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Ancistrolikokines E–H and related 5,8′-coupled naphthylisoquinoline alkaloids from the Congolese liana *Ancistrocladus likoko* with antiausterity activities against PANC-1 human pancreatic cancer cells†

Shaimaa Fayez,[a] Doris Feineis,[a] Virima Mudogo,[b] Suresh Awale *[c] and Gerhard Bringmann *[a]

A striking feature of the metabolite profile of *Ancistrocladus likoko* (Ancistrocladaceae) is the exclusive production of 5,8′-linked naphthylisoquinoline alkaloids varying in their OMe/OH substitution patterns and in the hydrogenation degree in their isoquinoline portions. Here we present nine new compounds of this coupling type isolated from the twigs of this remarkable Central African liana. Three of them, the ancistrolikokines E (**9**), E_2 (**10**), and F (**11**), are the first 5,8′-linked naphthyldihydroisoquinolines found in nature with *R*-configuration at C-3. The fourth new metabolite, ancistrolikokine G (**12**), is so far the only representative of the 5,8′-coupling type that belongs to the very rare group of alkaloids with a fully dehydrogenated isoquinoline portion. Moreover, five new *N*-methylated naphthyltetrahydroisoquinolines, named ancistrolikokines A_2 (**13**), A_3 (**14**), C_2 (**5**), H (**15**), and H_2 (**16**) are presented, along with six known 5,8′-linked alkaloids, previously identified in related African *Ancistrocladus* species, now found for the first time in *A. likoko*. The structural elucidation was achieved by spectroscopic analysis (HRESIMS, 1D and 2D NMR) and by chemical (oxidative degradation) and chiroptical (electronic circular dichroism) methods. The new ancistrolikokines showed moderate to good preferential cytotoxic activities towards pancreatic PANC-1 cells in nutrient-deprived medium (NDM), without causing toxicity under normal, nutrient-rich conditions, with ancistrolikokine H_2 (**16**) being the most potent compound.

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Introduction

Ancistrocladus likoko J. Léonard (Ancistrocladaceae)[1,2] is a widely occurring woody liana mainly growing along rivers and in lowland freshwater swamp forests in the North-Central part of the Democratic Republic of the Congo. It belongs to the small monogeneric Ancistrocladaceae family from palaeotropical Africa and Asia, comprising nearly 20 accepted species.[1–3] Phytochemically, *Ancistrocladus* plants are characterized by the presence of biosynthetically and structurally unique naphthylisoquinoline alkaloids.[4,5] These secondary metabolites are the first natural polyketide-derived isoquinoline alkaloids since not only the naphthalene part, but also the isoquinoline moiety originates from acetate-malonate units.[6–8] Depending on their individual structures, they display pronounced antiplasmodial,[9–12] antitrypanosomal,[9,13,14] antileishmanial,[9,14–16] antileukemic,[17–19] or anti-HIV activities.[20,21]

Previous phytochemical investigations on the roots and leaves of *A. likoko* had led to the isolation of six naphthylisoquinoline alkaloids,[22,23] *viz.*, korupensamine A (**1a**), the ancistrolikokines A–D (**2–4** and **6**), and ancistroealaine A (**7**), along with the biosynthetically related tetralone *cis*-isoshinanolone (**8**) (Fig. 1). All of those six alkaloids of *A. likoko* were based on the same coupling type, with the biaryl axis being located between C-5 and C-8′. Although this 5,8′-coupling site is quite frequently found in other Central African species like *e.g.*, in *A. ealaensis*[24] or *A. congolensis*,[25,26] and, in particular in the Cameroonian liana *A. korupensis*, which likewise produces a whole series of 5,8′-linked naphthylisoquinolines, among them also dimers,[20,27,28] no other plant produces 5,8′-coupled alkaloids exclusively.

In this paper, we report on the isolation and structural elucidation of further 5,8′-coupled naphthylisoquinoline

[a]*Institute of Organic Chemistry, University of Würzburg, Am Hubland, D-97074 Würzburg, Germany. E-mail: bringman@chemie.uni-wuerzburg.de*

[b]*Faculté des Sciences, Université de Kinshasa, B.P. 202, Kinshasa XI, Democratic Republic of the Congo*

[c]*Division of Natural Drug Discovery, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. E-mail: suresh@inm.u-toyama.ac.jp*

† Electronic supplementary information (ESI) available: Spectroscopic data include NMR (^{1}H, ^{13}C, ^{1}H,^{1}H-COSY, HSQC, HMBC, NOESY, and ROESY), HRESIMS, IR, and ECD spectra of compounds **5** and **9–16**. See DOI: 10.1039/c7ra11200a

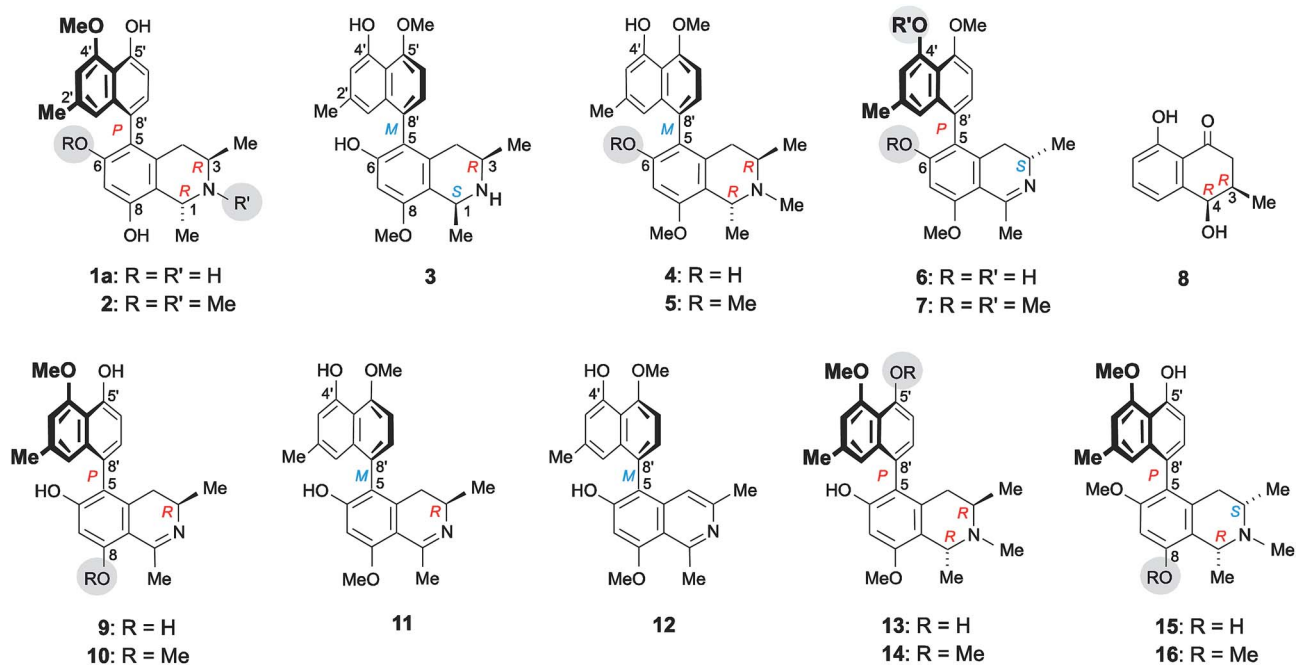

Fig. 1 Secondary metabolites isolated from the Central African liana *Ancistrocladus likoko*, among them the nine new ancistrolikokines E (**9**), E_2 (**10**), F (**11**), G (**12**), A_2 (**13**), A_3 (**14**), C_2 (**5**), H (**15**), and H_2 (**16**).

alkaloids from the twigs of *A. likoko*, comprising nine new compounds (Fig. 1) and six known[20,25–29] metabolites (Fig. 2); the latter had already been discovered in previous studies on related African *Ancistrocladus* species. Three of the new alkaloids, the ancistrolikokines E (**9**), E_2 (**10**), and F (**11**) are the first monomeric naphthyldihydroisoquinolines with *R*-configuration at C-3 found in nature. Such an entity has so far been found only as part of two dimers.[26,28] The fourth new alkaloid, ancistrolikokine G (**12**) (Fig. 1), is one of the very rare examples of fully dehydrogenated naphthylisoquinoline alkaloids that are optically active without the presence of stereogenic centers, since the only stereogenic element in **12** is the chiral biaryl axis. Prior to this work, only nine such naphthylisoquinolines have been identified in *Ancistrocladus* species, all of them from Asia.[16,30–33] Among these compounds, ancistrolikokine G (**12**) is the first representative that was isolated from an African liana and that is 5,8′-coupled. Furthermore, five new *N*-methylated naphthyltetrahydroisoquinoline alkaloids with a 5,8′-biaryl linkage were discovered in the twigs of *A. likoko*, named ancistrolikokines A_2 (**13**), A_3 (**14**), C_2 (**5**), H (**15**), and H_2 (**16**). As part of our ongoing studies on the antiproliferative potential of naphthylisoquinoline alkaloids towards cancer cell lines,[18,19,33] the new ancistrolikokines presented here, as well as related known,[20,25–29] but yet not investigated 5,8′-coupled alkaloids, were tested for their activities against PANC-1 human pancreatic cancer cells under nutrient-deprived conditions.

