Malettinins B−D: New Polyketide Metabolites from an Unidentified Fungal Colonist of *Hypoxylon* Stromata (NRRL 29110)

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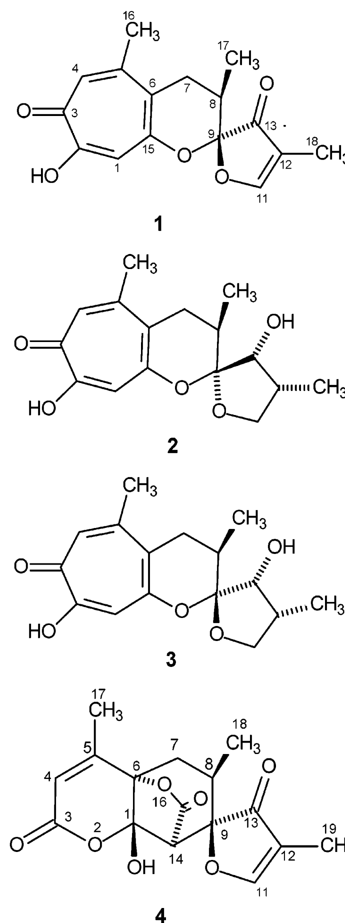
Malettinins B−D (**2**−**4**), three new antimicrobial polyketide-derived metabolites related to the previously reported malettinin A (**1**), have been obtained from nonsporulating cultures of an isolate of *Mycelia sterilia* MYC-155 (= NRRL 29110) collected from colonies of *Hypoxylon* stromata. Malettinins B (**2**) and C (**3**) are partially reduced analogues of malettinin A and were identified by analysis of NMR and MS data. Malettinin D (**4**) is biogenetically similar, but possesses a new ring system, and the structure of **4** was established by single-crystal X-ray diffraction analysis.

Mycoparasitic and fungicolous fungi are those that colonize the hyphae or survival structures of other species.[1,2] Damage to the host species occurring during the colonization process can be caused by antifungal agents produced by such colonists, thus suggesting their study as prospective sources of antifungal natural products. Our studies of mycoparasitic and fungicolous fungi have led to the isolation of a variety of new bioactive secondary metabolites.[3−8] One group of fungicolous isolates under investigation in this project are those that colonize nutrient-rich stromata[9] of wood-decay fungi. As part of this investigation, stromata of *Hypoxylon* sp. growing on dead Aspen logs in New Mexico were collected. One of the cultures isolated from stromatal filings of this *Hypoxylon* sp. specimen produced a nonsporulating *Mycelia sterilia* culture (MYC-155 = NRRL 29110). The organic extract from solid-substrate fermentation cultures of this isolate displayed potent antifungal activity. Thus, this culture was selected for chemical investigation. A new polyketide-derived tropolone (malettinin A; **1**) was initially isolated as the major active component.[8] Further studies have led to the identification of three additional related minor metabolites called malettinins B−D (**2**−**4**). Malettinins B (**2**) and C (**3**) are partially reduced analogues of **1** that retain the tropolone unit, while malettinin D (**4**) is biogenetically similar, but structurally different, incorporating a new ring system. This report describes the structure elucidation of malettinins B−D (**2**−**4**).



Results and Discussion

Malettinins A−D (**1**−**4**) were all obtained by processes involving Sephadex LH-20 column chromatography followed by reversed-phase HPLC. HRFABMS and NMR data established the molecular formula of malettinin B (**2**) as $C_{16}H_{20}O_5$. This formula contains four hydrogen atoms more than that of malettinin A (**1**), requiring two fewer unsaturations. Analysis of the ^{1}H and ^{13}C NMR data for malettinin B (Tables 1 and 2) revealed the presence of considerable structural similarities with malettinin A (**1**) that facilitated the structure elucidation of malettinin B (**2**). Of particular utility were the signals in the aromatic region (δ_H 6.76, 7.01) that were assigned to the tropolone protons. Diastereotopic methylene proton signals corresponding to H_2-7 (δ_H 2.54, 3.02) and the aryl methyl singlet (H_3-16; δ_H 2.36) were also present. However, the midfield region of the ^{1}H NMR spectrum was significantly different, as it showed several new signals for mutually coupled protons. In addition, the vinyl methyl doublet at δ_H 1.77 (J = 1.1 Hz) was replaced by a doublet at δ_H 1.04 (J = 6.6 Hz), and the downfield olefinic proton signal at δ_H 8.02 was absent. Three ^{13}C NMR signals in the downfield (sp^2) region in the spectrum of **1** (δ_C 173.4, 114.8, 198.8) were replaced by three sp^3 carbon signals in the spectrum of **2** (δ_C 74.5, 36.8, 76.6), corresponding to an oxygen-attached methylene

