Three New Metabolites from Marine-Derived Fungi of the Genera *Coniothyrium* and *Microsphaeropsis*

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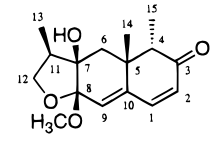
The marine sponges *Ectyplasia perox* and *Myxilla incrustans* were investigated for associated fungal strains. Among others, a *Coniothyrium* sp., from *E. perox*, and a *Microsphaeropsis* sp., from *M. incrustans*, were isolated, cultured, and investigated for their biologically active secondary metabolite contents. The new compound microsphaeropsisin (**1**) together with the known compounds (*R*)-mellein (**4**), (3*R*,4*S*)-hydroxymellein (**5**), (3*R*,4*R*)-hydroxymellein (**6**), and 4,8-dihydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**7**) were isolated from the *Microsphaeropsis* sp. From culture extracts of the *Coniothyrium* sp., the new compounds (3*S*)-(3′,5′-dihydroxyphenyl)butan-2-one (**2**) and 2-(1′(*E*)-propenyl)-octa-4(*E*),6(*Z*)-diene-1,2-diol (**3**), together with the six known metabolites (3*R*)-6-methoxymellein (**8**), (3*R*)-6-methoxy-7-chloromellein (**9**), cryptosporiopsinol (**10**), phenylethanol, (*p*-hydroxyphenyl)ethanol, and 2-(hydroxymethyl)furan, were obtained. All structures were determined using spectroscopic methods. With the exception of **3**, all compounds were tested for their antimicrobial properties, and all but **10** demonstrated significant antimicrobial activity in agar diffusion assays.

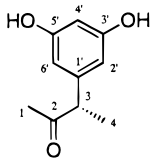
Marine macro-organisms are well-known for their production of diverse and unique biologically active metabolites.[1,2] The supply of these compounds is unfortunately often limited, e.g., due to their low concentrations in the producing organisms and the lack of profitable synthesis for these often complex natural products.[3] As a result of this, marine natural products research is now focusing more on marine microorganisms, mainly bacteria and fungi that can be cultured.[4,5] Obligate marine fungi[6] have been shown to produce some unique and biologically active metabolites.[7,8] Marine isolates of fungal genera usually encountered in terrestrial habitats have been obtained from various substrates, including algae,[9] sea hares,[10] sponges,[11] and sediments.[12] These fungi, which have been proven to be prolific sources of novel compounds, may represent specifically adapted strains of originally terrestrial genera.

In an attempt to investigate the diversity of fungi associated with marine sponges, we isolated more than 500 fungal strains from animals of six different locations and tested extracts of selected strains for their antimicrobial activity and their ability to inhibit HIV-1 reverse transcriptase and tyrosine kinase ($p56^{lck}$).[13] Two of the extracts with antimicrobial properties were derived from a *Coniothyrium* species (strain no. 193H77), isolated from the marine sponge *Ectyplasia perox* collected from the Caribbean Sea, Dominica, Windward Islands, and a *Microsphaeropsis* species (strain no. H5-50), associated with *Myxilla incrustans* from Helgoland, Germany. The observed antimicrobial activity together with TLC information promoted an investigation of the secondary metabolite production of these fungi. Additionally, literature data indicated these two genera to have received only moderate attention by the natural products community.[14,15]

The *Microsphaeropsis* species was cultivated on a solid biomalt medium. Successive workup of the EtOAc extract by vacuum−liquid chromatography (VLC) and normal- and reversed-phase (RP-18) HPLC yielded compounds **1** and **4**−**7**.



