DIONCOPHYLLINOL D, THE FIRST 4-HYDROXYLATED NAPHTHYLISOQUINOLINE ALKALOID, FROM THE LEAVES OF *TRIPHYOPHYLLUM PELTATUM*[1]

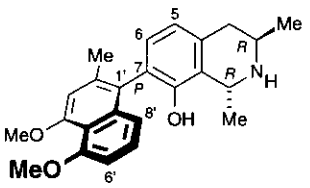
Gerhard Bringmann,*[a] Matthias Wenzel,[a] Markus Rückert,[a] Kristina Wolf,[a] Stefan Busemann,[a] Manuela Schäffer,[a] and Laurent Aké Assi[b]

[a]Institut für Organische Chemie der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany
[b]Centre National de Floristique, 08 B. P. 172, Abidjan 08, Ivory Coast

Dedicated to Professor Koji Nakanishi, on the occasion of his 75th birthday

Abstract - The first isolation of a 4-hydroxylated naphthylisoquinoline alkaloid, dioncophyllinol D (**5**), from the leaves of *Triphyophyllum peltatum* (Dioncophyllaceae), is described. Its structural elucidation is based mainly on NMR, degradative, and computational methods. With its rare 7,8'-linkage between the isoquinoline and naphthalene parts, **5** is one of the very few naphthylisoquinolines without a stable configuration at the biaryl axis.

The West African liana *Triphyophyllum peltatum* belongs to the small family Dioncophyllaceae, which consists of the three monotypic genera, *Triphyophyllum, Habropetalum*, and *Dioncophyllum.*[2] Previous phytochemical work on *T. peltatum* revealed the presence of a broad series of novel naphthylisoquinoline alkaloids.[3] Most of them, like the main alkaloid dioncophylline A (**1**),[4] are based on a 7,1'-linkage ('A-type') between the isoquinoline and the naphthalene parts, but the distinctly less frequent coupling types B (7,6'),[5] and C (5,1'),[6] and D (7,8')[3] have also been found. Different from the related Ancistrocladaceae-type alkaloids, all these Dioncophyllaceae alkaloids lack their presumably original oxygen at C-6.[3] On the other hand, *T. peltatum* likewise produces alkaloids with additional, not polyketide-derived oxygen functionalities, among them dioncopeltine A (**2**)[7] and its even higher oxidized cyclic analog, dioncolactone A (**3**),[7] as well as the 4-methoxylated, fully dehydrogenated naphthylisoquinoline dioncophyllacine A (*rac*-**4**).[8] Given the remarkable biological activities of *Triphyophyllum* alkaloids, *e.g.* the excellent antimalarial activity of dioncopeltine A (**2**) *in vitro*[9] and in *vivo*,[10] the search for further related alkaloids, in particular with (additional) free hydroxy functions, seems rewarding. In this paper, we report on the first isolation of a 4-hydroxylated naphthyltetrahydroisoquinoline alkaloid, dioncophyllinol D (**5**), exhibiting the very rare 7,8'-linkage.



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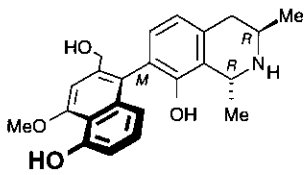

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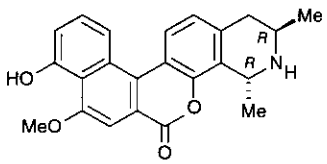

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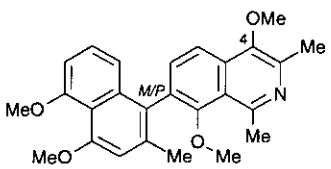

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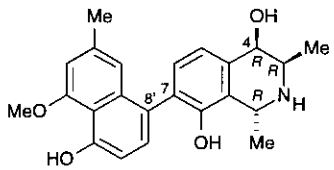

