Biosynthesis and Stereochemistry of Phlegmacin-Type Fungal Pigments

Michael Müller,[a] Kai Lamottke,[a] Wolfgang Steglich,*[a] Stefan Busemann,[b] Matthias Reichert,[b] Gerhard Bringmann,*[b] and Peter Spiteller[c]

Dedicated to Professor Heinz G. Floss on the occasion of his 70th birthday

Keywords: Biosynthesis / Biaryls / Fungal pre-anthraquinones / Oxidative phenolic coupling / Quantum chemical CD calculations

The absolute stereostructures of phlegmacins A_1 (**3a**) and B_1 (**3b**) were determined by biosynthetic studies, quantum chemical CD calculations, and NOE experiments. In *Cortinarius odorifer* these compounds are formed by regioselective oxidative dimerization of (*R*)-torosachrysone (*R*-**2**).

Introduction

Dimeric pre-anthraquinones, dihydroanthracen-1(2*H*)-ones, occur in several *Cortinarius*, *Dermocybe*, and *Tricholoma* species and are responsible for the spectacular colors of these toadstools.[1−3] According to the position of the C−C bond formed during oxidative coupling of the putative precursors atrochrysone (**1**) or torosachrysone (**2**), the dimers can be classified as belonging to the phlegmacin (**3**), atrovirin (**4**), and flavomannin (**5**) groups (Scheme 1).[2,4] Usually, only one type of regioisomers is observed for a certain species, whereas the configurations at the chiral axis and at the stereogenic centers are variable.

Phlegmacins A_1 (**3a**) and B_1 (**3b**) were first discovered in *Cortinarius* (*Phlegmacium*) *odorifer* Britz. (German: "Anis-Klumpfuß"), a common toadstool of spruce forests in mountainous regions.[5] Enantiomers of these fungal phlegmacins occur in the medicinal plant *Cassia torosa* (Fabaceae).[6] Closely related are the toxic peroxisomicines, 7,10′-coupled dimers of prechrysophanol,[7] which have been isolated from fruits and roots of *Karwinskia* species (Rhamnaceae).[8]

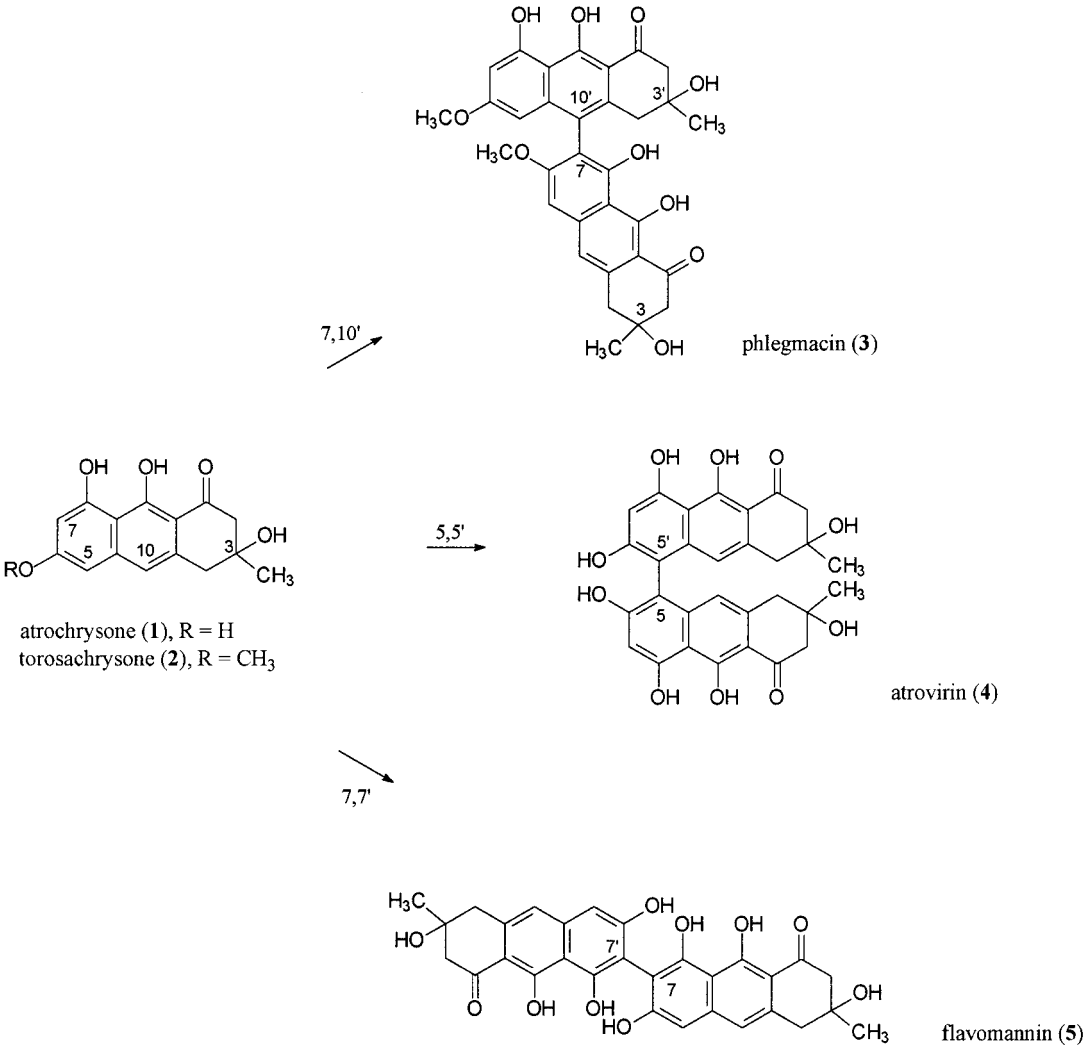
The polyketide-origin of the phlegmacins was demonstrated by Gill and co-workers[9] by feeding [2-^{13}C]acetate to fruit bodies of the Australian *Cortinarius sinapicolor*. The incorporation of two molecules of (*S*)-[*methoxy*-^{13}C]torosachrysone 8-*O*-β-D-gentibioside into the anhydrophlegmacin-9,10-quinone derivative present in this toadstool, established the formation of both halves of the molecules from torosachrysone as well as the (3′*S*) configuration of the dimer.

