# Ancistroealaines A and B, Two New Bioactive Naphthylisoquinolines, and Related Naphthoic Acids from *Ancistrocladus ealaensis*[1]

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*Received May 19, 2000*

Two new 5,8′-coupled naphthylisoquinoline alkaloids, ancistroealaines A (**1**) and B (**2**), eleutherolic acid (**3**), and two naphthoic acids, ancistronaphthoic acid A (**4**) and B (**5**), have been isolated from *Ancistrocladus ealaensis*. Their structures were determined by spectroscopic, chemical, and chiroptical methods. Ancistroealaine A (**1**) exhibited activity against *Leishmania donovani* and *Trypanosoma cruzi in vitro.*

*Ancistrocladus ealaensis* J. Léonard (Ancistrocladaceae),[2] a tropical liana indigenous to Central Africa, belongs to the small monogeneric family of the Ancistrocladaceae, which consists of ca. 20 species.[3] Prominent among the secondary metabolites of these plants and the closely related Dioncophyllaceae are mono- and dimeric naphthylisoquinoline alkaloids.[4] These natural biaryls are characterized by their unprecedented origin of isoquinoline alkaloids from acetate,[5] their promising biological activities,[6] and their intriguing chemotaxonomic implications.[7] Phytochemical investigations on *A. ealaensis* by Cavé et al. revealed the presence of five naphthylisoquinoline alkaloids, but neither have their relative or absolute configurations been assigned nor have any bioactivities been reported for these compounds.[8−10] Given the high antimalarial activities of some naphthylisoquinoline alkaloids from West African Dioncophyllaceae and Ancistrocladaceae species[11] and the likewise promising anti-HIV activities of the michellamines, dimeric naphthylisoquinoline alkaloids from the Cameroonian liana *Ancistrocladus korupensis*,[12] a thorough phytochemical investigation of the alkaloids of *A. ealaensis* from the Congo region and their pharmacological evaluation seemed rewarding. In this paper, we describe the isolation and structural elucidation of two new bioactive naphthylisoquinoline alkaloids, ancistroealaines A (**1**) and B (**2**), from *A. ealaensis*, and of three structurally related naphthoic acid derivatives, eleutherolic acid (**3**) and ancistronaphthoic acids A (**4**) and B (**5**) (Figure 1).

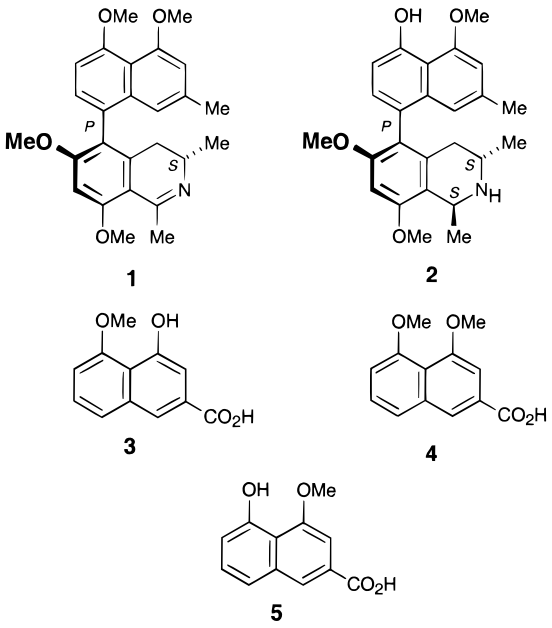


**Figure 1.** Natural products from *Ancistrocladus ealaensis.*

## Results and Discussion

*A. ealaensis* was collected in the Democratic Republic of Congo. Leaves, stem bark, and roots were air-dried, powdered, and successively extracted with petroleum ether, $CH_2Cl_2$, and MeOH. While the petroleum ether extract was found to contain the well-known[13] phytosterol $\beta$-amyrin,[14] the $CH_2Cl_2$ extract of the stem bark proved to be a rewarding source of novel metabolites. This extract was thus prefractionated by CC and finally resolved by preparative HPLC. The molecular formula of the first, quite lipophilic, compound, **1**, was $C_{26}H_{29}NO_4$, as deduced from HRMS. $^1$H NMR investigations indicated the presence of a naphthylisoquinoline alkaloid (Figure 2a). The position of the biaryl axis in the naphthalene moiety was suggested from the "normal", not high-field-shifted position of the $CH_3$-2′ signal ($\delta$ 2.31) and the spin system pattern of the aromatic protons (two doublets and three singlets), excluding the axis from being located at C-1′ or C-3′. Since only one out of four methoxy groups ($\delta$ 3.77, 3.96, 3.98, and 4.05) was high-field shifted, it had to be located at C-6 of the isoquinoline, which was confirmed by the HMBC interaction of that group with C-6 (Figure 2b). As a consequence of the chemical shifts of the methoxy groups, the axis could not be neighbored by $OCH_3$-4′ or $OCH_3$-5′, and thus, with respect to the naphthalene part, it had to be located at C-8′. This conclusion was further confirmed by ROESY interactions between $H_{eq}$-4 and H-1′ (Figure 2c), which, in turn, suggested a coupling at C-5 of the isoquinoline moiety. Again, this assumption was corroborated by HMBC interactions from both H-7 and H-7′ with C-5 and by the "normal" chemical shift of $OCH_3$-8 ($\delta$ 4.05), which clearly excluded the axis from being located at C-7. In conclusion, compound **1** is a 5,8′-coupled naphthylisoquinoline.

