New Azaphilones from the Inedible Mushroom *Hypoxylon rubiginosum*

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Received March 16, 2004

Fractionation of the methanol extract of the fruit bodies of the xylariaceus ascomycete *Hypoxylon rubiginosum* resulted in the isolation of three new azaphilone derivatives named rubiginosins A−C (**1**−**3**) and a new fatty acid named rubiginosic acid (**4**), together with three known compounds, entonaemin A (**5**), daldinin C (**6**), and orsellinic acid. Their structures were elucidated by 2D NMR, MS, IR, and UV spectra and chemical reaction. The absolute configuration of compound **3** was established by CD spectroscopy. The structure of **4** was confirmed by X-ray crystallographic analysis.

The genus *Hypoxylon* (Xylariaceae) currently comprises 150 species, of which about 25 are present in Europe.[1] Steglich et al. (1974) reported the isolation of mitorubrin and its derivatives from *Hypoxylon fragiforme*.[2] Later, hypoxylone[3] was isolated from *H. sclerophaeum* collected in France and hypoxylonols A and B[4] from the Japanese *H. truncatum*. In the course of our studies on the chemosystematics of the genus, we previously reported the isolation of a novel perylenequinone, truncatone,[5] in high concentration from the spider sex pheromones, (3*R*)-hydroxybutyric acid, its dimer and trimer from *H. truncatum*,[6] macrocarpones from *H. macrocarpum*,[1] and three cyclic azaphilones, daldinins C, E, and F, from *H. fuscum*.[7] The taxonomy, biogeography, and biology of the genus *Hypoxylon* was reviewed by Ju and Rogers.[8] In modern classification, not only the combination of morphological traits, colors of stromata pigments in KOH, and PCR-based methods relating to molecular taxonomy and phylogeny but also the chemical constituents of these fungi have been employed as chemical markers. These previous studies indicated that the secondary metabolites have a great chemotaxonomic significance in *Hypoxylon* and other Xylariaceae genera[1,7] In continuation of our studies, we now report the isolation of four new compounds (**1**−**4**) together with three known compounds from *H. rubiginosum* (Pers.: Fr.) Fr. (Xylariaceae).

Results and Discussion

The methanolic extract of stromata of *H. rubiginosum* was divided into 14 fractions via reversed-phase HPLC. This was followed by silica gel column chromatography to give four new compounds (**1**−**4**) and three known compounds (entonaemin A (**5**),[9] daldinin C (**6**),[5,10] and orsellinic acid[11]) as described in the Experimental Section.

Rubiginosin A (**1**) was obtained as an oil. FABMS gave a quasi-molecular ion peak at *m*/*z* 445 $[M + H]^+$, and HRFABMS indicated a molecular formula of $C_{23}H_{24}O_9$. Additionally, in the ^{1}H NMR spectrum, two meta-coupled aromatic protons were detected at δ 6.10 and 6.22 (d, J = 2.5 Hz), together with three olefinic proton signals. Three singlet methyl signals were also observed. The presence of a conjugated ketone was indicated by the IR and UV absorption bands at 1646 cm^{-1} and 377 nm and the signal at δ 195.8 in the ^{13}C NMR spectrum. The ^{1}H and ^{13}C NMR spectra suggested the presence of an azaphilone and orsellinic acid partial structures for **1**.[9] Furthermore, the ester linkage of orsellinic acid was determined at the C-6 position of the azaphilone moiety by the long-range correlation between H-6 and C-8′ (Figure 2). The relative configuration of **1** was deduced by the NOE correlation between H-6 and H-9, indicating that they were β-oriented. In addition, the double bond at C-10 was assigned as the *E*-form due to the coupling constant (15.3 Hz) and NOE correlation between H-10 and H-12. The spectral data of **1** resembled those of entonaemin A (**5**)[9] except for the presence of an acetoxy group at C-12, indicating that **1** was a monoacetate of entonaemin A. Acetylation of **1** resulted in formation of the triacetate of entonaemin A (**7**). The locations of three acetyl groups were clearly determined at C-12, C-2′, and C-4′ by the long-range correlations between the acetoxy groups and C-12, C-2′, and C-4′ in the HMBC spectrum. Moreover, the presence of a tertiary hydroxyl group at C-7 (δ 3.60, s) in **7** suggested that it was not acetylated. Therefore, the structure of rubiginosin A was determined to be 5,6,7,8-tetrahydro-7-hydroxy-3-[(1*E*)-3-acetoxy-1-propenyl]-7-methyl-8-oxo-1*H*-2-benzopyran-6-yl 2,4-dihydroxy-6-methylbenzoate (**1**).

