# Bioactive Isocoumarins Isolated from the Endophytic Fungus *Microdochium bolleyi*

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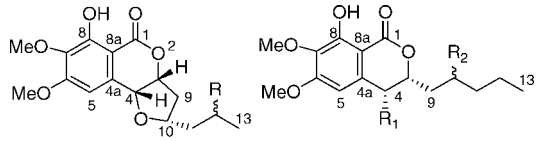
*Received February 11, 2008*

Three new isocoumarin derivatives (**2**−**4**) were isolated together with monocerin (**1**) from *Microdochium bolleyi*, an endophytic fungus from *Fagonia cretica*, a herbaceous plant of the semiarid coastal regions of Gomera. Compounds **2** and **3** are both 12-oxo epimers of **1**, and **4** is a ring-opened derivative of **1**. The structures were elucidated by detailed spectroscopic analysis and comparison with reported data. The absolute configurations were determined by a modified Mosher's method. Compounds **1**, **3**, and **4** showed good antifungal, antibacterial, and antialgal activities against *Microbotryum violaceum, Escherichia coli, Bacillus megaterium*, and *Chlorella fusca*. Compound **2** was moderately antifungal and antialgal.

Monocerin (**1**) and analogues have been isolated as antifungal, insecticidal, and phytotoxic secondary metabolites from several fungal sources including *Drechslera monoceras* (*Exserohilum monoceras*, *Helminthosporium monoceras*),[1] *Exserohilum turcum,*[2−4] and *Fusarium larvarum*.[5,6] It is a nonspecific toxin and also is a nonspecific inhibitor of seed germination.[3] Its biological action could be caused by interference with selected stages of the cell division cycle.[7] Monocerins have attracted the attention of synthetic chemists because of their unique structural feature, *cis*-fused furobenzopyranones, and their broad spectrum of activity.[8−12] Ring-opened derivatives, such as **5**, were isolated from *Fusarium larvarum*[5,6] and demonstrated toxic activity toward insects.[5,6,13] The total synthesis of several of these compounds (fusarentins) was completed by Simpson and co-workers.[9,14]

In our ongoing screening for biologically active secondary metabolites from fungi,[15] we investigated metabolites produced by the endophytic fungus *Microdochium bolleyi* (internal strain no. 8880), isolated from *Fagonia cretica*, a herbaceous plant of the semiarid coastal regions of Gomera. We have continued investigating endophytes from this biotope, since previous investigations had shown that a particularly high proportion of endophytes from plants from Gomera were biologically active.[16] The fungus was cultivated on biomalt agar medium. The crude ethyl acetate extract of the culture showed very good antifungal activity against *Microbotryum violaceum* and moderate algicidal activity against *Chlorella fusca*. Fractionation of the ethyl acetate extract led to the isolation and structural determination of the known monocerin (**1**), together with two new analogues and a ring-opened derivative (**2**−**4**). We herein report on the isolation, structural elucidation including relative and absolute configuration, and the results of some bioactivity tests on these compounds.




The fungus *Microdochium bolleyi* was cultivated on biomalt agar medium for 4 weeks at 21 °C and subsequently extracted with ethyl acetate. The crude extract was fractionated on a silica gel column, followed by Sephadex LH-20 column chromatography to yield a crude mixture containing **1**−**4**. Further silica column chromatography or preparative TLC gave the pure compounds.

The structure of monocerin (**1**), the major metabolite, was determined by detailed spectroscopic analysis and comparison with reported data.[1,5,8,12,17,18] In particular, the distinct NOE effect of H-4 with H-3 and H-10 in the NOESY and NOEDIFF spectra indicated that these protons had the same (*β*) *o*rientation. The optical rotation values ($[\alpha]^{20}_D = +46.6$ for **1**, literature[1,9,18] +48.2 to +53.0) confirmed the established structure as **1**, namely, (3*R*,4*R*,10*S*)-**1** as shown.

