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Dioncophylline E from *Dioncophyllum thollonii*, the first 7,3′-coupled dioncophyllaceous naphthylisoquinoline alkaloid☆

Gerhard Bringmann[a,*], Kim Messer[a], Kristina Wolf[a], Jörg Mühlbacher[a], Matthias Grüne[a], Reto Brun[b], Adriaan M. Louis[c]

[a]*Institut für Organische Chemie der Universität, Am Hubland, D-97074 Würzburg, Germany*
[b]*Schweizerisches Tropeninstitut, Socinstrasse 57, CH-4002 Basel, Switzerland*
[c]*Herbier National de Gabon, BP 4015, Libreville, Gabon*

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Dedicated to Professor Gottfried Blaschke on the occasion of his 65th birthday.

Abstract

The isolation and structural elucidation of dioncophylline E, a novel naphthylisoquinoline alkaloid from the rare West African liana *Dioncophyllum thollonii*, is described. The alkaloid displays an unusual 7,3′-linkage between the naphthalene and the isoquinoline portions. Due to the resulting medium degree of steric hindrance exerted by the *ortho*-substituents next to the biaryl axis, the compound undergoes slow rotation about the axis at room temperature and thus is the first such alkaloid that occurs as a mixture of two configurationally semi-stable atropo-diastereomers. Dioncophylline E exhibits good antimalarial activity against both chloroquine-sensitive and -resistant strains of *Plasmodium falciparum* while its antitrypanosomal activity against *Trypanosoma cruzi* is weak and that against *T. brucei rhodesiense* is moderate. Furthermore, four additional naphthylisoquinoline alkaloids previously known from the related plant species *Triphyophyllum peltatum*, have been identified in *D. thollonii*. © 2002 Published by Elsevier Science Ltd.

Keywords: *Dioncophyllum thollonii*; Dioncophyllaceae; Dioncophylline E; Structural elucidation; Stereochemistry; Antimalarial activity

1. Introduction

Within the Dioncophyllaceae, a very small family of palaeotropical lianas consisting only of three monotypic genera, the 'part-time' carnivorous (Bringmann et al., 1996b) plant *Triphyophyllum peltatum* is the phytochemically by far best investigated species (Bringmann et al., 1998a, b, c, 2000b). Its structurally, biosynthetically, and pharmaceutically remarkable secondary metabolites, the naphthylisoquinoline alkaloids, have been found throughout the Dioncophyllaceae and in the related Ancistrocladaceae (Bringmann and Pokorny, 1995), but never outside the two plant families. Thus, dioncophylline A (**1**, Fig. 1), an insect antifeedant (Bringmann et al., 1992b), polyketide-derived (Bringmann et al., 2000a) main metabolite of *T. peltatum* (Bringmann et al., 1990b), is characterized by the presence of two stereocenters and a stereogenic axis between the 7- and the 1′-position of the molecular portions. The antifungal (Bringmann and Pokorny, 1995) alkaloid dioncophylline B (**2**) (Bringmann et al., 1991b), by contrast, does not form stable atropisomers, but undergoes rapid rotation about its—now 7,6′-located—biaryl axis, which is neighbored by only two small OH groups, while dioncophylline C (**3**) (Bringmann et al., 1992a), a highly antimalarial (François et al., 1996) alkaloid with a 5,1′-coupling type, is again configurationally stable. These three natural biaryls—like the meanwhile ca. 16 further naphthylisoquinolines isolated from *T. peltatum* (Bringmann et al., 1998a, b, c, 2000b)—all have in common the characteristic combination of an *R*-configuration at C-3 and the absence of an oxygen function at C-6. These structural features classify them as 'Dioncophyllaceae-type alkaloids', in contrast to the 'Ancistrocladaceae-type' naphthylisoquinolines,

☆ Part 150 in the series "Acetogenic isoquinoline alkaloids". For part 149, see Bringmann et al. (2002a).

* Corresponding author. Tel.: +49-931-888-5323; fax: +49-931-888-4755.
E-mail address: bringman@chemie.uni-wuerzburg.de (G. Bringmann).

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which are *S*-configured at C-3 and oxygenated at C-6 (Bringmann and Pokorny, 1995).

Different from *T. peltatum*, very little is known phytochemically about the two other Dioncophyllaceae species. Early isolation work on *D. thollonii* (Lavault and Bruneton, 1980) revealed the presence of six naphthylisoquinolines, but their structures were not satisfactorily characterized, in particular with respect to the absolute configurations at the stereocenters (which were arbitrarily assumed to be *S*) and -axes (which were not recognized as stereogenic elements). Therefore and because of promising hints from LC-NMR investigations on crude plant extracts (Bringmann et al., 1999a), a reinvestigation of this plant seemed rewarding. Here we report on the isolation and structural assignment of the four known naphthylisoquinolines **1**, **4**, (*P*)-**5**, and its atropisomer (*M*)-**5** from *D. thollonii* and of the new compound dioncophylline E (**6**), the first 7,3′-coupled dioncophyllaceous alkaloid. In contrast to the very few other 7,3′-coupled Ancistrocladaceae alkaloids known (Govindachari et al., 1973; Ruangrungsi et al., 1985; Bringmann et al., 1998d), **6** is configurationally semistable at the axis. It is thus the first naphthylisoquinoline alkaloid that exists as a pair of slowly interconverting atropo-diastereomers, (*P*)-**6** and (*M*)-**6**, at room temperature.

