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Uredinorubellins and Caeruleoramularin, Photodynamically Active Anthraquinone Derivatives Produced by Two Species of the Genus *Ramularia*

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Both the phytopathogenic fungus *Ramularia collo-cygni* and the hyperparasite *R. uredinicola* biosynthesize a number of red and yellow anthraquinone derivatives called rubellins. The new compounds uredinorubellins I and II, which were isolated from *R. uredinicola*, contribute to understanding the biosynthesis pathway that leads from simple anthraquinones to the rubellins. In addition, we isolated for the first time such simple compounds as chrysophanol and helminthsporin from both *Ramularia* species. A blue compound isolated from the mycelium of *R. collo-cygni* was revealed to be a unique 9,4-anthracenedione derivative. Structure elucidation by ^{1}H and ^{13}C NMR of the new but unstable compound caeruleoramularin was possible only by feeding the fungus different labeled ^{13}C acetates. The photodynamic activity of the uredinorubellins was comparable to rubellin D, whereas chrysophanol and caeruleoramularin did not display such activity.

The genus *Ramularia* belonging to the hyphomycetes was described in morphological detail by Braun.[1] Recently a new species was found in Guilan Province in Iran and determined to be *R. uredinicola* Khodap. & U. Braun. This fungus lives as a hyperparasite on the uredosori of the rust fungus *Melampsora* sp., which attacks leaves of *Salix babylonica*.[2]

The phytopathogenic fungus *R. collo-cygni* (Sutton & Waller) initiates a leaf spot disease in barley and other cereals.[3,4] Due to early ripening of infected plants, the pathogen causes yield losses of up to 20%. Recently, we provided evidence that *R. collo-cygni* produces photodynamically active non-host-specific phytotoxins, namely, the anthraquinone derivatives rubellin D (**1**),[5] rubellin B (**2**),[6] and rubellin C (**3**).[7] Additionally, we elucidated the structure of rubellin A (**4**) and two new rubellins: 14-dehydrorubellin D and rubellin E. The latter is related to rubellins B and D but does not possess a lactone ring. Rubellins were also found in *R. uredinicola*.[8]

By [1-^{13}C]-acetate and [2-^{13}C]-acetate incorporation into the rubellins, we showed that the anthraquinone derivatives were biosynthesised via the polyketide pathway. In addition, the labeling pattern after feeding [U-$^{13}C_6$]-glucose demonstrated the existence of the fungal folding mode of the poly-β-keto chain.[8]

In this paper we report the isolation, structural elucidation, and bioactivity of novel compounds involved in the biosynthesis pathway of the rubellins. Additionally, a new anthracenedione derivative was isolated and its structure elucidated.

Results and Discussion

Searching for possible intermediates of the biosynthesis pathway of rubellins, we focused on minor components. The new compounds uredinorubellins I (**5**) and II (**6**) were subsequently isolated from a standing liquid culture of *R. uredinicola*.

The molecular masses of **5** and **6** were determined as 542 and 526 Da, respectively. Their masses are identical to those of rubellins B/D (**2**, **1**) and A/C (**4**, **3**), respectively. This analogy was supported by UV−vis spectral analysis: the red uredinorubellin I (**5**) reached maximum absorbance at 499 nm, the yellow uredinorubellin II (**6**) at 440 nm. This difference is probably due to the existence of an additional hydroxy group in the anthraquinone unit of the red compounds. The typical lactone absorption band observed in rubellins at 1735 cm^{-1} in the IR spectrum was not observed for either new compound.

^{1}H and ^{13}C NMR data (DEPT and HSQC) showed that in uredinorubellins I and II (**5**, **6**) the lactone group present in rubellins A/B (**4**, **2**) is replaced by a simple carbonyl group. As shown in Table 1, C-25 carbonyl carbons have higher chemical shifts (δ_C 202.8 and 202.9, respectively) and C-15 exists as a quaternary carbon atom. The key HMBC and COSY correlations are shown in Figure 1. In addition, coupling data from ^{1}H−^{1}H-COSY, ^{1}H−^{1}H-NOESY, and ^{13}C−^{1}H-correlation HMBC (Supporting Information) unambiguously confirm the structures of uredinorubellins I (**5**) and II (**6**).

