ent-Dioncophylleine A and Related Dehydrogenated Naphthylisoquinoline Alkaloids, the First Asian Dioncophyllaceae-Type Alkaloids, from the "New" Plant Species *Ancistrocladus benomensis*[1]

Gerhard Bringmann,*[,†] Michael Dreyer,[†] Hélène Kopff,[†] Heiko Rischer,[†,‡] Michael Wohlfarth,[†,§] Hamid A. Hadi,[⊥] Reto Brun,[▽] Harald Meimberg,[#] and Günther Heubl[#]

Institut für Organische Chemie der Universität Würzburg, Am Hubland, D-97074 Würzburg, Germany, Department of Chemistry, University of Malaya, 59100 Kuala Lumpur, Malaysia, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland, and Department Biology I, Section: Biodiversity Research, Systematic Botany, Ludwig-Maximilians University Munich, Menzinger Strasse 67, D-80638 München, Germany

Received November 19, 2004

Three new fully dehydrogenated naphthylisoquinoline alkaloids, the 7,1′-coupled *ent*-dioncophylleine A (**3a**), the likewise 7,1′-coupled 5′-*O*-demethyl-*ent*-dioncophylleine A (**4**), and the 7,8′-linked dioncophylleine D (**5**), have been isolated from the leaves of the recently described Malaysian highland liana *Ancistrocladus benomensis*. All of them lack an oxygen function at C-6; this so-called Dioncophyllaceae-type structural subclass had previously been found only in naphthylisoquinoline alkaloids from West and Central African plants. Moreover, compounds **3a** and **4** are the first fully dehydrogenated, i.e., only axially chiral, naphthylisoquinoline alkaloids of this type that are optically active; compound **5**, by contrast, is fully racemic, due to its configurationally unstable biaryl axis. The structural elucidation was achieved by spectroscopic and chiroptical methods. Biological activities of these alkaloids against different protozoan parasites are described.

Ancistrocladus benomensis Rischer & Bringmann is a newly discovered species of the monogeneric plant family Ancistrocladaceae,[2] which has recently been described botanically.[3] First phytochemical investigations on the bark of this Southwest-Malaysian highland liana have shown the presence of novel naphthylisoquinoline alkaloids.[4] This group of natural products is remarkable because of the presence of a usually rotationally hindered and thus stereogenic biaryl axis,[5] their biosynthesis, since these are the first acetogenic tetrahydroisoquinoline alkaloids,[6,7] and their pronounced antiinfective activities, e.g., against different protozoan pathogens, such as *Plasmodium*, *Leishmania*, and *Trypanosoma* species.[8−10] The discovery of naphthylisoquinoline alkaloids with unprecedented structural features, e.g., optically active fully aromatic derivatives with a hydroxymethyl group at C-3, such as ancistrobenomine A (**1**) and 6-*O*-demethylancistrobenomine A (**2**), previously isolated from the bark of *A. benomensis*, makes it rewarding to search for further such alkaloids in other organs of this phytochemically productive new plant species.

In this paper we report on the isolation, structural elucidation, and bioactivities of the remarkable, novel naphthylisoquinoline alkaloids from the leaves of *A. benomensis*, the 7,1′-coupled compounds *ent*-dioncophylleine A (**3a**), i.e., the configurationally stable enantiomer of the known[11] dioncophylleine A (**3b**), 5′-*O*-demethyl-*ent*-dioncophylleine A (**4**), and the 7,8′-linked dioncophylleine D (**5**). Compounds **3a**, **4**, and **5** belong to the heretofore infrequently isolated group of fully aromatic naphthylisoquinoline alkaloids. Among these, they are the first optically active and moreover the first Asian representatives of Dioncophyllaeae-type alkaloids (devoid of an oxygen at C-6), previously only found (albeit optically inactive) in Central and West African species,[5] and dioncophylleine D (**5**) is, moreover, the first naturally occurring 7,8′-coupled Dioncophyllaceae-type alkaloid at all.