In order to study the formation of phlegmacins A_1 and B_1, we synthesized several monomeric pre-anthraquinones and applied each of them to young fruit bodies of *C. odorifer* in their natural environment. After 6 days, the toadstools were harvested and the pigments investigated. The model compounds **6**−**8** used in this study were obtained by tandem Michael−Dieckmann condensations according to Weinreb and co-workers,[10,11] whereas the syntheses of (*R*)- and (*S*)-[*methoxy*-^{13}C]torosachrysone ((*R*)- and (*S*)-**2**) have been described earlier.[12]

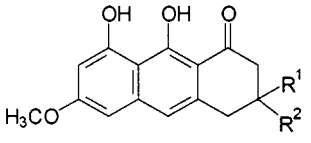
From the two enantiomers of [*methoxy*-^{13}C]torosachrysone *only* the (*R*)-form was incorporated into phlegmacins A_1 (**3a**) and B_1 (**3b**) in *C. odorifer*. Each of the stereoisomers, **3a** and **3b**, exhibited a ^{13}C-enrichment of about 3.5% for each methoxy group.[11] By contrast, the mixture of phlegmacins A_1 and B_1 obtained after feeding (*S*)-[*methoxy*-^{13}C]torosachrysone showed no enhancement of the methoxy signals in the ^{13}C NMR spectrum.[13] This indicates that both, phlegmacins A_1 and B_1, are (*R*)-configured at C-3 and C-3′ and differ only in their absolute configurations at the chiral axes. Feeding experiments with the simpler analogs **6**−**8** followed by analyses of the crude extracts by HPLC/MS revealed the absence of corresponding dimers.

[a] Department Chemie der Ludwig-Maximilians-Universität, Butenandtstr. 5−13, 81377 München, Germany
Fax: +49-(0)89-218077756
E-mail: wos@cup.uni-muenchen.de
[b] Institut für Organische Chemie, Universität Würzburg, Am Hubland, 97074 Würzburg, Germany
Fax +49-(0)931-8885323
E-mail: bringman@chemie.uni-wuerzburg.de
[c] Institut für Organische Chemie und Biochemie II, Technische Universität München, Lichtenbergstraße 4, 85747 Garching, Germany

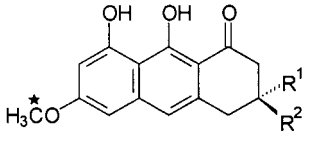
  DOI: 10.1002/ejoc.200400518



Scheme 1. Putative biosynthesis of dimeric pre-anthraquinones.



6, $R^1 = R^2 = H$
7, $R^1 = R^2 = CH_3$
8, $R^1 = CH_3$, $R^2 = H$



(*R*)-**2**, $R^1 = OH$, $R^2 = CH_3$
(*S*)-**2**, $R^1 = CH_3$, $R^2 = OH$
★ C: ^{13}C-label

In the case of *C. odorifer* the oxidative coupling proceeds with low atroposelectivity, providing a 2:1 mixture of isomers B_1 and A_1, whereas the related *C. auroturbinatus* forms phlegmacin B_1 with only traces of isomer A_1.[3] The coupling is highly substrate-specific and occurs neither with (*S*)-torosachrysone nor with the artificial analogs **6**–**8**, in which the >C(OH)CH_3 group in 3-position has been replaced by a CH_2, a >C$(CH_3)_2$, or a >CH(CH_3) group. The fact that *Cortinarius odorifer* and the Australian *C. sinapicolor*[9] produce phlegmacins with opposite configuration at C-3 and C-3′, respectively, reflects the occurrence of both enantiomers of torosachrysone in *Cortinarius* species.[14] Investigations to see whether – as in the biosynthesis of lignans in plants[15] – "dirigent proteins" are responsible for the observed stereo- and regioselectivities in the formation of fungal dihydroanthracenone dimers, are in progress.