The structures of both uredinorubellins support the hypothesis that the lower part originates from an anthraquinone unit. Therefore, it is likely that uredinorubellins I and II (**5**, **6**) are precursors that react in a Baeyer−Villiger oxidation event to generate the seven-membered lactones rubellins A and B (**4**, **2**), respectively. Conversion to the more stable five-membered lactones rubellins C and D (**3**, **1**) has been described previously.[8]

In light of these results, we hoped to identify the anthraquinone units that serve as primary precursors. Indeed, we were able to isolate two relatively apolar compounds from *R. uredinicola* mycelia. Their structures were identified as helminthosporin (**7**) and chrysophanol (**8**)[12−14] using chromatographic and spectroscopic methods. However, because of the symmetry conditions of helminthosporin (**7**), two ^{13}C signals in the NMR spectrum (C-8 and C-10a) were changed in comparison to the literature (Supporting Information).[13]

There is evidence that anthraquinones act as precursors to dimeric anthraquinones via oxidative coupling. For example, peroxidases catalyze the oxidation of alizarin and purpurin anthraquinones to the corresponding 3,30-bializarin and 3,30-bipurpurin, respectively. In addition, 2,20-biquinizarin is formed from quinizarin anthracenone.[15]

Such oxidative coupling of chrysophanol (**8**) between C-4 and C-5 allowed the biosynthesis of a dimeric anthraquinone, a precursor

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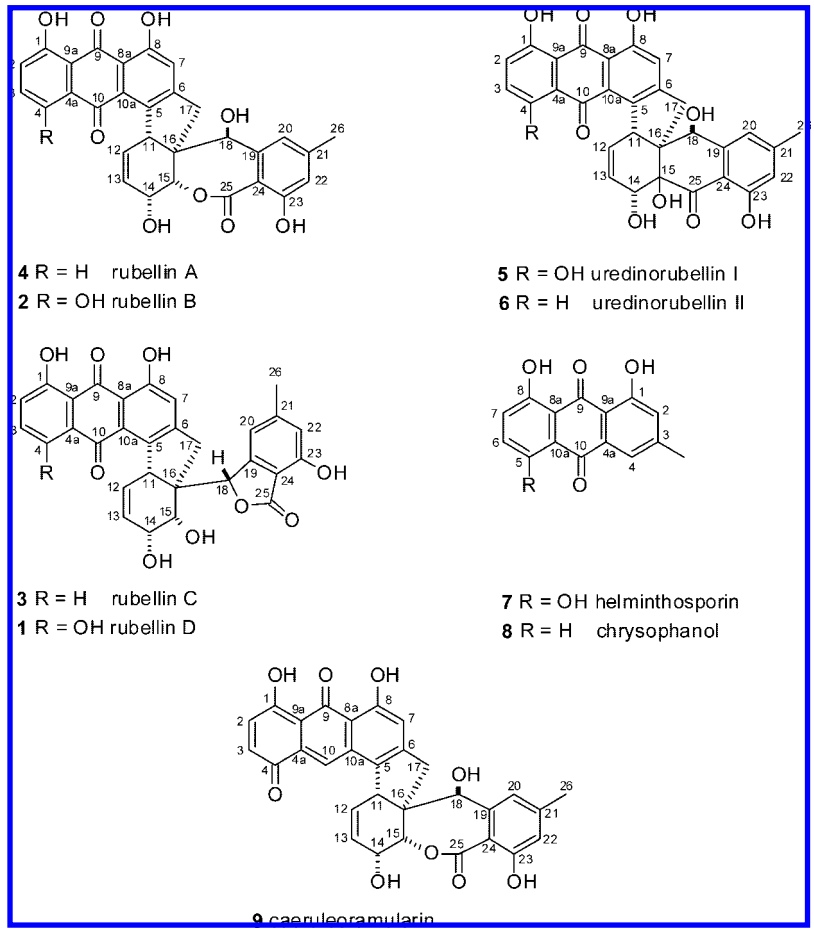
Chart 1



of uredinorubellin II (**6**). The corresponding precursor of uredinorubellin I (**5**) could be formed via an analogous reaction between helminthosporin (**7**) and chrysophanol (**8**). It should be noted that the C-atom numbering of rubellins by Arnone et al.,[16,17] which differs from the usual anthraquinone nomenclature, was used.

Uredinorubellins I and II (**5**, **6**), helminthosporin (**7**), and chrysophanol (**8**) were also detectable in cultures of *R. collo-cygni*, albeit at lower levels (see Table 2).