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Table 1. ^{1}H NMR Data for Malettinins B−D (**2**−**4**)

H #	**2**[a] δ_H (mult., *J* in Hz)	**3**[a] δ_H (mult., *J* in Hz)	**4**[b] δ_H (mult., *J* in Hz)
1	6.76 (s)	6.95 (s)	
4	7.01 (s)	7.06 (s)	6.22 (q, 1.8)
7a	3.02 (dd, 17, 6.2)	2.80 (dd, 17, 5.9)	2.55 (dd, 14, 6.3)
7b	2.56 (17, 3.8)[c]	2.52 (dd, 17, 13)[c]	2.05 (dd, 14, 11)
8	2.57 (m)	2.22 (m)	2.42 (m)
11a	4.18 (t, 7.8)	4.12 (dd, 8.8, 7.7)	8.32 (q, 1.2)
11b	3.64 (dd, 10, 7.8)	3.65 (dd, 8.8, 5.8)	
12	2.70 (m)	2.50 (m)	
13	3.90 (d, 4.8)	4.32 (d, 8.7)	
14			2.76 (s)
16	2.36 (s)	2.40 (s)	
17	1.01 (d, 6.6)	1.08 (d, 6.6)	2.10 (d, 1.8)
18	1.04 (d, 6.6)	1.21 (d, 7.2)	0.81 (d, 6.7)
19			1.72 (d, 1.2)

[a] Recorded in acetone-d_6 solution at 400 MHz. [b] Recorded in CD_3OD solution at 300 MHz. [c] These signals overlapped to some degree with neighboring signals in acetone-d_6 solution, so the *J* values listed were measured in $CDCl_3$ solution.

Table 2. ^{13}C NMR Data (δ_C) for Malettinins B−D (**2**−**4**)

C #	**2**[a]	**3**[b]	**4**[c]
1	112.6	113.2	105.0[d]
2	164.1	164.4	
3	173.0	171.7	162.5
4	125.0	125.0	121.6
5	151.2	149.8	152.4
6	120.2	122.2	81.3
7	33.9	32.0	33.8
8	28.8	29.9	33.3
9	113.1	106.2	87.7
11	74.5	73.7	175.8
12	36.8	34.7	116.3
13	76.6	73.0	204.5
14			53.1
15	160.3	158.3	168.9
16	27.2	26.9	
17	14.3	15.0	18.5
18	10.3	12.8	12.7
19			5.1

[a] Recorded in acetone-d_6. [b] Recorded in $CDCl_3$. [c] Recorded in CD_3OD. [d] Signal was particularly weak in the 1D spectrum, but correlations to this position were observed in the HMBC data.

unit and two methine units (one oxygenated) based on DEPT data. Comparison of DEPT results with the molecular formula indicated that malettinin B (**2**) contains two free OH groups, rather than one as in malettinin A. These observations suggested that the α,*β*-unsaturated ketone subunit in malettinin A (**1**) was reduced to the corresponding saturated alcohol moiety in malettinin B. Analysis of COSY, HMQC, and HMBC data confirmed the presence of the resulting tetrahydrofuran unit and enabled establishment of the structure. Of particular importance were the HMBC correlations from H_2-11 (δ_H 3.64, 4.18) to C-9 and from H-8 (δ_H 2.57) to C-13, which allowed connection of the $-OCH-CH(CH_3)-CH_2O-$ spin system to C-9, resulting in assignment of the tetrahydrofuran ring. The chemical shift of the doubly oxygenated carbon C-9 was significantly downfield-shifted (δ_C 113.1) relative to that of **1** (δ_C 101.1), and comparison with literature precedents indicated that this is consistent with the absence of the α-carbonyl group.[10,11] Given all these data, malettinin B (**2**) was identified as a reduced analogue of malettinin A.