1



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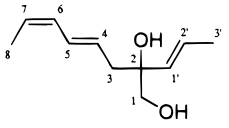

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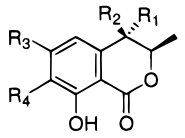
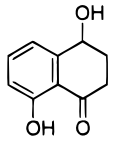
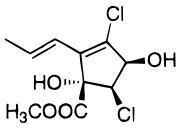


	R_1	R_2	R_3	R_3
4	H	H	H	H
5	OH	H	H	H
6	H	OH	H	H
8	H	H	OCH_3	H
9	H	H	OCH_3	Cl



7



10

The molecular formula of **1** was determined as $C_{16}H_{22}O_4$ by accurate mass measurement. ^{13}C NMR spectroscopy (^1H decoupled and DEPT) showed that three of the six elements of unsaturation, as indicated by the molecular formula of **1**, could be attributed to two carbon−carbon double bonds (126.3 ppm, d, C-2; 126.9 ppm, d, C-9; 140.9 ppm, s, C-10 and 143.9 ppm, d, C-1) and a carbonyl group (204.2 ppm, s, C-3); the molecule is thus tricyclic. After association of all ^1H NMR resonances with the ^{13}C NMR resonances of the directly bonded carbon atoms via a $^1H-^{13}C$ 2D NMR shift-correlated measurement (HMQC), the presence of a single OH group was evident (3.03 ppm, d, $J = 1.9$ Hz). ^1H and ^{13}C NMR spectra further revealed the presence of one methoxyl group (48.3 ppm), two methylene groups (42.1 ppm, t, C-6 and 72.8 ppm, t, C-12), one of which had to be

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Table 1. ^{1}H (300 MHz, $CDCl_3$) and ^{13}C (75.5 MHz, $CDCl_3$) NMR Spectral Data for Microsphaeropsisin (**1**)

position	δ_H	δ_C[a]	HMBC[b]	NOE[c]
1	6.89 (d, J = 9.9 Hz)	143.9 (d)	H-9	*d*
2	5.90 (d, J = 9.9 Hz)	126.3 (d)		*d*
3		204.2 (s)	H-1, H-4, H_3-15	
4	2.27 (q, J = 7.3 Hz)	53.5 (d)	H_3-14, H_3-15	*d*
5		39.7 (s)	H-1, H_2-6, H-9, H_3-14, H_3-15	
6α	1.87 (brd, J = 14.2 Hz)	42.1 (t)	O*H*-7, H-11, H-14	H-6*β*, H-11, H-12α, H_3-15
6*β*	1.68 (d, J = 14.2 Hz)			*d*
7	O*H* 3.03 (d, J = 1.9 Hz)	78.2 (s)	H-6*β*, O*H*-7, H-9, H-11, H-12α, H_3-13	H_3-13, H_3-14, OCH_3
8		101.3 (s)	H-6*β*, H_2-12, OCH_3	
9	6.24 (s)	126.9 (d)	H-1	H-1
10		140.9 (s)	H-1, H-4, H-6*β*, H_3-14	
11	2.10 (ddq, J = 6.5, 7.3, 8.4 Hz)	42.9 (d)	H_3-13	*d*
12α	4.13 (dd, J = 8.4, 8.8 Hz)	72.8 (t)	H_3-13	H-12*β*
12*β*	3.50 (dd, J = 6.5, 8.8 Hz)			H-12α
13	1.05 (d, J = 7.3 Hz)	14.4 (q)	H-11, H_2-12	O*H*-7, H-12*β*
14	1.39 (s)	27.4 (q)	H_2-6	H-4, H-6*β*, O*H*-7
15	0.98 (d, J = 7.3 Hz)	14.6 (q)	H-4	H-6α
OCH_3	3.43 (s)	48.3 (q)		O*H*-7, H-9

[a] Multiplicity deduced by DEPT. [b] Protons showing long-range couplings to carbon atoms. [c] Signals enhanced as observed by difference NOE measurements. [d] Resonance frequency not irradiated.