5

Leaves of *T. peltatum* were dried, powdered, and macerated with MeOH / 1N HCl (1:1). After removal of the organic solvent, the aqueous residue was re-extracted with $CHCl_3$. Column chromatography of this $CHCl_3$ extract on deactivated (NH_3) silica gel and separation by semipreparative reversed phase HPLC gave a pure yellow solid. Its ^1H NMR spectrum showed the typical signals for a naphthylisoquinoline alkaloid. Different *e.g.* from **1,** the coupling pattern of the aromatic protons, in particular the presence of four aromatic doublets and two singlets, suggests the biaryl axis to be positioned in the 6'- or 8'-position of the naphthalene part. The main difference compared to the ^1H NMR spectrum of **1**, however, is the lack of the typical diastereotopic protons at C-4 with expected chemical shifts of 2.7 (dd) and 3.4 (dd) ppm. Instead, a distinctly low-field shifted doublet (J = 2.2 Hz) is observed at 4.5 ppm (Figure 1a), hinting at the presence of a CH-X array at C-4, which is furthermore confirmed by the multiplicity of H-3 (δ 3.7 ppm, dq, J = 2.2 Hz, 6.9 Hz). From the chemical shift of H-4 (δ 4.5 ppm), an OH group can be assumed to be located at C-4. The presence of a novel 4-hydroxylated naphthyltetrahydroisoquinoline alkaloid is corroborated by HRMS, which delivers the molecular formula $C_{23}H_{25}NO_4$ (M^+, m/z = 379.177).

NOE interactions (Figure 1a) of H-5 with both, H-4 and H-6, suggest the biaryl axis to be located at C-7 of the isoquinoline part. This is confirmed by an HMBC interaction (Figure 1b) between H-6 and C-8', across the biaryl axis. The position of the biaryl linkage in the naphthalene part at C-8' is demonstrated by HMBC correlations (Figure 1b) between H-1' and C-8' as well as the aforementioned interaction between H-6 and C-8', which likewise rules out a coupling *via* C-6'. In agreement with this 7,8'-coupling, CH_3-2' exhibits NOE effects (Figure 1a) both with H-1' and H-3' thus excluding the axis to be positioned in that methyl-substituted ring of the naphthalene part, which is underlined by the normal, not high-field shifted absorption of CH_3-2' (δ 2.5 ppm). The methoxy group in the naphthalene part is located at C-4' (and not C-5') as evidenced by an NOE effect with H-3' (Figure 1a) and an HMBC interaction with C-4' (Figure 1b).

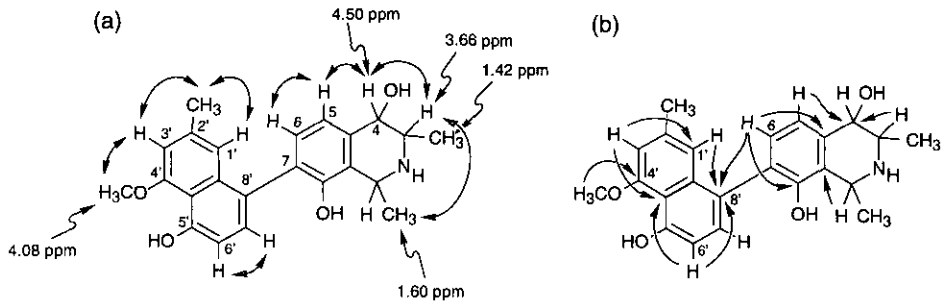

Figure 1. Constitution of the new alkaloid, from (a) selected ^{1}H NMR shifts (δ values in ppm) and NOE interactions, and (b) HMBC correlations.

The novel alkaloid with the very rare 7,8' ('D-type') coupling pattern and the unprecedented extra OH-group at C-4, subsequently named dioncophyllinol D, is the first naphthylisoquinoline alkaloid with three stereocenters at C-1, C-3, and C-4. From an NOE interaction between H-3 and CH_3-1 (Figure 2), which are thus both axial and *cis* to each other, a relative *trans*-configuration of the two methyl groups at C-1 and C-3 can be deduced. The small coupling constant (J = 2.2 Hz) between the axial proton at C-3 and H-4 indicates the latter to be equatorial, revealing a relative *cis*-configuration of OH-4 and CH_3-3. This is confirmed by another clear NOE interaction between H-3 and H-4, which excludes a *trans*-diaxial position of these two protons and thus an equatorial position of the oxygen substituent at C-4. For the given *cis*-configuration at C-3 and C-4, both *trans* relative to CH_3-1, a semiempirical conformational analysis (AM1[11] as implemented in VAMP 5.0[12]) indicates the preferred presence of a half-chair conformation as seen in Figure 2, in full agreement with the NOE data and coupling constants observed.