Malettinin C (**3**) proved difficult to separate completely from malettinin B (**2**), despite efforts that included employment of four different HPLC stationary phases and various mobile phases. However, it was possible to obtain a sample enriched in malettinin C in which the signals for the two compounds were readily distinguishable (approximately a 5:1 mixture), and it was therefore possible to solve the structure of malettinin C by analysis of this sample. MS and NMR data indicated that malettinin C is an isomer of malettinin B, and DEPT data indirectly revealed that it also possesses two exchangeable protons. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) were consistent with the presence of the same tropolone-dihydropyran unit found in **1** and **2**. The ^{13}C and ^{1}H NMR data for **3** (Tables 1 and 2) were very similar to those of **2**, except for some modest differences in shifts and/or *J* values for several signals. These data implied that **3** is a diastereomer of **2**, and this conclusion was verified by detailed analysis of 2D NMR data for **3**. Complete NMR assignments were made by analysis of COSY, HMQC, and HMBC data and supported by comparison to those of malettinin B (**2**).

Assignment of the relative stereochemistry of malettinins B (**2**) and C (**3**) proved to be challenging and was complicated by a shortage of relevant NOESY correlations useful in relating the stereochemistry of the tetrahydrofuran and dihydropyran rings, and by fortuitous overlap of key signals. The latter problem was overcome to some degree by analysis of each compound using different solvents. A considerable difference in the vicinal J_{H7-H8} values for the two compounds was observed (6.2 and 3.8 Hz for **2**; 5.9 and 13 Hz for **3**), indicating a stereochemical or conformational difference in the dihydropyran ring. The corresponding *J* values of **1** and its methanol adduct[8] showed close similarity with **3**, arguing that the relative stereochemistry at C-8 in **3** is likely to match that in **1**, placing H-8 in a pseudoaxial position in **3**, but not in **2**. The $J_{H12-H13}$ values for **2** and **3** were also significantly different (4.8 Hz for **2**; 8.7 Hz for **3**), suggesting a possible stereochemical difference in that region as well. Comparison of the $J_{H12-H13}$ value for **2** (4.8 Hz) with the literature value (4.5 Hz) for a compound incorporating a spiro-linked tetrahydrofuran-dihydropyran unit substituted with vicinal methyl and hydroxyl groups in a cis orientation (blazeispirol A)[12] showed excellent agreement. The value for **2** also lies in the 4.4−5.3 Hz range reported for several monocyclic *cis*-3-hydroxy-4-alkyltetrahydrofuran derivatives,[13] while that of **3** (8.7 Hz) is outside this range. A strong NOESY correlation observed between H-12 and H-13 in **2** is consistent with placement of both protons on the same face of the tetrahydrofuran ring. However, H-12 and H-13 also showed a similarly intense NOESY cross-peak in the data for **3**. Interestingly, analysis of *J* values published for another series of synthetic monocyclic tetrahydrofurans containing similarly disposed vicinal methyl and hydroxyl groups with both cis and trans geometry[14] revealed that the vicinal coupling constant between the adjacent methine protons can vary considerably with the relative stereochemistry at other positions around the ring. Specifically, in *trans*-3-hydroxy-4-methyl tetrahydrofurans bearing substituents at both the 2- and 5-positions, alteration of the relative configuration at C-2 resulted in a range of trans J_{H3-H4} values of at least 1.5−8.7 Hz depending on the substituent identities. One case with identical substituents differing in relative configuration only at C-2 gave a value of 3.6 Hz for the trans J_{H3-H4} in one diastereomer and 8.7 Hz for the other. Thus, consideration of *J* values alone in comparing the stereochemistry of **2** and **3** could be misleading. This conclusion, together with the NOE results noted above, suggested that the stereochemical difference between **2** and **3** could actually be at ketal carbon C-9. Such a change would also be consistent with a difference in *J* values observed for the dihydropyran ring. One other spectral difference between **2** and **3** was the presence of a