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10.1021/np000247+ CCC: $19.00 
Published on Web 09/08/2000

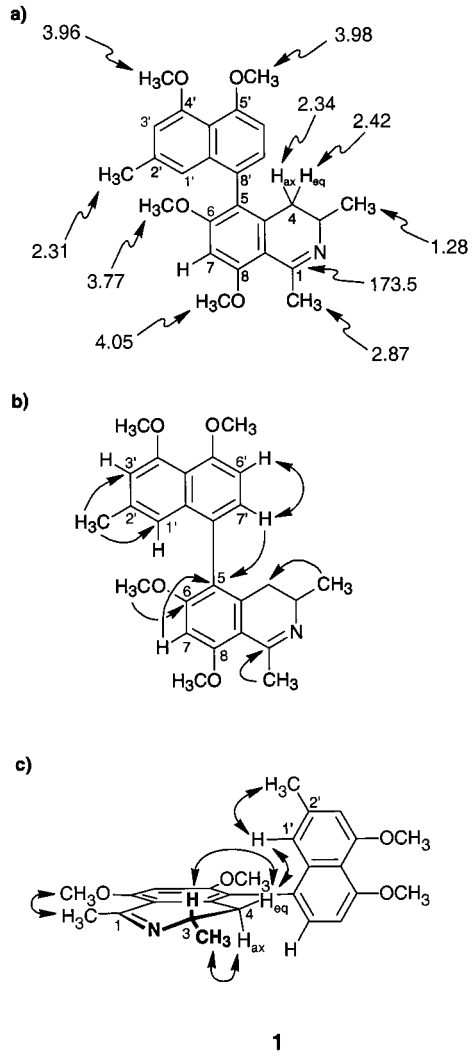


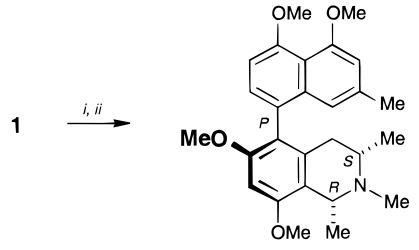

**Figure 2.** Selected NMR data of ancistroealaine A (**1**): $^1$H and $^{13}$C NMR shifts ($\delta$ values in ppm) (a), HMBC (single arrows) and H,H-COSY (double arrow) interactions (b) relevant for the constitution; relative configuration at the biaryl axis through ROESY interactions (c).

The presence of a double bond between C-1 and N was deduced from the chemical shift ($\delta$ 2.87) of the $CH_3$-1 signal in the $^1$H NMR spectrum and its multiplicity (singlet) and from the chemical shift of the C-1 peak ($\delta$ 173.5) in the $^{13}$C NMR spectrum. This assumption was further corroborated by the absence of an H-1 signal, which normally appears around 4 ppm in 1,2,3,4-tetrahydroisoquinolines.[4] Since no naphthylisoquinoline alkaloid with this constitution had been described, compound **1** was new and was thus given the name ancistroealaine A.

The absolute configuration of **1** at C-3 was determined by ruthenium-mediated oxidative degradation[15] followed by stereochemical analysis of the degradation products by gas chromatography with mass selective detection (GC-MSD) after derivatization with the *R*-enantiomer of Mosher's chloride. The formation of (*S*)-3-aminobutyric acid clearly showed the alkaloid to be *S*-configured at C-3.

The configuration at the biaryl axis relative to the now known configuration at C-3 was assigned as *P* by the already mentioned (see above) specific ROESY interaction between $H_{eq}$-4 and H-1′ (Figure 2c). The close similarity of the CD spectrum of **1** with that of the related *P*-configured naphthylisoquinoline ancistrobertsonine C (**6**) from *Ancistrocladus robertsonium*[16] again supported the axial *P*-configuration attributed to **1** above. Thus, ancistroealaine A must have stereostructure **1**.

**Scheme 1.** Partial-Synthetic Transformation of Ancistroealaine A (1) into Ancistrobertsonine C (**6**)[a]



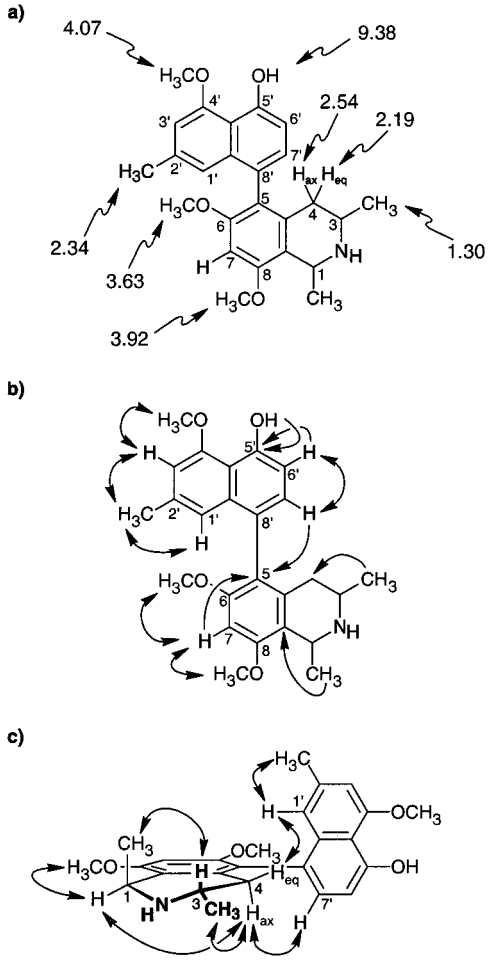

[a] (i) $NaBH_4$, MeOH, RT; (ii) HCHO, MeOH, RT; then $NaBH_4$, MeOH, RT.

