Ancistrocongolines A−D, New Naphthylisoquinoline Alkaloids from *Ancistrocladus congolensis*[1]

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Four new naphthylisoquinoline alkaloids, ancistrocongolines A−D (**4**−**7**) were isolated from *Ancistrocladus congolensis*, along with the known compound korupensamine A (**8**). Structural elucidation was achieved by chemical, spectroscopic, and chiroptical methods. Their biological activities against the pathogens of malaria, Leishmaniasis, Chagas disease, and African sleeping sickness were evaluated.

The Ancistrocladaceae and the closely related Dioncophyllaceae are the only plant families known to produce naphthylisoquinoline alkaloids, a rapidly growing class of structurally unique secondary metabolites,[2,3] which arise from a likewise unprecedented biosynthetic origin of tetrahydroisoquinoline alkaloids from acetate units.[4] For this reason, and because of various promising bioactivities,[5] among them antimalarial,[6] antileishmanial,[7] and antitrypanosomal[2] effects, the search for new representatives of this interesting class of compounds, by isolation or by partial or total synthesis,[8−11] is a rewarding goal.

A particularly noteworthy Ancistrocladaceae species is *Ancistrocladus congolensis*, for which previous work by Cavé's group[12] had revealed the presence of naphthylisoquinoline alkaloids. Some of them had been attributed most unusual structures, among them ancistrocongolensine (**1**) (Figure 1), which, in contrast to all other known naphthylisoquinoline alkaloids, was assumed to lack a methyl group at C-1, while the postulated structure of ancistrocongine (**2**) lacks both a methyl group at C-1 and the usual oxygen function at C-5′. Furthermore, the occurrence of the "conventional" (i.e., C-1 methylated and C-5′ oxygenated) naphthylisoquinoline alkaloid (−)-ancistrocladine (**3**)[13] and the isolation of a compound named "(+)-ancistrocladine", postulated as the "optical isomer" (i.e., the enantiomer) of **3**, resolved from **3** on an *achiral* phase were reported.[14] For none of these alkaloids previously found in *A. congolensis* were full absolute stereostructures established, neither for the stereogenic centers nor for the axes (whose chirality was not taken into consideration). With the collection of *A. congolensis* from the Province Orientale in the Congo basin, we got the opportunity to reinvestigate this phytochemically productive plant species. In this paper, we describe the isolation and complete structural elucidation of four new naphthylisoquinoline alkaloids from *A. congolensis*, named ancistrocongolines A−D (**4**−**7**), and the known compound korupensamine A (**8**) (Figure 1). We did not, however, detect the presence of alkaloids corresponding to **1** or **2** in *A. congolensis* or any related naphthylisoquinolines without a methyl group at C-1 or an oxygen function at C-5′. Ancistrocladine (**3**) or its "optical isomer" was also not detected.

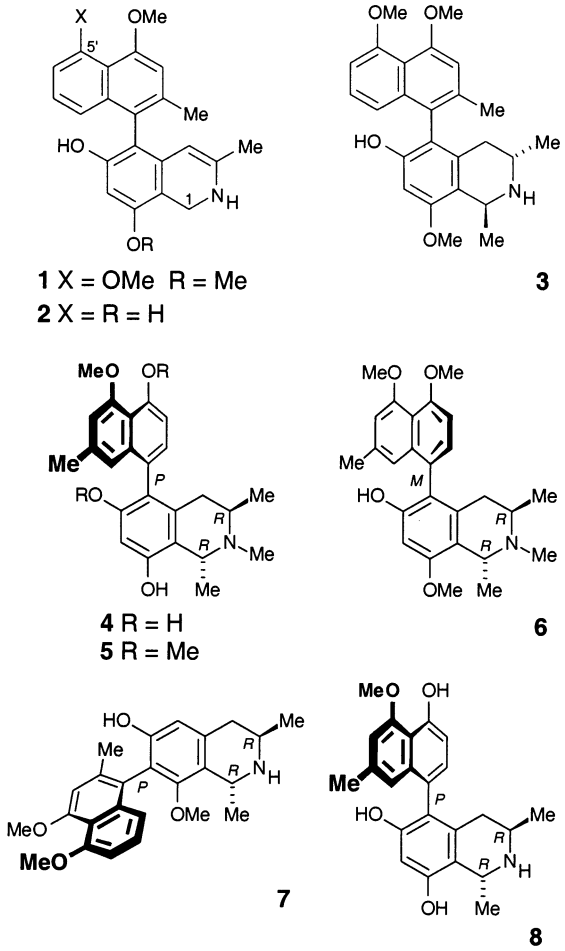

Figure 1. Natural products from *A. congolensis*: structures previously reported (**1**−**3**)[12] and the alkaloids ancistrocongolines A−D (**4**−**7**) and korupensamine A (**8**) described in this paper.

