

**DIMERIC MURRAYAFOLINE A, A POTENTIAL BIS-CARBAZOLE
ALKALOID: 'BIOMIMETIC' SYNTHESIS, ATROPOISOMER
SEPARATION, AND ANTIMALARIAL ACTIVITY¹**

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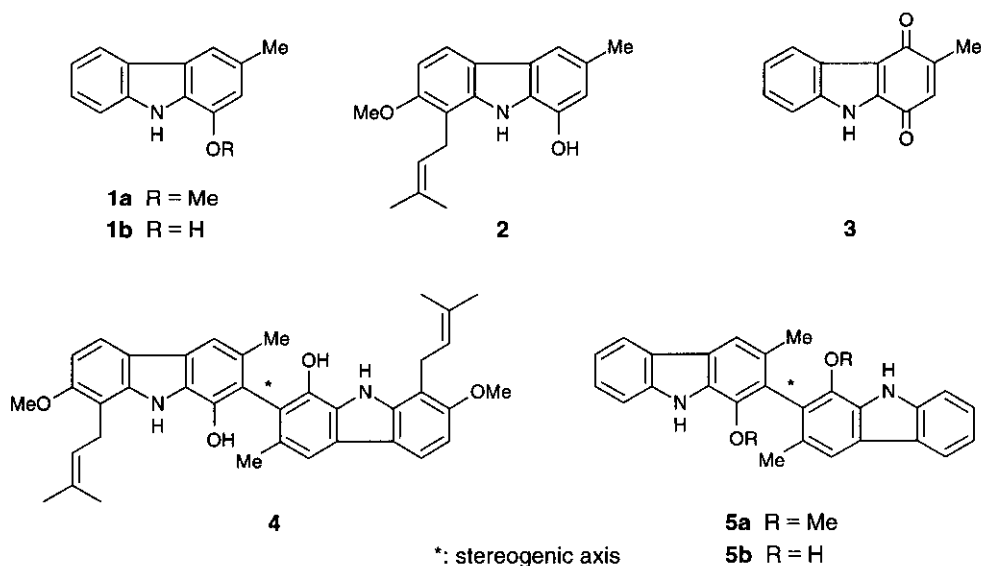
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Dedicated to Professor Rolf Huisgen, on the occasion of his 75th birthday

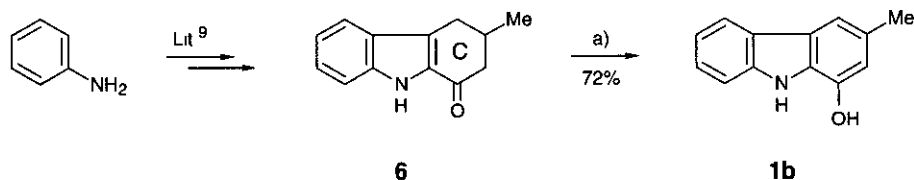
Abstract - The first total synthesis of a (potential) dimeric carbazole alkaloid is described. After an improved preparation of the monomeric building block, 'biomimetic dimerization' by oxidative phenolic coupling was best achieved with di-*tert*-butyl peroxide in chlorobenzene, leading to a highly selective formation of the 2,2'-coupled 'dimer', the parent framework of several naturally occurring dimeric carbazole alkaloids. For the atropo-enantiomer analysis, a chromatographic procedure on a chiral phase was developed. Both the mono- and the dimeric phenolic carbazoles displayed *in vitro* antimalarial activity against *Plasmodium falciparum*.

The genus *Murraya* (Rutaceae) consists of nine tropical plant species,^{2,3} many of which are used in folk medicine. Thus, *M. euchrestifolia*, which is endemic to Taiwan, is used as an analgetic for local anaesthesia, as well as for the treatment of eczema, rheumatism and dropsy.⁴ A phytochemical investigation of this species revealed the presence of a series of carbazole alkaloids, among them murrayafolines A (**1a**) and B (**2**).⁵ Moreover, related alkaloids like murrayaquinone A (**3**) and bis-murrayafoline B (**4**), apparently arising from phenolic precursors **1b** and **2**, by oxidative oxygenation resp. 'dimerization', were isolated from the same plant.^{5,6} Due to restricted rotation around the central biaryl axis, **4** should display the phenomenon of atropoisomerism. Regrettably, no attention has been paid to this stereochemical aspect during the structure elucidation work, so that an optical rotation for this natural biaryl was not measured.⁶ Nothing is known about the biological activity of such biscarb-

azole alkaloids as yet, either. This would, however, be most rewarding, as dimeric alkaloids may exhibit high biological activities completely different from the corresponding monomers.^{7,8} Thus, not only for an ultimate stereochemical attribution of biscarbazole alkaloids, but also for a pharmacological investigation of the natural products and their structural analogs, the elaboration of a first synthetic access to these biheteroaryls seemed rewarding. In this paper, we describe the preparation of the biscarbazoles (**5a**) ('dimeric murrayafoline A') and (**5b**), by a highly regioselective oxidative coupling of synthetic *O*-demethylmurrayafoline A (**1b**).