2. Results and discussion

Air-dried and powdered root material of *D. thollonii* was successively extracted with *n*-hexane and methanol, of which the latter was perforated with chloroform. This chloroform extract was then subjected to CC. By TLC comparison, the main Dragendorff-active compound resembled the well known (Bringmann et al., 1990a, c) alkaloid dioncophylline A (**1**), but NMR investigations revealed the presence of *two* compounds, apparently resulting from the co-occurrence of **1** and its 5′-*O*-demethyl analog **4** (see Fig. 1). They proved to be difficult to resolve because of their nearly identical chromatographic behavior on normal-phase silica gel (Bringmann et al., 1998c). This separation problem had previously been solved (Bringmann et al., 1998c) after conversion of the mixture into a pair of brominated and benzylated, now well separable, derivatives. In an attempt to resolve the mixture directly, without chemical modification, we took advantage of their possibly different partition coefficients in a two-phase mixture, by using high-speed countercurrent chromatography (HSCCC) (Ito and Conway, 1996). Indeed, after optimization of the solvent mixture ($CHCl_3$–MeOH–0.1 N HCl 6:3:3), 70% of a starting sample was easily separated even on a semipreparative scale, with complete recovery of the remaining unresolved material. The resulting two compounds thus obtained in a pure form indeed proved to be dioncophylline A (**1**) and 5′-*O*-demethyldioncophylline A (**4**), identical in all respects with authentic material from *T. peltatum* (Bringmann et al., 1998c). The example demonstrates the value of the HSCCC methodology as an entirely different separation principle, saving time (here three chemical protection and deprotection steps) and material (as compared to the unavoidable loss on normal separation columns e.g. with SiO_2 as the adsorbent).

In the region less polar than **1**, the second isolated main constituent of *D. thollonii* coeluted on TLC with *N*-methyldioncophylline A (**5**) and also exhibited the corresponding NMR signals, albeit with a (1:1)-peak doubling for some of the signals, e.g. for those of the *N*-methyl (2.54 and 2.55 ppm) and the 2′-methyl (2.17 and 2.20 ppm) groups, clearly indicating the presence of a mixture of two structurally very similar, apparently stereoisomeric naphthylisoquinolines. By our ruthenium-catalyzed oxidative degradation (Bringmann et al., 1996a), the stereocenters of the two isomers were shown to be *R*-configured, both at C-1 and C-3, suggesting the isomers to differ only by their axial configuration. As previously for a similar mixture of (*P*)-**5** and its likewise natural atropo-diastereomer (*M*)-**5** from *Ancistrocladus abbreviatus* (Bringmann et al., 1991a), resolution of (*P*)-**5** and (*M*)-**5** succeeded again by chromatography on a chiral phase (Chiralcel OD), albeit now with a slight adaptation of the separation conditions to the semipreparative HPLC column. As expected for atropisomeric naphthylisoquinoline alkaloids (even though diastereomeric ones), the CD spectra of the resulting pure substances were near-opposite to each other and the physical and spectral data were fully identical with those of the known (Bringmann et al., 1991a) alkaloids *N*-methyldioncophylline A [(*P*)-**5**] and *N*-methyl-7-*epi*-dioncophylline A [(*M*)-**5**] (see Fig. 1).

One of the more polar fractions obtained from CC seemed to contain one pure compound according to TLC. As described for (*M*)/(*P*)-**5** above, however, the NMR spectrum of this fraction again showed two sets of signals for two very similar naphthyl-1,3-dimethyltetrahydroisoquinoline structures. HPLC analysis gave two well separated peaks with retention times differing by ca. 10 min, even on a preparative scale. But the analysis of the two chromatographic fractions thus obtained, showed these samples to be fully identical: Both by HPLC and by NMR, again each of them consisted of the two previous compounds, indicating their interconversion at room temperature. This was unambiguously proven by an immediate investigation of the freshly resolved single peaks as obtained by rapid analytical HPLC analysis, now clearly showing a gradual equilibration of the two peaks, with a moderate atropisomerization rate at room temperature (see Fig. 2).

This interconversion could also be nicely followed by HPLC-NMR experiments in the stopped-flow mode, by