Results and Discussion

The methanolic extract of the stem material of *A. congolensis* was fractionated by high-speed countercurrent chromatography (HSCCC)[15] and subsequent HPLC separation to yield the known alkaloid korupensamine A[16] (**8**) and a less polar related compound. The near-identical UV absorption of that additional alkaloid as compared to that of **8**, as well as the MS fragmentation pattern with an $[M]^+$

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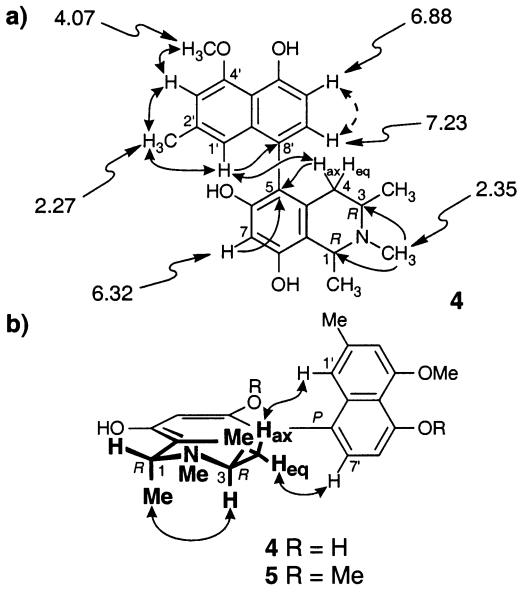

Figure 2. NMR results decisive for the structural attribution of **4**: chemical shifts, H,H-COSY (broken-line arrow), HMBC (single arrows), and ROESY (double arrows) interactions (a), as well as ROESY interactions for the determination of the relative configuration (b).

of 393 and a strong $[M - 15]^+$ peak resulting from the loss of a methyl group and with a molecular formula $C_{24}H_{27}NO_4$, as deduced from HRMS, indicated another representative of this class of compounds. The first assignment was confirmed by ^{1}H NMR, showing the typical chemical shifts of a naphthyl-1,3-dimethyltetrahydroisoquinoline with one methoxy function (δ 4.07), one *N*-methyl group at δ 2.35, and three singlets and two doublets in the aromatic region each corresponding to one proton (see Figure 2a). Given the presence of only two adjacent aromatic protons (as obvious from H,H-COSY experiments) at δ 6.88 and 7.23, this substitution pattern hinted either at a 6′- or an 8′-coupling site of the biaryl axis in the naphthalene moiety. This was supported by the "normal" chemical shift of the 2′-methyl group (δ 2.27), which would otherwise, with the axis neighboring the 1′- or the 3′-position, be high-field shifted.[2] The location of the linkage between the two molecular halves at C-8′ was established from an HMBC interaction between H-1′ and the quaternary carbon atom C-8′. The attribution of H-1′ in turn succeeded by ROESY interactions with H_{ax}-4 and by the ROESY series {H-1′ ↔ CH_3-2′ ↔ H-3′ ↔ OCH_3-4′}. In the isoquinoline portion, the axis is located at C-5, as unambiguously shown by HMBC effects of both H_{ax}-4 and H-7 (δ 6.32) with C-5, confirmed by the interaction of H-7′ with this carbon. The presence of an *N*-methyl group was proven by HMBC interactions of its CH_3 protons (δ 2.35) with both C-1 and C-3.

From a ROESY interaction between H-3 and CH_3-1 (Figure 2b) a relative *trans*-configuration of the two stereocenters at C-1 and C-3 was deduced. The absolute configuration at both of these stereocenters was established by GC-MSD analysis of the Mosher derivatives of *N*-methylalanine (derived from C-1) and *N*-methyl-3-aminobutyric acid (derived from C-3) obtained by a ruthenium-mediated oxidative degradation developed earlier (see Figure 3).[17]

Accordingly, the new alkaloid **4** is *R*-configured both at C-1 and C-3, in agreement with the relative *trans*-attribution discussed above. This in combination with the relative configuration at the axis, as evident from the ROESY interactions of H_{ax}-4 with H-1′ and H_{eq}-4 with H-7′ (see Figure 2b), indicates that the axis has to be *P*-configured.