This structural assignment was further confirmed by partial-synthetic preparation of **6** from **1** by *cis*-selective reduction (dr > 95:5) with $NaBH_4$ according to a procedure elaborated earlier,[17] followed by reductive *N*-methylation with formaldehyde−$NaBH_4$ to give **6** (Scheme 1). The product was identical with an authentic sample of natural **6**, by HPLC coelution and by CD and NMR spectra.

The second compound isolated had the molecular formula $C_{25}H_{29}NO_4$, as deduced by HRMS of the $[M - CH_3]^+$ peak. $^1$H NMR experiments and EIMS data ($M^+$ *m/z* 407) again indicated the presence of a naphthylisoquinoline alkaloid related to **1**, but having only three methoxy groups. In the naphthalene moiety the biaryl axis again was placed at C-8′ because of the "normal" $^1$H NMR chemical shift (Figure 3a) of the $CH_3$-2′ group ($\delta$ 2.34), the HMBC interaction of H-6′ with C-5′ (Figure 3b), and the spin system pattern of H-6′ and H-7′. The free OH group was located at C-5′ as deduced from HMBC interactions between OH and C-5′. In the isoquinoline moiety, coupling at C-7 was excluded by the HMBC interactions found between H-7 and both C-8 and C-6 (Figure 3b).

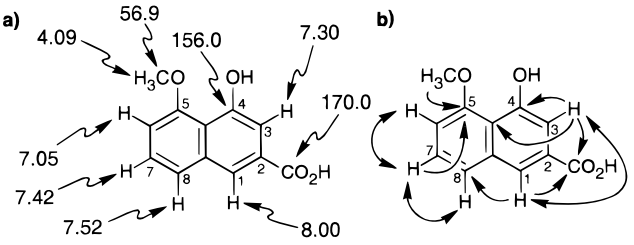
The ROESY interaction between $CH_3$-1 and H-3 (Figure 3c) revealed the relative configuration of C-1 vs C-3 to be *trans*. As for **1**, application of the oxidative degradation method established the absolute configuration at C-3 of the second alkaloid to be *S*, from which, given the relative *trans*-configuration as evident from NMR (see above), the absolute configuration of C-1 also had to be *S*. Again on the basis of that absolute stereoarray in the tetrahydroisoquinoline part, the configuration at the axis was determined as *P* by specific ROESY interactions between $H_{ax}$-4 and H-7′ and between $H_{eq}$-4 and H-1′. This absolute axial configuration was confirmed by the close resemblance of its CD spectrum with that of **6**. In conclusion, this new alkaloid, named ancistroealaine B, must have structure **2**.

Further resolution of the third CC fraction by preparative RP-HPLC yielded colorless crystals, homogeneous according to analytical TLC. In solution, the compound displayed a strong blue fluorescence. The molecular formula $C_{12}H_{10}O_4$ of this nonalkaloidal compound was deduced by HRMS. The $^1$H NMR spectrum showed the signals of one methoxy group ($\delta$ 4.09), two aromatic protons ($\delta$ 7.30 and 8.00) with a typical *meta* coupling, and three neighboring aromatic hydrogens ($\delta$ 7.05, 7.42, and 7.52). The $^{13}$C NMR spectrum revealed resonances corresponding to a methoxy group ($\delta$ 56.9), five tertiary and five quaternary aromatic carbons, and a signal typical of an aromatic carboxylic acid ($\delta$ 170.0). This, together with the molecular formula, indicated the presence of a hydroxy- and methoxy-substituted naphthoic acid. The methoxy group was located at C-5 by the HMBC interaction from that *O*-methyl group to C-5 and the HMBC interaction of that carbon atom with



**2**

**Figure 3.** Selected $^1H$ and $^{13}C$ NMR shifts ($\delta$ values in ppm) (a), HMBC (single arrows) and H,H-COSY interactions (double arrows) (b) relevant for the constitution; relative configuration at the stereogenic centers and at the biaryl axis of ancistroealaine B (**2**) through ROESY interactions (c).

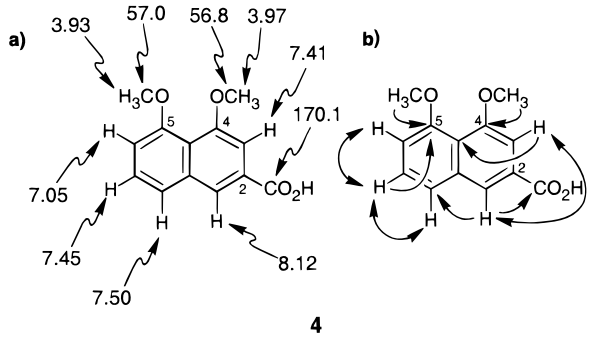


**3**

**Figure 4.** Selected NMR data of eleutherolic acid (**3**): $^1H$ and $^{13}C$ NMR shifts ($\delta$ values in ppm) (a), as well as HMBC (single arrows) and H,H-COSY (double arrows) interactions (b).

