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Jozimine B, a Constitutionally Unsymmetric, Antiplasmodial 'Dimer' of the Naphthylisoquinoline Alkaloid Ancistrocladine[1]

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Abstract—The first synthesis of a constitutionally unsymmetric bis-naphthylisoquinoline, the unnatural 'dimer' of the *Ancistrocladus* alkaloid ancistrocladine, jozimine B, is described. The constitution and the configuration at the new, central biaryl axis were established through extensive NMR investigations and quantum chemical CD calculations. Compared with ancistrocladine, its only weakly antiplasmodial monomeric precursor, jozimine B exhibits a distinctly enhanced antimalarial activity.

Introduction

Dimeric naphthylisoquinoline alkaloids constitute a new generation of bioactive compounds from tropical plants.[2,3] The most prominent representative of this class of quateraryls is michellamine B (**1**, see Fig. 1), a cross-coupling product of two atropo-diasteromeric monomeric naphthylisoquinoline alkaloids, korupensamines A and B.[4] Michellamine B (**1**) and other related dimeric naphthylisoquinolines of natural origin displays a good order of anticytopathic activity against HIV-1 and -2.[2,5] More recently, a heterodimeric coupling product, consisting of a 5,8′- and a 7,8′-coupled monomeric naphthylisoquinoline unit, has been isolated: korundamine A (**2**).[6] Besides likewise showing a good portion of anti-HIV activity, **2** is the first natural dimeric naphthylisoquinoline with an appreciable antimalarial activity against *Plasmodium falciparum* in vitro.[6] Since the detection of michellamines, a broad series of natural[7–14] and unnatural[15–21] dimeric naphthylisoquinolines have been synthesized, aiming at the discovery of hopefully more active and simultaneously less toxic structural analogs. A most promising strategy for this is the dimerization of naturally occurring and thus (in some cases) sufficiently available monomeric naphthylisoquinolines, even if the corresponding dimers have not yet been found in nature. As an example, dioncophylline A, the main alkaloid of the West African liana *Triphyophyllum peltatum* (Dioncophyllaceae), was dimerized to give jozimine A (**3**),[15] which, compared to its monomeric precursor, was found to exhibit a ca. 35-fold increased activity against *P. falciparum.* Jozimine C (**4**), prepared by oxidative 'dimerization' of dioncophylline C,[19] shows a good portion of anti-HIV activity, but its antimalarial activity is lower than that of its natural monomeric half (Fig. 1).

In this paper, we report on the *non*-phenolic oxidative coupling of ancistrocladine (**5**), a 5,1′-coupled naphthylisoquinoline alkaloid easily isolable *i.a.* from the Indian liana *Ancistrocladus heyneanus* (Ancistrocladaceae),[22] but also synthetically accessible,[23] to give *P*-jozimine B [(*P*)-**6**], along with its didehydro analog as a minor by-product. Jozimine B is the first constitutionally unsymmetric synthetic 'dimer'[24] of a naturally occurring monomeric naphthylisoquinoline and has a good portion of antimalarial activity, far higher than its monomeric precursor, ancistrocladine (**5**).

Results and Discussion

In contrast to the first total syntheses of michellamines[7,9] and of jozimine C (**4**), which had been efficiently built up by oxidative dimerization of appropriately protected monomeric precursors using silver(I) oxide, this reagent did not give any reaction in the case of ancistrocladine (**5**), apparently due to the lack of a free phenolic oxygen function in the naphthalene part. The use of lead tetraacetate in dichloromethane in the presence of boron trifluoride etherate, by contrast, directly on ancistrocladine (**5**), without any protective groups present, smoothly lead to one main product, accompanied by a small quantity of a minor additional product in a 95:5 ratio (see Scheme 1). The two compounds, although chromatographically nearly identical,

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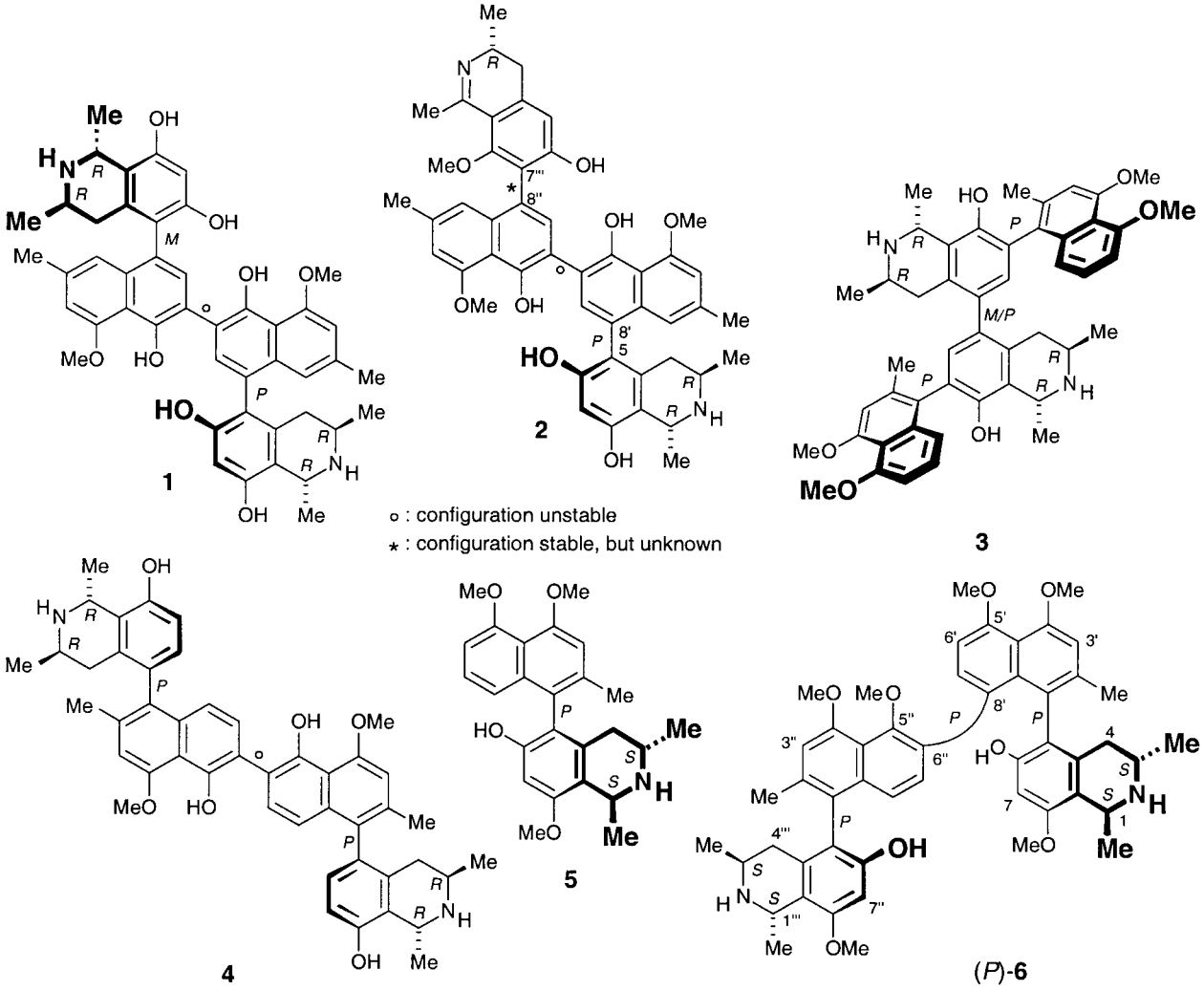


