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# Antiprotozoal dimeric naphthylisoquinolines, mbandakamines B<sub>3</sub> and B<sub>4</sub>, and related 5,8'-coupled monomeric alkaloids, ikelacongolines A–D, from a Congolese *Ancistrocladus* liana†

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From the leaves of a botanically and phytochemically as yet unexplored *Ancistrocladus* liana discovered in the rainforests of the Central region of the Democratic Republic of the Congo in the vicinity of the town of Ikela, six new naphthylisoquinoline alkaloids were isolated, viz., two constitutionally unsymmetric dimers, the mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4), and four related 5,8'-linked monomeric alkaloids, named ikelacongolines A–D (5a, 5b, 6, and 7). The dimers 3 and 4 are structurally unusual quateraryls comprising two 5,8'-coupled monomers linked via a sterically strongly constrained 6',1''-connection between their naphthalene units. These compounds contain seven elements of chirality, four stereogenic centers and three consecutive chiral axes. They were identified along with two known related compounds, the mbandakamines A (1) and B<sub>2</sub> (2), which had so far only been detected in two *Ancistrocladus* species indigenous to the Northwestern Congo Basin. In addition, five known monomeric alkaloids, previously found in related Central African *Ancistrocladus* species, were isolated from the here investigated Congolese liana, three of them belonging to the subclass of 5,8'-coupled naphthylisoquinoline alkaloids, whereas two compounds exhibited a less frequently occurring 7,8'-biaryl linkage. The stereostructures of the new alkaloids were established by spectroscopic (in particular HRESIMS, 1D and 2D NMR), chemical (oxidative degradation), and chiroptical (electronic circular dichroism) methods. The mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4) displayed pronounced activities *in vitro* against the malaria parasite *Plasmodium falciparum* and the pathogen of African sleeping sickness, *Trypanosoma brucei rhodesiense*.

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## Introduction

The evergreen rain forests and swamp regions in Central Africa, in particular in the Democratic Republic of the Congo, are one of the main centers of distribution of lianas belonging to the Ancistrocladaceae plant family.<sup>1–3</sup> Comprehensive data about the natural diversity of the genus *Ancistrocladus* within the Congo region, however, are still lacking.<sup>1–3</sup> Recent field trips in combination with phylogenetic and phytochemical

investigations clearly suggested the presence of further, hitherto unrecognized taxa,<sup>3–11</sup> along with the four botanically acknowledged *Ancistrocladus* species,<sup>1,2,12,13</sup> *A. congolensis* J. Léonard, *A. likoko* J. Léonard, *A. ealaensis* J. Léonard, and *A. ileboensis* Heubl, Mudogo & G. Bringmann. The latter species was discovered in the Southern Congo Basin, and described for the first time in 2010,<sup>12</sup> whereas the three other Congolese taxa were botanically acknowledged for the first time in 1949.<sup>13</sup> These three species are widely distributed in the North-Western and North-Central region of the Democratic Republic of the Congo.

It is all the more remarkable that phytochemical investigations on Central African *Ancistrocladus* plants have picked up speed only quite recently, during the past decade,<sup>4–11,14–21</sup> considering that intense isolation work on Asian as well as West and East African taxa had started much earlier, leading to the discovery of structurally unique mono- and dimeric naphthylisoquinoline alkaloids with promising antiparasitic and antiviral activities.<sup>22–27</sup> More recent phytochemical studies on *Ancistrocladus* lianas from the Congo region, including all of the four valid taxa<sup>14–21</sup> mentioned above, but also botanically as yet

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unidentified plants,<sup>4–11</sup> furnished a whole series of further novel-type naphthylisoquinoline alkaloids, among them *N,C*-coupled compounds<sup>9,10</sup> and, in particular, dimeric representatives with complex molecular architectures.<sup>4–8,14–16,18,21</sup> Some of these secondary metabolites attracted attention due to their extraordinary antiparasmodial<sup>6,8,15,16</sup> or antileishmanial<sup>10</sup> activities or, according to their individual structures, to their strong antiproliferative effects against human leukemia,<sup>16,18,20</sup> cervical HeLa,<sup>7,21</sup> or pancreatic cancer cells.<sup>7,9,17,19,21</sup>

Structurally most fascinating are the highly unsymmetric mbandakamine-type dimers like *e.g.*, mbandakamines A (**1**)<sup>4,16</sup> and B<sub>2</sub> (**2**)<sup>6</sup> (Fig. 1), which were discovered in a botanically as yet undescribed liana growing in the rainforests near the town of Mbandaka in the Northwestern part of the Democratic Republic of the Congo. The dimers **1** and **2** consist of two constitutionally and stereochemically nearly identical 5,8'-coupled monomeric halves, which are linked *via* the sterically highly crowded 6',1''-positions of their naphthalene units. The central axis of **1** and **2** is thus located in one of the *peri*-positions of the 'northwestern' naphthalene portion, neighboring one of the outer axes in the adjacent *peri*-position, which gives rise to a rotationally strongly hindered central binaphthalene linkage.<sup>4,6,16</sup> With the two outer axes being chiral, too, **1** and **2** possess three consecutive stereogenic axes, and, thus, together with four stereogenic centers in their two tetrahydroisoquinoline subunits, a total of seven elements of chirality.<sup>4,6,16</sup> The two mbandakamines exerted promising antiparasmodial and antitypanosomal activities *in vitro*,<sup>4,6</sup> and mbandakamine A (**1**) was also found to display strong cytotoxic activities against drug-sensitive (CCRF-CEM) and multidrug-resistant (CEM/ADR5000) human leukemia cells.<sup>16</sup>

Here, we describe the isolation and structural elucidation of two new mbandakamine-type dimers, mbandakamines B<sub>3</sub> (**3**)

and B<sub>4</sub> (**4**) (Fig. 1), from the leaves of an as yet unidentified Congolese *Ancistrocladus* liana. These two metabolites were found to co-occur in the plant with the mbandakamines A (**1**) and B<sub>2</sub> (**2**). Such mbandakamine-type dimers have so far been known to be present only in two *Ancistrocladus* species, *viz.*, in a botanically yet undescribed liana (three representatives)<sup>4,6</sup> and in *A. ealaensis* (four examples).<sup>16</sup> Both plants grow in the Mbandaka region.

Furthermore, we report on the identification of four new monomeric 5,8'-coupled naphthyltetrahydroisoquinolines, named ikelacongolines A–D (**5a**, **5b**, **6**, and **7**) (Fig. 1), in the leaves of this undescribed Central Congolese *Ancistrocladus* liana, along with three known and likewise 5,8'-linked alkaloids previously detected in the two Central African species *A. congolensis* and *A. likoko*, ancistrocongoline C (**8**)<sup>28</sup> and the ancistrollokines C (**9**)<sup>29</sup> and F<sub>2</sub> (**10**)<sup>17</sup> (Fig. 2). In addition, two naphthylisoquinoline alkaloids with a rare 7,8'-linkage, the ealamines A (**11**) and G (**12**)<sup>30</sup> (Fig. 2), which had been isolated for the first time from the twigs and leaves of *A. ealaensis* only recently, have now been found to be part of the metabolite pattern of the here investigated liana.

As part of our ongoing studies on the antiparasitic potential of naphthylisoquinoline alkaloids, the mbandakamines B<sub>3</sub> (**3**) and B<sub>4</sub> (**4**) and the ikelacongolines A–C (**5a**, **5b**, and **6**) were tested against the pathogens causing *malaria tropica*, leishmaniasis, Chagas' disease, and African sleeping sickness.

## Results and discussion

### Isolation and structural elucidation of mono- and dimeric naphthylisoquinoline alkaloids

HPLC-UV-MS-guided analysis of leaf samples of a botanically yet undescribed *Ancistrocladus* liana from the Central Congo

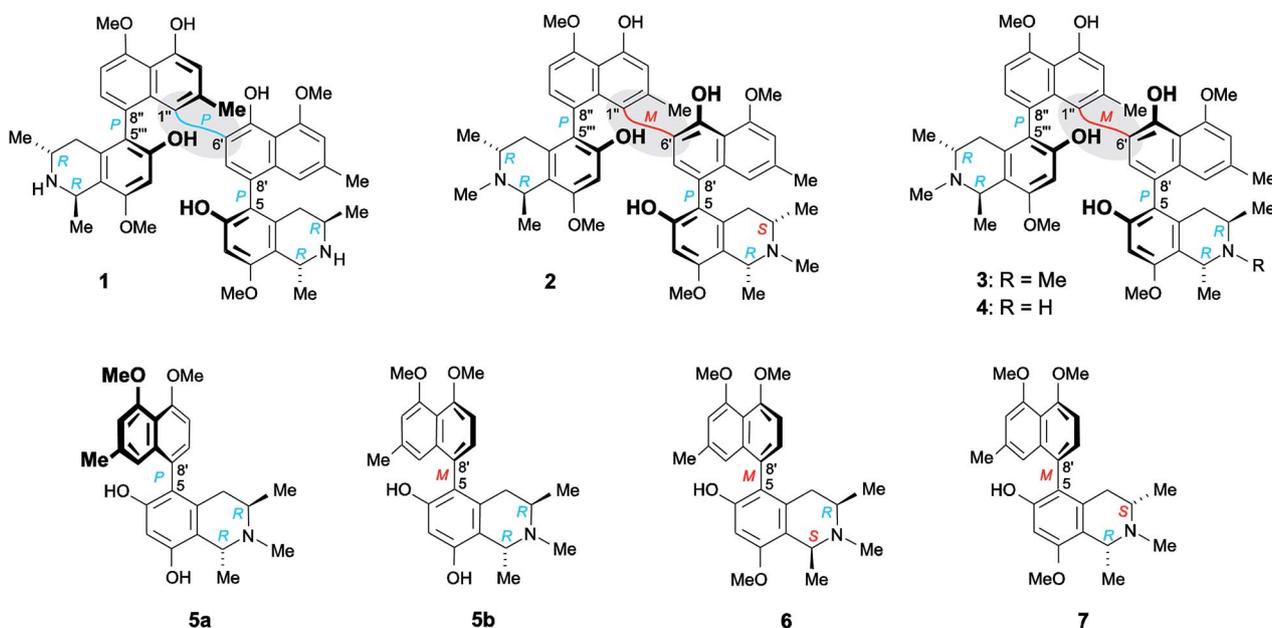


Fig. 1 Mbandakamine-type dimers and related 5,8'-coupled monomeric naphthylisoquinolines from the leaves of an unexplored Congolese *Ancistrocladus* liana: the two previously known<sup>4,6</sup> mbandakamines A (**1**) and B<sub>2</sub> (**2**) and the two new mbandakamines B<sub>3</sub> (**3**) and B<sub>4</sub> (**4**), which were isolated along with four new alkaloids, ikelacongolines A–D (**5a**, **5b**, **6**, and **7**).