attached to oxygen, two methine groups (2.10 ppm, m, H-11 and 2.27 ppm, q, J = 7.3 Hz, H-4) with adjacent methyl groups (0.98 ppm, d, J = 7.3 Hz, H_3-15 and 1.05 ppm, d, J = 7.3 Hz, H_3-13), one quaternary methyl group (1.39 ppm, s, H_3-14), and three quaternary carbon atoms (39.7 ppm, s, C-5; 78.2 ppm, s, C-7 and 101.3 ppm, s, C-8), including one substituted with an oxygen-containing functionality and one as part of an acetal moiety. From the ^{1}H−^{1}H COSY spectrum of **1** three fragments of the molecule could be deduced. Thus, coupling was observed between H_3-15 and H-4, H_3-13 and H-11, and further to H_2-12. The only other ^{1}H−^{1}H spin system involved the methine protons H-1 (6.89 ppm, d, J = 9.9 Hz) and H-2 (5.90 ppm, d, J = 9.9 Hz) and left C-9 and C-10 to form the second carbon−carbon double bond. A UV maximum at 280 nm indicated that the double bonds and the carbonyl group were conjugated. This deduction was confirmed via a ^{1}H−^{13}C HMBC measurement (Table 1), which revealed both the carbonyl group (C-3) and the $\Delta^{9,10}$ double bond to be joined to the $\Delta^{1,2}$ double bond. Long-range correlations between both H-4 and H_3-15 and C-3 established the connectivity of C-3 and C-4. Correlations between H_3-14 and C-4, C-5, C-6, and C-10 showed that C-14 was bound to C-5 and further C-5 to C-4, C-10 and C-6, thus establishing the first of the three rings. Further, long-range correlations between the hydrogens of the hydroxyl and the methoxyl group and C-7 and C-8, respectively, located the hydroxyl group at C-7 and the methoxyl group at C-8. As evidenced by its ^{13}C NMR chemical shift (101.3 ppm), C-8 is the carbon atom involved in the acetal moiety and must be further connected to the methylene group C-12 via the remaining oxygen atom. Further long-range correlations between O*H*-7 and C-6 and between both H_3-13 and H-11 and C-7 connected both C-6 and C-11 to C-7, leaving the acetal moiety (C-8) to be bound to both C-7 and C-9, thus completing the planar structure of **1**. The relative configurations at the five chiral centers were established by NOE difference measurements, the results of which are shown in Table 1. Irradiation at the resonance frequency of H_3-15 (0.98 ppm) enhanced only the signal of H-6α, whereas irradiation at 1.39 ppm (H_3-14) enhanced the signals of H-4, H-6*β*, and O*H*-7. Irradiation at 1.87 ppm (H-6α) enhanced the signals of H-11 and H_3-15; irradiation at 3.03 ppm (O*H*-7) enhanced the signals of H_3-13, H_3-14, and the methoxyl group. Further, irradiation at the resonance frequency of H_3-13 (1.05 ppm) enhanced the signals of H-12*β* and O*H*-7. Thus, H-4, H-6*β*, O*H*-7, H-12*β*, H_3-13, H_3-14, and the methoxyl group had to be on the same side of the molecule and H-6α and H_3-15 on the other side. Finally, irradiation at 6.24 ppm (H-9) enhanced the signal of H-1, confirming the double-bond assignments. Compound **1** is thus a new natural product of the eremophilane type for which we suggest the trivial name microsphaeropsisin.

Compounds **4**−**7** were identified by comparison of their spectroscopic data and optical rotations with published values as the known fungal metabolites (*R*)-mellein (**4**),[16,17] (3*R*,4*S*)-hydroxymellein (**5**),[18,19] (3*R*,4*R*)-hydroxymellein (**6**),[19,20] and 4,8-dihydroxy-3,4-dihydro-2*H*-naphthalen-1-one (**7**).[21,22] The observed optical rotation of zero for **7** suggests the isolate to be racemic.

The *Coniothyrium* species was grown in liquid shake culture and subsequently extracted with EtOAc. The extract was separated by VLC followed by normal- and reversed-phase HPLC to yield compounds **2**, **3**, and **8**−**10**, as well as phenylethanol, (*p*-hydroxyphenyl)ethanol, and 2-(hydroxymethyl)furan.

