called Ancistrocladaceae type, all of the compounds isolated bear an oxygen at C-6. Biological activities of these alkaloids against different protozoic pathogens are described.

The monogeneric plant family Ancistrocladaceae comprises nearly 30 species of lianas indigenous to the palaeotropical rain forests of Africa and Asia.[2] These plants are characterized by the presence of structurally, biosynthetically, and pharmacologically remarkable natural products, the naphthylisoquinoline alkaloids.[3] More than 100 alkaloids of this type have already been isolated, structurally assigned, and tested for their bioactivities, assisted by QSAR (quantitative structure−activity relationship) investigations,[4,5] showing great potential in particular with respect to antiprotozoal activities.[6−8] It thus seems highly rewarding to search for further such alkaloids in other, phytochemically still unexplored *Ancistrocladus* species, including possibly new, even botanically as yet undescribed species. We have recently discovered such a new species in the South of Malaysia, near the mountain Gunung Benom, at an altitude of 900 m above sea level. Morphological and first preliminary phytochemical investigations revealed that this liana is unequivocally different from the only known Malaysian species, *A. tectorius*;[9] it has therefore been given a name of its own, *Ancistrocladus benomensis*. The status of *A. benomensis* as a distinct species was confirmed in a molecular taxonomic study using comparative sequencing of the ITS region of nuclear rDNA and the *trnK* intron of cpDNA as well as Inter-Simple Sequence Repeat (ISSR) fingerprinting including 75 samples of *A. tectorius* from 24 locations in Southeast Asia.[10] We have meanwhile succeeded in cultivating the species and have recently obtained inflorescences, now permitting its scientific description, which is presently in progress.[11]

In this paper we report on the isolation, structural elucidation, and bioactivities of the remarkable, in part largely novel naphthylisoquinoline alkaloids from the bark of *A. benomensis*, the 5,1′-coupled compounds ancistrobenomine A (**1**), 6-*O*-demethylancistrobenomine A (**2**), and 5′-*O*-demethylancistrocline (**3**). Compounds **1** and **2** belong to the as yet very rare group of fully aromatic naphthylisoquinoline alkaloids that are optically active without the presence of stereogenic centers. Furthermore they are the first naphthylisoquinoline alkaloids whose methyl group at C-3 is functionalized by an additional hydroxy function; such a CH_2OH group at C-3 had previously been known only from the free, naphthalene-devoid isoquinoline alkaloid **4**, isolated from *A. tectorius*.[9] A compound similar to **1** and **2**, but without the benzylic oxygen at Me-3, viz., 6-*O*-methylhamateine (**5**), had also been known from *A. tectorius*.

Results and Discussion

Liquid−liquid separation and preparative HPLC of the CH_2Cl_2 extract of air-dried and ground bark material of *A. benomensis* yielded three nitrogen-containing compounds. Their UV and ^{1}H NMR spectra suggested that they were naphthylisoquinoline alkaloids.

HREIMS of the least polar compound established a molecular formula of $C_{25}H_{25}NO_5$. Its ^{1}H NMR spectrum looked different from the expected spectrum of a "normal" naphthyltetra- or naphthyldihydroisoquinoline alkaloid[12] because of the lack of resonances for the methyl groups at C-1 and C-3 between δ 1.0 and 1.8, the missing quartet of H-1 around δ 4.4, the lacking multiplet of H-3 in the region between δ 3.2 and 4.0, and the likewise unobserved signal of the diastereotopic geminal protons at C-4. The appearance of an additional aromatic singlet (δ 6.81), in turn, hinted at a fully dehydrogenated isoquinoline moiety, which was confirmed by significant downfield shifts of the ^{13}C NMR resonances for C-1, C-3, and C-4 (δ 158.6, 145.1, and 117.7, respectively; see Figure 2a). That additional

