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Ancistrotanzanine C and Related 5,1′- and 7,3′-Coupled Naphthylisoquinoline Alkaloids from *Ancistrocladus tanzaniensis*[1]

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Three new naphthylisoquinoline alkaloids, the 7,3′-coupled ancistrotanzanine C (**6**), the 5,1′-coupled *O*-methylancistrocladinine (**7**), and the likewise 5,1′-coupled *O*,*N*-dimethylancistrocladine (**8**, previously known only as a partial-synthetic compound), have been isolated from the highland liana *Ancistrocladus tanzaniensis*, along with the two known 7,3′-coupled naphthylisoquinoline alkaloids ancistrocladidine (**4**) and ancistrotectorine (**5**). All of the compounds are *S*-configured at C-3 and bear an oxygen at C-6, and thus belong to the so-called Ancistrocladaceae type, similar to **1**−**3** previously isolated from this newly discovered plant species. The structural elucidation was achieved by chemical, spectroscopic, and chiroptical methods. The biological activities of the alkaloids against the pathogens causing malaria tropica, leishmaniasis, Chagas' disease, and African sleeping sickness were evaluated.

The naphthylisoquinoline alkaloids comprise a rapidly growing class of remarkable natural products: structurally, because of the presence of a usually rotationally hindered and thus stereogenic biaryl axis,[2,3] biosynthetically, since these are the first acetogenic tetrahydroisoquinoline alkaloids,[4,5] and pharmacologically, because of their high antiinfective activities, e.g., against different protozoan pathogens, such as *Plasmodium*, *Leishmania*, and *Trypanosoma* species,[6−9] all of which cause severe, widespread tropical diseases.[10] The East African species *Ancistrocladus tanzaniensis* (Ancistrocladaceae),[11] which has been botanically described only recently,[12] has proven to produce a series of novel bioactive representatives of this interesting class of natural products.[13] Thus, the new alkaloid ancistrotanzanine A (**1**) is based on a hitherto unknown 5,3′-coupling type between the two molecular portions, the naphthalene and the isoquinoline parts, while ancistrotanzanine B (**2**) and ancistrotectoriline A (**3**), also obtained from the Southeast Asian species *A. tectorius*,[14] are both 5,8′-coupled.[13]

In this paper, we report on the isolation and structural elucidation of five further naphthylisoquinoline alkaloids from *A. tanzaniensis*, among them the already known alkaloids ancistrocladidine (**4**)[15,16] and ancistrotectorine (**5**),[17,18] and the new compound ancistrotanzanine C (**6**), which are all based on a 7,3′-linkage, and the 5,1′-coupled, likewise new alkaloids *O*-methylancistrocladinine (**7**) and *O*,*N*-dimethylancistrocladine (**8**); the latter had previously been prepared by partial synthesis.[19] The results mark a strong chemotaxanomic relationship of *A. tanzaniensis* with the only other known East African *Ancistrocladus* species, *A. robertsoniorum*,[20,21] and also with the Southeast Asian Ancistrocladaceae plants.

Results and Discussion

A. tanzaniensis was collected in Tanzania in the Uzungwa Mountains at 1200 m above sea level. Repeated column

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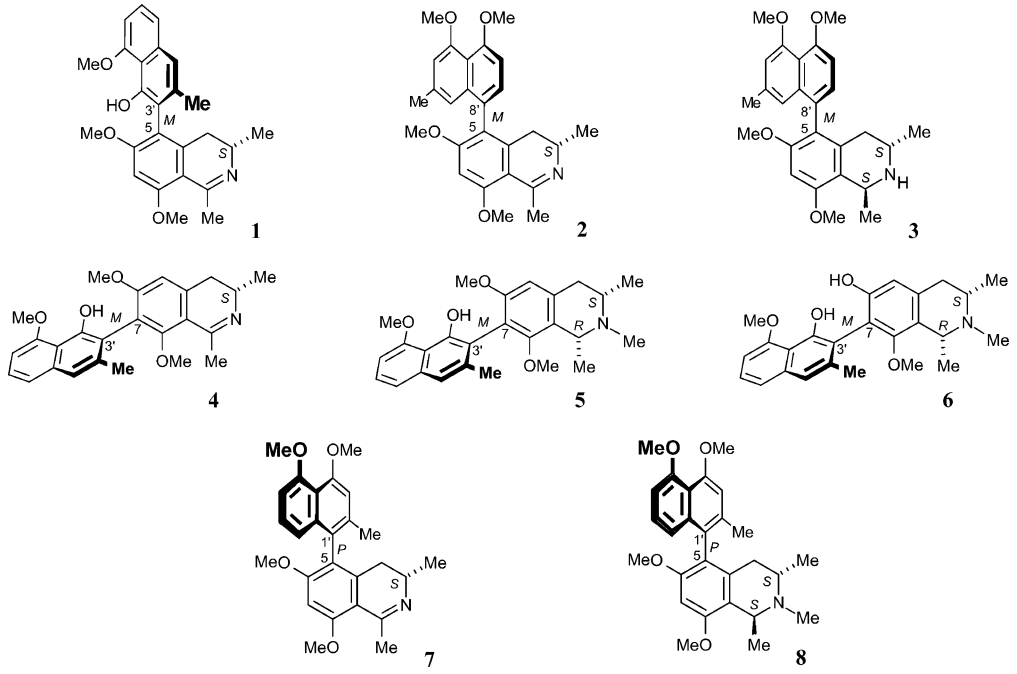