Figure 1. The natural bis-naphthylisoquinolines michellamine B (**1**) and korundamine A (**2**), the non-natural dimers jozimine A (**3**) and jozimine C (**4**), as well as the monomeric alkaloid ancistrocladine (**5**) and its new dimer, *P*-jozimine B [(*P*)-**6**)].

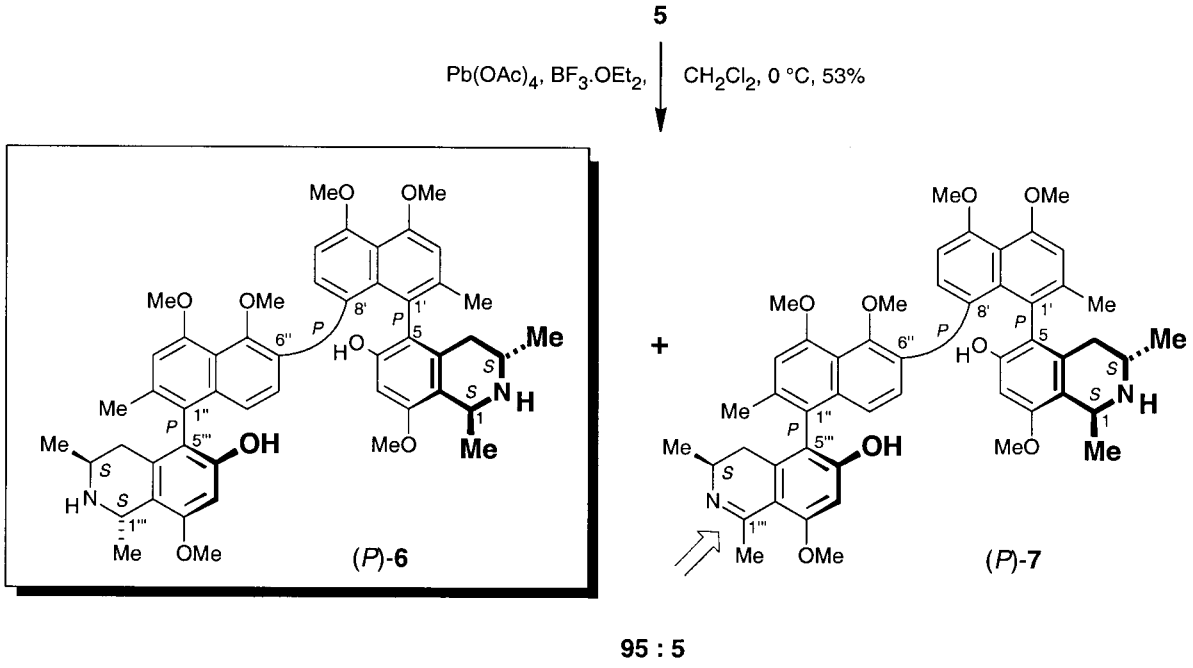
were successfully resolved by a series of consecutive chromatographic separations.

First NMR-spectroscopic investigations on the more rapidly eluting main product **6** gave unambiguous hints at the formation of a non-C_2 symmetric dimer of **5**, presumably coupled via the *ortho*- and *para*-positions of the distal naphthalene rings (i.e. via C-8′ and C-6″): The ^{1}H NMR spectrum (for selected chemical shifts, see Table 1) showed complete sets of signals for each of the two ancistrocladine portions (except for one missing H each); furthermore the protons at C-7′ and C-7″, which had appeared as doublets of doublets in **5** (δ=7.21 ppm), now gave (slightly high-field shifted) doublets (at 7.07 and 7.14 ppm, respectively), hinting at an—unsymmetric—coupling in these rings. A full attribution of all of the signals both of the starting material **5** and of each of the two moieties of the product succeeded by HMBC experiments (Fig. 2).

Of diagnostical value was the large deviation of the chemical shift of the methoxy group at C-5″ (3.42 ppm, see Table 1), which significantly differs from the corresponding signal of the starting material **5** (3.98 ppm) and from the likewise unaffected respective signal in the other ancistrocladine portion of the dimer (4.03 ppm). This strong high-field shift of nearly 0.6 ppm is in agreement with an *ortho–para* coupling of the two molecular moieties in **6**.

