



Ancistrobertsonines B, C, and D as well as 1,2-didehydroancistrobertsonine D from *Ancistrocladus robertsoniorum*¹

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Abstract

From the East African Liana *Ancistrocladus robertsoniorum*, four new naphthylisoquinoline alkaloids have been isolated using High Speed Countercurrent Chromatography (HSCCC) and High Performance Liquid Chromatography (HPLC). Their stereostructures were established by spectroscopic, chemical, and chiroptical methods. Two of the new compounds, ancistrobertsonines B and C, constitute *O*- and *N*-permethylated 5,1'-coupled and 5,8'-coupled regioisomeric naphthylisoquinolines, while ancistrobertsonine D and its 1,2-didehydro analogue are based on a 7,1'-coupling mode between the isoquinoline and naphthalene moieties. Biological tests revealed that some of the isolated alkaloids possess moderate antimalarial activities. © 1999 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Ancistrocladus robertsoniorum Léonard (Ancistrocladaceae) (Léonard, 1984; Bringmann, Haller, Bär, Isahakia & Robertson, 1994), a tropical liana indigenous to Kenya, belongs to the small monogeneric family of Ancistrocladaceae, which consists of 20 species (Gereau, 1997). As a rich source of second-

ary metabolites, the Ancistrocladaceae and the closely related Dioncophyllaceae produce a unique class of natural biaryls, the naphthylisoquinoline alkaloids, being remarkable with respect to their intriguing structures, their unprecedented biosynthesis, promising biological activities, and chemotaxonomic implications (Bringmann & Pokorny, 1995; Bringmann, François, Aké Assi & Schlauer, 1998). Early investigations on *A. robertsoniorum* showed this plant to form the naphthoquinone droserone in a crystalline form when wounded (Bringmann, Kehr, Dauer, Gulden, Haller, Bär et al., 1993; Peters et al., 1995). First systematic phytochemical investigations carried out recently by our group (Bringmann, Teltschik, Schäffer, Haller, Haller, Bär et al., 1998) revealed the presence of the 5,1'-coupled al-

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kaloid ancistrocladine (**1a**) and its atropisomer, hamatine (**1b**), as well as the 5,8'-linked naphthylisoquinolines ancistrobrevine B (**2**) and ancistrobertsonine A (**3**) as the major alkaloids of *A. robertsoniorum*. In this paper, the isolation of four minor naphthylisoquinolines, named ancistrobertsonines B (**4**), C (**5**), and D (**7**) as well as 1,2-didehydroancistrobertsonine D (**6**, alias 6-*O*-demethylancistrocladisine), i.e. 5,1'- and 7,1'-coupled alkaloids, is described. With the isolation of these four new metabolites, a total of eight naphthylisoquinoline alkaloids (of which five are new) have been identified from this species, allowing a more exact chemotaxonomic classification of this interesting plant (Fig. 1).

2. Results and discussion

A. robertsoniorum was collected in Kenya in November 1992 and in August 1993. For the extraction and separation of the alkaloids the same isolation

method as previously elaborated (Bringmann, Teltschik et al., 1998) was used, viz the extraction of the dried and ground plant material with 1 N H₂SO₄–MeOH (5:1), followed by liquid–liquid partition and further resolution by High Speed Countercurrent Chromatography (HSCCC) (Conway, 1990). In total, six fractions were obtained. The isolation and characterization of the major alkaloids **1a**, **1b**, **2**, and **3** of fractions 3–5 have already been described earlier (Bringmann, Teltschik et al., 1998). Further analysis of fractions 1, 3, and 6 yielded four Dragendorff-positive compounds.

¹H NMR investigations of fraction 1 showed the presence of two very similar, *N*-methylated Ancistrocladaceae-type naphthylisoquinoline alkaloids, which were separated by HPLC on a chiral stationary phase (Chiralcel OD) after purification by column chromatography and preparative TLC on silica gel. The molecular formula of both compounds was shown to be C₂₇H₃₃NO₄ by High Resolution Mass Spectrometry (HRMS).

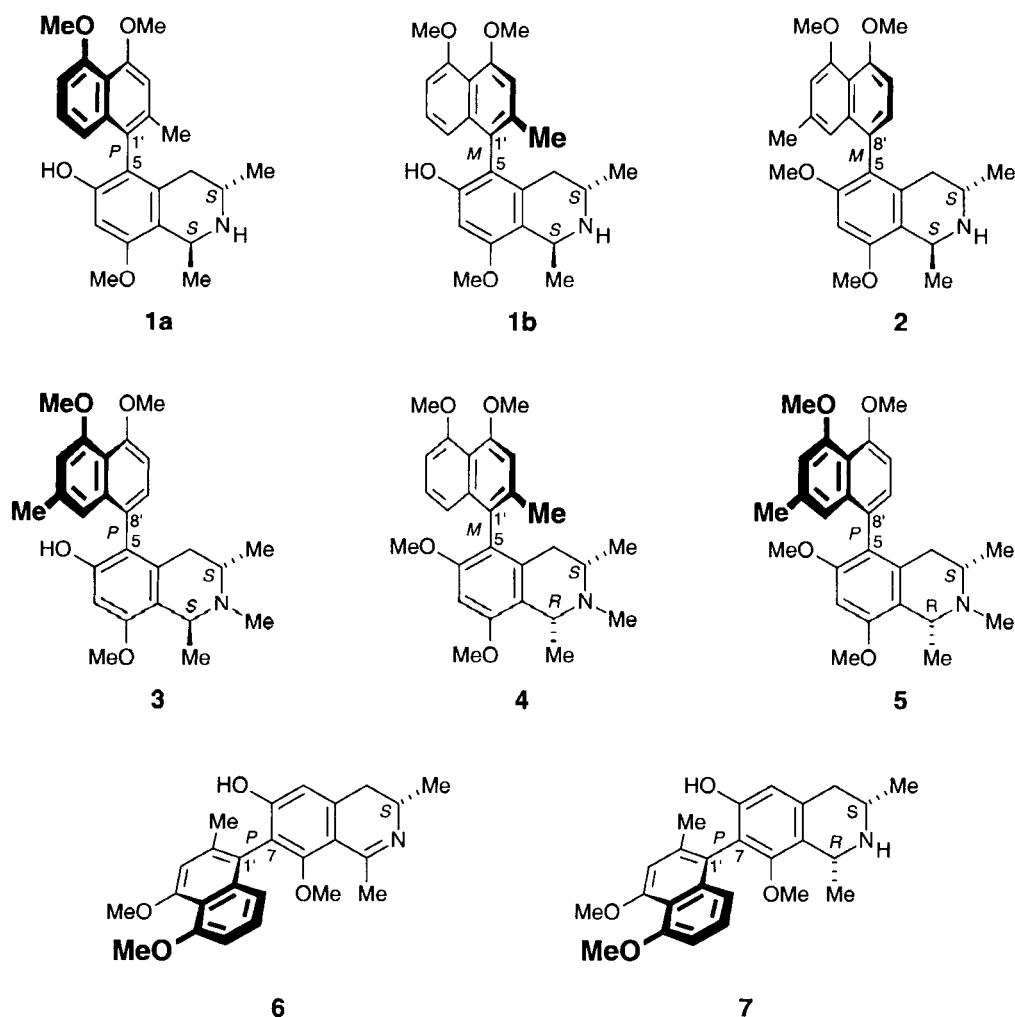
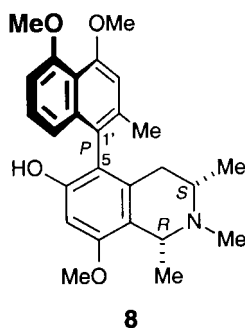


Fig. 1. Naphthylisoquinoline alkaloids of *A. robertsoniorum*.

The ^1H NMR spectrum of the slightly less polar alkaloid, named ancistrobertsonine B, resembled the spectrum of ancistrocline (**8**), a 5,1'-coupled alkaloid from *A. tectorius* (Bringmann & Kinzinger, 1992).



A first hint at the position of the biaryl axis in the naphthalene part of the molecule was obtained from the chemical shift of the Me-2' group (2.05 ppm) (see Fig. 2(a)). Like in **8**, this signal is high-field shifted, whereas the resonances of MeO-4' (4.00 ppm) and MeO-5' (3.97 ppm) remain in their 'normal' positions, excluding a 3'- and a 6'-position of the biaryl axis. A series of NOE and ROESY (Rotating Frame Overhauser Effect Spectroscopy) measurements (see Fig. 2(b)), as well as the spin system pattern also exclude the biaryl axis to be situated in the 6'-, 7'-, or 8'-position and confirm the coupling site to be C-1' in the naphthalene moiety.

