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Six naphthylisoquinoline alkaloids and a related benzopyranone from a Congolese *Ancistrocladus* species related to *Ancistrocladus congolensis* ☆

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Abstract

From the roots of a recently discovered *Ancistrocladus* taxon, with close affinities to *Ancistrocladus congolensis* regarding molecular ITS sequence data, six naphthylisoquinoline alkaloids, 5′-*O*-demethylhamatine (**2**), 5′-*O*-demethylhamatinine (**3**), 6-*O*-demethylancistroealaine A (**4**), 6,5′-*O*,*O*-didemethylancistroealaine A (**5**), 5-*epi*-6-*O*-methylancistrobertsonine A (**6**), and 5-*epi*-4′-*O*-demethylancistrobertsonine C (**7**), have been isolated, along with a likewise benzopyranone carboxylic acid, **8**. The structural elucidation succeeded by chemical, spectroscopic, and chiroptical methods. Their bioactivities were tested against protozoan parasites causing severe tropical diseases. Furthermore, eight known related alkaloids were identified.

Keywords: Congolese *Ancistrocladus* species; *Ancistrocladus congolensis*; Ancistrocladaceae; Structural elucidation; Naphthylisoquinoline alkaloids; 5′-*O*-Demethylhamatine; 5′-*O*-Demethylhamatinine; 6-*O*-Demethylancistroealaine A; 6,5′-*O*,*O*-Didemethylancistroealaine A; 5-*epi*-6-*O*-Methylancistrobertsonine A; 5-*epi*-4′-*O*-Demethylancistrobertsonine C; 2-Methyl-4-oxo-4-*H*-1-benzopyrane 5-carboxylic acid; Antiprotozoal activity

1. Introduction

The naphthylisoquinoline alkaloids (Bringmann and Pokorny, 1995) comprise more than 120 structurally, biosynthetically, and pharmacologically remarkable natural products. Some of them strongly inhibit proliferation of protozoan pathogens, such as *Plasmodium* (Bringmann, 2003), *Leishmania* species (Bringmann et al., 2003a), and *Trypanosoma* (Ponte-Sucre et al., 2007; Bringmann et al., 2003b; Bringmann and Feineis, 2000), which cause fatal widespread tropical diseases (Heby et al., 2003). Due to their antiparasitic properties, these alkaloids are promising candidates as lead structures for urgently needed new anti-infections drugs and make the search for further analogs a rewarding goal. The occurrence of these metabolites is as yet restricted to the small palaeotropical plant families. Dioncophyllaceae, with only three monotypic genera, *Habropetalum, Dioncophyllum*, and *Triphyophyllum* (Airy Shaw, 1951; Schmid, 1964), and the closely related Ancistrocladaceae with only one genus, *Ancistrocladus* (Taylor et al., 2005; Gereau, 1997). Including the 'new' species *Ancistrocladus benomensis* (Rischer et al., 2005), this genus comprises 17 species of lianas indigenous to the evergreen palaeotropical rainforests of Africa and South-East Asia

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(Taylor et al., 2005; Rischer et al., 2005). We have recently discovered an as yet not fully identified, possibly new *Ancistrocladus* species collected in the swamp region of the rainforest Yeteto in the vicinity of Ikela in the Democratic Republic of Congo. First phytochemical investigations on the leaf material of this plant species had shown the presence of a novel-type subfamily of the naphthylisoquinoline alkaloids (Bringmann et al., 2006), the first *N,C*-coupled naphthyldihydroisoquinolinium salts, ancistrocladinium A (**1**, see Fig. 1), and ancistrocladinium B and its atropodiastereomer (structures not shown).

In this paper, we describe the isolation, structural elucidation, and biotesting of six new, but again *C,C*-coupled, naphthylisoquinoline alkaloids from the roots of this Congolese taxon, viz. 5′-*O*-demethylhamatine (**2**), 5′-*O*-demethylhamatinine (**3**), 6-*O*-demethylancistroealaine A (**4**), 6,5′-*O*,*O*-didemethylancistroealaine A (**5**), 5-*epi*-6-*O*-methylancistrobertsonine A (**6**), 5-*epi*-4′-*O*-demethylancistrobertsonine C (**7**), and a benzopyranone carboxylic acid, **8**, along with eight related, but known alkaloids, among them one of the recently discovered *N,C*-coupled alkaloids, ancistrocladinium A (**1**) (Bringmann et al., 2006).

2. Results and discussion

Plant material of the presumably new *Ancistrocladus* species was collected in the rainforest Yeteto of the Ikela region in the Democratic Republic of Congo. The air-dried powdered root material was extracted with *n*-hexane, CH_2Cl_2, and MeOH:H_2O (9:1). The latter two extracts were each submitted to liquid–liquid separation, FCPC (fast centrifugal partition chromatography), and preparative reversed-phase HPLC, which permitted isolation of eight known naphthylisoquinolines (see Fig. 2) identified as ancistrocladinium A (**1**) (Bringmann et al., 2006), 6-*O*-demethylancistrobrevine A (**9**) (Bringmann et al., 1992; Bringmann and Pokorny, 1995), hamatine (**10**) (Govindachari et al., 1975), ancistrocladine (**11**) (Govindachari and Parthasarathy, 1971; Bringmann and Pokorny, 1995), hamatinine (**12**) (Anh et al., 1997), 6-*O*-methylhamatine (**13**) (Govindachari et al., 1975; Unger et al., 2004), 6-*O*-methylhamatinine (**14**) (Anh et al., 1997), and ancistroealaine A (**15**) (Bringmann et al., 2000a).

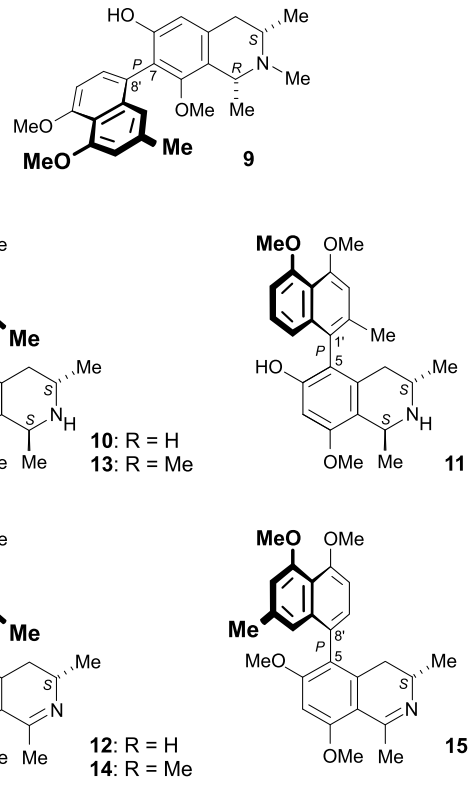


Fig. 2. Further known alkaloids, found for the first time in the Congolese *Ancistrocladus* species.

