Naphthylisoindolinone alkaloids: the first ring-contracted naphthylisoquinolines, from the tropical liana Ancistrocladus abbreviatus, with cytotoxic activity†‡§

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The West African liana Ancistrocladus abbreviatus is a rich source of structurally most diverse naphthylisoquinoline alkaloids. From its roots, a series of four novel representatives, named ancistrobrevolines A–D (14–17) have now been isolated, displaying an unprecedented heterocyclic ring system, where the usual isoquinoline entity is replaced by a ring-contracted isoindolinone part. Their constitutions were elucidated by 1D and 2D NMR and HR-ESI-MS. The absolute configurations at the chiral axis and at the stereogenic center were assigned by using experimental and computational electronic circular dichroism (ECD) investigations and a ruthenium-mediated oxidative degradation, respectively. For the biosynthetic origin of the isoindolinones from ‘normal’ naphthyltetrahydroisoquinolines, a hypothetic pathway is presented. It involves oxidative decarboxylation steps leading to a ring contraction by a benzilic acid rearrangement. Ancistrobrevolines A (14) and B (15) were found to display moderate cytotoxic effects (up to 72%) against MCF-7 breast and A549 lung cancer cells and to reduce the formation of spheroids (mammospheres) in the breast cancer cell line.

Introduction

Ancistrocladus abbreviatus Airy Shaw (Ancistrocladaceae) is a woody liana native to the coastal rainforests of West Africa.1–4 It is characterized by a plethora of structurally most diverse secondary metabolites belonging to the emerging class of naphthylisoquinoline alkaloids, with 280 as yet known representatives.5–9 From this tropical plant, no less than 69 such compounds have so far been isolated, many of them with unique molecular scaffolds.5,10–18 With 33 examples,5,10,11,13,14,17 naphthyltetrahydroisoquinoline alkaloids such as ancistrobrevine A (1), its 6-O-demethyl analogue 2,5,14 and ancistrobrevine D (3)5,11 (Fig. 1) constitute the major portion of compounds produced by the plant. Their molecular moieties are coupled via their 5,1′-, 5,8′-, 7,1′-, or 7,8′-positions. In all these metabolites, the biaryl axis is rotationally hindered, due to the presence of mostly bulky ortho-substituents, leading to the phenomenon of atropisomerism.5,7,19,20 Many of the alkaloids of A. abbreviatus are typical Ancistrocladaceae-type compounds (i.e., with S-configuration at C-3 and an oxygen function at C-6), such as compounds 1–3. But also metabolites with structural characteristics typical of alkaloids occurring in plants of the related Dioncophyllaceae family21–23 were isolated, viz., 3R-configured and lacking an oxygen function at C-6, such as dioncophyllinine A (4)24,25 and its N-methyl analogue 5,16 Even mixed, hybrid-type compounds like ancistrobrevine M (7; 3R, 6-OH)17 and dioncoline A (6; 3S, 6-H),17 possessing the opposite characteristics, were discovered in the plant. Ancistrocladus abbreviatus is the only Ancistrocladus species known to contain naphthylisoquinoline alkaloids of all these four subclasses. Likewise reported was the occurrence of nine naphthyldehydroytetroisoquinolines,5,12,14,15 six of which were 5,1′-coupled like ancistrobrevinid C (8),15 which was highly effective against the aggressive and metastatic growth of pancreatic cancer cells. The outstanding metabolite pattern of A. abbreviatus also comprises a unique series of seven alkaloids with a non-hydrogenated...
isoquinoline part, from all four coupling types mentioned above, with the biaryl axis as the only stereogenic element, among them ancistrobreveine D (9). Even though they are devoid of stereogenic centers, they are all optically active, occurring as enantiomerically pure compounds or as scalemic mixtures. The broad spectrum of alkaloids produced by A. abbreviatus is further enlarged by the occurrence of dimeric congeners like the C2-symmetric representative jozimine A2 (11; both halves 7,1′-coupled). In the roots of A. abbreviatus, three further symmetric and unsymmetric dimers were detected, with the parent compound jozimine A2 (11) being the most active agent against HT-29 colon cancer, HT-1080 fibrosarcoma, and MM.1S multiple myeloma cell lines. Likewise isolated were quinoid structures like ancistrobreviquinone A (10), possessing an ortho-naphthoquinone entity. Even more remarkable is the structure of the first 1-demethylated naphthylisoquinoline alkaloid, 1-nor-8-O-demethylancistro-breve H (12), lacking the otherwise generally present methyl group at C-1. This compound was isolated along with a series of unprecedented seco-alkaloids from the roots of A. abbreviatus, having undergone a cleavage of the heterocyclic ring, with an elimination of the entire C-1/Me-1 unit, among them ancistrosecoline D (13) (Fig. 1). This alkaloid exerted potent, and selective, cytotoxicity against HeLa cervical cancer cells by inducing apoptosis.

The ‘chemical creativity’ of A. abbreviatus is even larger, as shown in this report by the discovery of a series of metabolites constituting a novel subtype of axially chiral naphthylisoindolinone alkaloids, named ancistrobrevolines A–D (14–17). In these compounds, the six-membered heterocyclic part of the isoquinoline portion has undergone a ring contraction to give a five-membered heterocycle as part of an isoindolinone system, with loss of the whole C-3/Me-3 entity, which had so far been present in all other naphthylisoquinoline alkaloids. For the origin of the novel naphthylisoindolinones 14–17 in the plants, a biosynthetic concept is presented, involving the stepwise loss of two carbon atoms and a benzilic acid rearrangement, leading to the observed ring contraction. Ancistrobrevolines A (14) and B (15) showed

Fig. 1  Secondary metabolites produced by A. abbreviatus: the naphthyltetrahydroisoquinolines ancistrobrevine A (1) and its 6-O-methyl analogue 2, ancistrobrevine D (3), dioncophylline A (4) and its N-methyl congener 5, dioncoline A (6), and ancistrobrevine M (7), the ditydroisoquinoline ancistrobrevine C (8), the non-hydrogenated ancistrobreveine D (9), the naphthoquinone derivative ancistrobrevique H (10), the dimer jozimine A2 (11), the 1-unsubstituted 1-nor-8-O-demethylancistrobreve H (12), the ring-cleaved ancistrosecoline D (13), and the novel ancistrobrevolines A–D (14–17). – Ancistrocladaceae-type alkaloids (with 6-OR and 3S) are labeled in “blue–blue”, Dioncophyllaceae-type representatives (6-H and 3R) are marked in “red–red”, the hybrid-type (6-OR and 3R) ancistrobrevine M (7) in “blue-red”, and the only known17 inverse-hybrid-type (6 H, 3S) dioncoline (6) in “red-blue”. 

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moderate to good cytotoxic activities against lung and breast cancer cell lines.