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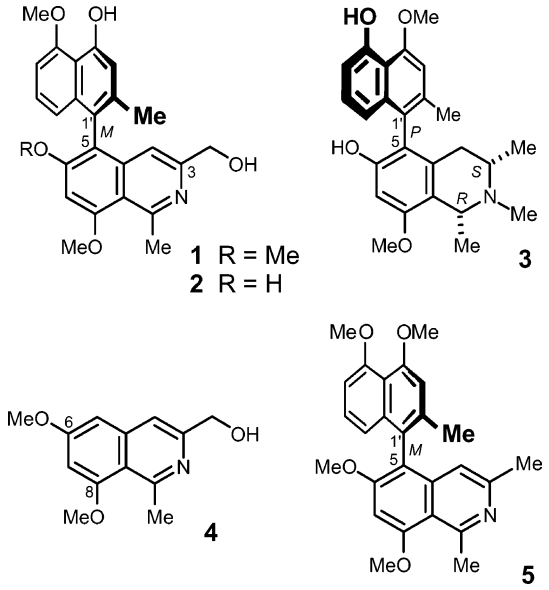

Figure 1. Structures of the new naphthylisoquinoline alkaloids **1**−**3**, isolated from *Ancistrocladus benomensis*, and the known 6,8-dimethoxy-3-hydroxymethyl-1-methylisoquinoline (**4**) and 6-*O*-methylhamateine (**5**) previously isolated from *A. tectorius*.

Table 1. Bioactivities of Compounds **1**−**3**

	IC$_{50}$ [μg/mL]		
	1	**2**	**3**
P. falciparum (strain: K1)	0.76	2.18	0.51
standard: chloroquine 0.041[a]			
T. cruzi	4.8	23.4	36.4
standard: benznidazole 0.55[a]			
T. b. rhodesiense	1.3	14.7	2.2
standard: melarsoprol 0.00095[a]			
L. donovani	>10	>30	18.8
standard: miltefosin 0.305[a]			
cytotoxicity L6 (MIC)	12.3	59.9	15.5

[a] IC$_{50}$ values in μg/mL.

aromatic proton showed an HMQC correlation with C-4 (not shown), which identified it as H-4; this was further supported by its NOESY interaction with CH_3-2′.

Three of the five oxygen functions were identified as methoxy groups from their three-proton singlets at δ 3.91, 4.22, and 4.12. These were located at C-6, C-8, and C-5′ due to their characteristic ^{1}H NMR shifts and their NOESY correlations of both OMe-6 and OMe-8 to H-7 and of OMe-5′ to H-6′ (Figure 2b). Thus, the two other oxygen functions remained to be hydroxy groups. One of the two OH groups, with the ^{1}H NMR signal at δ 9.52, was located at C-4′ by a NOESY correlation with H-3′. The second hydroxy group belonged to a hydroxymethylene group connected to C-3 of the isoquinoline moiety, as yet unprecedented in naphthylisoquinoline alkaloids. This structural feature had so far been known only for the free, naphthalene-devoid isoquinoline **4** from the also Southeast Asian species *A. tectorius*.[9]

In agreement with this assumption, the ^{1}H NMR spectroscopic data for the isoquinoline part of the new alkaloid, which was henceforth named ancistrobenomine A, were quite similar to those of **4**. The most notable difference was the multiplicity of the methylene protons, which appeared as a set of two geminal diastereotopic protons, due to the chirality of the molecule as a consequence of the rotationally hindered biaryl axis, showing doublets at δ 4.56 and 4.58, with a large coupling constant of 14.3 Hz. The signal at δ 60.4 was negative in the DEPT 135 spectrum, again confirming the respective carbon atom to possess only two

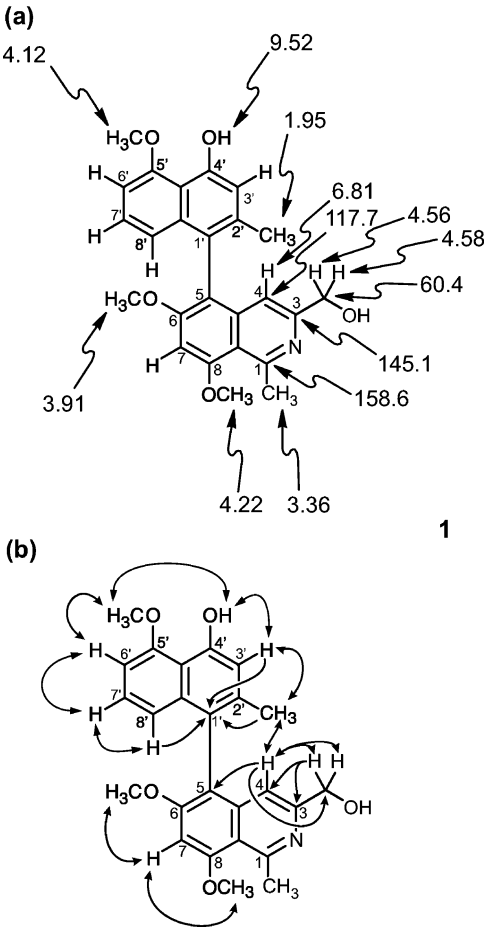

Figure 2. Selected NMR data of ancistrobenomine A (**1**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a), HMBC (single arrows), and NOESY (double arrows) (b) relevant for the constitution.