In contrast to ancistrocline (**8**), ancistrobertsonine B bears a methoxy (instead of a hydroxy) group at C-6. The signal of MeO-6 appears up-field shifted (3.62 ppm), while the resonance of MeO-8 remains unaffected at 3.92 ppm, which indicates the biaryl axis to be situated at C-5. This conclusion is confirmed by the high-field shift of the protons at C-4 (1.93 and 2.27 ppm) and Me-3 (1.00 ppm) (see Fig. 2(a)). In addition, a C-7 coupling site can be excluded because of ROESY interactions of H-7 with both MeO-6 and MeO-8 groups and Heteronuclear Multiple Bond Correlations (HMBC) of H-7 with C-6 and C-8 as shown in Fig. 2(b). The relative configuration of C-1 vs. C-3 was concluded to be *cis*, from the chemical shifts of H-1 (3.81 ppm) and H-3 (2.38 ppm) as well as from NOE interactions between these two spin systems. Further NOE experiments also showed an interaction between H-8' in the naphthalene part and H-4_{ax} (which was identified as such by the large coupling constant of $J_{ax}=9.4$ Hz over to H-3) in the isoquinoline part, proving both spin systems to be on the same side of the molecule as depicted in Fig. 2(c).

The absolute configuration at C-3 was determined by ruthenium-mediated oxidative degradation (Bringmann, God & Schäffer, 1996). The degradation products, 3-(*N*-methylamino)butyric acid and *N*-methylalanine (as well as their *N*-demethylated ana-

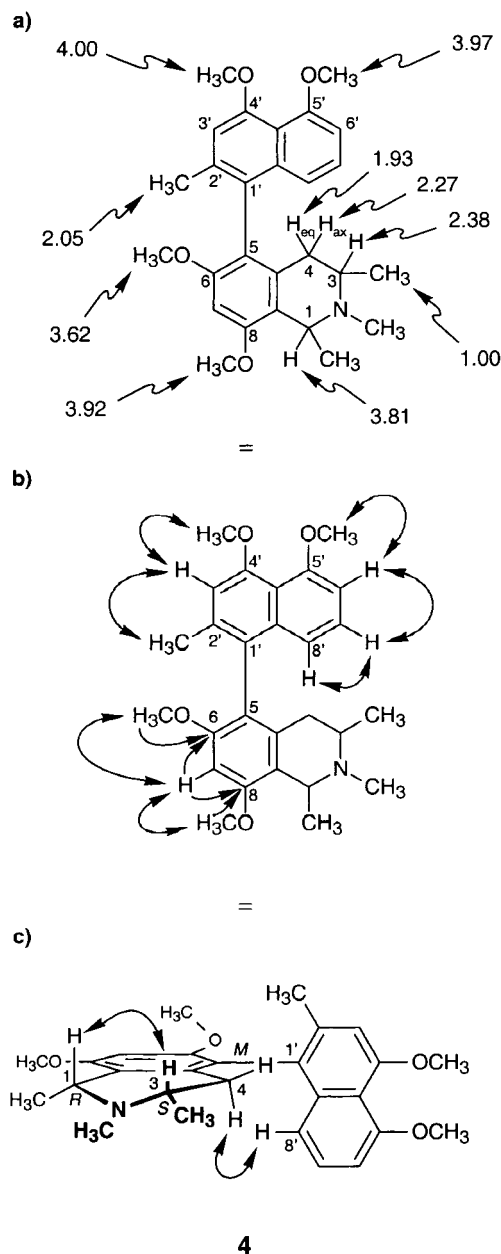


Fig. 2. Constitution of ancistrobertsonine B (**4**) from (a) selected ^1H NMR chemical shifts (δ values in ppm), as well as (b) NOE, ROESY (double arrows) and relevant HMBC (unidirectional arrows) interactions; its relative stereostructure (c) as determined by further characteristic NOE interactions.

logues) were stereochemically analyzed by Gas Chromatography with Mass Sensitive Detection (GC-MSD) after derivatization with *R*-Mosher chloride. The formation of 3-*S*-(*N*-methylamino)butyric acid showed the alkaloid clearly to be *S*-configured at C-3.

For *cis*-configured tetrahydroisoquinolines of this type less reliable information can be obtained for C-1, due to the formation of *N*-methylalanine from both of the two (here heterochiral) stereocenters, C-1 and C-3 (Bringmann, God & Schäffer, 1996). Therefore the ab-

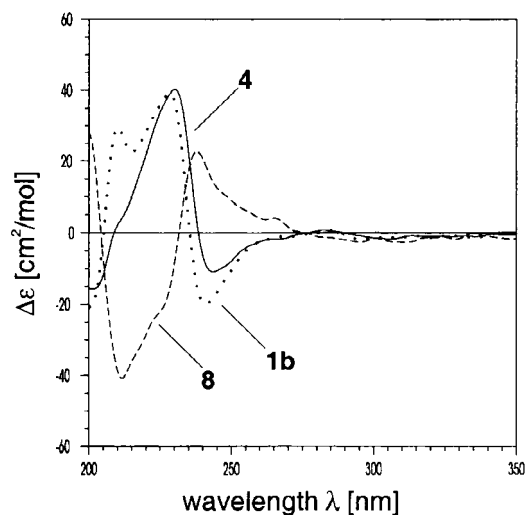


Fig. 3. CD spectra of ancistrobertsonine B (**4**) (—), hamatine (**1b**) (· · ·), and ancistrocline (**8**) (---).

solite configuration of C-1 was more conveniently deduced from the relative configuration of C-1 vs C-3 as established through NMR (see above) and the now known absolute configuration at C-3, unambiguously indicating C-1 to be *R*-configured.

Likewise based on the relative configuration as shown in Fig. 2(c) and the given absolute configuration at C-3, the axial configuration of ancistrobertsonine B was attributed as *M*. This result is corroborated by comparison of the CD spectrum with that of hamatine (**1b**, see Fig. 3), which also shows a first negative and a second positive Cotton effect, while the CD spectrum of the *P*-configured ancistrocline (**8**) is, as expected, virtually opposite. The new, thus *M*-configured alkaloid, ancistrobertsonine B, must consequently have stereostructure **4**.

The more polar compound from the same fraction, named ancistrobertsonine C, is a naphthylisoquinoline alkaloid with a similar ^1H NMR spectrum as compared to that of **4**. In contrast to **4**, however, a 'normal', i.e. not high-field shifted signal is observed for Me-2' (2.32 ppm), and a different multiplicity for the signals of the four aromatic protons (2 singlets and 2 doublets) in the naphthalene part of ancistrobertsonine C (see Fig. 4(a)). Like for ancistrobertsonine B, MeO-4' (3.97 ppm) and MeO-5' (4.00 ppm) were found to have 'normal' shifts, which exclude the biaryl axis to be located at C-3' or C-6'. This was confirmed by ROESY experiments (see Fig. 4(b)), showing interactions between H-1', Me-2', H-3' and MeO-4' as well as between MeO-5', H-6' and H-7', leaving only the 8'-position open for the biaryl axis. Like in ancistrobertsonine B (**4**), the up-field shift of the signals of MeO-6 (3.62 ppm), Me-3 (0.99 ppm), H_{eq}-4 and H_{ax}-4 (1.99 and 2.27 ppm) indicate these protons to be in close proximity of the biaryl axis, which must thus be

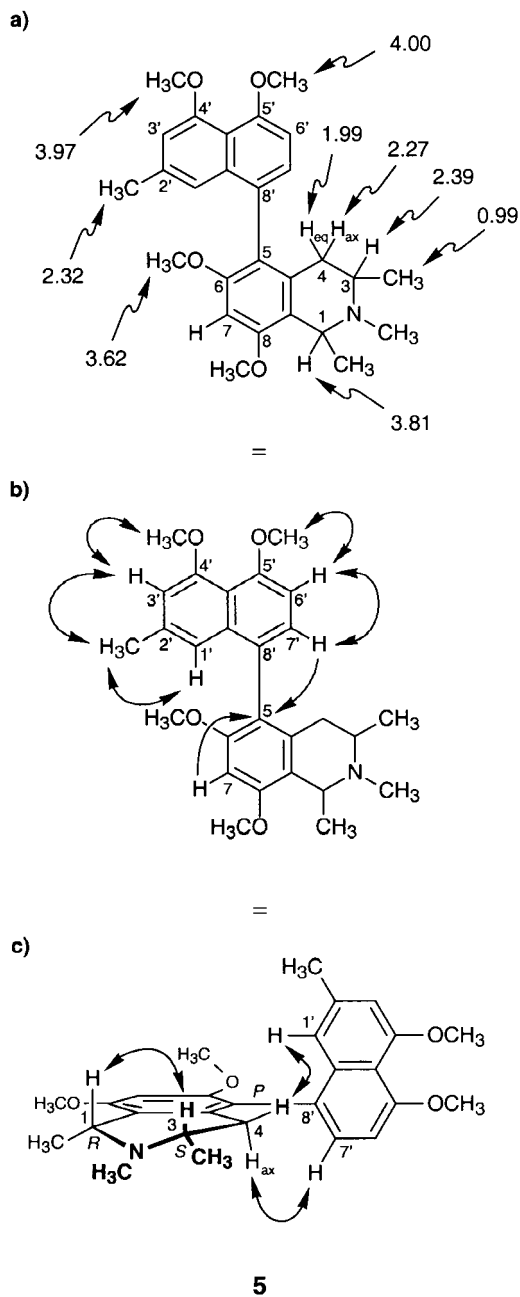


Fig. 4. Selected ^1H NMR shifts (δ values in ppm) (a), HMBC (single arrows) and ROESY interactions (double arrows) (b) relevant for the constitution; relative configuration at the stereogenic centers and the biaryl axis (c) of ancistrobertsonine C (**5**) through long-range NOE interactions.

located at C-5. The 5,8'-linkage of the two parts of the molecule is further confirmed by HMBC experiments, showing C-5 to have crosspeaks with H-7 in the isoquinoline part and H-7' in the naphthalene part (see Fig. 4(b)).

