# Pigments from the Puffball *Calvatia rubro-flava* − Isolation, Structural Elucidation and Synthesis

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*Dedicated to Professor Burchard Franck on the occasion of his 75th birthday*

**Keywords:** Natural products / Azo compounds / Chiral sulfoxides / Quantum chemical calculations / Enantioselective synthesis

The orange pigment rubroflavin (**1**) from the dried fruit bodies of *Calvatia rubro-flava* (Lycoperdaceae) owes its high optical rotation to a methanesulfinyl group directly attached to a 1,4-benzoquinone semicarbazone chromophore. Rubroflavin is present in fresh fungi in its leuco form **4**, which is easily oxidized to **1**. Thermal fragmentation of **1** yields (–)-3-methanesulfinyl-5-(methylthio)phenol (**6**), whose configuration was assigned as (*S*) by quantum mechanical calculations. This result is supported by CD comparison of **6** with (*S*)-4-(methanesulfinyl)toluene, and the synthesis of (*S*)-**1** from esters of deoxyrubroflavin (**8**) by stereoselective sulfoxidation. In the same manner, optically active (*S*,*S*)-oxyrubroflavin (**2**) and (*S*)-craniformin (**3**) were obtained. NMR measurements in different solvents indicate that **1** and the related 1,4-benzoquinone semicarbazones are in equilibrium with their azophenol tautomers.

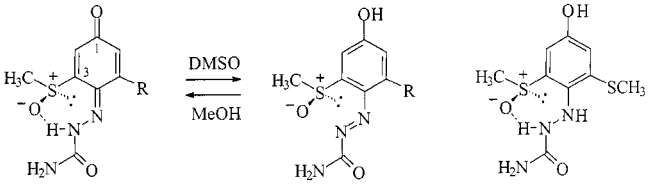
## Introduction

Several mushrooms and toadstools exhibit spectacular color reactions on bruising.[1] A remarkable example is the North American puffball *Calvatia rubro-flava* (Cragin) Lloyd (Lycoperdaceae) whose white fruit bodies stain immediately golden yellow when touched, and change to orange-brown on aging or drying. In this paper we describe the isolation, structural elucidation, and synthesis of the compounds responsible for these phenomena.[2,3]

## Results and Discussion

### Structural Elucidation and Chemistry

Chromatography of the methanol extract of air-dried fruit bodies of *C. rubro-flava* on Sephadex LH-20, followed by TLC yielded three orange pigments and a colorless leuco derivative. The main pigment rubroflavin (**1**) constitutes ca. 1% of the dry weight. On reduction with Zn/AcOH it yields leucorubroflavin (**4**), identical with the colorless compound isolated from the fungus. The easy reoxidation of **4** to rubroflavin (**1**) explains the color changes shown by the fruit bodies.




**1a** R = $SCH_3$ **1b**
**2a** R = $S(O)CH_3$ **2b**
**3a** R = $SO_2CH_3$ **3b**
**4**

In the $^1H$ NMR spectrum ([$D_6$]DMSO) of rubroflavin, two methyl singlets at $\delta_H = 2.25$ ($SCH_3$) and 2.64 ($SOCH_3$), two doublets for *meta*-coupled ring protons at $\delta = 5.97$ and 6.57, and two exchangeable signals at $\delta = 6.42$ (1 H) and 6.53 (2 H) are visible. The $^1H$-coupled $^{13}C$ NMR spectrum ($CD_3OD$) suggests a 2,6-disubstituted 1-imino-4-benzoquinone structure. Signals at $\delta_C = 180.8$ and 132.2 are typical for the C=O and C=N carbon atoms, respectively, and two $^3J = 7.5$ Hz couplings of the ring protons with the C=N carbon atom indicate the position of the substituents. In addition, an isolated carbon signal at $\delta_C = 166.4$ can be assigned to the carbonyl group of a semicarbazone moiety. The high optical rotation $[\alpha]_D^{22} = -2180$ (MeOH) of rubroflavin indicates that the chiral methanesulfinyl substituent ($\delta_H = 2.64$) is directly attached to the chromophore. From these results, structures **1a** and **4** can be deduced for rubroflavin and its leuco derivative, respectively. Interestingly, the $^{13}C$ NMR spectrum of **1** in [$D_6$]DMSO lacks the signal for the ring carbonyl group and is in agreement with the pres-

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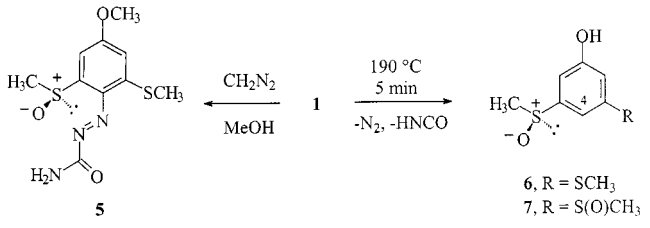
ence of the azophenol tautomer **1b**. This indicates an equilibrium between tautomers **1a** and **1b**, depending on hydrogen bond formation with the solvent.

Attempts to determine the molecular composition of rubroflavin by high-resolution EI MS were misleading at first. The spectra indicated a strong molecular ion peak at $m/z$ = 202 corresponding to the formula $C_8H_{10}O_2S_2$. Neither the three exchangeable protons in the $^1H$ NMR spectrum nor the presence of nine carbon signals in the $^{13}C$ NMR spectrum were thereby explained. Finally, the elemental analysis and FAB experiments with added NaCl provided the true molecular composition $C_9H_{11}N_3O_3S_2$. The misleading EI MS was found to be caused by thermal fragmentation of **1** in the ion source (vide infra).

Treatment of rubroflavin (**1**) in aqueous methanol with diazomethane in the presence of silica gel afforded methyl ether **5** (Scheme 1). Obviously, rubroflavin reacts under the methylation conditions as its azophenol tautomer **1b**.




Scheme 1. Methylation and thermolysis of rubroflavin (**1**)

Heating rubroflavin to 190 °C induces a smooth fragmentation to phenol **6** with formation of $N_2$ and HNCO. The same reaction takes place in the ion source of the mass spectrometer and explains the difficulties in observing the molecular ion of rubroflavin.

Due to electronic interactions of the chiral sulfoxide with the benzoquinone chromophore, rubroflavin (**1**) exhibits a complex CD spectrum (Figure 1) from which we were unable to assign the absolute configuration of the pigment. After thermolysis, however, the CD spectrum of the derived phenol **6** showed a single negative Cotton effect ($\Delta\varepsilon$ = −13.6) at 241 nm (Figure 2) which agreed well with that of (*S*)-methyl tolyl sulfoxide of established configuration.[4] To check this assignment, the CD spectrum of phenol **6** was investigated by quantum mechanical calculations (vide infra). The results obtained by this method strongly support the (*S*) configuration for **6**.

The orange-red oxyrubroflavin (**2**) was isolated as a minor pigment from the dried fruit bodies of *C. rubro-flava*. It was identified as the corresponding bis(sulfoxide) by the similar chemical shifts of the two methanesulfinyl groups ($\delta_H$ = 2.82 and 2.87; $\delta_C$ = 44.7 and 45.1) and the intense fragment ion peak at $m/z$ = 218 ($C_8H_{10}O_3S_2$) in the EI MS. The latter corresponds to phenol **7** that can be prepared by thermal fragmentation of oxyrubroflavin at 210 °C. Phenol **7** exhibits two triplets of equal intensity for 4 H in the $^1H$ NMR spectrum, indicating a 1:1 mixture of the (*S*,*S*) and

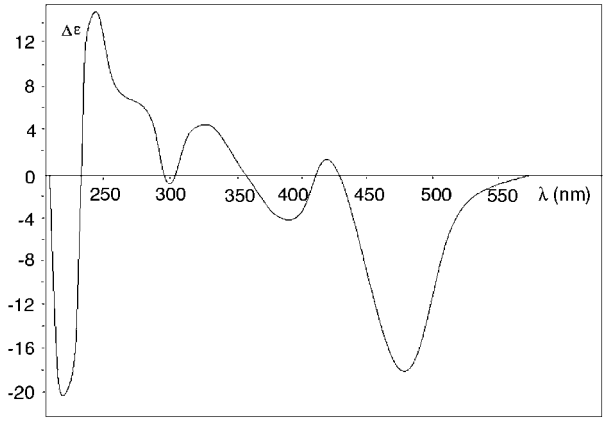



Figure 1. Experimental CD spectrum of rubroflavin (**1**) (in MeOH)