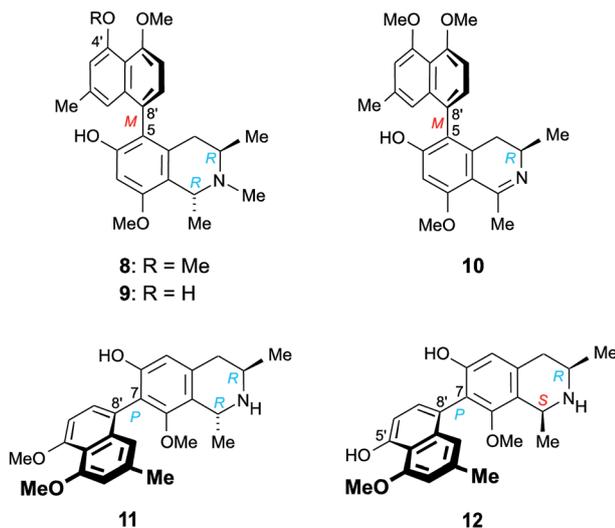


Fig. 2 Five known<sup>17,28–30</sup> 5,8'- and 7,8'-coupled naphthylisoquinoline alkaloids isolated from the as yet unidentified Congolese *Ancistrocladus liana*: ancistrocongoline C (8), ancistrolidikines C (9) and F<sub>2</sub> (10), and ealamines A (11) and G (12).

Basin, revealed the presence of secondary metabolites with UV and MS profiles typical of mono- and dimeric naphthylisoquinoline alkaloids. For the isolation of these compounds, ground material of air-dried leaves was exhaustively extracted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (1 : 1, v/v). The crude extract was suspended in H<sub>2</sub>O, and, after decantation and filtration, the solid was dissolved in MeOH. Fractionation and purification of the organic layer by reversed-phase HPLC provided six new naphthylisoquinoline alkaloids, along with seven known ones,<sup>4,6,16,17,28–30</sup> which had already been isolated from other Congolese *Ancistrocladus* species before.

One of the monomeric compounds was readily identified as the 5,8'-linked ancistrocongoline C (8) (Fig. 2). This alkaloid had so far been known only from the Central African species *A. congolensis*.<sup>28</sup> It is the 4'-O-methyl analog of the co-occurring alkaloid ancistrolidikine C (9), previously detected as a main constituent in the roots and twigs of *A. likoko*.<sup>17,29</sup> These two *N*-methylated naphthyltetrahydroisoquinoline alkaloids have now been isolated along with ancistrolidikine F<sub>2</sub> (10), which had been found for the first time in the twigs of *A. likoko* as one further example of the very small group of 5,8'-coupled naphthylidihydroisoquinolines with *R*-configuration at C-3.<sup>18</sup>

In addition, two known<sup>30</sup> 7,8'-linked alkaloids were detected in that unexplored Central African *Ancistrocladus liana*, viz., ealamines A (11) and G (12) (Fig. 2). They had previously been discovered in the leaves and twigs of the Congolese liana *A. ealaensis*.<sup>30</sup> 7,8'-Coupled compounds with *R*-configuration at C-3 have been detected in *Ancistrocladus* plants only rarely.<sup>15,30–32</sup> Up to now, such an entity has been found in a total of just 13 (out of over 250) naphthylisoquinoline alkaloids, among them four dimers. Likewise noteworthy is the fact that these metabolites have so far been identified only in two species, namely in *A. korupensis* from Cameroon (three compounds)<sup>31,32</sup> and in *A. ealaensis* (ten representatives).<sup>15,30</sup>

With their *R*-configuration at C-3 and an oxygen function at C-6, these five monomeric alkaloids 8–12 are typical 'hybrid-type', i.e., mixed Ancistrocladaceae/Dioncophyllaceae-type naphthylisoquinoline alkaloids.<sup>24</sup> This finding is of special interest from a geo- and chemotaxonomic point of view. The metabolite profiles of most of the *Ancistrocladus* plants from the Northwestern Congo Basin are likewise dominated by the presence of such hybrid-type alkaloids.<sup>4–7,14–18,28–30</sup> Asian and East African Ancistrocladaceae plants, by contrast, exclusively produce 3*S*-configured and 6-oxygenated compounds (known as 'Ancistrocladaceae-type' alkaloids),<sup>22–25</sup> whereas the only other plant family that likewise contains naphthylisoquinolines, the West African Dioncophyllaceae, solely forms alkaloids with *R*-configuration at C-3, always lacking an oxygen function at C-6 (hence categorized as 'Dioncophyllaceae-type' alkaloids).<sup>24,25</sup> Remarkably, from some of the species endemic to the Southern, Central, and Eastern Congo, metabolites exclusively belonging to the subclass of Ancistrocladaceae-type naphthylisoquinoline alkaloids were isolated,<sup>9–11</sup> whereas other species from these regions mainly produce Dioncophyllaceae-type compounds, along with a certain number of Ancistrocladaceae- and hybrid-type alkaloids.<sup>8,20,21</sup>

Isolation work on the leaf extract of this phytochemically unexplored Central Congolese *Ancistrocladus liana* also furnished two known,<sup>4,6,16</sup> constitutionally highly unsymmetric dimers with a 6',1''-coupled central biaryl axis, the mbandakamines A (1) and B<sub>2</sub> (2) (Fig. 1). These structurally unusual quateraryls had already been identified as constituents of the above-mentioned as yet undescribed Congolese *Ancistrocladus liana* from the rainforests in the Mbandaka region in the Northwestern Congo Basin.<sup>4,6</sup> Furthermore, only one further species, *A. ealaensis*, was known to likewise produce mbandakamine-type dimers, among them being mbandakamine A (1).<sup>16</sup> This finding further supports the close chemotaxonomic relationship of that as yet unidentified *Ancistrocladus* plant from the Ikela region in Central Africa to other Congolese *Ancistrocladus* species, in particular to those indigenous to the Northwestern part of the Democratic Republic of the Congo.

Furthermore, six new mono- and dimeric naphthylisoquinoline alkaloids were discovered in the leaves of that Central Congolese *Ancistrocladus liana*.

The molecular formula of the first new alkaloid, obtained as a pale-yellow amorphous solid, was C<sub>50</sub>H<sub>56</sub>N<sub>2</sub>O<sub>8</sub>, as deduced from HRESIMS. It was, thus, identical to that of the co-occurring dimer mbandakamine B<sub>2</sub> (2). The <sup>1</sup>H and <sup>13</sup>C NMR data (Table 1) of the isolated compound were almost identical to those of 2,<sup>6</sup> except for some slight shift differences, again showing a full set of signals, which indicated that this new dimer was – similar to 2 – unsymmetric. The <sup>1</sup>H NMR spectrum revealed the presence of eight aromatic protons, two methylene groups with diastereotopic protons, six methyl groups, two *N*-methyl functions, and four aromatic *O*-methyl groups. The 'southeastern' half of the new dimer displayed chemical shifts typical of an *N*-methylated naphthyltetrahydroisoquinoline with a 5,8'-biaryl linkage. It was established to be coupled *via* C-6' ( $\delta_C$  136.2) in the naphthalene subunit with a second, 'northwestern' part, which



Table 1  $^1\text{H}$  and  $^{13}\text{C}$  NMR data of mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4) in methanol-*d*<sub>4</sub> (150 MHz,  $\delta$  in ppm)

No.	3		4	
	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$	$\delta_{\text{H}}$ (J in Hz)	$\delta_{\text{C}}$
1/1'''	4.68, q (6.6)/4.55, q (6.8)	58.9/58.1	4.70, q (6.7)/4.51, q (6.7)	49.0/59.0
3/3'''	3.95, m/3.67, m	50.1/50.0	3.65, m/3.61, m	44.7/50.1
4 <sub>ax</sub> /4 <sub>ax</sub> '''	1.74, dd (18.2, 11.2)/1.84, dd (18.2, 11.8)	32.2/29.3	1.75, dd (17.9, 11.4)/1.86, dd (17.9, 5.1)	33.4/29.3
4 <sub>eq</sub> /4 <sub>eq</sub> '''	2.77, dd (18.3, 4.5)/1.84, dd (18.2, 5.1)		2.84, dd (17.8, 4.7)/1.86, dd (17.9, 11.5)	
5/5'''		119.5/123.0		119.7/122.9
6/6'''		156.3/155.7		156.7/155.7
7/7'''	6.65, s/5.64, s	97.9/98.3	6.66, s/5.63, s	98.0/98.3
8/8'''		158.0/157.0		157.8/157.1
9/9'''		115.6/112.4		114.8/112.3
10/10'''		134.0/130.8		134.0/130.6
1'/1''	6.56, s/—	118.7/126.5	6.54, s/—	118.8/126.5
2'/2''		137.0/139.5		136.9/139.4
3'/3''	6.81, s/6.87, s	108.1/114.8	6.81, s/6.88, s	108.1/114.8
4'/4''		158.0/155.6		158.2/155.6
5'/5''		152.6/158.2		152.6/158.2
6'/6''	—/7.01, d (8.1)	136.2/104.8	—/7.02, d (8.0)	136.2/104.9
7'/7''	6.83, s/6.97, d (8.0)	132.8/132.8	6.83, s/6.97, d (8.0)	132.7/132.8
8'/8''		126.5/126.5		126.5/127.3
9'/9''		137.0/137.9		136.9/137.9
10'/10''		115.6/115.9		115.6/115.9
1-CH <sub>3</sub> /1'''-CH <sub>3</sub>	1.49, d (6.5)/1.42, d (6.7)	17.3/18.9	1.56, d (6.7)/1.42 (6.6)	18.7/18.9
3-CH <sub>3</sub> /3'''-CH <sub>3</sub>	1.08, d (6.4)/1.08, d (6.4)	17.5/16.9	1.14, d (6.4)/1.08 (6.5)	19.2/16.9
N-CH <sub>3</sub> /N'''-CH <sub>3</sub>	2.54, s/2.41, s	35.7/34.7	—/2.42, s	—/34.6
8-OCH <sub>3</sub> /8'''-OCH <sub>3</sub>	3.93, s/3.42, s	56.1/55.7	3.94, s/3.41, s	56.2/55.7
2'-CH <sub>3</sub> /2''-CH <sub>3</sub>	2.30, s/2.06, s	22.1/22.7	2.29, s/2.07, s	22.1/22.7
4'-OCH <sub>3</sub>	4.06, s	57.0	4.06, s	57.0
5''-OCH <sub>3</sub>	4.16, s	56.9	4.16, s	56.9