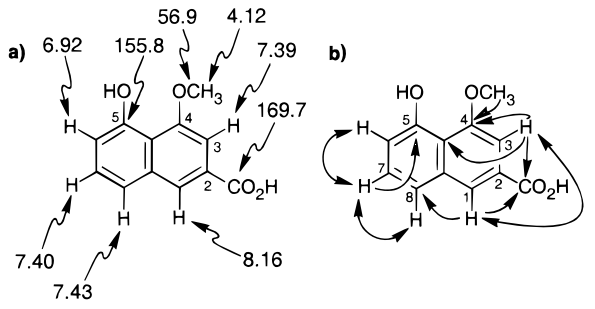
H-7. HMBC interactions between H-1/H-3 and the carboxy C atom also allowed for the localization of the carboxylic function, which is attached to C-2 (Figure 4). The hydroxy group was found to be in the 4-position, by the typical $^{13}C$ NMR chemical shift of C-4 ($\delta$ 156.0), located by its HMBC interaction with H-3. Following this, the spin system pattern and the HMBC experiments confirmed this compound to be 4-hydroxy-5-methoxy-2-naphthoic acid (**3**).

Naphthoic acid **3** had previously been described as a synthetic degradation product (named eleutherolic acid)



**4**

**Figure 5.** Selected $^1H$ and $^{13}C$ NMR shifts ($\delta$ values in ppm) (a), and HMBC (single arrows) and H,H-COSY (double arrows) interactions (b) of ancistronaphthoic acid A (**4**).



**5**

**Figure 6.** Selected $^1H$ and $^{13}C$ NMR shifts ($\delta$ values in ppm) (a), and HMBC (single arrows) and H,H-COSY (double arrows) interactions (b) of ancistronaphthoic acid B (**5**).

obtained from the natural product eleutherol,[18] but **3** itself had not been reported in nature.

Two additional compounds were isolated from the third CC fraction, showing a close structural relationship to **3** according to $^1H$ and $^{13}C$ NMR. Two three-proton singlets ($\delta$ 3.97, 3.93) in the $^1H$ NMR spectrum of the first one of these two compounds (Figure 5a) indicated the presence of an additional methoxy group compared with **3**, which was confirmed by the molecular formula $C_{13}H_{12}O_4$ deduced from HRMS and by the presence of two $OCH_3$ signals ($\delta$ 57.0, 56.8) in the $^{13}C$ NMR spectrum. HMBC experiments (Figure 5b) indicated this compound to possess structure **4** and, thus, to be the *O*-methyl analogue of **3**. Compound **4** is known as a synthetic compound, prepared from partial-synthetic **3**.[18] All physical and spectroscopic data of **4** fully matched those published earlier; its occurrence as a natural product, however, is reported here for the first time. Because of its close structural and biosynthetic relationship to the Ancistrocladaceous alkaloids (e.g., to ancistroealaines A (**1**) and B (**2**)), we have named it ancistronaphthoic acid A.

HRMS indicated the molecular formula $C_{12}H_{10}O_4$ for compound **5**. The $^1H$ and $^{13}C$ NMR spectra were similar to those of **3**, with only minor differences with regard to the chemical shifts (Figure 6a). The HMBC interactions of H-1 with C-8 and with the carboxy carbon allowed the placement of the carboxylic function at the 2-position. The methoxy group was found to be located at C-4, as indicated by the HMBC interaction (Figure 6b) from H-3 to both the carboxy carbon and C-4, together with the HMBC interaction between the $OCH_3$ protons and C-4. The OH group was placed at C-5 by the HMBC interaction between H-7 and C-5 together with the chemical shift of C-5 ($\delta$ 155.8), establishing this naphthoic acid to have structure **5**. Ancistronaphthoic acid B (**5**) has not been previously described in the literature.

**Table 1.** Bioactivities of the Compounds **1** and **2**

| | $IC_{50}$ [$\mu$g/mL] | |
|---|---|---|
| | **1** | **2** |
| *P. falciparum* (strain: K1) standard: chloroquine 0.065 | 1.2 | 0.52 |
| *P. falciparum* (strain NF54) standard: chloroquine 0.0044 | 4.0 | 0.79 |
| *T. cruzi* standard: benznidazole 0.3 | 2.35 | 17.6 |
| *T. b. rhodesiense* standard: melarsoprol 0.0063 | 3.46 | 2.03 |
| *L. donovani* standard: pentostam 49 | 4.1 | 10 |
| cytotoxicity L6 (MIC) standard: > 90 | >90 | 90 |
| cytotoxicity macroph. (MIC) standard: >30 | >30 | >30 |

The naphthoic acids **3**−**5** are apparently closely related to the isocyclic parts of the naphthylisoquinoline alkaloids, which are derived from acetate units, both for the naphthalene and the isoquinoline portions.[5] Until now, only some likewise "overoxidized" acetogenic naphthoquinones and tetralones, apparently derived from the same 4,5-dihydroxy-2-methylnaphthalene precursor, had been identified from Ancistrocladaceae and phylogenetically related plant sources.[4,19−26]

In the earlier work on *A. ealaensis*, the isolation and structural investigation of five naphthylisoquinoline alkaloids had been described by a French group.[8−10] None of those natural products, which had been attributed 5,1′- and 7,1′-coupled naphthylisoquinoline structures, however, seem to be identical with the 5,8′-coupled new naphthylisoquinoline alkaloids **1** and **2** described in this paper. The latter differ not only by their coupling type but also by their *O*-methylation pattern.

