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Bismurrayaquinone A: Synthesis, Chromatographic Enantiomer Resolution, and Stereoanalysis by Computational and Experimental CD Investigations[1]

Gerhard Bringmann*, Alfons Ledermann, Martin Stahl, and Klaus-Peter Gulden
Institut für Organische Chemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany

Abstract: The first total synthesis of a naturally occurring dimeric carbazole alkaloid, bismurrayaquinone A (**4**), is described, by oxidation of dimeric *O*-demethylmurrayafoline A, a still hypothetic natural product recently synthesized. By chromatography on a chiral phase, the two atropoenantiomers were separated. The absolute configurations of these two rotational isomers were elucidated by comparison of their calculated spectra with those measured experimentally.

INTRODUCTION

Dimeric carbazole alkaloids, *e.g.* bismurrayafoline B (**1**), constitute an interesting young class of biaryl constituents of Southeast Asian *Murraya* species, in which they co-occur together with related monomers like murrayafoline A (**2a**) and murrayaquinone A (**3**).[2] Recently, the first natural dimeric carbazolequinone, named bismurrayaquinone A (**4**), was isolated by Furukawa *et al.*[3] *Murraya* plants, *e.g. M. koenigii* (L.) SPRENG, are intensively used in India, as a flavoring agent in food and as a cure for skin eruptions.[4] Other species of the Rutaceae family, likewise rich sources of carbazole alkaloids, have also been used extensively in folk medicine.[5] Although some carbazole alkaloids are pharmacologically active,[6-8] the observed physiological effects could not yet be correlated to specific carbazole alkaloids.[9] For a thorough investigation of the biological activities of the alkaloids and their structural analogs, their synthetic availablility on a larger scale and their stereochemical investigation is desirable. Until recently, the aspect of atropisomerism for these obviously axially chiral dimeric carbazoles has not been taken into consideration, so that it is not even clear whether the isolated compounds are racemic or stereochemically pure. We have recently developed a first synthetic pathway to dimeric murrayafoline A (**5a**) and its bisphenolic analog **5b**.[10] The natural occurrence of these biscarbazole alkaloids is highly probable due to the presence of the monomer **2a** and its oxidation product **4** in *M. koenigii*.[3] We have shown that these first synthetic dimeric carbazoles, which exhibit moderate antimalarial activity, are chiral and can be resolved into their atropoenantiomeric antipodes.[10] In this paper, we report on the completion of the first total synthesis of bismurrayaquinone A (**4**), by oxidation of its presumable biogenetic precursor

5b. Furthermore, we describe the racemate resolution of **4** on a chiral chromatographic phase and the attribution of the axial configuration of its atropoenantiomers by comparison of their theoretical and experimental CD spectra.

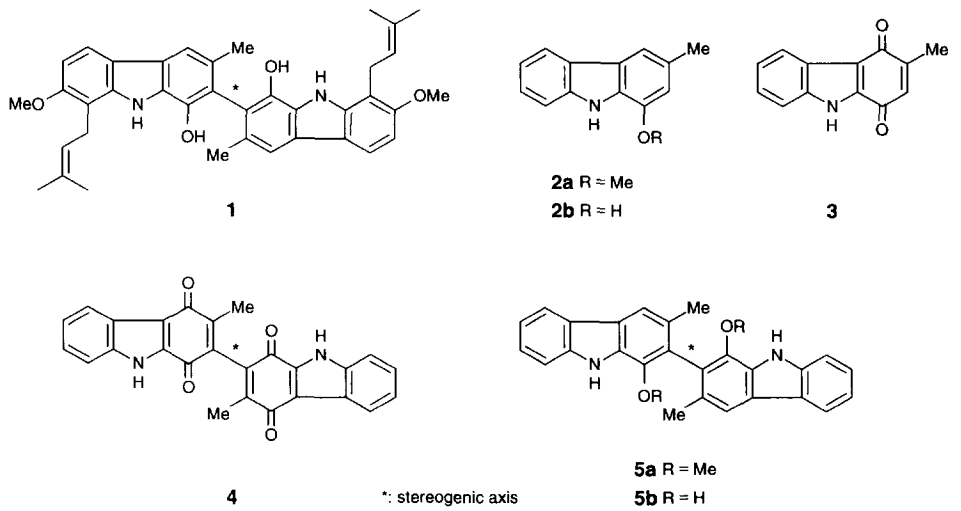
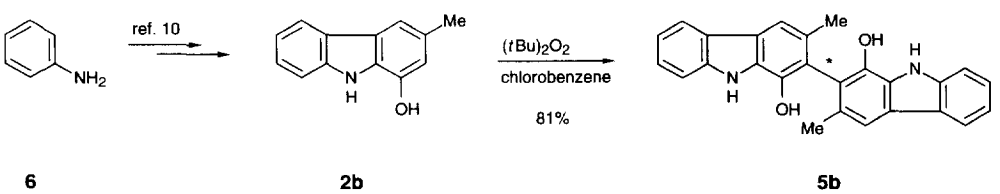


Fig. 1. Mono- and dimeric carbazole alkaloids and related compounds.