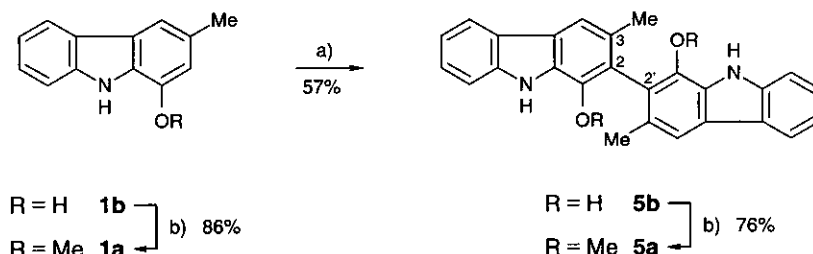


In the literature, a synthetic access to the 'monomer' **1b** had already been described, yet with low yields in the final aromatization of the C ring of carbazalone (**6**).⁹ By optimization of this critical dehydrogenation step (Scheme 1), we could improve the yield of **1b** from 45 %⁹ up to 72 %.



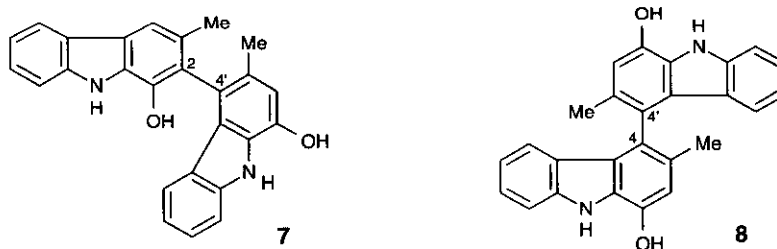
Scheme 1: Improved synthesis of the monomeric carbazole (**1b**). Reagents and conditions: a) 10% Pd/C, Ph₂O, 1,2,4-trimethylbenzene¹⁰, 220 - 230 °C, 4 h.

For the synthesis of the desired 2,2'-linked dimeric carbazoles (**5**) by oxidative coupling of the phenolic monomer (**1b**), problems may be expected from a possible formation of the corresponding regioisomeric 2,4'- or 4,4'-coupled products (**7**) or (**8**). Treatment of **1b** with di-*tert*-butyl peroxide as an oxidant gave essentially a single coupled product straightaway.



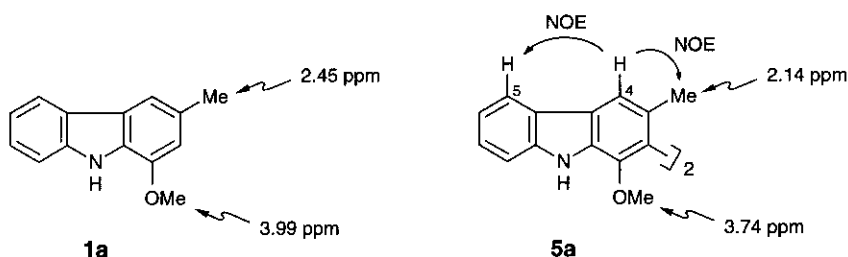
Scheme 2: The dimerization to biscarbazole derivatives. Reagents and conditions: a) $(t\text{-BuO})_2$, chlorobenzene, reflux, 3 h; b) CH_2N_2 / Et_2O , MeOH, 5 °C, 12 h (for **1b**), resp. 24 h (for **5b**).

The successful dimerization could be seen by the parent ion at m/z 392 in the mass spectrum and by the disappearance of the signal of H-2 ($\delta = 6.67$ ppm) in the ^1H nmr spectrum, as well as the high-field shift of the resonance of the methyl group at C-3 (2.14 instead of 2.45 ppm for **1b**). From this methyl signal, however, no conclusion could be drawn with respect to the coupling *site*, since also in the imaginable alternative products (**7**) and (**8**), the methyl groups would be adjacent to the biaryl axis and should thus be prone to the anisotropic influence of the aryl substituent.



