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ANCISTROHEYNINE A, THE FIRST 7,8′-COUPLED NAPHTHYLISOQUINOLINE ALKALOID FROM *ANCISTROCLADUS HEYNEANUS**

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Key Word Index—*Ancistrocladus heyneanus*; Ancistrocladaceae; ancistroheynine A; naphthylisoquinoline alkaloids; biaryls; atropisomerism; structural elucidation; antimalarial activity.

Abstract—The isolation and structural elucidation of a novel naphthylisoquinoline alkaloid, named ancistroheynine A, from the Indian liana *Ancistrocladus heyneanus* is described. The absolute configuration at the stereocentre was established by oxidative degradation to give *S*-3-aminobutyric acid. The axial configuration was unambiguously determined by long-range nuclear Overhauser enhancement (NOE) measurements, which covered the, as yet, longest distance within this class of compounds. Ancistroheynine A is the first 7,8′-coupled naphthylisoquinoline alkaloid found in *A. heyneanus*. Its accumulation in spherical inclusions is strongly enhanced in aging parts of the plant. It displays pronounced *in vitro* activity against *Plasmodium falciparum*, the most lethal human malaria parasite. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Ancistrocladus heyneanus is a tropical liana indigenous to the West Ghats in India. From the roots of this productive plant, Govindachari and his group isolated a series of naphthylisoquinoline alkaloids and, due to their brilliant pioneering work, established unambiguously their absolute stereostructures [2]. Thus, besides the main alkaloid ancistrocladine (**1**) [3], its methyl ether **2** [4] and its dehydro analogue, ancistrocladinine (**3**) [5], which likewise show a 5,1′-linkage between the two molecular moieties, were found, as well as the related, but 7,1′- and 7,3′-coupled, respectively, alkaloids ancistrocladisine (**4**) [6] and ancistrocladidine (**5**) [7]. All of their structures were fully confirmed by X-ray analyses [8] or by total synthesis [9, 10]. More recently, isoancistrocladine (**6**), previously known as the borohydride reduction product of **3** [3, 5], was isolated by our group [11]. It also belongs to the 5,1′-coupling type, but is the first naturally occurring *cis*-configured naphthylisoquinoline alkaloid with a free, i.e. non-methylated, secondary NH-function. The promising biological properties of the naphthylisoquinoline alkaloids [12], including antifungal, insect antifeedant and growth retardation, antiplasmodial and molluscicidal activities, make the search for further representatives of this intriguing class of compounds a rewarding task.

In the present paper, we report the isolation and structural elucidation of a novel alkaloid, termed ancistroheynine A (**7a**), from *A. heyneanus*. Its 7,8′-linkage makes it the first naphthylisoquinoline alkaloid being coupled via the less substituted naphthalene ring, from an Asian *Ancistrocladus* species. The antiplasmodial activity of **7a** was assayed against asexual erythrocytic *Plasmodium falciparum* stages *in vitro*.

RESULTS AND DISCUSSION

During light microscopic investigations of fresh stem material of *A. heyneanus*, we detected strikingly yellow-coloured solutes in single parenchyma cells throughout the whole cross-section (see Fig. 2a). A bright yellow fluorescence (see Fig. 2b) was observed upon irradiation with UV light (365 nm). The distribution of this yellow material was very dense in the pith and bark regions, so that an isolation of the substance from these tissue parts seemed most promising. The yellow solutes apparently accumulated in

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*Part 77 in the series 'Acetogenic Isoquinoline Alkaloids'. For Part 76, see ref. [1].