Besides the strongly separated signals for the methyl groups at C-1 (0.86 ppm) and C-1‴ (1.62 ppm), the first one of which is high-field shifted by ca. 0.8 ppm as compared to 1-Me of **5** (1.45 ppm), a largely different chemical shift of the proton at C-7 (5.90 ppm) as compared to the proton at C-7‴ (6.54 ppm) is obvious. The latter is high-field shifted by more than ca. 0.6 ppm compared to 7-H in **5** (6.49 ppm), which can only be explained by the ring current influence of the neighboring aromatic system.[25] A comparably strong shift by ca. 0.8 ppm, but now in the low-field direction, is found for one of the two diastereotopic methylene protons at C-4, viz. for the 4-H_{eq} (2.93 ppm) proton compared to 4‴-H_{eq} (2.15 ppm) (see Table 1). For further diagnostically significant divergent ^{1}H NMR shifts, see Table 1.

With the constitution of the coupling product established, the last required structural information was the attribution of the configuration at the newly generated biaryl axis, which



Scheme 1. Non-phenolic oxidative dehydrodimerization of ancistrocladine (**5**).

Table 1. Divergent chemical shifts of selected protons (δ-values in ppm) of the two molecular 'halves' of jozimine B [(*P*)-**6**)], compared to those in **5** as the 'monomeric' standard (shifts largely changed by the coupling, are marked in **bold**)

Jozimine B [(*P*)-**6**)] moieties coupled at				For comparison: Ancistrocladine (**5**)	
C-8′		C-6″			
7′-H	7.07	7″-H	7.14	7′-H	7.21
5′-OCH_3	4.03	5″-OCH_3	**3.42**	5′-OCH_3	3.98
1-CH_3	**0.86**	1‴-CH_3	1.62	1-CH_3	1.45
7-H	**5.90**	7‴-H	6.54	7-H	6.49
4-H_{eq}	**2.93**	4‴-H_{eq}	2.15	4-H_{eq}	1.96
8-OCH_3	**3.59**	8‴-OCH_3	3.89	8-OCH_3	3.86
2′-CH_3	**1.78**	2″-CH_3	2.04	2′-CH_3	2.15

succeeded by the interpretation of specific ROESY interactions (Fig. 3), mainly from 5″-OMe to 4-H_{eq}, from H-3″ to 1-Me, and, in addition, both from H-7″ and H-8″ to H-7. The latter again proved C-8″ to be 'free', not coupled, and thus further confirmed the 8′-6″-coupling mode of the two molecular halves, as did likewise the ROESY effect of H-7′ with 5″-OMe. Accordingly, the coupling product, named jozimine B, has to be attributed the full absolute stereostructure (*P*)-**6** (see Figs. 1 and 2). The other possible atropoisomer of jozimine B, (*M*)-**6** (not shown), i.e. with *M*-configuration at the new central axis, was not even found in traces.

The more slowly eluting minor compound **7** likewise shows a double set of signals. Of these, only one corresponds to an ancistrocladine moiety, while the other half of **7** is

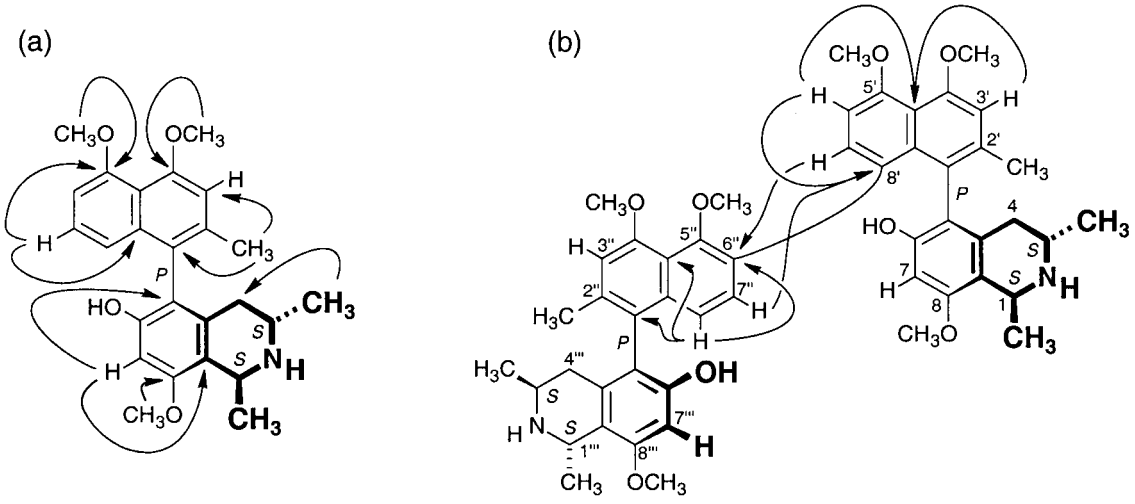


Figure 2. Constitution of jozimine B (**6**), by selected HMBC interactions found for *both* molecular moieties (a), and by additional specific ones within and between the two 'halves' of the molecule (b).