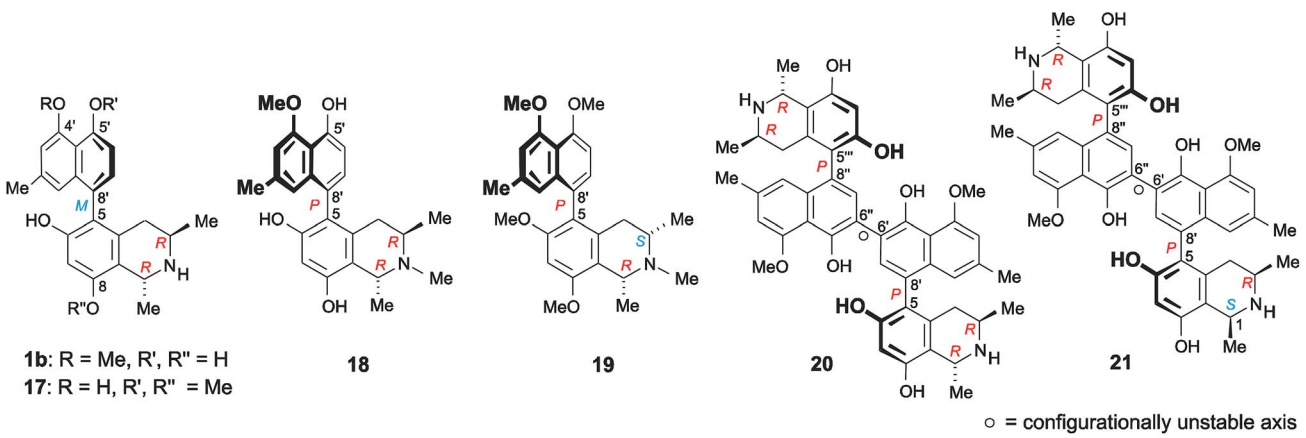

Fig. 2 Four known monomeric 5,8′-coupled naphthylisoquinoline alkaloids, the korupensamines B (**1b**) and E (**17**), ancistrocongoline A (**18**), and ancistrobertsonine C (**19**), and two known dimers, the michellamines A (**20**) and A_2 (**21**), identified for the first time in *Ancistrocladus likoko*.

Results and discussion

Isolation and structural elucidation of mono- and dimeric naphthylisoquinoline alkaloids

Air-dried and powdered twig material of *Ancistrocladus likoko* was exhaustively extracted with CH_2Cl_2–MeOH (1 : 1, v/v). The crude extract was dissolved in MeOH–H_2O and further purified by liquid/liquid partition with *n*-hexane, followed by repeated column chromatography (CC) on silica gel for fractionation. The subfractions thus obtained were subjected to preparative reversed-phase HPLC, permitting isolation of 15 metabolites, among them six known[20,25–29] 5,8′-coupled naphthylisoquinoline alkaloids (Fig. 2), which had already been discovered in other African *Ancistrocladus* species before.

One of these metabolites was korupensamine B (**1b**),[27] earlier identified in *A. korupensis* from Cameroon. This alkaloid is the atropo-diastereomer of the co-occurring compound korupensamine A (**1a**)[22] (Fig. 1). It was isolated from the twig extracts of *A. likoko*, together with two further 5,8′-coupled naphthylisoquinoline alkaloids, *viz.*, korupensamine E (**17**),[28] which had previously been found in *A. korupensis*, and ancistrocongoline A (**18**)[25] (Fig. 2), earlier detected in the Congolese species *A. congolensis*. With their *R*-configuration at C-3 and an oxygen function at C-6, **1b**, **17**, and **18** belong to the subclass of mixed Ancistrocladaceae/Dioncophyllaceae-type (*i.e.*, 'hybrid-type') naphthylisoquinolines. This is of special interest with respect to the chemotaxonomic relevance of naphthylisoquinoline alkaloids for the classification of the plants.

Southeast Asian and East African Ancistrocladaceae typically produce 3*S*-configured and 6-oxygenated compounds (*i.e.*, 'Ancistrocladaceae-type' alkaloids).[4,5,16–18,30–33] The only other plant family that likewise produces naphthylisoquinolines, the West African Dioncophyllaceae, exclusively contains alkaloids with *R*-configuration at C-3, always lacking an oxygen function at C-6 (*i.e.*, 'Dioncophyllaceae-type' alkaloids).[4,5,34] West African and some of the Central African *Ancistrocladus* plants produce both, typical Dioncophyllaceae- and Ancistrocladaceae-type alkaloids, and all possible hybrid forms thereof,[4,5,11,19] while 'Ancistrocladaceae/Dioncophyllaceae hybrid-type' alkaloids are frequently found in Central African species together with Ancistrocladaceae-type compounds.[4,5,20,22–28,35,36]

Thus, in view of the structures of the alkaloids found so far in *A. likoko*, this liana is a typical Central African species. Besides the hybrid-type alkaloids presented above, and the two 3*S*-configured metabolites ancistrolikokine D (**6**) and ancistroealaine A (**7**) (Fig. 1), a further, third Ancistrocladaceae-type compound was identified, *viz.*, ancistrobertsonine C (**19**)[29] (Fig. 2), which had earlier been found in the Kenyan species *A. robertsoniorum*. The isolation of the two known hybrid-type dimers michellamine A (**20**)[20,26] and michellamine A_2 (**21**),[26] previously detected only in *A. korupensis* and *A. congolensis*, strongly support the close chemotaxonomical relationship of *A. likoko* to *Ancistrocladus* species from West and Central Africa.

In addition, nine new 5,8′-coupled naphthylisoquinolines were discovered in the twigs of *A. likoko*. The molecular formula of the first new alkaloid, obtained as a dark-yellow solid, was $C_{23}H_{24}NO_4$, as deduced from HRESIMS. The compound displayed NMR signals typical of a naphthyl-1,3-dimethyldihydroisoquinoline as obvious from the downfield shift of the methyl group at C-1 (δ 2.80) in the ^{1}H NMR spectrum (Table 1) and its multiplicity (singlet), and from the low-field chemical shift of the ^{13}C NMR signal of C-1 (δ 174.7) (Table 2). This was further corroborated from the absence of an H-1 signal, which normally appears at δ 4.60–4.80 in naphthyltetrahydroisoquinoline alkaloids.[4,24,31–33] The ^{1}H NMR spectrum showed the chemical shifts of one methoxy function (δ 4.08) and five aromatic protons, *viz.*, three singlets and two doublets, each corresponding to one proton. Two of these protons (δ 6.67 and 6.80) were located *meta* to each other. This substitution pattern, with only two adjacent protons (δ 6.79 and 7.09) (Fig. 3A), hinted either at a 6′- or an 8′-position of the biaryl axis in the naphthalene portion. This assumption was supported by the 'normal' chemical shift of the methyl group at C-2′ (δ 2.32). The exact coupling site was established from the HMBC interaction between H-1′ and the quaternary carbon atom C-8′, thus clearly revealing that the naphthalene portion was coupled *via* C-8′. The attribution of H-1′ (δ 6.69) and H-3′ (δ 6.80), in turn, was assigned from the NOESY correlation sequence {H-1′ ↔

Table 1 ^{1}H NMR data of ancistrolikokines E (**9**), E_2 (**10**), F (**11**), and G (**12**) in methanol-d_4 (600 MHz, δ in ppm, *J* in Hz)

No.	9	10	11	12
3	3.75, m	3.76, m	3.68, m	
4_{ax}	2.21, dd (10.2, 16.6)	2.24, dd (10.2, 16.8)	2.47, dd (11.4, 16.8)	6.78, s
4_{eq}	2.63, dd (5.4, 16.7)	2.66, dd (5.4, 16.8)	2.39, dd (5.8, 16.8)	
7	6.53, s	6.66, s	6.64, s	6.94, s
1′	6.67, s	6.64, s	6.58, s	6.39, s
3′	6.80, s	6.81, s	6.68, s	6.67, s
6′	6.79, d (7.9)	6.80, d (7.8)	6.92, d (7.9)	7.00, d (7.9)
7′	7.09, d (7.8)	7.10, d (7.8)	7.07, d (7.9)	7.10, d (7.8)
1-CH_3	2.80, s	2.78, s	2.77, s	3.18, s
3-CH_3	1.18, d (6.6)	1.19, d (6.7)	1.24, d (6.7)	2.38, s
2′-CH_3	2.32, s	2.31, s, s	2.26, s	2.16, s
8-OCH_3		4.03, s	4.02, s	4.13, s
4′-OCH_3	4.08, s	4.08, s, s		
5′-OCH_3			4.10, s	4.14, s