Results and discussion

Isolation and structural elucidation of ancistrobrevolines A–D

Air-dried root bark material of *A. abbreviatus* collected in the Parc National de Taï in the Southwestern Côte d’Ivoire (Ivory Coast) was repeatedly extracted with MeOH. After filtration of the crude extract and evaporation of the solvent, the residual material was macerated with MeOH–H₂O (9 : 1, v/v), followed by liquid–liquid partitioning with *n*-hexane and fractionation by column chromatography on silica gel. HPLC-UV guided analysis of an alkaloid-rich fraction showed the presence of metabolites exhibiting UV spectra closely similar to those of naphthylisoquinoline alkaloids. They showed a first UV maximum at 224–229 nm, yet with an additional second UV maximum at 259–261 nm, resembling that of alkaloids with a fully dehydrogenated ring like ancistrobreveine D (9).

**Ancistrobrevoline A** (14). The first isolated compound had a molecular formula of C₂₅H₂₇NO₅, as indicated from its sodium adduct in HR-ESI-MS, C₂₅H₂₇NNaO₅ (m/z 444.17711 [M + Na]+). The ¹H and ¹³C NMR spectra (Table 1) showed predominantly signals typical of conventional naphthylisoquinoline alkaloids (Fig. 2A), in particular for the naphthalene part, with its usual 4′,5′-dimethoxy-2′-methyl substitution pattern, coupled via C-8′. The NMR data revealed a spin system with four aromatic methines, H-1′ (δ₁H 6.70), H-3′ (δ₁H 6.76), H-6′ (δ₁H 6.92), and H-7′ (δ₁H 7.16), a three-proton singlet (δ₁H, δ₂H, δ₃H 2.29, 22.0) evidencing the presence of an aryl-methyl group (Me-2′), and further singlets for two methoxy functions, MeO-4′ (δ₁H, δ₂H 3.92, 56.9) and MeO-5′ (δ₁H, δ₂H 3.95, 56.7). The two aromatic singlets (δ₁H 6.70 and 6.76) and an AB spin system of two adjacent protons (δ₁H 6.92 and 7.16) indicated that the coupling site of the biaryl axis was located in the methyl-free part of the naphthalene moiety, i.e., either at C-6′ or C-8′, which was in agreement with the normal, not high-field-shifted signal of Me-2′ (δ₁H 2.29). The NOESY correlation sequence {MeO-5′ ↔ H-6′ ↔ H-7′} excluded the biaryl axis from being located at C-3′, thus establishing C-8′ to be the axis-bearing carbon atom. This assignment was confirmed by HMBC long-range couplings from H-1′ (δ₁H 6.70) and H-6′ (δ₁H 6.92) to C-8′ (δ₁C 124.6), from H-7′ (δ₁H 7.16) to C-9′ (δ₁C 137.5), and from H-3′ (δ₁H 6.76) to C-1′ (δ₁C 118.8) (Fig. 2B).

Even the heterocyclic unit displayed, in part, the usual features as in other naphthylisoquinoline alkaloids, like the isocyclic benzene ring with the axis at C-7 (for the atom numbering applied, see ref. 26) and two methoxy functions at C-6 and C-8, as deduced from two singlets at δ₁H 3.67 and 3.24, each corresponding to three protons, and from an aromatic singlet appearing at δ₁H 7.21 (H-5) (Fig. 2A). The coupling pattern suggested the presence of a phenyl unit with no substituent at C-5, thus leaving C-7 (δ₁C 128.1) as the axis-bearing carbon atom. This assumption was corroborated by HMBC interactions of H-5

| Position | 14 (δ₁H in Hz) | 15 (δ₁C in ppm) | 16 (δ₁H in Hz) | 17 (δ₁C in ppm) |
|----------|----------------|----------------|----------------|----------------|
| 1        | 4.69, q        | 58.4           | 4.65, q        | 58.3           |
| 2        | 169.8          | 169.9          | 169.9          | 170.4          |
| 3        | 101.2          | 105.4          | 105.4          | 110.4          |
| 4        | 161.1          | 158.4          | 158.4          | 158.2          |
| 5        | 128.1          | 126.7          | 126.7          | 126.5          |
| 6        | 155.6          | 155.8          | 155.8          | 156.6          |
| 7        | 132.6          | 131.0          | 131.0          | 135.3          |
| 8        | 134.0          | 133.9          | 133.9          | 133.6          |
| 1'       | 6.70, s        | 118.8          | 6.80, s        | 119.1          |
| 2'       | 137.3          | 137.9          | 137.9          | 136.1          |
| 3'       | 6.76, s        | 109.6          | 6.77, s        | 109.7          |
| 4'       | 158.4          | 158.4          | 158.4          | 158.2          |
| 5'       | 158.3          | 158.5          | 158.5          | 158.6          |
| 6'       | 106.3          | 106.4          | 106.4          | 110.4          |
| 7'       | 130.3          | 130.6          | 130.6          | 126.7          |
| 8'       | 124.6          | 124.6          | 124.6          | 119.1          |
| 9'       | 137.5          | 137.4          | 137.4          | 138.1          |
| 10'      | 117.1          | 117.3          | 117.3          | 117.8          |
| 1-Me     | 1.54, d (6.6)  | 17.2           | 3.20, d (6.6)  | 17.2           |
| 2'-Me    | 2.29, s        | 22.0           | 2.30, s        | 22.0           |
| N-Me     | 3.15, s        | 27.1           | 3.13, s        | 27.1           |
| 6-OME    | 3.67, s        | 56.5           | 56.5           | 56.3           |
| 8-OME    | 3.24, s        | 60.6           | 3.23, s        | 60.5           |
| 4'-OME   | 3.92, s        | 56.9           | 3.92, s        | 56.9           |
| 5'-OME   | 3.95, s        | 56.7           | 3.96, s        | 56.7           |
A further most notable difference compared to usual naphthylisoquinolines was the presence of an additional oxygen function in that eastern part of the molecule. The $^{13}$C NMR data displayed a downfield-shifted three-proton singlet ($\delta_{\text{H}}$ 3.15) suggested the presence of an N-methyl group, which was in accordance with the observation of a NOESY correlation to the protons of the methyl group at C-1 and with an HMBC interaction from the protons of that methyl group (resonating at $\delta_{\text{H}}$ 3.15) to the aliphatic carbon atom C-1 ($\delta_{C}$ 58.4) (see structure III in Fig. 3).

The observed HMBC and NOESY interactions, while not fitting with the presence of an isoquinoline unit, gave conclusive evidence of a contracted ring in the new alkaloid, as a consequence of the loss of the carbon atom C-3, thus giving rise to a five-membered isoidolinone skeleton. This structural assignment presented in Fig. 3 (see structure V) was in agreement with the lack of the signal for C-3 in $^{13}$C NMR (normally resonating at $\delta_{C}$ 45–53) and with the appearance of a signal at 1673 cm$^{-1}$ in the IR spectrum, which is characteristic of a carbonyl group as part of an amide function. In conclusion, the new alkaloid had to be a 7,8′-coupled biaryl alkaloid, structurally differing from a conventional naphthylisoquinoline by its unprecedented isoidolinone part.