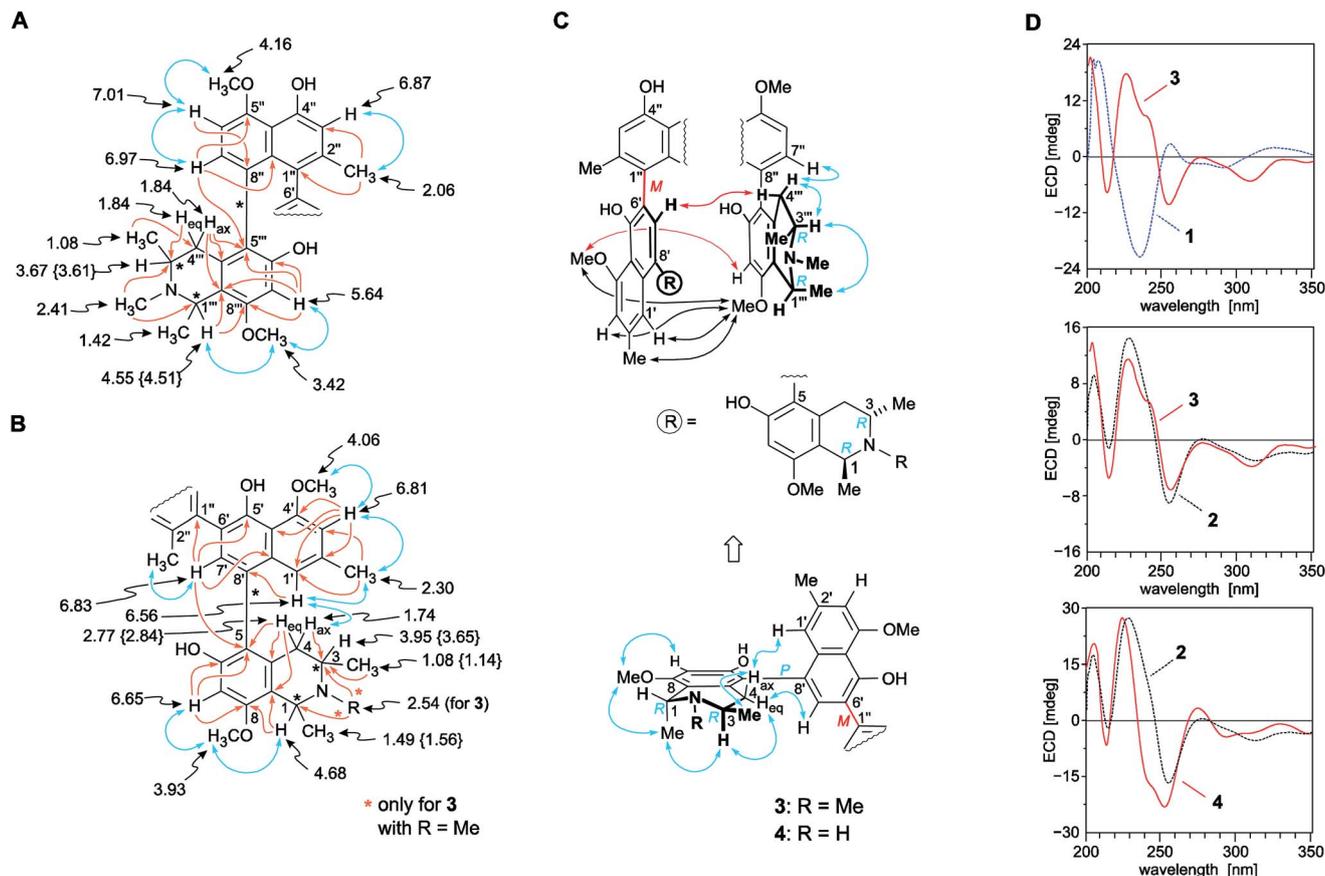
likewise consisted of a naphthyl-1,3-dimethyl-*N*-methyltetrahydroisoquinoline of the 5,8'-coupling type.

The first, 'southeastern' molecular portion (Fig. 3B) was found to possess four aromatic protons, with two singlets ( $\delta_{\text{H}}$  6.65 and 6.83) and a two-proton spin system in the naphthalene part exhibiting a *meta*-coupling pattern ( $\delta_{\text{H}}$  6.56 and 6.81). In the aliphatic region, it exhibited a quartet [H-1, ( $\delta_{\text{H}}$  4.68)], a multiplet [H-3, ( $\delta_{\text{H}}$  3.95)], two diastereotopic protons, H-4<sub>ax</sub> ( $\delta_{\text{H}}$  1.74) and H-4<sub>eq</sub> ( $\delta_{\text{H}}$  2.77), and two *O*-methyl groups ( $\delta_{\text{H}}$  3.93 and 4.06). In that part of the  $^1\text{H}$  NMR spectrum, also two three-proton doublets were monitored, typical of Me-1 ( $\delta_{\text{H}}$  1.49) and Me-3 ( $\delta_{\text{H}}$  1.08), and two three-proton singlets, one corresponding to an aryl-methyl group {Me-2', ( $\delta_{\text{H}}$  2.30)} in the naphthalene part, and the other one evidencing the presence of an *N*-methyl group ( $\delta_{\text{H}}$  2.54) in the isoquinoline subunit (Table 1). This finding was confirmed by HMBC interactions of the methyl protons with both, C-1 and C-3 (Fig. 3B). The location of the biaryl linkage at C-8' was established from an HMBC cross peak between H-1' ( $\delta_{\text{H}}$  6.56) and the quaternary carbon atom C-8' ( $\delta_{\text{C}}$  126.5). The attribution of H-1' was, in turn, corroborated by a NOESY interaction with H-4<sub>ax</sub> and by the NOESY correlation sequence {H-1'  $\leftrightarrow$  Me-2'  $\leftrightarrow$  H-3'  $\leftrightarrow$  OMe-4'}. In the tetrahydroisoquinoline portion, the coupling position was deduced to be C-5 ( $\delta_{\text{C}}$  119.5), as obvious from HMBC interactions from H-7 ( $\delta_{\text{H}}$  6.65), H-7' ( $\delta_{\text{H}}$  6.83), and H-4<sub>eq</sub> to C-5. (The two methoxy

groups at  $\delta_{\text{H}}$  3.93 and 4.06 showed NOESY interactions with H-7 and H-3' ( $\delta_{\text{H}}$  6.81), respectively, thus being located at C-8 in the isoquinoline subunit and at C-4' in the naphthalene part. Consequently, the remaining two oxygen functions had to be free hydroxy groups at C-6 and C-5' (Fig. 3B).

The  $^1\text{H}$  NMR spectrum (Table 1) of the northwestern molecular portion again displayed the typical chemical shifts of a 5,8'-coupled *N*-methylated naphthyltetrahydroisoquinoline with a methoxy group ( $\delta_{\text{H}}$  3.42) at C-8''' (Fig. 3A). This was confirmed by NOESY correlations in the series {H-7'''  $\leftrightarrow$  OMe-8'''  $\leftrightarrow$  Me-1'''  $\leftrightarrow$  H-3'''}, and by HMBC interactions from H-7''' ( $\delta_{\text{H}}$  6.97), H<sub>eq</sub>-4''' ( $\delta_{\text{H}}$  1.84), and H-7''' ( $\delta_{\text{H}}$  5.64) to C-5''' ( $\delta_{\text{C}}$  123.0), evidencing the coupling position of the biaryl axis in the isoquinoline subunit to be located at C-5'''. In contrast to the naphthalene part of the southeastern half, the signal pattern in the  $^1\text{H}$  NMR spectrum showed an *ortho* coupling of the two aromatic protons H-7'' and H-6'' ( $\delta_{\text{H}}$  7.01), no *meta*-coupled doublets next to the high-field shielded methyl group ( $\delta_{\text{H}}$  2.06) at C-2'', but only a singlet at H-3'' ( $\delta_{\text{H}}$  6.87), thus suggesting C-1'' and C-8'' to be axis-bearing carbon atoms. ROESY correlations between H-6'' and MeO-5'' ( $\delta_{\text{H}}$  4.16) revealed the naphthalene unit of the northwestern naphthylisoquinoline to possess a methoxy function at C-5'. HMBC interactions from H-7'' to C-5'' ( $\delta_{\text{C}}$  158.2) and C-9'' ( $\delta_{\text{C}}$  137.9) and from H-6'' to C-8'' established C-8'' to be quaternary and, thus, proving that the





**Fig. 3** Selected  $^1\text{H}$  NMR data (in methanol- $d_4$ ,  $\delta$  in ppm), HMBC (single orange arrows) and NOE (double blue arrows) interactions indicative of the constitutions of the (A) 'northwestern' and (B) 'southeastern' naphthylisoquinoline portions of the mbandakamines **B**<sub>3</sub> (**3**) and **B**<sub>4</sub> (**4**): the values of **4** that are different from those of **3** are given in {}. All other NMR data of **3** and **4** are nearly identical ( $\pm 0.02$  ppm); (C) selected ROESY correlations between the naphthalene subunit of the 'northwestern' half and the isoquinoline part of the 'southeastern' moiety establishing the 6',1''-coupling sites in the central binaphthalene core (black arrows) and the relative configuration at the centers and the two outer axes (blue arrows) and at the central biaryl axis (red arrows) of **3** and **4**; (D) confirmation of the absolute configuration of **3** and **4** at the central biaryl axis by comparison of their ECD spectra with those of the known<sup>4,6,16</sup> related mbandakamines **A** (**1**, central axis: *P*) and **B**<sub>2</sub> (**2**, central axis: *M*): in the case of **3** with the ECD curves of **1** (top) and **2** (center), and, in the case of **4**, with the ECD spectrum of **2** (bottom); for the structures of **1** and **2**, see Fig. 1.

isoquinoline and the naphthalene portions of the northwestern molecular half were coupled through C-5''' and C-8''' (Fig. 3A). The proximity of a second aryl substituent to the methyl group ( $\delta_{\text{H}}$  2.06) of the naphthalene subunit in the northwestern part of the dimer became evident from the strongly upfield-shifted signal of Me-2'', which revealed the central biaryl axis to connect the two molecular halves of the isolated alkaloid by a 6',1''-coupling of the two naphthalene subunits. This assignment was further proven by  $^3J$  HMBC interactions from H-3'', Me-2'', and H-7' to C-1'' (Fig. 3A and B), and from ROESY interactions between H-1', Me-2', H-3', and MeO-4' in the naphthalene unit of the northwestern naphthylisoquinoline and OMe-8''' in the isoquinoline portion of the southeastern half of the dimer (Fig. 3C). Thus, the central axis was located at C-1'', *i.e.*, in the *peri*-position neighboring one of the outer axes (*i.e.*, C-8'''), which led to an extremely high steric hindrance around that 6',1''-linked central biaryl axis.

ROESY cross peaks of the protons of Me-1 ( $\delta_{\text{H}}$  1.49) to H-3 ( $\delta_{\text{H}}$  3.95), and of Me-1''' ( $\delta_{\text{H}}$  1.42) to H-3''' ( $\delta_{\text{H}}$  3.67) assigned the

relative configuration of the methyl group at C-1 *versus* the one at C-3, and that at C-1''' *versus* the one at C-3''', to be *trans* to each other in both cases (Fig. 3C). The absolute configurations at C-3 and C-3''' in the two tetrahydroisoquinoline portions were established by ruthenium-mediated oxidative degradation with subsequent GC-MSD analysis of the resulting chiral products according to a procedure developed by our group earlier.<sup>33</sup> In the case of the isolated alkaloid dimer, this analytical method delivered only the *R*-enantiomers of 3-aminobutyric acid and of *N*-methyl-3-aminobutyric acid (both derived from C-3), thus, together with the relative *trans*-configuration assigned above, the absolute configurations at C-3, C-3''', C-1, and C-1''' were attributed to be all *R*. The configurations at the two outer axes in the monomeric halves of the new dimer relative to the stereocenters became evident from specific long-range ROESY interactions between H-1' and H-4<sub>ax</sub> and between H-7' and H-4<sub>eq</sub> in the southeastern portion and between H-7''' and H-4'''<sub>eq</sub> in the northwestern moiety (Fig. 3C). This, in combination with the absolute configurations at C-1 and C-3 and at C-1''' and C-3''', as



again determined by the oxidative degradation, clearly showed that the naphthalene–isoquinoline linkages both had to be *P*-configured.