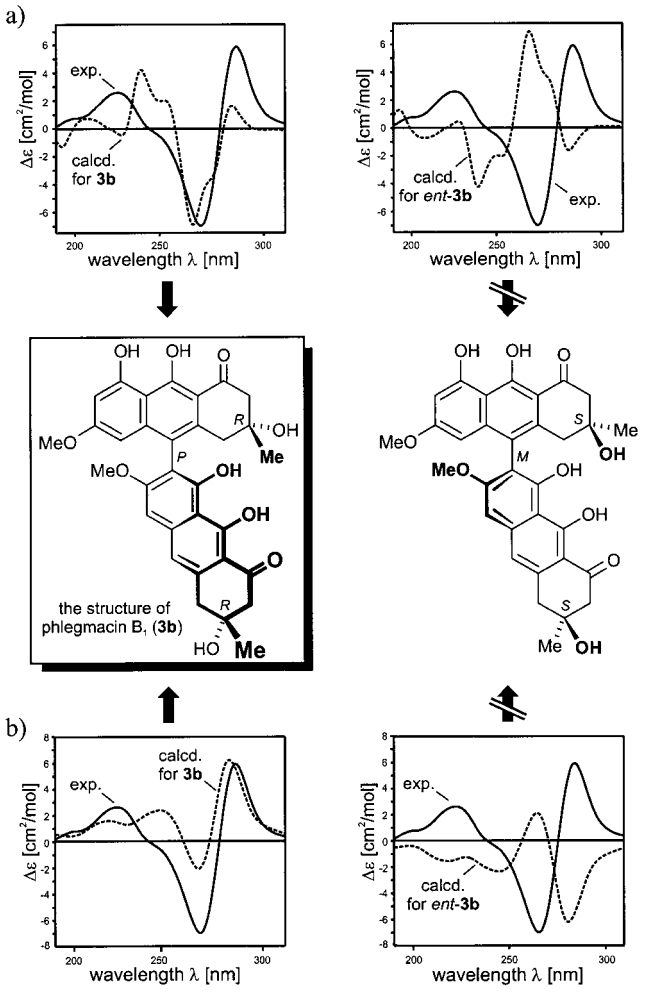
After revealing the absolute configuration at the stereogenic centers C-3 and C-3′, the configuration at the biaryl axes of phlegmacins A_1 and B_1 remained to be determined.

The circular dichroism (CD) spectra of dimeric pre-anthraquinones are dominated by the chiral axis, which causes the appearance of two strong Cotton effects centered around 275 nm. Dimers which exhibit a negative Cotton effect towards longer wavelengths (negative CD couplet) are designated as "type A", whereas in the CD spectra of "type B" isomers the sign of the first Cotton effect is positive (positive CD couplet).[2,3,16]

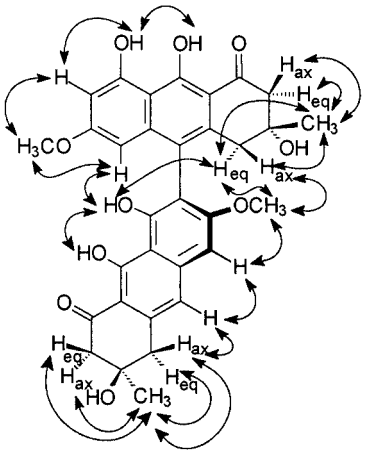
In order to establish the axial configuration of the phlegmacins we applied quantum chemical CD calculations[17,18] and compared the calculated CD curve with the experimental one obtained for phlegmacin B_1 (**3b**).[19] Given the above established absolute (*R*) configuration at both, C-3 and C-3′, and arbitrarily starting with the (*P*)-atropisomer, the (*P*,3*R*,3′*R*)-diastereomer of **3** was submitted to a conformational analysis by means of the semiempirical AM1[20] method, resulting in no less than 340 conformers within the relevant energetical range of 3 kcal/mol above the global minimum. For each optimized geometry a CD spectrum was computed using the CNDO/S-CI[21] approach as implemented in the program BDZDO/MCDSPD.[22] The single CD curves thus obtained were added up and weighted in accordance to their respective heat of formation, i.e., following the Boltzmann statistics, to give the overall theoretical spectrum, which was subsequently UV-corrected[17] in order to take systematic shifts into account. The comparison of the CD spectrum thus predicted for (*P*,3*R*,3′*R*)-**3** with the experimental CD curve of phlegmacin B_1 (**3b**) revealed a good agreement (see a, left part, in Scheme 2). By contrast, the theoretical CD curve for (*M*,3*S*,3′*S*)-**3**[23] (obtained by reflection of the spectrum calculated for *P*,3*R*,3′*R* at the zero line) showed a virtually opposite behavior (see a, right part, in Scheme 2), thus assigning phlegmacin B_1 (**3b**) to possess a *P*-configured chiral axis, and, in consequence, phlegmacin A_1 (**3a**) to be *M*-configured.

For a further confirmation of this structural assignment and in view of the molecular flexibility of the relevant chromophores, additional CD calculations for (*P*,3*R*,3′*R*)-**3** were performed, now based on molecular dynamics (MD) simulations using the MM3[24] force field as implemented in the molecular modeling package SYBYL[25] at a virtual temperature of 500 K and a simulation length of 500 ps. For the geometries extracted every 0.5 ps from the trajectory of motion single CD spectra were computed using again the CNDO/S-CI[21] method. Summing up of the resulting 1000 calculated CD spectra and subsequent UV correction[17] delivered the overall simulated CD curve, which again corresponded with the measured CD spectrum of **3b** (see b, left part, in Scheme 2), while the likewise predicted curve for (*M*,3*S*,3′*S*)-**3** (see above) was once again found to be virtually opposite (see b, right part, in Scheme 2). In consequence, both theoretical approaches resulted unambiguously in phlegmacin B_1 (**3b**) having a *P*-configured axis and hence phlegmacin A_1 (**3a**) being *M*-configured.