Results and Discussion

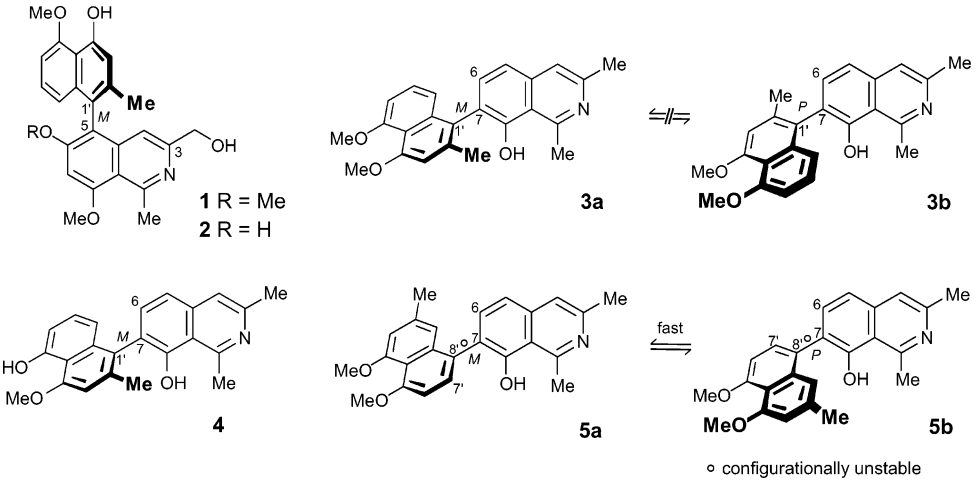
A. benomensis was collected in Southwest Malaysia on the mountain Gunung Benom, at an altitude of 900 m above sea level. Fast centrifugal partition chromatography (FCPC),[12] a new liquid−liquid chromatographic method, and preparative HPLC of a leaf extract yielded three nitrogen-containing compounds, **3a**, **4**, and **5**. Their UV spectra displayed a third maximum at around 370 nm, which is typical of fully dehydrogenated naphthylisoquinoline alkaloids.[4]

The most polar compound, **3a**, was found to possess the molecular formula $C_{24}H_{23}NO_3$, as deduced from HREIMS of *m/z* 373.1676. This molecular weight was unusually low for alkaloids isolated from Asian *Ancistrocladus* plants and suggested, together with the presence of only three oxygen atoms, the existence of a Dioncophyllaceae-type alkaloid, which is characterized by the lack of an oxygen function at C-6 (and the presence of an *R*-configuration at C-3, which is not relevant here). This assumption was corroborated by the occurrence of seven aromatic protons in the ^{1}H NMR spectrum, two more than in "normal" naphthyltetra- or naphthyldihydroisoquinoline alkaloids,[13] one of which was supposed to be the proton at C-6 (δ 7.62), and by the appearance of two doublets at δ 7.48 and 7.62 (Figure 1a). These were different from the doublets at δ 6.73 and 6.82, which belonged to a spin system of three neighboring protons, H-6′, H-7′ (pseudo-triplet at δ 7.21), and H-8′, thus to be located in the isoquinoline moiety. The last remaining aromatic proton was assigned to be located at C-4, as this additional singlet (δ 7.64), together with the lack of the

* To whom correspondence should be addressed. Tel: +49-931-888-5323. Fax: +49-931-888-4755. E-mail: bringman@chemie.uni-wuerzburg.de.
† Institut für Organische Chemie der Universität Würzburg.
‡ Present address: VTT Biotechnology, Tietotie 2, FIN-02044 Espoo, Finland.
§ Present address: BASF Aktiengesellschaft, GKA/S−E210, D-67056 Ludwigshafen, Germany.
⊥ University of Malaya.
▽ Swiss Tropical Institute.
Ludwig-Maximilians University Munich.

10.1021/np049626j CCC: $30.25
Published on Web 04/15/2005

Chart 1



two doublets of the aliphatic methyl groups at C-1 and C-3 around δ 1.1 and 1.9, the missing quartet of H-1 around δ 4.4, and the likewise not observed multiplet of H-3 in the region between δ 3.2 and 4.0, was typical of a fully dehydrogenated naphthylisoquinoline alkaloid.[4] The significant downfield shifts of the ^{13}C NMR resonances for C-1, C-3, and C-4 (δ 159.5, 142.0, and 121.6, respectively) and of the two methyl groups at C-1 and C-3 (δ 3.29 and 2.72) in the ^{1}H NMR unequivocally confirmed the presence of a fully dehydrogenated isoquinoline portion (Figure 1a).

From a NOESY correlation sequence in the series CH_3-3−H-4−H-5−H-6 and from HMBC interactions between H-4 and C-5 and, in turn, H-5 and C-4 (Figure 1b), the coupling position of the biaryl axis was deduced to be located at C-7 of the isoquinoline moiety. By these correlations, the assignment of H-4 to the singlet at δ 7.64 and the assumed location of a Dioncophyllaceae-type specific proton at C-6 (δ 7.61) were also confirmed (Figure 1b).