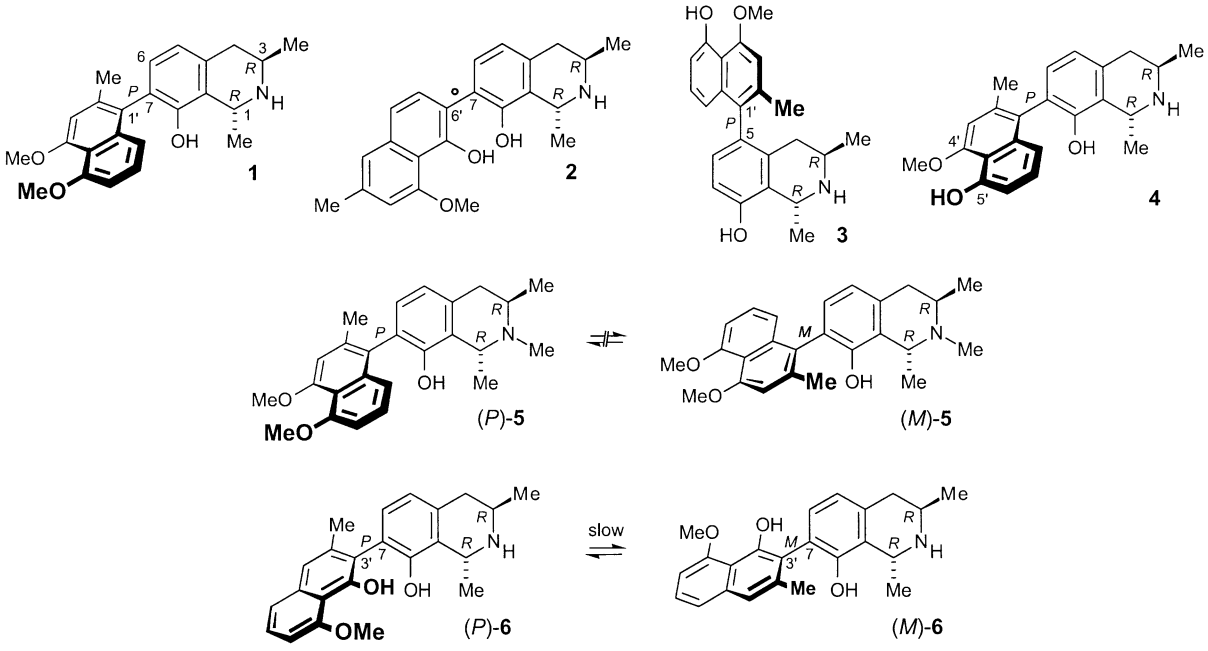

Fig. 1. Bioactive naphthylisoquinolines from Dioncophyllaceae: dioncophyllines A-C (**1–3**), 5′-*O*-demethyldioncophylline A (**4**), *N*-methyldioncophylline A and its 7-epimer [(*P*)-**5** and (*M*)-**5**], and the two configurationally semi-stable atropisomers of dioncophylline E (**6**) (○: configurationally unstable axis).

watching, e.g., the well-separated signals of the 1- and 3-methyl groups (not shown) of the two—apparently stereoisomeric—naphthylisoquinoline alkaloids present in that mixture. Unfortunately, some of the other NMR signals of these two species overlapped in the LC-NMR solvent system (CH_3CN–D_2O), which thus did not permit a secure attribution of the entire two sets of signals. Still, from the very similar NMR signals and also from HPLC-MS/MS experiments, giving rise to identical mass spectra for both peaks, it became clear that the two interconverting species were probably again atropodiastereomers. This was entirely unprecedented in so far as all other naphthylisoquinoline alkaloids as yet known, form rotational isomers that are—depending on the substitution patterns in the proximity of the biaryl linkage—either configurationally fully stable, such as, e.g., dioncophyllines A (**1**) and C (**3**), or rotate very rapidly at room temperature, as is the case for dioncophylline B (**2**). The apparent semi-stability of the axial configuration already gave a clear hint that this slowly atropisomerizing alkaloid had to be new and was apparently based on an unusual, if not novel, coupling type.

The ^1H NMR spectrum of that pair of interconverting new alkaloids exhibited signals typical of a naphthylisoquinoline with one methoxy substituent (4.00/4.04 ppm, Fig. 3a), suggesting the two other oxygen functions, as evident from the molecular formula of $C_{23}H_{25}NO_3$ (from HRMS of the $[M-CH_3]^+$ peak), to be hydroxy groups. The aromatic region showed the presence of a one-proton system and of groups of two and of three adjacent protons, by H,H-COSY

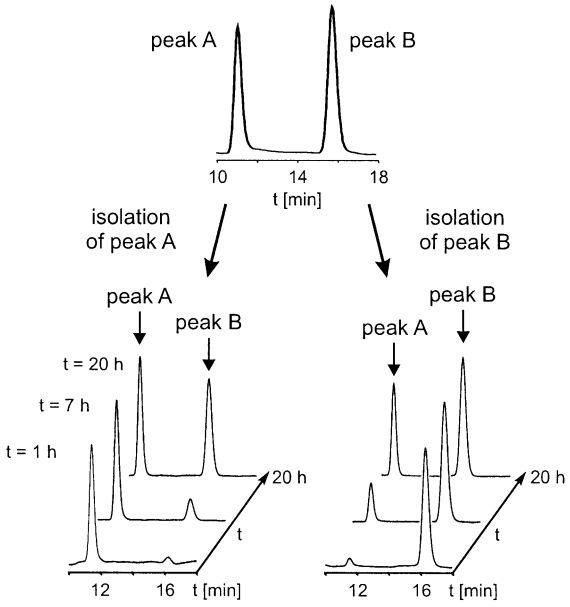

Fig. 2. Analytical HPLC analysis of the interconversion of peaks A and B of dioncophylline E (**6**) on reversed phase material (C18).

