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Jozipeltine A, a Novel, Unnatural Dimer of the Highly Hydroxylated Naphthylisoquinoline Alkaloid Dioncopeltine A[1]

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Abstract—The synthesis of jozipeltine A (**5**), the 6′-coupled constitutionally symmetric dimer of the highly antimalarial naphthylisoquinoline alkaloid dioncopeltine A (**4**), is described. After selective protection of two of the four OH- and NH-functionalities of **4**, the coupling succeeds oxidatively, with Ag_2O as the reagent. Deprotection gives the target molecule **5**, in only three steps from **4**. Jozipeltine A is the first naphthylisoquinoline dimer with oxygen functions in the side chain of the naphthalene part. Investigations on its antiplasmodial and antiviral activities provide valuable insight into structure–activity relationships within this promising class of bioactive quateraryls.

Introduction

Within the rapidly growing class of naphthylisoquinoline alkaloids,[2,3] their natural and unnatural dimeric analogs constitute a remarkable group of bioactive quateraryls. Thus, michellamine B (**1**, see Fig. 1) shows a high anticytopathic activity against HIV-1 and HIV-2.[4] Despite its non-negligible toxicity, it constitutes an interesting novel antiviral lead structure, which has triggered the search for new, hopefully better related quateraryls both by isolation[5,6] and chemical synthesis.[7–19] Besides the—sometimes tedious—total synthetic access, in particular their partial synthesis, preferentially by oxidative 'dimerization'[20] of appropriate monomeric naphthylisoquinoline alkaloids easily isolable from wild-growing or green-house cultivated plants or from cell cultures, is a rewarding alternative. This approach has already permitted the discovery that jozimine C (**2**), prepared by oxidative dimerization of dioncophylline C from *Triphyophyllum peltatum* (Dioncophyllaceae), is the as yet most active unnatural dimer of a naturally occurring monomeric naphthylisoquinoline alkaloid against HIV.[14] Jozimine A (**3**), obtained from natural dioncophylline A (likewise from *T. peltatum*) by Wittig–Klar reaction, exhibits an antimalarial activity significantly higher than that of its monomeric precursor, by a factor of 20,[8] and similar effects were found for jozimine B[18] and pindikamine A.[9] Although this activity enhancement through chemical dimerization has not been observed in each single case (e.g. for none of the anti-HIV active compounds **1**[2] or **2**[14]), even this 'negative' result delivered valuable information on structure–activity relationships. Particularly rewarding seemed a chemical dimerization of the naturally occurring naphthylisoquinoline dioncopeltine A (**4**): as one of the main metabolites of *T. peltatum*, it is easily available both, by isolation[21] and by stereocontrolled total synthesis,[22] and it has, together with dioncophylline C, the as yet highest antimalarial activity of all the numerous natural and unnatural naphthylisoquinoline alkaloids investigated so far, both in vitro[23] and in vivo.[24] Moreover, dioncopeltine A, with its additional benzylic oxygen function in the naphthalene part, possesses the same high number of free hydroxy functions as korupensamines A and B,[25] the molecular halves, e.g. of michellamine B (**1**). Thus, anti-HIV activity might be expected for its dimer **5**—but also an increased chemical instability. In this paper, we report on the first synthesis of jozipeltine A (**5**), by oxidative dimerization[26] of a partially protected derivative of **4**, and on its bioactivity.

Results and Discussion

The expected sensitivity of dioncopeltine A (**4**), due to its—otherwise extremely rare—additional benzylic oxygen function, anticipated the necessity of preparing an appropriately protected derivative of **4** prior to the coupling step. Indeed, treatment of genuine **4** with a variety of otherwise successful[20] oxidants (e.g. Ag_2O, $FeCl_3$, $K_3[Fe(CN)_6]$, and di-*tert*-butyl peroxide) gave complex reaction mixtures, apparently not worth investing tedious isolation work. The easily prepared known[27] *O*,*N*-bis-benzylated derivative **6** (see Scheme 1) of dioncopeltine A (**4**), however, when treated with Ag_2O under conditions previously elaborated