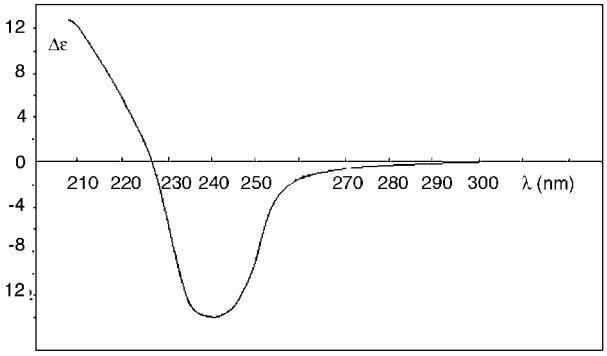
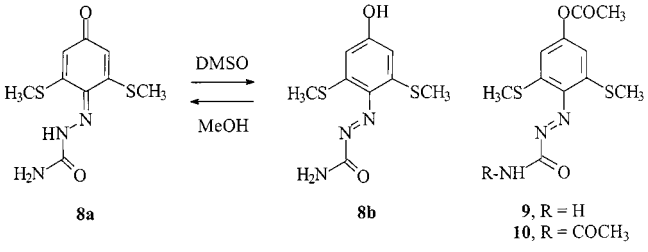



Figure 2. Experimental CD spectrum of thermolysis product **6** (in MeOH)

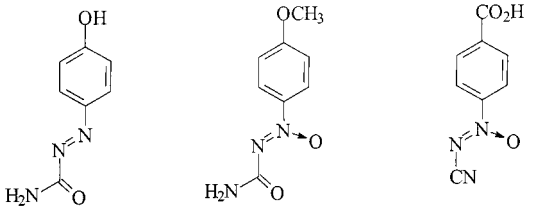
(*S*,*R*) diastereomers. A similar mixture of diastereomers was obtained by treatment of rubroflavin (**1**) with peracetic acid followed by thermolysis of the resulting oxyrubroflavin. Apparently, the oxyrubroflavin isolated from the fungus is formed from **1** by nonenzymatic autoxidation.




The second minor pigment deoxyrubroflavin (**8**) can be considered a biosynthetic precursor of rubroflavin (**1**). Like rubroflavin, its NMR spectra depend strongly on the solvent. In $CD_3OD$, only the $^{13}C$ NMR signals of the semicarbazone form **8a** are visible, whereas in [$D_6$]DMSO the signals correspond to the azophenol tautomer **8b**. Treatment of **8** with acetic anhydride and DMAP at 30 °C yielded the *O*-acetyl derivative **9**, whereas at 60 °C the *O*,*N*-diacetyl derivative **10** was formed. Oxidation of **8** with ammonium cerium(IV) nitrate (CAN)[5] gave a mixture of 53% (±)-rub-

roflavin and 12% (±)-oxyrubroflavin that could easily be separated by chromatography on Sephadex LH 20.

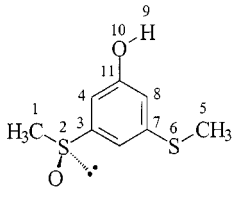
Rubroflavin (**1**) has been isolated by Takaishi et al.[6,7] also from *Calvatia craniiformis* (Schw.) Fr. In this fungus, **1** is accompanied by the sulfone craniformin (**3**), 4-hydroxyphenyl-1-azoformamide (**11**), and 4-methoxyphenyl-1-*O*,*N*,*N*-azoformamide (**12**). The unique semicarbazones and azo derivatives from *Calvatia rubro-flava* and *C. craniiformis* are biosynthetically closely related to the carcinostatic agent calvatic acid (**13**),[8] from cultures of *C. craniiformis* and *C. lilacina* (Mont. & Berk.) Lloyd.




**11** **12** **13**

## Quantum Mechanical Calculations

We now tried to determine the absolute configuration of the thermolysis product **6** by comparison of its experimental CD spectrum with the one calculated for its (*R*) isomer by quantum chemical semiempirical CNDO/2S methods.[9] To obtain molecular geometries to be used in the calculation of the CD spectrum, we performed geometry optimizations employing the semiempirical AM1 method.[10] Using a structure based on standard bond lengths and bond angles,[11] we obtained a starting geometry by energy optimization. Further conformers were then generated by systematic variation of the dihedral angles $\theta_1 = C_1S_2C_3C_4$, $\theta_2 = C_5S_6C_7C_8$, and $\theta_3 = C_4C_{11}O_{10}H_9$ (cf. Scheme 2) of the starting geometry. Starting from the optimized geometries obtained at fixed values of $\theta_3 = 0$ and 180°, we generated a total number of 2592 points by changing $\theta_1$ and $\theta_2$ in steps of 10°. For each pair of dihedral angles $\theta_1$ and $\theta_2$ we performed a complete geometry optimization of all remaining internal coordinates using the AM1 method, where the starting values of the structural parameters to be optimized have been taken from the preceding calculation.[12]




Scheme 2. Definition of dihedral angles in **6**

Boltzmann weights ($T$ = 298 K) for each of the points were calculated using the corresponding semiempirical heats of formation. The corresponding CD spectrum for each structure was calculated by employing the semiempirical CNDO/2S method as implemented in the BDZDO/MCD3SP program package.[13] In all cases, configuration interaction (CI) included 169 singly excited configurations, formed from the 13 highest filled orbitals and 13 lowest unoccupied orbitals. The rotational strengths were calculated using the origin-independent dipole-velocity formalism.[14] The calculated CD curve was obtained as a sum of Gaussians, centered at the wavelengths of the corresponding transitions, multiplied with the rotational strength.[15,16]

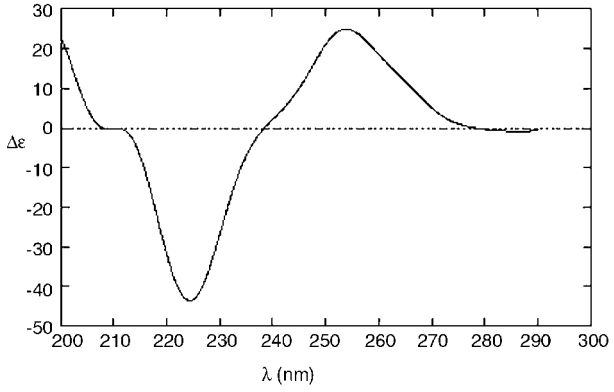



Figure 3. Calculated CD spectrum of thermolysis product **6**

The resulting CD curve of **6** is shown in Figure 3. In contrast to the experimental spectrum of **6** in Figure 2, the first calculated Cotton effect is positive.

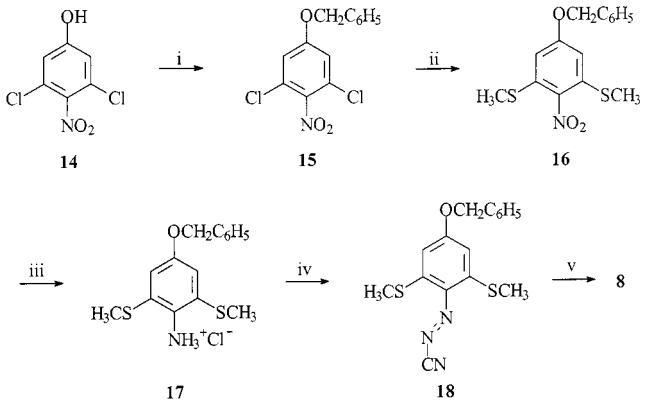
Since the spectrum has been calculated for the (*R*) isomer, we conclude that the configuration of the thermolysis product **6** is (*S*). Additional calculations[17] at the ab initio level showed that the $-S(=O)CH_3$ group is configurationally stable under the conditions of the thermolysis. The configuration of the stereogenic sulfur atom in rubroflavin (**1**) is therefore also (*S*).

## Syntheses of Rubroflavin (1) and Related Pigments

Our first synthetic target was deoxyrubroflavin (**8**), the apparent biosynthetic precursor of rubroflavin. The synthesis of **8**[18] commenced with nitrophenol **14**, which can easily be prepared by nitration of commercially available 3,5-dichlorophenol.[19] Compound **14** was transformed into the benzyl ether **15** by treatment with benzyl chloride (Scheme 3).