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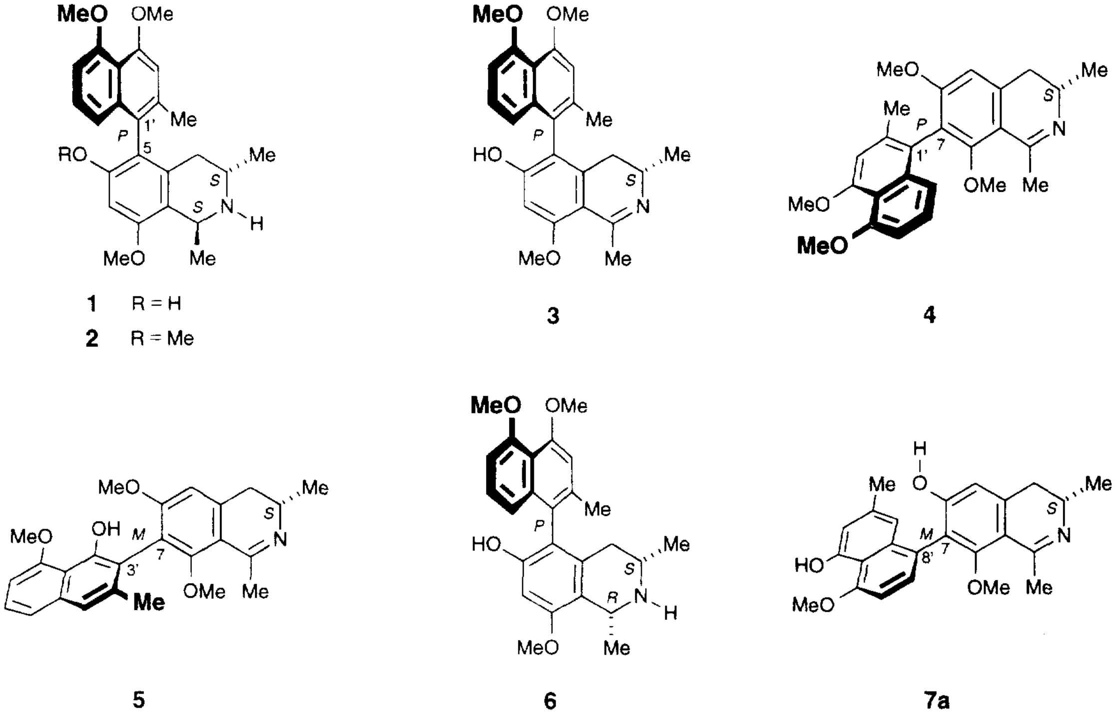


Fig. 1. Alkaloids of *A. heyneanus*.

spherical inclusions with diameters of *ca* 13–18 resp. 10 μm. The number of these inclusions increased when the cut shoots of young plants were left standing in water for 2–10 days. Since none of the known alkaloids **1**–**6** of *A. heyneanus* mentioned above shows a similar yellow color and fluorescence, the isolation of the respective chemical constituent(s) seemed most promising.

The easiest way to obtain substance concentrations sufficient for chemical analysis was to use tissue regions of the pith, since the concentration of disturbing solutes was low compared with other tissues, such as the xylem/phloem region. Pith tissue of the shoot of a *ca* two-year-old *A. heyneanus* plant that had been cultivated as described previously [12–14], was prepared by peeling off the bark and the xylem/phloem regions. The pith material was then powdered after deep freezing in liquid nitrogen. Extraction with methanolic HCl at room temperature with ultrasonic treatment and subsequent filtration, evaporation of the solvent and neutralization with NH_3, gave a crude extract. Column chromatography on silica gel, with dichloromethane–methanol (19:1) as eluent, delivered a bright yellow amorphous solid, which was homogeneous according to TLC. Alternatively, the powdered pith material was extracted with aqueous 0.1 M HCl and worked up as described above.

By high resolution mass spectrometry of its $[M - Me]^+$ peak, the isolated compound was found to correspond to the molecular formula $C_{24}H_{25}NO_4$. The ^1H NMR spectrum showed typical resonances of a naphthylisoquinoline alkaloid with a dihydroisoquinoline moiety, the singlet for the protons of the methyl group at C-1 (δ 2.18, Fig. 3a) being exchangeable in D_2O, as found for other dihydroisoquinolines [2, 15]. A distinct NOE effect (Fig. 3b) from this methyl group at C-1 to one of the two *O*-methyl signals (δ 3.79) indicates that this methoxyl function must be located at C-8. From its high-field shielding, compared with other aryl methoxyl groups (δ *ca* 4), it becomes clear that the naphthalene substituent with its ring current effect must be located at C-7 of the isoquinoline part.