In a previous study, we identified a blue compound in mycelia and culture filtrate during rubellin B production.[8] HRMS analysis revealed the empirical formula to be $C_{30}H_{22}O_9$. Although its molecular mass of 526 Da is identical to that of the yellow rubellins (**3**, **4**), obvious differences were apparent in the UV−vis spectrum. The shift of λ_{max} from 443 nm (rubellins A and C) to 564 nm for the new substance indicated an alteration of the chromophore. We named the new compound caeruleoramularin (**9**). As expected, treatment with NaOH resulted in a bathochromic shifting to 593 nm.

^{13}C NMR spectroscopy of the native caeruleoramularin (**9**) over extended periods was not possible because of its instability. Although measurements were possible at a lower concentration of 2 mg/mL, only very weak signals were obtained. In order to increase the strength of the signal, we fed differentially labeled ^{13}C precursors to the culture medium. Assuming that caeruleoramularin (**9**) and the rubellins (**1**−**4**) are structurally related and hence part of the same biosynthesis pathway, we used either [1-^{13}C]-acetate or [2-^{13}C]-acetate, with alternative feeding of both precursors, avoiding interfering $^{13}C-^{13}C$ couplings. In this way we successfully obtained the assignments of the corresponding signals in ^{1}H, ^{13}C, DEPT, and HSQC spectra (Table 1).

2D NMR spectra (HMBC, COSY, TOCSY, and NOESY, Supporting Information) supported our model of the structure of caeruleoramularin (**9**) in which the lower rings are identical with those of rubellins A (**4**) and B (**2**). However, major differences in the upper parts of caeruleoramularin (**9**), compared with the rubellins, were apparent. H-2 (δ_H 7.23) gave HMBC cross-peaks with C-3 (δ_C 140.7), C-9a (δ_C 113.6), and C-9 (δ_C 185.4); H-3 (δ_H 7.07) with the carbonyl C-4 (δ_C 183.8) and C-4a (δ_C 128.0). Additional HMBC couplings were observed, i.e., H-7 (δ_H 7.00) coupled with C-5 (δ_C 136.7), C-8 (δ_C 158.9), and C-16 (δ_C 53.0); H-11 (δ_H 4.00), with C-5 and C-6 (δ_C 136.7 and 150.7, respectively) and further C-atoms in the lower ring system. In the $^{1}H-^{1}H$-TOCSY spectrum coupling only between H-2 and H-3 was observed. H-10 (δ_H 7.80) plays a significant role in structure elucidation. HMBC cross-peaks were detectable with C-4, C-5, C-8a, and C-10a (δ_C 183.8, 136.7, 107.7, and 130.7, respectively); alleviated couplings with C-4a and C-9 (δ_C 128.0 and 185.4, respectively) were also observed. Further evidence of the anthracene-9,4-dione structure of caeruleoramularin (**9**) was generated by the NOESY spectrum. H-10 (δ_H 7.80) interacted with H-11 and H-12 (δ_H 4.00 and 5.60, respectively) in the lower part of the molecule. The key HMBC correlations and NOESY interactions are shown in Figure 1.

Per culture flask (100 mL phosphate-buffered medium) *R. collo-cygni* produced about 5 mg of caeruleoramularin (**9**) isolated from mycelium and culture filtrate.

Fain et al. have published several studies on the tautomerism of hydroxyanthraquinones.[18–20] Fluorescence excitation, electronic emission, and resonance Raman spectroscopy together with transformation analysis (methods described by Marzocchi et al.)[21] revealed the existence of anthracenedione tautomers with carbonyls in different rings; however it was not possible to isolate these isomers. In this study, caeruleoramularin (**9**), a 9,4-anthraquinoid, isolated as a pure substance from *R. collo-cygni* shows a related