In addition, seven new compounds were found, two of them in the CH_2Cl_2 and five in the MeOH:H_2O fraction.

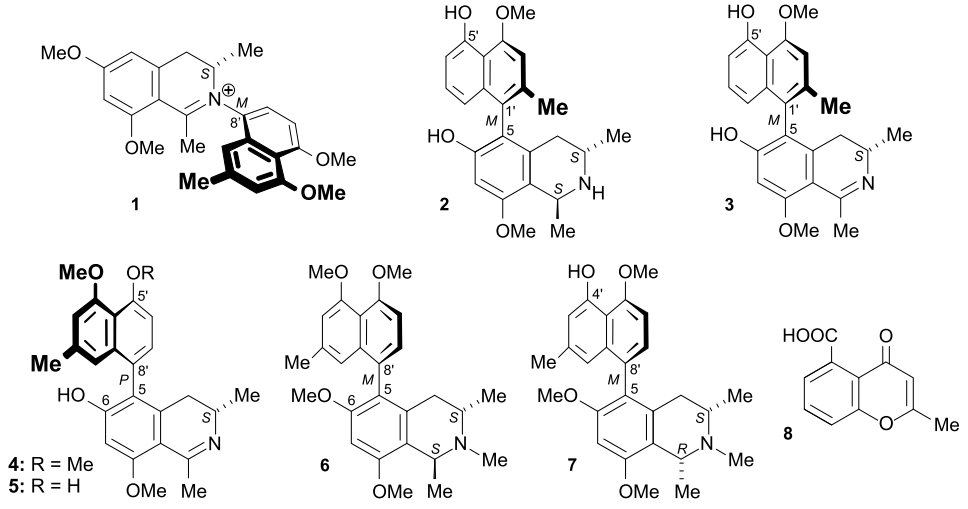


Fig. 1. The recently discovered *N,C*-coupled naphthylisoquinoline, ancistrocladinium A (**1**), and six new, but *C,C*-coupled alkaloids, **2**–**7**, and the benzopyranone carboxylic acid **8**, isolated from a Congolese *Ancistrocladus* taxon related to *A. congolensis*.

Their UV and ^{1}H NMR spectra indicated that six of them were naphthylisoquinoline alkaloids.

The molecular formula of the first, quite polar compound, **2**, was $C_{24}H_{27}NO_4$ as evidenced by HRESIMS. ^{1}H NMR investigations indicated the presence of a naphthyltetrahydroisoquinoline alkaloid with two aromatic methoxy groups, which were found to be normally shifted (3.93 and 4.13 ppm), thus excluding the possibility that they were in the proximity to the biaryl axis. The position of this axis in the naphthalene moiety was deduced from the high-field shifted position of the CH_3-2′ group (2.14 ppm) and the coupling pattern of the aromatic protons (one triplett, two doublets, and two singlets), indicating the axis to be located at C-1′ or C-3′. The latter could be excluded since the more down-field shifted methoxy group (4.13 ppm) was assigned as OCH_3-4′ by an NOE interaction with H-3′, which, in turn, showed an NOE correlation to CH_3-2′. Consequently the biaryl axis had to be located C-1′. This was further confirmed by the crosspeaks found in the NOESY spectrum in the series {H-8′–H-7′–H-6′} and by the multiple bond couplings from H-3′ and H8′ to C-1′.

The high-field shifted methoxy group of **2** resonating at 3.93 ppm was assigned to be located at C-8, since NOE correlations were observed for the signal with both, H-7 and CH_3-1. Therefore, the biaryl axis had to be located at C-5 in the isoquinoline portion, which was in agreement with HMBC couplings to C-5 (118.6 ppm) observed from both, the signals of the protons H_{eq}-4 and H-7. In conclusion, the new naphthylisoquinoline alkaloid was established to be 5,1′-coupled and to possess the constitution **2** shown in Fig. 3a, with free OH functions at C-6 and C-5′.

The relative configuration of the methyl groups at C-1 and C-3 of **2** was deduced to be *trans* to each other from NOE correlation between CH_3-1 (1.63 ppm) and H-3 (3.68 ppm) and also between H-1 (4.77 ppm) and CH_3-3 (1.19 ppm). The absolute configurations at C-1 and C-3 were determined as 1*S*,3*S* by ruthenium-catalyzed oxidative degradation (Bringmann et al., 1996) affording L-alanine and (*S*)-aminobutyric acid. An NOE interaction between H-3 and the methyl group at C-2′ (see Fig. 3b) revealed that these two spin systems are on the same side of the molecule, thus permitting assignment of the stereo array of the axis to be *M*-configured as shown in Fig. 3b. This result was corroborated by an NOE interaction between H-4_{ax} and H-8′. The new alkaloid thus possesses the structure **2** and is hence an analog of the co-occurring (see above) alkaloid hamatine (**10**) with a free OH group at C-5; it was therefore named 5′-*O*-demethylhamatine. The resemblance of the CD spectrum of **2** with that of **10**, in which the biaryl axis is also *M*-configured (Govindachari et al., 1977), further confirmed the above assigned absolute configuration.

The molecular formula ($C_{24}H_{25}NO_4$) of the second new alkaloid, compound **3**, was deduced from HRESIMS and from the ^{13}C NMR spectral data. With respect to NMR (see Table 1), the only differences between compound **2**

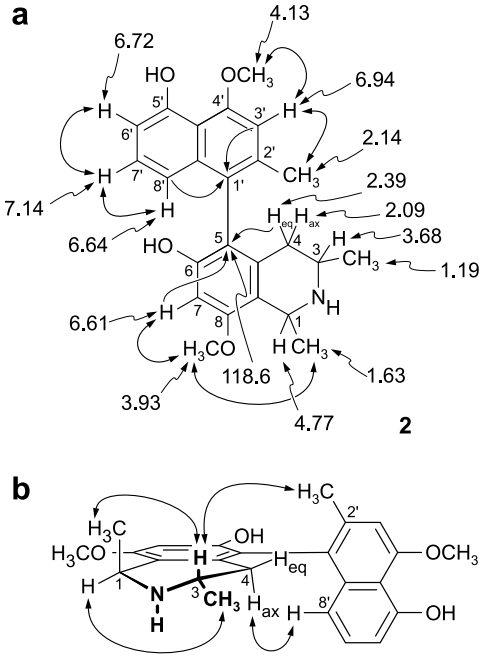


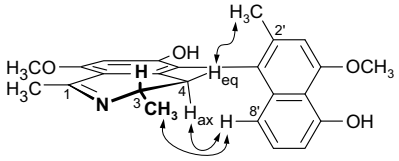
Fig. 3. Selected NMR data of 5′-*O*-demethylhamatine (**2**) indicative (a) of the constitution from ^{1}H and ^{13}C NMR shifts (ppm), and by HMBC (single arrows) and NOE (double arrows) interactions, and (b) of the relative configuration at the axis and the stereogenic centers, through NOE interactions.

and **3** within the NMR data were the lack of the H-1 quartet at ca. 4 ppm typical of tetrahydroisoquinolines of this type and the down-field shifted CH_3-1 signal at 2.78 ppm (Bringmann and Pokorny, 1995), which indicates the presence of a naphthyl-1,3-dimethyldihydroisoquinoline alkaloid.