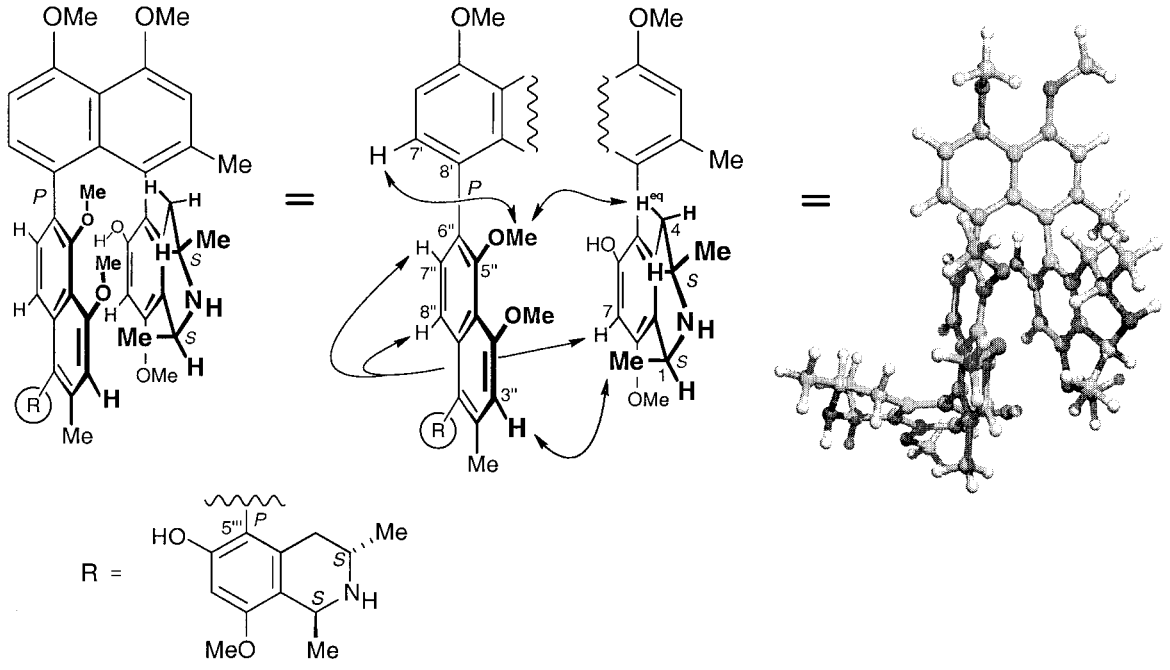


Figure 3. Selected ROESY interactions of *P*-jozimine B [(*P*)-**6**]: further proof of a 8′,6″-coupling and elucidation of the relative configuration at the central axis. The naphthalene part has been 'cut' for reasons of transparency.

Table 2. Selected chemical shifts (δ-values in ppm) indicative of the coupling site between the two molecular 'halves' of 1‴,2‴-dehydrojozimine B [(*P*)-**7**], compared to those in **5** and in ancistrocladinine (which was available from previous isolation work[27]) as the 'monomeric' standard (shifts largely changed by the coupling are marked in **bold**)

For comparison: ancistrocladine (**5**)		1‴,2‴-dehydrojozimine B [(*P*)-7] moieties coupled at				Ancistrocladinine (=1,2-dehydroancistrocladine)	
		C-8′		C-6″			
1-H	4.40	1-H	4.31	1‴-H	–	1-H	–
1-CH_3	1.45	1-CH_3	**1.12**	1‴-CH_3	**2.76**	1-CH_3	2.22
5′-OCH_3	3.98	5′-OCH_3	3.96	5″-OCH_3	**3.54**	5′-OCH_3	3.89
7-H	6.49	7-H	**5.78**	7‴-H	6.71	7-H	6.27
4-H_{eq}	1.96	4-H_{eq}	**2.70**	4‴-H_{eq}	2.34	4-H_{eq}	1.89
3′-H	6.80	3′-H	6.98	3″-H	6.83	3′-H	6.78
6′-H	6.81	6′-H	6.87	6″-H	–	6′-H	6.85
7′-H	7.21	7′-H	6.96	7″-H	7.02	7′-H	7.13
8′-H	6.87	8′-H	–	8″-H	6.65	8′-H	7.02
8-OCH_3	3.86	8-OCH_3	**3.47**	8‴-OCH_3	4.04	8-OCH_3	3.80
2′-CH_3	2.15	2′-CH_3	**1.85**	2″-CH_3	2.12	2′-CH_3	2.00

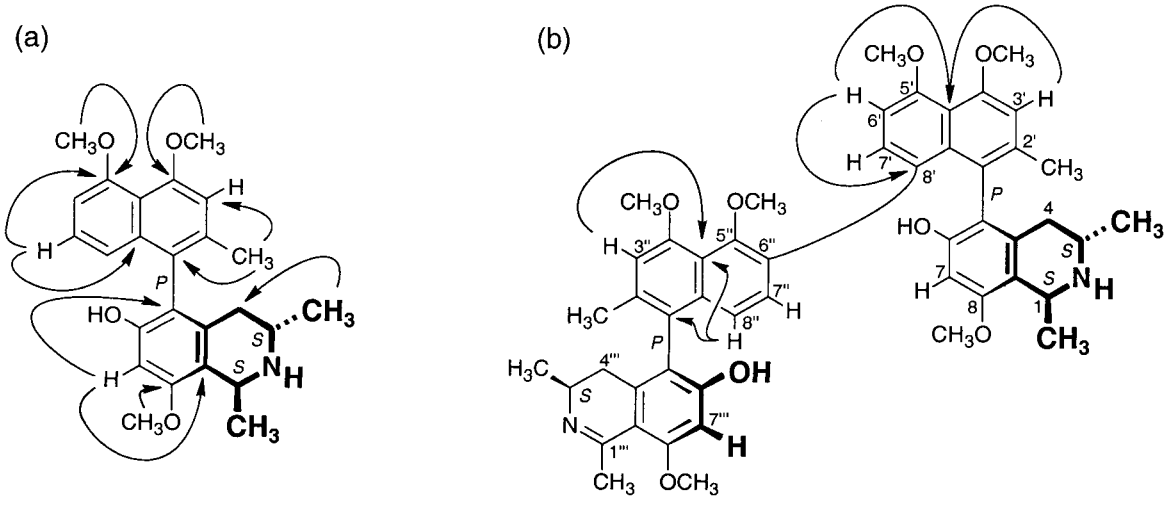


Figure 4. Constitution of 1‴,2‴-dehydrojozimine B [(*P*)-**7**], by selected joint HMBC interactions in each molecular half (a), and by specific ones in the two molecular moieties of the molecule (b).