Ancistroealaines A (**1**) and B (**2**) represent pure "Ancistrocladaceae-type" alkaloids, *S*-configured at C-3 and equipped with an oxygen function at C-6. With respect to these structural features, *A. ealaensis* resembles the Asian *Ancistrocladus* species[4,27−29] and the East African species *A. robertsoniorum*,[16,30] which all have been found to produce this subtype of naphthylisoquinoline alkaloids, exclusively. All of the West African *Ancistrocladus* species that have been investigated so far contain "Dioncophyllaceae-type" alkaloids (i.e., with *R*-configuration at C-3 and without oxygen at C-6)[4] and also all sorts of hybrid-type forms.[4,31−35]

Several naphthylisoquinoline alkaloids are known[4,6,11,24] to be highly active against *Plasmodium falciparum*, the pathogen of malaria, which causes more then one million deaths per year.[36] Naphthylisoquinolines **1** and **2** also showed significant antimalarial activities (Table 1). Antiplasmodial activity of **1** and **2** was more pronounced against the chloroquine-resistant K1 strain than against the chloroquine-sensitive strain NF54. It is noteworthy that the compounds studied exhibited cytotoxic effects on mammalian cells only at or above 90 $\mu$g mL$^{-1}$ (highest concentration tested). Thus, the antimalarial activity is specific against erythrocytic forms of *P. falciparum*. $\beta$-Amyrin, by contrast, showed no activity in this test system.

*Leishmania donovani* is the pathogen of visceral Leishmaniasis ("Kala Azar"), a tropical disease with presently more than 12 million infected people.[37] It is another protozoal parasite, related to *Trypanosoma cruzi*, the causative agent of Chagas disease, and *Trypanosoma brucei rhodesiense*, the pathogen of African sleeping sickness. In light of the interesting activities of some naphthylisoquinoline alkaloids against malaria,[4,6,11,24] compounds **1** and **2** were tested in vitro against *L. donovani*, *T. cruzi*, and *T. b. rhodesiense*. Compound **1** displayed good activity against *T. cruzi*, and it was found to be less active than the standard, benznidazole only by a factor of 8 (Table 1). It was only slightly active against *T. b. rhodesiense*. Compound **2** exhibited moderate activity, and $\beta$-amyrin was inactive against *T. cruzi* and *T. b. rhodesiense*. Against *L. donovani* (Table 1), we found good activity of naphthylisoquinoline **1**, which was even more active than the standard, pentostam. On the other hand, pentostam is not very active in vitro. Compound **1** revealed no evidence of cytotoxicity.

The promising in vitro activities against these pathogenic agents make the naphthylisoquinoline alkaloids an interesting new antileishmanial and antitrypanosomal lead, and further research into the activities of other naphthylisoquinoline alkaloids against these pathogens could prove to be rewarding.

## Experimental Section

**General Experimental Procedures**. Melting points were determined on a Reichert-Jung Thermovar hot plate and are uncorrected. Optical rotations were taken on a Perkin-Elmer 241MC polarimeter (25 °C, 10 cm cell); IR spectra on a Perkin-Elmer 1429 spectrophotometer; and CD spectra (25°, EtOH, 0.1 cm cell) on a Jasco J-715 spectropolarimeter. $^1$H NMR (600.13 MHz) and $^{13}$C NMR (150.9 MHz) spectra were measured on a Bruker DMX 600 instrument using $CDCl_3$ ($\delta$ 7.26 and 77.01) and $CD_3OD$ ($\delta$ 3.30 and 49.01) as 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). EIMS and HRMS were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments (70 eV). For TLC, precoated Si gel 60 $F_{254}$ plates (Merck, 5 × 10 cm), deactivated with concentrated aqueous $NH_3$ were used. Spots were detected under UV light. Column chromatography was carried out on Si gel 60 (60−200 mesh, Merck), deactivated with 5% aqueous $NH_3$. HPLC (anal): $\mu$-Bondapak $C_{18}$ column (Waters, 3.9 × 300 mm, 5 $\mu$m); flow 1 mL min$^{-1}$; detection, UV-254 nm; solvent, (A) $CH_3CN$, (B) $H_2O$ (0.1% trifluoroacetic acid); gradient, 0 min 5% A, 50 min 80% A. HPLC (prep): Delta-Pak $C_{18}$, 300 Å, 19 × 300 mm; flow, 6 mL min$^{-1}$, UV-detection (254 nm). (*R*)-MTPA-Cl was prepared from (*S*)-MTPA (Fluka Chemie AG, Deisenhofen, Germany) as described earlier.[15] Organic solvents were dried and distilled prior to use.

**Plant Material.** Leaves, stem bark, and roots of *A. ealaensis* were collected by one of us (V.M.) near Eala, Democratic Republic of Congo, in December 1996, and identified by Dr. J. Schlauer, University of Würzburg. A voucher specimen is deposited at the Herbarium Bringmann at the Institut für Organische Chemie (no. 21), Würzburg.

