Pergamon

Tetrahedron, Vol. 52, No. 42, pp. 13409-13418, 1996

0040-4020/96 $15.00 + 0.00

PII: S0040-4020(96)00812-5

Jozimine A ('Dimeric' Dioncophylline A), a Non-Natural Michellamine Analog with High Antimalarial Activity[1]

Gerhard Bringmann*, Wael Saeb, and Dagmar Koppler

Institut für Organische Chemie, Universität Würzburg, D-97074 Würzburg, Germany

Guido François*

Prins Leopold Instituut voor Tropische Geneeskunde, Nationalestraat 155, B-2000 Antwerpen, Belgium

Abstract: The synthesis of jozimine A, the first non-natural dimer of a naturally occurring monomeric naphthylisoquinoline alkaloid, is described, starting from dioncophylline A, as isolated from *Triphyophyllum peltatum*, resp. prepared by total synthesis. It is the first dimeric naphthylisoquinoline alkaloid that displays distinct antimalarial activity (IC_{50} = 0.075 µg/ml) against asexual erythrocytic stages of *Plasmodium falciparum*, which even exceeds the activity of its monomeric precursor, dioncophylline A (IC_{50} = 1.44 µg/ml), and thus constitutes a novel type of antimalarial quateraryls.

INTRODUCTION

The naphthylisoquinoline alkaloids[2] constitute a group of structurally intriguing and pharmacologically important natural products. Thus, dioncophylline A (**1**), an axially chiral biaryl alkaloid from the West African liana *Triphyophyllum peltatum*,[3,4] has been found to exhibit fungicidal,[5] insect growth retarding and antifeedant activity,[6] and, in particular, activity against the malaria parasites *Plasmodium falciparum*[7] and *P. berghei*.[8] This interesting class of compounds has recently been significantly extended by the isolation and structure elucidation of michellamines A (**2a**) and B (**2b**), the first naturally occurring dimeric naphthylisoquinoline alkaloids, from the 'new' Central African liana, *Ancistrocladus korupensis*.[9,10] Unlike all the numerous monomeric naphthylisoquinoline alkaloids known so far, these dimers exhibit a high antiviral activity against both HIV-1 and HIV-2, and michellamine B (**2b**) is presently in advanced preclinical development in the US.[11] While such antiviral activities can be attributed only to these dimeric naphthylisoquinolines **2a** and **2b**, antimalarial activity has so far been found only for monomeric representatives, such as dioncophylline A (**1**), and for the monomeric halves of michellamines, *e.g.* for korupensamine A (**3**).[7,12] The high anticytopathic

activity of michellamines has stimulated the elaboration of several total syntheses.[13-18] As michellamine B (**2b**) exhibits a certain toxicity,[10] the availability of structural analogs of this promising new anti-HIV lead, hopefully with still better pharmacological properties, is an urgent demand - either by isolation of further representatives from the same plants,[2] or, more rationally, by synthetic procedures. In this paper, we report on the first preparation of a non-natural dimer **4** ('jozimine A') of a natural monomeric naphthylisoquinoline alkaloid, and its unexpectedly high antimalarial activity against *P. falciparum*.

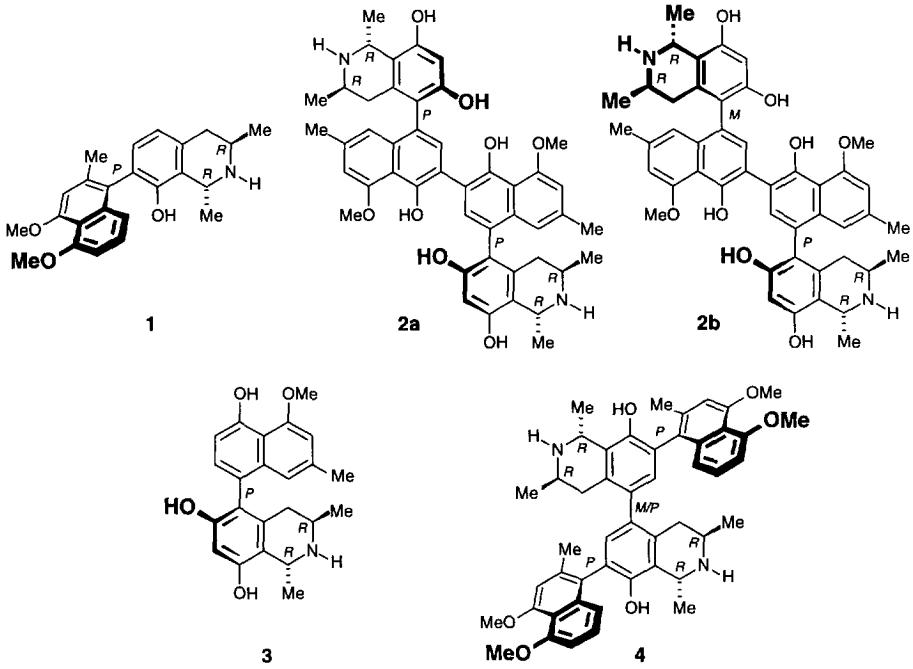
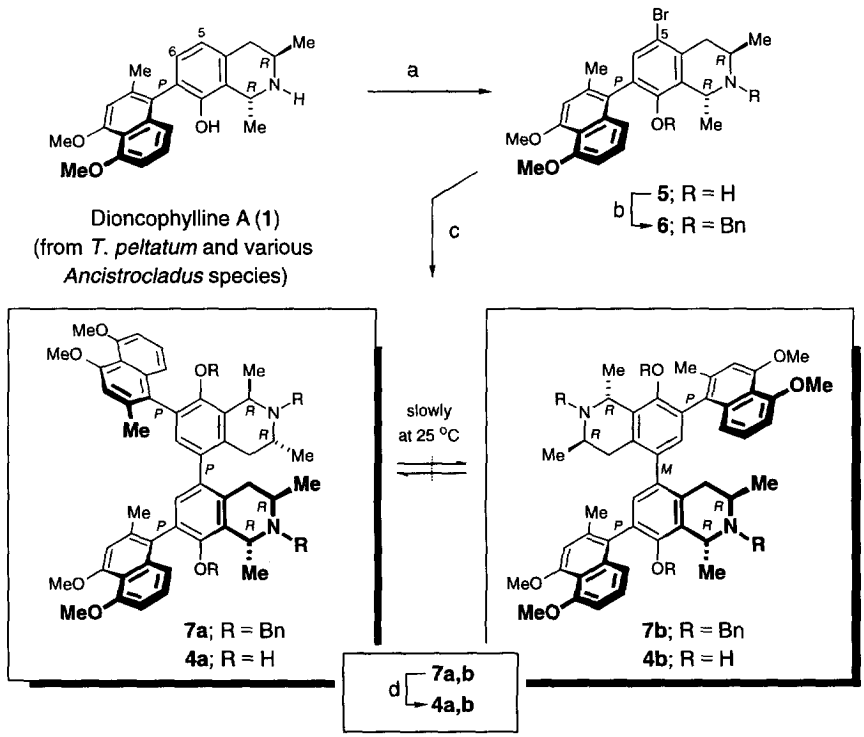