Exchange of the halogen atoms against methylthio residues was achieved by treatment of ether **15** with sodium methanethiolate in acetone. The corresponding bis(methylthio) derivative **16** was obtained in high yield as bright yellow crystals. The reduction of the nitro group in **16** was best performed with Sn/HCl, and yielded the amine hydrochloride **17** that could be transformed into the corresponding diazo cyanide **18** by diazotation and subsequent treatment with aqueous KCN at pH = 10.[20] At first, an orange precipitate of the *syn*-diazo cyanide was formed that changed to the dark red *anti* isomer after prolonged stirring.[20]

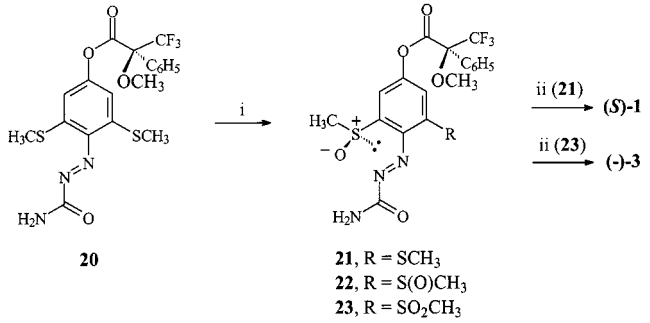
Transformation of diazo cyanide **18** into the desired semicarbazone needed some experimentation, since the usual methods ($HCl/Et_2O$, formic acid, polyphosphoric acid, or



Scheme 3. Synthesis of deoxyrubroflavin (**8**); reagents and conditions: i) $C_6H_5CH_2Cl$, aq. NaOH, EtOH (86%); ii) $CH_3SNa$, acetone (84%); iii) Sn, aq. HCl, MeOH (90%); iv) $NaNO_2$, HCl, $H_2O$, 0 °C, then $Na_2CO_3$, KCN (95%); v) $TiCl_4$, AcOH, $H_2O$ (73%)

KOH in *t*BuOH) caused loss of the side chain under formation of 3,5-bis(methylthio)phenol. The problem was solved by treatment of **18** with trifluoroacetic acid, or preferably $TiCl_4$/AcOH/$H_2O$.[21] In both cases, deoxyrubroflavin (**8**) was obtained in good yield. Our method allows for the synthesis of **8** from 3,5-dichloro-4-nitrophenol in gram quantities in 45% overall yield.

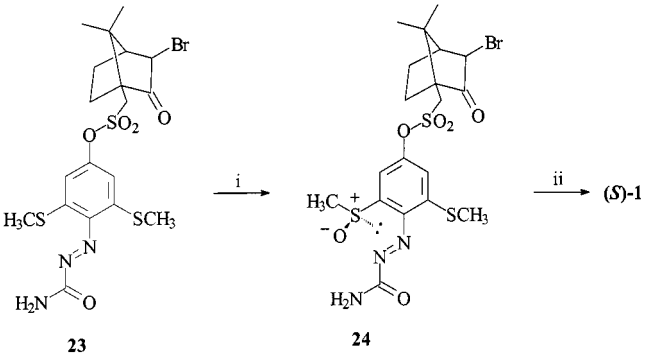
For the enantioselective oxidation of deoxyrubroflavin (**8**), the Kagan modification of the Sharpless epoxidation was selected.[22] Attempts to oxidize **8** directly at −20 °C with *tert*-butyl hydroperoxide (TBHP) in $CH_2Cl_2$ in the presence of Ti(O*i*Pr)$_4$, diethyl (*S*,*S*)-tartrate [(*S*,*S*)-DET], and 1 equiv. of water were unsatisfactory due to the low solubility of the substrate. Compound **8** was therefore converted into the lipophilic ester **19** by reaction with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*S*)-MTPA][23] in the presence of dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) (Scheme 4).[24]



Scheme 4. Diastereoselective sulfoxidation of ester **19**; reagents and conditions: i) *t*$BuO_2H$, Ti(O*i*Pr)$_4$, (*S*,*S*)-DET, $H_2O$, $CH_2Cl_2$, −20 °C; column chromatography on Sephadex LH-20; fractional crystallization from $CCl_4$ (**20**); ii) 0.2 N NaOH, MeOH

TLC-controlled oxidation of ester **19** under Kagan's conditions yielded 35% of rubroflavin ester **20** (*de* 67%), 22% of oxyrubroflavin ester **21** ($[\alpha]_D^{20} = -920$), and 8% of the sulfone derivative **22** (*de* 86%).[25] Whereas in the case of **20** and **22** the ratio of diastereomers could be determined from the $^{19}F$ and $^1H$ NMR spectra, the stereochemical composition of **21** remains obscure. After unsuccessful experiments to achieve the separation of the diastereomers by chromatography or HPLC on silica gel or chiral phases (Pirkle, Okamoto; cellulose triacetate, cyclodextrin), repeated fractionated crystallization from $CCl_4$ yielded the diastereomerically homogenous (*S*)-MTPA ester of (*S*)-rubroflavin (**20**). Mild alkaline saponification of **20** afforded optically pure (*S*)-rubroflavin (**1**) ($[\alpha]_D^{20} = -2140$), identical in every aspect with the natural product. (*S*)-Craniformin (**3**)[6] was obtained from **22** with 86% optical purity, $[\alpha]_D^{20} = -1034$ (MeOH), corresponding to an optical rotation of −1200 for the pure enantiomer. Unfortunately, the optical properties of natural craniformin were not reported in the original publication.[6]

Similar to the (*S*)-MTPA ester **19**, the camphorsulfonate **23** was obtained from **8** and (+)-3-bromocamphor-10-sulfonyl chloride with DMAP as catalyst (Scheme 5). In the case of **23**, the TLC-controlled enantioselective oxidation yielded the sulfoxide **24** with 72% *de* (Scheme 5). Fractionated crystallization of **24** from 2-propanol gave the pure diastereomer that was hydrolysed to (*S*)-rubroflavin, which was identical in every respect with the product obtained from ester **20**.



Scheme 5. Diastereoselective sulfoxidation of sulfonate **23**; reagents and conditions: i) *t*$BuO_2H$, Ti(O*i*Pr)$_4$, (*S*,*S*)-DET, $H_2O$, $CH_2Cl_2$, −20 °C; column chromatography on Sephadex LH-20; fractional crystallization from *i*PrOH; ii) 0.2 N NaOH, MeOH

## Conclusions

The enantioselective synthesis of rubroflavin (**1**) proves the structure of this unique natural product. The selective formation of the (*S*) enantiomer in the (*S*,*S*)-DET-directed oxidation of deoxyrubroflavin is predicted by Kagan's model for enantioselective sulfoxidations,[22] and agrees with the configuration of **1** derived from the quantum mechanical calculations. The optimization of the optical yields and the chromatographic separation of the diastereomeric esters are left for further investigations.

## Experimental Section

**General:** Melting points (uncorrected): Reichert Thermovar hot stage. − Optical rotations: Perkin−Elmer 214. − UV/Vis: Beckman 25 and Varian Cary 17. − CD: Circulardichrograph III NRS-

Jouan-Roussel at 20 °C. − IR: Pye Unicam SP 1100 and Perkin−Elmer 1420. − NMR: Bruker AC 200 and WM 400, solvent peak or TMS as internal standard. − MS: A.E.I. MS 30 and MS 50 with data system DS 50. EI MS spectra were obtained at 70 eV with direct inlet. − TLC: Merck, silica gel 60 PF, solvent systems (v/v): A: $CHCl_3$/MeOH, 2:1; B: $CHCl_3$/MeOH, 10:1; C: *n*BuOH/AcOH/$H_2O$, 4:1:1; D: *i*PrOH/$HCO_2H$/$H_2O$, 20:1:5. − Column chromatography: Silica gel 60 (40−63 μm, Merck) and Lobar RP-8 (size B, Merck). − Gel chromatography: Sephadex LH-20 (Pharmacia). − Analytical HPLC: Waters M 721, packing material Lichrosorb RP-8, 7 μm, flow rate 1 mL/min. Eluent A: $H_2O$/MeOH, 9:1; eluent B: $H_2O$/MeOH, 1:9. Linear gradient: 5 min: B 50%, within 10 min to B 100%, then 15 min B 100%. UV/Vis detection at 245 and 435 nm. Waters 600 E Pump and System Controller with Photodiode Array Detector 990+. All solvents were distilled before use. The anhydrous solution of TBHP in toluene was purchased from Fluka. − The elemental analyses were carried out by Fa. Pascher, Bonn, and the Microanalytical Laboratory at the University of Bonn. − *C. rubro-flava* was collected in September 1983 in a meadow in the Marshall Forest, Great Smoky Mountains, Georgia, USA (leg. et det. Dr. R. Baird).