**Extraction and Isolation.** The air-dried material (400 g stem bark; 300 g leaves) was ground and sequentially extracted with petroleum ether, $CH_2Cl_2$, and MeOH. The $CH_2Cl_2$ extract of the stem bark was prefractionated by CC ($CH_2Cl_2$−MeOH, 95:5). The first fraction was further resolved by HPLC to yield 32 mg of **1** and 12 mg of **2**. In the same way, 5.9 mg of **3**, 23.3 mg of **4**, and 8.7 mg of **5** were obtained from the third fraction. The petroleum ether extract of the leaves was fractionated by CC ($CH_2Cl_2$). The first fraction gave 80 mg of $\beta$-amyrin[14,38] after recrystallization from MeOH.

**Ancistroealaine A (1):** light yellow powder (MeOH); mp 94−96 °C; $[\alpha]^{25}_D$ −34.3° (*c* 0.55, EtOH); CD (EtOH) $\Delta\epsilon_{212}$ 7.05, $\Delta\epsilon_{230}$ −4.98, $\Delta\epsilon_{243}$ 4.13, $\Delta\epsilon_{317}$ −2.11; IR (NaCl) $\nu_{max}$ 2925 (m), 2853 (m), 1682 (s), 1633 (m), 1583 (s), 1294 (m), 1276 (m), 1200 (s), 799 (w) cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.28 (3H, d, $J$ = 6.8 Hz, $CH_3$-3), 2.31 (3H, s, $CH_3$-2′), 2.34 (1H, dd, $J$ = 16.9, 9.8 Hz, $H_{ax}$-4), 2.42 (1H, dd, $J$ = 16.9, 5.6 Hz, $H_{eq}$-4), 2.87 (3H, s, $CH_3$-1), 3.76 (1H, m, H-3), 3.77 (3H, s, $OCH_3$-6), 3.96 (3H, s, $OCH_3$-4′), 3.98 (3H, s, $OCH_3$-5′), 4.05 (3H, s, $OCH_3$-8), 6.51 (2H, s,

H-7, H-1′), 6.69 (1H, s, H-3′), 6.81 (1H, d, $J$ = 8.0 Hz, H-6′), 6.99 (1H, d, $J$ = 8.0 Hz, H-7′); $^{13}$C NMR ($CDCl_3$) $\delta$ 17.4 ($CH_3$-3), 22.1 ($CH_3$-2′), 24.2 ($CH_3$-1), 31.9 (C-4), 47.8 (C-3), 55.9 ($OCH_3$-8), 56.1 ($OCH_3$-6), 56.3 ($OCH_3$-4′), 56.6 ($OCH_3$-5′), 93.8 (C-7), 104.9 (C-6′), 108.4 (C-9), 108.9 (C-3′), 116.1 (C-8′), 116.5 (C-1′), 122.7 (C-5), 123.3 (C-10′), 128.7 (C-7′), 135.8 (C-9′), 137.1 (C-2′), 140.2 (C-10), 157.5 (C-5′), 157.7 (C-4′), 163.5 (C-8), 165.9 (C-6), 173.5 (C-1); EIMS $m/z$ 419 $[M]^+$ (100), 404 $[M - CH_3]^+$ (50), 388 $[M - OCH_3]^+$ (13), 209.5 $[M]^{2+}$ (7); HRMS $m/z$ 419.2099 (calcd for $C_{26}H_{29}O_4N$, 419.2097).

**Ancistroealaine B (2):** yellow powder (MeOH); mp 248 °C; $[\alpha]^{20}_D$ −16.7° ($c$ 0.71, EtOH); CD (EtOH) $\Delta\epsilon_{201}$ 4.49, $\Delta\epsilon_{226}$ −8.59, $\Delta\epsilon_{239}$ 5.82, $\Delta\epsilon_{285}$ −0.72; IR (NaCl) $\nu_{max}$ 3359 (s, NH, OH), 2926 (m), 2844 (m), 1682 (s), 1613 (m), 1587 (s), 1436 (m), 1258 (w), 1203 (s), 798 (w) cm$^{-1}$; $^1$H NMR ($CDCl_3$) $\delta$ 1.30 (3H, d, $J$ = 6.3 Hz, $CH_3$-3), 1.66 (3H, d, $J$ = 6.7, $CH_3$-1), 2.19 (1H, dd, $J$ = 17.9, 4.5 Hz, $H_{eq}$-4), 2.34 (3H, s, $CH_3$-2′), 2.54 (1H, dd, $J$ = 17.9, 11.6 Hz, $H_{ax}$-4), 3.47 (1H, m, H-3), 3.63 (3H, s, $OCH_3$-6), 3.92 (3H, s, $OCH_3$-8), 4.07 (3H, s, $OCH_3$-4′), 4.82 (1H, m, H-1), 6.50 (1H, s, H-7), 6.62 (1H, d, $J$ = 1.2 Hz, H-3′), 6.69 (1H, d, $J$ = 1.1 Hz, H-1′), 6.86 (1H, d, $J$ = 7.8 Hz, H-6′), 7.06 (1H, d, $J$ = 7.9 Hz, H-7′), 9.38 (1H, br. s, OH-5), 9.70 (1H, br. s, NH); $^{13}$C NMR ($CDCl_3$) $\delta$ 18.5 ($CH_3$-1), 18.6 ($CH_3$-3), 22.2 ($CH_3$-2′), 32.6 (C-4), 44.1 (C-3), 47.5 (C-1), 55.4 ($OCH_3$-8), 56.1 ($OCH_3$-6), 56.1 ($OCH_3$-4′), 94.2 (C-7), 106.3 (C-3′), 109.9 (C-6′), 113.6 (C-10′), 114.7 (C-9), 118.0 (C-1′), 120.8 (C-5), 123.8 (C-8′), 130.4 (C-7′), 132.4 (C-10), 135.3 (C-9′), 135.7 (C-2′), 154.1 (C-5′), 156.1 (C-8), 156.4 (C-4′), 158.0 (C-6); EIMS $m/z$ 407 $[M]^+$ (10), 406 $[M - H]^+$ (8), 392 $[M - CH_3]^+$ (100), 376 $[M - OCH_3]^+$ (5.5); HRMS $m/z$ 392.1862 (calcd for $C_{24}H_{26}O_4N$ $[M - CH_3]^+$, 392.1862).