Table 2 ^{13}C NMR data of ancistrolikokines E (**9**), E$_2$ (**10**), F (**11**), and G (**12**) in methanol-d_4 (150 MHz, δ in ppm)

No.	9	10	11	12
1	174.7	175.3	175.5	157.5
3	48.7	49.4	49.3	142.4
4	32.5	32.7	33.4	118.9
5	121.7	122.5	122.3	114.5
6	167.1	167.7	168.0	165.2
7	102.3	99.1	99.1	101.9
8	165.0	165.6	165.7	163.0
9	106.8	108.3	108.7	114.2
10	141.0	142.7	142.5	143.5
1′	118.3	118.5	116.3	116.6
2′	137.4	137.9	139.4	139.5
3′	107.2	107.7	113.3	113.4
4′	157.9	158.0	156.1	156.2
5′	155.5	156.2	157.9	158.2
6′	110.0	110.3	104.2	104.6
7′	130.7	131.1	130.0	130.9
8′	123.0	122.9	126.0	125.2
9′	136.6	136.9	136.6	137.4
10′	114.3	114.8	114.6	114.9
1-CH$_3$	24.2	24.8	24.6	23.6
3-CH$_3$	17.6	17.9	18.0	18.7
2′-CH$_3$	21.7	22.2	21.8	21.8
8-OCH$_3$		56.6	56.6	56.7
4′-OCH$_3$	56.7	56.8		
5′-OCH$_3$			56.7	56.5

Me-2′ ↔ H-3′ ↔ OMe-4′} (Fig. 3B), and from HMBC interactions, each, from H-1′ and H-3′ to Me-2′ (Fig. 3A). In the dihydroisoquinoline portion, the axis was located at C-5, as deduced from HMBC interactions from H-7′ and H-7 to C-5, and from H-4$_{eq}$ to C-5 (Fig. 3A). In conclusion, the new compound was a 5,8′-coupled naphthyldihydroisoquinoline with one methoxy group at C-4′ in the naphthalene half. The three other oxygen functions had to be free hydroxy groups at C-6, C-8, and C-5′.

The absolute configuration at C-3 in the dihydroisoquinoline portion was determined by ruthenium-mediated oxidative degradation as described earlier,[37] by stereochemical analysis of the resulting amino acids by gas chromatography with mass-selective detection (GC-MSD) after derivatization with the *R*-enantiomer of Mosher's acid chloride. The formation of *R*-3-aminobutyric acid unequivocally established the alkaloid to be *R*-configured at C-3.

The relative and, thus, absolute configuration at the biaryl axis was attributed to be *P*, by NOESY interactions between H-4$_{ax}$ and H-1′ on the one hand, and between H-4$_{eq}$ and H-7′ on the other (Fig. 3B). The assignment of the absolute axial configuration as *P* was confirmed by the fact that the ECD spectrum of the new metabolite was virtually opposite to that of the structurally closely related and likewise 5,8′-coupled, but *M*-configured 6-*O*-demethylancistectorine D (**22**)[18] (Fig. 3C).

Thus, the new alkaloid had the full absolute stereostructure **9** as shown in Fig. 1. It was henceforth named ancistrolikokine E, in continuation of the series of alkaloids previously isolated from *A. likoko*.[22,23] It is the first 5,8′-coupled naphthyldihydroisoquinoline found in nature with *R*-configuration at C-3.

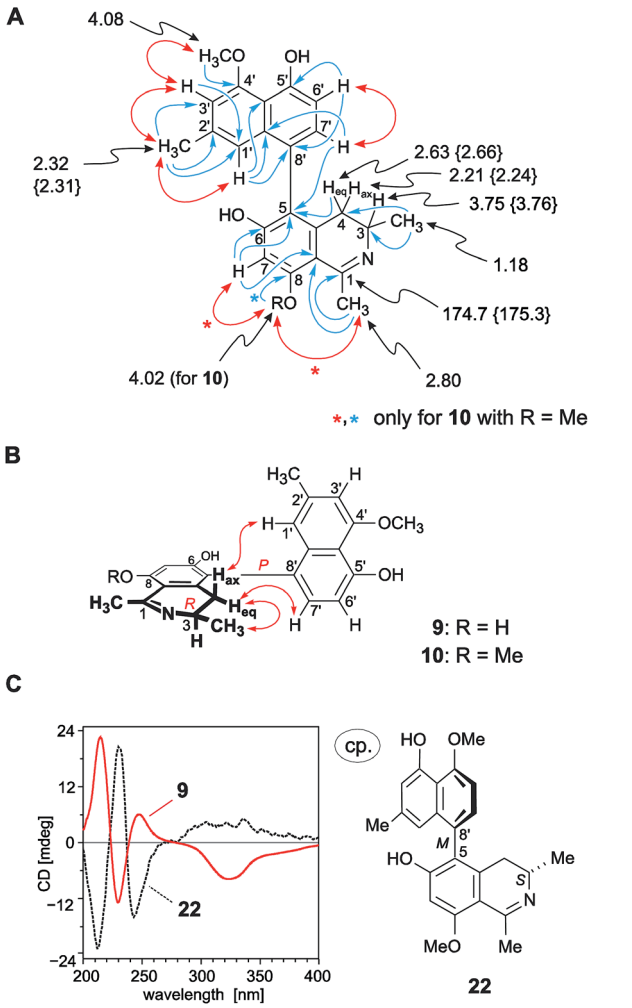

Fig. 3 (A) Selected ^{1}H and ^{13}C NMR data (δ in ppm): HMBC (single blue arrows) and NOESY (double red arrows) interactions of ancistrolikokines E (**9**) and E$_2$ (**10**); the values of **10** that are different from those of **9** are given in { }; (B) NOESY interactions indicative of the configuration at the biaryl axis of **9** and **10** relative to the stereogenic center at C-3; (C) confirmation of the absolute axial configuration of **9** by comparison of its ECD spectrum with that of the known[18] related 5,8′-coupled alkaloid 6-*O*-demethylancistectorine D (**22**).

The second new alkaloid, isolated from the twig extracts of *A. likoko*, showed ^{1}H and ^{13}C NMR data again suggesting the presence of a naphthyldihydroisoquinoline alkaloid belonging to the 5,8′-coupling type. It strongly resembled the constitution of the above-presented ancistrolikokine E (**9**), yet with a higher degree of *O*-methylation. According to HRESIMS, the new compound corresponded to a molecular formula of $C_{24}H_{26}NO_4$, and the NOESY correlation sequence {Me-1 ↔ OMe-8 ↔ H-7} (Fig. 3A) revealed the new metabolite to be equipped with a methoxy group (δ 4.03) at C-8 in the dihydroisoquinoline portion.

By the above-mentioned methods – the oxidative degradation, specific NOESY interactions across the biaryl axis (see Fig. 3B), and ECD spectroscopy (see the ESI†) – the new alkaloid was established to be *R*-configured at C-3 and *P*-configured at the axis, similar to ancistrolikokine E (**9**). The new metabolite

had the complete stereostructure **10** (Fig. 1). It is the 8-*O*-methyl analog of ancistrolikokine E (**9**), and was thus named ancistrolikokine E_2.

The molecular formula of the third new alkaloid, as established from the HRESIMS and ^{13}C NMR spectroscopic data (Table 2), was $C_{24}H_{26}NO_4$, and, thus, the same as that of ancistrolikokine E_2 (**10**). ^1H NMR investigations (Table 1) again indicated the presence of a 5,8′-coupled naphthyl-1,3-dimethyldihydroisoquinoline with two normally shifted methoxy groups (δ 4.02 and 4.10), the only difference between **10** and the new metabolite being the OH/OMe pattern in their naphthalene parts. In **10** the methoxy group was located at C-4′, while the new metabolite was equipped with a methoxy function at C-5′ as assigned by cross-peaks in the NOESY spectrum in the series {OMe-5′ ↔ H-6′ ↔ H-7′}. This was further confirmed by an NOE interaction between H-3′ and Me-2′, which, in turn, showed an NOE correlation to H-1′ (Fig. 4A).