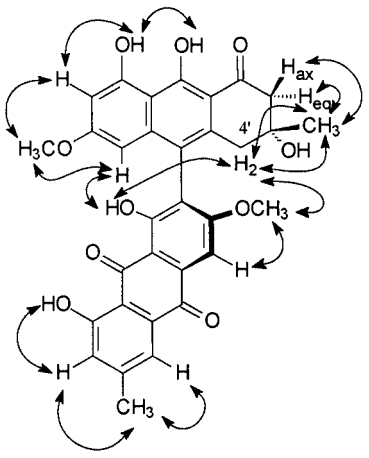
This assignment by quantum chemical CD calculations was fully confirmed by NOE experiments at 600 MHz. The ROESY spectrum of phlegmacin A_1 (**3a**) in $CD_2Cl_2/CDCl_3$ (1:1) showed cross signals between 4′-H_{eq} at δ 2.73 and the 8-OH proton at δ 9.96. In addition, correlations between 4′-H_{ax} at δ 2.68 and the 6-OCH_3 group at $\delta = 3.75$ and, to a minor extent, between 4′-H_{eq} and the 6-OCH_3 group were observed (Figure 1). In the case of phlegmacin B_1 (**3b**), the ROESY spectrum in $CDCl_3$ indicated a correlation between 4′-H_{ax} at $\delta = 2.83$ and the 8-OH proton at $\delta = 9.95$ and a correlation between 4′-H_{eq} at $\delta = 2.64$ and the 6-OCH_3 group at $\delta = 3.77$ ppm. The signals for 4′-H_{ax} and 4′-H_{eq} in the spectra of **3a** and **3b** could be distinguished by the ^4J W-coupling occurring between the pseudo-equatorial protons at C-4′ and C-2′. Furthermore, NOE correlations between each of the protons at C-2′ and C-4′ and the methyl group at C-3′ supported the chair conformation of the cyclohexenone ring with the methyl group adopting a pseudo-equatorial position.[8c–8e] The complete assignment of all proton and carbon NMR signals was based on COSY, ROESY, HSQC, and HMQC experiments (see Exp. Sect.).



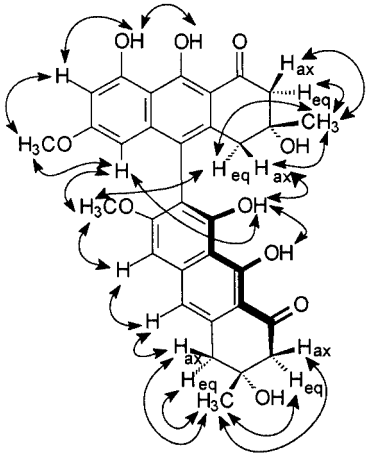
Scheme 2. Attribution of the absolute configuration of phlegmacin B_1 (**3b**) by comparison of the experimental CD spectrum (in MeOH) with the spectra calculated for (*P*,3*R*,3′*R*)-**3** and (*M*,3*S*,3′*S*)-**3**; a) according to the AM1-Boltzmann approach; b) following the MM3-MD method (the Δε values in the computed spectra were scaled to match the experimental ones; in each case, the scaling factor was the same for the entire spectrum)



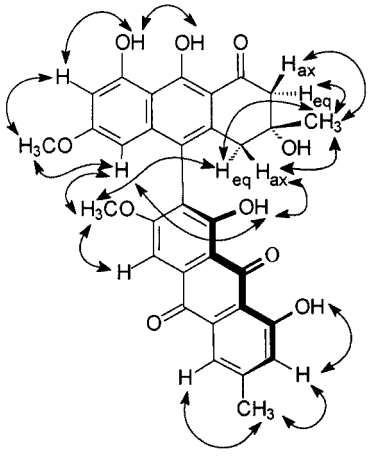
phlegmacin A_1 (**3a**)



anhydrophlegmacin-9,10-quinone A_1 (**9a**)



phlegmacin B_1 (**3b**)



anhydrophlegmacin-9,10-quinone B_1 (**9b**)

Figure 1. NOE correlations of phlegmacins A_1 ($CD_2Cl_2/CDCl_3$, 1:1) and B_1 ($CDCl_3$) at 300 K). NOEs between geminal protons are omitted for reasons of clarity.

Figure 2. NOE correlations of anhydrophlegmacin-9,10-quinones A_1 and B_1 ($CDCl_3$, 300 K). The signals of the 4′-protons of **9a** are unresolved. NOEs between geminal protons are not shown.

These results could only be explained by assigning the (*M*) and (*P*) configuration to phlegmacins A_1 and B_1, respectively. Since **3a** and **3b** are the enantiomers of the *Cassia* phlegmacins B_2 and A_2,[6] the latter should possess the (*P*,3*S*,3′*S*) and (*M*,3*S*,3′*S*) configuration, respectively. This attribution is in agreement with the co-occurrence of these pigments with (*S*)-torosachrysone.[26]

In a similar manner, the diastereoisomeric anhydrophlegmacin-9,10-quinones A_1 (**9a**) and B_1 (**9b**)[3] were investigated. The NOE correlations depicted in Figure 2 confirmed the conclusions obtained for the corresponding phlegmacin isomers.

Importantly, the stereoisomers of dimeric pre-anthraquinones exhibit diagnostic differences in the ^{1}H NMR spectra ("s*yn-anti* rule"),[3b,27] which were successfully applied by Gill and co-workers to establish the complete stereostructures of 5,5′- (atrovirin),[28] 5,10′- (pseudophlegmacin),[29] and 10,10′- (tricolorin)[30] dimers. In these cases, the occurrence of the 4′-methylene protons as a well-separated AB quadruplet ($\Delta\delta$ = 0.15−0.35 ppm) indicates a *syn* relationship between the OH group at C-3′ and the bulk of the second torosachrysone (anthraquinone) moiety. In contrast, more narrow signals ($\Delta\delta \leq 0.08$ ppm) are typical of an *anti* relationship of the two residues. Therefore, by knowing either the axial or central absolute configuration of a 5,5′- or 10,10′-torosachrysone dimer, the complete stereostructure can be derived from the ^{1}H NMR spectrum. The results of the present publication indicate, however, that this empirical "s*yn-anti* rule" has to be reversed for phlegmacins. The chemical shift differences of the 4′-methylene protons for phlegmacin A_1 ("s*yn*") and B_1 ("*anti*") are $\Delta\delta$ = 0.06 and 0.19 ppm, respectively, whereas the *syn-anti* rule would predict the opposite order.[31,32] This may be due to the smaller anisotropic effect of a torosachrysone (anthra-

quinone) residue connected through C-7 to the second half of the molecule, when compared with residues connected through C-5 or C-10 as is the case in atrovirins, tricolorins and pseudophlegmacins.