The oxidative degradation (Bringmann et al., 1996) determined the absolute configuration at C-3 of **3** as *S*, which, in combination with the above mentioned NOE interactions between H-8′ and the protons of the methyl group at C-3 and H_{ax}-4 (see Table 1) on the one hand, and between CH_3-2′ and H_{eq}-4 on the other, established the axis to be *M*-configured. This stereochemical assignment was confirmed by the very similar CD spectrum of **3** with that of the known (Anh et al., 1997), likewise co-occurring (see above) alkaloid hamatinine (**12**). Compound **3** itself had not previously been found in nature, and was henceforth named 5′-*O*-demethylhamatinine (**3**; for the structure see Fig. 1 and Table 1), since it is the respective analog of **12**.

The third and fourth new alkaloids, compounds **4** and **5**, showed a very similar chromatographic and spectroscopic behavior, but proved separable on reversed-phase HPLC material (RP C_{18}). The faster eluting alkaloid, compound **4**, was found to possess a molecular formula of $C_{25}H_{27}NO_4$, while compound **5** had a molecular formula of $C_{24}H_{25}NO_4$,as deduced from HRESIMS. The ^{1}H NMR spectra of the two peaks indicated that both molecules were, like compound **3**, naphthyl-1,3-dimethyldihydroisoquinoline alkaloids, but belonging to the 5,8′-coupling type. Furthermore, compound **4** had a methoxy

Table 1
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data together with some important HMBC and NOE correlations of **3** in MeOD; (the stereostructure shows the most important correlations indicative of the relative configuration)



3

Position	**3**			
	^{1}H	^{13}C	^3J HMBC (H → C)	ROESY, NOESY
1	–	175.0		–
3	3.75, m_c	49.1		3-Me, H-4$_{ax}$, H-4$_{eq}$
4$_{ax}$	2.21, *dd*, $J = 17.0$, 10.6 Hz	32.9		H-8′, 3-Me, H-4$_{eq}$, H-3
4$_{eq}$	2.49, *dd*, $J = 16.9$, 5.4 Hz	32.9	C-5	2′-Me, 3-Me, H-4$_{ax}$, H-3
5	–	121.4		–
6	–	168.5		–
7	6.66, *s*	99.7	C-5	8-OMe
8	–	166.1		–
9	–	108.4		–
10	–	142.6		–
1′	–	124.9		–
2′	–	137.0		–
3′	6.94, *s*	108.1		2′-Me, 4′-OMe
4′	–	157.7		–
5′	–	156.5		–
6′	6.73, *d*, $J = 7.6$, 1.0 Hz	110.9		H-7′
7′	7.18, *d*, $J = 8.3$, 7.8 Hz	129.2		H-6′, H-8′
8′	6.64, *dd*, $J = 8.3$, 1.0 Hz	116.8		H-7′, H-4$_{ax}$, 3-Me
9′	–	137.5		–
10′	–	115.2		–
1-Me	2.78, *s*	24.9		8-OMe
2′-Me	2.16, *s*	20.6		H-3′, H-4$_{eq}$
3-Me	1.20, *d*, $J = 6.3$ Hz	18.1		H-8′, H-3, H-4
4′-OMe	4.13, *s*	56.9		H-3′
8-OMe	4.04, *s*	56.8		1-Me, H-7

group located at C-5′, while compound **5** possessed a hydroxy-function at this position, like compounds **2** and **3**.

By the above mentioned methods – the oxidative degradation, specific NOE interactions across the biaryl axis (see Table 2), and CD spectroscopy – the new compounds **4** and **5** (for the structures see Fig. 1 and Table 2) were established to be *S*-configured at C-3 and *P*-configured at the axis. Compound **4**, which is the new 6-*O*-demethyl analog of the 6-methoxy alkaloid ancistroealaine A, was thus named 6-*O*-demethylancistroealaine A and in a similar way compound **5** was called 6,5′-*O*,*O*-didemethylancistroealaine A.

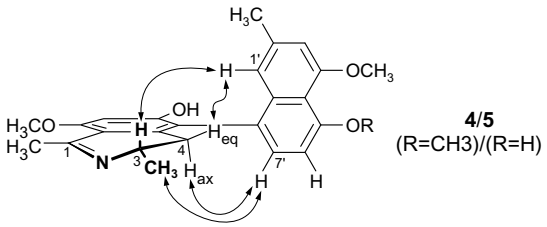
The fifth new alkaloid, compound **6**, was difficult to separate from 6-*O*-methylhamatinine (**12**) by reversed-phase HPLC; the resolution did, however, succeed by repeated FCPC runs. The alkaloid thus obtained in a pure form corresponded to a molecular formula of $C_{27}H_{33}NO_4$ according to HRESIMS. It showed the ^{1}H NMR spectrum of a fully *O*- and *N*-methylated naphthyltetrahydroisoquinoline alkaloid (see Fig. 1 and Table 3), with four methoxy groups (3.98, 3.95, 3.93, and 3.65 ppm), three *C*-methyl groups (2.27, 1.52, and 1.07 ppm), and a three-proton singlet resonating at 2.54 ppm, which is characteristic of an *N*-CH_3 group, and it likewise belonged to the 5,8′ coupling type.

Like in compound **2** the two methyl groups at C-1 and C-3 were *trans* to each other, and again C-1 and C-3 were *S*-configured, which was elucidated by the above mentioned degradation procedure. NOE interactions finally revealed the axis to be *M*-configured. This was confirmed by the almost mirror-image like CD spectrum of **6** compared to that of ancistrobertsonine A (Bringmann et al., 1998) (**16**, see Fig. 4), which is *P*-configured at the biaryl axis. This is in agreement with the fact that the biaryl chromophore usually dominates the CD spectrum (Bringmann and Busemann, 1998; Bringmann et al., 1999; Bringmann et al., 2003a). Compound **6** (for the structure see Fig. 1 and Table 3) had hitherto not been described in the literature and was, in accordance to its structural relationship to ancistrobertsonine A (**16**), named 5-*epi*-6-*O*-methylancistrobertsonine A.