Compound **2** was obtained as an optically active colorless oil. The molecular formula $C_{16}H_{20}O_7$ was established by HREIMS, indicating seven double-bond equivalents. The IR spectrum of **2** indicated OH (3603 $cm^{-1}$) and ester carbonyl (1737 $cm^{-1}$) functionalities and a pentasubstituted aromatic system (3027, 1668, 1600, 1430, 861 $cm^{-1}$). This evidence was in agreement with the observation of signals in the $^{13}C$ NMR and DEPT spectra (see Experimental Section) for four secondary oxygenated carbons ($\delta_C$ 81.2, d; 75.9, d; 74.7, d; 65.0, d), one ester carbonyl atom ($\delta_C$ 167.7, s), and six aromatic atoms ($\delta_H$ 158.7, s; 156.3, s; 137.4, s; 130.9, s; 104.5, d; 102.0, s), accounting for five double-bond equivalents. The remaining double-bond equivalents were due to two additional rings in the molecule.

The $^1H$ and $^{13}C$ NMR spectra of compounds **1** and **2** were similar, with differences observed in the side chain carbons: A methylene group ($\delta_C$ 19.0, t; $\delta_H$ 1.25, m, 1.33, m) in **1** was replaced by an oxo-methine ($\delta_C$ 65.0, d; $\delta_H$ 4.01, m) in **2**. The signals for the methyl group were shifted downfield with the proton triplet being replaced by a doublet ($\delta_C$ 24.0, q, $\delta_H$ 1.19, d, $J = 6.0$ in **2**; $\delta_C$ 13.9, q, $\delta_H$ 0.82, t, $J = 7.4$ in **1**). These facts suggested that **2** was a 12-hydroxy analogue of **1**. The proposed structure was confirmed by a proton spin system from H-4 to $H_3$-13, established by a $^1H-^1H$ COSY experiment and the distinct NOE enhancement of H-4 with H-3, H-9*β*, and H-10 observed in the NOESY and NOEDIFF spectra. The absolute configuration at C-12, bearing a secondary OH group, was determined as *R* by a modified Mosher method,[19−21] as shown in Figure 1. The absolute configuration cannot be based on comparison of the deviating optical rotations of **1** and **2** (see Experimental Section). However, it is reasonable to assume a biosynthetic relationship between monocerin (**1**) and the co-occurring isocoumarins **2**−**4**. On the basis of the established absolute configurations of **1** and **4** (see below), compound **2** was therefore tentatively determined to be (12*R*)-12-hydroxymonocerin.

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10.1021/np800095g CCC: $40.75 
Published on Web 05/30/2008

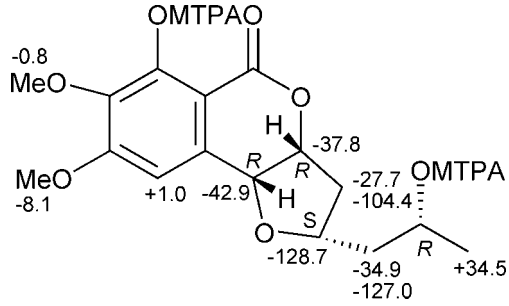


**Figure 1.** $\Delta\delta$ ($\delta_S - \delta_R$) values (in Hz) for the MTPA ester of **2**.

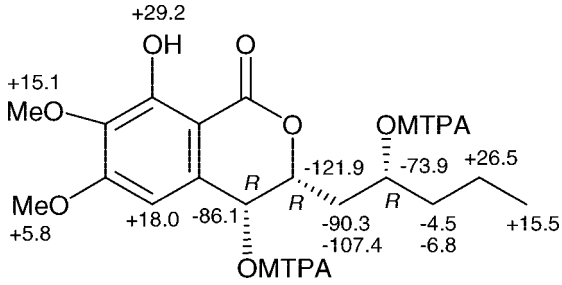


**Figure 2.** $\Delta\delta$ ($\delta_S - \delta_R$) values (in Hz) for the MTPA ester of **4**.

Compound **3** had the molecular formula $C_{16}H_{20}O_7$, the same as that of **2**. The IR and UV spectra of **3** resembled those of **2**, and the $^1$H and $^{13}$C NMR spectra of **3** were nearly identical to those of **2**. A difference was observed in the $^{13}$C NMR resonances of C-10 ($\delta$ 78.4 in **3**, 75.9 in **2**) and C-12 ($\delta$ 67.2 in **3**, 65.0 in **2**). The distinct NOE enhancement of H-4 with H-3, H-9$\beta$, and H-10 in NOESY and NOEDIFF spectra revealed the same polycyclic skeleton of **3** and **2**. The structural difference was thus attributed to the opposite configuration at C-12. Due to the probable biosynthetic relationship with monocerin (**1**) and isocoumarin **4**, compound **3** was determined to be the C-12 epimer of **2**, namely, (12*S*)-12-hydroxymonocerin.