RESULTS AND DISCUSSION

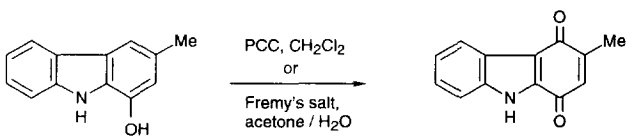
The development of synthetic pathways to dimeric carbazole alkaloids and the investigation of stereochemical aspects of these new natural products seems important for a thorough investigation of their biological activity, also concerning possible enantiomer-specific activities – without possibly difficult procurement from the tropical plant material. A good basis for a first preparation of bismurrayaquinone A (**4**) in the chemical laboratory should be the first total synthesis of **5b**, as recently achieved by the oxidative dimerization of the monomeric carbazole **2b**.[10] By further optimization (see Experimental), the yield of this biomimetic coupling reaction could be further improved to 81% (see Scheme 1).



Scheme 1. Improved preparation of **5b** by biomimetic oxidative coupling of **2b**.

For the scheduled oxidation of **5b** to the targed bisquinone **4**, we first of all optimized this reaction on the monomeric level, *e.g.* for the preparation of murrayaquinone A (**3**) from its presumable precursor

2b. In the literature, this oxidation had already been described with Fremy's salt as an oxidant, albeit in a most unsatisfying yield of 40%.[11] Better results had been obtained when starting from the isomeric 4-hydroxy-3-methylcarbazole.[12] Yet, due to the availability of the dimeric precursor **5b** only in its given substitution pattern, we performed the oxidation reactions with its monomeric analog **2b**, nonetheless. Another possibility for the oxidation of carbazoles is the use of pyridinium chlorochromate (PCC).[13] With this reagent Ramesh and Kapil were able to synthesize murrayaquinone B from murrayafoline B in 32% yield.[13] By a thorough optimization of the reaction conditions, we obtained yields of up to 73% using Fremy's salt as an oxidant and 44% by performing the reaction of **2b** with PCC in dichloromethane.

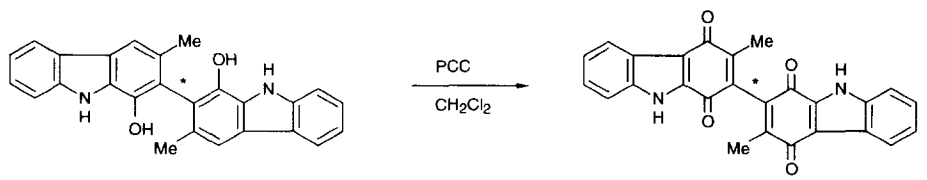


2b **3**

Scheme 2. Improved synthesis of murrayaquinone A (**3**) from its putative phenolic precursor **2b**.

In addition, the latter reaction gave an intensively orange-red colored by-product. By comparison with the data published in the literature,[3] the product was unambiguously identified as bismurrayaquinone A (**4**) - an unexpected first access to this natural product, although in an unacceptable 14% yield.

Consequently, both oxidation reaction conditions were then applied to the authentic *dimeric* substrate **5b**. Purification of the crude product was performed by column filtration over silica gel, whereas separation over florisil led to strongly decreased yields. Thus, whereas Fremy's salt gave only traces of **4**, optimum yields of up to 73% were obtained after oxidation of **5b** with PCC in dichloromethane.



5b **4**

Scheme 3. Completion of the first total synthesis of (still racemic) bismurrayaquinone A (**4**).

With this natural product now for the first time synthetically available, we could investigate its stereochemistry. For its chromatographic enantiomer resolution, analytical conditions similar to those elaborated for the related non-quinoid biscarbazoles **5a** and **5b**[10] proved to be optimal for the bisquinone **4**, again by using a Chiracel OF (Daicel Chem. Ind.) column as the stationary chromatographic phase (Fig. 2).