protons and its downfield shift corroborated the presence of a hydroxy function.

The position of this hydroxymethylene group at C-3 was assigned by NOESY interactions of both protons with H-4 and by HMBC correlations to C-3 and C-4. H-4 in turn showed an HMBC interaction with the carbon atom of the CH_2OH group (Figure 2b).

From the upfield shifted (δ 3.91) signal of the methoxy group of C-6, the biaryl axis was determined to be located at C-5 in the isoquinoline portion. This was in agreement with the presence of a proton at C-7 (i.e., the only other possible coupling position), as evident from the above-mentioned NOESY interactions with both OMe-6 and OMe-8.

The coupling position of the naphthalene moiety was clearly assigned to be at C-1′ because of the typical upfield shift[3] of the CH_3-2′ group (δ 1.95) and a continuous NOESY correlation sequence in the series CH_3-2′−H-3′−OH-4′−OCH_3-5′−H-6′−H-7′−H-8′. Furthermore, ^3J HMBC interactions from H-8′, H-3′, and CH_3-2′ to the quaternary carbon atom C-1′ confirmed the biaryl axis to be located at this position.

In conclusion, ancistrobenomine A is a 5,1′-coupled fully dehydrogenated naphthylisoquinoline alkaloid with an as yet unprecedented hydroxymethylene functionality at C-3. Its constitution is shown in Figure 2a.

With the lack of any stereogenic centers usually present at C-1 and C-3, the biaryl axis constitutes the only stereogenic element of ancistrobenomine A. Its absolute axial configuration was determined by comparison of its CD spectrum with that of the *P*-configured similar com-

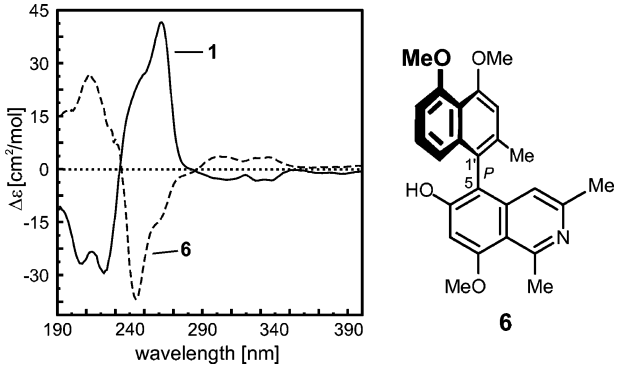

Figure 3. Comparison of the CD spectrum of **1** with that of the known *P*-enantiomer of ancistrocladeine (**6**) for the assignment of the absolute axial configuration of ancistrobenomine A (**1**).

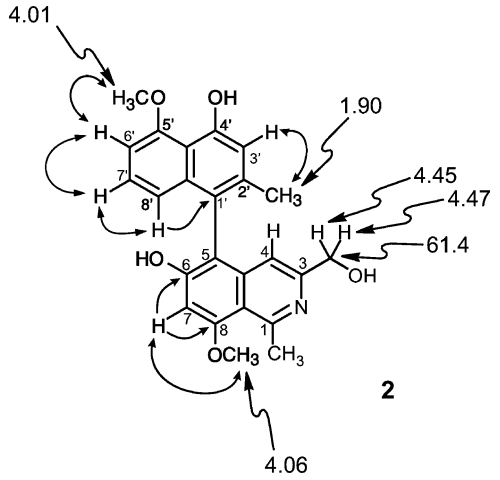

Figure 4. Selected NMR data of 6-*O*-demethylancistrobenomine A (**2**): ^{1}H and ^{13}C NMR shifts (δ in ppm), HMBC (single arrows), and NOESY (double arrows) relevant for the constitution.

pound ancistrocladeine[13] (**6**, Figure 3), a likewise 5,1′-coupled aromatic naphthylisoquinoline alkaloid, obtained only synthetically, by partial synthesis from ancistrocladine.[14,15] As its CD spectrum was found to be opposite of that of **1**, the biaryl axis of the latter was clearly evidenced to be *M*-configured (Figure 1).