The sixth new compound, **7**, had a molecular formula of $C_{26}H_{31}NO_4$ as deduced from HREIMS *m*/*z* $[M-CH_3]^+ =$ 406.2012. The ^{1}H NMR spectrum hinted at a great structural similarity with the above-investigated alkaloid **6** (see Fig. 1 and Table 3). The NOE interactions clearly showed that the only difference was the stereochemical orientation at C-1 and the OMe substitution pattern. The NOESY experiment furthermore revealed cross peaks between OCH_3–H-5′ (4.09 ppm) and H-6′, leaving the singlet at 9.37 ppm to be assigned to a free hydroxy group at C-4′. This conclusion was confirmed by HMBC showing a long-range coupling between C-3′ and the signal of the proton of the OH group at C-4′ (112.8). This was further corroborated by the oxidative degradation, which resulted in an *R*-configuration at C-1. Because of its structural relationship to the known (Bringmann et al., 1999) alkaloid ancistrobertsonine C (structure not shown) the new compound **7** (c.f. 1) was henceforth named 5-*epi*-4′-*O*-demethyl-ancistrobertsonine C.

Further resolution of the more polar FCPC fractions by preparative RP-HPLC yielded colorless crystals of a nitrogen-free metabolite, compound **8**. Its molecular formula ($C_{11}H_8O_4$) was deduced by HRESIMS. In the aromatic region the ^{1}H NMR spectrum showed an ABC-spin system corresponding to a 1,2,3-trisubstituted benzene ring. These signals at δ 7.50, 7.37, and 7.22 were attributed to H-7, H-8, and H-6, respectively (see Fig. 5a). This conclusion was also confirmed by NOESY experiments (see Fig. 5b).

Table 2
^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data together with some important HMBC and NOE correlations of **4** and **5** in MeOD; (the figure shows the most important interactions establishing the configuration at the axis relative to the stereocenter)

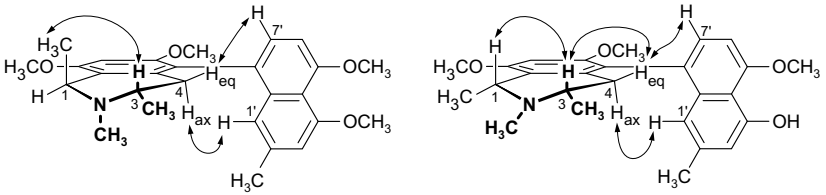


Position	**4**		**5**		**4** and **5**	**4** and **5**
	^{1}H	^{13}C	^{1}H	**5** ^{13}C	^{3}J HMBC (H → C)	ROESY, NOESY
1	–	175.8	–	175.7		–
3	3.70, *m*$_c$	49.5	3.70, *m*$_c$	49.5		H-1′
4$_{ax}$	2.48, *dd*, *J* = 16.9, 11.2 Hz	33.6	2.48, *dd*, *J* = 16.9, 11.2 Hz	33.7		H-7′
4$_{eq}$	2.41, *dd*, *J* = 16.8, 5.9 Hz	33.6	2.41, *dd*, *J* = 16.9, 5.9 Hz	33.7	C-5	H-1′
5	–	122.7	–	122.6		–
6	–	167.8	–	168.1		–
7	6.68, *s*	99.4	6.67, *s*	99.4	C-5	8-OMe
8	–	165.9	–	165.9		–
9	–	108.8	–	108.7		–
10	–	142.7	–	142.8		–
1′	6.71, *s*	118.1	6.72, *s*	118.8		2′-Me, H-3, H-4$_{eq}$
2′	–	138.5	–	138.2		–
3′	6.82, *s*	110.3	6.83, *s*	107.9		2′-Me, 4′-OMe
4′	–	159.0	–	158.2		–
5′	–	159.0	–	156.4		–
6′	6.94, *d*, *J* = 8.1 Hz	106.6	6.80, *d*, *J* = 7.8 Hz	110.4		H-7′, 5′-OMe
7′	7.11, *d*, *J* = 8.0 Hz	130.8	7.05, *d*, *J* = 7.8 Hz	131.7	C-5	3-Me, H-6′, H-4$_{ax}$
8′	–	125.0	–	123.1		–
9′	–	137.3	–	136.7		–
10′	–	117.6	–	115.0		–
1-Me	2.80, *s*	24.8	2.79, *s*	24.8		8-OMe
2′-Me	2.33, *s*	22.2	2.34, *s*	22.3		H-1′, H-3′
3-Me	1.25, *d*, *J* = 6.7 Hz	18.1	1.26, *d*, *J* = 6.7 Hz	18.2		H-7′
4′-OMe	3.94, *s*	57.1	4.10, *s*	57.0		H-3′
5′-OMe	3.96, *s*	56.9	–	–		H-6′
8-OMe	4.05, *s*	56.9	4.04, *s*	56.8		1-Me, H-7

In addition a singlet in the olefinic region, at 5.98 ppm, and another signal at 2.15 ppm were assigned as H-3 and an allylic methyl group, CH_3-2, respectively, interacting with each other by NOESY correlations. The ^{13}C NMR spectrum showed ten signals corresponding to the above assigned structural units and with the presence of two carbonyl groups corresponding to a methylbenzopyranone carboxylate. HMBC couplings of both, H-6 and H-7, with the carboxyl C-atom resonating at 174.3 ppm and of H-3 with the low-field shifted, quaternary C-5 atom (137.8 ppm), suggested the carboxylic acid to be located in the 5-position. This was further corroborated by the fact that H-7 is low-field shifted in comparison to the signals of H-6 and H-8, which excludes an otherwise imaginable alternative with the sequence {O-1–C-2–C-3–C-4} inverted. The second carbonyl C-atom (179.6 ppm) showed HMBC couplings with H-8 and H-3, which suggested the presence of a ketone function at C-4. All aromatic protons revealed long-range couplings with the quaternary carbon atoms at 158.2 and 120.7 ppm as evidenced by HMBC, thus indicating these to be present in the same ring system. The assignment of the signals to C-9 and C-10, respectively, was possible due to further long-range couplings between H-3 and C-10. The signal of C-9 (158.2 ppm) and of the last quaternary carbon atom, C-2 (169.2 ppm) were both found to be shifted to low-field, indicating the presence of an oxygen substitution. Thus, **8** possesses the structure of a 2-methyl-4-oxo-4*H*-1-benzopyrane 5-carboxylic acid shown in Fig. 5. Like each of the isoquinoline and naphthalene halves of the alkaloids co-occurring in the plants, **8** constitutes a decarboxylated hexaketide derivative (Bringmann et al., 2000b), with eleven (core) carbon atoms, yet based on a different folding type.