experiments. The group of two neighboring aromatic protons was easily located in the 5- and 6-positions (6.81, 7.05 and 7.06 ppm) of the isoquinoline moiety by HMBC (H-4′s → C-5, H-5 → C-4) and ROESY interactions (H-4′s ↔ H-5 ↔ H-6) (Fig. 3b). The position of the biaryl axis between the two molecular moieties was determined as follows: HMBC signals from H-6 to the quaternary carbon atom C-3′ in the naphthalene half of the molecule assigned the coupling site to be C-7, which

was further confirmed by interactions of H-5 with the likewise quaternary C-7. The coupling position in the naphthalene part was excluded to be in the methyl-free ring, because of the existence of the above mentioned spin system of three neighboring aromatic protons. Of the remaining two positions of the isoquinoline substituent, C-3′ or C-1′, the latter was ruled out from the fact that it bears the above-mentioned single aromatic proton, which was assigned by an HMBC interaction between H-8′ and this—hence tertiary—C-atom C-1′ and, vice versa, from H-1′ to C-8′, thus unequivocally locating the biaryl axis to be at C-3′. This attribution was further confirmed by HMBC correlations from H-1′ (7.24 and 7.27 ppm), HO-4′ (9.71 ppm), and H-6 (7.05 and 7.06 ppm) to C-3′. The 7,3′-linkage thus deduced was further supported by the high-field shifted signal of the CH_3-2′ group (2.14 and 2.15 ppm) as compared to that e.g. of the 7,6′-coupled alkaloid dioncophylline B (**2**), which exhibits a 'normal' shift of 2.46 ppm (Bringmann et al., 1991b). The methoxy function was proven to be located at C-5′, by HMBC interactions of the methyl protons with C-5′ and by ROESY signals with H-6′ (Fig. 3b).

As to the stereostructure, the relative configuration of the two methyl groups at C-1 and C-3 was deduced to be *trans* by a ROESY interaction between H-3 and CH_3-1 (Fig. 3b), in agreement with the typical (Bringmann et al., 1990a; Bringmann and Pokorny, 1995) ^{1}H NMR down-field shift for H-3 (3.69–3.77 ppm). The absolute configuration was investigated by oxidative degradation of the new compound to give (*R*)-3-aminobutyric acid (from C-3) and D-alanine (mainly from C-1), establishing 1*R*,3*R*-configuration, which is again in agreement with the relative 1,3-*trans* array proven above by NMR.

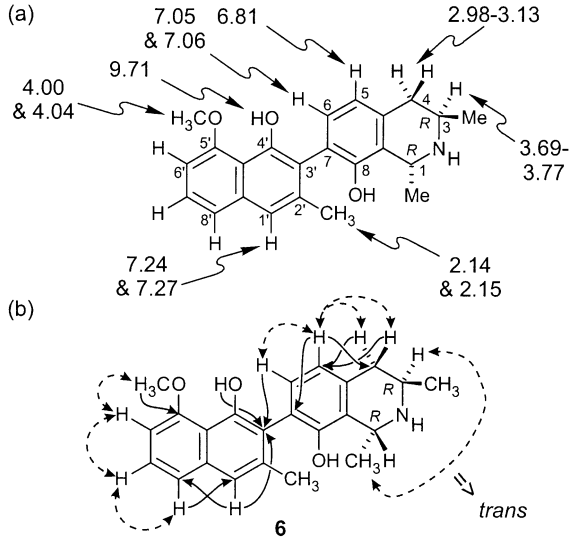

Fig. 3. Selected NMR data of dioncophylline E (**6**): ^{1}H NMR shifts for the co-occurring two atropisomers (a), decisive HMBC (single arrows), and ROESY interactions (broken line arrows) (b).

The new alkaloid is unique both constitutionally, being the first 7,3′-coupled 'Dioncophyllaceae-type' alkaloid (i.e. with *R*-configuration at C-3 and without an oxygen function at C-6) and stereochemically, because it is configurationally semi-stable at the axis, due to the unsubstituted C-6 position. The two only other 7,3′-linked naphthylisoquinoline alkaloids as yet known belong to the 'Ancistrocladaceae-type' (i.e. being 3*S*-configured and 6-oxygenated), viz ancistrocladidine (**7**) from *Ancistrocladus heyneanus* (Govindachari et al., 1973, 1975; Parthasarathy and Kartha, 1983) and *A. tectorius* (Meksuriyen et al., 1990) and ancistrotectorine (**8**), its *N*-methyl dihydro analog from *A. tectorius* (Ruangrungsi et al., 1985) and from *A. guineënsis* (Bringmann et al., 1998d), but those biaryls are configurationally fully stable at the axis (see Fig. 4).