The two methoxy groups at δ 3.94 and 3.99 showed NOESY interactions with H-6′ (δ 6.82) and H-3′ (δ 6.83), respectively, being located at C-5′ and C-4′ in the naphthalene half. The remaining third oxygen function thus had to be a hydroxy group at C-8.

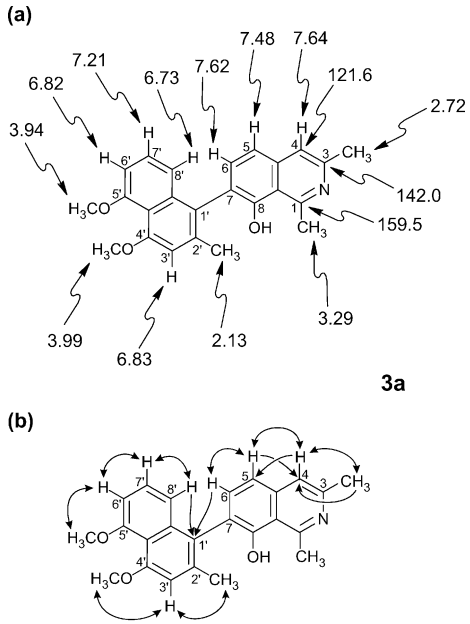


Figure 1. Selected NMR data of *ent*-dioncophylleine A (**3a**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a), HMBC (single arrows), and NOESY (double arrows) (b) relevant for the constitution.

The coupling position of the naphthalene moiety was clearly assigned to be at C-1′ because of the typical upfield shift[5] of the CH_3-2′ group (δ 2.13) and two NOESY correlation sequences in the series CH_3-2′−H-3′−OCH_3-4′ and OCH_3-5′−H-6′−H-7′−H-8′. Further evidence was given by ^{3}J HMBC interactions from H-8′ and H-6 to the quaternary carbon atom C-1′.

In conclusion, this 7,1′-coupled alkaloid had the constitution **3a** (Figure 1a), which was the same as that of dioncophylleine A (**3b**), previously obtained only by partial synthesis from dioncophylline A.[11]

Because of the lack of any stereogenic centers usually present at C-1 and C-3, the biaryl axis constitutes the only stereogenic element of **3a**. Thus, the absolute axial configuration was easily determined by comparison of its CD spectrum with that of the *P*-configured similar compound dioncophylleine A[11] (**3b**). The two spectra were found to be opposite of each other, which clearly indicated compound **3a** to be the enantiomer of **3b** and thus to be *M*-configured (Figure 2).

From these results, the new natural product had the full absolute stereostructure **3a** and was thus named *ent*-dioncophylleine A. For the investigation of the enantiomeric purity of *ent*-dioncophylleine A (**3a**), an HPLC analysis on a chiral phase (OD-H) was developed; this resulted in two peaks with a ratio of 7:93. CD spectra of the two peaks were recorded online, in the stop-flow mode, by HPLC-CD[14] coupling. The more rapidly eluting small peak showed a CD spectrum similar to that of dioncophylleine A (**3b**) and was thus the *P*-configured natural product, while the CD spectrum of the major one was fully opposite of that of **3b**, thus clearly revealing that the separated substances are

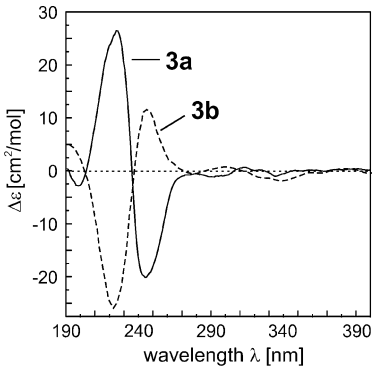


Figure 2. Comparison of the CD spectrum of **3a** with that of the known[12] *P*-enantiomer of dioncophylleine A (**3b**) for the assignment of the absolute axial configuration.

indeed enantiomers and assigning an *M*-axial configuration to the main enantiomer. While ancistrobenomines A (**1**) and B (**2**) had so far been the only nonracemic fully aromatic naphthylisoquinoline alkaloids, with no detectable traces of the other respective atropisomer,[4] *ent*-dioncophylleine A (**3a**) is the first fully dehydrogenated naphthylisoquinoline alkaloid that occurs in a *nearly* enantiopure form in the plants.