Figure 1. Selected mono- and dimeric naphthylisoquinolines with interesting pharmacological properties.

RESULTS AND DISCUSSION

Besides the chemical modification of isolated michellamines, either by derivatization, or by (rather tedious) total synthesis, the presently most promising approach to the directed preparation of novel dimeric naphthylisoquinolines should be the dimerization of naturally occurring monomeric alkaloids, since some of these are easily available, even on a multigram scale, from natural sources. As a first example for such a strategy, we chose dioncophylline A (**1**), because of its good availability from *Triphyophyllum peltatum*[3] and several *Ancistrocladus* species,[2,19,20] and because of its biological activities.[5-8]

Initial attempts to submit dioncophylline A (**1**) to oxidative conditions as previously used for the synthesis of michellamines,[14,15] gave complex reaction mixtures. For this reason, a reaction sequence bromination → protection → coupling → deprotection (Scheme 1) seemed most promising for the generation of a dimeric dioncophylline A. The presence of a free phenolic oxygen function at C-8 indeed allowed the bromination to be performed regiospecifically at C-5, despite the presence of the likewise electron-rich naphthalene system. This bromination was best done with tetrabutylammonium tribromide,[21] regioselectively leading to 5-bromodioncophylline A (**5**) in a good yield (85 %). That the bromination had indeed taken place at C-5, was clearly to be seen by the fact that the two dublets of aromatic protons at C-5 and C-6 had been replaced by a singlet for the remaining proton at C-6, which, in addition, did not give the NOE interaction with 4-H_{ax} as 5-H previously did in dioncophylline A (**1**); the identity of the remaining proton as 6-H was furthermore underlined by specific ROE interactions in the final target molecule **4** (see below). Protection of the two protic OH- and NH-functionalities was done by benzylation of 5-bromodioncophylline A (**5**) by the Claisen carbonate



Scheme 1. Reagents and conditions: a) N(*n*-Bu)$_4$Br$_3$, $CHCl_3$, NaOAc, 0 °C., 85 %; b) BnBr, K_2CO_3, acetone, reflux, 87 %; c) *t*-BuLi, 2-MeTHF, -95 °C; $CuCl_2$, -95 °C→ r.t., 14 %; d) H_2, Pd/C (10 %), EtOH, 99 %.

method,[22] with benzyl bromide in the presence of potassium carbonate in acetone, giving the fully protected dioncophylline A derivative **6** in 87 % yield. An X-ray structure analysis of **6** again confirms the correct position of the bromine substituent at C-5.[23]

The crucial step of the sequence, joining two dioncophylline A molecules together, was achieved according to the method of Wittig and Klar[24] - by lithiation of **6** with *tert*-butyllithium at -95 °C and subsequent transformation into the corresponding diaryl cuprate, which, upon warming to room temperature, reacted to the desired quateraryl **7** (see Scheme 1). The dimeric structure of the reaction product was demonstrated by the correct [M+]-peak in the mass spectrum (*m/z* 1112, 1%). Interestingly, also a relatively intensive peak at *m/z* 541 (17%) was detected, indicating the loss of two methyl groups to give a dikation [M^{++} - 2 CH_3]. Subsequent cleavage of all of the four *O*- and *N*-benzyl protective groups succeeded by catalytic hydrogenation to give the final target molecule **4**, the dimeric dioncophylline A ('jozimine A'[25]). Although the coupling yield is as yet very low (14%), this method allows the preparation of jozimine A in sufficient amounts for first biological studies (see below). Moreover, this interesting quateraryl is the only product formed in the reaction, besides extended hydrodehalogenation, leading to *O,N*-dibenzyldioncophylline A, which, by debenzylation to dioncophylline A (**1**), allows a recycling of the precious material.