From ^1H and ^{13}C nmr spectroscopy, the obtained dimeric product was found to be constitutionally symmetric, giving only one set of signals for both halves together, thus excluding the 'mixed' 2,4'-coupled product (**7**). After transformation of the bisphenolic coupling product to its dimethyl ether, the coupling site could clearly be shown to be the 2-position for both halves by the distinct upfield shift of the *O*-methyl resonance ($\delta = 3.74$ ppm), compared with the normal position of the signal for the monomeric analog **1a** ($\delta = 3.99$ ppm). This clearly hints at a direct proximity of the biaryl axis to the

oxygen functions as only in **5**, whereas for the *O*-methylation product of **8**, again the usual *O*-methyl resonance would have been expected. The 2,2'-coupling site is furthermore underlined by the presence of H-4, as evident by its NOE interaction with both H-5 and Me-3.



Besides the constitution of the dimeric products (**5**), also their stereochemistry with respect to the phenomenon of axial chirality is interesting, giving rise to (*P*)- and (*M*)-atropo-enantiomers. Therefore we have developed a stereoanalysis on a chiral chromatographic phase for these potential natural products. The best results were obtained on a Chiralcel OF (Daicel Chem.) column, using *n*-hexane / 2-propanol as a solvent mixture (see Figure 1).

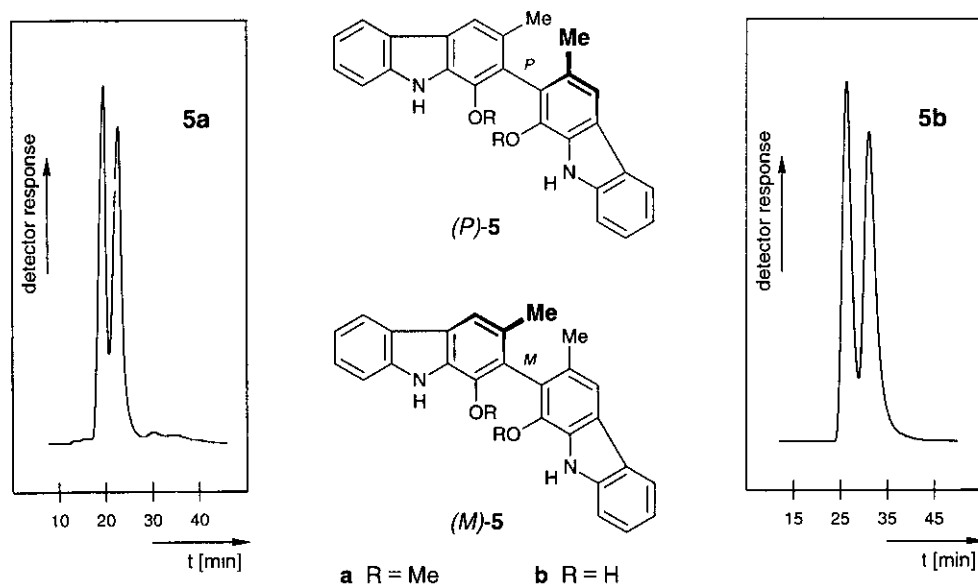


Figure 1: Chromatographic resolution of the racemic biscarbazoles (**5a**) (left) and (**5b**) (right); experimental conditions: adsorbant, Chiralcel OF (Daicel Chem.); solvent, *n*-hexane / 2-propanol (95 : 5) (for **5a**), resp. *n*-hexane / 2-propanol / formic acid (95 : 5 : 0.03) (for **5b**); flow rate, 1.0 ml/min; detection, 254.0 nm; temperature, 30 °C.

The highly selective formation of the 2,2'-coupling type (**5**) is remarkable, and can be seen only in partial agreement with related results for 3,5-dimethylphenol,¹¹ which, at higher temperatures, predominantly reacted to an *o,o'*-coupled product, whereas at lower temperature, mainly the *o,p'*-coupled product originated. By contrast, in the oxidative 'dimerization' of **1b**, no evidence for the formation of other regioisomeric coupling products like **7** or **8** of related polarity could be found, not even when decreasing the reaction temperature to 50 °C. The exclusive formation of the naturally occurring coupling type of **5** suggests that in the biogenesis of biscarbazole alkaloids, a similar reaction mechanism should be involved as in our (probably biomimetic) synthesis. It even seems that the formation of such dimers can occur spontaneously on exposure to air (and light), since we could observe that in tlc samples of **1b** dissolved in CH₂Cl₂ gradually the 'dimer' (**5b**) is formed, so that the question arises whether bismurrayafoline B is a genuine natural product or might just be formed during the isolation from *M. euchrestifolia*. Here again, an investigation of the possible enantiomeric purity of natural **4** will be most conclusive.