The FABMS of **2** gave a molecular ion peak at *m*/*z* 403 $[M + H]^+$, and its HRFABMS spectrum indicated a molecular formula of $C_{21}H_{22}O_8$. The IR and UV spectra showed the presence of a hydroxyl (3343 cm^{-1}), a conjugated ketone (1656 cm^{-1}, 382 nm), a conjugated ester (1721 cm^{-1}, 302 nm), and an aromatic (1621, 1530 cm^{-1}, 217 and 264 nm) group. Comparison of its NMR spectral data with those of **5**[9] indicated that **2** possessed an azaphilone and orsellinic acid unit. The only notable difference between **2** and **5** was the ester linkage of orsellinic acid and the azaphilone at C-7. This was proven by acetylation of **2** to afford the tetraacetate **8** with acetoxy groups located at C-6, C-12, C-2′, and C-4′, which were determined by the HMBC spectrum. The relative configurations of H-6 and H-9 were both determined to be β-oriented by the NOE correlation between H-6 and H-9 in the NOESY spectrum. The C-10 double bond was revealed to be the *E*-form by the coupling constant (J = 15.3 Hz) and NOE correlation between H-10 and H-12. From the above experimental data, rubiginosin B (**2**) was deduced to be 5,6,7,8-tetrahydro-6-hydroxy-3-

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10.1021/np040063l CCC: $27.50
Published on Web 07/08/2004

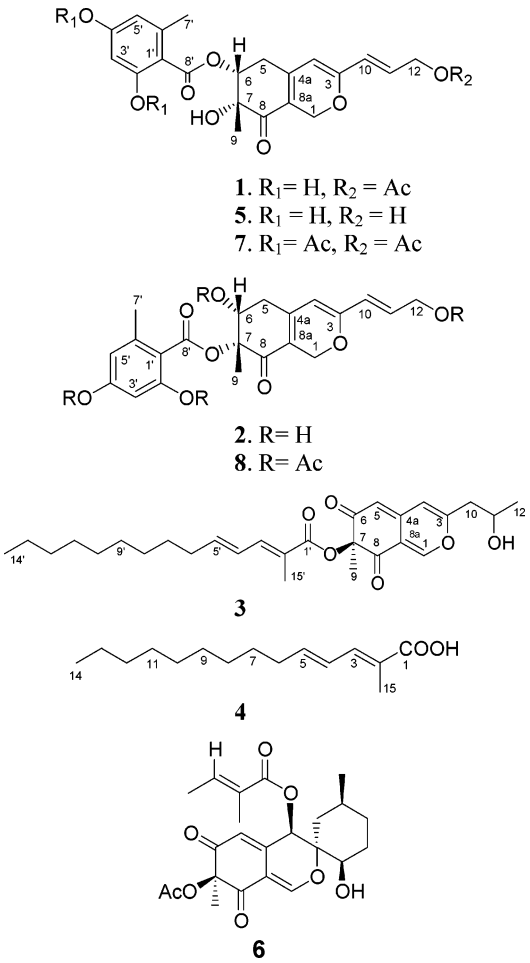

Figure 1. Structures of compounds **1**−**8**.