Compound **4** had the molecular formula $C_{16}H_{22}O_7$, indicating six double-bond equivalents. The IR and UV spectra of **4** were reminiscent of those of **1**, showing absorption bands indicating OH groups, carbonyl groups, and those typical of a pentasubstituted phenyl group. The observation of a chelated proton, resonating at $\delta$ 11.04 in the $^1$H NMR spectrum, indicated that rings A and B were unchanged. This was confirmed by a comparison of the $^{13}$C NMR signals of **1** and of **4** related to ring A. Signals of one ester carbonyl ($\delta_C$ 169.0, s) and six aromatic carbons ($\delta_H$ 158.8, s; 156.1, s; 136.9, s; 136.5, s; 102.9, d; 101.8, s) were in agreement with the corresponding values for **1**, accounting for five double-bond equivalents. The remaining double-bond equivalent was attributed to the fused pyranone (ring B).

Analysis of the $^1$H$-^1$H COSY spectrum readily established the proton spin system from H-4 to H$_3$-13 and assigned both secondary OH groups at C-4 and C-10. The small coupling constant ($^3J_{3,4}$ = 2.0) between H-3 and H-4 suggested a *cis* configuration of these protons, which was further confirmed by a NOE difference experiment. The absolute configuration of both secondary alcohol groups was determined by comparison of the NMR data of their MTPA esters. As shown in Figure 2, the negative $\Delta\delta$ ($\delta_S - \delta_R$) values for H-3, H-4, H$_2$-9, and H-10 corresponded to a typical $\Delta\delta$ pattern for diesters of *R,R*-1,4-diol with chiral anisotropic reagents as reported by Riguera and co-workers.[22] Therefore, the configuration of both chiral atoms at C-4 and C-10 was elucidated as *R*. As a consequence, the absolute configuration at C-3 was also determined as *R* on the basis of the established relative configuration of the ring system. Compound **4** was thus elucidated as (3*R*,4*R*,10*R*)-**4** as shown.

**Table 1.** Agar Diffusion Assays for Antibacterial, Antifungal, and Antialgal Activities (0.05 mg of substance)[a]

| compound | *Escherichia coli* | *Bacillus megaterium* | *Microbotryum violaceum* | *Chlorella fusca* |
|---|---|---|---|---|
| **1** | 10 | 6 | gi 23 | 8 |
| **2** | 0 | 0 | 7 | 6 |
| **3** | 8 | 6 | 9 | 10 |
| **4** | 10 | 7 | 9 | 10 |
| penicillin | 18 | 14 | 0 | 0 |
| tetracycline | 18 | 18 | gi 10 | 0 |
| nystatin | 0 | 0 | 0 | 20 |
| actidione | 0 | 0 | 35 | 50 |
| acetone | 0 | 0 | 0 | 0 |

[a] Radii of the zones of inhibition are given in mm. gi = growth inhibition; i.e., there was some growth within the zone of inhibition.

Compound **4** is structurally correlated to fusarentin 6,7-dimethyl ether (**5**) and analogues, a series of metabolites coisolated with moncerins from the fungus *Fusarium larvarum*.[5,6] Biosynthetic studies revealed that both groups of metabolites are of heptaketide origin, and the fusarentins may act as precursors to the monocerins.[17,18] The same biosynthetic relationship probably exists between the compounds identified in this study.