The promising antiprotozoal activities of several naphthylisoquinoline alkaloids against pathogens belonging to the genera *Plasmodium*, *Leishmania*, and *Trypanosoma*

Table 3
^{1}H and ^{13}C NMR data together with some important HMBC and NOE correlations of **6** (400 MHz, 100 MHz) in MeOD and **7** (600 MHz, 150 MHz) in $CDCl_3$; (the figure shows the most important correlations diagnostic for the relative configuration)



Position	**6**				**7**			
	^{1}H	^{13}C	^{3}J HMBC (H → C)	ROESY, NOESY	^{1}H	^{13}C	^{3}J HMBC (H → C)	ROESY, NOESY
1	4.49, *q*, $J = 6.8$ Hz	58.0	*N*-Me (^{4}J)	3-Me, *N*-Me	4.58, *q*, $J = 7.2$ Hz	58.8	*N*-Me (^{4}J)	H-3
3	3.54, m_c	49.4	*N*-Me (^{4}J)	1-Me	3.09, m_c	58.5	*N*-Me (^{4}J)	H-1
4_{ax}	2.01, *dd*, $J = 17.8$, 11.5 Hz	31.7		3-Me, H-1′	2.56, *dd*, $J = 16.9$, 8.0 Hz	30.0		3-Me, H-1′
4_{eq}	2.50, *dd*, $J = 18.3$, 4.3 Hz	31.7		H-7′	2.45, *dd*, $J = 16.9$, 5.0 Hz	30.0		H-7′
5	–	122.3		–	–	120.5		–
6	–	159.4		–	–	158.6		–
7	6.72, *s*	95.4	C-5	8-OMe, 6-OMe	6.57, *s*	94.4	C-5	6-OMe, 8-OMe
8	–	158.2		–	–	156.3		–
9	–	116.9		–	–	113.4		–
10	–	131.9		–	–	133.1		–
1′	6.57, *s*	118.3	C-8′	H-4_{ax}, 2′-Me	6.56, *s*	115.7	C-8′	H-4_{ax}, 2′-Me
2′	–	137.8		–	–	138.7		–
3′	6.77, *s*	110.0		2′-Me, 4′-OMe	6.76, *s*	112.8		2′-Me
4′	–	158.8		–	–	154.7		–
5′	–	158.0		–	–	156.0		–
6′	6.93, *d*, $J = 8.0$ Hz	107.0		5′-OMe	6.77, *d*, $J = 8.0$ Hz	102.7		5′-OMe
7′	7.09, *d*, $J = 7.9$ Hz	129.8	C-5	H-4_{eq}	7.02, *d*, $J = 8.0$ Hz	127.7	C-5	H-4_{eq}
8′	–	127.5		–	–	126.1		–
9′	–	137.7		–	–	135.7		–
10′	–	117.6		–	–	113.5		–
1-Me	1.52, *d*, $J = 6.8$ Hz	17.4		*N*-Me, 8-OMe, H-3	1.82, *d*, $J = 7.0$ Hz	19.3		*N*-Me, 8-OMe, 3-Me
2′-Me	2.27, *s*	22.1		H-1′, H-3′	2.31, *s*	21.9		H-1′, H-3′
3-Me	1.07, *d*, $J = 6.8$ Hz	18.2		H-4_{ax}, *N*-Me, H-1	1.41, *d*, $J = 7.0$ Hz	18.6		H-4_{ax}, *N*-Me, 1-Me
4′-OMe	3.93, *s*	57.0		H-3′	–	–		
5′-OMe	3.95, *s*	57.2		H-6′	4.09, *s*	56.0		H-6′
6-OMe	3.65, *s*	56.5		H-7	3.68, *s*	56.1		H-7
8-OMe	3.98, *s*	56.3		1-Me, H-7	3.95, *s*	55.5		1-Me, H-7
N-Me	2.54, *s*	35.9		H-1, 1-Me, H-3, 3-Me	2.91, *s*	43.2		H-1, 1-Me, H-3, 3-Me
4′-OH	–		–	–	9.37, *s*		C-3′	

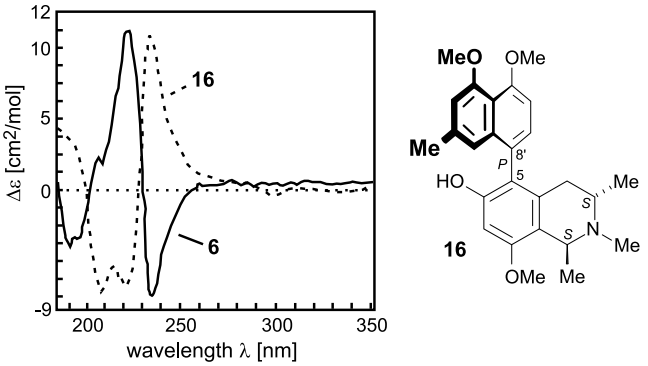


Fig. 4. Comparison of the CD spectrum of 5-*epi*-6-*O*-methylancistrobertsonine A (**6**) with that of the known related alkaloid ancistrobertsonine A (**16**) to confirm the absolute axial configuration of **6**.

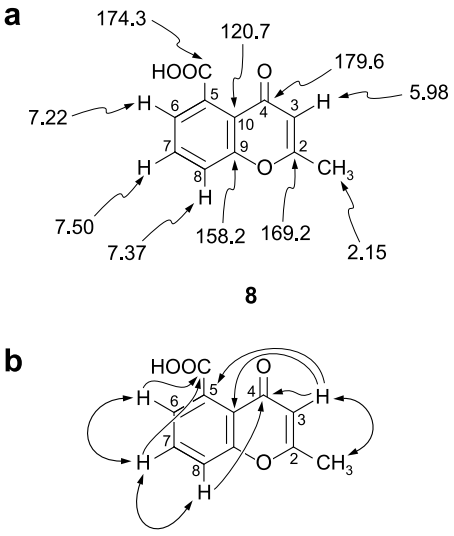


Fig. 5. Selected NMR data of 2-methyl-4-oxo-4*H*-1-benzopyrane 5-carboxylic acid (**8**): ^{1}H and ^{13}C NMR shifts (ppm) (a), HMBC (single arrows) and NOE (double arrows) interactions relevant for the constitution (b).