The molecular formula of **2** was established as $C_{10}H_{12}O_3$ by accurate mass measurement, indicating the molecule to have five elements of unsaturation. ^{1}H NMR measurements revealed the presence of two methyl groups (1.35 ppm, d, J = 6.8 Hz, H_3-4 and 2.08 ppm, s, H_3-1), the first with an adjacent methine group (3.63 ppm, q, J = 6.8 Hz, H-3) and the other connected to a double bond, two hydroxyl groups (5.19 ppm, brs), and three aromatic hydrogens (6.27 ppm, m). From the ^{13}C NMR and DEPT spectra a carbonyl group (209.3 ppm, s, C-2) and three quaternary aromatic carbons (143.2 and 2 × 157.3 ppm) were deduced. These findings, together with UV maxima at 217 and 280 nm suggested the presence of a 1,3,5-trisubstituted aromatic ring. The ^{13}C NMR chemical shifts of the ring carbons, ranging from 101.8 to 157.3 ppm, showed the typical influence of electronegative ring substituents. Thus, the two OH groups had to be phenolic, accounting for two of the three substituents on the aromatic ring. The ^{1}H NMR signal for the methyl group H_3-1 (2.08 ppm) appeared as a singlet, indicating it to be adjacent to the carbonyl group C-2, leaving the methine group to be positioned between it and the aromatic ring, thus giving **2**. Comparison of the ^{1}H NMR shifts of **2** with published values for the dimethoxyl derivative clearly substantiated the structure proposed for **2**.[23] The absolute configuration at C-3 was determined as *S* by comparison of the optical rotation of **2** (+124.0°) to those of similar 3-phenylbutan-

2-ones, e.g., −350° for (3*R*)-phenylbutan-2-one,[24] +340° for 3(*S*)-phenylbutan-2-one,[24] and +218° for (3*S*)-(*p*-methoxyphenyl)butan-2-one.[25] Thus, **2** is the new compound (3*S*)-(3′,5′-dihydroxyphenyl)butan-2-one.

Compound **3** was obtained as a clear oil. CIMS with NH_3 gave ions at *m/z* 182 [M^+] and 200 [$M^+ + NH_4$]. CIMS with isobutane resulted in ions at *m/z* 182 [M^+] and 165 [$MH^+ - H_2O$], while GC−EIMS yielded fragment ions at *m/z* 101 [$M^+ - C_6H_9$] and 151 [$M^+ - CH_2OH$]; the molecular formula of **3** is $C_{11}H_{18}O_2$. From a $^1H-^{13}C$ 2D NMR shift correlated measurement (HMQC) 16 hydrogens could be assigned to their directly bonded carbon atoms, revealing the presence of two methyl groups (13.3 ppm, C-8, and 17.8 ppm, C-3′), one methylene group (41.2 ppm, C-3), one methylene group adjacent to an oxygen (68.8 ppm, C-1), six olefinic methine groups (125.6 ppm, C-7; 126.5 ppm, C-2′; 127.4 ppm, C-4; 129.0 ppm, C-6; 129.7 ppm, C-5; and 133.3 ppm, C-1′), and one quaternary carbon atom (74.8 ppm, C-2) adjacent to oxygen. Thus, two hydroxyl groups had to be present within the molecule. From the $^1H-^1H$ COSY spectrum, the molecular fragments from C-3 to C-8 and C-1′ to C-3′ could be established. Coupling was observed between H-1′ (5.47 ppm, dd, $J = 1.6, 15.7$ Hz) and H-2′ (5.77 ppm, dq, $J = 6.8, 15.7$ Hz), and H-2′ and H_3-3′ (1.75 ppm, brd, $J = 6.8$ Hz). Further, H_2-3 (2.40 ppm, m) coupled with H-4 (5.62 ppm, ddd, $J = 7.6, 7.6, 15.2$ Hz), H-4 with H-5 (6.45 ppm, dd, $J = 11.4, 15.2$ Hz), H-5 with H-6 (5.99 ppm, brdd, $J = 10.8, 11.4$ Hz), H-6 with H-7 (5.45 ppm, dq, $J = 6.8, 10.8$ Hz), and H-7 with H_3-8 (1.75 ppm, brd, $J = 6.8$ Hz). HMBC $^1H-^{13}C$ long-range correlations observed between H_2-3, H-4, H-1′, and C-2 linked both of these fragments to the quaternary carbon atom C-2. The hydrogens of the remaining hydroxymethyl group C-1 showed long-range correlations to C-3 and, hence, also had to be connected to C-2. The configurations of the double bonds were determined to be 4*E*,6*Z*,1′*E* on the basis of the $^1H-^1H$ spin coupling constants ($J = 15.2, 10.8, 15.7$ Hz, respectively) and confirmed by NOE difference measurements. Irradiation at the ^1H NMR frequency of H_3-8 and H_3-3′ enhanced the signals of both H-5 and H-1′, confirming the *Z* configuration at $\Delta^{6,7}$ and the *E* configuration at $\Delta^{1',2'}$. Thus, **3** is the new compound 2-(1′(*E*)-propenyl)-octa-4(*E*),6-(*Z*)-diene-1,2-diol.

