ANCISTROGUINEINES A AND B AS WELL AS ANCISTROTECTORINE-NAPHTHYLISOQUINOLINE ALKALOIDS FROM ANCISTROCLADUS GUINEÈNSIS*†

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(Received in revised form 6 April 1997)

Key Word Index—Ancistrocladus guineensis; Ancistrocladaceae; leaves; ancistroguineine A; ancistroguineine B; ancistrotectorine; naphthylisoquinoline alkaloids; biaryls, naturally occurring; quercetin; structural elucidation.

Abstract—The isolation of three naphthylisoquinoline alkaloids from the leaves of Ancistrocladus guineensis is described. Their complete structures were established by spectroscopic, chiroptical and degradative methods. Thus, two hitherto unknown 5,8’-coupled naphthylisoquinolines, named ancistroguineines A and B, were isolated, constituting the first example of a pair of 3-epimeric naphthylisoquinoline alkaloids. Moreover, ancistrotectorine, a 7,3’-coupled alkaloid previously known only from the South-East Asian species Ancistrocladus tectorius, was isolated. Its absolute stereostructure was confirmed by oxidative degradation and by comparison of experimental and calculated CD spectra. © 1997 Elsevier Science Ltd

INTRODUCTION

Ancistrocladus guineensis Oliv., a liana indigenous to Nigeria and Cameroon [2], belongs to the small monogenic family of Ancistrocladaceae [3], which consists of ca 25 species. Like the closely related Dioncophyllaceae, the Ancistrocladaceae produce a unique class of natural products, the naphthylisoquinoline alkaloids [4]. These compounds are of high interest due to their unprecedented structures, promising biological activities and remarkable chemotaxonomical implications [4]. No phytochemical studies have so far been reported for A. guineensis. The investigation of its secondary metabolites would, however, be of great interest for chemotaxonomic reasons and, in particular, because of the pronounced pharmacological properties of the closely related species, A. korupensis [5, 6]. This liana grows in the same region and has been found to be the, as yet, only source of anti-HIV dimeric naphthylisoquinolines, the michellamines [7], and their antimalarial monomeric ‘halves’, korupensamines A (1a) and B (1b) [8]. In the present paper, we report on the isolation and structural elucidation of two new naphthylisoquinoline alkaloids from A. guineensis, ancistroguineines A (2) and B (3) (Fig. 1). The structure of the likewise isolated naphthylisoquinoline alkaloid ancistrotectorine (4), which is already known from the South East Asian species A. tectorius [9], was confirmed with respect to its absolute configuration by degradative methods, as well as experimental and theoretical CD investigations. In addition, the widespread [10] flavone, quercetin, was identified.

RESULTS AND DISCUSSION

Because of the expected occurrence of naphthylisoquinoline alkaloids in A. guineensis, an appropriate isolation method, as elaborated recently [1], was chosen, viz., extraction of the dried and ground plant material with 1N sulphuric acid–methanol (5:1) and subsequent resolution of the extract using High Speed Countercurrent Chromatography (HSCCC) [11]. Further separation of the HSCCC fractions by column chromatography yielded three Dragendorff-positive compounds. One of these substances, which co-occurred with the second, chromatographically very similar one in the third main HSCCC fraction, was shown to be a naphthylisoquinoline alkaloid by ’H NMR and mass
spectroscopic analysis, HR mass spectrometry indicating a molecular formula C_{24}H_{27}NO_{4}. From the close analogy of the 'H NMR data of the compound with those of the known [12] 5,8'-coupled alkaloid ancistrobrevine B (5) from _A. abbreviatus_, a similar molecular framework was to be expected.

Different from ancistrobrevine B (5), the molecule contains only two methoxy groups at C-4' and C-8, as located by Rotating Frame Overhauser Enhancement Spectroscopy (ROESY) measurements [Fig. 2(a)]. The position of the coupling site at C-5 of the isoquinoline moiety was deduced from the high-field shift of the Me-3 protons (δ 0.97) and the protons at C-4 (δ 1.98), resulting from the anisotropic effect caused by the naphthyl substituent. In the naphthalene part, the biaryl axis cannot be positioned in the methyl-substituted isocycling ring, because of the normal, i.e. not high-field shifted position of Me-2' (δ 2.36). The position of the axis at C-7' can be excluded by the multiplicity of the signals of the two aromatic protons of that ring (two doublets). A ROESY interaction between H-4 and H-1' indicates the proximity of these two molecular parts and establishes the biaryl axis to be located at C-8', thus excluding a 6'-coupling site.