The absolute configuration at the stereocenter of the new alkaloid at C-1 in the isoidolinone ring system was determined by a ruthenium-mediated oxidative degradation procedure (see the ESI†) developed by us earlier.$^{6,27}$ Although this periodate oxidation method had initially been designed for the degradation of 1,3-disubstituted tetra- or dihydroisoquinolines, not for isoidoline alkaloids, with their stable C-N bond, the reaction luckily succeeded in this case, too, affording the simple and easy-to-analyze chiral amino acid N-methyl-D-alanine, hence establishing the absolute configuration at the stereocenter as R.

Unfortunately, it was not possible to assign the configuration at the axis relative to the stereocenter at C-1 by NMR, because no significant long-range NOESY interactions across the biaryl axis were observed, like between Me-1 and/or H-1 in the isoidolinone ring and H-1′ or H-7′ in the naphthalene half. Apparently the small ring size of the heterocycle leads to enlarged distances between the substituent at C-1 and the respective H atoms attached to the naphthalene part. Therefore, quantum-chemical electronic circular dichroism (ECD) calculations were performed for both possible atropo-diastereomers.

The experimental ECD spectrum of the isolated naphthylisoindolinone (Fig. 4) was very similar to the one calculated for the P-isomer (in red) at the TD$\omega$B97XD3/def2-TZVP/B3LYP-D3/def2-TZVP level, while being virtually opposite to the one predicted for M (in blue), which unambiguously showed that the new compound was P-configured and, thus, had the full absolute stereostructure 14, as presented in Fig. 1.
This novel natural product was given the name ancistrobrevoline A.

**Ancistrobrevoline B (15).** The second new minor root bark metabolite was spectroscopically almost identical to ancistrobrevoline A (14), with only slightly different chemical shifts (Table 1), thus evidencing the presence of another naphthylisoindolinone, again 7,8′-coupled, as attributed by the observed key HMBC and NOESY interactions (see the ESI†). The 1H NMR spectrum displayed a three-proton singlet at δH 3.13, hinting at an N–Me group, and the 13C NMR data revealed the presence of a downfield-shifted signal at δC 169.9, typical of the amidic carbonyl entity of the isoindolinone unit. This was in accordance with a carbonyl signal at 1678 cm⁻¹ in the IR spectrum of the new metabolite. HR-ESI-MS showed a protonated molecular-ion peak [M + H]+ at m/z 408.18150 (calcd for C24H26NO5+, 408.18055) and an [M + Na]+ peak at m/z 430.16117 (calcd for C24H25NNaO5+, 430.16249), thus having 14 mass units less than 14, suggesting that the isolated new metabolite might be an O-demethylated analogue of 14. In agreement with this assumption, the 1H NMR spectrum displayed singlets for only three O-methyl units, resonating at δH 3.13, 3.92, and 3.96 (Table 1). They were assigned to be located at C-8, C-4′, and C-5′, based on NOESY interactions with Me-1/H-1, H-3′, and H-6′.

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Fig. 3  Decisive 1H and 13C NMR shifts (in methanol-d₄, δ in ppm) and key NOESY (double red arrows) and HMBC (single blue arrows) interactions in structures I, III, and V, evidencing the structural elements of the heterocyclic ring in ancistrobrevoline A (14). The two geminal protons at C-4, the proton and the methyl group at C-3 (underlined in pink in structure II), typical of usual naphthyltetrahydroisoquinoline alkaloids, were not observed in the 1H NMR spectrum of 14. Deletion of the carbon atom C-3 (marked in pink in structure IV) leads to the formation of the isoindolinone subunit of 14 as shown in structure V.

Fig. 4  Assignment of the absolute configuration of ancistrobrevoline A (14) by comparison of its experimental ECD spectrum (in black, taken in methanol) with the ones calculated (A) for the (1R,7P)-isomer (ECD curve in red) and (B) for 1R,7M (in pale blue) using TDDFT-B97XD3/def2-TZVP//B3LYP-D3/def2-TZVP.
respectively, similar as for anciestrobrevoline A (14) (Fig. 2). Thus, different from 14, one of the oxygen functions in the new metabolite was a free hydroxy group at C-6. The ECD spectrum of the new compound (see the ESI†) matched well that of anciestrobrevoline A (14), proving that the absolute configuration at the biaryl axis was again R, as for 14. Likewise as in the case of 14, the degradation of the new compound (see Fig. 4) evidenced the full stereostructure of the new compound as presented in Fig. 1, hence being the 6-O-demethyl analogue of 14. It was named anciestrobrevoline B.

Ancistrobrevoline C (16). The third new compound was obtained as a yellow amorphous powder. According to HRESIMS and 13C NMR, it had a molecular formula of C23H27NO2, as deduced from its monoprotonated molecular ion, [M + H]+, at m/z 408.17974, identical to that of anciestrobrevoline B (15). Likewise as in the case of 14, the degradation established the absolute configuration at the stereocenter at C-1 as R by the formation of N-methyl-d-alanine. The new alkaloid thus had the absolute stereostructure 15 as presented in Fig. 1, hence being the 6-O-demethyl analogue of 14. It was named anciestrobrevoline B.

Ancistrobrevoline C (16). The third new compound was obtained as a yellow amorphous powder. According to HRESIMS and 13C NMR, it had a molecular formula of C23H27NO2, as deduced from its monoprotonated molecular ion, [M + H]+, at m/z 408.17974, identical to that of anciestrobrevoline B (15). Again, the 1H NMR spectrum showed signals for three C-methyl groups (δH 1.55, 2.09, and 3.14), for three methoxy functions (δH 3.64, 3.92, and 3.97), and for one aliphatic proton (δH 4.58) and five aromatic ones (δH 6.81, 6.85, 6.91, 7.01, and 7.18) (Fig. 5A). This, together with an IR band at 1675 cm⁻¹ and a downfield-shifted signal at δC 170.4 in 13C NMR, characteristic of an amide carbonyl entity, indicated the new minor metabolite to be yet another naphthylisoindoline alkaloid. Different from 15, the new compound exhibited a spin pattern of the aromatic protons consisting of two singlets, one doublet, and two doublets of doublets. This was in agreement either with a 5,1'- or a 7,1'-coupling, of which the 5,1'-linkage was excluded due to HMBC interactions of H-5 (δH 7.01) to C-4 (δC 170.4) and C-7 (δC 120.5) and a NOESY correlation between H-5 and MeO-6 (δH 3.64) in the isoindolinone part (Fig. 5B). This assignment was confirmed by NOESY correlation sequences in the series {H-8' ↔ H-7' ↔ MeO-5'} and {MeO-4' ↔ H-3' ↔ Me-2'} and by HMBC cross-peaks of Me-2' (δH 2.09) and H-8' (δH 6.81) to C-1' (δC 58.3) in the naphthalene moiety. The three methoxy groups were determined to be located at C-6, C-4', and C-5', based on NOESY interactions with their neighboring protons, H-5, H-3', and H-6', respectively (Fig. 5B). Another oxygenated carbon atom was found to be located in the phenyl ring, at C-8 (δC 151.9), bearing a free hydroxy function. The ruthenium-mediated oxidative degradation established the v-enantiomer of N-methylalanine, establishing the absolute configuration at the stereocenter as R.