Based on two- and three-bond coupling in the heteronuclear multiple bond correlation (HMBC) spectrum (Fig. 3c), the position of the axis in the isoquinoline moiety is also confirmed by cross-peaks of the proton singlet of H-5 (δ 6.12) and the carbon signals of C-7 (δ 114.3) and C-4 (δ 35.7). The rotating frame Overhauser enhancement spectroscopy (ROESY)-type interactions of H_{eq}-4 (δ 2.68) and H_{ax}-4 (δ 3.42) to Me-3 (δ 1.20), and of H_{eq}-4 to H-5, confirm the constitution in the isoquinoline moiety. The oxygen function at C-6 must be a free hydroxyl group, which is deduced from the HMBC correlation of the HO-6 signal (δ variable, *ca* 5–6) with C-5 (δ 118.30).

Consequently, one of the two oxygen functions on the naphthalene nucleus must be a methoxyl and the other one a free hydroxyl group, the latter giving a sharp, low-field signal (δ 9.30) due to the chelation effect. In the HMBC spectrum, this hydroxyl group is correlated with C-3′ (δ 114.15), which in turn shows HMBC correlations with Me-2′ (δ 2.44) and H-1′ (δ 6.94). This indicates that the isoquinoline substituent cannot be coupled via C-1′ or C-3′ as found in the