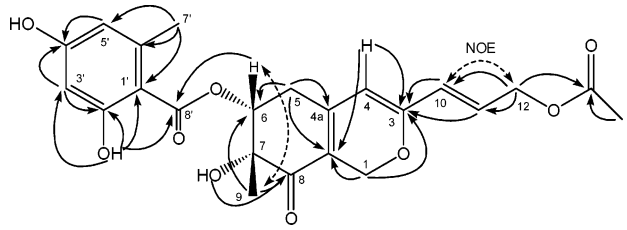

Figure 2. Important HMBC (arrows) and NOESY correlations of **1**.

[(1*E*)-3-hydroxy-1-propenyl]-7-methyl-8-oxo-1*H*-2-benzopyran-7-yl 2,4-dihydroxy-6-methylbenzoate.

The molecular formula of rubiginosin C (**3**) was determined to be $C_{28}H_{38}O_6$ by HRFABMS. Its ^{1}H NMR spectrum showed the presence of six olefinic protons, four methyls, nine methylenes, and one methine. The ^{13}C NMR spectrum indicated 28 carbon signals including two conjugated ketones, one conjugated ester, and one secondary carbon (δ 65.3 d). The ^{1}H and ^{13}C NMR data of **3** suggested the presence of an azaphilone structure, similar to daldinin A[5,10] except for the differences of the fatty acid at C-7 and the side chain at C-3. The side chain at C-3 was characterized to be 2-hydroxylpropane by the ^{1}H−^{1}H COSY correlations between H-11/H-10 and H-12 and by the long-range correlations between H-12 (δ 1.30, d, J = 6.3 Hz) and H-10/C-11, H-10 and H-11/C-3 in the HMBC spectrum. The fatty acid at C-7 was deduced to be 2-methyl-2*E*,4*E*-tetradecadienoic acid (**4**). The absolute configuration of **3** at C-7 was established by comparing its CD spectrum with those of daldinins A and B,[5,10] which showed positive (352 and 226 nm) and negative (286 nm) Cotton effects. These results

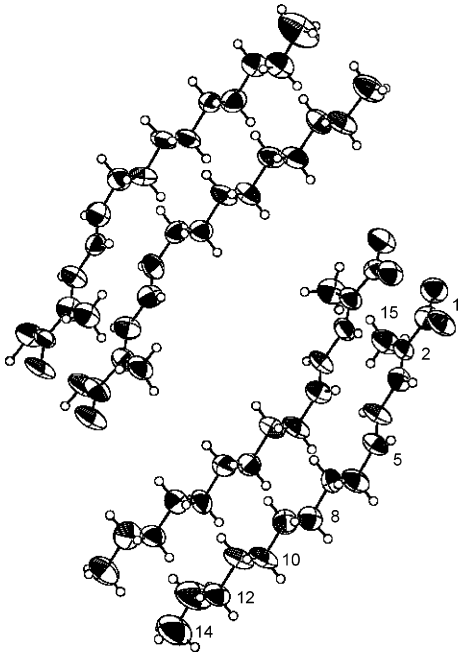

Figure 3. ORTEP drawing of **4**.

indicated that **3** possessed *S*-configuration at C-7. The absolute configuration at C-11 remains unclear. Thus, rubiginosin C (**3**) was found to be (7*S*)-7,8-dihydro-3-(2-hydroxy-1-propyl)-7-methyl-6,8-dioxo-6*H*-2-benzopyran-7-yl 2-methyl-2,4-tetradecadienoate.

Rubiginosic acid (**4**) was obtained as needles. Its molecular formula was determined to be $C_{15}H_{26}O_2$ by HREIMS. Its IR and UV spectra indicated the presence of a conjugated carboxylic group. Investigation of the ^{1}H−^{1}H COSY and HMBC spectra revealed that **4** was 2-methyl-2,4-tetradecadienoic acid, which was the same as the fatty acid partial structure of **3** at C-7. The double bonds were both determined to be *E* by the NOE correlations between H-3 and H-5, H-4, and H-15 in the NOESY spectrum. Further, the structure of **4** was confirmed by X-ray crystallographic analysis, and its ORTEP drawing is shown in Figure 3.[12] Consequently, rubiginosic acid (**4**) was found to be 2-methyl-2*E*,4*E*-tetradecadienoic acid. The occurrence of a fatty acid containing 15 carbon atoms is very rare in nature. Previously, (*E*,*Z*)-3,5- and (*E*,*E*)-3,5-tetradecadienoic acids were reported as synthetic products.[13]