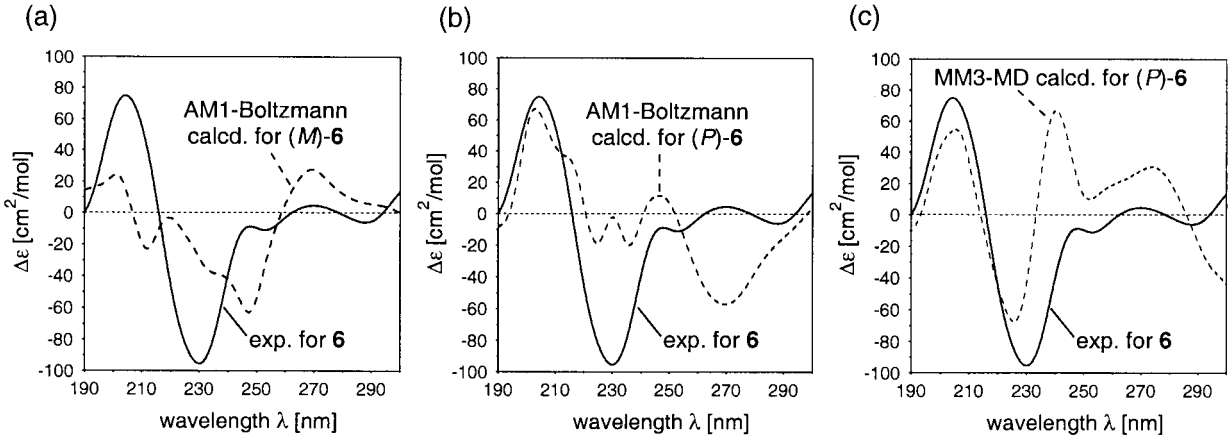


Figure 5. Confirmation of the absolute configuration at the central axis of **6** by comparison of the experimental CD spectrum with the spectra calculated for (*M*)- and (*P*)-**6**, based on a semiempirical conformational analysis with Boltzmann-weighting (a, b), and on MM3-MD simulations (c).

1,2-dehydrogenated, with a C═N double bond,[26] as evident from the lack of a proton at C-1‴ (see Table 2) and from the presence of a singlet for the methyl protons at C-1‴ at 2.76 ppm, instead of the previously present doublet at 1.45 ppm. Again, a complete attribution of the proton signals was achieved by HMBC measurements (Fig. 4).

Again, as for **6**, a symmetric coupling can be excluded for **7**. For an *ortho–ortho* product a distinct high-field shift would have been expected for *both* of the methoxy group at C-5′ and C-5″, as well as similar shifts for protons in analogous positions on the two connected naphthalene moieties, which, however, was not found. By contrast, the distinct high-field shift only for the methoxy groups at C-5″ (3.54 ppm, see Table 2), which is thus in the direct proximity of the new biaryl axis in contrast to the OMe group at C-5′ (3.96 ppm), and the significantly divergent chemical shifts of the protons of the two naphthalene parts (6′-H, 7′-H, 7″-H, and 8″-H; see Table 2) clearly hint at an unsymmetric *ortho–para* junction (i.e. 8′,6″-coupling) of the two naphthalene systems. From all this and from further significant proton shifts (see Table 2) it can be concluded that, at least from its constitution (except of course for the C═N double bond), the minor product **7** is identical to the main product **6**.

To likewise prove the *stereochemical* identity of the two products with respect to the configuration at the newly generated biaryl axis, turned out to be difficult, because—different from **6** (cp. Fig. 3, above)—configurationally diagnostical ROESY interactions could not unambiguously be stated for **7**. The only clear ROESY interaction between the two molecular halves, i.e. between the aromatic proton at C-7′ and the methoxy group at C-5″, gives additional support to the attribution of an *ortho–para* coupling between C-8′ and C-6″ as deduced above.

Decisive information on the absolute configuration at the central biaryl axis was thus expected from chiroptical investigations by means of quantum chemical CD-calculations.[28] Given the large molecular sizes of jozimine B and its dehydro product (*i.a.* with their three biaryl axes), they are, in total, quite flexible compounds. Starting with jozimine B, a random search algorithm based on molecular mechanics gave a series of conformers of both (*P*)- and (*M*)-**6**, which were further optimized using the semiempirical AM1 parameterization.[29] For this, only minimum structures with significantly different conformations were taken into consideration, so that finally 43 conformers of (*P*)-**6** and 23 conformers of (*M*)-**6** were obtained, of which, in turn, 13 and 8 conformers, respectively, whose energies were below a 'cut-off' of 3 kcal/mol above the respective global minimum. Starting with these energetically favored minimum structures, single CD spectra were calculated, Boltzmann-weighted according to the heats of formation of the respective conformers, and added up to the corresponding theoretical overall spectra, which were then submitted to our 'UV-correction'.[28] The theoretical spectrum thus obtained for (*P*)-**6** (see Fig. 5b) shows a relatively good agreement between the one calculated for *P* as compared to the experimental one, in particular in the deciding short-wavelength region, while the one calculated for (*M*)-**6** (structure not shown) is significantly different (see Fig. 5a).

The agreement for (*P*)-**6** is, however, not perfect, certainly due to the molecular flexibility of this large molecule. For the quantum chemical prediction of CD spectra of such non-rigid molecules, we have recently started using the combination of CD calculations with molecular dynamics (MD) simulations,[30] and have now applied this method to jozimine B. In this case, MD simulations using the MM3 force field were performed over a period of 500 ps, solving Newton's equations of motion in intervals of 2 fs. The extraction of molecular geometries was done every 0.5 ps. Of each of the 1000 structures thus obtained, single CD spectra were calculated, which, in contrast to the method described above, were averaged *arithmetically*, according to the timely distribution, to give a—now MD-based—overall CD spectrum for (*P*)-**6** as presented in Fig. 5c. Due to the considerably higher computational demand, the CD spectrum was calculated here only for the *P*-atropisomer of jozimine B, (*P*)-**6**, not for (*M*)-**6**. As can be seen, this spectrum shows a better agreement with the one experimentally obtained for jozimine B, thus confirming the conclusion of a *P*-configuration at the central axis of this new dimer, as already evident from the NOE-NMR investigations.