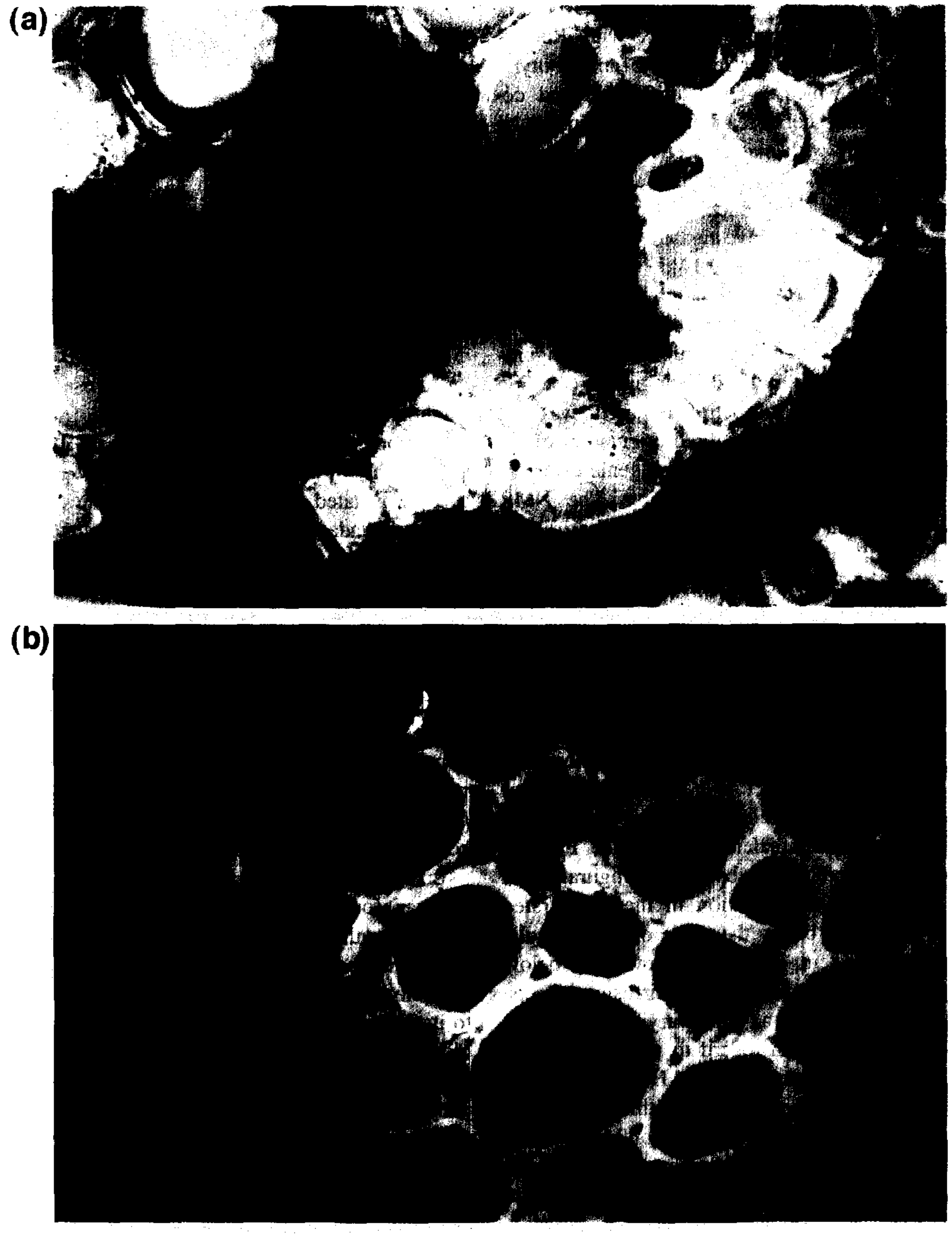


Fig. 2. (a) Bright field microscopic image of the pith region of *A. heyneanus* with yellow cell inclusions. Magnification × 472. (b) Corresponding image using fluorescence microscopy, showing yellow fluorescence of the inclusions. Magnification × 472.

other known constituents **1–6** of this species, but must be located in the methyl-free isocyclic ring. The linkage of the isoquinoline part to C-8′, and not to C-6′, is demonstrated by the NOE-deduced proximity of H-6′ (δ 6.72) both to the other aromatic proton at C-7′ (δ 7.09) and the above-mentioned naphthalene *O*-methyl group, which must thus be located at C-5′. This again is underlined by its 'normal', not high-field shifted, position (δ 4.13) in the spectrum.

The NMR data hint at the gross structure **7** for the isolated compound, thus indicating the presence of a new naphthylisoquinoline alkaloid, now named ancistroheynine A. Its constitution is in agreement with a complete assignment of all the ^{1}H and ^{13}C spectral data as accomplished using H,H-correlation spectroscopy (H,H-COSY), heteronuclear multiple quantum correlation (HMQC), and HMBC spectroscopy (Fig. 3c). A combination of these 2D experiments allowed further confirmation of the established substitution pattern of the two ring systems, as well as the very unusual 7,8′-coupling position. According to this constitution, ancistroheynine A has two stereogenic elements, the stereocentre (C-3) and the biaryl axis, which, due to the steric demand of the adjacent substituents, gives rise to atropisomerism. The absolute configuration at C-3 was elucidated using our oxidative degradation procedure

[16], which has recently been greatly improved [1]. Thus, oxidation of ancistroheynine A with $RuCl_3$–$NaIO_4$ in a phosphate buffer led to *S*-3-aminobutyric acid (Scheme 1), which, after esterification and derivatization with Mosher's reagent, was identified by gas chromatography with mass selective detector (GC-MSD) analysis. This clearly shows that ancistroheynine A is *S*-configured at C-3 which is typical (and thus expected) for all hitherto investigated alkaloids from Asian *Ancistrocladus* species [2, 12].

Given the absolute *S*-configuration at C-3, ancistroheynine A may be represented by one of the two atropoisomeric structures **7a**, i.e. with the *M*-configuration at the biaryl axis, or by its *P*-analogue **7b** (see Fig. 4). This last structural information required, was obtained by determination of the stereo-orientation of the naphthalene system relative to the stereogenic centre in the partially saturated heterocyclic ring. The NMR procedure had been established earlier for related representatives of this class of compounds, which in all previous cases, were tetrahydro- but not dihydroisoquinolines [18, 19]. Despite the long distance between significant nuclei on the naphthalene part and protons in the dihydroisoquinoline moiety that are located significantly out of the aromatic plane, and the general problems arising in such medium-sized molecules [20], we managed to find weak, but unambiguous steady-state NOE effects (1.1 and 1.7%, respectively) in optimized NOE difference experiments between H_{ax}-4 and H-1′ of the naphthalene moiety (see Fig. 4). Since (from the established absolute configuration at C-3 and the known conformation in the unsaturated ring) H_{ax}-4 is located below the plane of the isocyclic isoquinoline ring system, H-1′ must likewise be below that plane. In order to exclude spin diffusion effects, 1D-NOE difference spectra with varying irradiation times were measured. With regard to the fact that the minimum distance between H_{ax}-4 and H-1′ is *ca* 4–5 Å (according to conformational analyses), this NOE effect is most remarkable. It was further confirmed by a distinct cross-peak in the ROESY spectrum, thus allowing the first attribution of the axial configuration relative to the centre of a 7-coupled dihydroisoquinoline. Based on the absolute *S*-configuration at C-3 elucidated above, these NOE experiments clearly allowed establishment of the *M*-configuration and thus determine the full stereostructure **7a** of ancistroheynine A.