The constitution of the alkaloid was further corroborated by a series of significant long-range H,C-Heteronuclear Multiple Bond Correlation (HMBC) interactions [Fig. 2(b)].

Since none of the known naphthylisoquinolines [4] has the same constitution as the isolated alkaloid, the compound must be new and has subsequently been named ancistroguineine A. Like the related alkaloids 1a, 1b and 5, ancistroguineine A has a relative trans-configuration at C-1 vs C-3. This was deduced from the chemical shifts of H-1 (δ 4.34) and H-3 (δ 3.09), which lie in the typical range [4, 13] for trans-substituted 1,3-dimethyltetrahydroisoquinolines and, in particular, by a ROESY correlation of H-3 with the likewise pseudo-axial methyl group at C-1 [Fig. 2(c)].

For the elucidation of the absolute configuration at C-1 and C-3, our ruthenium-mediated oxidative degradation procedure [14], which has recently been further improved [15], was applied. From the S-configuration of the resulting Mosher-derivatives of both 3-aminobutyric acid (6) and alanine (7), the configurations at the two stereogenic centres of ancistroguineine A were established as S. This reveals a stereochemical relationship of the new alkaloid to ancistrobrevine B, which is likewise S,S-configured, rather than to korupensamines A (1a) and B (1b), which have the R,R-configuration.

The last stereochemical information required was the configuration at the biaryl axis, which, due to its restricted rotation, constitutes an additional stereo- genic element. Regrettably, a determination of the relative configuration at the biaryl axis, by (sometimes long-range) NOE or ROESY interactions between particular protons in the naphthalene part, specifically...
Fig. 2. Constitution 2 of ancistroguineine A as deduced from chemical shifts (δ values in ppm) and selected ROESY interactions (a) as well as HMBC correlations (b); its relative configuration at the stereocentres through chemical shifts and ROESY correlations (c).

with one of the diastereotopic protons at C-4 was not possible here. These two protons happen to be isochronic, so that the usually observable two signals overlap to give one doublet. By an investigation of the Circular Dichroism (CD), however, a clear attribution of the axial chirality of ancistroguineine A was possible. Thus, the virtually opposite CD spectra of the new compound and the M-configured alkaloid, ancistrobrevine B (5), indicate ancistroguineine A to be a 5-epi-5′-O-demethylancistrobrevine B.

The second, slightly less polar alkaloid, which was isolated from the same HSCCC fraction, was found to be a stereoisomer of 2 and, thus, a new compound, subsequently named ancistroguineine B. The position of the naphthyl substituent at C-5 of the isoquinoline moiety was indicated by the high-field shift of the Me-3 protons (δ 0.97, see Fig. 3(a)) and the protons at C-4 (δ 1.82 and 2.18). The coupling site in the naphthalene system was again found to be C-8′ from the ‘normal’ chemical shift of the Me-2′ protons (δ 2.35) combined with NOE and ROESY correlations between Heq-4 and H-7′ as well as Has-4 and H-1′ (see also below). Again, like in 2, the two methoxy groups are located at C-8 and C-4′, the remaining hydroxy groups being situated at C-6 and C-5′, as evident from NOE, ROESY and HMBC interactions [Figs. 3(a) and (b)].

Different from 2, however, the relative configuration at C-1 vs C-3 must be cis, which can clearly be seen from NOE and ROESY interactions between H-1 and H-3, indicating that these nuclei must be on the same side of the tetrahydroisoquinoline system [Fig. 3(c)]. An additional hint at the relative cis-configuration at C-1 vs C-3 is the chemical shift of H-1 (δ 4.31) and, in particular, of H-3 (δ 2.75), as well as the chromatographic behavior as compared with that of 2 (more rapid elution for cis- than trans-isomers within the series [16]).

In contrast to the trans-configured alkaloid ancistroguineine A (2), its cis-diastereomer ancistroguineine B allowed the determination of the relative configuration at centres vs axis by NMR inves-
tigations, because, in this case, the two diastereotopic protons at C-4 are clearly differentiated. Hence, from distinct ROESY interactions between H-n-4 and H-1' (which are also indicative of the constitution, see above), these two protons must be on the same side of the molecule. This is confirmed by an additional clear ROESY interaction between Heq-4 and H-7', which thus must likewise be in close mutual proximity.