The experimental CD spectrum of the dehydro compound **7**

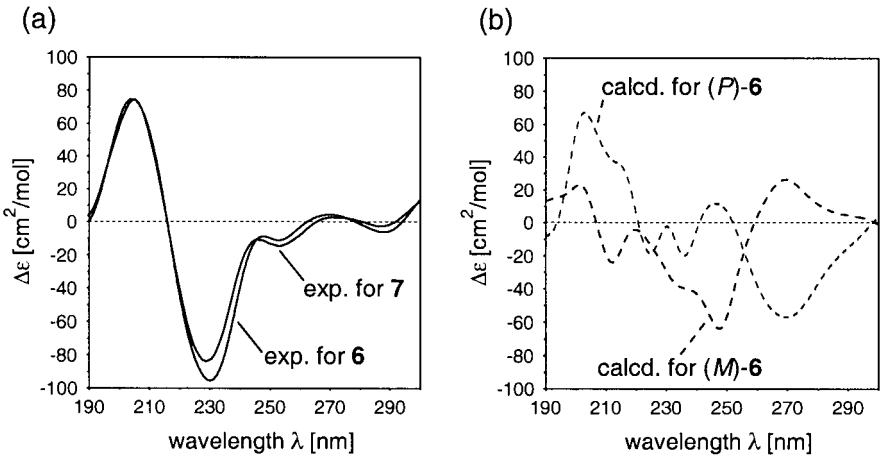


Figure 6. Close similarity of the experimental CD spectra of **6** and **7** (a) and comparison of the theoretical (AM1→Boltzmann) spectra for (*M*)- and (*P*)-**6** (b).

is virtually identical to that of **6** (see Fig. 6a). This clearly hints at an identical absolute configuration at the central biaryl axis of **6** and **7**. While a simple dehydrogenation at C-1/N-2 should thus have no major influence on the relative orientation of the main chromophores of the molecule to each other, an opposite configuration at the central biaryl axis would drastically change this orientation, leading to a distinctly different CD spectrum as can be seen by the comparison of the AM1-based→Boltzmann-weighted calculated spectra of *P*- and *M*-jozimines B in Fig. 6b, thus proving that the more slowly eluting compound **7** is the 1,2-dehydrogenated derivative of authentic *P*-jozimine B [(*P*)-**6**].

The structural investigations thus show that with a very high regio- and atropisomer-selectivity only the constitutionally unsymmetric, 6′,8″-coupled dimer (*P*)-**6** has been formed in the lead tetraacetate oxidation of ancistrocladine (**5**), along with negligible amounts of the constitutionally and stereochemically fully identical further oxidized dehydro product (*P*)-**7**. Other regio- or stereoisomeric products were not found even in traces.

Reasons for the high regioselectivity in the coupling step in favor of a constitutionally unsymmetric coupling product may be deduced from previous work on the lead tetraacetate non-phenolic oxidative coupling of anisol, for which a radical cation mechanism has been proposed.[31] Electron density calculations and the ESR spectrum of the methoxyphenyl radical cation show the unpaired electron to be mainly localized *para* to the methoxy group.[31] Assuming a similar situation in the coupling of **5**, the regioselectivity becomes understandable, with the reactive—but sterically highly hindered—*para*-position (C-8′), of one of the two molecules of ancistrocladine (**5**) then inevitably reacting with the sterically distinctly less hindered *ortho*-position (C-6″) of the second one. The high stereoselectivity in the coupling step should be due to the extremely high steric interactions required for an *M*-type coupling of the two ancistrocladine moieties, which would have to pass unattainably high coupling transition states.

With respect to the interest in configurationally stable and stereochemically homogeneous 1,8-diaryl-substituted naphthalenes,[32] the structure of jozimine B [(*P*)-**6**], which can now be produced so smoothly, in a regio- and stereoselective one-step reaction from easily available ancistrocladine (**5**), is even more remarkable.

Besides possessing a chemically and stereochemically interesting structure with three stereogenic axes and four stereocenters, *P*-jozimine B [(*P*)-**6**] displays significant antimalarial activities in vitro against chloroquine-resistant (IC_{50} [K1-strain]=0.10 μM) and sensitive (IC_{50} [NF 54-strain]=0.14 μM) strains of *Plasmodium falciparum*, and is thus nearly 35 times more active than the parent compound, ancistrocladine (**5**) (3.49 and 4.62 μM, respectively). The antimalarial activities of the minor product, (*P*)-**7**, are somewhat lower (0.28 and 0.47 μM, respectively), but still distinctly higher than those of the starting material, ancistrocladine (**5**).[33] Thus the drastic increase in antimalarial activity by chemical 'dimerization', as already shown for dioncophylline A to give jozimine A (**3**),[15] could, now once again, be verified here for ancistrocladine (**5**) to give jozimine B (**6**) and its dehydro analog **7**. This makes it rewarding to design, pepare and test further such dimers of naphthylisoquinoline alkaloids.

Experimental

Melting points were measured on a Reichert–Jung Thermovar hot-plate and are uncorrected. IR spectra were taken on a Perkin–Elmer 1420 infrared spectrophotometer, and reported in wave numbers (cm^{-1}). NMR spectra were recorded with a Bruker DMX 600 spectrometer. The chemical 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. Coupling constants, *J*, in Hertz. The CD spectra were recorded in ethanolic solution on a J-715 spectropolarimeter (JASCO, Gross–Umstadt, Germany) at room temperature within the range of 190–400 nm. HPLC separations: combination of a Waters 600E pump, a Nova–Pak C_{18} (Waters, 200×25 mm, 6 μm) column, and a Waters 996 photodiode array detector. The mass spectra were obtained on Finnigan MAT 8200 and MAT 90 mass spectrometers at 70 eV in the EI mode unless otherwise stated.