Table 1. ^{1}H and ^{13}C NMR Data of Uredinorubellins I and II (**5**, **6**) Recorded in THF-d_8 at 30 °C and Caeruleoramularin (**9**) Recorded in DMSO-d_6 at 25 °C

	uredinorubellin I (**5**)		uredinorubellin II (**6**)		caeruleoramularin (**9**)	
position	δ_C (ppm), mult.	δ_H (ppm)	δ_C (ppm), mult.	δ_H (ppm)	δ_C (ppm), mult.	δ_H (ppm)
1	159.1, C		164.2, C		169.4, C	
2	130.5, CH	7.36	124.9, CH	7.32	140.4, CH	7.32
3	130.0, CH	7.33	137.8, CH	7.77	140.7, CH	7.07
4	158.2, C		120.1, CH	7.82	183.8, C	
4a	114.2, C		135.3, C		128.0, C	
5	139.7, C		139.6, C		136.7, C	
6	156.3, C		156.4, C		150.7, C	
7	121.9, CH	7.05	121.5, CH	7.02	113.1, CH	7.00
8	164.6, C		164.4, C		158.9, C	
8a	115.8, C		115.7, C		107.7, C	
9	191.7, C		193.7, C		185.4, C	
9a	113.6, C		116.9, C		113.6, C	
10	189.0, C		183.4, C		119.3, CH	7.80
10a	129.9, C		129.8, C		130.7, C	
11	47.0, CH	5.37	46.8, CH	5.29	45.6, CH	4.00
12	128.0, CH	5.96	128.1, CH	5.92	129.2, CH	5.60
13	126.1, CH	5.83	125.9, CH	5.79	126.3, CH	5.60
14	67.7, CH	4.50	67.8, CH	4.50	64.5, CH	4.30
15	76.9, C		76.9, C		79.9, CH	4.19
16	51.1, C		51.2, C		53.0, C	
17	36.8, CH_2	4.34, 2.25	36.9, CH_2	4.31, 2.20	41.3, CH_2	4.80, 3.00
18	73.7, CH	4.15	73.7, CH	4.11	78.5, CH	4.70
19	144.5, C		144.5, C		142.2, C	
20	123.8, CH	6.67	123.7, CH	6.65	116.7, CH	6.83
21	149.9, C		149.8, C		143.6, C	
22	118.7, CH	6.79	118.7, CH	6.78	116.9, CH	6.74
23	165.2, C		165.2, C		157.2, C	
24	112.3, C		112.3, C		112.0, C	
25	201.8, C		201.9, C		168.6, C	
26	22.0, CH_3	2.32	21.09, CH_3	2.31	22.0, CH_3	2.30

structure. Natural products with such an anthracenedione structure have so far not been described.

Because of the well-known photodynamic activity of the rubellins[5,7,8] together with the data concerning the biological activity of anthraquinone derivatives,[9–11] we investigated the performance of these new compounds in several test systems, to ascertain whether their activity is caused by photodynamic effects.

The photodynamic action of the different compounds toward three mammalian cell lines (rabbit, human, and mouse) is presented in Figure 2. Only uredinorubellin I (**5**) influences cell viability in all cell lines, notably in human colon adenocarcinoma cells (HT29), which are not sensitive to any of the other substances. Both rubellin D (**1**) and uredinorubellin II (**6**) are effective on rabbit synovial cells (HIG-82) and mouse monocytes-macrophages (J774A.1), whereas the simple anthraquinone chrysophanol (**8**) and caeruleoramularin (**9**) are ineffective at concentrations of 2 μM. The antimicrobial activity of the isolated anthraquinone derivatives on *Staphylococcus aureus* is presented in Figure 3. This Gram-positive bacterium is a typical member of the microflora of wounds. Rubellin D (**1**) is highly active, uredinorubellins I and II (**5**, **6**) are less so,

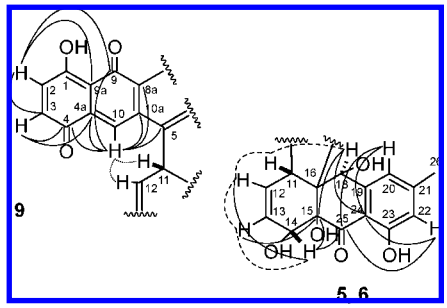


Figure 1. Key HMBC (continuous line), NOESY (dotted line), and COSY (dashed line) correlations of caeruleoramularin (**9**) and uredinorubellins I and II (**5**, **6**).