Compounds **8** and **9** were identified by comparison of their spectroscopic data and optical rotations with published values as (3*R*)-6-methoxymellein and (3*R*)-6-methoxy-7-chloromellein.[26] Phenylethanol, (*p*-hydroxyphenyl)-ethanol, and 2-(hydroxymethyl)furan were identified by NMR[27] and EIMS. Compound **10** was found to be cryptosporiopsinol by comparison of its ^1H and ^{13}C NMR data and optical rotation with published values.[28,29]

From the two fungal genera investigated, three new compounds (**1**−**3**) have been isolated. This result suggests that marine-derived fungal strains of the genera investigated are a source of new natural products and, in this sense, as interesting as their terrestrial counterparts. Sesquiterpenes of the eremophilane type have been reported mainly from higher plants[30] but are also encountered in fungi, e.g., in *Penicillium roqueforti*[31] and the marine mitosporic fungus *Dendryphiella salina*.[32] In most sesquiterpenes of the eremophilane type described to date, CH_3-14 and CH_3-15 are cis, indicating microsphaeropsisin (**1**) to be a rare eremophilane derivative in having these moieties trans.

Pentaketides of the mellein-type (**4**−**6**, **8**, and **9**) and the related compounds **7** and **10** are well-known fungal metabolites, most of them with biological (predominantly antimicrobial) activity. (−)-Mellein (**4**) was first described in 1933 from *Aspergillus melleus*.[33] Since then, both stereoisomers have been frequently reisolated from different fungi.[34,35] The mellein derivatives **5**, **6**, and **9** also represent typical fungal metabolites, first isolated from *Septoria nodorum* (**5**),[18] *Lasiodiplodia theobromae* (**6**),[20] and *Sporormia affinis* (**9**),[26] respectively. In contrast, the 6-methoxyl derivative **8** was first described from stored carrots[36] and has since been described both as a fungal metabolite[26] and as a phytoalexin[37] produced by cultured carrot cells in response to fungal infection. Cryptosporiopsinol (**10**) is a fungal metabolite biosynthetically related to mellein.[28,29] Compound **7** was first isolated from a fungus of the genus *Scytalidium*[21] and named 4,8-dihydroxytetralone. Stereoisomers have since then been reported not only from other fungi, e.g., as (+)-isosclerone from *Sclerotinia sclerotiorum*,[38] but also from the stem bark of the walnut tree *Juglans regia* as (−)-regiolone.[22] Phenylethanol and (*p*-hydroxyphenyl)ethanol are presumably decarboxylation and deamination products of phenylalanine and tyrosine and have been isolated from various fungi.[35]

