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# Mbandakamines A and B, Unsymmetrically Coupled Dimeric Naphthylisoquinoline Alkaloids, from a Congolese *Ancistrocladus* Species

**Gerhard Bringmann,*[,†] Blaise Kimbadi Lombe,[†,‡] Claudia Steinert,[†] Karine Ndjoko Ioset,[†] Reto Brun,[§,∥] Florian Turini,[#] Günther Heubl,[#] and Virima Mudogo[‡]**

*Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany, Faculté des Sciences, Université de Kinshasa, B.P. 202, Kinshasa XI, Democratic Republic of the Congo, Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland, University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland, and Department of Biology I, Biodiversity Research, Section Systematic Botany, Ludwig-Maximilians University Munich, Menzinger Strasse 67, D-80638 München, Germany*

*bringman@chemie.uni-wuerzburg.de*

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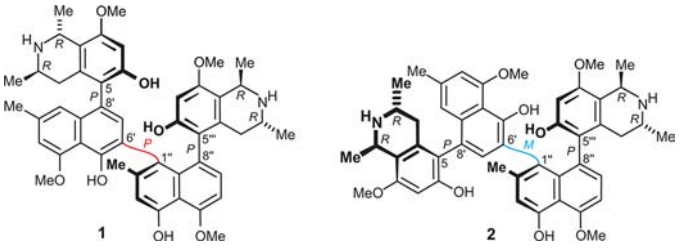
## ABSTRACT



**Mbandakamines A (1) and B (2), isolated from the leaves of an as yet unidentified Congolese *Ancistrocladus* species, are the first dimeric naphthylisoquinoline alkaloids with an unsymmetrically coupled central biaryl axis. Their novel 6′,1″-coupling type implies a hitherto unprecedented *peri*–*peri* coupling in one of the naphthalene parts, leading to the as yet highest steric hindrance at the central axis and a total of seven elements of chirality. Mbandakamine A exhibits good antimalarial activity.**

Naphthylisoquinoline alkaloids are remarkable natural products in many respects: structurally, biosynthetically, and pharmacologically.[1] They have so far been found only in the small palaeotropic families Dioncophyllaceae and Ancistrocladaceae. These unique alkaloids consist of two polyketide-derived[2] molecular halves, a naphthalene part and an isoquinoline portion, connected by a biaryl axis, which is, in most cases, rotationally hindered. Besides this element of axial chirality, the alkaloids carry up to three[3] stereogenic centers in the isoquinoline moiety. Likewise intriguing are the pharmacological properties of naphthylisoquinoline alkaloids, among them antimalarial activities *in vitro* and *in vivo*.[4] Even more fascinating are the dimeric naphthylisoquinolines,[5] since they not only possess at least double the number of elements of chirality[6,7] but also can exhibit pronounced anti-HIV activities,[5] like michellamine B, in contrast to all monomeric analogs tested so far.

[†] University of Würzburg.
[‡] Université de Kinshasa.
[§] Swiss Tropical and Public Health Institute.
[∥] University of Basel.
[#] Ludwig-Maximilians University Munich.

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These promising anti-HIV activities in combination with the unique structural features triggered several total syntheses of michellamine B[8] and the directed search for novel dimers in different *Ancistrocladus* species like *A. korupensis*[9] and *A. griffithii*.[10] More recently, by the use of LC-MS/MS-NMR-CD, we have discovered five new dimers in the Chinese species *A. tectorius*[7] and an additional one in a new, botanically yet undescribed *Ancistrocladus* species from the Democratic Republic of the Congo.[11] All of them were, for the first time, coupled *ortho* to the methyl group of the naphthalene part and, thus, had a rotationally hindered central axis, leading to the presence of three consecutive chiral biaryl axes. Furthermore, the new dimers exhibited excellent activities against *Plasmodium falciparum*, combined with a low cytotoxicity.[11] These unique structural and pharmacological properties of naphthylisoquinoline dimers made it highly rewarding to further search for such compounds in other *Ancistrocladus* species, particularly in botanically undescribed ones. From a recent collection near Mbandaka in the Congo Basin, plant material of a botanically as yet unidentified *Ancistrocladus* species became available. A genetic structure analysis based on microsatellite fingerprinting[12] showed that the specimen exhibited a genotype that only occurs in the Mbandaka region, not related to any yet botanically described taxon.