Experimental Section

General: NMR experiments were performed at a Bruker DMX 600 (^{1}H: 600 MHz, ^{13}C: 151 MHz) device. Chemical shifts are given in δ relative to $CHCl_3$ (δ_H = 7.26) and $CDCl_3$ (δ_C = 77.00). NOE experiments were carried out with the pulse program "roesyph" (mixing time 500−1000 ms).

Feeding Experiments: 5−10 mg of the respective ^{13}C-labelled precursor, dissolved in 0.25−0.5 mL DMSO, were injected via syringe into the stalks of three fruit bodies of *Cortinarius odorifer* growing in mixed forests near Garmisch-Partenkirchen and Wolfratshausen, Bavaria. After 6 days, the toadstools were harvested, during which time their cap diameter had increased from approximately 3 to 5 cm. The same technique was applied to the artificial precursors **6**, **7**, and **8**.

Isolation of Phlegmacins B_1 and A_1: After the feeding experiments, the fruit bodies (30 g) were immediately cut into fine pieces and shaken for 1 h in the dark with MeOH (200 mL). The extract was filtered, the filtrate evaporated to 50 mL, and the solution partitioned between EtOAc (200 mL) and H_2O (2 × 50 mL). The organic phase was dried (Na_2SO_4) and the solvent removed. The residue was chromatographed on Sephadex LH 20 (first column: eluent MeOH, second column: acetone/MeOH, 4:1), and the fractions containing the phlegmacins were collected. Pure anhydrophlegmacin-9,10-quinone A_1 (**9a**) (3.5 mg) was obtained after the second chromatography. Complete separation of the phlegmacin diastereomers by preparative TLC (SiO_2, toluene/ethyl formate/formic acid, 50:49:1) afforded phlegmacins A_1 (4 mg) and B_1 (14 mg) in NMR spectroscopically pure form. In the experiments with compounds **6**, **7**, and **8**, only the genuine dimers phlegmacins A_1 and B_1 could be detected by HPLC and MS.

Phlegmacin A_1 (3a): UV/Vis (EtOH): λ_{max} (ε) = 233 (44000), 276 (103500), 318 (sh, 12950), 332 (sh, 9700), 400 (18400) nm. CD (MeOH): λ_{max} (Δε) = 224 (−35.3), 267 (+94.9), 284 (−69.9), 333 (+4.0), 375 (−1.4), 415 (+3.7) nm. ^{1}H NMR (600 MHz, $CDCl_3$, 300 K): δ = 1.32 (s, 3 H, 3′-CH_3), 1.50 (s, 3 H, 3-CH_3), 1.89 (br, 2 H, 3-OH, 3′-OH), 2.72 (d, ^2J = 16.1, 1 H, 4′-H_{ax}), 2.78 (d, ^2J = 16.1, 1 H, 4′-H_{eq}), 2.78 (d, ^2J = 17.2, 1 H, 2′-H_{ax}), 2.87 (d, ^2J = 17.5, 2-H_{ax}), 2.90 (d, ^2J = 17.5, 1 H, 2-H_{eq}), 2.91 (d, ^2J = 17.2, 1 H, 2′-H_{eq}), 3.11 (d, ^2J = 16.1, 1 H, 4-H_{eq}), 3.14 (d, ^2J = 16.1, 1 H, 4-H_{ax}), 3.66 (s, 3 H, 6′-OCH_3), 3.78 (s, 3 H, 6-OCH_3), 6.12 (d, ^4J = 2.2, 1 H, 5′-H), 6.50 (d, ^4J = 2.2, 1 H, 7′-H), 6.72 (s, 1 H, 5-H), 7.02 (s, 1 H, 10-H), 10.00 (s, 1 H, 8-OH), 10.19 (s, 1 H, 8′-OH), 16.17 (s, 1 H, 9-OH), 16.65 (s, 1 H, 9′-OH) ppm. ^{1}H NMR (600 MHz, CD_2Cl_2/$CDCl_3$, 1:1, 300 K): δ = 1.27 (s, 3 H, 3′-CH_3), 1.45 (s, 3 H, 3-CH_3), 1.58 (br, 2 H, 3-OH, 3′-OH), 2.68 (d, ^2J = 16.1, 1 H, 4′-H_{ax}), 2.73 (d, ^2J = 16.1, 1 H, 4′-H_{eq}), 2.76 (d, ^2J = 17.5, 1 H, 2′-H_{ax}), 2.83 (d, ^2J = 17.5, 1 H, 2′-H_{eq}), 2.84 (m, 2 H, 2-H), 3.07 (d, ^2J = 16.0, 1 H, 4-H_{eq}), 3.11 (d, ^2J = 16.0, 1 H, 4-H_{ax}), 3.61 (s, 3 H, 6′-OCH_3), 3.75 (s, 3 H, 6-OCH_3), 6.09 (d, ^4J = 2.2, 1 H, 5′-H), 6.45 (d, ^4J = 2.2, 1 H, 7′-H), 6.72 (s, 1 H, 5-H), 7.00 (s, 1 H, 10-H), 9.96 (s, 1 H, 8-OH), 10.13 (s, 1 H, 8′-OH), 16.18 (s, 1 H, 9-OH), 16.63 (s, 1 H, 9′-OH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 300 K, an asterisk indicates ^{13}C-enrichment in the case of a positive feeding experiment): δ = 28.90 (3′-CH_3), 29.70 (3-CH_3), 41.52 (4′-C), 43.34 (4-C), 50.51 (2′-C), 51.09 (2-C), 55.17* (6′-OCH_3), 56.00* (6-OCH_3), 70.48 (3′-C), 71.14 (3-C), 98.33 (5-C), 99.50 (5′-C), 100.35 (7′-C), 108.18 (8a′-C or 9a′-C), 108.25 (8a′-C or 9a′-C), 108.33 (8a-C or 9a-C), 108.53 (9a-C or 8a-C), 111.49 (7-C), 117.66 (10-C), 120.26 (10′-C), 134.13 (4a′-C), 135.84 (4a-C), 140.61 (10a′-C or 10a-C), 140.65 (10a-C or 10a′-C), 156.09 (8-C), 160.69 (8′-C), 161.75 (6-C), 163.71 (6′-C), 165.90 (9-C), 166.93 (9′-C), 201.83 (1′-C or 1-C), 201.96 (1-C or 1′-C) ppm.