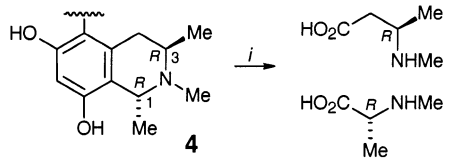

Figure 3. Oxidative degradation of **4** to give readily analyzable amino acids. (*i*) $RuCl_3$, $NaIO_4$; stereoanalysis of the Mosher derivatives of the methyl esters by GC-MSD.

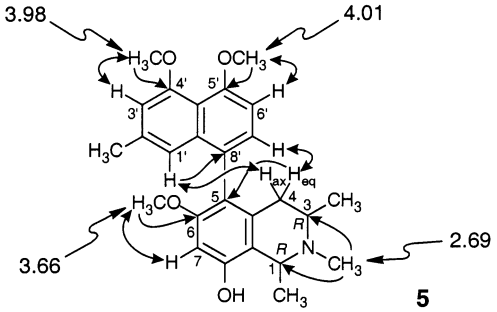

Figure 4. Selected NMR shifts and HMBC (single arrows) and ROESY (double arrows) interactions of **5**.

This matches with the nearly identical CD spectrum of **4** compared to that of korupensamine A (**8**), which is also *P*-configured at the biaryl axis, as firmly established by CD calculations[16] and by total synthesis.[9] This new *N*-methylated korupensamine A derivative **4** was named ancistrocongoline A. For additional proof of structure, **4** was prepared from synthetically available korupensamine A (**8**)[9] by *N*-methylation on an analytical scale under Eschweiler-Clarke conditions. The product proved to be fully identical with the newly identified alkaloid ancistrocongoline A (**4**) by its spectroscopic and chromatographic data.

The root bark of *A. congolensis* was extracted with acidified water (pH 2); the resulting extract was resolved by CC, MPLC, and HPLC to yield three additional pure compounds. The first one strongly resembled the above isolated korupensamine A (**8**) and ancistrocongoline A (**4**), with an aromatic proton pattern of two doublets and three singlets, again indicating a 6′- or 8′-coupling position in the naphthalene part. The exact coupling site was deduced from the HMBC correlation between H-1′ and C-8′, which was revealed to be a quaternary carbon, indicating the isoquinoline substituent at C-8′ (see Figure 4). With respect to the isoquinoline moiety, the naphthyl portion was established to be coupled to C-5 by HMBC interactions between H_{eq}-4 and H-7′ with the quaternary C-5. From the ^{1}H NMR signal at δ 2.69 and the HMBC interactions of this CH_3 group with both C-1 and C-3 it was evident that the new compound was again *N*-methylated but, in contrast to **4**, possessed three methoxy groups, with NMR resonances at δ 3.66, 3.98, and 4.01. Their positions were deduced from HMBC correlations to C-6, C-4′, and C-5′, respectively, in accordance with the ROESY interactions between OCH_3-4′ and H-3′, OCH_3-5′ and H-6′, and OCH_3-6 and H-7. This new compound was therefore named ancistrocongoline B (**5**). A ROESY effect between CH_3-1 and H-3 hinted at a relative *trans*-arrangement of the two methyl groups at C-1 and C-3, which was confirmed by the oxidative degradation (see above), clearly establishing an absolute 1*R*,3*R*-configuration for **5**. On the basis of the known configuration at the stereocenters, it was now possible to determine the relative and thus also the absolute configuration at the axis due to ROESY interactions between H_{ax}-4 and H-1′, and between H_{eq}-4 and H-7′, assigning a *P* descriptor to the linkage (see Figure 2b). This

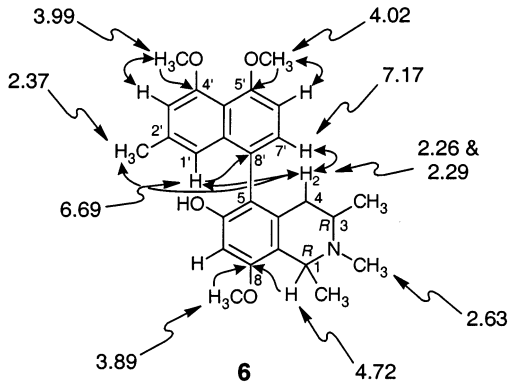

Figure 5. Constitution of **6** by NMR shifts and by HMBC (single arrows) and ROESY (double arrows) interactions.

result was confirmed by the nearly identical CD spectrum of **5** as compared to that of **8**.