Further morphological, genetic, and chemical investigations are presently being performed to clarify if the sample might be a new species. From this plant material, we now report on the isolation, structural elucidation, and bioactivities of mbandakamines A (**1**) and B (**2**), the first naturally occurring dimeric naphthylisoquinolines with an unsymmetric 6′,1″-coupling at the central axis, leading to an exceptionally high steric hindrance in the binaphthalene core.

Investigation of different plant extracts by LC-MS hinted at the presence of a dimeric naphthylisoquinoline alkaloid, occurring in substantial quantities, especially in the leaves. Reversed-phase preparative HPLC of the crude leaf extract (after maceration in chloroform) provided a dimer with a molecular formula of $C_{48}H_{52}N_2O_8$, as evident from HRESIMS. Its $^1$H NMR spectrum showed a full set of signals, indicative of an unsymmetric dimer, which excluded the presence of ancistrogriffithine A,[10] the only other known, but symmetric, dimer with this molecular formula, showing that the isolated dimer was new. It was henceforth named mbandakamine A (**1**).

One of the naphthylisoquinoline portions of **1**, the 'northwestern' part **1a** (see Figure 1), showed a *meta*-coupling pattern for the two aromatic protons H-1′ [$\delta_H$ 6.62 (1H, *pt*, $J$ = 1.2 Hz)] and H-3′ [$\delta_H$ 6.74 (1H, d, $J$ = 1.2 Hz)] and the sequential ROESY correlations H-1′−$CH_3$-2′ [$\delta_H$ 2.34 (3H, d, $J$ = 0.7 Hz)], H-3′−$OCH_3$-4′ [$\delta_H$ 4.09 (3H, s)], together with HMBC correlations of a high-field shifted aromatic proton [$\delta_H$ 6.44 (1H, s)] to two carbons belonging to the adjacent isoquinoline part ($\delta_C$ 120.5) and to the naphthalene portion ($\delta_C$ 127.8) of the second, 'southeastern' part **1b** of **1** (Figure 1, left). These findings suggested a 5,8′-coupling within the first naphthylisoquinoline moiety, **1a**, which was corroborated by the ROESY interactions of H-1′ with H-$4_{ax}$ [$\delta_H$ 2.52 (1H, dd, $J$ = 18.0, 11.7 Hz)] and of H-7′ [$\delta_H$ 6.44 (1H, s)] with H-$4_{eq}$ [$\delta_H$ 3.89 (1H, dd, $J$ = 18.1, 4.5 Hz)], and by HMBC correlations from H-$4_{eq}$, H-7 [$\delta_H$ 6.46 (1H, s)] and H-7′ to C-5 ($\delta_C$ 120.5) (Figure 1). In the isoquinoline part the HMBC interaction from H-1 [$\delta_H$ 4.79 (1H, q, $J$ = 6.7 Hz)], $CH_3$-1 [$\delta_H$ 1.57 (3H, q, $J$ = 6.7 Hz)], and H-7 to C-8 ($\delta_C$ 157.3), and the sequential ROESY interactions from H-1 to $OCH_3$-8 [$\delta_H$ 3.85 (3H, s)] to H-7, proved the methoxy group to be linked to C-8, and consequently, the remaining hydroxy group had to be at C-6 (Figure 1, left). In **1b**, the 'southeastern' naphthylisoquinoline part of **1** (Figure 1, right), an AB spin system of two aromatic protons [$\delta_H$ 7.00 and $\delta_H$ 7.05 (1H, d, $J$ = 7.9 Hz)], the ROESY series $OCH_3$-5″ [$\delta_H$ 4.15 (3H, s)]−H-6″ ($\delta_H$ 7.00)−H-7″ ($\delta_H$ 7.05),