The molecular weight of the second, least polar compound, **4**, was 14 u below that of *ent*-dioncophylleine A (**3a**), typical of the loss of CH_2 due to *O*-demethylation. HREIMS indicated a molecular formula of $C_{23}H_{21}NO_3$, which again showed the lack of an oxygen, as compared to an (expected) Ancistrocladaceae-type naphthylisoquinoline alkaloid, and a close similarity to the molecular formula of **3a**, suggesting this compound to be an *O*-demethyl derivative of *ent*-dioncophylleine A (**3a**). Indeed, all its NMR data (^{1}H NMR, NOESY, HMBC, HMQC) were—apart from little variations of the chemical shifts caused by the different solvents used, MeOD and $CDCl_3$—in good agreement with those of **3a**, revealing the presence of a fully aromatized 7,1′-coupled naphthylisoquinoline alkaloid with only one methoxy group at δ 4.15. This OCH_3 showed a NOESY interaction to the singlet of H-3′ (δ 6.98) and an HMBC correlation to C-4′, to which another long-range interaction of H-3′ was also observed. These findings, together with the lacking NOESY correlation of H-6′ (δ 6.76) to a possible methoxy group at C-5′, unequivocally assigned the only OCH_3 function to be at C-4′.

Thus, the second isolated natural product, **4**, was a likewise 7,1′-coupled fully aromatic naphthylisoquinoline with a methoxy group only at C-4′ and two hydroxy functions at C-5′ and C-8.

The absolute configuration at the biaryl axis was again determined to be *M*, by comparison of its CD data with those of **3a**, which were similar, revealing the compound to possess structure **4**, i.e., the—likewise new—5′-*O*-demethyl derivative of **3a**; it was thus named 5′-*O*-demethyl-*ent*-dioncophylleine A. In contrast to the 93:7 enantiomeric mixture of **3a/3b**, compound **4** was found to be enantiomerically pure by HPLC analysis on a chiral phase (the same as above), resulting in only one peak, the CD spectra of which recorded at different positions were all identical.

The polarity of the third compound, **5**, was between those of **3a** and **4**. It exhibited the same molecular weight as compound **3a** (*m/z* 373), and its HREIMS gave a molecular formula of $C_{24}H_{23}NO_3$, identical to that for *ent*-dioncophylleine A (**3a**). The ^{1}H NMR spectrum again showed the signals expected for a fully dehydrogenated Dioncophyllaceae-type naphthylisoquinoline alkaloid. The most significant differences as compared to the spectrum of **3a** were the "normal", i.e., not high-field shifted, methyl group at C-2′ (δ 2.30) and the lack of a pseudo-triplet of H-7′ at δ 7.35 (Figure 3a). Instead of the latter, only two doublets and an additional singlet were observed in the naphthalene moiety, suggesting the biaryl axis to be located at C-6′ or C-8′.

A NOESY correlation sequence in the series OCH_3-5′–H-6′–H-7′ (Figure 3b) excluded the carbon atom at position 6′ from being quaternary and thus to be the coupling position of the biaryl axis, whereas C-8′ lacked a proton. HMBC interactions from H-1′ and H-6 with C-8′ and from H-7′ and H-5 with C-7 determined both the location of the biaryl axis at C-8′ in the naphthalene moiety and at C-7 in the isoquinoline portion. This assignment was further confirmed by a NOESY correlation sequence in the series H-6–H-5–H-4–CH_3-3 and by crosswise HMBC interactions of H-5 with C-4 and H-4 with C-5, excluding C-5 from being the coupling position in the isoquinoline half.

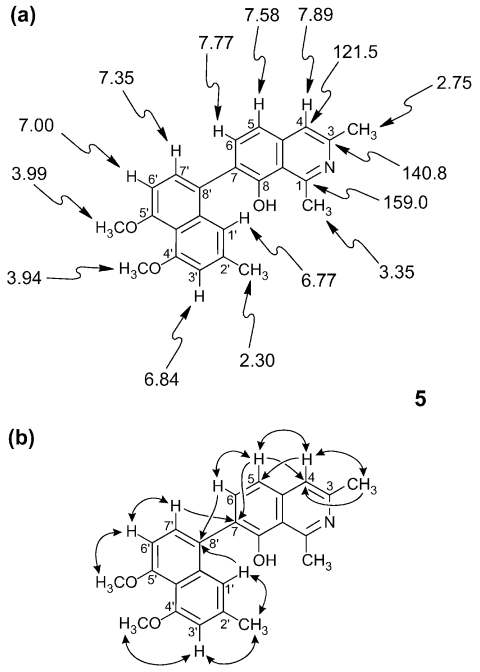
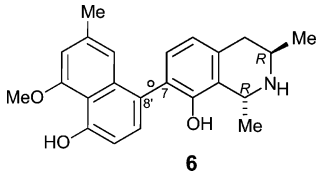


Figure 3. Selected NMR data of dioncophylleine D (**5**): ^{1}H and ^{13}C NMR shifts (δ in ppm) (a), HMBC (single arrows), and NOESY (double arrows) (b) relevant for the constitution.