Phlegmacin B_1 (3b): UV/Vis: same as **3a**. CD (MeOH): λ_{max} (Δε) = 223 (+42.0), 267 (−127.1), 284 (+104.6), 333 (−3.3), 369 (+1.7), 420 (+3.4) nm. ^{1}H NMR (600 MHz, $CDCl_3$, 300 K): δ = 1.32 (s, 3 H, 3′-CH_3), 1.50 (s, 3 H, 3-CH_3), 2.27 (br, 2 H, 3-OH, 3′-OH), 2.64 (dd, ^2J = 16.1, ^4J = 1.5, 1 H, 4′-H_{eq}), 2.80 (d, ^2J = 17.3, 1 H, 2′-H_{ax}), 2.83 (d, ^2J = 16.1, 1 H, 4′-H_{ax}), 2.85 (d, ^2J = 17.5, 1 H, 2-H_{ax}), 2.86 (dd, ^2J = 17.3, ^4J = 1.5, 1 H, 2′-H_{eq}), 2.88 (d, ^2J = 17.5, 1 H, 2-H_{eq}), 3.09 (d, ^2J = 16.1, 1 H, 4-H_{eq}), 3.13 (d, ^2J = 16.1, 1 H, 4-H_{ax}), 3.67 (s, 3 H, 6′-OCH_3), 3.77 (s, 3 H, 6-OCH_3), 6.17 (d, ^4J = 2.2, 1 H, 5′-H), 6.51 (d, ^4J = 2.2, 1 H, 7′-H), 6.72 (s, 1 H, 5-H), 7.01 (s, 1 H, 10-H), 9.95 (s, 1 H, 8-OH), 10.21 (s, 1 H, 8′-OH), 16.17 (s, 1 H, 9-OH), 16.66 (s, 1 H, 9′-OH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 300 K, an asterisk indicates ^{13}C-enrichment in the case of a positive feeding experiment): δ = 28.94 (3′-CH_3), 29.13 (3-CH_3), 41.34 (4′-C), 43.31 (4-C), 50.48 (2′-C), 51.02 (2-C), 55.25* (6′-OCH_3), 56.03* (6-OCH_3), 70.67 (3′-C), 71.12 (3-C), 98.43 (5-C), 99.75 (5′-C), 99.94 (7′-C), 108.16 (8a′-C or 9a′-C), 108.21 (9a-C′ or 8a′-C), 108.35 (8a-C), 108.56 (9a-C), 111.36 (7-C), 117.70 (10-C), 120.39 (10′-C), 134.09 (4a′-C), 135.84 (4a-C), 140.61 (10a′-C or 10a-C), 140.66 (10a-C or 10a′-C), 156.70 (8-C), 160.79 (8′-C), 161.44 (6-C), 163.65 (6′-C), 165.92 (9-C), 166.99 (9′-C), 201.76 (1′-C), 202.03 (1-C) ppm.

Anhydrophlegmacin-9,10-quinone A_1 (9a, from *C. odorifer*): ^{1}H NMR (600 MHz, $CDCl_3$, 300 K): δ = 1.34 (s, 3 H, 3′-CH_3), 1.88 (br, 2 H, 3-OH, 3′-OH), 2.48 (s, 3 H, 3-CH_3), 2.72 (m, 2 H, 4′-H), 2.80 (d, ^2J = 17.5, 1 H, 2′-H_{ax}), 2.90 (d, ^2J = 17.5, 1 H, 2′-H_{eq}), 3.70 (s, 3 H, 6′-OCH_3), 3.89 (s, 3 H, 6-OCH_3), 6.08 (s, 1 H, 5′-H), 6.52 (s, 1 H, 7′-H), 7.11 (s, 1 H, 2-H), 7.58 (s, 1 H, 5-H), 7.67 (s, 1 H, 4-H), 10.21 (s, 1 H, 8′-OH), 11.98 (s, 1 H, 1-OH), 12.41 (s, 1 H, 8-OH), 16.72 (s, 1 H, 9′-OH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 300 K, an asterisk indicates ^{13}C-enrichment in the case of a positive feeding experiment): δ = 22.19 (3-CH_3), 28.92 (3′-CH_3), 41.48 (4′-C), 50.42 (2′-C), 55.33* (6′-OCH_3), 56.58* (6-OCH_3), 70.42 (3′-C), 99.40 (5′-C), 100.02 (7′-C), 103.44 (5-C), 108.00 (9a′-C or 8a′-C), 108.12 (8a′-C or 9a′-C), 111.27 (7-C), 113.56 (9a-C), 118.41 (10′-C), 120.27 (8a-C), 121.50 (4-C), 124.81 (2-C), 132.95 (4a-C), 134.15 (4a′-C), 135.20 (10a-C), 139.64 (10a′-C), 148.84 (3-C), 160.96 (8′-C), 161.59 (8-C), 162.65 (1-C), 163.85 (6′-C), 164.24 (6-C), 167.40 (9′-C), 181.95 (10-C), 191.10 (9-C), 201.68 (1′-C) ppm.