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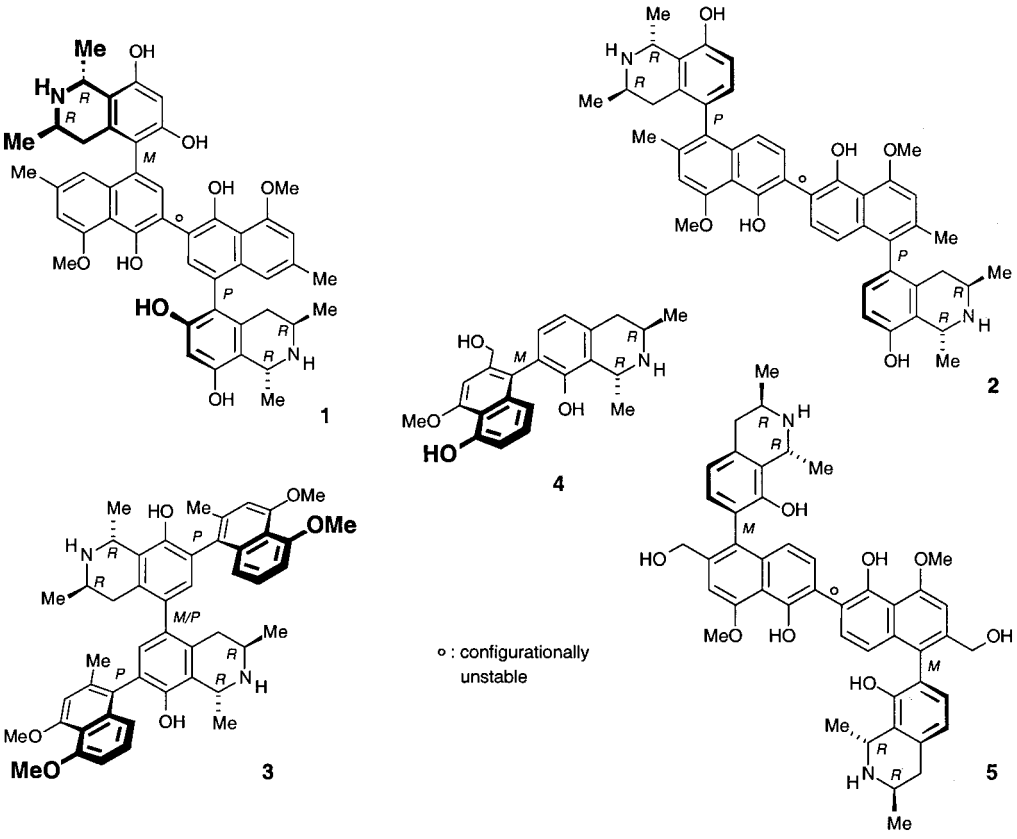
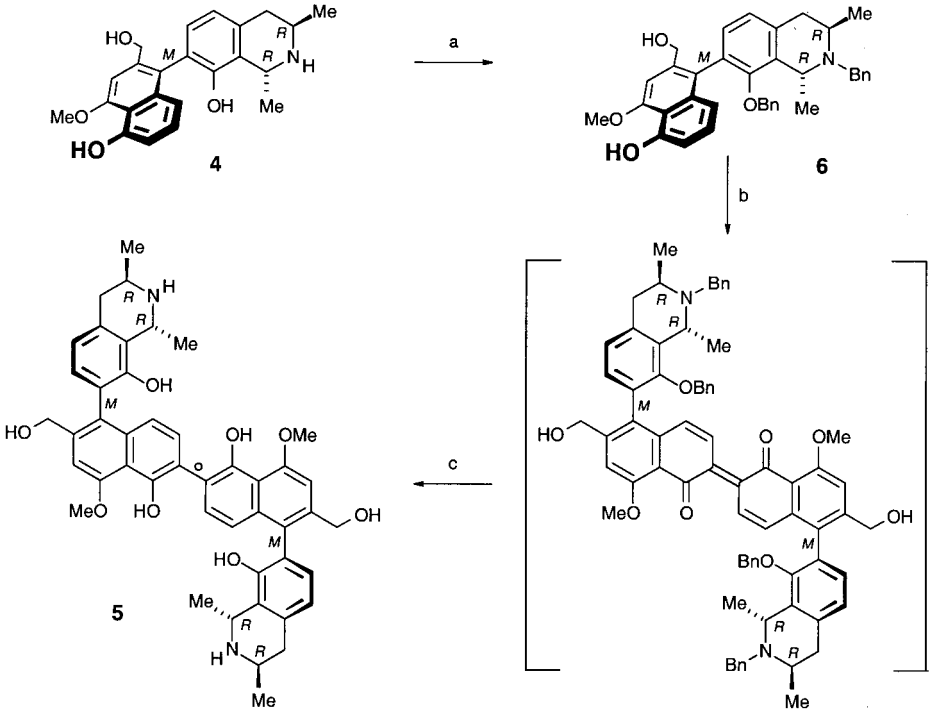


Figure 1. Bioactive dimeric naphthylisoquinolines **1**–**3**, as well as dioncopeltine A (**4**) and its novel dimer, jozipeltine A (**5**).