The fourth compound isolated had a molecular formula of $C_{26}H_{31}NO_4$, obtained by HRMS of the $[M - CH_3]^+$ peak. By its three methoxy groups (δ 3.89, 3.99, 4.02) and an *N*-methyl substituent (δ 2.63, see Figure 5), it resembled the above isolated ancistrocongoline B (**5**). The aromatic pattern of three singlets and two doublets indicated a 6′- or 8′-coupled naphthalene moiety. The exact coupling site was deduced to be C-8′ by an HMBC correlation between H-1′ and the quaternary C-8′. The coupling site in the isoquinoline portion was located at C-5 by HMBC interactions and by ROESY correlations that are possible only for this coupling type, viz., from H-1′, CH_3-2′, and H-7′ (δ 6.69, 2.37, and 7.17) to the protons at C-4 (δ 2.26 and 2.29). A significant difference in the NMR spectrum of the alkaloid as compared to that of **5** was in the chemical shift of the methoxy group in the isoquinoline half (δ 3.89 vs 3.66 as in **5**), hinting at the occurrence of this substituent at C-8, which was confirmed by HMBC correlations of H-1 (δ 4.72) and OCH_3-8 to C-8. A relative *trans*-configuration between the two stereocenters at C-1 and C-3 was evident from a ROESY interaction of CH_3-1 with H-3 (compare Figure 2b). This was again confirmed by the oxidative degradation, which established the absolute configuration as 1*R*,3*R*. An assignment of the relative axial configuration by ROESY interactions between the protons at C-4 and naphthalene substituents, as for the other alkaloids presented above, could not be done with confidence in this case, due to the close chemical shifts (δ 2.26 and 2.29) of the two protons at C-4 combined with the multiplicity of their signals (occurring as an ABX-type pattern), which did not allow a distinct attribution of the cross-peaks in the 2D-ROESY experiment to only one of the two diastereotopic protons. But the absolute configuration at the axis was clearly deduced as *M* by the mirror-image-like CD spectrum of **6** compared to that of korupensamine A (**8**) (see Figure 6). This new compound was henceforth named ancistrocongoline C (**6**).

The fifth compound isolated from *A. congolensis*, with a molecular formula of $C_{25}H_{29}NO_4$ obtained from EIMS and HRMS, exhibited the typical appearance of a naphthylisoquinoline in ^{1}H NMR, but clearly indicated a coupling type different from a 5,8′-linkage, due to the pattern of its five aromatic protons. With a spin system of three neighboring protons, giving rise to two doublets (δ 6.85 and 6.96) and one pseudo triplet (δ 7.20), and from their H,H-COSY interactions excluding the naphthalene to be connected to the isoquinoline moiety via its "methyl-free" ring, the position of the axis had to be in the ring that bears the methyl group (CH_3-2′ at δ 2.15) (see Figure 7). Whether

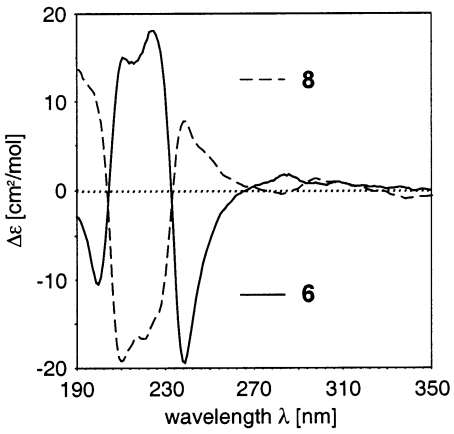

Figure 6. CD spectra of ancistrocongoline C (**6**) and korupensamine A (**8**).

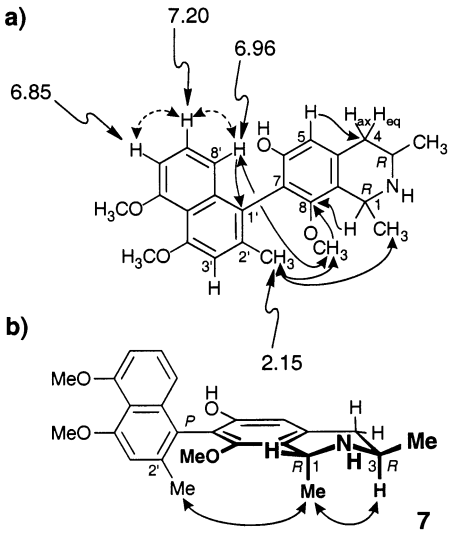

Figure 7. Constitution of **7** (a) by selected chemical shifts, H,H-COSY (broken-line arrows), HMBC (single arrows), and ROESY (double arrows) effects (b) and relative configuration by ROESY interactions.