The second methoxy group was again assigned to C-4′ by its NOESY correlation with H-3′, leaving the third oxygen function to be a hydroxy group at C-8. In conclusion, this third compound was a 7,8′-coupled isomer of **3a** with the constitution **5** shown in Figure 3.

An alpha D value of zero and the lack of any Cotton effects in the CD spectrum led to the assumption that **5** was a racemate of configurationally stable or, rather, unstable atropo-enantiomers. The latter seemed more probable since HPLC analysis on a chiral phase (OD-H) resulted in only one peak, even under most different chromatographic conditions. Furthermore, **5** is the dehydrogenated and *O*-methylated form of the likewise 7,8′-coupled, again configurationally unstable, naphthylisoquinoline dioncophylline D (**6**), a previously assumed



natural product prepared by total synthesis in our group.[15] In contrast to **3a**, i.e., the configurationally stable 7,1′-isomer, the lack of the *ortho*-substituted methyl group next to the axis of **5** leads to a low rotational barrier and hence to a quick interconversion of the two enantiomers at room temperature.

Alkaloid **5** is the first 7,8′-coupled Dioncophyllaceae-type alkaloid, i.e., the as yet only naturally occurring naphthylisoquinoline alkaloid with a coupling type "D",[5] thus henceforth named dioncophylleine D. It is likewise the first naphthylisoquinoline alkaloid that is subject to a rapid atropo-enantiomerization, making understandable its occurrence in a racemic form.

Alkaloids **3a**, **4**, and **5** exhibited weak antiplasmodial activities against the K1 strain of *P. falciparum* (resistant

to chloroquine and pyrimethamine) as well as weak antitrypanosomal activities against the pathogen of African sleeping sickness, *T. b. rhodesiense*, but no activity against *T. cruzi* (Chagas' disease).

Conclusions

The structures of the alkaloids from the leaves of *A. benomensis* in this paper fully confirm the exceptional position of this unique plant species visible already from the bark alkaloids:[4] Compounds **3a**, **4**, and **5** are again fully dehydrogenated naphthylisoquinolines and, moreover, the first optically active representatives based on 7,1′- and 7,8′-coupling types. Surprisingly, most of the alkaloids isolated from the leaves are devoid of an oxygen function at C-6 and thus belong to the Dioncophyllaceae type (the second criterion, an *R*-configuration at C-3, is not relevant here due to the sp²-hybridization); thus, compounds **3a**, **4**, and **5** are the first Asian representatives of this type, which had previously been restricted to West and Central African naphthylisoquinoline alkaloids exclusively.[5] In addition, dioncophylleine D (**5**) is the as yet only naturally occurring 7,8′-coupled Dioncophyllaceae-type alkaloid.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage microscope and are uncorrected. Optical rotations (25 °C, 10 cm cell) were measured on a Jasco P-1020 polarimeter. UV spectra were recorded on a Varian CARY 50 Conc UV−visible spectrophotometer, IR spectra were taken on a Jasco FT/IR-410 spectrometer, and CD spectra (25 °C, MeOH, 0.02 cm cell) were taken on a Jasco J-715 spectropolarimeter. ^{1}H NMR (400 MHz, 600 MHz) and ^{13}C NMR (100 MHz, 150 MHz) spectra were measured on Bruker Avance 400 and DMX 600 instruments, using $CDCl_3$ (δ 7.26 and 77.01) and CD_3OD (δ 3.31 and 49.15) as the solvents and internal ^{1}H and ^{13}C standards. Proton-detected, heteronuclear correlations were measured using HMQC (optimized for $^1J_{HC}$ = 145 Hz) and HMBC (optimized for $^nJ_{HC}$ = 7 Hz) pulse sequences. EIMS and HREIMS were determined on Finnigan MAT 8200 and Finnigan MAT 90 instruments (70 eV), respectively. FCPC separations were performed on Kromaton apparatus with a 1000 mL rotor. Preparative HPLC was carried out on a Symmetry C_{18} column (Waters, 19 × 300 mm, 7 μm), flow 11 mL min^{-1}, UV detection (233 nm), solvent (A) CH_3CN (0.05% trifluoroacetic acid), (B) H_2O (0.05% trifluoroacetic acid), linear gradient, 0 min 20% A, 25 min 55% A. (*R*)-MTPA-Cl was prepared from (*S*)-MTPA (Fluka Chemie AG, Deisenhofen, Germany) as described earlier.[16] Organic solvents were dried and distilled prior to use.