Figure 1. Structures of naphthylisoquinoline alkaloids **1**−**8** isolated from *Ancistrocladus tanzaniensis*.

chromatography and preparative HPLC of a CH_2Cl_2 extract of air-dried and ground leaves of *A. tanzaniensis* afforded five alkaloids. Their UV spectra suggested that they were naphthylisoquinoline alkaloids.

The two least polar compounds were easily identified as the known alkaloid ancistrocladidine (**4**), which had been previously isolated from *A. heyneanus*[15] and from *A. tectorius*,[16] and ancistrotectorine (**5**) from *A. tectorius*[17] and *A. guineënsis*.[18] Their spectroscopic, physical, and chromatographical data were identical to those reported in the literature.[15−18]

The other three alkaloids were found to be new. The most polar compound was found to possess a molecular formula of $C_{25}H_{29}NO_4$ as revealed from the number of signals in the ^{13}C NMR spectrum and from HREIMS. Its ^1H NMR spectrum contained all the signals expected for a naphthyltetrahydroisoquinoline alkaloid (Figure 2a) bearing methyl groups at C-1 (δ 1.45), N (δ 2.49), C-3 (δ 1.26), and C-2′ (δ 2.18), two methoxy groups (δ 3.33 and 4.03), two singlets for aromatic hydrogens (δ 6.58 and 7.29), and a system of three neighboring aromatic hydrogens. The appearance of the signals of the two diastereotopic protons at C-4 at a "normal" position (δ 2.63 and 2.73), i.e., not upfield shifted, strongly indicated the biaryl axis to be connected to C-7 of the isoquinoline part.[22] This assumption was substantiated by the upfield shifted resonance of the aromatic methoxy group at C-8 (δ 3.33). The position of this methoxy group was confirmed by its NOESY interaction with the methyl group at C-1 (δ 1.45), while the other OMe group was established to be at C-5′ by a NOESY interaction with H-6′ (Figure 2b). The *N*-methyl group (δ 2.49) was localized by HMBC interactions with both C-1 and C-3. NOESY correlations between H-5 with both H-4_{ax} and H-4_{eq} established C-5 to be devoid of a naphthalene substituent, which was further confirmed by HMBC interactions between H-5 and C-4. The biaryl axis was therefore assigned at C-7 of the isoquinoline part.

From the spin system of three neighboring aromatic protons, the coupling position of the naphthalene moiety was excluded to be in the methyl-free ring, which was further supported by the upfield shifted signal of the CH_3-2′ group (δ 2.18).[2] Of the remaining two positions of the

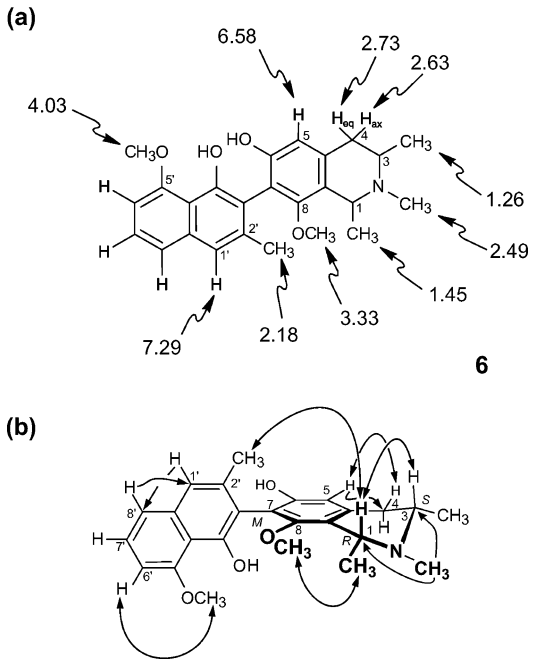

Figure 2. Selected NMR data of ancistrotanzanine C (**6**): ^1H NMR shifts (δ in ppm) (a); HMBC (single arrows) and NOESY (double arrows) interactions used for the determination of the relative configuration (b).

naphthalene substituent, C-3′ and C-1′, the latter was excluded since it bears the above-mentioned aromatic proton, which was assigned by an HMBC interaction between H-8′ and the (likewise tertiary and hence unsubstituted) aromatic C atom C-1′, and vice versa, from H-1′ to C-8′, thus unequivocally locating the biaryl axis at C-3′. This, in conclusion, established the naphthylisoquinoline alkaloid to be based on the as yet rare 7,3′-linkage and to possess the structure **6** shown in Figure 2a.