Table 2. Content of Metabolites from *R. collo-cygni* and *R. uredinicola* under Different Culture Conditions: (a) Shaking Liquid Culture (14 days); (b) Standing Liquid Culture (29 days); (c) Shaking Liquid Culture (18 days); (d) Standing Liquid Culture (35 days)

	R. collo-cygni		*R. uredinicola*	
	[mg/culture flask[a]]			
	a	b	c	d
helminthosporin (**7**)	<0.01	n.d.[b]	0.08	1.11
chrysophanol (**8**)	<0.01	n.d.	0.02	1.11
uredinorubellin I (**5**)	0.47	0.17	2.63	0.92
uredinorubellin II (**6**)	0.41	<0.01	1.10	1.07

[a] Calculated from mycelium and culture filtrate combined. [b] n.d. not detectable.

and caeruleoramularin (**9**) displays only mild antimicrobial activity against *S. aureus* DSM11729; the simple anthraquinone chrysophanol (**8**) has no effect. The Gram-negative bacterium *Pseudomonas aeruginosa*, also a member of the microflora of wounds, was not affected by the tested compounds (data not shown). It is important to note that in both experiments no antimicrobial effect was observed in darkness (data not shown), demonstrating the photodynamic activity of these compounds.

In terms of the relationship between structure and response, two conclusions can be drawn. First, the intensity of the photodynamic action is influenced by the lower part of the anthraquinone derivatives. Second, variations in the natural anthraquinone structure, such as in caeruleoramularin (**9**), diminish this activity. In order to support these conclusions, we compared the production of singlet oxygen in a photodynamic type II reaction. As shown in Figure 4, caeruleoramularin (**9**) is clearly less effective at inducing fatty acid peroxidation, as measured by ethane release from α-linolenic acid, than the related rubellin B (**2**).

Previous results, together with those presented in this study, strongly emphasize the need to focus future research on corre-

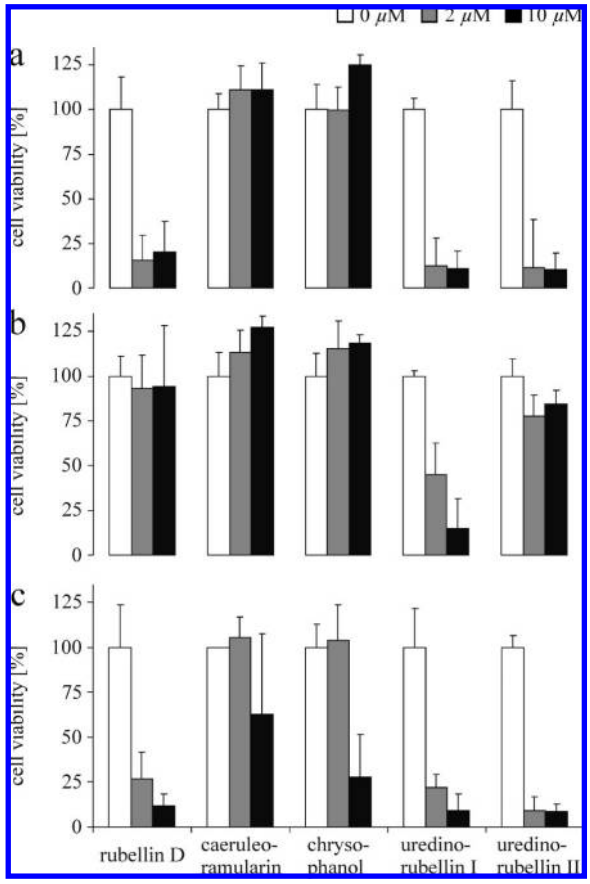


Figure 2. Percentage of cell viability influenced by rubellin D (**1**), caeruleoramularin (**9**), chrysophanol (**8**), uredinorubellin I (**5**), and uredinorubellin II (**6**) on the following cell lines: HIG-82 (a), HT29 (b), and J774A.1 (c). Illumination of 2.5 W/cm^2, 40 s. Data represent mean values (n = 4), and error bars represent standard deviations.