the coupling site is C-1′ or C-3′ became evident from an HMBC interaction between H-8′ and C-1′, showing C-1′ to be quaternary and hence the axis-bearing carbon. This finding was in agreement with ROESY correlations between H-8′ and OCH_3-8, and from CH_3-2′ to OCH_3-8 and CH_3-1, which were possible only if the alkaloid is 7,1′-linked. The coupling position to be C-7 in the isoquinoline part was evidenced on one hand by assigning the remaining aromatic proton to C-5 by HMBC effects between H-5 and C-4, and on the other hand by the two HMBC interactions of OCH_3-8 and H-1 with C-8. Oxidative degradation established a 1*R*,3*R*-configuration at the two stereocenters in the isoquinoline part of the molecule. Due to the known configuration at C-1, the assignment of the absolute configuration at the axis as *P* was possible by the already mentioned ROESY correlation between CH_3-2′ and CH_3-1 (see Figure 7b). This new compound was named ancistrocongoline D (**7**).

Because of the already mentioned promising bioactivities of several naphthylisoquinoline alkaloids with respect to their antimalarial, antileishmanial, and antitrypanosomal effects,[2,5−7] all new ancistrocongolines A−D (**4**−**7**) were tested for their in vitro activities against the corresponding pathogens (see Table 2). All of the compounds exhibit weak to moderate activities in these test systems, with most of the IC_{50} values being within a range of only 50−100 times higher than those of the standards. These findings, al-

Table 1. ^{1}H and ^{13}C NMR Spectral Data of Ancistrocongolines A−D (**4**−**7**)

	4[a,b]		**5**[a,]		**6**[a,b]		**7**[a,c]	
C/H no.	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	4.23 q (6.5)	55.4	5.1 q (6.4)	58.4	4.72 q (6.6)	57.8	4.73 q (6.7)	49.9
3	3.21 m	46.2	3.90 m	48.6	3.83 m	48.0	3.87 m	45.2
4	1.95 dd (17.8, 11.1), 2.19 dd (17.8, 4.6)	31.2	2.03 dd (19.0, 12.4), 2.59 dd (18.7, 4.8)	28.3	2.26 dd (18.7, 6.3), 2.29 dd (18.7, 10.7)	29.0	2.89 dd (17.4, 11.8), 3.15 dd (17.6, 4.9)	34.5
4a		134.8		130.1		130.6		133.1
5		116.9		119.6		117.5	6.58 s	111.4
6		152.4		158.4		154.4		157.4
7	6.32 s	100.2	6.92 s	98.6	6.59 s	97.5		120.0
8		154.3		155.3		157.3		157.2
8a		118.5		110.1		113.1		118.6
1′	6.77 s	118.2	6.58 s	116.5	6.69 s	115.9		123.5
2′		136.7		136.5		138.3		137.6
3′	6.64 s	106.9	6.68 s	108.5	6.75 s	109.3	6.91 s	110.4
4′		156.3		157.6		157.8		158.2
4′a		114.0		116.2		116.5		117.7
5′		154.9		156.8		158.4		158.7
6′	6.88 d (7.8)	109.8	6.88 d (8.0)	105.4	6.87 d (8.1)	105.2	6.85 d (7.4)	107.0
7′	7.23 d (7.8)	131.0	7.17 d (7.8)	128.4	7.17 d (7.8)	130.3	7.20 dd (8.4, 7.8)[d]	127.5
8′		121.8		125.5		121.1	6.96 d (8.5)	119.7
8′a		136.1		136.2		136.0		138.0
1-CH_3	1.44 d (6.7)	17.0	1.85 d (6.1)	19.1	1.73 d (6.6)	19.0	1.63 d (6.7)	19.4
N−CH_3	2.35 s	35.9	2.69 s	32.9	2.70 s	33.1		
3-CH_3	1.00 d (6.5)	19.1	1.31 d (6.3)	17.0	1.31 d (6.6)	16.8	1.51 d (6.4)	19.3
6-OCH_3			3.66 s	55.7				
8-OCH_3					3.89 s	55.6	3.07 s	60.5
2′-CH_3	2.27 s	22.1	2.31 s	22.1	2.37 s	22.2	2.15 s	20.8
4′-OCH_3	4.07 s	56.2	3.98 s	56.5	3.99 s	56.5	3.96 s	56.8
5′-OCH_3			4.01 s	56.3	4.02 s	56.3	3.92 s	57.0

[a] Signals were assigned by H,H-COSY, HMQC, HMBC, and NOESY spectra. [b] Spectra recorded in $CDCl_3$. [c] Spectra recorded in CD_3OD. [d] The signals are partly overlapped, resulting in a *pseudo* triplet.