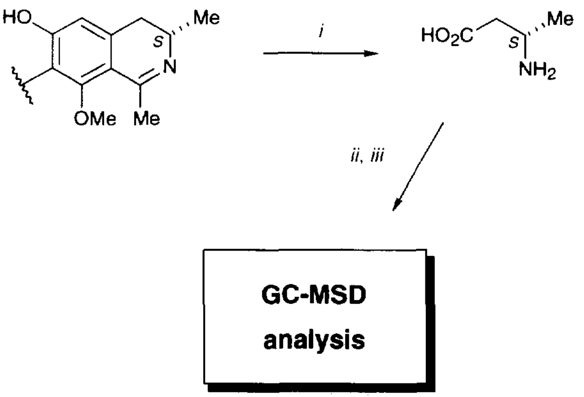
This absolute stereostructure of ancistroheynine A (**7a**) is also consistent with its CD spectrum, which is virtually opposite to that of the structurally closely related, near-enantiomeric naphthylisoquinoline alkaloid, yaoundamine A (**8**), which has been recently isolated from the Central African *Ancistrocladus* species, *A. korupensis* [12, and unpublished work] (Fig. 5).

Ancistroheynine A (**7a**) is the first 7,8′-coupled naphthylisoquinoline alkaloid isolated from an Asian *Ancistrocladus* species. From African *Ancistrocladus* plants, only three 7,8′-coupled alkaloids have previously been isolated, namely ancistrobrevine A [12, 21], an 'Ancistrocladaceae-type' alkaloid, dioncophylline D, which belongs to the 'Dioncophyllaceae type' [12] and the above-mentioned alkaloid, yaoundamine A (**8**), a characteristic 'hybrid-type' alkaloid [12, and unpublished work].

The correspondence of the yellow coloured and fluorescing solutes observed in light microscopy investigations, to this new alkaloid **7a**, is underlined by a comparison of localized Raman spectroscopy of a microscopical cross-section of the shoot of *A. heyneanus* with the Raman spectra of the pure isolated compound. [unpublished results].

Like some other naphthylisoquinoline alkaloids [22], **7a** was found to show antimalarial activity against asexual erythrocytic *P. falciparum* forms *in vitro*, with an IC_{50} value of 2.1 $\mu g\ ml^{-1}$.

Although ancistroheynine A (**7a**) was discovered due to its enhanced accumulation in spherical inclusions of older cuttings, it is also a 'regular' metabolite of *A. heyneanus*, hence a 'true' natural product occurring in plant material as collected in the tropical rain forest, yet only in traces. The increased formation of **7a** under these particular strenuous environmental conditions shows that the directed optimization of such conditions might deliver a rewarding source of new, hitherto



Scheme 1. Absolute configuration at C-3 of ancistroheynine A, as deduced from the oxidative degradation reaction. (i) $RuCl_3$, $NaIO_4$; (ii) esterification with MeOH; (*R*)-MTPA-Cl (Mosher's chloride, see ref. [17]).