Anhydrophlegmacin-9,10-quinone-B_1 (9b, from *C. auroturbinatus*):[3b] ^{1}H NMR (600 MHz, $CDCl_3$, 300 K): δ = 1.35 (s, 3 H, 3′-CH_3), 1.86 (br, 2 H, 3-OH, 3′-OH), 2.47 (s, 3 H, 3-CH_3), 2.66 (d, ^2J = 16.1, 1 H, 4′-H_{eq}), 2.82 (d, ^2J = 17.6, 1 H, 2′-H_{ax}), 2.83 (d, ^2J = 16.1, 1 H, 4′-H_{ax}), 2.88 (d, ^2J = 17.6, 1 H, 2′-H_{eq}), 3.70 (s, 3 H, 6′-OCH_3), 3.88 (s, 3 H, 6-OCH_3), 6.09 (s, 1 H, 5′-H), 6.51 (s, 1 H, 7′-H), 7.09 (s, 1 H, 2-H), 7.57 (s, 1 H, 5-H), 7.66 (s, 1 H, 4-H), 10.19 (s, 1 H, 8′-OH), 12.00 (s, 1 H, 1-OH), 12.36 (s, 1 H, 8-OH), 16.70 (s, 1 H, 9′-OH) ppm. ^{13}C NMR (151 MHz, $CDCl_3$, 300 K): δ = 22.16 (3-CH_3), 28.85 (3′-CH_3), 41.33 (4′-C), 50.55 (2′-C), 55.31 (6′-OCH_3), 56.69 (6-OCH_3), 70.57 (3′-C), 99.34 (5′-C), 100.02 (7′-C), 103.49 (5-C), 107.93 (9a′-C), 108.09 (8a′-C), 111.20 (7-C), 113.56 (9a-C), 118.49 (10′-C), 120.24 (8a-C), 121.48 (4-C), 124.75 (2-C), 132.94 (4a-C), 134.15 (4a′-C), 135.19 (10a-C), 139.66 (10a′-C), 148.83 (3-C), 160.93 (8′-C), 162.01 (8-C), 162.60 (1-C), 163.85

(6′-C), 164.00 (6-C), 167.34 (9′-C), 181.90 (10-C), 191.17 (9-C), 201.64 (1′-C) ppm.

Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 369 and Graduiertenkolleg 690 "Elektronendichte") and the Fonds der Chemischen Industrie. Thanks are due to Ms. Sabine Voß for her skilful technical assistance and to Dr. Bernhard Oertel and Dr. Monika Winner for stimulating discussions. M. M. gratefully acknowledges assistance and helpful discussions with Dipl.-Chem. E. Löw with respect to the synthesis and biosynthetic application of model compounds **7** and **8**.