Because of the new coupling type within the Dioncophyllaceae alkaloids, the new compound **6** was henceforth named dioncophylline E. For the attribution of the absolute axial configuration of the two respective atropo-diastereomers, (*M*)- and (*P*)-dioncophylline E [(*M*)- and (*P*)-**6**], an investigation of their circular dichroism (CD) seemed to be the best method since the two atropisomers (although diastereomers due to the additional existence of the stereogenic centers!) should deliver near-opposite CD spectra because the biaryl chromophore strongly dominates the CD behavior of such molecules (Bringmann and Busemann, 1998). The usual stand-alone CD measurements of the previously resolved dioncophylline E isomers turned out to be difficult because of their interconversion, so that the method of choice was a direct CD analysis in hyphenation with HPLC separation, as possible by the HPLC-CD coupling technique, which has recently been introduced into phytochemical analysis (Bringmann et al., 1999b). Indeed, on-line CD analysis of the two HPLC peaks obtained by resolution of dioncophylline E (**6**) yielded near-opposite CD-spectra (Fig. 5, bottom, full lines), again confirming this new alkaloid to consist of two rotational isomers that gradually interconvert at room temperature.

In addition to just demonstrating the existence of two atropisomeric forms, an interpretation of the CD spectra thus measured on line, became possible by a technique improved by our group earlier, the quantum

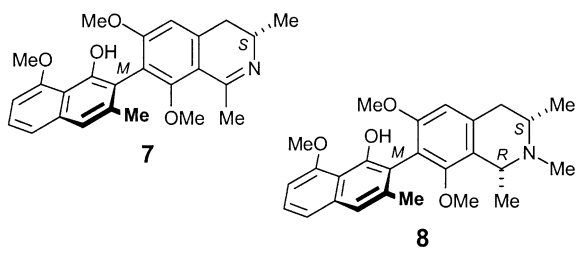

Fig. 4. The two other 7,3′-linked—but 6-oxygenated and thus configurationally stable—naphthylisoquinolines known to date.

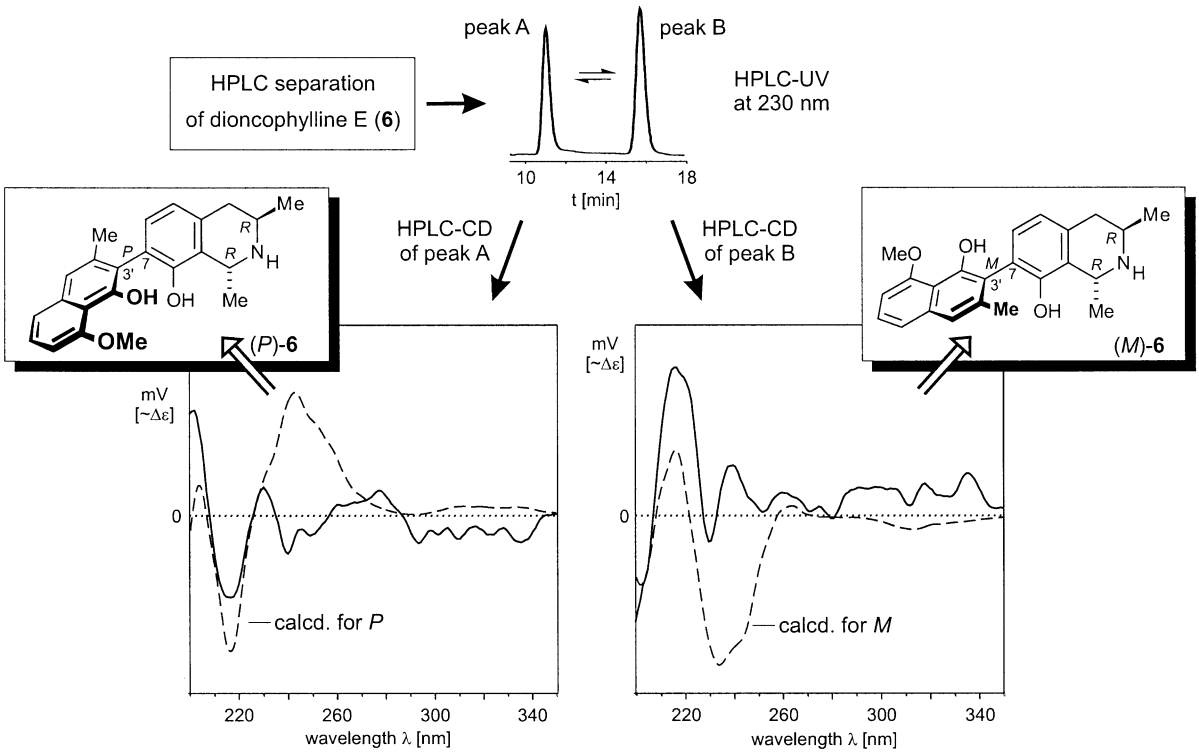

Fig. 5. LC-CD spectra (full lines) of the two rotational isomers of dioncophylline E (**6**) and assignment of the absolute configurations at the biaryl axes by comparison with the calculated MD-based CD spectra (broken lines).

chemical calculation of CD spectra based on molecular dynamics (MD) simulations (Bringmann et al., 2001). As seen in Fig. 5, the CD spectrum calculated for the *P*-atropisomer of **6** matches quite well with that of the more rapidly eluting peak A in the decisive region between 200 and 230 nm, while the experimental CD spectrum for the slower peak B on reversed-phased material is in good agreement with the one calculated for *M*, clearly indicating peak A to correspond to (*P*)-**6** and peak B to (*M*)-**6**. This assignment is also in agreement with the similarity of the CD spectrum of (*M*)-**6** with that of the—likewise 7,3′-coupled and *M*-configured—Ancistrocladaceae alkaloid ancistrotectorine (**8**, see Fig. 4).