Table 2. Bioactivities of the Compounds **4**−**8**

	[μg/mL]				
	4	**5**	**6**	**7**	**8**
P. falciparum (strain: K1)[a,b]	0.214	0.158	3.002	1.983	0.164
T. cruzi[a,c]	39.9	17.4	>90	30.1	14.5
T. brucei rhodesiense[a,d]	2.97	2.54	15.9	5.86	1.87
L. donovani[a,e]	n.d.[f]	18.8	19.3	>30	25.1
cytotoxicity: L-6[g]	90	33.4	>90	27.7	38.0

[a] IC_{50} value. [b] Artemisinin: $IC_{50} = 0.001$ μg/mL. [c] Benznidazole: $IC_{50} = 0.382$ μg/mL. [d] Melarsoprol $IC_{50} = 0.003$ μg/mL. [e] Pentostam: $IC_{50} = 47.2$ μg/mL. [f] Not determined due to cytotoxicity toward host cells. [g] MIC value.

though not reaching the good results of other naphthylisoquinolines,[2,5−7] give an important contribution to our structure−activity relationship investigations.[18,19]

In contrast to the five 5,1′-coupled alkaloids previously reported in *A. congolensis* by Cavé's group,[12] none of the 5,8′- and 7,1′-coupled compounds isolated in this work resembled those structures in their physical or NMR properties. All of the new ancistrocongolines (**4**−**7**) as well as korupensamine A (**8**) are representatives of the so-called "hybrid-type" naphthylisoquinolines,[2] i.e., with a 3*R*-configuration and an oxygen functionality at C-6, bearing both structural features of "Ancistrocladaceae-type" alkaloids (with 3*S*-configuration and oxygen at C-6), until now found in all Asian Ancistrocladaceae and in the East African species *Ancistrocladus robertsoniorum*,[20] and the "Dioncophyllaceae-type" ones (3*R*, no oxygen at C-6), as produced mainly by West African species such as *A. abbreviatus*.[21] With these phytochemical characteristics, *A. congolensis* strongly resembles *A. likoko*, the only other Central African (Democratic Republic of Congo) liana that produces 3*R*-configured alkaloids,[22] in contrast to *A. ealaensis* (also indigenous to the Congo basin), in which so far only pure "Ancistrocladaceae-type" (i.e., 3*S*-configured) alkaloids have been found.[7]

Experimental Section

General Experimental Procedures. Column chromatography was carried out using silica gel 60 (60−200, Merck) deactivated with 7.5% concentrated NH_3. MPLC: Lobar Column LiChroprep RP_{18} 25 × 310 mm, 8.0 mL min^{-1}. HPLC (anal.): Symmetry RP_{18} 4.6 × 250 mm (Waters), 0.5 mL min^{-1}. HPLC (semiprep): RCM 8 × 10 with Nova Pak C_{18} 7.8 × 200 mm (Waters), 1.2 mL min^{-1}; Symmetry RP_{18} 7.8 × 300 mm (Waters), 3.8 mL min^{-1}; Dynamax-60A NH_2 8 × 300 mm (Rainin), 2.0 mL min^{-1}. HPLC (prep): Prep LC 25 with Nova Pak C_{18} 25 × 210 mm (Waters), 8.0 mL min^{-1}. HSCCC (P.C. Inc.): "Tripple coil", 1.68 mm × 37.0 m (medium coil), 1.68 mm × 106.5 m (large coil), (H) → T, lower phase as mobile phase, forward rotation mode, 860−880 rpm. Melting points were measured on a Thermovar hot plate (Reichert-Jung) and are uncorrected. IR spectra were taken on a Jasco FT/IR-410 spectrometer, CD spectra on a Jasco J-715 spectropolarimeter, and optical rotations on a Perkin-Elmer 241MC polarimeter. ^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker DMX 600 either in $CDCl_3$ or CD_3OD with the solvent as the internal standard ($CDCl_3$, δ 7.26 and 77.01; CD_3OD, δ 3.30 and 49.02, respectively). Proton-detected, heteronuclear correlations were analyzed using HMQC (optimized for $^1J_{HC} = 145$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). NOE effects were measured using standard ROESY pulse sequences from the Bruker pulse program library. EIMS (70 eV) and HREIMS (70 eV) were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments. LC-ESI-MS/MS analyses were done with a Triple Stage Quadrupole TSQ 7000 mass spectrometer from Finnigan, equipped with an ESI interface and a Personal DECstation 5000/33 (Digital Equipment). Argon was used as the collision gas with a pressure of