previously found (Bringmann, 2003; Bringmann et al., 2003a; François et al., 1997, 1999) made it rewarding to screen the new compounds, **2**–**8**, but also the known, but not yet tested 6-*O*-demethylancistrobrevine A (**9**) (Table 4). All of the naphthylisoquinoline alkaloids (**2**–**7** and **9**) tested were found to exhibit weak antiplasmodial activities against the K1 strain of *Plasmodium falciparum* (resistant to chloroquine and pyrimethamine), though not reaching the good results of other naphthylisoquinolines (Bringmann, 2003; Bringmann et al., 2003a; François et al., 1997, 1999). The results are important contributions to our ongoing QSAR (Quantitative Structure-Activity Relationship) investigations within this class of compounds (Bringmann and Rummey, 2003; Stiefl et al., 2003). The antitrypanosomal activities exhibited by (**2**–**7** and **9**) were found to be moderate to weak against the pathogens of African sleeping sickness (*Trypanosoma brucei rhodesiense*), and Chagas' disease (*T. cruzi*). Compound **6** was found to exhibit activity against *Leishmania donovani* (visceral leishmaniasis), comparable to those of the most active naphthylisoquinolines tested (Bringmann et al., 2003a). The two *O*-demethyl derivatives (**4** and **5**) of the highly antileishmanially active ancistroealaine A (**15**) (Bringmann et al., 2000a), by contrast, exhibited only weak activity against *L. donovani*, thus indicating a high *O*-methylation rate as an important precondition for attaining good antileishmanial activity within the class of naphthylisoquinoline alkaloids. The likewise isolated *N*,*C*-coupled ancistrocladinium A (**1**) already described for leaf extracts of this *Ancistrocladus* plant species, had previously been shown to exhibit promising bioactivities (Bringmann et al., 2006), especially against leishmania. Therefore, more detailed bioactivity testings and synthetic studies in combination with structure-activity relationship investigations are presently in progress to further optimize the activity of such *N*,*C*-coupled naphthylisoquinoline alkaloids (Ponte-Sucre et al., 2007).

All of the compounds reported here, possess an oxygen function at C-6 and are *S*-configured at C-3, and thus, all of them, belong to the subclass of "Ancistrocladaceae-type" alkaloids, which is typical of naphthylisoquinoline alkaloids from South-East Asian (Bringmann et al., 2002b, 2005; Chen et al., 1981; Govindachari et al., 1975; Govindachari and Parthasarathy, 1970, 1971) and East African *Ancistrocladus* species (Bringmann et al., 1998, 1999, 2000a, 2002a, 2003a,c, 2004).

Thus, from a chemotaxonomic point of view, the investigated plant species seems to range between the East African species, to which it shows large similarities, and the other Central African species, in particular to *Ancistrocladus congolensis*; which is in agreement with the results of the molecular ITS sequence experiments (Heubl et al., 2006; Meimberg et al., 2000).

The variety of the compounds isolated indicate that the *Ancistrocladus* plants from Central Africa are a rich source of new naphthylisoquinoline alkaloids and give a strong chemotaxonomical evidence that this plant is a new species.

3. Experimental

3.1. General

IR spectra were taken on a Jasco FT/IR-410 spectrometer, UV spectra on a Varian Cary 50 spectrophotometer, CD spectra on a Jasco J-715 spectropolarimeter, and optical rotations on a Jasco P-1020 polarimeter. ^{1}H NMR (400 MHz, 600 MHz) and ^{13}C NMR (100 MHz, 150 MHz,) were recorded on a Bruker AMX 400 or on a DMX 600 instrument, using $CDCl_3$(δ 7.26 and 77.01) and CD_3OD (δ 3.31 and 49.15) as the solvents and the internal ^{1}H and ^{13}C standards. For the NOESY experiments the mixing time was set to 1 s. Proton-detected, heteronuclear correlations were analyzed using HMQC (optimized for $^{1}J_{HC} = 145$ Hz) and HMBC (optimized for $^{n}J_{HC} = 7$ Hz). EIMS (70 eV), HREIMS (70 eV), and

Table 4
Bioactivities of the compounds **2**–**9**

	IC_{50} (μg/ml)							
	2	**3**	**4**	**5**	**6**	**7**	**8**	**9**
P. falciparum (strain: K1)	1.0	2.8	1.8	2.1	1.9	2.6	>5	2.1
Standard: chloroquine 0.041[a] *T. cruzi*	18.2	15.2	10.5	6.4	14.6	4.7	21.5	9.5
Standard: benznidazole 0.53[a] *T. b. rhodesiense*	2.1	9.8	13.9	9.7	6.9	3.4	19.7	5.0
Standard: melarsoprol 0.0046[a] *L. donovani*	4.4	>30	21.2	17	1.6	12.1	>30	19.4
Standard: miltefosine 0.05[a] Cytotoxicity (L-6 cells)	70.2	83.3	>90	>90	68.0	43.8	>90	25.3

[a] All values in μg/ml.

HRESIMS were determined on Finnigan MAT 8200, Finnigan MAT 90 and Bruker micrOTOF instruments, respectively. HPLC (preparative): SymmetryPrep C_{18}, 19 × 300 mm; 7 μm (waters); UV-detection 190–600 nm (photodiode array detector); flow 11 ml min^{-1}; solvent (A) CH_3CN (0.05% trifluoroacetic acid), (B) H_2O (0.05% trifluoroacetic acid); using a linear gradient: 0 min 20% A, 25 min 55% A, with isocratic solvent systems: between 28% and 52% of solvent A, and an XTerra C_{18}, 19 × 300 mm column; 7 μm (waters); flow 11 ml min^{-1}; solvent (A) CH_3CN (8 mM NH_3) and (B) H_2O (8 mM NH_3); linear gradient: 0 min 40% A, 20 min 100% A. For fast centrifugal partitioning chromatography (FCPC), an apparatus from Kromaton was used, equipped with a 1000-ml rotor; UV-detection 254 nm; flow 15 ml min^{-1}; 900 rpm, descending mode with two different solvent systems; stationary phase, *n*-heptane–EtOAc (8:2), mobile phase, MeOH–H_2O (8:2), retention rate 75% and $CHCl_3$–EtOAc (6:2) as the stationary phase and MeOH–H_2O (2:6) as the mobile phase. Sephadex LH-20 material for column chromatography was used from Amersham Bioscience. (*R*)-MTPA-Cl was prepared from (*S*)-MTPA (Fluka Chemie AG, Deisenhofen, Germany) as described earlier (Bringmann et al., 1996). Organic solvents were dried and distilled prior to use.

3.2. Plant material

Root material of the *Ancistrocladus* plants was collected by one of us (V.M.) in the region Ikela of the rainforest Yeteto, in the Democratic Republic of Congo. A voucher specimen (No. 62) has been deposited at Herb. Bringmann, University of Würzburg.

3.3. Extraction and isolation

Five hundred and sixty gram of lyophilized root material were powdered and sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH–H_2O (9:1). The $CH_2Cl_2$and MeOH–H_2O extracts were concentrated *in vacuo* to give 20 g and 15 g of a crude residue, respectively. A portion of 2 g of the MeOH–H_2O extract were dissolved in methanol and directly submitted to preparative HPLC on the Symmetry-C_{18} column, using the gradient system, to give 8.4 mg of compound **7** (retention time 21.3 min). The remaining part of the MeOH–H_2O extract was resolved on Sephadex LH-20 using methanol as the solvent, to give three product-containing fractions. The first of these Sephadex fractions was further purified by preparative HPLC under acidic conditions (see Section 3.1), using a Symmetry-C_{18} column and an isocratic solvent system with 38% aqueous acetonitrile, giving 8.6 mg of compound **13**. The second Sephadex fraction was a mixture of several compounds and therefore further resolved by FCPC using *n*-heptane:EtOAc (2:8) as the organic layer and methanol:water (2:8) as the aqueous phase giving, in turn, two different fractions. From the first FCPC fraction, 7.1 mg of compound **3**, 7.7 mg of **4**, 3.3 mg of **5**, 3.3 mg of **9**, and 6.7 mg of **12** were isolated by isocratic elution with 38% aqueous acetonitrile and acidic conditions (see Section 3.1) using a preparative Symmetry-C_{18} HPLC column. The second FCPC fraction contained 2.5 mg of compound **2**, which was further purified by the same HPLC conditions as the compounds **3**–**5**, **9**, and **12**. The third Sephadex fraction obtained from the MeOH–H_2O extract was resolved by preparative HPLC with isocratic conditions using 28% aqueous acetonitrile with TFA (see Section 3.1) as the eluent, giving 5.5 mg of alkaloid **10** and 2.7 mg of compound **11**.