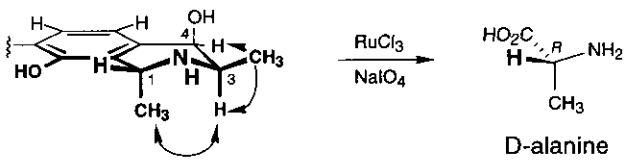

Figure 2. Relative and absolute configuration at the stereogenic centers of the tetrahydroisoquinoline part, through NOE interactions and degradation to give D-alanine.

For the determination of the absolute configuration, our efficient oxidative degradation procedure as recently further improved[13] was used. In this case, different from all the other naphthyltetrahydroisoquinoline alkaloids degraded so far, no 3-aminobutyric acid was to be expected because of the additional hydroxy function at C-4. Regrettably, 2-hydroxy-3-aminobutyric acid as a possible degradative product was not observed, either. Still, unambiguous results were obtained from the D-alanine analyzed, whose absolute configuration

clearly shows C-1 to be *R*-configured. From this and the relative configuration as elucidated above, the stereocenters C-3 and C-4 were deduced to have the *R*-configuration, too, as drawn in Figure 2.

The last remaining stereochemical information required, was the chirality at the biaryl axis, *i.e.* the question whether dioncophyllinol D is represented by the stereostructure *P*-**5** or *M*-**5** (Figure 3). A calculation of the CD behavior expected for these two imaginable atropo-diastereomeric forms of **5** using the previously most successful CNDO/S-CI method[14] and comparison of the theoretically predicted CD spectrum with the experimental one of the natural product, gave no significant agreement, neither for the *M*- nor for the *P*-atropisomer. The apparent reason is the low atropisomerization barrier due to the small steric demand of the *ortho*-substituents next to the biaryl axis. Probably for the same reason, extended NOE and ROE experiments, as successfully applied in other cases,[15] did not give any diagnostically valuable long-range interactions between the naphthalene and the isoquinoline parts as expected for *P*- or *M*-**5**. First calculations (AM1[11]) to determine the activation energy of the atropisomerization process confirm the supposition of a rapid dynamic rotation about the biaryl axis at room temperature ($\Delta H^{\ddagger} < 20$ kcal mol^{-1}). This assumption is supported by low temperature NMR experiments, leading to a clear decoalescence of the CH_3-1 protons at *ca.* 220 K and a rough estimation of the atropisomerization barrier at this temperature as *ca.* 11 kcal mol^{-1}.[16]

Consequently, dioncophyllinol D has the absolute stereostructure **5**, with all of the three stereocenters at C-1, C-3, and C-4 *R*-configured, whereas the axis is configuratively labile, leading to a rapid equilibrium *P*-**5** ⇌ *M*-**5** at room temperature.

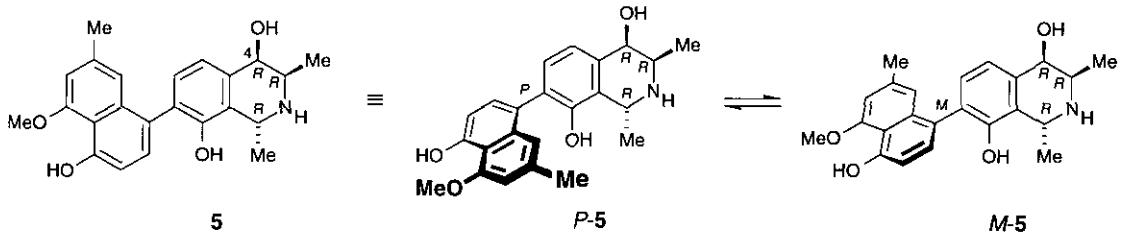

Figure 3. *P*- and *M*-atropisomeric forms of **5**, rapidly interconverting at room temperature.