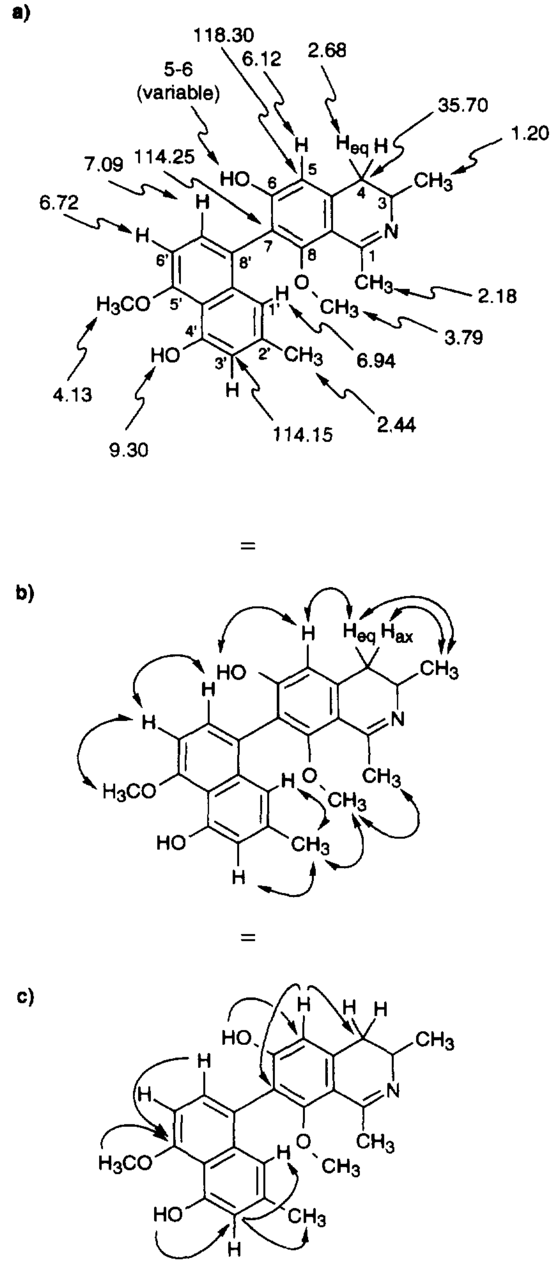


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Fig. 3. Constitution 7 of ancistroheynine A through (a) selected chemical ^{1}H and ^{13}C NMR shifts (δ values), (b) NOE (respectively ROESY) effects and (c) significant heteronuclear long-range couplings.

unknown alkaloids normally occurring in the plants only in trace amounts. The investigation of the inducing factors for the formation of this new compound and its optimization, are in progress.

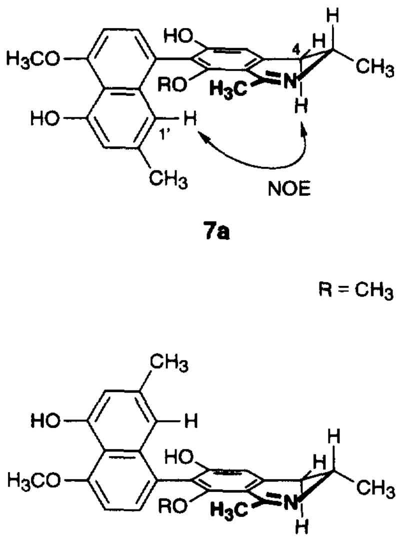
EXPERIMENTAL

General. Mps uncorr. Optical rotations: 25°, 10 cm cell, $CHCl_3$ (filtered through basic Al_2O_3). CD: 25°, EtOH. IR: KBr. ^{1}H NMR (600 MHz): $CDCl_3$, δ 7.26. Proton detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC} = 150$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). EIMS: 70 eV. CC: silica gel (60–200 mesh, Merck) by addition of 5% aq. NH_3; polyamide 6 (50–160 mesh, Machery-Nagel). TLC: precoated silica gel 60 F_{254} plates (Merck), deactivated with NH_3. Spots were visualized under UV light and by Dragendorff's reagent. HPLC: μBondapak C_{18} (10 μm, 3.9 mm × 30 cm, Waters), UV detection at 270 nm, flow 1 ml min^{-1}.

Plant material. Plants were cultivated in the Botanical Garden, University of Würzburg, from fully matured seeds of *A. heyneanus* Wall. ex J. Grah. collected in 1992 in India. Details regarding the cultivation were described earlier [13]. Shoots of 2-year-old plants were cut and kept standing in H_2O under conditions of high humidity for up to 10 days.

Extraction and isolation. Fresh pith material (15.2 g) from a shoot was ground after freezing in liquid N_2 and subsequently extracted with methanolic HCl at room temp. with ultrasonic assistance. The soln was evapd and the residue (156 mg), after neutralization with NH_3, subjected to CC on deactivated silica gel using CH_2Cl_2–MeOH (19:1) as eluent. Alternatively, the ground powder was extracted using 0.1 M aq. HCl at room temp. using ultrasonic assistance, giving a residue (105 mg).