The residue of the CH_2Cl_2 extract was split into two portions and both were further resolved by FCPC, but with different solvent systems. One part was treated like the second Sephadex fraction from the MeOH–H_2O extract with a solvent system consisting of *n*-heptane:EtOAc (2:8) and MeOH:H_2O (2:8) resulting in two FCPC fractions. Fraction one contained two alkaloids, which were separable by preparative HPLC under acidic conditions (acidified with TFA, see above) and an isocratic elution with 36% aqueous acetonitrile as the eluent, giving compounds **14** (3.0 mg) and **15** (11.2 mg). The second fraction also contained two different alkaloids, which were further resolved by isocratic preparative HPLC with 34% aqueous acetonitrile, providing 7.8 mg of **8** and again compound **13**. From the other part of the CH_2Cl_2 extract, two more alkaloids were isolated. FCPC with $CHCl_3$:EtOAc (6:2) and MeOH:H_2O (2:6) again furnished two fractions, the first one of these was further purified by isocratic preparative HPLC with 52% acidified aqueous acetonitrile, to give 1.2 mg of compound **1**, from the second fraction, 1.8 mg of compound **6** were isolated by using basic conditions described in Section 3.1, and an XTerra column for separation.

3.4. 5′-O-Demethylhamatine (**2**)

Colorless amorphous solid; m.p. 174–180 °C; $[\alpha]_D^{25}$ +26.0 (MeOH; *c* 0.10); UV (CH_2Cl_2) λ_{max} (log ε) nm: 337 (0.34), 322 (0.37), 309 (0.41), 237 (1.42); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{211}$ +7.8, $\Delta\varepsilon_{228}$ +13.2, $\Delta\varepsilon_{240}$ −6.1 (MeOH; *c* 0.12); IR (KBr) ν_{max} cm^{-1}: 2937 (w), 2848 (w), 1680 (s), 1610 (s), 1447 (m), 1427 (m), 1392 (m), 1363 (m), 1204 (s), 1136 (s), 838 (m), 801 (m); ^1H NMR (400 MHz, MeOD): δ 1.19 (3H, *d*, *J* = 6.3 Hz, CH_3-3), 1.63 (3H, *d*, *J* = 6.7 Hz, CH_3-1), 2.09 (1H, *dd*, *J* = 17.9, 4.9 Hz, H_{ax}-4), 2.14 (3H, *s*, CH_3-2′), 2.39 (1 H, *dd*, *J* = 18.0, 11.7 Hz, H_{eq}-4), 3.68 (1H, m_c, H-3), 3.93 (3H, *s*, OCH_3-8), 4.13 (3H, *s*, OCH_3-4′), 4.77 (1H, *q*, 6.8 Hz, H-1), 6.61 (1H, *s*, H-7), 6.64 (1H, *dd*, *J* = 8.3, 1.0 Hz, H-8′), 6.72 (1H, *dd*, *J* = 7.6, 1.0 Hz, H-6′), 6.94 (1H, *s*, H-3′), 7.14 (1H, *dd*, *J* = 8.3, 7.7 Hz, H-7′); ^{13}C NMR (100 MHz, MeOD): δ 18.8 (CH_3-1), 19.3 (CH_3-3), 20.7 (CH_3-2′), 33.0 (C-4), 45.2 (C-3), 49.3 (C-1), 56.2 (OCH_3-8), 56.9 (OCH_3-4′), 99.0 (C-7), 108.3 (C-3′), 110.6 (C-6′), 114.5 (C-9), 115.4 (C-10′), 117.0 (C-8′), 118.6 (C-5), 126.1 (C-1′), 128.9 (C-7′), 132.9 (C-10), 137.5 (C-9′), 136.9 (C-2′), 156.4 (C-5′), 157.1 (C-6), 157.4 (C-4′), 157.9 (C-8); EIMS *m*/*z* (rel. int.): 393 $[M]^+$ (9), 378 $[M-CH_3]^+$ (100), 362 $[M-OCH_3]^+$ (5); HRESIMS: *m*/*z* 394.2021 $[M+H]^+$ (calcd. for $C_{24}H_{28}NO_4^+$ 394.2012).

3.5. 5′-O-Demethylhamatinine (**3**)

Yellow amorphous solid; $[\alpha]_D^{25}$ +25.0 (MeOH; *c* 0.10); UV (CH_2Cl_2) λ_{max} (log ε) nm: 335 (0.30), 321 (0.33), 311 (0.34), 235 (1.05); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{213}$ −6.1, $\Delta\varepsilon_{230}$ +6.9, $\Delta\varepsilon_{245}$ −4.7 (MeOH; *c* 0.10); IR ν_{max} cm^{-1}: 2917 (w), 2851 (w), 1681 (s), 1626 (w), 1609 (w), 1576 (m), 1447 (w), 1426 (w), 1358 (w), 1325 (w), 1268 (w), 1203 (s), 1133 (s), 842 (m), 801 (m); for NMR data, see Table 1; EIMS *m*/*z* (rel. int.): 392 $[M+H]^+$ (100), 391 $[M]^+$ (73), 376 $[M-CH_3]^+$ (33), 360 $[M-OCH_3]^+$ (6); HRESIMS *m*/*z*: 391.1769 $[M]^+$ (calcd. for $C_{24}H_{25}NO_4^+$ 391.1778).

