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# Habropetaline A, an antimalarial naphthylisoquinoline alkaloid from *Triphyophyllum peltatum*☆

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Dedicated to Professor Dr. Meinhart H. Zenk on the occasion of his 70th birthday.

**Abstract**

The isolation, structural elucidation, and antiprotozoal activities of habropetaline A, a novel naphthylisoquinoline alkaloid from *Triphyophyllum peltatum*, are described. This alkaloid had previously only been identified on line, by the LC–MS/MS–NMR–CD triad, in the crude extract of the rare and difficult-to-provide related plant species *Habropetalum dawei*, whose small quantities available had not permitted to isolate the compound. As predicted by quantitative structure–activity relationship (QSAR) investigations, habropetaline A exhibits strong antimalarial activity against *Plasmodium falciparum*, while it is inactive against other protozoal pathogens (*Trypanosoma brucei rhodesience*, *T. cruzi*, and *Leishmania donovani*).


*Keywords:* *Triphyophyllum peltatum*; Dioncophyllaceae; Habropetaline A; Structural elucidation; Stereochemistry; Antimalarial activity

## 1. Introduction

Naphthylisoquinoline alkaloids constitute a rapidly growing class of mostly axially chiral natural biaryls biosynthetically originating from acetate units (Bringmann et al., 2000b). Some of these secondary metabolites from tropical lianas show remarkable antitrypanosomal (Bringmann and Feineis, 2000; Bringmann, 2003), antileishmanial (Bringmann et al., 2000a), fungicidal (Bringmann et al., 1992b), and, in particular, antimalarial activities. Among the most active of these compounds are dioncopeltine A (**1**) (François et al., 1997), dioncophylline B (**2**) (François et al., 1999), and dioncophylline C (**3**) (François et al., 1997), with excellent antiplasmodial activities in vitro and in vivo (Bringmann and Feineis, 2000). This makes the naphthylisoquinoline framework a promising novel antimalarial lead structure, and thus the search for further compounds of this type a rewarding task. Such alkaloids have so far been found only in the very small families Ancistrocladaceae, which consists of only one genus, *Ancistrocladus*, with ca. 23 species, and the Dioncophyllaceae, an even smaller family with only three monotypic genera, *Triphyophyllum*, *Habropetalum*, and *Dioncophyllum*. Nearly all of the most active naphthylisoquinoline alkaloids that have so far been found, in particular compounds **1**–**3** (Bringmann et al., 1991a, b, 1992a), have been isolated from *Triphyophyllum peltatum*, a rare, 'part-time carnivorous' Westafrican Dioncophyllaceae species. A phytochemical analysis of the other two species of this family, however, is hampered by their even poorer availability. With a few grams of material of *Habropetalum dawei* in hand, we have recently identified a new alkaloid, habropetaline A, right from a crude stem extract, using the analytical 'triad' HPLC coupled to MS/MS, NMR, and CD (Bringmann et al., 1999b), and have established its full absolute stereostructure as **4**. Its close similarity to dioncopeltine A (**1**) warranted its preparative availability for antimalarial testing, but unfortunately, enough material of *H. dawei* to provide sufficient quantities of **4** was not available. Quantitative structure–activity relationship (QSAR) (Bringmann and Rummey,

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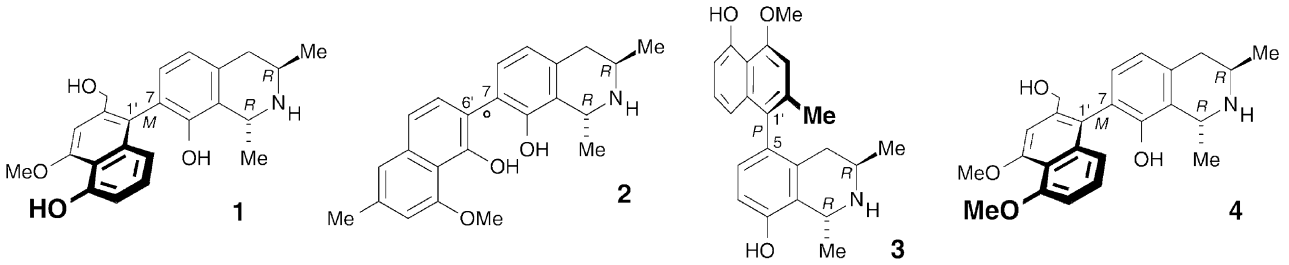