**Preparation of Ancistrobertsonine C (6) from 1.** To a solution of 7.0 mg (16.6 $\mu$mol) **1** in 2 mL of MeOH was added 3.7 mg (97.9 $\mu$mol) $NaBH_4$ carefully, and the reaction mixture was stirred at 25 °C for 48 h. After addition of 100 $\mu$L concentrated aqueous HCl and adjustment of the pH to 9 with concentrated aqueous $NH_3$ the solution was extracted with $CH_2Cl_2$, followed by removal of the organic solvent in vacuo. To a solution of the brown residue (5.8 mg, 13.8 $\mu$mol, 83.3%) in 1.5 mL of MeOH was added 10 $\mu$L of a 37% aqueous HCHO solution. After stirring the reaction mixture at 25 °C for 1.5 h, 20.0 mg of $NaBH_4$ was added, and the mixture was again stirred at 25 °C for an additional 2 h. The reaction was quenched with 100 $\mu$L concentrated aqueous HCl, the pH was adjusted to 9 with concentrated aqueous $NH_3$, and the solution was extracted with $CH_2Cl_2$. Removal of the solvent and purification of the crude product by CC ($CH_2Cl_2$−MeOH, 96:4) afforded **6** (3.1 mg, 7.1 $\mu$mol, 51.6%), identified by HPLC coelution experiments with an authentic sample of **6** and by $^1$H NMR and CD spectra.[16]

**Eleutherolic Acid (3):** colorless crystals (EtOH); mp 247 °C [ref 18 (EtOH): 248 °C]; UV $\lambda_{max}$ (log $\epsilon$) 218 (3.83), 234 (3.89), 308 (3.21), 348 nm (3.19); IR (NaCl) $\nu_{max}$ 3367 (s), 2921 (m), 2842 (m), 1688 (s), 1611 (m), 1585 (m), 1293 (s), 1236 (s), 793 (w) cm$^{-1}$; $^1$H NMR ($CD_3OD$) $\delta$ 4.09 (3H, s, $OCH_3$-5), 7.05 (1H, d, $J$ = 7.4, H-6), 7.30 (1H, d, $J$ = 1.5 Hz, H-3), 7.42 (1H, dd, $J$ = 8.0, 7.4 Hz, H-7), 7.52 (1H, d, $J$ = 8.0 Hz, H-8), 8.00 (1H, d, $J$ = 1.4 Hz, H-1); $^{13}$C NMR ($CD_3OD$) $\delta$ 56.9 ($OCH_3$-5), 107.5 (C-6), 110.4 (C-3), 118.3 (C-10), 122.5 (C-1), 123.8 (C-8), 124.2 (C-2), 127.9 (C-7), 137.5 (C-9), 156.0 (C-4), 157.5 (C-5), 170.0 (COOH); EIMS $m/z$ 218 $[M]^+$ (100), 203 $[M - CH_3]^+$ (42), 175 $[203 - CO]^+$ (42), 149 $[175 - C_2H_2]^+$ (50); HRMS $m/z$ 218.0573 (calcd for $C_{12}H_{10}O_4$, 218.0579).

**Ancistronaphthoic Acid A (4):** colorless crystals (benzene); mp 207 °C [ref 18 (benzene): 209 °C]; UV $\lambda_{max}$ (log $\epsilon$) 213 (3.39), 243 (3.63), 298 (2.73), 345 nm (2.75); IR (NaCl) $\nu_{max}$ 3390 (s), 3034 (m), 2926 (m), 1682 (s), 1586 (m), 1438 (m), 1199 (s), 1073 (s), 797 (w) cm$^{-1}$; $^1$H NMR ($CD_3OD$) $\delta$ 3.93 (3 H, s, $OCH_3$-5), 3.97 (3H, s, $OCH_3$-4), 7.05 (1H, d, $J$ = 7.1, H-6), 7.41 (1H, d, $J$ = 1.4 Hz, H-3), 7.45 (1H, dd, $J$ = 7.5, 7.1 Hz, H-7), 7.50 (1H, d, $J$ = 7.4 Hz, H-8), 8.12 (1H, d, $J$ = 1.3 Hz, H-1); $^{13}$C NMR ($CD_3OD$) $\delta$ 56.8 ($OCH_3$-4), 57.0 ($OCH_3$-5), 106.2 (C-3), 110.2 (C-6), 119.2 (C-2), 120.8 (C-10), 123.2 (C-8), 124.9 (C-1), 128.5 (C-7), 138.0 (C-9), 158.5 (C-5), 158.7 (C-4), 170.1 (COOH); EIMS $m/z$ 232 $[M]^+$ (100), 217 $[M - CH_3]^+$ (2); HRMS $m/z$ 232.0734 (calcd for $C_{13}H_{12}O_4$, 232.0736).