The structure of jozimine A (**4**) is related to that of michellamines - and is yet distinctly different: the two molecular halves, the monomeric naphthylisoquinolines, are linked *via* the isoquinoline, not *via* the naphthalene part, *i.e.* with a connectivity naphthalene-isoquinoline-isoquinoline-naphthalene. This has also stereochemical consequences, since, different from michellamines, now the inner axis can no longer rotating freely, which, due to the higher steric demand, gives rise to the existence of two atropodiastereomers, (*P*)- and (*M*)-jozimine A (**4a** resp. **4b**). By rapid HPLC separation, they can be resolved, but gradually isomerize back at room temperature (*e.g.* in methanol), to give again the initial *ca.* 1:1 ratio.

The configuration at the newly generated central biaryl axis of these nicely C_2-symmetric atropodiastereomers **4a** and **4b** was elucidated by an investigation of dipol-dipol interactions using the ROESY experiment.[26] Different from related systematic investigations on monomeric naphthylisoquinolines,[27] the experiments on jozimine A were hampered by the molecular symmetry of this dimer and the resulting magnetic equivalence of analogous nuclei of the two molecular halves - and by a partial atropisomerization of jozimine A during longer NMR experiments. Of diagnostical value was in particular the interaction of 6"-H specifically with one of the protons at C-4' of the chiral isoquinoline part (see Fig. 2). Thus, interaction of 6"-H and 4'-H_{ax} (which is 'above' the isoquinoline plane) of the less rapidly eluting atropisomer clearly establishes this isomer to be *P*-configured at the central axis - and thus to be represented by the structure **4a**. The more rapid isomer is revealed to be the *M*-atropisomer **4b** by a clear ROE interaction of 6"-H with 4'-H_{eq} (which is 'below' the isoquinoline plane). This attribution is furthermore confirmed by an additional specific interaction of that 4'-H_{eq} with 8'''-H. These interactions (*e.g.* 6"-H with 4'-H_{ax} for **4a**) and the likewise observed corresponding reverse interactions (*e.g.* 6'-H with 4"-H_{ax}) clearly concern interactions of protons of the one molecular half with those of the other dioncophylline A subunit (*i.e.* not 6'-H with 4'-H), since they are not observed in the monomeric precursor, dioncophylline A (**1**).

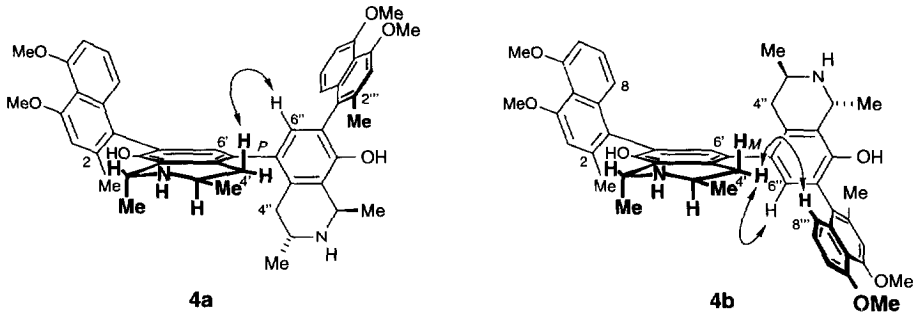


Figure 2. Selected ROE interactions relevant for the elucidation of the configuration at the central axis of **4a** and **4b** (for further ROE interactions, see Experimental).

Of particular interest was the biological activity of jozimine A. Although, regrettably, it did not show a particular antiviral activity comparable to that of michellamines,[28] it surprisingly displayed a high antimalarial activity against asexual erythrocytic stages of *P. falciparum*, with an IC_{50} value of 0.075 μg/ml. This high activity was entirely unexpected, since all hitherto investigated dimeric naphthylisoquinolines, namely the michellamines, are inactive towards *P. falciparum*.[12] By contrast, the new dimer, jozimine A (**4**), even exceeds the activity of its monomeric precursor, dioncophylline A (**1**) (1.44 μg/ml)[7] by a factor of *ca.* 20. Compared with its synthetic precursor, the new 5-bromodioncophylline A (**5**), which was likewise tested and found to show an IC_{50} value of 9.27 μg/ml, jozimine A (**4**) is more active even by a factor of >120.

Jozimine A is a prototype - both structurally and in relation to its antimalarial activity. This makes it even more rewarding to design, synthesize, and test further related and non-related unnatural dimers of naturally occurring - or artificial - monomeric naphthylisoquinoline alkaloids. This work is in progress.

EXPERIMENTAL

Melting points were measured on a Reichert-Jung Thermovar hot-plate and are uncorrected. NMR spectra were recorded with a Bruker AC 200, a Bruker WM 400, a Bruker AC 250, and a Bruker DMX 600 spectrometer. The chemicals shifts δ are given in parts per million (ppm) with the proton signals in the deuterated solvent as internal reference for ^1H and ^{13}C NMR. The coupling constants, *J*, are given in Hertz. HPLC separations: combination of a Waters M 510 HPLC pump, a U6K injector, and a reversed phase C_{18} column (Waters μBondapak™, 30 cm × 3.9 mm). Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter. IR spectra were taken on a Perkin-Elmer 1420 infrared spectrophotometer, and reported in wave numbers (cm^{-1}). Mass spectra were obtained on a Finnigan MAT 8200 mass spectrometer at 70 eV in the EI mode.