The formation of *R*-3-aminobutyric acid in the degradation procedure proved the new alkaloid to be *R*-configured at C-3. Long-range NOESY interactions between H-4_{ax} and H-7′ and between H-4_{eq} and H-1′ permitted assignment of the stereo array at the axis to be *M* as shown in Fig. 4A. This was in agreement with the ECD spectrum of the compound, which was nearly identical to that of the likewise *M*-configured 6-*O*-demethylancistectorine D (**22**)[18] (Fig. 4B). Hence, the new alkaloid possessed the stereostructure **11**. It was named ancistrolikokine F.

The fourth new alkaloid possessed a molecular formula of $C_{24}H_{24}NO_4$, as deduced from HRESIMS. The ^1H and ^{13}C NMR spectroscopic data (Table 1) and specific HMBC and ROESY interactions (see ESI†) of the new metabolite corresponded to the presence of a 5,8′-coupled naphthylisoquinoline alkaloid with an OH/OMe pattern identical to that of ancistrolikokine F (**11**). The ^1H NMR spectrum of the new metabolite implied the presence of a fully dehydrogenated isoquinoline portion. This was obvious from the lack of resonances for protons at C-1 and C-3 and the appearance of an additional aromatic singlet at δ 6.78, instead of the signals of the two diastereotopic geminal protons at C-4 usually observed for naphthyltetra- or naphthyldihydroisoquinoline alkaloids.[31–33] This finding was further supported by the significant downfield shifts of the signals of the two methyl groups at C-1 (δ 3.18) and C-3 (δ 2.38) in the ^1H NMR spectrum (Table 1) and of the ^{13}C NMR resonances of C-1, C-3, and C-4 (δ 157.5, 142.4, and 118.9, respectively) (Table 2).

Owing to the lack of stereogenic centers in the isoquinoline moiety, the biaryl axis was the only stereogenic element of the new alkaloid. Its absolute axial configuration (Fig. 5) was easily determined to be *M* due to its opposite ECD spectrum compared to that of the 5,1′-coupled ancistrocladeine (**23**),[38] a known, likewise fully dehydrogenated, but *P*-configured alkaloid previously obtained by semi-synthesis from ancistrocladine.[38,39] The new alkaloid thus possesses the structure **12**. It was named ancistrolikokine G.

HPLC analysis of this new metabolite of *A. likoko* on a chiral phase (Chiralcel® OD-H) resulted in only one peak, and the ECD spectra recorded at different positions of the peak (*e.g.* left or right slope) were all identical, suggesting that this fully dehydrogenated naphthylisoquinoline alkaloid **12** is apparently produced by *A. likoko* in an enantiomerically pure form.

Non-hydrogenated, fully aromatic naphthylisoquinoline alkaloids have as yet been found quite rarely in nature. While some of them were reported to occur in the plants in a completely racemic form,[4,32,40–43] other representatives of this subtype were found to be optically active.[16,30–33] Ancistrolikokine G (**12**) is the first naturally occurring such merely axially chiral alkaloid possessing a 5,8′-biaryl linkage – a remarkable finding with respect to the fact that the metabolite pattern of most of the Central African *Ancistrocladus* plants[20–28] is dominated by 5,8′-coupled – yet always hydrogenated – naphthylisoquinoline alkaloids.

Resolution of one of the more nonpolar CC fractions by preparative RP-HPLC yielded five further pure new naphthylisoquinoline alkaloids. The ^1H (Table 3) and ^{13}C NMR (Table 4) data and specific NOE correlations (Fig. 6A) of these

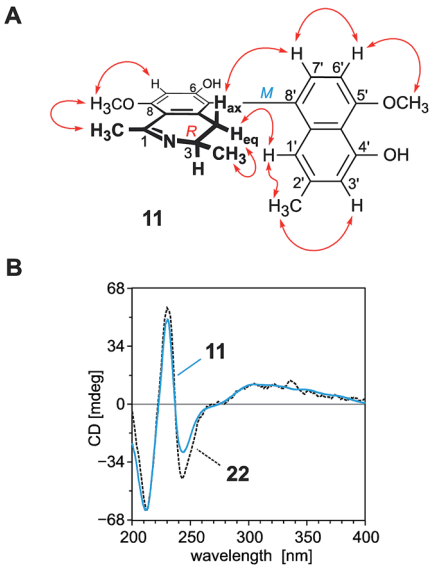

Fig. 4 (A) NOESY interactions indicative of the configuration at the biaryl axis of ancistrolikokine F (**11**) relative to the stereogenic center; (B) confirmation of the absolute axial configuration of **11** by comparison of its ECD spectrum with that of the known[18] related 5,8′-coupled alkaloid 6-*O*-demethylancistectorine D (**22**), for its structure, see Fig. 3.

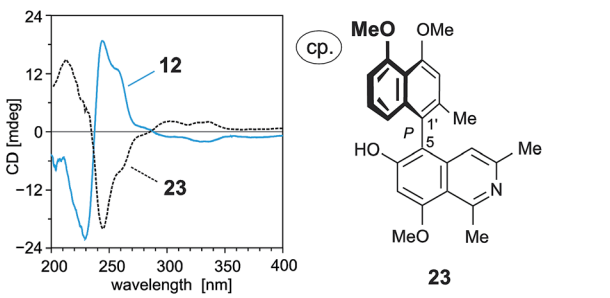

Fig. 5 Assignment of the absolute axial configuration of ancistrolikokine G (**12**), by comparison of its ECD spectrum with that of the known *P*-configured ancistrocladeine (**23**).[38,39]

Table 3 ^{1}H NMR data of ancistrolikokines A_2 (**13**), A_3 (**14**), C_2 (**5**), H_1 (**15**), and H_2 (**16**) in methanol-d_4 (600 MHz, δ in ppm, J in Hz)

No.	13	14	5	15	16
1	4.73, q	4.73, q	4.76, q	4.65, q	4.68, q
3	3.96, m	3.98, m	3.95, m	3.21, m	3.17, m
4_{ax}	2.03, dd (11.8, 18.5)	2.04, dd (11.7, 18.6)	2.04, dd (11.8, 18.3)	2.56, dd (11.4, 17.3)	2.59, dd (11.4, 17.3)
4_{eq}	2.64, dd (5.0, 18.6)	2.63, dd (4.9, 18.6)	2.25, dd (5.1, 18.3)	2.22, dd (2.9, 17.3)	2.24, dd (3.0, 17.3)
7	6.60, s	6.61, s	6.78, s	6.55, s	6.75, s
1′	6.61, s	6.60, s	6.51, s	6.61, s	6.58, s
3′	6.79, s	6.68, s	6.64, s	6.78, s	6.78, s
6′	6.81, d (7.8)	6.94, d (7.9)	6.90, d (7.9)	6.76, d (7.8)	6.76, d (7.8)
7′	7.11, d (7.8)	7.16, d (7.9)	7.06, d (7.9)	7.02, d (7.8)	7.03, d (7.8)
1-CH_3	1.67, d (6.7)	1.68, d (6.7)	1.70, d (6.7), s	1.77, d (6.6)	1.72, d (6.6)
3-CH_3	1.19, d (6.5)	1.19, d (6.5)	1.23, d (6.5)	1.19, d (6.5)	1.27, d (6.5)
N-CH_3	2.70, s	2.70, s	2.75, s	3.03, s	3.03, s
2′-CH_3	2.30, s	2.28, s, s	2.23, s	2.29, s	2.29, s
6-OCH_3			4.00, s	3.57, s	3.65, s
8-OCH_3	3.90, s	3.92, s	3.66, s		3.99, s
4′-OCH_3	4.08, s	3.92, s, s		4.08, s	4.08, s
5′-OCH_3		3.95, s	4.10, s		

Table 4 ^{13}C NMR data of ancistrolikokines A_2 (**13**), A_3 (**14**), C_2 (**5**), H_1 (**15**), and H_2 (**16**) in methanol-d_4 (150 MHz, δ in ppm)

No.	13	14	5	15	16
1	59.6	59.6	59.6	62.4	62.2
3	50.5	50.6	50.5	60.4	60.5
4	29.2	29.1	29.6	34.1	34.3
5	120.0	120.4	121.8	120.7	121.8
6	157.5	157.4	158.5	159.7	160.1
7	99.0	98.9	95.5	98.8	95.9
8	158.2	158.2	160.0	155.7	157.9
9	111.8	111.9	112.9	113.5	115.2
10	132.2	132.3	132.1	134.3	134.6
1′	118.4	117.5	116.3	119.0	119.0
2′	137.8	138.1	139.0	137.2	137.1
3′	107.4	109.9	113.0	107.5	107.6
4′	158.1	158.9	156.0	157.9	158.0
5′	155.9	158.4	157.2	155.7	155.8
6′	110.5	106.7	104.4	110.1	110.3
7′	131.3	130.2	129.4	131.5	131.6
8′	124.2	126.0	127.7	124.7	124.8
9′	137.1	137.6	136.8	136.8	137.0
10′	115.0	117.6	114.7	114.6	114.8
1-CH_3	19.1	19.0	19.2	19.3	19.8
3-CH_3	16.5	16.2	16.7	17.9	18.0
N-CH_3	33.8	33.9	34.1	41.2	41.6
2′-CH_3	22.1	22.0	21.8	22.0	22.3
6-OCH_3			56.3	55.8	56.4
8-OCH_3	56.1	56.1	56.2		56.4
4′-OCH_3	56.7	56.9		56.7	57.0
5′-OCH_3		56.7	56.6		

compounds hinted at the presence of naphthyltetrahydroisoquinolines, again belonging to the 5,8′-coupling type. The NMR data clearly revealed a great structural similarity of these metabolites, which only differed by their OH/OMe substitution patterns and the relative configuration in their tetrahydroisoquinoline portions.