Like for **4** the relative configuration at C-1 vs C-3 was again established as *cis* by NOE-measurements (see Fig. 4(c)): Irradiation of H-1 (3.81 ppm) resulted in a significant enhancement of the signal of H-3 (2.39

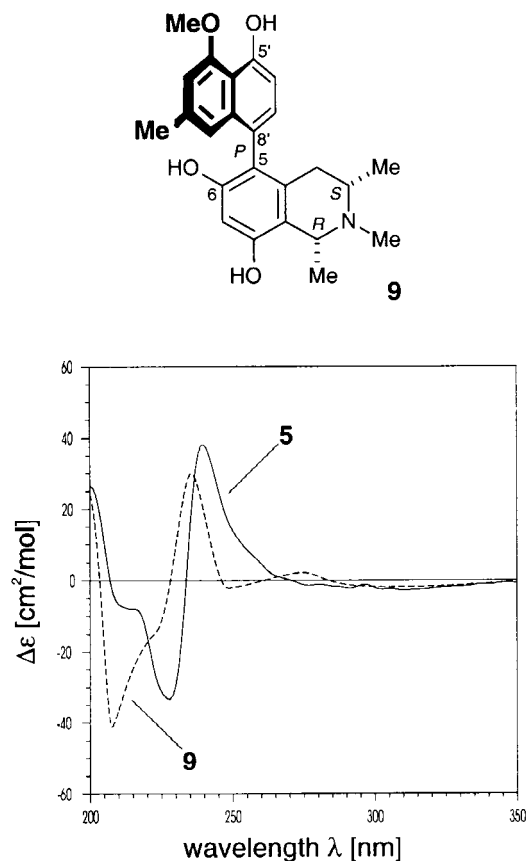


Fig. 5. CD comparison of ancistrobertsonine C (**5**) (—) and korupensamine D (**9**) (---).

ppm). This relative *cis*-configuration is in agreement with the chemical shifts of these two protons (see above), which are typical of *cis*-substituted 1,3-dimethyltetrahydroisoquinolines (Bringmann & Pokorny, 1995). Again, the absolute configuration at C-3 was established as *S*, by the above-mentioned degradation method with GC-MSD analysis of the Mosher derivative of 3-aminobutyric acid. With this fact and the NOE interaction between H-1 and H-3, the absolute configuration at C-1 unequivocally has to be *R*. The configuration of the biaryl axis as the third stereogenic element was determined as *P* by long-range NOE interactions between the protons H_{ax}-4 and H-7' as well as between H_{eq}-4 and H-1' (see Fig. 4(c)).

This axial *P*-configuration was further corroborated by comparison of the CD spectra of this new alkaloid, ancistrobertsonine C (**5**), with that of korupensamine D (**9**) (Hallock et al., 1994) (see Fig. 5), which is likewise 1*R*,3*S*,5*P*-configured. Due to the same configuration of the stereogenic elements the new alkaloid **5** also can be classified as 6,8,5'-*O,O,O*-trimethylkorupensamine D.

From the third HSCCC fraction, a polar yellow solid was obtained by preparative TLC and HPLC, which revealed to be a naphthylisoquinoline alkaloid,

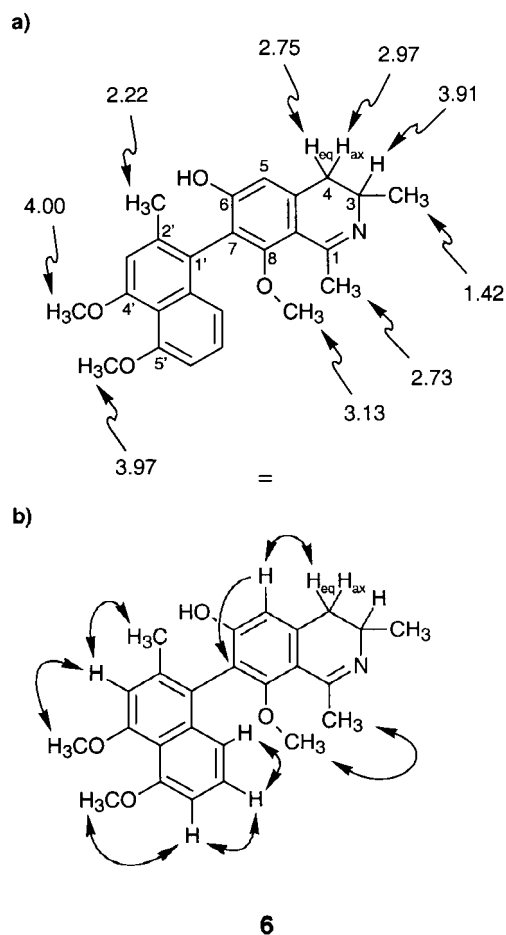
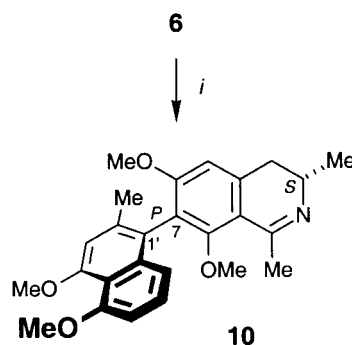


Fig. 6. Constitution **6** of 1,2-didehydroancistrobertsonine D from (a) selected ¹H NMR chemical shifts (δ values in ppm), as well as (b) ROESY and relevant HMBC interactions.

with a constitution related to that of ancistrocladisine (**10**, cf Scheme 1) from *A. heyneanus* (Govindachari, Parthasarathy & Desai, 1972) and *A. hamatus* (Desai et al., 1976).

The most obvious difference between these two compounds is the lack of an *O*-methyl group in the new alkaloid **6** compared with **10**, from which the molecular formula was deduced to be C₂₅H₂₇NO₄. This was con-



Scheme 1. Transformation of 1,2-didehydroancistrobertsonine (**6**) into ancistrocladisine (**10**). (i) CH₂N₂, MeOH–H₂O (9:1) (Organikum, 1988).

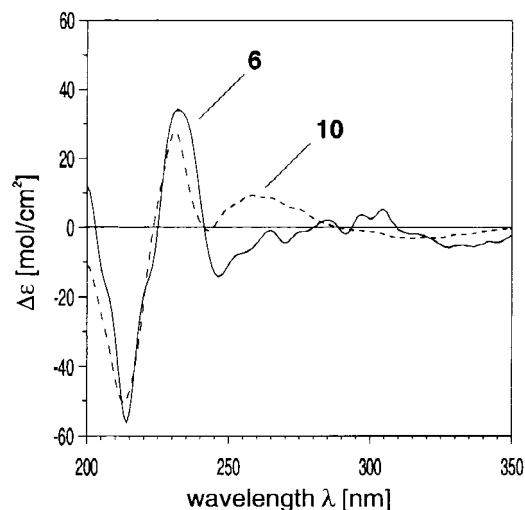


Fig. 7. CD comparison of 1,2-didehydroancistrobertsonine D (**6**) (—) and ancistrocladisine (**10**) (---).

firmed by HRMS analysis of the $[M]^+$ peak at m/z 405.