Thus, ancistrobenomine A has the full absolute stereostructure **1**. Still, the question remained whether **1** occurs enantiomerically pure in the plant. HPLC analysis on a chiral phase (OD-H) resulted in one peak, even under most different chromatographic conditions. This, in combination with the finding that the CD trace of the early and the late slopes of the peak measured online showed the same LC-CD[16] spectra, evidenced ancistrobenomine A to be enantiomerically pure.

HREIMS of the second and most polar alkaloid indicated a molecular formula of $C_{24}H_{23}NO_5$, which again showed the presence of an "additional" fifth oxygen atom. In comparison with the structure of **1**, the loss of 14 molecular units, i.e., CH_2, suggested that the alkaloid was an *O*-demethylated derivative of **1**. Indeed, its ^{1}H NMR spectrum was in good agreement with that of ancistrobenomine A (**1**), showing a fully aromatized naphthylisoquinoline alkaloid with the set of two protons (δ 4.45 and 4.47) and the downfield shifted ^{13}C NMR signal at δ 61.4 typical of the hydroxymethylene function at C-3 (Figure 4). The most significant difference—apart from little variations of the chemical shifts caused by the different solvents MeOD and $CDCl_3$—was the lack of the upfield shifted methoxy group at C-6 (δ 3.91) next to the biaryl axis of the new compound. This confirmed the above supposed *O*-demethylation at C-6, as the remaining two methoxy groups (δ 4.01 and 4.06) were assigned to be located at C-5′ and C-8 by NOESY correlations to H-6′ and H-7, respectively (Figure 4). In contrast to this only NOESY interaction, H-7 showed HMBC interactions with both C-8 and C-6, the signals of which were both downfield shifted, thus again giving evidence that an oxygen function remained at position 6, but now a hydroxy group instead of an OMe.

In the naphthalene moiety, the spin pattern of three neighboring protons (H-6′, H-7′, and H-8′) and the upfield shifted methyl group at C-2′ (δ 1.90) left C-1′ or C-3′ to be the coupling position of the biaryl axis. The latter was excluded by a NOESY correlation sequence in the series OCH_3-5′−H-6′−H-7′−H-8′ and CH_3-2′−H-3′. The HMBC interaction of H-8′ with the quaternary carbon C-1′ finally confirmed this assignment. Thus, at least from its constitution, the compound is the—likewise 5,1′-coupled—6-*O*-demethyl analogue of **1**.

The absolute configuration at the biaryl axis was determined to be *M* by comparison of its CD data with that of **1**, which were similar, revealing the compound to possess structure **2**, i.e., the—also new—6-*O*-demethyl derivative of **1**; it was thus named 6-*O*-demethylancistrobenomine A.

The third alkaloid, the polarity of which was between that of compounds **1** and **2**, gave a molecular formula of $C_{25}H_{29}NO_4$ by HREIMS. Its ^{1}H NMR spectrum was typical of that of an *N*-methylated naphthyltetrahydroisoquinoline alkaloid with two methoxy groups (δ 3.90 and 4.15) and four further methyl groups (δ 1.39, 1.81, 2.20, and 2.95, Figure 5a). The one resonating at δ 2.95 was characteristic[17] of an *N*-methyl group, which was easily corroborated by its NOESY correlations to all proximal protons, i.e., H-1, CH_3-1, H-3, and CH_3-3 (Figure 5b).

The coupling position of the naphthalene moiety was deduced to be at C-1′ or C-3′ due to the presence of three neighboring protons, i.e., in the methyl-free ring consisting of the spin pattern H-6′, H-7′, and H-8′, and due to the upfield shifted signal of the methyl group at C-2′ (δ 2.20). A coupling via C-3′ was excluded, because this carbon was found to carry a proton that showed NOESY correlations to CH_3-2′ and to one of the two methoxy groups (δ 4.15), which, in addition, was not upfield shifted and thus remote from the biaryl axis. This led to the assignment of this OMe to be located at C-4′. The C-1′ coupling position was further corroborated by HMBC interactions of protons H-3′ and H-8′ with the quaternary carbon atom at position 1′. It is of further diagnostical value that the likewise quaternary C-10′ is also seen from the above-mentioned two protons via ^3J HMBC correlations, and additionally from H-6′.