As to the stereostructure, the relative configuration at the two methyl groups at C-1 and C-3 was deduced from a NOESY interaction between H-1 and H-3 (see Figure 2b) revealing that these two protons—and thus also the two respective methyl groups—were *cis* diaxial. The absolute

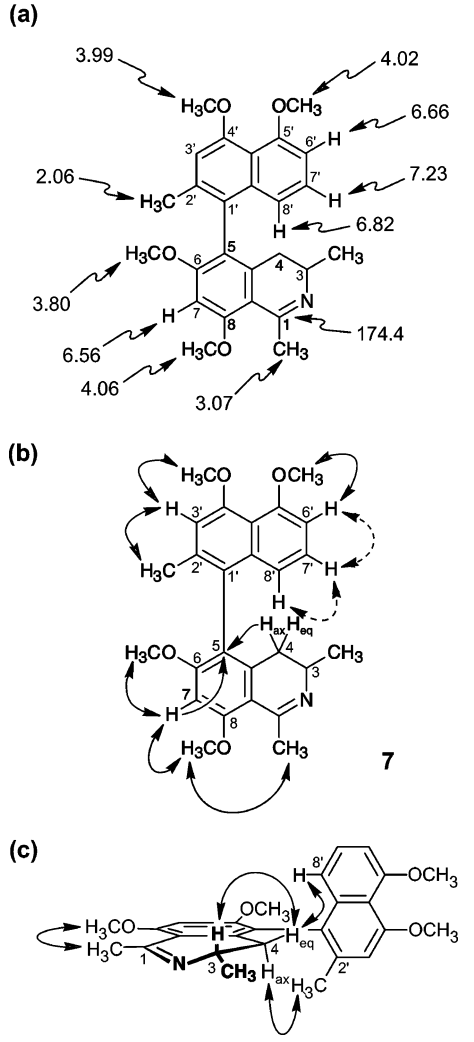

Figure 3. Selected NMR data of *O*-methylancistrocladinine (**7**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a); H,H-COSY (broken-line arrow), HMBC (single arrows), and NOESY (double arrows) interactions used for the determination of the relative configuration (b); configuration at the biaryl axis relative to the stereogenic center through NOESY interactions (c).

configurations at C-1 and C-3 were determined to be 1*R*,3*S* by ruthenium-catalyzed oxidation[23] giving *R*-*N*-methylalanine and (*S*)-3-methylaminobutyric acid as the main products. A NOESY correlation between H-1 and the methyl group at C-2′ (see Figure 2b) revealed that these two spin systems are on the same side of the molecule, which is, in conjunction with the above established absolute configuration, the "upper" side of the isoquinoline, thus allowing the assignment of the stereoarray at the axis as drawn in Figure 2b, i.e., *M*-configured. According to these findings the alkaloid had the full stereostructure **6**. It was thus new and was henceforth named ancistrotanzanine C (**6**). The alkaloid could also be addressed as the 6-*O*-demethyl analogue of ancistrotectorine (**5**). This conclusion was further corroborated by the CD spectrum of **6**, which, as expected, was very similar to that of **5**, in which the biaryl axis is also *M*-configured.[17,18]

The molecular formula of the fourth, slightly less polar new alkaloid was $C_{26}H_{29}NO_4$, as deduced from HREIMS. Its ^{1}H NMR data (see Figure 3) indicated the presence of a naphthyldihydroisoquinoline alkaloid substituted with four methoxy groups, resonating at δ 3.80, 3.99, 4.02, and 4.06. With a spin system of three neighboring protons, giving rise to two doublets (δ 6.66 and 6.82) and one doublet of doublets (δ 7.23), and from their H,H-COSY interactions, the position of the biaryl axis again had to be in the ring that bears the methyl group (CH_3-2′). The upfield shift of the latter (δ 2.06, Figure 3a) confirmed this result. Whether the coupling site was C-1′ or C-3′ became evident from NOESY correlations between CH_3-2′ and H-3′ and between H-3′ and OCH_3-4′ (Figure 3b). This finding was in agreement with NOESY correlations between H-8′ and H-4$_{eq}$ and between H-4$_{ax}$ and CH_3-2′ (Figure 3c), which were possible only if the alkaloid was 5,1′-linked. In the isoquinoline portion, the axis was indeed found to be located at C-5, as shown by HMBC correlations of both H$_{ax}$-4 and H-7 with the quaternary C-5 atom. The 5,1′-coupling type was further confirmed by the fact that only one out of four methoxy groups, OCH_3-6, was upfield shifted (δ 3.80). This was corroborated by NOESY interactions of H-7 (δ 6.56) with the protons of both of the two methoxy groups at C-6 and C-8 and of OCH_3-8 with CH_3-1 (Figure 3b). The presence of a double bond between C-1 and the nitrogen atom was deduced from the chemical shift (δ 3.07) of the CH_3-1 signal in the ^{1}H NMR spectrum, from its multiplicity (singlet), from the chemical shift of the ^{13}C NMR peak of C-1 (δ 174.4), and from the absence of an H-1 signal, which normally appears around δ 4.20 in 1,2,3,4-tetrahydroisoquinolines.[2] These findings permitted us to attribute the constitution **7** shown in Figure 3a to the alkaloid.