On the basis of these results, it was clear that stromata of *H. rubiginosum* contain chemical constituents different from other members of the genus, such as *H. fragiforme* and *H. howeianum*, which mainly contain mitorubrin type azaphilones. In any case, the relationship among these fungi is reinforced by the fact that mitorubrins and compounds of the entonaemin/rubiginosin type are clearly biogenetically related. These compounds may be useful to include as tools to evaluate further chemotaxonomic relationships in the so-called *H. rubiginosum* complex, a conglomerate of manifold tropical and temperate species that are difficult to identify on the basis of morphological characters alone.[8]

Interestingly, some of the new compounds obtained from *H. rubiginosum* resemble those of *Entonaema*, i.e., a genus that is believed to be closely related to *Hypoxylon* as inferred from morphological data and was recently shown to produce metabolites similar to those of the related genus *Daldinia*.[14] *Daldinia* and *Entonaema* genera mainly differ from *Hypoxylon* in their stromatal anatomy: stromata of *Entonaema* being gelatinous, hollow, and filled with liquid

when fresh, while fruit bodies of *Daldinia* are also gelatinous and characterized by internal concentric zones.[8] Since characteristics such as ascospore morphology and anamorphs appear to be rather uniform in all three aforementioned genera, it has been argued that they should not be kept separate.[15] In fact, the current study proves that *Entonaema* and particular species of *Hypoxylon* have a similar secondary metabolism in stromata, while *Daldinia* never contains mitorubrins[16] and is similar to *Entonaema* with regard to its metabolism in culture. Hence, the current results reinforce the view of many mycologists that all three genera should be maintained and also provide evidence that *Entonaema* may be most closely related to *H. rubiginosum* and allied species of *Hypoxylon*. Likewise, the isolation of daldinin C from an additional source in the Xylariaceae is in agreement with the close taxonomic relationship of these fungi, and like with the widespread binaphthyls,[14] its occurrence in this ascomycete family may be less specific.

Experimental Section

General Experimental Procedures. Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured on a JASCO DIP-1000 polarimeter with $CHCl_3$ as solvent. UV spectra were obtained on a Shimadzu UV-1650PC instrument in MeOH. CD spectra were measured on a JASCO J-725 spectrometer in MeOH. IR spectra were measured on a JASCO FT/IR-5300 spectrophotometer. The ^1H and ^{13}C NMR spectra were recorded on a Varian Unity 600 NMR spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), using either $CDCl_3$ or CD_3OD as solvent. Chemical shifts are given relative to TMS (δ 0.00) as internal standard (^1H) and δ 77.0 (ppm) from $CDCl_3$ and δ 49.0 (ppm) from CD_3OD as standards (^{13}C). Mass spectra including high-resolution mass spectra were recorded on a JEOL JMS AX-500 spectrometer. X-ray reflection data were measured on a DIP Image diffractometer using Mo Kα radiation (λ = 0.71073 Å). Column chromatography was carried out on silica gel 60 (0.2−0.5 mm, 0.04−0.063 mm, Merck).

Fungal Material. Fruit bodies of *Hypoxylon rubiginosum* were collected from bark and wood of *Fraxinus excelsior* at the Neandertal in Haan-Gruiten, North Rhine Westphalia, Germany, by one of the authors (M.S.), on April 16, 2003, and identified by the collectors by microscopic studies of the stromata, as well as of a corresponding culture made from the ascospores. A voucher specimen is deposited at the Mycological Herbarium of the Fuhlrott Museum, Wuppertal, Germany, as specimen Ww 4314. A duplicate specimen is also deposited at the personal herbarium of M.S. The fungus showed the typical characteristics of the genus and species as previously described by Petrini & Müller[17] as *H. rubiginosum* var. *rubiginosum*, which is in conformity with *H. rubiginosum* sensu Ju & Rogers.[8]