**Isolation of Pigments 1, 2, 8, and Leucorubroflavin (4) from *C. rubro-flava*:** Air-dried fruit bodies of *C. rubro-flava* (13.2 g) were cut and extracted exhaustively with MeOH. The extract was concentrated and the residue filtered through a short silica gel column ($CHCl_3$/MeOH, 2:1). Column chromatography of the solution on Sephadex LH 20 with MeOH yielded three colored zones. A bright yellow initial fraction contained oxyrubroflavin (**2**), leucorubroflavin (**4**), and some rubroflavin (**1**), the orange-yellow main fraction contained only **1**. The last, orange-colored fraction consisted of a mixture of **1** and deoxyrubroflavin (**8**). Preparative TLC was used to separate **1**, **2**, and **4** on silica gel (system A). Compound **4** appeared as narrow colorless zone (fluorescence quenching at 254 nm) below the main pigment, and was characterized without further purification. The lemon-yellow minor pigment **2** showed the lowest $R_f$ value and was purified by repeated preparative TLC on silica gel (system A) followed by column chromatography on Sephadex LH-20 (eluent MeOH). Pigment **1** exhibited an intermediate $R_f$ value and was obtained analytically pure by chromatography on Sephadex LH-20 (2 ×, eluent MeOH), followed by chromatography on a Lobar RP-8 column (eluent MeOH/$H_2O$, 1:1). Yields: 90 mg of **1** (0.75% of dry-weight), 5 mg of **2** (0.04%), 5 mg of **4** (0.04%), 2 mg of **8** (0.015%).

**Rubroflavin (1):** Red needles, monohydrate. − M.p. 184−185 °C (dec.). − $[\alpha]_D^{22}$ = −2180 (*c* = 0.07, MeOH). − $R_f$ (TLC) = 0.57 (system A); 0.33 (system B); 0.66 (system C); 0.73 (system D), yolk-yellow spot. − Analytical HPLC: $t_R$ = 3.9 min. − UV (MeOH): $\lambda_{max}$ (lg ε) = 214 nm (4.01), 243 (3.92), 252 (3.89), 275 (3.65), 295 (3.61), 435 (4.03), 460 (sh, 3.97). − CD (MeOH): $\lambda_{max}$ (Δε) = 221 (−21.65), 247 (+15.33), 265 (+7.72), 297 (−0.86), 325 (+4.61), 385 (−5.47), 425 (+1.07), 478 (−19.51) (Figure 1). − IR (KBr): $\tilde{\nu}$ = 3440 $cm^{-1}$ (s), 3250 (br., s), 2920 (m), 2850 (w), 1700 (s), 1670 (s), 1570 (ss), 1465 (m), 1430 (m), 1360 (w), 1285 (sh, ss), 1230 (ss), 1185 (sh, ss), 1070 (s), 1055 (s), 1010 (m), 970 (m), 930 (sh, w), 845 (s), 775 (w), 675 (w). − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.41 (br. s, 3 H), 2.84 (s, 3 H), 6.44 (dq, $^4J$ = 2.3, $^5J$ = 0.25 Hz, 1 H), 7.04 (d, $^4J$ = 2.3 Hz, 1 H). − $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 2.25 (br. s, 3 H), 2.64 (s, 3 H), 5.97 (d, $^4J$ = 2.3 Hz, 1 H), 6.42 (br., 1 H),* 6.53 (br., 2 H),* 6.57 (d, $^4J$ = 2.3 Hz, 1 H), *exchangeable with $D_2O$. − $^{13}$C NMR (100.6 MHz, $CD_3OD$): δ = 15.0 (Q, $^1J$ = 138.5 Hz, *C*$H_3$S), 44.7 (Q, $^1J$ = 140 Hz, *C*$H_3$SO), 115.4 (Dd, $^1J$ = 165, $^3J$ = 4.6 Hz), 116.9 (Dd, $^1J$ = 160, $^3J$ = 4.2 Hz), 132.2 ("t", $^3J$ = 7.3 Hz, C-4), 148.9 (m), 154.0 (m), 166.4 (s, *C*$ONH_2$), 180.8 (s, C-1). − $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO): δ = 14.7 (Q, $^1J$ = 140 Hz, *C*$H_3$S), 43.8 (Q, $^1J$ = 141 Hz, *C*$H_3$SO), 107.2 (Dd, $^1J$ = 166, $^3J$ = 4.5 Hz, C-6), 112.7 (Dd, $^1J$ = 164, $^3J$ = 4.0 Hz, C-2), 135.4 (t, $^3J$ = 7.0 Hz, C-4), 145.3 (m, C-5), 148.0 (m, C-3), 162.4 (t, $^2J$ = 2.0 Hz, C-1), 162.6 (s, *C*$ONH_2$). − (+)-FAB MS (glycerol + NaCl): *m*/*z* = 296 [$M^+$ + Na], 318 [$M^+$ − H + 2Na]. − EI MS: *m*/*z* (%) = 217 ($C_8H_{11}NO_2S_2$, 1.3), 202.0125 (calcd. for $C_8H_{10}O_2S_2$: 202.0122, 25), 187 ($C_7H_7O_2S_2$, 24), 186 ($C_8H_{10}OS_2$, 39), 156 ($C_7H_8O_2S$, 8), 153 ($C_8H_9OS$, 42), 139 ($C_7H_7OS$, 8), 107 ($C_7H_7O$, 9), 94 ($C_2H_6S_2$, 21), 79 ($CH_3S_2$, 6), 63 ($C_2H_7S$, 8), 60 ($CH_4N_2O$, 27), 47 ($CH_3S$, 10), 44 ($CONH_2$, 38), 43 (HNCO, 100), 42 (NCO, 19). − $C_9H_{11}N_3O_3S_2 \times H_2O$ (273.3 + 18): calcd. C 37.11, H 4.49, N 14.42, O 22.0, S 22.0; found C 37.22, H 4.24, N 14.21, O 19.4, S 19.7. − Sodium salt: $C_9H_{11}N_3O_3S_2Na$ (295.3): calcd. C 36.61, H 3.41, N 14.23; found C 36.58, H 3.89, N 13.95.

**Oxyrubroflavin (2) (1:1 Mixture of Diastereomers):** Amorphous, orange-red solid, dec. at 200−210 °C. − $[\alpha]_D^{22}$ = −860 (*c* = 0.1, MeOH). − $R_f$ (TLC) = 0.24 (system A), lemon-yellow spot. − UV (MeOH): $\lambda_{max}$ (lg ε) = 197 nm (4.19), 245 (3.98), 280 (sh, 3.88), 352 (4.01), 430 (3.18), 455 (sh, 3.12). − CD (MeOH): $\lambda_{max}$ (Δε) = 208 nm (+8.27), 238 (−1.41), 269 (+1.01), 316 (−0.63), 355 (+0.46), 393 (+0.43), 463 (−5.00). − IR (KBr): $\tilde{\nu}$ = 3340 $cm^{-1}$ (sh, m), 3040 (br., s), 2800 (m), 2695 (w), 1710 (sh, ss), 1690 (ss), 1587 (s), 1550 (s), 1475 (ss), 1420 (m), 1370 (ss), 1275 (s), 1240 (s), 1215 (ss), 1175 (m), 1125 (w), 1060 (s), 1040 (ss), 1010 (s), 960 (s), 940 (m), 890 (m), 850 (m), 750(w), 720 (w), 675 (m), 645 (w). − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.84 (s, 3 H), 2.89 (s, 3 H), 7.20 (s, 1 H), 7.21 (s, 1 H). − $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 2.68 (s, 3 H), 2.76 (s, 3 H), 6.89 (s, 1 H), 6.92 (s, 1 H), 7.10−7.40 (br., NH and $NH_2$). − $^{13}$C NMR (100.6 MHz, $CD_3OD$): δ = 44.7 (Q), 45.1 (Q), 118.4 (D), 118.5 (D) (signals of quat. C atoms not visible). − $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO): δ = 43.1 (Q, $^1J$ = 141 Hz,), 43.8 (Q, $^1J$ = 140 Hz), 112.6 (Dd, $^1J$ = 166, $^3J$ = 5.0 Hz, 2 C), 134.2 (t, $^3J$ = 6.5 Hz), 149.1 (m), 149.2 (m), 161.9 (s), 163.6 (t, $^3J$ = 2.0 Hz). − EI MS: *m*/*z* (%) = 234 ($C_8H_{10}O_4S_2$, 7), 233 ($C_8H_{11}NO_3S_2$, 3), 218 ($C_8H_{10}O_3S_2$, 27), 203 ($C_7H_7O_3S_2$, 21), 202 ($C_8H_{10}O_2S_2$, 15), 201 ($C_8H_9O_2S_2$, 6), 187.99 ($C_7H_8O_2S_2$, 5), 187.96 ($C_6H_4O_3S_2$, 23), 187 ($C_7H_7O_2S_2$, 18), 182 ($C_8H_6OS_2$, 3), 173 ($C_6H_5O_2S_2$, 18), 172 ($C_7H_8O_3S$, 12), 156 (4), 155 ($C_7H_7O_2S$, 7), 94 (28), 85 (10), 63 (17), 45 (30), 44 (42), 43 (HNCO, 100). − $C_9H_{11}N_3O_4S_2 \times 0.5\ H_2O$ (298.3): calcd. C 36.20, H 4.02, N 14.08; found C 36.48, H 4.08, N 14.14.