The absolute axial configuration of the new alkaloid was deduced by comparison of its experimental ECD spectrum (full line in blue) with that of anciestrobrevoline A (14, dotted line in black). The new alkaloid differed from 14 only by the position of the methyl group in the naphthalene part or, in other words, by the coupling type (7,1' versus 7,8'), and by the substitution pattern at C-6 and C-8 (OMe/OH versus OH/OMe in 14). The ECD spectrum of the new alkaloid was virtually opposite to the one of 14 (Fig. 5D), suggesting that the larger part of the naphthalene unit should be directed down, i.e., with an opposite stereoorientation as compared to 14 and 15. According to the Cahn-Ingold-Prelog priority rules, however, the descriptor was again assigned as P, i.e., formally the same as for 14 and 15. The new compound thus had the full stereostructure 16 as presented in Fig. 5D. It was named anciestrobrevoline C.

Ancistrobrevoline D (17). The fourth compound was isolated as a yellow amorphous solid with a molecular formula of
C₄₂H₃₃NO₄, as established from the corresponding [M + Na]+ adduct, C₂₉H₂₃NNaO₄, at m/z 400.15033. ¹H and ¹³C NMR data (Table 1) revealed the new metabolite to be yet another 7,1'-linked naphthylisoindolinone alkaloid, with a constitution strongly resembling that of the above-described ancistrobrevoline C (16). Similar to 16, it displayed a 4',5'-dimethoxy-substituted naphthalene portion, as deduced by NOESY cross-peaks between MeO-4' and H-3' and between MeO-5' and H-6'. Likewise observed in 1D and 2D NMR were the typical structural features of the heterocyclic ring in the isoindolinone moiety, as outlined in Fig. 5C. The molecular weight of the new metabolite, however, was lower than those of 14–16, hinting at the presence of only four oxygen atoms. This gave rise to the assumption that the new alkaloid might be derived from the subclass of dioncophyllaceae-type compounds, which are characterized by the lack of an oxygen function at C-6 (and the presence of an R-configuration at C-3, which, however, does not apply here). Indeed, the most significant difference in the NMR spectrum of the new metabolite compared to that of 16 was the appearance of an additional aromatic proton, which was supposed to be the hydrogen atom at C-6. The coupling pattern of the aromatic protons showed one singlet (H-3'), two doublets (H-6' and H-8'), and one doublet of doublets (H-7') in the naphthalene part (Table 1), and two doublets resonating at δH 7.10 and δH 7.37, located in the isoindolinone portion. This assignment was in accordance with COSY interactions between H-5 (δH 7.37) and H-6 (δH 7.10), which showed ortho-coupling to each other (J = 7.5 Hz), and it was also confirmed by HMBC cross-peaks from H-6 to C-1' (δC 126.1), C-10 (δC 133.6), and C-8 (δC 151.5) (Fig. 5C). Oxidative degradation⁵⁻⁷⁷ established the absolute configuration at the stereocenter at C-1 as R. The ECD spectrum of the new alkaloid (Fig. 5E) was virtually opposite to that of ancistrobrevoline C (16), while resembling that of ancistrobrevolines A (14) and B (15) (see ESI†), hence establishing the absolute axial configuration as P. The new alkaloid consequently had the full absolute stereostructure 17 as presented in Fig. 5E. It was henceforth named ancistrobrevoline D.

**Proposed biosynthetic origin of naphthylisoindolinone alkaloids**

The structures of the discovered four novel alkaloids 14–17, with their isoindolinone parts connected to the naphthalene moieties via a stereogenic axis, are unprecedented, and differ substantially from previously described artificial naphthylisoindolinones.²⁸⁻⁹⁰ In the field of ‘normal’, amino-acid derived benzylisoquinoline alkaloids, some few other, yet naphthalene-devoid isoindolinones are known like aristoyagonine (18)³⁰⁻³² and aristolactam I (19)³⁸,³¹,³⁴ (Fig. 6A). Biosynthetically, 18 and 19 are assumed to be formed from the corresponding benzyltetrahydroisoquinoline alkaloids, by 3,4-dioxxygenation, followed by a benzilic acid rearrangement, with loss of a carbon atom and ring contraction.²⁸⁻³⁴

In an analogous way, the four novel naphthylisoindolinones 14–17 (general partial structure IV, see Fig. 6B) might arise from the respective naphthyltetrahydroisoquinoline alkaloids I, via the corresponding 3,4-diketones III – yet with the additional necessity of eliminating the methyl group at C-3, maybe via II, or possibly via the 3-oxo analogues. The loss of the C-3 methyl group might occur by a stepwise oxidation to the respective carboxylic acid followed by decarboxylation. A similar elimination of a C-methyl group in naphthylisoquinoline alkaloids had already been observed at C-1 (see the structure of 1-nor-8-O-demethylvicristinebrev H, 12, in Fig. 1), as found to be produced by the same plant, A. abbreviatus,³⁸ and also oxygenations at Me-3 (see ref. 5, 35 and 36) and at C-4 (see ref. 5, 37 and 38) are known to occur in the biosynthesis of naphthylisoquinoline alkaloids. As in the case of 18 and 19, the resulting diketones III could then undergo a benzilic acid rearrangement, with loss of CO₂, to provide the isoindolinone unit IV.

**Cytotoxic activity of ancistrobrevolines A and B against breast and lung cancer cells**

Some of the mono- and dimeric naphthylisoquinoline alkaloids isolated from the West African liana A. abbreviatus display strong cytotoxic activities against various cancer cell lines, among them multiple myeloma,³⁸⁻³⁹ leukemia,³⁶⁻³⁹ cervical,¹⁵,¹⁶,⁴⁰ fibrosarcoma,³⁷ colon,³⁷ and pancreatic cancer cells.⁴¹⁻⁴⁴,⁴⁰ Investigations regarding the specificity and effectiveness of the compounds on the viability of those cancer cells indicated that the alkaloids may have a substantial therapeutic potential and can thus be regarded as promising candidates for drug development.

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Fig. 6 (A) Structures of the conventional, naphthalene-devoid isoindolinones, aristoyagonine (18) and aristolactam (19). (B) From ‘normal’ naphthyltetrahydroisoquinolines (I) to ancistrobrevolines (IV): Proposed biosynthetic pathway, with oxidation, decarboxylation, and ring contraction reactions.

[Diagram of structures and reactions]
And only recently, dioncophylline A (5), a main constituent of A. abbreviatus, and related compounds have been reported to exhibit pronounced antiproliferative activities in the submicromolar range against two breast cancer cell lines, MCF-7 and MDA-MB-231.\textsuperscript{41} Breast\textsuperscript{42,43} and lung\textsuperscript{44,45} cancer are among the most commonly diagnosed lethal cancer types worldwide. Although the death rate caused by cancer has been decreasing over the past years,\textsuperscript{46} as a result of improved diagnostic and therapeutic approaches to many cancer types, treatment of lung and breast cancer still suffers from a poor prospect to completely eradicate the tumor cells, which in turn leads to a recurrent and metastatic manifestation of the disease, and thus to high mortality rates.\textsuperscript{47–48} Therefore, the search for novel therapeutic agents to efficiently combat lung and breast cancer cells still remains an important task.