The ancistrocladisine-like constitution of the new alkaloid **6** was clearly deduced from NMR data (see Fig. 6(a)). The spin coupling pattern of H-3, H_{eq}-4 and H_{ax}-4, the signal of Me-3, which appears as a doublet, and the broad singlet of Me-1 (2.73 ppm) strongly indicated the presence of a naphthyl-3,4-dihydroisoquinoline. For the Me-1 signal a slow H/D-exchange was observed in D₂O or CD₃OD. The resonances of Me-3 (1.42 ppm), H-3 (3.91 ppm), H_{eq}-4 and H_{ax}-4 (2.75 and 2.97 ppm) did not appear high-field shifted. This fact and the ROESY interactions observed between H-4_{eq} and H-5 (see Fig. 6(b)) excluded the biaryl axis to be situated in the 5-position in the isoquinoline part of the molecule. Further analysis of the ROESY experiment (marked interaction between Me-1 and MeO-8, but no interactions between H-5 and any of the methoxy groups) revealed a HO-6/MeO-8 substitution pattern in the isoquinoline part. The high-field shift of MeO-8 (3.13 ppm) and the HMBC interaction between H-5 and C-7 (see Fig. 6(b)) clearly showed C-7 to be the coupling position in the isoquinoline part of the molecule. In the naphthalene part of the molecule the biaryl axis is attached to C-1', which is deduced from the high-field shift of the protons of Me-2' (2.22 ppm), the unaffected, i.e. not high-field shifted resonances of MeO-4' and MeO-5' (4.00 and 3.97 ppm) (see Fig. 6(a)), the spin system pattern observed for the aromatic protons, and ROESY interactions between Me-2', H-3' and MeO-4' as well as between MeO-5', H-6', H-7' and H-8' (Fig. 6(b)).

From the *S*-configuration of the Mosher derivative of the 3-aminobutyric acid formed in the oxidative degradation (Bringmann et al., 1996) of the alkaloid,

the configuration of its stereogenic center at C-3 was established as *S*.

The last structural information required was the configuration at the sterically hindered biaryl linkage. Attempts to elucidate the absolute configuration at the biaryl axis by long-range NOE interactions (Bringmann, Koppler, Scheutzw & Porzel, 1997) were not successful, whereas the comparison of the CD spectra of the new compound and the closely related alkaloid ancistrocladisine (**10**) (Govindachari et al., 1972; Bringmann, Pokorny, Stäblein & Aké Assi, 1993) gave a first hint at a *P*-configuration at the axis (see Fig. 7).

For an unambiguous elucidation of the axial chirality and of the full stereostructure of the new compound in general, the alkaloid was reacted with diazomethane (de Boer & Baker 1963; Organikum, 1988), to give its 6-*O*-methyl derivative (see Scheme 1), which proved to be fully identical with natural (from *A. heyneanus*) and synthetic (Bringmann & Reuscher, 1989) ancistrocladisine (**10**), in all its physical, spectroscopic, and chromatographic properties, thus confirming the full stereostructure of the novel alkaloid as **6**, i.e., 6-*O*-demethylancistrocladisine or (see below) 1,2-didehydroancistrobertsonine D (**6**).

Workup of the sixth fraction of the HSCCC separation by preparative TLC on silica gel and reversed phase HPLC yielded a pale yellow amorphous solid, homogeneous according to analytical TLC. By HRMS of the $[M-Me]^+$ peak, the compound, subsequently named ancistrobertsonine D, was found to correspond to the molecular formula C₂₅H₂₉NO₄. Again the constitution of this new naphthylisoquinoline was established by ¹H NMR and ¹³C NMR spectroscopy. A 7,1'-position of the axis was deduced i.a. from the high-field shifted signals of Me-2' (2.22 ppm) and MeO-8 (3.07 ppm) (see Fig. 8(a)), indicating these two groups to be adjacent to the biaryl axis, and ROESY interactions observed between Me-1 and MeO-8, Me-2', H-3', and MeO-4' as well as between MeO-5', H-6', H-7' and H-8' (see Fig. 8(b)). A 5,1'-coupling between the two halves can be excluded by the 'normal' chemical shift of H_{ax}-4 (2.54 ppm) and H_{eq}-4 (2.75 ppm) and by a ROESY interaction between H_{eq}-4 and the isolated aromatic proton (6.58 ppm), H-5 (see Fig. 8(a/b)).

The relative configuration at the centers and the axis was established by ROESY experiments. A weak, but significant ROESY interaction between H-1 (4.31 ppm) and H-3 (3.01 ppm) revealed the relative configuration of C-1 vs C-3 to be *cis*. The same experiment also showed a distinct interaction between Me-1 in the isoquinoline and Me-2' in the naphthalene part, indicating both groups to be on the same side of the molecule, which should thus have the (relative) stereostructure **7** shown in Fig. 8(c).

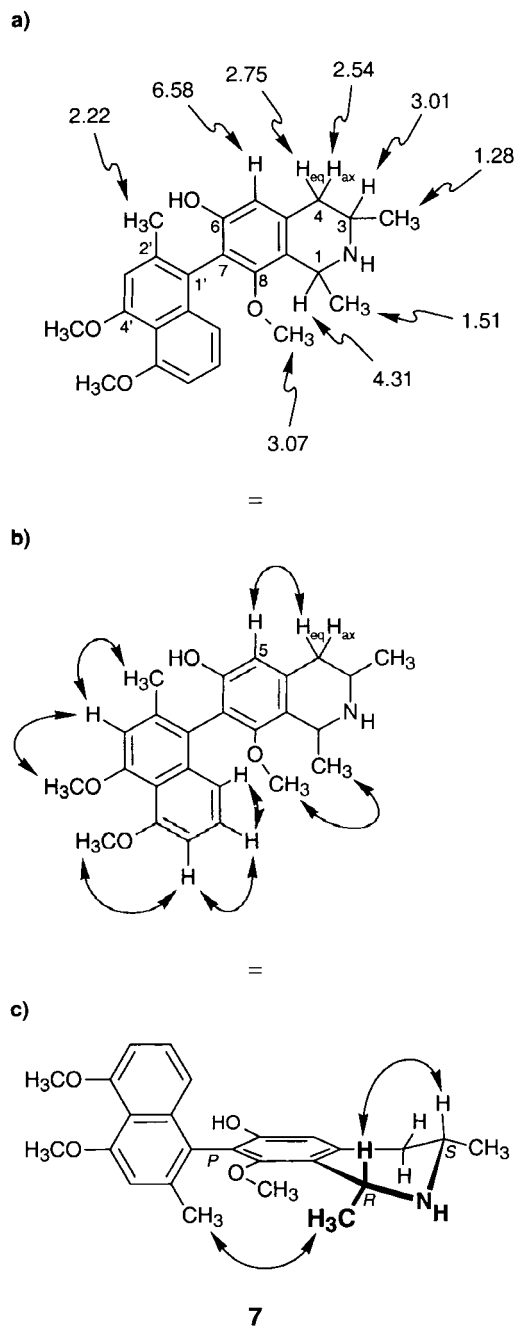


Fig. 8. Constitution **7** of ancistrobertsonine D from (a) selected ^1H NMR chemical shifts (δ values in ppm), as well as (b) ROESY interactions; its relative stereostructure (c) by further characteristic ROESY interactions.

From a ruthenium-mediated degradation experiment (Bringmann et al., 1996) the configuration at C-3 was established to be *S*. Given the relative *cis*-configuration

² The absolute stereostructure was further corroborated by quantumchemical circular dichroism calculations, by methods described earlier (Bringmann & Busemann, 1998)

Table 1

IC_{50} values of the compounds **2**, **3**, **4**, **5**, and **7** (isolated from *A. robertsoniorum*) as well as **10**, **11a**, and **11b** (partial synthetic origin) on chloroquine resistant (K1-strain) and susceptible (NF54-strain) strains of *Plasmodium falciparum*

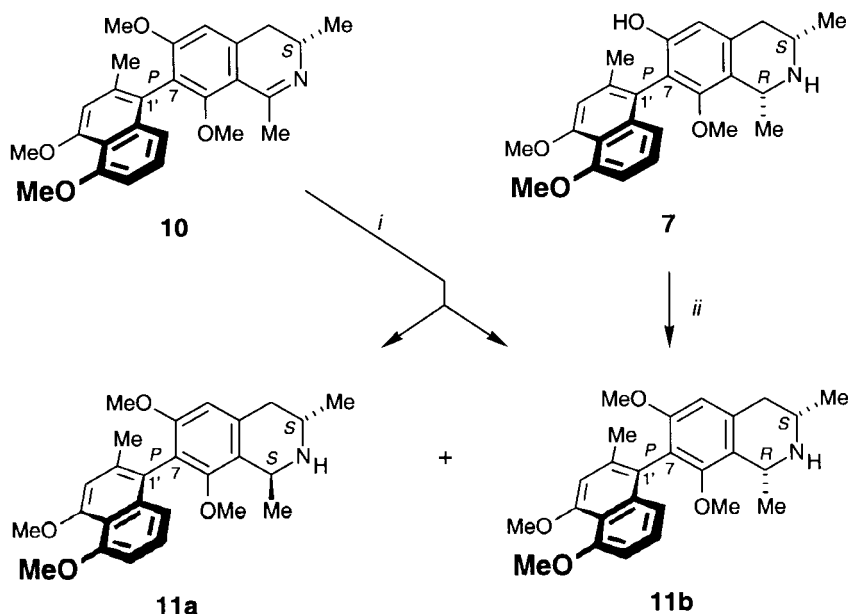
Compound	K1-strain IC_{50} [μM]	NF54-strain IC_{50} [μM]
2	2.0	4.7
3	15.9	> 23.7
4	9.0	> 23.0
5	4.5	10.1
7	—	4.8
10	1.4	5.9
11a	1.7	5.0
11b	1.0	4.9

as evident from NMR, the absolute configuration of C-1 must be *R* as depicted in Fig. 8(c).