5-Bromodioncophylline A (5). To a cooled (0 °C) solution of dioncophylline A (**1**) (100 mg, 265 µmol) in dry $CHCl_3$ (5 ml) containing 20% NaOAc, a solution of $N(n\text{-}Bu)_4Br_3$ (140 mg, 290 µmol) in dry $CHCl_3$ (5 ml) was added under N_2 and the mixture was stirred for 10 min. After addition of 5% aqueous $Na_2S_2O_3$ solution (5 ml), the aqueous layer was extracted with CH_2Cl_2. The extract was washed with brine and dried ($MgSO_4$). After removal of the solvent, the crude product was recrystallized from MeOH, to give **5** (102 mg, 85%) as colorless crystals: mp 225 °C; $[\alpha]_D^{18}$ = -69.9 (*c* = 0.72 in $CHCl_3$); IR (KBr): ν 3440, 3300, 2950, 2910, 2830, 1580, 1450, 1380, 1370, 1330, 1260, 1100, 1070, 960, 870, 810, 795, 765; ^1H NMR (250 MHz, $CDCl_3$): δ = 1.29 (d, *J* = 6.1 Hz, 3H, 3-CH_3), 1.45 (d, *J* = 6.8 Hz, 3H, 1-CH_3), 2.22 (s, 3H, 2'-CH_3), 2.35 (dd, *J* = 17.1, 11.8 Hz, 1H, 4-H_{ax}), 2.95 (dd, *J* = 17.1, 4.4 Hz, 1H, 4-H_{eq}), 3.37 (m_c, 1H, 3-H), 3.97 (s, 3H, 5'-OCH_3), 4.02 (s, 3H, 4'-OCH_3), 4.45 (q, *J* = 6.5 Hz, 1H, 1-H), 6.79 (s, 1H, 3'-H), 6.81 (d, *J* = 7.7 Hz, 1H, 6'-H), 6.97 (d, *J* = 7.6 Hz, 1H, 8'-H), 7.20 (s, 1H, 6-H), 7.25 (dd, *J* = 8.1, 8.5 Hz, 1H, 7'-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.95 (CH_3 at C-1), 20.28 (CH_3 at C-3), 21.08 (CH_3 at C-2'), 33.93 (C-4), 45.43 (C-1), 50.22, 51.80 (C-3), 56.77 (OCH_3 at C-4'), 56.82 (OCH_3 at C-5'), 75.01, 103.41, 109.21, 117.66, 119.30, 126.78, 127.12, 127.76, 128.10, 128.22, 128.82, 133.68, 136.45; MS: *m/z* (%) = 457/455 (15/14) [M^+], 442/440 (100/98) [M^+ - CH_3], 361 (3) [M^+ - CH_3 - Br], 221 (26), 220 (26); Anal. calcd. for $C_{24}H_{26}BrNO_3$ (456.35): C, 63.16; H, 5.73; N, 3.06. Found: C, 63.07; H, 5.66; N, 2.97.

5-Bromo-*N*-*O*-dibenzyldioncophylline A (6). A mixture of 5-bromodioncophylline A (**5**) (100 mg, 219 µmol), benzyl bromide (0.25 ml, 250 µmol), and K_2CO_3 (207 mg,1.50 mmol) was refluxed in dry acetone (10 ml) for 4 h. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on deactivated (7.5% NH_3) silica gel with petroleum ether / ethyl acetate (97:3) as eluent. The crude product was recrystallized from methanol, to give **6** (121 mg, 87%) as colorless crystals: mp 145 °C; $[\alpha]_D^{18}$ = +17.6 (*c* = 0.81 in $CHCl_3$); IR (KBr): ν 3020, 2960, 2910, 1575, 1445, 1375, 1335, 1000, 830, 750, 730, 690; ^1H NMR (200 MHz, $CDCl_3$): δ = 1.12 (d, *J* = 6.8 Hz, 3H, 3-CH_3), 1.22 (d, *J* = 6.7 Hz, 3H, 1-CH_3), 1.95 (s, 3H, 2'-CH_3), 2.37 (dd, *J* = 17.2, 11.2 Hz, 1H, 4-H_{ax}), 2.20 (dd, *J* = 17.2, 4.9 Hz, 1H, 4-H_{eq}), 3.25 (d, *J* = 13.7 Hz, 1H, N-CH_2Ph), 3.33-3.45 (m, 1H, 3-H), 3.68 (s, 2H, O-CH_2Ph), 3.70-3.91 (m, 2H, N-CH_2Ph and 1-H), 3.79 (s, 3H, 5'-OCH_3), 3.83 (s, 3H, 4'-OCH_3), 6.08-7.25 (m, 15H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.61 (CH_3 at C-1), 20.35 (CH_3 at C-3), 21.08 (CH_3 at C-2'), 34.00 (C-4), 45.89 (C-1), 50.42, 52.00 (C-3), 56.68 (OCH_3 at C-4'), 56.75 (OCH_3 at C-5'), 75.11, 105.43, 109.11, 118.96, 119.59, 126.98, 127.76, 128.13, 128.26, 128.56, 128.82, 133.62, 136.40, 137.03; MS: *m/z* (%) = 637/635 (3/5) [M^+], 622/620 (97/100) [M^+ - CH_3], 515/513 (9/7) [M^+ - CH_3 - C_7H_7O], 423/421 (9/7), 91 (56) [$C_7H_7^+$]; Anal. calcd. for $C_{38}H_{38}BrNO_3$ (636.59): C, 71.69; H, 6.01; N, 2.20. Found: C, 71.44; H, 5.96; N, 2.41.