For the elucidation of the absolute configuration of 3, again the improved oxidative degradation procedure was applied. The unequivocal identification of R-3-aminobutyric acid (6) (as its Mosher derivative) clearly establishes the absolute configuration at C-3 as R. Whereas, as for other cis-configured tetrahydroisoquinolines of this type [14, 15, 17], less reliable information can be deduced from the corresponding Mosher derivative of alanine (7), the absolute configuration at C-1 can unambiguously be seen from the relative cis-configuration established above, clearly indicating C-1 to be S-configured, as in 2.

From the absolute stereoarray in the tetrahydroisoquinoline part and the known relative configuration at centres vs axis, the absolute configuration at the biaryl linkage can be deduced as P (as depicted in the stereodrawing in Fig. 3(c)), i.e. with the methyl-substituted naphthalene part above the isoquinoline plane. This is further confirmed by comparison of the CD spectra of ancistroguineines A (2) and B (3), which show a qualitatively very similar appearance. Thus, ancistroguineine B is represented by the stereostructure 3 and can therefore likewise be considered as a 3-epi-ancistroguineine A or a 1-epi-8-O-methylkorupensamine A.

From the first HSCCC fraction, a distinctly less polar naphthylisoquinoline alkaloid was isolated by column chromatography. The [M]+ peak at m/z 421 in the mass spectrum and a high resolution measurement of the [M–Me]+ peak allowed us to establish its molecular formula as C_{26}H_{38}NO_{4}. Again, by extensive 1H NMR and 13C NMR spectroscopy, the constitution of this non-polar alkaloid was established. Its 7,3' coupling type was deduced in particular from the high-field shifted signals of Me-2' (δ 2.17), OMe-6 (δ 3.69) and OMe-8 (δ 3.33), indicating that all of these three groups were adjacent to the biaryl axis [Fig. 4(a)], and HMBC interactions between H-1' and C-8', as well as between OH-4' and C-3' [Fig. 4(b)]. A compound of the constitution shown in Fig. 4(a) and (b), including the rare 7,3'-site of the biaryl axis, named ancistrotectorine (4), has previously been isolated from the South East Asian species A. tectorius. Still, given the slightly divergent physical and spectroscopic data and the fact that authentic material of ancistrotectorine from A. tectorius is presently not available (Cordell, G. A., personal communication), it proved to be necessary to complete the structural elucidation by establishing the relative and absolute configurations at all of the three elements of chirality. Again, as for 2 and 3, the relative cis-configuration at C-1 vs C-3 was established through NOE interactions between H-1 and H-3 [Fig. 4(c)], which was again consistent with the chemical shifts of H-1 (δ 3.75) and H-3 (δ 2.56). The absolute configuration was elucidated by the usual oxidative degradation procedure, to be 1R,3S. This is again in agreement with the structure published for ancistrotectorine [9].

While in the literature [9] the absolute configuration
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Fig. 4. Selected NMR data (δ values in ppm) as well as NOE, ROESY and HMBC interactions indicative of the constitution (a, b) and the relative configuration (c) of ancistrotectorine (4).

at C-1 and C-3 for this alkaloid of A. tectorius had not been determined directly, the absolute axial configuration had been deduced by CD-comparison with a similar, likewise 7,3'-coupled alkaloid isolated previously [18, 19], named ancistrocladidine. From this, in combination with an X-ray structural analysis, the absolute configuration at the stereocentres had been concluded. Still, given the electronic difference between ancistrotectorine and ancistrocladidine—the latter being a dihydroisoquinoline—further evidence for the identity of ancistrotectorine from A. tectorius and the alkaloid described in this paper was highly desirable, as well as additional information on the stereostructure. Regrettably, in this case, no diagnostically useful long-range NOE interactions were observable, e.g. between Me-2' and significant protons within the tetrahydroisoquinoline part, e.g. with H-1, for the elucidation of the relative configuration at axis and stereocentres. For this reason, we applied the efficient method [20, 21] of calculating the CD spectrum of ancistrotectorine, both for the M-configured structure 4 and for its P-diastereomer. The good agreement of the experimental CD spectrum of the isolated alkaloid (and thus of ancistrotectorine from Cordell) with that of 4, which has M-configuration, and the near-opposite spectrum calculated for the P-atropodiastereomer of 4 show that the isolated compound indeed has the M-configuration at the biaryl axis and, therefore, the stereostructure 4. This ultimately establishes that the alkaloid from A. guineënsis is identical to authentic ancistrotectorine from A. tectorius, even without the availability of comparison material, and again corroborates the structural proposal by Cordell et al.