The first compound within this series of new metabolites had a molecular formula of $C_{25}H_{29}NO_4$, as evidenced by HRESIMS. It was found to possess two methoxy groups (δ 3.90 and 4.08), which were attributed to be located at C-4′ and C-8, three *C*-methyl groups (δ 1.19, 1.67, and 2.30), and a three-proton singlet resonating at δ 2.70, typical of an *N*-CH_3 group. From a ROESY correlation between H-3 and CH_3-1 (Fig. 6B), 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,[37] were found to be *R*, both for C-1 and C-3. On the basis of the known configuration at the stereocenters, the relative and thus also the absolute configuration at the biaryl axis was deduced from ROESY interactions between H_{ax}-4 and H-1′, and between H_{eq}-4 and H-7′, showing the axis to be *P*-configured (Fig. 6B, top). This attribution was further confirmed by the fact that the ECD spectrum of the isolated compound was opposite to that of the *M*-configured, and likewise 5,8′-coupled ancistrolikokine C (4)[22] (Fig. 6C, left). Thus, the new naphthyltetrahydroisoquinoline possessed the stereostructure **13** and was hence the 6-*O*-demethyl-8-*O*-methyl analog of ancistrolikokine A (**2**), which is a main alkaloid of *A. likoko*.[22] It was therefore named ancistrolikokine A_2. Alternately, the as yet unknown metabolite **13** might be addressed as the 8-*O*-methyl analog of the co-occurring ancistrocongoline A (**18**).[25]

The second new compound within this series of five *N*-methylated naphthylisoquinoline alkaloids isolated from the twig extract of *A. likoko* corresponded to a molecular formula of $C_{26}H_{31}NO_4$, according to HRESIMS. The most significant difference within the NMR data compared to those of **13** (Table 3) was an upfield-shifted signal of the methoxy group at C-5' (δ 3.95) monitored for the new compound. Like in **13**, the relative configuration at C-1 *versus* C-3 was deduced to be *trans* from specific HMBC and NOE interactions, and again the absolute configurations were assigned to be 1*R*,3*R* by ruthenium-mediated oxidative degradation.[37] ECD spectroscopy established the compound to be *P*-configured at the biaryl axis (see ESI†). The alkaloid thus had the stereostructure **14** as presented in Fig. 1. It was the new 5′-*O*-methyl analog of **13**, and

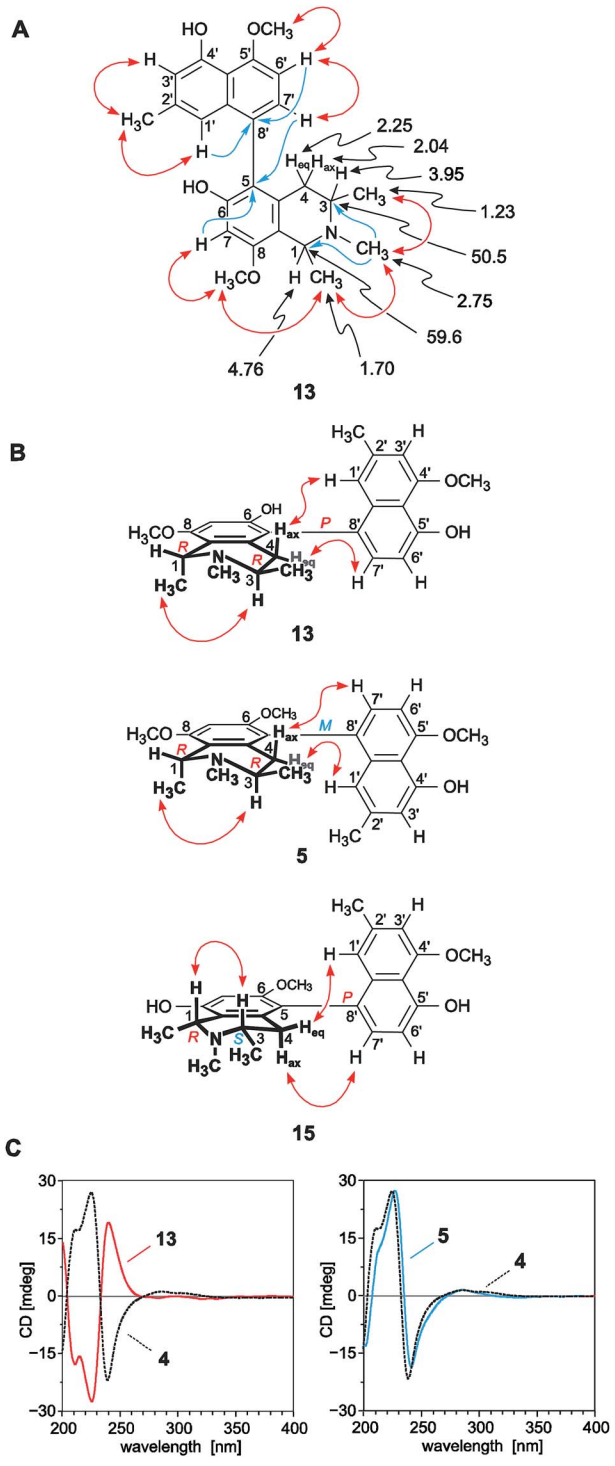

Fig. 6 (A) Selected ^{1}H and ^{13}C NMR data (δ in ppm), HMBC (single blue arrows) and NOE (double red arrows) interactions of ancistrolikokine A_2 (**13**); (B) NOESY correlations evidencing the relative configuration of **13** (top), ancistrolikokine C_2 (**5**) (middle), and ancistrolikokine H (**15**) (bottom) at the biaryl axis and at the stereogenic centers C-1 and C-3 in the isoquinoline part; (C) assignment of the absolute axial configuration of **13** (left) and **5** (right) by comparison of their CD spectra with that of the known[22] *P*-configured ancistrolikokine C (**4**).

was thus named ancistrolikokine A_3 (**14**). It could likewise be addressed as the 5′,8-*O*,*O*-dimethyl analog of the co-occurring ancistrocongoline A (**18**).[25]

HRESIMS analysis of the third of these five *N*-methylated naphthylisoquinoline alkaloids gave the same molecular formula of $C_{26}H_{31}NO_4$ as determined for ancistrolikokine A_3 (**14**). NMR measurements revealed that this new metabolite, different from **14**, possessed a free hydroxy function at C-4′ and a methoxy group at C-6 (Table 3). Similar to **13** and **14**, the two methyl groups at C-1 and C-3 were *trans* to each other, and again, both C-1 and C-3 were *R*-configured, as obvious from the results of the oxidative degradation.[37] Long-range NOE interactions between H_{ax}-4 and H-7′, and between H_{eq}-4 and H-1′, evidenced the biaryl axis to be *M*-configured (Fig. 6B, center). This was confirmed by the nearly identical ECD spectrum of the new alkaloid compared to that of the likewise *M*-configured ancistrolikokine C (**4**)[22] (Fig. 6C, right). The new compound thus had the stereostructure **5** as displayed in Fig. 1. It is the 6-*O*-methyl analog of the co-occurring ancistrolikokine C (**4**)[22] and was henceforth named ancistrolikokine C_2.