***N',N'',O',O''*-Tetrabenzyl-5',5''-bidioncophylline A (7a/b).** A 1.7 M solution of *t*-BuLi in *n*-hexane (110 µl, 187 µmol) in 2-methyltetrahydrofuran (2-MeTHF) (1 ml) was slowly added to a cold solution (-95 °C) of **6** (100 mg, 157 µmol) in 2-MeTHF (2 ml), and the mixture was stirred for 30 min at -95 °C. $CuCl_2$ was added and stirring was continued for 1 h at -95 °C. The solution was gradually warmed to room temperature and

stirred for 5 h. After addition of saturated NH_4Cl solution (2 ml), the mixture was extracted with CH_2Cl_2 and the organic extract was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified by chromatography on deactivated (7.5% NH_3) silica gel with petroleum ether / ethyl acetate (100:10) as an eluent. From methanol, a 50:50 atropisomeric mixture of **7a/7b** (24 mg, 14%) was obtained as a colorless powder; mp 108-110 °C; IR (KBr): ν 3020, 2990, 2980, 1590, 1270, 840, 810, 800, 750, 730, 700; ^1H NMR (600 MHz, $CDCl_3$) **7a** (less rapidly eluting[29] atropisomer with *P*-configuration at the central axis): δ = 1.15 (d, *J* = 6.5 Hz, 3H, 3-CH_3), 1.29 (d, *J* = 6.5 Hz, 3H, 1-CH_3), 2.10 (dd, *J* = 17.4, 4.4 Hz, 1H, 4-H_{eq}), 2.16 (s, 3H, 2'-CH_3), 2.30 (dd, *J* = 17.2, 10.8 Hz, 1H, 4-H_{ax}), 3.34 (d, *J* = 13.9 Hz, 1H, NC*H*$_2$Ph), 3.44 (m_c, 1H, 3-H), 3.75 (d, *J* = 13.9 Hz, 1H, NC*H*$_2$Ph), 3.89 (s, 2H, OC*H*$_2$Ph), 3.91 (s, 3H, 5'-OCH_3), 3.95 (s, 3H, 4'-OCH_3), 4.03 (q, *J* = 6.5 Hz, 1H, 1-H), 6.29-7.30 (m, 15H, Ar-H); **7b** (more rapidly eluting[29] atropisomer with *M*-configuration at the central axis): δ = 1.30 (d, *J* = 5.7 Hz, 3H, 3-CH_3), 1.31 (d, *J* = 5.7 Hz, 3H, 1-CH_3), 2.05 (s, 3H, 2'-CH_3), 2.37 (dd, *J* = 17.2, 11.4 Hz, 1H, 4-H_{ax}), 2.70 (dd, *J* = 17.3, 4.4 Hz, 1H, 4-H_{eq}), 3.48 (m_c, 1H, 3-H), 3.53 (d, *J* = 13.6 Hz, 1H, NC*H*$_2$Ph), 3.87 (d, *J* = 13.6 Hz, 1H, NC*H*$_2$Ph), 3.90 (s, 6H, 4' and 5' - OCH_3), 3.94 (d, *J* = 10.1 Hz, 1H, OC*H*$_2$Ph), 4.00 (d, *J* = 10.1 Hz, 1H, OC*H*$_2$Ph), 4.08 (q, *J* = 6.0 Hz, 1H, 1-H), 6.37-7.39 (m, 15H, Ar-H); ^{13}C NMR (150 MHZ, $CDCl_3$) atropisomer **7a** and **7b**: δ = 19.65 (CH_3 at C-1), 19.79 (CH_3 at C-1), 20.26 (CH_3 at C-3), 20.74 (CH_3 at C-3), 20.83 (CH_3 at C-2'), 20.83 (CH_3 at C-2'), 31.28 (C-4), 31.40 (C-4), 45.68 (C-3), 45.80 (C-3), 50.08 (C-1), 50.67 (C-1), 51.70, 51.80, 56.34 (OCH_3 at C-4'), 56.40 (OCH_3 at C-4'), 56.44 (OCH_3 at C-5'), 56.46 (OCH_3 at C-5'), 74.56, 74.75, 76.70, 76.92, 77.13, 104.87, 105.07, 109.05, 116.12 (C-3'), 116.19, 118.89, 119.16 (C-8'), 125.96 (C-9), 126.18 (C-9), 126.41, 126.57, 127.10, 127.23, 127.30, 127.73, 127.76, 127.78, 127.91, 128.07, 128.13, 128.42, 128.57, 129.55, 129.69, 131.09 (C-7'), 131.41 (C-7'), 132.73, 132.97, 133.11, 133.34, 135.52, 135.88, 135.90, 135.99, 136.58, 136.77, 137.17, 137.30, 140.71 (C-9'), 140.81 (C-9'), 154.06, 154.19 (C-8), 156.08, 156.11, 157.11, 157.23; MS: *m/z* (%) = 1112 (1) [M^+], 1097 (70) [M^+ - CH_3], 1006 (3) [M^+ - CH_3 - $C_7H_7^+$], 541 (17) [M^{++} - 2 CH_3], 91 (100) [$C_7H_7^+$]; Exact mass calcd. for $C_{75}H_{73}N_2O_6$ (M^+ - CH_3) 1097.546. Found: 1097.546.