Fig. 1. Bioactive naphthylisoquinolines from *T. peltatum*—a selection (○: configurationally unstable).

unpublished) investigations confirmed the expectation that **4** should exhibit high antiplasmodial activity, making the availability of the compound even more desirable. With the structure and the chromatographic behavior of this promising compound known, we have started a directed search for the alkaloid in *T. peltatum* from Ivory Coast (Fig. 1), since this plant was available to us from previous collections, but also from our successful propagation efforts, both as green plants and as tissue cultures (Bringmann and Rischer, 2001). In this paper we report on the preparative isolation of compound **4**, the confirmation of its structure as previously postulated from the on line investigations, and the experimental confirmation of its high antimalarial activity.

## 2. Results and discussion

Among the three Dioncophyllaceae species known to date, *Triphyophyllum peltatum* is phytochemically by far the best investigated one (Bringmann et al., 1998a, b, c, 2000c). Moreover, it has the highest content in naphthylisoquinoline alkaloids, while *H. dawei* and *D. thollonii* produce large amounts of related, but nitrogen-free naphthoquinones and tetralones (Lavault and Bruneton, 1980; Hanson et al., 1981; Bringmann et al., 1999b, c, d). Although no less than 15 novel naphthylisoquinoline alkaloids have so far been isolated from *T. peltatum*, an alkaloid corresponding to the structure **4** had not yet been found in this plant. With the chromatographic (and spectroscopic) properties of **4** known from the previous on line investigations on the extract of *H. dawei*, we could now identify this alkaloid likewise in *T. peltatum*, which allowed us the preparative isolation of the compound. For this purpose, the air-dried and powdered root material of *T. peltatum* was extracted with methanol. This extract was perforated with *n*-hexane and subsequently with chloroform. The chloroform extract thus obtained was pre-resolved on a short silica column and submitted to a preparative HSCCC separation. The combination of these two different separation principles permitted the isolation of a pure naphthylisoquinoline alkaloid slightly less polar than dioncopeltine A (**1**) (Bringmann et al., 1991b, 1999a) and previously unknown from *T. peltatum*, but now easily accessible. The $^1$H NMR data strongly resembled those of dioncopeltine A, with its significant C*H*$_2$OH protons at 4.43 ppm instead of those of the 'normal' $CH_3$-2′ group. The sole difference was the lack of the hydrogen bridged OH-5′ proton (between 8 and 10 ppm) and the presence of an extra methoxy signal at 3.92 ppm, indicating a 5′-*O*-methylated analog of dioncopeltine A, in agreement with the molecular composition of $C_{24}H_{27}NO_4$ obtained by HRMS of the $[M]^+$ peak. These findings were confirmed by the HMBC interactions of H-7′ (7.20 ppm) and the additional *O*–$CH_3$ group with the quaternary C-5′ atom at 158.53 ppm and a ROESY signal between the methoxy function and H-6′ (6.90 ppm) (see Fig. 2).

By an oxidative degradation procedure described previously (Bringmann et al., 1996), the absolute configuration at C-1 and C-3 was determined as 1*R*, 3*R*, in agreement with the relative *trans*-array deduced from both the chemical shift of H-3 (3.40 ppm) and the ROESY interaction between $CH_3$-1 and H-3. With the absolute configuration at the centers known, the axis was established to be *M*-configured by a long-range ROESY interaction (Bringmann et al., 1997) between the $CH_2$-2′ protons (4.43 ppm) and those of the methyl group at C-1 (1.49 ppm, Fig. 2b). This stereochemical assignment is in agreement with the nearly identical CD spectra of **4** and of dioncopeltine A (**1**), which is known to be *M*-configured *i.a.* from its total synthesis (Bringmann et al., 1999a). The structure of compound **4**, now named habropetaline A,[1] was found to be fully identical with that of the above-mentioned alkaloid previously identified in *H. dawei*, which had been structurally elucidated by LC–NMR, LC–MS/MS, and LC–CD directly from the crude plant extract (Bringmann et al., 1999b).