3.6. 6-O-Demethylancistroealaine A (**4**)

Yellow crystalline powder; m.p. 155–160 °C; $[\alpha]_D^{25}$ −63.2 (MeOH; *c* 0.10); UV (CH_2Cl_2) λ_{max} (log ε) nm: 334 (0.33), 321 (0.35), 316 (0.34), 310 (0.34), 232 (1.06); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{213}$ +9.2, $\Delta\varepsilon_{231}$ −7.2, $\Delta\varepsilon_{244}$ +5.5 (MeOH; *c* 0.10); IR ν_{max} cm^{-1}: 2925 (br), 2849 (w), 1675 (s), 1624 (w), 1582 (s), 1454 (w), 1411 (w), 1381 (w), 1353 (w), 1325 (m), 1295 (w), 1274 (m), 1247 (w), 1201 (m), 1133 (m), 1099 (w), 1080 (w), 968 (w), 834 (w), 800 (w); for NMR data, see Table 2; EIMS *m*/*z* (rel. int.): 405 $[M]^+$ (100), 390 $[M-CH_3]^+$ (45), 374 $[M-OCH_3]^+$ (10); HREISMS *m*/*z*: 406.2002 $[M+H]^+$ (calcd. for $C_{25}H_{28}NO_4^+$ 406.2013).

3.7. 6,5′-O,O-Didemethylancistroealaine A (**5**)

Yellow amorphous solid; $[\alpha]_D^{25}$ −68.6 (MeOH; *c* 0.10); UV (CH_2Cl_2) λ_{max} (log ε) nm: 335 (0.45), 325 (0.47), 321 (0.48), 316 (0.47), 313 (0.47), 235 (1.15); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{213}$ +8.9, $\Delta\varepsilon_{231}$ −4.7, $\Delta\varepsilon_{244}$ +3.8 (MeOH; *c* 0.10); IR ν_{max} cm^{-1}: 2925 (m), 2852 (w), 1681 (s), 1626 (m), 1580 (s), 1453 (m), 1400 (w), 1356 (m), 1328 (m), 1299 (w), 1263 (m), 1201 (s), 1131 (s), 835 (m), 800 (m); for NMR data, see Table 2; EIMS *m*/*z* (rel. int.): 392 $[M+H]^+$ (99), 391 $[M]^+$ (99), 376 $[M-CH_3]^+$ (40), 360 $[M-OCH_3]^+$ (7); HRESIMS *m*/*z*: 392.1861 $[M+H]^+$ (calcd. for $C_{24}H_{26}NO_4^+$ 392.1856).

3.8. 5-epi-6-O-Methylancistrobertsonine A (**6**)

Colorless amorphous solid; $[\alpha]_D^{25}$ +1.2 (MeOH; *c* 0.09); UV (CH_2Cl_2) λ_{max} (log ε) nm: 337 (0.26), 321 (0.33), 307 (0.40), 237 (1.29); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{227}$ +11.1, $\Delta\varepsilon_{240}$ −8.0 (MeOH; *c* 0.18); IR ν_{max} cm^{-1}: 2926 (s), 2854 (m), 1693 (s), 1585 (s), 1454 (m), 1388 (w), 1326 (m), 1255(m), 1204 (m), 1138 (m), 1096 (w), 1079 (w), 889 (w), 839 (m), 801(w); for NMR data, see Table 3; EIMS *m*/*z* (rel. int.): 435 $[M]^+$ (2), 420 $[M-CH_3]^+$ (100), 404 $[M-OCH_3]^+$ (7); HRESIMS *m*/*z*: 436.2477 $[M-H]^+$ (calcd. for $C_{27}H_{34}NO_4^+$ 436.2482).

3.9. 5-epi-4′-O-Demethylancistrobertsonine C (**7**)

Light-brown amorphous solid; $[\alpha]_D^{25}$ +15.7 (MeOH; *c* 0.12); UV (CH_2Cl_2) λ_{max} (log ε) nm: 307 (0.42), 230 (1.05); CD $cm^2\,mol^{-1}$: $\Delta\varepsilon_{230}$ +10.6, $\Delta\varepsilon_{242}$ −3.98 (MeOH; *c* 0.10); IR ν_{max} cm^{-1}: 3400 (br), 2960 (s), 2930 (s), 2850 (s), 1690 (s), 1612 (w), 1601 (m), 1595 (m), 1460 (w), 1410 (w), 1390 (m), 1310 (w), 1270 (s); for NMR data, see Table 3; EIMS *m*/*z* (rel. int.): 422 $[M+H]^+$ (1), 406 $[M-CH_3]^+$ (100), 391 $[M-OCH_3]^+$ (2); HREIMS *m*/*z*: 406.2012 $[M-CH_3]^+$ ($C_{25}H_{28}NO_4$ calcd. 406.2013).

3.10. 2-Methyl-4-oxo-4H-1-benzopyrane 5-carboxylic acid (**8**)

Colorless crystals (MeOH); m.p. 224 °C (subl.); UV (CH_2Cl_2) λ_{max} (log ε) nm: 321 (0.31), 312 (0.38), 280 (0.40), 233 (0.77) nm; IR ν_{max} cm^{-1}: 2923 (s), 2852 (m), 1714 (m), 1652 (s), 1597 (m), 1576 (w), 1539 (s), 1487 (w), 1455 (w), 1393 (m), 1366 (m), 1272 (w), 1201 (m), 1125 (w), 851 (w);^1H NMR (400 MHz, MeOD): δ 2.15 (3H, *s*, CH_3-2), 5.98 (1H, *s*, H-3), 7.22 (1H, *dd*, *J* = 7.3, 1.1 Hz, H-6), 7.37 (1H, *dd*, *J* = 8.5, 1.1 Hz, H-8), 7.50 (1H, *dd*, *J* = 8.5, 7.4 Hz, H-7); ^{13}C NMR (100 MHz, MeOD): δ 20.4 (CH_3-2), 111.3 (C-3), 120.0 (C-8), 120.7 (C-10), 125.3 (C-6), 135.1 (C-7), 137.8 (C-5), 158.2 (C-9), 169.2 (C-2), 174.3 (COOH-5), 179.6 (C-4); EIMS *m*/*z* (rel. int.): 160 $[M-COO]^+$ (100); HRESIMS *m*/*z*: $[M]^-$ 203.0350 (calcd. for $C_{10}H_7O_4^-$ 203.0350).

3.11. Known alkaloids isolated, **9**–**15**

The likewise isolated alkaloids 6-*O*-demethylancistrobrevine A (**9**), hamatine (**10**), ancistrocladine (**11**), hamatinine (**12**), 6-*O*-methylhamatine (**13**), 6-*O*-methylhamatinine

(**14**), and ancistroealaine A (**15**) were found to be identical in their spectroscopic, physical and/or chromatographic properties with data previously reported (Bringmann et al., 1992, 2000a, 2003d; Govindachari and Parthasarathy, 1971; Govindachari et al., 1975; Anh et al., 1997; Rizzacasa and Sargent, 1991).

3.12. Oxidative degradation procedure

The degradation, the derivatization of the resulting amino acids, and the subsequent GC–MSD analysis were carried out following a method developed earlier (Bringmann et al., 1996).

3.13. Biological experiments

Antiparasitic activities against the pathogens *P. falciparum*, *T. cruzi, T. brucei rhodesiense*, and *L. donovani* were assessed as described previously (Bringmann et al., 2000a).

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