With the absolute configuration at the stereocenters in the isoquinoline part established and the relative configuration of centers vs axis again known from NMR (see also Fig. 8(c)), the absolute configuration at the biaryl axis was unambiguously assigned as *P*, leading to **7** as the absolute stereostructure of ancistrobertsonine D. This structure, including its absolute configuration (*1R,3S,7P*)² was confirmed by synthetic transformations. Reduction of ancistrocladisine (**10**) with $\text{LiAlH}_4/\text{AlMe}_3$ gave two stereoisomeric tetrahydroisoquinolines, **11a** and **11b**, of which the *cis*-isomer **11b** proved to be identical with the *O*-methylation product of ancistrobertsonine D (**7**) by co-chromatography on reversed phase HPLC, physical (mp and $[\alpha]_D$), and spectroscopic (^1H NMR and CD) data (Scheme 2).

An alkaloid with the same constitution as **7** (cf in Fig. 8(a/b)), named ‘ancistrine’, had previously been isolated by Foucher, Pouset & Cavé, (1975) from the roots of the Central African liana, *Ancistrocladus ealaensis*. However, neither the relative nor the absolute configuration was determined for that alkaloid. Moreover, the physical (mp and $[\alpha]_D$) data and some characteristic ^1H NMR shifts of ‘ancistrine’ do not correspond to those of **7**, and no more authentic material of ‘ancistrine’ is available (A. Cavé, personal communication) so that, given the uncertain identity of ‘ancistrine’, ancistrobertsonine D (**7**) has to be regarded as a new compound.

In contrast to some other naphthylisoquinolines isolated from Anastrocladaceae and Dioncophyllaceae in West and Central Africa (Bringmann, François et al., 1998), the alkaloids **2**, **3**, **4**, **5**, and **7** isolated from *A. robertsoniorum* as well as the partial-synthetic compounds **10**, **11a**, and **11b** showed only moderate anti-malarial activities *in vitro*. Interestingly, antiplasmodial activity was more pronounced against the chloroquine resistant K1 strain and to a 2–5 fold



Scheme 2. Confirmation of the absolute configuration of the new alkaloid **7** by conversion into the *cis*-dihydro derivative **11b** of ancistrocladisine (**10**). (i) $\text{LiAlH}_4\text{--AlMe}_3$, THF (abs.) (Bringmann, Jansen & Rink, 1986) (ii) CH_2N_2 , $\text{MeOH--H}_2\text{O}$ (9:1) (Organikum, 1998).“

lesser extent against the susceptible NF54 strain of *Plasmodium falciparum* (see also Table 1). It is noteworthy that the studied compounds expressed cytotoxic effects on mammalian cells only at or above 90 $\mu\text{g/ml}$ (highest concentration tested). Thus, the anti-malarial activity, although only moderate, is specific against intraerythrocytic forms of *P. falciparum*. Compound **6** was not tested due to the low quantities isolated and because of its instability.

In summary, four new naphthylisoquinoline alkaloids were isolated from the East African Liana *Ancistrocladus robertsoniorum*. The new compounds **4**, **5**, **6** and **7** and the alkaloids **1a**, **1b**, **2** and **3** isolated previously from this plant are all *S*-configured at C-3 and have an oxygen function at C-6, thus representing pure ‘Ancistrocladaceae-type’ (Bringmann & Pokorny, 1995) alkaloids. In this respect *A. robertsoniorum* therefore resembles the Asian *Ancistrocladus* species, which have been found to produce ‘Ancistrocladaceae-type’ alkaloids only, whereas all the West and Central African species investigated so far produce ‘Dioncophyllaceae-type’ alkaloids (i.e. with *R* at C-3 and without oxygen at C-6), too, as well as ‘hybrid types’ (Bringmann & Pokorny, 1995). On the other hand, the identification of 5,8'-coupled alkaloids like **2**, **3**, and **5**, which have never been found in Asian *Ancistrocladus* species (Bringmann & Pokorny, 1995), reveals a clear chemotaxonomic relationship to the West and Central African representatives of this genus.

The work described here thus confirms the assumption (Bringmann, Teltschik et al., 1998) that *A. robertsoniorum* is the geographic and chemotaxonomic link

between African and Asian representatives of the Ancistrocladaceae family.

3. Experimental

3.1. General

Mps uncorr. Optical rotations: 25°, 10 cm cell, CHCl_3 . CD: 25°, EtOH. IR: KBr. ^1H NMR: (200 MHz, 400 MHz or 600 MHz) and ^{13}C NMR (63 MHz, 100 MHz or 150 MHz) were recorded in CDCl_3 , with the solvent as int. standard (CDCl_3 , δ 7.26 and δ 77.01, resp.). Proton detected, heteronuclear correlations were measured using Heteronuclear Multiple Quantum Correlation (HMOC, optimized for $^1J_{\text{HC}} = 150$ Hz) and HMBC (optimized for $^nJ_{\text{HC}} = 7$ Hz). EIMS: 70 eV. TLC: precoated silica gel 60 F₂₅₄ plates (Merck), deactivated with NH_3 . Spots were visualized under UV light and by Dragendorff's reagent. Prep. TLC: plates with a layer thickness of 2 mm and a concentration zone (Merck) were used. 80–100 mg samples were applied and recovered with 25% MeOH in CHCl_3 after resolu. HPLC (analytical): Novapak C₁₈ (4 μm , 3.9×150 mm, Waters), flow 1.0 ml min⁻¹; Chiralcel OD (10 μm , 4.6×250 mm, Daicel), flow 1.0 ml min⁻¹; HPLC (preparative): Novapak C₁₈ (6 μm , 7.8×200 mm, Waters), flow 4.0 ml min⁻¹; Novapak C₁₈ (6 μm , 25×200 mm, Waters), flow 12.0 ml min⁻¹; Chiralcel OD (10 μm , 10×250 mm, Daicel) flow 4.0 ml min⁻¹; UV detection 280 nm (tunable UV detector) and 200–400 nm (photodiode array detector). HSCCC: $\text{CHCl}_3\text{--MeOH--}$

0.1 M HCl (5:5:3), mobile phase: lower phase, (H) → T, Triple Coil, 1.7×950 mm (large coil), TLC detection (see above), flow 2.0 ml min^{-1} , 850 min^{-1} .

3.2. Plant material

Stems and leaves of *A. robertsoniorum* Léonard were collected in November 1992 and in August 1993 in the Buda Mafisini Forest in Kenya, identified by one of us (R.H.) and confirmed by Dr J. Schlauer (Würzburg). A voucher specimen has been deposited at the Herbarium Bringmann (No. 8), Würzburg.

3.3. Extraction and isolation

The air dried stems (1.1 kg) were ground and extracted exhaustively with 1 N H_2SO_4 -MeOH (5:1) as described earlier (Bringmann, Teltschik et al., 1998). After the removal of MeOH, the aq. soln was prefractonated by liquid-liquid partition using *n*-hexane, CHCl_3 , and (after basification with conc. NH_3 soln of pH 8) *n*-BuOH. ^1H NMR and the Dragendorff test proved the CHCl_3 fr. to be the only alkaloid containing fr. After evapn of the solvent the residue (ca 11.5 g) was subjected to HSCCC (1 g/run). Portions of 4 ml were collected, monitored by TLC, and combined. In the following, the isolation of the alkaloids in the order of increasing polarity is described.

3.4. Isolation of ancistrobertsonines B (4), and C (5)

For removal of the main part of brown by-products, fr. 1 from the HSCCC sepn was subjected to CC on silica gel, using methyl-*tert*-butylether as the eluent. Further purification on prep. TLC yielded 70 mg of a yellow oil as a mixt. of two alkaloids. Resolution of these compounds was finally achieved analytically and preparatively by HPLC on a chiral stationary phase (Chiralcel OD) using *n*-hexane-*i*-propanol-triethylamine (96:4:0.1) as eluent. After removal of the eluent under reduced pressure, the alkaloids were recrystallized separately from *n*-hexane-*i*-propanol to give 13 mg of 4 and 51 mg of 5.