**Deoxyrubroflavin (8):** Red powder, m.p. 168−169 °C. − $R_f$ (TLC) = 0.77 (system A), 0.66 (system B), 0.38 (EtOAc), yellow spot. − UV (MeOH): $\lambda_{max}$ (lg ε) = 212 nm (4.06), 232 (4.18), 253 (4.16), 278 (4.05), 310 (sh, 3.58), 387 (3.97). − IR (KBr): $\tilde{\nu}$ = 3460 $cm^{-1}$ (s), 3320 (m), 3080 (br., m), 2920 (w), 2790 (w), 1700 (ss), 1670 (sh, s), 1580 (s), 1545 (ss), 1450 (m), 1430 (s), 1350 (m), 1330 (s), 1230 (br., ss), 1205 (sh, ss), 1120 (m), 970 (m), 940 (m), 855 (m), 840 (s), 800 (m), 770, 720, 665, 635 (all w). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.32 (s, 3 H), 2.55 (s, 3 H), 4.80−5.20 (br., 2 H),* 6.15 (d, $^4J$ = 1.8 Hz, 1 H), 6.22 (d, $^4J$ = 1.8 Hz, 1 H), 10.41 (br. s, 1 H),* *exchangeable with $D_2O$. − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.39 (s, 6 H), 6.55 (s, 2 H). − $^{13}$C NMR (100.6 MHz, $CD_3OD$): δ = 15.6 (Q, $^1J$ = 138 Hz, 2 C), 109.9 (D, $^1J$ = 164 Hz, 2 C), 137.1 (t, $^3J$ = 7.0 Hz, 1 C), 147.9 (m, 2 C), 166.1 (s, 1 C), 180.7 (t, $^2J$ = 3.0 Hz, 1 C). − $^{13}$C NMR (100.6 MHz, [D$_6$]DMSO): δ = 14.9, 107.3, 136.1, 143.5, 161.1, 163.7. − EI MS: *m*/*z* (%) = 257 (15) [$M^+$], 242 ($C_8H_8N_3O_2S_2$, 7), 240 ($C_9H_8N_2O_2S_2$, 5), 213 ($C_8H_9N_2OS_2$, 8), 212 (5), 211 (11), 210 ($C_8H_8N_3O_2S$, 100), 201 ($C_8H_{11}NOS_2$, 10), 200 ($C_7H_8N_2OS_2$, 4), 198 ($C_8H_8NOS_2$, 11), 197

($C_8H_7NOS_2$, 4), 188 (4), 187 (5), 186 ($C_8H_{10}OS_2$, 53), 185 ($C_7H_7NOS_2$, 47), 184 ($C_7H_6NOS_2$, 12), 172 (4), 171 ($C_6H_7N_2S_2$, 13), 157 ($C_6H_7NS_2$, 20), 153 (29), 137 (5), 109 (12), 69 (41). − HRMS [$C_9H_{11}N_3O_2S_2$, $M^+$]: calcd. 257.0293; found 257.0288. − $C_9H_{11}N_3O_2S_2$ (257.3): calcd. C 42.01, H 4.31, N 16.33; found C 41.70, H 4.24, N 16.37.

**Leucorubroflavin (4):** Compound **4** is easily oxidized in air, and should be immediately characterized after chromatographic purification. − Colorless, amorphous solid; slightly soluble in MeOH, easily soluble in DMSO. − M.p. 167−170 °C (dec.). − $R_f$ (TLC) = 0.53 (system A), 0.46 (system C), strong quenching of fluorescence at 254 nm. − IR (KBr): $\tilde{\nu}$ = 3465 $cm^{-1}$ (m), 3350 (br. m), 3100 (br. m), 3020 (w, sh), 1732 (w, sh), 1705 (ss), 1600 (s), 1475 (m, sh), 1455 (s), 1380, 1365, 1335 (w), 1266 (ss), 1210 (ss), 1134 (s), 1015 (ss), 972 (m), 870 (w), 830 (m), 800 (w), 660 (w), 590 (w), 505 (m), 450 (w). − $^1$H NMR (400 MHz, [D$_6$]DMSO): δ = 2.55 (s, 3 H), 2.73 (s, 3 H), 6.91 (d, $^4J$ = 2.5 Hz, 1 H), 7.32 (d, $^4J$ = 2.5 Hz, 1 H), 7.80 (br. s, ca. 2 H, exchange with $D_2O$). − Pigment **1** is quantitatively transformed into **4** on shaking for 2 min with Zn/AcOH. Preparative TLC on silica (system A) yielded **4** as colorless crystals that were identical with the natural product.

**Rubroflavin Monomethyl Ether (5):** To a solution of **1** × $H_2O$ (10 mg, 0.35 mmol) in 3 mL of MeOH were added 10 drops of water and a large excess of a solution of diazomethane in ether. After stirring the mixture with some silica gel (Mallinckrodt) for 2.5 h at 20 °C, the excess of diazomethane was removed with $N_2$ and the solvent evaporated. The residue was purified on analytical TLC plates [silica 60 (Merck) with concentration zone, system A]. Yield: 7 mg (70%). − Orange-red needles from $CH_2Cl_2$. − M.p. 178−180 °C (dec.). − $[\alpha]_D^{20}$ = −1700 (*c* = 0.04, MeOH). − $R_f$ (TLC) = 0.76 (system A), orange-yellow spot. − UV/Vis (MeOH): $\lambda_{max}$ (lg ε) = 207 nm (4.03), 227 (sh, 3.90), 242 (3.89), 280 (3.86), 341 (3.68), 397 (sh, 3.51). − CD (MeOH): $\lambda_{max}$ (Δε) = 222 nm (−15.15), 246 (+9.68), 281 (−1.56), 296 (+2.65), 344 (−9.68), 395 (+3.90), 465 (−10.46). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.50 (br. s, 3 H), 2.86 (s, 3 H), 4.03 (s, 3 H), 6.90 (dq, $^4J$ = 2.6, $^5J$ = 0.2 Hz, 1 H), 7.62 (d, $^4J$ = 2.6 Hz, 1 H). − $^1$H NMR (400 MHz, [D$_6$]acetone): δ = 2.56, 2.77, 4.04 (each s, 3 H), 7.03, 7.49 (each d, $^4J$ = 2.6 Hz, 1 H). − $^{13}$C NMR (100.6 MHz, [D$_6$]acetone): δ = 15.4 (Q, $^1J$ = 140 Hz), 44.6 (Q, $^1J$ = 141 Hz), 56.6 (Q, $^1J$ = 146 Hz), 106.3 (Dd, $^1J$ = 168, $^3J$ = 5.0 Hz), 112.6 (Dd, $^1J$ = 163.5, $^3J$ = 5.0 Hz), 137.7 ("t", $^3J$ = 7.2 Hz), 146.6 (m, *J* < 2 Hz), 150.0 (m), 162.6 (br. s), 164.6 (qm, $^3J$ = 4.0 Hz). − EI MS: *m*/*z* (%) = 289 ($C_{10}H_{15}N_3O_3S_2$, $M^+$ + 2, 1), 287 (1) [$M^+$], 272 ($C_9H_{10}N_3O_3S_2$, 45), 257 (5), 256 ($C_9H_{10}N_3O_2S_2$, 5), 243 ($C_9H_{11}N_2O_2S_2$, 7), 231 ($C_9H_{13}NO_2S_2$, 6), 227 ($C_9H_{11}N_2OS_2$, 20), 217 ($C_8H_{11}NO_2S_2$, 9), 216 ($C_9H_{12}O_2S_2$, 82), 215 ($C_8H_{11}N_2OS_2$, 8), 214 (7), 213 (38), 202 ($C_8H_{10}O_2S_2$, 17), 201 ($C_8H_9O_2S_2$, 100), 200 ($C_8H_{10}NOS$, 29), 199 (7), 198 ($C_8H_8NOS_2$, 14), 184 ($C_8H_8OS_2$, 35), 183 (21), 170 ($C_8H_{10}O_2S$, 24), 168 (7), 167 ($C_8H_9NOS$, 24), 165 (15), 153 (13), 152 (20), 138 (22), 121 (31), 57 (34), 45 (30), 44 (38), 43 (HNCO, 31). − HRMS: [$C_{10}H_{13}N_3O_3S_2$, $M^+$]: calcd. 287.0399; found 287.0392. − $C_{10}H_{13}N_3O_3S_2$ (287.3): calcd. C 41.80, H 4.56, N 14.62; found C 42.13, H 4.82, N 14.18.