The isolated compounds **1**−**4** were tested in an agar diffusion assay for their antifungal, antibacterial, and algicidal properties toward *Microbotryum violaceum, Escherichia coli, Bacillus megaterium*, and *Chlorella fusca* (Table 1). Whereas compounds **1**, **3**, and **4** inhibited all four test organisms, compound **2** was antialgal against *Chlorella fusca* and antifungal against *Microbotryum violaceum*, but was not antibacterial. These activities are consistent with results of our previous investigations in which we compared the antifungal activities of endophytic fungi and fungi associated with algae from different marine habitats.[16] Isolates from the marine biotopes from the island of Gomera were particularly active in our tests.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 241 MC polarimeter at the sodium D-line. UV spectra were recorded on a UV-2101PC spectrophotometer; peaks are reported in nm. IR spectra were recorded on a Nicolet-510P spectrophotometer; peaks are reported in cm$^{-1}$. NMR spectra were recorded at 293 K on a Bruker Avance-500 spectrometer. Chemical shifts are reported in parts per million ($\delta$), using $CDCl_3$ as an internal standard with coupling constant (*J*) in Hz. $^1$H and $^{13}$C NMR assignments were supported by $^1$H$-^1$H COSY, HMQC, HMBC, NOESY, and NOEDIFF experiments. Mass spectra and high-resolution mass spectra were performed on a MAT 8200 mass spectrometer. Commercial silica gel (Merck, 0.040−0.063 mm) was used for column chromatography. Precoated silica gel plates (Merck, G60 F-254 or G50 UV-254) were used for analytical and preparative thin-layer chromatography (TLC), respectively. Microbiological methods and culture conditions are as described in the literature.[24,25]

**Culture, Extraction, and Isolation.** The endophytic fungus *Microdochium bolleyi*, internal strain No. 8880, was isolated following surface sterilization from *Fagonia cretica*, growing in Gomera, and was cultivated on 12 L of 5% w/v biomalt solid agar medium at room temperature for 28 days.[24,25] The culture was extracted with ethyl acetate to afford 5 g of a residue after removal of the solvent under reduced pressure. The extract was subjected to column chromatography (CC) on silica gel, eluted with a gradient of petroleum ether in ethyl acetate (90:10, 50:50, 0:100), to give 14 subfractions. Fraction 8 gave the main metabolite **1** (166.0 mg) after CC on Sephadex LH-20 ($CHCl_2$/MeOH, 9:1), followed by silica gel CC (400−600 mesh, $CH_2Cl_2$). Fraction 9 was fractionated using Sephadex LH-20 CC ($CHCl_2$/MeOH, 5:1) and then purified by preparative TLC ($CH_2Cl_2$/2-propanol, 50:1) to yield **4** (8.0 mg). Fraction 12 was first subjected to Sephadex LH-20 CC ($CHCl_2$/MeOH, 2:1) and then preparative TLC ($CH_2Cl_2$/2-propanol, 30:1) to give compounds **2** (2.5 mg) and **3** (3.4 mg).

**Monocerin (1):** colorless oil; $[\alpha]^{20}_D$ +46.6 (*c* 2.43, in MeOH); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 11.21 (1H, s, 8-O*H*), 6.53 (1H, s, H-5), 4.98 (1H, ddd, *J* = 6.1, 3.1, 1.0 Hz, H-3), 4.46 (1H, d, *J* = 3.1 Hz, H-4), 4.02 (1H, m, H-10), 3.86 (3H, s, 6-OC*H*$_3$), 3.79 (3H, s, 7-OC*H*$_3$), 2.52 (1H, ddd, *J* = 14.5, 8.5, 6.1 Hz, H-9$\beta$), 2.03 (1H, ddd, *J* = 14.5, 6.0, 1.0 Hz, H-9$\alpha$), 1.60 (1H, m, H-11b), 1.49 (1H, m, H-11a), 1.33 (1H, m, H-12b), 1.25 (1H, m, H-12a), 0.82 (3H, t, *J* = 7.4 Hz, H$_3$-13); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.8 (C, C-1), 158.6 (C, C-6), 156.0 (C, C-8), 137.2 (C, C-7), 131.3 (C, C-4a), 104.5 (CH, C-5), 101.9 (C, C-8a), 81.3 (CH, C-3), 78.5 (CH, C-10), 74.3 (CH, C-4), 60.6 ($CH_3$, 7-O*C*H$_3$), 56.2 ($CH_3$, 6-O*C*H$_3$), 39.0 ($CH_2$, C-9), 38.1 ($CH_2$, C-11), 19.0 ($CH_2$, C-12), 13.9 ($CH_3$, C-13); EIMS *m*/*z* 308.1 $[M]^+$, 265.0, 247.1, 209.0, 167.0, 148.0, 81.1, 41.1.