The last HSCCC fraction contained quercetin, a widespread flavone [10], which had previously also been isolated i.e., from A. heyneanus [22].

The results of this first phytochemical investigation of the Central African Ancistrocladus species, A. guineënsis, give interesting insights into the chemo- and, thus, geo-taxonomic context of this most remarkable liana. Thus, although ancistrotectorine (4) is a known compound, its isolation from A. guineënsis is the first report on a 7,3'-coupled naphthylisoquinoline alkaloid from an African Ancistrocladus species. From its S-configuration at C-3 and its oxygen function at C-6, it belongs to the pure Ancistrocladaceae-type alkaloids, which are typical of Asian [4] and East African [1] species of this family, but are far less frequently found in West and Central African lianas. By contrast, the new alkaloids ancistroguineines A (2) and B (3) show a 5,8'-coupling type, which is relatively common to Central and West African Ancistrocladus species, in particular to A. korupensis (cf. structures 1a and 1b) and A. abbreviatus (cf. structure 5), and even the
East African species, *A. robertsoniorum* [1], whereas no 5,8'-coupled naphthylisoquinolines have ever been isolated from Asian representatives of this interesting genus. Likewise, most remarkably, 3 is an *N*-unsubstituted naphthylisoquinoline alkaloid with a relative cis-configuration at C-1 vs C-3. This structural type has hitherto been found only in isoanstrocladine from the Indian species, *A. heyneanus*, yet with a 1R,3S-configuration; hence, 3 is the first 1S,3R-configured *N*-unsubstituted naphthylisoquinoline alkaloid. The rare occurrence of this '1,3-cis-NH array' possibly has to do with its instability compared with the corresponding trans-diastereomers [23, 24]. Furthermore, 2 and 3 are the very first example of a pair of 3-epimeric naphthylisoquinoline alkaloids, all other pairs of diastereomeric alkaloids from Ancistrocladaceae are either epimeric at the axis (1a and 1b) or at C-1. This shows the broad synthetic variability and creativity of Ancistrocladaceae plants and demonstrates that it is rewarding to investigate the structures of further co-occurring alkaloids and, in particular, the biogenetic factors determining their stereochemical formation. This work is in progress.

**CONCLUSION**

As regard the biological activity of the isolated compounds, the polar F1 fr. of the HSCCC elution did not show any activity against the *A. hydrophila* test organism. This work is in progress.

**EXPERIMENTAL**

**Isolation of ancistroguineine A** (2). The third fr., which consisted of two alkaloids with similar chromatographic behaviour, was resolved by CC on silica gel eluting with CHCl₃-MeOH-conc. NH₃, 95:5:0.1 to yield 2 as the more polar fr., which was obtained as needles (12.2 mg) from CHCl₃. Mp 202–204°. [α]₁₂⁵° +191.4° (CHCl₃, c 0.52). CD: Δε₂₃₅ -23.7, Δε₂₅₈ +15.8, Δε₂₇₅ -2.3 (EtOH, c 0.06). IR νmax cm⁻¹: 3390 (O=H), 1600 (C=O), 1575 (C=C), 1250 (C=O), 1108 (C=O). ¹H NMR (600 MHz, CDCl₃): δ 0.97 (3H, m, δ = 7.6 Hz, Me-3), 1.45 (3H, m, δ = 6.6 Hz, Me-1), 1.98 (2H, d, δ = 7.5 Hz, H-4), 2.36 (3H, s, Me-2'), 3.09 (1H, m, δ = 3.2 Hz, H-3), 3.85 (3H, s, OMe-8), 4.08 (3H, s, OMe-4'), 4.34 (1H, t, δ = 6.6 Hz, H-6'), 4.61 (1H, s, H-7), 6.67 (1H, s, H-3'), 6.83 (1H, s, H-1'), 6.88 (1H, d, δ = 7.9 Hz, H-6'), 7.16 (1H, d, δ = 7.8 Hz, H-7'). ¹³C NMR (150.9 MHz, CDCl₃): δ 21.5 (Me-1), 22.2 (Me-2'), 22.6 (Me-3'), 36.0 (C-4'), 42.3 (C-2), 47.2 (C-1), 55.2 (OMe-8), 56.2 (OMe-4'), 95.8 (C-7), 106.8 (C-3'), 110.0 (C-6'), 113.8 (C-10'), 117.3 (C-9'), 117.9 (C-7'), 121.0, 121.5 (C-8', C-5), 131.7 (C-7'), 135.4 (C-2'), 135.8, 136.8 (C-10, C-9'), 152.6, 155.0, 156.4, 156.6 (C-4', C-5', C-6, C-8). The ¹H attribution was achieved by HMOC and HMBC expts. EIMS: m/z (rel. int.): 393 [M⁺] (7), 379 [M-CH₃] + (27), 378 [M]+ (100). HRMS m/z 378.1711 [M-CH₃]+ requires: 378.1711.