The remaining aromatic proton at δ 6.61 was located to be at C-5 or C-7 in the isoquinoline portion. Of these two possible positions, the latter was assigned to be H-7 by its two HMBC correlations to downfield shifted carbon atoms at δ 158.7 and 156.9, indicating these positions to be oxygen-substituted. An additional NOESY interaction of this proton with the second methoxy group, thus located at C-6 or C-8, confirmed this assignment. The methoxy group was excluded to be at the 6-position, due to its NOESY correlation to CH_3-1. Furthermore, the chemical shift of δ 3.90 was typical of an OMe group located distal to the biaryl axis (in contrast to fully aromatic naphthylisoquinolines, where δ values of ca. 3.9 ppm may be indicative of the proximity of a biaryl axis, see below, while for tetrahydronaphthylisoquinolines, a high-field-shifted signal at ca. δ 3.60 would be expected for a methoxy function at C-6[12]). Therefore this naphthylisoquinoline was clearly assigned to be 5,1′-coupled and to be equipped with

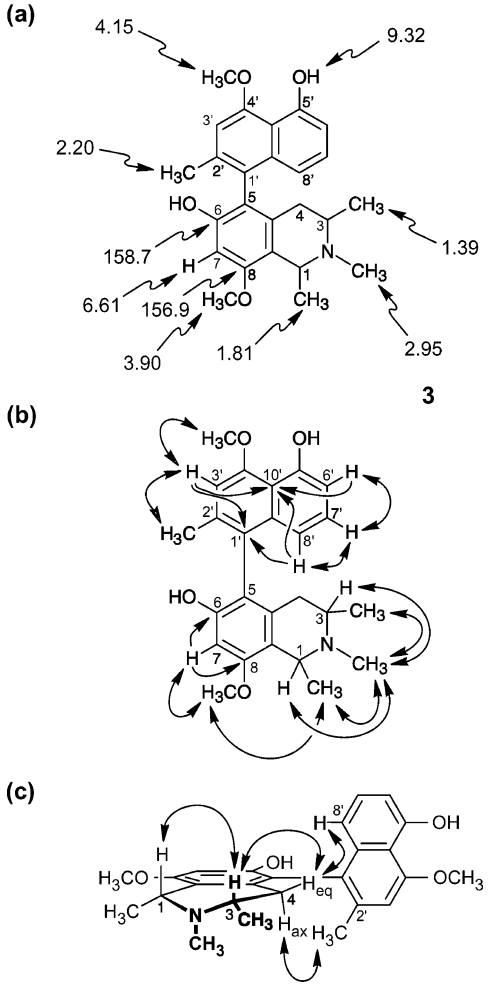

Figure 5. Selected NMR data of 5′-*O*-demethylancistrocline (**3**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a), HMBC (single arrows), and NOESY (double arrows) (b) relevant for the constitution; relative configuration at the biaryl axis and the stereogenic centers through NOESY interactions (c).

an *N*-methyl and two methoxy groups at C-4′ and C-8, leaving the other two downfield shifted and thus oxygenated carbon atoms, C-6 and C-5′, to bear free hydroxy groups.

Both the constitution and the spectroscopic data of this new natural product, except for the hydroxy group at C-5′, were in good agreement with those of the known alkaloid ancistrocline.[18,19]

The absolute configuration at C-3 was determined by ruthenium-mediated oxidative degradation.[20] The formation of (*S*)-3-methylaminobutyric acid proved the alkaloid to be *S*-configured at C-3. The relative configuration at C-1 versus C-3 was deduced to be *cis* from a NOESY interaction between H-3 and H-1 (Figure 5c), which, in conjunction with the absolute *S*-configuration at C-3, unambiguously indicated C-1 to be *R*-configured.