All compounds except **3**, which decomposed prior to its biological investigation, were tested for their antimicrobial activity in agar diffusion[39] and ELISA-based assays for HIV-1 reverse transcriptase and tyrosine kinase ($p56^{lck}$) inhibitory activity. While no compound showed a significant activity in the enzyme-inhibition assays, all compounds, except **10**, possessed moderate antimicrobial activity at the 50 μg level. The most commonly observed effect was antifungal activity: compounds **1**, **4**, **6**−**9**, phenylethanol, and (*p*-hydroxyphenyl)ethanol inhibited *Eurotium repens*; **1**, **2**, **5**−**7**, and phenylethanol inhibited *Ustilago violacea* and **2** inhibited *Mycotypha microspora*. The growth of *Bacillus megaterium* was inhibited only by 2-(hydroxymethyl)furan. The observed antimicrobial activity of nearly all isolated compounds and the relatively high abundance of some of these in the extract, e.g., mellein and the hydroxymelleins, explains the initially observed activity of the extracts.

Experimental Section

General Experimental Procedures. The general experimental procedures were carried out as previously described.[40]

Isolation and Taxonomy of the Fungal Strains. The *Coniothyrium* strain (strain no. 193H77) was isolated from the sponge *E. perox*, collected by divers using scuba from the waters around the Caribbean Island of Dominica, by inoculating small pieces of its inner tissue on cellulose agar: cellulose (10 g/L), yeast extract (1 g/L), benzylpenicillin (250 mg/L), streptomycin sulfate (250 mg/L), agar (15 g/L), ASW (800 mL/L). Artificial seawater (ASW) contained the following salts (g/L): KBr (0.1), NaCl (23.48), $MgCl_2 \cdot 6H_2O$ (10.61), $CaCl_2 \cdot 2H_2O$ (1.47), KCl (0.66), $SrCl_2 \cdot 6H_2O$ (0.04), Na_2SO_4 (3.92), $NaHCO_3$ (0.19), H_3BO_3 (0.03). The strain was identified as belonging to the genus *Coniothyrium* by Dr. S. Draeger, Institute for Microbiology, TU Braunschweig. Morphology on SNA agar: mycelium superficial, partly immersed, branched, septate, hyaline. Conidiomata pycnidial, 85−125 μm in diameter, globose, brownish black, superficial, unilocular, wall of dark brown textura angularis. Conidiogenous cells annellidic, ampulliform, 1.7−2.6 × 2.2−6.0 μm, hyaline. Conidia (1.3) 1.7 × 2.2−3.0 μm, brown, thick-walled, smooth, aseptate, cylindrical, apexes obtuse. The *Microsphaeropsis* strain (strain no. H5-50) was isolated from the sponge *M. incrustans* collected by scuba divers from the waters around Helgoland, German Bight, by inoculating small pieces of its inner tissue on glucose peptone yeast extract agar: glucose·H_2O (1 g/L), peptone from soymeal (0.5 g/L), yeast extract (0.1 g/L), benzylpenicillin (250 mg/L), streptomycin sulfate (250 mg/L), agar

(15 g/L), ASW (800 mL/L). The strain was identified as belonging to the genus *Microsphaeropsis* by Dr. R. A. Samson, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Morphology on SNA agar: mycelium superficial, partly immersed, branched, septate, hyaline. Conidiomata pycnidial, 65−110 μm in diameter, globose, dark brown, superficial, unilocular, ostiole slightly papillate, wall of olivaceous brown textura angularis. Conidiogenous cells phialidic, cylindrical, 1.5 × 4.5 μm. Conidia 1.3−1.7 × 3.0−4.3 μm, olivaceous brown, thin-walled, aseptate, smooth, cylindrical, some slightly allantoid, apexes obtuse. The genera *Coniothyrium* and *Microsphaeropsis* are related morphologically.[41] A species determination is often impossible under laboratory conditions. Both strains are deposited at the fungal culture collection of the Institute for Microbiology, Technical University of Braunschweig, Germany.