**Oxidation of 1 with Hydrogen Peroxide:** A solution of **1** × $H_2O$ (8 mg, 0.028 mmol) in 2 mL of acetic acid was stirred with 20 drops of 30% $H_2O_2$ for 1 h at room temperature. After evaporation of the solvent, the residue was purified by preparative TLC on silica gel (system A) followed by chromatography on a Sephadex LH-20 column (eluent MeOH). Yield: 3.6 mg (45%). The compound was identical with "natural" oxyrubroflavin (**2**) but exhibited a higher optical rotation: $[\alpha]_D^{22}$ = −1035 (*c* = 0.2, MeOH).

**Thermolysis of Rubroflavin (1):** Rubroflavin (4.5 mg) was decomposed in a stream of argon for 5 min at 190 °C. The product was dissolved in MeOH and purified by TLC on analytical silica gel 60 $F_{254}$ plates with $CHCl_3$/MeOH, 8:1. The $UV_{254}$-active zone ($R_F$ ≈ 0.40) was extracted with $CHCl_3$/MeOH (3:1) to yield, after evaporation of the solvent, 1 mg (30%) of (−)-(*S*)-3-methanesulfinyl-5-(methylthio)phenol (**6**). − Colorless, amorphous solid, grey spot on standing on TLC plates. − $[\alpha]_D^{20}$ = −200 (*c* = 0.01, MeOH). − UV ($CH_3OH$): $\lambda_{max}$ = 218 nm, 237, 255 (sh), 297. − CD ($CH_3OH$): $\lambda_{max}$ (Δε)= 226 (0), 241 nm (−13.63) (Figure 2). − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.48 (s, 3 H), 2.75 (s, 3 H), 6.80 ("dd", $^4J$ = 1.6, 1.6 Hz, 2 H), 6.98 ("dd", $^4J$ = 1.6, 1.6 Hz, 1 H). − EI MS: *m*/*z* (%) = 203 (19), 202 (95) [$M^+$], 188 (20), 187 (100), 186 (9), 156 (10), 141 (7), 139 (15), 124 (13), 85 (19), 45 (29). − HRMS [$C_8H_{10}O_2S_2$, $M^+$]: calcd. 202.0122; found 202.0105.

**Thermolysis of Oxyrubroflavin (2):** Compound **2** (2 mg) was decomposed under argon for 3 min at 210 °C. The product was dissolved in MeOH and separated by preparative TLC on silica gel $60_{F254+366}$ (eluent $CHCl_3$/MeOH, 6:1). The main zone ($R_F$ = 0.47) was extracted with $CHCl_3$/MeOH, 3:1 to yield (3*S*,5*RS*)-3,5-bis(methanesulfinyl)phenol (**7**) (0.5 mg) as a colorless solid. − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.82 (s, 6 H), 7.23 (m, 2 H), 7.41 (2 × t, 1 H) (1:1 mixture of diastereomers). − EI MS: *m*/*z* (%) = 220 (7), 219 (8), 218 (92) [$M^+$], 205 (6), 204 (7), 203 (80), 202 (10), 201 (11), 190 (9), 189 (12), 188 (100), 187 (24), 186 (6), 173 (7), 172 (26), 155 (15), 45 (41). − [$C_8H_{10}O_2S_2$, $M^+$]: calcd. 218.0072, found 218.0035.

***O*-Acetyldeoxyrubroflavin (9):** Compound **8** (51 mg, 0.2 mmol) was stirred at room temperature for 30 min with acetic anhydride (2 mL) and a few crystals of DMAP. After addition of water (5 mL), the mixture was stirred for additional 30 min. Then, the mixture was extracted with EtOAc, and the organic layers were dried with $Na_2SO_4$. Chromatography of the residue on silica gel with EtOAc/hexanes (1:1) yielded **9** (55 mg, 93%), red powder, m.p. 172 °C, $R_f$ (TLC) = 0.51 (EtOAc). − UV (MeOH): $\lambda_{max}$ (lg ε) = 200 nm (4.09), 237 (4.35), 255 (sh, 4.28), 275 (sh, 4.16), 385 (3.73). − IR (KBr): $\tilde{\nu}$ = 1760 $cm^{-1}$ (ss), 1700 (ss), 1600 (m), 1575 (s), 1540 (m), 1480 (s). − $^1$H NMR ($CDCl_3$): δ = 2.28 (s, 3 H), 2.41 (s, 6 H), 5.90 (br. s, 1 NH), 6.50 (br. s, 1 NH), 6.83 (s, 2 H). − EI MS: *m*/*z* (%) = 301 (1) [$M^+$ + 2], 299 (0.3) [$M^+$], 284 (2), 257 (39), 255 (27) 241 (7), 228 (8), 186 (28), 185 (27), 170 (51), 153 (19), 138 (27), 85 (7), 69 (9), 45 (19), 44 (33), 43 (100). − $C_{11}H_{13}N_3O_3S_2$ (299.3).

***O*,*N*-Diacetyldeoxyrubroflavin (10):** Acetylation, using the same procedure as for **9** but at 60 °C, afforded diacetate **10** (61 mg, 90%). Red powder, m.p. 155 °C, $R_f$ (TLC) = 0.57 (EtOAc). − UV (MeOH): $\lambda_{max}$ (lg ε) = 198 nm (4.19), 240 (4.34), 253 (4.28), 275 (sh, 4.08), 398 (3.76). − IR (KBr): $\tilde{\nu}$ = 1750 $cm^{-1}$ (ss), 1720 (ss), 1575 (m), 1545 (s), 1460 (s). − $^1$H NMR ($CDCl_3$): δ = 2.34 (s, 3 H), 2.43 (s, 6 H), 2.60 (s, 3 H), 6.81 (s, 2 H), 9.0 (br., NH). − EI MS: *m*/*z* (%) = 341 (3) [$M^+$], 340 (4), 323 (3), 269 (5), 255 (4), 227 (11), 200 (16), 186 (22), 151 (12), 85 (6), 59 (14), 45 (13), 44 (14), 43 (100). − $C_{13}H_{15}N_3O_4S_2$ (341.3).

**5-Benzyloxy-1,3-dichloro-2-nitrobenzene (15):** To a solution of 3,5-dichloro-4-nitrophenol (**14**)[19] (15.5 g, 75 mmol) in 30 mL of 10% aqueous NaOH and 100 mL of ethanol benzyl chloride (12.9 mL, 110 mmol) was added dropwise with stirring. After the addition, the mixture was heated for 30 min with a water bath and then worked up as usual. Yield 19.3 g (86%), pale yellow crystals, m.p. 54 °C, $R_f$ (TLC) = 0.78 ($CH_2Cl_2$). − $^1$H NMR ($CDCl_3$): δ = 5.10 (s, 2 H), 6.96 (s, 2 H), 7.45 (br. s, 5 H). − $C_{13}H_9Cl_2NO_3$ (298.1): calcd. C 52.37, H 3.04, N 4.70; found C 52.34, H 3.09, N 4.61.