[1] A compound named '5′-*O*-methyltriphyopeltine', with the same constitution as habropetaline A (**4**), had already been reported earlier (Lavault and Bruneton, 1980); from the divergent (or lacking) physical and spectroscopic data, from the missing stereochemical assignment at the axis and at the centers, and from the lack of authentic comparison material, it is unclear whether that compound previously isolated, is identical to the one described in this paper; for this reason, the alkaloid **4** had to be treated as new and thus has been given a new name, habropetaline A, after the name of the plant genus, *Habropetalum*, in which the compound had initially been found.

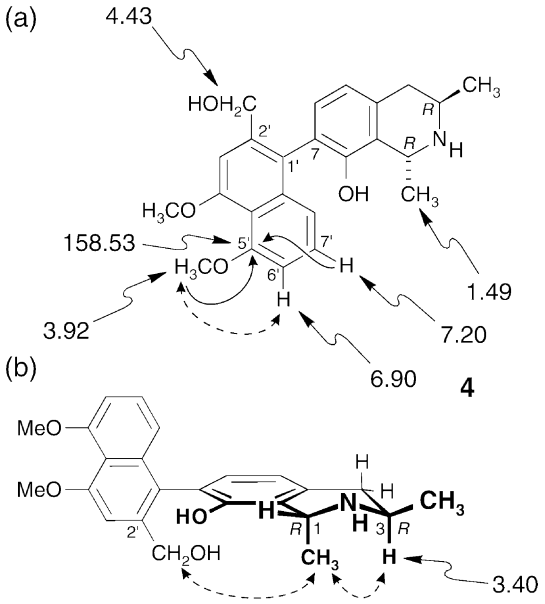


Fig. 2. Decisive $^{1}H$ and $^{13}C$ NMR shifts and correlations of habropetaline A (**4**) (a), ROESY interactions indicative of the relative configuration at the centers and at the axis (b) (HMBC: single arrows, ROESY: broken-line arrows).

This preparative availability of habropetaline A (**4**) now allowed to test its—predictedly good (Bringmann and Rummey, unpublished)—antiplasmodial activity. Like dioncopeltine A (**1**), the new alkaloid showed a very good effect against *Plasmodium falciparum*, without any cytotoxicity. With $IC_{50}$ values of 5.0 and 2.3 ng $ml^{-1}$ for the strains K1 (chloroquine and pyrimethamine resistant) and NF54 (sensitive to all known drugs), respectively, habropetaline A (**4**) is almost as active as the used standard artemisinine (K1: 1.2 ng $ml^{-1}$, NF54: 1.2 ng $ml^{-1}$), one of the most potent natural products used against this disease. It is thus as active as dioncopeltine A (**1**) (K1: 4.8 ng $ml^{-1}$, NF54: 3.3 ng $ml^{-1}$). No activity was found against *Trypanosoma brucei rhodesiense*, *T. cruzi*, and *Leishmania donovani*, other protozoal parasites causing tropical diseases. Further examinations with this promising compound will follow.

## 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. $^{1}H$ NMR (600 MHz) and $^{13}C$ NMR (150 MHz) were recorded on a Bruker DMX 600 in $CD_3OD$ with the solvent as the internal standard ($CD_3OD$: $\delta$ 3.30 and $\delta$ 49.02). Proton-detected, heteronuclear correlations were analyzed using HMQC (optimized for $^{1}J_{HC} = 145$ Hz) and HMBC (optimized for $^{n}J_{HC} = 7$ Hz). ROE effects were measured using 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. HSCCC: 'Triple coil', 1.68 mm×37.0 m (medium coil), 1.68 mm×106.5 m (large coil), (H)→T, lower phase as the mobile phase, forward elution mode. The absolute configurations of the stereocenters at C-1 and C-3 of **4** were determined by oxidative degradation as described previously (Bringmann et al., 1996).

### 3.2. Plant material

Material of *T. peltatum* was present from an earlier collection (02/1993) by one of us (LAA) in the Parc de Taï (West Ivory Coast). A voucher specimen (No. 02) has been deposited at Herb. Bringmann, University of Würzburg.