Cultivation. The *Coniothyrium* strain was cultivated in liquid shake culture (Biomalt agar with artificial seawater, biomalt (Vitaborn, Hameln, Germany) 20 g/L, 15 × 0.5 L, 50 U/min) at room temperature for 16 days. The *Microsphaeropsis* strain was cultivated on solid medium in penicillium flasks (Biomalt agar; biomalt 20 g/L, agar 6.8 g/L, 9 × 0.5 L) for 40 days at room temperature. Media were inoculated with small mycelia plugs from stock culture (*Coniothyrium* sp.) or a suspension of mycelia from small-scale cultivations (one Petri dish per five penicillium flasks) in water (*Microsphaeropsis* sp.).

Biological Activity. Antimicrobial testing was performed against the bacteria *Escherichia coli* (Migula) Castellani & Chambers (Gram negative) and *B. megaterium* de Bary (Gram positive), the fungi *U. violacea* (Pers.) Roussel (Ustomycetes), *E. repens* Corda (Ascomycetes), *M. microspora* Fenner (Zygomycetes), and *Fusarium oxysporum* Schltdl. (Fungi imperfecti), and the alga *Chlorella fusca* Shih Krauss (Chlorophyceae) using agar diffusion assays.[39] Applied amounts were 250 μg of extract or 50 μg of compound per test disk.

Extraction and Isolation. *Coniothyrium* sp.: Cultivation broth and mycelia were homogenized using a Waring blender, and the resulting mixture was extracted with EtOAc (3 × 5 L) to yield 1.75 g of a brown oil. The extract was fractionated by VLC (Si gel 60, gradient hexane to EtOAc) to yield five fractions. Fractions 3 (250 mg) and 4 (149 mg) were further fractionated by normal-phase HPLC (hexane/EtOAc 80/20) to yield **8** (5 mg, 0.67 mg/L medium), phenylethanol (12 mg, 1.6 mg/L medium), and 2-(hydroxymethyl)furan (17 mg, 2.3 mg/L medium). Further purification of one previous HPLC fraction by reversed-phase (C_{18}) HPLC (methanol/water 70/30) yielded **10** (5.1 mg, 0.68 mg/L medium). Fraction 5 (75 mg) was fractionated by normal-phase HPLC (hexane/EtOAc 70/30) to yield **2** (1.2 mg, 0.16 mg/L medium), **3** (1.3 mg, 0.17 mg/L medium), **9** (7.0 mg, 0.93 mg/L medium), and (*p*-hydroxyphenyl)ethanol (3.0 mg, 0.4 mg/L medium).

Microsphaeropsis sp.: Mycelia and medium were diluted with water (1.5 L) and homogenized using a Waring blender. The resulting mixture was extracted with EtOAc (3 × 3 L) to yield 0.93 g of brown oil. This extract was fractionated by VLC (Si gel 60, gradient hexane/EtOAc) to yield four fractions. Fraction 2 (145 mg) was further fractionated by normal-phase HPLC (hexane/EtOAc 70/30) to yield **1** (1.3 mg, 0.29 mg/L medium), **4** (20.8 mg, 4.6 mg/L medium), **5** (425 mg, 94 mg/L medium), and **6** (51 mg, 11 mg/L medium). Further purification of one previous HPLC fraction with reversed-phase (C_{18}) HPLC (methanol/water 75/25) yielded **7** (5 mg, 1.1 mg/L medium).

Microsphaeropsisin (1): colorless powder (1.3 mg); mp 152−154 °C; $[\alpha]^{20}_D$ −97.7° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 223 (2250), 280 (8560) nm; IR (film) ν_{max} 3515, 2925, 1665, 1245, 1205, 1180, 1025 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* [M$^+$] 278 (100), 247 (56), 221 (56), 203 (94), 162 (54), 134 (70); HREIMS *m/z* 278.151 (calcd for $C_{16}H_{22}O_4$ 278.152).