ROESY interactions were found across the central biaryl axis between H-7' and H<sub>ax</sub>-4''' and between OMe-4' and H-7''' (Fig. 3C), which were identical to those earlier observed for mbandakamine B<sub>2</sub> (2),<sup>6</sup> but complementary to those in mbandakamine A (1).<sup>4</sup> These interactions established the central biaryl axis of the new dimer to be *M*-configured.

This assignment was confirmed by the fact that the ECD spectrum of the new metabolite was virtually identical to that of the structurally closely related mbandakamine B<sub>2</sub> (2) (Fig. 3D, center), but nearly mirror-imaged to that of mbandakamine A (1) (Fig. 3D, top), which is *P*-configured at the central biaryl axis. Previous investigations<sup>4,8,16,21,34</sup> had already revealed that the chiroptical properties of such naphthylisoquinoline dimers are dominated by the orientation of the two strong naphthalene chromophores to each other, and, thus, by the configuration at the central biaryl linkage, although – as in the case of 1 and the new dimer – the stereocenters at all the other six stereogenic elements (two outer axes and four centers) were identical. Consequently, the new mbandakamine-type dimer isolated from the 'new' Central Congolese *Ancistrocladus* species had to possess the full stereostructure 3, and – as displayed in Fig. 1 – to be 1*R*,3*R*,5*P*,6'*M*,5'''*P*,3'''*R*,1'''*R*-configured. It was, hence, the 3-*epi*-analog of the co-occurring mbandakamine B<sub>2</sub> (2). According to its close structural relationship to 2, the new dimer 3 was given the name mbandakamine B<sub>3</sub>.

Resolution of a further alkaloid-enriched subfraction of the leaf extract provided a second new dimeric naphthylisoquinoline alkaloid. In <sup>1</sup>H and <sup>13</sup>C NMR analysis the compound showed full sets of signals (Table 1) similar to those of 3, again hinting at the presence of a mbandakamine-type dimer consisting of two 5,8'-coupled naphthyltetrahydroisoquinoline halves connected to each other *via* C-6' and C-1''. According to HRESIMS, the new compound possessed a molecular formula of C<sub>49</sub>H<sub>54</sub>N<sub>2</sub>O<sub>8</sub>, suggesting the presence of an *O*- or *N*-demethylated derivative of 3. The 1D and 2D NMR data clearly revealed the missing methyl group to be the one attached to the nitrogen atom in the southeastern portion of mbandakamine B<sub>3</sub> (3) (Table 1 and Fig. 3B), which was confirmed by the upfield shifts of C-1 (δ<sub>C</sub> 49.0) and C-3 (δ<sub>H</sub> 44.7) compared to those in 3 (δ<sub>C</sub> 58.9 and 50.1, respectively).

By the above-employed methods – the oxidative degradation, NMR investigations showing specific NOESY and ROESY interactions across the two outer biaryl axes and between the isoquinoline subunit of the southeastern molecular half and the naphthalene portion of the northwestern molecular moiety (Fig. 3B), and ECD spectroscopy (Fig. 3C, bottom) – the new dimer was assigned to be *R*-configured at all four stereogenic centers, *P*-configured at the two outer axes, and *M* at the central biaryl linkage, similar to mbandakamine B<sub>3</sub> (3). Thus, this second new dimer had the absolute stereostructure 4 as shown in Fig. 1. It was henceforth named mbandakamine B<sub>4</sub>.

In addition to these two mbandakamine-type dimers 3 and 4, four new monomeric 5,8'-coupled naphthylisoquinolines were isolated from the leaf extract. According to the <sup>1</sup>H and <sup>13</sup>C NMR

data (Tables 2 and 3), these alkaloids showed a great constitutional similarity, having in common a 4',5'-dimethoxynaphthalene portion and an *N*-methyl function in their isoquinoline halves, but clearly differing by the OH/OMe substitution patterns of their tetrahydroisoquinoline subunits, and by their elements of central and axial chirality.

The first new compound within this series of monomeric alkaloids was obtained as a yellow amorphous solid. It corresponded to a molecular formula of C<sub>25</sub>H<sub>29</sub>NO<sub>4</sub>, as deduced from HRESIMS. Given a spin system of five aromatic protons, with three singlets (δ<sub>H</sub> 6.50, 6.65, and 6.78) and two doublets due to the presence of a pair of adjacent protons (δ<sub>H</sub> 6.95 and 7.18) in the naphthalene part (Fig. 4A), this substitution pattern hinted either at a 6'- or an 8'-coupling site of the biaryl axis. HMBC interactions from H-1' (δ<sub>H</sub> 6.65) and H-6' (δ<sub>H</sub> 6.95) to the quaternary carbon atom C-8' (δ<sub>C</sub> 126.6) in combination with NOESY correlations in the series {H-1' ↔ Me-2' ↔ H-3' ↔ OMe-4'} and a cross peak between H-6' and H-7' (δ<sub>H</sub> 7.18) evidenced the tetrahydroisoquinoline moiety to be located at C-8' (Fig. 4A). The high-field shifted signals of the diastereotopic protons at C-4 (δ<sub>H</sub> 2.03 and 2.61) suggested the naphthalene half to be located at C-5 of the isoquinoline part. This assignment was confirmed by HMBC interactions of H-7', H-7 (δ<sub>H</sub> 6.50), and H<sub>eq</sub>-4 (δ<sub>H</sub> 2.61) to C-5 (δ<sub>C</sub> 119.7) (Fig. 4A). The <sup>1</sup>H NMR spectrum showed the chemical shifts of two methoxy functions (δ 3.93 and 3.95), which were attributed to be located at C-4' and C-5' in the naphthalene subunit due to NOESY interactions to H-3' and H-6', respectively. The two other oxygen functions thus had to be free phenolic hydroxy groups at C-6 and C-8 in the isoquinoline part. From the <sup>1</sup>H NMR signal at δ<sub>H</sub> 2.72, corresponding to three protons, and from the HMBC interactions of this methyl group with both C-1 and C-3, it was evident that the new compound was *N*-methylated.

From a NOESY correlation between H-3 (δ<sub>H</sub> 3.96) and Me-1 (δ<sub>H</sub> 1.72) (Fig. 4B, top), a relative *trans*-configuration of the two stereocenters at C-1 and C-3 was established. The absolute configurations, determined by ruthenium-mediated oxidative degradation,<sup>33</sup> were found to be *R* at both stereocenters. The relative and, thus, absolute axial configuration was deduced to be *P*, according to NOESY interactions between H-4<sub>ax</sub> (δ<sub>H</sub> 2.03) and H-1' on the one hand, and between H-4<sub>eq</sub> (δ<sub>H</sub> 2.61) and H-7' on the other (Fig. 4B). The results were in agreement with the ECD spectrum of the new metabolite, which was opposite to that of the known<sup>28</sup> (and co-occurring) 5,8'-coupled, but *M*-configured alkaloid ancistrocongoline C (8) (Fig. 4C, left). Thus, the new compound possessed the stereostructure 5a as presented in Fig. 1. It was named ikelacongoline A, after the Congolese town of Ikela, where the plant material had been collected. This alkaloid may also be referred to as the *N*-methylated analog of korupensamine C from the Cameroonian liana *A. korupensis*,<sup>26</sup> or as the 5'-*O*-methyl derivative of ancistrocongoline A,<sup>17,28</sup> a main metabolite of both of the two Congolese species *A. congolensis* and *A. likoko*.

The second new monomeric alkaloid had the same molecular formula of C<sub>25</sub>H<sub>29</sub>NO<sub>4</sub> as ikelacongoline A (5a), which was evidenced by HRESIMS measurements. It was again a 5,8'-linked *N*-methylated naphthyltetrahydroisoquinoline alkaloid,



Table 2 <sup>1</sup>H NMR data of ikelacongolines A (5a), B (5b), C (6), and D (7) in methanol-*d*<sub>4</sub> (600 MHz,  $\delta$  in ppm, J in Hz)

No.	5a	5b	6	7
1	4.72, q (6.7)	4.72, q (6.7)	4.65, q (6.6)	4.64, q (6.7)
3	3.96, m	3.92, m	3.15, m	3.23, m
4 <sub>ax</sub>	2.03, dd (18.6, 11.9)	2.49, dd (18.6, 12.2)	2.58, dd (17.3, 11.5)	2.23, dd (17.3, 11.0)
4 <sub>eq</sub>	2.61, dd (18.5, 4.9)	2.20, dd (18.8, 5.0)	2.21, dd (17.3, 2.9)	2.45, dd (17.3, 3.4)
7	6.50, s	6.48, s	6.60, s	6.62, s
1'	6.65, s	6.77, s	6.69, s	6.73, s
3'	6.78, s	6.79, s	6.80, s	6.79, s
6'	6.95, d (8.0)	6.93, d (8.0)	6.94, d (8.0)	6.94, d (8.0)
7'	7.18, d (7.9)	7.15, d (7.9)	7.16, d (7.9)	7.17, d (8.0)
1-CH <sub>3</sub>	1.72, d (6.7)	1.74, d (7.9)	1.73, d (6.6)	1.75, d (6.6)
3-CH <sub>3</sub>	1.24, d (6.6)	1.24, d (6.6)	1.27, d (6.5)	1.28, d (6.5)
N-CH <sub>3</sub>	2.72, s	2.77, s	3.02, s	3.01, s
8-OCH <sub>3</sub>			3.92, s	3.92, s
2'-CH <sub>3</sub>	2.30, s	2.32, s	2.31, s	2.32, s
4'-OCH <sub>3</sub>	3.93, s	3.93, s	3.93, s	3.93, s
5'-OCH <sub>3</sub>	3.95, s	3.95, s	3.96, s	3.96, s

Table 3 <sup>13</sup>C NMR data of ikelacongolines A (5a), B (5b), C (6), and D (7) in methanol-*d*<sub>4</sub> (150 MHz,  $\delta$  in ppm)

No.	5a	5b	6	7
1	60.0	60.1	62.1	61.7
3	50.8	50.8	60.3	59.9
4	29.4	29.9	34.0	33.1
5	119.7	119.6	119.9	120.0
6	157.2	157.2	157.5	157.2
7	102.6	102.6	99.0	99.3
8	156.4	156.4	157.2	157.5
9	110.9	111.0	114.3	114.0
10	132.2	132.1	134.7	134.7
1'	118.0	118.4	118.3	118.3
2'	138.2	138.0	137.9	137.9
3'	110.0	110.1	109.9	109.8
4'	159.0	158.8	158.7	158.8
5'	158.4	158.4	158.4	158.4
6'	107.0	106.9	106.6	106.6
7'	130.4	130.8	130.9	130.4
8'	126.6	126.7	126.1	126.0
9'	138.0	137.8	137.5	138.1
10'	117.8	117.8	117.4	117.6
1-CH <sub>3</sub>	19.1	19.3	19.8	20.0
3-CH <sub>3</sub>	16.7	16.9	17.9	17.9
N-CH <sub>3</sub>	34.2	34.2	41.4	41.4
2'-CH <sub>3</sub>	22.2	22.2	22.0	22.1
8-OCH <sub>3</sub>			56.1	56.1
4'-OCH <sub>3</sub>	57.1	57.1	56.9	56.9
5'-OCH <sub>3</sub>	56.9	56.9	56.7	56.7

with <sup>1</sup>H and <sup>13</sup>C NMR data almost identical to those of 5a, suggesting that the isolated compound possessed the same constitution as 5a. Significant differences in the NMR spectra of these two alkaloids were monitored only for the chemical shifts of the diastereotopic protons H<sub>ax</sub>-4 ( $\delta_{\text{H}}$  2.49) and H<sub>eq</sub>-4 ( $\delta_{\text{H}}$  2.20) (see Fig. 4A and Table 2) located next to the biaryl axis, thus hinting at the presence of two probably atropo-diastereomeric alkaloids in the leaves of this 'new' *Ancistrocladus* plant.