Recovery of dioncophylline A (1). Early chromatographic fractions of the above separation gave *N,O*-dibenzyldioncophylline A (64.5 mg, 74%) as colorless crystals: mp 69 °C; $[\alpha]_D^{18}$ = +55.8 (*c* = 0.52 in $CHCl_3$); IR (KBr): ν 3060, 3035, 3005, 2940, 2910, 2820, 1578, 1250, 1200, 1120; ^1H NMR (250 MHz, $CDCl_3$): δ = 1.24-1.30 (m, 6H, 1- and 3-CH_3), 2.07 (s, 3H, 2'-CH_3), 2.37 (dd, *J* = 17.2, 11.2 Hz, 1H, 4-H_{ax}), 2.20 (dd, *J* = 17.2, 4.9 Hz, 1H, 4-H_{eq}), 3.25 (d, *J* = 13.7 Hz, 1H, N-C*H*$_2$Ph), 3.33-3.45 (m, 1H, 3-H), 3.68 (s, 2H, O-C*H*$_2$Ph), 3.70-3.91 (m, 2H, N-C*H*$_2$Ph and 1-H), 3.79 (s, 3H, 5'-OCH_3), 3.83 (s, 3H, 4'-OCH_3), 6.08-7.25 (m, 15H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 20.12 (CH_3 at C-1), 20.49 (CH_3 at C-3), 21.00 (CH_3 at C-2'), 33.75 (C-4), 44.59 (C-1), 51.22, 51.88 (C-3), 55.76 (OCH_3 at C-4'), 55.79 (OCH_3 at C-5'), 74.60, 105.43, 108.41, 118.90, 119.09, 125.98, 126.97, 128.55, 128.99, 129.16, 129.62, 132.52, 136.45, 137.13, 137.22; MS: *m/z* (%) = 557 (4) [M^+], 542 (100) [M^+ - CH_3], 91 (43) [$C_7H_7^+$]; Anal. calcd. for $C_{38}H_{39}NO_3$ (557.5): C, 81.83; H, 7.04; N, 2.51. Found: C, 81.63; H, 7.23; N, 2.56. Hydrogenolysis of this compound in ethanol (2 ml) in the presence of Pd/C 10% (5 mg) for 1 h gave dioncophylline A (**1**) (42.8 mg, 98%), fully identical with authentic material.

***P*- and *M*-Jozimines A (4a resp. 4b; 5',5''-bidioncophylline A).** A mixture of **7a/7b** (6 mg, 5.3 µmol) was hydrogenated in ethanol (2 ml) in the presence of Pd/C 10% (5 mg) for 1 h. After filtration and evaporation of the solvent, the residue precipitated from CH_2Cl_2, to give a 50:50 atropisomeric mixture of **4a/4b** (4.0 mg, 99%) as a colorless powder of mp 220-223 °C, which was subsequently resolved by HPLC on a semipreparative C_{18} bonded reversed-phase column using a non-linear gradient (CF_3CO_2H buffer (pH = 2.5) / CH_3CN from 60:40 to 80:20) as the eluent. After removal of the solvent, **4a** and **4b** were obtained as colorless powders from CH_2Cl_2; *P*-jozimine A (**4a**, less rapidly eluting atropisomer): mp 223 °C, $[\alpha]_D^{20}$ = -130.8 (*c* = 0.03 in ethanol); ^{1}H NMR (600 MHz, MeOH-d_4): δ = 1.61 (d, *J* = 6.3 Hz, 3H, 3-CH_3), 1.83 (d, *J* = 6.7 Hz, 3H, 1-CH_3), 2.36 (s, 3H, 2'-CH_3), 2.66 (dd, *J* = 17.5, 11.6 Hz, 1H, 4-H_{ax}), 3.12 (dd, *J* = 17.5, 4.9 Hz, 1H, 4-H_{eq}), 3.90 (m_c, 1H, 3-H), 4.12 (s, 3H, 4'-OCH_3), 4.15 (s, 3H, 5'-OCH_3), 5.04-5.11 (m, 1H, 1-H), 6.98 (s, 1H, 6-H), 7.08 (d, *J* = 7.7 Hz, 1H, 6'-H), 7.10 (s, 1H, 3'-H), 7.16 (d, *J* = 8.3 Hz, 1H, 8'-H), 7.43 (dd, *J* = 7.7, 8.3 Hz, 1H, 7'-H); *M*-jozimine A (**4b**, more rapidly eluting atropisomer): mp 225 °C, $[\alpha]_D^{20}$ = -79.8 (*c* = 0.05 in ethanol), δ = 1.508 (d, *J* = 6.2 Hz, 3H, 3-CH_3), 1.90 (d, *J* = 6.7 Hz, 3H, 1-CH_3), 2.44 (s, 3H, 2'-CH_3), 2.81-2.84 (m, 2H, 4-H), 3.90 (m_c, 1H, 3-H), 4.09 (s, 3H, 4'-OCH_3), 4.16 (s, 3H, 5'-OCH_3), 5.04-5.11 (m, 1H, 1-H), 7.00 (s, 1H, 6-H), 7.02 (d, *J* = 8.1 Hz, 1H, 6'-H), 7.05 (d, *J* = 8.5 Hz, 1H, 8'-H), 7.13 (s, 1H, 3'-H), 7.31 (dd, *J* = 8.1, 8.5 Hz, 1H, 7'-H); all the data below were measured for the atropisomeric mixture **4a/4b**; NOE interactions found by the ROESY method, as observed for both **4a** *and* **4b** (600 MHz, spectral width 5000 Hz, 256 t_1 increments, 2 kHz spin-lock, 700 ms mixing time): 7-H/8-H, (resp. 7'''-H/8'''-H), 7-H/6-H, (7'''-H/6'''-H), 4-OCH_3/3-H, (4'''-OCH_3/3'''-H), 5-OCH_3/6-H, (5'''-OCH_3/6'''-H), 4'-H_{eq}/3'-H, (4''-H_{eq}/3''-H), 1'-CH_3/1'-H, (1''-CH_3/1''-H), 3'-CH_3/3'-H, (3''-CH_3/3''-H), 6'-H/8-H, (6''-H/8'''-H), 2-CH_3/6'-H, (2'''-CH_3/6''-H), 1'-CH_3/3'-H, (1''-CH_3/3''-H), 4'-H_{eq}/4'-H_{ax}, (4''-H_{eq}/4''-H_{ax}), 2-CH_3/3-H, (2'''-CH_3/3'''-H); for additional, atropisomer-specific NOE interactions, see Fig. 2); IR (KBr): ν 3400, 2910, 2870, 1710, 1580, 1450, 1380, 1260, 1120; ^{13}C NMR (150 MHz, MeOH-d_4) atropisomer **4a** and **4b**: δ = 20.64 (CH_3 at C-1), 20.76 (CH_3 at C-1), 22.13 (CH_3 at C-3), 22.22 (CH_3 at C-3), 23.33, (CH_3 at C-2'), 23.46 (CH_3 at C-2'), 36.17 (C-4), 36.98 (C-4), 47.48 (C-3), 47.58 (C-3), 52.14 (C-1), 52.48 (C-1), 59.42 (OCH_3 at C-4'), 59.45 (OCH_3 at C-4'), 59.54 (OCH_3 at C-5'), 59.57 (OCH_3 at C-5'), 109.72, 109.82, 112.94 (C-3'), 120.48, 120.56, 121.92 (C-8'), 125.73 (C-9), 125.90 (C-9), 128.31, 128.73, 130.22 (C-7'), 130.34 (C-7'), 133.51, 133.96, 134.59, 135.06, 136.22, 136.56, 140.02, 140.81 (C-9'), 141.11 (C-9'), 154.49 (C-8), 160.98 (C-5'), 161.04 (C-5'), 161.32 (C-4'), 161.49 (C-4'); MS: *m/z* (%) = 752 (24) [M^+], 737 (100) [M^+ - CH_3], 361 (54) [M^{++} - 2 CH_3]; Exact mass calcd. for $C_{47}H_{49}N_2O_6$ (M^+ - CH_3) 737.359. Found: 737.359.