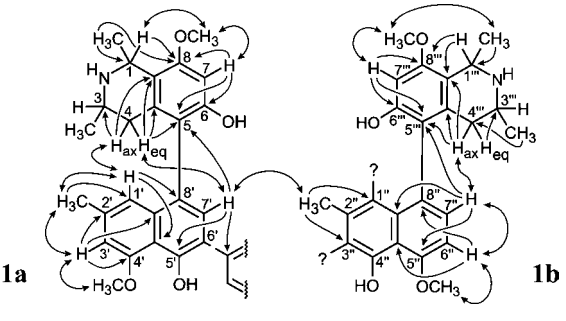


**Figure 1.** Key NOESY (double arrows) and selected HMBC (single arrows) correlations between the 'northwestern' (**1a**) and the 'southeastern' (**1b**) naphthylisoquinoline portions of mbandakamine A (**1**); for the $^1$H, $^{13}$C, and 2D-NMR data of **1**, see Table S2 in the SI.

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and HMBC correlations from H-7″ to C-5″ ($\delta_C$ 158.4) and to C-9″ ($\delta_C$ 138.1) and from H-6″ to C-8″ ($\delta_C$ 126.3) and to C-10″ ($\delta_C$ 116.2), revealed the presence of a naphthalene with a $OCH_3$-5″ group (Figure 1, right). The connection in the 8″-position to C-5‴ ($\delta_C$ 122.7) of the isoquinoline portion was evidenced by ROESY interactions between H-7″ and H-$4_{ax}$‴ [$\delta_H$ 2.42 (1H, dd, $J$ = 18.0, 12.0 Hz)], by HMBC correlations between H7″ and C-5‴, and was further corroborated by HMBC correlations from H-$4_{ax}$‴, H-$4_{eq}$‴ [$\delta_H$ 1.97 (1H, dd, $J$ = 18.0, 4.3 Hz)], and H-7‴ [$\delta_H$ 5.32 (1H, s)] to C-5‴. This showed that the second naphthylisoquinoline portion, **1b**, was 5,8′-coupled, too, i.e., 5‴,8″ in the molecular context of **1** (Figure 1, right). The HMBC correlations from H-1‴ [$\delta_H$ 4.64 (1H, q, $J$ = 6.7 Hz)] and H-7‴ to C-8‴ ($\delta_C$ 156.5) and the ROESY series $CH_3$-1‴ [$\delta_H$ 1.53 (3H, d, $J$ = 6.7 Hz)]−$OCH_3$-8‴ [$\delta_H$ 3.03, (3H, s)]−H-7‴ proved the methoxy group to be attached to C-8‴ and the hydroxy group to be at C-6‴ (Figure 1, right).

In the first, 'northwestern' moiety **1a**, the position of the inner biaryl axis was determined by the already mentioned HMBC correlation from H-7′ to a carbon ($\delta_C$ 127.8) belonging to a different naphthalene part, by the ROESY series H-1′−$CH_3$-2′−H-3′−$OCH_3$-4′ (Figure 1, left), and by a further ROESY interaction from H-7′ to $CH_3$-2″. This methyl group, located in the naphthalene part of the 'southeastern' naphthylisoquinoline moiety **1b**, exhibited an upfield shifted signal of 1.88 ppm (3H, s), indicating that a second shielding substituent, *viz*. the naphthalene portion of **1a**, had to be attached in a neighboring position of **1b**, i.e., at C-1″ or C-3″ (Figure 1, right). In addition to the mentioned correlations, numerous unexpected ROESY interactions between the naphthalene part of **1a** and the isoquinoline portion of **1b**, and even between the two isoquinoline moieties of **1a** and **1b**, were observed, e.g., from $OCH_3$-4′ to $OCH_3$-8‴, from $CH_3$-3 to $OCH_3$-8‴, and from H-$4_{ax}$ and H-$4_{eq}$ to H-7‴ (Figure 2). Such interactions had never been observed in any natural naphthylisoquinoline dimer, showing that the two naphthalenes had to be connected by a biaryl axis from C-6′ to C-1″, pressing parts of the two naphthylisoquinoline moieties into close spatial proximity. A likewise imaginable 6′,3″-coupling would have led to an arrangement in which the two naphthylisoquinoline moieties point in opposite directions. With its 6′,1″-coupling, mbandakamine A is the first natural dimeric naphthylisoquinoline alkaloid with a biaryl axis joining two naphthylisoquinoline halves in an unsymmetric way. In particular the 1″-position is extremely overcrowded, and the 'northwestern' naphthylisoquinoline **1a** is jammed between the rigid methyl group at C-2″ of **1b** and the bulky isoquinoline substituent at C-8″. This attachment of two bicyclic aryl substituents in the two neighboring *peri* positions 1″ and 8″ creates a unique array explaining the extraordinary chemical shifts of the protons H-7‴ and $OCH_3$-8‴.