NOESY cross peaks of the protons of Me-1 ( $\delta_{\text{H}}$  1.74) to H-3 ( $\delta_{\text{H}}$  3.92) established the methyl groups at C-1 and C-3 to be *trans* to

each other (Fig. 4B, bottom). The oxidative degradation procedure again furnished only the *R*-enantiomers of 3-aminobutyric acid and *N*-methyl-3-aminobutyric acid. In conjunction with the relative *trans*-configuration, the absolute configuration of the tetrahydroisoquinoline subunit had to be 1*R*,3*R*, similar as in the case of 5a. Given the identical constitution and the same configurations at C-1 and C-3, 5a and the new metabolite had to differ only by an opposite axial configuration. Long-range NOESY correlations between H<sub>ax</sub>-4 and H-7' and between H-4<sub>eq</sub> and H-1' (Fig. 4B, bottom) clearly revealed the new compound to be *M*-configured at the biaryl linkage. This finding was confirmed by the fact that the ECD spectrum was virtually opposite to that of 5a, but nearly identical to that of the likewise *M*-configured and 5,8'-coupled ancistrocongoline C (8) (Fig. 4C, left). Consequently, the new alkaloid possessed the stereostructure 5b and was hence the atropo-diastereomer of 5a. It was named ikelacongoline B.

According to HRESIMS analysis, the third new alkaloid had a molecular formula of C<sub>26</sub>H<sub>31</sub>NO<sub>4</sub>, thus possessing 14 mass units more than 5a and 5b. Similar to the latter, the compound again displayed NMR signals (see Tables 2 and 3) typical of an *N*-methylated 5,8'-coupled naphthyltetrahydroisoquinoline with two methoxy groups in the naphthalene part ( $\delta_{\text{H}}$  3.93 and 3.96). Unlike 5a and 5b, this alkaloid possessed a third methoxy function ( $\delta_{\text{H}}$  3.92) at C-8 in the isoquinoline portion as deduced from NOESY interactions between H-7 ( $\delta_{\text{H}}$  6.60) and OMe-8.

From an NOE correlation between H-1 ( $\delta_{\text{H}}$  4.65) and H-3 ( $\delta_{\text{H}}$  3.15) (Fig. 5A), the relative configuration at C-1 *versus* C-3 was established to be *cis*. Ruthenium-mediated oxidative degradation yielded 3-aminobutyric acid and its *N*-methyl derivative as their *R*-enantiomers, thus clearly establishing C-3 to be *R*-configured. In view of the above-assigned *cis*-array of the two methyl groups at C-1 and C-3, the absolute configuration of the stereogenic center at C-1 was deduced to be *S*.

Based on the long-range NOESY interactions from H-4<sub>ax</sub> ( $\delta_{\text{H}}$  2.58) to H-7' ( $\delta_{\text{H}}$  7.16), and from H-1' ( $\delta_{\text{H}}$  6.69) to H-4<sub>eq</sub> ( $\delta_{\text{H}}$  2.21) and H-3, the axial configuration was determined to be *M* (Fig. 5A). The assignment was further corroborated by the fact



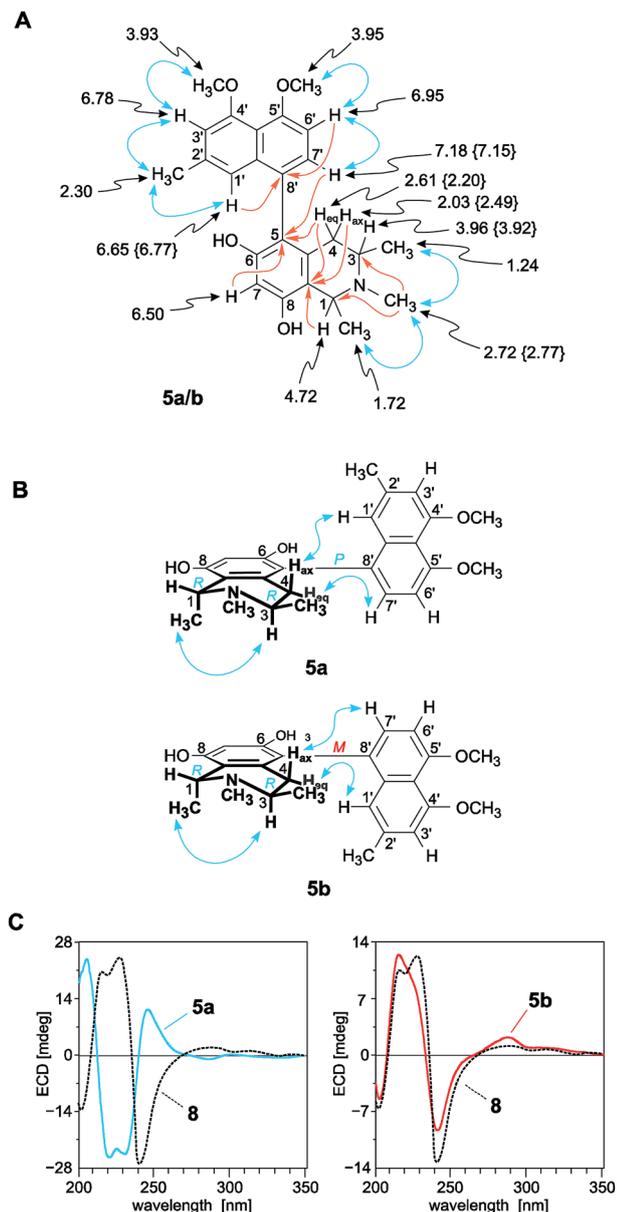


Fig. 4 (A) <sup>1</sup>H NMR data (in methanol-*d*<sub>4</sub>, δ in ppm), selected HMBC (single orange arrows) and NOE interactions (double blue arrows) of ikelacongolines A (5a) and B (5b): the values of 5b that are different from those of 5a are given in {}. All other values of 5a and 5b are nearly identical (±0.02 ppm); (B) NOESY correlations evidencing the relative configuration of 5a and 5b at the biaryl axis and at the stereogenic centers C-1 and C-3 in the tetrahydroisoquinoline subunit; (C) assignment of the absolute axial configuration of 5a and 5b, by comparison of their ECD spectra with that of the known,<sup>28</sup> co-occurring and structurally related alkaloid ancistrocongoline C (8); for the structure of 8, see Fig. 1.

that the ECD spectrum of the isolated alkaloid matched the ECD curves of the two related, likewise *M*-configured metabolites ikelacongoline B (5b) and ancistrocongoline C (8) nearly perfectly (see ESI<sup>†</sup>). This monomeric naphthylisoquinoline thus possessed the absolute stereostructure 6, as presented in Fig. 1, and, hence, was the new 1-*epi* analog of 8. It was named ikelacongoline C.

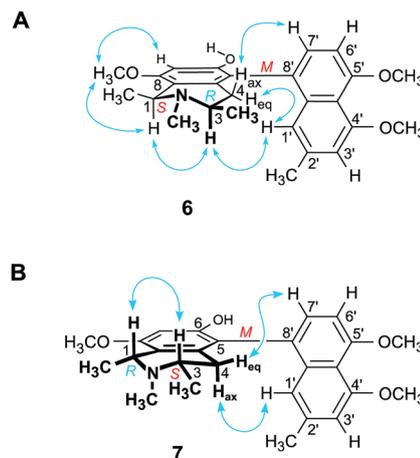


Fig. 5 NOESY interactions indicative of the configuration at the biaryl axis of (A) ikelacongoline C (6) and (B) ikelacongoline D (7) relative to the stereogenic centers in the tetrahydroisoquinoline subunit.

According to HRESIMS, the fourth isolated new metabolite had the same molecular formula of C<sub>26</sub>H<sub>31</sub>NO<sub>4</sub> as the above-investigated ikelacongoline C (6). Moreover, 1D and 2D NMR measurements hinted at a great structural similarity with 6, indicating that the two alkaloids differed only in the stereochemical orientation of the methyl groups at C-1 and C-3. The formation of *S*-*N*-methyl-3-aminobutyric acid by oxidative degradation proved the new alkaloid to be *S*-configured at C-3 (instead of *R* as assigned for 6). The relative configuration of C-1 versus C-3 was again deduced to be *cis*, due to a NOESY interaction of H-1 (δ<sub>H</sub> 4.64) with H-3 (δ<sub>H</sub> 3.23), which was axial, as obvious from the coupling constants of H<sub>ax</sub>-4 [δ<sub>H</sub> 2.23, (1H, *J* = 17.3, 11.0 Hz)] and H<sub>eq</sub>-4 [δ<sub>H</sub> 2.45, (1H, *J* = 17.3, 3.4 Hz)]. Thus, given the relative *cis*-configuration at C-1 and C-3, the absolute configuration at C-1 was determined to be *R*. On the basis of the resulting 1*R*,3*S*-stereoarray in the tetrahydroisoquinoline part, the absolute axial configuration was assigned to be *M* due to long-range NOE cross peaks between H-7' (δ<sub>H</sub> 7.17) in the naphthalene subunit and H<sub>eq</sub>-4 in the isoquinoline portion, and also between H-1' (δ<sub>H</sub> 6.73) and H<sub>ax</sub>-4 (Fig. 5B). Comparison of the ECD spectrum of the isolated alkaloid with that of 6, which was similar (see ESI<sup>†</sup>), further confirmed this new metabolite to possess the stereostructure 7, as shown in Fig. 1. The compound was named ikelacongoline D. With its constitution being identical to that of ancistroyafungine A,<sup>9</sup> an alkaloid isolated earlier from a botanically likewise as yet unidentified *Ancistrocladus* liana from the North-Central part of the Congo Basin, and with the same configuration at C-1 and C-3, but with *M*-configuration at the biaryl axis, ikelacongoline D might also be addressed as 5-*epi*-ancistroyafungine A.

The alkaloid pair 6/7 is most remarkable because their tetrahydroisoquinoline parts are enantiomeric to each other (1*S*,3*R* versus 1*R*,3*S*), while the axes are identically configured. The occurrence of such a pair of naphthylisoquinolines within one plant is as yet unprecedented.