Isolation of ancistroheynine A (**7a**). A second CC of the frs of the MeOH extract containing the yellow compound on silica gel with CH_2Cl_2–MeOH (19:1) as eluent gave **7a** (1.7 mg) as a yellow amorphous powder, which could not be recrystallized. The residue of the H_2O extract was neutralized and fractionated on polyamide with a gradient of MeOH–H_2O 1:1 → 1:0. Repeated HPLC with MeCN–0.1 M Pi buffer (pH 5.4)(1:1) afforded **7a** (1.6 mg). $[\alpha]_D^{25} = +68^\circ$ ($CHCl_3$; c 0.3). CD: $\Delta\varepsilon_{201}$ −44.7, $\Delta\varepsilon_{207}$ +32.5,



7a

R = CH_3

7b

Fig. 4. Determination of configuration at biaryl axis through long-range NOE experiments.

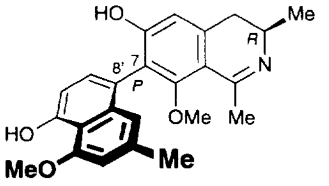


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Fig. 5. Structure of the 7,8'-coupled alkaloid yaoundamine A.

$\Delta\varepsilon_{212}$ + 13.9, $\Delta\varepsilon_{232}$ + 52.1, $\Delta\varepsilon_{249}$ − 33.4, $\Delta\varepsilon_{272}$ + 6.7, $\Delta\varepsilon_{319}$ − 16.0, $\Delta\varepsilon_{330}$ − 16.4, $\Delta\varepsilon_{341}$ + 5.4. IR ν_{max} cm^{-1}: 3420–3200 (OH), 2830 (OMe), 1595 (C=C), 1255 (C–O). ^{1}H NMR (600 MHz, CDCl$_3$): δ 1.20 (3H, *d*, J = 6.2 Hz, Me-3), 2.18 (3H, *s*, Me-1), 2.44 (3H, *s*, Me-2'), 2.68 (1H, *dd*, J_{gem} = 16.6 Hz, J_{eq} = 2.6 Hz, H$_{eq}$-4), 3.42 (1H, *dd*, J_{gem} = 16.6 Hz, J_{ax} = 5.6 Hz, H$_{ax}$-4), 3.64 (1H, *m*, H-3), 3.79 (3H, *s*, OMe-8), 4.13 (3H, *s*, OMe-5'), 6.02 (1H, *s*, OH-6), 6.12 (1H, *s*, H-5), 6.72 (1H, *d*, J = 8.0 Hz, H-6'), 6.86 (1H, *d*, J = 0.9 Hz, H-3'), 6.94 (1H, *d*, J = 0.9 Hz, H-1'), 7.09 (1H, *d*, J = 8.0 Hz, H-7'), 9.30 (1H, *s*, OH-4'). ^{13}C NMR (150 MHz, CDCl$_3$): δ 22.1 (Me-1), 22.4 (Me-2'), 29.7 (Me-3), 35.7 (C-4), 53.4 (OMe-8), 56.5 (OMe-5'), 68.9 (C-3), 101.9 (C-6'), 102.3 (C-7), 112.0 (C-1'), 114.2 (C-3'), 118.3 (C-5), 125.7 (C-7'), 131.8 (C-8'), 136.8 (C-10'), 137.1 (C-9), 137.6 (C-9'), 141.0 (C-10), 155.2 (C-4'), 155.3 (C-5'), 160.1 (C-6), 162.1 (C-1), 165.7 (C-8). An assignment of C-9 and C-9' might be interchangeable, because the correlations in the HMBC spectrum are overlapping, the signal for C-2' is probably overlapped by C-10'. MS *m/z* (rel. int.): 391 [M]$^+$ (61), 376 [M − Me]$^+$ (47), 360 [M − OMe] (5), 195.5 [M]$^{2+}$ (4); HRMS *m/z* 376.154 [M − Me]$^+$ ($C_{23}H_{22}NO_4$ requires 376.154).

Oxidative degradation of **7a**. Oxidative degradation of 1.3 mg **7a** with 9.0 mg NaIO$_4$ and RuCl$_3$ as catalyst was carried out in 0.4 ml of MeCN–CCl$_4$–0.1 M Na-Pi buffer (pH 6) (1 : 1 : 2). Esterification and derivatization of the resulting 3-aminobutyric acid with (*R*)-MTPA-Cl and the subsequent GC-MSD analysis on a nonpolar fused silica capillary column were performed according to an improved method [1].