**Compound 2:** colorless oil; $[\alpha]^{20}_D$ −12.7 (*c* 0.11 in $CH_2Cl_2$/MeOH, 1:1); UV ($CH_2Cl_2$/MeOH, 1:1) $\lambda_{max}$ (log $\epsilon$) 304 (3.18), 275.2 (3.66), 229.0 (3.99) nm; IR ($CHCl_3$) $\nu_{max}$ 3603, 3027, 1737, 1668, 1600, 1430, 1119, 861 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 11.24 (1H, s, 8-O*H*), 6.58 (1H, s, H-5), 5.05 (1H, ddd, *J* = 6.0, 3.0, 1.0 Hz, H-3), 4.56 (1H, d, *J* = 3.0 Hz, H-4), 4.41 (1H, m, H-10), 4.01 (1H, m, H-12), 3.94 (3H, s, 6-OC*H*$_3$), 3.90 (3H, s, 7-OC*H*$_3$), 2.65 (1H, ddd, *J* = 14.5, 8.5, 6.0 Hz, H-9$\beta$), 2.20 (1H, ddd, *J* = 14.5, 5.5, 1.0 Hz, H-9$\alpha$), 1.82 (1H, ddd, *J* = 14.0, 8.0, 3.5 Hz, H-11b), 1.73 (1H, ddd, *J* = 14.0, 9.0, 4.0 Hz, H-11a), 1.19 (3H, d, *J* = 6.0 Hz, H$_3$-13); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.7 (C, C-1), 158.7 (C, C-6), 156.3 (C, C-8), 137.4 (C, C-7), 130.9 (C, C-4a), 104.5 (CH, C-5), 102.0 (C, C-8a), 81.2 (CH, C-3), 75.9 (CH, C-10), 74.7 (CH, C-4), 65.0 (CH, C-12), 60.7 ($CH_3$, 7-O*C*H$_3$), 56.3 ($CH_3$, 6-O*C*H$_3$), 44.6 ($CH_2$, C-11), 39.5 ($CH_2$, C-9), 24.0 ($CH_3$, C-13); HREIMS *m*/*z* 324.12094 (calcd for $C_{16}H_{20}O_7$, 324.12094).

**Compound 3:** colorless oil; $[\alpha]^{20}_D$ +4.7 (*c* 0.17 in $CH_2Cl_2$/MeOH, 1:1); UV ($CH_2Cl_2$/MeOH, 1:1) $\lambda_{max}$ (log $\epsilon$) 275.2 (4.21), 229.4 (4.54) nm; IR ($CHCl_3$) $\nu_{max}$ 3603, 3021, 1731, 1674, 1600, 1457, 1166, 854 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 11.23 (1H, s, 8-O*H*), 6.57 (1H, s, H-5), 5.06 (1H, dd, *J* = 6.0, 3.0 Hz, H-3), 4.60 (1H, d, *J* = 3.0 Hz, H-4), 4.35 (1H, m, H-10), 3.98 (1H, m, H-12), 3.95 (3H, s, 6-OC*H*$_3$), 3.91 (3H, s, 7-OC*H*$_3$), 2.67 (1H, ddd, *J* = 14.5, 8.5, 6.0 Hz, H-9$\beta$), 2.22 (1H, dd, *J* = 14.5, 5.5 Hz, H-9$\alpha$), 1.84 (1H, ddd, *J* = 14.0, 9.0, 6.0 Hz, H-11b), 1.75 (1H, dt, *J* = 14.0, 4.0 Hz, H-11a), 1.18 (3H, d, *J* = 6.0 Hz, H$_3$-13); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 167.6 (C, C-1), 158.7 (C, C-6), 156.3 (C, C-8), 137.5 (C, C-7), 130.6 (C, C-4a), 104.5 (CH, C-5), 102.0 (C, C-8a), 80.8 (CH, C-3), 78.4 (CH, C-10), 75.0 (CH, C-4), 67.2 (CH, C-12), 60.7 ($CH_3$, 7-O*C*H$_3$), 56.3 ($CH_3$, 6-O*C*H$_3$), 44.8 ($CH_2$, C-11), 39.6 ($CH_2$, C-9), 23.6 ($CH_3$, C-13); HREIMS *m*/*z* 324.12096 (calcd for $C_{16}H_{20}O_7$, 324.12094).