Given the pronounced antiproliferative activities of some of the naphthylisoquinoline alkaloids such as dioncophylline A (5),\textsuperscript{17,39,41} jozimine A\textsubscript{2} (11),\textsuperscript{17,40} or ancistrobrevidine C (8),\textsuperscript{15} with their usual six-membered heterocyclic ring system, it seemed rewarding to likewise study the cytotoxic potential of the new ring-contracted naphthylisoindolinones. This was also promising in view of the fact that aristolactams (Fig. 6A) and some of their non-natural analogues,\textsuperscript{28,43} all equipped with an isoindolinone moiety similar to the one of ancistrobrevolines A–D (14–17), are known to exhibit remarkable anticancer properties too.\textsuperscript{34,49,50}

We here, therefore, report on the evaluation of ancistrobrevolines A (14) and B (15) for their growth-inhibitory effects against MCF-7 breast adenocarcinoma cells and against the non-small cell lung cancer (NSCLC) cell line A549. Due to the lack of isolated material of ancistrobrevines C (16) and D (17), only 14 and 15 were assessed.

These two alkaloids exhibited only low cytotoxic effects against MCF-7 breast cancer cells, as determined by the MTT assay (see Experimental Section). The results showed that exposure of MCF-7 cells to varying concentrations (10, 30, 50, 70, and 100 μM) of 14 and 15 resulted in growth-inhibitory activities of 19–38% (for 14) and 3.7–43% (for 15) (see Table 2). Although 14 and 15 did not reach the pronounced growth-inhibitory activities of the previously investigated naphthylisoquinoline alkaloids on breast cancer cells,\textsuperscript{41} the results are important contributions to our ongoing investigations on structure–activity (SAR) relationships.

Against A549 lung cancer cells, the cytotoxic activities of 14 and 15 were significantly higher, reaching ca. 8–72% and 36–68%, respectively (see Table 3), with IC\textsubscript{50} values of 34.6 μM (for 14) and 9.05 μM (for 15) (Fig. 7). Thus, the IC\textsubscript{50} value of ancistrobrevoline B (15) was markedly lower than that of ancistrobrevine A (14), showing a higher sensitivity of A549 lung cancer cells to the growth inhibition induced by 15, which further evidenced the pronounced structure–function diversity in naphthylisoquinoline alkaloids. Studies on the immortalized human mammary epithelial cell line MCF-10A\textsuperscript{53} revealed ancistrobrevine B (15) to display moderate cytotoxicity on these non-tumorigenic cells, which led to a selectivity index of 1.4 regarding the A549 cancer cell line, whereas ancistrobrevoline A (14) showed no selectivity towards the cancer cells compared to the normal epithelial MCF-10A cells (SI = 1).

**Cytotoxic activity of ancistrobrevolines A and B against breast and lung cancer cells**

The manifestation of breast cancer is strongly associated with the occurrence of a small atypical population of tumor-initiating cells that possess the capacity of unlimited propagation and multipotent differentiation.\textsuperscript{55–58} These cancer-stem-like cells have been identified in a variety of tumors and are under suspicion to be a causative factor of tumor growth and metastasis, eventually facilitating resistance to chemotherapies, and thus, leading to treatment failure and tumor recurrence.\textsuperscript{55} Therefore, targeting these cancer stem cells in breast cancer has become a promising approach.\textsuperscript{56}

Cultivation of human breast epithelial adenocarcinoma cells MCF-7 under non-adherent non-differentiating conditions as described in the Experimental section results in the generation

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### Table 2 Concentration-dependent cytotoxic activity of ancistrobrevolines A (14) and B (15) against breast cancer cells (MCF-7)

| Compound | Cytotoxicity ± SD\textsuperscript{a} (%) |
|----------|--------------------------------------|
|          | 10 μM | 30 μM | 50 μM | 70 μM | 100 μM |
| 14       | 19.1 ± 22.7 | 25.3 ± 17.0 | 27.1 ± 6.82 | 28.5 ± 12.2 | 37.7 ± 12.7 |
| 15       | 3.68 ± 11.5 | 17.6 ± 31.5 | 34.0 ± 8.47 | 37.4 ± 13.0 | 43.0 ± 6.73 |

\textsuperscript{a} The results are shown as mean values ± standard deviation (SD); the experiments were done in triplicate (n = 3).

### Table 3 Concentration-dependent antiproliferative activity of ancistrobrevolines A (14) and B (15) against lung cancer cells (A549)

| Compound | Cytotoxicity ± SD (%)\textsuperscript{a} |
|----------|--------------------------------------|
|          | 10 μM | 30 μM | 50 μM | 70 μM | 100 μM |
| 14       | 8.27 ± 10.3 | 26.4 ± 4.01 | 54.3 ± 3.47 | 58.0 ± 3.66 | 72.7 ± 2.88 |
| 15       | 36.4 ± 9.62 | 61.9 ± 2.11 | 63.8 ± 9.52 | 64.0 ± 1.69 | 68.3 ± 3.88 |

\textsuperscript{a} The results are shown as mean values ± standard deviation (SD); the experiments were done in triplicate (n = 3).
of discrete clusters of spheroids, termed mammospheres (Fig. 8A), which undergo asymmetric division and self-renewal, making them aggressive and highly metastatic. Before this background, we studied the effect of ancistrobrevolines A (14) and B (15) on the sphere formation potential of MCF-7 cells after exposure to 10, 30, 50, 70, and 100 μM of the new naphthylisoindolinone alkaloids. After 5 d of treatment, the size of the spheroids was captured using a phase-contrast microscope. As shown in Fig. 8, compounds 14 and 15 significantly reduced the size of the spheres in MCF-7 culture compared to the non-treated control group, where large spheroids were observed. A dose-dependent spheroid inhibitory activity of ancistrobrevolines A (14) and B (15) was found in MCF-7-derived mammospheres. The results indicate the reduction of stemness and self-renewal of MCF-7 cells in the presence of 14 and 15.

Experimental

General experimental procedures

Optical rotations were recorded using a JASCO P-1020-polarimeter (JASCO, Gross-Umstadt, Germany) operating with a sodium light source (λ = 589 nm). UV spectra were measured on a Shimadzu UV-1800 spectrophotometer, and ECD spectra were recorded under nitrogen atmosphere on a JASCO J-715 spectrometer at room temperature, using a standard cell (0.02 cm), then processed using the SpecDis software. IR spectra were measured on a JASCO FT/IR-410 spectrometer. 1D and 2D NMR spectra were taken on a Bruker DMX 600 instrument at ambient temperature, using methanol-d₄ as the solvent, with the 1H and 13C signals of the solvent (δH 3.31 and δC 49.15 ppm) as the internal reference. Chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are reported in Hertz (Hz). Multiplicities of NMR signals are denoted as singlet (s), doublet (d), doublet of doublets (dd), or quartet (q). Spectra were processed using an the MestReNova NMR (Mestrelab

Fig. 7  Treatment of lung cancer (A549) cells with ancistrobrevolines A (14) and B (15). Determination of IC₅₀ and log IC₅₀ values, and regression analysis of the effects of (A) 14 and (B) 15 (10, 30, 50, 70, and 100 μM each) on the A549 cells after 48 h of incubation; the results are expressed as mean ± SD.