**5-Benzyloxy-1,3-bis(methylthio)-2-nitrobenzene (16):** Sodium methanethiolate[26] (14 g, 200 mmol) was slowly added to **15** (18 g, 60 mmol) in 200 mL of dry acetone. The mixture was heated under reflux for 6 h, and then cooled to room temperature. The yellow solid was filtered off, washed with water and recrystallized from MeOH. Yield 16 g (84%), bright yellow crystals, m.p. 156 °C, $R_f$ (TLC) = 0.64 ($CHCl_3$). − $^1$H NMR ($CDCl_3$): δ = 2.36 (s, 6 H), 5.14 (s, 2 H), 6.62 (s, 2 H), 7.41 (s, 5 H). − EI MS: *m/z* (%) = 321 (35) [$M^+$], 308 (6), 230 (5), 92 (20), 91 (100), 65 (15). − $C_{15}H_{15}NO_3S_2$ (321.4): calcd. C 56.06, H 4.70, N 4.36; found C 55.80, H 4.54, N 4.25.

**4-Benzyloxy-2,6-bis(methylthio)aniline Hydrochloride (17):** Tin dust was added to a hot solution of **16** (16 g, 50 mmol) in 100 mL of MeOH and 100 mL of 50% aqueous HCl until the yellow color disappeared. The mixture was cooled with ice, made alkaline with diluted aqueous NaOH, and extracted with $Et_2O$. The extracts were washed with water, dried ($Na_2SO_4$) and treated with gaseous HCl to yield the crystalline hydrochloride (14.6 g, 90%), m.p. 116 °C, $R_f$ (TLC) = 0.37 ($CHCl_3$, free amine). − $^1$H NMR ($CD_3OD$): δ = 2.46 (s, 6 H), 5.14 (s, 2 H), 7.06 (s, 2 H), 7.49 (br. m, 5 H). − $C_{15}H_{17}NOS_2 \times HCl$ (327.9): calcd. C 54.95, H 5.53, N 4.27; found C 55.18, H 5.40, N 4.19.

**4-Benzyloxy-2,6-bis(methylthio)phenyldiazocyanide (18):** A saturated aqueous solution of $NaNO_2$ (3.2 g, 45 mmol) was added under vigorous stirring at 0 °C to **17** (13.1 g, 40 mmol) in 20 mL of 50% aqueous HCl, and the mixture was stirred at this temperature for 1 h. Then, the solution was buffered with aqueous $Na_2CO_3$, and a solution of KCN (10 g) in water was added dropwise over 30 min. The color of the stirred reaction mixture changed from yellow to red, and after 5 h the dark red precipitate was collected by filtration and recrystallized from EtOAc/hexanes. Yield: 12.6 g (95%), dark red needles, m.p. 167 °C, $R_f$ (TLC) = 0.65 (EtOAc), 0.43 ($CHCl_3$). − UV (MeOH): $\lambda_{max}$ (lg ε) = 205 nm (sh, 4.29), 238 (4.20), 258 (4.24), 275 (sh, 3.98), 338 (4.01), 415 (4.24), 500 (sh, 3.68). − $^1$H NMR ($CDCl_3$): δ = 2.31 (s, 6 H), 5.20 (s, 2 H), 6.54 (s, 2 H), 7.38 (s, 5 H). − EI MS: *m/z* (%) = 330 (4) [$M^+$ + 1], 329 (10) [$M^+$], 318 (8), 317 (13), 316 (61), 276 (20), 238 (11), 200 (49), 184 (7), 169 (11), 141 (8), 109 (7), 92 (37), 91 (100), 65 (30). − $C_{16}H_{15}NOS_2$ (329.4): calcd. C 58.33, H 4.59, N 12.75; found C 58.02, H 4.60, N 12.37.

**Deoxyrubroflavin (8):** To a vigorously stirred solution of **18** (329 mg, 1 mmol) in 10 mL of acetic acid was added $TiCl_4$ (0.44 mL, 4 mmol) at 20 °C. Water (0.3 mL) was then slowly added, and the stirring was continued for 48 h at 25−30 °C. The dark reaction mixture was poured on ice, and after addition of satd. aqueous soda, the resulting $TiO_2$ slurry was immediately extracted with EtOAc. The extracts were filtered through Celite and co-evaporated with toluene in vacuo (3 ×). The residue was then dissolved in MeOH and purified by chromatography on Sephadex LH-20 to yield **8** (187 mg, 73%) as a red powder. The physical and spectroscopic data were in agreement with those of the natural product.

**(*S*)-MTPA Ester of Deoxyrubroflavin (19):** To a solution of (*S*)-MTPA (468 mg, 2 mmol), DMAP (10 mg), and **8** (514 mg, 2 mmol) in 10 mL of DMF was added DCC (206 mg, 1 mmol) at 0 °C. The solution was kept at 0 °C for 30 min and then at 20 °C for 15 min. After the addition of water, the mixture was extracted with $CH_2Cl_2$. The extracts were dried with $MgSO_4$ and concentrated in vacuo. Flash chromatography on silica gel with $CH_2Cl_2$/MeOH (20:1) yielded **19** (833 mg, 88%). After recrystallization from 2-propanol 756 mg (80%) red crystals were obtained, m.p. 58−60 °C, $R_f$ (TLC) = 0.49 ($CH_2Cl_2$/MeOH 20:1), 0.58 (EtOAc). − $[\alpha]_D^{20}$ = −111 ($c$ = 0.05, $CHCl_3$). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.40 (s, 6 H), 3.60 (s, 3 H), 5.85 (br., NH), 6.48 (br., NH), 6.61 (s, 2 H), 7.48−7.73 (br. m, 5 H). − $C_{19}H_{18}F_3N_3O_4S_2$ (473.5).

**Stereoselective Sulfoxidation of 19:** An oven-dried flask with degassed $CH_2Cl_2$ (10 mL) was flushed with argon and charged via syringe at 20 °C with $Ti(OiPr)_4$ (0.3 mL, 1 mmol) and (2*S*,3*S*)-DET (0.34 mL, 2 mmol). After addition of water (18 μL, 1 mmol), the mixture was stirred until it became homogenous (15−20 min). Then, ester **19** (342 mg, 1 mmol) was added, and the mixture was cooled to −20 °C. After injection of a solution of TBHP (100 mg, 1.1 mmol) in toluene, the mixture was vigorously stirred and kept at −20 °C until an optimal formation of sulfoxide **20** was indicated by TLC. To avoid over-oxidation, $Me_2S$ (0.5 mL) was added during the workup. Then, the mixture was stirred for 1 h, warmed to 20 °C and quenched with water (1 mL). After continued stirring for 1 h, the $TiO_2$ was removed by filtration through Celite. The filter cake was carefully washed with EtOAc, and the combined organic phases were dried with $Na_2SO_4$. The solvent was evaporated in vacuo, and the products were separated by chromatography on Sephadex LH-20 (eluent MeOH) or silica gel ($CH_2Cl_2$/MeOH, 20:1).

**(*S*)-MTPA Ester of Rubroflavin (20):** Yield 186 mg (38%), mixture of diastereomers (*de* 67%). Repeated recrystallization from $CCl_4$ afforded the pure (*S*,*S*) diastereomer **20**. Red crystals, m.p. 75−80 °C, $R_f$ (TLC) = 0.23 ($CH_2Cl_2$/MeOH 20:1), 0.27 (EtOAc). − $[\alpha]_D^{20}$ = −932 ($c$ = 0.1, MeOH). − UV (MeOH): $\lambda_{max}$ (lg ε) = 205 nm (sh, 4.39), 262 (4.13), 330 (sh, 3.59), 395 (sh, 3.52). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.47, 2.88, 3.67 (each s, 3 H), 5.94, 6.48 (each br. s, NH), 7.13 (d, $^4J$ = 2.5 Hz, 1 H), 7.48 (m, 3 H), 7.63 (m, 2 H), 7.79 (d, $^4J$ = 2.5 Hz, 1 H). − $C_{19}H_{18}F_3N_3O_5S_2$ (489.5).

**(*S*)-MTPA Ester of Oxyrubroflavin (21):** Yield 111 mg (22%), mixture of diastereomers. Light red crystals, m.p. 100−103 °C, $R_f$ (TLC) = 0.44 (system B). − $[\alpha]_D^{20}$ = −978 ($c$ = 0.06, $CHCl_3$). − UV (MeOH): $\lambda_{max}$ (lg ε) = 197 nm (4.31), 235 (sh, 3.96), 260 (3.93), 330 (sh, 3.60), 345 (sh, 3.46), 440 (2.67). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.86, 2.94, 3.68 (each s, 3 H), 6.16, 6.71 (each br. s, NH), 7.40−7.70 (m, 5 H), 8.02, 8.08 (each s, 1 H, diastereomers). − $C_{19}H_{18}F_3N_3O_6S_2$ (505.5).