The absolute configuration at C-3 of **7** was again determined to be *S* by ruthenium-mediated oxidative degradation,[23] affording (*S*)-3-aminobutyric acid as the main product.

This, in combination with the relative configuration at the axis, as evident from the above-mentioned specific NOESY interactions between H-4$_{eq}$ and H-8′ and between CH_3-2′ and H-4$_{ax}$ (Figure 3c), showed that the axis had to be *P*-configured. In consequence, the compound had to possess the full stereostructure **7** as displayed in Figures 1 and 3c, thus being the as yet unknown 6-*O*-methyl analogue of the known[24] alkaloid ancistrocladinine. The *P*-configuration of *O*-methylancistrocladinine (**7**) at the biaryl axis was further corroborated by comparison of its CD spectrum with that of the corresponding atropodiastereomer, 6-*O*-methylhamatinine,[25] which was found to be opposite, as expected.[3,26]

The fifth compound, corresponding to a molecular formula of $C_{27}H_{33}NO_4$ ($M^+ = 435$ *m*/*z*) according to HREIMS, showed the ^{1}H NMR spectrum of a fully *O*- and *N*-methylated naphthyltetrahydroisoquinoline alkaloid, with four methoxy groups (δ 4.03, 3.98, 3.96, and 3.67), three *C*-methyl groups (δ 1.36, 1.78, and 2.12), and another three-proton singlet with a chemical shift (δ 2.96) characteristic of an *N*-CH_3 group[27] (Figure 4a). The position of the latter was clearly assigned by NOESY interactions with all proximal protons, i.e., with H-1, CH_3-1, H-3, and CH_3-3 (Figure 4b).

Like for *O*-methylancistrocladinine (**7**, see above), CH_3O-4′ (δ 4.03) and CH_3O-5′ (δ 3.98) were found to have "normal" shifts, which excluded the biaryl axis from being located at C-3′ or C-6′. This was confirmed by NOESY interactions in the series CH_3-2′−H-3′−CH_3O-4′ and CH_3O-5′−H-6′−H-7′−H-8′ (Figure 4b) and by the presence of three neighboring hydrogens at C-6′, C-7′, and C-8′, leaving only C-1′ open for the coupling site. As only one of the two remaining methoxy groups located at the isoquinoline part is upfield shifted, the naphthalene moiety should be coupled via C-5. This assumption was confirmed by HMBC correlations between H-7 and both C-6 and C-8 (Figure 4b), whose signals showed typical downfield shifts (δ 158.7 and 156.9, respectively) because the methoxy groups were

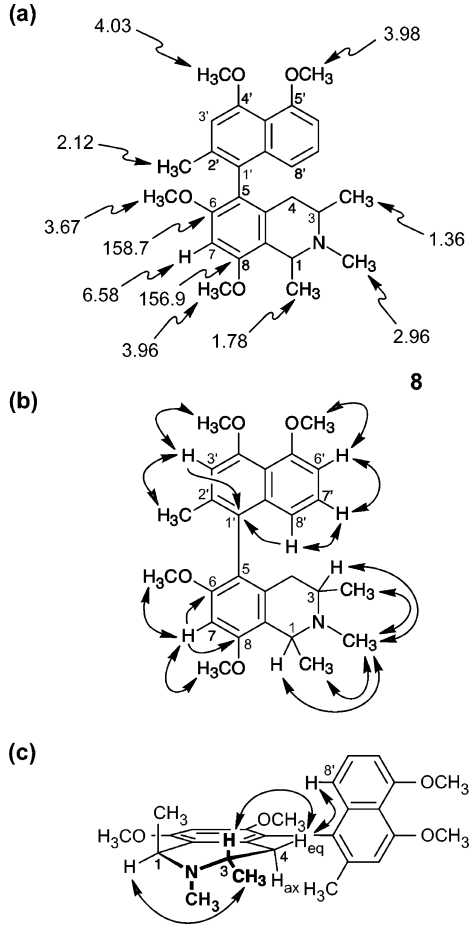

Figure 4. Selected NMR data of *O,N*-dimethylancistrocladine (**8**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a); HMBC (single arrows) and NOESY (double arrows) (b) relevant for the configuration; relative configuration at the biaryl axis and the stereogenic centers through NOESY interactions (c).