Fig. 8  Cytotoxic effects of ancistrobrevolines A (14) and B (15) on the formation of MCF-7-derived breast cancer stem-like cells (mammosphere). (A) Mammospheres derived from untreated MCF-7 cells as a control. (B–F) MCF-7 cells derived from mammospheres treated with 14 at concentrations of 10 (B), 30 (C), 50 (D), 70 (E), and 100 μM (F). (G–K) MCF-7 cells derived from mammospheres treated with 15 at concentrations of 10 (G), 30 (H), 50 (I), 70 (J), and 100 μM (K). The mammospheres were cultured for 5 d to form spheres in ultra-low attachment surface plates. The images of the mammospheres were taken at 10 × resolution using phase-contrast microscopy.
The air-dried powdered root bark material of *A. abbreviatus* was macerated in methanol (2 x 3 L) at 40 °C, followed by ultrasonication for 1 h. The extract was filtered, and the solvent was evaporated under reduced pressure to give a brownish solid residue, which was re-dissolved in 90% aqueous methanol and partitioned with *n*-hexane. The aqueous methanolic layer was dried under reduced pressure to yield 17.5 g of an alkaloid-rich fraction, which was directly subjected to fractionation over a silica gel column using a linear solvent system consisting of CH₂OH and CH₂Cl₂ with a gradient increase of CH₂OH (0 → 90%), giving rise to five naphthylisoindolinone-enriched fractions (F₁–F₅). The alkaloid-containing subfractions were purified on the X-Select HSS PFP column by applying the following method: 0 min: 68% B, 38 min: 88% B, affording 0.7 mg of ancistrobrevoline C (16) (retention time 22.0 min), 0.6 mg of ancistrobrevoline B (15) (retention time 25.3 min), 0.5 mg of ancistrobrevoline D (17) (retention time 26.4 min), and 1.1 mg of ancistrobrevoline A (14) (retention time 28.1 min).

### Plant material

Roots of *Ancistrocalus abbreviatus* Ayir Shaw (*Ancistrocalaceae*) were collected by one of us (late Prof. L. Aké Assi) in May 1996, in the Parc National de Taï, in the Southwest of the Ivory Coast. A voucher specimen (no. 3) has been deposited at the Herbarium Bringmann, Institute of Organic Chemistry, University of Würzburg.

### Chemicals and reagents

For the purification of the plant fractions by preparative HPLC, HPLC-grade solvents MeCN, MeOH, purchased from Sigma-Aldrich, were utilized. Spectroscopic-grade methanol was used, applying a flow rate of 10 mL min⁻¹; mobile phases: (A) 90% H₂O with 10% CH₃CN (0.05% trifluoroacetic acid) and (B) 90% CH₃CN with 10% H₂O (0.05% trifluoroacetic acid). For further purification, a Waters X-Select HSS PFP column (10 x 250 mm, 5 μm) was applied; mobile phases: (A) 90% H₂O with 10% CH₃OH (0.05% trifluoroacetic acid) and (B) 90% CH₃OH with 10% H₂O (0.05% trifluoroacetic acid).

### Extraction and purification of naphthylisoindolinoines

The air-dried powdered root bark material of *A. abbreviatus* (ca. 420 g) was macerated in methanol (2 x 3 L) at 40 °C, followed by ultrasonication for 1 h. The extract was filtered, and the solvent was evaporated under reduced pressure to give a brownish solid residue, which was re-dissolved in 90% aqueous methanol and partitioned with *n*-hexane. The aqueous methanolic layer was dried under reduced pressure to yield 17.5 g of an alkaloid-rich fraction, which was directly subjected to fractionation over a silica gel column using a linear solvent system consisting of CH₂OH and CH₂Cl₂ with a gradient increase of CH₂OH (0 → 90%), giving rise to five naphthylisoindolinone-enriched fractions (F₁–F₅). The alkaloid-containing subfractions were purified on the X-Select HSS PFP column by applying the following method: 0 min: 68% B, 38 min: 88% B, affording 0.7 mg of ancistrobrevoline C (16) (retention time 22.0 min), 0.6 mg of ancistrobrevoline B (15) (retention time 25.3 min), 0.5 mg of ancistrobrevoline D (17) (retention time 26.4 min), and 1.1 mg of ancistrobrevoline A (14) (retention time 28.1 min).

### Ancistrobrevoline A (14)

Yellow, amorphous powder; [α]D²⁰ +11.5 (c 0.05, MeOH); UV/Vis (MeOH) λmax (log ε) 196 (4.1), 213 (3.7), 229 (3.6), 261 (3.0), 306 (3.0) nm; ECD (c 0.10; MeOH) λmax (log ε) 213 (−2.7), 235 (−1.1), 249 (+1.5), 326 (+0.1) cm⁻¹ mol⁻¹⁻¹; IR (ATR) νmax 3400, 2967, 2929, 1673, 1585, 1463, 1421, 1383, 1198, 951, 808, 672 cm⁻¹⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; HR-ESI-MS m/z 444.17711 [M + Na]⁺ (calcd for C₂₅H₂₅NNaO₅, 444.17814).

### Ancistrobrevoline B (15)

Yellow, amorphous solid; [α]D²⁰ −8.3 (c 0.07, MeOH); UV/Vis (MeOH) λmax (log ε) 196 (4.1), 213 (3.5), 227 (3.4), 261 (2.8), 306 (2.8) nm; ECD (c 0.10; MeOH) λmax (log ε) 212 (−2.4), 232 (−1.1), 247 (+1.7), 301 (−0.1) cm⁻¹ mol⁻¹⁻¹; IR (ATR) νmax 2965, 1678, 1587, 1442, 1383, 1196, 1135, 836, 803, 673 cm⁻¹⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; HR-ESI-MS m/z 408.1815 [M + H]+ (calcd for C₂₄H₂₆NO₅, 408.1805), and m/z 430.1611 [M + Na]⁺ (calcd for C₂₅H₂₆NNaO₅, 430.1624).

### Ancistrobrevoline C (16)

Yellow, amorphous powder; [α]D²⁰ −18.9 (c 0.05, MeOH); UV/Vis (MeOH) λmax (log ε) 197 (4.1), 215 (3.3), 228 (2.4), 261 (2.8), 304 (2.7) nm; ECD (c 0.10; MeOH) λmax (log ε) 205 (−0.6), 225 (+1.5), 250 (−1.3), 300 (0.09) cm⁻¹ mol⁻¹⁻¹; IR (ATR) νmax 2927, 2850, 1675, 1589, 1439, 1387, 1264, 1200, 1135, 836, 805, 719 cm⁻¹⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; HR-ESI-MS m/z 408.1797 [M + H]+ (calcd for C₂₄H₂₅NO₅, 408.1805), and m/z 430.1608 [M + Na]⁺ (calcd for C₂₅H₂₆NNaO₅, 430.1624).