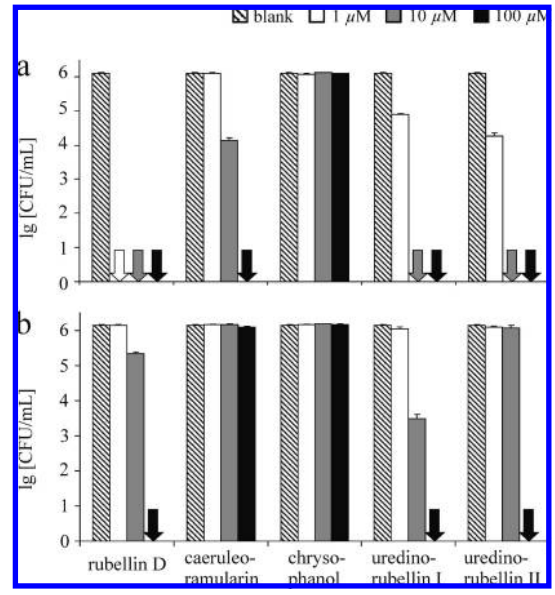


Figure 3. Antibiotic effects of rubellin D (**1**), caeruleoramularin (**9**), chrysophanol (**8**), uredinorubellin I (**5**), and uredinorubellin II (**6**) on *S. aureus* DSM11729 (ATCC 33592) and the MRSA strain *S. aureus* DSM1104 (ATCC 25923), a and b, respectively. Arrows indicate total killing of bacteria. Illumination of 2.5 W/cm^2, 40 s. Data represent mean values (n = 3), and error bars represent standard deviations.

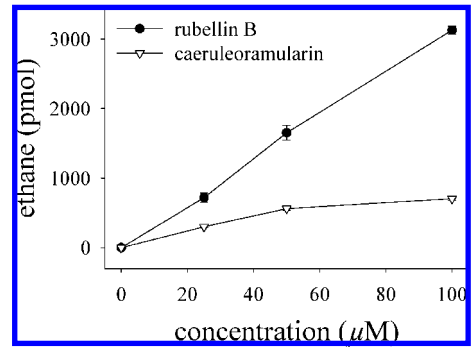


Figure 4. Ethane formation after 60 min from α-linolenic acid induced by different concentrations of rubellin B (**2**) and caeruleoramularin (**9**) at pH 5.0. Data represent mean values (n = 4), and error bars represent standard deviations.

sponding fungal polyketide synthase genes such as those involved in the synthesis of well-known secondary natural compounds.[22]

Experimental Section

General Experimental Procedures. UV−vis absorption spectra were recorded with a Shimadzu UV-160A spectrophotometer. HPLC was carried out using a Shimadzu equipment VP class system with a photodiode array SPD-M10A detector. NMR measurements were performed on a Bruker AVANCE 400 spectrometer using a 5 mm probe head with a z-gradient (^{1}H 400 MHz, ^{13}C 100 MHz). Data were acquired with Bruker standard pulse programs and processed using Bruker TOPSPIN software. HRESI mass spectra (with negative ionization) were recorded on a TSQ Quantum AM (ThermoElectron); the temperature of the ion source was 65 °C with samples in MeOH/H_2O (99:1). Other general procedures and instrumentation have been described previously.[5–8]

Fungal Material. Strain 43/3 was isolated from barley and identified by Dr. E. Sachs (BBA, Institute for Plant Protection in Field Crops and Grassland, Kleinmachnow, Germany) as *R. collo-cygni* Sutton & Waller. The hyperparasitic fungus *R. uredinicola* Khodap. & U. Braun was kindly provided by Prof. Dr. U. Braun (University of Halle-Wittenberg, Germany).

Cultivation and Isolation. Details concerning *R. collo-cygni* (strain 43/3) and *R. uredinicola*, as well as their cultivation, are reported by Miethbauer et al. Czapek-Thom medium was used as previously described.[8] For the improved production of caeruleoramularin (**9**) the following modifications were made: sucrose 10 g, KNO_3 2 g, KCl 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, Soerensen phosphate buffer (pH 5.4, 66 mM) per 1000 mL.

Culture filtrate was acidified to pH 4 and extracted with EtOAc; mycelia were extracted twice with the same organic solvent. A preliminary purification of the respective extracts was carried out by flash liquid chromatography on Si gel [EtOAc/MeOH/H_2O (100:17:13)] to remove hydrophilic waste and separate the blue from the red and yellow compounds.

The respective residues were redissolved in the appropriate mobile solvent. The isolation of caeruleoramularin (**9**), uredinorubellin I (**5**), and uredinorubellin II (**6**) was carried out by isocratic preparative HPLC (Shimadzu equipment VP class with photodiode array SPD-M10A) with MeOH/MeCN/H_2O (16:4:5), 0.5% HCOOH. Conditions: Phenomenex Luna C18(2), 250 × 21.2 mm, 10 μm, 100 Å; Security Guard Phenomenex C18(2), 10 × 10 mm, 10 μm, 300 Å; flow rate 6.5 mL/min. For chrysophanol (**8**) and helminthosporin (**7**), the following modifications were made: MeOH/MeCN (4:1)/H_2O (95:5), flow rate 8 mL/min. Rechromatographic runs were performed if necessary.