(3*S*)-(3′,5′-Dihydroxyphenyl)butan-2-one (2): colorless oil (1.2 mg); $[\alpha]^{20}_D$ +124.0° (*c* 0.12, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 217 (27 900), 280 (1170) nm; IR (film) ν_{max} 3250, 2920, 2850, 1670, 1605, 1455, 1155 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (3H, d, J = 6.8 Hz, H-4), 2.08 (3H, s, H-1), 3.63 (1H, q, J = 6.8 Hz, H-3), 5.19 (2H, brs, OH-3′/5′), 6.27 (3H, m, H-2′/H-4′/H-6′); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 16.9 (q, C-4), 28.3 (q, C-1), 53.5 (d, C-3), 101.8 (d, C-4′), 107.4 (d, C-2′/C-6′), 143.2 (s, C-1′), 157.3 (s, C-3′/C-5′), 209.3 (s, C-2); EIMS *m/z* [M$^+$] 180 (55), 149 (45), 137 (100); HREIMS *m/z* 180.078 (calcd for $C_{10}H_{12}O_3$, 180.079).

2-(1′(*E*)-Propenyl)-octa-4(*E*),6(*Z*)-diene-1,2-diol (3): colorless oil (1.3 mg); $[\alpha]^{20}_D$ −13.8° (*c* 0.13, $CHCl_3$); UV (EtOH) λ_{max} (ϵ) 235 (12 600) nm; IR (film) ν_{max} 3385, 2925, 2855, 1675, 1450, 1440, 1375, 1055, 1035, 985, 970 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.75 (6H, brd, J = 6.8 Hz, H-8/H-3′), 2.40 (2H, m, H-3), 3.48 (2H, m, H-1), 5.45 (1H, dq, J = 6.8, 10.8 Hz, H-7), 5.47 (1H, dd, J = 1.6, 15.7 Hz, H-1′), 5.62 (1H, ddd, J = 7.6, 7.6, 15.2 Hz, H-4), 5.77 (1H, dq, J = 6.8, 15.7 Hz, H-2′), 5.99 (1H, brdd, J = 10.8, 11.4 Hz, H-6), 6.45 (1H, dd, J = 11.4, 15.2 Hz, H-5); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.3 (q, C-8), 17.8 (q, C-3′), 41.2 (t, C-3), 68.8 (t, C-1), 74.8 (s, C-2), 125.6 (d, C-7), 126.5 (d, C-2′), 127.4 (d, C-4), 129.0 (d, C-6), 129.7 (d, C-5), 133.3 (d, C-1′); CIMS (NH_3) *m/z* 200 (100) [M$^+$ + NH_4], 182 (15) [M$^+$]; CIMS (isobutane) *m/z* 182 (15) [M$^+$], 165 (100) [MH$^+$ − H_2O]; GC−EIMS *m/z* 151 (8) [M$^+$ − CH_2OH], 101 (100) [M$^+$ − C_6H_9], 83 (92), 69 (62), 55 (86); HRMS sample decomposed prior to this measurement.

(3*R*)-6-Methoxy-7-chloromellein (9): white powder (7 mg); $[\alpha]^{20}_D$ −47° (*c* 0.3, $CHCl_3$); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 20.6 (q, *C*H$_3$), 34.8 (t, C-4), 56.5 (q, *C*H$_3$O), 75.7 (d, C-3), 101.1 (d, C-5), 102.9 (s, C-8a), 108.3 (s, C-7), 138.9 (s, C-4a), 159.2[a] (s, C-6), 160.8[a] (s, C-8), 169.5 (s, C-1); EIMS *m/z* [M$^+$] 244 (32), 242 (100), 226 (10), 224 (30), 200 (23), 198 (66); ^{1}H NMR data in agreement with literature values.[19] [a]Assignment may be interchanged.

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