### Ancistrobrevoline D (17)

Yellow, amorphous powder; [α]D²⁰ −24.0 (c 0.04, MeOH); UV/Vis (MeOH) λmax (log ε) 197 (4.0), 229 (3.2), 295 (2.6), 334 (2.5) nm; ECD (c 0.10; MeOH) λmax (log ε) 209 (−0.1), 226 (−2.7), 246 (+1.3), 286 (+0.08) cm⁻² mol⁻¹⁻¹; IR (ATR) νmax 2969, 2850, 1680, 1594, 1459, 1327, 1204, 1134, 836, 805, 719 cm⁻¹⁻¹; ¹H NMR and ¹³C NMR data, see Table 1; HR-ESI-MS m/z 400.1503 [M + Na]⁺ (calcd for C₂₃H₂₃NNaO₄, 400.1519).

### Oxidative degradation

The naphthylisoindoline alkaloids 14–17 (ca. 0.5 mg each) were subjected to a ruthenium(vii)-mediated periodate degradation,¹⁶,²⁶ followed by derivatization of the resulting amino acids with CH₃OH/HCl and (R)-x-trifluoromethylphenylacetyl chloride ([R]-MTPA-Cl, prepared from (S)-MTPA). The absolute configurations were assigned by GC on a dimethylpolysiloxane-coated capillary column coupled to a mass-selective detector and comparison with the corresponding derivatives of authentic amino acids of known absolute configuration.

### Computational details

For the calculation of the ECD spectra, a simplified approach was selected. Only the main conformers of the possible configurations of compound 14 were optimized with B3LYP-D₃/def2-TZVP.¹⁴ The conformational freedom of the methoxy groups was not taken into account because their influence on the ECD should be negligible. Excited states results were achieved utilizing TD-DFT/B97X-D/def2-TZVP(-f).⁶⁴ All calculations...
were done using ORCA 4.1.0.2.66 For processing of the data, SpecDis 1.71 was used69,68 with a UV shift of 18 nm, finally providing the computed ECD spectra, which were compared with the experimental one of 14.

Cell culture
Human breast (MCF-7) and lung (A549) cancer cells were purchased from the National Centre for Cell Sciences, Pune, India. Normal, non-tumoral breast cells (MCF-10A) were from ATCC, University Boulevard, Manassas, VA, USA. Breast and lung cancer cells were cultured in DMEM supplemented with 10% (v/v) FBS (heat-inactivated), 2 mM l-glutamine, 100 µg mL⁻¹ of streptomycin and 100 U mL⁻¹ of penicillin. The normal breast cells (MCF-10A) were maintained in DMEM/F12 media supplemented with FBS (10%), horse serum (5%), cholera toxin (100 ng mL⁻¹), EGF (20 ng mL⁻¹), hydrocortisone (0.5 mg mL⁻¹), and insulin (10 µg mL⁻¹) and incubated at 37 °C and 5% CO₂.

Cytotoxicity study using the MTT assay
Approximately 10⁴ cells/well were seeded in 96-well plates and kept in the incubator at 37 °C and 5% CO₂. The cells were kept overnight to allow them to adhere and were then fed with fresh medium containing different concentrations (10, 30, 50, 70, and 100 µM) of the test compounds, anisotroblevines A (15) and B (16). After incubation for 48 h, cell viability was assessed using the MTT assay.67 At the stipulated time following the treatment of the test sample, medium was aspirated; MTT (5 mg mL⁻¹) was added in each well and incubation was continued at 37 °C for 2 h. The plates were spun, supernatants were discarded, and purple-colored precipitates of formazan were dissolved in 100 µL of dimethylsulfoxide (DMSO). The color absorbance was recorded at 590 nm using a microplate reader (BioTek Instruments, Inc. USA). IC₅₀ calculations and regression analysis were performed using the GraphPad Prism 5.0 software.

The selectivity index (SI) of the test compounds was calculated using the following equation, where MCF-10A represents human normal cells, while A549 denotes lung cancer cells: SI = IC₅₀ normal cells/IC₅₀ cancer cells. The IC₅₀ value indicates the concentration of the test drug required for 50% inhibition of the cancer cells. SI values > 1 indicate that the compound is selective against the respective cancer cells.68

Mammosphere formation and drug treatment
Mammospheres derived from MCF-7 cells were formed by seeding the cells in 6-well ultra-low-attachment surface plates (Corning, Tewksbury, MA, USA) at a density of 2 × 10⁴ cells/well and cultured in serum-free DMEM-Ham’s F12 nutrient mixture (1 : 1, v/v), supplemented with 5 mg mL⁻¹ insulin, 0.5 mg mL⁻¹ hydrocortisone, 2% B-27 supplements, 10 ng mL⁻¹ fibroblast growth factor, and 20 ng mL⁻¹ epidermal growth factor. The cells were incubated at 37 °C and 5% CO₂ for 5 d to form mammospheres.69 To study the effect of anisotroblevines A (14) and B (15) on mammosphere formation, the cells were treated with different concentrations (10, 30, 50, 70, and 100 µM) of the agents and then allowed to form spheres for the next 5 d. The compound solutions were prepared using DMSO with its final non-toxic concentration (0.1%). An equal amount of DMSO was added to the non-treated control group. After 5 d, the mammospheres were photographed using phase-contrast microscopy, and the images were analyzed using the ImageJ analysis software.69

Conclusions
This paper describes the discovery of a structurally novel subclass of naphthylisoquinoline-related alkaloids, bearing a heterocyclic moiety that is ring-contracted to an isoindolinone entity. The results further highlight the “chemical creativity” of A. abbreviatus and demonstrate the outstanding phytochemical diversity of this productive West African liana. Already at the level of ‘normal’, standard-type naphthyltetrahydroisoquinoline alkaloids, the plant shows a remarkably broad variability,5,10,11,13,14,17 with no less than four out of seven known C,C- coupling types (5,1’, 7,1’, 5,8’, and 7,8’) and an otherwise very rare stereo-diversity, producing all four possible stereochemical combinations at C-1/C-3 (i.e. S,S, S,R, R,S, and R,R) (Fig. 1). In addition, it is the only plant that contains all four possible subclasses of naphthylisoquinoline alkaloids,5,17 namely Ancistroacladaceae- (6-OR, 3S), Dioncophyllaceae- (6-H, 3R), hybrid- (6-OR, 3R), and inverse hybrid-type (6-H, 3S) representatives, as outlined in Fig. 1.