For the elucidation of the absolute configuration of the bismurrayaquinone A enantiomers *(P)*-**4** and *(M)*-**4**, the investigation of their circular dichroism (CD) proved to be the method of choice.

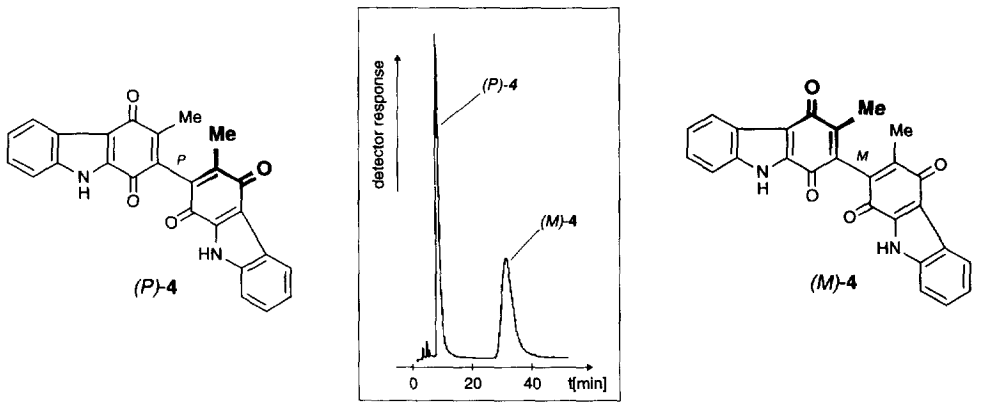


Fig. 2. Chromatographic resolution of a racemic mixture of synthetic bismurrayaquinones A, *(P)*-**4** and *(M)*-**4**; experimental conditions: adsorbant, Chiracel OF (Daicel Chem. Ind.); solvent, *n*-hexane / 2-propanol (65 : 35); for stereochemical attribution, see below.

The assumption that the two peaks in the chromatograms indeed represent enantiomeric substances, is confirmed by their opposite CD spectra, as illustrated in Fig. 3 (full lines). Although the structure of **4** with its two identical chromophores might be considered as ideal for an application of the exciton chirality method,[14] the assignment of "the *first Cotton effect*" in the experimental CD spectrum to one particular peak is not possible. Moreover, both the positions and the directions of the interacting degenerated transitions are not obvious either. Consequently, for the unambiguous stereochemical attribution of the two atropoenantiomers, we have applied a procedure optimized earlier in our group, *i.e.* the computational prediction of the CD spectra for the two enantiomers and comparison with the experimental ones (which, in this case, were even measured *after* the calculations had been performed). Different from theoretical CD investigations in the literature,[14,15] we calculate the CD spectra of a whole series of low-energy conformational species that all have to be taken into account at the given temperature. These CD spectra (which may vary drastically) then have to be averaged following a Boltzmann protocol, according to the calculated energies (AM1).

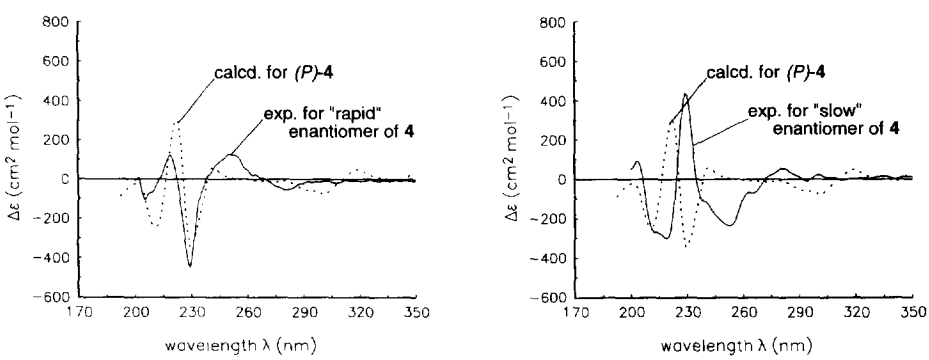


Fig. 3. Stereochemical attribution of the two bismurrayaquinone A enantiomers by comparison of experimental (in EtOH) and theoretical (CNDO/2S ⟹ Boltzmann averaging) CD spectra.