***P*-Jozimine B [(*P*)-6] and *P*-1‴,2‴-dehydrojozimine B [(*P*)-7].** Lead tetraacetate (32 mg, 72.14 μmol) in CH_2Cl_2 (2 ml) was added dropwise during 5 min to a stirred solution of ancistrocladine (**5**)[34] (15 mg, 36.85 μmol) and boron trifluoride diethyl etherate (50 μl) in CH_2Cl_2 (5 ml) at 0°C. Stirring was continued for 5 min at room temperature. The solution was poured into water (3 ml), the organic layer separated, and the aqueous phase extracted with CH_2Cl_2 (10 ml). The combined organic phases were washed ($NaHCO_3$) and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the crude product was first chromatographed on silica gel [CH_2Cl_2 / methanol (100:5)] and finally by preparative HPLC on RP_{18} material [H_2O–CF_3COOH buffer (pH=2.5) / MeOH (8:2), isocratic] to yield 15.8 mg (19.45 μmol, 53%) of **6**: mp dec. ≥229°C; $[\alpha]_D^{20}=-14.7$ (c=0.107 in $CHCl_3$); IR (KBr): $\tilde{\nu}$ 3390 (OH), 2940, 2910, 2820 (C–H), 1670, 1660, 1575 (C=C), 1555, 1545 (CH_3), 1235, 1110, 1100 (C=O), 795, 745; ^{1}H NMR (600.13 MHz, $CDCl_3$): δ=0.86 (d, J=6.2 Hz, 3H, 1-CH_3), 1.29 (6H, 3-CH_3 and 3‴-CH_3), 1.62 (d, J=6.2 Hz, 3H, 1‴-CH_3), 1.78 (s, 3H, 2′-CH_3), 1.99 (dd, J_{gem}=16.6 Hz, J_{ax}=12.0 Hz, 1H, 4-H_{ax}), 2.04 (s, 3H, 2″-CH_3), 2.15 (dd, J_{gem}=18.4 Hz, J_{eq}=4.8 Hz, 1H, 4‴-H_{eq}), 2.29 (dd, J_{gem}=18.0 Hz, J_{ax}=11.6 Hz, 1H, 4‴-H_{ax}), 2.93 (1H, 4-H_{eq}), 3.42 (s, 3H, 5″-OCH_3), 3.53 (m_c, 1H, 3‴-H), 3.57 (m_c, 1H, 3-H), 3.59 (s, 3H, 8-OCH_3), 3.89 (s, 3H, 8‴-OCH_3), 3.95 (s, 3H, 4″-OCH_3), 4.03 (s, 3H, 5′-OCH_3), 4.04 (s, 3H, 4′-OCH_3), 4.37 (q, J=6.7 Hz, 1H, 1-H), 4.82 (q, J=6.7 Hz, 1H, 1‴-H), 5.90 (s, 1H, 7-H), 6.54 (s, 1H, 7‴-H), 6.65 (d, J=8.5 Hz, 1H, 8″-H), 6.77 (s, 1H, 3″-H), 6.86 (d, J=8.2 Hz, 1H, 6′-H), 6.90 (s, 1H, 3′-H), 7.07 (d, J=8.1 Hz, 1H, 7′-H), 7.14 (d, J=8.5 Hz, 1H, 7″-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=16.56 (CH_3 at C-1), 18.47 (CH_3 at C-3), 18.51 (CH_3 at C-3‴), 18.60 (CH_3 at C-1‴), 19.68 (CH_3 at C-2″), 20.51 (CH_3 at C-2′), 31.79 (C-4‴), 32.42 (C-4), 44.37 (C-3‴), 45.13 (C-3), 48.10 (C-1‴), 48.20 (C-1), 55.28 (4″-OCH_3), 55.40 (8-OCH_3), 55.52 (8‴-OCH_3), 56.46 (4′-OCH_3), 56.65 (5′-OCH_3), 62.10 (5″-OCH_3), 96.67 (C-7), 97.10 (C-7‴), 104.80 (C-6′), 108.32 (C-3″), 110.09 (C-3′), 112.99 (C-10), 114.35 (C-10‴), 115.75 (C-5‴), 117.62 (C-9′), 118.54 (C-9″), 118.74 (C-8″), 118.94 (C-5), 119.78 (C-1″), 119.95 (C-1′), 127.64 (C-8′), 130.51 (C-7″), 131.24 (C-9‴), 132.09 (C-7′), 132.42 (C-9), 135.16 (C-10′), 135.24 (C-6″), 135.38 (C-10″), 137.94 (C-2″), 140.25 (C-2′), 153.01 (C-6), 153.09 (C-5″), 153.85 (C-6‴), 155.61 (C-8), 155.93 (C-4″), 156.61 (C-8‴), 157.76 (C-4′), 157.95 (C-5′); MS: m/z (%)=812 (2) [M^+], 797 (8) [M^+-CH_3], 392 (36) [$M^{+2}+H-2\cdot CH_3$], 391 (1) [$M^{+2}-2\cdot CH_3$]. Exact mass calcd for $C_{49}H_{53}N_2O_8$ (M^+-CH_3) 797.380. Found: 797.381.