Table 3. HMBC Data for Malettinins B–D (**2**–**4**)

position	**2** (H→ C#)	**3** (H→ C#)	**4** (H→ C#)
1	2, 3, 6, 15	2, 3, 6, 15	
4	2, 3, 5, 6, 16	2, 3, 5, 6, 16	3, 6, 17
7_{eq}	5, 6, 8, 15, 17	5, 6, 8, 9, 15	1, 5, 6, 8, 9
7_{ax}	5, 6, 8, 9, 15, 17	5, 6, 8, 9, 15, 17	1, 5, 6, 8, 9
8	6, 9, 13	6, 7, 9, 13, 17	7, 13, 18
11	9, 12, 13, 18	9, 12, 13, 18	9, 12, 13
12	11, 18	13, 18	
13	11, 18	8, 11, 12, 18	
14			1, 6, 8, 9, 13, 15
16	4, 5, 6	4, 5, 6	
17	7, 8, 9	7, 8, 9	4, 5, 6
18	11, 12, 13	11, 12, 13	7, 8, 9
19			11, 12, 13

strong NOESY correlation in **3** between H-13 and H_3-17 and a weaker correlation of H-13 with H-8, neither of which was observed in the data for **2**. Both of these correlations are consistent with the stereochemistry shown in **3** and would be less likely for a structure with the opposite relative stereochemistry at C-9. In an effort to secure additional stereochemically relevant information, NOESY data were recorded in different solvents and with different delay values, but further useful cross-peaks were not observed. On the basis of these considerations, the relative stereochemistry for **2** and **3** was proposed as shown, although these assignments are not considered conclusive. Molecular modeling calculations for the two structures (Spartan '02, Wavefunction, Inc.) were consistent with these assignments, as they supported the proposition that CH_3-17 would adopt a pseudoaxial orientation in **2** and a pseudoequatorial orientation in **3**, and also showed significant difference in the vicinal angle between H-12 and H-13 for the two structures, with that of **3** being significantly closer to 0°.

NMR and HRFABMS data indicated that malettinin D (**4**) contains the same number of carbon and hydrogen atoms as malettinin A (**1**), but possesses three additional oxygen atoms. Comparison of DEPT data and the molecular formula indicated the presence of one exchangeable proton. Analysis of the NMR data revealed significant differences relative to **1**–**3**, most notably, the absence of the tropolone moiety. ^1H and ^{13}C NMR data (Tables 1 and 2) revealed the presence of three methyl groups, one methylene unit, two methine groups, three quaternary sp^3 carbons (two of which are oxygenated), and four olefinic carbons, two of which are protonated. One of the protonated sp^2 carbons must be oxygenated on the basis of its considerable downfield shift (δ_C 175.8). In addition, the ^{13}C NMR spectrum showed the presence of a ketone carbon (δ_C 204.5), as well as two carboxyl groups (δ_C 162.5, 168.9). These data require a tetracyclic structure for malettinin D.

Analysis of HMQC and COSY data led to the identification of three isolated spin systems corresponding to the C7–C8–C18, C11–C12–C19, and C4–C5–C17 subunits of **4**. An isolated methine proton (H-14) was observed as a sharp singlet. Chemical shift considerations,[8,15,16] together with HMBC correlations (Table 3) of H-11 and H_3-19 with ketone carbonyl C-13 and of H-11 with quaternary carbon C-9, were consistent with the presence of the same furanone unit found in **1**, and correlations of H-8 to C-13 and of H_3-18 to C-9 again enabled connection of C-8 to C-9 of the furanone ring. However, in this case, C-9 was identified as an oxygenated quaternary carbon (δ_C 87.7), rather than a ketal carbon. Isolated methine H-14 showed correlations to C-8, C-9, and C-13, requiring linkage of C-14 to C-9. This proton also showed the only correlation to carboxyl carbon C-15 (δ_C 168.9), suggesting attachment of C-14 and C-15. HMBC correlations of H-4, H_2-7, H_3-17, and H-8 with the quaternary oxygen-bearing carbon C-6 (δ_C 81.2) allowed connection of the C-7–C-8–C-18 and C-4–C-5–C-17 systems through C-6. Correlation of H-4 with the carboxyl carbon C-3 (δ_C 162.5), together with the downfield shift of C-5 (δ_C 152.4), enabled connection of C-3 and C-4. Further correlations of H_2-7 and H-14 to C-1, and of H-14 to C-6, led to connection of doubly oxygenated carbon C-1 (δ_C 105.0) to C-6 and to linkage of C-1 and C-14. The resulting partial structure accounted for all but the exchangeable proton, which could be attributed to either a free hydroxy group or a free carboxylic acid unit. Consequently, ^1H NMR data were recorded using DMSO-d_6 as solvent, enabling observation of an exchangeable proton signal at δ_H 8.98. This signal showed HMBC correlations to C-1, C-6, and C-14. These results ruled out the possibility of a free carboxylic acid and reduced the number of candidate structures, but could not distinguish between two remaining possibilities. One structure (**4**) could be formed by connecting O-2 to C-1 and O-16 to C-6 to form δ-lactone and bicyclic γ-lactone rings, respectively. However, a structure arising from connection of O-2 to C-6 and O-16 to C-1 to form spiro γ-lactone and β-lactone rings fused to the cyclohexane ring could not be definitively ruled out.