located on these carbon atoms. From CH_3O-8, in turn, NOESY interactions with H-7 were found. Referring to the naphthalene part, the HMBC correlations from both H-3′ and H-8′ to the *peri*-position, C-1′, established this quaternary C atom as bearing the isoquinoline substituent. Thus, the 5,1′-coupling type could be assigned to the alkaloid and hence the configuration **8** shown in Figure 4a.

The absolute configuration at C-3 was again determined by ruthenium-mediated oxidative degradation.[23] 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 *trans* from a NOESY interaction between CH_3-3 and H-1 (Figure 4c), which, in conjunction with the absolute *S*-configuration at C-3, unambiguously indicated C-1 to be *S*-configured, too.

The absolute configuration at the axis was deduced from its CD spectrum, which showed a first positive and a second negative Cotton effect (Figure 5). From the good agreement of this CD spectrum with that of the known[28] 1-epimer of this compound, *O*-methylancistrocline (**9**, Figure 5), the alkaloid was assigned to be *P*-configured, too. A NOESY interaction between H-4$_{eq}$ and H-8′ fully confirmed the relative (and hence also the absolute) axial configuration (Figure 4c). The compound thus had the full absolute stereostructure **8** as shown in Figures 1 and 4c, i.e., that of a 1-*epi*-*O*-methylancistrocline or, in other words, the *O,N*-dimethyl analogue of the *trans*-configured alkaloid ancistrocladine.[19] This alkaloid had previously been prepared partial-synthetically from ancistrocladine,[19] but had never been found in nature.

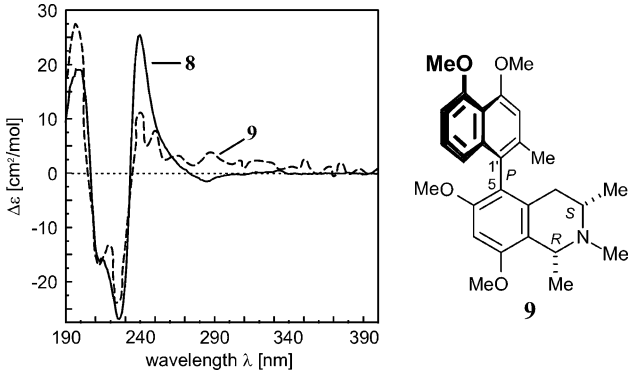

Figure 5. Comparison of the CD spectrum of *O,N*-dimethylancistrocladine (**8**) with that of the known *O*-methylancistrocline (**9**) to confirm the absolute axial configuration of **8**.

Table 1. Bioactivities of Compounds **4**−**8**

	IC$_{50}$ [μg/mL]				
	4	**5**	**6**	**7**	**8**
P. falciparum (strain: K1)[a]	0.3	0.7	0.1	2.2	3.6
standard: chloroquine 0.044[b]					
P. falciparum (strain: 3D7)[c]	1.9	9.1	4.2	5.4	34.1
standard: chloroquine 0.01[b]					
T. cruzi	23.4	4.3	14	60.5	65.3
standard: benznidazole 0.29[b]					
T. b. rhodesiense	2.0	4.3	1.3	5.2	5.4
standard: melarsoprol 0.0031[b]					
L. donovani	2.9	dnp[d]	dnp[d]	30	dnp[d]
standard: miltefosin 0.305[b]					
cytotoxicity L6 (MIC)	28.3	19.9	40.7	>90	42.9

[a] Basel. [b] All values in μg/mL. [c] Copenhagen. [d] Determination not possible due to higher toxicity to the host cell as compared to the parasite.

The pronounced antiprotozoal activities of naphthylisoquinoline alkaloids against pathogens belonging to the genera *Plasmodium*, *Leishmania*, and *Trypanosoma*[6−9] encouraged us to screen not only the new isolated compounds ancistrotanzanine C (**6**), *O*-methylancistrocladinine (**7**), and *O,N*-dimethylancistrocladine (**8**) but also the known ones, ancistrocladidine (**4**) and ancistrotectorine (**5**), against these parasites (Table 1).