Extraction and Isolation. Stromata of *H. rubiginosum* (11 g) were carefully detached from the plant substrate, ground to a fine powder in a mortar, and extracted with MeOH (2 × 30 min) in an ultrasonic bath. The combined extracts were filtered and concentrated in vacuo to yield an oily crude product (850 mg), which was separated by preparative HPLC on a MZ-Analysentechnik (Mainz, Germany) Kromasil MZ C18 column (7 μm, 250 × 40 mm) using 0.1% TFA−MeCN in a linear gradient from 30% to 100% MeCN in 100 min and continued at 100% MeCN until 185 min to give 14 fractions. Fractions 1 and 2 (12.4 mg), eluting at 40−50% MeCN, were further purified by reversed-phase column chromatography using MeOH−H_2O (1:1) to afford orsellinic acid (10.4 mg). Fractions 5 and 6 (270.9 mg), eluting at 60% MeCN, were rechromatographed on silica gel, with solvent system $CHCl_3$−MeOH−H_2O (25:2.5:0.1), to give **1** (171.6 mg) and a mixture (34.3 mg), which was further purified by a reversed-phase column, with MeCN−H_2O (1:1) as eluent, followed by normal-phase preparative HPLC using EtOAc as solvent to yield **2** (6.7 mg) and **5** (4.4 mg). Fraction 7 (32 mg), eluting at 70% MeCN, was purified by normal-phase preparative HPLC, with EtOAc as eluent, to obtain **6** (13.3 mg). Fraction 13 (36.0 mg) was chromatographed on silica gel, with solvent system $CHCl_3$−MeOH−H_2O (25:2.5:0.1), to afford **4** (27.5 mg) as white needles. Fraction 14 (72.8 mg), eluting at 90% MeCN, was subjected to silica gel column chromatography, using $CHCl_3$−MeOH−H_2O (25:2.5:0.1) as eluent, to obtain **3** (51.1 mg).

Rubiginosin A (1): oil; $[\alpha]_D^{20}$ +207.6° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (4.4), 223 (4.4), 266 (4.1), 304 (3.7), 377 (4.0) nm; CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 341 (+5.26), 296 (−1.83), 264 (+13.13), 226 (+10.87); IR (KBr) ν_{max} 3378, 1736, 1646, 1535, 1255 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 11.5 (1H, s, HO-2′), 6.47 (1H, td, J = 5.8, 15.3 Hz, H-11), 6.22 (1H, d, J = 2.5 Hz, H-3′), 6.10 (1H, d, J = 2.5 Hz, H-5′), 6.09 (1H, d, J = 15.3 Hz, H-10), 5.64 (1H, t, J = 3.0 Hz, H-6), 5.36 (1H, s, H-4), 5.09 (1H, brd, J = 12.6 Hz, H-1), 4.82 (1H, brd, J = 12.6 Hz, H-1), 4.71 (2H, d, J = 5.8 Hz, H-12), 3.95 (1H, brs, HO-7), 2.97 (1H, brd, J = 19.2 Hz, H-5), 2.79 (1H, brd, J = 19.2 Hz, H-5), 2.15 (3H, s, H-7′), 2.11 (3H, s, *CH*$_3$CO-12), 1.47 (3H, s, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.8 (C-8), 170.7 (C-8′ and CH$_3$*CO*-12), 165.5 (C-2′), 161.0 (C-4′), 159.7 (C-3), 147.6 (C-4a), 143.9 (C-6′), 131.1 (C-11), 124.9 (C-10), 113.9 (C-8a), 111.6 (C-5′), 105.4 (C-4), 105.0 (C-1′), 101.3 (C-3′), 76.1 (C-6), 74.4 (C-7), 63.8 (C-1), 63.5 (C-12), 32.0 (C-5), 24.3 (C-7′), 24.1 (C-9), 20.8 (*CH*$_3$CO-12); FABMS *m/z* 445 [M + H]$^+$; HRFABMS *m/z* 445.1453 (calcd for $C_{23}H_{25}O_9$, 445.1499).