Furthermore, 0.83 mg (1.02 μmol, 3%) of **7** were isolated: mp dec. ≥220°C; $[\alpha]_D^{20}=-31.0$ (c=0.215 in EtOH); IR (KBr): $\tilde{\nu}$ 3423 (OH), 3179 (C–H), 2363, 1679 (C=C), 1402, 1205, 1136 (C=O), 838, 803, 723; ^{1}H NMR (600.13 MHz, $CDCl_3$): δ=1.12 (d, J=6.8 Hz, 3H, 1-CH_3), 1.17 (d, J=6.7 Hz, 3H, 3‴-CH_3), 1.23 (d, J=6.5 Hz, 3H, 3-CH_3), 1.79 (dd, J_{gem}=16.8 Hz, J_{ax}=11.8 Hz, 1H, 4-H_{ax}), 1.85 (s, 3H, 2′-CH_3), 2.12 (s, 3H, 2″-CH_3), 2.16 (dd, J_{gem}=17.2 Hz, J_{ax}=9.8 Hz, 1H, 4‴-H_{ax}), 2.34 (dd, J_{gem}=17.2 Hz, J_{eq}=5.4 Hz, 1H, 4‴-H_{eq}), 2.70 (dd, J_{gem}=16.4 Hz, J_{eq}=4.4 Hz, 1H, 4-H_{eq}), 2.76 (s, 3H, 1‴-CH_3), 3.47 (s, 3H, 8-OCH_3), 3.54 (s, 3H, 5″-OCH_3), 3.55 (m_c, 1H, 3-H), 3.63 (m_c, 1H, 3‴-H), 3.94 (s, 3H, 4″-OCH_3), 3.96 (s, 3H, 5′-OCH_3), 3.98 (s, 3H, 4′-OCH_3), 4.04 (s, 3H, 8‴-OCH_3), 4.31 (q, J=6.8 Hz, 1H, 1-H), 5.78 (s, 1H, 7-H), 6.65 (d, J=8.6 Hz, 1H, 8″-H), 6.71 (s, 1H, 7‴-H), 6.83 (s, 1H, 3″-H), 6.87 (d, J=8.1 Hz, 1H, 6′-H), 6.96 (d, J=8.1 Hz, 1H, 7′-H), 6.98 (s, 1H, 3′-H), 7.02 (d, J=8.6 Hz, 1H, 7″-H); ^{13}C NMR (150.9 MHz, $CDCl_3$): δ=17.33 (CH_3 at C-1), 17.92 (CH_3 at C-3″), 19.19 (CH_3 at C-3), 20.47 (CH_3 at C-2″), 20.79 (CH_3 at C-2′), 24.82 (CH_3 at C-1‴), 32.64 (C-4‴), 33.68 (C-4), 45.48 (C-3), 48.92 (C-3″), 49.72 (C-1), 55.71 (8-OCH_3), 55.75 (4″-OCH_3), 56.68 (8‴-OCH_3), 57.08 (5′-OCH_3), 57.18 (4′-OCH_3), 62.88 (5″-OCH_3), 98.38 (C-7), 99.17 (C-7‴), 105.81 (C-6′), 108.23 (C-10‴ or C-5‴), 108.67 (C-3″), 111.52 (C-3′), 113.13 (C-10), 118.98 (C-9′), 119.67 (C-9″), 120.02 (C-8″), 120.72 (C-5), 121.95 (C-5‴ or C-10‴), 124.28 (C-1″), 125.52 (C-1′), 130.12 (C-8′), 131.71 (C-7″), 132.72 (C-7′), 132.80 (C-9), 136.24 (C-2″), 136.65 (C-6″), 136.69 (C-10″), 137.13 (C-10′), 138.94 (C-2′), 142.43 (C-9‴), 153.62 (C-5″), 155.56 (C-6), 156.39 (C-4″), 156.88 (C-8), 158.27 (C-4′), 158.57 (C-5′), 165.72 (C-8‴); MS: m/z (%)=795 (15) [M^+-CH_3], 390 (32) [$M^{+2}-2\cdot CH_3$]. Exact mass calcd for $C_{49}H_{51}N_2O_8$ (M^+-CH_3) 795.364. Found: 795.362.

Biological experiments

The activity against *P. falciparum* was tested by the semi-automated microdilution assay against intraerythrocytic forms derived from asynchronous stock cultures as previously described[35] with minor modifications.[36] Antimalarial activities were determined using two parasite strains: K1 (Thailand; resistant to chloroquine and pyrimethamine) and NF54 (an airport strain of unknown origin; susceptible to standard antimalarials) of *Plasmodium falciparum*. The activities are given as IC_{50} values (μM). Chloroquine was used as the standard (IC_{50} [K1]=0.125 μM, IC_{50} [NF54]=0.011 μM).

Computational

Conformational analyses

The conformational analyses of the dimeric naphthylisoquinolines were performed on Silicon Graphics OCTANE R10000 workstations by means of the AM1[29] parameterization as implemented in the program package VAMP 6.5,[37] starting from preoptimized geometries generated by the TRIPOS[38] force field using the RandomSearch algorithm.

Molecular dynamics

The MD simulation was carried out on a Silicon Graphics OCTANE R10000 workstation using the MM3[39] force field as implemented in the molecular modelling program package SYBYL.[40] The initial geometry of (*P*)-jozimine B [(P)-**6**] was generated and optimized with the TRIPOS[38] force field. There were no constraints applied to any internal coordinate, but the shake algorithm[41] was used. The temperature was kept constant around 300 K by coupling to an 'external thermal bath'.[42]

CD calculations

The wavefunctions required for the calculation of the rotational strengths for the electronic transitions from the ground state to excited states were obtained by CNDO/S-CI calculations[43] with a CI expansion including 576 singly occupied configurations and the ground state determinant. An increase of the CI level up to 784 occupied configurations showed no significant changes. These calculations were carried out on LinuX PentiumII workstations by the use of the BDZDO/MCDSPD[44] program package. All single CD spectra thus obtained were added up by the Boltzmann statistics according to the heats of formation, to give the calculated overall CD spectrum. For a better visualization, the rotational strengths were transformed into $\Delta\epsilon$ values and superimposed with a Gaussian band shape function.

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