Moreover, A. abbreviatus excels by the highest number of metabolic follow-up variations, resulting from – mostly oxidative – secondary modifications. Besides the phenol-oxidative coupling via the naphthalene portion, e.g. to give jozimine A₂ (13),17 with its three stereogenic axes, and a nearly complete series of its numerous atropo-diastereomers, it is the only plant that produces naphthylisoquinoline alkaloids whose isocyclic moiety is further oxygenated to form an ortho-naphthoquinone as in anistrebroviquinone A (10).15

Even more thrilling are the downstream variations of the tetrahydroisoquinoline portion, which can be dehydrogenated leading to the otherwise less frequently occurring 3,4-dihydroisoquinolines and to the (normally very rare) fully dehydrogenated naphthylisoquinoline alkaloids, with no less than seven representatives. Totally unprecedented even is the occurrence of 1-demethylated naphthylisoquinolines like 1-nor-8-O-demethylanistrebrovibreine H (12)18 and ring-cleaved representatives like anistrosecoline D (13)19 (Fig. 1) – and, as described here, the discovery of oxidatively ring-contracted alkaloids, delivering new molecular scaffolds within the class of naphthylisoquinoline alkaloids.

These first four naphthylisoindolines, anisotroblevines A-D (14-17), are all N-methylated, and they are all fully O-methylated in the naphthalene part and all are R-configured at C-1. In that respect they fit with the structural properties of the presumable precursors to 14, 15, 16, and 17, viz., anistrebrovireine A (1), its 6-O-demethyl analogue 2, anistrebrovibreine D (3), and N-methylidioncophylline A (5) (Fig. 1), respectively, which, in agreement with our biosynthetic concept, all co-occur in A. abbreviatus, where they are even among the main alkaloids. Despite their structural similarities, the new metabolites do vary
by their oxygenation degree at C-6, by the O-methylation degree at C-8, and by the stereo-orientation at the biaryl axis. This suggests that the structural requirements for the ring contraction are not particularly specific, evidencing that isoindolone formation in naphthylisoquinoline alkaloids is possibly a more general principle. The work shows that naphthylisoquinoline alkaloids are not just metabolically stable bioactive end products, but can also be substrates for further – mainly oxidative – modifications.

Among all investigated naphthylisoquinoline-producing plants, *A. abbreviatus* shows the broadest diversity of secondary metabolites. It thus occupies a phytochemically – and phylogenetically – outstanding position within the Ancistrocladaceae family, with a close relationship to Dioncophyllaceae lianas such as *Triphyophyllum peltatum* on the one hand and to geographically neighboring West and Central African Ancistrocladaceae plants on the other. Ancistrobrevoline A (14) and B (15) exhibited moderate antiproliferative activities against breast (MCF-7) and lung (A549) cancer cell lines. Furthermore, 14 induced a significant reduction of the formation of highly metastatic spheroid clusters in breast cancer-derived cancer-stem-like cells, worth further, more in-depth investigations on the antitumoral potential of naphthylisoquinoline alkaloids. The results presented here render phytochemical studies on the numerous further, as yet unidentified, trace compounds of *Ancistrocladus* plants and their biological evaluation rewarding goals.

**Conflicts of interest**

There are no conflicts to declare.

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**Notes and references**

1 C. M. Taylor, R. E. Gereau and G. M. Walters, *Ann. Mo. Bot. Gard.*, 2005, **92**, 360–399.
2 M. Cheek, *Kew Bull.*, 2000, **55**, 871–882.
3 H. K. Airy Shaw, *Kew Bull.*, 1949, **4**, 68–69.
4 H. K. Airy Shaw, *Kew Bull.*, 1950, **5**, 147–150.
5 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.
6 B. K. Lombe, D. Feineis and G. Bringmann, *Nat. Prod. Rep.*, 2019, **36**, 1513–1545.
7 N. Tajuddeen and G. Bringmann, *Nat. Prod. Rep.*, 2021, **38**, 2154–2186.
8 S. R. M. Ibrahim and G. A. Mohamed, *Fitoterapia*, 2015, **106**, 194–225.
9 X. F. Shang, C. J. Yang, S. L. Morris-Natschke, J. C. Li, X. D. Yin, Y. Q. Liu, J. W. Peng, M. Goto, J. Y. Zhang and K. H. Lee, *Med. Res. Rev.*, 2020, **40**, 2212–2289.
10 G. Bringmann, D. Lisch, H. Reuscher, L. Aké Assi and K. Günther, *Phytochemistry*, 1991, **30**, 1307–1310.
11 G. Bringmann, R. Zagst, H. Reuscher and L. Aké Assi, *Phytochemistry*, 1992, **31**, 4011–4014.
12 G. Bringmann, F. Pokorny, M. Stäblein, M. Schäffer and L. Aké Assi, *Phytochemistry*, 1993, **33**, 1511–1515.
13 G. Bringmann, R. Weirich, D. Lisch and L. Aké Assi, *Planta Med.*, 1992, **58**, A703–A704.
14 S. Fayez, D. Feineis, L. Aké Assi, M. Kaiser, R. Brun, S. Awale and G. Bringmann, *Fitoterapia*, 2018, **131**, 245–259.
15 S. Fayez, A. Cacciatore, S. Sun, M. Kim, L. Aké Assi, D. Feineis, S. Awale and G. Bringmann, *Bioorg. Med. Chem.*, 2021, **30**, 115950.
16 S. Fayez, D. Feineis, L. Aké Assi, E. J. Seo, T. Effert and G. Bringmann, *RSC Adv.*, 2019, **9**, 15738–15748.
17 S. Fayez, J. Li, D. Feineis, L. Aké Assi, M. Kaiser, R. Brun, M. A. Anany, H. Wajant and G. Bringmann, *J. Nat. Prod.*, 2019, **82**, 3033–3046.
18 S. Fayez, T. Bruhn, D. Feineis, L. Aké Assi, S. Awale and G. Bringmann, *J. Nat. Prod.*, 2020, **83**, 1139–1151.
19 G. Bringmann, C. Günther, M. Ochse, O. Schupp and T. Tasler, in *Progress in the Chemistry of Organic Natural Products*, ed. W. Herz, H. Falk, G. W. Kirby and R. E. Moore, Springer, Wien, New York, 2001, vol. 82, pp. 111–124.
20 J. E. Smyth, N. M. Butler and P. A. Keller, *Nat. Prod. Rep.*, 2015, **32**, 1562–1583.
21 H. K. Airy Shaw, *Kew Bull.*, 1951, **6**, 327–347.
22 S. Porembski and W. Barthlott, in *The Families and Genera of Vascular Plants. V. Flowering Plants – Dicotyledons, Malvales, Capparales and Non-betain Caryophyllales*, ed. K. Kubitzki and C. Bayer, Springer, Heidelberg, 2005, vol. 5, pp. 178–181.
23 G. Bringmann, G. François, L. Aké Assi and J. Schlauer, *Chimia*, 1998, **52**, 18–28.
24 G. Bringmann, M. Rübenacker, J. R. Jansen, D. Scheutzow and L. Aké Assi, *Tetrahedron Lett.*, 1990, **31**, 639–642.
25 G. Bringmann, J. R. Jansen, H. Reuscher, M. Rübenacker, K. P. Peters and H. G. von Schnering, *Tetrahedron Lett.*, 1990, **31**, 643–646.
26 For better comparability, the atom numbering follows the one applied for normal, intact naphthylisoquinolines, as also previously used for the *seco*-type ancistrosecolines13 like e.g., compound 13.
27 G. Bringmann, R. God and M. Schäffer, *Phytochemistry*, 1996, **43**, 1393–1403.