[1] W. Steglich, in *Pigments in Plants* (Ed.: F.-C. Czygan), 2nd ed., Gustav Fischer, Stuttgart, **1980**, pp. 393−412.
[2] M. Gill, W. Steglich, *Progr. Chem. Org. Nat. Prod.* **1987**, *51*, 1−317 and references cited in this review.
[3] [3a] W. Steglich, B. Oertel, *Sydowia* **1984**, *37*, 284−295. [3b] B. Oertel, *Dissertation*, University of Bonn (Germany), **1984**.
[4] *Römpp Encyclopedia Natural Products* (Eds.: W. Steglich, B. Fugmann, S. Lang-Fugmann), Thieme Verlag, Stuttgart, New York, **2000** and references cited therein.
[5] [5a] W. Steglich, E. Töpfer-Petersen, *Z. Naturforsch., Teil B* **1972**, *27*, 1286−1287. [5b] W. Steglich, E. Töpfer-Petersen, I. Pils, *Z. Naturforsch., Teil C* **1973**, *28*, 354−355.
[6] S. Takahashi, S. Kitanaka, M. Takido, U. Sankawa, S. Shibata, *Phytochemistry* **1977**, *16*, 999−1002.
[7] A. Yenesew, J. A. Ogur, H. Duddeck, *Phytochemistry* **1993**, *34*, 1442−1444.
[8] [8a] Review: A. Piñeyro-López, N. Waksman, *Studies in Natural Products Chemistry* **2000**, *22* (Bioactive Natural Products (part C)), pp. 555−606. [8b]V. R. Galindo, N. Waksman, *Nat. Prod. Lett.* **2001**, *15*, 243−251. [8c] NMR experiments: R. Ramírez-Durón, A. García-Luna, L. Garza-Ocañas, A. Piñeyro-López, N. Waksman de Torres, *Pharm. Biol.* **2002**, *40*, 440−447; X-ray structure: [8d] C. O. Rodriguez de Barbarin, N. A. Bailey, R. Ramírez-Durón, L. Martinez-Villarreal, A. Piñeyro-López, N. Waksman, *Ciencia UANL* **1998**, *1*, 37−42. [8e] Absolute configuration: A. Pérez, R. Ramírez-Durón, A. Piñeyro-López, N. Waksman, M. Reichert, G. Bringmann, *Tetrahedron* **2004**, *60*, 8547−8552.
[9] C. Elsworth, M. Gill, A. Giménez, N. M. Milanovic, E. Raudies, *J. Chem. Soc., Perkin Trans. 1* **1999**, 119−125.
[10] [10a] J. H. Dodd, S. M. Weinreb, *Tetrahedron Lett.* **1979**, *20*, 3593−3956. [10b] J. H. Dodd, R. S. Garigipati, S. M. Weinreb, *J. Org. Chem.* **1982**, *47*, 4045−4049. [10c] G. E. Evans, F. J. Leeper, J. A. Murphy, J. Staunton, *J. Chem. Soc., Chem. Commun.* **1979**, 205−206.
[11] M. Müller, *Dissertation*, University of Munich (Germany), **1995**.
[12] M. Müller, K. Lamottke, E. Löw, E. Magor-Veenstra, W. Steglich, *J. Chem. Soc., Perkin Trans. 1* **2000**, 2483−2489.
[13] K. Lamottke, *Dissertation*, University of Munich (Germany), **1999**.
[14] [14a] M. Gill, A. Gimenez, A. G. Jhingran, A. F. Smrdel, *Phytochemistry* **1989**, *28*, 2647−2650. [14b] A. Gimenez, *PhD thesis*, University of Melbourne (Australia), **1990**.
[15] L. B. Davin, H.-B. Nang, A. L. Crowell, D. L. Bedgar, D. M. Martin, S. Sarkanen, N. G. Lewis, *Science* **1997**, *275*, 362−366.
[16] The numbering indices denote the chronological order of description of the respective diastereomer.
[17] G. Bringmann, S. Busemann, in *Natural Product Analysis* (Eds.: P. Schreier, M. Herderich, H. U. Humpf, W. Schwab), Vieweg, Wiesbaden, **1998**, pp. 195−212.
[18] [18a] G. Bringmann, M. Dreyer, J. H. Faber, P. W. Dalsgaard, D. Staerk, J. W. Jaroszewski, H. Ndangalasi, F. Mbago, R. Brun, M. Reichert, K. Maksimenka, S. B. Christensen, *J. Nat. Prod.* **2003**, *66*, 1159−1165. [18b] G. Bringmann, J. Mühlbacher, C. Repges, J. Fleischhauer, *J. Comput. Chem.* **2001**, *22*, 1273−1278. [18c] S. G. Allenmark, *Nat. Prod. Rep.* **2000**, *17*, 145−155.
[19] For a recent review on the synthesis, biosynthesis and natural occurrence of biaryls, see: G. Bringmann, C. Günther, M. Ochse, O. Schupp, S. Tasler, *Progr. Chem. Org. Nat. Prod.* **2001**, *82*, 1−249.
[20] M. J. S. Dewar, E. G. Zoebisch, E. F. Healy, J. J. P. Stewart, *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.
[21] J. Del Bene, H. H. Jaffé, *J. Chem. Phys.* **1968**, *48*, 1807−1813.
[22] J. W. Downing, program package BDZDO/MCDSPD, Department of Chemistry and Biochemistry, University of Colorado, Boulder (USA); modified by J. Fleischhauer, W. Schleker, B. Kramer; ported to Linux by K.-P. Gulden.
[23] Given the extreme dominance of the chiroptical contributions of the chiral axis vs. the centers, additional CD calculations for the diastereomer (*M*,3*R*,3′*R*)-**3** appeared unnecessary.
[24] N. L. Allinger, Y. H. Yuh, J.-H. Lii, *J. Am. Chem. Soc.* **1989**, *111*, 8551−8566.
[25] SYBYL: Tripos Associates, 1699 St. Hanley Road, Suite 303, St. Louis, MO 63144.
[26] [26a] M. Takido, S. Takahashi, K. Masuda, K. Yasukawa, *Lloydia* **1977**, *40*, 191−194. [26b] Absolute configuration of (*S*)-torosachrysone: M. Endo, H. Naoki, *Tetrahedron* **1980**, *36*, 2449−2452.
[27] M. Gill, *Nat. Prod. Rep.* **1999**, *16*, 301−317 and references cited therein.
[28] M. Gill, P. M. Morgan, *Arkivoc* **2004**, 152−165 and references cited therein.
[29] M. S. Buchanan, M. Gill, P. Millar, S. Phonh-Axa, E. Raudies, J. Yu, *J. Chem. Soc., Perkin Trans. 1* **1999**, 795−801.
[30] K. Beattie, C. Elsworth, M. Gill, N. M. Milanovic, D. Prima-Putra, E. Raudies, *Phytochemistry* **2004**, *65*, 1033−1038.
[31] Due to these finding, the configurations assigned to the chiral axes of the phlegmacin pigments in refs. 3, 9, 11, and 27 should be reversed.
[32] In the case of phlegmacins, only the absolute configuration of the chiral center C-3′ can be determined by applying this modified "*syn-anti*" rule. The protons at C-4 are far too remote to experience the anisotropic effect of the chiral axis.

Received July 23, 2004