1.8 mTorr. The chromatographic separation was performed on a Symmetry RP_{18} column 2.1 × 150 mm (Waters), 0.2 mL min^{-1}. The absolute configurations of the stereocenters at C-1 and C-3 of the isolated naphthylisoquinolines were determined by Ru-mediated oxidative degradation, derivatization of the amino acids formed, and subsequent GC-MSD analysis as described previously.[17]

Plant Material. Stem and root bark of *A. congolensis* were collected by L. Nlandu at the Yandja-Rive (Forêt Lokokele), Democratic Republic of Congo, in June 1998. The material was identified by L. Nlandu and Dr. J. Schlauer, University of Würzburg. A voucher specimen has been deposited at Herb. Bringmann (No. 30), University of Würzburg.

Extraction and Isolation. The air-dried plant material (250 g stem) was ground and sequentially extracted with petroleum ether, CH_2Cl_2, and MeOH, yielding 0.55, 0.83, and 12.1 g of extract, respectively. The methanolic portion was further resolved into 13 fractions by preparative HSCCC (medium + large coil) using a quaternary solvent mixture of 5:3:5:3 $CHCl_3$−ethyl acetate−MeOH−0.1 M HCL, 4 mL min^{-1}. Fraction 11 was further purified by semipreparative HPLC on an amino phase column with 5:1 CH_2Cl_2−MeOH + 0.1% $(NH_4)_2CO_3$ (isocratic) and a linear gradient 0 min 95% to 35 min 55% CH_2Cl_2 to yield 6.7 mg of **8** (0.0027%). HPLC of fraction 12 with 5:1 CH_2Cl_2−MeOH + 0.1% $(NH_4)_2CO_3$ on amino phase yielded 2.8 mg of **4** (0.0011%).

Air-dried ground root bark (500 g) was extracted with 0.01 N aqueous HCl at room temperature, resulting in 10.0 g of extract. Prefractionation on silica gel (gradient: 7:3 CH_2Cl_2−petroleum ether, 100:0 to 1:1 CH_2Cl_2−MeOH) gave 10 fractions. Further chromatography of a 61% portion of the main fraction 2 on silica gel with CH_2Cl_2−MeOH (gradient 100:0 to 1:1), with subsequent HPLC on semipreparative C_{18} (Nova Pak, MeOH−H_2O−TFA, 50:50:0.1), yielded **7** (2.3 mg, 0.00075%). Another compound was purified by preparative HPLC (C_{18}, MeOH−H_2O−TFA, 50:50:0.1), resulting in 3.6 mg of **5** (0.0012%). The other part of main fraction 2 was successively further purified by MPLC (MeCN−H_2O−TFA, 40:60:0.1), chromatography on silica gel CH_2Cl_2−MeOH (gradient 100:0 to 5:1), and HPLC on a semipreparative C_{18} column (Symmetry) to yield **6** (1.4 mg, 0.00072%).

Ancistrocongoline A (4): amorphous, light brown powder; $[\alpha]^{23}_D$ +6.9° (*c* 0.05, $CHCl_3$); CD (EtOH) $\Delta\epsilon_{198}$ 16.3, $\Delta\epsilon_{210}$ −17.0, $\Delta\epsilon_{224}$ −14.2, $\Delta\epsilon_{238}$ 7.5, $\Delta\epsilon_{280}$ −0.5, $\Delta\epsilon_{300}$ 0.5, $\Delta\epsilon_{339}$ −0.9; IR (NaCl) ν_{max} 3747, 3313 (b), 2919, 2855, 1699, 1680, 1652, 1266, 1200, 1134, 772 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 393 $[M]^+$ (7), 392 $[M - H]^+$ (18), 378 $[M - CH_3]^+$ (100), 363 $[M - 2CH_3]^+$ (6), 348 $[M - 3CH_3]^+$ (30), 189 $[M - CH_3]^{2+}$ (10); HRMS *m/z* 392.1861 $[M - H]^+$ (calcd for $C_{24}H_{26}NO_4$, 392.1862).