Antimalarial assay. Compound **7a** was tested against asexual *Plasmodium falciparum* erythrocytic forms (strain NF 54, clone A1A9) *in vitro*, by measuring the incorporation of ^{3}H-hypoxanthine in the presence of the test substance after 42 hr of incubation at 37° [22].

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REFERENCES

1. Bringmann, G., God, R. and Schäffer, M. (1996) *Phytochemistry* (in press).
2. Govindachari, T. R. and Parthasarathy, P. C. (1977) *Heterocycles* **7**, 661.
3. Govindachari, T. R., Nagarajan, K., Parthasarathy, P. C., Rajagopalan, T. G. Desai, H. K. Kartha, G., Lai-chen, S.-m. and Nakanishi, K. (1974) *J. Chem. Soc., Perkin Trans.* 1. 1413.
4. Desai, H. K., Govindachari, T. R., Joshi, B. S., Parthasarathy, P. C., Ramachandran, K. S., Ravindranath, K. R., Sidhaye, A. R. and Viswanathan, N. (1976) *Indian J. Chem., Sect. B* **14**, 473.
5. Govindachari, T. R., Parthasarathy, P. C. and Desai, H. K. (1971) *Indian J. Chem.* **9**, 1421.
6. Govindachari, T. R., Parthasarathy, P. C. and Desai, H. K. (1972) *Indian J. Chem.* **10**, 1117.
7. Govindachari, T. R., Parthasarathy, P. C. and Desai, H. K. (1973) *Indian J. Chem.* **11**, 1190.
8. Parthasarathy, P.C. and Kartha, G. (1983) *Indian J. Chem., Sect. B* **22**, 590.
9. Bringmann, G., Jansen, J. R., Rink, H.-P. (1986) *Angew. Chem.* **98**, 917. (*Angew. Chem. Int. Ed. Engl.* **25**, 913).
10. Bringmann, G. and Reuscher, H. (1989) *Tetrahedron Letters* **30**, 5249.
11. Bringmann, G., Kinzinger, L., Ortmann, T. and de Souza, N. J. (1994) *Phytochemistry* **35**, 259.
12. Bringmann, G. and Pokorny, F. (1995) in *The Alkaloids* (Cordell, G. A., Ed.), Vol. 46, p. 127. Academic Press, New York.
13. Bringmann, G., Pokorny, F. and Zinsmeister, H.-D. (1991) *Der Palmengarten* **55/3**, 13.
14. Bringmann, G., Schneider, C., Pokorny, F., Lorenz, H., Fleischmann, H., Sankara Narayanan, A. S., Almeida, M. R., Govindachari, T. R. and Aké Assi, L. (1993) *Planta Med.* **59** (Suppl.), 623.
15. Bringmann, G., Pokorny, F., Stäblein, M., Schäffer, M. and Aké Assi, L. (1993) *Phytochemistry* **33**, 1511.
16. Bringmann, G., Geuder, T., Rübenacker, M. and Zagst, R. (1991) *Phytochemistry* **30**, 2067.
17. Dale, J.A. and Mosher, H.S. (1973) *J. Am. Chem. Soc.* **95**, 512.
18. Bringmann, G., Koppler, D., Porzel, A. and Scheutzow, D. (1994) *GIT Fachz. Lab.* **38**, 705.
19. Bringmann, G., Rübenacker, M., Koch, W., Koppler, D., Ortmann, T., Schäffer, M. and Aké Assi, L. (1994) *Phytochemistry* **36**, 1057.
20. Bothner-By, A. A., Stephens, R. L., Lee, J. T., Warren, C. D. and Jeanloz, R. W. (1984) *J. Chem. Soc.* **106**, 811.
21. Bringmann, G., Pokorny, F., Reuscher, H., Lisch, D. and Aké Assi, L. (1990) *Planta Med.* **56**, 496.
22. François, G., Bringmann, G., Phillipson, J. D., Aké Assi, L., Dochez, C., Rübenacker, M., Schneider, C., Wéry, M., Warhurst, D. C. and Kirby, G. C.