### 3.3. Extraction and isolation

#### 3.3.1. T. peltatum

Air-dried and ground root material (1.5 kg) was sequentially extracted with PE, $CH_2Cl_2$, and MeOH; the latter extract was again perforated with *n*-hexane and $CHCl_3$. Short CC of the chloroformic extract on silica with $CH_2Cl_2$–MeOH and HSCCC ($CHCl_3$–MeOH–0.1 N HCl 5:5:3 as the eluent system) yielded 20.3 mg of habropetaline A (**4**).

### 3.4. Habropetaline A (*4*)

Mp 225 °C. $[\alpha]^{25}_{D}$ −16.5° (MeOH; *c* 0.1). CD: $\Delta\varepsilon_{198}$ 13.1, $\Delta\varepsilon_{222}$ −41.0, $\Delta\varepsilon_{238}$ +31.7, $\Delta\varepsilon_{260}$ +2.7, $\Delta\varepsilon_{281}$ +12.2, $\Delta\varepsilon_{303}$ −2.3 (EtOH; *c* 0.01). IR $\nu^{KBr}_{max}$ $cm^{-1}$: 3516, 3410, 2958, 2924, 1593, 1460, 1431, 1390, 1338, 1300, 1261, 1201, 1127, 1099, 1076, 811, 765. $^{1}H$ NMR (600 MHz, $CD_3OD$): $\delta$ 1.28 (3H, *d*, $J = 6.3$ Hz, $CH_3$-3), 1.49 (3H, *d*, $J = 6.7$ Hz, $CH_3$-1), 2.59 (1H, *dd*, $J = 16.6$ Hz, $J = 11.0$ Hz, H-$4_{ax}$), 2.87 (1H, *dd*, $J = 16.8$ Hz, $J = 4.3$ Hz, H-$4_{eq}$), 3.40 (1H, *m*, H-3), 3.92 (3H, *s*, $OCH_3$-5′), 4.00 (3H, *s*, $OCH_3$-4′), 4.43 (2H, '*d*' (more exactly the two 'inner' peaks of the expected two doublets), '$J' = 3.2$ Hz, $CH_2$OH-2′), 4.44 (1H, *m*, H-1), 6.74 (1H, *d*, $J = 7.7$ Hz, H-5), 6.79 (1H, *d*, $J = 7.7$ Hz, H-6), 6.90 (1H, *dd*, $J = 7.8$ Hz, $J = 0.8$ Hz, H-6′), 6.92 (1H, *dd*, $J = 8.5$ Hz, $J = 0.9$ Hz, H-8′), 7.20 (1H, *dd*, $J = 8.5$ Hz, $J = 7.7$ Hz, H-7′), 7.24 (1H, *s*, H-3′). $^{13}C$ NMR (150 MHz, $CD_3OD$): $\delta$ 20.44 ($CH_3$-1), 22.14 ($CH_3$-3), 37.71 (C-4), 43.09 (C-3), 48.86 (C-1), 56.82 ($OCH_3$-5′), 57.03 ($OCH_3$-4′), 63.27 ($CH_2$OH-2′), 106.89 (C-3′), 107.76 (C-6′), 118.53 (C-4′a), 120.19 (C-8′), 121.54 (C-5), 123.87 (C-7), 125.90 (C-1′), 127.48 (C-7′), 128.37 (C-8a), 130.65 (C-6), 136.04 (C-4a), 138.34 (C-8′a), 140.15 (C-2′), 152.29

(C-8), 158.48 (C-4′), 158.53 (C-5′). The $^{13}C$ attributions were achieved by HMQC and HMBC experiments. EIMS *m/z* (rel. int.): 393 $[M]^+$ (20), 378 $[M–CH_3]^+$ (53), 360 $[M–CH_3–H_2O]^+$ (100). HREIMS *m/z*: 393.1939 $[M]^+$ ($C_{24}H_{27}NO_4$ requires 393.1940).

### 3.5. Biological experiments

Antiplasmodial activity was determined using the *P. falciparum* 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.

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