### Antiprotozoal activities of the mbandakamines B<sub>3</sub> and B<sub>4</sub> and the ikelacongolines A–C

Parasitic diseases such as malaria, African sleeping sickness, Chagas' disease, and leishmaniasis are still a major threat for millions of people in tropical and developing countries and, as a consequence, represent a serious obstacle to the social-economic progress in affected regions of Asia and Africa. The dramatic increase of parasite resistance to routinely used chemotherapeutics and the absence of vaccines aggravate the situation dramatically.<sup>35–37</sup> For this reason, several research groups from medicinal chemistry, pharmacy, and infection biology focus on the search of new effective agents for the treatment of tropical infectious diseases. In the recent years, intense phytochemical studies on African plants have led to the discovery of terpenoids, alkaloids, flavonoids, quinones, lignans, coumarins, and many other natural products displaying excellent anti-protozoal activities,<sup>38–45</sup> thus providing templates and new scaffolds for the development of a new generation of more potent drugs. Most promising sources for such lead compounds are *Ancistrocladus* species from Central Africa. Some of the known<sup>4–6,8,10,11,15,16,20,21,25,28,29,46,47</sup> mono- and dimeric naphthylisoquinolines exhibited pronounced inhibitory activities against *Plasmodium*, *Trypanosoma*, and *Leishmania* parasites, thus suggesting to likewise investigate the alkaloids of this Central Congolese liana presented here for their biological potential.

The two newly discovered mbandakamine-type naphthylisoquinoline dimers **3** and **4** exhibited excellent activities *in vitro* against both the chloroquine-sensitive (NF54) and the chloroquine-resistant (K1) strain of the malaria parasite *Plasmodium falciparum*, with half-maximum inhibition concentration values (IC<sub>50</sub>) of 39 nM (NF54) and even 6 nM (K1) for **3**, and 66 nM (NF54) and 26 nM (K1) for **4** (Table 4). Thus, their antiplasmodial activities were comparable to those of the structurally closely related – and likewise strongly active – mbandakamine B<sub>2</sub> (**2**),<sup>6</sup> and were in the same order of magnitude as those of the standard agents chloroquine and artemisinin (Table 4). Noteworthy was in particular the pronounced

antiplasmodial activities of **2–4** especially against the chloroquine-resistant K1 strain, without showing any cross-resistance. The new mbandakamine B<sub>3</sub> (**3**) was found to be one of the most active naphthylisoquinolines against the chloroquine-resistant strain K1 ever investigated. In addition, given the comparatively low cytotoxicity of **3** (6.64 μM), this dimer possessed a high selectivity index of *ca.* 1110. These findings made the mbandakamines B<sub>3</sub> (**3**) and B<sub>4</sub> (**4**) promising candidates for further biological evaluations, also with respect to the fact that they displayed good to strong activities against the pathogen of African sleeping sickness, *Trypanosoma brucei rhodesiense*, with IC<sub>50</sub> values of 0.476 μM for **3** and 0.211 μM for **4** (Table 4). Their inhibitory potentials, however, were slightly weaker than that of mbandakamine B<sub>2</sub> (**2**), which showed a pronounced effect *in vitro* in the lower nanomolar range (IC<sub>50</sub> = 5.0 nM),<sup>6</sup> stronger than that of any other of the numerous alkaloids tested so far.<sup>4–8,10,15,16,20,21,25,46,47</sup>

The inhibitory effects of the new monomeric alkaloids ikelacongolines A (**5a**), B (**5b**), and C (**6**) against the strains NF54 and K1 of *P. falciparum* were drastically lower compared to those of the dimers **2–4**. For lack of material, ikelacongoline D (**7**) was not tested. The compounds **5a**, **5b**, and **6** displayed only moderate antiplasmodial activities, with micromolar IC<sub>50</sub> values ranging from 1.41 to 8.10 (Table 4), and thus being 115 to 480 times less effective than the mbandakamines **2–4**.

These test results clearly demonstrated that the dimeric architecture of the mbandakamines had a significant impact on the bioactivity of 5,8'-linked naphthylisoquinoline alkaloids. A similar effect had previously also been observed for jozimine-type naphthylisoquinoline dimers of natural<sup>8,21</sup> and synthetic<sup>48–50</sup> origin consisting of two 7,1'-coupled – far less active – monomeric halves.

Against *Leishmania donovani*, the causative agent of visceral leishmaniasis, only very weak or virtually no activities were recorded for any of the investigated naphthylisoquinolines, while for the pathogen of Chagas' disease, *Trypanosoma cruzi*, a low, but unspecific inhibitory effect (SI < 1) was observed for the mbandakamines **2–4** (Table 4).

**Table 4** Antiparasitic activities of the mbandakamines B<sub>3</sub> (**3**) and B<sub>4</sub> (**4**), and the ikelacongolines A (**5a**), B (**5b**), and C (**6**) against *Plasmodium falciparum* (K1 and NF54 strains), *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense*, and *Leishmania donovani*, and cytotoxicity against rat skeletal myoblast (L6) cells

Compound	IC <sub>50</sub> <sup>a</sup> [μM]						Selectivity index <sup>b</sup> (K1/NF54)
	<i>P. falciparum</i> (K1 strain)	<i>P. falciparum</i> (NF54 strain)	<i>T. cruzi</i>	<i>T. brucei</i> <i>rhodesiense</i>	<i>L. donovani</i>	L6 cell (cytotoxicity)	
Standard	0.293 <sup>c</sup> 0.010 <sup>d</sup>	0.009 <sup>c</sup> 0.021 <sup>d</sup>	6.109 <sup>e</sup>	0.030 <sup>f</sup>	1.077 <sup>g</sup>	0.0096 <sup>h</sup>	—/— <sup>b</sup>
<b>2</b> <sup>i</sup>	0.004 <sup>i</sup>	0.017 <sup>i</sup>	2.98 <sup>i</sup>	0.005 <sup>i</sup>	94.2 <sup>i</sup>	1.37 <sup>i</sup>	342/80.6
<b>3</b>	0.006	0.039	15.1	0.476	65.5	6.64	1107/170
<b>4</b>	0.026	0.066	19.7	0.211	59.5	6.68	257/101
<b>5a</b>	2.87	6.38	130	47.2	>100	182	63.4/28.5
<b>5b</b>	2.75	8.10	72.2	15.3	>100	60.5	22.0/7.47
<b>6</b>	1.41	4.51	92.9	15.1	>100	49.9	35.4/11.1

<sup>a</sup> The IC<sub>50</sub> values are the means of two independent assays; the individual values vary by a factor of less than 2. <sup>b</sup> The selectivity index is calculated as the ratio of the IC<sub>50</sub> values concerning L6 cells to the IC<sub>50</sub> data relative to *P. falciparum*. <sup>c</sup> Chloroquine. <sup>d</sup> Artemisinin. <sup>e</sup> Benznidazole. <sup>f</sup> Melarsoprol. <sup>g</sup> Miltefosine. <sup>h</sup> Podophyllotoxin. <sup>i</sup> Values reported earlier, see ref. 6.



## Experimental

### General experimental procedures

Optical rotations were determined on a Jasco P-1020 polarimeter operating with a sodium light source ( $\lambda = 589$  nm). IR spectra were recorded on a Jasco FT/IR-410 spectrometer, UV spectra on a Shimadzu UV-1800 spectrophotometer, and ECD measurements were performed on a Jasco J-715 spectropolarimeter. The ECD data are reported in  $\Delta\epsilon$  values ( $\text{cm}^2 \text{mol}^{-1}$ ) at the given wavelength  $\lambda$  (nm), they were processed using SpecDis.<sup>51,52</sup> NMR spectra (600 MHz for  $^1\text{H}$  and 150 MHz for  $^{13}\text{C}$ ) were taken on a Bruker DMX 600 instrument, using methanol- $d_4$ , with the  $^1\text{H}$  or  $^{13}\text{C}$  signals of methanol ( $^1\text{H}$ ,  $\delta$  3.31 ppm,  $^{13}\text{C}$ ,  $\delta$  49.0 ppm) in the deuterated solvent as the internal reference. Chemical shifts ( $\delta$ ) are reported in parts per million (ppm), and coupling constants ( $J$ ) are given in hertz (Hz). NMR signal multiplicities are denoted as singlet (s), doublet (d), doublet of doublets (dd), quartet (q), or multiplet (m). Spectra were acquired and processed using the Topspin 3.5 software (Bruker Daltonics). HRESIMS measurements were performed in positive mode on a Bruker Daltonics micrOTOF-focus mass instrument. GC-MSD was done on a GCMS-QP 2010SE System (Shimadzu). Preparative HPLC was carried out on a Jasco System (PU-1580 Plus) in combination with UV/Vis detection at 200–680 nm (Jasco MD-2010 Plus diode array detector) at room temperature. For the isolation and purification of the constituents of the plant extracts, unless otherwise stated, SymmetryPrep™ C18 column (19 × 300 mm, 7  $\mu\text{m}$ , Waters) was used, flow rate 10 mL  $\text{min}^{-1}$ ; mobile phases: (A) 90%  $\text{H}_2\text{O}$  with 10% MeCN (0.05% trifluoroacetic acid) and (B) 90% MeCN with 10%  $\text{H}_2\text{O}$  (0.05% trifluoroacetic acid), using linear gradients (details given below). All organic solvents were of analytical-grade quality. Ultra-pure water was obtained from an Elga Purelab Classic system. (*S*)-MTPA was purchased from Sigma-Aldrich (Steinheim, Germany).

### Plant material

Leaves of the botanically as yet unidentified *Ancistrocladus* plant were collected by one of us (V. M.) in July 2006, in the vicinity of the village Leeke near the town of Ikela, Province Tshuapa, Democratic Republic of the Congo. A voucher specimen (No. 114) has been deposited at the Herbarium Bringmann, Institute of Organic Chemistry, University of Würzburg.

### Extraction and isolation

Air-dried leaves (*ca.* 300 g) were ground and exhaustively extracted with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  (1 : 1, v/v) at room temperature. The combined extracts were concentrated under reduced pressure to give 22.1 g of a crude residue. The solid extract was macerated in  $\text{H}_2\text{O}$ , with mechanical shaking (160 rpm), then filtered and dissolved in MeOH. After filtration, the raw methanolic extract was directly subjected to preparative HPLC on a SymmetryPrep™ C18 column, applying a linear gradient (0 min 20% B, 28 min 45% B), finally providing 11 fractions (F1–F11).

Fractions F1 (3.1 mg) and F2 (4.2 mg) were resolved by preparative HPLC, using an isocratic solvent system consisting of the mobile phases A and B (16 : 84, v/v) to give pure ikelacongoline B (**5b**) (2.7 mg) (retention time 25.4 min) and ikelacongoline A (**5a**) (3.5 mg) (retention time 28.9 min). From fraction F3 (2.6 mg), ealamine A (**11**) (1.7 mg) (retention time 18.9 min) was obtained, by isocratic HPLC using the solvents A and B (80 : 20, v/v).