With the unprecedented semi-stability of the axial chirality of dioncophylline E (**6**), it seemed rewarding to determine the atropisomerization barrier of this unusual novel naphthylisoquinoline alkaloid. This was achieved by measuring the decrease of the diastereomeric purity of freshly separated samples enriched in the respective *P*- or *M*-atropisomer (Fig. 6a). The rate constants at four different temperatures as determined from their logarithmic plots (Fig. 6b), delivered the corresponding enthalpy and entropy terms by means of analysis of slope and intersection with the coordinate axis according to procedures established in the literature (Frost and Pearson, 1964; Ernst, 1983).

The activation enthalpy $\Delta H^{\neq} = 81.9$ kJ mol^{-1} ($\Delta S =$ 60.0 J K^{-1} mol^{-1}) for the atropisomerization of (*P*)-**6** to (*M*)-**6** fits well with the slow interconversion of the two atropisomers as room temperature. It is likewise in a good agreement with the results from additionally performed quantum chemical calculations using the semi-empirical AM1 method as developed by Dewar et al. (1985), leading to a calculated value of $\Delta H^{\neq} = 107$ kJ mol^{-1}. The agreement between the predicted and the experimental values is satisfying, the deviation being due mainly to the fact that the calculations had to be done 'in vacuo', i.e. without regarding any solvent effects. Likewise in agreement with the experimentally measured atropisomeric equilibrium ratio of (*P*)-**6**:(*M*)-**6** of 43:57 at room temperature is the calculated stability difference of 0.8 kJ mol^{-1} in favor of the *M*-isomer.

Besides its new structural properties, dioncophylline E (**6**) is also pharmacologically interesting: While its antitrypanosomal activities against *Trypanosoma cruzi* (Chagas disease) and *T. brucei rhodesiense* (African sleeping sickness) are weak to moderate (18.4 and 0.73 μg ml^{-1}) as compared to the respective standards, benznidazole (0.68 μg ml^{-1}) and melarsoprol (0.002 μg ml^{-1}), it exhibits good activity against the pathogen of the malaria tropica, *Plasmodium falciparum*, with nearly identical IC_{50} values on the chloroquine-sensitive (NF54) and chloroquine-resistant (K1) strains. With values of only 22 and 21 ng ml^{-1}, the activity of the new alkaloid ranges within those of the most active naphthylisoquinoline alkaloids and is only by a factor of 5–10 weaker than that of the standards artemisine (2.8 and

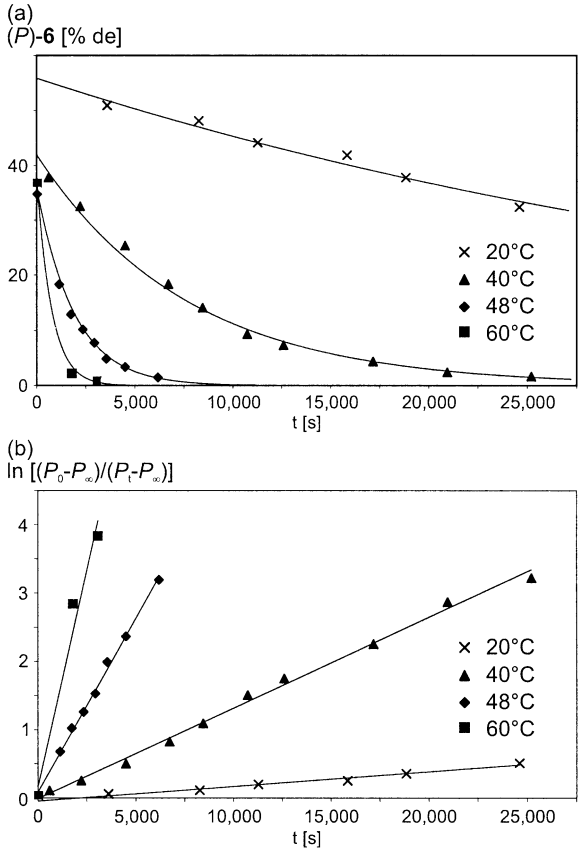

Fig. 6. Determination of the rotational barrier of dioncophylline E (**6**): Time-dependent decrease of the diastereomeric excess of peak A (a), logarithmic plots for the determination of the rate constants at different temperatures by the slope (b).

1.1 ng ml^{-1}, respectively) and chloroquine (4.4 and 65 ng ml^{-1}). These activities, including the even higher ones of a few other naphthylisoquinolines like dioncophylline C (**3**) (François et al., 1996; Bringmann and Feineis, 2000), dioncopeltine A (François et al., 1997), and habropetaline A (Bringmann et al., 2002b), make it rewarding to isolate or synthesize (Bringmann et al., 2000b) further new representatives of this novel class of bioactive compounds. This work is in progress.