The absolute configuration at the axis was deduced from the comparison of its CD spectrum with that of the known[21] 5′,6-*O*-dimethyl derivative of this compound, *O*-methylancistrocline, whose CD data were similar, assigning the alkaloid to be *P*-configured, too. NOESY interactions both between H-4$_{eq}$ and H-8′ and between H-4$_{ax}$ and CH$_3$-2′, in conjunction with the *S*-configuration at C-3, unequivocally confirmed the axial *P*-configuration (Figure 5c). This new compound thus had the full absolute stereostructure **3** as shown in Figures 1 and 5c and was henceforth named 5′-*O*-demethylancistrocline.

Alkaloids **1** and **3** exhibited moderate antiplasmodial activities against the K1 strain of *P. falciparum* (resistant to chloroquine and pyrimethamine). Weak antitrypanosomal activities were exhibited by compounds **1** and **3** against the pathogen of African sleeping sickness, *T. b. rhodesiense*, while **1** was nearly 1 order of magnitude less active than the standard drug against *T. cruzi* (Chagas' disease). Compound **2** was less active against all parasites as compared to compounds **1** and **3**.

Conclusions

According to the alkaloids present in the bark material, the "new" species *Ancistrocladus benomensis* is unequivocally different from the only accepted Malaysian species, *A. tectorius*, in many respects.[9]

It possesses the second and third optically active fully aromatic naphthylisoquinoline alkaloids, i.e., **1** and **2**, ever isolated as natural products, with 6-*O*-methylhamateine (**5**)[22] from *A. cochinchinensis* as the first and only known representative so far, while all other fully dehydrogenated naphthylisoquinolines had been reported to be racemic.[13,23]

Furthermore, these alkaloids possess a hydroxymethylene group, i.e., a novel functionality at C-3, previously reported only from free, naphthalene-devoid isoquinoline alkaloids from *A. tectorius*.[9]

The occurrence of these unprecedented naphthylisoquinoline alkaloids in *A. benomensis* is an indication that there may be other as yet undescribed species with promising bioactive compounds within the polymorphic taxon *A. tectorius* sensu Van Steenis[24] in Southeast Asia. For example, samples recently assigned to *A. griffithii* show characteristic dimeric naphthylisoquinolines, not yet found in other *A. tectorius* plants.[25,26] A taxonomic concept to split the *A. tectorius* complex into different species is currently in preparation, and molecular markers for species identification have been identified.[10] With these markers a species assignment of samples used for pharmaceutical investigations is facilitated.

Work on the chemical constitutents in the leaves of this productive "new" plant species is in progress.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotations (25 °C, 10 cm cell) were measured on a Jasco P-1020 polarimeter. UV spectra were recorded on a Varian CARY 50 Conc UV−visible spectrophotometer, IR spectra were taken on a Jasco FT/IR-410 spectrometer, and CD spectra (25 °C, MeOH, 0.02 cm cell) were taken on a Jasco J-715 spectropolarimeter. ^{1}H NMR (400 MHz, 600 MHz) and ^{13}C NMR (100 MHz, 150 MHz) spectra were measured on Bruker Avance 400 and DMX 600 instruments, using $CDCl_3$ (δ 7.26 and 77.01) and CD_3OD (δ 3.31 and 49.15) as the solvents and internal ^{1}H and ^{13}C standards. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7 Hz) pulse sequences. EIMS and HREIMS were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments (70 eV), respectively. Preparative HPLC was carried out on a Symmetry C_{18} column (Waters, 19 × 300 mm, 7 μm), flow 11 mL min^{-1}, UV detection (233 nm), solvent (A) CH_3CN (0.05% trifluoroacetic acid), (B) H_2O (0.05% trifluoroacetic acid), linear gradient, 0 min 20% A, 25 min 55% A. Organic solvents were dried and distilled prior to use.

Plant Material. Bark material of *A. benomensis* was collected by three of us (H.R., K.W., and H.A.H.) in the region Gunung Benom, Pahang, Malaysia, in April 2000 (export permit number WKL00/149). The botanical description of this

species is in progress.[11] Voucher specimens are deposited at Herb. Bringmann (no. 61), University of Würzburg, Germany.