3.5. Ancistrobertsonine B (4)

Mp $192\text{--}193^\circ$. $[\alpha]_{\text{D}}^{25} + 4^\circ$ (CHCl_3 ; *c* 0.09). CD: $\Delta\epsilon_{200} - 15.5$, $\Delta\epsilon_{202} - 15.6$, $\Delta\epsilon_{230} 40.4$, $\Delta\epsilon_{244} - 10.8$, $\Delta\epsilon_{282} + 0.8$, $\Delta\epsilon_{306} - 1.7$ (EtOH, *c* 0.02). IR $\nu_{\text{max}} \text{ cm}^{-1}$: 3050 ($=\text{C}-\text{H}$), 2962, 2930, 2820 (C-H), 1585, 1570, 1475 ($\text{C}=\text{C}$), 1258, 1200, 1070 (C-O). ^1H NMR (200 MHz, CDCl_3): δ 1.00 (3H, *d*, $J = 6.4$ Hz, Me-3), 1.32 (3H, *d*, $J = 6.4$ Hz, Me-1), 1.93 (1H, *dd*, $J_{\text{gem}} = 16.0$ Hz, $J_{\text{eq}} = 3.4$ Hz, $\text{H}_{\text{eq}}-4$), 2.05 (3H, *s*, Me-2'), 2.06 (1H, *dd*, $J_{\text{gem}} = 16.0$ Hz, $J_{\text{ax}} = 9.4$ Hz, $\text{H}_{\text{ax}}-4$), 2.39 (1H, *m*, H-3), 2.45 (3H, *s*, *N*-Me), 3.62 (3H, *s*,

OMe-6), 3.81 (1H, *q*, $J = 6.4$ Hz, H-1), 3.92 (1H, *s*, OMe-8), 3.97 (3H, *s*, OMe-5'), 4.00 (3H, *s*, OMe-4'), 6.50 (1H, *s*, H-7), 6.77 (1H, *dd*, $J_{\text{ortho}} = 7.9$ Hz, $J_{\text{meta}} = 1.1$ Hz, H-6'), 6.80 (1H, *s*, H-3'), 6.90 (1H, *dd*, $J_{\text{ortho}} = 8.3$, $J_{\text{meta}} = 1.1$ Hz, H-8'), 7.16 (1H, *dd*, $J_{\text{ortho}} = 8.3$ Hz, $J_{\text{ortho}} = 7.9$, H-7'). ^{13}C NMR (150 MHz, CDCl_3): δ 20.63 (Me-2'), 20.85 (Me-3), 22.40 (Me-1), 35.21 (C-4), 41.10 (*N*-Me), 46.89 (C-3), 55.32 (OMe-8), 56.05 (OMe-6), 56.41 (OMe-4'), 56.46 (OMe-5'), 57.12 (C-1), 93.80 (C-7), 105.37 (C-6'), 108.96 (C-3'), 116.32 (C-10'), 118.46 (C-8'), 118.85 (C-5), 125.38 (C-1'), 126.15 (C-9), 126.35 (C-7'), 134.85 (C-2'), 136.54 (C-10), 136.86 (C-9'), 156.03 (C-4'), 156.12 (C-8), 156.24 (C-6), 157.26 (C-5'). EIMS m/z (rel. int.): 435 $[\text{M}]^+$ (2), 420 $[\text{M}-\text{Me}]^+$ (100), 404 $[\text{M}-\text{OMe}]^+$ (7). HRMS m/z 435.241 $[\text{M}]^+$, ($\text{C}_{27}\text{H}_{33}\text{NO}_4$ requires: 435.241).

3.6. Ancistrobertsonine C (5)

Mp $148\text{--}149^\circ$. $[\alpha]_{\text{D}}^{25} - 3^\circ$ (CHCl_3 ; *c* 0.09). CD: $\Delta\epsilon_{200} 13.3$, $\Delta\epsilon_{216} - 4.0$, $\Delta\epsilon_{228} - 16.7$, $\Delta\epsilon_{240} + 19.0$, $\Delta\epsilon_{310} - 1.4$ (EtOH, *c* 0.02). IR $\nu_{\text{max}} \text{ cm}^{-1}$: 2950, 2920, 2820 (C-H), 1605, 1572 ($\text{C}=\text{C}$), 1270, 1250, 1090, 1070 (C-O). ^1H NMR (200 MHz, CDCl_3): δ 0.99 (3H, *d*, $J = 5.1$ Hz, Me-3), 1.47 (3H, *d*, $J = 6.4$ Hz, Me-1), 1.99 (1H, *dd*, $J_{\text{gem}} = 14.9$ Hz, $J_{\text{eq}} = 1.9$ Hz, $\text{H}_{\text{eq}}-4$), 2.27 (1H, *dd*, $J_{\text{gem}} = 14.8$ Hz, $J_{\text{ax}} = 10.9$ Hz, $\text{H}_{\text{ax}}-4$), 2.32 (3H, *s*, Me-2'), 2.39 (1H, *m*, H-3), 2.45 (3H, *s*, *N*-Me), 3.62 (3H, *s*, OMe-6), 3.81 (1H, *q*, $J = 6.4$ Hz, H-1), 3.93 (1H, *s*, OMe-8), 3.97 (3H, *s*, OMe-5'), 4.00 (3H, *s*, OMe-4'), 6.49 (1H, *s*, H-7), 6.68 (1H, *s*, H-3'), 6.69 (1H, *s*, H-1'), 6.84 (1H, *d*, $J_{\text{ortho}} = 8.0$, H-6'), 7.15 (1H, *d*, $J_{\text{ortho}} = 8.0$, H-7'). ^{13}C NMR (150 MHz, CDCl_3): δ 20.85 (Me-3), 21.99 (Me-2'), 22.48 (Me-1), 36.35 (C-4), 41.20 (*N*-Me), 55.18 (OMe-6), 55.54 (C-3), 56.06 (OMe-8), 56.24 (OMe-4'), 56.58 (OMe-5'), 57.36 (C-1), 93.57 (C-7), 105.22 (C-6'), 108.52 (C-3'), 115.89 (C-10'), 117.86 (C-1'), 120.31 (C-5), 120.52 (C-9), 126.48 (C-8'), 128.93 (C-7'), 135.77 (C-2'), 136.27 (C-9'), 137.17 (C-10), 156.03 (C-8), 156.41 (C-5'), 156.51 (C-6), 157.12 (C-4'). EIMS m/z (rel. int.): 435 $[\text{M}]^+$ (3), 420 $[\text{M}-\text{Me}]^+$ (100), 404 $[\text{M}-\text{OMe}]^+$ (5). HRMS m/z 435.241 $[\text{M}]^+$, ($\text{C}_{27}\text{H}_{33}\text{NO}_4$ requires: 435.241).

3.7. Isolation of 1,2-didehydroancistrobertsonine D (6)

Fr. 3 of the HSCCC sepn was further resolved by prep. TLC using CHCl_3 -MeOH (19:1) as eluent. Purification of the most polar compound on prep. HPLC with MeOH- H_2O (40:60) 10 min, (40:60 → 46:54) in 2 min, (46:54) 8 min, (46:54 → 52:48) in 5 min, (52:48) 10 min, (52:48 → 40:60) in 3 min, gave 7 mg of 6 as microcrystalline powder after recrystallization from MeOH- H_2O . Mp $185\text{--}187^\circ$. $[\alpha]_{\text{D}}^{25} - 30^\circ$ (CHCl_3 ; *c* 0.04). CD: $\Delta\epsilon_{200} - 119.0$, $\Delta\epsilon_{207} - 79.0$, $\Delta\epsilon_{210} - 83.0$, $\Delta\epsilon_{231} + 34.5$,