In the feeding experiment, either 2 g of sodium [1-^{13}C]-acetate or sodium [2-^{13}C]-acetate (both 99% ^{13}C) was added in 10 × 100 mL of culture medium. Isolation was carried out in the same manner as described above.

Analytical Conditions. Analytical HPLC (for details of equipment, see above) was carried out at 25 °C isocratic with MeOH/MeCN/H_2O (16:4:5), 0.5% HCOOH. Conditions: Phenomenex Luna C18(2), 250 × 4 mm, 5 μm, 100 Å; Security Guard Phenomenex C18(2), 3 × 4 mm; flow rate 0.6 mL/min. Quantification was performed with

calibration curves at the corresponding λ_{max}. TLC was done on Merck RP-18 F254s, solvent MeOH/H_2O (3:1).

Uredinorubellin I (5). Analytical HPLC (ret. time) 15.8 min; preparative HPLC (ret. time) 37.3 min; TLC (R_f) 0.08; UV (MeOH) λ_{max} (log ε) 233 (4.68), 255 (4.32), 272 (4.25), 339 (3.75), 499 (4.13) nm; FTIR (ATR) ν_{max} 3541 (OH), 3283, 2925, 2780, 1705, 1627, 1599, 1569, 1449, 1403 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS *m/z* 541.1110 (calcd for $C_{30}H_{21}O_{10}$ 541.1135).

Uredinorubellin II (6). Analytical HPLC (ret. time) 13.6 min; preparative HPLC (ret. time) 31.8 min; TLC (R_f) 0.12; UV (MeOH) λ_{max} (log ε) 227 (4.51), 253 (4.22), 338 (3.55), 440 (3.83) nm; FTIR (ATR) ν_{max} 3399 (OH), 3224, 2964, 2927, 2870, 1688, 1621, 1570, 1460, 1397 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS *m/z* 525.1185 (calcd for $C_{30}H_{21}O_9$ 525.1180).

Caeruleoramularin (9). Analytical HPLC (ret. time) 14.7 min; preparative HPLC (ret. time) 17.5 min; TLC (R_f) 0.45; UV (MeOH) λ_{max} (log ε) 225 (4.29), 302 (3.80), 564 (3.86) nm; FTIR (ATR) ν_{max} 3411, 2927, 2856, 1700(sh), 1665, 1619, 1580, 1454 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); HRESIMS *m/z* 525.1150 (calcd for $C_{30}H_{21}O_9$ 525.1186).

Bioassays. The photodynamic bioassays were carried out on the rabbit synoviocyte cell line HIG-82 (ATCC CRL-1832), human colon adenocarcinoma cell line HT29 (DSMZ ACC-299), and the mouse monocyte-macrophage cell line J774A.1 (DSMZ ACC-170), cultured in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 1% (vv^{-1}) penicillin (10 000 IU), and streptomycin (10 000 μg/mL). Cells were cultured to confluency in a humidified incubator with 5% (vv^{-1}) CO_2 in air at 37 °C. Compounds were dissolved in DMSO as a stock solution (2 mM) and kept in the dark at 4 °C. Further dilutions were performed in modified RPMI 1640 medium (without phenol red and supplemented with 10% FCS) to reach a final photosensitizer concentration of 2 or 10 μM. Three days before treatment, cells were seeded in microtiter plates (2×10^3 cells/well). Cells were incubated with fresh medium (modified RPMI 1640 medium) with 2 or 10 μM of the photosensitizer for 24 h before light exposure. Before photosensitization, cells were washed, incubated with modified RPMI 1640 medium, and subsequently illuminated at room temperature with a cold light source (KL 2500 LCD, Carl Zeiss AG) with 2.5 W/cm^2 for 40 s. Following illumination, cells were incubated in a humidified 5% CO_2 incubator at 37 °C for 24 h. Cell viability was assessed by the XTT assay.[23]

Antimicrobial assays were performed as described by Schastak et al.[24] with the following strains: *Staphylococcus aureus* DSM1104 (ATCC 25923), the MRSA strain *S. aureus* DSM11729 (ATCC 33592), and *Pseudomonas aeruginosa* DSM1117 (ATCC 27853). Illumination was carried out by cold light sources (see above) with 2.5 W/cm^2 for 40 s.