Extraction and Isolation. The air-dried material (190 g bark) was ground and extracted with 1.5 L of MeOH at room temperature. The MeOH extract was concentrated in vacuo to give 11.9 g of a residue, which was partitioned between MeOH/H_2O (90:10) and *n*-hexane. The MeOH/H_2O phase was reduced to ca. 10% of its volume in a vacuum and extracted with CH_2Cl_2. One gram of the dried CH_2Cl_2 extract was dissolved in MeOH and directly resolved using preparative HPLC with a Symmetry C_{18} column to give 1.1 mg of compound **2** (t_R = 19.3 min), 2.8 mg of compound **3** (t_R = 20.2 min), and 3.4 mg of compound **1** (t_R = 21.8 min).

Ancistrobenomine A (1): pale yellow powder; mp 270 °C (MeOH, dec); $[\alpha]_D^{25}$ −21.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 219 (1.79), 235 (2.09), 259 (1.95), 307 (0.85), 323 (0.81), 335 (0.85), 363 (0.58) nm; CD (MeOH) $\Delta\epsilon_{206}$ −25.2, $\Delta\epsilon_{212}$ −22.0, $\Delta\epsilon_{221}$ −27.6, $\Delta\epsilon_{261}$ +38.0; IR (NaCl) ν_{max} 3385, 3008, 2923, 2852, 1669, 1613, 1580, 1345, 1201, 1120, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.95 (3H, s, CH_3-2′), 3.36 (3H, s, CH_3-1), 3.91 (3H, s, OCH_3-6), 4.12 (3H, s, OCH_3-5′), 4.22 (3H, s, OCH_3-8), 4.56 (1H, d, *J* = 14.3 Hz, C*H*$_2$OH-3), 4.58 (1H, d, *J* = 14.3 Hz, C*H*$_2$OH-3), 6.49 (1H, d, *J* = 8.5 Hz, H-8′), 6.76 (1H, d, *J* = 7.7 Hz, H-6′), 6.81 (1H, s, H-4), 6.93 (1H, s, H-3′), 6.94 (1H, s, H-7), 7.10 (1H, dd, *J* = 7.7, 8.5 Hz, H-7′), 9.52 (1H, s, OH-4′); ^{13}C NMR ($CDCl_3$) δ 20.5 (CH_3-2′), 23.4 (CH_3-1), 56.3 (OCH_3-5′), 56.4 (OCH_3-8), 56.6 (OCH_3-6), 60.4 (*C*H_2OH-3), 96.3 (C-7), 103.7 (C-6′), 113.1 (C-3′), 113.9 (C-9), 114.1 (C-10′), 115.5 (C-5), 117.7 (C-4), 118.6 (C-8′), 119.4 (C-1′), 126.6 (C-7′), 135.8 (C-9′), 138.2 (C-2′), 140.9 (C-10), 145.1 (C-3), 154.9 (C-4′), 156.7 (C-5′), 158.6 (C-1), 162.2 (C-8), 163.9 (C-6); EIMS *m/z* 419 $[M]^+$ (100), 404 $[M - CH_3]^+$ (9), 388 $[M - OCH_3]^+$ (4); HREIMS *m/z* 419.1731 $[M]^+$ (calcd for $C_{25}H_{25}NO_5$, 419.1733).

6-*O*-Demethylancistrobenomine A (2): pale yellow amorphous solid; mp 169 °C (MeOH); $[\alpha]_D^{25}$ −34.4° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (0.44), 259 (0.28), 307 (0.07), 335 (0.08), 363 (0.05) nm; CD (MeOH) $\Delta\epsilon_{205}$ −24.4, $\Delta\epsilon_{213}$ −21.6, $\Delta\epsilon_{222}$ −27.1, $\Delta\epsilon_{259}$ +36.9; IR (NaCl) ν_{max} 3383, 2926, 1670, 1630, 1368, 1201, 1111, 753 cm^{-1}; ^1H NMR (MeOD) δ 1.90 (3H, s, CH_3-2′), 3.13 (3H, s, CH_3-1), 4.01 (3H, s, OCH_3-5′), 4.06 (3H, s, OCH_3-8), 4.45 (1H, d, *J* = 14.3 Hz, C*H*$_2$OH-3), 4.47 (1H, d, *J* = 14.3 Hz, C*H*$_2$OH-3), 6.53 (1H, d, *J* = 8.5 Hz, H-8′), 6.78 (1H, d, *J* = 7.7 Hz, H-6′), 6.79 (1H, s, H-3′), 6.82 (1H, s, H-4), 6.93 (1H, s, H-7), 7.03 (1H, dd, *J* = 7.7, 8.5 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 20.6 (CH_3-2′), 24.0 (CH_3-1), 57.3 (OCH_3-5′), 57.4 (OCH_3-8), 61.4 (*C*H_2OH-3), 102.8 (C-7), 105.3 (C-6′), 114.2 (C-3′), 114.4 (C-4), 114.5 (C-9), 114.7 (C-5), 115.1 (C-10′), 118.1 (C-8′), 120.0 (C-1′), 128.1 (C-7′), 136.2 (C-9′), 138.9 (C-2′), 141.2 (C-10), 145.2 (C-3), 155.6 (C-4′), 157.9 (C-5′), 158.4 (C-1), 163.6 (C-8), 164.1 (C-6); EIMS *m/z* 405 $[M]^+$ (100), 390 $[M - CH_3]^+$ (11), 374 $[M - OCH_3]^+$ (4); HREIMS *m/z* 405.1574 (calcd for $C_{24}H_{23}NO_5$, 405.1576).