for the synthesis of **1**[28] and **2**,[14] gave a deeply violet-colored reaction mixture, hinting at a successful dimerization reaction with over-oxidation to the corresponding diquinone.[29] Again, as in similar previous cases,[14,19,20,28] this over-reaction did not provide any inconveniences, since both in the course of a short column purification on silica gel with CH_2Cl_2/methanol or within the—anyhow required—hydrogenolytic debenzylation, the colorless respective



Scheme 1. Synthesis of jozipeltine A (**5**). *Reagents and conditions*: (a) BnBr (2.0 equiv.), K_2CO_3, acetone, reflux, 96%; (b) Ag_2O, 0.2% Et_3N in $CHCl_3$; (c) H_2, EtOH, Pd/C (10%), rt, 53% from **6**.

binaphthyl was obtained. Eventual purification by column chromatography and crystallization from CH_2Cl_2/methanol gave the target molecule **5**.

Besides being plausible from the intermediate over-oxidation to give the characteristic violet-colored diphenoquinone, the successful coupling in the 6′-position for both molecular halves and the resulting constitutionally symmetric structure of **5** were proven by a series of 1D- and 2D-NMR experiments. As for michellamine B (**1**) and jozimine C (**2**), the newly generated axis, which connects the respective 6′-positions of the two molecular halves, does not constitute an additional element of chirality.

The smooth course of the dimerization reaction by which jozipeltine A (**5**) is formed from its monomeric precursor, **4**, suggests that **5** might be a natural product too, possibly occurring in the same plants that likewise produce dioncopeltine A (**4**). A thorough LC–MS analysis of various extracts of *Triphyophyllum peltatum*, from which **4** can be isolated,[21] and the two other known[30] Dioncophyllaceae species, *Habropetalum dawei* and *Dioncophyllum thollonii*, however, revealed **5** not to be present in any of these plant species. This clearly shows that for the formation of **5** from **4**, appropriate enzymes (as indeed existing for other dimers in other plants[31]) are required—or specific reagents, as elaborated in this paper.

Remarkably, although showing some antiplasmodial activity against *Plasmodium falciparum* in vitro (K1=875 ng ml^{-1}, NF54=2530 ng ml^{-1}), jozipeltine A (**5**) is significantly less active than its monomeric precursor, dioncopeltine A (**4**) (K1=4.8 ng ml^{-1}, NF54=3.3 ng ml^{-1}). This gives rise to the assumption that only for compounds like dioncophylline A[8] and ancistrocladine,[18] which contain only one phenolic OH group each, the dimerization and thus doubling of the number of free OH groups increases the antiplasmodial activity. But for dioncophylline C (to give jozimine C, **2**)[14] and korupensamines A and B (leading to michellamine B, **1**),[32] which already contain two or even three free phenolic hydroxy groups per monomeric portion, the formation of dimers, now equipped with four or even six free phenolic OH groups, results in a decrease of antiplasmodial activity.[33] This provides valuable information for the design, synthesis, and biotesting of further, hopefully more active dimers. This work is in progress.

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 are 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 ^{1}H and ^{13}C NMR. Coupling constants, *J*, are reported in Hertz. The mass spectra were obtained on Finnigan MAT 8200 and MAT 90 mass spectrometers at 70 eV in the EI mode. HPLC-ESI-MS/MS analyses were performed with a triple stage quadrupole TSQ 7000 mass spectrometer equipped with an ESI interface (Finnigan MAT, Bremen, Germany), and a Personal DECstation 5000/33 (Digital Equipment, Unterföhring, Germany) with ICIS 8.1 software (Finnigan MAT). Nitrogen served both as sheath and auxiliary gas, argon as collision gas. Chromatographic separations were performed on a Symmetry C_{18} column (150×2.1 mm I.D., 5 μm) (Waters, Eschborn, Germany) with a binary gradient delivered by an Applied Biosystems 140b pump. Solvent A was 0.1‰ (v/v) TFA in water, solvent B was acetonitrile. HPLC was programmed as follows: 0 min 10% B, 25 min 50% B. The flow rate was set to 0.2 ml min^{-1} and the injection volume was 5 μl. Dioncopeltine A (**4**) was available both by isolation from *Triphyophyllum peltatum* (Dioncophyllaceae)[21] and from a stereoselective total synthesis.[22] The dibenzylated derivative **6** was prepared as described previously.[27]