3. Experimental

3.1. General

Mps: uncorr. IR spectra were taken on a Jasco FT/IR-410 spectrometer, CD spectra on a Jasco J-715 spectropolarimeter, and optical rotations on a Perkin-Elmer 241MC polarimeter. ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz) were recorded on a Bruker DMX 600 either in $CDCl_3$ or CD_3OD with the solvent as the int. standard ($CDCl_3$: δ 7.26 and δ 77.01, CD_3OD: δ 3.30 and δ 49.02). Proton-detected, heteronuclear correlations were analyzed using HMQC (optimized for $^1J_{HC} = 145$ Hz) and HMBC (optimized for $^nJ_{HC} = 7$ Hz). NOE and ROE effects were measured using NOESY and ROESY pulse sequences from the standard Bruker pulse program library. EIMS (70 eV) and HREIMS (70 eV) were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments. Analytical HPLC: Symmetry RP_{18} 4.6×250 mm (Waters). Semipreparative HPLC: Prep LC 25, Nova Pak 25×210 mm (Waters). Chiral HPLC: Chiralcel OD 4.6×250 mm and 10×250 mm (Daicel). HPLC-CD measurements were performed on a Jasco J-715 spectropolarimeter with a standard flow cell. CD spectra were recorded in the stop-flow mode. HSCCC: 'Triple coil', 1.68 mm×37.0 m (medium coil), 1.68 mm×106.5 m (large coil), (H)→T, lower phase as mobile phase, forward elution mode. The absolute configuration of the stereocenters at C-1 and C-3 of the isolated naphthylisoquinolines were determined by oxidative degradation as described previously (Bringmann et al., 1996a).

3.2. Plant material

Plant material of *D. thollonii* was collected by one of us (AML) in Rabi Kounga (Gabon) (10/1996). A voucher specimen (No. 01) has been deposited at Herb. Bringmann, University of Würzburg.

3.3. Extraction and isolation

D. thollonii: 1.2 kg of air-dried root material were ground and subsequently extracted with *n*-hexane and MeOH. The methanolic extract was perforated with *n*-hexane and $CHCl_3$, and the resulting chloroform extract prefractionated by CC with CH_2Cl_2–MeOH (9:1) as the eluent. The second fraction, containing Dragendorff-active compounds, was further resolved by CC with $CHCl_3$–MeOH (9:1/10:3/15:1).

3.4. Dioncophylline E (6)

Analytical HPLC: MeOH–H_2O 60:40, 0.1% TFA, 1 ml min^{-1}, 20 °C, 230 nm. Semipreparative HPLC: MeOH–H_2O 70:30, 0.1% TFA, 6 ml min^{-1}, 24 °C, 230 nm. Mp 59–60 °C. $[\alpha]_D^{25}$ −146° (MeOH; *c* 0.1). CD (faster atropisomer): $[\Phi]_{217}$ −3.3, $[\Phi]_{230}$ 0.8, $[\Phi]_{240}$ −1.7, $[\Phi]_{277}$ 0.7, $[\Phi]_{334}$ −1.2. CD (slower atropisomer): $[\Phi]_{216}$ 5.3, $[\Phi]_{230}$ −1.3, $[\Phi]_{240}$ 1.7, $[\Phi]_{280}$ −0.4, $[\Phi]_{335}$ 1.3. IR ν_{max}^{KBr} cm^{-1}: 3376, 2961, 2919, 2851, 1578, 1464, 1358, 1088, 805, 625. ^1H NMR (600 MHz, $CDCl_3$; 'double' values are due to partial spectral differences for the two atropisomeric forms of **6**): δ 1.58 and 1.59 (3H, *d*, $J = 6.0$ and 6.1 Hz, CH_3-3), 1.71 and 1.74 (3H, *d*, $J = 6.5$ and 6.7 Hz, CH_3-1), 2.14 and 2.15 (3H, *s*, CH_3-2′), 2.98–3.13 (2H, *m*, H-4), 3.69–3.77 (1H, *m*, H-3), 4.00 and 4.04 (3H, *s*, CH_3O-5′), 4.89–4.96 (1H, *m*, H-1) 6.74 and 6.77 (1H, *m*, H-6′), 6.81 (1H, *d*, $J = 7.9$ Hz, H-5), 7.05 and