5′-*O*-Demethylancistrocline (3): brownish amorphous solid; mp 213 °C (MeOH); $[\alpha]_D^{25}$ 61.7° (*c* 0.01, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (0.35), 227 (0.42), 307 (0.07), 319 (0.06), 335 (0.05) nm; CD (MeOH) $\Delta\epsilon_{197}$ +11.8, $\Delta\epsilon_{210}$ −11.3, $\Delta\epsilon_{219}$ −4.5, $\Delta\epsilon_{225}$ −5.5, $\Delta\epsilon_{239}$ +11.7; IR (NaCl) ν_{max} 3380, 3016, 2948, 2862, 2704, 1675, 1605, 1428, 1203, 1134, 758 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 (3H, d, *J* = 6.7 Hz, CH_3-3), 1.81 (3H, d, *J* = 6.7 Hz, CH_3-1), 2.15 (1H, dd, *J* = 17.4, 9.6 Hz, H_{ax}-4), 2.20 (3H, s, CH_3-2′), 2.79 (1H, dd, *J* = 17.4, 3.3 Hz, H_{eq}-4), 2.95 (3H, s, CH_3-N), 3.01 (1H, m, H-3), 3.90 (3H, s, OCH_3-8), 4.15 (3H, s, OCH_3-4′), 4.51 (1H, q, *J* = 6.7 Hz, H-1), 6.61 (1H, s, H-7), 6.63 (1H, dd, *J* = 8.3, 0.8 Hz, H-8′), 6.82 (1H, s, H-3′), 6.87 (1H, dd, *J* = 7.7, 0.8 Hz, H-6′), 7.24 (1H, dd, *J* = 8.4, 7.7 Hz, H-7′), 9.32 (1H, s, OH-5′); ^{13}C NMR ($CDCl_3$) δ 18.2 (CH_3-3), 19.8 (CH_3-1), 21.0 (CH_3-2′), 31.4 (C-4), 42.7 (*C*H_3-N), 56.1 (OCH_3-8), 56.9 (OCH_3-4′), 59.3 (C-3), 60.6 (C-1), 97.8 (C-7), 107.6 (C-3′), 111.1 (C-6′), 114.7 (C-10′), 115.3 (C-8′), 115.7 (C-9), 116.1 (C-5), 121.2 (C-1′), 129.9 (C-7′), 136.1 (C-9′), 139.4 (C-2′), 140.8 (C-10), 154.7 (C-6), 155.6 (C-5′), 157.1 (C-8), 157.3 (C-4′); EIMS *m/z* 407 $[M]^+$ (3), 392 $[M - CH_3]^+$ (100), 376 $[M - OCH_3]^+$ (43); HREIMS *m/z* 407.2095 $[M]^+$ (calcd for $C_{25}H_{29}NO_4$, 407.2097).

Oxidative Degradation of 3. The ruthenium(III)-catalyzed periodate degradation, derivatization of the resulting amino acids, and subsequent GC-MSD analyses were carried out as described previously.[20]

Biological Experiments. Antiparasitic activities against the pathogens *P. falciparum, T. cruzi, T. brucei rhodesiense,* and *L. donovani*, as well as cytotoxicities (rat skeletal myoblast L-6 cells), were assessed as described earlier.[27]

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