Rubiginosin B (2): oil; $[\alpha]_D^{20}$ +20.9° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 217 (3.5), 264 (3.2), 302 (2.9), 382 (3.1) nm; CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 340 (+0.33), 294 (−0.15), 264 (+1.07), 225 (+1.10); IR (KBr) ν_{max} 3343, 1721, 1656, 1621, 1530, 1172 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) δ 6.51 (1H, td, J = 4.4, 15.3 Hz, H-11), 6.21 (1H,d, J = 15.3 Hz, H-10), 6.20 (1H, d, J = 2.5 Hz, H-5′), 6.14 (1H, d, J = 2.5 Hz, H-3′), 5.51 (1H, s, H-4), 4.99 (1H, brd, J = 12.6 Hz, H-1), 4.81 (1H, brd, J = 12.6 Hz, H-1), 4.67 (1H, t, J = 4.7 Hz, H-6), 4.21 (2H, d, J = 4.4 Hz, H-12), 2.87 (1H, dd, J = 4.7, 18.7 Hz, H-5), 2.70 (1H, dd, J = 4.7, 18.7 Hz, H-5), 2.50 (3H, s, H-7′), 1.73 (3H, s, H-9); ^{13}C NMR (CD_3OD, 150 MHz) δ 192.7 (C-8), 170.2 (C-8′), 165.1 (C-2′), 163.6 (C-4′), 161.3 (C-3), 149.3 (C-4a), 145.5 (C-6′), 138.1 (C-11), 123.4 (C-10), 115.7 (C-8a), 112.4 (C-5′), 107.0 (C-1′), 105.8 (C-4), 102.0 (C-3′), 87.3 (C-7), 72.4 (C-6), 64.9 (C-1), 62.6 (C-12), 35.3 (C-5), 24.3 (C-7′), 19.9 (C-9); FABMS *m/z* 403 [M + H]$^+$; HRFABMS *m/z* 403.1411 (calcd for $C_{21}H_{23}O_8$, 403.1399).

Rubiginosin C (3): oil; $[\alpha]_D^{20}$ +52.1° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 225 (4.1), 270 (4.4), 332 (4.3) nm; CD (MeOH) λ_{ext} nm ($\Delta\epsilon$) 352 (+ 2.59), 286 (−1.51), 226 (+1.43); IR (KBr) ν_{max} 3424, 1701, 1668, 1638, 1551, 1454, 1109 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.88 (1H, d, J = 1.4 Hz, H-1), 7.34 (1H, dd, J = 0.8, 11.3 Hz, H-3′), 6.33 (1H, dd, J = 11.3, 14.6 Hz, H-4′), 6.20 (1H, d, J = 1.4 Hz, H-4), 6.12 (1H, m, H-5′), 5.55 (1H, t, J = 1.4 Hz, H-5), 4.15 (1H, m, H-11), 2.53 (2H, m, H-10), 2.19 (2H, dd, J = 7.4, 14.6 Hz, H-6′), 1.92 (3H, d, J = 0.8 Hz, H-15′), 1.58 (3H, s, H-9), 1.42 (2H, m, H-7′), 1.30 (3H, d, J = 6.3 Hz, H-12), 1.27−1.28 (12H, m, from H-8′ to H-13′), 0.88 (3H, t, J = 6.9 Hz, H-14′); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 193.6 (C-8), 193.3 (C-6), 167.8 (C-1′), 159.2 (C-3), 153.9 (C-1), 144.8 (C-5′), 142.5 (C-4a), 141.2 (C-3′), 125.9 (C-4′), 123.0 (C-2′), 115.2 (C-8a), 110.7 (C-4), 107.3 (C-5), 83.9 (C-7), 65.3 (C-11), 42.6 (C-10), 33.3 (C-6′), 31.9 (C-12′), 29.1−29.5 (from C-8′ to C-11′), 28.9 (C-7′), 23.5 (C-12), 22.6 (C-13′), 22.0 (C-9), 14.1 (C-14′), 12.3 (C-15′); FABMS *m/z* 471 [M + H]$^+$; HRFABMS *m/z* 471.2760 (calcd for $C_{28}H_{39}O_6$, 471.2747).