The molecular formula of the fourth new *N*-methylated alkaloid was $C_{25}H_{29}NO_4$, as established by HRESIMS. The NMR data were typical of a 5,8′-coupled naphthyltetrahydroisoquinoline equipped with two methoxy groups at C-4′ and C-6 (δ 4.08 and 3.57), leaving the other two oxygenated carbon atoms, C-5′ and C-8, to bear free hydroxy functions. The constitution and the spectroscopic data of this new natural product, except for the methoxy group at C-6, were in good agreement with those of the known[27] alkaloid korupensamine D from *A. korupensis*. From an NOE correlation between H-1 (δ 4.65) and H-3 (δ 3.21) the relative configuration at C-1 *versus* C-3 was deduced to be *cis* (Fig. 6B, bottom). The formation of *S*-*N*-methyl-3-aminobutyric acid by oxidative degradation[37] proved the new metabolite to be *S*-configured at C-3 and, given the relative *cis*-configuration at C-1 and C-3 in the tetrahydroisoquinoline moiety, indicated C-1 to be *R*-configured. NOESY interactions between H-4_{eq} and H-1′, and between H-4_{ax} and H-7′, in conjunction with the *S*-configuration at C-3, unequivocally confirmed the biaryl axis to be *P*-configured. This alkaloid thus had the full absolute stereostructure **15** as shown in Fig. 1 and was, hence, new. In continuation of the series of new alkaloids of the 5,8′-coupling type discovered in *A. likoko*, it was named ancistrolikokine H. This compound can likewise be addressed as 6-*O*-methylkorupensamine D.

The last isolated new alkaloid was found to possess a molecular formula of $C_{26}H_{31}NO_4$, showing **14** additional mass units compared to **15** (corresponding to an extra CH_2 portion). Its ^{1}H and ^{13}C NMR spectra were very similar to those of **15**, the only structural difference being the presence of a further *O*-methyl group in the isoquinoline moiety of the new metabolite. By NOESY measurements, this methoxy group was established to be located at C-8. By oxidative degradation and specific NOE interactions, the isoquinoline was, similar to **15**, again found to be 1*R*,3*S*-configured. Like for **15**, NOE interactions revealed the axis to be *P*-configured, corroborated by ECD spectroscopy (see ESI†). The results showed the compound to possess structure **16** as presented in Fig. 1, and, thus, to be the likewise new 8-*O*-

methyl analog of **15**; it was named ancistrolikokine H_2. The new metabolite can also be addressed as the 5′-*O*-demethyl derivative of the co-occurring known[29] alkaloid ancistrobertsonine C (**19**) (Fig. 2).

Preferential cytotoxicity of ancistrolikokines and related alkaloids against PANC-1 human pancreatic cancer cells

A series of **15** monomeric 5,8′-linked naphthylisoquinoline alkaloids (**1a**, **1b**, **3–5**, **9–11**, **13–19**), the dimer michellamine A (**20**), and the tetralone *cis*-isoshinanolone (**8**) from the twigs of *A. likoko* were investigated for their cytotoxic activities against the PANC-1 human pancreatic cancer cell line in normal nutrient-rich Dulbecco's modified Eagle's medium (DMEM) and nutrient-deprived medium (NDM), following the antiausterity strategy.[44,45]

Despite great research efforts to better understand the pancreatic tumor microenvironment, the prognosis of this severe malignant cancer disease still remains dismal. A particular problem is that treatment suffers from the rapid development of multidrug-resistance of the tumor cells towards conventional chemotherapeutic agents,[46,47] and from the fact that the pancreatic cancer cells show a high tolerance against extreme nutrition starvation, enabling them to proliferate aggressively under extremely hypovascular (austerity) and hypoxic conditions.[45,48] Accordingly, novel treatment strategies against pancreatic cancer are urgently needed.

The antiausterity approach[44,45] aims at the identification and development of new targeted agents[44,49–51] with preferential cytotoxicity to pancreatic tumor cells under nutrient-deficient conditions. The preferential cytotoxicites are represented as PC_{50} values, *i.e.*, the concentration at which 50% of the pancreatic cancer cells were killed in NDM without exhibiting toxicity at normal nutrient-rich medium.

All of the naphthylisoquinoline alkaloids tested displayed moderate to strong preferential cytotoxicities against the PANC-1 cell line, with micromolar PC_{50} values ranging from 4.89 to 110.1 μM (Table 5). Ancistrolikokine H_2 (**16**) exerted the most potent effects in NDM, with a PC_{50} value of 4.89 μM (Fig. 7). The test results showed that the OMe/OH substitution patterns of the isoquinoline portion seem to play a crucial role for the cytotoxic potential within this series of 5,8′-coupled naphthylisoquinoline alkaloids. Ancistrolikokine E (**9**), with two hydroxy functions at C-6 and C-8, showed only moderate preferential cytotoxicity (PC_{50}, 61.5 μM), whereas ancistrolikokine E_2 (**10**) with its mixed 6-OH/8-OMe-substituted dihydroisoquinoline subunit, displayed a very good activity against the PANC-1 cells (PC_{50}, 18.1 μM). This tendency was supported, on the one hand, by the likewise strong preferential cytotoxicity of the ancistrolikokines C_2 (**5**) and H_2 (**16**) (PC_{50}, 10.1 and 4.89 μM), equipped with two *O*-methyl groups at C-6 and C-8, and, on the other hand, by the only moderate to weak activities of the korupensamines A (**1a**) and B (**1b**) (PC_{50}, 110.1 and 94.9 μM), possessing a 6,8-dihydroxy substituted tetrahydroisoquinoline half. By contrast, a distinct influence of the oxygenation pattern of the naphthalene part at C-4′ and C-5′ (OMe/OH *vs.* OH/OMe) was not found, as obvious from nearly similar preferential cytotoxicities of **5** and **16** (PC_{50}, 10.1 and 4.89 μM) and **10** and **11** (PC_{50}, 18.1 and 20.8 μM).

Furthermore, the test results indicate that the degree of dehydrogenation in the isoquinoline portion had no significant impact on the biological activity of this series of 5,8′-linked naphthylisoquinoline alkaloids, since among the most active compounds (PC_{50} < 30 μM), there were naphthyldihydroisoquinolines like **10** and **11**, but also naphthyltetrahydroisoquinolines like **3–5** and **17**. *N*-Methylation of the alkaloids did not seem to be an important structural element either, as suggested by the nearly identical PC_{50} values observed for korupensamine E (**17**) (PC_{50}, 7.72 μM) and its *N*-methylated analog ancistrolikokine C (**4**) (PC_{50}, 7.11 μM).

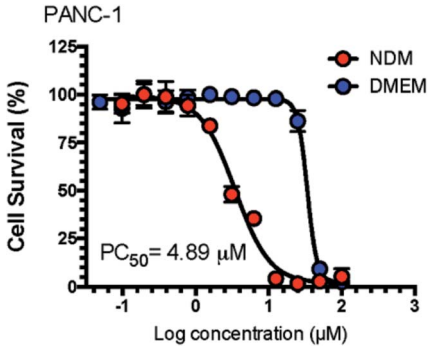

Fig. 7 Preferential cytotoxic activity of ancistrolikokine H_2 (**16**) against the PANC-1 human pancreatic cancer cell line in nutrient-deprived medium (NDM) and Dulbecco's modified Eagle's medium (DMEM).

Table 5 Preferential cytotoxicity of the monomeric naphthylisoquinoline alkaloids **1a**, **1b**, **3–5**, **9–11**, **13–19**, the dimer **20**, and the tetralone **8** on human pancreatic cancer PANC-1 cells in NDM

Compound	PC_{50}[a] (in μM)	Compound	PC_{50}[a] (in μM)
1a	110.1	**14**	40.2
1b	94.9	**15**	26.8
3	9.74	**16**	4.89
4	7.11	**17**	7.72
5	10.1	**18**	20.0
9	61.5	**19**	28.3
10	18.1	**20**	41.5
11	20.8	**8**	41.7
13	28.9	arctigenin[b]	0.475

[a] Concentration at which 50% of the cells were killed preferentially in NDM. [b] Used as a reference compound.

Experimental

General experimental procedures

Optical rotations were recorded on a Jasco P-1020-polarimeter operating with a sodium light source (λ = 589 nm). IR spectra were measured on a Jasco FT/IR-410 spectrometer, and UV spectra were taken on a Shimadzu UV-1800 spectrophotometer. ECD measurements were performed under nitrogen on a Jasco J-715 spectrometer. 1D and 2D NMR measurements were done

on a Bruker Avance III HD 600 MHz instrument, using methanol-d_4 (δ_H 3.31 and δ_C 49.15 ppm) as the solvent. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). Multiplicities of NMR signals are given as singlet (s), doublet (d), doublet of doublets (dd), quartet (q), or multiplet (m). Spectra were acquired and processed using an ACD/NMR processor (version 12.01) and the Topspin 3.2 software (Bruker Daltonics). HRESIMS measurements were performed in positive mode on a Bruker Daltonics micrOTOF-focus mass instrument. HPLC-DAD investigations were conducted on a Jasco LC-2000 Plus Series System. For LC-MS analysis, an Agilent 1100 Series System was used. 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 isolation and purification of the plant extracts, a SymmetryPrep C18 column (19 × 300 mm, 7 μm, Waters) was used, flow rate 10 mL min^{-1}; mobile phases: (A) 90% H_2O with 10% MeCN (0.05% trifluoroacetic acid) and (B) 90% MeCN with 10% H_2O (0.05% trifluoroacetic acid), using linear gradients (details given below). GC-MS analysis was carried out on a Shimadzu GC-MS-QP 2010 instrument. For maceration, a mechanical shaker was used at 160 RPM (rotations per minute). All organic solvents were of analytical-grade quality. Ultra-pure water was obtained from an Elga Purelab Classic system.