**Compound 4:** colorless oil; $[\alpha]^{20}_D$ −4.3 (*c* 0.58 in $CH_2Cl_2$/MeOH, 1:1); UV ($CH_2Cl_2$/MeOH, 1:1) $\lambda_{max}$ (log $\epsilon$) 306.0 (3.11), 273.4 (3.66), 229.8 (43.97) nm; IR ($CHCl_3$) $V_{max}$ 3603, 3027, 1737, 1674, 1620, 1515, 1457, 1116, 839 cm$^{-1}$; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 11.04 (1H, s, 8-O*H*), 6.56 (1H, s, H-5), 4.73 (1H, ddd, *J* = 5.5, 5.5, 2.0 Hz, H-3), 4.66 (1H, d, *J* = 2.0 Hz, H-4), 4.06 (1H, m, H-10), 3.94 (3H, s, 6-OC*H*$_3$), 3.89 (3H, s, 7-OC*H*$_3$), 2.23 (1H, ddd, *J* = 14.5, 7.5, 3.0 Hz, H-9b), 2.06 (1H, ddd, *J* = 14.5, 7.5, 5.5 Hz, H-9a), 1.57 (1H, m, H-11b), 1.54 (1H, m, H-11a), 1.49 (1H, m, H-12b), 1.40 (1H, m, H-12a), 0.97 (3H, t, *J* = 7.0 Hz, H$_3$-13); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 169.0 (C, C-1), 158.8 (C, C-6), 156.1 (C, C-8), 136.9 (C, C-7), 136.5 (C, C-4a), 102.9 (CH, C-5), 101.8 (C, C-8a), 79.5 (CH, C-3), 68.0 (CH, C-10), 66.5 (CH, C-4), 60.7 ($CH_3$, 7-O*C*H$_3$), 56.3 ($CH_3$, 6-O*C*H$_3$), 39.7 ($CH_2$, C-11), 37.6 ($CH_2$, C-9), 18.8 ($CH_2$, C-12), 13.9 ($CH_3$, C-13); HREIMS *m*/*z* 326.13652 (calcd for $C_{16}H_{22}O_7$, 326.13655).

**Esterification of 2 with MTPA Chloride.** Both (*S*)- and (*R*)-MTPA esters of **2** (**2***S*, **2***R*) were obtained by treatment of **2** (0.6 mg, respectively) with (*R*)- and (*S*)-MTPA chlorides (5 $\mu$L) in dry pyridine (0.5 mL) catalyzed with dimethylaminopyridine and stirred at room temperature overnight. The MTPA esters (1.25 mg, 89% yielding) were purified by a minicolumn chromatography on silica gel (300 mesh, petroleum ether/EtOAc, 5:1).

$^1$H NMR of **2***S*-MTPA ester (500 MHz, $CDCl_3$): $\delta$ 6.56 (1H, s, H-5), 5.25 (1H, m, H-12), 4.95 (1H, dd, *J* = 4.9, 3.1, H-3), 4.44 (1H, d, *J* = 3.1, H-4), 3.97 (3H, s, 6-OC*H*$_3$), 3.91 (1H, m, H-10), 3.91 (3H, s, 7-OC*H*$_3$), 2.35 (1H, ddd, *J* = 13.8, 8.7, 4.9, H-9$\beta$), 2.28 (1H, ddd, *J* = 13.0, 6.8, 2.9, H-11b), 2.04 (1H, dd, *J* = 13.8, 4.0, H-9$\alpha$), 1.90 (1H, ov, H-11a), 1.33 (3H, d, *J* = 6.3, H$_3$-13).