Jozipeltine A (5). A solution of 20.0 mg (35.7 μmol) **6** in 10 ml dry $CHCl_3$ containing 0.2% NEt_3 was treated with 300 mg (1.29 mmol) Ag_2O. After 6 h stirring at room temperature, the solvent was removed in vacuo and the residue purified by a short column chromatography on deactivated (5% NH_3) silica gel, with CH_2Cl_2/methanol (100:1) as the eluent. After evaporation of the solvent in vacuo, the deep-violet colored crude product was hydrogenated in ethanol (3 ml) in the presence of Pd/C 10% (5 mg) for 1 h. After evaporation of the solvent in vacuo, the residue was chromatographed on silica gel, with CH_2Cl_2/methanol (9:1) as the eluent, to yield 9.7 mg (12.8 μmol, 53%) of **5** as a colorless powder from CH_2Cl_2/methanol: mp dec.≥180°C; $[\alpha]_D^{20}$=+59.2 (*c*=0.06 in EtOH); IR (KBr): $\tilde{\nu}$ 3443 (OH), 2921 (C–H), 1683, 1627 (C=C), 1443, 1380, 1208, 1133; ^{1}H NMR (600 MHz, $CDCl_3$): δ=1.47 (d, *J*=6.4 Hz, 3H, 3-CH_3), 1.65 (d, *J*=6.7 Hz, 3H, 1-CH_3), 2.87 (dd, *J*=17.5, 11.6 Hz, 1H, 4-H_{ax}), 3.13 (dd, *J*=17.4, 4.6 Hz, 1H, 4-H_{eq}), 3.77 (m_c, 1H, 3-H), 4.17 (s, 1H, 4′-OCH_3), 4.42 (d, *J*=13.9 Hz, 1H, 2′-CH*H*OH), 4.45 (d, *J*=13.3 Hz, 1H, 2′-C*H*HOH), 4.77 (q, *J*=6.7 Hz, 1H, 1-H), 6.79 (d, *J*=8.7 Hz, 1H, 8′-H), 6.85 (d, *J*=7.8 Hz, 1H, 4-H), 6.95 (d, *J*=7.8 Hz, 1H, 5-H), 7.25 (d, *J*=8.7 Hz, 1H, 7′-H), 7.27 (s, 1H, 3′-H); ^{13}C NMR (150 MHz, $CDCl_3$): δ=17.2 (CH_3 at C-1), 18.6 (CH_3 at C-3), 29.3 (C-4), 33.9 (C-3), 43.2 (C-1), 55.5 (4′-OCH_3), 61.6 (CH_2-OH at C-2′), 103.6 (C-7′), 114.5, 115.9 (C-6), 120.1 (C-3′), 122.9, 124.7, 130.6 (C-8′ and C-5), 132.4, 135.6, 138.4, 151.0 (C-8), 151.2 (C-5′), 156.7 (C-4′); MS: *m/z* (%)=756 (1) [M^+], 741 (5) [M^+-CH_3], 378 (10) [M^{2+}], 363 (30) [$M^{2+}-2CH_3$]. Exact mass calcd. for $C_{45}H_{45}N_2O_8$ (M^+-CH_3) 741.318. Found: 741.318.

Biological experiments

Antiplasmodial activity was determined using the NF54 strain of *P. falciparum* (sensitive to all known drugs) and K1 strain (resistant to chloroquine and pyrimethamine). A modification of the [^{3}H] hypoxanthine incorporation assay[34] was used.[35] 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. The activities are given as IC_{50} values. Chloroquine was used as the standard (IC_{50} [K1]=42 ng ml^{-1}, IC_{50} [NF54]=18 ng ml^{-1}).

Plant material

Root and bark material of *Triphyophyllum peltatum* (Hutch. et Dalz.) Airy Shaw were available from previous collections in the West Ivory Coast in 1990.[21] Fresh leaves of *Triphyophyllum peltatum* were obtained from our plants cultivated[36] in the Botanical Garden Würzburg (Germany) in 1999. Twig material of *Habropetalum dawei* (Hutch. et Dalz.) Airy Shaw was from Sierra Leone (voucher specimen at Herb. Bring. No. 22), and twigs of *Dioncophyllum thollonii* Baill. from Gabon.[37]

Preparation of plant extracts

Five grams of dried plant material (roots, twigs or leaves) were ground and extracted for 2 h at room temperature with 100 ml acetonitrile/water (1:1, v/v) at pH 3 (TFA). The resulting solution was filtered and lyophilized to yield 200 to 500 mg of crude extract; 2 mg of this extract were redissolved in acetonitrile/water (1:9, v/v) and used for analysis without further treatment.

Trace analysis in Dioncophyllaceae plants

Jozipeltine A (**5**) was detected in a reference solution (5 ng/μl in acetonitrile/water 1:9, v/v) in positive mode by HPLC–MS in its doubly protonated form $[M+2H]^{2+}$ at *m/z* 379.4 at a retention time of t_R=15.3 min. However, no trace of **5** could be detected in any of the plant extracts investigated.

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