Ultimately, crystals of malettinin D suitable for single-crystal X-ray analysis were obtained by crystallization from 35% CH_3CN/H_2O. The X-ray crystallographic model of **4** (Figure 1) revealed the gross structure and allowed assignment of the relative stereochemistry. Malettinin D (**4**) possesses a previously unreported tetracyclic ring system with bridged γ-lactone ring and a central cyclohexane ring that adopts a slightly distorted chair conformation. The relative stereochemistry at C-8 and C-9 matches that assigned for the corresponding positions in malettinins A (**1**) and C (**3**).

Even though the structure of malettinin D (**4**) is significantly different from those of malettinins A–C (**1**–**3**), the

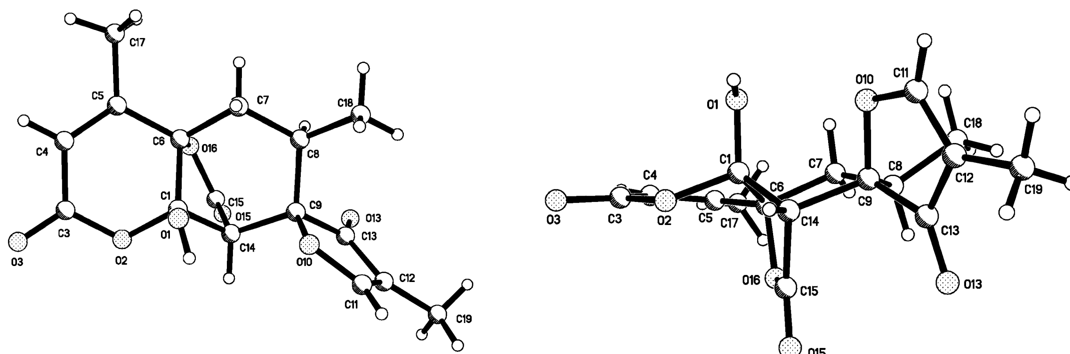

Figure 1. Two different perspective views of the final X-ray crystallographic model of malettinin D (**4**).

four compounds appear to be biogenetically related. A polyketide origin has been proposed for **1**[8] (and therefore presumably for **2** and **3**), with the tropolone unit perhaps arising via an aromatic ring expansion, as may be the case for other fungal tropolones.[17] A similar biosynthetic origin could be proposed for malettinin D (**4**) that would involve an oxidative cleavage of the tropolone ring.

Malettinins A−C (**1**−**3**) were largely responsible for the antifungal activity of the extract. Like malettinin A (**1**),[8] malettinins B and C (**2** and **3**) showed activity in assays against *Candida albicans* (ATCC 90029), causing zones of inhibition of 19 and 13 mm, respectively, at 250 μg/disk, while malettinin D (**4**) was inactive in the assay at this level. Malettinins B−D (**2**−**4**) all exhibited antibacterial activity against *Staphylococcus aureus* (ATCC 29213) and *Bacillus subtilis* (ATCC 6051) in standard disk assays at 200 μg/disk. Malettinin B (**2**) caused zones of inhibition of 24 and 26 mm, respectively. Malettinin C (**3**) showed somewhat weaker activity, as it exhibited zones of inhibition of 12 and 15 mm, and malettinin D (**4**) produced 10 and 14 mm zones.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in chloroform-*d*, acetone-d_6, or methanol-d_4, and chemical shifts were referenced relative to the corresponding chloroform (δ_H 7.24/δ_C 77.0), acetone (δ_H 2.04/δ_C 29.8), or methanol (δ 3.30/49.0) signals. ^{1}H NMR data were recorded at 300 MHz (Bruker AC-300), 400 MHz (Bruker DRX-400), or 600 MHz (Bruker AMX-600 MHz); ^{13}C NMR data were recorded at 90 MHz (Bruker WM-360) or 100 MHz (Bruker DRX-400). All 2D NMR data were recorded at 600 MHz (^{1}H dimension). The optical rotations were measured on a JASCO model DIP-1000 digital polarimeter. UV absorptions were recorded using a Beckman DU 640 or a Shimadzu UV-2101PC spectrophotometer. IR spectra were obtained with a Mattson Cygnus 25 FT spectrophotometer. Electron impact (EI) mass spectra were obtained with a VG Trio-1 quadrupole mass spectrometer operating at 70 eV using a direct inlet probe. FABMS, HRFABMS, and HREIMS data were recorded using a VG ZAB-HF double-focusing mass spectrometer.