Antimalarial assays. Asexual erythrocytic stages of *P. falciparum* (NF 54, clone A1A9) were continuously maintained *in vitro* following previously described methods.[7,30] The activities of **4** and **5** were assayed by measuring the incorporation of ^{3}H-labeled hypoxanthine by the parasites in their presence.[31,32] The corresponding IC_{50} values expressing the antiplasmodial activities were obtained after linearization on the sigmoid dose response curves and application of probit analysis.[33]

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (Graduiertenkolleg "Magnetische Kernresonanz *in vivo* und *in vitro* für die biologische und medizinische Grundlagenforschung" and SFB 251 "Ökologie, Physiologie und Biochemie pflanzlicher und tierischer Leistung unter Streß"), the UNDP / WORLD BANK / WHO Special Programme for Research and Training in Tropical Diseases (TDR), and the Fonds der Chemischen Industrie. The authors thank Tania Steenackers, Markus Rückert, and Ralf God for experimental help, as well as Roland Götz and Jörg Holenz for helpful discussions.

REFERENCES AND NOTES

1. "Acetogenic Isoquinoline Alkaloids", part 87; for part 86, see ref. 23. - "Antiprotozoal Activity of Naphthylisoquinoline Alkaloids", part 6; for part 5, see François, G.; Steenackers, T.; Timperman, G.; Aké Assi, L.; Haller, R. D.; Bär, S.; Isahakia, M. A.; Robertson, S. A.; Zhao, C.; De Souza, N. J.; Holenz, J. and Bringmann, G. *Int. J. Parasitol.*, in press.
2. (a) Bringmann, G.; Pokorny, F. in *The Alkaloids*; Cordell, G., Ed.; Academic Press: New York, Vol. 46, 1995, pp. 127-271. - (b) Bringmann, G. *Bull. Soc. Chim. Belg.* **1996**, in press.
3. Bringmann, G.; Rübenacker, M.; Jansen, J. R.; Scheutzow, D.; Aké Assi, L. *Tetrahedron Lett.* **1990**, *31*, 639-642.
4. Bringmann, G.; Jansen, J. R.; Reuscher H.; Rübenacker, M.; Peters, K.; von Schnering, H. G. *Tetrahedron Lett.* **1990**, *31*, 643-646.
5. Bringmann, G.; Aké Assi, L; Rübenacker, M.; Ammermann, E.; Lorenz, G. *German patent D. O. S. DE* 4117080 A1, November 26, 1992; *European Patent EP* 0515856 A1, December 02, 1992.
6. Bringmann, G.; Gramatzki, S.; Grimm, C.; Proksch, P. *Phytochemistry* **1992**, *31*, 3821-3825.
7. François, G.; Bringmann, G.; Phillipson, J. D.; Aké Assi, L.; Dochez, C.; Rübenacker, M.; Schneider, C.; Wéry, M.; Warhurst, D. C.; Kirby, G. C. *Phytochemistry* **1994**, *35*, 1461-1464.
8. François, G.; Bringmann, G.; Dochez, C.; Schneider, C.; Timperman, G.; Aké Assi, L. *J. Ethnopharmacol.* **1995**, *46*, 115-120.
9. Manfredi, K. P.; Blunt, J. W.; Cardellina II, J. H.; McMahon, J. B.; Panell, L. L.; Cragg, G. M.; Boyd, M. R. *J. Med. Chem.* **1991**, *34*, 3402-3405.
10. Boyd, M. R.; Hallock, Y. F.; Cardellina II, J. H.; Manfredi, K. P.; Blunt, J. W.; McMahon, J. B.; Buckheit, Jr., R. W.; Bringmann, G.; Schäffer, M.; Cragg, G. M.; Thomas, D. W.; Jato, J. G. *J. Med. Chem.* **1994**, *37*, 1740-1745.
11. Cragg, G. M.; Boyd, M. R.; Cardellina II, J. H.; Newman, D. J.; Snader, K. M.; McCloud, T. G. *Ethnobotany and the Search for New Drugs*; Wiley, Chichester (Ciba Foundation Symposium 185), 1994; pp. 178-196.