The formation of reactive oxygen species (ROS), namely, singlet oxygen and oxygen radicals, was determined by fragmenting α-linolenic acid and α-keto-4-thiomethylbutyric acid.[5]

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Supporting Information Available: Physicochemical properties and ^1H and ^{13}C NMR data of helminthosporin (**7**) and chrysophanol (**8**), respectively, as well as the complete 2D NMR correlations. This information is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Braun, U. *IHW-Verlag*; Eching, 1998; Vol. 2, pp 1−493.
(2) Khodaparast, A.; Braun, U. *Mycotaxon* **2005**, *91*, 357–359.
(3) Huss, H.; Sachs, E. *Der Pflanzenarzt.* **1998**, *51*, 15–18.
(4) Sachs, E. *Nachrichtenbl. Dtsch. Pflanzenschutzdienstes* **2000**, *52*, 160–163.
(5) Heiser, I.; Sachs, E.; Liebermann, B. *Physiol. Mol. Plant Pathol.* **2003**, *62*, 29–36.
(6) Miethbauer, S.; Heiser, I.; Liebermann, B. *J. Phytopathol.* **2003**, *151*, 665–668.
(7) Heiser, I.; Hess, M.; Schmidtke, K.; Vogler, U.; Miethbauer, S.; Liebermann, B. *Physiol. Mol. Plant Pathol.* **2004**, *64*, 135–143.
(8) Miethbauer, S.; Haase, S.; Schmidtke, K. U.; Günther, W.; Heiser, I.; Liebermann, B. *Phytochemistry.* **2006**, *67*, 1206–1213.
(9) Huang, Q.; Lu, G.; Shen, H.-M.; Chung, M. C. M.; Ong, C. N. *Med. Res. Rev.* **2007**, *27*, 609–630.
(10) Schenck, L. W.; Kuna, K.; Frank, W.; Albert, A.; Asche, C.; Kucklaender, U. *Bioorg. Med. Chem.* **2006**, *14*, 3599–3614.
(11) Koceva-Chyla, A.; Wieclawska, B.; Jóźwiak, Z.; Bryszewska, M. *Cell. Biol. Int.* **2006**, *30*, 645–652.
(12) Danielsen, K.; Aksnes, D. W.; Francis, G. W. *Magn. Reson. Chem.* **1992**, *30*, 359–360.
(13) Fokou, P. A. Dissertation, University of Bielefeld, Germany, 2006.
(14) Yagi, A.; Makino, K.; Nishioka, I. *Chem. Pharm. Bull.* **1977**, *25*, 1764–1770.
(15) Arrieta-Baeza, D.; Roman, R.; Vazquez-Duhalt, R.; Jimènez-Estrada, M. *Phytochemistry* **2002**, *60*, 567–572.
(16) Arnone, A.; Nasini, G.; Camarda, L.; Assante, A. *J. Chem. Soc., Perkin Trans. 1* **1986**, 255–260.
(17) Arnone, A.; Nasini, G.; Camarda, L.; Assante, A. *Gazz. Chim. Ital.* **1989**, *119*, 35–39.
(18) Fain, V. Y.; Zaitsev, B. E.; Ryabov, M. A.; Russ, J. *Gen. Chem.* **2003**, *73*, 657–663.
(19) Fain, V. Y.; Zaitsev, B. E.; Ryabov, M. A. *Chem. Nat. Compd.* **2005**, *41*, 146–152.
(20) Fain, V. Y.; Zaitsev, B. E.; Ryabov; M, A.; Russ, J. *Org. Chem* **2006**, *42*, 1469–1472.
(21) Marzocchi, M. P.; Mantini, A. R.; Casu, M.; Smulevich, G. *J. Chem. Phys.* **1998**, *108*, 534–549.
(22) Schümann, J.; Hertweck, C. *J. Biotechnol.* **2006**, *124*, 690–703.
(23) Roehm, N. W.; Rodgers, G. H.; Hatfield, S. M.; Glasebrock, A. L. *J. Immunol. Methods.* **1991**, *142*, 257–265.
(24) Schastak, S.; Gitter, B.; Handzel, R.; Hermann, R.; Wiedemann, P. *Methods Find. Exp. Clin. Pharmacol.* **2008**, *29*, 1–5.

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