Plant Material. Leaves of *A. benomensis* were collected by three of us (H.R., M.W., and H.A.H.) in the region Gunung Benom, Pahang, Malaysia, in April 2000 (Export permit number WKL00/149). The species has been botanically described recently.[3] Voucher specimens have been deposited at Herb. Bringmann (no. 61), University of Würzburg, Germany.

Extraction and Isolation. The air-dried material (138 g leaves) was ground and extracted with 1 L of MeOH/CH_2Cl_2 (1:1) at room temperature. This extract was concentrated in vacuo to give 2.07 g of a residue, which was dissolved in 15 mL of MeOH. Fast centrifugal partition chromatography (FCPC)[12] was used to separate out the chlorophyll of this leaf extract in only one run in a quantitative manner. The lower organic phase from the solvent system $CHCl_3$/EtOAc/MeOH/H_2O (5:3:5:3) was chosen as the mobile phase. The flow was set to 8 mL/min and the rotation speed to 1100 rpm. The stationary phase, which contained the alkaloids, was flushed out of the rotor with MeOH with reversed flow direction to give 205 mg of a yellow extract after evaporation of the solvent. It was dissolved in MeOH and directly resolved using preparative HPLC with a Symmetry C_{18} column to give 6.4 mg of compound **3a** (t_R = 18.4 min), 5.8 mg of compound **5** (t_R = 18.9 min), and 3.9 mg of compound **4** (t_R = 19.5 min).

Table 1. Bioactivities of Compounds **3a**, **4**, and **5**

	IC$_{50}$ [μg/mL]		
	3a	**4**	**5**
P. falciparum (strain: K1)	3.9	3.1	0.5
Standard: chloroquine 0.0016[a]			
T. cruzi	54.0	21.8	24.3
Standard: benznidazole 0.55[a]			
T. b. rhodesiense	5.6	1.4	4.7
Standard: melarsoprol 0.00095[a]			
cytotoxicity using L-6 cells	*b*	20.6	*b*

[a] All values in μg/mL. [b] Determination not possible.

***ent*-Dioncophylleine A (3a):** white solid; mp 265 °C (MeOH) (lit. 272 °C, EtOH);[15] $[\alpha]_D^{25}$ −29.7° (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (1.68), 307 (0.39), 371 (0.18) nm; CD (EtOH) $\Delta\epsilon_{198}$ −2.9, $\Delta\epsilon_{224}$ 26.0, $\Delta\epsilon_{244}$ −20.5; IR (NaCl) ν_{max} 3379, 3055, 2988, 2924, 2849, 1670, 1617, 1594, 1265, 1199, 1130, 1097, 1078, 896 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.13 (3H, s, CH_3-2′), 2.72 (3H, s, CH_3-3), 3.29 (3H, s, CH_3-1), 3.94 (3H, s, OCH_3-5′), 3.99 (3H, s, OCH_3-4′), 6.73 (1H, d, *J* = 8.2 Hz, H-8′), 6.82 (1H, d, *J* = 7.7 Hz, H-6′), 6.83 (1H, s, H-3′), 7.21 (1H, dd, *J* = 7.7, 8.2 Hz, H-7′), 7.48 (1H, d, *J* = 8.2 Hz, H-5), 7.62 (1H, d, *J* = 8.2 Hz, H-6), 7.64 (1H, s, H-4); ^{13}C NMR ($CDCl_3$) δ 18.7 (CH_3-3), 20.6 (CH_3-2′), 23.7 (CH_3-1), 56.5 (OCH_3-4′), 56.6 (OCH_3-5′), 106.4 (C-6′), 108.9 (C-3′), 116.5 (C-10′), 117.7 (C-9), 117.8 (C-8′), 118.6 (C-5), 121.8 (C-4), 122.4 (C-1′), 125.2 (C-7), 127.7 (C-7′), 136.7 (C-9′), 137.4 (C-2′), 140.2 (C-10), 140.4 (C-6), 142.1 (C-3), 155.7 (C-8), 157.6 (C-5′), 157.9 (C-4′), 159.2 (C-1); EIMS *m/z* 373 [M]$^+$ (100), 358 [M − CH_3]$^+$ (14), 342 [M − OCH_3]$^+$ (10); HREIMS *m/z* 373.1676 [M]$^+$ (calcd for $C_{24}H_{23}NO_3$, 373.1678).