$\Delta\epsilon_{244} + 9.0$, $\Delta\epsilon_{273} - 18.6$, $\Delta\epsilon_{302} - 3.7$ (EtOH, c 0.03). IR $\nu_{\max}$ cm^{-1} : 3380 (O–H), 2940, 2920, 2820 (C–H), 1570, 1490 (C=C), 1270, 1260, 1070 (C–O). ^1H NMR (200 MHz, CDCl_3): δ 1.42 (3H, d , $J = 6.1$ Hz, Me-3), 2.22 (3H, s , Me-2'), 2.73 (3H, s , Me-1), 2.75 (1H, dd , $J_{\text{gem}} = 16.5$ Hz, J_{ax} not measurable due to partial overlap by signal of Me-2', $H_{\text{ax-4}}$), 2.97 (1H, dd , $J_{\text{gem}} = 16.5$ Hz, $J_{\text{eq}} = 5.2$ Hz, $H_{\text{eq-4}}$), 3.13 (1H, s , OMe-8), 3.91 (1H, m_c , H-3), 3.97 (3H, s , OMe-5'), 4.00 (3H, s , OMe-4'), 6.80 (1H, s , H-3'), 6.81 (1H, s , H-5), 6.82 (1H, dd , $J_{\text{ortho}} = 8.4$ Hz, $J_{\text{meta}} = 1.3$ Hz, H-6'), 6.91 (1H, dd , $J_{\text{ortho}} = 8.4$ Hz, $J_{\text{meta}} = 1.3$ Hz, H-8'), 7.15 (1H, $pseudo-t$, $J_{\text{ortho}} = 8.2$ Hz, H-7'). ^{13}C NMR (150 MHz, CDCl_3): δ 17.49 (Me-3), 20.74 (Me-2'), 23.53 (Me-1), 34.35 (C-4), 48.25 (C-3), 56.36 (OMe-4'), 56.48 (OMe-5'), 60.36 (OMe-8), 106.35 (C-6'), 108.83 (C-3'), 111.56 (C-5), 111.96 (C-9), 116.64 (C-10'), 117.19 (C-8'), 117.87 (C-1'), 118.81 (C-7), 128.06 (C-7'), 136.16 (C-9'), 138.03 (C-2'), 140.61 (C-10), 157.94 (C-5'), 158.35 (C-4'), 162.99 (C-8), 163.40 (C-6), 174.18 (C-1). EIMS m/z (rel. int.): 405 $[\text{M}]^+$ (100), 390 $[\text{M}-\text{Me}]^+$ (91), 375 $[\text{M}-\text{OMe}+\text{H}]^+$ (11). HRMS m/z 405.194 $[\text{M}]^+$, ($\text{C}_{25}\text{H}_{27}\text{NO}_4$ requires: 405.194).

3.8. Isolation of ancistrobertsonine D (7)

From HSCCC fr. 6 ancistrobertsonine D was isolated by prep. TLC using *i*-PrOH–MeOH (19:1) as eluent. The crude alkaloid obtained was further purified by prep. HPLC with MeOH– H_2O (4:6) 5 min, (4:6 $\rightarrow$ 6:4) in 15 min, (6:4) 5 min, (6:4 $\rightarrow$ 4:6) in 1 min, (4:6) 10 min. Attempts to recrystallize the compound from different solvents being not successful, the alkaloid was solved in MeOH– H_2O . After the removal of MeOH under reduced pressure, the aq. soln was lyophilized, yielding 8 mg of an amorphous, yellow powder. Mp 133–134 $^{\circ}$ $[\alpha]_{\text{D}}^{25} + 82^{\circ}$ (CHCl_3 ; c 0.51); (Data for 'ancistrine' from *A. ealaensis* (Foucher et al., 1975): Mp 230–231. $[\alpha]_{\text{D}}^{20} - 35^{\circ}$). CD: $\Delta\epsilon_{200} + 32.5$, $\Delta\epsilon_{213} - 86.8$, $\Delta\epsilon_{239} + 68.8$, $\Delta\epsilon_{282} - 32.0$, $\Delta\epsilon_{305} + 8.7$ (EtOH, c 0.01). IR $\nu_{\max}$ cm^{-1} : 3400 (O–H), 2950, 2920, 2840 (C–H), 1600, 1585, 1570 (C=C), 1260, 1070 (C–O). ^1H NMR (200 MHz, CDCl_3): δ 1.28 (3H, d , $J = 6.3$ Hz, Me-3), 1.51 (3H, d , $J = 6.4$ Hz, Me-1), 2.22 (3H, s , Me-2'), 2.54 (1H, dd , $J_{\text{gem}} = 16.0$ Hz, $J_{\text{ax}} = 10.8$ Hz, $H_{\text{ax-4}}$), 2.75 (1H, dd , $J_{\text{gem}} = 16.0$ Hz, $J_{\text{eq}} = 3.7$ Hz, $H_{\text{eq-4}}$), 3.01 (1H, m_c , H-3), 3.07 (1H, s , OMe-8), 3.98 (3H, s , OMe-5'), 4.02 (3H, s , OMe-4'), 4.31 (1H, q , $J = 6.4$ Hz, H-1), 6.58 (1H, s , H-5), 6.82 (1H, dd , $J_{\text{ortho}} = 7.8$ Hz, $J_{\text{meta}} = 1.0$ Hz, H-6'), 6.86 (1H, s , H-3'), 7.06 (1H, dd , $J_{\text{ortho}} = 8.4$, $J_{\text{meta}} = 1.0$ Hz, H-8'), 7.29 (1H, $pseudo-t$, $J_{\text{ortho}} = 7.9$ Hz, H-7'). ^{13}C NMR (150 MHz, CDCl_3): δ 20.53 (Me-2'), 20.86 (Me-

3), 22.21 (Me-1), 37.67 (C-4), 49.16 (C-3), 50.49 (C-1), 56.33 (OMe-4'), 56.44 (OMe-5'), 59.71 (OMe-8), 105.82 (C-6'), 109.15 (C-3'), 110.38 (C-5), 116.39 (C-10'), 117.56 (C-7), 117.74 (C-8'), 119.39 (C-1), 122.80 (C-9), 127.52 (C-10), 127.71 (C-7'), 136.30 (C-9'), 138.43 (C-2'), 152.61 (C-6), 156.55 (C-8), 157.54 (C-4'), 157.64 (C-5'). EIMS m/z (rel. int.): 407 $[\text{M}]^+$ (8), 392 $[\text{M}-\text{Me}]^+$ (100), 377 $[\text{M}-\text{OMe}+\text{H}]^+$ (12). HRMS m/z 392.186 $[\text{M}-\text{Me}]^+$, ($\text{C}_{24}\text{H}_{26}\text{NO}_4$ requires: 392.186).

3.9. Oxidative degradation of 4, 5, 6 and 7

The degradation, the derivatization of the amino acids, and the subsequent GC-MSD analysis were carried out as described previously (Bringmann et al., 1996).

3.10. Preparation of ancistrocladisine (10) from 1,2-didehydro-ancistrobertsonine (6)

To a soln of 2.4 mg (6.0 μmol) of 1,2-didehydroancistrobertsonine (6) in MeOH– H_2O (9:1) freshly prepd CH_2N_2 in Et $_2\text{O}$ (de Boer & Baker, 1963) was added until the generation of N_2 ceased and the resulting mixt. remained yellow (Organikum, 1988). After addn of CH_2N_2 the mixt. was left to stand 10 min at room temp. to complete the reaction. After that time the solvent was removed under reduced pressure to yield 2.5 mg (6.0 μmol , quantitative yield) ancistrocladisine (10) after recrystallization from CH_2Cl_2 . Mp 176 $^{\circ}$; (Bringmann & Reuscher, 1989): 178 $^{\circ}$ (synthetic ancistrocladisine); (Govindachari et al., 1972): 178–180 $^{\circ}$ (natural product from *A. heyneanus*). $[\alpha]_{\text{D}}^{25} + 6^{\circ}$ (CHCl_3 ; c 0.04); (Bringmann & Reuscher, 1989): $+7.8^{\circ}$. CD: $\Delta\epsilon_{200} - 13.2$, $\Delta\epsilon_{215} - 45.0$, $\Delta\epsilon_{230} + 18.0$, $\Delta\epsilon_{248} + 10.9$, $\Delta\epsilon_{262} + 9.0$, $\Delta\epsilon_{312} - 2.7$ (EtOH, c 0.05). Chromatographic behaviour as well as IR, ^1H NMR and MS data were identical to those of natural and synthetic ancistrocladisine obtained earlier (Govindachari et al., 1972; Bringmann & Reuscher, 1989). ^{13}C NMR (100 MHz, CDCl_3): δ 21.00 (Me-3), 23.70 (Me-2'), 29.70 (Me-1), 34.91 (C-4), 56.29 (OMe-6), 56.29 (OMe-4'), 56.38 (OMe-5'), 60.44 (OMe-8), 62.99 (C-3), 96.32 (C-5), 105.47 (C-6'), 106.44 (C-3'), 108.57 (C-7), 113.81 (C-9), 116.15 (C-10'), 117.78 (C-1'), 118.39 (C-8'), 129.70 (C-7'), 133.00 (C-2'), 135.95 (C-2'), 144.50 (C-10), 157.14 (C-5'), 157.50 (C-4'), 157.86 (C-8), 167.54 (C-6), 178.10 (C-1).

3.11. Reduction of ancistrocladisine (10) (Bringmann et al., 1986)

Ancistrocladisine (60 mg, 0.14 mmol), AlMe_3 (76 mg, 2.1 mmol solved in 1.1 ml *n*-hexane) and LiAlH_4 (40 mg, 2.1 mmol) were added to 8.0 ml of cold

³ 'Melting point' of the amorphous powder after lyophilization.