Plant material

Twigs of *Ancistrocladus likoko* J. Léonard (Ancistrocladaceae) were collected by one of us (V. M.) in August 1997, in the Northern Congo Basin, in the area near the town of Yangambi, Province Orientale, Democratic Republic of the Congo. A voucher specimen (no. 16) has been deposited at Herbarium Bringmann, University of Würzburg.

Extraction and isolation

Air-dried twigs of *A. likoko* (200 g) were ground to a fine powder, and extracted three times with CH_2Cl_2/MeOH (1 : 1, v/v) at room temperature. The crude extract was concentrated *in vacuo* to give *ca.* 22.1 g of a solid residue, which was dissolved in MeOH/H_2O (9 : 1, v/v), and, after filtration, further purified by liquid/liquid partition with *n*-hexane to remove non-polar compounds. The raw extract (*ca.* 7.8 g) was directly subjected to column chromatography (CC) on silica gel using a gradient of CH_2Cl_2/MeOH (90 : 10 to 55 : 45) giving rise to 17 fractions (F1–F17).

Fraction 5 (F5, 80 mg) was resolved on a silica gel column with *n*-hexane/CH_2Cl_2 and CH_2Cl_2/MeOH gradients and further purified by preparative HPLC, applying a linear gradient (0 min 10% B, 16 min 55% B), finally providing *cis*-isoshinanolone (**8**) (1.0 mg) (retention time 15.1 min).

From fraction 9 (F9, 500 mg), six alkaloids were isolated by isocratic HPLC with 26% aqueous MeCN acidified with 0.05% trifluoroacetic acid, *viz.*, ancistrolikokine A_3 (**14**) (2 mg) (retention time 14.3 min), ancistrolikokine A_2 (**13**) (4.0 mg) (retention time 18.0 min), ancistrolikokine H (**15**) (3.0 mg) (retention time 20.2 min), ancistrobertsonine C (**19**) (2 mg) (retention time 28.5 min), ancistrolikokine H_2 (**16**) (5.0 mg) (retention time 39.8 min), and ancistrolikokine C_2 (**5**) (2.0 mg) (retention time 48.0 min).

Fraction 10 (F10, 1.07 g) was repeatedly subjected to CC on silica gel with *n*-hexane/CH_2Cl_2 and CH_2Cl_2/MeOH (90 : 10 to 55 : 45) as the eluent yielding 24 subfractions ($F10_1$–$F10_{24}$), which were further resolved by preparative HPLC. From $F10_{17}$, pure ancistrolikokine B (**3**) (3.0 mg) (retention time 11.7 min) and korupensamine E (**17**) (4.0 mg) (retention time 12.2 min) were obtained, by applying the following gradient: 0 min 25% B, 10 min 45% B, 19 min 49% B, 19 min 49% B, 25 min 55% B, 26 min 55% B. Purification of $F10_{21}$ under gradient conditions (0 min 10% B, 11 min 40% B, 27 min 45% B) furnished ancistrolikokine C (**4**) (5.0 mg) (retention time 16.2 min), while resolution of $F10_{16}$ using a modified solvent system (0 min 2% B, 5 min 10% B, 8 min 20% B, 12 min 35% B, 14 min 36.4% B, 24 min 37.5% B, 35 min 65%) yielded ancistrolikokine G (**12**) (0.6 mg) (retention time 21.1 min).

Fraction 12 (F12, 300 mg) was further purified by preparative HPLC, using the following gradient: 0 min 20% B, 20 min 37% B, 24 min 60% B, finally giving rise to ancistrocongoline A (**18**) (10.0 mg) (retention time 17.9 min), ancistrolikokine E_2 (**10**) (6.0 mg) (retention time 20.0 min), and ancistrolikokine F (**11**) (10.0 mg) (retention time 20.2 min).

From fraction 13 (F13, 1.6 g), korupensamine A (**1a**) (23 mg) (retention time 18.0 min) and korupensamine B (**1b**) (16 mg) (retention time 19.0) were obtained by HPLC under gradient conditions (0 min 10% B, 11 min 25% B, 27 min 55% B). Using a linear gradient (0 min 25% B, 12 min 30% B, 24 min 55% B), preparative HPLC of fraction 15 (F15, 170 mg) provided pure ancistrolikokine E (**9**) (3.0 mg) (retention time 20.8 min).

The most polar fraction 17 (F17, 1.1 g) was directly submitted to HPLC, using a linear gradient (0 min 20% B, 14 min 37% B, 16 min 47% B, 23 min 100% B) to give michellamine A (**20**) (2.0 mg) (retention time 12.0 min) and michellamine A_2 (**21**) (0.5 mg) (retention time 12.5 min).

Ancistrolikokine A_2 (13). Yellow, amorphous solid; $[\alpha]_D^{25}$ −15.6 (*c* 0.05, MeOH); UV (MeOH) (log ε): λ_{max} 230 (4.1) nm; ECD (*c* 0.1, MeOH) λ_{max} ($\Delta\varepsilon$): 194 (+2.5), 196 (+2.8), 210 (−3.0), 214 (−2.9), 224 (−4.9), 239 (+3.1), 281 (−0.1), and 336 (−0.1) nm; IR (ATR) ν_{max}: 3377, 2936, 2850, 1671, 1196, 1175, 1121, 1022, 831, and 719 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS: *m*/*z* 408.2177 $[M + H]^+$ (calcd for $C_{25}H_{30}NO_4$, 408.2173).

Ancistrolikokine A_3 (14). Yellow, amorphous solid; $[\alpha]_D^{25}$ −15.3 (*c* 0.05, MeOH); UV (MeOH) (log ε): λ_{max} 231 (4.0) nm; ECD (*c* 0.1, MeOH) λ_{max} ($\Delta\varepsilon$): 210 (−1.0), 225 (−1.6), 239 (+1.1), 282 (−0.03), and 328 (−0.03) nm; IR (ATR) ν_{max}: 3379, 2943, 1671, 1198, 1127, 1067, 833, 799, and 721 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS: *m*/*z* 422.2328 $[M + H]^+$ (calcd for $C_{26}H_{32}NO_4$, 422.2331).

Ancistrolikokine C_2 (5). Yellow, amorphous solid; $[\alpha]_D^{25}$ +1.9 (*c* 0.05, MeOH); UV (MeOH) (log ε): λ_{max} 233 (4.3) nm; ECD (*c* 0.1, MeOH) λ_{max} ($\Delta\varepsilon$): 195 (−1.1), 201 (−3.0), 211 (+2.5), 226 (+6.3), 240 (−4.3), 284 (+0.3), and 334 (−0.1) nm; IR (ATR) ν_{max}: 3377, 2947, 2839, 1671, 1196, 1131, 1086, 1021, 801, and 717 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; *m*/*z* 422.2326 $[M + H]^+$ (calcd for $C_{26}H_{32}NO_4$, 422.2331).

Ancistrolikokine E (9). Yellow, amorphous solid; $[\alpha]_D^{25}$ −14.1 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 230 (3.6) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 194 (−1.8), 196 (−1.0), 198 (+0.3), 214 (+23.8), 229 (−15.2), 246 (+6.8), 260 (+2.0), 293 (−1.2), 322 (−8.9), and 350 (−3.3) nm; IR (ATR) ν_{max}: 3115, 2946, 1674, 1577, 1432, 1260, 1190, 1132, 839, 798, and 722 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS: *m*/*z* 378.1733 $[M + H]^+$ (calcd for $C_{23}H_{24}NO_4$, 378.1705).

Ancistrolikokine E_2 (10). Yellow, amorphous solid; $[\alpha]_D^{25}$ −41.1 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 230 (3.8) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 191 (+4.4), 201 (+0.2), 212 (+1.3), 228 (−1.4), 243 (+0.7), and 331 (−0.4) nm; IR (ATR) ν_{max}: 3310, 2974, 1674, 1575, 1434, 1201, 1260, 1132, 837, 798, and 721 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS: *m*/*z* 392.1858 $[M + H]^+$ (calcd for $C_{24}H_{26}NO_4$, 392.1861).