With the complete constitution of mbandakamine A established, the relative and absolute configurations of the seven stereogenic elements (the four centers and three axes) remained to be assigned.

The relative configurations of the stereogenic centers in the isoquinoline parts were determined to be *trans* based on NOE interactions between H-3 and the protons of $CH_3$-1 and between H-3‴ and the protons of $CH_3$-1‴ (Figure 2). The absolute configurations at C-3 and C-3‴ were established to be *R* by Ru-mediated oxidative degradation,[13] which, in view of the relative *trans*-configuration, evidenced C-1 and C-1‴ to also be (*R*)-configured. ROESY correlations between H-$4_{eq}$ and H-7′ and between H-$4_{eq}$‴ and H-7″ (Figure 2) revealed the respective nuclei to be on the same side of the molecule, which, in combination with the absolute (*R*)-configurations at C-1 and C-3 and at C-1‴ and C-3‴, assigned both outer axes to be (*P*)-configured. ROESY correlations between the isoquinoline portions of **1a** and **1b** and between H-1‴ and $OCH_3$-4′ (Figure 2; for all ROESY correlations, see Table S2 in the Supporting Information (SI)) evidenced the central axis of mbandakamine to be (*R*)-configured also. Consequently, mbandakamine A was (1*R*,3*R*,1‴*R*,3‴*R*,*PPP*)-configured and thus possessed the full absolute stereostructure **1** as shown in the abstract graphic. The discovery of mbandakamine A with its unprecedented unsymmetric structure warranted a search for its other imaginable atropisomer at the central biaryl axis. Indeed, the directed screening of the extract for another dimer with the same mass yielded one further isomer as a candidate, with a significantly longer retention time. Unfortunately, this compound, which occurred in substantially smaller quantities (ca. 1:10), eluted together with a second metabolite in reversed-phase HPLC. Although we were not able to fully remove the interfering substance, we managed to elucidate the full absolute stereostructure of the second dimer.

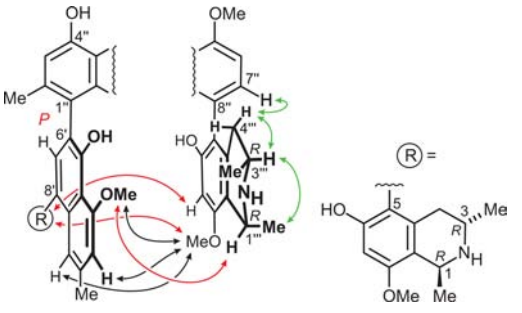


**Figure 2.** ROESY correlations between the naphthalene moiety of the 'northwestern' naphthylisoquinoline part **1a** (left) and the isoquinoline moiety of the 'southeastern' part **1b** (right), evidencing the 6′,1″-coupling (black arrows) and the relative configurations at the centers and the outer axes (green arrows) and at the central axis (red arrows) of mbandakamine A.