$^1$H NMR of **2***R*-MTPA ester (500 MHz, $CDCl_3$): $\delta$ 6.56 (1H, s, H-5), 5.26 (1H, m, H-12), 5.02 (1H, dd, *J* = 4.9, 3.0, H-3), 4.52 (1H, d, *J* = 3.0, H-4), 4.16 (1H, m, H-10), 3.99 (3H, s, 6-OC*H*$_3$), 3.91 (s, 3H, 7-OC*H*$_3$), 2.56 (1H, ddd, *J* = 13.9, 7.8, 4.9, H-9$\beta$), 2.53 (1H, ddd, *J* = 13.6, 7.8, 3.3, H-11b), 2.10 (1H, dd, *J* = 13.9, 5.0, H-9$\alpha$), 1.97 (1H, ddd, *J* = 13.6, 9.3, 4.0, H-11a), 1.26 (3H, d, *J* = 6.5, H$_3$-13).

**Esterification of 4 with MTPA Chloride.** Both (*S*)- and (*R*)-MTPA esters of **4** (**4***S*, **4***R*) were obtained by treatment of **4** (0.6 mg, respectively) with (*R*)- and (*S*)-MTPA chlorides (5 $\mu$L) in dry pyridine (0.5 mL), stirred at room temperature overnight. The MTPA esters (0.87 mg, 62% yielding) were purified by minicolumn chromatography on silica gel (300 mesh, petroleum ether/EtOAc, 7:1).

$^1$H NMR of **4***S*-MTPA ester (500 MHz, $CDCl_3$): $\delta$ 10.92 (1H, s, 8-O*H*), 6.72 (1H, s, H-5), 5.81 (1H, d, *J* = 1.6, H-4), 5.12 (1H, m, H-10), 4.25 (1H, br t, *J* = 6.9, H-3), 3.95 (3H, s, 6-OC*H*$_3$), 3.95 (3H, s, 7-OC*H*$_3$), 2.02 (1H, ddd, *J* = 15.8, 8.6, 6.5, H-9b), 1.83 (1H, ddd, *J* = 15.8, 7.4, 4.0, H-9a), 1.66 (1H, m, H-11b), 1.52 (1H, m, H-11a), 1.26 (2H, m, H$_2$-12), 0.91 (3H, t, *J* = 7.3, H$_3$-13).

$^1$H NMR of **4***R*-MTPA ester (500 MHz, $CDCl_3$): $\delta$ 10.87 (1H, s, 8-O*H*), 6.67 (1H, s, H-5), 5.94 (1H, d, *J* = 1.6, H-4), 5.27 (1H, m, H-10), 4.50 (1H, br t, *J* = 6.9, H-3), 3.94 (3H, s, 6-OC*H*$_3$), 3.92 (3H, s, 7-OC*H*$_3$), 2.24 (1H, ddd, *J* = 15.4, 7.8, 6.9, H-9b), 2.01 (1H, ddd, *J* = 15.4, 7.3, 4.6, H-9a), 1.67 (1H, m, H-11b), 1.53 (1H, m, H-11a), 1.26 (2H, m, H$_2$-12), 0.87 (3H, t, *J* = 7.3, H$_3$-13).

**Agar Diffusion Test for Biological Activity.** Compounds **1**−**4** were dissolved in acetone at a concentration of 1 mg/mL. Fifty microliters of the solutions (50 $\mu$g) was pipetted onto a sterile filter disk (Schleicher & Schuell, 9 mm), which was placed onto an appropriate agar growth medium for the respective test organism and subsequently sprayed with a suspension of the test organism.[25] The test organisms were the Gram-negative bacterium *Escherichia coli,* the Gram-positive bacterium *Bacillus megaterium* (both grown on NB medium), the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca* (both grown on MPY medium). Reference substances were penicillin, nystatin, actidione, and tetracycline. Commencing at the outer edge of the filter disk, the radius of the zone of inhibition was measured in millimeters. These microorgansims were chosen because (a) they are nonpathogenic and (b) they had in the past proved to be accurate initial test organisms for antibacterial, antifungal, and antialgal/herbicidal activities.