5′-*O*-Demethyl-*ent*-dioncophylleine A (4): white solid; mp 195 °C (MeOH); $[\alpha]_D^{25}$ −27.3° (*c* 0.05, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (1.69), 307 (0.41), 371 (0.21) nm; CD (EtOH) $\Delta\epsilon_{201}$ −3.7, $\Delta\epsilon_{229}$ 29.6, $\Delta\epsilon_{250}$ −21.7; IR (NaCl) ν_{max} 3394, 3012, 2948, 2851, 1667, 1608, 1524, 1199, 1122, 1082, 1078, 754, 729 cm^{-1}; ^{1}H NMR (MeOD) δ 2.17 (3H, s, CH_3-2′), 2.75 (3H, s, CH_3-3), 3.30 (3H, s, CH_3-1), 4.15 (3H, s, OCH_3-4′), 6.64 (1H, d, *J* = 8.3 Hz, H-8′), 6.76 (1H, d, *J* = 7.7 Hz, H-6′), 6.98 (1H, s, H-3′), 7.17 (1H, dd, *J* = 7.7, 8.3 Hz, H-7′), 7.61 (1H, d, *J* = 8.3 Hz, H-5), 7.66 (1H, d, *J* = 8.3 Hz, H-6), 7.90 (1H, s, H-4); ^{13}C NMR (MeOD) δ 18.6 (CH_3-3), 20.9 (CH_3-2′), 24.1 (CH_3-1), 57.0 (OCH_3-4′), 108.2 (C-6′), 111.3 (C-3′), 117.2 (C-10′), 119.2 (C-9), 119.6 (C-8′), 123.4 (C-5), 125.4 (C-4), 126.4 (C-1′), 126.8 (C-7), 129.3 (C-7′), 137.7 (C-9′), 137.9 (C-2′), 142.0 (C-10), 142.2 (C-6), 142.5 (C-3), 156.5 (C-8), 157.2 (C-5′), 158.4 (C-4′), 160.8 (C-1); EIMS *m/z* 359 [M]$^+$ (100), 344 [M − CH_3]$^+$ (29), 328 [M − OCH_3]$^+$ (9); HREIMS *m/z* 359.1514 (calcd for $C_{23}H_{21}NO_3$, 359.1522).

Dioncophylleine D (5): white amorphous solid; mp 212 °C (MeOH); $[\alpha]_D^{25}$ 0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 231 (1.62), 315 (0.41), 375 (0.17) nm; IR (NaCl) ν_{max} 3381, 2959, 2917, 2852, 1668, 1618, 1585, 1268, 1198, 1131, 1100, 1097, 830 cm^{-1}; ^{1}H NMR (MeOD) δ 2.75 (3H, s, CH_3-2′), 2.75 (3H, s, CH_3-3), 3.35 (3H, s, CH_3-1), 3.94 (3H, s, OCH_3-4′), 3.99 (3H, s, OCH_3-5′), 6.77 (1H, s, H-1′), 6.84 (1H, s, H-3′), 7.00 (1H, d, *J* = 8.0 Hz, H-6′), 7.35 (1H, d, *J* = 8.0 Hz, H-7′), 7.58 (1H, d, *J* = 8.4 Hz, H-5), 7.77 (1H, d, *J* = 8.4 Hz, H-6), 7.89 (1H, s, H-4); ^{13}C NMR (MeOD) δ 18.6 (CH_3-3), 22.1 (CH_3-2′), 24.2 (CH_3-1), 56.9 (OCH_3-5′), 57.1 (OCH_3-4′), 106.7 (C-6′), 110.3 (C-3′), 116.7 (C-7′), 117.9 (C-10′), 118.3 (C-6), 119.1 (C-1′), 120.6 (C-5), 123.4 (C-9), 126.1 (C-4), 128.2 (C-8′), 131.0 (C-7), 137.4 (C-9′), 138.7 (C-2′), 141.8 (C-10), 142.4 (C-3), 151.0 (C-8), 157.3 (C-4′), 159.1 (C-5′), 159.7 (C-1); EIMS *m/z* 373 [M]$^+$ (100), 358 [M − CH_3]$^+$ (11), 342 [M − OCH_3]$^+$ (11); HREIMS *m/z* 373.1676 [M]$^+$ (calcd for $C_{24}H_{23}NO_3$, 373.1678).