By the same methods as before for mbandakamine A (**1**), the constitution (HRMS, ROESY, HMBC) and the relative (ROESY) and absolute (oxidative degradation) configurations of all the stereocenters and configurations

(13) Bringmann, G.; God, R.; Schäffer, M. *Phytochemistry* **1996**, *43*, 1393–1403.

of the outer axes (ROESY) of the new dimer, **2**, were established (for the complete $^{1}H$, $^{13}C$, and 2D-NMR data, see Table S3 in the SI) to be the same as those in **1**, i.e., *R*-configuration at C-1, C-3, C-1‴, and C-3‴, and (*P*)-configuration for the outer biaryl axes. Thus, the only difference between the structures of **1** and **2** should indeed be the configuration at the central axis. By the ROESY correlations between H-7′ and H-$4_{ax}$‴ and between $OCH_3$-4′ and H-7‴ in **2** (Figure 3), which were complementary to those in **1** (see Figure 2), this axial configuration of **2** was evidenced to be *M* and, thus, opposite to that of **1**. Consequently, this compound **2**, now named mbandakamine B, was indeed the respective diastereomer of mbandakamine A (**1**).

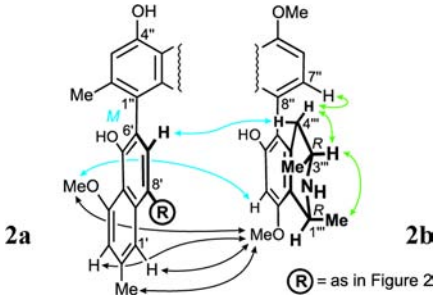


**Figure 3.** ROESY correlations between the naphthylisoquinoline halves **2a** and **2b** of mbandakamine B (**2**), indicative of the 6′,1″-coupling (black arrows) and the relative configurations at the centers and at the outer axes (green arrows) and at the central axis (blue arrows).

The most obvious spectral differences between **1** and **2** were the CD curves (Figure 4), which were virtually opposite, although **1** and **2** only differ in the configuration at the central axis, with all the other six stereogenic elements (two axes and four centers) being identical. Thus, as in the case of other naphthylisoquinoline dimers,[7] the CD spectrum is largely dominated by the strong binaphthalene chromophore, whose stereo-orienation is determined by the configuration of the central axis.

Mbandakamine A (**1**) exhibited significant *in vitro* antiplasmodial activities against the chloroquine-sensitive *Plasmodium falciparum* strain NF54, its diacetate salt ($IC_{50}$ = 0.043 $\mu$M) being even more effective than the free base ($IC_{50}$ = 0.13 $\mu$M). Mbandakamine B ($IC_{50}$ = 0.148 $\mu$M, diacetate) was three to four times less active against *Plasmodium falciparum*, showing the impact of axial chirality on the bioactivity.

(14) For a related, yet synthetic dimer with a similar molecular shape, but still smaller steric hindrance (8′,6″-coupled in the naphthalene part (and not 6′,1″)), see: Bringmann, G.; Saeb, W.; Kraus, J.; Brun, R.; François, G. *Tetrahedron* **2000**, *56*, 3523–3531.

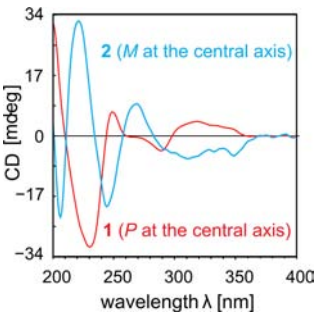


**Figure 4.** CD spectra of mbandakamines A (**1**) and B (**2**); for better comparibility the intensity of the curve of **2** is fitted to the one of **1**.

Mbandakamines A and B are unprecedented natural naphthylisoquinoline dimers, novel in many respects. Only six naphthylisoquinoline alkaloids are known so far to possess three consecutive chiral axes,[7,11] all of them being $C_2$-symmetric, in which the central axes connect the two naphthalenes in equivalent positions. Most remarkably, mbandakamines A and B, with their three sequential stereogenic axes, feature an unprecedented unsymmetric 1′,6″-coupling. As a consequence, they are the first naphthylisoquinoline dimers with two axes being located *peri*–*peri* to one another in one of the naphthalene portions. This dense coupling gives the central axes the highest steric load known from any, natural or synthetic,[14] dimeric naphthylisoquinoline.

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**Supporting Information Available.** Experimental procedures and complete spectral data of **1** and **2**, as well as biological tests, and genotype classification of the *Ancistrocladus* specimen. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.