Compounds **4** and **5** exhibited moderate and alkaloid **6** good antiplasmodial activities against the K1 strain of *Plasmodium falciparum* (resistant to chloroquine and pyrimethamine), but were far less efficient against the chloroquine-sensitive *P. falciparum* 3D7 strain. These findings, although not reaching the good results of other naphthylisoquinolines,[6−9] gave an important contribution to our ongoing QSAR (quantitative structure−activity relationship) investigations.[29,30]

Compound **4** was active against *Leishmania donovani*, which causes the widespread tropical disease visceral leishmaniasis; its activity in comparison to the highly active ancistrotanzanine B (**2**) (IC$_{50}$ = 1.6 μg/mL)[13] was weaker only by a factor of 2 and, in comparison to the positive control, miltefosin, by a factor of 10. By contrast, only weak or no antitrypanosomal activities were exhibited by compounds **4**−**8** against the pathogen of African sleeping sickness, *Trypanosoma brucei rhodesiense*, or against *T. cruzi* (Chagas' disease). The compounds were found to show only weak or no cytotoxicities.

All the compounds isolated from *A. tanzaniensis*, **4**−**8**, like those identified earlier, **1**−**3**, bear an oxygen at C-6 and have the *S*-configuration at C-3, characteristic of the alkaloids belonging to the "Ancistrocladaceae type".[2] The alkaloids from *A. tanzaniensis* thus belong to the same

group as those of the other East African *Ancistrocladus* species, *A. robertsoniorum*,[20,21] and the Southeast Asian species, such as *A. tectorius*.[14] By contrast, the Central and West African *Ancistrocladus* species are known to produce typical "Dioncophyllaceae-type" alkaloids (*R*-configurated at C-3 and devoid of an oxygen function at C-6)[31] and also mixed, hybrid-type alkaloids (e.g., with *R*-configuration at C-3 and oxygenated at C-6).[27]

A. tanzaniensis has revealed itself as a rewarding subject for phytochemical studies; it is now the plant species with the as yet highest number of 7,3′-coupled naphthylisoquinolines and likewise produces numerous 5,1′- and 5,8′-coupled alkaloids. This may be one of the reasons why it is this unusual "new" plant species in which the recently discovered coupling combination—the 5,3′-linkage—has been found for the first time.[13]

These results prove that the genus *Ancistrocladus* continues to be an unusually rich source of new naphthylisoquinoline alkaloids with new structural characteristics and promising bioactivities in the search for urgently needed new drugs against widespread fatal diseases such as malaria, leishmaniasis, Chagas' disease, and African sleeping sickness.

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 for compounds **4**, **5**, and **8** and on a Perkin-Elmer 241MC polarimeter for compounds **6** and **7**. 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, CH_3CN unless otherwise stated, 0.1 cm cell) were taken on a Jasco J-715 spectropolarimeter. ^1H 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 ^1H 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 JEOL JMS-HX/HX 110A and Finnigan MAT 90 instruments (70 eV), respectively. Preparative HPLC was carried out on a silica gel 60 column (Knauer, 18.5 × 327 mm, 5 μm), flow 6 mL min^{-1}, UV detection (307 nm), solvent CH_2Cl_2−MeOH−diethylamine (195:4:1), isocratic, or 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. (*R*)-MTPA-Cl was prepared from (*S*)-MTPA (Fluka Chemie AG, Deisenhofen, Germany) as described earlier.[20] Organic solvents were dried and distilled prior to use.

Plant Material. Leaves of *A. tanzaniensis* were collected by two of us (H.N. and F.M.) in the Uzungwa Mountains in Tanzania, in February 2000, and identified by C. Frimodt-Møller, University of Copenhagen, and Dr. H. Rischer, University of Würzburg. Voucher specimens are deposited at the Department of Medicinal Chemistry, Pharmaceutical University of Denmark, and at Herbarium Bringmann (no. 42), University of Würzburg, Germany.

Extraction and Isolation. The air-dried material (500 g leaves) was ground and sequentially extracted with petroleum ether, CH_2Cl_2, and MeOH. The CH_2Cl_2 extract was concentrated in vacuo to give 29.6 g of a residue, 2 g of which were dissolved in MeOH and directly resolved using preparative HPLC with a Symmetry C_{18} column to give 2.6 mg of compound **8** (t_R = 18.8 min), 2.1 mg of compound **5** (t_R = 22.5 min), and 3.8 mg of compound **4** (t_R = 23.0 min). The rest of the extract was fractionated by vacuum-liquid chromatography on silica gel 60H (1 kg, 90% < 45 μm, Merck) using CH_2Cl_2−MeOH−diethylamine (90:10:3, 1.5 L; 80:20:3, 1 L; 60:40:3, 1 L) as the eluent. The fraction eluted between 2 and 3.5 L was further fractionated by column chromatography on silica gel 60 (650 g, 60−200 mesh, Merck) using petroleum ether−EtOAc−diethylamine (90:10:3, followed by 80:20:3, 70:30:3, 60:40:3, 50:50:3, and 40:60:3, each 1.5 L). The fraction eluted with petroleum ether−EtOAc−diethylamine (70:30:3) was concentrated to give 910 mg of a residue, while the fraction eluted by petroleum ether−EtOAc−diethylamine (40:60:3) yielded 505 mg of a residue. The 910 mg fraction was purified by preparative HPLC on silica gel 60, to give 2.8 mg of compound **7** (t_R = 12.4 min), while the 505 mg fraction gave 12.4 mg of compound **6** (t_R = 7.6 min).