It is interesting to add that in our test system established earlier,¹² the phenolic compounds (**1b**) and (**5b**) showed clear antimalarial activities against asexual *Plasmodium falciparum* erythrocytic forms *in vitro*, with IC₅₀ values of 7.6 µg/ml and 10.8 µg/ml respectively, *i.e.* regardless of the mono- or dimeric nature of the substance. The *O*-methylated counterparts murrayafoline A (**1a**) and dimeric murrayafoline A (**5a**) however, were totally inactive in the same test system, with IC₅₀ values which exceeded 50 µg/ml. A detailed report of the antimalarial activities of a series of carbazoles will be published elsewhere.¹³

In contrast to the monomer (**1b**) and to the oxygenated and prenylated dimer bismurrayafoline B (**4**), the dimeric murrayafoline A (**5a**) and the corresponding bisphenolic compound (**5b**) have not been detected in nature, as yet. Very recently, however, a 2,2'-coupled dimer of murrayaquinone A (**3**) has been isolated from *M. koenigii*,¹⁴ making a natural occurrence of **5a/b** still more probable. Investigations to look for these potential natural biscarbazoles in *Murraya* species, as well as the application of the synthetic technique described here, to the preparation also of **4** and other related alkaloids, are in progress.

EXPERIMENTAL

General. Melting points were determined with a Kofler hot plate apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1420 infrared spectrophotometer. Mass spectra were

measured at 70 eV on a Finnigan MAT 8200 or on a Varian MAT CH7 mass spectrometer. ^1H and ^{13}C nmr spectra were recorded on a Bruker AC 250 (250 MHz) or on a Bruker AC 200 (200 MHz) spectrometer using CD_3OD ($\delta = 3.33$ ppm) and CDCl_3 ($\delta = 7.26$ ppm) as internal reference. The following abbreviations are used: d = doublet, dd = double doublet, m_c = central multiplet, s = singlet. Hplc analyses were carried out with a Knauer-364 pump, a Chiralcel OF column (Daicel Chem. Ind. Ltd., 25 x 0.46 cm) and an ERC-7215 UV detector.

1-Hydroxy-3-methyl-9H-carbazole (1b)

To a well dried mixture of **6** (1.00 g, 5.02 mmol) and 10% Pd/C (600 mg), prepared according to Linstead and Thomas,¹⁵ dry diphenyl ether (40 ml) and dry 1,2,4-trimethylbenzene (5 ml) were added under Ar. The stirred solution was degassed *in vacuo* with ultrasonic assistance and then heated to reflux at 220 - 230 °C for 20 h. After cooling to room temperature, the mixture was liberated from the non-polar solvent by filtration over SiO_2 with petroleum ether as an eluent. Further elution with CH_2Cl_2 / formic acid (99.9 : 0.1) and recrystallization from toluene / petroleum ether gave 721 mg (73%) of **1b** as nearly colorless needles: mp 156 °C (lit.,⁹ mp 158 °C). The spectroscopic data are in agreement with those reported in the literature.⁹

2,2'-Bis(1-hydroxy-3-methyl-9H-carbazole) (5b)

A solution of **1b** (200 mg, 1.01 mmol) in chlorobenzene (12 ml) and di-*tert*-butyl peroxide (148 mg, 1.01 mmol) was heated to reflux for 3 h. After cooling the solvent was evaporated *in vacuo* and the remaining solid was purified by chromatography on silica gel with CH_2Cl_2 / CHCl_3 / Et_2O (50 : 1 : 2) as an eluent. Recrystallization from toluene / petroleum ether (2 : 1) yielded 114 mg (57%) of **5b**: decomp. >250 °C; ir (KBr) 3480, 3390, 3020, 2800, 1545, 1480, 1440, 1375, 1330, 1240, 1220, 1090 cm^{-1} ; ^1H nmr (CD_3OD , 250 MHz) δ 8.04 (2H, d, $J = 7.8$ Hz, 5-H, 5'-H), 7.63 (2H, s, 4-H, 4'-H), 7.50 (2H, d, $J = 8.1$ Hz, 8-H), 7.36 (2H, dd, $J = 8.1$ Hz, $J = 7.1$ Hz, 7-H, 7'-H), 7.16 (2H, dd, $J = 7.8$, $J = 7.1$ Hz, 6-H, 6'-H), 5.46 (2H, s, Ar-OH), 2.14 (6H, s, Ar- CH_3); ^{13}C nmr (CD_3OD , 200 MHz) δ 141.71, 141.65, 129.98, 129.92, 126.22, 125.37, 124.70, 120.97, 120.18, 119.52, 113.51, 112.03, 20.44; ms m/z (rel. int.) 392 (M^+ , 100), 377 ($\text{M}-\text{CH}_3$, 10), 376 (10), 360 (10), 359 (21), 197 (45), 196 ($\text{M}-\text{C}_{13}\text{H}_{10}\text{NO}$, 58), 181 ($\text{M}-\text{C}_{14}\text{H}_{13}\text{NO}$, 7), 180 (11); HRms m/z Calcd (M^+) 392.152, obsd 392.153.