Rubiginosic acid (4): white needles (*n*-hexane−ether); mp 55−57 °C; UV (MeOH) λ_{max} (log ϵ) 263 (4.3); IR (KBr) ν_{max} 3400−2500, 1667, 1634, 1606, 1294, 1258, 973 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 7.28 (1H, dd, J = 0.8, 11.5 Hz, H-3), 6.35 (1H, dd, J = 11.5, 14.3 Hz, H-4), 6.14 (1H, m, H-5), 2.20 (2H, dd, J = 7.1, 14.3 Hz, H-6), 1.93 (3H, d, J = 0.8 Hz, H-15), 1.44 (2H, m, H-7), 1.25−1.30 (12H, from H-8 to H-13), 0.88 (3H, t, J = 7.1 Hz, H-14); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 173.7 (C-1),

144.8 (C-5), 141.0 (C-3), 125.9 (C-4), 123.9 (C-2), 33.4 (C-6), 31.9 (C-12), 29.2−29.5 (from C-8 to C-11), 28.9 (C-7), 22.7 (C-13), 14.1 (C-14), 12.2 (C-15); EIMS *m*/*z* 238 $[M]^+$; HREIMS *m*/*z* 238.1932 (calcd for $C_{15}H_{26}O_2$, 238.1933).

Crystal Data for 4. Data collection: DIP Image plate. Cell refinement: Scalepack (HKL). Data reduction: maXus.[18] Program used to solve structure: maXus.[19] Refinement: on F^2 full matrix least-squares. Diffractometer: DIP Image plate. A colorless crystal of $C_{15}H_{26}O_2$ having approximate dimensions 0.5 × 0.3 × 0.1 mm, MW 238, triclinic, P_1, $a = 10.383(2)$ Å, $b = 11.931(3)$ Å, $c = 13.358(4)$ Å, $\alpha = 72.275(10)°$, $\beta = 80.378(9)°$, $\gamma = 74.914(15)°$, $V = 1514.9(7)$ Å^3, $Z = 4$, Mo Kα radiation, $\lambda = 0.71073$Å, $\mu = 0.067$ mm^{-1}, 4793 reflections, 613 parameters; only coordinates of H atoms refined, $R = 0.0717$, $R_w = 0.1811$, $S = 1.022$.

Acetylation of Rubiginosin A (7). Rubiginosin A (**1**) (9.2 mg) in pyridine (1.5 mL) was acetylated with acetic anhydride (1.5 mL), and workup as usual afforded a diacetate (**7**) (8.6 mg) as an oil: $[\alpha]_D^{20}$ +27.1° (*c* 1.08, $CHCl_3$); UV (EtOH) λ_{max} (log ϵ) 223.8 (4.3), 379.8 (4.0) nm; IR (KBr) ν_{max} 3485, 1773, 1738, 1654, 1617, 1540, 1196 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.82 (1H, dd, $J = 0.8$, 2.2 Hz, H-5′), 6.77 (1H, d, $J = 2.2$ Hz, H-3′), 6.45 (1H, td, $J = 5.8$, 15.4 Hz, H-11), 6.09 (1H, td, $J = 1.7$, 15.4 Hz, H-10), 5.56 (1H, dd, $J = 2.5$, 3.3 Hz, H-6), 5.34 (1H, s, H-4), 5.05 (1H, dd, $J = 1.7$, 12.9 Hz, H-1), 4.83 (1H, td, $J = 1.4$, 12.9 Hz, H-1), 4.70 (2H, d, $J = 5.8$ Hz, H-12), 3.60 (1H, s, OH-7), 2.94 (1H, td, $J = 1.7$, 19.5 Hz, H-5), 2.73 (1H, brd, $J = 19.5$ Hz, H-5), 2.28 (3H, s, H-7′), 2.27 (3H, s, CH_3CO-2′), 2.24 (3H, s, CH_3CO-4′), 2.11 (3H, s, CH_3CO-12), 1.44 (3H, s, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 195.3 (C-8), 170.5 (CH_3CO-12), 168.8 (CH_3CO-4′), 168.6 (CH_3CO-2′), 165.3 (C-8′), 159.4 (C-3), 151.8 (C-4′), 149.3 (C-2′), 146.7 (C-4a), 139.4 (C-6′), 131.0 (C-11), 124.9 (C-10), 123.6 (C-1′), 121.3 (C-5′), 114.2 (C-8a), 114.1 (C-3′), 105.2 (C-4), 76.0 (C-6), 74.1 (C-7), 63.8 (C-1), 63.5 (C-12), 32.0 (C-5), 24.1 (C-9), 21.1 (CH_3CO-2′), 20.9 (CH_3CO-12), 20.8 (C-7′), 20.6 (CH_3CO-4′); FABMS *m*/*z* 529 $[M + H]^+$; HRFABMS *m*/*z* 529.1701 (calcd for $C_{27}H_{29}O_{11}$, 529.1710).