Ancistrocladidine (4): amorphous solid; $[\alpha]_D^{25}$ −122.3° (*c* 0.05, MeOH) (lit. −129.7°, *c* 0.06, $CHCl_3$);[16] spectroscopic data were identical to those of ancistrocladidine isolated from *A. heyneanus*[15] and *A. tectorius*.[16]

Ancistrotectorine (5): amorphous solid; $[\alpha]_D^{25}$ −4.1° (*c* 0.2, MeOH) (lit. −3.6°, *c* 0.2, EtOH);[18] spectroscopic data were identical to those of ancistrotectorine isolated from *A. tectorius*[17] and *A. guineënsis*.[18]

Ancistrotanzanine C (6): yellow oil; $[\alpha]_D^{25}$ −75.5° (*c* 0.01, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 231 (0.74), 307 (0.07) nm; CD (CH_3CN) $\Delta\epsilon_{220}$ +26.8, $\Delta\epsilon_{238}$ −17.5, $\Delta\epsilon_{265}$ −17.2, $\Delta\epsilon_{322}$ −8.1, $\Delta\epsilon_{337}$ −10.9; IR (NaCl) ν_{max} 3381, 2965, 1607, 1579, 1362, 1204, 1091 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (3H, d, *J* = 6.2 Hz, CH_3-3), 1.45 (3H, d, *J* = 6.5 Hz, CH_3-1), 2.18 (3H, s, CH_3-2′), 2.49 (3H, s, CH_3−N), 2.56 (1H, m, H-3), 2.63 (1H, dd, *J* = 15.5, 10.2 Hz, H_{ax}-4), 2.73 (1H, dd, *J* = 15.5, 3.3 Hz, H_{eq}-4), 3.33 (3H, s, OCH_3-8), 3.84 (1H, q, *J* = 6.5 Hz, H-1), 4.03 (3H, s, OCH_3-5′), 6.58 (1H, s, H-5), 6.76 (1H, dd, *J* = 8.0, 1.4 Hz, H-6′), 7.29 (1H, s, H-1′), 7.34 (1H, dd, *J* = 7.3, 8.3 Hz, H-7′), 7.38 (1H, dd, *J* = 8.3, 1.4 Hz, H-8′); ^{13}C NMR ($CDCl_3$) δ 20.3 (CH_3-2′), 21.4 (CH_3-3), 23.4 (CH_3-1), 37.9 (C-4), 41.8 (CH_3−N), 55.5 (C-3), 56.1 (OCH_3-5′), 57.0 (C-1), 60.2 (OCH_3-8), 103.7 (C-6′), 109.7 (C-5), 113.4 (C-10′), 114.7 (C-7), 115.0 (C-3′), 119.8 (C-1′), 121.2 (C-8′), 124.2 (C-9), 126.5 (C-7′), 136.6 (C-9′), 138.6 (C-2′), 137.4 (C-10), 156.1 (C-5′), 152.0 (C-4′), 152.1 (C-8), 155.7 (C-6); EIMS *m/z* 407 $[M]^+$ (100), 392 $[M - CH_3]^+$ (50), 377 $[M - (CH_3)_2]^+$ (17), 376 $[M - OCH_3]^+$ (11), 203.5 $[M]^{2+}$ (7); HREIMS *m/z* 407.2094 (calcd for $C_{25}H_{29}NO_4$, 407.2097).