12. Hallock, Y. F.; Manfredi, K. P.; Blunt, J. W.; Cardellina II, J. H.; Schäffer, M.; Gulden, K. P.; Bringmann, G.; Lee, A. Y.; Clardy, J.; François, G.; Boyd, M. R. *J. Org. Chem.* **1994**, *59*, 6349-6355.
13. Bringmann, G.; Götz, R.; Keller, P. A.; Walter, R.; Henschel, P.; Schäffer, M.; Stäblein, M.; Kelly, T. R.; Boyd, M. R. *Heterocycles* **1994**, *39*, 503-512
14. Bringmann, G.; Harmsen, S.; Holenz, J.; Geuder, T.; Götz, R.; Keller, P. A.; Walter, R.; Hallock, Y. F.; Cardellina II, J. H.; Boyd, M. R. *Tetrahedron* **1994**, *50*, 9643-9648.
15. Bringmann, G.; Götz, R.; Holenz, J.; Harmsen, S.; Walter, R. *Liebigs Ann. Chem.* **1996**, in press.
16. Kelly, T. R.; Garcia, A.; Lang, F.; Walsh, J. J.; Bhaskar, K. V.; Boyd, M. R.; Götz, R.; Keller, P. A.; Walter, R.; Bringmann, G. *Tetrahedron Lett.* **1994**, *35*, 7621-7624.
17. Hoye, T. R.; Chen, M.; Mi, L.; Priest, O. P. *Tetrahedron Lett.* **1994**, *35*, 8747-8750.
18. Hobbs, P. D.; Upender, V.; Liu, J.; Pollart, D. J.; Thomas, D. W.; Dawson, M. I. *J. Chem. Soc., Chem. Commun.* **1996**, *8*, 923-924.
19. Bringmann, G.; Zagst, R.; Reuscher, H.; Aké Assi, L. *Phytochemistry* **1992**, *31*, 4011-4014.
20. Hallock, Y. F.; Hughes, C. B.; Cardellina II, J. H.; Schäffer, M.; Gulden, K. P.; Bringmann, G.; Boyd, M. R. *Nat. Prod. Lett.* **1995**, *6*, 315-320.
21. Berthelot, J.; Guette, C.; Desbéne, P. L.; Basselier, J. J.; Chaquin, P.; Masure, D. *Can. J. Chem.* **1989**, *67*, 2061-2066.
22. Claisen, L.; Eisleb, O. *Justus Liebigs Ann. Chem.* **1913**, 401, 21-119.
23. Bringmann, G.; Saeb, W.; Peters, K.; Peters, E. M.; *J. Chem. Soc., Chem. Commun.*, submitted.
24. Wittig, G.; Klar, G. *Justus Liebigs Ann. Chem.* **1967**, 704, 91-108.
25. Although this dimeric dioncophylline A has as yet not been found in nature, it has been given a trivial name in order to avoid lenthy rational or half-rational nomenclature; this name is derived from the Kiswahili word '*jozi*', meaning 'pair' or 'couple'.
26. Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355-360.
27. Bringmann, G.; Koppler, D.; Scheutzow, D.; Porzel, A. *Magn. Reson. Chem.*, submitted.
28. Hallock, Y. F.; Cardellina II, J. H.; Boyd, M. R.; Saeb, W.; Bringmann, G., to be published.
29. Chromatographic attribution based on partial separation (d.r. *ca.* 75:25) on deactivated (7.5% NH_3) silica gel with CH_2Cl_2 / MeOH (9:1) as the eluent.
30. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673-675.
31. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710-718.
32. François, G.; Timperman, G.; Holenz, J.; Aké Assi, L.; Geuder, T.; Maes, L.; Dubois, J.; Hanocq, M.; Bringmann, G. *Ann. Trop. Med. Parasitol.* **1996**, *90*, 115-123.
33. Diem, K. in *Scientific Tables*; Sixth Edition; Documenta Geigy: Basel, 1962; pp. 54-55.

(*Received in Germany* 9 *August* 1996; *accepted* 8 *September* 1996)