Dimeric Murrayafoline A (2,2'-Bis(1-methoxy-3-methyl-9H-carbazole)) (5a)

A solution of **5b** (6.00 mg, 0.015 mmol) in methanol (1 ml) was treated with an excess of diazomethane in Et_2O at 5 °C for 24 h. After evaporation of the solvent *in vacuo*, the remaining solid was purified by chromatography over silica gel with CH_2Cl_2 / petroleum ether (1 : 1) as an eluent. Recrystallization from CH_2Cl_2 / petroleum ether (1 : 2) yielded 4.90 mg (76%) of **5a**: mp 272 °C; ir (CH_2Cl_2)

3440, 3125, 2940, 1600, 1095, 1010, 900 cm^{-1} ; ^1H nmr (CD_3OD) δ 8.07 (2H, d, $J = 7.8$ Hz, 5-H, 5'-H), 7.81 (2H, s, 4-H, 4'-H), 7.51 (2H, d, $J = 8.0$ Hz, 8-H, 8'-H), 7.39 (2H, m_c, 7-H, 7'-H), 7.17 (2H, m_c, 6-H, 6'-H), 3.74 (6H, s, Ar-OCH₃), 2.19 (6H, s, Ar-CH₃); ^{13}C nmr (CDCl_3 , 250 MHz) δ 141.67, 138.71, 130.54, 128.14, 126.31, 124.81, 123.59, 122.70, 119.32, 118.43, 115.53, 109.88, 58.91, 19.22; ms m/z (rel. int.) 420 (M^+ , 100), 405 ($\text{M}-\text{CH}_3$, 4), 390 ($\text{M}-\text{C}_2\text{H}_6$, 7), 389 ($\text{M}-\text{OCH}_3$, 6), 375 ($\text{M}-\text{C}_3\text{H}_9$, 22), 374 ($\text{M}-\text{C}_2\text{H}_6\text{O}$, 4), 373 (21), 359 ($\text{M}-\text{C}_3\text{H}_9\text{O}$, 7), 210 ($\text{M}-\text{C}_{14}\text{H}_{12}\text{NO}$, 10), 195 ($\text{M}-\text{C}_{15}\text{H}_{15}\text{NO}$, 7); HRms m/z Calcd (M^+) 420.184, obsd 420.185.

Murrayafoline A (1-Methoxy-3-methyl-9*H*-carbazole) (1a)

Under the same conditions *O*-methylation of **1b** (10.8 mg, 0.055 mmol) gave 10 mg (86%) of **1a** as a colorless oil. The spectroscopic data are in agreement with those reported in the literature.⁹

Chromatographic resolution of the racemic biscarbazoles 5a and 5b

Hplc analyses were carried out on a chiracel OF column (25 x 0.46 cm, Daicel Chem. Ind. Ltd.) at 30 °C; eluent: *n*-hexane / 2-propanol (95 : 5) (for **5a**), resp. *n*-hexane / 2-propanol / formic acid (95 : 5 : 0.03) (for **5b**); flow rate 1.0 ml/min; detection at 254.0 nm. The obtained R_f values are 19.55 min resp. 22.70 min for racemic **5a** and 26.25 min resp. 31.05 min for racemic **5b**.

Antimalarial Assays

Compounds (**1a**, **1b**, **5a**, and **5b**) were tested against asexual *Plasmodium falciparum* erythrocytic forms (strain NF 54) *in vitro*, by measuring the incorporation of ^3H -hypoxanthine in the presence of the test substance after 42 h of incubation at 37 °C, as recently described in detail for other alkaloids.¹² A complete description of the whole test series, including structurally related carbazoles, will be described elsewhere.¹³

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