Ancistrocongoline B (5): colorless solid; mp 161−162 °C (MeOH); $[\alpha]^{23}_D$ −12.0° (*c* 0.22, $CHCl_3$); CD (EtOH) $\Delta\epsilon_{201}$ 32.7, $\Delta\epsilon_{227}$ −28.4, $\Delta\epsilon_{242}$ 18.7, $\Delta\epsilon_{267}$ −0.9, $\Delta\epsilon_{302}$ 4.2, $\Delta\epsilon_{357}$ −4.2; IR (NaCl) ν_{max} 3250 (b), 2924, 2849, 1665, 1584, 1463, 1273, 1200, 1136, 719 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 421 $[M]^+$ (4), 407 $[M + H - CH_3]^+$ (27), 406 $[M - CH_3]^+$ (100); HRMS *m/z* 406.2017 $[M - CH_3]^+$ (calcd for $C_{25}H_{28}NO_4$, 406.2018).

Ancistrocongoline C (6): white-brown powder; mp 140−141 °C; $[\alpha]^{24}_D$ 128.1° (*c* 0.125, $CHCl_3$); CD (EtOH) $\Delta\epsilon_{200}$ −10.7, $\Delta\epsilon_{211}$ 15.2, $\Delta\epsilon_{224}$ 18.2, $\Delta\epsilon_{239}$ −19.6, $\Delta\epsilon_{286}$ 1.8; IR (NaCl) ν_{max} 3250 (b), 2924, 2849, 1665, 1584, 1463, 1273, 1200, 1136, 719 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 421 $[M]^+$ (1), 406 $[M - CH_3]^+$ (100), 203 $[M - CH_3]^{2+}$ (9); HRMS *m/z* 406.2022 $[M - CH_3]^+$ (calcd for $C_{25}H_{28}NO_4$, 406.2018).

Ancistrocongoline D (7): light brown powder; mp 232 °C; $[\alpha]^{24}_D$ −6.7° (*c* 0.52, $CHCl_3$); CD (EtOH) $\Delta\epsilon_{193}$ 14.2, $\Delta\epsilon_{210}$ −42.3, $\Delta\epsilon_{224}$ 5.0, $\Delta\epsilon_{231}$ −7.4, $\Delta\epsilon_{241}$ 4.7, $\Delta\epsilon_{257}$ 7.1, $\Delta\epsilon_{289}$ −13.9, $\Delta\epsilon_{311}$ 3.0; IR (NaCl) ν_{max} 3432 (b), 2924, 2846, 1671, 1613, 1457, 1423, 1393, 1338, 1260, 1202, 1130, 1098, 1070, 753 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 407 $[M]^+$ (5), 406 $[M - H]^+$ (5), 392 $[M - CH_3]^+$ (100), 196 $[M - CH_3]^{2+}$ (12); HRMS *m/z* 392.1862 $[M - CH_3]^+$ (calcd for $C_{24}H_{26}NO_4$, 392.1857).

Analytical Conversion of Korupensamine A (8) to Ancistrocongoline A (4). Methylation of **8** was done on a 1.0 mg (2.6 μmol) scale, with 3 equiv each of formic acid (98%), sodium formate (in 10 μL of H_2O), and formaldehyde (36%) in 100 μL of H_2O according to a procedure described in the literature.[23] The identity of ancistrocongoline A (**4**) formed was proven by HPLC-MS/MS (H_2O−MeCN, 95:5, 10 min 85:15, 30 min 1:99, 0.05% TFA), including the typical retro-Diels−Alder fragmentation[24] with a neutral loss of 57 u corresponding to C_3H_7N.

Korupensamine A (8). The isolated compound was identical in all respects to authentic materials of korupensamine A (**8**) previously isolated[16] and synthesized.[9]

Biological Experiments. *Plasmodium falciparum.* Antiplasmodial activity was determined using the *P. falciparum* strain K1 (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H]-hypoxanthine incorporation assay[25] was used.[26] 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 μL 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 μL per well with a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 days. For determination of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The color reaction that developed during the following 2−4 h in the presence of live cells was read photometrically at 540 nm. IC_{50} values were calculated from the sigmoidal inhibition curve.

Trypanosoma b. rhodesiense. Minimum Essential Medium (50 μL) supplemented according to Baltz et al.[27] 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 μL 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 μL) 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.[28] Fluorescence development was expressed as percentage of the control, and IC_{50} values were determined.

Rat Skeletal Myoblasts. Cytotoxicity was assessed using L-6 cells in RPMI 1640 medium with 10% FBS in microtiter plates. The assay protocol was very similar to the one used for *T. b. rhodesiense*. The effect of serial drug dilutions on the cells was determined with Alamar Blue and expressed as IC_{50} values.

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