**(*S*)-MTPA Ester of Craniformin (22):** Yield 41 mg (8%), mixture of diastereomers, 86% *de*. Orange-red powder, m.p. 83−85 °C, $R_f$ (TLC) = 0.20 (EtOAc). − $[\alpha]_D^{20}$ = −513 ($c$ = 0.07, $CHCl_3$). − UV (MeOH): $\lambda_{max}$ (lg ε) = 197 nm (4.35), 238 (3.98), 268 (3.93), 310 (3.75), 430 (2.70). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 2.92, 3.45, 3.70 (each s, 3 H), 6.56, 6.80 (each br. s, 1 NH), 7.51 (m, 3 H), 7.61 (m, 2 H), 8.15 (d, $^4J$ = 2.5 Hz, 1 H), 8.40 (d, $^4J$ = 2.5 Hz, 1 H). − $C_{19}H_{18}F_3N_3O_7S_2$ (521.5).

**(+)-3-Bromocamphor-10-sulfonic Acid Ester of Deoxyrubroflavin (23):** To **8** (514 mg, 2 mmol) in $CH_2Cl_2$ (20 mL) was added triethylamine (0.28 mL, 2 mmol), a few crystals of DMAP, and (+)-3-bromocamphor-10-sulfonyl chloride (726 mg, 2.2 mmol). The mixture was stirred for 20 min at room temperature and then concentrated in vacuo. Flash chromatography on silica gel with $CH_2Cl_2$/MeOH (10:1) yielded **23** (946 mg, 86%). Red crystals (EtOH), m.p. 100−101 °C, $R_f$ (TLC) = 0.54 (system B), 0.52 (EtOAc). − $[\alpha]_D^{20}$ = +82 ($c$ = 0.1, $CHCl_3$). − UV (MeOH): $\lambda_{max}$ (lg ε) = 191 nm (4.09), 230 (4.21), 252 (sh, 4.19), 273 (sh, 4.08), 377 (3.63). − CD (MeOH): $\lambda_{max}$ (Δε) = 308 nm (+2.11). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 1.02 (s, 3 H), 1.24 (s, 3 H), 1.55 (m, 1 H), 1.78 (m, 1 H), 2.00−2.55 (m, 3 H), 2.42 (s, 6 H), 3.28 (d, $^2J$ = 15.0 Hz, 1 H), 3.83 (d, $^2J$ = 15.0 Hz, 1 H), 4.62 (d, $^3J$ = 5.0 Hz, 1 H), 5.40 (br. s, NH), 6.40

(br. s, NH), 6.95 (s, 2 H). − $C_{19}H_{24}BrN_3O_5S_3$ (550.5): calcd. C 41.46, H 4.39, N 7.63; found C 41.35, H 4.46, N 7.41.

**(+)-3-Bromocamphor-10-sulfonic Acid Ester of Rubroflavin (24):** Stereoselective sulfoxidation of **23** (550 mg, 1 mmol) as described above yielded after chromatography **24** (198 mg, 35%) as a mixture of diastereomers (*de* 72%). − CD (MeOH): $\lambda_{max}$ ($\Delta\varepsilon$) = 226 nm (−1.79), 246 (+4.14), 270 (−1.87), 303 (+1.22), 338 (−1.06), 393 (+1.95), 465 nm (−3.82). − Repeated recrystallization from 2-propanol gave the pure (*S*,*S*) diastereomer **24**. Red powder, m.p. 194 °C, $R_f$ (TLC) = 0.37 (system B), 0.15 (EtOAc). − $[\alpha]_D^{20}$ = −713 (*c* = 0.15, $CHCl_3$). − UV (MeOH): $\lambda_{max}$ (lg ε) = 208 nm (4.23), 268 (4.17), 325 (sh, 3.54), 398 (3.50). − $^1$H NMR (400 MHz, $CDCl_3$): δ = 1.00, 1.25 (each s, 3 H), 1.72, 1.98, 2.17, 2.32, 2.38−2.55 (each m, 1 H), 2.50, 2.83 (each s, 3 H), 3.22, 3.81 (each d, $^2J$ = 15.0 Hz, 1 H), 4.59 (d, $^3J$ = 4.5 Hz, 1 H), 5.37, 6.23 (each br. s, NH), 7.41, 7.82 (each d, $^4J$ = 2.5 Hz, 1 H). − $C_{19}H_{24}BrN_3O_6S_3$ (566.5): calcd. C 40.28, H 4.27, N 7.42; found C 40.31, H 4.36, N 6.92.

**General Procedure for Ester Hydrolysis:** A solution of 0.2 N NaOH was added dropwise to the ester (0.5 mmol) in 50 mL of MeOH until the color of the solution changed from red to yellow. After complete hydrolysis (TLC control on silica gel, EtOAc), the solution was neutralized with diluted HCl. The solvent was then evaporated, and the aqueous residue extracted with EtOAc. The dried extracts were concentrated in vacuo and purified by chromatography on Sephadex LH-20 (MeOH) to afford the pure pigments in > 90% yield. The optically pure auxiliaries can be easily recovered by extraction of the acidified aqueous solution with EtOAc.

**(−)-(*S*)-Rubroflavin (1):** From pure (*S*,*S*)-**20**. Red needles, m.p. 183−185 °C (dec.) − $[\alpha]_D^{20}$ = −2140 (*c* = 0.1, MeOH). − Physical and spectroscopic data identical with those of the natural compound.

**Oxyrubroflavin (2):** From ester **21** (mixture of stereoisomers). Orange-red powder, m.p. 210 °C (dec.). − $[\alpha]_D^{20}$ = −920 (*c* = 0.1, MeOH). − Spectroscopic data identical with those of the compound isolated from *C. rubro-flava*.

**(−)-(*S*)-Craniformin (3):** From ester **22** (*ee* 86%). Orange-red powder, m.p. 168−170 °C, $R_f$ (TLC) = 0.17 ($CH_2Cl_2$/MeOH, 5:1), yellow spot. − $[\alpha]_D^{20}$ = −1034 (*c* = 0.05, MeOH). − UV (MeOH): $\lambda_{max}$ (lg ε) = 198 nm (4.29), 253 (3.96), 285 (sh, 3.77), 345 (3.96), 430 (3.67). − IR (KBr): $\tilde{\nu}$ = 3440 $cm^{-1}$ (s), 3340 (br., s), 2920 (m), 2850 (w), 1705 (br., ss), 1590 (s), 1490 (m), 1460 (m), 1425 (m), 1355 (m), 1300 (ss), 1280 (br., ss), 1205 (s), 1185 (s), 1145 (ss), 1135 (ss), 1070 (s), 1030 (m), 1005 (s), 970 (s), 885 (m), 790 (m), 770 (w), 745 (w). − $^1$H NMR (400 MHz, $CD_3OD$): δ = 2.87, 3.44 (each s, 3 H), 7.78, 7.89 (each d, $^4J$ = 2.0 Hz, 1 H). − EI MS: *m*/*z* (%) = 298 (5), 296 (6), 250 (11), 244 (6), 234 (36), 230 (11), 220 (13), 219 (54), 218 (100), 204 (10), 185 (6), 155 (15), 139 (36), 127 (10), 124 (9), 111 (10), 95 (12), 94 (14), 85 (15), 79 (10), 71 (12), 63 (18), 45 (17), 44 (16), 43 (41). − $C_9H_{11}N_3O_5S_2$ (305.3).

## Acknowledgments

This work was supported by the Deutsche Forschungsgemeinschaft. B. F. thanks the Fonds der Chemischen Industrie for a Promotionsstipendium. We are grateful to Dr. R. Baird for kindly supplying the fungi and Dr. G. Eckhardt, University of Bonn, for MS measurements.

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$$\Delta\varepsilon = \sum_{i=1}^{N} w_i \cdot \Delta\varepsilon_i,$$
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*N* is the number of located stationary points and Δε the superposition of Gaussians; $w_i$ and $E_i$ are the Boltzmann factor and the energy of the *i*th local minimum, respectively. The Gaussians were generated using an empirical half bandwidth of 7 nm at $e^{-1}$ of the maximum.
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Received December 27, 2000
[O00662]