In the present case, the CD calculations were performed for no less than 49 conformations, finally giving rise to the predicted overall CD spectrum. As seen in Fig. 3, the predicted CD spectrum, *e.g.* for *(P)*-**4**, clearly correlates with the experimental one of the more rapidly eluting bismurrayaquinone A enantiomer and exhibits a near-opposite CD behavior to that of the less rapidly eluting *M*-enantiomer. And *vice versa*, for the *M*-enantiomer, the predicted CD spectrum (not shown) analogously matches with the less rapidly eluting enantiomer. This clearly allows to attribute the *P*-configuration to the compound that elutes at 8.6 min, whereas the peak at 32.0 min is found to be the *M*-enantiomer.

The first synthetic access to bismurrayaquinone A (**4**) and its stereoanalysis, as described in this paper, open up the possibility of now investigating the as yet completely unknown stereochemical and pharmacological properties of this chemical constituent of *M. koenigii* in detail. This work is in progress.

EXPERIMENTAL AND COMPUTATIONAL PART

Experimental. The melting points were determined with a Kofler hot plate apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 infrared spectrophotometer. The ^{1}H NMR spectra were recorded on a Bruker AC 200 (200 MHz) using CD_3OD (δ = 3.33 ppm) or d_6-acetone (δ = 2.05 ppm) as internal reference. Mass spectra were recorded on a Finnigan MAT 8200 spectrometer. The following abbreviations are used: d = doublet, m_c = central multiplet, s = singlet, t = triplet. HPLC analyses were carried out with a Knauer-364 pump, a Chiracel OF column (Daicel Chem. Ind. Ltd., 25 x 0.46 cm) and an ERC-7215 UV detector. CD and UV spectra were recorded in ethanolic solution on a Jobin Yvon Model CD6 spectrograph at room temperature within the range of 200 – 350 nm.

*2,2'-Bis(1-hydroxy-3-methyl-9H-carbazole) (***5b***) (Improved protocol of a procedure described earlier*[10]*)*: A solution of 500 mg (2.54 mmol) **2b** in chlorobenzene (80 ml) and 371 mg (464 μl, 2.54 mmol) di-*tert*-butyl peroxide was heated to reflux. After 16 h, a second portion of 160 mg (200 μl, 1.10 mmol) of di-*tert*-butyl peroxide was added and the solution was refluxed for another 6 h in order to complete the dimerization. Purification of the crude product as described earlier,[10] yielded 401 mg (1.02 mmol, 81%) **5b**.

*Murrayaquinone A (***3***)*: Method A: A solution of 30 mg (0.15 mmol) 1-hydroxy-3-methyl-9*H*-carbazole in 7 ml of acetone was added dropwise to a well-stirred solution of 113 mg (0.42 mmol) Fremy's salt and 8 mg (0.06 mmol) KH_2PO_4 in 7 ml of H_2O, upon which the violet solution of Fremy's salt turned red-brown. After stirring for additional 30 min, the acetone was evaporated *in vacuo*. The resulting brown precipitate was filtered and washed with water. Purification by chromatography on silica gel with CH_2Cl_2 as an eluent yielded 24 mg (73%) of **3**; mp 249°C (lit.[3]: 246–247°C); IR (KBr): ν 3190 (br), 1650, 1620, 1590; ^{1}H NMR: δ = 2.18 (d, 3 H, J = 1.7 Hz), 6.51 (q, 1 H), 7.31-7.78 (m, 3 H), 8.25 (d, 1 H, J = 8.4 Hz), 9.16 (s, 1 H); all other spectroscopic data are in agreement with those reported in the literature.[11]

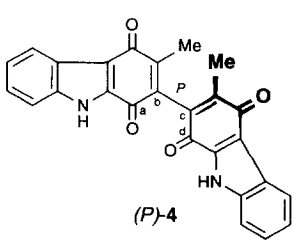
Method B: To a well-stirred solution of 216 mg (1.00 mmol) PCC in 30 ml of dry CH_2Cl_2 a solution of 50 mg (0.25 mmol) 1-hydroxy-3-methyl-9*H*-carbazole (**2b**) in 10 ml of dry CH_2Cl_2 was added. After 0.5 min, 100 ml of dry ether was added. The resulting black gum was filtered and washed two times with

20 ml of ether. The solvent was evaporated *in vacuo* and purification as mentioned above yielded 23 mg (44%) of **3** as an orange-colored powder; for spectral and physical data, see above. Furthermore, 7 mg (14%) of bismurrayaquinone A (**4**) were obtained; for spectroscopical and physical data, see below.