Fungal Material. The isolate of *Mycelia sterilia* was collected from colonies of *Hypoxylon* stromata found on dead Aspen logs near the Red River in Malette Meadow, New Mexico. Direct plating of stromatal filings was accomplished by sprinkling a small portion (100−200 mg) of the filings over the surface of each of two plates of dextrose-peptone-yeast extract agar (DPYA) containing streptomycin (25 mg/L) and tetracycline (1.25 mg/L). Plates were incubated in the dark at 25 °C for 5 days, and representative cultures of each colony type were isolated. After 7−12 days incubation, the tube cultures isolated from the stromatal filings were segregated into groups of species and maintained for identification and rice fermentation. One of these cultures, *Mycelia sterilia* MYC-155 (= NRRL 29110), produced a nonsporulating, dark olive-colored culture when grown on potato-dextrose agar (PDA) or corn meal. Fermentation and extraction was carried out as described previously.[8]

Isolation. This crude extract obtained from eight 500 mL fermentation flasks each containing 50 g of rice (1.5 g) was first partitioned between hexane (3 × 100 mL) and CH_3CN (50 mL), and the CH_3CN-soluble portion (1.0 g) was fractioned by Sephadex LH-20 column chromatography (30 g; 55 × 2.2 cm) eluting successively with 500 mL each of hexane−CH_2Cl_2 (1:4) and CH_2Cl_2−acetone (3:2 then 1:4). One fraction that was eluted with 1:4 hexane−CH_2Cl_2 (230 mg) consisted of malettinin A (**1**). Another column fraction eluted with 1:4 hexane−CH_2Cl_2 (120 mg) was subjected to Sephadex LH-20 column chromatography (50 × 1.5 cm) using the same gradient. The less polar fraction that was eluted with 1:3 hexane−CH_2Cl_2 (66 mg) was further purified by RP HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; flow rate, 2 mL/min; 20% to 45% CH_3CN in H_2O over 30 min) to provide malettinin B (**2**; 5 mg; t_R 24.6 min). Another fraction eluted with 1:4 hexane−CH_2Cl_2 (37 mg) was further purified by RP HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; flow rate, 2 mL/min; 20% to 30% CH_3CN in H_2O over 45 min) to provide malettinin C (**3**; 3 mg; t_R 22.3 min). A third fraction that was eluted from the original column with 1:4 hexane−CH_2Cl_2 (90 mg) was separated again by Sephadex LH-20 column chromatography (10 g; 30 × 1.5 cm) using the same gradient. The fraction that was eluted with 1:4 hexane−CH_2Cl_2 (33 mg) was subjected to RP HPLC (Alltech HS Hyperprep 100 BDS C_{18}; 10 × 250 mm; flow rate, 2 mL/min; 20% to 40% CH_3CN in H_2O over 45 min) to provide malettinin D (**4**; 4 mg; t_R 28.8 min).

Malettinin B (2): pale yellow oil; $[\alpha]_D$ −10° (*c* 0.1 g/100 mL, $CHCl_3$, 25 °C); UV λ_{max} (EtOH) 253 (ϵ 81 000), 360 (ϵ 23 000); IR ν_{max} ($CHCl_3$) 3383, 3200, 2972, 2940, 1630, 1593, 1445 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations (acetone-d_6, H-# ↔ H-#) H-4 ↔ H_3-16; H-7a ↔ H-7b, H_3-16 and H_3-17; H-7b ↔ H-7a, H_3-16, and H_3-17; H-8 ↔ H_3-17; H-11a ↔ H-11b, H-12, H-13, and H_3-18; H-11b ↔ H-11a, H-12, and H_3-18; H-12 ↔ H-11a, H-11b, H-13, and H_3-18; H-13 ↔ H-11a, H-12, and H_3-18; EIMS (70 eV) *m/z* 292 ([M]$^+$; rel int 1.1), *m/z* 274 ([M − H_2O]$^+$; rel int 6.3); FABMS (3-NBA) obsd *m/z* 293 [M + H]$^+$; HRFABMS obsd *m/z* 293.1389 [M + H]$^+$, calcd for $C_{16}H_{21}O_5$, 293.1389.