7.06 (1H, *d*, $J=7.8$ and 7.8 Hz, H-6), 7.24 and 7.27 (1H, *s*, H-1′), 7.29–7.37 (2H, *m*, H-7′ and H-8′), 9.71 (1H, *s*, OH-4′). ^{13}C NMR (150 MHz, $CDCl_3$): δ 17.8 and 18.1 (CH_3-1), 18.4 and 18.8 (CH_3-3), 20.6 and 20.8 (CH_3-2′), 33.8 and 33.8 (C-4), 44.1 and 44.4 (C-3), 48.1 and 48.1 (C-1), 56.2 and 56.2 (OCH_3-5′), 104.0 and 104.0 (C-6′), 113.3 and 113.3 (C-4′a), 116.9 and 117.2 (C-3′), 120.0 and 120.0 (C-1′), 120.6 and 121.2 (C-8a), 120.7 and 120.7 (C-5), 121.1 and 121.3 (C-8′), 122.0 and 122.4 (C-7), 126.6 and 126.6 (C-7′), 130.2 and 130.5 (C-6), 131.3 and 131.4 (C-4a), 136.3 and 136.5 (C-8′a), 137.4 and 138.1 (C-2′), 149.5 and 149.7 (C-8), 151.8 and 151.8 (C-4′), 156.0 and 156.0 (C-5′). The ^{13}C attributions were achieved by HMQC and HMBC experiments. EIMS *m*/*z* (rel. int.): 363 $[M]^+$ (5), 348 $[M-CH_3]^+$ (100), 333 $[M-2CH_3]^+$ (12). HREIMS *m*/*z*: 348.1604 $[M-CH_3]^+$ ($C_{22}H_{22}NO_3$ requires 348.1600).

3.5. Known naphthylisoquinoline alkaloids

Resolution of dioncophylline A (**1**) (Bringmann et al., 1990a,c) and 5′-*O*-demethyldioncophylline A (**4**) (Bringmann et al., 1998c) by preparative HSCCC ($CHCl_3$–MeOH–0.1 N HCl 6:3:3) yielded 5.1 mg and 5.0 mg of the respective pure compounds. Semi-preparative separation (*n*-hexane–*i*-PrOH 98:2, 0.1% NEt_3) of *N*-methyldioncophylline A and *N*-methyl-7-*epi*-dioncophylline A (Bringmann et al., 1991a) on a chiral phase resulted in 5.1 and 2.6 mg of the substances, reflecting a natural occurrence of 2:1. All substances were fully identical with the spectroscopic and physical properties reported in the literature (Bringmann et al., 1991a).

3.6. Oxidative degradation procedure

The oxidative degradation, the derivatization of the derived amino acids, and the subsequent GC-MSD analyses were carried out as described previously (Bringmann et al., 1996a).

3.7. Computational

The molecular dynamics simulation was performed on a SGI Octane (R10000) workstation using the TRIPOS force-field as implemented in the molecular modeling package SYBYL[1] with time steps of 2 fs. The molecule was weakly coupled to a virtual thermal bath at $T=300$ K (Berendsen et al., 1984), with a temperature relaxation time $t=0.28$ ps. The wavefunctions for the calculation of the rotational strengths for the electronic transitions from the ground state to the excited states were obtained by a CNDO/S-CI (Downing, 1992) calculation, in which the CI expansion takes into account the ground state and all *n* and π orbitals. These calculations were carried out on Linux *i*PII and *i*PIII workstations using the program package BDZDO/MCDSPD (Downing, 1992; Del Bene and Jaffé, 1968). For a better comparison of the theoretical CD spectrum with the experimental one, a Gaussian band shape function was generated over the calculated rotational strength values.

3.8. Biological experiments

Plasmodium falciparum: Antiplasmodial activity was determined using the strains K1 (resistant to chloroquine and pyrimethamine) and NF54 (sensitive to all known drugs). A modification of the [^{3}H]-hypoxanthine incorporation assay (Desjardins et al., 1979) was used (Ridley et al., 1996). Briefly, infected human red blood cells were exposed to serial drug dilutions in microtiter plates for 48 h at 37 °C in a gas mixture with reduced oxygen and elevated CO_2. [^{3}H]-Hypoxanthine was added to each well and after further incubation for 24 h the wells were harvested on glass fiber filters and counted in a liquid scintillation counter. From the sigmoidal inhibition curve the IC_{50} value was calculated. The assays were run in duplicate and repeated at least once.

Antitrypanosomal activities were determined as follows: *Trypanosoma cruzi*: Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well/100 µl in RPMI 1640 medium with 10% FBS and 2 mM L-glutamine. After 24 h 5000 trypomastigotes of *T. cruzi* [Tulahuen strain C2C4 containing the galactosidase (Lac Z) gene] were added in 100 µl per well with a serial drug dilution. The plates were incubated at 37 °C in 5% CO_2 for 4 d. For determination of the IC_{50} the substrate CPRG/Nonidet was added to the wells. The color reaction which developed during the following 2–4 h in the presence of live cells was read photometrically at 540 nm. IC_{50} values were calculated from the sigmoidal inhibition curve. *Trypanosoma b. rhodesiense*: Minimum Essential Medium (50 µl) supplemented according to Baltz et al. (1985) with 2-mercaptoethanol and 15% heat-inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions were added to the wells. Then 50 µl of trypanosome suspension (*T. b. rhodesiense* STIB 900) were added to each well and the plate incubated at 37 °C under a 5% CO_2 atmosphere for 72 h. Alamar Blue (10 µl) was then added to each well and incubation was continued for a further 2–4 h. The plate was then read using a Millipore Cytofluor 2300 at an excitation wavelength of 530 nm and an emission wavelength of 590 nm (Räz et al., 1997). Fluorescence development was expressed as percentage of the control, and IC_{50} values were determined.

[1] SYBYL: Tripos Associates, 1699 Hanley Road, Suite 303, St. Louis, MO 63144, USA.

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