**Acknowledgment.** We thank BASF AG and the Bundesministerium für Bildung and Forschung (BMBF), project no. 03F0360A, for supporting our research work, and Kathrin Meier and Tatjana Stolz for excellent technical assistance.

## References and Notes

(1) Aldridge, D. C.; Turner, W. B. *J. Chem. Soc., Sect. C* **1970**, *18*, 2598–2600.
(2) Robeson, D. J.; Strobel, G. A. *Agric. Biol. Chem.* **1982**, *46*, 2681–2683.
(3) Cuq, F.; Herrmann-Gorline, S.; Klaebe, A.; Rossignol, M.; Petitprez, M. *Phytochemistry* **1993**, *34*, 1265–1270.
(4) Lim, C.-H. *Agric. Chem. Biotechnol. (Engl Ed.)* **1999**, *42*, 45–47.
(5) Grove, J. F.; Pople, M. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2048−2051.
(6) Claydon, N.; Grove, J. F.; Pople, M. *J. Invertebr. Pathol.* **1979**, *33*, 364–367.
(7) Cuq, F.; Brown, S. C.; Petitprez, M.; Alibert, G. *Plant Cell Rep.* **1995**, *15*, 138–142.
(8) Mori, K.; Takaishi, H. *Tetrahedron* **1989**, *45*, 1639–1646.
(9) Dillon, M. P.; Simpson, T. J.; Sweeney, J. B. *Tetrahedron Lett.* **1992**, *33*, 7569–7572.
(10) Ko, B.-S. *Han'guk Nonghwa Hakhoechi* **1994**, *37*, 402–408.
(11) Mallareddy, K.; Rao, S. P. *Tetrahedron* **1996**, *52*, 8535–8544.
(12) Cassidy, J. H.; Farthing, C. N.; Marsden, S. P.; Pedersen, A.; Slater, M.; Stemp, G. *Org. Biomol. Chem.* **2006**, *4*, 4118–4126.
(13) Grove, J. F.; Pople, M. *Mycopathologia* **1981**, *76*, 65–67.
(14) McNicholas, C.; Simpson, T. J.; Willett, N. J. *Tetrahedron Lett.* **1996**, *37*, 8053–8056.
(15) Zhang, W.; Krohn, K.; Zia-Ullah; Flörke, U.; Pescitelli, G.; Di Bari, L.; Antus, S.; Kurtán, T.; Rheinheimer, J.; Draeger, S.; Schulz, B. *Chem. Eur. J.* **2008**, published online.
(16) Schulz, B.; Draeger, S.; dela Cruz, T.; Rheinheimer, J.; Siems, K.; Loesgen, S; Bitzer, J.; Schloerke, O.; Zeeck, A.; Ines Kock, I.; Hussain, H.; Dai, J.; Krohn, K. *Bot. Mar.* **2008**, accepted.
(17) Scott, F. E.; Simpson, T. J.; Trimble, L. A.; Vederas, J. C. *J. Chem. Soc., Chem. Commun.* **1984**, (12), 756–758.
(18) Axford, L. C.; Simpson, T. J.; Willis, C. L. *Angew. Chem, Int. Ed.* **2004**, *43*, 727–730.
(19) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

(20) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Org. Chem.* **1991**, *56*, 1296–1298.
(21) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(22) Seco, J. M.; Martino, M.; Quinoa, E.; Riguera, R. *Org. Lett.* **2000**, *2*, 3261–3264.
(23) Krohn, K.; Florke, U.; Rao, M. S.; Steingrover, K.; Aust, H.-J.; Draeger, S.; Schulz, B. *Nat. Prod. Lett.* **2001**, *15*, 353–361.
(24) Schulz, B.; Sucker, J.; Aust, H.-J.; Krohn, K.; Ludewig, K.; Jones, P. G.; Doering, D. *Mycol. Res.* **1995**, *99*, 1007–1015.
(25) Höller, U.; Wright, A. D.; Matthée, G. F.; König, G. M.; Draeger, S.; Aust, H.-J.; Schulz, B. *Mycol. Res.* **2000**, *104*, 1354–1365.

NP800095G