**Isolation of ancistroguineine B** (3). From the less polar CC fr. ancistroguineine B was isolated as an amorphous solid (9.4 mg). [α]₁₂⁵° -141.2° (CHCl₃, c 0.04). CD: Δε₃₅₀ -57.0, Δε₃₆₄ +9.9, Δε₃₅₂ -2.4, Δε₃₀₁ +1.4, Δε₃₄₁ -1.6 (EtOH, c 0.09). IR νmax cm⁻¹: 3390 (O=H), 1600, 1575 (C=C), 1250, 1105 (C=O). ¹H NMR (200 MHz, CDCl₃): δ 0.97 (3H, d, δ = 6.3 Hz, Me-3), 1.53 (3H, d, δ = 6.1 Hz, Me-1), 1.82 (1H, dd, δ = 16.1 Hz, J₆₇ = 10.6 Hz, H₆₇), 2.18 (1H, dd, δ = 16.5 Hz, J₆₇ = 19.9 Hz, H₆₇), 2.35 (3H, s, Me-2'), 2.75 (1H, m, H-3), 3.85 (3H, s, OMe-8), 4.08 (3H, s, OMe-4'), 4.31 (1H, q, δ = 6.3 Hz, H-3), 6.51 (1H, s, H-7), 6.66 (1H, d, δ = 1.2 Hz, H-2'), 6.85 (1H, d, δ = 1.1 Hz, H'-1'), 6.88 (1H, d, δ = 7.8 Hz, H-6'), 7.18 (1H, d, δ = 7.8 Hz, H-7'), 9.51 (1H, s, OH-5'). ¹³C NMR (150.9 MHz, CDCl₃): δ 21.1 (Me-1), 22.3 (Me-2'), 23.2 (Me-1), 36.6 (C-4), 48.2 (C-3), 49.7 (C-1), 55.1 (OMe-8), 56.2 (OMe-4'), 96.3 (C-7), 106.9 (C-3'), 109.6 (C-11'), 114.0 (C-10'), 117.3 (C-5), 118.5 (C-1'), 121.6 (C-9), 130.9 (C-7'), 136.4 (C-2'), 136.7 (C-4'), 137.3 (C-9'), 152.4 (C-6), 155.0, 156.4, 156.6 (C-4', C-5', C-6, C-8). The ¹H attribution was achieved by HMOC and HMBC expts. EIMS: m/z (rel. int.): 393 [M⁺] (7), 379 [M-CH₃] + (27), 378 [M]+ (100). HRMS m/z 378.1711 [M-CH₃]+ requires: 378.1711.

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**ACKNOWLEDGEMENTS**

The authors wish to thank their colleagues Dr. H. Bringmann, Dr. O. E. O. O. Gbajom, and Dr. C. A. O. Oke for helpful discussions, and the Research Fund of the University of Ibadan for a research grant and a Fellowship (to I. A. O.).
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Oxidative degradation of alkaloids. The degradation, the derivatization of the corresponding amino acids and the subsequent GC-MSD analysis were carried out as described in ref. [15].

Isolation of quercetin. The last HSCCC fr. yielded an amorphous, yellow solid (43.0 mg), identical to an authentic sample of quercetin (Sigma) with respect to ¹H, ¹³C, IR and MS data. Mp > 300 ° (decomp.); ref. [22] mp 310 °.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft (SFB 251 'Ökologie, Physiologie und Biochemie pflanzlicher und tierischer Leistung unter Streß' and Normalverfahren) and by the Fonds der Chemischen Industrie (financial assistance and generous fellowship to C.G.). Furthermore, we are indebted to Dr D. Koppler for fruitful discussions, Dr R. God for the GC-MSD analysis, F. Teltschik for orientating work in this field and M. Rückert for performing the NOE experiments.

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