Enantiomer Analysis by HPLC-CD. A racemate of dioncophylleine A (**3b**) was prepared by partial synthesis[15] from dioncophylline A to establish the chromatographic system on a chiral phase. The best resolution of the enantiomers succeeded on a Chiralcel OD-H column (Daicel, 250 × 4.6 mm, 5 μm), with *n*-hexane + 0.05% TFA (A) and *i*-PrOH + 0.05%

TFA (B) as the eluents, using the following gradient: 0 min 10% B, 10 min 10% B, 30 min 50% B, flow 0.5 mL min^{-1}. The UV trace was recorded at 233 nm and the CD trace at 225 nm. For LC-CD measurements, a 5 mm standard flow cell was used. The stop-flow CD spectra were recorded with a scan speed of 500 nm min^{-1} and a bandwidth of 0.5 nm.

Biological Experiments. Antiparasitic activities against the pathogens *P. falciparum, T. cruzi*, and *T. brucei rhodesiense* as well as cytotoxicity (L-6 cells, rat skeletal myoblasts) were assessed as described earlier.[18]

Acknowledgment. This work was supported by the Fonds der Chemischen Industrie, the Deutsche Forschungsgemeinschaft (project Br 699/7-1 and SFB 630 "New Agents against Infectious Diseases"), and by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR).

References and Notes

(1) Part 161 in the series Acetogenic Isoquinoline Alkaloids. For part 160, see ref 4.
(2) Gereau, R. E. *Novon* **1997**, *7*, 242−245.
(3) Rischer, H.; Heubl, G.; Meimberg, H.; Dreyer, M.; Hadi, A.; Bringmann, G. *Blumea*, in press.
(4) Bringmann, G.; Dreyer, M.; Rischer, H.; Wolf, K.; Hadi, H. A.; Brun, R.; Meimberg, H.; Heubl, G. *J. Nat. Prod.* **2004**, *67*, 2058−2062.
(5) Bringmann, G.; Pokorny, F. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 1995; Vol. 46, pp 127−271.
(6) Bringmann, G.; Wohlfarth, M.; Rischer, H.; Grüne, M.; Schlauer, J. *Angew. Chem.* **2000**, *112*, 1523−1525; *Angew. Chem., Int. Ed.* **2000**, *39*, 1464−1466.
(7) Bringmann, G.; Feineis, D. *J. Exp. Bot.* **2001**, *52*, 2015−2022.
(8) Bringmann, G.; Feineis, D. *Act. Chim. Thérapeut.* **2000**, *26*, 151−171.
(9) Bringmann, G.; Holzgrabe, U.; Hoerr, V.; Stich, G. *Pharmazie* **2003**, *58*, 343−346.
(10) Bringmann, G. In *Guidelines and issue for the discovery and drug development against tropical diseases*; Vial, H., Fairlamb, A., Ridley, R., Eds.; World Health Organisation: Geneva, 2003; pp 145−152.
(11) Fleischhauer, J.; Koslowski, A.; Kramer, B.; Zobel, E.; Bringmann, G.; Gulden, K. P.; Ortmann, T.; Peter, B. *Z. Naturforsch. B* **1993**, *48*, 140−148.
(12) http://www.kromaton.com.
(13) Bringmann, G.; Dreyer, M.; Faber, J. H.; Dalsgaard, P. W.; Stærk, D.; Jaroszewski, J. W.; Ndangalasi, H.; Mbago, F.; Brun, R.; Christensen, S. B. *J. Nat. Prod.* **2004**, *67*, 743−748.
(14) Bringmann, G.; Messer, K.; Wohlfarth, M.; Kraus, J.; Dumbuya, K.; Rückert, M. *Anal. Chem.* **1999**, *71*, 2678−2686.
(15) Fleischhauer, J.; Koslowski, A.; Kramer, B.; Zobel, E.; Bringmann, G.; Gulden, K.-P.; Ortmann, T.; Peter, B. *Z. Naturforsch.* **1993**, *48b*, 140−148.
(16) Bringmann, G.; Teltschik, F.; Michel, M.; Busemann, S.; Rückert, M.; Haller, R.; Bär, S.; Robertson, S. A.; Kaminsky, R. *Phytochemistry* **1999**, *52*, 321−332.
(17) Lavault, M.; Bruneton, J. *C. R. Acad. Sci., Ser. C* **1978**, *287*, 129−131.
(18) Bringmann, G.; Hamm, A.; Günther, C.; Michel, M.; Brun, R.; Mudogo, V. *J. Nat. Prod.* **2000**, *63*, 1465−1470.

NP049626J