Malettinin C (3): pale yellow oil; data recorded using ca. 5:1 mixture of **3** and **2**; $[\alpha]_D$ −18° (*c* 0.1 g/100 mL, $CHCl_3$, 25 °C); UV λ_{max} (EtOH) 253 (ϵ 46 000), 328 (ϵ 9800), 360 (ϵ 14 000); ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; NOESY correlations ($CDCl_3$, H-# ↔ H-#) H-4 ↔ H_3-16; H-7a ↔ H-7b and H_3-16; H-7b ↔ H-7a, H_3-16, and H_3-17; H-8 ↔ H-7a, H-13, and H_3-17; H-11a ↔ H-11b and H-12; H-11b ↔ H-11a, H-12, and H_3-18; H-12 ↔ H-11a, H-11b, and H-13; H-13 ↔ H-8, H-12, and H_3-17; EIMS (70 eV) *m/z* 292 ([M]$^+$; rel int 1.9), *m/z* 274 ([M − H_2O]$^+$; rel int 9.7); *m/z* 277 ([M − CH_3]$^+$; rel int 4.9); FABMS (thioglycerol) obsd *m/z* 293 [M + H]$^+$.

Malettinin D (4): pale yellow crystals; mp 198−200 °C; $[\alpha]_D$ −9.8° (*c* 0.10, $CHCl_3$, 27 °C); UV λ_{max} (EtOH) 210 (ϵ 4800), 268 (ϵ 2300); IR ν_{max} ($CHCl_3$) 3463, 2925, 2854, 1798, 1738, 1707, 1620, 1382, 990 cm^{-1}; ^{1}H NMR, ^{13}C NMR, and HMBC data, see Tables 1−3; HRFABMS (3-nitrobenzyl alcohol/NaI) obsd *m/z* 343.0780 (M + Na)$^+$, calcd for $C_{16}H_{16}O_7Na$, 343.0794.

Single-Crystal X-ray Diffraction Analysis of Malettinin D (4).[18] A pale yellow crystal (0.35 × 0.30 × 0.25 mm) obtained by crystallization from 35% CH_3CN−H_2O was selected for analysis. Data were collected on an Enraf-Nonius CAD4 diffractometer (Mo Kα radiation, λ = 0.71073 Å, graphite monochromator) at 215(2) K (N_2 cold gas stream) using ω−2ω scans (range 2.5−25.0°). Cell dimensions were determined from a least-squares analysis of 25 well-centered reflections to be a = 16.525(7) Å, b = 7.740(2) Å, c = 12.559(6) Å, α = 90°, β = 110.18(3)°, and γ = 90°. Intensity standards were measured at 2 h intervals. Net intensities were obtained by profile analysis of the 5628 data, and the calculated density is 1.411 mg/m^3. The systematic absence corresponded to space group $P2_1/n$. Equivalent data were averaged yielding 2641 unique data (R_{int} = 0.079, 2085 $F > 4\sigma(F)$). Data were corrected for Lorentz and polarization effects, but not for absorption. No change in intensity standards was noted. The computer programs from the MoLEN package were used for data reduction. The structure was solved using XS, a direct methods program, and refined by full-matrix least-squares performed with the SHELXL-97 computer program. Illustrations were made with the XP program, and tables were made with the XCIF program. All are in the SHELXTL v5.1 package. Thermal ellipsoids shown are at the 35% level. The hydroxyl and methyl H atom positions were determined using program constraints (fixed O−H and C−H distances and C−O−H, C−C−H, and H−C−H angles with program default values) that refine rotation about C−OH and C−CH_3 bonds. All non-hydrogen atoms were refined with anisotropic thermal parameters, and the final refinement, based on 2085 reflections, gave R_1 = 0.0477, R_2 = 0.1289 standard deviation.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **2**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Crystallographic data for malettinin D (**4**) have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 256110). 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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