The isolation of dioncophyllinol D (**5**) shows that the well-investigated plant *T. peltatum* is still a rewarding source of structurally novel alkaloids. While the oxygenation in the benzylic position of tetrahydroisoquinolines is a known metabolic step for conventional (*i.e.* amino acid-derived) tetrahydroisoquinolines,[17] a 4-hydroxylation in naphthyltetrahydroisoquinoline alkaloids is unprecedented and may give a hint at the order of oxygenation and dehydrogenation steps in the biogenesis of the fully aromatic, 4-methoxylated dioncophyllacines A (*rac*-**4**)[8] and B.[3] The biological activity of dioncophyllinol D (**5**) is under investigation.

EXPERIMENTAL

General. Melting points were determined with a Kofler hot plate apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 infrared spectrophotometer. Mass spectra were measured at 70 eV on a Finnigan MAT 8200 or on a Varian MAT CH7 mass spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker DMX 600 spectrometer using CD_3OD (δ 3.33 ppm) as the internal reference. HPLC purifications were carried out with a Waters 600E pump, a Nova-Pak C_{18} (Waters, 200 x 25 mm, 6 µm, integrated guard pak) column, and a Waters 996 photodiode array detector.

Plant Material. *T. peltatum* was collected and identified by one of us (L.A.A.) in the Parc de Taï, West Ivory Coast, in November 1991. Herbarium specimens are deposited at the Centre National de Floristique, Abidjan, and at the Institut für Organische Chemie, Würzburg.

Isolation of Dioncophyllinol D (5). Dried leaves of *T. peltatum* (40 g) were powdered and masserated for 2 d with 400 mL MeOH / 1N HCl (1:1) at rt with ultrasonic assistence. After removal of the MeOH, the aqueous residue was re-extracted with 1.5 L chloroform to yield 180 mg of a brownish crude extract, which was chromatographed over silica gel (60 g, deactivated with 7.5% NH_3) using CH_2Cl_2 / MeOH (95:5) as the eluent to yield a fraction of 22 mg containing the new compound. Further purification was done by semipreparative HPLC using a Nova-Pak C_{18} (200 x 25 mm, 6 µm) column with MeOH / H_2O (6:2) as the eluent to give 6 mg (0.015%) of **5** as a yellow solid, mp 209°C; α_D^{25} + 17° (c = 0.046, $CHCl_3$), IR (KBr) 3395, 3195, 3000, 1660, 1410, 1190, 1110 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 7.32 (1H, d, *J* = 8.6 Hz, 6'-H), 7.27 (1H, d, *J* = 8.6 Hz, 7'-H), 7.25 (1H, d, *J* = 8.1 Hz, 6-H), 7.24 (1H, s, 1'-H), 7.00 (1H, d, *J* = 8.1 Hz, 5-H), 6.85 (1H, d, *J* = 0.9 Hz, 3'-H), 4.66 (1H, q, *J* = 6.8 Hz, 1-H), 4.50 (1H, d, *J* = 2.2 Hz), 4.08 (3H, s, 4'-OCH_3), 3.66 (1H, dq, *J* = 2.2 Hz, 6.7 Hz, 3-H), 2.47 (3H, s, 2'-CH_3), 1.60 (3H, d, *J* = 6.8 Hz, 1-CH_3), 1.42 (3H, d, *J* = 6.7 Hz, 3-CH_3); ^{13}C NMR (CD_3OD, 150 MHz) δ 15.09 (3-CH_3), 17.21 (1-CH_3), 21.89 (2'-CH_3), 49.79 (3-C), 50.17 (1-C), 56.93 (4'-OCH_3), 67.07 (4-C), 108.40 (3'-C), 114.31 (4a'-C), 118.91 (8a'-C), 120.45 (6'-C), 121.69 (1'-C), 122.23 (8a-C), 122.78 (5-C), 128.58 (7-C), 131.20 (7'-C), 132.60 (6-C), 135.66 (5a'-C), 138.15, 138.22 (8'-C, 2'-C), 151.60 (8-C), 151.72 (5'-C), 157.51 (4'-C); MS *m/z* (rel. int.) 379 (M^+, 10.7), 364 (M-CH_3, 100), 348 (M-OCH_3, 11); HRMS *m/z* 379.177 (M^+, $C_{23}H_{25}O_4N$, requires 379.178).

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