**Ancistronaphthoic Acid B (5):** colorless crystals ($CHCl_3$); mp 238 °C; UV $\lambda_{max}$ (log $\epsilon$) 214 (3.83), 243 (4.11), 301 (3.10), 359 nm (3.27); IR (NaCl) $\nu_{max}$ 3358 (s), 2970 (m), 2929 (m), 1683 (s), 1585 (m), 1423 (s), 1236 (s), 1079 (s), 765 (w) cm$^{-1}$; $^1$H NMR ($CD_3OD$) $\delta$ 4.12 (3H, s, $OCH_3$-4), 6.92 (1H, dd, $J$ = 7.1, 1.7 Hz, H-6), 7.39 (1H, d, $J$ = 1.2 Hz, H-3), 7.40 (1H, dd, $J$ = 8.4, 7.1 Hz, H-7), 7.43 (1H, dd, $J$ = 8.4, 1.7 Hz, H-8), 8.16 (1H, d, $J$ = 1.2 Hz, H-1); $^{13}$C NMR ($CD_3OD$) $\delta$ 56.9 ($OCH_3$-4), 104.0 (C-3), 113.8 (C-6), 118.0 (C-10), 121.5 (C-8), 125.9 (C-1), 129.5 (C-7), 129.5 (C-2), 137.3 (C-9), 155.8 (C-5), 157.8 (C-4), 169.7 (COOH); EIMS $m/z$ 218 $[M]^+$ (100), 203 $[M - CH_3]^+$ (36), 175 $[203 - CO]^+$ (30); HRMS $m/z$ 218.0580 (calcd for $C_{12}H_{10}O_4$, 218.0579).

**Oxidative Degradation of 1 and 2.** The oxidative degradation, the derivatization of the derived amino acids, and the subsequent GC-MSD analyses were carried out as described previously.[15]

**Biological Experiments**. ***Plasmodium falciparum***. Antiplasmodial activity was determined using the NF54 strain of *P. falciparum* (sensitive to all known drugs) and the K1 strain (resistant to chloroquine and pyrimethamine). A modification of the [$^3$H] hypoxanthine incorporation assay[39] was used.[40] Briefly, infected human red blood cells were exposed to serial drug dilutions in microtiter plates for 48 h at 37 °C in a gas mixture with reduced oxygen and elevated $CO_2$. [$^3$H] hypoxanthine was added to each well and after further incubation for 24 h the wells were harvested on glass fiber filters and counted in a liquid scintillation counter. From the sigmoidal inhibition curve the $IC_{50}$ value was calculated. The assays were run in duplicate and repeated at least once.

***Trypanosoma cruzi***. Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 $\mu$L in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h 5000 trypomastigotes of *T. cruzi* (Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene) were added in 100 $\mu$L per well with 2× of a serial drug dilution. The plates were incubated at 37 °C in 5% $CO_2$ for 4 days. For measurement of the $IC_{50}$ the substrate CPRG/Nonidet was added to the wells. The color reaction that developed during the following 2−4 h was read photometrically at 540 nm. $IC_{50}$ values were calculated from the sigmoidal inhibition curve. Cytotoxicity was assessed in the same assay using noninfected L-6 cells and the same serial drug dilution. The MIC was determined microscopically after 4 days.

***Trypanosoma b. rhodesiense***. Minimum Essential Medium (50 $\mu$L) supplemented according to Baltz et al.[41] with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 $\mu$L of trypanosome suspension (*T. b. rhodesiense* STIB 900) was added to each well and the plate incubated at 37 °C under a 5% $CO_2$ atmosphere for 72 h. Alamar Blue (10 $\mu$L) was then added to each well, and incubation was continued for a further 2−4 h. The plate was then read using a Millipore Cytofluor 2300 at an excitation wavelength of 530 nm and an emission wavelength of 590 nm.[42] Fluorescence development was expressed as percentage of the control, and $IC_{50}$ values were determined.

***Leishmania donovani***. Mouse peritoneal macrophages were seeded in RPMI 1640 medium with 10% heat-inactivated FBS into Lab-tek 16-chamber slides. After 24 h *L. donovani* amastigotes (strain MHOM-ET-67/L82) were added at a ratio of 3:1 (amastigotes to macrophages). The medium containing free amastigotes was replaced by fresh medium 4 h later. The next day the medium was replaced by fresh medium containing different drug concentrations. The slides were incubated at 37 °C under a 5% $CO_2$ atmosphere for 96 h. Then the medium was removed, and the slides were fixed with MeOH and stained with Giemsa. The ratio of infected to noninfected macrophages was determined microscopically, expressed as percentage of the control, and the $IC_{50}$ value was calculated by linear regression.

**Acknowledgment.** This work was supported by the Deutsche Forschungsgemeinschaft (SFB 251 "Ökologie, Physiologie und Biochemie pflanzlicher und tierischer Leistung unter Stress"), by the BMBF (Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, project no. 0310722), and by the Fonds der Chemischen Industrie (fellowships to A.H. and C.G., and research funds). This investigation also received financial support from the UNDP/World Bank/WHO Special Program for Research and Training in Tropical Diseases (to R.B. and G.B.). We thank E. Ruckdeschel and Dr. K. Grüne for the NMR, Dr. G. Lange and F. Dadrich for the mass spectra, and Dr. J. Schlauer for the taxonomic classification of the plant material.

## References and Notes

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NP000247+