Fraction F5 (8.5 mg) was subjected to preparative HPLC on a Chromolith SemiPrep RP-18e column (Merck, 10 × 100 mm) applying a linear gradient (0 min 5% B, 6 min 15% B, 9 min 25% B, 12 min 35% B, 15 min 45% B) at a flow rate of 5 mL  $\text{min}^{-1}$ , affording pure mbandakamine A (**1**) (2.1 mg) (retention time 10.4 min) and an alkaloid-containing subfraction, which was further purified by preparative HPLC on a SymmetryPrep™ C18 column. Using an isocratic solvent system similar to the one described above, but with MeOH instead of MeCN, consisting of the solvents A' and B' (4.5 : 5 : 5, v/v), resolution of this subfraction at a flow rate of 10 mL  $\text{min}^{-1}$  furnished pure ikelacongoline C (**6**) (4.9 mg) (retention time 11.9 min) and ikelacongoline D (**7**) (1.3 mg) (retention time 14.0 min).

Purification of fraction F6 (9.1 mg) by preparative HPLC on a Chromolith SemiPrep RP-18e column under the conditions described above again yielded mbandakamine A (**1**) (3.6 mg) (retention time 10.4 min) and, additionally, ancistrocongoline C (**8**) (5.1 mg) (retention time 7.9 min).

From fractions F7 (8.7 mg) and F8 (8.3 mg), ealamine G (**12**) and ancistrolikokine F<sub>2</sub> (**10**) were isolated by preparative HPLC on a Chromolith SemiPrep RP-18e column under the conditions given above; the two compounds were further purified by semi-preparative HPLC on a Waters XSelect HSS PFP column (10 × 250 mm, 5  $\mu\text{m}$ ) using a mobile phase system consisting of the solvents A' and B' under linear-gradient conditions (0 min 50% B, 20 min 100% B) at a flow rate of 5 mL  $\text{min}^{-1}$ , thus finally giving rise to pure **12** (1.5 mg) (retention time 7.9 min) and pure **10** (1.3 mg) (retention time 10.2 min).

Fraction F9 (8.1 mg) was resolved on a Chromolith SemiPrep RP-18e column under the conditions described above, furnishing pure ancistrolikokine C (**9**) (5.1 mg) (retention time 9.5 min) and mbandakamine B<sub>4</sub> (**4**) (3.0 mg) (retention time 11.2 min).

Resolution of fraction F10 (9.3 mg) by preparative HPLC on a Chromolith SemiPrep RP-18e column, followed by chromatography on a SymmetryPrep C18 column, using the solvent systems A and B, with a linear gradient (0 min 20% B, 20 min 45% B), at a flow rate of 10 mL  $\text{min}^{-1}$  gave pure mbandakamine B<sub>3</sub> (**3**) (3.8 mg) (retention time 18.9 min) and mbandakamine B<sub>2</sub> (**2**) (5.1 mg) (retention time 19.4 min).

**Mbandakamine B<sub>3</sub> (3).** Pale-yellow amorphous solid;  $[\alpha]_{\text{D}}^{25} -19.1$  (*c* 0.08, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  226 (3.9) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 205 (−3.0), 218 (+6.1), 233 (+2.9), 249 (−3.9), 270 (−0.3), 289 (−1.0), 305 (−2.1), 329 (−0.4), 345 (−0.7), and 376 (+0.1) nm; IR (ATR)  $\nu_{\text{max}}$ : 3357, 2923, 2851, 1976, 1672, 1598, 1418, 1313, 1256, 1200, 1132, 1076, 1039, 833, 800, and 720  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; HRESIMS: *m/z* 813.41108 [ $\text{M} + \text{H}$ ]<sup>+</sup> (calcd for  $\text{C}_{50}\text{H}_{57}\text{N}_2\text{O}_8$ , 813.41114).



**Mbandakamine B<sub>4</sub> (4).** Pale-yellow amorphous solid;  $[\alpha]_{\text{D}}^{25} -22.5$  (*c* 0.09, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  229 (3.9) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 207 (−6.7), 218 (+9.9), 235 (−9.1), 240 (−9.6), 246 (−11.3), 271 (+1.6), 288 (−2.1), 302 (−1.8), 329 (−0.7), 350 (−1.8), 368 (+0.2), and 400 (+0.5) nm; IR (ATR)  $\nu_{\text{max}}$ : 33 534, 2940, 2848, 2013, 1983, 1674, 1446, 1334, 1201, 1134, 838, 800, and 723  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Table 1; HRESIMS:  $m/z$  799.3957  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{49}\text{H}_{55}\text{N}_2\text{O}_8$ , 799.3958).

**Ikelacongoline A (5a).** Yellow amorphous solid;  $[\alpha]_{\text{D}}^{25} -11.5$  (*c* 0.05, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  229 (3.5) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 196 (+15.1), 211 (−15.9), 217 (−14.6), 223 (−15.5), 238 (+7.2), 266 (+0.1), 280 (−0.6), 300 (+0.1), 334 (−0.4), and 350 (−0.1) nm; IR (ATR)  $\nu_{\text{max}}$ : 3373, 2943, 2015, 1673, 1604, 1585, 1524, 1490, 1456, 1434, 1392, 1298, 1264, 1201, 1137, 1091, 1041, and 722  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3;  $m/z$  408.21699  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{25}\text{H}_{30}\text{NO}_4$ , 408.21748).

**Ikelacongoline B (5b).** Yellow amorphous solid;  $[\alpha]_{\text{D}}^{25} -18.3$  (*c* 0.05, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  230 (3.6) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 198 (−6.7), 211 (+11.1), 215 (+9.8), 224 (+6.5), 237 (−8.8), 261 (+0.1), 284 (+1.7), 300 (+0.3), and 350 (−0.2) nm; IR (ATR)  $\nu_{\text{max}}$ : 3220, 2941, 2839, 1672, 1584, 1456, 1200, 1137, 837, 800, and 722  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; HRESIMS:  $m/z$  408.21747  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{25}\text{H}_{30}\text{NO}_4$ , 408.21748).

**Ikelacongoline C (6).** Brown amorphous solid;  $[\alpha]_{\text{D}}^{25} -24.4$  (*c* 0.05, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  231 (3.6) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 196 (−4.1), 210 (+12.4), 218 (+8.2), 222 (+8.6), 239 (−12.4), 277 (−0.3), 300 (+0.3), 318 (+0.5), and 351 (−0.1) nm; IR (ATR)  $\nu_{\text{max}}$ : 3379, 2941, 2839, 1671, 1583, 1542, 1449, 1363, 1198, 1021, 832, 800, and 720  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; HRESIMS:  $m/z$  422.23133  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{32}\text{NO}_4$ , 422.23133).

**Ikelacongoline D (7).** Brown amorphous solid;  $[\alpha]_{\text{D}}^{25} +44.3$  (*c* 0.05, MeOH); UV (MeOH) ( $\log \epsilon$ ):  $\lambda_{\text{max}}$  231 (3.6) nm; ECD (*c* 0.1, MeOH)  $\lambda_{\text{max}}$  ( $\Delta\epsilon$ ): 200 (−15.5), 214 (+14.7), 227 (+31.8), 243 (−11.2), 268 (+0.3), 285 (+0.9), 301 (−0.7), and 310 (−0.6) nm; IR (ATR)  $\nu_{\text{max}}$ : 3415, 2927, 2851, 2273, 1674, 1585, 1437, 1204, 1137, 841, 802, and 724  $\text{cm}^{-1}$ ;  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see Tables 2 and 3; HRESIMS:  $m/z$  422.23227  $[\text{M} + \text{H}]^+$  (calcd for  $\text{C}_{26}\text{H}_{32}\text{NO}_4$ , 422.23133).

### Known alkaloids isolated

The likewise isolated known monomeric and dimeric naphthylisoquinoline alkaloids mbandakamines A (1) and B<sub>2</sub> (2) (Fig. 1), ancistrocongoline C (8), ancistrollokine C (9), ancistrollokine F<sub>2</sub> (10), and ealamines A (11) and G (12) (Fig. 2), now isolated for the first time from the leaves of this as yet unidentified Central Congolese *Ancistrocladus* liana, were found to be identical in their spectroscopic, physical, and chromatographic behavior with the data reported previously.<sup>4,6,16,17,28–30</sup>

### Oxidative degradation

Following a procedure described earlier,<sup>33</sup> ca. 0.8 mg of mbandakamines B<sub>3</sub> (3) or B<sub>4</sub> (4), or ikelacongolines A (5a), B (5b), C (6), or D (7) were subjected to ruthenium(viii)-catalyzed

periodate oxidation, followed by conversion of the resulting chiral amino with MeOH/HCl to the respective methyl esters and Mosher-type derivatization with (*R*)- $\alpha$ -methoxy- $\alpha$ -trifluoromethylphenylacetyl chloride [(*R*)-MTPA-Cl, prepared from (*S*)-MTPA]. The absolute configurations of 3, 4, 5a, 5b, 6, and 7 at C-3 were assigned by gas chromatography coupled to mass-selective detection (GC-MSD) and comparison with the corresponding derivatives of the authentic amino acids of known absolute configuration.

### Antiprotozoal assay

The *in vitro* antiparasitic activities of the mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4), and the ikelacongolines A (5a), B (5b), and C (6) (Table 4) against the pathogens *Plasmodium falciparum* (NF54 and K1 strains), *Trypanosoma cruzi* (Tulahuen C4 strain, amastigotes), *Trypanosoma brucei rhodesiense* (STIB 900 strain, trypanomastigotes), and *Leishmania donovani* (MHOM-ET-67/L82, amastigotes), and the cytotoxicity against mammalian host cells (rat skeletal myoblast L6 cells) were determined as described previously.<sup>53</sup>

## Conclusions

Prior to this work, mbandakamine-type naphthylisoquinoline dimers<sup>4,16</sup> had been known only from two *Ancistrocladus* species indigenous to the Northwestern region of the Democratic Republic of the Congo. The as yet unidentified liana on which we report in this paper is the first *Ancistrocladus* plant discovered in the rainforests of the Central Congo Basin that was found to produce such mbandakamine-type compounds, too. From its leaves, four representatives were isolated, among them two new dimers, the mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4). They were identified together with a series of four new 5,8'-coupled monomeric naphthylisoquinoline alkaloids, named ikelacongolines A–D (5a, 5b, 6, and 7), and along with five alkaloids (8–12) known from previous phytochemical studies on related Congolese *Ancistrocladus* species;<sup>17,28–30</sup> three of them likewise belonged to the subclass of 5,8'-linked alkaloids, whereas two compounds were representatives of the quite rare 7,8'-coupling type. Particularly striking was the considerable number of *N*-methylated naphthylisoquinolines in this botanically and phytochemically so far unexplored plant, among them the two new mbandakamines B<sub>3</sub> (3) and B<sub>4</sub> (4) and the four new monomeric ikelacongolines A–D. More than half of the metabolites isolated from the leaf extract showed the presence of such an *N*-methyl group. The structures of these mono- and dimeric naphthylisoquinolines are chemotaxonomically quite significant, indicating a close phylogenetic relationship of this *Ancistrocladus* plant to other *Ancistrocladus* species from the Congo Basin, in particular to *A. ealaensis*<sup>15,16,30</sup> and another yet unidentified *Ancistrocladus* taxon<sup>4–6</sup> from the Northwestern part of the Democratic Republic of the Congo. These two species are likewise known to produce a high number of 5,8'-linked monomeric alkaloids along with mbandakamine-type dimers. Similar to the *Ancistrocladus* species presented here, their metabolite profiles are also dominated by the presence of mixed



Ancistrocladaceae/Dioncophyllaceae, *i.e.*, hybrid-type alkaloids, *i.e.*, with *R*-configuration at C-3 and an oxygen function at C-6.