*Bismurrayaquinone A (**4**)*: To a well-stirred solution of 178 mg (0.815 mmol) PCC in 25 ml of dry CH_2Cl_2, a solution of 64 mg (0.163 mmol) 2,2′-bis(1-hydroxy-3-methyl-9*H*-carbazole) (**5b**)[10] in 5 ml of dry CH_2Cl_2 was added. After 0.5 min, 150 ml dry ether was added and the supernatant liquid was decanted from the black gum, which was washed two times with 20 ml of ether. The combined organic layers were passed through a short column of silica gel and removal of the solvent yielded 50 mg (73%) of **4** as an orange-colored powder; decomp. >290°C; CD (EtOH): $\Delta\varepsilon_{205}$ -106.8, $\Delta\varepsilon_{219}$ +123.6, $\Delta\varepsilon_{229}$ -448.4, $\Delta\varepsilon_{250}$ +124.7, $\Delta\varepsilon_{281}$ -56.3; IR (KBr): ν 3290 (br), 1645 (s), 1625 (s); ^{1}H NMR (200 MHz, d_6-acetone): δ = 7.42 (m_c, 4 H), 7.67 (d, 2 H, J = 7.4 Hz), 8.22 (d, 2 H, J = 7.8 Hz), 11.82 (s, 2 H); the signal of the methyl groups at C-3 and C-3′ is overlapped by the solvent signal; in CD_3OD, it appears at δ = 2.06 (s, 6 H); MS (70eV): m/z (%) = 420 (M^+, 100), 406 (28), 405 (100), 392 (8), 391 (24), 377 (12), 375 (8), 288 (8); HMRS Calcd for $C_{26}H_{16}N_2O_4$: 420.111. Found: 420.111.

*Chromatographic resolution of racemic bismurrayaquinone A (rac-**4**)*: HPLC analyses were carried out on a Chiracel OF column (Daicel Chem. Ind. Ltd., 25 x 0.46 cm) at 30°C; eluent: *n*-hexane / 2-propanol (65 : 35); flow rate: 1.0 ml/min; detection at 260 nm. The obtained R_f values are 8.58 min for *(P)*-**4** and 31.95 min for *(M)*-**4**.

Computational. As a first step, the absolute energetic minimum conformation of bismurrayaquinone A (**4**) was searched by an AM1 calculation,[16] starting from a structure preoptimized by the TRIPOS force field.[17] An important structural parameter of this minimum structure is the dihedral angle ϑ(a,b,c,d) at the biaryl axis. That angle between the two molecular moieties was determined as ϑ = 98.7°.



(P)-**4**

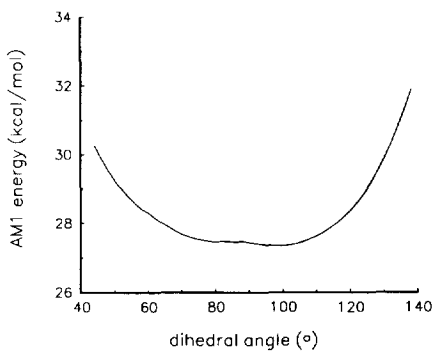


Fig. 4. Definition of the dihedral angle ϑ(a,b,c,d) at the axis of **4** and the energetic profile of the rotation of the two molecular halves around this axis as calculated for *(P)*-**4**.

From this point of lowest energy, two reaction paths were followed using VAMP 5.0:[18] The dihedral angle ϑ as the only fixed parameter was successively varied by steps of 2° in both directions, thus resulting in an energetic profile of the rotation around the biaryl axis, as shown in Fig. 4. For each of

the 49 particular conformations evaluated by this procedure, a theoretical CD spectrum was calculated using the origin-indepent formalism of the rotational strength:

$$R_{0\to a} = \Im\{\frac{e\hbar}{2\pi m\nu_a} < \psi_0|\nabla|\psi_a > \cdot < \psi_a|m|\psi_0 >\}.$$

The wavefunctions of the excited states were obtained by a CNDO/2S–CI calculation,[19] in which the CI expansion consists of 576 singly occupied configurations and the ground state determinant. In order to simulate the distribution of the ensemble of conformations over the conformational space at room temperature, all these single CD spectra were added up to the theoretical overall spectrum by the means of the Boltzmann statistic.

For a better optical comparison of the experimental spectra with the computational ones, we generated Gaussian band shape functions over the calculated rotational strength values according to the formula:

$$\sigma_{0\to a}(\lambda) = \frac{1}{\Delta m\sqrt{\pi}} \exp[-(\frac{\lambda - \lambda_a}{\Delta m})^2],$$

where Δm denotes the halfband width (5 nm).

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Dedicated to *Prof. P. von Ragué-Schleyer*, on the occasion of his 65th birthday.

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