(-78°) abs. THF under Ar. The mixt. was stirred vigorously while the temp. was held at -78° for 30 min. During a period of 4 h, the temp. was slowly raised to 0° . After that time, 12 ml of abs. THF and NaF (1.3 g, 30 mmol) were added and the mixt. was left to stir for another 5 min and hydrolyzed with 0.3 ml of H_2O . After additional stirring for 30 min, the precipitate was filtered off and washed with CH_2Cl_2 repeatedly. After removal of the solvent under reduced pressure, 30.5 mg of a mixt. of *trans*- and *cis*-1,2-dihydroancistrocladisine (**11a** and **11b**, respectively) were obtained. The resoln of this mixt. of epimers by preparative TLC [silica gel, $CHCl_3$ –MeOH (19:1)] yielded 18.4 mg (43.0 μ mol, 31%) of **11a** as the major product, along with 6.6 mg of **11b** (15.0 μ mol, 11%). The separated compounds were recrystallized from $CHCl_3$ –MeOH

3.11.1. *trans*-1,2-Dihydroancistrocladisine (**11a**)

Mp 260 – 262° ; (Bringmann & Reuscher, 1989) : 263 – 266° . $t_R = 18.9$ min (same elution profile as for the isolation of **7** was used). $[\alpha]_D^{25} = +43$ ($CHCl_3$, c 0.13); ref. (Bringmann & Reuscher, 1989) : $+49.7$. CD: $\Delta\epsilon_{200} - 28.6$, $\Delta\epsilon_{205} - 15.9$, $\Delta\epsilon_{212} - 33.6$, $\Delta\epsilon_{231} + 53.5$, $\Delta\epsilon_{245} - 3.6$, $\Delta\epsilon_{284} - 12.2$, $\Delta\epsilon_{305} + 0.9$ (EtOH, c 0.02). IR ν_{max} cm^{-1} : 3450 (O–H), 2960, 2920, 2850 (C–H), 1605, 1580, 1575 (C=C), 1260, 1075 (C–O). 1H NMR (200 MHz, $CDCl_3$): δ 1.54 (3H, d , $J = 6.4$ Hz, Me-3), 1.62 (3H, d , $J = 6.1$ Hz, Me-1), 2.19 (3H, s , Me-2'), 2.93–3.11 (2H, m , H_{ax-4} , H_{eq-4} ; J_{gem} , J_{ax} , J_{eq} not measurable due to peak overlap), 3.10 (3H, s , OMe-8), 3.61 (1H, m_c , H-3), 3.62 (3H, s , OMe-6), 3.96 (3H, s , OMe-5'), 4.00 (3H, s , OMe-4'), 4.63 (1H, q , $J = 6.4$ Hz, H-1), 6.49 (1H, s , H-5), 6.77 (1H, d , $J_{ortho} = 7.3$ Hz, H-6'), 6.79 (1H, s , H-3'), 6.89 (1H, d , $J_{ortho} = 7.6$ Hz, H-8'), 7.29 (1H, dd , $J_{ortho} = 7.6$ Hz, $J_{ortho} = 7.3$ Hz, H-7'). ^{13}C NMR (63 MHz, $CDCl_3$): δ 19.75 (Me-2'), 20.09 (Me-3), 20.94 (Me-1), 35.25 (C-4), 43.77 (C-3), 48.04 (C-1), 55.87 (OMe-6), 56.38 (OMe-4'), 56.53 (OMe-5'), 59.83 (OMe-8), 94.13 (C-5), 105.53 (C-6'), 106.20 (C-3'), 108.87 (C-7), 116.30 (C-10'), 118.57 (C-1'), 120.87 (C-8'), 122.64 (C-9), 126.30 (C-10), 132.67 (C-7'), 136.00 (C-9'), 136.49 (C-2'), 155.95 (C-6), 156.59 (C-8), 157.29 (C-4'), 157.69 (C-5'). EIMS m/z (rel. int.): 421 $[M]^+$ (2), 407 $[M-Me+H]^+$ (2), 406 $[M-Me]^+$ (8)

3.11.2. *cis*-1,2-Dihydroancistrocladisine (**11b**)

Mp 212 – 213° . $t_R = 16.9$ min (same elution profile as for the isolation of **7** was used). $[\alpha]_D^{25} = +77$ ($CHCl_3$, c 0.14). CD: $\Delta\epsilon_{200} + 5.0$, $\Delta\epsilon_{208} - 49.9$, $\Delta\epsilon_{227} + 28.3$, $\Delta\epsilon_{243} - 3.2$, $\Delta\epsilon_{261} + 4.9$, $\Delta\epsilon_{285} - 10.0$ (EtOH, c 0.04). IR ν_{max} cm^{-1} : 3400 (O–H), 2960, 2920, 2840 (C–H), 1600, 1580, 1575 (C=C), 1280, 1070 (C–O). 1H NMR (400 MHz, $CDCl_3$): δ 1.63 (3H, d , $J = 5.8$ Hz, Me-3), 1.78 (3H, d , $J = 6.4$ Hz, Me-1), 2.16 (3H, s , Me-2'), 2.83 (1H, dd , $J_{gem} = 13.7$ Hz, $J_{eq} = 3.7$ Hz, H_{eq-4}), 3.01

(3H, s , OMe-8), 3.12 (1H, dd , $J_{gem} = 13.7$ Hz, $J_{ax} = 7.8$ Hz, H_{ax-4}), 3.31 (1H, m_c , H-3), 3.60 (3H, s , OMe-6), 3.97 (3H, s , OMe-5'), 4.00 (3H, s , OMe-4'), 4.62 (1H, q , $J = 6.4$ Hz, H-1), 6.53 (1H, s , H-5), 6.77 (1H, d , $J_{ortho} = 8.3$ Hz, H-6'), 6.81 (1H, s , H-3'), 6.91 (1H, d , $J_{ortho} = 8.5$ Hz, H-8'), 7.29 (1H, dd , $J_{ortho} = 8.5$ Hz, $J_{ortho} = 8.3$ Hz, H-7'). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.07 (Me-2'), 21.75 (Me-3), 22.56 (Me-1), 37.83 (C-4), 49.98 (C-3), 51.13 (C-1), 56.28 (OMe-6), 56.78 (OMe-4'), 56.93 (OMe-5'), 60.02 (OMe-8), 95.53 (C-5), 105.08 (C-6'), 106.21 (C-3'), 108.60 (C-7), 108.84 (C-10), 118.20 (C-10'), 121.29 (C-8'), 125.28 (C-9), 126.39 (C-1), 134.39 (C-7'), 134.84 (C-9'), 135.76 (C-2'), 155.24 (C-6), 155.61 (C-8), 156.07 (C-4'), 156.64 (C-5'). EIMS m/z (rel. int.): 421 $[M]^+$ (14), 407 $[M-Me+H]^+$ (27), 406 $[M-Me]^+$ (100), 390 $[M-OCH_3]^+$ (8)

3.12. Methylation of ancistrobertsonine D (**7**)

O-methylation of 0.9 mg (2.0 μ mol) of ancistrobertsonine D (**7**) was performed in analogy to the preparation of ancistrocladisine (**10**) from **6** (see above), yielding 0.9 mg of pure 6-*O*-methylancistrobertsonine D after recrystallization from $CHCl_3$ –MeOH (2.0 μ mol, quantitative). Mp 215° . $t_R = 16.9$ min (same elution profile as for the isolation of **7** was used). $[\alpha]_D^{20} + 72$ ($CHCl_3$; c 0.12). CD: $\Delta\epsilon_{200} - 18.6$, $\Delta\epsilon_{208} - 50.0$, $\Delta\epsilon_{229} + 19.1$, $\Delta\epsilon_{246} - 10.9$, $\Delta\epsilon_{267} + 5.5$, $\Delta\epsilon_{281} - 10.9$, $\Delta\epsilon_{300} - 3.6$ (EtOH, c 0.04). IR, 1H NMR and MS data were fully identical to those of *cis*-1,2-dihydroancistrocladisine **11b** (see above). Due to the small amount of substance available, no ^{13}C NMR spectrum could be obtained.

3.13. Biological experiments

Activity against *P. falciparum* was tested by the semiautomated microdilution assay against intraerythrocytic forms derived from asynchronous stock cultures as previously described (Desjardins, Canfield, Haynes & Chulay, 1979) with minor modifications (Ridley et al., 1996). The strains used were K1 (Thailand; resistant to chloroquine and pyrimethamine) and NF54 (an airport strain of unknown origin; susceptible to standard antimalarials). Cytotoxicity was tested against rat skeletal muscle myoblast (L-6) cells as described previously (Kaminsky & Brun, 1998). The activities are given as IC_{50} values (μ M). Chloroquine was used as a standard [IC_{50} (K1) = 0.30 μ M, IC_{50} (NF54) = 0.01 μ M].

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