Acetylation of Rubiginosin B (8). Rubiginosin B (**2**) (3.2 mg) in pyridine (1.0 mL) was acetylated with acetic anhydride (1.0 mL), and workup as usual afforded a tetraacetate (**8**) (1.2 mg) as an oil: $[\alpha]_D^{20}$ +13.8° (*c* 0.41, $CHCl_3$); IR (KBr) ν_{max} 1743, 1614, 1581, 1370, 1219 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.86 (1H, dd, $J = 0.6$, 2.2 Hz, H-5′), 6.78 (1H, dd, $J = 0.6$, 2.2 Hz, H-3′), 6.43 (1H, td, $J = 5.8$, 15.7 Hz, H-11), 6.09 (1H, td, $J = 1.7$, 15.7 Hz, H-10), 5.64 (1H, t, $J = 4.9$ Hz, H-6), 5.31 (1H, s, H-4), 5.01 (1H, d, $J = 12.9$ Hz, H-1), 4.90 (1H, d, $J = 12.9$ Hz, H-1), 4.70 (2H, dd, $J = 1.7$, 5.8 Hz, H-12), 2.78 (1H, dd, $J = 5.8$, 18.4 Hz, H-5), 2.74 (1H, dd, $J = 4.9$, 18.4 Hz, H-5), 2.43 (3H, s, H-7′), 2.28 (3H, s, CH_3CO-4′), 2.25 (3H, s, CH_3CO-2′), 2.11 (3H, s, CH_3CO-12), 2.07 (3H, s, CH_3CO-6), 1.76 (3H, s, H-9); ^{13}C NMR ($CDCl_3$, 150 MHz) δ 188.4 (C-8), 170.2 (CH_3CO-6 and 12), 168.7 (CH_3CO-2′ and 4′), 164.3 (C-8′), 158.7 (C-3), 151.7 (C-4′), 149.2 (C-2′), 145.4 (C-4a), 139.5 (C-6′), 130.7 (C-11), 125.1 (C-10), 124.0 (1′), 121.2 (C-5′), 115.6 (C-8a), 114.3 (C-3′), 105.0 (C-4), 82.3 (C-7), 73.0 (C-6), 64.1 (C-1), 63.5 (C-12), 30.9 (C-5), 21.0 (CH_3CO-6 and 4′), 20.8 (CH_3CO-12 and 2′), 20.3 (C-7′), 18.3 (C-9); FABMS *m*/*z* 571 $[M + H]^+$; HRFABMS *m*/*z* 571.1766 (calcd for $C_{29}H_{31}O_{12}$, 571.1816).

Acknowledgment. The authors thank Ms Y. Okamoto, Dr. M. Tanaka, and Mr. S. Takaoka (TBU, Japan) for recording the mass, 600 MHz NMR, and X-ray spectra, respectively, and Dr. H. Wollweber, Wuppertal, Germany, for his help in locating and identifying the fungal material.

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(12) Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre (deposition number CCDC 229857). Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0) 1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).
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NP040063L