***O*-Methylancistrocladinine (7):** amorphous solid; mp 102 °C (MeOH); $[\alpha]_D^{25}$ −31.0° (*c* 0.04, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (0.22), 303 (0.04) nm; CD (CH_3CN) $\Delta\epsilon_{211}$ +17.1, $\Delta\epsilon_{231}$ −10.6, $\Delta\epsilon_{254}$ −1.2, $\Delta\epsilon_{296}$ −1.9; IR (NaCl) ν_{max} 2921, 2855, 1583, 1261, 1116 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (3H, d, *J* = 6.4 Hz, CH_3-3), 2.06 (3H, s, CH_3-2′), 2.24 (1H, dd, *J* = 17.4, 9.9 Hz, H_{ax}-4), 2.42 (1H, dd, *J* = 16.8, 5.4 Hz, H_{eq}-4), 3.07 (3H, s, CH_3-1), 3.75 (1H, m, H-3), 3.80 (3H, s, OCH_3-6), 3.99 (3H, s, OCH_3-4′), 4.02 (3H, s, OCH_3-5′), 4.06 (3H, s, OCH_3-8), 6.56 (1H, s, H-7), 6.66 (1H, d, *J* = 8.4 Hz, H-6′), 6.79 (1H, s, H-3′), 6.82 (1H, d, *J* = 7.8 Hz, H-8′), 7.23 (1H, dd, *J* = 8.1, 8.1 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 17.5 (CH_3-3), 20.4 (CH_3-2′), 24.6 (CH_3-1), 31.4 (C-4), 48.0 (C-3), 56.2 (OCH_3-8), 56.4 (OCH_3-6), 56.4 (OCH_3-5′), 56.6 (OCH_3-4′), 94.1 (C-7), 106.0 (C-6′), 108.3 (C-3′), 108.7 (C-9), 116.5 (C-10′), 116.9 (C-8′), 121.6 (C-5), 122.0 (C-1′), 127.5 (C-7′), 135.9 (C-2′), 136.0 (C-9′), 140.6 (C-10), 157.3 (C-4′), 158.0 (C-5′), 164.3 (C-8), 166.6 (C-6), 174.4 (C-1); EIMS *m/z* 419 $[M]^+$ (100), 404 $[M - CH_3]^+$ (48), 388 $[M - OCH_3]^+$ (12), 209.5 $[M]^{2+}$ (6); HREIMS *m/z* 419.2095 $[M]^+$ (calcd for $C_{26}H_{29}NO_4$, 419.2097).

***O,N*-Dimethylancistrocladine (8):** pale-yellow powder; mp 163 °C (dec) (lit. 183−185 °C, ether);[19] $[\alpha]_D^{25}$ −17.3° (*c* 0.03, MeOH) (lit. −21.0°, *c* 2.55, $CHCl_3$);[19] UV (MeOH) λ_{max} (log ϵ) 235 (1.86), 303 (1.32) nm; CD (MeOH) $\Delta\epsilon_{199}$ +19.6, $\Delta\epsilon_{226}$ −27.6, $\Delta\epsilon_{240}$ +26.2, $\Delta\epsilon_{285}$ −1.5; IR (NaCl) ν_{max} 2925, 2852, 2359, 1674, 1584, 1261, 1202, 1129 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, *J* = 6.7 Hz, CH_3-3), 1.78 (3H, d, *J* = 6.8 Hz, CH_3-1), 2.12 (3H, s, CH_3-2′), 2.22 (1H, dd, *J* = 17.3, 4.1 Hz, H_{eq}-4), 2.59 (1H, dd, *J* = 17.3, 9.5 Hz, H_{ax}-4), 2.96 (3H, d, *J* = 4.5 Hz, CH_3−N), 3.02 (1H, m, H-3), 3.67 (3H, s, OCH_3-6), 3.96 (3H, s, OCH_3-8), 3.98 (3H, s, OCH_3-5′), 4.03 (3H, s, OCH_3-4′), 4.60 (1H, q, *J* = 6.8 Hz, H-1), 6.58 (1H, s, H-7), 6.63 (1H, dd, *J* = 8.5, 1.0 Hz, H-8′), 6.79 (1H, dd, *J* = 8.0, 0.7 Hz, H-6′), 6.83 (1H, s, H-3′),

7.18 (1H, dd, J = 8.4, 7.8 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 18.7 (CH_3-3), 19.7 (CH_3-1), 20.8 (CH_3-2′), 30.8 (C-4), 43.2 (CH_3−N), 55.9 (OCH_3-8), 56.4 (OCH_3-6), 56.7 (OCH_3-4′), 56.9 (OCH_3-5′), 59.9 (C-3), 60.4 (C-1), 94.8 (C-7), 105.9 (C-6′), 109.3 (C-3′), 113.7 (C-9), 116.8 (C-10′), 117.5 (C-8′), 120.0 (C-5), 123.7 (C-1′), 127.0 (C-7′), 133.5 (C-10), 136.6 (C-9′), 136.6 (C-2′), 156.6 (C-4′), 156.9 (C-8), 158.1 (C-5′), 158.7 (C-6); EIMS m/z 435 $[M]^+$ (5), 420 $[M - CH_3]^+$ (100), 404 $[M - OCH_3]^+$ (6); HREIMS m/z 435.2406 $[M]^+$ (calcd for $C_{27}H_{33}NO_4$, 435.2409).

Oxidative Degradation of 6, 7, and 8. The ruthenium(III)-catalyzed periodate degradation, derivatization of the resulting amino acids, and subsequent GC-MSD analyses were carried out as described previously. [23]

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.[32]

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References and Notes

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