Furthermore, the new mbandakamines **3** and **4** have proven to be potent bioactive compounds displaying good to strong inhibitory effects against the pathogen of African sleeping sickness, *Trypanosoma brucei rhodesiense*, and, in particular, exerting excellent activities against chloroquine-sensitive (NF54) and chloroquine-resistant (K1) strains of the malaria parasite *Plasmodium falciparum*. With respect to the fact that the efficiency of many recommended therapeutic agents routinely used for the treatment of infectious diseases is threatened by the formation of an increasing resistance, the discovery of new agents is urgently needed. The promising activities of mbandakamine-type dimers warrant further investigations on their antiparasitic potential in particular, and on that of mono- and dimeric naphthylisoquinoline alkaloids in general. This work is in progress.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- C. M. Taylor, R. E. Gereau and G. M. Walters, *Ann. Mo. Bot. Gard.*, 2005, **92**, 360–399.
- M. Cheek, *Kew Bull.*, 2000, **55**, 871–882.
- F. G. Turini, C. Steinert, G. Heubl, G. Bringmann, B. K. Lombe, V. Mudogo and H. Meimberg, *Taxon*, 2014, **63**, 329–341.
- G. Bringmann, B. K. Lombe, C. Steinert, K. Ndjoko Ioset, R. Brun, F. Turini, G. Heubl and V. Mudogo, *Org. Lett.*, 2013, **15**, 2590–2593.
- B. K. Lombe, T. Bruhn, D. Feineis, V. Mudogo, R. Brun and G. Bringmann, *Org. Lett.*, 2017, **19**, 1342–1345.
- B. K. Lombe, T. Bruhn, D. Feineis, V. Mudogo, R. Brun and G. Bringmann, *Org. Lett.*, 2017, **19**, 6740–6743.
- B. K. Lombe, D. Feineis, V. Mudogo, R. Brun, S. Awale and G. Bringmann, *RSC Adv.*, 2018, **8**, 5243–5254.
- G. Bringmann, G. Zhang, T. Büttner, G. Bauckmann, T. Kupfer, H. Braunschweig, R. Brun and V. Mudogo, *Chem.–Eur. J.*, 2013, **19**, 916–923.
- S. M. Kavatsurwa, B. K. Lombe, D. Feineis, D. F. Dibwe, V. Maharaj, S. Awale and G. Bringmann, *Fitoterapia*, 2018, **130**, 6–16.
- G. Bringmann, I. Kajahn, M. Reichert, S. E. H. Pedersen, J. H. Faber, T. Gulder, R. Brun, S. B. Christensen, A. Ponte-Sucre, H. Moll, G. Heubl and V. Mudogo, *J. Org. Chem.*, 2006, **71**, 9348–9356.
- G. Bringmann, J. Spuziak, J. H. Faber, T. Gulder, I. Kajahn, M. Dreyer, G. Heubl, R. Brun and V. Mudogo, *Phytochemistry*, 2008, **69**, 1065–1075.
- G. Heubl, F. Turini, V. Mudogo, I. Kajahn and G. Bringmann, *Plant Ecol. Evol.*, 2010, **143**, 63–69.
- J. Léonard, *Bull. Soc. R. Bot. Belg.*, 1949, **82**, 27–40.
- G. Bringmann, C. Steinert, D. Feineis, V. Mudogo, J. Betzin and C. Scheller, *Phytochemistry*, 2016, **128**, 71–81.
- D. T. Tshitenge, D. Feineis, V. Mudogo, M. Kaiser, R. Brun and G. Bringmann, *Sci. Rep.*, 2017, **7**, 5767.
- D. T. Tshitenge, D. Feineis, V. Mudogo, M. Kaiser, R. Brun, E.-J. Seo, T. Efferth and G. Bringmann, *J. Nat. Prod.*, 2018, **81**, 918–933.
- S. Fayeze, D. Feineis, V. Mudogo, S. Awale and G. Bringmann, *RSC Adv.*, 2017, **7**, 53740–53751.
- S. Fayeze, D. Feineis, V. Mudogo, E.-J. Seo, T. Efferth and G. Bringmann, *Fitoterapia*, 2018, **129**, 114–125.
- S. Awale, D. F. Dibwe, C. Chandrasekar Balachadran, S. Fayeze, D. Feineis, B. K. Lombe and G. Bringmann, *J. Nat. Prod.*, 2018, **81**, 2282–2291.
- J. Li, R. Seupel, D. Feineis, V. Mudogo, M. Kaiser, R. Brun, D. Brännert, M. Chatterjee, E.-J. Seo, T. Efferth and G. Bringmann, *J. Nat. Prod.*, 2017, **80**, 443–458.
- J. Li, R. Seupel, T. Bruhn, D. Feineis, M. Kaiser, R. Brun, V. Mudogo, S. Awale and G. Bringmann, *J. Nat. Prod.*, 2017, **80**, 2807–2817.
- T. R. Govindachari and P. C. Parthasarathy, *Tetrahedron*, 1971, **27**, 1013–1026.
- T. R. Govindachari and P. C. Parthasarathy, *Heterocycles*, 1977, **7**, 661–684.
- G. Bringmann and F. Pokorny, The naphthylisoquinoline alkaloids, in *The Alkaloids*, ed. G. A. Cordell, Academic Press Inc, New York, 1995, vol. 46, pp. 127–271.
- S. R. M. Ibrahim and G. A. Mohamed, *Fitoterapia*, 2015, **106**, 194–225.
- Y. F. Hallock, K. P. Manfredi, J. W. Blunt, J. H. Cardellina II, M. Schäffer, K. P. Gulden, G. Bringmann, A. Y. Lee, J. Clardy, G. François and M. R. Boyd, *J. Org. Chem.*, 1994, **59**, 6349–6355.
- M. R. Boyd, Y. F. Hallock, J. H. Cardellina II, K. P. Manfredi, J. W. Blunt, J. B. McMahon, R. W. Buckheit Jr, G. Bringmann, M. Schäffer, G. M. Cragg, D. W. Thomas and J. G. Jato, *J. Med. Chem.*, 1994, **37**, 1740–1745.
- G. Bringmann, K. Messer, R. Brun and V. Mudogo, *J. Nat. Prod.*, 2002, **65**, 1096–1101.



- 29 G. Bringmann, C. Günther, W. Saeb, J. Mies, A. Wickramasinghe, V. Mudogo and R. Brun, *J. Nat. Prod.*, 2000, **63**, 1333–1337.
- 30 D. T. Tshitenge, PhD thesis, Julius-Maximilians-Universität Würzburg, 2017.
- 31 Y. F. Hallock, J. H. Cardellina II, M. Schäffer, M. Stahl, G. Bringmann, G. François and M. R. Boyd, *Tetrahedron*, 1997, **53**, 8121–8128.
- 32 Y. F. Hallock, J. H. Cardellina II, M. Schäffer, G. Bringmann, G. François and M. R. Boyd, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 1729–1734.
- 33 G. Bringmann, R. God and M. Schäffer, *Phytochemistry*, 1996, **43**, 1393–1403.
- 34 M. Xu, T. Bruhn, B. Hertlein, R. Brun, A. Stich, J. Wu and G. Bringmann, *Chem.–Eur. J.*, 2010, **16**, 4206–4216.
- 35 World malaria report 2015, [http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158\\_eng.pdf](http://apps.who.int/iris/bitstream/10665/200018/1/9789241565158_eng.pdf), accessed February 6, 2017.
- 36 A. Degarege, K. Fennie, D. Degarege, S. Chennupati and P. Madhivanan, *PLoS One*, 2019, **14**, e0211205.
- 37 T. K. Mackey, B. A. Liang, R. Cuomo, R. Hafen, K. C. Brouwer and D. E. Lee, *Clin. Microbiol. Rev.*, 2014, **27**, 949–979.
- 38 K. C. Chinsembu, *Acta Trop.*, 2015, **152**, 32–48.
- 39 P. A. Onguéné, F. Ntie-Kang, L. Likowo Lifongo, J. C. Ndom, W. Sippl and L. Meva'a Mbaze, *Malar. J.*, 2013, **12**, 449.
- 40 C. Ramahete, F. P. da Cruz, S. Mulhovo, I. J. Sousa, M. X. Fernandes, M. Prudêncio and M. J. U. Ferreira, *Bioorg. Med. Chem.*, 2014, **22**, 3887–3890.
- 41 C. N. Muthaura, J. M. Keriko, S. Derese and G. M. Rukunga, *Exp. Parasitol.*, 2011, **127**, 609–626.
- 42 M. A. Ibrahim, A. Mohammed, M. B. Isah and A. B. Aliyu, *J. Ethnopharmacol.*, 2014, **154**, 26–54.
- 43 C. J. D. Obbo, S. T. Kariuki, J. W. Gathirwa, W. Olaho-Mukani, P. K. Cheplogoi and E. M. Mwangi, *J. Ethnopharmacol.*, 2019, **229**, 127–136.
- 44 D. Zofou, F. Ntie-Kang, W. Sippl and S. M. N. Efange, *Nat. Prod. Rep.*, 2013, **30**, 1098–1120.
- 45 C. V. Simoben, F. Ntie-Kang, S. H. Akone and W. Sippl, *Nat. Prod. Bioprospect.*, 2015, **8**, 151–169.
- 46 E. Izumi, T. Ueda-Nakamura, B. Prado Dias Filho, V. F. Veiga Júnior and C. V. Nakamura, *Nat. Prod. Rep.*, 2011, **28**, 809–823.
- 47 M. M. Salem and K. A. Werbovetz, *Curr. Med. Chem.*, 2006, **13**, 2571–2598.
- 48 G. Bringmann, W. Saeb, D. Koppler and G. François, *Tetrahedron*, 1996, **52**, 13409–13418.
- 49 G. Bringmann, W. Saeb, M. Wohlfarth, R. Messer and R. Brun, *Tetrahedron*, 2000, **56**, 5871–5875.
- 50 G. Bringmann and S. Tasler, *Tetrahedron*, 2001, **57**, 331–343.
- 51 T. Bruhn, A. Schaumlöffel, Y. Hemberger and G. Bringmann, *Chirality*, 2013, **25**, 243–249.
- 52 T. Bruhn, A. Schaumlöffel, Y. Hemberger and G. Pescitelli, *SpecDis, Version 1.71*, [www.specdis-software.jimdo.com](http://www.specdis-software.jimdo.com), Berlin, Germany, 2017.
- 53 I. Orhan, B. Şener, M. Kaiser, R. Brun and D. Tasdemir, *Mar. Drugs*, 2010, **8**, 47–58.