Ancistrolikokine F (11). Yellow, amorphous solid; $[\alpha]_D^{25}$ +5.4 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 233 (3.7) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 193 (−0.9), 195 (−1.2), 211 (−6.8), 230 (+5.1), 243 (−3.0), 259 (−0.4), 303 (+1.2), 349 (+0.8), and 377 (+0.4) nm; IR (ATR) ν_{max}: 3387, 2936, 2842, 1671, 1574, 1194, 1181, 1127, 836, 798, and 717 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS: *m*/*z* 392.1864 $[M + H]^+$ (calcd for $C_{24}H_{26}NO_4$, 392.1861).

Ancistrolikokine G (12). Yellow, amorphous solid; $[\alpha]_D^{25}$ +3.9 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 229 (3.5) and 258 (3.2) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 191 (−3.0), 201 (−0.5), 211 (−0.3), 229 (−1.7), 244 (+1.5), 259 (+0.9), 270 (+0.1), and 330 (−0.1) nm; IR (ATR) ν_{max}: 3359, 2947, 1670, 1615, 1430, 1194, 1181, 1129, 1067, 835, and 719 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; HRESIMS: *m*/*z* 390.1702 $[M + H]^+$ (calcd for $C_{24}H_{24}NO_4$, 390.1705).

Ancistrolikokine H (15). Yellow, amorphous solid; $[\alpha]_D^{25}$ −13.3 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 233 (3.8) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 201 (+1.2), 211 (−4.2), 222 (−4.6), 237 (+5.2), 250 (+1.9), 256 (+1.2), 285 (−0.4), and 345 (+0.1) nm; IR (ATR) ν_{max}: 3367, 2921, 2854, 1670, 1201, 1132, 800, and 721 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS: *m*/*z* 408.2177 $[M + H]^+$ (calcd for $C_{25}H_{30}NO_4$, 408.2174).

Ancistrolikokine H_2 (16). Yellow, amorphous solid; $[\alpha]_D^{25}$ +8.9 (*c* 0.05, MeOH); UV (MeOH) (log *ε*): λ_{max} 229 (4.0) nm; ECD (*c* 0.1, MeOH) λ_{max} (Δ*ε*): 200 (+11.5), 210 (−11.0), 225 (−27.4), 238 (+25.0), 252 (+10.2), 285 (−1.6), 303 (−1.2), and 388 (−0.2) nm; IR (ATR) ν_{max}: 3385, 2947, 1673, 1325, 1198, 1179, 1127, 1023, 835, 798, and 719 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3 and 4; HRESIMS: *m*/*z* 422.2332 $[M + H]^+$ (calcd for $C_{26}H_{32}NO_4$, 422.2331).

Known alkaloids isolated

Six known naphthylisoquinoline alkaloids (Fig. 2), yet isolated for the first time from *A. likoko*, *viz.*, korupensamines B (**1b**) and E (**17**), ancistrocongoline A (**18**), ancistrobertsonine C (**19**), and michellamines A (**20**) and A_2 (**21**), were found to be identical in their spectroscopic, physical, and chromatographic behavior with the data reported previously.[20,25–29] Furthermore, korupensamine A (**1a**), ancistrolikokines B (**3**) and C (**4**), and *cis*-isoshinanolone (**8**) (Fig. 1), well-known main metabolites of *A. likoko*,[22,23] were isolated for biotesting.

Oxidative degradation

The ruthenium(III)-mediated periodate degradation of the ancistrolikokines A_2 (**13**), A_3 (**14**), C_2 (**5**), E (**9**), E_2 (**10**), F (**11**), H (**15**), and H_2 (**16**), the Mosher-type derivatization of the resulting amino acids using MeOH/HCl and *R*-α-methoxy-α-trifluoromethylphenylacetyl chloride (*R*-MTPA-Cl, prepared from *S*-MTPA), and the subsequent GC-MSD analysis were carried out as described earlier.[37]

Preferential cytotoxicity against PANC-1 cells

The monomeric naphthylisoquinoline alkaloids **1a**, **1b**, **3–5**, **9–11**, **13–19**, the dimer **20**, and the tetralone **8** (Table 5) were evaluated for their preferential cytotoxicity against PANC-1 human pancreatic cancer cells.[52] The PANC-1 (RBRC-RCB2095) human pancreatic cancer cell line was purchased from the Riken BRC cell bank and maintained in standard DMEM with 10% FBS supplement and stored at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air. Briefly, human pancreatic cancer cells were seeded in 96-well plates (1.5×10^4 per well) and incubated in fresh DMEM at 37 °C under 5% CO_2 and 95% air for 24 h. The cells were washed twice with PBS and the medium was changed to serially diluted test samples in both nutrient-rich medium (DMEM) and nutrient-deprived medium (NDM),[44] with a control and blank in each test plate. The composition of the NDM was as follows: 265 mg L^{-1} $CaCl_2 \cdot (2H_2O)$, 0.1 mg L^{-1} $Fe(NO_3)_3 \cdot (9H_2O)$, 400 mg L^{-1} KCl, 200 mg L^{-1} $MgSO_4 \cdot (7H_2O)$, 6400 mg L^{-1} NaCl, 700 mg L^{-1} $NaHCO_3$, 125 mg L^{-1} NaH_2PO_4, 15 mg L^{-1} phenol red, 25 mM L^{-1} HEPES buffer (pH 7.4), and MEM vitamin solution (Life Technologies, Inc., Rockville, MD, USA); the final pH was adjusted to 7.4 with 10% $NaHCO_3$. Arctigenin, the positive control in this study, was isolated from the seeds of *Arctium lappa*.[44] After 24 h of incubation of each of the investigated compounds in DMEM and NDM, the cells were washed twice with PBS and replaced by 100 μL of DMEM containing 10% WST-8 cell counting kit solution. After 3 h of incubation, the absorbance at 450 nm was measured on an EnSpire Multimode plate reader (PerkinElmer, Inc., Waltham, MA, USA). Cell viability was calculated from the mean values from three wells using the following equation:

$$\text{Cell viability (\%)} = [\text{Abs}_{(\text{test sample})} - \text{Abs}_{(\text{blank})}/\text{Abs}_{(\text{control})} - \text{Abs}_{(\text{blank})}] \times 100\%$$

Conclusions

Ancistrocladus likoko was revealed to be a rich source of predominantly monomeric 5,8′-linked naphthylisoquinoline alkaloids exhibiting a great structural variety with respect to their *O*-methylation patterns. While 15 of them are *R*-configured at C-3, thus belonging to the Ancistrocladaceae–Dioncophyllaceae hybrid type,[4,5] five of the alkaloids are Ancistrocladaceae-type metabolites,[4,5] *i.e.*, with *S*-configuration at C-3. Nine new representatives, among them the first alkaloid of this coupling type with a fully dehydrogenated isoquinoline

portion (compound **12**) and the first three naphthydihydroisoquinolines found in nature with *R*-configuration at C-3 (compounds **9–11**), have been isolated and structurally assigned from the twigs of *A. likoko*, together with six known[20,25–29] alkaloids previously identified in other African *Ancistrocladus* species. The structures of these naphthylisoquinolines are chemotaxonomically quite significant, corroborating the close phylogenetic relationship of *A. likoko* to other Central African *Ancistrocladus* species, in particular to *A. congolensis*[25,26] and *A. korupensis*,[20,27,28] which are likewise known to produce a high number of 5,8′-linked mono- and dimeric alkaloids. While the latter two species also produce naphthylisoquinolines with other coupling types like *e.g.*, 7,8′, 5,1′, or 7,1′,[25,26,53,54] all of the mono- and dimeric alkaloids isolated so far from *A. likoko* belong to the subclass of 5,8′-linked alkaloids exclusively. Thus, *A. likoko* occupies a special position within the *Ancistrocladus* genus, which, so far, comprise *ca.* 20 species: it is even the only taxon producing alkaloids solely with one same coupling type. The reason for this high coupling regioselectivity in the biosynthesis of naphthylisoquinolines in *A. likoko* is yet unknown.

Furthermore, this plant has become a promising source of potent bioactive compounds, with interesting preferential cytotoxicities against PANC-1 human cancer cells. The fact that pancreatic cancer is an aggressive malignant disease with a low survival rate, rapidly developing resistance to systemic therapies with conventional drugs, urgently demands the development of new anti-cancer agents. The promising activities of some of the ancistrolikokines warrant further investigations on the anti-cancer potential of naphthylisoquinoline alkaloids in general. More-in-depth studies towards this goal are presently in progress.

Conflicts of interest

There are no conflicts to declare.

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